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Anion-binding aromatic pentaamide macrocycles: Synthesis, high-affinity binding, transmembrane transport, and catalysis

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The precise spatial positioning of functional groups in biomacromolecules leads to astonishing binding, catalytic, and transport capabilities. In contrast, synthetic frameworks capable of convergently locking functional groups with minimized conformational uncertainty are highly desirable but rare. Here we report C₅-symmetric aromatic pentaamide macrocycles c5a-c synthesized in one pot from the corresponding monomers. The crystal structure of c5c reveals a fully constrained backbone that enforces ten alternating NH/CH hydrogen-bond donors and five large amide dipoles to point to the center of the macrocycle. With a highly electropositive cavity in a high-energy unbound state, macrocycles c5 bind anions in a 1:1 stoichiometry in solution, with *K_a* values up to 10⁷ M⁻¹ for halides and 10⁸ M⁻¹ for oxoanions. Macrocycle c5a was able to selectively transport chloride ions across lipid bilayers. The extraordinary binding of phosphate-related ions by c5 also enabled catalytic ester amidation owing to the stabilization of the corresponding transition states.

Molecular and supramolecular structures with multiple functional groups being convergently placed at defined positions occur ubiquitously in nature¹. By amassing multiple noncovalent forces^{2,3}, such structures exhibit sophisticated functions ranging from ligand binding and catalysis to mass transport and signal transduction that are still beyond the reach of synthetic constructs. For example, with a “selectivity filter” defined by aligned polar groups and dipoles, the potassium channel KcsA⁴ or the water channel aquaporin 1⁵ achieves astounding efficiency and exclusive selectivity in transporting potassium ions or water molecules. The remarkable power of convergent functional groups is also demonstrated by many ligand-binding proteins such as the sulfate-binding protein (SBP) from the gram-negative bacterium *S.*

*typhimurium*⁶. SBP binds the sulfate ion, an anion with a large hydration enthalpy (-258 kcal/mol), in high affinity ($K_a = 8.3 \times 10^6 \text{ M}^{-1}$ at pH 8.3) in water. By forming seven neutral hydrogen bonds with the sulfate ion, five involving the main chain peptide NH groups, one with a serine OH and another with the indole NH of a tryptophan residue, SBP demonstrates that neutral hydrogen bonds, if properly placed, can result in extraordinary binding affinity for an anion.

As a major class of guests, anions have attracted wide interest in molecular recognition because of their important roles in biology, medicine, catalysis, and the environment⁷. In contrast to cations, anions, with a variety of sizes and shapes, are challenging targets that remained a largely elusive species for synthetic receptors until recent years⁸. Anion recognition requires the convergent alignment of binding groups, especially hydrogen-bond donors. So far, typical anion receptors include linear or branched molecules carrying hydrogen-bond donors based on the NH groups of amides, ureas, pyrroles, amines, and ammoniums⁹. More recently, systems based on halogen bonds were also reported¹⁰⁻¹². With such hosts, the entropic costs for hydrogen-bond donors to converge on anions have to be compensated by the enthalpic gains from multiple non-covalent interactions¹³.

Efforts on arranging convergent functional groups with synthetically accessible structures have continued for quite a long time. A highly effective strategy for converging multiple functional groups is based on rigid structures such as molecular clefts^{14,15}, backbone-rigidified foldamers¹⁶⁻¹⁸, and rigid macrocycles¹²⁻²⁵. Such structures exhibit unique and often remarkable properties including selective and tight binding of guest species.

Shape-persistent macrocycles are especially powerful in converging functional groups and generating non-deformable cavities with defined sizes, based on which anion binders featuring high binding affinity and selectivity could be created. A rigid macrocycle reported by Flood *et al.* forms stable 2:1 sandwich complexes with large anions by orienting convergent cyanostilbene and phenyl CH groups²⁶. Another rigid macrocycle having convergent triazole and phenyl CH groups, also developed by Flood *et al.*, binds the chloride ion ($K_a = 1.3 \times 10^5 \text{ M}^{-1}$ in CH_2Cl_2) with a 1:1 stoichiometry²⁷. These systems, along with other examples such as those reported by Anslyn²⁸, Sessler^{25,29}, and Gale³⁰, demonstrate that rigid cyclic hosts with convergently organized hydrogen-bond donors, even those involving aromatic CH groups which are regarded as “weak” donors, can still achieve strong overall binding for anions.

Among hydrogen-bond donors, the amide NH functionality, with its exceptional hydrogen-bonding capability and ready synthetic accessibility, is much preferred in the design of synthetic anion receptors^{31,32}. Aromatic oligoamide macrocycles, with backbones comprised of aromatic residues linked by amide linkages, have persistent shapes that can recruit multiple binding elements together in a rigid relationship amenable to strong anion binding. Ideally, the backbones of such oligoamide macrocycles should not only be rigid but also be fully constrained, i.e., even rotation around each backbone aryl-amide single bonds should be hindered, so that local orientational ambiguity associated with the amide groups may be restricted or even eliminated. However, due to the structural limitations of the amide group, most known amide-based anion receptors have global to local conformational flexibility that has compromised both binding affinity and selectivity. In addition, incorporating amide NH units into anion receptors inevitably brings along amide C=O groups that incur undesired hydrogen-bonding interactions, leading to self-aggregation and poor solubility. As a result, few anion binders based on shape-persistent oligoamide macrocycles are known thus far.

Hamilton *et al.* published their seminal work on a rigid aromatic triamide macrocycle that projects three NH groups in a cavity of ~ 5 Å across and binds dihydrogen phosphate strongly ($K_a \approx 10^4$ M⁻¹ in DMSO-*d*₆)³³. This macrocycle, however, has a backbone in which the rotation of aryl-amide single bonds is not restricted. Consequently, either the C=O or the N-H group of a backbone amide linkage may point to the center of the macrocycle. While we have succeeded in construct aromatic oligoamide macrocycles with backbone amide C=O groups being rigidly placed into a convergent orientation²⁰, the inherent limitation of the amide group still precludes the creation of rigid aromatic oligoamide macrocycles capable of locking convergent NH groups for anion-binding. In fact, the creation of rigid hosts with binding sites containing only amide NH and phenyl CH groups as preorganized hydrogen-bond donors for anion binding remains a daunting conceptual and synthetic challenge with few feasible solutions. To address this challenge, we have now successfully designed and synthesized aromatic pentaamide macrocycles **c5** featuring a constrained backbone that, for the first time, locks multiple amide NH and CH groups into an unequivocally convergent placement. The non-deformable cavity of **c5** defined by these NH and CH donors, along with the positive ends of large amide dipoles that also point inward, is highly electropositive and predisposed for anion accommodation.

Results and discussion

Design of cyclic aromatic pentaamides **c5 featuring a fully constrained cyclic backbone with locked endocyclic CH and NH donors.** We recently reported a series of anion-binding aromatic oligoamide foldamers sharing general structure **1** (Fig. 1a)³⁵. The intramolecular hydrogen bonds incorporated into **1** serve to (1) partially rigidify the oligoamide backbone, (2) prevent the backbone amide C=O groups from engaging in additional H-bonding interaction, and (3) free the backbone amide NH groups to form hydrogen bonds with guest species. As shown in Fig. 1a, amide **1a**, i.e., the basic unit of **1**, has a large dipole moment (4.24 D) contributed by the two hydrogen-bonded amide groups, while amide **1b**, which lacks a sidechain amide group, has a smaller dipole moment (3.28 D). Thus, oligoamides **1** adopt a conformation in which the adjacent amide dipoles align in an energetically favorable antiparallel orientation. Similar to the handful of currently known anion-binding foldamers^{22,37-40}, oligoamides **1**, upon binding an anion, fold into conformation **1crs** in which the backbone NH groups, along with the positive ends of the amide dipoles, are oriented to define an electropositive cavity³⁵. The entropic barrier for **1** to adopt conformation **1crs** leads to modest anion binding affinities that are proportional to the number of NH groups³⁵.

Cyclizing oligoamides **1** yields the corresponding macrocycles. Based on the bond angles defined by the *sp*²-hybridized C and N atoms constituting the oligoamide backbone, macrocycles with five or six residues are most likely to form. Other smaller or larger macrocycles will be more strained and thus be more difficult to make. Optimization of **c5**, the five-residue macrocycle (Fig. 1b), with density functional theory (DFT) calculations revealed a slightly puckered conformation (Fig. S1a). In contrast, the analogous six-residue macrocycle has a significantly crinkled shape, indicating a strained structure (Fig. S1b). The cyclic backbone of **c5**, along with the intramolecular hydrogen bonds, hinders the rotation of the aryl-amide single bonds, which greatly reduces the conformational flexibility of the backbone. The intramolecular hydrogen bonds also prevent the amide groups from flipping 180°, forcing the amide NH and phenyl CH groups to point to the center of the macrocycle (Figs. 1b,c and S1a). The formation of **c5** is calculated to be 5.93 kcal/mol endothermic with respect to its acyclic counterpart (Fig. S2), which is a result of the repulsion between amide dipoles, and between the partially charged endocyclic NH and CH protons brought into

close proximity by the cyclized backbone. As confirmation, an energy decomposition analysis (EDA) reveals **c5** as being 16.91 kcal/mol less stable, but the system does gain stability through greater orbital interaction (-9.57 kcal/mol) when compared to the linear pentamer. Consequently, macrocycle **c5**, with its highly electropositive cavity, as shown with DFT computed Hirshfeld charges of +0.121 *e* and +0.037 *e* for NH and CH protons, respectively, is in a high-energy unbound state⁴¹ that is predisposed for binding anions.

Figure 1 | Linear oligoamide **1 and macrocycles **c5** consisting of basic residues derived from 5-amino-*N*-acylanthranilic acid. **a**, Linear oligoamides **1** with its energetically unfavorable crescent conformation **1crs**, and the overall electrical dipole moments of amides **1a** and **1b** in the depicted conformations from DFT calculations performed with the ADF³⁴ software package. **b**, Synthesis of macrocycles **c5a-c** based on a one-pot macrocyclization process. In **a** and **b**, the backbones of the linear and cyclic oligoamides are highlighted with thick bonds. **c**, Crystal structure of macrocycle **c5c** in which two of the five side chains are disordered with water molecules being hydrogen-bonded to the backbone NH groups.**

One-pot synthesis of macrocycles **c5.** The designed pentaamides **c5** are a series of new shape-persistent macrocycles that cannot be synthesized based on established amide-coupling chemistry³⁵. To synthesize **c5a**, a stepwise approach was first attempted. Using the amide-coupling strategy we developed recently³⁵, the linear precursor of **c5a**, prepared by starting from monomer **2a** in multiple steps, was cyclized to give **c5a** in an overall yield of ~22% (Scheme S1 and Supplementary Information). Subsequently, we were able to develop a much more efficient, one-pot synthesis of **c5a** by probing the conditions for the ring-opening reaction of the benzoxazinone monomer **2a** (Fig. 1b). In toluene, while heating **2a** (2 mM) and 4-(*N*, *N*-dimethylamino)pyridine hydrochloride (DMAP•HCl) and DMAP•HI under reflux led to **c5a** in 5% and 0%

yields, respectively, treating **2a** and DMAP•HBr (40 mol%) under the same condition gave **c5a** in 27% yield, indicating that Br⁻ served as a template for one-pot cyclization. Replacing DMAP•HBr with the 1:1 mixture of diphenyl phosphate (DPP) and DMAP (40 mol%) significantly improved the yield (~42%) of **c5a**, suggesting that the DPP anion is a more effective template than Br⁻. Based on the same one-pot procedure, macrocycles **c5b** and **c5c**, which carry sidechains having tri(ethylene glycol) (Tg) and allyl tails, were synthesized from monomers **2b** and **2c** in yields of 38% and 39%. The one-pot macrocyclization process has provided a simple and scalable route allowing further structural tuning and optimization, for instance, by adjusting the side chains and by screening additional anions as templates, based on which a variety of **c5** derivatives can be obtained in sufficient quantities.

Crystal structure of c5c and solution properties of c5a-c. Single crystals of **c5c** were obtained from CHCl₃:CH₃CN (1:1, v/v) by slow evaporation of solvent. The crystal structure of **c5c** confirms our design (Fig. 1c). Macrocyclic **c5c** has an overall planar conformation that resembles the energy-minimized structure of **c5** (Fig. S1a). Consistent with our design, all the endocyclic NH and CH groups of **c5c** point to the center of the macrocycle (Fig. 1c) and defines a cavity of ~6.8 Å (H to H) or ~4.6 Å (van der Waals). Dihedral angles from 0° to 40° are observed between the backbone amide groups and their flanking benzene rings, leading to a slightly puckered conformation that presumably alleviates the repulsion between the amide dipoles and between the partially positive endocyclic hydrogens. The intramolecular hydrogen bonds also enforce the coplanar alignment of the side chain amide groups and the benzene rings to which they are attached. In addition, three water molecules are hydrogen-bonded to amide protons *b* in the cavity.

Examining the packing of **c5c** in the solid state indicates that any two adjacent macrocycles have only a small portion, about one residue, of their backbones being stacked at ~3.4 Å (Fig. S3). The small contact surface between the neighboring molecules of **c5c** is likely caused by the need to avoid the repulsive interactions between the amide dipoles that would arise should the macrocycles stack face-to-face.

The lack of strong stacking interactions between the macrocyclic molecules is reflected by the overall good solubility of macrocycles **c5a-c** in solvents such as chloroform, which contrasts with the poor solubilities typical of most acyclic and cyclic aromatic oligoamides. In fact, macrocycle **c5a** is readily soluble in CHCl₃, CH₂Cl₂, DMF, and DMSO. With its polar side chains having the Tg tails, macrocycle **c5b** is not only soluble in many organic solvents, but also in water with a reasonable solubility (~1 mM).

The ¹H NMR spectra of macrocycles **c5a-c** in CDCl₃ (0.2 mM) reveal dispersed resonances (Fig. S4), lending further support for the limited self-aggregation of these macrocycles as indicated by the crystal structure of **c5c**. In DMSO-*d*₆:CDCl₃ with 1% to 100% (v/v) DMSO-*d*₆, the NMR signals of **c5a** (1 mM) remain sharp, with the resonance of sidechain amide protons *c* showing an upfield shift of -0.5 ppm, backbone amide protons *b* exhibiting a large downfield shift of 1.41 ppm, and that of the endocyclic CH proton *a* experiencing a very small downfield shift of 0.06 ppm (Fig. S5). These observations demonstrate that amide protons *b* are exposed to solvent and engage in hydrogen-bonding interactions with DMSO molecules while endocyclic phenyl protons *a* have minimal, if any, interaction with DMSO.

High-affinity anion recognition by macrocycles c5. We anticipate that macrocycles **c5**, with all the binding sites being highly preorganized, will serve as new anion receptors with hitherto unknown, versatile binding capability. The non-deformable cavity of **c5** may result in size-dependent anion binding. With the endocyclic NH and CH groups providing two different types of hydrogen-bond donors, macrocycles **c5**

may accommodate anions of markedly different properties. The size of the anion, the properties of the solvents, and the hydrogen-bonding capability of both the anions and solvents, are all important factors affecting anion binding.

Figure 2 | Complexes of macrocycle **c5 with halide ions.** **a**, Optimized structures of the complexes of **c5** with chloride, bromide, and iodide. In each complex, the CH/NH protons and the halide ion that are within van der Waals contact distances ($d_{H\cdots Cl^-} < 3.0$ Å; $d_{H\cdots Br^-} < 3.1$ Å; $d_{H\cdots I^-} < 3.3$ Å) are indicated by red dashed lines. **b**, 1H NMR spectra of **c5a** (0.1 mM) titrated with 0 to 5 equiv. of TBA•I in DMSO- d_6 :CDCl $_3$ (15:85,v/v). **c**, Job plot of **c5a** with TBA•I in DMSO- d_6 :CDCl $_3$ (15:85,v/v). **d**, The solvated cavity of **c5** with DMSO molecule(s) hydrogen-bonded to amide protons *b* (left) and the desolvated cavity of **c5** occupied by an anion (**X⁻**) which forms hydrogen bonds with amide protons *b* and phenyl protons *a* (right).

(i) *Binding of halides and perchlorate.* Differing in their diameters (Cl^- : $d = 3.4 \text{ \AA}$; Br^- : $d = 3.8 \text{ \AA}$, I^- : $d = 4.2 \text{ \AA}$) and hydrogen-bonding capability ($\text{Cl}^- > \text{Br}^- > \text{I}^-$), the spherical halides provide comparable substrates for examining the interaction between **c5** and anions. Fig. 2a shows the computationally optimized 1:1 complex of **c5** with the halides. I^- locates at the center of the macrocycle and is within van der Waals contact distances with all endocyclic CH and NH hydrogens. In contrast, Cl^- or Br^- occupies only part of the cavity that are within the van der Waals contact distances with two CH protons and two NH protons, respectively.

In $\text{DMSO-}d_6\text{:CDCl}_3$ (15:85, v/v, $\epsilon = 11$), in which **c5a** undergoes no self-aggregation as indicated by the lack of concentration-dependent shift of the ^1H resonances (Fig. S6), titrating **c5a** (0.1 mM) with tetrabutylammonium (TBA) halides (0 to 5 equiv.) caused phenyl protons *a* to downfield shift by 0.20 ppm with Cl^- , 0.13 ppm with Br^- , or 0.43 ppm with I^- (Figs. 2b and S7a,b). Given that protons *a* have almost no interaction with DMSO (Fig. S5), the halide-induced changes in chemical shift reflect the strengths of the C-H...halide hydrogen bonds, with the interaction of I^- with protons *a* being the strongest. The shifts of protons *a* also show that the halides are bound in the cavity of **c5a**.

Compared to protons *a*, the signal of amide protons *b* shifts very differently under the same conditions (Figs. 2a and S7). Cl^- induces a small (0.08 ppm) downfield shift, while Br^- causes a very small (-0.02 ppm) and I^- a much larger (-0.57 ppm) upfield shift. The seemingly irregular shifts of protons *b* induced by different halides can be explained by the competition between DMSO and the halides for hydrogen bonding. As shown in Fig. 2d, binding a halide to **c5a** expels the DMSO molecules from the cavity. Binding Cl^- leads to stronger hydrogen-bonding strength “felt” by protons *b*, causing the ^1H resonance to shift further downfield. On the other hand, displacing DMSO by Br^- and especially I^- , as weaker hydrogen-bond acceptors⁴², results in reduced hydrogen-bonding strengths sensed by protons *b*, leading to upfield shifts.

The shifts of protons *b* are consistent with the hydrogen-bond acceptor strengths of the three halides, i.e., Cl^- and I^- form the strongest and weakest hydrogen bonds, respectively, with protons *b*. In contrast, the downfield shifts of CH protons *a*, which clearly demonstrate that protons *a* sense enhanced hydrogen bonding strengths upon binding the halides, are inconsistent with the hydrogen-bonding capability of the halides. It is surprising that I^- , the weakest hydrogen-bond acceptor, causes the largest downfield shift of protons *a* and thus interacts most strongly with protons *a*, which implies that, different from typical hydrogen-bond donors, the CH units of **c5a** favor large anions with diffused charge density.

The titration data in $\text{DMSO-}d_6\text{:CDCl}_3$ (15:85, v/v) fit well into a 1:1 binding model using the online fitting tool Bindfit³⁶, giving association constants (K) of $(4.5 \pm 0.2) \times 10^3 \text{ M}^{-1}$, $(8.4 \pm 0.7) \times 10^3 \text{ M}^{-1}$, and $(2.7 \pm 0.3) \times 10^4 \text{ M}^{-1}$ for Cl^- , Br^- , and I^- , respectively. The 1:1 binding of **c5a** with each halide is confirmed by a Job’s plot (Figs. 2c and S8). The K values show that **c5a** binds the largest halide, I^- , with the highest affinity. The preference of **c5a** for large, weakly hydrogen-bonding anions is further demonstrated with perchlorate (ClO_4^-), a tetrahedral oxoanion having a slightly larger size ($d = 4.5 \text{ \AA}$) but being a weaker hydrogen-bond acceptor than I^- . Titrating **c5a** (0.1 mM) with TBA• ClO_4 (0-5 equiv.) in $\text{DMSO-}d_6\text{:CDCl}_3$ (15:85) induced a downfield shift (0.107 ppm) for phenyl protons *a*, but a large (-0.803 ppm) upfield shift for amide protons *b* (Fig. S7c), based on which a K of $(6.3 \pm 0.8) \times 10^4$ for ClO_4^- was obtained. The shifts of protons *a* and *b* are consistent with ClO_4^- being a weaker hydrogen acceptor than I^- . The larger K of ClO_4^- than that of I^- probably reflects the slightly larger size of the former, which matches the cavity of **c5a** better. Comparing the K values reveals selectivity ratios of 6 for I^-/Cl^- , and 3 for I^-/Br^- . With ClO_4^- the binding selectivity ratios increase to 14, 7, and 2 folds over Cl^- , Br^- , and I^- , respectively.

Ion binding has been believed to be electrostatically driven, on which solvent polarity, as roughly reflected by dielectric constants (ϵ), exerts a major impact. Halide binding by **c5a** was quantified by isothermal titration calorimetry (ITC) in DMSO:CHCl₃ (5:95, v/v, $\epsilon = 6.9$) and MeOH:CHCl₃ (5:95, v/v, $\epsilon = 6.2$), two mixed solvents with similar polarity. In DMSO:CHCl₃ (5:95), the K 's are from 10^4 to 10^5 M⁻¹ (Fig. S9 and Table S1), ~10 times of those in DMSO-*d*₆:CDCl₃ (15:85, $\epsilon = 11$), which demonstrates that lowering solvent polarity increases the affinity of **c5a** for halides. In MeOH:CHCl₃ (5:95), the K 's are around 10^6 M⁻¹ (Fig. S10 and Table S1), ~100 times of those in DMSO-*d*₆:CDCl₃ (15:85). Given the similar polarities of the two mixed solvents, the markedly enhanced affinity of **c5a** for halides in MeOH:CHCl₃ (5:95) was unexpected.

To gain further insights, compound **c5a** (0.1 mM) was subjected to ¹H NMR titration with TBA•halide (0-5 equiv.) in CD₃CN:CDCl₃ (80:20, v/v, $\epsilon = 31$), a mixed solvent that is much more polar than DMSO-*d*₆:CDCl₃ (15:85, v/v, $\epsilon = 11$). Downfield shifts of amide protons *b* and phenyl protons *a* are induced by all three halides (Fig. S12), indicating that both protons *a* and *b* experience enhanced hydrogen-bonding strength upon binding halides. The titration data fit into a 1:1 binding model, giving K values of $(9.7 \pm 1.1) \times 10^4$ M⁻¹ (for Cl⁻), $(5.0 \pm 0.5) \times 10^4$ M⁻¹ (for Br⁻), and $(6.0 \pm 0.9) \times 10^5$ M⁻¹ (for I⁻), which are 10 to 20 times of the K 's in DMSO-*d*₆:CDCl₃ (15:85). In CD₃CN:CDCl₃ (5:95, v/v, $\epsilon = 6.4$), the binding affinities (K_a 's) of **c5a** for the halides revealed by ITC reach 10^6 M⁻¹ for Cl⁻, and 10^7 M⁻¹ for Br⁻ and I⁻ (Table S1 and Figure S11). The enhanced affinities of **c5a** for the halides can be rationalized by the fact that, compared to DMSO, acetonitrile is a much weaker hydrogen-bond acceptor⁴³ and can be replaced from the cavity of **c5** at reduced energy costs. These results demonstrate that the hydrogen-bonding capabilities of solvents play a dominant role in determining the binding affinity of **c5** for the halides.

(ii) *High-affinity binding of strongly hydrogen-bonding oxoanions.* The high affinities of **c5a** for I⁻ and ClO₄⁻ indicate that weakly hydrogen-bonding anions with sizes matching the cavity of **c5** can achieve strong binding. We thus expect that anions with both suitable (matching) sizes and strong hydrogen-bonding ability can have drastically enhanced interaction with **c5** by maximizing their interaction with NH protons *b* and CH protons *a*.

Acetate (AcO⁻), diphenyl phosphate (DPP), and monomethyl phenylphosphonate (MPP) (Figure 3a), oxoanions that are very strong hydrogen-bond acceptors⁴³, were examined for their binding with **c5a** and **c5b**. In DMSO-*d*₆:CDCl₃ (15:85, v/v), upon adding less than one equiv. of the TBA salt of AcO⁻ or MPP, or the tetramethylammonium (TMA) salt of DPP, macrocycle **c5a** gave two sets of peaks for protons *a*, *b*, or *c* in the ¹H NMR spectrum (Figs. 3b and S7d,e), indicating a slow exchange between the free and bound forms of **c5a** on the NMR time scale. Binding affinities were determined with two-step competition ¹H NMR titration experiments by using Cl⁻ as the competitor (Supplementary Information and Fig. S13). Macrocycle **c5a** was found to tightly bind the three oxoanions in a 1:1 ratio. The larger DPP and MPP ($d \approx 4.3$ Å) show stronger binding with K values of $(1.6 \pm 0.1) \times 10^6$ M⁻¹ and $(1.1 \pm 0.1) \times 10^7$ M⁻¹, respectively, than that $((2.4 \pm 0.3) \times 10^5$ M⁻¹) of the smaller AcO⁻ ($d = 3.8$ Å), based on which selectivity ratios of 53, 356, and 2,444 for AcO⁻, DPP, and MPP, respectively, over Cl⁻ ($K = (4.5 \pm 0.2) \times 10^3$ M⁻¹) are found.

In CD₃CN:CDCl₃ (40:60, v/v, $\epsilon = 18$), competition ¹H NMR titrations with Cl⁻ ($K = (9.7 \pm 1.0) \times 10^4$ M⁻¹) as the competitor revealed the K 's for DPP and MPP as $(3.0 \pm 0.4) \times 10^7$ M⁻¹ and $(3.1 \pm 0.6) \times 10^8$ M⁻¹, respectively (Fig. S14), which give selectivity ratios of 310 and 3,200 for DPP and MPP, respectively, over Cl⁻. The interaction of **c5a** with the oxoanions, especially with the larger DPP and MPP, demonstrate that anions combining size-matching and strongly hydrogen-bonding capabilities, by involving the maximum

number of NH and CH donors and effectively outcompeting solvent molecules for hydrogen-bonding, can achieve remarkable binding affinity and selectivity.

Figure 3 | ^1H NMR spectra of **c5a.** **a**, Structures of three strongly hydrogen-bonding oxoanions. **b**, Spectra of **c5a** (0.1 mM) titrated with 0 to 2 equiv. of TBA-MPP, in 15:85 (v/v) DMSO- d_6 :CDCl $_3$ at 25 °C. **c**, Partial 2D (ROESY) spectra of the 1:1 mixture of **c5a** and TBA-MPP in DMSO- d_6 (20 mM, 500 MHz, mixing time = 0.4 s, 25 °C).

The superb anion binding ability of macrocycles **c5** was also demonstrated in highly competitive solvents. Based on ^1H NMR titrations, in DMSO- d_6 :CDCl $_3$ (70:30, v/v, $\epsilon = 34$), K values of $(2.4 \pm 0.1) \times 10^3 \text{ M}^{-1}$ for DPP and $(4.7 \pm 0.6) \times 10^4 \text{ M}^{-1}$ for MPP were obtained (Fig. S15). In the aqueous solvent mixture D $_2$ O:acetone- d_6 (20:80, v/v, $\epsilon = 33$), macrocycle **c5a** showed strong affinities for DPP ($K = (3.8 \pm 0.4) \times 10^4 \text{ M}^{-1}$) and MPP ($K = (1.7 \pm 0.1) \times 10^4 \text{ M}^{-1}$) (Fig. S16). Even in D $_2$ O:acetone- d_6 (60:40, v/v, $\epsilon = 56$), **c5b** was able to bind DPP with a K of $(5.4 \pm 0.4) \times 10^3 \text{ M}^{-1}$ (Fig. S17).

Two-dimensional (ROESY) NMR in DMSO- d_6 was performed on the 1:1 mixture of **c5a** and MPP to confirm the formation of complex **c5a**•MPP. ROEs between protons *a* of **c5a** and protons 1 and 2 of MPP were clearly detected (Fig. 3c), suggesting that MPP indeed is located inside the cavity of **c5a**. In contrast, no significant ROE could be found between the protons 1 and 2 of MPP and exterior protons *d* and *e* of **c5a**.

Selective transmembrane transport of halide ions. The low propensity of macrocycle **c5a** for self-aggregation, along with its good solubility in organic solvents, suggests that the molecules of **c5a** are unlikely to stack into channels when partitioned into the lipid bilayer. However, given its anion-binding capability, especially in low-polar solvents, macrocycle **c5a** may facilitate anion transport across the lipid bilayer by acting as a carrier. We thus performed vesicle-based fluorescence assays (Fig. 4a) to determine the transport rates of the halides, which allowed the transport selectivity to be compared. Macrocycle **c5a** mediated transmembrane transport of Cl $^-$, Br $^-$, and I $^-$, with the largest enhancement in Cl $^-$ transport (Fig. 4b), followed by Br $^-$ transport (Fig. 4c), and the smallest increase in I $^-$ transport, being observed (Fig. 4d). The total anion permeabilities (Fig. 4) of the halides mediated by **c5a** reveal that the transport of Cl $^-$, Br $^-$, and I $^-$ follows a selectivity order of 35.6:2.8:1, which is inversely related to the binding affinities of **c5a** for these three halides. The selective transport of Cl $^-$ across the cell membrane is biologically and medically significant⁴⁵. Natural chloride channels are known to be responsible for a broad range of functions⁴⁶, with a large spectrum of diseases being caused by defective Cl $^-$ channels⁴⁷. In this regard, macrocycle **c5a** provides an opportunity to develop synthetically tunable Cl $^-$ transporters to rectify ill functioned chloride

channels in order to reestablish transmembrane chloride conductance. One important application is the treatment for cystic fibrosis where Cl^- flux across epithelial cell membranes needs to be restored.

Figure 4 | Transmembrane transport of halide ions promoted by macrocycle c5a. **a**, Dissipation of the pH gradient, due to OH^-/Cl^- antiport or the functionally equivalent H^+/Cl^- symport, quenches emission of the pH-sensitive dye HPTS entrapped in liposome. **b**, **c**, **d**, Normalized time-lapse fluorescence emission curves of Cl^- , Br^- , and I^- transport facilitated by **c5a**, along with the control curves (in blue) measured with vesicles in the absence of **c5a**. The total anion permeability (ΔP_{X^-}) of each halide ion elicited by **c5a** is shown.

Catalytic ester amidation. With their tetrahedral shapes and negative charges, many phosphate-related anions similar to DPP or MPP have served as analogs of the negatively charged, tetrahedral transition states of nucleophilic acyl substitution (NAS) reactions^{49,50}. The high affinities of macrocycles **c5a** and **c5b** for DPP and MPP suggest that these macrocycles may be able to catalyze NAS reactions by binding and stabilizing the corresponding transition states. This intriguing possibility prompted us to probe the

amidation of methyl esters **3a-c** with *n*-octylamine, which gives amides **4a-c**, by using **c5a** as a likely catalyst (Fig. 5).

Figure 5 | The amidation of methyl esters **3a-c with *n*-octylamine.** **a**, The reactions were performed under neat reaction conditions with the molar ratio of ester:*n*-octylamine:DIEA of 1:1.5:2, and 1 mol% **c5a** at 60 °C. The amidation of **3a** was performed for 18 hrs, and those of **3b** and **3c** for 24 hrs. **b**, Time-dependent change in the concentrations of ester **3a** without **c5a** (control), with 1 mol% **c5a**, 5 mol% **c5a**, and 5 mol% + 10 mol% MPP monitored with ^1H NMR. The reactions were carried out with 0.5 M of **3a**, 0.75 M of *n*-octylamine, 1 M of DIEA, and 50 mM of $\text{Cl}_2\text{CHCHCl}_2$ (as the internal standard) in CDCl_3 at 50 °C.

As expected, the presence of **c5a** resulted in markedly increased conversion of esters **3a-c** into amides **4a-c** (Fig. 5). Esters **3a-c** were first reacted with *n*-octylamine at 60 °C for 18 hours under neat reaction conditions. In the presence of 1 mol% **c5a**, amides **4a**, **4b**, and **4c** were isolated in yields of 97%, 78% and 85%, respectively, which are significantly higher than the yields of 54%, 42%, and 31% of amides **4a-c** formed under the same conditions without **c5a** (Fig. 5a).

The amidation of ester **3a** was then monitored with ^1H NMR in CDCl_3 (Fig. 5b). Over a period of ~100 minutes, the conversion of ester **3a** was ~0.9% in the absence of **c5a**. Adding 1 mol% **c5a** results in increased conversion (~1.4%) of **3a**. The presence of 5 mol% **c5a** further increased the conversion of **3a** to ~6.5%. Including 10 mol% MPP and 5 mol% **c5a** in the reaction partially inhibited the conversion of **3a** to ~4.1%, indicating that binding to the cavity of **c5a** was responsible for the observed catalysis. The formation of amide **4a** was confirmed by NMR (Fig. S18). After ~11 hrs, more than 20% of **3a** reacted in the presence of 5 mol% **c5a**, while only ~3% of **3a** was converted in the absence of **c5a** (Fig. S19).

The catalytic amidation of ester **3a-c** clearly demonstrates the capability of macrocycle **c5a** in promoting a NAS reaction by, most likely, binding and stabilizing the negative transition state of such a reaction. While the catalytic reactivity shown by **c5a** has validated our hypothesis, organocatalysts with improved and eventually optimized efficiencies should be available based on the ready structural tunability of macrocycles **c5**.

Conclusions

Cyclic pentaamides **c5a-c** were synthesized from the one-pot macrocyclization of the corresponding monomers based on a novel amide coupling strategy we developed. These previously unknown shape-persistent macrocycles share a fully constrained backbone that rigidly locks endocyclic NH and CH groups into a convergent arrangement, leading to high-affinity binding of halides and oxoanions. Examining the binding affinities of **c5** for halide ions reveal size-dependent recognition favoring iodide, while the complexation of oxoanions show that anions with both better matched sizes and strong hydrogen-bonding capability achieved remarkable binding affinities, even in highly polar solvents containing high proportions of water. The superb capability of macrocycles **c5** to complex anions is demonstrated by the efficient and selective transport of chloride ions across lipid membranes and the intriguing catalytic amidation of esters brought about by **c5a**. The efficient, scalable one-pot synthetic procedure allows various derivatives of macrocycles **c5** to be prepared in sufficient quantities with ready structural tunability. Based on this solid platform, the development of the next-generation anion binders, transporters, and organocatalysts can be envisioned.

Methods

Vesicle-based halide transport assays. The assays were based on adding unilamellar 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) vesicles having a defined (100 nm) diameter and loaded with HPTS (30 μ M) in a buffer (10 mM HEPES, 100 mM NaX, pH 11.5) to a buffered solution (100 mM NaX, 10 mM HEPES at pH 6.5) containing **c5a** (2 μ M). The entrapped HPTS was excited at 450 nm ($\text{slit}_{\text{ex}} = 5$ nm) and the emission monitored at 510 nm ($\text{slit}_{\text{em}} = 5$ nm). The measured emission intensities were normalized against those of the same vesicles which were lysed with 0.1% Triton X-100 at the end of each experiment. Each of the normalized time-lapse fluorescence emission curves was fitted with the quantitative model according to the Goldman–Hodgkin–Katz flux theory (orange lines)⁴⁸, based on which the anion permeability of vesicles with or without **c5a** was determined. The total anion permeability (ΔP_{X^-}) of each halide ion elicited by **c5a** was calculated by subtracting the anion permeability measured with vesicles in the absence of **c5a**.

See the Supplementary Information for other information including synthetic steps, experimental conditions, analytical data, 1D and 2D NMR spectra, mass spectra, computational methods, and crystal structure.

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Author contributions

R.K.C. and R.B.R. designed and conducted the syntheses, and also performed the binding studies. Y.S., Y.L.Z., R.B.R., and Z.F.S. performed the vesicle-based assays on anion transport. R.K.C. carried out the ester amidation reactions. X. X. W. processed the X-ray data. D.P.M., L.S.S.B., K.R., and E.Z. performed the computational studies. T.S. helped with the NMR experiments. B.G. conceived and supervised the project. Z.F.S. and B.G. co-wrote the paper. All authors participated in discussion and editing of the manuscript.

Additional information

Supplementary information and chemical compound information are available in the online version of the paper. Correspondence and requests for materials should be addressed to B.G.

Competing interests

The authors declare no competing interests.

B. G. is a named inventor on a patent application (US PCT patent application PCT/US2021/050041) filed by The Research Foundation for the State University of New York on the anion-binding method described in this work.

Data Availability statement

Single-crystal X-ray structure data for macrocycle **c5c** (CCDC reference number: 2124841) can be obtained free of charge from the Cambridge Crystallographic Data Centre at www.ccdc.cam.ac.uk/data_request/cif. All other data generated or analysed during this study are included in this article and its supplementary information files.

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