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Organometallic Complexes with Biological Molecules: IX. Diorgano- and Triorgano-tin(IV)[*meso*-tetra (4-sulfonatophenyl)porphinate] Derivatives: Solid-state and Solution-phase Structural Aspects and *in vivo* Effects

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Diorgano- and triorgano-tin(IV) derivatives of *meso*-tetra(4-sulfonatophenyl)porphine (H_4TPPS) with general formula $(R_2Sn)_2$TPPS and $(R_3Sn)_4$TPPS ($TPPS^{4-}$ = [*meso*-tetra(4-sulfonatophenyl)porphinate]$^{4-}$, R = Me, Bu, Ph) have been obtained and their solid-state configuration inferred on the basis of IR and Mössbauer spectroscopy, while solution-phase studies have been carried out by ^{1}H and ^{13}C NMR in DMSO-d_6, together with determination of the *in vivo* cytotoxicity of the new derivatives towards embryonic development of *Ciona intestinalis*. In particular, octahedral and trigonal-bipyramidal eq-R_3Sn polymeric configurations are proposed, in the solid state, respectively for $(R_2Sn)_2$TPPS and $(R_3Sn)_4$TPPS complexes, with the arylsulfonate groups behaving as monoanionic bidentate bridging ligands.

The ^{1}H and ^{13}C NMR data lead to the conclusion that the metal-to-ligand ratio (2:1 or 4:1), binding site (the sulfonato-group oxygens), and the coordination polyhedron around the metal (*trans*-octahedral or trigonal-bipyramidal) found in the solid state are preserved in solution.

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INTRODUCTION

Owing to increasing interest in the antitumor activity of porphyrins and their metallic derivatives, many papers on their synthesis, structural aspects and *in vivo* activity have appeared in the literature in the last few years.[1–18] In all these reports metallic atoms were coordinated to the donor atoms of the porphyrin ring.

Only a few reports have been published recently, showing metallic or organometallic moieties coordinated to side-chain donor atoms.[19–21]

Investigations carried out by spectroscopic methods (namely IR, ^{1}H and ^{13}C NMR and, in the case of organotin(IV), Mössbauer) on diorganotin(IV)chloroprotoporphyrin IX,[19] platinum(II)-protoporphyrin[20] and organotin(IV)[*meso*-tetra(4-carboxyphenyl)porphinate][21] derivatives showed the involvement of the side-chain carboxylate oxygen atoms in coordinating the metal atoms. Furthermore, while the 15 platinum(II)–porphyrin complexes synthesized by Brunner *et al*. have been tested for their antitumor activity *in vivo* towards the MDA-MB 231 mammary

carcinoma cell line,[20] both the organotin(IV)-[*meso*-tetra(4-carboxyphenyl)porphinate][21] and diorganotin(IV)chloroprotoporphyrin IX derivatives[22] were tested for their cytotoxicity, respectively, towards immortalized mouse embryonal fibroblasts (NIH-3T3),[21] and towards early-developing embryos of *Anilocra physodes* (Crustacea, Isopoda),[22] showing that cytotoxicity of the parent organotin(IV) halide may be modulated by the use of appropriate ligands.

EXPERIMENTAL

Chemical materials and methods

$(R_2Sn)_2$TPPS and $(R_3Sn)_4$TPPS ($TPPS^{4-}$ = [*meso*-tetra(4-sulfonatophenyl)porphinate]$^{4-}$; R = Me, Bu, Ph) have been obtained as white solids by refluxing methanolic suspensions of R_2SnO or of R_3SnOH, freshly prepared by hydrolysis of the parent organotin(IV) chloride (a gift from Witco GmbH, Bergkamen), and free *meso*-tetra(4-sulfonatophenyl)porphine (Porphyrin Products, Logan, UT, USA).

The solids, recovered by filtration, were recrystalized from methanol or methanol–ether solutions and analyzed for C, H, N, Sn and S content (Table 1). C, H, N and S analyses were performed at the Laboratorio di Chimica Organica (University of Milan). Tin was determined as SnO_2, in our laboratory, according to standard method.[23]

IR spectra were recorded, as Nujol and hexachlorobutadiene mulls, on a Perkin-Elmer grating spectrometer, model 983G, between CsI windows. The spectra were analyzed using a Perkin-Elmer 3600 data station with Perkin-Elmer PE983 software.

The Mössbauer spectra were recorded with the apparatus described elsewhere.[19,21]

The NMR spectra were recorded with a Bruker AC250E spectrometer, operating at 5.87 T. The solvent, hexadeuterodimethylsulfoxide (DMSO-d_6) was a Merck (Darmstadt, Germany) UVASOL reagent (>99.9% deuteration) and was used as reference and for field-frequency lock. The spectra were recorded at room temperature (298 K) unless otherwise stated.

Biological materials and methods

Adult specimens of *Ciona intestinalis* (Urochordata) were collected from the Gulf of Palermo and Mazzara del Vallo, and identified according to Berrill.[24] Male and female gametes were removed from the gonoducts of dissected animals and transferred onto Syracuse dishes.

The eggs were reared in Millipore-filtered seawater while the sperms were diluted before insemination.

Soon after fertilization, eggs were incubated in the presence of light, in separate solutions, at different concentrations and exposure times, with the free *meso*-tetra(4-sulfonatophenyl)-porphine, TPPS, and with diorganotin(IV) and triorganotin(IV)[*meso*-tetra (4-sulfonatophenyl)-porphinate] derivatives, $(R_2Sn)_2$TPPS and

Table 1 Analytical data (% calculated values in parentheses) for $(R_2Sn)_2$TPPS and $(R_3Sn)_4$TPPS [R = Me, Bu, Ph; $TPPS^{4-}$ = [*meso*-tetra(4-sulfonatophenyl)porphinate]

Compound	C	H	N	S	Sn
$(Me_2Sn)_2$TPPS	46.05	3.69	4.63	9.97	19.70
	(46.90)	(3.10)	(4.56)	(10.44)	(19.33)
$(Bu_2Sn)_2$TPPS	49.90	4.82	3.87	8.94	17.50
	(49.86)	(4.63)	(4.15)	(9.50)	(17.60)
$(Ph_2Sn)_2$TPPS	55.09	3.48	3.84	8.59	16.00
	(55.30)	(3.13)	(3.79)	(8.68)	(16.07)
$(Me_3Sn)_4$TPPS	42.68	3.39	4.43	8.32	29.70
	(42.40)	(3.93)	(3.53)	(8.08)	(29.93)
$(Bu_3Sn)_4$TPPS	52.48	6.46	2.54	6.50	22.90
	(52.84)	(6.45)	(2.67)	(6.13)	(22.70)
$(Ph_3Sn)_4$TPPS	59.01	3.94	2.21	5.53	20.90
	(59.77)	(3.71)	(2.40)	(5.50)	(20.36)

$(R_3Sn)_4$TPPS, (R = Me, Bu, Ph), respectively (positive controls), and in simple seawater (negative controls).

Five experiments have been performed. For each experiment some of the eggs were transferred in the dialkyltin(IV)- and trialkyltin(IV)[*meso* - tetra(4 - sulfonatophenyl) - porphinate] derivative solutions and allowed to develop until the remainder, used as controls, were swimming larvae.

All the experiments were performed at 22 °C and the pH of the solutions obtained was controlled and maintained at the normal pH of seawater (7.76–7.78).

In vivo observations were made with a Leitz microscope and photographs were taken with a Leitz orthoplan microscope, using Ilford FP4 Plus film.

RESULTS AND DISCUSSION

Solid-state investigations

IR data

The coordination mode of the sulfonatophenyl groups of the [*meso*-tetra(4-sulfonatophenyl)porphinate]$^{4-}$ ligand (Fig. 1) towards the organotin(IV) moieties may be deduced from an accurate comparison of the IR spectra of the diorganotin(IV) and triorganotin(IV)[*meso*-tetra(4-sulfonatophenyl)porphinate]s [$(R_2Sn)_2$ TPPS and $(R_3Sn)_4$TPPS; R = Me, Bu, Ph] with those previously reported for organotin(IV)sulfonates, Me_3SnOSO_2R (R = Me, CF_3),[25] in particular with those of $Me_3SnOSO_2CH_3$ (Table 2). According to Yeats *et al.*,[25] the $RSnO_3^-$ group, as an ionic species with C_{3v} symmetry, should present, in its IR spectrum, six fundamental vibrations (three A_1 and three E), but owing to coordination and formation of more or less covalent bonds, the symmetry should be decreased to C_s, removing the degeneracy for the three E modes and increasing the number of

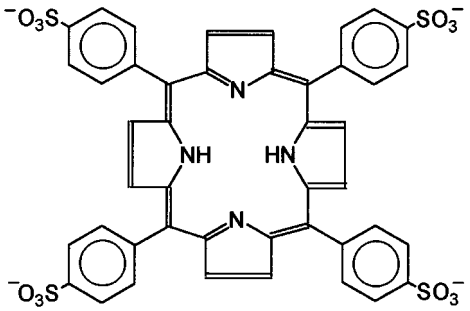

Figure 1 [*meso*-Tetra(4-sulfonatophenyl)porphinate]$^{4-}$ ligand, $TPPS^{4-}$.

Table 2 Relevant absorption bands of Me_3SnSO_3R[a] (R = CH_3, CF_3), H_4TPPS, $(R_2Sn)_2$TPPS and $(R_3Sn)_4$TPPS (R = Me, Bu, Ph) derivatives in the 4000–250 cm^{-1} region[b]

Assignment	$\nu(SO_3)$ (A″)	$\nu(SO_3)$ (A′)	$\nu(SO_3)$ (A′)	$\nu(SO_3)$ (A″)	$\nu(SO_3)$ (A′)	$\nu(SO_3)$ (A′)	Y-mode	$\rho(SO_3)$ (A″)	$\rho(SO_3)$ (A′)
$Me_3SnSO_3CH_3$	1266s	1112vs	1035s	562s	531s	516ms		352m, sh 346m, sh	275m, sh
$Me_3SnSO_3CF_3$	1319vs, b	1145s	1026s	633s	577ms	530m, sh		356ms 347s	317m
H_4TPPS	1228vs	1113vs	1029s	577s	549s	500w			
$(Me_2Sn)_2$TPPS	1227vs	1119vs	1033vs	581w	551m	502sh		366w 341w	281w
$(Bu_2Sn)_2$TPPS	1229vs	1122vs	1036vs	583w	550m	503w		363w 337w	279w
$(Ph_2Sn)_2$TPPS	1229vs	1120vs	1034vs	581w	551m	500sh	454s	364w 337w	283w
$(Me_3Sn)_4$TPPS	1220vs	1127vs	1041vs	584m	559m	490vw		360w 339w	280w
$(Bu_3Sn)_4$TPPS	1222vs	1119vs	1037vs	578m	549w	515m		360w 336w	260w
$(Ph_3Sn)_4$TPPS	1230vs	1111vs	1025vs	581m	550m	500vw	454s	365w 339w	276m

[a] See Ref. 25.
[b] Nujol and hexachlorobutadiene mulls; ρ, rocking; s, strong; m, medium; w, weak; sh, shoulder; b, broad; v, very.

fundamentals to nine (six A′ and three A″).[25]

Lowering of the symmetry to C_s could be explained both with monodentate and bidentate chelating or bridging behavior of the sulfonatophenyl groups, but the first of these hypotheses, for $(R_2Sn)_2$TPPS and $(R_3Sn)_4$TPPS, may be ruled out on the basis of the following reasons:

(1) As reported by Yeats *et al.*, at least two of the SO_3 stretchings, in the unidentate covalent $S_2O_5F_2$ and related compounds,[26–28] fall at higher wavenumbers than those of the diorganotin(IV)- and triorganotin(IV)[*meso*-tetra(4-sulfonatophenyl)porphinate]s.
(2) The IR data of both $(R_2Sn)_2$TPPS and $(R_3Sn)_4$TPPS are fully consistent with those previously reported for organotin(IV)sulfonates, in which bidentate bridging RSO_3 groups were proposed,[25–27,29,30] even though the band positions are slightly influenced by the different electronegativity of the R radical in RSO_3 groups. (For a deeper discussion, see Ref. 29).
(3) The Mössbauer parameter nuclear quadrupole splittings, ΔE, which will be discussed in the next section, are characteristic of polymeric species with octahedral and trigonal-bipyramidal environments, respectively in $(R_2Sn)_2$TPPS and in $(R_3Sn)_4$TPPS, suggesting the occurrence of bidentate sulfonatophenyl groups.

No SnC_2 stretchings could be identified in the region 600–400 cm^{-1} in the dialkyltin(IV) derivatives, neither were bands seen that were attributable to ν_{SnO}, while both for the diphenyltin(IV)- and triphenyltin(IV)[*meso*-tetra(4-sulfonatophenyl)porphinate]s the Y-mode Sn-Ph bands were present at $\approx$450 cm^{-1}.[31]

Mössbauer data

The experimental Mössbauer parameters isomer shift, δ (mm s^{-1}), and nuclear quadrupole splittings, ΔE (mm s^{-1}), for diorganotin(IV)- and triorganotin(IV)[*meso*-tetra(4-sulfonatophenyl)-porphinate]s measured at liquid–nitrogen temperature, are reported in Table 3, together with those of several related derivatives, measured at 80 K, for comparison.

The trend of the isomer shifts of diorganotin(IV)- and triorganotin(IV)[*meso*-tetra(4-sulfonatophenyl)porphinates]s reflects the difference in number and electronegativity of the organic radicals bonded to the tin(IV) atom, increasing from phenyltin(IV) to butyltin(IV) derivatives, and is in consonance with literature values for diorganotin(IV) and triorganotin(IV) derivatives.[32–34] The noteworthy differences from the isomer shifts of the organotin(IV)sulfonates previously reported in the literature,[25–27,29,30] four examples of which are reported in Table 3, would suggest a more covalent character[29] of the bonds in the organotin(IV)[*meso*-tetra(4-sulfonatophenyl)porphinate]s reported in this paper.

According to Yeats *et al.*,[29] a lower electronegativity of the SO_3 groups in [*meso*-tetra(4-sulfonatophenyl)porphinate]$^{4-}$ will cause a smaller withdrawal of p-electron density in the organotin(IV)[*meso*-tetra(4-sulfonatophenyl)porphinate]s, with concomitant smaller deshielding of the tin 5s electrons, resulting in the smaller δ values observed. On the other hand, the above-mentioned smaller imbalance in the p-orbital charge present in the organotin(IV)[*meso*-tetra(4-sulfonatophenyl)porphinate]s in comparison with the organotin(IV)sulfonates[25–27,29,30] will result in smaller nuclear quadrupole splittings (Table 3).

In conclusion, according to both IR and Mössbauer findings, we propose polymeric octahedral *trans*-R_2Sn configurations for diorganotin(IV)[*meso*-tetra (4-sulfonatophenyl)-porphinate]s (Fig. 2a), and polymeric trigonal-bipyramidal equatorial R_3Sn configurations for triorganotin(IV)[*meso*-tetra(4-sulfonatophenyl)-porphinate]s (Fig. 2b). In the proposed structure shown in Fig. 2(a), the formal 2+ charge on each diorganotin(IV) moiety is counterbalanced by the total 2− charge on four bridging sulfonato groups. The coordination polyhedron of tin(IV) should be formed by four oxygen atoms and two *trans*-R_2 groups. The same bonding situation should be envisaged for each of the sulfonato groups of the porphinate ligand, thus giving a complex polymeric structure, where all the diorganotin(IV) groups should be connected to each other by two bridging sulfonato groups belonging to two different [*meso*-tetra(4-sulfonatophenyl)porphinate] units.

Something similar can be hypothesized also for the triorganotin(IV) complexes, where, however, there should be only one sulfonato bridge between each pair of metal atoms, and two such bridges for each triorganotin(IV) moiety, counterbalancing the formal 1+ charge on the tin(IV) atom. The coordination polyhedron of the tin(IV)

Table 3 Experimental Mössbauer parameters,[a] isomer shift δ and nuclear quadrupole splittings $|\Delta E|_{exp}$ of $(R_2Sn)_2TPPS$, $(R_3Sn)_4TPPS$ [R = Me, Bu, Ph; $TPPS^{4-}$ = *meso*-tetra(4-sulfonatophenyl)porphinate] and of some related compounds

Compound	δ_{Sn} (mm s^{-1})	$\lvert\Delta E\rvert_{exp}$ (mm s^{-1})	Γ_1 (mm s^{-1})	Γ_2 (mm s^{-1})
$Me_2Sn(OSO_2CH_3)_2$[b]	1.52	5.05	0.98	1.00
$Me_2Sn(OSO_2C_6H_4CH_3)_2$[b]	1.51	4.85	1.01	1.01
$Me_3SnOSO_2CH_3$[c]	1.43	4.21	1.03	1.05
$Bu_3SnOSO_2C_6H_4CH_3$[d]	1.56	4.46	—[e]	—[e]
$(Me_2Sn)_2TPPS$	1.29	3.95	0.92	0.95
$(Bu_2Sn)_2TPPS$	1.39	3.83	0.89	0.90
$(Ph_2Sn)_2TPPS$	1.20	3.68	0.86	0.88
$(Me_3Sn)_4TPPS$	1.35	3.68	1.00	1.01
$(Bu_3Sn)_4TPPS$	1.49	3.74	0.82	0.82
$(Ph_3Sn)_4TPPS$	1.34	3.69	0.94	0.95

[a] Sample thickness ranged between 0.50 and 0.60 mg $^{119}Sn\ cm^{-2}$; isomer shift, $\delta \pm 0.03$ mm s^{-1} with respect to room-temperature $Ba^{119}SnO_3$; Γ_1 and Γ_2 values are the full width at half height of the resonant peaks, respectively at greater and lower velocity with respect to the centroid of the Mössbauer spectra; nuclear quadrupole splitting, $|\Delta E| \pm 0.02$ mm s^{-1}.
[b] See Ref. 29.
[c] See Ref. 25.
[d] Γ_1 and Γ_2 values are not reported. See Ref. 51.

atom should be a trigonal bipyramid with equatorial SnC_3 units and two axial oxygens, from two sulfonato groups belonging to two different ligand molecules.

Solution-state investigations

^{1}H NMR and ^{13}C NMR spectra

The ^{1}H and ^{13}C NMR spectral data of the complexes in DMSO-d_6 solution are collected in Tables 4 and 5, respectively. The choice of DMSO as the solvent, was dictated by the solubility of the compounds in water being too low for NMR detection. Chemical shifts are reported relative to TMS; the values of 2.62 ppm and 39.7 ppm were used for the ^{1}H and ^{13}C resonances of the DMSO reference at 298 K, while a value of 40.1 ppm was used for the carbon resonance of DMSO at 342 K.

The integrated intensities of the proton spectra are in agreement with stoichiometries found in the solid state.

The presence of the pyrrole ring NH resonance at high field ($\delta\ ca\ -2.8$ ppm) in the spectra of the complexes clearly indicates that the organotin(IV) moieties are bound to the sulfonato oxygens, in agreement with the findings from other techniques. The ^2J(Sn, ^{1}H) coupling constant, which can be used to calculate the C–Sn–C bond angle, θ, has been obtained only for the methyl derivatives. Equation [1] yields the θ value:[35]

$$\theta = 0.0161|^2J|^2 - 1.32|^2J| + 133.4 \qquad [1]$$

The calculated θ values are 182° and 119° for the trimethyltin(IV) and the dimethyltin(IV) complexes, respectively. These values correspond well to the *trans*-octahedral and trigonal-bipyramidal with equatorial alkyls geometries deduced for the di- and triorganotin(IV) complexes in the solid state by means of other techniques. It is difficult to believe that the polymeric structures proposed for the title compounds in the solid state are preserved in solution. One may then speculate that the triorganotin(IV) complexes become monomeric in solution, with four organometal moieties bound per porphinate ring through monodentate sulfonate groups, and achieve pentacoordination by binding a solvent molecule.

As far as the solution structure of the diorganotin(IV)[*meso*-tetra(4-sulfonatophenyl)-porphinate] derivatives is concerned, one can speculate that DMSO breaks sulfonato bridges, decreasing the rigidity of the polymeric structure and increasing the solubility of the complexes.

Each diorganotin(IV) unit should bind two monodentate sulfonato groups of two different ligand molecules, achieving hexacoordination by binding two solvent molecules.

The protons of the *meso*-phenyl substituents appear in the spectrum as a more or less resolved AB system, with typical vicinal coupling of *ca* 8 Hz. The assignment of the phenyl proton resonance has been made on the basis of literature data, which report for TPPS in DMSO (the authors do not indicate if TPPS was the free ligand or the salt) a reversal of the normal spectral position of the proton signals, with the *ortho*-protons appearing at lower field than the *meta*,[36] as a consequence of the very limited extent of monomer aggregation in this solvent, even at concentrations as high as 0.044 mol dm^{-3}.

The ^{13}C resonances (Table 5) of the porphyrin ring and of the *meso*-(4-sulfonatophenyl) groups were assigned starting from the data published for *meso*-tetraphenylporphyrin.[37,38] The ^{13}C substituent shifts found for the substitution toluene→*p*-toluenesulfonic acid or *p*-toluenesulfonyl chloride[39] were considered and are reported in Fig. 3, and the shifts calculated using these values for the *meso*-(4-sulfonatophenyl) groups have been compared with the experimental data for the assignment of the resonances, following the literature procedure.[37] The large downfield shift expected for the *p*-C carbon resonance allows the assignment of the lowest field signal to it. The experimental and calculated chemical shifts are shown in Fig. 3 for the free ligand.

Complexation to the organometallic moiety is not expected to induce large variations of the ^{13}C chemical shifts, since binding occurs at the

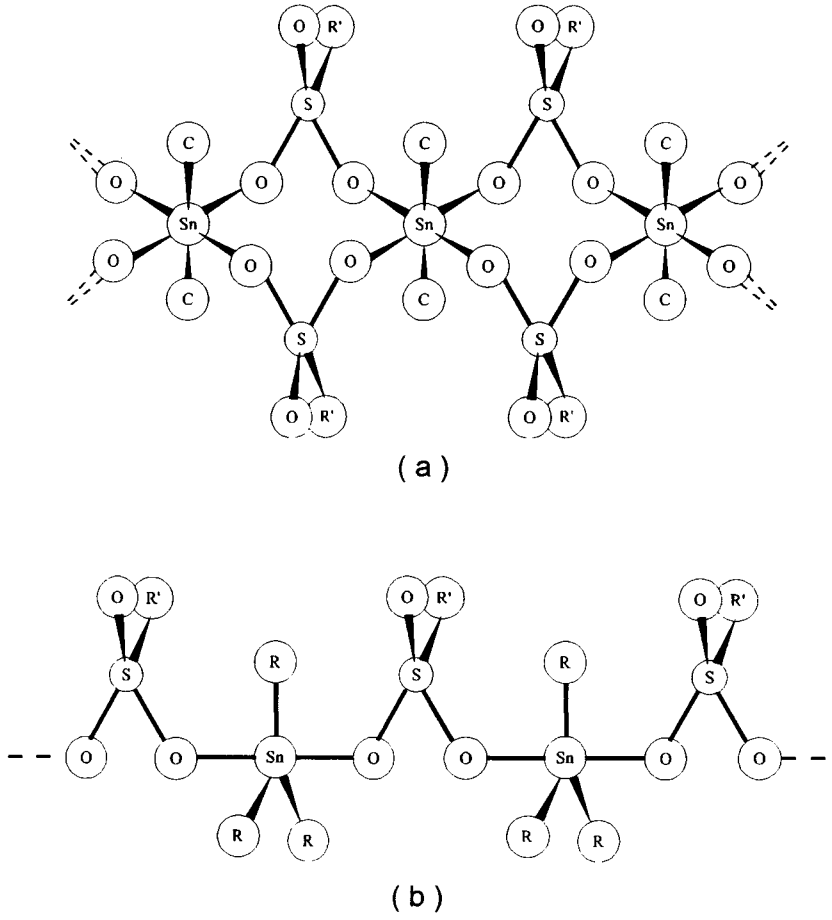

Figure 2 (a) Proposed polymeric octahedral configuration for $(R_2Sn)_2TPPS$ (R = Me, Bu, Ph) derivatives. (b) Proposed trigonal-bipyramidal configuration for $(R_3Sn)_4TPPS$ (R = Me, Bu, Ph) derivatives.

Table 4 ^{1}H NMR data for diorganotin(IV) and triorganotin(IV)[*meso*-tetra(4-sulfonatophenyl)porphinate] derivatives[a]

Assignment	H_4TPPS	$(Me_2Sn)_2$TPPS	$(Bu_2Sn)_2$TPPS	$(Ph_2Sn)_2$TPPS	$(Me_3Sn)_4$TPPS	$(Bu_3Sn)_4$TPPS	$(Ph_3Sn)_4$TPPS
N-H	−0.31b	−2.80b	−2.82	n.o.	−2.80b	−2.79	−2.75
m-H	8.40b	8.35 (J_{HH} 8.0)	8.27 (J_{HH} 7.97)	8.18b	8.20 (J_{HH} 7.97)	8.19 (J_{HH} 7.80)	8.20b (J_{HH} 7.9)
o-H	8.76b	8.21 (J_{HH} 8.0)	8.41 (J_{HH} 7.97)	8.29b	8.34 (J_{HH} 7.97)	8.33 (J_{HH} 7.80)	8.31b (J_{HH} 7.9)
β-Pyrrole	8.76b	8.99b	9.06	8.95	8.99	8.98	8.99
$H_{2',6'}$				—[b]			7.94 ($J_{Sn,H}$ 64.3)
$H_{3',5'}$				—[b]			7.7m
Others		1.08 ($^2J_{Sn,H}$ 109.5)	1.08t (J_{HH} 7.3) 1.52m, γ-CH_2 1.74–1.77m, b[c]		0.61 ($^2J_{Sn,H}$ 68.5)	0.97t (J_{HH} 7.1) 1.37m, b, γ-CH_2 1.67m, b, β-CH_2 1.19b, α-CH_2	

[a] Solvent DMSO-d_6; coupling constants are given in Hz; chemical shifts are relative to TMS. B_0 = 5.87 T. All measurements at room temperature.
[b] Broad overlapping signals, not interpretable.
[c] α- and β-CH_2; one broad Sn satellite was detected. Abbreviations: t, triplet; m, multiplet; b, broad; n.o., not observed.

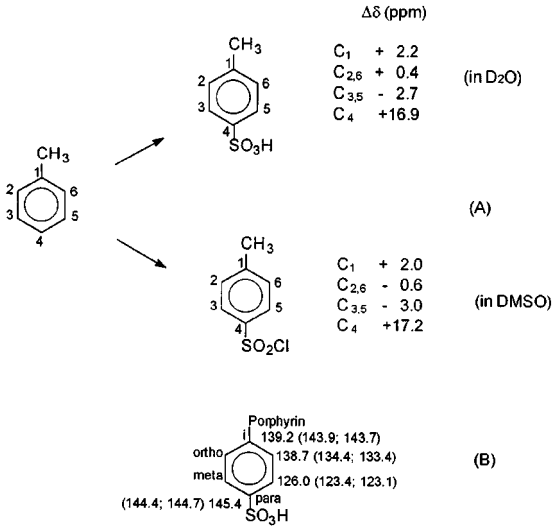

Figure 3 Calculation of the ^{13}C chemical shifts for the H_4TPPS ligand. (A) Substituent shifts observed following introduction of a *p*-sulfonato group on toluene.[39] (B) ^{13}C-NMR chemical shifts for a *meso*-(4-sulfonatophenyl) substituent of porphyrin, experimental and calculated (in parentheses) by applying the substituent shifts reported in A to the δ values of a *meso*-phenyl group.[37]

oxygens of the sulfonato group; and, indeed, for the [*meso*-tetra(4-carboxyphenyl)porphinate] ligand binding to organotins through the carboxyl oxygens, only minor changes of chemical shifts have been reported.[21] Thus, assignment of

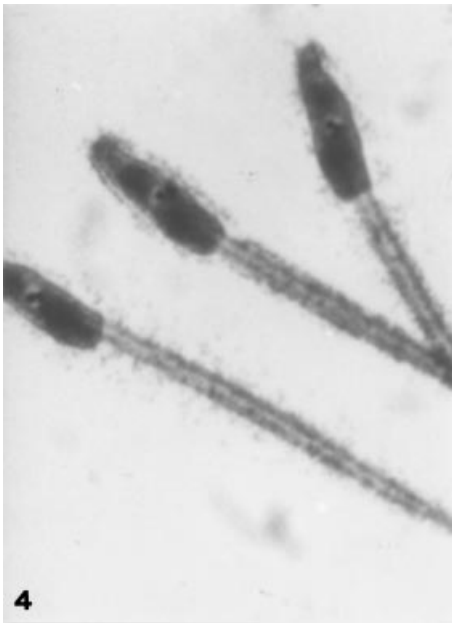

Figure 4 *Ciona intestinalis* swimming larvae.

Table 5 ^{13}C NMR data for diorganotin(IV)- and triorganotin(IV) [*meso*-tetra(4-sulfonatophenyl)porphinate] derivatives[a]

Assignment	H_4TPPS	$(Me_2Sn)_2$TPPS (342 K)	$(Bu_2Sn)_2$TPPS	$(Bu_2Sn)_2$TPPS (342 K)	$(Ph_2Sn)_2$TPPS	$(Me_3Sn)_4$TPPS	$(Bu_3Sn)_4$TPPS	$(Bu_3Sn)_4$TPPS (342 K)	$(Ph_3Sn)_4$TPPS (342 K)
p-C	149.69	147.66	147.70	147.56	148.04	147.91	147.69	147.78	147.96
α-C	145.33	146.3b	n.o.	145.87vb	n.o.	n.o.	n.o.	145.98vb	145.95
i-C	139.21	141.98	141.64	141.31	141.49	141.59	141.69	141.34	141.19
o-C	138.66	134.00	133.99	133.45	134.10b	133.98	133.97	133.49	133.45
β-C	128.61	131.53	131.54vb, sm	131.00b	131.58vb	131.75vb	131.58vb	131.04b	131.11
m-C	126.03	124.55	124.41	124.04	124.53	124.46	124.41	124.11	124.12
meso-C	121.78	119.91	119.88	119.48	120.04	119.94	119.87	119.57	119.62
CH_3		15.17sm				3.37vsm, b			
δ-CH_3			13.78	13.17			13.77	13.20	
γ-CH_2			25.74	25.19			26.78 (^3J 79.3)	26.22 (^3J 74.6)	
β-CH_2			27.40	26.90b			27.84 (^2J 27.3)	27.43 (^2J 25.8)	
α-CH_2			*ca* 34vvb	34vb			19.71vb (^1J *ca* 400)	19.14 (^1J 451)	
$C_{1'}$					n.o.				140.26
$C_{2',6'}$					135.06				135.82 (^2J 46.9)
$C_{3',5'}$					128.61				128.97 (^3J 71.2)
$C_{4'}$					129.5b				129.96

[a] Solvent DMSO-d_6; coupling constants are given in Hz; chemical shifts are relative to TMS. $B_0 = 5.87$ T. All measurements at room temperature except where indicated.

Abbreviations: m, multiplet; b, broad; v, very; sm, small; n.o., not observed; *i*, *ipso*; *o*, *ortho*; *m*, *meta*; *p*, *para*.

the carbon resonances for the complexes is straightforward.

In several cases, the resonances of the α- and β-pyrrole carbons are broadened or even not observed; this is always the case for the α-carbons of the complexes at 298 K. Therefore, the spectra were acquired also at a higher temperature (342 K) to sharpen the signals (Table 5). This behavior is connected with the tautomeric equilibrium between pyrrole-like (conjugated to β-carbons) and pyrrolenine-like (conjugated to nitrogen) α-carbons,[38] as expected when the pyrrole NH protons are present. Moreover, the position of the resonance at *ca* 146 ppm supports the existence of such tautomerism, since a pure pyrrolenine-like α-carbon should resonate at even lower field (150–152 ppm).[38]

The carbons directly bound to tin, when detected, appear as very small broad resonances, preventing the detection of tin satellites, for all complexes except $(Bu_3Sn)_4$TPPS at 342 K. For this complex, the ^1J(Sn, C) value (separate satellites are not observed for ^{119}Sn and ^{117}Sn) of 451 Hz yields a value of θ, calculated by Equation [2]:[40]

$$|^1J| = 11.4\theta - 875 \qquad [2]$$

to be 116°, indicating that in solution the complex retains the trigonal-bipyramidal geometry, with equatorial alkyls, found in the solid state.

The two- and three-bond tin–carbon couplings (Table 5), when available, follow the normal pattern with $^3J > {}^2J$,[41] and compare well with the literature data for phenyltin(IV) and butyltin(IV) complexes.[42–45] In conclusion, the presence of the solvent does not seem to affect the nature of the complexes formed by di- and tri-organotins with $TPPS^{4-}$.

Biological results

In order to evaluate possible cytotoxicity of the novel synthetic chemicals characterized above, we analyzed the development of *Ciona intestinalis* embryos after treatment with these chemicals, at different concentrations.

Negative controls

Embryo development seemed to parallel that regularly observed for fertilized eggs of *Ciona intestinalis*.[46]

In fact, development of these embryos resulted in two- to four-blastomere, gastrula and neurula stages after two, six and seven and a half hours, respectively. Moreover, after 24 h embryos were at the swimming larva stage (Fig. 4).

Fertilized eggs treated with free TPPS, $(R_2Sn)_2$TPPS and $(R_3Sn)_4$TPPS (R = Me, Bu, Ph)

Results obtained after analyses of embryo development are reported in Table 6:

(a) Treatment of fertilized eggs with free *meso*-tetra(4-sulfonatophenyl) porphine in 10^{-5} mol dm^{-3} solution resulted in undifferentiated embryos, while treatment with the same chemical at 10^{-7} mol dm^{-3} resulted in anomalous larvae occurring in solution.

(b) After incubation of fertilized eggs, both in 10^{-5} and 10^{-7} mol dm^{-3} $(Me_2Sn)_2$TPPS, embryo development was identical to that induced by the free *meso*-tetra(4-sulfonatophenyl)porphine, at the same concentrations.

(c) Treatment of fertilized eggs with $(Bu_2Sn)_2$TPPS at 10^{-5} mol dm^{-3} resulted in the arrest of development of the embryos, with 50% arrested at the four-cell stage and 50% at the 8–16-blastomere stage (Fig. 5). Similarly, an arrest of the development was observed after treatment of fertilized eggs with the same chemical at 10^{-7} mol dm^{-3}, i.e. 50% of the observed embryos were arrested at the neurula stage (neurulae showed an anomalous appearance, since they had open neural folds) (Fig. 5), and 50% at the larva stage (Fig. 6).

(d) Fertilized eggs incubated in $(Ph_2Sn)_2$TPPS at 10^{-5} mol dm^{-3} were all arrested in development at the four-blastomere stage (Fig. 7), while eggs incubated in a 10^{-7} mol dm^{-3} solution were arrested in development at the larva stage (larvae displayed anomalously, with twisted tails).

(e) Fertilized eggs treated with $(Me_3Sn)_4$TPPS at 10^{-5} mol dm^{-3} stopped developing at the early gastrula stage (Fig. 8). Treatment of fertilized eggs in 10^{-7} mol dm^{-3} solution produced either anomalous larvae (50%) or normal swimming larvae (50%) (Fig. 9).

(f) Treatment of fertilized eggs with either

Table 6 Results of development of fertilized *Ciona intestinalis* eggs incubated in solutions of H_4TPPS, $(R_2Sn)_2$TPPS and $(R_3Sn)_4$TPPS, [H_4TPPS = *meso*-tetra(4-sulfonatophenyl)porphine; R = Me, Bu and Ph] from the two-cell stage

		Development stage[a]							
		Blastomeres							
Compound	Concentration (mol dm^{-3})	2	4	8–16	Early gastrulae	Anomalous neurulae	Undifferentiated embryos	Anomalous larvae	Swimming larvae
H_4TPPS	10^{-5}						100		
	10^{-7}							100	
$(Me_2Sn)_2$TPPS	10^{-5}						100		
	10^{-7}							100	
$(Bu_2Sn)_2$TPPS	10^{-5}		50	50					
	10^{-7}					50		50	
$(Ph_2Sn)_2$TPPS	10^{-5}		100						
	10^{-7}							100	
$(Me_3Sn)_4$TPPS	10^{-5}				100				
	10^{-7}							50	50
$(Bu_3Sn)_4$TPPS	10^{-5}	100							
	10^{-7}		100						
$(Ph_3Sn)_4$TPPS	10^{-5}		50	50					
	10^{-7}		50	50					

[a] The controls give rise to 90% swimming larvae. Results are given as the percentage of developed or arrested eggs (average of five experiments).

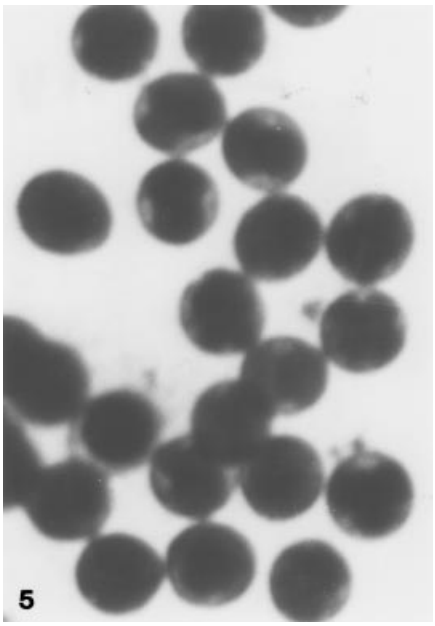

Figure 5 Two-cell stage incubated in 10^{-5} mol dm^{-3} $(Ph_2Sn)_2$TPPS solution. The two blastomeres had stopped developing.

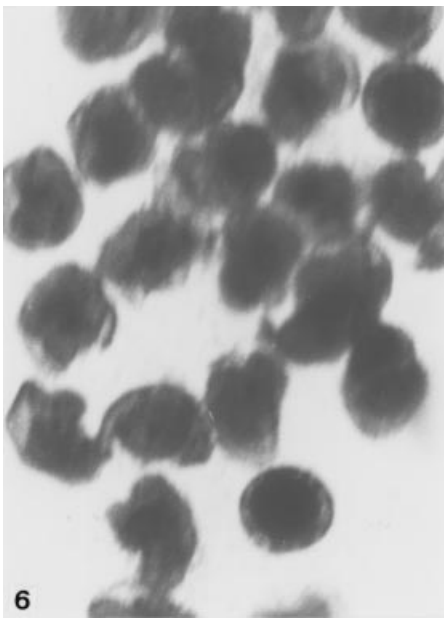

Figure 6 Anomalous larvae and neurulae obtained from two-cell stage incubated in 10^{-7} mol dm^{-3} $(Bu_2Sn)_2$TPPS solution.

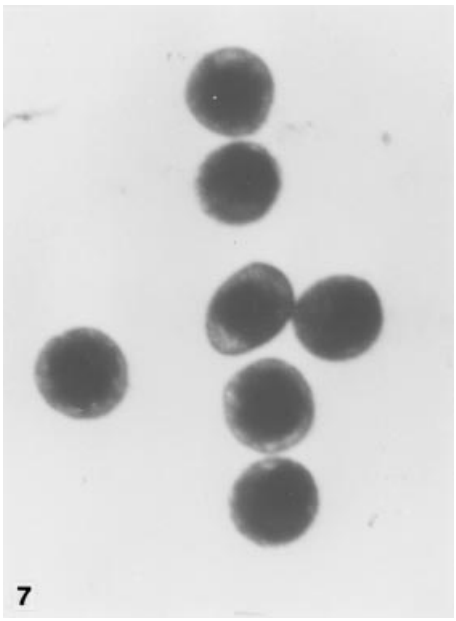

Figure 7 Anomalous 4, 16-cell stage incubated in 10^{-5} mol dm^{-3} $(Bu_2Sn)_2$TPPS solution.

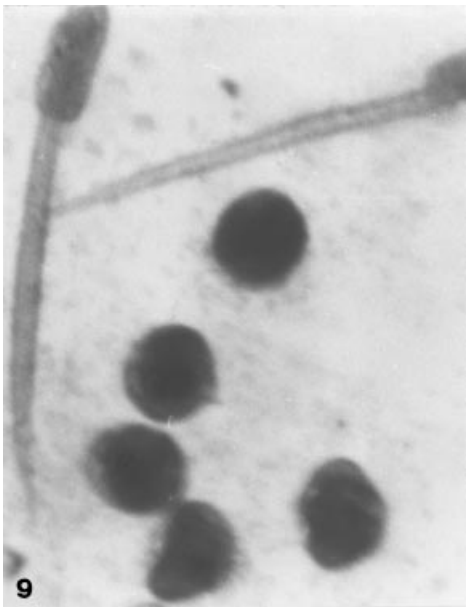

Figure 9 Normal and anomalous larvae derived from two-cell stage incubated in 10^{-7} mol dm^{-3} $(Me_3Sn)_4$TPPS solution.

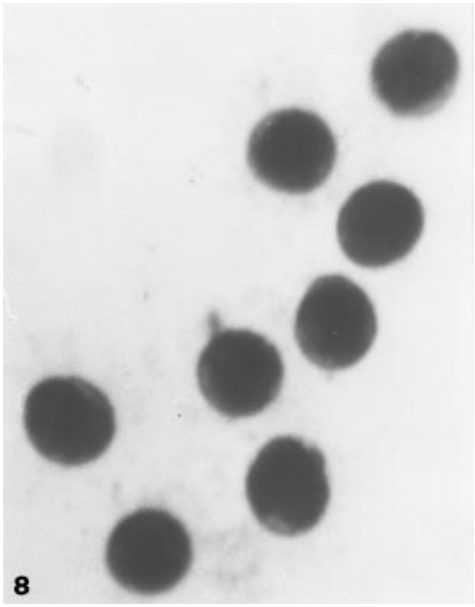

Figure 8 Anomalous early gastrulae derived from two-cell stage incubated in 10^{-5} mol dm^{-3} $(Me_3Sn)_4$TPPS solution.

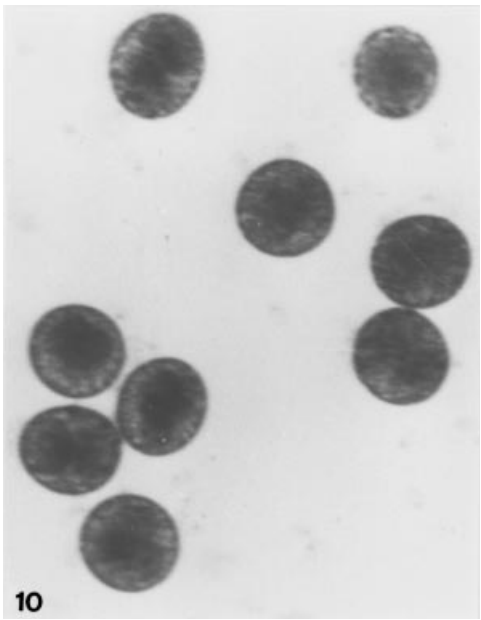

Figure 10 Anomalous two-cell stage incubated in 10^{-5} mol dm^{-3} $(Bu_3Sn)_4$TPPS solution.

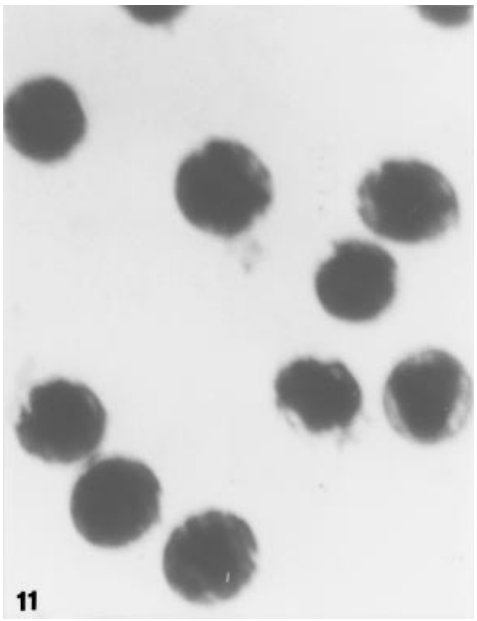

Figure 11 Anomalous four-cell stage derived from two-cell stage incubated in 10^{-5} mol dm^{-3} $(Bu_3Sn)_4$TPPS solution.

$(Bu_3Sn)_4$TPPS or $(Ph_3Sn)_4$TPPS at 10^{-5} mol dm^{-3} and 10^{-7} mol dm^{-3} resulted in the arrest of embryo development at the early stages of two to four blastomeres (Figs 10 and 11).

Bearing in mind that embryo development proceeds from two-, four-, eight- and 16-blastomere stages up to blastula, gastrula, neurula and larva stages, it can be deduced, as demonstrated elsewhere,[47] that triorganotin-(IV)[*meso*-tetra (4-sulfonatophenyl)porphinate]s, at both 10^{-5} and 10^{-7} mol dm^{-3} concentrations, are more toxic than the corresponding diorganotin(IV)[*meso*-tetra (4-sulfonatophenyl) porphinate]s. In fact, the former induced the arrest of embryo development at earlier stages (four and 8–16 blastomeres). A similar conclusion can be drawn by comparing results obtained from experiments carried out in each series of triorganotin(IV)- and of diorganotin(IV)[*meso*-tetra[4-sulfonatophenyl)porphinate]s. On going from the methyl to the butyl and phenyl organotin(IV)[*meso* - tetra(4 - sulfonatophenyl) porphinate] derivatives, the latter were more toxic. The lowest cytotoxicity was found after treatment of fertilized *Ciona intestinalis* eggs with free *meso*-tetra(4-sulfonatophenyl) porphine and dimethyltin(IV)[*meso*-tetra(4-sulfonatophenyl)porphinate], at both 10^{-5} and 10^{-7} mol dm^{-3} concentrations.

In conclusion, since arrest of development might have orginated from inhibition of mitotic spindle interaction with tubulin polymerization, as previously suggested by Mansueto *et al.*[48] and demonstrated by several other authors,[49,50] or from chromosome disorders during mitotic processes, it is desirable to perform a karyological analysis of embryos treated with the chemicals in this study.

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REFERENCES

1. R. P. Pandian and T. K. Chandrashekar, *J. Chem. Soc., Dalton Trans.* 119 (1993).
2. R. J. Balahura and R. A. Kirby, *Inorg. Chem.* **33**, 1021 (1994).
3. J. Wojaczynski and L. Latos-Grazynski, *Inorg. Chem.* **33**, 1054 (1994).
4. J. A. Gonzáles and L. J. Wilson, *Inorg. Chem.* **33**, 1543 (1994).
5. Y. H. Liu, M.-F. Bénassy, S. Chojnacki, F. D'Souza, T. Barbour, W. J. Belcher, P. J. Brothers and K. M. Kadish, *Inorg. Chem.* **33**, 4480 (1994).
6. B. Song and H. M. Goff, *Inorg. Chem.* **33**, 5979 (1994).
7. Y. Uemori, S. Takinami, A. Takahashi, H. Munakata, H. Imai, S. Nakagawa and E. Kyuno, *Inorg. Chim. Acta* **224**, 157 (1994).
8. J. Seth and D. F. Bocian, *J. Am. Chem. Soc.* **116**, 143 (1994).
9. L. R. Milgrom and R. J. Zuurbier, *Polyhedron* **13**, 209 (1994).
10. J. W. Buchler and F. Kunzel, *Z. Anorg. Allg. Chem.* **629**, 888 (1994).
11. J. Wojaczynski and L. Latos-Grazynski, *Inorg. Chem.* **34**, 1044 (1995).
12. T. Uno, A. Takeda and S. Shimabayashi, *Inorg. Chem.* **34**, 1599 (1995).
13. T. La, R. A. Richards, R. S. Lu, R. Bau and G. M. Miskelly, *Inorg. Chem.* **34**, 5632 (1995).
14. O. Q. Munro, H. M. Marques, P. G. Debrunner, K. Mohanrao and W. R. Scheidt, *J. Am. Chem. Soc.* **117**, 935 (1995).
15. L. M. Berreau and L. K. Woo, *J. Am. Chem. Soc.* **117**, 1314 (1995).
16. T. La and G. M. Miskelly, *J. Am. Chem. Soc.* **117**, 3613 (1995).
17. P. J. Marsh, J. Silver, M. C. R. Symons and F. A. Taiwo, *J. Chem. Soc., Dalton Trans.* 2361 (1996).

18. G. Ricciardi, L. De Benedetto and F. Lelj, *Polyhedron* **15**, 3183 (1996).
19. L. Pellerito, A. Pellerito, F. Maggio, M. Beltramini, B. Salvato and F. Ricchelli, *Appl. Organomet. Chem.* **7**, 79 (1993).
20. H. Brunner, F. Maiterth and B. Treittinger, *Chem. Ber.* **127**, 2141 (1994).
21. M. G. Mirisola, A. Pellerito, T. Fiore, G. C. Stocco, L. Pellerito, A. Cestelli and I. Di Liegro, *App. Organomet. Chem.* **11**, 499 (1997).
22. R. Vitturi, L. Pellerito, E. Catalano and M. R. Lo Conte, *Appl. Organomet. Chem.* **7**, 295 (1993).
23. W. P. Neumann, *The Organic Chemistry of Tin*, Interscience Publishers, London, 1970.
24. N. J. Berrill, *The Tunicata*, Ray Society, London, 1950.
25. P. A. Yeats, J. R. Sams and F. Aubke, *Inorg. Chem.* **10**, 1877 (1971).
26. R. J. Gillespie and E. A. Robinson, *Can. J. Chem.* **40**, 644 (1962).
27. P. A. Yeats, B. L. Poh, B. F. E. Ford, J. R. Sams and F. Aubke, *J. Chem. Soc. (A)* 2188 (1970).
28. H. A. Carter, S. P. L. Jones and F. Aubke, *Inorg. Chem.* **9**, 2485 (1970).
29. P. A. Yeats, J. R. Sams and F. Aubke, *Inorg. Chem.* **11**, 2634 (1972).
30. P. A. Yeats, B. F. E. Ford, J. R. Sams and F. Aubke, *J. Chem. Soc., Chem. Commun.*, 791 (1969).
31. D. H. Wiffen, *J. Chem. Soc.* 1350 (1956).
32. G. M. Bancroft and R. H. Platt, *Adv. Inorg. Chem. Radiochem.* **15**, 59 (1972).
33. N. N. Greenwood and T. C. Gibb, *Mössbauer Spectroscopy*, Chapman and Hall, London, 1971.
34. R. V. Parish, Structure and bonding in tin compounds. In: *Mössbauer Spectroscopy Applied to Inorganic Chemistry*, Long, G. J. (ed.), Plenum Press, New York, 1984, Vol. 1, p. 527.
35. T. P. Lockhart and W. F. Manders, *Inorg. Chem.* **25**, 892 (1986).
36. A. Corsini and O. Herrmann, *Talanta* **33**, 335 (1986).
37. R. J. Abraham, G. E. Hawkes, M. F. Hudson and K. M. Smith, *J. Chem. Soc., Perkin Trans. 2* 204 (1975).
38. L. R. Milgrom, *Tetrahedron* **3**, 879 (1984).
39. E. Breitmaier and W. Voelter, *Carbon-13 NMR Spectroscopy*, 3rd edn, VCH, Weinheim, 1990, Chapter 4.
40. T. P. Lockhart, W. F. Manders and J. J. Zuckerman, *J. Am. Chem. Soc.* **107**, 4546 (1985).
41. M. Bullpitt, W. Kitching W. Adcock and D. Doddrell, *J. Organomet. Chem.* **116**, 161 (1976).
42. T. N. Mitchell, *J. Organomet. Chem.* **59**, 189 (1973).
43. J. Holecek, M. Nádvornik, K. Handlír and A. Lycka, *J. Organomet. Chem.* **315**, 299 (1986).
44. F. Caruso, M. Giomini, A. M. Giuliani and E. Rivarola, *J. Organomet. Chem.* **466**, 69 (1994).
45. A. Pellerito, T. Fiore, A. M. Giuliani, F. Maggio, L. Pellerito, R. Vitturi, M. S. Colomba and R. Barbieri, *Appl. Organomet. Chem.* **11**, 601 (1997).
46. C. Mansueto, M. Lo Valvo, L. Pellerito and M. A. Girasolo, *Appl. Organomet. Chem.* **7**, 95 (1993).
47. F. Maggio, A. Pellerito, L. Pellerito, S. Grimaudo, C. Mansueto and R. Vitturi, *Appl. Organomet. Chem.* **8**, 71 (1994).
48. C. Mansueto, L. Pellerito and M. A. Girasolo, *Acta Embryol. Morphol. Exp.*, n.s. **10**, 237 (1989).
49. H. Faulstich, C. Stoumaros, K. H. Doenges and H. P. Zimmermann, *FEBS Lett.* **174**, 128 (1984).
50. P. R. Sager, R. A. Doharty and J. B. Holmsted, *Exp. Cell. Res.* **146**, 127 (1989).
51. S. J. Blunden and R. Hill, *Inorg. Chim. Acta* **87**, 83 (1984).

