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New organotin(IV) derivatives of dipeptides as models for metal–protein interactions: *in vitro* anti-tumour activity

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New organotin(IV) derivatives with general formulae R_2SnL, where R = *n*-Bu and L is the dianion of glycyltyrosine (H_2L-1), glycyltryptophane (H_2L-2), leucyltyrosine (H_2L-3), leucylleucine (H_2L-4), valylvaline (H_2L-5) and alanylvaline (H_2L-6) have been synthesized in 1:1 molar ratio by the reaction of Bu_2SnO with the respective dipeptide under azeotropic removal of water. Triphenyltin glycylleucinate was obtained by reacting Ph_3SnCl and sodium glycylleucinate with filtration of NaCl formed. The bonding and coordination behaviour in these derivatives are discussed on the basis of IR, multinuclear ^{1}H, ^{13}C and ^{117}Sn magnetic resonance and ^{119}Sn Mössbauer spectroscopic studies. These investigations suggest that all the ligands in R_2SnL act as dianionic tridentates coordinating through the COO^-, NH_2 and $N_{peptide}$ groups, whereas in $Ph_3Sn(HL)$ the ligand acts as a bidentate coordinating through the COO^- and NH_2 groups. The ^{119}Sn Mössbauer studies, together with the NMR data, indicate that, for the 1:1 monomeric derivatives, the polyhedron around tin in R_2SnL is a trigonal bipyramid with the butyl groups and $N_{peptide}$ in the equatorial positions, while the axial positions are occupied by a carboxylic oxygen and the amino nitrogen atom. In $Ph_3Sn(HL)$ the structure is intermediate between pseudotetrahedral and *cis*-trigonal bipyramidal, with the N_{amino} and two phenyl groups in the equatorial plane and the carboxylate oxygen and the third phenyl group in axial positions. All the complexes have been screened against seven cancer cell lines of human origin, viz. MCF-7, EVSA-T, WiDr, IGROV, M19, MEL A498 and H226. $Ph_3Sn(HL)$ displays the lowest ID_{50} values of the tin compounds tested and reported in this paper. Its activity is comparable to those of methotrexate and 5-fluorouracil. All the di-*n*-butyltin compounds exhibit lower *in vitro* anti-tumour activities than $Ph_3Sn(HL)$; however, they do provide significantly better activities than etoposide and *cis*-platin. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: organotin(IV); dipeptides; multinuclear magnetic resonance; IR spectroscopy; ^{119}Sn Mössbauer; anti-tumour activity

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INTRODUCTION

Metal ions are essential components for various physico-chemical processes occurring in living systems. Furthermore, they have potential use as metallopharmaceuticals exhibiting anti-tumour activity. The spectrum of their chemotherapeutic value has been widened since the modelling of *cis*-platin as the first metal-based anti-tumour drug,[1] and subsequently, of its analogues.[2] It is known that many drugs that inhibit the growth of tumour cells can be chelating agents and may act by interfering with the metalloenzymes that are necessary for the rapid growth of malignant cells.[3] The drug *cis*-platin can crosslink to two strands of the double helix of DNA, just as the bifunctional alkylating agents (nitrogen mustards) crosslink the DNA strands through the N-7 nitrogen atoms of the guanine bases and/or adjacent N-7 atoms of guanines in a single strand.[4] Prompted by the initial success of platinum chemotherapeutic metallopharmaceuticals, attention was first shifted to non-platinum chemotherapeutics starting from the basic *cis*-platin framework, with the aim to optimize the efficiency of such drugs. Among these, organotins have emerged as potential biologically active metallopharmaceuticals in the last two decades,[5,6] although their anti-tumour properties had been reported much earlier.[7] Also, organotins have been proposed as models for the interaction with the high-affinity site of ATPase (histidine only) and the low-affinity site of ATPase and haemoglobins (histidine and cystine).[8–10]

Because of the wide range of applications of organotins, several studies have been focused on the increasing amounts of both organic and inorganic tin present in the environment, the latter element having been evaluated as the third most important pollutant in the ecosystem. This has naturally raised the concern that tin may enter into the human food chain,[11] accumulate in the environment, and finally in biological systems.

The biological importance of organotins has been supported by studies concentrating on structure–activity correlations[12–19] that dealt mainly with structural aspects and anti-tumour activity, and also linked with possible tumorigenic activity. Indeed, butyltins present genotoxic effects[20,21] and may predispose animals to malignancy. The US EPA has classified phenyl-alkyltins, such as penbutotin oxide, as non-carcinogens in humans, but triphenyltin is the exception to this rule as a probable human carcinogen.[22] Speciation of organotins in biological systems has revealed two striking aspects of their behaviour, namely that the organotin moiety is an active species, able to link to biological molecules and to facilitate the transport of R_2Sn^{2+} to the target site, and that the highest activity can be due to dissociation of a chelating ligand as a part of the mechanism of inhibition.[23] Several studies have reported that ligands containing oxygen and nitrogen atoms as donor sites are often involved in compounds with potential anti-tumour activity.[24–30]

In order to obtain a better insight into how the metallic species behave inside biological systems, it is necessary to study their coordination behaviour with ligands that can occur in the biological medium, and hence to formulate structure–activity correlations to devise new derivatives with potential anti-tumour activity. This explains why attention has shifted towards metal derivatives of amino acids and peptides. In comparison with the organotin–amino acid systems,[31] only limited studies have been carried out on the interaction of organotins with peptides.[32–40]

In order to widen the scope of investigations on the coordination behaviour of ligands occurring in biological systems towards organotins, we carried out systematic studies of organotin(IV) derivatives of biologically relevant ligands,[30,31,40–44] with the final goal to develop new biologically active pharmaceuticals. Here, we report the synthesis and structural studies of some dibutyltin(IV) derivatives of dipeptides, viz. glycyl–tyrosine (Gly–Tyr), leucyl–tyrosine (Leu–Tyr), glycyl–tryptophane (Gly–Trp), valyl–valine (Val–Val), leucyl–leucine (Leu–Leu) and alanyl–valine (Ala–Val), and the triphenyltin(IV) derivative of glycyl–leucine (Gly–Leu). The structure of the complexes formed, with special focus on the possible modes of coordination, is discussed. Also, the anti-tumour activity of all the complexes is reported.

EXPERIMENTAL

All the reactions were carried out under an anhydrous atmosphere. Solvents were purified and dried before use. Dibutyltin(IV) oxide and triphenyltin(IV) chloride (E. Merck), as well as Gly–Tyr, Gly–Trp, Leu–Tyr, Leu–Leu, Val–Val, Ala–Val and Gly–Leu (Sigma) were used as received.

Synthesis of dibutyltin(IV) complexes of dipeptides

The complexes were prepared under anhydrous conditions by dropwise addition of a dry, hot methanol solution (30–40 ml) of dipeptide to a suspension of di-*n*-butyltin oxide. The mixture obtained was refluxed with constant stirring, giving a clear solution within 1 h. Refluxing was continued for at least 14–16 h with azeotropic removal of water. The excess of solvent was removed under reduced pressure. The oily product obtained was solidified, purified and crystallized by trituration with hexane (b.p.: 60–80 °C fraction from petroleum (E. Merck)). It was recrystallized from methanol–hexane.

Synthesis of triphenyltin(IV) glycylleucinate

Glycylleucine (1.5 mmol) was dissolved in the minimum amount (20 ml) of dry methanol. Sodium methoxide, prepared by dissolving sodium (1.2 equivalents) in dry methanol (15 ml), was then added. The resulting mixture was refluxed under constant stirring, giving a clear solution within 0.5 h. Refluxing was continued for another 3–4 h with constant stirring. A hot methanol solution (20 ml) of triphenyltin(IV) chloride (1.5 mmol) in 1 : 1 molar ratio was

added to the solution of the sodium salt of the dipeptide. The resulting mixture was further refluxed with constant stirring for another 6–7 h, and was then centrifuged and filtered in order to remove the sodium chloride formed. The excess of solvent was removed under reduced pressure. The oily mass obtained was solidified by trituration with petroleum ether (b.p.: 40–60 °C) and recrystallized from methanol–hexane.

Measurements

The melting points of the complexes synthesized were determined on a Toshniwal capillary melting point apparatus and were uncorrected. Carbon, hydrogen and nitrogen analyses of the dibutyltin(IV) complexes were carried out on a Perkin–Elmer, CHN-rapid elemental analyser at the Indian Institute of Technology, Delhi, India; those for the triphenyltin(IV) complex were carried out on a CHN analyser from Carlo Erba 1108, Heraeus, at the Central Drug Research Institute, Lucknow. The tin content in the complexes synthesized was determined gravimetrically as SnO_2.[40] Molar conductance measurements were carried out on the same instrument as reported previously.[40]

IR and far-IR spectra were recorded on a Perkin–Elmer 1600 series FTIR spectrophotometer from KBr discs in the range 4000–400 cm^{-1} and from CsI discs in the range 600–200 cm^{-1} at the Department of Chemistry, Indian Institute of Technology, Roorkee, India.

^{119}Sn Mössbauer spectra were recorded on a Mössbauer model MS-900 spectrometer, according to the procedure reported previously,[30,40–44] at the Department of Chemistry and Physics, University of District of Columbia, Washington, DC.

The NMR spectra were acquired on a Bruker Avance DRX250 instrument equipped with a Quattro probe, tuned to 250.13 MHz, 62.93 MHz and 89.15 MHz for ^{1}H, ^{13}C and ^{117}Sn nuclei respectively, and a Bruker AMX500 spectrometer. ^{1}H and ^{13}C chemical shifts were referenced to the standard Me_4Si scale from respectively residual ^{1}H and ^{13}C–^{2}H solvent resonances of chloroform ($CHCl_3$, 7.23 ppm, and $CDCl_3$, 77.0 ppm, for ^{1}H and ^{13}C nuclei respectively). The ^{117}Sn resonance frequencies were referenced to Ξ(^{117}Sn) 35.632 295.[45,46] 2D ^{1}H–^{13}C and ^{1}H–^{119}Sn[47,48] heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) spectra were acquired on a Bruker AMX500 spectrometer using the pulse sequence of the Bruker program library, including gradient pulses,[49] as described previously.[50]

Anti-tumour screening

The compounds were screened *in vitro* against seven human cancer cell lines that belong to the currently used anti-cancer screening panel of the NCI.[51] Test and reference compounds were dissolved to a concentration of 250 000 ng ml^{-1} in full medium, by 20-fold dilution of a stock solution containing 1 mg of compound per 200 µl aqueous solutions containing 1% of dimethylsulfoxide (DMSO) or ethanol using a literature procedure,[51,52] by the microculture sulforhodamine B (SRB) test. The experiment was started on day 0. On day 0, 150 µl of trypsinized tumour cells (1500–2000 cells/well) were plated in 96-wells flat-bottom microtitre plates (Falcon 3072, BD). The plates were preincubated for 48 h at 37 °C, 8.5% CO_2, to allow the cells to adhere. On day 2, a threefold dilution sequence of ten steps was made in full medium, starting from the stock solution. Every dilution was used in quadruplicate by adding 50 µl to a column of four wells. On day 7, the incubation was terminated by washing the plate twice with phosphate-buffered saline (PBS). Subsequently, the cells were fixed with 10% trichloroacetic acid in PBS and placed at 4 °C for 1 h. After five washings with tap water, the cells were stained for at least 15 min with 0.4% SRB dissolved in 1% acetic acid. After staining, the cells were washed with 1% acetic acid to remove the unbound stain. The plates were air-dried and the bound stain was dissolved in 150 µl 10 mM Tris-base. The absorbance was read at 540 nm using an automated microplate reader (Labsystems Multickan MS). Data were used for the construction of concentration–response curves and determination of the ID_{50} value by using the Deltasoft 3 software.

RESULTS AND DISCUSSION

Synthetic aspects

Dibutyltin(IV) oxide reacts with the dipeptides in equimolar ratio in dry methanol to give the complexes under azeotropic removal of water (Eqn (1)). The reaction of Ph_3SnCl with the sodium salt (formed according to Eqn (2)) of (Gly–Leu) in a 1 : 1 molar ratio led to the formation of the complex according to Eqn (3), where H_2L is as given in Scheme 1.

$$n\text{-}Bu_2SnO + H_2L \xrightarrow{1:1} n\text{-}Bu_2SnL + H_2O \quad (1)$$

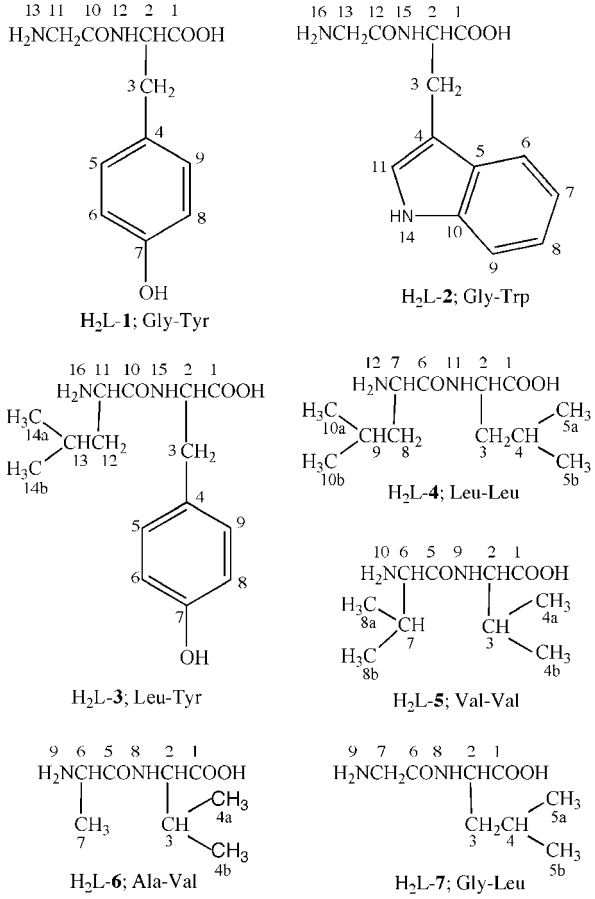
$$H_2L + NaMe \longrightarrow NaHL + MeOH \quad (2)$$

$$Pn_3SnCl + NaHL \xrightarrow{1:1} Ph_3Sn(HL) + NaCl \quad (3)$$

The reactions in Eqns (1)–(3) required 14–16 h of reflux. The resulting solids were obtained in good yields. The complexes are stable towards air and moisture, soluble in methanol and DMSO, but sparingly soluble in chloroform and other organic solvents. The analytical data of the complexes are presented in Table 1. From the data it can be inferred that the resulting complexes crystallized with 1 : 1 stoichiometry independently of the proportions of the organotin moiety and dipeptide used. The molar conductance of 10^{-3} M solutions of the complexes in methanol lie in the low range (0.0–0.1 Ω^{-1} cm^2 mol^{-1}), indicating their non-electrolytic nature.

IR spectral studies

Characteristic IR frequencies (cm^{-1}) and their assignments for the free dipeptides and their complexes are presented in Table 2. IR NH_2 stretching frequencies were used to distinguish coordinated from free amino groups. The position



Scheme 1.

of ν(N–H) bands is influenced by hydrogen bonding and by coordination of the nitrogen to tin.[31] In all the organotin(IV) dipeptide derivatives studied, very intense absorption bands in the range 3400–2950 cm^{-1}, due to the $\nu(NH_2)$ undergo a substantial lowering when compared with the free dipeptides (3475–2975 cm^{-1}), indicating coordination by the amino group to the central tin atom. Similar results have been reported for R_3SnAA (AA = amino acid)[31,41–44,53] and R_2SnL (H_2L = dipeptide).[34,35,40] For the H_2L-**1**, H_2L-**2**, H_2L-**3** and H_2L-**7** derivatives broadening occurs in the region 3500–3000 cm^{-1}, which indicates either overlapping of ν(OH) and ν(NH) vibrations, especially in the cases of H_2L-**1** and H_2L-**3** derivatives, or the presence of inter- and/or intramolecular hydrogen bonding.[31]

The carboxylate stretching frequencies have been utilized as a characteristic tool to confirm the mode of coordination through carboxylate oxygen, and also to identify the nature (monodentate, bidentate or bridging) of the bonding of the carboxylic group. The carboxylate groups in the organotin(IV) derivatives generally adopt a bridged structure in the solid state unless the organic substituents at the tin atom are bulky or the carboxylate group is branched at the α-carbon.[54] The IR absorption spectra indicate that ν_{as}(COO) values shown by these amino-coordinated compounds (1633–1613 cm^{-1}) get shifted to higher frequencies in comparison with free dipeptides (1590–1550 cm^{-1}), whereas the corresponding ν_s(COO) absorption frequencies (1409–1363 cm^{-1}) either remain at the same value or move to lower frequencies than in the free dipeptides (1405–1387 cm^{-1}). Strong interactions between the carboxylate carbonyl and the tin atom can thus be ruled out on this basis.[53] The magnitude of the $(\nu_{as} - \nu_s)$COO ($\Delta\nu$) separation, which has been shown to be useful in identifying structural features,[31] is larger in the amino-coordinated organotin(IV) derivatives ($\Delta\nu = 236 \pm 18$ cm^{-1}) than in the free dipeptides ($\Delta\nu = 175 \pm 25$ cm^{-1}) (Table 2). Further, the magnitudes of $\Delta\nu$ for all the derivatives have been found comparable to those obtained for R_3SnAA[31,41–44,53] and R_2SnL (H_2L = dipeptide),[40] indicating that the carboxylate

Table 1. Characteristic properties of di- and tri-organotin(IV) complexes of dipeptides

Compound	Complex [empirical formula]	Yield (%)	M.p. (°C)	Colour & physical state	Analysis (%): Found (calc.)			
					Sn	N	C	H
1	Bu_2SnL-**1** $[C_{19}H_{30}N_2O_4Sn]$	75	81–84	Light yellow solid	24.92 (25.30)	5.56 (5.97)	48.21 (48.64)	6.04 (6.44)
2	Bu_2SnL-**2** $[C_{21}H_{31}N_3O_3Sn]$	75	177–180	White solid	23.81 (24.11)	8.22 (8.54)	50.92 (51.25)	6.09 (6.35)
3	Bu_2SnL-**3** $[C_{23}H_{38}N_2O_4Sn]$	70	110–113	Cream solid	22.25 (22.60)	4.92 (5.33)	52.25 (52.59)	6.84 (7.29)
4	Bu_2SnL-**4** $[C_{20}H_{40}N_2O_3Sn]$	70	127–130	White solid	24.64 (24.98)	5.46 (5.89)	50.36 (50.55)	8.16 (8.48)
5	Bu_2SnL-**5** $[C_{18}H_{36}N_2O_3Sn]$	82	244–247	White solid	26.11 (26.55)	5.81 (6.26)	47.93 (48.34)	7.86 (8.12)
6	Bu_2SnL-**6** $[C_{16}H_{32}N_2O_3Sn]$	80	267–270	White solid	27.86 (28.32)	6.28 (6.69)	45.38 (45.85)	7.29 (7.69)
7	Ph_3Sn(HL-**7**) $[C_{26}H_{30}N_2O_3Sn]$	81	111–114	White solid	21.88 (22.09)	4.81 (5.21)	57.83 (58.14)	5.33 (5.63)

Table 2. Characteristic IR frequencies (cm^{-1}) of dipeptides and their di- and tri-organotin(IV) complexes[a]

Compound	Ligand complexe	ν(NH)	$\nu(CO_{amide})$	ν_{as}(COO)	ν_s(COO)	$\Delta\nu$	ν_{as}(Sn–C)	ν_s(Sn–C)	ν(Sn–O)	ν(Sn–N)/ ν(Sn ← N)
	H_2L-**1**	3417s 3233s 3167s	1668s	1559s	1388s	171	—	—	—	—
1	Bu_2SnL-**1**	3200s 3142s 2950s	1650s	1617s	1392s	225	675m	529m	566m	442m 405m
	H_2L-**2**	3426s 3283s 3092m	1683m	1567s	1397m	170	—	—	—	—
2	Bu_2SnL-**2**	3300s 3250s 2950m	1634s	1617s	1363m	254	658m	546m	509m	467w 410m
	H_2L-**3**	3433m 3250s 3108s	1675m	1590s	1394s	196	—	—	—	—
3	Bu_2SnL-**3**	3392s 3283s 2961m	1667m	1616s	1397m	219	587m	517m	573m	473m 408m
	H_2L-**4**	3255m 3096m	1650s	1589s	1393s	197	—	—	—	—
4	Bu_2SnL-**4**	3215s 3116s	1633s	1617s	1385s	232	625m	542m	581m	481m 418m
	H_2L-**5**	3467m 3167s 3083s	1659s	1583s	1387s	196	—	—	—	—
5	Bu_2SnL-**5**	3217m 3108m 2968s	1646s	1613s	1367s	246	610m	507w	550w	433w 414w
	H_2L-**6**	3317s 3042s 2975s	1667s	1550s	1400s	150	—	—	—	—
6	Bu_2SnL-**6**	3233m 3158m 2967m	1658s	1625s	1395m	230	584m	516w	533w	475w 458w
	H_2L-**7**	3402m 3242s 3065s	1691s	1558s	1405s	153	—	—	—	—
7	Ph_3Sn(HL-**7**)	3379s 3230s 3063s	1679s	1633s	1409s	224	570m	520m	574m	443m

[a] s, strong; m, medium; w, weak.

group acts as a monodentate ligand, and hence the possibility of ionic bonding and also bridging or chelation (which would give $\Delta\nu < 200\ cm^{-1}$) can be excluded.[31,40–44,53] Furthermore, the disappearance of a broad band in the spectra of the complexes in the region 2800–2200 cm^{-1}, which was present in all the free ligands as a weak intensity band, suggests the deprotonation of the free COOH group upon complexation.[31]

The appearance of a new band of medium intensity in the far-IR spectra of all the complexes in the region 581–509 cm^{-1}, which may be assigned to ν(Sn–O), further supports the bonding of COO group to the tin atom.[30,31,40]

In the derivatives studied, apart from the carboxylic oxygen and amino nitrogen as potential sites coordinating to the tin atom, the amide group also exhibits a strong

tendency to coordinate with the organotin(IV) moiety. Two characteristic bands, viz. amide I (essentially ν(C═O)) and amide II (ν(CN) + δ(NH)) as well as ν(NH), give the crucial information on the occurrence of metal coordination by the basic atoms of the amide group.[55,56] In all the organotin(IV) derivatives studied, an intense band of the amide I at 1670 ± 21 cm^{-1} in the free dipeptides undergoes a shift to a lower frequency (1679–1633 cm^{-1}) in the IR spectra of the dibutyltin(IV) derivatives upon complexation. This is probably due to the involvement of the peptide nitrogen (because of the deprotonation that has taken place) in bonding with tin, which lowers the bond order of the C═O (amide) group due to resonance stabilization.[40] Also, the lowering in $\nu(C{=}O)_{amide}$ (amide I) absorption frequency upon complexation suggests that the amide I group may be involved in the intramolecular/intermolecular hydrogen bonding and not in the coordination with tin.[31] Further, the amide II band gets shifted to lower frequency by ~35–60 cm^{-1} upon complexation in the case of dibutyltin(IV) derivatives **1–6** with respect to free dipeptides, which suggests that the amide nitrogen is the third coordinating site due to the deprotonation of the amide nitrogen. In the case of compound **7** (Ph_3Sn(HL-**7**)) this band remains unaffected, which indicates the non-participation of the C═O(amide) and NH (peptide) groups in the coordination to the Ph_3Sn(IV) moiety. It has been reported that the σ donor power of the peptide nitrogen is larger than that of the amino nitrogen in $Ph_2Sn(^-OOCCH_2N^-COCH_2NH_2)$ for which a valence bond structure is considered with resonance in peptide bonds only.[57] This is also in agreement with the crystallographic data of the coordinated glycylglycine in Ph_2Sn(Gly–Gly) with formal charges being $Q_{N_{pept}}$ –

Table 3. ^{1}H, ^{13}C and ^{117}Sn NMR data[a] for compounds **1–4** in methanol-d_4

	1		**2**		**3**		**4**	
Atom	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	179.7		180.5		180.2		180.8; 180.5	
2	58.9 [13]	4.39 [31]	59.4 [13]	4.55 [32]	59.0 [13]	4.37	56.3 [18]; 56.1 [18]	4.25 [18]; 4.21 [20]
3	36.4	3.12; 3.38	27.5	3.39; 3.81	36.4	3.14; 3.34	44.1; 43.6	1.45; 1.71
4	128.9		111.0		128.8		25.8	1.83
5	132.3	6.87	130.3		132.4	6.86 (d)	21.2; 21.4; 23.0; 23.2[c]	0.9–1.0[d]
6	116.4	6.67	119.5	7.49 (d)[b]	116.2	6.67 (t)	176.4 [35]	
7	157.6		122.4	7.13 (t)	157.7		54.8; 54.9	3.57; 3.40
8	116.4	6.67	120.1	7.05 (t)	116.2	6.67 (t)	44.3	1.39; 1.54
9	132.3	6.87	112.7	7.39 (d)	132.4	6.86 (d)	25.8; 26.1	1.76
10	173.9 [30]		137.9		176.8 [33]		23.6; 23.8; 24.2; 24.4[c]	0.9–1.0[d]
11	44.8	3.36; 3.50	125.1	7.03 (s)	54.9	3.46		
12			174.0 [31]		44.6	1.43; 1.88		
13			44.9	3.45; 3.53	26.0	1.87		
14					21.3; 23.9	1.00; 1.05		
Butyl α	20.4 [577/553]; 20.3 [595/571]	1.32 [54]; 0.56 [110]; 0.75 [47]	20.6 [577/551]; 19.7 [590/564]	1.30; 0.06 [100]; 0.37 [74]	20.7 [582/556]; 20.5 [582/556]	1.31; 0.52; 0.90	21.7; 20.9; 20.8; 20.2	0.8–1.7[e]
Butyl β	28.4; 28.2	1.22 [66]; 1.53 [87]	28.3 [34]; 27.8	1.53 [85]; 0.97	28.4 [34]; 28.3	1.24; 1.54	28.4; 28.3	
Butyl γ	27.7; 27.3	1.24; 1.33	27.7 [90]; 27.6 [100]	1.06; 1.33	27.7 [87]; 27.8 [104]	1.32; 1.24	27.6; 27.7; 27.8	
Butyl δ	14.0	0.85; 0.87	14.1; 14.0	0.80; 0.92	14.0	0.86; 0.89	13.9	0.9–1.0[d]
^{117}Sn	−123.7		−122.3		−134.6		−134.2; −140.4	

[a] $^1J(^{13}C–^{119/117}Sn)$ coupling constants are given between brackets; a single value indicates that the $^1J(^{13}C–^{119/117}Sn)$ coupling satellites are unresolved.
[b] Homonuclear proton–proton coupling multiplet abbreviations given in parentheses: s = singlet; d = doublet; t = triplet; all coupling values are 7 ± 1 Hz.
[c] Undetermined, permutable assignments, also between carbon atoms 5 and 10, because of overlapping pattern in proton spectrum.
[d] Several strongly overlapping triplets and doublets.
[e] Strongly overlapping patterns.

$Q_{O_{pept}} = -0.50$ and bond orders 1.50 for $(C-N)_{pept}$ and $(C-O)_{pept}$.[58]

The appearance of a pair of new bands of weak intensity in the region 481–405 cm^{-1} is assigned to ν(Sn–N) and ν(Sn ← N). This further confirms the coordination of the amino nitrogen as well as the peptide nitrogen to the organotin(IV) group,[30,31,40] whereas a single band due to ν(Sn ← N) at 443 cm^{-1} is observed for Ph_3Sn(HL-**7**).

The ν_{as}(Sn–C) and ν_s(Sn–C) bands in all the dibutyltin(IV) derivatives **1–6** were observed at 630 ± 50 cm^{-1} and 526 ± 20 cm^{-1} respectively. The presence of both bands suggests the existence of a bent C–Sn–C moiety in all these derivatives,[30,40] whereas in the case of **7** the corresponding ν_{as}(Sn–C) and ν_s(Sn–C) stretching absorptions were observed at 270 cm^{-1} and 220 cm^{-1} respectively.

Solution NMR spectral studies

^{1}H, ^{13}C and ^{117}Sn NMR chemical shifts and coupling constants obtained from methanol-d_4 solutions of compounds **1–6** are summarized in Tables 3 and 4. The assignment of the ^{13}C and ^{1}H resonances was based on ^{1}H–^{13}C HMQC and ^{1}H–^{13}C HMBC experiments and is in accordance with literature values for the amino acid residues.[59] The carbon atoms of the two *n*-butyl groups have pairwise different chemical shifts, indicating that they are diastereotopic. Also the $^1J(^{13}C-^{119/117}Sn)$ coupling constants are different for most diastereotopic *n*-butyl groups, but have orders of magnitude pointing to a distorted trigonal bipyramidal geometry. Calculation of the C–Sn–C angle from an empirical relationship established for dibutyl tin derivatives gives values between 130 and 134° for all derivatives.[60] Compounds **4** and **6** exhibit two ^{117}Sn chemical shifts and also a doubling of most of the ^{13}C and ^{1}H resonances, indicating that these compounds are dimeric in solution. Dimeric structure for di-*n*-butyl derivatives of tridentate ONO ligands are not unprecedented, as illustrated by di-*n*-butyltin pyridine-2-phosphonate-6-carboxylate.[61] The ^{117}Sn chemical shifts of all compounds vary between −122 and −145 ppm, which are typical values for a distorted trigonal bipyramidal geometry, encountered also in previous studies of diorganotin dicarboxylate derivatives.[62,63]

All these observations are in agreement with the structure proposed for the basic unit in Fig. 1, which is also proposed for the solid state from Mössbauer data.

Further evidence is found in the non-resolved $^2J(^{13}C-^{119/117}Sn)$ coupling constants of the carboxylic carbon atoms C-1, whereas a value of 30–34 Hz is measured for the coupling between the tin atom and the peptidic carbon atom. This value can be explained by a cumulative effect of ^3J and ^2J coupling pathways in the bicyclic structure proposed in Fig. 1. Moreover, ^{1}H–^{119}Sn HMQC spectra show correlations between the tin atom and the α-protons of both amino acids, which is only possible in a structure where tin is covalently bound to both, as illustrated in Fig. 1.

^{119}Sn Mössbauer spectral studies

The ^{119}Sn Mössbauer parameters have been utilized as a characterization tool for proposing the structure that a

Table 4. ^{1}H, ^{13}C and ^{117}Sn NMR data[a] for compounds **5, 6** and **7** in methanol-d_4

	5		**6**				**7**	
Atom	^{13}C	^{1}H	^{13}C		^{1}H		^{13}C	^{1}H
1	179.2		178.9	179.3			179.6	
2	62.5 [14]	4.23 [37]	62.7 [15]	62.4 [15]	4.13 [38]	4.18 [38]	55.0	4.35
3	33.9	2.19	33.4	33.9	2.24	2.18	44.0	1.52; 1.64
4	18.8; 19.8	0.89; 1.06	18.5; 20.3	18.9; 20.0	0.88; 1.09	0.90; 1.06	26.3	1.82
5	175.8 [31]		177.1 [34]	177.0 [32]			22.3; 23.7	0.96; 0.98
6	61.6	3.37	52.3	52.5	3.71	3.51	172.3	
7	31.8	2.52	19.6	20.2	1.39	1.45	45.4	3.83; 4.00
8	15.9; 20.0	0.90; 1.07						
Butyl α	21.1 [589/564]; 20.1 [584/559]	1.62; 1.34	21.7 [584/558]; 21.2 [595/570]; 21.1 [592/565]; 19.7 [585/559]		0.9–1.7[c]		i[b] 141.3	
Butyl β	28.6; 28.5	1.55; 1.74	28.4; 28.5				o[b] 137.8 [43]	7.80 [60]
Butyl γ	27.9[93]; 27.7[89]	1.36; 1.43	27.7; 27.8; 27.9				m[b] 129.8 [66]	7.48
Butyl δ	14.0; 14.0	0.91; 0.96	14.0		0.9–1.0[e]		p[b] 130.7 [14]	7.43
^{117}Sn	−139.9		−135.3, −145.3				−170[d]	

[a] $^1J(^{13}C-^{119/117}Sn)$ coupling constants are given between brackets. A single value indicates that the $^1J(^{13}C-^{119/117}Sn)$ coupling satellites are unresolved.
[b] Chemical shifts of phenyl groups; $^1J(^{13}C-^{119/117}Sn)$ coupling satellites non-visible for *ipso* carbon.
[c] Overlapping and complex superposition of first-order and non-first-order patterns.
[d] Very broad (±2000 Hz).
[e] Overlapping superposition of triplets.

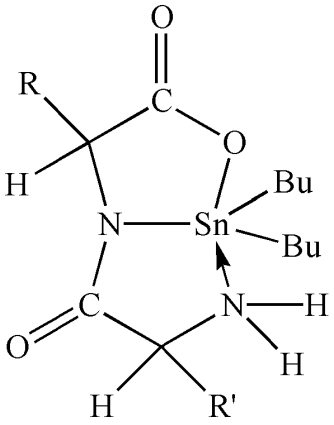


Figure 1. Coordination structure of dibutyltin(IV) complexes of dipeptides.

particular complex can adopt in the solid state. Whether coordination of the amino group nitrogen atom, bonding of the peptide nitrogen (apart from Ph_3Sn(HL-**7**)) and the carboxylic oxygen to tin lead to chelation or polymerization is discussed with reference to the ^{119}Sn Mössbauer data presented in Table 5.

The Mössbauer spectra of all the Bu_2Sn(IV) complexes exhibit a doublet centred in the isomer shift (IS) value range 1.19–1.30 mm s^{-1}. The quadrupole splitting (QS) values in the range 2.51–2.83 mm s^{-1} for Bu_2Sn(IV) complexes show that the electric field gradient around the tin nucleus is produced by the inequalities in the tin–peptide σ bonds[41,42] and is also due to the geometric distortions. The ρ (QS/IS) values (>2.0 in all the Bu_2Sn(IV) complexes) indicate a coordination number larger than four. Furthermore, the IS values are consistent with those of R_2Sn.trid (where trid$^{(2-)}$ represents 'planar' ligands with ONO and SNO donor atoms), whereas the QS values are in the range observed for Me_2SnONO-type complexes.[64] Earlier spectroscopic work of Herber and Barbieri[64] leads us to assume a trigonal bipyramidal type configuration for R_2Sn.trid, where

Table 5. ^{119}Sn Mössbauer data (80 K) of di- (**1**–**6**) and tri-organotin(IV) (**7**) dipeptido complexes[a]

Compound	QS (mm s^{-1})	IS (mm s^{-1})	ρ = QS/IS	τ_1(L)	τ_2(R)
1	2.80	1.25	2.24	0.43	0.47
2	2.81	1.29	2.18	0.42	0.44
3	2.83	1.30	2.18	0.44	0.47
4	2.58	1.22	2.11	0.40	0.47
5	2.55	1.23	2.07	0.47	0.48
6	2.51	1.19	2.11	0.44	0.50
7	1.43	1.11	1.29	1.01	1.04

[a] QS: quadrupole splitting; IS: isomeric shift relative to $BaSnO_3$ and tin foil (splitting: 2.52 mm s^{-1}); τ_1 (L): half line-width left doublet component; τ_2 (R): half line-width right doublet component (mm s^{-1}).

carbon atoms of the organotin moiety and ligand nitrogen are lying in equatorial position and OO or OS ligand atoms are axial, with ONO and ONS atom groups being located in a plane. The crystal and molecular structures of R_2Sn(Gly–Gly), where R = Ph, Me, *n*-Bu and Oct, show that the actual configurations are consistently distorted from the ideal trigonal bipyramid.[57,58] Thus, the tin atom configuration as shown in Fig. 1, in which two butyl groups and the peptide nitrogen atom are in equatorial positions, and the amino nitrogen and carboxylic oxygen atoms are axial, can again be proposed for the Bu_2SnL complexes in the solid state. Accordingly, these are then properly represented as glycyltyrosinato-/glycyltryptophanato-/leucyltyrosinato-/leucylleucinato-/valylvalinato-/alanylvalinato-/glycylleucinato-*O*,*N*,*N*(2-)dibutyltin(IV). Taking also into account the proposed structures in previous studies,[31,40] the above proposal is in complete agreement with both the QS values and the symmetry as well as the chelation constraints of the coordinating ligands.

It has been reported by Barbieri *et al*.[57] that the Sn–$N_{peptide}$ bond is shorter than the Sn–N_{amino} bond in R_2Sn(Gly–Gly), which may be due to the consistent s-character in the Sn–$N_{peptide}$ bond as well as its possible involvement into the π-delocalization of the peptide group. Further, on the basis of earlier crystallographic studies[58] of Ph_2Sn(Gly–Gly), it may be concluded that the single molecules of Bu_2SnL in the dibutyltin(IV) complexes studied may be bridged by hydrogen bonds between amino nitrogen and carbonyl oxygen of the peptide group and also by the carboxylate oxygen (not bound to tin). This intermolecular hydrogen bonding may thus be responsible for the low solubility of the complexes studied in common organic solvents. The ligating behaviour of the dipeptides towards R_2Sn(IV) moieties is then quite dissimilar from that of amino acids,[31] which is most likely due to the somewhat higher acidity of the peptide hydrogen atom.

The QS and IS values in the triphenyltin compound **7** are equal to 1.43 mm s^{-1} and 1.11 mm s^{-1} respectively. It has been reported[65,66] that the three conceivable (Fig. 2) five-coordinate isomers of R_3SnL complexes, where L is a bidentate ligand, have different QS values ranges, 1.7–2.3 mm s^{-1} for isomer (a), 3.0–3.9 mm s^{-1} for (b) and 3.5–4.1 mm s^{-1} for (c).

The QS value of compound **7**, i.e. 1.43 mm s^{-1}, is not really compatible with any of the five-coordinate structures of Fig. 2, which makes a pseudotetrahedral

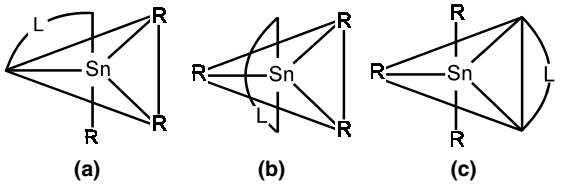


Figure 2. Structure of possible isomers for the R_3SnL (L = dipeptide anion).

Table 6. *In vitro* anti-tumoural activities (ID_{50}) of compounds **1**–**7**, in comparison with some reference compounds used clinically[a]

	ID_{50} in ng/ml												
Cell line	**1**	**2**	**3**	**4**	**5**	**6**	**7**	DOX	TAX	MTX	CPT	5FU	ETO
MEL A498	138	196	336	155	134	332	30	90	<3	37	2253	143	1314
EVSA-T	21	64	74	56	32	84	7	8	<3	5	422	475	317
H226	57	133	177	108	78	105	11	199	<3	2287	3269	340	3934
IGROV	25	72	118	90	46	199	6	60	<3	7	169	7	580
M19	73	182	205	150	89	139	16	16	<3	23	558	23	505
MCF-7	40	93	150	96	51	478	10	10	<3	18	699	18	2594
WiDr	284	424	420	295	265	332	8	11	<3	<3	967	<3	150

[a] See text for all line and reference compound definitions.

configuration (1.00–2.40 mm s^{-1}),[67] with, nevertheless, a realistically expectable very weak N→Sn coordination, the most plausible structure, i.e. actually close to structure (a) of Fig. 2 (arrangement in between *cis*-trigonal bipyramidal (1.7–2.3 mm s^{-1}) and pseudotetrahedral) (Fig. 3).

In vitro anti-tumour screening

Table 6 displays the *in vitro* anti-tumour activities of compounds **1**–**7** screened against seven cancer cell lines of human origin, MCF-7 (mammary cancer), EVSA-T (mammary cancer), WiDr (colon cancer), IGROV (ovarian cancer), M19 (melanoma), MEL A498 (renal cancer) and H226 (lung cancer). The ID_{50} values given in Table 6 are compared with those of some clinically used reference compounds,[52] doxorubicine (DOX), taxol (TAX), *cis*-platin (CPT), 5-fluorouracil (5FU), methotrexate (MTX) and etoposide (ETO).

Table 6 shows that the triphenyltin compound **7** displays the lowest ID_{50} values of the tin compounds tested and reported in this paper. Its activity is comparable to that of methotrexate, through with a much higher activity against cell line H226 than the latter; the activity of **7** is also comparably high when referring to 5 Fu, at least as far as cell lines IGROV, M19 and MCF7 are concerned, but more active against MEL A498, EVSA-T, H226 and less active against WiDr.

The di-*n*-butyltin compounds **1**–**6** exhibit lower *in vitro* anti-tumour activities than **7**, but they nevertheless provide significantly better activities than etoposide and *cis*-platin.

Further studies will state the possible relationship between anti-tumour and tumorigenic properties of the seven new di-*n*-butyltin/triphenyltin compounds; this is particularly true of compound **7**, because its triphenyltin moiety is known to be a probable human carcinogen.[22]

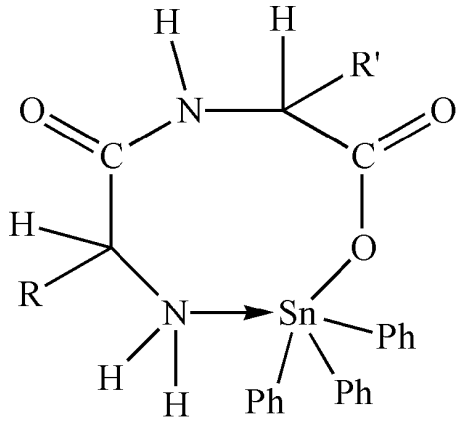


Figure 3. Coordination structure proposed for the triphenyltin(IV) complex **7**.

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