WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 3, 1832-1853. Research Article ISSN 2277– 7105

SYNTHETIC NUCLEIC ACIDS FROM THYMIDINE AND ORGANOTIN DIHALIDES AND THEIR ABILITY TO INHIBIT HUMAN CANCER CELL LINES INCLUDING PANCREATIC ACID AND GLIOBLASTOMAS CELL LINES

Loretta Chen[1], Jessica Frank[1], Charles E. Carraher, Jr.[*1], Michael R. Roner[2], Kimberly Shahi[2], Paul Slawek[1] and Francesca Mosca[1]

[1]Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431.

[2]University of Texas Arlington, Department of Biology, Arlington, TX 76010.

Article Received on
14 Jan. 2021,

Revised on 03 Feb. 2021,
Accepted on 24 Feb. 2021

DOI: https://doi.org/10.17605/OSF.IO/JYKNM

***Corresponding Author**
Dr. Charles E. Carraher Jr.
Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431.

ABSTRACT

Using the interfacial polymerization technique, synthetic nucleic acid polymers derived from thymidine and organotin dihalides were formed in moderate yield and chain length. Products were synthesized using the interfacial polycondensation process and commercially available reagents for easy reproducibility. Structural characterization of polymers was carried out using Infrared Spectroscopy (IR), and Matrix-assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-MS). Light scattering Photometry was used to determine chain length. The polymers showed good inhibition of a battery of human cancer cell lines including two breast, two pancreatic, and two glioblastoma brain cancer lines. All polymers tested were able to differentiate between healthy and malignant cells, showing excellent selectivity toward malignant cells.

KEYWORDS: thymidine, organotin dihalides, organotin polymers, synthetic nucleic acids, pancreatic cancer, breast cancer, glioblastomas brain cancer, MALDI MS, interfacial polymerization.

INTRODUCTION

Carraher and Millich were issued the first patent for the chemical synthesis of nucleic acids in 1971.[1] The nucleic acid was derived from thymidine and 2-deoxy-D-ribose. This was followed by the synthesis of additional related nucleic acid like polymers, [2-7] More recently, we focused on the synthesis metal-containing polymers due to their ability to inhibit a variety of viruses, bacteria, fungi, and cancers. These included a variety of metal-containing polymers including tin, [8-22] Group 4 and 5 metallocenes, [23-30] platinum and palladium [31-33] and Group 15 metals .[31-40]

Thymidine, (Figure 1), is one of the four bases present in DNA. Since thymidine has two reactive functional groups, as a difunctional compound with two hydroxyls on the sugar moiety, the di-functionality of this compound prevents crosslinking during polymerization. The other natural nucleic acids contain additional functional groups so complicated the synthesis of linear products.

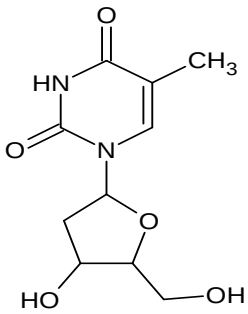

Figure 01: Thymidine structure.

Recently, we described in this journal the synthesis of Group 4 metallocene nucleic acid like polymers (Figure 2) and their ability to inhibit a wide range of human cancer cells.[41]

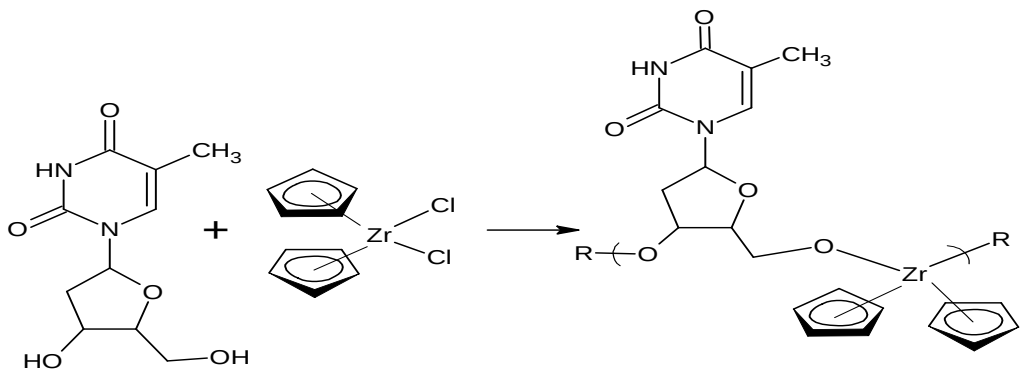

Figure 02: General repeat unit for the product of zirconocene dichloride and thymidine where R represents simple chain extension.

Here we describe the synthesis of the analogous organotin polymers (Figure 3).

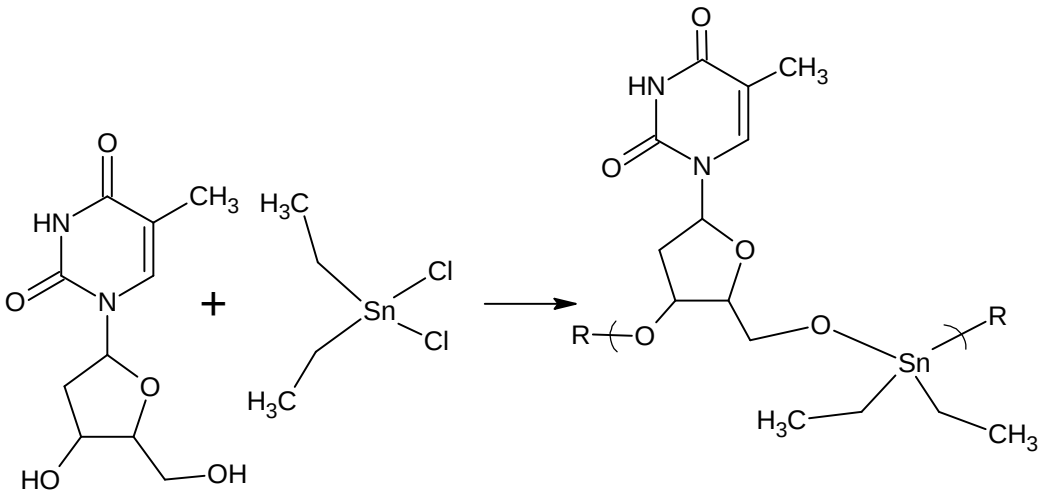

Figure 03: Reaction scheme for the synthesis of organotin nucleic acids derived from diethyltin dichloride.

While it has characteristics of a nucleic acid with the presence of thymidine in a polyether repeat unit, there is no guarantee that the chain will have the precise 3'-5' unit throughout the chain. Even so, as will be shown these products exhibit good inhibition of a wide group of human cancer cell lines including breast, pancreatic, and glioblastomas brain cancer cells.

Structurally, the polymers can be named as polyethers. We have synthesized a wide variety of organotin polyethers. [10,15, 42-50] These include the first water-soluble metal-containing polymers from reaction of poly(ethylene glycol) (also called poly(ethylene oxide)) with various organotin dihalides. [10,15,42-44]

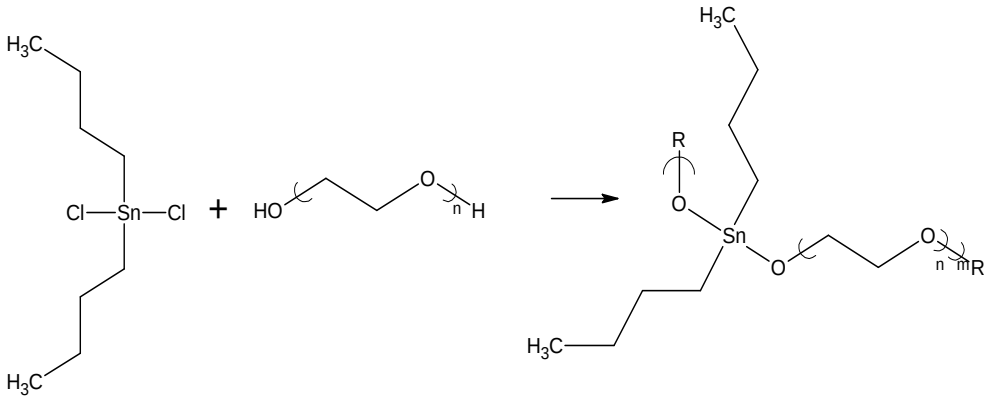

Figure 04: Synthesis of organotin poly(ethylene glycol) polymers.

EXPERIMENTAL

Reactions and Reactants: Reactions were carried out using the interfacial polycondensation technique. Briefly, an aqueous solution (30 ml) containing the thymidine (0.00300 mol) and sodium hydroxide (0.00600 mol) was transferred to a one-quart Kimax emulsifying jar fitted on top of a Waring Blender (model 1120; no load speed of about 18,000 rpm; reactions were carried out at room temperature, at about 25^{o}C). Stirring was begun and a hexane solution (30 ml) containing the organotin dichloride (0.00300 mol) was rapidly added (about 3-4 seconds) through a hole in the jar lid using a powder funnel. The resulting solution was blended for 15 seconds. The precipitate was recovered using vacuum filtration and washed several times with deionized water and hexane to remove unreacted materials and unwanted by-products. The solid was washed onto a glass petri dish and allowed to dry at room temperature.

Diphenyltin dichloride (1135-99-5), dimethyltin dichloride (753-73-1), thymidine (50-89-5) and dibutyltin dichloride (683-18-1) were purchased from Aldrich Chemical Co., Milwaukee, WS; diethyltin dichloride (866-55-7) was obtained from Peninsular Chemical Res., Gainesville, FL; and dioctyltin dichloride (3542-36-7), was obtained from Ventron Alfa Inorganics, Beverly, Mass.

Physical Characterization: Light scattering photometry was carried out employing a Brice-Phoenix Universal Light Scattering Photometer Model 4000. Infrared spectra were obtained employing attenuated total reflectance infrared spectroscopy utilizing a JASCO FT/IR-4100 fitted with an ATR Pro 450-s.

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. Fifty to two hundred shots were typically taken for each spectrum. Results employing trans-2-[3-(4-tert-butylphenyl)-2-propenylidene], malonitrile (DCTB) as the matrix are included in the present paper.

Cell Testing: The toxicity of each test compound was evaluated using a variety of cancer cell lines and with human normal embryonic lung fibroblast (WI-38) and mouse embryo-fibroblast (NIH/3T3) cell line as standards. Following a 24 hr incubation period, the test compounds were added at concentrations ranging from 0.0032 to 32 microg/mL and allowed

to incubate at 37°C with 5% CO_2 for 72 hrs. Following incubation, Cell Titer-Blue reagent (Promega Corporation) was added (20 uL/well) and incubated for 2 hrs. Fluorescence was determined at 530/590 nm and converted to % cell viability versus control cells.

All cytotoxicity values were calculated against a base-line value for each line that was generated from "mock-treatment" of the normal and tumor cell lines with media supplemented with all diluents used to prepare the chemotherapeutic compounds. For example, if the compounds were dissolved in DMSO and serial dilutions prepared in in Eagle's minimal essential medium, MEM, to treat the cells, then the mock-treated cells were "treated" with the same serial dilutions of DMSO without added chemotherapeutic compound. This was done to ensure that any observed cytotoxicity was due to the activity of the compound and not the diluents. For the studies reported here, the mock-treatment never resulted in a loss of cell viability of more than one percent, demonstrating that the activity observed was not due to cytotoxicity of any of the diluents used, but was due to activity of the tested compounds. When inhibition begins, the slop of the concentration/inhibition curve is steep until total inhibition occurs.

RESULTS AND DISCUSSION

Polymerization occurs employing the interfacial polymerization process developed by Morgan and coworkers at DuPont and enlarged by Carraher. [51-53] The interfacial synthetic technique is employed industrially in the production of aramid fibers and polycarbonates.[53,54] The rapidity of the reaction is a consequence of at least two factors. [53,54] First, the activation energy between acid chlorides and alcohols is about 20 kcal/mol compared with the typical reaction between acids and diols of about 40 kcal/mol. Second, the rapid stirring enlarges the interfacial surface on the order of 10,000 time. The reaction is rapid being completed within 10 seconds.

Table 1 contains the yield and chain length, DP, for the products as a function of the organotin moiety. Yield is in the minimum range. Chain length decreases as the size of the organic moiety on the tin increases consistent with size being an important factor in polymer chain growth.

Table 01: Yield and chain length as a function of the organotin dihalide for the thymidine polymers.

Organotin Moiety	Yield, %	Molecular Weight	Chain Length, DP
Me_2Sn	51	4.9×10^6	12,000
Et_2Sn	48	1.4×10^6	3,300
Bu_2Sn	58	1.5×10^6	3,200
Oc_2Sn	67	1.0×10^6	1,700
Ph_2Sn	85	5.6×10^6	11,000

Infrared Spectral Results: Table 2 contains band assignments for the diethyltin dichloride, thymidine, [55] and the resulting polymer. The polymer IR shows the absence of bands derived from the presence of the OH group as expected since it is now part of the connecting group Sn-O-C. The presence of bands characteristic of the thymidine are present including those derived from the C=O, N-H, and numerous C-H stretching, scissoring, wagging, rocking, and twisting associated bands. Diethyltin dichloride has seven characteristic bands, all presence in the IR of the polymer. The Sn-C is found in the monomer at 521 and 492 and in the polymer at 533 and 490. The Sn-O band is generally found about 1000. The presence of a new band found at 1009 assigned to the formation of the Sn-O linkage. The presence of the O-C linkage in the Sn-O-C grouping is generally found about 770. A new band is found at 777 and is assigned to this linkage. Thus, IR spectral results are consistent with the proposed organotin polyether linkage.

Table 02: Infrared band assignments for the monomers and polymer from diethyltin dichloride and thymidine.

Assignment	Et_2SnCl_2	Thymidine	Polymer
O-H		3641,3620,3482	
N-H		3428	3446
CH_2 st		2994	2990
CH_3 asym st	2979	2977	2975
CH_2 asym st	2962	2964, 2934	2947
CH_3 sym st	2929		2916
CH_2 sym st	2869		2868
C=O		1690,1665	1698,1657
CH_2 scissor bend	1448		1454
CH_3 op scissor		1435	1435
CH_2 wag		1172	1172
Sn-O-C st			1009
(Sn-)O-C			777
N-C op deformation		565	565
Sn-C asym st	521		533
Sn-C Sym st	492		490

MALDI MS Results: We have been employing MALDI MS to assist in the structural identification of our products. While MALDI MS was advertised as a method to obtain chain lengths, and molecular weights of polymers, it will do so only in certain cases. To be effective, the matrix and polymer must be soluble in volatile liquids that allow a "cocoon" to be made around the polymer. This casing both holds the polymer chain together and promotes the "volatilization" of the polymer allowing it to be ionized and analyzed. For most polymers this is not the case, so we utilize an approach that allows the MALDI MS to be used for samples that do not conform to this rquirememt. Polymers that posses heterochains tend to fracture under MALDI MS giving polymer chain fragments and it is the fragments rather, than entire chains, that are studied. The technique as employed by us has been recently reviewed. [55-59]

The following abbrevations are employed: T = thymidine fragment, Na = sodium (the sodium is present as a contaminant in the samples), U = one unit, 2U = 2 units, 3U = three units, etc.

A portion of the MALDI MS spectra for the polymer derived from dibutyltin dichloride and thymidine is given in Figure 5.

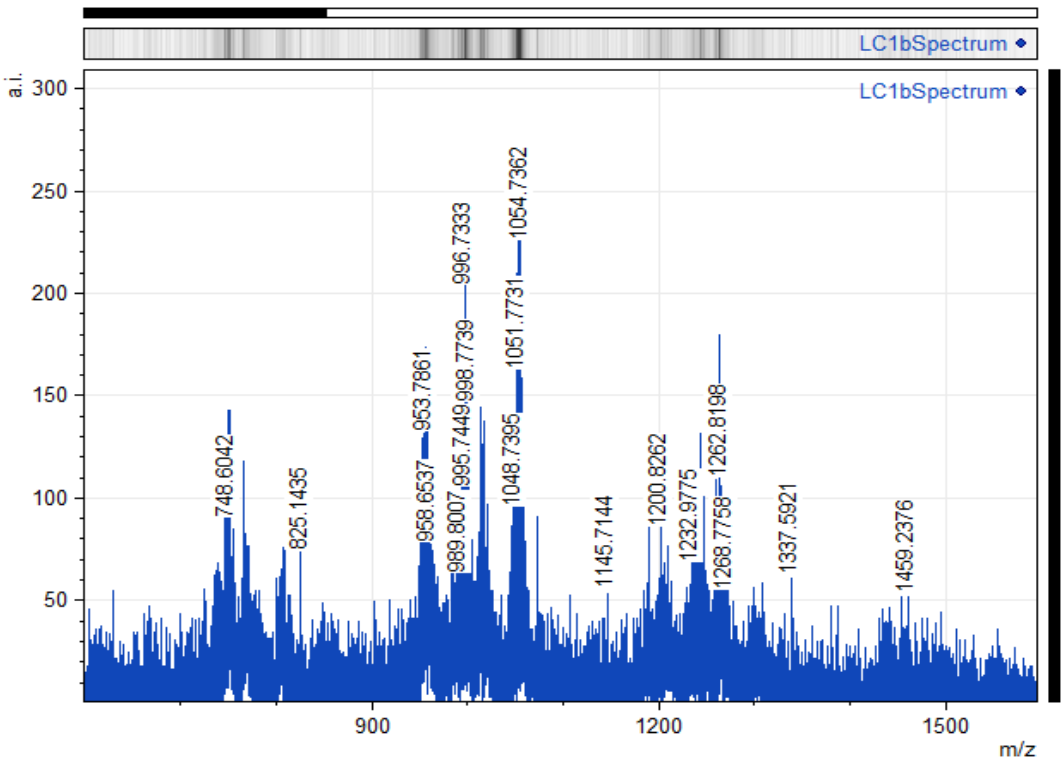

Figure 05: MALDI MS of the product of dibutyltin dichloride and thymidine over the approximate mass range of 600 to 1600 daltons.

The major ion fragments are given in Table 3. Ion fragments for up to nine units are found.

Table 03: Major ion fragments for the product from dibutyltin dichloride and thymidine over the mass range of 600 to 4500 daltons.

Ion fragment	(Tentative) Structureal Assignment
749	U+Bu_2Sn, O, Na
954	2U+Na-0
959	2U-0,Na
990	2U+0,Na
1145	2U+BuSn,Na
1201	2U+Bu_2Sn,0
1459	3U+0,Na
1896	4U
2132	4U+Bu_2Sn
2370	5U
2604	5U+Bu_2Sn
2844	6U
3080	6U+Bu_2Sn
3318	7U
3554	7U+Bu_2Sn
3792	8U
4032	8U+T
4268	9U

Each of the spectral bands is actually a combination of many bands associated with different masses from isotopes associated with the isotopes of tin as shown in Figure 6 for the band centered about 749 daltons. The length of each band is associated with the abundance of the particular isotope and its natural abundance shown in the left column. The relative abundance is given in Table 4 for this particular peak. The relative abundance is similar to that predicted shown in the next to the furthest left-hand column consistent with the presence of two tin atoms contained within the overall band at 749 daltons.

A portion of the MALDI MS spectra for the product derived from the reaction of dioctyltin dichloride and thymidine is given in Figure 6. The major ion fragments and their tentative identify are given in Table 3.

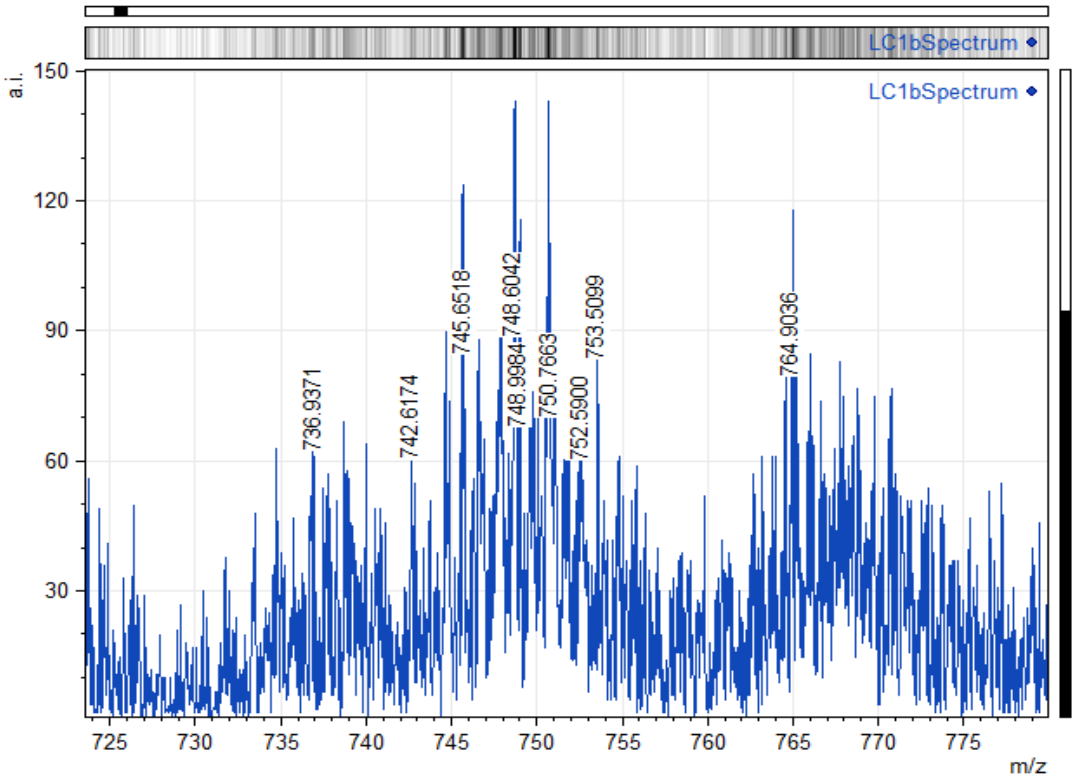

Figure 06: Portion of MALDI MS showing ion fragment bands for the polymer derived from the reaction of dibutyltin dichloride and thymidine.

Table 4 contains the ion fragment cluster analysis of two ions derived from the dibutyltin/thymidine product. The match is reasonable to the ion fragment cluster containing two tine atoms.

Table 04: Percentage relative abundances for two ion fragment clusters containing two tin atoms. Only ions with a (standard) relative abundance > 10% are given.

Standard			U+Bu_2Sn,O ,Na			2U+Na-0	
Da.	%-Rel. Abund.		Da.	%-Rel. Abund. Found		Da.	%-Rel. Abund. Found
232	12		743	12		948	12
233	13		744	13		949	14
234	43		745	42		950	42
235	35		746	34		951	34
236	94		747	92		952	91
237	51		748	52		953	52
238	100		749	100		954	100
239	35		750	34		955	36
240	81		751	82		956	81
242	32		753	31		958	30
244	22		755	21		960	23

Figure 7 contains a portion of the MALDI MS derived from the product of dioctyltin dichloride and thymidine.

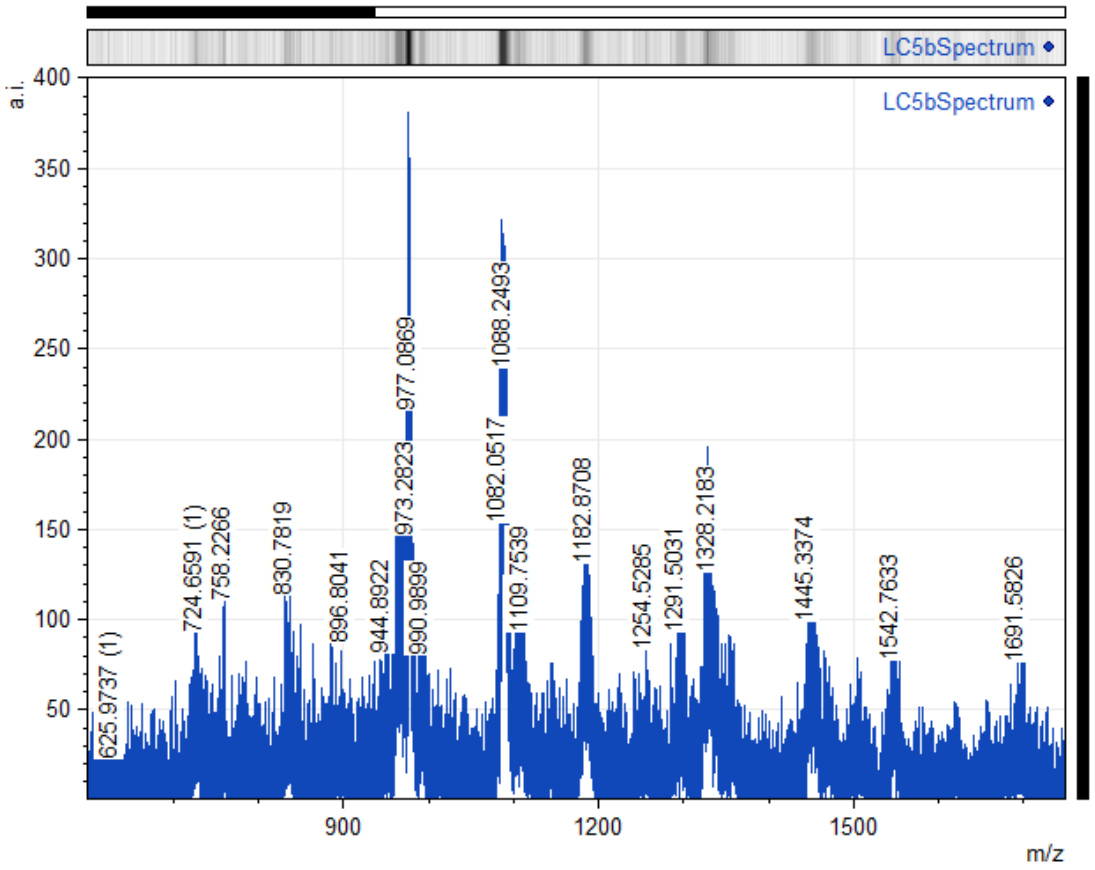

Figure 07: MALDI MS of the product from dioctyltin dichloride and thymidine over the approximate mass range of 700 to 1700 daltons.

Table 05: contains major ions present in the MALDI MS of the product of dioctyltin dichloride and thymidine.

m/e	(Tentative) Structural Assignment
626	U+O,Na
831	U+T
945	U+Oc_2Sn,0
973	U+Oc_2Sn,O,Na
1183	2U+O
1445	2U+O,Na
1542	2U+Oc_2Sn,O
1758	3U
1993	3U+T

2328	4U-O
2584	4U+T
2930	5U
3170	5U+T
3276	5U+Oc_2Sn
3539	6U+Oc_2Sn
3589	6U+Na
3862	6U+Oc_2Sn
4118	7U+O
4358	7U+A

Ion fragments are present containing up to seven units. Table 5 contains a relative abundance match for an ion fragment cluster containing a single tin. The relative isotope abundances are consistent with the presence of a single tin atom in the cluster.

Table 06: Percentage relative abundances for the ion fragment cluster assigned to U+T containing one tin atom. Only ions with a (standard) relative abundance > 10% are given.

Standard			Found	
Da.	**%-Relative Abundance**		**Da.**	**%-Relative Abundance**
116	45		45	827
117	24		23	828
118	75		77	829
119	26		28	830
120	100		100	831
122	14		15	833
124	17		18	835

Table 7 contains isotope abundance matches for two ion fragment clusters containing two tin atoms.

Table 07: Percentage relative abundances for ion fragment clusters containing two tin atoms.

Standard			2U+O			2U+O,Na	
Da.	**%-Rel. Abund.**		**Da.**	**%-Rel. Abund. Found**		**Da.**	**%-Rel. Abund. Found**
232	12		1177	13		1439	13
233	13		1178	15		1440	15
234	43		1179	44		1441	44
235	35		1180	34		1442	36
236	94		1181	94		1443	95
237	51		1182	53		1444	51
238	100		1183	100		1445	100
239	35		1184	35		1446	34
240	81		1185	84		1447	83
242	32		1187	34		1449	29
244	22		1189	24		1451	22

As in essentially all cases with metal-containing products, the major sites for chain breakage is at the hetero atoms as shown in Figure 8.

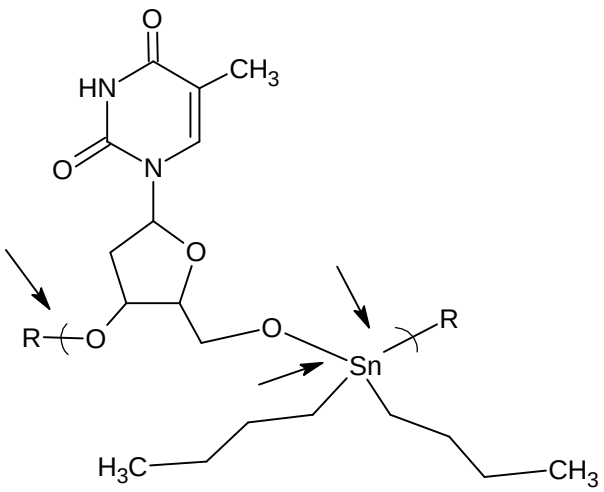

Figure 08: Sites where major chain scission occurs.

Cell Inhibition Results: Much of our recent effort is the synthesis of potential drugs for the purpose of evaluation of structure/cancer activity building a catalogue of results that will assist in further syntheses. The cancer cells employed in the current study are given in Table 9.

Table 09: Cell lines employed in the current study.

Strain Number	NCI Designation	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
CRL-1658	NIH/3T3	Mouse	Embryo-continuous cell line of highly contact-inhibited cells	Fibroblast
	U251	Human	Glioblastoma multiforme	Astrocytoma
	G55	Human	Glioblastoma	astrocytoma's
	AsPC-1	Human	Pancreatic cells	Adenocarcinoma
	PANC-1	Human	Epithelioid pancreatic cells	Carcinoma

Two values are typically taken when evaluating cell inhibition results. The first one is the amount of material that inhibits the particular cell line. It is generally given the name effective concentration, EC, and recorded as the amount necessary to inhibit 50% of the growth, EC_{60}. Physically it is described at the amount that induces a response halfway between the baseline and maximum. As inhibition begins, the slope of the concentration vs inhibition is steep and continues to total inhibition. Table 10 contains EC_{50} for monomers and polymers and the standard, cisplatin. Cisplatin is widely used in the treatment of a variety of cancers including brain tumors, ovarian cancer, testicular cancer, neuroblastoma, lung cancer, mesothelioma, bladder cancer, breast cancer, head and neck cancer and cervical cancer. It is quite toxic offering many unwanted side effects. Its high toxicity is shown from its low EC_{50} values for the standard WI-38 and NIH-3T3 cells.

The EC_{50} values for the polymers are generally less than those for the monomers. Thus, it is the combination and/or polymeric nature of the drug that results in the low EC_{50} values. Polymeric drugs present several potential advantages over traditional small, monomeric drugs, for the inhibition of cancer. To follow is a brief list that highlights some of these advantages.[43-49] To begin, the much larger size of polymers compared to monomeric drugs can aid in the retention of the polymer drug in the body. Polymer drugs inability to easily pass through biological membranes due to their larger size increases the retention time which allows more time for drug to take effect. Also, a result, of their larger size, polymers are filleted by the kidneys more slowly compared to small molecules reducing possible kidney

damage. Next, again due to their larger size, polymers may allow for greater binding possibilities to the target than a monomeric drug. Polymers offer the ability to bind more locations increasing their activity and effectiveness. Another possible advantage is that polymers exhibit the ability to incorporate multiple anti-cancer drugs into a single polymer chain. This ability to contain more than one anti-cancer agent is unique to polymers and may prove beneficial as it allows for a single drug to have varying modes of action to combat cancer. Additionally, polymers can have the ability to behave as a prodrug allowing them to enter the cell in an inactive form and slowly degrade into an active form inside the cell. The slow degradation of the polymer into the active form can be thought of a sort of time release drug. Large molecules, like polymers, may have the ability to collect to a greater extent in solid tumors compared to healthy tissues due to the fact that tumors have limited lymphatic drainage therefore, the polymer drug is able to be built up in the tumor. This effect of the polymers collecting in the tumors is known as the enhanced permeability and retention (EPR) effect. Polymeric drugs also have the advantage of greater design possibilities compared to a monomeric drug. Polymers may be synthesized with specific characterizations, chain length, polarity, monomeric components and cross-linking allowing polymers the possibility to maximize their anticancer activity. Finally, polymers may be coupled to molecules that have the ability to target a specific transport mechanism. The coupling of these molecules can be used as an escort to transport the polymer drug to a specific site without influencing the drugs activity.[35]

Of interest here are the two cancer cell lines where there are currently no decent chemo agents, pancreatic and glioblastomas brain cancers. In the United States about 32,000 individuals are diagnosed with pancreatic cancer yearly, with the average life span after diagnosis being less than one year. Worldwide, it is the fourth leading cause of cancer related death. In the current study the two most widely studied human pancreatic cancer cell lines are selected. AsPC-1 is an adenocarcinoma pancreatic cell line, which accounts for around 85% of the diagnosed human pancreatic cancers, and PANC-1 is an epithelioid carcinoma pancreatic cell line, accounting for about 10% of the human pancreatic cancer cases. For the current study, the organotin polymers displayed the ability to inhibit both cell lines, the best inhibition for PANC-1 being the diethyltin (0.13 µg/mL) and the best inhibition for AsPC-1 also being the diethyltin (0.12 µg/mL). This similarity in the inhibition of both cell lines is consistent with the idea that the polymers offer broad-spectra inhibition of other pancreatic cancer cell lines.

Similar to pancreatic cancer, the expected life expectancy after diagnosis for glioblastoma is less than 1 year. The polymers were tested against two glioblastoma cell lines. G55 is a new human glioblastoma cell line. It was developed by C. David James (Department of Neurological, University of California San Francisco). G55 is a human glioblastoma cell line that had been passed through nude mice and re-established as a stable xenograft cell line. The U251 cell line is one of the most used cancer cell lines. It is derived from a human malignant glioblastoma multiforme. Astrocytoma's are brain cancer that originates in astrocytes. High-grade astrocytoma's, called glioblastoma multiforme, are the most malignant of all brain tumors. The thymidine polymers inhibit both cell lines.

The polymers also inhibit all of the other tested human cancer cell lines.

Table 10: EC_{50} Concentrations (micrograms/mL) for the tested compounds. Values Given in ( ) are standard deviations for each set of measurements.

Sample	*WI-38*	*PANC-1*	*AsPC-1*	*U251*	*G55*
Me_2SnCl_2	0.22(.1)	0.80(.1)	0.71(.1)	0.91(.5)	1.2(.6)
Me_2Sn/TH	0.45(.05)	0.25(.02)	0.23(.02)	0.51(.04)	0.55(.04)
Et_2SnCl_2	0.20(.1)	0.48(.1)	0.90(.1)	1.1(.6)	1.3(.6)
Et_2Sn/TH	0.45(.05)	0.13(.02)	0.12(.02)	0.23(.04)	0.28(.04)
Bu_2SnCl_2	0.20(.05)	0.0032(.001)	0.012(.01)	1.0(.2)	1.0(.4)
Bu_2Sn/TH	0.46(.05)	0.24(.02)	0.22(.02)	0.42(.04)	0.44(.04)
Oc_2SnCl_2	0.30(.1)	0.85(.1)	0.85(.1)	1.3(.7)	0.95(.6)
Oc_2Sn/TH	0.44(.05)	0.23(.02)	0.22(.02)	0.44(.04)	0.49(.04)
Ph_2SnCl_2	0.25(.1)	0.71(.1)	0.83(.1)	0.68(.6)	0.89(.6)
Ph_2Sn/TH	0.43(.05)	0.20(.02)	0.22(.02)	0.35(.04)	0.37(.04)
Thymidine	1.7(.5)	1.7(.5)	1.8(.5)	1.8(.5)	1.8(.5)
Cisplatin	0.012(.01)	0.0023(.005)	0.0035(.005)	0.015(.01)	0.020(.01)

Sample	*PC-3*	*MDA-MB-231*	*HT-29*	*MCF-7*	3T3
Me_2SnCl_2	0.51(.1)	0.44(.1)	0.56(.1)	0.66(.1)	0.43 (.1)
Me_2Sn/TH	0.27(.02)	0.24(.02)	0.22(.02)	0.22(.02)	0.43(.05)
Et_2SnCl_2	0.61(.1)	0.64(.1)	0.71(.1)	0.77(.1)	0.46(.1)
Et_2Sn/TH	0.13(.02)	0.11(.02)	0.12(.02)	0.11(.02)	0.42(.05)
Bu_2SnCl_2	1.4(1.1)	1.4(1.3)	1.2(.1)	0.70(.06)	0.20 (.05)
Bu_2Sn/TH	0.23(.02)	0.25(.02)	0.21(.02)	0.23(.02)	0.45(.05)
Oc_2SnCl_2	0.55(.1)	0.65(.1)	0.65(.1)	0.70(.1)	0.56(.1)
Oc_2Sn/TH	0.21(.02)	0.22(.02)	0.21(.02)	0.22(.02)	0.45(.05)
Ph_2SnCl_2	0.82(.1)	0.76(.1)	0.56(.1)	0.68(.1)	0.66(.1)
Ph_2Sn/TH	0.24(.02)	0.24(.02)	0.21(.02)	0.21(.02)	0.42(.05)
Thymidine	1.8(.05)	1.8(.05)	1.8(0.5)	1.7(.5)	1.8(.5)
Cisplatin	0.0044(.004)	0.0029(.002)	0.0041(.003)	0.0057(.003)	0.015(.01)

The second value used to evaluate the ability of materials to inhibit cell growth is the ratio of the EC_{50} for the polymers divided into the EC_{50} for a standard cell line, here the WI-38 human call or NIH 3T3 cell line. The term chemotherapeutic index, CI_{50}, is often used to describe this ratio. It is generally accepted that WI-38 results are better at indicating ability of the tested compounds to prevent cancer growth in animals, and finally humans.

CI_{50} values based on only WI-38 cells are presented in Table 11. Large values are desired since it indicates that there is a preference for inhibiting the cancer cell lines in comparison to the standard cells.

There are a number of instances with the CI_{50} values are near and greater than two with CI_{50} values in the same vicinity as cisplatin.

Table 11: CI_{50} values for polymers and monomers based on data given in Table 10.

Sample	EC_{50}WI-38/ EC_{50}PNC-1	EC_{50}WI-38/ EC_{50}AsPC-1	EC_{50}WI-38/ EC_{50}PC-3	EC_{50}WI-38/ EC_{50}MDA
Me_2SnCl_2	0.28	0.31	0.43	0.50
Me_2Sn/TH	1.8	2.0	1.7	1.9
Et_2SnCl_2	0.83	0.81	0.91	0.91
Et_2Sn/TH	3.5	3.8	3.5	2.8
Bu_2SnCl_2	60	17	0.14	0.14
Bu_2Sn/TH	1.9	2.1	2.3	1.8
Oc_2SnCl_2	0.35	0.35	0.55	0.46
Oc_2Sn/TH	1.9	1.9	2.1	2.0
Ph_2SnCl_2	0.35	0.31	0.30	0.33
Ph_2Sn/TH	2.2	2.0	1.8	1.8
Cisplatin	5.2	3.4	2.7	4.1

Sample	EC_{50}WI-38/ EC_{50}U251	EC_{50}WI-38/ EC_{50}G55	EC_{50}WI-38/ EC_{50}HT-29	EC_{50}WI-38/ EC_{50}MCF-7
Me_2SnCl_2	0.45	0.31	0.39	0.39
Me_2Sn/TH	0.88	0.82	2.1	2.1
Et_2SnCl_2	0.34	0.29	0.67	0.71
Et_2Sn/TH	0.20	0.16	3.8	4.1
Bu_2SnCl_2	0.33	0.33	0.17	O,29
Bu_2Sn/TH	1.0	1.2	2.2	2.3
Oc_2SnCl_2	0.34	0.46	0.46	0.43
Oc_2Sn/TH	0.12	0.12	2.1	2.0
Ph_2SnCl_2	0.29	0.30	0.45	0.37
Ph_2Sn/TH	1.0	0.90	2.0	2.0
Cisplatin	0.80	0.57	2.9	2.1

The lowest CI_{50} values are for the brain cancer cell lines and even here the dibutyltin polymers shows values of 1 and 1.2. A number of the polymers have CI_{50} values of two and greater for the pancreatic cancer cell lines. This is consistent with the drugs showing a good ability to differentiate between the healthy and cancer cell.

In summary, all of the polymers show decent EC_{50} values in the tenths of a microgram/mL range and many exhibit CI_{50} values of two and greater. Thus, they show good inhibition of the tested human cancer cell lines.

SUMMARY

Synthetic nucleic acid polymers were synthesized in moderate yield from the interfacial polymerization of thymidine and organotin dihalides. Molecular weight decreases as the bulk on the organotin increases consistent with size having an influence on polymer formation. Infrared spectroscopy shows the formation of Sn-O bonds and absence of the R-OH group consistent with what is expected for the polyether formation. The polymers show good inhibition of a group of human cancer cell lines to the nanogram/mL range with good differentiation between the cancer and standard cell lines. They are a group of polymers that represent an additional structure for further study in the war against cancer.

REFERENCES

1. Carraher CE, Millich F. Buffering agents in interfacial phosphorous polyester process. US Pat, 1970; 3: 491-061.
2. Millich F, Carraher CE. Polyphosphate esters containing thymidine, 2-deoxy-D-ribose or xanthine. J Polym Sci, 1971; 9: 1715-1721.
3. Carraher CE, Powers D, Pandya B. Biologically active polyphosphate and polyphosphonate esters-nucleic acid analogs, In Biotechnology and Bioactive Polymers. NY: Plenum Press, 1994.
4. Millich F, Carraher CE. Interfacial synthesis of polyphosphonate and polyphosphate esters. I. Effects of alkaline medium. J Polym Sci, 1969; 1(7): 2669-2678.
5. Millich F, Carraher CE. Interfacial synthesis of polyphosphonate and polyphosphate esters. II. Dependence of yield and molecular weight on solvent volumes and concentrations, of comers in basic polymerization of hydroquinone and phenylphosphonic dichloride. J Polym Sci, 1970; 1(8): 163-169.
6. Millich F, Carraher CE. Interfacial synthesis of polyphosphonate and polyphosphate esters. III. Influences of solvents, diffusion rates, temperature, and other factors on yield

and molecular weight in the alkaline interfacial polycondensation of hydroquinone and phenylphosphonic dichloride. Macromolecules, 1970; 3: 253-256.

7. Carraher CE. Other phosphorus-containing polymers in Interfacial Synthesis. NY: Dekker, 1977.
8. Carraher CE. Organotin polymers, in Macromolecules Containing Metal and Metal-Like Elements, Vol. 4. Group IVB-Containing Polymers. NJ: Wiley, 2005.
9. Roner MR, Shahi KR, Barot G, Battin A, Carraher CE. Preliminary Results for the Inhibition of Pancreatic Cancer Cells by Organotin Polymers. J Inorg Organomet P., 2009; 19(3): 410-414.
10. Barot G, Shahi KR, Roner MR, Carraher CE. Synthesis, structural characterization, and ability to inhibit cancer growth of a series of organotin poly(ethylene glycols). J Inorg Organomet P., 2007; 17(4): 595-603.
11. Roner M, Carraher CE, Roehr J, Bassett K. Antiviral and anticancer activity of organotin polymers and reactants derived from norfloxacin and ampicillin. J Polym Mater, 2006; 23: 153-159.
12. Carraher CE, Sabir T, Roner M, Shahi K, Bleicher R, Roehr J. Synthesis of organotin polyamine ethers containing acyclovir and their preliminary anticancer and antiviral activity. J Inorg Organomet P., 2006; 16: 249-257.
13. Carraher CE, Naoshima Y, Nagao K, Roner MR, Zhao A, Barot G. Antibacterial, Yeast and Fungal Polymeric Materials Derived from Organotin-Containing Materials. J Polym Mater, 2011; 28(3): 303-336.
14. Carraher CE, Battin A, Shahi KR, Roner MR. Synthesis, structural characterization, and initial evaluation as anticancer drugs of dibutyltin polyamines derived from various 4,6-diaminopyrimidines. J Inorg Organomet P, 2007; 17(4): 631-639.
15. Carraher CE, Roner MR, Sooledo N, Moric-Johnson A, Miler L, Johnson J. Synthesis and Initial Cancer Cell Results of Organotin Polyethers Derived from the Anticoagulant Dicumarol. Int J Applied Pharm Biological Res, 2017; 2(5): 1-17.
16. Carraher CE, Roner MR, Campbell A, Moric-Johnson A, Miller L, Slawek P, Mosca F, Einkauf J, Haky J, Crichton R. Synthesis of Organotin Polyesters from Reaction of the Salt of D-Camphoric Acid and Organotin Dihalides and Initial Anticancer Activity. J Inorg Organomet Polym Mat, 2018; 28: 481-491.
17. Carraher CE, Roner MR, Patel D, Milller L, Moric-Johnson A, Slawek P, Mosca F, Frank J. Synthesis of organotin polymers from 2-ketoglutaric acid and their ability to inhibit the growth of human cancer cell lines. Hematology Medical Oncology, 2018; 3(1): 1-9.

18. Carraher CE, Roner MR, Frank F, Slawek P, Mosca F, Shahi K, Moric-Johnson A, Miller L. Organotin polymers for the control of pancreatic cancer. OBM Hepatology & Gastroenterology, 2019; 3(2): 1-10).
19. Carraher CE, Roner MR, Islam Z, Moric-Johnson A. Group 15 organotin containing polyamines from histamine and their ability to inhibit cancer cell lines from pancreatic, breast and other cancers. J Pharmacy & Pharmacetical Res, 2018; 2(1): 1-10.
20. Carraher CE, Roner MR, Lynch M, Moric-Johnson A, Miller L, Slawek P, Mosca F, Frank J. Organotin poly(ester ethers) from salicylic acid and their ability to inhibit human cancer cell lines. Journal of Clinical Research in Onology, 2018; 1: 1-11.
21. Carraher CE, Roner MR, Slawek P, Mosca F, Frank J, Miller L. Groups 4 and 15 and organotin condensation polymers for the treatment of cancers and viruses. MAMS, 2018; 1: 9-14.
22. Carraher CE, Roner MR, Frank J, Mosca F, Slawek P, Miller L. Inhibition of human glioblastomas brain cancer cell lines by metal-containing polymers. WJPR, 2019; 8(6): 123-139.
23. Carraher CE, Roner MR, Black K, Frank J, Moric-Jophnson A, Miller L. Polyesters from Reaction of 3,5-Pyridinedicarboxylic Acid and Group V-Containing Dihalides and Their Preliminary and Comparative Ability to Inhibit Cancer Cell Growth. International Journal of Applied Pharmaceutical and Biological Research, 2017; 2(4): 1-17.
24. Carraher CE, Roner MR, Campbell A, Moric-Johnson A, Miller L, Slawek P, Mosca F. Group IVB Metallocene Polyesters Containing Camphoric Acid and Preliminary Cancer Cell Data, International J. Polymeric Materials Polymeric Biomaterials, 2018; 67: 469-479.
25. Roner MR, Carraher CE, Jr., Shahi K, Ashida Y, Barot G. Ability of Group IVB metallocene polyethers containing dienestrol to arrest the growth of selected cancer cell lines. BMC Cancer, 2009; 9: 358.
26. Carraher CE, Roner MR, Shahi K, Ashida Y, Barot G. Synthesis, structural characterization, and anti-cancer evaluation of group IVB-metallocene polyethers containing the synthetic estrogen diethylstilbestrol. J Polym Mater, 2007; 24(4): 357-369.
27. Carraher CE. Condensation Metallocene Polymers. JIOMP, 2005; 15: 121-145.
28. Carraher CE, Truong NTC, Roner MR. Synthesis of Metallocene Poly (ether Esters) from Reaction with Glycyrrhetinic Acid. J Polym Mater, 2017; 34: 435-454.
29. Carraher CE, Roner MR, Black K, Frank J, Moric-Johnson A, Miller L, Russell F. Synthesis, Structural Characterization and Initial Anticancer Activity of Water Soluble

Polyethers From Hafnocene Dichloride and Poly(ethylene Glycols). JCAMS, 2017; 5(4): 254-268.

30. Carraher CE, Roner MR, Frank J, Moric-Johnson A, Miller L, Black K, Slawek P, Mosca F, Einkauf J, Russell F. Synthesis of Water Soluble Group 4 Metallocene and Organotin Polyethers and their Ability to Inhibit Cancer. Processes, 2017; 5,50: 1-13.
31. Roner MR, Carraher CE, Shahi K, Barot G. Antiviral Activity of Metal-Containing Polymers-Organotin and Cisplatin-Like Polymers. Materials. 2011; 4(6): 991-1012.
32. Carraher CE, Scott W, Schroeder J. Poly(cis-dihalodiamine platinum(II)) compounds: synthesis and biological activity. J Macromol Sci-Chem. 1981; (A15): 625-631.
33. Siegmann-Louda D, Carraher CE. Effect of poly(dichloro-2-chloro-p-phenylene-diamineplatinum(II) on normal and transformed cells. J Polym Materials, 1987; 4: 29-34.
34. Carraher CE, Roner MR, Mosca F, Moric-Johnson A, Miller L, Einkauf J, Russell F, Slawek P. Synthesis and Characterization, Including Cancer Cell Line Inhibition, of Group VA (Group 15)-Containing Polyesters from Reaction with Camphoric Acid. J Inorg Organomet Polym Mat, 2017; 27: 1627-1639.
35. Carraher CE, Roner MR, Black K, Frank J, Moric-Johnson A, Miller L. Polyesters from Reaction of 3,5-Pyridinecarboxylic acid and Group V-Containing Dihalides and Their Preliminary and Comparative Ability to Inhibit Cancer Cell Growth. Int J Applied Pharm Biological Res, 2017; 2(4): 1-17.
36. Carraher CE, Roner MR, Campbell A, Moric-Johnson A, Miller L, Slawek P, Mosca F. Group IVB metallocene polyesters containing camphoric acid and preliminary cancer cell activity. Int J Polym Mater Polym Biomat, 2018; 67: 469-479.
37. Carraher CE, Roner MR, Frank J, Black K, Moric-Johnson A, Miller L, Mosca F, Slawek P. Synthesis and initial anticancer activity of water and dimethyl sulfoxide soluble polyethers from zirconocene dichloride and poly(ethylene glycols). WJPR, 2019; 8(7): 63-84.
38. Carraher CE, Roner MR, Slawek P, Mosca F. Group 4 metallocene polymers-selected properties and applications, Inorganics, 2018; 6(65): 1-14.
39. Carraher CE. Organoantimony-containing polymers. J Polym Mater, 2008; 25(1): 35-50.
40. Sabir TS, Carraher CE. Synthesis of triphenylantimony and triphenylbismuth-containing polyether amines containing acyclovir. J Polym Mater, 2006; 23(4): 403-413.
41. Carraher CE, Roner MR, Barot G, Shahi K. Comparative anticancer activity of water-soluble organotin poly(ethylene glycol) polyethers. J. Polym. Mater, 2014; 31(2):123-133.

42. Carraher CE, Girish, Barot G, Vetter S, Nayak G, Roner MR. Degradation of organotin polyether derived from dibutyl dichloride and hydroxyl-capped poly(ethylene glycol) in trypsin and evaluation of trypsin activity employing light scattering photometry and gel electrophoresis. J. Chinese Adv. Mat. Soc, 2013: 1-6.
43. Carraher CE, Barot G, Shahi K, Roner MR. Influence of DMSO on the inhibition of carious cancer cells by water-soluble organotin polyethers. J. Chinese Adv. Mat. Soc, 2013; 1: 294-304.
44. Barot G, Shahi K, Roner MR, Carraher CE. Synthesis, anomalous fiber formation, and preliminary anticancer study of the organotin polyether derived from 2-butyne-1,4-diol. J. Polym. Mat, 2006; 23(4): 423-436.
45. Barot G, Roner MR, Naoshima Y, Nagao K, Shahi K, Carraher CE. Synthesis, structural characterization, and preliminary biological characterization of organotin polyethers derived from hydroquinone and substituted hydroquinones. J. Inorg. Organomet, Polym. 2009; 19: 12-27.
46. Carraher CE, Roner MR, Shahi K, Ashida Y, Barot G. J. Inorg. Organobet. Polym. 2008; 18: 180-188.
47. Carraher CE, Roner MR. Organotin polyethers as biomaterials, Materials. 2009; 2; 1558-1598.
48. Carraher CE, Roner MR, Barot G. Organotin-containing polyethers as potential anticancer drugs, Horizons in Cancer research. 2011; 43: 215-240.
49. Carraher CE, Barot G, Roner MR, Shahi K, Pellerito C, Fiore T, Scopelliti M, Pellerito L. Synthesis, structural analysis, fiber formation and preliminary anticancer characterization of the organotin polyether from dibutyltin dichloride and 2,5-dimmethyl-3-hexyne-2,5-diol, J. Polym. Mater. 2012; 29: 361-376.
50. Morgan PW. Condensation Polymers: By Interfacial and Solution Methods: NY; Wiley-Interscience, 1965.
51. Millich F, Carraher CE. Interfacial Synthesis. NY; Dekker, 1977.
52. Millich F, Carraher CE. Interfacial Synthesis. Vol. II. NY; Dekker, 1977.
53. Carraher CE. Polymer Chemistry, 10th edition. Taylor & Francis, 2018.
54. Carraher CE. Introduction to Polymer Chemistry, 4th Ed. Tylor & Francis, 2017.
55. Frank J, Carraher CE, Roner MR, Shahi K, Slawek P, Mosca F. Synthesis and Characterization of synthetic nucleic acids polymers derived from group 4 metallocene dichlorides and thymidine as anticancer agents, WJPR. 2020: 9(6): 2360-2380.
56. Belen M, Brandan SA. A structural and vibrational investigation on the antiviral

deoxyribonucleoside thymidine agent in gas and aqueous solution phases. International Journal of Quantum Chemistry, 2014; 114: 209-221.

57. Lummen G, Sperling H, Luboldt H, Otto T, Rubbe H. Cancer Chemotherapy Pharmacology, 1998; 42(5): 415-417.
58. Carraher CE, Roner MR, Carraher CL, Crichton R, Black K. Use of Mass Spectrometry in the Characterization of Polymers Emphasizing Metal-Containing Condensation Polymers. J Macromol Sci A, 2015; 52: 867-886.
59. Carraher CE, Sabir T, Carraher CL. Fundamentals of Fragmentation Matrix Assisted Laser Desorption/Ionization Mass Spectrometry, NY; Springer, 2008.