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## *Abstract:*

Water-soluble metallocene and organotin-containing polyethers were synthesized employing interfacial polycondensation. The reaction involved various chain lengths of poly(ethylene glycol), and produced water-soluble polymers in decent yield. Commercially available reactants were used to allow for easy scale up. The polymers exhibited a decent ability to inhibit a range of cancer cell lines, including two pancreatic cancer cell lines. This approach should allow the synthesis of a wide variety of other water-soluble polymers.

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Review

# Synthesis of Water-Soluble Group 4 Metallocene and Organotin Polyethers and Their Ability to Inhibit Cancer

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**Abstract:** Water-soluble metallocene and organotin-containing polyethers were synthesized employing interfacial polycondensation. The reaction involved various chain lengths of poly(ethylene glycol), and produced water-soluble polymers in decent yield. Commercially available reactants were used to allow for easy scale up. The polymers exhibited a decent ability to inhibit a range of cancer cell lines, including two pancreatic cancer cell lines. This approach should allow the synthesis of a wide variety of other water-soluble polymers.

**Keywords:** Group 4 metallocenes; anticancer; poly(ethylene glycol); pancreatic cancer; breast cancer; prostate cancer; interfacial polycondensation; Group 4 metallocene polymers; organotin polyethers

## 1. Polymer Solubility

Polymer solubility is both difficult and low compared with the solubility of smaller molecules, and is limited by the amount of suitable solvent, extent of solubility, and rapidness of solubility. By comparison, the solubility of metal-containing polymers is even more difficult. Over 50 years ago, Bailar, one of the pioneers of inorganic chemistry, described some problems associated with the solubility of metal-containing polymers [1]. Briefly, these are as follows. First, little flexibility is imparted by the metal ion or within its immediate environment; thus, flexibility must arise from the organic moiety. Flexibility increases as the covalent nature of the metal–ligand bond increases. Second, metal ions only stabilize ligands in their immediate vicinity; thus, the chelates should be strong and close to the metal ions. Third, thermal, oxidative, and hydrolytic stability are not directly related; polymers must be designed specifically for the properties desired. Fourth, metal–ligand bonds have sufficient ionic character to permit them to rearrange more readily than typical “organic bonds”. Fifth, polymer structure (such as square planar, octahedral, linear, and network) is dictated by the coordination number and stereochemistry of the metal ion or chelating agent. Lastly, employed solvents should not form strong complexes with the metal or chelating agent or they will be incorporated into the polymer structure and/or prevent a reaction from occurring. These problems are present with all metal-containing polymers.

Why is it important to have water solubility for polymers that are considered for use as drugs? Not all drugs must be water soluble to be useful. Many of the front-line drugs, such as ciprofloxacin, are not water soluble. Their solution testing is similar to what we employ in our non-water-soluble polymer testing. The drug is initially dissolved in dimethyl sulfoxide, DMSO, and then water is added

and the appropriate testing carried out. However, given the choice, water solubility is advantageous for two important reasons. First, it allows for versatility in the administration of the drug, including water-associated approaches. Second, it eliminates effects that may occur because of the presence of any non-water-solvent systems.

## 2. Poly(Ethylene Glycol) as a Synthetic Template

This paper is part of a focus on water-soluble polymers, and as such the emphasis is to describe approaches that have allowed metal-containing polymers to be water soluble. Recent activities involve the use of poly(ethylene oxide), PEG, to achieve water solubility for metal-containing polymers.

Poly(ethylene glycol) (also called poly(ethylene oxide)), PEG, is considered to be nontoxic and is currently employed in a number of medical-related treatments, including pill coatings and in many commercial laxatives [2,3]. It is intentionally attached to many materials, including drugs, to assist in their water solubility. When attached to certain protein medications, they produce a drug with a longer activity and with a reduced toxicity [4]. For instance, its incorporation into polymers is widely employed to increase the solubility of polymers of biologically important materials [5–7].

PEGs are generally designated some average molecular weight. Since the ethylene glycol unit,  $\text{CH}_2\text{CH}_2\text{O}$ , has a molecular weight of 44 Da, PEG 200 has about 4.5 ethylene glycol units, that is  $200/44$ . It should be noted that PEG is typically a mixture with the average being whatever the cited molecular weight is. Thus, PEG 200 is a combination of ethylene oxide repeat units, the most prevalent being four ethylene oxide units, molecular weight 176 Da, and five ethylene oxide units, molecular weight 220. The average is  $396/2$ , or approximately 200, which is what this particular product is sold as.

## 3. Synthesis of Metal-Containing Polymers

We have synthesized a variety of metal-containing polymers for different purposes. Our most recent purposes for their synthesis include their ability to be doped, producing near conductors [8–11], and their ability to inhibit unwanted pathogens and infectious agents, including bacteria, viruses, cancers, molds, and yeasts. Some of these efforts have been reviewed for platinum [12–15], organotin [15–19], Group 5 [20,21], Group 15 [22–24], uranium [25], ruthenium [26], and vanadocene-containing [27] polymers.

The majority of polymers made by us are dimethyl sulfoxide, DMSO, soluble at concentrations sufficient to allow for molecular weight analysis via light scattering photometry and cancer cell line and virus inhibition analysis.

For ease of treatment, water-soluble drugs offer greater ease and avoid possible side effects due to the presence of DMSO. Thus, we sought to employ PEG to achieve this. As noted before, PEG is known to assist biologically important molecules to become water soluble. PEG is relatively inexpensive and is considered nontoxic. Further, there are a number of PEG chains available with differing end groups and chain lengths such that tailoring the PEG is possible. In the future, we plan to employ this variability to design systems that allow a focus on Lewis bases that offer good ability to inhibit unwanted pathogens and infectious agents. We are at the initial juncture of this study. Because most of the literature studies employ simple dihydroxyl-capped PEG chains, these are the PEG chains currently being employed [4–7].

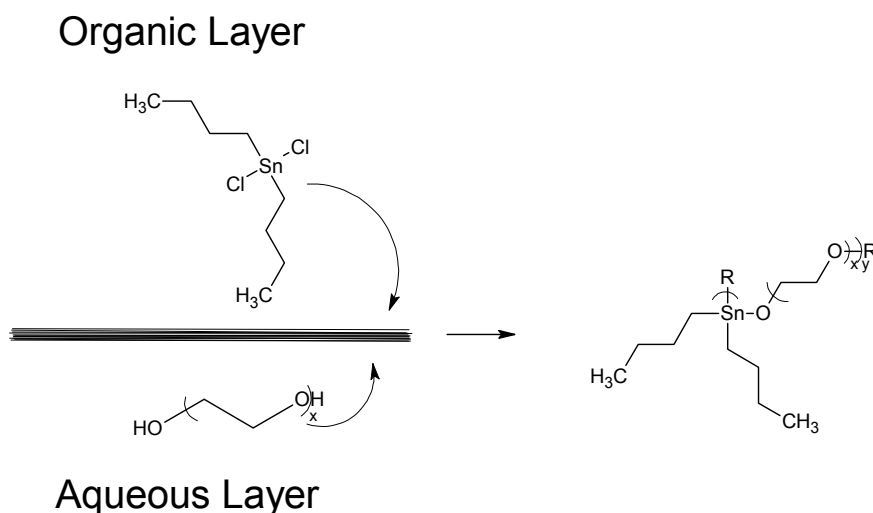
The general focus in this review is the formation of water-soluble polymers from products that are not traditionally water soluble. Included in this review is the use of these polymers to inhibit cancer cell growth.

The various water-soluble polymers described in this review are all synthesized employing commercially available materials, allowing for a ready scale up to ton quantities. While the ability to scale up is present, it is normally not straightforward [1]. The polymers are structurally characterized employing typical tools used to define their structure and are familiar to chemists. The main exception is the use of matrix assisted-laser desorption/ionization mass spectrometry, MALDI MS. MALDI MS is widely employed in biochemistry because the most important biological polymers act as if they are water soluble and the MALDI MS system is used for their analysis. However, most polymers

are not soluble in volatile liquids that allow an intimate mixture of the matrix material with the product. Thus, we developed a system that allowed non-soluble materials to be analyzed. In particular, organometallic structures, such as those employed by us, have poor stability when struck by the laser radiation employed in MALDI MS, with fragmentation typically occurring at the heteroatom sites in the polymer. This creates a fragmentation of the polymer chain, and it is these fragments that are employed to identify the basic repeat unit. This approach has been reviewed [28–30]. Even with this problem, we have been able to identify fragments to 30,000 Da, though we routinely look at ion fragment clusters from 500 to 5000 Da.

#### 4. Organotin Polyether Synthesis

The synthesis of water-soluble polymers was initially achieved employing organotin polyethers derived from reaction of the organotin dihalides with PEG as described in Figure 1 [31–38]. The system is described in some detail because it is the same system employed in the metallocene polyether system. The polymers were synthesized employing the interfacial polycondensation system, where the organotin dihalide was dissolved in an organic liquid, generally heptane, and the PEG was dissolved in water with sodium hydroxide added to neutralize the HCl formed from the reaction. The two phases were rapidly stirred, at about 18,000 rpm, at room temperature. Product was formed in about 15 s as a precipitate. While the product was water soluble, it was only soluble to about 0.25 g for 50 mL of water, so it was not totally soluble at the higher concentration of the reaction system of about one gram for 50 mL of water. The white solid was recovered and washed with heptane and several mL of water. The effectiveness of this simple cleanup procedure gives product without bands associated with heptane in their nuclear magnetic resonance spectra, NMR, and infrared spectra, IR. While molecular weight increases as the PEG chain length increases, molecular weight based on the number of repeat units decreases. This decrease in chain length may involve a decreased ability for the growing chains to readily locate PEG ends to react with. Reactions involving the interfacial system are generally rapid and complicated so that the time required for the reaction to occur is relatively short [1]. Also, the percentage yield generally decreases as the PEG length increases, presumably because the longer PEG chains have a greater water solubility and are more easily lost in the aqueous portion of the reaction system. The reaction aqueous phase and wash was tested and contains both unreacted PEG and organotin polyether. Table 1 contains the results for two of the PEG systems.



**Figure 1.** Description of the interfacial polycondensation system employed to synthesize water-soluble organotin polyethers, where *x* represents the PEG chain length and *y* represents the polymer's average degree of polymerization (PEG: Poly(ethylene glycol)).

**Table 1.** Sample results for the synthesis of water-soluble polyethers from reaction of dibutyltin dichloride with PEG. The specific reaction conditions are dibutyltin dichloride (3.00 mmole) in 30 mL heptane added to rapidly (18,000 rpm; no-load) stirred aqueous solutions (30 mL) containing diol (3.00 mmole) and sodium hydroxide (6.0 mmole) with stirring for 15 s (DMSO: dimethyl sulfoxide; DP: degree of polymerization).

PEG	%-Yield	Mol. Wt. DMSO	DP DMSO	Mol. Wt. Water	DP Water
PEG-400	61	$7.6 \times 10^4$	120	$7.4 \times 10^4$	120
PEG-10,000	6	$2.5 \times 10^5$	24	$2.2 \times 10^5$	22

Molecular weight was studied as a function of time for the product dissolved in DMSO and in water. In all cases, the molecular weight remained essentially unchanged for five weeks, with a molecular weight half-life greater than 60 weeks (that is the time for the molecular weight to become half of its original value). Thus, the organotin polyethers exhibit good solution stability in both DMSO and water, well within the time limits needed to accomplish the needed biological and physical measurements.

The polymers were characterized using typical analysis systems, including proton nuclear magnetic resonance spectrometry, various infrared spectroscopy systems, and MALDI mass spectrometry. IR shows the presence of bands characteristic of the formation of Sn-O consistent with the linkage between the PEG and organotin moiety having been formed. Further, IR shows the absence of the Sn-Cl band consistent with its elimination as the PEG reacts with the organotin halide.

Unlike most organic polymers, which do not possess elements that have atoms suitable to evaluate their presence through an investigation of their isotopic abundances, many of the products we work with have metals that do have such isotopes present. Tin has ten isotopes with seven isotopes present in amounts great than 5%, allowing the tin's presence to be determined using these isotopes. MALDI MS is used to make such determinations routinely. Because tin has isotopes, different ion fragments are created that have the same structural formula but vary by the particular tin isotope present. This creates what are referred to as spectral "fingerprints" characteristic of the natural abundance of these isotopes. Table 2 contains matches for ion fragment clusters that contain one and two ion atoms. The matches agree with what is expected, consistent with the presence of tin within these ion fragment clusters. Our focus is on ion fragments with masses of 500 Da and greater. Each of the ion fragment clusters above 500 (all ions are given in daltons, Da, or  $m/e = 1$ ) are actually clusters of ions that are produced because of the presence of tin atom(s) within each cluster.

Our purpose for synthesizing the polymers was to evaluate their biological properties, and to do so in DMSO and compare the results with the polymers dissolved in only water. Thus, DMSO was initially employed to dissolve the compounds and then water was added to give the desired concentration of the tested compound for one set of studies. An analogous set of studies originally dissolved the tested compounds in water, to which additional water was added to give the desired compound concentrations. This second set is referred to as the "water only" systems. The general biological procedure is described elsewhere [18,19].

Cancer is the leading cause of death globally. The cell lines employed in the current study are given in Table 3. They represent a broad range of important cancers. Two human pancreatic cancer cell lines and two human breast cancer cell lines are included.

**Table 2.** Isotopic abundance matches for two ion fragment clusters containing a single tin atom, top part, and two ion fragment clusters with two tin atoms, bottom part. Bu represents a butyl moiety.

Known for Sn		Bu <sub>2</sub> Sn <sub>2</sub> O		(OCH <sub>2</sub> CH <sub>2</sub> ) <sub>4</sub> OSnBu <sub>2</sub>		(CH <sub>2</sub> CH <sub>2</sub> O) <sub>5</sub> SnBr <sub>2</sub> ,Na	
m/e	Rel. Abu.	m/e	Rel. Abu. Found	m/e	Rel. Abu. Found	m/e	Rel. Abu. Found
116	45	260	40	418	43	477	30
117	24	261	21	419	29	478	25
118	75	262	71	420	75	479	79
119	26	263	29	421	30	480	28
120	100	264	100	422	100	481	100
122	14	266	19	424	19	483	20
124	17	268	20	426	16	485	20
Known for 2Sn		Bu <sub>2</sub> SnO(CH <sub>2</sub> CH <sub>2</sub> O) <sub>4</sub> SnBu <sub>2</sub>		OBu <sub>2</sub> SnO(CH <sub>2</sub> CH <sub>2</sub> O) <sub>4</sub> Bu <sub>2</sub> SnO			
m/e	Rel. Abu.	m/e	Rel. Abu. Found	m/e	Rel. Abu. Found		
232	12	627	9	664	18		
233	13	628	9	665	14		
234	46	629	46	666	42		
235	36	630	40	667	32		
236	94	631	88	668	94		
237	51	632	51	669	59		
238	100	633	100	670	100		
239	35	634	32	671	39		
240	81	635	82	672	81		
242	32	637	20	674	26		
244	22	639	10	676	21		

**Table 3.** Cell line characteristics and identification.

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
ATCC CRL-1658	NIH/3T3	Mouse	Embryo-continuous cell line of highly contact-inhibited cells	Fibroblast
ATCC CCL-1	L929	Mouse	Transformed	Fibroblast
ATCC CRL-8303	143	Human	Fibroblast	Bone
ATCC CCC-81	Vero	Monkey	Transformed	Africa Green Monkey kidney epithelial
ATCC CCL-75.1	WI-38 VA13 2RA	Human	Transformed	WI-38 Embryo lung fibroblast
ATCC CRL-8303	143	Human	Fibroblast	Bone osteosarcoma
ATCC CCL-81	Vero	Monkey	Transformed	African green monkey kidney epithelial
ATCC CCL-75.1	WI-38 VA13 2RA	Human	Transformed	WI-38 embryo lung fibroblast
	AsPC-1	Human	Pancreatic cells	Adenocarcinoma
	PANC-1	Human	Epithelioid Pancreatic cells	Carcinoma

We recently found that anticancer activity is brought about by the intact polymer and not through polymer degradation [31]. This is consistent with studies that show that polymers are stable in DMSO with half-chain lives, the time for the chain length to halve, generally in excess of 30 weeks [16–19]. In other studies, we found that the polymer drugs are cytotoxic and cell death is by necrosis [16–19].

Most organometallic compounds associate with polar solvents, such as DMSO, and the biological results may be influenced by the presence of the DMSO [36–38]. For similar organometallic polymers, the results on the influence of DMSO on the tumor were found to be minimal [32–34]. For the current study, the results in water and DMSO are similar, consistent with this [33,34].

Different measures are employed in the evaluation of compounds to control cancer growth. The two most widely employed measures are used in the present studies. The first measure employs as a measure effective concentration, EC. EC is the concentration dose needed to reduce the growth of a particular cell line. The concentration of a drug, antibody, or toxicant that induces a response halfway between the baseline and maximum after a specified exposure time is referred to as the 50% response concentration and is given the symbol  $EC_{50}$ . The second measure of the potential use of compounds in the treatment of cancer is the concentration of drug necessary to inhibit the standard cells compared to the concentration of drug necessary to inhibit the growth of the test cell line. The term chemotherapeutic index, CI, is employed for these measurements. The  $CI_{50}$  is the ratio of the  $EC_{50}$  for WI-38 cells divided by the  $EC_{50}$  for the particular test cell.

A recent focus is pancreatic cancer. Pancreatic cancer afflicts close to 32,000 individuals each year in the United States, with almost all dead within a year. It is the fourth-most leading cause of cancer death. Treatment for pancreatic cancer is rarely successful, as this disease typically metastasizes prior to detection. The pancreatic cancer cell lines we tested were AsPC-1, which is a human adenocarcinoma pancreatic cell line that represents about 80% of the human pancreatic cancers, and PANC-1, which is an epithelioid carcinoma pancreatic cell line which represents about 10% of the human pancreatic cancers. Both are human cell lines, and the pair is widely employed in testing for the inhibition of pancreatic cancer. The dibutyltin PEG polyethers exhibit excellent ability to inhibit both pancreatic cancer cell lines. The values in water and DMSO are similar, so only an average is given for the two products. For the PEG 400 dibutyltin polyether and the AsPC-1 Cell line, the  $EC_{50}$  value is 0.006 microgram/mL, in the nanogram/mL range, and the  $CI_{50}$  is 47. Values of 2 and greater are considered significant. For the PEG 10,000 dibutyltin product, the  $EC_{50}$  value is 0.06 microgram/mL and the  $CI_{50}$  value is 16. For the PAN-1 pancreatic cancer for the PEG 400 dibutyltin polymer cell, the  $EC_{50}$  value is 0.005  $\mu\text{g}/\text{mL}$  and the  $CI_{50}$  value is 56. Thus, the two values for the two different cancer cell lines are similar for the two different polymers, consistent with the possibility that the polymers will be active against other pancreatic cancers.

In summary, the organotin PEG polymers exhibit good inhibition towards pancreatic cancer cells when initially dissolved in DMSO or water. The aqueous solubility allows most forms of administration to be employed.

## 5. Group 4 Metallocene Polyethers

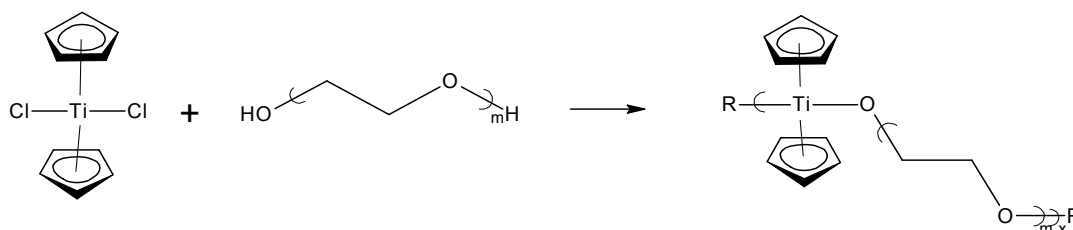
Group 4 metallocene-containing small molecules inhibit cancer cell growth. The mechanism by which they accomplish this differs from that found for cisplatin. This allows molecules from both groups to be employed as members of a “cocktail” intersecting cancer growth at differing junctures [39–44].

The first non-platinum metal to undergo clinical trial was titanocene dichloride [45]. While the activity of cisplatin involves interaction with deoxyribonucleic acid, DNA, the activity for titanocene dichloride is related to its ability to react with transferring [45,46].

A lack of solubility is a major problem limiting efforts to employ Group 4 metallocenes as anticancer agents [39–45]. The present effort involving PEG offers an avenue allowing metallocene-containing small and large molecules to be soluble.

We recently described the synthesis and initial cell line results for Group 4 metallocene polymers with structures analogous to the organotin polyethers [47–49] (Figure 2).





**Figure 2.** Synthesis of titanocene polyethers from reaction of titanocene dichloride and PEG, where R is a simple chain extension.

As in the case of the organotin products, these materials show good stability in solution for a month, with essentially no loss in molecular weight when dissolved in water or in DMSO.

Table 4 contains the yield, product molecular weights in water and DMSO, and average degree of polymerization, DP-number of repeat units for the product. The first column contains the particular metallocene and the molecular weight for the particular PEG followed by the number of PEG units in that particular PEG. The molecular weight and average chain length of the polymer decrease as the employed PEG length increases. This is the same as what occurred for the organotin products, and is again probably a consequence of the growing chains having increased difficulty in locating the end groups as the PEG chain length increases. The yield trends should be viewed with caution, because we have not yet perfected a good procedure for the recovery of the product. The infrared spectroscopy and proton NMR results are consistent with the proposed repeat unit. IR shows the formation of bands associated with the formation of the M-O linkage.

**Table 4.** Product yield and molecular weight as a function of PEG length and metallocene. Cp: cyclopentadiene group; MW: molecular weight.

Sample	Percentage Yield	MW (H <sub>2</sub> O)	MW (DMSO)	DP
Cp <sub>2</sub> Ti 200/4.5	68		$5.8 \times 10^6$	15,000
Cp <sub>2</sub> Ti 400/9	42		$1.0 \times 10^6$	850
Cp <sub>2</sub> Ti 1000/27	51	$1.8 \times 10^5$	$1.9 \times 10^5$	150
Cp <sub>2</sub> Ti 1500/34	52	$3.6 \times 10^4$	$3.7 \times 10^4$	21
Cp <sub>2</sub> Ti 2000/45	49	$3.4 \times 10^4$	$3.5 \times 10^4$	17
Cp <sub>2</sub> Ti 3400/77	46	$3.7 \times 10^4$	$3.7 \times 10^4$	11
Cp <sub>2</sub> Zr 200/4.5	24		$2.0 \times 10^6$	4700
Cp <sub>2</sub> Zr 400/9	15		$3.8 \times 10^5$	600
Cp <sub>2</sub> Zr 1000/27	30	$9.0 \times 10^4$	$9.1 \times 10^4$	75
Cp <sub>2</sub> Zr 4600/100	15	$6.1 \times 10^4$	$6.2 \times 10^4$	13
Cp <sub>2</sub> Zr 8000/180	34	$4.1 \times 10^4$	$4.2 \times 10^4$	5
Cp <sub>2</sub> Hf 200/4.5	32		$6.3 \times 10^6$	12,000
Cp <sub>2</sub> Hf 400/9	32	$5.3 \times 10^5$	$5.5 \times 10^5$	770
Cp <sub>2</sub> Hf 1000/27	24	$1.7 \times 10^5$	$1.9 \times 10^5$	150
Cp <sub>2</sub> Hf 4600/100		$9.3 \times 10^4$	$9.5 \times 10^4$	14
Cp <sub>2</sub> Hf 8000/180	38	$6.6 \times 10^4$	$6.8 \times 10^4$	8

As previously noted, because the metals have different natural abundance isotopes it is possible to do an isotope analysis using MALDI MS to compare the known isotopic relative abundances with the observed ion fragments. This analysis is routine and is consistent with the presence of the metals within the various ion fragments. Table 5 contains sample results for the isotopic abundances of ion fragments from the titanocene polymers. The results are in agreement with what is expected and consistent with the presence of the particular metal within the ion fragment clusters.



**Table 5.** Isotopic abundance matches for two ion fragment clusters containing a single titanium atom (top), and two ion fragment clusters containing two titanium atoms per cluster (bottom).

Known for Ti		O(CH <sub>2</sub> CH <sub>2</sub> O) <sub>4</sub> Cp <sub>2</sub> Ti(OCH <sub>2</sub> CH <sub>2</sub> ) <sub>4</sub>		O(CH <sub>2</sub> CH <sub>2</sub> O) <sub>4</sub> Cp <sub>2</sub> Ti(OCH <sub>2</sub> CH <sub>2</sub> ) <sub>4</sub>	
m/e	Rel. Abu.	m/e	Rel. Abu. Found	m/e	Rel. Abu. Found
46	11	583	10	539	11
47	11	584	11	540	11
48	100	585	100	541	100
49	8	586	8	542	7
50	7	587	6	543	7
Known for 2 Ti		(CH <sub>2</sub> CH <sub>2</sub> O) <sub>3</sub> Cp <sub>2</sub> Ti(OCH <sub>2</sub> CH <sub>2</sub> ) <sub>4</sub> Cp <sub>2</sub> TiO		(CH <sub>2</sub> CH <sub>2</sub> O) <sub>3</sub> Cp <sub>2</sub> TiO(CH <sub>2</sub> CH <sub>2</sub> O) <sub>4</sub> Cp <sub>2</sub> Ti(OCH <sub>2</sub> CH <sub>2</sub> ) <sub>4</sub>	
m/e	Rel. Abu.	m/e	Rel. Abu. Found	m/e	Rel. Abu. Found
94	22	688	22	875	23
95	21	689	20	876	21
96	100	690	100	877	100
97	16	691	18	878	20
98	15	692	16	879	18

One of our primary objectives is the synthesis of water-soluble drugs that inhibit the growth of pancreatic cancer. Table 6 contains results for the three water-soluble metallocene polymers. Cancer cell analysis requires a lower solubility for analysis compared with light scattering photometry. Thus, the reader may note that some cell line results are present in Table 6, but molecular weight values are not given in Table 4 because of the difference in solubility required to obtain molecular weight compared to cell line data.

Several observations are apparent. First, none of the metallocene dichlorides and PEGs offer inhibition to the limits tested. Second, the zirconocene polyethers offer the best inhibition of the pancreatic cancer cells based on having the lowest EC<sub>50</sub> values compared to the hafnocene and titanocene polyethers. Unlike the titanocene and hafnocene polyethers, there is a difference between the ability to inhibit the two cancer cell lines, with a greater, generally a ten-fold lower, ability to inhibit the PANC-1 cell line for the lower PEG chain lengths, but there are similar EC<sub>50</sub> values for the longer PEG products. The titanocene and hafnocene products exhibit inhibition rates that are similar to each another. For the present polymers, it is the zirconocene products that should have undergone clinical testing. Third, since neither the PEG or the metallocene dichloride exhibit an ability to inhibit pancreatic cell growth, it is the combination of PEG and metallocene that accounts for the ability to inhibit the pancreatic cancers.

In conclusion, water-soluble metallocene polymers have been synthesized. They exhibit decent inhibition of a battery of cancer cells according to the results for the pancreatic cancer cell lines presented.

**Table 6.** EC<sub>50</sub> results (micrograms/mL) for the water-soluble metallocene polyethers as a function of metallocene, metallocene/PEG polymer, and pancreatic cancer cell line. Reaction conditions were as follows: an aqueous solution containing PEG (0.00100 mole) and sodium hydroxide (0.00200) dissolved in 50 mL of water is added to a rapidly stirred (about 18,000 rpm) chloroform (50 mL) solution containing the metallocene dichloride (0.00100 mole). Stirring continued for 15 s at room temperature (about 28 °C)(EC: effective concentration).

Titanocene Results				Zirconocene Results				Hafnocene Results			
Compound	WI-38	PANC-1	AsPC-1	Compound	WI-38	PANC-1	AsPC-1	Compound	WI-38	PANC-1	AsPC-1
Cp <sub>2</sub> TiCl <sub>2</sub>	>32	>32	>32	Cp <sub>2</sub> ZrCl <sub>2</sub>	>32	>32	>32	Cp <sub>2</sub> Cp <sub>2</sub> HfCl <sub>2</sub>	>32	>32	>32
Cp <sub>2</sub> Ti/PEG 200	1.2	0.77	0.70	Cp <sub>2</sub> Zr/PEG 200	0.0019	0.17	0.011	Cp <sub>2</sub> Hf/PEG 200	0.45	0.52	0.59
Cp <sub>2</sub> Ti/PEG 400	0.95	0.61	0.64	Cp <sub>2</sub> Zr/PEG 400	0.0022	0.091	0.12	Cp <sub>2</sub> Hf/PEG 400	0.45	0.52	0.59
Cp <sub>2</sub> Ti/PEG 800	1.3	0.59	0.59								
Cp <sub>2</sub> Ti/PEG 1000	1.0	0.53	0.52	Cp <sub>2</sub> Zr/PEG 1000	0.0023	0.091	0.17	Cp <sub>2</sub> Hf/PEG 1000	0.45	0.52	0.59
Cp <sub>2</sub> Ti/PEG 1500	0.96	0.51	0.52								
Cp <sub>2</sub> Ti/PEG 2000	1.1	0.60	0.57								
Cp <sub>2</sub> Ti/PEG 3400	1.1	0.56	0.51								
				Cp <sub>2</sub> Zr/4600	0.0025	0.11	0.13	Cp <sub>2</sub> Hf/PEG 4600	0.45	0.52	0.59
CpTi/PEG 8000	1.1	0.62	0.53	Cp <sub>2</sub> Zr/8000	0.0029	0.13	0.18	Cp <sub>2</sub> Hf/PEG 8000	0.45	0.52	0.59

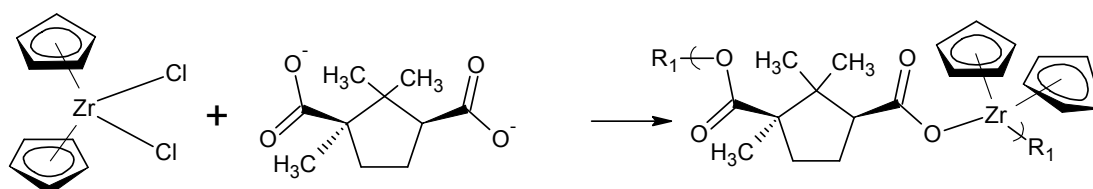
## 6. Future and Summary

PEG is widely employed industrially [50]. It is used in a number of laxatives, including MirLax, GlycoLax, and Movicol. It is also employed as a coating for many pills to allow the medication to pass through the harsh acidic stomach environment area unharmed. PEGs are employed as the soft segment in polyurethanes, and in other hard-soft copolymers as the soft portion. Some producers use it in toothpaste products as a dispersant, and it is employed as a lubricant in eye drops. Dr. Pepper adds PEG as an antifoaming agent. PEG has been used to help preserve wood-replacing water, giving the wood increased dimensional stability. Certain gene therapy vectors, such as viruses, can be coated with PEG to protect them from inactivation by immune systems and to de-target them from organs where they could build up and cause a toxic effect. Recently, they have been part of body armor combinations and to impart water solubility to certain electrically conductive polymers.

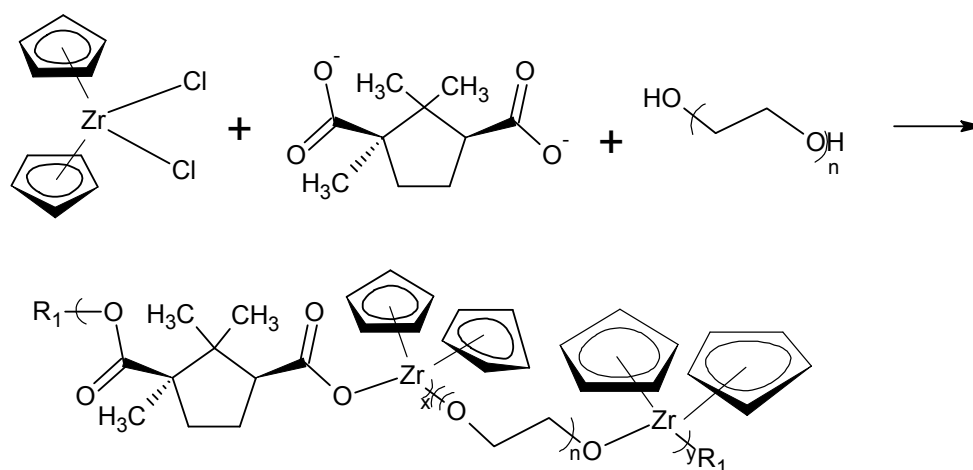
The use of PEG to enhance the aqueous solubility of materials includes macromolecules. It is well-established, but many venues remain. There remain a number of condensation polymers where aqueous solubility might be useful. These include commercial polymers, such as polyesters and nylons. There are also many speciality materials where water solubility would enhance their areas of potential use.

In our case, we have a number of polymers that exhibit a good ability to inhibit a range of cancer cell lines, viruses, and bacterial agents.

One product we plan to work towards making water soluble is a group of recently synthesized polyesters derived from the reaction of Group 4 metallocene dichlorides with the salts of camphoric acid (Figure 3) [51]. The zirconocene and hafnocene products exhibit good inhibition of a battery of cancer cell lines, including the pancreatic cancer cell lines described in Table 3, to the nanogram/mL range with  $CI_{50}$  values in the thousands. Our approach is to initially evaluate PEGs of varying length as co-monomers with camphoric acid to form products and then test the products for water solubility and ability to inhibit cancer cell lines (Figure 4).



**Figure 3.** Reaction scheme for the reaction between D-camphoric acid and zirconocene dichloride, where  $R_1$  represents a simple chain extension.



**Figure 4.** Reaction scheme for inclusion of PEG into the  $Cp_2Zr$ /camphoric acid product.

Some of these products exhibit good thermal stability to 1500 °C so that a simple CH analysis is not satisfactory for structural analysis [30]. Since neither of the Lewis bases have an element that is unique to it, we have begun using X-ray fluorescence spectroscopy to determine the percentage of metal in our products. From this information, the amount of both Lewis bases in the product can be determined.

The synthesis and study of water-soluble metal-containing polymers has begun. The results are promising, and allow for the synthesis of water-soluble products that exhibit good inhibition of a host of cancer cell lines. This is only the beginning of a long journey.

**Conflicts of Interest:** The authors declare no conflict of interest.

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