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Synthesis and Characterization of Linear and Star-Branched Poly(gamma-Stearyl-L-Glutamate).

Drew Scott Poche
Louisiana State University and Agricultural & Mechanical College

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Synthesis and characterization of linear and star-branched poly(γ-stearyl-L-glutamate)

Poché, Drew Scott, Ph.D.
The Louisiana State University and Agricultural and Mechanical Col., 1990
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Star Branched Poly(γ-stearyl-L-glutamate)

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in
The Department of Chemistry

by
Drew Scott Poche'
B.S., Southeastern Louisiana University, 1984
August 1990
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I would first like to thank my major Professor, Dr. William H. Daly. His dedication and enthusiasm for research, as well as his sharing of an unending supply of knowledge will long be remembered. Also, Professor Paul S. Russo, my minor advisor, deserves special thanks. He initiated the study which became my dissertation topic and was invaluable in the direction of the physical chemistry areas of this research. Thanks also goes to Dr. Frank Fronczek for solving the crystal structure described in Chapter 1. I would also like to thank Dr. Mark McLaughlin, Dr. Dewey Carpenter and Dr. Ezzat Younathan for serving as my committee members. I gratefully acknowledge the financial support of the Louisiana State University Department of Chemistry, a Research Corp. Grant, and the Coates Memorial Fund.

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I would also like to thank my family. My parents have always encouraged me and believed in my abilities and I am grateful for their help. I am also grateful to my wife’s parents who helped us in many ways throughout my time graduate school. I am dedicating this dissertation to Leah, my wife, who is my best friend and has given me unfailing support throughout this work.
It is appropriate to acknowledge specifically some individuals who helped with the work in Chapter 4 in light of the unusual way that the work was initiated. Dr. Paul S. Russo was first interested in linear PSLG and initiated the work on this novel polymer by assigning its synthesis and characterization as a project in a graduate polymer course. I was responsible for the synthesis of PSLG and, along with the students of the course, characterization of the polymer. As a result, dn/dc measurements of PSLG were accomplished by Ms. Mazidah Mustafah and Ms. Shoulan Yang. Ms. Mustafah also characterized with light scattering a low molecular weight PSLG. Marietta Aniano-Ilao did some early work on liquid crystallinity; the picture in Figure 4.7 resulted from one of her solutions. Dr. Soo Lee re-measured two Zimm plots. The Zimm plot of Figure 4.17B was obtained from solutions prepared by me and data collected by Dr. Lee.
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<td>aa</td>
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Abstract

The synthesis and characterization of linear and star branched poly(γ-stearyl-L-glutamate) (PSLG) is reported. A new synthesis for N-carboxyanhydride (NCA) monomers was developed by employing bis(trichloromethyl)carbonate (triphosgene) to functionalize the corresponding α-amino acid. Multi-functional primary amino initiators (central units) were synthesized to enable the production of three, four, six and nine arm star polymers by reaction of the central units with the desired NCA. Linear PSLG was produced by either sodium methoxide initiation or benzyl amine initiation of γ-stearyl-L-glutamate-N-carboxyanhydride (SLGNCA). The highest molecular weight polymers were produced from methoxide initiation. Other molecular weights can be produced from the same ratio of methoxide to monomer by varying the initial concentration of the monomer in the reaction.

The polymers were characterized by $^1$H and $^{13}$C nuclear magnetic resonance (NMR), infrared spectroscopy (IR), differential scanning calorimetry (DSC), gel permeation chromatography (GPC), dynamic and static laser light scattering (DLS and SLS), and to a lesser extent by light microscopy. Also, molecular models of both the linear and the star polymers were produced using SYBYL. DSC analysis reveals two endothermic transitions; the lower temperature transition corresponding to the melting of the side chains and the second transition corresponding to a liquid crystalline phase transition of the melted polymer. SLS yielded weight average molecular weight, radius of gyration, and 2nd virial coefficient. From these data and corresponding DLS measurements, other PSLG dimensions were calculated such as the diameter, the
hydrodynamic radius, and the length. A Mark-Houwink plot was constructed from the linear PSLG data. The lower functionality PSLG star polymers exhibit negative values for the 2nd virial coefficient when measured in tetrahydrofuran (THF) which is indicative of aggregation. DLS supported this interpretation of the virial coefficient. DSC thermograms also indicate a phase transition at about 68° C for the high molecular weight stars. GPC analysis of linear PSLG yielded a GPC calibration curve as well an indication of their polydispersity.
Chapter 1: Synthesis of $\gamma$-Stearyl-L-glutamate-N-carboxy anhydride: Application of Bis(trichloromethyl)carbonate
1.1 Introduction

Amino acids are the building blocks of proteins. Nature can assemble proteins with an enormous variety of amino acid sequences, molecular weights, and conformations. Each assembly gives rise to a protein with a specific role in carrying out some biochemical function. Since G.J. Mulder [1] first pointed out the significance of proteins in living matter in 1838, scientists have attempted to better understand proteins by isolating them from a natural source, degrading them to determine structure and amino acid sequence, and synthesizing them by any of a number of amino acid coupling reactions [2]. Thousands of publications relating directly to peptide synthesis have emerged over the last 100 years. Over 1000 publications in this area are cited in reference [2]. The Merrifield synthesis [3] of peptide chains is an elegant example of scientists' attempts to reproduce polymers that nature so routinely assembles. It is often the case that we cannot reproduce a naturally occurring protein by usual synthetic methods or the synthetic route is impractical. Thus, models of the protein of interest that are more practical or simpler to synthesize are produced. To this end, N-carboxyanhydrides (NCA) of α-amino acids, first reported by Leuchs [4], have developed into important monomers for the synthesis of proteins. Leuchs, a student of Emil Fischer, discovered a synthetic route to NCA derivatives and promptly found that they form peptides in the presence of nucleophiles such as water. Over the years, N-carboxyanhydrides have been used extensively for the synthesis of homopolypeptides (where they find the most extensive use), to incorporate peptides into other polymers such as polystyrene, to make polypeptides with blocks of repeating identical residues in the polymer chain, to add a
specific amino acid to the end of a protein, or to produce short peptide chains such as dimers or trimers [5-14]. Some interesting applications of NCA monomers include the synthesis of aspartame [15], of ribonuclease S-protein [16] where almost half of the 104 residues were attached with NCA monomers, and of high molecular weight homopolypeptides such as poly(γ-benzyl-L-glutamate) (PBLG) [17].

Figure 1.1 shows the structure of a generic α-amino acid and its NCA derivative with the side chain of the amino acid represented by an R- group. The anhydride ring serves to both protect and activate the amine and carboxylic acid function. Peptide bonds are formed when the nitrogen becomes "deblocked" by release of carbon dioxide. Depending on the R- group in the NCA derivative, the molecule will have varying degrees of solubility in organic solvents and impart changes in the physical properties of the resulting polymer. Most of the NCAs synthesized in this work are quite soluble in chloroform but L-alanine-NCA is not. While hexane is a non-solvent for practically all of the NCAs commonly reported in the literature, γ-stearyl-L-glutamate is somewhat soluble in hexane. Usually, NCA derivatives of α-amino acids are white crystalline solids but there are a few instances where the compound has been isolated as an oil [18]. When attacked by a nucleophile or strong base, the NCA can polymerize by loss of carbon dioxide to give a high molecular weight homopolypeptide. This chemistry will be covered in more detail in Chapter 2. Though unstable to excessive heat or moisture, the NCA monomer offers the most convenient route to the synthesis of high molecular weight homopolypeptides. The R- group or amino acid side chain can be any moiety that will not react with the reagent that ring closes the
amino acid or that will not react with the resulting NCA. These groups can, however, be present if protected or blocked by a suitable reagent that can then be removed after the polymer is synthesized [19]. The NCA monomers can be derived from D, L, or racemic amino acids, N-substituted amino acids, and \( \gamma \) - and \( \beta \)-amino acid-NCAs have been synthesized [20-22].

This chapter focuses on the synthesis of NCAs derived from L-amino acids or a racemic mixture. Of special interest is the synthesis of \( \gamma \)-stearyl-L-glutamate-NCA (SLGNCA) and the synthesis of the \( \alpha \)-amino acid \( \gamma \)-stearyl-L-glutamate (SLG) from L-glutamic acid. Several older techniques for glutamic acid modification were evaluated to test their applicability to the synthesis of this novel amino acid. The application of bis(trichloromethyl)carbonate (triphosgene) to \( \alpha \)-amino acids has provided a new, safer, and more convenient synthetic route to NCA monomers [23].

1.2 Approaches to the Synthesis of \( \gamma \)-Stearyl-L-glutamate

L-glutamic acid is an \( \alpha \)-amino acid with a carboxylic acid group in its side chain attached to the \( \gamma \)-carbon of the amino acid. This functional group is the target for selective modification by reacting it with stearyl alcohol to produce a long hydrocarbon side chain attached to the amino acid through an ester function. Several approaches were contemplated (Scheme 1.1). A straightforward approach to accomplish this task would be to react the stearyl alcohol directly with L-glutamic acid in the presence of an acid catalyst such as hydrochloric acid. This is the approach [24] typically used to synthesize \( \gamma \)-benzyl-L-glutamate (Scheme 1.1). That is, benzyl alcohol is used as the solvent and the reactant to produce the desired \( \gamma \)-ester. The problem with the reaction is that the \( \alpha \)-carboxylic acid function is also easily esterified in these conditions, giving a low yield
of the desired γ-ester. However, despite a low yield, the product is easily purified by dissolving the impure product in water and carefully adjusting the pH of the solution to 7-8 to form the amino acid zwitterion, causing the γ-benzyl-L-glutamate to crystallize. Since this is a quick, one step reaction, the low yield becomes less of a concern. Using this approach to produce γ-stearyl-L-glutamate, however, was not feasible. Stearyl alcohol is a solid that first has to be melted to use as the solvent, and it is troublesome to remove the large excess remaining at the end of the reaction. Also, being so non-polar, it is not a good solvent for L-glutamic acid, especially in the presence of HCl, which will form the amine hydrochloride salt. For these reasons, γ-stearyl-L-glutamate was never isolated from this reaction despite several attempts. The reaction was also attempted using chloroform as the solvent. It also failed, again due primarily to the insolubility of the amino acid in the solvent.

Another approach [25] that is useful for producing some γ-ester glutamic acid derivatives involves complexing the L-glutamic acid amino and α-carboxylic acid groups to copper(II) salts. As Scheme 1.1 shows, this ties up the α-carboxylic acid position and leaves the γ-position free to react as a nucleophile with alkyl halides. This is another approach useful for preparing γ-benzyl-L-glutamate but fails when long chain alkyl halides are applied. Because the reaction is run in aqueous media the long chain alkyl halides simply do not go into solution and react. This approach is generally useful for reactions in the side chains of α-amino acids that contain nucleophilic groups when it is desirable to leave the amino and α-carboxylic acid position untouched by the transformation. For example O-benzyl-tyrosine was prepared by this method [26]. By complexing L-tyrosine with copper sulfate in 2M NaOH, the hydroxyl group of the amino acid reacted with
benzylbromide to produce the desired ether linkage. This modified L-tyrosine was then used for the evaluation of triphosgene in the synthesis of its NCA derivative as discussed in Section 1.3 of this chapter.

The next approach for the synthesis of 7-stearyl-L-glutamate was to try to develop a reaction system where the reactants were both more organic soluble and reactive. One can make an amino acid more generally soluble in organic solvents by blocking the amine function with a suitable reagent so that zwitterion formation is no longer possible. As Scheme 1.2 shows, L-glutamic acid was reacted [27] with phthalic anhydride in dimethylformamide (DMF), forming N-phthaloyl-L-glutamic acid, 1. This compound can be made more reactive toward nucleophiles by forming the carboxylic acid anhydride by heating 1 with acetic anhydride. The resulting N-phthaloyl-L-glutamic anhydride, 2, has been shown by Sheehan and Bolhofer [28], to react with alcohols to form exclusively the γ-ester. For example, Dhar and Agarwal [29] has reacted 2 with cholesterol to form exclusively the γ-cholesteryl-L-glutamate without contamination from the α-ester. Alkoxides, however, being more reactive, are less selective and will give a mixture of γ- and α-esters [28] when reacted with 2. We have also found that amines will react with the γ-position predominately also. Figure 1.2 shows the carbonyl region of the 25 MHz 13C NMR spectra of 1, N-phthaloyl-γ-anilide-L-glutamate, and N-phthaloyl-γ-stearyl-L-glutamate. The γ-carbonyl of 1 has shifted from 173.4 ppm to 170.1 ppm in the γ-amide and in the γ-ester it has shifted from 173.4 to 172.2 ppm. The α-carbonyl is unshifted in both products. By recrystallizing 2 from acetone (slow evaporation), we were able to form crystals suitable for determining an x-ray structure. The crystals were large, clear, rectangular plates.
The crystal structure obtained for this compound, which to date is not reported in the literature, confirms that the γ-carboxylic acid group is in a sterically more favorable environment for nucleophilic attack. As Figure 1.3 shows, the phthalimide ring is nearly perpendicular to the anhydride ring, thus making attack at the α-carboxylic acid carbonyl by nucleophiles more difficult. As Table 1.3 shows, the C6N C2 C1 torsion angle is 96.3°, indicating that the two rings are only slightly skewed from the perpendicular. Also, the C2 N C9 O5 torsion angle indicates that C2 and O5 are only -6.5° from being eclipsed. Generally, branching or steric bulk at the α-position to an electrophilic site makes a nucleophilic attack more difficult. Tables 1.1, 1.2, 1.3, 1.4, and 1.5 give the bond distances, bond angles, torsion angles, O, N, C coordinates, and the H coordinates respectively for 2. Reaction of 2 with 1 equivalent of stearyl alcohol and one equivalent of triethylamine gave 4 in high yield. The triethyl amine serves to catalyze the reaction by first reacting as a nucleophile, making the reactive intermediate 3. The alcohol then attacks, with triethylamine as a good leaving group. The triethyl amine is not strictly necessary but without it, refluxing the reaction for more than 24 hours is necessary. When dodecyl amine was reacted with 2, triethylamine was also used in the reaction. However, its purpose here is to react as a base to neutralize the α-carboxylic acid released when the anhydride ring opens; thus the primary amine serves as a nucleophile only. An excess of the primary of amine could be used in reactions with a carboxylic acid anhydride (which was the procedure when aniline was reacted with 2) but because long hydrocarbon chain primary amines were applied they would be difficult to remove from the final product. Triethylamine is easily removed by washing the product.
Table 1.1. Bond Distances in Angstroms for N-phthaloyl-L-glutamic acid, 2.

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Table 1.2. Bond Angles in Degrees for N-phthaloyl-L-glutamic acid, 2.

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<td>C13</td>
<td>C12</td>
<td>-179.81 (0.35)</td>
</tr>
<tr>
<td>C7</td>
<td>C10</td>
<td>C11</td>
<td>C12</td>
<td>0.48 (0.59)</td>
</tr>
<tr>
<td>C10</td>
<td>C11</td>
<td>C12</td>
<td>C13</td>
<td>-1.19 (0.68)</td>
</tr>
<tr>
<td>C11</td>
<td>C12</td>
<td>C13</td>
<td>C8</td>
<td>0.97 (0.64)</td>
</tr>
</tbody>
</table>
Table 1.4 Coordinates for N-phthaloyl-L-glutamic acid, 2.

<table>
<thead>
<tr>
<th>Atom</th>
<th>x</th>
<th>y</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>0.7251 (2)</td>
<td>0.1002 (3)</td>
<td>0.7351 (2)</td>
</tr>
<tr>
<td>02</td>
<td>0.5397 (2)</td>
<td>0.806 (3)</td>
<td>0.7594 (2)</td>
</tr>
<tr>
<td>03</td>
<td>0.3515 (3)</td>
<td>0.2437 (4)</td>
<td>0.7862 (3)</td>
</tr>
<tr>
<td>04</td>
<td>0.6413 (2)</td>
<td>0.0026 (3)</td>
<td>0.4242 (3)</td>
</tr>
<tr>
<td>05</td>
<td>0.8142 (3)</td>
<td>0.4102 (3)</td>
<td>0.5784 (3)</td>
</tr>
<tr>
<td>N</td>
<td>0.7041 (2)</td>
<td>0.2081 (3)</td>
<td>0.5172 (2)</td>
</tr>
<tr>
<td>C1</td>
<td>0.6304 (3)</td>
<td>0.1613 (3)</td>
<td>0.6936 (2)</td>
</tr>
<tr>
<td>C2</td>
<td>0.5947 (3)</td>
<td>0.2131 (4)</td>
<td>0.5719 (3)</td>
</tr>
<tr>
<td>C3</td>
<td>0.5191 (4)</td>
<td>0.3408 (5)</td>
<td>0.5602 (3)</td>
</tr>
<tr>
<td>C4</td>
<td>0.4011 (3)</td>
<td>0.3249 (3)</td>
<td>0.6117 (3)</td>
</tr>
<tr>
<td>C5</td>
<td>0.4238 (3)</td>
<td>0.2501 (4)</td>
<td>0.724 (3)</td>
</tr>
<tr>
<td>C6</td>
<td>0.719 (3)</td>
<td>0.0961 (3)</td>
<td>0.4486 (3)</td>
</tr>
<tr>
<td>C7</td>
<td>0.8438 (3)</td>
<td>0.1169 (3)</td>
<td>0.415 (2)</td>
</tr>
<tr>
<td>C8</td>
<td>0.8966 (3)</td>
<td>0.2394 (4)</td>
<td>0.4611 (2)</td>
</tr>
<tr>
<td>C9</td>
<td>0.8073 (3)</td>
<td>0.3021 (4)</td>
<td>0.5277 (3)</td>
</tr>
<tr>
<td>C10</td>
<td>0.9043 (3)</td>
<td>0.0352 (5)</td>
<td>0.347 (3)</td>
</tr>
<tr>
<td>C11</td>
<td>1.0219 (4)</td>
<td>0.0815 (5)</td>
<td>0.3274 (3)</td>
</tr>
<tr>
<td>C12</td>
<td>1.0741 (3)</td>
<td>0.2023 (6)</td>
<td>0.3748 (3)</td>
</tr>
<tr>
<td>C13</td>
<td>1.0139 (3)</td>
<td>0.2851 (5)</td>
<td>0.4417 (4)</td>
</tr>
</tbody>
</table>
Table 1.5 Coordinates for hydrogen atoms in N-phthaloyl-L-glutamic acid, 2.

<table>
<thead>
<tr>
<th>Atom</th>
<th>x</th>
<th>y</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2</td>
<td>0.5342</td>
<td>0.1511</td>
<td>0.5271</td>
</tr>
<tr>
<td>H31</td>
<td>0.4921</td>
<td>0.3630</td>
<td>0.4803</td>
</tr>
<tr>
<td>H32</td>
<td>0.5715</td>
<td>0.4134</td>
<td>0.5990</td>
</tr>
<tr>
<td>H41</td>
<td>0.3371</td>
<td>0.2757</td>
<td>0.5578</td>
</tr>
<tr>
<td>H42</td>
<td>0.3696</td>
<td>0.4148</td>
<td>0.6232</td>
</tr>
<tr>
<td>H10</td>
<td>0.8669</td>
<td>-0.0494</td>
<td>0.3149</td>
</tr>
<tr>
<td>H11</td>
<td>1.0662</td>
<td>0.0284</td>
<td>0.2804</td>
</tr>
<tr>
<td>H12</td>
<td>1.1555</td>
<td>0.2306</td>
<td>0.3610</td>
</tr>
<tr>
<td>H13</td>
<td>1.0513</td>
<td>0.3699</td>
<td>0.4731</td>
</tr>
</tbody>
</table>

with dilute aqueous HCl. The amine blocking group was then removed by reaction of 3 with hydrazine [30] to yield the desired γ-stearyl-L-glutamate.

Unfortunately, hydrazine will also attack at the ester function, "undoing" the previous synthetic step, and reducing the yield and purity of the desired product. However, the product obtained from this approach was suitable for the formation of the NCA monomer. Indeed, the synthesis of γ-stearyl-L-glutamate-NCA (SLGNCA) was accomplished from this compound, conclusively demonstrating that attack at the γ-carboxylic acid function of 2 by stearyl alcohol is the predominant reaction.

The best approach and the one used most extensively in this research for the production of γ-stearyl-L-glutamate, was a procedure patented by Wasserman *et al.* [31] for reacting L-glutamic acid with long hydrocarbon chain alcohols in t-
butanol in the presence of sulfuric acid to give γ-esterified glutamic acid derivatives. Scheme 1.1 outlines the synthesis. The key to success in this approach is three-fold: 1) sulfuric acid as a catalyst is desirable because in addition to catalyzing the esterification the sulfate dianion forms a salt with two glutamic acid molecules through their protonated amine function. As Scheme 1.1 shows, this is effective in blocking the α-carboxylic acid position, leaving the γ-position open for attack; 2) t-butanol is an effective solvent, that dissolves both the stearyl alcohol and the glutamic acid salt; 3) The product can be cleanly re-crystallized from 1:1 n-butanol:water. To drive the reaction, a large excess of stearyl alcohol is used. The resulting γ-stearyl-L-glutamate is a white solid, surprisingly crystalline for a compound with so much hydrocarbon character. Despite the long hydrocarbon side chain, this compound is still insoluble in organic solvents (like a typical α-amino acid) and is certainly water insoluble.

1.3 Approaches to the Synthesis of γ-stearyl-L-glutamate-N-carboxyanhydride (SLGNCA)

Since Leuchs first reported NCA derivatives of α-amino acids, numerous procedures [4, 32-35] have been suggested for the preparation of these important cyclic monomers. Any synthesis of NCA derivatives must provide a clean route to the desired product so that material suitable for high molecular weight polymers can be produced. Scheme 1.3 outlines the various procedures reported to synthesize NCA derivatives. These synthetic routes sometimes involve more than one low yield step and the intermediates are often compounds hard to isolate or purify themselves. Leuchs applied phosphorous pentachloride as the ring closing reagent to carbamate derivatives of α-amino acids. Others have
similarly applied phosphorous tribromide or thionyl chloride to produce ring
closing. Fuchs [36] was the first to apply phosgene gas to effect ring closing and
Farthing [37a,b] modified his method in the 1950s. More recent approaches [38,
39, 40] involve one-step syntheses applying phosgene or a derivative of phosgene
directly to the α-amino acid to give the NCA. These approaches give high yields
of the NCA product and do not racemize the resulting NCA. Particularly
noteworthy is the Fuller et al. [41] application of phosgene in a solution of
toluene or benzene as a more convenient method for metering the amount of
phosgene used in the reaction. This method replaces the older phosgene applica-
tion method where phosgene gas is indiscriminately bubbled through the reaction
mixture to give the product. Avoiding an excess of phosgene is important to
prevent side reactions [24] that can inhibit later polymerization of the NCA or
make its purification more difficult. In fact, a procedure has been patented [42]
that uses less than one equivalent of phosgene and leaves unreacted α-amino acid
in the reaction mixture which must subsequently be removed by filtration. Still,
with Fuller’s method, an excess of phosgene is required and it is still dangerous to
handle the phosgene solutions. Because of this, phosgene substitutes have been
suggested. For example, trichloromethyl chloroformate (diphosgene) has been
suggested [43] but carbon black must be used in the process to catalyze dis-
association of the amino acid or the cyclization is inefficient.

The common denominator in all NCA syntheses, regardless of the ring closing
reagent used, is the generation of HCl as a by-product. Removal of HCl is
important because it can lead to side reactions during the NCA synthesis [44] and
it can reduce the molecular weight of the polypeptide later synthesized from the
NCA monomer [24]. It can be sparged out during the reaction with nitrogen but complete removal is usually accomplished later by repeated washing and recrystallization the NCA. There are several patented methods [45-47] for NCA purification, one of which includes passing a solution of the NCA through active columns. The more the NCA or a solution of it is handled, especially in a humid environment, the more likely it is to decompose to the parent amino acid or polymerize. NCAs were purified in this study by repeated recrystallizations and filtrations of dichloromethane solutions of the NCA through celite in the presence of sodium carbonate to remove traces of unreacted α-amino acid and HCl respectively. Usually, the NCA was used soon after its preparation.

Fuller's method had been used in our labs extensively [48] until bis(trichloromethyl)carbonate (triphosgene) was evaluated as a phosgene substitute. Triphosgene was first reported [49] in the 1880's. Its x-ray crystal structure was determined in 1971 [50], and it was recently evaluated as a general phosgene substitute by Eckert and Forster [51]. Triphosgene has proven in our hands to be an effective phosgene substitute for the synthesis of α-amino acid NCA derivatives [23]. In 1957, Hales et al. [52] postulated an intramolecular decomposition mechanism for triphosgene where a chlorine atom attacks as a nucleophile at the carbonyl carbon, giving three molecules of phosgene. Triphosgene is a crystalline solid that is safer and considerably easier to handle than phosgene. It was prepared by Eckert and Forster's method of exhaustively chlorinating dimethylcarbonate in carbon tetrachloride solution although recently Aldrich Chemical Company has made the compound commercially available. Its best features in the synthesis of NCA derivatives are: 1) because triphosgene delivers 3
equivalents of phosgene \textit{in situ} when attacked by a nucleophile, only 1/3 of an equivalent of it is necessary to give a complete reaction. No excess of phosgene is present in the reaction mixture. 2) like phosgene, it does not racemize the NCA product if an optically pure \(\alpha\)-amino acid starting material is used. 3) reaction conditions, times and yields are comparable to phosgene reactions to produce the NCA. 4) Triphosgene is soluble in the solvents commonly used for NCA recrystallization (usually THF or chloroform and hexane mixtures) which makes any residual reagent remaining easy to remove from the product.

Scheme 1.4 outlines the synthesis. Several \(\alpha\)-amino acids were evaluated. They were either the L-isomer or racemic mixtures. Triphosgene works by releasing two molecules of phosgene when its is attacked at the carbonyl carbon (this carbonyl group accounts for the third "phosgene" molecule or equivalent). The reaction, like phosgene itself, produces an HCl by-product that can be partially sparged out of the reaction with nitrogen. Some of it reacts with the amine function on the amino acid, making the hydrochloride salt. The triphosgene is added to a warm suspension of the \(\alpha\)-amino acid in tetrahydrofuran (THF). For DL-2-amino-stearic acid and \(\gamma\)-stearyl-L-glutamate, dissolution of the amino acid is rapid. As the amino acid side chain is shortened (thus making the amino acid less non-polar), the reaction times increase because the THF is less able to dissolve the amino acid hydrochloride salt. Also, as the amine function becomes protonated from the HCl by-product, it becomes less nucleophilic and is more sluggish in its reaction with triphosgene (or phosgene). Small increments of triphosgene added to the more sluggish reactions (such as the synthesis of L-alanine-NCA, Table 1.6, entry 7) did not appear to help increase yield or
decrease reaction time. Also, reaction times of over 5 hours lead to a discoloration that is difficult to remove from the product. Any small amount of unreacted amino acid starting material remaining was easily removed by filtering the warm reaction mixture. Purification of the product generally was effected by recrystallization from a 1:2 THF:hexane mixture. γ-Stearyl-L-glutamate, however, was recrystallized from hot hexane. Table 1.6 gives the amount of triphosgene used (in all cases, just over 1/3 of an equivalent was used), the percent yield, the melting point and the optical rotations of the resulting NCA. The melting points match literature melting points and are indicative of the purity of the product; optical rotations show that the NCA derivatives were not racemized by the reaction. Table 1.7 gives 100 MHz 'H NMR data. Figure 1.4 gives 100MHz 'H NMR spectrum and 25 MHz 13C spectrum of γ-stearyl-L-glutamate-N-carboxyanhydride, the NCA derivative of primary interest in this research.

Kricheldorf [53] has reported NMR data on many α-amino acid-NCA compounds. He has reported the non-equivalence of protons in several NCA compounds where, at first glance, the protons would seem chemically equivalent. In fact, when the spectra are recorded on instruments less than 100 MHz, the difference in chemical shift in these protons can not be detected [53]. Also, the non-equivalence is only evident in optically pure D or L-α-amino acid-NCAs. We observed the non-equivalence of the β-protons in the L-phenylalanine-NCA spectrum as well as the non-equivalence of the β-protons (same protons as in L-phenylalanine-NCA) in O-benzyl-L-tyrosine-NCA. Also, the protons in the two methyl groups in L-leucine-NCA. Kricheldorf attributes the non-equivalence of
Table 1.6 Results of the reaction of triphosgene (tp) with α-amino acids in THF from Ref. [23]

<table>
<thead>
<tr>
<th>amino acid (aa)</th>
<th>tp:aa</th>
<th>% yield(^a)</th>
<th>mp °C(^b)</th>
<th>Dissolution time, hr</th>
<th>[(\alpha)](^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ-stearyl-L-glutamate</td>
<td>1.04</td>
<td>89.5</td>
<td>77-78</td>
<td>&lt;1</td>
<td>-18.10(^e)</td>
</tr>
<tr>
<td>DL-2-amino stearic acid</td>
<td>1.07</td>
<td>81.8</td>
<td>98-99</td>
<td>1</td>
<td>----</td>
</tr>
<tr>
<td>γ-benzyl-L-glutamate</td>
<td>1.17</td>
<td>85.8</td>
<td>96-97</td>
<td>&lt;3</td>
<td>-19.11</td>
</tr>
<tr>
<td>O-benzyl-L-tyrosine</td>
<td>1.20</td>
<td>89.4</td>
<td>142</td>
<td>3(^e)</td>
<td>-88.45</td>
</tr>
<tr>
<td>L-phenylalanine</td>
<td>1.13</td>
<td>83.0</td>
<td>91-92</td>
<td>3</td>
<td>-108.30</td>
</tr>
<tr>
<td>L-leucine</td>
<td>1.16</td>
<td>66.8</td>
<td>78-79</td>
<td>d</td>
<td>-37.40</td>
</tr>
<tr>
<td>L-alanine</td>
<td>1.26</td>
<td>58.5</td>
<td>91-92</td>
<td>d</td>
<td>----</td>
</tr>
<tr>
<td>DL-valine</td>
<td>1.11</td>
<td>82.7</td>
<td>80-81</td>
<td>d</td>
<td>----</td>
</tr>
</tbody>
</table>

\(^a\) isolated yield
\(^b\) uncorrected melting points, Fischer-Johns hot stage
\(^c\) slight suspension remained
\(^d\) insoluble material removed by filtration after about 4 h.
\(^e\) filtered chloroform solutions; approximate concentration 1.0 g/dl
Table 1.7 100 MHz $^1$H NMR data for NCA derivatives from Ref. [23].

<table>
<thead>
<tr>
<th>NCA</th>
<th>Observed Chemical Shift (ppm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\gamma$-stearyl-L-glutamate</td>
<td>6.62 (s, N-H), 4.40 (t, $\alpha$-C-H), 4.09 (t, OCH$_2$-R), 2.56 (m, $\gamma$-CH$_2$), 2.20 (m, $\beta$-CH$_2$), 1.26 (s, alkyl-CH$_3$ chain), 0.88 (t, terminal methyl)</td>
</tr>
<tr>
<td>$\gamma$-benzyl-L-glutamate</td>
<td>7.37 (s, Ar-H), 6.71 (s, N-H), 5.15 (s, benzylic CH$_2$), 4.39 (t, $\alpha$-C-H), 2.61 (m, $\gamma$-CH$_2$), 2.14 (m, $\beta$-CH$_2$)</td>
</tr>
<tr>
<td>DL-2-aminostearic acid</td>
<td>6.33 (s, N-H), 4.35 (t, $\alpha$-C-H), 1.26 (s, alkyl CH$_2$ chain), 0.88 (t, terminal methyl)</td>
</tr>
<tr>
<td>O-benzyl-L-tyrosine</td>
<td>7.39 (m, Ar-H, O-benzyl), 7.25-6.89 (m, Ar-H, tyr), 5.91 (s, N-H), 5.04 (s, CH$_2$ benzylic), 4.46 (t, $\alpha$-C-H), 3.30-2.85 (m, $\beta$-CH$_2$)</td>
</tr>
<tr>
<td>L-phenylalanine</td>
<td>7.46-7.21 (m, Ar-H), 6.47 (s, N-H), 4.55 (m, $\alpha$-C-H), 3.36-2.88 (m, CH$_2$-benzylic)</td>
</tr>
<tr>
<td>L-leucine</td>
<td>7.03 (s, N-H), 4.34 (m, C-H), 1.82 (m, $\beta$-CH$_2$), 0.98 (dd, gem di-CH$_3$)</td>
</tr>
<tr>
<td>L-alanine</td>
<td>6.66 (s, N-H), 4.46 (q, $\alpha$-C-H), 1.60 (d, CH$_3$)</td>
</tr>
<tr>
<td>DL-valine</td>
<td>7.25 (s, N-H), 4.22 (d, $\alpha$-CH), 2.28 (m, $\beta$-CH), 1.06 (m, gem di-CH$_3$)</td>
</tr>
</tbody>
</table>

- spectra taken in CDCl$_3$ with tetramethylsilane as internal standard.

The $\beta$-CH$_2$ protons to the chirality of the $\alpha$-CH. Even though the $\alpha$-C, $\beta$-C bond allows rotation of the protons, they are never completely magnetically equivalent. This effect also results in a doublet-doublet splitting of the $\alpha$-CH$_2$ protons, an effect also observed in the aforementioned compounds.

1.4 Summary

An X-crystal structure of N-phthaloyl-L-glutamic anhydride, 2, illuminated previous literature reports of selective reactions of alcohols with 2. Using 2 as an
intermediate in the synthesis of \( \gamma \)-stearyl-L-glutamate was successful, but Wasserman's approach is by far the superior choice. It is one step, provides a good yield of the desired product, and is effectively purified by recrystallization from 1:1 n-butanol:water.

A safe, clean and facile synthesis of NCA derivatives of \( \alpha \)-amino acids was developed by employing triphosgene. It is an excellent substitute for phosgene gas for this application primarily because it only delivers one equivalent of phosgene gas in solution, thus avoiding excesses of the gas that complicate product purification. It is particularly useful for the synthesis of \( \gamma \)-stearyl-L-glutamate-NCA, the primary NCA of interest in this research.

1.5 Experimental

All solvents used were usually reagent grade when contamination from such impurities as water would not interfere with the synthesis. For NCA preparation, all solvents that came in contact with the NCA were dried over 4A molecular sieves at least overnight prior to use. The amino acids used in the triphosgene evaluation were purchased from Aldrich Chemical Company in a grade having greater than 99 % purity and were used as received. All other compounds used in the syntheses reported in this section were reagent grade.

The X-ray crystal structure of N-phthaloyl-L-glutamic anhydride was obtained on an Enraf-Nonius CAD4 diffractometer by Dr. Frank R. Fronczek. The instrument was equipped with Cu K\( \alpha \) (\( \lambda = 1.54184 \) Å) radiation and graphite monochromators. The structure was solved using Enraf-Nonius SDP software [54]. NMR spectra were recorded on an IBM NR/100 Bruker NMR spectrometer (100 MHz) at 298° K using tetramethylsilane as an internal standard. Infrared spectra
were recorded on a Perkin-Elmer 83E spectrometer. Optical activities of the NCA derivatives were measured with a Perkin-Elmer 24/MC polarimeter at room temperature and were run at IBM Almaden Research Center by Professor William H. Daly. Filtered chloroform solutions were used and the final concentration (1% solutions) of the NCA was determined gravimetrically by evaporation of the solvent. Melting points were measured on a Fisher-Johns hot stage melting point apparatus.

Synthesis

N-phthaloyl-L-glutamic acid, 1. L-glutamic acid (50 g, 0.34 mol) and 70 grams of phthalic anhydride (.47 mol) are mixed in 100 ml of DMF. After heating 3-4 hours at 130° C, the reaction was cooled to room temperature and poured into about 500 grams of cracked ice. The white solid that precipitated was suction filtered from the solution and recrystallized from water, yield: 46.1 grams (49% after recrystallization). 1 was titrated with 0.1032 N NaOH (standardized with potassium hydrogen phthalate) to determine the neutralization equivalent and from there the molecular weight of 1. The molecular weight determined was 276.6, calculated 277, a 0.14% error. 'H NMR in acetone-d6: 2.47 ppm (m, b-CH₂), 4.96 ppm (m, a-CH), 7.67 ppm (s, Ar-H). 13C NMR in acetone-d6: 24.15 ppm (s, b-CH₂), 29.26 ppm (s, γ-CH₂ under acetone multiplet), 51.24 ppm (s, α-CH), 167.58 ppm (s, phthalimide carbonyls), 169.83 (s, γ-carboxylic acid carbonyl), 173.26 ppm (α-carboxylic acid carbonyl). m.p. 196-197° C.

N-phthaloyl-L-glutamic anhydride, 2. N-phthaloyl-L-glutamic acid, 1, (20g, 0.072 mol) was heated to 120° C for about 1 hour in 75 ml of acetic anhydride. After
cooling, the acetic anhydride was stripped off under vacuum, the solid remaining was washed with ether and recrystallized from acetone by allowing the solution to slowly evaporate. In this way, crystals suitable for X-ray analysis were grown. Yield 17.7 grams (95 %). ¹H NMR in acetone-d₆: 2.47 ppm (m, β- and γ-CH₂), 4.96 ppm (m, α-CH), 7.67 ppm (s, Ar-H) ¹³C NMR in acetone-d₆: 167.58 ppm (s, phthalimide carbonyls), 166.10 ppm (s, carbonyl attached to α-CH in anhydride ring), 165.5 ppm (s, carbonyl attached to γ-CH₂ in anhydride ring). m.p. 199° C. See Figure 1.3 for X-ray crystal structure.

4-(N-phenylcarboxamido)-2-phthalimido-butanoic acid. Compound 2 (1 g, .0038 mol) was mixed with .71 grams (0.0076 mol) of aniline (that had been previously treated with charcoal) in 20 ml of acetone. After reacting at room temperature overnight the acetone was removed under vacuum and the remaining syrup was treated with aqueous 1N HCl. The resulting solid was recrystallized from water. Yield 1 gram (74 %). m.p. 108-110° C. ¹H NMR in acetone-d₆: 2.58 ppm (m, β- and γ- CH₂), 4.97 ppm (m, α-CH), 7.53-6.94 ppm (m, Ar-H due to aniline), 7.82 (s, Ar-H phthalimide ring), 9.1 ppm (s, broad, COOH). ¹³C NMR in acetone-d₆: 24.25 ppm (β-CH₂), 33.15 ppm (γ-CH₂), 51.40 ppm (α-CH), 119.25 ppm (Ar aniline), 123.03 ppm (Ar, phthalimide), 128.37 ppm (Ar phthalimide), 134.28 ppm (Ar aniline), 167.45 ppm (carbonyl phthalimide), 169.80 ppm (α-carbonyl COOH), 170.14 ppm (γ-carbonyl CONH). See Figure 1.2 for comparison of the ¹³C NMR carbonyl region of this compound with 1 and 2.

4-(N-dodecylcarboxamido)-2-phthalimido-butanoic acid. Compound 2 (4.3 grams, .017 mol) was mixed with 2.38 ml (.018 mol) of triethyl amine and 3.2 g (.017 mol) of dodecyl amine in 25 ml of acetone at room temperature. The mixture
immediately became warm and was allowed to stir for 1 hour. After evaporating off the acetone, the syrup solidified upon trituration with 2N HCl. The solid was vacuum dried at room temperature overnight. Yield: 6.7 grams (89%). m.p. 88-90°C. ¹H NMR: 0.87 ppm (t, terminal CH₃), 1.23 ppm (s, side chain CH₂), 2.32 ppm (m, b-, γ-CH₂), 3.14 ppm (m, CH₂N), 4.88 ppm (t, α-CH), 6.01 ppm (m, NH), 7.78 ppm (m, Ar-H). IR: 2920 cm⁻¹, str., aliphatic CH₂, 1790, 1720 cm⁻¹, phthalimido, 1620 cm⁻¹ amide.

γ-stearyl-L-glutamate. Compound 2 (9.65 g, 0.035 mol) and 10 grams of stearyl alcohol (0.037 mol) were dissolved in 50 ml of acetone. 5.2 ml (.044 mol) of triethylamine were added and the mixture refluxed overnight. The acetone was stripped off under vacuum and the gummy solid remaining was mixed with 50 ml of 1N HCl, causing a white, soap-like solid to form, m.p. 80-82°C. ¹H NMR of this material was consistent with γ-ester formation (compound 4) and the material was used directly in the amine deblocking step. It was dissolved in 150 ml of ethanol and 0.9 ml (0.028 ml) of hydrazine were added. After stirring 4.5 hours at 70°C, the solution was evaporated to dryness under vacuum. The purification method of Wasserman et al. for γ-stearyl-L-glutamate was used to cleanup the crude product. It was heated in 1:1 n-butanol:water and filtered hot to remove phthalhydrazide. After washing with methanol and ether it was again recrystallized and filtered. Overall yield of the γ-stearyl-L-glutamate was 1.6 grams (11%). m.p. 174-176°C. To further check the identity of this product, it was reacted with phosgene which successfully produced the NCA derivative.

γ-stearyl-L-glutamate-N-carboxyanhydride. γ-stearyl-L-glutamate, 1.6 g (.0037 mol), was suspended in 50 ml of THF and warmed to 45°C. Phosgene, (10 ml of
a 20 % solution in toluene) was then added, causing immediate dissolution.
After stirring for 45 minutes the reaction was sparged with nitrogen and poured
into 150 ml of hexane previously dried over molecular sieves. The volume of the
solution was reduced to half and the solution was refrigerated overnight. 0.5
grams (30 %) of the SLG-NCA monomer were collected. m.p. 77° C. 1H NMR:
0.87 ppm (t, terminal CH₃), 1.25 ppm (s, CH₂ groups in side chain), 2.19 ppm
(m, b-CH₂), 2.55 ppm (t, γ-CH₂), 4.09 ppm (t, CH₂ in side chain adjacent to γ-
ester), 4.39 ppm (t, α-CH), 6.75 ppm (s, broad, N-H). IR: 2980 cm⁻¹ , str., alipha-
tic CH₂, 1830, 1810 cm⁻¹ anhydride carbonyls.

O-benzyl-L-tyrosine. Tyrosine (20 g, 0.11 mol) was dissolved in about 100 ml of
2N NaOH. CopperII sulfate (13.6 g, 0.054 mol) were then added, making a deep
blue solution. The solution was warmed slightly and then cooled to room temper-
ature. 13.8 ml (.09 mol) of benzylbromide were added and the mixture stirred for
one hour. 400 ml of methanol were added and 50 ml more of 2N NaOH. The
resulting solid was filtered out and repeatedly triturated with 1N HCl to free the
amino acid from the Cu (II) ions. After washing with dilute ammonia, acetone,
and ether, the powdery solid was recrystallized from 80 % acetic acid. 7.8 grams
(26 %) of product were recovered. m.p. >220° C (decomp).

γ-stearyl-L-glutamate. As reported by Wasserman et al. [31]. L-glutamic acid (8.2
g, 0.055 mol), 60 grams of stearyl alcohol (.22 mol) and 85 ml of t-BuOH were
mixed and heated to 40° C. Sulfuric acid (6ml) were added dropwise and the
temperature was raised to 65° C. The reaction was stirred for about one hour,
during which time complete dissolution occurred. After removing the heat, 6 ml
(0.051 mol) of triethylamine were added, followed by 10 ml of water, 150 ml of
ethanol, and 17 ml of triethylamine. After standing 30 minutes, the white, finely divided solid was recovered by suction filtration and washed with 300 ml of hot methanol followed by a thorough ethyl ether wash. The solid was suspended in 500 ml of 1:1 n-butanol:water, heated to 93° C, held there until dissolution occurred, and then cooled to room temperature slowly. The shiny white leaflets of γ-stearyl-L-glutamate were isolated and washed with methanol and ethyl ether. Yield: 12.2 grams (56 %). m.p.165-167° C. IR:2830 cm⁻¹, aliphatic CH₂,1725 cm⁻¹, COOR, 1590 cm⁻¹, COOH.

**Bis(trichloromethyl)carbonate.** Dimethylcarbonate (25 ml, 0.22 mol) of dimethyl-carbonate were dissolved in 150 ml of carbon tetrachloride. Chlorine gas was passed slowly into the stirred solution irradiated with a 300 watt flood lamp. The HCl by-product is passed out of the reaction through a reflux condenser equipped with a tube that passes the reaction fumes through a sodium bicarbonate solution. The reaction can easily be followed by ¹H NMR. There will be no peaks in the spectrum of the suspension mixture when the reaction is complete. After about 48 hours, large amounts of crystalline material formed in the reaction. The reaction was then sparged out with nitrogen, the carbon tetrachloride evaporated under vacuum, and the resulting crystalline solid vacuum dried. Yield 61 grams (95 %). m.p. 80° C.

**NCA derivatives from α-amino acids using triphosgene.** The given α-amino acid (10 g) was suspended in 150 ml of THF. The reaction flask was fitted with a condenser and a tube connected at the end of the condenser to allow HCl or phosgene generated in the reaction to pass into a solution of concentrated ammonium hydroxide. After warming to 50° C, 1/3 of an equivalent of tri-
phosgene was added at once. The reaction cleared up as the reaction proceeded, the time for complete dissolution depending on the amino acid reacted (see Table 1.6). After dissolution or 4 hours of reaction time, the reaction mixture was concentrated to about one third to one half its original volume under vacuum and poured into twice its volume of hexane. If a small amount of unreacted amino acid remained, it was first removed by filtration. Crystallization began almost immediately after pouring into hexane. After refrigeration overnight, the crystals were filtered from the solution with suction filtration. The solid was then dissolved in chloroform or dichloromethane. Generally it was a little cloudy due to unreacted α-amino acid. This solution was filtered through celite after first adding a small amount of sodium carbonate. The filtrate was concentrated and poured into hexane and refrigerated. Recrystallization was repeated twice. See Table 1.6 and 1.7 for reaction time, yield, m.p. data and NMR data. N^5-CBZ blocked L-lysine was also used to evaluate triphosgene. Its NCA derivative was synthesized in ethyl acetate rather than THF. The yield and purity of N-CBZ-lysine-NCA was comparable to the NCAs reported in Table 1.6.
Figure 1.1. General structures of and α-amino acid and its N-carboxyanhydride derivative.

A α-amino acid

B NCA derivative with ring numbering system shown.
Scheme 1.1. Various approaches to the synthesis of \( \gamma \)-esterified L-glutamic acid derivatives.
CH₃(CH₂)₁₄CH₂O

No product
staeryl alcohol
HCl 70°C

xα staeryl alcohol

H₂SO₄ 60° 1 hr

No OH

Cu(OAc)₃

GLU
NaOH

CH₃(CH₂)₁₄CH₂O

NH₃⁺

CH(CH₃)OH

NH₃⁺

O

O

O

O

O

O

O
Scheme 1.2. Synthesis of SLG through the N-phthaloyl derivative of L-glutamic acid.
Figure 1.2. The carbonyl region of the 25 MHz $^{13}$C NMR spectra of 1 modified at the $\gamma$-position. Internal standard is tetramethylsilane, solvent is acetone-d$_6$.

A  N-phthaloyl-L-glutamic acid, 1.
  a: 167.53 ppm  b: 169.83 ppm  c: 173.26 ppm

B  4(N-phenylcarboxyamido)-2-phthalidimido-1-butanoic acid.
  a: 167.40 ppm  b: 169.90 ppm  c: 170.14 ppm

C  N-phthaloyl-$\gamma$-stearyl-L-glutamate.
  a: 167.50 ppm  b: 169.99 ppm  c: 172.16 ppm
Figure 1.3. X-ray crystal structure of 2. Recrystallized from acetone.
Scheme 1.3. Classical approaches used to synthesize NCA derivatives of \( \alpha \)-amino acids. Reference [2] reviews each method.
Lauchs approach:

\[
\text{RCH(O\text{CH}_3)_2CO}_2\text{Na} + \text{KOH} \rightarrow \text{RCH(O\text{CH}_3)_2CO}_2\text{K} + \text{KCH}_3
\]

\[
\text{RCH(O\text{CH}_3)_2CO}_2\text{K} + \text{NH}_2\text{NH}_2 \rightarrow \text{RCH(O\text{CH}_3)_2CO}_2\text{NH}_3\text{NH}_2
\]

\[
\text{RCH(O\text{CH}_3)_2CO}_2\text{NH}_3\text{NH}_2 + \text{HNO}_3 \rightarrow \text{RCH(O\text{CH}_3)_2CO}_2\text{NH}_3\text{HNO}_3
\]

Curtius approach:

\[
\text{RCH(O\text{CH}_3)_2CO}_2\text{K} + \text{KOH} \rightarrow \text{RCH(O\text{CH}_3)_2CO}_2\text{K} + \text{KCH}_3
\]

\[
\text{RCH(O\text{CH}_3)_2CO}_2\text{K} + \text{NH}_2\text{NH}_2 \rightarrow \text{RCH(O\text{CH}_3)_2CO}_2\text{NH}_3\text{NH}_2
\]

\[
\text{RCH(O\text{CH}_3)_2CO}_2\text{NH}_3\text{NH}_2 + \text{HNO}_3 \rightarrow \text{RCH(O\text{CH}_3)_2CO}_2\text{NH}_3\text{HNO}_3
\]

Fuchs approach:

\[
\text{RCH(O\text{CH}_3)_2CO}_2\text{K} + \text{KOH} \rightarrow \text{RCH(O\text{CH}_3)_2CO}_2\text{K} + \text{KCH}_3
\]

\[
\text{RCH(O\text{CH}_3)_2CO}_2\text{K} + \text{NH}_2\text{NH}_2 \rightarrow \text{RCH(O\text{CH}_3)_2CO}_2\text{NH}_3\text{NH}_2
\]

\[
\text{RCH(O\text{CH}_3)_2CO}_2\text{NH}_3\text{NH}_2 + \text{HNO}_3 \rightarrow \text{RCH(O\text{CH}_3)_2CO}_2\text{NH}_3\text{HNO}_3
\]

Farthing modification of Fuchs phosphorylation:
Scheme 1.4. Application of triphosgene to the synthesis of NCA derivatives of α-amino acids.
Figure 1.4.  NMR spectra of SLG-NCA. Internal standard is tetramethysilane, solvent is CDCl$_3$.

A  25 MHz $^{13}$C NMR.

B  100 MHz $^1$H NMR.
Chapter 2: Synthesis of Linear Poly(γ-stearyl-L-glutamate)
2.1 Introduction

Protein structure is classified into four levels: 1) the primary structure of a protein describes its monomer or amino acid residue sequence and the quantity of each residue, 2) the secondary structure describes the conformation of the backbone of the macromolecule, 3) the tertiary structure describes the three dimensional folding of the protein, and 4) the quaternary structure describes how polypeptide chains fit together, and how other prosthetic groups are bound to the protein. These structural levels are needed to fully describe the shape, size, and function of a protein. At the primary structural level, one can study the chemistry of the side chain of the repeat units. Changes in the side chains of a given polypeptide can impart solubility to an otherwise intractable polymer. Also, side chain differences can influence the secondary structure of the polypeptide. At the secondary structural level, there are the random, α-helical, and β-sheet conformations. There are also variations such as left or right handed helices and parallel or anti-parallel pleated sheets. These conformations impart differences in the physical properties of a polypeptide such as solubility, strength, and solution behavior. This dissertation is primarily concerned with the primary and secondary structural levels of glutamic acid homopolypeptides.

The α-helical structure was first proposed by Pauling and Corey [55]. Because of its stiffness, it has become a model for physical polymer chemists studying the behavior of rod-like polymers. This point is discussed further in Chapter 4. Poly(γ-alkyl or benzyl-L-glutamates) can be synthesized with a variety of molecular weights from the corresponding NCA monomer. Certainly the most studied of these polymers is poly(γ-benzyl-L-glutamate) (PBLG); the work is
summarized in reference [24], a monograph on PBLG with over 900 references. PBLG is soluble in a number of organic solvents, which facilitates the study of its physical properties. Its dilute solution behavior was first studied by Doty et al. [56] in 1956. Recent publications involving PBLG have included a variety of NMR studies [57-60] to determine secondary conformation and lyotropic liquid crystal orientation, polymerization initiated with Triton x-100 [61] to determine conformation of the chain when bound to a surfactant, use as an adsorbent for optical resolution [62], and various applications in medicinal chemistry [63-67]. The synthesis of PBLG starting from readily available L-glutamic acid, as mentioned in Chapter 1, is relatively straightforward [24].

Less studied at a fundamental level, but perhaps more commercially exploitable is poly(γ-methyl-L-glutamate) (PMLG). It has been suggested as a leather substitute, [68-72], as a protective coating [73], and as a GPC packing material [74]. All of these applications are possible due to the rod-like nature of PMLG. Poly(γ-stearyl-L-glutamate) (PSLG) has been of interest recently because of its ability to form thermotropic and lyotropic liquid crystals [75-78]. Wasserman et al. [31] suggested applications such as a mineral and vegetable oil thickener with improved lubricating properties, as a solid fuel, as an additive to improve the durability of waxes, and incredibly, as a component in the manufacture of "Napalm" bombs. Our own interest in PSLG includes not only its liquid crystalline behavior but also its solution behavior as a "fuzzy" rod-like polymer and its potential as a hydrophobic drug carrier. Also, although the synthesis of PSLG has been known for some years, no fundamental studies have been done to determine the best conditions for its production, nor has the
fundamental physical characterization of the polymer been completed. From the limited data available on PSLG, it was clear that the unique properties exhibited warrant further investigation.

All of the poly(glutamates) can be synthesized from their respective NCA monomers. Commercially available PMLG and PBLG can also be transesterified with the desired alcohol to give PXLG, where X designates the alcohol used for the transformation. NCA polymerization is generally initiated by primary amines or alkoxides. References abound [79-81, and citations therein] that describe the kinetics of the polymerization. Some aspects (such as termination) of NCA polymerization, however, are not fully understood although the type of NCA, the monomer concentration, the solvent, the temperature, and the reaction time are all known to influence not only the rate of polymerization but also the molecular weight of the polymer.

In this chapter, the focus is on approaches to the synthesis of PSLG with varying molecular weights [82]. Chapter 4 will describe the physical characterization of the various PSLG samples. The kinetics of the reaction were not studied in detail, but the conditions necessary for producing a given molecular weight were investigated. The polymers were synthesized using both primary amine and sodium methoxide initiation. The transesterification of PMLG with stearyl alcohol to give PSLG, which is the method reported in the literature [75] for producing the desired polymer, was also evaluated.

2.2 Synthesis by Amine Initiation

It is generally accepted that primary amines will initiate NCA monomers by
attack at the 5-position of the heterocyclic ring, (Figure 1.1) with the initiator remaining covalently attached to the chain it initiates (This feature of the mechanism is exploited to make star polymers, the synthesis of which is discussed in Chapter 3). Scheme 2.1 shows the mechanism of primary amine initiation. When the ring opens, a carbamate intermediate, 1, is formed that in some cases [83] is believed to propagate the chain with subsequent loss of CO$_2$. Alternatively, loss of CO$_2$ leaves a free primary amine that can attack another NCA monomer at the 5-position and hence propagate the chain. This mechanism is operative with aliphatic primary amines [84-86], primary diamines [87] and preformed, oligomeric peptides [88, 89]. In the latter case, a preformed, $\alpha$-helical peptide initiator accelerates the rate of polymerization. Some of the noteworthy and exploitable features of this mechanism are: 1) the molecular weight of the polymer can be predicted from the monomer $\{M\}$ to initiator ratio $\{I\}$; 2) the initiator may serve to covalently attach primary amine functionalized labels to the end of the polymer chain, and 3) lower molecular weight polymers are conveniently synthesized with primary amine initiation. Generally, $\{M\}:\{I\}$ ratios greater than 100 cause prohibitively slow rates of polymerization and the molecular weights are no longer predictable from the $\{M\}:\{I\}$ ratio. Primary amine initiation can give nearly monodisperse polymers [90] but the solvent choice is critical. Polar aprotic solvents such as dimethylformamide (DMF) favor monodisperse polymers whereas nonpolar solvents such as THF yield, by comparison, a more heterogenous molecular weight distribution. Exploiting this feature of the polymerization is limited to NCA monomers and their resulting polymers that are soluble in solvents like DMF. Unfortunately, PSLG and its
NCA monomer are not; the syntheses of PSLG were normally carried out in dichloromethane (DCM).

Figure 2.1 shows the results of polymerizing various concentrations of SLG-NCA in DCM using benzylamine as the initiator at an {M}:{I} ratio of 100. Within one day, all monomer has been consumed in these reactions. Based on the intrinsic viscosities, [η], higher molecular weight polymer is produced at the lower monomer concentrations. This can be explained in terms of end group accessibility. At higher concentrations, the polymers may aggregate, thus making their end groups less accessible and less reactive toward monomer. Direct comparison of the data in Figure 2.1 is complicated by the fact that only 84 % recovery is achieved at 2 % monomer concentration. All the other reactions had recoveries of at least 95 %. For reactions at 15 % and greater monomer concentrations, the reactions become thick within an hour of reaction time and the polymerization rate appears faster than the lower concentration reactions, as evidenced by a furious evolution of CO₂ from the reactions. If this is indeed a significant effect, the polymers produced from higher monomer concentrations should become increasingly polydisperse. Figure 2.2 shows the GPC traces of the polymers isolated from the reactions described above. Polydisperse polymer was obtained from the 2 % monomer sample only; there is a shoulder of low molecular weight polymer. This shoulder indicates that not all of the amino functions initiated at the same time. With primary amine initiations, this results in a heterogenous molecular weight distribution. The polymers resulting from the other reactions have practically identical GPC traces and identical retention times, indicating that increasingly higher monomer concentrations of SLG-NCA
monomer has little effect on the polydispersity and molecular weight of the resulting polymer.

It is known that in primary amine initiation [91], the reaction undergoes an induction period where the chains slowly build to molecular weight high enough to change conformation from a random coil to an α-helix. The transition is followed by rapid consumption of the monomer. The length of the induction period depends upon the \{M\}:{I} ratio; i.e. the lower this ratio is, the shorter the induction period. At 5 % monomer concentration, the monomer is consumed in less than an hour. Figure 2.3 shows, however, that at an \{M\}:{I} ratio of 200 and 5 % monomer concentration, there is a long induction period before measurable monomer consumption and the resulting polymer is no larger than one produced from an \{M\}:{I} ratio of 100 comparison of [η]. This lends support to the observation that in primary amine initiation, \{M\}:{I} ratios of >100 are not a reliable prediction of molecular weight [24].

Secondary [92] and tertiary [93] amines can be used to initiate NCA polymerization. Tertiary amines are known to initiate through the "active" monomer mechanism, discussed in Section 2.3, but secondary amines are known to undergo reaction by both of the mechanisms discussed in this Chapter. Secondary amines were not used in our PSLG synthesis but a reaction using tributylamine was investigated. An unexpected result was obtained from this synthesis. It was run at a 40 % monomer concentration in THF with an \{M\}:{I} ratio of 30. The monomer was not completely soluble; the initiator was added to the suspension. When the polymer was recovered, it was fractionated by partial precipitation with acetone and over 40 % of the polymer was recovered in the β-
sheet form as indicated by the IR spectrum in Figure 2.4; IR peaks at 1700, 1630 and 1530 cm\(^{-1}\) are indicative \([94]\) of the \(\beta\)-sheet conformation. The \(\beta\)-sheet formation indicates a low degree of polymerization (DP). In the case of glutamates, a DP of 5-10 is favorable for \(\beta\)-sheet formation. This material is clearly less soluble than \(\alpha\)-helical PSLG and forms gelatinous solutions in THF and THF-acetone mixtures. The % yield of the polymer was only 78 %, indicating nearly a quarter of the monomer did not react or that when the polymer was isolated by precipitation, a very low molecular weight fraction remained soluble and did not precipitate. Monomer insolubility is clearly undesirable if high molecular weight samples are desired.

2.3 Synthesis by Sodium Methoxide Initiation

Sodium methoxide initiation is known to produce higher molecular weight polymers than primary amine initiation, given the same \(\{M\}:\{I\}\) ratio of the two \([24]\). Scheme 2.2 shows the generally accepted mechanism for NCA polymerization initiated by strong bases such as sodium methoxide. An "active monomer" \([95, 96]\), intermediate 2, is formed when the methoxide removes the acidic proton from the nitrogen in the NCA, making a nucleophilic anion which can attack the 5-position of a second NCA. This resulting species, 3, can continue to propagate by adding monomer at the free amine. The growing polymer chain now has one reactive, cyclic end group which can either be attacked by the active monomer or couple with the amino end of another chain. That methoxide does not attack at the 5-position as a nucleophile has been demonstrated \([97]\) using \(^{13}C\) labeled sodium methoxide. No label was found in
the isolated polymer, thus indicating the initiator has not become covalently attached to the end of the polymer chains. To determine conditions necessary to produce a range of molecular weights by methoxide initiation, reactions similar to the ones described above using primary amine initiation were set up.

Polymerizations were run at varying monomer concentrations in DCM for 5 days using sodium methoxide in a \{M\}:${!}$ ratio of 100. Figure 2.5 summarizes the results. The lowest molecular weight polypeptide, based on $[\eta]$, formed when the reaction flasks were sealed. By allowing CO$_2$ to saturate the polymerization medium, the polymerization was inhibited. The presence of CO$_2$ influences the equilibrium between carbamate and free amine, which shifts the reaction. When the reactions are run open, the polymers become larger, showing that the evolution of CO$_2$ during the polymerization enhances the molecular weight.

Periodically sparging the reaction with N$_2$ to remove CO$_2$ enhanced the molecular weight somewhat in the lower monomer concentration reactions, but no effect was detected at the higher monomer concentration. However, at 15 % and greater monomer concentration, the reaction media was quite viscous and the limited diffusion of the polypeptide may have reduced the extent of chain coupling and thus the molecular weight of the product. To test this hypothesis, a reaction was run at 20 % monomer concentration for 1 day (all the monomer was reacted, $[\eta]=0.13$) and then was diluted to 5 % and allowed to stand an additional 4 days. The resulting polymer had an intrinsic viscosity of $[\eta]=0.32$. Since all of the monomer is consumed before dilution, the enhancement of molecular weight is undoubtedly due to chain coupling which either cannot occur or occurs at a much slower rate in reactions run at higher concentrations. The
highest molecular weight polymers were obtained when the reactions ran at low concentrations were rapidly concentrated on the rotary evaporator rather than directly precipitating the product. The concentration step was accompanied by rapid evolution of \( CO_2 \), indicating again that effective removal of \( CO_2 \) will promote the formation of the highest molecular weight polypeptides.

Chain coupling was also demonstrated by running a set of polymerizations for only 2-2.5 days. As Figure 2.6 shows, the trend of polymer size, \( [\eta] \), vs. initial monomer concentration in the reaction is reversed when compared to the reaction run for 5 days, with the higher polymers obtained from more concentrated solutions. Apparently, chain coupling has not occurred in the lower concentration samples by 2.5 days of reaction. This lends support [98] to the observation that aging of NCA polymerizations initiated by strong bases leads to an enhancement of the molecular weight. Figure 2.7 shows GPC traces of a 2 % monomer concentration reaction run for 2.5 days and one run for 5 days. Note the bimodal distribution of the polymer resulting from a 2.5 day reaction. After 5 days, the distribution is more narrow. In Chapter 4.6 a more detailed GPC analysis is described for polymers synthesized by methoxide initiation. Generally, they have a broader molecular weight distribution than the polymers synthesized by primary amine initiation.

That methoxide initiation is more rapid than primary amine initiation at the same \{M\}:\{I\} ratio can be seen by comparing Figure 2.3 with Figure 2.8. Like Figure 2.3, Figure 2.8 shows the disappearance (followed by IR) of the NCA with time for a reaction at 5 % monomer concentration and a \{M\}:\{I\} ratio of 200. There is no induction period (or a very short one) for the methoxide
initiated polymerization and the monomer is consumed in under 15 hours. Again, the lower the \( \{M\}:\{I\} \) ratio, the faster the monomer is consumed. This observation was shown by reacting a 2% monomer solution with methoxide in a \( \{M\}:\{I\} \) ratio of 200. After 2 days, no consumption of monomer was detected by IR. More methoxide was added, bringing the \( \{M\}:\{I\} \) ratio to 100. After 3 days more reaction the solution was concentrated on the rotary evaporator (again with copious bubbling of CO\(_2\)), and the resulting polymer had an intrinsic viscosity of \([n]=1.21\). Characterization of a selected molecular weight range of PSLG synthesized by methoxide initiation is discussed in Chapter 4.

2.4 Synthesis by Transesterification of PMLG

There are instances where investigators [75, 99] have reported the synthesis of PSLG by transesterification of the commercially available PMLG. This synthetic route has the advantage of being a one-step reaction. However, it is difficult to fully remove all methyl side chains without risking degradation of the polymer backbone. Thus, these polymers are more accurately described as copolymers of MLG and SLG. Data obtained from a study of these polymers should be used with caution to describe the physical behavior of PSLG because the residual, short methyl side chains could impart differences in the behavior of the polymer that would be absent with 100% stearyl side chains. Scheme 2.3 outlines the synthesis. A large excess of stearyl alcohol is used to drive the reaction to high substitution. We were only able to obtain 85-90% substitution of the methyl side chains as determined by \(^1\)H NMR. When conversion efficiency is reported by other workers, it has a similar value. Figure 2.9 shows the 100 MHz \(^1\)H NMR
spectrum of 4. The peak at 3.7 ppm is due to the methyl side chain. Although more tedious, synthesis directly from the monomer is the only way to ensure identical repeat units in the polymer and an absolute requirement for the study of PSLG at any fundamental level.

2.5 Summary

Conditions necessary for producing PSLG with \([\eta]\) ranging from 0.1-1.2 using the same solvent and \{M\}:\{I\} ratio. Initiation by primary amines is generally useful for producing PSLG with a DP under 100. Primary amine initiation of SLG-NCA shows almost no dependence of polymer size on increasing initial monomer concentration in the reaction. At a \{M\}:\{I\} ratio of 200, there is a long induction period prior to rapid monomer consumption.

Initiation with sodium methoxide produces higher molecular weight polymers than primary amine initiation due to the ability of polymer chains to couple in this reaction. Chain coupling is affected by the concentration of the polymer in the reaction, by reaction time, and by the presence of CO_2 in the reaction. There was no measurable induction period when methoxide was used as the initiator, even at an \{M\}:\{I\} ratio of 200.

Clean, reproducible PSLG samples are best synthesized starting from the monomer rather than attempting to fully transesterify commercially available PMLG or PBLG.

2.6 Experimental

All solvents for NCA polymerization were either HPLC grade previously dried over 4Å molecular sieves or Aldrich Gold Label quality. These solvents are
very low in water content. All polymerization flasks were flame dried immediately prior to use. The polymerizations were run under calcium sulfate drying tubes. PMLG was obtained from Polysciences, Inc. as a 10% ethylene-tetrachloride:ethylene-dichloride solution (30:70). All other reagents were obtained from Aldrich Chemical Company.

Intrinsic viscosities were measured at 30° C in THF using an Ubbeholde capillary viscometer. Solvent flow time exceeded 100 seconds. When necessary, the solutions were filtered through a 0.45 μm (Nalge) filter. The value [η] was obtained by extrapolating ηsp/c vs c and ηin vs c plots to zero concentration. IR and NMR spectra were obtained on the same instruments cited in Chapter 1. The NMR solvent was typically CDCl₃ with TMS as an internal standard unless otherwise noted.

GPC data were collected on a Waters HPLC instrument equipped a Phenogel 10 mixed bed column. A pre-column was in place which is designed to trap particulate matter in the sample to prevent its entry into the column. The polymer was detected with a refractive index detector using THF as the mobile phase and toluene as an internal standard. The flow rate of the mobile phase was 1 ml/min. The injection volume was 25 μl of about 5% w/v solutions. The data was collected and analyzed using Nelson Analytical GPC software.

Poly(γ-stearyl-L-glutamate):

Primary amine initiation. SLG-NCA (1.0 g, 0.0023 mol) was dissolved in DCM (the amount depending upon the concentration under investigation). 128 μl of a 2% v/v stock solution of benzyl amine in DCM (2.3 x 10⁻⁵ mol, {M}:{I} = 100) were added to the stirred monomer solution. After 3 days at
room temperature, the reactions were precipitated into 100 ml of acetone. The white solid was filtered out by gravity filtration and vacuum dried.

**Methoxide initiation.** SLG-NCA (1.0 g, 0.0023 mol) was dissolved in DCM. 5.4 μl of a 25 % solution of sodium methoxide in methanol were added at once to the stirred solution. The reaction was then allowed to stand for 5 days at room temperature. The polymer was isolated by precipitation into acetone. In some cases, it was first concentrated under vacuum. Yields in either of the above syntheses were typically 90 % or greater. m.p. 60-62° C. IR: 3290 cm⁻¹, NH amide, 2850 cm⁻¹, aliphatic CH₂, 1660 cm⁻¹, CONH, 1550 cm⁻¹, CONH. ¹H NMR: peaks are broad and poorly resolved until the α-helix is disrupted with trifluoroacetic acid. See Chapter 4.2 for a discussion of NMR data.

**Transesterification of PMLG.** A solution of PMLG (20 grams of a 10 % solution, i.e., 2 grams of polymer, 0.014 mol) in 70:30 ethylene-dichloride:ethylenetetrachloride were diluted with 50 ml of the same solvent mixture. 2.3 grams (0.012 mol) of p-toluenesulfonic acid and 32.4 grams (0.12 mol) of stearyl alcohol were added and the reaction was heated and stirred at 65° C for 5 days. The reaction was then poured into 1 liter of acetone to precipitate the polymer. Repeated precipitations of the polymer from DCM solutions were done to remove unreacted stearyl alcohol. The material was vacuum dried. By weight gain, the amount of substitution was 94 %. ¹H NMR (Figure 2.8) indicated a 85-90 % degree of substitution of the methyl groups. IR was identical to the PSLG synthesized above. No -OH group due to unreacted stearyl alcohol was found in the IR spectrum.
Scheme 2.1. Mechanism of primary amine initiation of NCA monomer.

Initiator remains bound to the chain it initiates.
Figure 2.1. Intrinsic viscosity, [$\eta$], vs. monomer concentration.

Reactions run in DCM at room temperature and

initiated with benzylamine. \{M\}:{I} = 100. Intrinsic

viscosities measured in THF at 30° C. Reactions at 20, 25, and 30

% were run 1 day. Reactions 2, 5, 10 and 15 % were run 3 days.
Figure 2.2. GPC traces of the polymers isolated from the benzylamine initiation of SLG-NCA at various monomer concentrations.

GPC traces run in THF at 1 ml/min. flow rate. Not shown is the internal standard toluene which elutes at 12.37 minutes. Elution time for each is 9.10 minutes except for A which eluted at 9.57 minutes.

A 2 % monomer, B 5 % monomer, C 10 % monomer, D 15 % monomer, E 20 % monomer, F 25 % monomer.
Figure 2.3. Benzyl amine initiation of SLG-NCA 5 % in DCM followed by IR spectroscopy. {M}:{I} = 200. An aliquot from the reaction was sequentially removed and cast onto a NaCl plate.

\[
\log(a/b) = \log \left[ \frac{\text{distance from zero % transmission to baseline of 1830 cm}^{-1} \text{ band}}{\text{distance from zero % transmission to top of 1830 cm}^{-1} \text{ band}}} \right] \div \left[ \frac{\text{distance from zero % transmission to baseline of 1735 cm}^{-1} \text{ band}}{\text{distance from zero % transmission to top of 1735 cm}^{-1} \text{ band}}} \right]
\]

1830 cm\(^{-1}\) band is NCA carbonyl.

1735 cm\(^{-1}\) band is ester carbonyl which is used to normalize the relative NCA or amide concentration since its concentration remains constant throughout the reaction.
○ amide appearance
△ NCA disappearance
Figure 2.4. IR spectrum of PSLG isolated in β-sheet form. Bands at 1635, 1535, and 1700 cm\(^{-1}\) are indicative of the conformation. Film on NaCl plate cast from DCM.
Scheme 2.2. Active monomer mechanism. Attack on the NCA ring by a strong base.
propagation by amino group

chain coupling, cyclic end
Figure 2.5. Intrinsic viscosity, $[\eta]$, vs. monomer concentration.

Reactions run in DCM at room temperature for 5 days with a
{M}:{I} ratio of 100 using methoxide initiation. Intrinsic viscosities
were determined in THF at 30° C.
○ rapid CO₂ removal before ppt.
× open vessel, N₂ sparge
□ open vessel
△ closed vessel
Figure 2.6. Comparison of polymer size obtained when the polymerization of SLG-NCA by methoxide is allowed to run 5 days instead of 2 days. See also the trend shown in Figure 2.5 plotted with open boxes.
Figure 2.7. GPC traces in THF at 1 ml/min. of 2 % monomer concentration in DCM initiated by methoxide, \( \{M\}:\{I\} = 100 \). Not shown is the internal standard toluene which elutes at 12.37 minutes.

A 2 day reaction

B 5 day reaction
Figure 2.8. SLG-NCA polymerization, 5% in DCM initiated with methoxide with a \{M\}:\{I\} = 200. Reaction was followed by periodically casting a sample of the reaction on a NaCl plate and running its IR spectrum. See Figure 2.3 for the explanation of log(a/b).
O amide appearance
△ NCA disappearance

\[ \log(a/b) \]

0 20 40 60 80 100 120 140

time h.
Scheme 2.3. Reaction of PMLG with stearyl alcohol to form PSLG.
\[ \text{xs stearyl alcohol} \quad \text{PTSA} \quad 60^\circ\text{C} \quad 1 \text{ week} \]

\[ x = 0.10 - 0.15 \quad y = 0.85 - 0.90 \]
Figure 2.9. $^1$H NMR spectrum of PMLG transesterified with stearyl alcohol. Internal standard was tetramethylsilane, solvent was CDCl$_3$. 
\[ \begin{aligned}
  x &= 0.10 - 0.15 \\
y &= 0.85 - 0.90
\end{aligned} \]
Chapter 3: Synthesis of Multi-functional Primary Amino Central Units: Applications for the Synthesis of Star Branched Poly(γ-stearyl-L-glutamate) and Poly(γ-benzyl-L-glutamate)
3.1 Introduction

Star polymers are macromolecules that contain polymer chains radiating outward from a central unit. That is, one end of each chain (or arm) is anchored on a small molecule that is the nucleus or center of the macromolecule. Given that polymer molecules can assume conformations that make them anywhere between completely flexible or random to completely stiff or rod-like, one can envision four different types of models for star polymers that can be synthesized given the proper central unit and monomer. Figure 3.1 shows a representation of each. A star polymer could have: 1) flexible arms and a flexible central unit, A, 2) flexible arms and a rigid central unit, B, 3) rigid arms and a rigid central unit, C, or 4) rigid arms and a flexible central unit, D. Model D of Figure 3.1 represents the star polymers synthesized in this research.

Star polymers have been of interest to researchers in recent years due to a desire to test theories that have been developed for many years to describe the behavior of star polymers in solution. Recently, Daoud and Cotton [100] has developed scaling theories to predict the shape and behavior of star polymers in solution and other mathematical treatments have been directed at confirming his predictions [101, 102]. However, some of these treatments, such as those performed by Croxton, do not support the Daoud and Cotton scaling predictions. Practically, star polymers are of interest [103] because of they exhibit lower processing or molding temperatures, but retain high strength. Hence, higher molecular weight polymers can be melt processed or extruded more easily if they are star branched rather than linear. Chapter 5 describes the applications of star polymers in more detail. Flory [104] developed equations predicting the
molecular weight distribution decades ago and the theories for predicting star polymer behavior that followed have only relatively recently been tested in the laboratory. Computer simulations [105-111] have been developed to predict star polymer behavior, such as how excluded volume interactions of star polymers with varying numbers of arms and DP affect the conformational property of the star. These studies are useful for developing theories that can later be disputed or verified through experimentation. Sometimes, though, the synthesis of a star polymer based upon a computer model represents such a formidable task that its synthesis and characterization in the lab is not practical. For example, a computer model [107] of a 50 arm star polymer may be useful for developing a theory on the macromolecule's physical properties, but its actual synthesis represents an extremely difficult task.

Good physical characterization of star polymers has been lacking or in conflict with theoretical predictions (besides the fact that the theories sometimes contradict each other) until the 1970's because of the difficulties in preparing representative samples. Sometimes branched polymers are formed during a polymerization process because some reactive functional group in the developing polymer backbone will initiate another chain. Such random processes form ill-defined, multi-chain macromolecules. In a 1948 paper [112], Schaefgen and Flory pointed out the need for the synthesis of multi-chain polymers that are consistent in the amount of branching and the molecular weight of the arms. In 1962, Morton et al. [113] reported the synthesis of monodisperse, 3 and 4 arm polystyrene. This method involves the synthesis of linear polystyrene chains by anionic polymerization techniques, producing in a "living" polymer which can be
coupled to a multi-functional central unit such as silicon tetrachloride.

Subsequent syntheses involve Morton's basic approach [114-122]. Hadjichristidis and Fetters [123] has successfully produced polystyrene star polymers with up to eighteen arms by applying such methods. Star polymers produced by this method have recently been made commercially available from Polysciences, Inc. These methods produce the cleanest and most monodisperse star polymers reported to date. Alternatively, and strategically similar to our synthesis of star polymers, one can form multi-functional central units and have the monomer propagate outward from the central point [124-126]. These syntheses usually involve such monomers as styrene, methylmethacrylate, isoprene, or 1,4-butadiene initiated by a delocalized carbanion to produce the arms of the star. The result is star polymers with random coil arms.

Synthesis of star polymers with poly(L-glutamate) arms from an NCA monomer and a multi-functional primary amino central unit gives a multi-chain polymer with rod-like arms in heliogenic solvents. Only one paper [127] has been published using NCA polymerization to produce a 3 arm star polymer. The initiator (diethylenetriamine) these workers used to polymerize BLGNCA can also cause the formation of linear chains due to the presence of a secondary amino group in the compound. The authors do not, however, mention this possibility. Although their intrinsic viscosity and optical rotatory dispersion data suggest a branched polymer with equal arm population, their light scattering data does not suggest that a clean, monodisperse star polymer had been produced.

Earlier work to produce branched polypeptides [128-132] used poly(L-lysine) as the "central unit." Thus, these polymers were really graft copolymers with short
grafts extending from a poly(L-lysine) backbone. A recent study [133] also makes use of poly(L-lysine) in this way. These polymers were of interest as models of protein systems. Dickstein [134-136] has synthesized rigid arm star polymers and studied the factors necessary to induce liquid crystalline behavior. His polymers contain long, flexible central units which allow the rod-like portion of the arms to align parallel in distinct layers. Star polymers with rod-like arms, however, do not dominate the literature.

The synthesis of star polymers reported here features SLGNCA and BLGNCA monomers reacted with multi-functional primary amino central units also synthesized in this work. In this chapter, the syntheses of the materials are described. In Chapter 5, the physical characterization of the polypeptides is described.

3.2 Synthesis of Multi-functional Primary Amino Central Units

As discussed in Chapter 2, one of the advantages of NCA initiation with primary amines is that the initiator remains bound to the polymer chain it initiates. This feature is exploitable in the synthesis of star polymers. Thus, if a compound is synthesized in which the number of primary amino functions is \( f \), then it is a potential initiator for synthesizing a star polymer with \( f \) arms. Our goal, then, was to produce compounds that had several pendant primary amino functions present.

The first objective was to synthesize an initiator with three active sites. Scheme 3.1 outlines the synthesis. The problem with this reaction was the difficulty isolating 1 with suitable purity as a free amine. The reduction [137]
from the amide to the amine is a low yield step and removing 1 from the mixture met with little success. As Scheme 3.2 shows, 1 can be synthesized through an azide intermediate, but again, its isolation and purification were never satisfactorily accomplished. The azide reduction step attempted is a modification [138a] of the Staudinger and Hauser [138b] reaction. The reactions leading up to the product are straightforward and easily accomplished. It was in the isolation of the product from the reduction steps [138a,b] which presented the difficulty. Both of these reductions, however, appeared to produce the desired amino functions, based on positive ninhydrin tests of the reaction mixtures. One other problem with the synthesis through an azide intermediate is that compounds with a high percentage of azide functions present a serious explosion hazard, which would discourage one from attempting to functionalize a compound with more than 3 azide units.

The reactions represented in Scheme 3.3 and 3.4 were the most successful in leading to multi-functional amino central units. These reactions require only nucleophilic attacks at carbonyl carbons or benzyl carbons; reactions which give a high yield of the desired product in each step. The reactions to produce the initiators 3a, 4a, 6a, and 9a were basically the same for each. For example, 1,3,5-tribromomethylbenzene [139] was reacted with 3 equivalents of the sodium salt of methanetriethyltricarboxylate to produce 9e. Compound 9e is also the first tier in Newkome et al. [140-142] arborol synthesis. 9e, as well as 4e and 6e, are all isolated as clear oils. 3e was isolated as a white, crystalline solid. 9e was then exhaustively amidated with a large excess of 1,3-propanediamine to yield the desired central units with 9 pendant amino functions. Any diamines with terminal
amino groups could in principle be used in this synthesis but 1,3-propanediamine
was used because it is sufficiently flexible to allow space between the arms in the
central unit and its boiling point is low enough to make removal of the excess
more convenient. The large excess of diamine is necessary to reduce coupling
reactions, both inter- and intra-molecular. Wilson and Tomalia of Dow Chemical
[143] have produced Starburst Dendrimers™, compounds containing high amounts
of branching with each branch capped with a diamine. Their reaction involves
the substitution of dozens of methyl ester groups in the same molecule with
ethylenediamine. They have found that large excesses of the diamine yield the
desired compound without bothersome side reactions predominating such as
polymerization or coupling of two units. In a like manner, the 3, 4, and 6
primary amino functionalized central units are also prepared. Analysis by fast
atom bombardment mass spectroscopy (FAB MS) gave the expected value (Table
3.1) for the mass of the parent peak except for 9a. After several failed attempts
our MS operator, Mr. Tom Mahier, was finally able to produce a spectrum of 9a
which showed a few lower molecular weight peaks but no peak at the expected
parent mass. The reason for poor results on this compound are presently not
known. HPLC runs of each central unit on a reversed phase C-8 column using
40:60 methanol:water as the mobile phase gave essentially one peak for each
chromatogram. Analysis of the UV spectrum at several points on the peak gave
the same spectrum, indicating there are no overlapping peaks. Also, hydrochloric
acid solutions of the central units were back-titrated with sodium hydroxide to
determine the number of active amino functions in the compounds. Table 3.1
shows the results. The calculated number of amino functions were obtained by
using the molecular ion molecular weight from FAB MS in the calculation
(except for 9a where the calculated value was used). In 4a, 6a, and 9a there
were fewer amino functions present than expected despite the fact that FAB MS
gave the expected molecular weight for 4a and 6a. This leads one to suspect that

Table 3.1 Results of the titration$^a$ of central units 3a, 4a, 6a, and 9a.

<table>
<thead>
<tr>
<th>central unit</th>
<th>calc. meq./g</th>
<th>obs. meq./g</th>
<th>calc.$^b$ # NH$_2$</th>
<th>obs. # NH$_2$</th>
<th>calc. mw</th>
<th>FAB MS$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1$^d$</td>
<td>9.33</td>
<td>9.72 ± 0.86</td>
<td>1</td>
<td>1.04 ± 0.09</td>
<td>---</td>
<td>d</td>
</tr>
<tr>
<td>3a</td>
<td>7.94</td>
<td>8.54 ± 0.22</td>
<td>3</td>
<td>3.23 ± 0.08</td>
<td>378</td>
<td>379</td>
</tr>
<tr>
<td>4a</td>
<td>7.49</td>
<td>6.77 ± 0.43</td>
<td>4</td>
<td>3.62 ± 0.23</td>
<td>534</td>
<td>535</td>
</tr>
<tr>
<td>6a</td>
<td>7.87</td>
<td>6.95 ± 0.49</td>
<td>6</td>
<td>5.30 ± 0.19</td>
<td>762</td>
<td>764</td>
</tr>
<tr>
<td>9a</td>
<td>8.47</td>
<td>7.16 ± 0.09</td>
<td>9</td>
<td>7.60 ± 0.09</td>
<td>1062</td>
<td>e</td>
</tr>
</tbody>
</table>

$^a$ Back titration of excess HCl in a solution of the central unit using NaOH and bromothymol blue indicator.

$^b$ Number expected, based on the syntheses.

$^c$ Parent peak + 1 .

$^d$ Benzylamine, run as a standard, molecular weight 107.16 .

$^e$ Used calculated value of 1062 to determine the calc. meq/g .

there is a small amount of material present in the central units that contain
unreacted ester functions. Therefore, 4a, 6a, and 9a were "recycled" back into a
reaction with 1,3-propanediamine neat. Surprisingly, titration of the products
isolated from this reaction gave identical results. The low titration values could
also reflect amino functions in the compounds that are unable to be titrated. The
high charge density required may limit the number of amino groups ionized in
dilute hydrochloric acid. Figure 3.2 and 3.3 give the $^1$H and $^{13}$C NMR spectra of the central units. Central units 4a and 6a show small amounts of unreacted ethyl ester functions are present. They are undoubtedly present in 9a but with the overwhelming presence of n-propylamine groups it is difficult to see the ethyl ester. The relative simplicity of the spectra indicates how symmetric these compounds are.

The central units are also quite reactive with ninhydrin, giving a deep blue solution indicative of the presence of primary amine functions. They are all isolated as white powders which are very hygroscopic, particularly 9a. Unfortunately, they are not generally soluble in common organic solvents. This is unfortunate because it is then more difficult to synthesize uniform star polymers when the initiator is insoluble in the reaction solvent. They are however, quite water soluble and to a lesser extent, methanol soluble. They also dissolve well in 1,3-propanediamine. Qualitatively, 4a appears to be the least soluble of the 4 central units. This is reasonable, if one considers the fact that it is a 1,4-disubstituted benzene ring, the least soluble disubstituted compound of the 1,2-, 1,3-, and 1,4- isomers. 3a is somewhat soluble in N-methylpyrrolidinone (NMP).

3.3 **Synthesis of Star Branched Poly($\gamma$-stearyl-L-glutamate) and Poly($\gamma$-benzyl-L-glutamate)**

Before synthesizing the star polymers, it is necessary to determine if each amino function in a given central unit will initiate a polymer chain. This was accomplished by synthesizing "star oligomers". That is, the initiator was added to BLGNCA monomer in a 5:1 ratio per amino function to create star polymers
with an arm DP of 5. Scheme 3.5 shows the synthesis using 3a as an example. These small macromolecules can then be analyzed by $^1$H NMR to determine if all active sites in the central unit reacted and to determine the arm DP of the star produced. Low DP arms allow the peaks due to the initiator to be clearly discernable in NMR. BLGNCA was chosen for the study because: 1) the $^1$H NMR peaks associated with PBLG do not interfere with peaks due to the central unit, 2) PBLG forms a $\beta$-sheet at a DP of about 6 and an $\alpha$-helix after a DP of about 11 is reached, [144] both of which can be identified by IR and $^1$H NMR to give a qualitative indication of how long the arms are, and 3) previous work [145] in our lab on PBLG-polysulfone graft copolymers established a correlation between PBLG chain length and chemical shifts in the $^1$H NMR spectrum. A DP of 5 for each arm will give an oligomer with random coil arms. Thus, bands due to the $\beta$-sheet or $\alpha$-helix should not be present in the IR spectrum of the star. Figure 3.4 shows the 200 MHz $^1$H NMR spectra of the resulting star oligomers synthesized by initiation with 3a, 4a, 6a, and 9a. The peak of interest in Figure 3.4 is at 4.0-4.6 ppm. This peak is due to the $\alpha$-CH in the PBLG backbone. In a random coil, this peak is located at about 4.6 ppm. In a completely $\alpha$-helical conformation, the peak is located at about 4.0 ppm. The fact that the peak is broad indicates a mixture of conformations and thus arms that are not monodisperse. Figure 3.5 shows the same NMR spectra as Figure 3.4 except about 10 % trifluoroacetic acid (TFA) has been added to interfere with intra- or intermolecular hydrogen bonding in the star and thus make the peaks more clearly resolvable. Also, because TFA is a helix breaking solvent, the polymers represented in Figure 3.5 are in a random conformation. These spectra were
used to calculate the arm DP. The peak at 4.6 ppm due the α-CH and the peak at 3.3 ppm due to the central unit were used to calculate the DP. Table 3.2 shows the results. They are very close to {M}:{I} ratio which indicate that all of the active sites in each central unit are initiating. If each active site were not initiating, the calculation would indicate a higher DP than expected because in the calculation it is assumed that the peak at 3.3 ppm is due to an unshifted CH$_2$ (γ- to the primary amine function) in the central unit and a completely shifted CH$_2$ (α- to the primary amine function) when the primary amino function is converted to an amide function. If there were unreacted amino functions then the peak at 3.3 ppm would represent fewer protons than we use in the calculation. For the 4, 6, and 9 arm oligomers, the initiator was added from a methanol stock solution. Methanol itself can act as an initiator in NCA polymerizations although it is slower to react than primary amino groups. The 'H NMR for these three compounds do indicate a small peak at about 3.7 ppm which could be due to methyl ester terminated polymer. The effects of methanol were investigated further and are discussed in Section 3.4 below.

Figure 3.6 shows the IR spectra of the star oligomers. Bands at 1660 cm$^{-1}$ and 1535 cm$^{-1}$ indicate a random peptide chain. Sharp bands at 1660 cm$^{-1}$ and 1550 cm$^{-1}$ indicate an α-helix. Sharp bands at 1630 cm$^{-1}$ and 1535 cm$^{-1}$ are indicative of the β-sheet conformation. Each oligomer seems to be a mixture of conformations but clearly the α-helix or β-sheet are not predominating. This result is also indicated by the NMR spectra of Figure 3.4. Thus, the oligomers synthesized contain the expected amount of branching but the length of each branch is not exactly the {M}:{I} ratio. That they are not monodisperse can be
explained by the observation that when a methanol solution of the initiator is added to the BLGNCA monomer in NMP, it initially precipitates and then goes into solution as it reacts. This is enough to cause polydispersity in the resulting polymer.

Table 3.2 Tabulated 1H NMR results from the analysis of oligomeric PBLG stars. Data is calculated from Figure 3.5.

<table>
<thead>
<tr>
<th>central unit</th>
<th>peak area α-CH</th>
<th>PA/no. H</th>
<th>calc.</th>
<th>obs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>55</td>
<td>42/12</td>
<td>5.0</td>
<td>5.2</td>
</tr>
<tr>
<td>4a</td>
<td>6.0</td>
<td>6.31/20.5</td>
<td>5.5</td>
<td>5.4</td>
</tr>
<tr>
<td>6a</td>
<td>1.4</td>
<td>1.3/30.2</td>
<td>5.6</td>
<td>6.1</td>
</tr>
<tr>
<td>9a</td>
<td>2.8</td>
<td>2.6/36.4</td>
<td>5.8</td>
<td>5.2</td>
</tr>
</tbody>
</table>

a PA is the peak area due to central unit at 3.3 ppm and no. H is the number of hydrogens under the peak (which have been corrected based on the titration data in Table 3.1).

b Corrected values based on the observed meq./g of amine from the titration data in Table 3.1.

PSLG stars were synthesized by two methods with a calculated arm DP of 50. By IR, the arms are clearly α-helical, the spectrum containing sharp, narrow, bands at 1660 cm⁻¹ and 1550 cm⁻¹. Thus, given that each amine function is initiating and the arms are rod-like, this synthesis produced star polymers represented by model d in Figure 3.1. Figure 3.7 shows the IR and 1H NMR spectra of 9 arm PSLG, which in every respect is identical not only to the other star PSLG synthesized but also to the linear PSLG. Of particular concern when the PSLG stars were produced was whether there would be room at the center of the star to accommodate repeat units with such long side chains (that is, is there
room at the center of the macromolecule to accommodate arms with a large
diameter). Steric crowding of the arms would certainly result in polydisperse
polymers. This possibility is further discussed in Chapter 5. Also, because
alcohols can cause NCA polymerization, the effects of methanol were also of
concern, the problem being that with a relatively high {M}:{I} ratio the methanol
would compete with the central units for monomer. This possibility is discussed
in section 3.4 below and further in Chapter 5. For comparison to the stars
produced in the presence of methanol, the star PSLG was also synthesized by
sonicating a suspension of the initiator in DCM and then adding the finely
suspended mixture to a DCM solution of the monomer. The reaction remains
somewhat cloudy throughout its duration, meaning that not all of the initiator is
reacting. This should result in the production of higher molecular weight stars.
Additionally, without methanol competing for monomer, a higher molecular
weight star was anticipated. In addition to IR and NMR analysis, the stars
produced by both methods were examined by DSC, intrinsic viscosity, GPC,
crossed polarized light microscopy and light scattering techniques, the data of
which are presented in Chapter 5.

3.4 Effect of Methanol in the polymerization

As mentioned above, methanol solutions of the central units are used in one
method for the polymerization of the star polymers. Because methanol is
nucleophilic there is a possibility that it could interfere with the polymerization
reaction by initiating chains. Generally, 1 ml of the initiator solution is added to
1 gram of the monomer, which would give a {MeOH}:{M} ratio of about 6.5
when BLGNCA is the monomer. Thus, if methanol can react rapidly with the monomer there would be essentially no polymer produced. Because a polymer is always isolated from these reactions it indicates that methanol does not completely interfere with a primary amine in its reactivity towards the NCA. To test this, a reaction was run with 1 gram of BLGNCA (0.0038 mol) and 1 ml (0.025 mol) of methanol in 20 ml of DCM. Aliquot of the reaction were removed, vacuum dried, and analyzed with ¹H NMR. Figure 3.8 shows the spectra obtained at 2 and 8 hours. The peaks at 4.6 and 3.9 are noteworthy. They are due to the α-CH in α-helical PBLG and the PBLG polymer in a random coil. The peak at 3.7 ppm is due to the methyl ester terminus of the PBLG produced. Its small peak area is indicative of polymer formation too. At 2 hours, the monomer has completely reacted based on the spectrum’s similarity to the 8 hour spectrum. That is, the α-CH peaks in both spectra have about the same area. Also, an IR spectrum taken at 7 hours shows a complete absence of NCA peaks. Thus, even though the methanol is in large excess, polymer is still formed. To verify that the peak at 3.9 ppm is due to an α-CH in a helix, TFA was added to the sample taken at 8 hours. As discussed earlier, TFA will break an α-helix and form a random chain, shifting the peak at 3.9 ppm to 4.6 ppm (this point is discussed in more detail in Chapter 4 in connection with NMR characterization of linear PSLG). As Figure 3.9 shows, the peak shifts completely to 4.6 ppm, the location of the α-CH in a random coil. The fact that polymer is forming means that as soon as an amino group is formed from the opening of the NCA ring it reacts rapidly with any monomer present. The implications are that if a low {M}:{I} ratio is used, methanol probably does not interfere to a large
extent. However, there does seem to be some methanol interference in the PBLG oligomer synthesis as the 'H NMR data in Figures 3.4 and 3.5 show (small peak at about 3.7 ppm). As the \{M\}:\{I\} ratio becomes larger (and the rate of reaction of the amino groups with NCA becomes slower), methanol could conceivably initiate enough polymer chains to create polydisperse polymer products, and cause the molecular weight of the desired star polymer to be severely reduced. This is more fully discussed in both Chapter 4 and 5 with regard to the GPC data obtained for both linear and star branched polymers.

3.5 Summary

Multi-functional, primary amino central units were synthesized and used to initiate SLGNCA and BLGNCA monomers to produce novel star polymers with rod-like arms. The data obtained from analysis of the central units were consistent with the expected structure. Each amino function or active site in the central unit reacts with monomer as shown by analysis of the resulting star oligomers by 'H NMR. A critical evaluation of these initiators, however, would indicate that if applied in methanol, they are not really ideal as NCA initiators primarily due to competition from the methanol. As the characterization data in Chapter 5 will demonstrate, application of the central units in heterogenous suspensions give a cleaner star product.

3.5 Experimental

All chemicals used in the synthesis were reagent grade and used as purchased
from Aldrich Chemical Company. For the polymerizations, the solvents were Aldrich Gold Label quality or grades low in water content (such as nano or HPLC grade) and the reaction flasks were flame dried prior to use.

Titrations were run using a 0.01086 N NaOH solution as the titrant which was standardized with potassium hydrogen-phthalate to a phenolphthalein endpoint. A 0.01622 N HCl solution, standardized with the NaOH solution above to a phenolphthalein endpoint, was used as the solvent for the central units. This solution was back-titrated with the NaOH solution to a bromothymolblue endpoint to yield the number of pendant amino functions in each central unit.

NMR spectra were recorded on an IBM Bruker AR-100 MHz or 200 MHz instrument as specified. The NMR solvent was CDCl₃ with TMS as an internal standard except for the central units which were recorded in D₂O with 3-(trimethyl-silyl)-1-propane sulfonic acid (TMPS) as an internal standard. IR spectra were recorded on a Perkin Elmer 283B instrument. Mass spectra for the central units were recorded on a Finnigan TSQ70 instrument by Mr. Tom Mahier using the fast atom bombardment mode. This technique gives M+1 or M+2 as the molecular ion peak. Elemental analysis on the central units were performed at Desert Analytics of Tucson, Arizona. The samples were dried at 100° C prior to analysis. HPLC chromatograms were run with the aid of Ms. Elizabeth Jordan using a Hewlett-Packard HP1090M model instrument. The column packing was a silica gel modified C-8 reverse phase material manufactured by Phenomenex. The mobile phase was 40:60 methanol:water.

1,3,5-tribromomethylbenzene. Mesitylene (34.7 ml,0.25 mol) was dissolved in 300 ml of carbon tetrachloride. 142 grams (0.75 mol) of N-bromosuccinimide were
added along with about 25 milligrams of AIBN. The mixture was refluxed with thorough stirring for about 14 hours under irradiation with a 250 watt lamp. The succinimide was then filtered out, the filtrate concentrated, and 2 times its volume of petroleum ether added. After refrigeration, white crystals formed that were isolated by filtration and recrystallized from petroleum ether: CHCl₃ to a constant melting point of 93-94°C. Yield 10g (11%). 100 MHz ¹H NMR: 4.5 ppm (s, benzylic CH₂), 7.35 ppm (s, aromatic H), consistent with the reported literature values.

1,3,5-tris(azidomethyl)benzene. 1,3,5-tribromomethylbenzene (1.3 g, 0.0036 mol) of were dissolved in 15 ml of DMF. 0.5 grams (0.011 mol) of NaN₃ were added and the reaction stirred and for 6-8 hrs at 100°C. The reaction was then poured into 50 ml of water and the water extracted with CHCl₃ to remove product. Evaporation of the solvent left a yellow oil. Yield 0.8 grams (92%). 100 MHz ¹H NMR. 4.40 ppm (s, CH₂), 7.26 ppm (s, aromatic H). IR: neat, film on NaCl plate, strong peak at 2100 cm⁻¹ due to N₃.

1,3,5-tris(aminomethyl)benzene, 1. The azido compound (0.8 g, 0.0033 mol) was dissolved in 50 ml of benzene. 1.79 ml (.0035 mol) of triethylphosphite were added slowly to the reaction at room temperature. After stirring overnight, the reaction was saturated with HCl gas and allowed to stand for two days more. A yellow syrup separated. The benzene was decanted off the syrup which was then dissolved in methanol. This solution was precipitated in ethyl ether to give a white, finely divided solid, which is the hydrochloride salt. The solid was dissolved in water, making a yellow solution, and the solution was treated with NaOH to give the free amine. Attempts to extract the free amine with ethyl
ether and ethyl acetate failed. The water solution was evaporated to dryness and the residue extracted with ethyl ether and DCM. A greenish syrup resulted. There was undoubtedly serious contamination from triethylphosphite by-products and the free amine is sufficiently water soluble so that extraction with organic solvents fail. Yield of the hydrochloride salt was 0.45 grams (51 %). 200 MHz 'H NMR in D$_2$O using TMPS as an internal standard: 4.16 ppm (s, CH$_3$), 7.46 ppm (s, aromatic H).

1,3,5-benzenetricarbonyltriamide. 1,3,5-benzenetricarbonyltrichloride (5 g, 0.019 mol) was added slowly to 25 ml of concentrated ammonium hydroxide at room temperature. The reaction was violent and rapid. A white paste formed. The reaction was diluted with water and suction filtered. The solid was water washed and acetone washed and then vacuum dried. Yield is quantitative. IR: KBr pellet, 1690 cm$^{-1}$ amide band. Attempts to reduce the amide functions to primary amine functions with borane-THF were probably successful but the product was not isolated in a form suitable as an NCA initiator.

triester, 3e. 1,3,5-benzenetricarbonyltrichloride (5 g, 0.019 mol) was dissolved in 100 ml of methanol. The solution became warm and immediately the triester precipitated. It was then concentrated and fully precipitated with water. The fine needles were removed by suction filtration and washed with a sodium bicarbonate solution and water. After vacuum drying, a yield of 4.5 grams (95 %) was obtained. m.p. 144-146$^\circ$ C. 100 MHz 'H NMR: 4.05 ppm (s, CH$_3$), 8.40 ppm (s, aromatic H). 25.13 $^{13}$C NMR: 52.51 ppm (s, O-CH$_3$), 131.21 ppm (s, Ar), 134.45 ppm (s, Ar), 166.5 ppm (s, carbonyl). IR: KBr pellet, 1735 cm$^{-1}$ ester band.

tetra, hexa, nona-ester, 4e, 6e, 9e. The general procedure outlined below is the
preparative method for all. The yields in each case are similar. In the synthesis of 6e and 9e, 1,3,5-tribromomethylbenzene is substituted for 1,4-dibromomethylbenzene. For 9e, diethylmalonate is substituted with methanetriethyltricarboxylate. 1,4-dibromomethylbenzene (5 g, 0.019 mol) was dissolved in 50 ml of benzene. 50 ml of a DMF solution of 5.7 ml (0.039 mol) of diethylmalonate and 2.5 grams (0.039 mol) of sodium ethoxide was added to the benzene solution. After heating for 70° C for 5 hours, the reaction was water washed and the organic layer dried with magnesium sulfate. Evaporation of the benzene yielded a clear oil. Unreacted starting material was removed by flash chromatography [146], eluting the product through silica gel first with benzene, then with ethyl acetate. The ethyl acetate fraction contained the product. Yield is typically 7.3 grams (78 %). NMR data below were collected on the 100 MHz instrument.

4e: 'H NMR: 1.25 ppm (t, \( \text{CH}_3 \)), 3.2 ppm (d, \( \text{Ar-CH}_2 \)), 3.7 ppm (t, -CH), 4.2 ppm (q, \( \text{CH}_2 \)), 7.15 ppm (s, aromatic H). \(^{13}\text{C} \) NMR: 13.91 ppm (\( \text{CH}_3 \)), 34.22 ppm (O-\( \text{CH}_2 \)), 53.74 ppm (benzylic \( \text{CH}_2 \)), 61.33 ppm (CH), 128.88 ppm (Ar), 135.50 ppm (Ar), 168.73 ppm (carbonyl). IR: neat, film on NaCl, 1740 cm\(^{-1}\), ester band.

6e: 'H NMR: 1.25 ppm (t, \( \text{CH}_3 \)), 3.2 ppm (d, \( \text{Ar-CH}_2 \)), 3.7 ppm (t, CH), 6.95 ppm (s, aromatic H). \(^{13}\text{C} \) NMR: 13.91 ppm (\( \text{CH}_3 \)), 34.37 ppm (O-\( \text{CH}_2 \)), 53.69 ppm (benzylic \( \text{CH}_2 \)), 61.34 ppm (CH), 127.66 ppm (Ar), 138.42 ppm (Ar), 168.62 (carbonyl). IR: neat, film on NaCl, 1740 cm\(^{-1}\) ester band.

9e: 'H NMR: 1.25 ppm (t, \( \text{CH}_3 \)), 3.43 ppm (s, \( \text{Ar-CH}_2 \)), 4.25 ppm (q, O-\( \text{CH}_2 \)), 7.0 ppm (s, aromatic H). \(^{13}\text{C} \) NMR: 13.91 ppm (\( \text{CH}_3 \)), 38.5 ppm (O-\( \text{CH}_2 \)), 61.9 ppm (benzylic \( \text{CH}_2 \)), 67.5 ppm (-C-), 132.0 ppm (Ar), 134.9 ppm (Ar), 166.2 ppm
(carbonyl). IR: neat, film on NaCl, 1740 cm\(^{-1}\) ester band.

3a, 4a, 6a, 9a: These are prepared the same way for each by the following method. About a 10 fold molar excess of 1,3-propane diamine was used for each ester function to substitute. The ester precursor was dissolved in benzene and then slowly poured into a benzene solution of the diamine at room temperature. The reaction was then warmed to 40-50\(^\circ\) C for 4-5 days under a calcium sulfate drying tube. A little of the product precipitated during the reaction. The reaction was then concentrated under vacuum and the syrup remaining was dissolved in a small volume of methanol. This solution was precipitated into ethyl ether. This operation was repeated three times and the resulting thick syrup was thoroughly ether washed and benzene washed. After vacuum drying at 50\(^\circ\) C for 36 hours, a solid formed. Yields are typically 50-60\% . IR showed the absence of ester functions. FAB MS: 3a, calc. 378, expt. 379. 4a, calc. 534, expt. 535. 6a, calc. 762, expt. 763.6. See Figure 3.2 and 3.3 for NMR spectra.

Elemental analysis:

3a. calc.: %C 57.14; %H 7.94; %N 22.22 . found: %C 56.40; %H 7.64; %N 21.32 .

4a. calc.: %C 58.43; %H 8.61; %N 20.97 . found: %C 56.48; %H 8.26; %N 18.91 .

6a. calc.: %C 56.69; %H 8.66; %N 22.05 . found: %C 55.15; %H 8.08; %N 18.24 .

9a. calc. %C 54.23; %H 8.48; %N 23.73 ; found: %C 55.02; %H 8.59; %N 20.62.

Oligomeric PBLG stars: BLGNCA (1 g, 0.0038 mol) was dissolved in 10 ml of NMP at room temperature. To make a star with an average arm DP of 5, the following ratios of monomer:initiator were used: 3 arm; \{M\}:{I} = 15. 4 arm;
\{M\}:/{I} = 20. 6 arm; \{M\}:/{I} = 30. 9 arm; \{M\}:/{I} = 45. The initiator was added in these ratios but corrections based on the observed meq./g from titration data were made when tabulating the data in Table 3.2. The initiator was added at once from a methanol stock solution except for the 3 arm reaction in which an NMP stock solution of the central unit was used. After stirring three days at room temperature, the reactions were precipitated into water and the collected solid vacuum dried 36 hours at 50° C. See Figures 3.4, 3.5, and 3.6 for the IR and NMR spectra. Yields are quantitative based on 'H NMR integration.

The methods outlined below are examples of how to prepare higher molecular weight branched PSLG. The full characterization of these materials is reported in Chapter 5.

**Star PSLG. Method I.** The stars with a DP = 50 for each arm are made in an identical fashion to the above synthesis expect that the monomer:initiator ratios are: 3 arm; \{M\}:/{I} = 150. 4 arm; \{M\}:/{I} = 200. 6 arm; \{M\}:/{I} = 300. 9 arm; \{M\}:/{I} = 450. PSLG stars were reacted in DCM with a monomer concentration of 5%. After 3 days, they were concentrated and precipitated into acetone. Their IR spectra indicate an α-helical conformation. For example, see Figure 3.7 for the 9 arm PSLG IR spectrum and 'H NMR spectrum. 'H NMR and IR spectra for the other star polymers are identical to the examples shown in Figure 3.7.

**Star PSLG. Method II.** The synthesis was run in DCM and the \{M\}:/{I} ratio was again 50. The reactions were typically one gram scale. The initiator was added to about 1-2 ml of DCM and then sonicated until a finely divided suspension resulted. The suspension was added to a rapidly stirred solution of monomer in DCM (5 %). After stirring for 3 days the reactions were
concentrated and the polymer precipitated into acetone. The product was isolated by filtration and vacuum dried. Yields were typically 95%. IR and NMR spectra were identical to the polymers obtained from Method I.

**Broken Rod PSLG.** SLGNCA (2.1 g, 0.00493 mol) was dissolved in 20 ml of DCM. A stock solution in DCM of 0.0195 g/ml of 1,6-hexanediamine was prepared. The desired amount of this initiator solution (in this case, 0.097 ml or 1.64 x 10^-5 mol of 1,6-hexanediamine, giving an {M}:{I} ratio of 150 per amino function) was added at once to the stirred monomer solution. After 7 days of reaction at room temperature, the reaction was concentrated on a rotary evaporator (with the evolution of CO₂ bubbles) to about 10 ml and then poured into 250 ml of stirred acetone to precipitate the polymer. The weight of recovered polymer was 1.5 g (80%).
Figure 3.1. Schematic representations of three arm star polymers.

Various combinations of flexible or rigid centers and arms are possible.
A B C D

- flexible center and arm
- rigid arm
- rigid center
Scheme 3.1. Approach to for the synthesis of a three arm initiator through an amide intermediate to produce a star polymer from NCA polymerization.
Scheme 3.2 Approach to for the synthesis of a three arm initiator through an azide intermediate for the production of a star polymer by NCA polymerization.
$$\text{Br} \quad \text{Br} \quad \text{Br} \quad \text{NaN}_3 \quad \text{DMF, 100°C} \quad \text{N}_3 \quad \text{N}_3 \quad \text{N}_3$$

1. Triethylphosphite
2. HCl
Scheme 3.3. Synthesis of the multi-functional ester precursors to the star central units. The reaction to produce 9e is the first tier in Newkome's [128-130] arborol synthesis.
Scheme 3.4. Synthesis of the multi-functional primary amino central units from the ester precursors shown in Figure 3.3.
3e $\xrightarrow{\text{NH}_2(\text{CH}_2)_2\text{NH}_2}$ 4-5 days 50° C

$\xrightarrow{\text{NH},}$

$\text{NH}_2$

$\text{NH}_3$

$\text{NH}_2$

4e

$\text{NH}_2$

$\text{NH}_3$

$\text{NH}_2$

6e

$\text{NH}_2$

$\text{NH}_2$

$\text{NH}_3$

$\text{NH}_2$

9e

$\text{NH}_2$

$\text{NH}_2$

$\text{NH}_2$
Figure 3.2. 200 MHz 'H NMR spectra of the central units 3a, 4a, 6a, and 9a. Internal standard was TMPS and the solvent was D$_2$O. Peak at 4.8 ppm is due to DHO.

A 3a, B 4a, C 6a, D 9a.
Figure 3.3. 50 MHz $^{13}$C NMR spectra of central units 3a, 4a, 6a, and 9a. Run in D$_2$O with TMPS as an internal standard. Peaks marked with an s represent the internal standard.

A 3a, B 4a, C 6a, D 9a.
Scheme 3.5. Synthesis of 3 arm PBLG oligomer from central unit 3a.
Figure 3.4. 200 MHz $^1$H NMR spectra of the PBLG oligomers. Spectra taken in CDCl$_3$ with TMS as an internal standard.

A 3 arm, B 4 arm, C 6 arm, D 9 arm.
Figure 3.5. 200 MHz 'H NMR spectra of the PBLG oligomers. Spectra were run in CDCl$_3$ with TMS as an internal standard. About 10% trifluoroacetic acid is present in these samples.

A 3 arm, B 4 arm, C 6 arm, D 9 arm.
Figure 3.6. IR spectra of the PBLG oligomers. The polymer was cast as a film from chloroform on a NaCl plate.

A 3 arm  B 4 arm  C 6 arm  D 9 arm.
Figure 3.7.  A IR spectrum of 9 arm PSLG. Film on NaCl plate.

B 200 MHz 'H NMR spectra of 9 arm PSLG. Both run in CDCl₃
with TMS as an internal standard. Upper: no TFA, Lower:
10 % TFA.

These spectra are representative of all of the PSLG stars
produced, regardless of the method.
Figure 3.8. 100 MHz $^1$H NMR spectra of PBLG produced by methanol initiation of BLGNCA monomer.

A Reaction at 2 hours, and B at 8 hours.
Figure 3.9. 100 MHz $^1$H NMR spectrum of PBLG produced from methanol initiation of BLGNCA after 8 hours of reaction in DCM at room temperature. TFA was added to the sample in CDCl$_3$ with TMS as an internal standard.
Chapter 4: Characterization of Poly(γ-stearyl-L-glutamate)
4.1 Introduction

Rod-like polymers lie near one extreme of two basic macromolecular geometries. Ideally, linear polymers can be completely flexible or freely jointed, completely stiff or rod-like, or, more realistically, somewhere between these two limits. Synthesis of an inflexible polymer chain represents an added challenge to the organic polymer chemist and provides a unique structure for study by the physical polymer chemist. Any group of atoms in the polymer backbone that allows flexibility about its bonds prevents the chain from being rod-like. Thus, there are limitations on the type of repeat units that can form a rod-like polymer. Repeat units that contain highly conjugated double bonds can form stiff polymer chains. An example of a polymer which obtains its stiffness through conjugation is polybenzobisthiazole (PBT) [147]. Polymer chains can also be rendered semi-inflexible if along the backbone there are repeating like charges (positive or negative) that repel one another. Polymers with amino or carboxylic acid groups in their repeat units are examples. Nature assembles rod-like molecules in the form of proteins that have an α-helical backbone. Though the bonds in a polyamide such as proteins are somewhat flexible, certain polypeptides fold into a helix stabilized by intramolecular hydrogen bonds. As long as the hydrogen bonds are not disrupted, the polymer maintains its rod-like character. As mentioned in Chapter 2, poly(L-glutamates) form such an α-helix, making them good models for studying the behavior of rod-like macromolecules. A further advantage lies in the fact that if the helix is disrupted with an appropriate solvent, a random chain polymer results. Thus, the polymer is a good candidate for comparing the behavior of rods and random chains. Other rod-like polymers
which have been the subject of numerous studies [148-156] are the polyisocyanates. These polymers, also, derive their stiffness from the formation of a helical backbone. Figure 4.1 shows the repeat units of various rod-like macromolecules.

Rod-like polymers are a key ingredient in the development of high performance, high strength materials [157-160]. They can orient themselves in solution in such a way (backbones parallel) that high uni-directional strength is obtained after spinning the solution into a non-solvent. Composites can be made by mixing rod-like polymers with random coil polymers at a concentration where the rod-like chains are aligned (anisotropic) and then spinning the polymer solution. The result is a composite with higher strength than material made from the random coil alone. The alignment of rod-like polymers often results in birefringent or liquid crystalline properties which also receive considerable attention. PSLG offers the opportunity to study these properties.

PBLG has long been an important model for the study of rod-like polymer behavior. Its mention here is appropriate because the extensive data collected on PBLG can serve as a useful guide for the study of structurally similar PSLG. Unlike many rod-like polymers which are intractable or insoluble, PBLG is generally soluble in organic solvents (while still maintaining its helical backbone), its stiffness approaches the ideal rod-like limit, and its side chain is easily modified. The rod-like properties of PBLG have been studied with respect to liquid crystal formation [161-163], solid state morphology [164-166], dilute solution properties [167-170], and the formation of gels [171-174].

As mentioned in Chapter 2, a family of poly(γ-alkyl-L-glutamates) has been
synthesized by several investigators [175-178] either by transesterification of
PMLG or PBLG or by polymerization of the appropriate NCA monomer. The
alkyl side chains impart not only good solubility to the polymer, but also induce
special physical properties, such as a lower melting point and the formation of
thermotropic and lyotropic liquid crystals. The studies on PSLG have focused
primarily on its ability to form liquid crystals both as a melt (thermotropic) and in
solution (lyotropic) [75-78]. More fundamental studies to determine its
dimensions and dynamics in solution have been lacking. Further, the PSLG used
in the liquid crystal studies has been synthesized by transesterifying PMLG with
stearyl alcohol, leaving methyl side chains in the resulting polymer; there is some
uncertainty as to what effect their presence has on the property of the polymer.

In this chapter, results are presented on the characterization of PSLG
synthesized as discussed in Chapter 2. The PSLG synthesized from the monomer
will be referred to as PSLG-xxK where xxK is the molecular weight in thousands
of daltons (i.e., PSLG-248K is a sample with a molecular weight of 248,000
daltons). The PSLG synthesized by trans-esterification of PMLG will be referred
to as PSLG-EX. The NMR studies included observation of the helix-coil
transition when a solution of PSLG was titrated with the helix breaking solvent
TFA. GPC chromatograms provided an indication of the polydispersity of the
PSLG samples studied. With DSC we were able to observe the endotherms due
to the melting point of the side chains and a thermotropic liquid crystal phase
transition. Polarized light microscopy allowed observation of the formation of
cholesteric liquid crystals, both thermotropic and lyotropic. With light scattering
we obtained the weight average molecular weight, the osmotic second virial
coefficient, the radius of gyration, the diffusion coefficient at zero concentration, and the hydrodynamic radius. Various calculations were made using the light scattering data including an estimation of the pitch/residue of the α-helix and the diameter of PSLG. Using intrinsic viscosity measurements and the weight average molecular weight obtained from static light scattering, a Mark-Houwink plot was constructed.

4.2 NMR Analysis

As mentioned in Chapter 3, one of the interesting and useful features of a 1H NMR spectrum of PSLG is the position of the α-CH peak. In the rod-like or helical conformation the α-CH is peak is poorly resolved at about 3.9 ppm; in the random conformation it sharpens and shifts to about 4.6 ppm. Mixtures of the conformations exhibit peaks at both positions. However, when the backbone is folded into an α-helix, it is difficult to detect by NMR the peaks due to the α-CH, β-CH₂, and γ-CH₂. With 200 MHz NMR, these peaks are noticeable but the β- and γ- methylenes overlap considerably. Somewhat unfortunate is the fact that the CH₂-O- peak due to the stearyl side chain is located at the same chemical shift as the α-CH of the backbone. This masks the presence of the α-CH although integration of the peak areas clearly demonstrates that the two peaks are overlapped. One other problem encountered when analyzing PSLG with 1H NMR is that the long aliphatic side chain CH₂ peaks (1.27 ppm) so dominate the other peaks in the spectra. Figure 4.2 shows a typical 200 MHz 1H NMR spectrum of PSLG. Note that all of the above features are present in the spectrum. One way around these difficulties is to break the helix with an
appropriate protic solvent. Trifluoroacetic acid (TFA) is the solvent typically used. It competes with the intramolecular hydrogen bonding responsible for holding the backbone in a stiff helix, thus breaking the helix and causing the polymer to assume a more random conformation. As Figure 4.2 shows, when a chloroform solution of PSLG contains about 10% TFA, the peaks become more cleanly resolved, and because the polymer is in a random conformation, the α-CH is shifted out from under the CH₂-O- peak to 4.6 ppm. These peak assignments for the α-CH in the random coil are consistent with observations [179, 180] of the α-CH in PBLG. A 50.25 MHz ¹³C NMR of PSLG run without added TFA does not show the peak due to the α-CH, the carbonyl in the backbone, or the β- and γ-carbons. After TFA is added, all of these peaks are present in the spectrum. Figure 4.3 shows the ¹³C spectra described above.

If small increments of TFA are added to the solution, one can determine the concentration necessary for complete helix disruption. Smith and Woody [181] followed the conversion of poly(γ-dodecyl-L-glutamate) (PDLG) from an α-helix to a random coil caused by TFA by using optical rotatory dispersion (ORD). Using PDLG with a molecular weight of 25 kg/mol, they determined that complete conversion to the random coil occurs at about 6% TFA. We carried out a similar experiment on PSLG-20K but followed the conversion of the helix-coil transition using NMR by observing the percentage of the α-CH peak that had shifted from 3.9 to 4.6 ppm as increasing amounts of TFA were added. This was done by comparing the areas obtained by integration of the peaks at 3.9 and 4.6 ppm. Figure 4.4 shows the results. Complete conversion of the helix was obtained at 5.66-6.54 % TFA, in good agreement with the results obtained on PDLG.
4.3 Liquid Crystalline Behavior and Differential Scanning Calorimetry

Liquids which show evidence of structure or alignment of their molecules can be called liquid crystals [182]. The liquid still takes the shape of its container but its molecules are ordered. Both melts and solutions can show liquid crystalline behavior, being broadly classified as thermotropic and lyotropic liquid crystals, respectively. There are usually common structural features in materials that display liquid crystalline behavior. Generally, if one wishes to design a molecule that will have liquid crystal characteristics, then the molecule must be geometrically highly anisotropic [182] and have these features: 1) it is usually narrow, sometimes with flat groups such as aromatic rings which extend the structure; 2) is rigid along one axis and 3) may be polar at one end and non-polar at the other end. Drawn in Figure 4.5 is cholesteryl benzoate, a molecule known since the 1880's to display liquid crystalline behavior in the melt [183-185]. Note that cholesteryl benzoate contains the structural features outlined above.

Liquid crystals can be broadly classified in three categories; each drawn schematically in Figure 4.6. They can be smectic, nematic, or twisted nematic which is also named cholesteric due to the fact that molecules containing the cholesterol moiety, i.e. cholesteryl benzoate, were among the first discovered to display such a liquid crystalline structure. The cholesteric liquid crystals are of particular interest here because PSLG displays this type of structure both as a melt and in solution. Cholesteric liquid crystals are twisted, stacked, planes of nematic structure, the twisted planes being due to anisotropic electrodynamic
forces that can be traced to a chiral center in the molecules. It is the chiral
center which makes the twisted stacking arrangement possible. In PSLG, the \( \alpha \)-
CH in the backbone is chiral (L form); because the polymer consists of a polar,
rigid backbone with long non-polar chains attached, the structure fits the general
pattern typical in compounds that display liquid crystallinity.

Liquid crystal textures can be viewed through a light microscope equipped
with crossed polarizers. The sample to be studied is placed on the microscope
stage between the crossed polarizers. When polarized light strikes the sample it
is rotated out of its original plane if the sample is birefringent. With an analyzer
in place (a polarizer adjusted so that it is perpendicular to the plane of the
polarized light striking the sample) between the sample and the eyepiece, the
rotated light is transmitted to the observer and the unrotated light is blocked.
Thus, if the sample is isotropic, it appears dark. If anisotropic, the observer will
see bright spots of light in the sample; particular patterns or textures will be
present depending upon the structure of the liquid crystal. Pictures of both
thermotropic and lyotropic liquid crystals of PSLG were taken and observations
on the texture of several solutions of varying concentration of PSLG in toluene
are tabulated in Table 4.1. From these observations, an A-point [162] (as defined
by Robinson) was determined for 248K PSLG. The A-point is the lowest
concentration at which the polymer solution develops an anisotropic phase. As
Table 4.1 shows, the onset of anisotropic solutions of PSLG-248K at 25\(^\circ\) C is
between 15.7 and 16.16 wt %. That is, the A-point for PSLG-248K is about 16 wt
%. The A-point can be predicted [186,187] by using Eq. 4.1 and 4.1a below.

\[
Flory: \quad \psi' = \frac{8}{x(1-2/x)} \quad \text{Eq. 4.1}
\]
Onsager: $\psi' = 4/x$ \hspace{1cm} Eq. 4.1a

$x$ is the axial ratio, $L/d$

$\psi'$ is the volume fraction

Using Eq. 4.1 and $d = 3.7$ nm (which is determined in section 4.6), the A-point is predicted to be 30 wt. %. Eq. 4.1a predicts 16.7 wt. %. Another calculation of the diameter (again, presented in section 4.6) yields 2.3 nm. Using this value to calculate $x$ gives 16.5 wt.% from Eq. 4.1 and 8.7 wt.% from Eq. 4.1a. These results lead to a dilemma in deciding which theory predicts the A-point with better accuracy. As will be discussed in section 4.6, the value for the diameter of PSLG in solution is more likely to be closer to 3.7 nm. If this is the case, the Onsager theory more closely predicts the A-point for PSLG than does the Flory equation.

Cholesteric liquid crystals display a "fingerprint" pattern of wavy lines with equal spacing if the distance between the pitches are greater than about 1 $\mu$m. Figure 4.7 shows a picture of the cholesteric liquid crystals viewed. The sample is about a 26 % toluene solution of PSLG-48K at 70º C. In Figure 4.7, the pitches were calculated to be 2.36 $\mu$m apart.

Two observations should be mentioned from these experiments. High molecular weight samples form lyotropic liquid crystals at much lower concentrations than low. Very low molecular weight samples (DP less than 100) don’t appear to display liquid crystalline behavior, at least not on the same time scale as the higher molecular weight samples (which show the behavior immediately upon melting). There is a minimum concentration necessary for the anisotropic solution [188] to form which is molecular weight dependent.
Concentrations lower than this critical value are isotropic.

Table 4.1. Observations\(^a\) of linear PSLG samples through crossed polarizers.

<table>
<thead>
<tr>
<th>mol. weight(^b)</th>
<th>L/d(^e)</th>
<th>film 25° C</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>248,000</td>
<td>26.3</td>
<td>melt</td>
<td>cholesteric pitches</td>
</tr>
<tr>
<td></td>
<td></td>
<td>melted</td>
<td>birefringent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>reheated</td>
<td>birefringent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.0 % sol'n 25° C</td>
<td>blank</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.2 %</td>
<td>blank</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.8 %</td>
<td>blank</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.2 %</td>
<td>blank</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.7 %</td>
<td>anisotropic and isotropic regions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16.2 %</td>
<td>areas of cholesteric pitches</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.7 %</td>
<td>strong birefringence</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.0 %</td>
<td>spherulites, strong birefringence, indication of cholesteric pitches</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21.6 %</td>
<td>strong birefringence</td>
</tr>
<tr>
<td>48,000</td>
<td>5.1</td>
<td>film</td>
<td>cholesteric pitches</td>
</tr>
<tr>
<td></td>
<td></td>
<td>melt</td>
<td>birefringent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>reheated</td>
<td>birefringent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26 % sol'n 70° C</td>
<td>cholesteric pitches, strong fingerprint pattern</td>
</tr>
<tr>
<td>20,000</td>
<td>2.1</td>
<td>film</td>
<td>blank</td>
</tr>
<tr>
<td></td>
<td></td>
<td>melt</td>
<td>blank</td>
</tr>
<tr>
<td></td>
<td></td>
<td>reheated</td>
<td>blank</td>
</tr>
<tr>
<td>209,000(^c)</td>
<td>22.2</td>
<td>film</td>
<td>birefringent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>melt</td>
<td>birefringent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>reheated</td>
<td>birefringent</td>
</tr>
</tbody>
</table>

\(a\) Films cast from toluene. Solutions in toluene wt/wt %.
\(b\) Molecular weights obtained from static light scattering.
\(c\) Trans-esterified PMLG; ca. 85 % SLG repeat units.
\(d\) Sample was cooled to room temperature and then reheated.
\(e\) L from Table 4.5. d = 3.7 nm.
Differential scanning calorimetry (DSC), is useful for observing such phenomena in polymers as melting points, phase transitions, decomposition points, or loss of small portions of the structure such as carbon dioxide or water. Liquid crystal phase transitions are also discernible in a DSC thermogram. Indeed, [189] DSC analysis is often used as a complementary tool to light microscopy for determining liquid crystalline phase transitions in polymers and low molecular weight materials. One of the effects of the long stearyl side chains is the dramatic decrease in the melting point of the polymer when compared to other poly(L-glutamates) such as PMLG or PBLG. PSLG melts at around 60° C, indicated by the DSC thermograms. Watanabe et al. DSC data indicate one endothermic transition in the thermogram of PSLG (produced by the transesterification of PMLG) at about 62° C which he assigns to both the melting point of the side chains and the onset of a thermotropic liquid crystal phase transition. Watanabe et al. DSC data on PXLG samples with shorter side chains (10, 12, 14, and 16 methylene groups), however, show two endothermic transitions, the lower temperature one being assigned as a melting point of the side chain, and the higher temperature (and smaller) transition being assigned as a liquid crystal phase transition. Both transitions shift to higher temperatures as the length of the side chain increases. Table 4.2 shows these results.

It seems unusual that PSLG would not exhibit a cleanly resolved, second transition. With our samples, we wished to use DSC techniques to verify Watanabe et al. DSC data. Also, crossed polarized light microscopy was used to aid in identification of the transitions observed in the DSC thermograms. Our PSLG produced from the modification of PMLG gave a thermogram with a single
endotherm, identical to Watanabe et al. results. However, PSLG synthesized from the monomer showed two endotherms, one at the expected 62° C and another, smaller transition at about 68° C. In lower molecular weight PSLG (DP less than 100), however, the second transition was absent. When the polymers were melted

Table 4.2. Watanabe et al. [75] DSC thermogram results.

<table>
<thead>
<tr>
<th>PXLG</th>
<th>1st transition °C</th>
<th>2nd transition °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>decyl</td>
<td>-26</td>
<td>30</td>
</tr>
<tr>
<td>dodecyl</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>tetradecyl</td>
<td>41</td>
<td>61</td>
</tr>
<tr>
<td>hexadecyl</td>
<td>54</td>
<td>64</td>
</tr>
<tr>
<td>octadecyl (stearyl)</td>
<td>62</td>
<td>--</td>
</tr>
</tbody>
</table>

a Synthesized from the corresponding NCA monomer.
b Made by ester exchange reaction using PMLG and the appropriate alcohol.

and observed through crossed polarizers at 70° C, they were highly birefringent, except for a low molecular weight PSLG which showed no anisotropic behavior upon melting. Figures 4.8, 4.9, 4.10, and 4.11 show the thermograms described above. Also included in these figures are light intensity data obtained by heating the polymers with the same temperature ramp as the DSC thermograms (2° C per minute) on a hot stage placed between crossed polarizers on a light microscope. The trace overlayed on the thermogram is the light intensity detected with change in temperature.

Before analyzing the thermograms, it is important to outline the potential
problems in analyzing the I vs. T traces as well as outline the potential complexity due to the unusual structure of PSLG. As the light intensity data were collected with crossed polarizers in place, the changing intensity is due to changes in crystallinity or order of the polymer with temperature. That is, if the sample becomes birefringent at any point in the heating or cooling cycle, an increase in detected light intensity will be observed. However, the light intensity observed can also be due to multiple scattering as the sample develops turbid spots upon cooling. These turbid spots could both increase or decrease the intensity of the light detected. The detector could pick up light which is scattered from these turbid spots. The other possibility also is that cloudy regions in the sample simply block the light that would otherwise be detected, resulting in a decrease in light intensity.

Thermal analysis of PSLG could be complicated by potentially complex side chain and backbone ordering. For example, the long side chains can crystallize between the backbones but the crystallites will not necessarily have the same crystal structure throughout the sample. This would have the effect of broadening the side chain melting transition, much like the broad melting range of an impure compound. Because the side chains are so long, the motion of the backbone may be uncoupled to the motion of the side chains when the sample melts. That is, the side chains could become a "solvent" for the backbones, allowing the polymer to move after the melting transition of the side chains. According to Watanabe et al. [75], only the last eight to ten methylenes of the side chain are involved in crystallization as the polymer is cooled, with the eight methylenes closest to the backbone maintaining an unordered structure. Given the complex thermal
behavior of PSLG, the interpretation of its DSC thermograms can be a difficult problem to solve.

Also, it is important to mention here, before a discussion of the thermal data, the "solution-drying history" of the polymer. Since PSLG forms lyotropic liquid crystals, the reaction solutions which are precipitated could very well be ordered solutions before precipitation (particularly the ones which were first concentrated, then precipitated). This means that the polymer will be, to one degree or another, somewhat crystalline (alignment of backbones) to begin with. During vacuum drying, crystallization of the solid can also occur. Low molecular weight samples of PSLG always precipitated as fine powders. The higher molecular weight material precipitated as either a fibrous material or a cloudy, film-like solid. These differences indicate a difference in ordering of the polymer which is molecular weight dependent and could explain differences in thermal behavior. For example, a polymer which is highly ordered may require more heat to break backbone-backbone interactions whereas an unordered solid may melt completely at a lower temperature.

Each polymer discussed below starts as a white solid, becomes clear in the melt, and remains clear upon cooling. These appearances affect how much light is detected as the sample undergoes thermal transitions.

Figure 4.8 shows the thermograms produced from analysis of PSLG-EX. On the first heating, the thermogram shows one endotherm. The light intensity trace shows that at the same temperature as the endotherm there is a rise in the intensity of light detected. Beyond the endotherm, the light intensity rises sharply, indicating that at about 70° C, the sample becomes highly birefringent.
Visual observations of the sample support this description. When PSLG-EX is held at 63° C and observed through crossed polarizers, there is no indication of birefringence, even though it is beyond the temperature of the endotherm. At 70° C, the sample is birefringent in all areas. The initial rise in the light intensity at about 59° C is probably due to the clearing of the sample as it melts and goes from an opaque solid to a clear liquid. Upon slow cooling, the polymer displays an exotherm at about 48° C, a transition due to the crystallization of the stearyl side chains. The fact that the transition temperature is somewhat lower than the melting temperature indicates that the side chains become supercooled during the cooling cycle. The sample stays clear after freezing and this clear solid is also birefringent. The light intensity trace shows a dramatic drop in intensity when the sample solidifies, but then a rapid recovery of the intensity. The recovery of intensity is puzzling but may be due to birefringence caused by the stearyl side chain crystallites which form when the polymer solidifies. This explanation is based on the behavior of the second heating thermogram. As the polymer is reheated, the light intensity drops, presumably because the side chains are now becoming disordered. When the side chains are fluid at about 58° C, the polymer backbones can align, giving rise to a sharp increase in the birefringent regions in the sample and thus the amount of detected light intensity. In the second heating, the endotherm appears reduced somewhat compared to the first heating (certainly, the transition is broader), indicating the polymer side chains are more crystalline before they have a thermal history. That is, fewer side chains crystallites have formed on the cooling cycle.

As mentioned previously, the PSLG samples synthesized from the monomer
display two endotherms in their heating thermograms, as Figure 4.9 shows. The thermograms shown in Figure 4.9 are from analysis of PSLG-248K. Again, the first endotherm is due to the melting of the side chain crystallites, the assignment of the second transition is somewhat more challenging but based on Watanabe’s data in Table 4.2, it is tempting to assign the second transition as the liquid crystal phase transition of the melt. When the polymer is melted and observed between crossed polarizers mounted on a light microscope, there is no visible birefringence at temperatures at or below 63° C, a temperature between the two transitions. At temperatures beyond the second transition, the polymer is highly birefringent. This indicates that the second transition is due to a phase transition of the melted polymer. The light intensity trace on the first heating cycle shows the rapid increase in light intensity detected when the side chains melt. The intensity drops and then recovers after the second transition, indicating that the polymer chains are becoming ordered after about 70° C. The second and thirdheatings show that first endotherm becomes poorly resolved and broad, indicative of poorly crystallized side chains after each cooling cycle. The light intensity data for these heating cycles show the rapid increase in light intensity between the two endotherms, which supports the assignment of the second transition as being due to an ordering of the polymer backbones. Also notable is the rapid decrease in light intensity as the polymer is heated from 30-55° C. The reason is the same as that described above for PSLG-EX. The small rise in light intensity between 58-61° C is probably due to clearing of the sample when the sidechains melt. Heating thermograms for PSLG-248K beyond the third heating cycle are identical to the third heating. The cooling thermograms for PSLG-248K are remarkably
sharp and also indicate that the sidechains are supercooled. The decrease in light intensity with cooling from 61-55° C is probably due to some cloudiness which develops as the sample cools. The sharp rise in light intensity at 45° C is described above for the PSLG-EX cooling thermogram.

Further support for the assignment of the 2nd transition as being due to polymer ordering is found in the thermograms of PSLG-20K. This low molecular weight PSLG displays only one transition in its thermogram and is not birefringent. Figure 4.10 shows the DSC thermograms for PSLG-20K. The polymer only has an axial ratio (length/width) of about 2.1 and was not expected to form liquid crystals. Generally, [190] axial ratios of 5-6 or greater are required to form liquid crystals. This polymer, when viewed between crossed polarizers, showed no birefringence at 70° C. The endotherm at 61° C is present in the first heating thermogram but the second endotherm is absent. This supports the assignment of the higher temperature transition as being due to a liquid crystal phase transition. The light intensity trace also shows that the intensity of light detected increases as the polymer melts. Upon cooling, there is no exotherm due to side chain recrystallization, meaning that when the polymer is cooled, the side chains stay randomly oriented. Upon reheating, there should be no melting transition present due to side chain crystallites, based on the lack of an exotherm in the cooling thermogram. As Figure 4.10 shows, no endotherm was detected. The lack of liquid crystalline behavior in this sample indicates that the rods are not aligning in a parallel manner. We can infer from the lack of the exotherm and endotherm described above then, that a parallel alignment of the backbones helps the sidechains to crystalize. This behavior is not evident in PSLG-20K.
In Table 4.3 the "area" under the transitions for PSLG-248K is recorded. These areas are proportional to the transition enthalpy and were obtained by plotting the thermograms on the same scale and then carefully cutting out and weighing the peaks. A plot of transition area vs. cycle no. is shown in Figure 4.12. There is essentially no difference in the enthalpy for the freezing of the side chains. However, when comparing the transitions of a first and third heating cycle, the area under the first transition has decreased and the area under the second transition has increased in the third heating cycle as Table 4.3 shows. The decreased area under the first transition in the third heating is consistent with the description of poorly crystallized side chains after a cooling cycle compared to the sample before it has a thermal history.

Table 4.3. Peak areas from PSLG-248K DSC thermograms.

<table>
<thead>
<tr>
<th>Cooling cycle no.</th>
<th>Exotherm Area (arbitrary units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26.90 ± 0.60*</td>
</tr>
<tr>
<td>2</td>
<td>27.87 ± 0.60</td>
</tr>
<tr>
<td>3</td>
<td>27.34 ± 0.40</td>
</tr>
<tr>
<td>4</td>
<td>26.50 ± 0.50</td>
</tr>
<tr>
<td>5</td>
<td>24.14 ± 1.40</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Heating cycle no.</th>
<th>1st Endotherm Area</th>
<th>2nd Endotherm Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47.27 ± 1.60</td>
<td>6.15 ± 0.20</td>
</tr>
<tr>
<td>3</td>
<td>37.49 ± 0.90</td>
<td>7.29 ± 0.60</td>
</tr>
</tbody>
</table>

a Error bar results plotting each thermogram 3 times and weighing the peak area from each.
Finally, Figure 4.11 shows the DSC thermograms of a PSLG sample (PSLG50K) which is "intermediate" in its thermal behavior. That is, upon first heating, only one endotherm is observed, much like the behavior of PSLG-20K. However, upon cooling, the exotherm is observed. During the second heating cycle, the first endotherm becomes broad and the second endotherm appears. There is apparently a "break-point" where, at a certain molecular weight, the second endotherm is no longer observed. For PSLG, this break appear to be at about 40-50K. It is in this molecular weight range too, that the axial ratio of PSLG is roughly at the minimal value where liquid crystallinity can still be expected according to Flory [187].

The DSC data for PSLG-248K can be pictorially summarized as shown in Figure 4.13. Before the polymer has a thermal history, it could be represented schematically by Figure 4.13 A. The "straight" side chains represent a high degree of crystallization, where the length of the side chains involved in the crystallite is the same (i.e. the crystallite represents a pure compound where all the molecules in the crystal are the same). After the first endothermic transition, the side chains become fluid, represented by the wavy side chains in Figure 4.13 B. However, the polymer as a whole at this point is solid, where there is no motion of the backbones. After the second endothermic transition, the sample becomes fluid and the polymer backbones can move or align as shown in Figure 4.13 C. This also explains why melt birefringence is not observed until a temperature several degrees above the melting point of the side chains. Upon cooling, at a temperature between the two transitions, the polymer can solidify while still having melted side chains as shown by the wavy side chains in Figure 4.13 D. At
the exothermic transition, the side chains crystallize, but their crystallites are poorly developed by comparison the polymer prior to heating, represented schematically in Figure 4.13 E by "bent" side chains. That is, the lengths of the side chains involved in the crystallites are different, much like having a long chain alcohol contaminated with shorter chain alcohols. This makes sense in terms of the clear nature of the sample after cooling. That is, if the side chains were crystallized more fully, the sample should be considerably more cloudy. Also, the fact the heating cycles beyond the first one exhibit a very broad side chain melting endotherm is indicative of "impure" stearyl crystallites.

4.4 Gel Permeation Chromatography

Gel permeation chromatography (GPC) is a technique used to separate macromolecules based upon their size. GPC column packing material typically consists of a crosslinked polystyrene network that contains pores of a known size. Columns containing increasingly smaller pore sizes are placed in series and the sample is eluted through each column to effect separation. Alternatively, one column which is packed with different pore sizes (mixed bed) may be used. The higher molecular weight polymers elute first because they are included in few or none of the pore sizes in the column(s). Small polymers elute more slowly because they are small enough to fit in most or every pore size and are thus held up on the column longer. GPC is a simple technique for quickly obtaining relative size information on polymers of the same type [191,192]. If molecular weights are determined by some other technique (such as light scattering), a GPC calibration curve can be constructed from the polymer of interest by plotting the log M vs. retention time and then other unknown molecular weight samples can
be run on the GPC under the same conditions to rapidly obtain a molecular weight. Typically, known molecular weight polystyrene standards which are quite monodisperse are used to construct a calibration curve. Then, any polymer that is a random coil can be compared to the calibration curve to obtain molecular weight and polydispersity information. Polymers other than random coils cannot be compared to the polystyrene standard curve because differences in their hydrodynamic volume make the comparison invalid. A universal constant can be used to correct for the structural differences, but it is generally a good idea to prepare a calibration curve from the polymer being studied to ensure accuracy from GPC data. Thus, molecular weights determined by static light scattering

Table 4.4 GPC data for linear PSLG.

<table>
<thead>
<tr>
<th>mol. weight *</th>
<th>retention time (min.)</th>
<th>GPC M(_w)</th>
<th>GPC M(_n)</th>
<th>M(_w)/M(_n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>248,000</td>
<td>7.70</td>
<td>254,900</td>
<td>90,300</td>
<td>2.82</td>
</tr>
<tr>
<td>175,000</td>
<td>7.93</td>
<td>150,600</td>
<td>75,700</td>
<td>1.99</td>
</tr>
<tr>
<td>148,000</td>
<td>8.17</td>
<td>156,800</td>
<td>78,900</td>
<td>1.99</td>
</tr>
<tr>
<td>126,000</td>
<td>8.40</td>
<td>100,200</td>
<td>64,600</td>
<td>1.55</td>
</tr>
<tr>
<td>93,000</td>
<td>8.63</td>
<td>67,200</td>
<td>50,100</td>
<td>1.34</td>
</tr>
<tr>
<td>47,000</td>
<td>8.87</td>
<td>51,900</td>
<td>40,900</td>
<td>1.27</td>
</tr>
<tr>
<td>38,200(b)</td>
<td>9.33</td>
<td>35,400</td>
<td>31,600</td>
<td>1.12</td>
</tr>
<tr>
<td>20,000</td>
<td>10.50</td>
<td>32,500</td>
<td>25,300</td>
<td>1.28</td>
</tr>
</tbody>
</table>

\(a\) Molecular weights used in calibration curve, Figure 4.14  
\(b\) Primary amine initiation. Value calculated from \{M\}:\{I\} ratio

(section 4.6) for several PSLG samples were used to construct a GPC calibration
curve. GPC analysis also gives a qualitative indication of the polydispersity of the sample. The broader the peak, the more polydisperse the sample. Figure 4.14 shows the GPC calibration curve obtained for PSLG using Nelson Analytical software. The molecular weights used for the curve were taken from the value obtained by light scattering after rounding it to the nearest thousand. This value generally has about a ±5% error associated with it but the GPC software only plots one number and not a range for the molecular weight and retention time. Figure 4.15 shows typical GPC chromatograms obtained for the calibration curve. Not shown is a toluene internal standard peak which consistently eluted at the same retention time. For the most part, the curves are a single, but somewhat broad, peak—indicative of a polydisperse sample. Using the calibration curve, the GPC data from the same samples that were used to construct the curve were run through the GPC software as "unknowns" so that their peak areas could be calculated and analyzed to obtain an indication of their polydispersity. Table 4.4 summarizes the results. The polydispersity index, $M_w/M_n$, was < 2 in all but one case. The primary amine initiated polymers had more narrow molecular weight distributions, having a polydispersity index < 1.2. Their chromatograms are shown in Chapter 2. The samples used to construct the calibration curve were all sodium methoxide in methanol initiated reactions with the exception of the sample plotted as 38200 molecular weight. It was a primary amine initiated polymer, with an $\{M\}:\{I\} = 100$. It was plotted to see how well the calculated molecular weight based on the $\{M\}:\{I\}$ ratio fit the curve.

The polydispersity of polymers initiated by sodium methoxide in methanol is undoubtedly due to a combination of two factors. Chains can be initiated by the
presence of methanol. Also, because the methoxide initiated polymerizations can enhance their molecular weight through chain coupling as discussed in Chapter 2, some of the chains that couple are not the same size or some do not couple at all. Thus, polymers formed in this process have more ways to become polydisperse than in primary amine initiated polymerizations. Unfortunately, the primary amine initiation does not produce high molecular weight polymers as mentioned in Chapter 2.

4.5 Laser Light Scattering

Static Light Scattering

Static light scattering (SLS) is a technique used for obtaining size and thermodynamic information about a polymer molecule. The measurement of the average intensity of light scattered by a polymer solution at various angles is the basis for SLS experiments ("average" intensity because the intensity fluctuates as the polymer chains diffuse. This is the basis for dynamic light scattering discussed later in this section). The molecular weight of the molecule is proportional to the intensity of the scattered light. That is, scattering increases with increasing molecular weight; SLS is an absolute measurement of molecular weight. Specifically, SLS provides a direct measurement of the weight average molecular weight, $M_w$. A weight average molecular weight is obtained because a given weight of larger molecular weight chains in the solution makes a greater contribution to the scattering intensity than an equal weight of smaller chains. The molecular weight average obtained is skewed toward the higher end of the distribution of the molecular weight of the sample. Given several sizes of a
particular polymer and a good solvent for light scattering, the bigger polymers are the easiest to analyze with SLS. This differs from techniques which rely on colligative properties for obtaining molecular weight. The success of these techniques, such as osmometry, depends upon the number density of solute particles present, not their mass density. The bigger the polymer, the fewer number of chains a given weight of it contains; hence, the techniques which depend upon colligative properties become difficult to use with higher molecular weight polymers. A number average molecular weight, Mn, is obtained from these techniques.

Scattering intensity is also dependent upon the concentration of the solution being measured and upon the scattering difference between the solution and the solvent itself. SLS experiments are typically done using dilute polymer solutions so that polymer-polymer interactions are minimized. This means that the intensity of scattered light is due to the sum of the scattering from individual polymer chains, not polymer clusters or aggregates where the scattering intensity from a given polymer chain would undergo interference by scattered light from other polymer molecules. The polymer solution must have a reasonably large differential index of refraction, dn/dc, in order to have an appreciable amount of scattering above the solvent scattering intensity. In fact, some polymer-solvent combinations are isorefractive [193]; that is, the polymer is "invisible" in a light scattering experiment because there is no difference between the refractive index of the solvent and the polymer solution.

For molecules as big as polymers, there is also angular dependence of the intensity of the light scattered. If the polymer dimensions are greater than about
1/20th of the wavelength of the light used in the experiment, it is no longer a "point" scatterer; i.e., the polymer will scatter light of different phases from different parts of the chain. Destructive or constructive interference of the light scattered from different parts of the chain will lead to differences in the intensity of the light detected at various angles. Because the shape of the molecule will influence the scattered intensity, measurement of the scattering at different angles gives information about the dimensions of the polymer, such as the radius of gyration, $R_g$. The influence on the scattered intensity which is due to phase interferences from the same molecule is designated by a function known as the particle form factor, $P(\theta)$. Figure 4.16 shows a plot of $1/P(\theta)$ vs. $q^2 R_g^2$, where the value $q = (4\pi n/\lambda_o)\sin\theta/2$. The value $n$ is the refractive index of the polymer solution, $\lambda_o$ is the wavelength of the incident light, and $\theta$ is the scattering angle. This plot shows how the shape of the particle influences the scattering intensity at a given angle. Note that at very low angles (or, as $1/P(\theta)$ approaches 1), the curves all coincide, meaning that polymer shape cannot be determined from low angle measurements alone. Only at high values of $qR_g$ can the shape of the polymer be determined. Sample polydispersity complicates the determination of the shape. Shape cannot be determined for samples with broad molecular weight distributions. The SLS experiments in this chapter were typically done in the range of 30-135°.

Finally, scattering increases with increasing concentration of polymer in solution; i.e., $I \propto cM$. If varying concentrations of a polymer solution are measured, the osmotic second virial coefficient, $A_2$, can be obtained. The magnitude of the second virial coefficient provides information on polymer-
polymer, polymer-solvent interactions.

To obtain weight average molecular weight, radius of gyration, and the second virial coefficient from an SLS experiment, measurement of several concentrations of polymer solution at several scattering angles is required. The standard procedure is to graphically represent the data on a Zimm plot [194] which provides the desired information about the polymer on a single plot. The equation below shows the relationship represented by the Zimm plot and shows that by extrapolating to $c = 0, \theta = 0$, and the point at which both $= 0$, the $M_w$, $R_g$, and $A_2$ can all be obtained.

$$Kc/R_s = 1/M_w(1 + (16\pi^2R_g^2/3\lambda^2)\sin^2\theta/2) + 2A_2c$$

where $K$ is an optical constant containing the differential index of refraction, $dn/dc$

$c$ is the concentration

$R_s$ is the Rayleigh ratio

$\lambda = \lambda_o/n$, $n$ is the refractive index of the solution.

Zimm plot is a plot of $Kc/R_s$ vs. $\sin^2\theta/2 + kc$, where $k$ is a scaling constant. At the limits of:

$c = 0$ and $\theta = 0$,

$M_w = R_s/Kc$, where the molecular weight is obtained at the y-intercept of the Zimm plot.

$c = 0$,

$R_g^2 = 3\lambda^2/16\pi^2$(slope $c=0$ line/intercept of $c=0$ line)

$\theta = 0$,

$A_2 = $ slope $\theta=0$ line/2

Necessarily, the $\theta = 0$ line and the $c = 0$ line intercept at an identical point on the y-axis.

The discussion below describes the results of static light scattering
experiments obtained for varying molecular weights of linear PSLG. The solvent used in these experiments was THF and the concentration range measured was typically 0.1-0.5 wt/vol. %. PSLG solutions in the concentration range studied display a $dn/dc$ value of $0.08 \pm 0.002$ at $\lambda_0 = 488.0$ nm.

Figure 4.17 shows Zimm plots of two linear PSLG samples; PSLG-96K and PSLG-155K. Table 4.5 summarizes the SLS results for several linear PSLG samples. With these data, it is possible to calculate the diameter of the polymer, the pitch/residue value of the $\alpha$-helix, and demonstrate that the polymer behaves as a stiff rod. The diameter of mutual exclusion can be calculated using the experimental $A_2$ data. The relationship used to make the calculation is shown below. This is the Onsager-Zimm-Schulz equation [195] which relates molecular dimensions to the second virial coefficient.

$$A_2 = \pi N_A d L^2 / 4M^2 \quad \text{Eq. 4.2}$$

where $N_A$ is Avogadro's number
$d$ is the diameter
$L$ is the length of the rod
$M$ is the molecular weight

Table 4.5 shows the results of the calculations. The length of the rod was calculated from the molecular weight assuming that the helical pitch/residue is 0.15 nm [55]. Therefore, the equation $L = 0.15M_w/382$ was used to calculate the length where 382 is the repeat unit molecular weight. An average value of $d = 3.7 \pm 0.6$ Å was obtained. Eq. 4.2 is only accurate when the length of the polymer is considerably longer than the diameter, i.e., infinitely thin rods. This restriction may account for the discrepancy in the value for the diameter obtained for the lower molecular weight PSLG's. The length of the rod can also be calculated
Table 4.5. Summary of data obtained for linear PSLG from SLS.

<table>
<thead>
<tr>
<th>M_w/10^5 daltons</th>
<th>Rg/nm</th>
<th>(A_2/10^4\ cm^2\ mol^{-2}\ [\eta]/dl\ g^{-1}\</th>
<th>L/nm</th>
<th>L_Rg/nm</th>
<th>d/nm</th>
<th>d_Rg/nm</th>
<th>h/nm</th>
<th>((M_2/M_{2+1})^x)</th>
<th>M_w^{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.09 ± 0.10</td>
<td>32.7 ± 2.0</td>
<td>1.92 ± 0.10</td>
<td>1.48</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.177</td>
<td>1.18</td>
</tr>
<tr>
<td>2.48 ± 0.12</td>
<td>33.2 ± 2.0</td>
<td>2.49 ± 0.13</td>
<td>1.21</td>
<td>97.4</td>
<td>115.0^d</td>
<td>3.4^e</td>
<td>2.4</td>
<td>0.199</td>
<td>1.33</td>
</tr>
<tr>
<td>1.85 ± 0.09</td>
<td>27.9 ± 1.6</td>
<td>2.33 ± 0.12</td>
<td>0.83</td>
<td>72.6</td>
<td>96.6</td>
<td>3.2</td>
<td>1.8</td>
<td>0.197</td>
<td>1.32</td>
</tr>
<tr>
<td>1.55 ± 0.08</td>
<td>23.2 ± 3.0</td>
<td>3.04 ± 0.21</td>
<td>0.64</td>
<td>61.1</td>
<td>80.4</td>
<td>4.2</td>
<td>2.4</td>
<td>0.197</td>
<td>1.31</td>
</tr>
<tr>
<td>1.21 ± 0.06</td>
<td>18.0 ± 2.0</td>
<td>1.96 ± 0.13</td>
<td>0.46</td>
<td>47.5</td>
<td>62.3</td>
<td>2.7</td>
<td>1.6</td>
<td>0.179</td>
<td>1.19</td>
</tr>
<tr>
<td>0.96 ± 0.05</td>
<td>13.0 ± 2.0</td>
<td>3.24 ± 0.18</td>
<td>0.25</td>
<td>37.7</td>
<td>45.0</td>
<td>4.5</td>
<td>3.1</td>
<td>0.179</td>
<td>--</td>
</tr>
<tr>
<td>0.47 ± 0.02</td>
<td>b</td>
<td>2.88 ± 0.16</td>
<td>0.16</td>
<td>18.5</td>
<td>--</td>
<td>4.0</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

**Ave.** 3.7±0.6 \ 2.3±0.5 \ 0.190±0.010

- **a** PSLG-EX
- **b** Polymer too small to reliably measure Rg.
- **c** \(L = M_w(0.15\ nm)/M_0\)
- **d** \(L_Rg = Rg(12^b)\)
- **e** Calculated using the value of L.
- **f** Calculated using the value of \(L_Rg\).
using \( L_{rg} = 12 R_g \). The \( R_g \) subscript is used only to distinguish between the two values. Table 4.5 shows the results. Using the length calculated in this manner in Eq. 4.2, a global average for \( d_{rg} \) is \( 2.3 \pm 0.5 \) nm. The fact that the value for the length is larger when calculated from \( R_g \) shows the effect polydispersity has on calculations of polymer dimensions. As will be discussed later in this section, the value of \( R_g \) obtained from light scattering is an average that weights the bigger polymers more heavily. For this reason, a larger value will be obtained for the length when its calculation is based on \( R_g \). The \( 3.7 \) nm value is probably a more reasonable estimate of the diameter of PSLG based on the Onsager equation. As shown below, when the polymer is treated as a solid cylinder, \( d = 2.3 \) nm. This result indicates that calculating the diameter from Eq. 4.2 using rod lengths based on \( R_g \) yields a value which is unreasonably low. Also, calculations from dynamic light scattering data discussed later in this section yield a hydrodynamic diameter of \( 3.6 \pm 0.2 \) nm, indicating that the larger value is a better estimate of the diameter of the rod in solution.

As mentioned above, Eq. 4.2 gives a diameter of mutual exclusion. The diameter can also be calculated [56] by assuming that PSLG is a solid cylinder. By cutting a cylinder out of the rod \( 1 \) A long and equating it to the linear mass density (i.e., the molecular weight of the repeat unit divided by its length, \( h \)), Eq. 4.3 below can be used to calculate the diameter. This value may or may not be the same as that obtained from Eq. 4.2. It depends on how dense the polymer chain actually is. For PBLG, the values are nearly identical [56].

\[
M_o/h = 1/4(\rho \pi d_p^2 N_A) \quad \text{Eq. 4.3}
\]
\[ d_p \] is the diameter. \( \rho \) subscript signifies the value is based on the density of PSLG.
\( h \) is the repeat length per monomer (1.5 Å)
\( \rho \) is the density of PSLG
\( M_0 \) is the repeat unit molecular weight

From densitometry experiments, the partial specific volume of PSLG was found to be 1.024 ml/g (\( \rho = 0.976 \) g/ml). From Eq. 4.3, \( d_p \) 2.3 nm, somewhat lower than \( d \). Since the diameter from Eq. 4.2 is somewhat larger than the solid cylinder diameter, it indicates that the side chains are probably extended and solvent permeable in solution; not nearly as compacted or folded back along the polymer backbone as a 2.3 nm diameter would suggest.

The diameter, \( d \), reported in Table 4.5 is in good agreement with a molecular model of linear PSLG constructed by the SYBYL molecular modeling software which is part of the LSU Macromolecular Computing and Analysis Facility. Figure 4.18 shows two views of the SYBYL structure with a DP of 20 (PSLG-DP20); a side view and an end-on view with the acid or initiator end facing out. It was minimized using the MAXIMIN2 routine with Tripos parameters. A noteworthy feature of the structure is the demonstration of how the stearyl side chains form a "sheath" around the \( \alpha \)-helical backbone by folding back along the chain with their terminal \( \text{CH}_3 \) end pointed toward the amino end of the polymer. If the diameter of the chain is measured at several points along the polymer a value of 4.1 ± 0.1 nm is obtained. The diameter is measured by allowing SYBYL to calculate the distance from a terminal \( \text{CH}_3 \) of a side chain protruding from one side of the helix to another \( \text{CH}_3 \) protruding from the other side of the helix. Measuring the diameter at the amino end of the chain gives a
value of $3.4 \pm 0.3$ nm. Another interesting feature of the model in Figure 4.18 is that at the acid end, the first few side chains are somewhat disordered. Apparently, the first few side chains in the early repeat units of PSLG are less crowded.

SYBYL is also capable of calculating the volume occupied by a compound. It does this by only calculating the volume that the atoms of the molecule occupy; space between atoms or groups in the molecule is not included. Using the value of the volume obtained by SYBYL we can again go back to a calculation of the solid cylinder diameter of PSLG. PSLG-DP20 has a volume of $V = 7.467$ nm$^3$ according to SYBYL. If we consider PSLG-DP20 as a simple solid cylinder with a length of $L_c = 3$ nm (where the subscript $c$ is used to signify cylinder and is used for clarity only), then we can calculate a value for the diameter of PSLG by using the equation for the volume of a cylinder, $V = \pi d_c^2 L_c$. From this equation, $d_c = 1.8$ nm. This means that if all the mass of PSLG-DP20 were "packed" into a cylinder without any empty space, the cylinder would only be 1.8 nm in diameter. If the SYBYL volume is correct, this value should be the same as the diameter obtained in Eq. 4.3. In light of the model in Figure 4.18 where there is 3-5 nm between terminal CH$_3$ groups in opposing stearyl side chains (the "diameter"), this result indicates that PSLG has a lot of "dead volume" in its chain.

SYBYL also indicates that the distance between 4 repeat units in the helix is .6015 nm, as shown in Figure 4.19. If we divide 0.6015 nm by 4 and then multiply by the known number of repeat units per turn which is 3.6, then the helical pitch is 0.5413 nm, in excellent agreement with the known value of 0.54 nm. The model indicates then that the long stearyl side chains do not seem to
bend the helix or change the pitch. It is worth mentioning, however, that application of modeling programs such as SYBYL have built-in "pitfalls". For example, the model shown in Figure 4.18 is not solvated. That is, no solvent-solute interactions are taken into account. Also, SYBYL uses a standard table of bond lengths, angles, etc. which may not always be applicable to the molecule of interest. When PSLG was built, the helical conformation was chosen—this means pre-existing bond lengths, angles, etc. are used to construct the helix. That is, SYBYL is given the "correct" conformation from the start. Upon, minimization, however, SYBYL will "break" or change a conformation if there is enough strain in the molecule.

Table 4.5 also shows that there is no particular dependence of $A_2$ on molecular weight. This is shown graphically in Figure 4.20 as a plot of $A_2$ vs. $M_w$. This is to be expected of rigid rods in the excluded volume limit and in good solvents. The plot is somewhat "noisy"; for this reason, a line was not drawn through the data points.

Using the $R_g$ data, a pitch/residue can be calculated by combining the equation above used to calculate the length and the relationship between the length of a rod and its $R_g$ value: $R_g^2 = L^2/12$. Thus $h$, the pitch/residue is $h = R_g(12^{1/2})382/M_w$. As mentioned previously, the known pitch/residue for a protein in an $\alpha$-helix is 0.15 nm. Table 4.5 summarizes the results of the calculations, which indicate an average value of 0.190 $\pm$ 0.010 nm. The average value from Table 4.5 is reasonably close to the expected value. Probably the deviation in this and other calculations made from the data obtained from these PSLG samples arise from the polydispersity of the samples. While these samples are
not terribly polydisperse (based on GPC data), calculations to obtain very precise
dimensions should ideally be made from data obtained from polymers with
Mw/Mn < 1.05. Other workers [196,197] have evaluated the pitch/residue
anywhere from 0.085-0.22 nm in PBLG. However, the dimension calculations
shown here based on these polymers appear to be fairly accurate. This indicates
that the molecular weight homogeneity of the PSLG samples studied was fairly
good. We can also obtain a value of h from a plot of Rg vs. Mw. In Figure 4.21,
the plot is shown. From the intercept of the line, h can be calculated. The
equation of this line is y = 1.32 ± .17 x 10^-4x + 1.81 ± 2.88. The slope of the line
equals h/M_w h. From this slope, h, the pitch/residue is 0.175 ± 0.022 nm, a little
closer to the known value than the global average in Table 4.5 predicts. We can
examine how polydispersity affects the calculation of h in the following way:
Light scattering gives a z average of <Rg^2>. That is, it weights the bigger
polymers more heavily in the average, resulting in an experimental Rg which is
always higher than the calculated value based on the weight average molecular
weight [198]. The difference in the experimental and calculated Rg is thus a
direct result of the polydispersity of the samples. We can show the discrepancies
caused by polydispersity by developing a different type of polydispersity index
which involves the z and z+1 average molecular weight. Because the higher
moments of the distribution are considered, the molecular weight distribution will
appear more homogeneous than the Mw/Mn obtained from GPC. Consider the
following:

<\text{Rg}^2> = \frac{M_z \cdot M_w \cdot h^2}{12M_w^2}

Then, \frac{(M_z M_{z+1})^h}{M_w} = 12^h \frac{\text{Rg} M_z}{h M_w} \text{ Eq. 4.4}
Using Eq. 4.4 and $h = 0.15$, the polydispersity index for each polymer was calculated which weights the bigger chains in the distribution more heavily. As Table 4.5 shows, the molecular weight distribution appears more narrow when we consider molecular weight averages that weight the bigger polymers more heavily. The fact that $(M_c M_a)^h M_w^{-1}$ is relatively close to 1 for each polymer when Eq. 4.4 is evaluated with $h=0.15$ indicates that the difference between this value and the experimental value of $h$ is due to sample polydispersity.

Because PSLG is an $\alpha$-helical polypeptide, it should behave as a rod in solutions that support the helix. As mentioned previously, $R_g$ is linearly related to $M_w$. In fact, rod-like polymer dimensions in general ($R_g$, length, $R_h$) are linearly related to the molecular weight which is not the case for gaussian chain polymers. This is because as the molecular weight of the rod-like polymer increases, the dimensions are increasing only in one direction or along one axis. In a random chain polymer, molecular weight increases also increase polymer dimensions but because the polymer can assume many conformations, the dimensions of the polymer necessarily don't "expand" or grow proportionately with the molecular weight. Thus, a plot of $R_g$ vs. $M_w$ should yield a straight line if the polymer is rod-like. Figure 4.21 shows this plot. The correlation coefficient of 0.982 is a little low and indicates that the straight line fit is a little "noisy". Another indication of rod-like behavior can be obtained by determining the Mark-Houwink $a$ value. The Mark-Houwink equation, $[\eta] = k M^a$, relates the intrinsic viscosity of a polymer to its molecular weight. Theoretically [199], $a = 0.5$ indicates that a random coil polymer was measured in a theta solvent, $a = 0.8$ is indicative of an expanded gaussian coil in the excluded volume limit, and $a > 1$
(upper limit of 1.8) indicates a stiff or rod-like polymer. A plot of the log[\eta] vs. log M gives the a value as the slope of the line and the k value as the y intercept. Figure 4.22 shows the Mark-Houwink plot obtained for linear PSLG. The intrinsic viscosity for each polymer was measured in THF and obtained by extrapolating \( \eta_{sp}/c \) vs. c and \( \eta_{inh} \) vs. c plots to zero concentration. Figure 4.27 shows a typical viscosity plot. The a value is \( 1.29 \pm 0.09 \), indicative of a stiff chain, although somewhat less stiff than PBLG which has an a value approaching the theoretical limit [169] in DMF. The k value, taken at the y-intercept is \( 1.29 \pm 0.35 \times 10^{-5} \text{ cm}^3\text{ g}^{-1} \). The Mark-Houwink equation for linear PSLG in the molecular weight range of 38,000 to 250,000 at 25° C is thus: \( [\eta] = 1.29 \pm 0.35 \times 10^{-5} M^{1.29 \pm 0.09} \). The molecular weight of PSLG-20K was determined by a "one angle Zimm plot"; i.e., five concentrations were measured at one scattering angle and the line extrapolated to the y-axis where the molecular weight was obtained. As mentioned previously, low molecular weight polymers are difficult to analyze by SLS techniques. Hence, because of the uncertainty in this point, it was not included in calculating the Mark-Houwink equation for the line.

As mentioned previously, \( R_g^2 = L^2/12 \) if the polymer behaves as a rod. For rods which are not infinitely thin, \( R^2/2 \), where R is the radius of the rod, is added to the equation. A plot of \( R_g \) vs. L should give a slope of 0.29 (i.e., \( 1/12^b \)) and an intercept of \( R/2^b \) if it approaches ideal rod-like behavior. Figure 4.23 shows the results. The slope of the line is \( 0.33 \pm 0.04 \) (i.e., \( 1/9.2^b \)) and an intercept of \( 1.73 \pm 2.81 \text{ nm} \). The \( y \)-intercept is \( R/2^b \); hence, we can calculate that for PSLG, \( R = 2.4 \text{ nm} \) with a large uncertainty due to the error in the \( y \)-intercept.

PSLG-EX was also analyzed by SLS. As mentioned in Chapter 2, the
substitution of stearyl chains appeared to be 85-90% based on \(^1\)H NMR integration. Using the manufacturer's molecular weight of 100,000 daltons, the molecular weight of PSLG-EX should be about 240,000 daltons, with a calculated Rg of 27.2 nm. The result obtained from a Zimm plot gave 209,500 ± 4900 with an Rg of 32.7 ± 0.2 nm and an A\(_2\) value of 1.923 ± 0.103 x 10\(^{-4}\) cm\(^3\)g\(^2\)mol. This sample of PSLG was not used for any of the calculations discussed above nor was it plotted with any of the data obtained for PSLG synthesized from the monomer. This material is more correctly considered a copolymer with about 1 or 2 methyl side chains for every 8 or 9 stearyl side chains. It was made primarily to evaluate the efficiency of synthesizing PSLG from PMLG. Clearly, this is easier than starting from L-glutamic acid and "working-up" to the polymer. However, despite others' claim (most notably Watanabe and co-workers [75]) that the side chains of PMLG can be completely substituted with stearyl alcohol, we were not able to accomplish this task. The data obtained from characterization of PSLG synthesized from PMLG or PBLG should be applied cautiously to the interpretation of the properties of PSLG.

**Dynamic Light Scattering**

While SLS measures the average intensity of scattered light from a polymer solution at a given angle, dynamic light scattering (DLS) techniques measure intensity fluctuations which occur as the polymer diffuses in solution. If one considers a simple case of only two scattering particles then we can consider how the scattering intensity fluctuations depend upon the distance between the two scatterers. As they diffuse in solution, the particles will be closer to each other at some time and farther away at another time so that the electric field of the
scattered light from each particle undergoes constructive or destructive interference. The interference pattern depends upon how far apart the scatterers are. Scattering intensity then, is time dependent. On the proper time scale, these intensity fluctuations are correlated and the correlation function which represents the intensity fluctuation is an exponential decay. Since scattering intensity depends upon the motion of the polymer in solution, DLS techniques allow for the calculation of how fast the polymer is moving in solution; that is, the diffusion coefficient. In fact, the easiest parameter to obtain from a DLS experiment is the diffusion coefficient. Because the diffusion of a polymer at finite concentration depends in part upon interactions between polymer molecules, DLS is a good technique for determining whether the polymer is aggregating. A book on DLS techniques [200] edited by Pecora gives a detailed description on the theory and experimental aspects used to successfully apply DLS in the study of macromolecules in solution. In the discussion below, the results of DLS experiments on linear PSLG are given. The solutions were the same ones used in the SLS experiments.

Figure 4.24 shows several plots which result after raw intensity data are fit with a cumulants [201] analysis. These plots are typical of the PSLG samples measured. The plot of $G \times 10^6$ vs. channel # is a representation of the amount of usable signal above the baseline. The channel # represents a point in the sample time where the intensity was measured. The higher the channel #, the longer the time. The function $G$ can be written as $G^{D}(\tau)$, the time autocorrelation function, which is introduced to characterize [202] the intensity fluctuations. The function is shown in Eq. 4.5.
\[ G^{(2)}(\tau) = B(1 + f|g^{(\nu)}(\tau)|^2) \]  \hspace{1cm} \text{Eq. 4.5}

where \( B \) is the baseline, \( f \) is a coherence factor which corrects for the fact that the photomultiplier tube is not a perfect point detector. Its value provides a ratio of useful signal to baseline scattering. The \( (2) \) superscript signifies that intensity, not electrical field, is being described. The capital \( G \), in this notation, signifies an unnormalized function.

Simply speaking, the greater the rise in the curve in the first 30 or so channels, the more usable signal there is. The more the polymer scatters, the easier it is to obtain a high signal above the baseline scattering intensity. The plot \( g^{(\nu)}(\tau) \) vs. \( \tau \times 10^4 \) is a representation of the exponential decay of the intensity with time. The function \( g^{(\nu)}(\tau) \) is called the normalized electric field autocorrelation function and is obtained by solving Eq. 4.5 for \( g^{(\nu)}(\tau) \).

\[ g^{(\nu)}(\tau) = (G^{(2)}(\tau)-B/Bf)^\nu \]  \hspace{1cm} \text{Eq. 4.6}

The lower case \( g \), in this notation, indicates the function is normalized. The \( (1) \) superscript denotes that the function refers to the electric field.

As Eq. 4.6 shows, \( g^{(\nu)}(\tau) \) is obtained by subtracting the baseline scattering out of the time autocorrelation function. It is this function that is of general interest in a DLS experiment because from it the mutual diffusion coefficient is obtained.

The plot of \( \log_e(g^{(2)}-1) \) vs. \( \tau \times 10^4 \) provides information on the polydispersity of the sample. If the plot is a straight line, it is an indication of a monodisperse sample. Curvature in the plot represents polydispersity. The error representation shown is the "noise" in each fit of the cumulants analysis. The x-axis in these plots is \( \tau \times 10^4 \) sec. The first cumulants analysis attempts to fit a line to the points in the \( \log_e(g^{(2)}-1) \) vs. \( \tau \times 10^4 \) plot. Typically, there is curvature in the points and a
straight line fit results in a large error of the fit. A 3rd cumulants fit gives the best values for diffusion coefficients because it will fit the points with a polynomial which is more representative of the curvature in the data. If there is little curvature (i.e., a polymer with Mw/Mn = 1) in the plot, 1st and 3rd cumulants analyses give values for the diffusion coefficient that are nearly the same. The height of the error bars represents the statistical error of measurement of g^{[2]}-1. The center of the bar indicates the difference between the theoretical fit and the collected data [202]. When each error bar is situated at zero error, X^2 = 1.

Two diffusion coefficients were determined from DLS experiments—the diffusion at zero concentration, D^o, and the mutual diffusion coefficient, D_m. The diffusion coefficient D_m for a given concentration is obtained from the scattered intensity of each concentration at one angle. A plot of D_m vs. c gives D^o as the y-intercept. Alternatively, the value of D_m for a given concentration can be obtained by measuring the solution at several angles and then plotting the values of r and q^2 obtained at each angle. The slope of the r vs. q^2 line gives D_m. The equation r = q^2D_m, where q = 4πn/λ_0(sinθ/2) (q is the magnitude of the scattering vector and r is the decay rate), is being plotted in this determination of D_m. Figure 4.25 shows the r vs. q^2 plots for linear PSLG samples. The error bars for the r values are generated by plotting the 1st and 3rd cumulants fits. Table 4.6 summarizes the diffusion coefficient data obtained for the PSLG samples. Note that the lower the molecular weight of the polymer (the smaller the polymer) then the larger the diffusion coefficient is (the faster the polymer diffuses). The D_m values were obtained from four or five concentrations
measured at 45°. Figure 4.26 shows the $D_m$ vs. $c$ plots. The slopes of the lines are positive, meaning the diffusion increases as the concentration increases. In a good solvent for the polymer, this is the expected trend. There are circumstances which cause the $D_m$ vs. $c$ slope to be negative. This can be an indication that the polymer is aggregating. This phenomenon will be discussed further in Chapter 5. The fact that for linear PSLG a positive $D_m$ vs. $c$ slope is obtained indicates that

Table 4.6 $D^o$ and $R_h$ for linear PSLG.

<table>
<thead>
<tr>
<th>Mw/gmol$^{-1}$</th>
<th>$D^o/10^{-7}$ cm$^2$s$^{-1}$</th>
<th>$R_h$/nm Eq. 4.7</th>
<th>$R_h$/nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>248,000</td>
<td>3.31$^a$</td>
<td>13.9$^b$</td>
<td>14.5$^c$</td>
</tr>
<tr>
<td>184,900</td>
<td>3.93</td>
<td>11.7</td>
<td>12.1</td>
</tr>
<tr>
<td>155,500</td>
<td>4.45</td>
<td>10.7</td>
<td>10.7</td>
</tr>
<tr>
<td>120,900</td>
<td>5.18</td>
<td>9.2</td>
<td>9.2</td>
</tr>
<tr>
<td>96,100</td>
<td>5.82</td>
<td>7.8</td>
<td>8.2</td>
</tr>
<tr>
<td>47,100$^d$</td>
<td>8.05</td>
<td>5.9</td>
<td>5.9</td>
</tr>
</tbody>
</table>

a  $D^o$ values are from plots of $D_m$ vs. $c$ using a fit of the 3rd cumulants data measured at $\theta=45^\circ$ and 25° C.

b  $R_h$ value is from 3rd cumulants fit using data collected from the lowest concentration (about 0.1-0.2%) at $\theta=45^\circ$.

c  $R_h$ value is calculated using $D^o$ in Eq. 4.7.

d  Data for PSLG-47K was obtained at $\theta=90$ and 25° C.

the polymer is not aggregated in THF at the concentration range studied. Also consistent with this result are the plots obtained when graphing $\eta_{sp}/c$ vs. $c$ and $\eta_{inh}$ vs. $c$ to obtain the intrinsic viscosity, $[\eta]$. If the polymer is unaggregated, both
plots are linear with their y-intercepts being equal. Curvature in these lines indicate association of the polymer chains. Figure 4.27 shows a typical intrinsic viscosity plot for linear PSLG. This plot is typical for a polymer dissolved in a good solvent. The star polymers discussed in Chapter 5 have increasing amounts of curvature with higher concentrations, indicating the polymer is aggregating. Further comparisons will be discussed in Chapter 5.

For rod-like polymers, there is a linear relationship between the diffusion coefficient and the molecular weight. A plot of $D^0$ vs. $1/M_w$ should be a straight line if the polymer is stiff. Figure 4.28 shows that for PSLG, the data is linear with a correlation coefficient of 0.995. The equation of the line is $y = 3.96 \pm 0.22 \times 10^{-4}x + 1.81 \pm 0.16$. Just as the SLS experiments described above indicated that PSLG is rod-like, the results of DLS experiments are consistent with this behavior.

Also shown in Table 4.6 is the value for the hydrodynamic radius for the polymer, $R_h$. It is calculated from Eq. 4.7, the Stokes-Einstein relationship [200], in which the diffusion of a spherical particle is a function of its radius. The value reported in column 3 of Table 4.6 is taken from 3rd cumulants analysis of the lowest concentration measured (about 0.1-0.2 %). The value at the lowest concentration is tabulated because if there are any polymer-polymer interactions that affect $R_h$ they will be less severe at the lowest concentration. However, in going from the highest (about 0.5-0.6 %) to the lowest concentration for each of these samples, there is little change in the value for $R_h$. As Eq. 4.7 shows, the Stokes-Einstein relationship relates $D^0$ to $R_h$, where the affects of polymer-polymer interactions on diffusion have been extrapolated out. In the limit of
dilute solutions, however, the value of $D_m$ obtained at a given concentration can be used in Eq. 4.7 to give a good estimate of $R_h$. The value in column 4 was calculated by hand using Eq. 4.7 below and the value of $D^\circ$ reported in column 2 of Table 4.6. The value of $R_h$ is the radius of an *equivalent sphere* which would display the same solution properties as the polymer in question (such as viscosity). The equation below is the Stokes-Einstein relationship:

$$D^\circ = \frac{kT}{6\pi\eta R_h} \quad \text{Eq. 4.7}$$

where $k$ is the Boltzmann constant  
$T$ is the temperature in K  
$\eta$ is the viscosity of the solution

Like other rod-like polymer dimensions, the hydrodynamic radius should scale linearly with molecular weight. Figure 4.29 shows a plot of $R_h$ vs. $M_w$, using $R_h$ from column 4 of Table 4.6. The equation for the line is $y = 4.19 \pm 0.09 \times 10^{-4} x + 4.20 \pm 0.16$ with a correlation coefficient of 0.999. Also, as Figure 4.30 shows, $R_g$ scales linearly with $R_h$. The equation of the line is $y = 3.20 \pm 0.40 \times 10^{-4} x - 11.95 \pm 4.51$ with a correlation coefficient of 0.990.

The ratio of $R_g$ and $R_h$ gives a value known as $\rho$ (not to be confused with density, which is typically symbolized with this character). The $\rho$ factor is one of three types of "shrinking" factors used to evaluate the degree of branching in polymer chains. This point will be discussed further in Chapter 5. For the present, we can calculate $\rho$ from the $R_g$ data in Table 4.5 and the $R_h$ data in Table 4.6 for linear PSLG. Table 4.7 shows the results of $\rho$ calculated from experimental data and evaluated from Eq. 4.10 below. The $\rho$ value can be calculated if one knows the length and hydrodynamic diameter, $d_n$, of the rod. From Kirkwood-Riseman [203] theory it is known that for rods,
\[ D^0 = (kT) \ln \left( \frac{L}{d_n} \right) / 3 \eta_0 L \]  
Eq. 4.8.

Since the value for \( D^0 \) was determined experimentally by DLS and the length of the rod can be calculated from Mw determined by SLS, we can solve Eq. 4.8 for \( d_n \) to obtain the hydrodynamic diameter. The results are shown in Table 4.7.

The expression \( \rho = \frac{R_g}{R_h} \), can be rewritten specifically for rod-like polymers by substituting \( L/12h \) for \( R_g \) and solving Eq. 4.7 for \( R_h \) and substituting it for \( R_h \).

Then,

\[ \rho = \frac{L}{12h} / kT / 6\pi \eta_0 D^0 \]  
Eq. 4.9

for rods. After substituting Eq. 4.8 into Eq. 4.9 we arrive at

\[ \rho = 2 \ln \left( \frac{L}{d_n} \right) / 12h \]  
Eq. 4.10.

As Table 4.7 shows, the hydrodynamic diameter determined here is essentially the same as the diameter determined by Eq. 4.2 using experimental \( A_2 \) values and rod lengths calculated from Mw. Figure 4.31 shows a plot of \( \rho \) vs. Mw for linear PSLG. The experimental values are somewhat higher than the calculated values, probably due to the fact that the length used in Eq. 4.10 is calculated from the weight average molecular weight and the experimental value of \( R_g^2 \) is

<table>
<thead>
<tr>
<th>polymer</th>
<th>( d_n / \text{nm} )</th>
<th>( \rho_{\text{exp}} )</th>
<th>( \rho ) (Eq. 4.10)(^a)</th>
<th>( \rho ) (Eq. 4.10)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSLG-96K</td>
<td>3.8</td>
<td>1.59 ± 0.16</td>
<td>1.32</td>
<td>1.43</td>
</tr>
<tr>
<td>PSLG-121K</td>
<td>3.7</td>
<td>1.96 ± 0.20</td>
<td>1.48</td>
<td>1.63</td>
</tr>
<tr>
<td>PSLG-155K</td>
<td>3.6</td>
<td>2.17 ± 0.22</td>
<td>1.63</td>
<td>1.79</td>
</tr>
<tr>
<td>PSLG-185K</td>
<td>3.7</td>
<td>2.31 ± 0.23</td>
<td>1.72</td>
<td>1.88</td>
</tr>
<tr>
<td>PSLG-248K</td>
<td>3.3</td>
<td>2.29 ± 0.23</td>
<td>1.95</td>
<td>2.05</td>
</tr>
</tbody>
</table>

Ave: 3.6 ± 0.2

\( a \) \( L \) calculated from Mw.

\( b \) \( L = 12hR_g \)
proportional to the z average molecular weight. If L is calculated using Rg, the calculated value of ρ is closer to the experimental value, as Table 4.7 shows.

4.6 Summary

Using SLS, DLS, and NMR techniques, PSLG has been shown to exhibit rod-like behavior in solutions of THF. The polymer is also unaggregated in the concentration range studied (0.1-0.5%). Using SLS data, rod dimensions were calculated; the diameter was in good agreement with a SYBYL molecular model and the pitch/residue was close to the anticipated value based on the α-helix of a polypeptide. DLS results were also consistent with a stiff polymer chain.

Linear PSLG displays lyotropic cholesteric liquid crystals in toluene. The melts are highly birefringent. DSC thermograms show two endothermic transitions in PSLG samples that display liquid crystallinity. The first transition has been assigned to the melting point of the stearyl side chain crystallites and the second to a liquid crystal phase transition.

4.7 Experimental

NMR spectra cited in this chapter were recorded on a IBM Bruker 200 MHz instrument using CDCl3 as the solvent and TMS as an internal standard. GPC data was collected as described in Chapter 2. DSC thermograms were measured with a Mettler FP 85 TA cell interfaced to an IBM compatible computer through a Mettler FP 80 central processor. In house software [204] controlled the temperature ramps which were 2° C/min. from 30-85° C for both the heating and the cooling cycles. Intrinsic viscosity data was collected using a Ubbelohde type viscometer in a water bath controlled at 30 ± 0.1° C. The solvent was HPLC
grade THF. Flow times were used to calculate reduced and inherent viscosities for each concentration measured. Plots of \( \eta_{sp}/c \) vs \( c \) and \( \eta_{mh} vs c \) extrapolated to zero concentration yielded the intrinsic viscosity, \([\eta]\). The samples for light microscopy were prepared by weighing PSLG and the appropriate amount of toluene into a vial. A 0.2 mm Vitrodynam cell was then loaded by placing an open end into the toluene solution and warming the solution. The other end of the cell is also open. After the solution creeped into the cell by capillary action (or pressure exerted by solvent vapor) the cell was flame sealed. The samples were studied after 3-5 days at the cited temperature. Temperatures above room temperature were maintained by mounting the sample on a Mettler FP 82 hot stage. The stage was then placed between crossed polarizers on an Olympus BH2 microscope.

The differential index of refraction, \( dn/dc \), for PSLG in THF was measured at 25° C and \( \lambda_0 = 488 \) nm over the same concentration range studied in the light scattering experiments. The instrument used for the measurement was a Brice-Phoenix differential refractometer. The instrument was calibrated and the instrument constant obtained by the measurement of aqueous KCl solutions. The value obtained for PSLG in THF assuming no dependence on molecular weight is \( dn/dc = 0.08 \pm 0.002 \).

The light scattering instrument used was designed by Professor Paul S. Russo and the necessary parts machined in the LSU machine shop by Mr. George Gascon. The laser source was a Lexel Model 95 Argon ion laser. For SLS experiments, the 488.0 nm (blue) line was used. For DLS experiments, the 514.5 nm (green) line was used. A Lauda RM-6 water bath circulated constant
temperature water through an insulated copper block in which the sample cell rested. An EMI-9863 phototube was used in the experiments. The instrument was also equipped with a Precision Pacific Model 126 photometer for signal magnification. The correlator used was a Langley-Ford Model 1096 which has 272 channels for data collection. The correlator is capable of operating in various modes to suit the experimental requirements. For example, a multi-tau mode is used for SLS experiments and an auto-correlate mode for DLS experiments. For light scattering, HPLC grade THF was used. It is low in water content (less than 0.05 %) and contains less particulate matter than other grades of THF. Because dust scatters light enormously and makes analysis of the polymer practically impossible, steps were taken to remove it. A dust free water supply was available for cleaning materials which come in contact with the polymer solutions to be analyzed. Tap water is purified through a Millipore Milli-R/Q purifier. The treated water has a resistivity of greater than 2.5 MΩcm. After passing the purifier, it is filtered through a Gelman 0.2 μm filter installed at the supply tap. Light scattering cells were soaked in Chromerge overnight. The Chromerge was thoroughly rinsed from the cells with dust free water from the source described above. The tubes were also scrubbed with a pipe cleaner using Alconox detergent and hot, purified water. They were then rinsed thoroughly and repeatedly (dozens of times) with the purified water. After rinsing, they were filled completely with dust free water, covered with aluminum foil, and sonicated no less than one hour. Again, the cells were rinsed several dozen times in dust free water and then checked by running a few milliliters of dust free water into them and then viewing them in the laser path at about 100x magnification. Dust
shows up as brightly moving "lines" in the laser light. When no dust was detected after about 30 seconds of observation, the cells were emptied, wrapped in aluminum foil and dried at about 100° C in a convection oven. Solutions of the polymer were made in the following manner: The polymer was weighed into a 5 ml volumetric flask. THF was filtered into the flask under a blanket of nitrogen using a 0.02 μm Anopore filter to make a stock solution. The stock solution was then filtered into a dust free test tube (cleaned as described above) using a 0.2 μm Nucleopore filter. THF was filtered into a dust free test tube using a 0.02 μm Anopore filter. These two tubes were checked in the laser for dust. It was typical to find a little dust present. These tubes were centrifuged for no less than one hour (sometimes overnight) at 7000 rpm to settle the dust. Dilutions of the polymer solution were made under a blanket of nitrogen using the filtered, centrifuged THF. Aliquots of the stock solution and THF were removed with Pipetman dial-type pipets carefully to avoid disturbing the settled dust. The dilutions were made directly into the clean cells and then immediately capped with a teflon lined screw cap. Teflon tape was then wrapped around the cap-tube interface to aid in sealing the tube. These solutions were analyzed after centrifuging at 7000 rpm for no less than one hour (sometimes overnight). Raw intensity data from DLS was analyzed using CORAN [205] which fits the data with cumulants [201] analyses. SLS data was obtained by evaluating Zimm plots generated from the average scattering intensity at 8 angles and 5 concentrations of polymer solution.
Figure 4.1. Repeat units of various rod-like or stiff molecules.

A PBT.

B PXLG, where the R group can be alkyl or aryl.

C Polyisocyanate, where the R group can be alkyl or aryl.

D Schematic representation of a polymer which has a like charge regularly repeating along the backbone.
Figure 4.2. 200 MHz 'H NMR of linear PSLG.

A without TFA.

B with about 10% TFA.
Figure 4.3. 50 MHz $^{13}$C NMR of PSLG.

A without TFA.

B with TFA.

Quartets at about 115 and 160 ppm are due to TFA.
Figure 4.4. Conversion of α-helix to random coil using increments of TFA followed by 1H NMR. The PSLG has a molecular weight of about 20,000 and was dissolved in CDCl₃ as a 10% w/v solution. The complete conversion to a random coil occurred at about 6% v/v TFA. TMS was the internal standard. The 200 MHz instrument was used.

A Spectrum without TFA present.

B Spectrum with 10% v/v TFA.
Figure 4.5. Cholesteryl benzoate, a molecule known to undergo a liquid crystalline phase transition in the melt. Noteworthy features of this molecule include the ester group at one end, the long hydrocarbon portion, and the high axial ratio.
Figure 4.6. Schematic representation of various liquid crystalline textures.

A Smectic.

B Nematic.

C Twisted nematic or cholesteric.
Figure 4.7. Photograph of a PSLG liquid crystal texture. Crossed polarizers were in place. This is a 26.5% w/w solution of PSLG-40K in toluene at 70° C. The objective lens was 10x. The distance between pitches is 2.36 µm. The bar marker is 11 µm.
Figure 4.8. DSC thermograms of PSLG-EX. The heating and cooling rates were 2° C/minute.

A  DSC thermogram.

B  Light intensity trace with crossed polarizers in place.

H1-first heating cycle, H2-second heating cycle, etc.

C1-first cooling cycle.
Figure 4.9. DSC thermograms of PSLG-248K. The heating and cooling rates were 2° C/minute.

A DSC thermogram.

B Light intensity trace with crossed polarizers in place.

H1-first heating cycle, H2-second heating cycle, etc.

C1-first cooling cycle, C2-second cooling cycle, etc.
Figure 4.10. DSC thermograms of PSLG-20K. The heating and cooling rates were 2° C/minute.

A DSC thermogram.

B Light intensity trace with crossed polarizers in place.

H1-first heating cycle, H2-second heating, etc.

C1-1st cooling cycle.
Figure 4.11. DSC thermogram of PSLG-50K. Heating and cooling ramps were 2° C/min.

H2-second heating cycle.
1st heating

1st cooling

---

H₂

exo-endo

exo-endo

T/C°
Figure 4.12. Area under the exotherm for PSLG-248K vs. cycle no. The area is proportional to the heat of fusion and the plot shows that there is little change through 5 cooling cycles. The area was determined by plotting the thermograms in triplicate on the same scale and then carefully cutting out and weighing each peak.
Figure 4.13. Schematic representation of PSLG-248K during a heating cycle followed by a cooling cycle. $T_1$ represents the temperature of the first endothermic transition; $T_2$ represents the temperature of the second endothermic transition. The negative sign after either represent the cooling cycle (i.e., approaching $T_1$ or $T_2$ from a higher temperature). The "straight" side chains represent a high degree of crystallization. The "wavy" lines represent that the side chains are fluid. The small arrows on each side of the polymer chain represent motion of the whole polymer molecule. The "bent" lines represent poorly crystallized side chains. Within the large circles is an "exploded" view of the side chains. The first circle represents high crystallinity. The second represents fluid side chains (no order). The third circle represents poorly organized side chain crystallization.
Figure 4.14. GPC calibration curve for linear PSLG. The curve was generated using Nelson Analytical Software.
Figure 4.15. Typical GPC chromatograms for linear PSLG. Flow rate was 1 ml/minute.

<table>
<thead>
<tr>
<th>retention time/minutes</th>
<th>Mw/10^3 daltons</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7.93</td>
</tr>
<tr>
<td>B</td>
<td>8.40</td>
</tr>
<tr>
<td>C</td>
<td>8.63</td>
</tr>
<tr>
<td>D</td>
<td>8.87</td>
</tr>
<tr>
<td></td>
<td>175-186</td>
</tr>
<tr>
<td></td>
<td>120-130</td>
</tr>
<tr>
<td></td>
<td>93-97</td>
</tr>
<tr>
<td></td>
<td>46-49</td>
</tr>
</tbody>
</table>
Figure 4.16. $1/P(\theta)$ vs. $q^2R_g^2$. This graph is only a sketch to show the effects different shapes on scattering intensity and does not represent real data or calculated points on the curves.
Figure 4.17. Zimm plots of PSLG. One plot (A) is for one of the lowest molecular weight PSLG samples measured. Despite this, the Zimm plot is quite good. Only the \( c = 0 \) line is a little noisy, indicating that the lower limit in the \( R_g \) that SLS can measure (at least with the 488.0 nm laser line), is being approached. The higher molecular weight samples have much better linear fits for their \( c = 0 \) line, as figure B shows. This is the Zimm plot for PSLG-155K.

A  \( M_w = 96,100 \pm 4,800 \) daltons

\[
R_g = 13.0 \pm 2.0 \text{ nm}
\]

\[
A_2 = 3.23 \pm 0.2 \times 10^{-4} \text{ cm}^3 \text{mol}^{-1} \text{g}^{-2}
\]

\( \theta/\text{deg}: 40, 50, 60, 90, 110, 120, 135 \)

\( c/\text{gml}^{-1}: 0.00254, 0.00381, 0.00507, 0.00634 \)

\( r_{c=0} = 0.571 \)

\( r_{\theta=0} = 0.999 \)

B  \( M_w = 155,500 \pm 8,200 \) daltons

\[
R_g = 23.2 \pm 3.3 \text{ nm}
\]

\[
A_2 = 3.04 \pm .21 \times 10^{-4} \text{ cm}^3 \text{mol}^{-1} \text{g}^{-2}
\]

\( \theta/\text{deg}: 40, 45, 50, 55, 60, 90, 110, 120, 135 \)

\( c/\text{gml}^{-1}: 0.00178, 0.00268, 0.00357, 0.00447 \)

\( r_{c=0} = 0.885 \)

\( r_{\theta=0} = 0.973 \)
Figure 4.18. SYBYL molecular model of PSLG with a DP of 20. The structure was run through the MAXIMIN2 routine for energy minimization.
Figure 4.19. SYBYL molecular model of the PSLG backbone showing only the backbone nitrogens. This model was generated by stripping off all of the atoms except N from the model in Figure 4.15.
Figure 4.20. $A_2$ vs. Mw. No clear trend is evident. If anything, there is a modest increase at lower molecular weight.
\[ A_2 / 10^{-4} \text{ cm}^3 \text{ mol}^{-2} \text{ g}^{-2} \] vs. \[ M_w / 10^5 \]
Figure 4.21. Rg vs. Mw. $y = 13.22 \pm 1.66x + 1.81 \pm 2.88$

$r = 0.982$
Figure 4.22. Mark-Houwink plot for linear PSLG. The Mark Houwink equation for PSLG is $[\eta] = 1.29 \pm 0.3 \times 10^{-5} M^{1.29 \pm 0.09}$.

The units on $K$ are cm$^3$/g.
Figure 4.23. $R_g$ vs. $L$. $L$ was calculated using: $L = \frac{M_w(0.15)}{382}$.

\[ y = 0.337 \pm 0.043x + 1.74 \pm 2.81 \]

\[ r = 0.985 \]
Figure 4.24. Typical plots generated after a cumulants analysis fit of the raw intensity data. These fits are for PSLG-185K and were measured at $\theta = 45$ and $c = 0.0066$ g/ml. $f(a)$ represents the amount of usable signal above the baseline scattering. It is a number between 0 and 1; the closer $f(a)$ is to 1, the larger the usable signal is. How closely $f(a)$ can be set to 1 depends ultimately on how well the polymer scatters. Poor scatters have low scattering above the baseline.
\[ G \times 10^{-6} = 9.814 \]

\[ \langle A \rangle > 0.173 \]

\[ \tau \times 10^4 \text{ (SEC)} \]

\[ \log_{10}(g^{1/2} - 1) \]

\[ \tau \times 10^4 \text{ (SEC)} \]

\[ g^{1/2} \text{ or } g^{1/4} - 1 \]

\[ \chi^2 \]

3rd Cumu \( \chi^2 = 1.087 \)

2nd Cumu \( \chi^2 = 1.248 \)

1st Cumu \( \chi^2 = 22.44 \)
Figure 4.25. \( r \) vs. \( q^2 \) plots for linear PSLG samples. These plots were obtained from data gathered on the highest concentration and at \( \theta = 30, 45, 60, \) and 90. The slope of the line is \( D_m \).

<table>
<thead>
<tr>
<th></th>
<th>( c/\text{gml}^{-1} )</th>
<th>( D_m/10^{-7} \text{ cm}^2\text{s}^{-1} )</th>
<th>( r )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>PSLG-96K</td>
<td>0.0063</td>
<td>6.50 ± 0.03</td>
</tr>
<tr>
<td>B</td>
<td>PSLG-121K</td>
<td>0.0040</td>
<td>5.33 ± 0.04</td>
</tr>
<tr>
<td>C</td>
<td>PSLG-155K</td>
<td>0.0045</td>
<td>4.20 ± 0.02</td>
</tr>
<tr>
<td>D</td>
<td>PSLG-185K</td>
<td>0.0066</td>
<td>3.90 ± 0.04</td>
</tr>
<tr>
<td>E</td>
<td>PSLG-248K</td>
<td>0.0059</td>
<td>3.49 ± 0.04</td>
</tr>
<tr>
<td>F</td>
<td>PSLG-EX</td>
<td>0.0040</td>
<td>3.37 ± 0.03</td>
</tr>
</tbody>
</table>
Figure 4.26. $D_m$ vs. $c$ plots for linear PSLG. The scattering angle was $\theta = 45$ at 25° C. The data shown are from 3rd cumulants analysis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$D^0/10^{-7}$ cm$^2$s$^{-1}$</th>
<th>$k_0/10^{-5}$ cm$^2$s$^{-1}$mlg$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A PSLG-96K</td>
<td>5.83</td>
<td>1.38</td>
</tr>
<tr>
<td>B PSLG-121K</td>
<td>5.18</td>
<td>0.35</td>
</tr>
<tr>
<td>C PSLG-155K</td>
<td>4.45</td>
<td>0.49</td>
</tr>
<tr>
<td>D PSLG-185K</td>
<td>3.94</td>
<td>0.60</td>
</tr>
<tr>
<td>E PSLG-248K</td>
<td>3.31</td>
<td>0.51</td>
</tr>
<tr>
<td>F PSLG-EX</td>
<td>3.14</td>
<td>0.63</td>
</tr>
</tbody>
</table>
45° scattering angle
\[ \Delta \text{ from slope of } \Gamma \text{ vs. } q^2 \text{ plot} \]
\(45^\circ\) scattering angle
\(\Delta\) from slope of \(\Gamma\) vs. \(q^2\) plot

\(D_m/10^{-7}\) cm² s⁻¹

\(c\) g ml⁻¹

\(\theta\) from \(\Gamma\) vs. \(q^2\) plot

\(\Delta\) from slope of \(\Gamma\) vs. \(q^2\) plot

\(D_m/10^{-7}\) cm² s⁻¹

\(c\) g ml⁻¹
45° scattering angle
\[\Delta \text{ from slope of } \Gamma \text{ vs. } q^2 \text{ plot}\]
Figure 4.27. Typical $\eta_{sp}/c$ or $\eta_{inh}$ vs. $c$ plot for linear PSLG, PSLG-248K. The y-intercept gives $[\eta]$. Both lines are linear least square fits. $\eta_{sp}/c$ vs $c$: y-int = 1.21.

$\eta_{inh}$ vs $c$: y-int = 1.26.
\[ \eta_{sp}/c \]
\[ \Delta \eta_{inh} \]
Figure 4.28. $D^\circ$ vs. $1/M_{\text{w}}$. The value $D^\circ$ is taken from the y-intercept of the $D_m$ vs. $c$ plots shown in Figure 4.26.

\[ y = 3.96 \pm 0.22x + 1.81 \pm 0.16 \]
\[ r = 0.995 \]
Figure 4.29. $R_h$ vs. $M_w$. $y = 4.19 \pm 0.09x + 4.20 \pm 0.16$

$r = 0.999$
Figure 4.30  \( R_g \) vs. \( R_n \)  \( y = 3.20 \pm 0.40x - 11.95 \pm 4.51 \)

\( r = 0.990 \)
Figure 4.31  $\rho$ vs. Mw.
\( \bigcirc \) calculated from Eq. 4.10
\( \triangle \) calculated from experimental values
Chapter 5: Characterization of Star Branched Poly(γ-stearyl-L-glutamate)
5.1 Introduction

Star polymers represent one of several possible unusual structural types. In addition to stars, there are structures known as ladders [206], combs [207], catenanes [208], and crosslinked networks. Figure 5.1 shows a schematic representation of each of these polymers. Each structure imparts special physical properties to the bulk material. Ladder polymers are most often produced by Diels-Alder type reactions [209]. The polymers formed are fused, repeating rings that make the polymer stiff and heat stable. Combs can be formed by grafting short oligomeric chains onto a linear polymer backbone. Comb polymers are useful in lowering the crystallinity of the polymer. For example, polyethylene is sometimes synthesized with a long hydrocarbon chain alkene comonomer to make short branches along the chain, thus making the polymer less crystalline and more tractable. Comb polymers have also been of interest [210] as liquid crystalline materials. In our labs [211], comb type polymers where the "teeth" of the comb are peptide grafts of varying length have been synthesized and have potential applications as optically active membranes and drug carrying vehicles. Catenanes are large, interlocking rings, resembling the links of a chain. Strictly speaking, catenanes are not polymeric but the interlocking rings are high molecular weight molecules. From a synthetic point of view, this assembly is the most challenging to produce. Recently [208], a catenane with 3 interlocking rings was synthesized in high yield, an amazing synthetic feat. Catenanes with more "chain links" are proposed by this synthetic effort. Cross-linked networks, if enough cross-links are present, will form gels [212]. Additionally, these materials are usually thermosets [213] (as in an automobile tire), having constant mechanical properties with
changing temperature. As mentioned in Chapter 3, star polymers have potential as valuable engineering materials because of the greater ease in processing the polymer as a melt or in concentrated solutions and their ability to impart multidirectional strength in composites. Thus, although linear polymers are presently the most commercially exploited and most frequently studied type of polymer (and certainly the easiest to synthesize), polymers with "specialized" microstructure are also promising as commercially exploitable materials. Recently, [214] a synthesis of star, comb and ladder type polymers by a group transfer polymerization technique was described.

While the introduction of Chapter 3 focused primarily on an overview of the recent synthetic efforts in star polymer production, it is appropriate to mention here some of the applications of these materials. Because of their comparatively lower melt and solution viscosity, applications to exploit this property have been evaluated. In addition to providing multi-directional strength in composites or strength comparable to their linear counterparts, they have been used as pressure sensitive adhesives [215], to improve the properties of tire tread [216], and to improve [217] the viscosity index of lubricating oil. Many star polymer studies [218-220] involve their diffusion in concentrated solution, in the melt, and in a linear polymer matrix. These studies should ultimately lead to an understanding of how star polymers can be exploited as moldable, processable, high performance materials both as a component in a composite or alone. Besides the computer studies aimed at predicting star polymer conformations mentioned in Chapter 3, neutron scattering [219a, 221, 222] and light scattering [223] techniques have been applied to both solutions (dilute and concentrated) and the
molten state of star polymers to determine their structure, dimension, and diffusion or movement in the given medium. Results are typically compared not only to theoretical predictions but to the behavior of linear polymers of the same molecular weight. Comparisons with linear polymers have led [224a,b,c,d] to the development of values such as the branching coefficient or shrinking factor, $g'$, which is defined by the ratio of the intrinsic viscosity of the star polymer to the intrinsic viscosity of the linear polymer with the same molecular weight. Determination of values such as these are useful in predicting the extent of branching in an unknown polymer.

Until recently, the literature has been dominated by star polymers made with polystyrene or polyisoprene arms (see references in Chapter 3). The microstructure of the star PSLG macromolecules synthesized in this research and discussed in Chapter 3 is unique because the arms of the star are rod-like. Rigid arm star polymers have received some attention recently [134] because of their potential in overcoming directional strength failures in composite materials. That is, a linear, rod-like polymer component in a composite gives superior strength in the direction of the long axis of the polymer but does little to increase strength when the stress is applied perpendicular to the direction of the long axis. Rigid arm star polymers have the ideal microstructure for enhancing strength in several directions because the arms of the star will lie in different directions or planes in the material. This is accomplished [134] by having long, flexible spacers or central units holding the rigid arms together. The arms can then align in planes, with each arm of a particular molecule having the potential to reside in a different plane.
The PSLG stars are also unusual because of the long hydrocarbon side chains emanating from each repeat unit. This type of repeating side chain imparts hydrophobicity to the structure and lowers the melting point of the polymer. If modified with a hydrophilic outer "shell", star PSLG could have applications as a drug carrying vehicle which mimics the behavior of a micelle.

The characterization of the polymers synthesized by the methods outlined in Chapter 3 is discussed here. The techniques and instrumentation used are the same as those used for the analysis of the linear PSLG described in Chapter 4. In this chapter, evidence for branching will be presented as well as comparisons between the behavior of linear and star branched PSLG. As the polymers were synthesized by two different techniques, the following designations will be made when referring to the star polymers: for the polymers synthesized with methanol as a co-solvent (Method I), \( f\)-PSLG-M1 ; for the polymers synthesized without the presence of methanol (Method II), \( f\)-PSLG-M2 , where the \( f \) designates the number of arms. Also, based on the titration data in Chapter 3, the number of arms will be designated as 3.0, 3.6, 5.2, and 7.6 for the 3, 4, 6 and 9 arm stars respectively. A two arm "star" or broken rod was also synthesized by reaction of SLGNCA with 1,6-hexanediameine. Because there was no methanol in this synthesis, discussion of this polymer will be included with the M2 series of star polymers. However, because its synthesis was completely homogenous, it is likely that the resulting polymer has a much narrower molecular weight distribution. It will be referred to simply as 2-PSLG.

5.2 GPC Characterization

When synthesizing star polymers, one of the goals is to produce branched
polymers with the desired number of arms without contamination from linear polymers. Syntheses of star polymers that involves initiation with a multifunctional initiator can produce the desired polymer without linear chains forming provided there are no competing initiators present which will form linear chains. As mentioned in Chapter 3, the initiators used for the synthesis of star PSLG have the unfortunate property of being generally insoluble in common organic solvents while being soluble in water and methanol. In order to control the DP of the arms of the star polymer, it is desirable to have a completely homogenous reaction where the monomer, initiator, and resulting polymer are completely soluble in the reaction solvent. Thus, the initiator was added in methanol to the DCM solution of monomer. As shown in Chapter 3, the methanol also initiates linear polymer chains. A useful tool for determining the extent of contamination by linear chains is GPC. A GPC column will effectively separate the linear and star species present, resulting in a bimodal chromatogram. As Figure 5.2 shows, the \( f \)-PSLG-M1 series is bimodal, with the peak at the lowest elution volume or time corresponding to contamination by linear polymer due to the presence of methanol. There are two noteworthy features of the chromatograms in Figure 5.2: the elution times and the size of the peak due to the linear polymer. The elution times are tabulated in Table 5.1. When the synthesis was planned, star polymers were desired with the same arm DP (DP 50) so that the only difference in each polymer was the number of arms. That is, the effective size of the polymer would remain unchanged but the star would become more dense with increasing number of arms. Thus, the expectation was that the peaks would elute at the same retention time on the GPC column despite the fact that the
Table 5.1 GPC elution times* for f-PSLG-M1 series.

<table>
<thead>
<tr>
<th>polymer</th>
<th>elution time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-PSLG-M1</td>
<td>8.87</td>
</tr>
<tr>
<td>3.6-PSLG-M1</td>
<td>8.87</td>
</tr>
<tr>
<td>5.3-PSLG-M1</td>
<td>8.87</td>
</tr>
<tr>
<td>7.6-PSLG-M1</td>
<td>8.53</td>
</tr>
</tbody>
</table>

a The internal standard was toluene; retention time = 12.37 minutes.

Molecular weight increases with increasing number of arms. Except for 7.6-PSLG-M1, the retention times are identical. This result is, however, rather surprising in this case because clearly some of the monomer has been consumed by the chains initiated by methanol. The fact that polymer 7.6-PSLG-M1 elutes faster (and is thus bigger) could be due to arms of unequal length attached to the center of the star. That is, due to crowding at the center of the star one arm may grow faster and longer than another. This point will be discussed in more detail in Section 3.3 below. The other noteworthy feature in the chromatograms is decreasing amounts of the linear polymer with increasing number of arms in the star polymer. This implies that the star polymers with more arms consume monomer faster than the stars with fewer arms. The denser star polymers could create a more favorable environment for the monomer; the monomer could be absorbed in the growing star polymer with a greater number of arms much more effectively than the less dense stars. Hence, this interaction causes the monomer to be more readily consumed by the higher arm star polymers.

As Figure 5.3 shows, the GPC chromatograms of the f-PSLG-M2 series
contain only one peak. Without methanol, only the star central units are initiating. With this heterogeneous synthesis, there was no expectation that stars with the same arm DP for each sample would be produced. In Table 5.2 the retention times for the f-PSLG-M2 series are shown. The 3.6 and 7.6 polymers of

Table 5.2. GPC elution times* for f-PSLG-M2 series.

<table>
<thead>
<tr>
<th>polymer</th>
<th>elution time / minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-PSLG</td>
<td>8.40</td>
</tr>
<tr>
<td>3-PSLG-M2</td>
<td>7.70</td>
</tr>
<tr>
<td>3.6-PSLG-M2</td>
<td>7.82</td>
</tr>
<tr>
<td>5.3-PSLG-M2</td>
<td>7.60</td>
</tr>
<tr>
<td>7.6-PSLG-M2</td>
<td>7.82</td>
</tr>
</tbody>
</table>

* Toluene was the internal standard; retention time = 12.37 minutes except for the 2-PSLG-M2 and 5.3-PSLG-M2 runs in which it eluted at 12.48 minutes.

differ. The similar retention times indicate that the star polymer dimensions are similar (not molecular weight). Light scattering techniques should show that these polymers have roughly similar $R_g$ and $R_h$. If retention times differ by 0.1-0.2 minutes, different sized polymers are indicated, based upon the results obtained with linear PSLG samples. The differences in size with star polymers that elute with retention times this close are, however, less pronounced. It is worth mentioning at this point that the elution times shown in Table 5.2 correspond to a linear PSLG molecular weight of about 190,000-220,000, using
the GPC calibration curve in Figure 4.13. SLS indicates that 5.3-PSLG-M2 has a molecular weight of about 470,000. The GPC data illustrates the relationship between molecular weight and star polymer dimensions; i.e., there are more monomer units packed into a smaller space. Thus, higher molecular weight star polymers elute at retention times corresponding to much lower molecular weight linear polymers because their size is similar. For this reason, branched polymers of any type, not just stars, cannot be correlated with linear polymer GPC calibration curves to obtain an accurate molecular weight estimate. As Table 5.2 shows, 2-PSLG elutes at 8.40 minutes. Using the GPC calibration curve in Figure 4.13, this corresponds to a molecular weight of about 100,000. SLS indicates that 2-PSLG has $M_w = 82,300$. This is an indication that the dimensions of 2-PSLG are not "shrunk" compared to its linear counterpart. That is, although there is a break in the middle of the rod, its dimensions are not seriously perturbed from a straight rod with $M_w = 82,300$.

Comparison of the $f$-PSLG-M1 series and $f$-PSLG-M2 series of polymers allows one to draw the following conclusions. Much larger star polymers can be made if no methanol is present in the synthesis. A difference in elution time of about 1 minute corresponds to a large molecular weight difference because the monomer density of the star polymers is very high. This conclusion was verified with SLS and is discussed in Section 5.6. Methanol obviously can compete with primary amine initiators when their concentration is comparatively low and thus promote the formation of linear polymer by-products. Synthesis by Method II leads to star polymers of differing and somewhat unpredictable sizes even if the same $\{M\}:\{I\}$ ratio is used for each. However, as the GPC traces show, the
bimodal molecular weight distribution is eliminated with the elimination of methanol, and a cleaner star polymer is produced.

5.3 Geometry and Dimension Considerations of Star PSLG: SYBYL Molecular Modeling

In Chapter 4 the dimensions of linear PSLG were calculated from laser light scattering data and SYBYL models. Linear PSLG is an arm on a star PSLG macromolecule. The star polymers we wish to synthesize can be assembled from the central units described in Chapter 3 and several linear chains to see how the pieces may fit together. Figure 5.4 shows SYBYL molecular models of star PSLG. The models contain 3, 4, 6, and 9 arms and an arm DP of 10. The arms are α-helical and were first minimized with the MAXIMIN2 routine using Tripos parameters. The arms were then attached to a maximin2 minimized structure of each central unit. Figure 5.4 also represents pictorially two general features [107] of star polymers: that the monomer density increases closer to the center of the star and that the monomer density increases with increasing number of arms. The 3, 4, and even the 6 arm star appear to have room between the arms although the six arm polymer is rather dense.

SYBYL modeling was used to address the question of whether 9 linear chains with a diameter of about 3.7 nm can be attached to a central point with an end to end distance of about 2 nm. The simple answer is yes, because SYBYL can not put together a model where there is no real space for each atom. That is, two portions of the molecule cannot share the same space. As the model in Figure 5.4 shows, there is intercalation of the hydrocarbon side chains between
the backbones of the arms in the star. The center of the 9 arm star is thus very
dense and hydrophobic. As the arms grow outward (arm DP higher than 10), the
monomer density is less severe and there is more room for the arms. However,
even though the model can be constructed with SYBYL, this does not mean that
in the synthesis each arm can grow at equal rates. With the kind of steric
crowding represented by the 9 arm model it is reasonable to speculate that one
or more arms may be somewhat longer than the rest. Section 5.6 below discusses
the possibility (or reality) of irregular arm length further based upon SLS data.
Also, the models in Figure 5.4 show that the stars, if regularly branched, can be
roughly described as hydrophobic spheres. This description of the star PSLG,
particularly the 6 and 9 arm species, lends support to the speculation made in
Section 5.2 above that the nonpolar monomer is absorbed by the growing star
macromolecule. A notable feature of the models in Figure 5.4 is that each
molecule is 8.3-8.8 nm across (diameter). So, provided the arm DP is the same,
the diameter of stars with differing number of arms remains the same but the
stars become "harder" or more dense with increasing arm number.

5.4 Intrinsic Viscosity

It has long been known that branching of various types will affect the
rheological properties of a polymer molecule. Branching of a polymer chain
always lowers the intrinsic viscosity of the polymer (when compared to a linear
polymer of the same molecular weight). For star polymers, various parameters
such as the g' value mentioned in Section 5.1 have been developed to attempt to
relate the easily measured [η] value to the number of branches.
Because there is obviously linear PSLG in the f-PSLG-M1 series which affects the \([\eta]\), the following discussion is in reference to the f-PSLG-M2 series. For this series, \([\eta]\) values indicate a reduction in solution viscosity when compared to linear polymers of the same molecular weight. This will be discussed more quantitatively in Section 5.6 where molecular weights from light scattering are reported. Specifically, in Section 5.6 the \(g'\) and \(g\) values will be calculated and compared with values cited in the literature. Figure 5.5 shows the \(\eta_{sp}/c\) vs \(c\) and \(\eta_{inh}\) vs \(c\) plots for each star polymer of the series. Not only do \([\eta]\) values give the first indication that there is branching in the polymers, but the plots also indicate that there may be aggregation occurring in the THF solutions of these particular polymers. Note the curvature in the plots, particularly in 3-PSLG-M2 and 3.6-PSLG-M2. Recall that linear PSLG solutions exhibit straight line relationships in plots of this type (Figure 4.26). Deviations from linear relationships in \([\eta]\) plots are usually interpreted as a qualitative indication of association or aggregation between the polymer molecules. That is, star PSLG, unlike its linear counterpart, appears to aggregate in THF. The 5.3-PSLG-M2 and 7.6-PSLG-M2 polymers appear to have less curvature in the same concentration range and in fact the \(\eta_{sp}/c\) vs \(c\) plot in both is arguably linear. Thus, these polymers, at the concentrations shown in the plots, are probably much less associated in THF than the fewer arm members of this series. 2-PSLG, however, displays no curvature in either line, indicating that it is unaggregated in THF. Note, too, that the concentrations measured for 2-PSLG were about twice those measured for the star polymers. It is tempting to conclude that "broken arm" PSLG does not aggregate; unfortunately, as SLS experiments will later show, its Mw is somewhat
lower than the star polymers. This leaves open the question of whether a higher molecular weight broken rod would aggregate. The observations made here then, may be due to molecular weight effects although 5.3-PSLG-M2 and 7.6-PSLG-M2 are the highest molecular weight polymers in the series and data presented later will argue in favor of these polymers being only weakly aggregated or unaggregated.

5.5 Differential Scanning Calorimetry and Liquid Crystalline Behavior

One of the purposes of pursuing the synthesis of star PSLG was to determine if, with branching, the liquid crystalline properties of PSLG remain the same, change, or are completely lost. The expectation was that star branched PSLG would be unable to form the lyotropic liquid crystal structure necessary for cholesteric liquid crystals. This does not mean, however, that this or other star polymers are not capable of forming anisotropic solutions. While it is true, based on their stiff backbones, that rod-like polymers with any type of repeat unit are capable of forming liquid crystals due to a parallel alignment of the rods, other molecular structures are known to form anisotropic solutions. For example, it is well known that [225-229] comb polymers form a variety of liquid crystalline structures depending upon the microstructure of the polymer molecule. In our own lab, concentrated solutions of polysulfone-PBLG graft copolymers have been shown to display highly birefringent characteristics in addition to multi-colored patterns between crossed polarizers [230]. Molecules that are sufficiently rigid or flat at the center (disc-like) form a relatively new type or class of liquid
crystalline structure known as columnar or discotic [231, 232] liquid crystals by stacking much like placing coins one on top of another. In fact, if a flat, rigid central unit were designed, discotic star polymers could be produced. Films of star copolymers with polyisoprene and polystyrene blocks have been shown [233, 234] to form crystalline regions where the polystyrene outer blocks intermolecularly order or align in a surrounding polyisoprene matrix.

Figure 5.6 shows the DSC thermograms of the f-PSLG-M2 series. The higher temperature endothermic phase transition is present, indicating that these polymers should display thermotropic liquid crystalline behavior if the assignment of the second transition made in Chapter 4 for linear PSLG is correct. When melts are viewed through crossed polarizers, each polymer of this series is highly birefringent. As Figure 5.6 shows, 2-PSLG, on the first heating cycle, does not display the higher temperature endotherm. The second heating cycle reveals the development of a "shoulder" on the high temperature side of the broad, first endotherm. The melt of 2-PSLG displays some birefringence, but it is weak by comparison to the polymers in the M2 series. A 30.8 % solution of 2-PSLG in toluene displays cholesteric pitches, the distance between them being 11.11 μm apart. The structure of the star polymers should not allow for the formation of cholesteric liquid crystals because there is no way for a nematic structure to organize with molecules of this shape. Solutions of these star polymers in toluene at about 22 % concentration and room temperature are highly birefringent but the 3 arm and 7.6 arm PSLG also display cholesteric pitches. This result is a good indication that the 7.6 arm PSLG has at least one arm that is considerably longer than the others. This arm can participate in the formation of cholesteric ordering
much like the linear PSLG. The same argument can be made for the three arm star although, because there is ample space between the arms, it could very well behave similarly to a "broken" rod, where cholesteric liquid crystal ordering is possible if its molecular weight is high enough. As mentioned above, 2-PSLG, despite being a somewhat lower molecular weight polymer than the stars, shows this effect. Figure 5.7 shows the pictures taken of the 22 % solutions at room temperature; the 3.6 and 5.3 arm polymer do not contain cholesteric pitches.

An interesting feature to note in the 3 arm and 7.6 arm liquid crystal structure is that the distance between the pitches is considerably longer than the linear PSLG samples discussed in Chapter 4. The distance between the pitches in the 3 arm star is 9.25 μm; the 7.6 arm star, 13.89 μm. This could mean that

Table 5.3. Liquid crystalline behavior for /-PSLG-M2 series.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>sample type</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>melt</td>
<td>weakly birefringent</td>
</tr>
<tr>
<td>3</td>
<td>30.8 % sol'n 25° C</td>
<td>cholesteric pitches</td>
</tr>
<tr>
<td>3.6</td>
<td>melt</td>
<td>birefringent</td>
</tr>
<tr>
<td>5.3</td>
<td>22 % sol'n 25° C</td>
<td>birefringent</td>
</tr>
<tr>
<td>7.6</td>
<td>melt</td>
<td>birefringent</td>
</tr>
<tr>
<td></td>
<td>22 % sol'n 25° C</td>
<td>well developed cholesteric pitches</td>
</tr>
</tbody>
</table>

a Melts observed at 70° C.
b Solutions in toluene w/w %.
the arms which are participating a parallel alignment are "forced" farther apart by
the shorter arms in the star. However, other possibilities for the difference exist;
for example, it could be simply a temperature effect since the linear PSLG was
observed a higher temperature than the stars. It is worth pointing out here that in
the thermograms for 3.6-PSLG-M2 and 5.3-PSLG-M2 the second transition,
relative to the first, appears smaller and poorly resolved than in the thermograms
for 3-PSLG-M2 and 7.6-PSLG-M2. This difference may account for differences
in the polymer's liquid crystalline behavior. Also, the first transition in the
thermogram for 5.3-PSLG-M2 is much broader than the same transition in the
other samples. This indicates that the stearyl side chains in 5.3-PSLG-M2 are less
crystalline than the side chains in the other samples.

Table 5.3 summarizes the liquid crystalline behavior for the $\theta$-PSLG-M2
series. The mechanism for the ordering in each of these polymers is undoubtedly
similar to that of the linear polymers (that is, some type of parallel alignment of
the backbones) based on the fact that the second phase transition occurs at the
same temperature and is about the same order of magnitude when compared to
the first transition in both types of polymers. This does not mean, however, that
the same type of liquid crystal structure must form.

5.6 Laser Light Scattering

Static Light Scattering

The star polymers of both series were analyzed using the same light
scattering techniques discussed in Chapter 4. The light scattering data, when
taken together with the observations discussed in preceding sections of this
chapter, give a clearer picture of the actual shape and size of the star PSLG synthesized. In this section, the discussion will focus primarily on the M2 series, where the complications of the bimodal molecular weight distribution in the M1 series are avoided.

The molecular weights of the M1 series are plotted vs. the number of arms in Figure 5.8. Also plotted is the calculated molecular weight based on the \( \{M\}:\{I\} \) ratio. This plot shows the severe reduction in molecular weight caused by the presence of methanol in the reaction. One other point to mention about these samples is that the \( A_2 \) value for each polymer is negative, indicative of aggregation.

Table 5.4 summarizes the data collected from SLS measurements of the M2 series. Again, these data were taken from the interpretation of a Zimm plot for each polymer. These Zimm plots are shown in Figure 5.11. The first observation to point out from Table 5.4 is that the molecular weight for this series of

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mw (daltons)</th>
<th>([\eta]) (dl/g)</th>
<th>( A_2/10^{-4} ) (cm(^3)-mol-g(^{-2}))</th>
<th>Rg (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-PSLG</td>
<td>82,300 ± 4,100</td>
<td>0.26</td>
<td>3.2 ± 0.2</td>
<td>13.4 ± 2.6</td>
</tr>
<tr>
<td>3</td>
<td>222,500 ± 11,100</td>
<td>0.48</td>
<td>-1.1 ± 0.1</td>
<td>31.1 ± 1.7</td>
</tr>
<tr>
<td>3.6</td>
<td>153,600 ± 7,800</td>
<td>0.52</td>
<td>-1.2 ± 0.1</td>
<td>34.2 ± 2.2</td>
</tr>
<tr>
<td>5.3</td>
<td>470,300 ± 23,600</td>
<td>1.10</td>
<td>1.3 ± 0.1</td>
<td>35.7 ± 1.9</td>
</tr>
<tr>
<td>7.6</td>
<td>330,300 ± 16,500</td>
<td>1.02</td>
<td>1.0 ± 0.1</td>
<td>37.3 ± 2.0</td>
</tr>
</tbody>
</table>

\( a \) Mw from \( c=0 \) extrapolation.
\( b \) From intercept of plots in Figure 5.5.
polymers is considerably higher than those of the M1 series. Clearly, the removal of methanol as a co-solvent eliminates the molecular weight reduction. These molecular weights are, however, much higher than the \( \{M\}:\{I\} \) ratio (which was always 50:1) predicts. This is undoubtedly because not all of the initiator is reacting in the heterogeneous reaction. Figure 5.8 also shows the molecular weight of the M2 series plotted against arm number. There is no particular trend but this is not unreasonable, due to the reasons discussed above.

**Treatment of SLS Data by theories designed for Star Polymers With Gaussian Coil Arms**

At this point, a short digression is necessary to explain further the \( g \) and \( g' \) values mentioned briefly in Sections 5.1 and 5.4. These values will be used to analyze the data presented in Table 5.4. The early theoretical work of Zimm and Kilb [235] attempted to quantify size reductions (both geometric and hydrodynamic) by developing a shrinking factor \( g \) or \( g' \) as mentioned earlier. The equations for a Gaussian coil in a theta solvent, for these factors are:

\[
g = \frac{<Rg^2_B>}{<Rg^2_L>} \quad \text{and} \quad \text{Eq. 5.1}
\]

\[
g' = \frac{[\eta]_B}{[\eta]_L} \quad \text{Eq. 5.2}
\]

where the subscripts \( b \) and \( l \) represent branched and linear polymer, respectively.

Theoretically, for a star polymer with arms of varying length:

\[
g = \frac{6f}{(f + 1)(f + 2)} \quad \text{Eq. 5.3}
\]

where \( f \) is the number of arms.

For a star with equal arm length,
\[ g = \frac{(3f - 2)}{f^2} \quad \text{Eq. 5.4} \]

The factors \( g \) and \( g' \) are related in the following [236] manner: \( g' = g^\omega \), where \( \omega \) has been predicted to be 0.5-1.5, depending on the theoretical treatment [235] (for example, whether the polymer is treated as a free draining \( \omega = 1 \) or non-free draining case \( \omega = 0.5 \)). It should emphasized that the equations above were derived for gaussian coil star polymers and that application of Eq. 5.3 and 5.4 to star polymers with stiff arms may not be valid. In fact, when \( f \) equals 1 or 2, Eq. 5.3 and 5.4 are reduced to 1 which is sensible for a random or gaussian coil chain.

A rod-like "star" polymer with \( f = 2 \) (broken rod) will not have the same \([\eta]\) or \( R_g \) as a stiff linear chain if there is sufficient flexibility between the arms. How much these values differ for a rod and a broken rod depend upon the stiffness of the central unit of the broken rod. Eq. 5.3 and 5.4 are to be applied cautiously to the PSLG star polymers. However, empirical data can be used in Eq. 5.1 and 5.2 and comparisons between the values obtained here and in the literature can be made. Another important point to make here concerns the effects of polydispersity on the calculations. Because the effects of branching on dimensional and, especially, hydrodynamic parameters increase rather slowly [104] with increased branching, polydispersity can mask the effects of branching. Also, lower molecular weight star polymers tend to show less deviation in their behavior from their linear counterparts. In their 1948 paper [112], which was among the first attempts to produce well defined star polymers, Shaefgen and
Flory make these points; their own star branched nylon samples were subject to both the effects of polydispersity and finite molecular weight.

**Treatment of Rg Data for Star Polymers With Rigid Arms**

For stars with rod-like arms, an equation can also be developed for the \( g \) factor in terms of \( f \). Consider the following:

As mentioned in Chapter 4, a rod has \( R_g^2 = L^2/12 \), where \( L \) is the length of the rod. If we consider the "two arm" star shown above, we can define its total length \( L \) as \( 2l \), where \( l \) is the length of an arm.

Hence, \( R_g^2(f=2) = (2l)^2/12 = l^2/3 \).

For a 2 arm star, \( R_g^2 = L^2/(2)^23 \).

The \( R_g \) for the 3 arm star shown above can be evaluated as follows:

\[
R_g^2 = 3\int_{0}^{l} x^2 dx/3\int_{0}^{l} dx = l^2/3.
\]

Substituting \( l = L/3 \) into the equation gives

\[
R_g^2 = L^2/(3)^23 \text{ for a 3 arm star.}
\]

Similarly, \( R_g^2 = L^2/(4)^23 \) for a four arm star, etc. We can write in general,

\[
R_g^2(f) = L^2/3^f.
\]
Since \( g = \langle Rg^2 \rangle / \langle Rg^2_r \rangle \), then \( g_{rod} = (L^2/3f)/(L^2/12) \)

Therefore, \( g_{rod} = 4/f \) \quad \text{Eq. 5.5.}

This equation assumes equal arm lengths, a completely rigid polymer, and is defined only for \( f > 2 \).

Eq. 5.5 was developed assuming that there is no difference between a two arm "star" (broken rod) and a linear chain (and there is no difference if the center of the broken rod is stiff). As mentioned before, the difference between the two structures will depend upon how much flexibility there is between the arms of the broken rod.

**Analysis of \( f \)-PSLG-M2 with \( f > 4 \)**

The \( Rg \) data in Table 5.4 for \( f > 4 \) can be analyzed with the following calculations using \( Rg \) values for the star's linear counterpart evaluated from the \( Rg \) vs. \( Mw \) plot in Figure 4.20. The 5.3-PSLG-M2 sample has a \( Rg \) of 35.7 nm. A linear polymer of the same molecular weight has \( Rg = 64 \) nm. Using Eq. 5.1, the \( g \) value for the 5.3 arm star is 0.31. Using the Mark-Houwink equation developed for linear PSLG in Chapter 4, \([\eta] = 2.69 \text{ dl/g}\) for a linear polymer of 470,300 molecular weight. The \( g' \) value for 5.3-PSLG-M2 is then 0.41. The results obtained for 7.6-PSLG-M2 are very interesting and shed light on the shape of this molecule. The \( Rg \) reported in Table 5.4 is much higher than would be expected for a star polymer with the degree of branching anticipated. A linear PSLG of 330,300 molecular weight has \( Rg = 45.5 \) nm. Therefore, \( g = 0.67 \). This \( g \) value should be lower than the value obtained for 5.3-PSLG-M2. The fact that the \( Rg \) is not reduced as much as expected indicates the arms of this star are not
uniform. That is, there are at least one or two arms that are considerably longer than the others. The g' value for this polymer is 0.60, using $[\eta] = 1.70$ for a linear polymer of the same molecular weight. A structure of this type is also consistent with the observed liquid crystalline behavior discussed in Section 5.5. A long arm could align to form a cholesteric structure much like the linear PSLG samples do. The intrinsic viscosity, because of the magnitude that it is reduced, further suggests that the polymer could be roughly described as a "broken" rod with shorter branches near the center. If there were only one arm considerably longer than the rest (so that the other, shorter arms were near the end of the long arm, i.e., a "stick with a ball on the end" shaped polymer), then it is unlikely that the intrinsic viscosity would be reduced to the extent that it is.

Analysis of $f$-PSLG-M2 with $f<4$

Closed association

The polymers 3-PSLG-M2 and 3.6-PSLG-M2 represent two cases where the polymers appear to be aggregated (viscosity plots and a negative $A_2$ value). The analysis discussed below is only a speculative interpretation of the effects of aggregation on the polymer dimensions measured by SLS techniques. The aggregation problem makes an absolute interpretation of the data obtained for these polymers difficult to achieve. We can look at the aggregation as a so-called closed association [237]. Aggregation or association of this type by definition means that there are two distinct species in equilibrium with one another. For example, a unimer (i.e., a single polymer molecule) can be in equilibrium with a dimer (i.e., association of two polymer molecules). This
particular description is at one extreme of an association model where there is no "in-between" equilibrium conditions or mixtures of several sizes of multimers. In the analysis below, the values for \([\eta]\) are assumed to be those resulting from unaggregated polymer. That is, the aggregation effect is assumed to be extrapolated out.

For 3.6-PSLG-M2, the molecular weight obtained from the Zimm plot appears to be a measure of a single, unaggregated polymer while the Rg is not. That is, the Rg is far too large to correspond to even a linear PSLG of molecular weight 153,500. From the Mark-Houwink plot in Chapter 4, the \([\eta]\) of a linear PSLG with a molecular weight of 153,500 is 0.63 dl/g. Hence, the g' value for 3.6-PSLG-M2 is 0.52/0.63 or 0.82. The Rg for linear PSLG with a molecular weight of 153,500 is 22.1 nm. A reasonable value of g can be obtained by dividing the measured Rg of 34.2 nm by two (which infers that the \(R_{g_{\text{aggregate}}} = 2R_{g_{\text{unimer}}}\) and then applying Eq. 5.1. Thus, g = 0.60 for 3.6-PSLG-M2. The assumption of a dimerized aggregate as a cause for the high experimental Rg in 3.6-PSLG-M2 can be rationalized in the following manner: If we assume the star is non-draining we can approximate the g value and the Rg of the star by using Zimm and Kilb's [235] result, \(g' = g^*\). Using the g' value of 0.82 we just obtained for 3.6-PSLG-M2, g = 0.67. By inserting this value of g and 22.1 nm for the Rg of linear PSLG into Eq. 5.1, \(<R_{g_b}> = 18.1 \text{ nm}\). If we divide the experimental value obtained for 3.6-PSLG-M2 by 18.1 nm, the result is 1.89 which suggests a dimerized aggregation within the context of the admittedly speculative assumptions.

We can analyze the experimental results from 3-PSLG-M2 in this way: We
will assume here that both Mw and Rg are aggregate dimensions. If we use the g value evaluated from Eq. 5.5 then g = 0.44; but, as Table 5.5 shows, the experimental g factor for 3.6-PSLG-M2 and 5.3-PSLG-M2 is about 50 % higher than the calculated value. Since the synthesis of the stars were the same (and hence the uniformity of the branching is likely to be the same) we will apply the same difference to the calculated value of g for 3-PSLG-M2, then g = 0.44/0.50 = 0.88. Again, using the non-draining ω value of 0.5, g' = 0.94. The Mw of 3-PSLG-M2 is determined by using g' = 0.94 and [η]b = 0.48 in Eq. 5.2 and solving for [η]i ([η]i = 0.51). Using the Mark-Houwink equation for linear PSLG, the molecular weight is estimated as Mw = 130,000. PSLG with Mw = 130,000 has Rg = 19.0 nm. Now, from Eq. 5.1 we can determine that Rg = 17.8 nm for 3-PSLG-M2. If we divide the experimentally determined Rg value (31.1 nm) by 17.8, we obtain 1.75, which roughly indicates a dimerized aggregate. Dividing the experimental Mw by 130,000 gives 1.71. In Figure 5.9, a schematic representation of the four star polymers is shown, based upon the data interpretation discussed above. Table 5.5 tabulates the numbers calculated above. If Eq. 5.5 correctly predicts the value of g, then the fact that the experimental values of g are higher indicate that the arms of these stars are not uniform. This result is consistent with the method of synthesis for the M2 series.

There were several assumptions made in the above analysis that make the results obtained here seem, at best, marginal. The association in these systems is probably not as simple as a unimer-dimer equilibrium. A more reasonable interpretation of the SLS is presented below based on another type of aggregation mode.
The evaluation of 2-PSLG is straightforward since the polymer is unaggregated. From the Mark-Houwink plot for linear PSLG, PSLG with $M_w = 82,300$ has $[\eta] = 0.28$. Hence, the $g'$ value for 2-PSLG is $0.26/0.28$ or $0.93$. That is, the intrinsic viscosity is only reduced by about 7 percent for the broken rod. This indicates that the polymer is rather stiff despite the six methylene units which "break" the helical backbone. Certainly, more flexibility in the center would cause a greater reduction in $[\eta]$. If then, the preferred conformation of 2-PSLG has on average, a 180° angle between the arms and a fairly narrow distribution about this angle, then the $R_g$ should not be much affected by the break in the backbone. Linear PSLG of $M_w = 82,300$ has a $R_g = 12.7$ nm. As Table 5.4 shows, 2-PSLG has a $R_g = 13.4$ nm. This gives $g = 1.05$ for 2-PSLG. This value indicates that the break in the helix does not seriously affect the dimensions of the polymer. Also, this result supports the approach taken to develop Eq. 5.5.

**Open Association**

Another way to analyze the aggregation observed in the star polymers is to consider the aggregation as being "open" [237]. That is, in open association, there are several equilibrium conditions in solution together so that a unimer + unimer may be in equilibrium with a dimer, a unimer + dimer in equilibrium with a trimer, and so forth. This association results in several solute sizes co-existing and would have the effect of making the polymer molecular weight distribution appear quite broad. Additionally, it would have the effect of making the $R_g$ of the polymer appear abnormally large. As mentioned in Chapter 4, $R_g$ is an average in which the larger species are weighted more heavily; hence, the
measured $R_g$ of an aggregating system may appear much larger than the measured molecular weight would indicate.

The Zimm plots obtained for the samples in this chapter were evaluated in the same manner as the ones obtained for linear PSLG in Chapter 4. That is, "obviously" bad concentration or angle data were removed to give the most "normal" Zimm plot possible (in most cases throughout this work, however, little data had to be "tossed out"). The point here is that curvature in the Zimm plots is easy to misinterpret or overlook altogether, if it is not too severe. However, closer examination of the SLS data obtained for the aggregating polymers reveals that there is curvature in the "virial lines" (lines at constant angle, revealing the concentration effects) of their Zimm plots. For the Zimm plots shown in Figure 5.11, the curvature is not immediately evident, particularly for 3.6-PSLG-M2 in part because some data was removed to construct the whole plot. If the virial lines at zero angle from 3-PSLG-M2 and 3.6-PSLG-M2 are plotted on an expanded scale, as shown in Figure 5.12, the curvature in the lines is evident. As Elias [237] points out, curvature in $1/(M_w)_\text{app}$ (where $M_w_\text{app} = R_g/Kc$) vs. c plots of the type shown in Figure 5.12 is an indication of open association. If these lines are extrapolated to zero concentration, a value for the molecular weight can be obtained. Using the y-intercepts in Figure 5.12, then, 3-PSLG-M2 has $M_w = 191,700$ daltons and 3.6-PSLG-M2 has $M_w = 118,100$ daltons. These molecular weights are lower than those evaluated from the Zimm plots in Figure 5.11, indicating that the effects of the aggregation has been extrapolated away, at least partly. The curvature is also present in the other virial lines. The plots shown in Figure 5.13 were measured at 120° and 135° scattering angles. Note that the
same type of curvature is present. It is also possible to extract a molecular weight from the intercept of these lines, one that presumably results from the scattering of the smaller molecular weight species preferentially. At high scattering angles, [237] the scattering of the smaller molecular weight species (for example, a single polymer molecule) is more readily observable because $P(\theta)$ becomes increasingly smaller with increasingly bigger aggregates at high angles. That is, scattering of the bigger aggregates at high angles becomes weak. Hence, extrapolating these lines to zero concentration should give a more accurate determination of the unimer molecular weight. From Figure 5.13 then, the molecular weight of 3-PSLG-M2 can be evaluated as $M_w = 139,300 \pm 2,000$ daltons; for 3.6-PSLG-M2, $M_w = 102,000 \pm 2,700$ daltons. These molecular weights are probably closer to the actual molecular weight of the unimer. It is

Table 5.5. Calculation of $g$ and $g'$ based on viscosity and SLS data for $f$-PSLG-M2 series.

<table>
<thead>
<tr>
<th>$f$</th>
<th>$g'$</th>
<th>$g^{*}$</th>
<th>$g$ (Eq. 5.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-PSLG</td>
<td>0.93</td>
<td>1.05 ± 0.10</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>b</td>
<td>b</td>
<td>0.44</td>
</tr>
<tr>
<td>3.6</td>
<td>b</td>
<td>b</td>
<td>0.31</td>
</tr>
<tr>
<td>5.3</td>
<td>0.41</td>
<td>0.31 ± 0.03</td>
<td>0.14</td>
</tr>
<tr>
<td>7.6</td>
<td>0.60</td>
<td>0.67 ± 0.07</td>
<td>0.07</td>
</tr>
</tbody>
</table>

a 10% error bars are placed on the $g$ values because there is about a 10% error in the measurement of $R_g$ by SLS.

b A value is not reported in this table due to the complications of aggregation.
not unusual to find curvature in Zimm plot lines if aggregation occurs. Burchard [238] has reported Zimm plots of polyvinylcarbanilate in diethyl ketone with severe curvature in the lines which became less pronounced at higher temperatures.

**Aggregation Tendency**

As the 3 and 3.6 arm polymers have negative $A_2$ values, it indicates that THF is a poor solvent for the polymers and that they tend to aggregate in THF. This supports the interpretation of the curved $\eta_sp/c$ vs. $c$ plots as being due to aggregation. DLS data presented below indicates that 3.6-PSLG-M2 is not as strongly associated as 3-PSLG-M2. The trend in these polymers is toward increasing numbers of arms causing less association. 2-PSLG, however, has a positive $A_2$ value comparable to the values for linear PSLG. If $A_2$ is plotted vs. $f$ as in Figure 5.10, a "tendency to aggregate" trend can be presented with the sharp drop in the curve representing aggregation at $f = 3$ and the sharp rise in the curve representing less tendency to aggregate as $f$ exceeds 5. The $A_2$ value plotted for linear PSLG ($f = 1$) is the average of the values reported in Table 4.5. Why the lower arm stars aggregate and the higher arms do not is presently unknown. However, a reasonable suggestion is that there is no room between chains for the aggregation to occur in higher arm stars. For 7.6-PSLG-M2, however, its non- or weak aggregating properties are probably due to the fact that its behavior more closely resembles a linear or broken-rod PSLG polymer, based on its $R_g$ from SLS and its liquid crystalline behavior. In the 3 and 3.6 arm stars an entropy argument can be used to explain the aggregation. One may consider that in a rod, there is only two aggregation "structures" (excluding end-end
aggregation); parallel (identical end groups pointed in the same direction) or anti-parallel (identical end groups pointed in opposite directions). A three arm star can aggregate by pairing or aligning one of its arms with any of the other arms of another molecule. This leads to several possible combinations of arm pairs and hence greater possibility for such pairing to occur. Several aggregation "structures" then lead to an increase in entropy of the system.

Dynamic Light Scattering

For the PSLG star polymers, DLS experiments were carried out in the same manner and approximately the same concentration range as the linear PSLGs discussed in Chapter 4. The DLS data confirm aggregation of the 3 and 3.6 arm star of the M2 series and provide a hydrodynamic radius that can be compared to a linear PSLG of the same molecular weight.

Figure 5.14 shows the plots obtained after cumulants analysis of 3-PSLG-M2 measured at each concentration (ca. 0.1-0.5 %). A noteworthy feature of these plots is the rise off of the baseline of the exponential decay. As the sample is diluted, the curve finally decays to the baseline. Curves not decaying to the baseline indicate that there is a "slow mode" present or a signal detected that does not exponentially decay. This phenomenon can be caused by aggregation and the fact that the curves "bottom-out" closer to the baseline with successive dilutions supports the possibility that aggregation in the solutions is causing the slow mode. Occasionally, a sample of linear PSLG would show small increments of rise above the baseline (not as much as shown in Figure 5.14), but there was no particular correlation with sample dilution. The non-exponentially decaying
species in those cases were probably small amounts of dust in the sample.

Figure 5.15 shows the $D_m$ vs. $c$ plots for each of the star polymers in the M2 series. The points in these plots are taken from 3rd cumulants data. Note the severe negative slope in the 3 and 3.6 arm plots. As mentioned in Chapter 4, this is an indication of an aggregating polymer. As the negative $A_2$ value indicates, the polymer-polymer interactions in these solutions is much stronger than polymer-solvent interactions. Thus, with increasing concentration, the polymer diffuses more slowly in the direction along a gradient leading to a lower concentration; that is, it is poorly solvated. The $D_m$ vs. $c$ plots for the 5.3 and 7.6 arm polymer, are flat to slightly positive, indicating that they are, if anything, only weakly aggregated and the aggregation does not occur in less concentrated solutions. 2-PSLG has a $D_m$ vs. $c$ slope comparable to the linear PSLG.

Tabulated in Table 5.6 are the results of DLS experiments performed on the M2 series. Note in column 3 that the $R_h$ value was taken from the highest concentration (about 0.5%). If this value is compared to the value calculated in column 4 it is apparent that the aggregated stars have a much larger deviation between the two values. The calculated value of $R_h$ in column 4 assumes all of the polymer-polymer interactions have been extrapolated out ("zero" concentration). Thus, the large deviation for the 3 and 3.6 arm stars suggests that the polymer aggregates in the concentration range studied. The close agreement between the $R_h$ value at "zero" concentration and at the highest measured concentration for the 5.3 and 7.6 arm star suggest (though does not prove) that the polymer is not aggregating in this concentration range. In Figure 5.16 the $\Gamma$ vs. $q^2$ plot for the M2 series are shown. Though the fits for these plots are still
linear, the error bars are large at each point by comparison to linear PSLG $r$ vs. $q^2$ plots. This shows the large deviation between a 3rd and 1st cumulants fit of these data. Large deviations in 1st and 3rd cumulants analyses is indicative of a broad molecular weight distribution. This lends support to the open association model discussed earlier. Again, the $r$ vs. $q^2$ plot for 2-PSLG is comparable to the plots obtained for linear PSLG.

### Table 5.6. $D^o$, $D_m$, and $R_h$ data for $f$-PSLG-M2 series.

<table>
<thead>
<tr>
<th>polymer</th>
<th>$D^o/10^7$ cm$^2$s$^{-1}$</th>
<th>$R_h$/nm</th>
<th>$R_h$/nm Eq. 4.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-PSLG</td>
<td>7.3$^a$</td>
<td>6.5$^o$</td>
<td>6.5$^e$</td>
</tr>
<tr>
<td>3</td>
<td>3.2</td>
<td>20.5</td>
<td>14.8</td>
</tr>
<tr>
<td>3.6</td>
<td>4.4</td>
<td>16.7</td>
<td>10.8</td>
</tr>
<tr>
<td>5.3</td>
<td>2.0</td>
<td>23.8</td>
<td>23.7</td>
</tr>
<tr>
<td>7.6</td>
<td>3.0</td>
<td>14.3</td>
<td>15.9</td>
</tr>
</tbody>
</table>

- $D^o$ values come from the intercept of $D_m$ vs. c plots measured at $\theta = 45^\circ$ and 25$^\circ$ C.
- $R_h$ is taken from the 3rd cumulants data at the highest concentration and $\theta = 45^\circ$.
- $R_h$ is calculated using $D^o$ in column 2 in Eq. 4.7.

### Treatment of DLS Data for Gaussian Coil Star Polymers

Besides, the $g$ factor described above, there is another shrinking factor used to determine the effects of branching on a polymer structure. This factor, defined by Stockmayer and Fixman [239], is the ratio of the hydrodynamic radius of a star to the hydrodynamic radius of a linear polymer with the same molecular weight, called $h$. 
\[ h = \frac{(R_h)_b}{(R_h)_i} \quad \text{Eq. 5.6} \]

Theoretically [239], for arms of equal length,
\[ h = f^{0.5}[(2-f)+2^{0.5}(f-1)]^{-1} \quad \text{Eq. 5.7} \]

Again, Eq. 5.7 has been developed for Gaussian chain polymers in a theta solvent but data can be used in Eq. 5.6 regardless of the geometry of the arms of the star. According to currently available data [223], the hydrodynamic radius of a Gaussian polymer is much less affected by branching than its Rg is, at least for small amounts of branching. Simply speaking, for a given star polymer, \( h > g \).

For example, for a three arm polystyrene star in cyclohexane, \( g = 0.90 \) and \( h = 0.97 \) [224b]. Eq. 5.7 gives \( h \) equal to 0.94 for a 3 arm star; stars containing arms of varying length will have \( h \) values higher than predicted by this equation.

Huber, Burchard, and Fetters [223] report an \( h \) value of 0.84 for a 12 arm polystyrene star (Eq. 5.7 predicts 0.86) in cyclohexane (a theta solvent). In other words, it takes 12 branches to reduce the \( R_h \) by only about 16%.

TREATMENT OF DLS DATA FOR RIGID ARM STARS

The small effect of branching on \( R_h \) can be shown by calculating the friction coefficient, \( f \), for a rod-like polymer and a star with rigid arms. The factor \( f \) is defined as the ratio of the frictional force a polymer feels as it moves in solution and its velocity. Eq. 5.8 below, developed by Kirkwood and Riseman [240], shows the dependence of the friction coefficient on the size of the polymer.

\[ f = \frac{6 \pi \eta b n}{(1 + b/n \sum 1/r_i)} \quad \text{Eq. 5.8} \]

where \( \eta \) is the solvent viscosity
\( b \) is the repeat unit length
\( n \) is the number of repeat units
\[ \sum \frac{1}{r_{ij}} \] is the reciprocal of the total sum of the distance between each repeat unit.

\[ f = \frac{kT}{D^0}, \quad f \propto R_h \]

where \( k \) is Boltzmann's constant

\( T \) is the absolute temperature

Figure 5.17 shows a plot of \( f/f_0 \) vs \( n \) where \( f_0 \) is the friction coefficient of a single repeat unit. The plot shows data calculated from Eq. 5.8 for a rigid rod and a 4 arm star with rigid arms assuming that the angle between the arms is 90°. Note that there is little difference in the friction coefficient until the molecular weight (or number of repeat units) is fairly high. The value of \( f \) will, however, shrink faster with higher amounts of branching. As mentioned previously with respect to Schaefgen and Flory's work [112], it is easy to see how polydispersity could mask the effects of branching, particularly when evaluating \( h \). In Appendix I, the IBM Basic programs written to calculate \( \sum 1/r_{ij} \) and \( f \) are shown with sample output and assumptions made about the structures in order to do the calculations.

**Analysis of DLS Data for \( f \)-PSLG-M2 with \( f > 4 \)**

Using the equation that fits the plot of \( R_h \) vs. \( M_w \), the \( R_h \) value for a linear PSLG of molecular weight 470,300 is 23.9 nm. Thus, an \( h \) value of 0.99 results using the value of \( R_h \) determined from DLS measurements. A linear polymer of 330,500 molecular weight has an \( R_h \) value of 18.1 nm. This gives an \( h \) value of 0.88. It is interesting that the \( h \) value for 7.6-PSLG-M2 is reasonably close to the value obtained for 2-PSLG. This further supports the model drawn in Figure 5.9 for 7.6-PSLG-M2, with two arms considerably longer than the others. Table 5.7
summarizes the $h$ values obtained from DLS data. It appears, from these particular PSLG samples, that branching under 8 or 9 arms has little impact on the hydrodynamic radius. That is, a linear sample of the same molecular weight has a similar $R_h$.

**Analysis of DLS Data for f-PSLG-M2 with $f<4$**

Using the data tabulated in Table 5.6, the $h$ values can be determined for the PSLG stars. As before, the analysis presented in this section for PSLG stars with $f<4$ is a speculative interpretation and represents an attempt to determine what effect aggregation has on the behavior and dimensions of the polymer. The discussion below is based on a simple, closed association model. It is worth mentioning here that open association and hence a broad molecular weight distribution may explain why there is so little difference in the value of $h$ between the star polymers.

In the discussion of the intrinsic viscosity-molecular weight relationships earlier in this section, we concluded that the 3-PSLG-M2 has a molecular weight of about 130,000. Again, using the equation which fits the plot of $R_h$ vs. $M_w$ for linear PSLG shown in Figure 4.28, $R_h = 9.7$ nm. Again, we will assume that the DLS data in column 3 indicates a "dimerized" 3 arm star. The reason for this assumption is because we have just shown that $f$ (and hence $R_h$) is not affected much by branching. This means that the 3 arm star should have a similar $R_h$ to its linear counterpart. The $R_h$ value reported in column 3 of Table 5.6 is from the highest concentration measured for 3-PSLG-M2. It is at this concentration where the affects of aggregation will be the greatest. The value of 20.5 nm is
about twice the value of linear PSLG with a molecular weight of 130,000 (9.7 nm). Hence, dividing the value of $R_h$ for the 3 arm PSLG from column 3 in Table 5.6 by two, the $R_h$ value for 3-PSLG-M2 is 10.2 nm. Therefore, the $h$ value calculated for 3-PSLG-M2 is 10.2/9.7 or 1.05. Recall that a molecular weight of 153,500 was determined from SLS for 3.6-PSLG-M2. The $R_h$ for 153,500 molecular weight linear PSLG is 10.6 nm. If this value and the $R_h$ value for 3.6-PSLG-M2 in column 4 of Table 5.6 is used in Eq. 5.6 (assuming the effects of aggregation have been extrapolated out), then $h$ is equal to 1.02 for 3.6-PSLG-M2.

The value for $h$ for 2-PSLG is 6.5 nm/7.6 nm or 0.85, where 7.6 nm is the $R_h$ value for linear PSLG with a molecular weight of 82,300. For a broken rod, it appears that $R_h$ shrinks "faster" than for the star polymers. However, as mentioned before, 2-PSLG has a narrower molecular weight distribution than the stars. It is possible that the true shrinking of $R_h$ for the star polymers is masked by polydispersity, especially since the $R_h$ shrinking is a small effect to begin with.

**Calculation of $\rho$ values for \(f\)-PSLG-M2**

Finally, as Table 5.7 shows, $\rho$ values were calculated using Eq. 5.9 below.

Eq. 5.10 is the theoretical [241] equation which shows the dependence of $\rho$ on the number of arms for Gaussian coil stars in a theta solvent. Generally, the $\rho$ factor should be greater than 1 but should decrease with increasing arm number and be less than the $\rho$ value of its linear counterpart of the same molecular weight.

\[
\rho = \frac{R_g}{R_h} \quad \text{Eq. 5.9}
\]
\[ \rho = \left[ \left( \frac{3f-2}{f \pi} \right)^{0.5} \left[ 2-f + 2^{0.5}(f-1) \right] \right] / 3f \quad \text{Eq. 5.10} \]

The \( \rho \) value for each polymer is: for 2-PSLG, \( \rho = 13.4/6.5 \) or 2.06; for 3-PSLG-M2, \( \rho = 15.6 \text{ nm} / 10.2 \text{ nm} \) (i.e. 20.5 nm/2 from Table 5.6, column 4) or 1.53; for 3.6-PSLG-M2, \( \rho = 17.1 \text{ nm}/10.8 \text{ nm} \) or 1.58; for 5.3-PSLG-M2, \( \rho = 35.7 \text{ nm} / 23.7 \text{ nm} \) or 1.51; for 7.6-PSLG-M2, \( \rho = 37.3 \text{ nm} / 15.9 \text{ nm} \) or 2.34. Again, for the 3 and 3.6 arm polymer the assumptions made for the calculations of the \( g \) and \( h \) factors were made (aggregation effects). Also shown in Table 5.7 is the calculated value of \( \rho \) for a linear PSLG of the same molecular weight using equation 4.10 and calculating \( L \) in that equation from the molecular weight. Note

Table 5.7. \( h \) and \( \rho \) factors calculated from \( R_h \) values obtained from DLS. The samples are from the \( f \)-PSLG-M2 series.

<table>
<thead>
<tr>
<th>polymer</th>
<th>Mw/10^6 daltons</th>
<th>( h )</th>
<th>( \rho )</th>
<th>( \rho ) (linear)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-PSLG</td>
<td>0.82</td>
<td>0.85</td>
<td>2.06</td>
<td>1.27</td>
</tr>
<tr>
<td>3</td>
<td>1.30 (1.39)*</td>
<td>c</td>
<td>c</td>
<td>1.53 (1.57)</td>
</tr>
<tr>
<td>3.6</td>
<td>1.53 (1.02)*</td>
<td>c</td>
<td>c</td>
<td>1.63 (1.39)</td>
</tr>
<tr>
<td>5.3</td>
<td>4.70</td>
<td>0.99</td>
<td>1.51</td>
<td>2.27</td>
</tr>
<tr>
<td>7.6</td>
<td>3.30</td>
<td>0.88</td>
<td>2.34</td>
<td>2.07</td>
</tr>
</tbody>
</table>

a \( \rho \) value calculated from Eq. 4.10. The value for \( L \) used in this equation was calculated from Mw.

b Values calculated from the intercept of the plots in Figure 5.13.

c Value is not reported in this table due to the complications of aggregation.

that the values obtained for the stars are lower than the value obtained for their linear counterpart. The difference between the two values increases with
branching expect for 7.6-PSLG-M2. The high $p$ value for this sample again reflects an unequal molecular weight distribution of the arms. The $p$ value for 2-PSLG is higher than the value for its linear counterpart. This seems unusual but reflects the fact that the $R_g$ has not changed but $R_h$ has shrunk in comparison to its linear counterpart. This is in contrast to the general observation made previously that branching has a greater effect on $R_g$ than on $R_h$. On the other hand, 2-PSLG is not a branched polymer—hence, it is likely that its behavior would be different from star polymer behavior. Again, the effects of branching on $R_h$ may be masked by polydispersity in the star samples studied.

5.7 Summary

Based on light scattering and intrinsic viscosity data, the PSLG samples described in this chapter are star branched but the branching appears to be non-uniform. The 3 and 3.6 arm PSLG of the M2 series (synthesized without methanol as a co-solvent) are aggregated in THF, even in the low concentration range studied. The 3.6-PSLG-M2 sample appears to be less strongly aggregated. The 5.3 and 7.6 arm samples, by comparison to the lower arm members of the series, are either very weakly aggregated where the association between polymers is broken by the dilutions done in the experiment or are non-aggregated. The broken rod sample, 2-PSLG appears unaggregated in THF. The shrinking factors $g'$, $g$, $h$, and $p$ were determined for the polymers. Based on Eq. 5.5 the branching appears to be non-uniform. The $h$ values reflect that there is little change in the star's $R_h$ when compared to its respective linear counterpart. For 2-PSLG, however, $R_h$ appears be reduced by about 15% compared to its linear
counterpart. The $\rho$ values obtained for the star PSLG were reduced compared to the value obtained for linear PSLG of the same molecular weight.

5.8 Experimental

The experiments in this chapter were carried out in the same fashion as described in the Experimental Section of Chapter 4.
Figure 5.1. Schematic representation of unique macromolecular structures.


E Crosslinked polymers.
Figure 5.2. GPC traces of the f-PSLG-M1 series. Flow rate was 1 ml/minute. See Table 5.1 for elution times and Figure 5.8 for molecular weight range.

A 3 arm. B 3.6 arm. C 5.3 arm. D 7.6 arm.
Figure 5.3. GPC traces for the f-PSLG-M2 series. The flow rate was 1 ml/minute. See Table 5.2 for elution times and Table 5.4 for molecular weight.

A 2-PSLG. B 3 arm. C 3.6 arm. D 5.3 arm. E 7.6 arm.
Figure 5.4. SYBYL models for the PSLG stars. Each arm is helical with a DP of 10. The central unit and arms of the star were minimized with the Maximin2 routine and then joined together as shown. The diameter of each of these models is about 8.5 nm.

A 3 arm. B 3.6 arm. C 5.3 arm. D 7.6 arm.
Figure 5.5. $\eta_{sp}/c$ and $\eta_{inh}$ vs. $c$ plots for the f-PSLG-M2 series. The solvent was THF and the temperature 30° C. The y-intercept gives $[\eta]$. Data in plots A, D and E are linear least square fits. Lines in plots B and C are fitted by a second degree polynomial.

A 2-PSLG. $\eta_{sp}/c$ vs $c$: y-int = 0.26, $\eta_{inh}$ vs $c$: y-int = 0.26.

B 3 arm. $\eta_{sp}/c$ vs $c$: y-int = 0.45, $\eta_{inh}$ vs $c$: y-int = 0.50.

C 3.6 arm. $\eta_{sp}/c$ vs $c$: y-int = 0.53, $\eta_{inh}$ vs $c$: y-int = 0.52.

D 5.3 arm. $\eta_{sp}/c$ vs $c$: y-int = 1.10, $\eta_{inh}$ vs $c$: y-int = 1.13.

E 7.6 arm. $\eta_{sp}/c$ vs $c$: y-int = 0.96, $\eta_{inh}$ vs $c$: y-int = 1.02.
\( \) \( \Delta \eta_{\text{inh}} \)

\( \) \( \bigcirc \eta_{\text{ap}}/c \)

\( c/\text{gdl}^{-1} \)

\( \eta_{\text{sd}}/c \) or \( \eta_{\text{inh}} \)
Figure 5.6. DSC thermograms for the f-PSLG-M2 series. The traces shown here are a first heating and cooling cycle. The heating and cooling ramp was 2° C/minute.

A 2-PSLG. B 3 arm. C 3.6 arm. D 5.3 arm. E 7.6 arm. H2-second heating cycle.
heating trace
cooling trace

B

exoe
endo

C

exoe
endo
Figure 5.7. Pictures of f-PSLG-M2 series taken with crossed polarizers in place. These were approximately 22 wt. % in toluene at room temperature, except for 2-PSLG which was 31 wt %. The 10x objective lens was in place. The actual magnification is shown with a scale bar on the photographs.

A 2-PSLG. The distance between pitches is 11.1 μm.

B 3 arm. The distance between pitches is 9.2 μm.

C 3.6 arm.

D 5.3 arm.

E 7.6 arm. The distance between pitches is 13.9 μm.

The bar markers are 100 μm.
Figure 5.8. This plot shows the deviation from the expected molecular weight when methanol is used as a co-solvent in the star polymer synthesis and when it is absent.
$M : I$ ratio $M_w$

- $\bigcirc$ M1 series
- $\triangle$ M2 series
Figure 5.9. Schematic representation of the f-PSLG-M2 series polymers, based upon the characterization described in Chapter 5.

A 3 arm. B 3.6 arm. C 5.3 arm. D 7.6 arm.
Figure 5.10. This plot, $A_2$ vs. $f$, is a graphic representation of PSLG's tendency to aggregate with change in branching.
Figure 5.11. Zimm plots of the f-PSLG-M2 series polymers. The data obtained from these plots are provided in Table 5.4.

A  2-PSLG.  \( \theta/\text{deg: } 40, 45, 50, 60, 90, 110, 120, 135 \)
\( c/\text{gml}^{-1}: 0.00202, 0.00303, 0.00404, 0.00505 \)
\( r_{c=0} = 0.571 \)
\( r_{\theta=0} = 0.950 \)

B  3 arm.  \( \theta/\text{deg: } 40, 45, 60, 90, 110, 120, 135 \)
\( c/\text{gml}^{-1}: 0.00058, 0.0012, 0.0017, 0.0023, 0.0029 \)
\( r_{c=0} = 0.992 \)
\( r_{\theta=0} = -0.797 \)

C  3.6 arm.  \( \theta/\text{deg: } 40, 45, 50, 60, 90, 110, 120, 135 \)
\( c/\text{gml}^{-1}: 0.00182, 0.00273, 0.00455 \)
\( r_{c=0} = 0.995 \)
\( r_{\theta=0} = -0.943 \)

D  5.3 arm.  \( \theta/\text{deg: } 40, 45, 50, 60, 90, 110, 120, 135 \)
\( c/\text{gml}^{-1}: 0.00094, 0.00283, 0.00377, 0.00472 \)
\( r_{c=0} = 0.978 \)
\( r_{\theta=0} = 0.998 \)

E  7.6 arm.  \( \theta/\text{deg: } 40, 45, 50, 60, 90, 110, 120, 135 \)
\( c/\text{gml}^{-1}: 0.00091, 0.00182, 0.00273, 0.00364, 0.00455 \)
\( r_{c=0} = 0.991 \)
\( r_{\theta=0} = 0.936 \)
Figure 5.12. Zero-angle virial lines of: A 3-PSLG-M2, y-intercept = 35.72; and B 3.6-PSLG-M2, y-intercept = 57.98.
Concentrations for plot A were: 0.0029 g/ml, 0.0023 g/ml, 0.0017 g/ml, and 0.0012 g/ml. Concentrations for plot B were: 0.0046 g/ml, 0.0027 g/ml, 0.0018 g/ml, and 0.0009 g/ml.
Figure 5.13. Virial lines for: A 3-PSLG-M2 and B 3.6-PSLG-M2.

A  
  y-intercept of 120° line: 48.44 .
  y-intercept of 135° line: 49.89 .

B  
  y-intercept of 120° line: 65.35 .
  y-intercept of 135° line: 68.95 .

See Figure 5.12 legend for concentrations measured.
$\Delta 120^\circ$ scattering angle

O $135^\circ$ scattering angle

\[ \text{Graph showing } Kc/R_0/10^{-5} \text{ g mol}^{-1} \text{ vs. } c/10^{-3} \text{ g ml}^{-1} \]
$\triangle 120^\circ$ scattering angle

$\bigcirc 135^\circ$ scattering angle

\[ \frac{Kc}{R_0} / 10^{-5} \text{ gml}^{-1} \]

\[ c / 10^{-3} \text{ gml}^{-1} \]
Figure 5.14. Graphic representation of cumulants analysis of raw DLS data obtained for 3-PSLG-M2. $\theta = 45^\circ$ and $25^\circ$ C.

A 0.0029 g/ml. B 0.0023 g/ml. C 0.0017 g/ml
D 0.0012 g/ml. E 0.0006 g/ml.

See discussion in Chapter 4 for description of these plots.
\[ f(A) \geq 0.068 \]

**Graphs:**

1. **Top Left:**
   - Graph showing CHANNEL # vs. \( G \times 10^{-7} \)
   - Indicates a constant or nearly constant value.

2. **Top Right:**
   - Graph showing \( \tau \times 10^4 \) (SEC) vs. g or g' vs. 1
   - Different curves indicating various rates or decay rates.

3. **Bottom Left:**
   - Graph showing \( \tau \times 10^4 \) (SEC) vs. \( \log(g - g_0) \)
   - Shows exponential decay.

4. **Bottom Right:**
   - Graph showing fit of \( g - g_0 \) with \( \chi^2 \)
   - Indicates the goodness of fit with \( \chi^2 = 0.8827 \), \( 4.258 \), and 109.5 for 1st, 2nd, and 3rd cumulants, respectively.
$I > .054$

$X = 10^4$ (SEC)

$X = 66.99$

$X = 4.343$

$X = 114.0$
D

\[ f(A) > 0.047 \]

\[ \text{1ST CUMU} \quad X^2 = 48.04 \]

\[ \text{2ND CUMU} \quad X^2 = 2.243 \]

\[ \text{3RD CUMU} \quad X^2 = 0.9363 \]
E

\( G \times 10^{-7} \)

\( f(A) > 0.33 \)

\( E \)

\( \tau \times 10^4 \) (SEC)

1ST CUMU \( \chi^2 = 5.908 \)

2ND CUMU \( \chi^2 = 0.926 \)

3RD CUMU \( \chi^2 = 0.935 \)

ERROR OF FIT TO \( g_0 = 1 \)

\( \log_2 (g_0 - 1) \)

\( \tau \times 10^4 \) (SEC)

CHANNEL #
Figure 5.15. $D_m$ vs. c plots for the $f$-PSLG-M2 series. The points plotted are from 3rd cumulants analysis at $\theta = 45$ and 25°.

C. The data represented by the triangle are $D_m$ obtained from the slope of the $\Gamma$ vs. $q^2$ plots in Figure 5.16. The point is lower because the slope of the $\Gamma$ vs. $q^2$ plot results from fitting the line through both 3rd and 1st cumulants data.

<table>
<thead>
<tr>
<th></th>
<th>$D^\circ/10^{-7}$ cm$^2$s$^{-1}$</th>
<th>$k_e/10^{-5}$ cm$^2$s$^{-1}$mlg$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2-PSLG</td>
<td>7.28</td>
</tr>
<tr>
<td>B</td>
<td>3 arm</td>
<td>3.16</td>
</tr>
<tr>
<td>C</td>
<td>3.6 arm</td>
<td>4.17</td>
</tr>
<tr>
<td>D</td>
<td>5.3 arm</td>
<td>1.94</td>
</tr>
<tr>
<td>E</td>
<td>7.6 arm</td>
<td>2.67</td>
</tr>
</tbody>
</table>
$D_m$ measured at $45^\circ$

$\Delta D_m$ from slope of $\Gamma$ vs. $q^2$ plot
45° scattering angle
Δ $D_m$ from $\Gamma$ vs. $q^2$ plot

![Graph B](image)

$D_m/10^{-7}$ cm$^2$·s$^{-1}$ vs. $c/10^{-3}$ gml$^{-1}$

45° scattering angle
Δ $D_m$ from $\Gamma$ vs. $q^2$ plot

![Graph C](image)

$D_m/10^{-7}$ cm$^2$·s$^{-1}$ vs. $c/10^{-3}$ gml$^{-1}$
$45^\circ$ scattering angle
Δ $D_m$ from $\Gamma$ vs. q' plot

$D_m/10^{-7}$ cm$^2$ s$^{-1}$

$C/10^{-3}$ gml$^{-1}$

$45^\circ$ scattering angle
Δ $D_m$ from $\Gamma$ vs. q' plot

$D_m/10^{-7}$ cm$^2$ s$^{-1}$

$C/10^{-3}$ gml$^{-1}$
Figure 5.16. $\Gamma$ vs. $q^2$ plots for the f-PSLG-M2 series.

<table>
<thead>
<tr>
<th></th>
<th>c g/ml</th>
<th>$D_m/10^{-7}$ cm$^2$s$^{-1}$</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.0051</td>
<td>7.45 ± 0.06</td>
<td>0.999</td>
</tr>
<tr>
<td>B</td>
<td>0.0029</td>
<td>2.08 ± 0.08</td>
<td>0.999</td>
</tr>
<tr>
<td>C</td>
<td>0.0036</td>
<td>3.49 ± 0.01</td>
<td>0.999</td>
</tr>
<tr>
<td>D</td>
<td>0.0047</td>
<td>1.81 ± 0.00</td>
<td>0.999</td>
</tr>
<tr>
<td>E</td>
<td>0.0046</td>
<td>2.98 ± 0.03</td>
<td>0.999</td>
</tr>
</tbody>
</table>

Error bars on $D_m$ result from using the 1st and 3rd cumulants fit in the plots.
Figure 5.17. Friction coefficient, $f$, vs. number of repeat units, $n$ for a 4 arm star in 2 dimensions with the shape of a plus (+) sign. The friction coefficient for one repeat unit, $f_o$, is divided into $f$ to give $f/f_o = 1$ for 1 repeat unit and hence a y-intercept of 1. See Appendix 1 for a description of the programs written to calculate $f$. 
linear rod
4 arm star (rod arms)
Chapter 6: Future Directions
6.1 Synthesis

As a maximum molecular weight of only about 250,000 was achieved in this work for linear PSLG, it would be desirable to produce PSLGs with molecular weights somewhat higher, perhaps upwards of a half million. This could be accomplished by continuing to initiate SLGNCA with sodium methoxide, but without methanol present as an initiator solvent. The reaction would be heterogenous initially, but should become homogenous immediately, since only small quantities of methoxide are needed. In fact, even though the reaction is initially heterogenous, workers [242] still studied the reaction kinetics of BLG-NCA reacted with solid sodium methoxide as an initiator. Without the methanol present, the reaction should not only produce higher molecular weight polymer but will undoubtedly have a narrower molecular weight range although a heterogenous reaction is not ideal for producing monodisperse polymers.

The star polymer synthesis definitely has room for improvement. The initiators synthesized in this work suffer primarily from solubility problems. Unfortunately, this property limits their usefulness. Also, it would be more useful in the study of star polymers with rod-like arms if a rod with a smaller diameter were used for the arms. PBLG or PMLG arms are better candidates for model stars with rod-like arms except PMLG's usefulness is somewhat limited by its poor or limited solubility. The other common synthetic technique should be employed to make these star polymers; namely, the coupling of linear chains to a multi-functional coupling agent. Linear PXLG chains could be synthesized with a primary amine. This ensures an amino function at the end of the chain and gives the most narrow molecular weight distribution. The linear PSLG could then be
coupled to reagents such as the examples shown in Scheme 6.1. The compound in Scheme 6.1 labeled (A) is commercially available. Also shown in Scheme 6.1 is the synthesis of a six arm coupling reagent. Note that it bears a similarity to the reactions used to produce the initiators discussed in Chapter 3. However, using a diol rather than a diamine allows the introduction of triflate groups into the molecule. The methylene groups between the alcohol functionalities serve as a "spacer" so that there is sufficient room between the arms of the star polymer. The triflate anion is an outstanding leaving group and the coupling compounds should react easily with the amino end of the polymer, making a secondary amino link between the center of the star and the arms. This step could be run in the presence of a weak base to prevent protonation of the amino functions on the end of the chains. The linear polymer chains would be in excess to ensure all active sites of the central unit are reacted; Also, if in excess, they could act as "proton sponges" themselves rather than introducing a base into the reaction. One of the drawbacks to this approach of star polymer synthesis is that linear chains always contaminate the star polymer. The unreacted linear chains would have to be removed by fractionation; perhaps a THF solution of the polymer could be fractionally precipitated with acetone to effect the separation. This would be similar to the fractionation discussed in Chapter 2 to remove the -sheet component of the polymer mixture. With ester linkages holding the arms to the central unit, a selective hydrolysis could be accomplished which would cleave the arms of the polymer from the central point of attachment without degrading the arms themselves. GPC analysis of the resulting polymer would give an indication of the molecular weight distribution of the arms.
6.2 Characterization

If linear PSLG were synthesized with a higher molecular weight, then a value for the persistence length could be more reliably obtained for PSLG. Also, it would be desirable to have higher molecular weight points on the Mark-Houwink curve to ensure that the slope of the line remains constant in the higher range. Also, with higher molecular weight PSLG, interesting macrostructures such as strong films and gels could be produced.

The PSLG samples already synthesized should be characterized more fully with light microscopy. By studying several concentrations of one polymer at a given temperature, the A-point can be determined for a given molecular weight of PSLG. The A-point is the concentration at which the solution is consists of both an isotropic and anisotropic phase. This could be determined for several molecular weight samples and compared to data already available on PBLG. Any differences in behavior would undoubtedly be due to the long hydrocarbon side chains of PSLG, the only structural difference between it and PBLG.

Another valuable experiment using light microscopy would be to create a phase diagram for mixtures of PSLG and a Gaussian coil polymer such as polystyrene dissolved in THF or toluene (a thermodynamically good solvent for polystyrene). Work in Russo's [243] group has produced a similar diagram for the PBLG-polystyrene-pyridine system. What may be particularly interesting and informative in the PSLG-polystyrene system is whether the polymer-polymer phase separation occurs at a different concentration than in the PBLG-polystyrene system; that is, will the long hydrocarbon side chains help in "dissolving" the polystyrene and thus keep the two polymer components mixed.
Because LSU is equipped to do diffusion experiments with FRAP (Fluorescence Recovery After Photobleaching) instrumentation, it would be interesting to label PSLG with a fluorescein derivative and study its diffusion behavior in a concentrated solution, a gel, another polymer matrix or study the diffusion of the label itself in solutions of PSLG. Another interesting diffusion experiment would be to follow the motion of a labeled linear PSLG molecule through a matrix of star branched PSLG to determine if the diffusion is faster or slower when compared to motion in a linear polymer matrix; i.e., whether the labeled polymer is more easily entangled in a star branched polymer matrix.

One of the advantages to synthesizing the star branched polymers as outlined in section 6.1 is that the molecular weight of the linear polymer component in the synthesis (one arm of the star) can be determined separately by SLS and then the molecular weight of the star polymer can be similarly determined. With this information, the true degree of branching can be obtained by dividing the molecular weight of the star polymer by the molecular weight of the linear polymer (i.e., by the molecular weight of one arm). Continuing work with the star branched PSLG is necessary; particularly important is finding a solvent that the polymer will not aggregate in at low concentrations but that also has a relatively high dn/dc value so that the polymer solutions will scatter light appreciably in SLS and DLS experiments. With unaggregated systems, the shrinking factors $g$, $h$, and $\rho$ can be more reliably determined. The shrinking factors determined for these polymers (stars with rod-like arms) can then be compared to the values obtained for gaussian coil stars already reported in the literature.
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Appendix I. Kirkwood-Riseman Modeling of Rod-like and Rigid Arm Star Polymers.
The calculation of $\sum \sum 1/r_{ij}$ in Eq. 5.5 by hand is tedious for small polymers and impossible for "real" polymers where the DP is large. However, a relatively simple computer program which sums the required number of times can easily evaluate the term. The higher the DP, the longer it takes for the computer to do the calculations. For example, a polymer with a DP of 500 requires the computer to do 500 loops 500 times. The program shown in Figure A.1 was used to calculate the friction coefficient for a rod-like polymer consisting of n beads.

Figure A.1. IBM BASIC code for calculating $f$ for a rod-like polymer.

5 'AUTHOR(S): DREW POCHE'
6 'DATE: MARCH 5, 1990
9   DIM X(2000), DP(20)
11 INPUT "HOW MANY RODS DO YOU WANT TO CALCULATE:";M
13 PRINT "ENTER THE DP OF EACH ROD:"
14 FOR S = 1 TO M
16   INPUT DP(S)
17 NEXT S
19 FOR L = 1 TO M 'repeat for M number of rods
20   SUM = 0
30   FOR I = 0 TO DP(L)-1
40     X(I) = I 'x coordinate
50 NEXT I
60   FOR I = 0 TO DP(L)-1
70     FOR J = 0 TO DP(L)-1
80       IF I = J THEN 120
90     Z = (X(I)-X(J))^2 'distance between two beads
100   R = 1/Z^0.5
110   SUM = SUM + R 'SUM holds the value of $\sum \sum 1/r_{ij}$
120 NEXT J
130 NEXT L
135 F = 6*3.1415927*0.00453*DP(L)/(1 + SUM/DP(L)) 'Eq. 5.5
140 PRINT "DP = "; DP(L), "1/R_{ij} = ";SUM, "f = ";F
150 NEXT L
160 END

In Figure A.2 a model of the rod-like polymer is shown where the solid beads
represent a repeat unit.

Figure A.2. Rod-like polymer with 13 repeat units. The first monomer is set at the origin of an x,y axis. The 13th bead will then be located at position (12,0) on the "plot".

(0,0) . . . . . . . . . . (12,0)

Lines 30-50 in Figure A.1 set the monomer positions on an x-y axis, where X(I) is the x coordinate. The y coordinate will always be zero for a rod-like array of beads so that the distance formula simplifies to the equation in line 90. Lines 60-130 calculate the distance between each bead (where the "bond" length is given an arbitrary value of 1) and sum the distances to give $\Sigma 1/r_{ij}$. The storage location named SUM contains this value. Line 135 contains the Kirkwood-Riseman equation (Eq. 5.5) where $\eta = 0.00453$ and the value for b is set to 1.

Figure A.4 shows the program which does the same calculation for a four arm star. Figure A.3 shows a model of the four arm star for which f is calculated in Figure A.4.

Figure A.3. Four arm star model with 13 beads. The arm DP is considered to be 4 in this model so that the center bead is accounted for. The center of the star is at the origin of an x-y axis.

(0,3) c
  .
  .
  .
 b (-3,0) . . . . . (3,0) a
  .
  .
 (0,-3) d
In Figure A.4, code lines 20-234 set up the positions of the beads where X(I) and Y(I) are the x and y coordinates. Lines 232-234 specifically set the center bead at (0,0). Lines 20-60 set the positions of the beads in arm a. Lines 70-110 set the positions of the beads in arm b. Lines 120-170 set the positions of the beads in arm c. Lines 175-230 set the positions of the beads in arm d. The K increments assign a number to the bead which is its "distance" from the origin (again, the distance between beads is arbitrarily set to 1). For the star in Figure A.3 the four loops mentioned above will set the positions of three beads in each loop. With one bead at the origin, this is a total DP of 13. If an arm DP of 100 is entered into the program, the star is evaluated as having 99 beads in each arm plus one center bead for a total DP of 397. The code in lines 240-280 calculate \( \sum \sum 1/r_{ij} \), where the equations in lines 270 and 272 evaluate the distance between two points, \( c^2 = a^2 + b^2 \). Line 295 calculates f for the star; the value NC in the equation is the total number of beads present.

Figures A.5 and A.6 show sample output for each program.
'AUTHORS: DREW POCHE' AND LEAH POCHE'

'DATE: MARCH 5-13, 1990

DIM X(1000), Y(1000), DP(20) 'X and Y are x-y coordinates

INPUT "HOW MANY STARS DO YOU WANT TO CALCULATE:";M

PRINT "ENTER THE DP OF ONE ARM FOR EACH STAR:"

FOR S = 1 TO M

INPUT DP(S)

NEXT S

FOR L = 1 TO M

K = 1 'K will set the distance from the bead to the orgin

FOR I = 1 TO DP(L) - 1

X(I) * K

K = K + 1

Y(0) = 0

NEXT I

K = -1

FOR I = DP(L) TO DP(L)*2 - 2

X(I) = K

Y(I) = 0

K = K -1

NEXT I

K = 1

FOR I = DP(L)*2 - 1 TO DP(L)*3 - 3

X(I) = 0

Y(I) = K

K = K + 1

NEXT I

K = -1

FOR I = DP(L)*3 - 2 TO DP(L)*4 - 4

X(I) = 0

Y(I) = K

K = K + 1

NEXT I

NC = DP(L)*4 - 3 'NC is the total number of beads

X(NC) = 0

Y(NC) = 0

SUM = 0

FOR I = 1 TO NC

FOR J = 1 TO NC

IF I = J THEN 280

Z = (X(I) - X(J))^2 + (Y(I) - Y(J))^2

R = Z^0.5

SUM = SUM + (1/R) 'Holds the value of \sum 1/r_i

NEXT J

NEXT I
295 $F = \frac{(6 \times 3.141728 \times 0.00453 \times NC)}{(1 + \text{SUM}/NC)}$  
'Eq. 5.5
300 PRINT "DP (4 ARM STAR):";NC, "1/Rij =";SUM, "f =";F
310 NEXT L
320 END

Figure A.5. Sample output for the program listed in Figure A.1.

HOW MANY RODS DO YOU WANT TO CALCULATE: 9
ENTER THE DP OF EACH ROD:
 1
 12
 24
 48
 120
 240
 480
 600
 800

DP = 1  
 1/Rij = 0.0  
f = 0.085388

<table>
<thead>
<tr>
<th>DP</th>
<th>1/Rij</th>
<th>f</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>50.47705</td>
<td>0.196807</td>
</tr>
<tr>
<td>24</td>
<td>133.2461</td>
<td>0.312782</td>
</tr>
<tr>
<td>48</td>
<td>332.0450</td>
<td>0.517663</td>
</tr>
<tr>
<td>120</td>
<td>1048.503</td>
<td>1.052282</td>
</tr>
<tr>
<td>240</td>
<td>2428.773</td>
<td>1.842936</td>
</tr>
<tr>
<td>480</td>
<td>5523.465</td>
<td>3.277026</td>
</tr>
<tr>
<td>600</td>
<td>7172.884</td>
<td>3.954756</td>
</tr>
<tr>
<td>800</td>
<td>10025.89</td>
<td>5.047965</td>
</tr>
</tbody>
</table>
Figure A.6. Sample output for the program listed in Figure A.4.

HOW MANY STARS DO YOU WANT TO CALCULATE: 7
ENTER THE DP OF ONE ARM FOR EACH STAR:
5
10
20
40
60
100
200

DP (4 ARM STAR): 17

<table>
<thead>
<tr>
<th></th>
<th>1/Rij</th>
<th>f</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>297.2141</td>
<td>0.349764</td>
</tr>
<tr>
<td>77</td>
<td>746.3807</td>
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</tr>
<tr>
<td>157</td>
<td>1764.331</td>
<td>1.095460</td>
</tr>
<tr>
<td>237</td>
<td>2869.583</td>
<td>1.543879</td>
</tr>
<tr>
<td>397</td>
<td>5233.622</td>
<td>2.390144</td>
</tr>
<tr>
<td>797</td>
<td>11645.80</td>
<td>4.359112</td>
</tr>
</tbody>
</table>
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Vita

Drew S. Poche' was born on November 8, 1962 in New Orleans, Louisiana. He attended public schools in Metarie, Louisiana until his family moved to Covington, Louisiana in 1975. He graduated from Covington High School in 1980 and began undergraduate school at Southeastern Louisiana University. During undergraduate school he was employed by V-labs, Inc. of Covington, Louisiana. After obtaining a B.S. degree in Chemistry in December of 1984 and an Associates degree in Computer Science in May of 1985, he began graduate school at Louisiana State University in the fall semester of 1985. He is currently a candidate for the Ph.D. degree with a major in Organic Chemistry and a minor in Physical Chemistry. His research area is in the field of Polymer Chemistry with special emphasis on the synthesis and properties of rod-like polymers.

In August of 1987 he married Leah Hatch.
Candidate: Drew S. Poche

Major Field: Chemistry

Title of Dissertation: Synthesis and Characterization of Linear and Star Branched Poly(γ-stearyl-L-glutamate)

Approved:

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