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Potentiometric and spectroscopic evidence for co-ordination of dimethyltin(IV) to phosphate groups of DNA fragments and related ligands

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The co-ordination of dimethyltin(IV) to 5′-GMP, 5′-ATP and 5′-d(CGCGCG)$_2$ and to their sugar constituents (D-ribose and 2-deoxy-D-ribose) was investigated in aqueous solution by means of potentiometric titration and ^{1}H and ^{31}P NMR spectroscopic methods. The results showed that in acidic media the phosphate groups can provide suitable sites for metal ion co-ordination, while the hydroxy groups of the sugars or the sugar moieties of the two nucleotides play a role in this process at higher pH. The base moieties of 5′-GMP and 5′-ATP were not co-ordinated to dimethyltin(IV). The stability constants of the complexes formed in the above systems were determined by pH-metric titration. The data revealed a stronger co-ordination ability of the triphosphate as compared with that of the monophosphate. The comparison of the stability constants of the D-ribose and 2-deoxy-D-ribose complexes showed that more stable species were formed when neighbouring alcoholic hydroxy groups were available for co-ordination. The observed chemical shift changes of the ^{31}P NMR resonances, as compared with those measured for the metal-free systems, demonstrated that the phosphate groups of the DNA fragment 5′-d(CGCGCG)$_2$ chains act as binding sites for dimethyltin(IV) between pH 4.5 and 7. The 1- and 2-D ^{1}H NMR spectra indicated that the base and sugar moieties do not participate in the co-ordination process under these conditions.

Introduction

The biological, including the antitumour activity[1] of organotin(IV) compounds has been well demonstrated. Diorganotin(IV) derivatives, and mainly those of dialkyltin(IV), appear to be the most active.[2] In attempts to seek a correlation between antitumour activity and structure, it is generally accepted that the co-ordination of organic ligands would facilitate transport across membranes, while the antitumour activity would be exerted by the diorganotin(IV) moieties[2,3] dissociated from the complexes. The latter would interact with nucleic acids, similarly as in the case of the widely used anticancer drug cisplatin, [Pt(NH$_3$)$_2$Cl$_2$]. Moreover, metal ion–nucleic acid complexes could also play a significant role in transport processes across membranes,[4] affecting both the kinetics and thermodynamics of the interaction with the phase boundary.

Studies were recently published on the interactions between organotin(IV) compounds and native RNA or DNA,[5] while others investigated analogous interactions with model compounds that can imitate the possible binding moieties of native nucleic acids.[6] The mechanism of action of cisplatin is fairly well documented relative to that for organotin(IV) compounds. In spite of the efforts that have been made in this field, the question remains unsolved as to which parts of the nucleic acids are favoured by organotin(IV) cations: are they able to bind to nucleic acids in some way and influence or prevent their replication, or might their interaction cause the unwinding of nucleic acids? The three possible binding sites are the donor nitrogens of the bases, the phosphate oxygens of the chain and the sugar oxygens of the nucleotides.

We earlier discussed the co-ordination chemistry of diethyltin(IV),[7] di-*n*-butyltin(IV)[8,9] and dibenzyltin(IV)[10] with non-protected carbohydrates, flavonoids[8] and 2-polyhydroxyalkylthiazolidine-4-carboxylic acids (PHTAc).[11] The co-ordination symmetry and local structure of the complexes have been determined by means of Mössbauer and FTIR spectroscopy[7–11] and EXAFS.[12] The formation equilibria and solution structures of the diethyltin(IV) complexes of PHTAc,[11] *N*-D-gluconylamino acids[13] and L-cysteine and its derivatives[14] and of the dimethyltin(IV) complexes of several monosaccharides[15] have also been investigated.

In order to obtain more information about the molecular basis of the interactions between organotin(IV) species and biologically important molecules, *e.g.* 5′-GMP, 5′-ATP and the hexamer 5′-d(CGCGCG)$_2$ and their sugar moieties, *e.g.* D-ribose and 2-deoxy-D-ribose, systematic equilibrium and spectroscopic studies of dimethyltin(IV) with the latter systems were undertaken in solution. The complex formation constants were determined by pH-metric titration, while the bonding sites of the organotin(IV) species were measured by means of ^{1}H and ^{31}P NMR methods.

Experimental

Materials

The ligands (5′-ATP, 5′-GMP, D-ribose and 2-deoxy-D-ribose) were obtained from Sigma-Aldrich and used without further purification. The 5′-d(CGCGCG)$_2$ DNA oligomer was purchased from Oswel DNA Service and purified by Sephadex gel filtration. The sequence is a palindrome and readily forms a duplex. For the NMR measurements, the lyophilised sample and the other ligands were dissolved in distilled water containing 10% of D_2O. Dimethyltin(IV) dichloride (Fluka) solution was standardised potentiometrically by pH-metric titration, with a Titrisol NaOH standard solution (Merck). All other reagents were Reanal products of analytical grade.

pH-Metric measurements

The co-ordination equilibria were investigated by potentiometric titration in aqueous solution ($I = 0.1$ M, $NaClO_4$, and $T = 298 \pm 0.1$ K) in an automatic titration set including a Dosimat 665 (Metrohm) autoburette, an Orion 710A precision digital pH-meter and an IBM-compatible PC. The Orion 8103BN semimicro pH glass electrode was calibrated[8] *via*

$$E = E_0 + K\cdot\log[H^+] + J_H[H^+] + J_{OH}K_w[H^+]^{-1} \quad (1)$$

the modified Nernst eqn. (1), where J_H and J_{OH} are fitting parameters in acidic and alkaline media for the correction of experimental errors, mainly due to the liquid junction and to the alkaline and acidic errors of the glass electrode; $K_W = 10^{-13.75}$ M^2 is the autoprotolysis constant of water.[9] The parameters were calculated by a non-linear least squares method.

The species formed in the systems were characterised by the generalised equilibrium process (2), while the formation con-

$$pM + qL \overset{\beta_{M_pL_qH_{-r}}}{\rightleftharpoons} M_pL_qH_{-r} + rH$$

or

$$pM + qL + rOH \overset{\beta_{M_pL_q(OH)_r}}{\rightleftharpoons} M_pL_q(OH)_r \quad (2)$$

stants for these generalised species are given by eqn. (3) and

$$\beta_{M_pL_qH_{-r}} = \frac{[M_pL_qH_{-r}][H]^r}{[M]^p[L]^q} = \frac{M_pL_q(OH)_r](K_w)^r}{[M]^p[L]^q[OH]^r} = \beta_{M_pL_q(OH)_r}(K_w)^r \quad (3)$$

were calculated *via* the computer program PSEQUAD[10] where M denotes dimethyltin(IV) and L the non-protonated ligand molecule (D-ribose, 2-deoxy-D-ribose, 5′-GMP^{3-} or 5′-ATP^{4-} in the cases of the ligands studied) and H refers to the overall protonation state of the complex. Charges are generally omitted for clarity.

The protonation constants were determined from four or five titrations (70–110 data points per titration, the ligand concentrations varying from 2×10^{-3} to 2×10^{-2} M). The complex stability constants were also determined from four to seven independent titrations in each system. The dimethyltin(IV)-to-ligand ratio varied from 1:1 to 1:5 (from 1:1 to 1:10 for the 2-deoxy-D-ribose–metal system). The metal ion concentration varied from 6×10^{-4} to 7×10^{-3} M, and the ligand concentrations generally from 8×10^{-4} to 6×10^{-2} M.

NMR measurements

The ^{1}H NMR spectra were recorded either on a Bruker AM-400 (Medical University, Szeged) or on a Bruker DRX-600 (University of Bergen) spectrometer, with 16–32 K data points. A suitable signal-to-noise ratio was achieved after the collection of 64–16 transients. During the recycling delay the water resonance was suppressed. The ^{31}P spectra were run on the Bruker DRX-600 spectrometer. The chemical shifts are given relative to phosphoric acid. One hundred and twentyeight transients were collected for each spectrum. The 2-D NOESY spectra were recorded in the phase-sensitive mode with quadrature detection, using the States-TPPI method. The data were collected in 2k complex points in F2 and 512 points in F1, with 32 transients averaged for each t1 increment. A relaxation delay of 2 s between each transient was used. The spectral width was 12 000 Hz and the probehead temperature was held at 285 K. The 3-9-19 Watergate technique was used for solvent suppression.[16] The NOESY spectra were recorded with a mixing time of 0.2 s. The NMR data were processed on a Silicon Graphics Indy computer, using the program XWIN-NMR (Bruker). All the NMR spectra of the systems containing sugars or mononucleotides were recorded at room temperature (298 K), but the 1-D measurements on the hexamer were carried out at lower temperature (278 K) in order to obtain better signals in the imino region.

Table 1 Formation constants of the species formed by the hydrolysis of $(CH_3)_2Sn^{IV}$ and complex-formation constants (log β and log K) for the complexes in the D-ribose– and 2-deoxy-D-ribose–$(CH_3)_2Sn^{IV}$ systems, measured at 25 °C at an ionic strength of 0.1 M $NaClO_4$ (estimated errors in parentheses)

(*a*) Hydrolysis	
β [M(OH)]	−3.12(1)
β [M(OH)$_2$]	−8.33(1)
β [M(OH)$_3$]	−19.33(1)
β [M(OH)$_2$]	−4.83(4)
β [M(OH)$_3$]	−9.69(2)

(*b*) Complex formation	D-Ribose	2-Deoxy-D-ribose
β (MLH$_{-3}$)	−15.72(1)	−17.22(1)
β (ML$_2$H$_{-4}$)	−24.90(1)	−27.09(1)
log $K_{MLH_{-3}}$ [a]	−7.39	−8.89
log $K_{MH_2H_{-4}}$ [b]	−9.18	−9.87
log $K^{corr.}_{MLH_{-3}}$ [c]	−19.61	−21.56

[a] Calculated *via* eqn. (4). [b] Calculated *via* eqn. (6). [c] Calculated *via* eqn. (5).

Results and discussion

Potentiometric and ^{1}H NMR studies of the systems containing sugars and dimethyltin(IV)

The hydrolysis of dimethyltin(IV) has been investigated in a variety of media by several authors.[17–19] We performed potentiometric titrations to obtain these data at the ionic strength of 0.1 M $NaClO_4$ we usually apply. We detected the formation of species with the same composition and similar stability to those reported by Arena *et al.*[18] and Burger and co-workers.[15] The observed species are M(OH), M(OH)$_2$, M(OH)$_3$, M$_2$(OH)$_2$ and M$_2$(OH)$_3$ (the overall stability constants are shown in Table 1). Introduction of other polynuclear hydroxo complexes, such as M$_3$(OH)$_4$, M$_4$(OH)$_5$ and M$_4$(OH)$_6$, into the set of species led to no improvement in the fitting.

The preliminary results showed that some nucleotide complexes of dimethyltin(IV) are water-soluble in the wide pH range suitable for pH-metric titration. Two nucleotides (5′-GMP and 5′-ATP) and two sugars (D-ribose and 2-deoxy-D-ribose) were therefore selected, and their interactions with dimethyltin(IV) were investigated potentiometrically.

Representative pH *vs.* H$_{-1}$ [mol OH$^-$/mol dimethyltin(IV)] curves, calculated from the differences between the titration curves of the ligand–dimethyltin(IV) systems and of the ligands, normalised to the total metal concentrations, are plotted in Fig. 1 [(A) D-ribose and 2-deoxy-D-ribose, (B) 5′-GMP and 5′-ATP]. The formation constants obtained, together with some calculated log K values, are given in Table 1. The titration curves revealed that complexes with a 1:1 ligand-to-metal ratio were formed. There was no evidence of the presence of polynuclear species in solution. The species formed in solutions of different pH were found to be in different protonation states (see Table 1).

The pH *vs.* H$_{-1}$ curves of the dimethyltin(IV)–D-ribose and –2-deoxy-D-ribose systems proved to be different at pH > 8 from the curve representing the processes of cation hydrolysis. This observation conforms to previous findings on the systems containing dimethyltin(IV) and several monosaccharides (D-glucose, 2-deoxy-D-glucose, D-sorbitol, and D- and L-arabinose) or the disaccharide D-saccharose.[15] Although the alcoholic hydroxy groups of the carbohydrates are very weak acids (p$K \approx$ 12–14),[20] metal-promoted deprotonations of these groups take place above pH 8,[21] or in the case of the D-fructose–dimethyltin(IV) system even at pH ≈ 4.5,[15] resulting in the formation of MLH$_{-x}$ (x = 2 or 3). The species distribution curves of the dimethyltin(IV)–D-ribose (ratio 1:5) and –2-deoxy-D-ribose

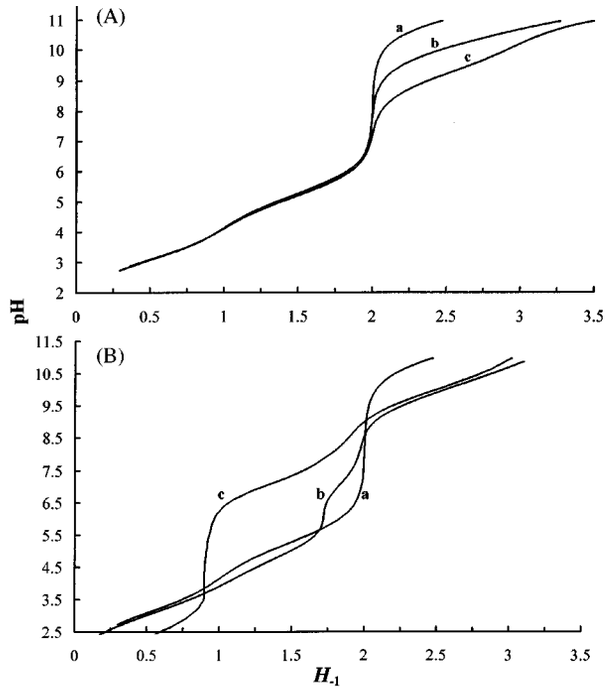


Fig. 1 pH *vs.* H_{-1} curves for the different systems. (A) Dimethyltin(IV)–D-ribose (1:5) (c) and dimethyltin(IV)–2-deoxy-D-ribose (1:10) (b) compared with the hydrolysis of the metal ion (a); $[(CH_3)_2Sn^{2+}] = 0.005$ M. (B) Dimethyltin(IV)–5′-ATP (1:5) (c) and –5′-GMP (1:5) (b) compared with the hydrolysis of the metal ion (a); $[(CH_3)_2Sn^{2+}] = 0.0008$ M.

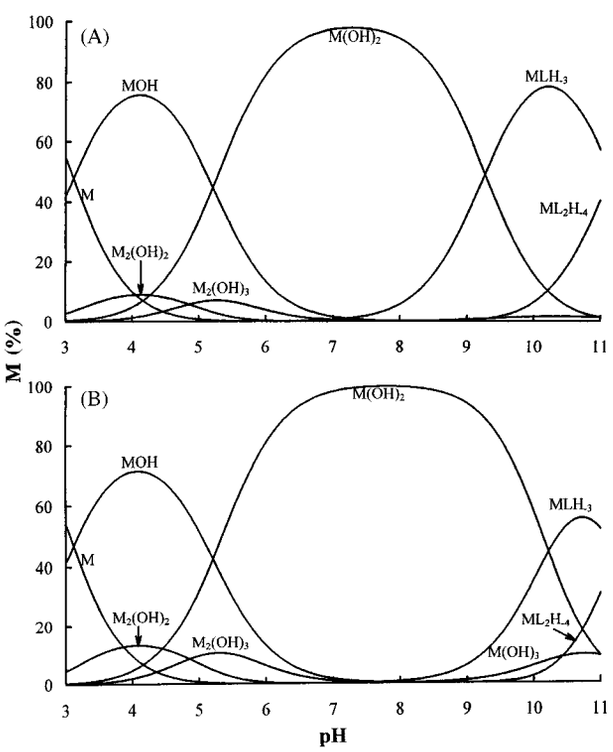


Fig. 2 Species distribution diagrams for the dimethyltin(IV)–D-ribose (A) and –2-deoxy-D-ribose (B) systems in molar ratios of 1:5 (A) and 1:10 (B). The dimethyltin(IV) concentrations are 0.003 (A) and 0.005 M (B)

(ratio 1:10) systems, calculated from the pH-metric measurements [Fig. 2(A),(B)], show that in the physiological pH range in solutions containing 0.005 M dimethyltin(IV) the hydrolysis product $M(OH)_2$ predominates. On increase of the pH of the solutions, further deprotonation (hydroxide ion consumption) processes take place in both systems, leading to the complexes MLH_{-3} and ML_2H_{-4}. The presence of the latter complex can

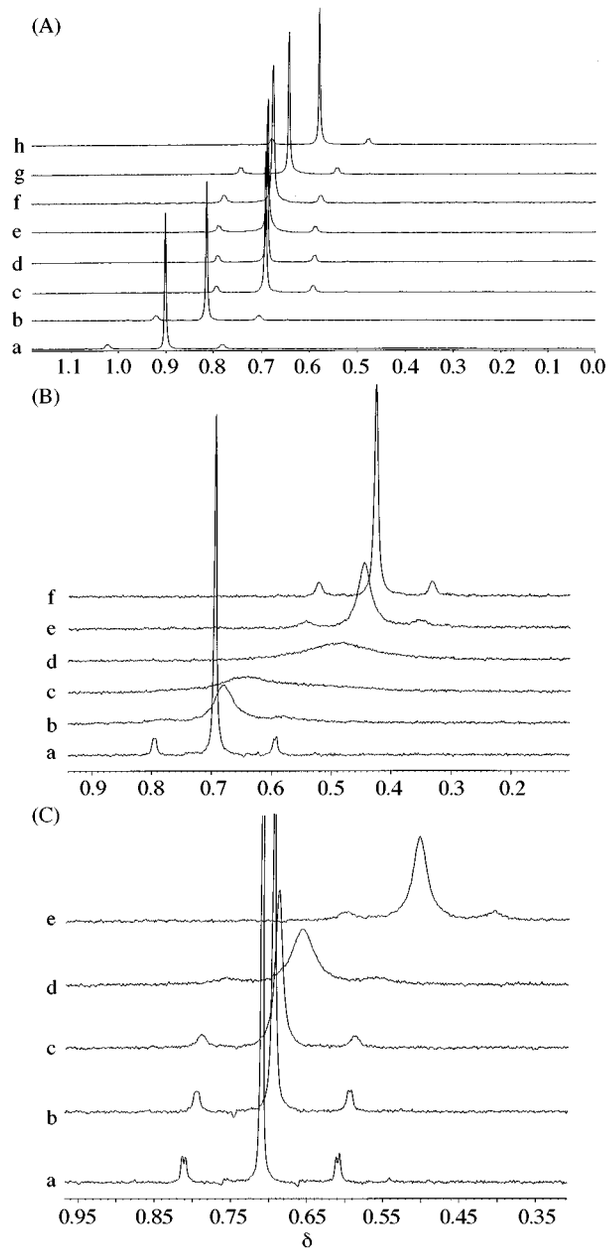


Fig. 3 The ^{1}H NMR spectra of dimethyltin(IV) without ligands (A), dimethyltin(IV)–D-ribose 1:5 (B) and dimethyltin(IV)–2-deoxy-D-ribose 1:10 (C), as a function of pH in the methyl proton range. Curves: (A) a–h, pH 2.95, 4.95, 7.62, 8.72, 9.30, 9.99, 10.58 and 11.07, respectively; (B) a–f, pH 7.68, 8.73, 9.29, 9.99, 10.57 and 11.13; (C) a–e, pH 7.50, 8.74, 9.31, 10.00 and 11.08.

be demonstrated only by varying the metal-to-ligand ratio systematically.

D-Ribose–dimethyltin(IV) system. The pH-dependent ^{1}H NMR spectra [Figure 3(A),(B)] reveal slight differences between the two series [that containing only dimethyltin(IV), and that containing D-ribose and dimethyltin(IV)] at around pH 7.5. The signals of the sugar (not shown) and also the methyl protons of dimethyltin(IV) are somewhat broadened at this pH. Owing to the presence of the many possible sugar anomers in the solution, a precise assignment of the different sugar protons would be rather difficult. The observed line broadening of the signals can be explained by the presence of very small amounts of complex species. Increase of the pH results in an extra base-consuming process, and the species MLH_{-3} is formed. This shows that at least one alcoholic hydroxy group is deprotonated and co-ordinated to dimethyltin(IV). Comparison of the stability constants of the D-ribose complexes with those obtained for

Table 2 Measured chemical shifts and two-bond coupling of methyl protons in the systems studied as a function of pH

	δ			$^2J(^{119}Sn–C–^1H)$/Hz		
pH	Hydrolysis	$(CH_3)_2Sn^{2+}$–D-ribose	$(CH_3)_2Sn^{2+}$–2-deoxy-D-ribose	Hydrolysis	$(CH_3)_2Sn^{2+}$–D-ribose	$(CH_3)_2Sn^{2+}$–2-deoxy-D-ribose
2.95	0.902	0.904	0.905	98.96	99.71	99.46
4.99	0.814	0.809	0.814	87.37	87.12	87.88
7.62	0.693	0.685	0.690	82.09	82.09	82.09
8.73	0.690	0.670	0.685	82.09	—	81.83
9.30	0.688	0.629	0.678	82.09	—	—
9.99	0.677	≈0.470	0.647	82.09	—	—
10.57	0.643	0.435	—	82.09	—	—
11.09	0.580	0.417	0.494	82.09	≈77.05	—

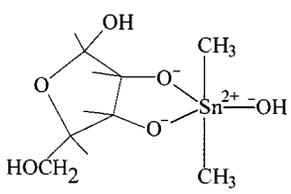
the 2-deoxy-D-ribose–dimethyltin(IV) system [on the basis of eqn. (4)] suggests the five-membered chelate co-ordination of

$$MH_{-2} + L \rightleftharpoons MLH_{-3}^{-} + H^+$$
$$\log K_{MLH_{-3}} = \log \beta_{MLH_{-3}} - \log \beta_{MH_{-2}} \quad (4)$$

D-ribose to the metal ion, which is not possible in the latter system. The stability difference is more expressed if we adjust these stabilities by the acidity constants of the two sugars [eqn. (5)] which were determined earlier by means of calorimetric

$$\log K^{corr.}_{MLH_{-3}} = \log K_{MLH_{-3}} - pK_{sugar} \quad (5)$$

titrations ($pK = 12.22$ and 12.67 for D-ribose and 2-deoxy-D-ribose, respectively).[20b] The almost two orders of magnitude difference between the corrected stability constants supports the different modes of co-ordination of the two ligands.



At higher pH a further base-consuming process takes place. The results obtained by varying the ligand excess systematically (from 1:1 to 1:10) suggested formation of the bis complex ML_2H_{-4}. This can be either a parent complex in which the central dimethyltin(IV) is surrounded by four deprotonated alcoholic hydroxy groups (two from each D-ribose) and the two methyl groups, resulting in an octahedral structure, or a mixed-ligand hydroxo complex where two hydroxide ions, two deprotonated alcoholic hydroxy groups and the two methyl groups are situated in the co-ordination sphere. The former structure may be more likely because of the higher stability of D-ribose complexes as compared with those of 2-deoxy-D-ribose on the basis of eqn. (6) (Table 1), where the formation of chelate or dichelate complexes is not possible.

$$MLH_{-3} + L \rightleftharpoons ML_2H_{-4}^{2-} + H^+$$
$$\log K_{ML_2H_{-4}} = \log \beta_{ML_2H_{-4}} - \log \beta_{MLH_{-3}} \quad (6)$$

The ^1H NMR spectra of the system containing D-ribose and metal ion in a ratio of 5:1 indicate a chemical exchange process between two dimethyltin(IV) units. This process is reflected by the strong line broadening of the ^1H signals of the methyl groups of the dimethyltin(IV) centres [Figure 3(A),(B)]. The above-mentioned two forms are probably the hydrolysis product $M(OH)_2$ and the complex MLH_{-3}. The most likely binding sites are the 2′,3′ *cis*-hydroxy groups, as found for the dibutyltin(IV)–D-ribose complex prepared in the solid state from methanol.[8,22] The measured chemical shifts and the coupling constants are listed in Table 2.

2-Deoxy-D-ribose–dimethyltin(IV) system. In the case of 2-deoxy-D-ribose, the processes are somewhat different. As a consequence of the steric arrangement of the hydroxy groups there is no possibility for the formation of chelate rings. In the mixed-ligand hydroxo complex MLH_{-3}, the metal ion is probably co-ordinated to two hydroxide ions and one ligand, *via* one deprotonated hydroxy group. At high pH formation of the bis complex is also detectable. The ^1H NMR spectra of this system show that at >pH ≈ 9 the signals of the sugar protons are broadened relative to those of the ligand without metal ion, indicating the interaction of dimethyltin(IV) and alcoholic hydroxy group(s) of the sugar. As in the case of D-ribose, it is preferable to follow the complex-formation processes *via* the signals of the methyl protons of the organotin(IV) [Fig. 3(A),(C)]. The same phenomenon is observed as in the case of D-ribose, but only above pH ≈ 9. Owing to their low stability, the complexes formed in the 2-deoxy-D-ribose–dimethyltin(IV) system do not predominate even at high pH, which correlates well with the distribution curve.

The complex-formation constants determined by potentiometric titration and calculated by means of eqns. (4) and (6) are listed in Table 1. Comparison of the stability constants for the species MLH_{-3} and ML_2H_{-4} in the two systems shows the above-discussed stability differences. The same stability sequence can be seen from the calculated H_{-1} *vs.* pH curves. The curve of the system containing D-ribose exhibits a significant difference from the curve of the hydrolysis process above pH ≈ 8. There is a smaller difference at higher pH (above ≈9) in the case of the deoxy sugar.

Potentiometric and NMR studies of the systems containing 5′-GMP or 5′-ATP and dimethyltin(IV)

Several investigations have been carried out[23,24] on the protonation and complex-formation properties of this family of ligands, and a few studies have also been published on the interactions between organotin(IV) and nucleotides.[6] It has been found that the base moieties of the pyrimidine nucleotides do not display affinity towards any of the metal ions studied, including dimethyltin(IV). On the other hand, the nitrogen atoms of the purine nucleotides act as strong donors for different types of metal ions. In some cases there is a possibility for the formation of an (inner- or outer-sphere) macrochelate ring between the phosphate oxygen and N7 through the metal ion.[24b] Co-ordination of the phosphate groups of different nucleotides to dimethyltin(IV) has been demonstrated by several authors, but there is no evidence for the co-ordination of donor atoms other than phosphate oxygens. Deprotonation of N1 of 5′-GTP and its co-ordination to dimethyltin(IV) was suggested on the basis of thermodynamic data obtained by calorimetric titration, but no spectroscopic evidence is available for this process.[6a] On the other hand, in alkaline solution, the sugar moieties of the nucleotides are likely to be good co-ordination sites for different metal ions [*e.g.* copper(II)[25–27] or antimonate(III)[28]], as indicated by EPR and NMR spectroscopy[25] and by EXAFS[26] and X-ray diffraction[27,28] measurements.

Table 3 Protonation constants (log β and pK) for the ligands and complex-formation constants (log β and log K values) for the complexes in the 5′-GMP– and 5′-ATP–$(CH_3)_2Sn^{IV}$ systems, measured at 25 °C at an ionic strength of 0.1 M $NaClO_4$ (estimated errors in parentheses)

	5′-GMP	5′-ATP
$\beta(H_4L)$	19.4(3)	—
$\beta(H_3L)$	18.23(1)	12.2(1)
$\beta(H_2L)$	15.75(1)	10.59(1)
$\beta(HL)$	9.50(1)	6.60(1)
pK_{H_4L}	1.2	—
pK_{H_3L}	2.48	1.6
pK_{H_2L}	6.25	3.99
pK_{HL}	9.50	6.60
$\beta(MLH_2)$	—	14.29(2)
$\beta(MLH)$	14.81(3)	11.96(2)
$\beta(ML)$	10.13(2)	7.98(1)
$\beta(MLH_{-1})$	—	1.32(2)
$\beta(MLH_{-2})$	−6.29(5)	—
$\beta(MLH_{-3})$	−15.80(1)	−15.92
$\beta(M_2LH)$	—	15.17(7)
log K_{ML}	—	7.98
log K_{MLH} [a]	5.31	—
log $K_{MLH_{-3}}$ [b]	−7.47	−7.59

[a] Calculated *via* eqn. (7). [b] Calculated *via* eqn. (9).

Potentiometric titration was used to determine the protonation constants of the ligands and to describe the systems containing dimethyltin(IV) together with the nucleotides. Multinuclear NMR spectroscopy was also applied to follow these processes, and to determine the structures of the complexes formed in solution.

The protonation constants obtained for 5′-GMP and 5′-ATP together with the determined log β and some calculated log K values of the complexes formed, are presented in Table 3. These protonation constants are in good agreement with values published earlier.[29] From the potentiometric titration data on the two nucleotides in the systems containing both ligands and metal ions, the same types of pH *vs.* H_{-1} curves were obtained as from the two sugars as ligands [Fig. 1(B)]. There are significant differences between the curves of the systems containing ligands and metal in 5 : 1 ratio and the curve of the hydrolysis of dimethyltin(IV).

5′-GMP–dimethyltin(IV) system. The pH *vs.* H_{-1} curve of this system starts to differ from the analogous curve of the hydrolysis of the metal ion above pH 3. This is well demonstrated by the concentration distribution curve of the metal ion [Fig. 4(A)], where formation of the complex MLH was observed in this pH region. When the metal ion concentration is higher than 0.001 M a white precipitate is formed above pH 3. The hydrolysis products of dimethyltin(IV) are soluble at this concentration, and there was no precipitation during the titration of 5′-GMP. This white precipitate is therefore probably due to the interaction between the ligand and the metal ion that results in the formation of a neutral, water-insoluble complex [MLH = $(CH_3)_2$Sn(GMP-H)]. The evaluation of the pH-dependent ^{31}P NMR spectra confirms the co-ordination of the phosphate group to organotin(IV) in this pH region. The process of deprotonation of the phosphate group of 5′-GMP causes a continuous downfield shift of the phosphorus signal as the pH increases. (Only one set of signals can be observed, due to fast exchange between the "free" and bound ligand in the whole pH region.) On the addition of dimethyltin(IV) to the solution a similar tendency was found, but the co-ordination of the ligand to the dimethyltin(IV) *via* the phosphate group is unambiguously described by the significant upfield shift of the signals, relative to the resonances of the "free" ligand. The stability constant of the phosphate-co-ordinated MLH complex [according to eqn. (7)] clearly reflects the strong affinity of

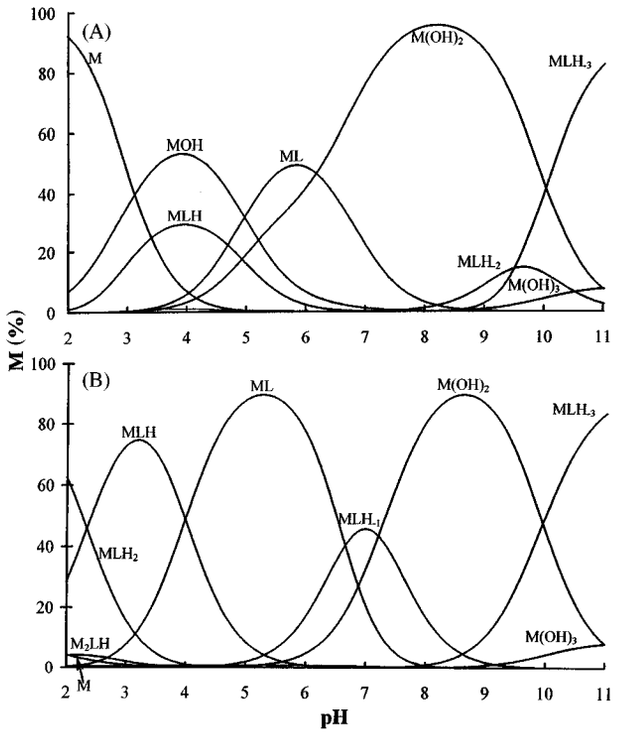


Fig. 4 Species distribution diagrams for the dimethyltin(IV)–5′-GMP (A) and –5′-ATP (B) systems in 1 : 5 molar ratio. The dimethyltin(IV) concentrations are 0.0008 (A) and 0.001 M (B).

$$M^{2} + HL^{2-} \rightleftharpoons MLH$$
$$\log K_{MLH} = \log \beta_{MLH} - pK_{LH} \qquad (7)$$

dimethyltin(IV) for the phosphate group. In fact, this stability constant (log K = 5.31) is greater than those obtained for many transition metal ions[24a] and is almost comparable to the stability of aluminum(III)–nucleoside monophosphate complexes.[30a]

Above pH 4 the next deprotonation step can be readily followed in the pH *vs.* H_{-1} curves and in the concentration distribution curves. This process can very probably be assigned to the deprotonation of a water molecule co-ordinated to the dimethyltin(IV), resulting in the formation of a mixed-ligand hydroxo complex ML [ML = $(CH_3)_2$Sn(OH)(GMP,H)$^-$]. This means that the hydrolysis of dimethyltin(IV) is shifted toward higher pH by approximately 1.5 pH units due to the co-ordination of 5′-GMP. Above pH 7 the hydrolysis product $M(OH)_2$ is the predominant species, and the phosphate group of the nucleotide is no longer able to compete with the hydroxide ions. [The pH *vs.* H_{-1} curve reaches a value of 2 at around pH 8, with an inflection point, confirming the predominance of $M(OH)_2$ (Fig. 1(B)]. The release of the 5′-GMP from the co-ordination sphere of the dimethyltin(IV) at around pH 7–8 is also indicated by the ^{31}P NMR spectra, since there is no significant difference between the phosphorus signals of the metal-free and metal-containing solutions.

There are six sets of signals in the pH-dependent ^{1}H NMR spectra of 5′-GMP. They belong to the base proton H8, the doublet of H1′ and H3′, H4′, H5′ and H5″ of the sugar moiety in the sequence of descending δ values. The deprotonation of N7 results in a continuous upfield shift of the proton H8 of the "free" ligand. In the presence of dimethyltin(IV) the same upfield shift is observed but at a slightly lower pH. Although this might suggest some interaction between the organotin(IV) and the base part of the ligand we could find no direct evidence for it. Up to pH 7 no changes can be observed in the various sugar proton resonances.

At around neutral pH the hydrolysis product $M(OH)_2$ is the only species revealed by potentiometry and ^{1}H NMR spectroscopy. On increase of the pH further processes take place,

resulting in the species MLH_{-2} followed by MLH_{-3}. The ^1H NMR spectra point to interactions between the alcoholic hydroxy groups of 5′-GMP and the metal ion. Several processes may cause changes in the NMR spectra (and the pH *vs.* H_{-1} curve): (i) the metal ion can be co-ordinated to the sugar moiety through protonated or deprotonated alcoholic hydroxy groups (the stability constants obtained suggest the deprotonation of these groups); (ii) deprotonation of the alcoholic hydroxy groups by either a water elimination mechanism or an extra base-consuming process; (iii) in parallel, deprotonation of N1 of the base moiety of the ligand at around pH 9.5. The pH *vs.* H_{-1} curve of the 5′-GMP–dimethyltin(IV) system rises more rapidly than that of the hydrolysis [Fig. 1(B)]. The complex formed by the end of these processes can be described by the formula MLH_{-3}, which has the same protonation state as found in the systems containing only sugars as ligands. The ^1H NMR spectra provide very clear evidence for the formation of species other than hydrolysis products. All the sugar protons are affected by the co-ordination of the dimethyltin(IV). These effects are much more marked in the system containing the metal and the ligand in equimolar ratio. The signals of the sugar protons are strongly broadened and shifted upfield at higher pH, as a result of the appearance of the complex species in solution. In the system containing the ligand and the metal in a ratio of 5:1 the same changes can be observed in the δ range of the methyl protons as for the sugars. A new signal appears at around δ 0.46, which indicates the presence of another organotin(IV)-containing species in the solution. In consequence of the slow exchange between the hydrolysis product $M(OH)_2$ and the complex species MLH_{-3}, two broad signals are present in the pH range 9–10.5. At pH 11.2, all the organotin(IV) is complexed by the ligand, and only the species MLH_{-3} is present. This results in one sharp signal. On the other hand, the pH-dependent ^{31}P NMR spectra indicate no phosphate oxygen co-ordination above pH 7.

A similar log K value can be calculated for the sugar-bound complexes MLH_{-3} or MLH_{-2} (N1 is protonated in the latter species) as in the case of the two simple sugars, from either of eqns. (8) or (9), where HL^{2-} is N1-protonated 5′-GMP, while

$$MH_{-2} + HL^{2-} \rightleftharpoons MLH_{-2}^{3-} + H^+$$
$$\log K_{MLH_{-2}} = \log \beta_{MLH_{-2}} - \log \beta_{MH_{-2}} - pK_{LH} \quad (8)$$

$$MH_{-2} + L^{3-} \rightleftharpoons MLH_{-3}^{4-} + H^+$$
$$\log K_{MLH_{-3}} = \log \beta_{MLH_{-3}} - \log \beta_{MH_{-2}} \quad (9)$$

L^{3-} is the non-protonated ligand. The determined log K value (−7.47) is very similar to that obtained for D-ribose (see Table 1) which supports the dialcoholate mode of co-ordination. [The composition of MLH_{-3} is therefore very probably $(CH_3)_2Sn(OH)(GMP - 2H)^{4-}$.]

5′-ATP–Dimethyltin(IV) system. The pH *vs.* H_{-1} curve of this system, generated from the titration curves, exhibits a marked difference from that for the hydrolysis of dimethyltin(IV) in the acidic pH range up to ≈3.7 [Fig. 1(B)]. This difference can be explained by the deprotonation and co-ordination of the phosphate groups to the metal ion, which prevents the hydrolysis of dimethyltin(IV). The 5′-ATP provides much stronger binding sites for the metal ions than 5′-GMP because of the larger negative charge on the three phosphate groups, and the possibility of the formation of six-membered chelate rings. This is also suggested by the large difference between the log K values of the two phosphate-co-ordinated species (see Table 3). The larger donor ability of the triphosphate group than that of the monophosphate is clearly reflected by the pH *vs.* H_{-1} curves of the 5′-ATP- and 5′-GMP-containing systems. Owing to the presence of four possible oxygen donor atoms in the triphosphate group, a binuclear species M_2LH is also detected in small amount, especially in the equimolar system [Fig. 4(B)]. No precipitation occurs throughout the whole pH range as the distribution of the charges is different from that for 5′-GMP.

Between pH 4 and 8 further deprotonation processes take place. The pK values of these deprotonation steps suggest that these can be assigned to the deprotonation of N1 (pK = 3.98) and to a hydrolysis step (pK = 6.66). A very similar value was obtained for the N1 deprotonation of 5′-ATP (Table 3), indicating that the complex-formation processes have little effect on the protonation state of the mentioned group. In the presence of 5′-ATP the formation of the hydrolysis products is shifted about 3.5 pH units higher as compared with the pH measured in the ligand-free system (even higher than in the case of 5′-GMP). Consequently, this ligand binds the metal ion strongly, and it is able to compete with the hydroxide ion present in higher concentration than can 5′-GMP. The stability constant determined for the formation of ML [certainly $(CH_3)_2Sn(ATP)^{2-}$] is notably greater than those for many metal ions.[24b] Only a few examples can be found for the formation of analogous equimolar complexes of nucleoside triphosphates with log K values of a similar order of magnitude.[6a,30b] Aluminium(III) is reported to form stable equimolar complexes with 5′-ATP (log K = 7.92),[30b] while N1-protonated 5′-GTP binds dimethyltin(IV) with log K = 7.80, as shown by means of potentiometric and calorimetric titrations.[6a]

Three groups of signals are present in the pH-dependent ^{31}P NMR spectra of 5′-ATP. The resonances can readily be assigned on the basis of the different splitting patterns. The signal with the largest chemical shift is the resonance of the γ phosphorus atom, and the group with the smallest shift is that of the β one. The signals are shifted downfield with increasing pH, due to the deprotonation processes on the phosphate oxygens. Between pH 2 and 8 the signals of the system containing 5′-ATP and metal ion are shifted upfield relative to the resonances of the solution containing only ligand, as a consequence of the co-ordination of dimethyltin(IV) (Fig. 5). All three phosphorus resonances are affected strongly as the distribution of charges changes on the phosphate moiety. The further small chemical shifts observed for all phosphorus atoms (compared with the ligand) above pH 9.5 probably originate from the indirect intramolecular (through water molecules) interactions between the central ion and the negatively charged phosphate groups.

Seven groups of signals are present in the pH-dependent ^1H NMR spectra of 5′-ATP, which can be assigned to the H8 and H2 resonances of the adenine ring, the doublet of H1′ and H3′, H4′, H5′ and H5″ of the sugar moiety, in sequence of descending δ values. The changes in chemical shift of the base protons reflect the deprotonation of N1. The addition of metal ion to the solution does not affect the sugar proton resonances and has only a very slight upfield effect on the base proton H2 signal, but causes a more expressed upfield shift on the H8 signal. We do not suggest the direct co-ordination of dimethyltin(IV) to N7, since earlier studies have shown that direct N7 co-ordination results in a large downfield shift.[31] On the other hand, we cannot exclude formation of the outer-sphere macrochelate between the triphosphate moiety and the base nitrogen N7 (direct co-ordination of the metal ions to the phosphates and through-water co-ordination to N7), as shown for many metal ions.[24b] A possible structure of the complex ML is shown below. The binding mode is probably trigonal bipyramidal, where a water molecule occupies the fifth site around the dimethyltin(IV) centre, or the above-mentioned macrochelate.

The signals of the methyl protons in dimethyltin(IV) are shifted markedly downfield as compared with the corresponding signals of the hydrolysis product, which is a result of the stronger phosphate co-ordination than in the case of 5′-GMP. The $^2J(^{119,117}Sn–^1H)$ two-bond coupling constants [pH 6.25, δ 0.862, $^2J(^{119}Sn–^1H)$ = 92.0 Hz] are larger than those obtained in the same pH range for the hydrolysis [pH 6.25, δ 0.740,

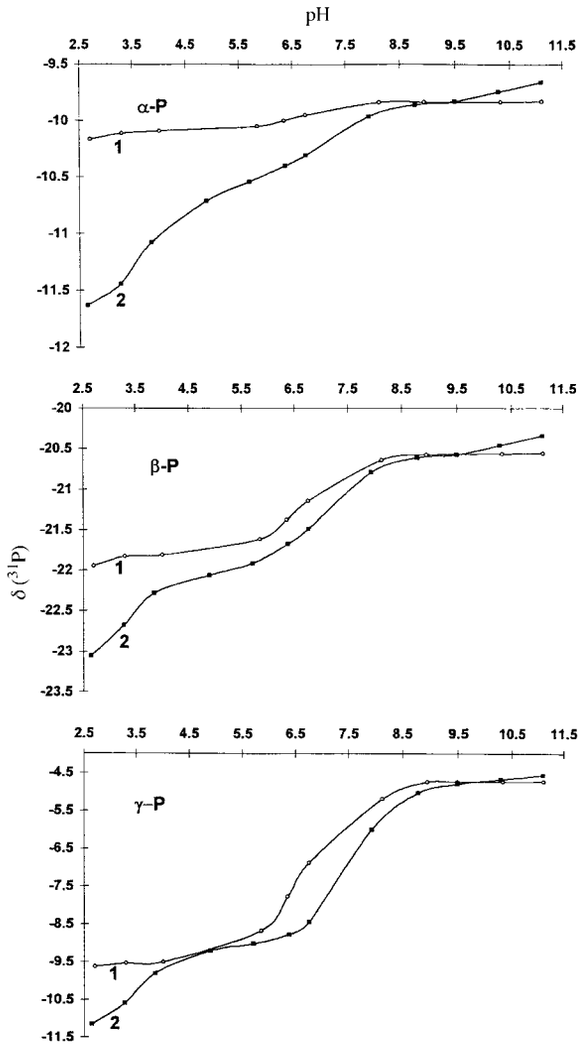
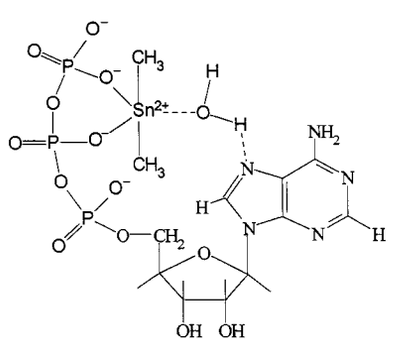


Fig. 5 pH-Dependent ^{31}P chemical shifts of the different (α,β,γ) phosphorus atoms of 5′-ATP in the presence (2) and the absence (1) of dimethyltin(IV). Dimethyltin(IV) : 5′-ATP = 1 : 1.



$^2J(^{119}Sn–^1H) = 84.1$ Hz], which means a slight distortion of the environment of the dimethyltin(IV) centre.

Above pH 8 the hydrolysis product $M(OH)_2$ predominates and deprotonation of the sugar moiety takes place at approximately the same pH as in the case of 5′-GMP [Fig. 4(B)]. The deprotonation leads to the species MLH_{-3} [very probably $(CH_3)_2Sn(OH)(ATP - 2H)^{5-}$], in which the ligand is co-ordinated to the dimethyltin(IV) in a bidentate manner through the sugar moiety. The deprotonation of the sugar hydroxy groups at relatively low pH causes a rapid rise in the pH *vs.* H_{-1} curve in the basic pH range. (The same phenomenon was observed for 5′-GMP.) The calculated log K value (similarly to eqn. 9) for the formation of MLH_{-3} also supports bidentate D-ribose type co-ordination of the sugar part of the ligand to the dimethyltin(IV).

The pH-dependent ^1H NMR spectra reveal these changes both in the ligand and around the dimethyltin(IV). Strong line broadening is observed for all the sugar protons. Characteristic changes can be seen in the δ range of H1′. The doublet starts to broaden at around pH 7.5 and a new signal appears at higher fields. This may be caused by the slow exchange between the bound and free 5′-ATP molecules. Above pH 8 the H3′ resonance is not observed in the spectra, in consequence of the shift of the water resonance. The H4′ signal is shifted upfield and the splitting pattern of H5′ and H5″ changes drastically. The slow exchange between the hydrolysis product $M(OH)_2$ and the metal ion co-ordinated to 5′-ATP results in the presence of two very broad signals in the chemical shift range of the methyl protons of dimethyltin(IV). At high pH the species MLH_{-3} becomes predominant, and consequently the signal of the methyl protons of the complexed dimethyltin(IV) is narrow, indicating the formation of stable species.

^1H and ^{31}P NMR studies of the 5′-d(CGCGCG)$_2$–dimethyltin(IV) system

The co-ordination ability of the 5′-d(CGCGCG)$_2$ hexamer towards dimethyltin(IV) was investigated by means of ^1H and ^{31}P 1-D and ^1H NOESY 2-D NMR spectroscopy. The assignments of the ^1H resonances of the hexamer were published earlier.[32] The 1-D ^1H and ^{31}P spectra of the hexamer were measured at neutral pH (≈7) and increasing dimethyltin(IV) concentration from 0 up to a metal-to-hexamer ratio of 12 : 1 or 24 : 1. In the first case a constant ionic strength (0.2 M $NaClO_4$) was applied to stabilise the duplex structure in solution. The measurements were repeated without using salt for the stabilisation, in order to try to avoid the possible disadvantages of the high concentration of ions for the co-ordination of dimethyltin(IV). This change did not influence the stability of the duplex, indicating the extreme stability of the 5′-d(CGCGCG)$_2$ sequence. Addition of dimethyltin(IV) to the hexamer did not induce any changes either in the NOESY maps or in the 1-D ^1H spectra. The signals of the phosphate groups of the hexamer did not indicate any chemical shift changes and the linewidths were the same at any metal-to-ligand ratio used. Consequently, at neutral pH, dimethyltin(IV) is not able to bind to any part of the hexamer molecule.

It might be envisaged that the extreme stability of this sequence (because of the six C-G base pairs) can cause it to be inert towards metal ion co-ordination. Thus, temperature-dependent 1-D ^1H and ^{31}P NMR measurements were performed to establish whether the organotin(IV) is able to co-ordinate to the molecule when the strong connection between the two strands is weakened or when it reaches the single strand state. The measurements were started at 278 K and the last spectrum was recorded at 353 K. Dramatic changes occurred in both the ^1H and the ^{31}P NMR spectra when the temperature was increased, before and after the melting point of the duplex. A very characteristic change was the collapse of the imino signals of the bases. However, no difference could be observed between the spectra recorded in the presence and the absence of dimethyltin(IV). Furthermore, after the solution that contained metal ion and hexamer in a ratio of 24 : 1 was cooled to 278 K exactly the same ^1H and ^{31}P NMR spectra were obtained as before the heating procedure. It is clear that at neutral pH the hexamer–organotin(IV) interaction is insensitive to temperature. No interaction was observed at all.

The pH-dependent ^1H and ^{31}P NMR spectra above and below pH 7 were also measured. The change of pH slightly affected the shape of the ^1H spectra; there were large changes only in acidic solution and at around pH 11. In acidic solutions a white precipitate was formed at around pH 4.5, even without metal ion. At around pH 11 the imino signals started to diminish, due to the collapse of the double helix structure, but this

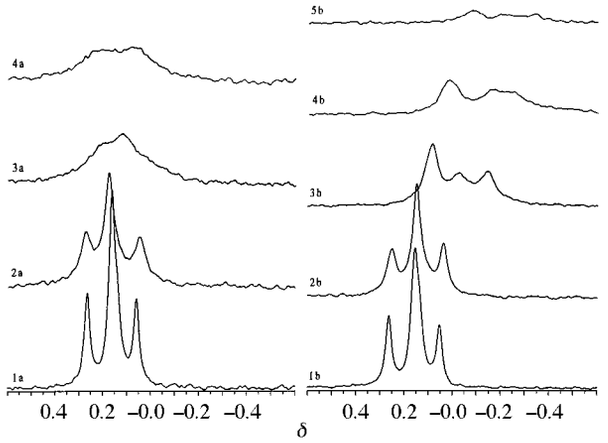


Fig. 6 pH-Dependent ^{31}P NMR spectra of the 5′-d(CGCGCG)$_2$ system in the absence (a) and the presence (b) of dimethyltin(IV). [5′-d(CGCGCG)$_2$]:[dimethyltin(IV)] = 6:1. Curves: 1a–4a, pH 7.01, 6.26, 4.84 and 3.87, respectively; 1b–5b, pH 7.08, 6.05, 4.87, 4.51 and 4.13.

did not depend on the concentration of dimethyltin(IV) in the solutions. The same spectra were measured in the presence and the absence of organotin(IV). Above pH 7, an increase of pH did not have any significant effect on the ^{31}P spectra. However, when the pH was decreased to the point where precipitation started, a significant upfield shift of the ^{31}P signals was observed, at a 6:1 ratio of dimethyltin(IV) to hexamer. This is in contrast with what was observed in the case of the free hexamer (Fig. 6). A similar effect was found at ratios of 3:1 and 12:1. These changes were very similar to those observed for the dimethyltin(IV)–mononucleotide systems. From the spectra it is difficult to ascribe the effect to any specific phosphate in the hexamer. The reason for this may be that the assembly of bases does not give a structure and/or an electron-rich "hot spot" that will favour the co-ordination of dimethyltin(IV).[33] Hence, native DNA or RNA might be expected to contain better binding sites than the sequence herein. Nevertheless, significant effects on the phosphates are observed to be induced by dimethyltin(IV), and, even though bonding takes place only below pH 7, this might be of biological significance. Lamm and Pack[34] have shown that the pH near the surface of DNA can be up to three units lower than the bulk pH.

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