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JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Diaxial Diureido Decalins as Compact, Efficient, and Tunable Anion Transporters

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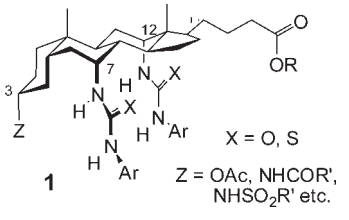
Supporting Information

ABSTRACT: Decalins bearing two axial −NHCONHAr substituents and an ester-linked alkyl side chain have been synthesized and studied as anion receptors and transporters. The design relates to steroid-based "cholapods" but is more compact and less intrinsically lipophilic. Transport rates depend on both NHAr and the alkyl side chain. High activities can be achieved; with optimal substitution, chloride-nitrate exchange across vesicle membranes is measurable at transporter/lipid ratios as low as 1:250 000.

Transmembrane anion transport attracts increasing interest from supramolecular chemists.[1] Although many small molecules are known to promote cation transport across cell membranes, there is still a shortage of corresponding agents for anions. The transport of inorganic anions (mainly chloride and carbonate) is an important biological process, and defects in natural anion channels underlie a number of major genetic disorders[2] (notably cystic fibrosis[3]). Synthetic anionophores might be used to treat these "channelopathies" by replacing the missing activity, and would certainly be valuable as research tools.

Among the growing variety of synthetic anion transporters, there are systems thought to act as static channels,[4] mobile carriers,[5] and relays.[6] However, despite the range of structures and mechanisms, activities have tended to remain quite low. Thus studies in vesicles (the most common experimental platform) are often carried out at transporter lipid ratios ≥1:100.[7] In this context, the cholapods[8] **1** are exceptional. These steroid-derived molecules are powerful anion receptors, showing K_a up to 10^{11} M^{-1} for chloride in chloroform.[9] Their high affinities, combined with lipophilicity, allow them to act as efficient anion carriers.[10] Activities are excellent at transporter/lipid = 1:25 000, and measurable at ratios as low as 1:250 000.



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Although successful, the cholapods inherit potential disadvantages from their steroid precursors. First, their molecular weights are >700, too high to be drug-like[11] (still less "lead-like"[12]) and possibly large enough to retard movement across a membrane. Second, they are very lipophilic, with calculated[13] logP around 8 or more. While transporters need to partition from water into membranes, this value may be too high; certainly it is well above the limit of 5 for drug-like molecules,[11] and is likely to impede delivery to target membranes. In response to these issues, we now report a second family of powerful anion transporters based on a smaller, less lipophilic scaffold. Conceptually related to the cholapods, the new system is complementary in matching activities while offering a different set of molecular and physical properties.

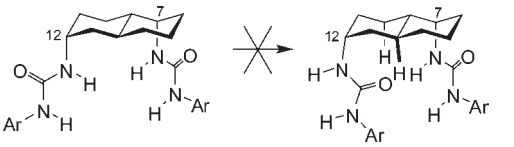


Figure 1. The core decalin unit in cholapods **1**. The steroid numbering is retained and the system has been reoriented for clarity. Rotation about axial C−N is restricted due to potential steric clashes between urea oxygen and axial CH groups. This preorganizes the NH groups for binding and prevents counterproductive intramolecular C═O···HN hydrogen bonding. Inward-directed 7- and 12-NH groups are observed in crystal structures of **1**.[14]

At the core of cholapods **1** is a *trans*-decalin system substituted with two axial urea groups. This unit provides 4 H-bond donors which are favorably positioned for anion binding due to restricted rotation about the axial C−N bonds (Figure 1). The third substituent Z can also act as an H-bond donor, but this is less important; variants with Z = OAc can be highly active.[10a,10c] We therefore proposed that a "stripped-down" analogue with a minimal *trans*-decalin scaffold might be equally effective.

While the arrangement in Figure 1 could be accessible, the achiral design **2** seemed more practical. As discussed below, an efficient synthesis could be envisaged via Hagemann's esters **3**. Restricted rotation about the axial C−N bonds in **2** provides the same advantages as for **1**. Indeed, molecular modeling confirmed that intramolecular hydrogen bonding should be strongly disfavored in the free receptors.[15] Meanwhile, as illustrated in Figure 2, calculated structures for **2**·Cl^- featured realistic NH···Cl^- distances[16] and apparently unstrained conformations. The molecular weight for **2a** is 464, with calculated logP = 4.51. The ester group presents an opportunity for varying properties without affecting the binding site. Systems related to **2** have been discussed as potential oxoanion receptors,[17] but no syntheses have been reported.

Received: August 23, 2010
Published: January 21, 2011

dx.doi.org/10.1021/ja1076102 | J. Am. Chem. Soc. 2011, 133, 1614–1617

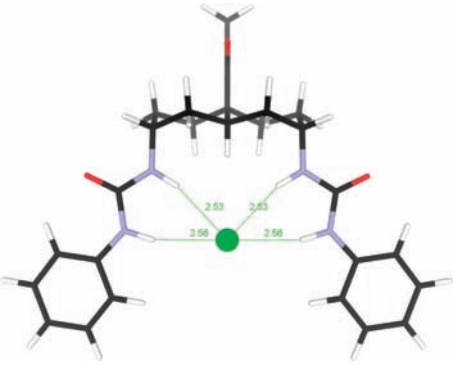
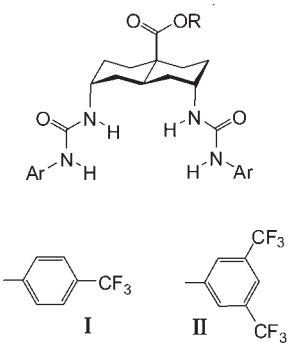


Figure 2. *Ab initio* calculated structure for **2a** binding chloride anion.[18] All four N−H and the Cl^- are roughly coplanar, with H···Cl^- ~2.5 Å.

To construct bis-ureas **2**, we first sought a route to appropriate bis-amino scaffolds. As shown in Scheme 1, the bis-Boc-protected diaminodecalins **6** could be prepared from Hagemann's esters **3**.[18] Conversion of **3** to **4**, following methodology of Jones,[19] was followed by stereoselective reduction, mesylation, and azide displacement to give **5**.[20] Azide reduction and protection as Boc gave **6**, convenient and safe intermediates for long-term storage. The sequence was performed for R = Me and Et. In the latter case, the starting material **3** (R = Et) was commercially available; in the former case, ester **3** (R = Me) was prepared in a single step from methyl acetoacetate and paraformaldehyde.

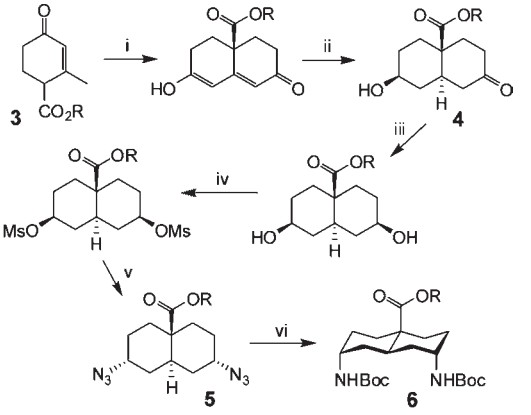


2a R = Me, Ar = Ph
2b R = Me, Ar = I
2c R = Me, Ar = II
2d R = Et, Ar = Ph
2e R = Et, Ar = I
2f R = Et, Ar = II
2g R = Octyl, Ar = Ph
2h R = Octyl, Ar = I
2i R = Octyl, Ar = II

Removal of the Boc protection in **6** (R = Me), followed by reaction with appropriate aryl isocyanates, gave **2a**−**c**. Similar treatment of **6** (R = Et) gave **2d**−**f**. We chose to focus on trifluoromethyl-substituted arylurea groups, because the CF_3 units increase H-bond donor power without causing solubility problems. Octyl-substituted ureas **2g**−**i** were also prepared, to provide variants with increased lipophilicity. Demethylation of **2a**−**c** with LiBr in DMF gave the corresponding carboxylic acids, then O-alkylation with octyl iodide/K_2CO_3/DMF gave the octyl esters. An NOE spectrum of **2c** showed a strong correlation between the CHN*H* signal and that for the decalin ring junction C*H*, consistent with the expected conformation.[18] The cholapods **1a**−**c** [Z = OAc, X = O, R = Me. Ar = Ph, **I** and **II**, respectively] were synthesized as comparisons, using previously reported procedures.[10a]

Decalins **2** were first assessed as anion receptors. ^{1}H NMR titrations were performed for ethyl esters **2d**−**f** against $Et_4N^+Cl^-$ in $CDCl_3$. The NH signals could not be followed due to broadening, but appeared at high chemical shifts (δ = 8.7−9.2 for ArN*H*, 7.1−7.2 for CHN*H*) after 1 equiv of Cl^- had been added. No further movement was observed after addition of further Cl^-. These data are consistent with strong 1:1 complex formation

Scheme 1. Synthesis of Intermediates 6[a]



[a] Reagents and conditions: (i) ethyl 3-chloropropionate, NaOMe/MeOH, 27−37%. (ii) H_2 (4 atm), Pd/C, diethyl ether, 65−69%. (iii) $NaBH_4$, MeOH, 0 °C, 91−96%. (iv) MsCl, DIPEA, MeOH, 70%. (v) NaN_3/DMF, 100 °C, 24 h, 72−77%. (vi) Zn, AcOH, then $(Boc)_2O$, DIPEA, MeOH, 62%.

involving all four NH groups, as shown in Figure 2. The aromatic CH protons also moved during the titrations, and could be followed throughout. Again no change was observed after 1 equiv of substrate, implying strong 1:1 complexation. However, movements before this point were not linear with concentration, implying that complexes with 2:1 receptor/chloride stoichiometry could also be formed. Indeed, modeling revealed convincing structures for such species.[18] To estimate binding strengths, we used Cram's extraction technique,[21] as previously applied to the cholapods.[22,9b] Although errors are introduced by the mixed stoichiometries, the results suggested that the steroid and decalin scaffolds are roughly equally effective. Thus, apparent affinities for decalins **2g**−**i**[23] to $Et_4N^+Cl^-$ in chloroform were 7 × 10^6, 10^8, and 6.2 × 10^8 M^{-1}, respectively, while the corresponding values for **1a**,[10a] **1b**, and **1c** were 1.5 × 10^7, 1.8 × 10^8, and 7.7 × 10^8 M^{-1}.[18] As expected, electron-withdrawing Ar groups increased affinities in both series.

Anion transport properties were assayed in large unilamellar vesicles (200 nm mean diameter) composed of 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) and cholesterol in a 7:3 ratio. Decalins **2** or cholapods **1** were preincorporated[24] in the vesicle membranes at transporter/lipid mole ratios of 1:2500 to 1:250 000. Following previously reported methodology,[10b] the vesicles were prepared with internal and external aqueous $NaNO_3$ (225 mM) and internal lucigenin (1 mM). After addition of sodium chloride (25 mM) to the suspension, the influx of Cl^- was followed through the decay in lucigenin fluorescence. Dynamic Light Scattering measurements confirmed that the vesicles remained intact during the experiments.[18]

Selected traces from the experiments are shown in Figure 3. Diureidodecalins **2** were found to be effective transporters of chloride, and presumably also of nitrate (which must be counter-transported to preserve electrical neutrality). As expected, activity increased with electron-deficiency of Ar, in line with the binding results (Figure 3a). Less predictably, effectiveness was quite strongly dependent on the ester group R. As shown in Figure 3b for Ar = 4-trifluoromethylphenyl, activities increased in the order R = Me < Et < Octyl. The most active of this series, octyl ester **2h**, was slightly more effective than the comparable

dx.doi.org/10.1021/ja1076102 |*J. Am. Chem. Soc.* 2011, 133, 1614–1617

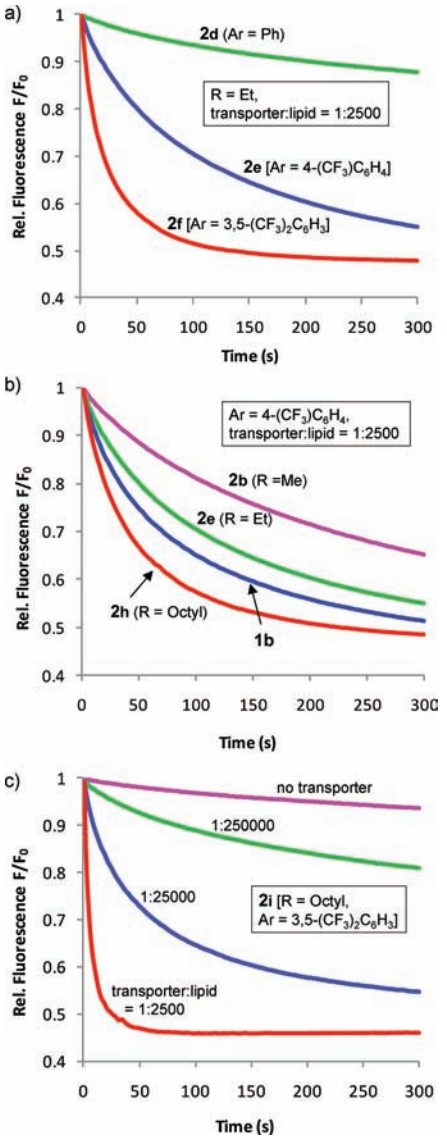


Figure 3. Chloride transport by **2** into vesicles containing $NaNO_3$ (225 mM) and lucigenin: (a) varying Ar (R = Et, transporter/lipid = 1:2500); (b) varying R (Ar = 4-trifluoromethylphenyl, transporter/lipid = 1:2500). Data for cholapod **1b** are also shown. (c) Varying the transporter.lipid ratio for **2i**, the most powerful of the transporters studied.

cholapod **1b**. A similar pattern was observed for Ar = 3,5-bis-(trifluoromethyl)phenyl (i.e., **2c** < **2f** < **1c** < **2i**; see Figure S16). It might be supposed that transporters with smaller ester substituents are leaching from the membranes into the aqueous phase, and are thus present in smaller amounts. However, experiments with Me ester **2c** disproved this hypothesis; treatments expected to promote leaching by lowering the aqueous concentration of **2c** (dilution of vesicles with water, passage through Sephadex) had no effect on transport rates.[18] It therefore seems that the longer side chain assists either by positioning the decalin favorably within the membrane or by promoting movement from one face to another. The most active of the decalins, **2i**, was effective at very low loadings. As shown in Figure 3c, transport rates were substantially higher than background even at transporter/lipid = 1:250 000.

Finally, variation of the counter-transported anion was used to explore anion selectivity.[5d,10a] When vesicles containing **2i** were prepared using aqueous Na_2SO_4, chloride influx was not detected. This implies that sulfate is not transported, so that charge accumulates in the vesicles and chloride transport is inhibited. On the other hand, vesicles prepared with $NaHCO_3$ behaved similarly to those containing nitrate, implying that bicarbonate (or carbonate) can be transported. Like cholapods[10a] and certain other systems,[5d] the decalins can therefore mimic the effect of biological chloride/carbonate exchangers.

In conclusion, we have shown that readily accessible diaxial diureidodecalins **2** can serve as compact analogues of cholapod anionophores. The work confirms the value of axial urea groups for anion recognition, and provides a second family of molecules capable of chloride transport at very low loadings. With a smaller, less lipophilic scaffold than the cholapods, variations on **2** can provide new insights into the requirements for anion transport, and new opportunities for optimizing activity.

■ ASSOCIATED CONTENT

Supporting Information. Details of experimental procedures, molecular modeling and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

Financial support from the BBSRC (BBS/S/E/2006/13241), the EPSRC (EP/F03623X/1) and the Pakistan HEC is gratefully acknowledged.

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(23) Octyl esters were used for optimal solubility in the organic phase.

(24) Transport activity was also observed when receptors **2** were added as solutions in methanol to preformed vesicles. However, rates were often lower than observed for preincorporated **2**, depending on the choice of Ar and R and also on the exact method of addition. Details of these experiments will be published separately.