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Steroids as organising elements in anion receptors

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Contents

Abstract

The steroid nucleus has proved useful in various areas of supramolecular chemistry, acting as a building-block for extended, well-defined molecular architectures, and a scaffold for preorganised arrays of functionality. This article discusses its applications in the area of anion recognition, where cholic acid (**2**) has been especially valuable. The three secondary hydroxyl groups on **2** are nicely arranged for H-bond donation to a single anion, and may be converted into stronger neutral or positively-charged H-bond donors. Macrocyclic and acyclic receptors derived from **2** have been used to bind inorganic anions, carboxylates and nucleic acids.

Keywords: Anion recognition; Supramolecular chemistry; Steroids; Enantioselectivity; Hydrogen bonding; Ionophores

1. Introduction

Receptor design is governed largely by the concept of preorganisation, the creation of a binding site of well-defined shape which complements the structure of the target species. Nature achieves this goal with flexible, linear molecules which fold into particular conformations. This approach, however, is not yet realistic for the supramolecular chemist. Modelling software cannot be relied upon to predict the conformations of complex linear structures, and the creative exploitation of folding is still more difficult. In practice, the design of synthetic receptors is strongly reliant on rigid subunits which can be used to define clefts and cavities, and can serve as scaffolds for organised arrays of functional groups.

When supramolecular chemists require a rigid unit, the most obvious choice is usually an aromatic ring. Indeed, benzenoid structures often seem to dominate books and reviews on the subject [1]. However, fused alicyclic systems may be equally rigid and can play a complementary role. They even have certain intrinsic advantages; for example, they are usually chiral and possess two valencies (and thus two orientations) at each non-bridging centre. In the general case, such aliphatic systems may require elaborate syntheses, but one particular variant is readily available. The steroid nucleus consists of four fused rings covering an area of ca. 10×6 Å. It is widely available in nature as (of

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course) a single enantiomer. In some versions it is quite inexpensive; notable examples are cholesterol (**1**) (currently 0.16£ g^{-1} from Aldrich Chemical Co.) and cholic acid (**2**) (currently 0.25£ g^{-1}). Cholesterol (**1**) is limited in usefulness by its low degree of functionalisation. One end is furnished with a hydroxyl group and an alkene unit, but the other presents a shield of hydrocarbon. However, cholic acid (**2**) is more promising. The nucleus is substituted with three secondary hydroxyl groups, while the side-chain is terminated by a carboxyl. The hydroxyl groups are fairly evenly spaced around the periphery and usefully, from the point of view of receptor design, are co-directed. The *cis*-AB ring junction imparts a curved profile (again useful for receptor design), and assists in differentiating between the hydroxyl groups. The 3α group is rendered equatorial and thus less hindered than the axial 7/12α hydroxyls. Also, a CH_2 unit is placed in a 1,3-diaxial relationship with the 7α-OH, so that the latter becomes somewhat more hindered than the 12α-OH.

For some years, we and others have been exploring the potential of steroids as scaffolds in supramolecular chemistry, with special emphasis on the exploitation of cholic acid (**2**) [2–5]. A substantial part of this work has been in the area of anion recognition, where the steroid can contribute by providing a scaffold on which to mount polar functional groups, and lipophilic surfaces to promote solubility in non-polar media [6]. This review of the area is divided into two parts, covering: (a) electroneutral anion receptors derived from cholic acid (**2**); and (b) positively charged receptors derived from **2** and other steroidal starting materials.

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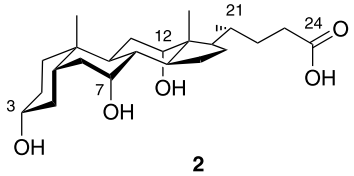

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2. Electroneutral anion receptors based on cholic acid

Anion recognition [7,8] has been studied since the early days of supramolecular chemistry. Indeed, the first anion receptors were described in 1968 [9], within a year of the first report on crown ethers. However, the areas of anion and cation binding developed rather differently. The early cationophores (crown ethers, cryptands, spherands etc.) were electroneutral, compatible with organic solvents, and often capable of solubilising cations in non-polar environments. The early anion receptors were polycationic and essentially restricted to water. It is only quite recently, perhaps in the last 10–12 years, that much attention has been paid to the design of electroneutral, lipophilic anion-binding molecules. Neutral anion receptors have several potential applications, in parallel with their cation-binding counterparts. They may be capable of membrane transport, and therefore biological activity. They may be used in sensing devices such as ion-selective electrodes, and might act as novel phase transfer catalysts, solubilising inorganic salts by binding the anion as opposed to the cation.

One obvious approach to electroneutral anionophores is the deployment of neutral H-bond donor functionality around a central binding site. However, the execution of this strategy is non-trivial. Anions are generally larger than cations, while convergent H-bond donor centres must be spaced more widely than, for example, electron pair donors [compare Fig. 1(a and b)]. Both factors imply that large, elaborate frameworks may be required. Moreover, most neutral H-bond donor groups are also H-bond acceptors, raising the possibility of intramolecular H-bonding within the receptor.

The steroid nucleus provides an attractive solution to these problems. It is extended, and thus able to support well-separated functional groups, and rigid, such that these groups cannot easily interact with each other. Of the readily-available steroids, cholic acid (**2**) is especially useful [2,3]. Its curved profile and co-directed hydroxyl groups naturally lend themselves to the creation of macrocyclic or cleft-type architectures with polar interiors and apolar exteriors. The hydroxyl groups may be differentiated and converted to a range of other functional groups, while the side-chain carboxyl affords further possibilities.

Cholic acid has been converted into a series of electroneutral anionophores targeted at inorganic anions. These may be divided into two categories; acyclic and macrocyclic receptors which exploit the H-bonding properties of (at least some of) the secondary hydroxyls, and acyclic podands in which all the hydroxyl groups have been modified to give alternative binding functionality. These two classes are considered in Sections 2.1 and 2.2 below.

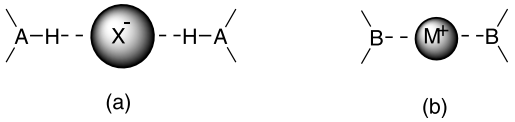
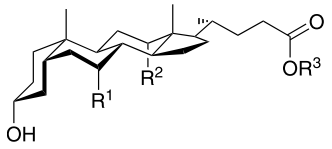


Fig. 1. Binding of: (a) a spherical anion by H-bond donor groups A–H; and (b) a cation by electron pair donor atoms B. The spacing between atoms A is considerably greater than that between atoms B.

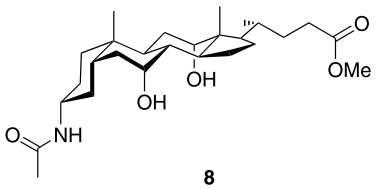
2.1. Hydroxysteroid anion receptors derived from cholic acid

Hydroxyl groups are H-bond donors, so it might be expected that simple ester derivatives of **2** would have anion-binding properties. Indeed, the addition of tetrabutylammonium (TBA) bromide, tosylate, mesylate, or hydrogen phenylphosphonate to methyl cholate (**3**) in benzene-d_6 caused significant changes in steroidal C*H* chemical shifts [10,11]. In particular, protons on the α (lower) face of the steroid moved downfield by up to 0.55 p.p.m., clearly suggesting anion binding to this face. In the case of TBA tosylate, it was possible to analyse the data using a 1:1+2:1 (substrate–receptor) binding model. The derived association constants K_a were 220 M^{-1} for 1:1 binding, and 50 M^{-1} for the equilibrium between 1:1 and 2:1 stoichiometries. Binding was also studied by observing the solubilisation of TBA hydrogen phenylphosphonate in non-polar media. For example, octyl cholate (**4**) was able to solubilise a full equivalent of phosphonate in benzene–hexane 1:1, whereas methyl deoxycholate (**5**) and chenodeoxycholate (**6**) dissolved just 25 and 9 mol%, respectively. In the case of methyl lithocholate (**7**) where only one hydroxyl group is left, no solubilisation was observed.



3 R^1 = OH, R^2 = OH, R^3 = Me
4 R^1 = OH, R^2 = OH, R^3 = n-C_8H_{17}
5 R^1 = H, R^2 = OH, R^3 = Me
6 R^1 = OH, R^2 = H, R^3 = Me
7 R^1 = H, R^2 = H, R^3 = Me

Although these simple esters complexed anions in benzene or less polar solvents, no binding could be detected in $CDCl_3$. However, complex formation in this more competitive solvent could be achieved by 'mutating' the 3α-OH into –NH–CO, as in **8**. The latter showed measurable affinities for TBA chloride and bromide in $CDCl_3$. The NMR titration data for both salts against receptor **8** were consistent with 1:1 complex formation, giving $K_a = 53$ and 36 M^{-1}, respectively [11,12].



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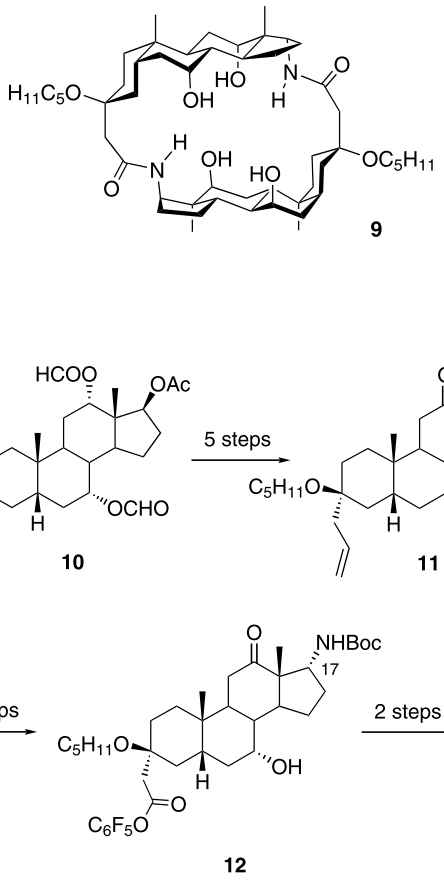
Further, significant increases could be obtained using a more elaborate, cyclodimeric architecture. The macrocycle **9** was synthesized from **2** as shown in Scheme 1. Firstly, the C21–C24 side-chain was degraded to give the 17β-acetate (**10**). The acetate was converted to tosylate, a short spacer+externally-directed solubilising group were introduced via a 'silyl-modified Sakurai' at C3, and a keto group was generated at C12. This last (apparently perverse) conversion was necessary because steric hindrance from any 12α-OR substituent seemed to block nucleophilic displacement of the 17β-tosylate. However, treatment of ketone **11** with N_3^- gave the corresponding 17α azide in good yield. Reduction/protection of the azido group and cleavage/activation of the C=C bond then gave **12**, and cyclodimerisation followed by reduction of the C12 keto group resulted in macrocycle **9**. The 'cryptand' **9** was specifically designed to encapsulate small spherical anions such as fluoride or chloride. ^{1}H-NMR titrations against TBA halides revealed downfield movements ($\Delta\delta$) of the N*H* signals and of some steroidal C*H*, thought to correspond to protons on the interior surface of the cavity. The data was consistent with 1:1 complex formation, with binding constants of 3320, 990 and 250 M^{-1} to TBA fluoride, chloride and bromide, respectively [12]. Semi-empirical MO calculations (AM1) supported the hypothesis that, at least, fluoride or chloride could be bound within the

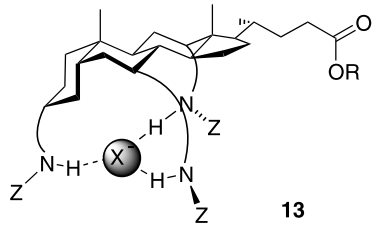


Scheme 1. Synthesis of macrocycle **9**.

cavity. Neither anion caused significant distortion of the carbon framework, while bromide caused some widening of the C7 portal [13]. However, the minimisations confirmed that the ovoid structure of **9** is not quite ideal for halide binding. The NH···HN distance is somewhat too long, such that the two amides cannot both form strong H-bonds to a bound anion. In accord with this prediction, the limiting N*H* $\Delta\delta$ were somewhat smaller for **9** than for **8**, despite the higher affinities shown by the former.

2.2. Steroidal podands with multiple NH groups

As H-bond donors in supramolecular chemistry, NH units are far more versatile than OH groups. The third valence on the nitrogen can be exploited structurally, and can also be used to tune the H-bond donor strength. These advantages were exploited in a second series of steroid-based anion receptors, represented schematically by **13**. Instead of constructing elaborate frameworks derived from more than one steroid unit, synthetic effort was focussed on modifying the functional groups on a single molecule of cholic acid. In the resulting 'cholapods', multiple NH units converge on a binding site beneath the α-face of the steroid. The NH groups may belong to amides, sulfonamides, carbamates, ureas or thioureas. NH acidity, and therefore H-bond donor strength, can be controlled by variation of electron withdrawing groups Z.

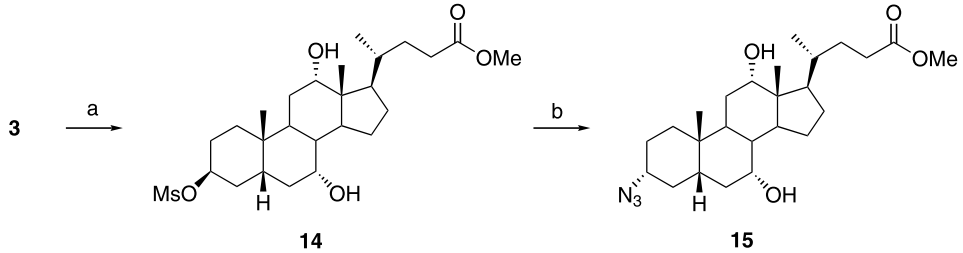


13

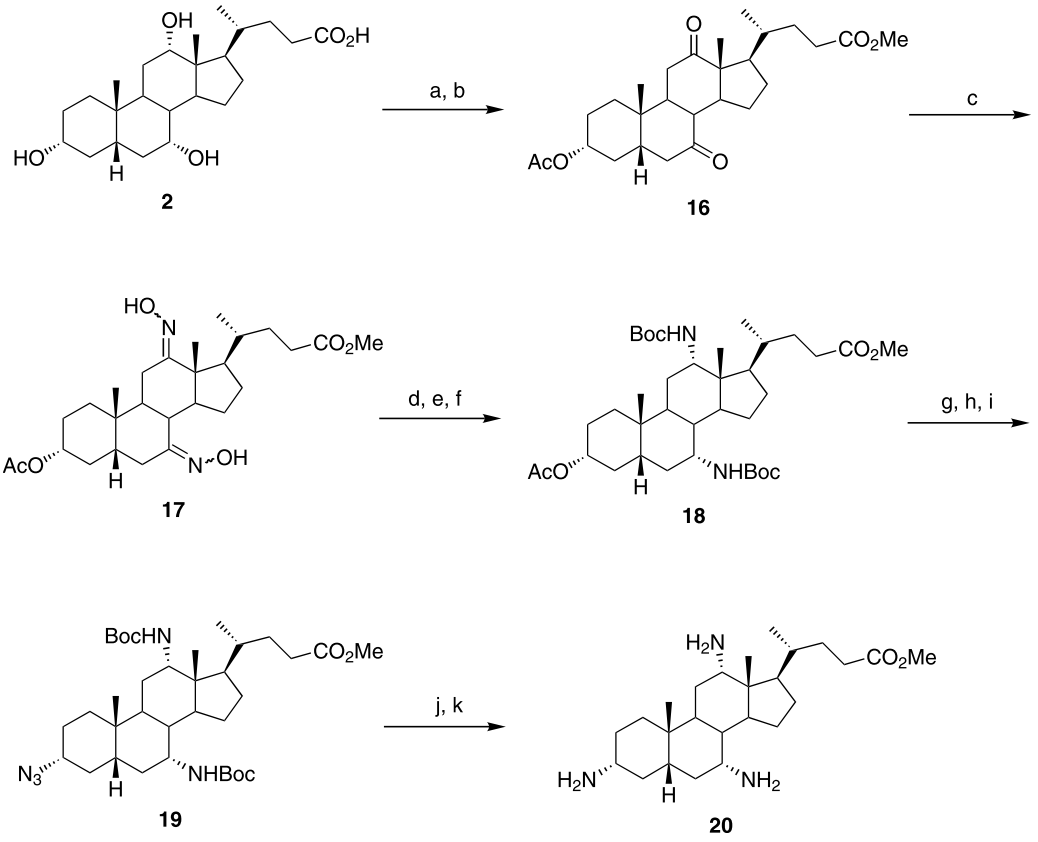
Key intermediates for the synthesis of this new generation of receptors are dihydroxy azide (**15**) and bis-(*t*-Boc-amino) azide (**19**). A convenient synthesis of **15** is shown in Scheme 2. In the first step, methyl cholate (**3**) is subjected to an unusual Mitsunobu procedure in which methanesulfonic acid is employed as the acidic/nucleophilic component to give **14** [14,15]. Displacement of the mesylate with azide then gives **15**. It is notable that no protection of the hydroxyl groups on 7 and 12 positions is required; the Mitsunobu reaction appears to be selective for the equatorial hydroxyl group and is thus entirely regioselective.

Azide **19** is notable as a precursor of triamine **20**, the 'aza-equivalent' of methyl cholate. The first synthesis of **20** was unrealistic for large-scale use, consisting of 17 steps and suffering from a low overall yield [16]. A few years later a shorter and practical synthesis was developed [17]. The pathway starting from cholic acid is outlined in Scheme 3. Esterification and selective protection of the 3α-position followed by oxidation of the remaining hydroxyl groups afforded diketone **16**. The amino groups on C7 and C12 were introduced by oximation–reduction. Catalytic hydrogenation of dioxime **17** took place almost exclusively from the β-face to give α-directed amino and/or hydroxylamino groups. This mixture was further reduced to diamine by Zn/AcOH and then protected to give bis-carbamate **18**. The acetoxy group was cleaved by methanolysis, and the resulting alcohol subjected to the double-displacement procedure discussed earlier (Mitsunobu, with methanesulfonate as nucleophile, followed by azide anion). The product **19** could be transformed into **20** as shown, but was more usually exploited as a differentially-protected triazacholate, separately addressable at positions 3 and 7/12.

The first cholapod anionophores were the sulfonamido bis-carbamate **21** and the tris-sulfonamide **22** [18]. Both systems provide quite well-defined binding sites for anions. In **21**, free rotation is possible about the C3–N bond, but the carbamate NH groups are preorganised to some extent through: (a) restricted rotation about the axial C7/C12–O bonds; and (b) the preference for *Z*,*Z*-conformations across the carbamate units [19]. In **22**, restricted rotation about the C7/C12–N bonds (Fig. 2) holds the NH groups firmly in place for anion recognition. NMR titrations revealed the binding constants shown in Table 1, significantly higher than those for **9**.



Scheme 2. Reagents and conditions: (a) Ph_3P, DEAD, MsOH, DMAP; (b) NaN_3, DMPU.



Scheme 3. Reagents and conditions: (a) *p*-TsOH, AcOMe; (b) K_2CrO_4, AcOH; (c) $H_2NOH \cdot HCl$, AcONa, MeOH; (d) H_2, $PtO_2 \cdot H_2O$, AcOH; (e) Zn, HOAc; (f) $(Boc)_2O$, $NaHCO_3$ aq., THF; (g) Na_2CO_3, MeOH; (h) Ph_3P, Et_3N, $MeSO_3H$, DEAD, THF; (i) NaN_3, DMF; (j) H_2, Pd/C (10%), $(Boc)_2O$, EtOH; (k) TFA, CH_2Cl_2.

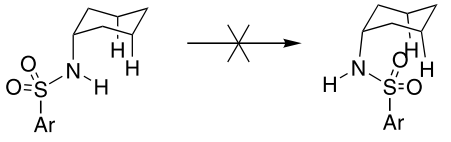
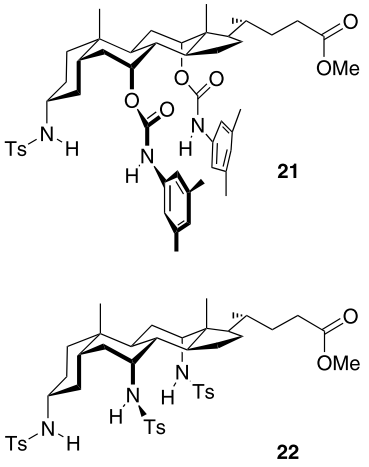


Fig. 2. Restricted rotation about the C7/C12–N bonds.



The cholapod architecture clearly had potential for further development. Electron-withdrawing substituents could be used to increase H-bond donor potency, and further NH groups could be added. However, progress was hindered by problems of measurement; at K_a ca. 10^5 M^{-1}, receptor **22** had already attained the upper limit compatible with NMR titrations. While measurements might have been possible in more polar solvents, chloroform provides a better model for certain media of interest, for example, biological membranes or the polymer materials used in ion-selective electrodes. Continued use of chloroform would also facilitate comparisons with the earlier, weaker receptors.

A solution was found in Cram's extraction-based methodology, whereby binding constants are determined from the ability of the receptor to extract a hydrophilic substrate from water into an organic

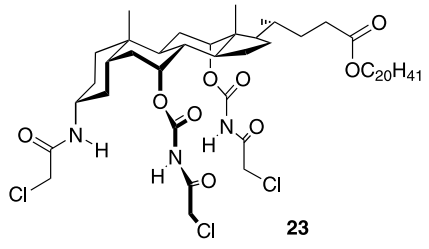
Table 1
Association constants (K_a, M^{-1}) of **21** and **22** with TBA salts in $CDCl_3$ (^{1}H-NMR titrations) [18]

Anion	Complex with **21**	Complex with **22**
F^-	15,400	not determined
Cl^-	7200	92,000
Br^-	7200	9200
I^-	930	525
TsO^-	865	950

medium [20]. The method is applicable to very high binding constants, because receptor saturation (i.e. quantitative complex formation) can be avoided by lowering substrate concentration in the aqueous phase. A disadvantage is that the method relies on knowledge of the distribution constant K_d of the substrate between aqueous and organic phases (in the absence of receptor). This can be difficult to measure, especially for the hydrophilic substrates most suitable for use with powerful receptors.

Cram's method was adapted for anion binding with the aid of receptors **23**–**28** [21]. C_{20} side-chains were incorporated to prevent loss of receptor into the aqueous phase, and electron-withdrawing substituents were expected to increase affinities. Tetraethylammonium (TEA) salts were found to be useful substrates except that, as feared, their K_d values were mostly too low for direct measurement. Instead, K_d for TEACl and TEABr were calculated indirectly through measurements on **23**, the weakest receptor of the series. ^{1}H-NMR titrations in water-saturated $CDCl_3$ (the medium of relevance to the extraction experiments) gave K_a values of 16,500 and 8400 M^{-1}, respectively. Extraction experiments gave extraction constants K_e for the two salts, and K_d values could then be determined from the relationship linking these quantities ($K_a = K_e/K_d$).

Once the K_d values were established, binding constants could be measured for the full series of second-generation cholapods. The results are presented in Table 2. Considering **21** as the starting point, the introduction of two trifluoromethyl groups (in **24**) caused at least a 10-fold increase in binding constants [22]. Replacement of the tosyl group by *p*-nitrophenylsulfonyl (in **25**) gave a further large increase, and *p*-nitrophenyl groups at all three centres (in **26**) raised the binding constants above 10^7 M^{-1}. A fourth nitro group (in **27**) had a deleterious effect (probably because the *o*-NO_2 interfered with the binding site) and tris-*p*-nitro-sulfonamide (**28**) was, disappointingly, an order of magnitude less potent than **26**.



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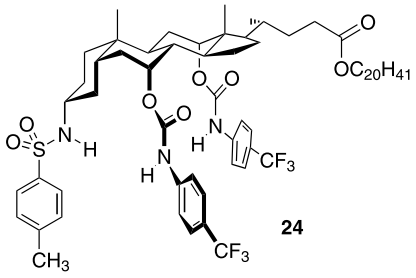

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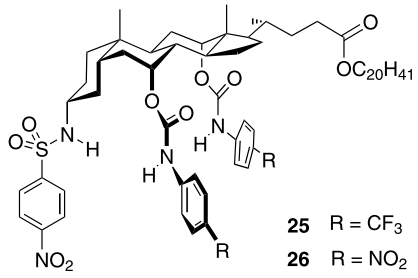
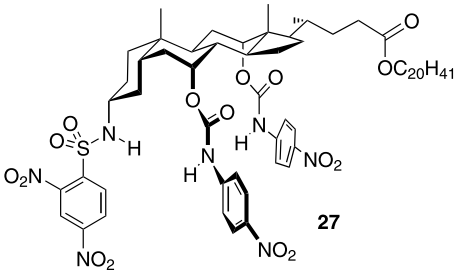


25 R = CF_3
26 R = NO_2



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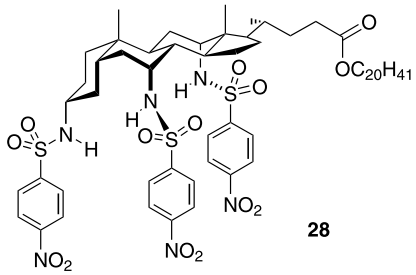

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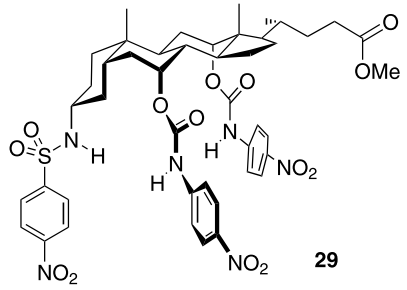
Table 2
Association constants (K_a, M^{-1}) of **24**–**28** with TEA chloride and bromide in water-saturated $CHCl_3$ (extraction measurements) [21]

Receptor	K_a, TEACl (M^{-1})	K_a, TEABr (M^{-1})
24	9.0×10^4	1.07×10^5
25	5.5×10^6	6.9×10^6
26	3.4×10^7	2.9×10^7
27	7.3×10^6	5.1×10^6
28	3.2×10^6	1.1×10^6

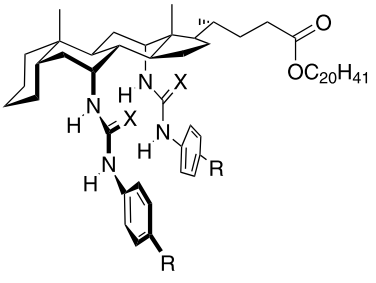


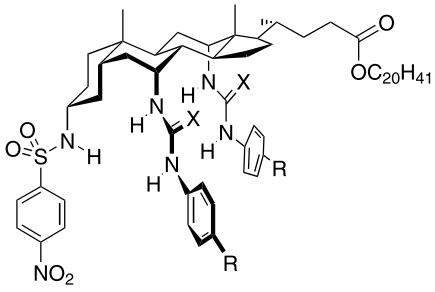
29

NMR studies of cholapod-anion complexes have consistently supported the original binding concept, represented by **13**. Nonetheless, confirmation by X-ray crystallography has long been sought. No complex has yet yielded suitable crystals, but a structure was obtained for **29** (the Me ester analogue of **26**) as an acetone solvate. As shown in Fig. 3, a well-defined binding site is occupied by a molecule of the acetone. Although only one host–guest hydrogen bond is observed, the three NH groups converge and may thus be preorganised for anion recognition.

In a third series of cholapods **30**–**36** the number of H-bond donors was raised to four and five [23]. Bis-(thio)ureas **30**–**33** were prepared from intermediate **37**, while **34**–**36** were obtained from **19**. As expected, the additional H-bond donor groups caused further increases in binding constants. The results are shown in Table 3. Again, the influence of electron withdrawing substituents is clearly manifest. The sequence also illustrates the slight advantage of thiourea over urea groups in anion recognition. The affinities of the stronger receptors are exceptional; at the time of writing, the binding constant of 10^{11} M^{-1} for **36**+TEACl is the highest published value for an electroneutral, purely organic anionophore.

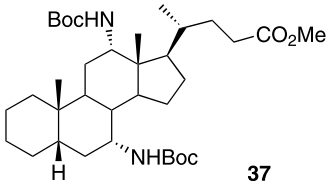
The advent of these powerful and highly lipophilic anionophores raises the possibility of new applications in areas such as membrane chemistry. For example, in a collaboration between our group and that of B.D. Smith, it has recently been shown that cholate bis-(phenylureas) **30** and **38** serve as 'translocases' for phospholipids [24]. The steroids promote the shuttling of polar, phosphate-containing head-groups of phospholipids across vesicle and cell membranes. The urea side-chains are essential for strong binding of the phosphate head-group as in **39** and apparently cannot be replaced by simple amide, alcohol or amine moieties.





30 R = H, X = O
31 R = CF_3, X = O
32 R = NO_2, X = O
33 R = NO_2, X = S

34 R = CF_3, X = O
35 R = NO_2, X = O
36 R = NO_2, X = S



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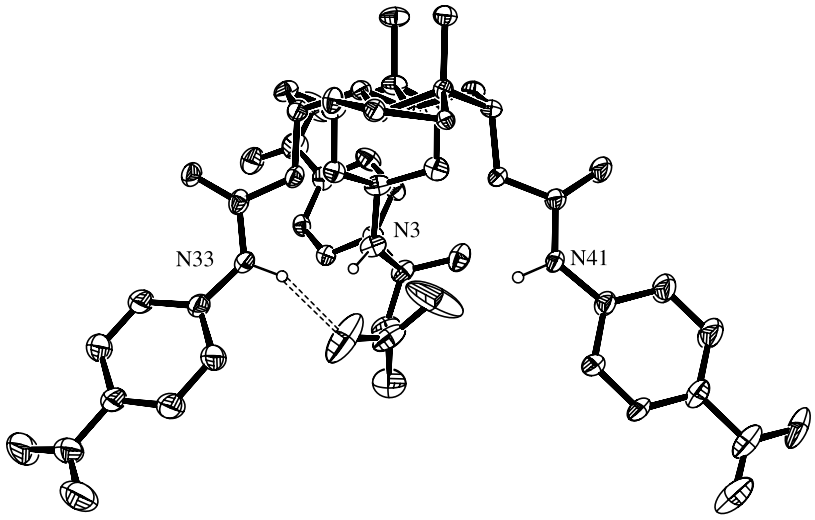
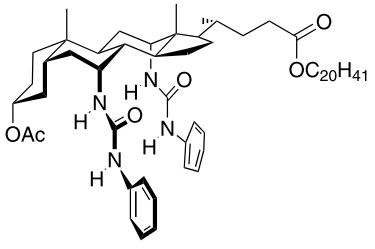


Fig. 3. Structure of **29** in the crystal [21]. Hydrogens bound to carbon, the steroidal side-chain (C20–C24) and chloroform solvent are omitted for clarity. The structure is viewed down the long axis of the steroid nucleus, from the C20 end. The proposed anion-binding site is occupied by a molecule of acetone solvent, hydrogen-bonded to N33–H (steroidal C7–OCONH).

Table 3
Association constants (K_a, M^{-1}) of **30**–**36** with TEA chloride and bromide in water-saturated $CHCl_3$ (extraction measurements) [23]

Receptor	K_a, TEACl (M^{-1})	K_a, TEABr (M^{-1})
30	1.62×10^7	9.79×10^6
31	2.83×10^7	1.84×10^7
32	4.77×10^8	2.26×10^8
33	1.05×10^9	3.24×10^8
34	4.58×10^9	2.63×10^9
35	6.60×10^{10}	1.68×10^{10}
36	1.03×10^{11}	2.59×10^{10}



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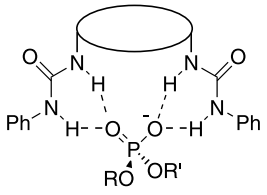
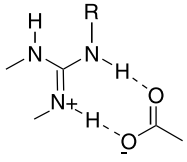


39

environment created by other substituents. Cholic acid, once again, is an attractive starting material for such systems. Elaboration of the secondary hydroxyl groups can lead to a variety of receptors in which three independent contacts are made with a substrate. A series of cations **41**–**46** were designed as enantioselective extractants of chiral carboxylates from aqueous into organic media [26,15]. The lipophilic steroidal skeleton was expected to ensure that the complexes would favour organic media, while the 3α-guanidinium unit and carbamate NH groups would cooperate in binding the carboxylate. Chiral discrimination would result from the steroidal superstructure, and would presumably be enhanced by differential substitution at positions 7 and 12. A six-membered guanidinium ring was preferred to the five-membered analogue, mainly because of its greater stability.



40

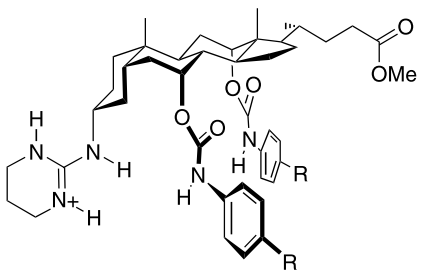
3. Steroid-based anion receptors with net positive charge

3.1. Steroidal guanidinium cations as anion receptors

The guanidinium cation is well-established as a centre for carboxylate recognition, through formation of electroneutral, H-bonded complexes **40**. High affinities are observed, even in quite polar solvents [25]. Placed on a steroidal scaffold, a guanidinium group can therefore position a carboxylate within a well-defined (and chiral)

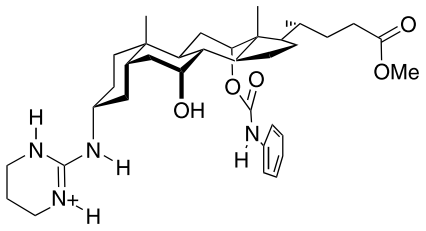
The receptors **41** and **42** were prepared from azide **15** (Scheme 4). After reduction of the azido group at C3 the guanidinium moiety was introduced via a stepwise procedure (alternative single-step methods having failed; see later). The obtained diol **48** was reacted with phenyl isocyanate to yield **41**. Receptor **42** was prepared following a similar pathway, although in this case the carbamates were inserted early in the sequence.

For the less symmetric steroids **43**–**46** a longer sequence was necessary as indicated in Scheme 5. In



41 R = H

42 R = CF_3



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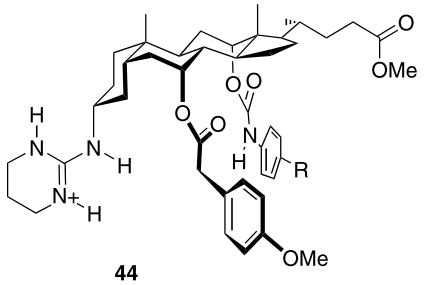

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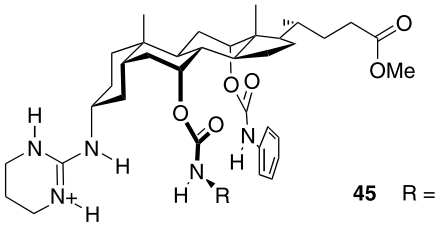
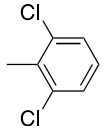


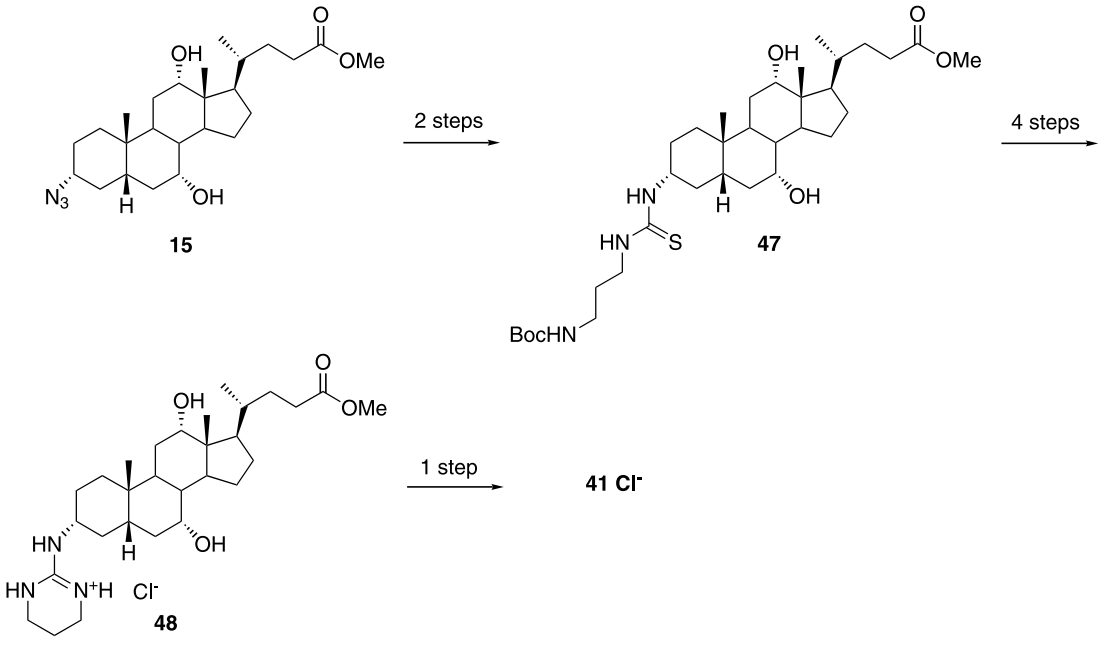
45 R = Bu^t

46 R =

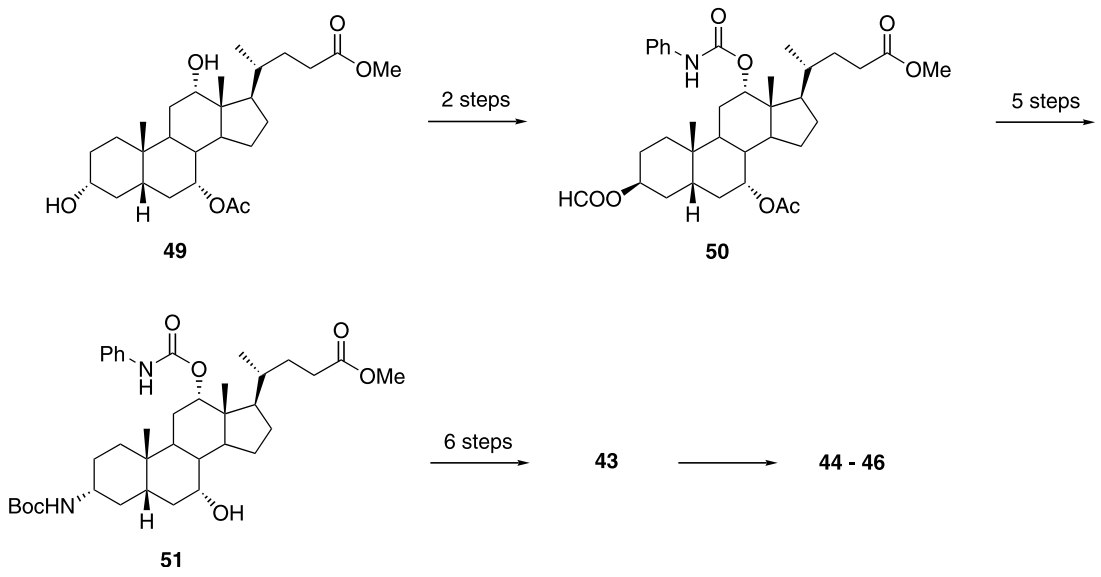


this case the 3α-N was introduced via a conventional Mitsunobu (formate as nucleophile). The guanidinium unit was introduced via the stepwise procedure discussed above. The receptors **44**–**46** were synthesized from **43** by derivatisation of the hydroxyl group with the appropriate acid chloride (for **44**) or isocyanate (for **45** and **46**).

Receptors **41**–**46** proved capable of extracting a range of *N*-acyl amino acids from aqueous phosphate buffer into chloroform. The extraction efficiency and the



Scheme 4. Synthesis of guanidinium **41**.



Scheme 5. Synthesis of receptors **43**–**46**.

enantioselectivity could both be followed by NMR, the receptor acting as a chiral shift reagent for the determination of enantiomeric excess. Selected results are shown in Table 4. Quite good enantioselectivities were obtained for the symmetrically derivatised receptors **41** and **42**, with *N*-acetyl amino acids as substrates. The results were insensitive to the bulk of the amino acid side-chain; the receptors performed as well with the alanine as with the valine or phenylalanine derivatives. However, the hydrophilic asparagine derivative resisted extraction. *N*-Boc amino acids were extracted with greater efficiencies but lower selectivities. Replacement of the acetyl group by a Boc-group in the substrate increased the extraction efficiency but lowered the enantioselectivity. Against expectation, the less symmetric receptors **43**–**45** gave *lower* selectivities, while **46** behaved much as **41** and **42**. This result emphasizes the subtle nature of enantioselective recognition, and the danger of relying on simple design concepts. Receptor **46** was also used to extract lipophilic amino acids, probably as amino–carboxylates, although selectivities were low.

A model for the binding geometry was derived from NMR-spectroscopy and calculations. Monte Carlo Molecular Mechanics (MCMM) searches were performed on the complexes of **46** with *N*-acetyl-L-valinate (Fig. 4a) and *N*-acetyl-D-valinate (Fig. 4b). In either case, both carbamate NH groups and two guanidinium NH groups were predicted to act as H-bond donors to substrate oxygens. Accordingly, downfield movements were observed for four of the five receptors N*H* resonances on formation of the L-valinate complex.

Table 4
Extraction efficiencies and enantioselectivities (L:D) shown by receptors **41**–**46** in extraction experiments [26,15]

Receptor	Substrate	Extraction efficiency (%)	Enantioselectivity (L:D)
41	*N*-Ac-DL-alanine	52	7:1
41	*N*-Ac-DL-Phenylalanine	87	7:1
41	*N*-Ac-DL-valine	71	7:1
41	*N*-Ac-DL-asparagine	~0	–
41	*N*-*t*-Boc-DL-valine	98	1:1
41	*N*-*t*-Boc-DL-histidine	66	3.5:1
42	*N*-Ac-DL-alanine	41	10:1
42	*N*-Ac-DL-valine	63	9:1
42	*N*-Ac-DL-phenylalanine	90	9:1
43	*N*-Ac-DL-valine	35	2:1
44	*N*-Ac-DL-valine	76	1:1
45	*N*-Ac-DL-valine	69	4:1
46	*N*-Ac-DL-valine	89	9:1
46	DL-phenylalanine	35	2:1

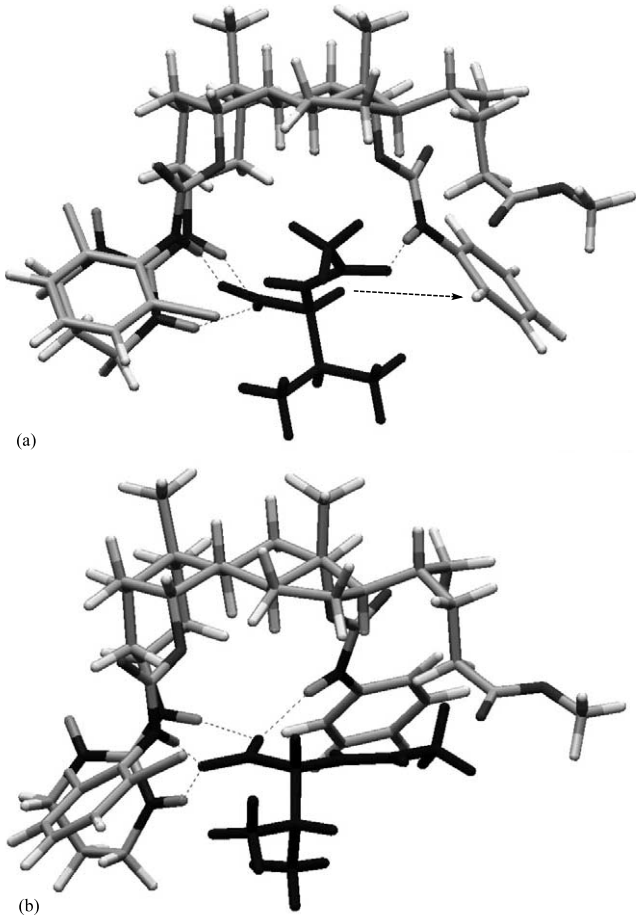
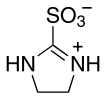


Fig. 4. Structure of: (a) **46** · *N*-Ac-L-valinate; and (b) **46** · *N*-Ac-D-valinate derived from computer-based molecular modelling [15]. The substrate anions are coloured black, intermolecular hydrogen bonds are shown as broken lines. The arrow in (a) represents the observed intermolecular NOE. Although these protons are ca. 3.8 Å from each other in the model, they can be brought within 2.2 Å by rotation about the N–Ph bond.

The major difference between the two complexes relates to the 12-carbamoyl N*H* of **46**. In the case of the L-valinate complex (Fig. 4a), this hydrogen is bound to the acetyl oxygen of the substrate. However, in the D-valinate complex (Fig. 4b), the 12-N*H* binds to a carboxylate oxygen in a distorted, higher-energy arrangement (consistent with the experimental preference for the L-enantiomer). Further NMR evidence, notably an intermolecular NOE (see Fig. 4a), supported the models.

Enantioselective extraction, as shown by **41**–**46**, raises the possibility of enantioselective transport through a non-polar barrier. This phenomenon is potentially attractive as a method for separating enantiomers. A particular advantage is the 'catalytic' nature of the process. A single molecule of receptor can shuttle many substrate molecules between donor and acceptor aqueous phases. Transport studies were undertaken using receptor **53**, based on **42** but with two minor modifications [27]. Firstly, to avoid loss of receptor from organic into aqueous phase, a lipophilic C_{20} side-chain was introduced. Secondly, the six-membered ring guanidinium moiety was replaced by a five-membered ring. The latter could be introduced through a single-step procedure involving reaction of the 3α-NH_2 with reagent **52**, and proved to have no significant disadvantages; extractions with methyl ester **54** confirmed that the enantioselectivity was not degraded. In experiments with a 'U-tube' apparatus, guanidinium **53** proved capable of transporting *N*-acetyl-DL-phenylalanine through CH_2Cl_2 with nearly 70% enantiomeric excess. About 20 equivalents of the substrate were transferred during the experiment. Receptor **53** was also used in a 'hollow-fibre membrane' separator, an apparatus for large-scale aqueous–organic–aqueous transport. Lower e.e.'s were obtained in this case, probably because the apparatus enforced a change in the non-polar solvent (from CH_2Cl_2 to 2.5% octanol in hexane). Nevertheless, the results obtained for both experiments raise hopes for industrial-scale separations in the future.



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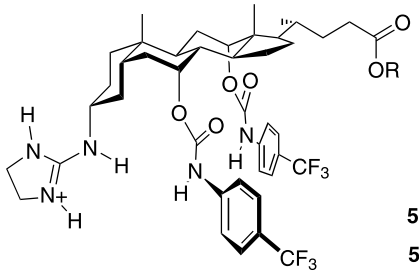
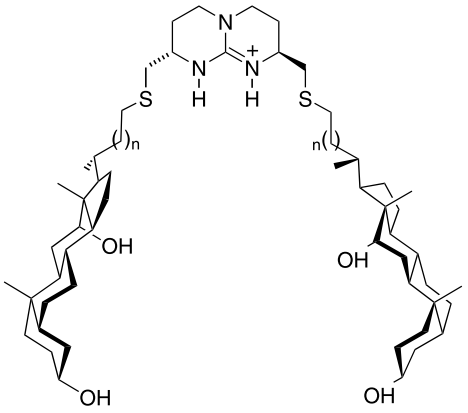


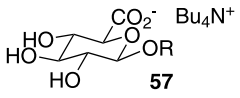
53 R = $C_{20}H_{41}$

54 R = CH_3

Two other groups have combined guanidinium units with steroids to create preorganised receptors. In **55** and **56**, de Mendoza and co-workers employed a bicyclic guanidinium core and side-arms derived from deoxycholic acid [28]. The targets were uronate salts such as **57** and **58**. Association constants in acetonitrile–chloroform mixtures were measured via NMR-titrations. The steroidal units in **56** had little effect on binding, but higher affinities were measured for less flexible **55**. The Kalesse group used a framework derived from corticosterone to position guanidinium units relative to an imidazole, as in **59** [29,30]. The imidazole was expected to act as a general base, promoting cleavage of bound RNA. Compound **59** was capable of cleaving RNA models, although accelerations over background were only moderate.



55 n = 1
56 n = 2



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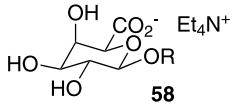

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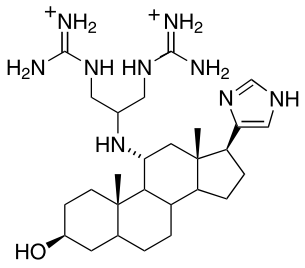

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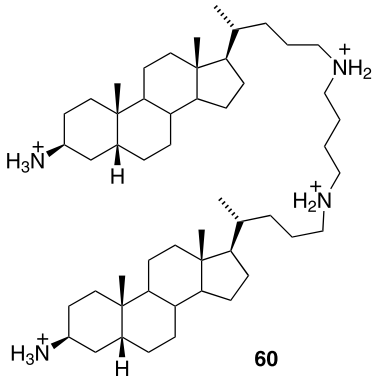
3.2. Protonated aminosteroids

Finally, the steroidal framework has been used to organise ammonium centres, along with other polar functional groups, in systems such as **60**–**63**. Dimeric tetra-ammonium **60** belongs to a series of compounds, derived from deoxycholic acid, lithocholic acid or cholesterol, which were studied by Burrows and co-workers as ligands for DNA [31–33]. Compound **60** was especially effective, showing higher affinities than the natural DNA-complexer spermine [31]. Related compounds were used by Blagbrough and co-workers, for similar purposes [34–36]. 'Facial amphiphiles' **61** [37] and **62** [38] have been shown to act as cytofectins, promoting the delivery of DNA from liposomes to cell



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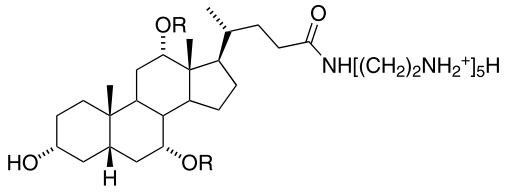
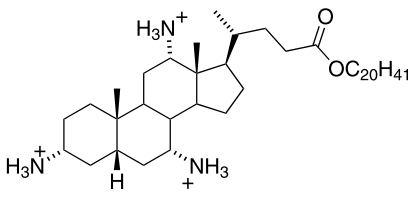


61 R = α-D-glucosyl



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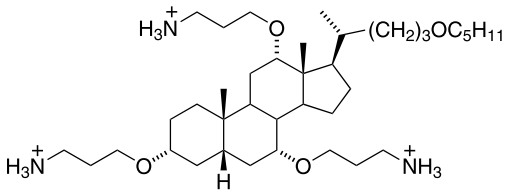

63

nuclei [39]. Facial amphiphile **62** has also been shown to promote 'non-leaky' fusion of liposomes [38]. Tris-ammonium cation **63**, and relatives, permeabilise the membranes of Gram-negative bacteria and possess antibiotic activity [40]. Although, the foregoing compounds have not been studied specifically as anion receptors, their interactions with the phosphate groups in DNA and membrane lipids is presumably critical for their behaviour. Other steroidal amines have been shown to discharge pH gradients across the membranes, probably by transporting H^+X^- [41–43].

4. Conclusion

Steroid-based anion receptors have shown exceptional binding constants, encouraging enantioselectivities and a number of other activities. Much structural space remains unexplored, especially in the series derived from cholic acid (**2**), and further advances can be expected. Priorities for future work will be: (a) the control of selectivities in binding inorganic anions; (b) the demonstration of new properties for steroid-based anionophores, that might parallel the behaviour of cation-binding receptors; and (c) the achievement of truly useful enantioselectivities in binding carboxylates. It is notable that the podand architecture of **21**–**36** and **41**–**46** is amenable to variation through combinatorial chemistry [44,45], and this technique may expand the scope and effectiveness of steroid-based anion recognition in years to come.

Acknowledgements

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