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SYNTHESIS AND CATION-BINDING ABILITIES OF NOVEL POLYQUINANE CROWN ETHERS CONTAINING A BIS-ACETAL ETHER FUNCTIONALITY

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Abstract: New macrocyclic ether hosts bearing a 'bis-acetal ether' functionality and a lipophilic posterior have been synthesised from the readily available cis,syn,cis-triquinane dione 1 and ethylene glycols in one step. Complexation characteristics and ability of these host molecules to translocate cations across phospholipid bilayer vesicles or liposomes has been studied.

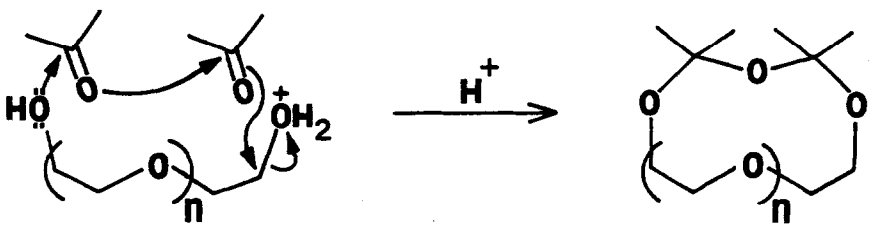
Ever since their discovery two decades ago, myriad modifications of the Pedersen's original crown ethers have been synthesised and their complexing and related properties extensively investigated.[1] The structural changes in crown ethers have centred around alterations in the distribution, number and type of the donor atoms in the macrocycle, incorporation of rigidifying rings and functionalities and addition of lipophilic groups. Understandably, these modifications have been sought to achieve greater complexing ability and higher selectivity. The variation in the location of the donor oxygen atoms in the crown ether can be attained by replacing one (or more) of the $-O-CH_2-CH_2-O-$ groups by either $-O-CH_2-(CH_2)_{n+2}-O-$ or $-O-CH_2-O-$ groupings. The latter functionality when present in the ring leads to macrocyclic polyether acetals and these have been recently synthesised and investigated to a limited extent.[2] However, if two acetal moieties appear in tandem within the crown ether, an $-O-CH_2-O-CH_2-O-$ ('bis-acetal ether') functionality is generated. The resulting 'bis-acetal ether' containing crown ethers can be expected to exhibit interesting cation binding but their synthesis and reactivity has not received attention.[3] Herein, we report the synthesis of some novel macrocyclic polyethers, built on a lipophilic, convex, polyquinane backbone and incorporating the bis-acetal ether functionality.

Synthesis

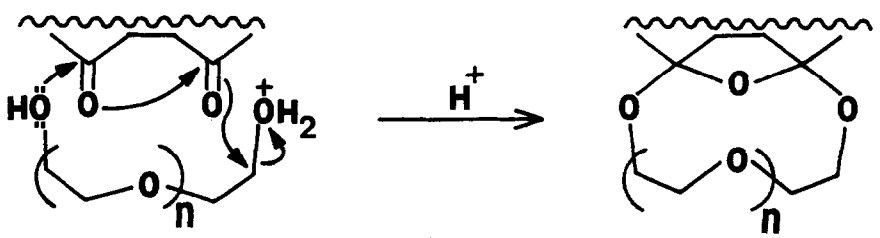
The most direct way to the synthesis of bis-acetal ether macrocyclic ethers is through the stitching together of two carbonyl groups with the appropriate polyethylene glycol as shown in Scheme 1. However, practical realisation of such an intermolecular process is

Scheme 1

Intermolecular



Intramolecular

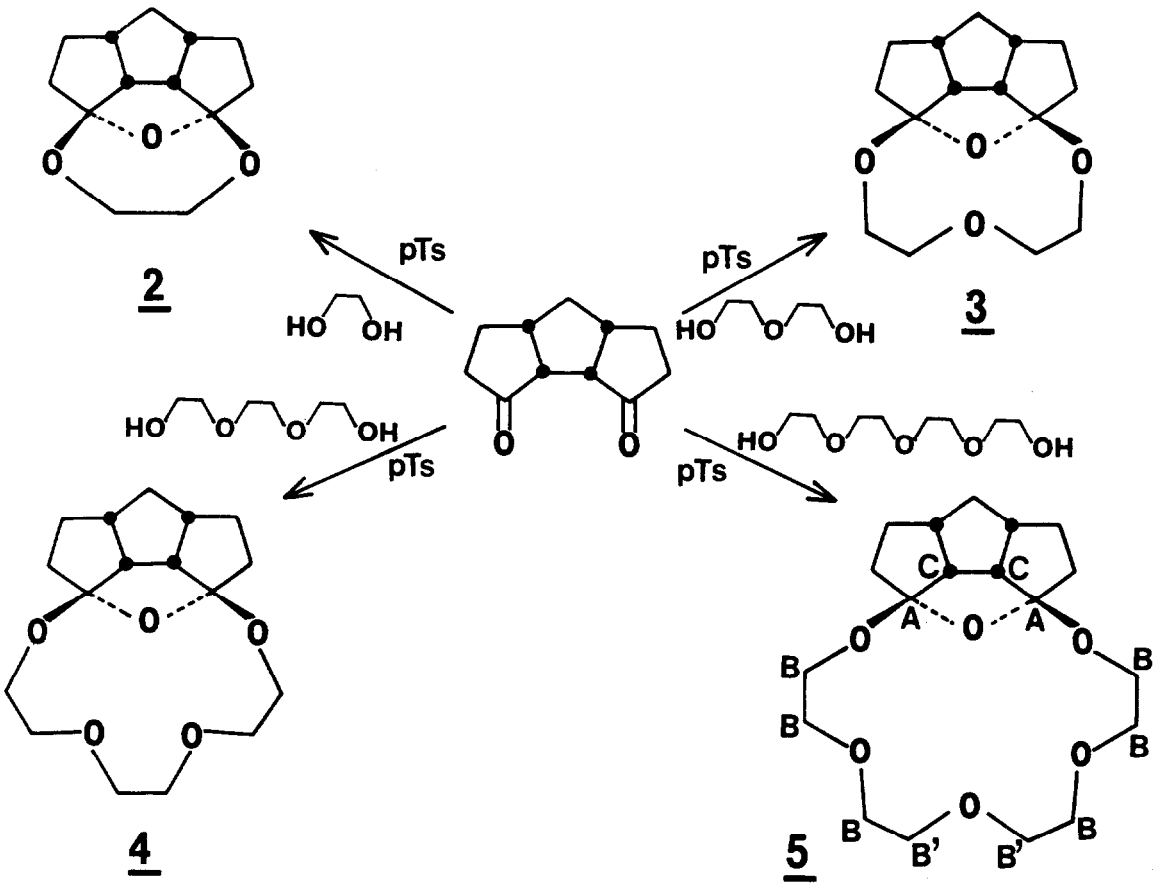


disfavoured on several counts including side reactions. One way to overcome these problems is to have the two carbonyl groups built-in close proximity in the same molecule for an intramolecular transacetalisation using the desired polyethylene glycol, Scheme 1. In a recent study, we have described the synthesis of all cis-triquinane dione 1, in four convenient steps from readily available staring materials and demonstrated that the two carbonyl groups in it are in close proximity. The tricyclic dione 1[4] seemed to be an ideal substrate for intramolecular stitching of the two carbonyl groups through intramolecular transacetalisation reaction.

Reaction of 1 with ethylene glycol in the presence of p-toluenesulphonic acid (p-TSA) gave 2 in 85% yield as the sole product of the reaction. The structure of 2 was clearly delineated through its 7 line ^{13}C NMR spectrum (C_2 symmetry) and the presence of resonances at δ 122.8 & 67.6 due to acetal and ether bearing carbon atoms, respectively. In a similar manner diethylene glycol, triethylene glycol and tetraethylene glycol with 1 furnished 3, 4 and 5, respectively. Structures 3-5 were unambiguously assigned on the basis of spectral

data summarised in Table 4 in the experimental section. With the ready availability of 4(M5) and 5 (M6) bearing five and six donor oxygen atoms, respectively, in the novel 'bis-acetal ether' arrangement, it was decided to examine their ion-binding properties in relation to 15-crown-5 and 18-crown-6.

Scheme 2



Cation-binding Studies

It was of interest to monitor the abilities of M5 and M6 to complex with alkali and alkaline earth cations and the complexation constants, the structural changes that might occur upon ion binding, their abiliity to phase transfer metal salts into organic solvents and to translocate cations across phospholipid bilayer vesicles or liposomes.

In the first set of experiments, the interaction between alkali metal picrates and the macrocycles was studied. A solution of M5 or M6 in dichloromethane (DCM) was shaken up with an aqueous solution of the alkali picrate for several minutes. The two layers were separated and the absorbance of the picrate in the two layers measured. The experimental protocol followed was largely that of Pedersen[5] and of Ferguson et al[6]. From the changes in

the absorbance values of the aqueous layer before and after phase transfer the percentage extraction into the DCM layer was calculated. Table 1 presents the results of such phase transfer effected by M5 and by M6. It is clear from Table 1 that M5 is not an efficient ion-binding agent. None of the six picrates gets transferred into the organic layer to any significant extent, the best being K^+ to the extent of 7%. The higher homolog M6 is however able to transfer significant amounts of sodium and potassium picrates into DCM. Amongst the salts tried, Na^+ is preferred the most, followed by K^+ while NH_4^+ is transferred to about 8%. The other salts tried were not transferred. M6 was studied further.

Table 1

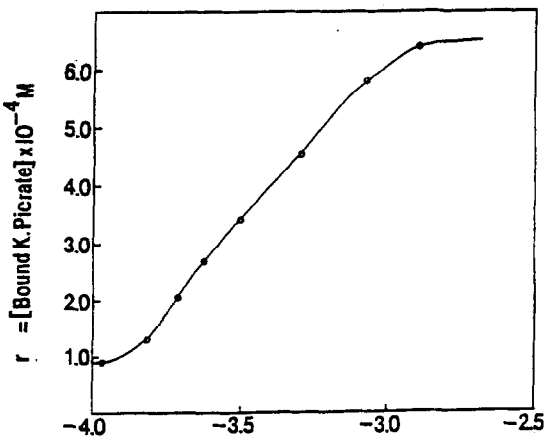
Efficiency of phase transfer of metal picrates from water to dichloromethane by M5 and M6

	Li^+	Na^+	K^+	NH_4^+	Ca^{2+}/Mg^{2+}
M5	-	1%	7%	-	-
M6	-	56%	42%	8%	-

Figure 1 shows the binding isotherm of K picrate with M6 at room temperature. The bound salt concentration was estimated from the absorbance of the organic layer after equilibration and the free salt concentration from that of the aqueous layer. As can be seen, the curve levels off at a stoichiometry of about one K^+ per M6 molecule. The binding is thus 1:1 in the complex. The stability constant of the complex, calculated from the

Fig. 1

Bjerrum plot of the binding of potassium picrate by 10^{-3} M M6 in DCM



a) $r = \dfrac{nKA}{1 + KA}$

b) $\log K = -\log A$ at $r = \dfrac{n}{2}$

curve, is 3 x 10^{3} M^{-1} (log K = 3.47). This value is comparable to the value of log K = 3.58 seen for the complexation of K^{+} by cyclohexyl-15-crown-5 and log K = 3.71 for its complexation with the Na^{+} ion.[7] The molecule M6 is thus comparable to the 15-crown-5 derivatives in its complexing properties.

Table 2 lists the ^{13}C NMR spectral chemical shifts and spin-lattice relaxation times(T_1) of the various carbon atoms of M6 in the free state and when it is complexed to KSCN in $CDCl_3$ solutions. Interestingly, not all the ethylene oxide carbons are affected in the same way in their chemical shift values upon ion binding. The chemical shift value of the carbon atoms designated as B' is downfield shifted in contrast to those of the B type carbons, and similar to those of the ring junction carbons A and C. This differential behaviour suggests that the metal ion is not symmetrically placed so that it can affect all liganding atoms the same way. A conformational rearrangement is indicated upon ion binding. It appears likely that complexation occurs not by placing the metal ion in the central cavity but perhaps in a "wraparound" fashion, since the chemical shift values of carbons A, B' and C are affected differently from those of carbons B. The spin-lattice relaxation times (T_1 values shown in Table 2) of all the carbon atoms are not remarkably altered though they are uniformly decreased, suggesting restriction in the motion of all the various liganding moieties uniformly. The loss in mobility however, is not drastic, consistent with the low value of the stability constant of the complex.

Table 2

^{13}C NMR chemical shifts and relaxation times (T_1) of the carbon atoms of M6 in the presence and absence of bound KSCN in $CDCl_3$

	δ, ppm		Δ δupon	T, Sec**	
Nucleus	with KSCN	M6 alone	KSCN binding*	M6 + KSCN	free M6
A	123.59	123.12	0.47 df	10.45	11.26
B	70.04	71.22	1.18 uf	0.56	0.75
	68.56	70.39	0.83 uf	0.60	0.85
	67.69	70.02	2.33 uf	0.55	0.82
B'	63.79	63.48	0.31 df	0.57	0.78
C	60.08	60.19	0.11 df	0.81	1.17
ring outer carbons	45.69	45.46	0.23 df	0.92	1.13
	37.61	37.79	0.18 uf	0.43	0.56
	36.22	36.42	0.20 uf	0.47	0.65
	31.90	32.06	0.16 uf	0.52	0.69

* uf = upfield and df = downfield

**by the inversion recovery method

Comparison of the structures of M5 and M6 with the corresponding crown ethers 15-crown-5 and 18-crown-6 respectively would lead to the expectation that M5 complex preferentially with Na^+ and M6 with K^+. The reason that this expectation is not borne out lies in the mutual steric disposition of the various liganding oxygen atoms. There are two types of complexing oxygen atoms in the molecule - the ethylene oxide type, similar to those in the crowns, and the heterocyclic ring oxygen. The latter is sterically more constrained and out of plane of the former. In addition there is also the steric factor imposed by the nonpolar tricyclic ring system. The two types of oxygens would thus be expected to bind to a central metal ion in different orientation, and the stength and selectivity of binding would be different from that of the crown ethers. This is perhaps why M6 resembles 15-crown-5 in its slight preference of Na^+ over K^+, and why M5 is an inefficient complexone.

We also monitored the ability of M5 and M6 to translocate metal ions across phospholipid vesicles. The assay method used was that of Uratani and Cramer.[8] Briefly, 1 ml of a 12 mg/ml solution of egg phosphatidylcholine (EPC) in chloroform was evaporated into a thin film and the film dispersed in 1 ml of pH 7.4 buffer containing 5 mM HEPES and 120 mM of the desired alkali halide (say NaCl) and sonicated briefly to yield unilamellar liposomes that contained the alkali halide solution inside as well as outside. When a different salt (say KCl) was desired to be on the outside, the liposomes were dialyzed or gel filtered using 120 mM KCl. This produces EPC liposomes with 120 mM NaCl inside and 120 mM KCl outside. To 50 λ of this suspension was added 5 λ of a solution of 10^{-5} M 8-anilinonaphthalene-1-sulfonate (ANS) in methanol. ANS inserts in the outer half of the bilayer and its fluorescence intensity is sensitive to the voltage across the bilayer or the transmembrane diffusion potential. Now when an ionophore is added to the liposome, it would translocate ions across and cause a voltage difference across the bilayer, which would be

Table 3

Translocation preferences of ionophores across liposomes

Ionophore used	Fluorescence intensity change when			Transport preference
	Na^+ in, K^+ out	Na^+ in, NH_4^+ out	Na^+ in, Li^+ out	
Valinomycin	increase (+ +)	decrease (-)	decrease (-)	$K^+ > Na^+$
gramicidin A	decrease (- -)	decrease (- -)	decrease (- -)	Na^+
M6	increase (+)	decrease (-)	decrease (-)	$K^+ > Na^+$
M5	-	-	increase (+)	Li^+

Note : When Na^+ is transported out, emission intensity decreases and when K^+ is transported in, it increases, since ANS is on the outer surface of the bilayer.

registered as a change in the fluorescence intensity of ANS at 480 nm. Table 3 shows that the results of such ion transport experiments using valinomycin (K^+ ionophore) gramicidin A (Na^+ ionophore), M6 and M5. 2 λ of a methanolic solution of the ionophore (final

Table 4

Compound	Time & Yield	mp °C	^{1}H NMR (100 MHz) δ,$CDCl_3$	^{13}C NMR (25 MHz) δ,$CDCl_3$	Analytical data	Mass m/z, 70ev
2	3 h, 85%	140 (bp. at 0.1 mm)	4.2-3.5(4H, m), 3.0-2.5(4H, m), 2.3-1.2(10H, m)	122.8, 67.6, 58.8, 46.5, 38.2, 37.8, 31.1	Calcd. for $C_{13}H_{18}O_3$, C, 70.24; H, 8.16 Found: C, 70.50; H, 8.18	M^+ 222
3	24 h, 23%	165	4.0-3.4(8H, m) 3.1-1.2(14H, m)	123.5, 71.0, 63.2 60.0, 45.6, 37.8, 36.6, 32.0	Calcd. for $C_{15}H_{22}O_4$ C, 67.64; H, 8.33 Found: C, 67.82; H, 8.45	M^+ 266
4	10 h, 70%	95	4.1-3.3(12H, m) 3.2-1.2(14H, m)	123.2, 71.6, 71.0, 63.9, 60.2, 45.4, 37.7, 36.4, 31.9	Calcd. for $C_{17}H_{26}O_5$ C, 65.78; H, 8.44 Found: C, 65.92; H, 8.43.	M^+ 310
5	10 h, 80%	115	3.8-3.3(16H, m) 3.0-1.2(14H, m)	123.0, 71.1, 70.2, 69.9, 63.2, 60.0, 45.2, 37.6, 36.2, 31.82	Calcd. for $C_{19}H_{30}O_6$ C, 64.38; H, 8.53 Found: C, 64.12; H, 8.54	M^+ 354

concentration 1 μM) was added to the liposome containing ANS and the emission intensity followed upon exciting at 370 nm in a spectroflurimeter. Valinomycin is seen to transport K^+ inside and when there is no K^+, it transports Na^+. Gramicidin A is seen to be a Na^+ ionophore. M6 behaves similar to valinomycin, though less efficiently since the intensity changes were less than what was seen with the latter. Interestingly, however, M6 prefers K^+ slightly over Na^+ in bilayer translocation while it transfers Na^+ more efficiently in phase transfer experiments using picrates. M5 is seen to be a weak translocator of Li^+, as expected from its structure.

Experimental

Tricyclo[6.3.0.$0^{2,6}$]undecan-3,11-dione 1 was prepared according to the procedure described by us previously[4]:

General Procedure for the synthesis of macrocyclic ethers 2-5:

Into a 100 ml RB flask filled with a Dean-Stark water separator, reflux condensor and mercury seal, the dione (500 mg, 31 mmol), corresponding ethylene glycol (1 ml) and p-toluenesulphonic acid (5 mg) in dry benzene (50 ml) were placed. The reaction mixture was refluxed until all the dione had reacted (tlc) and then the mixture was diluted with more benzene (50 ml), washed with aq.$NaHCO_3$, brine and dried over anhydrous Na_2SO_4. The crude product obtained after removal of the solvent was purified by column chromatography to remove less polar impurities. The appropriate fraction containing the required product was crystallised from dichloromethane-hexane. Details of characterisation of the compounds 2-5 are given in Table 4.

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