Supramolecular Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713649759

An artificial sodium ion channel from calix[4]arene in the 1,3-*alternate* conformation

Olasupo Lawal[a]; Khayzuran S. J. Iqbal[a]; Ali Mohamadi[a]; Parichehr Razavi[a]; Howard T. Dodd[a]; Marcus C. Allen[a]; Samreen Siddiqui[a]; Flavia Fucassi[a]; Peter J. Cragg[a]
[a] School of Pharmacy and Biomolecular Sciences, University of Brighton, Brighton, UK

To cite this Article Lawal, Olasupo , Iqbal, Khayzuran S. J. , Mohamadi, Ali , Razavi, Parichehr , Dodd, Howard T. , Allen, Marcus C. , Siddiqui, Samreen , Fucassi, Flavia and Cragg, Peter J.(2009) 'An artificial sodium ion channel from calix[4]arene in the 1,3-*alternate* conformation', Supramolecular Chemistry, 21: 1, 55 — 60
To link to this Article: DOI: 10.1080/10610270802528307
URL: http://dx.doi.org/10.1080/10610270802528307

Supramolecular Chemistry
Vol. 21, Nos. 1–2, January–March 2009, 55–60

Taylor & Francis
Taylor & Francis Group

An artificial sodium ion channel from calix[4]arene in the 1,3-*alternate* conformation

Olasupo Lawal, Khayzuran S.J. Iqbal, Ali Mohamadi, Parichehr Razavi, Howard T. Dodd, Marcus C. Allen, Samreen Siddiqui, Flavia Fucassi and Peter J. Cragg*

School of Pharmacy and Biomolecular Sciences, University of Brighton, Brighton, UK

(*Received 7 July 2008; final version received 3 October 2008*)

The synthesis of a calix[4]arene with pendent polyether substituents is described. The compound was shown by NMR to bind Na^+ when in the 1,3-*alternate* conformation and computational simulations suggest metal binding within the calixarene annulus. ^1H NMR indicated that the complexed cation attracts water and the formation of a stable dihydrate was also indicated by *in silico* methods. Lipid bilayer experiments confirmed that the 1,3-*alt* calixarene functions as an artificial transmembrane ion in the presence of Na^+ but not K^+.

Keywords: calixarenes; polyethers; artificial ion channels; electrophysiology; ion transport

Introduction

From the earliest days of supramolecular chemistry, researchers have exploited the biomimetic potential of macrocycles and related molecules. Similarities between crown ethers and the ionophores valinomycin and nonactin helped to explain the ion transporting ability of the former (*1*). Likewise, enterobactin, a highly selective bacterial siderophore that targets Fe^{III} in aqueous solution with a binding constant approaching 10^{50}, inspired a range of tripodal chelating agents (*2*). On a broader front, calixarenes, cyclam derivatives and podands have all been considered as frameworks from which artificial metalloenzymes could be constructed (*3*).

Many analogues of naturally occurring macrocycles succeed because they bind guests strongly with high specificity; however, it is often just as important to facilitate highly specific binding that is environmentally reversible. In Nature, examples can be found in transmembrane ion channels (*4–6*). These are generally composed of large proteins having an affinity for cell surfaces with regions that can pass through the cellular phospholipid bilayer. The transmembrane region of the protein (or aggregation of proteins) forms a pore, through which ions travel, which must be continuous through the bilayer if ions are to flow continuously. This necessitates a concerted structure between 3 and 6 nm. The ion transport that results is controlled through two main mechanisms: filtering and gating.

Currently, much of our detailed understanding of ion channel protein behaviour relies on electrophysiological studies and the few high-resolution crystal structures that are available. A vast majority of these are of proteins selective for simple cations (*7*) such as the K^+ channels KcsA (*8*) and MthK (*9*), the Mg^{2+} transporter MgtE (*10*), Na^+/K^+ channels (*11, 12*) and an acid-sensing ion channel, ΔASIC1 (*13*). These structures in turn often act as models for other, less well-characterised, channel proteins such as the K^+-selective region of the protein product of the hERG (human *ether-á-go-go*) gene (*14*). Despite the wealth of information concerning the physiological activity of natural transmembrane proteins, there are extensive deficiencies in our detailed knowledge of ion transport mechanisms. For example, structural and dynamic evidence from the voltage-gated K^+ channel KcsA indicates that as hydrated ions approach the entrance to the pore, which often resembles a funnel, they are stripped of their solvent shell as they reach a constrictive filter (*8, 15*), yet whether this is a general principle remains unknown. The same deficiencies exist in our knowledge of the channel-gating processes that control the flow of ions in response to a range of external stimuli.

Synthetic ion channel models allow individual aspects of transmembrane transport to be probed, in particular the structural changes that can alter specificity or transport rate. Artificial analogues of cation channels based on crown ethers first started appearing in the 1980s (*16*) and have since been refined to include membrane-spanning substituents with high specificity (*17, 18*) and even voltage-dependent gating (*19*). Rigid macrocycles have also been employed in cation channel mimetic compounds. Resorcin[4]arenes with undecyl and heptadecyl substituents conduct both Na^+ and K^+, with selectivity for K^+ over Na^+ by a factor of 3, but only for derivatives with longer substituents (*20*). Similar results were reported for other resorcin[4]arenes (*21*) but calixarenes, in particular

*Corresponding author. Email: p.j.cragg@bton.ac.uk

ISSN 1061-0278 print/ISSN 1029-0478 online

DOI: 10.1080/10610270802528307
http://www.informaworld.com

calix[4]arene, have latterly been seen as potential ion-selective filters around which ionophore or channel frameworks can be constructed (*22, 23*). Calix[4]arene–cholic acid conjugates have been shown to transport Na^+ across vesicle membranes with the rate constant for the 1,3-*alt* conformer an order of magnitude higher than the *cone* (*24*). This agrees with other examples that suggest 1,3-*alt* calix[4]arenes exhibit enhanced ionophoricity (*25*). In this work, we report on the synthesis and behaviour of an amphiphilic 1,3-*alt* calix[4]arene derivative designed to probe the influence of conformation on metal ion selectivity and transmembrane transport.

Experimental

Unless otherwise stated, all materials and solvents are from Sigma-Aldrich (Gillingham, Dorset, UK). NMR spectra were recorded on a Bruker AM-360 instrument at 360 and 90 MHz for ^1H and ^{13}C, respectively. Chemical shifts were referenced to tetramethylsilane. Elemental analysis of polyether-containing compounds is often inconsistent due to variable levels and composition of included solvent. For this reason high-resolution electrospray ionisation mass spectrometry was used to determine the compounds' identities as the technique is known to be a good method of analysis for supramolecular complexes (*26*). HR-MS were recorded on a Bruker micrOTOF instrument operating in the positive mode. Calix[4]arene (**1**) was prepared from 4-*t*-butylcalix[4]arene according to the literature (*27*).

Triethylene glycol tosylate, monomethyl ether (2)

Triethylene glycol, monomethyl ether (16.1 ml, 16.4 g, 0.1 mol) was dissolved in a mixture of tetrahydrofuran (50 ml) and aqueous sodium hydroxide (5.7 g, 0.14 mol in 30 ml H_2O) and stirred in an ice bath until the solution temperature fell below 5°C. A solution of *p*-toluenesulphonyl chloride (20.7 g, 0.11 mol) in tetrahydrofuran (30 ml) was added to the stirred solution at a rate that kept the temperature below 5°C. Once the addition was complete, the solution was left to stir for a further 1 h below 5°C, poured onto iced water (75 ml water, 75 g ice) and stirred until all the ice melted. Most of the tetrahydrofuran was removed by rotary evaporation and the product extracted into dichloromethane (3 × 30 ml). The organic extract was dried over calcium chloride, filtered and the solvent removed by rotary evaporation. Triethylene glycol tosylate, monomethyl ether, **2**, was obtained as a colourless oil. Yield: 27 g (87%); ^1H NMR ($CDCl_3$) δ: 7.80 (d, $J = 8.6$, 2H Ar*H*), 7.35 (d, $J = 8.6$, 2H Ar*H*), 4.16 (t, 2H, —CH_2OTs), 3.50–3.75 (m, 10H, —CH_2CH_2O—), 3.39 (s, 3H, —OCH_3), 2.45 (s, 3H, TsCH_3); ^{13}C NMR ($CDCl_3$) δ: 144.8, 132.9, 129.5, 127.8, 72.4, 71.8, 71.0, 70.6, 70.1, 69.7, 58.8, 21.5; ESI HR-MS *m/z* found: 341.1056, calculated: 341.1035 $[M + Na]^+$.

Triethylene glycol iodide, monomethyl ether (3) (*28*)

Triethylene glycol tosylate, monomethyl ether, **2**, (10 g, 0.03 mol) was dissolved in acetone (125 ml). Potassium iodide [25 g (5 equiv.)] was added and refluxed overnight. The solution was cooled to room temperature and filtered to remove inorganic salts. The solvent was removed under reduced pressure and the residue dissolved in a mixture of ethyl acetate (75 ml) and distilled water (75 ml). The mixture was separated and the ethyl acetate phase washed with water (30 ml) and then saturated sodium thiosulphate (50 ml). The organic phase was dried over magnesium sulphate, filtered and solvent removed to give triethylene glycol iodide, monomethyl ether, **3**, as a colourless oil. Yield: 5.2 g (61%); ^1H NMR ($CDCl_3$) δ: 3.76 (t, 2H, —CH_2OCH_3), 3.65–3.70 (m, 6H, —CH_2CH_2-O—), 3.54–3.60 (m, 2H, —OCH_2CH_2I), 3.39 (s, 3H, —OCH_3), 3.26 (t, 2H, —OCH_2CH_2I); ^{13}C NMR ($CDCl_3$) δ: 72.5, 71.6, 70.5 (× 2), 70.1, 59.0, 3.3; ESI HR-MS *m/z* found: 296.9998, calculated: 296.9964 $[M + Na]^+$.

*Tetrakis(triethylene glycol monomethyl ether) calix[4]arene, 1,3-***alternate** *conformer (1,3-alt 4)*

Following the method of Verboom et al. (*29*), a suspension of calix[4]arene, **1**, (0.50 g, 1.15 mmol [98%]), anhydrous caesium carbonate (0.32 g, 2.3 mmol) and iodotriethylene glycol monomethyl ether, **3**, (1.26 g, 4.6 mmol) was refluxed in anhydrous acetonitrile (100 ml) under nitrogen for 7 days. After cooling to room temperature, the solvent was removed under reduced pressure and the solids taken up in dichloromethane (30 ml). The suspension was washed with 1 M hydrochloric acid (2 × 10 ml) and brine (10 ml), the organic phase separated and solvent removed under reduced pressure. The crude material was subjected to flash chromatography on silica (BDH Laboratory Supplies), eluting with ethanol, and fractions with an R_f of 0.84 were combined. Removal of solvent left a viscous residue that slowly solidified. The residue was dissolved in hot diethyl ether and left in a freezer overnight. A beige powder, isolated by filtration, was shown to be tetrakis(triethylene glycol monomethyl ether) calix[4]arene in the 1,3-*alternate* conformation (1,3-*alt* **4**). Solvent was removed from the filtrate to yield a mobile oil containing an intractable mixture of mono-, tri- and tetrasubstituted calix[4]arenes in the *cone* conformer. Yield of 1,3-*alt* **4**: 139 mg (12%); m.p.: 80.3–80.5°C; ^1H NMR ($CDCl_3$) δ: 7.05 (d, $J = 7.56$, 8H, Ar*H*), 6.62 (t, $J = 7.56$, 4H, Ar*H*), 3.85 (m, 8H, —CH_2OAr), 3.75 (s, 8H, ArCH_2Ar), 3.73–3.67 (m, 20H, —CH_2CH_2O—), 3.60–3.54 (m, 20H, —CH_2CH_2O—), 3.38 (s, 12H, —OCH_3); ^{13}C NMR ($CDCl_3$) δ: 155.7, 133.5, 129.9, 121.7, 72.0, 71.2,

70.8, 70.7, 70.6, 70.4, 59.1, 35.0; ESI HR-MS *m/z* found: 527.2638, 1031.4727, calculated: 527.2621 $[M + 2 Na]^{2+}$, 1031.5344 $[M + Na]^{+}$.

NMR binding studies

Compound 1,3-*alt* **4** was dissolved in $CDCl_3$ and the ^1H and ^{13}C spectra recorded at 25°C. An excess of solid $NaPF_6$ was added and the spectra recorded again after 2, 27, 87 min and 18.5 h. An analogous experiment was undertaken with NH_4PF_6.

Molecular modelling

Cone and 1,3-*alt* conformers of **4** were drawn with ChemDraw and exported into Spartan'06 (*30*). Full geometry optimisation was undertaken using restricted HF/PM3 methods from energy minimised structures initially calculated using the MMFF94 molecular mechanics force field. The Na^+ complex of 1,3-*alt* **4** and its dihydrate were constructed using options from the *Build* menu. For the initial dihydrate structures, two water molecules were introduced in close proximity to the RHF/PM3 geometry-optimised metal complexes prior to further geometric refinement by the RHF/PM3 method. In general, 450–500 iterative cycles were required to optimise the Na^+·1,3-*alt* **4** RHF/PM3 model geometry with a further 130 cycles to optimise that of the dihydrate.

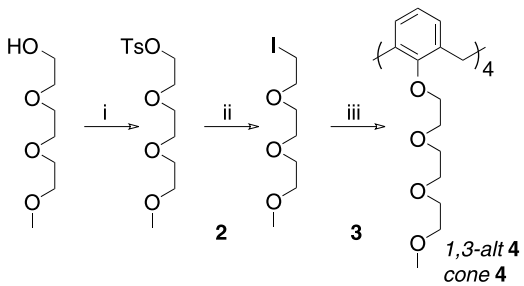
Planar bilayer experiments

The general method used is described elsewhere (*31*). Pure synthetic lipids (Avanti Polar Lipids, Birmingham, AL, USA) were dispersed in chloroform and stored at −70°C under nitrogen. Lipid bilayers were formed from a dispersion of 15 mg/ml 1-palmitoyl-2-oleoyl phosphatidylethanolamine and 15 mg/ml 1-palmitoyl-2-oleoyl phosphatidylserine in *n*-decane, which was drawn across a 0.25 mm diameter hole in a polystyrene cup separating two solution-filled chambers, designated cis and trans. The cis chamber (to which the calixarene was added) was held at ground, and the trans chamber was clamped at −50 mV using a BLM-120 patch clamp amplifier (Bio-Logic, Echirolles, France) equipped with a 10 GB (10 GΩ) bilayer headstage (Warner Instruments, Hamden, CT, USA). The sign of the membrane potential refers to the trans chamber, and currents are defined as positive when cations flow from trans to cis. Transmembrane currents were low-pass filtered at 300 Hz (4 pole Bessel) digitised at 10 kHz and recorded directly to disk via a CED Micro 1401 Mark II AD interface. Membrane capacitance was measured by differentiating a triangular wave input of 0.2 kHz. Only bilayers that had a resting conductance of less than 10 pS and an initial capacitance of at least 150 pF were used. For cation transport studies, bilayers were bathed in symmetrical Ringer's solutions containing either 150 mM NaCl or 150 mM KCl, 10 mM HEPES, 1 mM EGTA, 1.05 mM $CaCl_2$, 1 mM $MgCl_2$ and 50 μM free calcium. All recordings were made at room temperature and analysed offline using WinEDR v2.3.9 software (Strathclyde electrophysiological software).

Results and discussion

We have previously reported model compounds for a sodium cation filter (*32*) and transmembrane channel (*33*) that have three-fold symmetry. Both encapsulate Na^+ within a cavity defined by six oxygen atoms, presumably due to a preference for an octahedral coordination environment, and cations in the filter model are additionally linked by coordinated water. Interestingly, this trimeric motif, unique so far in ion channel architecture, has recently been found in the high-resolution structure of an H^+-activated ion channel (*13*). In the case of K^+, both binding (*34*) and transport (*21*) appear to require ligands with eight binding sites in the cubic arrangement also found in the filter region of the K^+-selective transmembrane protein KcsA (*8*). To date, well-known examples of metal transporting calix[4]arenes and related resorcin[4]arenes are almost exclusively restricted to K^+.

Calixarene 1,3-*alt* **4** was originally prepared through reaction of the tosylated polyether, **2**, with de-*t*-butylated calix[4]arene, **1**, using the appropriate metal carbonate; however, the easily synthesised iodo derivative, **3**, was found to give a cleaner reaction (Scheme 1). Initially, we attempted to synthesise both *cone* **4** and 1,3-*alt* **4** using potassium and caesium carbonate, respectively. Workup of the reaction mixtures isolated solid 1,3-*alt* **4** in both cases. In addition to mass spectral evidence, the NMR spectra showed a singlet at 3.75 ppm in the ^1H spectrum which correlated with a peak at 35.0 ppm in the ^{13}C spectrum in agreement with the literature values for 1,3-*alt* calixarenes (*35*). Mass spectra of the remaining oily residues confirmed them to be mixtures of mono-, tri- and tetrasubstituted calix[4]arenes with the corresponding



Scheme 1. Synthesis of calixarenes: (i) TsCl, $NaOH_{(aq)}$, 1,4-dioxane, 0°C; (ii) KI, acetone, reflux; (iii) calix[4]arene, K_2CO_3 or Cs_2CO_3, CH_3CN, reflux.

m/z values of 593.2, 885.4 and 1031.5 a.u. for their sodium salts. NMR confirmed that these compounds were in the *cone* conformer: a pair of doublets at 4.45 and 4.25 ppm in the ^{1}H spectrum correlated with a peak at 31.9 ppm in the ^{13}C spectrum. Given the difficulty in separating the *cone* components attention was focused on 1,3-*alt* **4**.

To our surprise Na^+, but not K^+, inclusion in 1,3-*alt* **4** was inferred from changes in the ^{1}H and ^{13}C NMR spectra (Figure 1 and Table S1 in the Supporting Information). The most significant feature of the ^{1}H spectrum was the deshielding of the aromatic protons by 0.16 and 0.05 ppm. This effect was mirrored in the ^{13}C spectrum, where the associated carbons moved by 1.17 and 0.35 ppm. The shielding was increased for the phenolic carbon (−0.34 ppm) together with the protons on the methylene bridge (−0.03 ppm) and those of the first carbon of the lower rim substituents. The methylene bridge gave rise to a singlet at 3.75 ppm in the absence of Na^+. Over the course of an 18 h experiment, the peak was initially shielded (−0.11 ppm) before coalescing with ethyleneoxy resonances at 3.71 ppm. Upon addition of $NaPF_6$, a new peak appeared as a broad feature between 7.9 and 7.5 ppm (Figure S10 in the Supporting Information). It is probable that this was from trace water present in the Na^+ salt. The peak became progressively deshielded until, after 18 h, it had sharpened and was centred at 10.5 ppm. Integration indicated that it corresponded to four protons for each calixarene, consistent with two water molecules to every Na^+·1,3-*alt* **4** complex. Taken together, these changes suggested that the cation was not associated with the polyether region of the molecule but resided inside the annulus of the calixarene initially interacting most strongly with the aryl carbons and protons on the methylene bridge. Over time, two water molecules coordinate to the bound cation down the axis of the calixarene. Hydration was evident within 2 min of the salt being added but took almost 20 h to reach completion. As a control, the experiment was repeated using NH_4PF_6 over a 96 h period. Protons on the methylene bridge and, crucially, all those associated with the polyether region of the molecule were deshielded by 0.05–0.07 ppm as would be expected if the cation was interacting with the polyether substituents. No evidence of bound water was observed.

In the absence of crystallographic evidence, a computer simulation was used to gain some insight into the probable position of a Na^+ guest within the calixarene host. We have previously shown that even simple molecular mechanics geometry optimisations can give models of host–guest complexes that compare favourably with information drawn from NMR binding studies (*36*) and that gas phase PM3 calculations can provide good models for inclusion phenomena in solution (*37*).

Computer models of 1,3-*alt* **4** and *cone* **4**, generated as described in the Experimental section, suggested stable extended structures in both cases with *cone* **4** in the 'flattened cone' conformation having C_{2v} symmetry and 1,3-*alt* **4** in the expected 1,3-*alternate* S_4 conformation as shown in Figure 2. Molecular modelling of the Na^+ complex was undertaken using semi-empirical methods (restricted HF/PM3), where two starting positions were considered for Na^+. In these, the cation was either placed

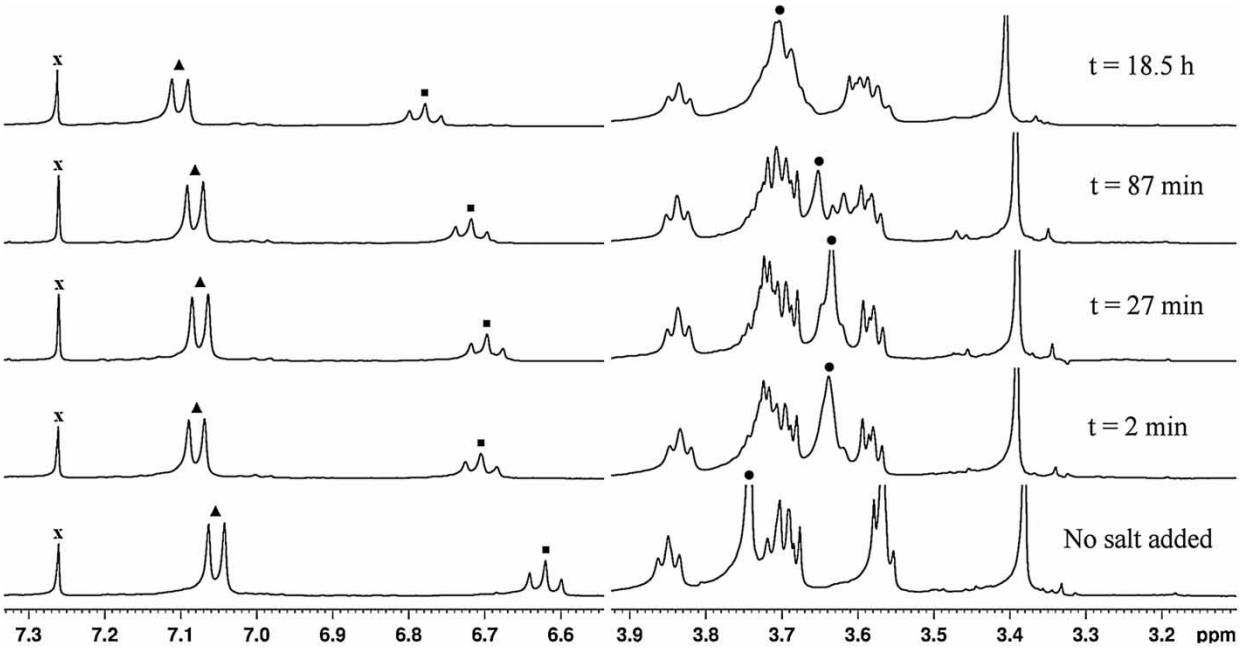


Figure 1. Time-dependent changes in the ^{1}H spectrum of 1,3-*alt* **4** following addition of $NaPF_6$ (▲ = aromatic protons in positions 3 and 5; ■ = aromatic protons in position 4; ● = methylene bridge protons; x = chloroform).

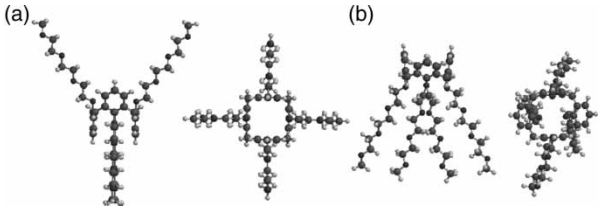


Figure 2. Simulated ligand structures: (a) 1,3-*alt* **4** (side and top); (b) *cone* **4** (side and top).

close to the macrocyclic annulus or near the ends of two polyether substituents. In the former case (structure I), the cation was drawn into the macrocyclic annulus with no polyether–cation interactions. In the latter case (structure II), the cation bound by the macrocycle was also enfolded by one polyether substituent to give a coordination environment reminiscent of a cation–calixcrown complex. The heats of formation for the two structures (-2120 kJ mol^{-1} for I *vs.* -2169 kJ mol^{-1} for II), illustrated in Figure 3, were within 2.5%. Structure I had the closest contacts between Na^+ and four coplanar carbon atoms at the lower rim in good agreement with the density functional calculations for the Na^+·1,3-*alt* calix[4]arene complex (*38*). More importantly, it was consistent with the NMR shifts in the bridging methylene and aromatic protons following the addition of Na^+. Geometry-optimised dihydrate structures were generated from structures I and II of the Na^+·1,3-*alt* **4b** complex as shown in Figure 3. Structure I optimised with water molecules along the axis of the calixarene at distances of 2.7–2.8 Å from the cation. Na^+ had other close contacts from phenolic ether oxygen atoms at 2.6 Å and two aromatic centroids at 2.7 Å. In structure II, one water molecule adopted an axial position 2.8 Å from the cation while the other displaced the polyether and bridged between the ether oxygen atoms and the cation. The heats of formation were within 2% of each other (-2625 kJ mol^{-1} for structure I *vs.* -2678 kJ mol^{-1} for structure II). Perhaps surprisingly, these calculations indicated that even if polyether interactions were important in the formation of Na^+·1,3-*alt* **4b** complexes, water molecules could displace the more weakly bound ethers in the cation's primary coordination sphere.

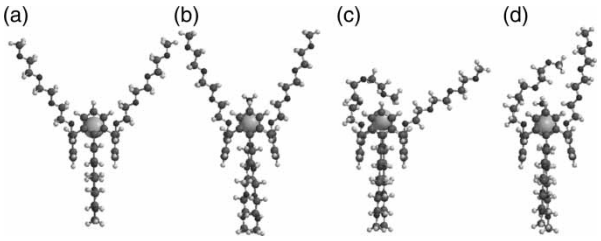


Figure 3. Simulated complex structures: (a) 1,3-*alt* **4**·Na^+, structure I; (b) 1,3-*alt* **4**·Na^+·2 H_2O, structure I; (c) 1,3-*alt* **4**·Na^+, structure II; (d) 1,3-*alt* **4**·Na^+·2 H_2O, structure II.

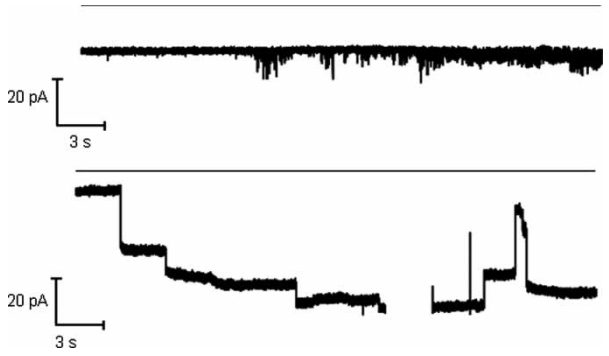


Figure 4. Transmembrane activity of 1,3-*alt* **4**: with K^+ (upper trace) and Na^+ (lower trace, with minor signal dropout).

Given that 1,3-*alt* **4** approaches 3 nm in length when fully extended, its potential to act as an artificial transmembrane ion channel was investigated using a planar lipid bilayer method. Solutions of 1,3-*alt* **4** were added to a planar lipid bilayer bathed in biomimetic Ringer's solution containing either K^+ or Na^+ at a concentration of 150 mM.[1] In total, five recordings were made with K^+ and three with Na^+ using a new bilayer in each case. Insertion of 1,3-*alt* **4** was indicated by a perturbation of the membrane shortly after the compound was introduced though no occurrences of the membrane busting were observed as a result. Figure 4 shows a typical example of bilayer activity following insertion of 1,3-*alt* **4**. All recordings of experiments involving K^+ showed the level of membrane activity increasing with time as in the upper trace of Figure 4 though no features corresponding to channels were observed. In two of the experiments, where Na^+ was present, channel-like features were recorded: in a third, an increasing current exhibiting some stepped features was observed but the bilayer burst without giving a long-lasting channel-like response. Where channel formation was observed, it usually appeared 10 s after the addition of 1,3-*alt* **4** and lasted for up to 60 s. Several examples of these stepped features were detected during the course of both successful experiments. The lower trace in Figure 4 shows a section from a typical experiment involving Na^+. The steps were on the scale of 100 pS implying insertion of several calixarenes in a manner that we have described with regard to a calix[6]arene derivative (*33*). The integrity of the bilayer held throughout the experiments.

Conclusions

The synthesis of 1,3-*alt* **4** has been described and the compound shown by NMR to bind Na^+. Over time, the cation also attracted water, present in trace amounts in the Na^+ salt, to form a dihydrate. Computational simulations agree with the NMR data in predicting Na^+ binding within the annulus of the calixarene. The models

indicate that this may be facilitated by interactions with oxygen atoms in a polyether substituent. Simulation of a hydrated complex mirrored the observation that two water molecules were incorporated in the complex during the NMR experiment performed in $CDCl_3$. The process of metal binding and hydration would be expected to be significantly faster when 1,3-*alt* **4** was inserted in a bilayer bathed by water on both sides.

Lipid bilayer experiments confirmed that 1,3-*alt* **4** functions as an artificial transmembrane ion channel in the presence of Na^+ but not K^+. Why does this calixarene act as a Na^+ channel when other compounds related to calix[4]arenes are K^+ selective? The answer lies in its conformation. Shinkai has proposed that the 1,3-*alternate* conformer of calix[4]arene provides an extended electron-rich environment that is attractive to monovalent cations (*39*). Evidence from NMR experiments and molecular simulations suggests that Na^+ is in its preferred six-coordinate environment in 1,3-*alt* **4** interacting with two water molecules, two phenolic oxygens and two aromatic rings. This is consistent with the current thinking on cation channel mechanisms that indicate that conductance occurs as water-linked chains of partially dehydrated cations pass through channel selectivity filters (*8, 15*). Calix[4]arene 1,3-*alt* **4** is therefore ideally suited as a synthetic ion channel for Na^+.

Supporting information

NMR spectra for compounds **2**, **3**, 1,3-*alt* **4** and *cone* **4**, 1,3-*alt* **4**·Na^+·2 H_2O together with HRMS spectra for 1,3-*alt* **4** and *cone* **4** are available as supplementary information.

Acknowledgements

We thank the University of Brighton for financial support (for K.S.J.I. and F.F.).

Note

1. A similar bilayer experiment in Na^+ rich media carried out using the crude mixture of *cone* compounds, including *cone* **4**, also demonstrated insertion into the lipid membrane but no transmembrane ion current was observed [data not shown]. As the *cone* compounds are *ca.* 1.7 nm when fully extended it is probable that they are unable to span the bilayer and therefore cannot form conducting channels.

References

(1) Takaki, U.; Hogen Esch, T.E.; Smid, J. *J. Am. Chem. Soc.* **1971**, *93*, 6760–6766; McLaughlin, S.G.A.; Szabo, G.; Ciani, S.; Eisenman, G. *J. Membrane Biol.* **1972**, *9*, 3–36.
(2) Raymond, K.N.; Isied, S.S.; Brown, L.D.; Fronczek, F.R.; Nibert, J.H. *J. Am. Chem. Soc.* **1976**, *98*, 1767–1774.
(3) Kirby, A. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 706–728.
(4) Cragg, P.J. *Sci. Prog.* **2002**, *85*, 219–241.
(5) Liang, X.; Campopiano, D.J.; Sadler, P.J. *Chem. Soc. Rev.* **2007**, *36*, 968–992.
(6) Anson, L. *Nature* **2006**, *440*, 439–489.
(7) Zhorov, B.S.; Tikhonov, D.B. *J. Neurochem.* **2004**, *88*, 782–799.
(8) Doyle, D.; Morais Cabral, J.; Pfuetzner, R.A.; Kuo, A.; Gulbis, J.M.; Cohen, S.L.; Chait, B.T.; MacKinnon, R. *Science* **1998**, *280*, 69–77.
(9) Jiang, Y.; Lee, A.; Chen, J.; Cadene, M.; Chait, B.T.; MacKinnon, R. *Nature* **2002**, *417*, 515–522.
(10) Hattori, M.; Tanaka, Y.; Fukai, S.; Ishitani, R.; Nureki, O. *Nature* **2007**, *448*, 1072–1075.
(11) Shi, N.; Ye, S.; Alam, A.; Chen, L.; Jiang, Y. *Nature* **2006**, *440*, 570–574.
(12) Morth, J.P.; Pedersen, B.P.; Toustrup-Jensen, M.S.; Sørensen, T.L.-M.; Petersen, J.; Andersen, J.P.; Vilsen, B.; Nissen, P. *Nature* **2007**, *450*, 1043–1049.
(13) Jasti, J.; Furukawa, H.; Gonzales, E.B.; Gouaux, E. *Nature* **2007**, *449*, 316–323.
(14) Farid, R.; Day, T.; Friesner, R.A.; Pearlstein, R.A. *Bioorg. Med. Chem.* **2006**, *14*, 3160–3173.
(15) Noskov, S.Y.; Bernèche, S.; Roux, B. *Nature* **2004**, *431*, 830–834.
(16) Dock, A.C.; Moras, D.; Behr, J.P.; Lehn, J.-M. *Acta Crystallogr. C* **1983**, *39*, 1001–1005.
(17) Fyles, T.M.; James, T.D.; Kaye, K.C. *J. Am. Chem. Soc.* **1993**, *115*, 12315–12321.
(18) Gokel, G.W. *Chem. Commun.* **2000**, 1–9.
(19) Hall, C.D.; Kirkovits, G.J.; Hall, A.C. *Chem. Commun.* **1999**, 1897–1898.
(20) Tanaka, Y.; Kobuke, Y.; Sokabe, M. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 693–694.
(21) Wright, A.J.; Matthews, S.E.; Fischer, W.B.; Beer, P.D. *Chem. Eur. J.* **2001**, *7*, 3474–3481.
(22) Iqbal, K.S.J.; Cragg, P.J. *Dalton Trans.* **2007**, 26–32.
(23) de Mendoza, J.; Cuevas, F.; Prados, P.; Meadows, E.S.; Gokel, G.W. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 1534–1537.
(24) Kobuke, Y.; Nagatani, T. *J. Org. Chem.* **2001**, *66*, 5094–5101.
(25) Baklouti, L.; Harrowfield, J.; Pulpoka, B.; Vicens, J. *Mini. Rev. Org. Chem.* **2006**, *3*, 355–384.
(26) Schalley, C.A. *Mass Spectrom. Rev.* **2001**, *20*, 253–309.
(27) Gutsche, C.D.; Levine, J.A.; Sujeeth, P.K. *J. Org. Chem.* **1985**, *50*, 5802–5806.
(28) Loiseau, F.A.; Hill, A.M.; Hii, K.K. *Tetrahedron* **2007**, *63*, 9947–9959.
(29) Verboom, W.; Datta, S.; Asfari, Z.; Harkema, S.; Reinhoudt, D.N. *J. Org. Chem.* **1992**, *57*, 5394–5398.
(30) Spartan' 06, Wavefunction Inc., Irvine, CA, USA.
(31) Hardy, S.P.; Richie, C.; Allen, M.C.; Ashley, R.H.; Granum, P.E. *BBA Biomembranes* **2001**, *1515*, 38–43.
(32) Cragg, P.J.; Allen, M.C.; Steed, J.W. *Chem. Commun.* **1999**, 553–554.
(33) Iqbal, K.S.J.; Allen, M.C.; Fucassi, F.; Cragg, P.J. *Chem. Commun.* **2007**, 3951–3953.
(34) Matthews, S.E.; Schmitt, P.; Felix, V.; Drew, M.G.B.; Beer, P.D. *J. Am. Chem. Soc.* **2002**, *124*, 1341–1353.
(35) Jaime, C.; de Mendoza, J.; Prados, P.; Nieto, P.D.; Sánchez, C. *J. Org. Chem.* **1991**, *56*, 3372–3376.
(36) Marcos, P.M.; Ascenso, J.R.; Cragg, P.J. *Supramol. Chem.* **2007**, *19*, 199–206.
(37) Sheehan, R.; Cragg, P.J. *Supramol. Chem.* **2007**, *20*, 443–451.
(38) Macias, A.; Norton, J.E.; Evanseck, J.D. *J. Am. Chem. Soc.* **2003**, *125*, 2351–2360.
(39) Ikeda, A.; Shinkai, S. *J. Am. Chem. Soc.* **1994**, *116*, 3102–3110.