Selective cation binding with *cis,cis*-1,3,5-trioxycyclohexyl based ligands: application to ion transport and electrochemical detection and assessment of complexation by electrospray mass spectrometry

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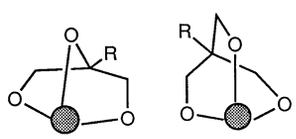
The synthesis is reported of two sets of oxa-amide ionophores based on 2-phenylglycerol and *cis,cis*-cyclohexane-1,3,5-triol, with ligand coordination numbers of four, five and six. Ionophores based on the hexadentate cyclohexyl triamide 9 show excellent Na^+/K^+ selectivity ($-\log K^{pot}_{Na,K} = 3.1$), and the pentadentate analogue 10 shows good Li^+/Na^+ selectivity ($-\log K^{pot}_{Li,Na} = 2.2$). Solution NMR and liquid–liquid extraction studies confirmed the formation of 1:1 complexes and IR studies pinpointed the presence of amide binding. Ligands based on 2-phenylglycerol exhibited good Ca^{2+} selectivity which was highest for the hexadentate triamide, 6. Detailed ESMS studies revealed similar trends in ion-binding, performed under controlled conditions.

Introduction

The characterisation of effective new ionophores for use in the measurement of intracellular or extracellular cation concentrations remains a subject of considerable current interest.[1–3] Much attention is focused on the development of neutral ionophores that may be used in potentiometric ion-selective electrodes. The design of suitable ionophores is guided by a combination of factors: the ionophore must be sufficiently lipophilic so as not to leach from a polymeric membrane into the aqueous analyte; it must possess sufficiently rapid kinetics of complexation to ensure a stable response within seconds and it should exhibit sufficient selectivity in binding and transporting the target ion so that its response is nernstian over a large range of activities in the presence of potential interferent ions.

Guidelines for the design of suitable ionophores emerge from a consideration of the complexation chemistry of the ions involved. Thus, for ions of relatively high charge density, such as lithium, amide carbonyl oxygens are particularly good donors.[1] Smaller ions—such as lithium and magnesium—tend to form more energetically stable complexes with ligands that engender six-ring chelates (compared to five-ring analogues),[4] while larger ions (*e.g.* K^+ *vs.* Li^+/Na^+) prefer to take up higher coordination numbers in their complexes. With these thoughts in mind and as a continuation of a systematic study of the efficacy of appropriate podands and macrocyclic ligands for selective cation complexation, we report the synthesis and evaluation of two sets of ionophore of varying denticity. The first extends our work on podands based on a trioxa-system.[5] Whilst the trioxa-triamide **1** exhibited reasonably good Na^+/K^+ selectivity ($-\log K^{pot}_{Na,K} = 2.64$)[5] compared to the structurally similar ligand **2** reported by Simon[2] ($-\log K^{pot}_{Na,K} = 2.3$), it was felt that the juxtaposition of three six-ring chelates involving the ether oxygens may have been giving rise to some degree of strain in the putative six-coordinate 1:1 complex[4,5] (Scheme 1). Furthermore, in some structurally analogous work involving cryptands, it had been noted that when there were three carbons around the bridgehead separating two successive oxygen atoms *e.g.* **3**, complex stabilities with Na^+/K^+ were much lower[6] and selectivities much poorer than related cryptands, *e.g.* **4** and **5** based on a glycerol sub-unit.[7,8] Accordingly, we have examined podands **6**–**8**, derived from 2-phenylglycerol, varying the coordination number from six to four by replacing amide groups with butyl chains (Scheme 1).



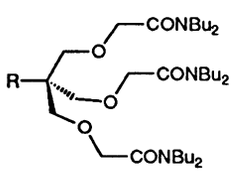
Scheme 1

For the purposes of comparison, we have examined the behaviour of **6**–**8** in relation to ligands possessing similar denticities but based on a *cis,cis*-1,3,5-trioxycyclohexyl sub-unit. Ligands **9**–**11** will normally adopt a chair conformation with the three substituents in equatorial sites. Interconversion between this conformer and the alternative conformer wherein the substituents adopt axial sites will be rapid and should be promoted by the presence of a suitable metal ion (Scheme 2). Following ion-binding, ligands **9**–**11** may favour the binding of smaller cations[9] since cooperative ligation of the ether oxygens engenders three six-membered chelate rings in a pseudo-adamantyl array.

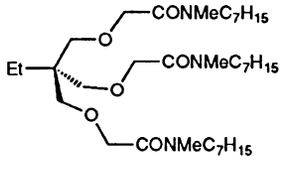
Results and discussion

Ionophore synthesis

All of the trioxa ionophores **6**–**11** were prepared *via* the corresponding triols by stepwise alkylation methods. A Wittig reaction with 2-acetoxyacetophenone **12** yielded 3-acetoxy-2-phenylpropene **13** which was oxidised to the corresponding 1,2-diol, **14**, by *N*-methylmorpholine*N*-oxide (NMO) under OsO_4 catalysis. Treatment of this diol with NaOMe–MeOH yielded 2-phenyl-1,2,3-trihydroxypropane **15** as a colourless solid. Alkylation of the triol (NaH, THF, reflux, 6 d) with *N*,*N*-dibutyl-2-chloroethanamide **18** gave the triamide derivative **6** in modest yield after purification by chromatography on neutral alumina. In the synthesis of the mono- and di-butyl ethers **7** and **8**, the corresponding mono-butyl ether **22** and mono-amide **21** were prepared by rather different reaction sequences. Reaction of the triol **15** with 2,2-dimethoxypropane under acid catalysis afforded the ketal **16** which was alkylated with 1-bromobutane (NaH–THF) to give the product ether. Deprotec-



1 a R = Et
b R = Ph



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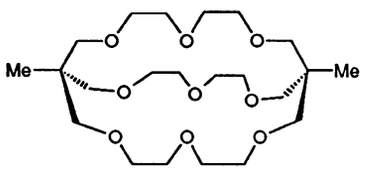

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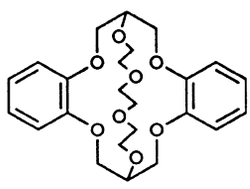

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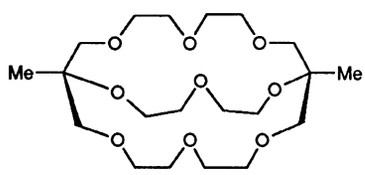

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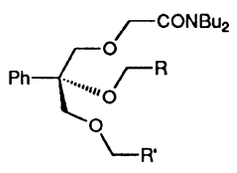
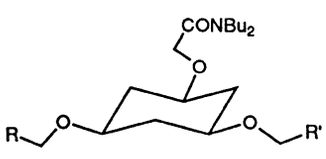
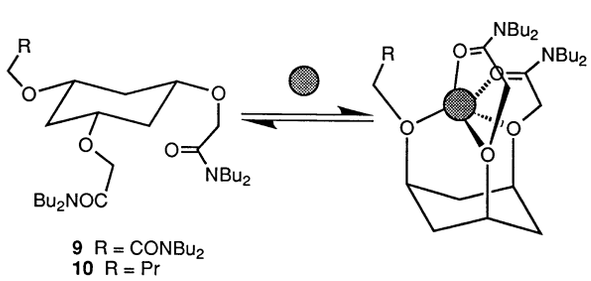


6 R = $CONBu_2$ = R'
7 R = $CONBu_2$; R' = Pr
8 R = Pr = R'



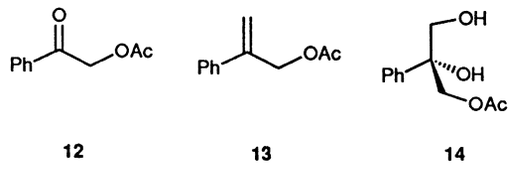
9 R = $CONBu_2$ = R'
10 R = $CONBu_2$; R' = Pr
11 R = Pr = R'



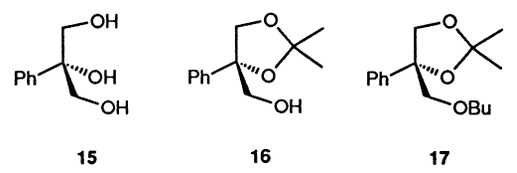
9 R = $CONBu_2$
10 R = Pr

Scheme 2

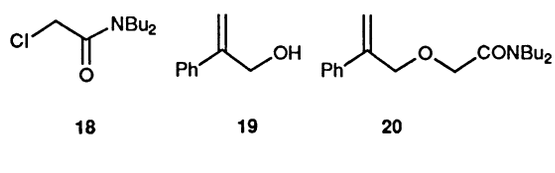
tion was sought under a variety of conditions (*e.g.* aqueous oxalic acid, TsOH–H_2O; Ts = *p*-$MeC_6H_4SO_2$) but competitive dehydration occurred to give the alkyl ether **23**. With hindsight, alternative methodology for deprotection of the diol (*e.g.* using CBr_4–PPh_3)[10] may have averted acid-catalysed elimination. The route which was eventually used in the synthesis of **7** and **8** involved the intermediacy of 2-phenylpropenol, **19**. Alkylation of this alcohol with BuBr or $ClCH_2CONBu_2$ (NaH, THF) yielded the intermediate ethers **23** and **20** respectively and oxidation with NMO and OsO_4 gave the desired 1,2-diols **22** and **21**. Alkylation with the appropriate electrophile then afforded the target ligands **7** and **8**.



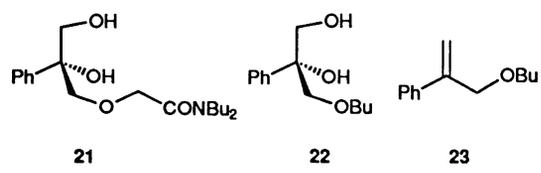
12 **13** **14**



15 **16** **17**

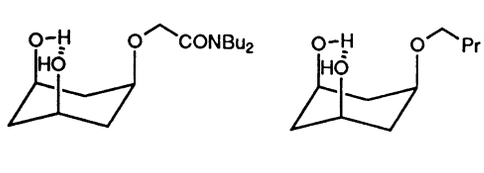


18 **19** **20**



21 **22** **23**

The C_3-symmetric triamide **9** was prepared by direct alkylation of anhydrous *cis*,*cis*-cyclohexane-1,3,5-triol (NaH, KI, $ClCH_2CONBu_2$, THF). The preparation of the related ligands **10** and **11** was undertaken in a low-yielding, two-step alkylation sequence. Attempts to selectively protect the 1,3-diol moiety in *cis*,*cis*-cyclohexane-1,3,5-triol under a variety of conditions [*e.g.* $(MeO)_2CMe_2$, H^+; PhCHO, $ZnCl_2$; cyclohexanone, H^+] were stymied by the poor solubility of the triol precursor in a range of solvents. Mono-alkylation of the triol proceeded in dimethylformamide (DMF) in modest yield however: reaction with $ClCH_2CONBu_2$ (DMF, NaH, 80 °C, 2 d) afforded the mono-amide **24** in 22% yield, while treatment with 1-bromobutane (DMF, NaH, 5 d, 80 °C) allowed the isolation of the monobutyl ether **25** in a 37% isolated yield following chromatographic purification. Subsequent alkylation of these diols gave the desired ionophores **10** and **11**.



24 **25**

Electrode response studies

The behaviour of ligands **6**–**11** as ionophores for the detection of selected Ia/IIa cations was compared in standard polymeric membrane ion-selective electrodes (ISEs). The selectivity coefficients for the plasticised PVC-based membrane electrodes were measured using a fixed interference method at 298 K for **6**–**8**, and at 310 K for **9**–**11**. For the evaluation of these selectivity coefficients, a 0.1 mol dm^{-3} solution of the chloride salt of the interferent ion was used. The results of these studies for potentiometric ISEs incorporating **6**–**8** are shown in Table 1.

Table 1 Selectivity coefficients and electrode response parameters for ISEs based on ionophores **6**–**8** and **1b** (310 K)

Ligand	Primary ion	Calibration: Slope/ mV	Calibration: Limit of detection/ mol dm^{-3}	Selectivity: p$K_{i,j}^{pot}$ Na	K	Mg	Ca	Li
Monoamide **8**	Li	59.1	$10^{-4.5}$	0.19	0.2	1.8	0.7	—
	Mg	25.5	$10^{-4.3}$	−0.9	−0.8	—	0.1	—
	Ca	27.5	$10^{-4.7}$	−0.7	−0.7	2.9	—	—
Diamide **7**	Na	61.5	$10^{-5.1}$	—	0.8	1.4	0.5	—
	Ca	30.8	$10^{-6.0}$	3.1	3.7	4.5	—	3.4
Triamide **6**	Na	56.0	$10^{-4.4}$	—	0.9	1.3	0.5	—
	Ca	30.0	$10^{-5.1}$	2.4	3.3	4.7	—	2.1
1b[5]	Ca	30.0	$10^{-5.2}$	2.3	3.2	4.8	—	2.0

Table 2 Selectivity coefficients and electrode response parameters for ISEs based on **9**–**11** (298 K)

Ligand	Primary ion	Calibration: Slope/ mV	Calibration: Limit of detection/ mol dm^{-3}	Selectivity: p$K_{i,j}^{pot}$ Na	K	Mg	Ca	Li	NH_4
Monoamide **11**	Na	60.0	$10^{-4.8}$	—	0.6	1.3	0.8	0.1	0.5
	Li	56.0	$10^{-5.1}$	0.7	1.2	2.1	1.3	—	1.1
Diamide **10**	Na	57.2	$10^{-4.8}$	—	1.7	1.9	0.5	0	1.5
	Li	57.0	$10^{-5.6}$	2.2	3.8	3.6	2.1	—	3.6
	Ca	27.4	$10^{-5.2}$	0.6	3.6	4.2	—	−0.9	3.3
Triamide **9**	Na	55.5	$10^{-5.9}$	—	3.1	2.7	0.8	0.1	3.0
	Li	58.4	$10^{-5.8}$	0.8	3.4	3.2	1.3	—	3.4
	Ca	27.7	$10^{-6.6}$	−0.6	3.9	4.0	—	−0.9	3.6

Using the diamide **7** and the triamide **6**, good selectivity and sensitivity for Ca^{2+} were observed: a nernstian slope with a limit of detection of $10^{-6.0}$ mol dm^{-3} was found and selectivities over Li^+, Na^+, K^+, Mg^{2+} were good, particularly with the diamide-based sensor. The sensor based on **6** behaved in a very similar manner (slope, detection limit, selectivity) to the parallel electrode incorporating **1b**,[5] (Table 1) indicating that the loss of one methylene group had not perturbed selectivity in ion-binding. Reduction of the ligand coordination number had a pronounced effect on ion selectivity only in the transition from a potential ligand coordination number of five to four. This was illustrated by the dramatic reduction in selectivity and deterioration in response characteristics for Ca^{2+}, and by the very modest enhancement in selectivity for Li^+.

Similar experiments were conducted with plasticised PVC-based membrane electrodes incorporating **9**–**11** (Table 2). Fast response times (<10 s) were observed for all of the electrodes examined, and the combination of the presence of the three focused ether oxygen lone-pairs which give rise to three six-ring chelates on ion binding and the strongly dipolar amide donors gave rise to some pronounced selectivity in response to the small, relatively charge-dense lithium ion. This effect was most marked with the five-coordinate ligand **10**, which exhibited selectivities over K^+, Mg^{2+} and Ca^{2+} of $10^{3.8}$, $10^{3.6}$ and $10^{2.1}$ respectively. Of particular interest was the good selectivity found in the presence of 0.1 mol dm^{-3} Na^+. The measured value of $10^{2.2}$ is one of the highest found for a non-macrocyclic ionophore[1] (*cf.* $10^{3.25}$ for the most selective Li^+ sensor based on a diamide-substituted 14-crown-4 ionophore[1a]).

Marked selectivity for sodium over potassium was observed with sensors based on the triamide **9**. This selectivity is clearly attributable to the donor atom and donor number preferences of the smaller, more charge-dense Na^+ ion. The measured selectivity for Na^+ over K^+ of $10^{3.1}$ (Table 1), obtained as with all the reported data using the relatively polar plasticiser-nitrophenyl octyl ether, constitutes the highest reported sodium ion selectivity in a membrane-based potentiometric Na^+ sensor.[2d] The claim that certain calixarene-based sensors impart a Na^+/K^+ selectivity of over 1000[12] has recently been shown[2d] to be incorrect. All other sodium-selective sensors, including

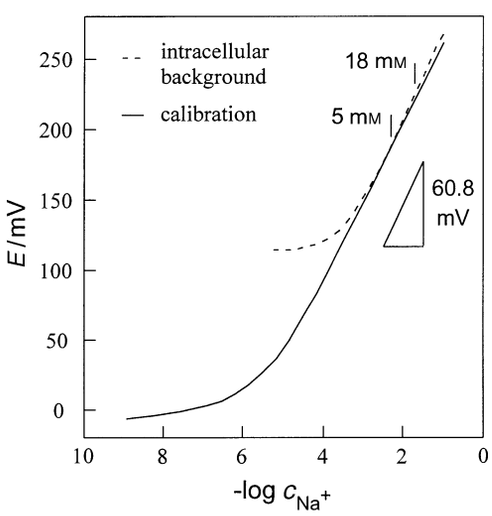


Fig. 1 Response curve for Na^+ obtained with the electrode based on **9** in the presence of interfering ions, of concentrations similar to those found in intracellular fluids (310 K; 120 mmol dm^{-3} K^+, 3 mmol dm^{-3} Mg^{2+}, 0.4 μmol dm^{-3} Ca^{2+})

those based on monensin,[13] bis-crown,[14,2d] hemispherand[15] and calixarene[12,16,17] derivatives have Na^+/K^+ selectivities of less than 10^3:1. Given that the Li^+ ion (except in patients being treated for depression with Li_2CO_3) is not a biologically significant ion, and that intracellular Ca^{2+} concentrations are typically much less than 0.1 mmol dm^{-3}, this ionophore may be of interest for the selective determination of sodium concentration in intracellular fluids. Typically, the intracellular Na^+ concentration is small compared to that of the coexisting K^+ ion (Na^+ *ca.* 10; K^+, 120 mmol dm^{-3}), so that significant sodium selectivity is needed for an accurate analysis. The calculated required selectivity coefficient of $-\log K_{Na,K}^{pot}$ in Na^+ assay with < 1% error is −3.5.[18] Using an electrode incorporating the cyclohexyltriamide ionophore **9**, the response curve for Na^+ has been measured in the presence of a simulated intracellular

Table 3 IR and NMR studies of complexation with ligands **6**, **7**, **9** and **10** (CH_3OH or CD_3OD; 293 K)

	$\Delta\nu_{CO}/cm^{-1}$[a]				
Ligand	Li^+	Na^+	K^+	Ca^{2+}	log K_{CaL}[b]
6	—	3	1	18	3.2
7	—			24	3.0
10	14	10	2	14	3.6
9	19	12	0	12	>5.5

[a] IR spectra were run with an M : L ratio of 5 : 1; [ligand] = 5×10^{-2} mol dm^{-3}. $LiClO_4$, $Ca(ClO_4)_2$, $NaCF_3SO_3$ and KI were used as the metal salts. The ligand exhibits a carbonyl stretching frequency of 1647 cm^{-1} in MeOH solution (CaF_2 cells). [b] Estimated from a fitting of the ^{13}C NMR titration curve.

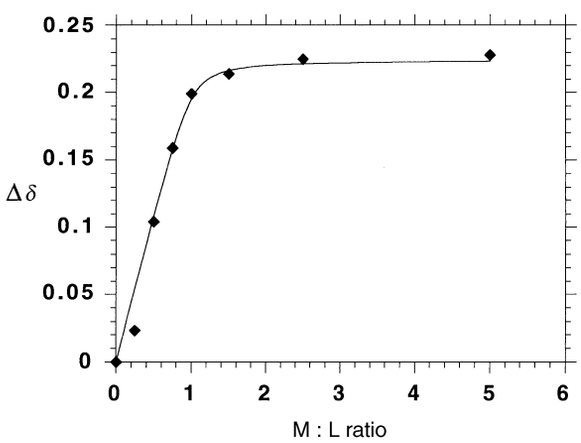


Fig. 2 Variation of the change in the ^{13}C NMR chemical shift for the OCH_2CO resonance in ligand **10**, with added $Ca(ClO_4)_2$ in CD_3OD (293 K, [**10**] = 5×10^{-2} mol dm^{-3})

background of interfering ions (Fig. 1). Given that the typical intracellular Na^+ concentration range is between 5 and 18 mmol dm^{-3}, the electrode is able to measure the sodium concentration with nernstian response in this region.

Spectroscopic investigation of solution complexation

Aspects of the complexation behaviour of selected ionophores have been studied by solution IR, NMR spectroscopic and electrospray mass spectrometric methods. IR studies carried out in methanol solution (293 K, 5×10^{-2} ligand, 25×10^{-2} mol dm^{-3} metal salt) using CaF_2 cells were used to establish the presence of amide carbonyl ligation in this competitive coordinating solvent (Table 3). Amongst the podands based on 2-phenylglycerol, only the calcium complexes of **6** and **7** exhibited clear evidence of carbonyl ligation as shown by the reduction in the carbonyl IR stretching frequency. With the cyclohexyl-based ligands **9** and **10**, the amide was clearly coordinated to the metal ion in the Li^+, Na^+ and Ca^{2+} complexes, but not in the K^+ complexes. For the calcium complexes of **6**, **7**, **9** and **10**, a ^{13}C NMR titration was carried out in CD_3OD (293 K) in order to establish the stoichiometry of complexation and semi-quantitatively assess the strength of the binding interaction. Incremental addition of $Ca(ClO_4)_2$ to a solution of **9** in CD_3OD (101 MHz) gave separate ^{13}C signals for the free and bound ligand carbons up to a stoichiometry of 1 : 1, when only resonances due to the complexed ligand were observed. Such behaviour is consistent with formation of a relatively thermodynamically stable 1 : 1 complex (log $K > 5.5$) as the exchange between free and bound complex is slow on the NMR timescale. For the other calcium complexes examined, the rate of exchange between 'free' and 'bound' complexes was sufficiently fast to allow the monitoring of the

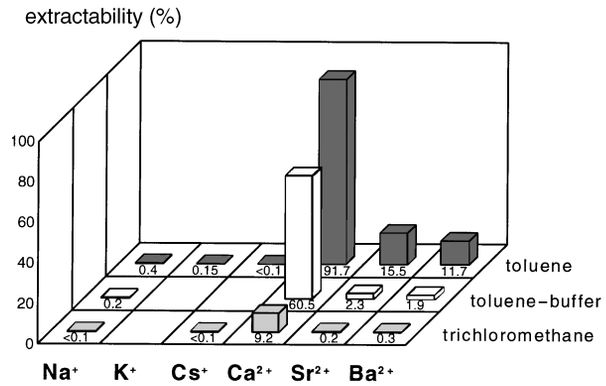


Fig. 3 Extractability of metal ions with ligand **9**; [$M(NO_3)_2$] = 10^{-4} mol dm^{-3}, [picric acid] = 5×10^{-3} mol dm^{-3}, pH = 5.2 (0.1 mol dm^{-3} NaOAc/HCl buffer), [**9**] = 10^{-3} mol dm^{-3} in $CHCl_3$ or PhMe

coordination shift as a function of Ca^{2+} : ligand ratio. Estimates of the equilibrium constant for 1 : 1 complex formation are given in Table 3, based on a fitting of the binding curve obtained by plotting $\Delta\delta_C$ *vs.* Ca^{2+} : ligand ratio.[19] A representative example is given in Fig. 2, which depicts the change in the ^{13}C chemical shift for the OCH_2CO carbon resonance in **10** as a function of added $Ca(ClO_4)_2$. In this case, the equilibrium constant was estimated to be $10^{3.6(2)}$, which is significantly lower than that found with the hexadentate triamide **9** ($>10^{5.5}$), and rather similar to the values estimated with **6** and **7** ($10^{3.2}$ and $10^{3.0}$ respectively).

The electrode response studies had highlighted the selectivity of **10** for Li^+ (over Na^+) and of **9** for Na^+ (over K^+). Analysis of the ^{13}C NMR titration curves following addition of $LiClO_4$ or $NaCF_3SO_3$ (293 K, CD_3OD) suggested very weak complexation with estimated equilibrium constants for 1 : 1 complex formation of the order of unity and 20 respectively.

Liquid–liquid extraction behaviour of ligands 9 and 1b

Given that the ionophore in membrane-based ion-selective electrodes serves to transport selectively the target ion from an aqueous phase into a relatively fluid, plasticised PVC matrix, relevant and useful information about the efficacy of ionophores may be obtained by examining the phase transfer of various metal ions from aqueous to different lipophilic organic phases.[20] With ligand **9**, and also **1b**, liquid–liquid extraction investigations were performed in micro-reaction vials in buffered aqueous media (298 K, pH 5.2) and toluene or chloroform. The hexadentate ligand **9** showed some evidence for Na^+/K^+ selectivity in extraction (Fig. 3), although the most pronounced selectivity in extraction and the most efficient example involved transport of the Ca^{2+} ion (over Sr^{2+}, Ba^{2+}, Na^+, K^+ and Cs^+). Information about the overall stoichiometry of complexation was also obtained by measuring the ratio of cation concentrations in the organic and aqueous phase (D_M) as a function of the ligand concentration in the organic solvent (c_L), Fig. 4. The data obtained with slopes of unity were consistent with preferential 1 : 1 complex formation[20] for Ca^{2+}, Sr^{2+}, Ba^{2+} and Na^+, as had been surmised from the NMR titration data in CD_3OD.

Similar experiments were undertaken with ligand **1b**—which behaved in a parallel manner to **6** in electrode response studies. Again preferential Ca^{2+} extraction was defined (Fig. 5), and for both Ca^{2+} and Sr^{2+} analysis of the log D_M *vs.* c_L response was consistent with 1 : 1 complex formation. This observed calcium selectivity in the extraction studies accords with the selectivity defined by the electrode response work with **6** and **1b**.[5]

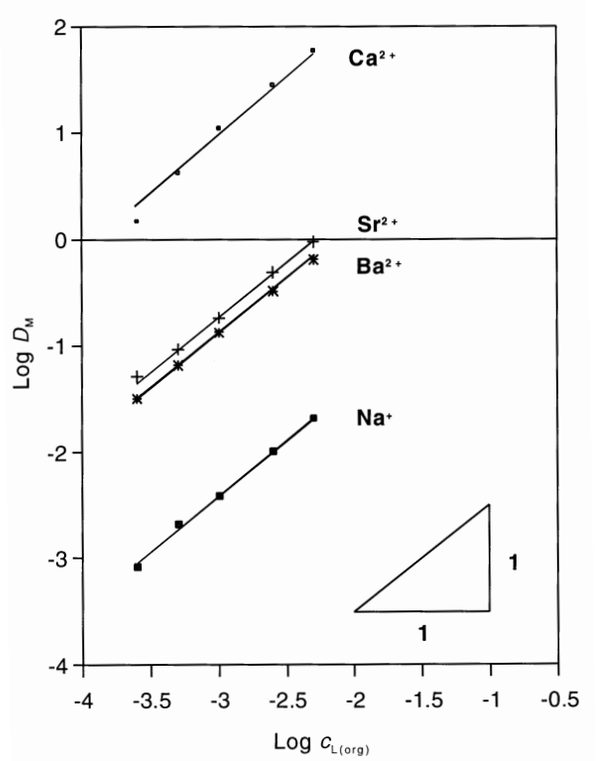


Fig. 4 Variation of log D_M with ligand concentration (c_L) for the extraction of Ca^{2+}, Sr^{2+}, Ba^{2+} and Na^+ with ligand **9**; $[M(NO_3)_n] = 10^{-4}$ mol dm^{-3}, [picric acid] = 5×10^{-3} mol dm^{-3}

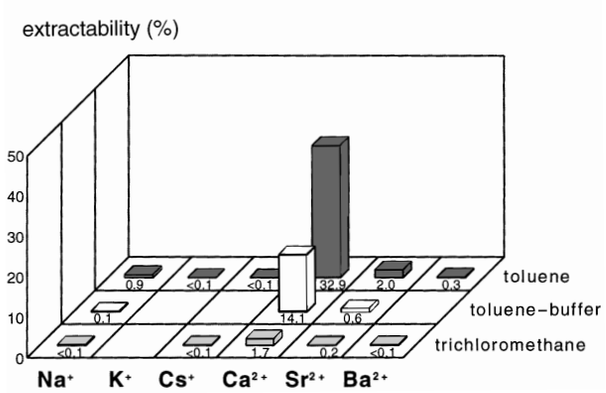


Fig. 5 Extractability of metal ions with ligand **1b**; $[M(NO_3)_2] = 10^{-4}$ mol dm^{-3}, [picric acid] = 5×10^{-3} mol dm^{-3}, pH = 5.2 (NaOAc–HCl buffer), [**1b**] = 10^{-3} mol dm^{-3} in $CHCl_3$ or PhMe

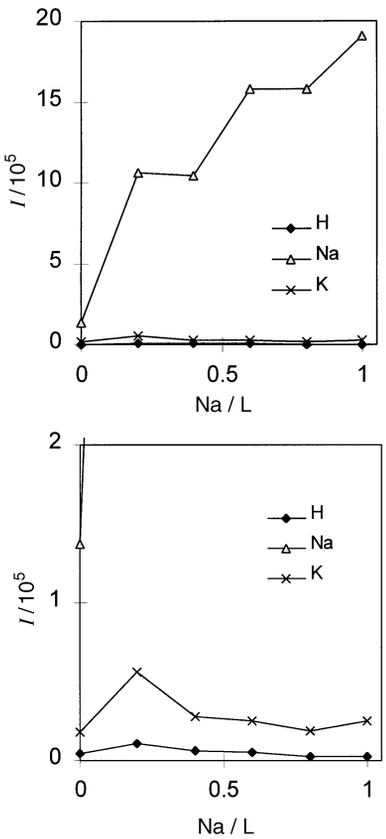


Fig. 6 Variation of complex peak intensities in ESMS as a function of Na^+:**9** ratio (AnalaR MeOH, [**9**] = 10^{-4} mol dm^{-3})

Electrospray (ES) mass spectroscopic studies

The use of electrospray mass spectrometry in complexation and supramolecular chemistry is becoming commonplace since it offers the possibility of giving information about the distribution of molecular or ionic species in solution.[21–23] The use of the atmospheric pressure inlet may allow information to be gained about the equilibrium (if fast kinetics of exchange exist) distribution of species in solution provided that no distortion of the species distribution occurs during ionisation (if appropriate), desolvation, transfer through the ES interface and in the mass analyser and detector—which is usually a function of the *m*/*z* value.[23] The latter two factors may be studied experimentally and appropriate conditions established, *e.g.* by varying the accelerating cone voltage, that minimise fragmentation and inhibit any discrimination.[24] On the other hand, a detailed understanding of the ES ionisation and desolvation process is lacking.[25–28] It is quite likely that given the observed correlation of the ESMS response factor for the ionic species present in solution with species solvation energies,[23] there may exist suitable examples where the ESMS technique may be applied with some confidence in studying solution speciation, provided that suitable control experiments are performed.

Our own studies focused on the ligands **9** and **10**. The hexadentate ligand had shown selectivity for Na^+ in the electrode response and extraction studies, while **10** demonstrated good Li^+/Na^+ discrimination in the ISE work. Following an analysis of the IR and NMR solution studies carried out in methanol, it was immediately apparent that [**9**·Na]$^+$ and [**9**·K]$^+$ had very different solution structures: in the potassium complex amide binding was absent, whereas in the 1:1 sodium complex it had been established that the three amide carbonyls were binding cooperatively. (Single band at 1633 cm^{-1}, coordination shift of 0.9 ppm for the carbonyl in the ^{13}C NMR spectrum.) Therefore, there were likely to be at least three methanol molecules bound to the K^+ ion in its complex with **9**, whereas the sodium complex may have none. This would inevitably lead to marked differences in the energy of desolvation which is an important factor that has been shown to determine ESMS response factors.[23]

A preliminary set of experiments was carried out with the triamide **9**, comparing the [Na·**9**]$^+$ peak height to that for [H·**9**]$^+$ and the impurity peak due to [K·**9**]$^+$. The sodium concentration was varied from 10^{-5} to 10^{-4} mol dm^{-3}, with [**9**] = 10^{-4} mol dm^{-3} using distilled AnalaR methanol with a 30 V cone voltage. The comparison of the intensity of the [**9**·Na]$^+$ peak compared to [**9**·H]$^+$ and [**9**·K]$^+$ (Fig. 6) reveals a general increase in the sodium complex signal accompanied by a diminution in the other peaks. Previous workers have suggested that by carrying out similar experiments in FABMS, 'ionisation efficiencies' may be estimated by comparing the intensity ratio of the sodium *vs*. potassium complex against the protonated ligand *vs*. potassium

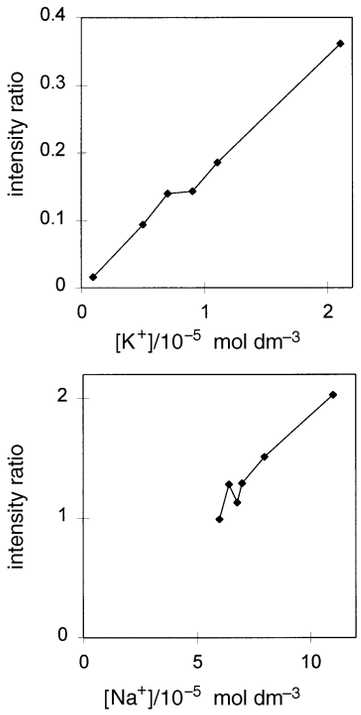


Fig. 7 Relative peak intensity for $[Na^+\cdot\mathbf{9}]^+$ and $[K\cdot\mathbf{9}]^+$ compared to $^+N(C_8H_{17})_4$ as a function of Na^+/K^+ concentration (Aristar MeOH, $[\mathbf{9}] = 10^{-5}$ mol dm^{-3})

complex.[29] Important assumptions in this work were that increases in sodium concentration would lead to proton displacement and a diminution in the peak intensity for the protonated ligand. However, the effective proton concentration was uncontrolled; the impurity $[K^+]$ may be constant for a given data set, but again is uncontrolled. In addition both protons and potassium ions are competing for the ligand in the solvent, even when the ligand is in excess. The experiment highlights the problem of finding an inert standard to allow quantitative inter-comparisons of spectral peak heights by normalising the performance of the mass spectrometer and the spraying/ionisation process. Ideally, such a standard should have a similar *m*/*z* ratio and a similar solvation energy to the ions of interest, it should not interfere with complex formation and its peak intensity should be independent of sample composition. Rather than using the K^+ impurity as an internal standard, experiments therefore were carried out with the tetra-alkylammonium ions $^+N(C_8H_{17})_4$ and $^+N(C_{10}H_{21})_4$, varying in turn the Na^+ and K^+ ion concentrations with a fixed ligand concentration of 10^{-5} mol dm^{-3} (30 V cone voltage, Aristar MeOH, 100 μdm^3 loop). Results shown in Fig. 7 which are corrected for a Na^+ background of 6×10^{-5} and K^+ of 1×10^{-6} mol dm^{-3}, suggest that in comparison to the $^+N(C_8H_{17})_4$ 'internal standard', the change in the peak intensity for Na^+ and K^+ complexes as a function of Na^+ or K^+ concentration is similar (slopes are 0.18 for K^+, 0.17 dm^3 mol^{-1} for Na^+). However, inspection of the mass spectral peak heights showed that as the Na^+ (or K^+) concentration was increased, the intensity of the $^+NR_4$ standard (both R = C_8H_{17} and $C_{10}H_{21}$) did not remain constant, but decreased. Similar observations in ESMS have been noted by Kebarle[28] and undermine the use of a non-competitive ion as a standard.

The outcome of these two sets of experiments highlighted the need to assess the concentration of interferent ions in the ESMS analysis. To a first approximation, it was concluded that the species $[Na\cdot\mathbf{9}]^+$ and $[K\cdot\mathbf{9}]^+$, notwithstanding their different solvation states in MeOH, possessed ionisation efficiencies in the ESMS experiment that were similar. Attention was then focused on the optimisation of experimental conditions (cone voltage, ligand/ion concentrations) in order to try and develop a working protocol for the ESMS assessment of selectivity in ion binding by the synthetic ionophores under investigation.

Table 4 ESMS determined selectivity parameters for ligand **9** (11 V cone voltage, 4 kV capillary voltage, $[\mathbf{9}] = 10^{-5}$ mol dm^{-3}, 100 μdm^3 injection loop)

M^+	I_{Na^+} [a]	I_{M^+}	I_{Na^+}/I_{M^+} [b]
K^+	28.0	3.03	9.3(1.1)
Rb^+	28.5	1.17	24.6(1.5)
Li^+	12.0	26.1	0.46(0.03)

[a] Each figure given is the mean for three sets of experiments, for each of which the given peak intensity represents the mean of *ca*. 80 scans in the plateau region of the ion-current. [b] Esd in parenthesis.

Table 5 ESMS determined selectivity parameters for valinomycin[a] (110 V cone voltage, Aristar MeOH, [valinomycin] = 10^{-5} mol dm^{-3}, 100 μdm^3 injection loop)

M^+	I_K	I_M	I_K/I_M
Li	45.0	0.14	330
Na	52.1	2.60	20.3
Rb	16.8	36.1	0.47

[a] Relative peak intensities found in EtOH were $I_{Na} = 1.0$, $I_K = 4.7$, $I_{Rb} = 12.9$.[30]

A pragmatic ESMS method for assessing ion selectivity

When the ligand **9** was present in relatively high concentrations in solution, *i.e.* $\geqslant 10^{-4}$ mol dm^{-3}, many more 'impurity' peaks were evident. Reducing the ligand concentration had the effect of lowering the intensity of (or removing) any peaks due to trace metal complexes, and in addition it reduced the tendency to observe 'cluster ions', such as L_nCa, (n = 2–6, evident at 30 V cone voltage, $[\mathbf{9}] = 10^{-4}$ mol dm^{-3}). At concentrations of **9** of 10^{-5} mol dm^{-3} this effect was suppressed. Maximal peak intensities for singly charged ions were obtained at a cone voltage of 110 V (60 V for doubly charged ions): at this voltage there was still no evidence for significant fragmentation. Competition experiments were then carried out for pairs of ions ($[\mathbf{9}] = 10^{-5}$; $[M^+] = 10^{-4}$ mol dm^{-3}), allowing equimolar concentrations of the ions to compete for a deficiency of the ligand, a procedure that has been used in earlier work.[29] The results (Table 4), using ligand **9** show the expected trend in ion-selectivity that had been deduced from the electrode response studies. For ligand **9**, the selectivity order was Li > Na > K > Rb. Obviously the selectivity ratios found by this ESMS method, Li/Na = 2.2, Na/K = 9.3 whilst qualitatively in agreement with the selectivity coefficients determined by potentiometry ($-\log K^{pot}_{Li,Na} = 0.8$; $-\log K^{pot}_{Na,Ka} = 3.1$) cannot sensibly be quantitatively linked. A control experiment using valinomycin—a well known K^+ ionophore with a selectivity over sodium in binding of *ca*. 10^4—was performed under identical conditions. The results (Table 5) again show good qualitative agreement in the trend for ion-binding (Rb > K > Na > Li) and are broadly similar to those recently reported using a related method.[30]

For the diamide **10**, maximal peak intensities were observed with a 90 V cone voltage and again the pronounced Li^+ selectivity observed accords with the electrode response work (Table 6 *cf*. Table 1). Comparing the Li/Na selectivity for ligands **9** and **10**, the ESMS work suggested a selectivity for Li^+ for **10** that was only twice that found for **9** whereas in a working electrode this difference was a factor of 25.

Table 6 ESMS determined selectivity parameters for **10** (90 V cone voltage, [**10**] = 10^{-5} mol dm^{-3}, 4 kV capillary voltage, Aristar MeOH, 100 μdm^3 injection loop)

M^{+a}	I_{Li}[b]	I_M	I_{Li}/I_M
Na^+	13.3	3.22	4.1
K^+	11.1	0.41	27
Rb^+	13.1	0.22	58
M^+	I_{Na}	I_M	I_{Na}/I_M
Li	3.14	13.1	0.24
K	9.05	1.47	6.2
Rb	11.7	0.70	16.6

[a] [M^+] = 10^{-4} mol dm^{-3}. [b] Peak intensities are the mean of *ca*. 80 scans on the ion-current plateau region.

Summary

The precise details of the processes involved in electrospray ion production remain ill-defined. Whether the mass spectrum obtained can be assumed to be a direct representation of the sample solution equilibria seems doubtful. Counterions dissociate, the droplet gains translational and vibrational energy, solvent molecules are removed leading to a concentration of various species in the droplet. It has not been established whether formation of the ion observed in the mass spectrum is the result of a final droplet explosion or is linked to 'evaporation' of solvent molecules from the droplet surface. These different processes presumably favour ion-formation in different ways depending upon the charge density, polarity, solvation energy and *m*/*z* ratio of the species involved.

Notwithstanding the complications arising from the electrospray process and the journey of the charged droplet to the mass spectrometer, by using ligands of opposite ion-binding order, these experiments have shown that a reliable qualitative order of complexing ability may be obtained that can be correlated to the order established in ion-selective electrode studies. The numerical selectivity ratios are dependent on experimental parameters and comparative studies must be made with due caution.

Conclusion

All of these solution complexation studies highlight the selectivity of ligands **9** and **10** for lithium. The formation of six-ring chelates on ion-binding aids Li/Na selectivity which is maximised in the tuning of coordination number preference for ligand **10**, a pentacoordinate diamide. Ligand **9** exhibits very good Na/K selectivity and the selectivity ratio determined by ISE measurements ($10^{3.1}$) is the highest observed for a neutral ionophore under such controlled conditions. For measurements of [Na^+] in intracellular fluids lacking significant Ca^{2+} concentration (<0.5 μmol dm^{-3}), this ionophore may prove to be of some practical use.

Experimental

Electrospray mass spectrometry

Spectra were recorded with a VG Platform II (Fisons Instruments) employing Mass Lynx software. Samples were presented as solutions in methanol (Aristar Grade, BDH unless otherwise stated) at a flow rate of 0.01 cm^3 min^{-1}.

Solutions were made up in glassware or PMP volumetric flasks. Samples were prepared using Gilson Pipetman micropipettes, in polypropylene Eppendorf tubes. Glassware was washed with hot nitric acid, thoroughly rinsed with deionized water and dried. Plastics were washed with methanol or propanol followed by deionized water and then dried at 35 °C in air. The spectrometer was operated in positive ion mode, with a capillary voltage of 4 kV and source temperature of 60 °C. Mass-scale calibration employed NaI. [HPLC grade methanol (Fisons) was used as the background (carrier) solvent into which the samples were 'injected'.] Cone voltages and analyte concentrations were varied according to the nature of the experiment (see text) and ranged from 30 to 110 V, and from 10^{-4} to 10^{-7} mol dm^{-3}.

The solvent flow was maintained using a Hewlett-Packard HPLC instrument that was directly linked to the ES mass spectrometer. The sample was inserted into the flow using an injection valve with a 10 or 100 μl steel loop and transported to the electrospray capillary through a silica tube. The injection valve and tubing were flushed through with a variety of solvents from dichloromethane to deionized water prior to experiments being performed.

Liquid–liquid extraction investigations were performed in microreaction vials (0.5 cm^3 of each phase) at 298(±1) K in the system metal nitrate–picric acid without buffer and in aqueous sodium acetate–HCl with buffer (pH 5.2), respectively, with ligand **9** (or **1b**)–organic solvent (PhMe or $CHCl_3$). After a shaking time of 30 min equilibrium was achieved and all samples were centrifuged. The determination of metal concentration in both phases was carried out radiometrically using the γ-radiation measurement of ^{22}Na, ^{42}K, ^{85}Sr, ^{133}Ba and ^{137}Cs in a NaI(Tl) scintillation counter (Cobra II, Canberra-Packard), and the β-radiation of ^{45}Ca in a liquid scintillation counter (Tricarb 2500, Canberra-Packard). The radioisotopes were supplied by Medgenix Diagnostics GmbH, Rathingen.

Potentiometric studies

The membranes were prepared by dissolving 1.3% ionophore, 65.6% NPOE (Fluka), 32.8% PVC (high molecular mass, Fluka) and 0.5% sodium tetrakis[3,5-bis(trifluoromethyl)-phenyl]borate in distilled tetrahydrofuran and casting the solution in a glass ring resting on a sheet of plate glass.

Slow evaporation was achieved by weighting down a pad of filter papers on top of the ring. Discs 7 mm in diameter were cut from these master membranes and mounted in standard electrode bodies supplied by Fluka. The inner filling solution was a 10^{-3} mol dm^{-3} solution of the analyte. The electrodes were conditioned in 10^{-2} mol dm^{-3} analyte solution. Calibrations and selectivity measurements were performed using a constant volume dilution technique in a thermostatted small volume cell (*ca*. 2.3 cm^3). A **T**-shaped thermostatted liquid junction configuration was used in which the analyte solution flowed over a capillary containing a saturated KC bridge solution in contact with a saturated calomel reference electrode (Russell pH Ltd). Measurements were recorded by a buffer amplifier interfaced to a Keithley 197 multimeter and a chart recorder (Kipp and Zonen).

Selectivity coefficients were determined by the fixed interferent method.[11,18] Solutions were made up in deionised water. AnalaR NaCl, KCl, LiCl, $MgCl_2.6H_2O$ and $CaCl_2$ (1 mol dm^{-3} volumetric solution) were obtained from BDH, Microselect NH_4Cl from Fluka and RbCl (99.99%) from Aldrich.

Syntheses

All reactions were carried out in apparatus which had been oven-dried and cooled under argon. All solvents were dried by distillation from an appropriate drying agent and water was purified from the 'Purite' system. Alumina refers to Merck Alumina activity II–III that was soaked in ethyl acetate for at least 24 h prior to use. Silica refers to Merck silica gel F60 (230–400 mesh). Analytical and preparative HPLC was performed on a Varian Vista 5500 or Star 5065 instrument fitted with a C_{18} reverse phase column ('Dynamax'). ^{1}H and ^{13}C NMR spectra were obtained with a Bruker AC 250 operating at 250.13 and 62.90 MHz respectively, Varian Gemini 200 operating at 200 and 50.1 MHz respectively, Varian XL 200 operating at 200.1 MHz, and a Varian VXR 400S operating at 400.1 MHz. All chemical shifts are given in ppm relative to the residual solvent resonance and coupling constants (*J*) are in Hz.

Mass spectra were recorded on a VG 7070E, operating in FAB, EI^+ or DCI ionization modes as stated. Electrospray mass spectra were recorded using a VG Platform (Fisons instruments) operating in ES^+ mode or were performed by the EPSRC Mass Spectrometry service at Swansea.

Accurate mass spectrometry was performed by the EPSRC Mass Spectrometry service. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer as a thin film or KBr disc, or as a solution in MeOH using CaF_2 cells, as stated. UV spectra were recorded on a UVIKON 930 spectrometer. Combustion analysis was performed using an Exeter Analytical Inc CE440 elemental analyser. Metal concentrations were determined by atomic absorption spectroscopy using a Perkin-Elmer 5000 atomic absorption spectrophotometer. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected.

2-Acetoxyacetophenone 12. 2-Bromoacetophenone (10 g, 0.05 mol) and potassium ethanoate (6 g, 0.06 mol) were dissolved in ethanoic acid (50 ml) and the mixture was boiled under reflux for 14 h. The solvent was removed, the residue dissolved in dichloromethane (75 ml), filtered, washed with water (3 × 25 ml), and dried (K_2CO_3). The solvent was removed to yield orange crystals, 8.06 g (91%), mp 45–47 °C. $\delta_H(CDCl_3)$ 2.22 (3 H, s, CH_3), 5.24 (2 H, s, CH_2), 7.54 (3 H, dt, arom.), 7.90 (2 H, dd, arom.). $\delta_C(CDCl_3)$ 20.90 (*C*H_3), 66.51 (*C*H_2), 128.15 (*meta* arom. *C*H), 129.26 (*ortho* arom. *C*H), 134.28 (*para* arom. *C*H), 134.57 (arom. *C*), 170.75 (*C*O_2), 192.66 (*C*OPh). ν_{max}(KBr disc)/cm^{-1} 1226 (CO), 1742 (CO_2). *m*/*z* (CI, dichloromethane) 179 (M^+, 43%), 105 (PhCO, 100%) (Found: C, 66.95; H, 5.70. $C_{10}H_{10}O_3$ requires C, 67.03; H, 5.63%).

3-Acetoxy-2-phenylpropene 13. Methyltriphenylphosphonium bromide (10.1 g, 0.283 mol) and potassium *tert*-butoxide (3.17 g, 0.283 mol) were suspended in tetrahydrofuran (150 ml) and stirred under N_2 at 40 °C for 1 h. 2-Acetoxyacetophenone (4.85 g, 0.270 mol) was added and the mixture boiled under reflux for 46 h under N_2, evaporated to dryness, and extracted with hexane (3 × 75 ml). The solvent was removed and the residue chromatographed on silica, eluting with 3:1 dichloromethane–hexane (R_f = 0.65), to give a yellow oil, 3.85 g (79%). $\delta_H(CDCl_3)$ 2.08 (3 H, s, CH_3), 4.99 (2 H, s, CH_2), 5.37 (1 H, d, =CH_E, *J* 1), 5.57 (1 H, d, =CH_Z, *J* 1), 7.39 (5 H, m, arom.). $\delta_C(CDCl_3)$ 21.43 (*C*H_3), 66.25 (*C*H_2), 115.72 (=*C*H_2), 126.56 (*ortho* arom. *C*H), 128.61 (*para* arom. *C*H), 129.04 (*meta* arom. *C*H), 138.55 (=C), 143.09 (arom. *C*), 171.27 (*C*O). ν_{max}(thin film)/cm^{-1} 1740 (C=O), 1228 (CO). *m*/*z* (CI, dichloromethane) 177 (M^+ + 1, 100%), 194 (M^+ + 18, 29%).

3-Acetoxy-1,2-dihydroxy-2-phenylpropane 14. 3-Acetoxy-2-phenylpropene (3.17 g, 18.0 mmol) and *N*-methylmorpholine *N*-oxide (NMO) (2.22 g, 19.0 mmol) were dissolved in *tert*-butyl alcohol (20 ml) and 2.5% w/v solution of osmium tetroxide in *tert*-butyl alcohol (0.6 ml) was added. The solution was stirred for 5 d and further NMO (0.5 g, 4.3 mmol) and 2.5% w/v osmium tetroxide solution (0.6 ml) added. After another 2 d sodium sulfite (2.0 g) was added, the mixture was stirred for 1 h and methanol (50 ml) was added. The mixture was filtered through Celite and the solvent removed. Water (10 ml) was added, and the pH reduced to 2 using 1 mol dm^{-3} hydrochloric acid. The product was extracted with dichloromethane (2 × 50 ml), dried (K_2CO_3), and the solvent removed. The residue was chromatographed on silica, eluting with 3:1 dichloromethane–hexane to remove fast running impurities and the product removed with ethyl acetate (R_f = 0.8), to give a colourless oil, 1.26 g (46%). $\delta_H(CD_3OD)$ 2.03 (3 H, s, CH_3), 3.77 (2 H, dd, CH_2OH), 4.41 (2 H, d, CH_2O, *J* 1), 7.35 (3 H, m, arom.), 7.55 (2 H, m, arom.). $\delta_C(CD_3OD)$ 21.29 (*C*H_3), 68.37 (*C*H_2OH), 69.52 (*C*H_2O), 77.02 (*C*), 127.37 (*ortho* arom. *C*H), 128.78 (*para* arom. *C*H), 129.56 (*meta* arom. *C*H), 143.67 (arom. *C*), 173.25 (*C*O). ν_{max}(thin film)/cm^{-1} 1731 (CO_2), 1247 (CO). *m*/*z* (CI, methanol) 193 ($M^+ + 1 - H_2O$, 100%), 228 (M^+ + 18, 11%) (Found: C, 67.79; H, 7.26. $C_{11}H_{14}O_4$ requires C, 68.04; H, 7.22%).

2-Phenyl-1,2,3-trihydroxypropane 15. Sodium (5 mg) was dissolved in methanol (30 ml) and 3-acetoxy-1,2-dihydroxy-2-phenylpropane (1.16 g, 5.52 mmol) was added. The solution was stirred for 14 h, and passed through acidic cation exchange resin, washing with methanol. The solvent was removed under reduced pressure and the residue chromatographed on silica eluting with ethyl acetate (R_f = 0.2) to give a white solid, 0.77 g (83%), mp 44–46 °C. $\delta_H(CDCl_3)$ 3.62 (2 H, br s, CH_2OH), 3.80 (2 H, d, CH_2OH, *J* 11.5), 3.98 (2 H, d, CH_2OH, *J* 11.5), 4.35 (1 H, s, CO*H*), 7.48 (5 H, m, arom.). $\delta_C(CD_3OD)$ 68.23 (*C*H_2OH), 78.17 (C*C*H_2), 127.39 (*ortho* arom. *C*H), 128.30 (*para* arom. *C*H), 129.32 (*meta* arom. *C*H), 144.52 (arom. *C*). ν_{max}(KBr disc)/cm^{-1} 3262 (OH), 696 (Ar–H). *m*/*z* (CI, methanol) 186 (M^+ + 18, 100%), 168 (M^+, 16%) (Found: C, 64.56; H, 7.52. $C_9H_{12}O_3$ requires C, 64.25; H, 7.20%).

2,2-Dimethyl-4-(hydroxymethyl)-4-phenyldioxolane 16. Under argon 2-phenyl-1,2,3-trihydoxypropane (500 mg, 3.0 mmol), 2,2-dimethoxypropane (1.5 ml, 10.4 mmol), and pyridinium *p*-toluenesulfonate (10 mg, 0.04 mmol) were dissolved in acetone (30 ml). The reaction was boiled under reflux through 4 Å molecular sieves for 8 h. The solvent and excess dimethoxypropane were removed under reduced pressure and the residue purified by column chromatography on silica eluting with 4:1 hexane–ethyl acetate (R_f = 0.25) to yield a colourless oil, 410 mg (75%). $\delta_H(CDCl_3)$ 1.37 (3 H, s, CH_3); 1.58 (3 H, s, CH_3); 3.76 (2 H, m, CH_2OH); 4.16 (1 H, d, CH_2O, *J* 9); 4.41 (1 H, d, CH_2O, *J* 9); 7.36 (5 H, m, arom. C*H*). $\delta_C(CDCl_3)$ 26.40 (*C*H_3); 27.54 (*C*H_3); 68.85 (*C*H_2OH); 71.87 (*C*H_2O); 85.87 (Ph*C*); 110.78 (*C*Me_2); 125.80, 128.01, 128.84 (arom. *C*H); 142.90 (arom. *C*). ν_{max}(thin film)/cm^{-1} 1060 (C–H, arom.), 3448 (OH). *m*/*z* (CI, Dichloromethane) 208 (M^+, 13%), 177 (M–CH_2OH, 100%) (Found: C, 69.41; H, 7.88. $C_{12}H_{16}O_3$ requires C, 69.19; H, 7.75%).

4-(Butoxymethyl)-2,2-dimethyl-4-phenyldioxalane 17. Under argon the alcohol **11** (180 mg, 0.86 mmol) was dissolved in tetrahydrofuran (10 ml) and sodium hydride (25 mg, 1.04 mmol) and 1-bromobutane (0.141 g, 1.03 mmol) was added. The mixture was boiled under reflux for 24 h, additional sodium hydride (25 mg, 1.04 mmol) and 1-bromobutane (70 mg, 0.51 mmol) was added, and the reaction boiled under reflux for another 24 h. Tetrahydrofuran (25 ml) was added and the reaction filtered through Celite, the solvent and excess 1-bromobutane removed under vacuum to yield an oil, 228 mg (88%). $\delta_H(CDCl_3)$ 0.89 (3 H, t, CH_2CH_3, *J* 7); 1.26–1.39 (5 H, m, CH_3 + CH_2CH_3); 1.48–1.61 (5 H, m, CH_3 + $CH_2CH_2CH_2$); 3.44 (2 H, m, OCH_2CH_2); 3.54 (2 H, q, CCH_2O, *J* 12); 4.12 (1 H, d, $COCH_2C$, *J* 8); 4.46 (1 H, d, $COCH_2C$, *J* 8); 7.39 (5 H, m, Arom. C*H*). $\delta_C(CDCl_3)$ 14.39 (CH_2CH_3); 19.76 (CH_2CH_3); 26.73 (CCH_3); 27.42 (CCH_3); 32.13 ($CH_2CH_2CH_3$); 72.12, 72.23 and 76.40 (CH_2O); 84.79 (Ph*C*); 110.61 (CCH_3); 126.11, 127.63 and 128.42 (arom. *C*H); 143.84 (arom. *C*). ν_{max}(thin film)/cm^{-1} 2932 (C–H stretch), 1114 (arom. C–H). *m*/*z* (CI, dichloromethane) 264 (M^+, 100%) (Found: C, 72.53; H, 9.32. $C_{16}H_{24}O_3$ requires C, 72.69; H, 9.15%).

***N*,*N*-Dibutylchloroethanamide 18.** Under argon chloroacetyl chloride (5.98 g, 53 mmol) was dissolved in diethyl ether (50 ml) and cooled to −70 °C. Dibutylamine (15 g, 116 mmol) was dissolved in diethyl ether (50 ml) and added slowly over 30 min at lower than −40 °C. The reaction was allowed to warm to room temperature, filtered, the filtrate washed with hydrochloric acid (1 mol dm^{-3}, 2 × 25 ml), dried ($MgSO_4$), and the solvent removed to yield a colourless oil, 10.21 g (95%). $\delta_H(CDCl_3)$ 0.96 (6 H, m, CH_3CH_2), 1.27–1.63 (8 H, m, CH_2), 3.30 (4 H, t, CH_2N), 4.08 (2 H, s, CH_2Cl). $\delta_C(CDCl_3)$ 13.44 (*C*H_3), 19.72, 29.09 and 30.83 (*C*H_2), 41.04 (*C*H_2CO), 45.58 and 47.67 (*C*H_2N), 165.61 (*C*O). *m*/*z* (CI, dichloromethane) 206 (M^+ + 1).

1,2,3-Tri(*N*,*N*-dibutylcarbamoylmethoxy)-2-phenylpropane 6. Under argon 2-phenyl-1,2,3-trihydoxypropane (550 mg, 3.27 mmol) and potassium iodide (1.08 g, 6.54 mmol) were dissolved in tetrahydrofuran (25 ml) and sodium hydride (0.15 g, 6.25

mmol) and *N*,*N*-dibutylchloroethanamide (0.80 g, 3.9 mmol) were added. The mixture was boiled under reflux for 6 d, after 2 and 4 d additional sodium hydride (0.15 g, 6.25 mmol) and *N*,*N*-dibutylchloroethanamide (0.80 g, 3.9 mmol) were added. After cooling to room temperature additional tetrahydrofuran (50 ml) was added, the reaction was filtered through Celite and the residue purified by column chromatography on alumina eluting with hexane–ethyl acetate (3:1 to 5:2) (R_f = 0.2 in 3:2 hexane–ethyl acetate) to yield a brown oil, 608 mg (28%). δ_H 0.93 (18 H, m, CH_3); 1.28 (12 H, m, CH_3CH_2); 1.48 (12 H, m, $CH_3CH_2CH_2$); 3.05 (4 H, t, NCH_2); 3.27 (8 H, m, NCH_2); 3.94 (2 H, d, CCH_2O, *J* 10); 4.07 (2 H, d, CCH_2O, *J* 10); 4.18 (2 H, s, CH_2CO); 4.21 (2 H, s, CH_2CO); 4.31 (2 H, s, CH_2CO); 7.31 (3 H, m, arom. C*H*); 7.55 (2 H, d, arom. C*H*). δ_C 14.17, 14.26 and 14.31 (*C*H_3, rotamer); 20.34, 20.48 and 20.67 (*C*H_2CH_3, rotamer); 30.12, 31.28 and 31.46 ($CH_2CH_2CH_2$, rotamer); 45.96, 46.09, 46.92, 46.98 and 47.11 (N*C*H_2, rotamer); 63.47 (CO*C*H_2CO); 69.47 and 70.02 (C*C*H_2O); 74.85 (CH_2CO-*C*H_2CO); 81.92 (Ph*C*); 127.69, 128.29 and 128.85 (arom. *C*H); 139.06 (arom. *C*); 168.94, 169.08 and 169.73 (*C*=O, rotamer). ν_{max}(thin film)/cm^{-1} 1648 (NC=O), 1119 (C–O). *m*/*z* (CI, dichloromethane) 677 (M^+ + 1, 26%), 128 (NBu_2, 100%). *m*/*z* (MH^+) found 676.527; $C_{39}H_{69}N_3O_6$ + 1 requires 676.526.

3-Hydroxy-2-phenylprop-1-ene 19. Under argon, sodium (20 mg) was dissolved in dry methanol (100 ml) and 3-acetoxy-2-phenylprop-l-ene (13.0 g, 97 mmol) was added. The mixture was stirred for 48 h, the solvent removed, the residue dissolved in dichloromethane (100 ml), washed with water (50 ml), dried (K_2CO_3), and purified by column chromatography on silica, eluting with 3:1 dichloromethane:hexane (R_f = 0.25), yielding a yellow oil, 8.0 g (81%). δ_H($CDCl_3$) 4.55 (2 H, d, CH_2O, *J* 5); 5.36 (1 H, d, =CH_E), *J* 1); 5.47 (1 H, d, =CH_Z, *J* 1); 7.40 (m, 5 H, arom. CH). δ_C($CDCl_3$) 64.9 (*C*H_2OH); 112.9 (=*C*H_2); 126.7, 128.5 and 129.1 (arom. *C*H): 139.4 (*C*=); 147.8 (arom. *C*). ν_{max}-(thin film)/cm^{-1} 3346 (O–H), 1025 (O–C) (Found: C, 80.70; H, 7.36. $C_9H_{10}O$ requires C, 80.56; H, 7.51%).

3-(*N*,*N*-Dibutylcarbamoylmethoxy)-2-phenylprop-1-ene 20. 3-Hydroxy-2-phenylprop-l-ene (1.34 g, 10 mmol) and potassium iodide (20 mg) were dissolved in dry tetrahydrofuran (50 ml) under argon. Sodium hydride (300 mg, 12.5 mmol) and *N*,*N*-dibutylchloroethanamide (2.47 g, 12 mmol) were added and the reaction boiled under reflux. After 24 h additional sodium hydride (100 mg, 4.2 mmol) and chloroethanamide (0.25 g, 4.2 mmol) were added and the reaction mixture was boiled under reflux for a further 12 h. The reaction was filtered, the solvent removed and the product purified by column chromatography on silica, eluting with 4:1 hexane–ethyl acetate (R_f = 0.4 in 3:1 hexane–ethyl acetate), to yield a yellow oil, 2.20 g (73%). δ_H($CDCl_3$) 0.79 (6 H, q, CH_2CH_3, *J* 7); 1.14 (4 H, m, CH_2CH_3, *J* 7); 1.36 (4 H, m, CH_2CH_2, *J* 6); 3.01 (2 H, t, NCH_2, *J* 7.8); 3.19 (2 H, t, NCH_2, *J* 7.8); 4.03 (2 H, s, CH_2O); 4.37 (2 H, s, CH_2O); 5.24 (1 H, d, =C*H*, *J* 1); 5.43 (1 H, d, =C*H*, *J* 1); 7.17 (m, 3 H, arom. C*H*); 7.37 (m, 2 H, arom. C*H*). δ_C($CDCl_3$) 14.2 and 14.3 (*C*H_3, rotamer); 20.4 and 20.6 (*C*H_2CH_3, rotamer); 30.1 and 31.4 (*C*H_2CH_2, rotamer); 45.8 and 47.1 (N*C*H_2, rotamer); 69.2 and 73.3 (*C*H_2O); 115.3 (=*C*H_2); 126.4, 128.2 and 128.7 (arom. *C*H); 138.8 (=*C*); 144.1 (arom. *C*); 168.9 (*C*=O). ν_{max}(thin film)/cm^{-1} 1645 (C=O). *m*/*z* (CI, dichloromethane) 304 (M^+ + 1, 100%) (Found: C, 75.27; H, 9.59. $C_{10}H_{29}NO_2$ requires C, 75.31; H, 9.65%).

1-Butoxy-2,3-di(*N*,*N*-dibutylcarbamoylmethoxy)-2-phenylpropane 7. Under argon 3-butoxy-1,2-dihydroxy-2-phenylpropane (160 mg, 0.70 mmol) and potassium iodide (20 mg) were dissolved in dry tetrahydrofuran (20 ml) and sodium hydride (40 mg, 1.67 mmol) and *N*,*N*-dibutylchloroethanamide (345 mg, 1.67 mmol) were added. The mixture was boiled under reflux for 24 h and then additional sodium hydride (20 mg, 1.36 mmol) and chloroethanamide (100 mg, 0.49 mmol) were added. After refluxing for a further 6 d the reaction was filtered through Celite, the solvent removed and the product purified by column chromatography on silica eluting with 6:1 hexane–ethyl acetate (R_f = 0.25 in 3:1 hexane–ethyl acetate) to give a pale yellow oil, 90 mg (23%). δ_H($CDCl_3$) 0.86 (15 H, m, CH_3); 1.28 (10 H, m, CH_2CH_3); 1.46 (10 H, m, CH_2CH_2); 2.98–3.45 (10 H, br m, NCH_2 + OCH_2); 3.83 (1 H, d, OCH_2, *J* 10.3); 3.84 (1 H, d, OCH_2, *J* 10.0); 3.97 (1 H, d, OCH_2, *J* 10.3); 4.03 (1 H, d, OCH_2, *J* 10.0); 4.13 (2 H, s, OCH_2); 4.18 (2 H, d, OCH_2, *J* 2.6); 7.35 (3 H, br m, arom. C*H*); 7.50 (2 H, m, arom. C*H*). δ_C($CDCl_3$) 14.3 and 14.4 (*C*H_3); 19.8, 20.5 and 20.7 (*C*H_2CH_3); 30.2, 31.4 and 32.2 (*C*H_2CH_2); 45.9, 46.0, 46.9 and 47.2 (N*C*H_2, rotamer); 64.2 (O*C*H_2); 70.8, 71.9, 73.8 and 75.0 (O*C*H_2); 82.1 (*C*); 127.1, 128.0 and 128.6 (arom. *C*H); 140.1 (arom. *C*); 169.0 and 169.5 (*C*=O). ν_{max}(thin film)/cm^{-1} 1648 (C–O). *m*/*z* (CI, dichloromethane) 563 (M^+ + 1, 100%) (Found: C, 70.30; H, 10.72; N, 4.76. $C_{33}H_{58}N_2O_5$ requires C, 70.42; H, 10.39; N, 4.98%).

3-(*N*,*N*-Dibutylcarbamoylmethoxy)-1,2-dihydroxy-2-phenylpropane, 21. 3-(*N*,*N*-Dibutylcarbamoylmethoxy)-2-phenylprop-l-ene (320 mg, 1.05 mmol), NMO (128 mg, 1.10 mmol) and 2.5% w/v osmium tetroxide in *tert*-butyol alcohol (0.5 ml) were stirred in *tert*-butyl alcohol (20 ml) under argon for 6 d. Dichloromethane (45 ml) and sodium hydrogen sulfite (1 g) were added and the suspension stirred for 30 min. The mixture was filtered, the solvent removed and the product purified by column chromatography on silica using a gradient elution of 2:1 to 1:1 hexane–ethyl acetate (R_f = 0.4 in 1:1 in hexane–ethyl acetate) to give a yellow oil, 190 mg (54%). δ_H($CDCl_3$) 0.84 (6 H, t, CH_2CH_3, *J* 6.0); 1.18 (4 H, m, CH_2CH_3); 1.40 (4 H, m, CH_2CH_2); 2.95 (2 H, t, NCH_2, *J* 8.0); 3.24 (2 H, t, NCH_2, *J* 8.0); 3.53 (1 H, d, OCH_2, *J* 11.4); 3.74 (2 H, dd, OCH_2, *J* 9.4 and 20.4); 3.86 (1 H, d, OCH_2, *J* 11.4); 4.11 (2 H, s, OCH_2); 7.22 (3 H, m, Arom. C*H*); 7.48 (2 H, dd, Arom. C*H*, *J* 8 and 1.2). ν_{max}(thin film)/cm^{-1} 3360 (O–H), 1640 (C=O). *m*/*z* (EI) 338 (M^+ + 1, 100%) (Found: C, 67.65; H, 9.11; N, 4.31. $C_{27}H_{47}NO$ requires C, 67.63; H, 9.27; N, 4.15%).

3-Butoxy-1,2-dihydroxy-2-phenylpropane 22. *N*-Methylmorpholine *N*-oxide (0.35 g, 3.0 mmol) and 3-butoxy-2-phenylprop-l-ene (0.57 g, 3.0 mmol) were dissolved in *tert*-butyl alcohol (10 ml), 2.5% w/v OsO_4 in *tert*-butyl alcohol (0.5 ml) was added and the reaction was stirred under argon for 7 d. Sodium metabisulfite (1 g) was added and after stirring for 1 h the reaction was filtered through Celite, the solvent removed, and the product purified by column chromatography on silica, eluting with 3:1 hexane–ethyl acetate (R_f = 0.25), yielding a yellow oil, 0.34 g (58%). δ_H($CDCl_3$) 0.92 (3 H, t, CH_3, *J* 7); 1.37 (2 H, m, CH_2CH_3); 1.44 (2 H, m, $CH_2CH_2CH_3$); 3.49 (2 H, t, O$CH_2CH_2CH_3$, *J* 11.5); 3.64 (1 H, d, OCH_2, *J* 9.1); 3.69 (1 H, d, OCH_2, *J* 6.5); 3.85 (1 H, d, OCH_2, *J* 9.1); 3.95 (1 H, d, OCH_2, *J* 11.5); 7.35 (3 H, m, arom. C*H*); 7.49 (2 H, m, arom. C*H*). δ_C($CDCl_3$) 14.36 (*C*H_3); 19.74 (*C*H_2CH_2); 32.01 (*C*$H_2CH_2CH_2$); 69.20 (O*C*H_2CH_2); 72.28 (C*C*H_2O); 76.13 (*C*OH); 77.24 (*C*H_2OH); 125.74, 127.93 and 128.78 (arom. *C*H); 142.09 (arom. *C*) ν_{max}(thin film)/cm^{-1} 3416 (OH), 1098 (C–O). *m*/*z* (CI, dichloromethane) 242 (M^+ + 18, 21%), 207 (M^+ − OH, 100%) (Found: C, 69.92; H, 8.67. $C_{13}H_{20}O_3$ requires C, 69.61; H, 8.99%).

1,2-Dibutoxy-3-(*N*,*N*-dibutylcarbamoylmethoxy-2-phenylpropane 8. 3-(*N*,*N*-dibutylcarbamoylmethoxy)-1,2-dihydroxy-2-phenylpropane (120 mg, 0.36 mmol) was dissolved in dry tetrahydrofuran (20 ml) under argon and sodium hydride (20 mg, 0.80 mmol) and 1-bromobutane (109 mg, 0.78 mmol) added. The mixture was boiled under reflux for 48 h. After 24 h additional sodium hydride (10 mg, 0.42 mmol) and 1-bromobutane (54 mg, 0.39 mmol) were added. The reaction was allowed to cool to room temperature, dichloromethane (35 ml) added, the mixture was filtered through Celite, and the solvent and excess 1-bromobutane removed under reduced pressure to yield a yellow oil, 120 mg (69%). δ_H($CDCl_3$) 0.89 (2 H, m, CH_3); 1.26 (8 H, m, $CHCH_3$); 1.48 (8 H, m, CH_2CH_2); 3.08 (2 H, t, OCH_2CH_2); 3.25–3.48 (6 H, m, OCH_2CH_2 + NCH_2); 3.72 (1 H, d, OCH_2C, *J* 7.5); 3.83 (2 H, d, OCH_2C, *J* 6.0); 3.91 (1 H, d, OCH_2C, *J* 7.5);

4.12 (2 H, s, OCH_2CO); 7.28 (3 H, m, arom. C*H*); 7.43 (2 H, m, arom. C*H*). δ_C($CDCl_3$) 14.3, 14.4 and 14.5 (*C*H_3); 19.8, 20.5 and 20.7 (*C*H_2CH$_3$); 30.1, 30.4, 32.1 and 33.0 (CH$_2$*C*H$_2$CH$_2$); 45.8 and 46.9 (N*C*H$_2$); 63.4 (O*C*H$_2$CO); 71.5, 71.9, 73.3 and 74.4 (O*C*H$_2$); 80.6 (*C*); 127.4, 127.7 and 128.4 (arom. *C*H); 141.3 (arom. *C*); 169.2 (*C*O). ν_{max}(thin film)/cm^{-1} 1648 (C=O), 1112 (C–O). *m*/*z* (CI, dichlromethane) 450 (M^+ + 1, 100%). *m*/*z* (MH^+) found 450.3583; $C_{27}H_{47}NO_4$ + 1 requires 450.3583.

3-Butoxy-2-phenylpropene 23. 3-Hydroxy-2-phenylprop-l-ene (1.34 g, 10 mmol) was dissolved in dry tetrahydrofuran (30 ml) under argon and sodium hydride (0.30 g, 12.5 mmol) and 1-bromobutane (1.51 g, 11.0 mmol) were added. After boiling under reflux for 24 h additional sodium hydride (0.10 g, 4.2 mmol) and 1-bromobutane (0.50 g, 3.6 mmol) were added and the reaction mixture was boiled under reflux for a further 36 h. The solvent was removed, the residue dissolved in dry dichloromethane (30 ml), filtered through Celite, and the solvent and volatile materials removed under vacuum to yield a yellow oil, 1.4 g (74%). δ_H($CDCl_3$) 0.91 (3 H, t, CH_3CH_2, *J* 7.3); 1.36 (2 H, m, CH_2CH_3, *J* 6.9); 1.59 (2 H, m, OCH_2CH_2, *J* 8.0); 3.50 (2 H, t, OCH_2CH_2, *J* 6.4); 4.37 (2 H, s, OCH_2); 5.35 (1 H, s, =CH_2); 5.53 (1 H, s, =CH_2); 5.53 (1 H, s, =CH_2); 7.32 (3 H, m, arom. CH); 7.51 (2 H, m, arom. CH). δ_C($CDCl_3$) 14.50 (CH_3); 19.99 (CH_2); 32.43 (CH_2); 70.58 (OCH_2); 73.32 (OCH_2); 114.37 (=CH_2); 126.63, 128.25 and 128.85 (arom. CH); 139.52 (=C); 145.14 (arom. C). ν_{max}(thin film)/cm^{-1} 1094 (C–O). *m*/*z* (CI, dichloromethane) 189 (M^+, 100%) (Found: C, 82.52; H, 9.81. $C_{13}H_{18}O$ requires C, 82.61; H, 9.60%).

***cis*,*cis*-1,3,5-Tri(*N*,*N*-dibutylcarbamoylmethoxy)cyclohexane 9.** Anhydrous *cis*,*cis*-cyclohexane-1,3,5-triol (0.5 g, 3.8 mmol) was dissolved in dry DMF (20 ml) under argon. Sodium hydride (0.3 g, 12.5 mmol), potassium iodide (2.0 g, 16.0 mmol) and *N*,*N*-dibutylchloroethanamide (2.4 g, 11.7 mmol) were added and the reaction heated at 60 °C for 6 d. Every 2 d further sodium hydride (0.3 g, 12.5 mmol), potassium iodide (2.0 g, 16.0 mmol) and *N*,*N*-dibutylchloroethanamide (2.4 g, 11.7 mmol) were added. The excess sodium hydride was quenched with water (1 ml) and the mixture filtered. The solvent and volatile materials were removed under vacuum (160 °C, 0.1 mm Hg) and the residue purified by column chromatography on alumina, using a gradient elution of 2:1 to 4:1 ethyl acetate–hexane (R_f = 0.3, 4:1 ethyl acetate–hexane), yielding a brown oil, 0.49 g (20%). δ_H($CDCl_3$) 0.94 (18 H, m, CH_3C); 1.24–1.35 (15 H, m, CH_2 + CH_{eq}); 1.47–1.57 (12 H, m, CH_2); 2.54 (3 H, m, CH_{ax}); 3.16–3.31 (12 H, dt, CH_2N); 3.38 (3 H, m, C*H*O); 4.17 (6 H, s, CH_2O). δ_C($CDCl_3$) 13.83 (*C*H_3); 20.08, and 22.22 (*C*H_2CH$_3$, rotamer); 29.60 and 30.97 (N*C*H_2CH$_2$, rotamer); 37.54 (*C*H_2); 45.56 and 46.78 (NCH_2, rotamer); 66.97 (CHO); 73.36 (CH_2O); 168.70 (C=O). ν_{max}(thin film)/cm^{-1} 1645 (NC=O), 1102 (C–O). *m*/*z* (CI, dichloromethane) 640 (M^+ + 1, 100%), 188 (OCH_2CONBu_2, 95%). *m*/*z* (MH+) found 640.527; $C_{30}H_{60}N_3O_6$ requires 640.526.

***cis*,*cis*-1-(*N*,*N*-Dibutylcarbamoylmethoxy)3,5-dihydroxycyclohexane 24.** Anhydrous *cis*,*cis*-cyclohexane-1,3,5-triol (250 mg, 1.89 mmol) and potassium iodide (314 mg, 1.89 mmol) were dissolved in DMF (5 ml) under argon. Sodium hydride (45 mg, 1.89 mmol) and *N*,*N*-dibutylchloroethanamide (389 mg, 1.89 mmol) were added and the reaction mixture heated at 80 °C for 2 d. The solvent and volatile material were removed by distillation (120 °C, 0.05 mm Hg), the residue dissolved in ethyl acetate (30 ml), filtered through Celite, the solvent removed and the product purified by column chromatography on alumina using a gradient elution with ethyl acetate to 5:95 methanol–ethyl acetate (R_f = 0.25 in 5:95 methanol–ethyl acetate) to yield a pale-yellow oil, 122 mg (22%). δ_H($CDCl_3$) 0.87 (6 H, m, CH_3); 1.26 (4 H, m, CH_2CH$_3$); 1.46 (7 H, m, CH_2CH$_2$ + CH_{ax}); 2.13 (3 H, m, CH_{eq}); 3.14 (2 H, br t, NCH_2); 3.44 (1 H, m, C*H*O); 3.65 (2 H, m, C*H*OH); 4.13 (2 H, s, OCH_2); 4.21 (2 H, br s, O*H*). δ_C($CDCl_3$) 13.73 (*C*H_3); 19.99 and 20.11 (*C*H_2CH$_3$, rotamer); 29.48 and 30.85 (*C*H_2CH$_2$, rotamer); 39.14 and 42.34 (ring *C*H_2); 45.61 and 46.72 (N*C*H_2, rotamer); 65.58 and 67.48 (*C*HO); 74.62 (O*C*H_2CO); 169.25 (*C*O). ν_{max}(thin film)/cm^{-1} 1634 (CON), 3386 (OH). *m*/*z* (CI, dichloromethane) 302 (M^+ + 1, 100%). *m*/*z* (MH^+) found 302.2321; $C_{16}H_{31}NO_4$ + 1 requires 302.2331.

***cis*,*cis*-1-Butoxy-3,5-dihydroxycyclohexane 25.** Anhydrous *cis*,*cis*-cyclohexane-1,3,5-triol (250 mg, 1.89 mmol) was dissolved in DMF (5 ml) under argon, sodium hydride (45 mg, 1.89 mmol) and 1-bromobutane (285 mg, 2.08 mmol) were added and the reaction mixture was heated at 80 °C for 5 d. The solvent and volatile materials were removed under vacuum and the product purified by column chromatography on alumina using a gradient elution of ethyl acetate to 95:5 ethyl acetate–methanol (R_f = 0.8 in 90:10 ethyl acetate–methanol) to give a colourless oil, 0.13 g (37%). δ_H($CDCl_3$) 0.88 (3 H, t, CH_3); 1.18–1.58 (7 H, m, CH_2CH$_3$ + CH_2CH$_2$ + CH_{ax}); 2.21 (3 H, m, CH_{eq}); 3.26 (1 H, m, C*H*OBu); 3.43 (2 H, t, OCH_2); 3.58 (2 H, m, C*H*OH). δ_C($CDCl_3$) 13.84 (*C*H_3); 19.23 (*C*H_2CH$_3$); 31.95 (*C*H_2CH$_2$); 40.11 and 43.26 (ring *C*H_2); 65.78 (*C*HOH); 68.44 (*C*HOCH_2); 73.24 (O*C*H_2). ν_{max}(thin film)/cm^{-1} 3372 (OH), 1024 (C–O). *m*/*z* (CI, dichloromethane) 189 (M^+ + 1, 100%). *m*/*z* (MH^+) found 189.1491; $C_{10}H_{20}O_3$ + 1 requires 189.1491.

***cis*,*cis*-1,3-Dibutoxy-5-(*N*,*N*-dibutylcarbamoylmethoxy)-cyclohexane 10.** *cis*,*cis*-1-(*N*,*N*-Dibutylcarbamoylmethoxy)-3,5-dihydroxycyclohexane (80 mg, 0.26 mmol) was dissolved in anhydrous DMF (5 ml) under argon and sodium hydride (20 mg, 0.83 mmol) and 1-bromobutane (0.114 g, 0.83 mmol) added. The reaction mixture was stirred at 60 °C for 2 d when additional sodium hydride (20 mg, 0.83 mmol) and 1-bromobutane (0.114 g, 0.83 mmol) were added. After a further 2 d of stirring at 60 °C the solvent and excess 1-bromobutane were removed under vacuum, the residue dissolved in dichloromethane (25 ml), washed with water (2 × 10 ml), dried (K_2CO_3), filtered and the solvent removed under reduced pressure. The product was purified by column chromatography on alumina eluting with dichloromethane (R_f = 0.3) to yield a colourless oil, 67 mg (62%). δ_H($CDCl_3$) 0.90 (12 H, m, CH_3); 1.27 (11 H, m, CH_2CH$_3$ + CH_{ax}); 1.48 (8 H, m, CH_2CH$_2$); 2.31 (3 H, m, CH_{eq}); 3.32 (6 H, m, ring CH_2 + CC*H*OCH_2); 3.43 (2 H, t, NCH_2); 3.59 (1 H, m, C*H*OCH_2); 4.14 (2 H, s, CH_2CO). δ_C($CDCl_3$) 13.85 and 19.30 (*C*H_3); 20.06 and 20.21 (*C*H_2CH$_3$); 29.56 and 30.99 (NCH_2*C*H_2, rotamer); 32.09 (OCH_2*C*H_2); 37.99 and 38.32 (ring *C*H_2) 45.45 and 46.84 (NCH_2, rotamer); 67.38 and 68.25 (*C*HO); 73.04 and 73.68 (OCH_2); 168.94 (CO). ν_{max}(thin film)/cm^{-1} 1643 (CON), 1093 (C–O). *m*/*z* (CI, dichloromethane) 414 (M^+ + 1, 9%); 358 (M − Bu, 100%). *m*/*z* (MH^+) found 414.3583; $C_{24}H_{47}NO_4$ + 1 requires 414.3583.

***cis*,*cis*-1-Butoxy-3,5-bis(*N*,*N*-dibutylcarbamoylmethoxy)-cyclohexane 11.** *cis*,*cis*-1-Butoxy-3,5-dihydroxycyclohexane (100 mg, 0.51 mmol) was dissolved in dry tetrahydrofuran (10 ml) under argon, sodium hydride (30 mg, 1.25 mmol) and *N*,*N*-dibutylchloroethanamide (0.231 g, 1.25 mmol) were added and the reaction mixture was boiled under reflux for 2 d. Additional sodium hydride (30 mg, 1.25 mmol) and *N*,*N*-dibutylchloroethanamide (0.231 g, 1.25 mmol) were added and the reaction boiled under reflux for a further 6 d. The solvent was removed under reduced pressure and the excess chloroethanamide removed by distillation (0.1 mmHg, 150 °C). The residue was dissolved in dichloromethane (50 ml), filtered through celite and the solvent removed under reduced pressure. The product was purified by column chromatography on alumina eluting with 3:1 hexane–ethyl acetate (R_f = 0.3 in 2:1 hexane–ethyl acetate) to yield a colourless oil, 90 mg (32%). δ_H($CDCl_3$) 0.87 (15 H, m, CH_3); 1.23 (13 H, m, CH_2CH_2 + CH_{ax}); 1.47 (10 H, m, CH$_2$CH_2); 2.42 (3 H, m, CH_{eq}); 3.11–3.28 (3 H, m, NCH_2 + C*H*O); 3.29–3.42 (3 H, m, OCH_2 + C*H*O); 4.11 (4 H, s, CH_2CO). δ_C($CDCl_3$) 13.72 and 19.18 (*C*H_3); 19.96

and 20.09 (CH_2CH_3); 29.47 and 30.86 (NCH_2CH_2, rotamer); 31.96 (OCH_2CH_2); 37.46 and 37.83 (ring CH_2); 45.38 and 48.69 (NCH_2); 67.09 and 68.22 (CHO); 72.71 and 73.41 (OCH_2); 168.73 (CO). ν_{max}(thin film)/cm^{-1} 1646 (NCO), 1093 (C–O). *m*/*z* (CI, dichloromethane) 528 ($M^+ + 1$, 100%). *m*/*z* (MH^+) found 527.443; $C_{30}H_{58}N_2O_5 + 1$ requires 527.442.

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