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A fully automated pH–NMR titration set-up for protonation studies

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A fully automated pH–NMR titration set-up, consisting of a Bruker 250 MHz NMR instrument and a potentiometric titration unit, has been built so that pH titrations with simultaneous recording of ^{1}H and ^{31}P NMR spectra at each titration point can be run. The new set-up has been tested by studying the protonation of three diazacrown ethers having dangling phosphonate groups. From the fitting of the pH dependences of the chemical shifts of the ^{31}P and ^{1}H signals the protonation constants as well as the chemical shifts of the individual protonated species were obtained using the program HYPNMR. The main advantages of the new set-up are the relatively small amount of substance (0.05 mmol) needed for a single titration and the fact that once started the system needs no operator during the whole titration time (about 20 h).

The determination of protonation and complex stability constants and thus the study of speciation in solution have reached a mature stage. Many powerful programs for the calculation of equilibrium constants from potentiometric pH titrations have been developed[1,2] and reviewed.[3] Although based on different mathematical approaches, all of them allow one to test different combinations of equilibrium constants to fit the experimental data and therefore find the "best" model which describes the system. With the "best" model the speciation, *i.e.* the concentrations of the species as a function of pH, can be calculated.

Similarly spectrophotometric titrations have been put on line with computers, so that fully automated data collection has become possible.[4] The fitting of the large amount of data can be done with programs,[5] which give at the same time the spectral properties (absorption maxima λ_{max} and molar absorptivities ε) and the equilibrium constants. Especially powerful are programs, which separate linear (ε values) from non-linear (K values) parameters so that entire spectra as a function of pH can be used for the calculation. A further important development and help in the interpretation of spectrophotometric titrations is the evolving factor analysis,[6] which allows one to determine the minimum number of species necessary to fit the data before any assumption on the model and use of the law of mass action has to be made.

If the system studied is relatively simple, generally one experimental method gives the final answer in this type of studies and the calculation has become more or less routine. However, for more complicated systems which include a large number of species, in particular dinuclear and polynuclear species, the use of one method is generally not sufficient to elucidate all detail and to give a complete picture. The combination of two or more techniques becomes then necessary, as examples from the literature have clearly shown.[7]

In addition, if one is not only interested in stabilities, stoichiometries and speciation but also in the structure of the species in solution, spectroscopic methods become of paramount importance. Beside UV–VIS spectroscopy (see above) NMR studies can also give detailed structural information; NMR is being used more and more frequently for the determination of protonation and stability constants[8–10] but the amount of time and substance needed generally does not allow the collection of a large number of data. In general many of these studies use the pH dependence of the chemical shifts in a qualitative way.

For NMR measurements there have been attempts to automate data collection during a titration[11] and programs have been developed for the calculation of equilibrium constants.[12,13] However, a complete and consistent set-up with a quantitative evaluation and tests against classical methods has not been presented. We have therefore developed now a fully automated pH–NMR titration set-up, which allows one to obtain at each titration point ^{31}P and ^{1}H NMR spectra with the same solution.

Experimental

pH–NMR instrument

The set-up for the automatic pH–NMR titration consists of a pH titration unit and a 250 MHz Bruker NMR instrument equipped with a flow-through probe. The titration unit is run by a PC (286-AT) which controls the pH-meter (Metrohm 654), the burette (Metrohm 655) and the pump. The flow-through probe is a commercially available LC-probe (Bruker PH LCTXO250SB P/C-H-D-5 O) with a 5 mm cuvette and capillaries (0.25 mm) as inlets and outlets. The probe is equipped for ^{13}C, ^{1}H and ^{31}P nuclei. The coupling of the two instruments was done by installing a pump (Neusager NF 10, TTE, 20 ml min^{-1}), which circulates the solution from the thermostatted titration vessel through the probe, and by connecting the ASPECT3000 workstation of the NMR instrument with the PC through a RS232 communication interface (Fig. 1).

Once both computers have been initialized and the relevant running parameters have been introduced, the PC starts the titration by measuring and controlling the stability of the pH. If the pH is constant the pump is turned off and a message is sent through the RS232 interface to the ASPECT3000 which then starts the recording of one or more NMR spectra. This work done, a message is sent back (through the RS232) to the PC which turns on the pump, adds the next amount of base, measures and controls the pH, whereby the cycle is closed. Once the last titration point is reached the titration data (pH, ml) and the NMR spectra are stored and the job terminated.

The programs necessary to run the set-up were taken from

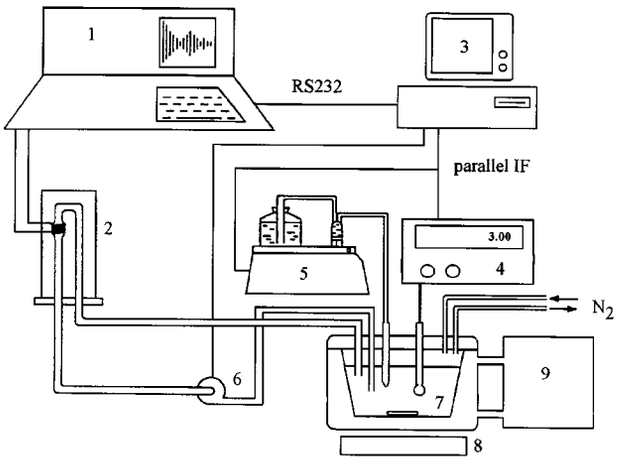


Fig. 1 Automated pH–NMR titration set-up: 1, Bruker NMR spectrometer (250 MHz); 2, flow-through probe for ^{1}H, ^{31}P, ^{13}C (Bruker); 3, 286-AT personal computer; 4, pH-meter (Metrohm 654); 5, burette (Metrohm 655); 6, pump (Neuburger, NF10, TTE); 7, titration vessel with pH-electrode, N_2 inlet and outlet; 8, magnetic stirrer; 9, thermostat.

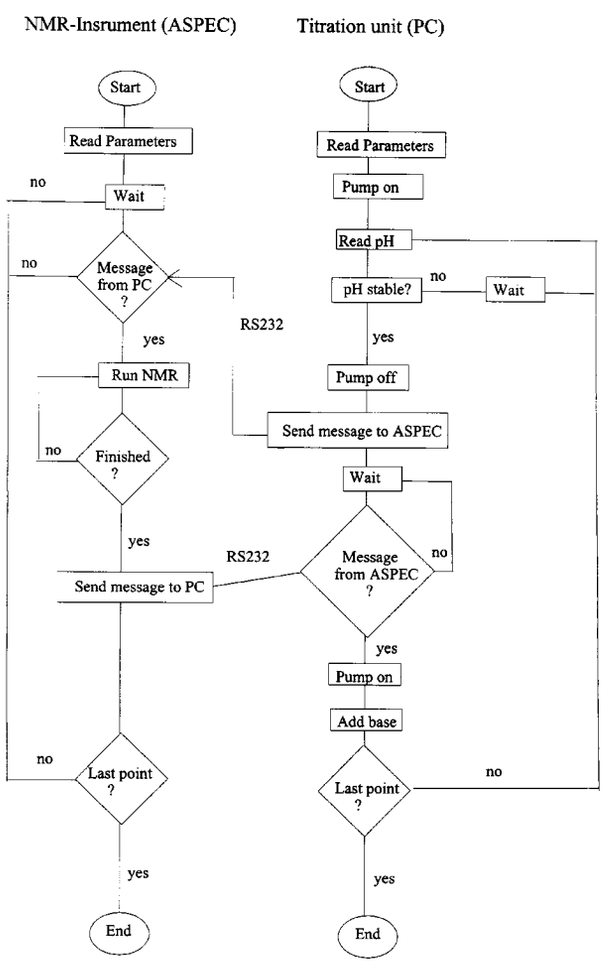


Fig. 2 Flow chart of the two programs running the automated pH–NMR titration set-up.

the literature for the ASPECT3000[11] and for the PC the program TITFIT[2] was modified so that the pump can be controlled and the communication interface RS232 to the ASPECT3000 can be activated, resulting in a new program TITNMR (written in turbo basic for DOS). The flow chart of the two programs and their coupling is given in Fig. 2.

Solutions of the ligands (5×10^{-3} mol dm^{-3}) for the pH–NMR titrations were made up in D_2O/H_2O (20%) for the ^{31}P measurements and in D_2O (99.8%) for the combined ^{1}H and ^{31}P NMR spectra. As base $[Et_4N]OH$ in water was used for the ^{31}P experiments and NaOD in D_2O for the combined spectra. pH Values measured in D_2O were calculated from the equation pH = pD − 0.4.[14] The ^{1}H chemical shifts are referred to 3-(trimethylsilyl)propanesulfonate and the ^{31}P shifts to 5% H_3PO_4 in D_2O, both used as external standards. In the fully automatic titration 10 ml of the ligand solution in the thermostatted vessel were titrated with 0.005 ml base addition up to 0.3 ml total base. For a combined ^{1}H and ^{31}P NMR measurement it takes about 20 h to run a complete titration.

Potentiometric measurements

pH Titrations were run on the automatic titrator previously described,[15] consisting of a Metrohm 605 pH-meter, a Metrohm 665 burette, a thermostatted titration vessel, and a 286-AT PC controlling the set-up. Calibration of the electrode and control titrations to check the calibration were done as previously described.[15] The activity coefficient of the proton, a_H, and the pK_w value were determined separately to be 0.95 and 13.92, respectively.

Ligand hydrochloride (1×10^{-3} mol dm^{-3}) was dissolved in 0.1 mol dm^{-3} $[Et_4N]NO_3$ and titrated with 0.1 mol dm^{-3} $[Et_4N]OH$, the exact concentration of which was determined using potassium hydrogenphthalate. Ligand solution (20 ml) was titrated with 0.01 ml base increments up to 1 ml total addition. The fitting of the curves was done with the program TITFIT,[2] whereby σ_{ml} was smaller than 2×10^{-3} ml. The results are mean values of two separate titrations.

Syntheses

The ligands were synthesized from their parent macrocycles by addition of CH_2O and H_3PO_3 and crystallized as hydrochlorides.[16]

4,10-Bis(phosphonomethyl)-1,7-dioxa-4,10-diazacyclododecane 1. Yield 65.5% (Found: C, 28.66; H, 6.63; Cl, 6.44; N, 6.62; P, 14.80; H_2O, 8.11. Calc. for $C_{10}H_{24}N_2O_8P_2 \cdot 0.75HCl \cdot 1.8H_2O$: C, 28.46; H, 6.77; Cl, 6.30; N, 6.64; P, 14.68; H_2O, 7.96%). ^{1}H NMR (D_2O): δ 3.89 (t, OCH_2, $^{3}J_{HH} = 4.56$); 3.45–3.67 (t, 4 NCH_2) and 3.495 (d, 2 NCH_2P, $^{2}J_{PH} = 12.17$ Hz). ^{13}C NMR (D_2O): δ 67.0 (OCH_2); 58.5 (d, NCH_2) and 55.7 (d, NCH_2P, $^{1}J_{PC} = 136.7$ Hz). ^{31}P NMR (D_2O): δ 11.01.

7,13-Bis(phosphonomethyl)-1,4,10-trioxa-7,13-diazacyclopentadecane 2. Yield 60.5% (Found: C, 30.28; H, 6.35; Cl, 13.76; N, 5.84; P, 13.40. Calc. for $C_{12}H_{28}N_2O_9P_2 \cdot 1.8HCl$: C, 30.52; H, 6.37; Cl, 13.51; N, 5.93; P, 13.12%). ^{1}H NMR (D_2O): δ 4.05–3.8 (m, 8H, NCH_2); 3.8–3.6 (m, 12H, OCH_2) and 3.47 (d, 4H, NCH_2P, $^{2}J_{PH} = 12.4$ Hz). ^{13}C NMR (D_2O): δ 72.46 (OCH_2-*C*H_2O); 66.02, 65.69 (O*C*H_2CH_2N); 58.07, 56.59 (OCH_2*C*H_2N) and 53.02 (d, NCH_2P, $^{1}J_{PC} = 8.4$ Hz). ^{31}P NMR (D_2O): δ 11.48.

7,16-Bis(phosphonomethyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane 3. Yield 49.4% (Found: C, 32.22; H, 7.05; Cl, 6.90; N, 5.27; P, 11.90; H_2O, 6.99. Calc. for $C_{14}H_{32}N_2O_{10}P_2 \cdot 2H_2O \cdot 1HCl$: C, 32.16; H, 7.13; Cl, 6.78; N, 5.36; P, 11.85; H_2O, 6.83%). ^{1}H NMR (D_2O): δ 3.84 (t, 4 NCH_2CH_2O), 3.69 (s, 4 OCH_2CH_2O); 3.62 (t, 4 NCH_2CH_2O) and 3.39 (d, 4 NCH_2P, $^{2}J_{PH} = 12.63$ Hz). ^{13}C NMR (D_2O): δ 71.2 (OCH_2CH_2O), 65.0 (O*C*H_2CH_2N); 55.8 (d, OCH_2CH_2N, $^{3}J_{PC} = 2.3$ Hz) and 52.1 (d, NCH_2P, $^{1}J_{PC} = 138$ Hz). ^{31}P NMR (D_2O): δ 11.53.

Results and discussion

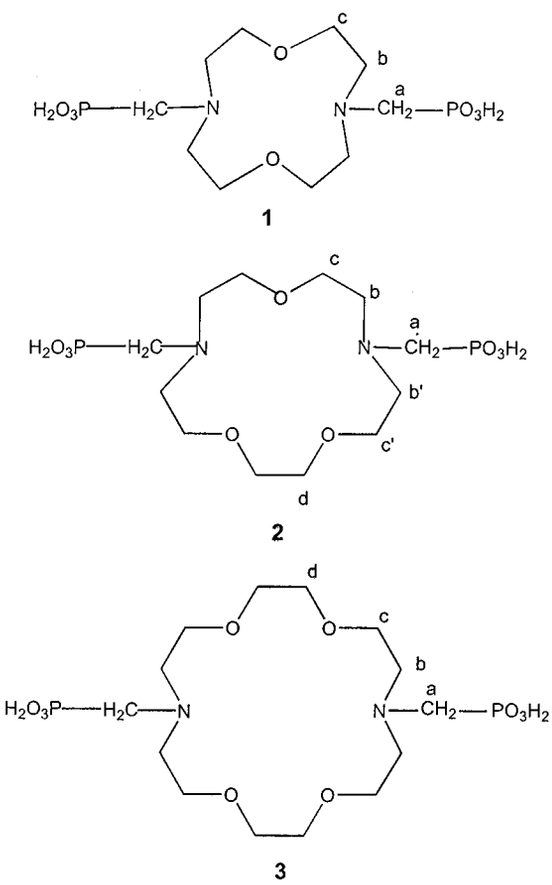
To test the new system, the three ligands **1**–**3** were studied, since they allow one to measure ^{1}H as well as ^{31}P NMR spectra as a function of pH.

To obtain the protonation constants to which the results of our new set-up could be compared with, the compounds were

Table 1 Protonation constants of the bis(phosphonomethyl)aza-crowns **1**–**3**

Ligand	log K_1	log K_2	log K_3	log K_4	Method
1	11.04(3)	9.36(2)	5.80(1)	4.56(1)	Pot.[a]
	10.70(1)	9.54(1)	5.81(8)	4.48(3)	$^1H/^{31}P$[b]
2	10.87(1)	9.76(1)	6.03(1)	4.69(1)	Pot.[a]
	10.30(4)	9.94(3)	5.43(1)[c]	4.38(1)	$^1H/^{31}P$[b]
	10.82	9.81	5.96	4.73	Pot.[d]
3	10.55(2)	9.25(2)	5.76(1)	4.85(1)	Pot.[a]
	10.51(3)	9.28(3)	5.64(3)[c]	4.61(6)	^{31}P[b]
	10.58(1)	9.35(1)	5.58(1)[c]	4.67(1)	$^1H/^{31}P$[b]
	10.96	9.35	5.76	4.89	Pot.[d]

[a] At 25 °C, $I = 0.1$ mol dm^{-3} ($[NMe_4]NO_3$). [b] At 25 °C, no control of ionic strength. [c] Calculated keeping δ_{LH3} fixed. [d] At 25 °C, $I = 0.1$ mol dm^{-3} ($[NMe_4]Cl$).[9]



first titrated under controlled conditions using $[Et_4N]OH$ as base in the fully automatic potentiometric titrator described previously.[15] The data were fitted with TITFIT[2] giving two high and two low log K^H values (Table 1). The results for **2** and **3** are in good agreement with the literature.[9] Interesting is that $\Delta\log K_{1,2} = \log K_1 - \log K_2$ and $\Delta\log K_{3,4} = \log K_3 - \log K_4$ are for all ligands distinctly larger than the statistical value of 0.6, indicating that the protonation of the two ammonium and phosphonate groups are not independent from each other.

In a second experiment a combined pH–NMR titration of compound **3** in 20% D_2O/H_2O and $[Et_4N]OH$ as base was run. After each base addition to the titration solution the pH was checked for stability, then the ^{31}P NMR spectrum was recorded. The chemical shifts of the ^{31}P signal as a function of pH [Fig. 3(a)] were determined using the peak search subroutine of WinNMR.[17] These data were transferred to HYPNMR[13] and the fitting gave four log K^H values (Table 1) in addition to the chemical shifts of the individual species (δ_{LHn}). Since log K_3 and log K_4 strongly correlate the calculation was only possible and converged after having fixed δ_{LH3} to the mean value

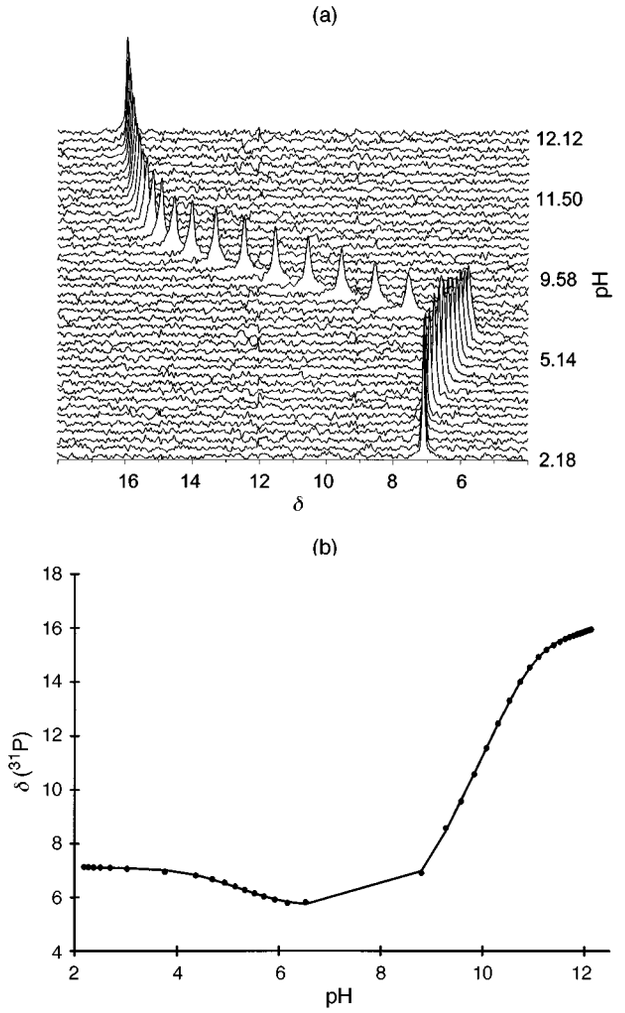


Fig. 3 (a) The ^{31}P NMR spectra of compound **3** as a function of pH. (b) δ_P as a function of pH: •, experimental points; —— function calculated with the parameters in the text.

between δ_{LH2} and δ_{LH4}. The quality of the fitting can be seen in Fig. 3(b). The log K^H values compare well with those obtained from potentiometry although measured in 20% D_2O. The change in chemical shift from δ_{LH4} 7.2 to δ_{LH2} 5.5 is a clear indication that the first two acidic protons (log K_3 and log K_4) stem from the deprotonation of the hydrogenphosphonate groups (PO_3H^-), whereas the change from δ_{LH2} 5.5 to δ_L 16.5 reflects the deprotonation of the ammonium groups.[18]

A third series of experiments consisted in the pH titrations of compounds **1**–**3** with simultaneous ^1H and ^{31}P NMR recording. In this case the titrations were done in D_2O with NaOD as base. After each addition of NaOD the pH was checked for stability, then the ^1H and ^{31}P spectra were recorded. The amount of data so obtained is very large and ideal for simultaneous fitting of all results at once, an option included in HYPNMR.[13] The results, corrected from pD to pH, are also shown in Table 1. The chemical shifts of each protonated species for ^{31}P and ^1H signals are given in Table 2. Here again the value of δ_{LH3} had to be fixed to the mean value of δ_{LH2} and δ_{LH4} because of the strong correlation between log K_3 and log K_4. For the three ligands **1**–**3** the ^{31}P signals behave all in the same way and therefore the order of protonation or deprotonation is as discussed above for **3** (Figs. 4–6). The ^1H signals show some additional interesting points. The largest pH-induced shift is observed for the doublet belonging to the CH_2 group in α position to the phosphonate group. In the pH region in which log K_1 and log K_2 play a role as well as in the alkaline region where log K_3 and log K_4 are important a continuous shift to higher frequency is found. The signal corresponding to the CH_2 groups beside

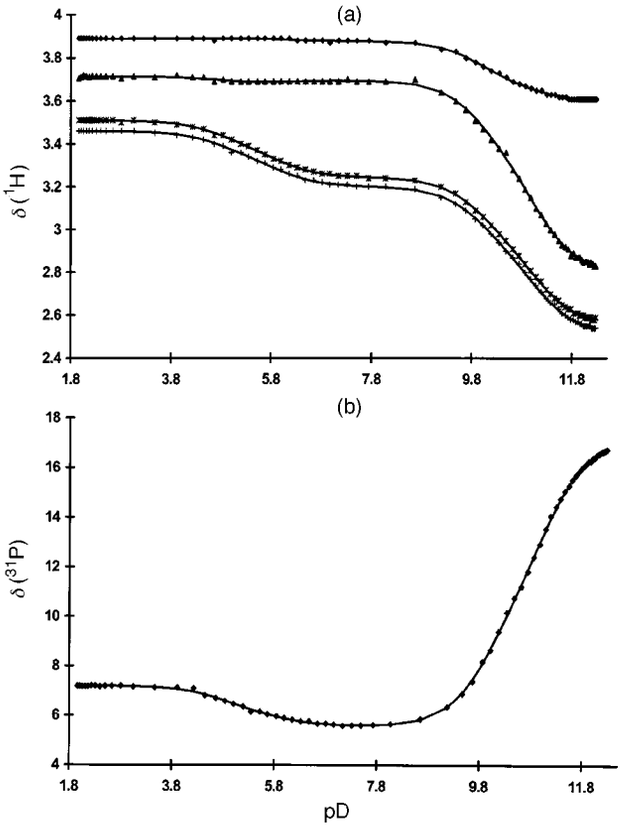


Fig. 4 (a) δ_H and (b) δ_P of compound **1** as a function of pH. (a) ◆ Hc, ▲ Hb, ∗ Ha, + Ha′ experimental points, —— function calculated with the parameters in the text. (b) ◆ experimental points, —— function calculated with the parameters in the text.

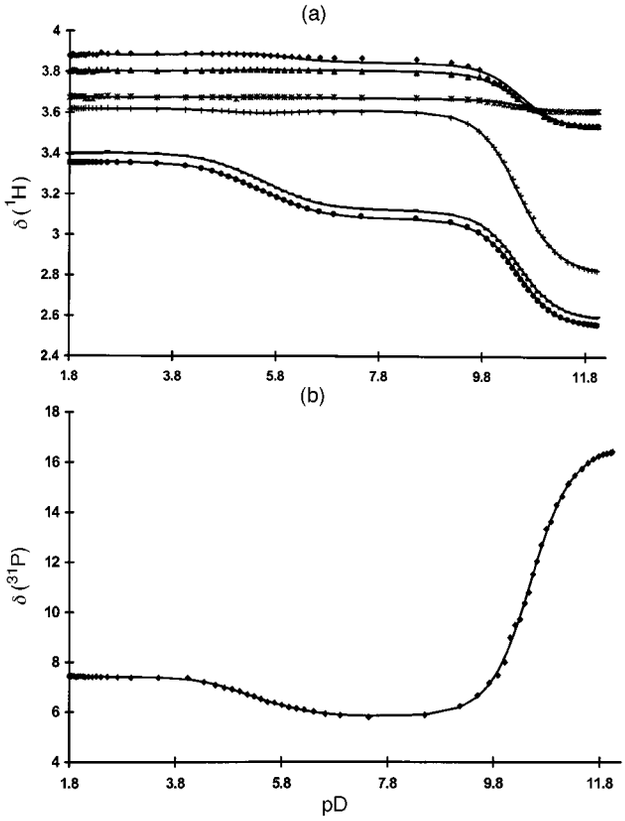


Fig. 5 (a) δ_H and (b) δ_P of compound **2** as a function of pH. (a) ∗ Hd, ◆ Hc′, ▲ Hc, + Hb, − Ha′, ● Ha experimental points, —— function calculated with the parameters in the text. (b) ◆ experimental points, —— function calculated with the parameters in the text.

Table 2 Chemical shifts of the protonated species

Ligand		δ_P	δ_{Hd}	$\delta_{Hc'}$	δ_{Hc}	δ_{Hb}	δ_{Ha}	$\delta_{Ha'}$
1	H_4L	7.20			3.89	3.71	3.51	3.46
	H_3L	5.97			3.89	3.68	3.35	3.32
	H_2L	5.57			3.88	3.70	3.24	3.20
	HL	10.61			3.68	3.36	2.96	2.92
	L	17.17			3.60	2.80	2.55	2.51
2	H_4L	7.43	3.89	3.80	3.67	3.62	3.41	3.36
	H_3L^a	6.61	3.87	3.81	3.68	3.61	3.26	3.22
	H_2L	5.81	3.87	3.81	3.68	3.60	3.14	3.10
	HL	11.59	3.60	3.66	3.62	3.22	2.81	2.77
	L	16.63	3.54	3.53	3.61	2.80	2.59	2.55
3	H_4L	7.15	3.91		3.76	3.69	3.45	3.41
	H_3L^a	6.36	3.90		3.76	3.69	3.32	3.23
	H_2L	5.56	3.91		3.76	3.69	3.18	3.15
	HL	11.16	3.78		3.71	3.32	2.92	2.88
	L	16.13	3.41		3.66	2.93	2.65	2.61

[a] δ_{LH3} values fixed in the calculation.

the ammonium is only slightly shifted in the acidic part of the titration, but "feels" the deprotonation at high pH. The protons further away from the protonation sites show even smaller, sometimes practically no, shifts.

Whereas the log K_2–log K_4 values compare well with the potentiometric results, the log K_1 values obtained from NMR measurements are distinctly smaller. This is a consequence of the fact that in these measurements we use NaOD as base which involves the possibility of Na^+ complexation. This has been observed in similar compounds.[10] An exception is ligand **3** with the largest ring which seems not to interact with Na^+ at all and thus gives an unchanged log K_1 value.

The ^{1}H NMR spectrum of compound **1** at high pH consists of a doublet and two well resolved triplets as expected for a rapid inversion of the macrocyclic ring. At low pH, however, the magnetic degeneracy of the methylene groups adjacent to the

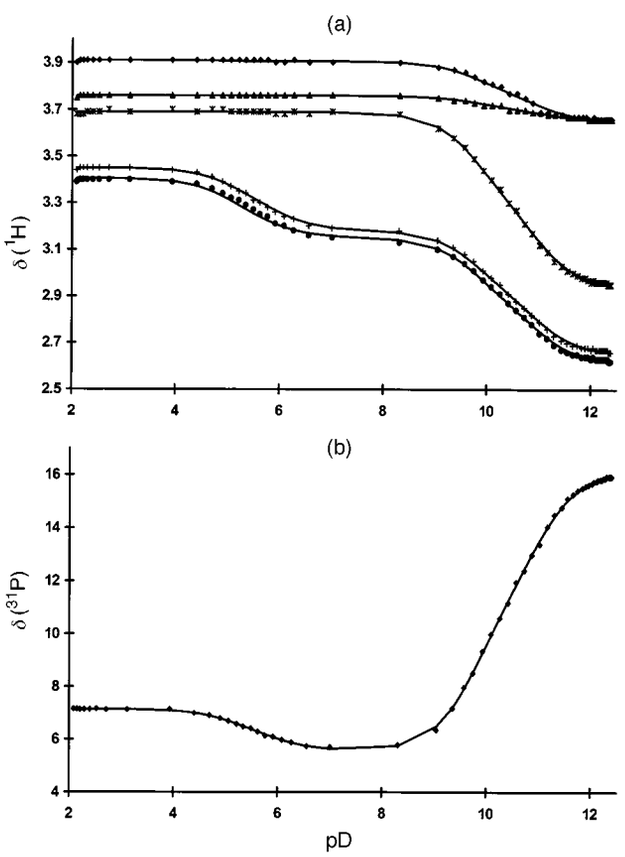


Fig. 6 (a) δ_H and (b) δ_P of compound **3** as a function of pH. (a) ◆ Hd, ▲ Hc, ∗ Hb, + Ha, ● Ha′ experimental points, —— function calculated with the parameters in the text. (b) ◆ experimental points, —— function calculated with the parameters in the text.

nitrogens is lifted, two triplets separated by 0.09 ppm being observed. This is consistent with a more rigid structure of the macrocycle, in which intramolecular hydrogen bonds are formed as discussed for similar compounds.[10]

In conclusion we can say that the fully automated pH–NMR titration set-up is an ideal instrument to follow chemical shifts as a function of pH. It allows one simultaneously to record spectra of different nuclei on the same solution and thus to collect a large amount of data from which not only the log K^{H} values but also the chemical shifts of the single species can be determined. For a complete titration with simultaneous ^{1}H and ^{31}P NMR spectra recording we need about 0.05 mmol of each compound. There are of course some limitations. First is the use of NaOD instead of $[Et_4N]OH$, when running ^{1}H spectra. The Na^{+} ion can in some cases form complexes with the ligands studied and thus interfere with the measurements of the log K^{H} values. Secondly, overlapping protonation constants are strongly correlated and their determination is only possible when one of the δ_{LH_n} values is fixed. On the positive side is the fact that from the δ_{LH_n} values one can specifically determine the protonation sites at a molecular level (micro constants). In the ligands here studied it is clear that the most basic sites are the amino nitrogens and that the phosphonate groups follow. The combination of potentiometric and NMR titration is thus a very powerful method for a detailed study of equilibria and structures in solution.

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