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SYNTHESIS AND CHARACTERIZATION OF POLYETHERS FROM ISOMANNIDE AND GROUP 4 METALLOCENE DICHLORIDES AND THEIR ABILITY TO INHIBIT HUMAN CANCER CELLS

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

[1]Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431 and Florida Center for Environmental Studies, Palm Beach Gardens, FL 33410.

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

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

ABSTRACT

Polymers were formed in moderate yield and chain length from the interfacial polymerization of isomannide and Group 4 metallocene dichlorides. The products were synthesized from commercially available materials, so reproduction should be straight forward. The polymers were structurally characterized employing IR and NMR spectroscopy, MALDI MS and light scattering for chain length determination. The polymers showed good inhibition of a battery of human cancer cell lines including two breast and two pancreatic cancer cell lines with excellent ability to differentiate between the healthy cells and cancer cells. The polymers spontaneously formed flexible fibers with aspect ratios of over 1000.

KEYWORDS: isomannide, Group 4 metallocene polymers, titanocene polymers, zirconium polymers, hafnocene polymers, spontaneous fiber formation, pancreatic cancer, breast cancer, MALDI MS, interfacial polymerization.

INTRODUCTION

We have been engaged in the synthesis of a variety of metal-containing polymers including Group 4 metallocene-containing polymers for a variety of purposes.[1-12] Most recently one focus is on the synthesis and characterization of Group 4 metallocene-containing polymers as anticancer, antiviral, antimould, and antibacterial agents.[13-27] Some of this activity has

involved employing biologically active agents as the Lewis base with the idea that this biological activity might impart biological characteristics to the polymer that might enhance the ability to inhibit unwanted growth in bacteria, viruses, and cancers.

Isomannide (Fig. 1) is a natural product originally derived from the secretions of the flowering ash. Isomannide (CAS 641-74-7) has a variety of names including the IUPAC name (3*R*,3*aR*,6*R*,6*aR*)-2,3,3*a*,5,6,6*a*-hexahydrofuro[3,2-b]furan-3,6-diol. It is commercially produced from glucose in large amounts. Its structure is given in Fig. 1. It is also known as mannitol from its resemblance to the Biblical food. It is a sugar alcohol derived from sugar by reduction. It is a heterocyclic compound derived from the double dehydration of hexitols as mannitol and is similar in structure to isoidide and isomannide. The selective dehydration of mannitol to isomannide occurs in good yield with the use of zeolite.[28] Thus, it is a "green material" not derived from petro chemicals. Pseudo-peptides derived from isomannide are inhibitors of serine proteases.[29] Clinically it is used to reduce acutely raised intracranial pressure after a head trauma until a more definitive treatment is prescribed.[30,31] It is also used to open the blood-brain barrier by temporarily shrinking the endothelium cells that make up the barrier. Thus, it is routinely used for delivering drugs to the brain.[30,31] It has other medical uses. In foods, is it employed as a sweetener for persons with diabetes.

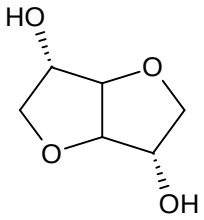

Figure 01: Structure of isomannide.

Isomannide offers rigidity and non-toxicity and is touted as a good intermediate for inclusion into polycondensation polymers. There has been some effort at including isomannide into polymers. A number of polyesters and polycarbonates have been prepared from isomannide.[32-40] Much of this effort has been reviewed.[32] For instance, Hans Kricheldorf and coworkers produced a number of polymeric materials from reaction of isomannide with dialkylcarbonates, diphosgene, co-condensations of isosorbide and isomannide, producing a variety of polycarbonates.[32]

Some of the syntheses have been achieved employing the interfacial polycondensation of isosorbide bischloroformate with various diphenols.[41-43] Many of these products are cholesteric liquid-crystalline materials with interesting optical properties.

We recently reported in this journal the synthesis of organotin polyesters from the reaction of organotin dihalides and isomannide.[10] Here we briefly describe the synthesis of Group 4 metallocene polyethers (Fig. 2) from the reaction of isomannide with Group 4 metallocene dihalides.

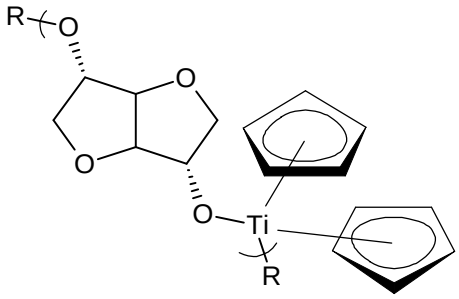

Figure 02: Structure of repeat unit from the reaction of Group 4 metallocene dichlorides, here titanocene dichloride, with isomannide.

EXPERIMENTAL

Reactions and Reactants

Reactions were carried out using the interfacial polycondensation technique such that the isomannide-containing phase and reaction are maintained under nitrogen because of the instability of isomannide to base in the presence of oxygen. Briefly, an aqueous solution (30 ml) containing the isomannide (0.00300 mol) and sodium hydroxide (0.0060 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 chloroform solution (30 ml) containing the metallocene 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. Dilute (0.1 molar HCl) is added to neutralize any remaining base. The nitrogen blanket is no longer necessary. The precipitate was recovered using vacuum filtration and washed several times with deionized water and heptane to remove unreacted materials and unwanted by-products. The solid was washed onto a glass petri dish and allowed to dry at room temperature.

Titanocene dichloride (1271-19-8), isomannide (69-65-8), and zirconocene dichloride (1291-32-3) were purchased from Aldrich Chemical Co., Milwaukee, WS; and hafnocene dichloride (12116-66-4) was obtained from Ventron Alfa Inorganics, Beverly, Mass. The reactants were used as received.

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 alpha-cyano-4-hydroxycinnamic acid are included in the present paper. The solid product along with solid matrix were mixed together employing copper spheres giving a fine powder that was employed to obtain the spectra. Infrared spectra were obtained employing attenuated total reflectance infrared spectroscopy utilizing a JASCO FT/IR-4100 fitted with an ATR Pro 450-s. ^{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 human normal 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. When inhibition begins, the slop of the concentration/inhibition curve is steep until total inhibition occurs.

RESULTS AND DISCUSSION

Product Yield and Chain Length

Polymerization occurs through the interfacial polymerization process developed by Morgan and coworkers at DuPont and enlarged by Carraher.[44-47] The interfacial synthetic technique is employed industrially in the production of aramid fibers and polycarbonates. The rapidity of the reaction is a consequence of at least two factors.[48,49] 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.

Product yield and chain length are given in Table 1. Yields are generally moderate to good. The products from zirconocene and hafnocene are white, as expected since both reactants are white. The product from titanocene is cream to yellow colored because titanocene dichloride has two color sites. The Ti-Cl is a bright red color that vanishes as the reaction occurs after about 10 seconds turning to a cream color from the Cp-Ti color site. The products are low to moderate polymers with chain lengths ranging from 45 for the hafnocene polymer to 1600 for the zirconocene product. There appears to be no trend with respect to chain length. We often find that yield is Zr=Hf>Ti. The explanation for this is often in terms of the hard/soft concept where the yield becomes greater as the metal becomes softer.

Table 01: Product yield and chain length, degree of polymerization, DP for the product of metallocene dichlorides and isomannide.

Metallocene	Yield (%)	Molecular Weight	DP
Cp_2Ti	24	6.5×10^4	200
Cp_2Zr	52	5.9×10^5	1600
Cp_2Hf	50	2.0×10^4	45

Infrared Spectral Results

Infrared spectra were obtained for the monomers and products. Table 2 contains results of this spectroscopy emphasizing the bands associated with the metallocene moiety. All bands are given in wavenumbers. The OH associated bands are absent as expected because of the formation of the M-O linkage. CH bands associated with the metallocene and isomannide are all present in the products as are other bands associated with both monomers. M-O bands are found at 420 and 345 below the capability of the employed instrumentation. Various scissoring, wagging and stretching bands derived from isomannide are observed.[50,51] Bands derived from the metallocene are found associated with various stretching and modes.[6],[13],[15-22],[24] Thus, IR is consistent with the formation of the ether band.

Table 02: Infrared spectral bands for titanocene dichloride, isomannide and metallocene polymers.

Band Assignment	Isomannide	Cp_2TiCl_2	Cp_2Ti Polymer	Cp_2Zr Polymer	Cp_2Hf Polymer
OH St	3402				
CH St Arom		3103	3110	3101	3100
CH Sym St Alip	2992,2958		2953,2926	2954,2925	2956,2920
CH Assym St Alip	2891		2853	2851	2851
CH_2Scissor	1468		1486	1484	1490
Cp C-C St		1440	1441	1442	1440
CH_2 Scissor	1412		1414	1417	1396
C-C St		1371	1370	1374	1375
Def Scissor OH, CH	1197				
CO St in CHOH	1083		1080	1086	1086
CH ip Wag		1014	1011	1086	1014

Isomannide IR spectral assignments taken from literature.[44,45] Metallocene IR assignments are also taken from literature.[13],[15-22],[24]

Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrophotometry

We have been investigating the solid-state fragmentation of various polymers employing MALDI TOF MS emphasizing metal-containing polymers for use in the structural identification of these polymers. While MALDI MS was touted as a method to analyze polymer molecular weight, it suffers in that for this to occur matrix must be in intiment contact with the polymer sample and for this to occur the matrix and sample must be soluble in a highly volative solvent. For most polymers this is not the case so we utilize an approach that allowed 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.[16],[52,53]

MALDI MS was carried out on the products over the general range of 500 to 5000 Da. All mass values are given in Da. Alpha-cyano-4-hydroxycinnamic acid was used as the matrix. Because it has an acid and hydroxyl group within it, reactions with the titanocene moiety occur and ion fragment clusters containing such interfeering ions are present. These have been eliminated in identifying the major ions found in the spectra. This problem has been discussed elsewhere.

A portion of the MALDI MS for the titanocene/isomannide polymer is given in Figure 3. Each of the metal-containing ion fragment clusters above 400 are clusters of ions that are produced because of the presence of titanium atom(s) within each cluster. Because titanium has isotopes, different ion fragments result that have the same structural formula but vary by the titanium isotope present. This creates what is often referred to as spectral “fingerprints” characteristic of the natural abundance of these isotopes. This will be discussed for the zirconium and hafnium-containing polymers. Even so, the metal-containing fragments given in the following tables are clusters of such ion fragments. Table 3 contains the most abundant ions and ion fragment clusters given in Figure 3. Figure 3 contains a MALDI M for the product of titanocene dichloride and isomannide and table 3 contains the assignments for the major ion fragments found for this product. The following abbreviations are employed in describing the ion fragment clusters where U is one unit, 2U is two units, I is isomannide minus two protons. Sodium is a common contaminant.

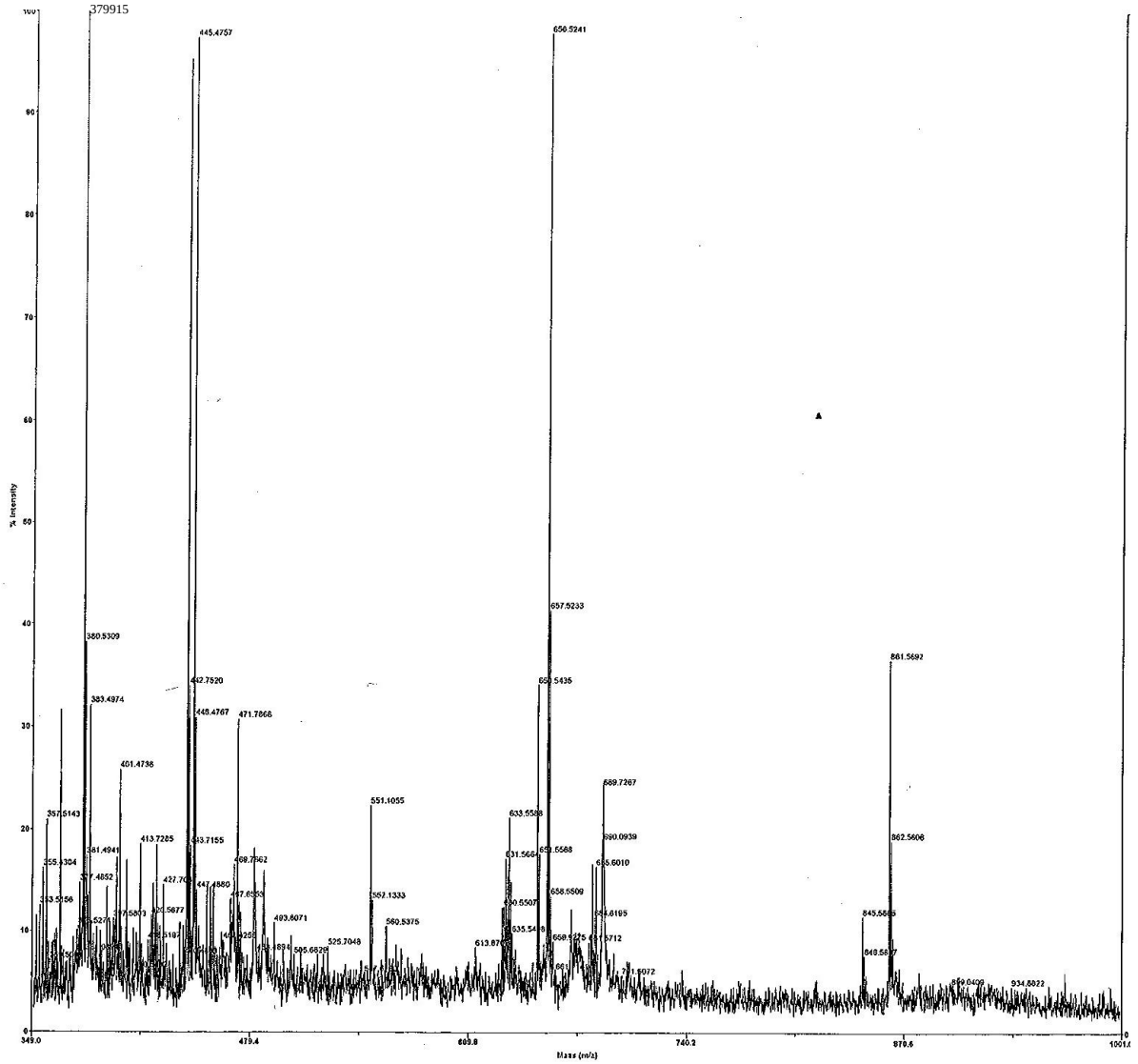

Figure 03: MALDI MS for the product of titanocene dichloride and isomannide over the mass range of 350 to 1000 Da.

Table 03: Most abundant ion fragment clusters derived from the products of isomannide and titanocene dichloride from 400 to 2500 Da.

Mass (Da)	(Tentative) Assignment		Mass (Da)	(Tentative) Assignment
401	U+I-Cp		1222	4U-Cp
505	U+Cp_2Ti		1349	4U+I-2O
525	U+ Cp_2Ti,Na		1420	4U+I-O
551	U+ Cp_2Ti,2O,Na		1623	5U+O
633	2U-O		1780	5U+ Cp_2Ti
845	2U+ Cp_2Ti,Na		1981	6U+O,Na
899	3U-Cp		2088	6U+I
940	3U-Cp+O,Na		2223	7U-O
1095	3U+I-O			

Ion fragment clusters to seven units are found. Also, there is often found the loss of the Cp group from the metallocene. This is common for metallocene polymers and indicates a particular site where chain scission occurs that is accompanied with loss of the Cp moiety.

All three metallocene metal atoms have isotopes. Thus, isotope abundance matches are possible. Titanium has five isotopes. Table 4 contains an isotopic abundance match for two ion fragments. The matches are reasonable and consistent with the presence of the titanium atom present in the fragments.

Table 04: Isotopic abundanxe matches for two ion fragment clusters containing two titanium atoms each. Isotope abundances >5 are given.

Known-Two Ti		U+ Cp_2Ti,2O,Na		2U+ Cp_2Ti,Na	
Da	**Rel. Abund.**	**Da**	**Rel. Abund**	**Da**	**Rel. Abund**
94	22	549	23	843	24
95	21	550	21	844	22
96	100	551	100	845	100
97	16	552	15	846	17
98	15	553	15	847	15

Table 5 contains the major ion fragments for the product of isomannide and zirconocene dichloride.

Table 05: Major ion fragment clusters for the product of zirconocene dichloride and isomannide over the range of 350 to 2000 Da.

Mass (Da)	(Tentative) Assignment		Mass (Da)	(Tentative) Assignment
379	U+O		927	2U+CpZr,O,Na
401	U+O,Na		996	2U+Cp_2Zr,2O,Na
423	U+2O,Na		1034	3U-Cp
445	U+I-Cp		1097	3U
734	2U		1169	3U+I-Cp
763	2U+O,Na		1874	5U-Cp,2O,I
862	2U+I			

Zirconium has four isotopes with greater than 5% relative abundance. Table 6 contains an isotopic abundance match for an ion fragment cluster containing a single zirconium atom. The match is consistent with the presence of zirconium in the ion fragment.

Table 06: Isotopic abundance matches for one ion fragment clusters containing a single zirconium atom.

Known for One Zr		U+O	
Da	**Rel. Abundance**	**Da**	**Rel. Abundance**
90	100	379	100
91	22	380	20
92	33	381	34
94	33	383	33

The major ion fragments for the polymer from hafnocene dichloride and isomannide are given in Table 7.

Table 07: Major ion fragment clusters for the product of hafnocene dichloride and isomannide over the range of 350 to 800 Da.

Mass (Da)	(Tentative) Assignment
361	Cp_2Hf+2O,Na
424	U-Cp+Na
477	U+Na
493	U+Na,O
718	U+CpHf,Na
867	2U-Cp+Na

As noted before, bond scission occurs at the heteroatom sites, mainly the metal-containing moiety as shown in Figure 4.

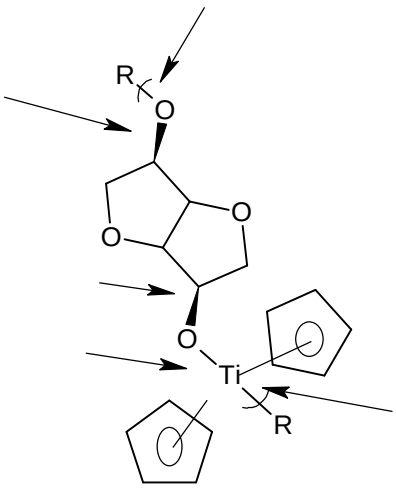

Figure 04: Sites of major bond scission for the metallocene polymers, here for the titanocene moiety.

MALDI MS results are consistent with the proposed polymer repeat unit.

Proton NMR Spectroscopy

Proton NMR spectroscopy was carried out on all of the products and monomers. All NMRs of the metallocene dichlorides show a band about 6.3 (all locations are given in ppm) associated with the Cp groups.[21-27] This is found in the NMR spectra for the polymers. The hydroxyl proton found about 7.75 is missing as expected in the polymers. Isomannide has several protons in varying environment.[54,55] These are given in Figure 5. For the titanocene polymers these appear about A 4.9; B 4.4; C 4.1. In comparison to isomannide itself these bands are at A 5.1; B 4.5; and C 4.1. Thus, there is a mild shift but proton locations for all of the protons are present.

Because of the poor polymer solubility in d6 DMSO further analysis is not viable.

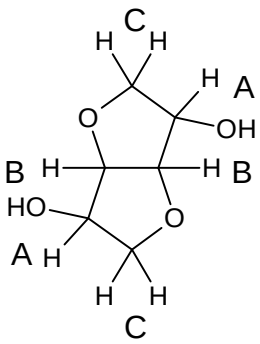

Figure 05: Structural location of protons associated with isomannide.

Cancer tumor analysis results

As noted before, a major purpose in synthesizing metal-containing polymers is to investigate their ability to inhibit unwanted pathogens and infectious agents here the focus is cancer. Table 8 contains the cell lines employed in the current study.

Table 08: Cancer 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
	AsPC-1	Human	Pancreatic cells	Adenocarcinoma
	PANC-1	Human	Epithelioid pancreatic cells	Carcinoma

The two most widely used approaches employed to evaluate cell line effectiveness are used in the present study. The first measures the concentration dose needed to reduce the growth of the cell line. The term effective concentration, EC, is used here. The amount of a tested material that induces inhibition halfway between the baseline and maximum dose is referred to as the 50% response concentration and given the symbol EC_{50}. EC_{50} values for the monomers and polymers, including values for cisplatin as a standard, are given in Table 9. Cisplatin is a widely used anticancer drug. It is toxic as indicated by the low EC_{50} values for the two standard cell lines, NIH 3T3 and WI-38. In combating cancer cisplatin, and other related platinum drugs, exhibits many unwanted side effects in patients including loss of hair, loss of taste, numbness, vomiting, kidney damage, hearing loss, etc.[56] Even so, it remains among the most widely employed drugs for the treatment of cancer.[56]

Table 09: EC_{50} Concentrations (micrograms/mL) for the tested compounds. Values given in ( ) are standard deviations for each set of measurements.

Sample	NIH-3T3	*WI-38*	*PANC-1*	*AsPC-1*
Cp_2TiCl_2	1.8(.2)	2.2(.1)	0.45(.3)	0.51(.05)
Cp_2Ti/IS	40(6)	44(5)	1.9(1)	2.2(.9)
Cp_2ZrCl_2	1.8(.2)	0.94(.1)	0.38(.3)	0.44(.05)
Cp_2Zr/IS	22(4)	20(3)	2.0(1)	3.0(1)
Cp_2HfCl_2	1.7(.2)	1.2(.2)	0.22(.2)	0.18(.01)
Cp_2Hf/IS	40(5)	44(5)	1.7(1)	2.9(1)
Cisplatin	0.0044(.004)	0.0029(.002)	0.0041(.003)	0.0057(.003)

Sample	*PC-3*	*MDA-MB-231*	*HT-29*	*MCF-7*
Cp_2TiCl_2	0.48(.05)	0.35(.05)	0.54(.06)	0.47(.05)
Cp_2Ti/IS	6.6(2)	7.0(4)	8.0(3)	11(4)
Cp_2ZrCl_2	0.25(.03)	0.27(.03)	0.31(.02)	0.33(.05)
Cp_2Zr/IS	9.2(4)	4.4(1)	5.1(2)	2.2(1)
Cp_2HfCl_2	0.25(.03)	0.27(.03)	0.31(.02)	0.33(.05)
Cp_2Hf/IS	4.1(1)	11(4)	17(5)	6.2(3)
Cisplatin	0.0044(.004)	0.0029(.002)	0.0041(.003)	0.0057(.003)

The polymer inhibits all the tested cell lines. The EC_{50} values for the polymers are larger than those for the monomers and cisplatin. Isomannide exhibits no inhibition to any of the tested cell lines to the concentration limit tested.

The following focuses on the ability of the polymers to inhibit specific cancer cell lines. There is no effective treatment of pancreatic cancer once it metastasizes. In the USA yearly about 32,000 new cases are diagnosed. Within a year almost all die. Worldwide, pancreatic cancer is the fourth leading cause of cancer death. Inhibition of pancreatic cancer cell lines is

difficult compared with other human cancer cell lines. Even so, we found several metallocene-containing polymers that exhibit good ability to inhibit pancreatic cancer. In the current study the two most widely studied human pancreatic cancer cell lines are employed. The tested cell lines are AsPC-1 which is an adenocarcinoma pancreatic cell line, which accounts for about 80% of the diagnosed human pancreatic cancers, and PANC-1 which is an epithelioid carcinoma pancreatic cell line, accounting for about 10% of the human pancreatic cancer cases. In the current study, the metallocene polymers show decent inhibition of both cell lines with the inhibition similar for the two cell lines. This similarity is consistent with the idea that the polymers may offer broad-spectra inhibition of other pancreatic cancer cell lines.

In the present study a matched pair of breast cancer cell lines were used. 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 other studies a major difference in the ability to inhibit these two cell lines by the organotin polymers was found.[57] It has not been found for metallocene-containing polymers. Here, the inhibition between the two breast cell lines is similar.

Generally, the NIH and WI-38 values are similar consistent with the ability to use either in evaluating drug effectiveness.

Table 10: CI_{50} values for monomers and polymers derived from data given in Table 9 based on WI-38 data.

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	EC_{50}WI-38/ EC_{50}HT-29	EC_{50}WI-38/ EC_{50}MCF-7
Cp_2TiCl_2	4.9	4.3	4.6	6.3	4.1	4.1
Cp_2Ti/IS	28	20	6.7	6.3	5.5	4.0
Cp_2ZrC_2	2.5	2.1	3.8	3.5	3.0	2.8
Cp_2Zr/IS	10	6.7	3.0	2.9	2.5	1.8
Cp_2HfC_2	5.5	6.7	4.8	4.4	3.9	3.6
Cp_2Hf/S	24	14	9.8	3.6	2.4	6.5
Cisplatin	5.2	3.4	2.7	4.1	2.9	2.1

The second calculation for measuring anticancer effectiveness is the chemotherapeutic index, CI, where the CI_{50} is the ratio of the EC_{50} for the standard cell line NIH/3T3 or WI-38 cells divided by the EC_{50} for the test cell. When there is a difference between the NIH/3T3 and WI-38 results, the WI-38 results are considered a better measure. The CI_{50} results are shown employing WI-38 cells as the standards in Table 10. Values greater than one are desirable in

this measure since it indicates that there is a preference for inhibiting the cancer cell lines in comparison to the standard cells. In the present case, all the polymers and metallocene monomers are greater than one. The CI_{50} values for the polymers are similar to the cisplatin values. The greater toxicity to the WI-38 cell (Table 5) is consistent with the greater toxicity of cisplatin compared to normal cells. This is also reported in other studies.[56]

Overall, the CI_{50} values for the polymers are greater compared to cisplatin and the metallocene monomers. Further, the titanocene polymers are generally greater compared to the zirconocene and hafnocene polymers.

The results for the breast cancer are similar. For the pancreatic cancer, the values for the PNC are a little larger than for the AsPC call lines. The significance for this is not known but the CI_{50} values for both pancreatic cancer cell lines is large, generally greater than ten. As noted before, cisplatin is known for exhibiting many intense negative side-effects[56] so it is positive that the current isomannide polymers exhibit good CI_{50} values.

The two cell lines most used as standards are the NIH/3T3 and WI-38 cell lines. NIH/3T3 cells are mouse embryo fibroblast cells. They are part of a group of cell lines that are 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. While NIH/3T3 cells are often favored because of ease of handling aided by an infinite life span, results from WI-38 cells are given greater importance when there is a difference. Here, the polymer results are similar for the two cell lines.

Thus, the current polymers show good inhibition of the cancer cell lines based on EC_{50} values, and outstanding ability to differentiate cancer cell line growth based on CI_{50} values. These results indicate that further study is merited for the polymers, especially with respect to pancreatic cancer.

SUMMARY

Polyethers have been produced by the interfacial polymerization of Group 4 metallocene dichlorides and isomannide in moderate yield and chain length. Synthesis rapidly occurs

through use of the interfacial polymers that is employed industrially to produce polycarbonates and aromatic nylons using commercially available reactants. Thus, ready synthesis to produce gram to kilogram amounts is reasonably straightforward. Structural characterization was accomplished using NMR, IR, MALDI MS and is consistent with the proposed polyether structure. The polyethers exhibit good inhibition of all tested cancer cell lines including human breast and pancreatic cancer cell lines.

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