OBC
www.rsc.org/obc

# Rapid NMR screening of chloride receptors: uncovering catechol as a useful anion binding motif †

**David K. Smith***
*Department of Chemistry, University of York, Heslington, York, UK YO10 5DD.*
*E-mail: dks3@york.ac.uk*

***Received 29th August 2003, Accepted 25th September 2003***
***First published as an Advance Article on the web 1st October 2003***

**This paper reports the application of a high-throughput $^1$H NMR screening method to the evaluation of potential anion receptors, and the results of the screening indicate that catechol is a surprisingly good host for chloride anions.**

Over recent years, there has been intensifying interest in the design, synthesis and investigation of receptors for anions.[1] This interest is partly driven by the undoubted importance of anions in biological systems.[2] A large number of neutral organic receptors capable of binding anionic guests have been developed using amides, ureas and pyrroles as hydrogen bond donor groups to bind the anion.[3] The thermodynamic affinity of these receptors for anions has often been determined by NMR titration techniques,[4] but such experiments are time-consuming, tedious and require excessive amounts of instrument time. In this paper we illustrate how a calibrated competitive approach, recently developed by us to investigate potassium cation binding,[5] can rapidly indicate the approximate affinities of a range of receptors for a given anion – in this case chloride. We illustrate that applying this methodology to anion binding enables efficient receptors to be rapidly identified. Using this screening method we have discovered that catechol (**15**) is a remarkably good host for chloride anions (Fig. 1).

In order to screen a series of compounds for chloride binding, it was first necessary to calibrate a binding event against which the other receptors would compete. Given that simple amides are well-known to bind anionic guests through the formation of N–H $\cdots$ anion hydrogen bonds,[3] we decided to use commercially available compound **1** as a reference receptor. Compound **1** was titrated against tetrabutylammonium chloride (TBACl) in $CD_3CN$ in the usual way (maintaining [**1**] constant) and the resonances for the N–H and *ortho* Ar–H protons were followed (Fig. 2). Fitting the data for both protons to a 1 : 1 binding profile using HypNMR,[6] gave a reproducible binding constant of 31 $M^{-1}$ (±10%). The data fitted 1 : 1 stoichiometry unambiguously, and there was no evidence for complexes of higher stochiometry being formed under the conditions of the NMR titration. Using the binding constant value, it is possible to calculate the concentration of complex present at each point in the titration,[5] and there is a straight line relationship between [**1**·$Cl^-$] and the observed chemical shifts of N–H and Ar–H, allowing calibration graphs for the binding event to be generated (Fig. 3 illustrates the calibration graph for the N–H proton).

Binding constants can then be determined for novel receptors of interest in the following way. A single NMR spectrum is measured, in which the sample contains a known concentration of reference receptor **1** (the same as in the calibration titration), a defined amount of TBACl, and a known concentration of the receptor of interest. If the receptor of interest is able to compete with compound **1** for the chloride anions, the chemical shifts of the N–H and Ar–H protons of compound **1** will be perturbed (see Fig. 4 for an example of how this is manifested in the NMR spectra). The perturbed chemical shift values can be used with the calibration graph to determine the concentration of complex **1**·$Cl^-$ present in the competition. Using the method explained in detail previously,[5] and outlined in the ESI †, it is then possible to use this value to calculate the binding constant for the receptor of interest.

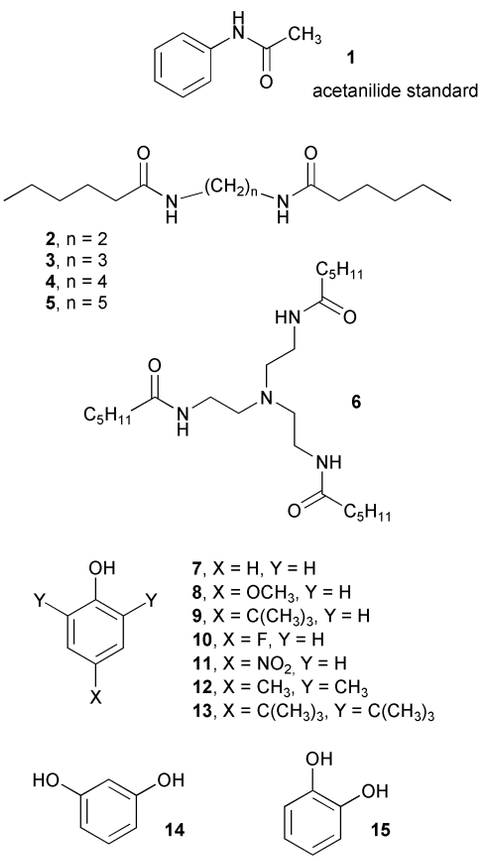


**Fig. 1** Compounds screened for chloride binding.

Only a single NMR spectral measurement is necessary, and therefore this method is much faster than a traditional NMR titration (although the competition measurements were repeated to reduce errors). It should be noted that the binding constants from this method only provide an approximate guide (*ca.* ±25%) given the limited information used for their determination. They are, however, of considerable use for high throughput screening purposes, as they clearly indicate which receptors are best able to compete with reference receptor **1**.

We were concerned about the possibility that both compound **1** and the receptor of interest may simultaneously bind to the anionic guest forming a 1 : 1 : 1 complex, hence invalidating the screening method. However, under the conditions at which

† Electronic supplemenary information (ESI) available: calibration graphs for the binding process between receptor **1** and chloride, and a worked example illustrating the use of this calibrated competitive method to determine the binding constant between receptor **15** and chloride. See http://www.rsc.org/suppdata/ob/b3/b310455a/

DOI: 10.1039/b310455a

 

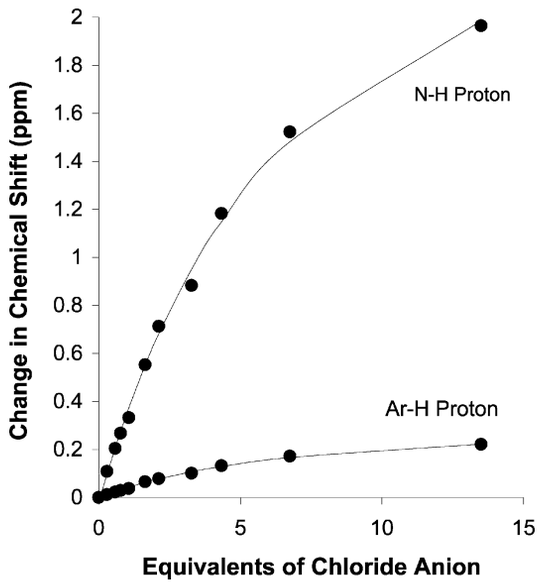


**Fig. 2** NMR titration curves for acetanilide (**1**, 3.67 mM) on the addition of tetrabutylammonium chloride (solvent: $CD_3CN$).

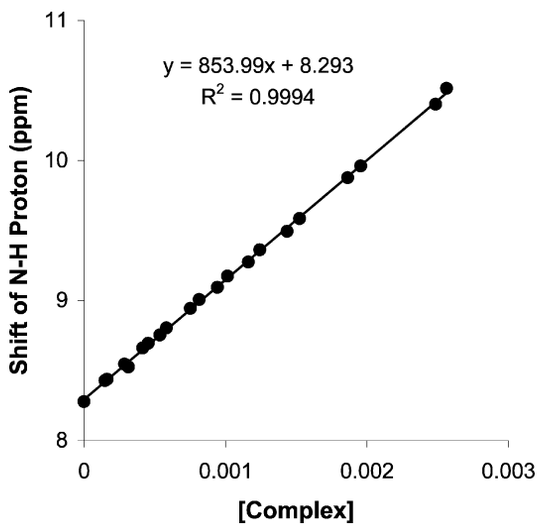


**Fig. 3** Calibration graph for compound **1** being perturbed by chloride anions. The graph relates the NMR shift of the N–H proton and the concentration of complex **1**.$Cl^-$ that is present in solution.

the experiments were performed, and in the control NMR titrations, only 1 : 1 complexes were ever observed, and there was never any evidence for the presence of complexes of higher stoichiometry, with multiple receptor molecules binding to a single anion.

To illustrate the application of high throughput sreening to the determination of thermodynamic data for anion binding, compounds **2**–**15** were screened for their ability to bind chloride anions. Receptors **2**–**6** were synthesised‡ to help validate the screening approach, as similar compounds have been previously reported by Werner and Schneider.[7] Commercially available phenols **7**–**15** were also screened, as such compounds should be capable of binding anions *via* the formation of O–H ··· anion hydrogen bonds. The approximate binding constants determined using the calibrated competition experiments are presented in Table 1.§

### Amide containing receptors 2–6

As expected,[7] tripodal receptor **6** with three amide groups bound anions more effectively than bidentate receptors **2**–**5**. Compounds **2**–**5** showed moderate chloride binding – it should be noted that the aliphatic N–H groups have lower acidity than the aromatic N–H of the reference compound, and should form weaker hydrogen bonds with the guest anion. Hence it is not surprising that receptors **2**–**5** do not offer much improvement over the reference receptor. On comparing receptors **2**–**5**, which each contain two N–H groups, compound **2** which has the shortest spacer chain is the most effective receptor. The relative magnitudes of these $K_{app}$ values are consistent with the results of Werner and Schneider (in $CDCl_3$)[7] and this gives us some confidence in applying the high-throughput assay. The decrease in binding strength from receptor **2** to **5** is indicative of the fact that the chelate effect for anion binding plays a greater role in chloride binding when the spacer chain is shorter. As has previously been pointed out, this is primarily due to entropic factors.[7]

**Table 1** Apparent, approximate binding constants ($K_{app}$) for receptors **2**–**15** with chloride anions (calculated from the competition studies in $CD_3CN$ – see ESI and ref. 5)

| Competing receptor | $K_{app}/M^{-1}$ ($CD_3CN$) |
|---|---|
| **2** | 45 |
| **3** | 30 |
| **4** | 20 |
| **5** | 12 |
| **6** | 125 |
| **7** | 48 |
| **8** | 40 |
| **9** | 50 |
| **10** | 95 |
| **11** | 555 |
| **12** | <1 |
| **13** | <1 |
| **14** | 145 |
| **15** | 1015 |

### Phenolic receptors 7–15

The results obtained with phenols **7**–**15** were particularly interesting. Compound **7** (phenol) was able to compete with calibrant **1** and showed a fair affinity for chloride anions. It is worth noting that a number of previous receptors for anions have utilised aliphatic hydroxy groups,[8] and phenols have also been implicated in anion binding.[9–12] Hong and co-workers in particular have made use of azophenols in a range of chromogenic molecules designed for selective anion sensing.[13] Interestingly, tyrosine is also known to play a key biomolecular role in binding chloride anions in proteins through hydrogen bond formation, indicating the importance of phenol–anion interacions in the hydrophobic encapsulated interiors of proteins.[14] Phenols have not, however, previously been thoroughly or systematically investigated in the field of anion binding – unlike the amide functional group, which has seen extensive exploitation.[3] Obviously, there is considerable scope for the determination of electronic, steric and structural effects on the anion binding ability of simple phenolic compounds – and some of these effects are elucidated below, as a consequence of the results from our high throughput screening approach.

As might be expected, increasing the acidity (and consequently the H-bond donor ability) of the phenolic O–H group enhances its ability to interact with an anion, and hence the binding strength. Receptors **10** (fluorophenol, –I effect of fluoride group) and **11** (nitrophenol, –M effect of nitro group) both exhibit significantly enhanced chloride binding when compared with unfunctionalised phenol. Such electronic effects on the strength of hydrogen bond formation are well known when binding anions using amide and urea based receptors.[15] It is noteworthy that nitrophenol in particular shows effective binding ($K_{app}$ = 555 $M^{-1}$), as such units have previously been used in anion sensors.[12]

On the other hand, increasing the steric hindrance close to the O–H group effectively prevents chloride binding. Com-

pounds **12** (2,4,6-trimethylphenol) and **13** (2,4,6-tri-*tert*-butylphenol) which have bulky groups in the *ortho* positions to the phenolic O–H group are unable to compete effectively with reference receptor **1** and hence do not exhibit significant chloride anion binding ability (Table 1). Such steric effects play a dramatic role in anion binding as a consequence of the relatively large size of anionic guests (in particular compared with their cationic counterparts).

Compound **14** (resorcinol) possesses two O–H groups and can hence chelate the anion using two hydrogen bonds. This increases the strength of binding three-fold when compared to monodentate **7** (phenol). This increase in binding is particularly noteworthy given that the second O–H group will have an unfavourable +M mesomeric effect on the individual hydrogen bond strength. The most remarkable receptor, however, is compound **15**, (catechol) which binds chloride over twenty times more strongly than simple phenol **7** ($K_{app}$ = 1015 $M^{-1}$). This strong binding was immediately evident during the screening process, as catechol induced the largest shift in the N–H proton of acetanilide in the presence of chloride anion, hence alerting us to the relatively high affinity of this complex (Fig. 4). This result indicates that the *ortho* arrangement of the O–H groups in catechol is much more appropriate for binding chloride anions in a chelate mode than the *meta* arrangement of the same groups in resorcinol. Furthermore, it is worth noting that the rigid arrangement of phenolic O–H groups on the aromatic skeleton of catechol is significantly better than the more flexible combination of two (or even three) amide N–H groups in compounds **2**–**6**.

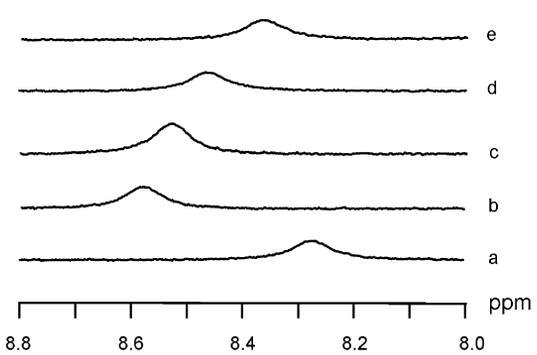


**Fig. 4** Extracts from the NMR spectra of: (a) **1** (3.67 mM); (b) **1** (3.67 mM) and TBACl (3.57 mM); (c) **1** (3.67 mM), TBACl (3.57 mM) and **7** (phenol) (4.46 mM); (d) **1** (3.67 mM), TBACl (3.57 mM) and **14** (resorcinol) (5.65 mM); (e) **1** (3.67 mM), TBACl (3.57 mM) and **15** (catechol) (5.66 mM). This figure illustrates the relative abilities of these phenols to compete with receptor **1** for chloride anions, with catechol **15** removing the majority of the chloride anions from reference receptor **1**.

To ensure that this approach to finding higher affinity receptors was indeed valid in this case of anion binding, we performed further analysis on the best receptor (**15**, catechol) using a traditional $^1$H NMR titration with TBACl (Fig. 5). As can be seen from the binding profile, the stoichiometry of the complex is clearly 1 : 1. Furthermore, the catechol O–H protons remained visible throughout the titration and this indicates that the process which occurs is not simply anion induced catechol deprotonation. The binding constant determined from the titration data (using HypNMR)[6] was high, with the precise value ($K_a$ = 1575 $M^{-1}$) showing reasonable agreement with that determined using our rapid screening approach (given the limitations of NMR titrations when used with sharp binding profiles, and our calibrated competitive assay).

In summary, this communication illustrates that a high-throughput method[5] can be applied to anion binding in order to rapidly assess approximate binding constants. Furthermore, this approach has generated an interesting lead structure (catechol) for further investigation in anion recognition.

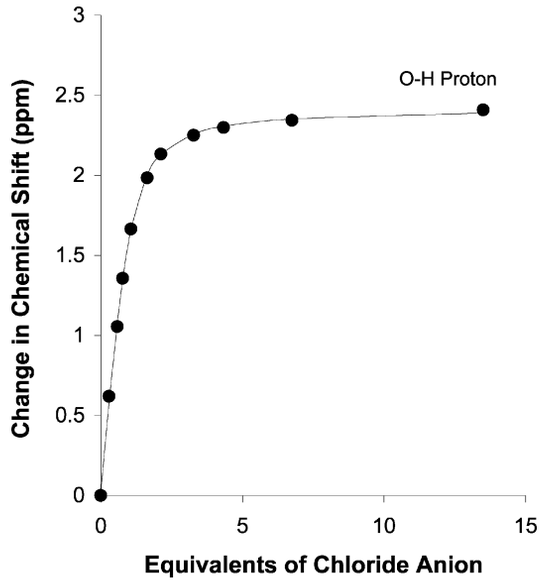


**Fig. 5** NMR titration curves for catechol (**15**, 3.71 mM) on the addition of tetrabutylammonium chloride (solvent: $CD_3CN$).

Although there have been a few previous reports of interactions between anions and a catechol framework, the improvements offered by this struture over simple phenol have not previously been emphasised.[11,12] The binding constant obtained for catechol with chloride anions in $CD_3CN$ is surprisingly high when the structural simplicity of this building block is taken into consideration. Catechol based building blocks are widespread in both synthetic and natural systems (*e.g.* dopamine, siderophores *etc.*) and we are confident that such moieties will go on to find widespread application in anion receptors. In addition, the ability of this type of structure to bind anions may be of considerable biomolecular significance. Our future work will continue to focus on the application of high throughput methods to supramolecular chemistry and on the exploitation of catechol-containing receptors with selectivities for a variety of different anions.

I would like to thank all the current members of my research group for their help, and for putting up with having me back in the lab for a while! EPSRC provides funding for our research in the field of anion binding (GR/N 25046).

## Notes and references

‡ Compounds **2**–**6** were synthesised using standard methodology and all analytical data were in full agreement with the structures indicated.
§ It is important that the two receptors do not significantly perturb each others NMR spectra through mutual hydrogen bonding, otherwise this calibrated competitive method is invalid. Attempts to use this method in $CDCl_3$ completely failed for this reason. In $CD_3CN$, however, the receptors are reasonably well solvated and did not significantly perturb each others NMR spectra – the only major shifts being due to competition for the chloride anion.

1 For a general review see: (*a*) P. D. Beer and P. A. Gale, *Angew. Chem., Int. Ed.*, 2001, **40**, 487–516; (*b*) For a review of recent research see: P. A. Gale, *Coord. Chem. Rev.*, 2003, **240**, 191–221; (*c*) For a book see: *Supramolecular Chemistry*, eds A. Bianchi, E. Garcia-España and K. Bowman-James, Wiley-VCH, New York, 1997.
2 For illustrative examples: (*a*) C.-H. Tai, P. Burkhard, D. Gani, T. Jenn, C. Johnson and P. F. Cook, *Biochemistry*, 2001, **40**, 7446–7452; (*b*) B. T. Burlingham and T. S. Widlanski, *J. Org. Chem.*, 2001, **66**, 7561–7567; (*c*) F. M. Ashcroft, *Ion Channels and Disease*, Academic Press, San Diego and London, 2000.
3 There are some excellent reviews of this area: (*a*) K. Choi and A. D. Hamilton, *Coord. Chem. Rev.*, 2003, **240**, 101–110; (*b*) C. R. Bondy and S. J. Loeb, *Coord. Chem. Rev.*, 2003, **240**, 77–99; (*c*) J. L. Sessler, S. Camiolo and P. A. Gale, *Coord. Chem. Rev.*, 2003, **240**, 17–55.
4 L. Fielding, *Tetrahedron*, 2000, **56**, 6151–6170.

5 R. E. Heath, G. M. Dykes, H. Fish and D. K. Smith, *Chem. Eur. J.*, 2003, **9**, 850–855.
6 C. Frassineti, S. Ghelli, P. Gans, A. Sabatini, M. S. Moruzzi and A. Vacca, *Anal. Biochem.*, 1995, **231**, 374–382.
7 F. Werner and H.-J. Schneider, *Helv. Chim. Acta*, 2000, **83**, 465–478.
8 (*a*) A. P. Davis and J.-B. Joos, *Coord. Chem. Rev.*, 2003, **240**, 143–156; (*b*) J. M. Coterón, F. Hacket and H.-J. Schneider, *J. Org. Chem.*, 1996, **61**, 1429–1435; (*c*) S. Kondo, T. Suzuki and Y. Yano, *Tetrahedron Lett.*, 2002, **43**, 7059–7061.
9 Phenolic groups on calixarenes can interact with anions – see for example: (*a*) A. Arduini, G. Giorgi, A. Pochini, A. Secchi and F. Ugozzoli, *J. Org. Chem.*, 2001, **66**, 8302–8308; (*b*) K. Ito, H. Miki and Y. Ohba, *Yakugaku Zasshi*, 2002, **122**, 413–417.
10 A crystal structure has been reported of phenol bound to an anion: R. Goddard, H. M. Herzog and M. T. Reetz, *Tetrahedron*, 2002, **58**, 7847–7850.
11 Catechols and resorcinols have been bound as guest molecules *via* interactions with a host which contains phosphonate anions: T. Schrader, *J. Org. Chem.*, 1998, **63**, 264–272.
12 Phenol-anion and catechol-anion interactions have been used in anion sensors: H. Miyaji and J. L. Sessler, *Angew. Chem., Int. Ed.*, 2001, **40**, 154–157.
13 (*a*) D. H. Lee, K. H. Lee and J. I. Hong, *Org. Lett.*, 2001, **3**, 5–8; (*b*) D. H. Lee, H. Y. Lee, K. H. Lee and J. I. Hong, *Chem. Commun.*, 2001, 1188–1189; (*c*) K. H. Lee, H. Y. Lee, D. H. Lee and J. I. Hong, *Tetrahedron Lett.*, 2001, **42**, 5447–5449; (*d*) C. Lee, D. H. Lee and J. I. Hong, *Tetrahedron Lett.*, 2001, **42**, 8665–8668.
14 R. M. Wachter, D. Yarbrough, K. Kallio and S. J. Remington, *J. Mol. Biol.*, 2000, **301**, 157–171.
15 T. R. Kelly and M. H. Kim, *J. Am. Chem. Soc.*, 1994, **116**, 7072–7080.