BIS(METHYLPHOSPHONIC ACID) DERIVATIVES OF 1,4,8,11-TETRAAZACYCLOTETRADECANE (CYCLAM). SYNTHESIS, CRYSTAL AND MOLECULAR STRUCTURES, AND SOLUTION PROPERTIES

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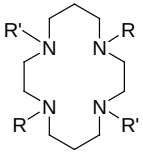
Dedicated to Professor Josef Loub who established laboratory of X-ray diffraction methods at the Faculty of Science, on the occasion of his 70th birthday.

Cyclam derivatives with methylphosphonic acid arms in position 1,8 and substituent R = H, Me, CH_2Ph in positions 4 and 11 are synthesised by Mannich reaction of an appropriate cyclam derivative, formaldehyde and phosphonic acid/diethyl phosphite followed by removal of protecting benzyl groups from nitrogen atoms. Mono(methylphosphonic acid) derivative of cyclam can be obtained by a similar route. Crystal structures of four phosphonic acid derivatives show the same ring conformation and orientation pendants due to strong intramolecular hydrogen bonds between phosphonate oxygen atoms and protonated nitrogen atoms adjacent over ethylene chains. The hydrogen bonds are stable even in aqueous solution. Activation parameters for destabilisation of the conformation are estimated from temperature-dependent NMR measurement. The protonation constants determined confirm the expected high basicity of the compounds and its dependence on the nitrogen atom substituents. The enhanced basicity of the nitrogen atoms non-bonded to methylenephosphonic acid moiety, is explained by the presence of the strong hydrogen bonds.

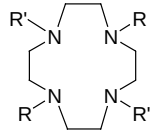
Key words: Azacrown compounds; Macrocycles; Cyclam; Tetrazacyclotetradecane; Phosphonic acids; Crystal structure; Conformation analysis; Basicity; Potentiometry; NMR spectroscopy; Protonation constants; Hydrogen bonds.

Polyazacycles with coordinating pendant arms are superior ligands for transition metal ions and lanthanides[1,2]. They form thermodynamically very stable complexes and show high selectivities to metal ions[1–3]. Polydentate ligands, such as 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid

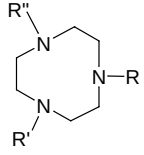
(**1a**, H_4teta) and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (**2a**, H_4dota), form thermodynamically and kinetically very stable complexes even with labile metal ions as the first-row transition-metal divalent ions or trivalent lanthanides[2]. The kinetic stability is caused, primarily, by convenient steric arrangement of the ligands that can fully wrap a metal ion and protect it from solvent molecules or other reagents. On the other hand, the ligands show low selectivity to metal ions[2]. The selectivity can be enhanced by replacing acetate pendants by with other groups such as hydroxyalkyl or acetamide pendants[3].



1a, R = R' = CH_2CO_2H H_4teta
1b, R = R' = $CH_2PO_3H_2$ H_8tetp
1c, R = R' = $CH_2P(Ph)O_2H$ H_4tetp^{Ph}



2a, R = R' = CH_2CO_2H H_4dota
2b, R = R' = $CH_2PO_3H_2$ H_8dotp
2c, R = R' = $CH_2P(Ph)O_2H$ H_4dotp^{Ph}
2d, R = H, R' = CH_2CO_2H H_2do2a
2e, R = H, R' = $CH_2PO_3H_2$ H_4do2p



R = R' = R'' = CH_2CO_2H,
$CH_2P(Me)O_2H$ or
$CH_2P(Et)O_2H$

The properties of macrocyclic ligands have been explored when designing the magnetic resonance imaging (MRI) contrast agents[4] based on Gd^{3+} or diagnostic and/or therapeutic radiopharmaceuticals utilising metal radionuclides[5]. In search for other ligands with similar or better properties than common acetate derivatives, research has also been focused on synthesis and investigation of azamacrocycles with phosphonic or phosphinic acid pendant arms. Complexes with the phosphorus ligands exhibit higher selectivity in complexation and sufficient thermodynamic stability[6]. Kinetic properties of the complexes are comparable with their acetate analogs[7]. The most studied compounds were lanthanide complexes of 1,4,7,10-tetraazacyclododecane (cyclen) derivatives. The investigations has led to an NMR shift reagent[8] for alkali metal ions $[Tm(dotp)]^{5-}$, where H_8dotp = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(methylphosphonic acid) (**2b**), and temperature[9] or pH (ref.[10]) MRI probes based on H_8dotp or

on phosphinic acid derivatives of cyclen. Phosphinic acid derivatives of 1,4,7-triazacyclononane selectively complex Mg^{2+} over Ca^{2+} and can be used for determination of Mg^{2+} concentration *in vivo*[11].

Derivatives of cyclam were investigated much less than those of cyclen. Only one derivative containing four methylenephosphonic acid arms (H_8tetp, **1b**) was studied[12,13]. It was found that the ligand contains two very basic ring nitrogen atoms[12,13] showing some selectivity for larger metal ions[6b]. It was suggested that all the arms cannot be coordinated to the same transition metal ions due to the bulkiness of the phosphonate moiety and, therefore, full coordination ability of the ligand cannot be utilised. We assume that the limitation can be overcome by synthesis of cyclam-based ligands with only two phosphonic acid arms with four nitrogen and two oxygen donor atoms, which should be particularly suitable for complexation of octahedral cations of the first transition-metal row. The synthesis and investigation of the 1,8-disubstituted cyclam ligands H_4L are the subject of the present paper.

EXPERIMENTAL

General

Starting amines **3a**–**3c** (for structures, see Scheme 1) were prepared by the literature procedures[14,15]. Reactive paraformaldehyde was filtered off from an old aqueous formaldehyde solution and dried in desiccator over P_2O_5. Palladium (10%) on active carbon purchased from Aldrich (Catalog No. 33,010-8) and all other chemicals available from commercial sources (Fluka, Aldrich, Avocado and Lachema) were used as obtained. Solvents were dried by established procedures[16]. Water for determination of protonation constants was purified using Milli-Q (Millipore) purification system. TLC was performed on Silufol silica gel sheets (Kavalier, Votice, Czech Republic) in the mixtures propan-2-ol–25% aqueous NH_3–water 7 : 3 : 3 (A), EtOH–25% aqueous NH_3 15 : 1 (B), and propan-2-ol–25% aqueous NH_3–water 15 : 3 : 3 (C) using ninhydrin detection. Silica gel 60 (0.040–0.065 mm, Merck), sulfonate (Dowex 50, Fluka) and carboxylate (Amberlite CG50/N1, Rohm & Haas) cation exchangers and an anion exchange resin (Dowex 1, Fluka) were used for column chromatography.

Elemental analyses were done in the Institute of Macromolecular Chemistry of Academy of Sciences of the Czech Republic (Prague). Melting points were determined using a Kofler hot-stage apparatus (Boetius) and are uncorrected. NMR spectra were recorded on a Varian Unity Plus at 400 MHz for ^1H, 169 MHz for $^{31}P\{^1H\}$ and 100 MHz for ^{13}C with internal references TMS for $CDCl_3$ solutions and *t*-BuOH for D_2O solutions and external reference 85% H_3PO_4. Hydrogen atom assignments were based on 2D NMR experiments. For NMR titration experiments, see below. Temperature was controlled by a VT-regulator, containing a thermocouple calibrated using MeOH and $HOCH_2CH_2OH$ according to a literature procedure[17]. Thermogravimetric (TG) analysis was done at the Prague Institute of Chemical Technology on a Stanton Redcroft apparatus at 25–300 °C in air (10 °C min^{-1}).

Chemicals and Stock Solutions for Potentiometric Titrations

The stock solution of nitric acid (≈0.03 mol dm^{-3}) was prepared by passing a solution of recrystallised potassium nitrate through a Dowex 50W-8 column in the H^+ form because of traces of NO and NO_2 present in the concentrated acid. Carbonate-free KOH solutions (≈0.2 and ≈0.95 mol dm^{-3}) were standardised against potassium hydrogen phthalate and HNO_3 solution against the ≈0.2 M KOH solution. Samples of the ligands for preparation of stock solutions were dried to constant weight ($H_4L^1 \cdot 9H_2O$ at 120 °C for 2 h; $H_4L^2 \cdot 8H_2O$ at 100 °C for 4 h; $H_4L^3 \cdot 6H_2O$ at 110 °C for 2 h; $H_4L^4 \cdot 5.5H_2O$ at 110 °C for 1 h; $H_4L^5 \cdot 4H_2O$ at 110 °C for 1.5 h). Ligand stock solutions were prepared by dissolving the dried solids in water except of **4a** (H_4L^1) which was dissolved in the standard KOH solution (≈1.8 equivalents) followed by addition of water. Analytical concentrations of the ligands determined from the weights of the dried ligands were in accordance with their concentrations determined together with refinement of protonation constants using OPIUM program.

Potentiometric Titrations

Titrations were carried out in a vessel thermostatted at 25 ± 0.1 °C, at an ionic strength $I(KNO_3)$ = 0.1 mol dm^{-3} and in the presence of extra HNO_3 in the –log $[H^+]$ range 1.8–12.1 (except for the titrations with **4a** (H_4L^1), which is insoluble below pH about 5) using a PHM 240 pH-meter, a 2-ml ABU 900 automatic piston burette and a GK 2401B combined electrode (Radiometer). The initial volume was 5 cm^3 and the concentration of all the ligands was 0.004 mol dm^{-3}. Titration for each system was carried out at least four times, each titration consisting of about 40 points. An inert atmosphere was ensured by constant passage of argon saturated with the vapor of the solvent used in measurements. Water ion product, pK_w = 13.78, was taken from ref.[18]. The protonation constants β_n calculated are concentration constants and are defined by $\beta_n = [H_nL]/([H]^n[L])$ ($pK_1 = \log \beta_1$; $pK_n = \log \beta_n - \log \beta_{n-1}$). The constants (with standard deviations) were calculated with program OPIUM (ref.[19]). The program minimises the criterion of the generalised least squares method using the calibration function

$$E = E_0 + S \log [H^+] + j_1[H^+] + j_2K_w/[H^+] ,$$

where the additive term E_0 contains the standard potentials of the electrodes used and contributions of inert ions to the liquid-junction potential, S corresponds to the Nernstian slope, the value of which should be close to the theoretical value and $j_1[H^+]$ and $j_2[OH^-]$ terms are contributions of the H^+ and OH^- ions to the liquid-junction potential. It is clear that j_1 and j_2 cause deviation from a linear dependence between E and –log $[H^+]$ only in strong acid and strong alkaline solutions. The calibration parameters were determined from titration of standard HNO_3 with standard KOH before any titration of ligands to give a calibration-titration pair used for calculations of the constants.

NMR Titrations

^1H and ^{31}P NMR titration experiments for determination of the highest protonation constants of **4e** (H_4L^5) (–log $[H^+]$ range 12.4–13.5, about 30 points) were carried out under the conditions of potentiometric titrations (H_2O, 0.1 M KNO_3, 25 °C, 0.004 M ligand), however, with no control of ionic strength at the last points above –log $[H^+]$ 13. A coaxial capillary

with D_2O was used for the lock and water signal was presaturated. The ^{31}P NMR spectra were recorded with no ^1H decoupling. Protonation contants were calculated with OPIUM from δ_P of phosphonic acid group and δ_H of the NCH_2P moiety. NMR titrations over the whole pH region were done at 25.0 °C in H_2O with presaturation of water signal, at concentration of ligands 0.03 mol dm^{-3} using a coaxial capillary with D_2O for the lock. pH (approximate range 0–14) of the solutions was adjusted with aqueous KOH or aqueous HCl and measured with a pH-meter calibrated with standard buffers[20].

Variable–Temperature NMR Measurements

^1H NMR spectra for determination of activation parameters of fluxional behavior of the cyclam ring were followed in the range of 0–90 °C in water with presaturation of water proton signal and with D_2O in a coaxial capillary for lock. Compound **4e** (H_4L^5) was measured at pH 10.97 (100% formation of $(H_2L^5)^{2-}$). Temperature-dependent spectra were treated with software package G-NMR to give the rate constant of the exchange. The rate constants were used for calculation of the activation parameters.

Crystal Structure Determinations

The diffraction-quality crystals of compounds **4b**·$8H_2O$ (H_4L^2·$8H_2O$), **4c**·$6H_2O$ (H_4L^3·$6H_2O$) and **4e**·$4H_2O$ (H_4L^5·$4H_2O$) were grown from aqueous solutions by slow vapour diffusion of acetone; the crystals of **4a**·$9H_2O$ (H_4L^1·$9H_2O$) were obtained from a hot aqueous solution by slow cooling. The picked-up crystals were mounted on glass fibres in random orientation with epoxy glue. Using a CAD4 diffractometer (Enraf–Nonius, λ = 0.71073 Å) diffraction data for **4a**·$9H_2O$ (H_4L^1·$9H_2O$) and **4b**·$8H_2O$ (H_4L^2·$8H_2O$) were collected at 293(1) K, for **4c**·$6H_2O$ (H_4L^3·$6H_2O$) and **4e**·$4H_2O$ (H_4L^5·$4H_2O$) at 150(1) K. The lattice parameters of the studied compounds were always determined from 25 reflections (θ-intervals: for **4a**·$9H_2O$ (H_4L^1·$9H_2O$) 13.0–14.0°; for **4b**·$8H_2O$ (H_4L^2·$8H_2O$) 14.0–15.1°; for **4c**·$6H_2O$ (H_4L^3·$6H_2O$) 13.0–14.0°; for **4e**·$4H_2O$ (H_4L^5·$4H_2O$) 11.0–12.5°). The intensities were collected by the ω–2θ scan; three standard reflections were always measured after 1 h (mean variation 5.9% for **4a**·$9H_2O$ (H_4L^1·$9H_2O$), 2.8% for **4b**·$8H_2O$ (H_4L^2·$8H_2O$), 1.6% for **4c**·$6H_2O$ (H_4L^3·$6H_2O$) and 2.5% for **4e**·$4H_2O$ (H_4L^5·$4H_2O$). Lorenzian-polarisation correction was used for all compounds using program JANA 98 (ref.[21]); absorption corrections were not applied. The structures were solved by the Patterson and Fourier method or by direct methods, and refined by full-matrix least-squares techniques (SHELXS86 (ref.[22]), SHELXL97 (ref.[23])). Scattering factors for the neutral atoms used were included in the program SHELXL97 (ref.[23]). The structure of **4b**·$8H_2O$ (H_4L^2·$8H_2O$) was refined as racemic twin (SHELXL97, ref.[23]). The hydrogen atoms were found and refined isotropically in structures of **4b**·$8H_2O$ (H_4L^2·$8H_2O$), **4c**·$6H_2O$ (H_4L^3·$6H_2O$) and **4e**·$4H_2O$ (H_4L^5·$4H_2O$). All H atoms of macrocyclic ring, but no hydrogen atoms of solvate water molecules and of phosphonic acid groups, were found and refined in structure of **4a**·$9H_2O$ (H_4L^1·$9H_2O$). Some H atoms of the phenyl group were found and also refined, but the remaining ones were located in theoretical position in the structure of **4a**·$9H_2O$ (H_4L^1·$9H_2O$). Table I gives pertinent crystallographic data of all structures. The *R* value obtained for structure of **4a**·$9H_2O$ (H_4L^1·$9H_2O$) is rather high due to a poor quality of the crystals. Thus, e.s.d. values of bonding parameters are about three times higher than for the other structures, however, structure of the compound appears to be correct. Crystallographic parameters of the structures determinated are given in Table I. Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallo-

TABLE I

Experimental data for the X-ray difraction studies of compounds $H_4L^1 \cdot 9H_2O$, $H_4L^2 \cdot 8H_2O$, $H_4L^3 \cdot 6H_2O$ and $H_4L^5 \cdot 4H_2O$

Parameters	**4a**·9H_2O ($H_4L^1 \cdot 9H_2O$)	**4b**·8H_2O ($H_4L^2 \cdot 8H_2O$)	**4c**·6H_2O ($H_4L^3 \cdot 6H_2O$)	**4e**·4H_2O ($H_4L^5 \cdot 4H_2O$)
Formula	$C_{26}H_{60}N_4O_{15}P_2$ [a]	$C_{20}H_{54}N_4O_{14}P_2$	$C_{14}H_{46}N_4O_{12}O_2$	$C_{12}H_{38}N_4O_{10}P_2$
M_w	730.72[a]	636.61	524.49	460.40
T, K	293(1)	293(1)	150(1)	150(1)
Crystal dimension, mm	0.18 × 0.25 × 0.48	0.18 × 0.42 × 0.50	0.18 × 0.55 × 0.73	0.07 × 0.07 × 0.55
Colour and shape	colorless irregular	colorless plate	colorless plate	colorless needle
Crystal system	monoclinic	triclinic	triclinic	orthorhombic
Space group	*C2/c*(no.15)	*P1*(no.1)	*P-1*(no.2)	*Pbca*(no.61)
a, Å	11.045(9)	8.8361(7)	7.857(2)	8.030(3)
b, Å	21.143(14)	10.1124(7)	8.2558(8)	16.660(5)
c, Å	15.897(4)	10.4054(8)	10.7117(9)	16.220(6)
α, °	90	81.002(6)	69.471(8)	90
β, °	97.98(5)	73.665(6)	76.25(1)	90
γ, °	90	66.116(6)	71.88(1)	90
U, Å^3	3 676.4(5)	814.9(1)	611.7(2)	2 170(1)
Z	4	1	1	4
D_c, g cm^{-3}	1.320[a]	1.297	1.424	1.409
λ, Å	0.71073	0.71073	0.71073	0.71073
μ, mm^{-1}	0.186	0.198	0.242	0.255
F(000)	1 488	344	284	992
θ range of data collection, °	1.93–25.14	2.04–24.97	2.05–24.97	2.44–23.98
Index ranges	–12, 13; –25, 25; –18, 0	–9, 10; –11, 12; –11, 12	–8, 9; 0, 9; –11, 12	0, 9; 0, 19; 0, 15
Number of reflections measured	6 356	5 276	2 144	1 988
R_σ	0.1815	0.015	0.011	0.029
Number of refletions observed [$I > 2\sigma(I)$]	2 361	4 811	2 021	1 340
Number of independent reflections	3 271	5 128	2 144	1 701
R_{int}	0.2112	–	–	–
Coefficients A, B in weighting scheme[b]	0.1919, 47.3939	0.0429, 0.0833	0.0397, 0.2763	0.0438, 1.4830
Data, restraints, parameters	3 271, 0, 271	5 1128, 0, 575	2 144, 0, 238	1 701, 0, 204
Goodness-of-fit on F^2	1.152	1.028	1.078	1.087
Final R, R' indices [$I \geq 2\sigma(I)$][c]	0.1701, 0.4376	0.0244, 0.0633	0.0263, 0.0708	0.0326, 0.0794
Maximum shift/e.s.d.	0.244	0.128	0.000	0.001
Largest difference peak and hole, e Å^3	1.13, –0.97	0.217, –0.214	0.330, –0.321	0.356, –0.333

[a] Theoretical values, some H-atoms were not found. [b] $w = 1/[\sigma^2(F_o^2) + (A \cdot P)^2 + B \cdot P]$, where $P = (F_o^2 + 2F_c^2)/3$ (SHELXL97, ref.[23]). [c] $R = \Sigma|F_o - F_c|/\Sigma|F_c|$; $R' = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$ (SHELXL97, ref.[23]).

graphic Data Centre as supplementary publication number CCDC-143863 (**4a**·$9H_2O$; $H_4L^1 \cdot 9H_2O$), -143865 (**4b**·$8H_2O$; $H_4L^2 \cdot 8H_2O$), -143866 (**4c**·$6H_2O$; $H_4L^3 \cdot 6H_2O$) and -143864 (**4e**·$4H_2O$; $H_4L^5 \cdot 4H_2O$). Copies of the data can be obtained free of charge on application to CCDC, e-mail: deposit@ccdc.cam.ac.uk.

Syntheses

Caution! *Mixture of aqueous HCl and formaldehyde can produce highly carcinogenic* $(ClCH_2)_2O$*!*

1,8-Dibenzyl-1,4,8,11-tetraazacyclotetradecane-4,11-di(methylphosphonic Acid) (**4a**, H_4L^1)

Hydrated hydrochloride of **3a** (2.00 g, ≈3.5 mmol) was dissolved in water (40 ml), alkalinised with 15% aqueous NaOH (20 ml) and extracted with 4 × 40 ml of chloroform. The organic phases were dried (anhydrous Na_2SO_4), evaporated and the residue was dissolved in dry benzene (70 ml). Diethyl phosphite (0.99 g, 7 mmol) was added, the flask was equipped with a magnetic stirrer and Dean–Stark apparatus. Dry paraformaldehyde (0.31 g, 11 mmol) was slowly added (30 min) to the refluxing mixture. Then the mixture was left under reflux for 1.5 h. The reaction was followed using TLC (B): the amount of the starting amine (R_F 0.35) decreased and *N*-monophosphonated cyclam (R_F 0.6) together with *N*,*N''*-diphosphonated cyclam (R_F 0.9) was detected. The mixture was left to cool down, evaporated on a rotary evaporator and the residue was dissolved in aqueous HCl (1 : 1, 50 ml); unlike amine **3a**, the residue was soluble in hydrochloric acid. Phosphorous acid (1.00 g, 12 mmol) was added and the mixture was refluxed for 1 h. Aqueous formaldehyde (4.0 ml of 35%, 0.5 ml portions) was then added under reflux over 4 h and the mixture was left under reflux for another 4 h. The mixture was cooled and white precipitate (crude hydrochloride of the product) was filtered off, washed with ethanol and acetone, and dried on filter. The solid was suspended in water (15 ml) and dissolved in a minimum amount of the concentrated aqueous ammonia added dropwise. The slightly yellow solution was introduced onto a Dowex 50 column (H^+-form; 25 × 4 cm). Acids were eluted with water and the product was obtained on elution with 10% aqueous ammonia. Fractions containing the product were evaporated and excess of ammonia was removed by co-distillation with water (three times). The pure product was obtained after recrystallisation from boiling water (60 ml). The yield of **4a**·$9H_2O$ ($H_4L^1 \cdot 9H_2O$) was 1.75 g (70%). TLC (A): R_F 0.5; m.p. 168–169 °C. For $C_{26}H_{60}N_4O_{15}P_2$ (730.7) calculated: 42.74% C, 8.28% H, 7.67% N; found: 42.70% C, 8.13% H, 7.62% N. ^{1}H NMR (1 M KOD): 1.71 br m, 4 H ($CH_2C\mathbf{H}_2CH_2$); 2.57 m, 4 H ($BnNC\mathbf{H}_2CH_2CH_2$); 2.57 d, 4 H, ^2J(P-H) = 11.6 ($NC\mathbf{H}_2P$); 2.73 t, 4 H, ^3J(H-H) = 7.6 ($CH_2CH_2C\mathbf{H}_2N{\sim}P$); 2.77 m, 4 H ($BnNC\mathbf{H}_2CH_2$); 2.91 m, 4 H ($C\mathbf{H}_2CH_2N{\sim}P$); 3.71 s, 4 H ($NC\mathbf{H}_2Ph$); 7.36–7.43 m, 10 H ($C_6\mathbf{H}_5$). $^{31}P\{^1H\}$ NMR (1 M KOD): 15.87. TG analysis: The compound dehydrates in the range 50–100 °C; weight loss 22.36% (theory for 9 H_2O 22.20%); the anhydrous compound is decomposed above 270 °C.

1,4,8,11-Tetraazacyclotetradecane-4,11-di(methylphosphonic Acid) (**4e**, H_4L^5)

The nonahydrate of phosphonic acid **4a** (H_4L^1; 2.00 g, 2.7 mmol) was suspended in dry THF (50 ml) and hexamethyldisilazane (HMDSA, 20 ml) was added. The mixture was refluxed for 1 h (the suspension dissolved during 10 min). After cooling, volatile matters were removed in vacuum with bath temperature 50 °C to give a yellow-brown oil. The residue was dissolved in dry THF (50 ml) and benzyl chloroformate (6.0 ml, 43 mmol) was added. The mix-

ture was stirred at room temperature for 36 h (optimum time) and EtOH (5 ml) and concentrated aqueous NH_3 (5 ml) was added dropwise to hydrolyse silyl ester groups and excess of chloroformate. After 30 min volatile matters were removed on a rotary evaporator and the residue was dissolved in 30% HBr/AcOH (35 ml), stirred at room temperature for 1 h and evaporated. The dark residue was mixed with water (30 ml) and the solution was extracted with chloroform (20 ml) to remove non-polar impurities. The water solution was introduced onto a Dowex 50 W × 8 column (H^+-form; 30 × 3 cm) and elution with water and 10% aqueous NH_3 afforded the product. The fractions containing desired ligand were evaporated to give the crude product which contained some partly dephosphonylated macrocycle (^{31}P NMR analysis) and several minor ninhydrin positive compounds. It was purified by chromatography on carboxylic cation exchange resin (Amberlite in H^+-form; 30 × 5 cm; elution with water). The pure product was eluted after approximately 600 ml of water. The fractions containing product were evaporated, the resulting solid was dissolved in warm water (30 ml) and the product crystallised on slow addition of acetone (150 ml) and standing in a refrigerator overnight. Colorless needles were filtered off, washed with acetone and dried in air. The yield of **4e**·4H_2O (H_4L^5·4H_2O) was 1.01 g (81%). TLC (A): R_F 0.4; m.p. 289–290 °C (dec.). For $C_{12}H_{38}N_4O_{10}P_2$ (460.4) calculated: 31.29% C, 8.32% H, 12.17 % N; found: 31.73% C, 8.47% H, 12.06% N. ^{1}H NMR (1 M KOD): 1.78 m, 4 H ($CH_2C\mathbf{H}_2CH_2$); 2.67 t, 4 H, ^3J(H-H) = 5.2 ($CH_2C\mathbf{H}_2NH$); 2.73 t, 4 H, ^3J(H-H) = 5.6 ($CH_2CH_2C\mathbf{H}_2NH$); 2.76 d, 4 H, ^2J(P-H) = 10.0 ($NC\mathbf{H}_2P$); 2.81 t, 4 H, ^3J(H-H) = 5.8 (P~$NC\mathbf{H}_2CH_2CH_2$); 2.90 t, 4 H, ^3J(H-H) = 5.2 (P~$NC\mathbf{H}_2CH_2$). ^{31}P{^{1}H} NMR (D_2O): 21.80; ^{31}P NMR (H_2O, borate buffer pH 9.18): 19.72 d, ^2J(P-H) = 10.5. TG analysis: The compound dehydrate in the range 80–100 °C; weight loss 15.98% (theory for 4 H_2O 15.65%); the anhydrous compound is decomposed above 290 °C.

4-Benzyl-11-methyl-1,4,8,11-tetraazacyclotetradecane-1,8-di(methylphosphonic Acid) (**4b**, H_4L^2) and 4,11-Dimethyl-1,4,8,11-tetraazacyclotetradecane-1,8-di(methylphosphonic Acid) (**4c**, H_4L^3)

Appropriate amine (**3b** or **3c**, respectively; 4.4 mmol) and diethyl phosphite (1.80 g, 13.3 mmol) were dissolved in dry benzene (50 ml) in a flask equipped with the Dean–Stark apparatus. Paraformaldehyde (0.53 g, 17.7 mmol) was added into the refluxing solution in small portions over 2 h. The reaction mixture was evaporated to give a yellow oil which was dissolved in aqueous HCl (1 : 1) and the solution was refluxed for 2 h. Phosphorous acid (1.00 g, 12.2 mmol) was added and 37% aqueous formadehyde solution (4.0 ml, 50 mmol) was added in portions (0.5 ml) into the refluxing mixture over 4 h. The resulting cloudy solution was refluxed for 8 h. The cooled mixture was evaporated to dryness and acids were removed on Dowex 50 W × 8 (150 ml) by elution with water followed by elution with 10% aqueous ammonia. The fractions containing product were collected, evaporated and dissolved in a minimum amount of water and transferred onto an Amberlite 50/AR column (carboxylic ion exchanger; 300 ml). The product was eluted with ≈600 ml of water. The fractions containing product were evaporated to dryness, the residue was dissolved in water (30 ml) by heating and decolorised with charcoal. The pure product was precipitated on slow addition of acetone and standing overnight in a refrigerator. It was filtered off, washed with acetone and dried on filter. The yield of **4b**·8H_2O (H_4L^2·8H_2O) 1.35 g (85%). TLC (A): R_F 0.5; m.p. 171–172 °C. For $C_{20}H_{54}N_4O_{14}P_2$ (636.6) calculated: 37.73% C, 8.54% H, 8.80% N; found: 37.91% C, 8.41% H, 8.83% N. ^{1}H NMR (1 M KOD): 1.70 br, 4 H ($CH_2C\mathbf{H}_2CH_2$); 2.26 s, 3 H ($NC\mathbf{H}_3$); 2.50–2.57 m, 4 H ($MeNC\mathbf{H}_2CH_2CH_2$ + $BnNC\mathbf{H}_2CH_2CH_2$); 2.58–2.61 m, 2 H

(MeNC**H**$_2$CH$_2$); 2.59 and 2.60 d + d, 2 H + 2 H, ^2J(P-H) = 12 + 12 (NC**H**$_2$P); 2.68–2.77 m, 4 H (BnNC**H**$_2$CH$_2$ + BnNCH$_2$CH$_2$C**H**$_2$); 2.79 t, 2 H, ^3J(H-H) = 8.0 (MeNCH$_2$CH$_2$C**H**$_2$); 2.84–2.91 m, 4 H (P~NC**H**$_2$CH$_2$); 3.70 s, 2 H (PhC**H**$_2$N); 2.37–2.45 m, 5 H (C$_6$**H**$_5$). ^{31}P{^{1}H} NMR: 21.58 and 21.26 (D_2O); 15.94 (1 M KOD). TG analysis: The compound dehydrates in the range 40–100 °C; weight loss 21.17% (theory for 8 H_2O 22.64%); the anhydrous compound decomposed above 280 °C.

The yield of **4c**·6H_2O (H_4L^3·6H_2O) 1.42 g (80%). TLC (A): R_F 0.4; m.p. 256–259 °C (dec.). For $C_{14}H_{46}N_4O_{12}P_2$ (524.5) calculated: 32.05% C, 8.84% H, 10.68% N; found: 32.44% C, 8.54% H, 10.49% N. ^{1}H NMR (1 M KOD): 1.71 m, 4 H (CH$_2$C**H**$_2$CH$_2$); 2.23 s, 6 H (NC**H**$_3$); 2.50 t, 4 H, ^3J(H-H) = 6.8 (CH$_2$CH$_2$C**H**$_2$NMe); 2.55 t, 4 H, ^3J(H-H) = 7.7 (CH$_2$C**H**$_2$NMe); 2.62 d, 4 H, ^2J(P-H) = 12.0 (NC**H**$_2$P); 2.76 t, 4 H, ^3J(H-H) = 8.2 (P~NC**H**$_2$CH$_2$CH$_2$); 2.87 t, 4 H, ^3J(H-H) = 7.7 (P~NC**H**$_2$CH$_2$). ^{31}P{^{1}H} NMR (D_2O): 21.59; ^{31}P NMR (H_2O, borate buffer pH 9.18): 18.77 t, ^2J(P-H) = 10.4. TG analysis: The compound dehydrates in the range 80–120 °C in two distinct steps; weight loss 20.01% (theory for 6 H_2O 20.60%); the anhydrous compound decomposed above 260 °C.

4-Methyl-1,4,8,10-tetraazacyclotetradecane-1,8-di(methylphosphonic Acid) (**4d**, H_4L^4)

Compound **4b**·8H_2O (H_4L^2·8H_2O; 0.25 g, 0.4 mmol) was dissolved in water (8 ml) and acidified with concentrated formic acid (1 ml). Pd/C (10%, 0.05 g) was added and the mixture was stirred and kept under pressure of hydrogen in a rubber balloon for 48 h. The suspension was filtered and the filtrate evaporated to dryness. The residue was dissolved in water (5 ml) and precipitated by slow addition of acetone. This procedure was repeated once again to obtain pure product. It was filtered, washed with acetone and dried in air. The yield of **4d**·5.5H_2O (H_4L^4·5.5H_2O) 0.19 g (95%). TLC (A): R_F 0.4; m.p. 256–260 °C (dec.). For $C_{13}H_{43}N_4O_{11.5}P_2$ (501.5) calculated: 31.11% C, 8.66% H, 11.17% N; found: 31.17% C, 8.75% H, 11.32% N. ^{1}H NMR (1 M KOD): 1.72 br, 4 H (CH$_2$C**H**$_2$CH$_2$); 2.21 s, 3 H (NC**H**$_3$); 2.50 t, 2 H, ^3J(H-H) = 6.4 (CH$_2$CH$_2$C**H**$_2$NMe); 2.53 t, 2 H, ^3J(H-H) = 7.6 (CH$_2$C**H**$_2$NMe); 2.61 d, 2 H, ^2J(P-H) = 11.6 (Me~NC**H**$_2$P); 2.63 d, 2 H, ^2J(P-H) = 11.2 (H~NC**H**$_2$P); 2.69–2.72 m, 6 H (NHC**H**$_2$CH$_2$ + MeNCH$_2$CH$_2$C**H**$_2$ + NHC**H**$_2$CH$_2$CH$_2$); 2.76 t, 2 H, ^3J(H-H) = 8.2 (NCH$_2$CH$_2$C**H**$_2$); 2.84–2.88 m, 4 H (P~NC**H**$_2$CH$_2$). ^{31}P{^{1}H} NMR (1 M KOD): 16.24 (Me~NCH$_2$P); 16.36 (H~NCH$_2$P). TG analysis: The compound dehydrates in the range 30–110 °C in two distinct steps; weight loss 20.00% (theory for 5.5 H_2O 19.76%); the anhydrous compound decomposed above 260 °C.

Diethyl 4,11-Dibenzyl-1,4,8,10-tetraazacyclotetradecane-1-(methylphosphonate) (**5**) and Tetraethyl 4,11-Dibenzyl-1,4,8,10-tetraazacyclotetradecane-1,8-di(methylphosphonate) (**6**)

Diethyl phosphite (1.15 g, 7.8 mmol) and amine **3a** (2.00 g, 5.2 mmol) were dissolved in dry benzene (60 ml) in a flask equipped with the Dean–Stark apparatus. Under reflux and vigorous stirring, paraformaldehyde (0.31 g, 10.4 mmol) was added in small portions to the solution over 3 h and the mixture was refluxed for 4 h. The reaction was followed by TLC (B). It showed high conversion of the starting amine **3a** to diethyl ester **5** and tetraethyl ester **6** as major components and to a minor component with R_F about 0.2. The mixture was evaporated and separated by chromatography on silica (B) to give tetraethyl ester **6** (R_F 0.9), diethyl ester **5** (R_F 0.6) and starting amine **3a** (R_F 0.35).

Pure diester **5** was obtained as a clear oil. Yield 1.16 g (42% based on starting amine **3a**). For $C_{29}H_{47}N_4O_3P$ (530.7) calculated: 65.57% C, 8.94% H, 10.54% N; found: 65.30% C, 8.76% H, 10.20% N. ^{1}H NMR ($CDCl_3$): 1.26 t, 6 H, ^3J(H-H) = 7.2 (OCH_2CH_3); 1.70 m, 2 H ($CH_2CH_2CH_2$); 1.81 br, 2 H, ($CH_2CH_2CH_2$); 2.44–2.48 m, 2 H; 2.51–2.56 m, 4 H; 2.61 br, 2 H; 2.71 br, 2 H; 2.77 br, 4 H; 2.82 d, 2 H, ^2J(P-H) = 9.2 (NCH_2P); 2.85 br m, 2 H; 3.62 br d, 2 H (NCH_2Ph); 4.01 m, 4 H ($POCH_2CH_3$); 7.2–7.4 m, 5 H (C_6H_5). $^{31}P\{^1H\}$ NMR ($CDCl_3$): 24.6.

Pure oily tetraester **6** afforded after chromatographic purification (see above) was dissolved in acetone (10 ml) and water (5 ml). Acetone was slowly evaporated on a rotary evaporator to give **6** as tiny white leaves insoluble in remaining water. It was centrifuged, washed with water and dried in a vacuum desiccator over P_2O_5. The yield of **6** was 0.94 g (27% based on **3a**). M.p. 83–84 °C. For $C_{34}H_{58}N_4O_6P_2$ (680.8) calculated: 59.98% C, 8.59% H, 8.23% N; found: 60.29% C, 8.51% H, 8.56% N. ^{1}H NMR ($CDCl_3$): 1.26 t, 12 H, ^3J(H-H) = 7.2 (OCH_2CH_3); 1.66 m, 4 H ($CH_2CH_2CH_2$); 2.47 t, 4 H, ^3J(H-H) = 6.6; 2.59 t, 4 H, ^3J(H-H) = 6.4; 2.70 t, 4 H, ^3J(H-H) = 7.0; 2.77 d, 4 H, ^2J(P-H) = 8.2 (NCH_2P); 2.81 t, 4 H, ^3J(H-H) = 6.4; 3.55 s, 4 H (CH_2Ph); 4.03 m, 8 H ($POCH_2CH_3$); 7.20–7.35 m, 10 H (C_6H_5). $^{31}P\{^1H\}$ NMR ($CDCl_3$): 24.1.

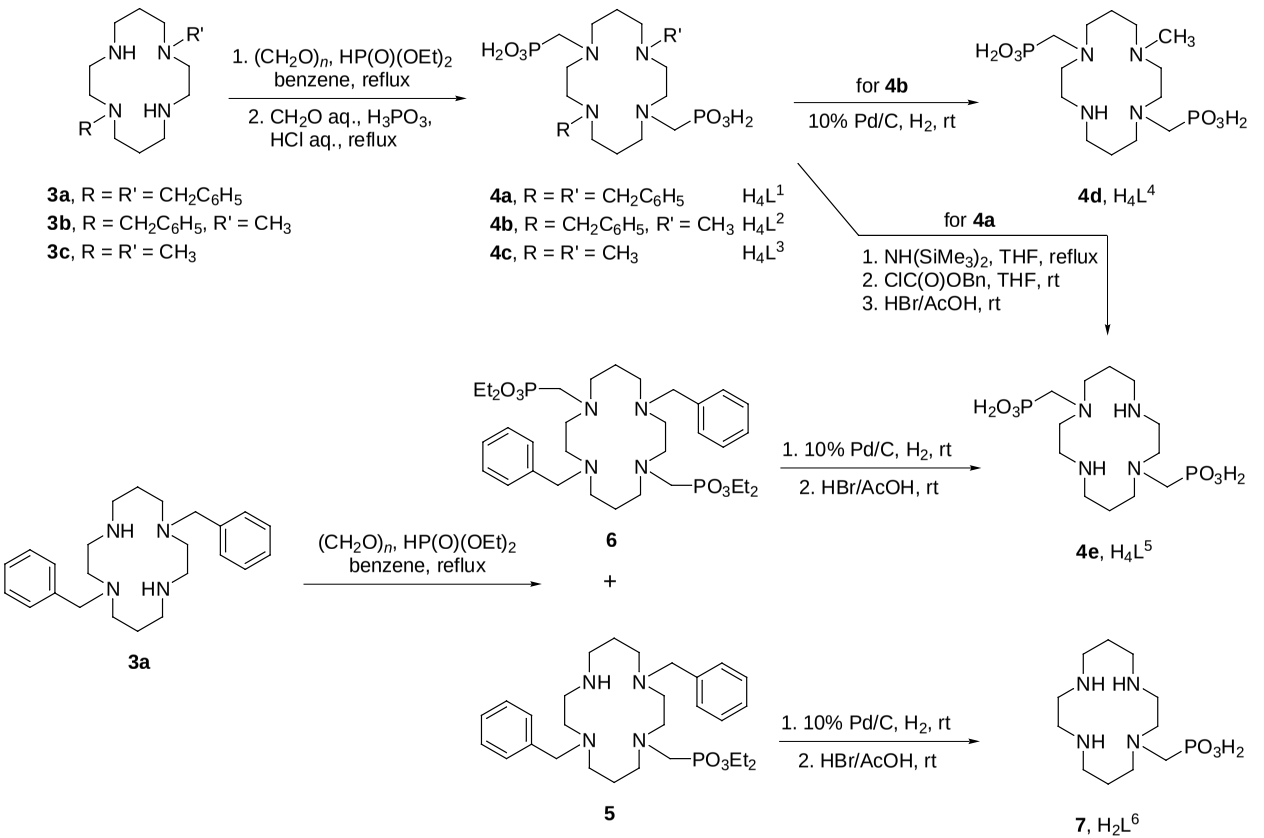
1,4,8,11-Tetraazacyclotetradecane-1-(methylphosphonic Acid) (**7**, H_2L^6)

Diester **5** (500 mg, 0.94 mmol) was dissolved in EtOH (10 ml), acidified with concentrated formic acid (1 ml) and 10% Pd/C (0.1 g) was added. The suspension was stirred and kept under pressure of hydrogen in a rubber balloon for 48 h. Palladium was filtered off and ethanol was evaporated *in vacuo*. The residual oil was dissolved in 30% HBr/AcOH (5 ml) and stirred overnight. The solvent was evaporated and the residue was dissolved in water (2 ml), decolorised with charcoal and evaporated to dryness. The solid residue was dissolved in concentrated HBr (2 ml) and the product was precipitated with EtOH to give 570 mg of $H_2L^6 \cdot 3.5HBr \cdot 2.5H_2O \cdot 0.5C_2H_5OH$ (95%). TLC (A): R_F 0.2; m.p. 195–198 °C (dec.). For $C_{12}H_{38.5}Br_{3.5}N_4O_6P$ (645.6) calculated: 22.31% C, 6.02% H, 43.31% Br, 8.67% N; found: 22.48% C, 5.42% H, 42.25% Br, 8.91% N. ^{1}H NMR (D_2O): 2.01 and 2.24 2 × m, 4 H ($CH_2CH_3CH_2$); 2.79 t, 2 H, ^3J(H-H) = 6.4; 2.81 d, 2 H, ^2J(P-H) = 11.2 (NCH_2P); 3.04–3.06 m, 2 H; 3.30 t, 2 H, ^3J(H-H) = 5.6; 3.34–3.39 m, 4 H; 3.52–3.63 m, 8 H. $^{31}P\{^1H\}$ NMR (D_2O): 19.70.

RESULTS AND DISCUSSION

Syntheses

Arms bearing phosphonate group were attached using the Mannich reaction between appropriate macrocyclic amine, formaldehyde and a phosphorus precursor with P–H bond (Scheme 1). The optimum way to reduce the extent of the main side reaction, reductive *N*-methylation of the secondary amine[24], seems to use aqueous HCl (1 : 1) as a solvent at a moderate temperature[25]. Unfortunately, 1,8-dibenzylcyclam **3a** was almost insoluble in aqueous HCl (1 : 1). Therefore, Mannich reaction of **3a** under anhydrous



3a, R = R' = $CH_2C_6H_5$
3b, R = $CH_2C_6H_5$, R' = CH_3
3c, R = R' = CH_3

4a, R = R' = $CH_2C_6H_5$ H_4L^1
4b, R = $CH_2C_6H_5$, R' = CH_3 H_4L^2
4c, R = R' = CH_3 H_4L^3

4d, H_4L^4

6

4e, H_4L^5

3a

5

7, H_2L^6

SCHEME 1

conditions had to be used. It should be noted that the quality of paraformaldehyde used is a crucial point for success of such reactions. The most reactive form found was obtained from old aqueous formaldehyde solution; commercial samples were less suitable. The reaction in benzene proceeded in two steps. With excess of paraformaldehyde and diethylphosphite in the reaction, reductive methylation took place in the extent of 10–15%. Unfortunately, tetraethyl ester **6** could not be separated from *N''*-methyl *N*-monophosphonylated ester (N-C**H**$_3$ 3.1 ppm in $CDCl_3$), as well as free acid **4a** (H_4L^1) contaminated with *N''*-methyl derivative (obtained after hydrolysis in reflux in aqueous HCl (1 : 1) for 12 h or treatment with 30% HBr/AcOH for 12 h). However, the pure tetraester **6** together with diester **5** were obtained if the reaction was stopped before consuming of the starting amine. Finally, acid **4a** (H_4L^1) was obtained by the reaction in benzene and followed by phosphonylation in water. The reaction in benzene was stopped after partial phosphonylation and esters in the mixture were *in situ* hydrolysed; thus, intermediates became soluble in 1 : 1 hydrochloric acid, and the preparation was finished in this solvent with excess of H_3PO_3 and formaldehyde. Surprisingly, it was observed that pure diethyl ester of **5** was extensively decomposed to the starting amine **3a** and (hydroxymethyl)phosphonic acid in hydrolysis in refluxing with HCl (1 : 1) or using Me_3SiBr in acetonitrile at room temperature. In spite of decomposition of intermediate **5**, synthesis of **4a** (H_4L^1) in aqueous HCl was successful as **4a** was again consumed in the Mannich reaction. Compounds **4b** (H_4L^2) and (**4c**, H_4L^3) were prepared under similar conditions (Scheme 1).

The protecting benzyl group is usually removed using hydrogenation, which was commonly used in synthesis of simple (aminoalkyl)phosphonic or phosphinic acids. Unfortunately, **4a** (H_4L^1) is soluble only in hot water, in cold water above pH 5 or in warm formic acid. Attempts to remove benzyl groups in **4a** (H_4L^1) by transfer hydrogenation with HCOOH on 10% Pd/C at 40 °C as well as hydrogenation in neutral water solution led to cleavage of the C–P bond and quantitative recovery of 1,8-dimethylcyclam. Another method for removal of benzyl group from amines is the reaction with chloroformates[26]. Preliminary results on deprotection of tetraethyl ester **6** using chloroformates indicated that a number of by-products were reduced. The desired product was finely obtained using trimethylsilyl ester prepared *in situ* from the free acid and hexamethyldisilazane. The use of silyl ester group seems to be critical for the deprotection as no conditions were found for clean deprotection of esters **5** and **6** with ClCOOEt or ClCOOBn. Bonds in the N–C–P moiety in esters **5** or **6** are weakened by influence of the electronegative amine nitrogen atoms whereas the silyl

group is an electropositive electron-donating group, thus, strengthening the N–C–P bonds. Bis(phosphonic acid) **4e** (H_4L^5) could be easily separated from small amounts of by-products by chromatography on a carboxylate cation exchange resin as they are not eluted from the resin with water. Because of high solubility of **4b** (H_4L^2) in acidified water, its benzyl group was easily removed by hydrogenation on 10% Pd/C to give **4d** (H_4L^4).

In search for optimum synthesis of pure ester **6**, we also obtained pure ester **5**. Both esters **5** and **6** were hydrogenated in EtOH to remove benzyl groups; subsequently, the esters were hydrolysed without characterisation with 30% HBr/AcOH to give hydrobromides of **4e** (H_4L^5) and **7** (H_2L^6), respectively. This procedure is a route for synthesis of a new ligand **7** (H_2L^6) but is not convenient for synthesis of **4e** (H_4L^5) due to a low overall yield.

Crystal and Molecular Structures

Solving structures, we wanted to confirm protonation of the nitrogen atoms and conformation of the rings. Of the compounds studied, **4a** (H_4L^1), **4b** (H_4L^2), **4c** (H_4L^3) and **4e** (H_4L^5) were found to form single crystals suitable for X-ray analysis. Molecular structures of **4a** (H_4L^1), **4b** (H_4L^2), **4c** (H_4L^3) and **4e** (H_4L^5) are shown with the numbering scheme in Fig. 1 and the crystal packing of **4a**·$9H_2O$ ($H_4L^1 \cdot 9H_2O$) and **4e**·$4H_2O$ ($H_4L^5 \cdot 4H_2O$) are depicted in Figs 2 and 3. Tables II and III list selected bond distances and angles for all the compounds.

In the crystalline state, all the compounds exist as zwitterions with two protonated ring nitrogen atoms not bearing the methylphosphonic acid moiety. The structures of symmetrically substituted molecules contain the centres of symmetry. A comparison of bond distances and angles in the rings shows that the ring conformation for all the structures is virtually the same as the common (3,4,3,4)-*B* conformation of the cyclam ring[2] which was also observed for **1c**·$4H_2O$ ($H_4tetp^{Ph} \cdot 4H_2O$) and $(AdNH_3)_2$**1c**·$6H_2O$ ($(AdNH_3)_2(H_2tetp^{Ph}) \cdot 6H_2O$)[30]. As observed in structures of the mentioned phosphinic acid derivatives, the ring conformations are stabilised by hydrogen bonds N–H···O2 in the range of 2.64–2.87 Å. In addition, the N1···N4 distances range from 2.82 to 2.93 Å, which would point to additional hydrogen bonds. However, the angles N1–H···N4 are less than 110° (100–110°) and, hence, we assume only its weak contribution to stabilisation of the conformation. The shortest intramolecular hydrogen bonds were found in dimethyl derivative **4c** (H_4L^3, Table III).

The coordination around the phosphorus atoms significantly deviates from the regular tetrahedron. In the structures, the P–O distances are

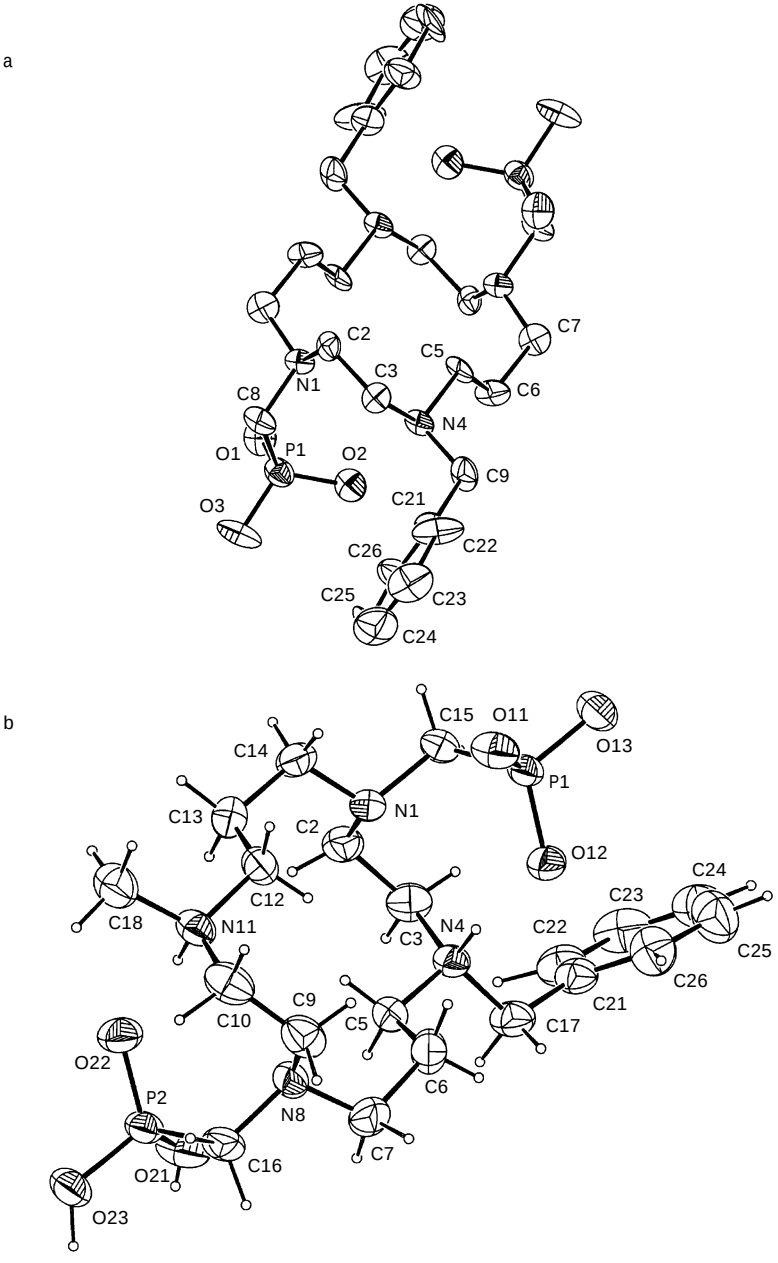
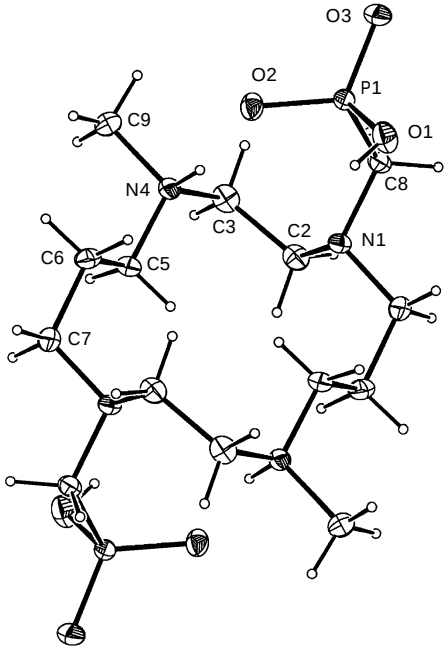


FIG. 1

Molecular structure of **4a** (H_4L^1) (a), **4b** (H_4L^2) (b), **4c** (H_4L^3) (c) and **4e** (H_4L^5) (d) with the numbering scheme

c



d

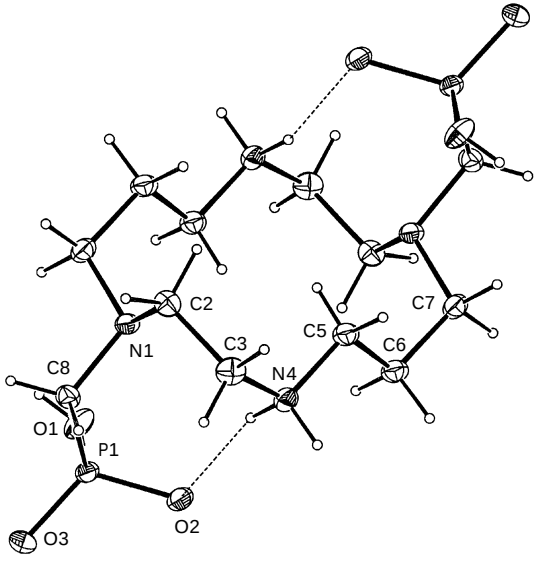


FIG. 1
(*Continued*)

TABLE II
The geometry on phosphorus atoms (selected bond lengths, Å and angles, °)

Parameter	**4a** (H_4L^1)	**4b** (H_4L^2; P1)	**4b** (H_4L^2; P2)	**4c** (H_4L^3)	**4e** (H_4L^5)
P–O(H)	1.535(7)	1.546(2)	1.537(2)	1.577(1)	1.563(2)
P–O···(N)	1.534(7)	1.502(2)	1.496(2)	1.508(1)	1.514(2)
P–O	1.486(7)	1.532(2)	1.532(2)	1.501(1)	1.505(2)
P–C	1.798(8)	1.809(2)	1.800(3)	1.818(1)	1.808(2)

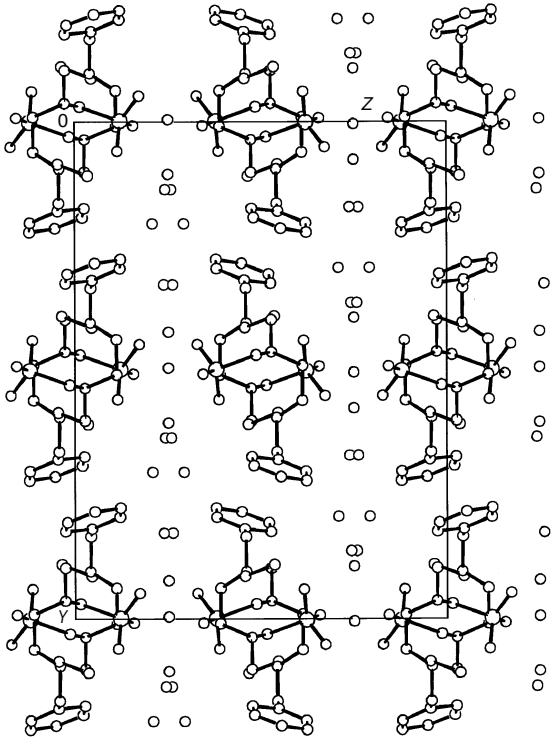


FIG. 2
Crystal packing of **4a**·$9H_2O$ ($H_4L^1 \cdot 9H_2O$)

strongly influenced by hydrogen bonds and also by protonation. The molecules in **4b**·8H_2O (H_4L^2·8H_2O) and **4e**·4H_2O (H_4L^5·4H_2O) are linked into chains through intermolecular hydrogen bond of about 2.55 Å between the P–O–H group and the P=O moiety of another molecule. In structures of **4a**·9H_2O (H_4L^1·9H_2O) and **4c**·6H_2O (H_4L^3·6H_2O), ligand molecules are linked through water molecules with H-bond distances 2.61–2.84 Å. These differences in the linking are shown in Figs 2 and 3 where crystal packing of **4a**·9H_2O (H_4L^1·9H_2O) and **4e**·4H_2O (H_4L^5·4H_2O) are depicted.

Protonation Constants

Protonation constants determined in this work are presented in Table IV and dissociation constants of our ligands are compared with those of related compounds in Table V. It is known that fully substituted acetate[2,27] and phosphonic acid[16,28] derivatives of cyclen, and partly also cyclam, form quite stable complexes with alkali metal ions (especially with Na^+) and, therefore, they would be titrated in the presence of non-complexing back-

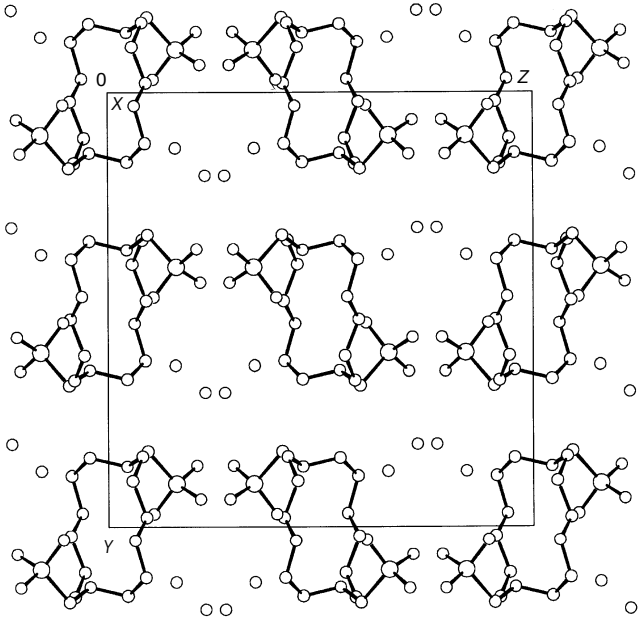


FIG. 3
Crystal packing of **4e**·4H_2O (H_4L^5·4H_2O)

TABLE III
Selected hydrogen bonds and angles

Parameter	**4a** (H_4L^1)	**4b** (H_4L^2; 1,4)	**4b** (H_4L^2; 8,11)	**4c** (H_4L^3)	**4e** (H_4L^5)
			Intramolecular bond		
d(N···O), Å	2.72(1)	2.732(4)	2.701(4)	2.645(2)	2.875(4)
(N–H···O), Å	–	171(1)	166(1)	174.7(8)	160(1)
			Intermolecular bonds		
			(P)O–H···$O^{\#}$–(P)		
d(O···$O^{\#}$), Å	–	2.536(4)	2.546(4)	–	2.554(4)
(O–H···$O^{\#}$), °	–	176(1)	168(1)	–	165(1)
			(P)O–H···O_w		
d(O···O_w), Å	2.65(1)	–	–	2.620(3)	–
(O–H···O_w), °	–	–	–	174.7(2)	–

$^{\#}$ Atom of a neighbouring molecule.

TABLE IV
Protonation constants of bis(methylphosphonic acid) cyclam derivatives (0.1 M KNO_3, 25 °C)

Constants	**4a** (H_4L^1)	**4b** (H_4L^2)	**4c** (H_4L^3)	**4d** (H_4L^4)	**4e** (H_4L^5)
log β_1	10.53(2)	10.87(2)	11.47(1)	–	–
log β_2	21.212(7)	22.289(4)	23.642(3)	24.82(2)	26.41(1)[a]
log β_3	28.31(1)	29.53(1)	30.840(6)	32.57(4)	33.19(1)
log β_4	34.75(1)	35.918(8)	37.166(5)	38.16(4)	38.55(2)
log β_5		37.51(2)	38.69(1)	39.79(4)	39.70(3)
log β_6		38.5(1)	39.54(8)		

[a] Determined using NMR titration (see Experimental).

ground cations like tetraalkylammonium ions. The complexation is mainly caused by arrangement of pendant arms suitable for such complexation and, for phosphonic acid derivatives, also by high negative charge of anions. In this case, because of structure of the ligands (cyclam skeleton) and strong intramolecular hydrogen bonds, we can suppose that K^+ used in the background electrolyte does not form so stable complexes to influence values of protonation constants determined here. In addition, the high values of pK_1 and pK_2 themselves point to no interactions of K^+ with the ligands. The last two constants **4e** (H_4L^5) with values of pK_A > 12.5 were determined by means of NMR titration experiments. The acid constants of **4a** (H_4L^1) could not be determinated due to insolubility of the compound below pH ≈ 5.

On the basis of a comparison with literature data for similar ligands[15,16,30,31], of the values of the constants, of molecular structures of **4a**

TABLE V
Comparison of dissociation constants of bis(methylphosphonic acid) derivatives of cyclam and similar ligands

Ligand	pK_1	pK_2	pK_3	pK_4	pK_5	pK_6
4a(H_4L^1)	10.53	10.68	7.10	6.44		
4b(H_4L^2)	10.87	11.42	7.24	6.38	1.60	1.0
4c(H_4L^3)	11.47	12.17	7.20	6.33	1.52	0.85
4d(H_4L^4)	24.82		7.75	5.59	1.63	
4e(H_4L^5)	26.41		6.78	5.36	1.15	
1b(H_8tetp)[a]	26.1		8.82	7.75	6.25	5.42
1a(H_4teta)[b]	10.52	10.17	4.09	3.35		
1c(H_4tetp^{Ph})[c]	9.85	9.94	1.85			
2b(H_8dotp)[a]	13.7	12.2	9.28	8.09	6.12	5.22
2e(H_4do2p)[d]	12.80	10.72	8.47	6.39		
2a(H_4dota)[e]	11.74	9.76	4.68	4.11	2.37	
2d(H_2do2a)[f]	11.38	9.62	3.95	2.62		
2c(H_4dotp^{Ph})[c]	11.44	7.27	2.75	1.45		

[a] Refs[11b,16] (0.1 M (Me_4N)NO_3, 25 °C). [b] Ref.[29a] (0.1 M (Me_4N)NO_3, 25 °C). [c] Ref.[30] (0.1 M KNO_3, 25 °C). [d] Ref.[31] (0.1 M KCl, 25 °C). [e] Ref.[29c] (0.1 M (Me_4N)NO_3, 25 °C). [f] Ref.[32] (0.1 M Me_4NCl, 25 °C).

(H_4L^1), **4b** (H_4L^2), **4c** (H_4L^3), **4e** (H_4L^5) and of NMR titrations of **4c** (H_4L^3) and **4e** (H_4L^5) (see below), we suggest a protonation scheme for the bis(methylphosphonic acid) ligands. The first two protons are bound to the opposite nitrogen atoms similarly to **2b** (H_8dotp)[16,28], **1b** (H_8tetp)[16] and the tetraazatetraacetic macrocyclic ligands[33]. However, there are two non-equivalent nitrogen atoms and we assigned protonation sites to the nitrogens without phosphonic acid arms. Protonation of both nitrogen atoms is a highly concerted process as the abundance of monoprotonated form HL^{3-} is low for all the ligands (Fig. 4).

With the dissociations constants now discussed, we noticed the reversed order of pK_1 and pK_2 of **4a** (H_4L^1), **4b** (H_4L^2) and **4c** (H_4L^3) (Table V). The effect, however unsual, is a simple mathematical consequence of low abundance of HL^{3-} species in the equilibria and it can be explained by the distribution diagrams in Fig. 4. It could be seen that species $(HL^3)^{3-}$ is present at maximum abundance about 13%. With such abundance, the curve for the species crosses curves representing fully deprotonated $(L^3)^{4-}$ and diprotonated $(H_2L^3)^{2-}$ forms at –log [H^+] values (equal to values of pK_1 and pK_2) in points corresponding to the reverse order of constants. The mathematics has another two implications: (i) dissociation constants pK_1 and pK_2 would have an equal value only if abundance of the monoprotonated species were just 33% and (ii) dissociation constant pK_2 cannot be found if the maximum abundance of the HL^{3-} form is less than about 10% as, in such case, –log [H^+] (and the pH-metric titration curve) is influenced by the pres-

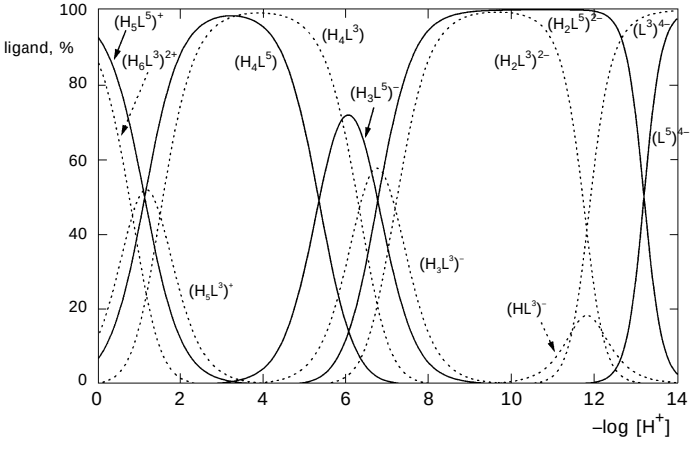


FIG. 4
Distribution diagrams of **4c** (H_4L^3, dotted line) and **4e** (H_4L^5, full line)

ence of the monoprotonated species only marginally and its influence on the $[H^+]$ change is below the sensitivity limit of potentiometry. The last example applies to **4d** (H_4L^4) and **4e** (H_4L^5) as only one constant over two deprotonations (protonations) could be determined. The reverse order of dissociation constants has been found for acid constants of cyclam itself[34] and its phenylphosphinic acid derivative **1c** (H_4tetp^{Ph})[30] and only one constant was also determined for last two deprotonations of **1b** (H_8tetp)[16]. The effect seems to be a characteristic feature of cyclam derivatives probably due to alternative arrangement of the ethylene and trimethylene chains between the nitrogen atoms in the ring. The protonated species of tetraazamacrocycles with acid pendant arms are stabilised by intramolecular hydrogen bonds (see crystal structures and ref.[30]).

The next two protons are shared by both the phosphonic moieties to give the other relatively stable zwitterion with overall charge equal to zero (Fig. 4). The next proton(s) should be bound to the remaining nitrogen atoms of the (aminomethyl)phosphonate moiety. The suggestion of nitrogen protonation by the fifth (and sixth) proton(s) is supported by a decrease in ^{31}P NMR shift of **4c** (H_4L^3) and **4e** (H_4L^5) at pH below 2 (see NMR part). The fifth proton could also be attached to the double-bonded phosphoryl oxygen, which is, however, inconvenient if we assume the presence of the intramolecular hydrogen bond. The suggestion is supported by determination of molecular structure of $(H_6L^3)^{2+}$ in crystal of **4c**·$2HCl·4H_2O$ ($H_4L^3·2HCl·4H_2O$)[35]. In the cation, all the ring nitrogen atoms are protonated and one proton is bound to each phosphonic acid moiety. A conformation of ring is changed and the intramolecular hydrogen bonds discussed above are not present.

Comparison of pK_A values of the ligands with acetate[29], phosphonic[16,28,31] and phosphinic[30,36a] acid derivatives of tetraazamacrocycles confirms the expected high overall basicity of phosphonic acid compounds. The higher basicity should imply high thermodynamic stability of complexes with appropriate metal ions. Constants of **4e** (H_4L^5) are very close to the corresponding constants of **1b** (H_8tetp; pK_1, pK_2, pK_5 and pK_6) which suggests a similar protonation scheme of both ligands. The values of the second protonation constants ($\log \beta_{21}$, protonation of both nitrogen atoms) of the ligands change with electronic effects of the substituents on the nitrogens without the methylphosphonic acid moiety ($(NH)_2$ > (NH)(NMe) > $(NMe)_2$ > (NBn)(NMe) > $(NBn)_2$), which is an indirect proof of the above protonation scheme. We could not assign sites of protonation in the asymmetric ligands due to a low abundance of HL^{3-} species but we can assume that the N–H group in $(L^4)^{4-}$ and N–Me group in $(L^2)^{4-}$ are protonated first.

NMR Titrations

Dependence of NMR spectra on pH should confirm protonation sites expected for this kind of ligands. Changes in chemical shifts for different nuclei can be used for assignment of sites of protonations[37]. It is well documented that δ_P of simple (α-, β- and γ-aminoalkyl)phosphonic acids[38], phosphonic acid complexones[39] and macrocycles[28,31,40] or phosphinic acids[14,36a,41] decreases with deprotonation of the phosphorus moiety with a steep increase in the phosphorus chemical shift while removing the last proton from the protonated α-amino group. The steep increase in δ_P is explained by breaking the intramolecular hydrogen bond(s) between protonated amino group and phosphonic (phosphinic) acid moiety[38]. The smallest value of δ_P in the titration curve pH–δ_P is assigned to the $NH^+(CH_2)_nP(O)(R)O^-$ (R = O^-, alkyl, or aryl; n = 1–3) zwitterionic moiety in which the hydrogen bond is stabilised to the largest extent. In ^{1}H NMR spectra, successive removal of protons from any acid or basic groups in amino acids usually causes a monotonic decrease in δ_H of an adjacent CH_n groups[37–40].

Measurements of NMR spectra in dependence on pH for the three most interesting compounds **4c** (H_4L^3), **4d** (H_4L^4) and **4e** (H_4L^5) show the presence of dynamic equilibria on NMR timescale at room temperature after attachment of two protons to the ligands (see Fig. 5 for **4e**). Because of the dynamic effects, ^{13}C NMR spectra could not be obtained and only partial assingment of ^{1}H NMR spectra could be made in neutral or acid solutions. The spectra are well resolved and could be fully assigned only in strongly alkaline solutions. The phosphorus NMR signals are sharp down to acid pH becoming progressively broader below pH about two (the effect is more pronounced for the dimethyl derivative).

The phosphorus chemical shift of both the symmetrical compounds (Fig. 6) increases in pH region 10–13 with attachment of the first two protons as well as in pH region 5–8 where two other protons are bound. The direction of the δ_P changes points to the protonation of phosphorus atoms; however, the phosphonate moiety is protonated only in neutral pH region. The most basic sites are nitrogen atoms. Protonation of the nitrogen atoms not bearing the methylphosphonic acid group was confirmed by the pH–δ_H dependence of the *N*-Me protons of **4c** (H_4L^3, Fig. 6) as well as by the ligand structure in the solid state. The chemical shift of the methyl group moves downfield with decreasing pH (pH 10–13) and is almost constant down to acid pH with only a small downfield shift due to protonation of distant phosphonic acid groups at neutral pH. The δ_H of methylene in the NCH_2P

moiety of the **4d** (H_4L^4, Fig. 6) and **4e** (H_4L^5, not shown) in alkaline solutions surprisingly decreases with lowering pH. The change is opposite to the common trend for δ_H of methylene in the NCH_2P moiety on protonation of the amine or phosphonate groups and could be caused by some conformational changes if we assume formation of intramolecular hydrogen bonds similar to those found in the solid state. The presence of the hydrogen bond can also explain the change of δ_P in the alkaline region as "protonation" of the phosphonic acid moiety through the hydrogen bond. The doublet of methylene protons in the NCH_2P moiety could not be resolved at neutral pH for the acids. A similar preferable protonation of the secondary amine groups was observed in aqueous solution for **2d** (H_2do2a) with the concomitant rigidifying of the structure of the diprotonated spe-

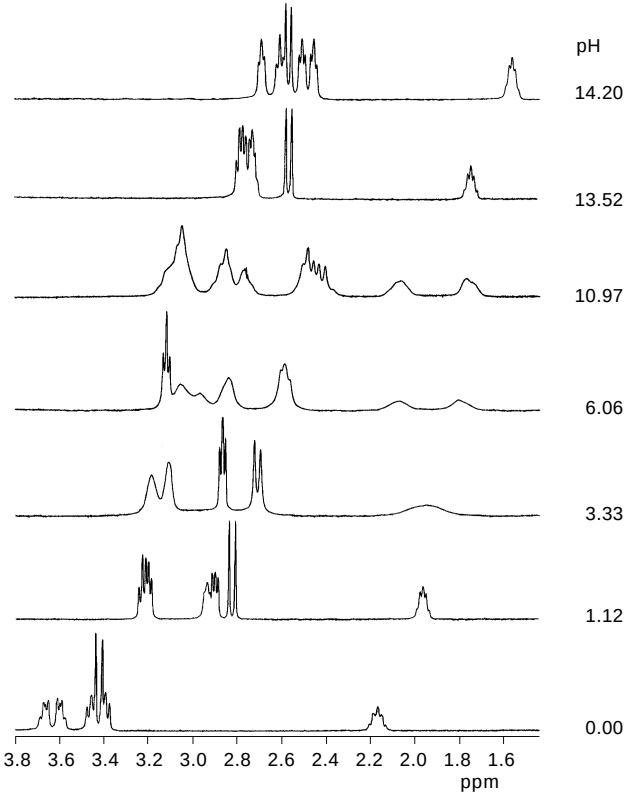


FIG. 5
^{1}H NMR spectra of **4e** (H_4L^5) at different pH and at 25 °C

cies[32]. However, **2e** (H_4do2p) is protonated in solution first on the tertiary nitrogen atoms of (aminomethyl)phosphonic acid groups forming again a structure stabilised by hydrogen bonds[31]. In both the compounds, the next two protons are attached to the phosphonic acid arms.

In acid solutions, δ_P steeply drops and the ^{31}P NMR signal becomes progressively broader. At the same time, the signal of NCH_2P protons of **4e** (H_4L^5) is shifted downfield much more than that of methyl protons **4c**

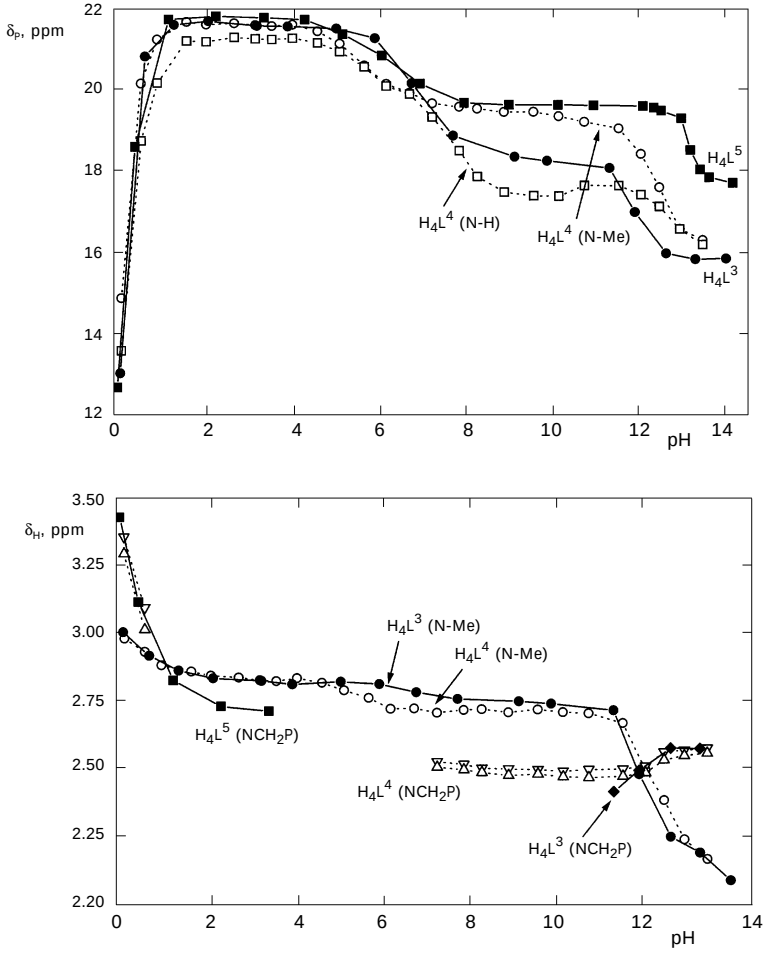


FIG. 6

Dependence of ^{31}P (a) and ^{1}H (b) chemical shifts on pH for **4c** (H_4L^3: ● (N–Me) and ◆ (NCH_2P)), **4d** (H_4L^4: ❑ (N–H), ❍ (N–Me) and Δ, ∇ (NCH_2P)) and **4e** (H_4L^5: ■ (NCH_2P)); for abundances of species, see Fig. 4

(H_4L^3) (for H_4L^3, the signal of NCH_2P protons could not be assigned). The changes could be explained by protonation of the nitrogen atom of the NCH_2P moiety (downfield shift of δ_P) with possible formation of a new or other hydrogen bond between the protonated α-amino and phosphonic acid groups (broadening of the signals) similar to that suggested for simple (α-aminoalkyl)phosphonic acids[38] or macrocyclic methylphosphonic acid derivatives[28,31,40]. Protonation of all ring nitrogens was observed in $(H_6L^3)^{2+}$ in the solid state (see above)[35].

For the unsymmetrical derivative **4d** (H_4L^4), the dependence of ^1H and ^{31}P NMR spectra on pH exhibits the same features as the spectra disscused above. The changes in the most alkaline region corresponding to NCH_2P moieties are similar. The third proton (pH 7–9) is probably attached to the phosphonate group adjacent to the secondary amino group as only its ^{31}P NMR signal is changed and the signal of the methyl group is not influenced (Fig. 6). In pH region 4–6.5, the signals of the methyl group as well as that of the adjacent phosphorus atom move to higher δ, which indicates protonation of the phosphonic group. This can be explained by different strengths of the stabilising hydrogen bonds. The hydrogen bonds are shorter in **4c** (H_4L^3) than in **4e** (H_4L^5) (Table III) and, therefore, they withdraw more the electron density from the adjacent phosphonic groups. Consequently, the phosphonic group close to *N*-Me became less basic.

Broadening of ^1H NMR signals after protonation of two nitrogen atoms suggests some kind of dynamic equilibria. Therefore, in a preliminary study, we measured NMR spectra of **4e** (H_4L^5) at pH 10.97 in water (100% fomation of $(H_2L^5)^{2-}$) at different temperatures (0–90 °C, 10 °C steps). Coalescence of geminal protons of the central methylene group (δ_H around 2.0 and 1.8 ppm) in the trimethylene chain was observed between 50 and 60 °C. We were able to determine activation parameters of the process ($\Delta H^{\#}$ = 82(3) kJ mol^{-1} and $\Delta S^{\#}$ = 44(10) kJ mol^{-1} K^{-1} with E_a = 85(3) kJ mol^{-1}) by treatment of the variable–temperature data. If we assume that the process corresponds to rupture of the hydrogen bonds discussed above, then, $\Delta H^{\#}$ and $\Delta S^{\#}$ suggest a strong hydrogen bond and a transition from a highly ordered state, respectively. The high value of the activation energy is also in agreement with the high strength of hydrogen bonds present in the $(H_2L^5)^{2-}$ species.

The results presented here support our previous interpretation of solution behaviour for **2c** (H_4dotp^{Ph}) and **1c** (H_4tetp^{Ph})[30] where the presence of some stable conformations of the ligands was suggested and explained tentatively by the presence of an intramolecular hydrogen bond of the same kind like here and by hydrophobic interactions between phenyl substitu-

ents. But, at that time we did not have in hand direct confirmation of the presence of such hydrogen bonds in aqueous solutions.

CONCLUSIONS

We found a suitable route for synthesis of mono- and bis(methylphosphonic acid) derivatives of cyclam employing benzyl protection for secondary amino groups. Lability of (N–)C–P and N–C(–P) bonds during deprotection procedures was observed. Structures of the phosphonic acid ligands in the solid state reveal the presence of strong intramolecular hydrogen bonds between the phosphonic moiety and amino group over the adjacent ethylene chain. The hydrogen bond is present even in aqueous solution. It was confirmed by values of protonation constants as well as titration and variable–temperature NMR experiments. High basicity of our phosphonate ligands is a consequence of the hydrogen bond and not of spreading of electron density of the highly charged phosphonate group, which was used as an explanation of higher basicity of the amino group in simple (α-aminoalkyl)-phosphonic acids[42]. Intramolecular hydrogen bond was presented as an explanation of high protonation constants of phosphonic acid analogues of H_3nta and H_4edta[39c]. High basicity of the ligands suggests a high thermodynamic stability of their metal complexes; kinetic stability of the complexes should be also enhanced due to convenient steric arrangement and hexadenticity of the ligands. Investigations of complexing properties of the ligands are under way.

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