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Synthetic K^+/Cl^--Selective Symporter across a Phospholipid Membrane

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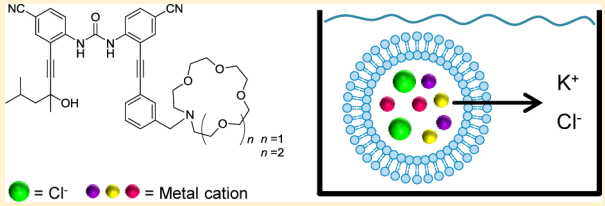
Supporting Information

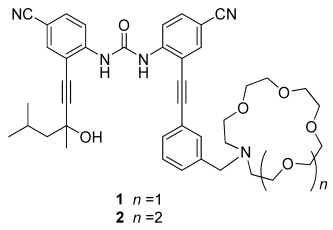
ABSTRACT: Synthetic molecules which selectively transport sodium or potassium chloride across a lipid membrane have been prepared. The salt carriers consist of two heteroditopic binding sites, an anion-binding cavity with three hydrogen bond donors and an azacrown ether for binding an alkali metal cation. The association constants between the carriers and chloride ion have been enhanced by 1 order of the magnitude in the presence of sodium or potassium ion in 10% (v/v) CD_3OH/CD_3CN, due to the formation of a contact ion-pair between the bound cation and chloride as demonstrated by the single-crystal X-ray structure of a sodium chloride complex. A series of transport experiments have demonstrated that the synthetic molecule functions as a mobile carrier of transporting salts via M^+/Cl^- symport. Among alkali metal chlorides, the carrier with an 18-azacrown-6 exhibits a strong selectivity toward potassium chloride, while the carrier with a 15-azacrown-5 displays a moderate selectivity for sodium chloride.

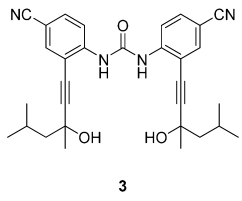


INTRODUCTION

The cellular membrane transport of chemical species is a key process in biological systems. Polar ions and molecules hardly pass through cell membranes, and their transport can be facilitated by transmembrane proteins that function as channels or transporters. A variety of cation−anion cotransporters have been previously identified, such as Na−K−Cl, Na−Cl, and K−Cl symporters that can transport both cations and anions in the same direction simultaneously.[1] These symporters play important roles in biological processes such as neuronal proliferation and differentiation, synaptic plasticity, and cell-to-cell communications, and consequently, they are closely associated with Bartter syndrome, Gitelman syndrome, deafness, renal tubular acidosis, and thyroid diseases. The working principle of some symporters is highly attractive in that the transport of an ion against its concentration gradient can be driven by coupling the movement of another ion with a favorable electrochemical gradient. For example, sodium/phosphate[2] and sodium/iodide symporters[3] have been known to use the transmembrane sodium ion gradient as the driving force for the cellar uptake of phosphate and iodide, respectively. This might be a useful approach to alleviate the abnormal intracellular accumulation of a specific ion, e.g., chloride in cystic fibrosis.

A large number of synthetic mobile carriers have been reported in recent years,[4,5] but synthetic salt transporters capable of tranporting both cations and anions across a phospholipid membrane are extremely rare. Smith et al. demonstrated for the first time in 2003 that a ditopic macrobicycle was able to transport NaCl and KCl through a POPC bilayer.[6] Another was reported by Gale and co-workers who described that calix[4]pyrrole derivatives could selectively transport larger alkali metal salts such as RbCl and CsCl.[7,8] Herein, we describe new synthetic symporters **1** and **2** that consist of two cooperative binding sites for a metal cation and an anion[9] and transport alkali metal chlorides via M^+/Cl^- symport across a POPC membrane. In particular, **2** transports potassium chloride with unprecedentedly high selectivity, while **1** displays moderate selectivity for sodium chloride.





RESULTS AND DISCUSSION

We previously described synthetic chloride transporters, mimicking the hydrogen bonding mode observed in the X-ray crystal structure of a ClC chloride channel, which could transport chloride ions across a POPC membrane via a Cl^-/NO_3^- antiport.[10] With further modification, compound **3** with the *p*-cyano substituents was found to be the most effective Cl^-/NO_3^- antiporter in the series. The symporters **1** and **2** have been designed based on this molecule, and an azacrown ether as a metal binding site is introduced in a way of achieving a contact-ion pair between the bound cation and anion.

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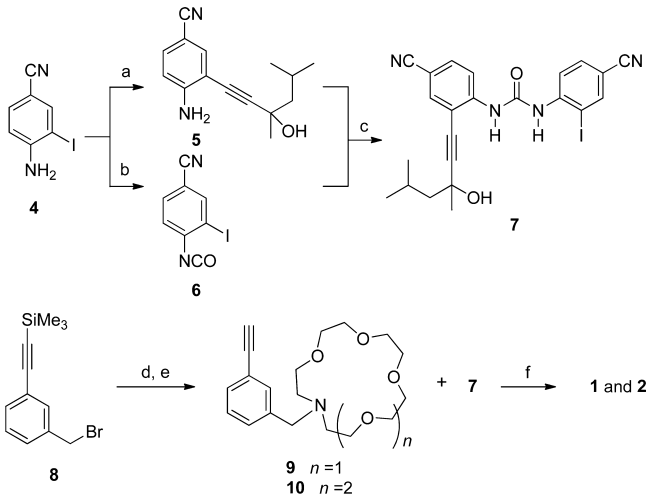
The syntheses of **1** and **2** are outlined in Scheme 1. 4-Cyano-2-iodoaniline (**4**) was converted to compound **5** via

Scheme 1[a]



[a]Reagents and conditions (a) 3,5-dimethylhex-1-yn-3-ol, Pd(Ph_3P)$_2Cl_2$, CuI, Et_3N/THF, 55 °C, 99%; (b) triphosgene, Et_3N, EtOAc, rt → 60 °C; (c) 1,2-dichloroethane, rt, 34% (for two steps); (d) 1-aza-15-crown-5 or 1-aza-18-crown-6, *N*,*N*-diisopropylethylamine, 63−80%; (e) tetra-*n*-butylammonium fluoride, CH_2Cl_2, 93−98%; (f) Pd(Ph_3P)$_2Cl_2$, CuI, Et_3N/THF, 45−50 °C, 50−84%.

Sonogashira reaction[11] with 3,5-dimethylhex-1-yn-3-ol, and compound **4** was also treated with triphosgene to give the corresponding isocyanate **6**. Compounds **5** and **6** were coupled to afford urea **7** which was subjected to Sonogashira reaction with **9** and **10** providing symporters **1** and **2**, respectively.

The binding properties of **1** with sodium chloride were first investigated by ^{1}H NMR spectroscopy (Figure 1). Addition of

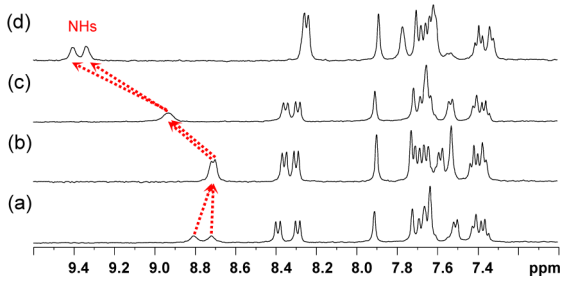


Figure 1. Partial ^{1}H NMR spectra (400 MHz, 10% (v/v) CD_3OH/CD_3CN, 25 °C) of (a) free **1** and (b) in the presence of $NaClO_4$ (2 equiv), (c) Bu_4NCl (2 equiv), and (d) both $NaClO_4$ (2 equiv) and Bu_4NCl (2 equiv).

sodium perchlorate alone led to negligible change in the chemical shifts for the NH signals of **1** in 10% (v/v) CD_3OH/CD_3CN (Figure 1b).[12] Upon addition of tetrabutylammonium chloride (2 equiv), the NH signals were shifted downfield by $\Delta\delta$ = ∼ 0.2 ppm as a result of hydrogen bonding with chloride ion (Figure 1c). The combination of both sodium perchlorate and tetrabutylammonium chloride gave rise to more pronounced changes ($\Delta\delta$ = 0.4−0.5 ppm) in the ^{1}H NMR chemical shifts of **1** (Figure 1d). In addition, carrier **2** also showed identical spectral behaviors toward potassium chloride (see the Supporting Information, Figure S1). These results clearly suggest that symporters **1** and **2** bind both an alkali metal cation and chloride ion in a positive cooperative manner.

The quantitative information on the binding properties was obtained from ^{1}H NMR titrations (Table 1). The association

Table 1. Association Constants (K_a ± 10%, M^{-1}) between Symporters 1 and 2 with Ions (Chloride, Sodium, and Potassium Ions) at 25 ± 1 °C

			K_a (M^{-1})	
ion[a]	solvent (in CD_3CN)	additive	**1**	**2**
Cl^-	10% CD_3OH	none	72	57
	10% CD_3OH	$NaClO_4$[b]	870	580
	10% CD_3OH	KPF_6[b]	710	660
Na^+	50% CD_3OD	none	630	2900
K^+	50% CD_3OD	none	200	>2 × 10^4

[a]For titration experiments, tetrabutylammonium chloride, sodium perchlorate, and potassium hexafluorophosphate were used. [b]2 equiv of salt was added.

constants (K_a) of **1** and **2** with chloride ion are 72 and 57 M^{-1} in 10% (v/v) CD_3OH/CD_3CN, respectively. The association constants are greatly increased by 1 order of the magnitude in the presence of either sodium perchlorate (2 equiv) or potassium hexafluorophosphate (2 equiv)[12] due to the electrostatic interactions between the bound metal ion (Na^+ or K^+) and chloride. In addition, symporter **1** with a 15-azacrown-5 unit binds sodium ion (K_a = 630 M^{-1}) slightly stronger than potassium ion (K_a = 200 M^{-1}) in 50% (v/v) CD_3OD/CD_3CN. On the other hand, symporter **2** with an 18-azacrown-6 unit shows much higher binding affinity toward potassium ion (K_a > 2 × 10^4 M^{-1}) than sodium ion (K_a = 2900 M^{-1}).

The formation of a contact ion-pair between sodium and chloride ions in the complex was determined by the single-crystal X-ray structure (Figure 2). Slow diffusion of *n*-hexane

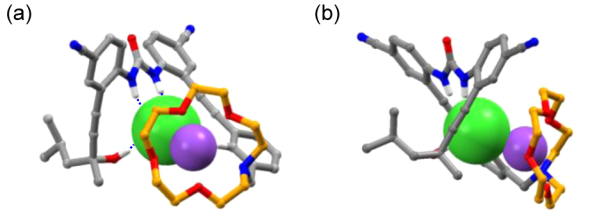


Figure 2. Two views of X-ray crystal structure of a complex between **1** and sodium chloride. The chloride ion (green) is held by hydrogen bonding with two NH protons and one OH proton, and the sodium ion (purple) is coordinated to the 15-aza-crown-5 cavity. In the complex, NaCl is bound as a contact-ion pair. Here, hydrogen atoms except for NH and OH protons and a lattice-included solvent are omitted for clarity.

into an *n*-hexane/CH_2Cl_2 solution of **1** and sodium chloride gave single crystals of complex **1**·NaCl which were suitable for X-ray diffraction analysis. As anticipated, the chloride ion is held by hydrogen bonding with two NHs and one OH. The N···Cl distances are 3.33 and 3.15 Å, while the O···Cl distance is 3.15 Å. The sodium ion is coordinated to the 15-azacrown-5 cavity with a N···Na distance of 2.73 Å and an O···Na distance averaging 2.44 Å. In addition, a contact ion-pair between the bound ions is observed with a Na···Cl distance of 2.689(2) Å,[13] which provides additional electrostatic interactions for the

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stabilization of the complex. It is worth noting that the bound sodium chloride is completely encircled by the organic surfaces of symporter **1**, which may speed up the movement of the complex in the lipid membrane during the transport.

The transport experiments were conducted with large unilamellar vesicles composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC)[6] using a chloride-selective electrode. First, the transport ability of **1** with a 15-azacrown-5 was examined with vesicles containing sodium chloride (500 mM in 5 mM phosphate buffer at pH = 7.2) which were immersed in an isotonic solution of sodium nitrate (Figure 3).

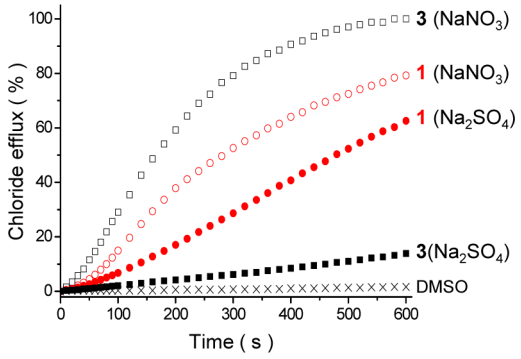
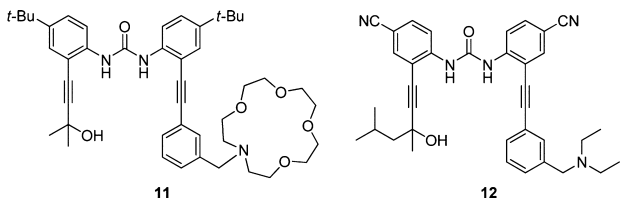


Figure 3. Chloride efflux facilitated by symporter **1** and reference **3** (2 mol % to lipid) from vesicles to a $NaNO_3$ or Na_2SO_4 solution. Vesicles were loaded with sodium chloride (500 mM in 5 mM phosphate buffer at pH = 7.2), and the chloride efflux was measured using a chloride selective electrode.

The efflux of chloride ion is negligible for 600 s without any carrier, but there is appreciable efflux of chloride ion upon addition of **1**. There are two possible pathways for the chloride efflux; Na^+/Cl^- symport and Cl^-/NO_3^-antiport. To distinguish them, transport experiments were repeated in a sodium sulfate solution (166 mM in 5 mM phosphate buffer at pH = 7.2) because the hydrophilic sulfate ion is known to resist transport across a POPC membrane.[14] As seen in Figure 3, the chloride efflux was still effective although the rate decreased slightly, meaning that the chloride transport occurs mostly by Na^+/Cl^- symport. In other words, **1** facilitates the transport of sodium chloride salt across a POPC membrane. This explanation is supported by the fact that compound **3** transports chloride ion very efficiently by Cl^-/NO_3^- antiport in a $NaNO_3$ solution but negligibly in a Na_2SO_4 solution (Figure 3). In addition, reference molecules **11** and **12** with the partially modified binding site for either Na^+ or Cl^- displayed much lower activities of transporting chloride ions by Na^+/Cl^- symport relative to **1** (Figure 4). This result confirms that both cation- and anion-binding sites of **1** play important roles in the transport of sodium chloride salt across a POPC membrane.



11 **12**

The transport experiments[15] were also carried out in vesicles composed of POPC/cholesterol in different ratios, 80:20 and 70:30 (see the Supporting Information, Figure S12).

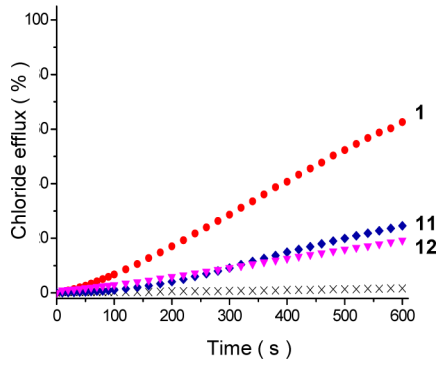


Figure 4. Chloride efflux facilitated by **1**, **11**, and **12** (2 mol % of each compound to lipid) from POPC vesicles (500 mM in 5 mM phosphate buffer at pH = 7.2) to a Na_2SO_4 solution (166 mM in 5 mM phosphate buffer at pH = 7.2). Detergent was added to lyse the vesicles after 600 s, and chloride efflux was measured using a chloride selective electrode.

Cholesterol with a rigid molecular framework has been assumed to reduce the fluidity of membranes, thus interrupting the movement of carriers and their complexes inside the lipid bilayer of vesicles.[16] The transport activity of **1** was reduced as the cholesterol ratio increased, implying that **1** functions as a mobile carrier for transporting sodium chloride salt. Finally, the transport selectivity was examined using a series of vesicles containing alkali metal halides (LiCl, NaCl, KCl, RbCl, and CsCl) in a sodium sulfate solution. As expected, **1** showed the highest transport activity toward sodium chloride (Figure 5).

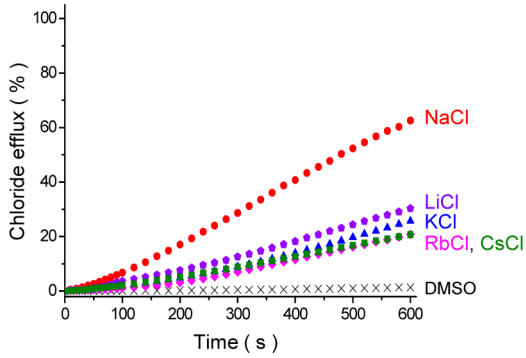


Figure 5. Comparison of salt-transport abilities of **1** (2 mol % to lipid). Vesicles were loaded with each salt (500 mM in 5 mM phosphate buffer at pH = 7.2) and the chloride efflux into a Na_2SO_4 solution (166 mM in 5 mM phosphate buffer at pH = 7.2) was measured using a chloride selective electrode.

Next, the transport behaviors of **2** were examined, and important observations were as follows. First, the transport experiments were repeated three times using vesicles containing potassium chloride suspended in a sodium sulfate solution. Under these conditions, the membrane transport of ions may occur via K^+/Cl^- symport, K^+/Na^+ antiport, and/or Cl^-/SO_4^{2-} antiport. To reveal the contribution of each process, the efflux rates of both potassium and chloride ions were measured using potassium- and chloride-selective electrodes, respectively (Figure 6a). The efflux rate of potassium ions is similar to but slightly higher than that of chloride ion. This result indicates that K^+/Cl^- symport is dominant, but K^+/Na^+ antiport also occurs. Second, the transport rate of chloride ions is higher in a sodium sulfate (166 mM) solution than in a

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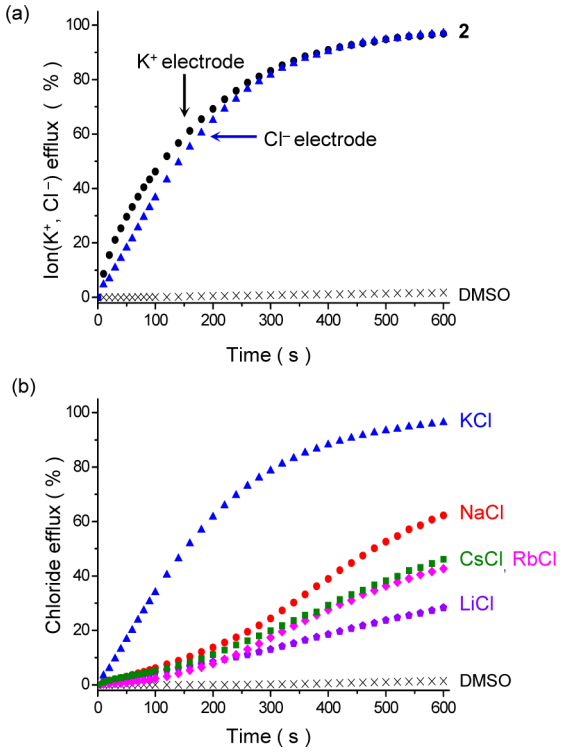

Figure 6. (a) Potassium ion and chloride effluxes facilitated by symporter **2** from vesicles loaded with KCl to a Na_2SO_4 solution, which were measured using a potassium and chloride selective electrodes, respectively. (b) Comparison of salt transport abilities of symporter **2** (2 mol % to lipid). Vesicles were loaded with each salt (500 mM in 5 mM phosphate buffer at pH = 7.2), and the chloride efflux into a Na_2SO_4 solution (166 mM in 5 mM phosphate buffer at pH = 7.2) was measured using a chloride selective electrode.

sodium nitrate (500 mM) solution (see the Supporting Information, Figure S13), which is opposed to the observation with **1**. This is unexpected because under the latter conditions Cl^-/NO_3^- antiport is possible in addition to K^+/Cl^- symport. The reason is not clear at this moment, but a possibility is that two extravesicular solutions may have different electrochemical potentials. On the other hand, the rates of chloride efflux are independent of the kind of cations in the extravesicular sulfate solutions (Na_2SO_4, K_2SO_4, and Cs_2SO_4) (see Supporting Information, Figure S13). Third, the KCl transport efficacy of **2** is much higher than the NaCl transport activity of **1**. This could be attributed to the difference in the binding affinities of the two pairs; Na^+ and a 15-azacrown-5 unit in **1** and K^+ and an 18-azacrown-6 unit in **2**. As summarized in Table 1, the association constant between **2** and potassium hexafluorophosphate is too large to be measured accurately by the ^{1}H NMR titration (K_a > 2 × 10^4 M^{-1}) in 50% (v/v) CD_3OD/CD_3CN but the association constant between **1** and sodium perchlorate is much smaller (K_a > 2900 M^{-1}). Finally, and most importantly, symporter **2** gave unprecedentedly high selectivity for potassium chloride among all alkali metal chlorides (LiCl, NaCl, KCl, RbCl, and CsCl).

CONCLUSIONS

Synthetic mobile carriers that can transport alkali metal halides such as NaCl and KCl via M^+/Cl^- symport across a POPC lipid membrane. The carriers bind alkali metal salts with the formation of a contact ion-pair as demonstrated by the X-ray crystal structure of a sodium chloride complex. In addition, carrier **1** with a 15-azacrown-5 transports sodium chloride selectively across a POPC membrane, while carrier **2** with an 18-azacrown-6 exhibits a high transport selectivity toward potassium chloride. It has been nicely demonstrated for the first time that the transport activity and selectivity can be tuned by careful modification of the cation- and anion-binding sites. This approach may allow us to develop a synthetic mimic that can transport an ion against the concentration gradient as seen in biological systems,[2,3] which may be useful for future applications in the treatment of various channelopathies.

EXPERIMENTAL SECTION

General Information and Methods. All chemicals were purchased from commercial suppliers and used without further purification unless otherwise specified. Air-sensitive reactions were carried out under nitrogen or argon. Triethylamine (Et_3N) and tetrahydrofuran (THF) were purchased as anhydrous grade. Dichloromethane (CH_2Cl_2) was purified by drying over calcium hydride (CaH_2), followed by distillation. Silica gel 60 (230−400 mesh) was used for column chromatography. NMR chemical shifts were reported using residual protonated solvent peaks (for ^{1}H NMR spectra, CD_3CN 1.94 ppm; CD_3OH and CD_3OD 3.31 ppm; acetone-d_6 2.05 ppm; DMSO-d_6 2.50 ppm and for ^{13}C NMR spectra, DMSO-d_6 39.52 ppm; acetone-d_6 29.84 ppm, 206.26 ppm).

Compound 5. A Schlenk flask containing compound **4**[17] (2.15 g, 8.81 mmol), $Pd(PPh_3)_2Cl_2$ (56 mg, 0.08 mmol, 0.01 equiv), and CuI (15 mg, 0.08 mmol, 0.01 equiv) was evacuated under vacuum and filled with N_2. Degassed Et_3N (44 mL), THF (44 mL), and 3,5-dimethyl-1-hexyn-3-ol (3.8 mL, 3 equiv) were added in order, and the solution was stirred at 55 °C for 10 h. The mixture was filtered through Celite and concentrated, and the residue was dissolved in CH_2Cl_2. The solution was washed with brine and saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (silica gel, 1:2.6 EtOAc/CH_2Cl_2) to afford **5** (2.11 g, 99%) as an oily liquid: ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) 7.48 (s, 1H), 7.37 (d, 1H), 6.85 (d, 1H), 5.82 (s, 2H), 4.47 (s, 1H), 2.05 (m, 1H), 1.68 (d, 2H), 1.61 (s, 3H), 1.03 (d, 6H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 153.4, 136.4, 133.6, 119.9, 114.7, 108.1, 102.1, 99.1, 77.9, 68.3, 52.7, 31.5, 25.8, 24.6; IR (thin film) ν 3469 (NH), 3353 (OH), 2219 (C≡N) cm^{-1}; GC−MS m/z calcd for $C_{15}H_{18}N_2O$ $[M]^+$ 242.1, found $[M]^+$ 242.2; HR-MS m/z calcd for $C_{15}H_{18}N_2NaO^+$ $[M + Na]^+$ 265.1317, found $[M + Na]^+$ 265.1310.

Compound 7. An ethyl acetate solution (15 mL) of **4** (1.1 g, 4.5 mmol) and Et_3N (125 μL, 0.2 equiv) was added dropwise to a solution of triphosgene (0.67 g, 0.5 equiv) in EtOAc (15 mL). After being stirred for 1 h at room temperature, the solution was heated at reflux for 4.5 h. The solution was concentrated, and the residue was carefully washed with dry *n*-hexane to remove excess triphosgene. Without further purification, the crude product **6**[18] was dissolved in 1,2-dichloroethane (40 mL), to which was added compound **5** (1.20 g, 1.1 equiv). The solution was stirred for 17 h at room temperature. After concentration, the residue was purified by flash column chromatography (silica gel, initially CH_2Cl_2 and then 9:1 CH_2Cl_2/EtOAc) to afford compound **7** (0.79 g, 34% for two steps) as white solids: mp 226−230 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.27 (s, 1H), 8.95 (s, 1H), 8.36 (m, 1H), 8.03 (m, 2H), 7.80 (m, 3H), 5.36 (s, 1H), 1.97 (m, 1H), 1.63 (d, 2H), 0.96 (d, 6H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 152.3, 145.0, 144.3, 143.8, 136.7, 133.6, 133.5, 123.0, 121.8, 118.8, 118.0, 115.0, 109.0, 107.2, 104.1, 89.6, 77.1, 68.6, 52.8, 31.2, 25.9, 24.8; IR (thin film) ν 3371 (NH and OH overlapped), 2958 (ArCH), 2225 (C≡N), 1719 (C═O) cm^{-1}; MALDI-TOF m/z calcd for $C_{23}H_{21}IN_4O_2$ $[M + Na]^+$ 535.1, $[M + Na]^+$ found 535.2. Anal. Calcd for $C_{23}H_{21}IN_4O_2$: C, 53.9; H, 4.1; N, 10.9. Found: C, 53.9; H, 4.1; N, 11.0.

Compound 9. Compound **8** prepared from 3-iodobenzyl alcohol following literature procedures.[19,20] 1-Aza-15-crown-5 (0.45 g, 2.06

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mmol, 1.1 equiv) was dissolved in *N,N*-diisopropylethylamine (10 mL), and **8** (0.5 g, 1.87 mmol, 1 equiv) was added. The solution was stirred for 8 h at 40 °C. After being cooled to room temperature, the solution was concentrated. The residue was purified with flash column chromatography (silica gel, 4% Et_3N/EtOAc) to give precursor **9** with a trimethylsilyl (TMS) group as an ivory oil (80%): ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 7.43 (s, 1H), 7.32 (m, 3H), 3.59 (s, 2H), 3.52 (m, 16H), 2.62 (t, 4H), 0.22 (s, 9H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 142.2, 132.9, 131.1, 130.2, 129.2, 123.9, 106.5, 94.1, 72.0, 71.5, 71.1, 70.1, 61.0, 55.5, −0.1; IR (thin film) ν 2955 (ArH), 2152 (C≡C) cm^{-1}; GC−MS *m*/*z* calcd for $C_{22}H_{35}NO_4Si$ $[M]^+$ 405.2, found $[M]^+$ 405.3.

To a CH_2Cl_2 solution (15 mL) of precursor **9** (0.59 g, 1.45 mmol) in an ice−water bath was added tetra-*n*-butylammonium fluoride (1 M solution in THF, 1.53 mL, 1.05 equiv), and the solution was stirred for 1 h. After concentration, the residue was purified with flash column chromatography (silica gel, 4% Et_3N/EtOAc) to afford **9** (0.45 g, 93%) as an ivory oil: ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 7.44 (s, 1H), 7.33 (m, 3H), 4.17 (s, 1H), 3.60 (s, 2H), 3.54 (m, 16H), 2.63 (t, 4H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 142.1, 132.9, 131.2, 130.2, 129.2, 123.0, 84.5, 79.0, 71.9, 71.3, 70.9, 70.7, 60.9, 55.4; IR (thin film) ν 2861 (ArH), 2105 (C≡C) cm^{-1}; GC−MS *m*/*z* calcd for $C_{19}H_{27}NO_4$ $[M]^+$ 333.2, found $[M]^+$ 333.3. Anal. Calcd for $C_{19}H_{27}NO_4$: C, 68.4; H, 8.2; N, 4.2. Found: C, 68.4; H, 8.2; N, 4.2.

Compound 10. Compound **10** was prepared using **8** and 1-aza-18-crown-6, following the procedures described for the synthesis of **9**: pale yellow oil (92 mg, 62% for two steps); ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) 7.50 (s, 1H), 7.37 (m, 3H), 4.14 (s, 1H), 3.69 (s, 2H), 3.59 (m, 20H), 2.37 (t, 4H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 142.1, 132.8, 131.1, 130.1, 129.1, 122.9, 84.5, 78.9, 71.6, 71.44, 71.40, 71.1, 70.7, 60.2, 54.8; GC−MS *m*/*z* calcd for $C_{21}H_{31}NO_5$ $[M]^+$ 377.2, found $[M]^+$ 377.2. Anal. Calcd for $C_{21}H_{31}NO_5$: C, 66.8; H, 8.3; N, 3.7. Found: C, 66.8; H, 8.2; N, 3.8.

Compound 1. A dry Schlenk flask containing compound **7** (0.66 g, 1.29 mmol), $Pd(PPh_3)_2Cl_2$ (45 mg, 0.05 equiv), and CuI (12.3 mg, 0.05 equiv) was evacuated under vacuum and filled with N_2. Degassed Et_3N (5 mL), THF (6 mL), and a THF solution (5 mL) of **8** (450 mg, 1.05 equiv) were added. The mixture was stirred at 45−50 °C for 7.5 h. After being cooled to room temperature, the mixture was filtered through Celite with CH_2Cl_2. The organic solution was washed with brine and saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (silica gel, 1:5 EtOAc/CH_2Cl_2 (1:5) and 5% MeOH/acetone) to afford **1** (0.78 g, 84%) as ivory solids: mp 63−65 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.47 (s, 1H), 9.44 (s, 1H), 8.38 (d, 1H), 8.15 (d, 1H), 8.07 (s, 1H), 7.82 (m, 3H), 7.59 (d, 2H), 7.41 (m, 2H), 5.37 (s, 1H), 3.64 (s, 2H), 3.54 (m, 16H), 2.66 (t, 4H), 1.91 (m, 1H), 1.58 (d, 2H), 1.47 (s, 3H), 0.92 (d, 6H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 152.2, 144.4, 144.2, 142.1, 137.4, 136.7, 133.7, 133.4, 132.8, 131.5, 130.7, 129.3, 122.6, 121.7, 120.8, 118.8, 118.7, 114.8, 114.1, 106.9, 106.8, 103.9, 98.6, 83.1, 77.1, 71.8, 71.3, 70.9, 70.6, 68.4, 60.8, 55.4, 52.7, 31.1, 25.8, 24.7; IR (thin film) ν 3235 (OH), 3158 (NH), 2923 (ArH), 2346 (C≡C), 2226 (C≡N), 1707 (C═O) cm^{-1}; HR-MS *m*/*z* calcd for $C_{42}H_{47}N_5O_6$ $[M + H]^+$ 718.3605, found $[M + H]^+$ 718.3605. Anal. Calcd for $C_{42}H_{47}N_5O_6$: C, 70.3; H, 6.6; N, 9.8. Found: C, 70.0; H, 6.8; N, 9.7.

Compound 2. Compound **2** was prepared in 50% yield following the procedure described for the synthesis of **1**: mp 44−46 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) 8.91 (s, 1H), 8.87 (s, 1H), 8.53 (d, 1H), 8.36 (d, 1H), 7.97 (s, 1H), 7.78 (s, 1H), 7.77 (d, 1H), 7.74 (d, 1H), 7.67 (s, 1H), 7.56 (d, 1H), 7.50 (d, 1H), 7.39 (t, 1H), 4.49 (s, 1H), 3.74 (s, 2H), 3.58 (m, 20H), 2.75 (t, 4H), 2.00 (m, 1H), 1.67 (d, 2H), 1.53 (s, 3H), 1.00 (d, 6H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 152.29, 142.35, 137.42, 136.78, 133.78, 133.47, 132.90, 131.50, 130.83, 129.39, 122.70, 121.81, 120.95, 118.83, 118.79, 107.14, 106.91, 104.03, 98.66, 98.63, 83.15, 77.24, 71.71, 71.53, 71.19, 70.76, 68.50, 60.33, 55.02, 52.81, 32.38, 31.19, 25.86, 24.75, 23.36, 14.43, 11.77; IR (KBr pellet) ν 3402 (NH and OH overlapped), 2395 (C≡C), 2254 (C≡N), 1749 (C═O) cm^{-1}; HR-MS *m*/*z* calcd for $C_{44}H_{51}N_5O_7$ $[M + H]^+$ 762.3867, found $[M + H]^+$ 762.3864. Anal. Calcd for $C_{44}H_{51}N_5O_7$: C, 69.4; H, 6.8; N, 9.2. Found: C, 69.4; H, 6.7; N, 9.1.

Compound 3. Compound **3** was prepared in 79% yield following literature procedures:[21] white solids; mp 169−171 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.12 (s, 2H), 8.21 (d, 2H), 7.83 (s, 2H), 7.80 (d, 2H), 5.37 (s, 2H), 1.94 (m, 2H), 1.61 (d, 4H), 1.50 (s, 6H), 0.95 (d, 12H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 151.4, 143.1, 136.1, 132.7, 121.0, 118.2, 113.9, 105.4, 103.4, 76.2, 66.8, 51.6, 30.5, 24.5, 24.2; IR (KBr pellet) ν 3647 (OH), 3446 (NH), 2785 (ArH), 2391 (C≡C), 2281 (C≡N), 1751 (C═O) cm^{-1}; MALDI-TOF *m*/*z* calcd for $C_{31}H_{34}N_4O_3$ $[M + Na]^+$ 533.3, found $[M + Na]^+$ 533.6. Anal. Calcd for $C_{31}H_{34}N_4O_3$: C, 72.9; H, 6.7; N, 11.0. Found: C, 72.8; H, 6.75; N, 11.0.

Compounds **11** and **12** were prepared following procedures described previously for the preparation of compound **1** and **2** from the corresponding precursors.[22]

Compound 11: white solid (yield 84%); mp 66−67 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) 8.29 (s, 1H), 8.18 (s, 1H), 8.16 (s, 1H), 8.04 (d, 1H), 7.61 (s, 1H), 7.54 (d, 1H), 7.48 (d, 1H), 7.44 (d, 2H), 7.37 (m, 3H), 4.49 (s, 1H), 3.70 (s, 2H), 3.59 (m, 16H), 2.74 (t, 4H), 1.5 (s, 6H), 1.32 (d, 18H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 153.3, 153.2, 146.2, 142.1, 138.9, 138.7, 132.8, 131.3, 130.1, 130.0, 129.6, 129.3, 127.6, 127.1, 123.7, 121.7, 121.2, 121.1, 113.8, 113.4, 101.7, 95.9, 86.2, 78.3, 72.0, 71.4, 71.0, 70.8, 61.0, 55.5, 34.9, 34.8, 32.2, 31.7, 31.6; IR (thin film) ν 3239 (NH and OH overlapped), 2957 (ArH), 2340 (C≡C), 1696 (C═O) cm^{-1}; MALDI-TOF *m*/*z* calcd for $C_{45}H_{59}N_3O_6$ $[M + H]^+$ 738.5, found $[M + H]^+$ 738.6. Anal. Calcd for $C_{45}H_{59}N_3O_6$: C, 73.2; H, 8.1; N, 5.7. Found: C, 72.9; H, 8.15; N, 5.5.

Compound 12: pale ivory solid (yield 59%); mp 69−71 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) 8.54 (s, 1H), 8.52 (s, 1H), 8.37 (d, 1H), 7.98 (d, 1H), 7.97 (s, 1H), 7.77 (m, 3H), 7.61 (s, 1H), 7.56 (d, 1H), 7.47 (d, 1H), 7.39 (t, 1H), 3.75 (s, 1H), 3.60 (s, 2H), 2.53 (m, 4H), 1.99 (m, 1H), 1.67 (d, 2H), 1.53 (s, 3H), 1.01 (m, 12H); ^{13}C NMR (100 MHz, acetone-d_6) δ (ppm) 152.2, 144.3, 144.2, 142.1, 137.3, 136.6, 133.7, 133.4, 132.6, 131.4, 130.1, 129.3, 122.6, 121.5, 120.8, 118.7, 118.6, 114.7, 114.1, 106.9, 106.7, 103.7, 98.4, 83.1, 77.2, 68.4, 57.8, 52.7, 47.4, 30.4, 25.7, 24.6, 12.1; IR (thin film) ν 3335 (NH), 3280 (OH), 2346 (C≡C), 2227 (C≡N), 1719 (C═O) cm^{-1}; MALDI-TOF *m*/*z* calcd for $C_{36}H_{37}N_5O_2$ $[M + H]^+$ 572.3, found $[M + H]^+$ 572.4. Anal. Calcd for $C_{36}H_{37}N_5O_2$: C, 75.6; H, 6.5; N, 12.3. Found: C, 75.8; H, 6.7; N, 12.0.

^{1}H NMR Titrations. A stock solution of **1** or **2** (8.0×10^{-4} M) in 10% (v/v) CD_3OH/CD_3CN or 50% (v/v) CD_3OD/CD_3CN was prepared with or without 2 equiv of each salt ($NaClO_4$ or KPF_6). Using this solution as a solvent, a guest stock solution of tetrabutylammonium chloride (5.0×10^{-2} M for anion titrations) or sodium perchlorate/potassium hexafluorophosphate ($5.0–9.0 \times 10^{-3}$ M, for cation titrations) was prepared. Aliquots of the guest solutions (from 10 to 500 μL, 10−12 data points) were added to the stock solution, and the spectrum was recorded after each addition. The association constant (K_a, M^{-1}) was determined by nonlinear least-squares fitting of the titration curves[23] plotting chemical shift changes of appropriate ^{1}H NMR signals (see the Supporting Information) against molar equivalents of guests.

Transport Experiments.[6,15] A chloroform solution (15 mL) of 1-palmitoyl-2-oleoylphosphatidylcholine (POPC, 30 mg) was evaporated under reduced pressure to give a thin film. The lipid film was dried under high vacuum overnight and then was rehydrated by vortexing with a 1 mL of MCl (LiCl, NaCl, KCl, RbCl, or CsCl) solution (500 mM NaCl, 5 mM phosphate buffer at pH 7.2). The lipid suspension was then subjected to nine freeze−thaw cycles and was allowed to age for 30 min at room temperature. The liquid suspension was extruded 23 times through a 200 nm polycarbonate membrane using an extruder. Nonencapsulated salts were removed by dialysis three times.

A series of vesicles were suspended in an external solution (500 mM $NaNO_3$ or 166 mM Na_2SO_4, K_2SO_4, or Cs_2SO_4, buffered to pH 7.2 with 5 mM sodium phosphate salts). The lipid concentration per sample was 1 mM. An aliquot of the DMSO solution of a symporter (4

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mM) was added, and the ion release from vesicles was monitored using an ion-selective electrode. The initial reading was considered as 0% release of an ion and the reading after the addition of 10% Triton X-100 to the lipid solution at 600 s was considered as 100% release. Transport experiments were performed on a pH/ISE meter, and the chloride or potassium ion efflux was measured using a chloride or potassium ion selective electrode.

X-ray Crystal Analysis. Finely ground NaCl (3 equiv) salts were suspended in a CH_2Cl_2 solution of **1**, and the suspension was stirred overnight and filtered. To the solution was added a small amount of *n*-hexane until a precipitate was formed. The solution was transferred into a test tube wherein *n*-hexane was carefully layered, and the test tube was sealed to prevent evaporation. The tube was allowed to stand for 2 weeks at room temperature, affording single crystals suitable for X-ray diffraction analysis.

[**1**·NaCl]: $C_{43}H_{49}Cl_3N_5NaO_6$ (M = 861.21); triclinic, space group *P*-1 (no. 2), a = 12.3245(5) Å, b = 12.5220(6) Å, c = 16.8567(8) Å, α = 99.5940(14)°, β = 110.9510(13)°, γ = 104.0750(14)°, V = 2262.27(18) Å^3, Z = 2, T = 300.0 K, μ(Mo Kα) = 0.262 mm^{-1}, D_{calc} = 1.264 g/mm^3, 89757 reflections measured ($4.7 \leq 2\Theta \leq 52.0$), 8905 unique ($R_{int}$ = 0.1047, R_6 = 0.0565) which were used in all calculations. The final R_1 was 0.0776 ($I > 2\sigma(I)$), and wR_2 was 0.2289 (all data).

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures; details of binding studies and transport experiments; X-ray details; crystallographic data (CIF); ^{1}H and ^{13}C NMR spectra of new compounds. 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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