

Ciprofloxacin Polymers Derived from Diallyltin and Divinyltin Dihalides

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ABSTRACT

The initial synthesis of condensation polymers from newly available divinyltin dichloride and diallyltin dichloride and ciprofloxacin is accomplished employing a modified interfacial polycondensation process. The polymeric (DP are about 2,000) products are rapidly (less than 15 seconds) formed in good yield. Infrared spectroscopy shows the formation of Sn-O and Sn-N bonds as predicted from the proposed structure. F-MALDI MS also shows ion fragments containing organotin in the predicted isotopic abundances as well as ion fragments containing both organotin and ciprofloxacin. The polymers exhibit low toxicity towards healthy WI-38 cells with variable toxicities towards a variety of cancer cell lines.

KEY WORDS: *Ciprofloxacin, organotin, organotin polymers, diallyltin, divinyltin, F-MALDI MS, organotin polyamine esters, MALDI mass spectroscopy, infrared spectroscopy, colon cancer, bone cancer, prostate cancer, pancreatic cancer*

INTRODUCTION

Tin has the largest number of its organometallic derivatives in commercial use than any other metal.^[1] The topic of organotin polymers has

been recently reviewed.^[2] Organotin products, including those based on organotin polymers developed by Carraher, are mainly employed today in the thermal stabilization of poly(vinyl

chloride) based polymers. Others are employed in agriculture and industry to make transparent films and in the prevention of mildew, yeast, and bacteria growth.

As early as the 1970's organotin compounds have been recognized as active anticancer reagents.^[3-6] Polymer-containing drugs can act either in control release of the drug(s) or as a drug itself. In the war against cancer, polymeric drugs have unique advantages over small monomeric drugs. Tumor-associated vasculature is frequently hyperpermeable to plasma proteins and other macromolecules.^[7,9] These leaky vasculatures and limited lymphatic drainage, typical of tumors and missing in normal tissue, result in the accumulation of macromolecules, e.g., polymeric drugs in the interstitial space of a large variety of tumors, known as the 'enhanced permeability and retention effect' (EPR). Polymeric drugs then can release the cytotoxic therapeutic agents in the intracellular fluid of the tumor tissue in a sustained and stable way or may act as the drug itself. This targeted drug-delivery reduces the side-effects to other parts the body. Related to a control release mechanism, the continuous release of bioactive agents is crucial for cell-cycle-specific drugs as well as for reducing drug resistance. In other work by us, we have also found that polymers may also offer another advantage. Healthy cells have their autoimmune systems fully functional whereas cancer cells are compromised with respect to their autoimmune systems. Thus systems such as those that are related to p53 may be inactivated allowing polymeric drugs to be effective at combating the cancer whereas within healthy cells, these

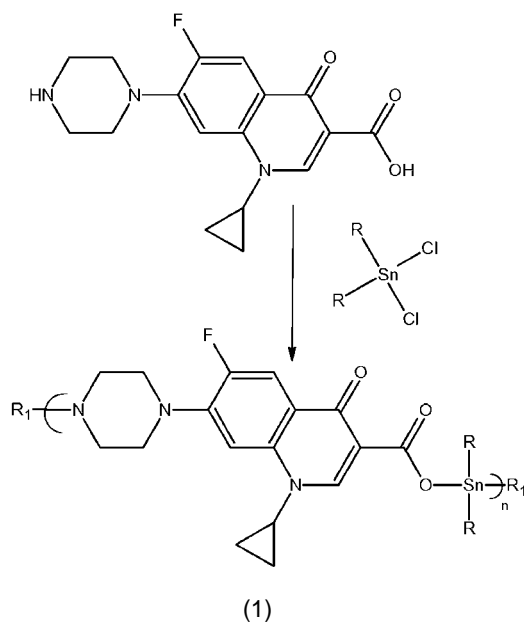
defense mechanisms are active and can combat the intrusion of unwanted molecules more effectively.^[10]

We recently reported the synthesis of ciprofloxacin-containing organotin polymers **(1)**.^[11] We have also reported on their antiviral activity,^[12-13] antibacterial activity^[14] and their ability to inhibit Balb 3T3 cells as a prelude to identifying their potential as anticancer drugs.^[15] We are currently continuing to study their use as anticancer and antiviral drugs.

The organotin compounds exhibit good inhibition of Balb 3T3 cells. The best results were found for the dibutyl derivative which showed a GI_{50} of 0.1 micrograms/mL and the diphenyltin derivative with a GI_{50} value of 0.5 micrograms/mL. By comparison, cisplatin, the most widely used anticancer drug, showed a GI_{50} value of about 0.5 micrograms/mL. Thus, both the dibutyltin and diphenyltin-ciprofloxacin polymers showed GI_{50} values in the same range as cisplatin and merit further study as anticancer drugs.^[16]

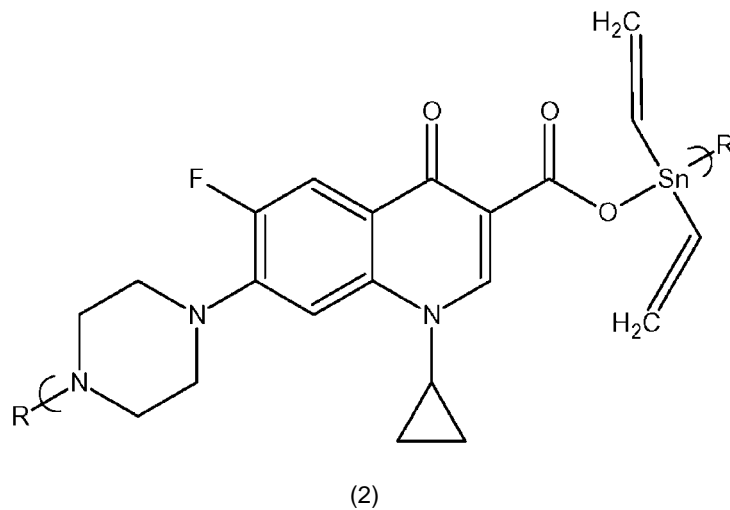
While ciprofloxacin shows little antiviral activity, the organotin polymers exhibit some activity against the Vaccinia WR virus.^[12,13] Some of the products also exhibited good inhibition of various bacteria including *S. Aureus* and *Ps. Aeruginosa*.^[14]

Recently, we have also begun publishing specific physical characterization data related to these organotin-ciprofloxacin polymers. This includes their characterization involving Mossbauer^[16] and IR^[17] for various products, not covered here, and various MS techniques.^[18,19]



Here, we report on the synthesis of corresponding products except those derived from divinyltin dichloride and diallyltin dibromide. These products offer an added option of creation of crosslinked products employing the organotin double bonds. This

is the initial report of the synthesis of condensation polymers derived from these two organotin dihalides since they have only become commercially available recently. A representative structure of the repeat unit for these products is given in (2) for the divinyltin dichloride polymer.



EXPERIMENTAL

Reagents

Chemicals were used as received. Ciprofloxacin hydrochloride (85721-33-1) was obtained from ICN Biochemicals, Aurora, OH. Divinyltin dichloride (7532-85-6) and diallyltin dibromide (17381-88-3) were purchased from Gelest, Morrisville, PA.

Synthesis

The polymers were synthesized employing a modified classical interfacial system.^[2] Briefly, 2.00 mmols ciprofloxacin hydrochloride were suspended in 20 mL water containing 6.00 mmols NaOH. Acetone (10 mL) was added to the suspension. The suspension was dissolved after about two minutes shaking. This solution was added to the blender. Stirring (18,500 rpm no load) was begun and the organic phase containing 2.00 mmoles organotin dihalide in 20 mL heptane was rapidly (less than 10 s) added. Stirring continued for about 30s. A white precipitate formed. The solid was removed using suction filtration. The solid was washed repeatedly with heptane and water removing unreacted materials, salts, and unreacted base. The product was washed onto a glass Petri dish and allowed to dry. Solubility was determined employing about 0.1 mg of solid in 3 mL of liquid and observing over a period of a month. Disappearance of the solid indicated that the product was soluble. In cases where partial solubility was indicated, amount of dissolved material was determined by recovering, drying, and weighting the remaining solid.

Instrumental

FTIR spectra were obtained employing KBr pellets using a Mattson Instruments galaxy Series 4020 FTIR using 32 scans and an instrumental resolution of 4 cm⁻¹. Light scattering photometry was performed employing a Brice Phoenix 4000 Light Scattering Photometer. High resolution electron impact positive ion matrix assisted laser desorption ionization time of flight, HR MALDI-TOF, mass spectrometry was carried out employing a

Voyager-DE STR BioSpectrometer, Applied Biosystems, Foster City, CA. The standard settings were used with a linear mode of operation and an accelerating voltage of 25,000 volts; grid voltage 90% and an acquisition mass range of 2000 to 100,000. Two hundred shots were typically taken for each spectrum. Several matrix materials were employed but here only results employing α -cyano-4-hydroxycinnamic acid are included.

Biological Characterization

Each of the cell lines listed in Table 13 were obtained from NCI and maintained in MEM-Eagles supplemented with 10% fetal bovine serum at 37°C in a 5% CO₂ atmosphere.

For testing of the compounds, cells were harvested, counted, and plated into 96-well plates at 1x10⁴ cells per well in MEM-Eagles supplemented with 10% fetal bovine serum, and incubated for 24 hs at 37°C in a 5% CO₂ atmosphere. A stock solution of the compound was prepared in DMSO at a known concentration. On day two the cell media was removed and replaced with MEM-Eagles supplemented with 10% fetal bovine serum and the indicated drug concentrations. Forty-eight hours later the cells were assayed for proliferation using the CellTiter 96® Aqueous One Solution Cell Proliferation Assay by Promega Corporation. Assays were performed by adding a small amount of the CellTiter 96 Aqueous One Solution Reagent directly to culture wells, incubating for 1–4 hs and then recording absorbance at 490nm with a 96-well plate reader. The quantity of formazan product as measured by the amount of 490nm absorbance is directly proportional to the number of living cells in culture.

RESULTS AND DISCUSSION

Synthesis

The product was formed in good yield employing the modified interfacial polycondensation technique. The classical interfacial

technique employs water as the solvent in the aqueous phase. Ciprofloxacin was not soluble in water even with added base to neutralize the carboxylic acid functional group. We developed a solvent system that allowed solubility of the ciprofloxacin and also was immiscible with the organic phase solvent, here heptane. The system employed addition of acetone to a suspension of ciprofloxacin. This effectively dissolves the ciprofloxacin allowing the desired reaction to occur.

The products were not soluble in DMF, DMA, acetone, chloroform, heptane and water. They have slight solubility in DMSO sufficient to allow medical tests to be run in DMSO but not sufficient to allow light scattering photometry to be run or to allow solution NMR to be carried out on the products. The products were soluble in HMPA. Thus, light scattering photometry was run in HMPA and the results appear in Table 1. Most organotin polymers produced in a similar fashion except employing only water as the aqueous phase solvent have chain lengths below 500, generally on the order of several hundred.^[2] The DPs for the present products are in the range of 2,000. The presence of the acetone is believed to allow the production of longer chains presumably because the growing chains have a greater solubility in the acetone-water mixture. Precipitation of growing polymer chains is believed to be the molecular weight limiting step for many interfacial polymerizations.^[2,10]

Table 1. Product yield and molecular weight

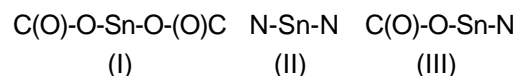
Organotin	% Yield	dn/dc	Mol. Wt.	DP
Divinyltin	100	0.209	1.14 x 10 ⁶	2300
Diallyltin	70	0.265	1.08 x 10 ⁶	2000

Vibrational Spectroscopy

Infrared vibration assignments were made through comparison of monomer and polymer IR spectra and utilizing literature citations.

The IR spectra, Figures 1 and 2, of the two polymers are similar as expected since they differ in only the presence of an additional methylene unit for the diallyltin product in comparison to the divinyltin product. IR data is given in Tables 2 and 3 for selected bands. In the region above 3000 (all band assignments are given in cm⁻¹) the spectra have bands from both the organotin moiety and the ciprofloxacin. Peaks at about 3500 assigned to the O-H stretching and about 3370 assigned to the N-H stretching are missing for the polymer consistent with the protons being replaced with the organotin moiety. The region between 3000 to 900 contains mainly peaks assigned to the ciprofloxacin moiety. The peak at about 2617 is almost the same location for all of the organotin polymers and is assigned to the carbonyl group contained within the quinoline ring.^[17]

The polymer actually can have three repeat units about the organotin moiety as shown below.



In past studies, products containing structure (I) take on an octahedral structure about the tin atom with the carbonyl-oxygen backbonding onto the tin.^[2] Structures (II) and (III) are found to occur as traditional tetrahedral geometries.^[2] The octahedral structure is referred to as a bridged structure whereas tetrahedral structures are referred to as nonbridged structures. These structures can be identified employing the location(s) of the carbonyl-

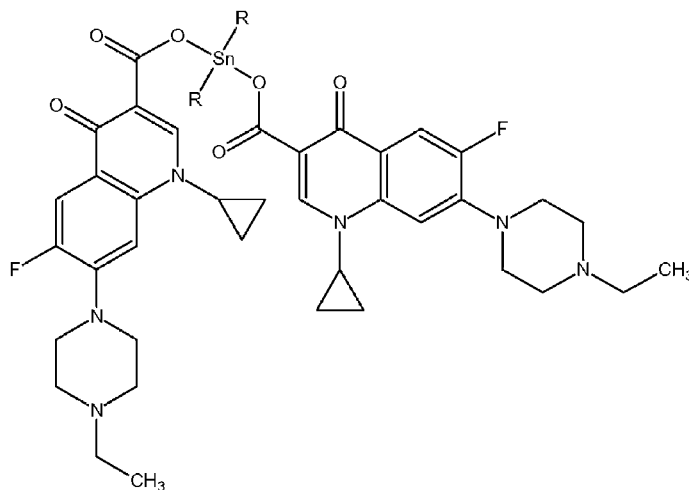
associated bands.^[2] Non-bridged structures show an asymmetrical stretching associated band about 1650 and a weaker symmetrical stretching band near 1360. Bridged structures show the corresponding bands at about 1580 and 1400. The structure assignment is complicated because of the presence of the ring ketone found at about 1620. Even so, the polymer shows bands at about 1578 and additional bands absent at about 1650 consistent with an octahedral structure about the tin indicating that the predominance of structures about the tin being of forms I and II, referred to as the symmetrical structures. Mossbauer spectroscopy on similar structures except where the alkyl group is butyl, methyl, and phenyl is in agreement with this.^[17,20]

The presence of mainly the symmetrical structures is consistent with initial reaction preferentially occurring at either the amine or the carboxylic grouping. If the reaction occurs within the aqueous phase then it might be expected that reaction initially occurred preferentially at the carboxylic site since it is more

nucleophilic. Counter, if reaction occurs within the organic layer then the initial reaction would favor reaction with the amines. Reaction with enrofloxacin (**3**) occurs giving about a 100% yield whereas the analogous reaction with the ester of ciprofloxacin gives a low yield. This is consistent with products of form I being initially formed.

Ordinary esters have two bands in the 1300 to 1050 region. The band about 1300 is sometimes stronger than the carbonyl band. While these C(O)-O-C stretching bands do not vary as much as in alcohols, ethers, and acids, the position of the band due to the C(O)-O stretching is dependent on the nature of both the acidic and the alcoholic components. Similar bands are found for alkyltin carboxylates and dibutyltin diacetate and dibutyl dilaurate. The two bands at about 1270 and 1070 are assigned to the Sn-O-C(O) grouping.

In general, Sn-C bands occur within the 600-500 and 530-470 regions for the asymmetric and symmetric stretching. The intensities of these bands vary considerably. Based on the



(3)

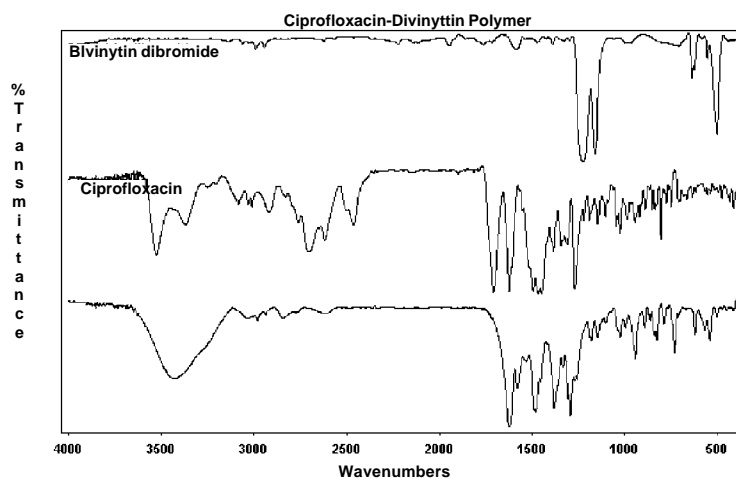


Fig. 1. FTIR spectrum of ciprofloxacin-divinytin polymer (bottom) along with the spectrum of divinytin dibromide (top) and ciprofloxacin (middle)

TABLE 2. IR Absorption band assignments for divinytin dichloride, ciprofloxacin, and the polymer derived from their reaction

Band Assignment	Divinytin Dichloride	Cipro	Polymer
O-H Carbonyl St.		3526	
N-H St.		3372	
C-H of = CH Sym. St.	2991		2982
N-H St. (various)		2762,2701 2618,2462	
C=O Carboxylic Acid St.		1708	
C=O Ketone St.		1624	1625
C=O Ester Asym. St.		1579	
In-plane Ring St.		1495	1489
Asym. Ring St.		1449	1456
Dimeric OH In-Plane Bend Coupled With C-O St.		1418	
C-F St.		1309	1305
Ester		1292	
C-O of =C-O-Group St.		1272	1269
C-O-Sn St.			1072
C-F St.		1045	1037
Sn-O		943	
2° Amine Wagging		804	
Sn-N St.		731	
Sn-C Asym. St.		554	541
Sn-C Sym. St.		504	503

simple spectrum of divinyltin dichloride and diallyltin dibromide these bands are assigned to about 550 and 500. This is consistent with the location for most of the dialkyltin monomers.^[2] Both bands are found for the organotin monomers and polymers.

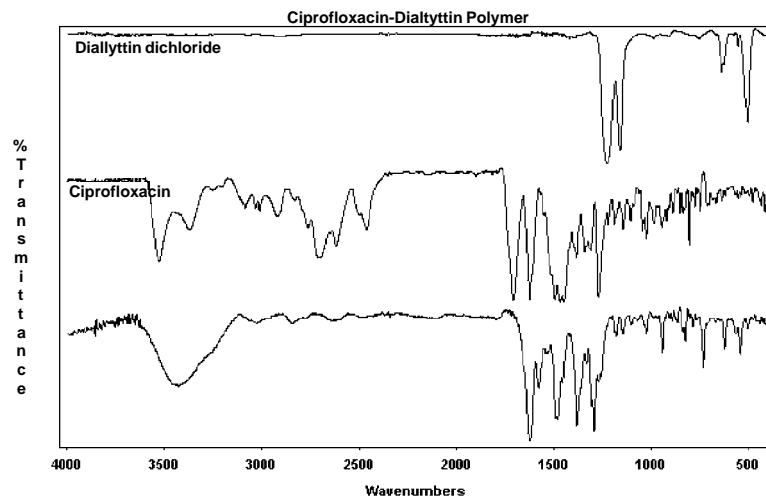


Fig. 2. FTIR spectrum of ciprofloxacin-divinyltin polymer (bottom) and of diallyltin dichloride (top) and ciprofloxacin (middle)

TABLE 3. IR Absorption band assignments for diallyltin dichloride, ciprofloxacin, and the polymer derived from their reaction

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		2618,2462	
C=O Carboxylic Acid St.		1708	
C=O Ketone St.		1624	
C=O Ester Asym. St.		1579	
In-plane Ring St.		1495	1489
Asym. Ring St.		1449	1456
Dimeric OH In-Plane Bend Coupled With C-O St.		1418	
C-F St.		1309	1309
Ester		1292	
C-O of =C-O- Group St.		1272	1269
C-O-Sn St.		1069	
C-F St.		1045	1034
Sn-O		941	
2° Amine Wagging		804	
Sn-N St.			731
Sn-Cl	630		
Sn-C Asym. St.	554		541
Sn-C Sym. St.	504		505

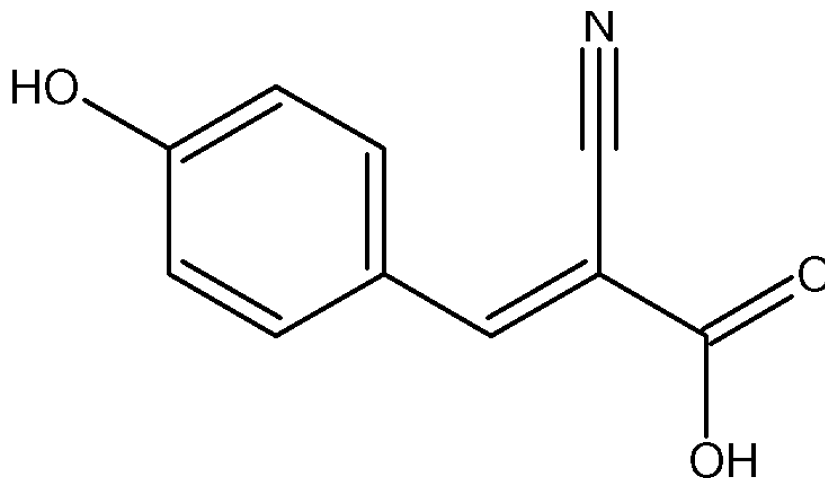
The location of the Sn-O vibrational assignment is varied reportedly occurring over a range of 1000 to 300. It is believed that this variation may be due to the presence of symmetrical and asymmetrical vibrations where only one is often assigned. Even so, a new band appears at about 940 and is assigned due to the presence of the Sn-O. This new band is also found for the monomeric enrofloxacin product.

The Sn-N absorption band, like the Sn-O band, is reported to occur over a wide range from 800 to 400. A new band appears at about 730 and is assigned to the Sn-N moiety. The enrofloxacin product does not have a band here whereas the product from the ester of ciprofloxacin does.

F-MALDI MS

Fragmentation MALDI MS, F-MALDI MS, was conducted on the products. The basics for this technique were described in this journal.^[21] Results appear in Tables 4 to 12 and Figures 3-10. Abbreviations are employed in Tables 4 to 12; thus, U= one unit; 2U= two units; V = vinyl, CH₂=CH- ; Al = Allyl; B = Sn(CH=CH₂)₂; A = ciprofloxacin; Pip = piperazine; Cp = cyclopentane.

For comparison, the F-MALDI MS for the matrix, α -cyano-4-hydroxycinnamic acid, 4, shows ion fragments at 145 (matrix minus CO₂), 189 (matrix parent ion), and 212 (matrix + Na). None of these ion fragments are found in the spectra of either product.



(4)

The F-MALDI MS for the lower masses for the product of divinyltin dichloride and ciprofloxacin is given in Figure 3. Table 4 contains the major

ion fragments found in the range of 150 to 1,200 daltons.

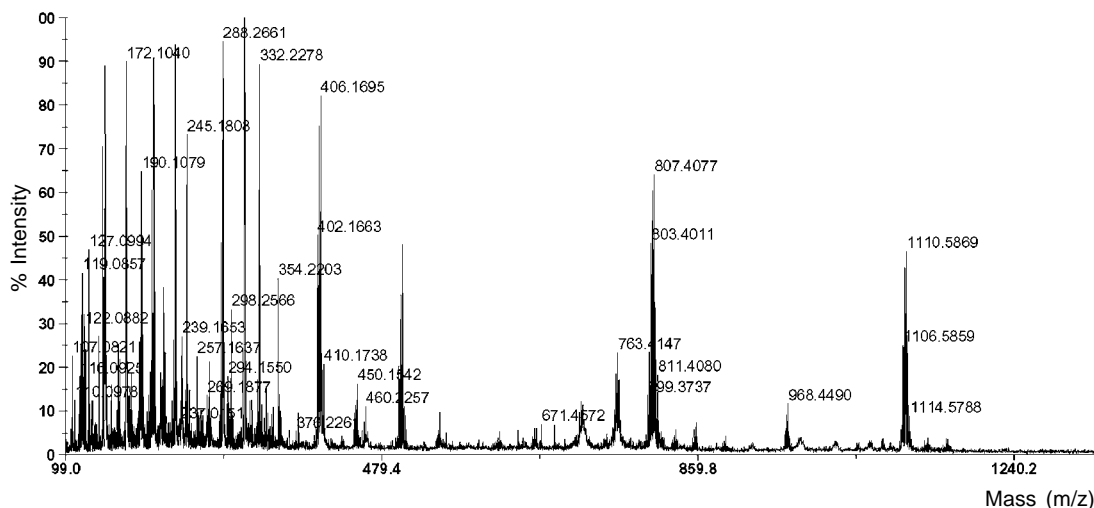


Fig. 3. F-MALDI TOF MS for the product of divinyltin dichloride and ciprofloxacin over the range of 100 to 1,200 daltons

TABLE 4. F-MALDI TOF MS results for the product of divinyltin dichloride and ciprofloxacin for the mass range of 150 to 1,200 daltons from ion fragment clusters shown in Fig. 3

m/e	(Tentative) Assignment	m/e	(Tentative) Assignment
172	Sn	190	Sn,O
245	A-cp,CO ₂	288	A-CO ₂
314	A-O	406	U-2V,CO ₂
450	U-2V	504	U
763	U+Sn,Pip	807	U+Sn,Pip,CO ₂
857	U+Sn,2Pip	968	2U-CO ₂
1110	2U+Sn,O-Pip	1151	2U+Sn,Pip,CO ₂ -6V

Tables 5 to 7 contain isotopic abundance matches for ion fragments containing one, two, and three tin atoms. The matches are reasonable given the low intensities of the

various tin isotopes. Figure 4 contains an expansion of the range for mass values given in Table 5. Figure 5 is the expanded mass range for assignments given in Table 6 for ion fragment

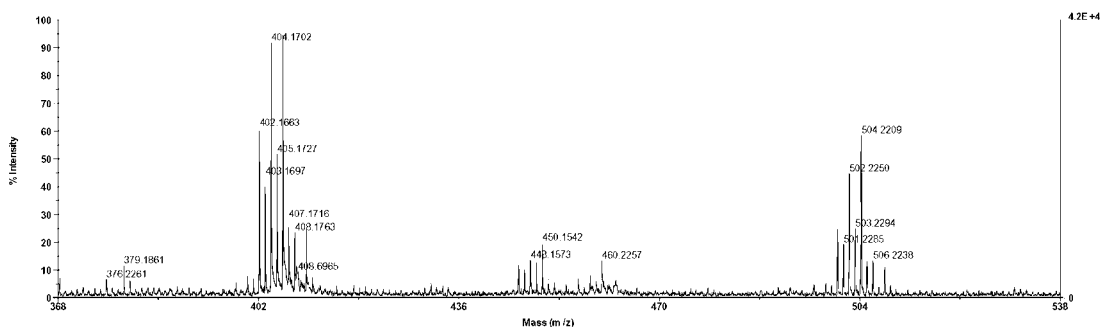


Fig. 4. Expanded F-MALDI MS for the mass range given in Table 5 for the ion fragment clusters assigned to U-2V,CO₂ and one unit (U)

TABLE 5. Isotopic abundance patterns for selected one tin-containing ion fragments derived from the product of divinyltin dichloride and ciprofloxacin (% intensity >5%)

Ion Fragment →		U-2V,CO ₂		One Unit	
Ion Mass	Abundance Predicted (%)	Ion Mass	Abundance Found (%)	Ion Mass	Abundance Found (%)
116	44	402	60	500	43
117	24	403	40	501	20
118	74	404	80	502	76
119	26	405	50	503	30
120	100	406	100	504	100
122	14	408	22	506	17
124	18	410	25	508	15

clusters containing two tin atoms assigned to U+Sn,Pip,CO₂ and U+Sn,Pip. Figure 6

contains an expanded F-MALDI MS for the ion fragment cluster described in Table 7 and

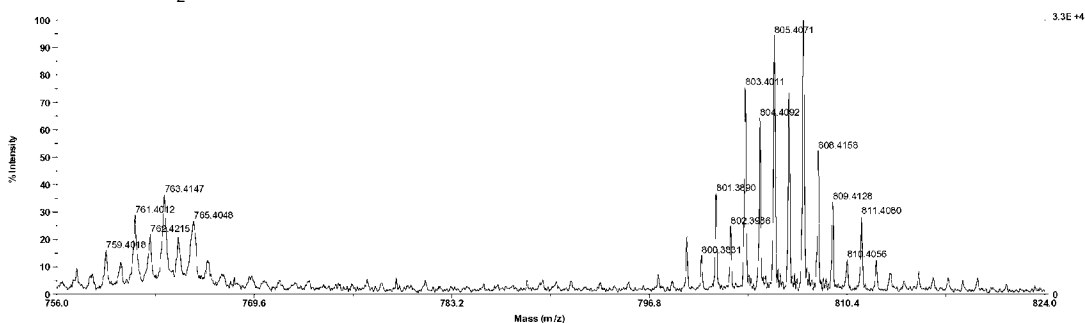


Fig. 5. Expanded mass range for assignments given in Table 6 for ion fragment clusters containing two tin atoms assigned to U+Sn,Pip,CO₂ and U+Sn,Pip.

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TABLE 6. Isotopic abundance patterns for the product of divinyltin dichloride and ciprofloxacin containing two tin-containing ion fragments (% intensity >10%)

Ion Fragment →		U+Sn,Pip,CO ₂		U+Sn,Pip	
Ion Mass	Abundance Predicted (%)	Ion Mass	Abundance Found (%)	Ion Mass	Abundance Found (%)
232	17	801	17	758	16
233	14	802	14	759	14
234	46	803	60	760	45
235	36	804	50	761	34
236	94	805	96	762	80
237	51	806	70	763	50
238	100	807	100	764	100
239	35	808	45	765	40
240	81	809	30	766	80
242	32	811	28	768	32
244	22	813	10	770	16

assigned to 2U+Sn-V. Table 8 and Figure 7 contain ion fragments obtained within the

higher range of 20,000 to 80,000 daltons. The assignments are suggestive at best given the great number of lower mass ion fragments that

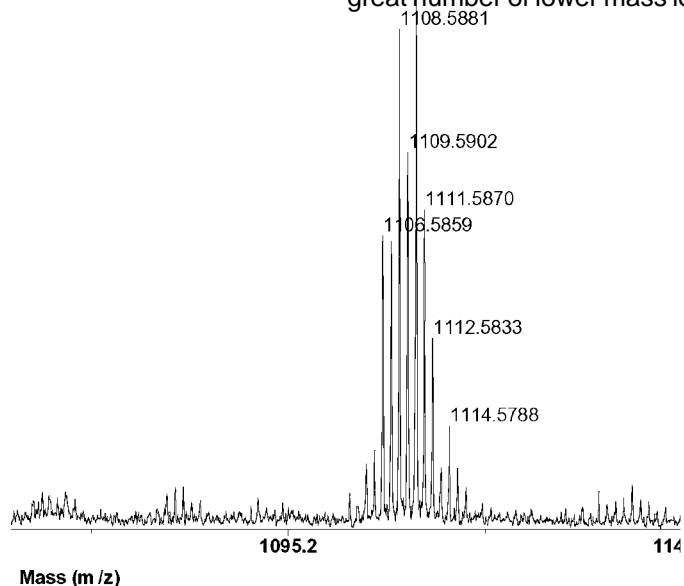


Fig. 6. Expanded F-MALDI MS for the ion fragment cluster described in Table 7 assigned to 2U+Sn-V

TABLE 7. Isotopic abundance patterns for the product of divinyltin dichloride and ciprofloxacin and selected three tin-containing ion fragments (% intensity >10%)

Ion Fragment→		2U+Sn,Pip-6V		2U+Sn-V	
Ion Mass	Abundance Predicted (%)	Ion Mass	Abundance Found (%)	Ion Mass	Abundance Found (%)
350	20	1105	14	1145	18
351	20	1106	14	1146	20
352	47	1107	55	1147	42
353	42	1108	57	1148	44
354	79	1109	85	1149	90
355	60	1110	65	1150	60
356	100	1111	100	1151	100
357	57	1112	55	1152	50
358	88	1113	35	1153	30
359	37	1114	15	1154	24
360	63	1115	25	1155	24
361	17	1116	12	1156	17
362	34	1117	8	1157	22
364	19	1119	5	1159	15

have been stripped of one or more Sn-vinyl moiety. The sensitivity of Sn-alkyl moieties to bond scission by UV radiation is documented. Because the MALDI MS instrument employs laser UV radiation as the energy source it is not surprising that such bond scission is found. Such loss of Sn-alkyl groups is found employing similar organotin polymers containing the ciprofloxacin moiety except

where the alkyl group is methyl, ethyl, propyl, and butyl. By comparison, other organotin polymers show limited Sn-alkyl bond scission that occurs at the site of chain breakage. It is not currently known why the ciprofloxacin products are so sensitive to the employed UV radiation.

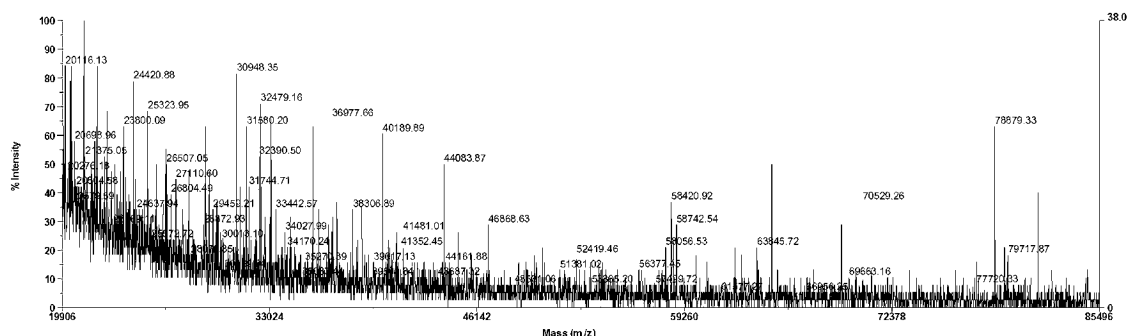


Fig. 7. F-MALDI TOF MS for the product of divinyltin dichloride and ciprofloxacin over the range of 20,000 to 80,000 daltons

TABLE 8. F-MALDI TOF MS results for the product of divinyltin dichloride and ciprofloxacin for the range of 20,000 to 80,000 daltons

m/e	(Tentative) Assignment	m/e	(Tentative) Assignment
21295	42U-B	22754	45U-V
30948	62U-B,O	36978	73U+A
44084	88U-Pip	58421	115U+B
70529	140U+B,CO ₂ ,O	78879	CO ₂

Table 9 and Figure 8 contain the lower mass range ion fragments obtained from the diallyltin-ciprofloxacin product. A number of the ion fragments are the same as those found for the divinyltin product, Table 4 and Fig. 3, and

represent ion fragments clusters derived from only the ciprofloxacin (as expected) and higher mass ion fragments containing tin ion fragment clusters that are stripped of all allyl groups

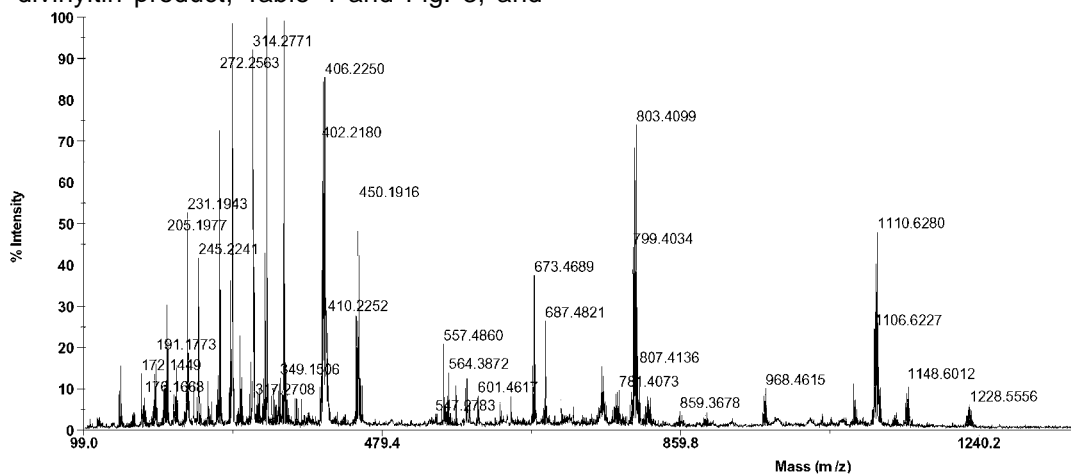


Fig 8. Low mass F-MALDI MS for the product of diallyltin dichloride and ciprofloxacin

TABLE 9. F-MALDI TOF MS results for the product of diallyltin dichloride and ciprofloxacin for ion fragments within the range of 150 to 1,300 daltons

m/e	(Tentative) Assignment	m/e	(Tentative) Assignment
272	A-F,cp	288	A-CO ₂
354	A,Na	406	U-2Al,CO ₂
450	U-2Al	557	U,Na
671	U+Sn,Pip,O-4Al	687	U+A-2Al,Pip
803	U+A-Al,O	891	2U-4Al
1111	2U+Sn,Pip-6Al	1151	2U+Sn,Pip,CO ₂ -6Al
1228	2U+A-4Al		

Tables 10 and 11 and Figures 9-11 contain isotopic abundance matches for ion fragments containing one and three tin atoms. Again, the matches are reasonable given the low abundances of the particular ion fragments. Even so, the patterns clearly are consistent with the presence of one and three tin atoms.

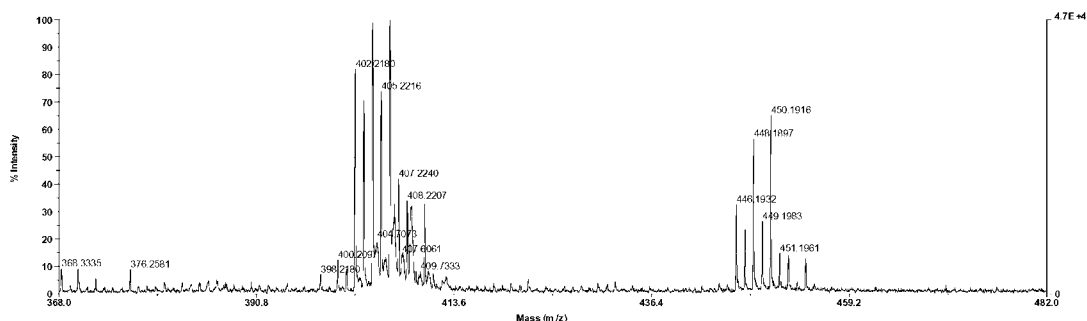


Fig. 9. Expanded F-MALDI MS for the ion fragment clusters at 406 (U-2Al,CO₂) and 450 (U-2Al) Da. described in Table 10

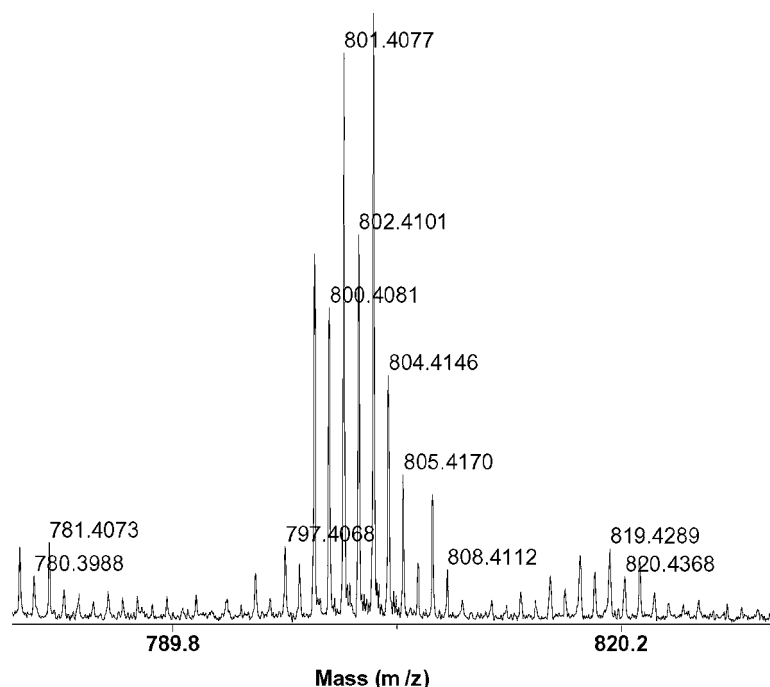


Fig. 10. Expanded F-MALDI MS for the product of diallyltin dichloride and ciprofloxacin for the ion fragment cluster at about 803 daltons assigned a structure of U+A-Al,O and described in Table 10

TABLE 10. Isotopic abundance patterns for selected one tin-containing ion fragments derived from the product of diallyltin dichloride and ciprofloxacin (% intensity >10%)

Ion Fragment→		U-2Al,CO ₂		U-2Al		U+A-Al,O	
Ion Mass	Abun. Pred. (%)	Ion Mass	Abun. Found (%)	Ion Mass	Abun. Found (%)	Ion Mass	Abun. Found (%)
116	44	402	65	446	45	799	50
117	24	403	40	447	30	800	40
118	74	404	80	448	75	801	90
119	26	405	50	449	30	802	50
120	100	406	100	450	100	803	100
122	14	408	20	452	15	805	20
124	18	410	20	454	15	807	18

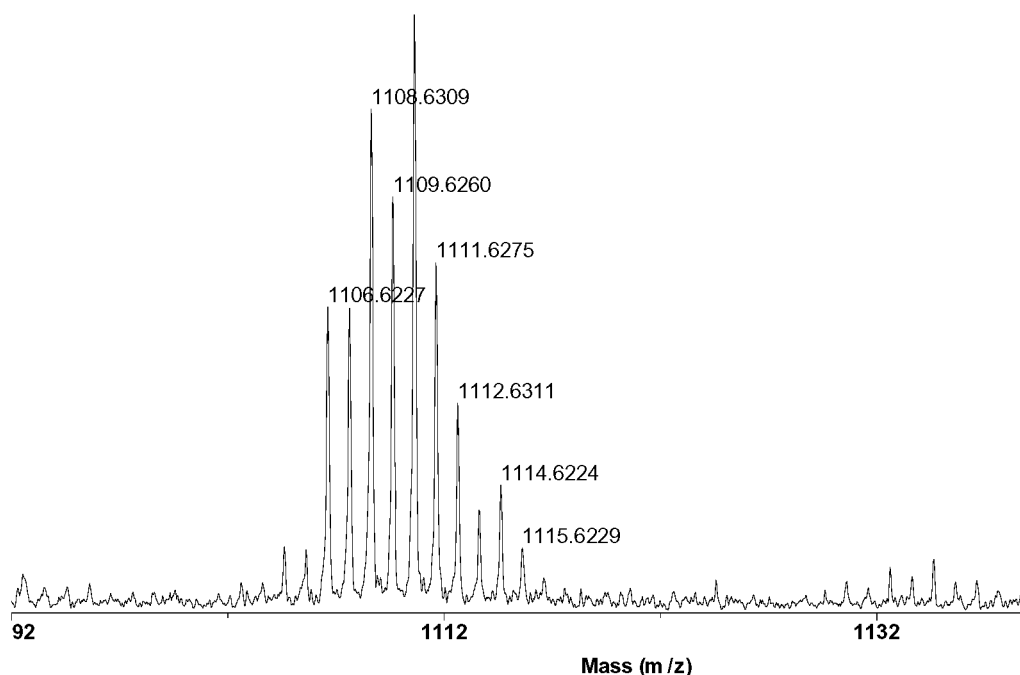


Fig. 11. Expanded F-MALDI MS for the product of diallyltin dichloride and ciprofloxacin for the ion fragment cluster centering about 1110 daltons assigned to the three tin containing structure $2U+Sn,Pip-6Al$ described in Table 11

TABLE 11. Isotopic abundance patterns for the product of diallyltin dichloride and ciprofloxacin and selected three tin-containing ion fragments (% intensity >10%)

Ion Fragment→		2U+Sn,Pip-6Al		2U+Sn,Pip,CO2-6Al	
Ion Mass	Abundance Predicted (%)	Ion Mass	Abundance Found (%)	Ion Mass	Abundance Found (%)
350	20	1105	14	1145	20
351	20	1106	14	1146	20
352	47	1107	43	1147	40
353	42	1108	45	1148	42
354	79	1109	84	1149	85
355	60	1110	60	1150	60
356	100	1111	100	1151	100
357	57	1112	57	1152	50
358	88	1113	35	1153	30
359	37	1114	15	1154	25
360	63	1115	30	1155	25
361	17	1116	15	1156	15
362	34	1117	12	1157	15
364	19	1119	10	1159	12

Table 12 and Figure 12 contain the ion fragments derived for the diallyltinciprofloxacin product in the higher mass range. As noted for

the divinyltin product, the assignments must be considered as suggestive at best but are consistent with the product being polymeric.

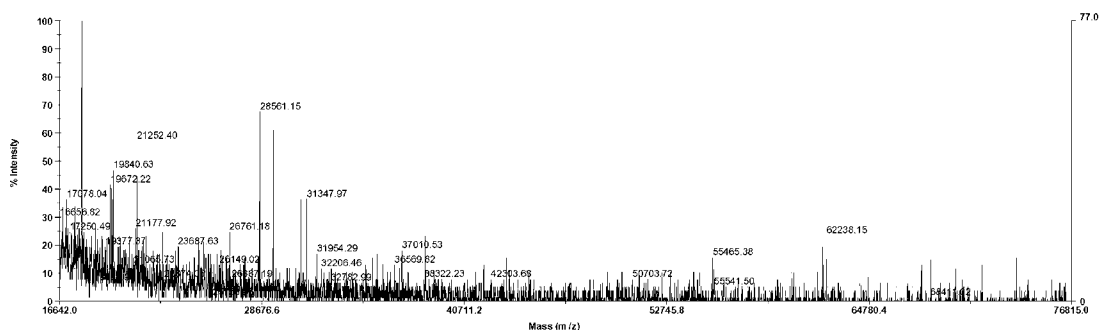


Fig. 12. F-MALDI TOF MS for the product of diallyltin dichloride and ciprofloxacin over the range of 15,000 to 80,000 daltons

TABLE 12. F-MALDI TOF MS results for the product of diallyltin dichloride and ciprofloxacin for the range of 20,000 to 80,000 daltons

m/e	(Tentative) Assignment	m/e	(Tentative) Assignment
21252	40U+CO ₂	28561	54U-Pip+O
31348	59U+Pip	37011	70U-Pip,O
55465	104U+A	62238	117U+B-Al

Thus, F-MALDI is consistent with the proposed structure and the polymeric nature of the products.

Cancer Cell Lines

More organotin compounds have been tested as potential anticancer agents than any other family of compounds.^[1-3] While this is true, with many demonstrating good activity against cancer cell lines, the structural features required for biological activity have not been defined. Both diorganotin and triorganotin compounds can be active, but the most effective groups are normally butyl and less so phenyl. The nature of the ligands in active compounds can also vary enormously. Active organotin compounds do appear to share certain characteristics including available coordination positions on the tin and low hydrolytic cleavage of the Sn-alkyl (or Sn-aryl) bonds. There also appears to be a balance between lipophilic properties needed for crossing the cell membrane and hydrophilic character required to display activity in an aqueous environment.^[22] Thus, while the dimethyltin and diethyltin moieties offer greater hydrophilic character and dilauryltin and dioctyltin units offer greater lipophilic character, the dibutyltin and diphenyltin units appear to offer the necessary balance.

The mechanism(s) of action of tin-containing compounds is not well-understood. Organotin

compounds can bring about numerous cellular effects, including accumulation of organotin in the Golgi apparatus and endoplasmic reticulum; changes in membrane order and phospholipid synthesis; inhibition of DNA, RNA and protein synthesis; enhancement of cell proliferation; changes in cAMP levels and calcium mobilization; inhibition of mitochondrial and Na⁺ – K⁺ ATPases; and activation of enzymes associated with apoptosis.^[23-34] Organotin compounds that exhibit antitumor activity have been shown to bind to and unwind DNA,^[31] effect cell death by necrosis,^[32] and increase extracellular calcium influx and generate reactive oxygen species leading to apoptosis.^[33] Thus, there appears to be many cellular targets and numerous mechanisms of action for organotin compounds. There are also differences in the general toxic effects caused by tin-containing substances. Simple organotin compounds, like triethyltin chloride and dibutyltin dichloride, are known to bring about nervous system and immune system damage.^[34] More complicated compounds, with good antitumor effects, that contain substituted benzoates or steroidcarboxylates showed significant toxicity in the form of paralysis and gastrointestinal disturbances.^[35] However, other organotin compounds with good antitumor activity containing phenanthroline, lupinyl-sulfide, or carnosine showed very little general toxicity.^[32,36] Therefore, a number of organotin compounds are promising effective anticancer agents but do so with varying unwanted side effects through different mechanisms.

Several diverse organotin polymers have been shown to exhibit the ability to inhibit cell growth including the dibutyltin analogue of

carboplatin,^[37,38] polyoxaalkyltin compounds,^[37,38] and the triphenyltin derivative of a substituted p-tolylamide-2-acid, all of which have monomer units linked by oxygen bridges. Because tin has a tendency to coordinate with a variety of ligands, tin compounds may form a wide variety of polymeric species. Here we add to the number of likely organotin anticancer candidates.

The tested cell lines represent a broad range of cancers including breast, bone, prostate, pancreas, and colon. Drug solutions were prepared by dissolving the drug in DMSO and then addition of water to achieve the necessary concentration to be tested. The organotin polymers that are soluble in water were also dissolved in water and water added to achieve the necessary drug concentrations. Standard dilutions are employed decreasing concentration until the GI_{50} is determined.

Different measures are employed in the evaluation of cell line results. Here we use the

two most widely employed- GI_{50} values which is the lowest concentration where growth is inhibited by 50% and the Chemotherapeutic Index, CI_{50} with is a measure of the amount needed to inhibit 50% cell growth, GI_{50} , for the cancer cell line divided by the amount needed to inhibit 50% cell growth for one of the healthy cell lines, here WI-38 cell line. It is to be noted that different researchers generally emphasize one of these measures over the other with neither measure universally accepted. Thus, results from both of these measures are presented. Table 14 contains the GI_{50} results. For comparison with a standard cancer drug cisplatin, the most widely used anticancer drug, was included in the tests and results are also included in Table 14. Further, one human healthy cell line is included in the study, the WI-38 cell. The dibutyltin dichloride monomer was also tested and results added for comparison. Finally, since this is the first report for the divinyltin and diallyl polymers, results for ciprofloxacin and dibutyltin are also added for comparison.

TABLE 13. Cell line Characteristics and Identification

Strain #	NCI Design.	Species	Tumor Origin	Histological Type
3465	PC-3	Human	Prostate	Carcinoma
7233	MDA MB-231	Human	Pleural effusion breast	Adenocarcinoma
1507	HT-29	Human	Recto-sigmoid-colon	Adenocarcinoma
7259	MCF-7	Human	Pleural effusion-breast	Adenocarcinoma
ATCC CCL-75	WI-38	Human	Normal embryonic lung	Fibroblast
ATCC CRL-1658	NIH3T3	Mouse	Continuous cell line of highly contact-inhibited cells	Embryo-fibroblast
AsPC-1		Human	Pancreatic	Carcinoma
PANC-1		Human	Pancreatic	Carcinoma

TABLE 14. GI₅₀ concentrations (micrograms/mL) for organotin polyamines for tested cell lines. Values given in () are standard deviations for each set of measurements

Sample	WI-38	3T3	PC-3	MDA	HT29
Dibutyltin Dichloride	0.15(0.05)	0.20(.05)	1.40(.11)	1.40(.12)	1.20(.11)
Ciprofloxacin Cipro/Divinyl- tin	19.5(1.3)	2.35(0.25)	40.8(3.9)	31.1(2.9)	56.0(2.1)
Cipro/Diallyl- tin	40.0(3.8)	4.50(4.60)	42.2(4.4)	26.4(2.6)	52.1(5.7)
Cipro/Dibutyl- tin	40.4(4.3)	3.85(3.5)	38.9(3.3)	28.8(3.0)	50.1(5.5)
Cisplatin	1.60(0.20)	0.25(0.03)	43.5(3.6)	2.20(0.20)	9.20(1.05)
Sample	MCF-7	AsPC-1	PANC-1		
Dibutyltin Dichloride	0.70(.06)	>0.15	>0.15		
Ciprofloxacin Cipro/Divinyl- tin	19.3(2.1)	40.5(4.0)	41.3(4.0)		
Cipro/Diallyl- tin	30.4(3.5)	40.5(3.8)	44.0(4.4)		
Cipro/Dibutyl- tin	30.4(3.5)	40.5(3.8)	44.0(4.4)		
Cisplatin	7.50(0.96)	36.6(3.3)	38.7(3.7)		
Cisplatin	3.00(.28)	>0.05	>0.05		

The divinyltin and diallyltin ciprofloxacin polymers show a relatively low toxicity to the WI-38 healthy cells which is positive since low toxicity to healthy cells is desirable. Cisplatin is over 2,500 times as toxic towards the healthy cell line compared with the divinyltin and diallyltin ciprofloxacin polymers. The dibutyltin ciprofloxacin polymer is about 25 times as toxic in comparison to the divinyltin and diallyltin ciprofloxacin polymers. Thus, the new polymers

exhibit a low toxicity towards the healthy WI-38 cells. On a concentration level, the dibutyltin/ciprofloxacin polymer shows good inhibition towards the 3T3, MDA, HT29 and MCF-7 cell lines consistent with the dibutyltin, in general, offering inhibition at lower concentration in comparison to other alkyltin moieties. The divinyltin and diallyltin ciprofloxacin polymers show only moderate ability to inhibit any of the cell lines except the

3T3 where it exhibits good inhibition at a relatively low concentration. None of the products exhibit good low concentration of the pancreatic cell lines consistent with the well known resistance of these cell lines to essentially all chemo drugs.

The second measure is the 50% chemotherapeutic index, CI_{50} . The chemotherapeutic index is the concentration of the compound that inhibits the growth of the tumor cell by 50% divided by the concentration of the

compound that inhibits the growth of the normal cell (WI-38) by 50%. Larger values are desired since they indicate that a larger concentration is required to inhibit the healthy cells in comparison to the cancer cells or stated in another way, larger values indicate some preference for inhibiting the cancer cells in preference to the normal cells. In general, CI_{50} values larger than 2 are considered significant. CI_{50} values are given in Table 15. Those CI_{50} values equal to and greater than two are highlighted by bold type for ready identification.

TABLE 15. Chemotherapeutic Index-50% for the samples for each cell line.

Sample	WI-38/ WI-38	WI-38/ 3T3	WI-38/ PC-3	WI-38/ MDA	WI-38/ HT-29
Dibutyltin-Dichloride	1	0.75	0.11	0.11	0.13
Ciprofloxacin	1	8.3	0.01	0.63	0.35
Cipro/Divinyltin	1	8.9	0.95	1.5	0.77
Cipro/Diallyltin	1	11	1.0	1.4	0.80
Cipro/Dibutyltin	1	6.4	0.04	0.72	0.17
Cisplatin	1	0.003	0.02	0.02	0.008
Sample	WI-38/ MCF-7	WI-38/ AsPC-1	WI-38/ PANC-1		
Dibutyltin-Dichloride	0.21	<1	<1		
Ciprofloxacin	1.0	0.48	0.47		
Cipro/Divinyltin	1.3	1.0	0.91		
Cipro/Diallyltin	1.3	1.0	0.91		
Cipro/Dibutyltin	0.21	0.04	0.04		
Cisplatin	0.005	<0.3	<0.3		

The only values greater than two are found for the 3T3 cells. In comparison to cisplatin, the CI_{50} values for the polymers are generally greater, but not generally greater than two. Thus, while the new polymers exhibit low toxicity to the healthy WI-38 cells, they do not show outstanding CI_{50} or GI_{50} values. Even so, this low toxicity towards healthy cells may be useful in other applications including use as antiviral agents. A number of ciprofloxacin-containing polymers have shown the ability to inhibit a number of viruses. Future testing is warranted in select situations but not as a general purpose anticancer drug.

An additional question concerns the influence of simply having the dibutyltin moiety present in a polymer. Table 16 contains the CI_{50} values normalized against the CI_{50} value for dibutyltin dichloride. This ratio should offer some measure of the effectiveness of having the dibutyltin moiety within the polyamine polymer.

No norm has been established for this kind of comparison but it is informative that most (93%) of the values are 1 or greater with many of the values much greater than 2 consistent with the advantage of having the organotin moiety as part of the polymer structure. All of the divinyltin and diallyltin ciprofloxacin polymers have values greater than 5.

SUMMARY

Organotin polyamine esters have been formed in good yield from reaction of divinyltin dichloride and diallyltin dibromide with ciprofloxacin employing a modified interfacial reaction system. IR spectroscopy, MALDI MS spectrophotometry, and light scattering photometry are consistent with the assigned structure. IR spectroscopy shows the formation of new bands consistent with the presence of Sn-O and Sn-N linkages. F-MALDI MS spectrophotometry shows ion fragment clusters that

contain one, two, and three tin atoms. Light scattering photometry shows that the products are polymeric with weight average molecular weights in the vicinity of 10^6 . The products show reasonable inhibition of cancer cells from a variety of cancers but not as good as found for dibutyltin and diphenyltin derived products. The best values are found against 3T3 cells, often employed as an indicator of additional antitumor behavior. Along with low GI_{50} values, the two polymers also show good CI_{50} values towards the 3T3 cells. The GI_{50} concentrations for the polymers in the range of 4 micrograms/mL are in the same range as found for cisplatin, the most widely employed anticancer agent.

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