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New organotin(IV) complexes with L-Arginine, N_α-*t*-Boc-L-Arginine and L-Alanyl-L-Arginine: Synthesis, structural investigations and cytotoxic activity

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ABSTRACT

Novel diorganotin(IV) derivatives of L-Arginine (HArg), N_α-(*tert*-Butoxycarbonyl)-L-Arginine (Boc–Arg–OH) and L-Ala-L-Arg (H_2Ala–Arg), $H_2NC(=NH)NH(CH_2)_3CH(NHR')CO_2H$, where R′ = H in HArg, R′ = $C(O)OC(CH_3)_3$ in Boc–Arg–OH, R′ = $H_2NCH(CH_3)CO$ in H_2Ala–Arg and triorganotin(IV) derivatives of Boc–Arg–OH have been synthesized and structurally characterized. The complexes were investigated by FT-IR and ^{119}Sn Mössbauer in the solid state and by ^{1}H, ^{13}C, ^{119}Sn and ^{1}H–^{1}H COSY NMR spectroscopy, in solution. The spectroscopic characterization leading to the proposed molecular structures was accomplished on the basis of these experiments. L-Arginine appears to behave as a chelating ligand through carboxylate and -NH_2 groups in $Me_2Sn(Arg)_2$, while in N_α-*t*-Boc-L-Arginine complex, the N_α-protected amino group being exempted from coordination, only the carboxylate groups are effectors of bonding to the organometallic moieties. FT-IR spectra give a clear indication that guanidino groups in all the complexes are not involved in coordination, since ν(C=N–H) frequency of the terminal guanidino group is fairly constant and unshifted relative to the free ligand. The biological activity of organotin(IV)-complexes was also investigated by use of human HT29 colorectal carcinoma cells. The cytotoxic activity of the compounds was determined by the MTT quantitative colorimetric assay, capable of detecting viable cells in comparison with that exerted by cisplatin. A marked cytotoxic activity for nearly all complexes, is evident being higher than that exerted by cisplatin, while no significant improvement of activity was observed for $Me_2Sn(Arg)_2$ and Me_2Sn(Ala–Arg), which was confirmed by IC_{50} values. Then, we assessed whether the cytotoxicity induced by organotin(IV) complexes was associated with the induction of apoptosis. Light microscopy analysis, performed to study the morphological changes induced in HT29 cells, confirmed the results obtained with MTT test. No significant morphological alterations were observed in HT29 cells after treatment with Me_2Sn(Ala–Arg) and Me_2Sn(L-Arg)$_2$. Cells treated with $^nBu_2Sn(Boc–Arg)_2$, $^nBu_2Sn$(Ala–Arg), $^nBu_3Sn$(Boc–Arg) and Me_3Sn(Boc–Arg), appeared rounded, isolated and detached from culture substrate, indicating the commitment to apoptotic cell death.

1. Introduction

Proteomics, besides elucidating the cellular functions of proteins in both normal and pathological processes, is applied in target identification and drug discovery [1]. Selection of a suitable target whose biological activity can be directly linked to a pathological process is the first step in the development of new small molecule therapeutics. In the field of complexes of organometallic ions with proteins (or simple aminoacids and peptides) the task of combining in one molecule a DNA binding moiety (peptide) and a reactive domain represented by an antitumor active organometallic moiety, is strongly appealing. Moreover, the imposition by (organo)metallic ions of conformational constraints on peptidic ligands can possibly lead to molecules whose structure acts as a template orienting side chains in such a way that they act as recognition elements towards a receptor substrate.

Tethered oligoarginine conjugates, where the peptide is attached to a rhodium or ruthenium intercalator, bind and with photoactivation selectively cleave DNA. The presence cell-penetrating peptide oligoarginine is found to increase the nonspecific binding affinity of functionalized intercalator for both matched and mismatched DNA [2,3].

The aim of this work is to synthesize complexes of Arginine, effector of recognition, with organotin(IV) ions (R_2Sn^{2+} and R_3Sn^+, R = Me, ^{n}Bu) which are known to possess antitumour [4],

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antimicrobial [5], anti-inflammatory activities [6]. L-Arginine is an essential aminoacid with a side-chain guanidino group, which is strongly basic and protonated in a wide range of pH values and serves as a biological recognition site through hydrogen bonding [7]. Molecular recognition, enzymatic reactions and protein structures are connected with the properties of arginine. Recognition of DNA for initiation of DNA transcription, as in Zinc finger domain, are exerted by arginine [8]. A literature review [9] demonstrates that L-Arginine, the only substrate of the NO production, affects cardiovascular system (blood vessels and heart); Arg residues, *e.g.* Arg376 and Arg182 called as "arginine finger", have a profound effect on synthetic or hydrolytic processes of ATP in the rotational mechanism of F_0F_1-ATPase [10]. Moreover, experiments performed "in vivo" and "in vitro" also suggest that L-Arginine may have a complex antiaggregatory, anticoagulatory and profibrinolytic effect. Therefore, a novel therapeutic potential of L-Arginine should be taken into consideration [11].

Recently, interests in organotin(IV) carboxylates are increasing due to their possible medical uses as antitumor agents [12]. Novel organotin(IV) complexes of L-Arginine (HArg), N_α-*t*-Boc-Arginine (Boc–Arg–OH) along with complexes with the dipeptide, L-Alanyl-L-Arginine (H_2Ala–Arg) were synthesized. In all the complexes, the ligand is expected to be the recognition determinant of the specimen towards DNA, while the organometallic moieties, which are known to possess antitumor activity [13], might show an increased biological activity. The Arg-Gly-Asp (RGD) tripeptide sequence has been identified as a major cell adhesion recognition motif, in this context it appears that protection of the amino terminal group of RGD by Boc increases the inhibition of secondary cataract versus unprotected RGD [14].

2. Experimental

2.1. Materials and methods

^{n}Bu$_2$SnO and ^{n}Bu$_3$SnOCH$_3$ (Aldrich), Me$_3$SnOH (Alfa Aesar), L-Arginine and N_α-*t*-Boc-L-Arginine (Fluka) and L-Ala-L-Arg (Bachem) were used without further purification. Methanol was distilled over magnesium and benzene dried according to the literature. Me$_2$SnO was obtained by hydrolysis of Me$_2$SnCl$_2$ dissolved in water by treatment with 25%(w/w) aqueous ammonia solution. Elemental microanalyses for C, H and N were carried out by the Laboratorio di Microanalisi, University of Padova, Italy.

Thermogravimetric measurements were performed from room temperature to 800 °C, with a Mettler TA-4000 system operating in a pure nitrogen atmosphere.

The Mössbauer (nuclear γ resonance) spectrometers, the related instrumentation and data reduction procedures were as previously described [15]. A 10 mCi Ca^{119}SnO$_3$ source (RITVERC GmbH, St. Petersburg, Russia) was employed. The isomer shifts (δ) are relative to room temperature Ca^{119}SnO$_3$. Infrared spectra (nujol mulls, CsI windows) were recorded with an FT-IR spectrometer Perkin-Elmer Spectrum One. ^{1}H, ^{13}C spectra in CD$_3$OD, DMSO-d_6 and D$_2$O, were recorded at 298 K with Bruker Avance 300 operating at 300 MHz for ^{1}H and at 63 MHz for ^{13}C. ^{119}Sn NMR experiments were carried out at 298 K at 111.92 MHz in CD$_3$OD solution with a spectral width of 400 ppm. ^{1}H and ^{13}C resonances were calibrated on tetramethylsilane as reference. For ^{119}Sn NMR spectra, in CD$_3$OD, tetramethyltin(IV) was employed as external reference.

2.2. Biological studies

2.2.1. Chemicals and reagents

Stock solutions of the compounds were prepared in DMSO and just prior to treatment freshly diluted in the culture medium. The final concentration of DMSO never exceeded 0.1% which is a concentration that was experimentally determined to have no discernible effect on employed cell line.

2.2.2. Cell lines and culture conditions

Human colon-rectal carcinoma HT29 cells were cultured in RPMI 1640 medium, supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS) and 2.0 mM glutamine, at 37 °C in a humidified atmosphere containing 5% CO_2. In order to assess cell growth and morphology, 10^4 cells/well were seeded in 96-well plates. After plating, cells were allowed to adhere overnight and then treated with the compounds or with the same amount of DMSO employed as vehicle (control cells).

2.2.3. Cell viability assay

The effect of the compounds on cell viability was determined by the MTT quantitative colorimetric assay [16]. This method is based on the reduction of the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) into purple formazan by mitochondrial dehydrogenases of living cells. Dye absorbance in viable cells was measured at 570 nm with 630 nm as a reference wavelength, using an ELISA microplate reader (OPSYS MR, Dynex Technologies) against lysis buffer as a blank. Values reported in Fig. 7a are expressed as the percentage of the viability with respect to vehicle-treated control cells. All experiments were repeated at least five times and each experimental condition was repeated in triplicate wells in each experiment.

2.3. Synthesis

The reaction of R$_2$SnO (R = Me, ^{n}Bu) with HL = HArg and Boc–Arg–OH, H_2L = H_2Ala–Arg in a 1:2 and 1:1 molar ratio, Me$_3$SnOH/^{n}Bu$_3$SnOCH$_3$ with HL = Boc–Arg–OH, led to the formation of the complexes according to Eqs. (1)–(3), respectively:

$$R_2SnO + 2HL \rightarrow R_2Sn(L)_2 + H_2O, \quad (1)$$

$$R_2SnO + H_2L \rightarrow R_2Sn(L) + H_2O, \quad (2)$$

$$R_3SnOH/R_3SnOCH_3 + HL \rightarrow R_3Sn(L) + H_2O/CH_3OH. \quad (3)$$

2.3.1. *Me$_2$Sn(L-Arg)$_2$* (***1***)

A solution of Me$_2$SnO (0.329 g, 2 mmol) in benzene was added to a solution of HArg (0.697 g, 4 mmol) in hot methanol. The mixture was refluxed for 4 h. The solution was filtered on a sintered glass filter and the white solid washed with dichloromethane and stored in a vacuum dessicator. $C_{14}H_{32}N_8O_4Sn$ (***1***); dec. 189 °C; M = 495.17 g/mol; Anal. Calc.: C, 33.96; H, 6.51; N, 22.63. Found: C, 33.70; H, 7.17; N, 21.96%. IR data (CsI, cm^{-1}): 3341 msh, 3263m, 3087m ν(NH); 1679s ν(−C=NH); 1645s ν_{as}(COO$^-$), 1422m ν_s(COO$^-$), $\Delta\nu$ = 224; 586m ν_{as}(Sn–C), 516w ν_s(Sn–C); 436m ν(Sn–N). ^{119}Sn Mössbauer data: δ = 1.08, ΔE = 2.69, $\Gamma\pm$ = 0.90 mm s^{-1}.

2.3.2. *^{n}Bu$_2$Sn(Boc–Arg)$_2$·2H$_2$O* (***2***) *Me$_2$Sn(Ala–Arg)·0.5H$_2$O* (***5***)

A solution of Bu$_2$SnO (0.497 g, 2 mmol) or Me$_2$SnO (0.329 g, 2 mmol) in dry methanol was added to a solution of Boc–Arg–OH (1.097 g, 4 mmol) or H_2Ala–Arg (0.491 g, 2 mmol), in dry methanol. The mixture was refluxed for 4 h. The solvent was reduced under vacuum to a small volume; an oil was obtained in both cases which gave a white solid product when dried *in vacuo* and washed with a a mixture of methanol/petroleum ether (1:3, v/v). $C_{30}H_{64}N_8O_{10}Sn$ (***2***); m.p. 159–162 °C. M = 815.60 g/mol; Anal. Calc.: C, 44.18; H, 7.91; N, 13.74. Found: C, 44.02; H, 8.12; N, 12.96%. Selected IR data (CsI, cm^{-1}): 3346m, 3163m ν(NH); 1608s ν(−C=NH) + ν_{as}(COO$^-$), 1393m ν_s(COO$^-$), $\Delta\nu$ = 247; 595m ν_{as}(Sn–C); 565m ν_s(Sn–C). ^{119}Sn Mössbauer data: δ = 1.00, ΔE = 2.11, $\Gamma\pm$ = 0.81 mm s^{-1}. $C_{11}H_{27}N_5O_4Sn$ (***5***); dec. 194 °C. M = 401.06 g/mol; Anal. Calc.: C,

32.94; H, 6.03; N, 17.46. Found: C, 33.70; H, 6.24; N, 17.86%. Selected IR data (CsI, cm^{-1}): 3343mbr, 3172mbr ν(NH); 1612sbr $\nu(-C{=}NH) + \nu_{as}(COO^-)$, 1403m $\nu_s(COO^-)$, $\Delta\nu = 209$; 568m ν_{as}(Sn–C); 530m ν_s(Sn–C). ^{119}Sn Mössbauer data: $\delta = 1.08$, $\Delta E = 2.69$, $\Gamma\pm = 0.90$ mm s^{-1}.

2.3.3. ^{n}Bu$_2$Sn(Ala–Arg)·1.5H$_2$O (**6**)

A solution of ^{n}Bu$_2$SnO (0.497 g, 2 mmol) in dry methanol (20 mL) was added to a methanolic solution of H$_2$Ala–Arg (0.491 g, 2 mmol); the mixture was refluxed for 4 h. The excess of solvent was reduced under vacuum to a small volume and the white solid precipitated by addition of diethyl ether, which was filtered off, washed with diethyl ether and dried *in vacuo*. $C_{17}H_{39}N_5O_4Sn$ (**6**); m.p. 109–112 °C. M = 512.24 g/mol; Anal. Calc.: C, 40.58; H, 7.61; N, 13.92. Found: C, 40.18; H, 7.56; N, 13.20%. Selected IR data (CsI, cm^{-1}): 3341mbr, 3172mbr, 3076mbr ν(NH); 1608sbr $\nu(-C{=}NH) + \nu_{as}(COO^-)$, 1402m $\nu_s(COO^-)$, $\Delta\nu = 206$; 582m ν_{as}(Sn–C); 537w ν_s(Sn–C). ^{119}Sn Mössbauer data: $\delta = 1.19$, $\Delta E = 2.80$, $\Gamma\pm = 0.79$ mm s^{-1}.

2.3.4. Me$_3$Sn(Boc–Arg)·CH$_3$OH (**3**) and ^{n}Bu$_3$Sn(Boc–Arg)·CH$_3$OH (**4**)

A solution of Boc–Arg–OH (0.549 g, 2 mmol) in dry methanol (20 mL) was added to a solution Me$_3$SnOH (0.362 g, 2 mmol) or ^{n}Bu$_3$SnOCH$_3$ (0.642 g = 0.56 ml, 2 mmol). After stirring the solution overnight, the excess of solvent was reduced under vacuum to a small volume in a rotary evaporator, a thick oil was obtained in both cases. These were treated with diethyl ether and petroleum ether and white solids were formed. The complexes were washed with a mixture of methanol/petroleum ether (1:3 v/v). $C_{14}H_{32}N_4O_5Sn$ (**3**); m.p. 119–122 °C. M = 469.17 g/mol; Anal. Calc.: C, 38.40; H, 7.30; N, 11.94. Found: C, 37.94; H, 7.00; N, 11.57%. Selected IR data (CsI, cm^{-1}): 3343sbr, 3164sbr ν(NH); 1683sbr $\nu(-C{=}NH)$; 1631m $\nu_{as}(COO^-)$, 1406m $\nu_s(COO^-)$, $\Delta\nu = 225$; 545m ν_{as}(Sn–C); 512w ν_s(Sn–C). ^{119}Sn Mössbauer data: $\delta = 1.25$, $\Delta E = 3.08$, $\Gamma\pm = 0.91$ mm s^{-1}. $C_{24}H_{52}N_4O_5Sn$ (**4**); m.p. 144–147 °C. M = 595.41 g/mol; Anal. Calc.: C, 48.41; H, 8.80; N, 9.41. Found: C, 47.89; H, 8.66; N, 10.07%. Selected IR data (CsI, cm^{-1}): 3344sbr, 3221mbr, 3090mbr ν(NH); 1683 sbr $\nu(-C{=}NH)$; 1627mbr $\nu_{as}(COO^-)$, 1417m $\nu_s(COO^-)$; $\Delta\nu = 207$; 513m ν_{as}(Sn–C). ^{119}Sn Mössbauer data: $\delta = 1.38$, $\Delta E = 3.11$, $\Gamma\pm = 1.14$ mm s^{-1}.

Several attempts to crystallize this compound in suitable form for the X-ray analysis failed. In absence of structural data, the coordination environment of the tin center is indicated on the basis of infrared and Mössbauer spectra [17].

3. Results and discussion

3.1. Solid state studies

3.1.1. IR spectra and Mössbauer data

The IR spectra of the complexes have been examined in comparison with the spectra of the free ligands. The X-ray structure of L-Arg dihydrate and infrared spectra including assignments have been reported [18,19]. The structures of the organotin(IV) complexes of the amino acids and dipeptide raise the question: which are the coordination sites to the tin atom (amino group nitrogen, carboxyl oxygen atoms and peptidic N–H or carbonyl groups), which coordination numbers result from these interactions and the detailed coordination geometry about the tin atom. The isomer shift (δ) data are typical of organotin(IV) derivatives [20,21]. The narrowness of the linewidths, Γ, suggests the occurrence of single tin sites in each compound.

3.1.2. Complex with L-Arginine

The position of ν(N–H) bands the amino groups is influenced by hydrogen bonding and by coordination of the nitrogen to tin. Coordinated amino groups show a substantial lowering of the N–H stretching frequencies along with an enhancement of their intensities.

The ligand exists in a zwitterionic form, in the solid state [22]. Vibrational frequencies associated with coordinated NH_2 group, in Me$_2$Sn(L-Arg)$_2$, show a shift to lower frequencies relative to the free ligand indicating coordination of the amino group to the central tin atom. Two bands typical of stretching ν(N–H) of primary amines are detected at 3341 and 3263 cm^{-1}. The asymmetric vibration $\nu_{as}(COO^-)$ is consistently shifted to higher frequencies in Me$_2$Sn(L-Arg)$_2$ (**1**), $\nu_{as}(COO^-)$ band at 1618 cm^{-1} in the free ligand shifts to 1645 cm^{-1}, while the symmetric $\nu_s(COO^-)$ remains at the same value. Mössbauer spectrum of Me$_2$Sn(L-Arg)$_2$ (**1**) is characterized by a quadrupole splitting value which is typical of both tetrahedral and *cis*-R_2 octahedral structures, but the *cis*-R_2 octahedral structure (Fig. 1) may be inferred since the aminoacid coordinates to the tin atom through the carboxylate and the amino groups.

3.1.3. Complexes with N_α-(tert-Butoxycarbonyl)-L-Arginine

Inhibition of coordinating ability of the protected amino group (-NH-Boc) in α, which is consistent with its lower basicity, is evidenced by a band at 3344 cm^{-1} in the IR spectra of N_α-*t*-Boc-L-Arg, which remains unaltered in ^{n}Bu$_2$Sn(Boc–Arg)$_2$ (**2**), Me$_3$Sn(Boc–Arg) (**3**) and ^{n}Bu$_3$Sn(Boc–Arg) (**4**) indicating Boc group's non-involvement in coordination. The ν(C=NH) vibration which is present in the spectra of the ligands HArg and Boc–Arg–OH (at 1679 cm^{-1} and 1681 cm^{-1}, respectively) and assigned [19] to the guanidyl group appears unaffected in the spectra of the amino acid complexes reported above, indicating lack of involvement of the guanidinic group in coordination to the organometallic ion. A carboxylate ion, RCO_2^-, can coordinate to metal ions in a number of ways, as a unidentate ligand, as a chelating ligand, as a bridging bidentate ligand or as a monatomic bridging ligand, either alone with additional bridging or in arrangement involving chelation and bridging. For the triorganotin(IV) derivatives, $\nu_{as}(COO^-)$ vibration is shifted to a small extent in the opposite direction. $\Delta\nu$ [$\Delta\nu = \nu_{as}(COO^-) - \nu_s(COO^-)$] value for ^{n}Bu$_2$Sn(Boc–Arg)$_2$ (**2**) is 247 cm^{-1}, and larger than in the free ligands, which suggests that carboxylate groups are coordinating in a monodentate fashion [23]. A different pattern is presented in the case of the triorganotin(IV)-Boc–Arg derivatives, were $\Delta\nu = 225$ cm^{-1} and $\Delta\nu = 207$ cm^{-1} are significantly less than the free ligand value ($\Delta\nu = 243$ cm^{-1}). Mössbauer spectrum of ^{n}Bu$_2$Sn(Boc–Arg)$_2$ (**2**) is characterized by quadrupole splitting value [$\Delta E = 2.11$ mm s^{-1}] which is typical of both *cis*-R_2 octahedral and tetrahedral structures. The tetrahedral arrangement is foreseeable for compound ^{n}Bu$_2$Sn(Boc–Arg)$_2$, in which the tin atom is unidentately coordi-

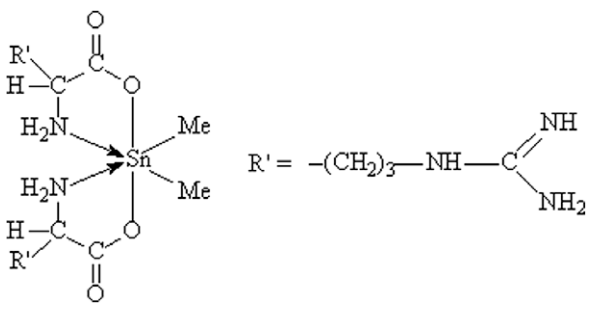


Fig. 1. Structure of Me$_2$Sn(L-Arg)$_2$.

nated by the carboxylate groups (Fig. 2a). Trialkyltin(IV) derivatives of aminoacids are generally characterized by polymeric structures with carboxylate groups bridging planar R_3Sn units [24]. Δ values for such compounds are in the range 3.3–3.9 mm s^{-1}. Discrete structures, with unidentate coordination of the carboxylate group, are normally adopted by triphenyl- or tricyclohexyltin(IV) derivatives as a consequence of the steric hindrance of the organic groups. Such compounds show Δ values ranging from 2.3 to 3.0 mm s^{-1}. The Δ values of Me_3Sn(Boc–Arg) [ΔE = 3.08 mm s^{-1}] and $^n Bu_3Sn$(Boc–Arg) [ΔE = 3.11 mm s^{-1}], fit well with the literature data for monomeric, distorted tetrahedral structures (Fig. 2b) taking into account that the partial quadrupole splitting of the alkyl group is larger than that of the phenyl group. In such case, discrete structures would be imposed by the steric hindrance of the ligand which bears the large Boc substituent on the α-amino group.

3.1.4. Complexes with L-Alanyl-L-Arginine

In the diorganotin(IV) complexes of dipeptide H_2Ala–Arg, information on the occurrence of metal coordination by the basic atoms of the amide group may be obtained from the infrared frequencies of the modes amide I [ν(C=O)] and amide II [ν(CN) + δ(NH)] [17].

The zwitterionic forms of the dipeptides in the solid state present asymmetric and symmetric COO^- stretching vibrations, which are shifted to higher frequencies, in the complexes. In Me_2Sn(Ala–Arg) (***5***) and $^n Bu_2Sn$(Ala–Arg) (***6***) the broad bands at 1612 cm^{-1} and 1608 cm^{-1}, respectively, probably arise from the overlap of amide I which is shifted to lower frequencies and $\nu_{as}(COO^-)$ bands relative to the vibrations in the free ligand at 1635 cm^{-1} and 1548 cm^{-1}.

The IR data support the notion that the dipeptide chelate the metal ion, bonding through the terminal amino and carboxylate groups and a deprotonated peptide nitrogen, yielding pentacoordinated tin(IV) complexes, forming five-membered rings (Fig. 3), while no evidence is offered for the involvement of the side-chain (guanidyl) donor atoms in bonding. Further, the Mössbauer quadrupole splitting measured for compounds Me_2Sn(Ala–Arg) [ΔE = 2.69 mm s^{-1}] and $^n Bu_2Sn$(Ala–Arg) [ΔE = 2.80 mm s^{-1}] are consistent with the *cis*-R_2 trigonal-bipyramidal structures [25]. Relative to the Mössbauer structural investigations in the solid state, due to the high electronegativity of oxygen and nitrogen

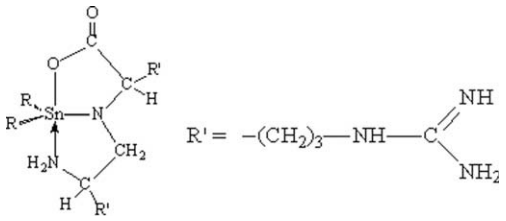


Fig. 3. Structure of R_2Sn(Ala–Arg) where R = Me, ^{n}Bu.

atoms, the Q.S. is mainly governed by ∠C–Sn–C bond angle [26]. The ∠C–Sn–C bond angle has been calculated by using Parish's relationship: Q.S. = 4[*R*][1 – (3/4)sinθ]^{½}, where θ = ∠C–Sn–C and [*R*] is partial splitting (p.q.s.) for alkyl groups bonded to tin, the reported p.q.s. value for alkyl groups being −1.03 mms^{-1}, closely related to those reported for diorganotin(IV) derivatives of dipeptides. The experimental Δ values for structurally characterized dialkyltin(IV) derivatives are spread over a wide range, from 2.5 to 3.2 mm s^{-1}, and the observed trends appear to be essentially correlated to the ∠C–Sn–C bond angle which correspondently varies from 123° to 144°. The comparison of the data obtained in this work with those reported in the literature led us to estimate the ∠C–Sn–C bond angles of compounds *(5)* and *(6)* which resulted approximately 125° and 130°.

The geometry of the di- and triorganotin(IV) group may be related to the tin–carbon stretching modes which occur in the 600–500 cm^{-1} region of the infrared spectra [17]. Two vibrational modes, ν_{as}(Sn–C) and ν_s(Sn–C), due to a significantly distorted trigonal-planar SnC_3 structure are present in Me_3Sn(Boc–Arg) (***3***) complex. In $Me_2Sn(Arg)_2$ (***1***), the presence of both ν_{as}(Sn–C) and ν_s(Sn–C) bands suggests a bent C-Sn-C fragment. For the dipeptide complexes, Me_2Sn(Ala–Arg) (***5***) and $^n Bu_2Sn$(Ala–Arg) (***6***), the presence of both vibrations, is consistent with the proposed trigonal bipyramidal structure.

3.2. TGA analysis

The thermal decomposition of the synthesized complexes $^n Bu_2Sn$(Boc–Arg)·2H_2O, Me_3Sn(Boc–Arg)·CH_3OH, $^n Bu_3Sn$(Boc–Arg)·

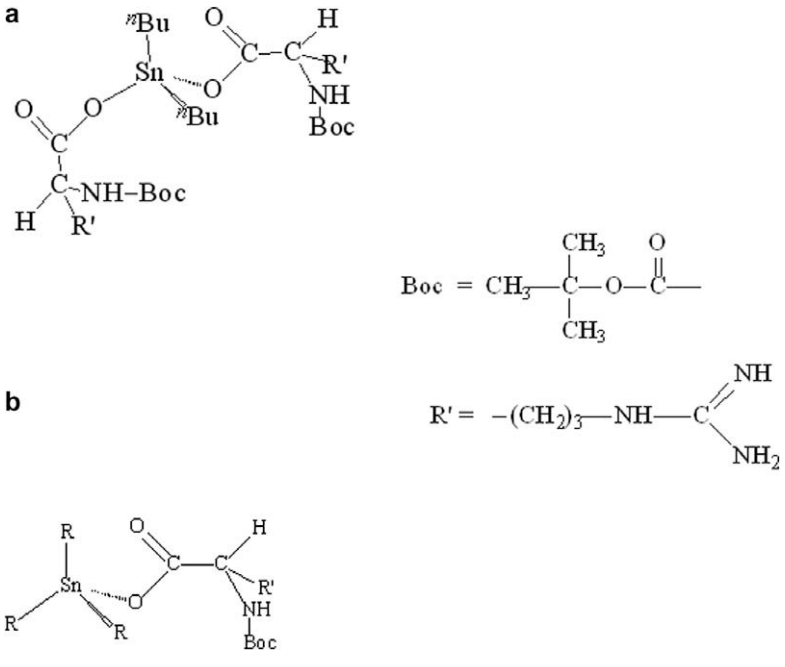


Fig. 2. Structure of (a) $^n Bu_2Sn(Boc–Arg)_2$ (b) R_3Sn(Boc–Arg) where R = Me, ^{n}Bu.

CH_3OH, $Me_2Sn(Ala–Arg)\cdot 0.5H_2O$ and $^nBu_2Sn(Ala–Arg)\cdot 1.5H_2O$ was investigated by thermogravimetry (TGA) and differential TGA (DTG) techniques to identify the attached solvent molecules, in order to confirm the proposed stoichiometry and to support the conclusions of the various spectroscopic techniques [27]. Thermogravimetric analyses were performed from room temperature to 800 °C. No solvent molecule was found for $Me_2Sn(Arg)_2$. $Me_3Sn(Boc–Arg)\cdot CH_3OH$ undergoes a mass loss of 6.58% in the 30.20–110.80 °C range corresponding to the 6.82% value expected for 1 mol CH_3OH and $^nBu_3Sn(Boc–Arg)\cdot CH_3OH$ undergoes a mass loss of 5.74% (calculated 6.04%) in the range 67.43–101.89 °C for 1 mol CH_3OH per formula unit.

The percent weight loss for $^nBu_2Sn(Boc–Arg)\cdot 2H_2O$ is 3.63% (calculated 4.41%) in the range 24.64–118.71 °C corresponding to 2 mol of water and for $^nBu_2Sn(Ala–Arg)\cdot 1.5H_2O$ is 5.01% (calculated 5.27%) in the range 32.34–139.22 °C for the latter corresponding to 1.5 mol water.

In $Me_2Sn(Ala–Arg)\cdot 0.5H_2O$ complex the mass loss 2.51% (calculated 2.24%) in the range 32.33–139.22 °C is equivalent to 0.5 mol of water per formula unit. The residue obtained was, in all cases, SnO_2, as evidenced by FT-IR spectrum and tin content. The temperatures at which the water molecules have been lost, rule out any involvement in coordinating the tin(IV) atom, therefore water molecules could be involved in hydrogen bonding in the complexes.

4. Solution state studies

4.1. ^1H and ^{13}C NMR spectra

The resonances for the individual amino acids were assigned according to Wüthrich [28] and literature data [11]. Structural information for the complexes in solution has been acquired by ^1H, ^{13}C, ^{119}Sn and $^1H–^1H$ COSY spectra. In Table 1 ^1H N.M.R. spectra are reported for the free ligands and the complexes. Solubility for all specimen was very poor in all solvents and the goal of obtaining concentrations higher than 10^{-3} mol/L proved to be frustrating.

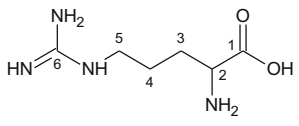
4.2. $R_2Sn(IV)^{2+}$ complexes with L-Arginine

For the $Me_2Sn(Arg)_2$ complex surprisingly solubility was larger in D_2O than in DMSO. Relative to the free zwitterionic form of the ligand, all resonances are shifted to higher field on complexation as

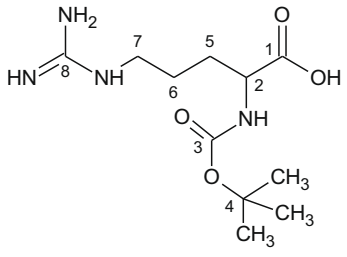
Table 1
^1H chemical shifts[a] (δ) for ligands and di- and triorganotin(IV) complexes.

	Compound (solvent)	δ (ppm)[a]
	L-Arg (D_2O)	**H-2**: 3.76 (t,1H); **H-3**: 1.89 (dt, 2H); **H-4**: 1.66 (m, 2H); **H-5**: 3.23 (t, 2H)
1	Me_2Sn(L-Arg)$_2$ (D_2O)	**H-2**: 3.32(t, J = 5.9, 1H); **H-3+ H-4**: 1.72–1.51 (m, 4H); **H-5**: 3.18 (t, J = 6.5, 2H); **H-α**: 0.66 [82.5 Hz][b] θ_1 = 134.1°; [78.9 Hz][b] θ_2 = 129.5°
	Boc–Arg (CD_3OD)	**H-2**: 3.97 (t, J = 5.8, 1H); **H-5 + H-6**: 1.73–1.54 (m, 3H)[c]; **H-5**: 1.80 (m, 1H); **H-7**: 3.18 (t, J = 6.1, 2H); **Boc** [d]: 1.43(s, 9H); **NH**Arg: 7.70 (br,1H):, **NH_2**Arg: 7.04 (br, 2H)
2	$Bu_2Sn(Boc–Arg)_2$ (CD_3OD)	**H-2**: 3.93 (t, J = 6.1, 1H); **H-5 + H-6**: 1.86–1.70 (m,br, 4H); **H-7**: 3.14 (t, J = 6.3, 2H); **Boc**[d]: 1.40(s, 9H); **H-α**: 1.25 (m, 4H); **H-β**:1.66 (dd, 4H); **H-γ**: 1.35(m, 4H); **H-**δ: 0.92(dt, 6H)
4	$Bu_3Sn(Boc–Arg)$ (CD_3OD)	**H-2**: 3.92 (t, J = 5.8, 1H); **H-5 + H-6**: 1.58 (m, br, 4H); **H-7**: 3.13 (t, J = 6.7, 2H); **Boc**[d]: 1.38(s, 9H); **H-α**:1.09(t, 4H); **H-β**:1.55 (t, 4H); **H-γ**:1.32(q, 4H); **H-δ** = 0.88 (t, J = 7.3, 9H)
	Boc–Arg (DMSO-d_6)	**H-2**: 3.62 (t, J = 6.1, 1H); **H-5 + H-6**: 1.70–1.43 (m,4H)[c]; **H-7**: 3.03 (t, 2H); **Boc**[d]: 1.36(s) (m, 9H); **NH**Arg: 7.61 (br,1H):, **NH_2**Arg: 7.40 (br, 2H)
4	$Bu_3Sn(Boc–Arg)$ (DMSO-d_6)	**H-2**: 3.65 (dt, 1H); **H-5 + H-6**: 1.69–1.42 (m, 4H); **H-7**: 3.04 (t, J = 6.5, 2H); **Boc**[d]: 1.36(s, 9H); **NH**Arg + **NH_2**Arg: 7.65 (br, 3H); **H-α**: 1.10 (t, 4H); **H-β**: 1.52 (dd, 4H); **H-γ**: 1.26 (dd, J = 14.6 Hz, 7.3 Hz, 4H); **H-δ**: 0.96 (t, 9H)
	Boc–Arg (D_2O)	**H-2**: 3.88 (br, 1H); **H-5 + H-6**: 1.67–1.30 (m, br, 4H); **H-7**: 3.18 (t, 2H); **Boc**[d]: 1.40(s, 9H)
3	$Me_3Sn(Boc–Arg)$ (D_2O)	**H-2**: 3.90 (t, 1H); **H-5 + H-6**: 1.63 (m,br, 4H); **H-7**: 3.20 (t, 2H); **Boc**[d]: 1.42(s, 9H); **H-α**: 0.42 (s, 9H) [64.2 Hz][b], θ_1 = 115.0°
	Ala–Arg (CD_3OD)	**H-2**: 4.23 (t, 1H); **H-3**: 1.84 (dd, 2H), 1.72 (dd, 2H); **H-4**: 1.62 (q, 2H); **H-5**: 3.17 (t, 2H); **H-8**: 3.84 (q, J = 7.0 Hz, 1H); **H-9**: 1.44 (d, J = 7.0 Hz, 3H)
5	$Me_2Sn(Ala–Arg)$ (CD_3OD)	**H-2**: 4.21 (t, J = 5.5 Hz, 1H); **H-3**: 1.77 (dd, 2H), 1.64 (dd, 2H); **H-4**: 1.52 (q, 2H); **H-5**: 3.19 (dd, J = 15.5 Hz, 7.8 Hz, 2H); **H-8**: 3.57 (q, J = 7.0 Hz, 1H); **H-9**: 1.42 (d, J = 7.0 Hz, 3H); **H-α$_1$**: 0.81 [81.0 Hz][b] θ_1 = 132.2°; [75.0 Hz][b] θ_2 = 125.0°; **H-α$_2$**: 0.70 [84.0 Hz][b] θ_1 = 130.6°; [78.0 Hz][b] θ_2 = 128.5°
6	$Bu_2Sn(Ala–Arg)$ (CD_3OD)	**H-2**: 4.20 (t, J = 5.5, 1H); **H-3**: 1.67 (m, 4H); **H-4**: 1.59 (q, 2H); **H-5**: 3.22 (dd, 2H); **H-8**: 3.48 (q, J = 7.0, 1H); **H-9**: 1.43 (d, 3H); **H-α** = 1.17 (t, J = 7.0, 4H); **H-β** = 1.54 (m, 4H); **H-γ** = 1.38(m, 4H); **H-δ**: 0.93(dt, J = 12.2 Hz, 7.3 Hz, 6H)
5	$Me_2Sn(Ala–Arg)$ (DMSO-d_6)	**H-2**: 3.88 (t, J = 5.1 Hz, 1H); **H-3**: 1.76 (dd, 2H), 1.60 (dd, 2H); **H-4**: 1.45 (q, 2H); **H-5**: 3.00 (dd, J = 7.0 Hz, 2H); **H-8**: 3.37 (q, 1H); **H-9**: 1.28 (d, J = 7.0 Hz, 3H); (**NH_2**Ala + **NH_2** guanidyl + **N-*H*** guanidyl): 8.92 (br, 1H), 7.90 (br, 5H); **H-α$_1$**: 0.67 [80.8 Hz][b] θ_1 = 131.9°; [78.5 Hz][b] θ_2 = 128.9°; **H-α$_2$**: 0.55 [79.0 Hz][b] θ_1 = 129.6°
6	$Bu_2Sn(Ala–Arg)$ (DMSO-d_6)	**H-2**: 3.87 (t, J = 5.1 Hz, 1H); **H-3**: 1.77 (dd, 2H), 1.57 (dd, 2H); **H-4**: 1.45 (dd, J = 10.2 Hz, J = 7.6 Hz, 2H); **H-5**: 3.00 (dd, J = 13.9, 7.2, 2H); **H-8**: 3.39 (dd, J = 14.0, 7.0, 1H); **H-9**: 1.28 (d, 3H); **H-β** + **H-γ** = 1.40–1.18 (m, 8H); (**NH_2**Ala + **NH_2** guanidyl + **N-*H*** guanidyl): 8.86 (br, 1H), 7.82 (br, 5H); **H-α** = 1.09 (t, J = 7.0, 4H); **H-δ**: 0.88 (dt, J = 11.4 Hz, 7.2 Hz, 6H)

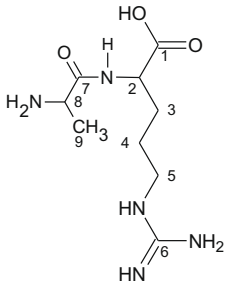
Abbreviations: s, singlet; d, doublet; dd, double doublet; t, triplet; dt, double triplet; m, multiplet; q, quartet; sbr, singlet broad.



L-Arg



Boc-Arg



Ala-Arg

[a] In ppm from TMS.
[b] $^2J(^{119}Sn,^1H)$ and $^2J(^{117}Sn,^1H)$ = coupling constants and θ_n = C–Sn–C bond angle [31].
[c] Overlapping of $1CH_2$ (Boc–Arg) + 2 CH (Boc–Arg) [11].
[d] Boc = *tert*-Butoxycarbonyl.

previously found by Nath et al. [29] for organotin(IV)–amino acid complexes. L-Arginine conformation has been reported as a partly folded extended state of the zwitterionic form of the basic amino acid with the positive charge distributed equally over three side chain nitrogen atoms in its planar guanidinium group in aqueous solution [30], hence both resonances for β-CH_2 and γ-CH_2 are present at 1.89 ppm and 1.66 ppm, respectively. While sharpness of resonances rules out ligand exchange for the complex in solution, the appearance of closely spaced resonances for both CH_2 groups, can be due to a modified conformation of the coordinate ligand where the guanidyl group is present, relative to the free aminoacid, leading to the equivalence of β-CH_2 and γ-CH_2.

As for the coordination of the organometallic moiety, a single resonance is present at 0.66 ppm. Lockhart and Manders' relationship [31] between C–Sn–C bond angles and ^2J can be applied. $^2J(^{119}Sn, ^1H)$ being 82.5 Hz, a C–Sn–C bond angle of 134.1° was calculated. The dramatic difference between C–Sn–C bond angle in the solid state and solution phase, is accounted by the larger mobility of complex molecule in aqueous solution relative to the solid state.

In $Me_2Sn(Arg)_2$ complex, ^{13}C NMR in D_2O, resonances due to the carboxylate groups are shifted to higher frequencies relative to the free ligand, while guanidyl C=NH resonance along with resonances of the alkyl groups appear to be virtually unshifted with the notable exception of α-CH which are shifted to higher field by ~2 ppm and β-CH_2 by 1 ppm in the opposite direction. These data agree with coordination by carboxylate and amino groups to the organometallic moiety as in the solid state.

4.3. $R_2Sn(IV)^{2+}$ and $R_3Sn(IV)^{2+}$ with N_α-(tert-butoxycarbonyl)-L-Arginine

Coordination of di- and triorganotin(IV), with N_α-*t*-Boc–Arginine (Boc–Arg–OH) has been investigated to monitor the effect of reduced basicity of the α-amino group and potential coordinating ability of the guanidyl group. In Tables 1 and 2, ^1H and ^{13}C NMR relevant resonances for the complexes are presented along with those of the free ligand for comparison. NMR resonances of $^nBu_2Sn(Boc–Arg)_2$ in CD_3OD show only minor shifts relative to the free ligand, which is consistent with the coordination to the organometallic moiety only through the carboxylate group. ^{119}Sn NMR spectrum, in CD_3OD, was also acquired. The most remarkable aspect of this spectrum at room temperature is the appearance of two resonance signals at −168 and −170 ppm, which gives a clear indication of pentacoordinated moieties. The significant variance with the solid state (Mössbauer) structural information (i.e. tetrahedral geometry), along with the lack of ^2J derived C–Sn–C angle from the proton NMR spectrum, stresses the relevance of the ^{119}Sn NMR information, clearly indicating an equilibrium between tetrahedral and pentacoordinated carboxylate bonded specimen: monodentate ligand ⇄ chelate ligand. $^nBu_3Sn(Boc–Arg)$ in CD_3OD shows the equivalence of β-CH_2 and γ-CH_2 in a multiplet centered at 1.58 ppm, with a remarkable upfield shift following coordination. The $^1H–^1H$ COSY spectrum of $^nBu_3Sn(Boc–Arg)$ is reported in Fig. 4. $Me_3Sn(Boc–Arg)$ in D_2O showing a close similarity of resonances with free ligand, suggests the bonding only of the carboxylate group to the organometallic moiety, and ^{13}C NMR shows only minor variations. The close similarity of resonances for free ligand Boc–Arg–OH, $^nBu_2Sn(Boc–Arg)_2$, $Me_3Sn(Boc–Arg)$ and $^nBu_3Sn(Boc–Arg)$ is possibly due to the extensive hydrogen bonding which is expected to be present in all specimen.

4.4. $R_2Sn(IV)^{2+}$ complexes with L-Alanyl-L-Arginine

The complexes under investigation are obtained by means of a neutralization reaction leading to a variation of charge distribution in arginine as evidenced in DMSO-d_6 solution by ^1H NMR spectra. C=NH, (NH_2Ala + NH_2 guanidyl + N–H guanidyl) resonances are present at 8.92 ppm, 1H, and 7.90 ppm, 5H, for $Me_2Sn(Ala–Arg)$; at 8.86 and 7.82 ppm for $^nBu_2Sn(Ala–Arg)$, indicating a novel conformation of the dipeptide, presumably adopting an extended conformation as suggested by Xian et al. [30]. The $^1H–^1H$ COSY spectra of the $Me_2Sn(Ala–Arg)$ (***5***) and $^nBu_2Sn(Ala–Arg)$ (***6***) are reported in Figs. 5 and 6, respectively. As expected, the resonance due to peptidic N–H is absent, since in the complex coordination takes place with ionization of peptidic group. The solution behaviour of the

Table 2
^{13}C chemical shifts[a] (δ) for ligands and di- and triorganotin(IV) complexes.

	Compound (solvent)	δ (ppm)
	L-Arg (D_2O)	**C-1**: 177.2; **C-2**: 57.1; **C-3**: 30.3; **C-4**: 25.1; **C-5**: 41.6
1	Me_2Sn(L-Arg)$_2$ (D_2O)	**C-1**: 181.9; **C-2**: 55.8; **C-3**: 30.9; **C-4**: 24.7; **C-5**: 41.3; **C-**α: 2.3
	Boc–Arg[b] (CD_3OD)	**C-1**: 179.7; **C-2**: 56.7; **C-3**: 157.9; **C_{quat}-4**: 80.2; **C-5**: 31.5; **C-6**: 26.5; **C-7**: 42.2; **C-8**: 158.9; **C-Boc**: 29.0
2	$Bu_2Sn(Boc–Arg)_2$ (CD_3OD)	**C-1**: 179.4; **C-2**: 56.6; **C-3**: 157.8; **C_{quat}-4**: 80.3; **C-5**: 31.8; **C-6**: 26.3; **C-7**: 42.3; **C-8**: 158.8; **C-Boc**: 28.9; **C-**α: 23.4; **C-**β: 29.1; **C-**γ: 28.5; **C-**δ: 14.2
3	$Bu_3Sn(Boc–Arg)$ (CD_3OD)	**C-1**: 179.3; **C-2**: 56.4; **C-3**: 157.8; **C_{quat}-4**: 80.3; **C-5**: 31.6; **C-6**: 26.4; **C-7**: 42.2; **C-8**: 158.8; **C-Boc**: 29.4; **C-**α: 16.9; **C-**β: 28.4; **C-**γ: 26.4; **C-**δ: 14.2
2	$Bu_2Sn(Boc–Arg)_2$ (DMSO-d_6)	**C-1**: 175.7; **C-2**: 54.6; **C-3**: 157.3; **C_{quat}-4**: 77.4; **C-5**: 30.0; **C-6**: 25.2; **C-7**: 34.7; **C-8**: 154.8; **C-Boc**: 28.2; **C-**α: 18.6; **C-**β: 30.1; **C-**γ: 25.2; **C-**δ: 13.9
3	$Bu_3Sn(Boc–Arg)$ (DMSO-d_6)	**C-1**: 175.8; **C-2**: 54.5; **C-3**: 157.3; **C_{quat}-4**: 77.4; **C-5**: 30.1; **C-6**: 25.2; **C-7**: 41.4; **C-8**: 154.9; **C-Boc**: 28.3; **C-**α: 16.9; **C-**β: 27.5; **C-**γ: 26.7; **C-**δ: 13.7
	Boc–Arg (D_2O)	**C-1**: 179.3; **C-2**: 55.6; **C-3**: 156.8; **C_{quat}-4**: 81.0; **C-5**: 28.9; **C-6**: 24.5; **C-7**: 40.7; **C-8**: 157.6; **C-Boc**: 27.6
4	$Me_3Sn(Boc–Arg)$ (D_2O)	**C-1**: 179.9; **C-2**: 56.0; **C-3**: 157.3; C_{quat}-4: 81.2; **C-5**: 29.3; **C-6**: 24.8; **C-7**: 41.3; **C-8**: 158.0; **C-Boc**: 28.1; **C-**α: −2.21
	Ala–Arg (CD_3OD)	**C-1**: 178.3; **C-2**: 55.8; **C-3**: 31.1; **C-4**: 26.4; **C-5**: 42.2; **C-6**: 158.9; **C-7**: 172.5; **C-8**: 50.8; **C-9**: 18.6
5	$Me_2Sn(Ala–Arg)$ (CD_3OD)	**C-1**: 180.0; **C-2**: 56.4; **C-3**: 31.7; **C-4**: 25.9; **C-5**: 42.3; **C-6**: 158.8; **C-7**: 177.0; **C-8**: 52.1; **C-9**: 19.9; **C-**α: 0.6, −0.8
6	$Bu_2Sn(Ala–Arg)$ (CD_3OD)	**C-1**: 180.2; **C-2**: 56.9; **C-3**: 31.8; **C-4**: 26.0; **C-5**: 42.2; **C-6**: 158.9; **C-7**: 177.3; **C-8**: 52.4; **C-9**: 21.3; **C-**α: 20.5, 20.1; **C-**β: 28.6, 28.4; **C-**γ: 27.9, 27.8; **C-**δ: 14.1
	Ala–Arg (DMSO-d_6)	**C-1**: 175.3; **C-2**: 53.2; **C-3**: 29.9; **C-4**: 25.2; **C-5**: 40.8; **C-6**: 157.4; **C-7**: 172.5; **C-8**: 50.1; **C-9**: 21.4
5	$Me_2Sn(Ala–Arg)$ (DMSO-d_6)	**C-1**: 175.1; **C-2**: 54.4; **C-3**: 29.8; **C-4**: 24.0; **C-5**: 40.6; **C-6**: 157.3; **C-7**: 173.4; **C-8**: 50.1; **C-9**: 19.1; **C-**α: 0.4, −0.4
6	$Bu_2Sn(Ala–Arg)$ (DMSO-d_6)	**C-1**: 175.7; **C-2**: 54.6; **C-3**: 30.1; **C-4**: 25.0; **C-5**: 40.5; **C-6**: 157.4; **C-7**: 173.6; **C-8**: 50.4; **C-9**: 18.4; **C-**α: 19.4, 19.3; **C-**β: 26.8, 26.7; **C-**γ: 26.1, 26.0; **C-**δ: 13.5

$Sn-\overset{\alpha}{C}H_3$; $Sn-\overset{\alpha}{C}H_2-\overset{\beta}{C}H_2-\overset{\gamma}{C}H_2-\overset{\delta}{C}H_3$

[a] In ppm from TMS.

[b] Boc = *tert*-Butoxycarbonyl.

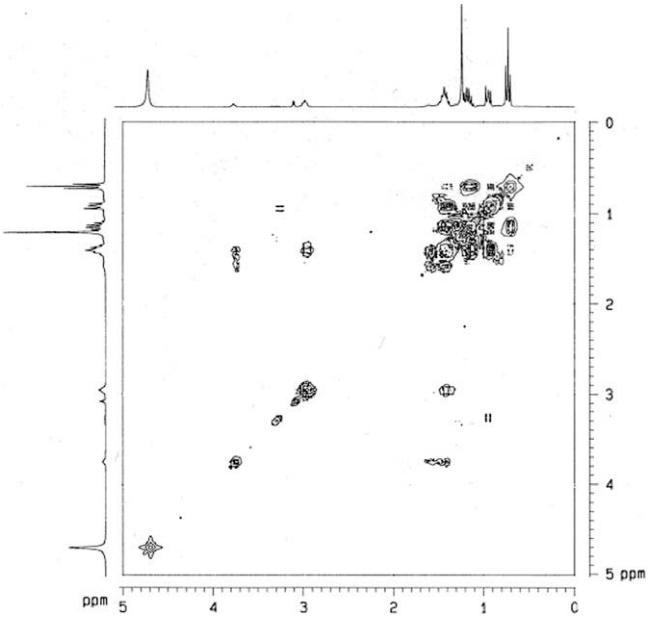


Fig. 4. ^{1}H–^{1}H COSY spectrum for $^{n}Bu_3Sn$(Boc–Arg).

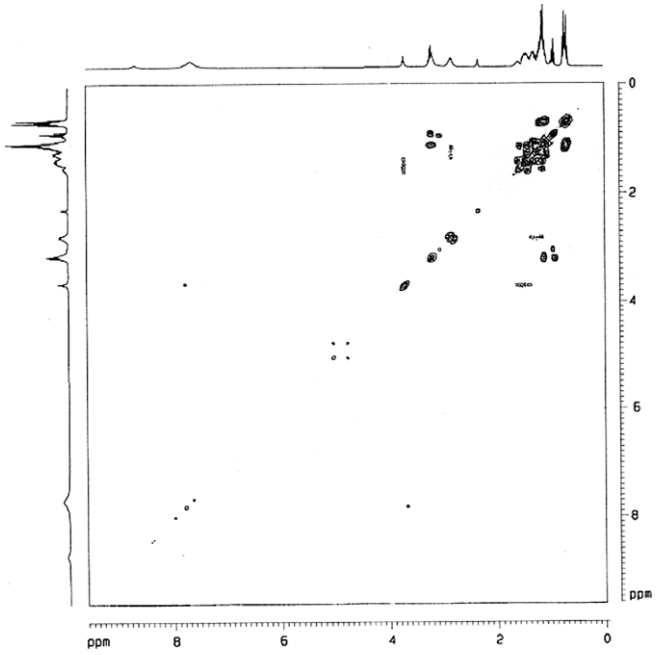


Fig. 6. ^{1}H–^{1}H COSY spectrum for $^{n}Bu_2Sn$(Ala–Arg).

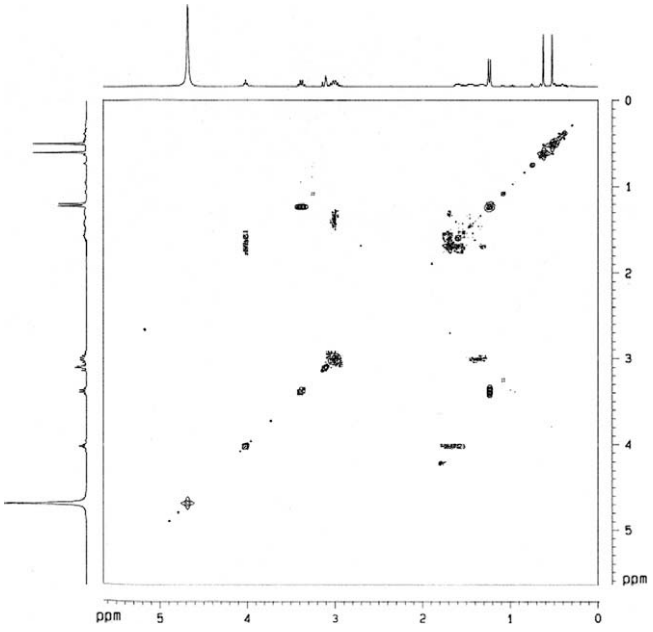


Fig. 5. ^{1}H–^{1}H COSY spectrum for Me_2Sn(Ala–Arg).

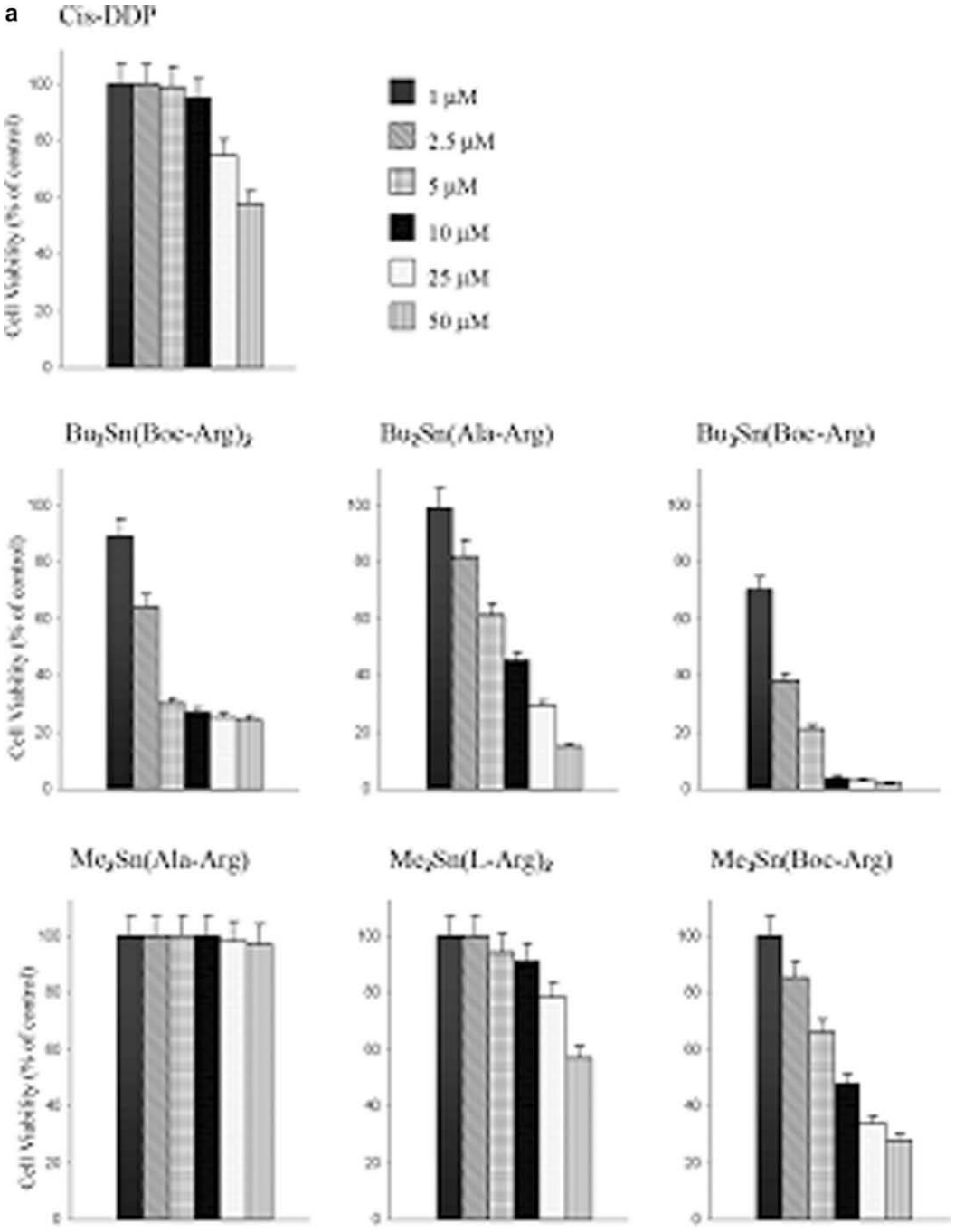
complexes (stability and/or dissociation in CD_3OD and DMSO-d_6 solvents) has been monitored and structural details, namely C–Sn–C angles, obtained by evaluating $^{2}J(^{119}Sn, ^{1}H)$ by use of Lockhart and Manders' relationship [31]. The C–Sn–C angle has been calculated for Me_2Sn(Ala–Arg) (Table 1) both in DMSO-d_6 and CD_3OD solvents and closely agrees with that found for the complex in the solid state by use of the literal point-charge model, which closely supports the idea that a trigonal–bipyramidal structure is present both in the solid phase and in solution. Me_2Sn(Ala–Arg) C–Sn–C bond angle closely agree with those reported for dimethyltin-dipeptides obtained by us for Met–His [27], Trp–Ala, Trp–Trp and His–Tyr complexes [32]. As for Met–His derivatives, the Ala–Arg complexes reported in this work are examples of amphiphilic peptide complexes. As bioactivity of proteins is depending on conformation, along with construction of "peptide-amphiphiles" creating novel biomaterials with distint protein-like structures [33,34], it is tempting to suggest that this Ala–Arg complexes may represent new leads for a class of amphiphilic organometallic complexes.

Higher frequencies shifts of significant value, are only observed for α-H(ala) resonance of Me_2Sn- and $^{n}Bu_2Sn$-complexes in both solvents, which gives an indication of involvement of alanine's amino group in coordination to the organometallic moieties. For the $^{n}Bu_2Sn$(IV)-derivatives, diagnostic values of $^{n}J(^{119}Sn, ^{1}H)$ coupling constants could not with confidence be evaluated which prevented the acquisition of C–Sn–C bond angles.

Relative to the free dipeptide, the shifts of ^{13}C NMR resonances due to metal coordination have been monitored. A general pattern emerges in the complexes, namely a shift to lower field for arginine (COO^{-}) group, alanine carbonyl peptidic (CO) group in CD_3OD; with the notable exception of the remaining ^{13}C NMR resonances which are virtually unshifted also in DMSO-d_6, possibly due to extensive hydrogen bonds in both free ligand and complexes. It is noteworthy that in both solvents guanidyl C=NH resonance being invariant, no evidence is given for involvement of this group in bonding to the organometallic moiety. ^{13}C and ^{1}H NMR spectra of Me_2Sn(Ala–Arg) and $^{n}Bu_2Sn$(Ala–Arg) in both solvents, present a pattern which is peculiar for the several organotin(IV) dipeptide complexes we previously investigated [27,32], namely, the chirality of the complexes is responsible for the appearance of two magnetically non-equivalent R_2(Sn)-resonances.

Considering the conformation of the complexes both in the solid state and in solution, as previously observed for arginine, whereas in the free ligand the peptide backbone is predominantly present in a flexible extended form, in the complexes chelation by amino, peptide nitrogens and carboxylate groups provides a stiff backbone; therefore it is attached side-chain interactions which play a crucial role in determining which conformation is preferred.

The ^{119}Sn resonance in the ^{119}Sn NMR spectra of Me_2Sn(Ala–Arg), recorded in CD_3OD, exhibit a single chemical shift at –120 ppm which is characteristic of pentacoordinated organotin(IV) complexes with dipeptides [17]. Unfortunately, the scarce



b

IC_{50} (μM)

Cis-DDP	58
$Bu_2Sn(Boc\text{-}Arg)_2$	3.5
$Bu_2Sn(Ala\text{-}Arg)$	18.5
$Bu_2Sn(Boc\text{-}Arg)$	2
$Me_2Sn(L\text{-}Arg)_2$	56
$Me_2Sn(Boc\text{-}Arg)$	9.5

Fig. 7. (a) Effects of organotin(IV)complexes or cisplatin on HT29 cell viability. Cells were treated for 24 h with the compounds, at the indicated concentrations. Cell viability and IC_{50} value were estimated by MTT assay, as reported in Section 2.1. Results are expressed as the percentage of viable cells with respect to untreated controls. Data represent the average ±SD of five independent experiments performed in triplicate. (b) IC_{50} values relative to the indicated compounds.

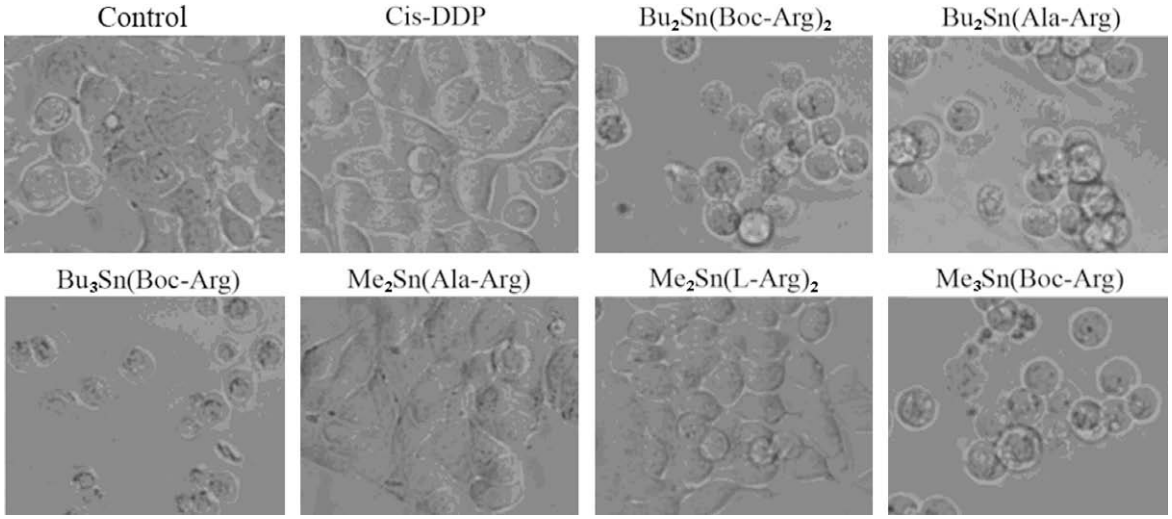


Fig. 8. Morphological changes induced in HT29 cells by organotin(IV)-complexes or cisplatin treatment. Cells were treated for 24 h with 10 M of drugs and observed by light microscopy. Data are representative of three independent experiments.

solubility of the remaining complexes prevented the acquisition of suitable ^{119}Sn NMR spectra.

5. Biological studies

5.1. The effects of the organotin(IV)-complexes on HT29 cells viability

Scattered information is available on organotin(IV)-complexes antitumor activity; a series of related derivatives of aminoacids and peptides have been found to exhibit promising antiproliferative activity *in vitro* and *in vivo* [17,35]. All dimethyltin(IV) compounds exhibit lower *in vitro* cytotoxic activity than di- and tributyltin(IV) derivatives. This pattern is reproduced in the compounds presented in this work. To examine the effects of organotin(IV)-complexes, in comparison with that exerted by cisplatin, on human HT29 colorectal carcinoma cells, monolayer cultures were treated for 24 h with various concentrations (1–50 μM) of the drugs and cell viability was evaluated by MTT assay (Fig. 7a) to measure mitochondrial enzyme activity, as reported in Material and Methods. All the compounds, except Me_2Sn(Ala–Arg) which results completely inefficacious, clearly reduced cell viability in a dose-dependent manner. Moreover, we observed that the cytotoxicity trend was in the order $^{n}Bu_3Sn$(Boc–Arg) > $^{n}Bu_2Sn$(Boc–Arg)$_2$ > $^{n}Bu_2Sn$(Ala–Arg) > Me_3Sn(Boc–Arg), apparently butyltin(IV)-derivatives have displayed higher activity following a general behavior among organotins. For all these complexes, cytotoxic activity was higher than that exerted by cisplatin, while that of Me_2Sn(L-Arg)$_2$ was similar. For these compounds the maximum reduction in cell viability (60–85%) was reached at the concentration of 50 μM. The comparison of IC_{50} values evidenced that Boc–Arg complexes, and in particular the butyltin(IV) derivatives, were the most efficacious (Fig. 7b). Experiments performed to assay the possible cytotoxicity of free ligands employed to synthesize the organotin(IV)-complexes demonstrated that these ligands were not able to exert any effect on HT29 cell viability (not shown). Light microscopy analysis, performed to study the morphological changes induced in HT29 cells by 24 h of treatment with 10 μM of organotin(IV)-complexes or cisplatin, confirmed the results obtained with MTT test. In fact, as showed in Fig. 8, no evident morphological alterations were observed in HT29 cells after treatment with cisplatin, Me_2Sn(L–Arg)$_2$ and Me_2Sn(Ala–Arg) whereas, when treated with $^{n}Bu_2Sn$(Boc–Arg)$_2$, $^{n}Bu_2Sn$(Ala–Arg), $^{n}Bu_3Sn$(Boc–Arg) and Me_3Sn(Boc–Arg), cells appeared rounded, isolated and detached from culture substrate, indicating the commitment to apoptotic cell death.

In conclusion, a tentative qualitative structure–activity relationship indicates that butyltin(IV)-derivatives are more active than dimethyltin(IV), and surprisingly Boc–Arg–OH derivatives being more active than L-Ala-L-Arg in Bu_2Sn-complexes.

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