ABSTRACT

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HELICAL AND HIGHER STRUCTURAL ORDERING IN POLY(2-METHOXYSTYRENE)/BIOCOMPATIBILITY AND CELL ADHESION STUDIES OF POLY(2-METHOXYSTYRENE)

Advisor: Professor Ishrat Khan

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Poly(2-methoxystyrene) has been synthesized by a variety of polymerization pathways such as anionic, free radical, and helix–sense anionic. The polymers were casted on glass slides and cell adhesion, cell morphology, and cell growth were observed using HeLa Ovarian Cancer cell culture. Tissue culture modified poly(styrene) (TCPS) was used as a control. HeLa cell growth and adhesion was established onto all of the polymers but at different levels of preference. In all cases, the HeLa cells showed the best growth on (-) and (+) optically active helical poly(2-methoxystyrene) (+) and (-) P2MS. Remarkably, the HeLa Ovarian Cancer cells displayed a chiral preference for (+) optically active helical P2MS.

HELICAL AND HIGHER STRUCTURAL ORDERING IN POLY(2-METHOXYSTYRENE)/BIOCOMPATIBILITY AND CELL ADHESION STUDIES OF POLY(2-METHOXYSTYRENE)

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LIST OF ABBREVIATIONS

2-methoxystyrene	2MS or 2-MeOSt
Poly(3-methyl-4-vinylpyridine)	P3M4VP
Poly(2-methoxystyrene)	P2MS or P2-MeOSt
Triphenylmethyl methacrylate	TrMA
High performance liquid chromatography	HPLC
(+) or (-)-2,3,-dimethoxy-1,4-bis(dimethylamino)butanes	(+) or (-) DDB
(-)-Sparteine	(-)-Sp
(S)-(+)-1-(2-pyrrolidinylmethyl)pyrrolidine	(+)-PMP
(S)-(-)-methyl benzyl methacrylate	(S)-(-)-MBMA
N,N-Diphenylethylenediamine	DPEDA
Poly(ethylene oxide)	PEO
Poly(ethylene glycol)	PEG
(R) or (S)- Mandelic Acids	(R) or (S) – MA
Poly-((4-carboxyphenyl)acetylene)	P4CPA
Poly(3-carboxyphenyl isocyanate)	P3CPI
Circular Dichroism	CD
Induced Circular Dichroism	ICD
Tissue Culture Poly(styrene)	TCPS
2,2'-Azobisisobutyronitrile	AIBN
n-Butyl lithium	n-BuLi
Tetrahydrofuran	THF
Nuclear Magnetic Resonance	NMR
Free radically prepared poly(2-methoxystyrene)	FRP2MS
Anionically prepared poly(2-methoxystyrene)	ANPMS
Molecular Weight	MW

CHAPTER I

INTRODUCTION

Proteins are macromolecules central to life.[1] Nearly all biological processes involve the specialized functions of one or more protein molecules. Proteins function to produce other proteins, control all aspects of cellular metabolism, regulate the movement of various molecular and ionic species across membranes, convert and store cellular energy, and carry out many other activities. Essentially all of the information required to initiate, conduct, and regulate each of these functions must be contained in the structure of the protein. For example, the protein collagen is found in tendons and ligaments in the human body. Collagen's structure consists of three helices entwined together that form what is called a super helix. The key to the strength and functional role of collagen is the higher structural order of the macromolecule i.e. the formation of the super helix. As we embark upon a new millennium, it is important to recognize and address the bio-hazardous issues, therapeutic demands, and technological challenges that will accompany the rapidly changing and growing society in which we live. Therefore, as scientists strive to develop a new generation of bio-materials for any number of biological applications, it is necessary to synthesize polymers possessing higher structural orders, which may be capable of generating relevant biological properties and functions.

The helix is one of the fundamental, dissymmetric shapes. The helix was developed in natural systems in the early stages of evolution and used as the structural motif for the

molecules of life (DNA and RNA) and as an important conformation element that enforces long-range order in other biomacromolecule. Helicity received attention in organic chemistry after the discovery of chirality at the end of the last 1800s, but molecules with extended helical structures have been described only recently. Examples are the copper phenantholine-based helices reported by Lehn and the self-organized quadruple helices from amphiphilic molecules synthesized by Fuhrhop.[2] In polymer chemistry, helical architectures have been studied since the pioneering work of Natta, Pino, and others.[3] Most isotactic polymers have short range helical conformation in solution. These are dynamic rather than static structures and the direction of the helical twist is very sensitive to small changes in polymer side chain structure and the type of solvent.[4] The helical conformations are really interconverting between the left and right-handed forms.

Polymers that maintain stable helical structures in solution, as do biomacromolecules, are very rare, but are of great interest since they can display optical activity due solely to main-chain conformation. Furthermore, they may be used as versatile building blocks for the construction of novel chiral supramolecular architectures as chiral catalysts, chiral hosts, chiral adsorbents.[5,6,7]

Helical polymers which offer the possibility to change their chiro-optical properties through a change in their main-chain conformation via some type of external stimuli, such as irradiation, change in temperature, or change in concentration, are also of great interest as well.[8,9] The helical conformational change can be detected in the absorption region of the polymer chain by circular dichroism spectroscopy. The overall change in

the chiro-optical properties can be determined far from the absorption region by measuring the optical rotation.[10] These polymers are especially interesting for various medicinal and optical data storage applications.[11-18]

To date, there are many examples of synthetic macromolecules, which have been demonstrated to have well-defined helical or secondary structures. Among the most popular are the bulky methacrylates which may be polymerized by anionic polymerization to obtain predominantly one-handed helical optically active polymers e.g., poly(triphenylmethyl methacrylate) and poly(diphenyl-2-pyridylmethyl methacrylate).[19-22] The helical polymers are predominantly isotactic and the helical conformation is maintained because the bulky side groups do not readily permit helix-to-helix interconversion. However, stereomutation has been observed for the methacrylates at higher temperatures. It was thought for a substantial amount of time that these bulky side groups were needed in order to carry out helix sense selective polymerization of vinyl monomers. In a 1958 paper, Nobel Laureate Natta showed that ortho substituted isotactic polystyrenes existed in favored helical conformations in the solid state. The ortho group is key in the steric stabilization of the helical conformation. In 1998, the successful preparation of optically active helical poly(3-methyl-4-vinylpyridine) was reported by Khan and Ortiz.[23] It was found that a methyl group specifically placed close to the polymer backbone does not readily permit helix-to-helix interconversion. Therefore, the Natta reports in the 1950s raised the possiblility of plausibly preparing helical polymers using vinyl monomers which are structurally quite simple compared to the bulky methacrylates. The optically active helical poly(3-methyl-4-vinylpyridine) is

stable in the solid state at room temperature and in the solution at –78 °C. Novak has also shown for poly(carboiimides) that the introduction of sterically demanding groups near the polymer chain can increase inversion barrier.[24,25] Recently, Okamoto and coworkers have reported the preparation of a higher structurally ordered polymer using an ortho substituted styrene, [(S)-2-(1-pyrrodinylmethyl)-1pyrrodinylmethyl]styrene.[26]

Other examples of helical polymers are poly(isocyanates),[27-32] poly(N,N'-disubstituted acrylamides),[33-34] poly(chloral),[35-36] and synthetic peptides. [37-40] A number of papers have recently reported on synthetic β-peptides with stable secondary structures or helical conformations.[41] The secondary helical structures of these β-peptides are possible because of intramolecular hydrogen bonding, whereas the optically active helical isotactic poly(bulky methacrylates) adopt the helical conformation because of steric reasons, i.e., lowest energy conformation is the helix.

This dissertation reports on the successful preparation of optically active helical poly(2-methoxystyrene) (P2MS) and confirms and further demonstrates that very bulky side groups may not be necessary to carry out helix sense selective polymerization of vinyl monomers. It confirms the relevance of carrying out helix sense selective polymerization of monomers with fairly simple structures, such as 2-methoxystyrene. The motivation for this work results from a number of observations including the fact that in the solid state isotactic α-olefins and certain substituted styrenes are known to be in a low energy helical conformation with trans-gauche (...TGTG...) type backbone conformation.[42-43] In this study, the helix-sense-selective polymerization of 2-methoxystyrene was successfully carried out. In addition, a method for preparation of

optically active helical poly(2-methoxystyrene) from a racemic mix of helices was also developed. Further bio-compatibility studies were done to demonstrate and confirm the validity of poly(2-methoxystyrene) as a relevant polymeric material for various biomedical applications.

CHAPTER II

BACKGROUND

2.1 Optical Activity

Optical activity, defined as the rotation of the plane of plane-polarized light passing through a material, is a property found in molecules and supramolecular structures which lack a plane of symmetry. The type of optical activity of most frequent concern to chemists derives from a carbon atom or another atom, such as nitrogen, phosphorous or silicon, with four different substituents. Optical activity of compounds containing asymmetric carbon atoms is generally measured in solution and expressed as degrees of rotation per unit concentration per unit length. However, many types of crystals also exhibit optical activity in the solid state. These crystals are of two major types. The first type includes crystals of small molecules, which are themselves molecularly dissymmetric and show optical activity in solution, such as camphor and sucrose. The second and more interesting type is composed of crystals, not containing optically active small molecules or ions, whose optical activity is produced only by the dissymmetry of crystal structure itself and which disappears upon destruction of the crystal structure.

The silicon and oxygen atoms of alpha-quartz are arranged in helices with the d- and l- form containing helices of opposite chirality. This dense packing of dissymmetric helices in the solid state results in very high optical activity along the optical axes of single crystals of d- and l-alpha-quartz, with the optical rotation ranging form about 20/mm to nearly 400/mm depending on the wavelength. Optical activity is found in

quartz, because left- and right-handed helices are dissymmetric, whereas carbon atoms with four different substituents, the usual causes of optical activity in organic compounds, are asymmetric.

2.2 Optical Activity in Polymers

Many investigators observed the phenomenon of optical activity associated with asymmetric carbon atoms in small molecules. The first recorded work to incorporate optical activity in a synthetic polymer through asymmetric carbon atom is that of Walden, who polymerized the bis-(-)-2-methylbutyl ester of methylenesuccinic acid to form a polymer no different in optical rotation from the starting monomer. Many subsequent attempts to synthesize optically active polymers, however, failed because of uncertainty as to the true definition of an asymmetric carbon atom, particularly in polymers, and as to what structures were capable of producing optical activity. Several attempts were made to produce optically active ordinary vinyl monomers through polymerization of optically active monomers and subsequent removal of asymmetric side chains. But, after the optically active side chains or initiator residues were removed, the remaining polymer was not optically active.

The absence of optical activity in these polymers was explained by showing that asymmetric carbon atoms could not be generated from ordinary vinyl monomers (CH2CXY) in high polymers. The CXY carbon atoms in the polymer center of a heterotactic triad, between two CH2CXY differing in configuration only, were shown to be not asymmetric but pseudoasymmetric and incapable of contribution to the optical activity. The central carbon atoms in a tactic triad could be regarded as asymmetric

only insofar as the two substituents forming the polymer chain were of different lengths, which could contribute to optical activity but only in the lowest molecular weight polymers or oligomers.

Isotactic polymers were prepared from a number of chiral alpha olefins. In most cases, optical rotation per monomer unit for the polymer was found to be considerably higher than molar optical rotation for low molecular weight with saturated model compounds. The amount of excess rotation for the polymer was found to vary with distance of the asymmetric carbon atom from the chain backbone, reaching a maximum for poly(4-methyl-1-hexene) and becoming quite low as asymmetric atom was moved several carbons from the chain. The observed excess optical rotation in such polymers was found to increase with decreasing solubility and presumably increasing isotacticity of the polymer.

As a result of many of the above observations, Pino and coworkers have proposed that these polymers exist in solution primarily in the helical conformation of one screw sense, with the helicity strongly contributing to the optical rotation. They considered helicity of the polymer chain in solution as an important contributing factor in causing the excess optical rotations observed for these polymers. However, in contrast to the long and rigid helices observed for polypeptides, the helical regions in these polyolefins and similar polymers were considered to be short-range and constantly coiling and uncoiling, although the total helical content of a polymer solution would remain constant over time at constant conditions. Furthermore, it was thought that the excess optical rotation noted in the polymers was not due primarily to optical rotation by the

chiral helices themselves. Rather it was felt that the steric effect of the adjacent helix forced the substituents around the asymmetric carbon atoms to assume preferred conformations, which in turn increased the observed optical rotation. The specific rotation of certain highly crystalline polyolefins was found to be quite different in solution from that in the crystalline or solid-state, in which the helical forms exist exclusively in the crystalline or solid state.

2.3 Isotactic Polymers

Isotactic polymers, due to energy considerations, crystallize into helical forms. However, if these polymers are prepared with optically inactive or racemic initiators, equal amounts of left- and righthanded helices will be formed. The overall effect of this in the crystal structure is crystallization of a 50:50 mixture of the two types of helices in all isotactic polymers except for certain polymers which contain asymmetric carbon atoms in the side chains; normal isotactic polymers have crystal structures containing equal numbers of left- and right-handed helices in the unit cell. Hence, the crystals do not show optical activity based on helical dissymmetry. Furthermore, even if a helical polymer without assymmetric carbon atoms were initiated asymmetrically to give an excess of one form of helix, the specific rotation in solution could not then be measured, since the optical activity would be destroyed upon dissolution as the dissymmetric helices rearranged to random coils. Hence, for isotactic polymers in solution, optical activity can be demonstrated only when the equilibrium of left-handed helix; right-handed helix is shifted by some characteristic of the molecule such that one sense of helix predominates over the other. The standard means of accomplishing this in the past has been by

introducing asymmetric groups into the side chains of polymers such that one helical arrangement became more stable than the other because of better packing of the side chains. However, if a polymer could be synthesized having the following criteria, the existence of optical activity due purely to helicity in the polymer would appear possible.

(1) The polymer must have a rigid helical structure. It must be totally isotactic with no helix reversals, or else there must be a significant length of helix between the initiation site and the first reversal, so that the amounts of right – and left-handed helix formed will not be identical and optical rotation will be caused by the presence of one form of helix predominantly or exclusively, as is the case in quartz.

(2) The optical activity of the polymer must be measured in the solid state. If measurement in solution is attempted, the optical activity would be destroyed by uncoiling of the helices and re-equilibration to equal proportion of the two helical forms. For the same reason, the solid polymer cannot be contacted with a solvent in which it is soluble before optical activity is measured.

The polymer, which would seem best to fit these criteria are polymers containing side groups which are very bulky and adjacent the polymer chain, and which could be obtained in the isotactic form.

2.4 Optically Active Helical Polymers: Secondary Structure

Polymers possessing secondary structure contain chiral organizations which are present as a helical conformation of the polymeric backbone.[44] Again, it was first recognized in the 1950s that polymerization of substituted olefins could lead to

formation of polymers with helical conformation.[45] Pino and Loernzi and various others first reported experimental evidence for such structures in solution in 1960. [46-52] The properties of these helical polymers are highly dependent on the helix inversion barrier. The screw sense of a particular strand is stable at room temperature, when the helical inversion barrier is high (> ~ 85kJ*mol^{-1}), whereas the two screw senses may be in equlibrium when the inversion barrier is lower.[24] In non-chiral side chained polymers, the left- and right-handed helices are enantiomers, having equal free energies. In polymers with chiral side chains, the helices are diastereomers and consequently have different free energies. Polymers with low helix inversion barriers have dynamic properties which can be used to build chiral architectures that respond to interactions with small molecules, light, or subtle changes in monomer composition or temperature.[8,9] However, when the helix inversion barrier of the polymer is high, the helical conformation is formed under kinetic conditions, which implies that upon incorporation into the growing chain, each monomer contributes to the helix, and is sterically locked into its conformation.

2.5 Synthesis of Optically Active Helical Polymers

Most naturally occurring polymers such as proteins, nucleic acids, and polysaccharides are optically active. These polymers often possess a specific conformation or higher-order structure, which is essential in order to exert their sophisticated functions in living systems. In synthetic polymer chemistry, one of the most challenging tasks is to construct functional polymeric systems that will be as

effective as those in living systems. Today, optically active synthetic polymers have become of great interest and importance in this respect.

The development of optical activity of polymers can be typically prepared by several methods. The methods include: (1) modification of optically active naturally occurring polymers, (2) polymerization of optically active monomers, (3) enantiomer-selective polymerization, (4) asymmetric synthesis polymerization, (5) screw-sense-selective polymerization, and (6) inducing helical conformations.

Many useful polymers can be prepared by the chemical modifications of natural polymers including proteins and polysaccharides.[53] For an example, the reaction of microcrystalline cellulose with phenyl isocyanate derivatives affords a series of cellulose phenyl carbamste derivatives, which can be used as chiral stationary phases.[54]

In enantiomer-selective polymerization, one antipode of a racemic monomer is preferentially polymerized to give an optically active polymer. The first example of enantiomer-selective polymerization was reported on racemic propylene oxide initiated by an Et_2Zn-optically active alcohol system by Tsuruta.[55] Since then, many studies have been performed on the enatiomer-selective polymerization of cyclic monomers such as epoxides, episulfides, amino acid N-carboxylic acid anhydrides, and methacrylates.[20]

Synthetic optically active polymers have applications in chiral chromatographic separations and in catalysts for asymmetric induction. High-performance liquid chromatography (HPLC) with a chiral stationary phase is often used to analyze or to separate a racemic mixture. Compared with low molecular -weight optically active stationary phases, the polymeric ones are attractive and interesting, since the higher-

order structures of these polymers may play an essential role in the optical resolution. Figure 1 shows some typical optically active polymers (1-5) used for chiral stationary phases.[56-58]

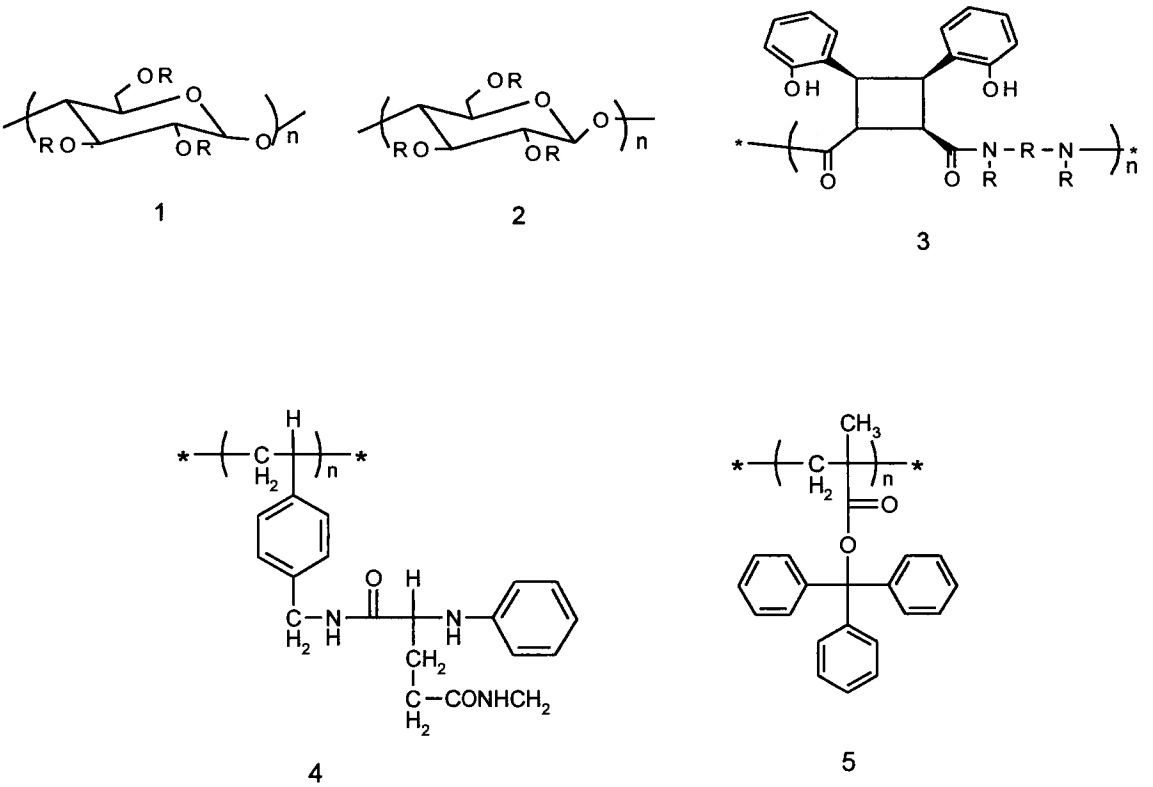


Figure 1: Examples of Optically Active Helical Polymers

Polymeric optically active catalysts have a number of advantages, unlike the corresponding low-molecular-weight ones, such as easy recovery of the normally quite expensive optically active catalysts, simple product purification, and the possibility of carrying out a continuous flow reaction and flow membrane reactor type syntheses.

As shown in the above examples of applications of optically active polymers, the specific conformation of these polymers is considered to play an important role in designing sophisticated functions of the polymers. The optical activity of polymers is

attributed to configurational and/or conformational chirality. Configurationally optically active polymers possess chiral centers in their main chains or in the side groups. On the other hand, conformationally optically active polymers show optical activity derived from a chiral conformation such as helical structure, even without the presence of any chiral centers in the macromolecule.

2.6 Asymmetric Polymerizations

Asymmetric polymerization, in which chirality is introduced to a polymer chain via polymerization reaction, is one of the ways of synthesizing optically active polymers. The interest in asymmetric polymerization is not only because it is an effective way of synthesis but also because it provides an insight into polymerization mechanisms.

Many examples of such polymerizations have been reported since the Ziegler-Natta Catalyst was discovered.[59] With regard to reaction process and structure of the obtained polymers, asymmetric polymerizations can be divided into three categories: (1) Enantiogenic polymerization (polymerization with asymmetric synthesis or asymmetric induction), (2) Enantioasymmetric (enantiomer- or asymmetric-selective or stereo-selective) polymerization, and (3) Helix-sense-selective (atropogenic) polymerization. The polymerization of categories 1 and 3 are distinguished from the polymerization of 2, because they include an asymmetric synthesis process generating a chiral center (configuration) or conformation.

Category 1 is based on the idea that an optically active polymer can be obtained if chiral centers are introduced in to the polymer backbone by the polymerization or co-polymerization reaction. Various attempts have been made to synthesize this type of

optically active polymers. Polymerizations of dienes and cyclic olefins are typical examples belonging to this category. However, in most cases, the optical yields of the polymers cannot be estimated, except for those of substituted dienes such as polysorbates which can be ozonolyzed into a chiral diacid.[60]

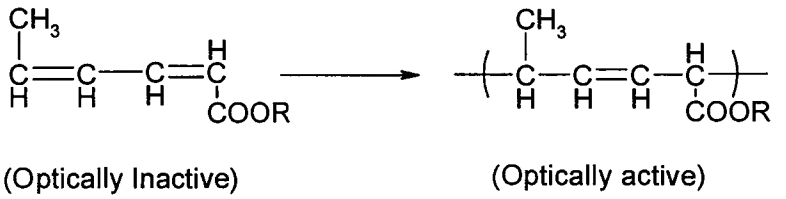


Figure 2: Optically inactive and optically active substituted dienes

Category 2, enantioasymmetric polymerization, is a type of polymerization in which one of a pair of enantiomers is polymerized preferentially. This is a kind of kinetic resolution of enantiomers. Numerous studies have been performed on the enantioasymmetric polymerization of a number of racemic monomers, including cyclic and olefinic compounds. The enantiomer selectivities observed in these polymerizations did not exceed 70% and were particularly low in the polymerization of olefinic monomers. In 1977, a highly enatioasymmetric polymerization with selectivity over 90% was attained for the first time. This was the polymerization of racemic methacrylates such as alpha-methyl methacrylate using Grignard reagent-(-)-Sparteine complexes.[61] The enantiomer excess (ee) of the polymerized monomers at an early stage of the polymer was 93% and the residual monomer was almost optially pure after 65-70% of the total monomer had been polymerized.

Category 3 involves polymerizations in which polymers having chirality due to helicity are produced. Naturally occurring polymers such as proteins and DNA possess characteristic helical structures which are stabililized through hydrogen bonds. The helix is one of the most fundamental conformations of macromolecules. The right-handed helix and left-handed helix are mirror images of each other. Therefore, helices can be chiral even if no other chiral centers are present. It is well known that stereoregular vinyl polymers often exist in a helical conformation in the solid state. For example, Natta et al.[62] showed that isotactic polypropylene is a mixture of equal amounts of right- and left- handed helices although such helical structures are not stable in solution because of the thermodynamic tendency to assume a random conformation in solution (from lack of barriers to rotation). This result suggests that even a vinyl polymer may possess a stable helical structure if one can introduce a substituent on a side group which is bulky enough to prevent uncoiling of the helical polymer chain produced in the polymerization process. The existence of this type of optically active vinyl polymer was first reported in the literature for the atropogenic (helix-sense-selective) polymerization of triphenylmethyl methacrylate with chiral anionic initiators.[63] In such a helical polymer, the right-handed helix and left-handed helix are energetically almost equivalent, whereas in most optically active polymers, including proteins and DNA, one of the helices is energetically more favorable.

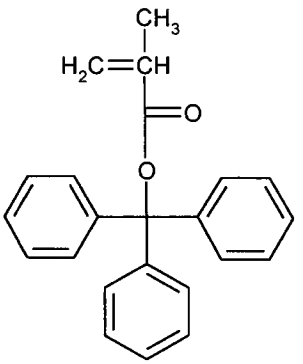


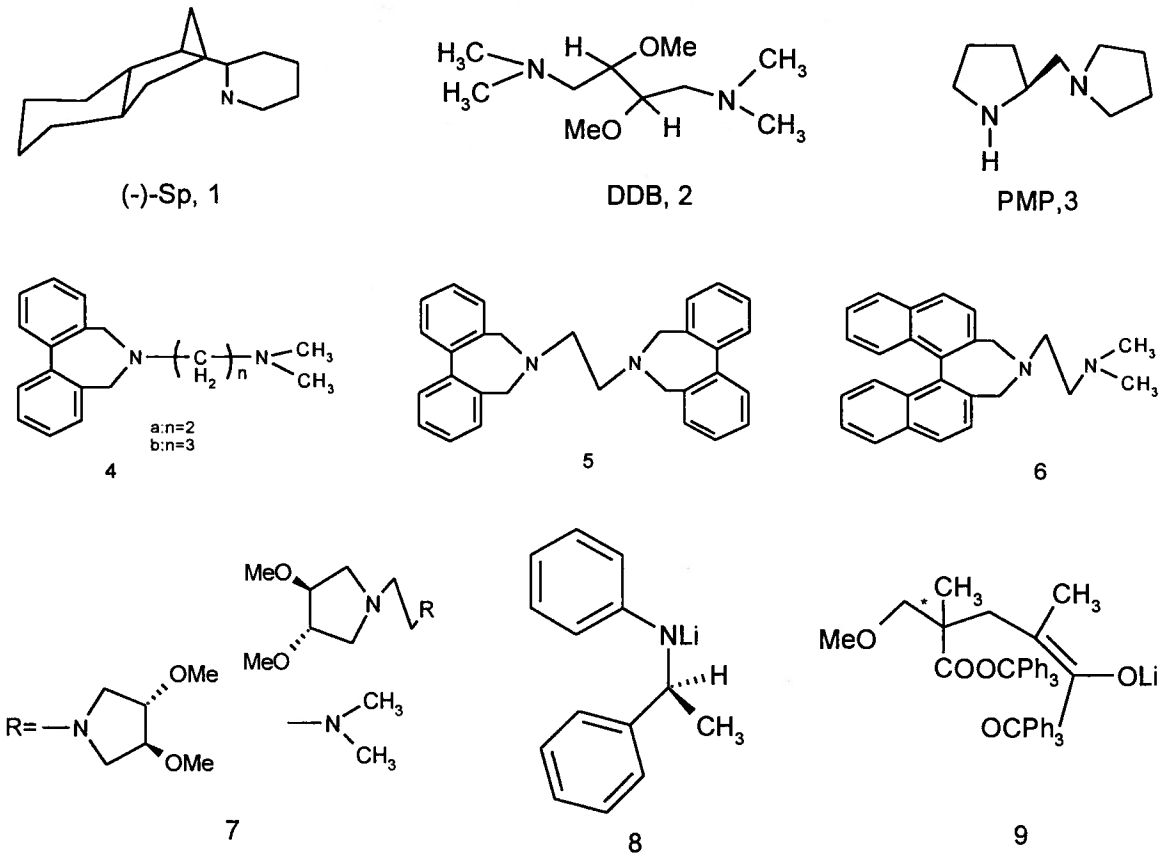
Figure 3: Triphenylmethyl methacrylate

2.6.1 Helix-sense-selective Polymerization of Triphenylmethyl Methacrylate (TrMA)

Triphenylmethyl Methacrylate gives a highly isotactic, optically active polymer with one-handed helical structure by anionic polymerization with optically active initiators at low temperatures.[64-72] This polymer is the first example of an optically active vinyl polymer whose chirality arises exclusively from its helical conformation of the main chain. The optical activity is lost when the polymer is converted into the methyl ester, indicating that the helical conformation is maintained by sterical repulsion of bulky trityl groups.

The polymer is prepared by polymerization with the complexes of achiral organolithiums with optically active ligands or optically active organolithiums. (-)-Sparteine ((-)-Sp,**1**),[64, 65, 67, 68, 72] (S,S)-(+)- and (R,R)-(-)-2,3,-dimethoxy-1,4-bis(dimethylamino)butanes ((+)- and (-)-DDB, **2**),[66,68] (S)-(+)-1-(2-pyrrolidinylmethyl)pyrrolidine ((+)-PMP, **3**),[68] and the ligands bearing biphenyl or binaphthyl moiety 4a,b and 5-7[69-72] (Chart 1) are effective for the complex formation

Figure 4: Examples of Optically Active Chiral Ligands



with achiral organolithiums, and a lithium amide. (**8**)[64, 65] Additionally, a lithum enolate having the structure of a TrMA-dimer anion (**9**)[73] can directly afford optically active poly(TrMA) without a chiral ligand. Polymerizations of TrMA with the complexes of 9-fluorenyllithium (FlLi) with (-)-Sp, (+)-DDB, and (+)-PMP have been carried out and all yielded polymer with high optical activity and possessed almost perfectly isotactic configuation were obtained.[68] Optical activity increased with DP. Chromatographic optical resolution of mixture of right- and left-handed helical poly(TrMA)s ($[\alpha]^{25}_{D}$ –82°) prepared with Li(R)-N-(1-phenyl)amilide in tetrahydrofuran

gave a fraction of high optical activity ($[\alpha]^{25}_{D}$ –365 °) which is considered to contain pure one-handed helical polymer.[74] One handed helical poly(TrMA) shows high optical activity also in the solid state.[75] Copolymerization of TrMA with (S)-(-)-MBMA also gives an optically active polymer which contains isotactic TrMA sequences with helical conformation.[76, 77]

The stereochemical mechanism of TrMA polymerization have been investigated in detail.[67, 68] In the asymmetric polymerization of TrMA, a small amount of oligomers with low optical activity is produced in addition to the high optically active polymer. It had been assumed that oligomer anions with certain specific stereostructure might predominantly add monomers to give a polymer and the others might remain as oligomers until the completion of polymerization.[65] In order to obtain information on this point, Okamoto and coworkers carried out the asymmetric oligomerization of TrMA with the complexes of FlLi with (-)-Sp, (+)-DDB, and (+)-PMP and made a detailed analysis on the oligo-methyl methacrylate. Optical rotation and circular dichroism absorption of the same positive sign confirmed that the polymers obtained with these initiators possessed the helical conformation of the same screw sense.[78-81]

2.6.2 Synthesis of Optically Active Helical Poly(3-methyl-4-vinyl pyridine)

It was demonstrated in 1998 that very large bulky side groups, such as triphenyl, may not be necessary to carry out helix sense selective polymerization of vinyl monomers. The successful preparation of optically active helical poly(3-methyl-4-vinylpyridine) by L. Ortiz and I. Khan confirmed this notion.[82] Their work resulted from the fact that in the solid-state isotactic α-olefins and certain substituted styrenes

are known to be in a low energy helical conformation with a trans-gauche (...TGTG...) type backbone conformation and the previous reported work done in the preparation of isotactic poly(3-methyl-2-vinylpyridine) by anionic polymerization.[83] The isotacticity of the polymer is probably a result of a favored helical conformation caused by the non-bonded interaction between the 3-methyl group and the penultimate pyridine group.

Asymmetric anionic polymerizations of the monomer, 3-methyl-4-vinylpyridine, with $DPEDA^{(-)}Li^{(+)}$/optically active ligand complexes were carried out in toluene at – 78°C. With the (+)DDB/$DPEDA^{(-)}Li^{(+)}$ initiating complex, polymers with specific rotations at - 78°C of (–) 4.00 were obtained; while the (-)DDB/$DPEDA^{(-)}Li^{(+)}$ initiating complex produces polymers with specific rotations at - 78°C of (+) 14.00. At – 4°C, the optical activity of both the (+) and the (-) poly(3-methyl-4-vinylpyridine) decreased with time to zero rotation. The loss of optical activity is thought to have been lost because of helix-to-helix interconversion resulting in racemization. While in the solution at – 4°C, mutarotation was observed; the polymers are stable (i.e., no change in optical activity) in the solid state at room temperature or lower. The polymers are stable in the solid state when stored in the refrigerator for several months without any loss of optical activity. Helix-to-helix interconversion was not observed in the solution when stored at –78°C. It was concluded that the above observations favor some sort of higher structure, most likely secondary helical structure, and the secondary helical structure is giving rise to the optical activity. Because of rapid helix-to-helix interconversion or reversal in solution at room temperature and the time required to obtain a CD spectra, it was not possible to obtain CD spectra of the (+) and (-)

polymers in the solution. The conformation of (+) and (-) P3M4VP, however, may be locked in an elastomeric solid matrix of poly(ethylene oxide) (PEO, MW 600,000) and poly(ethylene glycol) (PEG, MW 3000).[84] The CD spectra of the composites show mirror image Cotton effect signals at 212 nm and 223 nm, indicating the formation of enantiomeric higher structural order. The helical conformation of the (+) and the (-) P3M4VP is stable in the PEO/PEG matrix at room temperature. Therefore, even in instances where the activation of energy of the helix-to-helix interconversion is small such that the left- and the right-handed helices are rapidly equilibrating in solution, it is possible to readily lock in the helical conformation in a solid matrix. In the solid composite, the helix-to-helix interconversion process is not a molecular process but a process that requires the segmental motion or rearrangement of the neighboring polymer chains such that conformational reorientation required for helix-to-helix interconversion is possible. When the (+) and (-) P3M4VP/PEO/PEG composites are heated to 60°C for 30 minutes, the Cotton effect signals are lost indicating either helix-to-helix interconversion resulting in racemization or helix-to-coil transition resulting in loss of the helical structure. These observations suggest that the optically activity arises because of secondary structure and is not due to the presences of chiral center(s) in the polymer chain.

Molecular modeling studies were carried out to determine the preferred conformation of poly(3-methyl-4-vinylpyridine). The oligomers of 3-methyl-4-vinylpyridine were modeled using the Spartan series of programs. Molecular mechanics calculations were performed with MM3, and optimized geometries were further

optimized with the PM3 semi-empirical method. Models were built of the mm triads, mmm tetrads and mmmm pentads, in both helical and non-helical form, the geometries were optimized by MM3 and PM3.[85] Both MM3 and PM3 calculations support that helical isotactic tetramers and pentamers are more stable than the non-helical forms. The results of the calculations are shown in table 1.

Table 1: Calculated energy (Kcal/mole) of 3-methyl-4-vinylpryidine oligomers

Oligomer	Strain Energy (MM3)	Hf (PM3)
mmm helix	75.7	54.2
mmm non-helix	83.6	60.8
mmmm helix	98.2	75.5
mmmm non-helix	100.1	80.5

Only small differences in energy were found for the different isomers of 3-methyl-4-vinylpyridine dimers and trimers, as the end groups were able to undergo conformation changes that affected the optimized energy of the entire molecule. Additionally, no stable helix was found for the rrr tetrad, which supported their premise that poly(3-methyl-4-vinylpyridine) has an isotactic configuration.

Systematic conformation search was carried out on two isotactic enantiomeric oligomers, (2R, 4S, 6R, 8S, 10R, 12S)- 2, 4, 6, 8, 10, 12- hexa(3-methyl-4-pyridyl)tetradecane, abbreviated as *Enantiomer A* and (2S, 4R, 6S, 8R, 10S, 12R)- 2, 4, 6, 8, 10, 12-hexa(3-methyl-4-pyridyl) tetradecane, abbreviated as *Enantiomer B*, using the Alchemy Series of programs.[85] The conformation search results displayed the presence of a low energy left-handed helical conformation for enantiomer B and a low energy right-handed helical conformation for enantiomer A. The conformation search

indicates that the helical twist starts with the third repeat unit in the oligomer. The systematic conformation search was carried out by specifying the number of rotatable bonds (n bonds) and the rotation increment in degrees in the oligomers. The systematic conformation search examined the total number of conformations, N, and since the only one increment to all rotatable bonds was set, N is calculated by the following expression: $N = [360/delta]^{n\ bonds}$. For each conformation achieved by this set of rotations, all internal atomic distances along with the potential energy of a conformation were computed. The conformational studies suggested that it is reasonable to conclude that the secondary structure of isotactic poly(3-methyl-4-vinylpyridine) is most likely helical.

Ultimately, conformational analysis suggested that the position of the 3-methyl group in the polymer chain and orientation of the methyl groups in the same direction is very important for the helical conformation to be stable. If the methyl groups are randomly oriented, the helical conformations will be less favorable. The mechanism for the mode of the monomer addition to the living polymer chain end can also be concluded from this observation. Monomer addition in a conformation close to the S-trans would maintain the methyl group orientation, i.e. same direction, and hence, the growing helical conformation. MM3 calculations indicate that monomer conformations close to S-trans are significantly lower in energy than conformations close to S-cis.

2.7 Other Examples of Helical Polymers

Macromolecules that possess higher order structure or helical conformation essentially fall into two categories. The categories are high energy barrier and low

energy barrier helical polymers. Again, the properties of the two categories of helical polymers are highly dependent on the helix inversion barrier, which generally determines if a helical screw sense of a particular strand is stable at room temperature (high barrier) or if the two helical screw senses may be in equilibrium (lower barrier), thus resulting in either the presence or absence of optical rotation. [24]

2.7.1 Low Helix Inversion Barrier Helical Polymers

Poly(isocyanates), which are also called Nylon 1, are stiff helical polymers. Their stiffness is a result of the partial double bond of the backbone amide bonds. Poly(isocyanates) are the most extensively investigated of all helical polymers in the last 20 years. Goodman and Chen are responsible for most of the pioneering and ground work, while Green is attributed with the more refined studies about these polymers.[86, 87]

The helical conformation of poly(butyl isocyanate) was confirmed in the late 1960's by X-ray crystal structure analysis.[88] The conformational analysis yielded a 3/8 helix. The helical conformation is also maintained in solution for poly(alkyl isocyanates) and poly(aryl isocyanates). When an achiral monomer is polymerized, left- and right-handed helices are formed, which are distributed throughout the polymer chains and are dynamically interconverting.

The helical sense of the polymer can be controlled by a variety of means such as inclusion of a chiral monomer, chiral solvent, or the a chiral initiator for the polymerization. Green and coworkers have demonstrated that the helical moiety of poly(alky isocyanate)s is highly sensitive to slight chiral biases in the monomer. They

showed that substitution of a proton for a deuterium is sufficient to induce a preferential helical sense in the resulting polymers.[87,89]

It has also been demonstrated that poly(n-hexyl isocyanate) displayed sensitivity in chiral solvents. Poly(n-hexyl isocyanate) has a persistence length of 20-40 nm for the helical segment depending on the solvent in which the measurements were carried out.[90] It was determined that poly(n-hexyl isocyanate) dissolution in the chiral solvent, (S)-1-chloro-2-methylbutane, changes the persistence length and resulted in an excess of one helical sense.[91] It was also reported that circular dichroism spectra of these polymers decrease upon the addition of an achiral or racemic solvent.[92]

A very popular area of interest that is receiving a great deal of attention is in the switching the helical sense of polyisocyanates. The extreme sensitivity of the helices due to slight chiral influences is responsible for this behavior. In 1996, Zentel and Maxein, polymerized optically active poly(isocyanate)s containing azo chromophores to give polymers with a preferred, predominant helical twist and photoswitchable functions.[93] Their studies indicated that upon photoswitching the chromophore form the trans to the cis state, the percentage of right-handed (P) helical segments increases and the observed CD effect for the polymer reverses. Studies where films consisting of copolymers of the photochromic isocyanate and n-hexyl isocyantes embedded in a matrix of poly(methyl methacrylate) exhibited stable photochromic switching; However, upon irradiation, the helical sense was reversed.[94]

Yoshio Okamoto has also prepared helical poly(isocyanates).[95] Okamoto and coworkers have demonstrated that helical poly(isocyanate) can be prepared utilizing

optically active anionic initiator. The one-handed helical structure of the polymer is dictated by the initiator. The polymers are sufficiently rigid to maintain the helical conformation in solution. Okamoto also reported the polymerization of chiral aromatic polymers with helical preference. These polymers displayed optical rotation, which were temperature dependent, of $[\alpha]^{25}_{365} - 1969^{o}$. Remarkably, the polymer even adopts a perfect helical conformation in solution at room temperature.

Bruce Novak's work with poly(guanidine)s or poly(carbodiimide)s represent another example of low inversion barrier helical polymers. Novak's studies involved preparing optically active poly(guanidines) from achiral carboimides using copper complexes [Cu(OMe)Cl] equipped with chiral bisoxazoline ligands.[96] The copper complex displayed preferential solubility in the (R)-monomer versus the (S)-monomer and resulted in faster polymerizations and yielded polymer with higher optical rotations. Polymerizations of racemic mixtures of carboimides with copper complexes [Cu(OMe)Cl] equipped with chiral bisoxazoline ligands yielded polymers with optical rotations as high as – $[\alpha]$ 49^{o} after 1.5 hours with percent yields of 12%, which ultimately yields polymers with an enantiomeric excess of one helix sense.

In 1967, Ciardelli and coworkers synthesized the first chiral poly(acetylene) by polymerizing (S)-4-methyl-1-hexyne.[97] Poly(acetylene)s equipped with chiral pendages may be prepared by ring-opening metathesis polymerization.[98] Poly(acetylene)s fall into the category of chiral π-conjugated polymers. Intriguingly, a small number of chiral π-conjugated polymers display strong optical activity in the π-π^* transition, which is attributed to the presence of secondary structure or helical conformation in

solution. Other examples of conjugated polymer with a helical main chain are poly(phenylvinylene), oligo(β-pyrrole), poly(p-phenylene), poly(thiophenes), and poly(aniline)s.[99-104]

Poly(aniline)s are unique in that they are prepared by electrochemical polymerization in the presence of a chiral dopant. Polymers prepared in the presence of enantiomeric counterions give rise to circular dichroism spectra with completely different signs. However, these signals are lost completely upon deprotonation of the polymer which suggests that the helicity is due to hydrogen bonding along with electrostatic interactions with the counterions.

Another example of polymers that possess helical conformation with low energy inversion barrier is the poly(silane)s. Poly(silane)s are linear polymers of silicon in which σ-electrons in the polymer backbone are delocalized. The σ-conjugation gives rise to electronic effects that indirectly influence the conformation of the polymer backbone, which in turn affects the optoelectronics properties of the polymer.[105] Poly(silane) chains are very flexible and possess no distinct conformational preferences. However, upon introduction of enantiopure substituents in the dialkyldiclorosoliane monomers, polysilanes can be prepared displaying a preferred handedness of the helical backbone.[106-107, 108-114] Fujiki and coworkers synthesized a large number of chiral poly(silane)s using the Wurtz synthesis and demonstrated that the optoelectronic properties of such helical polymers are related to their conformational properties and that such properties can be regulated by temperature and the solvent.[108, 109,113]

In poly[((S)-2-methylbutyl)(6,9,12-trioxytetradecyl)silane], it was found that the predominance of left-handed helical segments tightened and increased in length upon cooling. In poly(diaryl-silanes), it has been reported that reversible control over the helix sense is possible, in which the helix reversal occurred upon heating.[115,116]

Another example of helical polymers that possess low helix inversion barrier polymers are the isotactic vinyl polymers, which often possess a helical conformation in the solid state. These polymers do not maintain helical conformation in solution at room temperature due to the lack of bulky substituents. Helix-helix reversal takes place rapidly and no optical rotation is observed. Again, Khan and Ortiz reported the preparation of optically active helical poly(3-methyl-4-vinylpyridine) by anionic polymerization at –78°C.[78]

2.7.2 High Helix Inversion Barrier Helical Polymers

Helical polymers that possess a high helix inversion barrier are the sterically restricted poly(methacrylate ester)s. Again, Okamoto and coworkers reported the successful preparation via anionic polymerization of poly(triphenylmethyl methacrylate) at low temperature in the presence of an optically active initiator. The polymerization results in the formation of a highly isotactic, highly stable, optically active polymer. The stable helical conformation of the backbone in these polymers, unlike isotactic vinyl polymers, is a result of steric interactions between the bulky trityl groups. However, a loss of optical activity occurs upon their conversion to methyl ester groups. The helical conformation of the poly(methacrylate) uncoils or undergoes helix-to-helix interconversion. Again, poly(methacrylate ester)s have been extensively

investigated and by Okamoto and his group.[117, 118] These polymers have not only been prepared by anionic pathways, but have also been prepared by cationic, free-radical, and Ziegler-Natta techniques. Nakano and Okamoto have recently reported the use of a cobalt(II)-salophen complex in the free-radical polymerization of a methacrylate ester. The polymerization resulted in the preparation of an highly isotactic polymer with an excess of a helical sense.

Okamoto has reported the preparation of high helix inversion polymers via anionic polymerization of N, N'-disubstituted acrylamides. These polymers are highly crystalline and the tacticity of these polymers are considered isotactic. It has also been reported that N, N'- diphenylacrylamide, which contain bulky substituents, afford optically active polymers in the anionic polymerization with chiral initiators.[117,118]

Phthalocyaninatopoly(siloxane)s are another example of helical polymers with high helix inversion barriers. Engelkamp and coworkers have reported that dihydroxyphthalocyanatosilicon modified with eight (S)-citronellol side groups can be polymerized using methods developed for n-alkyl derivatives.[119] At an estimated polymerization degree of 27, the helices do not show any sign of helix reversal up to temperatures of 120°C and was found to be strongly CD active.

Poly(aldehyde)s are another example of high helix inversion barrier helical polymers. Poly(aldehyde)s are characterized by an oxymethylene repeat unit, and depending on the substituents present, a helical conformation can be present. Vogl and coworkers have reported on a number of these compounds.[111-113,120-121] The most extensively studied poly(aldehyde) is poly(chloral). Poly(chloral) that is prepared by

anionic polymerization is very isotactic and insoluble in organic solvents. A 4/1-helical conformation with a helical pitch of .51 nm was obtained using X-ray diffraction analysis. No conformational studies have been performed on poly(chloral) due to their insolubility in solution. However, molecular mechanics calculations, NMR spectroscopic studies, and crystallographic studies imply similar characteristics for well defined oligomers.

Preparation of high helix inversion barrier helical polymers of poly(isocyanide)s have also been reported. Poly(isocyanide)s are unique in that each carbon atom in the polymer backbone has a substituent connected to it. Millich proposed that poly(isocyanide)s would have helical conformation due to the restricted rotation around the single bonds on the polymer backbone.[122] The Ni(II) catalyst polymerization reported by Nolte and coworkers confirmed Millich's proposal.[123] The transition metal catalyst allowed the polymerization of the sterically challenged poly(tert-butyl isocyanide) and provided the first example of an optically active polymer which is primarily based on the stable helical conformation of the backbone.[124] The nickel(II)-catalyzed polymerization of isocyanides proceed remarkably fast, considering the steric crowding that is introduced upon preparation of the polymer chain. The driving force of the reaction is the conversion of the divalent carbon in the monomer into a tetravalent carbon in the polymer. The proposed mechanism for this polymerization is called the merry-go-round mechanism. When the isocyanides are mixed with the Ni(II) catalyst, a square-planar complex is formed. The easy formation of tightly coiled helix is explained by the pre-organizing effect of the nickel center.

2.8 Foldamers: Low Molecular Weight Chiral Molecules with Preferred Helical Conformations

Foldamers represent a new class of compounds and are defined by Samuel Gellman as being polymers "with a strong tendency to adopt a specific compact conformation.[125] Foldamers are further defined as oligomers of modest length that in solution display such specific conformational preference. Foldamers are remarkable in that they not only represent polymers with higher order conformational preference, but that are also bio-compatible. Scientists have adopted as a research interest the synthetic equivalents of helices and β-sheets, the two most common structural elements found in Nature. Poly(amino acid)s are probably the most simple example of how structural information in the polymer backbone can facilitate the folding of the molecules in a predefined manner. The research groups of Samuel Gellman and Dieter Seebach have provided methods and guidelines for the construction of defined helical architectures from these compounds.

Instead of α-amino acids found in natural proteins, the Gellman and Seebach systems are of composed of β-amino acid units.[126]

Gellman's research has been focused on preparing helical structures with long term stability utilized conformationally rigid monomers.[127] Gellman and coworkers utilized nuclear magnetic resonance, circular dichroism, and X-ray crystallography to confirm that chains made up of six β-amino-acid units such as in the case of trans-2-aminocyclohexanecarboxylic acid, form well defined helices in methanol solution and in the solid state.[128] Molecular modeling calculations of trans-2-

aminocyclohexanecarboxylic acid have particular preferences for the formation of a 14-helix and a 12-helix, respectively.[29] The helix is stabilized by hydrogen bonds between every third unit. The 14-helix has a length of 5.0 A per turn, and an almost perfect three-fold symmetry owing to its having 3.0 residues per turn. The standard α-helix found in proteins has 3.6 residues and 5.6 A turn. The solution structure was determined using NMR. NMR was used to measure the time it took for the amide hydrogen atoms to exchange with hydrogens from the solvent. The amide hydrogens usually exchange within minutes unless they are taking part in strong hydrogen bonds, but the two interior hydrogen bonded NH's in Gellman's 14-helix took more than two days to exchange. These extremely slow exchange rates demonstrate the remarkable stability of the folded structure.

Dieter Seebach and coworkers have reported the successful development of hexa-β-peptides. Hexa-β-peptides form helical structures in water as well as in organic solvents.[129] Unlike Gellman's work, the use of cyclic monomers that impose conformational restriction in the backbone was not a prerequisite for formation of these helical structures. However, Gellman's group later demonstrated that the incorporation of cyclic β-amino acids n the sequence does have a positive effect on the stabilization of the helix.[130] Seebach and coworkers have also showed that β-sheets and turns can be prepared and that the 14-helix can also be prepared through the use of γ-amino acids.[131-132]

Other foldamers that have been reported are the vinylogous amino acids, the β-sulfonopeptides, and the vinylogous sulfonopeptides.[133-136] Foldamers related to the

aforementioned work include Nielsen's peptide nucleic acids.[137] Peptide nucleic acids represent a class of flexible oligomeric DNA analogues in which the base pairs are carried by a peptide backbone. These molecules adopt a more rigid conformation and form a double helical structure when cyclohexyl derived amino acids are included.[138] Hamilton and coworkers have reported the preparation of foldamers from anthranillic acid and pyridine-2,6-dicarboxylic acid moieties which use hydrogen bonds between nearest neighbor groups to structurally harden their structure and maintain helical conformation.[139-140]

Another class of foldamers that do not rely on hydrogen bonding to achieve the appropiate folding of oligomeric compounds are Lokey and Iverson's oligomeric complexes of dialkoxynaphthalene and naphthalenetetracarboxylicdiimide. Lokey and Iverson utilized the attractive aromatic electron donor-acceptor interactions between the two compounds to achieve oligomeric folding. These oligomers are also called "aedamers" and give pleated structures in solution which yield a plum color due to a broad charge-transfer absorption bands in the visible region.[141]

Moore and coworkers reported the folding of oligo(m-phenyleneethylene)s into helical stacks that are driven by solvophobic effects.[142] Oligo(m-phenyleneethylene)s exhibit a large degree of conformational freedom because rotation is possible around the ethynylene linkers, which allows switching to a helical conformation. The pendant oligo(ethylene oxide) chained aromatic backbone ensures solubility in a large variety of solvents.

2.9 Induced Optically Active Helical Polymers

Another method for preparing conformationally rigid helical polymers is called helix induction. Helix induction involves starting with a racemic helical mixture and complexing with enantiomeric small molecules, thus, resulting in the formation of an induced helical structure with a predominance of a single-screw sense. The complexation causes the formation of a diastereomeric pair and a "chaperoned" or "induced" shift of the equilibrium towards the lower energy diastereomer. The relatively low activation energy of the helix-to-helix interconversion is what permits helicity enrichment via helical induction.[143]

Helical induction by acid-base interaction has been reported to be highly effective for helicity selection. The first example of the prevailing helix formation of an optically inactive polymer ascribed to acid-base interactions is in the case of the poly(acetylene)s. It was found that poly(1, 1-diethylpropargylamine) can form an induced helix complexation with optical active acids, (R) – and (S)-mandelic acids.[144] Achiral poly(isocyanates), poly(guanidines) and poly(organophosphazene)s also have been induced to a helical conformation using chiral acid-base interactions.[145-149]

Yoshio Okamoto and coworkers have demonstrated the helicity induction of poly(3-carboxyphenyl isocyanate) by chiral acid-base interactions.[150] The induced circular dichroism spectrum of P3CPI was measured in the presence of a chiral amine. The CD spectrum was taken in a mixture of methanol/ethanol because in pure methanol, no ICD was observed. However, a rather intense CD peak was observed at 263 nm. The CD pattern is similar to those of the optically active poly(phenyl

isocyante)s with a one-handed helical conformation, which suggests that a prevailing one-handed helical structure is induced on P3CPI through an acid-base interaction. Green and coworkers reported a similar observation where a prevailing one-handed conformation of poly(n-hexyl) isocyanate) is driven by a minute chiral solvation energy. [151-156] The peak intensity gradually decreased with time. This is due to the decomposition of the polymer through the formation of cyclic trimer.

Eiji Yashima and coworkers have reported the helical induction of achiral, stereoregular poly-((4-carboxyphenyl)acetylene), P4CPA, into a prevailing one-handed helix upon complexation with chiral amines. In their study, they monitored P4CPA in the presence of optically active amines in DMSO via circular dichroism spectroscopy. The magnitude of the cotton effects reflected the configuration of chiral amines and the magnitude of the ICD increased with an increase in the bulkiness of the chiral amines. Yashima noted that the intense ICD is due to the hydrogen bond formation of the hydroxy group to a carboxy residue of the polymer, as well as the acid base interactions. The dynamic nature of the helical polymer induced by optically active amines was also investigated through CD titration experiments. The optical activity of the polymers disappeared instantly when the complex was exposed to a stronger acid, such as trifluoroacetic acid. P4CPA is freed to give an original, optically inactive polymer. This indicates that the induced helix of the P4CPA is dynamic in nature and the helix-sense can be controlled with a rather small amount of the chiral amino alcohol with opposite configuration.

Boronic acids are known to form a complex with diol-containing compounds including carbohydrates, and boronates immobilized in polymer matrices. Aromatic boronic acids have been used as synthetic carriers of carbohydrates and ribonucleosides for selective membranes transport. Mikami and Shinkai have prepared a class of polymers formed by polycondensation of diboronic acid and chiral tetraalcohols. The non-covalent intramolecular interaction of this D-mannitol based polymer, between the amines and boron atoms imposed sp^3 hybridization on boron, resulting in an induced helical conformation of the polymer, according to calculations.[157]

Helicity selection of a single-screw sense starting with a racemic mixture of helical poly(3-methyl-4-vinylpyridine) by helix induction has also been reported on by Khan and Ortiz.[158] Complexes of racemic helical poly(3-methyl-4-vinylpyridine/(R) and (S) – mandelic acid were prepared via acid-base interactions in tetrahydrofuran, water, methanol, and deuterated water. Complexes with mandelic acid to monomer repeat ratios of 1:2, 1:1.5, 1:1, and 5:1 were prepared. TheP3M4VP/(R)- or (S) complexes were prepared by stirring .5 grams of P3M4VP with the appropriate amount of (R)- or (S)-mandelic acid for 8 hours at room temperature. The solution was poured onto poly(ethylene) plates and dried under vacuum to obtain the CD samples. The weight average molecular weights (Mw) of the polymers, relative to poly(styrene) standards, used in preparing the complexes were between 30,000 and 35,000. The $[\alpha]^{20}_D$ of (R)- and (S)-mandelic acids were -153° and +154°, respectively, in H_2O. Helicity selection chaperoning was observed for complexes prepared in THF, water, and methanol at mandelic acid to monomer repeat unit ratios of 1:1, 1:1.5, and 1:2. The most well-

defined circular dichroism spectra were observed for the 1:2 complexes. Increasing the MA content to a ratio of 5:1 resulted in the loss of mirror image cotton effect signals attributable to the helical conformation.

The ICD spectra in the solid state of P3M4VP/(R)- and (S)-MA complexes, prepared in THF at a MA to monomer repeat unit ratio of 1:2, show two mirror image Cotton effect signals at 211 and 214 nm, indicating formation of enantiomeric helical structures. Helicity induction was also observed for complexes prepared in THF, H_20, and CH_3OH at MA to monomer repeat unit ratios of 1:1, 1:1.5, and 1:2.

Khan and coworkers have also reported induced helical poly(3-methyl-4-vinylpyridine via acid-base interactions of the achiral polymer with various D and L amino acids.[159] The induced circular dichroism (ICD) spectra of P3M4VP/D and L-alanine complexes in methanol/water displayed cotton effect signals at 278.4 nm, 274.8 nm, and 270.8 nm, thus indicating helical induction of enantiomeric secondary structures. The complexes have also been carried out in water. Khan and coworkers determined the formation of the induced helical structures or complexes to be rapid. The acid-base interaction between P3M4VP and L-alanine in methanol/water shows an intense cotton effect signal within 30 minutes. Once the helical poly(3-methyl-4-vinylpyridine) is induced, the complex is stable at room temperature.

2.10 Bio-compatible Polymers

Bio-materials that are capable of supporting cell growth and cell adhesion are of great demand and interest. Applications for such materials are for tissue engineering (replacement and regeneration), drug delivery, as well as for substrates for cell culture.

Synthetic polymeric materials play an important role in tissue engineering and are being applied in conducting, guiding, and inducing tissue formation and in blocking tissue interaction.[160,161] Synthetic polymers for these applications should have nontoxicity, the ability to interact specifically with appropiate cells, and good biocompatibility.

The development of synthetic bio-materials that support cell growth and adhesion is an active area of interdisciplinary research, which depends on cell function, flow conditions, and polymer surface characteristics such as wettability (the ability to form H bonds), charge, and roughness.[162] The importance of polymer surface wettability for cell adhesion was reported in 1960, was later confirmed in other studies.[162-164] Cell adhesion and proliferation preferentially occurs onto water wettable or H-bond forming substrates like tissue culture poly(styrene) (TCPS) and is promoted by the adsorption of some serum proteins from the culture medium.[162]

Serum derived fibronectin adsorption (which is somewhat different from the cellular fibronectin) onto various surfaces has been shown to depend on the wettability of the surfaces and on the serum concentration of the culture medium.[162, 165-175] Competition between the adsorption of fibronectin (Fn) and other serum proteins such as albumin (Alb), immunoglobulin G (IgG), lipoproteins, α-1-trypsin, and α-2-macrogolubulin reasonably explains the dependence of Fn adsorption on the serum concentration.[162, 165-175]

Cell adhesion and proliferation onto surfaces in the absence of serum protein is promoted by the cellular secretion of protein(s) like Fn.[176,177]

Such proteins as fibronectin, vitronectin, and the collagens, as well as active fragments of such proteins, have been used to modify synthetic materials in order to enhance their ability to support cell adhesion, spreading, and proliferation.[178-183] Hydrophilic polymers have also been used to improve the surface properties of synthetic materials used for biological and biomedical applications.[184-186] One such hydrophilic polymer used to improve surface properties is poly(ethylene oxide), which is well known for its ability to repel proteins and has been used to modify synthetic materials to provide them with a barrier to reduce protein adsorption, as well as improving biocompatibility.[186,187] Chemically modifying the surface chemistry of synthetic materials has also been used to enhance cell attachment. Polystyrene which has been chemically modified to enhance cell attachment (TCPS) for cell culture of most mammalian cells is very well known.[188-198] The importance of the chemical nature of the polymer surface upon initial cell adhesion and proliferation is shown in that hydrophobic surfaces do not support cell attachment. Unmodified poly(styrene) does not support cell attachment efficiently. It has been determined that cells attach to TCPS and to attach poorly to unmodified polystyrene. This results from an effect of surface chemistry upon the adsorption of serum and cellular proteins onto the polymer surface. TCPS has a higher oxygen content than does poly(styrene), and on this basis, it has been proposed that hydroxyl, carboxyl, and carbonyl groups on the surface improve cell adhesion.[188, 191, 199, 197, 200, 201]

Although TCPS might be a cell compatible biointerface and generally well known for excellent cell growth, serious problems have been reported. From the evaluation of the mRNA expression of inflammatory cytokines, an inflammatory reaction was observed

not only on TCPS but also on common polymer materials such as polyurethane and poly(ethylene terephthalate).[202] Therefore, cell-compatible materials, which do not induce any inflammatory reactions, have to be prepared.

CHAPTER III
EXPERIMENTAL SECTION

3.1 Apparati

3.1.1 High-Vacuum Line

The High-Vacuum Line is used to provide the vacuum for the polymerization reactions. The high vacuum line consisted of a high vacuum producing mechanical pump, a low temperature liquid nitrogen trap, and a nitrogen line. The polymerization reaction vessels were connected to the high vacuum line through three-way stopcocks (See Figure 5).

3.1.2 Apparatus for the Purification of Chiral Ligands/Monomer: (+)-DDB, (-)-DDB, and (2MS)

The apparatus utilized for the distillation, purification, and isolation of the chiral ligands and the monomer were prepared by attaching a side flask (100 ml) and four breakseals utilizing glass blowing techniques (See Figure 6). The chiral ligands and monomer were dried over CaH_2 for 48 hours under a nitrogen atmosphere before distillation. After the ligands and/or monomer was distilled into the lower part of the apparatus under vacuum, the ligands and/or monomer were then sealed in the corresponding amount in the ampoules.

3.1.3 Apparatus for Polymerization

Anionic polymerizations were performed in a specially prepared 100 ml round flask as shown in Figure 7. Two breakseal ampoules, containing distilled 2MS and (+) or (-) DDB were connected to the flask. The glassware was designed so that the polymerization could be run continuously under vacuum for long periods of time at low temperatures. A magnetic stirrer bar was also included in the flask. A side arm was attached for the injection of n-butyl lithium. The apparatus was attached to the high-vacuum line through the joint.

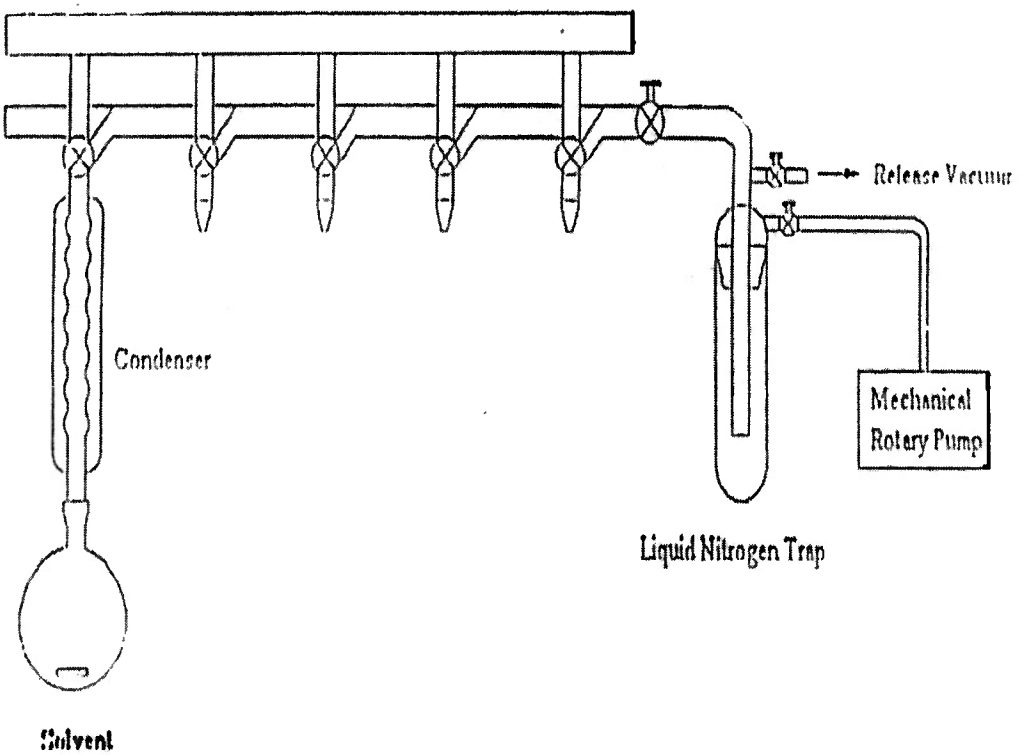


Figure 5: The High Vacuum Line

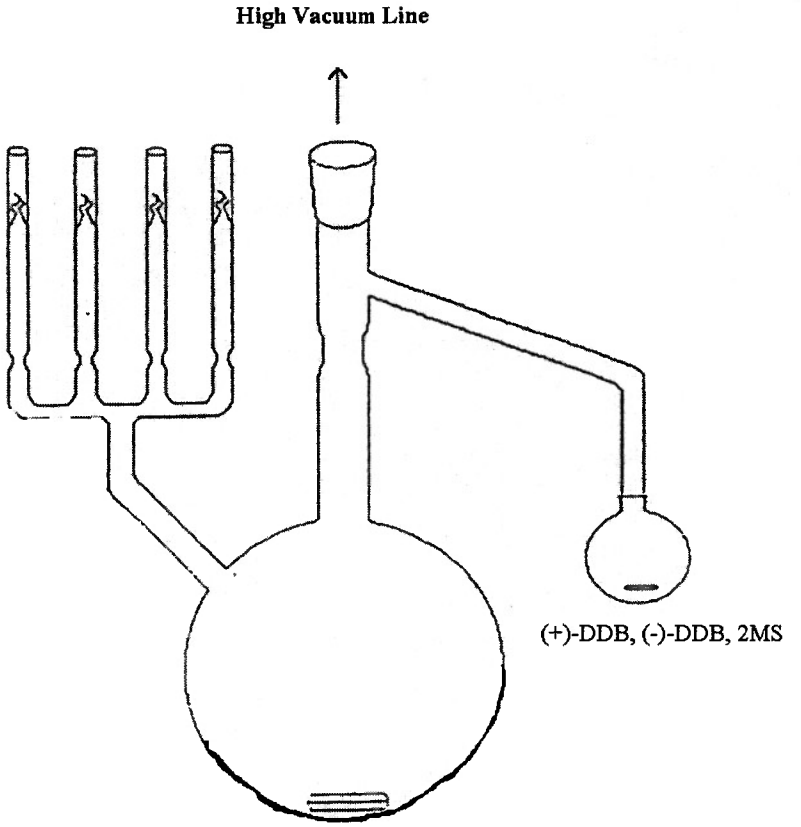


Figure 6: Apparatus for the Distillation of (+)DDB, (-)DDB, or 2MS

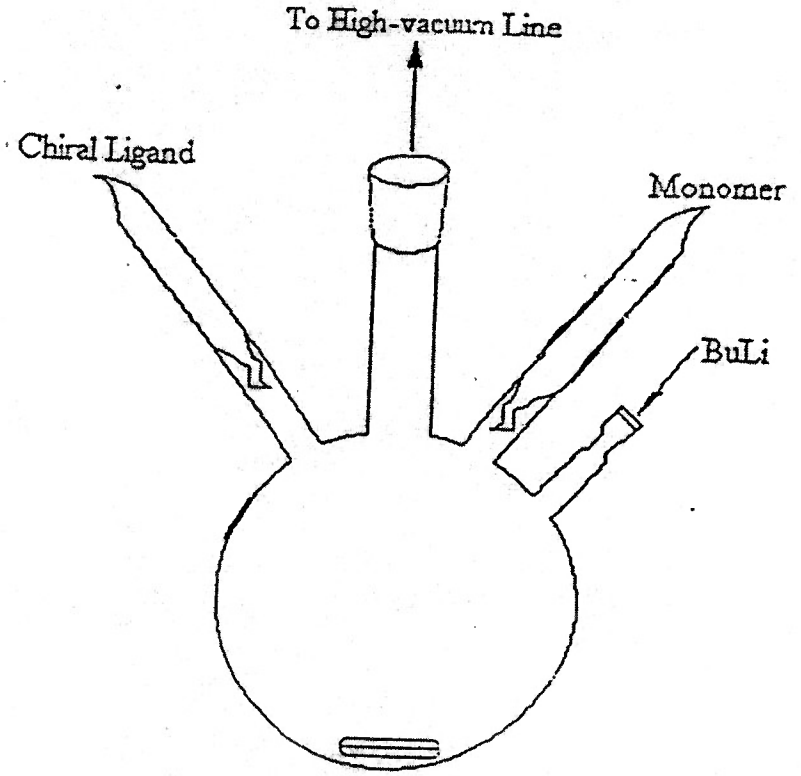


Figure 7: Apparatus for Polymerizations

3.2 Reagents

2,2'-Azobisisobutyronitrile, AIBN

AIBN: Aldrich (F.W. 164.21, mp 103-105°, solid, toxic) was purified by recrystallization.

t-Butyl Catechol

t-Butyl catechol: Aldrich, 97%, F.W. 166.22, M.P. 52-55°C, B.P. 285°C.

n-Butyl lithium

n-Butyl lithium (Aldrich, 2.0 M solution in cyclohexane, M.P. 64.06, d .775) was utilized as received.

Calcium Hydride

Calcium hydride: Aldrich (-40 mesh to +4 mesh, 95%, F.W. 42.10, d 1.900)

Chloroform,

Chloroform: Aldrich (99.8%, FW 119.38, M.P. –63°, B.P. 60.5-61.5°, d 1.492)

Copper

Copper: Aldrich (F.W. 63.54, d 8.940, -40 mesh)

Diethyl ether

Diethyl ether (Aldrich, Anhydrous, 99+%, F.W. 74.12, B.P. 34.6°C, d 0.706) was used as received.

(R, R)-(-)-2,3-Dimethoxy-1,4-bis(dimethylamino)butane, (-) DDB

(R, R)-(-)-2,3-Dimethoxy-1,4-bis (dimethylamino) butane (DDB) (Aldrich, 96%, F.W. 204.32, B.P. 62-64°C/3mm, d 0.896) was dried by distilling over CaH_2 under vacuum and sealed into breakseals just before use.

(S, S)-(+)-2,3-Dimethoxy-1,4-bis(dimethylamino)butane, (+) DDB

(S,S)-(+)-2,3-Dimethoxy-1,4-bis(dimethylamino)butane, (-) DDB, (Aldrich (96%, F.W. 204.32, B.P. 62-64°C/3mm, n^{20}_D 1.4343, d 0.896) was dried by distilling over CaH_2 under reduced nitrogen pressure and separated into breakseals just before use.

(R)-Mandelic acid

Mandelic acid (Aldrich, F.W. 152.15, M.P. 131-133°, $[\alpha]^{20}_D$ –153°, c 2.5 M in H_20) was used as received.

(S)-Mandelic acid

Mandelic acid (Aldrich, F.W. 152.15, M.P. 131 – 134°, $[\alpha]^{20}_D$ +154, c 2.8 M in H_20) was used as received.

Methanol

Methanol (Aldrich, 99%, A.C.S reagent, F.W. 32.04, B.P. 64.7°, d 0.791, F.P. 52°F) was used as received.

2-methoxycinnamic acid

2-methoxycinnamic acid (Aldrich, F.W. 178.19, M.P. 183-186°C) was used as received.

Poly(ethylene glycol)

Poly(ethylene glycol) (PEG) (Aldrich, d 1.204, crystalline powder, M_n 3400, Viscosity (210°F) 90 centistokes, T_m 62°C) was used as received.

Poly(ethylene oxide)

Poly(ethylene oxide) PEO, (Aldrich, M_v 600,000, T_m = 65°C) was used as received.

2-propanol

2-propanol: (Fisher, histological grade, F.W. 60.10)

Quinoline

Quinoline, (Fisher, F.W. 129.16, F.P. 92°C, B.P. 108-110°C, M.P. –17°C, d 1.093) was dried over CaH_2 for 24 hours under a nitrogen atmosphere and distilled just before use.

Sodium Carbonate

Sodium Carbonate: (F.W. 105.99, M.P. 851°C, d 2.532)

Tetrahydrofuran

Tetrahydrofuran (THF) (Fisher certified, F.W. 72.11, B.P. 65.8 – 66.1°, d 0.855) was refluxed and distilled from either calcium hydride or sodium and benzophenone just before use.

Toluene

Toluene (Fisher certified, F.W. 92.14, M.P. –93°, B.P. 110°, d .865) was purified by reflux and distillation from calcium hydride just before use.

3.3 Procedure

3.3.1 Monomer Synthesis

The monomer, 2-methoxystyrene, was synthesized using the method of Okamura and co-workers (See Figure 10).[203] The monomer, 2-methoxystyrene, is obtained in 33% yield from a decarboxylation reaction of 2- methoxycinnamic acid.

After the decarboxylation reaction was completed, the product was purified via the following method: The product was taken up in fifty milliliters of ethyl ether in a seperatory funnel, neutralized with the equivalence of cold, diluted hydrochloric acid, and extracted. Next, the extracted organic layer was taken up into an additional seperatory funnel and washed several times with water and small amounts of diluted hydrochloric acid. Both steps were repeated until a good portion of the product was recovered and the base, quinoline, was neutralized. The organic layer was then placed over anhydrous sodium carbonate and allowed to stir for 6 hours and filtered via vacuum filtration technique. The ethyl ether was then evaporated off of the product and was then dried over CaH_2 for several hours, distilled, and analyzed via ^{1}H and ^{13}C NMR Spectroscopy; (See Figures 8 and 9): H^{1} NMR ($CDCL_3$) δ: 7.8 (t), 7.6 (t), 7.2 (m), 7.0 (t), 6.8 (t), 5.8 (d), 5.4 (d), 3.8 (s): C^{13} NMR ($CDCL_3$), Aromatic ring carbons: C-1 126.9, C-2 156.9, C-3 114.5, C-4 128.9, C-5 120.7, C-6 126.6, -CH= 131.8, $-CH_2=$ 111.0, OCH_3 55.5, $CDCl_3$ 77.0.

Figure 8: ^{1}H NMR spectrum of 2-methoxystyrene

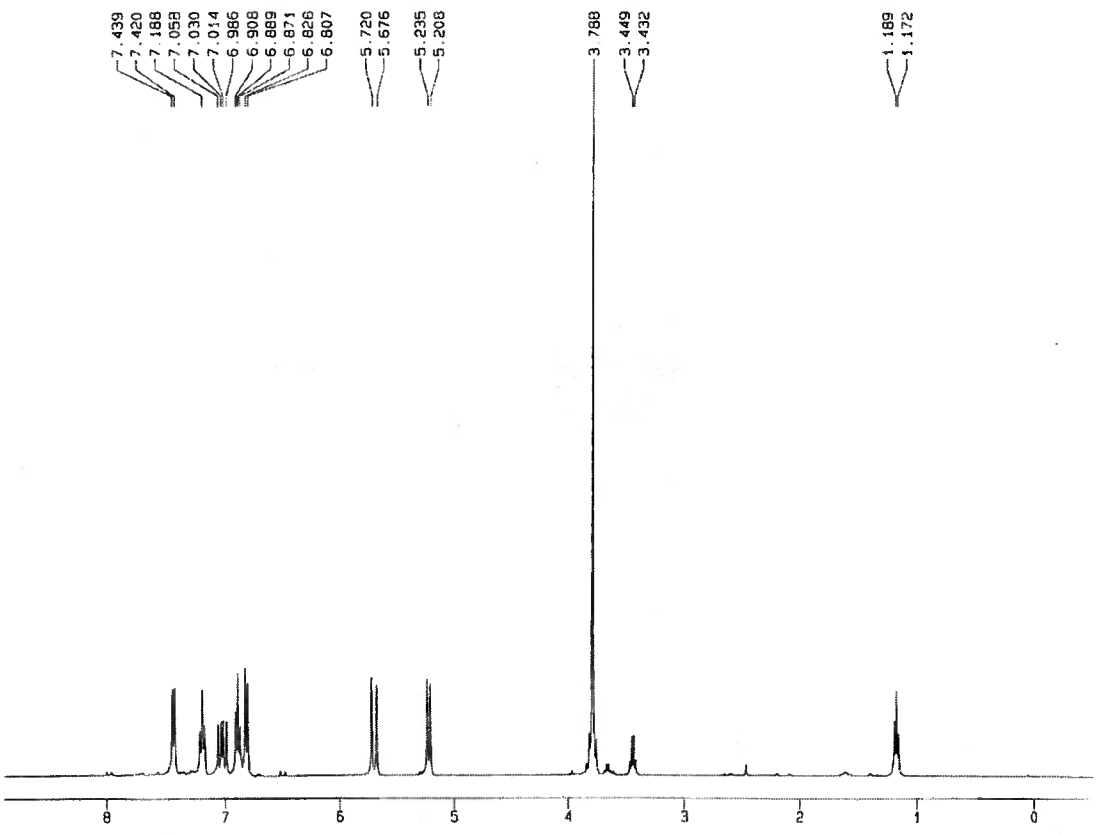
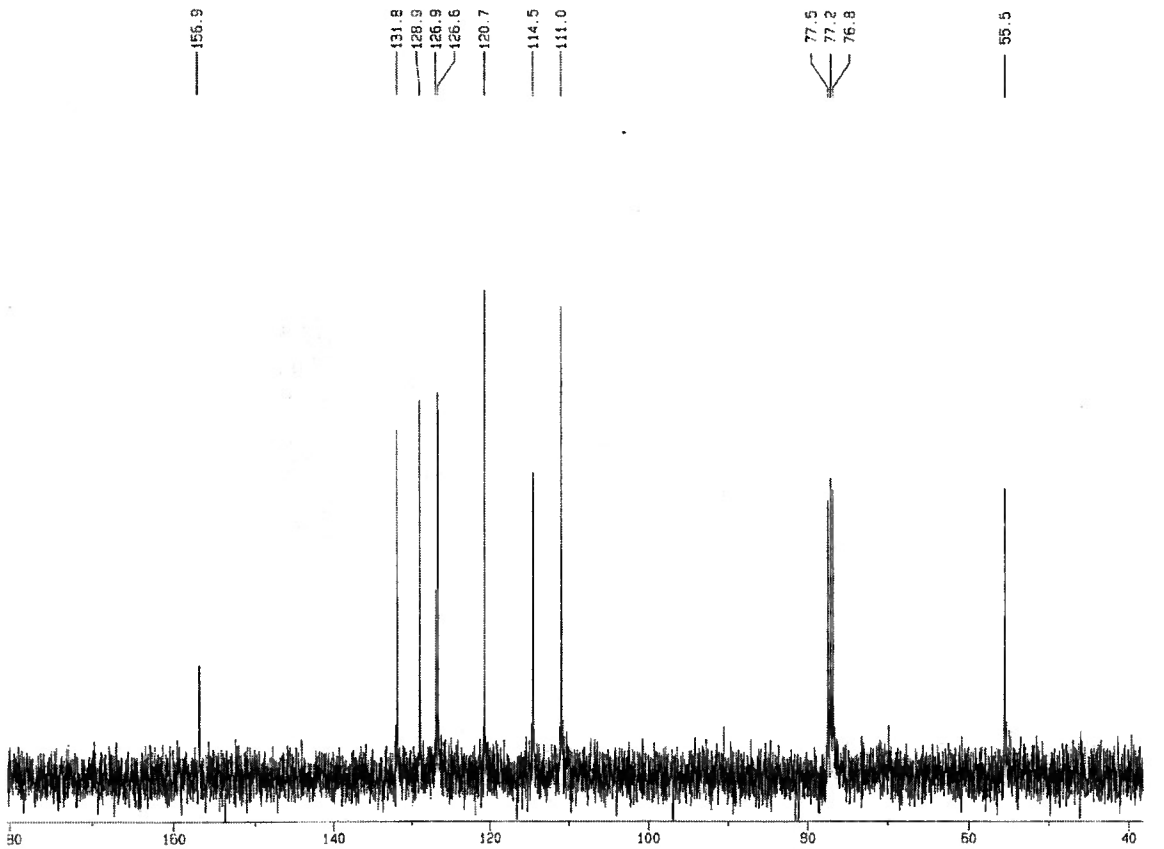


Figure 9: ^{13}C NMR spectrum of 2-methoxystyrene



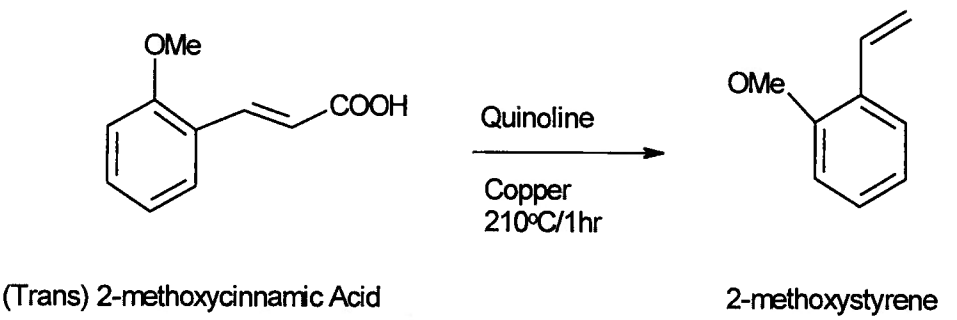


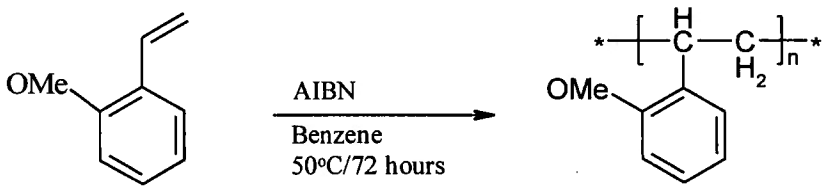
Figure 10: Synthesis of the Monomer

3.3.2 Polymer Synthesis

A series of polymers were prepared via anionic living polymerization pathways. Anionic polymerizations are highly sensitive to impurities and moisture; thus, all reagents used were dried over various drying agents and purified via vacuum distillation methods. Polymers were also prepared by free radical polymerization methods.

3.3.2.1 Free-Radical Polymerization

In a 250 ml ampoule was placed 0.1 g of recrystallized 2,2'-Azobisisobutyronitrile, AIBN. Two ml of benzene, which was dried overnight over calcium hydride, was distilled onto the AIBN under vacuum. Next 0.5 ml of the monomer, 2-methoxystyrene, was added via syringe. The system was sealed under vacuum and the mixture was stirred in an oil bath at 50°C for 72 hours. The polymer was precipitated in methanol, filtered and dried overnight in a vacuum oven. (See Figure11)



2-methoxystyrene

Poly(2-methoxystyrene)

Figure 11: Free-radical polymerization of 2-methoxystyrene

3.3.2.2 Anionic Polymerization

Ten ml of toluene (or tetrahydrofuran), which was dried overnight over calcium hydride, was injected into the polymer reaction vessel under nitrogen. Then the appropriate amount (depending on the desired M.W. of the polymer) of n-BuLi was added into the reaction vessel via syringe under nitrogen. The reaction vessel was then closed under vacuum and the mixture was allowed to stir for 30 minutes at room temperature. Next, the reaction mixture was cooled to the desired temperature and the corresponding amount of the monomer, 2-methoxystyrene, was added through a breakseal to the catalyst (either tolyl lithium) solution. The reaction was allowed to stir for 48 or 72 hours and terminated with methanol or chilled hexanes. The polymer was recovered by precipitation into methanol (See Figure 12). Polymerization in tetrahydrofuran (using n-BuLi) was carried out at –78°C for 2 hours.

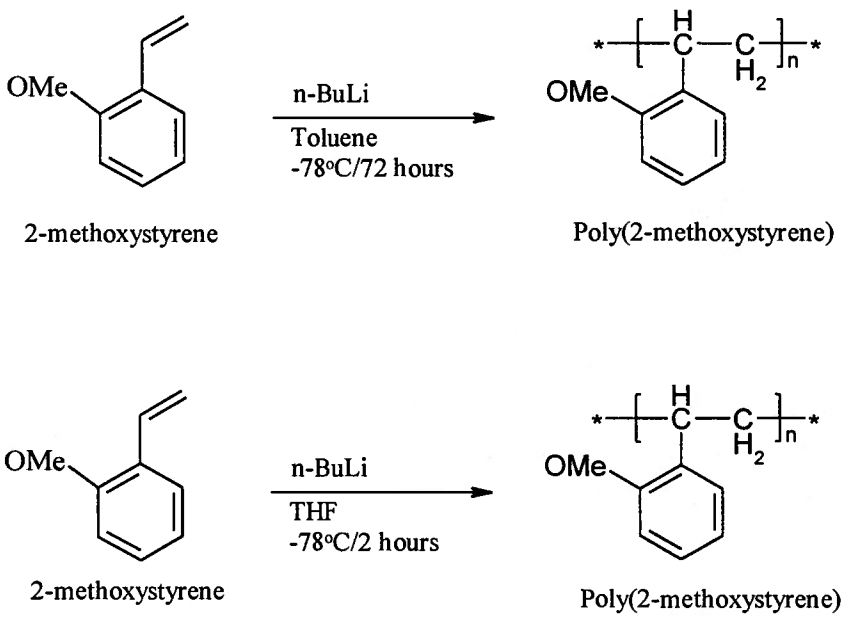


Figure 12: Anionic Polymerization of 2-methoxystyrene

3.3.3 Polymerization of P2MS with Optically Active Initiators

The polymerizations utilizing optically active initiators were similar to the method reported by Okamoto (See Figure 13).[64-72] All anionic polymerizations were carried out using high vacuum breakseal techniques. The purity of the monomer was checked by ^{1}H and ^{13}C NMR Spectroscopy. The chiral anionic initiating n-BuLi - (+) or (-) DDB [(+) or (–) 2,3-dimethoxy-1,4 bis(dimethylamino) butane) complexes were prepared by first reacting n-BuLi in toluene at room temperature: this was followed by the addition of the (+) or (-) – 2,3-dimethoxy-1,4 bis(dimethylamino) butane [(+)-DDB or (-)-DDB] chiral ligand at room temperature, the complexation was allowed to proceed for 1 hour. The optical purities of the (-) DDB and (+) DDB were about the same, with $[\alpha]_D^{20}$ values of –14.4 and +14.5, respectively were observed. The molar ratio of the chiral ligand to the lithio anion used was 1:1. This was to insure a 1:1 complex formation. After preparation of the chiral initiating complexes, the breakseal containing the 2-methoxystyrene was broken, and was added to the initiator solution maintained at –78°C. The ratio of the monomer to the initiator was determined by the M.W. of the target polymer. The reactor was sealed and the polymerization was allowed to take place for 72 hours at –78°C. The dark yellow solution was terminated by CH_3OH. Poly(2-methoxystyrene) was purified via evaporation due to its inability to precipitate solely into cold methanol. The solid polymer was dried in a vacuum oven at room temperature for 12 hours.

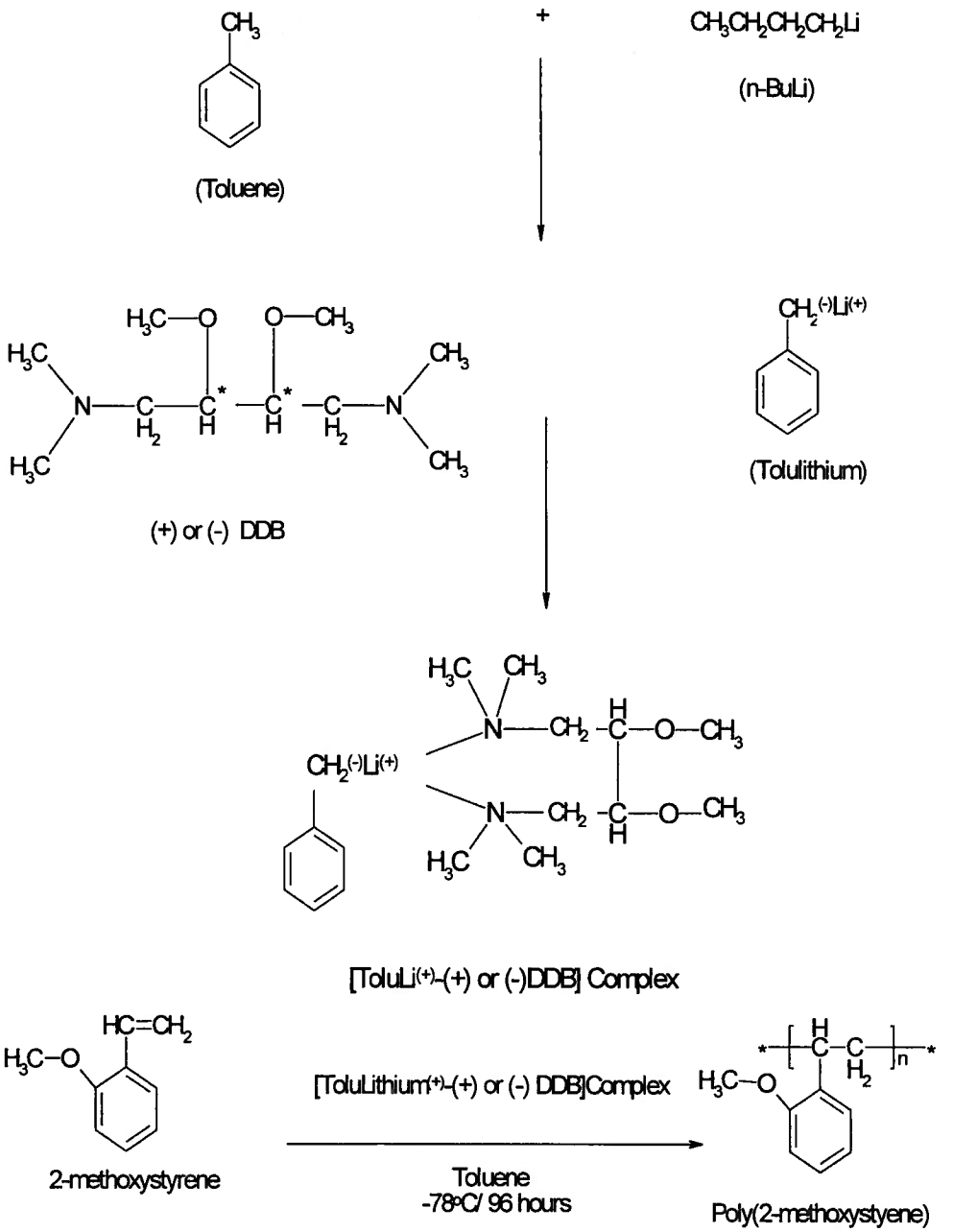


Figure 13: Polymerization of Poly(2-methoxystyrene) with Chiral initiators

3.3.4 Preparation of P2MS/Mandelic acid Complexes

Poly(2-methoxystyrene) was complexed with (R) and (S) Mandelic acid w/w ratios (P2MS:MA) of 1:1, 1:1.5, 1:2, 1:5, and 1:10 in THF. The mixtures were allowed to stir for 24 hours. CD measurements were carried out in solution and solid-state.

3.3.5 Preparation of (+) and (-) Helical P2MS/PEO/PEG Composites

(+) and (-) Poly(2-methoxystyrene) was dissolved in THF and co-precipitated with .05 g of PEO and .05 of PEG in 1:1:1 ratio by weight into chilled hexanes. The precipitate was transferred onto a poly(ethylene) IR card and the solvent was immediately evaporated in a vacuum oven at room temperature. CD measurements were carried out on the solid samples.

3.3.6 Biocompatibility Studies of Poly(2-methoxystyrene)

HeLa Ovarian Cancer cells were used for the characterization of the preliminary cell adhesion and cell growth. The Hela Cells (human ovarian cancer cells) (ATTC) that were used in this study were cultured in RPMI-1640 medium (Cellgro, USA), supplemented with 5% fetal bovine serum (FBS) and 1% antibiotic solution.

Five milligrams of each of the polymers were dissolved in 5 ml of anhydrous THF and casted on glass slides and allowed to dry. The polymer coated glass slides were then placed into the bottom of 12-well tissue culture modified poly(styrene) plates (Corning Incorporated, USA). The polymer coated glass slides were then sterilized by 70% alcohol and allowed to dry for several hours. One ml of cell suspension with a cell concentration of about 2.0 X 10^4 / ml were added to each well. In this study, TCPS or

tissue culture modified poly(styrene) was the control. The cell attachment and growth was observed by microscopy (Nikon-TMS, Japan) and digital photomicrographs were recorded (Sony-CCD-IRIS, Japan).

For the counting of cells at the end of cultured periods, media were taken out from culture plate. The cells were then detached by trypsin-EDTA digestive treatment for 10 min. Trypsinization was stopped by addition of medium. The cells were collected by centrifugation, resuspended, and counted by a hemacytometer. Values were an average of at least three cell countings.

3.4 Characterization

3.4.1 ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR)

^{1}H and ^{13}C NMR spectra were obtained on a Bruker ARX 400 spectrometer at room temperature in $CDCl_3$ and at high temperatures in o-dichlorobenzene- d_4. Tetramethylsilane (TMS) and Hexamethyldisilane (HMDS) were used as the internal standards.

3.4.2 Gel Permeation Chromatography (GPC)

The molecular weight measurements were performed using a Waters GPC-150C system. Two high porosity cross-linked poly(styrene) GPC columns were connected in series. Toluene or tetrahydrofuran were used as mobile phases. The flow rate was 1.0 ml/min. The GPC measurements were carried out at 30°C. The concentration was 0.1%. A set of narrow molecular weight distribution poly(styrene) standards were used as standards.

3.4.3 Differential Scanning Calorimetry (DSC)

Thermal studies were obtained using a Pyris Differential Scanning Calorimeter. The samples were packed in a aluminum pan holder and scanned under a nitrogen atmosphere.

3.4.4 Polarimetry

Optical rotation was measured by a Rudolph Autopol III polarimeter in a cell with a pathlength of 1.0 dm and at a wavelength of 589 nm.

3.4.5 Circular Dichroism

Conformational studies of the polymers were studied using a JASCO-J-720 spectropolarimeter at room temperature.

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Stereospecific Polymerization of 2-methoxystyrene

The polymerizations of 2-methoxystyrene were carried out with n-BuLi as an initiator in THF at temperatures of 0°C and -78°C and with n-BuLi as an initiator in toluene at temperatures of 30°C, 0°C, -20°C, and -78°C. Additionally, polymerizations of 2-methoxystyrene were carried out with AIBN as an initiator in benzene at a temperature of 50°C. The results are shown in Table 2.

Table 2. Polymerizations of 2-methoxystyrene under different conditions[a]

Initiator	solvent	temp(°C)	time(h)	yield(%)	*Mw*	*Mn*
AIBN	benzene	50	72	55	6000	3270
n-BuLi	THF	-78	2.5	100	4000	3400
n-BuLi	Toluene	-78	120	traces	----	----
n-BuLi	Toluene	0	48	75	19,000	11,000
n-BuLi	Toluene	-20	72	75	3800	2840
n-BuLi	Toluene	30	24	90	8400	5230

a. GPC in THF using Waters Styragel columns HR3 at flow rate of 1.0 ml/min. Molecular weighs are relative to polystyrene standards.

The polymer obtained with n-BuLi in toluene at low temperature displayed similar physical properties to those reported in the literature.[204] The polymers displayed melting temperature (T_m) was 275-295°C, thus suggesting that the polymer is partially crystalline. However, a glass transition temperature (T_g) was not observed for the polymer. The fact that a glass transition temperature is not observed would indicate that either the glassy amorphous domain is small (< 300 Å) or that the overall amorphous content of the polymer is not sufficient for observation by DSC. Techniques such as DMTA (Dynamic Mechanical Thermal Analysis) and/or $T_{1\rho}$ measurements may be necessary to make further conclusion regarding the polymer morphology.

The NMR spectrum of P-2-MeOSt prepared with n-BuLi in THF at -78°C is shown in Figure 14. The integration ratios of the peaks at 7.1, 3.5, 2.5, and 1.9 ppm are 4:3:1:2, which respectively correspond to the phenyl, methoxy, methine, and methylene groups of P-2-MeOSt.

Figure 15 shows the ^{1}H NMR spectra of the methoxy groups of the polymers prepared under various conditions. The methoxy peaks show stereochemical resolution of pentads or higher. The stereochemistry of the polymers obtained in THF and toluene is a function of the polymerization temperature. The polymers obtained in THF at temperatures of -78°C and 0°C show different stereoregularities. The polymer obtained with n-BuLi in toluene displayed a peak at 3.17 ppm, which broadened in area and decreased in intensity at an elevation of temperature. This peak was assigned as the mmmm (isotactic) pentad by Okamoto and coworkers [204] In addition to NMR spectroscopy Okamoto used X-Ray diffraction to justify the assignment of this peak as the isotactic pentad.

Assignments of the fine peaks of the methoxy resonance of the NMR spectra of the prepared polymers were attempted. Assignments were made following the analysis made by Okamoto and co-workers.[205] The work reported by Okamoto demonstrated that the methoxy resonances were resolved into ten peaks, corresponding to ten configurationally different pentad sequences.[206] However, the spectra obtained at 400 MHz NMR shows stereochemical splitting of the methoxy protons at heptad or higher n-ad level of sequences; the methoxy resonances resolve into 20 peaks or more. This is easily accounted by the fact that a higher powered NMR spectrometer is being utilized in our case i.e. a stronger magnetic field would insure greater resolution of proton peaks, which would mean the addition of more proton peaks. Despite the addition of more peaks, our results were consistent with Okamoto's pentad findings. Therefore, we employed the same method of analysis in assigning our polymers. The spectra were divided into groups. The peaks at 3.10 - 3.29 ppm were assigned to the isotactic triad (I); which was split into three components (3.14, 3.17, and 3.22 ppm) by pentad sequences. The peaks at 3.32, 3.34, 3.35, 3.38 ppm were assigned to the heterotactic triad and peaks at 3.02 and 3.04 ppm to the components of the syndiotactic triad. The fractions I, H, and S were determined by the relative intensities of the corresponding absorptions or absorption areas (Integration).

In Table 3, the fractions of the triad sequences in the polymer obtained under different conditions are listed. For comparison, the stereoregularities (or tacticity) of the polymers that were prepared by the radical and anionic (in THF and toluene) polymerizations are also shown. The tacticity of the polymers obtained in THF as well as in toluene, were both greatly affected by the temperature. The polymer prepared in

THF decreased in isotacticity and increased in heterotacticity at an elevation of temperature. Similar observations were made of the polymers prepared in toluene. However, the polymer prepared in toluene at 0° C was more isotactic than the polymer prepared in THF at 0°C. This, perhaps, has to do with the nature of the ion-pairing in the solvents. In toluene, the ion-pairs are expected to be tighter, and the possibility of aggregation of the polymerization center is substantially higher than in THF. In THF, one would expect the formation of solvent-separated ion-pair to be the dominant active species. In Okamoto's report, he demonstrated, through the use of Bovey single-σ plots and Chujo's equation [$\Delta\varepsilon = -kT \times \ln(4IS/H^2)$], the validity of these findings.[214] Okamoto states that in the polymerization involving 2-methoxystyrene and n-BuLi in toluene, the ether groups of the monomer units at the chain end and/or the adding monomer may interact with the lithium counterion, and this interaction should be the cause for isotactic polymerization at low temperatures. However, the energy of the interaction may be so small that the stereospecificity of the polymerization decreases with a small rise of the reaction temperature. Okamoto's results showed that $\Delta\varepsilon$ is zero in the polymerization in THF regardless of the temperature. However, $\Delta\varepsilon$ in the polymerization in toluene with n-BuLi decreased with a decrease in the polymerization temperature, suggesting that the penultimate effect exists and increases with decreasing temperature.

Figure 14: ^{1}H NMR spectrum of P-2-MeOSt in $CDCL_3$

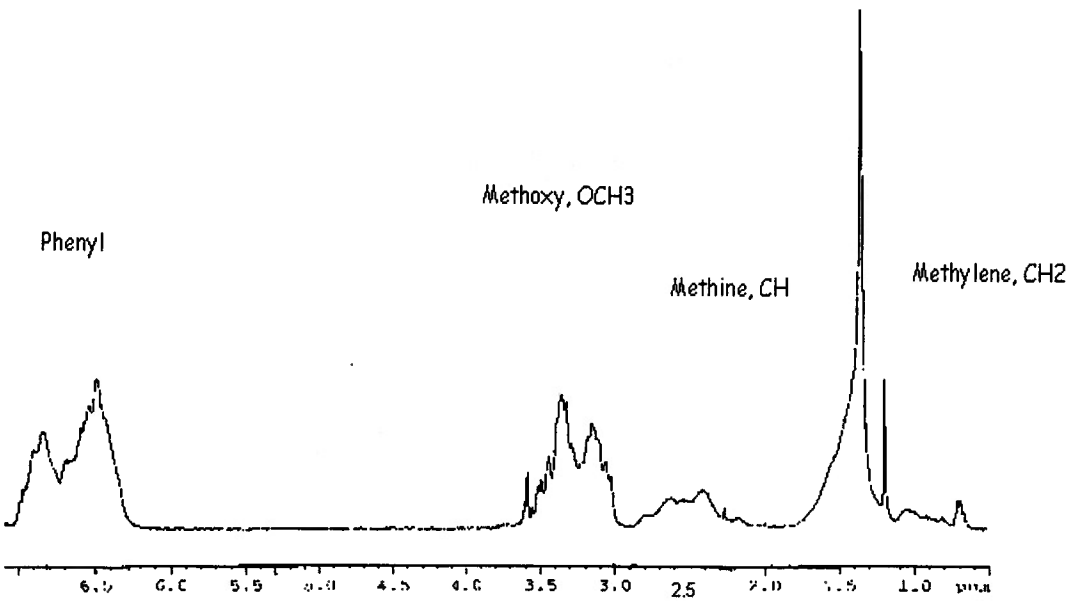
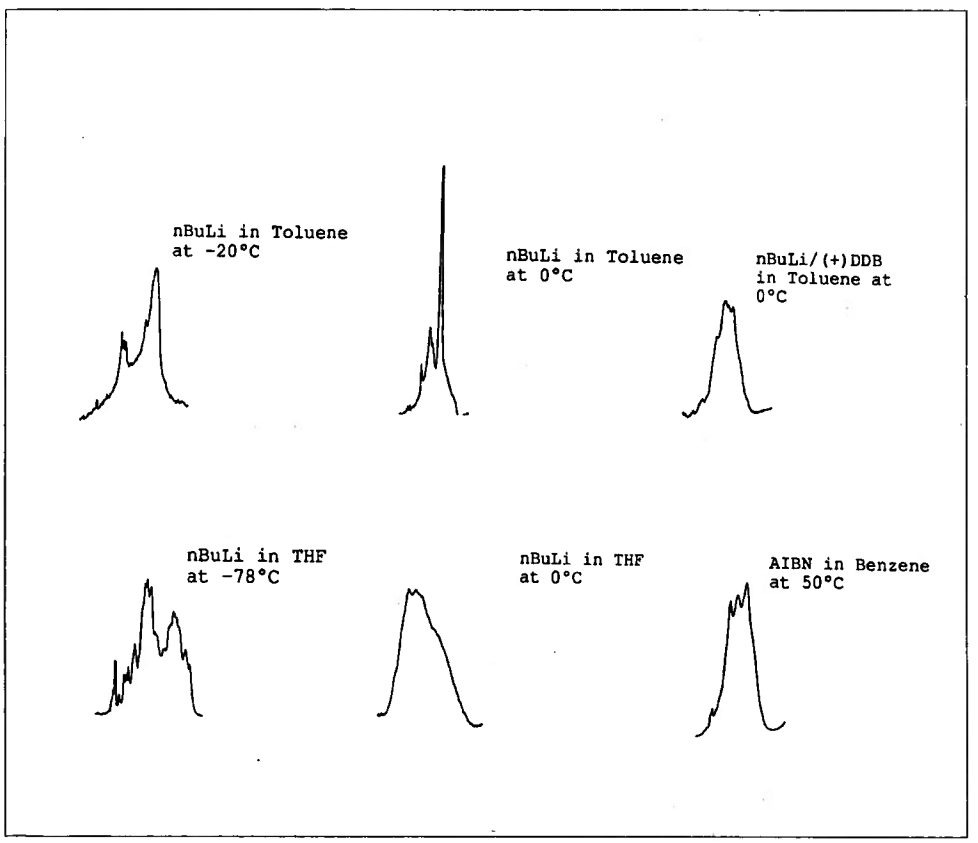


Figure 15. ^{1}H NMR Spectra of methoxy region of P-2-MeOSt



4.2 Optically Active Helical Poly(2-methoxystyrene)

Asymmetric anionic polymerizations of 2-methoxystyrene with n-BuLi/optically active ligand complexes were carried out - 78°C in toluene and the results are tabulated in Table 3. The polymers prepared at -78°C had to be precipitated in cold methanol and in cold hexanes, and yields of the purified polymer were very small. Direct analysis by ^{1}H NMR of the polymeric reaction mixture upon termination by methanol indicated small amounts of the residual monomer; i.e., most of the monomer under these conditions is converted to polymer. The small yields for the purified polymer is a result of (1) the optical rotation measurement process, where half of the reaction mixture is precipitated and half of the reaction method is saved in its original state and (2) small yields are expected for an anionic polymerization involving the monomer, 2-methoxystyrene, in toluene at –78°C initiated by n-BuLi.[207]

Table 3. Tacticity of Poly(2-methoxystyrene) Obtained Under Different Conditions

Initiator	solvent	temp(°C)	yield(%)	Tacticity, %		
				H	*I*	*S*
AIBN	benzene	50	45	29	52	18
n-BuLi	THF	-78	95	37	47	13
n-BuLi	THF	0	50	55	41	3
n-BuLi	Toluene	0	75	32	50	22
n-BuLi	Toluene	-20	75	23	62	14

H-Heterotactic, I-Isotactic, S-Syndiotactic

The optical rotations of the polymers were measured in two ways: (a) as soon as the polymer was terminated at –78°C by CH_3OH, a sample was taken out of the reaction flask and the rotation measured, and (b) the rotation was measured after the polymers were precipitated via evaporation and placed in tetrahydrafuran. The $[\alpha]^{-78}_{589}$ values

determined by both methods were different. The optical rotations taken by method "a" for the prepared polymers were relatively stable and those taken by method "b" were unstable.

Table 4 shows the optical rotations of the prepared polymers using method "a". With the (+) DDB/n-BuLi initiating complex, polymers with $[\alpha]^{-78}_{589} \sim$ (+) 78.00 are obtained; while the (-) DDB/n-BuLi initiating complex produced polymers with $[\alpha]^{-78}_{589} \sim$ (-) 12.00. An explanation for the difference in the observed optical rotations for the (+) and the (–) polymers may be plausible because of the the presence of absolute chiral centers in the polymer backbones. In these systems, it is thought and well accepted that these type of helical polymers solely display optical activity due to their low energy helical conformation as opposed to true chiral centers. However, the

Table 4. Anionic Polymerization of 2-methoxystyrene with the Complexes of n-BuLi with the optically active ligands (+) or (-) DDB (Method A)[a-c]

Run	chiral ligand	temperature	time(h)	$[\alpha]^{-78C}_{589}$	*Mw*
Mn					
1	(+)DDB	-78	96	(+) 78	---
2	(+)DDB	-78	96	(+) 73	58,000
3	(-)DDB	-78	96	(-) 12	---
4	(-)DDB	-78	96	(-) 15	333,000

a. GPC in THF using Waters Styragel HR3 at a flow rate of 1.0 mL/min.
b. Molecular weights are relative to polystyrene standards.
c. Indetermination value for [α] is +/- 0.1

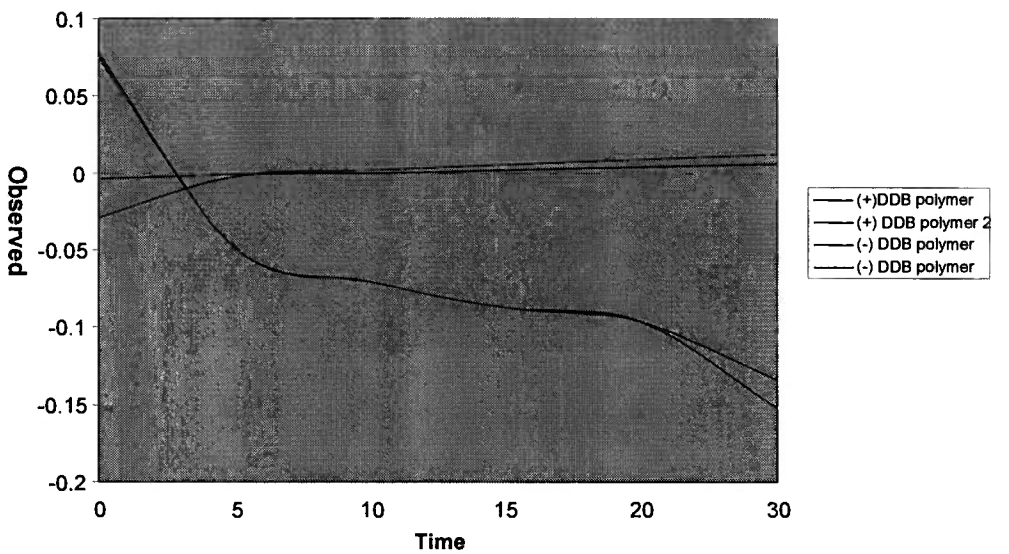
presence of true chiral centers in the backbones of these polymers may influence the type of twist sense and/or the direction of the helical twist, which will in turn reflect the

observed (overall or summative) optical rotations yielded by these polymers because of all possible conformations present in the sample. Evidence is present that demonstrates that poly(alkyl isocyanate)s adopt a chain conformation with long blocks of left- and right-handed helices separated by kinked and mobile helical reversals. It is the low population of these reversals which leads to an extreme sensitivity of these polymers to chiral information that minute chiral forces give rise to large excesses of one helical sense and therefore to small or large optical rotations.[209] It has been shown for a polyisocyanate chain that the large [α] arises from the helical conformation, where the left-handed helix exists in some excess over the right-handed helix due to small free energy favoring the former helix ($2\Delta G_h$).[210,211]

Again, the optical rotations taken by method "b" were unstable and very much different from the optical rotations taken of the polymers using method "a". The reason for this occurrence is not clear at the moment, but it may be due to intramolecular and intermolecular interactions of the polymers i.e. the possible formation and dissociation of different type of intermolecular or intramolecular polymeric complexes. Under method "a" conditions, the system may be at a state of thermodynamic equilibrium and clearly free of any stimuli that may perturb the system, which explains the stability of the optical rotations exhibited by the polymers under these conditions. However, there is clear perturbence of the system using method "b". The polymers were isolated through high vacuum evaporation as opposed to the conventional means of precipitating polymers in methanol. The ligand prepared polymers are soluble in methanol. The optical rotations of the polymers determined by using method "b",

were measured over various periods of time; the measurments were made at room temperature in tetrahydrofuran. In Figure 16, the observed rotation versus time (minutes) for the polymer prepared with the (+) DDB/n-BuLi initiating complex and the polymers prepared with the (-) DDB/n-BuLi initiating complex in toluene are shown. The (+) DDB prepared polymer displayed an initial observed rotation at (+) .073 and

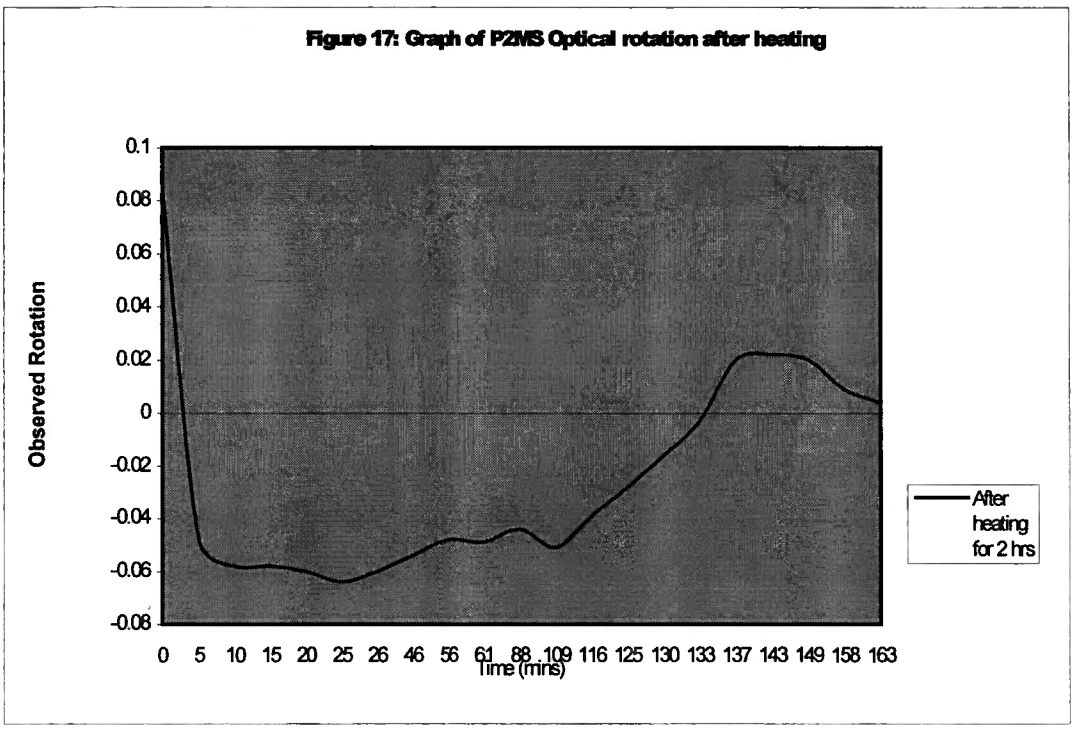
Figure 16. Graph of Optical Rotation of polymers of 2MS with Chiral Ligands



later maximized at (-) .152. The (-) DDB prepared polymer displayed an reading of (+) .011. It is important to mention that for each polymer, the final observed optical rotation over a period of few hours eventually reached zero or resulted in the formation of an optically inactive system (see Figure 17). The behavior observed while monitoring the optical rotations of these polymers using method "b", resembles that of a polymer changing its conformation, perhaps coiling and/or uncoiling or experiencing

a helix to helix interconversion resulting in racemization or going through a change in aggregation state, with each aggregation state froming a different chiral structure. The loss and recovery of optical rotation exemplified in these polymers may be explained by the possibility of absolute chiral centers being present in the polymer backbone. It is normally accepted that in vinyl homopolymers only psuedo assymmetric centers are present because of the difficulty of assigning priorities to two groups making up the polymer chain at the substituted carbon. However, at the polymer chain end such assignment is relatively simple and the presence of absolute chiral centers is a possibility in the instances when the polymers are prepared by chiral initiator. Furthermore, it is accepted that these type of helical polymers solely display optical activity due to their low energy helical conformation as opposed to true chiral centers. However, the presence of true chiral centers in the backbones (close to the chain ends) of these polymers may influence the type of twist sense and/or the direction of the helical twist, which will in turn reflect the observed optical rotations yielded by these polymers. The chiral centers would serve as a component for memory recall of the conformation of these polymers in different environments. For instance different temperatures may have a preference for different overall conformations of the polymers and the absolute chiral center would be the element which would guide the formation of stable conformations at a particular temperature. To test the validity of the above explanations, a heating study was done using the (+)DDB prepared polymer. In Figure 17, the (+) DDB prepared polymer was heated for 1 hr at 70°C and immediately placed into the polarimeter. The overall optical rotation of the polymer was zero. However, when the polymer sample was removed from the plane of polarized light (polarimeter)

and put into a freezer (-72°C) for several days, the optical activity of the polymer for the most part returned. There was, however, a slight difference in maximum optical rotation of the polymer, but this was to be expected due to the potential loss of various

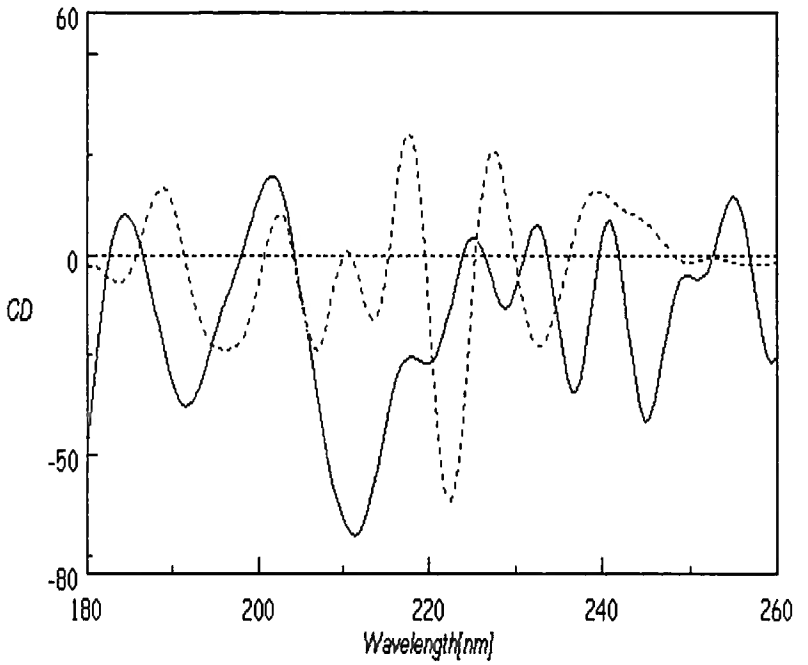


mechanical or chemical properties normally accompanied at the onset of segmental motion in polymers.

The optical rotation of the polymer is stable in their solid state at room temperature or lower, and the solid polymer may be stored in the refridgerator for several months without any loss in optical activity. The optical rotations are also stable at – 78° C in solution. These observations favor some sort of higher structure, most likely a secondary helical structure, giving rise to optical activity. Because of the rapid helix-to-

helix interconversion in the solution at room temperature, and the time required to obtain a CD spectra, we were not successful in obtaining a solution CD spectra showing in any cotton effect signals indicative of higher structural order (see Figure 18).

Figure 18: CD spectra of (+) and (-) Optically Active Helical Poly(2-methoxystyrene) in solution (THF) at 25°C

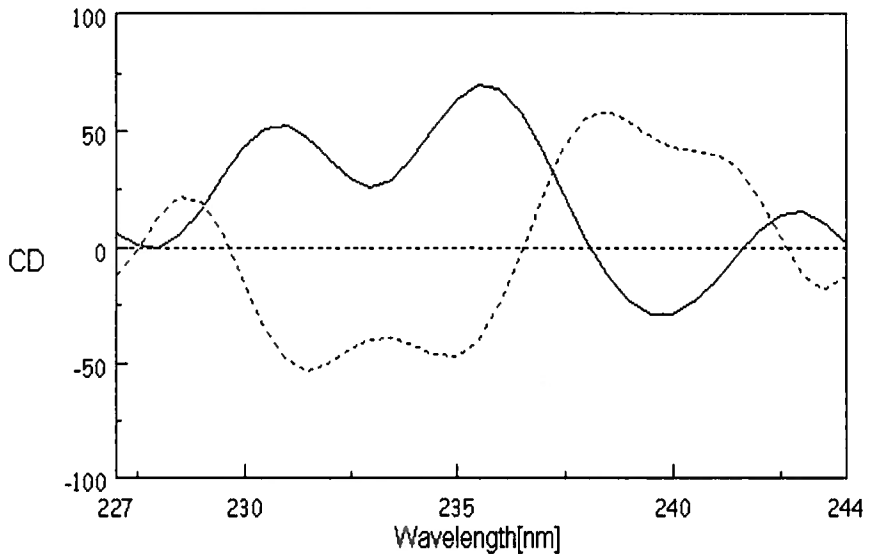


——— (+) O.A.H. Poly(2-methoxystyrene)
---------- (-) O.A.H. Poly(2methoxystyrene)

The helical conformation of (+) and (-) P2MS, however, may be locked in a solid matrix of poly(ethylene oxide) (PEO, MW 600,000) and poly(ethylene glycol) (PEG, MW 2000). The (+) and (-) P2MS/PEO (MW 600,000)/PEG (MW 2000) [1:1:1 by weight ratio] composites were prepared by carrying out the helix sense polymerization to produce approximately .05 grams of the (+) or the (-) P2MS at –78°C. The polymerizations were immediately followed by the addition of the .05 grams of PEO

and .05 grams of PEG into the solution at –78°C. The polymeric mixture was then casted on poly(ethylene) IR plates, evaporated, and dried in a vacuum oven at room temperature. The CD spectra show mirror image cotton effect signals at 233 and 239 nm with an isobestic point indicating the formation of enantiomeric higher structural order (see Figure 19).[23] The reason the composite spectra do not align exactly is

Figure 19: CD spectra of (+) and (-) Optically Active Helical Poly(2-methoxystyrene) in PEO/PEG Matrix at 25°C



——— **(+) O.A.H. Poly(2-methoxystyrene)**
---------**(-) O. A. H. Poly(2-methoxystyrene)**

because of the helix to helix interconversion experienced by the polymers during the method of isolation used to make the polymeric matrix. Precipitation could not be used to make the composite matrix because (+) and (-) P2MS are both soluble in methanol and hexane. The (+) and (-) P2MS, PEO, and PEG remained in solution until all the

solvent could be evaporated, thus, allowing time for the polymer's conformation to shift slightly as a result of the solution temperature reaching room temperature. Nevertheless, even in instances where the activation energy of the helix-to-helix interconversion is small, such that the left- and the right-handed helices are rapidly equilibrating in solution, it is possible to readily lock in the helical conformation in a solid matrix. When the (+) and (-) P2MS/PEO/PEG composites are heated to 70°C for 1 hour, the cotton effects are lost indicating either helix-to-helix interconversion resulting in racemization or helix-to-coil transition resulting in loss of the helical structure. These observations suggests that the optical activity (overall) is because of some secondary structures and not due to the presence of a true (absolute) chiral center present in the polymer's backbone.

The optical activity of the (+) and (-) polymer shows reversible optical rotation or a secondary structure reversibility on heating and cooling the polymers. This phenomenon for the (+) poly(2-methoxystyrene) prepared by the (+)DDB/tolylithium complex is shown in Figure 20. The polymer initially shows an (+) optical rotation, but, in 25 minutes, the rotation changes to (-) optical rotation. At room temperature (eventually) or upon heating the polymers to 40 °C, the optical activity of the polymers decrease to almost zero. However, on cooling the polymer sample to -71° C for 24 hours, the polymers regain their original optical rotation. The polymer has been successfully cycled several times upon heating and cooling. This occurrence is demonstrated in Figure 21. The phenomenon of the reappearance of the original optical rotation is most likely due to the presence of true asymmetric center(s) in the polymer's backbone. The true asymmetric center(s) induces the secondary chirality giving rise to dynamic and

reversible optical rotation. This process is totally reversible suggesting that the polymers have a memory for the secondary structure.

Figure 20: Thermal Studies of (-) Helical P2MS prepared at -78 C

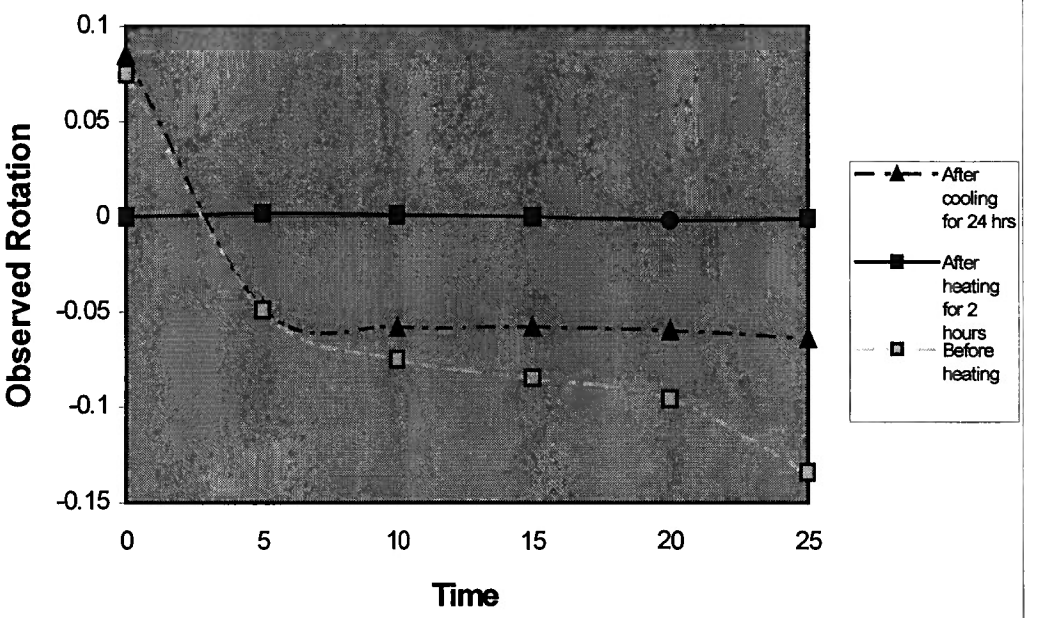
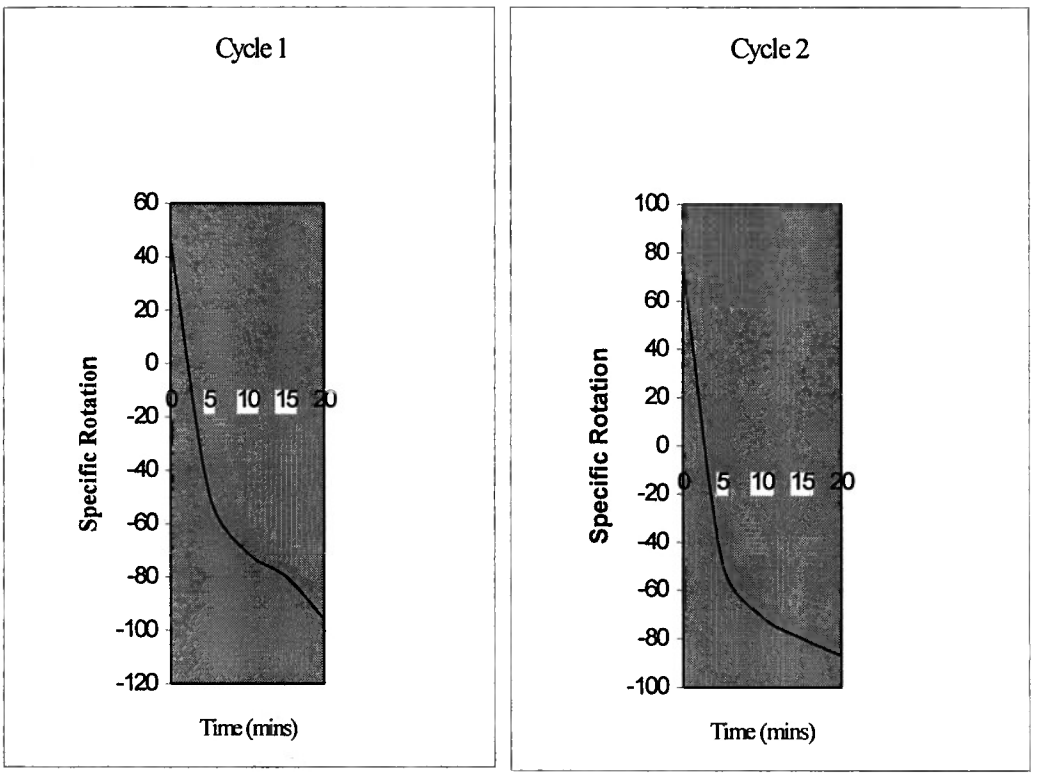


Figure 21: Cycling Studies of (+) Helical P2MS prepared at –78°C



Asymmetric polymerizations of 2-methoxystyrene with the complexes of n-BuLi/(+) or (-) DDB were done at 0°C. The results are listed in Table 5. The yield of the polymerization was more than 60%. These polymers unlike those prepared at –78°C were insoluble in methanol and were able to be precipitated. The polymers were filtered and allowed to air dry for 12 hours at room temperature. The optical rotations of the polymers were not as high or as dynamic as those of the polymers prepared at

–78°C. (+) Poly(2-methoxystyrene) had an average [α] initial specific rotation at (+) 3.0 and a loss of optical rotation normally preceded in about 30 minutes time at room temperature. The polymers insolubility into methanol and lower optical rotation are probably due to lower molecular weights [212] and an expected decrease in

Table 5. Anionic Polymerization of 2MS with the n-BuLi/ (+) or (-) DDB complexes at 0°C[a]

run	chiral ligand	temperature	time(h)	*Mw*	*Mn*
1	(+)DDB	0	48	4110	4020
2	(-) DDB	0	48	4000(T)	4000(T)

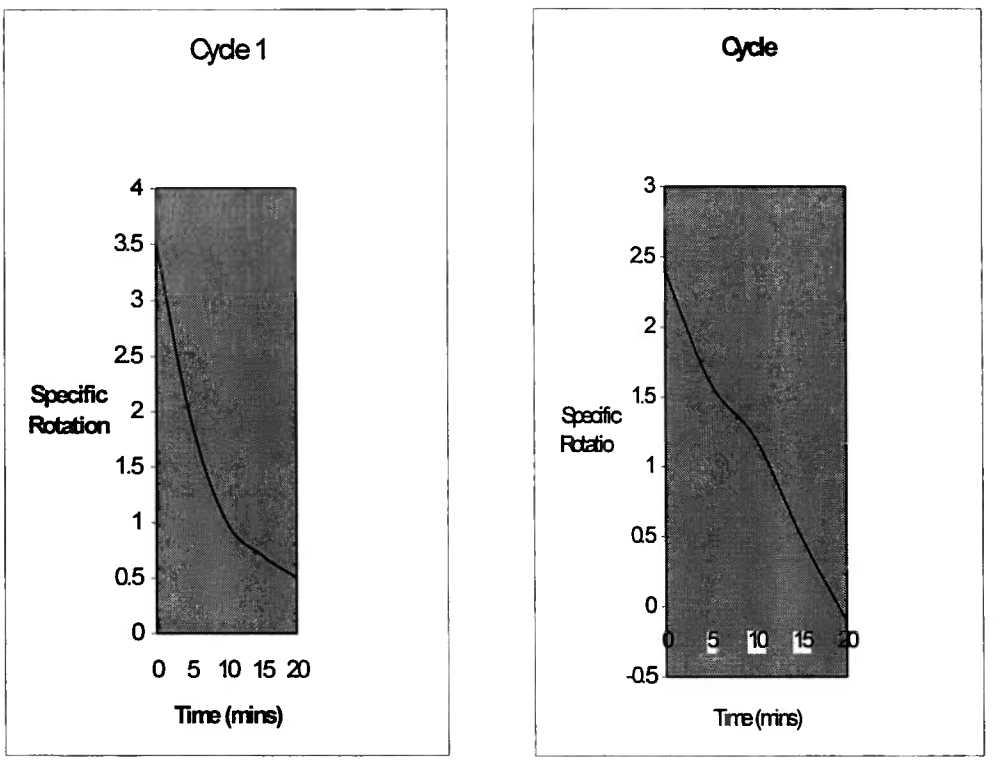
a. **GPC in THF using Waters Styragel HR3 at a flow rate of 1.0 mL/min. Molecular weights are relative to polystyrene standards**

isotacticity. The isotactic content for the chiral polymer made at 0°C was 65%. The polymers made at –78°C are thought to be mostly isotactic (90% or more). We know that an elevation of polymerization temperature will result in a decrease in isotacticity.[205] Both factors ultimately would have a drastic effect on the overall helicity, crystallinity, and higher structural order of the polymer, thus resulting in a difference in physical and chemical properties. Despite the loss in isotacticity and overall helicity, the polymers does not lose their reversibility. This confirms the notion that the polymers optical rotation is a direct result of the polymers helicity and their reversibility is a direct result of the asymmetric centers present on the backbone of the polymer.

Table 6. Tacticity of (+) -P2MS vs. racemic P2MS both prepared at 0°C

Initiator	solvent	temp(°C)	yield(%)	Tacticity, %		
				H	*I*	*S*
n-BuLi	Toluene	0	75	32	50	22
n-BuLi/(+)DDB	Toluene	0	80	20	65	15

Figure 22: Cycling Studies of (+) Helical P2MS prepared at 0°C

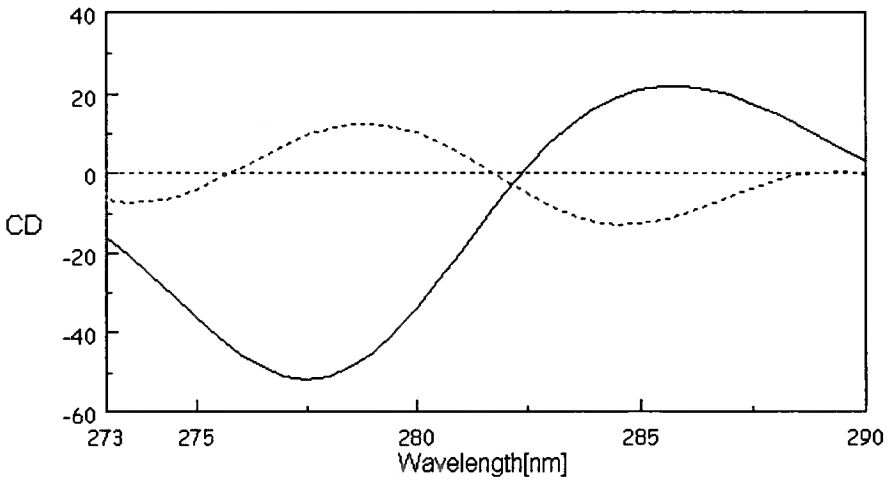


4.3 Induced Helical Poly(2-methoxystyrene)

An effective and very current method for preparing conformationally rigid helical polymers is the formation of induced helical structure of a single-screw sense starting with achiral synthetic polymers. Achiral poly(isocyanates), poly(guanidines) and poly(acetylenes), and poly(organophosphazene)s have been "chaperoned" or induced to a helical conformation using chiral acid-base interactions.[145-149]

Again, helicity selection of a single-screw sense starting with a racemic mixture of helical poly(2-methoxystyrene) by chiral chaperoning has been demonstrated. Complexes of racemic helical poly(2-methoxystyrene)/(R) and (S) - mandelic acid were prepared by intramolecular interactions in tetrahydrofuran and toluene. Complexes with mandelic acid to monomer repeat ratios of 1:2, 1:1.5, 1:1, and 5:1 were prepared. The P2-MeOSt/(R)- or (S) complexes were prepared by stirring .5 grams of P2-MeOSt with the appropriate amount of (R)- or (S)-mandelic acid for 8 hours at room temperature. The samples were prepared for analysis in two ways. One way involved the sample solution being poured onto poly(ethylene) plates and dried under vacuum to obtain the CD samples. The second way involved taking the sample solutions and placing them into a Jasco sample holder made of quartz for CD analysis. The best CD spectra were observed using the second way of preparation. The weight average molecular weights (Mw) of the polymers, relative to poly(styrene) standards, used in preparing the complexes were 19,000. The $[\alpha]^{20}_{D}$ of (R)- and (S)-mandelic acids were -153° and +154°, respectively, in H_2O and THF. Helicity selection chaperoning was observed for complexes prepared in both THF and toluene at mandelic acid to monomer repeat unit

Figure 23: ICD spectra of P2MS/(R) and (S) Mandelic Acid complex in 1:1 ratio in THF at 25°C



ratio's of 1:1, 1:1.5, and 1:2, the most well defined Circular Dichroism spectra were observed for the 1:1 complexes (See Figure 23).

4.4 Biocompatibility and Cell Adhesion Studies of Poly(2-methoxystyrene)

Table 7 lists the four different polymers that are utilized in the biocompatibility studies. The polymerizations of 2-methoxystyrene were carried out with n-BuLi as an initiator in toluene at a temperature of -20°C and with AIBN as an initiator in benzene at a temperature of 50°C. Asymmetric anionic polymerizations of 2-methoxystyrene with n-BuLi/optically active ligand complexes were carried out at 0°C.

The polymers obtained displayed similar physical properties. They all showed insolubility in n-hexane, solubility in toluene and THF, and partial solubility in water. The polymers displayed melting temperature, Tm, was 275-295°C and displayed no

distinctive glass transition temperature (Tg), thus demonstrating that the polymers possess high crystallinity.

The stereoregularity of the polymers was investigated by means of NMR spectroscopy. The methoxy resonance of the spectrum split into more than 20 components due to tactic heptads. Table 2 displays the fractions of triad sequences content of the polymers. Tacticity analysis studies indicated that the four polymers are all predominantly isotactic, with (+) and (-) poly(2-methoxystyrene) being the most isotactic.

Chiral polymers, (+) and (-) helical poly(2-methoxystyrene) (P2MS), which were prepared through asymmetric pathways at 0°C, display small optical rotations of (+)2.5 and (-)-- (measurement unavailable), respectively. The optical rotations are stable below temperatures of –78°C; however, at room temperature a loss of optical activity occurs over a period of 30 minutes . The optical rotation is reversible upon cooling. The phenomenon of the reappearance of the original optical rotation is most likely due to the presence of true asymmetric center(s) in the polymer's backbone and has been discussed earlier in the case of the polymers prepared at –78°C. The true asymmetric center(s) induces the secondary chirality giving rise to reversible optical rotation.[213]

HeLa Ovarian Cancer cells were used for the preliminary evaluation of cell adhesion and cell growth. The Hela Cells (human ovarian cancer cells) (ATTC), that were used in this study, were cultured in RPMI-1640 medium (Cellgro, USA), supplemented with 5% fetal bovine serum (FBS) and 1% antibiotic solution.

Table 7. Polymerizations of 2-methoxystyrene under different conditions[a]

Initiator	solvent	temp(°C)	time(h)	yield(%)	*Mw*	*Mn*
AIBN	benzene	50	72	55	6000	3270
n-BuLi	toluene	-20	48	75	19,000	11,000
n-BuLi/(+) DDB	toluene	0	48	85	4110	4020
n-BuLi/(-) DDB	toluene	0	48	10	4000(T)	4000(T)

a. GPC in THF using Waters Styragel columns HR3 at flow rate of 1.0 ml/min. Molecular weighs are relative to polystyrene standards.

Five (5 mg) milligrams of each of the polymers were dissolved in 5 ml of anhydrous THF, casted on glass slides, and allowed to dry. The polymer-coated glass slides were then placed into the bottom of 12-well tissue culture modified poly(styrene) plates (Corning Incorporated, USA). The polymer-coated, glass slides were then sterilized by 70% alcohol and allowed to dry for several hours. One milliliter of cell suspension with a cell concentration of about 2.0 X 10^4 / ml were added to each well. In this study, TCPS or tissue culture modified poly(styrene) was the control. The cell attachment and growth were observed by microscopy (Nikon-TMS, Japan) and digital photomicrographs were recorded (Sony-CCD-IRIS, Japan).

After 24 hours, cell growth and adhesion were observed for all polymers, but there seemed to be no distinct difference among the different polymers (Figure 18). The number of cells after 72, 120, and 168 hours on the films is shown in Table 8. After 168 hours, the number of HeLa cells on TCPS was 42.7 X 10^4 cells/cm^2. On (+)P2MS, (-) P2MS, free radically prepared poly(2-methoxystyrene) (FRP2MS), and anionically prepared poly(2-methoxystyrene), the number of cells were 27.5 X 10^4 , 22.0 X 10^4, 22.0 X 10^4, 15.4 X 10^4 , and 15.1 X 10^4 respectively. In all cases, the Hela cells showed the best growth on (+) optically active helical poly(2-methoxystyrene) (+) P2MS It is truly surprising that poly(2-methoxystyrene) supports the attachment and growth of Hela cells. In light of the fact that polystyrene does not support such cellular activity. The difference is most likely due to the presence of the methoxy group in the backbone of the polymer. The methoxy group at the ortho position of the benzene ring may plausibly bring hydrophilicity/wettability to the polymer surface of poly(2-methoxystyrene) and

Table 8. Tacticity of 2-methoxystyrene polymers

Initiator	solvent	temp(°C)	yield(%)	Tacticity, %		
				mr	*mm*	*rr*
AIBN	benzene	50	45	29	52	18
n-BuLi	toluene	0	75	32	50	22
n-BuLi/(+)DDB	toluene	0	80	20	65	15

Table 9: Number of HeLa Cells on the Sample Surfaces after 3 days, 5 days and 7 days of Incubation

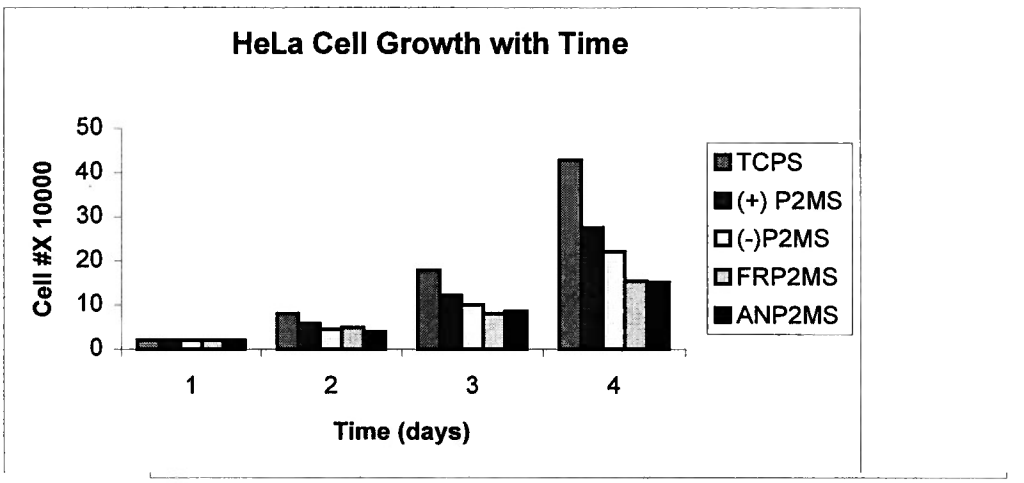
Sample	Number ($x10^4$) after 3 days	Number ($x10^4$) after 5 days	Number ($x10^4$) after 7 days
TCPS	8.0 ± 0.9	17.8 ± 3.0	42.7 ± 4.0
(+) P2MS	5.8 ± 0.3	12.1 ± 0.5	27.5 ± 2.0
(-) P2MS	4.5± 0.3	10.0 ± 0.3	22.0 ± 0.4
FRP2MS	4.8 ± 0.2	8.0 ± 0.2	15.4 ± 0.9
ANP2MS	3.9 ± 0.2	8.5± 1.3	15.1 ± 2.0

may be instrumental in cell attachment to take place. Cell adhesion and growth in synthetic materials depend upon the wettability or the presence of H bond groups forming within a particular system. The adsorption of serum proteins onto the surfaces of the various polymers is the result of hydrogen bond formation and in the case of poly(2-methoxystyrene) the methoxy group may participate in hydrogen bonding. The concentration of serum proteins onto the surfaces of the various polymers is directly proportional to the amount of attached cells and/or cell growth on each polymer.

The tacticity of the polymers and surface concentration of proteins seem to correlate with increased cell adhesion. In Table 8, the tacticity of the polymers are listed. The assignments were made following the analysis made by Okamoto and co-workers.[212] The work reported by Okamoto demonstrated that the methoxy resonances were resolved into ten peaks, corresponding to ten configurationally different pentad sequences.[213] The fractions mm, mr, and rr were determined by the relative intensities of the corresponding absorptions or absorption areas (integration). The more isotactic the polymer, the more cell growth and adhesion that is experienced. This is demonstrated in that free radically prepared poly(2-methoxystyrene), FRP2MS, is slightly more isotactic than anionically prepared poly(2-methoxystyrene), ANP2MS. Cell growth and adhesion were greater in F2MS than ANP2MS. It seems that the Hela Ovarian Cancer cells display a recognition for stereoregularity, which in turn is connected to the concentration of proteins that are bound to the polymer surfaces.

Molecular weight may also have an adverse effect on cell growth as well. In a report by Jeffrey Hubbell and coworkers, it was shown that the more the increase in molecular weight of poly(ethylene glycol) PEG in a lactide-PEG network, a well-known

Figure 24: HeLa Cell Growth On P2MS with Time



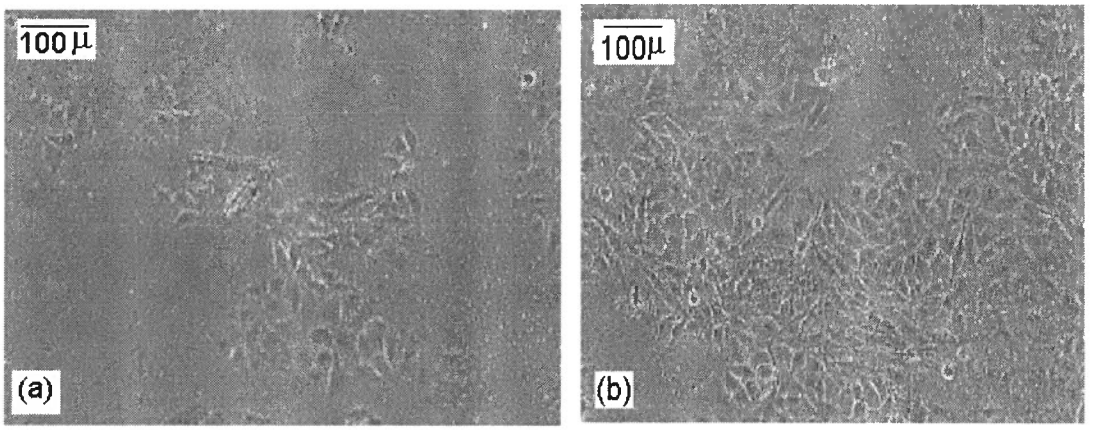
biocompatible bio-material, the more increased resistance to cell adhesion.[214] Cell adhesion in FRP2MA is slightly greater than ANP2MS. This could also be likely due to the fact that the molecular weight of ANP2MS is higher than FRP2MS. Increased molecular weights in poly(2-methoxystyrene) may change the overall surface properties of the polymer, thus changing the overall wettability of the polymer, which in turn will adversely affect cell growth and adhesion. Further studies are currently under way.

In all cases, the Hela cells showed the best growth on (+) and (-) optically active helical poly(2-methoxystyrene) (+) and (-) P2MS. The roles that molecular weight and tacticity play in this occurrence seem to be minimal at this time. The isotacticity of (+)P2MS and (-) P2MS is relatively the same for the two chiral polymers but is slightly higher than ANP2MS and FRP2MS. This offers some insight to the explanation as to why the Hela cells show preference for (+) and (-) P2MS. The molecular weights of all the polymers are all relatively low, except for ANP2MS whose molecular weight is significantly higher.

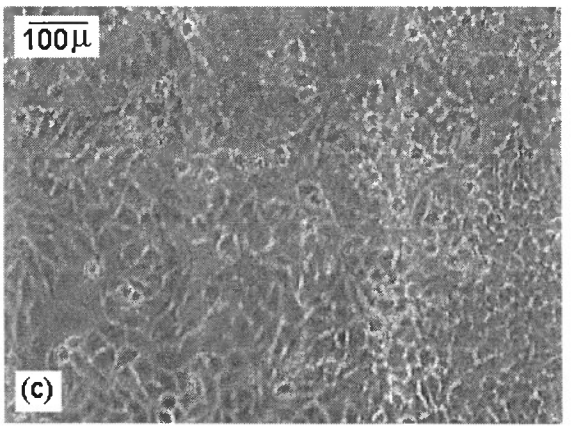
The two most notable factors governing the preference of Hela cells for (+) and (-) P2MS over ANP2MS and FRP2MS is chirality and higher structural order (helicity). (+) and (-) P2MS are unique synthetic polymers in that they possess optical rotation arising from helicity. However, ANP2MS and FRP2MS do not possess optical rotation nor helicity. Most naturally occurring polymers such as proteins, nucleic acids, and polysaccharides are optically active and often possess a specific conformation or higher-order structure arising from the optical activity, which is essential in order to exert their sophisticated functions in living systems. The presence of secondary structure and chirality in these polymers could be very well responsible for the

excellent cell growth and adhesion experienced on the surfaces of (+) and (-) P2MS. It can even be further said that the Hela cells prefer (+) P2MS over (-) P2MS, which exemplifies a chiral profiling ability found in living cells. The HeLa cells demonstrate a type of chiral preference for (+) optically active helical P2MS, which is quite remarkable in that this type of behavior in living cells toward a synthetic bio-material has never been reported.

Figure 25: HeLa Cell Growth on P2MS



3days 5 days



7 days

CHAPTER V

CONCLUSIONS

Optically active (right-handed and left-handed) helical poly(2-methoxystyrene) has been prepared by the asymmetric helix-sense selective polymerization at 0°C and -78°C by anionic living polymerization using chiral initiating complexes. At – 78° C in toluene as the solvent, the (+) DDB/n-BuLi initiating complex results in the formation of polymers with $[\alpha]^{-78}_{589}$ ~ (+) 78.00; while the (-) DDB/n-BuLi initiating complex produced polymers with $[\alpha]^{-78}_{589}$ ~ (-) 12.00. The observed optical rotations were stable at – 78° C but at room temperature, the optical activity of the polymers decreased over time to an observed optical rotation of zero. However, on cooling the polymer samples to -71° C for 24 hours, the polymer regains its original optical rotation. The reversibility of the optical rotations suggests that the bulk of the observed rotation is because of secondary structure, most likely helical structure. The observation of opposite optical rotations suggests that enantiomeric secondary structures are formed. The CD spectra of (+) and (-) P2MS/PEO (MW 600,000)/PEG (MW 2000) [1:1:1 by weight ratio] composites show mirror image cotton effect signals at 233 and 239 nm with an isobestic point indicating the formation of enantiomeric higher structural order. Helix sense selecltive polymerizations of 2-methoxystyrene with the complexes of n-BuLi/(+) or (-) DDB were done at 0°C. The optical rotations of the polymers were not as high or as dynamic as those of the polymers prepared at –78°C. (+) Poly(2-

methoxystyrene) had an average [α] initial specific rotation at (+) 3.0 and observed for the low temperature polymers, the optical activity is dynamic suggesting that the rotation is because of higher structure or secondary helical structure. The difference between the observed rotations is most likely explained by the difference in the stereoregularities of the polymers prepared at the two different temperatures. The helical polymers prepared at 0°C have an isotactic triad content of 65%. The polymers prepared at –78°C are mostly isotactic (>90% triad content). Greater isotactic content should result in the formation of a better (longer persistence length of the helical conformation) and a thermodynamically more favored system.

Additionally, cell adhesion and growth have been established on the surfaces of a number of different poly(2-methoxystyrene)s. Cell adhesion and growth were observed on polymers prepared by free-radical methods (FRP2MS), polymers prepared by living anionic methods (ANP2MS), and on the chiral or helical polymers. Cell adhesion on FRP2MA is slightly greater than ANP2MS. This is likely due to the fact that the molecular weight of ANP2MS is higher than FRP2MS. Higher molecular weights in poly(2-methoxystyrene) may change the overall surface properties of the polymer, thus changing the overall wettability of the polymer which may adversely affect cell growth and adhesion. In all cases, the Hela cells showed the best growth on (+) and (-) optically active helical poly(2-methoxystyrene) (+) and (-) P2MS. The two most notable factors governing the preference of Hela cells for (+) and (-) P2MS over ANP2MS and FRP2MS is chirality and higher structural order (helicity). This is the first report of an effect of chirality of a synthetic polymeric material on cell adhesion and growth.

REFERENCES

1. (a) McMurray, J. Organic Chemistry, 4th Edition, Brooks/Cole, New York, **1996**. (b) Garrett, R. H.; Grisham, C.M. Biochemistry, Saunders, New York, **1995**.
2. "Frontiers in Supramolecular Organic Chemistry", ed. H.J. Schneider and H. Durr, VCH, Weinheim, **1991.**
3. M. Farina, *Top. Stereochem.*, **1987**, *17*, 1.
4. G. Wulff, *Angew. Chem., Int. Ed. Engl.*, **1989**, *28*, 21.
5. Drenth, W.; Nolte, R. J. M., *Acc. Chem. Res.*, **1979**, *12*, 30.
6. Ute, K.; Hirase, K.; Kashimoto, H.; Hatada, K.; Vogl, O., *J. Am. Chem. Soc.*, **1991**, *113*, 6305.
7. Okamoto, Y.; Yashima, E., *Prog. Polym. Sci.*, **1990**, 263
8. Maxein, G.; Zentel, R., *Macromolecules*, **1995**, *28*, 8438
9. Fujiki, M., *J. Am. Chem. Soc.*, **2000**, *122*, 3336
10. Mueller, M.; Zentel, R., *Macromolecules*, **1994**, *27*, 4404
11. Feringa, B. L.; Jager, W. F.; DeLange, B., *Tetrahedron*, **1993** *49*, 8267
12. Feringa, B.L.; Huck, N. P. M.; Schoevoors, A. M., *Adv. Mater.*, **1996**, *8*, 681
13. Feringa, B. L.; Huck, N. P.; Jager, W.F.; De Lange, B., *Science*, **1996**, *273*, 1686
14. Zhang, M.; Schuster, G. B., *J. Phys. Chem.*, **1992**, *96*, 3063

15. Suarez, M.; Devadoss, C.; Shuster, G. B., *J. Phys. Chem.*, **1993**, *97*, 9299

16. Udayakumar, B. S.; Schuster, G. B. *J. Org. Chem.* **1993**, *58*, 4165

17. Zhang, M.; Schuster, G. B., *J. Am. Chem. Soc.*, **1994**, *116*, 4852

18. Zhang, Y.; Schuster, G. B., *J. Org. Chem.*, **1994**, *59*, 1855

19. Okamoto, Y. *Macromol. Symp.* **1996,** *101*, 345.

20. Okamoto, Y.; Nakano, T. *Chem Rev.* **1994**, *94*, 349

21. Okamoto, Y.; Ishikura, M.; Hatada, K.; Yuki, H., *Polym. J.*, **1983**, *15*, 851

22. Nakano, T.; Okamoto, Y.; Hatada, K., *J. Am. Chem. Soc.*, **1992**, *114*, 1318.

23. Ortiz, L.; Khan, I.M., *Macromolecules*, **1998**, *31*, 5927

24. Novak, B.M.; Goodwin, A.; Schlitzer, D.; Tatten, T.E.; Deming, T.., *Poly. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)*, **1996**, *37(2)*, 446

25. Goodwin, A., Novak, B.M., *Macromolecules*, **1994**, *27*, 4404

26. Habaue, S., Ajiro, H. Okamoto, Y., *J. Polym. Sci. Poly. Chem.*, **2000**, *38*, 4088

27. Nolte, R.J.M.; van Beijen, A. J. M.; Drenth, W. *J. Am. Chem. Soc.* **1974**, 96, 5932.

28. Kamer, P. C. J.; Nolte, R. J. M.; Drenth, M. M.; Drenth, W. *J. Am. Chem. Soc.* **1988,** 110, 6818.

29. Green, M. M.; Gross, R. A.; Grosby, C., III; Schilling, R. C. *Macromolecules* **1988**, 21, 1839.

30. Lifson, S.; Andreola, C.; Peterson, N. C.; Green, M. M.; Reidy, M. P.; Johnson, R. J.; Darling, G.; O'Leary, L. J.; Wilson, G. *J. Am. Chem. Soc.* **1989**, 11, 6452.

31. Okamoto, Y.; Matsuda, M.; Nakano, T.; Yashima, E. *J. Polym. Sci., Polym. Chem.* **1994**, 32, 309.

32. Maeda, K.; Matsuda, M.; Nakano, T.; Okamoto, Y. *Polym. J.* **1995**, 27, 141

33. Okamoto, Y.; Adachi, M.; Shohi, H.; Yuki, H. *Polym. J.* **1981**, 13, 175.

34. Okamoto, Y.; Hayashida, H.; Hatada, K. *Polym. J.* **1989**, 21, 543

35. Corley, L.S.; Vogl, O. *Polym. Bull.* **1980**, 2, 211

36. Vogl, O.; Jaycox, G. D. *Polymer*, **1987**, 28, 2179

37. Seebach, D.;Overhand, M.;Kuhule, F. N. M; Martinoni, B.; Oberer, L.; Hommel, V.; Widmer, H. *Helv. Chim. Acta.* **1996**, *79*,913.

38. Seebach, D.; Ciceri, P.E; Overhand, M.; Jaun, B.; Rigo, D.; Oberer, L.; Hommel, V.; Amstutz, R.; Widner, H. *Helv. Chimica. Acta.* **1996**, *79*, 2043.

39. Iverson, B.L. *Nature*, **1997**, *385*, 113

40. Apella, D.H.; Christiansun, L.A.;Karle, I.L.; Powell, D.R.; Gellman, S.H., *J. Am. Chem. Soc.* **1996**, *111*, 12011.

41. Bovey, F.A.; Jelinski, L. W., " *Chain Structure and Conformation of Macromolecules*", Academic Press, **1992**.

42. Natta, G.; Danusson, F.; Sianesi, D., *Makromol. Chem.* **1958**, *28*, 253.

43. Yuki, H.; Okamoto, Y.; Kuwae, Y.; Hatada, Koichi. *J. Polym. Sci: Part A,* **1969**, *7*, 1946.

44. (a).Takei, F.; Yanai, K.; Chitsuka, K.; Takahasi, S. *Chem.-Eur. J.*, **2000**, *6*, 983. (b). Cornelissen, J.; Rowan, A.; Nolte, R. and Sommerdijk, N. *Chem. Rev.*, **2001**, *101*, 4039.

45. Natta, G.; Corradini, *Nuovo Cimento*, **1960,** 10 Suppl. 153, 9.

46. Pino, P.; Lorenzi, G.P, J. Am. Chem. Soc., 1960, 82, 4745.

47. Pino, P.; Chiradelli, G.; Lorenzi, G.; Natta, G. *J. Am. Chem. Soc.*, **1962**, *84*, 1487.

48. Pino, P.; Chiradelli, G.; Lorenzi, G.P. *J. Am. Chem. Soc.*, **1963**, *85*, 3888.

49. Pino, P. *Adv. Polym. Sci.*, **1965**, 4, 983.

50. Pino, P. Salvadori, P.; Chiellini, E.; Luisi, P.L. *Pure Appl. Chem.*, **1968**, *16*, 469.

51. Farima, M., Peraldo, M. Natta, G. *Agnew Chem.*, **1965**, *77*, 149.

52. Schultz, R.; Kaiser, E. *Adv. Polym. Sci.*, **1965**, *4*, 236.

53. Ouchi, T.; Ohya, Y., *Macromolecular Design of Polymeric Materials*. Hatada, K.; Kitayama, T.; Vogl, O., (Eds), pp. 351-378. Marcel Dekker, New York (**1997**).

54. Okamoto, Y.; Kawashima. M.; Hatada, K., *J. Am. Chem. Soc.*, **1984**, *106*, 5357.

55. Tsuruta, *J. Polym. Sci., Part D*, **1972**, *6*, 179

56. Okamoto, Y.; Nagamura, Y.; Fukumoto, T.; Hatada, K., *Polym. J.*, **1991**, *23*, 1197

57. Kobayashi, K.; Kakimoto, M.; Imai, Y.; *Polym. J.*, **1994**, *26*, 763

58. Okamoto, Y.;Yashima, E. *Macromolecular Design of Polymeric Materials*, Hatada, K., Kitayama, T., Vogl, O., (Eds), pp. 731, Marcel Dekker, New York (**1997**)

59. Kawamura, T.; Uryu, T., Matasuzaki, K. *Makromol. Chem.*, **1982**, *183*, 151.

60. Selfgny, .E (Ed.), *Optically Active Polymers*, D. Reidel, Dordrecht (**1979**).

61. Okamoto, Y.; Ohta, K.; Yuki, H. *Chem Lett.*, 617, (**1977**)

62. Natta, G.; Rarina, M. *Makromolek. Chem.*, **1961**, *43*, 251

63. Natta, G.; Pino, P.; Mazzanti, G; Corradini, P.; Gianni, U., *Atti Accad. Nazl. Lincei*, **1955**, *19*, 397

64. Okamoto, Y.; Suzuki, K.; Ohta, K.; Hatada, K.; Yuki, H. *J. Am. Chem. Soc.*, **1979**, *101*, 4673

65. Okamoto, Y.; Suzuki, K.; Yuki, H. *J. Polym. Sci., Polym . Chem. Ed.*, **1980**, *18*, 3043

66. Okamoto, Y.; Shohi, H.; Yuki, H. *J. Polym. Sci., Polymer. Lett. Ed.*, **1983**, *21*, 601

67. Okamoto, Y.; Yahima, E.; Naknao, T.; Hatada, K. *Chem. Lett.*, **1987**, 759

68. Nakano, T.; Okamoto, Y.; Hatada, K. *J. Am. Chem. Soc.*, **1992**, *114*, 1318.

69. Kanoh, S.; Suda, H.; Kawaguchi, N.; Motoi, M. *Makromol. Chem.* **1986**, 187, 53

70. Kanoh, S.; Kawaguchi, N.; Sumino, T.; Hongo, Y.; Suda, H. *J. Polym. Sci., Part A, Polym. Chem.* **1987**, *25*, 1603

71. Kanoh, S.; Kawaguchi, N.; Sumino, T.; Motoi, M.; Suda, H. *Polym. J.* **1988**, *20*, 539

72. Wulff, G. Sczepan, R.; Steigel, A. *Tetrahedron Lett.* **1986**, *27*, 1991

73. Wulff, G.; Vogt, B.; Petzold, *J. Polym. Mat. Sci. Eng. (Am. Chem. Soc.)* **1988**, *58*, 859

74. Okamoto, Y.; Okamoto, I.; Yuki, H. *J. Polym. Sci. Polym. Lett. Ed.* **1981**, *19*, 451

75. Bartus, J.; Vogl, O.; *Polym. Bull.* **1992**, *28*, 203

76. Yuki, H.; Ohta.; Okamoto, Y.; Hatada, K. *J. Polym. Sci. Polym. Lett. Ed.* **1981**, *19*, 451

77. Ohta, K.; Okamoto, Y.; Hatada, K.; Yuki, H. *J. Polym. Sci., Polym. Chem. Ed.* **1979**, *17*, 2917

78. Okamoto, Y.; *Macromol. Symp.*, **1996**, *101*, 345

79. Okamoto, Y.; Nakano, T. *Chem Rev.*, **1994**, *94*, 349

80. Okamoto, Y.; Ishikura, M.; Hatada, K.; Yuki, H. *Polym. J.*, **1983**, *15*, 851

81. Nakano, T.; Okamoto, Y.; Hatada, K.; *J. Am. Chem. Soc.*, **1992**, *114*, 1318

82. Ortiz, L.; Khan, I. *Macromolecules*, **2000,** *41(1),* 895

83. Khan, I.M.; Hogen-Esch, T.E.; *Macromolecules*, **1987**, *20*, 2335-2340

84. The composites were prepared using **PEO** (**MW** 600, 000) and **PEG** (**MW** 3000) to obtain a thermoplastic elastomeric material. Elastomeric materials are easy to fabricate for CD measurements. Temperature dependent racemization studies were not carried out but since segmental motion seems

to be necessary for helix-to-helix interconversion, the racemization in the solid state should be possible above the glass transition temperature of the polymer matrix. Rate of racemization should increase with increasing temperature. Detailed DSC studies have not been undertaken, PEG is a good plasticizing agent, and additionally because PEO has a melting temperature around 60°C, the crystalline content of the composite at this temperature is small.

85. Alchemy 32 Version 2.05, Tripos, Inc. St. Louis, MO, USA

86. Goodman, M.; Chen, S., *Macromolecules*, **1970**, *3*, 395. (b). Goodman, M.; Chen, S., *Macromolecules*, **1971**, 4, 625.

87. Green, M., Peterson, N.; Sato, T.; Teramoto, A.; Cook, R.; Lifson, S.; *Science*, **1995**.

88. Shmueli, V.; Traub, W.; Rosenbeck; *J. Polym. Sci.*, **1969**, *7*, 515.

89. Gu, H.; Nakamura, Y.; Sato, T.; Teramoto, A.; Green, M.; Andreola, C.; Peterson, N.; Lifson, S.; *Macromolecules*, **1995**, *28*, 1016.

90. Cook, R.; Johnson, R.; Wade, C.; O'Leary, D.; Munoz, B.; Green, M.; *Macromolecules*, **1990**, *23*, 3454.

91. Green, M.; Khatri, C.; Peterson, N. *J. Am. Chem. Soc.*, **1993**, *115*, 4941.

92. Khatri, C.; Pavlova, Y.; Green, M.; Morawitz, H. *J. Am. Chem. Soc.*, **1997**, *119*, 6991.

93. (a). Mueller, M.; Zentel, R. *Macromolecules*, **1996**, *29*, 1609. (b). Mayer, S.; Maxein, G.; Zentel, R. *Macromolecules*, **1998**, *31*, 8522.

94. Mayer, S.; Zentel, R. *Macromol. Rapid. Commun.*, **2000**, *21*, 927.

95. Okamoto, Y.; Matsuda, M.; Nakano, T.; Yashima, E. *Polym. J.*, **1993**, *25*, 391.

96. Shibayama, K.; Siedel, S.; Novak, B. *Macromolecules*, **1997**, *30*, 3159.

97. Ciardelli, F.; Lanzillo, S.; Pieroni, O. *Makromol. Chem.*, **1967**, *103*, 1.

98. Moore, J.; Gorman, C.; Grubbs, R. *J. Am. Chem. Soc.*, **1991**, *113*, 1704.

99. Harlev, E.; Wudl, F., *Synthesis and Optical Properties of Chiral Poly[2, 5-bis(3, 7-dimethyl-6octenoxy)-1, 4-phenylene vinylene].* In conjugated polymers and related materials, The Connection of Chemical and Electronic Structure, Proceedings of the 81st Nobel Symposium; Salaneck, W.R. Lundstrom, IO.; Ranby, B., Eds.; Oxford University Press. Oxford, UK, **1993**; p139.

100. Magnus, P.; Danikiewicz, W.; Katoh, T.; Huffman, J.; Folting, K. *J. Am. Chem. Soc.*, **1990**, 112, 2465.

101. Majidi, M.; Kane-Maguire, L.; Wallace, G. *Polymer*, **1994**, *35*, 3113.

102. Majidi, R.; Kane-Maguire, L.; Wallace, G. *Polymer*, **1995**, *36*, 3597.

103. Majidi, M.; Kane-Maguire, L.; Norris, I.; Wallace, G.; Ashraf, S. *Synth. Meth.*, **1997**, *84*, 115.

104. Havinga, E.; Bouman, M.; Meijer, E.; Pomp, A.; Simenon, M. *Synth. Meth.*, **1994**, *66*, 93.

105. Teramae, H.; Takeda, K. *J. Am. Chem. Soc.*, **1989**, *111*, 1281.

106. (a). Terunuma, D.; Nagumo, K.; Kamata, N.; Matsuoka, K.; Kuzuhara, H. *Chem. Lett.*, **1998**, 681-682.

107. Nakashima, H.; Fujiki, M.; Koe, J.; *Macromolecules*, **1999**, *32*, 7707-7709.

108. Fujiki, M. *J. Am. Chem. Soc.*, **1996**, *118*, 11345-11346.

109. Fujiki, M. *Appl. Phys. Lett.*, **1994**, *65*, 3251-3253.

110. Ebihara, K.; Koshishara, S.; Yoshimoto, M.; Maeda, T.; Ohnishi, T.; Koinuma, H.; Fujiki, M. *Jpn. J. Appl. Phys.* **1997**, *36*, L1211-L1213.

111. Furukawa, K.; Ebata, K.; Fujiki, M. *Adv. Mat.*, **2000**, *12*, 1033.

112. Fujiki, M.; Toyoda, S.; Yuan, C.; Takigawa, H. *Chirality*, **1998**, *10*, 667.

113. Fujiki, M. *J. Am. Chem. Soc.*, **1994**, *116*, 6017-6018.

114. Fujiki, M. *J. Am. Chem. Soc.*, **1994**, *116*, 11976-11981.

115. Koe, J.; Fujiki, M.; Motonaga, M.; Nakashima, H. *Chem. Commun.*, **2000**, 389-390.

116. Koe, J.; Fujiki, M.; Motonaga, M.; Nakasima, H., *Macromolecules*, **2001**, 34, 1082.

117. Okamoto, Y.; Nakano, T. *Chem. Rev.*, **1994**, *94*, 349.

118. Okamoto, Y.; Yashima, E. *Prog. Polym. Sci.*, **1990**, 263.

119. Engelkamp, H.; van Nostrum, C.; Picken, S.; Nolte, R.; *M. Chem. Commun.*, **1998**, 979.

120. Sikorski, P.; Cooper, S.; Atkins, E.; Jaycox, G.; Vogl, O. *J. Polym. Sci., Part A: Polym. Chem.*, **1998**, *36*, 1855.

121. Hatada, K.; Jaycox, G.; Vogl, O.; In Macromolecular Design of Polymeric Materials Series: Plastics Engineering; Hatada, K.; Jaycox, G.; Vogl, O.; Eds.; Marcel Dekker: New York, **1997**; Chapter 11, p 181.

122. Millich, F., *Chem. Rev.*, **1972**, 72, 101.

123. Nolte, R.; Drenth, W.; In New Methods for Polymer Synthesis; Migs, W.J., Ed.; Plenum Press: New York, **1992**; Chapter 9, p 273.

124. Nolte, R.; van Beijen, A.; Prenth, W. *J Am. Chem. Soc.*, **1972**, *96*, 5932.

125. Gellman, S. *Acc. Chem. Res.*, **1998**, *31*, 173-180.

126. Inai, Y.; Hasegawa, K.; Hirabayashi, T.; Yokoka, I. *Polym. J.*, **1996**, *28*, 5, 440.

127. Dado, G.; Gellman, S. *J. Am. Chem. Soc.*, **1994**, *116*, 1054.

128. Appella, D.; Cristianson, L.; Karle, I.; Powell, D.; Gellman, S. *J. Am. Chem. Soc.*, **1996**, *118*, 13071.

129. Hintermann, T.; Seebach, D. *Chimia*, **1997**, *51*, 224-248.

130. Apella, D.; Borchi, J.; Durell, S.; Gellman, S. *J. Am. Chem. Soc.*, **1999**, *121*, 2309-2310.

131. Seebach, D.; Abele, S.; Gademann, K.; Jaun, B. *Agnew, Chem. Int. Ed.*, **1999**, *38*, 1595-1597.

132. Seebach, D.; Sifferlen, T.; Mathieu, P.; Hane, A.; Krell, C.; Bierbaum, D.; Abele, S. *Helv. Chim. Acta.*, **2000**, *83*, 2849.

133. Hagihora, M.; Anthony, N.; Stout, T.; Cardy, J.; Schneiber, S. *J. Am. Chem. Soc., 1992*, **114**, 6568.

134. Gude, M.; Piarulli, V.; Potenza, D.; Salom, B.; Gennari, C. *Tetra. Lett.*, **1996**, *37*, 8589.

135. Gennari, C.; Salom, B.; Potenza, D.; Williams, A. *Agnew. Chem.*, **1994**, *33*, 446.

136. Gennari, C.; Salom, B.; Potenza, D.; Longari, c.; Fioranvanzo, E.; Carugo, O.; Sardone, N. *Chem-Eur. J.*, **1996**, *2*, 664.

137. Nielsen, P., *Acc. Chem. Res.*, **1999**, *32*, 624-630.

138. Lagriffoule, P.; Wittung, P.; Erikson, M.; Jensen, K.; Norden, G.; Buchart, O.; Nielsen, P. *Chem.-Eur. J.*, **1997**, *3*, 912-919.

139. Hamuro, Y.; Geib, S.; Hamilton, A. *Agnew. Chem.*, **1994**, *33*, 446.

140. Hamuro, Y.; Geib, S.; Hamilton, A. *J. Am. Chem. Soc.*, **1996**, *118*, 7529.

141. Lokey, R.; Iverson, B. *Nature*, **1995**, *375*, 303.

142. Nelson, J.; Saven, J.; Moore, J.; Wolynes, P. *Science*, **1997**, *227*, 1793.

143. Ohshiro, N.; Shimizu, A.; Okumura, R.; Takei, F.; Onitsuka, K.; Takahashi, S.; *Chem. Lett.*, **2000**, 786.

144. Takei, F.; Yanai, K.; Onitsuka, K.; Takahashi, S., *Chem.-Eur. J.*, **2000**, *6*, 983.

145. Maeda, K.; Yamamoto, N.; Okamoto, Y.; *Macromolecules*, **1998**, 31, 5924-5926.

146. Schlitzer, D.; Novak, B.M.; *J. Am. Chem. Soc.*, **1998**, 120, 2196-2197.

147. Yashima, E.; Matsushima, T.; Okamoto, Y. *J. Am. Chem. Soc.* **1995**, 117, 11596-11957.

148. Yahima, E.; Nimura, T.; Matsushima, T.; Okamoto, Y. *J. Am. Chem. Soc.* **1996**, 118, 9800-9801.

149. The usage of the term "chaperoning" is appropriate in these systems. Starting with a racemic mixture of helical polymer, an optically active chaperone will be utilized to carry out a helicity selection process.

150. Yashima, E.; Maeda, Y.; Matsushima, T.; Okamoto, Y. *Chirality*, **1997**, *9*, 593.

151. Sommerdijk, N.; Buynsters, P.; Pistorius, A.; Wang, M.; Feiters, M.; Nolte, R.; Zwanenburg, B. *J. Chem. Soc.; Chem. Commun.*, **1994**, 1941.

152. Sommerdijk, N.; Buynsters, P.; Pistorius, A.; Wang, M.; Feiters, M.; Nolte, R.; Zwanenburg, B. *J. Chem. Soc., Chem. Commun.*, **1994**, 2739.

153. Sommerdijk, N.A.; Lambermon, M.; Nolte, R.; Zwanenburg, B. *Chem. Commun.*, **1997**, 455.

154. Sommerdijk, N.; Zwanenburg, B. *Chem. Commun.*, **1997**, 1423.

155. Sommerdijk, N.; Buynsters, P.; Akdemir, H.; Geurts, D.; Pistorius, A.; Feiters, M.; Nolte, R.; Zwanenburg, B. *Chem.-Eur. J.*, **1998**, *4*, 127.

156. Buynsters, P.; Feiters, R.; Ten Holte, P.; Nolte, R.; Pistorius, A.; Sommerdijk, N.; Ver Haegen, A.; Zwanenburg, B. *J. Materi. Chem.*, **2001**, *11*, 269-277.

157. Mikami, M.; Shinkai, S. *Chem. Lett.*, **1995**, 603.

158. Ortiz, L.; Pratt, L.; Smitherman, K.; Sannigrahi, B.; Khan, I.; *ACS Symposium Series,* **2002,** *812,* 55.

159. Sannigrahi, B.; Khan, I.; (*Currently being submitted to Biomacromolecules, 2003*)

160. Hubbell, J.A; Langer, R.; *Chem. Eng. News*, **1995**, *42*.

161. Hubbell, J.A.; *Bio/Technology*, **1995**, *13*, 565

162. (a). Van Wachem, P.B., Vreriks, C.M, Beugeling, T., Feijen, J., Detmers, J.P., van Aken, W.G. *Journal of Biomedical Materials Research*, **1987**, *21*, 701. (b). Grinnell, P.; *Int. Rev. Cytol.*, **1978**, *53*, 65,

163. Weiss, L.; *Int. Rev. Cytol.*, **1960**, *9*, 187.

164. Baier, R.E.; *Science,* **1968**, *162*, 1360.

165. Ruoslahti, E.; Pierschbacher, M.; Haymain, E.G.; Engvall, E.; *TIBS*, *188* ,**1982**.

166. Grinnell, F.; Phan, T.P.; *J. Cell. Phys.*, **1968,** *116*, 289.

167. Grinnell, F.; Feld, M.K.; *J. Biol. Chem.*, **1982**, *257*, 9, 4888.

168. Grinnell, F.; *Biocompatible Polymers, Metals, and Composites*, M. Seyder, Ed. Technomic Publ. Co., (**1983**), pp. 673 – 699

169. Curtis, A.S.G.; Forrester, J.V.; *J. Cell Biology*, **1983**, *97*, 1500.

170. Knox, P.; *J. Cell. Sci.*, **1984**, *71*, 51.

171. Bently, K.L.; Klebe, J.; *J. Biomed. Mat. Res.*, **1985**, *19*, 757.

172. Burrill, P.H.; Bernandini, I.; Kleinman, H.K.; Kretchmer, N.; *J. Supramol. Struct. Cell. Biochem.*, **1981**, ***16***, 385.

173. Horbett, T.A.; Schway, M.B.; Ratner, B.D.; *J. Colloid Interface Sci.*, **1985**, *104*, 28.

174. Curtis, A.S.G., Forrester, J.V.;*J. Cell Sci.*, **1984**, *71*, 17.

175. Feijen, J.; Beugeling, T.; Bantjes, A.; C. Th. Smit Sibenga, *Adv. Phys.*, **1979**, *3*, 100.

176. Grinnell, F.; Feld, M.K.; *Cell*, **1979**, *17*, 117.

177. Jaffe, E.A.; Mosher, D.F.; *J. Exp. Med.*, **1978**, *147*, 1779.

178. Tinois, E. Gaucherand, M.; Dumas, H.; Tardy, M.; Thivolet, J.; *Exp. Cell Res.*, **1991**, *193*, 310.

179. Sugawara, T.; Matsuda, T.; *J. Biomed. Mater. Res.*, **1995**, *29*, 1047.

180. Kesler, K.A.; Herring, M.B.; Arnold, M.P.; Glover, J.M.; Park, H.M.; Helmus, M.N.; Bendick, P.J.; *J. Vasc. Surg.*, **1986**, *3*, 58.

181. Koyayashi, H.; Ikada, Y.; *Curr. Eye. Res.*, **1991**, *10*, 899.

182. Brandley, B.K.; Schnaar, R.I.; *Anal. Chem.*, **1988**, *172*, 270.

183. Brandley, B.K.; Schnaar, R.L.; *Develop. Biol.*, **1989**, *135*, 74.

184. Feast, W.J.; Munro, S.K.; *Polymer Surfaces and Interfaces; Eds. John Wiley and Sons; New York* , **1987**.

185. Feast, W.J.; Munro, S.; Richards, R.W.; *Polymer Surfaces and Interfaces II; Eds. John Wiley and Sons* ,**1995**.

186. (a). Neff, J., Caldwell, K., Tresco, P., *J Biomedical Material Resources*, **1998,** *40*, 511. (b). Lee, J.H.; Kopeckoua, P.; Kopeckoua, J.; Andrade, J.D.; *Biomaterials*, **1990**, *11*, 455.

187. Gombotz, W.R.; Guanghui, W.; Horbett, T.A; Hoffmann, A.S.; *J. Biomed. Polym. Res.*, **1991**, *25*, 1547.

188. (a). Steele, J., Dalton, B., Johnson, G., Underwood, P., *J. of Biomedical Materials Research*, **1993**, *27*, 927. (b). Amstein, C.F.; Hartmann, P.A.; *J. Clin. Microbiolo.*, **1975**, *2*, 46.

189. Bently, K.L.; Klebe, R.J.; *J. Biomed. Mater. Res.*, **1985**, *19*, 757.

190. Chinn, J.A.; Horbett, T.A.; Ratner, B.D.; Schway, M.B.; Haque, Y.; Hauschika, S.L.; *J. Colloid Interface, Sci.*, **1989**, *127*, 67.

191. Curtis, A.S.G.; Forrester, J.V.; McInnes, C.; Laurie, F.; *J. Cell Sci.*, **1983**, *97*, 1500.

192. Ertel, S.I.; Ratner, B.D.; Horbett, T.A.; *J. Biomed. Mater. Res.*, **1990**, *24*, 1637.

193. Grinnell, F.; Feld, M.K.; *J. Biomed. Mater. Res.*, **1981**, *15*, 363.

194. Grinnell, F.; Feld, M.K.; *J. Biol. Chem.*, **1982**, *254*, 4888.

195. Grinnell, F.; Phan, T.V., *J. Cell Physiol.*, **1983**, *116*, 289.

196. Matsuda, T.; Litt, M.; *J. Polymer. Sci., Polym. Chem. Ed.*, **1974**, *12*, 489.

197. Ramsey, W.S.; Hertl, W.; Nowlan, E.D.; Binkowski, N.J.; *In Vitro*, **1984**, *20*, 802.

198. Underwood, P.A., Bennett, F.A.; *J. Cell Sci.*, **1989**, *93*, 641.

199. Lydon, M.J.; Minett, T.W.; Tighe, B.J.; *Biomaterials*, **1985**, *6*, 396.

200. Curtis, A.S.G.; Forrester, J.V.; Clark, P.; *J. Cell. Sci.*, **1986**, *86*, 9.

201. Ertel, S.I., Chikoti, A.; Horbett, T.A.; Ratner, B.D.; *J. Biomat. Sci., Polymer Ed.*, **1991**, *3*, 163.

202. (a). Watanabe, J., Eriguchi, T., Ishihara, K., *Biomacromolecules*, **2002**, *3*, 1109. (b). Sawada, S.; Shindo, Y.; Watanabe, A.; Iwasaki, Y.; Kato, S.; Akashi, M.; Ishihara, K.; Nakabayashi, N.; *Trans. Soc. Biomater.*, **1999**, *25*, 231.

203. Imanishi, Y.; Higashimura, T.; Okamura, S. *Makromol. Chem.*, **1964**, *70*, 68

204. Natta, G.; Danusson, F.; Sianesi, D. *Makromol. Chem.*, **1958**, *28*, 253.

205. Yuki, H.; Okamoto, Y.; Kuwae, K.; Hatada, N. *J. Polym. Sci.: Part A*, **1969**, *7*, 1946.

206. Bovey, F.; Tiers, G. *J. Polym. Sci.*, **1960**, *44*, 73.

207. Okamoto, Y; Suzuki, K.; Hatada, K.; Yuki, H. *J. Am. Chem. Soc.* 101, 4783, **1979**

208. J. Lie, G.B. Schuster, K. Cheon, M. Green, J. Selinger, *J. Am. Chem. Soc*, **2000**, *122*, 2603.

209. Green, M., Peterson, N., Sato, T., Teramoto, A., Cook, R.; Lifson, S.; *Science*, **1995**, *268*, 1860.

210. Lifson, S., *J. Am. Chem. Soc.*, **1989**, *111*, 8850.

211. Okamoto, N., Mukaida, F., Gu, H., Sato, Takahiro, Teramoto, A., Peterson, N., Andreola, C., Green, M., Lifson, S., *Macromolecules*, **1996**, *29*, 2878-2884.

212. (a) Hong, G., Nakamura, Y., Sato, T.; Teramoto, A.; Green, M.; Andreola, C.; Peterson, N., *Macromolecules*, **1995**, *28*, 1016-1024 (b) Ute, K., Fujunishi, Y., Jha, S., Cheon, K., Munoz, B., Hatada, K., Green, M., *Macromolecules*, **1999**, *32*, 1304-1307

213. Gordon, K.; Negi, S.; Khan, I.; *Polymer Preprint* (Am. Chem. Soc., Div. Polym. Chem.), 2001, 42(2)

214. Han, D.K.; Hubbell, J.; *Macromolecules*, **30**, 6077, (1997).