Synthesis and Binding Properties of Amide-functionalised Polyaza Macrocycles

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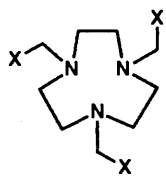
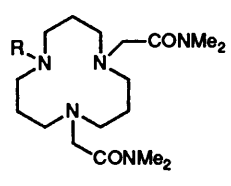
A series of amide *N*-functionalised coronands has been prepared based on parent [9]-N_3, [12]-N_3, [12]-N_2O_2, and [12]-N_4 polyazamacrocycles. Complexation with alkali and alkaline earth cations, particularly Li^+, Na^+, and Ca^{2+}, has been monitored by using ^{13}C NMR and IR spectroscopy, fast atom bombardment mass spectrometry, calorimetric, and potentiometric analysis in aqueous and alcoholic media. Particularly strong complexation in water has been observed for Ca^{2+} with 1,4,7,10-tetrakis(*N*,*N*-dimethylacetamido)-1,4,7,10-tetra-azacyclododecane, (**9**) (log K_s = 6.82-[H_2O, 298 K]), and selective Ca^{2+} complexation was observed with 1,7-dioxa-4,10-bis(dimethylethanamido)-4,10-diazacyclododecane, (**7**).

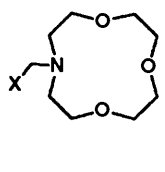
It is well established that the introduction of ligating amide substituents into polyaza[1] and polyoxa[2] macrocycles leads to enhanced discrimination in cation binding in favour of cations of high charge density (*e.g.* Li^+, Mg^{2+}, and Ca^{2+}). In order to achieve high free energies of complexation the ligands should be pre-organised (as far as possible) so that conformational changes on complexation—which contribute to both the enthalpy and entropy of complexation—are minimised. This idea has been demonstrated excellently in the binding of Li^+ and Na^+ by spherand-6.[3] For more flexible monocyclic ligands exhibiting faster kinetics of complexation, the minimisation of conformational change upon complexation is more difficult to achieve. However, both the nine-membered triazacyclononanes and the twelve-membered tetra-aza- and diaza-dioxa-cyclododecanes adopt similar ring conformations as free ligands and in their complexes, *i.e.* well-defined, 'square', [3.3.3] and [3.3.3.3] conformations.[4] Such behaviour may be contrasted, for example, with the larger [18]-O_6 ligand in which unfavourable negative entropies of complexation are typically observed with metal cations. For these reasons a series of amide-functionalised coronands, (**1**)–(**9**), has been synthesised in an attempt to synthesise ligands capable of forming reasonably strong complexes that discriminate in favour of Li^+ and Ca^{2+}.



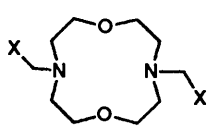
(**1**) X = $CONMe_2$
(**2**) X = CH_2CONMe_2



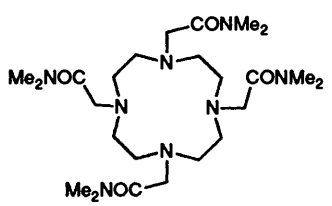
(**3**) R = CH_2CONMe_2
(**4**) R = H



(**5**) X = CH_2CO_2Me
(**6**) X = $CONMe_2$



(**7**) X = $CONMe_2$
(**8**) X = CH_2CONMe_2

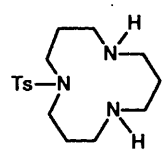


(**9**)

At the outset, the co-ordination number preference of 6 for Li^+ suggested that (**1**), (**3**), and (**7**) may favour Li^+/Na^+, and the tendency of Ca^{2+} to form octadentate complexes hinted that (**9**) should be selective for it.[5]

Results and Discussion

Ligand Syntheses.—Alkylation of the parent polyamines with *N*,*N*-dimethyl α-bromoethanamide[6] under basic conditions (either Cs_2CO_3–EtOH or K_2CO_3–CH_3CN) afforded direct syntheses of ligands (**1**), (**3**), (**6**), (**7**), and (**9**) in yields of 54, 60, 72, 85, and 55% respectively. The compounds (**2**), (**5**), and (**8**), which possess a C_3 substituent on nitrogen, were prepared conveniently by conjugation addition of *N*,*N*-dimethylpropenamide (or methyl propenoate) with the parent amines in dry methanol. Finally compound (**4**) which possesses one free secondary amine was prepared from the monotosylamide (**10**) by alkylation with *N*,*N*-dimethyl-α-bromoethanamide followed by detosylation[7] (HBr–AcOH–PhOH).

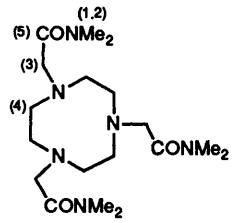


(**10**)

^{13}C *NMR and IR Studies of Complexation.*—Admixture of increasing amounts of solid anhydrous lithium chloride to (**1**) in CD_3OD:$CDCl_3$ (2:1) led to changes in the chemical shift of each ligand carbon atom (Table 1). Discrete lines for free and complexed ligand were observed at stoicheiometries intermediate between 0.1:1 and 1:1 (salt:ligand) consistent with slow kinetics (on the NMR timescale) of cation exchange. Resonances due to the free ligand had completely disappeared when one equivalent of LiCl had been added indicating that a 1:1 (or *n*:*n*) complex was formed. The shift in the amide carbonyl resonance (Δδ 1.02 ppm) is suggestive of simultaneous participation in binding of all three amide oxygens. With ligand (**2**), a very different behaviour was observed: 'averaged' signals for free and complexed ligand were discerned in formation of the 1:1 complex, (Figure 1), suggestive of formation of a weaker complex. Furthermore, both the ring methylene and the

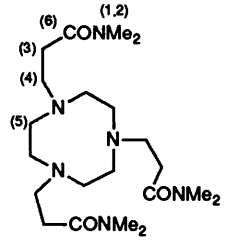
Table 1. ^{13}C NMR Co-ordination shifts ($\Delta\delta$) for (**1**), (**2**), and (**3**) following complexation with LiCl [298 K, CD_3OD:$CDCl_3$ (2:1)] and for (**9**) following complexation with $CaCl_2$.

(*a*)



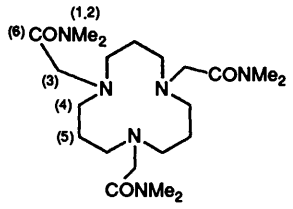
	Δδ/ppm
C-1	0.47
C-2	0.74
C-3	1.36
C-4	3.98
C-5	1.02

(*b*)



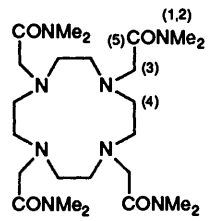
	Δδ/ppm
C-1	0.05
C-2	0.14
C-3	1.05
C-4	0.07
C-5	0.02
C-6	0.07

(*c*)



	Δδ/ppm
C-1	0.10
C-2	0.70
C-3	3.55
C-4	3.35
C-5	4.20
C-6	0.20

(*d*)



	Δδ/ppm
C-1	0.53
C-2	0.47
C-3	0.78
C-4	2.23
C-5	1.30

exocyclic CH_2N carbon resonances were minimally perturbed on complexation indicative of little or no interaction (on average) in solution of the ring nitrogen lone pairs. For the [12]-N_3 cycle (**3**), discrete resonances were observed again during formation of the 1:1 complex. The resonances for both free ligand and the complex were equally broadened, *e.g.* $\omega_{\frac{1}{2}}$ 80 Hz [(298 K, 62.1 MHz) for C_3 and C_5] when there was a 1:1 mixture of free ligand and complex. The nature of this exchange broadening was not studied further. Finally complexation of (**9**) with $CaCl_2$ under the same conditions was observed (Table 1). A 1:1 complex was formed with discrete, sharp resonances being discerned for ligand and complex at intermediate stoicheiometries. The relatively large amide carbonyl shift ($\Delta\delta_c$ 1.30) on complexation suggested that all four amides participated in binding to Ca^{2+} in the 1:1 complex.

Conformation of amide participation in binding was provided by recording IR spectra of thin films of the ligand and complex [as evaporated films (from MeOH) of their thiocyanate salts]. Complexation with (**1**), (**3**), and (**9**) was accompanied by a shift (to lower frequency) of the carbonyl stretching frequency by 26, 31, and 29 cm^{-1} respectively. With (**2**) only a shift of 2 cm^{-1} was noted, but using LiCl a shift of 15

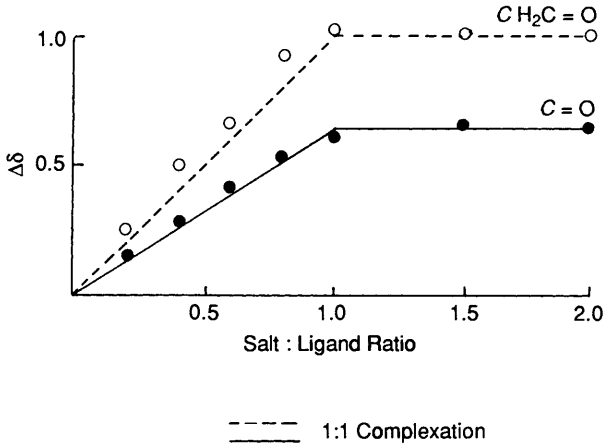


Figure 1. ^{13}C NMR co-ordination shifts for CH_2CO (upper: O) and CO (lower: O) carbon atoms of ligand (**2**) following incremental addition of solid LiCl [298 K, CD_3OD:$CDCl_3$ (2:1)].

cm^{-1} was observed: presumably thiocyanate is a competitive ligand under these conditions with this weaker ionophore.

Binding of lithium with the oxa–aza coronands (**5**)–(**8**) was also examined by ^{13}C NMR spectroscopy. In methanol solution, averaged signals were observed upon addition of LiCl to (**5**) and a very shallow titration curve was obtained, typical of a relatively weak complex (log K < 3) (Figure 2). With (**6**), (**7**), and (**8**) sharper titration curves were obtained with limiting chemical shifts being observed after addition of one equivalent of LiCl. However, at lower salt concentrations marked deviations from the expected curve were noted consistent with intermediate formation of weaker 2:1 ligand/Li^+ complexes.[8]

Binding of calcium to (**6**), (**7**), and (**8**) revealed that (**6**) formed a relatively strong 2:1 complex with a sharp curve bend at 1:2 salt:ligand ratio. This is not unexpected given calcium's co-ordination number preference[9] (8 not 5) and its desire to bind amide oxygen rather than ether oxygen. With (**7**) and (**8**) cation exchange was slow (298 K, CD_3OD, 62.1 MHz) and discrete, sharp signals were observed for the carbons of the ligand and of the complex, with a limiting shift at 1:1 stoicheiometry. In each case the amide carbonyl co-ordination shift was 1.2 ppm, indicative of strong amide binding. While the carbonyl resonance of (**7**) and (**8**) shifted to higher frequency on complexation, the CH_2O and CH_2N signals for (**7**) and (**8**) shifted to lower frequency, *e.g.* for (**7**) with added Ca^{2+}, ($\Delta\delta$ −2.6 [CH_2N ring], −1,0 [CH_2N sidearm] ppm). It is difficult, however, to attribute the direction and magnitude of the observed shifts to a particular factor: the conformational change experienced by the ligand is one cause and the change in the magnetic shielding caused by the introduction of a proximate cationic centre is another. The relative rates of dissociation of Ca^{2+} ion were studied further using 1:1 mixtures of free ligand and complex. For (**8**), signals due to both species had not coalesced at 330 K (CD_3OD, 62.1 MHz) implying a barrier to decomplexation of at least 63 kJ mol^{-1}. In aqueous solution, coalescence was observed with $\Delta G_c^{\ddagger}$ 54 (±0.5) kJ mol^{-1} compared with ΔG_c 65 (±0.5) kJ mol^{-1} for the calcium complex of (**7**). Complexation of IIa cations by macrocyclic ionophores is usually weaker in water than in methanol by between two and three orders of magnitude. Assuming that a high barrier to cation dissociation parallels strong complexation,[10] then the calcium complex of (**7**) is the stronger.

FABMS Studies of Complexation.—Fast atom bombardment mass spectroscopy has been promulgated as an effective technique for determining the selectivity of ligands for different

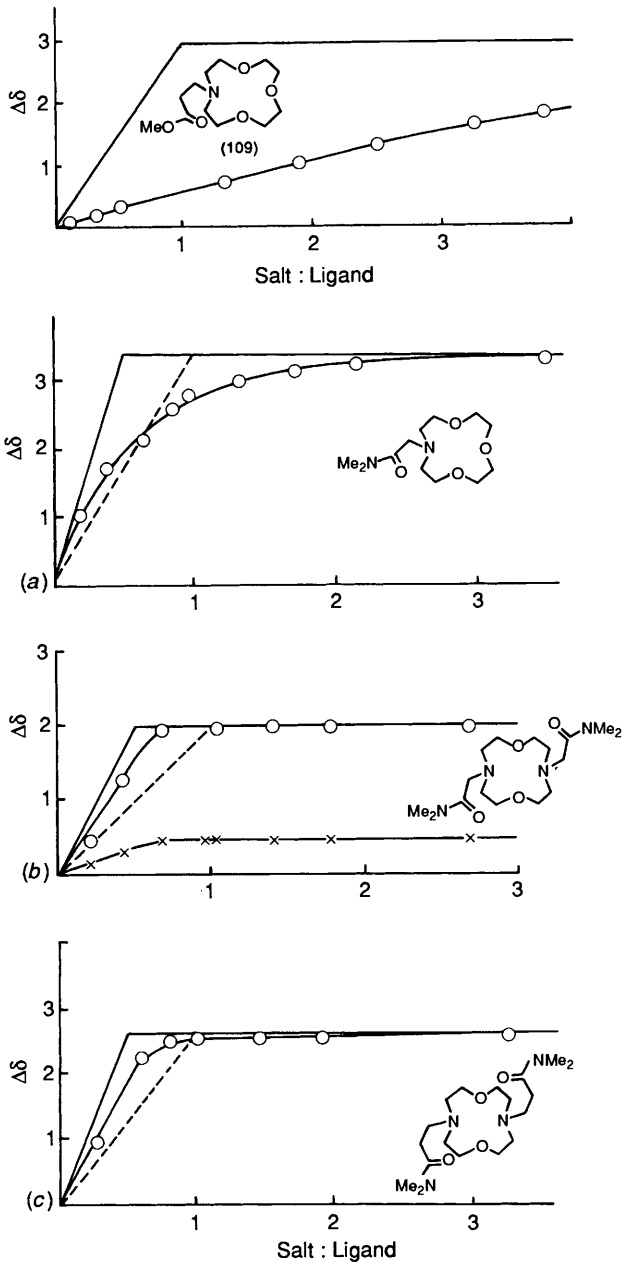


Figure 2. ^{13}C NMR co-ordination shifts (Δδ) for (**5**) (CH_2O), (**6**) (CH_2O), (**7**) (CH_2O + CH_2N ring), and (**8**) (CH_2N ring) incremental salt (LiCl) addition (298 K, CD_3OD). The limiting chemical shift value is fitted visually to the experimental curve.

metal cations.[11] Indeed it has been proposed that it offers a method for semi-quantitatively measuring equilibrium stability constants. Aqueous solutions of lithium, sodium, potassium, rubidium, and caesium chlorides were prepared and mixed (each 1.25×10^{-2} mol dm^{-3}). To this solution an equal volume of the ligand in methanol (1.25×10^{-2} mol dm^{-3}) was added together with an equal volume of glycerol as the FAB matrix. Thus, the cations compete for a deficiency of the ligand in this fixed interference method. Cation selectivities were evaluated (see Experimental for details) and the results expressed in terms of the selectivity factor, S, equation (1).

$$S = \log\left[\frac{I(L + M')}{I(L + M'')}\right] \quad (1)$$

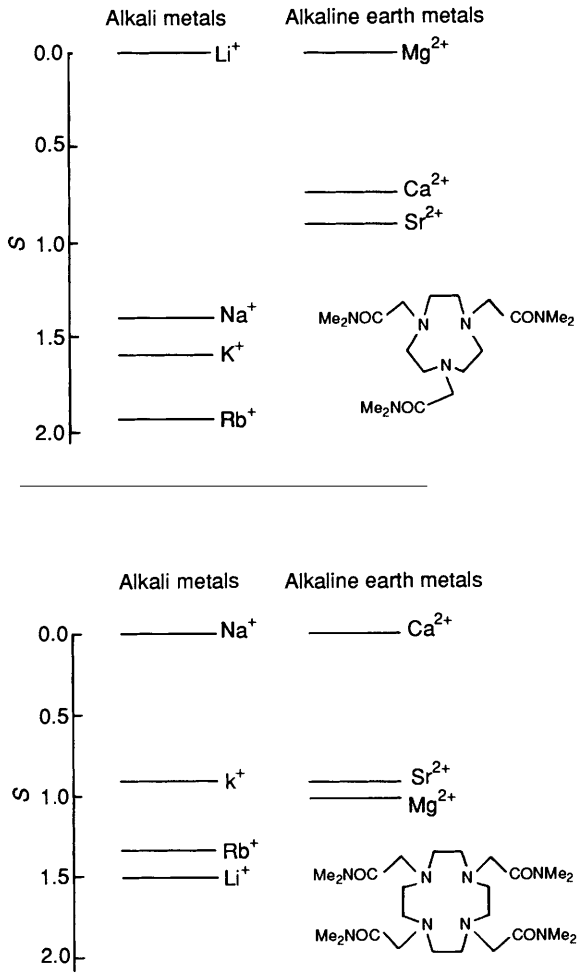


Figure 3. Fast atom bombardment mass spectroscopic selectivity values, (S), for complexation of cations with ligands (**1**) and (**9**) (8 keV, Xe atoms, 1:1:1 methanol:water:glycerol).

Where $I(M' + L)$ = average signal intensity for ligand and cation selectively bound. $I(M'' + L)$ = average signal intensity for less selectively bound species.

Selectivities for complexation of alkali and alkaline earth cations with (**1**) and (**9**) revealed that (**1**) was Li^+ and Mg^{2+} selective (Figure 3), while (**9**) was Na^+ and Ca^{2+} selective. In the hexaco-ordinate state Mg^{2+} and Li^+ have very similar ionic radii and both favour binding to donors with high dipole moments. Further the sterically limited bite of the [9]-N_3 nitrogen lone-pair density favours binding of smaller cations. With the [12]-N_4 system the situation changes to favour co-ordination of slightly larger cations and this tendency is enhanced by the ability of Na^+ and Ca^{2+} to take up co-ordination numbers greater than six. Such behaviour has been noted previously with 1,4,7,10-tetrakis(hydroxyethyl)-1,4,7,10-tetra-azacyclododecane[4] in which a preference for Na^+ and Ca^{2+} co-ordination was discerned. Ligands (**2**), (**3**), and (**4**) showed little evidence of binding under the given experimental conditions and only weak signals for the lithium and sodium complexes could be observed. In these cases the protonated ligand gave the major mass spectroscopic peak.

With ligands (**7**) and (**8**), selective complexation of Li^+ was evident with (**8**) apparently being the more selective ligand (Figure 4). Complexation of the monovalent mixed $[L{\cdot}CaCl]^+$ species was also evident although little can be concluded from this observation. Certainly, measurements of equilibrium

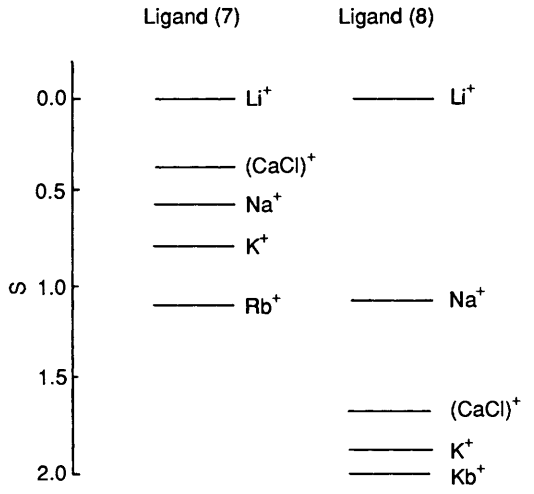


Figure 4. Fast atom bombardment mass spectroscopic selectivity values (*S*) for complexation of cations with ligands (**7**) and (**8**) (8 keV, Xe, 1:1:1 methanol:glycerol:water).

constants in methanol (see below) showed that ligand (**7**) formed the more stable Li complex so that experiments such as these may offer little more than a useful guide to the relative rate of cation binding in protic media. The experimental set-up is certainly not appropriate for conclusions to be confidently made about the relative equilibrium stabilities of complexes.

Calorimetric Experiments.—Complexation of (**5**), (**7**), and (**8**) in methanol was studied in detail by measuring the enthalpy and equilibrium stability constant for cation binding using standard calorimetric methods. Complexation of Ia and IIa cations with both (**5**) and 1,7-dioxa-4,10-diazacyclododecane afforded weak complexes (low log *K* values or not measurable) while (**8**) and particularly (**7**) formed stronger complexes by virtue of favourable amide oxygen–cation interactions. Contributions to the favourable reaction enthalpies observed with (**7**) and (**8**) come from several sources. These ligands both adopt 'endo–endo' conformations in the uncomplexed state [as revealed by the crystal structure [1a] of (**8**)]. This minimises the conformational re-ordering required during complexation. Such a situation may be contrasted with larger aza–oxa cycles, *e.g.* [18]-N_2O_4 or [222]-cryptand which favour an exo–exo conformation for the free ligand, so that energy is required to invert both nitrogen centres prior to complexation.[10,12,13] Differential ligand and cation solvation energies obviously need to be considered and the Li^+ ion in particular is highly solvated in methanol. This differential cation solvation effect is more dramatically revealed in the contribution to the reaction entropy of the change in the translational entropy of the liberated solvent molecules. Large positive ΔS values are observed for the complexation of the more strongly solvated small cations. This trend is particularly well shown in complexation of (**7**) (Table 2), and the strong complexation of Li^+ by (**7**) is primarily related to this favourable ΔS term notwithstanding the smallest enthalpy of complexation measured in this series. Further contributions to the reaction entropy come from changes in the ligand internal entropy because of orientational, rigidity and conformational changes on complexation. In the final complexed state, some of the ligand flexibility is lost resulting in an unfavourable negative entropy term. This effect will be more accentuated with (**8**) [compared with (**7**)] as it possesses longer more flexible aza-substituents, and is perhaps reflected in the less favourable ΔS terms.

Overall, ligand (**8**) shows little discrimination in binding Ia cations (log *K* values are very similar) and prefers Sr^{2+} and Ca^{2+}. This lack of discrimination masks the marked but compensating differences in ΔH and ΔS contributions along the series. Cations which are too small or too large to bind the focussed lone pairs of the ligand may require a more dramatic ligand conformational change (probably involving unfavourable torsional and angle strain) in order to optimise binding interactions. With ligand (**7**), selectivity in favour of Li^+ and Ca^{2+} is evident. Indeed for Ca^{2+}, assuming that $T\Delta S$ *ca.* 0, then log *K ca.* 8.0 and this ligand exhibits a marked preference—at equilibrium—to bind calcium ions.

Aqueous Potentiometric Measurements of pK_a *and log* K.—Successive acid dissociation constants for ligands (**1**), (**3**), (**4**), and (**9**) were determined by pH-metric titration followed by iterative data analysis using SCOGS-2 and SUPERQUAD. Similar values were obtained for the pK_as of each ligand compared to their respective parent polyazamacrocycles (Table 3). With the [12]-ring ligands (**3**) and (**4**), the diminution of pK_{a1} and pK_{a2} compared with triazacyclododecane itself is most probably due to less effective solvation of the protonated species (LH^+ and LH^{2+}) arising from unfavourable steric interactions with the *N*-substituents limiting solvent hydrogen bonding. In addition the putative intramolecular bifurcated hydrogen bond[18] is likely to be most strong in the symmetrical ligand (**3**) and the parent amine, which exhibit the highest first pK_a.

Metal complexation constants were determined also using potentiometric titration methods followed by iterative data analysis. Values are given (Table 4) for the binding of Li^+ and Na^+ with (**1**) and (**3**) and for Li^+, Na^+, and Ca^{2+} with (**9**). Ligands (**1**) and (**3**) were only moderately selective for Na^+ and Li^+ respectively—in contrast to the selectivity of (**1**) for Li^+ determined using FABMS. However, it should be noted that these stabilities are relatively high for simple monocyclic complexes of Li^+ and Na^+ in aqueous media. Indeed, the Li^+ complex of (**9**) (log K_s 5.23) is more stable than that of the macrobicycle, 4,10-dimethyl-1,4,7,10,15 penta-azabicyclo [5.5.5] heptadecane for which log *K* 4.8.[19] It is the most stable lithium azamacrocyclic complex in water for which reliable data have been reported.

With ligand (**9**), a marked preference to bind Ca^{2+} is evident, although this is less pronounced than the preference for Ca^{2+} observed with (**7**) in methanol. The fraction of the free aquo ion $(M^{n+})_{aq}$ is given by equation (2). M^{n+} = concentration of free

$$\alpha_0 = \frac{[M^{n+}]}{C_m} \quad (2)$$

metal aquo ion, C_m = total concentration of metal ion. Given equation (3) we arrive at equation (4).

$$K_{ML} = \frac{[ML^+]}{[M^+][L]} \quad (3)$$

$$\alpha_0 = \left(\frac{[M^+]}{[M^+]} + \frac{[ML^+]}{[M]^+}\right)^{-1} = \frac{1}{1 + K_{ML}[L]} \quad (4)$$

Hence α_0 may be calculated for a range of ligand concentrations and thus the percentage of metal cation bound at each ligand concentration may be calculated: % metal bound = $(1 - \alpha_0) \times 100$. For ligand (**9**), the % of Li^+, Na^+, and Ca^{2+} bound as a function of ligand concentration was calculated (Figure 5). The slight selectivity for sodium over lithium, despite the amide donor preference favouring lithium binding, is most likely a reflection of the ability of Na^+ to take up at least hepta co-ordination in its complexes with [12]-N_4 coronands, whereas Li^+ will be constrained to 5 or 6 co-ordination and a reduced enthalpy of complexation.

Table 2. Enthalpies of complexation and stability constants (298 K, CH_3OH) for reaction of 5, 7, and 8[a,b] with captions.

Ligand	Li^+	Na^+	K^+	Rb^+	Ca^{2+}	Sr^{2+}	Ba^{2+}	Ag^+
(5) log K	2.71	—	—	—	2.72	—	—	—
$-\Delta H$	3.00	20.80	13.10	—	6.80	11.90	19.10	49.90
$T\Delta S$	12.40	—	—	8.70	—	—	—	—
(7) log K	5.38	4.72	3.85	3.08	>5.50	>5	4.94	>5
$-\Delta H$	12.70	26.00	25.70	22.70	46.60	35.80	33.00	59.10
$T\Delta S$	17.90	0.80	−3.80	−5.20	—	—	−4.90	—
(8) log K	2.99	3.01	3.03	3.08	4.10	4.36	3.30	>5
$-\Delta H$	23.80	37.60	30.60	11.00	45.90	19.90	44.50	82.50
$T\Delta S$	−6.80	−20.50	−13.40	6.50	−22.60	−4.90	−25.70	—

[a] The parent 1,7-dioxa-4,10-diazacyclododecane gave measurable values only for complexation with Ag^+ (log K = 6.51, $-\Delta H$ 31.9 kJ mol^{-1}, $T\Delta S$ +5.1 kJ mol^{-1}) and Ba^{2+} (log K 2.34, $-\Delta H$ = 13.3, $T\Delta S$ −4.4 kJ mol^{-1}). [b] Stability constants for complexation of 1,4,7,10-tetrakis (hydroxyethyl)-1,4,7,10-tetrazacyclododecane in 9:1 CH_3OH–H_2O are reported to be Li^+: log K 2.4; Na^+, log K 3.6; K^+, log K 2.0; Ca^{2+}, log K 6.9.[14]

Table 3. Dissociation constants for protonation of ligands (1), (3), (4), and (9) 298 K, I = 0.1 NMe_4NO_3, ±0.05).[a]

Ligand	pK_{a1}	pK_{a2}	pK_{a3}
(1)	10.30	7.39	3.25
(3)	11.20	7.15	—
(4)	10.30	6.10	—
(9)	11.30	10.25	—
Triazacyclononane[b]	10.60	6.88	<2.50
Diethylenetriamine[b]	9.70	8.98	4.25
Triazacyclododecane[c]	12.60	7.60	—
Tetrazacyclotetradecane[d]	11.00	9.87	—

[a] Values of σ and ψ^2 were typically 2 and 6 in superquad data analysis: values of pK_a shown are the mean of three readings. [b] Data from reference 15. [c] Data from reference 16. [d] Reference 17.

Table 4. Stability constants for complexation of (1), (3), and (9) with cations (298 K, I = 0.1 NMe_4NO_3).

Ligand	Li^+	Na^+	Ca^{2+}
(1)	3.91	4.22	—
(3)	4.21	4.02	—
(9)	5.23	5.84	6.80

[a] Values given are the mean of (3) determinations (±0.05).

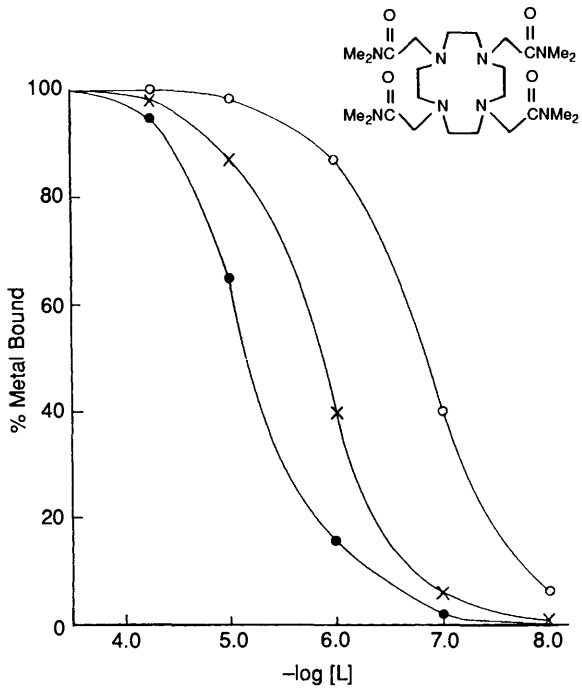


Figure 5. Variation in the percentage of Li^+ (●), Na^+ (×), and Ca^{2+} (○) bound by ligand (9) (298 K, 0.1 mol dm^{-3} NMe_4NO_3) with concentration of ligand.

Experimental

Proton and carbon NMR spectra were recorded on a Bruker AC 250 (250.13 MHz and 62.1 MHz) spectrometer. Chemical shifts are quoted to higher frequency of $SiMe_4$ as internal standard and are given in ppm, with coupling constants in Hz. IR spectra were recorded on a Perkin-Elmer 580A Infrared Spectrophotometer, and mass spectra were recorded either in the ei, ci, dci, or fab mode using a VG 7070E spectrometer. TLC was effected using Merck 60 7354 or 9385 for flash chromatography. HPLC analyses were carried out with a Varian 5500 instrument using both ion-exchange (TSK DEAE) or reverse-phase (Hypersil 5005) columns for analytical or semi-preparative work typically using aqueous acetate–CH_3CN gradient elution. Compounds that did not give correct combustion microanalyses were examined for their purity by TLC and/or HPLC and were ≥96% homogeneous.

^{13}C NMR Experiments.—Titration curves were obtained in 2:1 CD_3OD–$CDCl_3$ solution (3 cm^3) of the ligands and the solid alkali salts [LiCl (BDH), $CaCl_2$ (Aldrich)]. After each addition of salt, the ^{13}C NMR chemical shift (relative to TMS) was measured at 298 K using a Bruker AC250 instrument operating at 62.1 MHz for the carbon nucleus.

Fast Atom Bombardment Mass Spectroscopic Experiments.—The stainless steel tip of a FAB-probe was coated with a thin layer of the analytical solution (3 mm^3). Positive FABMS was performed using a primary ion atom beam of xenon (8 keV) on a VG 7070E mass spectrometer coupled to a VG II-250 data system. At an accelerating voltage of 6 kV the mass range m/z 20–2 000 was scanned as 3 s per decade (scan cycle time 10 s). Twenty successive spectra of each analytical solution were acquired and scans 5 to 15 inclusive were averaged to afford the final spectrum. Two runs were performed for each analytical solution and the values were averaged to obtain selectivity values for each ligand. The selectivity factor, S is given by equation (5), where I(ligand + M^+) represents the experimental signal intensity (mean of scans 5 to 15 typically).

$$S = \log\left[\frac{I(\text{Ligand} + M^+)}{I(\text{Ligand} + M^{1+})}\right] \quad (5)$$

pH-Metric Titration Experiments.—Apparatus and instrumentation. The titration cell was a double-walled glass vessel of approximately 5 cm^3 capacity. The temperature of the system was maintained at 25 °C using a Techne Tempette Junior TE-8J. The solutions in the titration cell were stirred using a magnetic stirrer. An automatic burette (Mettler DL20) of 1 cm^3 capacity was used and the pH was measured using a Corning 0001854 combination microelectrode. The titrations were controlled and the data stored using a BBC microprocessor. The burette functions (volume increments, total volume delivered, and time interval allowed for equilibration between each reading) were controlled by the use of Basic software stored on a disc. The data was transferred to the MTS mainframe using KERMIT. This data was subsequently analysed by two non-linear least-squares programs SCOGS2 and SUPERQUAD.[20]

Measurement of acid dissociation constants. The combination microelectrode was calibrated by using two buffers: (*i*) 0.05 mol dm^{-3} KHPh—pH 4.008, 25 °C; (*ii*) 0.025 mol dm^{-3} KH_2PO_4, 0.025 mol dm^{-3} Na_2HPO_4—pH 6.865m 25 °C. Stock ligand solutions were made up containing 0.001 mol dm^{-3} ligand, 0.00*N* mol dm^{-3} nitric acid (*N* = number of amine nitrogen atoms in the ligand) and 0.1 mol dm^{-3} tetramethylammonium nitrate to ensure constant ionic strength in deionised water (25 cm^3) (MilliQ). The titrant ligand solution (3 cm^3) was placed in the titration vessel which was fitted with a Teflon cap with three apertures for the combination electrode, burette tube, and nitrogen bubbler. The titrant was tetramethylammonium hydroxide whose exact molarity (0.070 mol dm^{-3}) was determined by titrating against 0.1 mol dm^{-3} HCl. Three separate titrations were performed on each ligand and the results were analysed by methods previously outlined.

Measurement of metal complexation constants. Titrant ligand solutions were made up containing 0.001 mol dm^{-3} ligand, 0.001 mol dm^{-3} cation, 0.00*N* mol dm^{-3} (*N* = number of amine nitrogen atoms in the ligand), and 0.1 mol dm^{-3} tetramethylammonium nitrate. The titrant once again was tetramethylammonium hydroxide (0.070 mol dm^{-3}) and as before titrations were performed under an atmosphere of nitrogen. Three separate titrations were made for each particular ligand–cation combination and data was analysed as before. The cations used were chloride salts, lithium chloride and sodium chloride solutions (BDH) and 0.1 mol dm^{-3} calcium chloride solution (BDH).

Calorimetric Experiments.—Materials. The salts used were $LiClO_4$ (Ventron), $NaNO_3$ (Merck), KI (Merck), RbI (Merck), $Ca(NO_3)_2$ (BDH), $SrBr_2$ (Ventron), $AgNO_3$ (Merck), and $Ba(ClO_4)_2$ (Merck). The solvent used was MeOH [0.01% H_2O (Merck)].

Stability constants and reaction enthalpies were determined using a Tronac Model 450 calorimeter. A solution of the ligand (0.05–0.025 mol dm^{-3}) was added slowly to a solution containing a salt (1.5–4 × 10^{-3} mol dm^{-3}). The heat *Q* produced during titration was related to the reaction enthalpy ΔH after correction for all non-chemical heat effects as shown by equation (6).

$$Q_t = \Delta n_t \Delta H \quad (6)$$

Where Δn_t is the number of moles of the complex formed at time, *t*. During the titration Δn_t varies since it is a function of the stability constant, *K*, equation (7).

$$M^{x+} + L = ML^{x+} : K = \frac{[ML^{x+}]}{[M^{x+}][L]} \quad (7)$$

No values for the stability constant can be calculated from the thermogram if log $K > 5.5$. In other cases, the salt concentration in the reaction vessel was so high (2–3 × 10^{-2} mol dm^{-3}) that every added molecule of the ligand forms a complex. In this case Δn_t is constant during the titration and only values of the reaction enthalpy can be obtained from the thermogram.

Syntheses.—N,N-*Dimethybromoacetamide.* Synthesised according to the method of Weaver and Whaley.[6]

1,4,7-*Tris*(N,N′-*dimethylacetamido*)-1,4,7-*triazacyclononane* (**1**). 1,4,7-Triazacyclononane (250 mg, 1.93 mmol) was stirred in dry acetonitrile (20 cm^3) under an atmosphere of nitrogen. Potassium carbonate (0.828 g, 6 mmol) and *N,N′*-dimethylbromoacetamide (1 g, 6 mmol) were added, and the mixture was heated to reflux for 48 h. The mixture was then cooled to room temperature and the inorganic salts were filtered off and the filtrate was evaporated under reduced pressure. The waxy red–brown solid was taken up into the minimum amount of 1 mol dm^{-3} hydrochloric acid and extracted with dichloromethane (5 × 10 cm^3). The acid aqueous layer was then basified to pH 14 using 1 mol dm^{-3} potassium hydroxide solution and extracted with dichloromethane (5 × 10 cm^3). The combined basic extracts were washed with distilled water (2 × 10 cm^3), dried over potassium carbonate and evaporated *in vacuo* to yield product—a red–brown viscous oil. The product was purified by column chromatography using alumina with a gradient from dichloromethane to 2% methanol–dichloromethane as eluant (R_f product = 0.3–1% MeOH–CH_2Cl_2). This yielded a clear viscous oil (400 mg, yield 54%). $\delta_H(CDCl_3)$ 2.88 (12 H, s, CH_2N), 2.92 (9 H, s, CH_3N), 3.05 (9 H, s, CH_3N), and 3.42 (6 H, s, CH_2CO); $\delta_C(CDCl_3)$ 35.4 (CH_3N), 36.9 (CH_3N), 55.5 (CH_2N), 59.9 (CH_2CO), and 171.0 (C=O); IR ν_{max}(thin film): 1 645 (C=O); *m/z* 385 (100, $M^+ + 1$) and 300 (60, $-CH_2CONMe_2$) Found: 384.2860; $C_{18}H_{36}N_6O_3$ requires M^+ 384.2849.

1,4,7-*Tris*(N,N′-*dimethylpropanamido*)-1,4,7-*triazacyclononane* (**2**). 1,4,7-Triazacyclononane (180 mg, 1.43 mmol) was stirred in anhydrous methanol (15 cm^3) under an atmosphere of nitrogen. Dimethylacrylamide (0.85 g, 8.6 mmol) was added and the mixture was refluxed under nitrogen for 3 h. The mixture was cooled to room temperature and the solvent was evaporated under reduced pressure. The residue was taken up into the minimum amount of 1 mol dm^{-3} hydrochloric acid and further with dichloromethane (5 × 10 cm^3). The acid aqueous layer was then basified to pH 14 using 1 mol dm^3 potassium hydroxide solution and extracted with dichloromethane (5 × 10 cm^3). The combined basic extracts were washed with distilled water (2 × 10 cm^3), dried over potassium carbonate and evaporated *in vacuo* to yield crude product. The product was purified by column chromatography using alumina with a gradient from dichloromethane to 2% methanol–dichloromethane as eluant (R_F product 0.45—CH_2Cl_2–2% MeOH). This yielded a clear viscous oil (325 mg, yield 53%). $\delta_H(CDCl_3)$ 2.43 (6 G, t, *J* 7.5 Hz, CH_2CO), 2.70 (12 H, s, CH_2N ring), 2.82 (6 H, t, *J* 7.5 Hz, CH_2N sidearm), 2.86 (9 H, s, CH_3N), and 2.97 (9 H, s, CH_3N); $\delta_C(CDCl_3)$ 31.7 (CH_2CO), 35.3, 37.4 (CH_3N), 54.4 (CH_2N ring), 55.7 (CH_2CN sidearm), and 172.1 (C=O); IR ν_{max}(thin film): 1 637 (C=O); *m/z* 427 (100, $M^+ + 1$), 426 (M^+, 10), 328 (40, $M^+ - CH_2CH_2CONMe_2$), and 228 (20, $2CH_2CH_2CONMe_2$) Found: 426.3310; $C_{21}H_{42}N_6O_3$ requires M^+ 426.3318.

1,5,9-*Tris*(N,N′-*dimethylethanamido*)-1,5,9-*triazacyclododecane* (**3**). 1,5,9-Triazacyclododecane (250 mg, 1.46 mmol) was stirred in dry ethanol (20 cm^3) under a nitrogen atmosphere. To this was added caesium carbonate (1.42 g, 4.4 mmol) and *N,N′*-dimethylbromoacetamide (0.73 g, 4.4 mmol) and the mixture was refluxed under nitrogen for 24 h. The mixture was then allowed to cool, and the ethanol was separated off under reduced pressure. The off-white waxy residue was taken up into dichloromethane and filtered. The

filtrate was then evaporated under reduced pressure. The residue was taken up into the minimum amount of 1 mol dm^{-3} hydrochloric acid and extracted with dichloromethane (5 × 10 cm^3). The acid aqueous layer was then basified with 1 mol dm^{-3} potassium hydroxide and extracted with dichloromethane (5 × 10 cm^3). The basic extracts were washed with distilled water (2 × 10 cm^3), dried over $MgSO_4$ and concentrated *in vacuo* to yield crude product. The product was purified by passing down an alumina column using a gradient from dichloromethane to 2% methanol–dichloromethane as eluant (R_F product 0.55—CH_2Cl_2–2% MeOH) to yield a clear viscous oil (365 mg, yield 60%). $\delta_H(CDCl_3)$ 1.63 (6 H, q, *J* 5.9 Hz, $CH_2CH_2CH_2$), 2.61 (12 H, t, *J* 5.8 Hz, CH_2N ring), 2.96 (9 H, s, CH_3N), 3.10 (9 H, s, CH_3N), and 3.27 (6 H, s, CH_2CO); $\delta_C(CDCl_3)$ 21.5 ($CH_2CH_2CH_2$), 35.2, 36.8 (CH_3N), 46.6 (CH_2N), 57.7 (CH_2CO), and 170.5 (C=O); IR ν_{max}(Thin Film): 1 646 (C=O); *m/z* 427 (100, $M^+ + 1$), 426 (5, M^+), 342 (30, $M^+ - CH_2CONMe_2$) Found: 426.3207; $C_2H_{42}N_6O_3$ requires M^+ 426.3318.

1-*Amino*-5,9-*bis*(N,N-*dimethylethanamido*)-1,5,9-*triazacyclododecane* (**4**), was synthesised as described in ref. 7.

10-*Methoxycarbonylethyl*-1,4,7-*trioxa*-10-*azacyclododecane* (**5**). 1,11-Diodo-3,6,9-trioxaundecane (18.5 g, 0.145 mol) and 3-aminomethylpropanoate hydrochloride (6.5 g, 0.047 mol) in dry acetonitrile (500 cm^3) containing anhydrous sodium carbonate (18 g) were heated under reflux with stirring for 48 h under an atmosphere of nitrogen. After cooling of the mixture to room temperature, the mixture was filtered, the solvent removed under reduced pressure, and the residue chromatographed on basic alumina, eluting with dichloromethane. Evaporation of the eluant gave a residue which was acidified with concentrated hydrochloric acid (5 cm^3) in methanol (20 cm^3). The excess hydrochloric acid was removed by evaporation as an azeotrope with methanol, the residue was crystallised from propan-2-ol at −5 °C to give 10-*methoxycarbonylethyl*-1,4,7-*trioxa*-10-*azacyclododecane hydrochloride* as a yellow crystalline solid, 2.53 g (22%), m.p. 135 °C, ν_{max}(KBr) 1 725 (C=O); $\delta_H(D_2O)$ 3.00 (2 H, t, *J* 6.7 Hz, CH_2C=O), 3.63 (2 H, t, 6.7 Hz, CH_2N side-arm), 3.78 (3 H, s, OCH_3), and 3.44–4.02 (16 H, m, CH_2O and CH_2N ring); *m/z* 262 (M^+, 10%). A molar equivalent quantity of solid tetramethylammonium hydroxide was added to a solution of 10-methoxycarbonylethyl-1,4,7-trioxa-10-azacyclododecane hydrochloride in dichloromethane. After stirring the solution for 15 min the bright-yellow colour diminished considerably, the solution was decanted from the residual solid hydroxide. Evaporation of the dichloromethane gave an oil which was redissolved in a small volume of dichloromethane and passed over a small quantity of alumina to give 10-*methoxycarbonylethyl*-1,4,7-*trioxa*-10-*azacyclododecane* as a colourless oil (Found: M^+, 261.1569 ± 0.002; $C_{12}H_{23}NO_5$ requires M^+, 261.1569); $\delta_H(CDCl_3)$ 2.48 (2 H, *J* 7.2 Hz, CH_2C=O), 2.70 (4 H, t, *J* 4.8 Hz, CH_2N ring), 2.86 (2 H, t, *J* 7.2 Hz, CH_2N side-arm), and 3.62–3.68 (15 H, m, OCH_3 and CH_2O's); $\delta_C(CDCl_3)$ 32.13 (CH_2C=O), 50.98 (CH_2N ring), 51.62 (CH_2N side-arm), 54.63 (OCH_3), 69.81, 69.98, and 70.83 (CH_2Os), and 172.55 (C=O).

10-(N,N-*Dimethylethanamide*)-1,4,7-*trioxa*-10-*azacyclododecane* (**6**). *N,N*-Dimethylbromoacetamide (0.2 g, 1.2 mmol) was added to a solution of 1,4,7-trioxa-10-azacyclododecane (160 mg, 0.9 mmol) in dry acetonitrile (15 cm^3) containing anhydrous sodium carbonate (0.3 g) and the resulting mixture was stirred and heated under reflux under a nitrogen atmosphere for 48 h. After cooling of the mixture to 20 °C the solid was removed by filtration and the filtrate evaporated under reduced pressure. The crude residue was taken up in the minimum amount of dilute hydrochloric acid (0.2 mol dm^{-3}) and the aqueous solution was washed with chloroform (5 × 25 cm^3). The aqueous layer was treated with potassium hydroxide solution and the product was extracted with chloroform (5 × 25 cm^3). The combined organic layers were evaporated under reduced pressure to a small volume, the solid was removed by filtration through a small bed of alumina. Evaporation of the filtrate gave 10-(N,N-*dimethylethanamido*)1,4,7-*trioxa*-10-*azacyclododecane* as a pale-yellow oil (168 mg, 72%) (Found: M^+, 260.173 50 ± 0.002; $C_{12}H_{24}N_2O_4$ requires M^+, 260.173 61; $\delta_C(CDCl_3)$ 35.58 (CH_3), 36.71 (CH_3), 54.22 (CH_2N), 54.92 (CH_2N), 69.87, 70.31, and 70.98 (CH_2O), and 170.60 (C=O).

4,10-*bis*(N,N-*Dimethylethanamido*)1,7-*dioxa*-4,10-*diazacyclodecane* (**7**). *N,N*-Dimethylbromoacetamide (0.57 g, 3.44 mmol) was added to a solution of 1,7-dioxa-4,10-diazacyclododecane (112) (0.30 g, 1.72 mmol) in dry acetonitrile (25 cm^3) containing anhydrous sodium carbonate (0.38 g) and the mixture was heated under reflux under an atmosphere of nitrogen with stirring for 48 h. After cooling of the mixture to 20 °C the mixture was filtered and the solvent removed under reduced pressure. The crude residue was taken up in the minimum volume of dilute hydrochloric acid (0.2 mol dm^{-3}) and washed with chloroform (5 × 25 ml). The aqueous layer was basified with potassium hydroxide solution and the product was extracted with chloroform (5 × 25 cm^3). The product was further purified by chromatography on alumina, eluting with dichloromethane–methanol (methanol increasing from 0 to 2%). Evaporation of the organic eluates gave 4,10-*bis*(N,N-*dimethylethanamido*)-1,7-*dioxa*-4,10-*diazacyclododecane* as a crystalline residue, 0.50 g (85%), m.p. 135 °C (Found: M^+, 344.243 393 ± 0.002; $C_{16}H_{32}N_4O_4$ requires M^+m 344.242 356; ν_{max}(nujol) 1 641 (C=O stretch) cm^{-1}; $\delta_H(CDCl_3)$ 2.89 (8 H, t, *J* 4.64 Hz, CH_2N), 2.93 (6 H, s, NCH_3), 3.06 (6 H, s, NCH_3), 3.45 (4 H, s, NCH_2C=O), and 3.57 (8 H, t, *J* 4.56 Hz, CH_2O); $\delta_C(CDCl_3)$ 35.6 (NCH_3), 36.42 (NCH_3), 54.69 (CH_2N), 57.41 (NCH_2C=O) 68.74 (CH_2O), and 179.23 (C=O).

4,10-*bis*(N,N-*Dimethylpropanamido*)-1,7-*dioxa*-4,10-*diazacyclododecane* (**8**). *N,N*-Dimethylpropenamide (1.46 g, 0.015 mol; freshly distilled) was added to a solution of 1,7-dioxa-4,10-diazacyclododecane (0.50 g, 0.287 mol) in methanol (20 cm^3), and the stirred mixture was heated under reflux, under a nitrogen atmosphere for 4 h. After cooling of the mixture to 20 °C the mixture was filtered and the solvent removed under reduced pressure. The crude residue was taken up in the minimum volume of dilute hydrochloric acid (0.1 mol dm^3) and washed with chloroform (5 × 25 cm^3). The aqueous layer was basified with potassium hydroxide solution and the product extracted into chloroform (5 × 25 cm^3). The solution was reduced to small volume under reduced pressure. Chromatography on basic alumina eluting with dichloromethane–methanol (methanol increasing from 0 to 2%) gave a pale yellow oil which was crystallised from toluene with a trace of ether present to give 4,10-bis(*N,N*-dimethylpropanamido)-1,7-dioxa-4,10-diazacyclododecane as colourless plates, m.p. 82 °C (Found: M^+, 372.2755 ± 0.001; $C_{18}H_{36}N_4O_4$ requires M^+, 372.2752); $\delta_H(CDCl_3)$ 2.49 (4 H, t, *J* 7.63 Hz, CH_2C=O), 2.65 (8 H, t, *J* 4.67 Hz, CH_2N ring), 2.83 (4 H, t, *J* 7.64 Hz, CH_2N side-arm), 2.89 (6 H, s, CH_3's), and 3.55 (8 H, t, *J* 4.67 Hz, CH_2O ring); $\delta_C(CDCl_3)$ 31.88 (CH_3), 37.96 (CH_3), 53.36 (CH_2N ring), 55.83 (CH_2N side-arm), and 70.11 (CH_2O), and 172.63 (C=O).

X-Ray Crystal Data.—$C_{18}H_{36}N_4O_4$; Monoclinic, a = 8.578 (2), b = 9.673 (2), c = 13.154 (4) Å, β = 107.63 (2)°; V = 1 040.2 $Å^3$, Z = 2, D_x = 1.19 g cm^{-3}, $F(000)$ = 204, μ(Mo-K_α) = 0.8 cm^{-1}. Space group determined uniquely from the systematic absences as $P2_1/n$ (alt $P2_1/c$, No. 14). The structure was determined by direct methods. Isotropic followed by anisotropic refinement of the non-hydrogen atoms with the hydrogens in geometrically idealised positions converged with R = 0.030 and R_w = 0.029 for 880 observed reflections

measured on a CAD4 diffractometer. Data have been deposited previously with the Cambridge Data Centre.[1a]

N,N'-*Dimethyl*-1,4,7,10-*tetra-acetamido*-1,4,7,10-*tetra-aza-cyclododecane* (**9**). 1,4,7,10-Tetra-azacyclododecane (200 mg, 1.2 mmol) was stirred in dry ethanol (5 cm^3) under a nitrogen atmosphere. To this was added caesium carbonate (1.05 g, 3.6 mmol) and *N,N'*-dimethylbromoacetamide (0.60 g, 3.6 mmol) and the mixture was boiled under reflux for 24 h. Crude product was obtained by an identical procedure to that of ligand (**3**) and the product was purified by column chromatography using alumina with a gradient from dichloromethane to 3% methanol–dichloromethane as eluant (R_F product = 0.25 2% MeOH–CH_2Cl_2). This yielded a clear viscous oil (340 mg, yield 55%). $\delta_H(CDCl_3)$ 2.47 (16 H, broad, s, CH_2N), 2.91 (12 H, s, CH_3N), 2.96 (12 H, s, CH_3N), and 3.28 (8 H, broad, s, CH_2CO); $\delta_C(CDCl_3)$ 35.5, 36.2 (CH_3N), 51.5 (CH_2N), 55.1 (CH_2CO), and 171.1 (C=O); IR ν_{max} cm^{-1} (Thin Film): 1 645 (C=O), *m/z* 513 (100, $M^+ + 1$), 428 (14, $M^+ - CH_2CONMe_2$), and 257 (10, $M^+ - 3CH_2CONMe_2$) Found: M^+ 512.3790 ± 0.0005; $C_{24}H_{48}N_8C_4$ requires 512.3798.

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References

1 (*a*) K. E. Matthes, D. Parker, H. J. Buschmann, and G. Ferguson, *Tetrahedron Lett.*, 1987, 5573; (*b*) H. Tsukube, H. Adachi, and S. Morosawa, *J. Chem. Soc., Perkin Trans. 1*, 1989, 1537.
2 P. E. Nicholson, R. Kataky, and D. Parker, *Tetrahedron Lett.*, 1989, 4559; P. E. Nicholson, R. Kataky, and D. Parker, *J. Chem. Soc., Perkin Trans. 2*, 1990, 321. For examples with acyclic ionophores see: E. Metzger, R. Aeschmann, M. Egli, G. Suter, R. Dohner, D. Ammann, M. Dobler, and W. Simon, *Helv. Chim. Acta*, 1986, **66**, 1821.
3 G. M. Lein and D. J. Cram, *J. Chem. Soc., Chem. Commun.*, 1982, 301.
4 J. Dale, *Ior. J. Chem.*, 1980, **20**, 3; S. Busen, J. Dale, P. Groth, and J. Krane, *J. Chem. Soc., Chem. Commun.*, 1982, 1172; J. C. Boeyens and S. M. Dobson, in 'Stereochemical and Stereophysical Behaviour of Macrocycles,' ed. I. Bernal, Elsevier, Amsterdam, 1987, Ch. 1.
5 The most stable Ca^{2+} complex observed [log K_s = 17.2 (H_2O, 298 K)] has been reported for 1,4,7,10-tetrazacyclododecane-tetra-acetic acid (DOTA): R. Delgado and J. J. R. Frausto da Silva, *Talanta*, 1982, **29**, 815; H. Stetter and W. Frank, *Angew. Chem., Int. Ed. Engl.*, 1976, **15**, 686.
6 W. E. Weaver and W. M. Whaley, *J. Am. Chem. Soc.*, 1947, **69**, 515.
7 I. M. Helps, D. Parker, K. J. Jankowski, J. Chapman, and P. E. Nicholson, *J. Chem. Soc., Perkin Trans. 1*, 1989, 2079.
8 M. J. Calverley and J. Dale, *Acta. Chem. Scand., Ser. B*, 1982, **36**, 241.
9 C. K. Schaner and O. P. Anderson, *J. Am. Chem. Soc.*, 1987, **109**, 3647; R. Hilgenfeld and W. Saenger, *Top. Curr. Chem.*, 1982, **101**, 1.
10 D. Parker, *Adv. Inorg. Chem. Radiochem.*, 1983, **26**, 1.
11 R. A. W. Johnstone and M. E. Rose, *J. Chem. Soc., Chem. Commun.*, 1983, 1268; J. Meili and J. Seibl, *Int. J. Mass Spectrom. Ion Phys.*, 1983, **46**, 367.
12 H. J. Buschmann, *Inorg. Chim. Acta*, 1985, **102**, 95 and references cited therein.
13 H. J. Buschmann, in 'Stereochemical and Stereophysical Behaviour of Macrocycles,' ed. I. Bernal, Elsevier, Amsterdam, 1987, Ch. 2.
14 E. Amble and J. Dale, *Acta. Chem. Scand., Ser. B*, 1979, **33**, 698.
15 R. R. Smith and A. E. Martell, 'Critical Stability Constants,' vol. 2, Plenum, New York, 1974.
16 L. J. Zompa, *Inorg. Chem.*, 1978, **17**, 2531.
17 A. P. Kengger, L. Hertli, and T. A. Kaden, *Helv. Chim. Acta*, 1978, **61**, 2296.
18 A. Bencini, A. Bianchi, A. Borselli, M. Ciampolini, M. Micheloni, N. Nardi, P. Paoli, B. Valtancoli, S. Chimichi, and P. Dapporto, *J. Chem. Soc., Chem. Commun.*, 1990, 174.
19 R. W. Alder, *Chem. Rev.*, 1989, **89**, 1215.
20 P. Gans, A. Sabatini, and A. Vacca, *J. Chem. Soc., Dalton Trans.*, 1985, 1196.

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