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THE PREPARATION AND PROPERTIES OF NEUTRAL DIAMIDE IONOPHORES FOR GROUP IIA METAL CATIONS

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Abstract—The preparation of a series of neutral ligands featuring ether and *N*-methyl-*N*-carbethoxypentylamide groups is described. These ligands as well as related ones bearing other diamide groups are shown to selectively chelate Group IIA cations by picrate extraction from water to methylene chloride. The changes in UV absorption of aromatic rings and amide groups in the ligands upon titration with metal salts in methanol allow the estimation of the stoichiometry of complexation and the ordering of cation binding. The observed selectivity sequences of cation extraction and binding are briefly discussed. Preliminary proton and ^{13}C NMR studies on the effect of addition of Group IIA cation salts to several of the ligands in methanol suggest that most of the complexation occurs at the central ether and amide groups. ^{13}C NMR T_1 changes by the Inversion Recovery Fourier Transform method are in agreement with the cation-induced shift data.

Naturally occurring macrocyclic and acyclic ionophores are involved in the selective transport of essential metal cations across biological membranes.[2a,b] Synthetic ionophores are of interest in that they provide model systems[2c] which can be varied greatly in structure. They can solubilize metal cations in lipid-like solvents and are useful in applications such as cation analysis, catalysis, organic synthesis and the study of the mechanisms of ion transport across membranes.

Previously, an acyclic 1,2-ethylenedioxydiacetamide system was found to show selective Group IIA cation complexation.[2c] It was recently reported that aromatic and alicyclic analogues of this system display a wider range of selective binding properties in ion sensitive electrodes.[1a] *N*-Methyl-*N*-carbethoxyundecylamides were originally used to enhance the lipophilic character of the potential ionophores and to give additional chelation sites. The Group IA and Group IIA cation selectivity of these ligands is somewhat less than that exhibited by the corresponding *N,N*-dipropylamides.[1a] The latter compounds are less soluble in the usual organic solvents than are the *N*-methyl-*N*-carbethoxyundecylamides and a compromise was sought via the use of the shorter and readily available *N*-methyl-*N*-carbethoxypentylamides **5–9V** which are prepared from the acid chlorides **1–4**. The ion selective behavior of **5–9V** is about the same as that of the *N,N*-dipropylamides **5–9PR** but their slight water solubility makes them less desirable for incorporation into liquid-membrane electrodes.[1b] The syntheses of the carbethoxypentylamides are described together with the complexation properties of these and related ligands using picrate extraction and differential UV spectroscopy.

RESULTS

The reaction of acid chlorides **1–4** with *N*-methyl-*N*-carbethoxypentylamine **10** gives the amides **5–9V**. Previously, the *N,N*-dipropylamides **5–9PR** had been prepared in the same manner.[1a]

We have also prepared the *N*-methyl-*N*-octadecyl amide **5XVIII** which can perhaps be more directly compared with the corresponding dipropyl amide than can the ω-carbethoxyalkyl amides. Octadecyl amides are expected to be more lipophilic than are dipropyl amides, and they may thus enhance cation transfer through membranes.

Extraction of metal cation picrates by the ligands

The extraction of metal cation picrates from water into an organic phase by a potential ligand provides a rapid screening of the selectivity of cation complexation of that ligand and a method for the comparison of the relative chelating abilities of different ligands.[4] Figure 1 (for **7PR**) illustrates the general appearance of the picrate extraction curve. Table 1 summarizes the relative extractability of cations by various ligands. The *cis* ligands **7PR** (Table 2) and **7V** (data not shown) behave similarly and extract cations in the order: $Ca^{2+} > Ba^{2+} \sim Mn^{2+} > Sr^{2+} \gg La^{3+} > Mg^{2+} > Na^{+}$. Thus the carbethoxypentyl group in **7V** did not enhance the ability of the ligand to extract cations. Both **7PR** and **7V** show a high M^{2+}/M^{+}

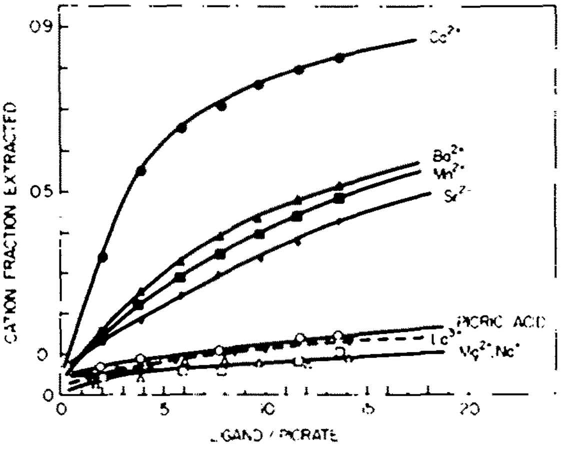
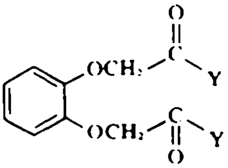
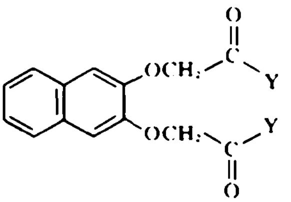


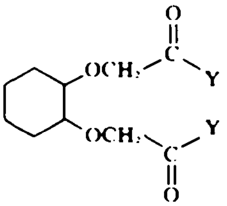
Fig. 1. The extraction of cation picrates from water to methylene chloride by **7PR** at cation concentration of 10^{-2} M (data of Table 1).



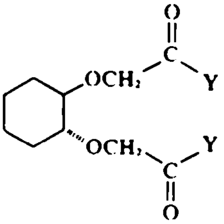
1 $Y = Cl$
5PR $Y = -N(C_2H_5)_2$
5V $Y = -\underset{\displaystyle CH_3}{\underset{|}{N}}(CH_2)_3CO_2C_2H_5$
5XVIII $Y = -\underset{\displaystyle CH_3}{\underset{|}{N}}(CH_2)_{17}CH_3$



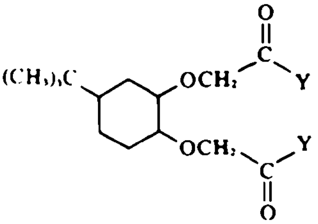
2 $Y = Cl$
6PR
6V



3 $Y = Cl$
7PR
7V



4 $Y = Cl$
8PR
8V



3a $Y = Cl$
9PR
9V

Table 1. Cation picrate extraction by ligands[a]

	Fraction of cation extracted					
Ligand	Ca^{2+}	Sr^{2+}	Ba^{2+}	Mg^{2+}	Mn^{2+}	Picric[b] Acid
7PR	0.84	0.43	0.50	0.15	0.50	0.14
8PR	0.37	0.15	0.19	0.05	0.50	0.06
7V	0.64	0.25	0.30	0.19	0.29	0.21
5PR	0.06	0.11	0.12	0.04	0.05	0.05
6PR	0.06	0.10	0.12	0.05	0.05	0.06
5XVIII	0.10	0.12	0.13	0.09	0.09	0.11
9PR	0.70	0.28	0.43	0.07	0.31	0.07

[a] Experimental procedures as described except: done between equal volumes (1.5 ml) of water and CH_2Cl_2 at 26°. Ligand = 7.0×10^{-4} M, picric acid = 6.86×10^{-5} M, cation = 1.0×10^{-2} M using MCl_2. The error limit is estimated to be less than 0.01 for extraction performed in triplicate.

[b] Fraction extracted = 0.04 without ligand. Done on a Cary 14 spectrophotometer.

selectivity even in methylene chloride (dielectric constant = 9). Such selectivity should be enhanced in solvents of higher polarity.[4] Incorporation of a *t*-butyl group into **7PR** produces a conformationally more rigid molecule; **9Pr** is somewhat less potent in its ability to extract Group II cations, but the selectivity sequence is retained. Thus some flexibility is apparently desirable in the *cis*-cyclohexanedioxy moiety. The *trans* isomer, **8PR**, extracts less Group IIA cations than the *cis*-isomer **7PR**. The 1,2-phenylenedioxy ligand **5PR** and the naphthalenedioxy ligand **6PR** extract cations to a lesser extent than **8PR** and do so in the order: $Ba^{2+} \sim Sr^{2+} > Ca^{2+} > Mg^{2+} \sim Mn^{2+}$.

The *trans*-ligand **8V** extracts cations in the order: $Mn^{2+} > Ca^{2+} > Ba^{2+} > Sr^{2+} > Mg^{2+} > Na^{+}$ (Table 3). The large extent of Mn^{2+} extraction is interesting since Mn^{2+} has been suggested as a substitute cation for Ca^{2+} (radius 1.00 Å) even though its ionic radius (0.80 Å) is smaller.[6] Ligands **7PR** and **7V** extract Ca^{2+} much more effectively

Table 2. Cation picrate extraction by **7PR**[a]

	Fraction of cation extracted							
Ligand/picric acid ratio	Ca^{2+}	Ba^{2+}	Mn^{2+}	Sr^{2+}	Mg^{2+}	Na^{+}	La^{3+}	Picric[b] acid
1.95	0.33	0.15	0.14	0.11	0.05	0.03	0.05	0.06
3.90	0.55	0.25	0.22	0.18	0.05	0.04	0.065	0.08
5.85	0.65	0.32	0.29	0.24	0.06	0.06	0.08	0.10
7.80	0.71	0.39	0.34	0.30	0.065	0.07	0.10	0.10
9.75	0.76	0.43	0.39	0.33	0.07	0.075	0.12	0.13
11.70	0.80	0.48	0.44	0.37	0.08	0.08	0.13	0.13
13.65	0.83	0.51	0.49	0.43	0.10	0.09	0.13	0.13

[a] Between equal volumes of water and CH_2Cl_2. Picric acid = 7.17×10^{-5} M, cation = 10^{-2} M using MCl_2.

[b] Fraction extracted = 0.04 without ligand.

Table 3. Cation picrate extraction by **8V**[a]

Ligand/picric acid ratio	Fraction of cation extracted					
	Mn^{2+}	Ca^{2+}	Ba^{2+}	Sr^{2+}	acid[b]	Mg^{2+}
2.00	0.07	0.07	0.05	0.05	0.04	0.04
4.01	0.11	0.11	0.08	0.06	0.04	0.04
6.00	0.15	0.12	0.08	0.07	0.05	0.04
8.02	0.19	0.15	0.09	0.08	0.05	0.04
10.02	0.21	0.18	0.10	0.08	0.05	0.04
12.02	0.25	0.21	0.11	0.10	0.05	0.04
14.03	0.29	0.23	0.12	0.11	0.05	0.04

[a] As for Table 2.

[b] Fraction extracted - 0.04 without ligand.

(76–85% of the total Ca^{2+} is extracted by 10–16 fold excess of ligand to picric acid at 10^{-2} M cation) than does dicyclohexyl-18-crown-6 (**11**, 10% of Ca^{2+} extracted by a 20-fold excess of ligand to picric acid at 10^{-2} M cation). Our ligands are generally selective for the complexation of the Group IIA cations while the crown ethers complex both Group IA and IIA cations.[7]

In picrate extraction measurements, the assumption is made that only picrate anions are transferred and not Cl^- which is used in the *in situ* preparation of the metal cation picrates. This was shown to be valid for the extraction of monovalent cation picrates with the actins by Eisenman *et al.*[4a] Data for divalent cation picrates have not been available. Since the estimate of the extent of cation transfer could be complicated by the transfer of picric acid, we measured the extent of picric acid transfer by each ligand. Although the transfer of picric acid is not insignificant, it is occasionally somewhat higher than the transfer of some picrates such as magnesium picrate (see Tables 2 and 3). This suggests that our ligands have affinity for protons as has been shown in ion-selective membrane measurements.[1a] The following data helped to validate the use of the picrate extraction method for divalent cations.

The transfer of $^{45}CaCl_2$ from water to methylene chloride without picric acid by **7PR** was less than 5% of that obtained in the presence of picric acid (see Experimental and Table 4). In the presence of picric acid, such extractions gave picrate/Ca^{2+} transfer ratios of 2.0. Furthermore, the percent of Ca^{2+} transferred by **7PR** with calcium picrate alone or with calcium picrate-$CaCl_2$ containing varying amounts of excess Cl^- varied by only 3%. Much larger variation is expected if Cl^- were transferred. Thus the use of picrate transfer to estimate the amount of divalent cation transfer in our systems seems to be justified.†

The complexation of various cations with **5PR** was studied in anhydrous methanol, a solvent for which there is much data on the complexation of crown ethers and other molecules.[8,9] The single broad absorption of **5PR** at 272–273 nm changes to a doublet at 269, 273 nm upon its complexation with various cations (Fig. 2), a situation reminiscent of the behavior of dibenzo-18-crown-6 and other catechol-derived crown ethers.[9a] A qualitative estimate of the extent of complexation of **5PR** with various salts is obtained by the method of Pedersen.[9a] Thus, the UV spectrum of **5PR** is taken in the presence of a large (usually fifty-fold) excess of the salt in anhydrous methanol so as to maximize the possibility of complexation. Although different anions are used, both our data and the crown ether work[9a] suggest that anion differences are negligible as long as the salt is soluble in methanol and does not itself absorb in the 275 nm region. The following cations form complexes: Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Cd^{2+}, Na^+, La^{3+}, Mn^{2+} and Cu^{2+}. Weak complexation of **5PR** is found with Co^{2+} and Al^{3+} and very weak complexation with Li^+, K^+ and Ni^{2+}. No complexation is found with NH_4^+, Zn^{2+} or Hg^{2+}. The effect of water on some of these complexes can be determined by comparing the above results with those obtained in 95%

Table 4. Two-phase extraction of $^{45}Ca^{2+}$ by **7PR** with and without picric acid

Conditions	Conc. **7PR**	Total $^{45}Ca^{2+}$	Species transferred[a]		
			Picrate	Net $^{45}Ca^{2+}$ [b]	Mole ratio Pic$^-$/$^{45}Ca^{2+}$
A. with	1.51×10^{-3} M	1.76×10^{-6} mol	3.44×10^{-6} mol	1.66×10^{-6} mol	2.07
picric	3.02×10^{-3}	2.60×10^{-6}	4.71×10^{-6}	2.52×10^{-6}	1.87
acid	6.04×10^{-3}	2.99×10^{-6}	5.65×10^{-6}	2.90×10^{-6}	1.95
B. without	1.51×10^{-3} M	0.098×10^{-6} mol			
picric	3.02×10^{-3} M	0.080×10^{-6}			
acid	6.04×10^{-3}	0.087×10^{-6}			

[a] From water to methylene chloride.

[b] Obtained by A-B.

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†We attempted to calculate extraction equilibrium constants using the method of Haynes and Pressman,[4c] modified for the equilibria involved in the extraction of divalent cations. The treatment gave upward curves instead of straight lines. It was suggested that this may be explained by a transition of extraction mechanisms from one involving cation-ionophore-anion$^+$ + anion$^-$ at lower ionophore concentrations to one involving cation-ionophore-(anion)$_2$ at higher ionophore concentrations.[8] Therefore it was decided to delay the estimation of picrate extraction equilibrium constants until more detailed work could be done in the future.

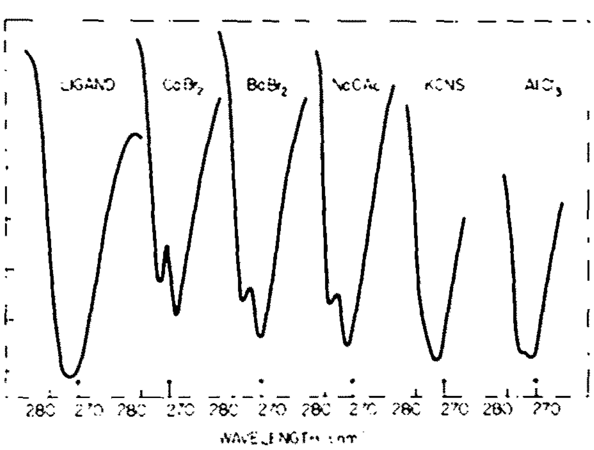


Fig. 2. Changes in UV absorption of **5PR** (3.4×10^{-4} M in methanol) upon the addition of 50-fold excess of the indicated salt, using a Cary 118 spectrophotometer.

ethanol. In the presence of 5% water, the Ca^{2+}, Sr^{2+} and Cd^{2+} complexes remain stable, the Ba^{2+} and Na^+ complexes are partially decomposed, while Mg^{2+} does not complex with **5PR** at all.

Further data on the relative stability of isolable preformed complexes of **5PR** to water are given in Table 5. The ordering is: $Ca^{2+} > Sr^{2+} > Ba^{2+} \gg Mg^{2+}$. More detailed information can be obtained by the addition of incremental amounts of a salt to **5PR**. It is found that the spectrum of the complex forms fully at 0.9–1.2 equivalents of salt with no further significant change for the calcium, strontium and barium complexes (Fig. 3). The sodium complex starts to form at 10:1 (salt/ligand) and does not fully form until 40 equivalents of NaI have been added.

Differential UV spectroscopy was utilized to obtain the stoichiometry of complexation.[10] In this method, ligand is initially present in both beams of the spectrophotometer and the differential spectrum of the complex is recorded as a function of added increments of a salt to the sample cell until there is no further change in the UV spectrum (see Fig. 4). The data thus obtained for **5PR** and **6PR** were analysed by the mole-ratio method[11] to give the stoichiometry of chelation. It was thus found that **5PR** or **6PR** gave 1:1 stoichiometry with the bromides of calcium, strontium, or barium in methanol if the concentration of ligand was kept at a maximum value of 5×10^{-4} M. The stoichiometry of complexation of **5PR** with lanthanum tribromide was 2 ligand: 1 cation. In order to estimate formation constants

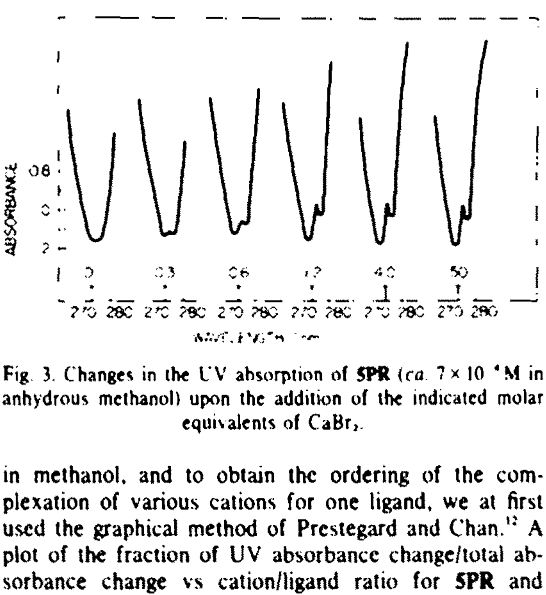


Fig. 3. Changes in the UV absorption of **5PR** (*ca.* 7×10^{-4} M in anhydrous methanol) upon the addition of the indicated molar equivalents of $CaBr_2$.

in methanol, and to obtain the ordering of the complexation of various cations for one ligand, we at first used the graphical method of Prestegard and Chan.[12] A plot of the fraction of UV absorbance change/total absorbance change vs cation/ligand ratio for **5PR** and $CaBr_2$ at a ligand concentration of 9×10^{-5} M generated a smooth curve with all of the data points within the theoretical 1:1 limit[12] and a K_f of 1.1×10^5 M^{-1}. Higher concentration of **5PR** gave the initial parts of the curve at the limit (at 5×10^{-4} M, Fig. 5) or outside of the limit (at 6.67×10^{-4} M), suggesting the presence of complexes of higher stoichiometry (2:1, etc.), especially at the beginning of the cation addition, when the excess of ligand is

Table 5. Stability of complexes of **5PR** and **6PR** in methanol toward water

Complex[a]	Behavior in methanol-water mixture
5PR. $MgBr_2$	Decomposes completely with 3.0% water
5PR. $CaBr_2$ or $Ca(SCN)_2 \cdot 4H_2O$	Decomposes with 30–40% water. Some complexation still present in 50% water (by differential UV)
5PR. $SrBr_2$	Decomposes with 40% water. Slight amount of complexation left.
5PR. $BaBr_2$	Decomposes completely with 30% water.
6PR. $CaBr_2$	Decomposes completely with 35% water.
6PR. $Ba(SCN)_2 \cdot 2H_2O$	Decomposes completely with 10% water.
6PR. $BaBr_2$	Decomposes completely with 30% water.

[a]Either isolated or judged present in solution by the differential UV method. CH_3OH-H_2O mixtures were prepared at constant complex concentration for proper UV spectral comparison.

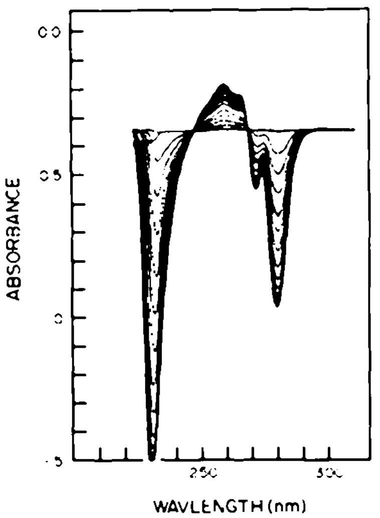


Fig. 4. Differential UV titration of **5PR** (6.67×10^{-4} M in anhydrous methanol) with 0.1 molar equivalents of $CaBr_2$ until no further change is found (5.1-fold excess).

at a maximum.[13] Both **5PR** (at 5×10^{-4} M, Fig. 5) and **6PR** (at 4×10^{-4} M) give complexation ordering with the respective bromides of: $Ca^{2+} \gg Sr^{2+} > Ba^{2+}$. Formation constants for all of these cases could be calculated by computer estimation of "eta" values as previously defined.[12] These K_f values, however, were all 20–30% higher than those calculated at lower concentrations using Scatchard plots, and they are presumably less accurate.

The presence of 3% water lowered the K_f for **5PR** + $CaBr_2$ by *ca.* 60% (from 1.1×10^5 M^{-1} to *ca.* 4×10^4 M^{-1}), beyond the error of the method which is estimated to be 20–30%. All of the K_f values are "apparent" since activity coefficients of $CaBr_2$ (and the other salts) are unknown in methanol.[14]

The possible ambiguities of stoichiometry encountered in the above studies as well as theoretical considerations[15] encouraged the use of more dilute solutions. The ligands **5PR** and **6PR** give 1:1 stoichiometry with ligand concentration at or below 2.45×10^{-4} M, whereas the ligands **7PR** and **8PR** give more complicated behavior.[16]

The importance of the basic system featuring two oxygens and two amide groups to the complexation ability of our systems is illustrated by the following data. No complexation of $CaBr_2$ in methanol (as judged by the UV spectroscopy technique utilizing a large excess of salt) is given by catechol, dimethyl 1,2-phenylenedioxydiacetate, 1,2-phenylenedioxydiacetonitrile or phenoxyacetic acid *N,N*-dipropylamide. Diethyl *trans*-1,2-cyclohexanedioxydiacetate in methylene chloride does not extract calcium picrate from water while cyclohexyloxyacetic acid *N,N*-dipropylamide extracts only a very small amount of calcium picrate.

The addition of the halides or thiocyanates of the Group IIA cations to our ligands cause induced proton and ^{13}C NMR shifts. Such cation-induced shifts can be used to estimate complexation sites.[17a] Our preliminary results[17b] show, for example, that the addition of $CaCl_2$ (0.9 equivalents) to **7V** and **8V** in methanol causes mainly downfield shifts of varying magnitude. These range from very little (0 or 0.01 ppm) at the ethyl protons to larger shifts (− 0.47 ppm) at the methine and methylene protons adjacent to the ether oxygens. The shifts are gradual and can be followed by incremental addition of the salts to the ligand in methanol-d_4 or deuterochloroform. Similarly, the fully proton-decoupled ^{13}C NMR spectra of **7V** and **8V** show cation-induced shifts which are largest for the carbons of the cyclohexane ring and the *N*-methyls of the amide groups. The relatively larger shifts around the ether and amide groups, as compared with the carbethoxypentyl groups, suggest that there is more cation complexation at the former sites.[13]

Another approach to the study of complexation sites on a ligand is the determination of the effect of the complexation of a ligand upon the spin lattice relaxation times (T_1) of the individual carbons. The T_1 values for all of the carbons of **7V** were determined before and after the addition of calcium or barium thiocyanate (two equivalents) by the Inversion Recovery Fourier Transform (IRFT) method.[18] As shown in Table 6, the overall effect of complexation is a general reduction of the T_1 values for each of the carbons in both complexes compared to the corresponding carbon in the free ligand. This anticipated result can be attributed to two factors. There is an increase in viscosity of the solution upon salt addition.[18b] This is known to increase the correlation time (τ_c) required for the rotation of the molecule for carbons which relax by dipole–dipole (DD) relaxation mechanisms. Since T_1 (DD) is inversely related to τ_c, this effect results in shorter T_1 values. Complexation with a metal cation gives a ligand more "bulk" which also serves to decrease the rate of rotational motion, and increase τ_c, since the heavier complex tumbles more slowly than the lighter ligand.[18]

It is significant that the T_1 values of some of the

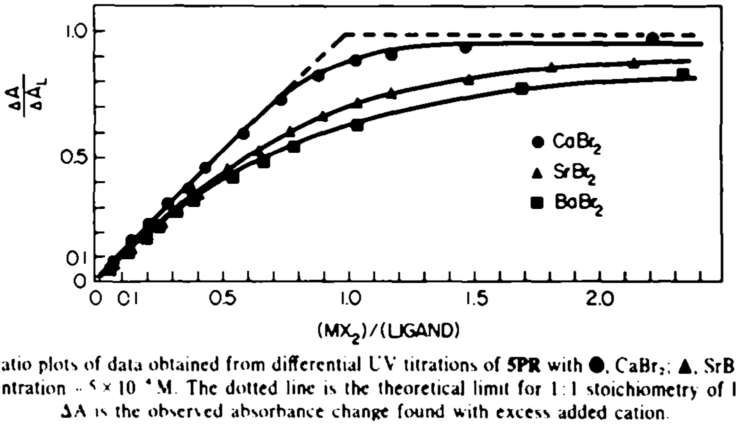
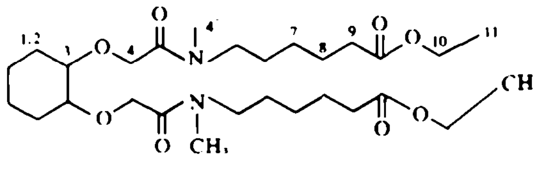


Fig. 5. Mole-ratio plots of data obtained from differential UV titrations of **5PR** with ●, $CaBr_2$; ▲, $SrBr_2$; ■, $BaBr_2$. Ligand concentration = 5×10^{-4} M. The dotted line is the theoretical limit for 1:1 stoichiometry of ligand/cation. ΔA is the observed absorbance change found with excess added cation.

Table 6. ^{13}C Spin-lattice relaxation times and ratios of T_1 values for 7V and its Ca^{2+} and Ba^{2+} complexes in methanol[a]



7v

Carbons:	1,2	3	4	4'	7	8	9	10	11
				T_1 values (s)					
Free ligand	0.35	0.41	0.55	2.50	0.73	1.01	1.31	3.80	3.72
Ligand + Ca^{2+} added (2 equiv)	0.09	0.09	0.13	0.50	0.27	0.37	0.53	1.50	1.79
Ligand + Ba^{2+} added (2 equiv)	0.09	0.11	0.13	0.62	0.18	0.28	0.45	1.24	1.61
				Ratio of T_1 values					
Lig/Lig + Ca^{2+}	3.9	4.7	4.2	5.0	2.7	2.7	2.5	2.5	2.1
Lig/Lig + Ba^{2+}	4.0	3.9	4.3	4.0	4.1	3.7	2.9	3.1	2.3

[a] Ligand (0.5 M) repetition time 15 sec.; 128 scans; pulse interval times; infinity, 6.5, 3.0, 1.0, 0.5, 0.25, 0.1 sec. Complexes repetition times: 8 sec., 256 scans; interval times: infinity, 3.0, 1.5, 1.0, 0.5, 0.2, 0.1, 0.01 sec. T_1 values calculated from semilog plots of $A_0 - A$ vs pulse interval time.[17b,21]

carbons in 7V decrease more than do others (Table 6). Thus the addition of $Ca(SCN)_2$ to 7V causes a decrease of four or five fold in the T_1 values of carbons next to the ether oxygens, the N-methyl carbons and the ring methylenes. The other T_1 values are decreased only two or three fold. It is believed that the changes in T_1 values for carbons 4 and 4' arise from the change in the degree of segmental motion[19] resulting from complexation with calcium. During complexation the greatest restriction to segmental motion is expected to occur at or near those sites of ion-dipole interaction. This results in the "anchoring" of the chain at these sites, effectively restricting segmental motion. The result is enhanced shortening of the T_1 values at these sites, in addition to shortening caused by greater viscosity and bulk. The effect of this anchoring decreases down the chain, going from a factor of 2.7 at carbon 7 to 2.5 at carbon 9 to 2.1 at the terminal methyl. The continuous and consistent decrease in T_1 ratios suggests that the restriction of segmental motion as a result of complex formation is smaller farther away from the ring. Thus, the results again suggest that complexation under these conditions does not involve the ester group.

The barium complex shows similar results but the overall effect on T_1 values along the chain is greater than in the calcium complex. This suggests that there is greater restriction to segmental motion in the barium than in the calcium complex. The larger barium cation sterically hinders the rotation of the side chain more than does the smaller calcium cation, assuming the same stoichiometry of binding for both cations.† The reasons for the large change in T_1 values for the ring methylenes upon complexation are not fully understood but presumably include restriction of motion.

The overall changes in T_1 values of 2.1–5.0 are less in methanol-d_4 than they would be in a non-hydrogen bonding solvent.[18c,19b,20] T_1 values for the carbonyl carbons are not included since the relatively long relaxation times of such carbons (10–30 s)[18c,20] precluded their inclusion in our study.

In summary, all of the NMR methods used suggest that the major part of the complexation of 7V or 8V with calcium or barium cations in methanol occurs at the ether oxygens and amide carbonyls with very little complexation at the ester groups. Further work on the detailed nature of the NMR changes for our ligands upon interaction with various cations is in progress.

DISCUSSION

The extraction of cation picrates is mainly useful for the qualitative comparison of the relative ability of different ligands to transfer cations from water to an organic phase and for the relative ordering of cation transfer for a given ligand. The aromatic ring-containing ligands, **5PR** and **6PR**, transfer the Group IIA cations in the order: $Ba^{2+} \sim Sr^{2+} > Ca^{2+} > Mg^{2+}$, the same ordering found for their selectivity when incorporated in ion-selective liquid membrane electrodes by Ammann *et al.*[3e] A rationalization for this selectivity sequence is that it is related in an inverse manner to the free energies of hydration of the cations,[22] i.e. the most strongly hydrated cation, Mg^{2+}, is the most difficult to transfer from water to an organic phase. Furthermore, this ordering is generally found for the binding of these cations to large anions (usually of strong acids); the larger cations can pack larger anions around themselves more easily.[6] Perhaps such an argument can be extended to our chelated cations with the relatively large picrate counter ions. The situation is more complicated, however, for the ligands **7PR** and **8PR** which transfer: $Ca^{2+} > Ba^{2+} > Sr^{2+}$ and $Ca^{2+} > Ba^{2+}, Sr^{2+}$, respectively, but show ion-selective electrode behavior[2a] in the ordering: $Ca^{2+} > Sr^{2+} > Ba^{2+} \gg Mg^{2+}$ for both ligands. Among the reasons for the non-correspondence of the ordering obtained by these different methods which

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† Steric effects, for example, have been used to explain why the methyl group in 9-methylanthracene (no preferred conformation, $T_1 \sim 14$ s) has a larger T_1 value than the methyl group in 1-methylnaphthalene ($T_1 = 5.8$ s) which is forced into a preferred conformation by the *peri*-hydrogen.[18d]

involve organic phase/water interfaces may be the differing polarities of the organic phase, the different stoichiometries of binding that probably pertain in these methods,[23] and the influence of ion-pairing especially in the picrate method.[4a]

Assuming the yet unproven complexation of amide oxygen rather than nitrogen, the "cavity" formed by the ether oxygens and amide oxygens in a square planar array is measured by CPK models to be 2.4 Å for **5PR** and 2.0–2.2 Å for **7PR** and **8PR**. The flexible nature of the "cavity" observed does not allow firm conclusions.

It is of interest that the *cis*-ligand **7PR** transfers more cation picrate than does the *trans*-ligand **8PR**. Too rigid a structure causes a small decrease in the amount and selectivity of cation transfer (compare **7PR** with its 4-*tert*-butyl derivative **9PR**). As anticipated, the aromatic ring-containing ligands **5PR** and **6PR**, which have less basic ether oxygens, transfer much less cation picrate. A similar situation occurs with dibenzo-18-crown-6 vs dicyclohexyl-18-crown-6.[4b]

The stability of preformed complexes of **5PR** to water is in the ordering: $Ca^{2+} > Sr^{2+} > Ba^{2+} \gg Mg^{2+}$. This stability ordering is also found for the complexation of the cation dibromides in anhydrous methanol with **5PR** and **6PR** where the stoichiometry is 1:1. Our results indicate that this ordering also holds for **7PR** although the stoichiometry of binding is not 1:1 but more complicated. This ordering may indicate a field strength effect (stronger binding by the smaller and more intensely charged ions) with deviation for Mg^{2+} because of its small radius which does not allow efficient "packing" of all of the necessary chelation sites (the "radius ratio" effect[6]). It is interesting that the ordering $Ca^{2+} > Sr^{2+} > Ba^{2+} \gg Mg^{2+}$ is found for proteins such as troponin,[6] extra-cellular enzymes,[6] ligands such as 2,2′-ethylenedioxy-bis-[ethyl-(iminodiacetate)],[6] and for 3-oxadiacetic acid.[24] However, it is not yet possible to predict ordering or to form general conclusions about the ordering for these diamide ligands. We note that Kirsch and Simon[23] have obtained other ordering sequences for their acyclic ionophores[2c,3a] which are not that structurally different from **7PR** or **8PR**.

In summary, our new class of ethylenedioxydiacetamide ligands have complexation selectivity for Group IIA cations vs many others cations. The ordering of selectivity within Group IIA, as determined by binding studies in methanol, follows the sequence found for troponin and other peptides.

EXPERIMENTAL

Most of the solvents used were dried by distillation over phosphorus pentoxide, calcium hydride or lithium aluminum hydride. Organic solutions were dried over magnesium sulfate. ^1H NMR spectra were recorded on a Varian A-60A spectrometer at 60 MHz, using TMS as an internal standard. Mass spectra were taken on a DuPont CEC 21–492 mass spectrometer. Infrared spectra were recorded on Perkin Elmer 300 or 467 spectrophotometers. UV spectra were recorded on Perkin Elmer 402 or Cary 14 spectrophotometers in anhydrous methanol or as otherwise indicated. TLC was done on Brinkmann silica gel HF_{254} or Eastman Kodak silica gel and alumina precoated plates. The solvents were diethyl ether, chloroform, triethylamine (solvent A) or diethyl ether, benzene, triethylamine (solvent B) in 1:1:1 ratio (by volumes). M.ps were taken on a Thomas–Hoover "Unimelt" apparatus and are uncorrected, as are the boiling points.

N-*Methylcaprolactam* **12** was obtained in 75% yield by the reaction of caprolactam with dimethyl sulfate (1.3 equivalents) in benzene at reflux for 16 h, followed by conversion of the resultant *O*- and *N*-methylated products to **12** by further reflux for 6 h after partial purification: b.p. 105° (10 mm) [lit.[25] b.p. 120° (19 mm)]; IR (neat) 1630 cm^{-1}; NMR ($CDCl_3$) δ 1.5–1.57 (*m*, 6, CH_2), 2.3–2.5 (m, 2, N–CH_2), 2.9 (s, 3, CH_3), 3.2–3.4 (m, 2, CH_2C-0).

N-*Methyl*-N-5-*carbethoxypentylamine*. HCl **10**. Reaction of **12** with conc HCl at reflux for 28 h, followed by a benzene azeotrope to remove water and partial removal of solvent gave N-*methyl*-N-5-carboxypentylamine. HCl in 87% yield: m.p. 84–86°; IR (nujol) 1710 cm^{-1}. Reaction of the acid with HCl gas in ethanol at 5° for 1.5 h gave **10** in 81% yield: m.p. 87–91°; IR (Nujol) 1740 cm^{-1}; MS (70 eV) *m/e* 173 (M^+–HCl), 158, 129, 128, 101, 100, 86, 73, 69, 58, 44; NMR (DMSO-d_6) δ 1.2 (t, 3, CH_3CH_2), 1.1–2.0 (m, 6, CH_2), 2.3 (t, 2, NCH_2), 2.5 (s, 3, CH_3N), 2.8 (m, 2, OC(=O)CH_2), 4.1 (q, 2, CH_2CH_3).

1,2-*Phenylenedioxydiacetic acid* N - *methyl* - N - 5 - *carbethoxypentylamide* **5V** (83%) was obtained as a thick oil from the reaction of 1,2-phenylenedioxydiacetyl chloride[3a] with *N* - methyl - *N* - 5 - carbethoxypentylamine hydrochloride (two equivalents) and triethylamine (four equivalents) in CH_2Cl_2 at 4–6° (ice-bath) over 30–50 min with stirring. In a general procedure, solvent was removed after 1–2.5 h, triethylamine hydrochloride was precipitated with diethyl ether, and the filtrate was concentrated to give the product. Any special treatment is described. For **5V**: IR (neat) 1640, 1710 cm^{-1}; NMR ($CDCl_3$) δ 6.90 (s, 4, aryl), 4.75 (s, 4, OCH_2C=O), 4.08 (q, 4, CH_2CH_3), 3.4 (br t, 4, NCH_2), 2.9, 3.03 (2s, 6 NCH_3), 2.15, 2.30 (2s, 4, CH_2C=O), 1.4 (m, 12), 1.2 (t, 6, CH_3CH_2); TLC R_f 0.35 ($CHCl_3$: $(C_2H_5)_2O$: $(C_2H_5)_3N$, 1:1:1, solvent A); MS (70 eV) *m/e* (rel intensity) 536 (M^+, 9), 491 (17), 336 (100), 306 (3), 214 (67), 200 (20), 186 (35), 172 (65). Calc. for $C_{28}H_{44}N_2O_8$: 536.31094. Found: 536.31067; UV max (95% C_2H_5OH) 272 nm (ε 1720).

cis - 1,2 - *Cyclohexanedioxydiacetic acid* N-*methyl*-N-5-*carbethoxypentylamide* **7V**. Liquid, 85% as above: IR (neat) 1730, 1640 cm^{-1}; NMR (CCl_4) δ 1.2 (t, 6, CH_3CH_2), 0.9–1.8 (m, 20), 2.2 (t, 4, CH_2C=O), 2.9 (d, 6, NCH_3), 3.3 (m, 4, CH_2N), 4.15 (s, 4, CH_2O), 4.1 (q, 4, CH_2CH_3), 3.55 (m, 2, CH–O); MS (70 eV) *m/e* (rel intensity) Calc. for $C_{28}H_{50}N_2O_8$: 542.3567. Found: 542.3572; 542 (M^+, 7), 497 (8), 342 (4), 231 (100), 200 (28), 186 (30), 172 (36), 129 (53); TLC R_f 0.5 (solvent B).

trans - 1,2 - *Cyclohexanedioxydiacetic acid* N - *methyl* - N - 5 - *carbethoxypentylamide* **8V**. Liquid 85% from *trans* - 1,2 - cyclohexanedioxydiacetyl chloride[3a] as above; IR (neat) 1730, 1640 cm^{-1}; NMR (CCl_4) δ 1.2 (t, 6, CH_3CH_2), 0.9–1.2 (m, 20), 2.2 (t, 4, CH_2C=O), 3.0 (d, 6, NCH_3), 3.4 (m, 4, CH_2N), 4.15 (S, 4, CH_2O), 4.1 (q, 4, CH_2CH_3), 3.6 (m, 2, CH–O); MS (70 eV) *m/e* (rel intensity) Calc. for $C_{28}H_{50}N_2O_8$: 542.3567. Found: 542.3572; 542 (M^+, 6), 497 (16), 342 (9), 328 (44), 231 (100), 200 (32), 186 (30), 172 (30), 129 (44); TLC R_f 0.45 (solvent B).

2,3 - *Naphthalenedioxydiacetic acid* N - *methyl* - N - 5 - *carbethoxypentylamide* **6V**. Obtained as a brown oil (1.3 g, from 2,3-naphthalenedioxydiacetyl chloride[3a] as above) which was chromatographed on silica gel (50 g) and eluted with diethyl ether: triethylamine: benzene (1:1:1-solvent B) to give 1.2 g (77% of liquid **6V**: TLC R_f 0.35 (solvent B); IR (neat) 1730, 1650 cm^{-1}; NMR (CCl_4) δ 1.2 (t, 6, CH_3CH_2), 0.8–1.8 (m, 12), 2.1 (m, 4, CH_2C=O), 2.9 (d, 6, NCH_3), 3.2 (m, 4, NCH_2), 4.0 (q, 4, CH_2CH_3), 4.7 (s, 4, OCH_2), 7.0–7.8 (m, 6, aryl); MS (70 eV) *m/e* (rel intensity) 586 (M^+, 14), 541 (14), 386 (100), 214 (60), 185 (52), 172 (36), 129 (55), 115 (26), 87 (88), calc. for $C_{32}H_{46}N_2O_8$ 586.3254, found 486.3234.

cis - 4 - t - *Butyl* - cis - 1,2 - *cyclohexanedioxydiacetic acid* N - *methyl* - N - 5 - *carbethoxypentylamide* **9V**. Liquid, 41% from the reaction of *cis* - 4 - *t* - butyl - *cis* - 1,2 - cyclohexanedioxydiacetyl chloride **3a** and *N* - methyl - *N* - 5 - carbethoxypentylamine hydrochloride as for **7V**: TLC R_f 0.5 (solvent B); IR (neat) 1730, 1640 cm^{-1}; NMR (CCl_4) δ 0.9 (s, 9, *t*-butyl H), 1.2 (t, 4, CH_3CH_2), 0.8–2.1 (m, 19), 2.3 (t, 4, CH_2C=O), 2.9 (d, 6, NCH_3), 3.8 (m, 2, OCH), 3.3 (m, 4, NCH_2), 4.07 (q, 4, CH_2CH_3), 4.0, 4.12 (2s, 4, OCH_2) MS (70 eV) *m/e* (rel intensity 598 (m^+, 11), 583 (4), 553 (15), 455 (6), 398 (4), 384 (16), 340 (11), 310 (72), 244 (16), 232 (100), 230 (96), 215 (27), 200 (76), 186 (36), 172 (47), 160 (54), 154 (19), 128 (92); Calc. for $C_{32}H_{58}N_2O_8$: 598.4193. Found: 598.4182.

1,2 - *Phenylenedioxydiacetic acid* N - *methyl* - N - *octadecylamide* **5XVIII**. Obtained in 24% yield as above from 1, *N*-methyloctadecylamine (2.0 equivalents), and triethylamine (2.5 equivalents). After removal of most of the triethylamine hydrochloride, the product (which has a tendency to gel) was washed with 9% HCl, water and dried to give a pale yellow solid, m.p. 49–52°, which was recrystallized from benzene and ethanol to give a white solid: m.p.: 55–58°; IR (Nujol) 1680, 1700 cm^{-1}; TLC (EtOAc) 1 spot (R_f 0.4); NMR (CCl_4) δ 0.9 (t, 6, CH_3), 1.1–1.4 (m, 64, CH_2) 2.9, 3.1 (d, 6, CH_3N), 3.3 (br s, 4, CH_2N), 4.65 (s, 4, $OCH_2C{=}O$), 7.0 (s, 4, aryl). Anal. Calc. for $C_{48}H_{88}N_2O_4$: C, 76.13; H, 11.71; N, 3.70. Found: C, 76.37; H, 11.90; N, 3.64.

Picrate extraction procedures

The method was essentially that of Frensdorff.[4b] Aqueous solutions were made up from standardized stock solutions of NaOH, KOH and other inorganic reagents and picric acid. Calcium picrate, for example, was prepared either by neutralizing picric acid with $Ca(OH)_2$ at 7×10^{-3}M, with $CaCl_2$ added to a final [Ca^{2+}] of 10^{-2}M, or by adding excess $CaCl_2$ to picric acid. The ionophores were dissolved in methylene chloride. Equal volumes of the two solutions in stoppered centrifuge tubes were mixed in a multi-purpose rotator at 80 rpm for about 5 min to ensure complete equilibration. Centrifugation was needed for complete phase separation. The extractions were conducted at 25 ± 1.0°. Equilibrium picrate concentrations in both phases were determined with a Perkin Elmer 402 spectrophotometer. The two methods of determining picrate in the organic phase (the direct value and the value obtained by difference from the aqueous phase) agreed well and the organic phase value was usually used. Picrate extinction coefficients in water (14500 M^{-1} cm^{-1} at 354 nm) or methylene chloride (18000 M^{-1} cm^{-1} at 370 nm) were determined in separate runs. Extraction of sodium picrate (from NaOH-picric acid) with CH_2Cl_2 gave no detectable picrate absorption in the organic phase. (see data in Tables 1–3).

The data in Table 4 were obtained using test tubes containing an aqueous solution of 7.0×10^{-3} M picric acid and 1.0×10^{-4} M $CaCl_2$ with a trace amount of $^{45}CaCl_2$ (1.0 ml) to which was added methylene chloride (1.0 ml, with or without dissolved **7PR**). The test tubes were capped, vortexed vigorously, rotated at 40 rpm for 15 min, and then centrifuged using a table-top centrifuge for *ca.* 5 min. Aliquots of the aqueous layer were taken for measurement of picrate absorbance at 356 nm with a Cary 14 spectrophotometer and for radioactivity counting with a Beckman LS 250 liquid scintillation counter. The net amount of Ca^{2+} transferred to the organic phase in the presence of picric acid was obtained by subtracting the amount of Ca^{2+} transferred in the absence of picric acid from the amount of Ca^{2+} transferred in its presence (see Table 4).

Titration methods

Anhydrous $CaBr_2$, $SrBr_2$, $BaBr_2$ and $MgBr_2$ (ROC/RIC) and other salts were used as received and kept under nitrogen in a desiccator. For qualitative studies and the determination of stoichiometry of binding by mole-ratio methods (Fig. 3–5), ligand solutions in anhydrous methanol were prepared by diluting a 0.1 M stock solution to the expected suitable concentration. For the differential spectra (Fig. 4), ligand solution was placed in each 1-cm cuvet. Cation solution (0.01 M, 0.1 M or 1.0 M) was added via a 10-μl syringe so that volume changes could be neglected. After each addition, the cuvet was thoroughly shaken before the UV spectrum was recorded with a Perkin-Elmer 402 spectrophotometer. Addition was to the reference cell for convenience since the ligand cation complexes have lower absorbance than the ligand in the 280 nm wavelength region. For the data in Fig. 2, 50-fold excess of salts was used directly. Mole-ratio plots (as in Fig. 5) were constructed according to Prestegard and Chan.[12] Estimates of the apparent K_f values were done by comparing the experimental curves with those generated by an IBM 1130 computer for different "eta" values.[15]

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REFERENCES

[1a] Yeshiva University, [b] Queens College, [c] Ramapo College.

[2a] P. B. Chock and E. O. Titus, *Progress in Inorg. Chem.* (Edited by S. J. Lippard), Vol. 18, pp. 287–382. Wiley, New York (1973); [b] V. Prelog, *Pure Appl. Chem.* **25**, 197 (1971); [c] W. Simon, W. E. Morf and P. Ch. Meier, *Structure and Bonding*, Vol. 16, pp. 113–160. Springer Verlag, New York (1973).

[3a] D. Ammann, R. Bissig, M. Güggi, E. Pretsch, W. Simon, I. J. Borowitz and L. Weiss, *Helv. Chim. Acta* **58**, 1535 (1975); [b] W. Simon, ETH Zurich, private communication.

[4a] G. Eisenman, S. Ciani and G. Szabo, *J. Memb. Biol.* **1**, 294 (1969); [b] H. K. Frensdorff, *J. Am. Chem. Soc.* **93**, 4684 (1971); [c] D. H. Haynes and B. C. Pressman, *J. Memb. Biol.* **18**, 1 (1974); [d] K. H. Wong, K. Yagi and J. Smid, *J. Memb. Biol.* **18**, 379 (1974); [e] M. Bourgoin, K. H. Wong, J. Y. Hui and J. Smid, *J. Am. Chem. Soc.* **97**, 3462 (1975); [f] A. Sadakane, T. Iwachido and K. Toei, *Chem. Soc. Japan* **48**, 60 (1975).

[5a] W. E. Morf and W. Simon, *Helv. Chim. Acta* **54**, 2683 (1971); [b] E. Pretsch, D. Ammann and W. Simon, *Res./Devel.* **25**, 20 (1974).

[6] R. J. P. Williams, *Quart. Rev.* **24**, 331 (1970).

[7] C. J. Pedersen and H. K. Frensdorff, *Agnew. Chem. Int. Ed. Engl.* **11**, 16 (1972).

[8] Prof. D. Haynes, private communication.

[9a] C. J. Pedersen, *J. Am. Chem. Soc.* **89**, 2495 (1967); [b] **92**, 391 (1970); [c] H. K. Frensdorff, *J. Am. Chem. Soc.* **93**, 600 (1971); [d] J. M. Lehn, *Structure and Bonding*, Vol. 16, pp. 1–69. Springer Verlag, New York (1973).

[10] D. R. Pfeiffer, P. W. Reed and H. A. Lardy, *Biochem.* **13**, 4007 (1974).

[11] D. A. Skoog and D. M. West, *Fundamentals of Analytical Chemistry*, pp. 680–682, 2nd Edn, Holt Rinehart & Winston, New York (1963).

[12a] J. H. Prestegard and S. Chan, *Biochem.* **8**, 3921 (1969); [b] *J. Am. Chem. Soc.* **92**, 4440 (1970).

[13] R. Büchi and E. Pretsch, *Helv. Chim. Acta* **58**, 1573 (1975), have concluded from ^{13}C NMR studies that 1,2-ethylenedioxydiacetic acid bis-*N*-methyl-*N*-11-carbethoxyundecylamide gives 1:1 and 2:1 complexation of ligand with $CaCl_2$ in methanol at a ligand concentration of *ca.* 0.2 M.

[14] For related data on the activities of $CaCl_2$ and $CaBr_2$ in water, see: [a] H. G. McLoed, A. R. Gordon, R. A. Robinson and R. H. Stokes, *Ann. New York Acad. Sci.* **51**, 593 (1948); [b] R. A. Robinson, *J. Am. Chem. Soc.* **69**, 2244 (1947); [c] **70**, 1870, 1874 (1948).

[15] Theoretical treatments of curve fitting of molecular complexation data by: [a] J. Reuben, *J. Am. Chem. Soc.* **95**, 3534 (1973) and [b] D. Derenleau, *J. Am. Chem. Soc.* **91**, 4044 (1969) conclude that the best data is obtained between 0.2 and 0.8 of $\Delta A/\Delta A_{t}$; the curve shapes are concentration dependent and low concentrations (such as $1/K_f$) are needed to avoid molecular aggregation.

[16] T-C. Wun, R. Bittman and I. J. Borowitz, *Biochemistry*, **16**, 2074 (1977).

[17a] D. E. Williamson and G. W. Everett, Jr., *J. Am. Chem. Soc.* **97**, 2397 (1975); [b] L. Weiss, Ph.D. Thesis, Yeshiva University (1975).

[18a] R. L. Vold, J. S. Waugh, M. P. Klein and D. E. Phelps, *J. Chem. Phys.* **48**, 3831 (1968); [b] R. Freeman and H. D. Hill, *J. Chem. Phys.* **51**, 3140 (1969); [c] E. Breitmaier, K. H. Spohn and S. Berger, *Angew. Chem. Int. Ed. Engl.* **14**, 144 (1975); [d] G. C. Levy, *Acct. Chem. Research* **6**, 161 (1973).

[19a] A. Allerhand, D. Doddrell, V. Glushko, D. W. Cochran, E. Wenkert, P. J. Lawson and F. R. N. Gurd, *J. Am. Chem. Soc.* **93**, 544 (1971); [b] D. Doddrell and A. Allerhand, *J. Am. Chem. Soc.* **93**, 1558 (1971).

[20] R. Buchi, E. Pretsch and W. Simon, *Tetrahedron Letters* 1709 (1976).

[21] A. Abragam, *The Principles of Nuclear Magnetism*, p. 64. Oxford University Press, London (1961).

[22a] R. Noyes, *J. Am. Chem. Soc.* **84**, 513 (1962); [b] **86**, 971 (1964).

[23] N. N. L. Kirsch and W. Simon, *Helv. Chim. Acta* **59**, 357 (1976).

[24] M. Miyazaki, Y. Shimoishi, H. Miyasa and K. Toei, *J. Inorg. Nucl. Chem.* **36**, 2033 (1974).

[25a] R. E. Benson and T. L. Cairns, *J. Am. Chem. Soc.* **70**, 2115 (1943); [b] H. Lüssi, *Chimia* **27**, 65 (1973).