Binding and Transport of Alkali Metal Ions by Synthetic Analogues of Nactins

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Our new synthesis of 32-linked macrocycles, models of nactin ionophores, has been extended to more complex structures. The complexation properties of these macrocycles were determined using the method of extraction of alkali metals from an aqueous phase. Extraction constants of some compounds are much higher than those measured for nonactin and for dibenzo-18-crown-6, whereas the association constants measured for the same alkali metals are very weak. There is no complexation selectivity towards the cations, only a slight preference for lithium is observed. These synthetic ionophores are able to transport alkali metal ions through a non-polar barrier, but without clear selectivity. The measured rates are lower than those obtained for nonactin and dibenzo-18-crown-6, the decomplexation being the rate-determining step.

Ionophores are molecules which have the property of transporting ions across biological membranes, an important function at the cellular level. Essential activities of a living organism depend on the relative concentrations of alkali cations outside and inside the cell.[1] Several antibiotics can selectively induce ion transport across the cellular membrane, amongst them the nactins.[2] Nactins are, structurally, 32-membered macrocycles containing eight oxygen atoms in the ring, four being ether oxygens and the remaining four belonging to ester groups (Figure 1). The main difference between nactins and synthetic

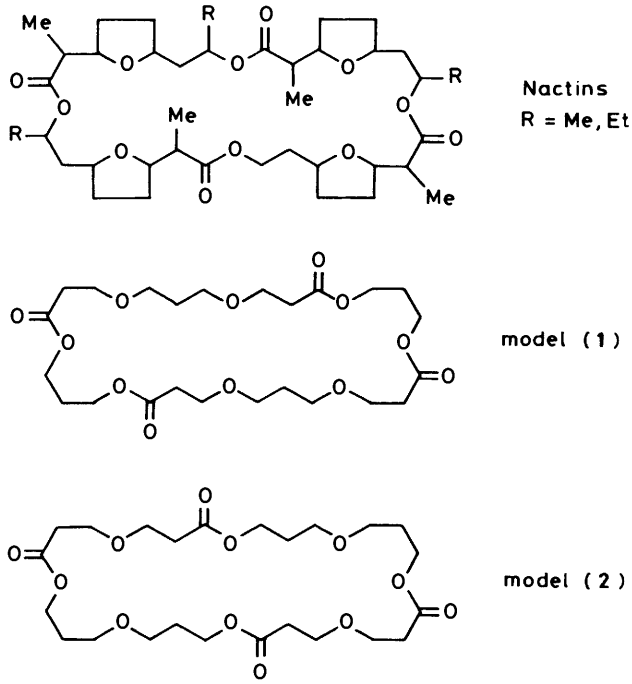


Figure 1.

ionophores (crown ethers and cryptates)[3] is that in the natural compounds the oxygen atoms are linked by three-carbon bridges while in the synthetic derivatives they are linked by two-carbon bridges. A further important difference is that nactins contain ester groups.

Our aim was to synthesize simple models of nactin macrotetrolides, avoiding the rather long and tedious synthesis of the natural antibiotics.[4] Two preceding communications[5,6] have described the new synthetic ways developed to prepare two models which differ in the arrangement of both ester and ether functions (Figure 1).

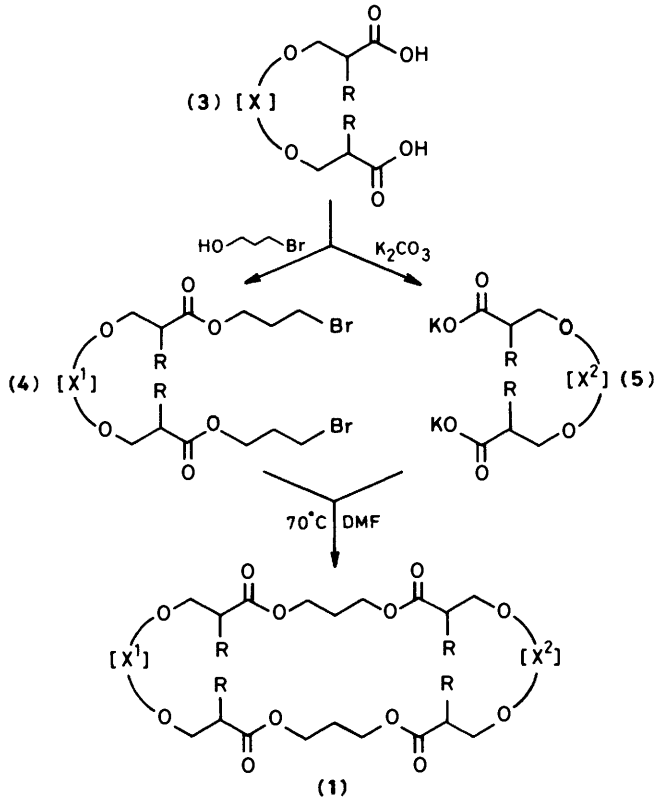
With a limited number of compounds representing the two models, it was possible to distinguish those structural features needed to improve the ionophore properties of our macrocycles. For instance, we have shown the prime importance of the position of the carbonyl groups in the ring. Type (**2**) models, closer to natural nactins, are far better carriers, being less flexible than our preliminary models. To overcome this last defect, the synthesis of type (**2**) macrocycles has been extended to give derivatives containing either single or double heterocyclic centres; these give more rigid structures.

Having thus obtained ten model compounds, we determined their complexation properties using the liquid–liquid extraction method. Other results obtained by spectroscopy (n.m.r. and u.v.) and by conductimetry are also described. Additionally, to complete the study of the ionophore properties of our molecules, transport rates across an organic phase (artificial membrane) have also been determined.

Results and Discussion

Synthesis.—Scheme 1 depicts the synthetic procedure used to obtain compounds (**1a**—**d**).[5,7] It is remarkable that, with the exception of (**1c**) which is a crystalline solid (m.p. 137 °C), the remaining macrocycles are oils, and difficult to purify; they needed several column chromatographic purifications and pentane extractions in order to obtain analytical samples. However, the yields of purified compounds are quite satisfactory (25—50%) if one bears in mind the size of the ring.

Compounds (**2**) are obtained following the procedure shown in Scheme 2 [(**2b**) and (**2c**) have already been described].[6,7] The different steps are easily performed and the cyclization reaction, based on a double substitution of bromine atoms or tosyl groups by carboxylic salts, is highly reproducible. The yield of cyclic compounds compared with that of by-products (probably linear polymers) is always high (75—90%). The purification of the oily macrocyles is difficult, but yields of pure compounds (25—50%) are significantly higher than those described in the literature for macrocyles of comparable size.[8–10]



Scheme 1.

Macrocycle (**2a**), symmetrical owing to the lack of ring substituents, will be used as a reference compound for type (**2**) models. All other compounds of this series have two methyl [phenyl for (**2c**)] groups on the ring carbons. These two chiral centres give rise to two diastereoisomers, referred to as (**A**) and (**B**), which have been routinely separated by column chromatography on silica gel. Isomer (**B**), which contains the complexation properties, remains adsorbed on silica, and is subsequently extracted with methanol in a Soxhlet apparatus. To date, we have been unable to assign the *meso* and the racemic structure to these compounds. They have identical ^1H and ^{13}C n.m.r. spectra because the chiral centres are too far apart.[11] An examination of molecular models shows that the racemic diastereoisomer can adopt a conformation that favours the complexation more easily than the *meso* one. Therefore, the compounds that we have labelled **A** and **B** should correspond to the *meso* and racemic forms respectively.

To introduce some rigidity into type (**2**) macrocycles, one (**2e**—**f**) or two (**2d**) furan rings, or a tetrahydrofuran ring (**2h**) were incorporated in the structure. For this last compound, the synthesis starts from the *exo*-7-oxa[2.2.1]bicycloheptane-2,3-dioic acid, obtained by hydrolysis of the corresponding anhydride.[12] The oxygen bridge is on the same side as the carboxylic groups, which will favour the existence of a binding cavity. An attempt to obtain a macrocycle with the oxabicycloheptane part near the methyl groups [the tetrahydrofuran analogue of (**2e**)] failed. The diacid dichloride (**14**) does not react with the ω-bromo alcohol (**6**; R = Me).

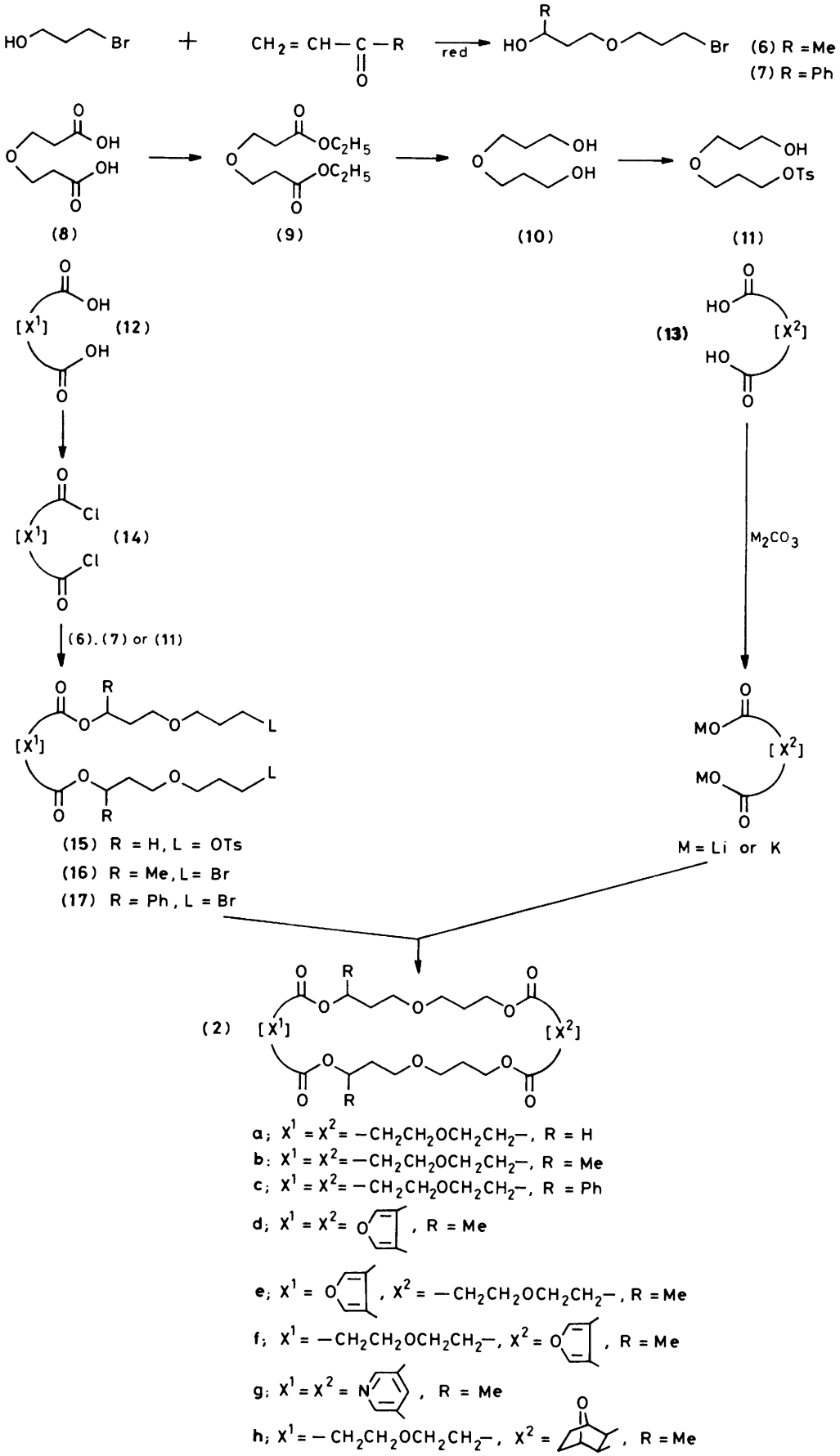
Complexation Properties.—These properties have been assessed by the 'liquid–liquid extraction method'. This method allows the quantitative determination of the cation-binding capability of cyclic polyethers in conditions approaching those found in biological systems. Thus, an aqueous solution of alkali nitrate containing 0.01% of the corresponding picrate is brought in contact with a chloroform solution of the macrocycle. The latter complexes the cation, and the picrate anion, more soluble in chloroform than the nitrate,[13] is transferred into the organic layer. The extraction equilibrium constants (K_e) can be calculated from the variation of the picrate concentration in one of the two phases. For this purpose, we have used the theory proposed by Eisenman *et al.*[14] for the determination of (K_e) in the case of the nactin ionophore antibiotics, and a programme of non-linear regression.

The results obtained with type (**1**) macrocycles and with (**A**) diastereoisomers of type (**2**) are not significant: the observed variations in concentration are weak and close to the experimental error. In contrast, the **B** diastereoisomers of compounds (**2b**—**h**) and the macrocycle (**2a**) exhibit complexation properties and their extraction equilibrium constants are reported in Table 1. (K_e) Values are generally much larger than those of dibenzo-18-crown-6 and of nonactin determined under the same experimental conditions. However, the values are close to those described for crown ethers of size similar to our macrocycles.[15–18] Generally, lithium is complexed preferentially to the other alkali cations (Table 2), with the exception of compounds (**2b**), (**2h**), and remarkably (**2c**). The Li^+/Cs^+ selectivity is very high for macrocycles (**2a**) and **B**-(**2d**) but, in contrast to nactins, there is no relationship between the selectivity and the diameter of the Na^+, K^+, and Rb^+ ions.

Compound (**2a**), unsubstituted on the ring carbons, is able to extract alkali cations from an aqueous phase, whereas the macrocycle (**1a**), its equivalent in the type (**1**) series, is not, thus proving the importance of the oxygen sequence. The presence of two methyl groups α to the ester oxygen considerably improves the extractability. This is probably a consequence of a more rigid structure caused by hindered rotations about the O–CH(Me) bond. This observation agrees with the semi-empirical calculations of Shanzer *et al.*[19] on the ionophore properties of macrocycles. The inclusion of a furan ring in the structure has different effects depending on its position. In the case of **B**-(**2e**) the furan is near the methyl groups (Scheme 2) and the cation-binding ability towards Li^+ is 10 times higher than that of **B**-(**2b**). This is a result of a less flexible structure with planar ester oxygens and with more closely wrapped side-chains. When the furan ring is on the opposite side to the methyl groups **B**-(**2f**), the macrocycle loses its extractability (Table 1). The molecular model of **B**-(**2f**) shows the furan ring outside the cavity with its heterocyclic oxygen unable to participate in the complexation. When two furan rings are present in both positions, **B**-(**2d**), its behaviour is intermediate between that of **B**-(**2e**) and **B**-(**2f**).

When the furan ring in **B**-(**2f**) is replaced by an *exo*-oxabicyclotetrahydrofuran ring **B**-(**2h**), with a more favourable geometry for complexation, the K_e constants increase, but remain considerably lower than those of **B**-(**2e**). Introduction of two pyridine rings **B**-(**2g**) produces an effect comparable with that of two furan rings **B**-(**2d**); the aromatic rings deprive the macrocycle of some of its flexibility, but there is no contribution from the 'outside' nitrogen lone pairs.

Physicochemical and Spectroscopic Studies.—In order to have an independent estimation of the interaction between


(6) R = Me
(7) R = Ph
(8)
(9)
(10)
(11)
(12)
(13)
(14)
(6), (7) or (11)
M2CO3
(15) R = H, L = OTs
(16) R = Me, L = Br
(17) R = Ph, L = Br
M = Li or K
(2)
a; X1 = X2 = −CH2CH2OCH2CH2−, R = H
b: X1 = X2 = −CH2CH2OCH2CH2−, R = Me
c; X1 = X2 = −CH2CH2OCH2CH2−, R = Ph
d; X1 = X2 = , R = Me
e; X1 = , X2 = −CH2CH2OCH2CH2−, R = Me
f; X1 = −CH2CH2OCH2CH2−, X2 = , R = Me
g; X1 = X2 = , R = Me
h; X1 = −CH2CH2OCH2CH2−, X2 = , R = Me

Scheme 2.

Table 1. Extraction equilibrium constants (K_e/l mol^{-1}) for the (**B**) diastereoisomers of type (**2**) models. For comparison, the (K_e) values of dibenzo-18-crown-6 (DB18C6) and of nonactin, determined in the same conditions, are given

Ligand	Li^+	Na^+	K^+	Rb^+	Cs^+
(2a)	1.6	0.6	1	0.9	0.01
B-(2b)	52.5	0.9	37	2.7	59.5
B-(2c)	1.6	11.5	2.6	7.8	37
B-(2d)	141	50.5	82	123	1.1
B-(2e)	484	164	283	283	25
B-(2f)	0.001	0.001	0	0	0
B-(2g)	9	2.4	3.3	4.6	0.4
B-(2h)	0.43	0.9	0.2	0.9	0.01
DB18C6	0.003	0.001	1	0.2	0.001
Nonactin	0.001	0.003	4.3	0.9	0.001

Table 2. Selectivity in the extraction of alkali ions from an aqueous phase for **B-(2)** compounds, DB18C6, and nonactin

Ligand	Order of selectivity	Li^+/Cs^+ selectivity ratio
B-(2a)	$Li^+ > K^+ > Rb^+ > Na^+ > Cs^+$	160
B-(2b)	$Cs^+ > Li^+ > K^+ > Rb^+ > Na^+$	0.9
B-(2c)	$Cs^+ > Na^+ > Rb^+ > K^+ > Li^+$	0.04
B-(2d)	$Li^+ > Rb^+ > K^+ > Na^+ > Cs^+$	128
B-(2e)	$Li^+ > K^+ = Rb^+ > Na^+ > Cs^+$	19
B-(2f)	$Li^+ \simeq Na^+ > Rb^+ \simeq Cs^+ \simeq K^+$	
B-(2g)	$Li^+ > Rb^+ > K^+ > Na^+ > Cs^+$	22
B-(2h)	$Na^+ = Rb^+ > Li^+ > K^+ > Cs^+$	43
DB18C6	$K^+ > Rb^+ > Li^+ > Na^+ \simeq Cs^+$	3
Nonactin	$K^+ > Rb^+ > Na^+ > Li^+ \simeq Cs^+$	1

ligands **B-(2)** and the alkali ions, different physicochemical and spectroscopic properties were measured.

Conductimetry has often been employed to determine the association constants of some cyclodepsipeptides[20,21] and many cyclic polyethers.[22–24] We have applied Evans' method[22] to **B-(2b)** using potassium picrate in acetonitrile. Surprisingly enough, we have not observed a significant modification of the equivalent conductancy, even working at different concentrations.

^1H and ^{13}C N.m.r. spectra of the most powerful complexants **B-(2b)**, **B-(2d)**, and **B-(2e)** are not modified when alkali salts are added to the solution, whereas those of nactins[25,26] and synthetic ionophores[27] are strongly affected, thus allowing the calculation of thermodynamic complexation values.

Electronic absorption spectroscopy is a further useful tool for study of the interaction between ionophores and metals. The method is based on the modification of the wavelength absorption of the counterion (generally the picrate anion) when the alkali cation is desolvated by the ligand. Thus, Smid *et al.*[28] have demonstrated the interaction between benzo-15-crown-5 and potassium picrate using tetrahydrofuran as solvent. Following this procedure, we have studied the couple **(2e)**/lithium picrate. In tetrahydrofuran, lithium picrate shows two absorption bands at 347 and 403 nm. The addition of increasing quantities of the diastereoisomer **A** does not produce any modification of the spectra, in accordance with its lack of lithium-binding ability. The same experiment, carried out with the **B** diastereoisomer, is reported in Figure 2. Three relevant facts can be noted: (i) a bathochromic shift of the 403 nm band, the maximum effect being obtained for a macrocycle/picrate ratio of 10 ($\Delta\lambda$ = 30 nm); (ii) the absence of an isosbestic point; (iii) a hypochromic effect for larger concentrations of **B-(2e)**. The first observation is similar to that reported by Smid[28] and corresponds to a free pair of ions, the Li^+ being entirely complexed by the macrocycle. The second pointed to the existence of several complexes in equilibrium. Finally, the hypochromic effect was unexpected. A possible explanation is that the picrate anion, when separated from the small lithium cation, increases its basicity enough to associate with the acidic protons α to the carbonyl group *via* hydrogen bonds (it is known that the absorbance of picric acid is much lower than that of the picrate anion).

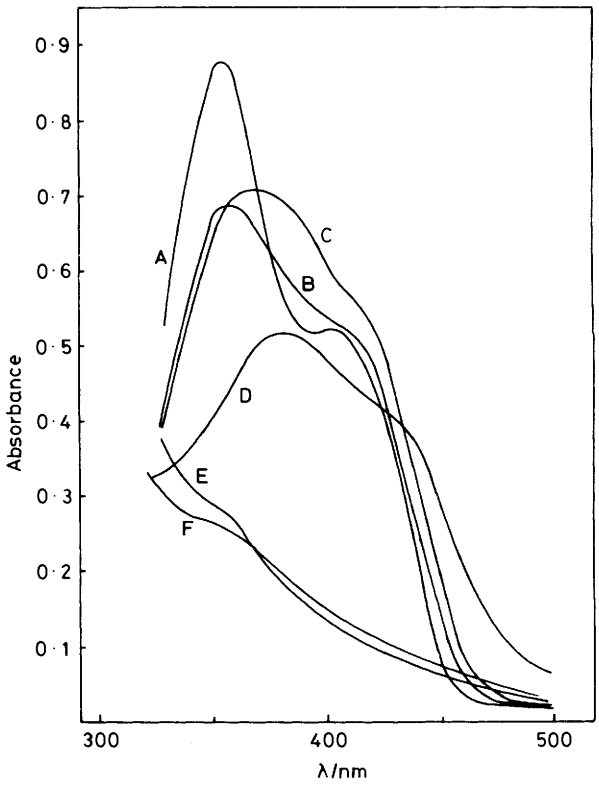


Figure 2. Electronic absorption spectra of a solution of lithium picrate in tetrahydrofuran. [**B-(2e)**]/[Picrate] ratios: 0. 1, 2, 20, 50 for A, B, C, D, E, F respectively

To sum up the last two sections, it may be noted that ion selectivity still remains a property of the natural ionophores. The products we have prepared, like the synthetic ionophores of Sumitomo *et al.*[29] have only a moderate selectivity. Our compounds, even after the last modifications, remain too flexible and probably exist in solution in several conformations of similar energy, but with different cavities, which can enclose ions of different size. To explain both the effect on the electronic spectra and the absence of effect on the conductance (which implies that the alkali ion has not lost its mobility) it is necessary to assume that the cation, perhaps accompanied by its solvent cage, interchanges quickly between different binding conformations of the same macrocycle.

Alkali Metal Transport through an Organic Phase.—To evaluate the ionophore properties of models (**1**) and (**2**) we have measured the transport rate of alkali ions between two aqueous phases through an organic one. With respect to its selectivity, it has been shown that this method gives transport results comparable with those obtained from cellular membranes or phospholipid bilayers.[30] The values presented in Table 3 were obtained by the already described method,[7] which uses picrate as the anion and chloroform as the organic phase.

Generally, **B** diastereoisomers of model (**2**) [and compound **(2a)**] have higher transport rates than **A** diastereoisomers and model (**1**) compounds. Concerning selectivity, the macrocycles present marked differences in the Rb^+/K^+ ratio, ranging from

11 A-(**2c**) to 0.3 **B**-(**2d**). With respect to the absolute values of rate constants, those of the worst carriers are similar to the rates measured by Kokube *et al.*[31] for polytetrahydrofuran macrocycles with three-carbon bridges between the oxygen atoms. On the other hand our best carriers are clearly inferior to nonactin (potassium) and dibenzo-18-crown-6 (save for lithium) (Table 3).

Conclusions.—Comparing the two parts of this study, extraction and transport, it is important to note that, for a given ion, there is no relationship between the extraction equilibrium constant and the rate transport constant. For instance, the largest transport rates correspond to macrocycles, (**2a**), **B**-(**2c**), **B**-(**2g**), having moderate extraction constants. This lack of relationship between binding and transport properties has been found both in natural ionophores[3] as well as in synthetic ones.[31] The rate-determining step in transport could be either the complexation or the decomplexation rate in the water–chloroform interphases (diffusion in a stirred organic phase is very rapid). We have compared (**2a**) and **B**-(**2h**) with nonactin (Figures 3 and 4). In all cases the maxima of complexation are

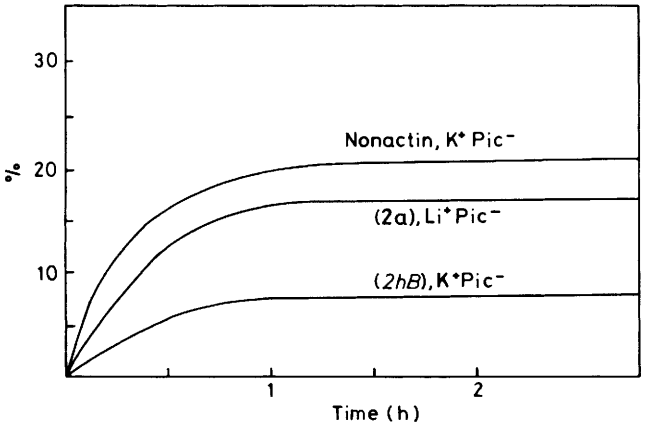


Figure 3. Complexation experiments at 25 °C

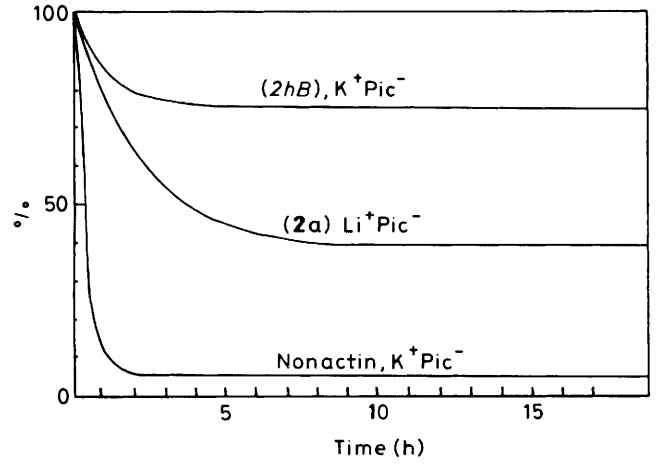


Figure 4. Decomplexation experiments at 25 °C

reached in *ca.* 1 h. However for decomplexation, the equilibrium is attained in 2 h for nonactin, 5 h for **B**-(**2h**) and 8 h for (**2a**). Moreover, if the decomplexation is complete for the natural product, only 25% of **B**-(**2h**) and 55% of (**2a**) are decomplexed at the equilibrium. Thus, for the compounds we have synthesized, the rate-limiting step in transport is decomplexation. This slow step together with the considerable flexibility of the macrocycles can explain the poor selectivity found in the extraction experiments.

Table 3. Transport rates (10^{-7} mol l^{-1} h^{-1}) of alkali ions through a chloroform phase

Ligand	Isomer	Li^+	Na^+	K^+	Rb^+	Cs^+
(**1a**)		0.8	1.2	1.4	6.3	1.8
(**1c**)			3.4	1.7	4.0	1.7
(**2a**)		81	74	81	75	78
(**2b**)	(A)	0.3	0.5	0.9	1.7	0.6
	(B)	45	54	43	51	36
(**2c**)	(A)		1.5	0.6	6.4	0.9
	(B)	17	71	78	71	13
(**2d**)	(A)	8	2	4	7	6
	(B)	45	25	60	19	41
(**2e**)	(A)	27	24	35	36	19
	(B)	6	4	5	17	7
(**2f**)	(A)	0.9	3	3	2	1
	(B)	6	6	8	7	10
(**2g**)	(B)	45	30	29	21	41
(**2h**)	(A)	20	15	16	13	17
	(B)	27	14	22	19	27
DB18C6		61	200	1 980	1 800	870
Nonactin				1 700		

Experimental

Complexation and Transport Measurements.—The electronic absorption spectra were recorded on a CARY 14 instrument. The extraction measurements were carried out for solutions with [macrocycle]–[metallic picrate] ratios between 0 and 50. These solutions were prepared from two starting solutions: the first one contained 25×10^{-4} mol l^{-1} of **B**-(**2e**) and 0.5×10^{-4} mol l^{-1} of lithium picrate in THF, and the second one contained 0.5×10^{-4} mol l^{-1} of lithium picrate in THF.

Conductimetric measurements were carried out in a U cell with platinum electrodes immersed in a thermostat at 25 ± 0.1 °C, coupled with a Tacussel resistivimeter. [Macrocycle]–[potassium picrate] ratios of 0, 0.25, 0.50, 0.75, 1.00, 1.25, and 1.50 were studied. These mixtures were obtained by taking between 0 ml and 1 ml from a solution containing 10^{-4} mol l^{-1} of macrocycle [DB18C6 or **B**-(**2b**)] and 10^{-3} mol l^{-1} of potassium picrate, and diluting it up to 25 ml with a 10^{-3} mol l^{-1} solution of potassium picrate in acetonitrile.

The method and the apparatus used for the determination of transport rates of alkali ions through chloroform has been described previously.[7]

Extraction Equilibrium Constants.—The starting solutions were: (i) an aqueous solution containing a mixture of nitrate (10^{-2} mol l^{-1}) and picrate (2×10^{-4} mol l^{-1}) of a given alkali ion; (ii) 7×10^{-4} mol l^{-1} of the macrocycle in chloroform. Each one of the above solutions (10 ml) was introduced into a cylindrical cell of 20 mm diameter. The two-phase system was vigorously stirred during 5 min, and afterwards centrifuged. To determine the concentration variation in the aqueous phase, the absorbance at 355 nm (ε 1.45×10^4) was measured. The different quantities necessary to calculate (K_e)[14] were determined. Each (K_e) is the mean value of three independent measurements.

Complexation and Decomplexation Experiments: Figures 3 and 4.—Solutions and apparatus are identical to the above described for (K_e) measurements. Complexation: 10 ml of the

chloroform solution of the macrocycle and 10 ml of the aqueous solution of alkali picrate were magnetically stirred at a slow and constant rate (60 r.p.m.) at 25 °C. The decrease in picrate concentration was followed by u.v. analysis. Decomplexation: identical volumes (10 ml) of chloroform and aqueous solutions were vigorously stirred during 5 min, and afterwards centrifuged. The chloroform layer was carefully withdrawn and placed in contact with 10 ml of distilled water. After magnetic stirring (60 r.p.m.), the quantity of picrate in the aqueous phase was titrated spectrophotometrically (at 355 nm).

Synthesis and Physicochemical Properties.—The ^1H n.m.r. spectra were recorded on a Varian EM-360A instrument at 60 MHz. The chemical shifts are reported in δ relative to internal TMS. The ^{13}C n.m.r. spectra were obtained at 20 MHz on a Varian FT80A, with $SiMe_4$ as internal standard, using recording conditions already described.[11]

The synthesis of macrocycles (**1a**—**d**), (**2b**), and (**2c**) has been described previously.[5,6] The FAB and chemical ionization mass spectra of the macrocycles described in this paper agree with the proposed structures and will be published elsewhere.[32] Only the molecular peaks will be given here.

3-*Oxaheptane*-1,7-*diol* (**10**).—This was obtained by reduction of the diester (**9**), prepared in turn by esterification of the diacid (**8**).[16] To a suspension of lithium aluminium hydride (25.6 g, 0.674 mol) in ether (370 ml) was added, under nitrogen, 370 ml of an ethereal solution of the diester (**9**) (37 g, 0.17 mol). The rate of dropwise addition should be rapid enough to produce a gentle reflux. When the addition was complete, the stirring was maintained during 2 h under nitrogen. A mixture of 210 g of Celite and 210 g of sodium sulphate decahydrate was added in small portions, ether being added when the mixture became too thick. The solids were filtered off, washed several times with chloroform, and the combined filtrates evaporated under reduced pressure. By distillation (b.p. 98—102 °C at 0.01 mmHg), the diol (**10**) (16 g, 71%) was obtained; δ($CDCl_3$) 1.81 (2 × 2 H, quint), 3.55 (2 × 2 H, t), and 3.70 (2 × 2 H, t); *m/z* 134 (Found: C, 54.0; H, 10.2. $C_6H_{14}O_3$ requires C, 53.7; H, 10.5%).

3-*Oxaheptanediol Monotoluene*-p-*sulphonate* (**11**).—The diol (**10**) (2.68 g, 0.02 mol) in pyridine (50 ml) was ice cooled. A solution of *p*-toluene-sulphonic acid chloride (3.82 g, 0.02 mol) in chloroform (30 ml) was added dropwise. After 2 h of stirring, the solution was acidified with 10M-hydrochloric acid and the organic layer was separated, dried ($MgSO_4$), and evaporated. The residue was purified by chromatography (silica gel; eluant chloroform) to give (**11**) as an oil (2.5 g, 23%); δ($CDCl_3$) 1.70 (2 H, quint), 1.80 (2 H, quint), 2.40 (3 H, s), 3.38 (2 H, t), 3.50 (2 H, t), 3.58 (2 H, t), 4.05 (2 H, t), 7.23 (2 H, d), and 7.70 (2 H, d); *m/z* 288 (Found: C, 54.3; H, 6.9. $C_{13}H_{20}O_5S$ requires C, 54.5; H, 7.0%).

Bis-3-(3-*tosylpropoxy*)*propyl* 3-*Oxaheptanedioate* [(**15**), $X^1 = O(CH_2CH_2)_2$].—Silver cyanide (1.1 g, 7.5 mmol), the sulphonic ester (**11**) (2.1 g, 7.5 mmol), and anhydrous benzene (8 ml) were introduced into a flask containing nitrogen. To the stirred mixture at room temperature the diacid dichloride (**14**)[33] (1.49 g, 7.5 mmol) was added. Stirring was continued for 24 h at 20—25 °C, and the mixture was then filtered. The precipitate was washed with benzene and the filtrate was distilled under reduced pressure to remove the solvent. The residue (2.0 g, 78%) was an oil; δ($CDCl_3$): 1.76 (2 × 2 H, quint), 2.40 (2 × 3 H, s), 2.50 (2 × 2 H, t), 3.35 (2 × 2 H, t), 3.45 (2 × 2 H, t), 3.70 (2 × 2 H, t), 4.06 (2 × 2 H, t), 7.30 (2 × 2 H, d), and 7.71 (2 × 2 H, d); *m/z* 702 (Found: C, 55.5; H, 7.0. $C_{32}H_{46}O_{13}S_2$ requires C, 55.0; H, 6.6%).

Bis-4-(3-*bromopropoxy*)*butyl Furan*-3,4-*dicarboxylate* (**16**; X^1 = –C=CH·O·CH=C–).—Furan-3,4-dicarboxylic acid (2 g, 11.9 mmol) and thionyl chloride (20 ml) were mixed and heated at 50—60 °C until gas evolution stopped. The excess of thionyl chloride was removed under reduced pressure and the diacid dichloride thus obtained, together with the alcohol (**6**)[6] (5.02 g, 23.8 mmol), silver cyanide (3.2 g, 23.8 mmol), and anhydrous benzene (15 ml) were stirred for 24 h at 20—25 °C; the mixture was then filtered. The precipitate was washed with benzene and the filtrate was distilled under reduced pressure to remove the solvent. The residue was purified by chromatography (silica gel; eluant acetone) to give (**16**) as an oil (5.7 g, 75%); δ($CDCl_3$) 1.33 (2 × 3 H, d), 2.00 (4 × 2 H, m), 3.41 (2 × 2 H, t), 3.47 (2 × 2 H, t), 3.50 (2 × 2 H, t), 5.21 (2 × 1 H, m), and 7.89 (2 × 1 H, s); δ ^{13}C ($CDCl_3$) 20.2, 30.7 32.8, 36.0, 67.3, 68.2, 69.4, 118.9, 148.4, and 161.1; *m/z* 542 (Found: C, 43.9; H, 5.6. $C_{20}H_{30}Br_2O_7$ requires C, 44.3; H, 5.6%).

Pyridine-3,5-*dicarbonyl Dichloride.*—The corresponding diacid (1 g, 6 mmol) in 10 ml of dichloromethyl–methyl ether containing a small amount of zinc chloride was heated under reflux for 1.5 h. After filtration and evaporation of the filtrate, the residue was an oil that crystallised with time, m.p. 72 °C (90%). The compound was used without further purification.

Bis-4-(3-*bromopropoxy*)*butan*-2-*yl Pyridine*-3,5-*dicarboxylate* (**16**; X^1 = –CH=CH·C=CH·N=C–).—Following an identical procedure to that used for (**16**; X1 = –C=CH·O·CH=C–), the crude ester was subjected to two successive chromatographic purifications (silica gel), first with chloroform as eluant and second using methanol. The pure product was an oil (53%); δ($CDCl_3$) 1.40 (2 × 3 H, d), 2.01 (4 × 2 H, quint), 3.40 (2 × 2 H, t), 3.46 (2 × 2 H, t), 3.53 (2 × 2 H, t), 5.33 (2 × 1 H, m), 8.76 (1 H, s), and 9.30 (2 × 1 H, s); δ ^{13}C ($CDCl_3$) 20.3, 30.5, 32.8, 36.0, 67.2, 68.2, 68.2, 70.4, 126.5, 137.8, 153.6, and 163.8; *m/z* 553 (Found: C, 45.5; H, 5.7; N, 2.1. $C_{21}H_{31}Br_2NO_6$ requires C, 45.6; H, 5.6; N, 2.5%).

7-*Oxa*[2.2.1]*bicycloheptane*-2,3-*dioic Acid* (**13**; X^2 = –CH·O·CH·CH_2·CH_2·CH·CH).—Water (500 ml) was added to the corresponding anhydride (20 g, 108 mmol).[12] The pH was adjusted to 10 with sodium hydroxide and the mixture was stirred for 24 h at room temperature. The solution was then acidified (pH 4) with 10% hydrochloric acid and afterwards concentrated under reduced pressure. The residue was dissolved in acetone and the sodium chloride filtered off. Evaporation of acetone yields an oil that crystallized with time (87%), m.p. 128—130 °C. The product was purified by crystallization in acetone–chloroform (1:1) (Found: C, 51.7; H, 5.2. $C_8H_{10}O_5$ requires C, 51.6; H, 5.4%).

Macrocycles (**2a**, **2d**—**h**): *General Prodecure.*—The diacid (**13**) (6.5 mmol) was dissolved in ethanol (15 ml) containing water (1.5 ml). An aqueous solution of 20% lithium carbonate was added until pH 7 [in the case of (**2a**) and (**2e**), potassium carbonate was used]. The solvents were removed under reduced pressure, the residue was dissolved in anhydrous dimethylformamide (100 ml), and compounds (**15**)—(**17**) (6.5 mmol) were added dropwise to the stirred mixture. The stirring was continued for 22 h at 70—80 °C. The solvent was evaporated and the residue extracted with chloroform. The chloroform extract was washed with a small quantity of water and dried ($MgSO_4$). By evaporation of the solvent an oily product was obtained.

Compound (**2a**) was purified by chromatography (silica gel; eluant acetone) followed by several extractions with pentane.

For the other macrocycles (**2d**—**h**) chromatography over silica gel with acetone as eluant [benzene for (**2h**)] gave the oily diastereoisomer **A**. The diastereoisomer **B** was extracted with methanol from silica gel in a Soxhlet. The purification of each diastereoisomer was carried out by successive extractions with pentane. In the following, yields are given after purification.

(**2a**) (43%); δ($CDCl_3$) 1.86 (4 × 2 H, quint), 2.52 (4 × 2 H, t), 3.58 (4 × 2 H, t), 3.70 (4 × 2 H, t), and 4.13 (4 × 2 H, t); *m/z* 520 (Found: C, 55.1; H, 7.6. $C_{24}H_{40}O_{12}$ requires C, 55.4; H, 7.7%).

(**2d**) [41% **A**, 8% **B**]; δ($CDCl_3$) 1.32 (2 × 3 H, d), 1.88 (4 × 2 H, m), 3.42 (4 × 2 H, t), 4.33 (2 × 2 H, t), 5.18 (2 × 1 H, m), and 7.90 (4 × 1 H, s); *m/z* 536 (Found: C, 58.2; H, 6.3. $C_{26}H_{32}O_{12}$ requires C, 58.2; H, 6.0%).

(**2e**) [43% **A**, 16% **B**]; δ($CDCl_3$) 1.36 (2 × 3 H, d), 1.91 (4 × 2 H, m), 2.56 (2 × 2 H, t), 3.35 (2 × 2 H, t), 3.38 (2 × 2 H, t), 3.72 (2 × 2 H, t), 4.13 (2 × 2 H, t), 5.26 (2 × 1 H, m), and 7.78 (2 × 1 H, s); *m/z* 542 (Found: C, 57.4; H, 6.9. $C_{26}H_{38}O_{12}$ requires C, 57.6; H, 7.1%).

(**2f**) [22% **A**, 2.5% **B**]; δ($CDCl_3$) 1.23 (2 × 3 H, d), 1.86 (4 × 2 H, m), 2.50 (2 × 2 H, t), 3.40 (2 × 2 H, t), 3.50 (2 × 2 H, t), 3.71 (2 × 2 H, t), 4.33 (2 × 2 H, t), 5.03 (2 × 1 H, m), and 7.93 (2 × 1 H, s); *m/z* 542 (Found: C, 57.5; H, 7.0. $C_{26}H_{38}O_{12}$ requires C, 57.6; H, 7.1%).

(**2g**) [13% **A**, 17% **B**]; δ($CDCl_3$) 1.36 (2 × 3 H, d), 1.96 (2 × 2 H, m), 3.46 (2 × 2 H, t), 3.53 (2 × 2 H, t), 4.40 (2 × 2 H, t), 5.30 (2 × 2 H, m), 8.68 (2 × 1 H, s), and 9.03 (4 × 1 H, s); *m/z* 558 (Found: C, 59.9; H, 6.0; N, 4.8. $C_{28}H_{34}N_2O_{10}$ requires C, 60.2; H, 6.1; N, 5.0%.

(**2h**) [18% **A**, 11% **B**]; δ($CDCl_3$) 1.26 (2 × 3 H, d), 1.86 (2 × 2 H, s), 2.52 (2 × 2 H, t), 2.72 (2 × 1 H, s), 3.43 (2 × 2 H, t), 3.60 (2 × 2 H, t), 3.70 (2 × 2 H, t), and 5.00 (2 × 1 H and 2 × 2 H, m); *m/z* 572 (Found: C, 59.0; H, 7.4. $C_{28}H_{44}O_{12}$ requires C, 58.7; H, 7.7%).

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