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SYNTHESIS AND INITIAL ANTICANCER ACTIVITY OF WATER AND DIMETHYL SULFOXIDE SOLUBLE POLYETHERS FROM ZIRCONOCENE DICHLORIDE AND POLY (ETHYLENE GLYCOLS)

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ABSTRACT

The initial synthesis of DMSO and water-soluble zirconocene polyethers through the reaction of zirconocene dichloride and various poly(ethylene glycols) employing commercially available reactants is described. Infrared, proton NMR, and MALDI MS results are consistent with the zirconocene-containing polyether structure. The materials are polymeric, with chain lengths ranging from 4700 to 5 with moderate product yield. They exhibit good inhibition of a variety of cancer cell lines including two pancreatic cancer cell lines.

KEYWORDS: metal containing polymers, PEG polymers, zirconocene-containing polymers, cancer, pancreatic cancer, water soluble polymers, MALDI MS.

INTRODUCTION

We have synthesized a variety of metal-containing polymers. These efforts have been recently reviewed for organotin-containing,[1,2] platinum-containing,[3] Group 15 metal-containing,[4] and Group 4 metallocene-containing polymers.[5] Here, focus is on the synthesis of zirconocene-containing polyethers. We initially published the synthesis of zirconocene-containing polymers in 1972,[6-8] and shortly thereafter, the formation of zirconocene polyethers[9] analogous to those described in the present paper but not based on reaction with poly(ethylene glycol) diols but rather based on small molecules such as hydroquinone and 2-

butyne-1,4-diol. Recently, efforts with metallocene-containing polymers emphasized the synthesis of these polymers as electrically-conductive materials[10] and as anticancer agents.[11-16]

Zirconocene dichloride and related Group 4 molecules have a distorted tetrahedral geometry about the metal atom. While employed in the formation of many materials, their largest use is as soluble stereoregular catalysts allowing the synthesis of a wide variety of stereoregular polymers including polyethylenes and polypropylenes.[17,18] Cotton and Wilkinson[19] describe Group 4 metallocene dichlorides as 9-coordinate bonding species (the hybrid orbitals being derived from one-s, three-p, and five-d orbitals). Each pi-Cp ring involves three hybrid orbitals. The three remaining orbitals consist of two equivalent spd(x^2-y^2), dz^2 orbitals (overlapping with the two chloride atoms) and one sp orbital, which is vacant and believed to be responsible for the catalytic activity of the Group 4 metallocenes and possibly some of their biological activity.[17]

Titanocene dichloride was the first-non-platinum metal-containing compound to undergo clinical trial.[20-26] The mechanism by which it inhibits cell growth is complex and not fully understood, but is believed to be related to the ability of the metallocene to interact with the protein transferrin.[26-27] This mechanism is different than that for cisplatin, among the most widely employed anticancer agents. Having a mechanism different than that for cisplatin is an advantage since it allows the metallocene to be part of a "mixture of drugs" delivered to patients that will intersect cancer growth at different sites decreasing the chances that resistant cells will be formed.

Because of the known anticancer activity for titanocene-containing small molecules we decided to focus on the potential anticancer activity of polymers containing the Group 4 metallocene moiety. We began synthesizing various Group 4 metallocene-containing polymers and found they exhibit good anticancer activity against a wide variety of cancer cell lines.[2][11-16] The advantages of employing metal-containing polymeric drugs has recently been reviewed.[2]

Poly(ethylene glycol) (also called poly(ethylene oxide)), PEG, is considered non-toxic and is employed in a number of medical-related treatments, including as pill coatings and in many laxatives.[30-32] It is intentionally attached to materials, including drugs, to assist in their water-solubility. When attached to certain protein-medications, they allow the drug a longer-

activity with reduced toxicity.[30] Its incorporation into polymers is widely employed to increase the solubility of polymers.[31,32]

The initial formation of water-soluble organotin polymers was recently achieved through the reaction of organotin dihalides with various poly(ethylene glycols) (Fig. 1).[33-35] These polymers exhibited good inhibition of a variety of cancer cell lines.[2][33-35]

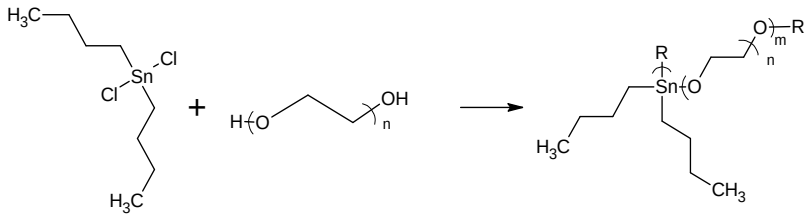

Figure 01: Repeat unit for the water-soluble organotin polyethers where R represents simple chain extension; "n" represents the number of ethylene oxide units; and "m" is the average number of polymer repeat units.

A major problem with titanocene containing monomeric materials is their poor or lack of solubility.[20-26] This hinders continued efforts to synthesize and study potential anticancer drugs based on the presence of the titanocene moiety. We recently described the synthesis of DMSO and water-soluble titanocene-containing polyethers produced through reaction of titanocene dichloride with a variety of hydroxyl-terminated poly(ethylene glycols) (Fig. 2).[36] These polymers are the first water-soluble titanium-containing polymers. The polymers offer decent ability to inhibit a wide variety of cancer lines.[36]

Here, is described the initial incorporation of PEG in the polymer backbone through reaction of the end-groups with zirconocene dichloride (Fig. 3), forming the first zirconocene-containing water soluble polymers.

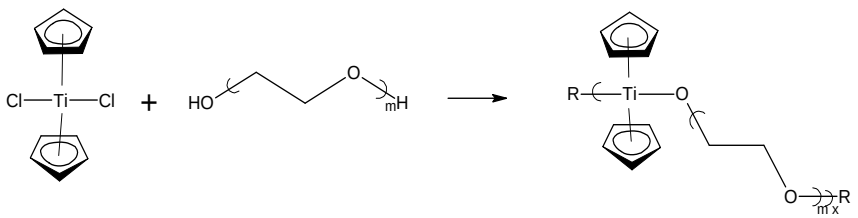

Figure 2: Repeat unit for the proposed unit structure for the reaction between titanocene dichloride and poly(ethylene glycols) where R represents simple chain extension; "m" represents the number of ethylene oxide units; and "x" is the average number of polymer repeat units.

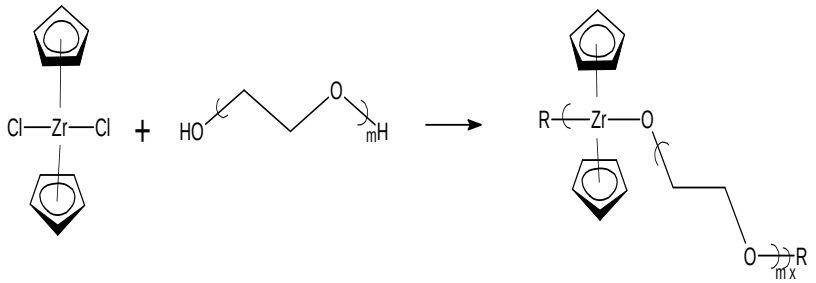

Figure 03: Repeat unit for the proposed unit structure for the reaction between zirconocene dichloride and poly(ethylene glycols) where "m" represents the number of ethylene oxide units; and "x" is the average number of polymer repeat units.

EXPERIMENTAL

Synthesis: Reactions were carried out using the interfacial polycondensation technique. Briefly, an aqueous solution (50 ml) containing the PEG (0.00100 mol) and sodium hydroxide (0.0020 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 about 25^{o}C). Stirring was begun and a chloroform solution (50 ml) containing the zirconocene dihalide (0.00100 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 chloroform to remove unreacted materials and unwanted by-products. The solid was washed onto a glass petri dish and allowed to dry at room temperature.

The various poly(ethylene glycols) (25322-68-3) and zirconocene dichloride (12636-72-5) were purchased from Aldrich Chemical Co., Milwaukee, WS. The reactants were used as received.

Physical Characterization: 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 500 to 2,500. Fifty to two hundred shots were typically taken for each spectrum. A graphite matrix was employed. Graphite from a number 2 pencil was marked on the sample holder and sample placed onto the graphite mark.

Infrared spectra were obtained employing attenuated total reflectance infrared spectroscopy utilizing a Thermo Scientific Nicolet iS5 FTIR equipped with an id5 ATR attachment. Polymer molecular weight was determined using light scattering photometry. Light scattering photometry was carried out employing a Brice-Phoenix Universal Light Scattering Photometer Model 4000 with the polymers dissolved in DMSO or water. ^{1}H NMR spectra were obtained in d_6 DMSO employing Varian Inova 400 MHz and Varian 500 MHz spectrometers.

Cell Testing: The toxicity of each test compound was evaluated using a variety of cancer cell lines, and with normal human embryonic lung fibroblast (WI-38) and mouse embryo-fibroblast (NIH/3T3) cell line as standards. Following a 24 h 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 h. Following incubation, Cell Titer-Blue reagent (Promega Corporation) was added (20 uL/well) and incubated for 2 h. Fluorescence was determined at 530/590 nm and converted to % cell viability versus control cells.

All cytotoxicity values are 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 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 cytotoxicity observed 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. Standard dilutions are employed beginning with the most concentrated with essentially total inhibition occurring to the most dilute where little or no inhibition occurs. The inhibition curve is always sigmoid and the EC_{50} determined at the midpoint of the curve. Once inhibition begins the concentration difference between the initial inhibition and final total inhibition is small with the region between initial inhibition to final total inhibition essentially linear.

RESULTS AND DISCUSSION

Yield and Chain Length: Zirconocene polyethers were formed in moderate yield (Table 1). The products are low to high polymers with polymer molecular weight decreasing as the PEG chain length increases possibly related to the increasing difficulty of the metallocene-active end-group or metallocene itself locating the PEG end group as the distance between the hydroxyl groups increases.

Reaction is rapid occurring within 15 seconds which is common for interfacial polymerization reactions. Rapid reaction is the consequence of the lower activation energies for reactions involving acid chlorides such as the zirconocene dichlorides with alcohols with an activation energy of about 20 kcal/.mol compared with typical condensation reactions where the activation energy is generally about 40 kcal./mol.[31,32] Further, rapid stirring of about 18,000 rpm is employed creating a large interface between the two reaction phases. The interfacial reaction system was developed by Morgan and enlarged by Carraher and is commercially employed to synthesize aromatic nylons and polycarbonates (31,32).[31,32] Thus, it is an industrially employed process for the production of polymers.

Table 01: Product yield and chain length as a function of PEG chain length.

Sample	Percentage Yield	MW (H_2O)	MW (DMSO)	DP
Cp_2Zr/200	24		2.0×10^6	4700
Cp_2Zr/400	15		3.8×10^5	600
Cp_2Zr/1000	30	9.0×10^4	9.1×10^4	75
Cp_2Zr/4600	15	6.1×10^4	6.2×10^4	13
Cp_2Zr/8000	34	4.1×10^4	4.2×10^4	5

As noted before, a major problem with most monomeric titanocene-derived products is poor solubility.[20-26] For the current products, all of the zirconocene polymers are soluble in DMSO and all but the PEG 200 and 400 are soluble in water. Producing water soluble products is one of the aims of the current research, and it has been achieved.

Molecular weight was determined in both DMSO and water and is essentially the same in both solvents consistent with solubility in water not requiring degradation of the polymer. The lack of solubility for the PEG 200 and 400 products is not unexpected since they offer the smallest portion of the water-solubilizing ethylene oxide unit.

Infrared Spectral Results: Infrared spectra were taken of the reactants and products over the range of 4000 to 400 cm^{-1}. All bands are given in cm^{-1}. Band assignments are given in Table 2 for PEG 200, zirconocene dichloride and the polymer derived from reaction between the zirconocene dichloride and PEG 200. Briefly, the C-H aromatic stretch for the C-H in the cyclopentadiene moiety found at 3104 is found at 3175 for the product. Bands from the PEG derived moiety assigned to the C-H asymmetric and symmetric stretching from the PEG itself are found at 2920, 2890, 2885, and 2865 are found in the polymer about 2952, 2940, 2887, and 2860. Additional bands assigned to wagging, scissoring, and rocking are also found as are a number of combination bands (Table 2).

Bands assigned to the Zr-O stretch in metallocene polyethers are generally assigned to about 440 for the asymmetric stretch and 345 for the symmetrical stretch. The higher new band is found at about 442.[11-16] The second band is below the capability of the instrument employed in the present study. An additional band assigned to the Zr-O-C unit is assigned to be about 1050.[11-16],[33-36] In this area there are two bands, one at 1100 is assigned to the O-C stretch in the PEG and a second new band about 1061 is tentatively assigned to the Zr-O-C formation. Thus vibration spectroscopy is consistent with the assigned repeat unit.

Table 02: Infrared band assignments for zirconocene dichloride, PEG 200, and the product of zirconocene dichloride and PEG200.

Band Assignment	Cp_2ZrCl_2	PEG 200	Polymer
C-H St	3104		3175
C-H Asym St		2920	2952
C-H Sym St		2890,2885,2865	2940,2887,2860
CH_2 Scissor		1470,1463,1453	1470, 1457
Cp(C-C St)	1440		1443
CH_2 Wag		1406	1400
C-C St	1371		1366
CH_2 Twist		1283,1244	1295,1264
C-O St, CH_2 Rock		1149	1131
C-O St		1102	1100
C-C St,C-O St, CH_2 Rock		1062	1061
CH ip Wag	1014		1005
CH_2 Rock,CH_2 Twist		963	957
CH_2 Rock, C-C St		947	949
CH_2 Rock, C-C, C-O St		887	886
C-H op St	873,827		874,830

As expected, the bands associated with the zirconocene moiety diminish as the PEG length increases.

MALDI MS: Mass spectra were obtained for the samples. In order to analyze high molecular weight products, the materials must be soluble in a volatile liquid such as water and acetone matching the matrix and polymer solubility allowing intimate mixing of the matrix and polymer. Since this is generally not the case with most polymers, MALDI MS is not able to produce entire polymer chain ions. Thus, we developed an alternative approach that focuses on analyzing lower molecular weight fragments. The precise technique has been reviewed and focuses on analysis of the fragments rather than on the entire chain.[37-39] While the PEG materials are sold as having a particular molecular weight, in reality this is only an approximate molecular weight and should be checked. For the PEG 200 this was done. For PEG 200 the two most common PEG chains have four, $HO(CH_2CH_2O)_4H$, and five, $HO(CH_2CH_2O)_5H$, ethylene oxide units with the combination having a mass of approximately 200 daltons. This is consistent with the results given in Table 3 where the most common ion fragment clusters have four and five ethylene oxide units. Results for the PEG 200 product are given. Masses are described in terms of m/e = 1 or daltons. Spectra were obtained using two modes, the reflective which is employed favoring precision of results and the linear mode favoring detection of higher mass ion fragments. Figures 4 and 5 contain MALDI MS spectra for the product for linear and reflective modes. The assignment of bands is not as straightforward as with many compounds since the PEG is a mixture of chain lengths. Even so, we were able to assign tentative general structure to the major ion fragment clusters. These assignments are given in Table 3. Recently we have been employing graphite as the matrix material because we found that it gives good results with few interfering ion fragments produced above 500 mass which is the typical lower mass range employed in our studies.

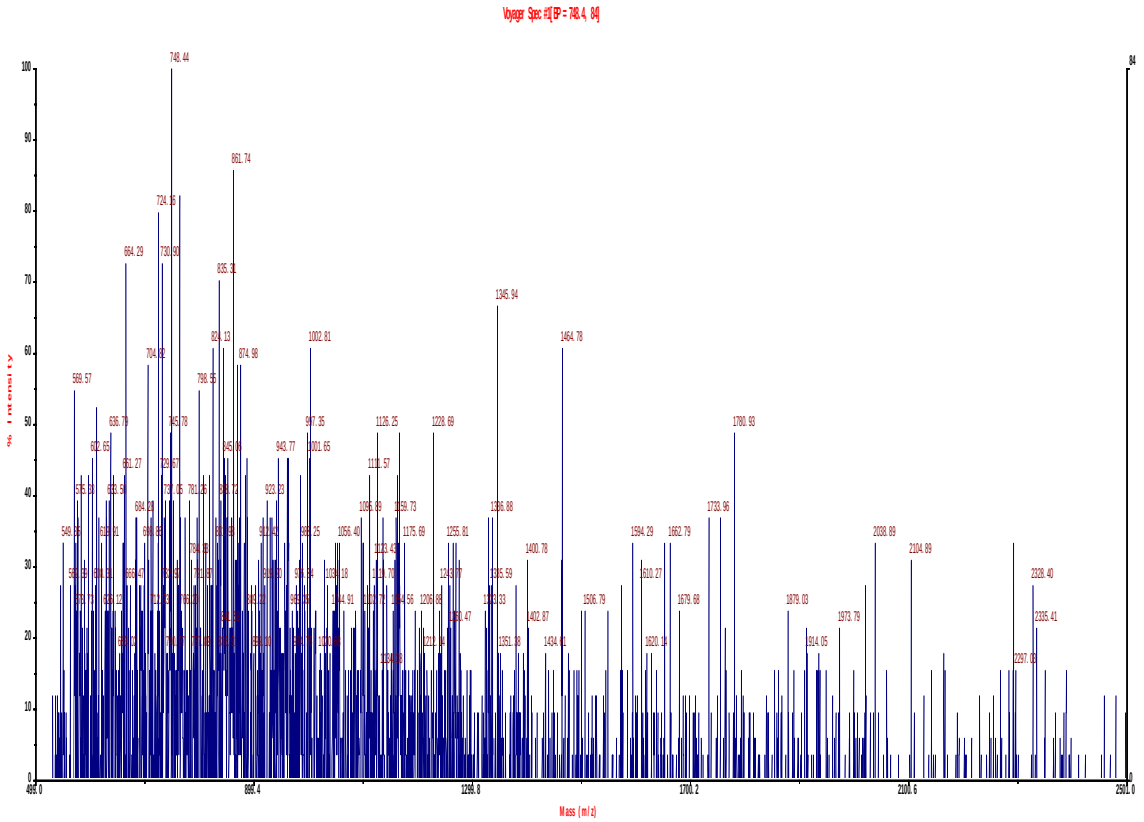

Figure 04: MALDI MS for the PEG 200/ zirconocene dichloride product over the approximate mass range of 500 to 2500 Da for the linear mode.

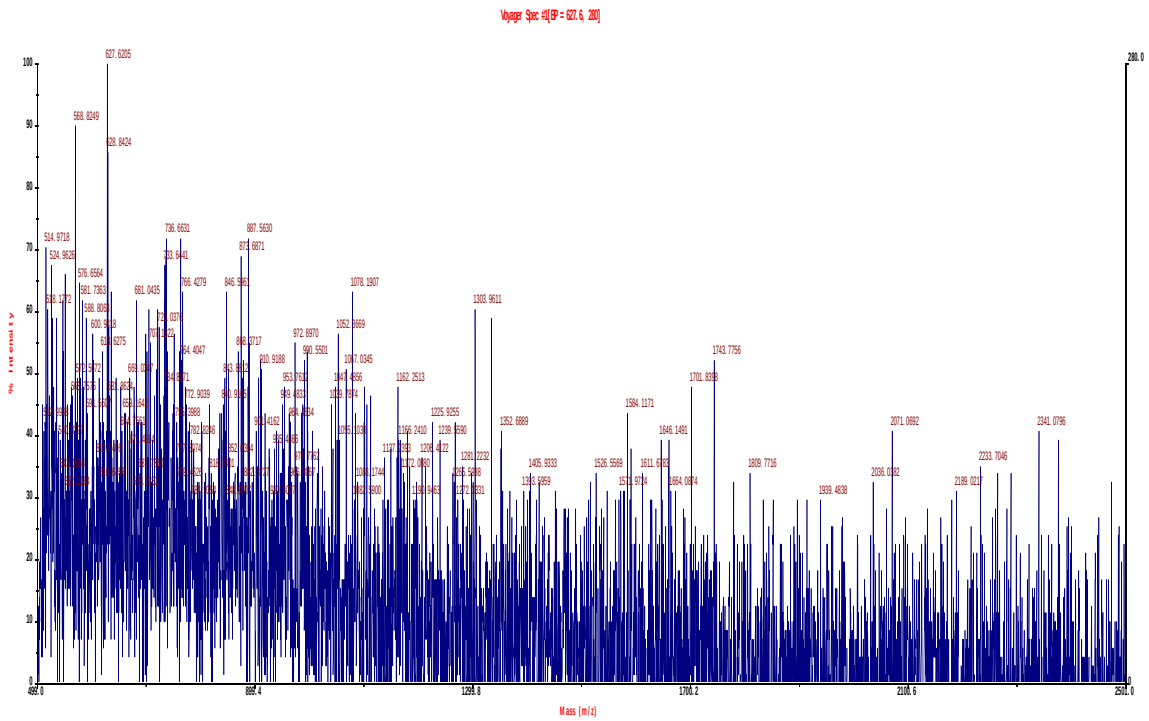

Figure 05: MALDI MS for the PEG 200/ zirconocene dichloride product over the approximate mass range of 500 to 2500 Da for the reflective mode.

Tentative assignments were made for the most abundant ion fragment clusters. Sodium is a common contaminant.

Table 03: Major ion fragments obtained for the product of PEG200 and zirconocene dichloride.

(Tentative) Assignment	Ion fragments for the Reflective Mode	Ion fragments for the Linear Mode
$(CH_2CH_2O)_4Cp_2Zr(CH_2CH_2O)_4$	569	570
$(CH_2CH_2O)_4Cp_2ZrO(CH_2CH_2O)_5$	627	
$(CH_2CH_2O)_5Cp_2Zr(CH_2CH_2O)_5$		664
$Cp_2ZrO(CH_2CH_2O)_5Cp_2ZrOCH_2CH_2O$		724
$Cp_2ZrO(CH_2CH_2O)_5Cp_2ZrOCH_2CH_2O$	737	
$Cp_2ZrO(CH_2CH_2O)_5Cp_2ZrO(CH_2CH_2O)_2$-O*		748
$Cp_2ZrO(CH_2CH_2O)_5Cp_2ZrO(CH_2CH_2O)_4$-O*		835
$Cp_2ZrO(CH_2CH_2O)_5Cp_2ZrO(CH_2CH_2O)_4$	846	
$Cp_2ZrO(CH_2CH_2O)_5Cp_2ZrO(CH_2CH_2O)_4$		862
$Cp_2ZrO(CH_2CH_2O)_5CpZrO(CH_2CH_2O)_5$-O *	874	874
$Cp_2ZrO(CH_2CH_2O)_5CpZrO(CH_2CH_2O)_5$	913	
$[Cp_2ZrO(CH_2CH_2O)_5]_2$ Na		944
$O(CH_2CH_2O)_4Cp_2Zr(OCH_2CH_2)OCp_2Zr$	991	
$[Cp_2Zr(OCH_2CH_2)_4]_2$ OCp_2Zr		1003
$(CH_2CH_2O)_4[Cp_2Zr(OCH_2CH_2)_5]_2$	1062	
$O(CH_2CH_2O)_4[Cp_2Zr(OCH_2CH_2)_5]_2$	1078	
$O(CH_2CH_2O)_5[Cp_2Zr(OCH_2CH_2)_5]_2$		1112
$OCp_2Zr(OCH_2CH_2)_5]_2Cp_2ZrO$		1126
$OCp_2Zr(OCH_2CH_2)_5]_2Cp_2ZrOCH_2CH_2$	1162	
$(CH_2CH_2O)_4[Cp_2Zr(OCH_2CH_2)_5]Cp_2Zr$	1226	1229
$(CH_2CH_2O)_4[Cp_2Zr(OCH_2CH_2)_5]_2Cp_2ZrO$	1304	
$O[Cp_2ZrO(CH_2CH_2O)_5]_3$		1346
$[Cp_2ZrO(CH_2CH_2O)_4]_3Cp_2Zr$	1406	
$[Cp_2ZrO(CH_2CH_2O)_5][Cp_2ZrO(CH_2CH_2O)_4]_2Cp_2Zr$		1455
$[Cp_2ZrO(CH_2CH_2O)_4]_4$	1591	1594
$[Cp_2ZrO(CH_2CH_2O)_5]_2[Cp2ZrO(CH_2CH_2O)_4]_2$	1661	1663
$[Cp_2ZrO(CH_2CH_2O)_5]_3Cp_2ZrO(CH_2CH_2O)_4$		1734
$O[Cp_2ZrO(CH_2CH_2O)_5]_3Cp_2ZrO(CH_2CH_2O)_4$	1745	
$[Cp_2ZrO(CH_2CH_2O)_4]_4$ $(Cp_2ZrC5)_4O$		1781
$[Cp_2ZrO(CH_2CH_2O)_4]_4Cp_2Zr$	1810	
$[Cp_2ZrO(CH_2CH_2O)_4]_4Cp_2ZrO$		1879
$[Cp_2ZrO(CH_2CH_2O)_4]_5$		1974
$O[Cp_2ZrO(CH_2CH_2O)_5][Cp_2ZrO(CH_2CH_2O)_4]_4$	2036	2039
$[Cp_2ZrO(CH_2CH_2O)_5]_2[Cp_2Zr(OCH_2CH_2)_4]_3$	2071	
$[Cp_2ZrO(CH_2CH_2O)_5]_3[Cp_2Zr(OCH_2CH_2)_4]_2$		2105
$OCp_2ZrO(CH_2CH_2O)_5]_4Cp_2Zr(OCH_2CH_2)_4O$	2189	
$[Cp_2ZrO(CH_2CH_2O)_5]_5O$	2233	
$(CH_2CH_2O)_4[Cp_2Zr(OCH_2CH_2)_5]_4O)Cp_2Zr$ $OCH_2CH_2)_4$		2328
$CH_2CH_2O)_4[Cp_2Zr(OCH_2CH_2)_5]_4O)Cp_2Zr$ $OCH_2CH_2)_4O$		2335
$OCH_2CH_2O)_4[Cp_2Zr(OCH_2CH_2)_5]_4O)Cp_2Zr$ $OCH_2CH_2)_4O$	2341	

*Minus one ethylene oxide oxygen at the end of the ethylene oxide chain.

Chain lengths to 5 repeat units are found. It appears that the PEG moiety remains largely intact and that chain scission generally occurs with breakage about the Cp_2Zr moiety (Fig. 6). Sites of chain cleavage typically occur at heteroatom sites for MALDI MS and especially for metal-containing polymers.[1,2] The particular order that the C4 and C5 ethylene oxide moieties appear in the table is not significant.

Each of the ion fragment clusters contain ion fragments containing different zirconium isotopes. Zirconium has four isotopes present in a relative abundance greater than ten percent. Table 4 contains isotopic abundances for two ion fragment clusters each containing two zirconium atoms. The matches are reasonable consistent with the presence of two zirconium atoms in these ion fragment clusters.

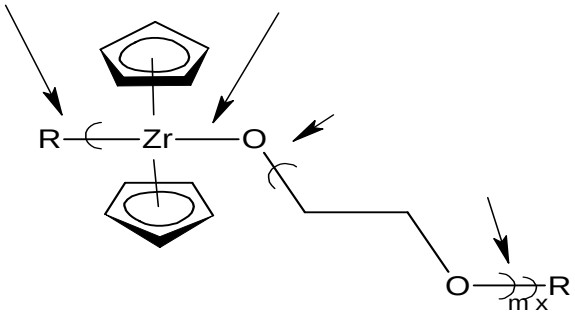

Figure 6: Most prevalent sites for bond cleavage.

Table 04: Isotopic abundance matches for two ion fragment clusters containing two zirconium atoms.

Known for Zr		**$Cp_2ZrO(CH_2CH_2O)_5Cp_2ZrO(CH_2CH_2O)_4$-O**		**$Cp_2ZrO(CH_2CH_2O)_5Cp_2ZrO(CH_2CH_2O)_4$**	
m/e	Rel. Abund.	m/e	Rel. Abund. Found	m/e	Rel. Abund. Found
90	100	835	100	862	100
91	22	836	24	863	20
92	33	837	32	864	34
94	33	839	33	866	34

Proton NMR: NMR was conducted employing d-6 DMSO for the various products. Results for the product from PEG 1000 are given. The zirconocene dichloride has one band at about 6.6 ppm. PEG has bands at about 3.6-3.5 ppm. The polymer shows bands about 6.53 and 6.44 ppm from the zirconocene moiety (cyclopentadiene unit) and 3.6 to 3.3 ppm assigned to the

ethylene units from the PEG-derived moiety. The two bands derived from the zirconocene moiety probably are the result of the two cyclopentadiene rings existing in slightly different environments. The NMR bands in the polymer and monomers are similar consistent with polymer formation having minimal effect on the NMR. These results are consistent with other studies.[40,41]

Cancer Cell Line Results: Cancer is the leading cause of death globally. The cell lines employed in the current study are given in Table 5. They represent a broad range of important cancers.

Table 05: Cell lines employed in the current study.

Strain #	NCI Desig.	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	Embyro-continuous cell line of highly contact-inhibited cells	Fibroblast
	AsPC-1	Human	Pancreatic cells	Adenocarcinoma
	PANC-1	Human	Epithelioid pancreatic cells	Carcinoma

Different evaluative measures are typically employed in the evaluation of compounds to control cancer growth. In other studies, we found that the polymer drugs are cytotoxic and cell death is by necrosis.[2][42] We recently found that the anticancer activity is brought about by the intact polymer and not through polymer degradation.[2][42] 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.[2]

The two most widely employed evaluative measures are used in the present study. The first involves the concentration dose needed to reduce growth of a particular cell line. Several names are associated with this concentration. The term effective concentration, EC, is employed here. 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}. Table 6 contains EC_{50} values for the present compounds and monomers. Cisplatin, among the most widely employed chemo-agents, is included as a standard. Consistent with other studies the zirconocene dichloride is relatively non-toxic[11-16][33-36] as are the PEGs. Even so, as noted before the

ability of various Group 4 metallocene small molecules to inhibit cancer growth is well established.[17-23] Low values are desired since they show that the cancer is inhibited at low concentrations.

Table 06: EC_{50} values (micrograms/mL) for the tested cell lines for zirconocene polymers and monomer, and cisplatin. Values given in () are the standard deviations.

Compound/DMSO	NIH-3T3	WI-38	PANC-1	AsPC-1
Cp_2ZrCl_2	>32	>32	>32	>32
Cp_2Zr/PEG 200	0.0015(.001)	0.0015(.001)	0.094(.06)	0.015(.01)
Cp_2Zr/PEG 400	0.0012(.001)	0.0012(.001)	0.012(.02)	0.013(.013)
Cp_2Zr/PEG 1000	0.0011(.001)	0.0016(.001)	0.115(.1)	0.11(.09)
Cp_2Zr/PEG 46000	0.0011(.001)	0.0019(.001)	0.14(.2)	0.094(.1)
Cp_2Zr/PEG 8000	0.0014(.001)	0.0011(.001)	0.094(.01)	0.122(.2)
Cisplatin	0.015(.01)	0.019(.01)	0.0023(.005)	0.0035(.005)

Compound/DMSO	PC-3	MDA	MCF-7	HT-29
Cp_2ZrCl_2	>32	>32	>32	>32
Cp_2Zr/PEG 200	0.034(.002)	0.040(.002)	0.022(.02)	0.039(.02)
Cp_2Zr/PEG 400	0.029(.002)	0.035(.02)	0.029(.02)	0.084(.02)
Cp_2Zr/PEG 1000	0.16(.1)	0.17(.1)	0.99(.02)	0.12(.09)
Cp_2Zr/PEG 4600	0.098(.1)	0.12(.1)	0.16(.1)	0.14(.1)
Cp_2Zr/PEG 8000	0.13(.1)	0.12(.1)	0.14(.1)	0.15(0.2)
Cisplatin	0.0044(.004)	0.0029(.002)	0.0041(.003)	0.0057(.003)

Compound/H_2O	NIH-3T3	WI-38	PANC-1	AsPC-1
Cp_2ZrCl_2	>32	>32	>32	>32
Cp_2Zr/PEG 200	0.0010(.001)	0.0019(.001)	0.17(.1)	0.011(.02)
Cp_2Zr/PEG 400	0.0009(.001)	0.0022(.001)	0.091(.1)	0.12(.1)
Cp_2Zr/PEG 1000	0.0009(.001)	0.0023(.001)	0.091(.1)	0.17(.1)
Cp_2Zr/PEG 46000	0.0015(.001)	0.0025(.001)	0.11(.2)	0.13(.1)
Cp_2Zr/PEG 8000	0.0017(.001)	0.0029(.001)	0.13(.2)	0.18(.1)
Cisplatin	0.015(.01)	0.019(.01)	0.0023(.005)	0.0035(.005)

Compound/H_2O	PC-3	MDA	MCF-7	HT-29
Cp_2ZrCl_2	>32	>32	>32	>32
Cp_2Zr/PEG 200	0.11(.1)	0.13(.1)	0.18(.1)	0.17(.1)
Cp_2Zr/PEG 400	0.15(.1)	0.19(.1)	0.11(.1)	0.089(.1)
Cp_2Zr/PEG 1000	0.15(.1)	0.15(.1)	0.13(.1)	0.087(.06)
Cp_2Zr/PEG 4600	0.19(.1)	0.089(.1)	0.099(.1)	0.12(.1)
Cp_2Zr/PEG 8000	0.14(.1)	0.17(.1)	0.16(.2)	0.14(.01)
Cisplatin	0.015(.01)	0.019(.01)	0.0023(.005)	0.0035(.005)

In general, the values of the samples initially dissolved in DMSO and those exposed to only water are similar. In Tables 6 and 7 these two are noted as Compound/DMSO for samples originally dissolved in DMSO after which an aqueous solution is added while

Compound/Water describes results when the samples are originally dissolved in water and no DMSO is added. While it is known that most organometallic compounds associate with polar solvents such as DMSO and that the biological results can be influenced by the presence of the DMSO[1-4][43-46] this appears not to be the case here. It is noteworthy that neither reactant inhibits any of the cancer cell lines, yet the polymer combination offers good inhibition of all of the tested cancer cell lines.

The values with respect to the length of the PEG chain are similar. Chain lengths in water are not given in Table 1 for the product from PEG 200 and 400 yet cancer cell data is given in water for all of the compounds. The reason for this is that the minimum solubility needed to determine chain length is 10^{-3} g/mL while the greatest solubility is 3.2 x 10^{-5} g/mL for the cancer cell data collection. Thus, the solubility employed to determine the cancer cell data is much less and the polymers are soluble at this concentration level.

Several observations with respect to individual cell lines results are appropriate. The pair of breast cancer cell lines deserve special comment. They represent a matched pair of cell lines. The MDA-MB-231 (strain number 7233) cells are estrogen-independent, estrogen receptor negative while the MCF-7 (strain line 7259) cells are estrogen receptor (ER) positive. In some studies involving organotin polymers we found there was a marked difference between the ability to inhibit the two cell lines dependent on polymer structure.[2,3][47] In these studies polymers containing a Lewis base that possesses the O-Phenylene moiety, such as hydroquinone and hydroquinone derivatives[2] and diethylstilbestrol,[47] exhibit a relatively greater ability to inhibit the MDA-MB-231 cells in comparison to the MCF-7 cells presumably because the MCF-7 cells react with the drugs removing them from inhibiting the MCF-7 cells whereas those structures, such as the PEG in the present study, that do not contain this structural moiety showed little difference between the ability to inhibit the two cell lines.[3]

The PC-3 results are of interest because this particular prostate cell line is viewed as among the most resistant of the prostate cancer cell lines.[48] The present polymers all exhibit good inhibition of the PC-3 cells. The polymers also exhibit good inhibition of the colon cancer HT-29 cell line.[41]

One of our recent focuses regards pancreatic cancer. Pancreatic cancer afflicts close to 32,000 individuals each year in the United States and 168,000 worldwide, and nearly all patients die

from the ravages of their disease within 3 to 6 months after detection. It is the fourth leading cause of cancer death worldwide behind lung (1.3 million deaths/year), stomach (1 million deaths/year), and liver (660,000 deaths/year). Treatment of pancreatic cancer is rarely successful as this disease typically metastasizes prior to detection. There is no chemotherapy for metastasized pancreatic cancer. We recently described the ability of a number of metallocene polymers to inhibit pancreatic cancer.[11][36] Because pancreatic cancer does not have a generally accepted "cure" one of our current focuses is on the synthesis of materials that might be successful in combating pancreatic cancer. The cell lines tested are AsPC-1 which is an adenocarcinoma pancreatic cell line and PANC-1 which is an epithelioid carcinoma pancreatic cell line. Both are human cell lines and the pair is widely employed in testing for inhibition of pancreatic cancer. For the current study, the polymers exhibit good inhibition of both cell lines in water only and when originally dissolved in DMSO.

Another evaluative measure of the potential use of compounds to inhibit cancer cell growth is the comparison of the ratio of the EC_{50} for the NIH/3T3 (or simply 3T3) or WI-38 cells divided by the EC_{50} for the particular test cell. This value is one of a group called a chemotherapeutic index, CI_{50}. High values are desired since this indicates that inhibition occurs towards the cancer in comparison to the healthy cells.

The CI_{50} values for polymers are given in Table 7. Superimposed in this data is a second study. Two cell lines are typically employed in the evaluation of the effectiveness of compounds to arrest the growth of tumor cell lines. These two cell lines are the NIH 3T3 and WI-38 cell lines. We have begun comparing these two cell lines as biomarkers to study the effectiveness of compounds to inhibit the growth of various tumor cell lines. NIH 3T3 cells are mouse embryo fibroblast cells. They are part of a group of cell lines referred to as partially transformed cells in that they are immortal unlike normal cells. They retain other characteristics of normal cells such as being contact-inhibited. Relative to most normal cells they are robust and easily maintained.

WI-38 cells are normal embryonic human lung fibroblast cells. They have a finite life time of about 50 replications. Compared to NIH 3T3 cells, they are more fragile and difficult to maintain for long periods of time. Thus, NIH 3T3 cells are often favored because of ease of handling aided by an infinite life span.

In the current study, the polymer results give similar CI_{50} values using WI-38 and NIH 3T3 cells as the standard so the use of either cell standard is acceptable. As a quick guide, one can simply look at the ratio of EC_{50} 3T3/EC_{50} WI-38 (or its inverse), so that when this ratio is near one there will be little difference in the CI_{50} values calculated using either cell as standard. For this study, while the ratios of activity for the WI-38/3T3 are not greatly different, they do vary. When there is a difference, the WI-38 cell line results are accepted as being more predictive of live-animal results so greater confidence is given to their results.[49] Thus, for the present study, greater confidence should be given to the WI-38 results. But, for the standard cisplatin, this is not the case where there are large differences. Thus, for the PANC-1 cell line, a CI_{50} of 3.5 is calculated for the 3T3 cells but for the WI-38 standard cell line a CI_{50} value of only 0.044 or a difference of about 100.

Table 7: CI_{50} values determined from data given in Table 6 for samples originally dissolved in DMSO (first set) and water (second set).

Compound/DMSO	EC_{50}3T3/ EC_{50}WI-38	EC_{50}WI-38/ EC_{50}3T3	EC_{50}3T3/ EC_{50}PANC-1	EC_{50}WI-38/ EC_{50}PANC-1
Cp_2Zr/PEG 200	1.0	1.0	0.016	0.016
Cp_2Zr /PEG 400	1.0	1.0	0.10	0.10
Cp_2Zr /PEG 1000	0.69	1.5	0.010	0.14
Cp_2Zr /PEG 4600	0.58	1.7	0.0080	0.014
Cp_2Zr /PEG 8000	1.3	0.79	0.015	0.012
Cisplatin	0.80	1.3	6.5	8.3

Compound/DMSO	EC_{50}3T3/ EC_{50}AsPC-1	EC_{50}WI-38/ EC_{50}AsPC-1	EC_{50}3T3/ EC_{50}PC-3	EC_{50}WI-38/ EC_{50}PC-3
Cp_2Zr/PEG 200	0.10	0.10	0.044	0.044
Cp_2Zr /PEG 400	0.092	0.092	0.041	0.041
Cp_2Zr /PEG 1000	0.010	0.015	0.007	0.010
Cp_2Zr /PEG 4600	0.011	0.020	0.011	0.019
Cp_2Zr /PEG 8000	0.011	0.0090	0.011	0.0085
Cisplatin	0.86	0.01	1200	15

Compound/DMSO	EC_{50}3T3/ EC_{50}MDA	EC_{50}WI-38/ EC_{50}MDA	EC_{50}3T3/ EC_{50}MCF-7	EC_{50}WI-38/ EC_{50}MCF-7
Cp_2Zr /PEG 200	0.0040	0.0040	0.068	0.068
Cp_2Zr /PEG 400	0.034	0.034	0.040	0.041
Cp_2Zr/PEG 1000	0.0060	0.0090	0.011	0.0016
Cp_2Zr /PEG 4600	0.0092	0.016	0.0069	0.012
Cp_2Zr /PEG 8000	0.012	0.0092	0.010	0.0079
Cisplatin	5.2	6.6	2.6	5.3

Compound/DMSO	EC_{50}3T3/ EC_{50}HT-29	EC_{50}WI-38/ EC_{50}HT-29
Cp_2Ti/PEG 200	0.038	0.038
Cp_2Ti/PEG 400	0.014	0.014
Cp_2Ti/PEG 1000	0.0092	0.014
Cp_2Ti/PEG 4600	0.0079	0.014
Cp_2Ti/PEG 8000	0.0093	0.0022
Cisplatin	3.7	4.6

Compound/H_2O	EC_{50}3T3/ EC_{50}WI-38	EC_{50}WI-38/ EC_{50}3T3	EC_{50}3T3/ EC_{50}PANC-1	EC_{50}WI-38/ EC_{50}PANC-1
Cp_2Zr/PEG 200	0.53	1.9	0.0060	0.0011
Cp_2Zr /PEG 400	0.41	2.4	0.0098	0.024
Cp_2Zr /PEG 1000	0.31	2.6	0.0099	0.025
Cp_2Zr /PEG 4600	0.60	1.7	0.014	0.023
Cp_2Zr /PEG 8000	0.59	1.7	0.013	0.022
Cisplatin	0.80	1.3	6.5	8.3

Compound/H_2O	EC_{50}3T3/ EC_{50}AsPC-1	EC_{50}WI-38/ EC_{50}AsPC-1	EC_{50}3T3/ EC_{50}PC-3	EC_{50}WI-38/ EC_{50}PC-3
Cp_2Zr/PEG 200	0.10	0.17	0.0091	0.017
Cp_2Zr /PEG 400	0.0075	0.018	0.0060	0.15
Cp_2Zr /PEG 1000	0.0053	0.014	0.0060	0.015
Cp_2Zr /PEG 4600	0.0012	0.019	0.0079	0.013
Cp_2Zr /PEG 8000	0.0094	0.016	0.012	0.021
Cisplatin	0.86	0.01	1200	15

Compound/H_2O	EC_{50}3T3/ EC_{50}MDA	EC_{50}WI-38/ EC_{50}MDA	EC_{50}3T3/ EC_{50}MCF-7	EC_{50}WI-38/ EC_{50}MCF-7
Cp_2Zr /PEG 200	0.0077	0.015	0.0056	0.011
Cp_2Zr /PEG 400	0.0047	0.012	0.0082	0.020
Cp_2Zr/PEG 1000	0.0047	0.012	0.0082	0.020
Cp_2Zr /PEG 4600	0.017	0.028	0.015	0.025
Cp_2Zr /PEG 8000	0.010	0.017	0.011	0.018
Cisplatin	5.2	6.6	2.6	5.3

Compound/H_2O	EC_{50}3T3/ EC_{50}HT-29	EC_{50}WI-38/ EC_{50}HT-29
Cp_2Zr/PEG 200	0.0059	0.011
Cp_2Zr/PEG 400	0.010	0.25
Cp_2Zr/PEG 1000	0.010	0.026
Cp_2Zr/PEG 4600	0.013	0.021
Cp_2Zr/PEG 8000	0.012	0.021
Cisplatin	3.7	4.6

The CI_{50} values given in Table 9 for the polymers are all less than one indicating that there is not a preference to inhibition by the polymers compared to the WI-38 or 3T3 cells.

There is not agreement as wither EC_{50} or CI_{50} values are better indicators for activity in live animals. For the present study, all of the polymers exhibit good inhibition of all the cancer cell lines based on EC_{50} values.

The fact that the polymers are both DMSO and water-soluble offer other researches an alternative to creating soluble polymeric and small molecules metallocene materials to test as anticancer drugs.

CONCLUSIONS

Water and DMSO soluble polymers were synthesized based on the interfacial reaction between zirconocene dichloride and various PEGs. Product yield is moderate with chain length decreasing as the length of the PEG increases presumably due to the increased difficulty of PEG end chains to find the metal-containing moiety to react with. IR shows formation of the Zr-O bond and MALDI MS the formation of ion fragment clusters to five units long with metal isotopic abundances consistent with the presence of the metal atom within the cluster.

The polymers exhibit good inhibition of the tested cancer cell lines when initially dissolved in DMSO or dissolved in water alone. The tested cell lines include two pancreatic, two breast, one prostate, and one colon cancer cell lines. The EC_{50} values are similar for the water alone and DMSO initially used solutions and as the PEG chain length varies. Along with offering materials exhibiting good inhibition of the cancer cell lines, they also offer other researchers an avenue to create metallocene polymers and small molecules with better solubility.

The products are formed employing commercially available reactants and a system that is used industrially to produce aramids and polycarbonates allowing ready scale-up.

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