Towards a redox-active artificial ion channel

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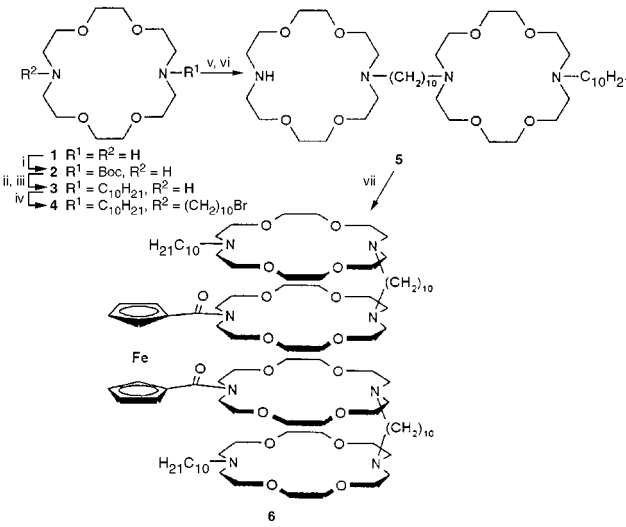
The synthesis, characterisation and properties of an artificial ion channel containing a redox-active ferrocene unit are described.

During the last ten years substantial efforts have been made to mimic the action of natural ion channels[1] by the design and synthesis of model systems which would span natural (or artificial) lipid bilayers.[2–6] Generally speaking, the artificial channels consist of head groups (*e.g.* macrocyclic polyethers) to selectively capture specific cations, connected by long aliphatic chains designed to span the bilayers and often including a 'relay unit' which is sometimes another macrocyclic ether.[7] Alternative head groups include calixarenes[8,9] which, at least in one case,[9] support the concept of the 'billiard ball' effect, in essence, the charge repulsion theory of multiple ion transport.[10] In addition, synthetic proteins have been designed to mimic natural proteins, albeit with a single span rather than the multiple spans believed to exist in natural systems.[11] It is known that natural K^+ channels fall into two distinct categories.[12] The first is that of voltage-gated (K_v) channels which open and close ('gate') in response to the potential across the cell membrane. They have six trans-membrane domains, the fourth one of which has a positive charge every third residue and hence acts as the voltage sensor. The second category is that of the inward rectifier (K_{ir}) which consists of two trans-membrane domains connected by a loop which allows transport of K^+ ions in one direction only against the natural concentration gradient. This paper reports the synthesis, characterisation and properties of a one-domain artificial ion channel containing a redox-active centre (a ferrocene unit) in the middle of the channel to act as a 'filter'[10] and to influence the passage of cations by changing the oxidation state of the ferrocene during ion transport. The only redox-mediated channels of which we are aware are two C-terminus ferrocene derivatives of the *natural* peptide alamethicin which were shown to undergo dramatic changes in channel activity across lipid bilayers on *chemical* oxidation with excess ceric ion.[13]

The overall synthetic strategy, based on the inspirational work of Gokel *et. al.*,[2–5] was to link two macrocyclic units (as head groups) *via* long aliphatic chains to a central ferrocene unit which would also act as a cation relay. The resultant channel was designed to be approximately 30 Å long, the thickness of a typical biological membrane. The hydrophobic nature of the aliphatic chains was expected to promote lipid solubility and variation of the ring size in the head groups was expected to control cation selectivity. The precise synthetic sequence, shown in Scheme 1, afforded the target channel as a viscous orange oil in an overall yield of 16% from *N*-Boc-diaza-18-crown-6. Each compound in the synthetic sequence was purified by chromatography (Al_2O_3) and characterised by high resolution mass spectrometry and a combination of ^1H and ^{13}C NMR using DEPT (135).[14]

Cyclic voltammetry (CV) studies in MeCN as solvent with Bu_4NClO_4 (0.2 M) as the supporting electrolyte and using a glassy carbon working electrode gave rather curious results. A strong oxidation wave was observed at *+727* mV but the corresponding reduction wave failed to appear over a range of scan rates from 20–200 mV s^{-1} (Fig. 1).† Ferrocene amides of the type incorporated in the channel normally give a well-behaved electrochemical response[15] and the results suggest some sort of fast decomposition of the oxidised channel on the glassy carbon electrode or a rapid conformational change which inhibits the reduction process.‡ This feature of the electrochemistry is currently being investigated further. Introduction of two of the biologically important cations (Na^+ and Ca^{2+}) in increasing stoichiometric ratio from 1:1 to 1:10 ($L:M^{n+}$) produced perturbations of the oxidation wave and the results are shown in Table 1. During the initial additions of Na^+ (up to 1:3 stoichiometry) anodic shifts of various magnitudes were observed which is consistent with coordination of the Na^+ ion by the amide functions of the ferrocene unit. Addition of further equivalents of Na^+, however, produced a reversal of the shifts to slightly more cathodic values. It is difficult to rationalise this observation but one explanation may be that as the channel fills with cations, charge repulsion may reduce coordination by the



Scheme 1 *Reagents and conditions*: i, Boc_2O, 1,4-dioxane, 44%; ii, $Br(CH_2)_9CH_3$, Na_2CO_3, KI, PrCN, 80%; iii, TFA, CH_2Cl_2, 94%; iv, $Br(CH_2)_{10}Br$, Na_2CO_3, KI, PrCN, 56%; v, **2**, Na_2CO_3, KI, PrCN, 67%; vi, TFA, CH_2Cl_2, 94%; vii, 1,1′-bis(chlorocarbonyl)ferrocene, Et_3N, toluene, 58%.

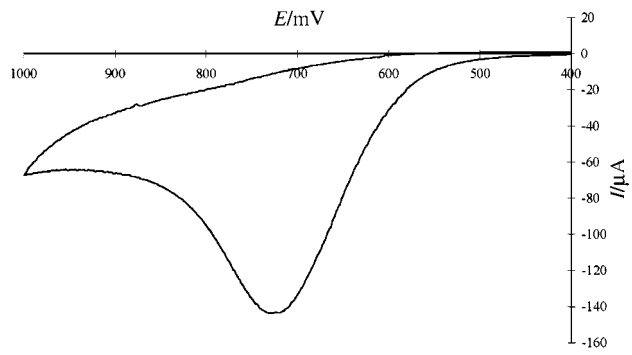


Fig. 1 Anodic voltammetric response of **6** (5×10^{-3} M in MeCN/0.2 M Bu_4NClO_4); working electrode: glassy carbon; counter electrode: Pt wire; reference electrode: Ag/AgCl; scan rate: 50 mV s^{-1}.

Table 1 Electrochemical data for metal complexes formed from ligand **6**

	Metal[a]			
	Na^+		Ca^{2+}	
Ratio metal : **6**	E_A[b]/mV	ΔE/mV	E_a[b]/mV	ΔE/mV
free **6**	727	—	727	—
1 : 1	—[c]	—[c]	755	28
2 : 1	758	31	792	65
3 : 1	782	55	867	140
4 : 1	775	48	874	147
10 : 1	757	30	884	157

[a] Triflate salts. [b] Determined in MeCN containing 0.2 M Bu_4NClO_4 as the supporting electrolyte. Solutions of **6** were 5×10^{-3} M and potentials were determined with reference to an Ag/AgCl, working electrode: glassy carbon; scan rate: 50 mV s^{-1} E_A represents the anodic voltammetric response. [c] Not recorded.

amide carbonyls, thus *reducing* the 'through bond' electron withdrawal from the ferrocene centre. With Ca^{2+}, however, the shift continued to be anodic throughout the concentration range, which may be due to the stronger coordination of the amide functions with the higher charge density Ca^{2+} ion. It should also be noted that addition of excess cations regenerated, at least in part, the reversibility of the CV wave.

An 'inside-out' patch excised from cells derived from Hamster brain§ was exposed to a solution of the synthetic channel and then tested for K^+ transport with potentials ranging from +60 to −60 mV across the membrane. The results against a control are shown in Fig. 2. Analysis of the data shows clear evidence of ion transport *promoted* by the artificial channel at both negative and positive pipette potentials. In addition, changing the potential across the patch from −60 to +60 mV results in a reduction of the transmembrane current (*i.e.* rectification) as the inside of the cell changes from negative to positive potential. For example, at −80 mV the average single channel current amplitude was −4.0 pA (equivalent to a conductance of 50 pS) whereas at +80 mV the same average current was +2.4 mA (30 pS). The results suggest the incorporation of an artificial channel with a degree of voltage control over cation transport. It is conceivable, however, that **6** is in some way activating endogenous channels within the biological membrane and hence lipid bilayer experiments are in progress to check this possibility. The details of this work will be published at a later stage but for now we report that **6**, together with several analogues, has shown similar channel activity in lipid bilayers to that reported by Gokel for non-redox-active artificial channels.[7,16,17] Thus the results obtained with the biological membrane may be viewed with more confidence as an example of an *artificial* channel with a degree of redox control over cation transport, one step nearer to the natural system.

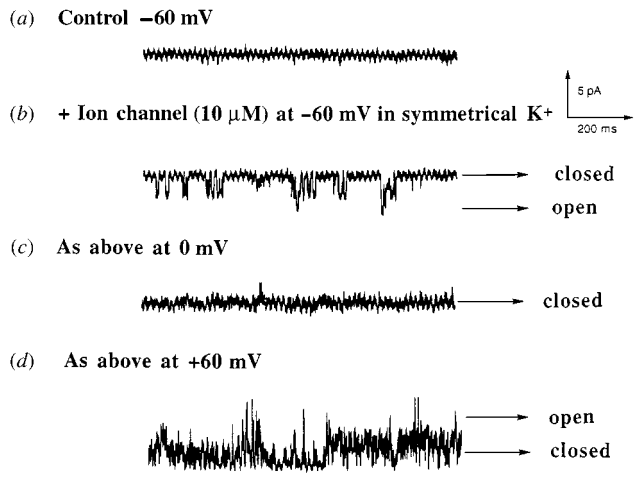


Fig. 2 Recordings of K^+ transport across an 'inside-out' patch when exposed to a solution of **6** (at 10 μM) and with a potential difference across the channel varying from −60 to +60 mV.

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Notes and references

† The analogous cobalticenium hexafluorophosphate channel (to be reported later) gave well-behaved, fully reversible electrochemistry.
‡ Similar results were obtained using a Pt working electrode except that the current response was much lower for the same concentration.
§ Hypothalamic neurons were acutely dissociated from coronal hampster (5–15 day old) brain slices and superfused with a solution containing NaCl (140 mM) , KCl (2.5 mM), $CaCl_2$ (3 mM), glucose (3 mM) and HEPES (pH 7.4, 10 mM). Channel currents were recorded from inside-out patches using standard techniques. Fire-polished, borosilicate glass pipetttes were fabricated with a two-stage pull and filled with a solution containing: KCl (125 mM) , NaCl (10 mM), $CaCl_2$ (3 mM), glucose (3 mM) and HEPES (pH 7.4, 10 mM) which typically had resistances of 5–10 MΩ. After excision, the patches were held at indicated pipette potentials and the cell bathing solution was replaced with the above pipette solution. The artificial channel solution (in DMSO) was dissolved in the bathing solution (usually at 10 μM) and introduced to the patch at equivalent flow rates to the control. All currents were acquired using an Axoclamp 200A interfaced to a computer running pClamp6 software (Axon Instr.). Data were fitted at 1 kHz with a low-pass 8-pole Bessel filter, sampled at 4 kHz and analysed using pClamp6.

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14 All compounds gave NMR data consistent with the proposed structures. *Selected data* for **2**: [HRMS-FAB, $(M + H)^+$] calc. for $C_{17}H_{35}N_2O_6$: 363.2495; found 363.2505. For **3**: [HRMS-FAB, $(M + H)^+$] calc. for $C_{22}H_{46}N_2O_4$: 403.3500; found 403.3536. For **4**: [HRMS-FAB, $(M + Na)^+$] calc. for $C_{32}H_{65}BrN_2O_4Na$: 643.4025; found 643.4010: For **5**. [HRMS-FAB, $(M + Na)^+$] calc. for $C_{44}H_{89}N_4O_8Na$: 824.6578; found 824.6548: For **6**. [HRMS-FAB, $(M + Na)^+$] calc. for $C_{100}H_{186}FeN_8O_{18}Na$: 1866.3132; Found 1866.3040.
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