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Conformationally Flexible Cleft Receptor for Chloride Anion Transport

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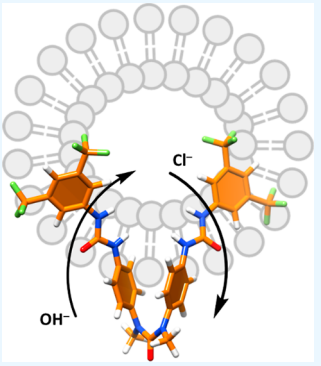
ABSTRACT: The design and synthesis of a cleft-shaped bis-diarylurea receptor for chloride anion transport is reported in this work. The receptor is based on the foldameric nature of *N*,*N*′-diphenylurea upon its dimethylation. The bis-diarylurea receptor exhibits a strong and selective affinity for chloride over bromide and iodide anions. A nanomolar quantity of the receptor efficiently transports the chloride across a lipid bilayer membrane as a 1:1 complex (EC_{50} = 5.23 nm). The work demonstrates the utility of the *N*,*N*′-dimethyl-*N*,*N*′-diphenylurea scaffold in anion recognition and transport.



INTRODUCTION

Anions play crucial roles in physiology, industry, and the environment. The design and synthesis of selective receptors for recognition, transport, extraction, and sequestration of specific anions is one of the grand challenges in supramolecular chemistry.[1−7] There is a growing interest in the development of small molecules that can transport chloride anions across lipid bilayer membranes.[8−12] These transporter molecules promise a potential cure for cystic fibrosis, a life-shortening genetic disease caused by the malfunction of an anion channel protein, namely, the cystic fibrosis transmembrane conductance regulator.[13,14] Such transporters have been proposed as potential therapeutic agents for cancer as well by altering the highly acidic pH of the cancer cells.[15−20]

The design of anion receptors with necessary hydrogen or halogen bonding functional groups relies on either macrocycles and cages or acyclic flexible molecules, clefts, podants, and foldamers with preorganized binding pockets.[6,7,21] Despite the significant advances made thus far, there is a continuous search for synthetic anion receptors that are easy to synthesize, with strong affinity and selectivity toward chloride and further ability to transport across the lipid bilayer membrane. One of the major challenges is that efficient chloride anion transporters often require challenging synthesis.

We report the design and synthesis of a cleft-shaped receptor based on an *N*,*N*′-dimethyl-*N*,*N*′-diphenylurea scaffold. *N*,*N*′-diphenylurea is linear in conformation, but *N*,*N*′-dimethylation induces a cleft shape due to the preference of the methyl groups in cis−cis conformation relative to the urea carbonyl.[22−25] This feature has not been exploited in the design of synthetic receptors for halide anion recognition and transport so far. We functionalized the scaffold by extending it with further aryl urea moieties to form a cleft-shaped bis-diarylurea anion receptor, with a central *N*,*N*′-dimethylurea (**1**, Scheme 1). The terminal aryl groups were substituted with two CF_3 groups in meta positions with respect to the urea groups, which are known to enhance the hydrogen bonding strength of the adjacent urea N−H protons.[26] Importantly, the receptor was easily accessible by a simple synthetic route. The receptor forms a hydrogen bond with a chloride anion in a 1:2 stoichiometry, but in a noncooperative manner, contrary to our expectations. However, the receptor efficiently transported chloride across lipid vesicles by forming a 1:1 host−guest complex, as suggested by Hill analysis.

RESULTS AND DISCUSSION

Synthesis and Molecular Structures of 1. The synthesis of receptor **1** is presented in Scheme 1. The methylation of the well-known *N*,*N*′-di(4-nitro)phenylurea (**2**) afforded the desired intermediate *N*,*N*′-di(4-nitro)phenyl-*N*,*N*′-dimethylurea (**3**). The reduction of the nitro groups into amines (**4**), followed by their conversion into isocyanates (**5**) and subsequent reaction with 3,5-bistrifluoromethylaniline gave the desired receptor **1** in good yields.

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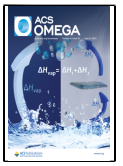

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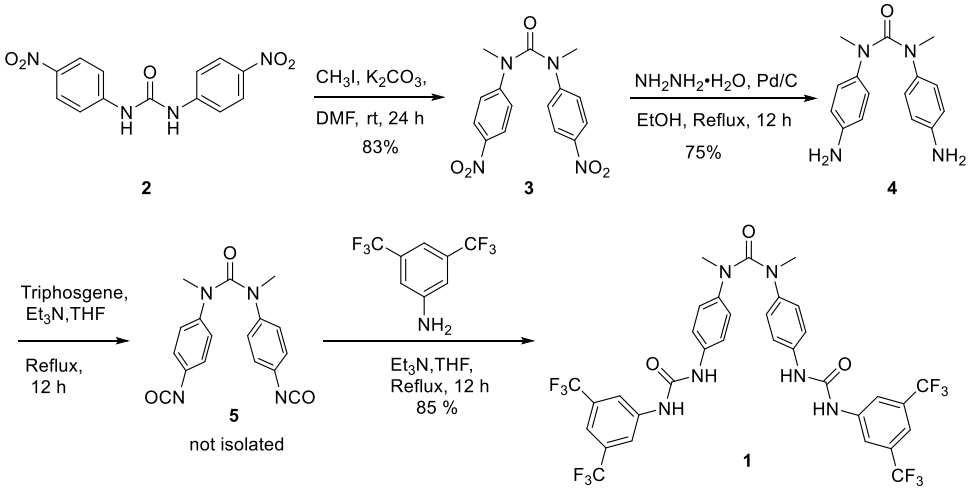
Scheme 1. Synthesis of Receptor 1



The molecule crystallized readily in CH_3CN and in DMSO, as solvates. Single-crystal X-ray diffraction (SCXRD) analysis confirmed that **1** adopts cleft conformation but with different interplanar distances between the inner and outer aryl rings. In the DMSO solvate of **1**, the two urea groups form bifurcated hydrogen bonds with DMSO, and **1** exists as a compact cleft (Figure 1a) with short interplanar distances between the two pairs of aryl rings (3.77 and 4.85 Å). On the other hand, **1** adopts a twisted cleft conformation in the CH_3CN solvate (Figure 1b) with interplanar distances of 3.84 and 11.32 Å between inner and outer aryl rings, respectively. The twisting was triggered by intermolecular hydrogen bonding between the N−H donors of one urea group and the C═O acceptor of another urea group in **1** (Figure S17). The other urea group forms a hydrogen bond with CH_3CN.

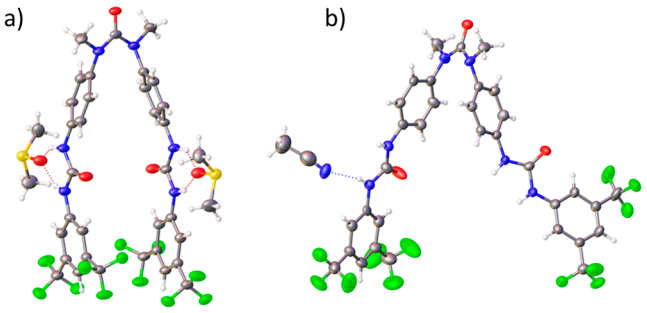


Figure 1. SCXRD-determined molecular structures of **1** as a (a) DMSO solvate and (b) CH_3CN solvate.

Recognition of Halide Anions. We examined the ability of **1** to recognize halide anions using ^{1}H NMR spectroscopy by titrating chloride, bromide, and iodide ions (0−10 eq) as their tetra-*n*-butylammonium (TBA) salts, independently. The results were analyzed to determine the binding constants (K_a) and host−guest equilibrium (1:1 or 1:2 or 2:1) between the receptor and the anion in solution using the "BindFit" program.[27−31] We selected the equilibrium binding models based on the visual inspection of the fit quality, the stochastic distribution of the residuals, the covariance of the fit parameters, and the correlation between the stepwise association constants and the resonance shifts of the complexes (Table S7).

The initial titration of TBA-chloride against **1** (5 mM) in DMSO-d_6 resulted in moderate shifts of the urea N−H protons ($\Delta\delta$ = 0.58 and 1.01 ppm, Figure S23), but the aromatic protons did not show any perturbations. Reasonable fits with moderate affinities were obtained for the binding models of 1:1 (K_a = 49 M^{-1}), 1:2 (K_{11} = 591 M^{-1}; K_{12} = 46 M^{-1}), and 2:1 (K_{11} = 100 M^{-1}; K_{21} = 25 M^{-1}), implying the possible formation of multiple complexes coexisting in solution (Table S8 and Figures S24−S32). We were prompted by this result to examine the binding affinity in a moderately polar solvent. Accordingly, we performed the titration of TBA-chloride (50 mM) against **1** in CD_3CN/DMSO-d_6 (98:2 v/v). The addition of chloride caused significant shifts in the resonance of the N−H protons of **1** ($\Delta\delta$ = 1.65 and 2.16 ppm, Figure 2a). The shifts reached a plateau upon 2 equivalents of TBA-chloride. A closer inspection of the resonance shifts of the aromatic protons suggested that the resonances of the inner aryl rings (*e* and *d*, Figure 2b) exhibited moderate upfield followed by downfield shifts. The difference in the shifts was not great (δ = 6.82 to 6.77 to 6.82 for the ortho-proton and δ = 7.23 to 7.21 to 7.30 for the meta-proton), but such shifts indicated the formation of a higher-order complex rather than a 1:1 complex. Notably, the α-methylene protons of the TBA cation did not show any shift in the resonance position, thereby indicating a lack of ion-pair formation. The two urea N−H protons and the two inner aryl protons were analyzed for a global fit against the 1:2 binding model. The analysis showed a good fit for the "full 1:2" binding model (Table S9; Figures 2b and S35) in comparison with other binding models. The dominance of K_{11} = 2.6 × 10^4 M^{-1} (with a ±13% error) over K_{12} = 550 M^{-1} (with a ±6% error) indicates the presence of negative cooperativity. This means that the binding of the first chloride to one of the urea groups reduces the affinity of the other urea group for the second chloride. Our attempts to fit the data with a 1:1 binding model (Figure S34) showed that K_a = 2.5 × 10^3 M^{-1} with a minimum error of ±5%. However, the covariance of fit, which measures the accuracy of the model in the regression analysis, suggested that a 1:2 binding model (4.11 × 10^{-4} vs 1.78 × 10^{-3}) is a more reliable fit for the data. Notably, the 1:2 model has a stochastic distribution of residuals for the urea N−H protons and a perfect fit for the inner aryl protons (Figure 2b), which is not the case for the aryl protons in the 1:1 model (Figure S34).

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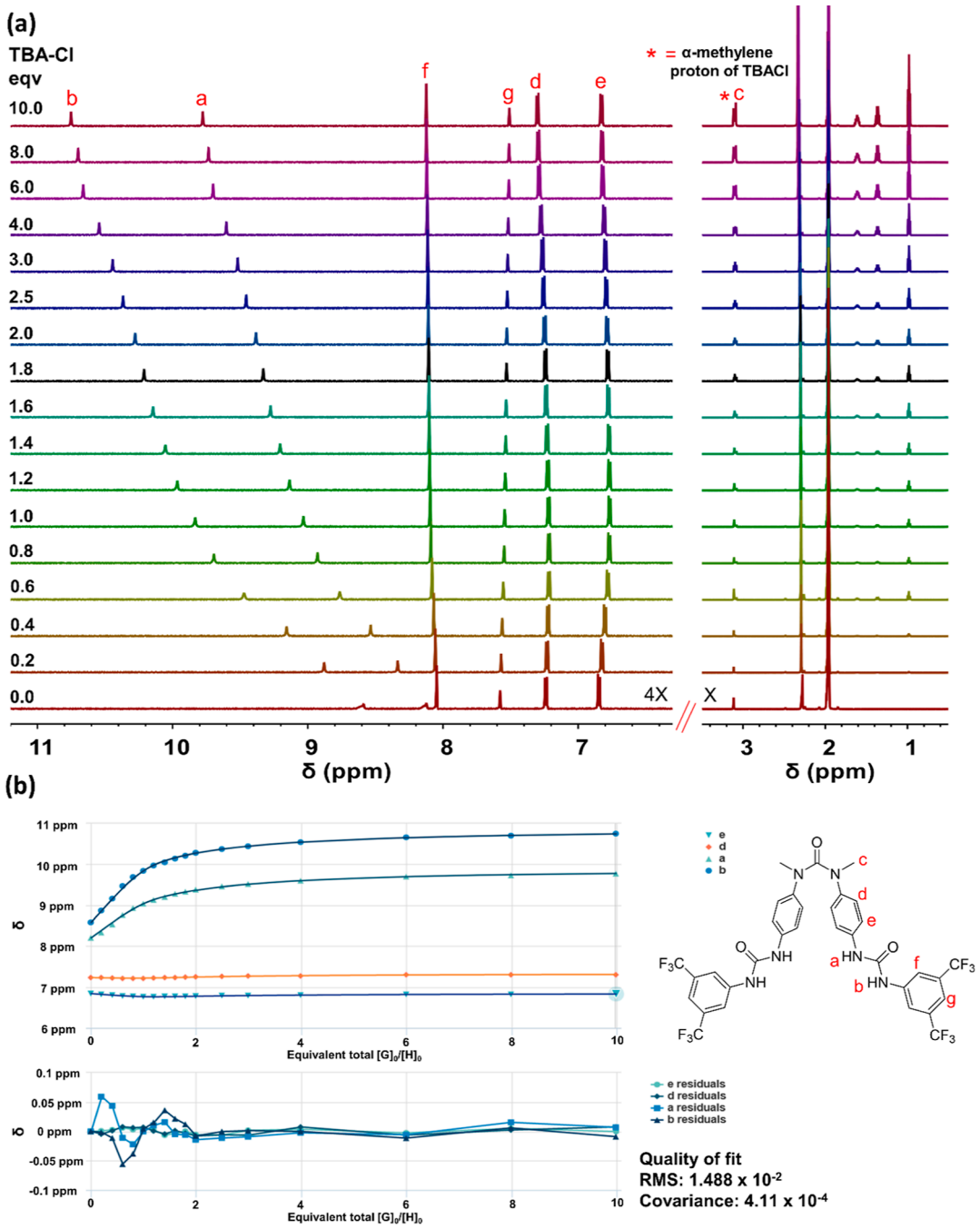

Figure 2. (a) ^{1}H NMR (600 MHz, CD_3CN/DMSO-d_6, 98:2 v/v) titration plot of **1** with TBA-chloride. The equivalents of anions added are indicated in individual traces. (b) Plots of the chemical shifts of protons vs equivalents of TBA-chloride added and fitted to a 1:2 binding model (b) and their residual distribution plots (*TBACl in the figure).

Mass spectrometry analysis of the mixture of **1** and TBA-chloride showed the presence of peaks at *m/z* 425.1572 for the 1:2 complex and *m/z* 815.4166 for the 1:1 complex, indicating the feasible formation of the 1:2 complex. However, when the anion was mixed with either 0.5 or 1 equivalents, only a 1:1 complex formation was observed in the gas phase.

Fortunately, we obtained crystals of receptor **1** with TBA-chloride easily and performed SCXRD analysis to determine the structure. A notable aspect of the complex structure is that a binding pocket in the cleft region is created by the two diarylurea moieties of **1** swinging apart (10−11 Å) and occupied by a TBA cation (Figure 3a). Each diarylurea group of **1** then forms hydrogen bonds with two symmetry-independent chloride anions (Figure 3a, $d_{N\cdot H\cdot\cdot Cl}$− = 2.30−2.34 Å, and $\theta_{N-H\cdots Cl}$− = 155 to 162°). The proximity of α-methylene protons of TBA cations further stabilizes the chloride anions. These C−H groups of TBA are sufficiently acidic to provide hydrogen bonding and anion stabilization,[32] which was not observed in the solution-state ^{1}H NMR binding studies.

To evaluate the possibility of forming a 1:1 complex between **1** and chloride, we used dispersion-corrected DFT (PBE0/def2-svp) methods to perform geometry optimizations. The optimized structure (Figure 3b) showed that **1** can bind

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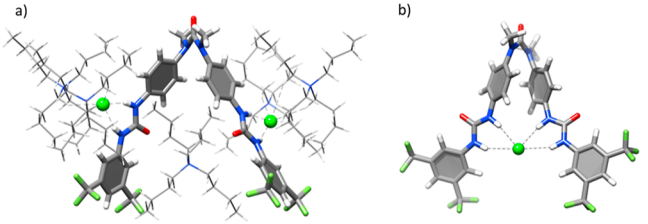

Figure 3. (a) SCXRD-determined complex structure of $[2(TBA)^+]$-$[\mathbf{1}.2Cl^-]$. The TBA cations are shown in wire-frame mode for clarity. Each urea group hydrogen-bonds with two independent chloride anions, which are surrounded by two TBA cations. Note another TBA cation inside the cleft area of **1**. (b) Geometry-optimized structure of the 1:1 complex of **1** and chloride by the dispersion-corrected DFT (PBE0/def2-svp) method.

with chloride anions in a reasonable manner in the cleft pocket by using the urea N−H groups for hydrogen bonding ($d_{N-H\cdots Cl^-}$ = 2.34−2.64 Å and $\theta_{N-H\cdots Cl^-}$ = 155 to 162°).

A similar ^{1}H NMR titration of TBA-bromide against **1** caused downfield shifts of up to 1.1 and 1.4 ppm ($\Delta\delta$) for the two urea N−H resonances of **1**, respectively (Figure S43). Notably, the aromatic protons did not undergo any shifts at all. The titration results fit well for the "full 1:2" binding model (Figure S45) with moderate affinity. The remarkable difference between the stepwise binding constants of K_{11} (3.0×10^3 M^{-1} with ±2% error) and K_{12} (5 M^{-1} with ±0.1% error) indicated the negative cooperativity associated with the binding event.

The ^{1}H NMR titration of TBA iodide against **1** resulted in moderate shifts of 0.29 and 0.4 ppm ($\Delta\delta$) for the two urea N−H resonances of **1**, without reaching saturation even after adding 10 equivalents of iodide. The data were fitted with a 1:1 model, although a sigmoidal residual distribution plot

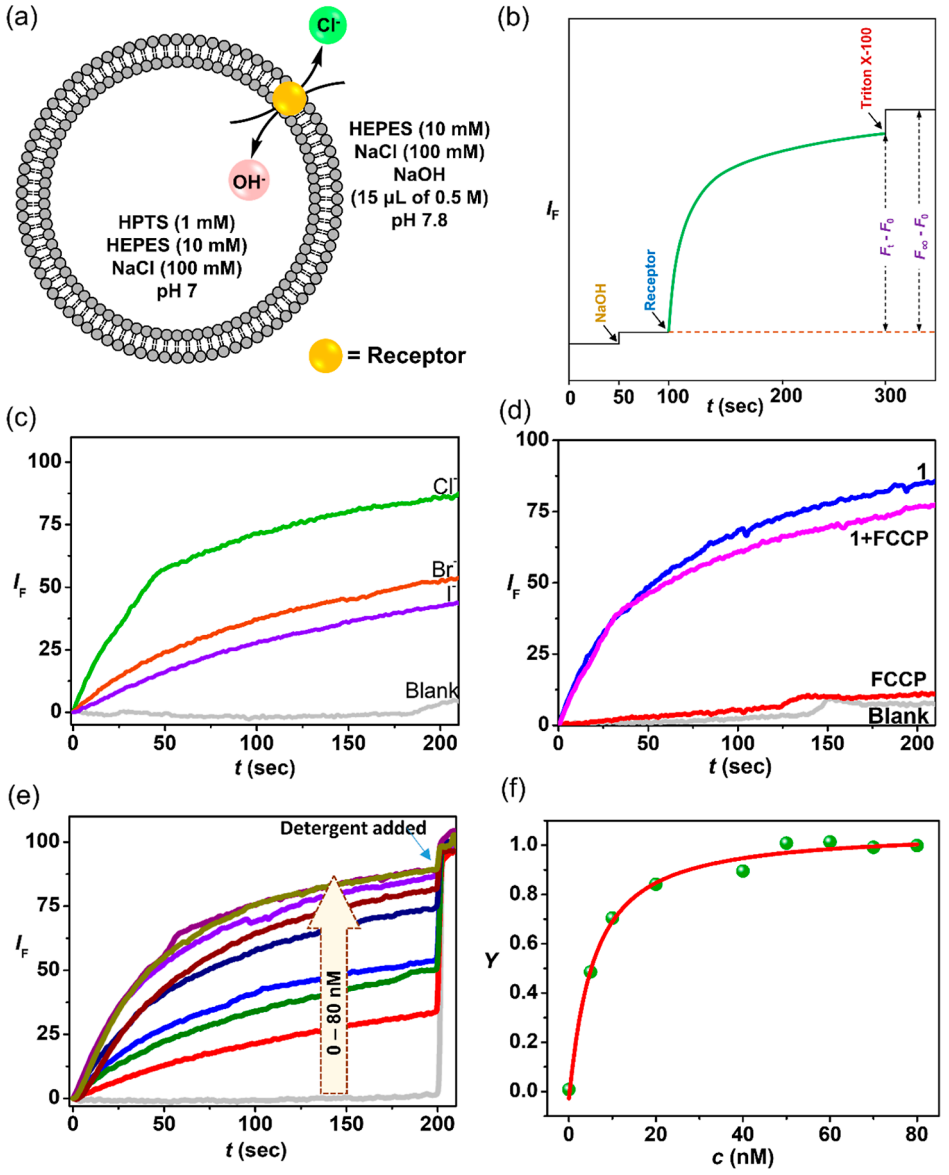

Figure 4. (a,b) Cartoon representations of the HPTS assay to examine chloride transport across LUVs. (c) Selectivity in the transport of anions in LUVs, mediated by **1**, measured by the HPTS assay. (d) HPTS assay with **1** vs **1** and FCCP-protonophore to identify the chloride/hydroxide exchange across LUVs mediated by **1**. (e) Concentration-dependent HPTS assay to measure the anion transport across LUVs facilitated by **1** and (f) corresponding Hill analysis.

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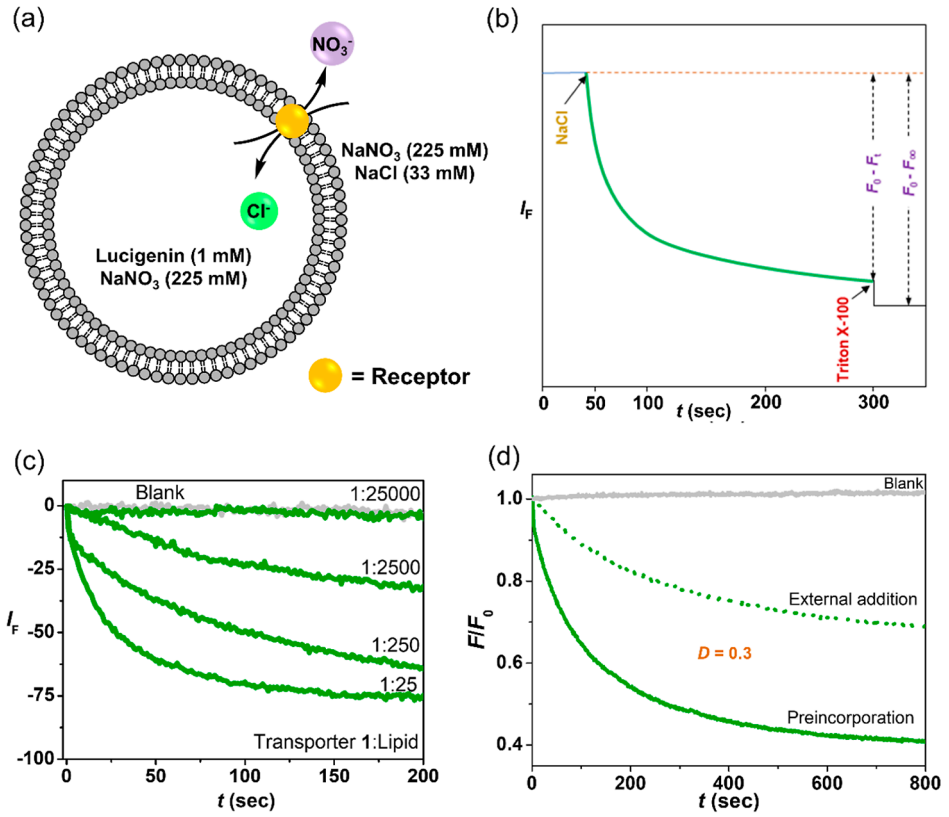

Figure 5. (a,b) Cartoon representations of the lucigenin assay and analysis to measure chloride/nitrate exchange across LUVs. (c) Emission intensity profiles of lucigenin encapsulated in LUVs upon chloride transport mediated by **1** with different ratios of **1** vs lipid. At the transporter-to-lipid ratio of 1:250, the decay half-life ($t_{1/2}$) of relative emission intensity is 97 s and the initial rate of change of relative fluorescence, I, is 0.00352 s^{-1}. (d) Lucigenin assay with preincorporated vs postincorporated receptor **1** in a 1:250 ratio in the LUVs to measure the deliverability of the chloride anion, which is found to be 0.3.

suggested weak binding ($K_{1:1}$ = 338 M^{-1} with ±13% error, Figure S54). The other binding models did not fit the data well (Figures S55−S62; Table S11).

Transport of Anions. We wondered whether **1** could transport chloride anion across a lipid bilayer membrane, having identified that it binds with chloride with moderate affinity. To test this hypothesis, we prepared large unilamellar vesicles (LUVs, ca. 200 nm in diameter) with egg-yolk phosphatidylcholine (EYPC) and cholesterol by following the standard protocols (see the Supporting Information) and performed 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS, a pH-sensitive dye) assay as illustrated in Figure 4a,b.[33] The vesicles (EYPC-LUVs⊃HPTS) were filled with either NaCl or NaBr or NaI and HPTS. The emission profiles of HPTS were monitored at different pH levels.

In a typical experiment, 1950 μL of HEPES buffer (10 mM HEPES, 100 mM NaCl, pH = 7.0) was added to a clean and dry fluorescence cuvette, followed by the addition of 50 μL of EYPC-LUVs⊃HPTS (EYPC, 62.5 μM). The cuvette was placed in the fluorescence instrument with slow stirring conditions by a magnetic stirrer equipped in the instrument (at t = 0 s). The time course of HPTS fluorescence emission intensity (F_t) was observed at λ_{em} = 510 nm upon being excited at λ_{ex} = 450 nm. At t = 50 s, a pH gradient of 0.8 was induced in the vesicle solution by adding NaOH (15 μL, 0.5 M), followed by **1** to facilitate anion exchange across the bilayer membrane of LUVs, and the change in emission intensity was monitored (Figure 4b). At t = 100 s, transporter molecules of different concentrations were added, and finally, at t = 300 s, 20 μL of 20% Triton X-100 was added to lyse vesicles and destroy the pH gradient.

For data analysis and comparison, time (X-axis) was normalized between the point of transporter addition (i.e., t = 100 s was normalized to t = 0 s) and the end point of the experiment (i.e., t = 300 s was normalized to t = 200 s). Fluorescence intensities, at specific time t (F_t), were normalized to fractional emission intensity (I_F) using the equation $I_F = [(F_t - F_0)/(F_\infty - F_0)] \times 100$, wherein F_0 is the fluorescence intensity just before the transporter addition (at t = 0 s) and F_∞ is the fluorescence intensity at saturation after complete leakage (at t = 300 s).

Excitedly, the results showed that **1** promoted anion exchange in the order of $Cl^- > Br^- > I^-$ (Figure 4c). The role of the sodium cation was ruled out by conducting analogous experiments with NaCl, KCl, and LiCl, which showed comparable outcomes (Figure S67). Furthermore, when **1** was combined with a protonophore, namely, carbonyl cyanide-4-(trifluoromethoxy)-phenylhydrazone (FCCP), the transport was reduced subsequently (Figures 4d and S68). The result confirms that **1** does not facilitate the symport of H^+ and OH^- but only the exchange of OH^- for Cl^- in the HPTS assay.[12,33] Overall, the combined results convincingly support that **1** facilitates chloride transport across the lipid bilayer membrane via a Cl^-/OH^- antiport mechanism.

A series of similar anion transport experiments mediated by **1** at different concentrations were performed, as shown in Figure 4e, and analyzed using the Hill plot[34] by following the equation $Y = Y_\infty + (Y_0 - Y_\infty)/[1 + (c/EC_{50})^n]$, where Y_0 is the

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fluorescence intensity just before the transporter addition (at t = 0 s), Y_∞ is the fluorescence intensity with excess transporter concentration, c is the concentration of the receptor/transporter to obtain the effective concentration EC_{50} that achieves 50% of the maximum transmembrane transport activity, and n is the Hill coefficient. The analysis revealed that **1** can mediate transmembrane chloride transport in a nanomolar quantity with an EC_{50} value of 5.23 nM (Figure 4f). Furthermore, a Hill coefficient value of n = 1.085 suggested that **1** formed a 1:1 complex with chloride in the lipid membrane.

The chloride anion transport activity of **1** across lipid bilayer membranes was further investigated by using the lucigenin assay (Figures 5a,b and S69−S71).[35] A series of LUVs were prepared by encapsulating a chloride-sensitive lucigenin dye in an aqueous $NaNO_3$ solution with EYPC lipids that were preincorporated with receptor **1** at different receptor-to-lipid ratios. In a typical experiment, 1200 μL of the $NaNO_3$ solution was added to a fluorescence cuvette containing 800 μL of EYPC/cholesterol- LUVs⊃1-lucigenin (EYPC, 400 μM). The cuvette was placed in the fluorescence instrument under a slow stirring condition using a magnetic stirrer equipped with the instrument. The time course of lucigenin fluorescence emission intensity (F_t), when excited at 450 nm, was observed at 535 nm, at t = 0 s.

The experiment was initiated by adding NaCl (33 μL, 2 M) to the cuvette at t = 50 s to create the chloride gradient between the intra- and extravesicular system. The quenching of lucigenin emission by chloride influx, mediated by **1**, was used to measure the chloride/nitrate (Cl^-/NO_3^-) anion exchange across the lipid membrane in the LUVs. Finally, a solution of 20 μL of 20% Triton X-100 was added at t = 850 s (15 min) to lyse those vesicles, resulting in the destruction of the chloride gradient. Fluorescence intensities (F_t), at time t, were normalized to fractional emission intensity (I_f) using the equation, $I_F = [(F_t - F_0)/(F_0 - F_\infty)] \times 100$, wherein F_0 is the fluorescence intensity just before the addition (at 0 s) and F_∞ is the fluorescence intensity at saturation after complete leakage (at 250 s). The quenching of lucigenin emission intensity depended on the receptor-to-lipid concentration ratio, which suggested that **1** enabled the Cl^-/NO_3^- exchange across the lipid bilayer (Figures 5c and S71).

We analyzed transport data by plotting emission intensity against time and fitting it to a single exponential function. From the fit, we estimated the decay half-life ($t_{1/2}$) and the initial rate of change of relative fluorescence (I) for **1** at a receptor-to-lipid ratio of 1:250. The estimated values were 97 s and 0.00352 s^{-1}, respectively. These results indicate that **1** is a moderate transporter.

Subsequently, we measured the deliverability of **1** by adding it externally to LUVs, which were prepared without preincorporating with the receptor. We compared the results with those obtained when **1** was preincorporated into LUVs at a transporter-to-lipid ratio of 1:250. The externally added **1** exhibited a 30% efficiency to facilitate Cl^-/NO_3^- exchange in comparison with preincorporated **1** in the lipid bilayer membrane of LUVs (Figures 5d and S75). The associated half-life ($t_{1/2}$) and initial rate of change of relative emission (I) for the external addition were 204 s and 0.00107 s^{-1}, respectively.

Interestingly, the preincorporated **1** mediated the exchange of chloride/bicarbonate (Cl^-/HCO_3^-) at a receptor-to-lipid ratio of 1:25 when the lucigenin assay was performed with LUVs filled with $NaHCO_3$ instead of a $NaNO_3$ solution (Figure S74). The results also confirmed that **1** could transport not only chloride but also bicarbonate anions across the lipid membrane to a certain degree.

CONCLUSIONS

In conclusion, we have demonstrated that a readily accessible bis-urea cleft receptor **1** acts as an efficient chloride anion transporter. Although the receptor binds with the chloride anion by forming a 1:2 complex in solution and in solid states, as evidenced by ^{1}H NMR and SCXRD analyses, it forms the expected 1:1 complex with the chloride anion to transport across the lipid membrane in nanomolar quantity (EC_{50} = 5.23 nM). The results demonstrate the utility of the foldameric *N*,*N*′-dimethyl-*N*,*N*′-diphenylurea moiety in designing synthetic supramolecular receptors and transporters for anions..

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c01146.

Synthesis and characterization of **1**, NMR-based anion binding studies, anion transport analysis, anion extraction analysis, mass spectrometry-based anion binding studies, and SCXRD analysis (PDF)

Crystallographic data for CCDC 2159196 (CIF)

Crystallographic data for CCDC 2159194 (CIF)

Accession Codes

CCDC 2159193, 2159194, and 2159196 contain the supplementary crystallographic data of this work. This can be obtained from https://www.ccdc.cam.ac.uk/structures/

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Notes

The authors declare no competing financial interest.

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