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Functionalized hydrazide macrocycle ion channels showing pH-sensitive ion selectivities

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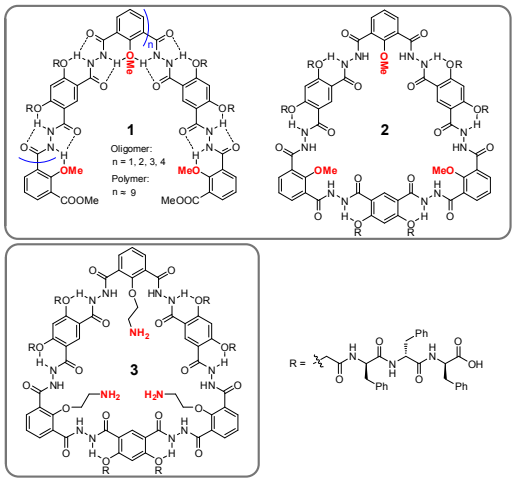
Transmembrane channels formed by functionalized hydrazide macrocycles are reported. The different pH values of buffer solution have a significant effect on the K^+/Cl^- selectivity of the macrocycles. This unique transport behavior is mainly induced by the different distribution of charges in the tubular channels under various pH values.

Transmembrane transport of charged species across biological membranes is crucially important for cellular pH regulation, metabolite neuronal exchange, nerve and muscle excitation. Natural ion channels are complex proteins that can form porous structures in biological membranes to mediate the transport of ions.[1] Inspired by the wide-ranging functions of natural ion channels, chemists have made great efforts to constructing synthetic mimics for gaining insights into the mechanism of natural channels,[2] and developing new technologies in separation, purification and sensing.[3] Currently, non-regulable artificial channels have been extensively studied.[4] In order to make artificial channels possess more similar qualities to their natural prototype, several regulable artificial channels have also been described.[5] Variety signals, such as voltage,[6] light,[7] pH[8] and the binding of ligands[9] were used to regulate the ion-transport properties of artificial channels. In spite of important advances, the design of new unimolecular channel responsive to external stimulus which can be easily synthesized is still a challenge.

Recently, Li and Hou have demonstrated that hydrogen-bonded aromatic hydrazide foldamers can efficiently mediate the transmembrane transport of cations by forming a helical tube across lipid bilayers (Scheme 1, **1**).[10] To further explore the influence of the backbone cavity on the transporting process, they have reported several shape-persistent aromatic hydrazide macrocycles which exhibit excellent membrane-insertion capacity and high NH_4^+/K^+ transport selectivity (Scheme 1, **2**).[11] Compare to the helical channel, the macrocyclic channel can be easily synthesized from one-step macrocyclization. And the macrocyclic channels have rigid backbone, nano-scaled cavity and multiple modifiable sites. All of these make it possible to modify the backbones of the macrocycles with inward-pointing functional groups, by which regulable macrocyclic channels may be developed.



Scheme 1 Structures of compounds **1–3**.

Herein we have reported the new aromatic hydrazide macrocycles **3** and their ion-transport properties (Scheme 1, **3**). For these macrocycles, three inward-pointing amino groups were introduced into the backbone cavity, and the Phe-based tripeptide chains were used to enhance membrane-incorporation ability of aromatic frameworks. In this paper, we showed that the new macrocycles can efficiently mediate the transmembrane transport of ions. The pH of buffer solution has a significant effect on the ion selectivity of the macrocycles, due to the presence of the amino groups.

We prepared aromatic hydrazide macrocycles **3** from the one-step macrocyclization of corresponding 4,6-dialkoxyisophthalohydrazide **4** and diacyl chloride precursors **5**

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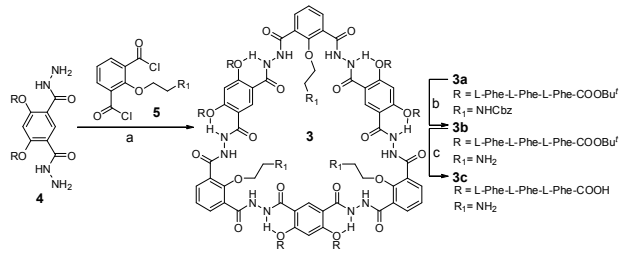
(see ESI).[11-12] Amino group deprotection of **3a** afforded compound **3b**. Carboxybutyl hydrolysis of **3b** yielded the compound **3c**. We also synthesized Compound **2** as control compound.



Scheme 2 Synthesis of compound **3** (a) Et_3N, DMA, r.t.; (b) H_2, Pd/C, MeOH, r.t.; (c) TFA, DCM, r.t.

To evaluate the incorporating ability of these macrocycles into lipid bilayers, the proton-transport activities of **3b, 3c** and **2** on vesicles were investigated using HPTS assay.[9c] Briefly, a suspension of Large Unilamellar Vesicles (LUVs) composed of egg yolk L-α-phosphatidylcholine (EYPC) entrapping pH-sensitive dye 8-hydroxypyrene-1,3,6-trisulfonate (HPTS, 0.1 mM, pH = 7.2) was first prepared. Then, a pH gradient across the membranes was introduced by addition of the vesicle solution to a buffer (pH = 6.0). By measuring the fluorescent intensity, the H^+ transport activity of these macrocycles was gauged. After a solution of **3b**, **3c**, or **2** in DMSO (x = 0.1%, molar ratio relative to lipid, represented by x) was injected into the vesicle solution, the fluorescence intensity of HPTS was increased significantly and reached 26%, 60%, 84% respectively (Fig. 1a), which supports the hypothesis that the macrocycles were involved in the H^+ transport across the membrane of the vesicles.

By fitting the dose–response curves with Hill equation, the effective concentration needed for 50% activity (EC_{50}) were determined to be 0.248% (**3b**), 0.033% (**3c**) and 0.0037% (**2**), respectively (Fig. S21, ESI).[13] The EC_{50} value of **3c** was remarkably higher than that of **2**. Because of their identical peripheral structure**,** this result can only be attributed to the different inward-pointing groups of **3c** ($-OCH_2CH_2NH_2$) and **2** (–OMe). It seems plausible that the decreased proton-transport activity of **3c** is due to the steric hindrance of alkylamino groups, which affected the passage of H^+ through the inner pore of the channel. This possibility has been ruled out by the additional chloride-transport expermients. The chloride-transport experiments were assessed on LUVs containing lucigenin (LG, 2 mM, pH = 6.2), a dye whose fluorescence emission is quenched by chloride ion.[14] The results are shown in Fig. 1b. After a solution of **3b**, **3c**, or **2** in DMF (x = 0.1%) was added to the vesicles, the fluorescence intensity of LG was significantly enhanced and reached 36%, 92%, 87% respectively. The EC_{50} values were determined to be 0.134% (**3b**), 0.0095% (**3c**) and 0.016% (**2**) (Fig. S22, ESI). It was found that the EC_{50} value of **3c** was lower than that of **2**, which means **3c** has a higher chloride-transport activity. Thus, the decreased proton-transport activity of **3c** is not caused by the steric hindrance of alkylamino groups. In buffer solution (pH = 6.2), compound **3c** possesses a positively charged inner cavity, due to the protonation of the inward-pointing amino groups. This positive charge likely leads to electrostatic interactions that repulsively with protons but attractively with chloride ions.

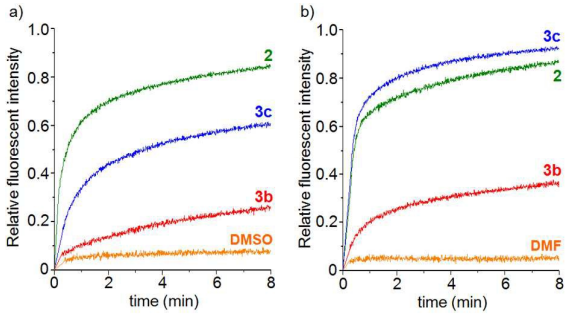


Fig. 1 (a) Changes in the fluorescent intensity of HPTS (λ_{ex} = 460 nm, λ_{em} = 510 nm) in vesicles with time after addition of macrocycles **2** and **3** (x = 0.1%). (b) Changes in the fluorescent intensity of LG (λ_{ex} = 372 nm, λ_{em} = 503 nm) in vesicles with time after addition of macrocycles **2** and **3** (x = 0.1%).

In addition, for the vesicular transport of proton and chloride ion texts, the EC_{50} values of 3**c** was obviously lower than that of **3b**, which indicated that the hydrophilic terminal carboxylic acid groups of the **3c** might enhance the membrane-incorporation ability of this compound.

To investigate the ion-transport activity of these macrocycles, the patch-clamp experiments on planar lipid bilayer were further performed.[15] For these experiments, two compartments containing KCl solution (1.0 M) were separated by a planar lipid bilayer composed of diphytanoylphosphatidylcholine (diPhyPC). A solution of **3b**, **3c**, or **2** in DMSO was added to the *cis* compartment which was grounded, to reach a final concentration of 0.5 μM. After addition of compounds, regular square–like, long-lived single-channel currents were observed, when a clamped voltage +100 mV was applied across the planar lipid bilayer (Fig. 2). These results provide strong evidence that the macrocycles could incorporate into the lipid bilayer and form ion channels.[16]

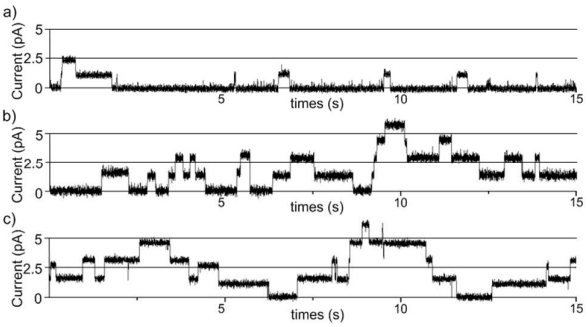


Fig. 2 Current traces (15 s) of (a) **3b**; (b) **3c** and (c) **2** all at 0.5 μM in the planar lipid bilayer at +100 mV in a symmetrical KCl solution (1.0 M).

The current–voltage (I–V) plots were obtained from the patch clamp experiments at different voltages (Fig. 3). All of these I–

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V plots displayed a linear relationship in the range of −100 to +100 mV. Their corresponding conductances (γ) for K^+ were calculated to be 11.3 ± 0.2 (**3b**), 14.6 ± 0.2 (**3c**) and 16.0 ± 0.3 (**2**) pS. These γ values are comparable to the previously reported value (γ= 20 ± 0.2 pS) for gramicidin A[17], showing the high efficiency of these macrocycles in transporting K^+.

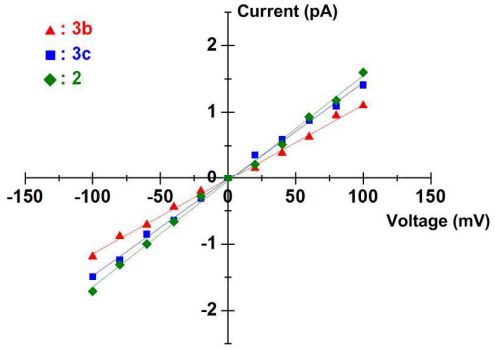

Fig. 3 *I-V* plots of macrocycles (0.5 μM) in the planar lipid bilayer in a symmetrical KCl solution (1.0 M).

Having established that macrocycles **3b**, **3c** and **2** mediates the efficient transport of K^+ ions through a channel mechanism, the patch-clamp experiments were further used to probe their ion selectivity. Alkaline cations selectivities were first tested by repetition of the patch-clamp experiment with different M^+/K^+ gradients under neutral conditions. From the corresponding *I–V* curves, we could determine their reversal potentials (ε_{rev}) for the respective cations and the permeability (*P*) ratios using the Goldman−Hodgkin−Katz equation.[18] All the channels exhibited an Eisenman-I selectivity towards alkaline cations ($Cs^+ > Rb^+ > K^+ > Na^+$) (Fig. S24-25, ESI). These findings indicated that the cations moved across the cavity of the macrocyclic channels after being dehydrated.[19]

In these new macrocyclic channels, multiple amino and carboxyl groups were introduced at backbone cavity and channel opening, respectively. We postulated that the pH value of electrolyte may influence the ion selectivity of the macrocyclic channels. To inspect and verify this possibility, the K^+/Cl^- selectivity of these macrocycles were confirmed by measuring the *I–V* curves under different pH values. Briefly, the saline solutions (0.3M and 1M) were adjusted to pH 4.0, 7.0, or 10.0. Then, the saline solutions were added to the both side of the bilayer (diPhyPC), *trans* chamber: KCl (1.0 M), *cis* chamber: KCl (0.3 M). The P_{K^+}/P_{Cl^-} values were obtained from Goldman−Hodgkin−Katz equation as described before. The results are shown in Fig. 4 (see ESI Fig. S26-28 for the details of experiment). For compound **3b** and **3c**, the K^+/Cl^- selectivity decreased considerably with the decrease of the pH value of electrolyte. At pH 10.0, the P_{K^+}/P_{Cl^-} values of **3b** and **3c** was determined to be 9.3 and 7.4, which revealed that the two macrocyclic samples were mainly transport K^+ under alkaline condition. At pH 7.0, the P_{K^+}/P_{Cl^-} values of the two macrocycles were reduced to 4.9 and 3.7. At pH 4.0, **3b** and **3c** displayed lower P_{K^+}/P_{Cl^-} values (2.8 and 2.3). This result implies that the macrocyclic channels have a signficantly reduced transport activity toward K^+ under acidity condition, which is associated with enhanced Cl^- transport activity. At pH 4.0, 7.0 and 10.0, there was no significant change in K^+/Cl^- selectivity of compound **2.**

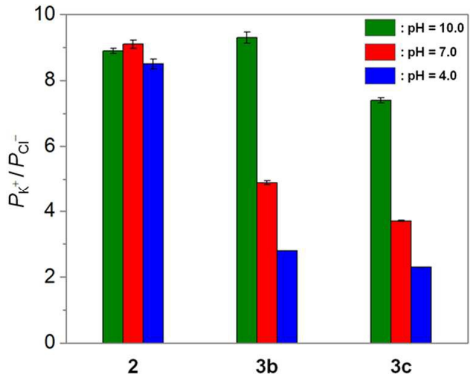

Fig. 4 P_{K^+}/P_{Cl^-} of **2**, **3b** and **3c** under different pH conditions.

In order to explain the pH effect on ion selectivity in this macrocyclic-channel system, a proposed model was established (Fig. 5a). This tubular model possesses three inward-pointing amino groups in the inner cavity, and multiple carboxyl groups at channel openings. At pH 10.0 (Fig. 5b), the deprotonated carboxyls of channel **3c** created two negatively charged channel openings. These openings offer an electrostatic barrier for the entry of Cl^- ions into the channel pore, which was preferred to K^+ ions. Under neutral conditions, compound **3c** was ionized to form internal salts. Compound **3c** displayed lower selectivity for K^+/Cl^-, due to the decrease of negative charge density at channel openings and positively charged inner cavity. At pH 4.0 complete protonation of the carboxyls and inward-pointing amino groups of compound **3c** further increased the affinity of the Cl^- ions and resulted in the lowest K^+/Cl^- selectivity. The γ values for K^+ of **3c** at pH 4.0 and 10.0 were also support this proposed model (Fig. S29, ESI).

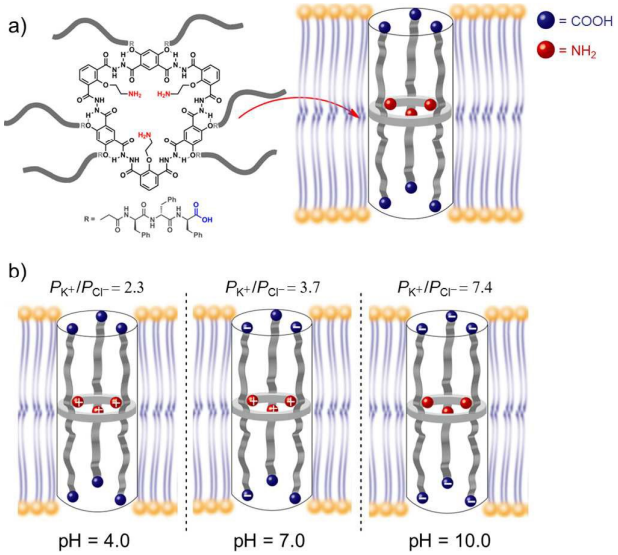

Fig. 5 (a) A schematic diagram for the transmembrane channels formed by **3c** in the lipid bilayer. (b) Illustration of

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charge changes occurring in the macrocyclic channels upon variations of pH value.

In summary, we have developed a class of pH-sensitive ion channels by introducing three inward-pointing amino groups into the cavity of the macrocyclic channels. These highly efficient unimolecular channels could be readily constructed from one-step macrocyclization. The protonation and deprotonation of multiple amines and carboxyls in the channels upon pH variation change the charge distribution, which contributes to the pH-sensitive ion selectivity. This finding illustrated that the design of new smart artificial channels may be easily achieved by introducing multiple inward-pointing functional groups into the backbone cavity of aromatic hydrazide macrocycles. Currently we are modifying backbone cavity with multiple mercapto groups, by which redox-responsive macrocyclic channels may be developed.

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