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Making a Match for Valinomycin: Steroidal Scaffolds in the Design of Electroneutral, Electrogenic Anion Carriers

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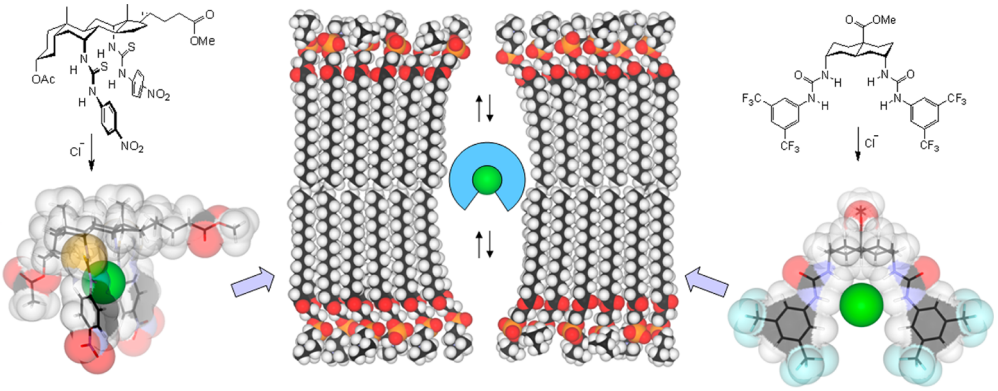

The natural product Valinomycin is a well-known transmembrane cation carrier. Despite being uncharged, this molecule can extract potassium ions from water without counterions and ferry them through a membrane interior. Because it only transports positive ions, it is electrogenic, mediating a flow of charge across the membrane. Equivalent agents for anions would be valuable research tools and may have therapeutic applications, especially in the treatment of "channelopathies" such as cystic fibrosis. However, no such molecules have been found in nature.

In this Account, we describe our research toward synthetic and rationally designed "anti- Valinomycins". As our core approach to this problem, we used the steroid nucleus, provided by cholic acid, as a scaffold for the assembly of anion receptors. By positioning H-bond donors on this framework, especially urea and thiourea groups in conformationally constrained axial positions, we created binding sites capable of exceptionally high affinities (up to 10^{11} M^{-1} for $R_4N^+Cl^-$ in chloroform). The extended hydrocarbon surface of the steroid helped to maintain compatibility with nonpolar media. When we tested these "cholapods" for chloride transport in vesicles, they provided the first evidence for electrogenic anion transport mediated by electroneutral organic carriers: in other words, they are the first authenticated anti-Valinomycins. They also proved active in live cells that we grew and assayed in an Ussing chamber.

In subsequent work, we have shown that the cholapods can exhibit very high activities, with transport observed down to carrier/lipid ratios of 1:250 000. We also understand some of the effects of structure on the activity of these molecules. For example, in most cases, powerful transporters also act as powerful receptors. On the other hand, some modifications which favor binding do not promote transport. We gained functional advantages by cyclizing the cholapod architecture, which encloses the anion binding site. We could also simplify the structure without compromising function. A steroid-inspired *trans*-decalin framework has proved highly effective and may lead to agents with practical advantages. Changing an ester side-chain in this system revealed a surprising effect, whereby increased length and/or lipophilicity resulted in substantially raised activity. Although much remains to be discovered about these anionophores, their high activities and intrinsic tuneabilities bode well for applications. In future work, we plan to develop and exploit these molecules as tools for biophysical research and to explore the possibility of useful biological activity.

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Introduction

Valinomycin **1** (Figure 1a) has been a useful and influential molecule. It is a natural product with strong biological activity but, unlike most such compounds, it does not exert its effect through action on a biomacromolecule. Instead it mimics a biological process normally mediated by proteins, namely, the transport of cations across cell membranes. Unwanted cation transport is severely disruptive to cells, hence Valinomycin's toxic and antibiotic effects. Indeed, Valinomycin is the archetypal example of a group of agents which work in this way, the ionophore (ion-carrying) antibiotics.[1]

The key to Valinomycin's activity is its ability to act as a potassium ion receptor, forming a complex with a hydrophobic exterior.[2] This allows it to bind K^+ across the water–membrane interface, to carry it across the apolar membrane interior, and to release it on the far side (Figure 1b).[3] Surprisingly to many chemists, this neutral molecule extracts only the cation and does not require a counterion; its action is thus electrogenic, resulting in a change of potential across the membrane. It is, moreover, highly cation-selective, with a strong preference for K^+ and no acidic/basic groups which might provide a mechanism for H^+ transport. Its overall effect is thus simple (only K^+ transport) and predictable, leading to widespread use as a research tool in biology. Its theoretical influence has been equally important. Lehn's early research on cryptands was inspired by Valinomycin,[4] so this intriguing molecule may take some credit for the birth of supramolecular chemistry.

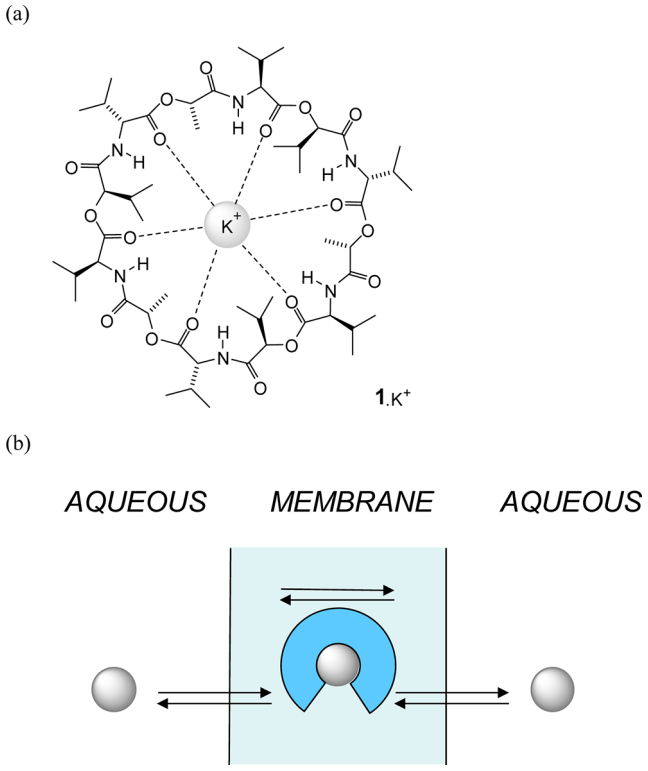


FIGURE 1. (a) Classic ionophore Valinomycin **1**, shown binding to its substrate K^+, and (b) the mobile carrier mechanism for ion transport as employed by Valinomycin and related natural products.

Given the importance of Valinomycin and its relatives, one is bound to ask if its action could be inverted. Can we access an "anti-Valinomycin", that is, an electroneutral, electrogenic carrier for biologically relevant *anions* (most obviously chloride)? There are several motivations. Aside from the theoretical interest, such a molecule would certainly be useful in research, complementing Valinomycin in biological and biophysical investigations. It might also show useful biological activity. In particular, there are genetic diseases which result from malfunctioning or missing anion channels, the most common being cystic fibrosis (CF).[5] Anion transporters might be used to alleviate the symptoms of these conditions through "channel-replacement therapies".[6]

As Valinomycin is naturally derived, one might hope to find its counterpart among secondary metabolites. Curiously, no such molecule has been discovered. A few natural products are known to promote anion transport, an example being the prodigiosins.[7] However, all contain basic centers which can be protonated to form salts with anionic substrates. While this facilitates the passage of anions through the membrane interior, it also raises the possibility of H^+X^- (nonelectrogenic) cotransport. To achieve simple anion transport, with no other effect, it seems we must resort to design and synthesis.

Some years ago, we embarked on a program in synthetic supramolecular chemistry, aimed at anion receptors which might serve as anti-Valinomycins. Herein we describe how this research progressed to give, among other results, the first examples of electrogenic anion transport mediated by neutral organic molecules operating via the mobile carrier mechanism.

Anion Recognition by Neutral Molecules: The Role of Cholic Acid

The first requirement of an anti-Valinomycin is that it should serve as an electroneutral anion receptor. When we first surveyed this area in the early 1990s, anion recognition was already an established area of supramolecular chemistry.[8] However, most effort had focused on cationic receptors, and there were still rather few electroneutral examples. Success had been met by using Lewis acidic metal centers,

but the metals (e.g., Sn, Hg) were not ideal for biological applications.[9,10] Some elegant systems employing oriented dipoles were unfortunately very weak.[11,12] The most promising approach seemed to be the use of neutral H-bond donor groups, pioneered mainly by Reinhoudt and coworkers.[13,14] However, even here affinities were modest. For example, the tren-based tris-tosylamide **2** (Z = Ts)[13] (Figure 2a) was found to bind $Bu_4N^+Cl^-$ with $K_a \sim 4000\ M^{-1}$ in $CDCl_3$ (a noncompetitive solvent for H-bond formation).[15] Typical cation receptors, including Valinomycin, are considerably more powerful.[16] To separate a chloride ion from both water and counterion would surely require fairly strong binding, and it did not seem likely that **2** could succeed.

While Reinhoudt's receptors provided a good starting point, their designs were not ideal. In particular they possessed flexibility which limited preorganization and, critically, allowed the formation of intramolecular hydrogen bonds (cf. Figure 2b). What was needed, it seemed, was a rigid scaffold that would position and separate the H-bond donors. We had become interested in the use of readily available steroids as components for supramolecular chemistry,[17] especially the inexpensive cholic acid **3**. Aside from its rigid framework, this starting material possesses a well-spaced set of differentiable functional groups, apparently suitable for conversion to H-bond donor arrays. Indeed the OH groups already present can serve as such, being codirected and incapable of interacting with each other. The irregular hydrophobic upper surface was also a useful feature if one wished to design membrane-soluble systems.

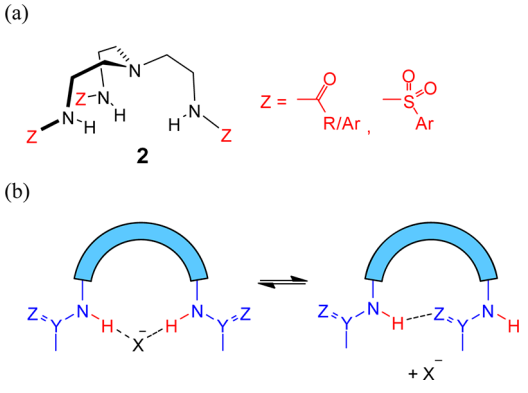
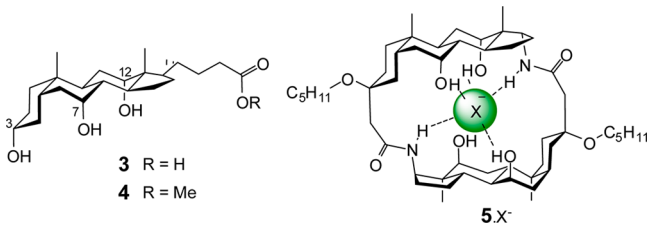


FIGURE 2. (a) Pioneering anion receptors **2** from the Reinhoudt group. (b) The problem faced by these and other systems based on electroneutral H-bond donor groups. Most such groups also contain H-bond acceptors, allowing intramolecular interactions which destroy the binding site.



One's first thought might be that simple esters of **3** could act as receptors or transporters, through H-bond donation from the OH groups to anionic substrates. We tested this and found, for example, that methyl cholate **4** bound $Bu_4N^+TsO^-$ appreciably in deuterobenzene ($K_a \sim 200\ M^{-1}$ for the 1:1 complex).[18] Unfortunately, no interaction was detected in $CDCl_3$, so this seemed unlikely to be useful. Better results were obtained with the "cryptand" **5**, which showed K_a in chloroform of 1000 and 3000 M^{-1} for $Bu_4N^+Cl^-$ and $Bu_4N^+F^-$, respectively.[19] However, despite the (to us) attractive structure, this system was no improvement on the much simpler **2**.

The lesson learnt from this work was that careful positioning of weak H-bond donors (simple OH and amides) was not the most profitable approach. Instead, designs should be built around strong H-bond donors, with potential for adjustment if necessary. We therefore decided to follow Reinhoudt more closely, using a single steroidal scaffold as a core for podand-type receptors. Illustrated in Figure 3, these "cholapods" seemed promising from several viewpoints. A wide variety should be accessible, among which many should be compatible with simple inorganic anions (including chloride, our principle target). Tuning of H-bond donor power should be relatively easy, especially by modifying terminal groups Z. Solubility could be controlled by variation of the side-chain ester.

Most importantly, the steroidal scaffold would confer a high level of preorganization. First, and obviously, the legs would be anchored at precisely defined positions. Second, most designs would benefit from more subtle conformational effects. The 7- and 12-substituents in the cholapods would be axial (as in cholic acid), and this places severe restrictions on bond rotation. In particular, for NHZ groups,

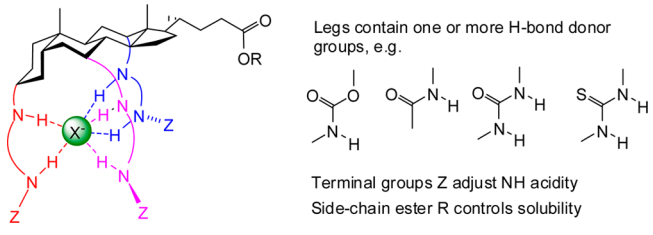


FIGURE 3. Cholapod approach to neutral anion receptors.

there is a strong preference for conformations where the NH is directed inward, under the body of the steroid. This is illustrated in Figure 4a for a NHAc group, for which the calculated energy difference corresponds to $K \approx 10^5$. In a cholapod such as **14** (see below) this orients the 7,12-legs for anion binding and, perhaps more significantly, precludes intramolecular H-bonding between the two groups (see Figure 4b). The C3 substituent is free to rotate but in many cases (including **14**) is incapable of reaching an H-bond donor. The cholapod design thus solves the problem posed in Figure 2b, providing a means of exploiting neutral H-bond donors without the penalty of intramolecular interactions.

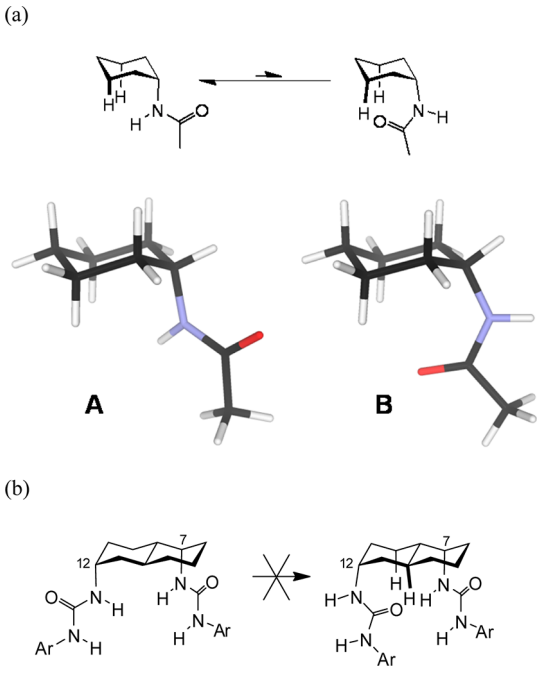


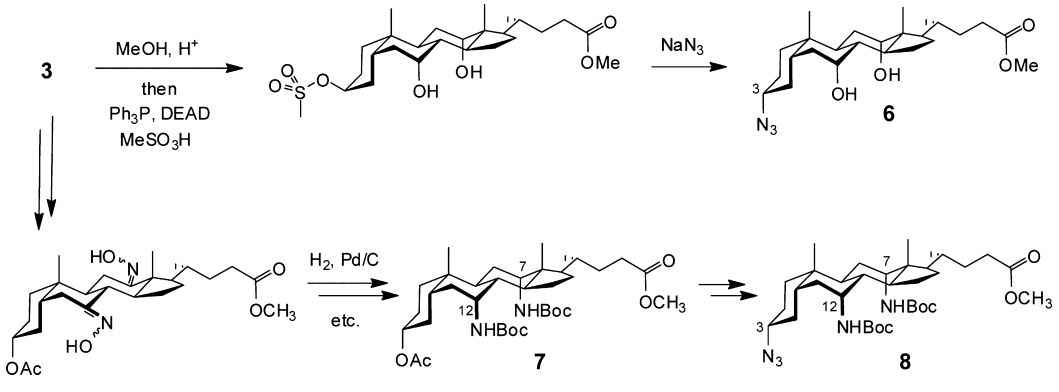
FIGURE 4. (a) Restricted rotation about an axial C-NHAc bond. Ab initio calculations predict that conformation A (NH-in) is more stable than conformation B by 28.4 kJ mol^{-1}. (b) The consequence for a 7,12 bis-ureidocholapod such as **14** (only the central trans-decalin is shown). The binding conformation (left) is enforced, and intramolecular H-bonding (right) is forbidden.

Considering specific designs for cholapods, it became clear that little could be achieved by simple derivatization of cholic acid; all the more promising structures possessed at least one NH group directly attached to the steroid. Our first priority was therefore to synthesize a suite of protected aminosteroids for use as intermediates. A useful early discovery was the transformation of cholic acid **3** into the 3α azide **6** via a novel variant of the Mitsunobu reaction (Scheme 1).[20] Replacing all hydroxyl groups took longer,[21] but eventually we found an efficient procedure, based on stereoselective 7,12-oxime hydrogenation to give bis-Boc-protected **7** followed by the Mitsunobu azidation to give bis-NHBoc-azide **8**.[22,23]

From **6**–**8**, we were able to prepare a good range of cholapods with potential as anion receptors. Early results were encouraging, if not spectacular. In **9** (Figure 5), a tosylamide and two carbamate groups resulted in $K_a = 7000$ M^{-1} for $Bu_4N^+Cl^-$ in $CDCl_3$ (measured by NMR titration).[24] In **10**, three tosylamides raised this figure to 100 000 M^{-1}, a useful advance on the 4000 M^{-1} measured for **2** (Z = Ts) (see above).

The next move, clearly, was to add further H-bond donors (e.g., by employing urea groups) as well as electron-withdrawing units to increase NH acidity. Here we benefited from the extended, irregular nature of the scaffold. Ureas may have excellent potential for anion binding, but they also promote insolubility in nonpolar media. In the case of the cholapods, we were confident that (a) solubilities would be relatively high and (b) any problems could be solved by changing the side-chain ester. Before proceeding, however, we faced a difficult decision. The binding constant of 10^5 M^{-1} obtained for **10** was around the highest that could be measured by ^{1}H NMR titration. How would we assess the binding power of stronger receptors? One solution was to change to a more polar and competitive solvent (e.g.,

SCHEME 1. Synthesis of Protected Aminosteroidal Intermediates



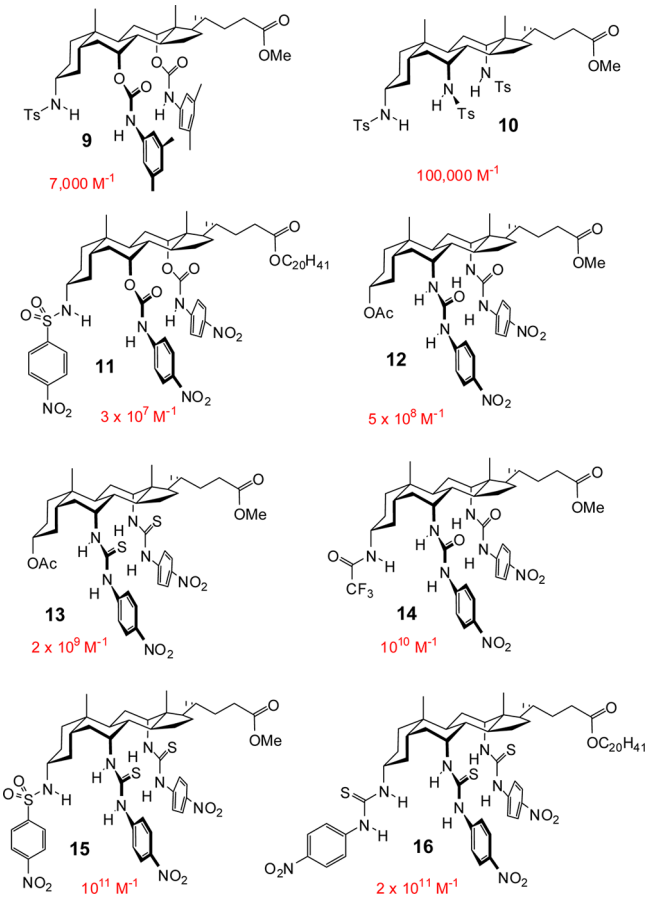


FIGURE 5. Cholapod anion receptors, with binding constants to $R_4N^+Cl^-$ in chloroform (red text). The values for **9** and **10** were measured by ^1H NMR titration in $CDCl_3$, with $Bu_4N^+Cl^-$ as substrate. The values for **11**–**16** were measured by extraction with $Et_4N^+Cl^-$ as substrate, and refer to water-saturated $CHCl_3$ as solvent.

DMSO). However, in the longer term we hoped to employ our receptors in an apolar environment, the membrane interior. It was not clear that their behavior in DMSO would be relevant for this application. The alternative was to continue in chloroform, but in this case a new method was needed. Fortunately, D. J. Cram had faced a similar situation in work on cation receptors and had developed a procedure based on the extraction of ion pairs from water.[25] The method is uniquely suitable for measuring high binding constants, because receptor saturation may be avoided by choice of counterion (picking one which is more difficult to extract) and also by diluting the substrate solution. For anion binding, we found that tetraethylammonium salts were suitable substrates, and proceeded to calibrate the method for Cl^-, Br^-, I^-, NO_3^-, AcO^-, ClO_4^-, and $EtSO_3^-$.[26,27] The approach does have disadvantages. Assumptions are made about stoichiometry and so forth for which, unlike titration with curve-fitting, there is no internal check. Also, tetraalkylammonium

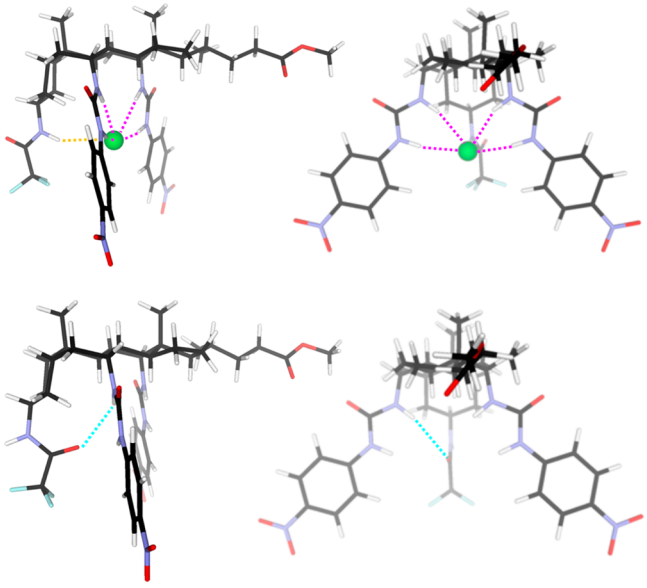
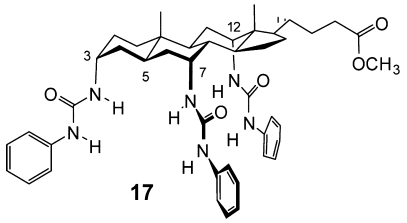


FIGURE 6. Modeling cholapod receptor **14**. Top: Complex **14**.Cl^- shown from two viewpoints. Chloride (green) is bound by four hydrogen bonds in the range 2.4–2.6 Å to urea N*H* (magenta), with a further interaction to the CF_3CONH (2.9 Å, orange). Bottom: On removal of the chloride, followed by Monte Carlo molecular mechanics, the urea positions are almost unchanged. The CF_3CONH rotates to bring the carbonyl closer to the urea NH groups, but the shortest O···H distance (cyan) is 3.1 Å. At best, this represents a very weak H-bond.

salts are strongly associated in chloroalkane solvents,[28] so the affinities presumably refer to tight ion pairs rather than separated anions. On the other hand, extraction ability is critical in the search for anion carriers, so a method based on phase transfer has particular relevance.

Once this assay was available, the potential of the cholapod design could be realized. Steroids **11**–**16** in Figure 5 represent a small sample of the structures that were prepared and tested.[26,27,29] As we had hoped, affinities rose quickly once ureas (and especially thioureas) and nitrophenyl groups were introduced. Indeed it proved remarkably easy to achieve very high binding constants of 10^{11} M^{-1} and above. One of our compounds, the eicosyl ester analogue of **15**, was also tested using voltammetry at the ITIES (interface between two immiscible electrolyte solutions), in a collaboration with R. Dryfe (University of Manchester). Unusually, this method gives the binding constant to the isolated anion, not the salt. The value obtained was still higher, at 5×10^{12} M^{-1} for chloride in 1,2-dichloroethane.[30] This increase is perhaps unsurprising, given the lower polarity of the solvent and absence of interference from a counterion. Modeling reinforced the view that preorganization, conferred by the steroidal framework, was a major factor in the success of these receptors. For example, as shown in Figure 6, cholapod

14 is able to form four hydrogen bonds to chloride of ~2.5 Å (close to the expected value),[31] supplemented by a weaker but significant interaction (2.9 Å) to the 3-NHCOCF$_3$ group. When the chloride is removed, the ureas undergo very little movement, implying that strain in the complex was minimal. The NHCOCF$_3$ rotates, attempting to form H-bonds with the urea NH groups. However, it can only achieve a CO···HN distance of 3.1 Å, implying a very weak interaction. Even this freedom can be removed by inverting steroidal C5, so that all the functionalized positions become axial. Indeed, tris-urea **17** was synthesized and found to be 2–5 times more powerful than the corresponding C5-epimer.[32] However the synthetic effort was considerable and this path was not explored further.



Cholapods as Anion Transporters

At this stage, we knew that we could make chloroform-soluble receptors with chloride affinities rising to very significant levels. The prospects for an "anti-Valinomycin" seemed good enough to begin direct testing. We were not equipped to perform this ourselves, but fortunately we were collaborating with the group of B. D. Smith in the University of Notre Dame. They had already shown that cholapods could act as "synthetic flippases", capable of transporting phospholipid head-groups through membrane interiors.[33,34] The transport of inorganic anions was a natural extension.

Membrane transport is often studied in synthetic phospholipid vesicles, using analytical techniques which can detect concentration changes in the entrapped volume or the external medium. Some studies had already taken place in other laboratories, working on synthetic channels and positively charged systems.[35–38] We decided to adopt an approach used by Gokel et al.,[37] in which a chloride salt is trapped in the vesicles and transporter-induced efflux is detected with a chloride-selective electrode (Figure 7). Note that in all such systems there must be some process which maintains charge neutrality on both sides of the membranes. If the transporter is electrogenic (affecting only anions) and working alone, it must also promote influx of the external anion X^- (antiport mechanism). The selection of X^- is therefore potentially important. We chose nitrate, which is more lipophilic than chloride (therefore easier

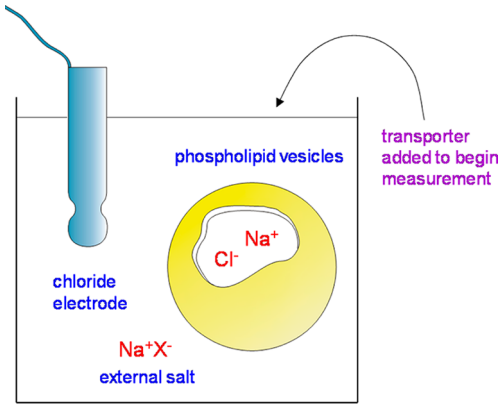


FIGURE 7. Ion selective electrode (ISE) assay for anion transport. Vesicles were ~200 nm diameter, composed of POPC and cholesterol in the ratio 7:3.

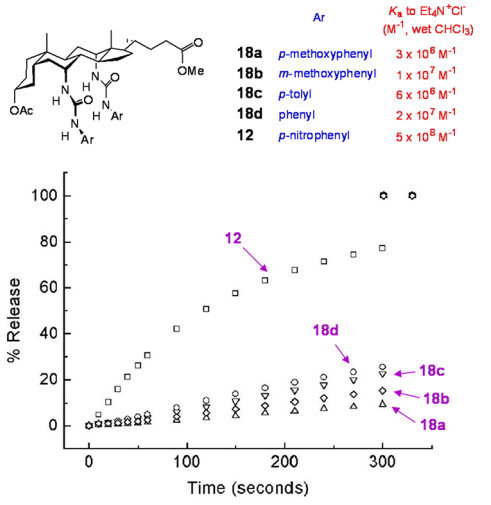


FIGURE 8. Chloride release from vesicles promoted by bis-ureidocholapods (cholapod/lipid = 1:250).

to transport)[39] and a good substrate for most cholapod receptors.[27]

Initial experiments were performed on a series of bis-ureidocholapods, **18a–d** and **12** (Figure 8).[40] Lacking a H-bond donor at position 3, these compounds were not the strongest receptors we had made; **12**, the most powerful, bound $Et_4N^+Cl^-$ with K_a of just $5 \times 10^8\ M^{-1}$. Nonetheless, as shown in Figure 8, the results were positive. The molecules clearly promoted chloride efflux to a degree which, as expected, correlated roughly with anion affinity. The effect of **12** was especially dramatic, with 80% chloride release after 5 min. Importantly, the rate of transport was sensitive to the external anion X^-. Replacement of nitrate with the hydrophilic sulfate halted transport, proving that neutrality was maintained by the antiport mechanism and that the cholapods were therefore electrogenic. For $X^- = HCO_3^-$, choride efflux was observed at an intermediate level,

implying that bicarbonate was transported but less well than chloride. Several tests indicated the mobile carrier mechanism rather than self-assembled channels.[40,41] In summary, the cholapods were electroneutral organic molecules acting as electrogenic anion carriers; by the standards we had set ourselves, true "anti-Valinomycins".

There was obvious interest in discovering whether the cholapods, like Valinomycin, could exert their effect in live cells. To test this, we engaged the help of another collaborator, D. N. Sheppard of the University of Bristol Department of Physiology. His group employed the Ussing chamber method, in which a live epithelium is grown on a filter support and placed between electrodes. Endogenous mechanisms create a potential difference across the epithelium, and agents which induce conductivity allow current to flow. As shown in Figure 9, cholapod **12** produced a clear response. THF, the vehicle used to deliver the cholapod, also gave a signal, but the difference was substantial (and statistically significant).[40]

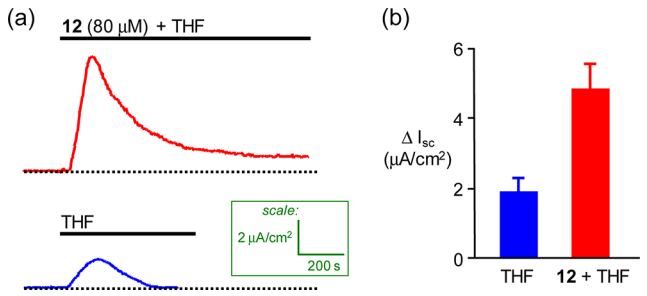


FIGURE 9. Chloride current generated in Madine Darby kidney cell (MDCK) epithelia by cholapod **12**. (a) Evolution of short-circuit current (I_{sc}) with time on addition of **12** in THF, compared to THF (representative traces). (b) Averaged values for peak ΔI_{sc}.

As **12** was not our best receptor, it seemed unlikely it would prove the best transporter. We therefore began a more comprehensive program of testing. However, fairly quickly it was realized that the ISE method had limitations. To perform well, a transporter had to reach the vesicle membranes and it seemed that delivery of some cholapods was poor. Of course, "deliverability" would be a critical parameter for a practical anionophore, but the ability to measure intrinsic transport ability was also important. We therefore needed a method which allowed the transporter to be preincorporated in the vesicles if desired. In response to this problem, the Smith

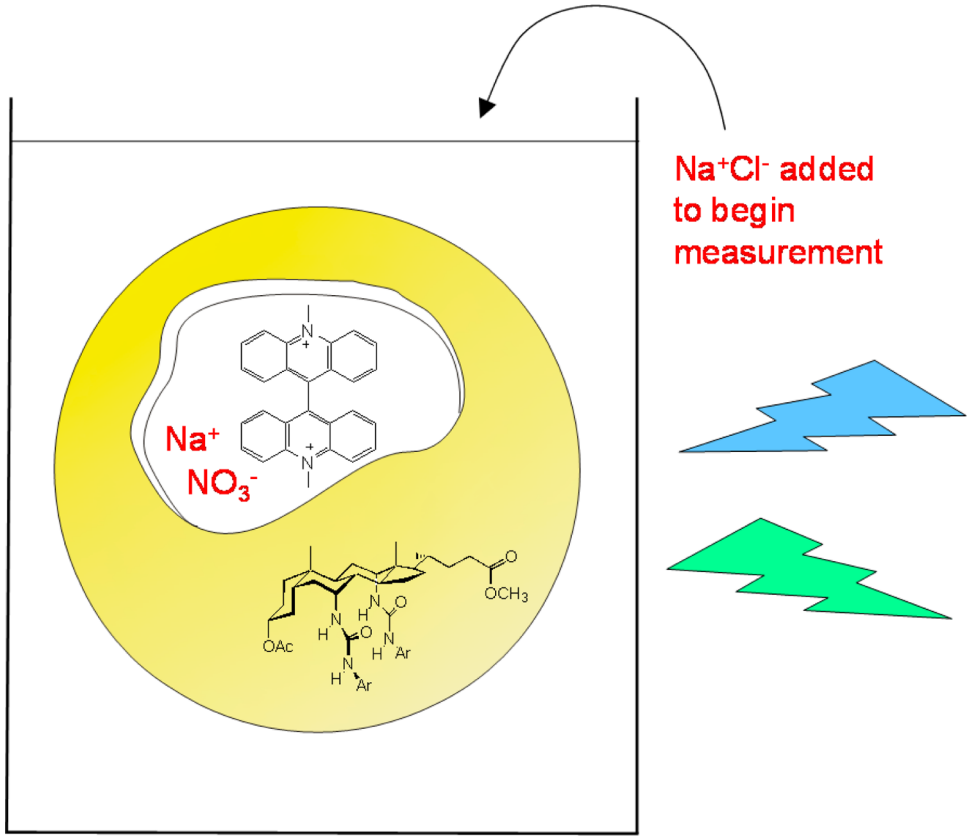


FIGURE 10. Lucigenin-based assay for anion transport. The vesicles entrap $NaNO_3$ and lucigenin, and the vesicle membranes contain transporter (which may be incorporated during vesicle formation, or added to preformed vesicles). After addition of NaCl, chloride influx is monitored by following the reduction in lucigenin fluorescence.

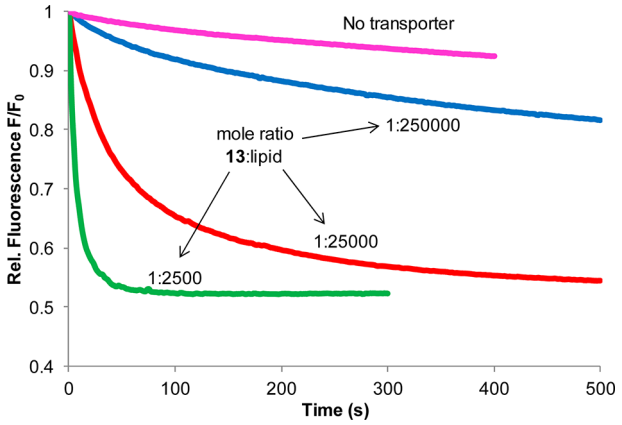


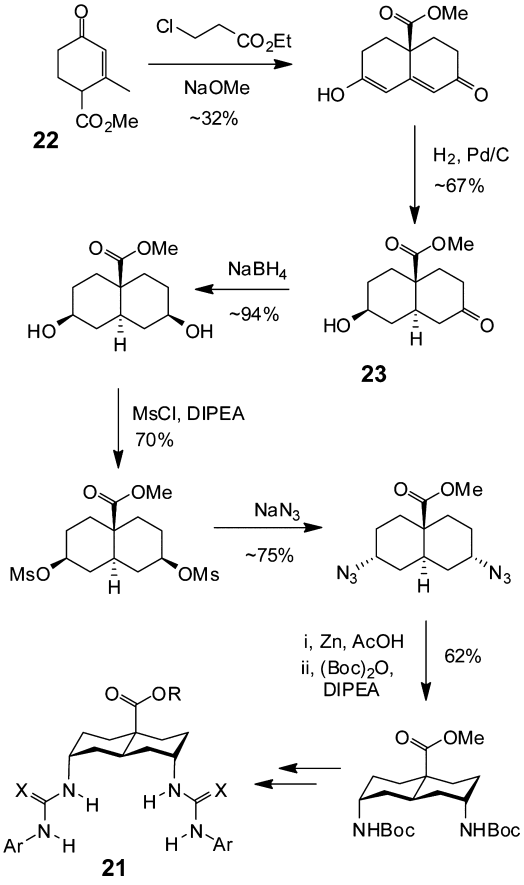
FIGURE 11. Fluorescence output traces from transport experiments employing cholapod **13** (lucigenin method, see Figure 10).

group developed a versatile assay in which chloride transport *into* the vesicles was monitored.[42] As shown in Figure 10, this was achieved through use of lucigenin, a fluorescent dye which is subject to quenching by chloride anions. The experiment is initiated through addition of chloride, so the transporter can be present during vesicle formation.

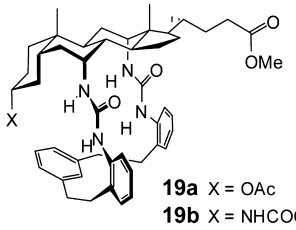
The lucigenin method was used to test a panel of 16 different cholapods, including **12**–**15** (Figure 5).[41] In some respects, the results were puzzling. In keeping with the earlier data (Figure 8), there seemed to be a general correlation between binding power and transport ability. Thus, all of **13**–**15** were superior to **12**. On the other hand, there were several exceptions. In particular, the effect of the substituent at steroidal C3 was difficult to understand. For example, OAc does not contribute to binding, but proved relatively favorable for transport. Whatever the underlying reasons, the best cholapods could be remarkably efficient. Traces due to **13**, the most active from this series, are shown in Figure 11. It can be seen that measurable transport was observed even at cholapod/lipid = 1:250 000. At this loading, we estimate that most vesicles will contain just one or two molecules of transporter.

As binding power was clearly not the only factor in play, we considered other options. Overall lipophilicity did not seem a likely candidate, as all cholapods seemed hydrophobic enough to locate exclusively in membranes. Indeed, cholapods with methyl and eicosyl ester side-chains showed identical activities.[41] An interesting thought was that enclosure of the bound chloride, fully separating it from water, might facilitate passage through the membrane. Valinomycin is noted for encapsulating potassium,[2] and this may be important for its activity. We therefore synthesized the cyclic "cholaphane" transporters **19** and applied the

SCHEME 2. Synthesis of Decalin-Based Transporters **21**



lucigenin assay. Both were significantly more active than acyclic analogues (**19a** by a factor of 20).[43] This approach is synthetically challenging, and further work may not be profitable in the short term. However ultimately, we believe, the most effective transporters may exhibit this feature.



19a X = OAc
19b X = $NHCOCF_3$

Steroid-Inspired Anion Carriers: Diaxial Diureidodecalins

Despite the success of the cholapods, the steroidal scaffolds confer some disadvantages. A practical anionophore should be deliverable to cell membranes, and must therefore be dispersible in water. Very high lipophilicities may therefore be counterproductive. High molecular weights are also disadvantageous, especially if one aims for orally active agents.[44] The steroids are both large and hydrophobic, so that typical cholapods possess MW >700 and clogP ≥ 8.

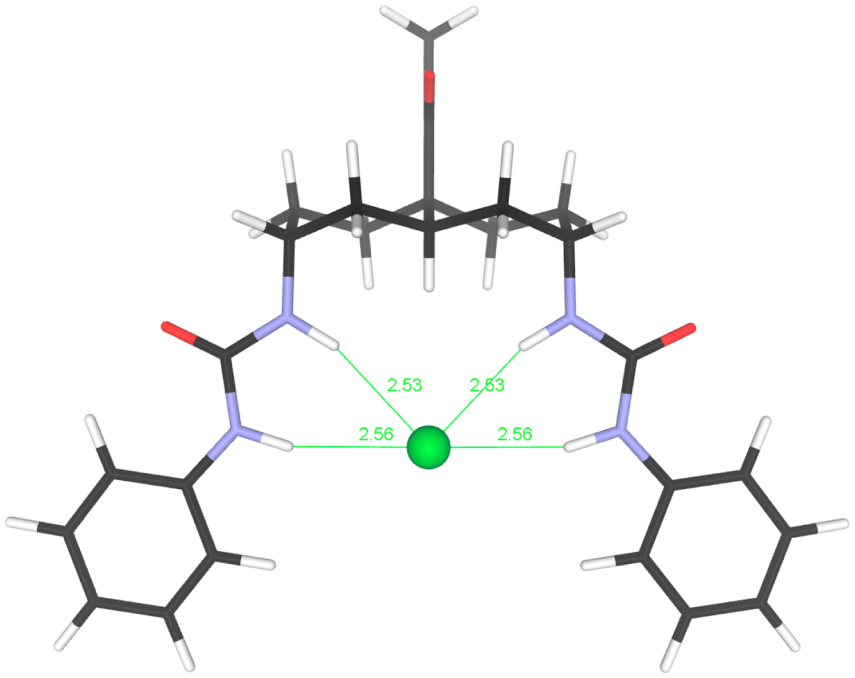
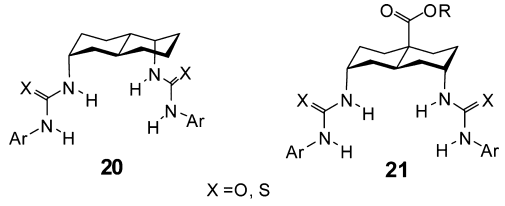


FIGURE 12. Ab initio calculated structure for **21** (R = Me, X = O, Ar = Ph) binding chloride. NH···Cl$^-$ distances range from 2.53 to 2.56 Å.

However, parts of their frameworks seemed dispensable, so we therefore considered "stripped down" analogues.

In particular, it was clear that most of the cholapods' binding/transport activity derived from the central *trans*-decalin portion **20**. While **20** seemed an interesting design for compact analogues of cholapods, the synthesis might not be straightforward. On the other hand, we realized that the closely similar **21** should be readily available. Hagemann's esters such as **22** could be transformed to decalins such as **23**, following the lead of Jones and Dodds[45] (Scheme 2). Conversion of **23** to **21** would require just functional group transformations. The ester group in **21** would allow tuning of lipophilicity and solubility. Calculations confirmed that the positioning of the ureas was fully compatible with chloride binding (Figure 12).



A series of diureidodecalins **24**–**26** (Figure 13) was synthesized via the route shown in Scheme 2.[46] Binding to chloride in chloroform was difficult to quantify, as NMR titrations indicated multiple stoichiometries. However the application of Cram's extraction method gave apparent

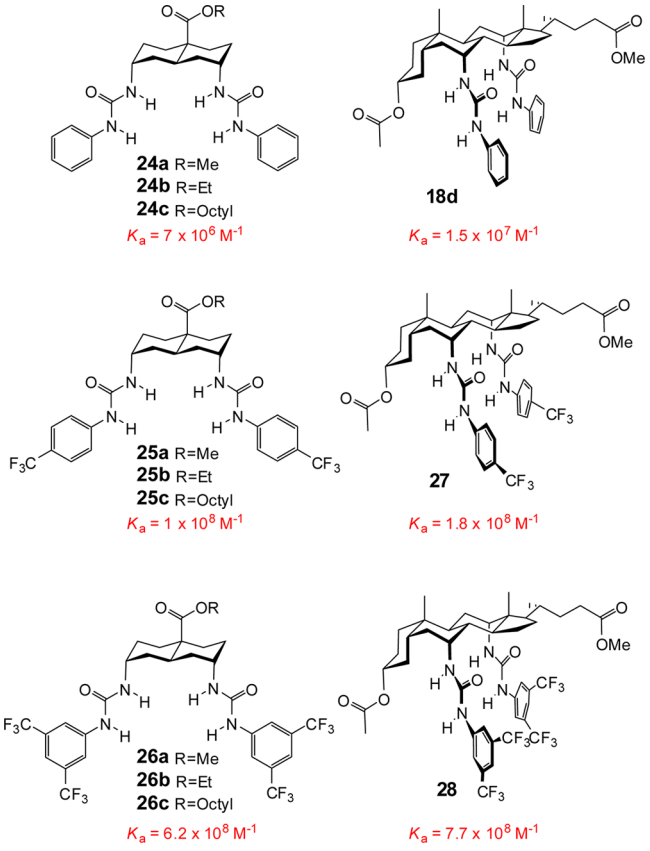


FIGURE 13. Decalin-based receptors and cholapod analogues. Apparent binding constants to $Et_4N^+Cl^-$ in wet chloroform, obtained using Cram's extraction method, are shown in red. For **24**–**26**, the measurements were performed on the octyl esters.

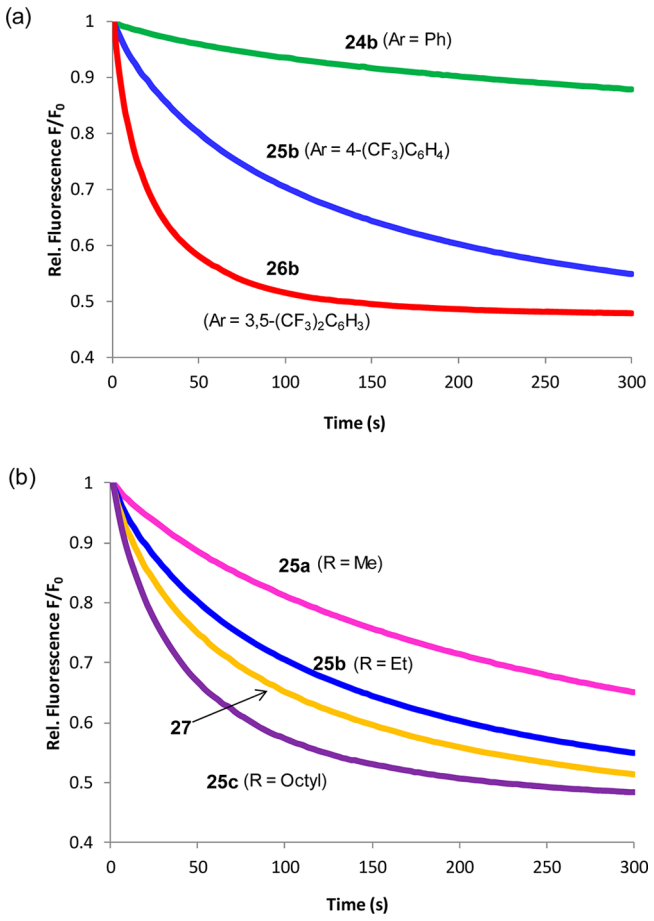
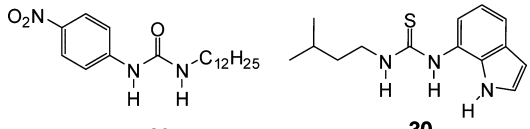


FIGURE 14. Fluorescence output traces from transport experiments (lucigenin method) employing decalin-based receptors **24**–**26** and cholapod **27** preincorporated into vesicles in a 1:2500 receptor/lipid ratio. (a) Variation of urea HNAr. (b) Variation of ester OR and comparison with cholapod.

affinities which tallied well with those of comparable cholapods (Figure 13). The nine variants were then tested for anion transport by the lucigenin method (Figure 10). As expected, they showed good activity which increased in parallel with the anion affinities (Figure 14a). More surprisingly, their performance was strongly affected by the side-chain ester. Increasing the length by just one carbon (Me to Et) yielded significant improvements, while the octyl esters were still more effective (Figure 14b). This would be easy to understand if the less lipophilic methyl esters were leaching from the vesicle membranes, but control experiments showed that this was not the case. Instead it seems that the length of the side-chain, or perhaps the overall lipophilicity of the system, is important in determining intrinsic transport activity. At present, we have no detailed explanation for this phenomenon. Nonetheless, it was pleasing to find that the octyl esters were very effective in absolute terms, showing measurable transport (again) down to decalin/lipid =1:250 000.[46] Indeed, they were slightly more active than the corresponding cholapods, as illustrated in Figure 14b. From a practical viewpoint, it is perhaps unfortunate that the most active decalins are also the largest and most lipophilic (i.e., the least different from the cholapods). However, the compact and tunable nature of this design allows room for many variations, and we believe that the decalin scaffold provides an excellent platform for future development.

Conclusions

The rational design of functional molecules is still a challenging task, especially where the function concerned has little or no precedent. However, it may not be unrealistic, and this Account describes one case where, arguably, it was possible to succeed. At the start of this program, there was no electroneutral organic molecule known to act as an electrogenic anion carrier, at the end there were examples with very promising activities. The route taken was not necessarily the most efficient, in that significant effects could have been achieved by simpler structures. For example, when performing controls for the cholapod work, we ourselves found that urea **29** was moderately active (though ~50 times less so than **12**).[41] More recently, others have shown that systems such as **30** and **31** are also quite effective.[47,48] Nonetheless, the activities of the cholapods and decalins remain exceptional and are likely to increase further. Current work focuses on the demonstration and application of these molecules in biological contexts. We have real hope that one or more will emerge as practical tools for biophysical research. For therapeutic applications, there are further challenges (e.g., delivery to relevant cell membranes, retention in membranes), but if these problems can be solved the impact could be considerable.



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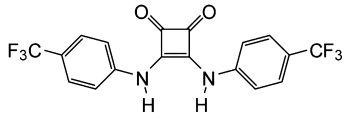

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Hennie Valkenier was born in Roden, The Netherlands in 1983. She obtained her B.Sc., M.Sc., and Ph.D. in Chemistry from the University of Groningen. Her Ph.D. research with Kees Hummelen focused on π-conjugated molecular wires and switches and their electrical conductance when embedded in various junction geometries. She is currently working with Anthony Davis at the University of Bristol on the synthesis and testing of anion carriers for biomedical applications.

Anthony P. Davis gained a B.A. in Chemistry from Oxford University in 1977, and then stayed for a D.Phil. under Dr. G. H. Whitham and postdoctoral work with Prof. J. E. Baldwin. In 1981, he moved to the ETH Zürich as a Royal Society European Exchange Fellow working with Prof. A. Eschenmoser, and then in 1982 was appointed Lecturer in Organic Chemistry at Trinity College, Dublin. In September 2000, he moved to the University of Bristol, where he is Professor of Supramolecular Chemistry in the School of Chemistry. His research interests include the supramolecular chemistry of carbohydrates and anions, and the study of steroid-based nanoporous crystals.

FOOTNOTES

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The authors declare no competing financial interest.

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