

## 238. Lipophilic Di- and Triamides as Ionophores for Alkaline Earth Metal Cations

by Daniel Erne, Nada Stojanac<sup>a</sup>), Daniel Ammann, Peter Hofstetter, Ernő Pretsch and Wilhelm Simon

Department of Organic Chemistry, Swiss Federal Institute of Technology, CH-8092 Zürich

<sup>a</sup>) Department of Chemistry, University of New Brunswick, P.O. Box 4400, Fredericton, N.B., Canada E3B 5A3

(19.IX.80)

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### Summary

Different electrically neutral lipophilic di- and triamides were prepared and their ion selectivity in membranes studied. In membranes the ionophore *N,N'*-diheptyl-*N,N'*-dimethyl-succinamide prefers  $\text{Ca}^{2+}$  over  $\text{Mg}^{2+}$  by a factor of 20 but rejects  $\text{Na}^+$  and  $\text{K}^+$  in respect to  $\text{Mg}^{2+}$  by a factor of 100 and 10, respectively. This selectivity suffices to perform useful intracellular  $\text{Mg}^{2+}$  activity studies.

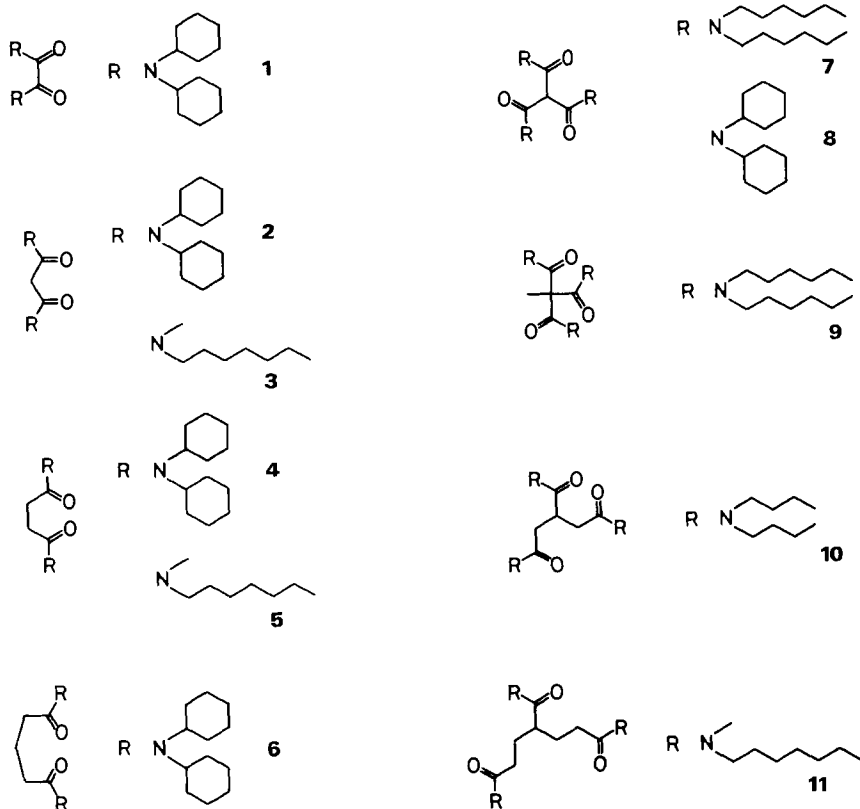
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**Introduction.** - The monocarboxylic polyether antibiotic-6016 has been reported to be a  $\text{Mg}^{2+}$  selective ionophore which displays preferential extraction from an aqueous into an organic phase of  $\text{Mg}^{2+}$  over  $\text{Ca}^{2+}$  and  $\text{Ba}^{2+}$  [1]. We were, however, unable to detect potentiometrically such selectivities when the antibiotic was incorporated in solvent polymeric membranes. In these membranes  $\text{Ca}^{2+}$  was clearly favoured over  $\text{Mg}^{2+}$ . Similar results were obtained for the antibiotic A 23187 which was claimed to be  $\text{Ca}^{2+}$  selective in extraction [2] as well as in transport systems [3]. The potentiometrically determined selectivity sequence is, however,  $\text{Ba}^{2+} > \text{Ca}^{2+} > \text{Mg}^{2+}$  [4]<sup>1)</sup>.

Despite major efforts it has so far been impossible to prepare a single membrane system showing a high selectivity for  $\text{Mg}^{2+}$  over  $\text{Ca}^{2+}$ . According to model calculations such a preference for  $\text{Mg}^{2+}$  might be achieved by an octahedral coordination of the cation with the O-atoms present in the high-dipole-moment ligand groups [8]. A number of 3-oxapentane diamides were prepared and tested in membranes [9] to see whether the suspected 1:2 cation/ligand complexes were formed by a hexadentate coordination. Although *N,N,N',N'*-tetracyclohexyl-3-oxapentane diamide does indeed form 1:2  $\text{Mg}^{2+}$ /ligand complexes [9] [10], in membranes it induces a selectivity of about  $10^4$  for  $\text{Ca}^{2+}$  over  $\text{Mg}^{2+}$ . This can be explained by the observed formation of highly lipophilic 1:3  $\text{Ca}^{2+}$ /ligand complexes [9].

<sup>1)</sup> A comparable selectivity for  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  as well as the analytically relevant suppression of alkali metal cations [5] is achieved by using liquid membrane electrodes based on certain lipophilic  $\beta$ -diketones [6] or organic phosphates [7].

## Scheme



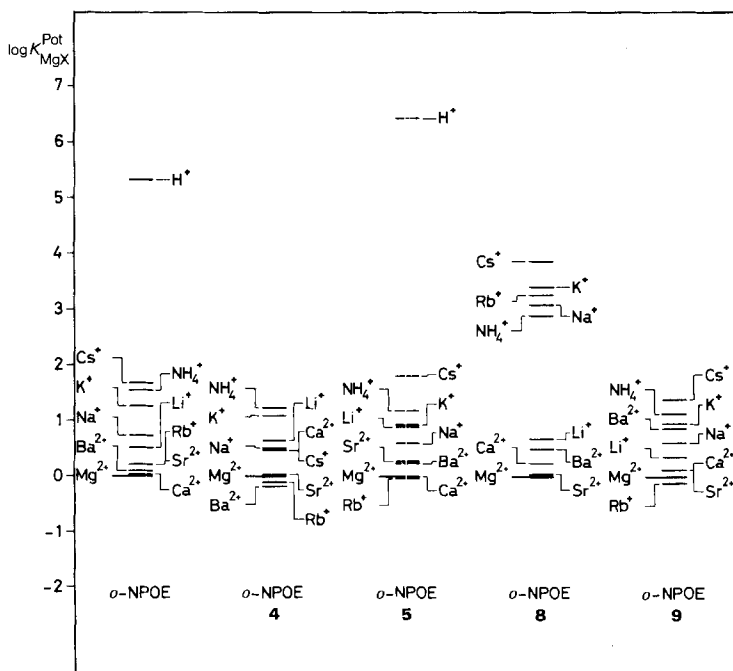
Studies of the interaction of simple diamides [11], as well as co-polyoxamides [12], especially with transition- and B-metals have been described. We report here on the preparation of new, lipophilic di- and triamides (see *Scheme*) and on their behaviour in membranes with respect to A-cations. These ligands can be assumed to form 1:3 and 1:2  $Mg^{2+}$ /ligand complexes having an octahedral coordination of  $Mg^{2+}$  with the ligand O-atoms. Thus they can be considered as possible ionophores for  $Mg^{2+}$ .

**Results and Discussion.** - The selectivities induced in solvent polymeric membranes by some of these ligands are presented in *Figures 1* and *2*. The selectivity factors,  $K_{MgX}^{Pot}$ , represent the membrane's preference for the ion X relative to  $Mg^{2+}$  [13]. Most of the ligands tested in polyvinyl chloride membranes plasticized with *o*-nitrophenyl octyl ether (*o*-NPOE) did not show large selectivity changes in the absence of potassium tetrakis(*p*-chlorophenyl)borate (KTPCIPB) (see *e.g.* columns 2 and 3 in comparison to 1 in *Fig. 1*). The compounds 7 and 8 (column 4 in *Fig. 1*) are an exception to this. Unexpectedly they induced some selectivity for  $Cs^+$ . The selectivity sequence of membranes with 8 roughly corresponds to

that which is typical for cation exchange membranes (see column 1 in *Fig. 2*) [14]. This is somewhat surprising since **8** does not seem to be present in the deprotonated form in substantial concentration in the membrane phase. No exchange of the proton in the  $\alpha$ -position to the three carbonyl groups was detectable when a solution of compound **8** in  $\text{CDCl}_3$  was equilibrated with  $\text{D}_2\text{O}$ . The substitution of this  $\alpha$ -proton in ligand **7** by a methyl group (**9**) leads, however, to a loss of the preference for large alkali metal cations (column 5, *Fig. 1*).

In the absence of anionic sites (e.g.  $\text{TpClPB}^-$ ), ligands **4** and **5** show no activity in membranes. When, however, the ligands **4** or **5** are added to membranes in the presence of such sites, dramatic selectivity changes compared with ligand-free membranes containing  $\text{TpClPB}^-$  are observed (columns 3 and 4 in comparison to column 1 in *Fig. 2*). The compounds **2** and **3** behave similarly. The diamides **1** (column 2 in *Fig. 2*) and **6** are not effective in such membranes. Under the same experimental conditions, the triamides **10** and **11** (columns 5 and 6 in *Fig. 2*) induce a greater loss in the preference for  $\text{Mg}^{2+}$  than membranes containing ligand **5** (column 4 in *Fig. 2*).

As indicated in *Figure 3*, compound **5** indeed behaves as an ionophore for  $\text{Mg}^{2+}$  in solvent polymeric membranes. Although the membranes exhibit a preference for  $\text{Ca}^{2+}$  over  $\text{Mg}^{2+}$  by a factor of about 20 (*Fig. 2*), the selectivity over alkali-metal cations and other alkaline-earth ions would be sufficient to perform useful measurements of  $\text{Mg}^{2+}$  activities in representative intracellular environments ( $< 10^{-5} \text{ M}$



*Fig. 1.* Selectivity factors,  $\log K_{\text{MgX}}^{\text{Pot}}$ , for solvent polymeric membranes with *o*-nitrophenyl octyl ether (*o*-NPOE) as membrane solvent. Ligand-free membranes (column 1) are compared with membranes containing different ligands (separate solution method, 0.1 M solutions of the chlorides, 20–22°).

$\text{Ca}^{2+}$ ,  $0.01\text{ M Na}^+$ ,  $0.1\text{ M K}^+$ ,  $\sim 0.001\text{ M Mg}^{2+}$ ). The corresponding microelectrodes have been realized [15].

The dependence of the EMF of an electrode cell assembly based on ligand **5** on the pH of the sample solution is given in *Figure 4*. The results show that at physiological pH values  $\text{Mg}^{2+}$  activities in the  $10^{-3}\text{ M}$  range can be measured without any interference by  $\text{H}_3\text{O}^+$  ions.

The interaction of the ionophore **5** with  $\text{Mg}^{2+}$  was corroborated by  $^{13}\text{C}$ -NMR spectroscopy (*Fig. 5*). Since the solubility of  $\text{Mg}(\text{SCN})_2$  in  $\text{CDCl}_3$  in the absence of ligand is negligible, the maximum amount of salt soluble in the presence of a ligand corresponds to the highest metal/ligand ratio for the complex. The chemical shifts induced by  $\text{Mg}(\text{SCN})_2$  would show a linear dependence on the metal/ligand ratio if a single kind of complex were formed. In the presence of ligand **5**  $\text{Mg}(\text{SCN})_2$  is soluble up to a molar metal/ligand ratio of 2:3 (*Fig. 5, C*). The non-linearity of the induced chemical shifts as a function of the metal/ligand ratio and the change in the splitting of the signals of the carbonyl C-atoms (*Fig. 5, B and C*) indicate that there must exist at least one other complex besides the 2:3 form.

This work was partly supported by the Swiss National Science Foundation. We thank Prof. Dr. N. Ōtake, University of Tokyo, for his kind gift of a small sample of the antibiotic-6016.

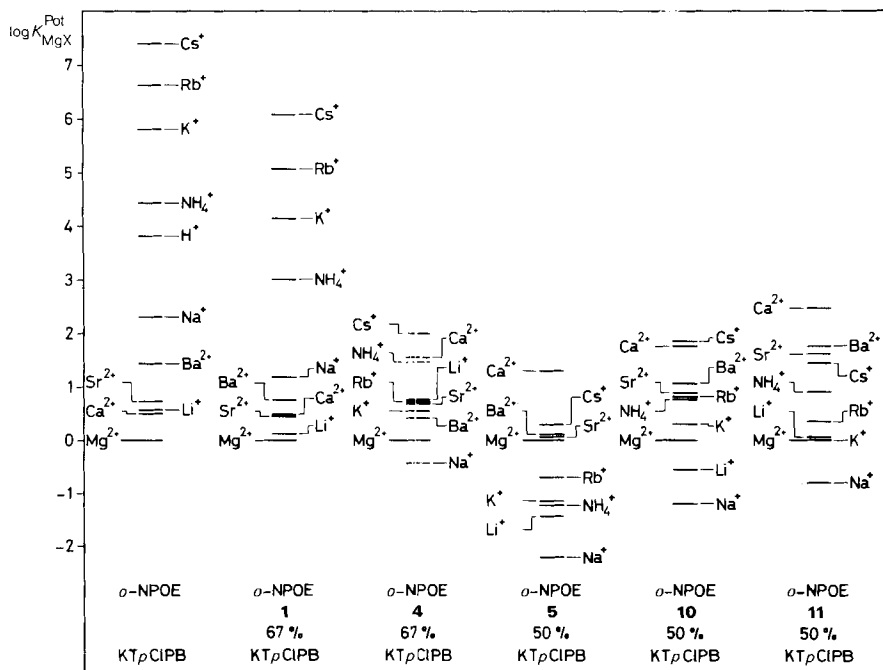


Fig. 2. Selectivity factors,  $\log K_{\text{MgX}}^{\text{Pot}}$ , for solvent polymeric membranes with *o*-nitrophenyl octyl ether (*o*-NPOE) as membrane solvent and incorporated lipophilic anionic sites (potassium tetrakis(*p*-chlorophenyl)borate (KTpCIPB), mol-% given). Ligand-free membranes (column 1) are compared with membranes containing different ligands (separate solution method,  $0.1\text{ M}$  solutions of the chlorides,  $20\text{--}22^\circ$ ).

## Experimental Part

**EMF.-Measurements.** - The membrane preparation and measuring technique are described in detail elsewhere [6] [16]. Membrane composition: 1-2 wt.-% ligand, 33 wt.-% polyvinyl chloride and 65-66 wt.-% *o*-nitrophenyl octyl ether (*o*-NPOE). The molar ratio of ligand to potassium tetrakis(*p*-chlorophenyl)borate (KTpCIPB) is given in Figure 2.

**<sup>13</sup>C-NMR.-Measurements.** - <sup>13</sup>C-NMR. spectra (Fig. 5) were recorded on a Bruker HXS-360 spectrometer at 90.5 MHz. Chemical shifts are reported in  $\delta$  (ppm) relative to TMS as internal standard.

**Preparation of the ligands.** - For recording procedures and abbreviations see [17]. The synthesis of ligand 6 was described earlier [9]. - *General procedure for the preparation of ligands 1-5.* The acid chloride (1 mol-equiv.) was reacted with the amine (4 mol-equiv.) in toluene at room temperature over night. The precipitated amine hydrochloride was filtered off and the solvent evaporated under vacuum. The residue was dissolved in chloroform of diethyl ether and the organic phase washed with 0.2M HCl, 0.2M NaOH, and with H<sub>2</sub>O. The crude product was further purified as indicated.

*N,N,N',N'*-Tetracyclohexyl-oxamide (1). The crude product was crystallized from diethyl ether/methylene chloride (45%), m.p. 196-198°. - IR. (CHCl<sub>3</sub>): 1630. - <sup>1</sup>H-NMR. (CDCl<sub>3</sub>): 1.0-3.5 (br., 44 H, 4 C<sub>6</sub>H<sub>11</sub>). - MS.: 416 (46, M<sup>+</sup>), 333 (40), 251 (4), 208 (86), 180 (36), 126 (71), 83 (100).

C<sub>26</sub>H<sub>44</sub>N<sub>2</sub>O<sub>2</sub> (416.65) Calc. C 74.95 H 10.64 N 6.72% Found C 74.76 H 10.53 N 6.86%

*N,N,N',N'*-Tetracyclohexyl-malonamide (2). Yield of the crude product: 30%. A portion was recrystallized from diethyl ether, m.p. 183-184°. - IR. (CHCl<sub>3</sub>): 1630. - <sup>1</sup>H-NMR. (CDCl<sub>3</sub>):

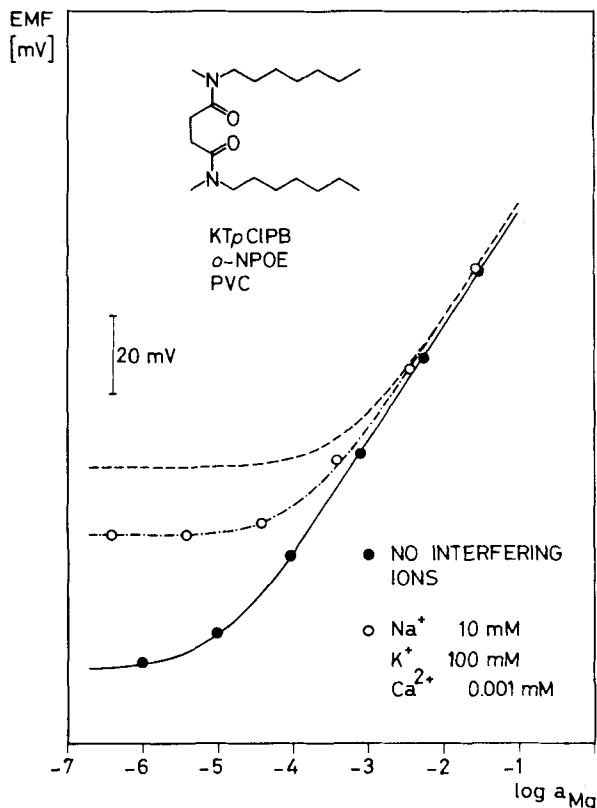


Fig. 3. EMF response of the solvent polymeric membrane electrode cell assembly to different Mg<sup>2+</sup> activities in the sample solution. Response in pure MgCl<sub>2</sub> solutions: lower trace; Mg<sup>2+</sup> response at constant ion background: middle trace; Mg<sup>2+</sup> response computed using selectivity data of Figure 2 (column 4) and actual slope of the electrode response at the constant intracellular ion background indicated: upper trace; experimental values: dots.

0.9-3.9 (br., 44 H, 4 C<sub>6</sub>H<sub>11</sub>); 3.35 (s, 2 H, COCH<sub>2</sub>CO). - MS.: 430 (7, M<sup>+</sup>), 347 (11), 265 (10), 250 (3), 208 (5), 180 (100), 138 (9), 98 (19), 83 (35).

C<sub>27</sub>H<sub>46</sub>N<sub>2</sub>O<sub>2</sub> (430.67) Calc. C 75.30 H 10.77 N 6.50% Found C 75.18 H 10.71 N 6.56%

*N,N'*-Diheptyl-*N,N'*-dimethyl-malonamide (3). Yield of crude product: 50%. A portion was distilled at 128-135°/0.04 Torr. - IR. (CHCl<sub>3</sub>): 1632. - <sup>1</sup>H-NMR. (CDCl<sub>3</sub>): 0.9 (t, 6 H, 2 CH<sub>2</sub>CH<sub>3</sub>); 1.1-1.7 (br., 20 H, 2 (CH<sub>2</sub>)<sub>5</sub>); 2.92, 3.02 and 3.03 (3 s, 6 H, 2 NCH<sub>3</sub>); 3.35 (t, 4 H, 2 NCH<sub>2</sub>); 3.45 (s, 2 H, COCH<sub>2</sub>CO). - MS.: 326 (13, M<sup>+</sup>), 311 (2), 297 (7), 283 (6), 269 (8), 255 (8), 242 (7), 229 (2), 198 (23), 170 (5), 156 (52), 128 (100).

C<sub>19</sub>H<sub>38</sub>N<sub>2</sub>O<sub>2</sub> (326.52) Calc. C 69.89 H 11.73 N 8.58% Found C 69.83 H 11.65 N 8.44%

*N,N,N',N'*-Tetracyclohexyl-succinamide (4). The crude product (2%) was recrystallized from diethyl ether. - IR. (CHCl<sub>3</sub>): 1620. - <sup>1</sup>H-NMR. (CDCl<sub>3</sub>): 1.0-3.8 (br., 44 H, 4 C<sub>6</sub>H<sub>11</sub>); 2.65 (s, 4 H, COCH<sub>2</sub>CH<sub>2</sub>CO). - MS.: 444 (10, M<sup>+</sup>), 363 (2), 362 (2), 280 (2), 265 (27), 264 (94), 236 (9), 183 (7), 182 (55), 181 (39), 180 (100), 154 (5), 138 (17), 100 (53), 83 (47).

C<sub>28</sub>H<sub>48</sub>N<sub>2</sub>O<sub>2</sub> (444.70) Calc. C 75.63 H 10.88 N 6.30% Found C 75.65 H 10.88 N 6.23%

*N,N'*-Diheptyl-*N,N'*-dimethyl-succinamide (5). The crude product (68%) was purified by flash chromatography (40 kPa) on silica gel with ethyl acetate/hexane 4:1 as eluent. - IR. (CHCl<sub>3</sub>): 1630. - <sup>1</sup>H-NMR. (CDCl<sub>3</sub>): 0.9 (t, 6 H, 2 CH<sub>2</sub>CH<sub>3</sub>); 1.1-1.7 (br., 20 H, 2 (CH<sub>2</sub>)<sub>5</sub>); 2.65 (m, 4 H, COCH<sub>2</sub>CH<sub>2</sub>CO); 2.95 and 3.0 (2 s, 6 H, 2 NCH<sub>3</sub>); 3.3 (2 t, 4 H, 2 NCH<sub>2</sub>). - MS.: 340 (8, M<sup>+</sup>), 256 (4), 212 (100), 184 (9), 156 (11), 128 (41), 86 (3), 57 (16).

C<sub>20</sub>H<sub>40</sub>N<sub>2</sub>O<sub>2</sub> (340.55) Calc. C 70.54 H 11.84 N 8.23% Found C 70.60 H 11.86 N 8.20%

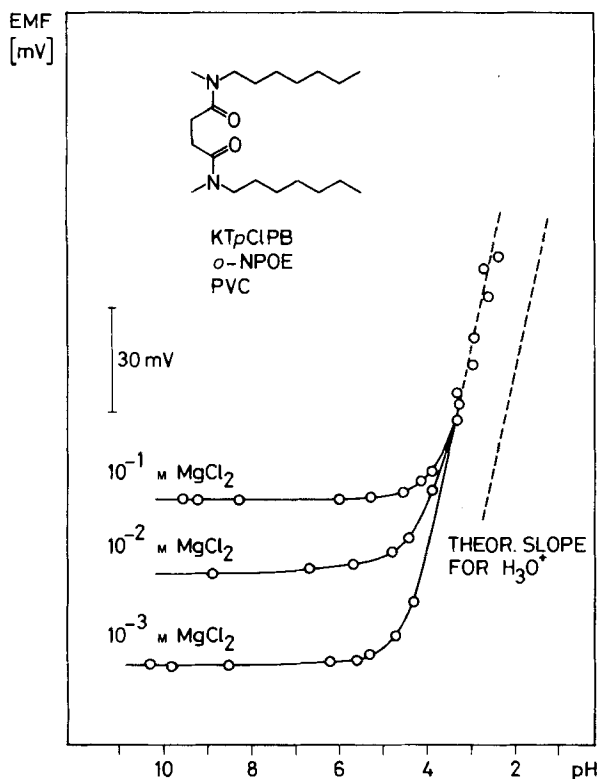


Fig. 4. Dependence of the EMF of the solvent polymeric membrane electrode cell assembly on the pH of the sample solution at different MgCl<sub>2</sub> concentrations. The pH values were adjusted by adding diluted KOH- or HCl-solutions to the sample. Experimental data (dots) are not corrected for changes in liquid junction potential and activity coefficients.

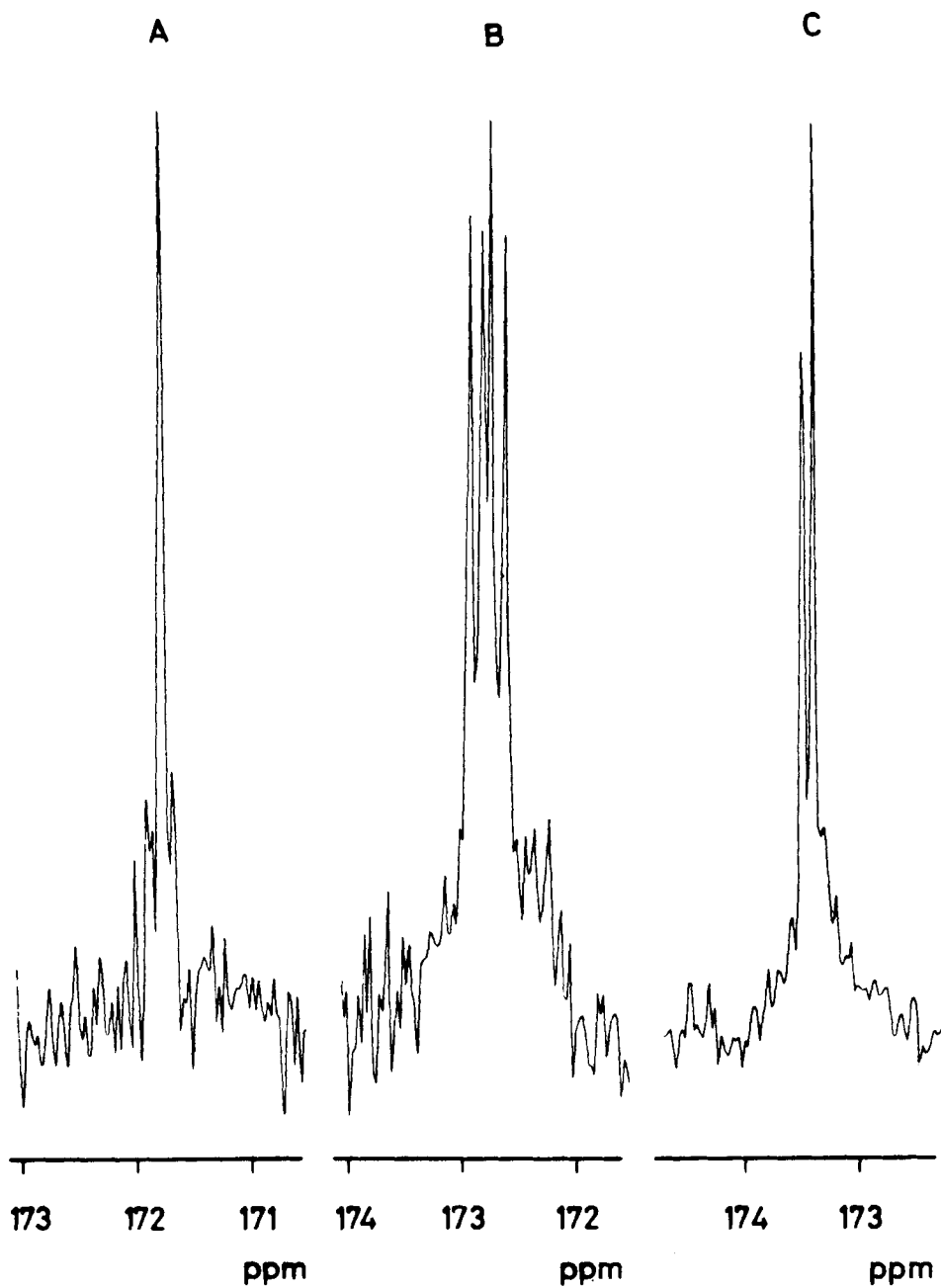


Fig. 5. Carbonyl region of the  $^{13}\text{C}$ -NMR. spectra of ligand 5 in  $\text{CDCl}_3$ . A: 0.74M 5, no  $\text{Mg}(\text{SCN})_2$ ; B: 0.65M 5, 0.22M  $\text{Mg}(\text{SCN})_2$ ; C: 0.68M 5, 0.47M  $\text{Mg}(\text{SCN})_2$ .

N,N,N',N',N'',N''-Hexahexyl-methanetricarboxamide (7) (65%). Reaction between 0.23 g (1 mmol) ethyl methanetricarboxylate (*Fluka, purum*), 1.3 g (7.2 mmol) dihexyl amine (*Fluka, techn.*) and 0.07 g (0.75 mmol) 2-hydroxypyridine (*Fluka, pract.*) as catalyst [19] yielded after 10 h under nitrogen at 160° 0.5 g (0.8 mmol) of 7. Crystallization from ethyl acetate afforded 0.42 g (0.65 mmol) of pure 7, m.p. (0.75 mmol) 2-hydroxypyridine (*Fluka, pract.*) as catalyst yielded after 10 h under nitrogen at 160° 6(CH<sub>2</sub>)<sub>4</sub>CH<sub>3</sub>; 3.1 and 3.2 (2 m, 12 H, 6 NCH<sub>2</sub>); 4.6 (s, 1H, CH). – MS.: 649 (4, M<sup>+</sup>), 465 (69), 438 (30), 254 (15), 212 (22), 185 (19), 184 (100), 114 (22), 85 (21), 43 (41).

C<sub>40</sub>H<sub>79</sub>N<sub>3</sub>O<sub>3</sub> (650.08) Calc. C 73.90 H 12.25 N 6.46% Found C 73.75 H 12.16 N 6.42%

N,N,N',N',N'',N''-Hexacyclohexyl-methanetricarboxamide (8). Reaction between 0.23 g (1 mmol) ethyl methanetricarboxylate (*Fluka, purum*), 1.3 g (7.2 mmol) dicyclohexyl amine (*Fluka, purum*) and 0.07 g (0.75 mmol) 2-hydroxy-pyridine (*Fluka, pract.*) as catalyst [19] afforded after 9 h under nitrogen at 160° 0.55 g (0.86 mmol) of crude material (86%). Several crystallizations from ethyl acetate yielded 8, m.p. 149–151°. – IR. (CHCl<sub>3</sub>): 1650. – <sup>1</sup>H-NMR. (CDCl<sub>3</sub>): 1.5 (br. m, 60 H, 30 CH<sub>2</sub>); 2.8 (br. m, 6 H, 6 NCH); 4.55 (s, 1H, CHCO). – MS.: 637 (3, M<sup>+</sup>), 554 (8), 430 (4), 250 (11), 208 (7), 181 (21), 180 (100), 168 (4), 138 (6), 126 (8), 98 (17), 83 (44), 55 (16).

C<sub>40</sub>H<sub>67</sub>N<sub>3</sub>O<sub>3</sub> · H<sub>2</sub>O Calc. C 73.24 H 10.60 N 6.41%  
 (637.99 + 18.02) Found „ 73.18 „ 10.67 „ 6.46%

Note that such ion carriers of rather high molar mass may tenaciously retain solvent molecules (see also [17][18]).

N,N,N',N',N'',N''-Hexahexyl-ethane-1,1,1-tricarboxamide (9). To a stirred solution of 0.34 g (0.52 mmol) of 7 in 45 ml dimethyl formamide (*Fluka, puriss. p.a.*) at 40–50° under nitrogen was added a suspension of 0.03 g (0.62 mmol) NaH (*Fluka, purum*) in 5 ml dimethyl formamide. After 1 h 0.09 g (0.62 mmol) methyl iodide (*Fluka, puriss. p.a.*) was added and stirring continued for 7 h at 40°. After cooling the mixture was diluted with diethyl ether and the precipitate filtered off. The filtrate was washed with water and the solvent removed *in vacuo*. The crude product (20%) was purified by preparative plate chromatography with ethyl acetate/hexane 1:1 as eluent: 0.07 g (0.1 mmol) of 9. – IR. (CHCl<sub>3</sub>): 1623. – <sup>1</sup>H-NMR. (CDCl<sub>3</sub>): 0.85 (m, 18 H, 6 CH<sub>2</sub>CH<sub>3</sub>); 1.25–1.5 (br. m, 48 H, 6 (CH<sub>2</sub>)<sub>4</sub>CH<sub>3</sub>); 3.2 (br. m, 12 H, 6 CH<sub>2</sub>N). – MS.: 663 (2, M<sup>+</sup>), 479 (42), 478 (19), 451 (21), 268 (18), 212 (32), 184 (100), 85 (36), 83 (17), 43 (70).

C<sub>41</sub>H<sub>81</sub>N<sub>3</sub>O<sub>3</sub> (664.11) Calc. C 74.15 H 12.29 N 6.33% Found C 73.42 H 12.22 N 6.09%

N,N,N',N',N'',N''-Hexabutyl-propane-1,2,3-tricarboxamide (10). To a solution of 4.7 g (36 mmol) dibutyl amine (*Fluka, puriss*) and 10 g (100 mmol) triethyl amine (*Fluka, puriss. p.a.*) in 150 ml toluene were added at 0° 2.8 g (12 mmol) propane-1,2,3-tricarboxyl chloride (obtained from the corresponding triacid with thionyl chloride in benzene) dissolved in 50 ml toluene. The reaction mixture was stirred at room temperature over night. The precipitated triethyl amine hydrochloride was filtered off and the solution washed with 0.2M HCl, 0.2M NaOH, and H<sub>2</sub>O. The solvent was evaporated under vacuum and the residue (7%) purified by chromatography on silica gel with chloroform as eluent. – IR. (liq.): 1640. – <sup>1</sup>H-NMR. (CDCl<sub>3</sub>): 0.9 (m, 18 H, 6 CH<sub>2</sub>CH<sub>3</sub>); 1.05–1.75 (br. m, 24 H, 6 (CH<sub>2</sub>)<sub>2</sub>); 2.5 (m, 4 H, 2 CHCH<sub>2</sub>); 3.3 (m, 13 H, 6 NCH<sub>2</sub>, CH). – MS.: 509 (27, M<sup>+</sup>), 381 (100), 353 (57), 339 (18), 224 (59), 128 (80), 57 (69).

C<sub>30</sub>H<sub>59</sub>N<sub>3</sub>O<sub>3</sub> (509.81) Calc. C 70.68 H 11.66 N 8.24% Found C 70.86 H 11.55 N 8.11%

N,N',N''-Triheptyl-N,N',N''-trimethyl-pentane-1,3,5-tricarboxamide (11). A mixture of 2.9 g (10 mmol) ethyl pentane-1,3,5-tricarboxylate (*EGA, 98–99%*), 9.3 g (72 mmol) *N*-heptyl-*N*-methyl-amine (*Fluka, purum*) and 2.9 g (30 mmol) 2-hydroxy-pyridine (*Fluka, pract.*) as catalyst [19] was heated for 75 h at 160° under nitrogen. The crude mixture (4%) was purified by column chromatography on silica gel using first ethyl acetate and in a second run ethyl acetate/methanol 9:1 as eluents: 0.23 g (0.4 mmol) of 11. – IR. (CHCl<sub>3</sub>): 1630. – <sup>1</sup>H-NMR. (CDCl<sub>3</sub>): 0.9 (t, 9 H, 3 CH<sub>2</sub>CH<sub>3</sub>); 1.1–1.6 (br., 30 H, 3 (CH<sub>2</sub>)<sub>5</sub>); 1.85 (m, 4 H, 2 CHCH<sub>2</sub>CH<sub>2</sub>); 2.25 (m, 5 H, 2 CH<sub>2</sub>CO and CHCO); 2.86, 2.88, 2.92 and 3.02 (4 s, 9 H, 3 NCH<sub>3</sub>); 3.25 (br., 6 H, 3 NCH<sub>2</sub>). – MS.: 537 (6, M<sup>+</sup>), 409 (18), 381 (8), 367 (40), 354 (20), 280 (6), 238 (16), 224 (6), 198 (27), 191 (35), 134 (100).

C<sub>32</sub>H<sub>63</sub>N<sub>3</sub>O<sub>3</sub> (537.87) Calc. C 71.46 H 11.81 N 7.81% Found C 71.27 H 11.70 N 7.96%



## REFERENCES

- [1] N. Ōtake & M. Mitani, *Agric. Biol. Chem.* **43**, 1543 (1979).
- [2] D. R. Pfeiffer, P. W. Reed & H. A. Lardy, *Biochemistry* **19**, 4007 (1974).
- [3] D. T. Wong, J. R. Wilkinson, R. L. Hamill & J.-S. Hong, *Arch. Biochem. Biophys.* **156**, 578 (1973).
- [4] A. K. Covington & N. Kumar, *Anal. Chim. Acta* **85**, 175 (1976).
- [5] P. C. Meier, D. Erne, Z. Cimerman, D. Ammann & W. Simon, *Mikrochim. Acta*, in press.
- [6] D. Erne, W. E. Morf, S. Arvanitis, Z. Cimerman, D. Ammann & W. Simon, *Helv.* **62**, 994 (1979).
- [7] J. W. Ross, jr., in 'Ion-Selective Electrodes' (R. A. Durst (Ed.)), NBS Special Publ. 314, 1969, p. 57.
- [8] W. Simon, W. E. Morf & D. Ammann, in 'Proceedings of the International Symposium on Calcium-Binding Proteins and Calcium Function in Health and Disease', Cornell University, Ithaca, N.Y., USA, June 5–9, 1977, in 'Calcium-Binding Proteins and Calcium Function' (R. H. Wasserman, R. A. Corradino, E. Carafoli, R. H. Kretsinger, D. H. MacLennan and F. L. Siegel (Eds.)), North-Holland, New York, Amsterdam, Oxford 1977, p. 50.
- [9] E. Pretsch, D. Ammann, H. F. Osswald, M. Guggi & W. Simon, *Helv.* **63**, 191 (1980).
- [10] K. Neupert-Laves & M. Dobler, in preparation.
- [11] M. L. Good & T. H. Siddall, III., *J. Inorg. Nucl. Chem.* **30**, 2679 (1968).
- [12] S. Siggia, A. H. Beeber & O. Vogl, *Anal. Chim. Acta* **96**, 367 (1978).
- [13] G. G. Guilbault, R. A. Durst, M. S. Frant, H. Freiser, E. H. Hansen, T. S. Light, E. Pungor, G. Rechnitz, N. M. Rice, T. J. Rohm, W. Simon & J. D. R. Thomas, *Pure Appl. Chem.* **48**, 127 (1976).
- [14] R. Scholer & W. Simon, *Helv.* **55**, 171 (1972).
- [15] F. Lanter, D. Erne, D. Ammann & W. Simon, *Anal. Chem.*, in press.
- [16] P. C. Meier, D. Ammann, W. E. Morf & W. Simon, in 'Medical and Biological Applications of Electrochemical Devices' (J. Koryta (Ed.)), J. Wiley & Sons, Chichester, New York, Brisbane, Toronto 1980, p. 13.
- [17] D. Erne, N. Stojanac, D. Ammann, E. Pretsch & W. Simon, *Helv.* **63**, 2264 (1980).
- [18] J. Šenkyr, D. Ammann, P. C. Meier, W. E. Morf, E. Pretsch & W. Simon, *Anal. Chem.* **51**, 786 (1979).
- [19] H. T. Openshaw & N. Whittaker, *J. Chem. Soc. (C)* **1969**, 89.