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Coordination Chemistry Reviews 250 (2006) 2939–2951
COORDINATION CHEMISTRY REVIEWS
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Review

Anion binding and transport by steroid-based receptors

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Received 27 March 2006; accepted 18 May 2006
Available online 27 May 2006

Contents

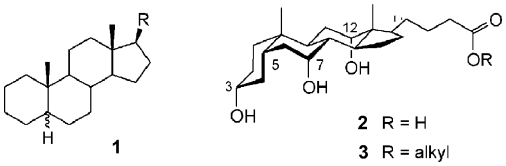
Abstract

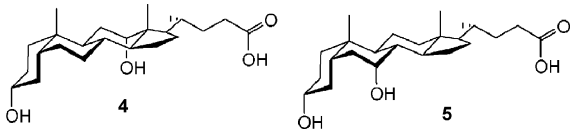
The steroid nucleus is well-established as a scaffold for anion receptors. The bile acids are especially useful, providing inexpensive starting points with helpful substitution patterns. This article describes developments since an earlier review in 2003. Included are podand and cyclic structures, uncharged and positive receptors, and various arrays of H-bond donor and other binding functionality. Applications have been found in anion sensing, selective extractions, transport across bilayer membranes, and the discovery of antibiotics.

Keywords: Anion recognition; Anion transport; Supramolecular chemistry; Steroids; Cholic acid; Hydrogen bonding

1. Introduction

The steroid nucleus **1** has been quite widely adopted as a building block for supramolecular chemistry [1]. It is large and rigid, and thus suitable for creating extended architectures with well-defined conformations. It is capable of a large range of substitution patterns, arising from both regio- and stereo-chemical variation, and is thus well adapted to serve as a scaffold for functional group arrays. It is readily accessible in several forms from natural sources, and these starting materials can generally be modified with good control, aided by the asymmetry of the structure and the long history of steroidal synthetic chemistry. Finally it is chiral, and therefore capable in principle of enantiodiscrimination.





Of the available steroidal starting materials, the most versatile are the bile acids, such as cholic acid (**2**), deoxycholic acid (**4**)

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doi:10.1016/j.ccr.2006.05.008

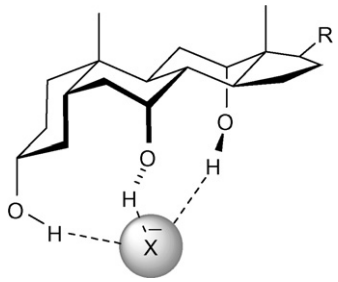
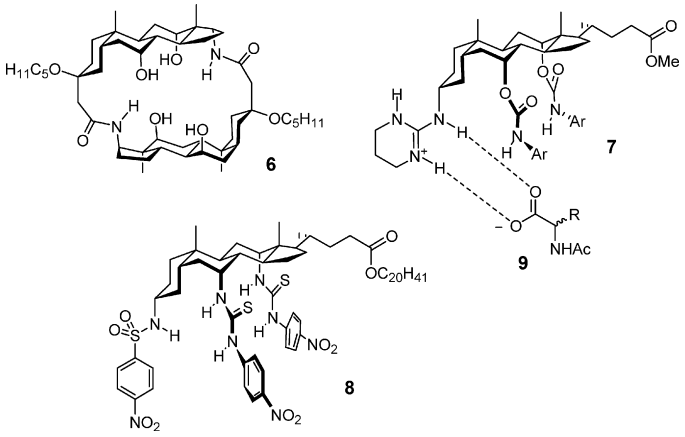


Fig. 1. Anion binding by cholic acid derivatives. H-bond donor strength can be tuned (e.g. OH changed to NHZ or NHCONHR) to generate more powerful receptors.

and chenodeoxycholic acid (**5**) [1a,e]. Obtained from the digestive systems of vertebrate organisms, these polyhydroxylated acids display useful levels of functionalisation distributed fairly evenly around the steroidal nucleus. Most are 5β-steroids (hence possessing *cis*-AB ring junctions), with α-directed hydroxyl groups. Their profiles are thus curved, with concave, functionalised α surfaces. This architecture is generally suitable for the binding of polar species, and especially so for binding anions. The spacing between the secondary hydroxyl groups in **2** is such that they can serve as H-bond donors to anionic substrates (Fig. 1) but cannot form (competing) intramolecular H-bonds. Thus cholate esters **3** can themselves act as anion receptors, although quite weak ones [2]. More significantly, **2** and other bile acids may be elaborated into structures with more powerful binding functionality (both charged and uncharged), and with more complex architectures (both podand and macrocyclic). Examples are the macrocycle **6** [3], and "cholapods"[1] **7** [4] and **8** [5]. These compounds can have interesting and sometimes impressive anion-binding properties. Macrocycle **6** encapsulates halide anions in $CDCl_3$, while cations **7** discriminate between enantiomers of carboxylates **9**. Receptor **8** binds chloride anion in chloroform with the exceptional K_a of 10^{11} M^{-1}, far higher than any published figure for a non-steroidal system.



These and other systems were discussed in an earlier review, published in this Journal in 2003 [6]. Since then a number of new designs have been reported, extending the scope and refining the binding properties of steroid-based anion receptors. One purpose of the present article is to summarise this work (see Section 2). Perhaps more importantly, there have been major developments in the use of steroid-based systems for anion transport. The steroid nucleus is not the only framework which can be used to organise arrays of H-bond donors, but it is exceptional in its compatibility with non-polar environments. Indeed the outstanding affinities displayed by **8** and its relatives arise partly from their solubility in chloroform—it is likely that other systems possess similar intrinsic binding power, but cannot be studied in such apolar (i.e. uncompetitive) media. Steroidal systems are thus especially suitable for "phase-transfer" applications, including transport across biological membranes. Anion transport had previously been demonstrated for positively charged steroids, and there have been further variations on this theme. More exceptionally, it has been shown that *electroneutral* cholapods can act as anion carriers, the first organic counterparts to well-known cation carriers such as Valinomycin. These steroid-based anion transporters are discussed in Section 3 of the article.

2. Anion recognition by steroid-based receptors

As discussed above, the steroid nucleus provides an excellent scaffold for organising arrays of anion-binding functionality. Considering the lipophilic nature of the steroid skeleton, it is not surprising that derived anion receptors have mostly been designed for operation in non-polar solvents. They have thus tended to exploit electroneutral binding functionality, mainly H-bond donors. Accordingly, the majority have possessed no net charge, and can therefore act either as receptors for salts (typically $R_4N^+X^-$) in bulk solution, or for anions at interfaces (e.g. in ion selective electrodes). Section 2.1 describes progress in the application of these neutral H-bond donor systems to simple achiral substrates. As an alternative to H-bond donors, it is possible to use electron deficient carbonyl groups, which can bind through reversible formation of covalent bonds. This interesting development is outlined in Section 2.2. A new type of system possessing net positive charge, and therefore capable of anion exchange, is discussed in Section 2.3. Lastly the enantioselective recognition of anions by steroidal receptors is updated in Section 2.4.

2.1. Electroneutral receptors for inorganic and simple organic anions; arrays of H-bond donors

Most new work has involved cholapods, exemplified by **8**. This architecture has several advantages. Firstly, a number of bile acids are readily available to serve as starting materials. Cholic acid (**2**) is especially useful, being the second least expensive steroid (after cholesterol) and possessing three functionalised positions. Secondly, as already illustrated for **2** (Fig. 1), the functional groups in the common bile acids are conveniently positioned for the creation of binding sites. Thirdly, the nature of the binding sites is readily varied. It is easy to introduce a range of "legs", containing different numbers and arrangements of H-bond donor groups. Notably, each leg can be terminated by -NHZ, a "tuneable" H-bond donor. By varying the electron-

[1] The term "cholapod", first introduced in Ref. [5], is used to denote podand-type receptors based on the bile acid (cholanoyl) skeleton.

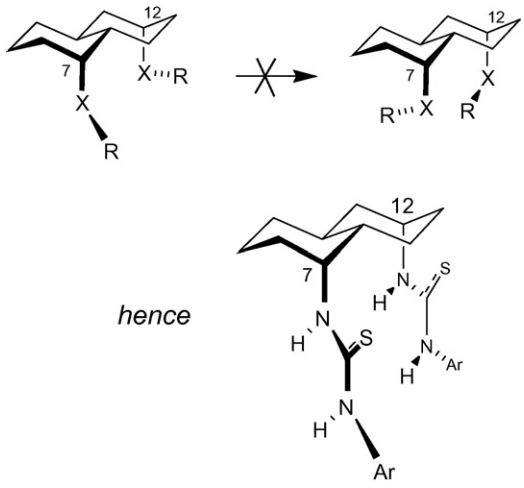


Fig. 2. Conformational constraints imposed on axial 7α and 12α groups, and the resulting dispositions of the thiourea groups in **8**. X = O or NH, R = any group other than H.

withdrawing ability of Z the acidity of NH can be altered, and thus the H-bond donor strength. Finally the options for intramolecular H-bond formation are limited in most cholapod designs. This is especially important for systems relying on neutral H-bond donors, because most such groups (amides, hydroxyls, sulfonamides, etc.) are also H-bond acceptors. A key feature is the axial disposition of the steroidal 7α and 12α positions. As illustrated in Fig. 2 a typical substituent XR, in either of these positions, is conformationally constrained by potential 1,3-diaxial interactions. Groups R are forced to diverge and are thus kept separate. Moreover, for X = NH, the hydrogen is inward-directed and available for anion binding. Thus, for example, the 7α and 12α thiourea groups in **8** are almost perfectly preorganised for anion recognition (Fig. 2). The same constraints do not apply to 3α substituents, such as the sulfonamide in **8**, and this does allow intramolecular H-bonds to form between donors at 7α and acceptors at 3α. However, in most cases these interactions seem to be strained and fairly weak.

The earlier work leading to **8** had focussed on optimising affinities, with chloride and bromide as standard substrates. More recently, attention has turned to selectivity. The potential variety of H-bond donor arrays suggested that cholapods could, in principle, be targeted at specific anion geometries. A study was performed on the series of cholapods shown in Fig. 3 [7,8]. These receptors possess between 3 and 6 H-bond donor groups, with seven different geometrical arrangements (**10/11**; **12**; **13**; **14**; **8/15–18**; **19/20**; **21**). Binding constants were measured to seven anions in $CHCl_3$, with Et_4N^+ as countercation, using a method based on Cram's extraction procedure [9]. Briefly, the receptor is dissolved in chloroform and shaken with an aqueous solution of $Et_4N^+X^-$ substrate. The amount of substrate extracted, combined with the partition coefficient in the absence of receptor, may be used to calculate the binding constant. The method is simple to operate and has an unusually high "dynamic range". Receptor saturation can be avoided by lowering the concentration of substrate in the aqueous phase. There is thus, in principle, no upper limit to the binding constants which can be measured.[2] Operation with multivalent anions is not straightforward, however, so in this case only monovalent anions were employed.

The measurements provided an unusually large and comprehensive collection of data for a family of receptors, binding several anions, studied by a single technique. The results are shown in Table 1, presented as binding constants to chloride (first column, indicating the general strength of a receptor) and selectivities versus chloride (remaining columns). The first point to note is the range of affinities. Binding constants to chloride range from $\sim 10^4$ (**10**) to $\sim 10^{11}$ (**8** and **18**). Secondly, the selectivities vary considerably, but not according to the simple, geometry-determined pattern one might have expected. Indeed, some of the most dramatic differences involve receptors with the *same* arrangement of H-bond donors. For example, in the series **8/15–18**, the acetate/chloride ratio varies between 0.24 and 3.8, while the perchlorate/chloride ratio changes by a factor of ~70. In general, there is a good correlation between binding *strength* of a receptor, and its *selectivity for strongly bound anions*; in other words, the spread of numbers is much greater for **8** or **18** than for **15**. Although unexpected, this result should perhaps have been predicted. Empirical equations for hydrogen bond strengths are well established [10], taking the form:

$$\Delta G^\circ_{\text{binding}} = c_1\alpha\beta + c_2$$

where α and β are parameters representing the strength of the H-bond donor and acceptor, while c_1 and c_2 are constants. Moving to a stronger H-bond donor (increasing α) has a multiplicative effect on all binding energies to all substrates. Thus, *differences* between binding energies increase as well as absolute values. Differences between binding energies translate to selectivities (ratios of binding constants), so these also increase as the H-bond donors become stronger. This "affinity-selectivity principle" could be quite general, applying wherever H-bonding or electrostatic effects dominate recognition. It does not seem to have been noticed in practice, at least in the field of anion recognition, possibly because it requires a collection of similar receptors with widely differing affinities studied by a common technique. The cholapods, combined with Cram's extraction method, are the first system to provide such a dataset.

Although the affinity-selectivity effect clouds the role of receptor geometry, the arrangement of H-bond donors is certainly important. Thus, the set of cholapods with 4 H-bond donors (**12–14**) show similar affinities but quite widely differing selectivities (e.g. figures for acetate: **12** = 0.23, **13** = 11).

Electroneutral anion receptors are usually studied with salts as substrates, the positive counterions being non-coordinating R_4N^+ or R_4P^+. In some respects it is more interesting to measure binding constants to unpaired anions; such values are, for example, more relevant to the transport properties discussed in Section 3. Unpaired anions are not, of course, available in bulk, but can be studied at interfaces using electrochemical techniques.

[2] This is not true of most titration methods, where spectrometer sensitivity limits the range of systems which can be addressed. For further discussion see Ref. [7].

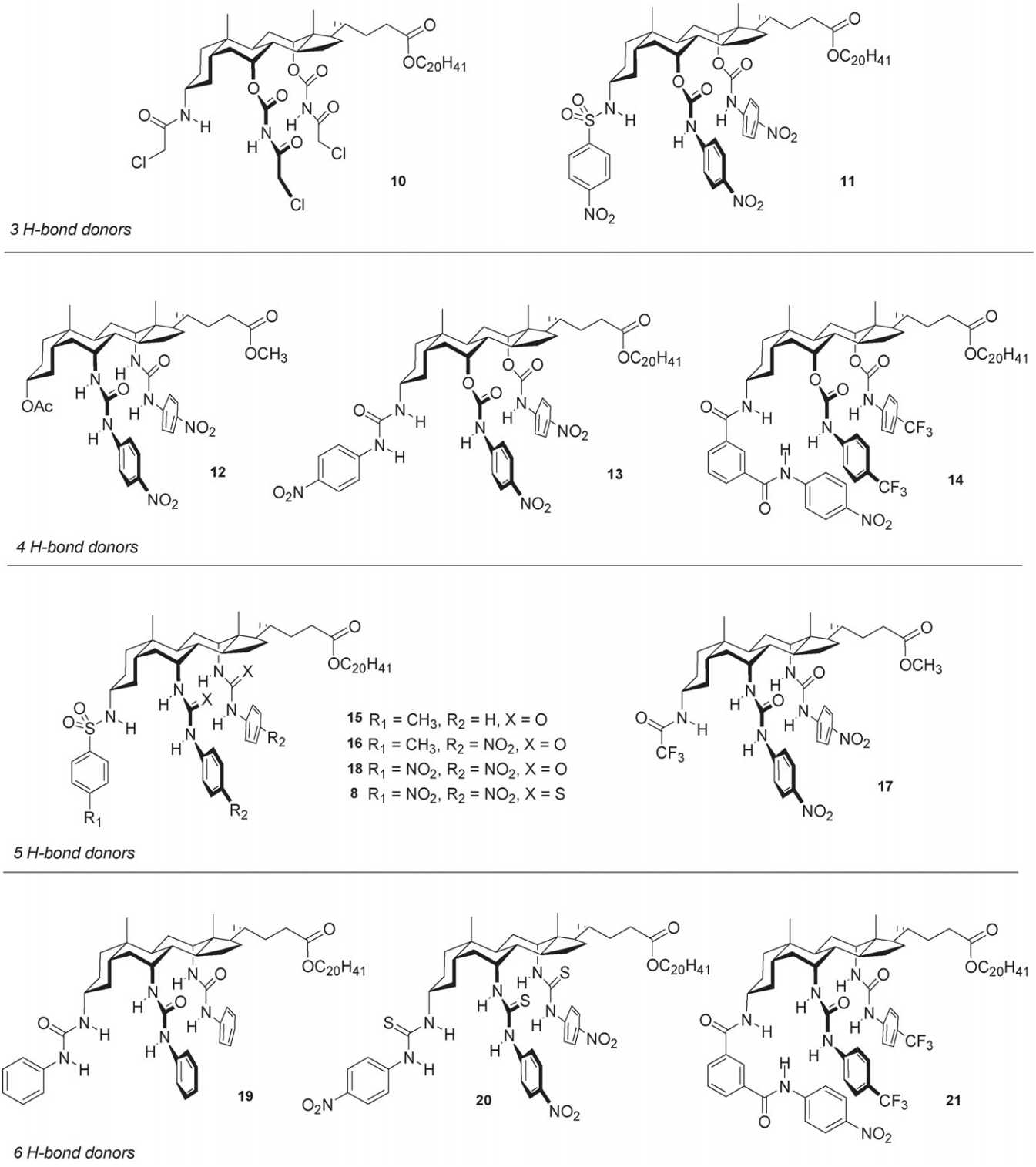


Fig. 3. The cholapod anion receptors studied in Ref. [7].

Cholapod **8** was investigated by voltammetry at the "interface between two immiscible electrolyte solutions" (ITIES), the solvents being 1,2-dichloroethane and water [11]. The binding constants to fluoride, chloride and bromide in the dichloroethane were found to be 10^{12}; 5×10^{12}; 2×10^{11} M^{-1}, respectively. Considering the change in solvent and omission of the counterion, these figures agree quite well with those obtained by the extraction method.

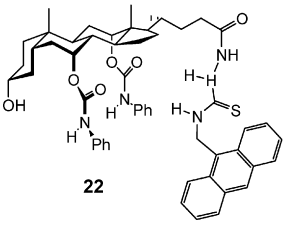
The group of Chan have used the cholapod architecture as the basis for fluorescent anion sensors. Anthracenyl thiourea units were introduced as photoinduced electron transfer (PET) reporters in the side chain or steroidal C3, as in **22**–**24**. Recep-

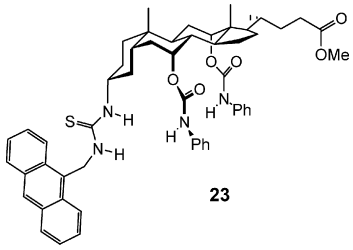
Table 1
Association constants for the binding of tetraethylammonium salts to receptors **8**, **10–21** in water-saturated chloroform, expressed relative to the values for $Et_4N^+Cl^-$

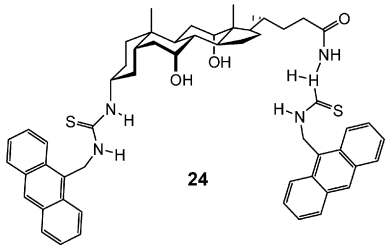
Receptor	K_a ($Et_4N^+Cl^-$) (M^{-1})	Selectivities (normalized to Cl^-)[a]						
		Cl^-	Br^-	I^-	NO_3^-	AcO^-	ClO_4^-	$EtSO_3^-$
Three H-bond donors								
10	1.6×10^4	1.0	0.51	[b]	2.9	5.8	[b]	0.45
11	1.1×10^8	1.0	0.36	0.13	0.72	9.7	0.081	0.13
Four H-bond donors								
12	5.2×10^8	1.0	0.11	0.020	0.32	0.23	0.0018	0.45
13	6.2×10^7	1.0	0.56	0.17	1.0	11	0.11	0.58
14	1.1×10^8	1.0	0.50	0.085	0.79	[b]	0.042	[b]
Five H-bond donors								
15	5.3×10^7	1.0	0.70	0.17	0.39	0.24	0.042	0.81
16	1.5×10^9	1.0	0.64	0.12	0.44	0.97	0.025	0.43
17	1.2×10^{10}	1.0	0.47	0.077	0.28	2.2	0.011	0.30
18	6.8×10^{10}	1.0	0.23	0.050	0.21	3.8	0.0061	0.23
8	1.1×10^{11}	1.0	0.26	0.018	0.079	1.9	0.00061	0.035
Six H-bond donors								
19	2.7×10^8	1.0	0.50	0.083	0.60	0.51	0.022	0.80
20	1.8×10^{11}	1.0	0.25	0.028	0.10	0.71	0.00055	0.19
21	1.5×10^{10}	1.0	0.56	0.034	0.58	5.4	0.0069	0.44

Gaps between rows indicate changes in receptor geometry.
[a] $K_a(Et_4N^+X^-)/K_a(Et_4N^+Cl^-)$.
[b] Not determined.

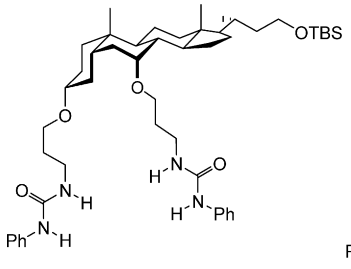
tors **22** and **23** were employed in CH_3CN, showing strongly decreased fluorescence in the presence of carboxylates and dihydrogen phosphate ($K_a = 10^3$ to $10^5\ M^{-1}$) [12]. Bis-thiourea **24** was employed as a receptor for dicarboxylates in an aqueous solvent system (MeOH–H_2O, 1:1). The decreases in fluorescence were rather low (~20%), but Job plots confirmed 1:1 stoichiometry, and the binding constants were high considering the polar solvent system. For example, a value of $6 \times 10^6\ M^{-1}$ was measured for L-glutamate [13].

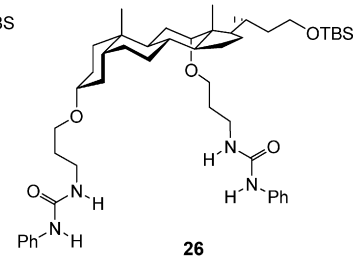


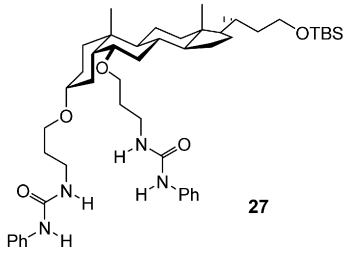




Kim and Kim have studied a number of cholapods with longer, more flexible legs. Receptor **25**, derived from chenodeoxycholic acid (**5**), bound a number of monovalent anions in $CDCl_3$ with K_a in the range 300–3000 M^{-1} [14]. This molecule was also employed in ion-selective electrodes which responded to most anions above 10^{-3} M but without showing unusual selectivity [15]. Receptors **26** (from deoxycholic acid (**4**)) and **27** (from hyodeoxycholic acid) were tested in the more competitive solvent DMSO [16]. As expected the binding constants were mostly lower (e.g. 90 M^{-1} for either receptor + TBA chloride), but **27** showed interesting selectivity for fluoride ($K_a = 15{,}000\ M^{-1}$).

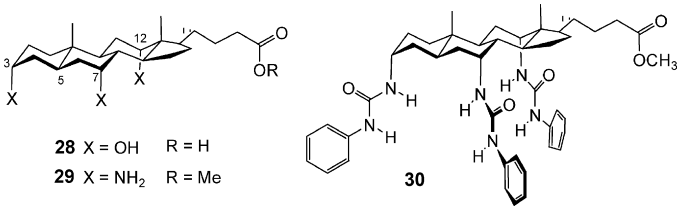




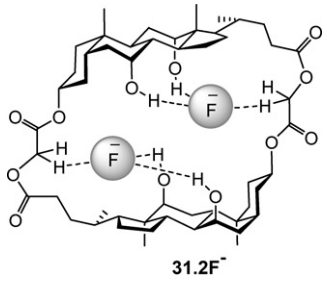


As discussed previously, the axial disposition of the 7α and 12α bonds is a key advantage of the cholapod architecture (cf.

Fig. 2). The 3α bond, however, is equatorial in receptors derived from the common bile acids. If the configuration at C5 could be inverted, to give an all-trans polycyclic structure, the 3α position would also become axial. Receptors derived from allocholic acid (**28**) would have even higher levels of preorganisation. This possibility has been explored very recently. Cholic acid (**2**) was converted to triamine **29** and thence to the tris-urea **30** [17]. The axial positioning of all three legs in **30** means that *no* intramolecular H-bonds are possible within this molecule. "Allocholapod" **30** was compared to its 5β-analogue **19**, for which weak H-bonding is predicted between the 3α urea C=O and the 7α NH groups. As expected the all-trans stereochemistry led to significant, though modest, increases in binding constants (e.g. factors of 3 and 5 for chloride and acetate, respectively).

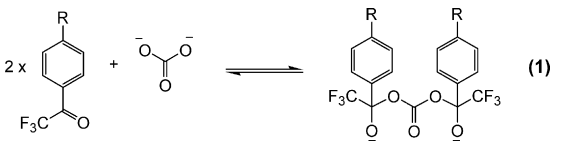


Finally, the possibility of using bile acids to create macrocyclic receptors (*cf.* **6**) has not been forgotten. The group of Maitra have prepared the cyclodimeric structure **31** in two steps from cholic acid (**2**), and have shown by NMR that it binds $Bu_4N^+F^-$ in $CDCl_3$ with 1:2 stoichiometry ($K_{a1} \approx 2000\,M^{-1}$, $K_{a2} \approx 250\,M^{-1}$) [18]. Complexation is thought to take place through formation of two OH···F^- and one CH···F^- hydrogen bonds, as shown. Direct evidence for the CH···F^- interaction is provided by ^1H NMR spectra, in which the chemical shift for one CH moves strongly downfield on titration with the substrate.



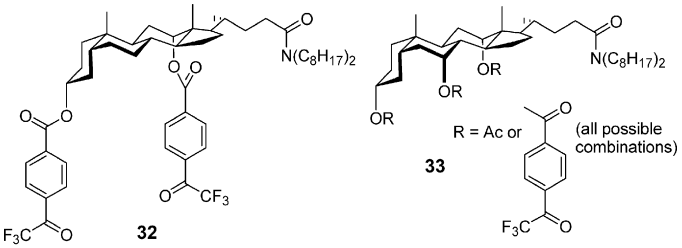
2.2. Anion recognition through covalent bond formation

Although supramolecular chemists are usually concerned with non-covalent bonding, the reversible formation of covalent bonds can be a practical alternative. In the case of anion recognition, it has long been known that carbonate-selective ion selective electrodes (ISEs) can be prepared using trifluoroacetylbenzene (TFAB) derivatives, thought to form adducts with carbonate as shown in Eq. (1) [19]:



(1)

Pyung and co-workers proposed that selectivity could be increased by mounting the trifluoroacetyl moieties on a bile acid scaffold, so that they would be appropriately positioned. They therefore converted deoxycholic acid (**4**) into bis-(trifluoroacetylbenzoate) (**32**), and tested its ability to extract carbonate from water into dichloromethane (Bu_4N^+ was present to serve as counterion) [20]. UV spectroscopy showed significant changes in the absorption of the aromatic unit, which did not occur for controls with single TFAB units. A further series of compounds **33** was prepared from cholic acid (**2**) [21]. Again the receptors with multiple TFAB units responded to carbonate, while the compounds with just one TFAB (and two acetates) showed no changes.



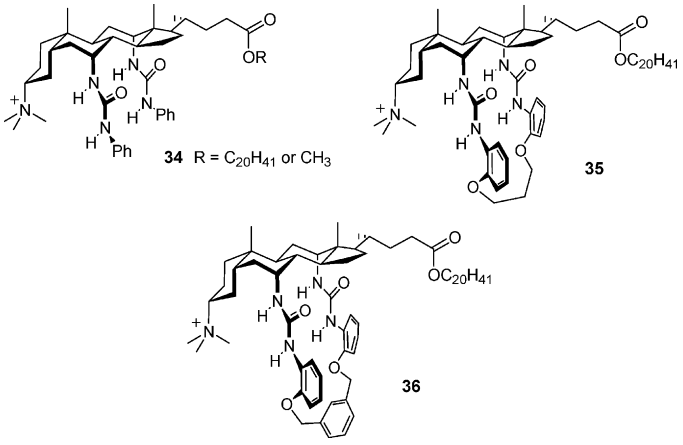
Receptors **32** and **33** were incorporated in ISEs by Nam and co-workers [22–24,15]. The compounds with 2 or 3 TFABs showed significantly greater selectivities for carbonate than those with just one of these units. The deoxycholic-based **33** was the most successful [23]. The derived electrode was used for the analysis of seawater, and shown to be accurate and convenient in this "real-life" application.

2.3. Positively charged cholapods; "smart phase transfer agents"

Although cholapods are best suited to non-polar solvents, this does not mean they must always be electrically neutral. We have recently introduced a new series of receptors in which the steroid framework is used to position both H-bond donors and a quaternary ammonium group. These structures may be related to the simple, lipophilic quaternary ammonium cations which are commonly used as phase transfer agents. Essentially, we have added structured binding sites to generate analogues with more sophisticated properties ("smart phase transfer agents").

Our prototype was cholapod **34**, in which an NMe_3^+ unit is combined with the well-established 7α,12α bis-ureido binding site [25]. Although this receptor is probably quite powerful, we felt that its most interesting property would be its selectivity. Quaternary ammonium salts are generally capable of extracting anions from water into non-polar media, with selectivities determined by lipophilicity as expressed in the classic Hofmeister series (typically $I^- > Br^- \approx NO_3^- > Cl^- > AcO^- > SO_4^- > PO_4^-$) [26]. Modelling suggested that receptors **34** would provide an almost ideal binding site for chloride or bromide, so that "contra-Hofmeister" extractions in favour of these anions might be possible. The extraction preferences of **34**(R = eicosyl) were easily measured in anion exchange experiments, and compared to tetraoctylammonium (TOA) as a simple quaternary ammonium control.

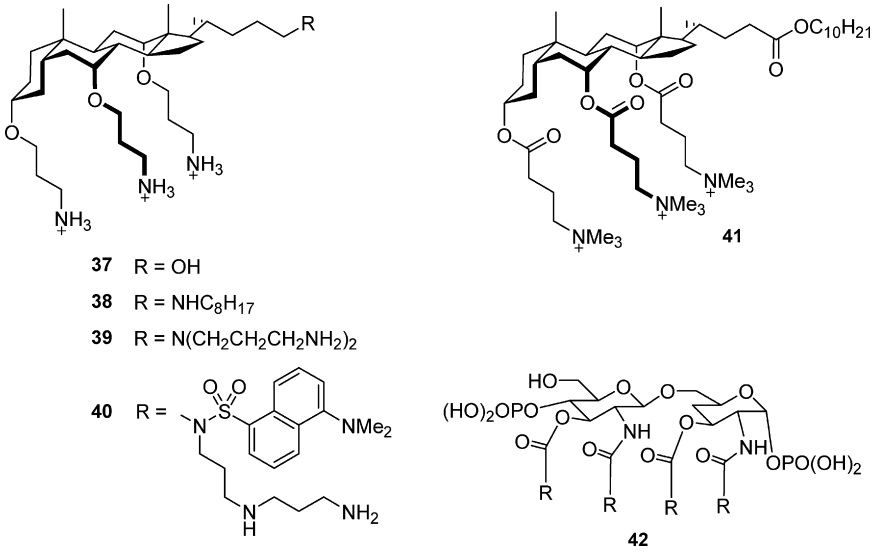
The results showed a substantial *attenuation* of the Hofmeister effect (i.e. quantitatively lower preferences for extraction of lipophilic anions), but little *reordering*. An X-ray crystal structure of **34**(R = Me)·$MeSO_3^-$ (Fig. 4) suggested an explanation. Although the binding site is not designed for methanesulfonate, movement of the "legs" allows all four NH groups to form strong hydrogen bonds.



Accordingly, we designed the cyclic analogues **35** and **36**, in which movement of the legs was restricted and binding sites were more enclosed [27]. The three-carbon bridge in **35** draws the legs together, potentially favouring smaller anions, while the five-carbon link **36** essentially "freezes" the ureas in the same positions as for **34**. Receptor **36** was thus expected to show much improved selectivities for chloride and bromide. Results from anion exchange experiments, along with those from **34** and TOA, are summarised in Tables 2 and 3. Table 2 shows the raw data, while in Table 3 these figures are manipulated to reveal more clearly the underlying preferences of the receptors.

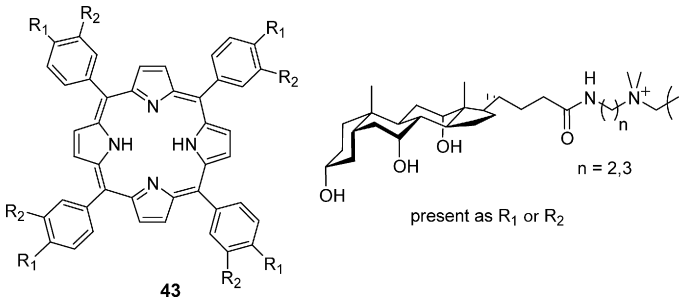
As mentioned above, receptor **34** attenuates the effect of anion lipophilicity (small spread of numbers in Table 2, relative to TOA), but does not strongly favour a particular substrate geometry. In contrast, receptor **36** is remarkably selective for chloride and bromide. Meanwhile the smaller cavity of **35** shows a distinct preference for acetate. The cyclisation strategy has thus proved highly effective for controlling selectivity in cationic cholapods, and may prove equally useful in the electroneutral series.

Cholic acid has also been transformed into polycationic cholapods, such as **37**–**41**. These molecules are cationic facial amphiphiles, with geometries reminiscent of certain cationic peptide antibiotics such as Polymyxin B (PMB) [28]. They do indeed possess antibacterial activity, associated with their ability to bind to and disrupt bacterial membranes. Steroids **37**–**39** were studied by Savage and co-workers [28]. Compound **37** was inactive against Gram-negative bacteria but, interestingly, was able to promote the activity of other antibiotics. Presumably it is able to bind to the outer cell wall, permeabilise it to some extent, but not pass through. The more lipophilic **38** was directly effective against Gram-negative organisms, probably because it can penetrate the outer cell wall. Both **37** and **38** also showed some haemolytic activity, indicating toxicity towards human cells. In contrast, the more cationic **39** retained antibacterial activity but did not lyse blood cells, up to quite high concentrations. The selectivity may be due to the charge differences between prokaryotic and eukaryotic cells, the former being more anionic. However later work employing fluorescent analogues such as **40** suggested that the cationic cholapods may specifically bind Lipid A units **42**, key anionic components of Gram-negative outer membranes [29]. In either case, the success of these compounds seems to be directly related to their anion-binding properties. Unlike PMB the cholapods **37**–**39** are also active against Gram-positive organisms, possibly through a second mechanism of action. The tris-quaternary ammonium cholapod **41** has been studied in less detail, but is also effective against both Gram-negative and Gram-positive bacteria [30].



Kolehmainen et al. have also described a family of positively charged, steroid-based receptors [31]. Their system **43** involves a porphyrin with four appendages derived from cholic acid, each bearing a positive charge. These molecules were studied in water–methanol mixtures, showing no interaction with inorganic

anions but binding nucleotides quite strongly ($K_a \approx 10^6\ M^{-1}$ for AMP and ATP). Presumably hydrophobic interactions were important in these cases.



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2.4. Enantioselective recognition by cholapods

Steroid-based receptors have obvious potential for enantioselective recognition. In earlier work it was demonstrated that guanidinium-substituted cholapods **7** were capable of enantioselective extraction of *N*-acyl amino acids [4], and also of enantioselective transport across bulk liquid membranes [32]. Ureas can serve as neutral surrogates of guanidinium groups, and are often more convenient to synthesize and handle. Accordingly, the bis-carbamoylurea **44** was prepared as an analogue of **7**, and also the regioisomeric **45** and **46** [33]. The receptors were applied to the extraction of "pseudoracemates" of *N*-acetylphenylalanine, **47** + **48**, with tetraethylammonium counterions. Isotope-labelling of **47** allowed measurement of enantioselectivities by LCMS. All three compounds showed the same stereochemical preference as guanidinium cations **7** preferring the L-enantiomer **47**. Selectivities were ~5:1, 4:1 and 3:1 for **44**, **45** and **46**, respectively. This level of enantiodiscrimination is slightly lower than for **7**,[3] but is still encouraging. Elaboration and variation of **44**–**46** should be quite straightforward, and it is likely that the resulting libraries would contain receptors with far higher selectivities.

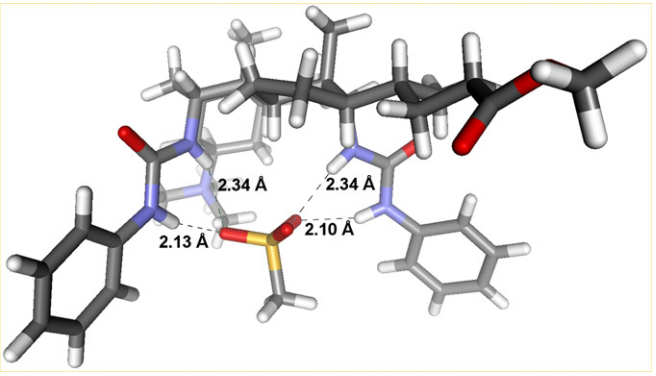


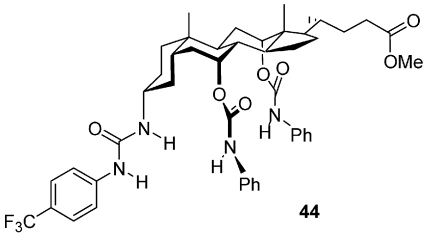
Fig. 4. X-ray crystal structure of **34**(R = Me)·$MeSO_3^-$, showing NH···O hydrogen bonding distances.

Table 2
Equilibrium constants *K* for quaternary ammonium-mediated anion exchange between water and chloroform, with $EtSO_3^-$ as reference anion[a]

	TOA	**34**	**35**	**36**
AcO^-	0.012	0.047	2.6	1.1
$EtSO_3^-$	1	1	1	1
Cl^-	1.2	4.9	19	920
Br^-	22	24	19	3200
NO_3^-	31	6.0	68	250
I^-	1600	98	81	970
PF_6^-	7700	13	120	94

TOA: tetraoctylammonium.
[a] Receptor in chloroform was shaken with an aqueous solution of $NaEtSO_3$ and NaX. The equilibrium position was determined by ^1H NMR integration ($EtSO_3^-$ vs. receptor). Values for $EtSO_3^-$ are 1 by definition.

The binding properties of **7** have been exploited by theoretical chemists interested in predicting enantioselectivities. The system was used as a test for the MINTA (mode integration approach) method for calculating binding free energies [34]. The computations were able to reproduce the ordering of selectivities from a series of the receptors, although the absolute values were



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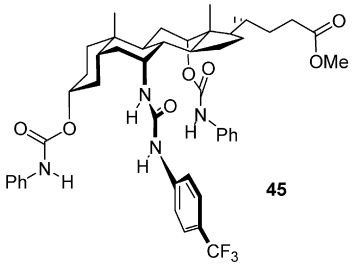

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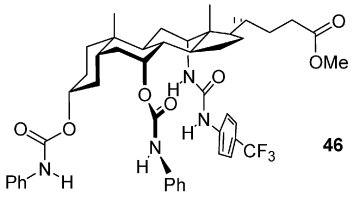

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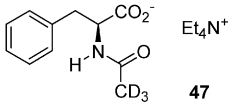

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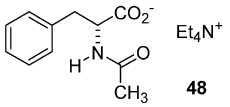

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[3] For example, 9:1 for **5** (Ar = C_6H_4-*p*-CF_3); see Ref. [4b].

Table 3
Intrinsic substrate preferences for **34**–**36**, as represented by $K_{receptor}/K_{TOA}$, normalized to PF_6^-[a]

	TOA[a]	34	35	36
AcO^-	1	2300	14000	7500
$EtSO_3^-$	1	590	65	81
Cl^-	1	2500	1000	66000
Br^-	1	640	56	12000
NO_3^-	1	110	140	670
I^-	1	37	3.4	52
PF_6^-	1	1	1	1

[a] For each receptor-anion combination, K (Table 1) is divided by the corresponding figure for TOA-anion. For each receptor, all ratios are then divided by that for PF_6^-. All values involving TOA and PF_6^- are therefore 1 by definition.

mostly exaggerated. Finally, several groups have appreciated the potential of bile acid-based recognition units in chiral chromatography [35]. The carboxyl-terminated side chain provides a straightforward means of attachment to the stationary phase, and even simple, symmetrically functionalised derivatives possess chiral, polar binding sites. A variety of phases have been produced and tested, mostly against neutral polar molecules but occasionally with anionic substrates [35c].

3. Anion transport by steroid-based systems

3.1. Electroneutral anion carriers

Simple inorganic ions such as Na^+ or Cl^- interact well with water but very poorly with non-polar media. An apolar environment, such as a non-polar organic solvent or the interior of a cell membrane, can thus act as a barrier to ionic species. However, ions can be transported across such barriers by agents which are themselves compatible with the apolar medium, and can interact with their substrates to facilitate passage. Ion transport is especially relevant to biology. The control of ion traffic across biological membranes is vital for living organisms, and is achieved by a variety of protein transporters and channels. Interference with these systems can lead to profound biological effects. There are, for example, many naturally derived antibiotics which function by promoting ion transport and destroying concentration differences. It is intriguing, however, that almost all these molecules act on *cation*s. There are few natural products capable of anion transport, and their activities are not fully understood [36]. There is thus much interest in synthetic anion transporters, which could serve as tools for the biosciences and might show novel and useful biological effects. One possibility is that chloride transporters could be used to treat patients with cystic fibrosis (CF), and related genetic diseases [37]. These conditions are due to defective chloride channel proteins, whose activities could in principle be replaced by synthetic systems.

A number of anion transport mechanisms can be distinguished, illustrated in Fig. 5. For transport through bulk organic solvents, the species which passes through the non-polar phase must be electrically neutral. This can be achieved by placing a positive charge in the receptor, (Fig. 5, process (a)), placing a lipophilic counterion in the organic phase (process (b)), or

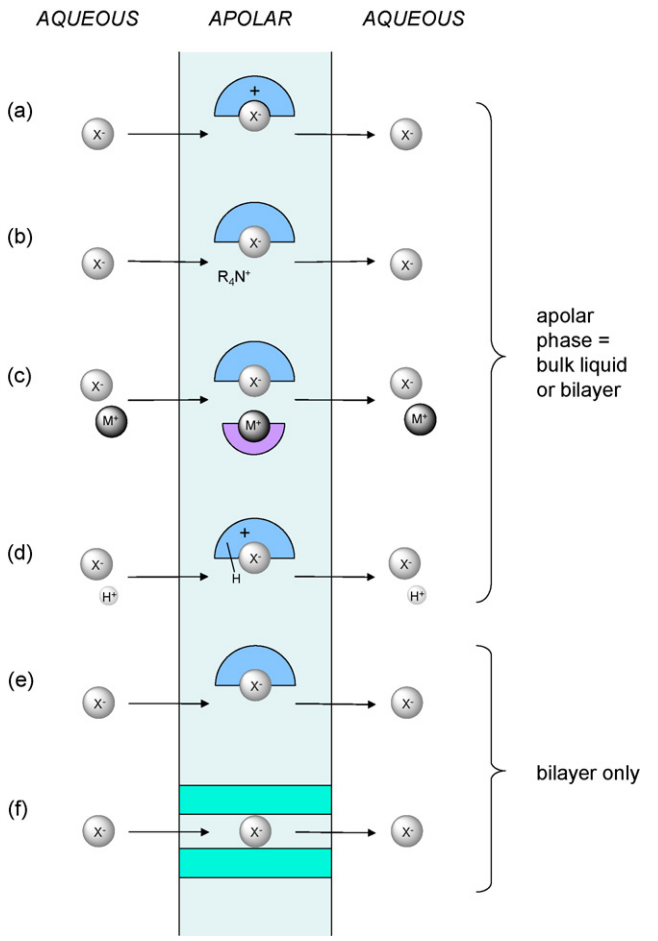


Fig. 5. Modes for anion transport across apolar barriers. (a) Transport by positively charged receptor. (b) Transport by neutral receptor, in presence of lipophilic cation. (c) Cotransport of anion and cation, employing complementary receptors. (d) Cotransport of anion and proton, employing basic anion receptor. (e) Transport by electroneutral receptor. (f) Transport through channel.

cotransporting counterions (process (c) or (d)). For transport across the thin layer of hydrocarbon in a biological membrane (~30 Å) two further mechanisms are possible; transport by a neutral carrier (process (e)), or a synthetic channel (process (f)). In these cases the species making the crossing is charged, but this is feasible because of the narrowness of the barrier.

With a view to treating cystic fibrosis, we were interested in realising process (e). This mode of transport has a number of advantages. Firstly, unlike (c) or (d) it mimics the overall effect of the channels missing from CF patients. Secondly, unlike (a) or (b), it does not involve lipophilic cations, which are often toxic. Thirdly, while synthetic channels (as in (f)) might offer higher transport rates, we felt that a carrier might be easier to understand and optimise. In addition, process (e) presented an intriguing challenge. Anion transport by positively charged species, as in (a)–(d), is not especially difficult; if a cation is mobilised in an organic phase, an anion of some type is virtually forced to join it. In contrast, an electroneutral carrier must work to *separate* anion and cation, as well as extracting the anion from water. Though difficult, this should not be impossible; cation transporters such

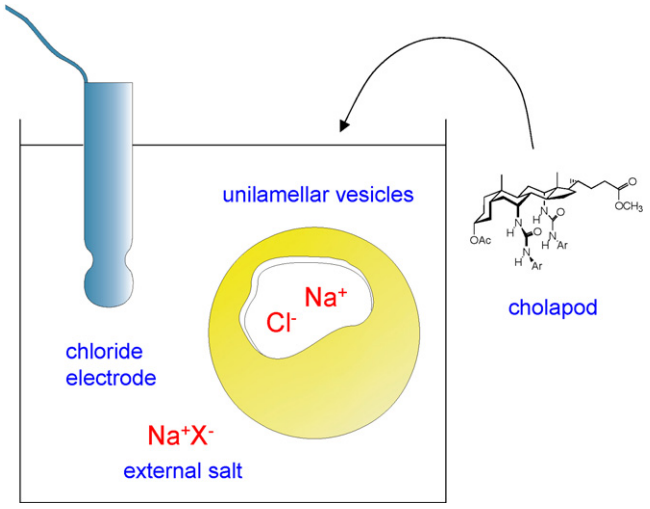


Fig. 6. Testing bis-ureidocholapods **49–52** and **12** for anion transport. NaCl aq. is encapsulated in vesicles, which are suspended in a solution of a second salt (NaX). Transport is initiated by addition of the cholapod, and detected using a chloride-selective electrode.

as Valinomycin were known to operate via polarity-inversion of process (e).

As described in earlier sections, the electroneutral cholapods show very high affinities for anions while maintaining compatibility with non-polar organic solvents. They thus seemed good candidates for "(e)-type" transporters. In collaboration with the group of B.D. Smith, the bis-ureas **49–52** and **12** were tested using the protocol illustrated in Fig. 6 [38]. The cholapods did prove to be effective transporters. Importantly, their activities depended strongly on the external anion X^-. For $X = NO_3$, transport proceeded rapidly, for $X = HCO_3$ lower rates were observed, and for external sulfate transport was blocked completely. These results showed that transport was "electroactive"; anions were crossing the membrane without accompanying cations, causing a net flow of charge and a change in potential. Without back-transport of a different anion, a potential gradient was building up and preventing further movement. Clearly nitrate could be transported by the receptor, but sulfate could not. Processes (c) and (d) were thus excluded, leaving only (e) and (f). Further experiments favoured (e) over channel formation (f). For example, the dependence of transport rates with receptor concentration was less than linear, the opposite of that expected for self-assembled channels (single molecules of the cholapods are too small to form channels by themselves). Also transport was halted by cooling the vesicles below their gel-phase transition temperature, suggesting that receptor mobility was necessary. Interestingly, evidence from crystallography suggests that some cholapods *can* form channels [39]. However, the pores are exceptionally wide (up to 14 Å), unlikely to be anion-selective, and probably unrelated to the anion transport properties.

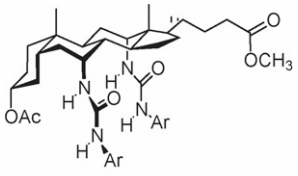
Transport rates correlated well with binding constants, as shown in Table 4. The most powerful receptor **12** was also the best transporter, achieving equilibrium across the vesicle membrane on a time-scale of 5–10 min. Receptor **12** was also applied to live cells, in collaboration with the group of D.S. Sheppard. Cells were grown on a filter support and tested in an Ussing

Table 4
Chloride transport and anion binding by diureidocholapods

Compound	Initial rate of efflux[a] (%/s)	K_a ($Et_4N^+Cl^-$) (M^{-1})[b]	K_a ($Et_4N^+NO_3^-$) (M^{-1})[b]
49	0.031	3.4×10^6	2.1×10^6
50	0.051	1.2×10^7	8.8×10^6
51	0.075	6.3×10^6	5.1×10^6
52	0.085	1.5×10^7[c]	1.0×10^7[c]
12	0.52	5.2×10^8	1.7×10^8

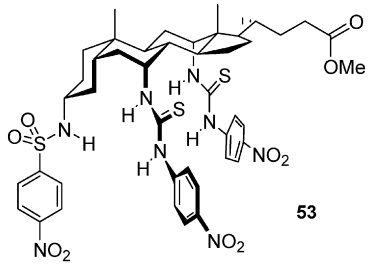
[a] See Fig. 6. External anion = NO_3^-.
[b] In water-saturated $CHCl_3$; measured by extraction, as described in Section 2.1.
[c] Determinations made on the corresponding eicosyl ester, to avoid overlap of NMR signals.

chamber. Addition of **12** promoted a flow of current, ascribed to receptor-mediated chloride transport [38].



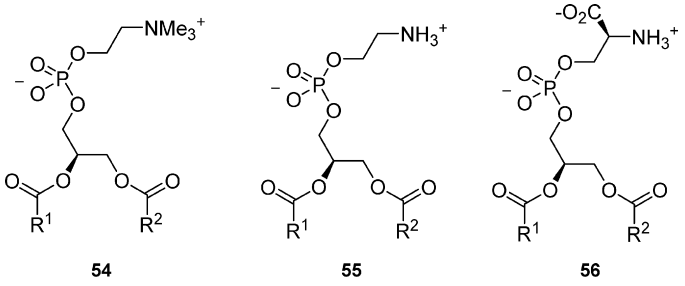
49 Ar = 4-methoxyphenyl
50 Ar = 3-methoxyphenyl
51 Ar = 4-methylphenyl
52 Ar = phenyl
12 Ar = 4-nitrophenyl

The data in Table 4 suggested that even higher transport efficiencies could be achieved with other cholapods—receptor **12** is by no means the strongest available. Early experiments with **53** (the Me ester analogue of **8**) were disappointing. Despite high affinities (e.g. $10^{11}\ M^{-1}$ for $Et_4N^+Cl^-$), it performed poorly in the chloride electrode test (Fig. 6). However, close inspection of the mixtures suggested that the receptor might be precipitating from the medium and not reaching the membranes. Accordingly, a new test was developed, shown in Fig. 7. The method is based on a fluorescent dye, lucigenin, which is quenched by chloride ions. The key advantage is that *inward* transport can be detected, so that the experiment can be initiated by addition of chloride to the external solution. This allows the receptor to be incorporated in the vesicles when they are made. The tests revealed that **53** is indeed an effective transporter, and is significantly more active than **12** [40]. Increasing affinities will not always lead to improved transport, as the release of chloride will at some stage become rate determining. However, the experiments suggest that we have not yet reached this point, and that higher affinities may yield further improvements.

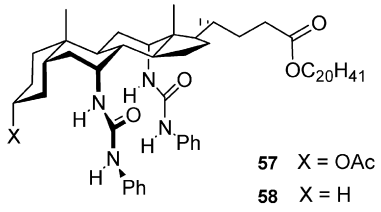


53

A second important natural phenomenon, related to anion transport, is the translocation of phospholipid head-groups through bilayer membranes. Typical phospholipids are the phosphatidylcholines **54**, the phosphatidylethanolamines **55** and the phosphatidylserines **56**. The polar head-groups of these molecules are incompatible with membrane interiors, so that translocation from one face to the other (flip-flop) is intrinsically a slow process. In nature, translocation can be induced by protein "flippases", which sometimes use energy to create asymmetrical distributions.



54 **55** **56**



57 X = OAc
58 X = H

Receptors which bind to the phosphate units in **54–56** can serve as "synthetic flippases", promoting translocation by shielding the hydrophilic PO_2^- units from the membrane interior [41]. Cholapods seemed well adapted for this purpose, and a number were tested. The bis-ureido systems **57** and **58** proved highly effective [42]. Incorporated in vesicles at 5 mol%, they induced flip-flop of a fluorescent analogue of **54** with $t_{1/2} \approx 15$ min, a major improvement on earlier systems. Not unexpectedly, the more hydrophilic **55** and **56** were translocated somewhat slower. Systems which reverse this selectivity are discussed in the next section.

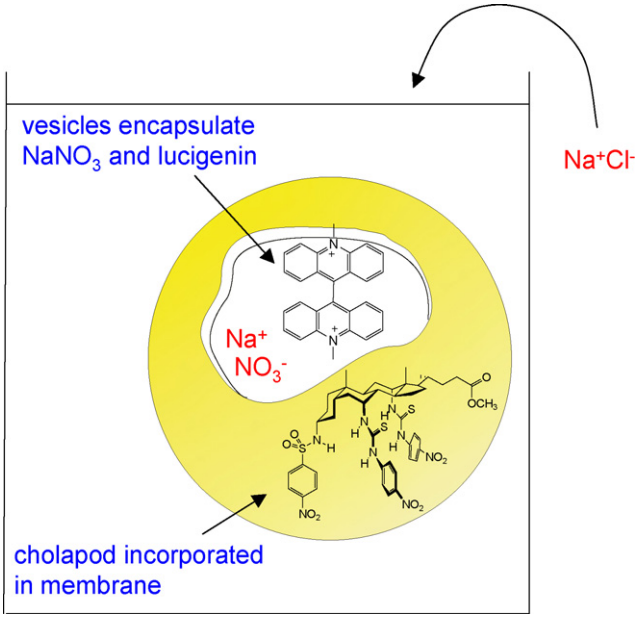
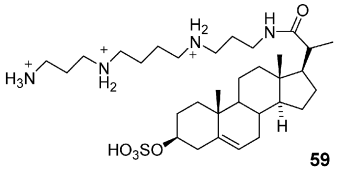


Fig. 7. Testing cholapod **53** for anion transport. Vesicles are formed from lipid + cholapod, encapsulating $NaNO_3$ and lucigenin (a chloride-sensitive fluorescent dye). Transport is initiated by addition of NaCl, and detected by monitoring the decrease in lucigenin fluorescence.

3.2. *Positively charged transporters*

The lipophilic steroid skeleton can also be useful in transporters which exploit electrostatic interactions (e.g. Fig. 5 processes (a)–(d)). In early work, the Regen and Matile groups studied systems such as **59** [43] and **60** [44], respectively. Both were found to discharge pH gradients across membranes, and were assumed to form pores capable of anion transport (with cotransport of H^+ or countertransport of OH^-). More recently, Regen has described the guanidinium **61** [45]. This species is based on the "molecular umbrella" concept, in which cholic acid units are positioned at the end of flexible linkers such that they can fold around a polar substrate. In the folded conformation the hydroxyl groups "solvate" the substrate while the apolar steroid surfaces are directed outwards. The ensemble can pass through the membrane interior and release the substrate on the other side. Thus, **61** was applied to ATP transport, exploiting the guanidinium–phosphate interaction. The Regen group has also described a number of other molecular umbrellas which operate through reversible covalent bond formation [46].



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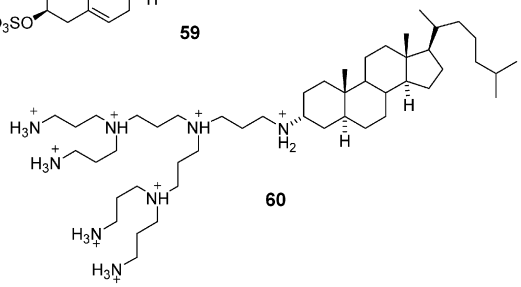

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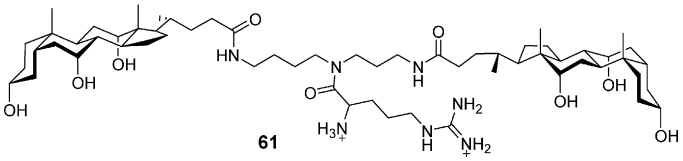

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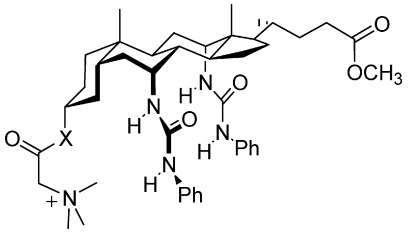
Quaternary ammonium cholapods such as **34–36** are potential anion transporters, via Fig. 5 process (a). In fact, preliminary experiments suggest that this class of compound is not effective for membrane transport of chloride (although further work might reverse this conclusion). A possible reason is that the receptor–chloride complexes are too tightly bound, and that anion release from the membrane is therefore too slow. On the

other hand, **34**($R = C_{20}H_{41}$) did transport anions through bulk chloroform, showing modest selectivity for chloride [25]. Also, quaternary ammonium cholapods have proved useful in the "flippase" studies. As discussed above, electroneutral cholapods **57** and **58** had favoured the neutral head-groups in phosphatidylcholines (PC, **54**). It seemed possible that positive cholapods would prefer the anionic head-groups of phosphatidylserines (PS, **56**). There are particular reasons for promoting the translocation of PS. In healthy cells the PS is concentrated on the inner membrane by an active transport system. Equilibration, causing PS to appear on the outer membrane, could have significant biological effects (including the promotion of apoptosis) [47]. A number of cationic cholapods were tested for PS translocation, including the previously described **34**($R = CH_3$) and the related compounds **62** [47] and **63** [48]. Both **62** and **63** were active, with significant selectivity for PS versus PC. The effect was also demonstrated in live cells (erythrocytes). Surprisingly, the slightly shorter receptor **34** was almost completely inactive, suggesting that specific geometric effects are important.



62 X = O
63 X = NH

4. Conclusion

Steroid-based structures have made a distinctive contribution to anion recognition. As revealed in this article, development proceeds apace and interest is expanding; aside from the author's group, around 20 other laboratories have become involved. The range of receptor structures has been extended, and new applications in sensing and transport have been reported. The potential of steroid-based receptors in biology is especially promising. If anion recognition is to be translated into biological activity, the membrane-compatible steroidal framework has definite advantages. Progress will certainly continue, and the prospect of truly useful activity (perhaps antibiotic, perhaps against cystic fibrosis) may not be too distant.

Acknowledgements

The author thanks his co-workers and collaborators, whose names appear in the references, for their contributions to the research described in this article. Funding for our programme has been provided by EPSRC, BBSRC, the European Commission and the University of Bristol.

References

[1] (a) E. Virtanen, E. Kolehmainen, Eur. J. Org. Chem. (2004) 3385;
(b) X.X. Zhu, M. Nichifor, Acc. Chem. Res. 35 (2002) 539;
(c) P. Wallimann, T. Marti, A. Furer, F. Diederich, Chem. Rev. 97 (1997) 1567;
(d) Y.X. Li, J.R. Dias, Chem. Rev. 97 (1997) 283;
(e) A.P. Davis, R.P. Bonar-Law, J.K.M. Sanders, in: Y. Murakami (Ed.), Comprehensive Supramolecular Chemistry, vol. 4, Supramolecular Reactivity and Transport: Bioorganic Systems, Pergamon, Oxford, 1996, p. 257;
(f) A.P. Davis, Chem. Soc. Rev. 22 (1993) 243.
[2] A.P. Davis, J.J. Perry, R.S. Wareham, Tetrahedron Lett. 39 (1998) 4569.
[3] A.P. Davis, J.F. Gilmer, J.J. Perry, Angew. Chem. Int. Ed. Engl. 35 (1996) 1312.
[4] (a) A.P. Davis, L.J. Lawless, Chem. Commun. (1999) 9;
(b) L.J. Lawless, A.G. Blackburn, A.J. Ayling, M.N. Pérez-Payán, A.P. Davis, J. Chem. Soc., Perkin Trans. 1 (2001) 1329.
[5] A.J. Ayling, M.N. Pérez-Payán, A.P. Davis, J. Am. Chem. Soc. 123 (2001) 12716.
[6] A.P. Davis, J.-B. Joos, Coord. Chem. Rev. 240 (2003) 143.
[7] J.P. Clare, A.J. Ayling, J.B. Joos, A.L. Sisson, G. Magro, M.N. Pérez-Payán, T.N. Lambert, R. Shukla, B.D. Smith, A.P. Davis, J. Am. Chem. Soc. 127 (2005) 10739.
[8] The syntheses of these receptors require scaffolds in which steroidal hydroxyl groups have been replaced by $-NH_2$. For improved preparations of key intermediates see:
(a) V. del Amo, L. Siracusa, T. Markidis, B. Baragana, K.M. Bhattarai, M. Galobardes, G. Naredo, M.N. Pérez-Payán, A.P. Davis, Org. Biomol. Chem. 2 (2004) 3320;
(b) V. del Amo, K. Bhattarai, M. Nissinen, K. Rissanen, M.N. Pérez-Payán, A.P. Davis, Synlett (2005) 1319.
[9] E.P. Kyba, R.C. Helgeson, K. Madan, G.W. Gokel, T.L. Tarnowski, S.S. Moore, D.J. Cram, J. Am. Chem. Soc. 99 (1977) 2564;
see also:
A.J. Ayling, S. Broderick, J.P. Clare, A.P. Davis, M.N. Pérez-Payán, M. Lahtinen, M.J. Nissinen, K. Rissanen, Chem. Eur. J. 8 (2002) 2197.
[10] M.H. Abraham, J.A. Platts, J. Org. Chem. 66 (2001) 3484;
see also:
C.A. Hunter, Angew. Chem. Int. Ed. 43 (2004) 5310.
[11] R.A.W. Dryfe, S.S. Hill, A.P. Davis, J.B. Joos, E.P.L. Roberts, Org. Biomol. Chem. 2 (2004) 2716.
[12] L. Fang, W.H. Chan, Y.B. He, D.W.J. Kwong, A.W.M. Lee, J. Org. Chem. 70 (2005) 7640.
[13] S.Y. Liu, L. Fang, Y.B. He, W.H. Chan, K.T. Yeung, Y.K. Cheng, R.H. Yang, Org. Lett. 7 (2005) 5825.
[14] K.S. Kim, H.S. Kim, Bull. Korean Chem. Soc. 25 (2004) 1411.
[15] J.H. Shim, I.S. Jeong, M.H. Lee, H.P. Hong, J.H. On, K.S. Kim, H.S. Kim, B.H. Kim, G.S. Cha, H. Nam, Talanta 63 (2004) 61.
[16] K.S. Kim, H.S. Kim, Tetrahedron 61 (2005) 12366.
[17] K.M. Bhattarai, V. del Amo, G. Magro, A.L. Sisson, J.-B. Joos, J.P.H. Charmant, A. Kantacha, A.P. Davis, (2006) 2335.
[18] S. Ghosh, A.R. Choudhury, T.N.G. Row, U. Maitra, Org. Lett. 7 (2005) 1441.
[19] M.E. Meyerhoff, E. Pretsch, D.H. Welti, W. Simon, Anal. Chem. 59 (1987) 144.
[20] N.J. Pyun, J.H. Chu, Y.M. Jun, D.J. Kim, Bull. Korean Chem. Soc. 20 (1999) 112.
[21] H.J. Pyun, J.H. Chu, W. Yoon, Y.M. Jun, D.J. Kim, Bull. Korean Chem. Soc. 20 (1999) 179.
[22] H.J. Lee, I.J. Yoon, C.L. Yoo, H.J. Pyun, G.S. Cha, H. Nam, Anal. Chem. 72 (2000) 4694.
[23] Y.S. Choi, L. Lvova, J.H. Shin, S.H. Oh, C.S. Lee, B.H. Kim, G.S. Cha, H. Nam, Anal. Chem. 74 (2002) 2435.
[24] F.Y. Song, J.H. Ha, B. Park, T.H. Kwak, I.T. Kim, H. Nam, G.S. Cha, Talanta 57 (2002) 263.
[25] A.L. Sisson, J.P. Clare, L.H. Taylor, J.P.H. Charmant, A.P. Davis, Chem. Commun. (2003) 2246.
[26] F. Hofmeister, Arch. Exp. Path. Pharmakol. 24 (1888) 247;
M.G. Cacace, E.M. Landau, J.J. Ramsden, Quart. Rev. Biophys. 30 (1997) 241.
[27] A.L. Sisson, J.P. Clare, A.P. Davis, Chem. Commun. (2005) 5263.
[28] P.B. Savage, Eur. J. Org. Chem. (2002) 759.

[29] B.W. Ding, N. Yin, Y. Liu, J. Cardenas-Garcia, R. Evanson, T. Cirsak, M.J. Fan, G. Turin, P.B. Savage, J. Am. Chem. Soc. 126 (2004) 13642.
[30] H.M. Willemen, L. de Smet, A. Koudijs, M.C.A. Stuart, I. Heikamp-de Jong, A.T.M. Marcelis, E.J.R. Sudholter, Angew. Chem. Int. Ed. 41 (2002) 4275.
[31] E. Kolehmainen, J. Koivukorpi, E. Sievanen, V. Král, Supramol. Chem. 17 (2005) 437.
[32] B. Baragaña, A.G. Blackburn, P. Breccia, A.P. Davis, J. de Mendoza, J.M. Padron-Carrillo, P. Prados, J. Riedner, J.G. de Vries, Chem. Eur. J. 8 (2002) 2931.
[33] L. Siracusa, F.M. Hurley, S. Dresen, L.J. Lawless, M.N. Pérez-Payán, A.P. Davis, Org. Lett. 4 (2002) 4639.
[34] J.M. Hayes, M. Stein, J. Weiser, J. Phys. Chem. A 108 (2004) 3572.
[35] Leading Refs.:
(a) A. Iuliano, A. Ruffini, Tetrahedron Asymm. 16 (2005) 3820;
(b) L. Vaton-Chanvrier, H. Oulyadi, Y. Combret, G. Coquerel, J.C. Combret, Chirality 13 (2001) 668;
(c) T. Takeuchi, J. Chu, T. Miwa, Chromatographia 47 (1998) 183.
[36] Prodigiosins:
A. Fürstner, Angew. Chem. Int. Ed. 42 (2003) 3582;
Pamamycins:
E.J. Jeong, E.J. Kang, L.T. Sung, S.K. Hong, E. Lee, J. Am. Chem. Soc. 124 (2002) 14655.
[37] See for example:
J.M. Boon, B.D. Smith, Curr. Opin. Chem. Biol. 6 (2002) 749;
J.L. Sessler, S. Camiolo, P.A. Gale, Coord. Chem. Rev. 240 (2003) 17;
J.R. Broughman, L.P. Shank, W. Takeguchi, B.D. Schultz, T. Iwamoto, K.E. Mitchell, J.M. Tomich, Biochemistry 41 (2002) 7350.
[38] A.V. Koulov, T.N. Lambert, R. Shukla, M. Jain, J.M. Boon, B.D. Smith, H.Y. Li, D.N. Sheppard, J.B. Joos, J.P. Clare, A.P. Davis, Angew. Chem. Int. Ed. 42 (2003) 4931.
[39] A.L. Sisson, V. del Amo Sanchez, G. Magro, A.M.E. Griffin, S. Shah, J.P.H. Charmant, A.P. Davis, Angew. Chem. Int. Ed. 44 (2005) 6878.
[40] B.A. McNally, A.V. Koulov, B.D. Smith, J.B. Joos, A.P. Davis, Chem. Commun. (2005) 1087.
[41] J.M. Boon, B.D. Smith, J. Am. Chem. Soc. 121 (1999) 11924.
[42] T.N. Lambert, J.M. Boon, B.D. Smith, M.N. Pérez-Payán, A.P. Davis, J. Am. Chem. Soc. 124 (2002) 5276.
[43] M. Merritt, M. Lanier, G. Deng, S.L. Regen, J. Am. Chem. Soc. 120 (1998) 8494.
[44] N. Sakai, S. Matile, Tetrahedron Lett. 38 (1997) 2613.
[45] V. Janout, B.W. Jing, I.V. Staina, S.L. Regen, J. Am. Chem. Soc. 125 (2003) 4436.
[46] Leading Refs.:
V. Janout, B.W. Jing, S.L. Regen, J. Am. Chem. Soc. 127 (2005) 15862.
[47] J.M. Boon, T.N. Lambert, A.L. Sisson, A.P. Davis, B.D. Smith, J. Am. Chem. Soc. 125 (2003) 8195.
[48] K.M. DiVittorio, T.N. Lambert, B.D. Smith, Bioorg. Med. Chem. 13 (2005) 4485.