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Synthesis and spectroscopic characterization of new triorganotin(IV) complexes with the bis(1-methyl-1*H*-imidazol-2-ylthio)acetate ligand: effects on trout erythrocyte components

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Triorganotin(IV) derivatives containing the anionic ligand bis(1-methyl-1*H*-imidazol-2-ylthio)acetate $[(S\text{-}tim)_2CHCO_2]^-$ were synthesized from the reaction between R_3SnCl acceptors (R = Me and Ph) and the sodium salt of the ligand. Mono-nuclear complexes of the type $[(S\text{-}tim)_2CHCO_2]SnR_3$ were obtained, which were fully characterized by elemental analyses and FT-IR in the solid state, and by NMR (^1H, ^{13}C and ^{119}Sn) spectroscopy and electrospray ionization mass in solution. The toxic effects shown by these compounds on trout erythrocyte components showed that the toxicity of the organotin(IV) complexes depends on the nature and on the lipophilicity of the substituents on the metal centre. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: organotin(IV) compounds; scorpionate ligands; methimazole; tin-119 NMR; electrospray ionization mass spectroscopy; trout erythrocyte; Hb; hemolysis; DNA damage; comet assay

Introduction

Organotin compounds are of interest in view of the considerable structural diversity they possess; this aspect has attracted the attention of a number of researchers and in recent years a multitude of structural types have been discovered.[1–3] In addition many organotin compounds have been tested for their *in vitro* activity against a large variety of tumor lines and have been found to be as effective or better than traditional heavy metal anticancer drugs such as *cis*-platin.[4,5] In addition, organotin compounds have found many applications in industry (as wood preservatives, marine antifouling paints and stabilizers for PVC) and in agriculture (as fungicides and biocides)[6] with the consequence that considerable amounts of the organotins have entered various ecosystems.[7] Many studies[8,9] have reported the toxic effects of organotin compounds as contaminants of marine and freswater ecosystems, and it has been demonstrated that, depending on the nature and the number of the organic groups bound to the tin cation, some organotins show specific toxic effects to different organisms even at very low concentrations.[10]

Recently, we have reported the synthesis and the spectroscopic characterization of new poly(pyrazolyl)borate[11,12] and poly(imidazolyl)borate[13,14] complexes containing organotin(IV) acceptors. We have attempted to develop the chemistry of organotin compounds bearing co-ligands of ambidentate character. The primary impetus has been to comprehend competitive coordination modes of poly(azolyl)borate ligands to the tin atom and to find a rationale related to the stability and structural motifs of this class of compounds.[15]

As an extension of this research field, we have developed the chemistry of some new organotin carboxylates obtained by the interaction of a number of organotin(IV) halides with new polyfunctional *S*,*N*,*O*-ligands, containing two pyridine groups and other biologically relevant hydrophilic moieties, such as carboxylate groups.[16]

In recent years a number of authors[17–26] have synthesized *S*,*N*-ligands of the type $(CH_2)_n(SAz)_2$, based on a nitrogenated aromatic ring system such as benzimidazole or pyridine. These ligands are able to coordinate by both S and the neighbouring N atom, and hence to form stable chelate rings of five or more atoms.[27–34]

Bearing in mind the above, we have developed a strategy for producing a new class of monoanionic and polyfunctional *N*,*O*,*S*-ligands of considerable coordinative flexibility. Towards this end, we report here the synthesis and characterization of some new complexes obtained from the interaction of a number of

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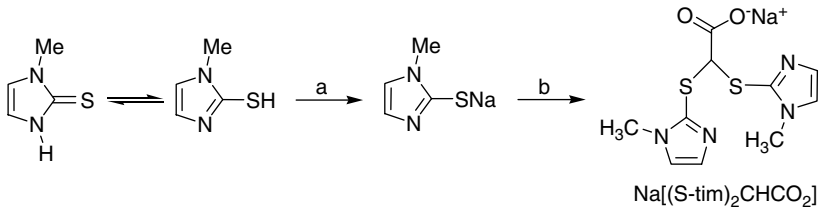


Figure 1. Synthesis of the sodium bis(1-methyl-1*H*-imidazol-2-ylthio)acetate ligand, Na[(S-tim)$_2$CHCO$_2$], starting from 2-mercapto-1-methylimidazole. Conditions: (a) NaOH (1 equiv.) in ethanol solution, r.t., 12 h; (b) dibromoacetic acid (0.5 equiv.), NaOH (1 equiv.), reflux, 6 h.

triorganotin(IV) halides with the sodium bis(1-methyl-1*H*-imidazol-2-ylthio)acetate ligand, Na[(S-tim)$_2$CHCO$_2$] (Fig. 1).

With the aim of explaining the role of anionic substituents on tin(IV) in the reactivity of some organotin compounds vs different cellular components, here we report the results obtained using trout erythrocytes as a biological system. In particular, the effects of the organotin(IV) compounds, {[(S-tim)$_2$CHCO$_2$]Sn(CH$_3$)$_3$} and {[(S-tim)$_2$CHCO$_2$]Sn(C$_6$H$_5$)$_3$}, were studied by following the hemolytic process, evaluating the stability of trout hemoglobins and investigating the nuclear DNA status.

Results and Discussion

Synthesis and characterization of the triorganotin(IV) compounds

Complexes **1** and **2** were synthesized by metathetic reaction of Na[(S-tim)$_2$CHCO$_2$] with (CH$_3$)$_3$SnCl or (C$_6$H$_5$)$_3$SnCl in chloroform solution at room temperature [equation (1)].

$$\mathrm{Na[(S\text{-}tim)_2CHCO_2] + R_3SnCl} \xrightarrow[\mathrm{r.t.}]{\mathrm{CHCl_3}} \mathrm{\{[(S\text{-}tim)_2CHCO_2]SnR_3\} + NaCl} \quad (1)$$

$$\mathbf{1}: \mathrm{R = CH_3}$$

$$\mathbf{2}: \mathrm{R = C_6H_5}$$

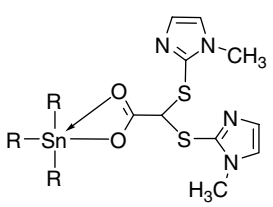
The derivatives {[(S-tim)$_2$CHCO$_2$]Sn(CH$_3$)$_3$}, **1**, and {[(S-tim)$_2$CHCO$_2$]Sn(C$_6$H$_5$)$_3$}, **2**, are reasonably stable and they show a good solubility in methanol, acetone, acetonitrile and chlorinated solvents, and they are insoluble in water and *n*-hexane.

Complexes **1** and **2** were characterized by analytical and spectral data. The infrared spectra carried out on the solid samples (nujol mull) showed all the expected bands for the ligands and the tin moieties: weak absorptions in the range 3107–3116 cm^{-1} are due to the azolyl ring C–H stretchings and medium to strong absorptions near 1510 cm^{-1} are related to ring 'breathing' vibrations. The presence of the COO moiety in derivatives **1** and **2** is detected by intense broad absorptions at 1639–1656 cm^{-1} and 1308–1325 cm^{-1}, due to the asymmetric and symmetric stretching modes, respectively; the shift to blue with respect to the sodium salt of the ligand ($\nu_{asym}CO_2$ = 1615 cm^{-1}) was observed upon complex formation. The magnitude of $\nu_{asym}CO_2 - \nu_{sym}CO_2$ ($\Delta\nu$) separation can be used to explain the type of carboxylate structure present in the solid state.[35,36] $\Delta\nu$ values for **1** and **2** are greater than 300 cm^{-1}, which is characteristic of bidentate coordination compounds.

In the far-IR region, medium to strong absorptions appeared upon coordination, due to stretching modes of Sn–O and Sn–C.[37] The absence of Sn–Cl stretching vibrations in the spectra of **1** and **2** confirms the substitution of the chloride in the formation of the complexes. In the far-IR spectra absorptions tentatively assigned to Sn–O were detected in the range 322–468 cm^{-1}. The Sn–C stretching frequencies were medium or strong absorptions in the range 507–550 cm^{-1} for the alkyl derivative **1** and in the range 245–278 cm^{-1} for the aryl derivative **2**; these absorptions agree well with the trends previously observed in similar organotin(IV) complexes of polyfunctional S,N,O-donor ligands.[38]

In the ^{1}H NMR spectra of complexes **1** and **2** in CDCl$_3$ solution (see Experimental section), the signals due to the 2-mercapto-1-methylimidazolyl rings were always deshielded with respect to those in the spectra of the free donor, confirming the existence of the complexes in solution; the signals due to the *CH*COO group exhibited significant downfield shift (from 5.05 ppm in the free ligand to 5.17–5.32 ppm in the complexes): this is suggestive of a strong bonding of the tin atom to the carboxylate group of the complexes. The room-temperature ^{1}H NMR spectra of derivatives **1** and **2** exhibited only one set of signals for the protons of the imidazolyl rings of the ligands. The tin–hydrogen, $^2J(^{119}\mathrm{Sn},^1\mathrm{H})$, and tin–carbon, $^1J(^{119}\mathrm{Sn},^{13}\mathrm{C})$, coupling constants in various cases could be correlated with the percentage of *s*-character, which the Sn atom present in the Sn–C bond and hence $^2J(^{119}\mathrm{Sn},^1\mathrm{H})$ and $^1J(^{119}\mathrm{Sn},^{13}\mathrm{C})$ may give information about the coordination number of tin.[39,40] In the trimethyltin(IV) derivative **1** the tin–proton coupling constant $^2J(\mathrm{Sn},^1\mathrm{H})$ was 61.1 Hz, falling in the range of penta-coordinated trimethyltin(IV) species. The tin–carbon coupling constants of compound **1**, $^1J(^{117}\mathrm{Sn},^{13}\mathrm{C})$ and $^1J(^{119}\mathrm{Sn},^{13}\mathrm{C})$, were 433 and 473 Hz, respectively; on the basis of Lockarts's equation[39,40] the Me–Sn–Me angle was estimated to be about 118°, suggesting in solution a distorted pentacoordinate environment of the tin atom (Fig. 2). The ^{119}Sn chemical shifts of the triorganotin(IV) derivatives **1** and **2**, at 34.52 and −95.83 ppm, respectively, were in accordance with those of penta-coordinate triorganotin(IV) complexes involving *S*-, *O*- or *N*-donors.[38,41,42]

Electrospray ionization is considered a 'soft' ionization technique. Consequently, few ions are produced, usually the molecular ion plus some adduct ions from the mobile phase solutions.[43,44]



1. R = CH$_3$
2. R = C$_6$H$_5$

Figure 2. Proposed structure of derivatives **1** and **2**.

ESI-MS is particularly suitable for study of labile organotin systems in solution. In the discussion of the mass spectra of the triorganotin(IV) derivatives, only the most abundant ion of the isotope cluster will be mentioned.

An interesting fragmentation pattern was detected in the positive- and negative-ion spectra of derivatives **1** and **2**, dissolved in methanol solution and detected at a fragmentation voltage of 30 V. For derivative **1** significant fragments at *m/z* 448 (70%) and *m/z* 470 (80%) in the positive-ion spectra were attributable to the species $[\{[(S\text{-tim})_2CHCO_2]Sn(CH_3)_3 + H\}]^+$ and $[\{[(S\text{-tim})_2CHCO_2]Sn(CH_3)_3 + Na\}]^+$; peaks due to clusters containing two triorganotin fragments associated with one or two molecules of the ligand were detected at *m/z* 611 (40%) and *m/z* 917 (100%). The instability of the trimethyltin(IV) derivative in methanol solution was demonstrated by the presence in the negative-ion spectrum of a fragment at *m/z* 283 (20%) due to the free ligand $[(S\text{-tim})_2CHCO_2]^-$ and of a major peak at *m/z* 239 (100%) due to the decarboxylate species $[\{[(S\text{-tim})_2CHCO_2] - CO_2\}]^-$. For derivative **2** the main peak in the positive-ion spectrum at *m/z* 634 (100%) was attributed to the complex $[\{[(S\text{-tim})_2CHCO_2]Sn(C_6H_5)_3\} + H]^+$. The instability of the triphenyltin(IV) derivative **2** in methanol solution was demonstrated by the presence in the positive-ion spectrum of a fragment at *m/z* 241 (80%) due to the decarboxylate ligand $[\{[(S\text{-tim})_2CHCO_2] - CO_2 + 2H\}]^+$. Analogously, in the negative-ion spectrum a fragment at *m/z* 421 (100%) was attributed to the free triorganotin(IV) acceptor $[(C_6H_5)_3SNCL + Cl]^-$.

Effect of triorganotin(IV) compounds on different trout erythrocyte components

It is known that, different from mammals and birds, multiple hemoglobin components are present in fish erythrocytes. This multiplicity may be related to the fact that hemoglobins have to provide oxygen for different purposes, namely metabolic demands and the operation of the swim bladder. In the case of erythrocytes from *Salmo irideus* trout, four different hemoglobin components are present and are named according to their anionic mobility, HbI, HbII, HbIII and HbIV. Two of these hemoglobin components, HbI and HbIV, have been widely studied.[45] They have very different oxygen-binding properties: HbI is characterized by the presence of cooperative phenomena and complete absence of the pH and organic phosphate effect, while in HbIV oxygen affinity and cooperativity depend on pH and organic phosphates (Root effect). A peculiar characteristic of fish hemoglobins is represented by their autoxidation rate; they are less stable with respect to human hemoglobin either as purified proteins or in the whole cell,[46] and then it is possibile to follow the process at relatively short time periods.

Figure 3 shows the time course of HbI autoxidation when the protein at a concentration of 0.8 mg/ml was incubated at 30 °C and pH 7.7. The presence of $\{[(S\text{-tim})_2CHCO_2]Sn(CH_3)_3\}$, **1**, and $\{[(S\text{-tim})_2CHCO_2]Sn(C_6H_5)_3\}$, **2**, at a concentration 100 μM reduced the rate of oxidation of HbI by stabilizing the ferrous state (Fe^{2+}) of the protein. A similar protective effect was observed when the experiment was carried in the presence of triphenyltin chloride, indicating that the effect was not influenced by the anion type. Also $(CH_3)_3SnCl$ was able to reduce the autoxidation rate more or less to the same extent (data not shown). It was not possibile to perform similar experiments with HbIV (the trout Hb component that is characterized by the presence of the Root effect) because the presence of some of these organotin

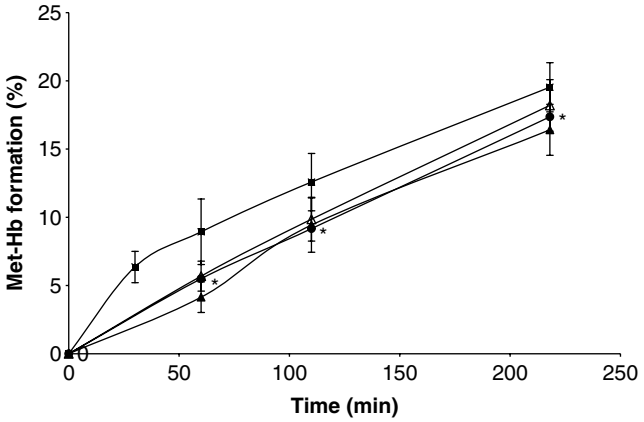


Figure 3. Formation of Met-Hb obtained incubating HbI (0.8 mg/ml) in potassium-phosphate buffer 0.1 M at pH 7.7 and $t = 30$ °C in presence of 100 μM organotin(IV) complexes (■, control; ▲, Ph_3SnCl; △, Ph_3SnL, **2**; ●, Me_3SnL, **1**; L = $[(S\text{-tim})_2CHCO_2]^-$). *$p < 0.05$ with respect to control.

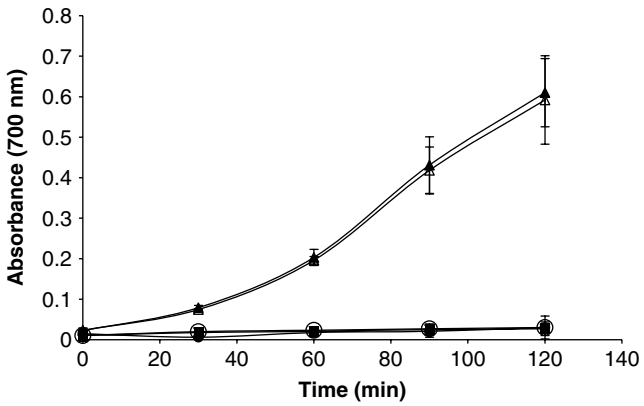


Figure 4. Effect of Sn-complexes (50 μM) on HbIV (1 mg/ml) incubated in potassium-phosphate buffer 0.1 M at pH 7.7 and $t = 30$ °C (■, control; ▲, Ph_3SnCl; △, Ph_3SnL, **2**; ○, Me_3SnCl; ●, Me_3SnL, **1**; L = $[(S\text{-tim})_2CHCO_2]^-$).

compounds (Ph_3SnCl, Ph_3SnL, L = $[(S\text{-tim})_2CHCO_2]^-$) in this case enhances protein denaturation. The influence that 50 μM of these organotin compounds has on the time of onset of HbIV precipitation used at the concentration of 1 mg/ml is shown in Fig. 4. Ph_3SnCl and Ph_3SnL, **2**, accelerate the precipitation process in HbIV and it begins immediately after adding the two triphenyl derivatives, while Me_3SnL and Me_3SnCl, at least during the time of our experiment, do not modify the rate of precipitation. This result obtained on HbIV supports the hypothesis that the toxicity of organotin compounds probably depends on the nature and the lipophilicity of the organic substituents (phenyl or methyl groups) on tin(IV), but the nature of the anion (Cl or L) seems to have no significant effect on the activity of the compounds, probably due to the dissociation of the complexes in hydrolytic conditions.

By varying properly pH and temperature, it is possible using trout erythrocytes suspended in isotonic medium to follow *in vitro* the hemolytic event over a relatively short time[47] and to investigate the effect of organotins on this process. The influence of a fixed amount (30 μM) of these organotins on the rate of hemolysis in trout erythrocytes during incubation in isotonic buffer at 35 °C and pH 6.3 was also investigated. Figure 5 shows the time course of the hemolysis. The susceptibility to hemolysis increased in the presence of the organotin complexes even if this increase was

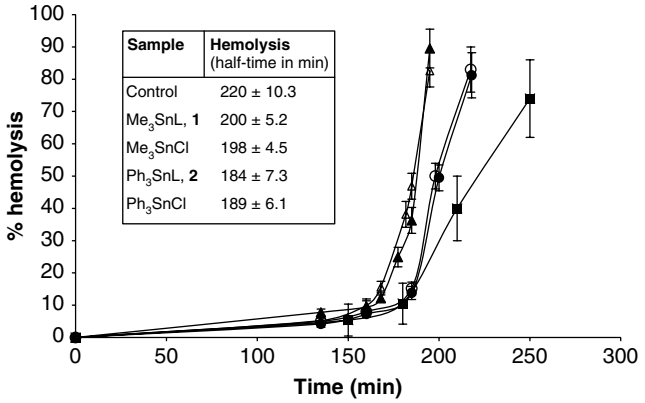


Figure 5. Time course of hemolysis of trout erythrocyte suspensions (Hb = 30 mg/ml) incubated in isotonic buffer pH 6.3 and $t = 35\,^{\circ}C$ in the presence of 30 μM organotin(IV) complexes (■, control; ▲, Ph_3SnCl; △, Ph_3SnL, **2**; ○, Me_3SnCl; ●, Me_3SnL, **1**; L = $[(S\text{-tim})_2CHCO_2]^-$).

minor with $\{[(S\text{-tim})_2CHCO_2]Sn(CH_3)_3\}$, **1**, and Me_3SnCl. The half-life ($t/2$) of hemolysis (expressed as the time necessary for 50% hemolysis to occur) determined from Fig. 5 is reported in the inset.

The 'comet assay' or single-cell gel electrophoresis was performed on trout erythrocyte suspension to explore whether the organotin(IV) compounds under study influenced the DNA status in these nucleated cells. This test has become increasingly popular for the measurement of DNA damage in individual cells and consists of embedding cells in agarose, followed by lysis, electrophoresis and staining to visualize DNA damage using fluorescence microscopy. Cells with increased DNA damage display an increased migration of genetic material in the direction of the electrophoresis. The extent of DNA damage is quantified by measuring the displacement of the genetic material between the cell nucleus ('comet head') and the resulting 'tail'. The parameters used as an index of DNA damage are tail length, tail intensity and tail moment and they are calculated by computerized image analysis. The 'comet assay' was performed on trout erythrocyte suspensions incubated in the presence of the organotin(IV) compounds (10 μM at 27 °C and pH 7.8 for 30 min). Table 1 clearly shows that all the considered parameters (tail length, tail intensity and tail moment) after 30 min incubation in the presence of Ph_3SnCl and Ph_3SnL, **2**, remarkably increased. This result seems to indicate that the presence of the two triphenyltin(IV) can induce single-strand breaks and/or alkali-labile sites. From the same table it can be noted that the presence of Me_3SnL, **1**, induces only a slight increase in comet parameters (DNA damage) in comparison with the control. However the different effect shown between Me_3SnCl and Me_3SnL indicates an influence of the type of anion contrary to what occurs in the other parameters that we have considered.

In conclusion, the genotoxic effects shown by $(C_6H_5)_3SnCl$ and $\{[(S\text{-tim})_2CHCO_2]Sn(C_6H_5)_3\}$, **2**, but not by $\{[(S\text{-tim})_2CHCO_2]Sn(CH_3)_3\}$, **1**, support the hypothesis that the nature and lipophilicity of the substituents on tin(IV) are important in explaining the toxicity of organotin compounds. The genotoxic effect of Me_3SnCl does not exclude a different involvment due to the anion.

Table 1. Mean values (±SEM) of tail length, tail intensity and tail moment in trout erythrocyte suspensions (Hb = 30 mg/ml) incubated in isotonic buffer at pH 7.8 and $t = 27\,^{\circ}C$ for 30 min in the presence of 10 μM organotin(IV) complexes

Sample	Time 0 min	Time 30 min
Tail length		
Control	8.97 ± 0.26	9.4 ± 0.23
Me_3SnCl	7.25 ± 0.30	10.91 ± 0.22[a,b]
Me_3SnL (**1**)	8.83 ± 0.26	10.28 ± 0.30[a,b]
Ph_3SnCl	8.27 ± 0.28	11.81 ± 0.40[a,b]
Ph_3SnL (**2**)	9.06 ± 0.34	11.93 ± 0.35[a,b]
Tail intensity		
Control	11114 ± 612	12671 ± 630
Me_3SnCl	11286 ± 580	14720 ± 650[a]
Me_3SnL (**1**)	11037 ± 585	9792 ± 506[b]
Ph_3SnCl	10280 ± 762	14929 ± 907[a]
Ph_3SnL (**2**)	10884 ± 998	12656 ± 663
Tail moment		
Control	0.87 ± 0.05	0.97 ± 0.06
Me_3SnCl	0.81 ± 0.05	1.25 ± 0.08[a,b]
Me_3SnL (**1**)	0.83 ± 0.06	1.03 ± 0.08
Ph_3SnCl	0.79 ± 0.06	1.42 ± 0.09[a,b]
Ph_3SnL (**2**)	0.85 ± 0.08	1.57 ± 0.10[a,b]

[a] $p < 0.05$ with respect to 0 min.
[b] $p < 0.05$ with respect to control 30 min.

Experimental

Chemistry

All syntheses and handling were carried out under an atmosphere of dry oxygen-free dinitrogen, using standard Schlenk techniques or a glove box. All solvents were dried, degassed and distilled prior to use. Elemental analyses (C, H, N, S) were performed in-house with a Fisons Instruments 1108 CHNS-O Elemental Analyser. Melting points were taken on an SMP3 Stuart Scientific Instrument. IR spectra were recorded from 4000 to 100 cm^{-1} with a Perkin-Elmer System 2000 FT-IR instrument. IR annotations used: m = medium, mbr = medium broad, s = strong, sbr = strong broad, w = weak. ^1H-, ^{13}C- and ^{119}Sn-NMR spectra were recorded on an Oxford-400 Varian spectrometer (400.4 MHz for ^1H, 100.1 MHz for ^{13}C and 149.3 MHz for ^{119}Sn). NMR annotations used: m = multiplet, s = singlet, sbr = broad singlet. Electrospray mass spectra (ESIMS) were obtained in positive- or negative-ion mode on a Series 1100 MSD detector HP spectrometer, using a methanol mobile phase. The compounds were added to the reagent-grade methanol to give solutions of approximate concentration 0.1 mM. These solutions were injected (1 μl) into the spectrometer via an HPLC HP 1090 Series II fitted with an autosampler. The pump delivered the solutions to the mass spectrometer source at a flow rate of 300 μl min^{-1}, and nitrogen was employed as both a drying and a nebulizing gas. Capillary voltages were typically 4000 and 3500 V for the positive- and negative-ion mode, respectively. Confirmation of all major species in this ESIMS study was aided by comparison of the observed and predicted isotope distribution patterns, the latter calculated using the IsoPro 3.0 computer program.

Synthesis

All reagents were purchased from Aldrich and used without further purification. The ligand $Na[(S\text{-tim})_2CHCO_2]$ was prepared

in accordance with the literature methods (Fig. 1; Pellei M, Alidori S, Benetollo F, Gioia Lobbia G, Mancini M, Gioia Lobbia GE, Santini C, unpublished work).

*{[(S-tim)$_2$CHCO$_2$]Sn(CH$_3$)$_3$} (**1**)*

To a chloroform solution (50 ml) of $(CH_3)_3SnCl$ (0.199 g, 1.0 mmol), Na[(S-tim)$_2$CHCO$_2$] (0.306 g, 1.0 mmol) was added at room temperature. After addition, the reaction mixture was stirred for 4 h and then filtered; the solvent was removed under vacuum and the residue was washed with chloroform–*n*-hexane (1 : 5). The product was dried, and re-crystallized from chloroform–diethyl ether. Yield 65%. ^{1}H NMR ($CDCl_3$, 293 K): δ0.55 [s, 9H, Sn-C*H*$_3$, ^2J(Sn-^{1}H) = 61.1 Hz], 3.72 (s, 6H, C*H*$_3$), 5.17 (s, 1H, C*H*COO), 6.96 (sbr, 2H, 5-C*H*), 7.05 (sbr, 2H, 4-C*H*). ^{13}C NMR ($CDCl_3$, 293 K): δ − 0.53 [s, Sn-CH$_3$, ^1J(^{117}Sn,^{13}C) = 433 Hz, ^1J(^{119}Sn,^{13}C) = 473 Hz], 34.34 (*C*H$_3$), 59.98 (*C*H), 123.64 (5-*C*H), 129.55 (4-*C*H), 138.98 (*C*HCOO), 170.32 (CH*C*OO). ^{119}Sn NMR ($CDCl_3$, 293 K): 34.52 (s). IR (nujol, cm^{-1}): 3107w (CH), 1639s ($\nu_{asym}CO_2$), 1511m (C═C + C═N), 1325sbr ($\nu_{sym}CO_2$), 550s, 507m, (Sn–C), 468w; 427w, 336mbr (Sn–O). ESIMS (major positive-ions, CH$_3$OH), *m/z* (%): 448 (70) [{[(S-tim)$_2$CHCO$_2$]Sn(CH$_3$)$_3$ + H}]$^+$, 470 (80) [{[(S-tim)$_2$CHCO$_2$]Sn(CH$_3$)$_3$ + Na}]$^+$, 611 (40) [{[(S-tim)$_2$CHCO$_2$][Sn(CH$_3$)$_3$]$_2$}]$^+$, 917 (100) [{[(S-tim)$_2$CHCO$_2$]Sn(CH$_3$)$_3$}$_2$ + Na]$^+$. ESIMS (major negative-ions, CH$_3$OH), *m/z* (%): 239 (100) [(S-tim)$_2$CHCO$_2$] − CO$_2$]$^-$, 283 (20) [(S-tim)$_2$CHCO$_2$]$^-$, 731 (50) [{[(S-tim)$_2$CHCO$_2$]$_2$Sn(CH$_3$)$_3$}]$^-$. Anal. calcd for $C_{13}H_{20}N_4O_2S_2Sn$: C, 34.92; H, 4.51; N, 12.53; S, 14.34%. Found: C, 34.52; H, 4.61; N, 12.28; S, 14.05%.

*{[(S-tim)$_2$CHCO$_2$]Sn(C$_6$H$_5$)$_3$} (**2**)*

To a chloroform solution (50 ml) of $(C_6H_5)_3SnCl$ (0.385 g, 1.0 mmol), Na[(S-tim)$_2$CHCO$_2$] (0.306 g, 1.0 mmol) was added at room temperature. After addition, the reaction mixture was stirred for 4 h and then filtered; the solvent was removed under vacuum and the residue was washed with chloroform–*n*-hexane (1 : 5). The yellow product was re-crystallized from chloroform–*n*-hexane. Yield: 63%. ^{1}H NMR ($CDCl_3$, 293 K): δ3.50 (s, 6H, C*H*$_3$), 5.32 (s, 1H, C*H*COO), 6.83 (sbr, 2H, 5-C*H*), 7.03 (sbr, 2H, 4-C*H*), 7.44–7.71 (m, 15H, Sn-C$_6$*H*$_5$). ^{13}C NMR ($CDCl_3$, 293 K): δ34.42 (*C*H$_3$), 59.86 (*C*H), 123.04 (5-*C*H), 129.87 (4-*C*H), 130.85, 131.29, 136.98, 137.86 (Sn-*C*$_6$H$_5$), 139.19 (*C*HCOO), 169.56 (CH*C*OO). ^{119}Sn NMR ($CDCl_3$, 293 K): −95.83(s). IR (nujol, cm^{-1}): 3116w (CH), 1656s ($\nu_{asym}CO_2$), 1508m (C═C + C═N), 1308sbr ($\nu_{sym}CO_2$), 456s (Ph), 444m, 429m, 383m, 322w (Sn–O), 278s, 245s (Sn–C). ESIMS (major positive-ions, CH$_3$OH), *m/z* (%): 241 (80) [(S-tim)$_2$CHCO$_2$ − CO$_2$ + 2H]$^+$, 634 (100) [{[(S-tim)$_2$CHCO$_2$]Sn(C$_6$H$_5$)$_3$} + H]$^+$. ESIMS (major negative-ions, CH$_3$OH), *m/z* (%): 421 (100) [Sn(C$_6$H$_5$)$_3$Cl + Cl]$^-$. Calcd for $C_{28}H_{26}N_4O_2S_2Sn$: C, 53.10; H, 4.14; N, 8.85; S, 10.13%. Found: C, 52.90; H, 4.28; N, 8.69; S, 9.90%.

Biological Studies

Methods

Samples

Red blood cells were obtained from *Salmo irideus*, an inbred strain of trout, provided by the fish farm 'Eredi Rossi Silvio' Sefro MC, Italy. The fish were kept in tanks containing water from Scarsito river, a tributary of Potenza, and fed with commercial fish food, obtained from Hendrix S.p.A (Mozzecane, VR, Italy). Blood was extracted by puncturing the lateral tail vein. After washing with isotonic medium at pH 7.8 (0.1 M phosphate buffer 0.1 M NaCl, 0.2% citrate, 1 mM EDTA) the cells were suspended in the desired isotonic buffer.

Met-Hb formation

Preparation of trout hemoglobin components was carried out as previously described.[48] The desired amount of the triorganotin(IV) complexes, Ph$_3$SnCl, Ph$_3$SnL, **2**, and Me$_3$SnL, **1**, L = [(S-tim)$_2$CHCO$_2$]$^-$, dissolved in ethanol, was added to hemoglobin solution. Since all the organotin(IV) derivatives used here were dissolved in ethanol, control experiments were performed in this solvent. The rate of met-Hb formation was followed in a Cary 219 spectrophotometer in the visible region; reference value (i.e. complete oxidation) was estimated by addition of ferricyanide. Absorbance at 700 nm was followed as an index of turbidity to monitor the onset of hemoglobin precipitation.

Hemolysis

In order to evaluate the hemolysis rate, the erythrocytes were suspended in isotonic medium at pH 6.3 and t = 35 °C. The degree of hemolysis was determined spectrophotometrically at 540 nm as previously described[47] in either the presence or the absence of the desired amount of complexes **1** and **2**, Ph$_3$SnCl and Me$_3$SnCl dissolved in ethanol. In particular this was determined as 100 × A/10 × A^* × 100% where A is the optical density of Hb present in the supernatant after centrifugation of red cell suspension and A^* × 100% is the optical density of the red cell suspension after complete lysis with 10 vols of distilled water at zero time incubation.

Single-cell gel electrophoresis (comet assay)

The 'comet' assay was performed on trout erythrocytes (1 × 10^6 cells/ml) incubated in a pH 7.8 isotonic buffer at 27 °C for 30 min in the presence and absence (control) of 10 μM ethanol solution of the derivatives Ph$_3$SnCl, Ph$_3$SnL, Me$_3$SnCL and Me$_3$SnL. After incubation, the erythrocytes were suspended in 0.7% low melting agarose in PBS and pipetted on microscope slides pre-coated with a layer of 1% normal melting agarose. The agarose with the cell suspension was allowed to set on the pre-coated slides at 4 °C for 10 min. Subsequently, another top layer of 0.7% low melting agarose was added and allowed to set at 4 °C for 10 min. The slides were then immersed in lysis solution (1% sodium *n*-lauroyl-sarcosinate, 2.5 M NaCl, 100 mM Na$_2$EDTA, 10 mM Tris HCl pH 10, 1% Triton X-100 and 10% DMSO) for 1 h at 4 °C in the dark, in order to lyse the embedded cells and to permit DNA unfolding. After incubation in lysis solution, slides were exposed to alkaline buffer (1 mM Na$_2$EDTA, 300 mM NaOH buffer, pH > 13) for 20 min; in this condition RNA was completely degraded. The slides were subjected to 20 min electrophoresis at 25 V in the same alkaline buffer and finally washed with 0.4 M Tris HCl buffer (pH 7.5) to neutralize excess alkali and to remove detergents before staining with ethidium bromide (2 μg/ml).

Cells were examined with an Axioskop 2 plus microscope (Carl Zeiss, Germany) equipped with an excitation filter of 515–560 nm and a magnification of ×20. Imaging was performed using a specialized analysis system ('Metasystem' Altlussheim, Germany) to determine tail moment (TM), a parameter correlated to the degree of DNA damage in the single cell.

Experiments were replicated three times and data (at least 150 scores per sample) are the mean values plus/minus the standard error of the mean (SEM). Statistical comparisons were performed using the Student *t*-test and differences were regarded as statistically significant when $p < 0.05$.

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