Multinuclear 1D and 2D NMR Investigations on the Interaction between the Pyrimidic Nucleotides 5'-CMP, 5'-dCMP, and 5'-UMP and Diethyltin Dichloride in Aqueous Medium

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The interactions between diethyltin dichloride and 5′-CMP, 5′-dCMP, and 5′-UMP in aqueous solution were investigated by multinuclear 1D and 2D NMR techniques including ^{119}Sn, ^{15}N and ^{31}P nuclei. These studies were combined with electrospray mass spectrometry, infrared spectroscopy, solid state ^{13}C, ^{31}P and ^{117}Sn CP-MAS NMR, and elemental analysis. As demonstrated by ^{31}P-^{1}H HOESY spectroscopy, the diethyltin moiety interacts with the phosphate group of the pyrimidic mononucleotides in the pH range 0.5–3.5. Compound **8** (X = Cl), the solid isolated in this pH range from 5′-CMP, contains two tin atoms bridged by one oxygen and one chlorine atom, each tin atom being linked to the phosphate group of a nucleotide moiety. For 5′-UMP the solid isolated (**12**) has a dimeric structure with two different tin atoms; it can be formed by dimerization of compound **11** with the elimination of two water molecules. As demonstrated by ^{1}H-^{119}Sn HMQC correlation NMR data and the $^{2}J(^{119}\text{Sn–O–}^{117}\text{Sn})$ coupling constant of 156 Hz, the diethyltin moiety reacts with the O(2′) and O(3′) oxygen atoms of the nucleotides to form a dimeric diethyldioxastannolane at pH > 9.0. Between pH 5.0 and 9.0, no evidence for any interaction between the diethyltin moiety and the nucleotides was found.

Introduction

Many organometallic compounds exhibit interesting antitumoural activity against several human cancer cell lines.[1] The well-known complex cisplatin, $Pt(NH_3)_2Cl_2$, clinically used in cancer chemotherapy, proved to interact mainly with the N7 atoms of two adjacent guanines in the same DNA strand.[2–5] For several years, we have been interested in the synthesis and characterisation of organotin complexes which, in some cases, display higher antitumour activities in vitro than cisplatin.[6] In contrast to platinum compounds, very little is known about the origin of the antitumoural activity of organotin compounds although, as for cisplatin, DNA was proposed to be the target.[7] Some work has already been performed on the interactions between organotins and DNA or its precursors. Barbieri et al. have investigated the solid resulting from the reaction of ethanolic organotins [$SnR_2Cl_2(EtOH)_2$ and $SnR_3Cl(EtOH)$] with aqueous DNA or mononucleotide solutions. However, only ^{119}Sn Mössbauer spectroscopy was used.[8–10] The condensate was assumed to be a diorganotin species interacting with two vicinal phosphate groups of DNA. Li et al. have investigated the interaction of $(C_2H_5)_2SnCl_2$(phen) (phen = phenanthroline) with 5'-dGMP in aqueous medium using [*trans*-$en_2Os(\eta\text{-}H_2)](CF_3SO_3)_2$, a versatile ^{1}H NMR probe. They also studied solid mixtures of the same diethyltin dichloride complex with 5'-AMP, 5'-CMP, and 5'-GMP dissolved in DMSO, using ^{1}H and ^{31}P NMR and UV spectroscopy.[11a] More recently, Hadjiliadis et al.[11b] studied the interaction of the purine nucleotides 5'-IMP and 5'-GMP with Et_2SnCl_2 using various techniques, including ^{1}H, ^{13}C and ^{31}P 1D NMR and Mössbauer spectroscopy. Finally, a very recent paper described the investigation of the interaction between dimethyltin dichloride and 5'-GMP, 5'-ATP and 5'-$d(CGCGCG)_2$ by potentiometric titrations and ^{1}H and ^{31}P NMR spectroscopy.[11c] All these authors concluded that the organotin moiety reacts with the phosphate group of the nucleotides. However, these interactions have not been investigated further using spectroscopic techniques that provide more detailed structural data, such as ^{119}Sn NMR spectroscopy. Potentially, this technique is an ideal tool of investigation, comparable to ^{195}Pt NMR spectroscopy for platinum compounds.[12]

This study investigates the interaction between a diorganotin dichloride, Et_2SnCl_2, and pyrimidic nucleic acid precursors within a wide pH range in aqueous medium. The three mononucleotides investigated, cytidine-5'-monophosphate (5'-CMP), 2'-deoxycytidine-5'-monophosphate (5'-dCMP) and uridine-5'-monophosphate (5'-UMP), were selected on the basis of their increased solubility when they are complexed to tin (Figure 1).

First, we monitored the ^{31}P chemical shift of the mononucleotides in aqueous solution as a function of pH in the presence or absence of diethyltin dichloride and the ^{119}Sn chemical shift of a mixture of the latter with the mononu-

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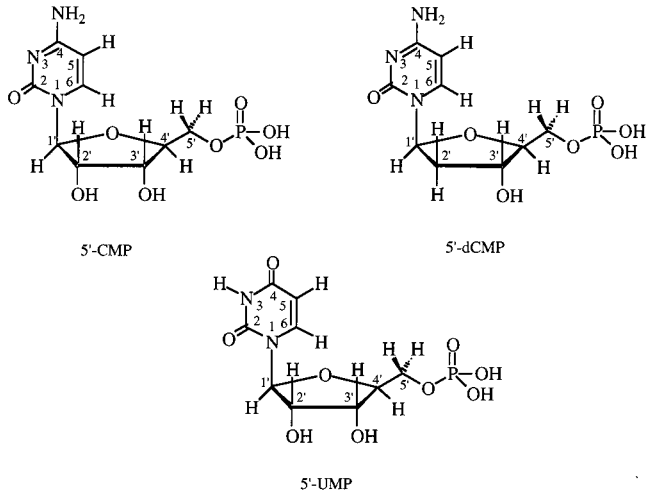


Figure 1. Structures and numbering scheme of the 5'-CMP, 5'-dCMP and 5'-UMP mononucleotides

cleotides. The differences in ^{31}P chemical shift titration profiles are used as indicators for an interaction between diethyltin dichloride and the mononucleotides. Subsequently, the structural features of the interaction are characterised by 1D and 2D NMR correlation techniques involving ^{1}H, ^{13}C, ^{15}N, ^{31}P and ^{119}Sn nuclei, completed by ^{35}Cl NMR data where appropriate. Proton detected ^{1}H-^{119}Sn HMQC[13–15] and phosphorus detected ^{31}P-^{1}H HOESY[16,17] have been reported to be very efficient for the characterisation of organotin complexes in solution.[18] Whenever precipitation occurs, the solid is isolated and characterised by solid state ^{13}C, ^{31}P, and ^{117}Sn CP-MAS NMR and IR spectroscopy as well as elemental analysis. None of the solids isolated provided crystals suitable for X-ray diffraction analysis.

Results and Discussion

Aqueous Chemistry of Diethyltin Dichloride

Figure 2 shows the different intermediates (in their monomeric form, for convenience) generated upon hydrolysis of Et_2SnCl_2 as mentioned in the literature.[19–21] The NMR data collected during this preliminary investigation are suitable references for the interpretation of data obtained in the presence of nucleotides.

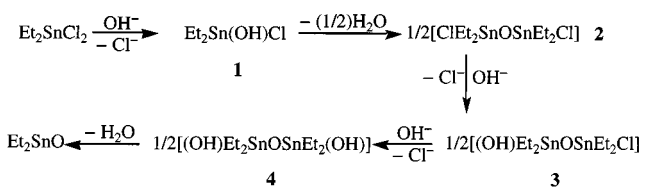
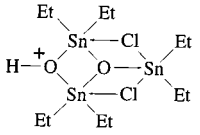
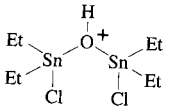


Figure 2. Different species formed under hydrolysis of Et_2SnCl_2 in aqueous solution

Table 1. Electrospray mass spectrometry data of a MeOH/H_2O (90/10) solution of Et_2SnCl_2 at pH 2.2



5 + H^+



2 + H^+

m/z	Assignment
653	**5** + H_3O^+
635	**5** + H^+
441	**2** + H^+
277	$[Et_2SnCl(CH_3OH)_2]^+$
245	$[Et_2SnCl(CH_3OH)]^+$

Table 2. Experimental elemental analysis data for the solid compound isolated from an aqueous solution of Et_2SnCl_2 at pH 2.8 as well as calculated data for the proposed structure

	%C	%H	%Cl	%Sn
Et_2SnCl_2 isolated at pH 2.8	21.8	4.3	14.0	52.2
$[ClEt_2SnOSnEt_2Cl]$ (**2**)	21.8	4.6	16.1	53.9

The pH of a 0.5 M aqueous solution of Et_2SnCl_2 is ca. 2.2. A precipitate is generated when the pH of the supernatant is raised above 2.2 with aqueous NaOH. Below pH 2.2, a clear solution is observed. An electrospray mass spectrum of a mixture obtained by adding methanol to an aqueous solution of Et_2SnCl_2 (pH 2.2) with a final MeOH/H_2O ratio of 90/10 reveals two species. On the basis of data from Dakternieks et al.[22a] these species can be assigned to compounds **5** and **2** (Table 1). The fragment detected at *m/z* 441 in the electrospray mass spectrum is the protonated form of intermediate **2** (Figure 2).

The other fragment ions correspond to $[Et_2SnCl]^+$ solvated by one or two methanol molecules or to hydrated **5** (Table 1). These results clearly indicate that in solution at pH 2.2 only one chloride anion dissociates from diethyltin dichloride.

The precipitate obtained at pH 2.8 was isolated. Its elemental analysis data (Table 2) indicate that it basically corresponds to compound **2**, probably contaminated by **3**, considering the slight chlorine deficit.

The ^{117}Sn NMR spectrum of this sample in $CDCl_3$ at 89.150 MHz displays eight resonances between $\delta = -87$ and $\delta = -182$. They can be divided into two pairs of equally intense resonances **B** and **C** and one set of four equally intense resonances **A**. Set **B** ($\delta = -91.7$ and -139.9) displays one pair of satellites with a coupling of 67 Hz. It is assigned to intermediate **2** which is described by Otera[22b] as a dimer for R = Me, Et and Bu with similar ^{119}Sn parameters for R = Et:[22b] $\delta(^{119}Sn1) = -140.6$; $\delta(^{119}Sn2) = -91.8$; $^{2}J(^{119}Sn–O–^{117/119}Sn) = 73$ Hz. Set **C** ($\delta = -157.0$ and -176.2) displays at least one pair of $^{2}J(^{117}Sn–O–^{117/119}Sn)$ satellites with a coupling of 212 Hz, others being unclear due to a low signal-to-noise ratio. The ^{117}Sn resonances of

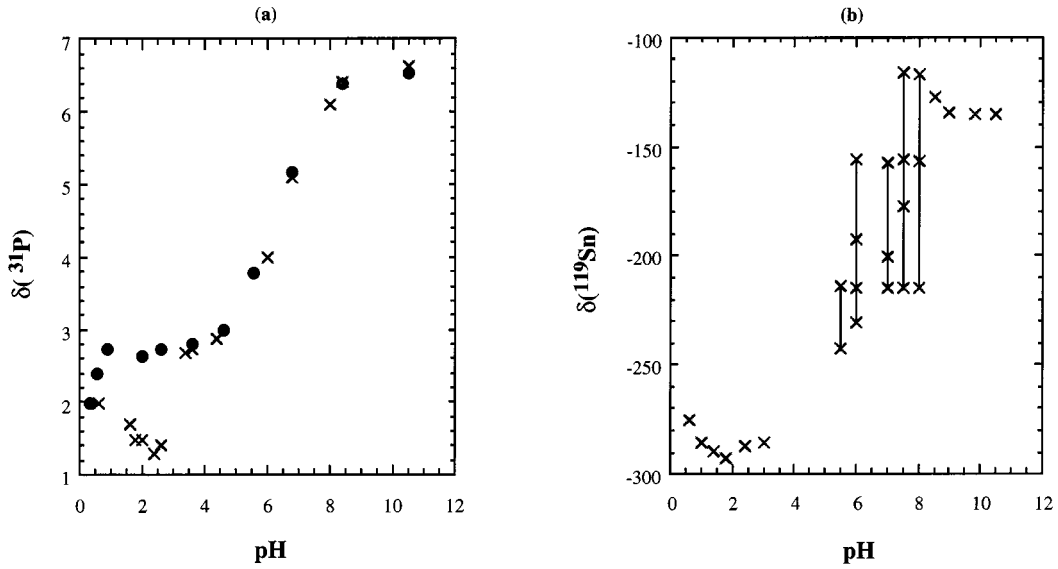


Figure 3. (a) ^{31}P NMR titration curve of 5'-CMP and a mixture of 5'-CMP and Et_2SnCl_2; full circles represent free 5'-CMP; crosses correspond to 5'-CMP/Et_2SnCl_2; (b) ^{119}Sn NMR titration curve for 5'-CMP/Et_2SnCl_2; multiple points related by straight lines for a given pH value account for the presence of multiple resonances

set **A** (δ = –87.9, –130.9, –151.8, and –181.8) display a pair of signals with a coupling constant of 98 Hz and another pair with a coupling constant of 175 Hz. Although the species generating **A** and **C** cannot be identified, these NMR data point to a mixture of intermediates including **3** and **4**, possibly aggregated to various degrees during hydrolysis of Et_2SnCl_2 depicted in Figure 2.

Interactions of Diethyltin Dichloride with the Nucleotides in Acidic Medium

Above pH 2.2, precipitation occurs from the solutions of the three 2:1 mixtures of 5'-CMP/Et_2SnCl_2, 5'-dCMP/Et_2SnCl_2, and 5'-UMP/Et_2SnCl_2. Consequently, samples for NMR experiments in solution are obtained from the supernatant of this precipitate. Because in acidic medium the three nucleotides behaved similarly in the presence of Et_2SnCl_2, only the mixture 5'-CMP/Et_2SnCl_2 is discussed in detail.

In the pH range 0.5–3.5, the addition of 0.5 equivalent of diethyltin dichloride to 5'-CMP, 5'-dCMP and 5'-UMP causes the ^{31}P resonance of the phosphate group of the nucleotides to shift to lower frequencies, with the maximum change in chemical shift in the pH range 2.0–2.5. The titration curve of the ^{31}P chemical shift is displayed in Figure 3a for 5'-CMP and 5'-CMP/Et_2SnCl_2.

This ^{31}P chemical shift modification can be traced back to an interaction of diethyltin dichloride with the phosphate group of the three nucleotides. ^{1}H-^{119}Sn, ^{1}H-^{31}P, ^{31}P-^{119}Sn, and ^{13}C-^{31}P HMQC experiments[23,24] performed on 5'-CMP/Et_2SnCl_2 at pH 2.2 failed to show any correlation peaks between the resonances of the organotin moiety and those of 5'-CMP. However, this does not formally exclude the interaction between diethyltin dichloride and the phosphate group of 5'-CMP. Indeed, the coupling can be very small or even nonexistent and undetectable depending on the bond strength.[25] The ^{31}P-^{1}H HOESY (^{31}P detected Heteronuclear Overhauser Spectroscopy) technique[16–18] which correlates nuclei through dipolar couplings was previously used by Barnham et al.[26] to characterise the Pt–NH···5'-phosphate intermolecular interaction in the complex [Pt(en)(5'-GMP–N7)$_2$]·9H_2O. This technique provided a 2D spectrum of 5'-CMP/Et_2SnCl_2 at pH 2.2 with four ^{31}P-^{1}H NOE cross-peaks, two from the CH_2 and CH_3 protons of diethyltin dichloride and two from the H5' and H6 protons of 5'-CMP. Similar results were obtained for the mixtures of the two other nucleotides with Et_2SnCl_2, i.e. 5'-dCMP/Et_2SnCl_2 and 5'-UMP/Et_2SnCl_2 at pH 2.2. With the ratio ($\gamma^1H/\gamma^{31}P$) > 2.38 in mind, the distances between the ethyl protons and the phosphorus atom of the three nucleotides cannot exceed 4 Å, proving that the tin moiety interacts with the phosphate group of the nucleotide. ^{31}P-^{1}H-NOE's are always positive, irrespective of the correlation time.[27] Hence, weak or missing NOE's cannot be assigned to a zero cross point of the NOE effect. The observed cross peak between the H6 proton and the ^{31}P nucleus of 5'-CMP, 5'-dCMP, and 5'-UMP arises from the *anti* conformation of the base relative to the sugar unit which is also known for free 5'-GMP.[28]

As assessed by the ^{119}Sn chemical shifts and the $^{1}J(^{13}C$–$^{119}Sn)$ values,[29,30] the tin atom is six-coordinated in all three mixtures, i.e. 5'-CMP/Et_2SnCl_2, 5'-dCMP/Et_2SnCl_2, and 5'-UMP/Et_2SnCl_2 at pH 2.2 (Table 3). Empirical correlations between C–Sn–C angles and coupling constants involving the ^{119}Sn nucleus[31] (Table 3) provide C–Sn–C angle values typically ranging from 150° to 170°, which are in agreement with distorted *trans*-octahedral, six-coordinate geometries at the tin atom.

As shown by ^{13}C, ^{31}P, and ^{117}Sn CP-MAS NMR, the solid isolated from the mixture 5'-CMP/Et_2SnCl_2 (2:1) at pH 2.2 contains both Et_2Sn and 5'-CMP moieties. The ^{31}P CP-MAS NMR spectrum exhibits 5'–CMP two resonances at δ = –11 and δ = –12 which are low frequency shifted

Table 3. ^{119}Sn chemical shifts (ppm) and coupling constants (Hz) determined at pH 2.2 for the three nucleotides in the presence of Et_2SnCl_2 in the molar ratio 2:1

	5'-CMP/Et_2SnCl_2	5'-dCMP/Et_2SnCl_2	5'-UMP/Et_2SnCl_2
$\delta(^{119}Sn)$ (D_2O)	–287	–278	–298
$\delta(^{117}Sn)$ (solid)	–274	*	–262 and –281
$^1J(^{13}C–^{119}Sn)$	850	834	876
$^2J(^1H–^{119/117}Sn)$	96	93	92
C–Sn–C angle[a]	172°	166°	163°
C–Sn–C angle[b]	152°	150°	154°
C–Sn–C angle[c]	160°	158°	162°

[a] Calculated from the empirical relationships of Howard et al.:[31a] Θ (deg) = 2.28 [$^2J(^1H–^{119}Sn)$] – 46.4. – [b] Lockhart et al.:[31b] Θ (deg) = 0.088 [$^1J(^{13}C–^{119}Sn)$] + 76.754. – [c] Holecek et al.:[31c] Θ (deg) = 0.1 [$^1J(^{13}C–^{119}Sn)$] + 74.675; * irreproducible, contaminated by Et_2SnO and other unidentified hydrolysis products.

with respect to the single one in solution at ca. $\delta = 1$. The assignment of the resonances in the ^{13}C CP-MAS spectrum is made by comparison with the solution ^{13}C chemical shifts of 5'-CMP/Et_2SnCl_2 at pH 2.2. In solution, ^1H-^{13}C HMBC and HMQC[23,24,32] spectra allowed the assignment of the ^{13}C and ^1H resonances that is further supported by literature data.[33]

Table 4. Comparison of the solid and solution state ^{13}C chemical shifts at pH 2.2 for the mixtures 5'-CMP/Et_2SnCl_2 (2:1) and 5'-UMP/Et_2SnCl_2 (2:1)

	5'-CMP/Et_2SnCl_2		5'-UMP/Et_2SnCl_2	
	solid	solution	solid	solution
C4	163.9	159.2	163.1	166.6
C2	154.0	148.5	152.2	152.2
C6	141.7	144.1	139.4	142.0
C5	98.9	95.3	102.3	103.0
			100.2	
C1'	87.2	89.8	93.1	89.3
	85.2			
C4'	83.4	83.4	84.4	83.5
			81.5	
C2'	76.2	74.2	75.2	74.3
	75.3			
C3'	72.9	69.2	71.8	70.1
	72.1		71.3	
C5'	68.0	63.8	67.6	65.1
CH_2	19.9	25.6	19.7	27.0
	18.4		19.0	
	17.8		17.2	
CH_3	9.2	9.2	9.7	9.8
			9.2	

Table 4 shows that some of the solid state ^{13}C resonances are duplicated, one is even triplicated; this is a result of the nucleotide moieties being nonequivalent, possibly because of chirality, packing effects, and/or polymorphism. The ^{13}C chemical shifts of the tin ethyl CH_2 group, the pyrimidic and the C5' carbons deviate most from those in the liquid state. For the pyrimidic carbons, this variation can be explained by a different H-bonding network in the solid state, as previously seen for several nucleotides by IR spectroscopy.[34] The ^{117}Sn NMR CP-MAS spectrum exhibits a single isotropic shift at $\delta = –274$ in the range typical for six-coordinate tin atoms in solution. These CP-MAS NMR data, combined with the solution NMR data, suggest that the tin environments in the solid and solution states are similar.

The vibrational data (Table 5) account exclusively for the existence of a tin–phosphate interaction, the ring vibration frequencies not being affected by the addition of Et_2SnCl_2. As suggested above for the variation of the pyrimidic ^{13}C chemical shifts, the shift of the $\nu(C(2)=O)$ frequency [$\Delta\nu = \nu$(5'-CMP/Et_2SnCl_2) – ν(5'-CMPNa_2) = 23 cm^{-1}] can be explained by different H-bonding networks.[34] The Raman spectrum displays two characteristic bands at 434 and 568 cm^{-1} that were tentatively assigned to the $\tilde{\nu}$(Sn–O–P) and $\tilde{\nu}$(Sn–O–Sn) vibration frequencies, respectively.[35–37]

On the basis of all previous data, four tin–phosphate complexes (Figure 4) are conceivable for the solid-state structure obtained from mixing 5'-CMP and Et_2SnCl_2. Unexpectedly, elemental analysis data from samples of the complex depend on the acid (HCl or HBr) used for the adjustment of the pH of the supernatant from which this solid precipitates; nevertheless, these data are self-consistent and reproducible (Table 6). With HCl, the chlorine detected originates not only from Et_2SnCl_2 but also, and even predominantly, from the excess HCl. However, chlorine is also detected when HBr is used in excess. In the latter case, the solid analysed contains two complexes, one only with bromine (ca. 80%) and one only with chlorine (ca. 20%). Structures **6**, **7** (X = Cl, OH), as well as structure **9** (X = OH) (Figure 4) can easily be rejected on the basis of the chlorine elemental analysis. Structure **8** (X = Cl) is favoured over structure **9** (X = Cl) because the calculated carbon and tin elemental data match the experimental data better for the former than for the latter (Table 6). A thermogravimetric analysis of the solid detects a weight loss of ca. 7% up to 170 °C, prior to a more substantial and continuous loss above 190 °C. The initial low weight loss is compatible with the expected loss of one equivalent of HCl and two equivalents of water, an observation which again matches structure **8** better than **9** (X = Cl). Structure **8** is proposed as a hydrochloric salt with a Sn–Cl–Sn bridge, which is known for various organotin oxides,[38,39] and a hydrogen bond between the chloride anion and the oxygen bridging the two

Table 5. IR data of free 5'-CMP and 5'-UMP as well as IR and Raman (*) data of the mixtures 5'-CMP/Et_2SnCl_2 and 5'-UMP/Et_2SnCl_2 in the solid state in KBr pellets (cm^{-1})

Tentative assignment	5'-CMPNa_2[a]	5'-CMP/Et_2SnCl_2[a]	5'-UMPNa_2[a]	5'-UMP/Et_2SnCl_2[a]
$\tilde{\nu}$(C2=O)	1653s	1730s, 1676s	1683s[b]	1708s, 1664m[b]
$\tilde{\nu}$(ring)	1540m	1540m	1472m	1466m
	1491m	1491m		
$\tilde{\nu}(PO_3)$asym + $\tilde{\nu}$[CO(sugar)]	1110s	1121s	1126s, 1091s	1120s, 1078m
	1082s	1075s		
$\tilde{\nu}(PO_3)$sym	978s	995m	976m	1009m, 1007m, 994m, 960w
		961m		
$\tilde{\nu}$(C–Sn)asym*	–	548m	–	548m
$\tilde{\nu}$(C–Sn)sym*	–	511s	–	511s
$\tilde{\nu}$(Sn–O–P)*	–	434w	–	436w
$\tilde{\nu}$(Sn–O–Sn)*	–	568w	–	568w

[a] s = strong, m = medium, w = weak, $\tilde{\nu}$ = stretching. – [b] $\tilde{\nu}$(C2=O) + $\tilde{\nu}$(C4=O).

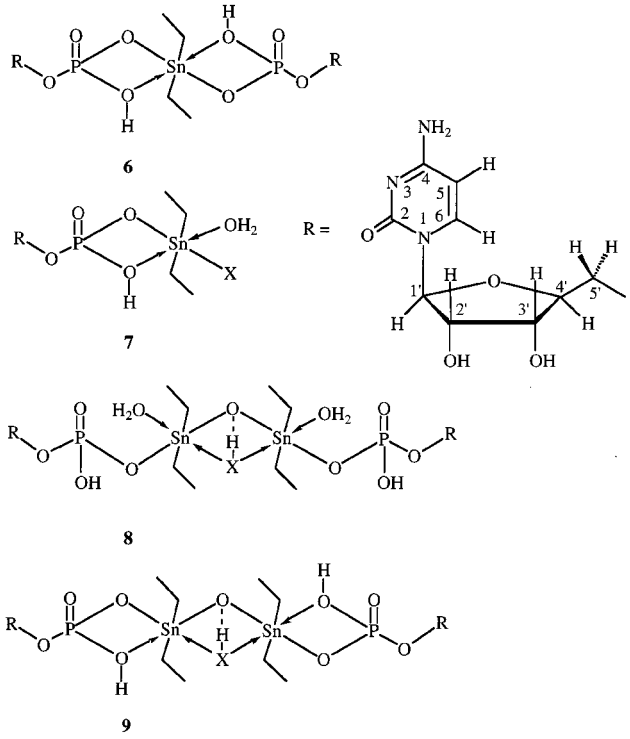


Figure 4. Potential structures for the solid isolated from the mixture 5'-CMP/Et_2SnCl_2 at pH 2.2 and assessed against the data (X = OH, Cl, or Br)

tin atoms. When prepared from the HBr solution, the compounds appear to be of type **8** with X being either Cl or Br.

As supported by similar ^{13}C and $^{117/119}Sn$ CP-MAS and solution NMR data, the environments of the tin atom in solution and in the solid state are proposed to be similar for 5'-CMP/Et_2SnCl_2. Thus, the structure of the complex in solution should be closely related to structure **8** (X = Cl), even if some differences between the solution and solid state ^{31}P and ^{13}C chemical shifts of the CH_2 group suggest local structure differences, possibly due to different H-bridge network or packing effects.

The ^{35}Cl solution NMR data of the supernatant shed some further, albeit indirect light on this proposal. It is well-known that the ^{35}Cl NMR signal of the chloride anion is very sharp and displays a chemical shift around $\delta = 0$.[40] In contrast, covalently bound chlorine generally fails to provide a useful ^{35}Cl signal since it is smeared out in the noise. The supernatant of the solid with proposed structure **8** falls into the latter category. Provided that **8** has identical structures in the supernatant and the solid, this structure implies that one chloride ion has been split off from the initial Et_2SnCl_2, while the second is covalently bound. The absence of any visible ^{35}Cl resonance in a solution of **8** implies the presence of covalently bound chlorine (as indicated by elemental analysis), necessarily being in fast exchange with the ionic chloride which must also be present.

Mixtures of Et_2SnCl_2 with the nonphosphorylated analogue of 5'-CMP, cytosine, also give rise to precipitates at pH 2–5; this occurs to an even larger extent than for 5'-CMP/Et_2SnCl_2. Indeed, the ^1H NMR spectra of the supernatants show that only small amounts of compounds containing a diethyltin moiety are present in solution, as assessed from a comparison of the integrations of the H6 and Et_2Sn protons. The precipitate was isolated; its ^{13}C CP-MAS NMR spectrum presents only ^{13}C resonances from the diethyltin moieties and none from the nucleobase. The ^{117}Sn CP-MAS NMR spectrum of the cytosine-derived

Table 6. Experimental elemental analysis data for the solid compounds isolated from 5'-CMP/Et_2SnCl_2 and 5'-UMP/Et_2SnCl_2 mixtures at pH 2.2 as well as calculated data for potential structures of Figures 4 and 5; the experimental data result from the average over three samples prepared independently with HCl or HBr; for 5'-UMP/Et_2SnCl_2, identical experimental results were obtained with HCl and HBr

	%C	%H	%N	%Cl	%Br	%P	%Sn
5'-CMP/Et_2SnCl_2[a]	28.4	4.7	7.5	3.1	–	5.6	21.2
5'-CMP/Et_2SnCl_2[b]	27.6	4.5	7.2	0.7	5.0	5.3	20.2
6	27.0	4.2	7.3	–	–	10.7	20.5
7 (X = Cl)	28.3	4.6	7.6	6.4	–	5.6	21.5
7 (X = OH)	29.2	4.9	7.9	–	–	5.8	22.2
8 (X = Cl)	28.7	4.7	7.7	3.3	–	5.7	21.9
8 (X = Br)	27.6	4.5	7.4	–	7.1	5.5	21.0
8 (X = OH)	29.2	4.9	7.9	–	–	5.8	22.2
9 (X = Cl)	29.7	4.5	8.0	3.4	–	5.9	22.6
9 (X = Br)	28.5	4.3	7.7	–	7.3	5.7	21.7
9 (X = OH)	30.2	4.7	8.1	–	–	6.0	23.0
5'-UMP/Et_2SnCl_2	31.0	4.3	5.5	–	–	5.9	23.0
10	29.2	4.7	5.2	–	–	5.8	22.2
11	30.2	4.5	5.4	–	–	6.0	23.0
12	30.7	4.4	5.5	–	–	6.1	23.4

[a] Prepared with HCl. – [b] Prepared with HBr.

Table 7. Experimental elemental analysis data for the solid compound isolated from cytosine/Et_2SnCl_2 at pH 2.2 as well as calculated data for the proposed structure

	%C	%H	%Cl
cytosine/Et_2SnCl_2	23.1	5.0	8.4
$[(OH)Et_2SnOSnEt_2Cl]$	22.8	5.0	8.4

sample displays two isotropic ^{117}Sn chemical shifts of comparable intensities at $\delta = -164$ and $\delta = -178$. In agreement with this, the elemental analysis data of this sample as well as the two equally intense ^{117}Sn resonances observed match the formula $[(OH)Et_2SnOSnEt_2Cl]$ (Table 7) that corresponds to intermediate **3** in the hydrolysis scheme of Figure 2. In line with Otera's finding on 3-chloro-1-hydroxytetraorganodistannoxanes (R = *n*Bu), this structure should be dimeric.[22b] In fact this compound, which is also soluble in $CDCl_3$, has a ^{117}Sn NMR spectrum which displays the same eight resonances as species A, B, and C, albeit with different relative populations, as in the sample isolated by precipitation at pH 2.8 from pure aqueous Et_2SnCl_2. Therefore, it probably includes intermediates **2**, **3** and **4**. Since essentially the same hydrolysis products are observed as with pure Et_2SnCl_2, it can be concluded that at least no peculiar interaction between the Et_2Sn moiety and cytosine occurs (Figure 2). In particular, this rules out any interaction between the Et_2Sn moiety and the nucleobase functionalities.

A solid was also isolated from a mixture of 5'-UMP and Et_2SnCl_2 at pH 2.2. The ^{31}P CP-MAS NMR spectrum exhibits two resonances at $\delta = -7$ and $\delta = -12$; they are shifted to low frequency with respect to the single one in solution at ca. 0.2 ppm. The ^{117}Sn CP-MAS NMR spectrum exhibits two isotropic resonances of equal intensity at $\delta = -262$ and $\delta = -281$ in the range corresponding to six-coordinate tin atoms. The vibrational data of 5'-UMP/Et_2SnCl_2 also unambiguously reflect a tin–phosphate interaction (Table 5). In contrast to 5'-CMP/Et_2SnCl_2, the elemental analysis data of 5'-UMP/Et_2SnCl_2 (Table 6) are independent of the acid used for adjusting the pH of the solution from which the solid originates, no chlorine or bromine being detected. These data are fairly consistent with structure **10** and even better for structure **11** (Table 6 and Figure 5). Unlike **8**, the thermogravimetric analysis of this solid does not indicate loss of H_2O or HCl, supporting structure **11** rather than **10**. However, the two different isotropic chemical shifts observed in the ^{117}Sn CP-MAS spectrum are in disagreement with both structures **10** and **11**; therefore, structure **12** can be proposed (Figure 5). It contains two different tin atoms and can be formed by dimerization of structure **11** with elimination of two water molecules. Among the three structures proposed, **12** agrees best with the elemental analysis data (Table 6) and is the only one to fit all the NMR data.

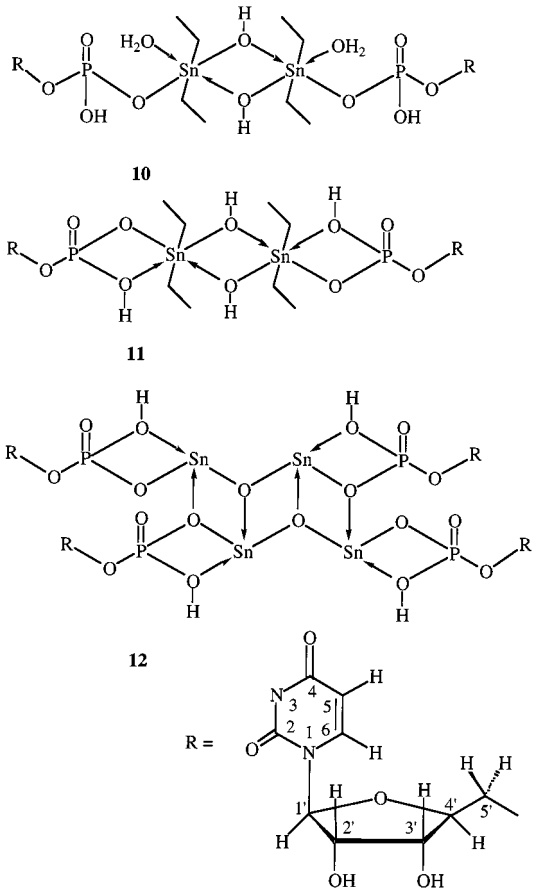


Figure 5. Potential structures for the solid isolated from the mixture 5'-UMP/Et_2SnCl_2 at pH 2.2 and assessed against the data; in structure **12**, the ethyl groups bonded to the tin atoms have been omitted for clarity

The tendency of the 5'-dCMP/Et_2SnCl_2 mixtures to precipitate is lower than that of 5'-CMP and 5'-UMP, with precipitation only occurring at higher pH's. Moreover, no reproducible elemental composition and solid state NMR data could be obtained as the solid appears to contain hydrolysis products of Et_2SnCl_2 as well as free and complexed 5'-dCMP with $\delta(^{31}P) = 0.6$ and –9.2.

Interaction of Diethyltin Dichloride with the Nucleotides at pH > 9.0

At pH 9.0, the 1D ^{119}Sn NMR spectrum of the mixture 5'-CMP/Et_2SnCl_2 (2:1) exhibits a single ^{119}Sn resonance at $\delta = -137$ (Figure 4), characteristic of R_2SnO_2 compounds in the presence of a donor giving rise to a five-coordinate tin.[31,41] The ^{13}C NMR spectrum exhibits a pair of signals for each carbon atom of the sugar unit, one ^{13}C resonance of each pair arising from free 5'-CMP. Upon addition of one more equivalent of diethyltin dichloride (5'-CMP/Et_2SnCl_2 = 1:1) the ^{13}C resonances corresponding to free 5'-CMP disappear but the 1D ^{119}Sn NMR spectrum displays a second very small ^{119}Sn signal at $\delta = -202$. The latter increases as the amount of Et_2SnCl_2 is further increased. The mixture 5'-UMP/Et_2SnCl_2 displays similar behaviour; in contrast, the mixture 5'-dCMP/Et_2SnCl_2 in any

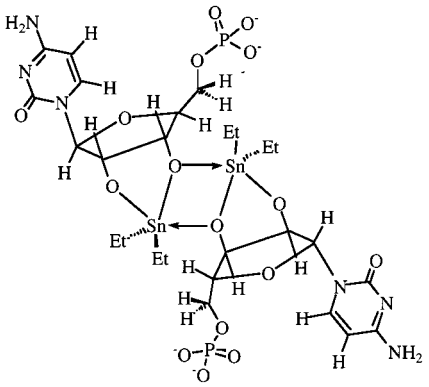


Figure 6. Structure proposed for the complex generated in mixture 5'-CMP/Et_2SnCl_2 at pH 10.5

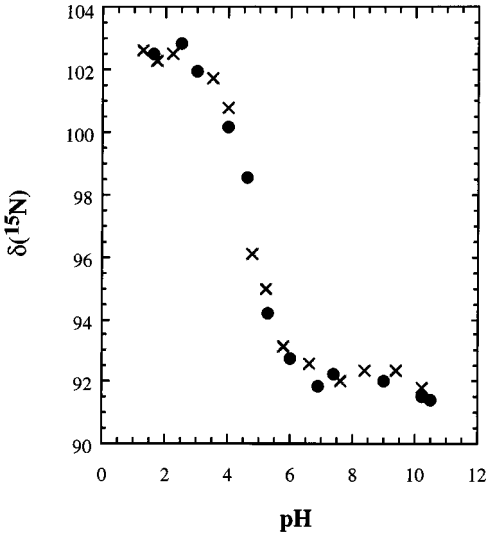


Figure 7. ^{15}N NMR titration curve as obtained for the N3 nitrogen atom of ^{15}N-enriched 5'-CMP and 5'-CMP/Et_2SnCl_2

Table 8. Chemical shifts (ppm) and coupling constants (Hz) for the three nucleotides studied at pH 10.5 in the presence of Et_2SnCl_2

	$\delta(^{119}Sn)$	$^1J(^{13}C–^{119/117}Sn)$	$^2J(^1H–^{119/117}Sn)$
5'-CMP/Et_2SnCl_2	–137	667	81
5'-dCMP/Et_2SnCl_2	–202	757	[a]
5'-UMP/Et_2SnCl_2	–137	665	79

[a] Nonobservable due to broad signal.

ratio exhibits a single ^{119}Sn resonance at δ = –202. Furthermore, the ^{119}Sn nuclei associated with the two ^{119}Sn resonances (δ = –137 and –202) are in chemical exchange, as demonstrated by the presence of intense cross-peaks in the 2D ^{119}Sn EXSY spectrum[42,43] recorded from the mixture 5'-CMP/Et_2SnCl_2. A 2D ^1H-^{119}Sn HMQC spectrum of this mixture reveals no correlations between the ^{119}Sn resonance at δ = –202 and the proton resonances of the 5'-CMP moiety. In contrast, the ^{119}Sn resonance at δ = –137 displays ^1H-^{119}Sn HMQC correlations with the H2' and H3' proton resonances of the sugar unit, as well as satellites corresponding to a $^2J(^{119}Sn–O–^{117/119}Sn)$ coupling constant of 156 Hz.

These ^1H-^{119}Sn HMQC correlations result from $^3J(^1H–^{119}Sn)$ couplings through the pathways H2'–C2'–O2'–Sn and H3'–C3'–O3'–Sn and can be traced to the formation of bonds between the tin atom and the O(2') and O(3') oxygen atoms of the sugar unit (Figure 6). The ^{119}Sn chemical shift of δ = –137, the $^1J(^{13}C–^{119}Sn)$ of 667 Hz (Table 8), and the $^2J(^{119}Sn–O–^{117/119}Sn)$ of 156 Hz, combined with the ^1H-^{119}Sn HMQC data, are consistent with a five-coordinate tin atom in a dimeric R_2SnO_2 diethyldioxastannolane as shown in Figure 6, one oxygen atom of the diol group of the sugar unit being tricoordinated.[41] Similarly, dimeric dibutylstannylene acetals have ^{119}Sn chemical shifts between δ = –115 and –150 in five-membered rings with pentacoordination at the tin atom (Table II of ref.[41b]).

A ^{35}Cl NMR spectrum of the mixture at pH 10.5 confirms that no covalently bonded chlorine is involved, as supported by the very narrow ^{35}Cl resonance at δ = 0[40] which indicates that this element is only present as a chloride anion. The ^{119}Sn resonance at δ = –202 starts appearing when the tin to nucleotide ratio exceeds 1:1 in a mixture of Et_2SnCl_2 with substrates which contain a diolic sugar unit, i.e. 5'-CMP, 5'-UMP, cytidine, and D-ribose. All of these substrates display a resonance around δ = –135 to –138, arising from the tin diolate complex being formed first. In contrast, the δ = –202 signal is the only resonance observed for a mixture of Et_2SnCl_2 with substrates which do not contain a diolic sugar unit, i.e. 5'-dCMP and cytosine. These observations unambiguously rule out that the second ^{119}Sn resonance at δ = –202 is due to any interaction between the tin moiety and functional groups of the pyrimidic unit, with or without a hydrogen bonding network. This is confirmed by ^{15}N NMR data obtained from ^{15}N enriched 5'-CMP/Et_2SnCl_2 mixtures (2:1, 1:1, 1:2) which did not reveal any deviation of the ^{15}N chemical shift of the N3, NH_2 or N1 atoms in the presence of diethyltin dichloride, with respect to free 5'-CMP. By way of an example, the titration curve of the $^{15}N3$ resonance of such a mixture is shown in Figure 7.

As in the acidic medium, the ^{119}Sn resonance at δ = –202 can only originate from some hydrolysed species on the hydrolysis pathway of Et_2SnCl_2 (Figure 2). Further evidence for this is the fact that the ^{119}Sn NMR spectrum of the supernatant of the mixture obtained from Et_2SnCl_2 in D_2O at pH 10.5 exhibits the very same resonance at δ = –202. By comparing the ^{117}Sn CP-MAS NMR spectrum with the one of pure Et_2SnO (δ = –166), the precipitate that was isolated from this mixture was identified as Et_2SnO (δ = –165), the anisotropy patterns being identical. As expected, the supernatant of this mixture exhibits a ^{35}Cl

resonance at a slightly higher chemical shift (δ = 4) than for completely anionic chloride (δ = 0, NaCl). This can originate from an equilibrium between intermediate **3**, with one chlorine atom covalently bonded to the tin atom, and intermediate **4**, from which chlorine can only be released as chloride. Compound **4** is the direct precursor of Et_2SnO which is indeed the solid detected; it can be formed directly by simple loss of a water molecule. Therefore, the species at δ = –202 is most likely intermediate **4** in the hydrolysis scheme of Et_2SnCl_2 (Figure 2).

Interaction of Diethyltin Dichloride with 5'-CMP, 5'-dCMP and 5'-UMP at pH 5.5–9.0

Several ^{119}Sn resonances appear in the ^{119}Sn NMR spectra of 5'-CMP/Et_2SnCl_2 in this pH range (Figure 3). These resonances are all very broad, needing a long acquisition time to obtain a reasonable signal-to-noise ratio. Due to the broadness of the resonances, ^1H-^{119}Sn HMQC correlations could not be detected between the proton resonances of the 5'-CMP moiety and the ^{119}Sn resonance. Attempts to reduce the line width of the ^{119}Sn resonances by recording a ^{119}Sn spectrum at lower temperatures failed, the resonances remaining still too broad. Likewise, 5'-CMP/Et_2SnCl_2 mixtures prepared in CD_3OD/D_2O (50:50) turned cloudy below 273 K.

Similar ^{119}Sn resonance frequencies, at least within experimental error, are found for all three nucleotides investigated in the pH range 5.5–9.0. For 5'-dCMP/Et_2SnCl_2, the ^{119}Sn resonance around δ = –202 is observed at pH 6.5.

These findings again suggest that all the ^{119}Sn resonances arise from hydrolysis products of Et_2SnCl_2, rather than from Et_2SnCl_2 interacting with the nucleotides. The broad ^{119}Sn resonances are in agreement with the associated species being in moderately slow exchange on the ^{119}Sn NMR time scale, since no evidence for multiple species was found in the ^1H and ^{13}C spectra (this indicates that these species, whatever they maybe, are in fast exchange on the ^1H and ^{13}C NMR time scales but in slow exchange on the ^{119}Sn NMR time scale). Unfortunately, 2D ^{119}Sn EXSY experiments which were aimed at supporting the presence of exchange were unsuccessful; this was due to broad linewidths and/or a low signal-to-noise ratio.

The solid isolated from the mixture 5'-CMP/Et_2SnCl_2 (1:1) around pH 7.0 mainly reveals a single isotropic ^{117}Sn chemical shift at δ = –167 in its CP-MAS spectrum. A comparison of its chemical shift anisotropy pattern with that of a pure sample of Et_2SnO reveals that this solid is the latter compound. However, a CP-MAS ^{117}Sn pattern from another unidentified minor species with much lower intensity (ca. 10%) is also observed at pH 7.0.

Conclusions

From pH 2.0–3.0, the Et_2Sn moiety from Et_2SnCl_2 is involved in bonding with the phosphate group of the three nucleotides studied. This interaction was characterised by a ^{31}P-^1H HOESY spectrum. At the same pH, the precipitated solid was isolated and structurally characterised. Among several possible structures, only one satisfied all data including the elemental analysis. This structure contains two tin atoms bridged by one oxygen and one chlorine atom, each tin atom being linked to the phosphate group of the nucleotide. Between pH 3.0 and 5.5 the absence of visible ^{119}Sn resonances precludes a conclusion.

Around neutral pH (5.5–9.0), no evidence for the Et_2Sn moiety interacting with the nucleotide was found, Et_2SnCl_2 giving rise to several hydrolysis products that are in mutual chemical exchange with one another.

In a 1:1 nucleotide/Et_2SnCl_2 mixture at pH > 9.0, Et_2SnCl_2 reacts with the O(2') and O(3') oxygen atoms of the sugar unit of the nucleotide as demonstrated by the presence of two HMQC cross-peaks H2'–Sn and H3'–Sn. With an excess of Et_2SnCl_2, a second ^{119}Sn resonance appears at δ = –202, which, however, does not correlate with any proton resonance of the nucleotides. This species is one of the hydrolysis products of Et_2SnCl_2, as further indicated by the absence of any ^{15}N chemical shift effect on the pyrimidic moieties that should have originated from the presence of a tin unit in the neighbourhood of nitrogen atoms.

The corresponding pH value limits for the interactions of the purine nucleotides 5'-IMP and 5'-GMP with Et_2SnCl_2 were: pH < 4 for the interaction through the phosphate group, pH > 9.5 for the interaction through the O(2') and O(3') oxygen atoms, and no observed interaction for pH 4–9.5.[11b]

These results are in contrast to the mode of interaction of cisplatin that interacts with the nitrogen atoms of purines (N7 of guanosine) and pyrimidines (N3 of cytidine).[44] However, they are similar to those reported for vanadium complexes which have been shown to interact with the phosphate group of mononucleotides at acidic pH, to hydrolyse at neutral pH and to react with the O(2') and O(3') oxygen atoms of the sugar unit of mononucleotides at basic pH.[45]

Experimental Section

Preparation of NMR Samples: The samples were typically prepared by dissolving the nucleotides (0.1 mmol) in D_2O (0.2 mL, 99.9% D, Aldrich). A solution of diethyltin dichloride (0.05 mmol) in D_2O (0.3 mL) was added dropwise. This resulted in a clear solution with a pH of approximately 6. The pH was adjusted by the dropwise addition of deuterated hydrochloric acid or sodium hydroxide solutions (0.1 M). Whenever generated, the precipitate was filtered and thoroughly washed with water and diethyl ether prior to drying in vacuum over P_2O_5 and further analysis (see Results and Discussion). The elemental analyses were performed by the "Laboratoire Central d'Analyse du CNRS", Vernaison, France. ^{15}N enriched (98% isotopic purity) 5'-CMP was purchased from CIL (MA).

NMR Experiments: All spectra were recorded at 303 K on a Bruker AMX500 spectrometer equipped with a digital lock and operating at 500.13, 202.46, 125.77, 186.50, 50.68, and 49.00 MHz for ^1H, ^{31}P, ^{13}C, ^{119}Sn, ^{15}N, and ^{35}Cl nuclei, respectively. ^{31}P chemical shifts were referenced to an aqueous solution of H_3PO_4 (85%). The ^{119}Sn chemical shifts were referenced to Ξ = 37.290665 MHz.[46a] The

^{35}Cl chemical shifts were referenced to a solution of NaCl (1 M, $\delta = 0$). The ^{15}N chemical shifts were referenced to $\Xi = 10.136767$ MHz.[46b]

Broad band ^{1}H-decoupled ^{13}C and ^{119}Sn spectra were recorded using the Bruker pulse sequences with standard delays. The 1D and 2D ^{1}H-^{119}Sn HMQC experiments consisted of gradient enhanced-versions of the standard HMQC pulse sequence processed in the magnitude mode and implemented as described elsewhere.[32] The 2D ^{31}P-^{1}H HOESY[16,17] experiments were performed by recording 128 FIDs of 1 K data points, 144 scans each, with an acquisition time of 0.2 s, a relaxation delay of 1.6 s and a mixing time of 1 s. The 2D ^{1}H-^{15}N HMQC spectra were typically obtained by recording 64 FIDs of 4 K data points, 32 scans each, with an acquisition time of 0.5 s and a relaxation delay of 1.5 s. The 2D ^{119}Sn EXSY spectra were typically obtained by recording 24 FIDs of 4 K data points, 3,200 scans each, with an acquisition time of 0.1 s, a relaxation delay of 1 s and a mixing time of 10 ms.

All CP-MAS NMR spectra[47] were recorded on a Bruker AC250 and, in later stages, on a Bruker DRX250 spectrometer operating at 101.26, 62.90, and 89.15 MHz for ^{31}P, ^{13}C, and ^{117}Sn nuclei, respectively. The spectrometer was equipped with a 7 or 4 mm MAS broad-band probe. The matching conditions for Hartmann-Hahn cross-polarization[47] (pulse length 5 μs) and the chemical shift reference for the ^{117}Sn nucleus were set with (*cyclo*-$C_6H_{11})_4Sn$ [$\delta = -97.35$ relative to $(CH_3)_4Sn$]. The ^{13}C chemical shift reference frequency was set with adamantane. The ^{117}Sn spectra were typically obtained by acquiring 32 K data points over a spectral width of 166.7 kHz, a 2 ms contact time and a relaxation delay of 2 s with 10,000 to 20,000 scans. The isotropic chemical shifts were identified by recording two spectra with sufficiently different spinning rates.[47] The ^{13}C CP-MAS spectra were obtained by acquiring 4 K data points over a spectral width of 17 kHz, a 2 ms contact time and a relaxation delay of 4 s with 1,000 to 10,000 scans. The ^{1}H-decoupled ^{31}P MAS spectra were obtained by acquiring 4 K data points over a spectral width of 20 kHz and a relaxation delay of 4 s with 100 to 1,000 scans.

Infrared Spectroscopy: Infrared spectra were acquired from KBr pellets in the Fourier transform mode from a Perkin–Elmer System 2000 FT-IR spectrometer. The Raman spectra were acquired in the Fourier-transform mode from a Perkin–Elmer System 2000 NIR FT-RAMAN spectrometer using a Raman dpy2 beam with 310 mW power.

Electrospray Mass Spectrometry: The electrospray mass spectra were recorded in the cationic and anionic modes on a Micromass Quattro II instrument coupled to a Masslynx system (ionisation in an electric field of 3.5 kV; source temperature 80 °C; source pressure 1 atm.; analyser pressure 10^{-5} mbar).[48] Cone voltages were 15 V. Calculated and experimental isotopic distributions were compared using the shareware program MassCluster v.2.1. for Apple Macintosh. The *m*/*z* ratios reported in Table 1 and Figure 3 correspond to the peak with the highest intensity of the isotopic distribution patterns.

Thermogravimetric Analysis: The thermogravimetric analysis was made on a Perkin–Elmer TGA-7 thermogravimetric analyser by heating the sample from 40 to 500 °C at 10 °C/min under a nitrogen atmosphere.

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