Encapsulated binding sites—synthetically simple receptors for the binding and transport of HCl†

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We report a receptor with an encapsulated amine/amide binding site, which binds HCl with high affinity in organic media—the rate of HCl transport through an apolar phase is controlled by the degree of encapsulation of the HCl binding site.

The prodigiosins are a class of natural products with potent anti-cancer activity and the ability to transport HCl through cell membranes modulating cellular pH, using a basic amine to bind H^+ and an array of N–H groups to hydrogen bond to the chloride anion.[1] There has therefore been considerable recent interest in the development of synthetic receptors for HCl. In 2002, Sidorov and co-workers reported a calix[4]arene amide derivative which formed ion channels and mediated HCl transport.[2] In 2005, Sessler and co-workers made a synthetic prodigiosin mimic which included a basic imidazole unit to bind H^+ and a bis-pyrrole in order to provide hydrogen bonds to Cl^-.[3] These derivatives also exhibited anti-cancer activity. In elegant work, Gale and co-workers have also developed HCl binding and transport systems based on the combination of an imidazole and either a pyrrole-amide or a 2,6-dicarboxamidopyridine unit.[4] Greater pre-organisation and HCl binding affinity led to a higher membrane-transport flux. Calixarenes functionalised with spermidine amines have also recently been used for HCl transport.[5]

For some time, we have been interested in the development of synthetically simple yet effective anion receptors.[6] Inspired by the work described above, we began to consider whether we could take an alternative approach towards HCl receptors. Our design strategy involved the incorporation of a basic amine to bind H^+, along with multiple amides capable of binding Cl^-. A large number of anion receptors have been reported based on 'tren' (tris(2-aminoethyl)amine), as this commercially available trigonal scaffold can readily be functionalised to yield three anion binding hydrogen-bonding amide or urea groups close to the central core.[7] Furthermore, tren receptors have been demonstrated to show 'flippase' activity—translocating anionic phospholipid analogues through a cell membrane.[8] It was argued that in this case, the tertiary amine unit may play a role in transporting the countercation through the membrane. Although it is known that amines can encourage the binding of acidic anions,[9] there have been few other attempts to harness the basicity of the central tertiary amine of tren-amides. In one crystallographic study, Suresh and co-workers reported the X-ray structures of HCl complexes of tren-based amide *N*, *N*′, *N*″-tris[(2-aminoethyl)-3-nitro-benzamide].[10] In the X-ray structures the amine was protonated, and Cl^- was bound to multiple receptors through N–H(amide)···anion and ArC–H···anion hydrogen bond interactions.

We synthesised receptors **1–8** (Fig. 1). We hoped that by generating binding sites with different degrees of encapsulation, we should be able to tune the behaviour of the receptors. Full details of synthesis and characterisation can be found in the ESI†.

Initially, the ability of these synthetically simple receptors to bind to anions as their tetrabutylammonium salts was investigated in $CDCl_3$: DMSO-d_6 (9 : 1) by NMR titration methods, with the data being fitted to a 1 : 1 model. Receptor **1**, which has the most deeply encapsulated binding site was screened against a range of anions (Table 1). Unsurprisingly, the receptor showed the strongest binding with fluoride, the most charge dense anion, with the binding strength decreasing for chloride and bromide. Surprisingly, receptor **1** bound Cl^- more effectively than $H_2PO_4^-$. Normally the more basic $H_2PO_4^-$ anion is more effectively bound than Cl^-—only in rare cases has this affinity order been reversed, usually in sterically congested macrocyclic binding sites.[11]

Receptors **2** and **3** were studied further to probe this behaviour (Table 1). Both of these receptors bound $H_2PO_4^-$ in preference to Cl^-. It is notable that $H_2PO_4^-$ binding remained fairly constant across the three receptors, whereas chloride binding became less favourable for receptors **2** and **3** than it was for receptor **1**. This suggests that the internal cavity of receptor **1** is actually particularly well suited for chloride

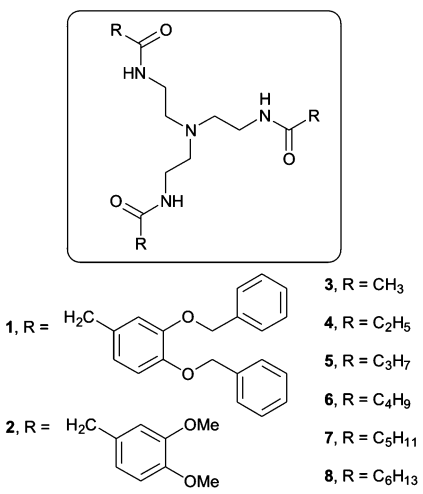

Fig. 1 Receptors **1–8** investigated in this paper.

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Table 1 Binding affinities, K, for some of the synthetic receptors with tetrabutylammonium anion salts in CD_3CN : DMSO-d_6 (9 : 1). Titration data were fitted to 1 : 1 stoichiometry, K values determined at 298 K with units of mol^{-1} dm^3. Missing data were not determined. All data are averaged over multiple runs with errors ±15%

Receptor	Cl^-	$H_2PO_4^-$	F^-	BzO^-	Br^-	NO_3^-
1	550	280	1090	250	35	<1
2	150	305	—	—	—	—
3	130	260	—	—	—	—
6	120	—	—	—	—	—
8	75	—	—	—	—	—

binding. It is possible that ArC–H···anion interactions[10] or anion–π interactions[12] also contribute to chloride binding. Alternatively the enhanced binding may be a consequence of the aromatic-ether organic shell providing a more polar local microenvironment, better able to stabilise bound Cl^-.[13]

We also determined the chloride binding affinities for simple aliphatic chain functionalised receptors **6** and **8**. We observed that as the receptor became more heavily encapsulated, the affinity for Cl^- decreased—receptor **8** only had about half the binding affinity of receptor **3**. The flexible substituents in these receptors therefore appear to hinder chloride binding—this supports the hypothesis that the specific nature/structure of the organic shell in receptor **1** plays a key role in enhancing chloride anion binding.

We then carried out NMR titrations of the receptors with HCl (as a solution in Et_2O). On the addition of HCl, the amide protons of the receptors flattened into the baseline, and new peaks corresponding to the amide protons in the HCl complex appeared further downfield—indicative of kinetically slow binding. Furthermore, a peak assigned to the protonated tertiary amine appeared at *ca.* 10 ppm. Job plot analysis was performed, and surprisingly, a 1 : 2 (host : guest) stoichiometry was observed (Fig. S1, ESI†). We hypothesise that the tetrahedral geometry of the central amine means that $N–H^+$ is directed away from the three amide N–H groups (in agreement with X-ray studies).[10] This gives rise to two different chloride binding sites in solution (Fig. 2). This proposal is in agreement with previously published crystallographic studies on the interactions between protonated tren-amine derivatives and anionic guests—in which, protonation of the tertiary amine led to the formation of complexes with a range of anion binding sites.[14] We propose that in the first binding site, H^+ protonates the nitrogen, and an associated chloride ion is held in close proximity by electrostatic attraction. In the second binding site, the chloride binds to the amide protons, (again with an associated proton, perhaps also interacting with the C=O groups). To probe this further, we investigated the binding of HBF_4. In this case, the binding stoichiometry was closer to 1 : 1, with the maximum in the Job plot being observed at 0.55 (Fig. S1, ESI†). This suggests that protonation of the tertiary amine still occurs, but BF_4^- is less able to interact with the amide groups in the 'cavity' of the receptor. We also investigated the ability of these receptors to bind benzoic acid. No binding was observed, proving that the acid must be sufficiently strong for recognition to take place.

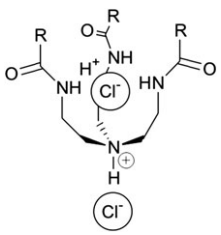

Fig. 2 Schematic 1 : 2 (host : guest) binding model for receptor **1** with HCl.

We analysed the HCl binding of these receptors by integration of the NMR spectra in order to yield apparent equilibrium constants (K_{app}) for the 1 : 2 binding event (Table 2). It should be noted that these K_{app} values include multiple binding events, and only give a general indication of the relative strength of interaction. Receptor **1** binds HCl most effectively, while the less deeply encapsulated receptors **2** and **3** are less effective—in this case by an order of magnitude. Receptor **8**, with longer alkyl substituents than receptor **3**, is a less effective HCl receptor. These studies indicate that similar factors influence the binding of HCl as for the binding of tetrabutylammonium chloride: *i.e.*, receptor **1** has high HCl affinity, potentially due to specific anion···shell interactions, or the development of a unique microenvironment.

We screened the ability of these receptors to transport HCl through an apolar phase using a simple U-tube. The receptor was dissolved in dichloromethane (10 mM), and placed at the base of the U-tube. In the left-hand-arm of the tube was aqueous HCl (pH 0.9) and in the right-hand-arm was deionised water (Fig. S3, ESI†). The transport of HCl was monitored by following the pH change in the right-hand-arm over four hours. In the absence of receptor, there was no transport of HCl, and pH was constant (Fig. 3 and 4, circles). On using triethylamine (Et_3N) as a control 'receptor', there was once again no transport of HCl (Fig. 3, triangles). Indeed, conversely, the pH in the right-hand-arm increased—indicating that Et_3N partly partitions from CH_2Cl_2 into the aqueous phase but is unable to transport HCl. Receptors **1** and **2**, however, gave effective transport of HCl—as observed by the significant decrease in pH (Fig. 3, squares and diamonds). Within error, both receptors exhibited similar rates of HCl transport. This is interesting, as their affinities for HCl were different by an order of magnitude. This demonstrates that binding strength is not the only factor which controls ion transport.

The ability of receptors **3–8** to transport HCl through the U-tube was studied (Fig. 4). Only the longer chain receptors **7** and **8** were able to rapidly transport HCl through CH_2Cl_2 (Fig. 4, squares and triangles). Receptor **6** was also able to

Table 2 Apparent binding affinities, K_{app}, for some of the synthetic receptors with HCl (dissolved in Et_2O) in CD_3CN : DMSO-d_6 (9 : 1). Titration data were fitted to 1 : 2 (receptor : HCl) stoichiometry, K_{app} values determined by spectral integration at 298 K with units of mol^{-2} dm^6. All data are averaged over multiple runs with errors ±15%

Receptor	HCl
1	1.3×10^6
2	2.3×10^5
3	1.4×10^5
8	9.2×10^4

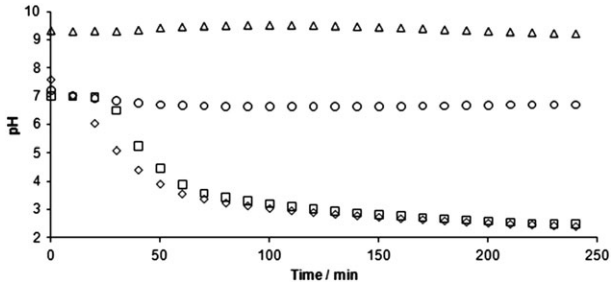

Fig. 3 U-tube transport results for HCl transport illustrating the pH change in the right-hand-arm of the apparatus over time. Circles represent no receptor, triangles represent Et_3N. Squares represent receptor **1** and diamonds receptor **2**.

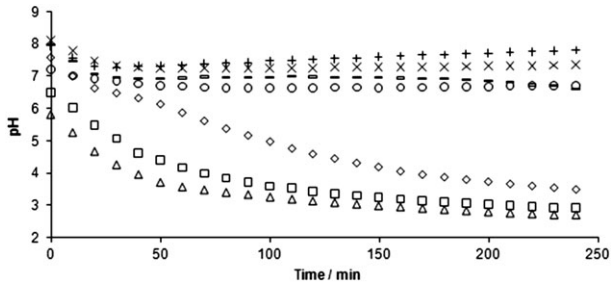

Fig. 4 U-tube transport results for HCl transport illustrating the pH change in the right-hand-arm of the apparatus over time. Circles represent no receptor. Receptors are represented by plus signs (**3**), crosses (**4**), dashes (**5**), diamonds (**6**), squares (**7**) and triangles (**8**).

slowly transport HCl, although the rate was significantly lower (Fig. 4, diamonds). This clearly demonstrates that those HCl receptors with a greater degree of core encapsulation are more effective transporters—irrespective of the inherent HCl binding affinity. This is in agreement with the observation that both receptors **1** and **2** transport HCl, even though they have different HCl affinities. We propose that the enhanced HCl transport exhibited by the more encapsulated receptors is a consequence of their ability to effectively shield the bound HCl from the apolar environment, coupled with the preference of these less polar receptors to remain within the apolar phase. These observations are in agreement with the hypothesis that the best transport agents do not necessarily have the highest affinities for the target—a degree of binding is required in order to facilitate transport, but most importantly, the receptor must screen the ions being transported from the surrounding apolar phase and then release the ions once transport is complete.[15]

In summary, this paper reports synthetically simple receptors with encapsulated binding sites, which have high affinities for HCl. These compounds can be considered as synthetic prodigiosin mimics. Receptor **1**, with its binding site encapsulated within an aromatic ether shell, has the highest affinity both for TBACl and HCl—we propose that this is a consequence of favourable interactions/environment between the organic shell and the bound chloride anion. We demonstrate that well-encapsulated tren-amide binding sites can be used to transport HCl through an apolar phase, with the encapsulating shell playing an active role in assisting HCl transport. We suggest that such simple compounds may have interesting membrane-transport abilities and intriguing biological activities.

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