Supramolecular umpolung: converting electron-rich resorcin[4]arenes into potent CH-bonding anion receptors and transporters

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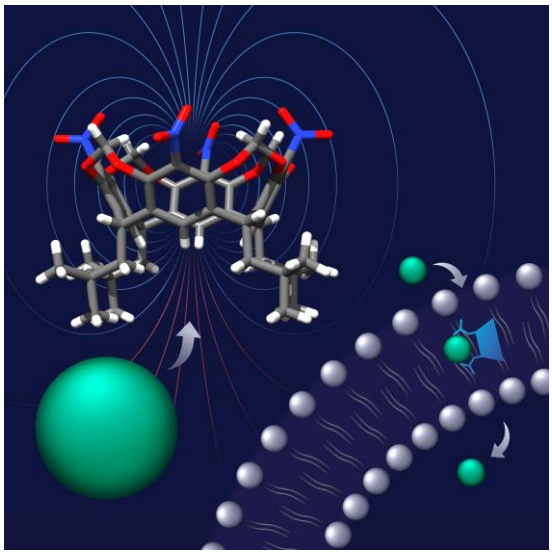

SUMMARY

CH-hydrogen bonding anion receptors constitute an emerging class of anion sensors and transporters which, owing to their proteolytic and pH resistance, have great potential for biological applications. However, CH groups, being weak hydrogen bond donors, require additional enhancement of their binding properties. In this work, we demonstrate a new enhancement strategy that relies on the generation of large dipole moments by a combination of electron-donating and electron-withdrawing substituents. More specifically, the substitution of electron-rich resorcin[4]arenes with electron-withdrawing nitro groups in the upper rim induces a dipole moment of 15.8 D and concentrates electrostatic potential at the lower rim, leading to strong CH···anion binding at this remote site. As a result, tetranitroresorcin[4]arenes, available in just two synthetic steps, acquire high affinities towards halides ($K_a(Cl^-)$ = 1.36 × 10^5 M^{-1} in THF), remarkable tolerance to water, and selective chloride transport activity (EC_{50} = 0.012 mol%). A pivotal role of the seemingly innocent alkyl chains surrounding the binding site in making the receptors resistant to competitive aqueous/organic solvent mixtures and enabling anion transport is also demonstrated.

Anion receptor, calixarene, nitroarene, CH hydrogen bonds, macrocyclic compound, supramolecular chemistry, artificial receptor, bilayer transporter

INTRODUCTION

Anions, due to their ubiquity and technological and biological relevance, constitute important targets for the construction of molecular receptors capable of their capture, sensing, and transport.[1] To ensure efficient binding, anion receptors are typically equipped with strong hydrogen bond donors (NH, OH),[2] halogen bond donors (*e.g.* polarized I atoms),[3,4] or positively charged groups,[5,6] which are known to strongly interact with anions. CH-based anion receptors are inherently weaker, but offer unique selectivity, high stability, and lipophilicity, which make them appealing for certain applications.[7,8] In particular, their lipophilic nature and high resistance to deprotonation make them promising candidates for transmembrane anion carriers.[9,10,11]

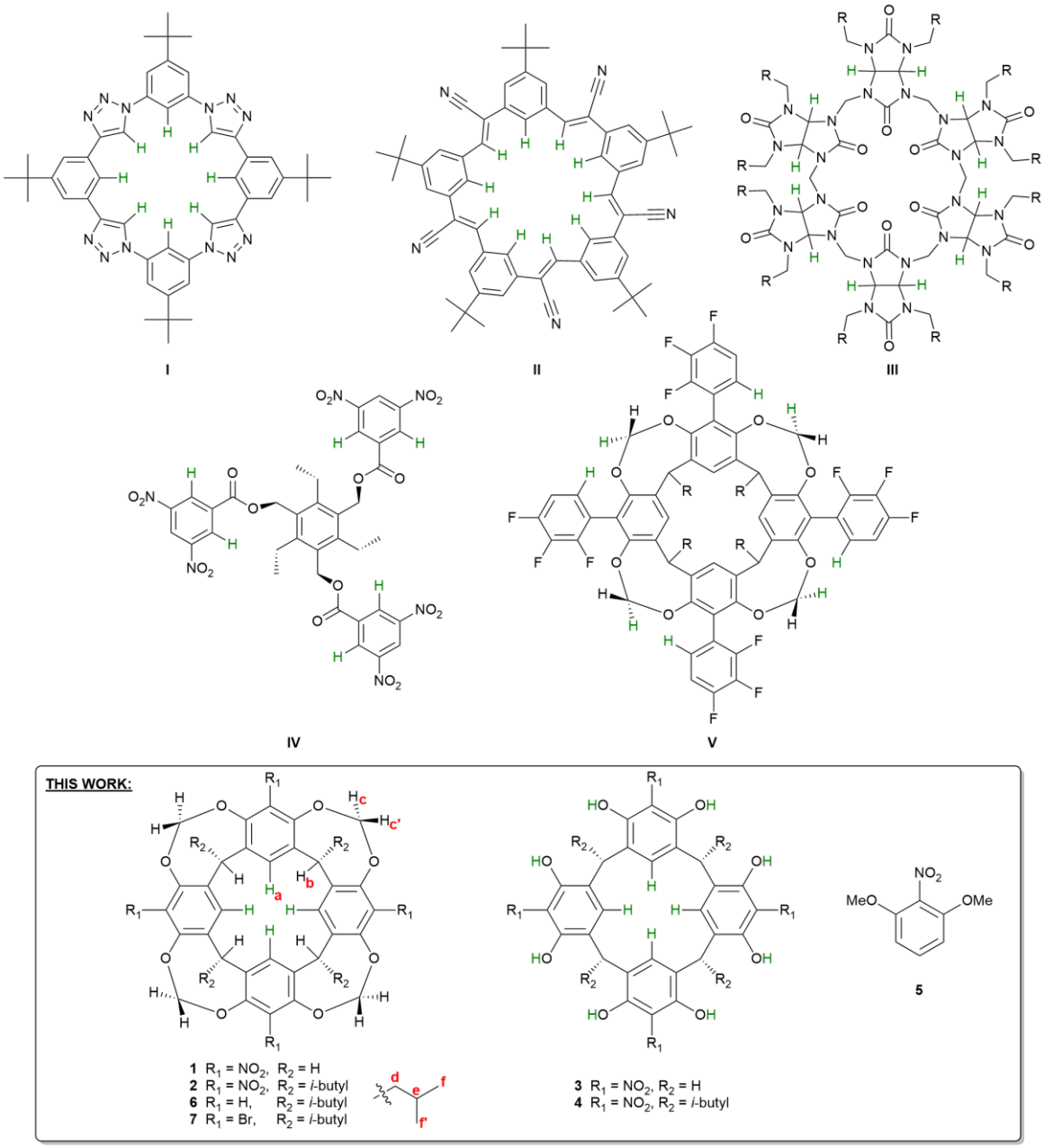

Figure 1. The previously reported and currently designed CH-based anion receptors (hydrogen atoms engaged in CH···anion interactions are marked with green).

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The main challenge in the design of CH-bonding anion receptors stems from the fact that the interaction of a single uncharged CH group with an anion is weak.[12,13] Auspiciously, CH groups are highly abundant and congested in the skeletons of organic molecules, which enables chelate/multivalency effects to enhance binding. Even with these advantages additional polarization of C-H bonds by several strongly electron-withdrawing groups (EWGs), as well as strict elimination of electron-donating groups (EDGs), is considered a prerequisite for any relevant CH···anion binding affinity. Using the principle of enhanced polarization, a range of CH-based anion receptors were recently obtained.[14,15,16,17,18,19,20,21] Some of them, *e.g.* **I-V** (Fig. 1),[17,22,23,24,25] exhibited remarkably high affinity, while others demonstrated the ability to operate in an aqueous environment[26,27] and/or unique transport properties.[9,10,11]

In this work, we demonstrate a novel and nonintuitive strategy for the construction of CH-bonding anion receptors that relies on a combination of electron-donating and electron-withdrawing groups and leads to the inversion of binding preferences. More specifically, we show that resorcinarenes, which are easily available macrocycles and are well established as cation receptors, can be converted into remarkably efficient anion receptors by a simple substitution. This substitution, together with favorable geometrical features of resorcinarenes, generates a large dipole moment, which is crucial to the concentration of electrostatic potential in a single binding site. Furthermore, we show that the seemingly trivial alkyl chain substitution around the anion binding site endows the receptors with remarkable water resistance and enables highly efficient anion transport across lipid bilayers.

RESULTS AND DISCUSSION

Design and theoretical calculations

Resorcin[4]arenes are cone-shaped phenolic macrocycles, well known for their electron-rich cavities and affinity for organic cations.[28,29,30] While some of them have also been shown to bind anions, they acted in these cases as classic OH hydrogen bond donors.[31,32,33] In the absence of OH groups (*e.g.* in *O*-alkylated derivatives), no significant interactions of resorcinarenes with anions could be expected. Nevertheless, the Dalcanale group noticed weak interactions of large anions (PF_6^-) with acetal CH groups in the upper rim,[34] and some of us found weak but non-negligible interactions of anions with **7** *via* aromatic CH groups in the lower rim.[35] The latter observation was particularly surprising because resorcinols are classic electron-rich aromatic rings that have not been previously considered capable of CH···anion interactions.

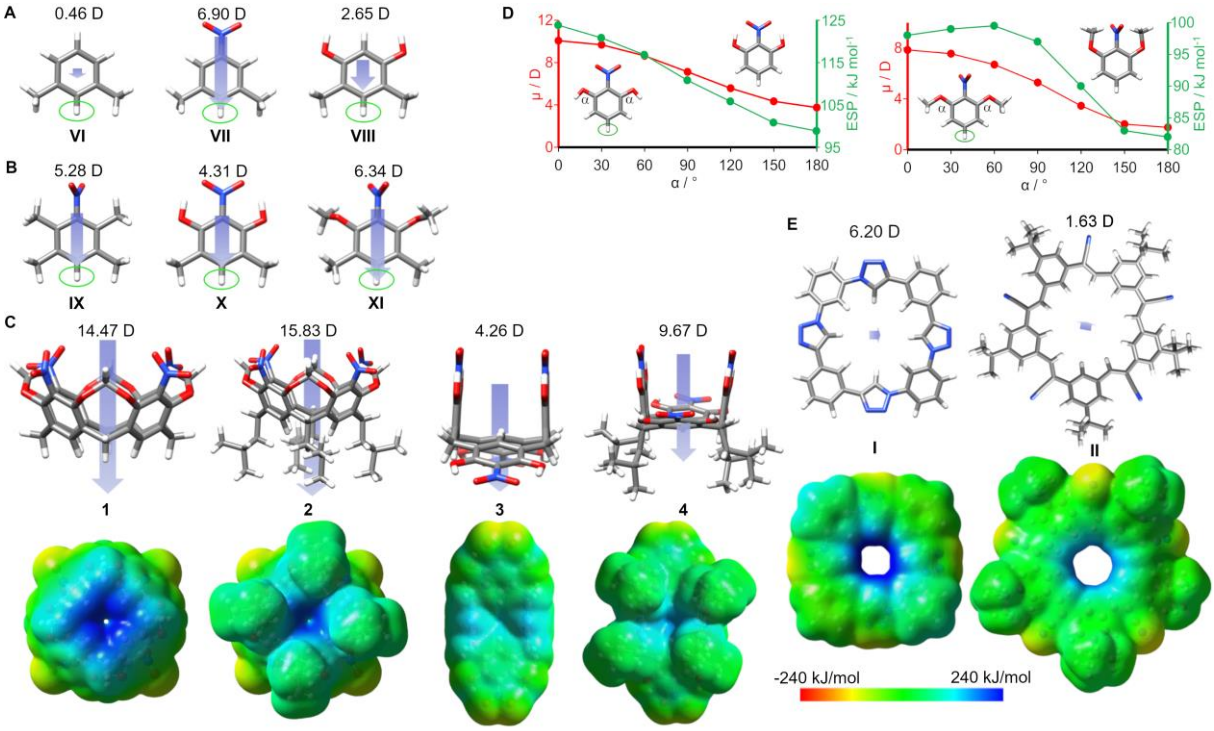

Figure 2. Theoretical calculations. The results of DFT calculations of the geometry, dipole moments (μ), and electrostatic surface potential (ESP) for model monomers **VI-VIII** (A); model monomers **IX-XI** (B); designed receptors **1**-**4** (C); and reference compounds **I** and **II** (E); dependence of the dipole moment μ and ESP values at H_a on the dihedral angle α (D). All calculations at DFT B3LYP/dgdzvp level in THF (PCM model), ESP mapped onto isosurfaces at ρ = 0.005 e/au^3, H_a atoms marked with green circles.

Intrigued by this observation we aimed to rationally enhance anion binding *via* the aromatic CH groups at the lower rim of resorcin[4]arenes, and for this purpose, we designed receptors **1**-**4** (Fig. 1, H_a marked in green). We assumed that the *para*-positioned -NO_2 groups in **1**-**4** would reduce the electron density at H_as by both resonance and induction effects,[17] while the -OCH_2/OH groups would donate electrons, but mostly to their *ortho*- and *para*- positions. However, the net effect of these contributions is difficult to predict based on qualitative arguments, and therefore we performed DFT calculations (DFT B3LYP/dgdzvp in THF) [36] for a series of model compounds (**VI**-**XI**, Fig. 2A-B), newly designed receptors (**1**-**4**, Fig. 2C), and previously known receptors (**I**, **II**, Fig. 2E), to analyze structural determinants of the two parameters that are relevant for anion binding: dipole moment (μ) and electrostatic surface potential (ESP).

Comparison of ESP values at the corresponding H_a atoms of model monomers **VI**-**VIII** (Fig. 2A), reveals the expected trend, *i.e.* the decrease of the ESP value in electron-rich 2,6-dimethylresorcinol **VIII** (+32 kJ mol^{-1}) as compared to 1,3-dimethylbenzene **VI** (+43 kJ mol^{-1}), and substantial increase of the ESP in electron-deficient 1,3-dimethyl-2-nitrobenzene **VII** (+100 kJ mol^{-1}). For **IX** (Fig. 2B), which contains two additional weakly electron donating methyl substituents, the ESP value at H_a decreases substantially (+82 kJ mol^{-1}) as compared to **VII** (+100 kJ mol^{-1}). Less expectedly, for **X** and **XI**, which contain stronger electron donating *meta* substituents (-OH or -OMe), the ESP values remain high (+97 kJ mol^{-1} for **X** and +92 kJ mol^{-1} for **XI**). Moreover, these values proved indifferent to the twist of the NO_2 group (in **X**, the -NO_2 group is coplanar with the aromatic ring, while in **XI** it is perpendicular). Instead, they depend on the rotation of OH/OMe groups (*i.e.* on torsion angle α, Fig. 2D). The highest ESP values are reached for α = 0-30° in **X** and α = 0-100° in **XI**, and their angular dependences are similar to the trends observed for the dipole moments. These observations suggest that the positive potential at H_a originates mostly from the inductive effects of electronegative atoms (O and N) in the upper part of the ring. -OH and -OMe substituents in *meta* positions do not reduce the positive ESP significantly, which implies that these EDG substituents are not detrimental for CH···anion interactions.

Macrocycles **1** and **2** consist of four **XI** units (Fig. 2C) and feature fixed cone conformations and -OAlk groups positioned at $\alpha \approx 100°$, optimal for the high ESP at H_a. The ESP values of **1** and **2** reach +240 kJ mol^{-1} in the apex area and remain high also in the vicinity of the neighboring aliphatic groups. The ESP values calculated for the macrocycles are much higher than for their components (**XI**), highlighting the importance of convergence. It is also important to note that **1** and **2** have large dipole moments (μ = 14.5 D for **1** and μ = 15.8 D for **2**) directed vertically, which is a consequence of their cone geometry (components perpendicular to the main axis cancel out, while the vertical components sum up). To estimate the potential of **1** and **2** as anion receptors, we compared them with the 'golden standards' in the area, that is the tetratriazole macrocycle **I**[22]and cyanostar **II**[23] (Fig. 2D). Calculations performed at the same level of theory indicate that the ESP values for **1** and **2** are similar to that of **II** (+240 kJ mol^{-1}) and only slightly lower than that of **I** (+270 kJ mol^{-1}).

In contrast to **1** and **2**, macrocycles **3** and **4**, consisting of four **X** units, adopt boat conformations, with all the OH groups positioned at α = 180° and engaged in intramolecular hydrogen bonds with -NO_2 groups. Although **X** (the building block of **3** and **4**) has ESP values similar to **XI** (the building block of **1** and **2**), the geometrical features of the macrocycles **3** and **4** preclude convergence and reduce the overall dipole moments. Both effects are responsible for low and scattered ESP values in **3** and **4**, making them less promising as anion receptors.

Synthesis

Tetranitroresorcinarenes **1** and **2** were obtained in two steps from commercially available substrates (see SI). First, the condensation of 2-nitroresorcinol with formaldehyde in aqueous NaOH gave **3** in 75 % yield.[37,38] Analogous condensation with isovaleraldehyde led to the formation of **4** as a mixture of regioisomers rccc-**4** and rcct-**4**. The required rccc-**4** (all aliphatic tails down) was isolated by column chromatography in a 45 % yield. Next, both tetranitroresorcinarenes **3** and rccc-**4** were *O*-bridged using bromochloromethane in DMF/Cs_2CO_3, to give **1** (45 %) and **2** (35 %), respectively. Compounds **5**-**7** were obtained according to the literature procedures.[39]

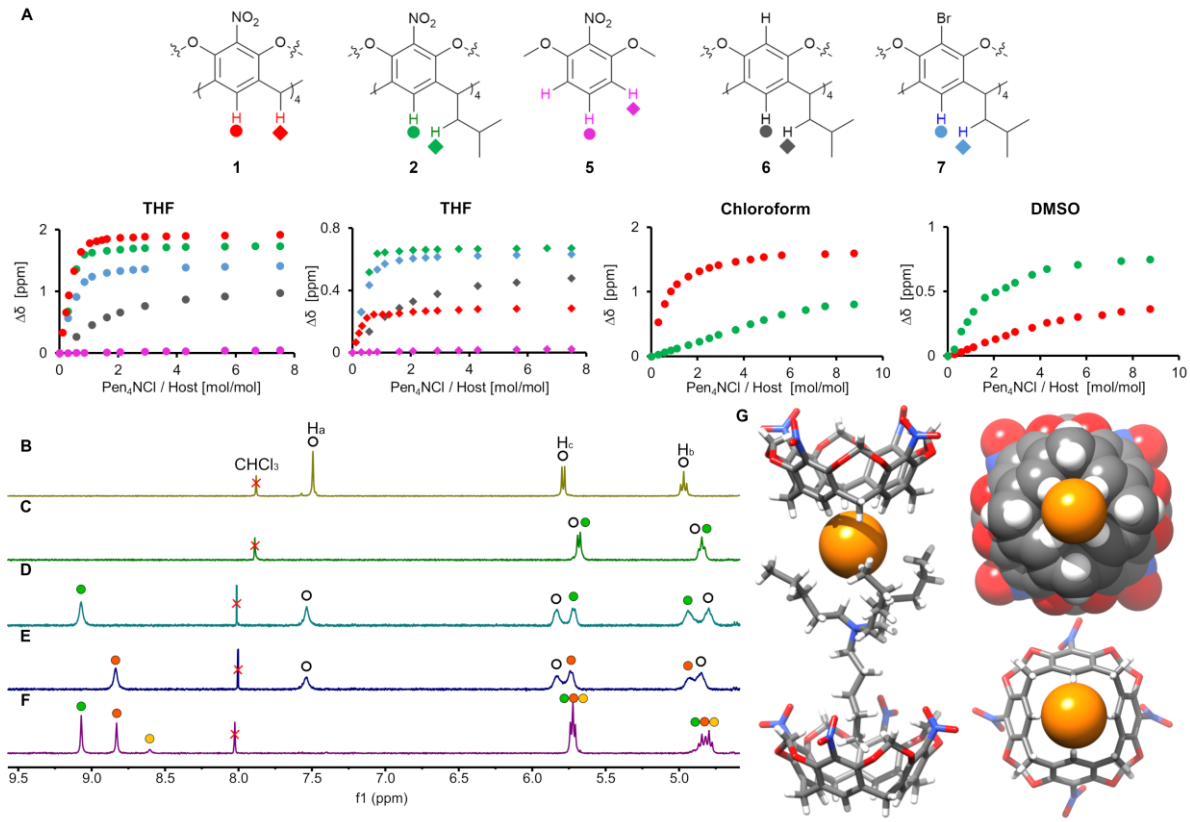

Figure 3. Anion binding by resorcinarenes. (A) Chemical shift changes for marked signals during ^{1}H NMR titrations of receptors (hosts) with Pen_4NCl in various solvents (C_{host}= 6.7 mM). (B-F) ^{1}H NMR spectra of **2** (B); **2** + Pen_4NCl (0.5 equiv.) at 300 K (C); **2** + Pen_4NCl (0.5 equiv.) at 255 K (D); **2** + Pen_4NBr (0.5 equiv.) at 255 K (E); **2** + Pen_4NCl + Pen_4NBr +But_4NI (1 equiv. each) at 255 K (F); all spectra in THF-d_8, 600 MHz, º free **2**, • **2**⊃Cl^-, • **2**⊃Br^-, • **2**⊃I^-, × residual $CHCl_3$). (G) X-ray crystal structure of **1**⊃Pen_4NBr (grey – C, light grey –H, red – O, blue – N, orange – Br, CCDC 2265717).

Anion binding

The binding properties of **1**-**7** towards Cl^- were investigated by ^{1}H NMR titrations in THF-d_8, using Pen_4NCl as a titrant. In the case of **1**, the addition of Pen_4NCl resulted in large downfield shifts of H_a ($\Delta\delta$ = + 1.9 ppm) and H_b signals directed towards the lower rim ($\Delta\delta$ = +0.30 ppm), while the remaining signals were almost completely indifferent (Fig. 3A). In case of **2**, Cl^- binding shifted signals from H_a ($\Delta\delta$ = + 1.7 ppm) and from the lower rim aliphatic hydrogens H_c ($\Delta\delta$ = +0.6 ppm). Interestingly, large changes (+7 ppm) were also observed in the ^{13}C NMR spectrum of **2**, namely at the signal corresponding to the carbon atom involved in C-H···anion interaction. These changes indicate that for both **1** and **2**, Cl^- binding takes place in the lower rim, *i.e.* at the apex of the cone.

The affinity and selectivity of **1** and **2** towards various anions (as *n*-Pen_4N^+ or *n*-Bu_4N^+ salts) were studied in various solvents and solvent mixtures (Table 1). In THF, **1** and **2** bind Cl^- and Br^- anions with affinities that exceed the detection limit of ^{1}H NMR titration. The K_as determined by UV titrations reach values as high as $1.36\cdot10^5$ M^{-1} for Cl^- binding by **2**, and $6.9\cdot10^4$ M^{-1} by **1**. Complexes of **2** with halides show slow kinetics of complexation on the ^{1}H NMR timescale, in line with their high stability. At ambient temperature, the signals of the interacting protons disappear (Fig. 3C) during ^{1}H NMR titrations and re-appear at different positions after >1 equiv. of $Pen_4NCl/Br/I$ is added (see SI). Upon lowering the temperature to 255 K, two sets of signals that correspond to free receptor **2** and its complex (**2**⊃X^-) become clearly visible throughout the entire titration (Fig. 3D-E and SI). In the ^{1}H NMR spectrum of a solution containing an equimolar mixture of **2**, Pen_4NCl, Pen_4NBr, and But_4NI (6.7 mM each) at 255 K, there are three sets of signals corresponding to **2**⊃Cl^-, **2**⊃Br^- and **2**⊃I^-, at ratios 0.47:0.42:0.11 (Fig. 3F). This selectivity order (Cl^-≈Br^->>I^-) corresponds well to the respective K_a values. It should be noted that the difference between the affinity to Cl^- and Br^- is small despite the much stronger hydrogen bond accepting ability of Cl^-. This suggests that the soft nature of CH bond donors and geometrical features of **2** favor softer and larger Br^- anion.

Table 1. Association constants (K_a)

Receptor	Anion	Solvent	K_a / M^{-1} [a]	Error / %	LogK_a
1	Cl^-	THF	69 000 [b]	(11 %)	4.84
	Br^-	THF	87 000 [b]	(7 %)	4.94
	I^-	THF	1 800	(39 %)	3.26
	ClO_4^-	THF	1 500	(7 %)	3.18
	PF_6^-	THF	400	(3 %)	2.61
	Cl^-	THF/water 9:1	36	(1 %)	1.55
	Br^-	THF/water 9:1	100	(3 %)	2.00
	I^-	THF/water 9:1	150	(1 %)	2.18
	ClO_4^-	THF/water 9:1	240	(4 %)	2.37
	Cl^-	acetone	4 300	(18 %)	3.64
	Cl^-	chloroform	950	(15 %)	2.99
	Cl^-	DMSO	18	(2 %)	1.24
2	Cl^-	THF	136 000 [b]	(10 %)	5.14
	Br^-	THF	63 000 [b]	(5 %)	4.80
	I^-	THF	29 000	(26 %)	4.46
	NO_3^-	THF	4 412	(15 %)	3.65
	ClO_4^-	THF	1 900	(9 %)	3.28
	Cl^-	THF/water 9:1	500	(3 %)	2.70
	Br^-	THF/water 9:1	2 500	(6 %)	3.40
	I^-	THF/water 9:1	11 500	(22 %)	4.05
	ClO_4^-	THF/water 9:1	3 800	(7 %)	3.58
	Cl^-	chloroform	< 10	(2 %)	0.80
	Br^-	chloroform	16	(3 %)	1.20
	Cl^-	DCM	81	(3 %)	1.91
	Cl^-	DMSO	130	(6 %)	2.11
	Cl^-	acetone	>136 000	-	>5.14
	Cl^-	THF/MeOH 6:4	120	(1 %)	2.07
	Cl^-	THF/DCM 4:6	500	(4 %)	2.71
	Cl^-	MeOH/DCM 4:6	9	(2 %)	0.94
4	Cl^-	THF	-[c]	-	-
5	Cl^-	THF	< 10	(1 %)	<1
6	Cl^-	THF	144	(1 %)	2.16
7	Cl^-	THF	6 700[b]	(3 %)	3.83

K_a - 1:1 association constants determined by titration of receptors **1**-**5** with *n*-Pen_4X or *n*-Bu_4NX salts, fitted with the Bindfit program.[40]
[a] Determined by ^1H NMR titrations, if not noted otherwise.
[b] Determined by UV titrations.
[c] No changes in lower-rim H_a signals were detected, instead, OH signals were shifted downfield, indicating interference of anions with the intramolecular hydrogen bonding system (*e.g.* Fig. S81).

Comparison of **2** with receptors **6** and **7** that have similar geometry but possess either -H (**6**, μ = 1.5 D, ESP(H_a) = **117** kJ mol^{-1}) or -Br (**7**, μ = 4.3 D, ESP(H_a) = 163 kJ mol^{-1}) substituents in the upper rim points to the crucial role of the electron withdrawing nitro substituent. For **6**, Cl^- binding affinity is three orders of magnitude lower, while for **7** it is 20 times lower than for **2**. Multivalency and convergence are also crucial, as illustrated by binding affinities of **4** and **5**. Almost no binding was observed for the acyclic monomer **5**. Macrocycle **4**, which adopts a boat conformation, also binds anions but considerably in a weak manner, and uses OH groups from the upper rim rather than CH_as from the lower rim. This is in agreement with the DFT calculations, which show much lower ESP values for **I** and no accumulation of the ESP at H_as of **4** (Fig. 2C).

X-ray structures

The solid-state structures of **1** (as THF, AcOEt or Et_2O solvates, Figs. S93-S95), **2** (as THF or $CHCl_3$ solvates, Figs. S96-S97) and **1**⊃Pen_4NBr (Figs. 3G, S92) were determined by X-ray crystallography. The solid-state structures of receptors **1** and **2** confirm the geometrical features suggested by the calculations, namely the out-of-plane twist of the -NO_2 groups (60-90°angle between the nitro group and the plane of the phenyl ring) and the α angles of *ca.* 100°. The structure of **1**⊃Pen_4NBr confirms the suggested binding mode, with Br^- located at the apex of the cone. The anion forms four almost linear CH_a···Br^- hydrogen bonds (C···Br 3.90 Å, CH_a···Br 2.97 Å, C-H···Br angle 165°), whereas the distance to CH_b is larger and has less favorable geometry (C···Br 4.55 Å, CH_b···Br 3.86 Å, C-H···Br angle 129°), suggesting that the role of CH_b moieties in anion binding by **1** is only supportive. It should also be noted that the Br^- anion is nestled between the arms of the

Pen_4N^+ cation, with short distances to CH_2 groups at α, γ and δ positions (CH···Br distances of 3.15-3.36 Å). In the structure of **1**⊃Pen_4NBr, there is an additional crystallographically independent molecule of **1**, which has no short contact with the anion and hosts one of the aliphatic arms of Pen_4N^+ in the cavity.

Atypical solvent dependence

Solvent polarity is the main parameter that modulates the stability of complexes. For complexes that are based on hydrogen bonds, the affinity decreases with increasing solvent polarity ($\Delta G \sim 1/\varepsilon$).[41] Although the correlation is not linear in the whole range of ε, the trend is typically preserved.[42] Water is one of the most strongly competing solvents due to its high ε and both strong hydrogen bond donating and accepting ability, which implies effective solvation of both anions and receptors.[43] On the other extreme, for receptors that bind lipophilic anions through classical and nonclassical hydrophobic effects, organic solvents are proven to be destructive.[44,45,46,47,48,49,50,51]

We found that the solvent dependence for **2** is different and that the typical trends mentioned above are violated (Table 1). For example, the K_a (**2**, Cl^-) in acetone (ε = 20.1) is higher than in THF (ε = 7.6), while in DCM (ε = 8.9) it is three orders of magnitude lower. Furthermore, anion binding in chloroform, which is the least polar among the tested solvents (ε = 4.8), is negligible. Particularly remarkable is the strength and selectivity of complexation in THF:water 9:1. This mixture of organic solvent and water should be destructive for anion binding regardless of the binding mechanism. We found that the addition of water indeed decreases the binding constant for the most hydrophilic anions, but the value of K_a (**2**, Cl^-) = 500 M^{-1} remains remarkably high considering such a competitive environment and a receptor based only on weak CH hydrogen bond donors. At the other extreme, the affinity for hydrophobic I^-, K_a (**2**, I^-) > 10^4 M^{-1}, is also unprecedented, considering the 90% content of the organic solvent in the mixture. To explain the solvent dependence and the high affinity of **2** towards anions, we have to consider its unique structural features: hydrophobic binding site positioned in the hydrophobic, semi-open pocket and the overall high dipole moment of the molecule. Thus, the high dipole moment provides an attractive force that drives anions into the binding site through a lower rim binding funnel, composed of CH groups possessing diffuse partial positive charges. This hydrophobic binding funnel makes polar molecules (water) and molecules with a significant fraction of positively polarized H atoms on their surfaces (THF, acetone, DMSO) unwanted guests in the cavity, which explains the relative resistance of anion binding by **2** to these solvents. On the contrary, chloroform, which has a diffuse negative charge at the surface originating from three C-Cl bonds, is highly competitive. The semi-open cavity may also be beneficial because binding may not require full desolvation of the anions (the most energy-demanding is the removal of the last water molecules from the solvation sphere).[52]

The anion binding characteristics of **1**, which is devoid of the hydrophobic pocket, agree with the above reasoning, and, contrary to **2**, the solvent dependence of anion binding by **1** is more typical (*e.g.*, lowering of K_a values for all anions was observed in THF:water 9:1, acetone and DMSO). Particularly illustrative are the opposite effects of chloroform and DMSO on chloride binding by **1** and **2** (Fig. 2A). In chloroform, Cl^- binding by **2** is strongly diminished (due to competitive interactions with hydrophobic walls), while for **1** strong binding of Cl^- is still observed. In DMSO, the effects are opposite: Cl^- binding by **2** is effective because DMSO cannot compete for the alkyl chains-buried binding site, whereas for **1**, DMSO reduces Cl^- binding to less than 20 M^{-1}, because it can easily access the binding site.

Anion transport across lipid bilayers

The strong affinity of **1** and **2** for halides, together with their high lipophilicity, prompted us to study their ability to transport chloride anions across lipid bilayers. In biological systems, anion transport through lipophilic membranes plays important roles in many essential biochemical processes, such as cellular respiration, signal transduction, pH regulation, and removal of metabolic products.[53] In living organisms, this task is performed by numerous specialized anionophores, and their failure often leads to severe diseases, such as cystic fibrosis, anemia, congenital myotonia, deafness, diabetes, and cancer.[53,54] Accordingly, considerable research efforts have been devoted to the development of artificial anionophores, motivated by the prospect of curing these diseases through the displacement of dysfunctional proteins.[55,56,57,58] This is challenging though, because the nonselective transport of ions might perturb complex ion homeostasis to the extent that the transporters become toxic. In particular, most NH-bonding transporters discharge proton gradients that are critical for maintaining life functions.[59] It is therefore particularly important to develop highly selective, non-protonophoric anion transporters. One of the most promising approaches to achieving this goal is based on the use of receptors that are highly resistant to deprotonation,[9,60,61] such as the CH-hydrogen bonding receptors described herein.

In this context, we first evaluated the Cl^- transport activity of **1**, **2**, **6**, and **7** in Cl^-/NO_3^- exchange, using large unilamellar vesicles (LUVs, ~200 nm, POPC:cholesterol 7:3, Fig. 4A) as a model membrane system.[9,10] The kinetics of the exchange were followed by chloride-induced quenching of lucigenin emission (entrapped inside LUVs) in response to chloride pulses. Receptors **1**, **2**, **6**, and **7** were incorporated into lipid bilayers either during (pre-incorporation method) or after (post-incorporation method) the formation of LUVs; in the latter case,

the transporters were added to the suspension of vesicles as a solution in THF. The results show no significant transport activity for the receptors applied by the post-incorporation method. However, upon pre-incorporation, resorcin[4]arene **2** exhibited significant Cl^-/NO_3^- exchange activity, in striking contrast to **1**, **6**, and **7**, which were still inactive (Fig. 4B).

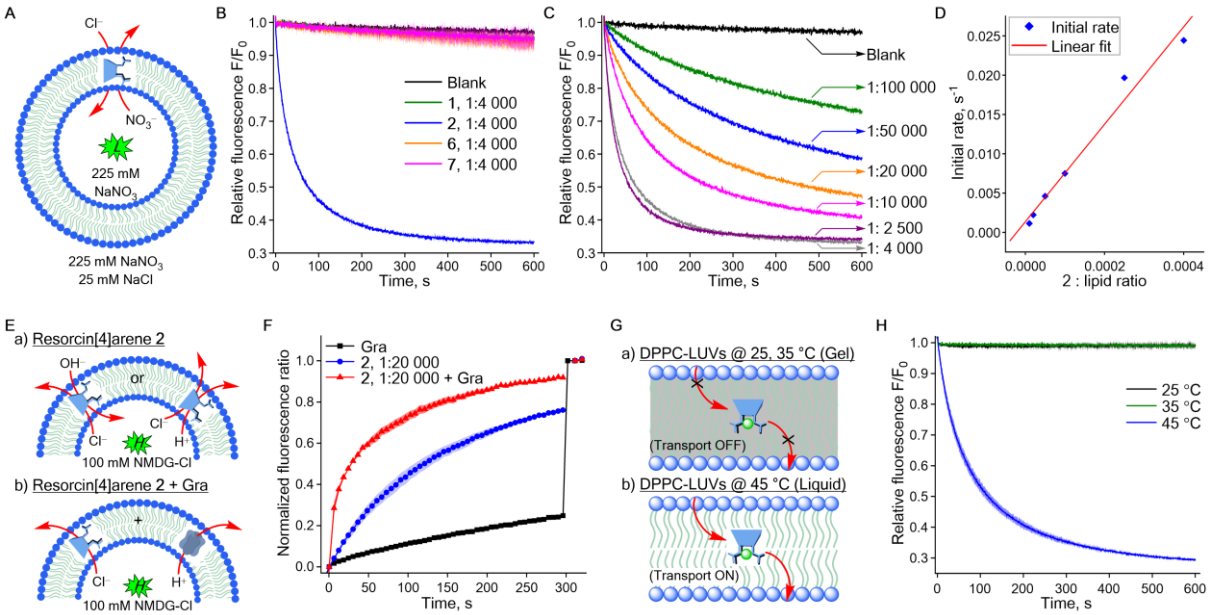

Figure 4. Cl^- transport by resorcin[4]arene receptors. (A) Schematic representation of POPC/cholesterol=7/3 LUVs with entrapped chloride sensitive dye, lucigenin, that were used for measuring Cl^-/NO_3^- exchange. (B) Change in the relative fluorescence F/F_0 due to the transport of chloride anions into POPC/cholesterol=7/3 LUVs by resorcin[4]arenes **1**, **2**, **6**, and **7**, pre-incorporated in the lipid membrane at 1:4000 transporter-to-lipid molar ratio. (C) Change in the relative fluorescence F/F_0 due to the transport of chloride anions into POPC/cholesterol=7/3 LUVs by resorcin[4]arene **2** preincorporated in the lipid membrane at 0.001 – 0.04 mol% transporter-to-lipid molar ratios. (D) The plot of initial rates from the transport of chloride anions into POPC/cholesterol=7/3 LUVs by resorcin[4]arene **2** pre-incorporated in the lipid membrane, as a function of transporter-to-lipid molar ratio and their linear fit. (E) Schematic representation of POPC/cholesterol=7/3 LUVs with entrapped pH-sensitive dye HPTS for measuring Cl^- ion transport in NMDG-Cl medium. (F) Change in normalized fluorescence ratio for the transport of Cl^- anion in NMDG-Cl medium into POPC/cholesterol=7/3 LUVs by resorcin[4]arene **2**, preincorporated in the lipid membrane at 1:20000 transporter-to-lipid molar ratio (0.005 mol% of the transporter with respect to combined lipids). (G) Schematic representation of DPPC bilayers below (25 and 35 °C) and above (45 °C) the phase transition temperature of the DPPC lipid. (H) Change in the relative fluorescence F/F_0 for the transport of chloride anions into DPPC LUVs with entrapped lucigenin dye by resorcin[4]arene **2** preincorporated in the lipid membrane at 1:20000 transporter-to-lipid molar ratio (0.005 mol% of transporter with respect to the DPPC lipid). Medium: 225 mM $NaNO_3$.

To quantify the transport activity of **2**, we performed a series of experiments varying the **2**-to-lipid molar ratio (Fig. 4B).[62] This gave us the specific initial transport rate [*I*] = 88 s^{-1}, which is the highest among the CH-based transporters reported to date (much higher chloride transport activity was observed for fluorinated bambusurils in Cl^-/HCO_3^- antiport by Valkenier et. al.,[9] but the bambusurils were less active than **2** in Cl^-/NO_3^- antiport), and the EC_{50} value of 0.012 mol% (corresponding to 1:8333 transporter-to-lipid molar ratio), which confirmed the superior activity of **2** in Cl^-/NO_3^- exchange. Linear concentration dependence of initial transport rates (Fig. 4D) together with the Hill coefficient n = 1.0 ± 0.6 suggest that **2** acts as a monomer, and therefore likely operates as a carrier rather than as a channel, which is consistent with the fact that a single molecule of resorcinarene **2** (0.88 nm in hight) is too small to span the lipid bilayer (3.5-4 nm in thickness).[9,63] The carrier mechanism was further corroborated by experiments in which the fluidity of the lipid membrane was varied, either by increasing cholesterol content,[64] or utilizing temperature-dependent fluidity of DPPC bilayers.[65] Both experiments confirmed that transporter **2** operates as a carrier. For example, upon increasing the cholesterol content from 30 mol% to 45 mol%, a marked decrease in the transport rate was observed from (*I*) = 0.020 s^{-1} to (*I*) = 0.004 s^{-1} (see SI). Furthermore, in DPPC LUVs, which exhibit a phase transition at around 41°C, no transport activity of **2** was observed at 25°C and 35°C (*i.e.* below the phase transition temperature), but the activity was restored at 45°C, that is upon melting the membrane (Fig. 4H).

To further elucidate the mechanism of chloride transport by **2**, Cl^-/SO_4^{2-} exchange was attempted. Due to its double charge and extreme hydrophilicity, SO_4^{2-} is considered unable to pass through lipid bilayers without the help of specialized transporters, and therefore unlikely to exchange with Cl^- *via* an antiport mechanism.[66,67] Somewhat surprisingly, we observed a small (approx. 10%), albeit significant drop in fluorescence intensity within the first *ca.* 100 s, followed by a plateau (Fig. S120). We hypothesized that this is due to H^+/Cl^- co-transport,

which generates an H^+ gradient across the membrane and, in this way, prevents further chloride influx. Alternative mechanisms, such as Cl^-/SO_4^{2-} exchange and Na^+/Cl^- co-transport, would lead to full equilibration between the interior and exterior of the LUVs. The latter two mechanisms were also ruled out based on HPTS assays performed in sodium gluconate and in *N*-methyl-D-glucamine sulfate solutions (see SI).[68,69] The H^+/Cl^- co-transport in sodium sulfate solution was further confirmed using vesicles loaded with a pH-sensitive dye, HPTS, instead of lucigenin.[70] Upon injection of NaCl, a decrease of the intravesicular pH was observed, indicative of the transport of H^+ along Cl^- into the vesicles (Fig. S121).

Since the H^+/OH^- transport is toxic to most living cells, it was of interest to check whether **2** is capable of transporting Cl^- selectively, *i.e.*, to equilibrate the Cl^- gradient faster than the H^+ gradient. To test this, we studied Cl^- transport in buffered *N*-methyl-D-glucamine hydrochloride (NMGD-Cl) solution, using HPTS assay.[9,71] The NMDG and its cation are considered to be too hydrophilic to be transported across the bilayer, and therefore no pH equilibration should be observed if the transport takes place through electrogenic mechanism only (due to the lack of any charge compensation mechanism). Contrary to these expectations, the activity of **2** under these conditions was significant. This is in agreement with the results described above, which showed that **2** can facilitate H^+/Cl^- symport (or functionally equivalent OH^-/Cl^- antiport). However, the activity of **2** was significantly (16 times) enhanced in the presence of gramicidin, a natural cationophore known to very rapidly transport H^+ (and Na^+) across lipid membranes. Apparently, the onset of the fast charge compensation mechanism speeds up the chloride transport by **2**, meaning that **2** transports Cl^- much faster than H^+ or OH^-.

Another topic of great physiological relevance is the ability of artificial transporters to facilitate Cl^-/HCO_3^- exchange – a process that is crucial for vital biological functions, such as respiration and digestion.[72,59] To investigate this, we measured chloride transport by **2** in sodium bicarbonate solution, using the lucigenin method.[9,10] As shown in Fig. S127, the chloride influx under these conditions is slower than in sodium nitrate, with a specific initial rate [I] = 9.2 s^{-1}, *i.e.* almost 10 times lower than for chloride/nitrate exchange. This is likely due to the soft nature of **2** and hence reduced affinity to hard anions, such as HCO_3^-.

Finally, since some resorcin[4]arenes are known to bind alkali metal cations, we were interested to see if there is any influence of cations on the transport activity of **2**. To verify this, we compared the results of the lucigenin assays using cesium salts instead of sodium salts.[64] The results were the same within the experimental error (see SI), indicating that alkali metal cations are unlikely to play any significant role in the Cl^-/NO_3^- exchange process. This is in agreement with the aforementioned lack of activity in the Na^+ transport experiment performed in a sodium gluconate medium (see SI).

Based on all transport experiments, it can be concluded that **2** is a very efficient and selective Cl^- transporter, operating *via* the carrier mechanism. The H^+ (or OH^-) co-transport was not completely avoided, but the selectivity of **2** for Cl^- transport is high (16-fold), which bodies well for future medicinal applications of this and other similar transporters.

Also, it is worth noting the striking difference in transport activity between **2** and the other three resorcinarenes, **1**, **6**, and **7**. Resorcin[4]arene **1** differs from **2** only in the absence of alkyl substituents in the lower rim. Apparently, the substituents play a key role in Cl^- transport across the lipophilic membrane, likely by surrounding the anion. On the other hand, alkyl substituents alone do not grant resorcin[4]arenes any significant anion transport ability, as shown by **6** and **7**. Due to the lack of nitro substituents in the upper rim, the latter two compounds have much lower dipole moments and much weaker chloride affinity than **2**; apparently too low to extract anions from the aqueous phase.

Conclusions

In conclusion, we have developed a new class of anion receptors and transporters based on the convergent array of CH hydrogen bond donors augmented with strong dipole-anion interactions. The effectiveness of the new receptors is comparable with the current golden standards in the field. Uniquely, their CH hydrogen bond donors come from the aromatic core of resorcin[4]arenes, substituted with both electron-withdrawing $-NO_2$ and electron-donating -O-alkyl groups. This design breaks the standard rules for the design of CH-bonding anion receptors, because the -O-alkyl groups would traditionally be expected to counterbalance the polarization of CH hydrogen bond donors. Despite this, in the current case, the inductive effects, the confocal position of the donors, and additional stabilization originating from optimally oriented dipole moments of the molecule result in unexpectedly high effectiveness in anion binding and transmembrane transport. These findings open new directions in the development of anion receptors because they demonstrate that electron-rich aromatic rings can also support strong anion binding.

Furthermore, we have also shown that simple alkyl substitution around the anion binding site renders the receptors remarkably water-tolerant and grants them outstanding anion transport properties. For example, the affinity of **2** toward iodide exceeds 10^4 M^{-1} even in the presence of 10% water in THF, while its activity in Cl^-/NO_3^- exchange across lipid bilayers surpasses the activity of previously described CH-bonding transporters. Resorcin[4]arene **2** shows also remarkable $Cl^- > OH^-$ selectivity in transport experiments, likely due to the soft nature of its CH donors and their resistance towards deprotonation. Such selectivity is highly desirable in medicinal applications because it is believed to minimize the toxicity of transporters.

The newly designed receptors have the advantages of simplicity, modularity, and synthetic availability: their synthesis involves only two metal-free steps and starts from commercially available and inexpensive reagents. We envision that through modifications of the lower rim substituents, their selectivity can be further tuned to bind anions of various shapes and in a variety of environments. Importantly, the synthetic procedures for such modifications are well established, because resorcin[4]arenes have been extensively used in supramolecular chemistry for many years and lower rim modifications have been utilized to control their solubility. In light of the current findings, lower rim modifications of resorcin[4]arenes can take new directions, and the existing synthetic know-how can result in unusually fast progress in this field.

EXPERIMENTAL PROCEDURES

Resource availability

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Agnieszka Szumna (agnieszka.szumna@icho.edu.pl).

Materials availability

There are restrictions to the availability of the reagents generated in this study because of the limited amounts synthesized.

Data and code availability

Crystallographic data are available free of charge from the Cambridge Crystallographic Data Centre under references CCDC 2265717-2265722. All other data supporting the findings of this study are available in the manuscript or supplementary materials.

Experimental procedures

Details of synthetic procedures, NMR spectra, titration protocols, DFT calculation details and coordinates for the optimized structures, detailed protocols, and results of anion transport studies can be found in Supplemental Information.

SUPPLEMENTAL INFORMATION

Supplemental Information includes experimental procedures, spectra, coordinates for optimized structures, and results of anion transport studies, Figures S1–S130.

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AUTHOR CONTRIBUTIONS

E.R.A. – investigation (synthesis, solution measurements, NMR experiments); D.M. – investigation (transport experiments); P.C. investigation (NMR experiments); H.J. investigation (theoretical calculations); O.D. investigation (X-ray structures); M.J.C. – conceptualization, funding acquisition, supervision, original draft; A.S. – conceptualization, funding acquisition, supervision, original draft. All authors contributed to visualization and writing, review & editing.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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