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SYNTHESIS, CHARACTERISATION AND ANTI-TUMOUR ACTIVITY OF BIS(POLYFLUOROALKYL)TIN DIHALIDES

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Summary

The synthesis and characterisation of fifteen new organotin compounds containing at least two $CF_3(CF_2)_5CH_2CH_2$ groups bound to tin are described. The activity of six of these compounds towards the murine P388 lymphocytic leukaemia tumour is given.

Introduction

Many organotin compounds have been tested by the National Cancer Institute. The percentage of active compounds against P388 lymphocytic leukaemia in mice is very high : up to 50% for complexed diorganotin dihalides, and 48% for uncomplexed R_2SnX_2 compounds (1).

Riess and Le Blanc mention the possibility of using perfluoro chemicals as a vehicle for the transport of drugs to tumours (2). They cite the tendency of $C_8F_{17}Br$ to concentrate in the macrophages of malignant tumours as indicative of this possibility.

Therefore a series of organotin compounds of the type $[CF_3(CF_2)_5CH_2CH_2]_2SnX_2$ (with X = Cl, Br) and their bipyridyl- and ortho-phenanthroline complexes were prepared and their anti-tumour activity (3) examined.

Synthesis of (3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)tin compounds

Direct synthesis of Rf_2SnI_2[::], 1

Impure Rf_2SnI_2, 1, can be prepared from RfI and metallic tin with LiI as catalyst and $CH_3CH_2OCH_2CH_2OH$ as co-catalyst and solvent as described for analogous syntheses (4).

The obtained mixture was analyzed by converting the formed Rf_2SnI_2 and Rf_3SnI into the corresponding Rf_2SnR_2 and Rf_3SnR compounds (R = CH_3 or CH_3CH_2) by means of the Grignard reagent RMgX. It could be shown that this direct synthesis gave with a

:: The polyfluoroalkyl group $CH_2CH_2(CF_2)_5CF_3$ used in this work will be called Rf from now on.

total yield of less than 40%,a mixture containing about 87% Rf_2SnI_2, 1 and 13% Rf_3SnI, 2.

Synthesis of Rf_2SnPh_2, $Rf_2SnPhCl$, Rf_2SnCl_2 and Rf_2SnBr_2

Rf_2SnPh_2, 7, was prepared following the classical scheme ($2RfMgI \xrightarrow{Ph_2SnCl_2} Rf_2SnPh_2$) with a yield of 80%. It can be readily transformed into $Rf_2SnPhCl$, 8, Rf_2SnCl_2, 9 and Rf_2SnBr_2, 10 : $Rf_2SnPh_2 \xrightarrow{X_2} Rf_2SnPhX \xrightarrow{X_2} Rf_2SnX_2$.

Synthesis of complexes of Rf_2SnCl_2 and Rf_2SnBr_2 with bipyridyl and with o-phenanthroline

Mixing of Rf_2SnX_2 with either bipyridyl or o-phenanthroline dissolved in diethylether yielded almost pure adducts in quantitative yields (5).

NMR spectra of 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyltin compounds

NMR spectra of the Rf_2Sn moeity

The chemical shifts and coupling constants of the protons of the polyfluorooctyl groups $C(H_A)_2\ C(H_B)_2(CF_2)_5CF_3$ are given in table 1

TABLE 1

Chemical shifts and coupling constants of the protons of the $C(H_A)_2C(H_B)_2(CF_2)_5CF_3$ groups (solvent : $CDCl_3$; TMS as internal standard ; 270 MHz).

	Compounds	δ_A ppm	$^2J(H_A\text{-}Sn)$ Hz	$^3J(H_AH_B)$ Hz	δ_B ppm	$^3J(H_BF)$ Hz	$^3J(H_BSn)$ Hz
3	Rf_2SnEt_2	0.976	-	-	2.233	-	-
4	Rf_3SnEt	1.111	-	8.60	2.282	17.8	-
5	Rf_2SnMe_2	0.992	-	-	2.212	-	-
6	Rf_3SnMe	1.06	-	8.26	2.256	18.1	-
7	Rf_2SnPh_2	1.466	-	-	2.337	-	-
8	$Rf_2SnPhCl$	1.660	55.4	8.08	2.490	18.0	65
9	Rf_2SnCl_2	1.967	64.7	7.57	2.627	17.9	111
10	Rf_2SnBr_2	2.036	59.2	7.65	2.584	17.7	105
11	Rf_2SnCl_2-o-phen	1.372	-	-	2.642	-	-
12	Rf_2SnCl_2-bipy	1.336	-	-	2.574	-	-
13	Rf_2SnBr_2-o-phen	1.504	-	-	2.646	-	-
14	Rf_2SnBr_2-bipy	1.475	-	-	2.573	-	-
15	Rf_4Sn (in CD_2Cl_2)	1.20	52.1	8.14	2.37	17.8	-

The NMR spectra of the ligands in the complexes with o-phenanthroline and bipyridyl

If the protons of o-phenanthroline are denoted A, B, C and D the spectra of free and of complexed phenanthroline can be lucidly presented as in table 2 below.

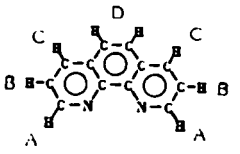
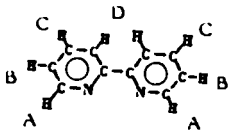


TABLE 2

Chemical shifts and coupling constants in free and complexed o-phenanthroline.

	δ_A ppm	$^3J(H_AH_B)$ Hz	$^4J(H_AH_C)$ Hz	δ_B ppm	$^3J(H_BH_C)$ Hz	δ_C ppm	δ_D ppm
free o-phen	9.192	4.34	1.77	7.630	8.09	8.242	7.782
11	9.780	4.83	1.42	8.109	8.19	8.734	8.173
13	9.926	4.83	1.53	8.110	8.20	8.745	8.181

The chemical shifts of and coupling constants between the protons of bipyridyl,



denoted by A, B, C and D, of free and of complexed bipyridyl are given in table 3.

TABLE 3

Chemical shifts and coupling constants in free- and complexed bipyridyl.

	δ_A ppm	$^3J(H_AH_B)$ Hz	$^4J(H_AH_C)$ Hz	$^5J(H_AH_D)$ Hz	δ_B ppm	$^3J(H_BH_C)$ Hz	$^4J(H_BH_D)$ Hz	δ_C ppm	$^3J(H_CH_D)$ Hz	δ_D ppm
free bipy	8.662	4.82	1.77	0.83	7.285	7.46	1.19	7.797	8.02	8.373
12	9.276	5.02	1.56	0.00	7.816	7.50	0.00	8.237	8.03	8.363
14	9.645	4.85	1.50	0.00	7.822	5.90	1.50	8.284	6.09	8.375

Mass spectra of 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyltin compounds

The analysis of the 70 eV mass spectra of the tetraorganotin compounds 3-7 is summarised in table 4

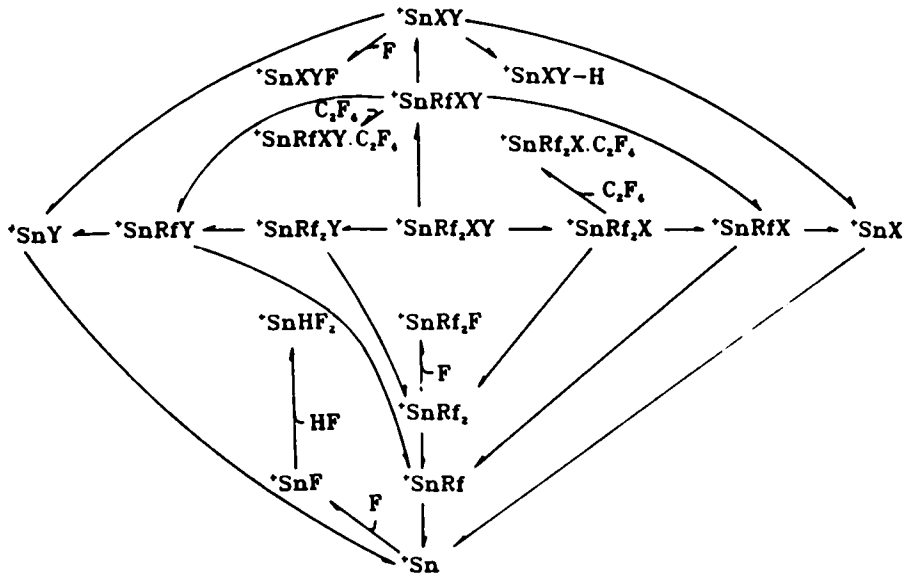
TABLE 4

Monoisotopic 70 eV mass spectra of compounds 3-7.

X / Y / Rf_2XYSn / ion	Me / Rf / Rf_3MeSn		Et / Rf / Rf_3EtSn		Me / Me / Rf_2Me_2Sn		Et / Et / Rf_2Et_2Sn		Φ / Φ / $Rf_2Φ_2Sn$	
	m/z::	I	m/z::	I	m/z::	I	m/z::	I	m/z::	I
^+Sn	120	-	120	-	120	-	120	3	120	6
^+SnF	139	11	139	37	139	7	139	25	139	10
^+SnX	135	18	149	5	135	12	149	20	197	19
^+SnRf	467	2	467	9	467	0.3	467	2	467	-
$^+SnHF_2$	159	3	159	14	159	-	159	48	159	-
$^+SnXY-H$	481	4	495	2	149	-	177	2	273	1
$^+SnRfX-H$	481	4	495	2	481	1	495	1	543	-
^+SnXYF	501	5	515	6	169	100	197	44	293	100
^+SnRfXF	501	5	515	6	501	-	515	2	563	1
$^+SnRf_2F$	833	14	833	4	833	-	833	-	833	-
^+SnRfXY	829	100	843	100	497	58	525	100	621	76
$^+SnRf_2X$	829	100	843	100	829	62	843	-	891	0.2
$^+SnRf_2Y$	1161	-	1161	22	829	62	843	-	891	0.2
$^+SnRfXY \cdot C_2F_4$	929	1	943	1	597	-	625	-	721	0.4
$^+SnRf_2X \cdot C_2F_4$	929	1	943	1	929	-	943	-	991	-
$^+SnRf_2XY$	1176	7	1189	-	844	-	872	-	868	-

:: m/z is the mass of the most intense ion in all instances presented here, I is the intensity in % of the base peak.

These results may be explained as follows



The 70 eV mass spectrum of $Rf_2SnPhCl$, 8 can be explained by an analogous fragmentation pattern : beside Sn^+ (3%), SnF^+ (10%), $SnCl^+$ (51%) and $SnCl_2F^+$(3%), the following tin-containing fragment-ions were observed : $PhSn^+$(43%), $PhSnClF^+$(100%), $SnRfPhCl^+$ minus F_2 (0.3%), $SnRfPhCl^+$ minus HF (1%), $SnRfPhCl^+$(24%), $SnRfPhF^+$(1%) and $SnRf_2Ph^+$ (0.6%).

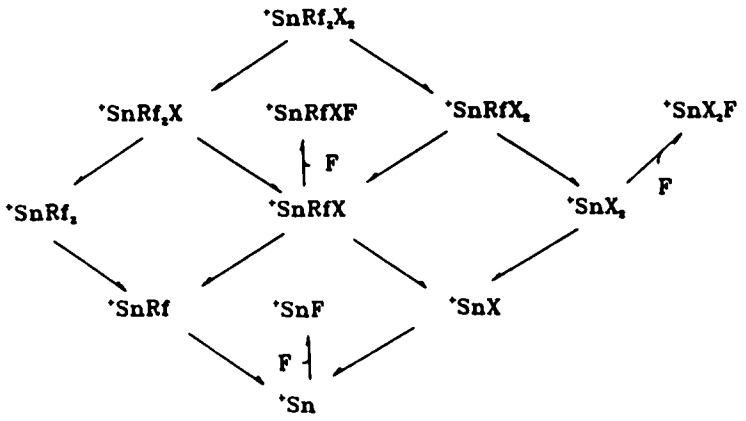
A digest of the mass spectra of two bis(polyfluorooctyl)tin dihalides is presented in table 5.

TABLE 5

Monoisotopic 70 eV mass spectra compounds 9 and 10.

X / ion	Cl		Br	
	m/z	I	m/z	I
^+Sn	120	2	120	6
^+SnF	139	13	139	25
^+SnX	155	100	199	100
$^+SnX_2$	190	5	278	6
$^+SnX_2F$	219	3	297	6
^+SnRf	467	3	467	1
^+SnRfXF	521	2	565	0.1
$^+SnRfX_2$	537	55	625	17
$^+SnRf_2X$	849	4	893	1

The following fragmentation pattern agrees with this digest :



The main difference between the fragmentations proposed for the polyfluoroalkyltin compounds (this work) and those observed for more usual alkyltin compounds (for illustration, see ref. 8 to 10) is that $RSnH_2^+$ or $RR'SnH^+$ ions are virtually imperceptible for polyfluoroalkyltin compounds whereas they are usually intense for more common alkyltin compounds. Furthermore, ions like SnXY minus H or SnRfX minus H (see table 4) are rarely detected among alkyltin fragments. The polyfluoroalkyltin derivatives on the contrary, yield appreciable amounts (>1%) of such fragments.

The mass spectra of the complexes 11-14 are all but identical to those of the uncomplexed diorganotin dihalides.

Anti-tumour activity

The in vivo anti-tumour activity of compounds 9-14 against P388 lymphocytic leukaemia in CDF_1 mice was evaluated under the auspices of the US National Cancer Institute, in accordance with standard protocols for primary screening (7).

Whereas compounds 10 (NSC 377950), 11 (NSC 377949), 12 (NSC 377948), 13 (NSC 377952) and 14 (NSC 377951) proved to be inactive (T/C[::] ranging between 100 and 110% for doses of 240-60 mg/kg day), compound 9 (NSC 377947) exhibits marginal activity (T/C = 125% for 240 mg/kg.day and about 120% for lower doses of 120 and 60 mg/kg.day).

Work is in progress to prepare water-soluble $RfR'SnX_2$ compounds. It has indeed been suggested (11) that poor water-solubility might account for the limited activity observed for all organotin compounds tested as yet.

[::] Ratio of medium survival times (days) of treated (T) and untreated (C) mice ; a compound is considered to be indicative of activity, if a reproducible T/C $\geq$ 120% is obtained.

Experimental part

Direct synthesis of Rf_2SnI_2

2.4 g tin (50 mmol), 0.8 g LiI as catalyst, 20 ml ethoxyethanol as cocatalyst and solvent and 19 g RfI (40 mmol) were mixed and refluxed under stirring for 3 h. in an oil bath at 135-150°C. After cooling and standing overnight, the reaction mixture was filtrated. Ortho-xylene was then added and the azeotrope ethoxyethanol-xylene evaporated under reduced pressure at 40°C, yielding 15 g of crude Rf_2SnI_2. 7.5 g of this mixture were added to the Grignard reagent prepared from 3 g ethylbromide (28 mmol) and 1 g Mg (42 mmol) in 50 ml diethyl ether. After hydrolysis with an NH_4Cl solution, the organic layer was washed first with a 5% $Na_2S_2O_3$ solution, then twice with demineralized water. It was then dried with anhydrous Na_2SO_4. The solvent was then evaporated under reduced pressure and 5.34 g of a liquid was obtained. Column chromatography on Al_2O_3 with hexane yielded first 4.04 g Rf_2SnEt_2, 3 (3.9 mmol, n_D^{20} = 1.3593)and then 0.84 g Rf_3SnEt, 4 (0.71 mmol, n_D^{20} = 1.3487).

The 270 MHz ^1H NMR spectra of compounds 3 and 4 in $CDCl_3$ revealed the expected signals for the ethyl groups[::] : $\delta(CH_2)$ = 1.020 and 1.075 ppm respectively ($^3J(^1H-^1H)$ = 8.0 and 7.8 Hz respectively ; $\delta(CH_3)$ = 1.209 and 1.229 ppm respectively ; $^3J(H-^{119/117}Sn)$ = 76.7/73.4 and 80.8/77.4 respectively.

Substituting methylmagnesium iodide for the ethylmagnesiumbromide, we obtained from the same weight of crude Rf_2SnI_2, 2.76 g Rf_2SnMe_2, 5 (3.3 mmol ; n_D^{20} = 1.3506) and 0.62 g Rf_3SnMe, 6 (0.53 mmol ; n_D^{20} = 1.3453).

Compounds 5 and 6 presented methyl singlets at 0.159 and 0.214 ppm respectively with $^2J(HSn)$ couplings of 53.9/51.7 and 52 Hz respectively.

Synthesis of Rf_2SnPh_2, 7

The Grignard reagent RfMgI was prepared from 40 g RfI (84.4 mmol) and 4 g Mg (167 mmol) in diethyl ether. Thereto was added 10.3 g diphenyltin dichloride (30 mmol) dissolved in 50 ml Et_2O. After refluxing the mixture for 1 h., hydrolysis and work up (vide supra), 33.5 g of a yellow oil was obtained.

A first column chromatography on Al_2O_3 with hexane resulted in 1.4 g Rf_2 (mp. 44°C), 12.7 g of mixed fractions and 16.9 g pure Rf_2SnPh_2, 7 (n_D^{20} = 1.4147). Repeated column chromatography on the mixed fractions yielded 5.6 g Rf_2 and 7.1 g 7. Rf_2 can be partially eliminated from Rf_2SnPh_2 poor and Rf_2 rich mixtures by recrystallyzing Rf_2 from hexane, thus concentrating Rf_2SnPh_2 in the mother liquors.

Synthesis of $Rf_2SnPhCl$, 8

An ice-cooled suspension of 7.4 g Rf_2SnPh_2, 7 (7.7 mmol) in 25 ml MeOH was titrated with a solution of chlorine in MeOH until the mixture cleared up. The solvent and the formed chlorobenzene were evaporated at 55°C under reduced pressure yielding 7.1 g of a solid which turned out to be NMR-pure $Rf_2SnPhCl$ (8) (mp 68-70°C ; after recrystallization from n-hexane).

[::] Overlapping of the methylene signals with those of the Rf group made it hazardous to try and estimate the $^2J(HSn)$ coupling constant.

Synthesis of Rf_2SnCl_2, 9

A suspension of 7.2 g Rf_2SnPh_2, 7 in 25 ml methanol was titrated with a solution of Cl_2 in methanol (vide supra) and a second equivalent of chlorine was added. The addition of excess chlorine proved necessary to cause the complete disappearence of the NMR phenyl signals. Recrystallization from n-hexane yielded pure 9 (mp 88-90°C).

Synthesis of Rf_2SnBr_2, 10

A methanolic bromine solution was used to titrate a suspension of 6.9 g Rf_2SnPh_2, 7 in 20 ml methanol until the suspension cleared up and a lasting brown colour was observable. Removal of methanol, bromobenzene and the excess bromine by evaporation under reduced pressure resulted in 7 g Rf_2SnBr_2, 10, which was found to be NMR pure (mp 54-57°C after recrystallization from hexane).

Syntheses of the complexes of Rf_2SnCl_2 and Rf_2SnBr_2 with bipyridyl and with o-phenanthroline

The complex of Rf_2SnCl_2 with o-phenanthroline was prepared by mixing a solution of 2 g of 9 (2.26 mmol) in 10 ml Et_2O and a solution of 0.41 g 1,10-phenanthroline (2.27 mmol) in 50 ml Et_2O. The mixture was refluxed for 15 minutes and evaporated yielding almost pure Rf_2SnCl_2-o-phen, 11, in quantitative yield.

The other complexes $Rf_2SnX_2 \cdot B$ were prepared analogously :

TABLE 6

Melting points of four complexes of Rf_2SnX_2 with bidentate nitrogen bases.

	X	B	mp (recrystallization solvent)
11	Cl	o-phen	101-105°C (methanol)
12	Cl	bipy	113-115°C (ethanol)
13	Br	o-phen	125-128°C (methanol)
14	Br	bipy	123-125°C (ethanol)

Synthesis of Rf_4Sn, 15

To the Grignard reagent, prepared from 13.5 RfI (28.5 mmol) and 1.2 g Mg (50 mmol) was added 1.42 g $SnCl_4$ (5.42 mmol) dissolved in 50 ml dry benzene. After refluxing the mixture for 1 hour and allowed to stand overnight, the usual work up yielded 4 g of a yellow oil. Column chromatography of this oil on Al_2O_3 with hexane gave first the dimer Rf_2 (1.42 g). Further elution with hexane, then with CH_2Cl_2 yielded 1.27 g Rf_4Sn, 15 (0.84 mmol, NMR pure, $n_D^{20} = 1.3422$).

Instruments

The NMR spectra were recorded on a Bruker HX 270 instrument.

The mass spectra were recorded on an MS 902 S instrument of AEI (source temperature : 200°C ; pressure : 10^{-7} torr) coupled to a NOVA computer, and analysed using a computer program, ISOMAS (6), allowing conversion of the m/z intensity listing into the monoisotopic spectrum.

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References

(1) V. L. Narayanan, Strategy for the discovery and development of novel anticancer agents, in "Structure-activity relationships of anti-tumour agents", D.N. Reinhoudt, T.A. Connors, H.M. Pinedo, K.W. van de Poll, ed., Martinus Nijhoff, The Hague (1983), p. 5.

(2) J.G. Riess and M. Le Blanc, Pure and Appl.Chem., 54 (1982), 2383.

(3) M. Gielen, "Anti-tumour active organotin compounds", in "Tin as a vital nutrient : implications in cancer prophylaxis and other physiological processes", ed. N. Cardarelli, Uniscience Program, CRC Press Inc. (1985) and references cited theirein.

(4) A. François, "Optimalisatie van de rechtstreekse synthese van di-n-butyltin dibromide", Afstudeerwerk, Free University of Brussels V.U.B., (1980).

(5) D.L. Alleston and A.G. Davies, J.Chem.Soc., 1962, 2050.

(6) I. Vanden Eynde and M. Gielen, unpublished results.

(7) R.I. Geran, N.H. Greenberg, M.M. MacDonald, A.M. Schumacher and B.J. Abbott, Protocols for screening chemical agents and natural products against animal tumors and other biological systems, Cancer Chemoth. Rep. 3 (1972), 1-103.

(8) M. Gielen and K. Jurkschat, Org.Mass Spectrom, 18 (1983).

(9) S. Boué, M. Gielen and J. Nasielski, Bull.Soc.Chim.Belg., 77 (1968), 43.

(10) S. Boué, M. Gielen, J. Nasielski, J.P. Lieutenant and R. Spielmann, Bull.Soc.Chim.Belg., 78 (1969), 135.

(11) G. Atassi, Anti-tumour and toxic effects of silicon, germanium, tin and lead compounds, Rev. Si, Ge, Sn, Pb cpds, in press.