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Substrate Discrimination by Cholapod Anion Receptors: Geometric Effects and the "Affinity−Selectivity Principle"

John P. Clare,[†] Alan J. Ayling,[†] Jean-Baptiste Joos,[†] Adam L. Sisson,[†] Germinal Magro,[†] M. Nieves Pérez-Payán,[‡] Timothy N. Lambert,[§] Rameshwer Shukla,[§] Bradley D. Smith,*[,§] and Anthony P. Davis*[,†]

Contribution from the School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, U.K., Department of Chemistry, Trinity College, Dublin 2, Ireland, and Department of Chemistry and Biochemistry and Walther Cancer Research Center, University of Notre Dame, Notre Dame, Indiana 46556

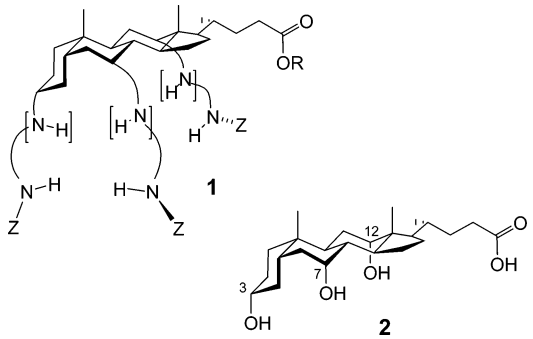
Received April 14, 2005; E-mail: Anthony.Davis@bristol.ac.uk

Abstract: Cholapod anion receptors can achieve high affinities while maintaining compatibility with nonpolar media. Previously they have been shown to transport anions across cell and vesicle membranes. In the present work, the scope of the architecture is expanded and structure−selectivity relationships are investigated. Eight new receptors have been synthesized, with up to six H-bond donor centers. Using Cram's extraction method, these compounds plus five known examples have been tested for binding to seven monovalent anions (tetraethylammonium salts, wet chloroform as solvent). Association constants in excess of 10^{10} M^{-1} have been measured for several pairings. Selectivities vary with receptor geometry, as expected. More remarkably, they also depend on receptor strength: more powerful receptors show a wider range of binding free energies, and therefore a greater spread of $K_a(X^-)/K_a(Y^-)$. This "affinity−selectivity" effect can be derived from empirical relationships for H-bond strengths, and could prove widely operative in supramolecular chemistry.

Introduction

Over the past 10−15 years, the study of anion recognition has become a major preoccupation of supramolecular chemistry.[1] Interest is reinforced by the importance of anions in biology, the prospect (still largely unfulfilled) of biological activity,[2] and the possibility of applying anion receptors in sensors[1c,3] and separations.[4] Although early work focused on positively charged systems,[5] increasing attention has been paid to electroneutral anion receptors.[6] Neutral receptors tend to be compatible with nonpolar media, and thus useful for "phase-transfer" applications. For example, they may be exploited in ion-selective electrodes or other sensing devices based on organic−aqueous interfaces.[7] In principle, they may also locate in cell membranes and mediate anion transport, mirroring the action of cationophores such as valinomycin.[8]

A common approach to electroneutral anion receptors is the organization of neutral H-bond donor units around a central binding site.[6a−d] Clearly, this strategy requires scaffolds that position the donors appropriately. As most H-bond donor groups are also acceptors,[9] it may also be necessary to prevent (or at least limit) intramolecular H-bonding between different parts of the receptor. The "cholapod" architecture **1** provides an effective solution.[10] As a podand, it is readily tunable: once the core unit has been prepared, the "legs" can be varied quite easily to generate a range of structures. In particular, terminal groups Z can be chosen to optimize NH acidity, controlling



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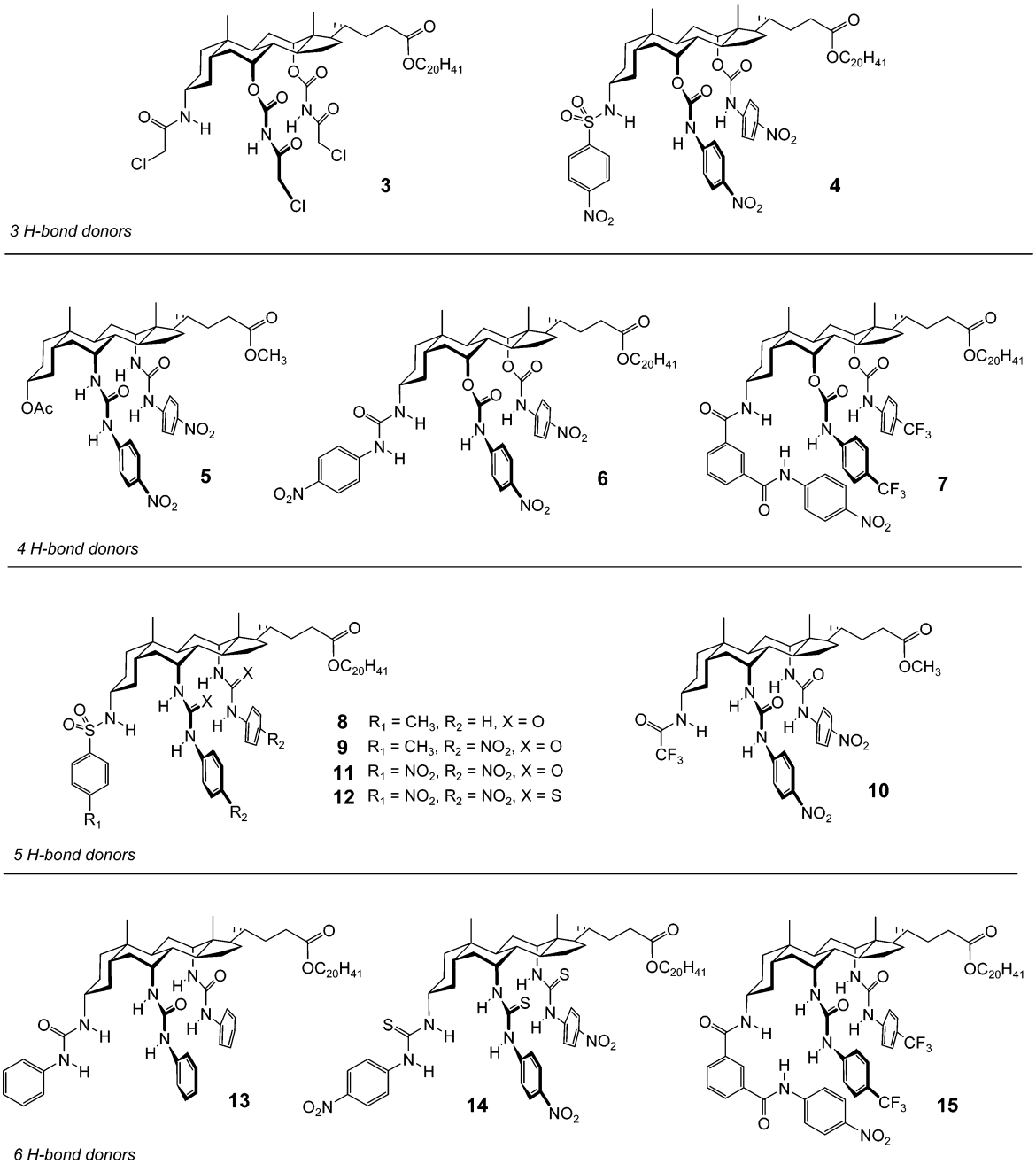
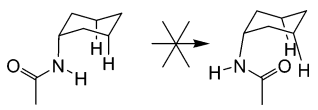
[†] University of Bristol.
[‡] Trinity College.
[§] University of Notre Dame.

(1) (a) Bianchi, A.; Bowman-James, K.; Garcia-España, E. *Supramolecular Chemistry of Anions*; Wiley-VCH: New York, 1997. (b) Schmidtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609. (c) Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 487. (d) Gale, P. A. *Coord. Chem. Rev.* **2003**, *240*, 1. (e) Gale, P. A. *Coord. Chem. Rev.* **2003**, *240*, 191.
(2) A few biologically active natural products show anion recognition properties, examples being the vancomycin group of antibiotics (carboxylate recognition: see Williams, D. H.; Bardsley, B. *Angew. Chem., Int. Ed.* **1999**, *38*, 1173), the prodigiosins (HCl transporters: see Furstner, A. *Angew. Chem., Int. Ed.* **2003**, *42*, 3582), and the pamamycins (anionophores: see Jeong, E. J.; Kang, E. J.; Sung, L. T.; Hong, S. K.; Lee, E. *J. Am. Chem. Soc.* **2002**, *124*, 14655). However, cation recognition is far better established as a mode of biological activity.
(3) Suksai, C.; Tuntulani, T. *Chem. Soc. Rev.* **2003**, *32*, 192. Martinez-Manez, R.; Sancenon, F. *Chem. Rev.* **2003**, *103*, 4419. Wiskur, S. L.; Ait-Haddou, H.; Lavigne, J. J.; Anslyn, E. V. *Acc. Chem. Res.* **2001**, *34*, 963.
(4) *Fundamentals and Applications of Anion Separations*; Moyer, B. A., Singh, R. P., Eds.; Kluwer: Dordrecht, 2004. Roundhill, D. M.; Koch, H. F. *Chem. Soc. Rev.* **2002**, *31*, 60. Levitskaia, T. G.; Marquez, M.; Sessler, J. L.; Shriver, J. A.; Vercouter, T.; Moyer, B. A. *Chem. Commun.* **2003**, 2248. Kavallieratos, K.; Sachleben, R. A.; Van Berkel, G. J.; Moyer, B. A. *Chem. Commun.* **2000**, 187.
(5) Llinares, J. M.; Powell, D.; Bowman-James, K. *Coord. Chem. Rev.* **2003**, *240*, 57.

Chart 1. Receptors Prepared and/or Tested during the Course of This Work



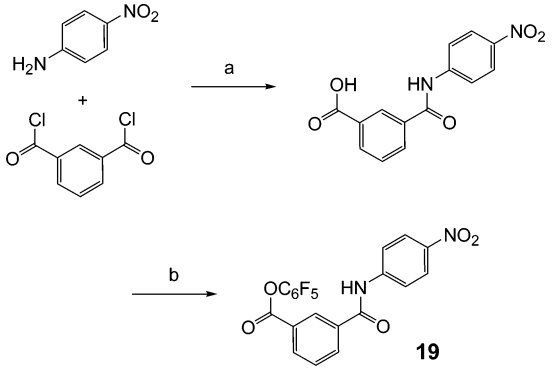
H-bond donor power and receptor affinities. The steroidal skeleton, derived from cholic acid **2**, provides a rigid framework for defining the binding site. The spacing between the three substituents (positions 3α, 7α, and 12α) is sufficient to prevent intramolecular hydrogen bonding in **2**, and limits inter-leg contacts in **1**. The axial orientation of the 7α and 12α substituents provides further preorganization. For example, a −NH−CO group in either position is fixed in a conformation which projects the NH underneath the steroid nucleus; rotation is prevented by potential 1,3-diaxial interactions (eq 1). Finally, the lipophilic nature of the steroid can be reinforced by choice of ester group R. Cholapods are thus well-suited to operation in nonpolar media, including the interior of cell membranes.



Variation of the cholapod structure has led to a wide range of binding constants. While early examples showed $K_a \approx 10^4$

(6) (a) Sessler, J. L.; Camiolo, S.; Gale, P. A. *Coord. Chem. Rev.* **2003**, *240*, 17. (b) Choi, K. H.; Hamilton, A. D. *Coord. Chem. Rev.* **2003**, *240*, 101. (c) Bondy, C. R.; Loeb, S. J. *Coord. Chem. Rev.* **2003**, *240*, 77. (d) Antonisse, M. M. G.; Reinhoudt, D. N. *Chem. Commun.* **1998**, 443. (e) Wedge, T. J.; Hawthorne, M. F. *Coord. Chem. Rev.* **2003**, *240*, 111.
(7) Buhlmann, P.; Pretsch, E.; Bakker, E. *Chem. Rev.* **1998**, *98*, 1593.
(8) Dobler, M. Molecular recognition: receptors for cationic guests. In *Comprehensive Supramolecular Chemistry*, Vol. 1; Gokel, G. W., Ed.; Pergamon: Oxford, 1996; p 267.
(9) An important exception is the pyrrole unit, as exploited by Sessler in the calixpyrroles (see ref 6a).
(10) (a) Davis, A. P.; Perry, J. J.; Williams, R. P. *J. Am. Chem. Soc.* **1997**, *119*, 1793. (b) Ayling, A. J.; Pérez-Payán, M. N.; Davis, A. P. *J. Am. Chem. Soc.* **2001**, *123*, 12716. (c) Ayling, A. J.; Broderick, S.; Clare, J. P.; Davis, A. P.; Pérez-Payán, M. N.; Lahtinen, M.; Nissinen, M. J.; Rissanen, K. *Chem. Eur. J.* **2002**, *8*, 2197. (d) Davis, A. P.; Joos, J.-B. *Coord. Chem. Rev.* **2003**, *240*, 143.

Scheme 1. Preparation of **19**[a]



[a] Reagents and conditions: (a) THF, then aqueous NaOH; (b) DCC, C_6F_5OH, THF.

M^{-1} for $R_4N^+Cl^-$ in chloroform,[10a] this figure was later raised to 10^{11} M^{-1} in the case of sulfonamido-bis-thiourea **12** (Chart 1).[10b,11] Access to such high affinities prompted investigations of anion transport. A number of cholapods were shown to promote translocation of chloride ions across vesicle and cell membranes.[12] Several lines of evidence, including a correlation of effectiveness with binding constants, suggested a carrier mechanism. Experiments also showed that transport was electroactive, i.e., that the anions were unaccompanied by cations and could thus support a flow of current (in the manner of natural chloride channels[13]). The cholapods are probably unique in this combination of properties, at least among purely organic systems; other transporters require countercations,[14] are themselves positively charged,[15] or are thought to operate by channel mechanisms.[15c−e,16] As electroneutral molecules, they should avoid the toxicity problems associated with cationic amphiphiles and may be realistic candidates for treatment of diseases caused by deficiencies in natural chloride transport (notably cystic fibrosis).[17] They have also been shown to act as phospholipid "flippases", a second potential mode of biological activity.[18]

(11) An even higher figure of 5×10^{12} M^{-1} was measured for unpaired chloride ions in 1,2-dichloroethane using a voltammetric method. See: Dryfe, R. A. W.; Hill, S. S.; Davis, A. P.; Joos, J.-B.; Roberts, E. P. L.; Fisher, A. C. *Org. Biomol. Chem.* **2004**, *2*, 2716.

(12) Koulov, A. V.; Lambert, T. N.; Shukla, R.; Jain, M.; Boon, J. M.; Smith, B. D.; Li, H. Y.; Sheppard, D. N.; Joos, J. B.; Clare, J. P.; Davis, A. P. *Angew. Chem., Int. Ed.* **2003**, *42*, 4931. McNally, B. A.; Koulov, A. V.; Smith, B. D.; Joos, J.-B.; Davis, A. P. *Chem. Commun.* **2005**, 1087.

(13) Dutzler, R.; Campbell, E. B.; Cadene, M.; Chait, B. T.; MacKinnon, R. *Nature* **2002**, *415*, 287.

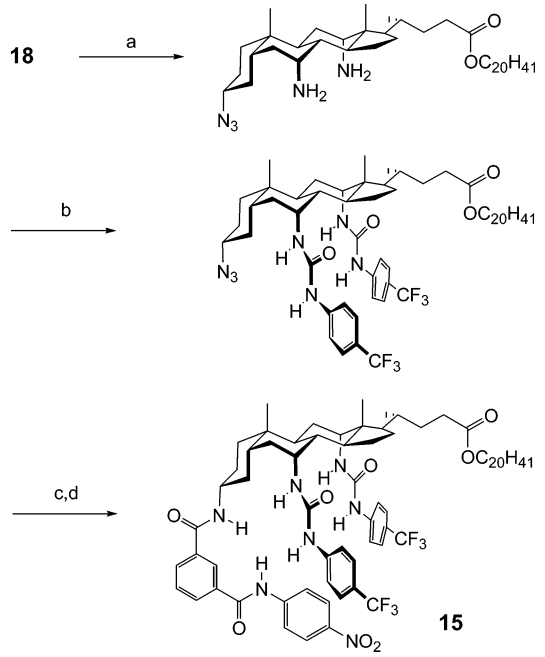
(14) Koulov, A. V.; Mahoney, J. M.; Smith, B. D. *Org. Biomol. Chem.* **2003**, *1*, 27. Chrisstoffels, L. A. J.; de Jong, F.; Reinhoudt, D. N. *Chem. Eur. J.* **2000**, *6*, 1376. Chrisstoffels, L. A. J.; de Jong, F.; Reinhoudt, D. N.; Sivelli, S.; Gazzola, L.; Casnati, A.; Ungaro, R. *J. Am. Chem. Soc.* **1999**, *121*, 10142.

(15) (a) Sessler, J. L.; Allen, W. E. *Chemtech* **1999**, *29*, 16. (b) Janout, V.; Jing, B. W.; Staina, I. V.; Regen, S. L. *J. Am. Chem. Soc.* **2003**, *125*, 4436. (c) Merritt, M.; Lanier, M.; Deng, G.; Regen, S. L. *J. Am. Chem. Soc.* **1998**, *120*, 8494. (d) Sakai, N.; Matile, S. *Tetrahedron Lett.* **1997**, *38*, 2613. (e) Sakai, N.; Sorde, N.; Das, G.; Perrottet, P.; Gerard, D.; Matile, S. *Org. Biomol. Chem.* **2003**, *1*, 1226. (f) Dietrich, B.; Fyles, T. M.; Hosseini, M. W.; Lehn, J.-M.; Kaye, K. C. *J. Chem. Soc., Chem. Commun.* **1988**, 691.

(16) Sidorov, V.; Kotch, F. W.; Abdrakhmanova, G.; Mizani, R.; Fettinger, J. C.; Davis, J. T. *J. Am. Chem. Soc.* **2002**, *124*, 2267. Sidorov, V.; Kotch, F. W.; Kuebler, J. L.; Lam, Y. F.; Davis, J. T. *J. Am. Chem. Soc.* **2003**, *125*, 2840. Schlesinger, P. H.; Ferdani, R.; Liu, J.; Pajewska, J.; Pajewski, R.; Saito, M.; Shabany, H.; Gokel, G. W. *J. Am. Chem. Soc.* **2002**, *124*, 1848. Schlesinger, P. H.; Ferdani, R.; Pajewski, R.; Pajewska, J.; Gokel, G. W. *Chem. Commun.* **2002**, 840. Schlesinger, P. H.; Djedovic, N. K.; Ferdani, R.; Pajewska, J.; Pajewski, R.; Gokel, G. W. *Chem. Commun.* **2003**, 308. Djedovic, N.; Ferdani, R.; Harder, E.; Pajewska, J.; Pajewski, R.; Schlesinger, P. H.; Gokel, G. W. *Chem. Commun.* **2003**, 2862.

(17) Welsh, M. J.; Ramsey, B. W.; Accurso, F.; Cutting, G. R. In *The Metabolic and Molecular Basis of Inherited Disease*; Scriver, C. R., Beaudet, A. L., Sly, W. S., Valle, D., Eds.; McGraw-Hill Inc.: New York, 2001; p 5121.

Scheme 2. Synthesis of **15**[a]



[a] Reagents and conditions: (a) CF_3CO_2H, CH_2Cl_2, then aqueous $NaHCO_3$, EtOAc; (b) *p*-$CF_3C_6H_4NCO$, CH_2Cl_2, Et_3N, DMAP; (c) Me_3P, THF, then H_2O; (d) **19**, Et_3N, THF.

Thus far, research on cholapods has focused on optimizing affinities, especially for chloride. For many purposes, including applications in biology, discrimination between anions may be equally important. The cholapod architecture is exceptionally "tunable"—a variety of "legs" may be appended to the basic steroidal scaffold to give a wide range of binding sites. Control of the number and spacing of NH groups might be expected to result in clear preferences for particular anions.[19] To test this hypothesis, we have now studied a series of 13 cholapod receptors binding seven monovalent anions. The receptors, eight of which are new, cover a range of geometries, with between three and six H-bond donors. The results confirm that the shape of the binding site can strongly affect selectivities. Less predictably, they also reveal that *affinities* can moderate selectivities in a systematic fashion. This "affinity−selectivity" effect could prove quite general, and may serve as a useful design tool for controlling binding preferences in supramolecular chemistry.

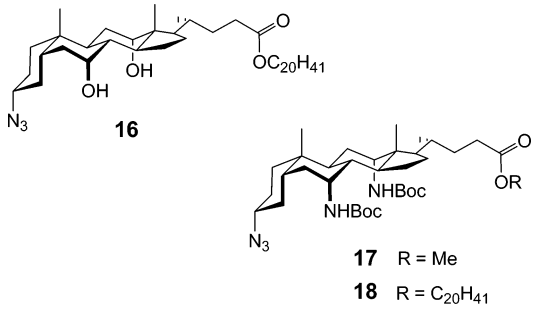
Results and Discussion

Receptor Design and Synthesis. The receptors studied in this work are shown in Chart 1, organized according to the number of H-bond donors in their binding sites. Bis-carbamates **3** and **4**, with three H-bond donors, have been reported previously;[10c] receptor **3** plays a central role in our method for measuring binding constants (see below). Among the set of cholapods with four H-bond donors, the bis-urea **5** was reported as a chloride transporter,[12] while ureido-bis-carbamate **6** is new,

(18) Lambert, T. N.; Boon, J. M.; Smith, B. D.; Pérez-Payán, M. N.; Davis, A. P. *J. Am. Chem. Soc.* **2002**, *124*, 5276. Boon, J. M.; Lambert, T. N.; Sisson, A. L.; Davis, A. P.; Smith, B. D. *J. Am. Chem. Soc.* **2003**, *125*, 8195. Smith, B. D.; Lambert, T. N. *Chem. Commun.* **2003**, 2261.

(19) Hay, B. P.; Dixon, D. A.; Bryan, J. C.; Moyer, B. A. *J. Am. Chem. Soc.* **2002**, *124*, 182. Hay, B. P.; Gutowski, M.; Dixon, D. A.; Garza, J.; Vargas, R.; Moyer, B. A. *J. Am. Chem. Soc.* **2004**, *126*, 7925. Hay, B. P.; Firman, T. K.; Moyer, B. A. *J. Am. Chem. Soc.* **2005**, *127*, 1810.

but related to a previously disclosed enantioselective carboxylate receptor.[20] Receptor **7** is novel, its design representing a marriage of the cholapod architecture with Crabtree's isophthalamide system.[21] Molecular modeling suggested that, in an unstrained conformation, the four H-bond donors can adopt a roughly tetrahedral arrangement around a spherical anion, with H···X^- distances of 2.7−2.8 Å. Receptors **5**−**7** provide an interesting series in which the same number of NH groups, with roughly similar donor potential, are arranged in quite different geometries. The receptors with five donors, by contrast, possess the same array of NH groups but with different acidities. Cholapods **11** and **12** were previously reported,[10b] while **8**−**10** are new. The final group, bearing six H-bond donors, are also new. Two geometrically similar compounds, the tris-(thio)ureas **13** and **14**, are complemented by a second isophthalamide, **15**. Both eicosyl and methyl esters were employed; aside from effects on solubility, the side chain is not expected to moderate the binding properties.[22] The new receptors **6**−**10** and **13**−**15** were prepared from intermediates **16**,[10c] **17**,[23] and **18**[10b] through deprotections and reactions with iso(thio)cyanates, acylating agents, and/or sulfonyl chlorides, as described in the Supporting Information. Pentafluorophenyl ester **19** (Scheme 1) was used to introduce the isophthalamide units in **7** and **15**. The synthesis of **15**, shown in Scheme 2, is illustrative.



Measurements of Binding Constants. The high affinities achievable by cholapod receptors present measurement difficulties. ^{1}H NMR titration, the most common and convenient approach, is limited to K_a values below ca. 10^5 M^{-1}. Above this limit, the chemical shift movements are essentially linear with concentration of added guest, halting after addition of one equivalent. Binding constants of, for example, 10^6 and 10^{10} M^{-1} yield almost identical profiles. The issue arises with all titration methods, although the limit is higher for more sensitive techniques which may be operated at lower concentrations.

When faced with this problem, a common response is to change the medium for the binding experiment, employing a more competitive solvent system. For anion recognition by hydrogen bonding, dimethyl sulfoxide (DMSO) is often appropriate. However, for our studies on cholapods we have two reasons for avoiding this approach. First, a distinguishing feature of the cholapod architecture is its lipophilicity, and thus its potential for membrane transport. Being designed to operate in nonpolar media, the molecules should be studied therein; results in DMSO might bear little relation to transport properties, due to self-association (or insolubility) in less-polar media. Second, and especially in the present work, we wish to compare a range of receptors and substrates under identical conditions. Accordingly, we have adopted the extraction-based method of Cram[24] for this program. As discussed previously,[10b,c] the receptor is dissolved in chloroform and equilibrated with an aqueous solution of $Et_4N^+X^-$, where X^- is the target anion. The amount of salt extracted is estimated by ^{1}H NMR integration, allowing the extraction constant K_e to be determined (eq 2; H = Host, $HEt_4N^+X^-$ = complex, assumed to be a tight ion pair in chloroform).

$$K_e = \frac{[HEt_4N^+X^-]_{org}}{[H]_{org}[Et_4N^+]_{aq}[X^-]_{aq}} \quad (2)$$

The distribution constant K_d of the substrate between water and chloroform in the absence of receptor must also be determined (eq 3).

$$K_d = \frac{[Et_4N^+X^-]_{org}}{[Et_4N^+]_{aq}[X^-]_{aq}} \quad (3)$$

The association constant K_a for the formation of the complex in the organic phase (water-saturated chloroform) is then given by eq 4.

$$K_a = \frac{[HEt_4N^+X^-]_{org}}{[H]_{org}[Et_4N^+X^-]_{org}} = \frac{K_e}{K_d} \quad (4)$$

As in titration methods, quantitative complex formation poses a potential problem: if the receptor extracts ∼1 equiv of substrate, $[H]_{org}$ tends to zero and K_e becomes impossible to estimate. However, in this case the issue can be addressed by reducing the concentration of substrate in the aqueous phase. The extraction method can therefore cope with a wide range of affinities. It is also very simple to operate, once K_d for a substrate has been established. Disadvantages are that K_d may not be trivially accessible (see below) and that the method is subject to various uncertainties. For example, the receptor may aggregate in the organic phase, depressing the level of extraction and leading to an underestimate of K_a. For these reasons, the K_a values presented in this paper should be considered "apparent".

In previous research, we had employed the extraction method with just two substrates, tetraethylammonium chloride and bromide. For the present work we wished to study a wider range of substrates, and thus needed to measure the corresponding distribution constants K_d. The anions chosen were acetate, ethanesulfonate, nitrate, iodide, and perchlorate. Di- and trivalent anions were avoided because of potential problems in interpreting spectra and analyzing extraction data. As before,[10c] two methods were used to determine K_d. The first, method A, was based on direct measurement of the amounts of salt transferred

(20) Siracusa, L.; Hurley, F. M.; Dresen, S.; Lawless, L. J.; Pérez-Payán, M. N.; Davis, A. P. *Org. Lett.* **2002**, *4*, 4639.
(21) Kavallieratos, K.; deGala, S. R.; Austin, D. J.; Crabtree, R. H. *J. Am. Chem. Soc.* **1997**, *119*, 2325. Kavallieratos, K.; Bertao, C. M.; Crabtree, R. H. *J. Org. Chem.* **1999**, *64*, 1675.
(22) Direct comparisons between methyl and eicosyl esters have been made in several cases, revealing no substantial differences.
(23) del Amo, V.; Siracusa, L.; Markidis, T.; Baragaña, B.; Bhattarai, K. M.; Galobardes, M.; Naredo, G.; Pérez-Payán, M. N.; Davis, A. P. *Org. Biomol. Chem.* **2004**, *2*, 3320.
(24) Kyba, E. P.; Helgeson, R. C.; Madan, K.; Gokel, G. W.; Tarnowski, T. L.; Moore, S. S.; Cram, D. J. *J. Am. Chem. Soc.* **1977**, *99*, 2564. Timko, J. M.; Moore, S. S.; Walba, D. M.; Hiberty, P. C.; Cram, D. J. *J. Am. Chem. Soc.* **1977**, *99*, 4207.

Table 1. K_d Values for Extraction of Tetraethylammonium Salts from Water into $CHCl_3$, Determined by Direct (A) and Indirect (B) Methods[a]

	K_d, M^{-1}	
anion	method A	method B
ClO_4^-	*1.1 × 10⁻² b*	
I^-	*8.4 × 10⁻³ b*	
Br^-	2.7 × 10⁻⁴ *c,d,e*	*2.2 × 10⁻⁴ d*
NO_3^-	2.0 × 10⁻⁴ *c,f*	*1.9 × 10⁻⁴*
$EtSO_3^-$		*2.9 × 10⁻⁵*
Cl^-	1.2 × 10⁻⁵ *d,g*	*1.3 × 10⁻⁵ d*
AcO^-	*h*	*8.5 × 10⁻⁷*

[a] Italicized figures were employed to calculate the K_a values in Table 2. $T = 303$ K. For experimental details, see Supporting Information. [b] Amount extracted determined by weighing, averaged over four experiments. All values were within ±3% of the mean. [c] Amount extracted determined by ^1H NMR with internal standard (1,1,2,2-tetrachloroethane). [d] Value from ref 10c. [e] Average of three experiments, values within ±9% of the mean. [f] Average of eight experiments, values within ±20% of the mean. [g] Average of five experiments, values within ±40% of the mean. [h] Method gave widely fluctuating values.

to the organic phase in large-scale chloroform−water extractions. Typically, an aqueous solution of salt (50 mL, 0.5 M) was equilibrated with chloroform (500 mL, presaturated with water), the phases were separated, and the chloroform was passed through hydrophobic filter paper and then evaporated. For the more hydrophobic salts $Et_4N^+I^-$ and $Et_4N^+ClO_4^-$, the residue could be weighed accurately. In other cases the quantities extracted were very low and the salts absorbed water from the atmosphere, so that weighing was more difficult. Instead, the amount extracted was determined by ^1H NMR integration against an internal standard (1,1,2,2-tetrachloroethane). The second approach, method B, involved determination of K_e for a reference receptor extracting the chosen substrate, and then an independent measurement of K_a by ^1H NMR titration. K_d was then obtained through rearrangement of eq 4. Chloroacetyl derivative **3** was used as reference; with three moderately acidic NH groups, this receptor was sufficiently powerful to perform the extractions but not too strong for study by NMR titration.

The results from both methods, including the previously published values for $Et_4N^+Cl^-$ and $Et_4N^+Br^-$, are summarized in Table 1. For perchlorate and iodide, method A gave excellent reproducibility, and the resulting values were therefore adopted for the remainder of this work. However, as the anions became more hydrophilic, the method became less consistent (see Table 1 footnotes). Method B, though also subject to error, proved less anion-dependent and was therefore relied upon for bromide, nitrate ethanesulfonate, chloride, and acetate. Despite the uncertainties, agreement between the two methods was good where both were applied.

Once the K_d values were available, the extraction method was applied to receptors **4**−**15**. The results, together with the NMR-derived values for **3**, are shown in Tables 2 and 3. Table 2 gives the full set of binding constants, while Table 3 presents the data in relation to the K_a values for tetraethylammonium chloride. To assist interpretation, the receptors in Table 3 are divided according to the number of H-bond donors, and then further grouped where donor geometries are (or appear to be) identical (as in **3**+**4**, **8**−**12**, and **13**+**14**). Within these latter groupings, the receptors are ordered according to their affinities to tetraethylammonium chloride. The data reveal selectivity variations both within and between these groups, as discussed in the following section.

Given the uncertainties surrounding the extraction method, it seemed prudent to corroborate at least some of the results in Table 3 using an independent technique. NMR competition methods may be used to obtain K_a ratios, even when the absolute binding constants are too high for direct measurement.[25] Essentially, the simple 1:1 binding equilibrium is replaced by an exchange equilibrium, eq 5. It is readily shown that K_{exch}, the constant for this process, is equal to the ratio of the formation constants for the two complexes, K_{AB} and K_{AC} (eq 6).

$$AB + C \overset{K_{exch}}{\rightleftharpoons} AC + B \tag{5}$$

$$K_{exch} = \frac{[AC][B]}{[AB][C]} = \frac{K_{AC}}{K_{AB}} \tag{6}$$

Competition for A between B and C keeps the equilibrium in balance over a range of concentrations, allowing accurate measurements of K_{exch}. The situation is readily exploited if both B and C are NMR-active, allowing direct estimates of [B]/[AB] and [C]/[AC].[26] Matters are less straightforward if, as in the present case, only a single component may be observed. A full analysis must take into account the presence of five species ([A], [B], [C], [AB], and [AC]), requiring the solution of cubic equations.[27] However, for very strong binding, and with B or C in excess over A, it is possible to assume that [A] = 0.[28] In this case eq 5 is the only process that needs to be considered, and mathematical analysis is simpler (see Supporting Informa-

Table 2. Association Constants K_a (M^{-1}) for the Binding of Tetraethylammonium Salts to Receptors **3**−**15** in Water-Saturated Chloroform, Measured by Extraction unless Otherwise Indicated[a]

receptor	Cl^-	Br^-	I^-	NO_3^-	AcO^-	ClO_4^-	$EtSO_3^-$
3	1.6 × 10⁴ *b*	8.4 × 10³ *b*	*c*	4.7 × 10⁴ *b*	9.5 × 10⁴ *b*	*c*	7.3 × 10³ *b*
4	1.1 × 10⁸	4.1 × 10⁷	1.4 × 10⁷	8.1 × 10⁷	1.1 × 10⁹	9.1 × 10⁵	1.5 × 10⁷
5	5.2 × 10⁸	5.8 × 10⁷	1.0 × 10⁷	1.7 × 10⁸	1.2 × 10⁸	9.6 × 10⁵	2.3 × 10⁸
6	6.2 × 10⁷	3.5 × 10⁷	1.0 × 10⁷	6.6 × 10⁷	6.6 × 10⁸	6.6 × 10⁶	3.6 × 10⁷
7	1.1 × 10⁸	5.7 × 10⁷	9.8 × 10⁶	9.1 × 10⁷	*c*	4.9 × 10⁶	*c*
8	5.3 × 10⁷	3.7 × 10⁷	9.0 × 10⁶	2.1 × 10⁷	1.3 × 10⁷	2.2 × 10⁶	4.3 × 10⁷
9	1.5 × 10⁹	9.6 × 10⁸	1.8 × 10⁸	6.6 × 10⁸	1.5 × 10⁹	3.7 × 10⁷	6.4 × 10⁸
10	1.2 × 10¹⁰	5.4 × 10⁹	9.0 × 10⁸	3.2 × 10⁹	2.6 × 10¹⁰	1.3 × 10⁸	3.5 × 10⁹
11	6.8 × 10¹⁰	1.6 × 10¹⁰	3.4 × 10⁹	1.4 × 10¹⁰	2.6 × 10¹¹	4.1 × 10⁸	1.6 × 10¹⁰
12	1.1 × 10¹¹	2.7 × 10¹⁰	1.9 × 10⁹	8.5 × 10⁹	2.0 × 10¹¹	6.2 × 10⁷	3.7 × 10⁹
13	2.7 × 10⁸	1.4 × 10⁸	2.2 × 10⁷	1.6 × 10⁸	1.4 × 10⁸	6.0 × 10⁶	2.2 × 10⁸
14	1.8 × 10¹¹	4.3 × 10¹⁰	4.9 × 10⁹	1.8 × 10¹⁰	1.3 × 10¹¹	9.8 × 10⁷	3.4 × 10¹⁰
15	1.5 × 10¹⁰	8.5 × 10⁹	5.2 × 10⁸	8.8 × 10⁹	8.2 × 10¹⁰	1.1 × 10⁸	6.6 × 10⁹

[a] $T = 303$ K. For experimental details, see Supporting Information. Errors for the extraction protocol are estimated at ±15% in most cases, assuming that the binding model is correct and excluding uncertainties in K_d. [b] Obtained using ^1H NMR titration. [c] Not determined.

Table 3. Association Constants for the Binding of Tetraethylammonium Salts to Receptors **3**−**15** in Water-Saturated Chloroform, Expressed Relative to the Values for $Et_4N^+Cl^-$

		selectivities (normalized to Cl^-)[a]						
receptor	K_a (M^{-1}) ($Et_4N^+Cl^-$)	Cl^-	Br^-	I^-	NO_3^-	AcO^-	ClO_4^-	$EtSO_3^-$
		Three-H-Bond Donors						
3	1.6×10^4	1.0	0.51	*b*	2.9	5.8	*b*	0.45
4	1.1×10^8	1.0	0.36	0.13	0.72	9.7	0.081	0.13
		Four-H-Bond Donors						
5	5.2×10^8	1.0	0.11	0.020	0.32	0.23	0.0018	0.45
6	6.2×10^7	1.0	0.56	0.17	1.0	11	0.11	0.58
7	1.1×10^8	1.0	0.50	0.085	0.79	*b*	0.042	*b*
		Five-H-Bond Donors						
8	5.3×10^7	1.0	0.70	0.17	0.39	0.24	0.042	0.81
9	1.5×10^9	1.0	0.64	0.12	0.44	0.97	0.025	0.43
10	1.2×10^{10}	1.0	0.47	0.077	0.28	2.2	0.011	0.30
11	6.8×10^{10}	1.0	0.23	0.050	0.21	3.8	0.0061	0.23
12	1.1×10^{11}	1.0	0.26	0.018	0.079	1.9	0.00061	0.035
		Six-H-Bond Donors						
13	2.7×10^8	1.0	0.50	0.083	0.60	0.51	0.022	0.80
14	1.8×10^{11}	1.0	0.25	0.028	0.10	0.71	0.00055	0.19
15	1.5×10^{10}	1.0	0.56	0.034	0.58	5.4	0.0069	0.44

[a] $K_a(Et_4N^+X^-)/K_a(Et_4N^+Cl^-)$. [b] Not determined.

Table 4. Selectivities[a] for $Et_4N^+Cl^-$ vs $Et_4N^+EtSO_3^-$, As Measured by Extraction and ^1H NMR Competition Titration

receptor	^1H NMR competition	extraction
8	1.4	1.2
11	3.1	4.4
12	16	29

[a] $K_a(Et_4N^+Cl^-)/K_a(Et_4N^+EtSO_3^-)$.

tion). Titrations of C into A + (excess) B, following NMR signals due to A, may thereby be used to obtain K_{AC}/K_{AB}.

Competition titrations were performed for three receptors, **8**, **11**, and **12**, for which substantial selectivity variations had been observed (vide infra). Water-saturated $CDCl_3$ was used as solvent to allow proper comparison with the extraction data. The method requires, of course, substantial differences between the NMR spectra of the two complexes (AB and AC in eq 5). This condition was satisfied for chloride and ethanesulfonate as guest anions. On addition of $Et_4N^+Cl^-$ to a preformed cholapod·$Et_4N^+EtSO_3^-$ complex, the signal due to the 3α-NH moved upfield by up to 0.6 ppm, while the other NH signals moved downfield by varying amounts. The data were analyzed by nonlinear curve-fitting, using an Excel spreadsheet adapted for the method as indicated above. The 3α-NH protons proved the easiest to follow accurately, giving consistently good fits (see Supporting Information). The resulting values for $K_a(Et_4N^+Cl^-)/K_a(Et_4N^+EtSO_3^-)$ are given in Table 4, where they are compared with the corresponding figures for the extraction experiments. Agreement is not exact, but is reasonable considering the potential errors and the very different methods employed. The same selectivity trend is observed in each case.

Discussion. The data in Table 2 confirm the high affinities achievable by cholapod anion receptors; many of the values exceed 10^{10} M^{-1}, and several are above 10^{11} M^{-1}. As expected, binding constants tend to increase with the number of H-bond donor groups, and also with their acidities. The tris-*p*-nitrophenylthiourea **14** joins previously disclosed **12** in binding chloride with $K_a > 10^{11}$ M^{-1}.

Regarding selectivities, it should first be noted that preferences revealed for any one receptor are not, by themselves, of great value. Anions vary in "stickiness", depending on their H-bond acceptor properties. Thus, the fact that all the receptors prefer chloride to iodide is of minor significance; chloride is more basic than iodide, and thus a better H-bond acceptor (and more hydrophilic). The interest lies in the comparison between rows in Table 3, where structure-determined selectivity differences may be perceived.

Surveying these figures, an interesting and unforeseen trend emerges. Discussions of selectivity usually focus on receptor *geometry*[29] (e.g., for anion recognition, the arrangement of H-bond donor groups[6a−d,19]). This factor is important for the cholapods, as discussed below. However, Table 3 reveals a second effect which is equally significant: the selectivities are strongly influenced by the *strength* of the noncovalent bonding. Receptors **3**−**15** include three sets (**3**+**4**, **8**−**12**, and **13**+**14**) for which geometries are identical (or closely similar), while affinities vary due to changes in NH acidity. For all these groupings it can be seen that, as binding constants to Cl^- rise, the selectivity figures for Br^-, I^-, NO_3^-, ClO_4^-, and $EtSO_3^-$ all decrease, while those for AcO^- rise. *In short, the tighter binding receptors show increased preferences for those anions which form stronger hydrogen bonds.* In some cases the differences are substantial. For example, in the series **8**−**12** the iodide/chloride ratio decreases from 0.17 in **8** to 0.018 in **12**, roughly an order of magnitude. Between the same two receptors, the perchlorate/chloride ratio decreases from 4×10^{-2} to 6×10^{-4}. Chloride/perchlorate selectivity thus increases by a factor of ∼70. There are a few anomalies, some of which could be due to experimental uncertainties, but overall the trend seems secure. Variation of selectivity with binding strength has also been observed for oligopyrrole receptors,[30] but this appears to be the first time that the trend has been revealed in a wide range of receptors and anions.

The behavior is consistent, in large part, with studies on the strength of hydrogen bonds,[31] which have led to empirical relationships of the form

$$\mathrm{Log}\, K = c_1 \alpha_2^{\mathrm{H}} \beta_2^{\mathrm{H}} + c_2 \qquad (7)$$

Equation 7 gives K, the 1:1 association constant mediated by a hydrogen bond, in terms of a parameter α_2^{H} which represents H-bond donor strength, a parameter β_2^{H} representing H-bond acceptor strength, and constants c_1 and c_2 which depend on the solvent. For two anions X and Y, it follows that

$$\mathrm{Log}\left(\frac{K_{\mathrm{X}}}{K_{\mathrm{Y}}}\right) = \mathrm{Log}\, K_{\mathrm{X}} - \mathrm{Log}\, K_{\mathrm{Y}} = c_1 \alpha_2^{\mathrm{H}} (\beta_{2\,\mathrm{X}}^{\mathrm{H}} - \beta_{2\,\mathrm{Y}}^{\mathrm{H}}) \qquad (8)$$

For a series of H-bond donors of different α_2^{H}, binding X and Y, the terms c_1, $\beta_{2\,\mathrm{X}}^{\mathrm{H}}$, and $\beta_{2\,\mathrm{Y}}^{\mathrm{H}}$ are constant. $\mathrm{Log}(K_{\mathrm{X}}/K_{\mathrm{Y}})$

(25) Fielding, L. *Tetrahedron* **2000**, *56*, 6151.
(26) Alper, J. S.; Gelb, R. I.; Laufer, D. A.; Schwartz, L. M. *Anal. Chim. Acta* **1989**, *220*, 171. Whitlock, B. J.; Whitlock, H. W. *J. Am. Chem. Soc.* **1990**, *112*, 3910. Jansen, R. J.; de Gelder, R.; Rowan, A. E.; Scheeren, H. W.; Nolte, R. J. M. *J. Org. Chem.* **2001**, *66*, 2643.
(27) Boss, R. D.; Popov, A. I. *Inorg. Chem.* **1985**, *24*, 3660. Wang, Z. X. *FEBS Lett.* **1995**, *360*, 111. See also: Wilcox, C. S.; Adrian, J. C., Jr.; Webb, T. H.; Zawacki, F. J. *J. Am. Chem. Soc.* **1992**, *114*, 10189.
(28) De Jong, F.; Reinhoudt, D. N.; Smit, C. J. *Tetrahedron Lett.* **1976**, 1375. De Boer, J. A. A.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1985**, *107*, 5347.
(29) Cram, D. J. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 1039.

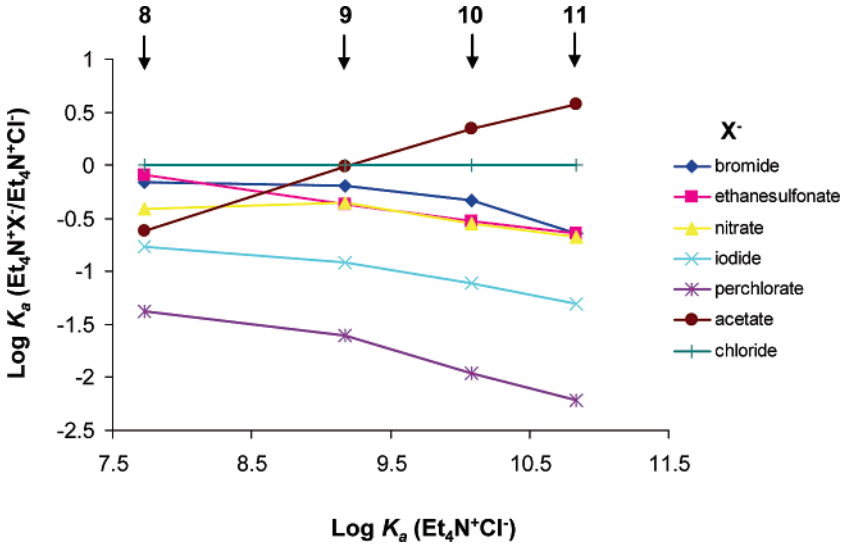


Figure 1. Log $K_a(Et_4N^+X^-)/K_a(Et_4N^+Cl^-)$ plotted vs Log $K_a(Et_4N^+Cl^-)^-$ for receptors **8**−**11**. Data from Table 3.

therefore increases linearly with α_2^H. Alternatively, from eq 7, α_2^H can be expressed in terms of K and β_2^H for one of the anions (e.g. Y):

$$\alpha_2^H = \frac{\text{Log } K_Y - c_2}{c_1 \beta_{2Y}^H} \qquad (9)$$

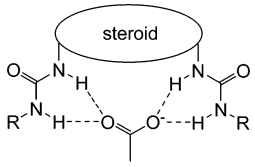
Therefore,

$$\text{Log}\left(\frac{K_X}{K_Y}\right) = \frac{(\beta_{2X}^H - \beta_{2Y}^H)(\text{Log } K_Y - c_2)}{\beta_{2Y}^H} \qquad (10)$$

Equation 10 predicts that a plot of Log(K_X/K_Y) vs Log K_Y should be linear, with slope and intercept dependent on ($\beta_{2X}^H - \beta_{2Y}^H$), i.e., the difference in H-bond acceptor strength between the two anionic substrates. For the structurally similar receptors **8**−**11**,[32] the corresponding plots are shown in Figure 1 (Y = Cl^-). The traces are indeed roughly linear, with slopes which reflect the "stickiness" of each anion. The intercept for the acetate plot is anomalous; eq 10 predicts that lines for different anions should diverge without crossing.[33] However, the above argument is developed for a single H-bond donor, effectively an "unstructured" receptor, and the situation with cholapods will certainly be more complex.

Equations 8 and 10 express an "affinity−selectivity principle" which should apply, at least, to all molecular recognition based on hydrogen bonding. Put simply, if a receptor discriminates between guests primarily through intrinsic differences in H-bond strengths, a more powerful receptor will exhibit a wider range of selectivity ratios. This occurs because, as binding free energies increase, so do differences between them. For example, the plots in Figure 1 show that the spread in selectivities for receptor **11** is substantially wider than the spread for the weaker binding receptor **8**. The source of the effect is the form of eq 7, wherein the parameters for donor and acceptor are *multiplied* in calculating the binding free energy. Similar equations should pertain wherever interactions are largely electrostatic in nature. If electrostatics control most aspects of molecular recognition, as argued by some workers,[31b,34] the principle could be quite general.

The importance of receptor strength tends to obscure our original target, the role played by binding site geometry. Nonetheless, it is clear that geometric factors can be very significant. The most obvious example is the variation in acetate/chloride selectivities. It is useful to compare receptors **4**, **5**, **6**, and **8**, all with quite similar affinities for chloride [$K_a(Et_4N^+Cl^-) = 5.3 \times 10^7 - 5.2 \times 10^8\ M^{-1}$]. For **5** and **8**, $K_a(Et_4N^+AcO^-)/K_a(Et_4N^+Cl^-) \approx 0.2$, while for **4** and **6** the corresponding values are close to 10. *Acetate/chloride selectivity is thus changed by a factor of ~50.* The key difference between these structures is that **5** and **8** possess ureas on both C7 and C12, a motif which seems to disfavor acetate binding (relative to chloride). This result is difficult to explain; the 7,12-bis-urea motif would seem well-organized for complexing AcO^- as in **20**.[35] However, it does confirm that adjusting the array of H-bond donors can produce dramatic changes in selectivity, even in non-macrocyclic architectures.



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Conclusions

Cholapod anion receptors are capable of high affinities while retaining compatibility with nonpolar organic media. In the present work we have explored the scope of the architecture and extended binding studies to a range of seven monovalent

(30) Anzenbacher, P.; Try, A. C.; Miyaji, H.; Jursikova, K.; Lynch, V. M.; Marquez, M.; Sessler, J. L. *J. Am. Chem. Soc.* **2000**, *122*, 10268. For a related theoretical study, see: Blas, J. R.; Marquez, M.; Sessler, J. L.; Luque, F. J.; Orozco, M. *J. Am. Chem. Soc.* **2002**, *124*, 12796.

(31) (a) Abraham, M. H.; Platts, J. A. *J. Org. Chem.* **2001**, *66*, 3484. (b) Hunter, C. A. *Angew. Chem., Int. Ed.* **2004**, *43*, 5310.

(32) Values for receptor **12** are offset from these plots, possibly due to geometric and/or chemical differences between ureas and thioureas.

(33) The constant c_2 is negative (see ref 31b), so the intercept is positive when X is more strongly bound than Y ($\beta_{2X}^H > \beta_{2Y}^H$), and vice versa.

(34) Hunter, C. A.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1990**, *112*, 5525.

(35) This conclusion is supported by modeling (Macromodel 7.1, MMFFs force field, chloroform solvation). Acetate is found to make four H-bonds of roughly equal length to **5**, without causing obvious distortion.

anions. Among the 13 receptors studied, the structures include between three and six H-bond donors sited in urea, thiourea, sulfonamide, carbamate, trifluoroacetamide, and isophthalamide binding units. The substrates are representative of most common anion geometries. The method used to obtain binding constants, extraction of Et_4N^+ salts from water into chloroform, is not without disadvantages. Errors are increased by the need to measure K_d, and the binding model is assumed rather than tested in each case. Against this, the quantity measured (extraction into a nonpolar medium) is directly relevant to the main potential application (transport across a nonpolar barrier). Moreover, the method is rapid and straightforward, and it possesses an unusual dynamic range. It has thus been possible to obtain values for 87 substrate−receptor combinations, in a single solvent system, ranging from 10^4 to 10^{11} M^{-1}.

The results allow three conclusions to be drawn. First, high binding constants ($K_a > 10^{10}$ M^{-1}) are achievable with several types of cholapods, especially those bearing five or six H-bond donors. Second, the arrangement of H-bond donors does indeed affect the selectivity, quite dramatically in some cases (although the rationalization of these effects may not be straightforward). *Third, and most interestingly, selectivities are quite strongly affected by the inherent binding strength of the receptor.* As affinities rise, the spread of binding constants also increases, so that selectivities for the more strongly bound guests tend to increase. Though unanticipated, the effect is consistent with physical-organic studies on hydrogen bonding and can be generalized as the "affinity−selectivity principle". As far as we know, this principle has not previously been articulated, possibly for lack of wide-ranging, comparable sets of data. The cholapods, allied with the extraction method, have now provided such a dataset. Meanwhile, this study has further demonstrated the potential of cholapods as powerful, selective, and lipophilic anion receptors.

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Supporting Information Available: Synthetic procedures for receptors **6**−**10** and **13**−**15**, experimental details for the measurements of binding constants, and mathematical analysis for the NMR competition titrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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