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SYNTHESIS OF STEREOREGULAR POLY[ALKYL MALOLACTONATES]
AND THE APPLICATION OF POLY[β -MALIC ACID] IN DRUG DELIVERY SYSTEMS

A Dissertation Presented

by

STEPHEN C. ARNOLD

Submitted to the Graduate School of the
University of Massachusetts in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

February 1987

Polymer Science and Engineering

Stephen C. Arnold



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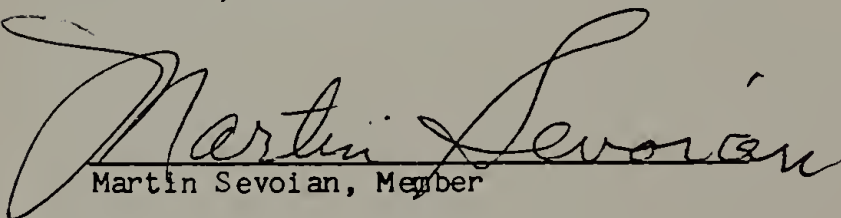
by


STEPHEN C. ARNOLD

Approved as to style and content:


Robert W. Lenz, Chairman of Committee


David A. Tirrell, Member


Martin Sevoian, Member


Edwin L. Thomas, Department Head
Polymer Science and Engineering

Dedicated with affection
to my wife Nina

Badlands, you gotta live it everyday
Let the broken hearts stand
As the price you've gotta pay
We'll keep pushin' til it's understood
And these Badlands start treating us good

--Bruce Springsteen

ACKNOWLEDGEMENTS

I want to extend my gratitude to my advisor Professor Robert W. Lenz for his infinite patience and moral support during my tenure at the University of Massachusetts. I want to thank my committee members Professor David A. Tirrell for his helpful suggestions and Professor Martin Sevoian for his interest in the project. Special thanks go to Professor Dr. Helmut Ringsdorf for his guidance and hospitality during my stay at the Johannes Gutenberg University in Mainz, West Germany, and to Professor Melvin J. Hatch for his contribution to my undergraduate education and training in organic chemistry.

I have had the opportunity to meet many talented people and make many friends during my graduate studies. In particular, I want to acknowledge Menas Vratsanos and Dave Kinning for their friendship which I hope will endure after we have graduated. I want to thank my various lab partners, Dani Fisher, Joe Daly, and Rich Gross, for tolerating my many idiosyncrasies and constant sarcasm in the laboratory. I also had the chance to make some good friends in Germany. I am especially grateful to Hubert Bader, Micheal Haups, and Bernd Reck for their fellowship. Finally, I want to thank my parents for their advice and support during my long college education and my wife, Nina, for her love, patience, and determination, especially during the times when I was working far away from her.

ABSTRACT

Synthesis of Stereoregular Poly(alkyl malolactonates) and the Application of Poly(β -malic acid) in Drug Delivery Systems

February 1987

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B.S., New Mexico Institute of Mining and Technology

M.S., Ph.D., University of Massachusetts

Directed by: Professor Robert W. Lenz

Optically pure methyl, ethyl, isopropyl, and benzyl (R)-malolactonate were prepared from (S)-malic acid in a six step synthesis and were bulk polymerized using tetraethylammonium benzoate as the initiator to yield high molecular weight, crystalline polyesters. The optical purities of methyl and benzyl (R)-malolactonate were determined by 300 MHz proton NMR spectroscopy of the β -lactone complexed with the chiral europium shift reagent, $\text{Eu}(\text{hfbc})_3$. Enantiomeric excesses of 100 percent were found.

The bulk polymerizations of alkyl (R)-malolactonates were heterogeneous because the resulting poly[alkyl (S)-malates] were insoluble in their monomers. The highest number average molecular weights were 41,000 for poly[methyl (S)-malate], 20,000 for poly[ethyl (S)-malate], 65,000 for poly[isopropyl (S)-malate], and 73,000 for poly[benzyl (S)-malate]. Poly[(S)- β -malic acid] was prepared from

poly[benzyl (S)-malate] by the catalytic hydrogenolysis of the pendent benzyl esters. The stereoregular poly[alkyl (S)-malates] were also crystalline with the following final melting points: 198°C (Bz), 182°C (H), 156°C (Me), 152°C (Et), and 131°C (iPr).

A copolymer was prepared from ethyl and benzyl (R)-malolactonate with a copolymer composition of 76 mole % ethyl (S)-malate and 24 mole % benzyl (S)-malate and with a number average molecular weight of 32,000. Another copolymer of ethyl (S)-malate and (S)-malic acid was obtained by the catalytic hydrogenolysis of the benzyl esters of poly[ethyl-co-benzyl (S)-malate].

These stereoregular poly[alkyl (S)-malates] are interesting new biomaterials. Since they are crystalline and water insoluble, they could be used to make sutures, protheses, and other implants that would eventually biodegrade into (S)-malic acid, an intermediate in the Krebs cycle, and the corresponding alcohol. Poly[(S)- β -malic acid] could be used as a biodegradable polymeric drug carrier, and copolymers of (S)-malic acid and its esters could be used in bioerodible drug delivery systems.

Polyacid-coated liposomes were prepared from dipalmitoylphosphatidylglycerol and either poly[acrylic acid] or poly[L-glutamic acid] bearing 5 mole percent pendent cholesteryl esters that inserted into phospholipid bilayer and anchored the polymer to the liposome. The absorption of the polymer onto multilamellar liposomes of dipalmitoylphosphatidylglycerol was detected by high-sensitivity differential scanning calorimetry and raised the phase transition

temperature of this lipid. These polyacid-coated liposomes could be used in liposomal drug delivery systems in which an entrapped drug would be released slowly into the bloodstream, because it would have to diffuse across a negatively charged, polyacid-coated, phospholipid bilayer.

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CHAPTER I

INTRODUCTION

Drug design is a difficult task requiring expertise in chemistry, biochemistry, biology, medicine, and biomedical engineering, and drug research is a broad area involving the synthesis and biological evaluation of new drugs and development of new drug delivery systems. In the area of drug delivery, polymer chemistry is an integral part of controlled release technology. Controlled drug delivery has been developed in response to the shortcomings of conventional drug forms such as tablets, capsules, and injections.

When a tablet is taken, the concentration of the drug in the bloodstream may initially rise above the therapeutic range in which the drug concentration is sufficient to produce the desired therapeutic effect and into the toxic range where the drug may cause unwanted side effects like headaches, nausea, and skin rashes. Over the next several hours, as the drug is metabolized, its concentration in the bloodstream falls within the therapeutic range, and then, below the therapeutic range, where the drug is pharmacologically ineffective, and another tablet must be taken to increase the concentration of the drug. In this way, the concentration of the drug fluctuates back and forth spending only about fifty percent of the time in the therapeutic range. On the

other hand, controlled drug release involves the slow release of biologically active substances in the therapeutic range usually from a polymer matrix or through a polymer membrane. Controlled drug delivery eliminates many side effects, because the concentration of the drug never reaches the toxic level, and it utilizes the drug more efficiently than conventional methods, because the concentration of the drug falls within the therapeutic range for eighty to ninety percent of the time (see Figure 1).

In 1975, Ringsdorf described a model of a polymeric drug carrier which is shown in Figure 2 (1). A drug is attached to the polymer with or without a spacer. Both the polymer and the spacer can be either biostable or biodegradable. The spacer is used to make the bound drug more accessible to the biological environment. Other molecules or functional groups can also be incorporated into the polymer to change its solubility or to transport it to specific cells. In fact, the coupling of a drug to a polymer may change the toxicity and metabolism of the drug and may thereby reduce the amount of the drug required for an effective treatment.

Many polymeric drug carriers have been based on biostable polymers such as poly(acrylic acid), poly(methacrylic acid), and the alternating copolymer of divinylether and maleic anhydride (2-7). In general, these drug delivery systems have been toxic because of the accumulation of the biostable polymer in the host. This fact has lead to the use of biodegradable polymers as drug carriers. However, there are very few polymers that are both biodegradable and functionalizable. The

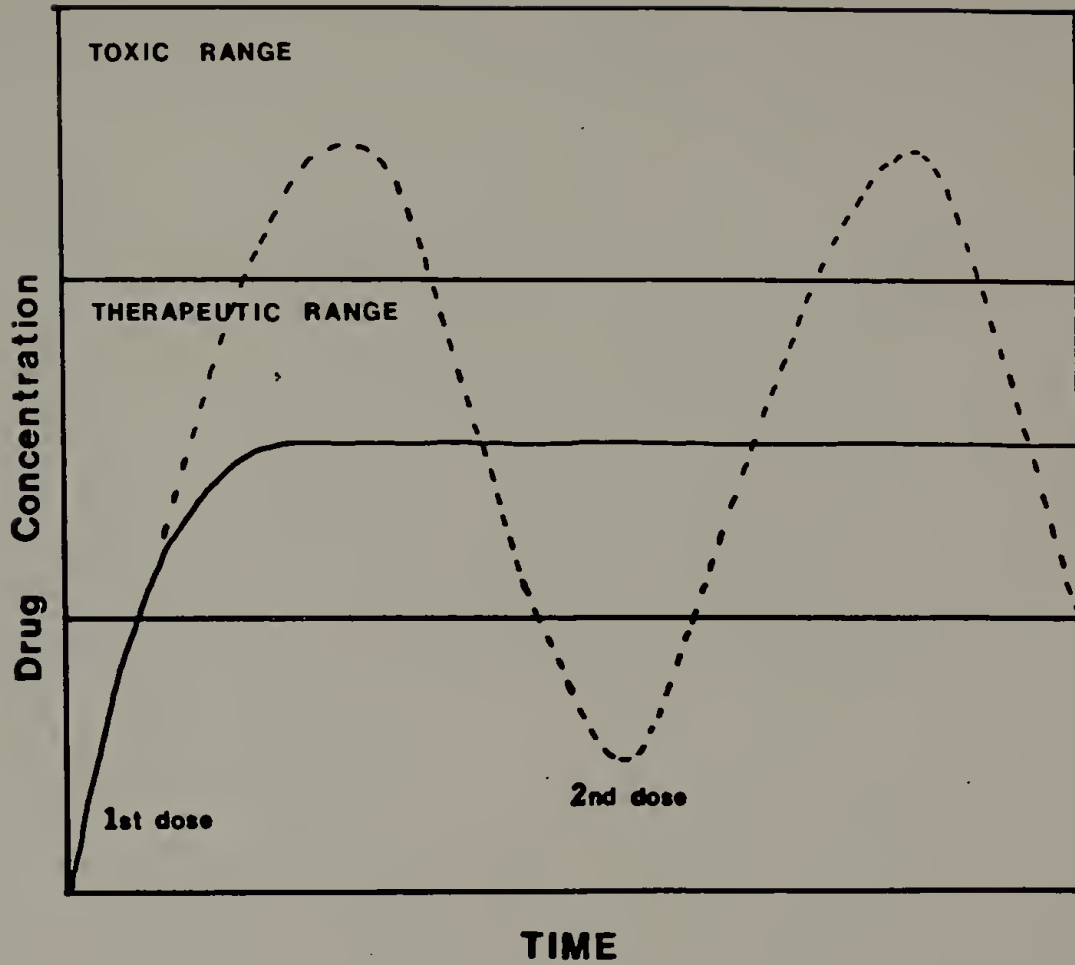


Figure 1. Conventional and Controlled Drug Delivery

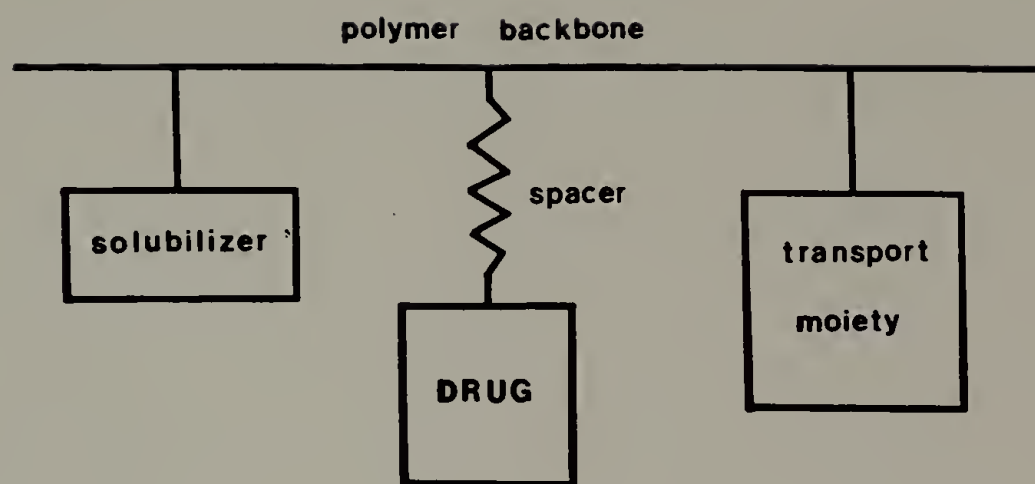


Figure 2. Ringsdorf's Model of a Polymeric Drug Carrier

polypeptides poly(N-hydroxyalkyl-L-glutamine) and poly(L-glutamic acid) have been used as biodegradable drug carriers (8-10). The degradation product from these polypeptides is L-glutamic acid which is readily absorbed by normal cells. Clearly, there is a real need to prepare more biodegradable, functionalizable polymers for biomedical application.

Biodegradable polymers have been used in other kinds of controlled drug delivery. Poly(glycolide), poly(L-lactide), and copolymers of glycolide and lactide have been used in bioerodible drug delivery systems in which the polymer matrix is impregnated with an unbound drug that is slowly released as the polymer swells and degrades into glycolic and lactic acid (11-12). L-Lactic acid is a natural product of muscle metabolism and is disposed of by normal pathways after the release of the drug. In this case, long term toxicity is avoided by building the polymer matrix from naturally occurring, nontoxic molecules.

In our laboratory, we have been developing biodegradable, functionalizable polymers based on malic acid which is another naturally occurring compound. The polymers of interest are poly(β -malic acid), poly[alkyl malates], and copolymers of malic acid and its esters. These polymers are designed to be practical alternatives to polypeptides and polylactides in drug delivery applications. In the rest of this chapter, the synthesis of these polymers is reviewed, and liposomal drug delivery is discussed with particular emphasis on the absorption of polymers onto phospholipid vesicle membranes.

A. Synthesis and Polymerization of Racemic Alkyl Malolactonates

In 1979, Vert and Lenz first reported the synthesis of amorphous poly[(R,S)- β -malic acid], and Johns continued these investigations and improved the overall synthesis which is shown in Figure 3 (13-17). Racemic (R,S)-bromosuccinic acid was converted into its anhydride by refluxing acetyl chloride. Distilled (R,S)-bromosuccinic anhydride was reacted with benzyl alcohol to form a mixture of monobenzyl (R,S)-bromosuccinates. This ester mixture was then dissolved in aqueous sodium bicarbonate and stirred vigorously in the presence of benzene. One isomer was converted into racemic benzyl malolactonate by an internal S_N2 reaction. The lactonization presumably occurred in the aqueous solution, and the β -lactone was extracted into the benzene phase before it decomposed. The other isomer was recovered by acidification of the aqueous solution, extraction into ether, and recrystallization from cyclohexane (18). Purified benzyl malolactonate was bulk polymerized using a weak base initiator such as tetraethylammonium benzoate. Poly[benzyl (R,S)-malate] was converted into amorphous poly[(R,S)- β -malic acid] by the catalytic hydrogenolysis of the pendent benzyl esters without polymer degradation.

Johns found that the bulk polymerization of racemic methyl, ethyl, and isopropyl malolactonate monomers with tetraethylammonium benzoate yielded low molecular weight polymers with number average molecular weights below 5,000 and that the bulk polymerization of racemic benzyl malolactonate with either tetraethylammonium benzoate or triethylamine

gave polymers with number average molecular weights between 7,000 and 25,000 (19). It was suggested that low molecular weight polymers were formed because of chain transfer to monomer, chain termination with reactive impurities, or monomer decomposition. Evidence for chain transfer to monomer included the observation of double bond absorptions in both the proton and carbon-13 NMR spectra of the polymers. Chain transfer to monomer would only result in a double bond at one end of some of the polymer chains, and therefore, the concentration of double bonds in a polymer sample would be low (except for oligomers) and would not be observed in the NMR spectra. Consequently, the most convincing evidence was found in the low molecular weight poly[methyl, ethyl, and isopropyl (R,S)-malates]. Evidence for chain transfer to reactive impurities included the correlation of polymer molecular weight with monomer purity for benzyl (R,S)-malolactonate.

The synthesis of poly[benzyl (R,S)-malate] was repeated in this study with the results listed in Table 1. The yields and molecular weights varied from batch to batch which is consistent with the hypothesis that low molecular weight polymers are formed because of chain termination with small amounts of reactive impurities, since the purity of the monomer varied from batch to batch. No double bond absorptions were observed in the proton and carbon-13 NMR spectra of these polymers. In fact, sometimes, the crude benzyl malolactonate polymerized during its distillation, probably initiated by some impurity. The number average molecular weight of these "thermally" polymerized polymers was between 3,000 and 5,000.

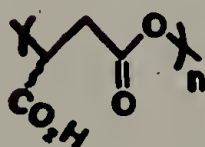
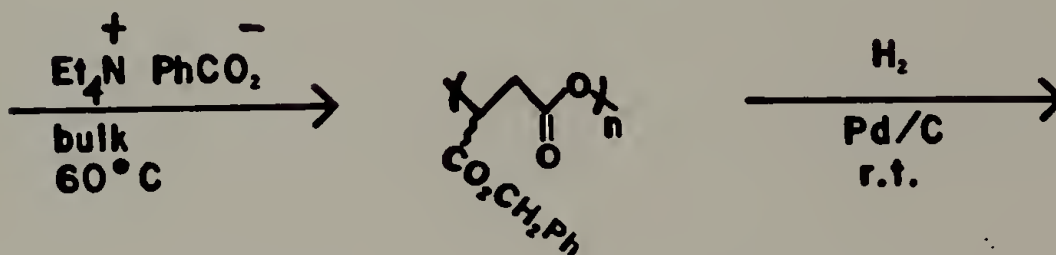
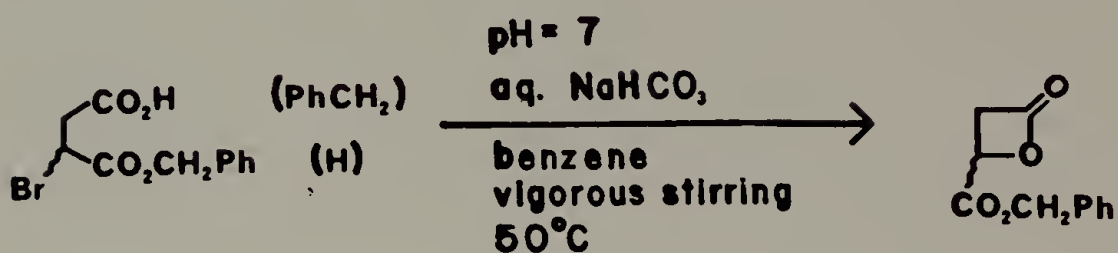
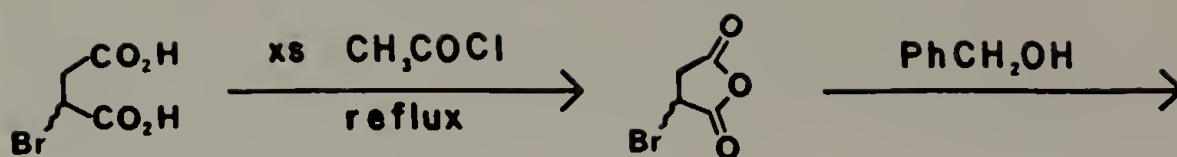


Figure 3. Synthesis of Amorphous Poly[(R,S)-malic acid]

Table 1

Yields and Molecular Weights of Poly[benzyl (R,S)-malate]

Yield ^a	M_n^b	M_w^b	M_w/M_n^c
68 %	21,000	22,000	1.1
52	18,000	20,000	1.1
54	17,000	19,000	1.1
58	15,000	17,000	1.1
68	13,000	15,000	1.1
74	12,000	13,000	1.1
75	9,000	11,000	1.2
80	7,900	13,000	1.6
81	5,300	6,200	1.2
69	3,900	4,700	1.2

^aBy bulk polymerization at 60°C for 7-15 days using tetraethylammonium benzoate as the initiator, 100 % conversion by infrared spectroscopy, mole ratio of monomer to initiator of 10^3 - 10^4 , polymer insoluble in 90::10 (v/v) methanol and acetone, supernatant cloudy.

^bMolecular weights were determined by gel permeation chromatography using polystyrene standards.

^cNarrow molecular weight distributions are probably caused by fractionation during polymer precipitation.

In a series of polymer preprints, some of the biological properties of amorphous poly[(R,S)- β -malic acid] have been reported. Poly[(R,S)- β -malic acid] was shown to degrade into malic acid under physiological conditions (20). Poly[(R,S)- β -malic acid] was dissolved in phosphate-buffered saline pH = 7.5. The molecular weight was monitored by gel permeation chromatography, and (S)-malic acid was detected by an enzymatic reaction. The half-life in terms of molecular weight was determined at three different temperatures (that is, the time required to reduce the molecular weight by fifty percent). At 50°C, degradation was rapid, and the half-life was 10 hours. At 37°C, degradation was slower, and the half-life was 110 hours. At 25°C, degradation was much slower, and the half-life was greater than 560 hours. These hydrolysis data demonstrated two complementary properties of poly[(R,S)- β -malic acid]. Firstly, poly[(R,S)- β -malic acid] degraded into its nontoxic monomer, malic acid, and secondly, poly[(R,S)- β -malic acid] degraded slowly enough to be used as a biodegradable polymeric drug carrier. Furthermore, poly[(R,S)- β -malic acid] was shown to be relatively nontoxic and nonantigenic (21,22). In one study, no mice died after being treated with poly[(R,S)- β -malic acid] so that no LD50 was calculated. However, in another study, a LD50 of 3,300 milligrams per kilogram of body weight was reported. No immune response (antibody generation) was detected against poly[(R,S)- β -malic acid], although this observation may be the result of the low molecular weight and the short exposure time of the polymer.

B. Synthesis and Polymerization of Optically Active Alkyl Malolactonates

Two syntheses of optically active poly[benzyl malolactonate] have been reported. Wojcik prepared optically active benzyl malolactonate by the mercury(II) catalyzed lactonization of a β -hydroxy thioester which is shown in Figure 4 (23). His benzyl malolactonate was probably

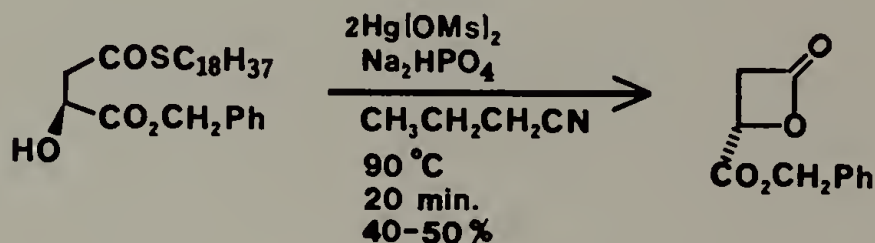


Figure 4. Mercury(II) Catalyzed Lactonization of α -Benzyl
 β -Thiostearyl (S)-Malate

of low bulk and optical purity because the lactonization reaction was not clean. His optically active benzyl malolactonate was an unstable white solid which melted between 35° and 37°C . Optically pure benzyl malolactonate is now known to be a colorless liquid (24). Furthermore, the reaction conditions were so harsh (high temperature and excess Lewis acid) that the benzyl malolactonate would not be expected to survive for very long (hence a short reaction time). The workup of the lactonization reaction only involved solvent evaporation and extraction with ether. This procedure would not have removed the byproduct, mercury(II) stearylthiolate, which is certainly soluble in ether.

Mercury(II) iodide is soluble in ether. Consequently, only low molecular weight polymers with number average molecular weights below 5,000 were obtained, because the purity of the monomer was low.

In another synthesis, Guerin and coworkers prepared optically active poly[benzyl malolactonate] by the method developed for the synthesis of poly[benzyl (R,S)-malolactonate], by using optically active (S)-bromosuccinic acid which was prepared in high optical purity from (S)-aspartic acid by a diazotization--nucleophilic substitution reaction (25). As shown in Figure 5, the mechanism of this reaction involves a neighboring group effect, an intermediate α -lactone, and two inversions at the chiral center. It would not be surprising if some racemization occurred in this reaction either by the decomposition of the diazonium salt into a carbocation or by the S_N2 reaction between the product, (S)-bromosuccinic acid, and excess sodium bromide. Moreover, Shelton and coworkers found that the lactonization of optically active β -bromobutyric acid occurred with 18 percent racemization (26). They postulated that racemization occurred by the S_N2 reaction between the unreacted, optically active β -bromobutyric acid and the bromide anions liberated during the lactonization reaction. Consequently, it would not be surprising if some racemization occurred in the lactonization of optically active benzyl bromosuccinate by the same mechanism. Guerin and coworkers determined the optical purity of their benzyl malolactonate by proton NMR spectroscopy using a chiral europium shift reagent and reported an enantiomeric excess of 80 percent, indicating that some racemization had indeed occurred. Low molecular weight

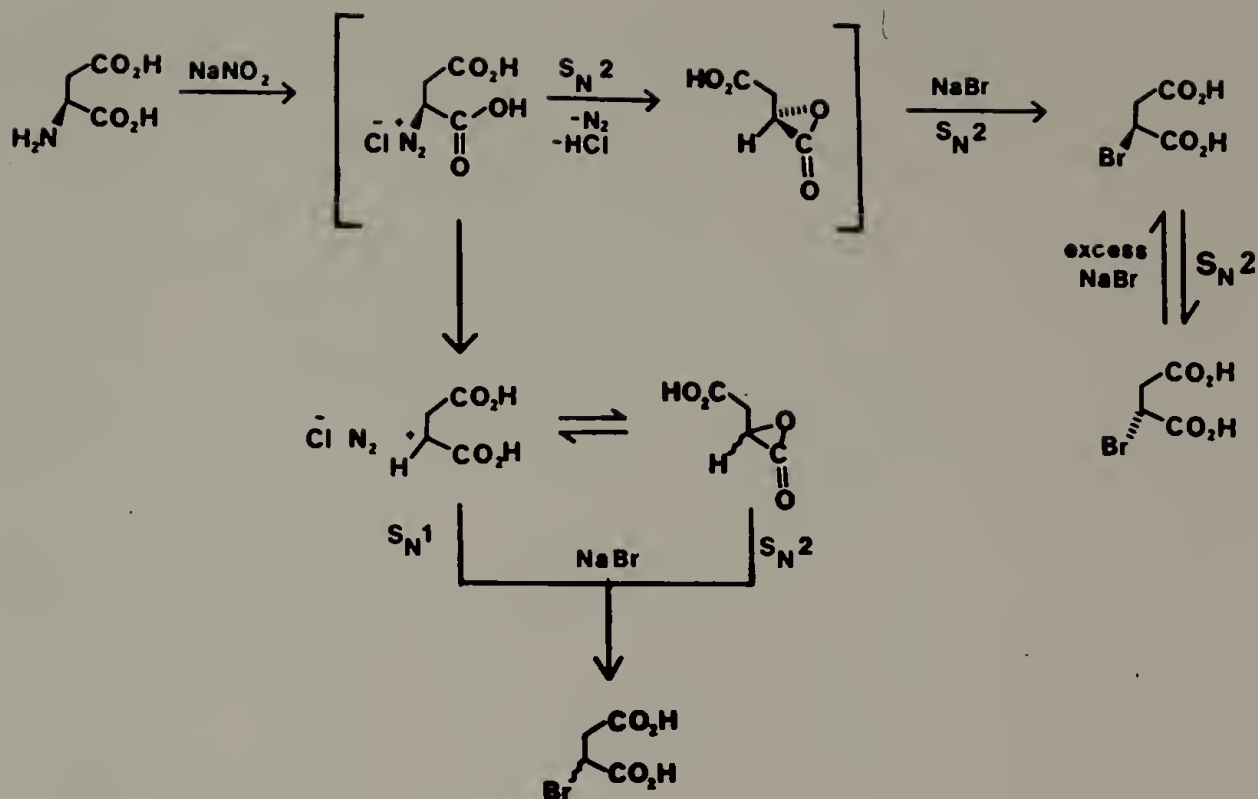


Figure 5. Synthesis of (S)-(-)-Bromosuccinic Acid from (S)-(+)-Aspartic Acid by a Diazotiation--Nucleophilic Substitution Reaction

poly[benzyl malolactonate] was prepared from this monomer, $M_{GPC} = 6,000$, and the polymer was also crystalline with a melting range between 120° and 160°C .

In this study, it was possible to prepare highly stereoregular poly[alkyl malolactonates] from optically pure monomers by the multistep synthesis shown in Figure 6. In this scheme, (S)-malic acid was first converted into its dibenzyl ester to protect the carboxyl groups during the subsequent mesylation reaction. Dibenzyl (S)-malate was reacted with mesyl chloride and pyridine to form dibenzyl (S)-O-mesylmalate by a S_N2 displacement reaction at the sulfur atom. Because dibenzyl (S)-O-mesylmalate contains a sulfur atom, catalytic hydrogenolysis could not be used to cleave the benzyl ester protecting groups as sulfur poisons the palladium catalyst. Consequently, the benzyl esters were cleaved with hydrobromic acid in acetic acid to form (S)-O-mesylmalic acid, the key intermediate product.

(S)-O-Mesylmalic acid was converted into its anhydride using trifluoroacetic anhydride. (S)-O-mesylmalic anhydride was then reacted with one equivalent of alcohol to form a mixture of monoalkyl (S)-O-mesylmalates. This ester mixture was dissolved into aqueous sodium bicarbonate and stirred vigorously in the presence of an organic solvent. One isomer was converted into optically pure alkyl (R)-malolactonate by an internal S_N2 reaction. The lactonization reaction presumably occurred in the aqueous solution, and the β -lactone was extracted into the organic layer before it decomposed. Purified alkyl (R)-malolactonate monomers were bulk polymerized with tetraethylammonium

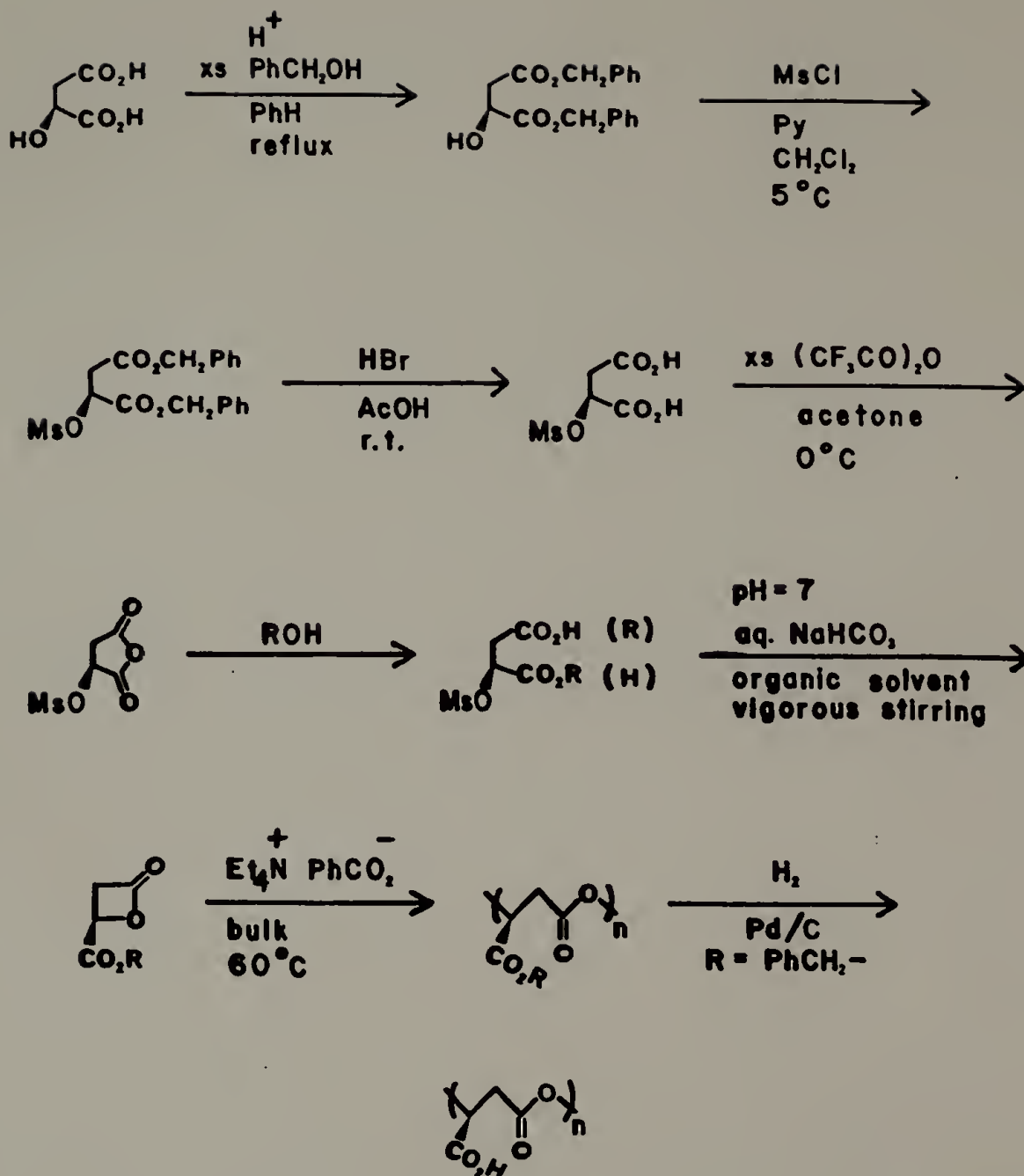


Figure 6. Synthesis of Stereoregular Poly[alkyl (S)-malates]

benzoate as the initiator into the corresponding crystalline polyesters. Optically active poly[(S)- β -malic acid] was then prepared from poly[benzyl (S)-malate] by the catalytic hydrogenolysis of the pendent benzyl esters.

This synthetic strategy resembles that used for the preparation of racemic alkyl malolactonates except that a mesylate leaving group was used instead of a bromide. The chiral center only undergoes two intentional inversions, one during the lactonization and another during the polymerization, so that polymers of high optical purity were obtained with the same absolute configuration as the starting material, malic acid. Although both enantiomers are commercially available, (S)-malic acid was generally used so that poly[alkyl (S)-malates] were formed. In drug delivery applications, these polymers would hydrolyze back to (S)-malic acid which is an intermediate in the Krebs metabolic cycle. In the next chapter, the synthesis and polymerization of optical pure alkyl malolactonates are discussed in detail.

C. Liposomal Drug Delivery

Vesicle transportation is an important biological process. Coated vesicles transport material between organelles and between cells. Vesicles are involved in the endocytosis of foodstuffs (receptor-mediated endocytosis) and the exocytosis of hormones (storage and secretion of insulin). Liposomal drug delivery systems try to imitate this process by encapsulating drugs inside synthetic phospholipid

vesicles. Liposomal drug delivery has been reviewed (27,28). The basic idea is to entrap drugs inside vesicles that are designed to slowly release the drug into the bloodstream (controlled release), or that are designed to transport the drug to certain cells. In practice, both kinds of vesicle have been difficult to prepare.

Liposomal drug delivery has several advantages. Liposomes are nontoxic and biodegradable, because they are composed of naturally occurring phospholipids. Liposomes are versatile, because their biological activity can be varied by changing the lipid composition. In fact, drug targeting is possible by modifying the outer surface of the liposome so that it is recognized only by certain cells. Liposomes also have several disadvantages. Liposomes are not always biostable. Liposomes exchange phospholipids and cholesterol with cell membranes and with high-density lipoproteins (29-33). They also absorb serum proteins and platelets (34-36). The result of these biological interactions is the destruction of the liposome and the sudden release of the entrapped material. Biostability and drug leakage are the major problems in liposomal drug delivery. Moreover, intravenously injected liposomes are naturally targeted to the reticuloendothelial system in the liver, spleen, and bone marrow (37). Drug targeting must overcome this natural liposome tissue distribution.

Polymerized liposomes have been prepared from lipids that undergo free radical polymerization (4,38). Polymerized liposomes are mechanically stable and impermeable. Entrapped material does not leak out. They are also not biodegradable or nontoxic, and therefore, they

are not suitable for drug delivery. However, Regen has recently reported the synthesis of polymerized liposomes that are potentially biodegradable (39,40). The polymerization was based on the thiol-disulfide redox cycle and was reversible under laboratory conditions. These vesicles are probably suitable for drug delivery.

Sunamoto has described another method of preparing stable, biodegradable liposomes. His method involved coating the liposome with polysaccharides bearing pendent palmitoyl or cholesteryl substituents that inserted into the bilayer and anchored the polymer onto the liposome (41-43). The polysaccharides were tightly bound to the liposomes and remained attached after gel filtration. The coated liposomes were less permeable and less susceptible to phospholipase D degradation than uncoated liposomes. Furthermore, the polysaccharide-coated liposomes had a remarkably different tissue distribution than conventional liposomes. They accumulated in the spleen and lung.

In this study, cholesterol was chemically bound to poly(acrylic acid) and poly(L-glutamic acid) using dicyclohexylcarbodiimide as the coupling agent. Polyacid-coated liposomes were prepared by vortex agitation of a phospholipid film into a buffered solution of the polymer. Polymer absorption onto the liposome raised the thermal phase transition temperature of dipalmitoylphosphatidylglycerol, and this interaction was detected by high-sensitivity differential scanning calorimetry as discussed in the following chapter.

D. Footnotes

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CHAPTER II

RESULTS AND DISCUSSION

A. Synthesis of Optically Pure Alkyl Malolactonates

As shown in Figure 7A, (S)-O-trifluoroacetylmalic anhydride was prepared by reacting powdered (S)-malic acid with excess trifluoroacetic anhydride under an inert atmosphere. The reaction was rapid and quantitative, and the yield was 85 percent after recrystallization. However, (S)-O-trifluoroacetylmalic anhydride could not be used in the preparation of optically pure alkyl malolactonates, because the trifluoroacetate leaving group hydrolyzed instantly in aqueous sodium bicarbonate before any β -lactone had formed (1). In contrast, the synthesis of (S)-O-mesyilmalic anhydride from (S)-malic acid required four steps, because the reaction of (S)-malic acid with mesyl chloride and pyridine yielded a brown mixture of carboxylate and sulfonate esters instead of the anhydride. Brewster and Ciotti found that mesyl chloride and pyridine converted acids and alcohols into the corresponding esters through an intermediate anhydride (2). The mechanism of this reaction is shown in Figure 7B, and the probable reaction sequence for (S)-malic acid is shown in Figure 7C. Obviously, under these reaction conditions, pyridinium carboxylates are more nucleophilic than alcohols, and

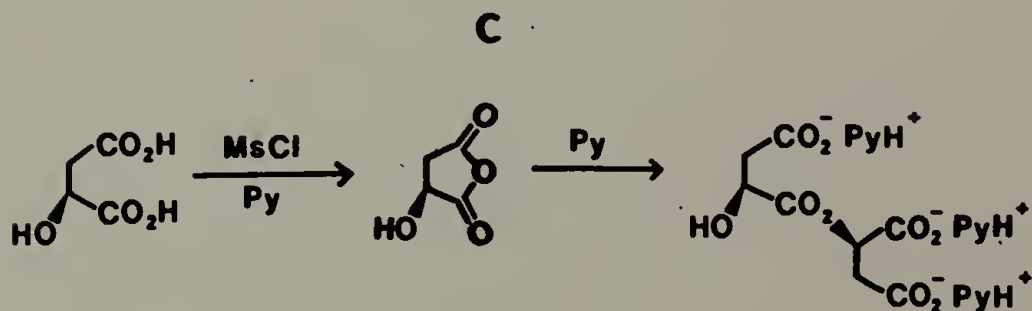
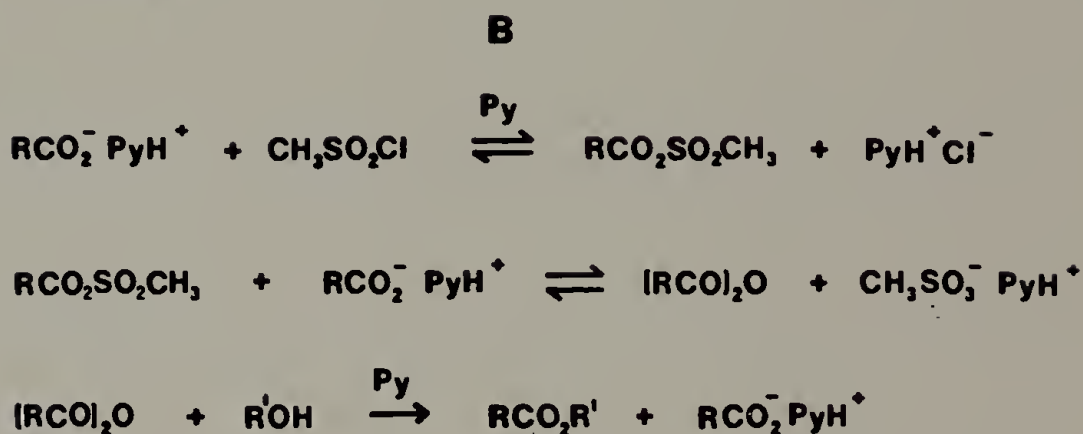
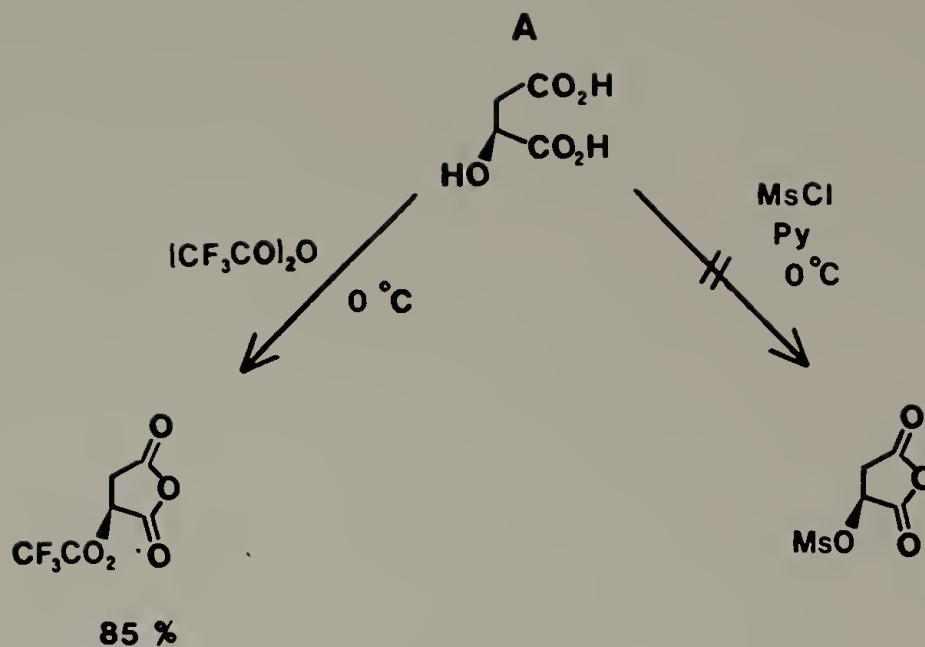
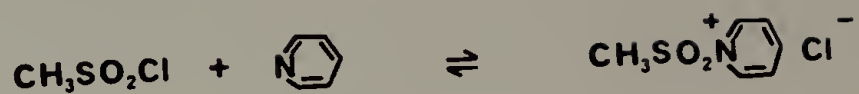


Figure 7. Malic Anhydride Derivatives

therefore, they react faster than alcohols with mesyl chloride. On the other hand, carboxylic acids and alcohols must react with trifluoroacetic anhydride at comparable rates; otherwise, trifluoroacetylmalic anhydride would not have been formed. (S)-Malic anhydride was the intermediate product which reacted with another molecule of (S)-malic acid to form oligomers of malic acid. The terminal hydroxy group of these oligomers was eventually converted into a mesylate group. Similar results were obtained when tetrahydrofuran was used as the solvent and/or when methanesulfonic anhydride was used as the mesylating agent.

(S)-O-Mesylmalic acid was prepared from (S)-malic acid in three steps. (S)-Malic acid was first converted into its dibenzyl ester to protect the carboxyl groups during the subsequent mesylation reaction. The simple acid-catalyzed esterification of (S)-malic acid with benzyl alcohol in benzene was a clean reaction with yields of 85 to 90 percent. A tenfold excess of benzyl alcohol was used to reduce the self-esterification of (S)-malic acid, and the water formed during the esterification was removed by azeotropic distillation. Sulfuric acid was found to be the best catalyst. Methanesulfonic acid and paratoluenesulfonic acid gave lower yields of dibenzyl (S)-malate. Spectroscopic evaluation of dibenzyl (S)-malate by infrared, proton NMR, and carbon-13 NMR spectroscopy showed that the reaction had occurred without dehydration--no double bond absorptions were observed in these spectra.

Nucleophilic Catalysis



Nucleophilic Substitution

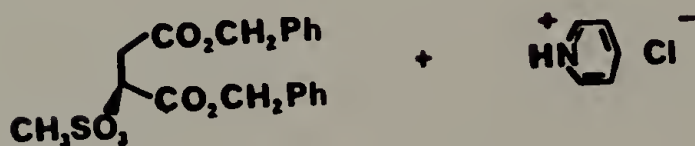
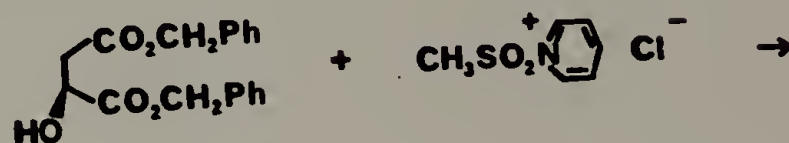


Figure 8. Mesylation of Dibenzyl (S)-Malate

Benzyl ester protecting groups were used because they could be formed without any side reactions. Trimethylsilyl ester protecting groups could not be formed cleanly, presumably because silylation was not selective. Both the carboxyl and hydroxy groups of (S)-malic acid reacted with trimethylchlorosilane and pyridine at comparable rates. Although dimethyl (S)-malate is a common chiral starting material in the synthesis of many naturally occurring compounds, it was not used in the synthesis of optically pure alkyl malolactonates, because the methyl ester protecting groups would have to be removed by hydrolysis (3,4). Hydrolysis of dimethyl (S)-O-mesylmalate under basic conditions would be followed by elimination, and therefore, fumaric acid would probably be the major product, and hydrolysis of dimethyl (S)-O-mesylmalate under acidic conditions would probably require a temperature high enough to cause the hydrolysis of both the methyl esters and the mesylate. Finally, tertiary butyl esters were not even considered as possible protecting groups, because they would probably decompose under the weakly acidic reaction conditions of mesylation.

Dibenzyl (S)-malate was reacted with 1.2 equivalents of mesyl chloride and 2.0 equivalents of pyridine in methylene chloride to form dibenzyl (S)-O-mesylmalate in nearly quantitative yield by a S_N2 displacement reaction at the sulfur atom as shown in Figure 8 (5). Pyridine was used as a nucleophilic catalyst and as an acid scavenger. The mixture of mesyl chloride and pyridine was aged for 15 minutes at room temperature before the methylene chloride solution of dibenzyl (S)-malate was added. The color of the mixture changed from yellow to dark

red during the aging process in which the nucleophilic catalysis equilibrium was established.

A mesylate leaving group was used instead of a tosylate, because mesylates would crystallized better than the corresponding tosylates, making purification by recrystallization possible, and because the mixture of monoalkyl (S)-O-mesylmalates would have a higher solubility in aqueous sodium bicarbonate than the corresponding monoalkyl (S)-O-tosylmalates in the lactonization reaction. Both of these properties are the result of the smaller size of the mesylate group. Crossland and Servis found that when pyridine was replaced by triethylamine in the mesylation reaction, the mechanism changed. An intermediate sulfene was formed which reacted with the substrate (6). This method was not used to prepare dibenzyl (S)-O-mesylmalate, because excess triethylamine must be used and might cause the formation of dibenzyl fumarate by an E2 elimination.

(S)-O-Mesylmalic acid was prepared by the debenzylation of dibenzyl (S)-O-mesylmalate by hydrobromic acid in acetic acid under nonhydrolytic conditions (7). A 70 percent yield of recrystallized (S)-O-mesylmalic acid was obtained in two crops of crystals. The reaction occurred readily with a twofold excess of hydrobromic acid and without any side reactions. No acetolysis was observed. The ultraviolet spectra of dibenzyl (S)-O-mesylmalate and (S)-O-mesylmalic acid are shown in Figure 9A. The disappearance of the phenyl absorption at 258 nm ($\epsilon = 330 \text{ L mol}^{-1} \text{ cm}^{-1}$) after treatment with hydrobromic acid was consistent with the complete removal of the benzyl ester protecting groups. The weak

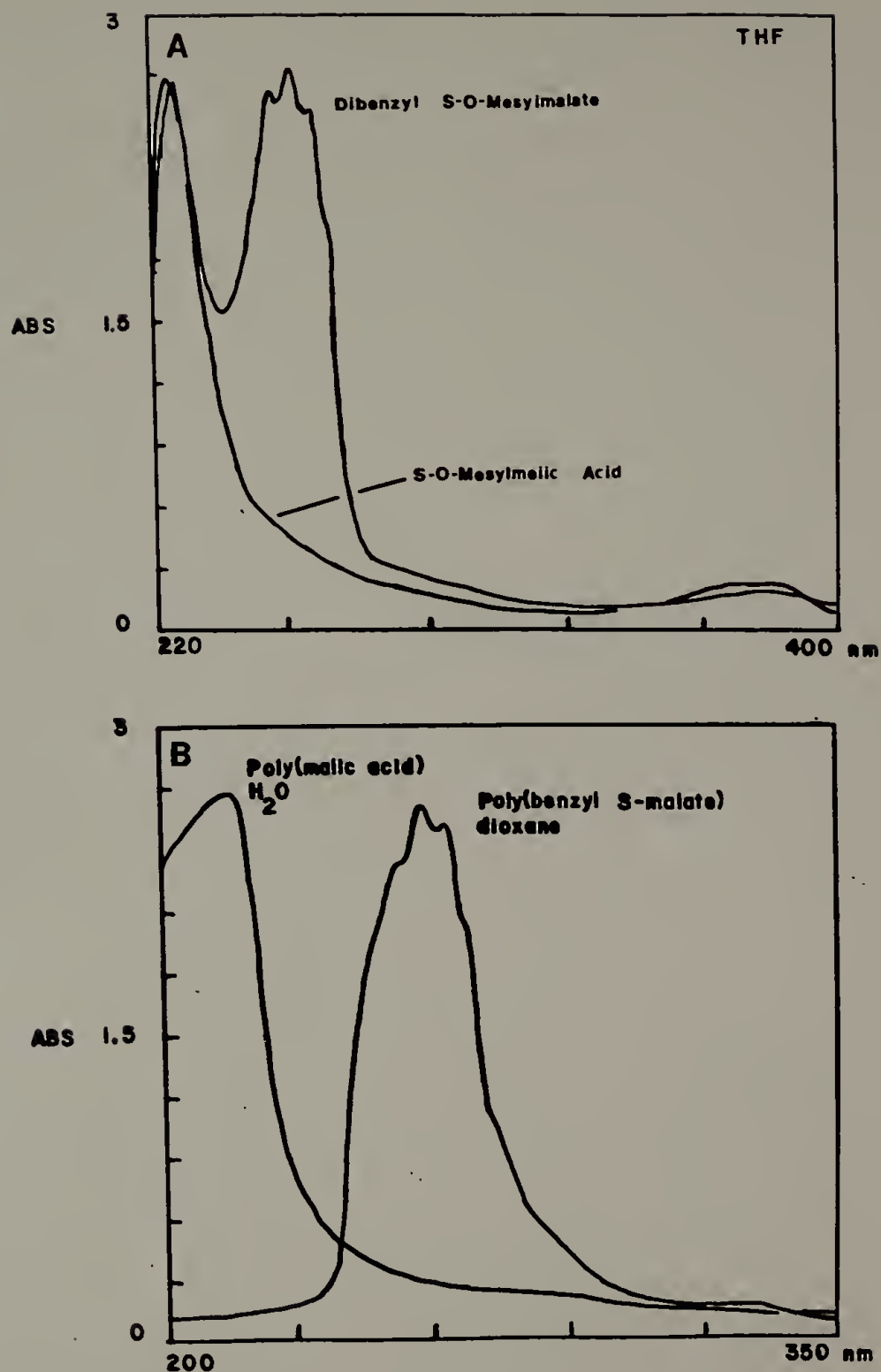


Figure 9. Ultraviolet Spectra of the Debenzylation of (A) Dibenzyl (S)-Malate and of (B) Poly[benzyl (S)-malate]

mesylate absorption band around 380 nm ($\epsilon = 1-20 \text{ L mol}^{-1} \text{ cm}^{-1}$) was responsible for the yellow color of the solid mesylate compounds and for their dark red solutions.

The conversion of (S)-malic acid into (S)-O-mesylmalic acid was also carried out successfully without isolating the intermediate products. The crude dibenzyl (S)-malate was dissolved immediately into methylene chloride and converted into dibenzyl (S)-O-mesylmalate, and then, the crude dibenzyl (S)-O-mesylmalate was dissolved into 30 weight percent hydrobromic acid in acetic acid and converted into (S)-O-mesylmalic acid. By not isolating the intermediate products, (S)-malic acid was transformed into (S)-O-mesylmalic acid in four working days and isolated in 75 percent yield overall.

The catalytic hydrogenolysis of dibenzyl (S)-O-mesylmalate proceeded to about 50 percent conversion before the palladium on carbon catalyst was poisoned. If more palladium on carbon catalyst was added, it was rapidly deactivated by the reaction mixture. The product was a mixture of water soluble [(S)-malic acid] and water insoluble [dibenzyl (S)-malate and monobenzyl (S)-O-mesylmalates] compounds. The benzyl ester protecting groups of dibenzyl (S)-O-mesylmalate were also not removed by catalytic transfer hydrogenolysis in which the palladium catalyst is supplied with hydrogen from either formic acid or ammonium formate (8,9).

(S)-O-Mesylmalic anhydride was prepared by adding an acetone solution of (S)-O-mesylmalic acid into a twofold excess of trifluoroacetic anhydride at 0°C . A 90 percent yield of recrystallized

(S)-O-mesylmalic anhydride was obtained in two crops of crystals. The monoalkyl (S)-O-mesylmalates were formed by adding one equivalent of alcohol directly to the crystals of (S)-O-mesylmalic anhydride under an inert atmosphere. Methanol reacted vigorously with (S)-O-mesylmalic anhydride, and the reaction vessel was cooled in an ice bath until the initial exotherm had subsided to prevent any thermal decomposition. The viscous, yellow reaction mixture was stirred at room temperature for 6 hours and then at 50°C for 12 hours to drive the alcoholysis to completion. Ethyl and benzyl alcohol reacted more slowly than methanol with (S)-O-mesylmalic anhydride, but after 6 hours at room temperature, both reaction mixtures were viscous yellow liquids, while isopropyl alcohol reacted very slowly, and after 6 hours at room temperature, some unreacted crystals of (S)-O-mesylmalic anhydride were still visible in the reaction mixture. In any case, after the reaction mixtures were stirred at 50°C for 12 hours, no (S)-O-mesylmalic anhydride was detected by infrared spectroscopy. These mixtures of monoalkyl (S)-O-mesylmalate were used in the lactonization reactions without purification.

The alcoholysis of (S)-O-mesylmalic anhydride should yield an unequal mixture of half esters, because the carbonyl adjacent to the mesylate group would be activated toward nucleophiles by the electron withdrawing power of the mesylate group. The proton NMR spectra of the mixtures of the monomethyl, monoethyl, and monoisopropyl (S)-O-mesylmalates were surprising clean and not representative of any half ester mixture. For example, the mixture of monomethyl (S)-O-mesylmalates should have two methyl peaks--one for each half ester. Its

proton NMR spectrum had one large peak at $\delta 3.84$ and another small peak at $\delta 3.76$ that were assigned to the two methyl esters. Integration of these two peaks indicated that the mixture was composed of 95 percent of the α -monomethyl (S)-O-mesylmalate and 5 percent of the β -monomethyl (S)-O-mesylmalate. Integration of the other mixtures of monoalkyl (S)-O-mesylmalates was not possible either because of spin-spin coupling (ethyl and isopropyl) or because of poor resolution (benzyl). For comparison, Johns found that the alcoholysis of racemic bromosuccinic anhydride gave a half ester mixture composed of 75 percent of α -monoalkyl (R,S)-bromosuccinate and 25 percent of β -monoalkyl (R,S)-bromosuccinate which is consistent with the lower electron withdrawing power of the bromine substituent (10,11).

The mixture of monoalkyl (S)-O-mesylmalates was dissolved into aqueous sodium bicarbonate pH = 7.0-7.5 and stirred vigorously in the presence of methylene chloride at 35°C for the methyl, ethyl, and isopropyl compounds and in the presence of benzene at 45°C for the benzyl compound. As shown in Figure 10, the α -monoalkyl (S)-O-mesylmalate isomer undergoes an internal $\text{S}_{\text{N}}2$ reaction, and the β -lactone is extracted into the organic layer before it decomposes. The reaction temperature was kept as low as possible so that the side reactions of decarboxylative elimination of the half ester and the hydrolysis of the β -lactone would not compete favorably with lactonization. This two phase reaction system yielded relatively pure crude β -lactone monomers, because many of the impurities stay in the aqueous solution.

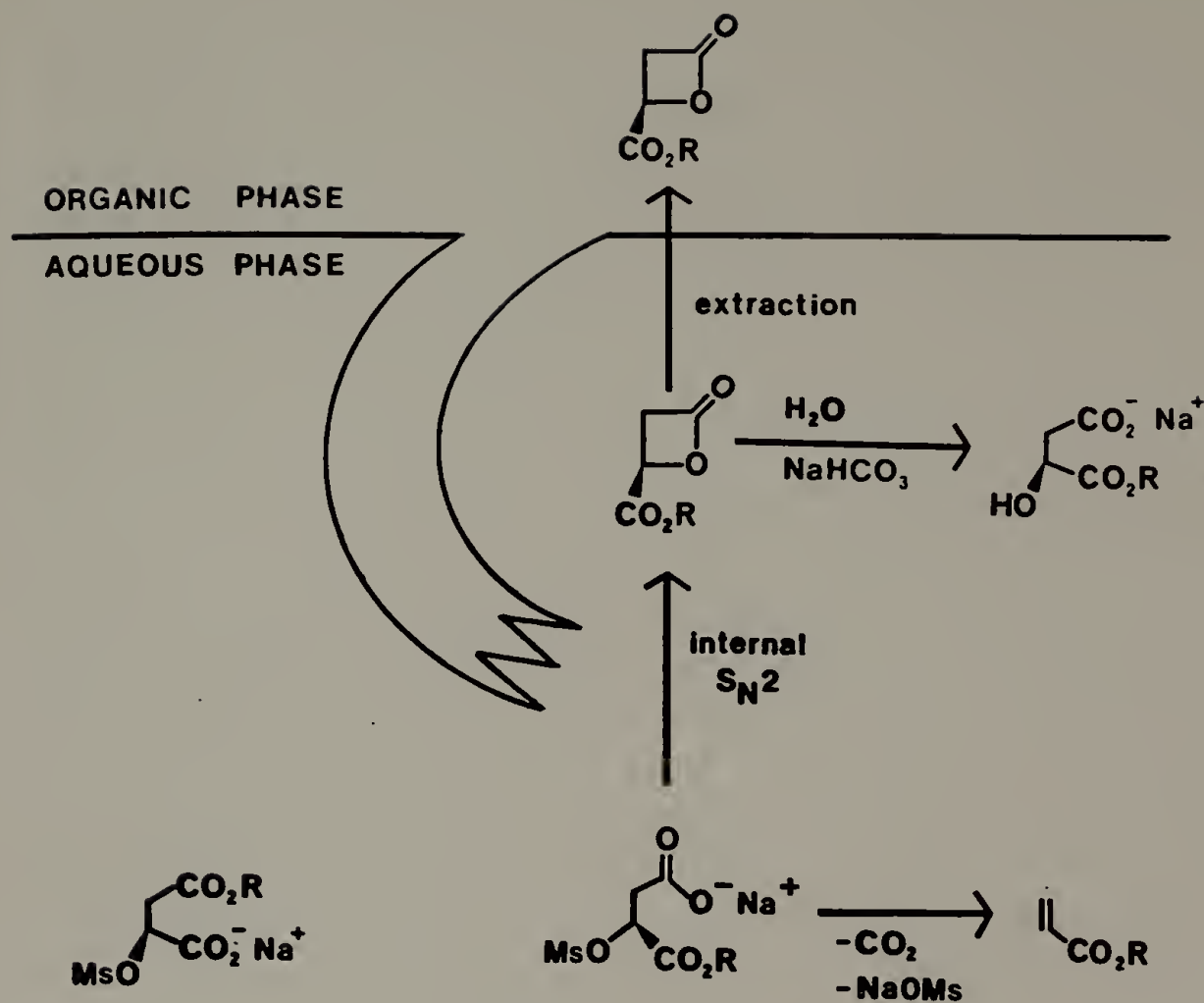


Figure 10. Lactonization of Monoalkyl (S)-O-Mesylmalates

The synthesis of optically pure alkyl malolactonates was the first time that a mesylate was used as the leaving group in the synthesis of a β -lactone. Table 2 shows the hydrolysis data of some alkyl bromides and mesylates (12). The mesylates appear to react about one order of magnitude faster than the bromides in S_N2 reactions and about two orders of magnitude faster in the borderline S_N1 - S_N2 reactions involving the isopropyl derivatives. Consequently, the replacement of a bromide with a mesylate would not affect the rate of lactonization considerably. However, mesylates are much less nucleophilic than bromides, so that racemization during the lactonization of the monoalkyl (S)-O-mesylmalates by the S_N2 reaction between unreacted monoalkyl (S)-O-mesylmalate and mesylate anions liberated during the lactonization reaction would not occur. This simple fact was the main reason why the synthetic strategy shown in Figure 6 was successful!

Table 2

Hydrolysis Data of Alkyl Bromides and Alkyl Mesylates				
Substrate	ΔH^\ddagger , kcal/mol	ΔS^\ddagger , cal/mol $^\circ\text{C}$	Rate Constant, $k_1 \times 10^6 \text{ sec}^{-1}$	
			25 $^\circ\text{C}$	50 $^\circ\text{C}$
MeOMs	21.2	-11.7	5.07	84.5
EtOMs	21.1	-12.3	3.94	71.5
nPrOMs	20.7	-14.4	3.05	51.6
iPrOMs	22.1	- 1.3	197	7400
MeBr	23.0	-10.1	0.407	11.1
EtBr	23.4	- 8.9	0.384	10.9
nPrBr	22.1	-13.6	0.280	7.11
iPrBr	24.4	- 1.4	3.82	110

B. Optical Purity

Lanthanide shift reagents have been used in the elucidation of complex NMR spectra of organic compounds (13-15). Lanthanide shift reagents are usually composed of a paramagnetic lanthanide cation and chelating, enolate ligands. The chelating ligands are necessary to stabilize the coordination compound so that it can be isolated in high purity. Organic molecules with weakly basic functional groups can bind to the lanthanide shift reagent and increase its coordination number. The paramagnetic cation changes the local magnetic field around the binding site and thereby changes the chemical shifts of the nuclei around the binding site. These induced chemical shifts are caused by both contact (through bond) and dipolar (through space) interactions depending on the metal, its charge, and the type of ligand bonding. Under the right conditions, a meaningless bundle of overlapping NMR signals is transformed into a well resolved spectrum when a lanthanide shift reagent is added.

Prud'homme and coworkers determined the optical purity of β -(1,1-dichloroethyl)- β -propiolactone by proton NMR spectroscopy using a chiral europium shift reagent (16,17). The same method was used in this study to determine the optical purities of methyl (R)-malolactonate and benzyl (R)-malolactonate. As shown in Figure 11 for methyl malolactonate, the alkyl malolactonates form diastereomeric coordination compounds with the chiral europium shift reagent, tris[3-heptafluorobutyryl]-(+)-camphorato]europium(III), $\text{Eu}(\text{hfbc})_3$. The alkyl malolactonates bind

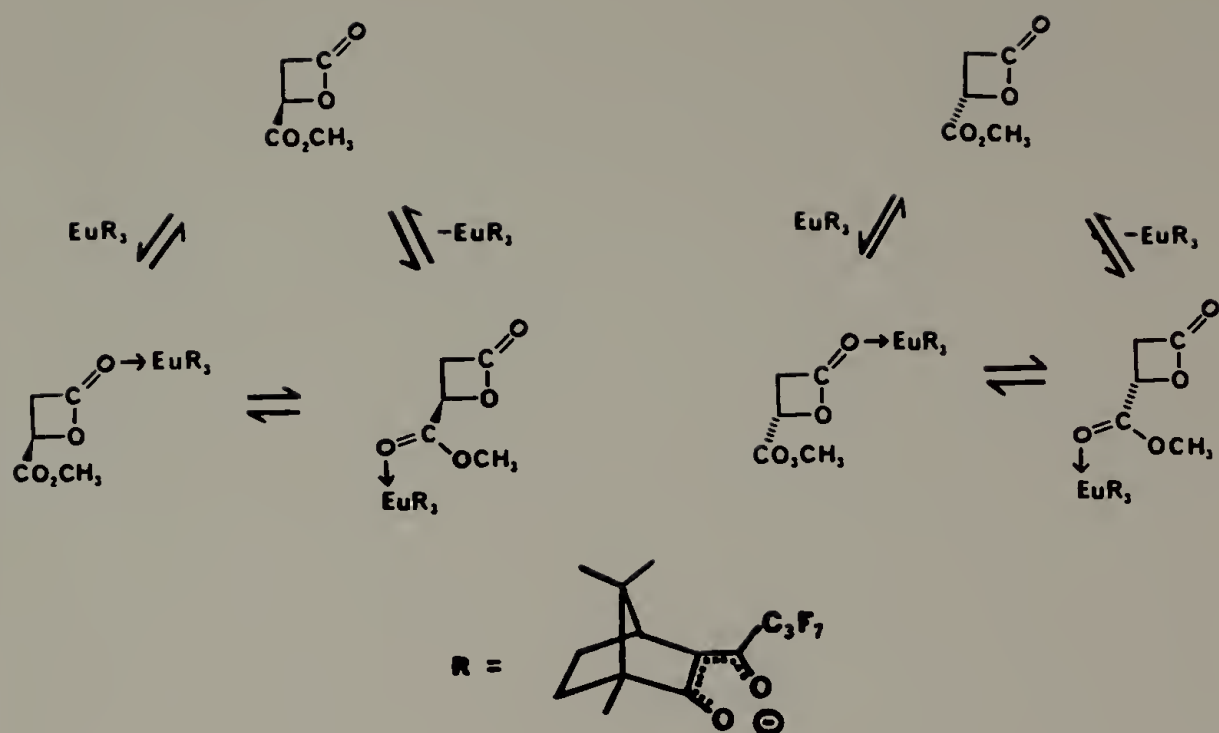


Figure 11. Optical Purity by Proton NMR Spectroscopy

reversibly to the $\text{Eu}(\text{hfbc})_3$ at both the beta-lactone and the pendent ester carbonyls. The enantiomers interact differently with the chiral europium shift reagent resulting in the separation of their spectra, and the optical purity of the sample is obtained by the integration of the appropriate peaks. One enantiomer may have a larger binding constant to the chiral europium shift reagent and therefore have larger induced chemical shifts, or the resulting diastereomeric coordination compounds may have different geometries and therefore different induced chemical shifts. Since all of these equilibria are fast on the NMR time scale, the observed NMR spectrum is the average spectrum of the complexed and uncomplexed enantiomers of alkyl malolactonate. In fact, the observed NMR spectrum also depends on any variable that affects the equilibria including the optical purity and concentration of the β -lactone, the concentration of the chiral europium shift reagent, the solvent and its moisture content, and the temperature. It is important to use dry europium shift reagents and solvents, because water competes with the substrate for ligand sites on the chiral coordination compounds.

Figure 12 shows the 300 MHz proton NMR spectra of methyl (R)-malolactonate and benzyl (R)-malolactonate. These two monomers were chosen for the optical purity experiments, because they both had sharp singlets in their proton NMR spectra which could be used to determine the optical purity of the samples without complication due to spin-spin coupling and overlapping peaks. Methyl (R)-malolactonate had a singlet at $\delta 3.86$ for the methyl protons, and benzyl (R)-malolactonate had a singlet at $\delta 5.22$ for the methylene protons of the benzyl group. These

alkyl malolactonates also showed typical ABX spectra for the methylene and methine protons of the β -lactone ring (18,19). Methyl (R)-malolactonate had a doublet of quartets at δ 3.71 that overlapped with the singlet of the methyl group for the methylene protons and a quartet at δ 4.89 for the methine proton. Benzyl (R)-malolactonate had a doublet of quartets at δ 3.62 for the methylene protons of the β -lactone and a quartet at δ 4.83 for the methine proton. Figure 13 shows the 300 MHz proton NMR spectrum of methyl malolactonate in the presence of $\text{Eu}(\text{hfbc})_3$. The concentration of methyl malolactonate was 0.32 M, and the molar ratio of β -lactone to chiral europium shift reagent was 7. $\text{Eu}(\text{hfbc})_3$ interacted with racemic methyl malolactonate and split the methyl singlet into two distinct peaks centered downfield at δ 4.20 and separated by 15 Hz, while under the same conditions, the optically active methyl malolactonate showed no splitting at δ 4.15. Similarly, Figure 14 shows the 300 MHz proton NMR of benzyl malolactonate in presence of $\text{Eu}(\text{hfbc})_3$. The concentration of benzyl malolactonate was 0.19 M, and the molar ratio of β -lactone to chiral europium shift reagent was 4. $\text{Eu}(\text{hfbc})_3$ interacted with racemic benzyl malolactonate and split the methylene singlet of the benzyl group into two distinct peaks centered downfield at δ 5.73 and separated by 30 Hz, while under the same conditions, the optically active benzyl (R)-malolactonate showed no splitting at δ 5.52. Based on these data, the optically active alkyl (R)-malolactonates were determined to be optically pure within experimental error. This fact means that the synthetic strategy shown in Figure 6 not only works chemically, but it also works without

Figure 12

300 MHz Proton NMR Spectra of (A) methyl (R)-malalactonate and of (B) benzyl (R)-malolactonate

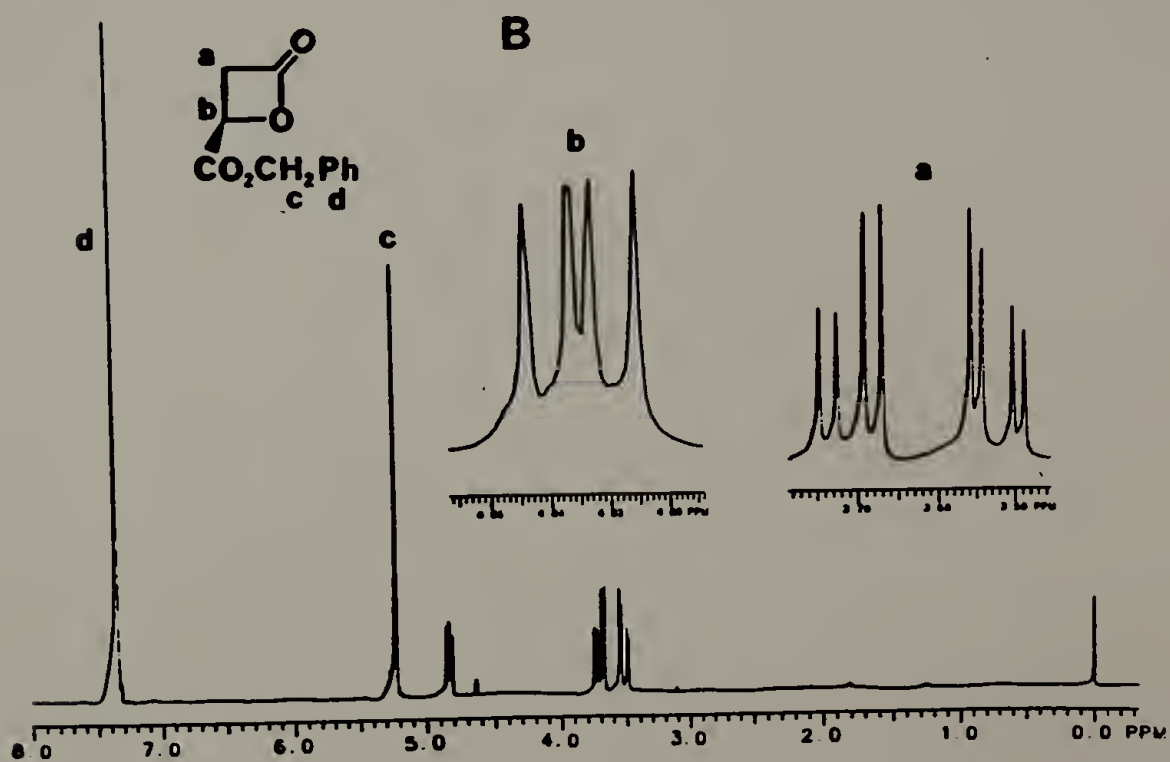
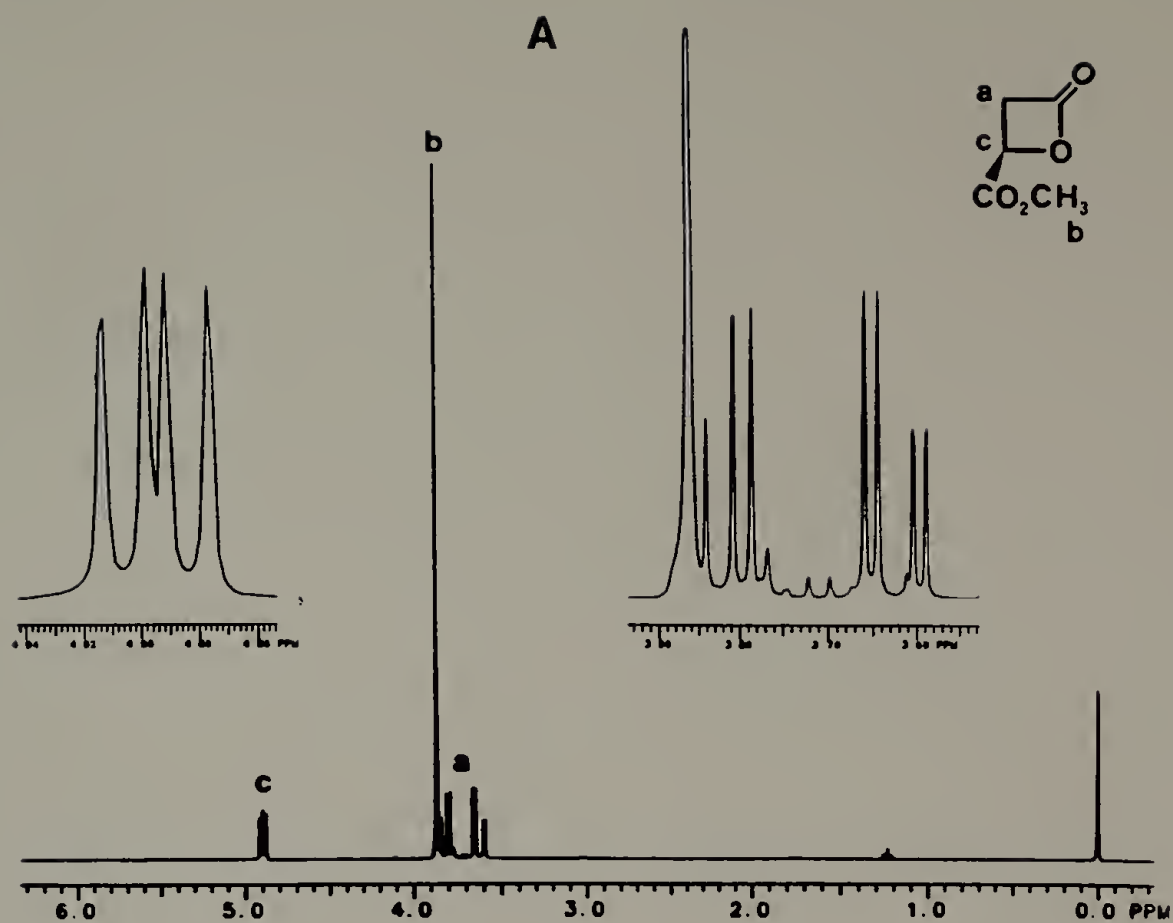


Figure 13

300 MHz Proton NMR Spectra of Methyl Malolactonate in the presence of the chiral europium shift reagent tris[3-(heptafluorobutyryl)-(+)-camphorato]europium(III). (A) racemic methyl malolactonate and (B) optically active methyl malolactonate under the same conditions.

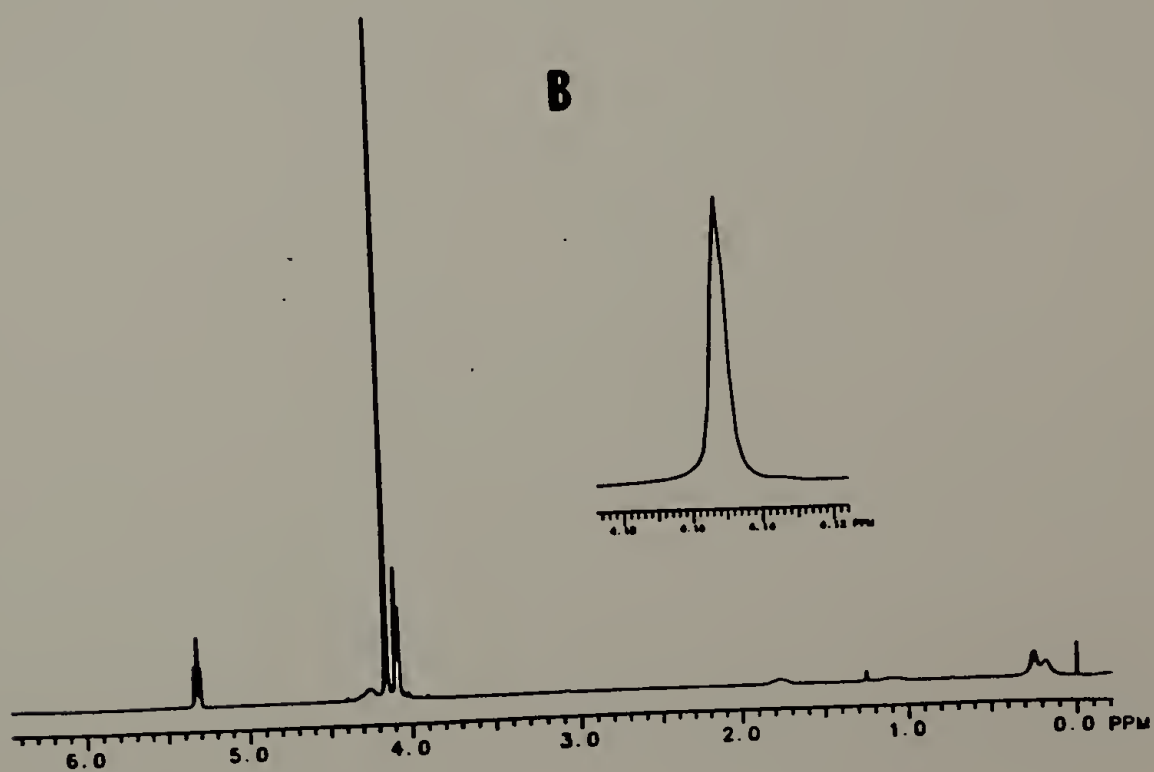
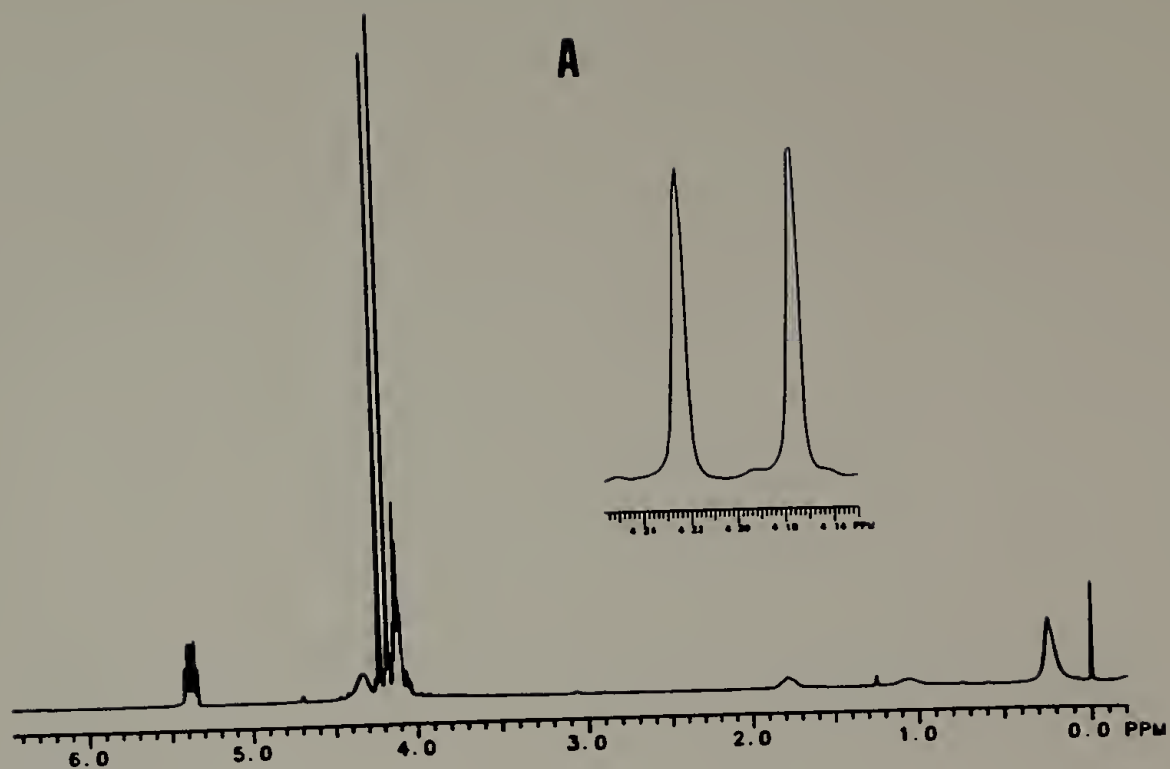


Figure 14

300 MHz Proton NMR Spectra of Benzyl Malolactonate in the presence of the chiral europium shift reagent tris[3-(heptafluorobutyryl)-(+)-camphorato]europium(III). (A) racemic benzyl malolactonate and (B) optically active benzyl malolactonate under the same conditions.

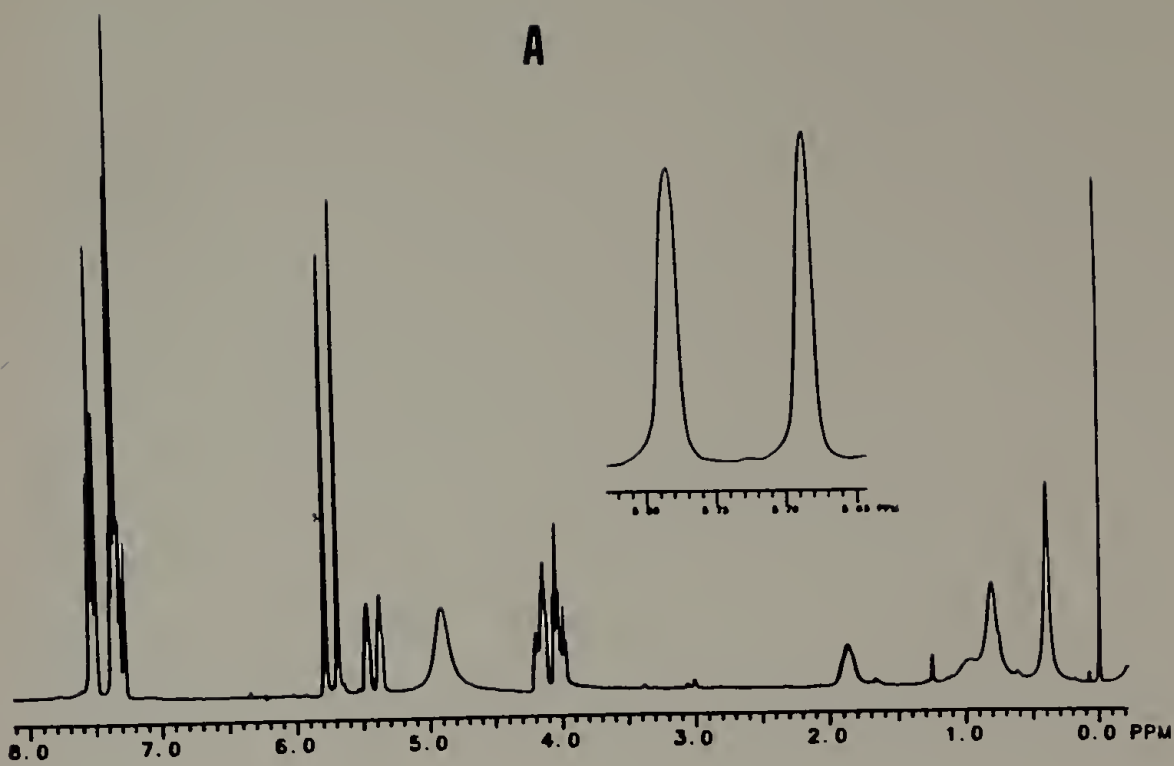
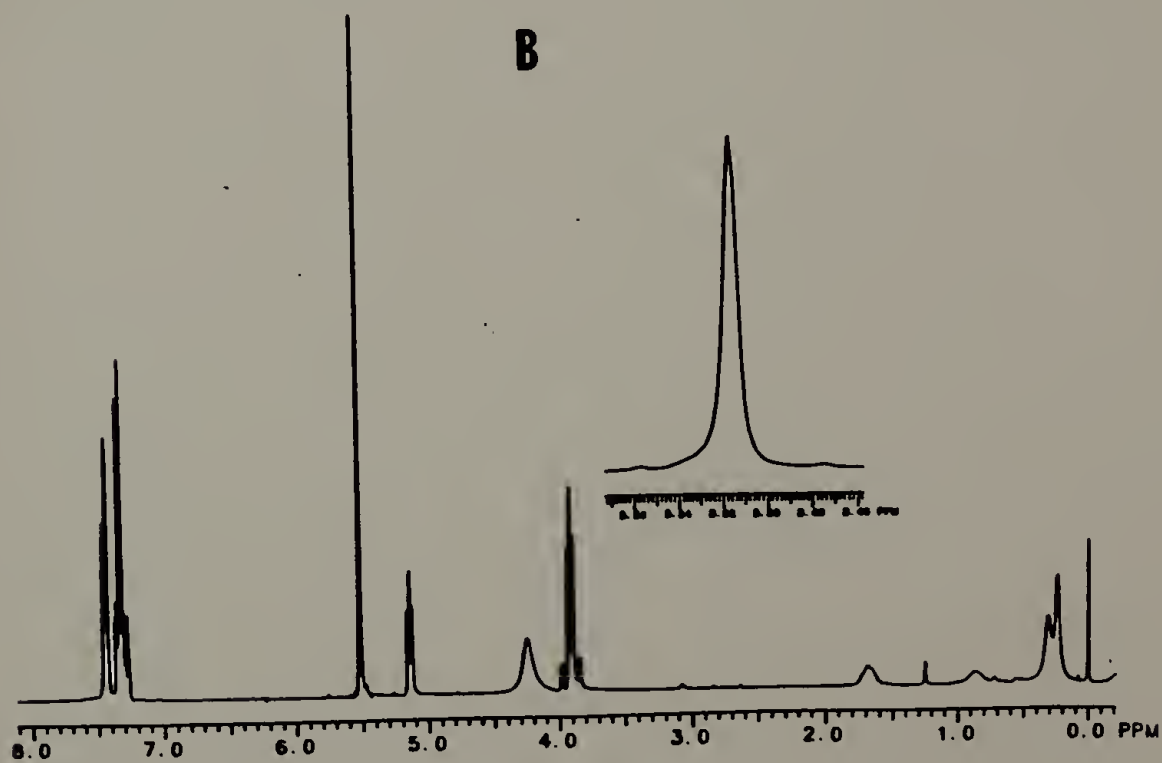
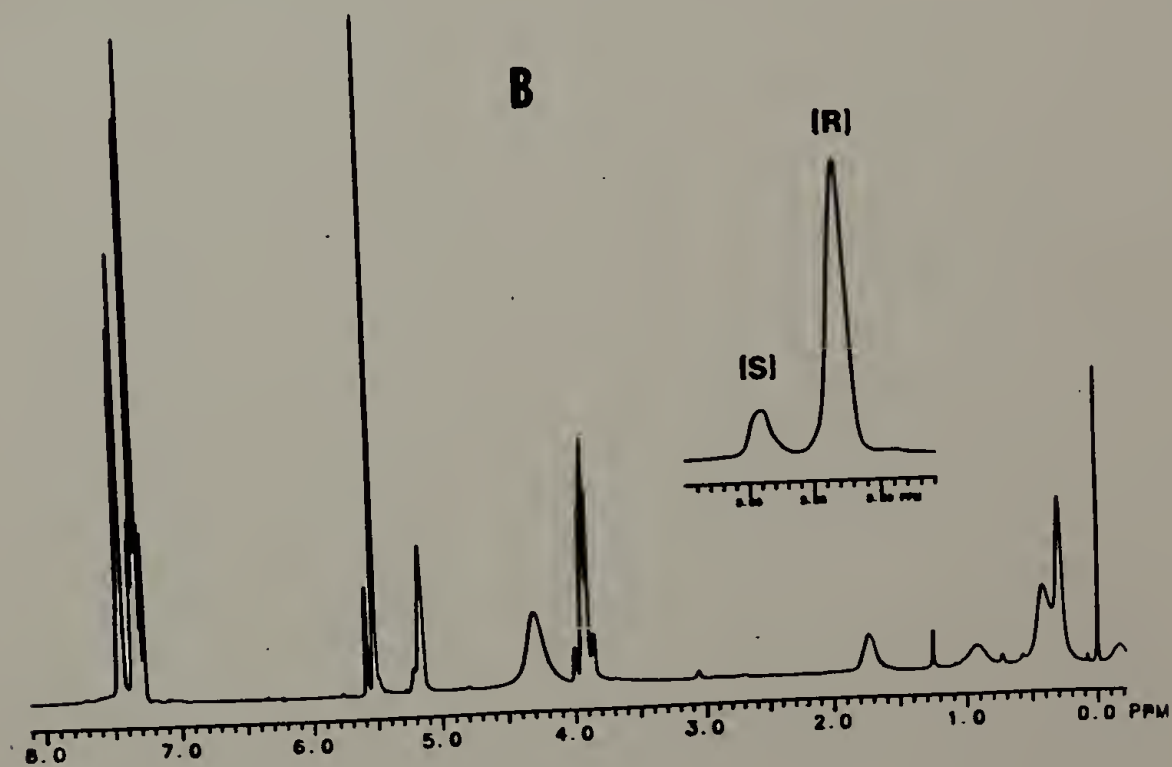
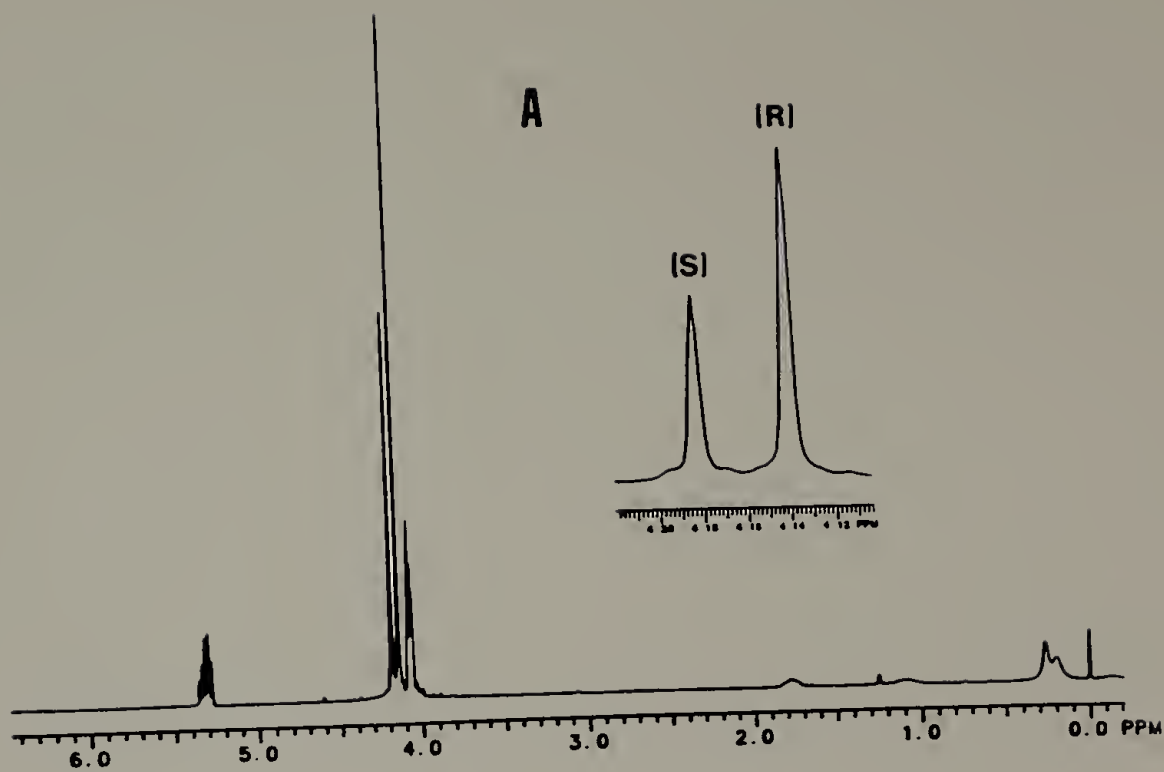
A**B**

Figure 15

300 MHz Proton NMR Spectra of a Mixture of Racemic and Optically Active Alkyl Malolactonate in the presence of the chiral europium shift reagent tris[3-(heptafluorobutyryl)-(+)-camphorato]europium(III). (A) methyl malolactonate and (B) benzyl malolactonate.



racemization. The experimental error was calculated from the integration error observed for the racemic monomer and was found to be 3 percent. Figure 15 shows the 300 MHz proton NMR spectra of a mixture of racemic and optically pure methyl malolactonate and a mixture of racemic and optically pure benzyl malolactonate. In both cases, the intensity of the upfield signal in the doublet representing the enantiomeric composition increased and was thereby identified as the (R) enantiomer.

C. Polymerization of Optically Pure Alkyl Malolactonates

β -Propiolactone monomers have been polymerized by anionic, cationic, and organometallic initiators (20-29). However, alkyl malolactonates are the only known β -substituted β -propiolactones that are polymerized by anionic initiators (30,31). This reactivity is related to the mechanism of the polymerization which is shown in Figure 16. Both the initiation and propagation steps are S_N2 reactions at the beta carbon atom. With β -alkyl β -propiolactones, the reaction site is sterically hindered by the alkyl group, and this steric hinderance is sufficient to prevent their polymerization by anionic initiators. With alkyl malolactonates, the carboalkoxy group increases the reactivity of the monomer enough to offset its steric hinderance and allows their polymerization by anionic initiators. It is well known that α -halocarbonyls are often the most reactive substrates in S_N2 reactions, because the charge in the transition state is delocalized into the α -carbonyl as shown in Figure 17A (32). In this way, the transition state

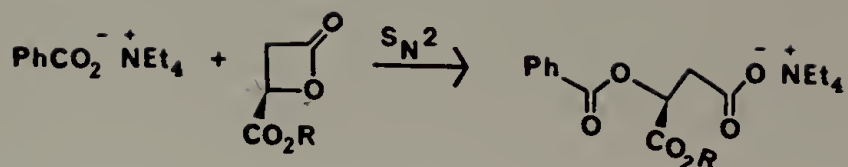
is stabilized, the activation energy is reduced, and the reaction is facile. For example, α -chloroacetone reacted 35,000 times faster with potassium iodide in acetone than n-butyl chloride (33). Alkyl malolactonates are similar to α -halocarbonyls: the leaving group is adjacent to the carbonyl. Hence, the transition state is stabilized, the activation energy is reduced, and polymerization is possible. Ring strain energy is also released during the polymerization.

There are important stereoelectronic considerations in S_N2 reactions alpha to a carbonyl. For charge to be delocalized into the α -carbonyl, the attacking nucleophile must approach the substrate parallel to the lobes of the π orbital of the α -carbonyl. This configuration allows for the most orbital overlap and therefore for the most stabilization in the transition state. Barrett and Trachtenbury found some evidence for this hypothesis (34). The reaction in Figure 17B, in which the iodide must approach in the plane of the ring for backside displacement and therefore perpendicular to the lobes of the π orbital of the α -carbonyl, had an activation enthalpy 20 kcal per mole higher than the reaction in Figure 17C, in which the iodide could approach parallel to the lobes of the π orbital of the α -carbonyl. This configurational restraint of α -carbonyl substrates may affect the rate of polymerization of alkyl malolactonate monomers (to be discussed later).

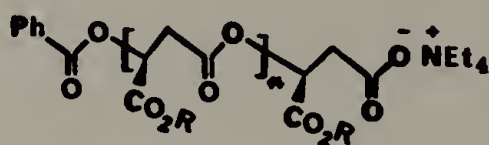
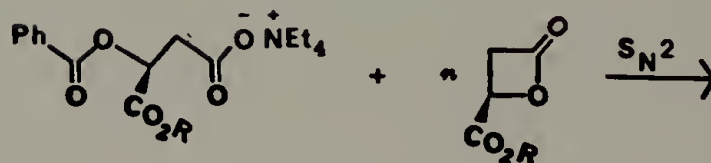
Optically pure alkyl malolactonates were polymerized in the bulk at 60°C using tetraethylammonium benzoate as the initiator with the results listed in Table 3 and Table 4. Tetraethylammonium benzoate was used

because the initiation reaction was clean, and the resulting benzoate ester was stable under the polymerization conditions. Triethylamine, another common anionic initiator, was not used, because the resulting charged Mannich base might undergo a β -elimination reaction sometime during the polymerization reaction and generate two types of propagating center--a zwitterion and a triethylammonium carboxylate. The polymers were isolated in high yields, with high molecular weights, and with broad molecular weight distributions. The homopolymerizations were heterogeneous, because the crystalline polyesters were insoluble in their monomers. Initially, the polymerization mixture was a colorless liquid. Then, after about fifteen minutes, it became cloudy white and heterogeneous, and after several hours, it had solidified into a hard, white plug of polymer. The poly[alkyl (S)-malates] were isolated by crushing the plug of polymer into small pieces with a hammer, dissolving those pieces in refluxing dioxane, and adding the resulting solution dropwise into a tenfold excess of 50::50 (v/v) ether and hexane for the methyl, ethyl, and isopropyl polymers and into a tenfold excess of ether for the benzyl polymer with stirring. The poly[alkyl (S)-malates] were isolated as fine white powders by suction filtration, washed with ether, and dried in vacuo. The molecular weights were higher than those previously obtained from the polymerization of racemic alkyl malolactonates (35,36). One of the polymerizations of benzyl (R)-malolactonate had a low yield of 60 percent, because the polymerization tube was overfilled and cracked during the polymerization reaction, presumably under the pressure created by the volume expansion

INITIATION



PROPAGATION



CHAIN TRANSFER

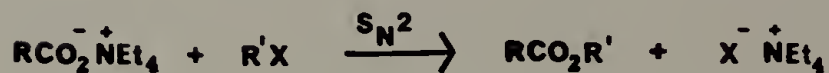
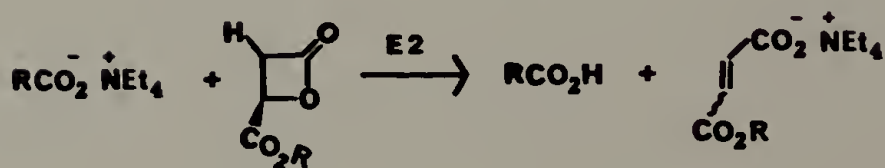
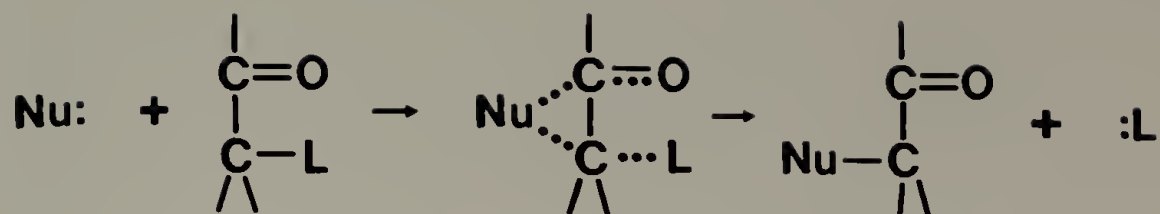
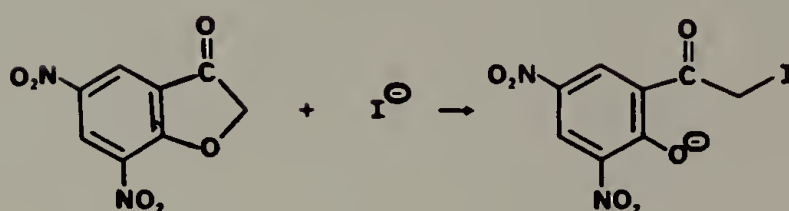


Figure 16. Anionic Polymerization Mechanism of Alkyl (R)-Malolactonates

A

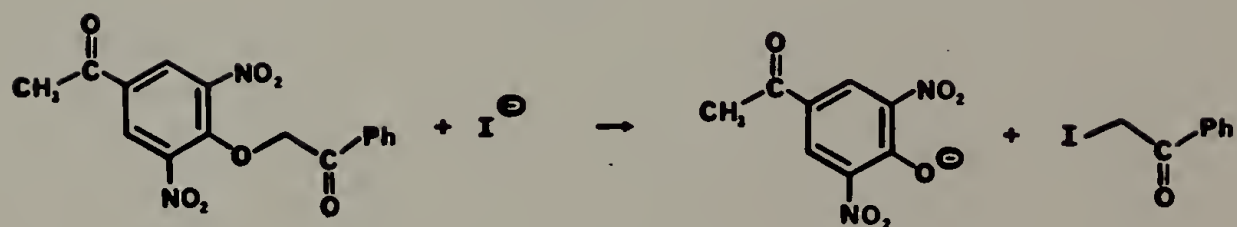


B



$$\Delta H^\ddagger = 31.3 \text{ kcal/mol}$$

C



$$\Delta H^\ddagger = 10.2 \text{ kcal/mol}$$

Figure 17. Stereoelectronic Considerations in Nucleophilic Substitution Reactions Alpha to a Carbonyl

Table 3
Yields and Molecular Weights of the Poly[alkyl (S)-malates]

Alkyl Group, R	Polymer ^a	M_n^b	M_w^b	M_w/M_n^b
	Yield			
Me	89 %	17,000	29,000	1.7
Me	97	41,000	41,000	2.0
Me	91	29,000	59,000	2.0
Et	82	20,000	39,000	1.9
Et	92	14,000	35,000	2.5
Et	85	20,000	38,000	1.9
iPr	82	43,000	85,000	2.0
iPr	82	65,000	130,000	2.0
iPr	83	43,000	110,000	2.6
Bz	85	13,000	21,000	1.6
Bz	90	40,000	92,000	2.3
Bz	60	61,000	180,000	2.9
Bz	89	42,000	110,000	2.6
Bz(R) ^c	75	56,000	110,000	2.0
Bz(R)	94	73,000	160,000	2.2
Et/Bz	83	32,000	72,000	2.2
Et/H	--	10,000	18,000	1.8

^a By bulk polymerization at 60°C for 5-7 days using tetraethylammonium benzoate as the initiator, 100 % conversion by infrared spectroscopy, mole ratio of monomer to initiator of 10³-10⁴.

^b Molecular weights were determined by GPC using polystyrene standards.

^c (R) configuration in the polymer.

Table 4

Specific Rotations of Alkyl (R)-Malolactonates
and Poly[alkyl (S)-malates]

Alkyl Group, R	Alkyl (R)-Malolactonate		Poly[alkyl (S)-malate]	
	$[\alpha]_D^a$	Temperature	$[\alpha]_D$	Temperature
Me	+5.8 ^o	28°C	-16.7 ^{ob}	32°C
Et	+2.9	26	-19.0 ^b	32
iPr	+3.9	25	-22.8 ^b	32
Bz	+8.2	25	+ 5.7 ^c	29

^a Measured in tetrahydrofuran at a concentration of 0.10 g/mL.

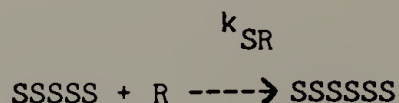
^b Measured in chloroform at a concentration of 0.100 g/mL.

^c Measured in N-methyl pyrrolidinone at a concentration of 0.10 g/mL.

accompanying the polymerization of benzyl (R)-malolactonate.

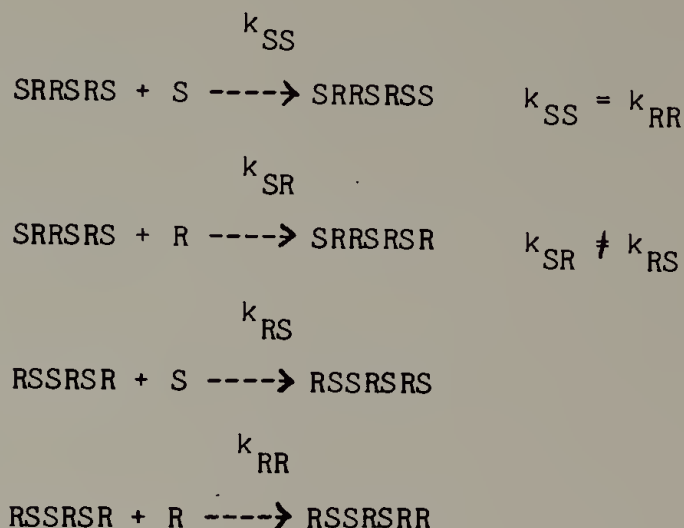
The copolymerization of ethyl and benzyl (R)-malolactonate appeared to be homogeneous, because the reaction mixture remained transparent during the copolymerization. A high molecular weight copolymer was obtained which suggests that the heterogeneous nature of the homopolymerizations was not responsible for the higher molecular weight polymers obtained in this study. Most likely, the absence of some reactive impurities, because of the use of a new synthetic route, or an increase in the rate of polymerization, because of some favorable stereoelectronic interactions between the propagating center and the optically pure monomer, was responsible for the higher molecular weight polymers obtained.

The latter hypothesis relates back to the configurational requirements on α -carbonyl substrates in S_N2 reactions. It claims that the rate of polymerization of optically pure alkyl malolactonates may be higher than the rate of polymerization of racemic alkyl malolactonates. In the polymerization of optically pure alkyl (R)-malolactonates, the propagating center always has the opposite (S) configuration as depicted below.



Only one rate constant is necessary. In contrast, the polymerization of racemic alkyl malolactonates can be considered as the

copolymerization of the two enantiomers, and therefore, assuming no penultimate effects, three different propagation reactions are possible with three different rate constants as shown below.



Clearly, the rates of polymerization are different, but whether the rate of polymerization of optically pure alkyl malolactonates is higher than the rate of polymerization of racemic alkyl malolactonates is not known. The absolute configuration of the propagating center and perhaps of the penultimate group may affect the geometry of the transition state of the polymerization reaction and thereby affect the amount of charge delocalization into the carbonyl of the pendent alkyl ester.

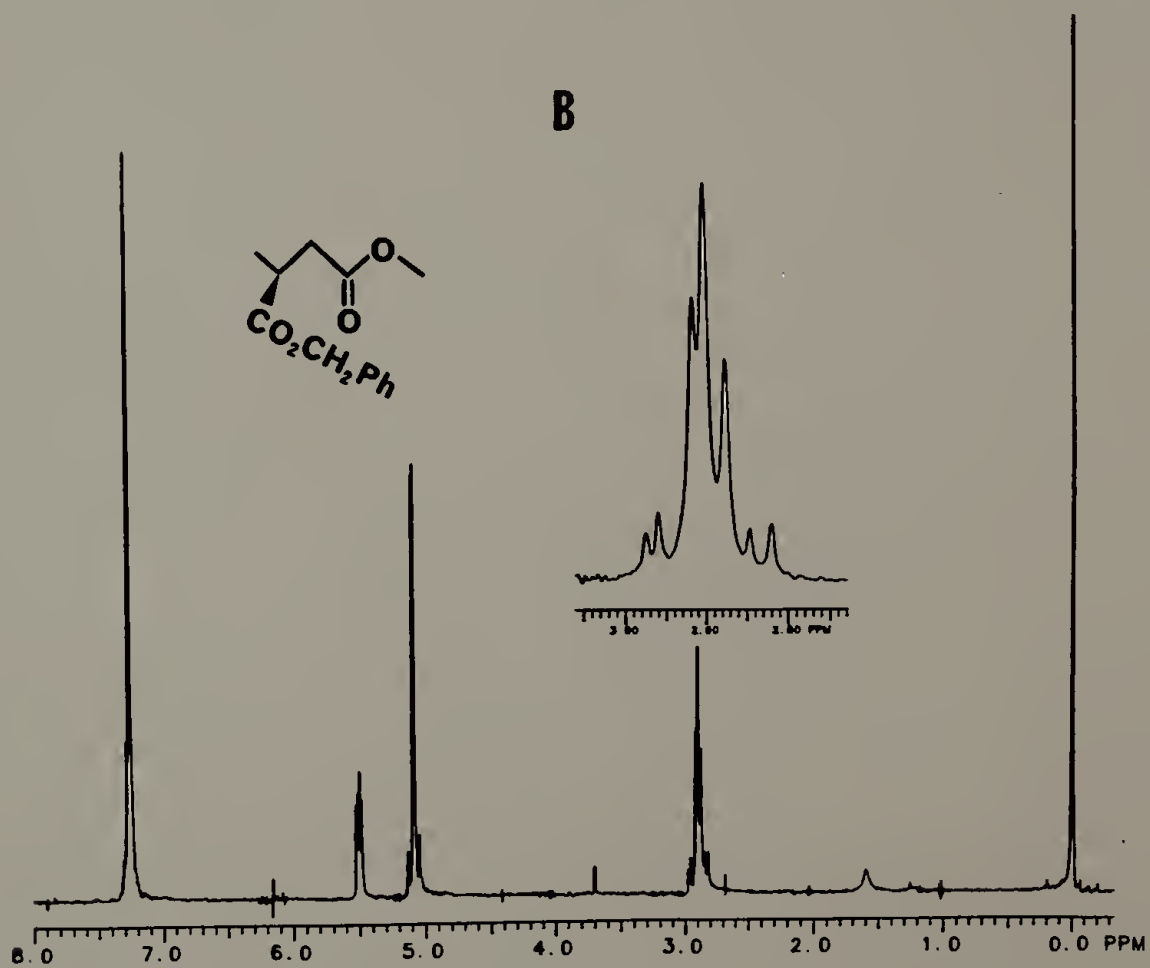
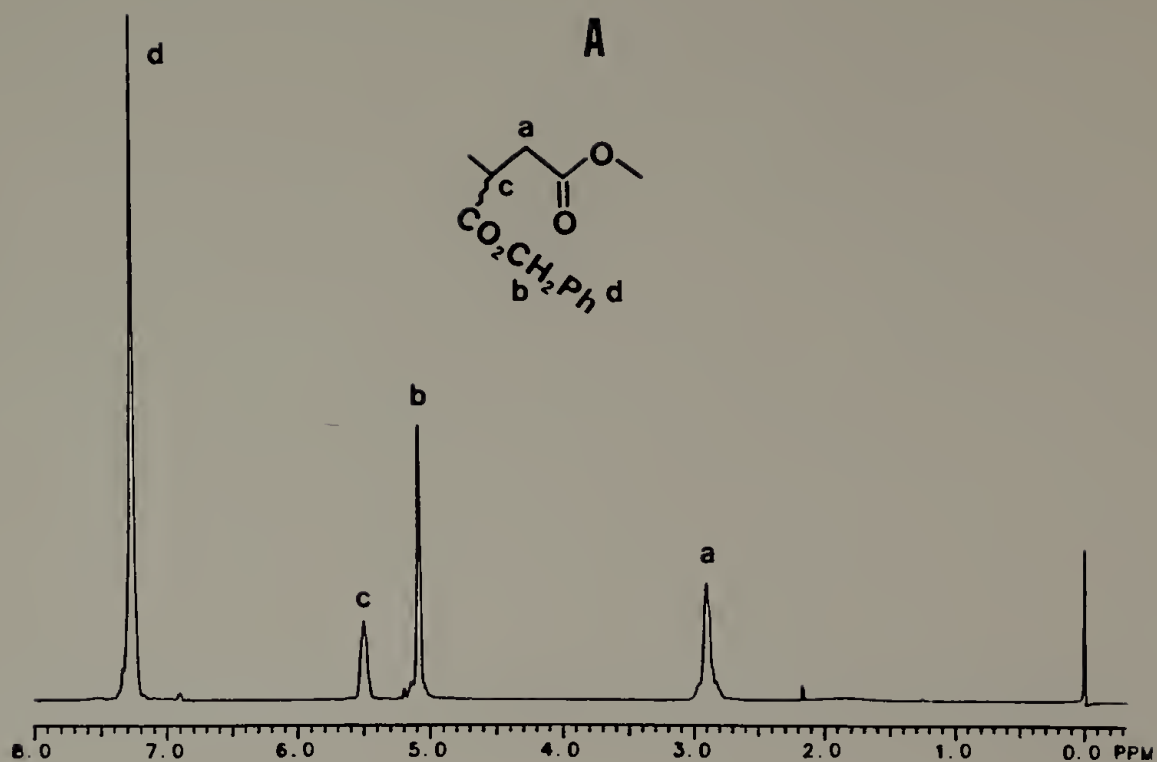
The polymerization of optically pure alkyl (R)-malolactonate formed isotactic polymers, while the polymerization of racemic alkyl malolactonates formed atactic polymers. This difference in tacticity was not observed in the carbon-13 NMR spectra of poly[benzyl (S)-malate] and poly[benzyl (R,S)-malate]; the spectra were identical. In contrast,

dyad tacticity has been observed in some atactic poly[β -alkyl- β -propiolactones] by carbon-13 NMR spectroscopy (37). However, the difference in tacticity was evident in the proton NMR spectra of the two polymers. As shown in Figure 18, the atactic poly[benzyl (R,S)-malate] showed only broad singlets, while the isotactic poly[benzyl (S)-malate] showed a typical ABX spectrum for the methylene (multiplet) and methine (quartet) protons of the polymer backbone.

The other poly[alkyl malates] showed the same tacticity effects in their proton NMR spectra. Atactic polymers had broad, overlapping signals (10), and isotactic polymers had sharp absorptions with all of the expected spin-spin coupling patterns (38). The most interesting case was poly[isopropyl malate] because the methyl groups of the pendent isopropyl ester are diastereotopic and therefore magnetically nonequivalent. Consequently, stereoregular poly[isopropyl (S)-malate] had a triplet at $\delta 1.25$ ($J = 6$ Hz) for these diastereotopic methyl groups, while atactic poly[isopropyl (R,S)-malate] had a broad doublet. The atactic structure must have caused enough chemical shift anisotropy in the pendent isopropyl esters to obscure the diastereotopic splitting pattern. It was also interesting to note that isopropyl (R)-malolactonate had a doublet of doublets at $\delta 1.31$ ($J = 3$ Hz) for the diastereotopic methyl groups which moved upfield and overlapped into a 1::2::1 triplet in poly[isopropyl (S)-malate]. The diastereotopic methyl groups were also evident in the carbon-13 NMR spectrum of poly[isopropyl (S)-malate]. The methyl absorption at $\delta 21.6$ was a doublet with $\Delta\delta = 12$ Hz.

Figure 18

300 MHz Proton NMR Spectra of (A) poly[benzyl (R,S)-malate] and (B) poly[benzyl (S)-malate] both polymerized by tetraethylammonium benzoate in the bulk at 60°C.



Poly[(S)- β -malic acid] was prepared from poly[benzyl (S)-malate] by the catalytic hydrogenolysis of the pendent benzyl esters. The only problem that was encountered was the insolubility of poly[benzyl (S)-malate] in common organic solvents at room temperature. Heating was always necessary to dissolve the polymer. Usually, upon cooling, the poly[benzyl (S)-malate] crystallized out of solution. N-Methyl pyrrolidinone was the only solvent that kept appreciable amounts of the polymer in solution at room temperature after cooling.

The hydrogenolysis was carried out in N-methyl pyrrolidinone at 40°C. The crude poly[(S)- β -malic acid] was isolated as a white powder by precipitation into ether, but it still contained some N-methyl pyrrolidinone which was difficult to remove completely. The crude polymer was washed for 24 hours with ether in a Soxhlet extractor, and then, reprecipitated from a methanol solution into ether. This workup procedure gave relatively pure poly[(S)- β -malic acid] in about 60-65 percent yield. The ultraviolet spectra of poly[benzyl (S)-malate] and poly[(S)- β -malic acid] are shown in Figure 9B. The disappearance of the phenyl absorption at 260 nm is consistent with the complete removal of the benzyl ester protecting groups. The infrared spectrum of poly[(S)- β -malic acid] showed a carbonyl stretch at 1600 cm^{-1} which is more characteristic of a carboxylate than a carboxylic acid. Inter- and intrachain hydrogen bonding may be responsible for this observation. The infrared spectrum of the copolymer of ethyl (S)-malate and (S)-malic acid also showed a carbonyl stretch at 1600 cm^{-1} for the (S)-malic acid repeat unit. Poly[(S)- β -malic acid] can be used as a biodegradable

polymeric drug carrier. Drugs can be chemically bound to the polymer by pendent ester and amide bonds and then slowly released in the biological environment as the polymer degrades into (S)-malic acid which is an intermediate in the Krebs cycle.

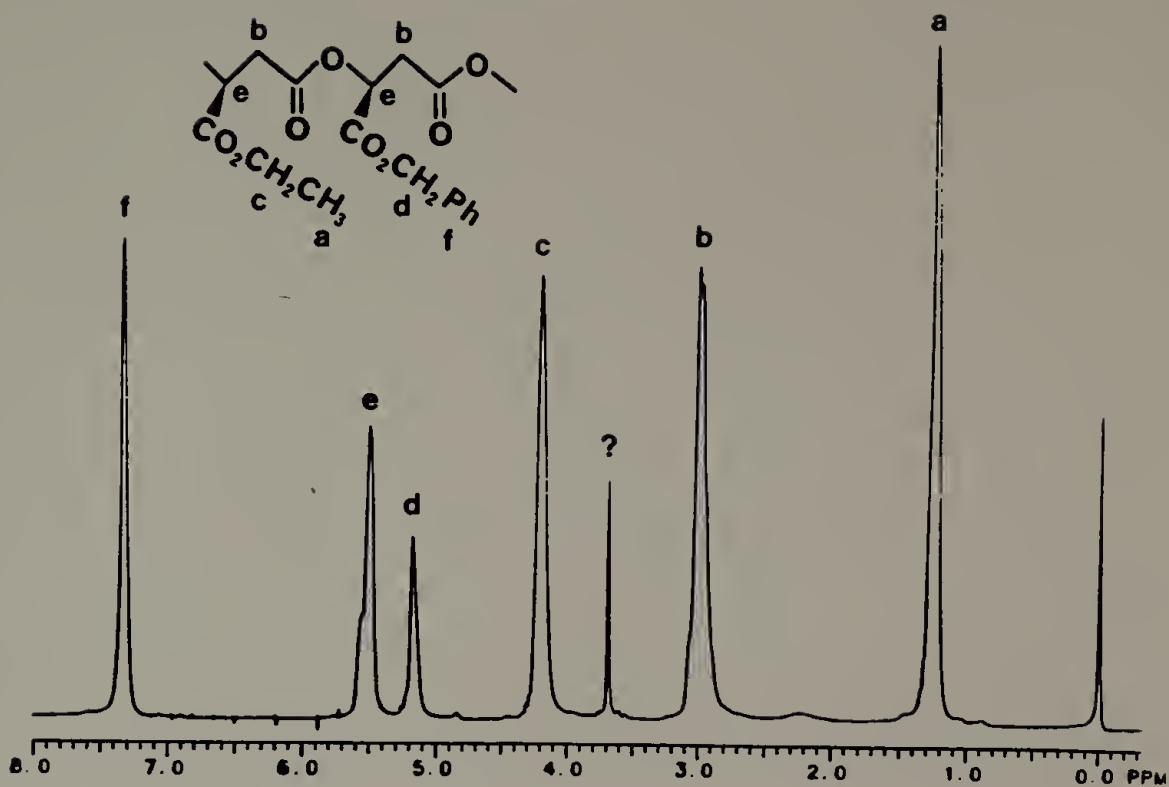
D. Copolymerization of Ethyl and Benzyl (R)-Malolactonate

Ethyl and benzyl (R)-malolactonate were copolymerized in the bulk at 60°C using tetraethylammonium benzoate as the initiator. The monomer feed composition was 80 mole percent ethyl (R)-malolactonate and 20 mole percent benzyl (R)-malolactonate. As previously mentioned, the copolymerization was homogeneous, and the copolymer was isolated in 83 percent yield. The copolymer was a rubbery material and was readily converted into a copolymer of ethyl (S)-malate and (S)-malic acid by catalytic hydrogenolysis of the benzyl esters in a mixture of ethyl acetate and 95% ethanol. Figure 19 shows the 300 MHz proton NMR spectra of poly[ethyl-co-benzyl (S)-malate] and poly[ethyl (S)-malate-co-(S)-malic acid]. The spectrum of poly[ethyl-co-benzyl (S)-malate] showed only broad singlets indicating a random distribution of monomer units. Integration of the methylene peaks of the pendent ethyl and benzyl ester groups showed that the copolymer composition was 76 mole percent ethyl (S)-malate and 24 mole percent benzyl (S)-malate which is within 4 percent of the monomer feed composition. In contrast, the spectrum of poly[ethyl (S)-malate-co-(S)-malic acid] showed the expected spin-spin coupling pattern in the pendent ethyl ester signals, and the coupling

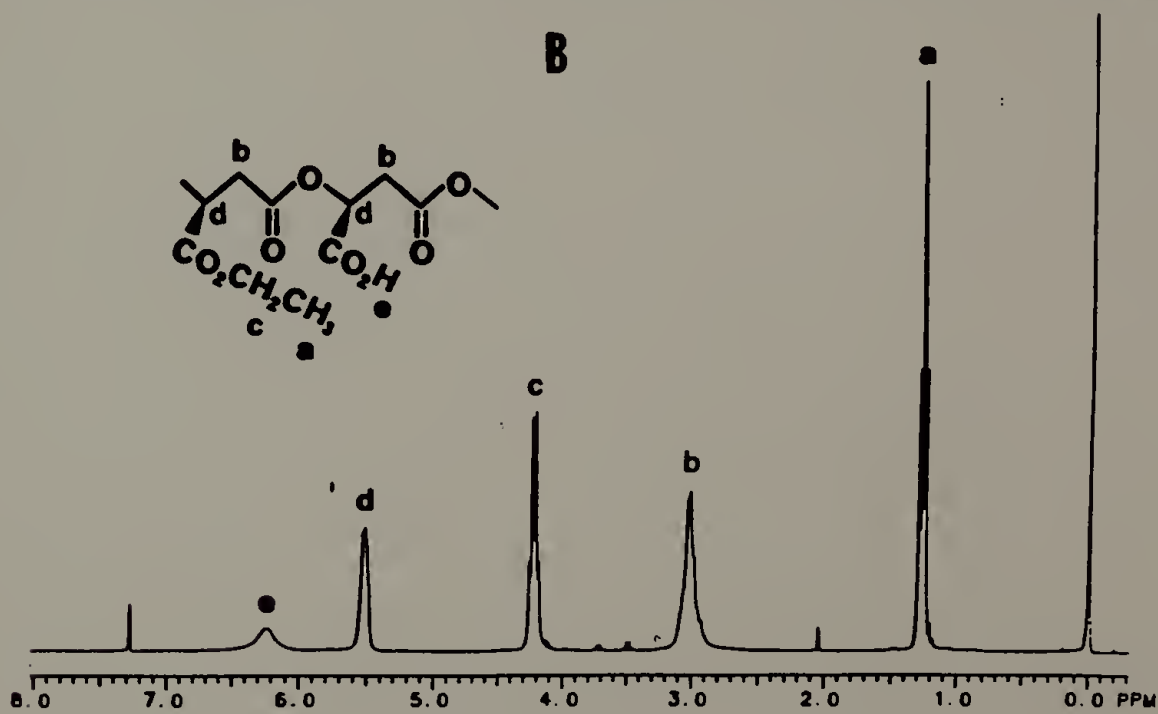
Figure 19

300 MHz Proton NMR Spectra of (A) poly[ethyl-co-benzyl (S)-malate] and (B) poly[ethyl (S)-malate-co-(S)-malic acid].

A



B



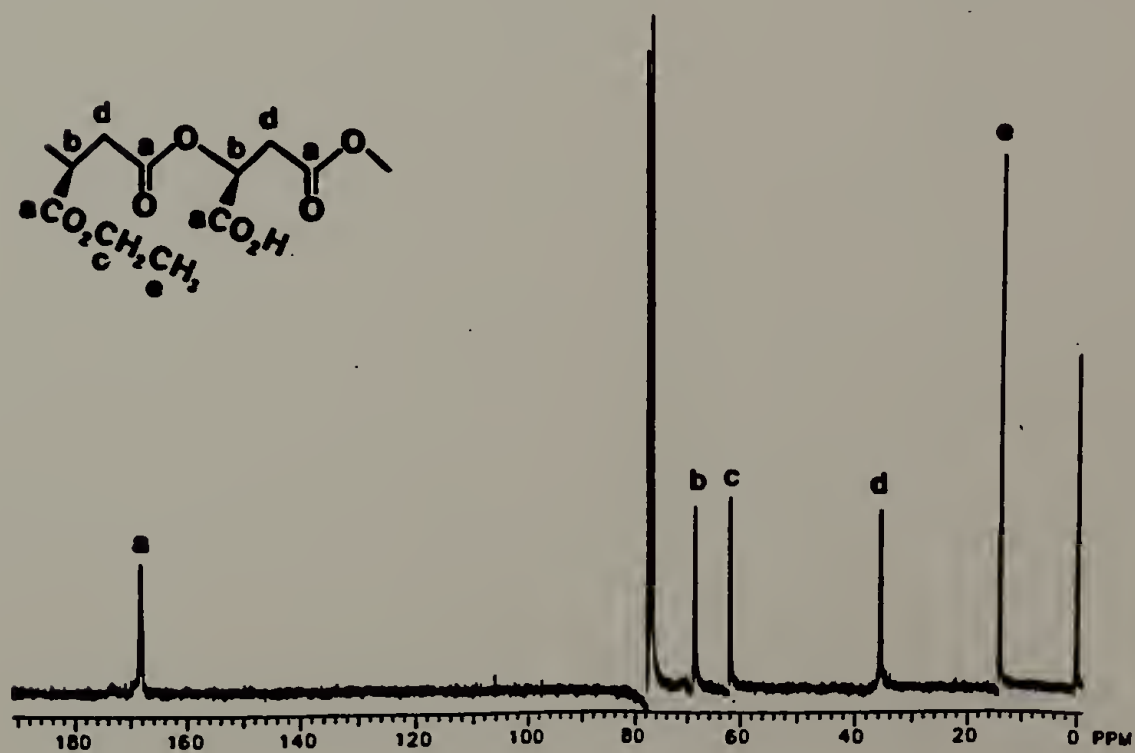
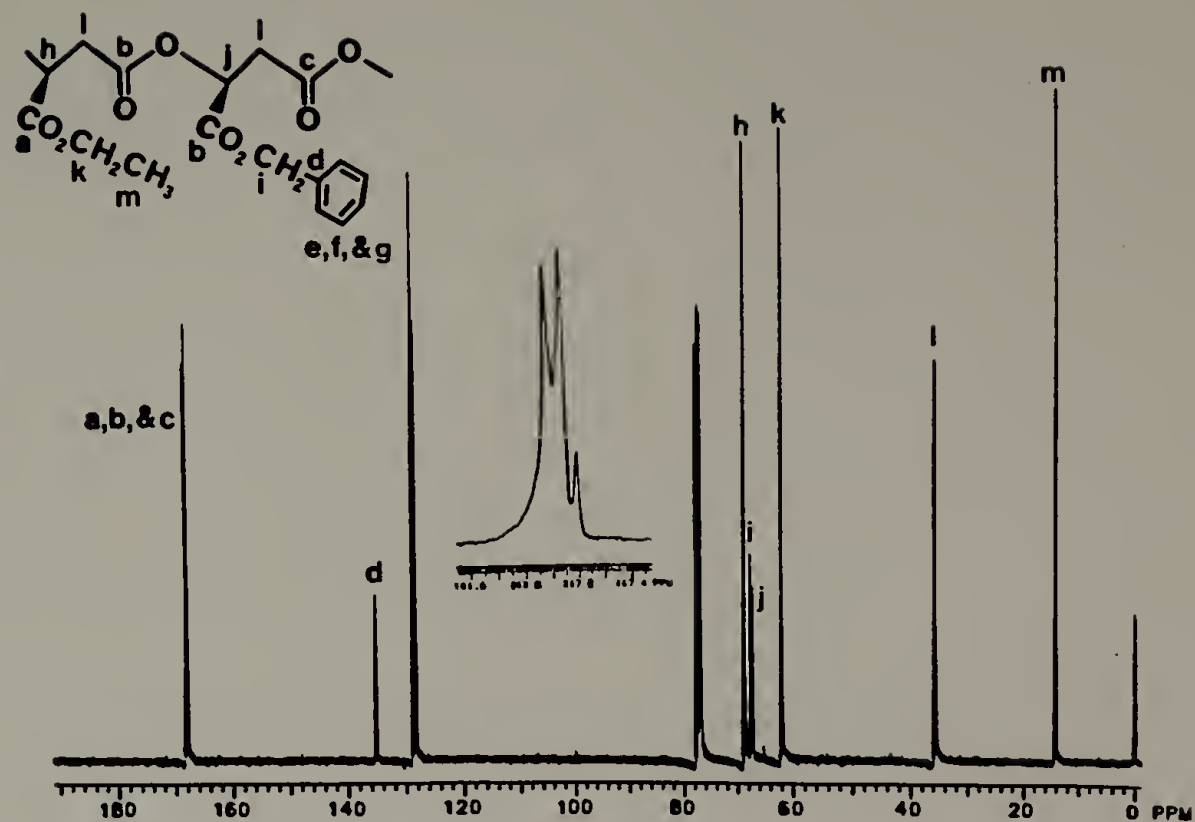
constants were almost the same as those in the homopolymer poly[ethyl (S)-malate]. Obviously, the benzyl (S)-malate repeat units in the copolymer created enough chemical shift anisotropy in the ethyl (S)-malate repeat units to obscure the spin-spin coupling pattern of the pendent ethyl ester signals, while the (S)-malic acid repeat units in the copolymer did not perturb the local magnetic field of the pendent ethyl esters very much.

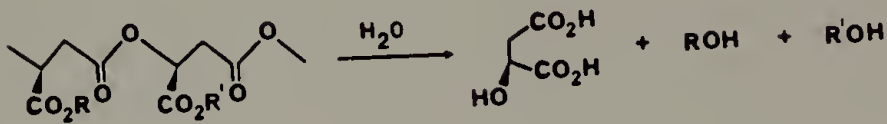
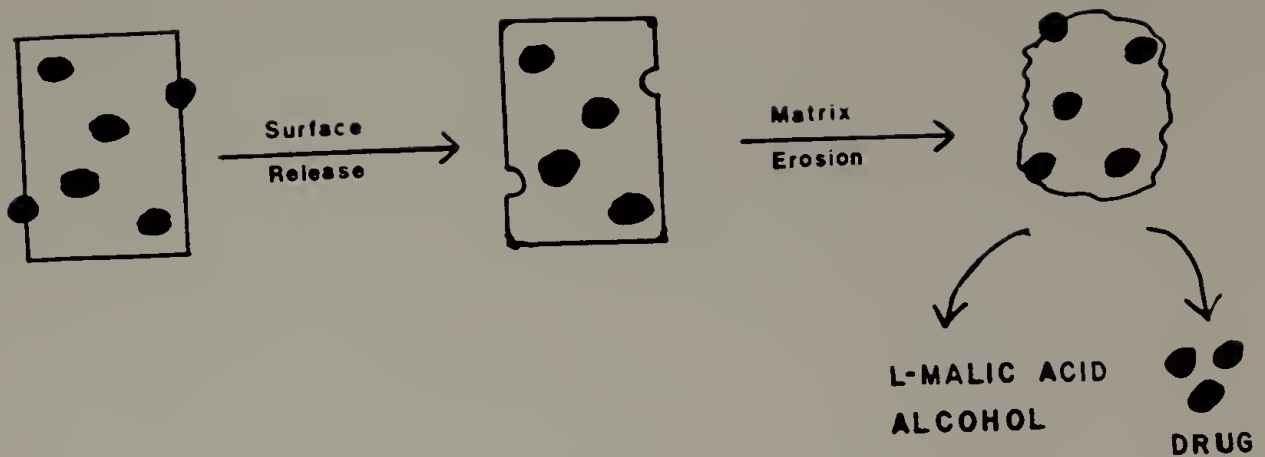
Figure 20 shows the carbon-13 NMR spectra of poly[ethyl-co-benzyl (S)-malate] and poly[ethyl (S)-malate-co-(S)-malic acid]. The copolymer composition of poly[ethyl-co-benzyl (S)-malate] was detectable in the carbonyl part of the spectrum which showed three peaks. The peaks were assigned with reference to the homopolymers and were separated by only 0.30 ppm. The copolymer composition of poly[ethyl (S)-malate-co-(S)-malic acid] was not detectable in the carbonyl region of the spectrum which showed only one broad peak. These spectra show that the carbonyl region is sometimes sensitive to the copolymer composition.

The copolymer of ethyl (S)-malate and (S)-malic acid is an interesting new biomaterial. It could be used in bioerodible drug delivery systems in which the drug is bound to the polymer matrix instead of being simply embedded in the polymer matrix. In this way, the drug could be released over a longer period of time. Figure 21 shows how this new kind of bioerodible drug delivery system would work. The copolymer matrix would eventually degrade into ethanol and (S)-malic acid. The (S)-malic acid would be absorbed by the host in the Krebs metabolic pathway.

Figure 20

70.4 MHz $^{13}\text{C}\{^1\text{H}\}$ NMR Spectra of (A) poly[ethyl-co-benzyl (S)-malate] and (B) poly[ethyl (S)-malate-co-(S)-malic acid].





R = H or alkyl

R' = drug

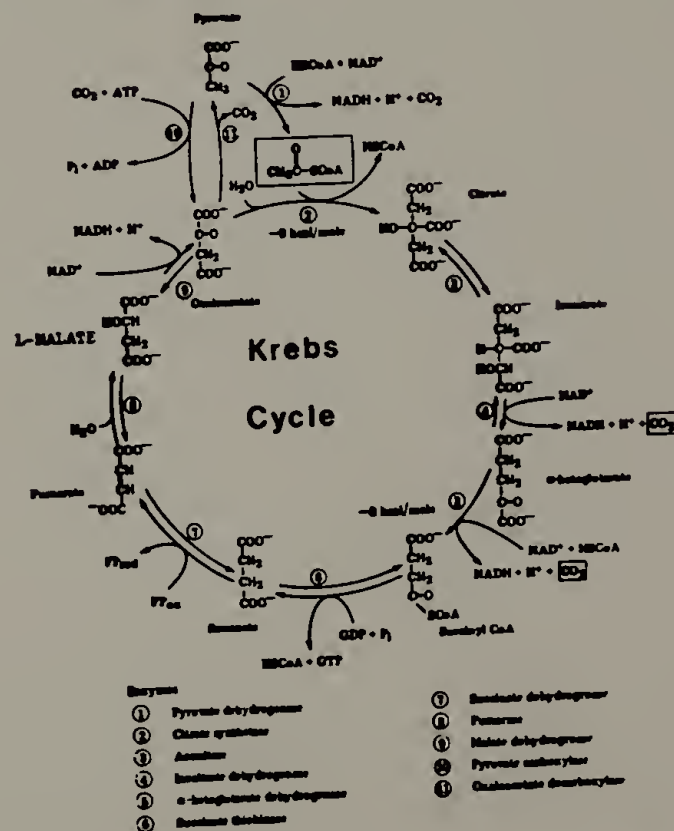


Figure 21. Bioerodible Drug Delivery Systems

E. Thermal Analysis

The stereoregular poly[alkyl (S)-malates] were all crystalline polyesters, and their thermal properties are shown in Table 5. Only poly[(S)- β -malic acid] decomposed on melting. The melting points of these polymers depended on the alkyl group in the following order Bz > H > Me ~ Et > iPr, and the enthalpies of fusion also depended on the alkyl group Bz > Et > Me > iPr. The most dramatic changes occurred with the bulky isopropyl group which must not pack well into the crystalline lattice of the polymer.

Figure 22 shows the DSC thermograms of poly[benzyl (S)-malate]. The first heating scan only showed a sharp melting transition between 198-173°C. After being cooled rapidly from the melt, the second heating scan showed a glass transition temperature of 40°C and a recrystallization exotherm at 113°C. Obviously, poly[benzyl (S)-malate] was quenched into an amorphous state that was able to recrystallize upon heating. The first heating scans of the other poly[alkyl (S)-malates] also showed sharp melting transitions of the same shape as poly[benzyl (S)-malate]. However, after being cooled rapidly from the melt, poly[methyl (S)-malate] and poly[ethyl (S)-malate] melted with lower enthalpies of fusion, and poly[isopropyl (S)-malate] was amorphous. No recrystallization exotherms were observed on the second heating scans. These data indicate that the poly[alkyl (S)-malates] were isolated in a highly crystalline form by precipitation.

Table 5
Thermal Analysis of the Poly[alkyl (S)-malates]^a

<u>Alkyl Group, R</u>	<u>Melting Range (peak), °C</u>	<u>Enthalpy of Fusion, cal/g</u>
H	182-147 (168) d	----
Me	156-119 (150)	9 (+/- 1)
Et	152-122 (147)	11
iPr	131-97 (123)	5
Bz	198-173 (190)	13

^aThe melting transitions were obtained on the first heating scan and were independent of the molecular weight of the polymer.

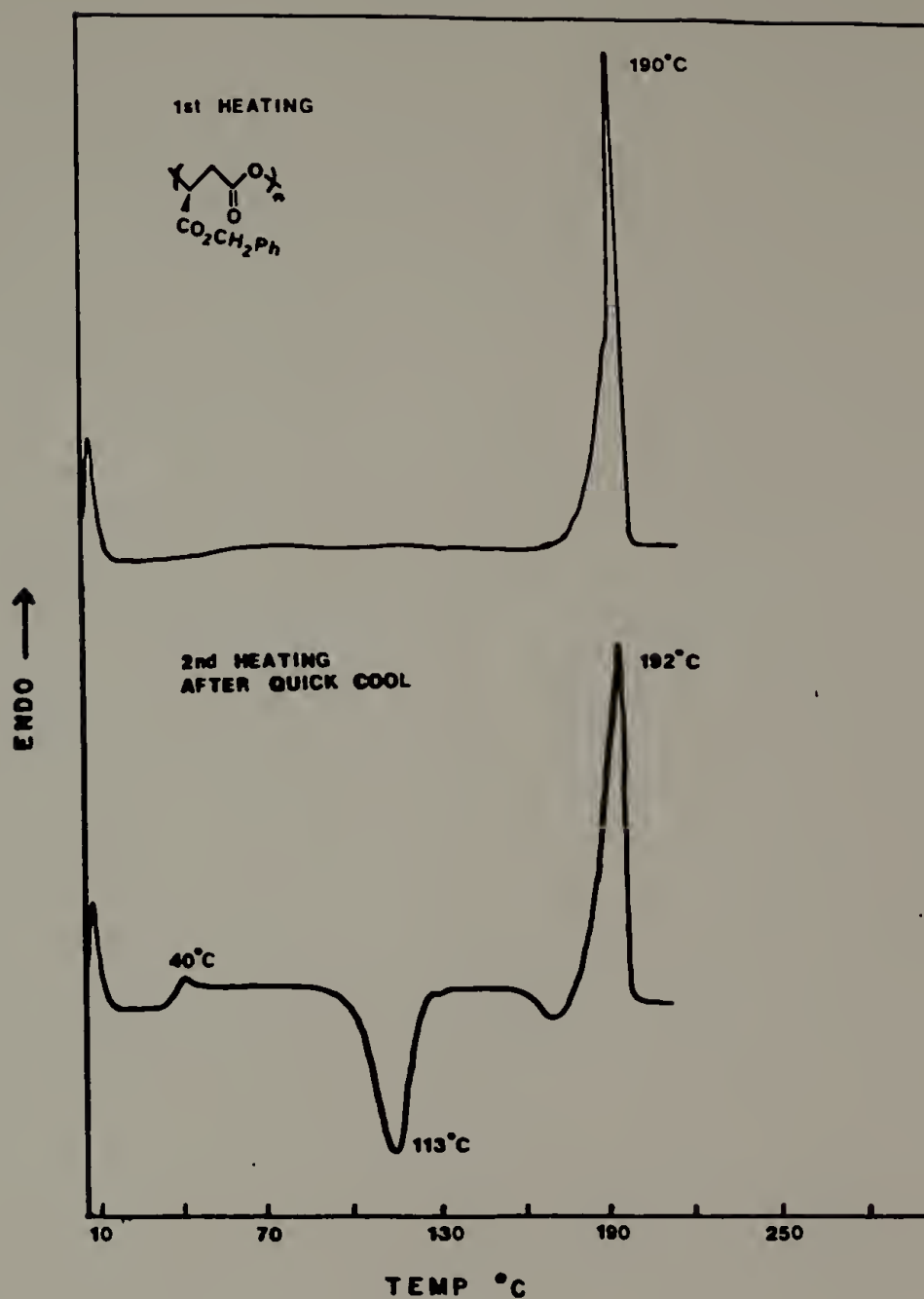


Figure 22. DSC Thermogram of Poly[benzyl (S)-malate]

Grenier and Prud'homme have reported that a racemic mixture of enantiomeric poly[α -methyl- α -ethyl- β -propiolactones] formed a stereocomplex that melted at a higher temperature and had larger spherulites than the enantiomerically pure polyesters (39). Some of their data is shown in Figure 23. Both poly[(R)- α -methyl- α -ethyl- β -propiolactone] and poly[(S)- α -methyl- β -ethyl- β -propiolactone] melted at 164°C, while a racemic mixture of these stereopolymers melted at 202°C with the same enthalpy of fusion as the enantiomerically pure polyesters. In this study, a racemic mixture of enantiomeric poly[benzyl malates] was formed which melted at a lower temperature and with a lower enthalpy of fusion than the enantiomerically pure polymers after annealing. Poly[benzyl (S)-malate] was prepared from (S)-malic acid, and poly[benzyl (R)-malate] was prepared from (R)-malic acid. A racemic mixture of these stereopolymers was formed either by precipitation from a hot chloroform solution into ether or by solution evaporation. The DSC thermograms of the racemic mixture of enantiomeric poly[benzyl malates] are shown in Figure 24, and the corresponding thermal data are listed in Table 6. Both poly[benzyl (R)-malate] and poly[benzyl (S)-malate] melted around 197°C, while a racemic mixture of these stereopolymers melted around 182°C with about three fifths of the enthalpy of fusion as the enantiomerically pure polyesters.

In the first case, the enantiomeric polymer chains of poly[α -methyl- α -ethyl- β -propiolactone] obviously packed better with chains of the opposite absolute configuration than with chains of the same absolute configuration. Consequently, the racemic mixture of the

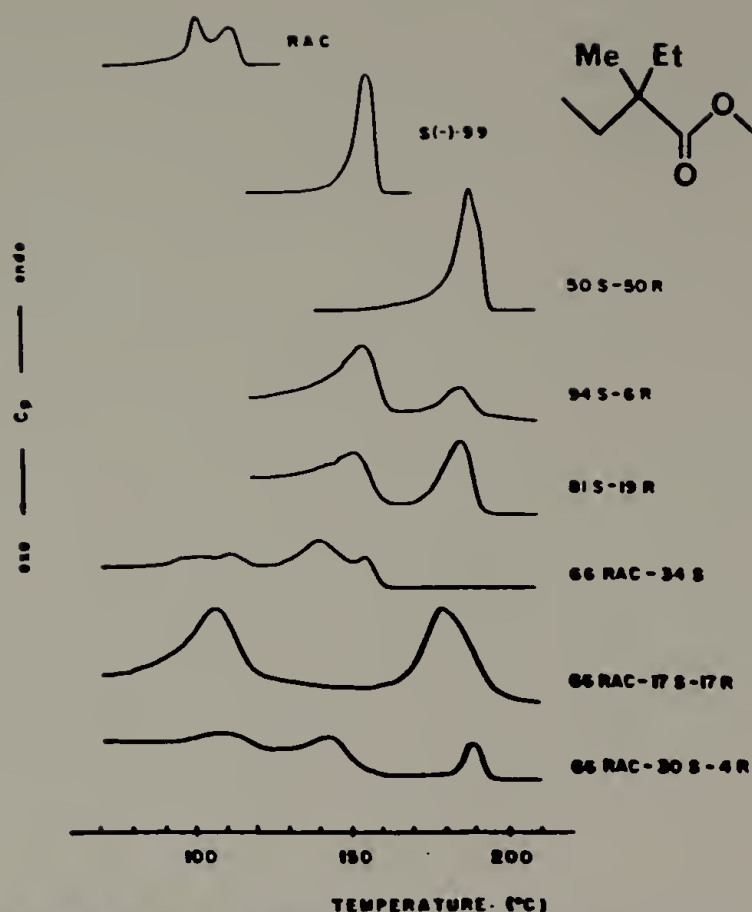


Fig. 5. Melting endotherms of PMEPL-RAC, PMEPL-S(-)-99, and of several representative binary and ternary mixtures.

TABLE I
Solid-State Characterization of PMEPL Samples: Racemic (RAC), Isotactic [S(-) and R(+)],
and Racemate (50S-50R)

Properties	RAC	S(-)	R(+)	50S-50R
T_f (°C)	121	164	163	202
ΔH_f (J/g)	27	60	60	60
R_n^a (μm)	30	4.2	4.6	≈ 500

^a Radius of the spherulites.

Figure 23. Thermal Data of Poly[α -methyl- α -ethyl- β -propiolactone]. Taken from J. Polym. Sci. Polym. Phys. Ed. 22 577 (1984).

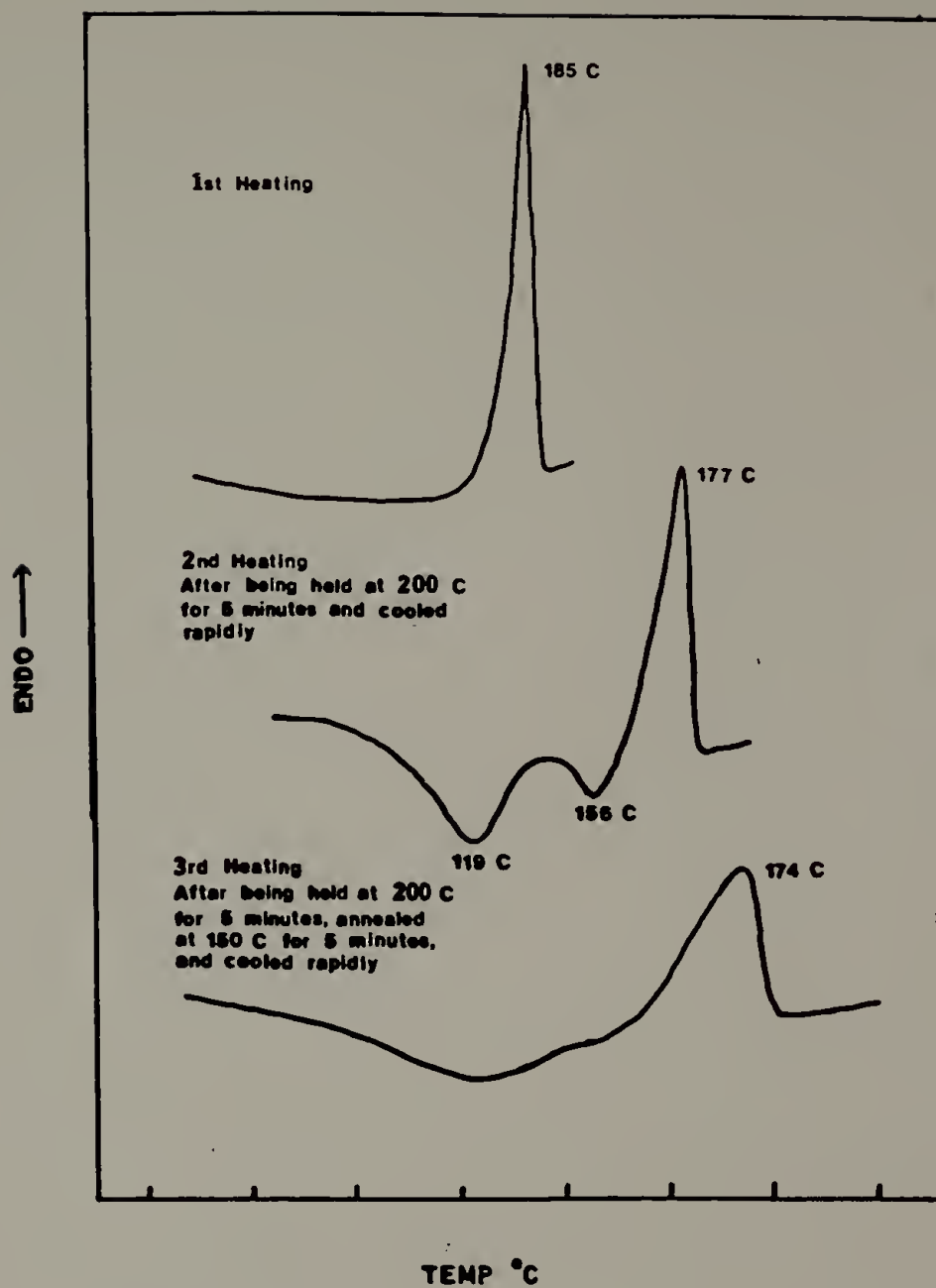


Figure 24. DSC Thermograms of a Racemic Mixture of Poly[benzyl (S)-malate] and Poly[benzyl (R)-malate]

Table 6
Poly[benzyl malate]

	(R,S)				
	(S)	(R)	1st	2nd	3rd
M_n^a	40,000	56,000			
M_w^a	92,000	110,000			
$[\alpha]_D^{29}{}^b$	+5.7°	-5.6°		0°	
ΔT	198-173 °C ^c	195-175 ^c	193-162 ^c	187-157 ^d	182-150 ^e
T(max)	190 °C	188	185	177	174
ΔH	13(+/- 1) cal/g	12	13	13	7.8

^a Molecular weights were determined by GPC using polystyrene standards.

^b Measured in N-methyl pyrrolidinone at a concentration of 0.10 g/mL.

^c First heating.

^d Second heating after being held at 200 °C for 5 minutes and cooled rapidly.

^e Third heating after being held at 200 °C for 5 minutes, annealed at 150 °C for 5 minutes, and cooled rapidly.

stereopolymers packed into a more highly ordered crystalline lattice and melted at a higher temperature. This behavior is common with low molecular weight compounds like malic acid. Racemic malic acid melts at 120°C , while (S)-malic acid melts at 100°C (40). In the second case, the enantiomeric polymer chains of poly[benzyl malate] packed better with chains of the same absolute configuration than with chains of the opposite configuration so that the racemic mixture of these stereopolymers packed into a less highly ordered crystalline lattice and melted at a lower temperature with a lower enthalpy of fusion.

F. Polyacid-Coated Liposomes

Polyacid-coated liposomes were prepared from polyacids bearing pendent cholesteryl esters which inserted into the lipid bilayer and anchored the polymer onto the surface of the liposome as shown below in Figure 25.

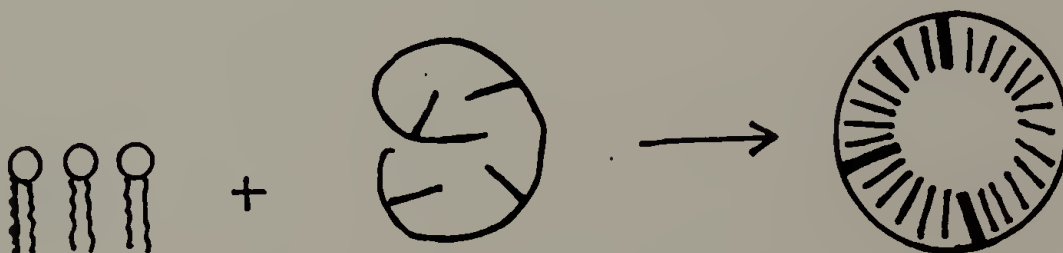


Figure 25. Polyacid-Coated Liposomes

The structure of these polyacid-coated liposomes would be expected to depend on the phospholipid, the polyacid and its molecular weight, the pH and ionic strength of the solution, and the number of cholesteryl anchors on the polymer chain. In this preliminary study, two phospholipids, dipalmitoylphosphatidylglycerol (DPPG) and dipalmitoylphosphatidylcholine (DPPC), and two polyacids, poly[acrylic acid] (PAA) and poly[L-glutamic acid] (PLGA), were used to determine the conditions necessary for polymer absorption onto phospholipid vesicle membranes. Figure 26 shows the chemical structure of these compounds. DPPG is a negatively charged lipid that can act as a hydrogen bond donor and acceptor with respect to an absorbed polymer, and DPPC is a zwitterionic lipid that can only act as a hydrogen bond acceptor. PAA was prepared by the free radical polymerization of acrylic acid in a 20 weight percent solution of dioxane at 60°C using azobisisobutyronitrile as the initiator. The molecular weight was controlled by the concentration of the initiator and the addition of butanethiol. PAA was atactic with an unknown amount of chain branching. In contrast, PLGA was prepared by the debenzilation of poly[γ-benzyl L-glutamate] and was a stereoregular, linear polyamide.

As shown in Figure 27, cholesterol was chemically bound to these polyacids using dicyclohexylcarbodiimide as the coupling agent and 4-dimethylaminopyridine as the nucleophilic catalyst in dimethylformamide solution. The coupling reaction was rapid and clean. After 12-24 hours, the reaction mixture was chilled, and the byproduct, dicyclohexylurea, was removed by suction filtration. The polymers were

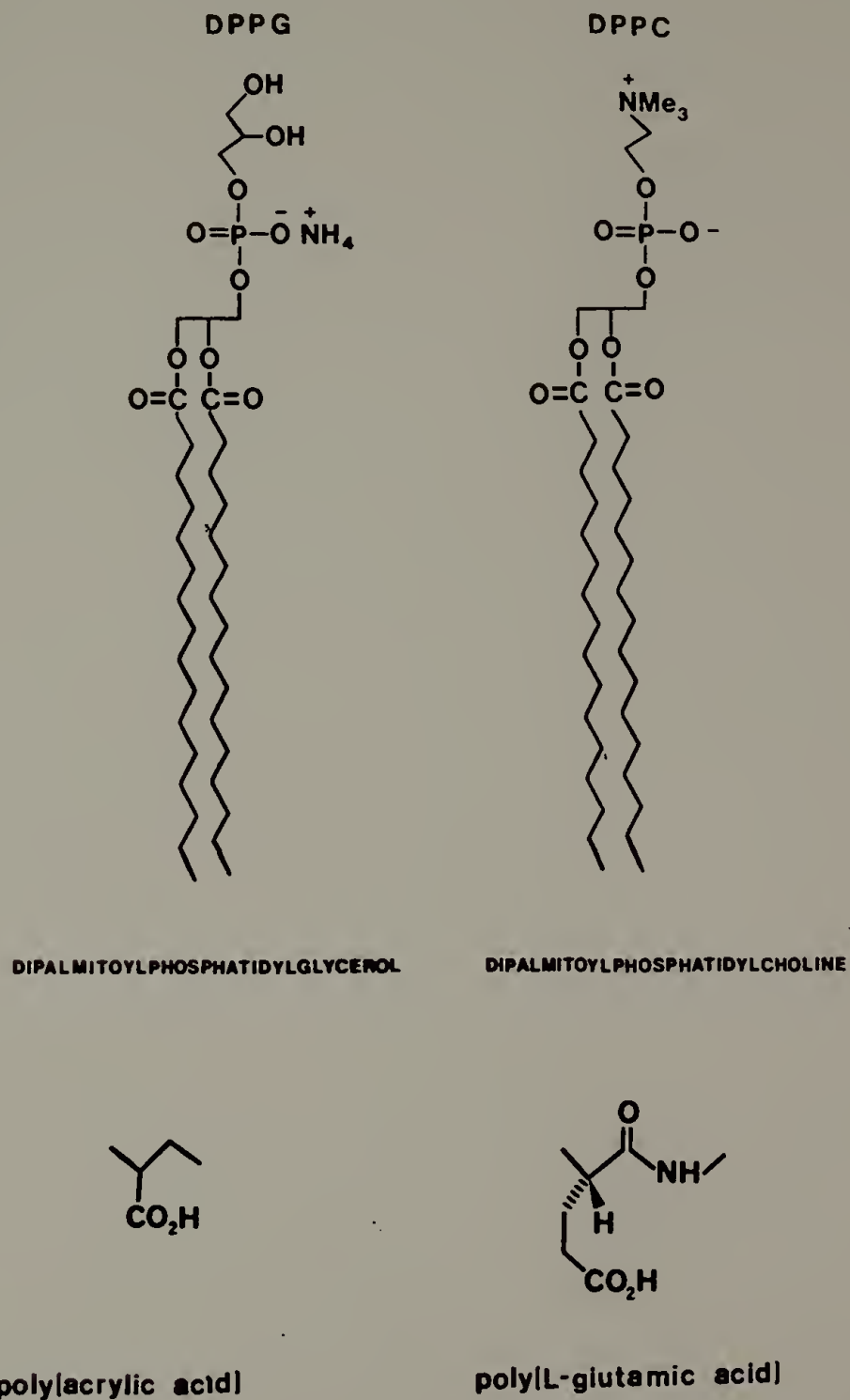


Figure 26. Structures of the Phospholipids and Polyacids

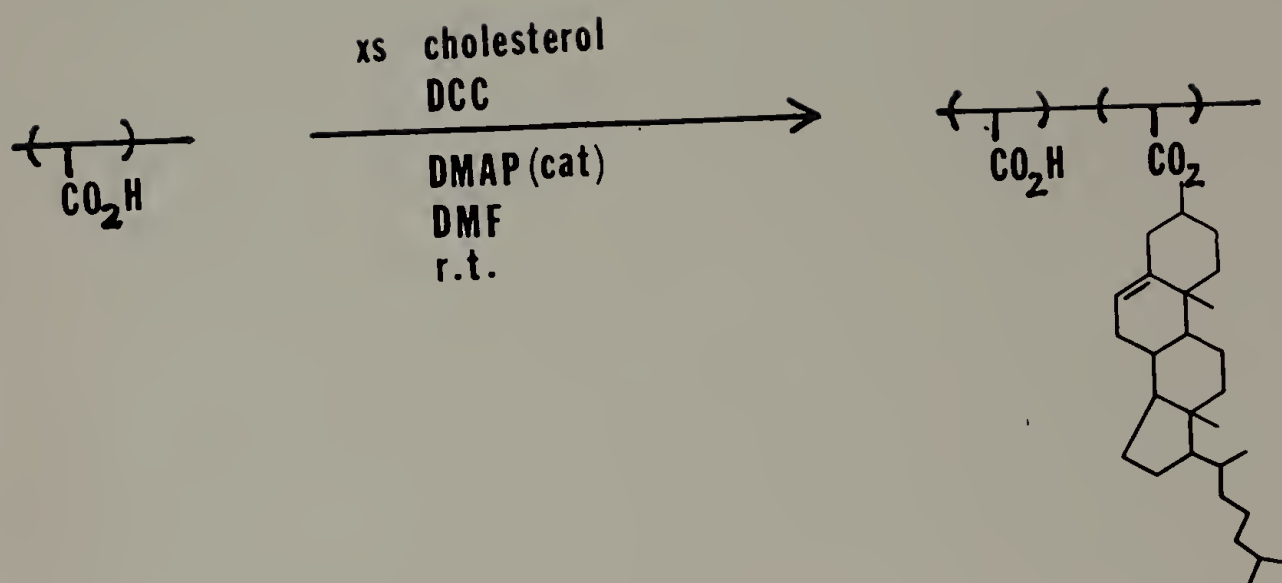


Figure 27. Coupling Cholesterol to Polymers

isolated by precipitation into chloroform to remove the excess cholesterol and then by precipitation into ether. The final product contained no free cholesterol as determined by thin layer chromatography.

Multilamellar polyacid-coated liposomes were prepared by mixing a dry lipid film into a buffered polymer solution. The concentration of both the phospholipid and the polymer was 1 mg/mL. The temperature of the lipid suspension was 55°C which is above the phase transition temperature (to be discussed in the next section) of both DPPG and DPPC.

The mechanism of the formation of polyacid-coated liposomes is not known in any detail, but it must involve the interaction of polymer coils in aqueous solution with a lipid film. Consequently, the conformation of the polymer in solution probably affects the structure of the resulting polyacid-coated liposomes. Aqueous solutions of the polyacids with pendent cholesteryl esters were sudsy, indicating that the polymers might be micellar. The ultraviolet spectrum of sudan red, a water insoluble dye, in absolute ethanol is shown in Figure 28A. Sudan red had an absorption at 520 nm which could be used to detect the solubilization of the dye in aqueous polymer solutions without overlapping with the carbonyl absorption bands of the polymer. As shown in Figure 28B, 0.10 M solutions of PAA with 5 and 15 mole percent pendent cholesteryl esters solubilized sudan red, as detected by an absorption at 550 nm and by the red color of their solutions, while PAA did not dissolve any sudan red. The pH of these polymer solutions was 4.1. The polymers with pendent cholesteryl esters were prepared from

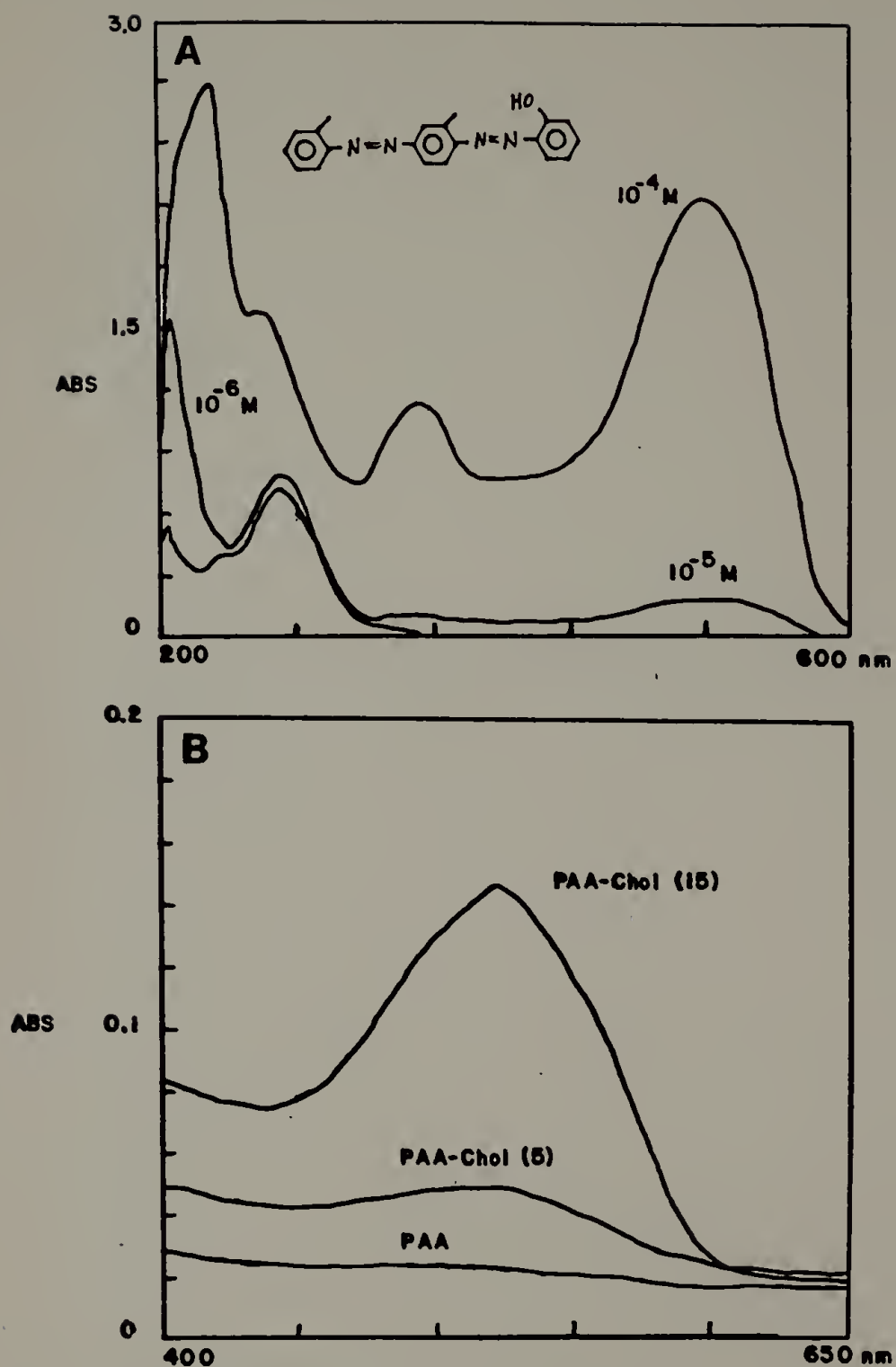


Figure 28. Solubilization of Sudan Red in Aqueous Polymer Solutions

the same batch of PAA which had a number average molecular weight of 50,000. Obviously, the poly(acrylic acid)s with pendent cholesteryl esters were micellar with the dye dissolving into the hydrophobic domains of the polymer coil. Similar results were obtained at neutral pH where the carboxyl groups were almost completely ionized, although the absorptions at 550 nm were reduced. In contrast, neither PLGA nor PLGA with 5 mole percent pendent cholesteryl esters dissolved any sudan red at neutral pH.

Both PAA and PLGA undergo pH dependent conformational changes in aqueous solution. The polymer coil of PAA expands slowly between pH = 2 and pH = 4 as the carboxyl groups are neutralized (41). PLGA undergoes a sharp helix-coil transition between pH = 5 and pH = 6 depending on the ionic strength of the solution. These conformational transitions were certainly modified by the pendent cholesteryl esters, but since they were far away from the physiological pH range in which the polyacid-coated liposomes were studied, they were ignored in the interpretation of the DSC thermograms of the polyacid-coated liposomes discussed in the next section.

G. High-Sensitivity Differential Scanning Calorimetry

A suspension of multilamellar phospholipid vesicles is characterized by a highly cooperative, reversible, thermal phase transition which is readily detected by high-sensitivity differential scanning calorimetry (43-45). As shown in Figure 29, it is a gel to

liquid crystalline phase transition, and it is accompanied by a lateral expansion and a decrease in the thickness of the bilayer. The phase transition temperature of both DPPG and DPPC is 41°C . In the preparation of lipid suspensions, the temperature must be above the phase transition temperature of the phospholipid, because the lipid molecules can only reassemble into multilamellar vesicles when they are in the liquid crystalline phase where they have lateral mobility.

Cholesterol affects the thermal phase transition of phospholipids (43-46). The addition of cholesterol to the membrane usually changes the phase transition temperature, broadens the melting endotherm, and reduces the enthalpy of fusion. At high cholesterol levels, the thermal phase transition disappears completely. Consequently, if the pendent cholesteryl esters are able to insert into the phospholipid bilayer and anchor the polyacid onto the liposome, the phase transition temperature should change, the melting endotherm should broaden, and the enthalpy of fusion should decrease, neglecting any polymer-lipid interactions that would certainly occur. A possible structure of a polyacid-coated membrane is shown in Figure 30. The polyacid could hydrogen bond to the polar head groups on the surface of the liposome and/or could be incorporated into the bilayer. Clearly, high-sensitivity differential scanning calorimetry is a priori an ideal technique to detect the absorption of polyacids with pendent cholesteryl esters onto phospholipid vesicle membranes.

Cholesterol also controls the fluidity and therefore the permeability of the lipid bilayer (43-46). Cholesterol increases the

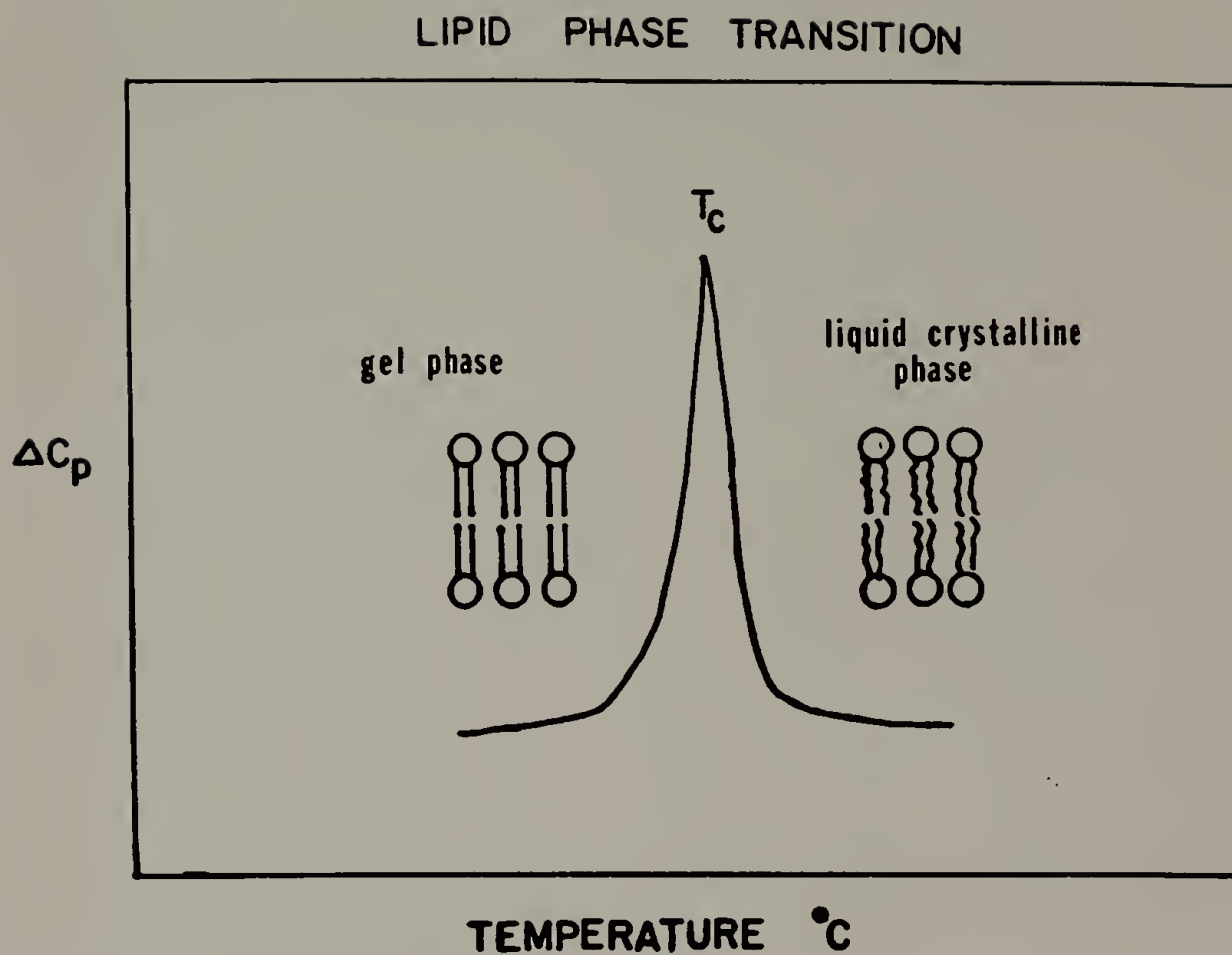


Figure 29. DSC Thermogram of Phospholipid Vesicle Membranes

Polyacid-Coated Bilayer

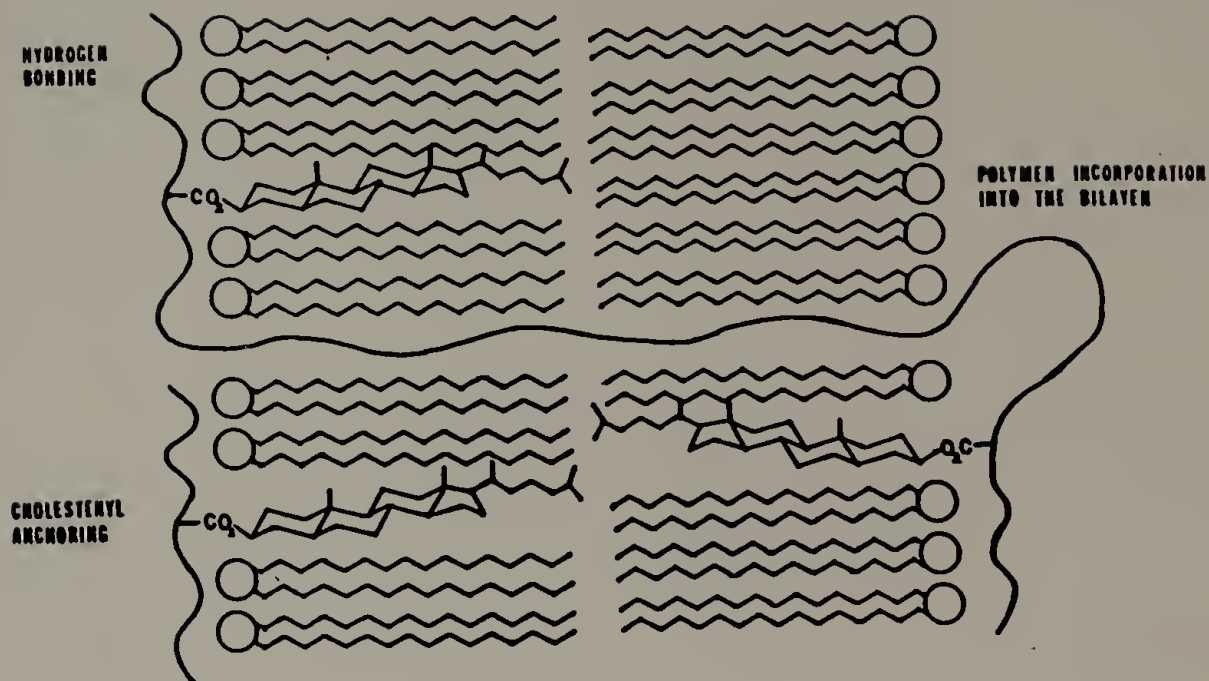


Figure 30. A Possible Structure of Polyacid-Coated Phospholipid Bilayers

fluidity of the membrane below the phase transition temperature by preventing the crystallization of the hydrocarbon chains into a rigid gel and decreases the fluidity of the membrane above the phase transition temperature by reducing the mobility of the hydrocarbon chains. Consequently, the pendent cholesteryl anchors of an absorbed polymer could modify the permeability of the membrane. Moreover, in liposomal drug delivery applications, the absorbed polyacid could make the membrane biologically and mechanically stable and could act as a charged barrier which would decrease the rate of release of negatively charged, entrapped material.

Figure 31A shows the melting endotherm of DPPG suspended in 50 mM Tris-HCl buffer. The melting endotherm was broadened, and the melting enthalpy was reduced as the pH was lowered. The phase transition temperature, however, remained constant at 40.8°C . Figure 31B shows the melting endotherm of a mixture of 90 mole percent DPPG and 10 mole percent cholesterol under the same conditions. The addition of cholesterol lowered the phase transition temperature by 1°C to 39.8°C and rendered the DPPG liposomes sensitive to pH. The melting endotherm was broadened, and the melting enthalpy was reduced more than in pure DPPG liposomes as the pH was lowered, but at $\text{pH} = 6.3$, the liposomes were disrupted and crystals of DPPG and cholesterol formed. The reason for this pH sensitization is not known, but perhaps some phase separation occurred allowing the cholesterol to crystallize out of the phospholipid bilayer and thereby destroying the vesicles.

In Figure 31C, the melting endotherms of DPPG suspended in buffered solutions of PAA and PAA bearing 5 mole percent pendent cholesteryl esters at pH = 7.1 are shown. The number average molecular weight of these polymers was 50,000. Under these conditions, no significant interactions between DPPG and PAA were evident, and the melting endotherm was essentially identical to that of DPPG in the absence of the polymer. In contrast, the addition of PAA with pendent cholesteryl esters raised the main melting temperature by about 6°C to 47.1°C . The melting endotherm was broadened substantially, and the melting enthalpy was reduced. A minor peak at 43.5°C was also observed. As shown in Figure 31D, similar results were obtained for PLGA and PLGA with 5 mole percent pendent cholesteryl esters at pH = 8.0. The viscosity average molecular weight of these polymers was 47,000. In this case, the addition of PLGA with pendent cholesteryl esters raised the main melting temperature by about 10°C to 50.8°C . The melting endotherm was broadened, and the melting enthalpy was reduced. A minor peak at 48.7°C was also observed.

One explanation of these thermal effects is that the pendent cholesteryl esters inserted into the phospholipid bilayer and anchored the polymer onto the surface of the liposome. This cholesteryl anchoring would be a hydrophobic interaction between the polymer and the lipid, but it would be reinforced by hydrogen bonds between the phospholipid and the absorbed polyacid. PLGA with pendent cholesteryl esters interacted with DPPG at a higher pH than PAA with pendent cholesteryl esters because PLGA could hydrogen bond better than PAA to

the surface of the liposome. PLGA could hydrogen bond by both the pendent carboxylate anions and by the polyamide backbone, while PAA could only hydrogen bond by the pendent carboxylate anions.

Takigawa and Tirrell have found that branched poly[ethylenimine] also raised the melting temperature of DPPG in 50 mM Tris-HCl buffer at pH = 7.4, but only by 2°C (47). The melting endotherm was broadened, and the melting enthalpy was reduced. In this case, a branch of poly[ethylenimine] could have inserted into the phospholipid bilayer and anchored the polymer to the liposome. This hydrophobic interaction would be reinforced by hydrogen bonds between the polyamine and the phosphodiester.

Figure 32 shows the pH dependence of the melting endotherms of DPPG and DPPC suspended in solutions of PAA or PLGA with 5 mole percent pendent cholesteryl esters. The melting temperature of DPPG increased, and the melting temperature of DPPC remained constant as the pH of the polymer solutions was lowered. In both cases, increasingly broad transitions with lower enthalpies were observed as the pH was lowered. Under these conditions, no significant interactions between DPPC and the polymers were evident. Similar behavior was found with poly[methacrylic acid] and poly[α -ethylacrylic acid] over certain pH ranges (48,49).

Figures 33A, 33B, and 33D show the pH dependence of the melting endotherms of DPPG and DPPC suspended in buffered solutions of PAA and PLGA. The important point to note is that these polyacids absorbed onto the DPPG liposomes and raised the phase transition temperature of this phospholipid in the same way as the polyacids with pendent cholesteryl

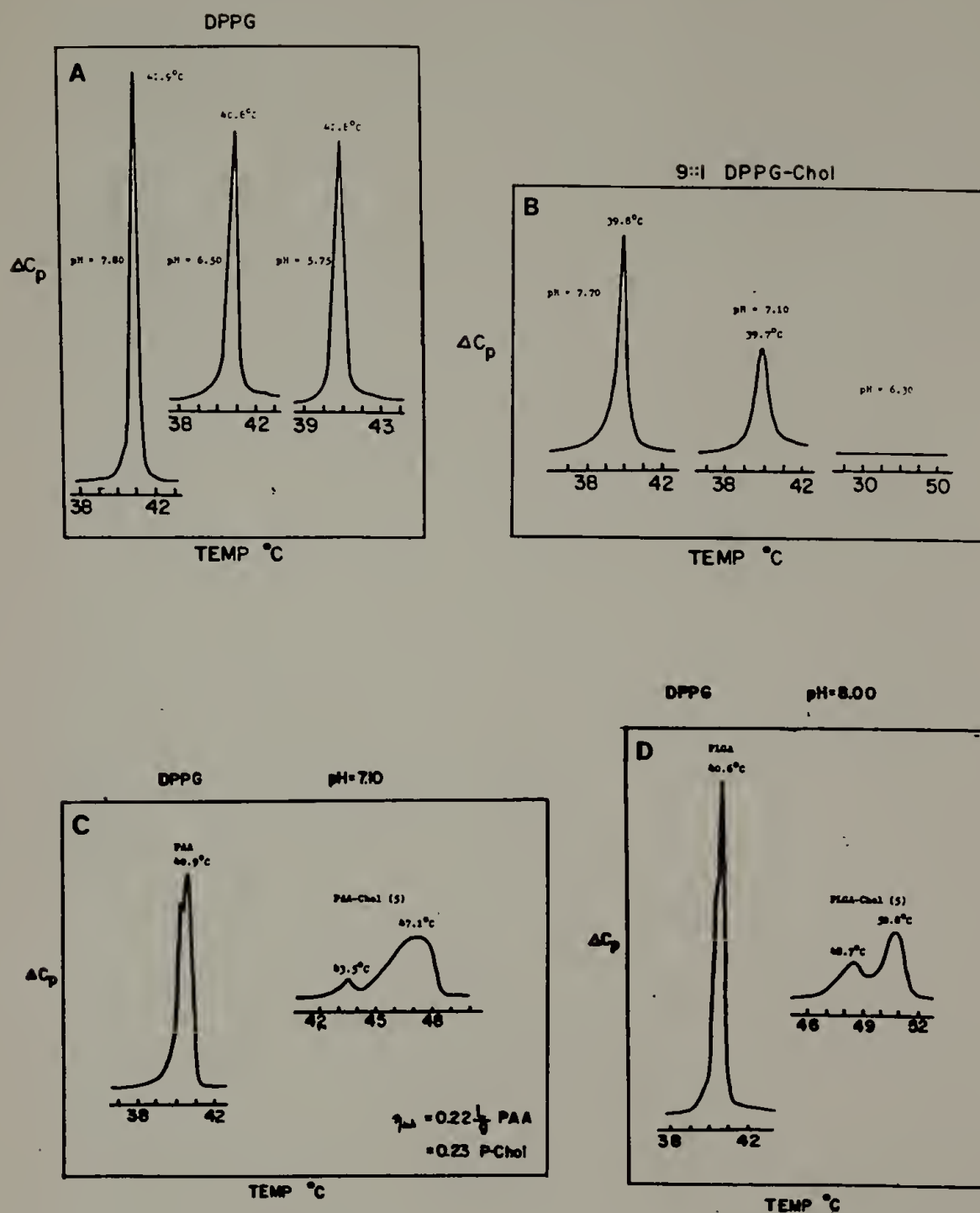


Figure 31. DSC Thermograms of DPPG (A), of a mixture of 90 mole % DPPG and 10 mole % cholesterol (B), of DPPG in the presence of PAA and PAA-Chol (5) (C), and of DPPG in the presence of PLGA and PLGA-Chol (5) (D)

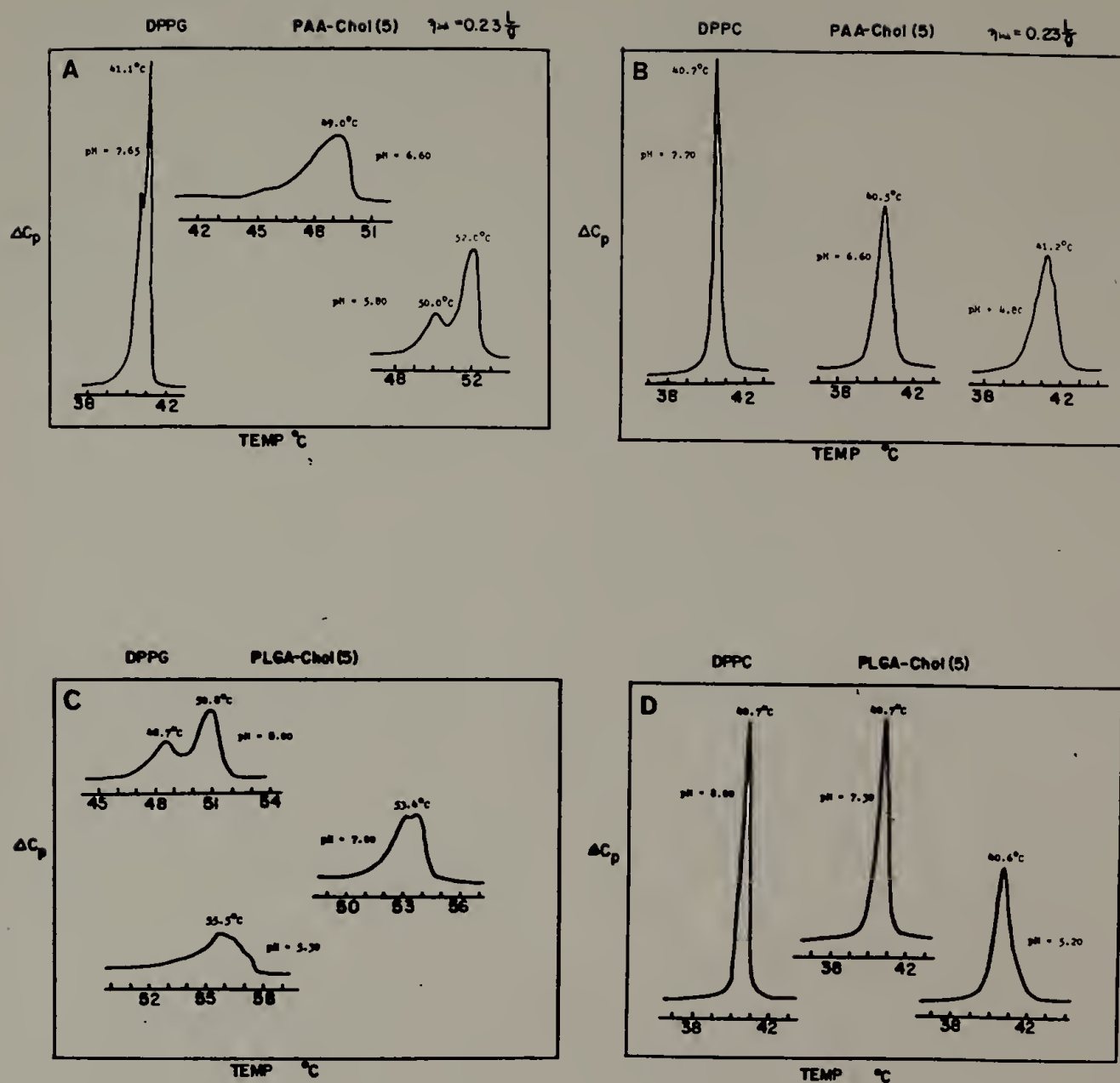


Figure 32. DSC Thermograms of DPPG (A) and DPPC (B) in the presence of PAA-Chol (5) and of DPPG (C) and DPPC (D) in the presence of PLGA-Chol (5) at different pH's

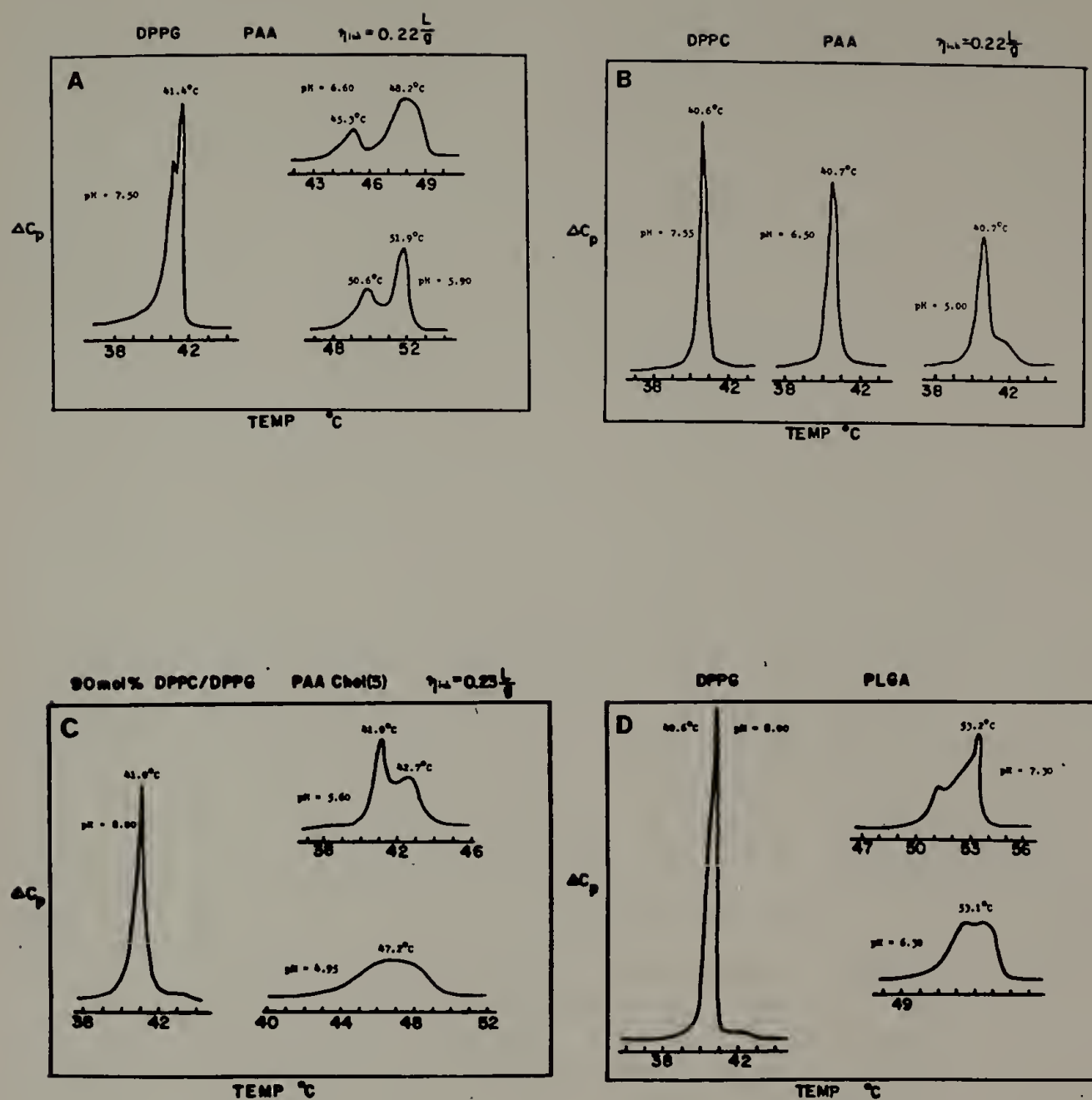


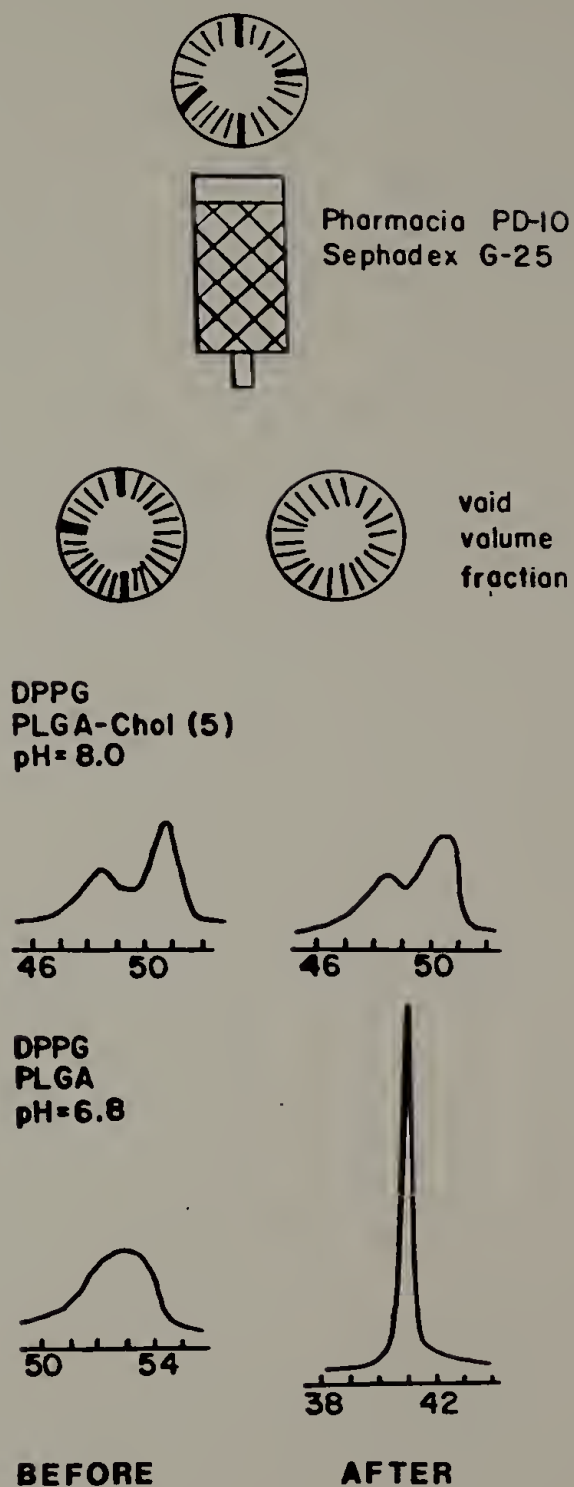
Figure 33. DSC Thermograms of DPPG (A) and DPPC (B) in the presence of PAA, of a mixture of 90 mole % DPPC and 10 mole % DPPG in the presence of PAA-Chol (5) (C), and of DPPG in the presence of PLGA (D)

esters did except at lower pH's. Hence, pendent cholesteryl anchors only enhanced the natural absorption of these polyacids onto DPPG liposomes. Figure 33C shows the pH dependence of the melting endotherm of a mixture of 90 mole percent of DPPC and 10 mole percent of DPPG suspended in a solution of PAA with 5 mole percent pendent cholesteryl esters. In contrast to pure DPPC liposomes (see Figure 32B), the melting temperature was raised by about 6°C to 47.2°C at $\text{pH} = 5.0$. Obviously, 10 mole percent of DPPG was sufficient to make the polymer absorb onto a predominantly DPPC surface.

H. Cholesteryl Anchoring

One measure of the strength of cholesteryl anchoring is whether or not the polyacid-coated liposomes survive column chromatography. The conditions can be varied to test the stability of the polyacid-coated liposomes toward a wide range of substances. In this study, a simple strength test was devised using a Pharmacia PD-10 column of Sephadex G-25. As shown in Figure 34, the polyacid-coated liposomes suspended in 50 mM Tris-HCl were passed over the PD-10 column and collected in the void volume fraction. Melting endotherms were obtained before and after chromatography and compared. The melting endotherm of the polyacid-coated liposomes prepared from PLGA with 5 mole percent pendent cholesteryl esters before chromatography was identical to that after chromatography. Apparently, the polymer was tightly bound to the liposomes. In contrast, the melting endotherm of the polyacid-coated

Figure 34. Chromatographic Evidence for Cholesteryl Anchoring



liposomes prepared from PLGA before chromatography was not the same as the melting endotherm observed after chromatography. In this case, the absorbed polyacid was pulled off of the liposome by the column, because it was not anchored to the liposome by a cholesteryl ester, and only uncoated liposomes were collected in the void volume fraction. Polyacid-coated liposomes prepared from PAA bearing 5 mole percent cholesteryl esters also failed this cholesteryl anchoring test which means that PLGA with pendent cholesteryl esters was indeed more tightly bound than the corresponding PAA polymer, as suggested by its absorption behavior.

I. Footnotes

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CHAPTER III

RECOMMENDATIONS FOR FUTURE WORK

In this study, the anionic polymerization of optically pure alkyl malolactonates gave higher molecular weight polymers than those previously reported for the anionic polymerization of racemic alkyl malolactonates (1). The reason for this difference in molecular weight is not known. Two explanations were given: (1) the bulk purities of the optically pure monomers might be higher than those of the racemic monomers, because of the new synthetic strategy, so that chain termination with reactive impurities would be reduced, or (2) the rates of polymerization of the optically pure monomers might be higher than those of the racemic monomers, because of some favorable stereoelectronic interactions between the propagating center and the alkyl malolactonate monomer. This latter hypothesis could be tested by preparing both enantiomers of an alkyl malolactonate by the method shown in Figure 6 and polymerizing them under the same conditions. A racemic mixture of alkyl malolactonate would also be prepared from equal amounts of the two enantiomers and polymerized along with the optically pure monomers. Since the bulk purity of the three samples would be roughly the same, the difference in the molecular weights of the polymers could

be attributed to the difference in the rates of polymerization of the optically pure and racemic monomers.

The synthetic strategy used in this dissertation is also applicable to the synthesis of other optically pure β -lactone monomers from optically pure β -hydroxy acids. The carboxyl group is first converted into its benzyl ester, and then, the hydroxy group is transformed into a mesylate leaving group. The benzyl ester protecting group is removed by hydrobromic acid in glacial acetic acid, and the resulting β -mesylate carboxylic acid is converted into the optically pure β -lactone by an internal S_N2 reaction. $-\zeta_p^2$

Racemic benzyl (R,S)-malolactonate has been polymerized by a triethylaluminum-water catalyst into a high molecular weight polymer (1,2). Optically pure benzyl (R)-malolactonate could be used to study the mechanism of this polymerization reaction by comparing the thermal and optical properties of the polymers obtained from the optically pure and racemic monomer for a given catalyst composition. In fact, polymers of different optical purities could be prepared by the anionic polymerization of unequal mixtures of the enantiomers of benzyl malolactonate. These polymers could also be compared to those obtained from the triethylaluminum-water catalyst.

The synthesis of polyacid-coated liposomes in this dissertation merits further investigation. In particular, the permeability across the polyacid-coated bilayer could be studied by dye release experiments in which a self-quenching, fluorescent dye is entrapped inside the liposome and slowly leaks out of the liposome at a rate that is

dependent on the structure of the bilayer (3,4). Since the entrapment procedure involves column chromatography and since some polyacid-coated liposomes do not survive column chromatography, the polymer would have to be added to purified, dye containing liposomes in those cases. Polyacid-coated liposomes that do not survive chromatography over Sephadex G-25 may survive chromatography over Sephadex CM C-25, because the column packing is negatively charged and would repel the negatively charged polyacid-coated liposomes.

Footnotes

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CHAPTER IV

EXPERIMENTAL SECTION

A. Synthesis of Stereoregular Poly(alkyl malolactonates)

1. Reagents

(S)-Malic acid, (R)-malic acid, mesyl chloride, hydrobromic acid in acetic acid, and trifluoroacetic anhydride were purchased from Aldrich Chemical and used without purification. Tetraethylammonium benzoate was purchased from Eastman Kodak and recrystallized from a mixture of hot tetrahydrofuran and dimethylformamide. Benzyl alcohol was stirred over calcium hydride for 12 hours and then vacuum distilled at 40-43°C (10^{-3} torr). Methanol, ethanol, isopropanol, and pyridine were stirred over calcium hydride for 12 hours and then distilled under dry argon. Methylene chloride was refluxed over activated 3 Å molecular sieves for 12 hours and then distilled under dry argon. Acetone was stirred over anhydrous potassium carbonate for 4 hours and then distilled under dry argon. Chloroform was stirred over phosphorous pentoxide for 2 hours and then distilled under dry argon. Tetrahydrofuran was distilled from sodium benzophenone ketyl under dry argon. Dimethylformamide was stirred over calcium hydride for 12 hours and then distilled from barium

oxide under reduced pressure at 45-50°C (ca. 30 torr). A one piece still with a 45 cm column and a recirculation loop was used for all of the distillations.

2. Dibenzyl (S)-Malate

0.40 mL (7.3 mmol) of concentrated sulfuric acid were added dropwise to a mixture of 775 mL (7.5 mol) of distilled benzyl alcohol and 500 mL of spectroscopic grade benzene with stirring in a two liter Erlenmeyer flask. This solution was poured into a two liter round bottom flask containing 100 grams (0.75 mol) of powdered (S)-(-)-malic acid and a magnetic stir bar. A 25 mL Dean-Stark trap filled with benzene and a reflux condenser were attached. The mixture was stirred and heated with a heating mantle until a stable reflux was obtained at 100°C. Water was removed by azeotropic distillation. After 3 hours, 27-30 mL of water (1.5-1.7 mol) were collected, and the hot reaction mixture was poured into a four liter beaker containing one liter of saturated sodium bicarbonate and stirred for 2 hours to remove the sulfuric acid catalyst and to cool the reaction mixture down. The mixture was transferred into a three liter separatory funnel, and the organic layer was washed once with 500 mL of 5% (w/v) solution of sodium bicarbonate and once with 500 mL of brine and then dried over anhydrous magnesium sulfate. After 1 hour, the magnesium sulfate was removed by suction filtration, and the solution transferred into a two liter round bottom flask containing a magnetic stir bar. A one piece still and a

heating mantle were installed, the benzene was distilled off using an aspirator vacuum, and then, the excess benzyl alcohol was removed under reduced pressure at 55-65°C (0.01 torr). The liquid residue was dissolved in 500 mL of ether, washed twice with 250 mL of 5% (w/v) solution of sodium bicarbonate, twice with 250 mL of water, and once with 250 mL of brine, dried over anhydrous magnesium sulfate, and decolorized with activated charcoal. After 1 hour, the suspension was filtered through celite, and the filtercake was washed with 100 mL of ether. The filtrate was transferred into a one liter round bottom flask, and the ether was removed on a rotary evaporator. The residue was dried in vacuo at room temperature for 6 hours. The product was a colorless liquid which crystallized readily in the freezer at -15°C. 210 grams of dibenzyl (S)-malate were isolated as a white solid (90% yield). MP = 29-32°C. Elemental Analysis: Theoretical: 68.8%C, 5.73%H; Found: 69.0%C, 5.67%H. $[\alpha]_D^{23} = -17.2^\circ$ (c = 10.5 g/100 mL, tetrahydrofuran). IR Spectrum # 1: 3550-3200 cm^{-1} (OH stretch--hydrogen bonded); 1720 cm^{-1} (C=O stretch); 1250 cm^{-1} , 1200 cm^{-1} , and 1150 cm^{-1} (C-O stretch); 740 cm^{-1} and 690 cm^{-1} (phenyl ring bending); thin film on a KBr plate. UV Spectrum: $\lambda(\text{max}) = 258 \text{ nm}$, $\epsilon = 560 \text{ L mol}^{-1} \text{ cm}^{-1}$, in tetrahydrofuran, phenyl absorption band. 300 MHz ^1H NMR Spectrum # 2: (a) δ 2.79-2.95 multiplet (2H), $|J_{AB}| = 17 \text{ Hz}$, $\Delta\delta_{AB} = 15 \text{ Hz}$; (b) δ 3.33-3.37 doublet (1H), $J = 5.1 \text{ Hz}$; (c) δ 4.51-4.56 quartet (1H); (d) δ 5.09 singlet (2H); (e) δ 5.16 singlet (2H); (f) δ 7.35 singlet (10H); 5 wt. % solution in deuterated chloroform. 50.4 MHz $^{13}\text{C}\{^1\text{H}\}$ NMR

Spectrum # 1: (a) 173.2, (b) 170.3, (c) 135.4, (d) 135.0, (e) 128.3-128.6 four peaks, (f) 67.5, (g) 67.3, (h) 66.7, (i) 38.7, 10 wt. % solution in deuterated chloroform.

3. Dibenzyl (S)-O-Mesylmalate

203 grams (0.65 mol) of dibenzyl (S)-malate were placed in a one liter Erlenmeyer flask, dissolved in 650 mL of dry methylene chloride, and chilled in the freezer at -15°C . Meanwhile, 65 mL (0.85 mol) of dry mesyl chloride and 104 mL (1.3 mol) of distilled pyridine were mixed in a one liter Erlenmeyer flask and aged 15 minutes at room temperature. The reaction was slightly exothermic, and the mixture changed color during the aging process from yellow to dark red. The mixture of mesyl chloride and pyridine was then chilled in the freezer. When both mixtures were cold, the methylene chloride solution of dibenzyl (S)-malate was poured into the mixture of mesyl chloride and pyridine, and the Erlenmeyer flask was placed in the refrigerator at 5°C . Pyridine was used as a nucleophilic catalyst and as an acid scavenger. The mesylation reaction was monitored by thin layer chromatography and was judged complete after 6 hours (silica gel, chloroform). The workup was simplified by allowing time for the byproduct, pyridinium chloride, to crystallize out of solution. After 18 hours, long needle crystals of pyridinium chloride were removed by suction filtration, and the filtercake was washed twice with 50 mL of methylene chloride. The dark red filtrate was transferred into a three liter separatory funnel,

washed twice with 500 mL of 1.0 M hydrochloric acid and once with 500 mL of brine, dried over anhydrous magnesium sulfate, and decolorized with activated charcoal. After 1 hour, the suspension was filtered through celite, and the filtercake was washed with 100 mL of methylene chloride. The filtrate was transferred into a one liter round bottom flask, and the methylene chloride was removed on a rotary evaporator. The dark red liquid was transferred into a one liter beaker where it crystallized rapidly. After drying in vacuo at room temperature for 12 hours, 243 grams of dibenzyl (S)-O-mesylmalate were isolated as a yellow solid (96% yield). MP = 60-63°C. Elemental Analysis: Theoretical: 58.2%C, 5.10%H, 8.16%S; Found: 58.7%C, 4.93%H, 8.06%S. $[\alpha]_D^{25} = -28.1^\circ$ (c = 9.97 g/100 mL, tetrahydrofuran). IR Spectrum # 2: 1730 cm^{-1} (C=O stretch); 1350 cm^{-1} (antisym. SO_2 stretch); 1260 cm^{-1} (C-O stretch); 1170 cm^{-1} (sym. SO_2 stretch); 750 cm^{-1} and 700 cm^{-1} (phenyl ring bending); thin film cast from a methylene chloride solution onto a KBr plate. UV Spectrum: $\lambda(\text{max}) = 258 \text{ nm}$, $\epsilon = 330 \text{ L mol}^{-1} \text{ cm}^{-1}$, phenyl absorption band; $\lambda(\text{max}) = 382 \text{ nm}$, $\epsilon = 21 \text{ L mol}^{-1} \text{ cm}^{-1}$, weak mesylate absorption band--which is responsible for the yellow solid and red solution, in tetrahydrofuran. 300 MHz ^1H NMR Spectrum # 3: (a) $\delta 2.99$ - 3.03 multiplet (2H); (b) $\delta 3.04$ singlet (3H); (c) $\delta 5.12$ doublet (2H); (d) $\delta 5.18$ doublet (2H); (e) $\delta 5.38$ - 5.44 quartet (1H); (f) $\delta 7.35$ singlet (10H); 5 wt. % solution in deuterated chloroform. 70.4 MHz $^{13}\text{C}\{^1\text{H}\}$ NMR Spectrum # 2: (a) 168.4, (b) 167.7, (c) 135.1, (d) 134.6, (e) 128.4-128.7 six peaks, (f) 73.9, (g) 68.1, (h) 67.3, (i) 38.9, (j) 36.9, 10 wt. % solution in deuterated chloroform.

4. (S)-O-Mesylmalic Acid

230 grams (0.59 mol) of dibenzyl (S)-O-mesylmalate were placed in a one liter Erlenmeyer flask containing a magnetic stir bar and dissolved in 100 mL of dry methylene chloride. 510 mL (2.4 mol HBr) of 30 wt. % hydrobromic acid in acetic acid were added in a hood with good ventilation. The reaction mixture was stirred at room temperature for 6 hours and then transferred into a one liter round bottom flask. A one piece still and a heating mantle were installed, and the volatile materials removed using an aspirator vacuum, keeping the temperature below 50°C (sometimes a mechanical pump was necessary). The collection flask was chilled in an ice-salt bath. When most of the volatile materials had been removed, the product came out of solution as an oil, and the distillation was stopped. It should not be distilled to dryness because it decomposes thermally! The mixture was poured into a beaker and allowed to cool down to room temperature, and then, the supernatant liquid was decanted off. (Caution: the supernatant liquid contained benzyl bromide, a severe lachrymator.) The yellow oily solid was dissolved in 100 mL of hot acetone, diluted with 100 mL of chloroform, and placed in the freezer for crystallization. After 18 hours, (S)-O-mesylmalic acid was isolated by suction filtration, washed once with 50 mL of cold chloroform, and dried in vacuo at room temperature for 4 hours. 60.0 grams of (S)-O-mesylmalic acid were isolated as a light yellow solid. The filtrate was decolorized with activated charcoal and dried with anhydrous magnesium sulfate. After 1 hour, the suspension

was filtered through celite, and the filtercake was washed with 100 mL of acetone. The filtrate was transferred into a 500 mL round bottom flask and concentrated on a rotary evaporator. The residue was diluted with about 60 mL of chloroform, seeded with a crystal, and placed back in the freezer. After 18 hours, another 15.4 grams of (S)-O-mesylmalic acid were isolated (70% yield overall). Sometimes, the second crop of (S)-O-mesylmalic acid was reddish orange instead of light yellow and required another recrystallization from a mixture of acetone and chloroform. MP=88-95°C with decomposition. Elemental Analysis: Theoretical: 28.2%C, 3.77%H, 15.1%S; Found: 28.6%C, 3.71%H, 15.3%S. $[\alpha]_D^{26} = -52.5^\circ$ (c = 10.1 g/100 mL, tetrahydrofuran). IR Spectrum # 3: 3600-2400 cm^{-1} (OH stretch); 1720 cm^{-1} (C=O stretch); 1350 cm^{-1} (antisym. SO_2 stretch); 1180 cm^{-1} (sym. SO_2 stretch); thin film cast from an ethyl acetate solution onto a KBr plate. UV Spectrum: $\lambda(\text{max}) = 378 \text{ nm}$, $\epsilon = 13 \text{ L mol}^{-1} \text{ cm}^{-1}$, weak mesylate absorption band, in tetrahydrofuran. 300 MHz ^1H NMR Spectrum # 4: (a) δ 2.98-3.17 doublet of quartets (2H), $|J_{AB}| = 17 \text{ Hz}$, $\Delta\delta_{AB} = 20 \text{ Hz}$, $J_{AX} = 10 \text{ Hz}$, $J_{BX} = -7 \text{ Hz}$; (b) δ 3.25 singlet (3H); (c) δ 5.37-5.43 quartet (1H); (d) δ 9.5-9.9 broad singlet (2H); 5 wt. % solution in deuterated methanol. 70.4 MHz $^{13}\text{C}\{^1\text{H}\}$ NMR Spectrum # 3: (a) 172.2, (b) 171.3, (c) 75.7, (d) 38.7, (e) 37.7, 10 wt. % solution in deuterated methanol.

The conversion of (S)-malic acid into (S)-O-mesylmalic acid was also carried out successfully without isolating the intermediate products. The liquid dibenzyl (S)-malate was not vacuum dried, but dissolved immediately into dry methylene chloride and converted into

dibenzyl (S)-O-mesylmalate. Then, during the workup, the dibenzyl (S)-O-mesylmalate was not allowed to crystallize, but dissolved immediately into the 30 wt. % hydrobromic acid in acetic acid and converted into (S)-O-mesylmalic acid. By not isolating the intermediate products, (S)-malic acid was converted into (S)-O-mesylmalic acid in four working days and isolated in 75 % yield overall.

The debenzylation of dibenzyl (S)-O-mesylmalate was the most difficult reaction in the synthesis of poly[alkyl (S)-malates] to carry out safely because of the use of hydrobromic acid and the formation of benzyl bromide. The following techniques were used to reduce the hazards associated with these chemicals: (1) all manipulations were carried out in the hood, (2) disposable gloves were always worn, (3) all glassware was rinsed thoroughly with acetone in the hood before being cleaned in the sink, (4) all dirty paper towels and disposable gloves were enclosed in plastic storage bags before being thrown out in the garbage, and (5) all chemical waste was stored in four liter glass bottles that were placed in plastic buckets (secondary containment) under the hood. Chemical waste containing benzyl bromide was treated with triethylamine to convert the dangerous benzyl bromide into the innocuous triethylbenzylammonium bromide. Triethylamine was also used to clean up small spills of benzyl bromide.

5. (S)-O-Mesylmalic Anhydride

130 grams (0.61 mol) of (S)-O-mesylmalic acid were dissolved in 260 mL of dry acetone and transferred into a 250 mL pressure equalizing addition funnel which was attached to a one liter, three neck, round bottom flask that was equipped with an overhead stirrer and thermometer and contained 285 mL (1.8 mol) of trifluoroacetic anhydride. A rubber septum was inserted into the addition funnel and wired down, and the reaction vessel was purged with dry argon. The round bottom flask was chilled in an ice-salt bath, and the (S)-O-mesylmalic acid solution was added slowly with stirring under dry argon, keeping the temperature near 0°C. An inert atmosphere was maintained over the reaction mixture by a balloon filled with dry argon. A balloon was slipped over the end of a two inch section of vacuum hose and tied down with a rubber band. A needle adapter was inserted into the other end of the hose and wired down. A one inch 20 gauge needle was screwed into the luer lock and inserted through the septum in the addition funnel. The reaction vessel was purged with dry argon, and then the venting needle was removed. When the balloon was full of dry argon, the argon inlet needle was removed leaving an inert argon atmosphere inside the reaction chamber. The addition of (S)-O-mesylmalic acid took 1 hour, after which the slurry was stirred for another hour at 0°C, then the ice bath was removed, and the slurry allowed to warm to room temperature. The overhead stirrer, thermometer, and addition funnel were replaced by stoppers, and the volatile materials were removed by evaporation. A

light yellow solid remained. The crude anhydride was recrystallized from 200 mL of hot dry acetone. After some crystals had formed, the flask was placed in the freezer overnight. The crystals were isolated by suction filtration, washed twice with 25 mL of chloroform, and dried under parafilm for 15 minutes in the Buchner funnel. Residual trifluoroacetic acid was removed by drying in vacuo at room temperature for 1 hour. 103.0 grams of (S)-O-mesylmalic anhydride were isolated as a light yellow solid (86% yield). A second crop of crystals was obtained by concentrating the dark red filtrate and diluting with some chloroform. After some crystals had formed, the flask was placed in the freezer overnight. Another 4.2 grams of (S)-O-mesylmalic anhydride were isolated (90% yield overall). MP = 94-98°C. Elemental Analysis: Theoretical: 30.9%C, 3.09%H, 16.5%S; Found: 30.9%C, 3.01%H, 16.4%S. $[\alpha]_D^{24} = -31.4^\circ$ (c = 10.2 g/100 mL, tetrahydrofuran). IR Spectrum # 4: 1850 cm^{-1} (shoulder, antisym. C=O stretch); 1780 cm^{-1} (sym. C=O stretch); 1320 cm^{-1} (antisym. SO₂ stretch); 1150 cm^{-1} (sym. SO₂ stretch); KBr pellet. UV Spectrum: $\lambda(\text{max}) = 378 \text{ nm}$, $\epsilon = 2 \text{ L mol}^{-1} \text{ cm}^{-1}$, weak mesylate absorption band, in tetrahydrofuran. 300 MHz ¹H NMR Spectrum # 5: (a) $\delta 3.35$ singlet (3H); (b) $\delta 3.40\text{-}3.75$ doublet of quartets (2H), $|J_{AB}| = 19 \text{ Hz}$, $\Delta\delta_{AB} = 68 \text{ Hz}$, $J_{AX} = 10 \text{ Hz}$, $J_{BX} = -6 \text{ Hz}$; (c) $\delta 5.92\text{-}6.00$ quartet (1H); 5 wt. % solution in deuterated acetone. 70.4 MHz ¹³C{¹H} NMR Spectrum # 4: (a) 169.2, (b) 167.6, (c) 73.6, (d) 39.2, (e) 36.5, 10 wt. % solution in deuterated acetone.

6. Monomethyl (S)-O-Mesylmalates

98.4 grams (0.51 mol) of (S)-O-mesylmalic anhydride were placed into a 250 mL round bottom flask containing a magnetic stir bar. A rubber septum was inserted and wired down. The flask was purged with dry argon. A rubber septum was inserted into a 100 mL graduated cylinder, and distilled methanol was added from a storage flask through a transfer needle under dry argon. The graduated cylinder containing the methanol was tared on a scale, and 16.2 grams (0.51 mol) of methanol were added into the reaction flask through a transfer needle under dry argon with stirring. The reaction was very exothermic. After a few minutes, the slurry was warm and fluid, and the flask was placed into an ice bath to prevent any thermal decomposition. After thirty minutes, the exotherm had subsided, and the ice bath was removed. The viscous reaction mixture was stirred for 6 hours at room temperature and then for 12 hours at 50°C. The ester mixture was a viscous yellow liquid and was stored in the freezer. Elemental Analysis: Theoretical: 31.9%C, 4.42%H, 14.2%S; Found: 32.1%C, 4.47%H, 14.1%S. IR Spectrum # 5: 3600-2400 cm^{-1} (OH stretch), 1720 cm^{-1} (C=O stretch), 1350 cm^{-1} (antisym. SO_2 stretch); 1160 cm^{-1} (sym. SO_2 stretch); thin film on a KBr plate. 300 MHz ^1H NMR Spectrum # 7: (a) δ 3.00-3.13 multiplet (2H); (b) δ 3.19 singlet (3H); (c) δ 3.84 singlet (3H); (d) δ 5.37-5.41 quartet (1H); (e) δ 9.10-9.25 broad singlet (1H); 5 wt. % solution in deuterated chloroform.

7. Monoethyl (S)-O-Mesylmalates

98.4 grams (0.51 mol) of (S)-O-mesylmalic anhydride were placed into a 250 mL round bottom flask containing a magnetic stir bar. 23.4 grams (0.51 mol) of distilled ethanol were added slowly under dry argon from a graduated cylinder as previously described. After thirty minutes, the reaction mixture was fluid and was stirred for 6 hours at room temperature (the slurry became a viscous liquid) and then for 12 hours at 50°C. The ester mixture was a viscous yellow liquid and was stored in the freezer. Elemental Analysis: Theoretical: 35.0%C, 5.00%H, 13.3%S; Found: 34.8%C, 5.02%H, 13.0%S. IR Spectrum # 6: 3600-2400 cm^{-1} (OH stretch); 1720 cm^{-1} (C=O stretch); 1350 cm^{-1} (antisym. SO_2 stretch); 1160 cm^{-1} (sym. SO_2 stretch); thin film on a KBr plate. 300 MHz ^1H NMR Spectrum # 8: (a) δ 1.21-1.40 triplet (3H), $J = 7.0$ Hz; (b) δ 3.04-3.19 multiplet (2H); (c) δ 3.19 singlet (3H); (d) δ 4.28-4.31 quartet (2H), $J = 7.0$ Hz; (e) δ 5.34-5.38 quartet (1H); (f) δ 9.10-9.25 broad singlet (1H); 5 wt. % solution in deuterated chloroform.

8. Monoisopropyl (S)-O-Mesylmalates

85.1 grams (0.44 mol) of (S)-O-mesylmalic anhydride were placed into a 250 mL round bottom flask containing a magnetic stir bar. 26.4 grams (0.44 mol) of distilled isopropanol were added under dry argon from a graduated cylinder as previously described. The alcoholysis was slow. The slurry was stirred for 6 hours at room temperature (some

anhydride crystals were still visible) and then for 12 hours at 50°C. The ester mixture was a viscous yellow liquid and was stored in the freezer. Elemental Analysis: Theoretical: 37.8%C, 5.51%H, 12.6%S; Found: 37.9%C, 5.52%H, 12.3%S. IR Spectrum # 7: 3600-2400 cm^{-1} (OH stretch); 1720 cm^{-1} (C=O stretch); 1350 cm^{-1} (antisym. SO_2 stretch); 1160 cm^{-1} (sym. SO_2 stretch); thin film on a KBr plate. 300 MHz ^1H NMR Spectrum # 9: (a,b) δ 1.28-1.31 doublet of doublets (6H), $J = 3.0$ Hz, diastereotopic methyl groups; (c) δ 2.95-3.10 multiplet (2H); (d) δ 3.19 singlet (3H); (e) δ 5.05-5.20 septet (1H), $J = 6.3$ Hz; (f) δ 5.30-5.34 quartet (1H); (g) δ 9.0-9.15 broad singlet (1H); 5 wt. % solution in deuterated chloroform.

9. Monobenzyl (S)-O-Mesylmalates

105.8 grams (0.55 mol) of (S)-O-mesylmalic anhydride were placed into a 250 mL round bottom flask containing a magnetic stir bar. 59.0 grams (0.55 mol) of distilled benzyl alcohol were added slowly under dry argon from a graduated cylinder as previously described. The mixture was stirred at room temperature for 6 hours (the slurry became a viscous liquid) and then for 12 hours at 50°C. The ester mixture was a viscous yellow liquid and was stored in the freezer. However, on one occasion, when the monobenzyl (S)-O-mesylmalate ester mixture was accidentally stored at room temperature for three days, it had started to crystallize. After five days at room temperature, it had crystallized into a light yellow waxy solid. MP = 46-63°C. The solid monobenzyl

(S)-O-mesylmalate ester mixture was more difficult to use than the liquid mixture in the lactonization reaction, because it was hard to remove from the round bottom flask, and because it dissolved slowly into aqueous sodium bicarbonate. Elemental Analysis: Theoretical: 47.7%C, 4.64%H, 10.6%S; Found: 47.6%C, 4.32%H, 10.4%S. IR Spectrum # 8; 3600-2400 cm^{-1} (OH stretch); 1720 cm^{-1} (C=O stretch); 1350 cm^{-1} (antisym. SO_2 stretch); 1160 cm^{-1} (sym. SO_2 stretch); 730 cm^{-1} and 690 cm^{-1} (phenyl ring bending); thin film on a KBr plate. 300 MHz ^1H NMR Spectrum # 10: (a) δ 3.00-3.14 multiplet ; (b) δ 3.11 singlet (a and b integrated to 5H); (c) δ 5.20-5.25 doublet (2H), $J = 2.4$ Hz; (d) δ 5.38-5.42 quartet (1H); (e) δ 7.35 singlet (5H); (f) δ 9.10-9.22 broad singlet (1H); 5 wt. % solution in deuterated chloroform.

10. Methyl (R)-Malolactonate

50 grams (0.22 mol) of monomethyl (S)-O-mesylmalates were dissolved into 500 mL of distilled water in a one liter beaker with stirring and then slowly neutralized with 20-23 grams (0.24-0.27 mol) of sodium bicarbonate. The final pH was between 7.0 and 7.5. This aqueous solution was added to a two liter, three neck, round bottom flask that was equipped with an overhead stirrer, thermometer, and reflux condenser, heated with a heating mantle, and contained 500 mL of warm methylene chloride. If the aqueous solution was light pink, it was first washed with 250 mL of ether in a one liter separatory funnel to remove the pink impurity before being added to the reaction flask. In

fact, washing the aqueous solution of monomethyl (S)-O-mesylmalates would probably be a good idea regardless of the color of the solution, because it would remove any water insoluble impurities before the lactonization reaction had occurred, and therefore, the crude β -lactone would be formed in higher purity. The mixture was stirred vigorously for 5 hours at 35°C . The reaction mixture was transferred into a one liter separatory funnel, and the organic layer was washed twice with 250 mL of a 5 % (w/v) solution of sodium bicarbonate, twice with 250 mL of distilled water, and once with 250 mL of brine, and dried over anhydrous magnesium sulfate. After 1 hour, the magnesium sulfate was removed by suction filtration and washed with 50 mL of methylene chloride. The filtrate was transferred into a one liter round bottom flask, and the methylene chloride was removed by evaporation. The crude β -lactone was purified by vacuum distillation using a short path still which is shown in Figure 35. 6.6 grams of methyl (R)-malolactonate were collected as a colorless liquid. Typical yields were 5-7 grams. BP = $50-52^{\circ}\text{C}$ (10^{-3} torr). Elemental Analysis: Theoretical: 46.2%C, 4.62%H; Found: 46.1%C, 4.65%H. $[\alpha]_{\text{D}}^{28} = +5.8^{\circ}$ ($c = 10.3$ g/100 mL, tetrahydrofuran). IR Spectrum #9: 1840 cm^{-1} (lactone C=O stretch); 1730 cm^{-1} (ester C=O stretch); 1210 cm^{-1} (C-O stretch); thin film on a KBr plate. 300 MHz ^1H NMR Spectrum # 11: (a) $\delta 3.58-3.90$ doublet of quartets (2H), $|J_{\text{AB}}| = 17$ Hz, $\Delta\delta_{\text{AB}} = 58$ Hz, $J_{\text{AX}} = 6$ Hz, $J_{\text{BX}} = -5$ Hz; (b) $\delta 3.86$ singlet (3H); (c) $\delta 4.87-4.92$ quartet (1H); 5 wt. % solution in deuterated chloroform.

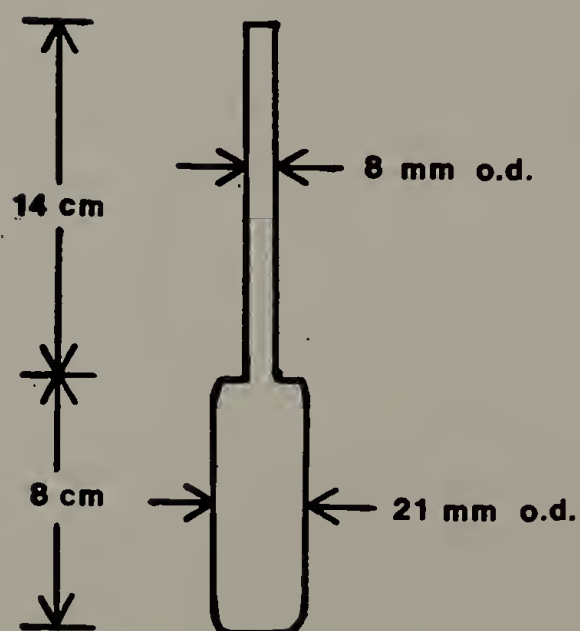
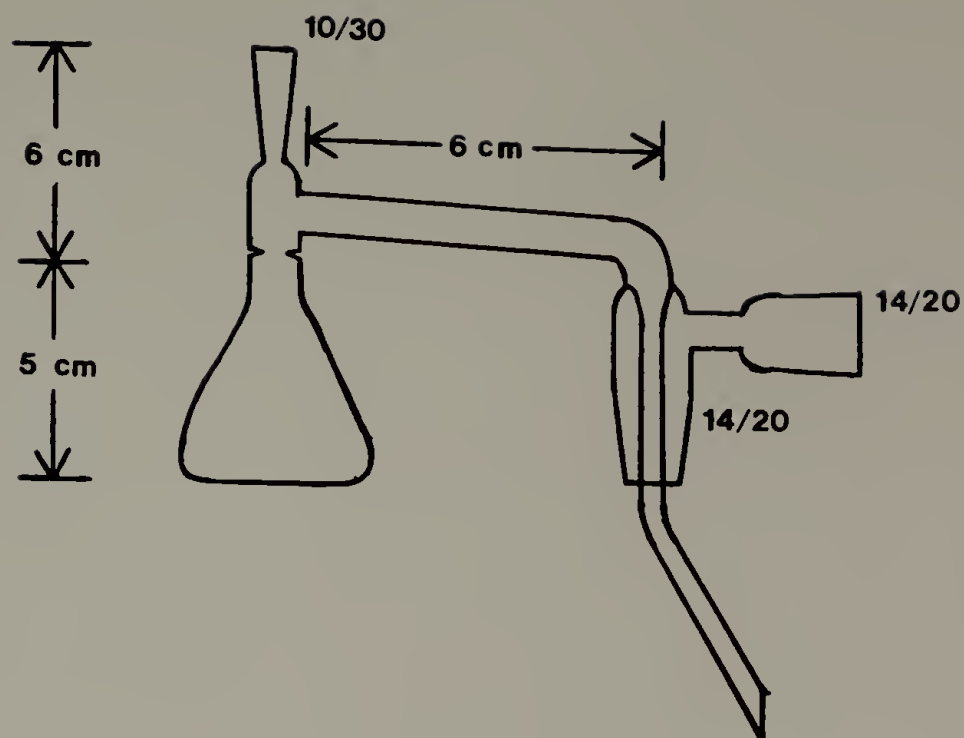


Figure 35. Design of the one piece still used to purify the alkyl malooactonates, and the dimensions of the polymerization tube

11. Ethyl (R)-Malolactonate

Ethyl (R)-malolactonate was prepared by the same method used for the synthesis of methyl (R)-malolactonate. Typical yields were 9-12 grams. BP = 60-62°C (10^{-3} torr). Elemental Analysis: Theoretical: 50.0%C, 5.56%H; Found: 50.0%C, 5.60%H. $[\alpha]_D^{26} = +2.9^\circ$ (c = 10.3 g/100 mL, tetrahydrofuran). IR Spectrum # 11: 1850 cm^{-1} (lactone C=O stretch); 1750 cm^{-1} (ester C=O stretch); 1200 cm^{-1} (C-O stretch); thin film on a KBr plate. 300 MHz ^1H NMR Spectrum # 13: (a) δ 1.36-1.31 triplet (3H), $J = 7.3$ Hz; (b) δ 3.56-3.87 doublet of quartets (2H), $|J_{AB}| = 16$ Hz, $\Delta\delta_{AB} = 63$ Hz, $J_{AX} = 7$ Hz, $J_{BX} = -4$ Hz; (c) δ 4.27-4.34 quartet (2H), $J = 7.1$ Hz; (d) δ 4.85-4.90 quartet (1H); 5 wt. % solution in deuterated chloroform.

12. Isopropyl (R)-Malolactonate

Isopropyl (R)-malolactonate was also prepared by the same method used for the synthesis of methyl (R)-malolactonate except that only about 40-45 grams of monoisopropyl (S)-O-mesylmalates dissolved into 500 mL of distilled water. Upon neutralization with sodium bicarbonate, all 50 grams dissolved. Typical yields were 10-14 grams. BP = 70-72°C (10^{-3} torr). Elemental Analysis: Theoretical: 53.2%C, 6.33%H; Found: 53.4%C, 6.10%H. $[\alpha]_D^{25} = +3.9^\circ$ (c = 10.1 g/100 mL, tetrahydrofuran). IR Spectrum # 13: 1850 cm^{-1} (lactone C=O stretch); 1740 cm^{-1} (ester C=O stretch); 1210 cm^{-1} and 1100 cm^{-1} (C-O stretch); thin film on a KBr

plate. 300 MHz ^1H NMR Spectrum # 15: (a,b) δ 1.30-1.33 doublet of doublets (6H), $J = 3.0$ Hz (some overlap), diastereotopic methyl groups; (c) δ 3.52-3.87 doublet of quartets (2H), $|J_{AB}| = 17$ Hz, $\Delta\delta_{AB} = 67$ Hz, $J_{AX} = 7$ Hz, $J_{BX} = -4$ Hz; (d) δ 4.82-4.86 quartet (1H); (e) δ 5.06-5.21 septet (1H), $J = 6.3$ Hz; 5 wt. % solution in deuterated chloroform.

13. Benzyl (R)-Malolactonate

50 grams (0.17 mol) of monobenzyl (S)-O-mesylmalates were placed into a one liter beaker, and 500 mL of distilled water were added. 20-23 grams (0.24-0.27 mol) of sodium bicarbonate were added slowly with stirring until the monobenzyl S-O-mesylmalates had dissolved completely. The final pH was between 7.0 and 7.5. This solution was added to a two liter, three neck, round bottom flask that was equipped with an overhead stirrer, thermometer, and reflux condenser, heated with a heating mantle, and contained 500 mL of warm spectroscopic grade benzene. If the aqueous solution was light pink, it was first washed with 250 mL of ether in a one liter separatory funnel to remove the pink impurity before being added to the reaction flask. In fact, washing the aqueous solution of benzyl (S)-O-mesylmalates would probably be a good idea regardless of the color of the solution, because it would remove any water insoluble impurities before the lactonization reaction had occurred, and therefore, the crude β -lactone would be formed in higher purity. The mixture was stirred vigorously at 45°C for 4 hours. The reaction mixture was transferred into a one liter separatory funnel, and

the organic layer was washed twice with 250 mL of a 5 % (w/v) solution of sodium bicarbonate, twice with 250 mL of water, once with 250 mL of brine, and dried over anhydrous magnesium sulfate. After 1 hour, the magnesium sulfate was removed by suction filtration and washed with 50 mL of benzene. The filtrate was transferred into a one liter round bottom flask, and the benzene was removed by evaporation. The crude β -lactone was poured into a 600 mL beaker containing a magnetic stir bar and 200 mL of a saturated sodium bicarbonate solution and was stirred for 1 hour. The two phase system was transferred into a 500 mL separatory funnel and washed three times with 150 mL of ether. The ether layers were combined and dried over anhydrous magnesium sulfate. After 1 hour, the magnesium sulfate was removed by suction filtration and washed with 50 mL of ether. The filtrate was transferred into a one liter round bottom flask, and the ether was removed by evaporation. The crude product was purified by vacuum distillation using a short path still. 14.2 grams of benzyl (R)-malolactonate were isolated as a colorless, dense liquid. In fact, the benzyl (R)-malolactonate condensed into small dense droplets which slowly travelled down the still and into the collection flask. Typical yields were 8-14 grams. BP = 100-110°C (10^{-3} torr). Elemental Analysis: Theoretical: 64.1%C, 4.85%H; Found: 63.8%C, 4.90%H. $[\alpha]_D^{25} = +8.2^\circ$ (c = 10.1 g/100 mL, tetrahydrofuran). IR Spectrum # 13: 1850 cm^{-1} (lactone C=O stretch); 1740 cm^{-1} (ester C=O stretch); 1210 cm^{-1} and 1100 cm^{-1} (C-O stretch); thin film on a KBr plate. 300 MHz ^1H NMR Spectrum # 17: (a) δ 3.48-3.77 doublet of quartets (2H), $|J_{AB}| = 17$ Hz, $\Delta\delta_{AB} = 53$ Hz, $J_{AX} = 7$ Hz, $J_{BX} =$

-4 Hz; (b) δ 4.80-4.87 quartet (1H); (c) δ 5.22 singlet (2H); (d) δ 7.33 singlet (5H); 5 wt. % solution in deuterated chloroform. 70.4 MHz $^{13}\text{C}\{^1\text{H}\}$ NMR Spectrum # 5: (a) 168.0, (b) 166.0, (c) 134.6, (d) 128.8, (e) 128.7, (f) 128.5, (g) 67.8, (h) 65.3, (i) 43.4, 10 wt. % solution in deuterated chloroform.

The yield of alkyl (R)-malolactonate depended on how carefully the vacuum distillation was carried out. The alkyl (R)-malolactonates were thoroughly degassed, and the diffusion pump was allowed to pump down on the system for at least one hour before the distillation was attempted. The one piece still was cleaned scrupulously. Before each distillation, the still was soaked in a base bath [5 M KOH in 50 % (v/v) aqueous ethanol] for about 12 hours, rinsed with acetone, dried in the oven, rinsed thoroughly with trimethylchlorosilane, dried in the oven, rinsed with water and then acetone, and dried again in the oven. This treatment gave a clean, unreactive, hydrophobic glass surface so that the alkyl (R)-malolactonate would not decompose or polymerize during the distillation.

14. Polymerization (General Procedure)

Tetraethylammonium benzoate was recrystallized from a mixture of tetrahydrofuran and dimethylformamide and then dissolved in absolute ethanol. A specified volume of this stock solution was injected into a clean, flame-dried polymerization tube which is shown in Figure 35. The tube was attached to a vacuum manifold by a short piece of vacuum hose,

and the ethanol was removed under reduced pressure. The tube was disconnected from the manifold and tared on a scale, and then, freshly distilled alkyl (R)-malolactonate was injected into the tube, and its weight was recorded. A magnetic stir bar was added, the tube was attached to the vacuum line, and the monomer was degassed for about ten minutes. The tube was frozen in a liquid nitrogen bath and sealed with a torch under vacuum. The contents of the tube were allowed to thaw, and then, the tube was wrapped in heavy-duty aluminium foil and placed in an oil bath at 60°C. After 5-7 days, the tube was broken, and the hard white plug of polymer was wrapped in paper towel and crushed into small pieces with a hammer. The polymers were dissolved in refluxing dioxane (c = 1g/10mL), filtered to remove some pieces of glass and paper towel, and added dropwise to a tenfold amount of 50::50 (v/v) ether and hexane for the methyl, ethyl, and isopropyl polymers or to a tenfold amount of ether for the benzyl polymer with stirring. The polymers precipitated as fine white powders and were isolated by suction filtration, washed with ether, and dried in vacuo at room temperature for 4 hours.

15. Tetraethylammonium Benzoate

5.0 grams (20 mmol) of tetraethylammonium benzoate were placed into a one liter Erlenmeyer flask that had a 24/40 ground glass joint top. 360 mL of dry tetrahydrofuran was added, and a rubber septum was inserted and wired down. The flask was flushed out with dry argon, the

suspension was heated with a hot air gun, and 2.0 mL of dry dimethylformamide was injected into the flask with swirling to dissolve the tetraethylammonium benzoate. The flask was placed in the freezer for recrystallization. After four hours, 3.2 grams of tetraethylammonium benzoate were isolated by suction filtration under dry argon (64 % yield). A stock solution was prepared by dissolving 2.77 grams of recrystallized tetraethylammonium benzoate into 50.0 mL of anhydrous ethanol ($c = 55 \text{ mg/mL}$).

16. Poly[methyl (S)-malate]

Elemental Analysis: Theoretical: 46.2%C, 4.62%H; Found: 46.4%C, 4.56%H. $[\alpha]_D^{32} = -16.7^\circ$ ($c = 10.4 \text{ g/100 mL}$, chloroform). IR Spectrum # 10: 1750 cm^{-1} (C=O stretch); 1280 cm^{-1} , 1210 cm^{-1} , and 1160 cm^{-1} (C-O stretch); thin film cast from a chloroform solution onto a KBr plate. 300 MHz ^1H NMR Spectrum # 12: (a) $\delta 2.92\text{--}3.11$ multiplet (2H), $|J_{AB}| = 17 \text{ Hz}$, $\Delta\delta_{AB} = 17 \text{ Hz}$; (b) $\delta 3.77$ singlet (3H), (c) $\delta 5.51\text{--}5.58$ quartet (1H); 5 wt. % solution in deuterated chloroform. 70.4 MHz $^{13}\text{C}\{^1\text{H}\}$ NMR Spectrum # 6: (a) 168.6, (b) 168.0, (c) 68.4, (d) 52.8, (e) 35.5, 10 wt. % solution in deuterated chloroform. Melting range = $156\text{--}119^\circ\text{C}$. Peak melting point = 150°C . Enthalpy of fusion = $9 (+/- 1) \text{ cal/gram}$.

17. Poly[ethyl (S)-malate]

Elemental Analysis: Theoretical: 50.0%C, 5.56%H; Found: 50.2%C, 5.55%H. $[\alpha]_D^{32} = -19.0^\circ$ (c = 10.1 g/100 mL, chloroform). IR Spectrum # 12: 1740 cm^{-1} (C=O stretch); 1280 cm^{-1} , 1190 cm^{-1} , 1150 cm^{-1} , and 1060 cm^{-1} (C-O stretch); thin film cast from a chloroform solution onto a KBr plate. 300 MHz ^1H NMR Spectrum # 14: (a) $\delta 1.25\text{--}1.30$ triplet (3H), $J = 7.2\text{ Hz}$; (b) $\delta 2.90\text{--}3.10$ multiplet (2H), $|J_{AB}| = 17\text{ Hz}$, $\Delta\delta_{AB} = 18\text{ Hz}$; (c) $\delta 4.18\text{--}4.26$ quartet (2H), $J = 7.0\text{ Hz}$; (d) $\delta 5.49\text{--}5.53$ quartet (1H); 5 wt. % solution in deuterated chloroform. 70.4 MHz $^{13}\text{C}\{^1\text{H}\}$ NMR Spectrum # 7: (a) 168.1, (b) 168.0, (c) 68.5, (d) 61.9, (e) 35.5, (f) 14.0, 10 wt. % solution in deuterated chloroform. Melting range = $152\text{--}122^\circ\text{C}$. Peak melting point = 147°C . Enthalpy of fusion = 11 (+/- 1) cal/gram.

18. Poly[isopropyl (S)-malate]

Elemental Analysis: Theoretical: 53.2%C, 6.33%H; Found: 53.1%C, 6.18%H. $[\alpha]_D^{32} = -22.8^\circ$ (c = 10.4 g/100 mL, chloroform). IR Spectrum # 14: 1740 cm^{-1} (C=O stretch); 1280 cm^{-1} , 1200 cm^{-1} , 1160 cm^{-1} , 1100 cm^{-1} , and 1050 cm^{-1} (C-O stretch); thin film cast from a chloroform solution onto a KBr plate. 300 MHz ^1H NMR Spectrum # 16: (a,b) $\delta 1.23\text{--}1.28$ triplet (6H), $J = \text{ca. } 6.0\text{ Hz}$, overlapping diastereotopic methyl groups; (c) $\delta 2.90\text{--}3.08$ multiplet (2H), $|J_{AB}| = 17\text{ Hz}$, $\Delta\delta_{AB} = 15\text{ Hz}$; (d) $\delta 4.98\text{--}5.13$ septet (1H), $J = 6.3\text{ Hz}$; (e) $\delta 5.40\text{--}5.50$ quartet (1H); 5 wt. % solution in deuterated chloroform. 70.4 MHz $^{13}\text{C}\{^1\text{H}\}$ NMR Spectrum # 9:

(a) 168.1, (b) 167.7, (c) 69.9, (d) 68.7, (e) 35.4, (f) 21.62, (g) 21.58, 10 wt. % solution in deuterated chloroform. The diastereotopic methyl groups [(f) and (g)] were resolved and separated by 12 Hz. Melting range = 131-97°C. Peak melting point = 123°C. Enthalpy of fusion = 5 (+/- 1) cal/gram.

19. Poly[benzyl (S)-malate]

Elemental Analysis: Theoretical: 64.1%C, 4.85%H; Found: 63.9%C, 4.92%H. $[\alpha]_D^{29} = +5.7^\circ$ (c = 10.1 g/100 mL, N-methyl pyrrolidinone). IR Spectrum # 16: 1750 cm^{-1} (C=O stretch); 1290 cm^{-1} , 1260 cm^{-1} , and 1060 cm^{-1} (C-O stretch); 740 cm^{-1} and 700 cm^{-1} (phenyl ring bending); KBr pellet. A coherent film could not be cast from a chloroform solution onto a KBr plate. 300 MHz ^1H NMR Spectrum # 18: (a) δ 2.81-2.99 multiplet (2H); (b) δ 5.08-5.09 doublet (2H); (c) δ 5.48-5.52 quartet (1H); (d) δ 7.26 singlet (5H); ca. 1 wt. % solution in deuterated chloroform. Poly[benzyl (S)-malate] was not very soluble in chloroform at room temperature. 50.3 MHz $^{13}\text{C}\{^1\text{H}\}$ NMR Spectrum # 9: (a) 168.0, (b) 167.9, (c) 135.2, (d) 128.7, (e) 128.5, (f) 128.3, (g) 68.6, (h) 67.6, (i) 35.5, ca. 3 wt. % solution in deuterated chloroform at 50°C. The 50.3 MHz carbon-13 NMR spectrum of poly[benzyl (S)-malate] was identical to the 70.4 MHz carbon-13 NMR spectrum #10 of poly[benzyl (R,S)-malate]. Melting range = 198-173°C. Peak melting point = 190°C. Enthalpy of fusion = 13 (+/- 1) cal/gram.

20. Hydrogenolysis of Poly[benzyl (S)-malate]

2.0 grams of poly[benzyl (S)-malate] (9.7 mmol repeating units) were dissolved in 50 mL of spectroscopic grade N-methyl pyrrolidinone with heating in a 100 mL round bottom flask containing a magnetic stir bar. 1.0 gram of 10 wt. % palladium (0.94 mmol palladium) on activated carbon was added, and the round bottom flask was attached to the hydrogenolysis apparatus shown in Figure 36. The suspension was stirred at 40°C, and hydrogen was admitted. After 3 hours, no more hydrogen was being consumed so the palladium catalyst was removed by suction filtration through celite. The filtrate was centrifuged for two hours to remove some fine carbon powder, decanted into a separatory funnel, and then added dropwise into 500 mL of ether with stirring. The polymer was isolated by suction filtration, washed twice with 50 mL of ether, and dried in vacuo at room temperature for 4 hours. 1.1 grams of polymer were isolated (100% yield). However, this sample contained some N-methyl pyrrolidinone. The polymer was washed with ether in a Soxhlet extractor for 24 hours and dried in vacuo at room temperature for 4 hours. The polymer was dissolved in 25 mL of warm methanol and added dropwise into 250 mL of ether with stirring. The polymer was isolated by suction filtration and dried in vacuo at room temperature for 4 hours. 0.70 grams of polymer were isolated (64% yield). Elemental Analysis: Theoretical: 41.4%C, 3.45%H; Found: 41.9%C, 3.91%H. $[\alpha]_D^{21} = -36.7^\circ$ (c = 3.19 g/100 mL, water). IR Spectrum # 18: 3150-2400 cm^{-1} (OH stretch--hydrogen-bonded); 1740 cm^{-1} (ester C=O

stretch); 1600 cm^{-1} (carboxyl C=O stretch--hydrogen bonded); 1270 cm^{-1} and 1190 cm^{-1} (C-O stretch); powdery white film cast from an ethanol solution onto a KBr plate. 300 MHz ^1H NMR Spectrum # 19: (a) $\delta 2.83\text{--}3.05$ multiplet (2H); (b) $\delta 5.25\text{--}5.35$ quartet (1H); 1 wt. % solution in deuterium oxide. Melting range = $182\text{--}147^\circ\text{C}$ with decomposition. Peak melting point = 168°C .

21. Hydrogenolysis Apparatus and its Operation

The hydrogenolysis apparatus is shown in Figure 36. The reaction mixture containing the polymer solution and palladium catalyst was first degassed using an aspirator by closing valves 1 and 2 and opening valves 3, 4, and 5. After about two minutes, degassing was stopped by closing valves 3 and 4. Now, the entire system was perged with hydrogen. Valves 1 and 2 were opened, and hydrogen was admitted and displaced the water in the graduated cylinder. Valve 1 was closed, valve 3 was carefully opened, and hydrogen was removed by the aspirator. The system was perged again with hydrogen. The system was then filled with hydrogen, and valve 1 was closed. The reaction chamber was filled with hydrogen and the catalyst was loaded with hydrogen by opening valve 4. When the consumption of hydrogen stopped, valve 4 was closed, and the system was evacuated and filled again with hydrogen. Valve 1 was closed, valve 4 was opened, valve 5 was vented, and the aspirator was turned off during the hydrogenolysis. The consumption of hydrogen was

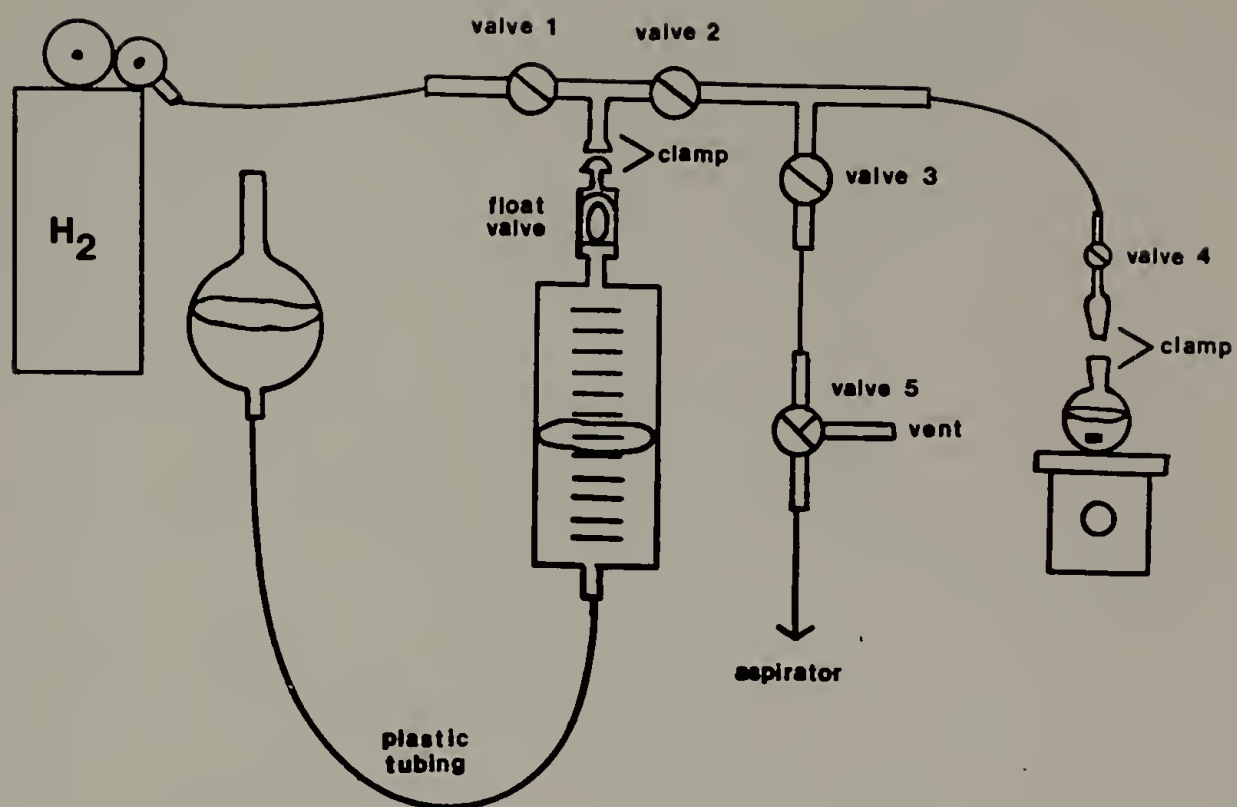


Figure 36. Hydrogenolysis Apparatus

monitored by water displacement. When no more hydrogen was being consumed, the entire system was evacuated using the aspirator. Valve 2 was closed after the water had filled the graduated cylinder. After about two minutes of degassing, the system was vented by turning valve 5, the reaction flask was removed, and the reaction mixture was worked-up. Note: The float valve was incorporated into the design of this hydrogenolysis apparatus so that water would never be accidentally sucked into the reaction flask either by the aspirator or by a reaction left unattended.

22. Copolymerization of Ethyl and Benzyl (R)-Malolactonate

9.20 grams (63.9 mmol) of freshly distilled ethyl (R)-malolactonate and 3.50 grams (17.0 mmol) of freshly distilled benzyl (R)-malolactonate were injected into a polymerization tube containing 10 milligrams (0.040 mmol) of tetraethylammonium benzoate and a magnetic stir bar. The monomer feed composition was 80 mole % ethyl (R)-malolactonate and 20 mole % benzyl (R)-malolactonate. The monomers were mixed on a vortex genie, the tube was attached to a vacuum line, and the mixture was degassed for 10 minutes. The tube was frozen in a liquid nitrogen bath and sealed with a torch under vacuum. The contents of the tube were allowed to thaw, and then, the tube was wrapped in heavy-duty aluminium foil and placed in an oil bath at 60°C. After 7 days, the tube was broken, and the polymer was dissolved in 120 mL of refluxing dioxane, filtered to remove some pieces of glass, and added dropwise into 1200 mL

of 50::50 (v/v) ether and hexane solution with stirring. The polymer was rubbery and adhered to the wall of the beaker. The solvent was decanted off, and the product was dried in vacuo at room temperature for 6 hours. 10.5 grams of copolymer were isolated (83% yield). IR Spectrum # 19: 1750 cm^{-1} (C=O stretch); 1280 cm^{-1} , 1160 cm^{-1} , and 1050 cm^{-1} (C-O stretch); 700 cm^{-1} (phenyl ring bending); thin film cast from a chloroform solution onto a KBr plate. 300 MHz ^1H NMR Spectrum # 20: (a) $\delta 1.15\text{--}1.35$ broad singlet (methyl); (b) $\delta 2.85\text{--}3.12$ broad singlet (methylene of the polymer); (?) $\delta 3.69$ s; (c) $\delta 4.08\text{--}4.30$ broad singlet (methylene of the ethyl group); (d) $\delta 5.09\text{--}5.25$ broad singlet (methylene of the benzyl group); (e) $\delta 5.41\text{--}5.65$ broad singlet (methine); (f) $\delta 7.24\text{--}7.42$ broad singlet (phenyl); 5 wt. % solution in deuterated chloroform. By integrating the methylene groups of the pendent ethyl and benzyl esters, a copolymer composition of 76 mole % ethyl (S)-malate and 24 mole % benzyl (S)-malate was calculated. Note that spin-spin coupling was not observed in the signals of the ethyl group. 70.4 MHz $^{13}\text{C}\{^1\text{H}\}$ NMR Spectrum # 11: (a) 168.1, (b) 168.0, (c) 167.8, (d) 135.2, (e) 128.7, (f) 128.5, (g) 128.2, (h) 68.7, (i) 67.6, (j) 67.1, (k) 61.9, (l) 35.7, (m) 14.0, 10 wt. % solution in deuterated chloroform.

23. Hydrogenolysis of Poly[ethyl-co-benzyl (S)-malate]

5.0 grams of copolymer (7.6 mmol benzyl esters) were dissolved into 50 mL of ethyl acetate and then diluted with 25 mL of 95% ethanol in a 250 mL round bottom flask containing a magnetic stir bar. 2.0 grams of

10 weight % palladium (1.9 mmol palladium) on activated carbon were added. The flask was attached to the hydrogenolysis apparatus, the suspension was stirred, and hydrogen was admitted. After 2 hours, no more hydrogen was being consumed, so the palladium catalyst was removed by suction filtration through celite. The filtrate was centrifuged for one hour to remove some fine carbon powder, decanted into a separatory funnel, and then added dropwise into 750 mL of 50::50 (v/v) ether and hexane with stirring. The polymer was isolated by suction filtration and dried in vacuo at room temperature for 4 hours. 1.9 grams of polymer were isolated as a white powder. IR Spectrum # 20: 3240 cm^{-1} (OH stretch--hydrogen bonded); 1740 cm^{-1} (ester C=O stretch); 1600 cm^{-1} (carboxyl C=O stretch--hydrogen bonded); 1260 cm^{-1} , 1140 cm^{-1} , and 1030 cm^{-1} (C-O stretch); thin film cast from a chloroform solution onto a KBr plate. 300 MHz ^1H NMR Spectrum # 21: (a) δ 1.20-1.32 triplet, $J = 7.2$ Hz (methyl); (b) δ 2.88-3.12 broad singlet (methylene of the polymer); (c) δ 4.15-4.30 quartet, $J = 6.8$ Hz (methylene of the ethyl group); (d) δ 5.45-5.60 broad singlet (methine); (e) δ 6.1-6.4 broad singlet (carboxylic acid); 5 wt. % solution in deuterated chloroform. In contrast to poly[ethyl-co-benzyl (S)-malate], note that spin-spin coupling was observed in the signals of the ethyl group and that the coupling constants were almost the same as those in poly[ethyl (S)-malate]. 70.4 MHz $^{13}\text{C}\{^1\text{H}\}$ NMR Spectrum # 12: (a) 168.3-168.0 broad singlet, (b) 68.7, (c) 61.9, (d) 35.7, (e) 14.0, 10 wt. % solution in deuterated chloroform.

24. (S)-O-Trifluoroacetylmalic Anhydride

10.0 grams (74.6 mmol) of powdered (S)-(-)-malic acid were placed into a 100 mL round bottom flask containing a magnetic stir bar. The flask was chilled in an ice bath, and a 100 mL pressure equalizing addition funnel filled with 40 mL (284 mmol) of trifluoroacetic anhydride was attached. The entire system was purged with dry argon. Trifluoroacetic anhydride was then added rapidly with stirring under dry argon. An inert atmosphere was maintained over the reaction mixture by a balloon filled with dry argon as previously described in the synthesis of (S)-O-mesyilmalic anhydride. The (S)-(-)-malic acid reacted and dissolved into the reaction solution usually within ten minutes, although the dissolution time varied from batch to batch. When the reaction was slow, the ice bath was removed temporarily until the (S)-malic acid started to react and dissolve. It was also observed that the rate of reaction and dissolution increased as the moisture content of the (S)-(-)-malic acid increased. The solution was stirred for one hour at 0°C, after which the volatile materials were removed by evaporation, and a white solid was obtained. The crude anhydride was recrystallized from 160 mL of 50::50 (v/v) hot benzene and chloroform. After some crystals had formed, the flask was placed in the refrigerator overnight. The product was isolated by suction filtration, washed once with 25 mL of cold carbon tetrachloride, and dried under parafilm in the Buchner funnel for 10 minutes. 13.5 grams of (S)-O-trifluoroacetylmalic anhydride were isolated as colorless needle crystals (85 % yield). This

reaction was easily scaled up. MP = 80-83°C. Elemental Analysis: Theoretical: 34.0%C, 1.42%H, 26.9%F; Found: 33.9%C, 1.35%H, 26.3%F. $[\alpha]_D^{24} = -33^\circ$ (c = 13.1 g/100 mL, tetrahydrofuran). IR Spectrum # 17: 1870 cm^{-1} (shoulder, antisym. anhydride C=O stretch and trifluoroacetate C=O stretch); 1800 cm^{-1} (sym. anhydride C=O stretch); 1240 cm^{-1} and 1150 cm^{-1} (C-O and C-F stretches); KBr pellet. 300 MHz ^1H NMR Spectrum # 6: (a) δ 3.53-3.75 doublet of quartets (2H), $|J_{AB}| = 19$ Hz, $\Delta\delta_{AB} = 33$ Hz, $J_{AX} = 11$ Hz, $J_{BX} = -8$ Hz; (b) δ 6.30-6.36 quartet (2H); 5 wt. % solution in deuterated acetone. 121.6 MHz ^{19}F NMR Spectrum: δ 1.30 singlet downfield from an external standard of 0.5 % (v/v) of aqueous trifluoroacetic acid, 5 wt. % solution in deuterated acetone.

25. Optical Purity

Tris[3-(heptafluorobutyryl)-(+)-camphorato]europium(III), $\text{Eu}(\text{hfbc})_3$, was purchased from Aldrich Chemical and used without purification.

A stock solution of $\text{Eu}(\text{hfbc})_3$ was prepared by dissolving 275 milligrams (0.23 mmol) into 4.50 mL of spectroscopic grade carbon tetrachloride and 0.50 mL of deuterated benzene. The deuterated benzene contained 1 % (v/v) tetramethylsilane and was used to spin lock the instrument. Samples were prepared by dissolving either 42 milligrams (0.32 mmol) of methyl malolactonate or 39 milligrams (0.19 mmol) of benzyl malolactonate into 1 mL of this stock solution [55 milligrams (0.046 mmol) of $\text{Eu}(\text{hfbc})_3$]. The procedure was identical for both the

racemic and optically active alkyl malolactonates, and the 300 MHz ^1H NMR spectra were recorded immediately after dissolution. The chiral europium shift reagent, $\text{Eu}(\text{hfbc})_3$, interacted with the racemic methyl malolactonate and split the methyl ester singlet into two distinct peaks separated by 15 Hz, while under the identical conditions, the optically active methyl (R)-malolactonate showed no splitting. Similarly, $\text{Eu}(\text{hfbc})_3$ interacted with the racemic benzyl malolactonate and split the methylene singlet of the benzyl group into two distinct peaks separated by 30 Hz, while under the same conditions, the optically active benzyl (R)-malolactonate showed no splitting. Based on this data, the optically active alkyl (R)-malolactonates were determined to be optically pure within experimental error. The experimental error was calculated from the integration error observed for the racemic monomer and was found to be 3 %. In both racemic mixtures, the upfield signal was identified as the (R) enantiomer by mixing the racemic and optical pure samples together and observing which peak grew in size.

26. Characterization

Melting points were determined on a capillary tube apparatus and were uncorrected. Elemental analyses were performed by the Microanalysis Laboratory of the University of Massachusetts. The specific rotations were measured on a Kern polarimeter at ambient temperature using a sodium lamp and a sample tube of one decimeter. Infrared spectra were recorded on a Perkin-Elmer 1312 spectrometer, and

ultraviolet spectra were recorded on a Beckmann DU-7 spectrometer. 300 MHz ^1H NMR spectra were recorded on a Varian XL-300 using tetramethylsilane as an internal standard. 70.4 MHz $^{13}\text{C}\{^1\text{H}\}$ NMR spectra were also recorded on a Varian XL-300, and 50.3 MHz $^{13}\text{C}\{^1\text{H}\}$ NMR spectra were recorded on a Varian XL-200 using tetramethylsilane as an internal standard. 121.6 MHz ^{19}F NMR spectra were recorded on a Varian XL-300 using 0.5 % (v/v) aqueous trifluoroacetic acid as an external standard. The molecular weights of these polymers were determined by gel permeation chromatography using a Waters Liquid Chromatograph Model 201 with ultrastryagel columns (10^6 , 10^5 , 10^4 , 10^3 , 500 Å) in chloroform at a flow rate of 1.50 mL/min. The chromatograph was calibrated with polystyrene standards. The thermal analysis of the polymers was obtained on a Perkin-Elmer DSC-4 at a heating rate of 20°C/min. The instrument was calibrated with naphthalene and indium. The melting transitions reported in this section were obtained on the first heating scan and were independent of the molecular weight of the polymer.

27. ABX Spin Analysis

All of these malic acid derivatives have an ABX spin system in their proton NMR spectrum (1,2). The AB portion of an ABX spectrum is usually composed of two overlapping quartets as shown in Figure 37. The absolute value of the coupling constant $|J_{AB}|$ is obtained from the doublet spacing in both quartets. The distance between alternate peaks in the quartets is equal to $2D_+$ or $2D_-$ where

$$2D_{+/-} = [\{\Delta\delta_{AB} \pm 1/2(J_{AX} - J_{BX})\}^2 + J_{AB}^2]^{1/2}$$

The distance between the midpoints of the quartets is equal to half the sum of J_{AX} and J_{BX} . The X portion of an ABX spectrum is usually composed of six peaks centered at δ_X as shown in Figure 37. The distance between the two tallest peaks is equal to the sum of J_{AX} and J_{BX} , the distance between the outermost peaks is equal to twice the sum of D_+ and D_- , and the distance between the innermost peaks is equal to twice the difference of D_+ and D_- .

For the compounds with two completely resolved quartets, the values of $|J_{AB}|$, $2D_+$, $2D_-$, and $J_{AX} + J_{BX}$ were calculated from the AB portion of the spectrum. Then, the two equations ($2D_{+/-}$) were solved for the two unknowns $\Delta\delta_{AB}$ and $J_{AX} - J_{BX}$. The values of J_{AX} and J_{BX} were determined by solving the sum and difference of J_{AX} and J_{BX} simultaneously. The values of $|J_{AB}|$ and $\Delta\delta_{AB}$ were confirmed by a decoupling experiment which is described below.

For the compounds with a multiplet in the AB portion of the spectrum, the ABX spectrum was converted into a AB spectrum by decoupling the X proton from the A and B protons, and then, the values of $|J_{AB}|$ and $\Delta\delta_{AB}$ were determined from the resulting AB spectrum. An AB spectrum is composed of four peaks as shown in Figure 37. The inner peaks are more intense than the outer peaks. The $|J_{AB}|$ is obtained from the doublet spacing, and the $\Delta\delta_{AB}$ is obtained from the following equation

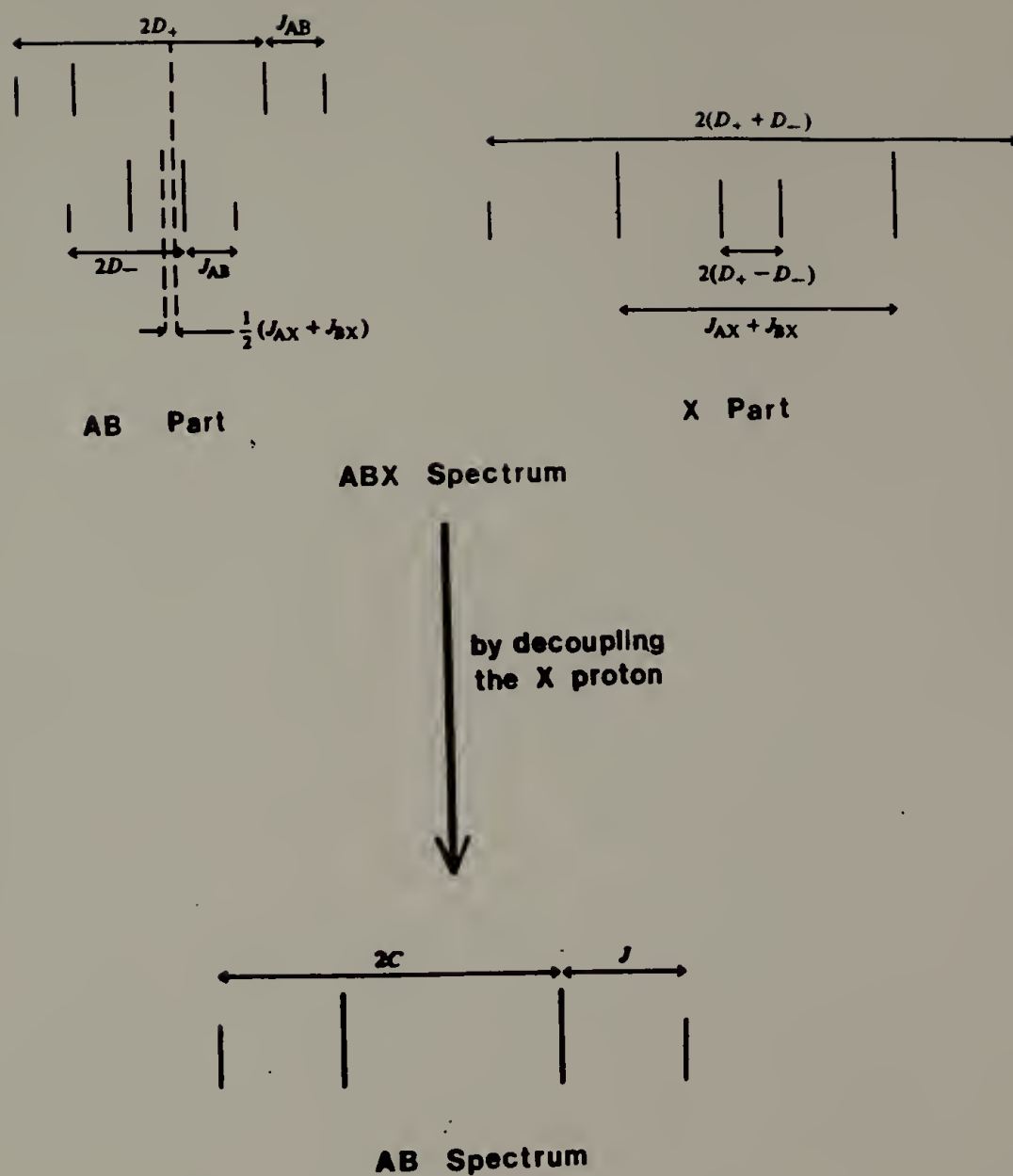


Figure 37. Peak Spacings for an ABX Spectrum and for an AB Spectrum

$$\Delta\delta_{AB} = [(2C)^2 - (J_{AB})^2]^{1/2}$$

where 2C is the distance between alternate peaks in the spectrum.

B. Liposome Work

1. Reagents

Acrylic acid, cholesterol, 4-dimethylaminopyridine, and N-hydroxysuccinimide were purchased from Aldrich Chemical. Acrylic acid was distilled under reduced pressure at 48°C using an aspirator vacuum. Cholesterol was recrystallized from a mixture methanol and chloroform, 4-dimethylaminopyridine was recrystallized from benzene, and N-hydroxysuccinimide was used without purification. Azobisisobutyronitrile was purchased from Fluka Chemical and recrystallized from ether. Dipalmitoylphosphatidylglycerol, dipalmitoylphosphatidylcholine, protein gel filtration standards, and poly(L-glutamate) were purchased from Sigma Chemical and used without purification. Coumarin 4 was purchased from Eastman Kodak and used without purification. Diphtheria toxoid was purchased from the Massachusetts Department of Public Health and purified by dialysis. Dioxane was distilled from sodium benzophenone ketyl under dry argon. Dimethylformamide was stirred over calcium hydride for 12 hours and then distilled from barium oxide under reduced pressure at 45-50°C (ca. 30 torr). Water was distilled from potassium permagnate.

2. Poly(acrylic acid)

30 mL (0.44 mol) of freshly distilled acrylic acid and 130 mL of distilled dioxane were transferred into a 500 mL, three neck, round bottom flask that was equipped with an overhead stirrer, thermometer, and rubber septum. The solution was deoxygenated by bubbling dry argon through the stirred solution and was heated to 55°C with a heating mantle. Then, 55 milligrams (0.34 mmol) of recrystallized azobisisobutyronitrile was dissolved into 1 mL of dioxane and injected into the reaction mixture. The temperature increased because of the polymerization reaction and leveled off at 70°C after about 25 minutes. After 4 hours, the viscous reaction solution was transferred into a 250 mL separatory funnel and added dropwise into 1000 mL of ether with stirring. The supernatant liquid was decanted off, and the polymer was redissolved into 150 mL of methanol, filtered using a Buchner funnel, and added dropwise into 1500 mL of ether with stirring. The polymer was isolated by suction filtration, washed with 100 mL of ether, and dried in vacuo at 70°C for 6 hours. 25 grams of poly(acrylic acid) were obtained (79 % yield). Elemental Analysis: Theoretical: 50.0%C, 5.56%H. Found: 50.0%C, 5.94%H. $\eta_{inh} = 2.2$ dL/g [$c = 0.2$ % (w/v) in dimethylformamide at 30°C]. The molecular weight was determined by gel permeation chromatography in 90 % (v/v) aqueous tetrahydrofuran using polystyrene standards. $M_n = 50,000$, $M_w = 66,000$, $M_w/M_n = 1.3$. The

molecular weight was reduced by using more azobisisobutyronitrile (0.1-1.0 mole percent) as the initiator and/or by using some n-butyl mercaptan (0-1.0 mole percent) as a chain transfer agent.

3. Cholesterol

75 grams (0.19 mol) of cholesterol were dissolved into a mixture of 500 mL of methanol and 150 mL of chloroform with heating. The hot solution was gravity filtered into a one liter Erlenmeyer flask and allowed to stand at room temperature for 2 hours and then at 5°C for another 2 hours. Needle crystals of cholesterol were collected by suction filtration, washed with 50 mL of cold methanol, and dried in vacuo at room temperature for 4 hours. 64 grams of cholesterol were isolated (86 % yield). Elemental Analysis: Theoretical: 83.7%C, 11.9%H. Found: 83.5%C, 12.0%H.

4. 4-Dimethylaminopyridine

100 grams (0.82 mol) of 4-dimethylaminopyridine were dissolved into 300 mL of hot benzene, gravity filtered into a 500 mL Erlenmeyer flask, allowed to stand at room temperature for 2 hours and then at 5°C for another 2 hours. Tan platlets of 4-dimethylaminopyridine were collected by suction filtration, washed with 25 mL of cold benzene, and dried in vacuo at room temperature for 4 hours. 65 grams of product were

isolated (65 % yield). Elemental Analysis: Theoretical: 68.9%C, 8.20%H, 23.0%N. Found: 68.9%C, 8.47%H, 22.7%N.

5. Dicyclohexylcarbodiimide

Dicyclohexylcarbodiimide was vacuum distilled. The middle fraction was collected at 85-87°C (0.01 torr). Dicyclohexylcarbodiimide was stored in the freezer at -15°C under dry argon. Elemental Analysis: 75.7%C, 10.7%H, 13.6%N. Found: 76.1%C, 11.0%H, 13.6%N.

6. Coupling Cholesterol to Poly(acrylic acid)

4.48 grams (62.2 mmol) of poly(acrylic acid), 41.5 milligrams (0.34 mmol) of recrystallized 4-dimethylaminopyridine, and 2.66 grams (6.87 mmol) of recrystallized cholesterol were placed into a 250 mL round bottom flask containing a magnetic stir bar. A rubber septum was inserted and wired down. 75 mL of distilled dimethylformamide were transferred into the flask under dry argon. The mixture was stirred until everything had dissolved. Then, 0.657 grams (3.19 mmol) of distilled dicyclohexylcarbodiimide were dissolved into 10 mL of dimethylformamide and injected into the reaction mixture. Dicyclohexylurea started to crystallize out of solution after 30 minutes. 4-Dimethylaminopyridine was used as a nucleophilic catalyst. After about 24 hours, the solution was chilled in the freezer at -15°C for several hours, and then, the dicyclohexylurea was removed by suction

filtration. The filtercake was washed with 5-10 mL of methanol. The filtrate was transferred into a 250 mL separatory funnel and added dropwise into 900 mL of chloroform with stirring to remove the excess cholesterol. The polymer was isolated by suction filtration, washed with 50 mL of ether, redissolved into 60 mL of methanol, suction filtered using a Buchner funnel, and added dropwise into 700 mL of ether with stirring. The cloudy supernatant liquid was decanted, and the polymer was dried in vacuo at 50°C for 12 hours. 3.40 grams of polymer were isolated. No free cholesterol was detected in the product by thin layer chromatography (silica gel, chloroform). $\eta_{inh} = 2.3 \text{ dL/g}$ [$c = 0.2 \%$ (w/v) in dimethylformamide at 30°C].

7. Coupling Cholesterol to Poly(L-glutamic acid)

1.00 gram of poly(L-glutamate) $M_v = 47,000$ was dissolved into 20 mL of distilled water and slowly acidified with concentrated hydrochloric acid with stirring. Poly(L-glutamic acid) precipitated out of solution as a fine white powder. The final pH was 2.0. The suspension was allowed to agglutinate and settle in the refrigerator. After about 6 hours, the polymer was isolated by suction filtration, washed with 25 mL of methanol, and dried in vacuo at 50°C for 4 hours. 0.84 grams of poly(L-glutamic acid) were obtained.

0.84 grams (6.5 mmol repeating units) of poly(L-glutamic acid), 0.30 grams (0.78 mmol) of recrystallized cholesterol, and 4.5 milligrams (37 μmol) of recrystallized 4-dimethylaminopyridine were placed into a

100 mL round bottom flask containing a magnetic stir bar. A rubber septum was inserted and wired down. 25 mL of distilled dimethylformamide was transferred into the flask under dry argon, and the mixture was stirred until everything had dissolved. Then, 87 milligrams (0.42 mmol) of distilled dicyclohexylcarbodiimide were dissolved into 10 mL of dimethylformamide and injected into the reaction mixture. After 24 hours, the solution was chilled in the freezer for 2 hours and then filtered to remove some small particles of dicyclohexylurea. The filtrate was transferred into a 250 mL separatory funnel and added dropwise into 250 mL of chloroform with stirring. The polymer was allowed to settle overnight and then isolated by suction filtration using a fritted glass funnel and washed with 10 mL of chloroform. The polymer was redissolved into 20 mL of dimethylformamide and added dropwise into 200 mL of ether with stirring. The polymer was allowed to settle, isolated by suction filtration, and dried in vacuo at room temperature for 10 hours. The polymer was dissolved into 20 mL of a 0.50 M sodium hydroxide solution, and the aqueous solution was suction filtered and transferred into cellulose dialysis tubing (molecular weight cutoff = 1000 daltons). The tube was dialyzed against 1800 mL of distilled water pH = 7.0 in a two liter Erlenmeyer flask for three days with stirring. The water was changed twice daily. The aqueous polymer solution (pH = 7.0) was then freeze-dried. 0.62 grams of polymer were isolated. No free cholesterol was detected in the product by thin layer chromatography (silica gel, chloroform).

8. Coupling N-Hydroxysuccinimide and Coumarin 4 to Poly(acrylic acid)

Diphtheria toxoid, a protein antigen, was chemically bound to poly(acrylic acid) by using pendent N-hydroxysuccinimide active esters as the coupling agents. The coupling reaction was carried out in aqueous sodium bicarbonate. The polymer was also labelled with a fluorescent dye, coumarin 4, so that the polymer could be detected in vitro and in vivo at low concentrations by fluorescence spectroscopy. The procedure is shown in Figure 38. N-Hydroxysuccinimide and coumarin 4 were first attached to poly(acrylic acid) using dicyclohexylcarbodiimide as the coupling agent, and then, the resulting copolymer was used to bind the diphtheria toxoid in aqueous solution. This protein coupling method could also be used with different proteins and/or with different polymers.

2.90 grams (40.3 mmol repeating units) of poly(acrylic acid), 1.30 grams (11.3 mmol) of N-hydroxysuccinimide, 94.0 milligrams (0.534 mmol) of coumarin 4, and 15.0 milligrams (0.123 mmol) of 4-dimethylaminopyridine were placed into a 100 mL round bottom flask containing a magnetic stir bar. A rubber septum was inserted and wired down. 30 mL of distilled dimethylformamide were transferred into the flask under dry argon. The mixture was stirred until everything had dissolved. Then, 2.70 grams (13.1 mmol) of distilled dicyclohexylcarbodiimide were dissolved into 10 mL of dimethylformamide and injected into the reaction mixture. Dicyclohexylurea started to crystallize out of solution after 3 minutes. After 20 hours, the

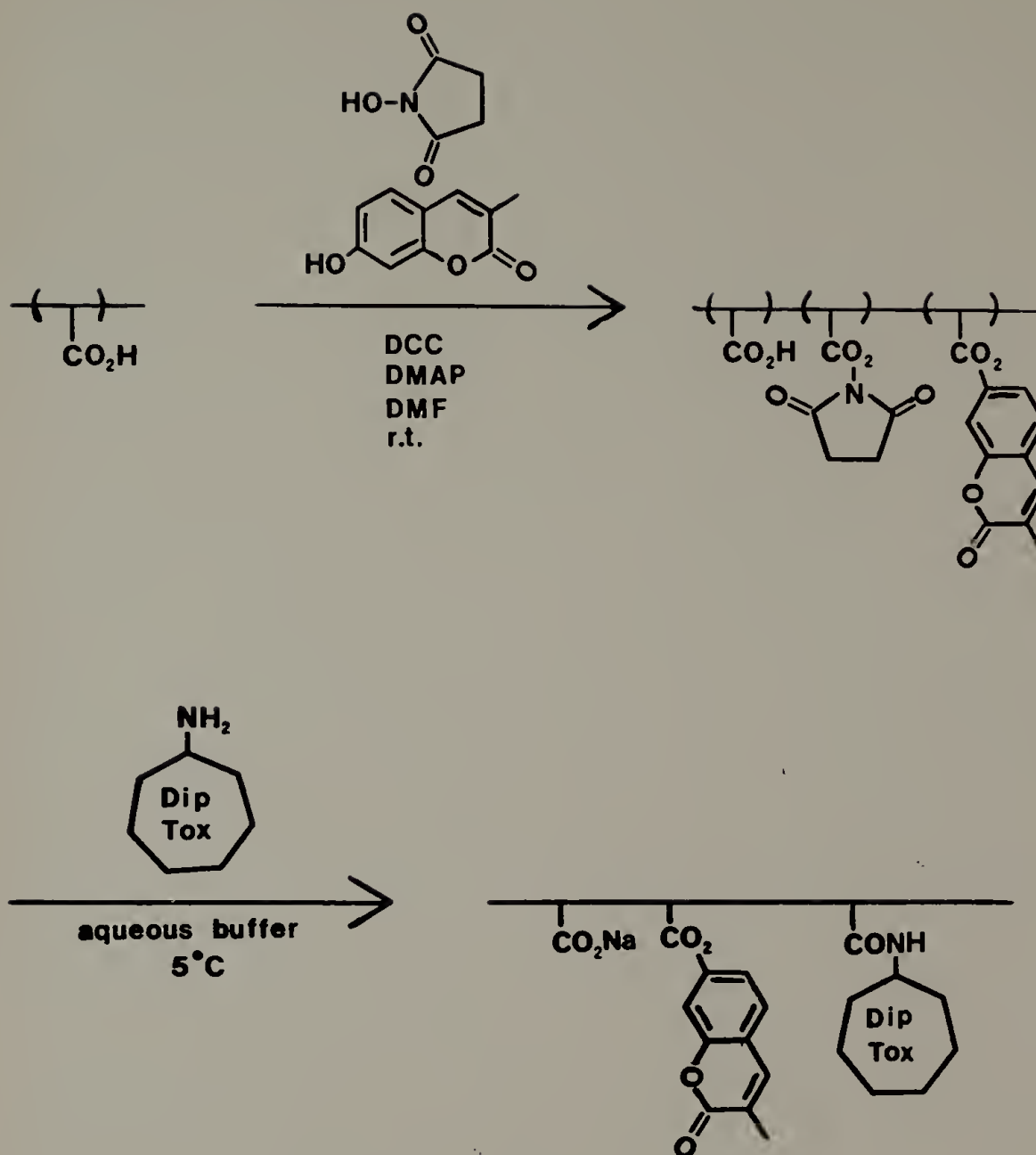


Figure 38. Coupling Diptheria Toxoid to Poly[acrylic acid] Bearing Pendent N-Hydroxysuccinimide Active Esters

reaction mixture was chilled in the freezer for five hours, and then, the dicyclohexylurea was removed by suction filtration. The filtercake was washed with 5 mL of dimethylformamide, and the filtrate was added dropwise into 360 mL of chloroform with stirring. The dicyclohexylurea was washed with 50 mL of ether and dried in vacuo at room temperature for 6 hours. 2.90 grams (12.9 mmol) of dicyclohexylurea were isolated. The polymer was allowed to settle, isolated by suction filtration, and washed with 20 mL of ether. The polymer was redissolved into 30 mL of dimethylformamide and added dropwise into 300 mL of ether with stirring. The polymer was isolated by suction filtration and dried in vacuo at room temperature for 6 hours. 2.00 grams of copolymer were isolated. The copolymer dissolved into aqueous sodium bicarbonate, and the resulting solution fluoresced blue under ultraviolet radiation from a small ultraviolet lamp ($\lambda = 366$ nm). Elemental Analysis (neglecting the coumarin dye and polymer fractionation): Theoretical: 49.8% C, 4.69% H, 5.04% N; Found: 50.5% C, 6.41% H, 5.77% N.

9. Coupling Diptheria Toxoid to Poly(acrylic acid)

The commercial diptheria toxoid solution was transferred into cellulose dialysis tubing and dialyzed against a 0.10 M sodium bicarbonate solution for 24 hours (molecular weight cutoff = 10,000). The buffer was changed every 6-8 hours. The concentration of the diptheria toxoid was determined by ultraviolet spectroscopy. The

absorbance at 280 nm was equal to the concentration of the protein in milligrams per mL.

139 milligrams of copolymer ($M_n = 7,000$) and 140 milligrams of sodium bicarbonate were dissolved into 4.0 mL of a 0.10 M sodium bicarbonate solution with stirring. 1.0 mL of purified diphtheria toxoid solution ($c = 3.0$ mg/mL, molecular weight = 62,000) was added to the reaction mixture and stirred at 5°C for 6 hours. Then, the reaction mixture was transferred into cellulose dialysis tubing (molecular weight cutoff = 1000 daltons) and dialyzed against one liter of a 0.10 M sodium bicarbonate solution for 24 hours with stirring. The buffer was changed every 6-8 hours. 1.0 mL of the dialyzed reaction mixture was passed over a Sepharose CL-6B column (diameter = 1 cm, length = 30 cm) that had been calibrated with protein standards. 0.50 mL elution fractions were collected, and their fluorescence was measured. The fluorescence spectra were recorded on a Perkin Elmer Model 650-10S Fluorescence Spectrophotometer at a scanning rate of 30 nm/min ($\lambda_{\text{ex}} = 290$ nm, $\lambda_{\text{em}} = 350-520$ nm). The protein-polymer complex was detected just after the void volume of the column by plotting the fluorescence intensity versus elution volume (molecular weight = 400,000).

Diphtheria toxoid might also be attached to a polymer in an inert organic solvent using dicyclohexylcarbodiimide as the coupling agent if the diphtheria toxoid could be freeze-dried and dissolved into the inert organic solvent without the loss of any biological activity. This procedure may work better than the aqueous coupling of the diphtheria toxoid to a polyacid bearing pendent N-hydroxysuccinimide active esters.

10. Liposome Preparation

10.0 grams of phospholipid were placed into a 25 mL round bottom flask and dissolved into 1 mL of chloroform. The chloroform was removed on the rotary evaporator leaving behind a thin lipid film. The lipid film was dried in vacuo for 30 minutes to remove any residual chloroform. 10.0 mL of polymer solution, prepared by dissolving 10.0 milligrams of polymer into 10.0 mL of 50 mM Tris-HCl buffer pH = 8.00, were added. If the polymer was insoluble in the buffer solution, then it was added with the phospholipid and incorporated into the lipid-polymer film. The lipid film usually peeled away from the wall of the flask and floated around in the polymer solution. The lipid suspension was placed into a hot water bath until the temperature was 55°C, and then, it was mixed on a vortex genie for 2 minutes. The lipid suspensions were allowed to cool down to room temperature before analysis.

11. High-Sensitivity Differential Scanning Calorimetry

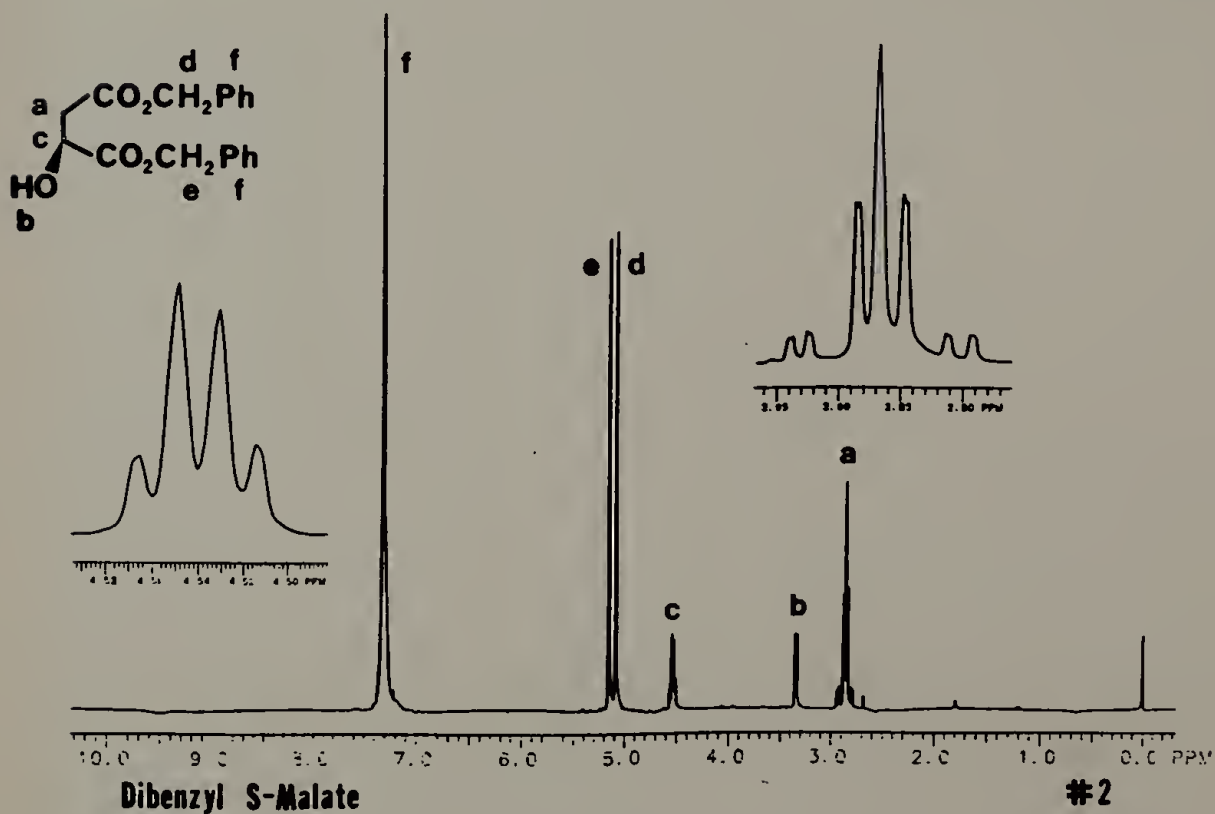
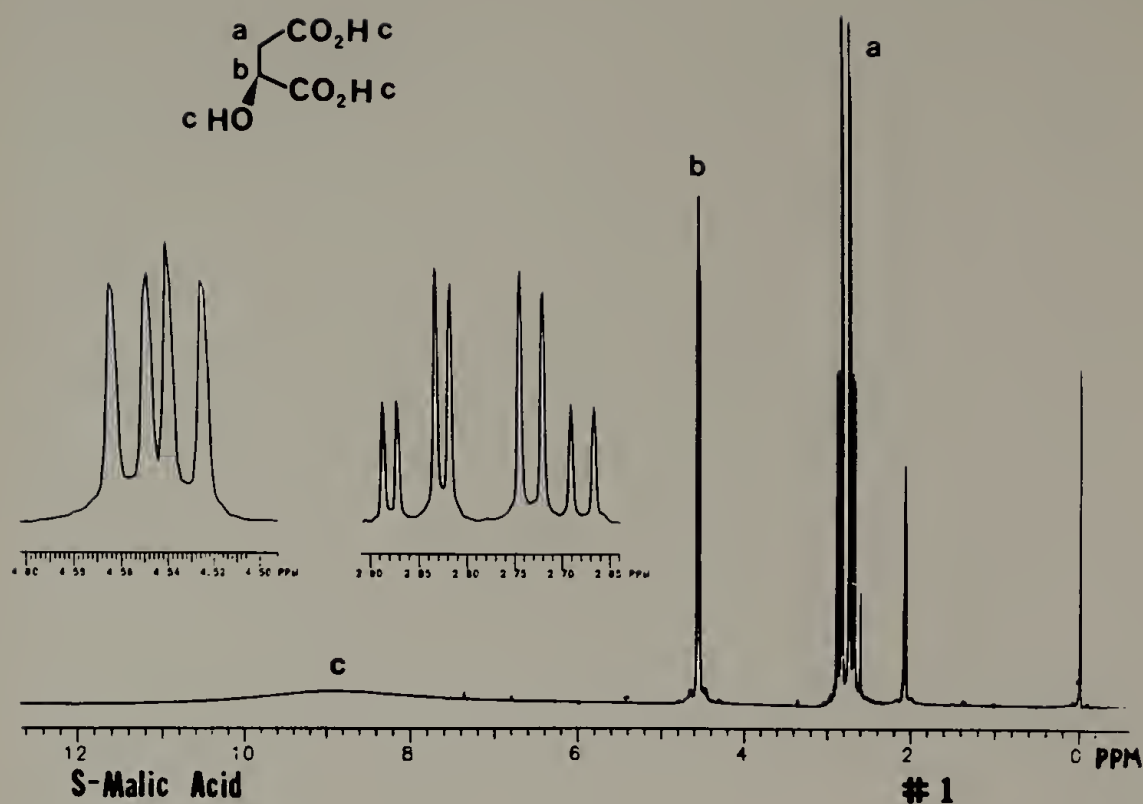
Calorimetric scans were recorded on a Microcal MC-1 scanning microcalorimeter at a heating rate of 15°C per hour. Samples were degassed for 10 minutes before being injected into the calorimeter. The lipid and polymer concentrations were always 1.0 milligram per mL. The pH was changed by the addition of concentrated hydrochloric acid. The lipid suspensions were reheated on a hot water bath to 55°C and remixed

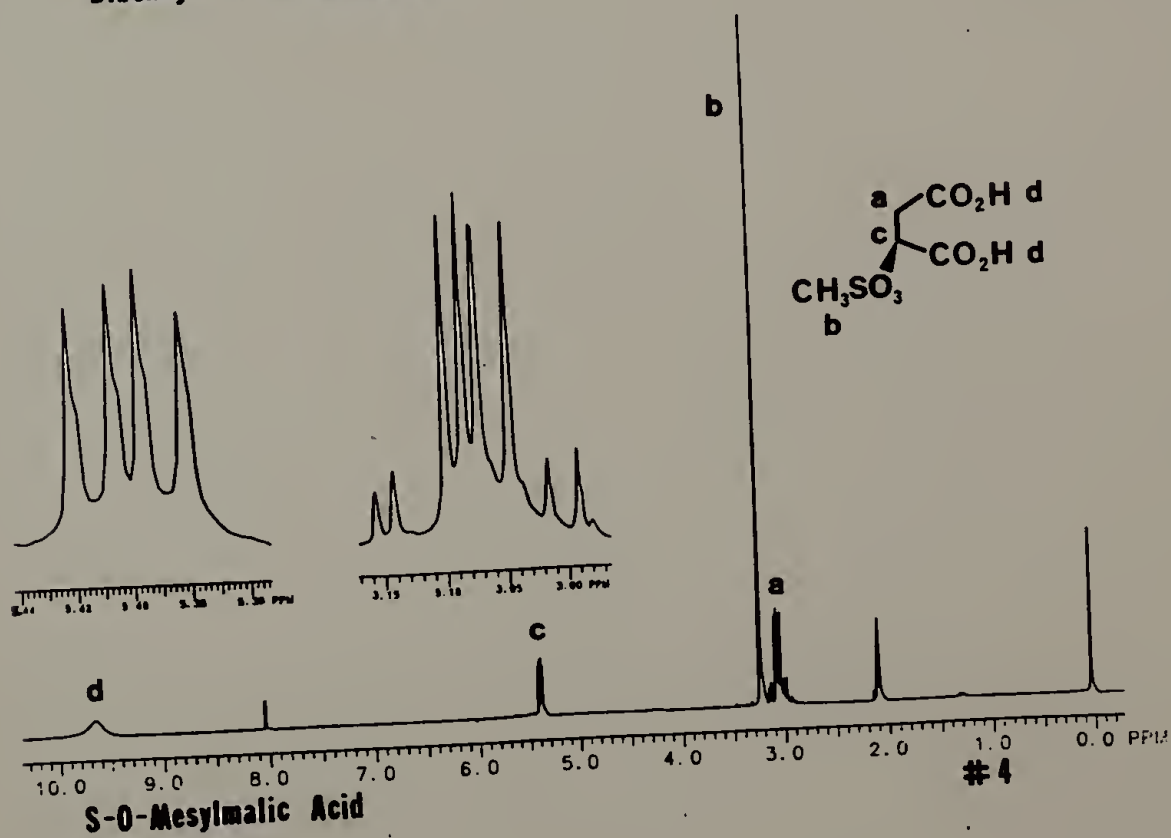
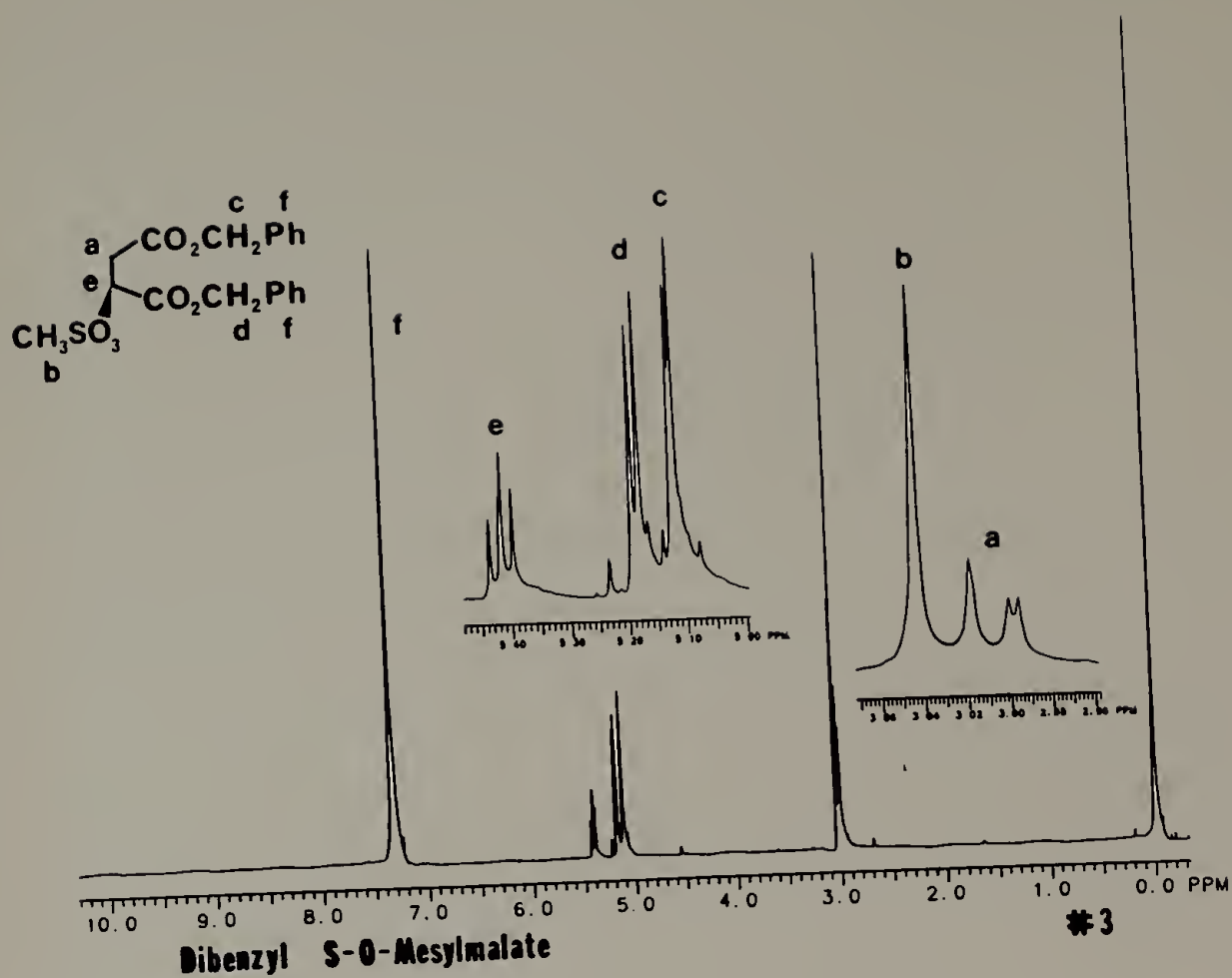
on a vortex genie for 2 minutes after the addition of concentrated hydrochloric acid. The lipid suspensions were allowed to cool down to room temperature, and the pH was measured again.

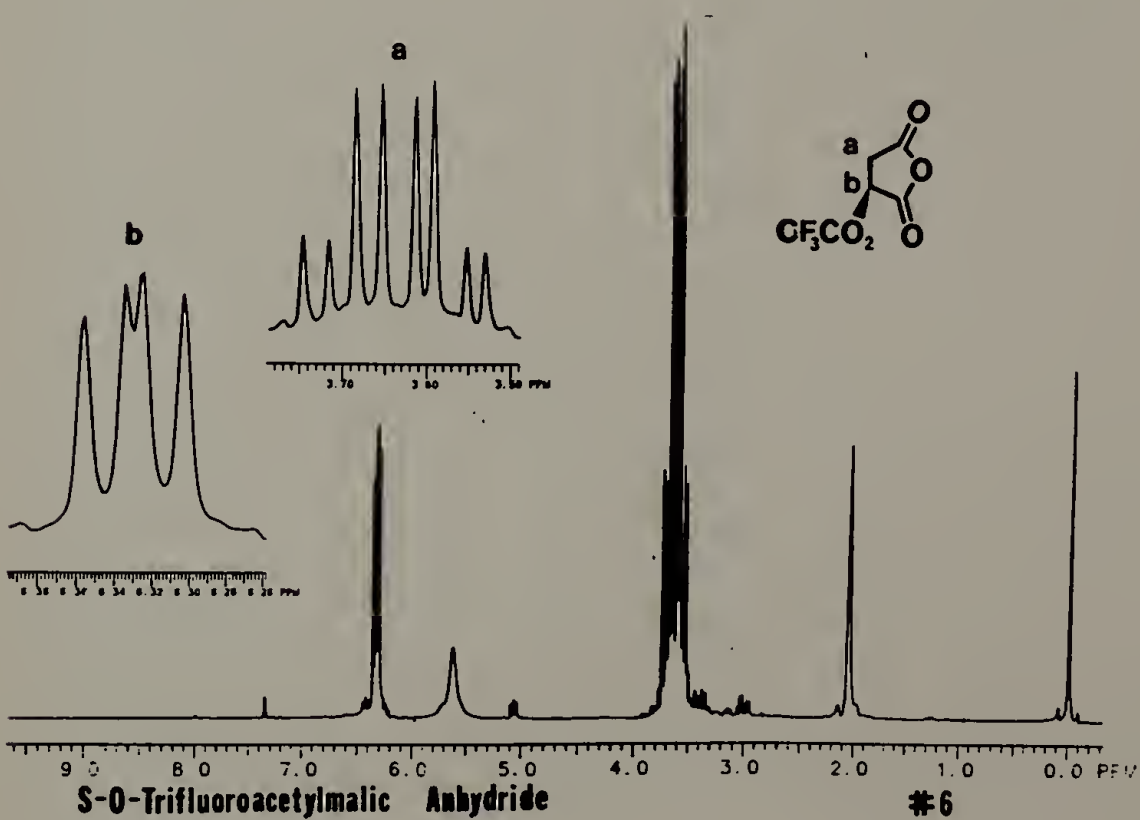
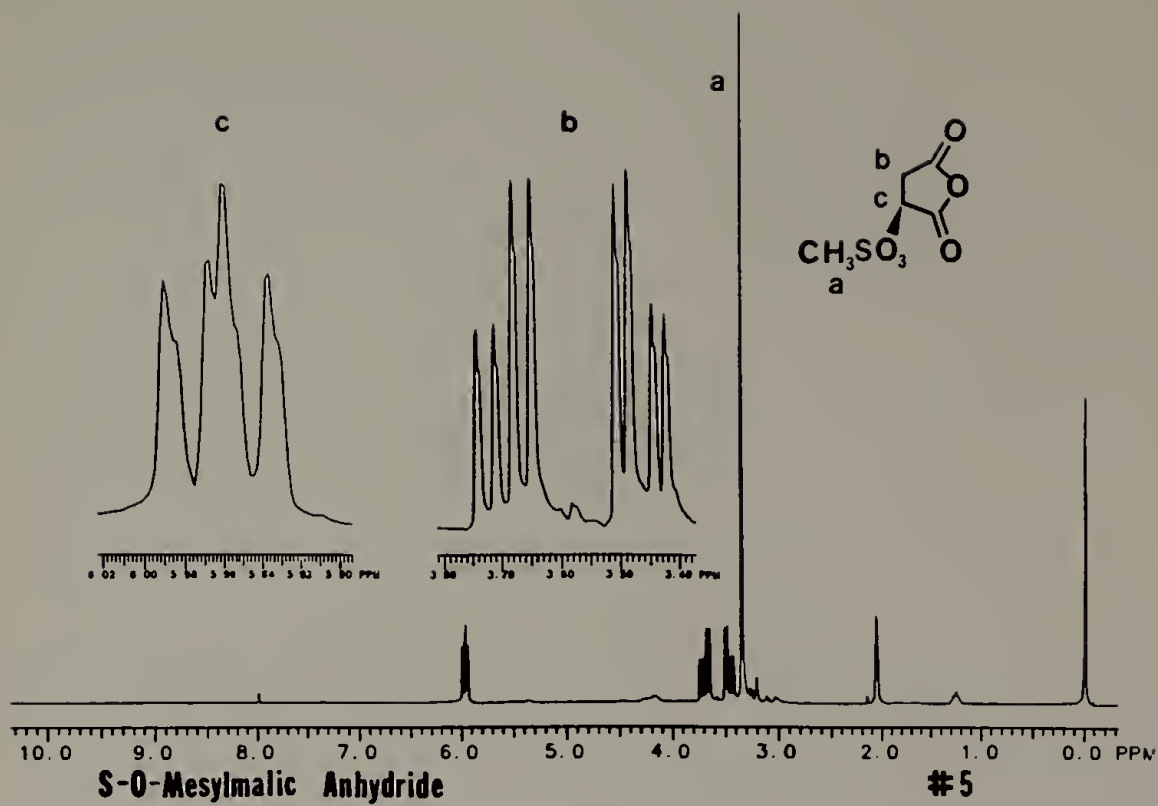
Footnotes

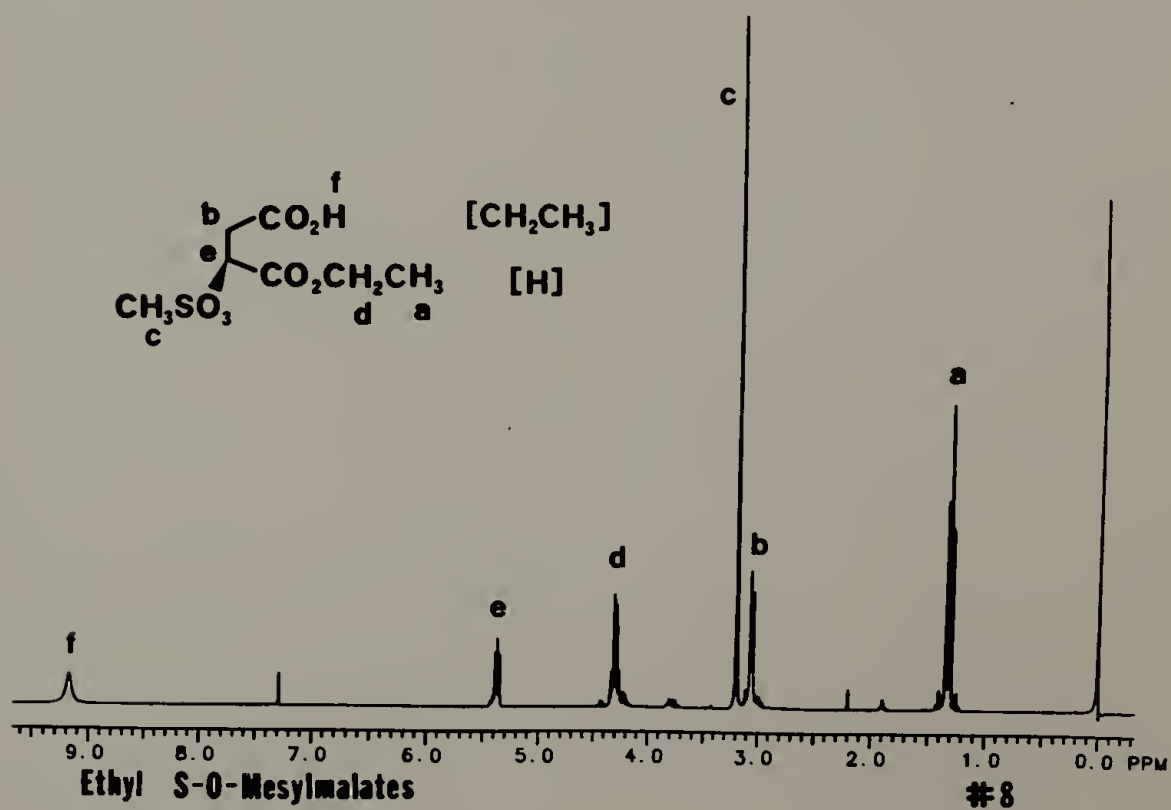
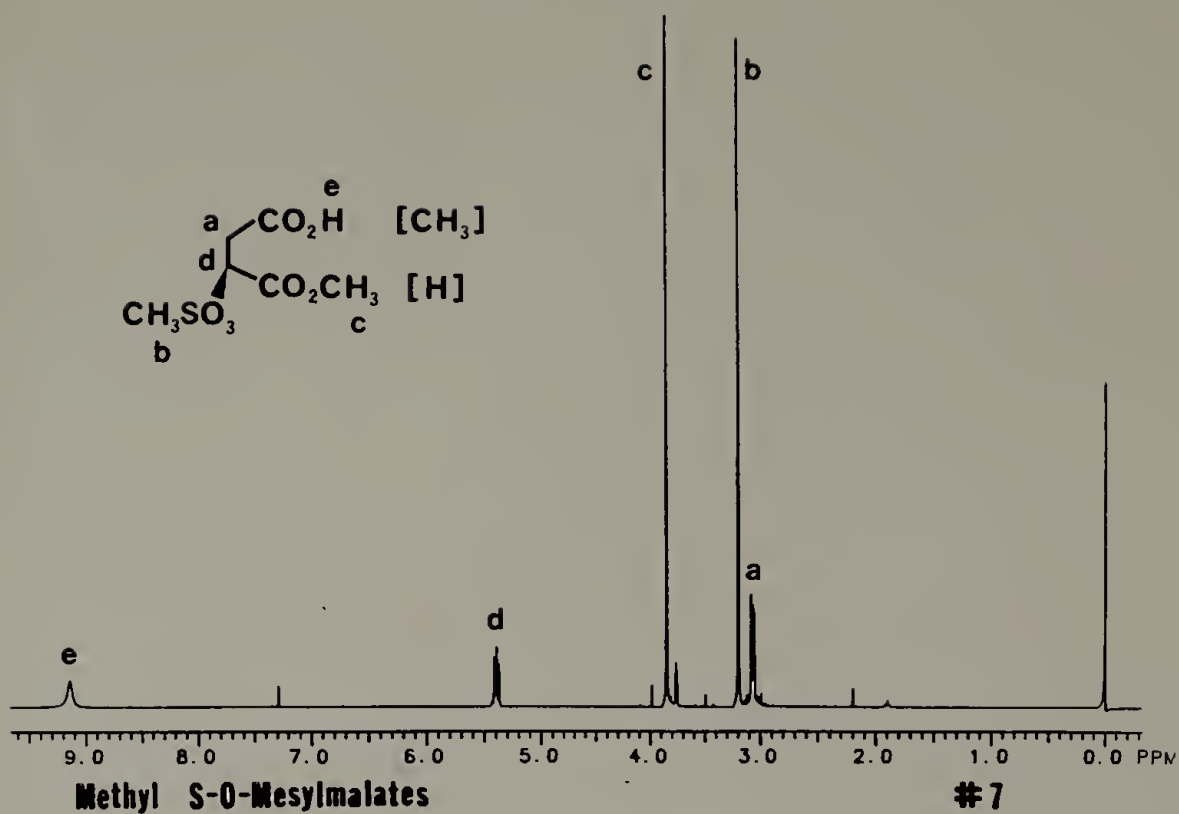
1. J.B. Lambert, H.F. Shurvell, L. Verbit, R.G. Cooks, and G.H. Stout, Organic Structural Analysis, MacMillan Publ. Comp., N.Y., 1976. Equation 4-24 in Chapter 4 is missing a squared sign. J_{AB} should be J_{AB}^2 in the formula.
2. E.W. Garbisch, J. Chem. Ed. 45 311, 402, and 480 (1968).

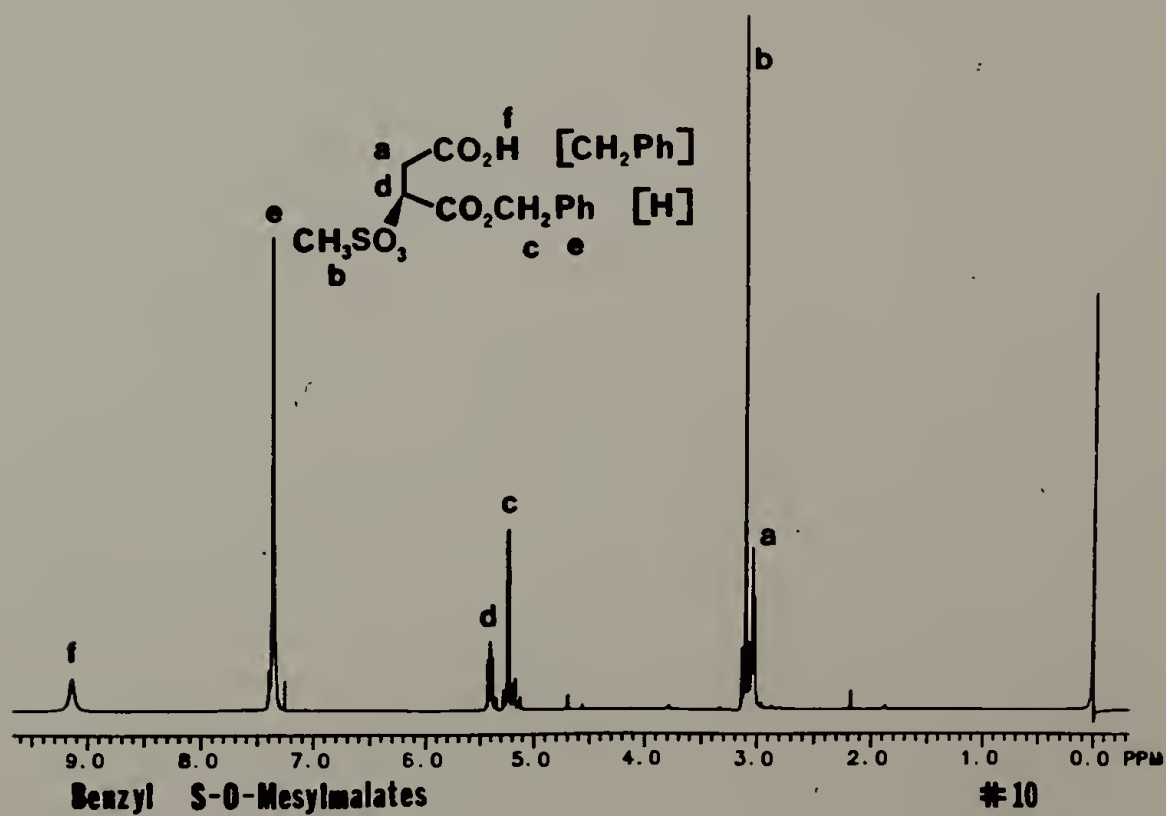
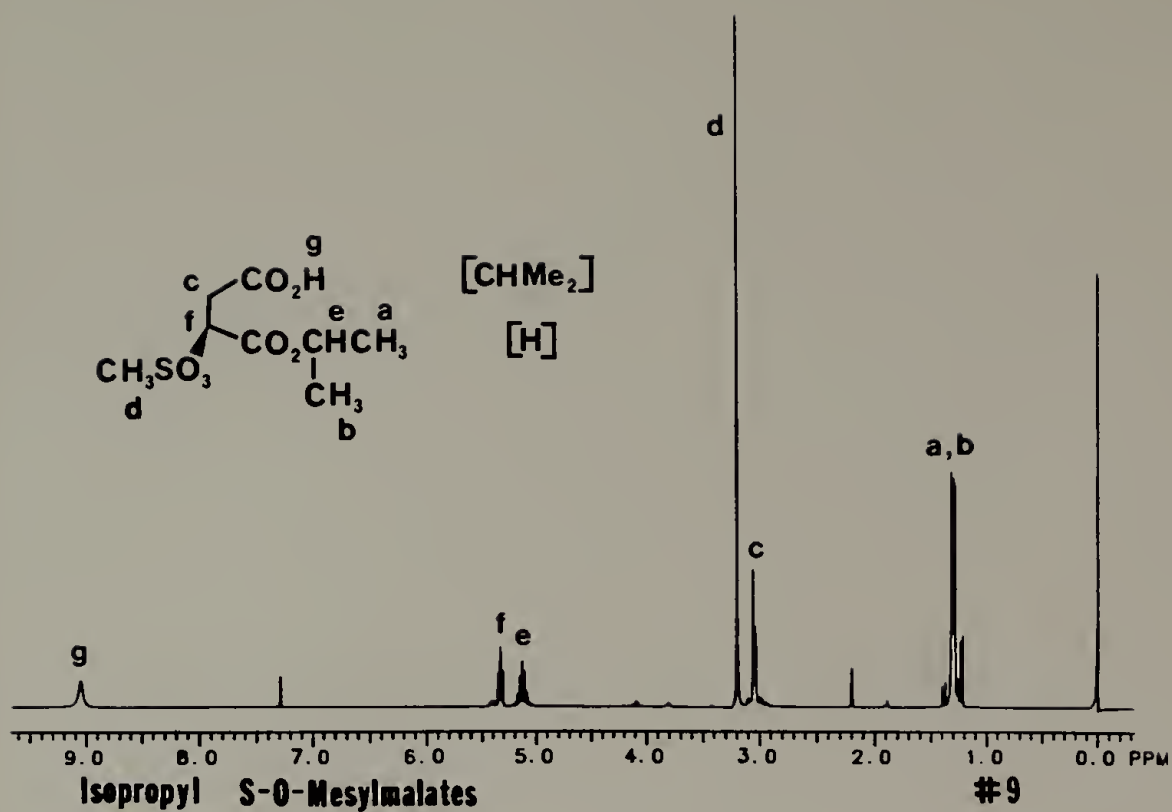
APPENDIX A
PROTON NUCLEAR MAGNETIC RESONANCE SPECTRA

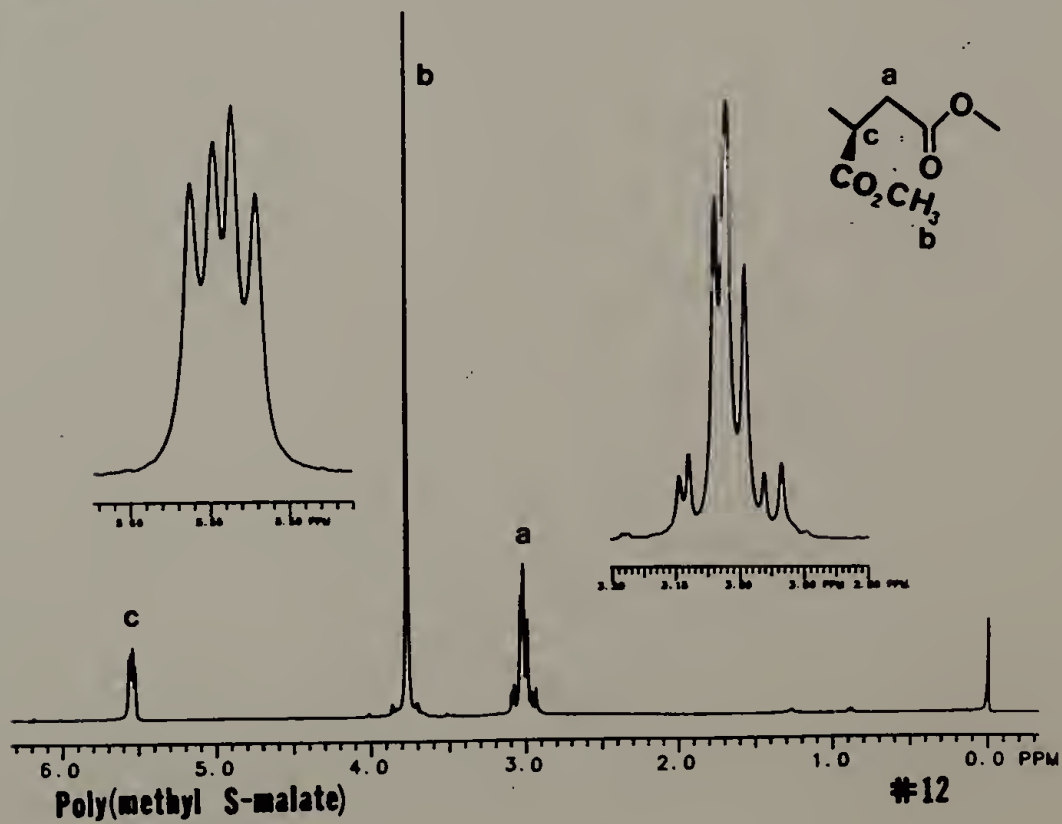
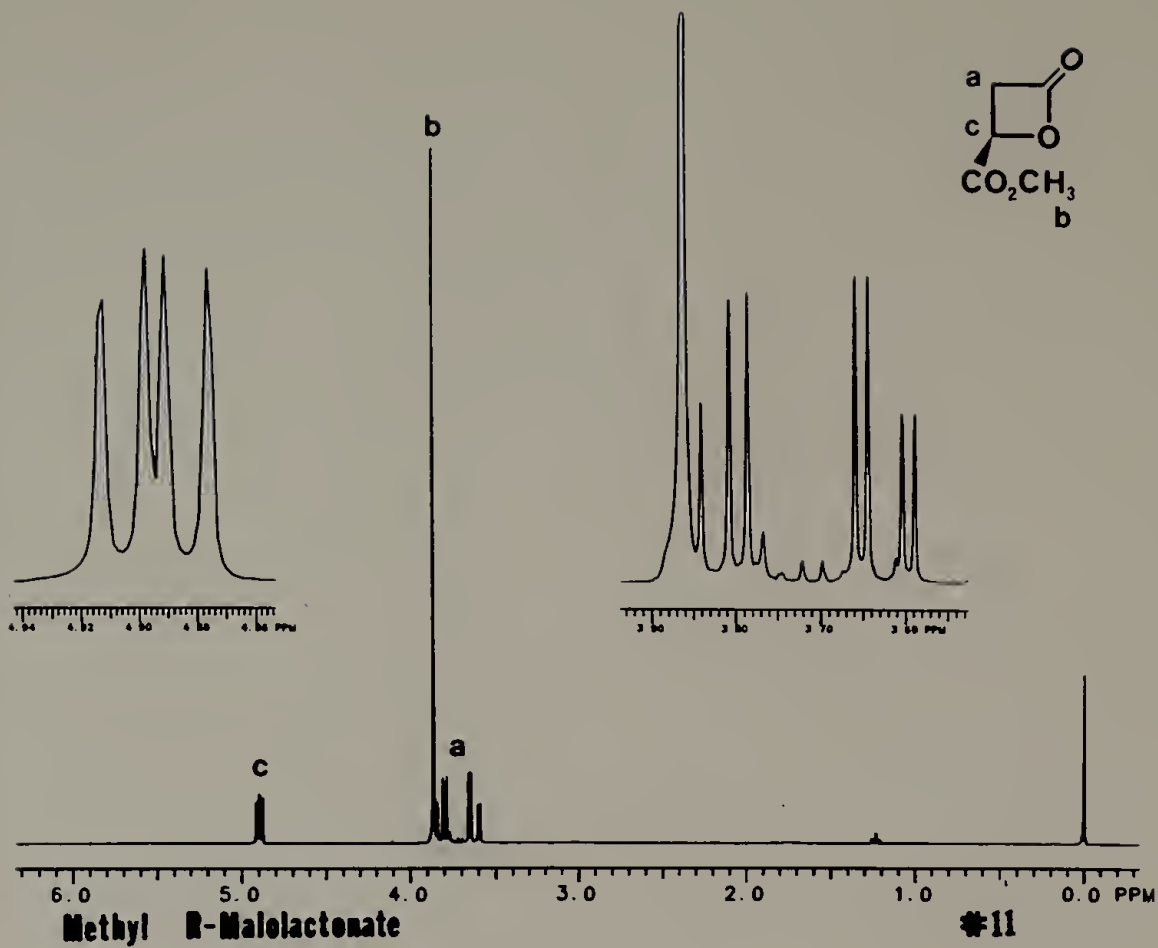


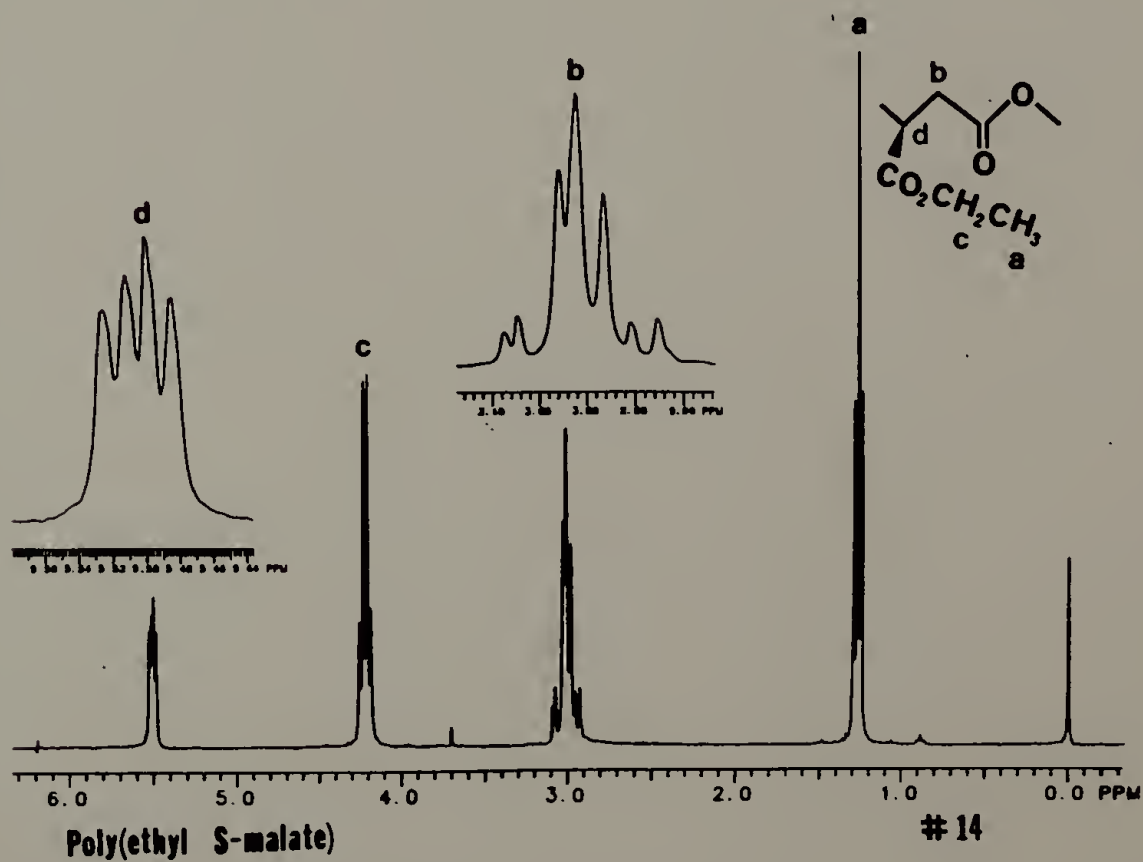
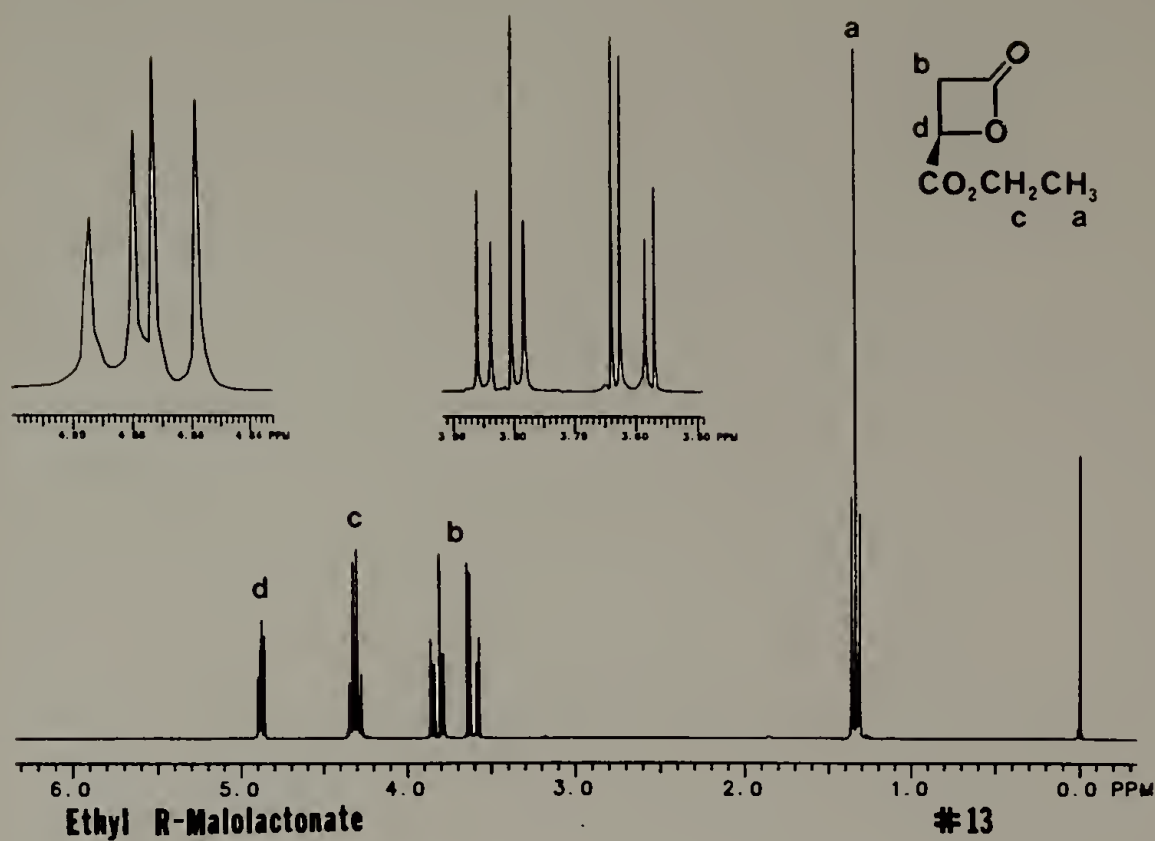


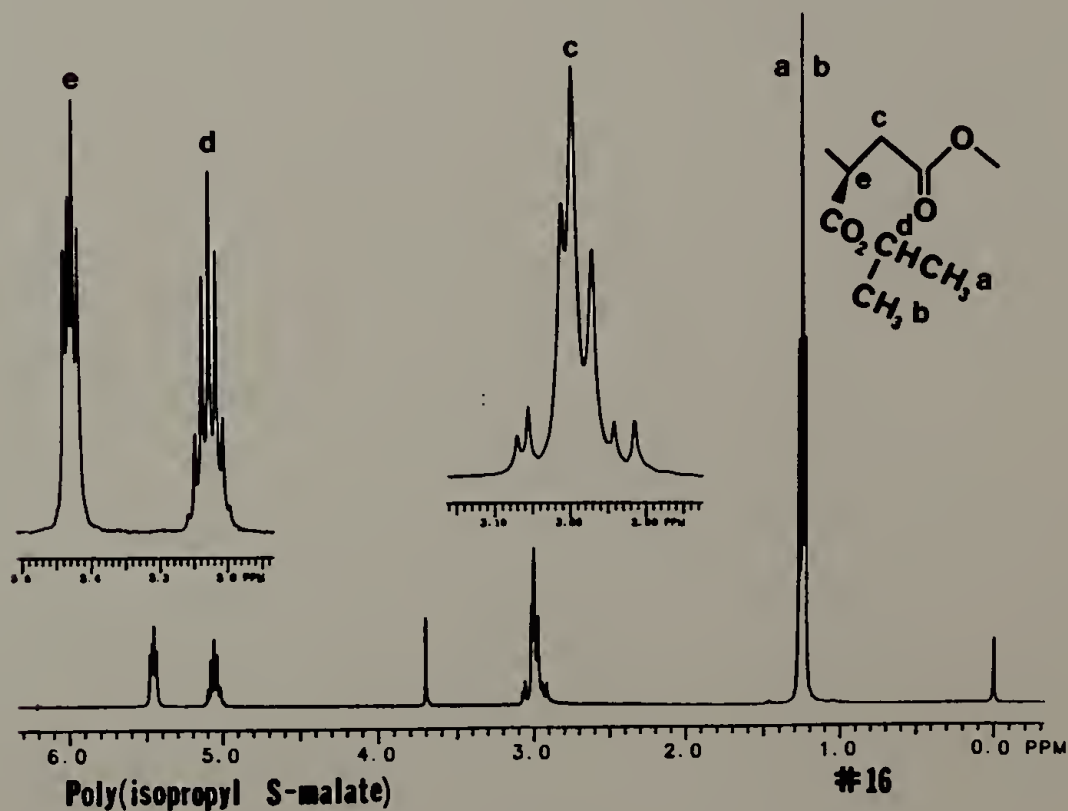
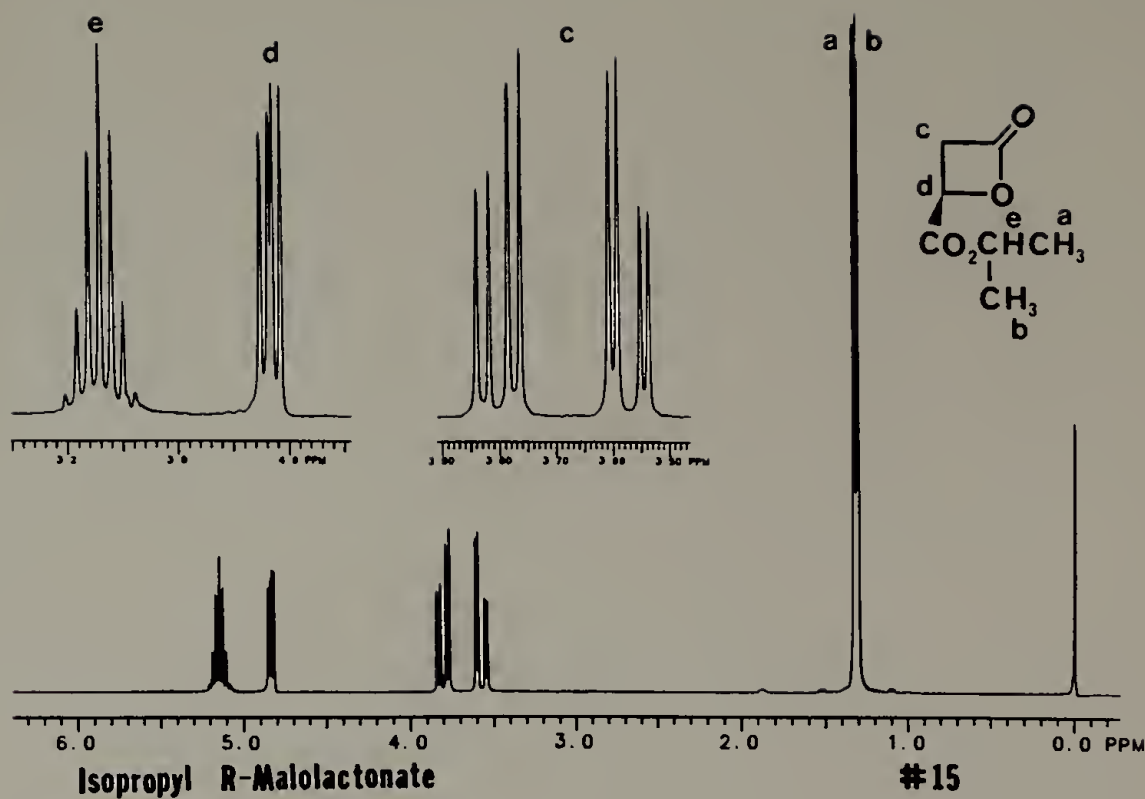


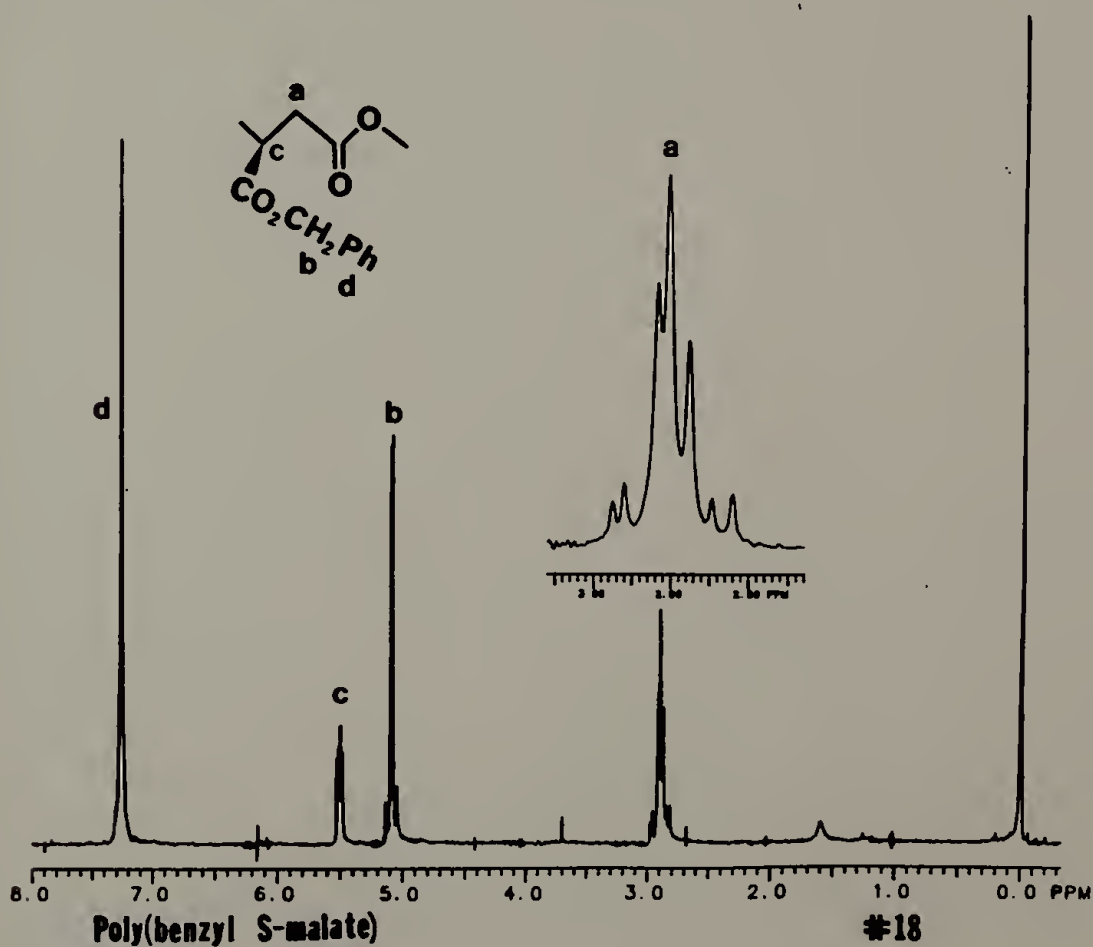
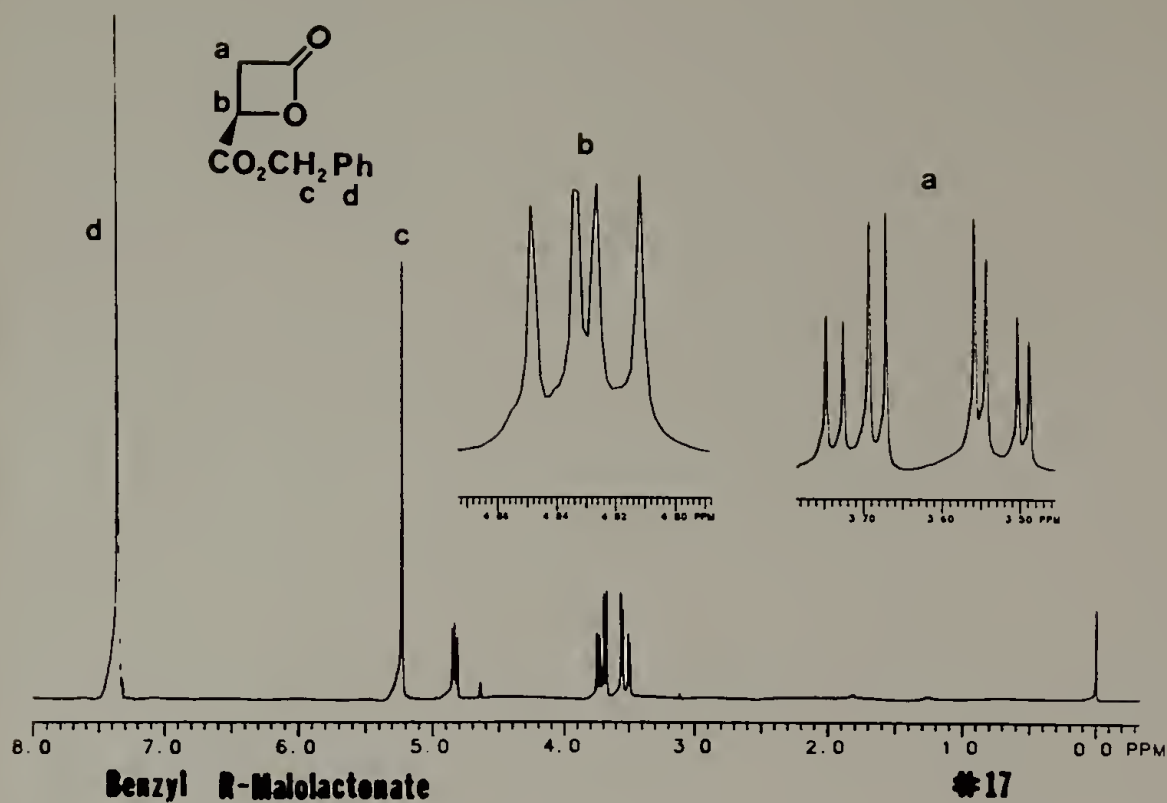


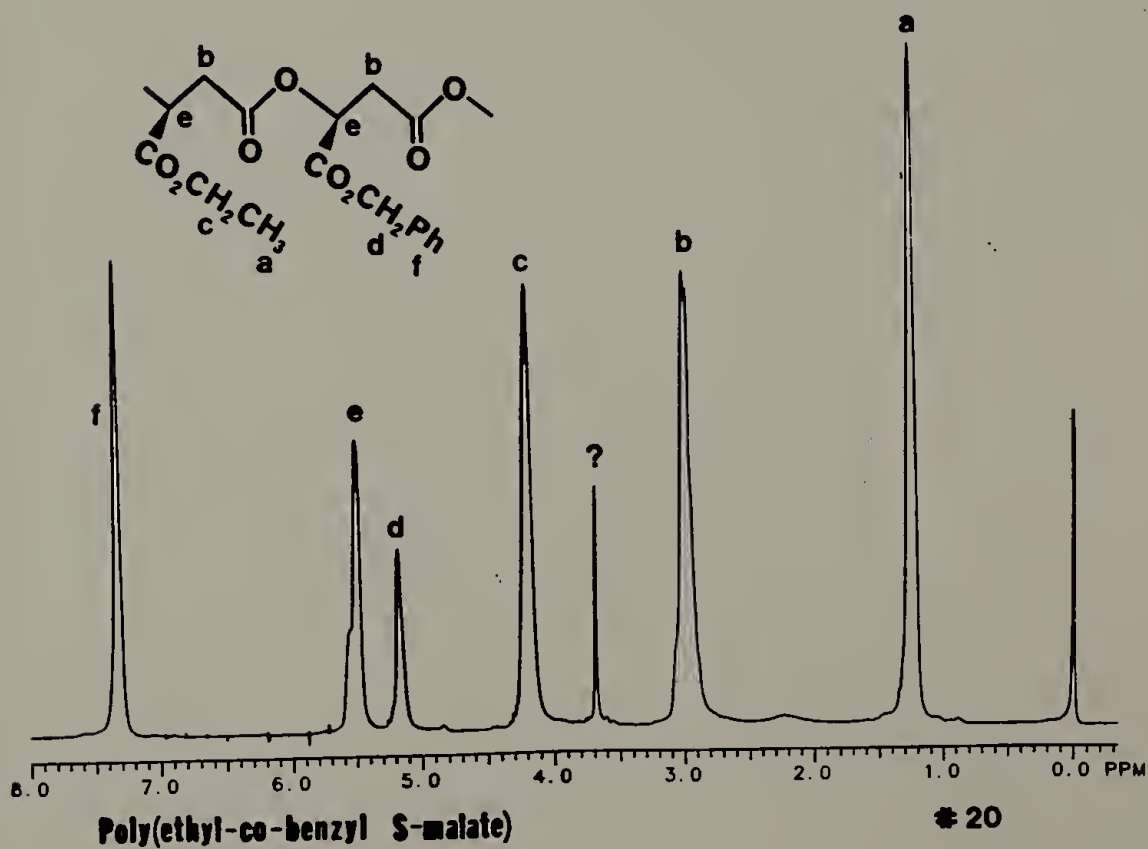
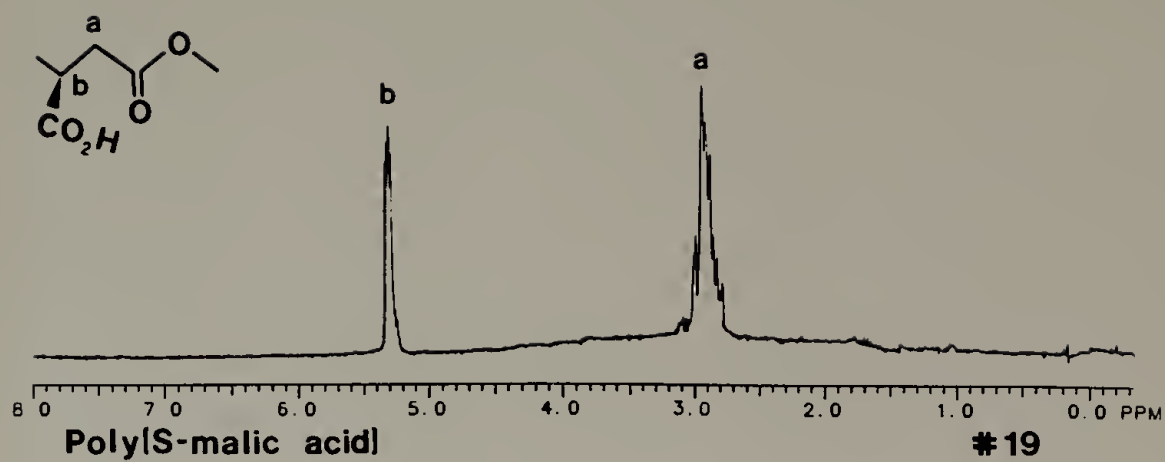


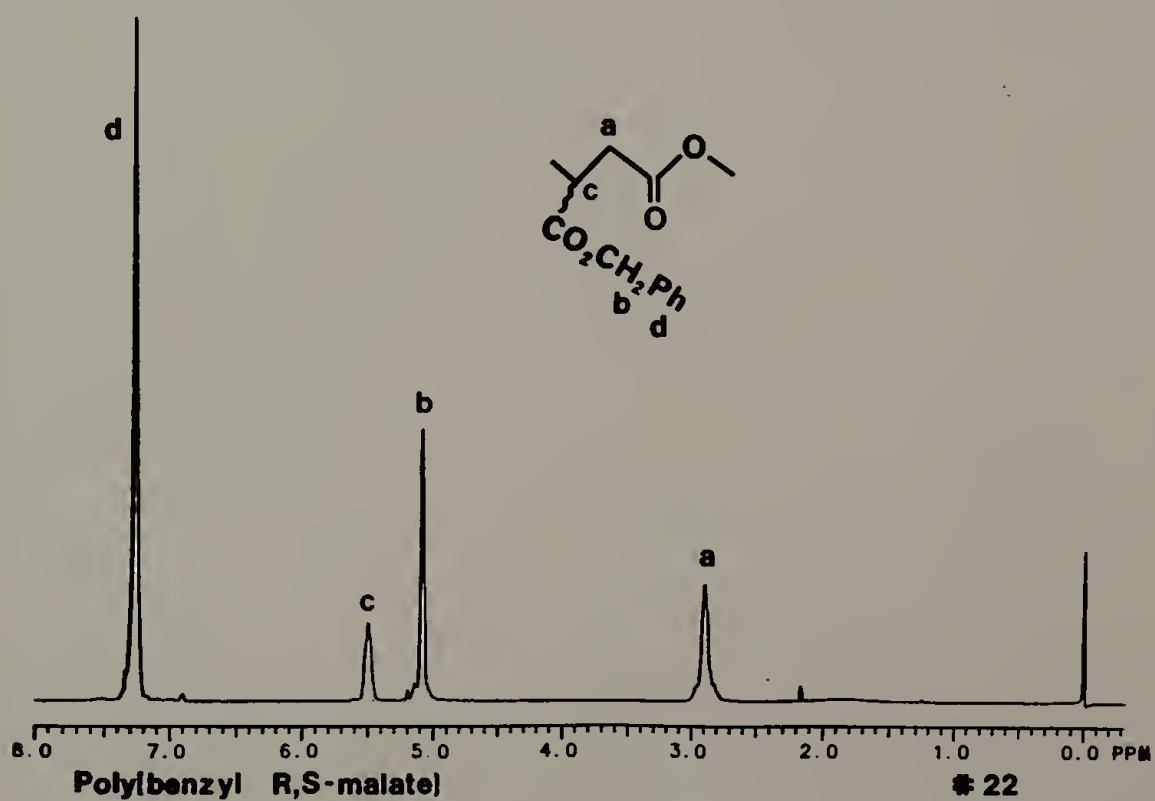
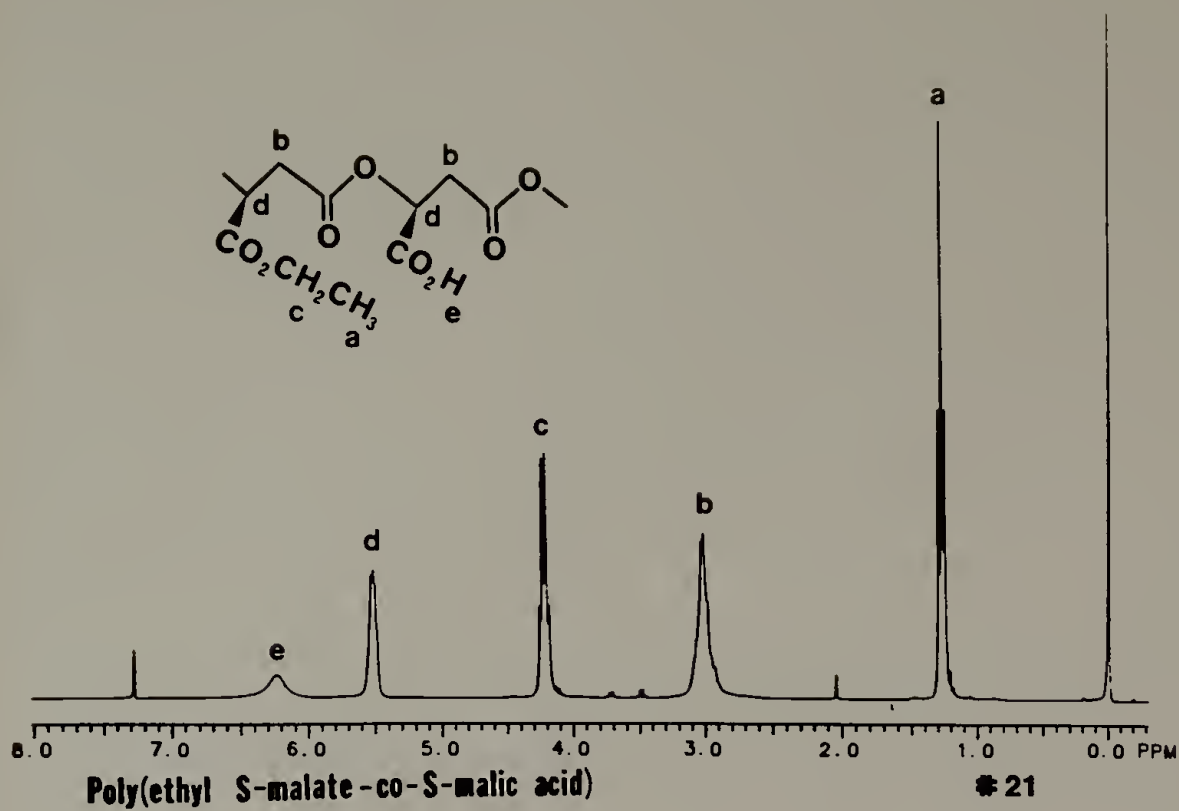




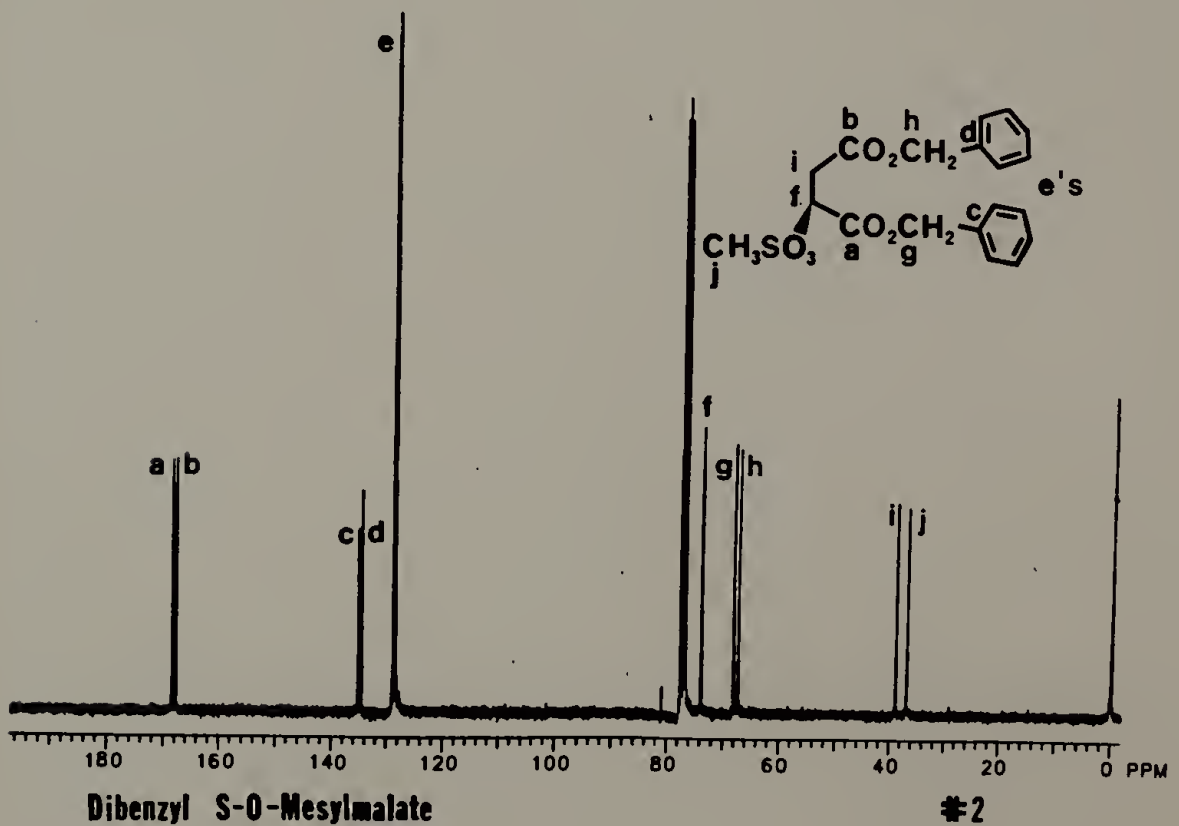
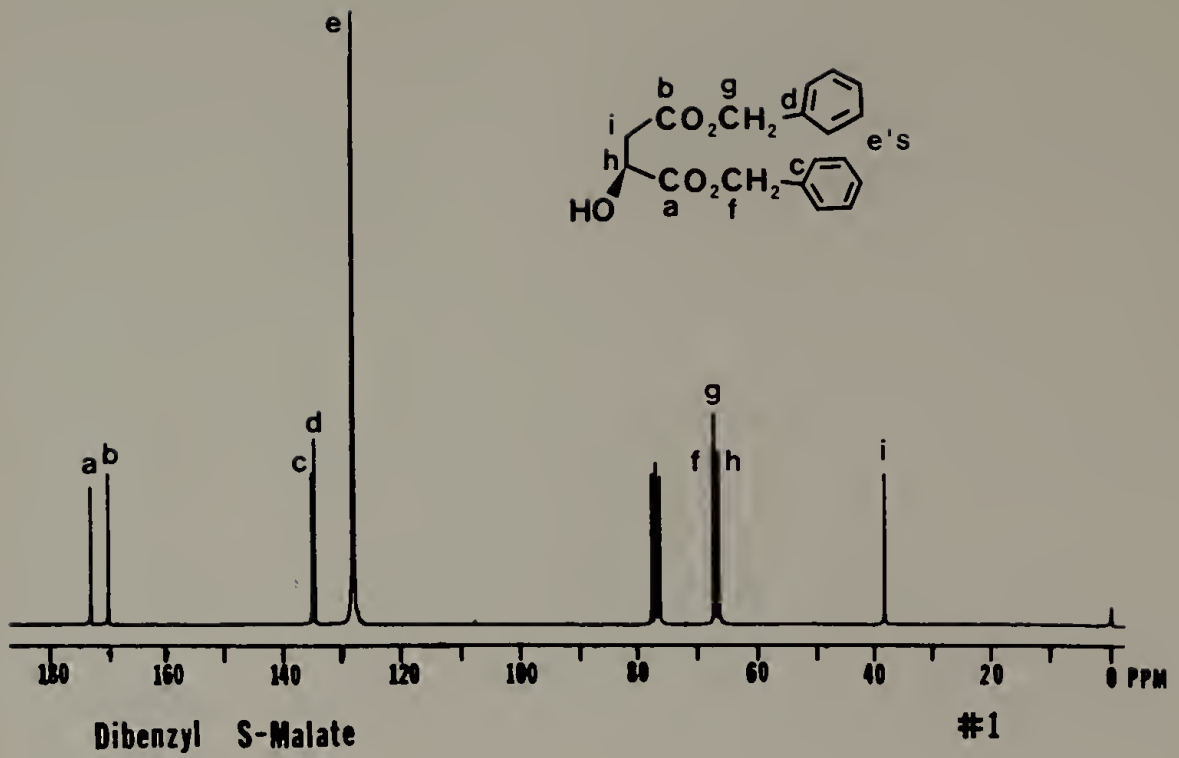


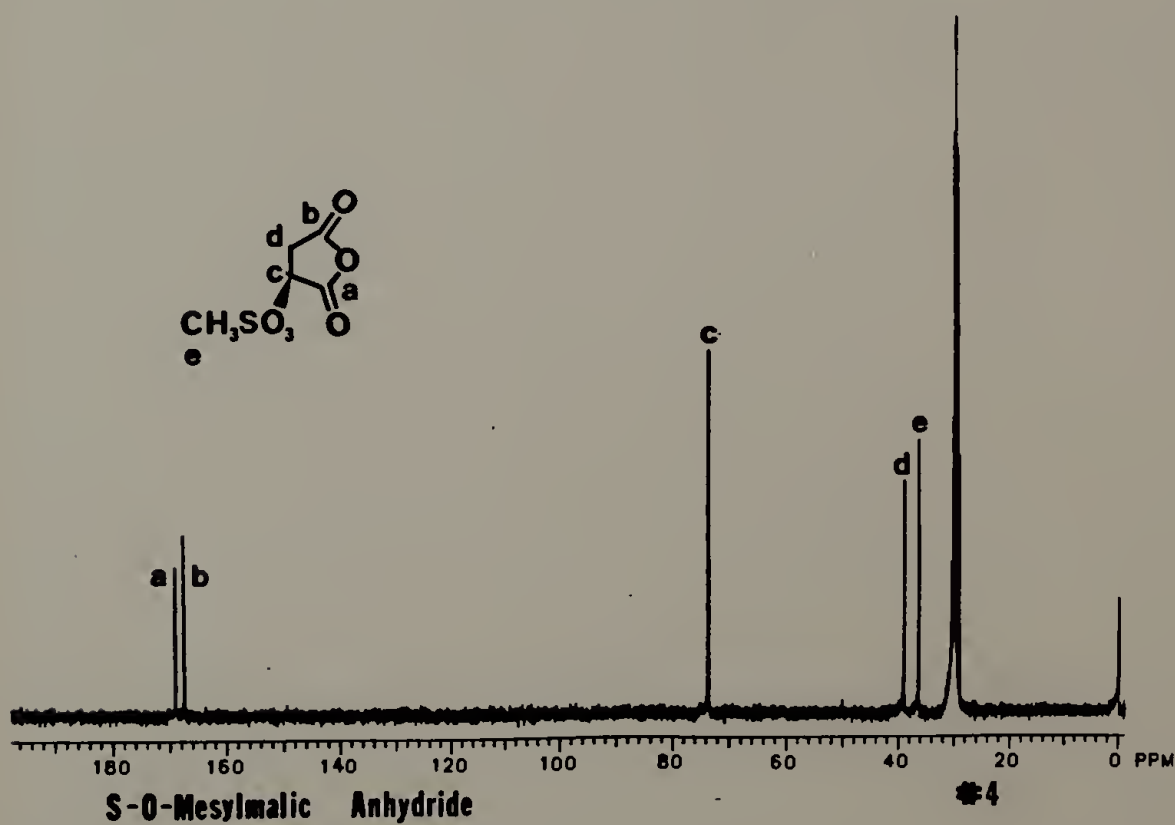
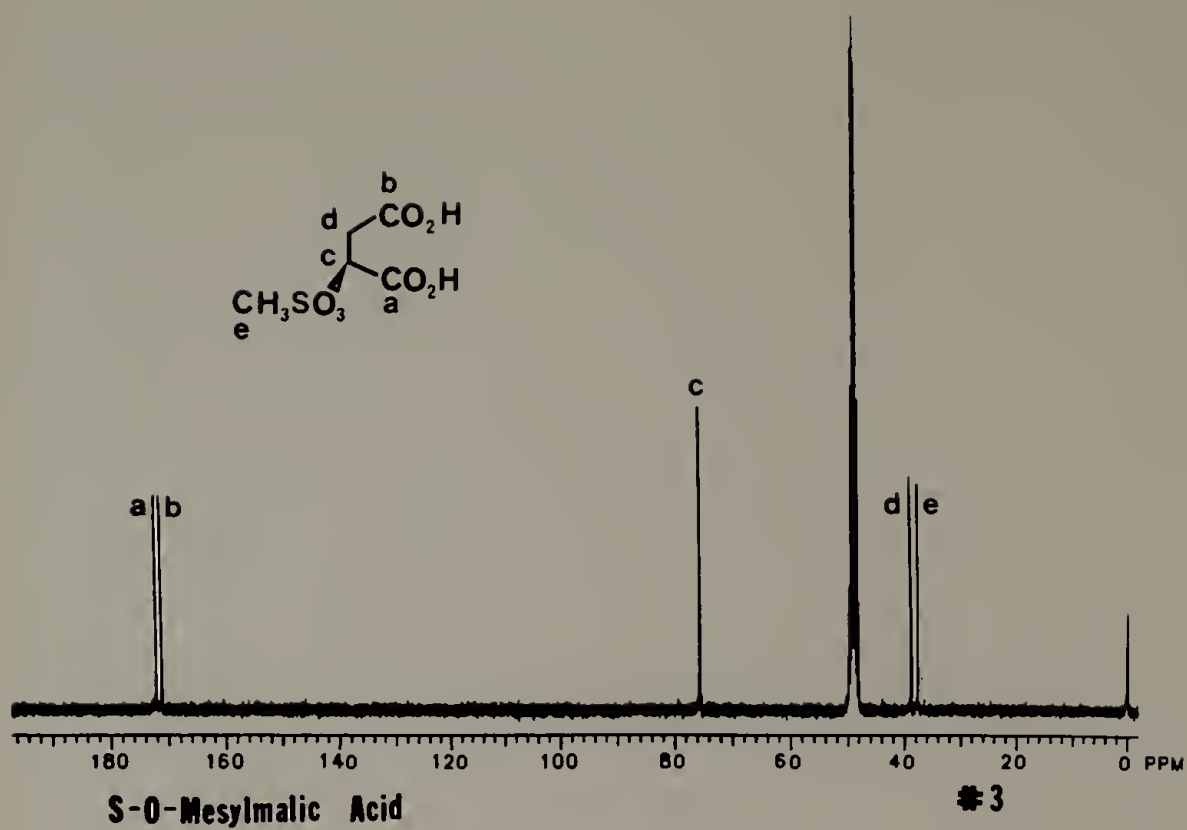


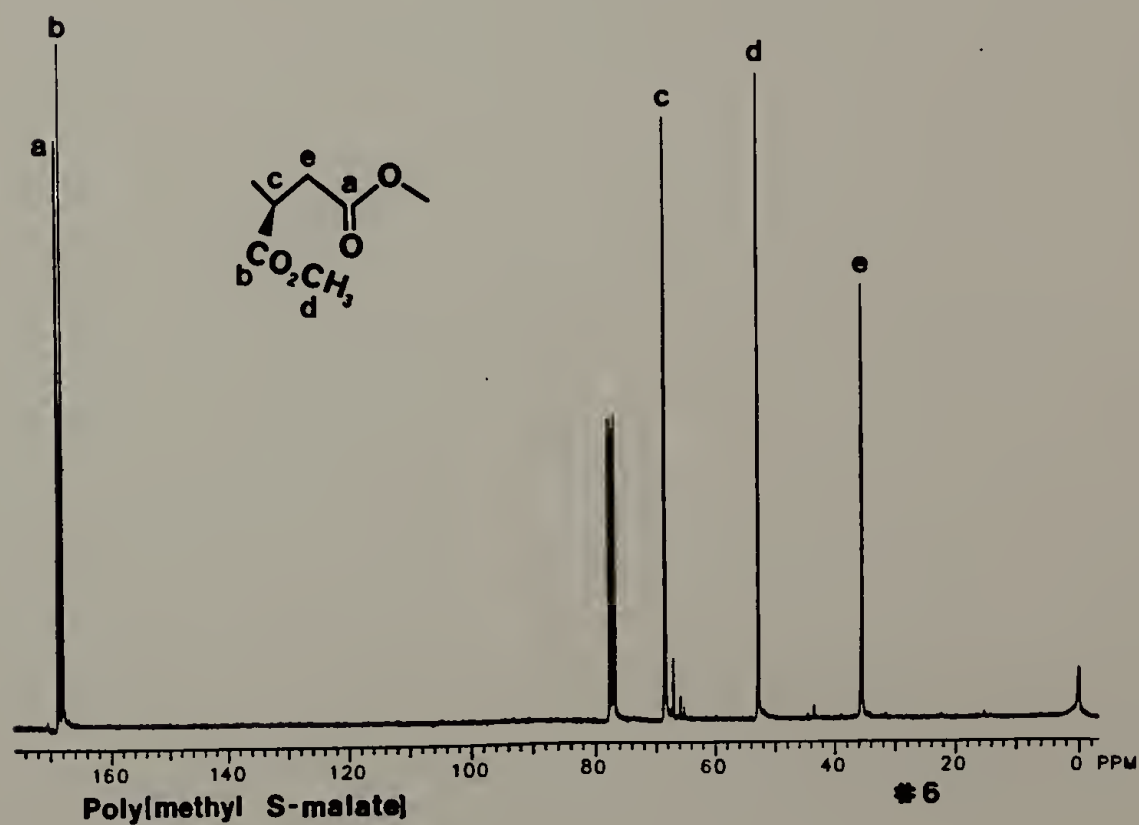
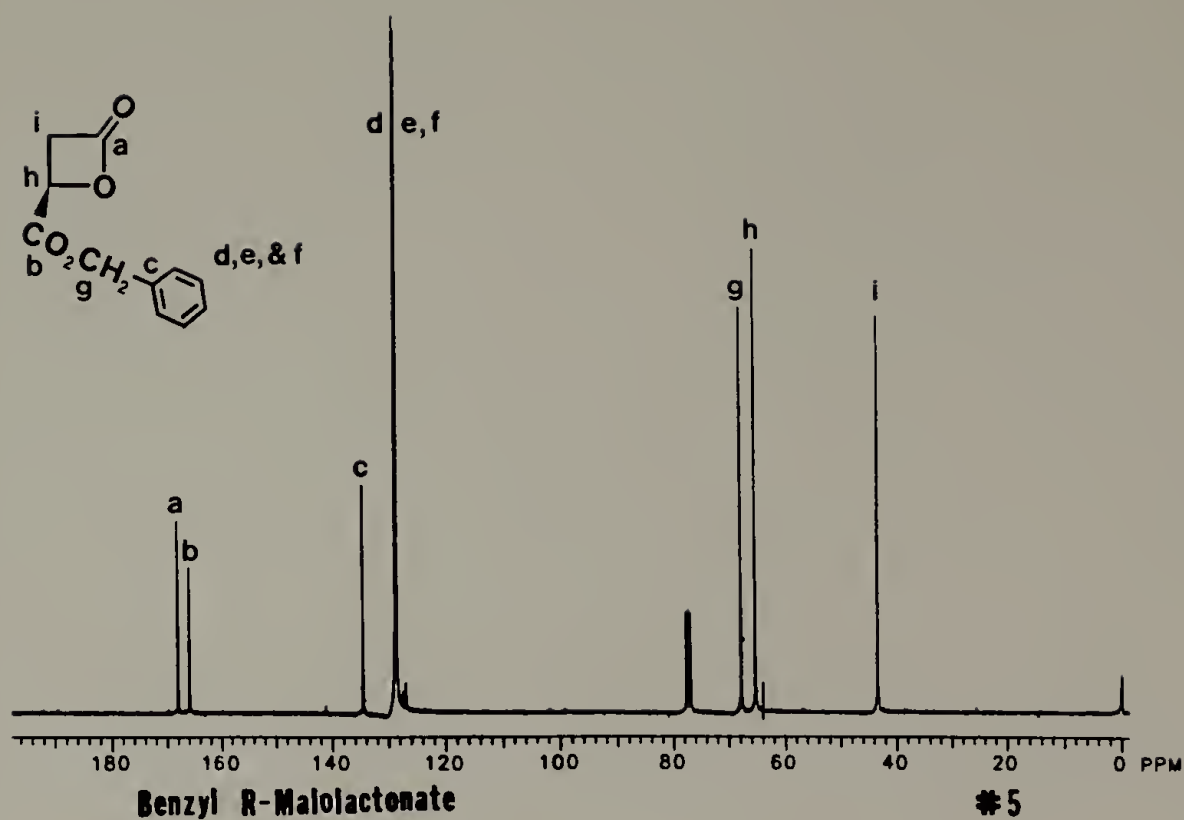


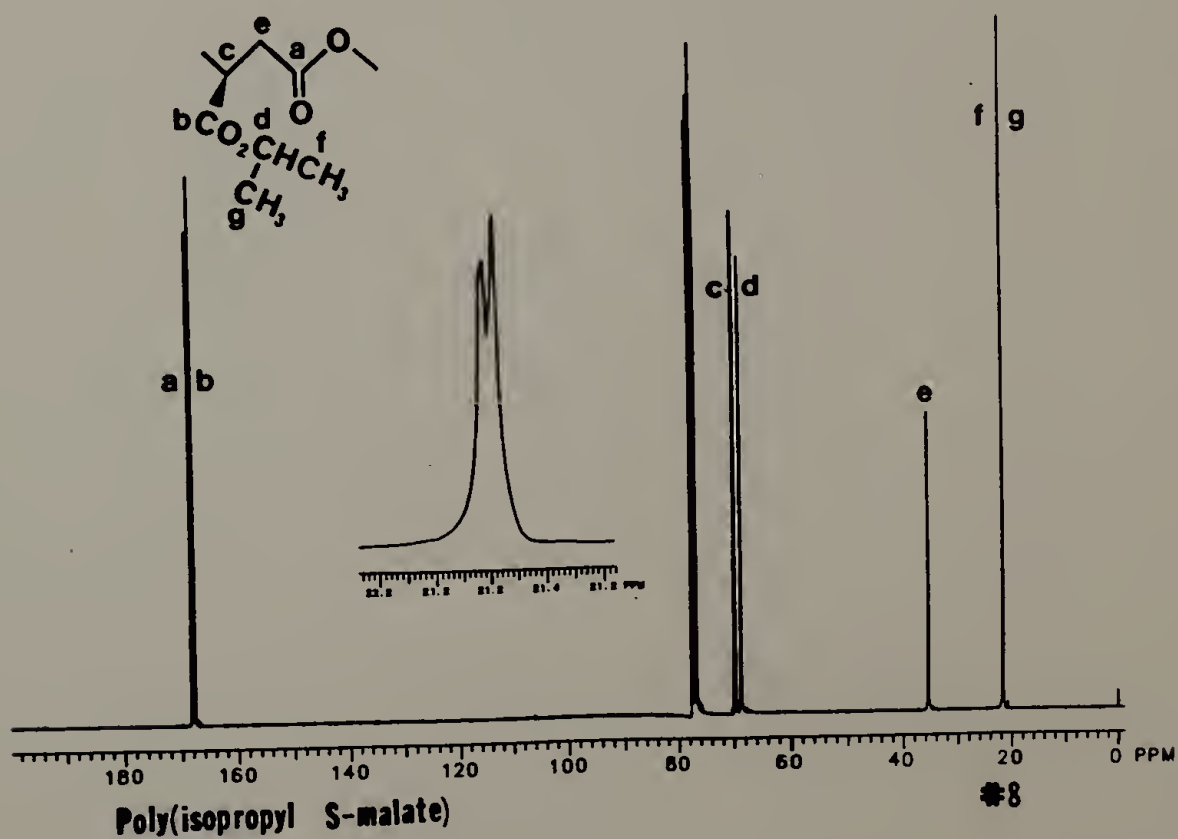
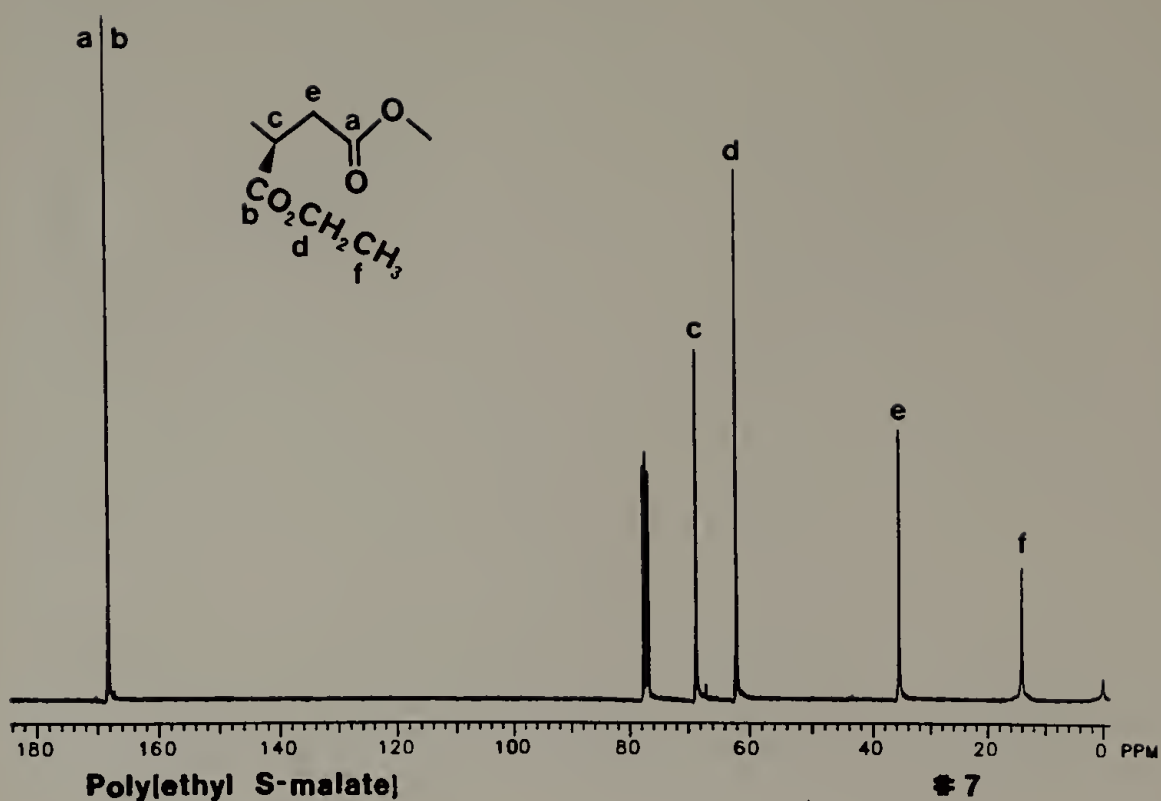


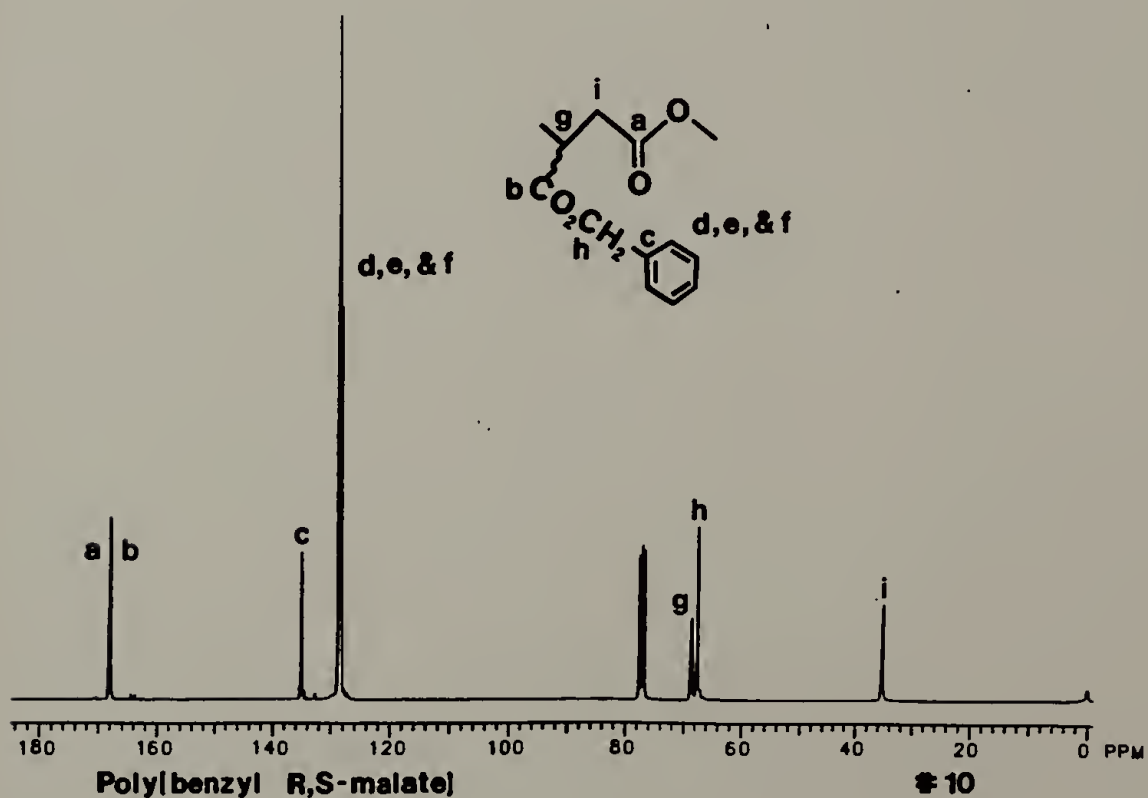
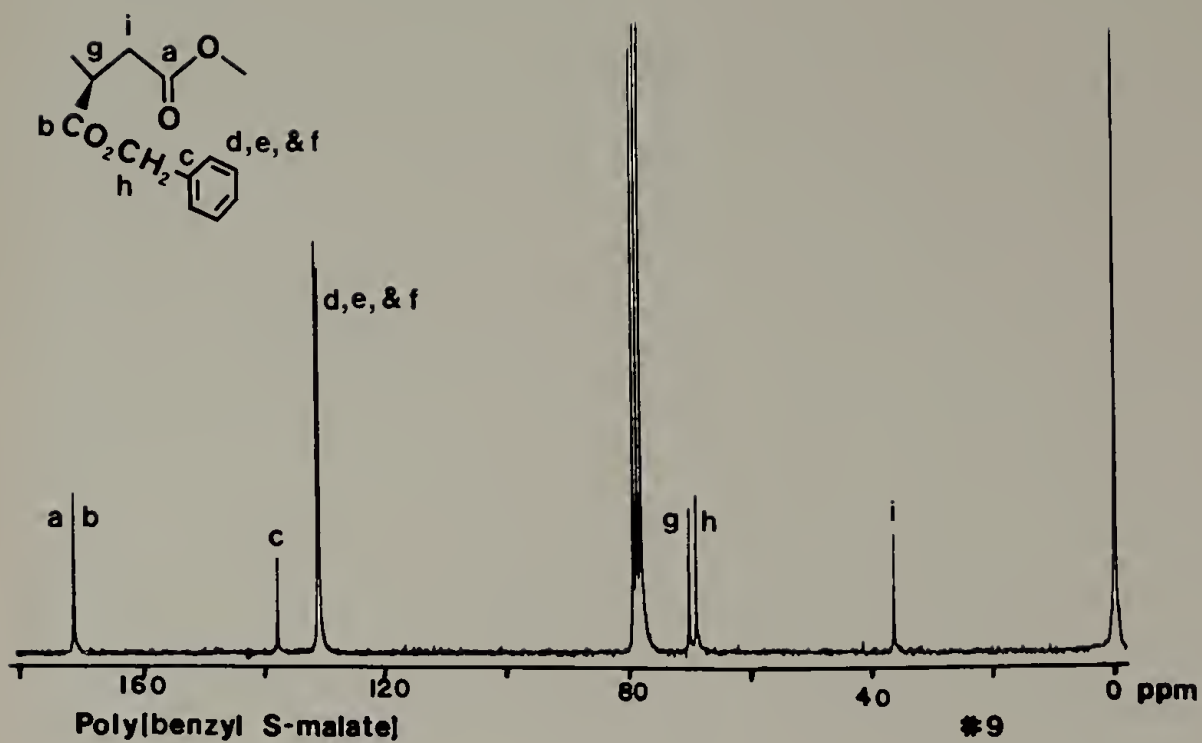
APPENDIX B
CARBON 13 NUCLEAR MAGNETIC RESONANCE SPECTRA

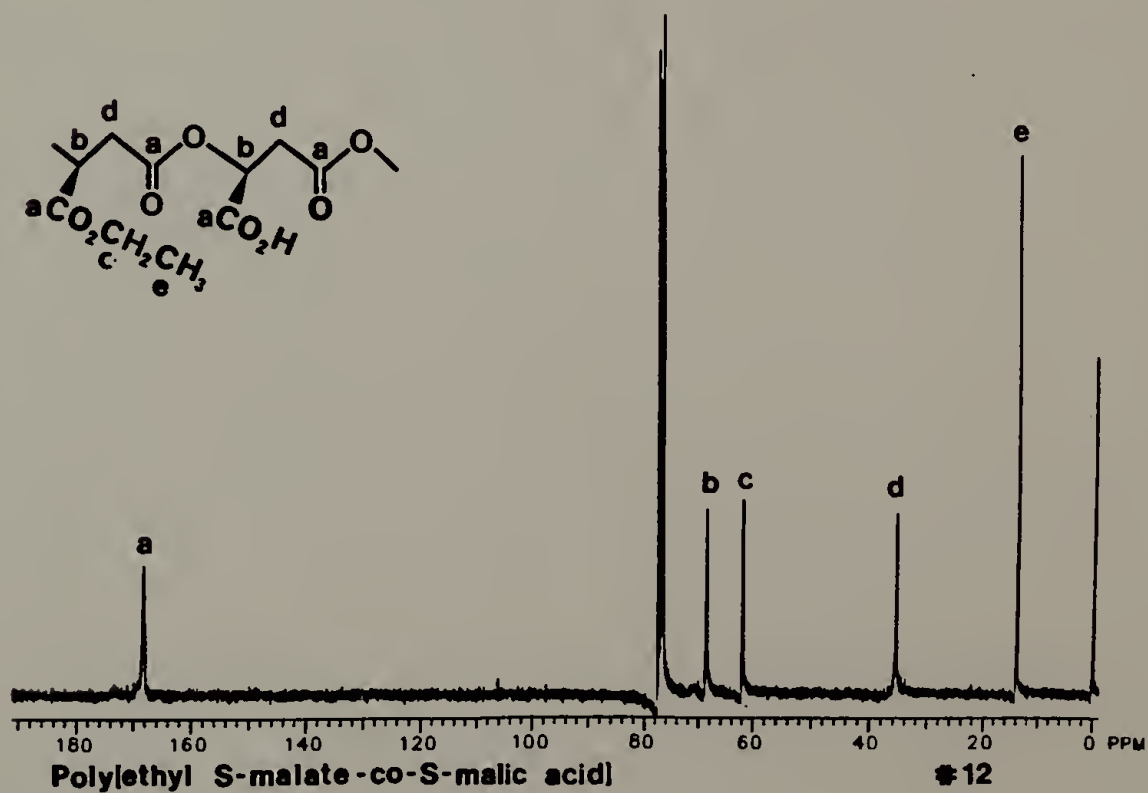
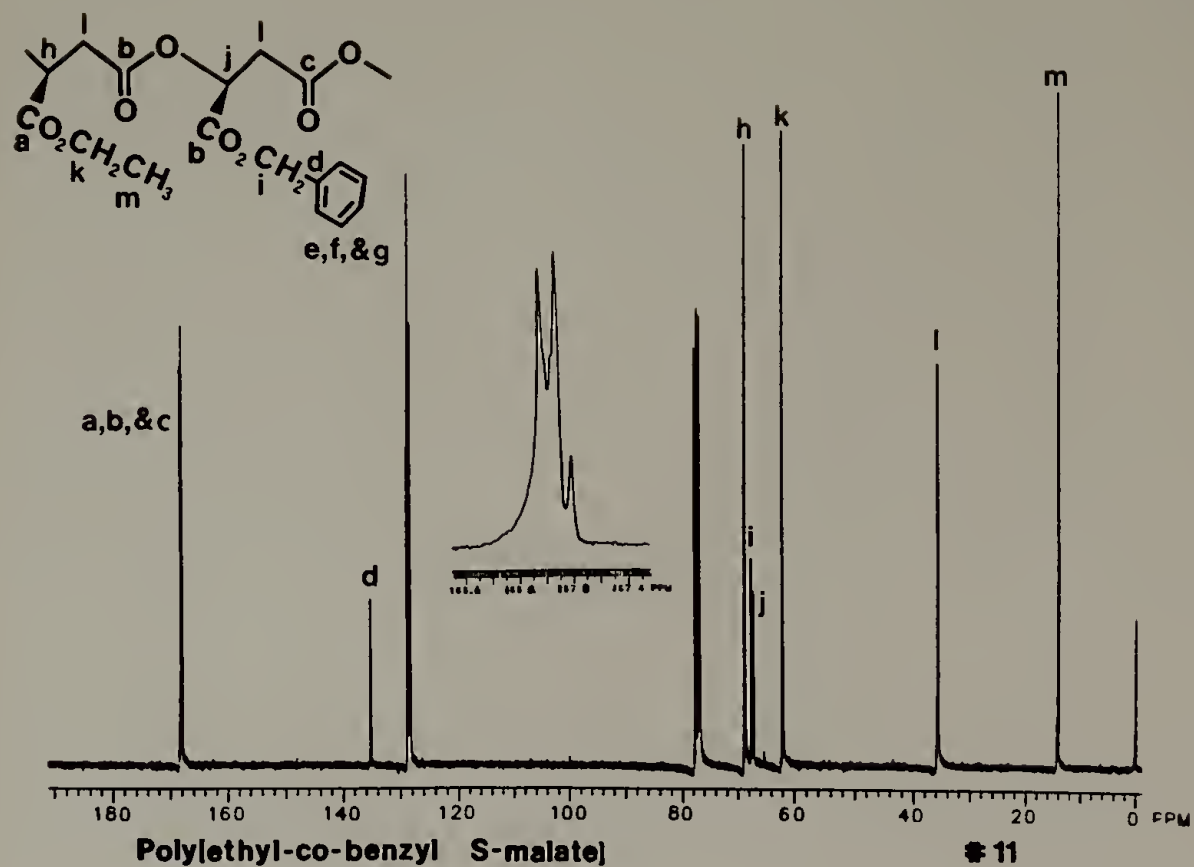




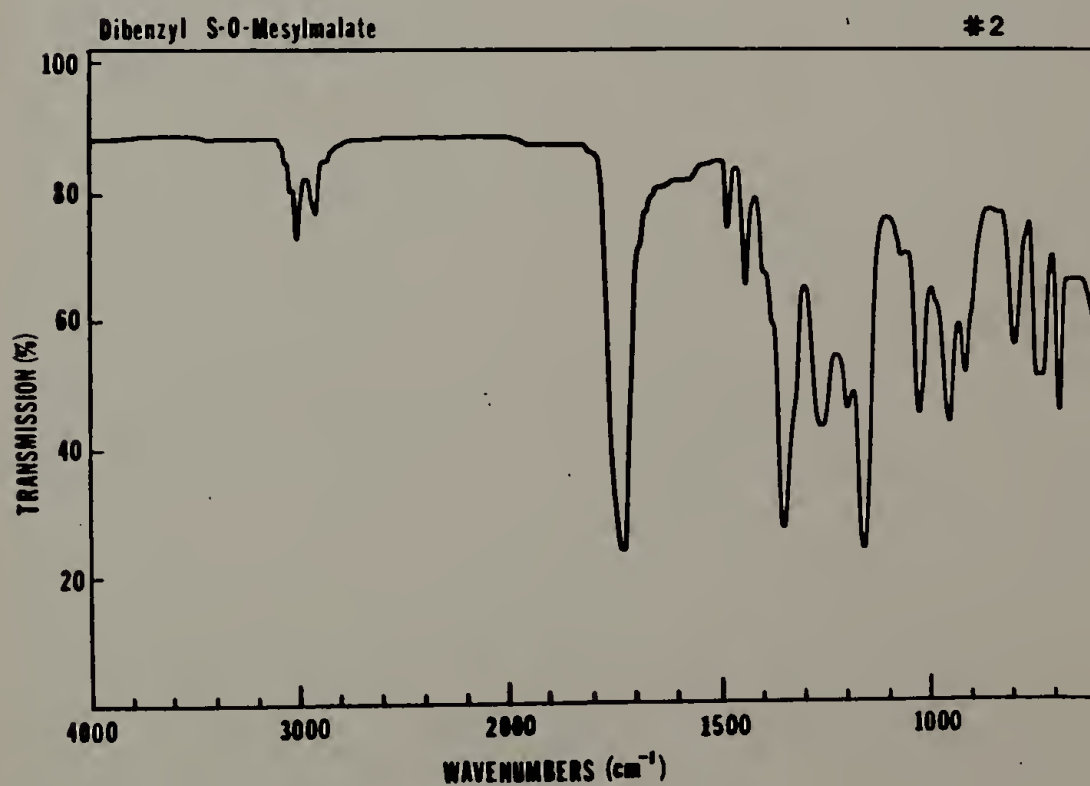
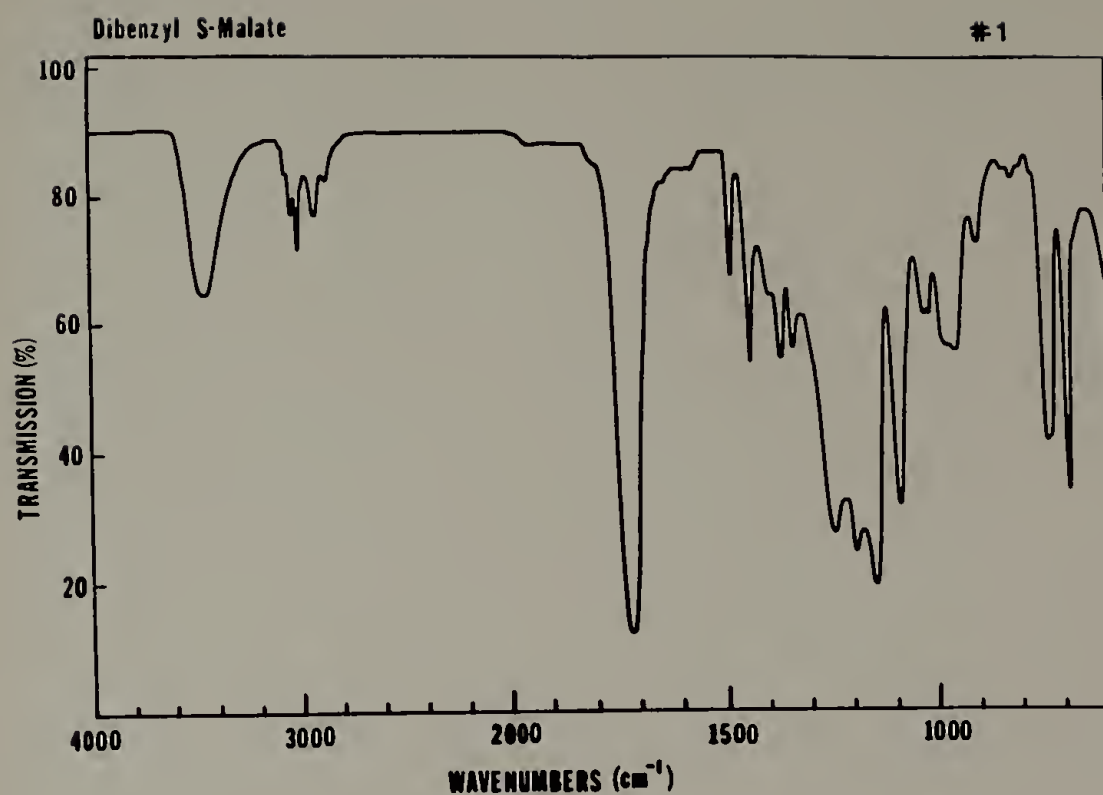


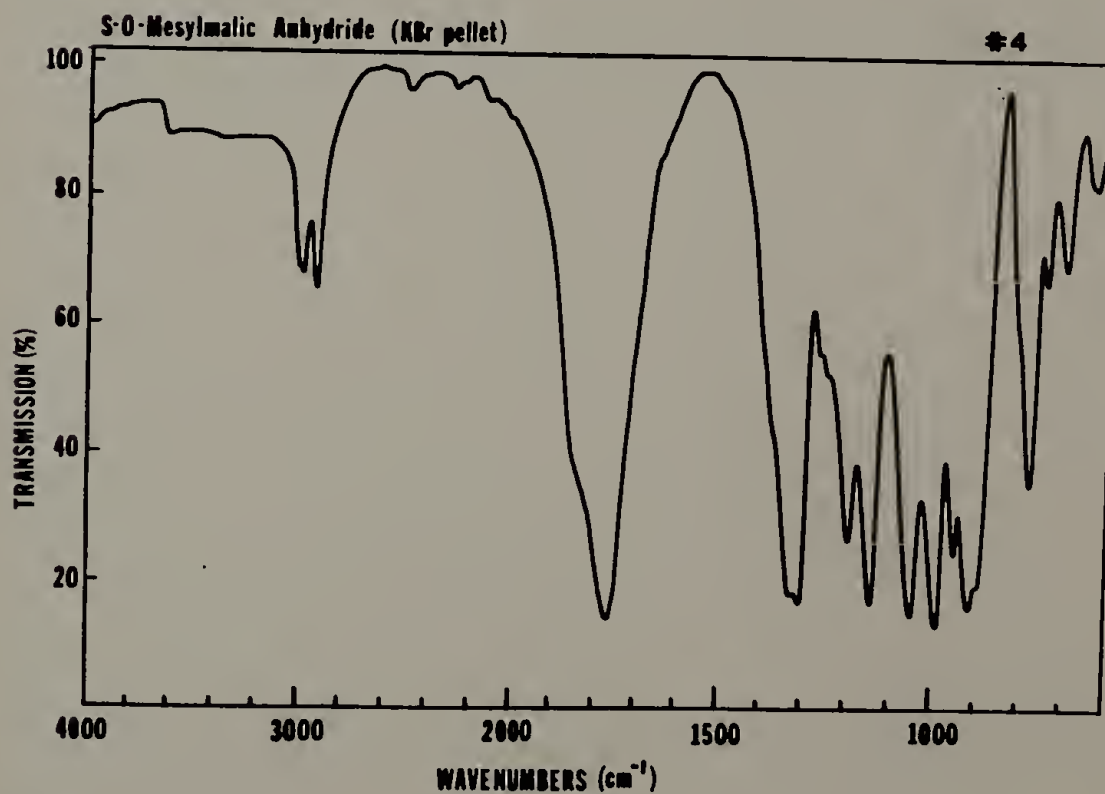
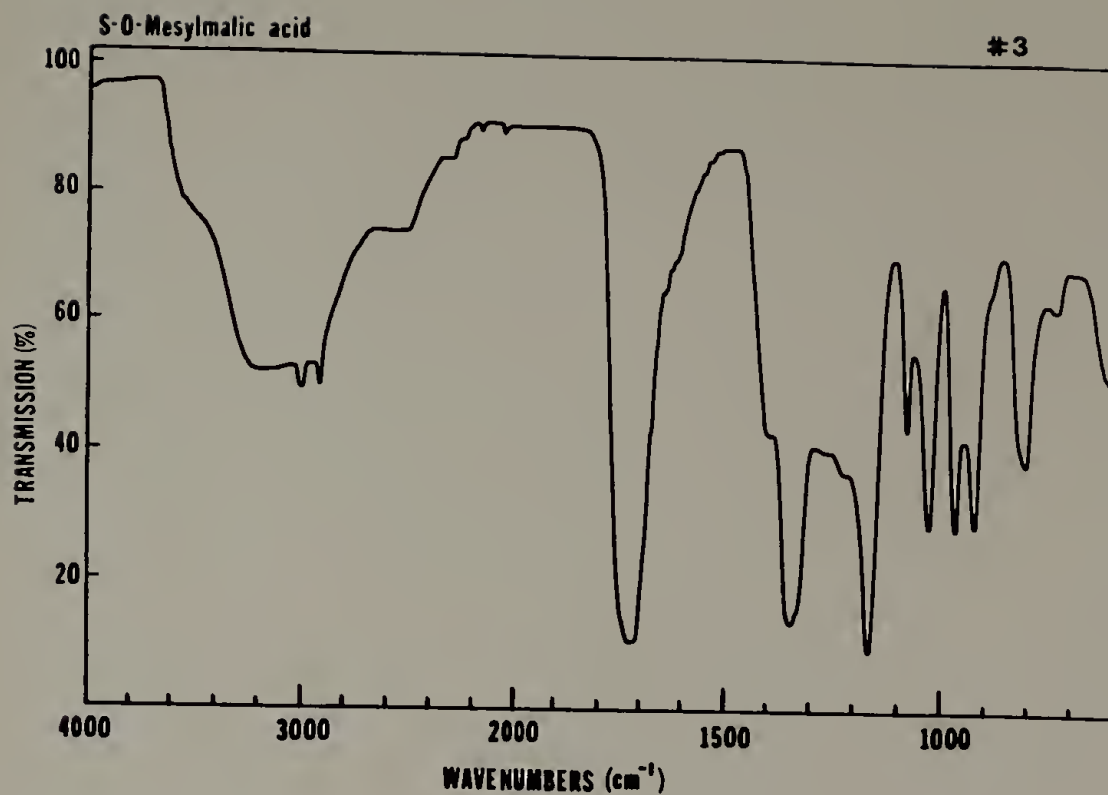


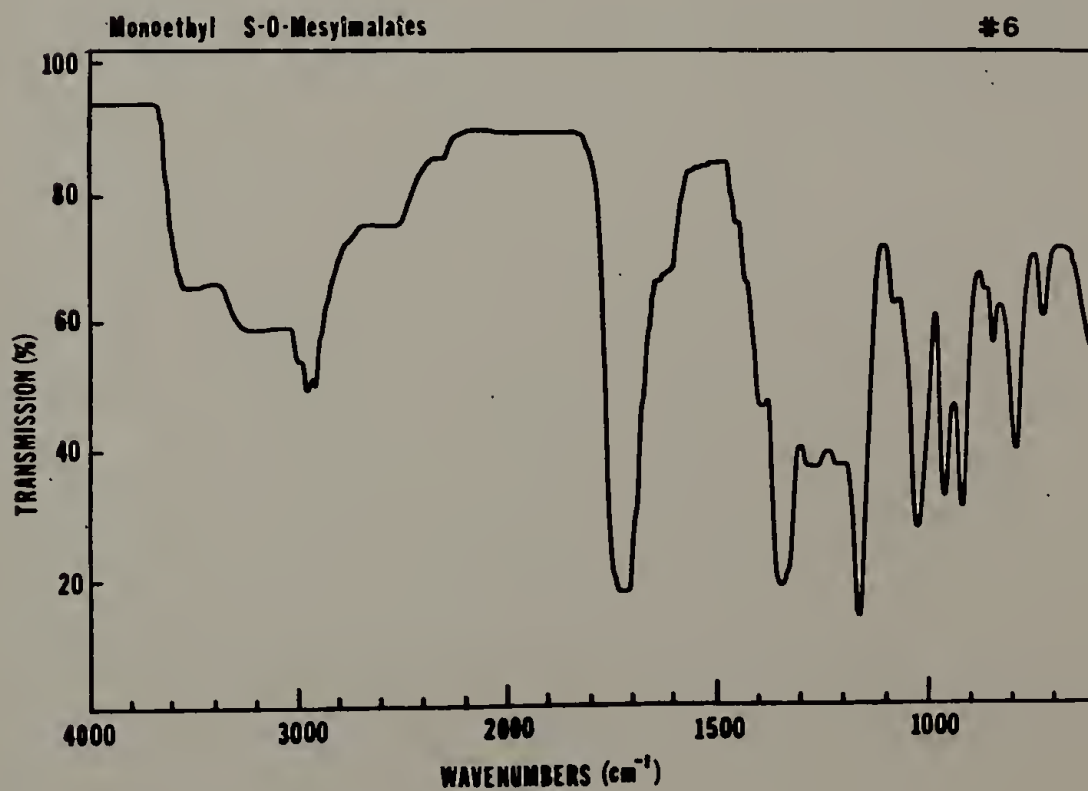
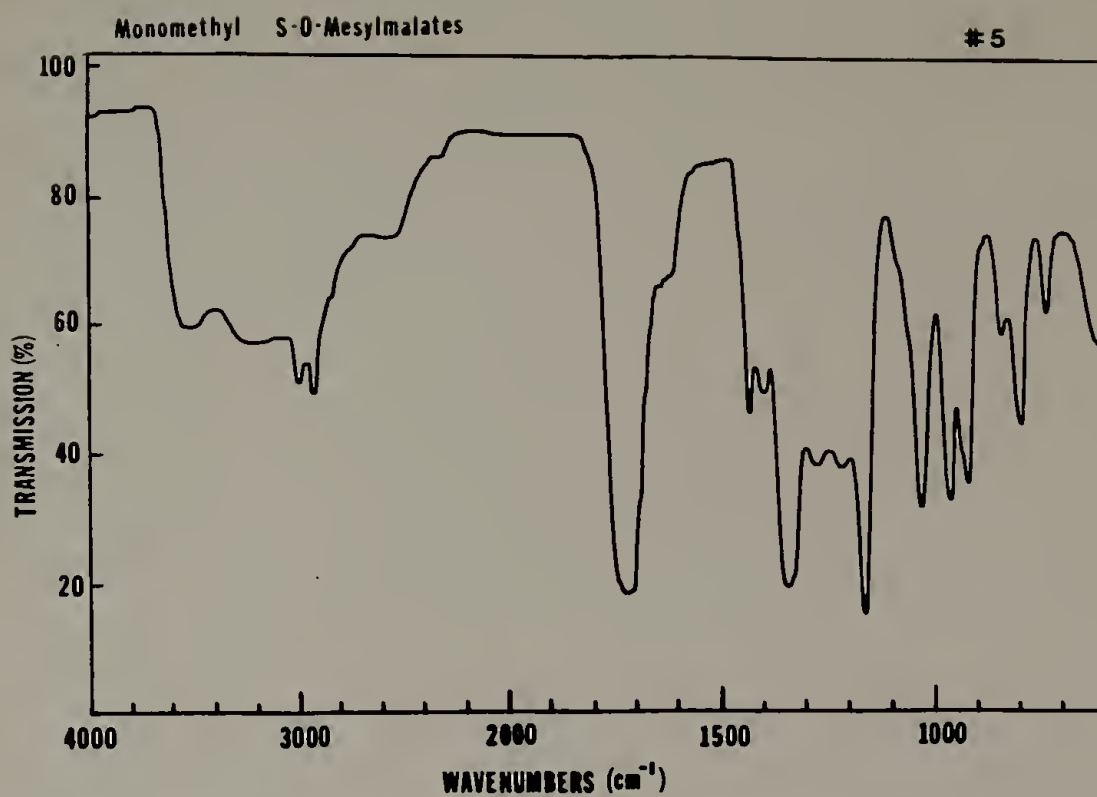


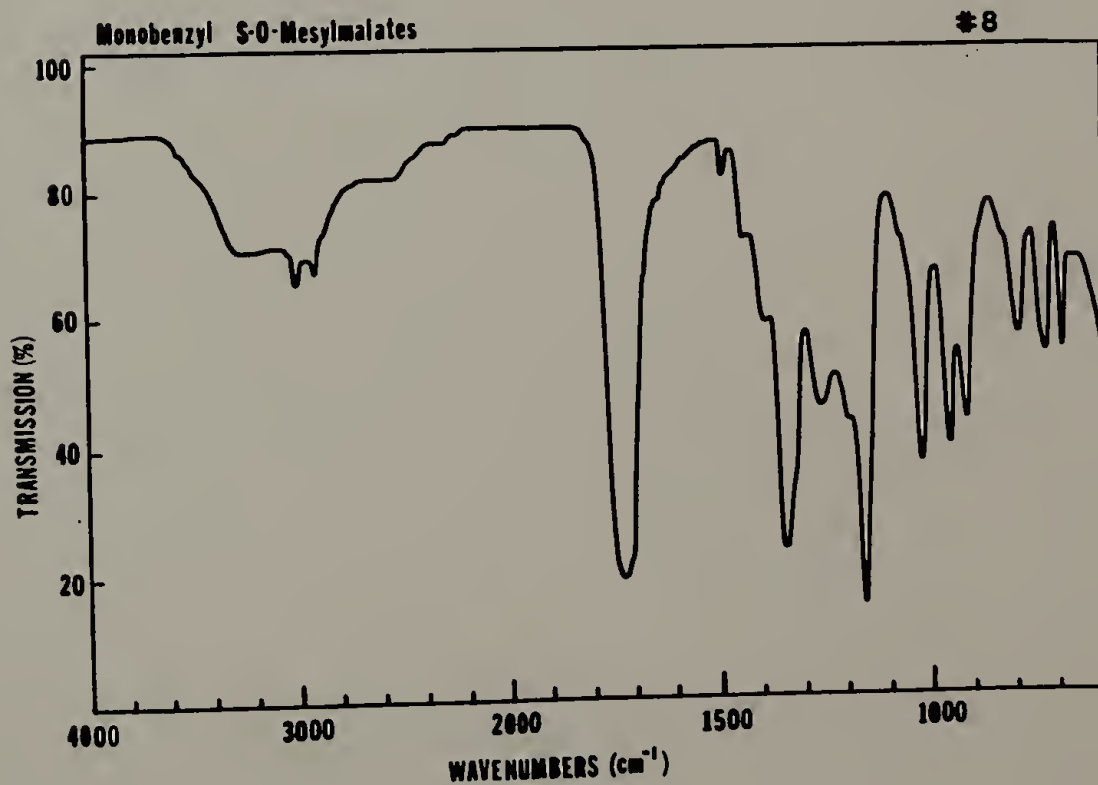
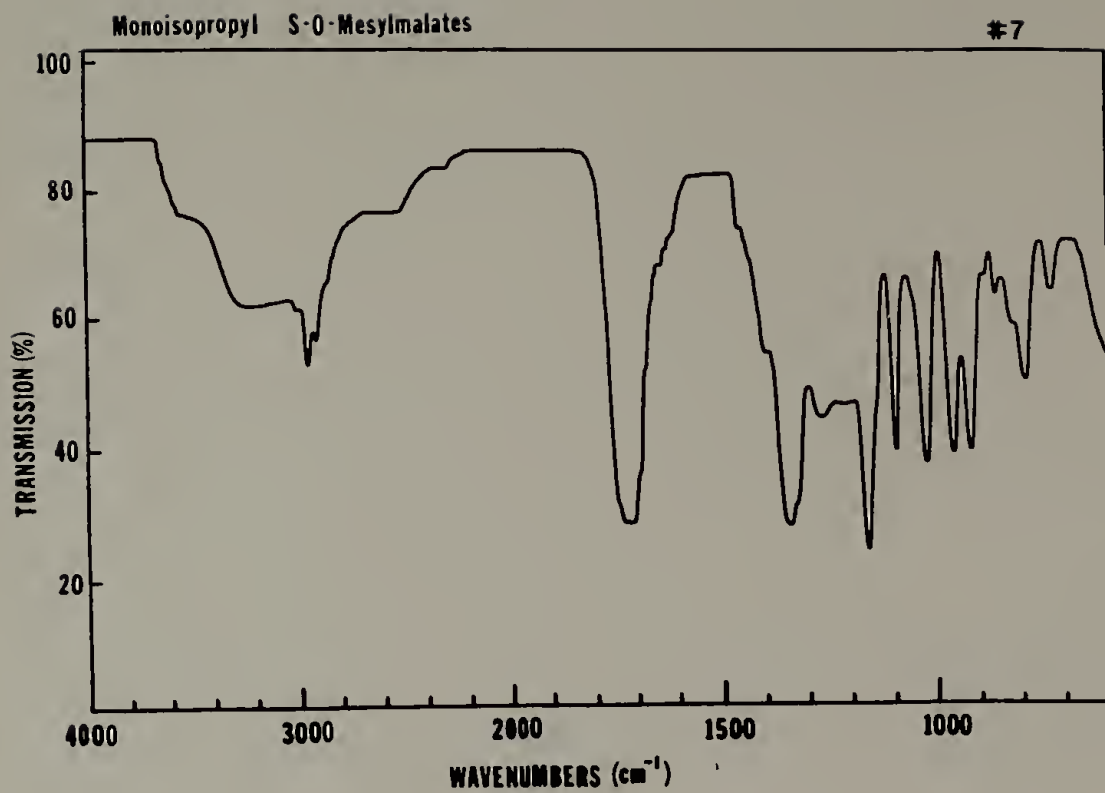


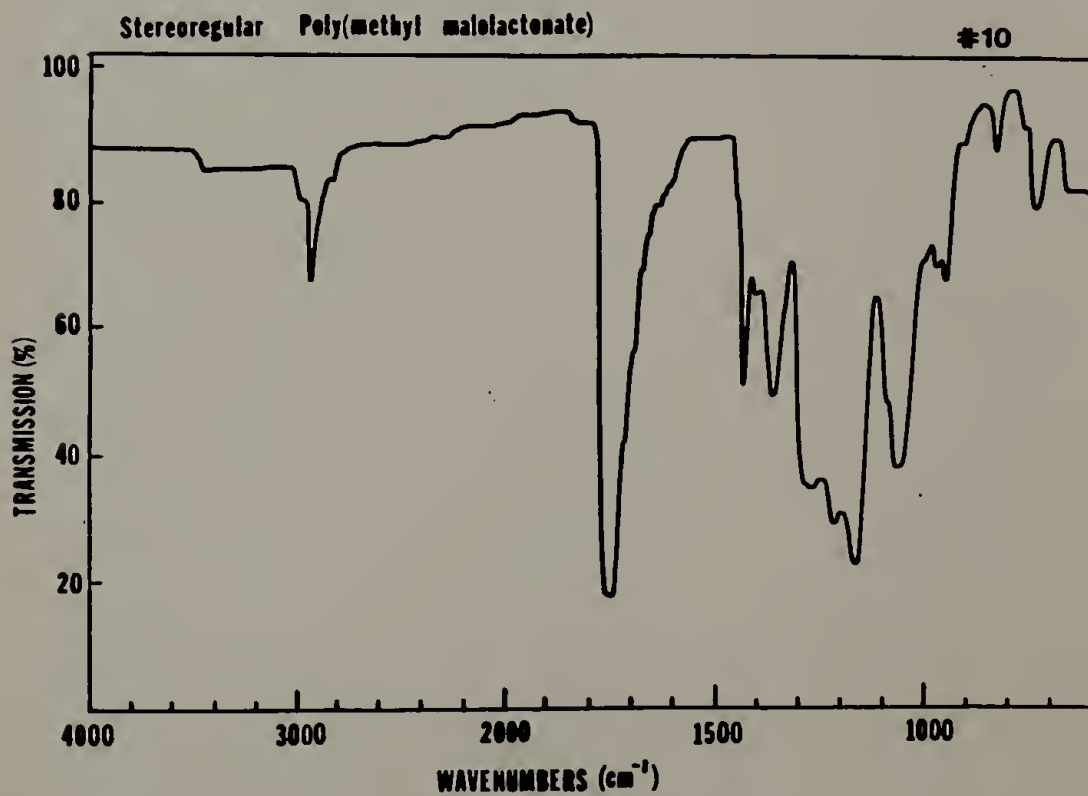
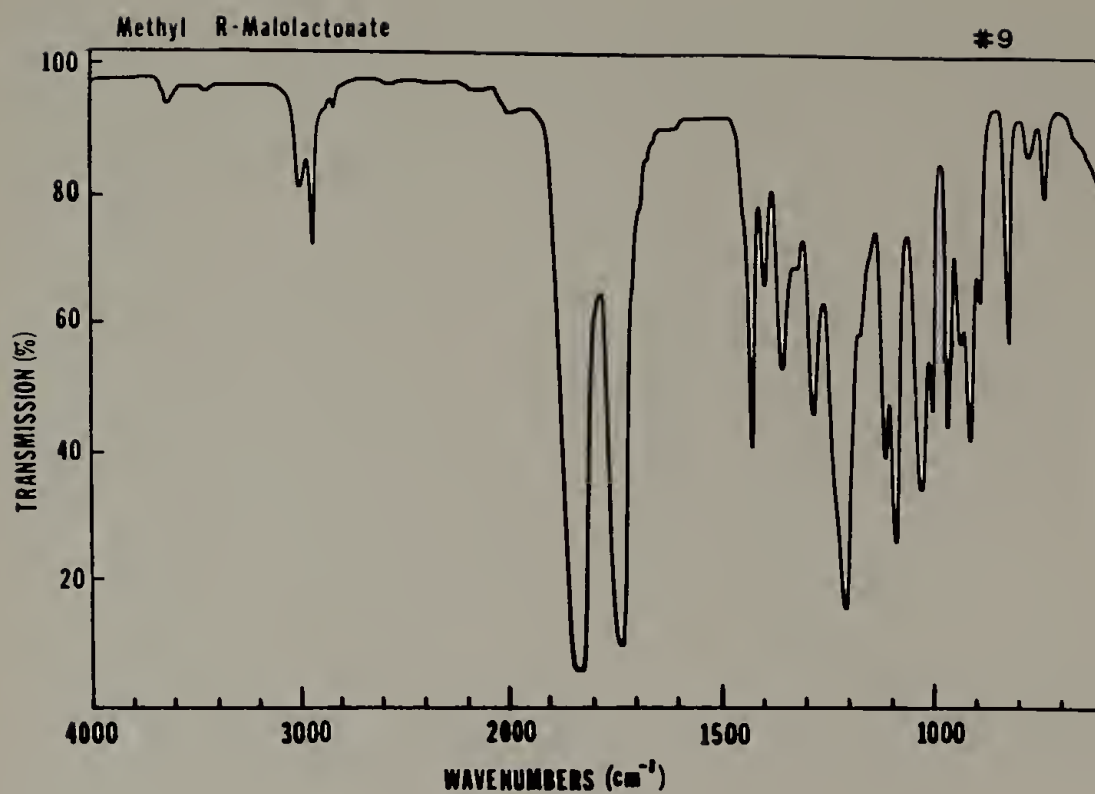
APPENDIX C
INFRARED SPECTRA

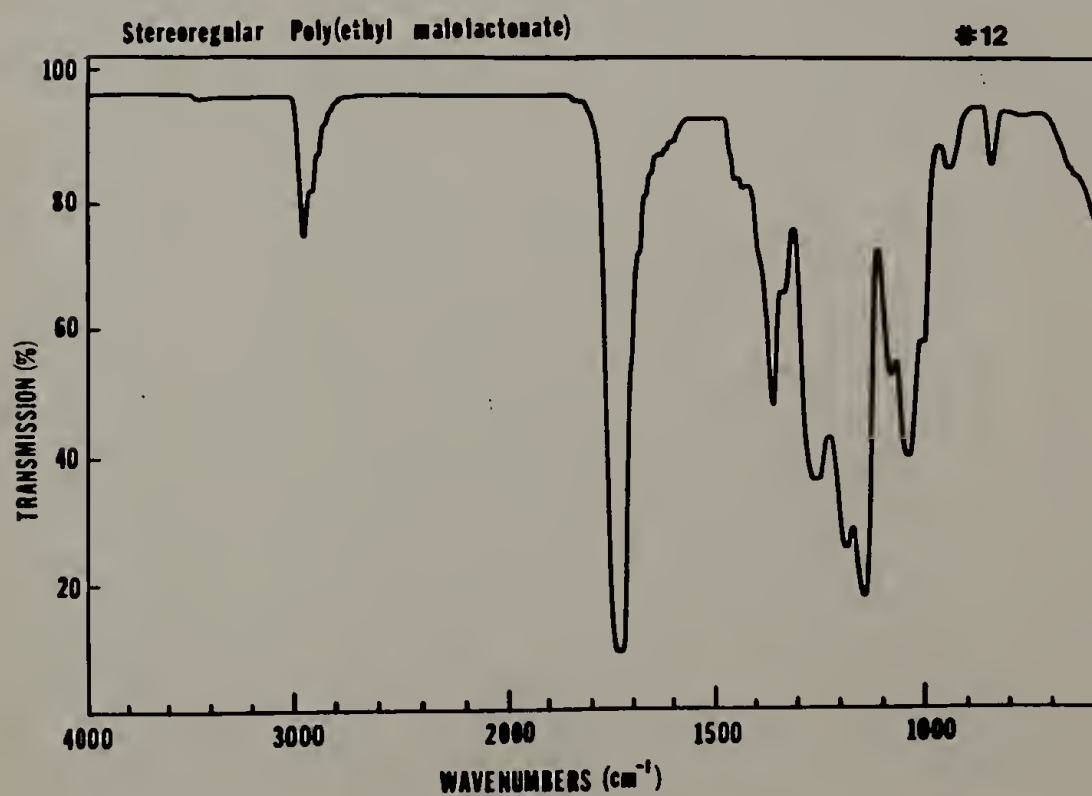
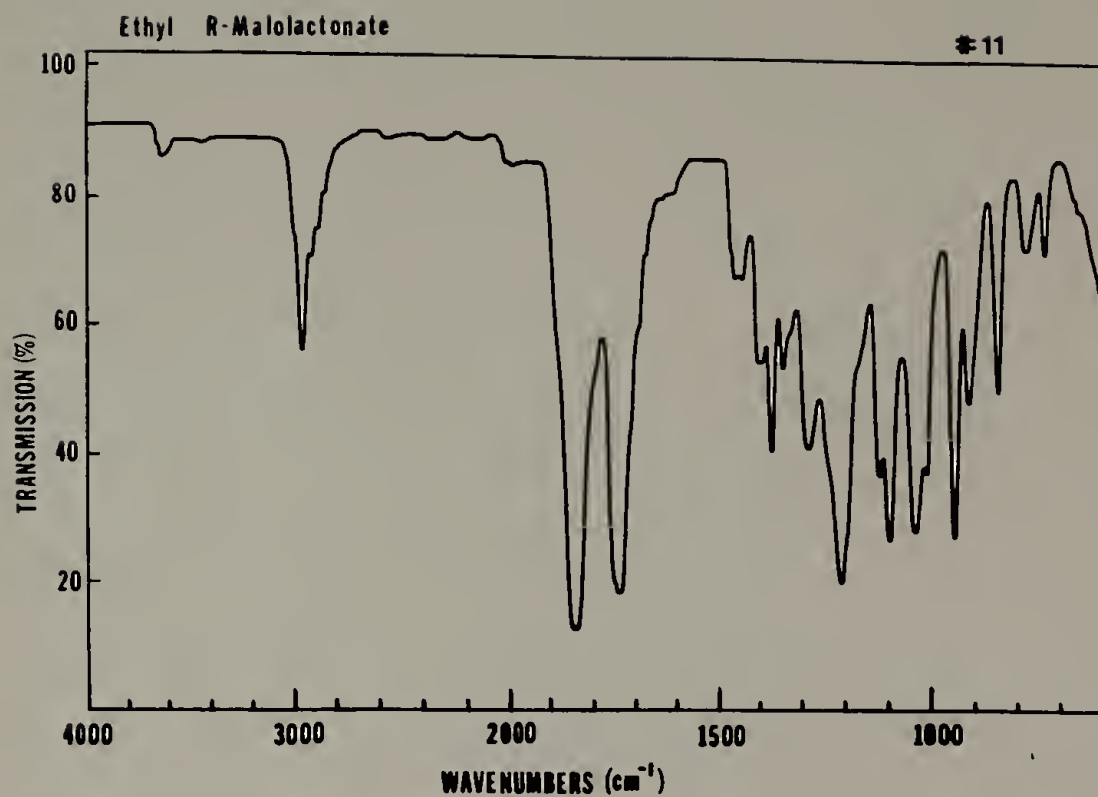


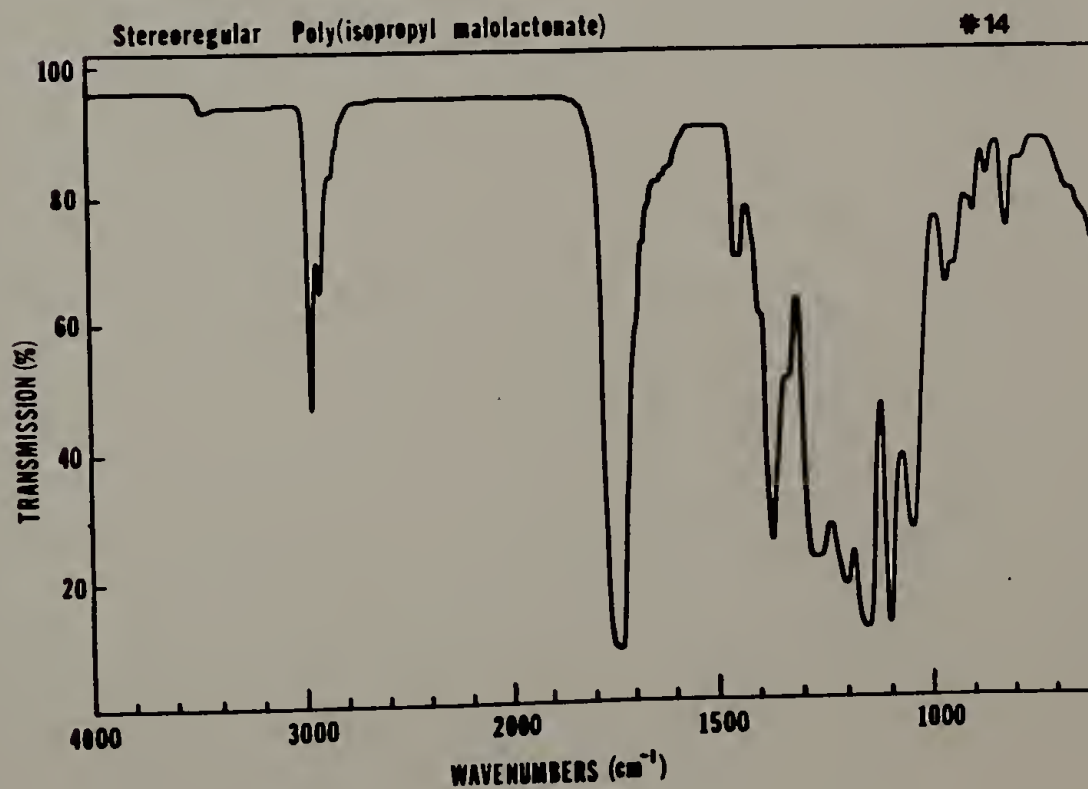
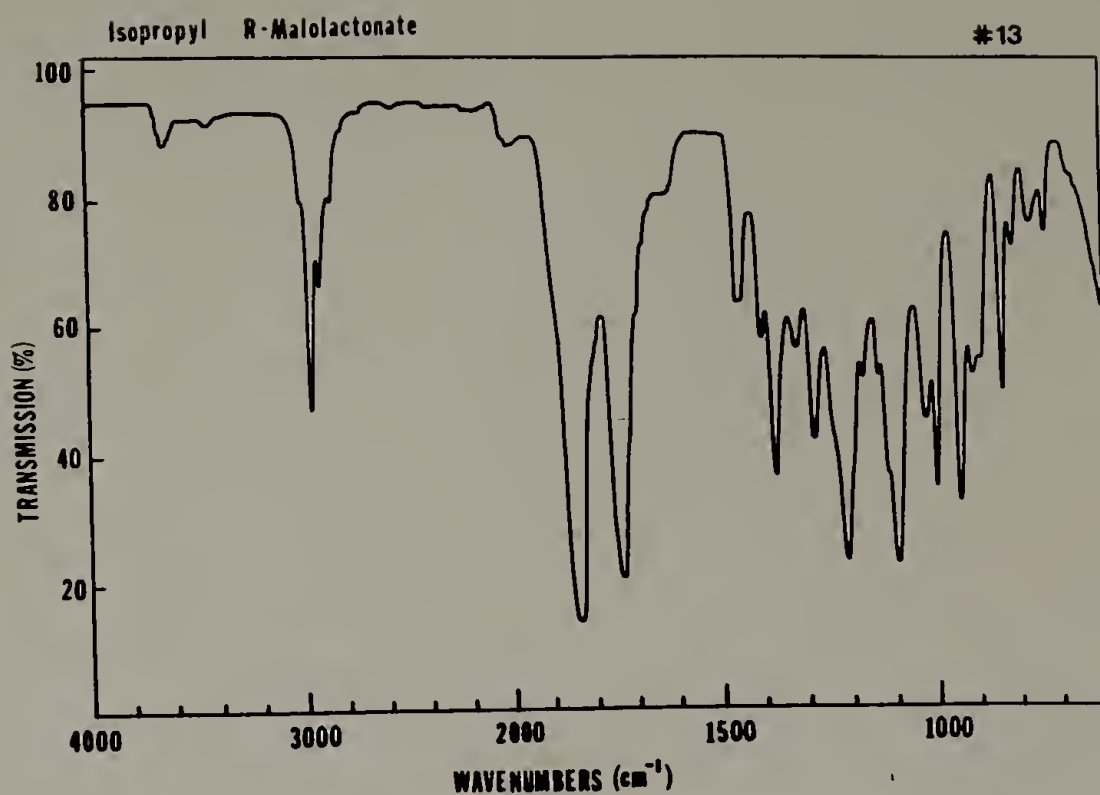


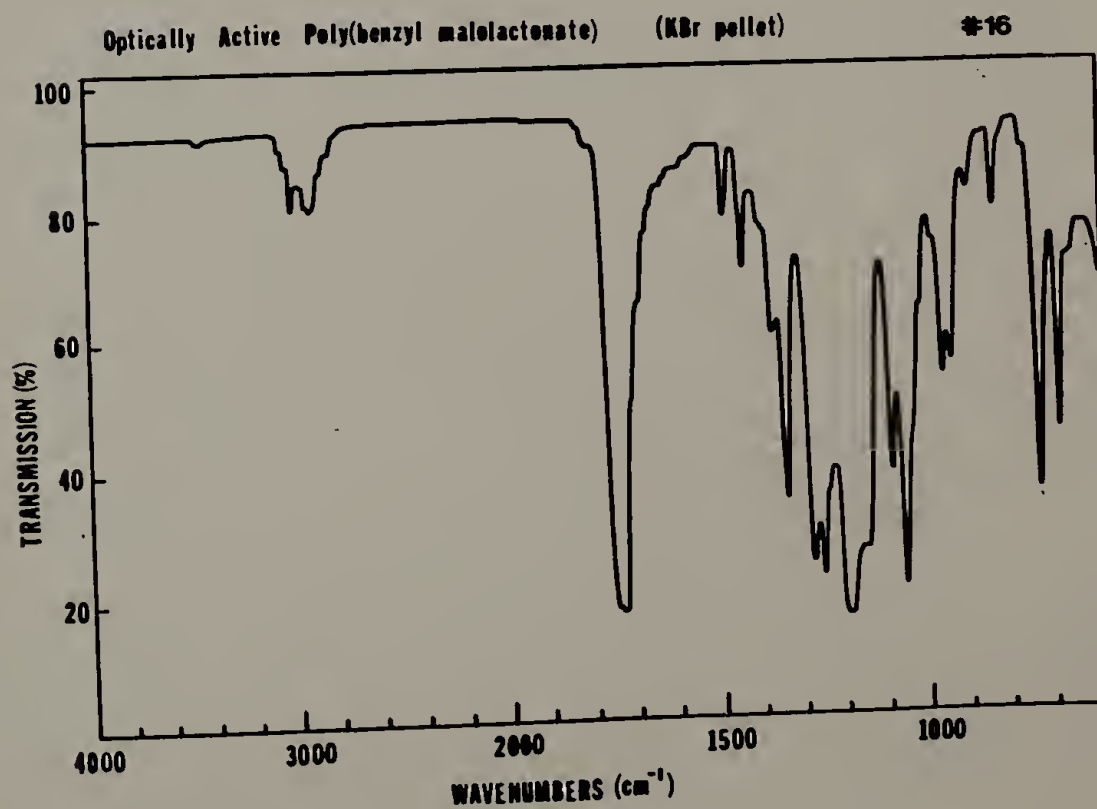
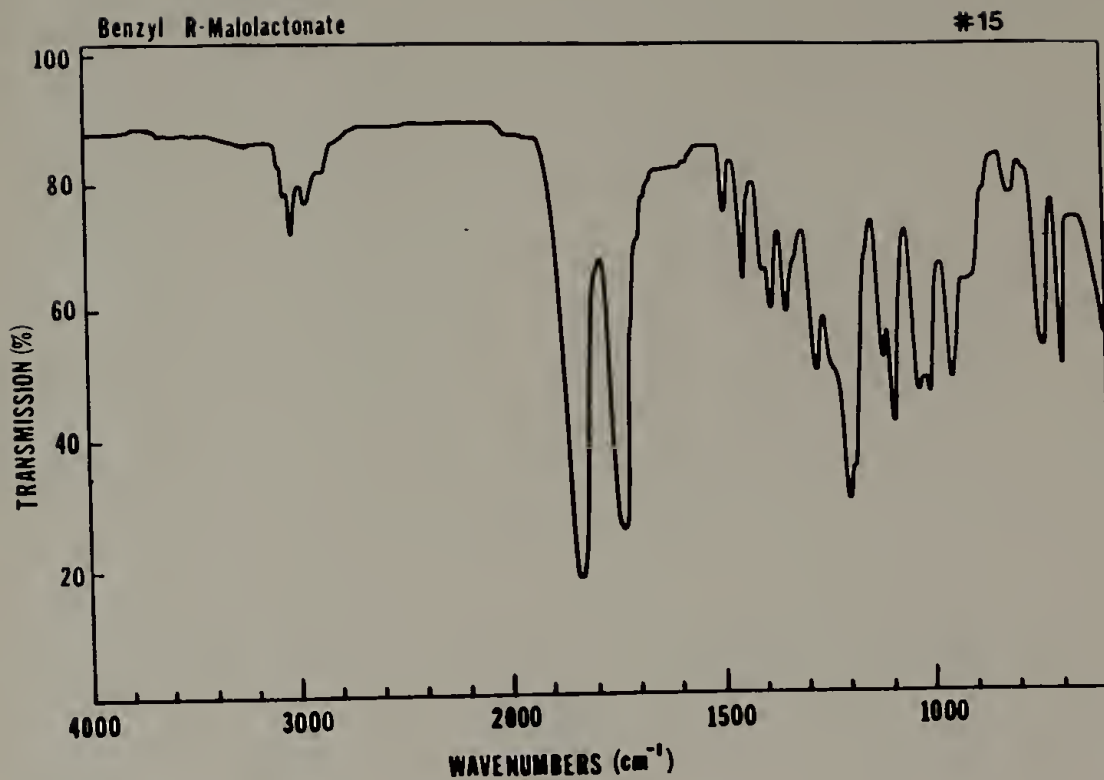


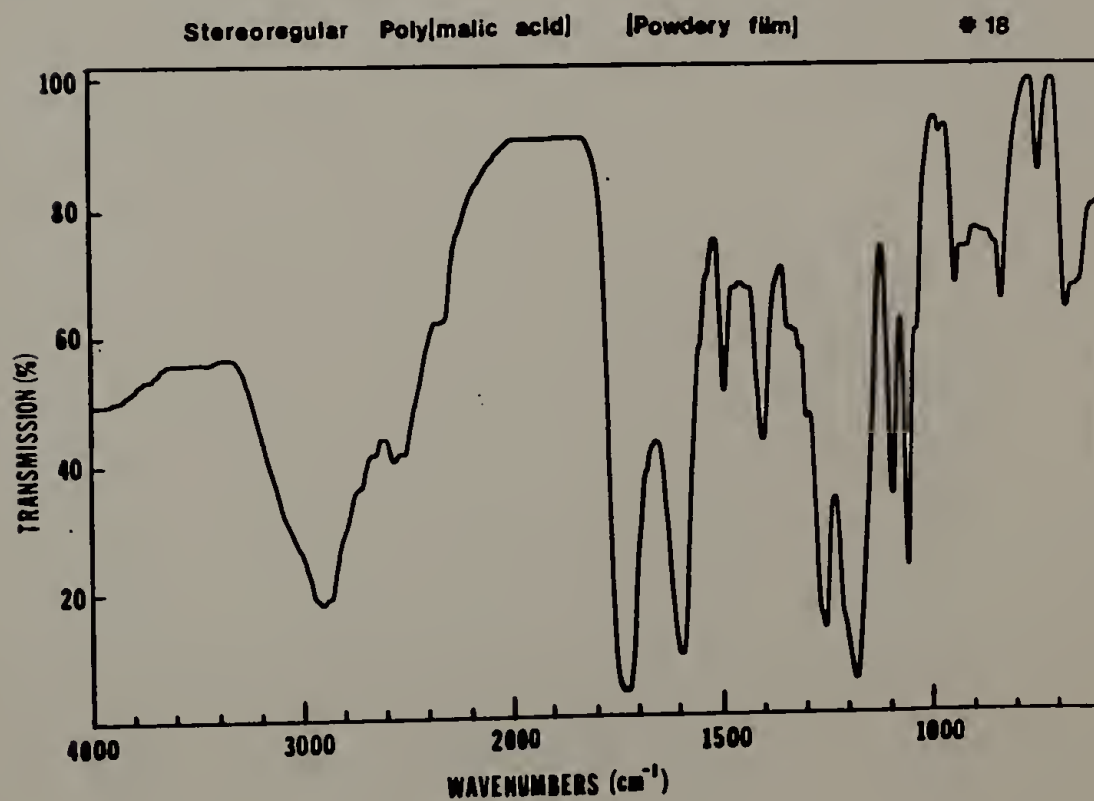
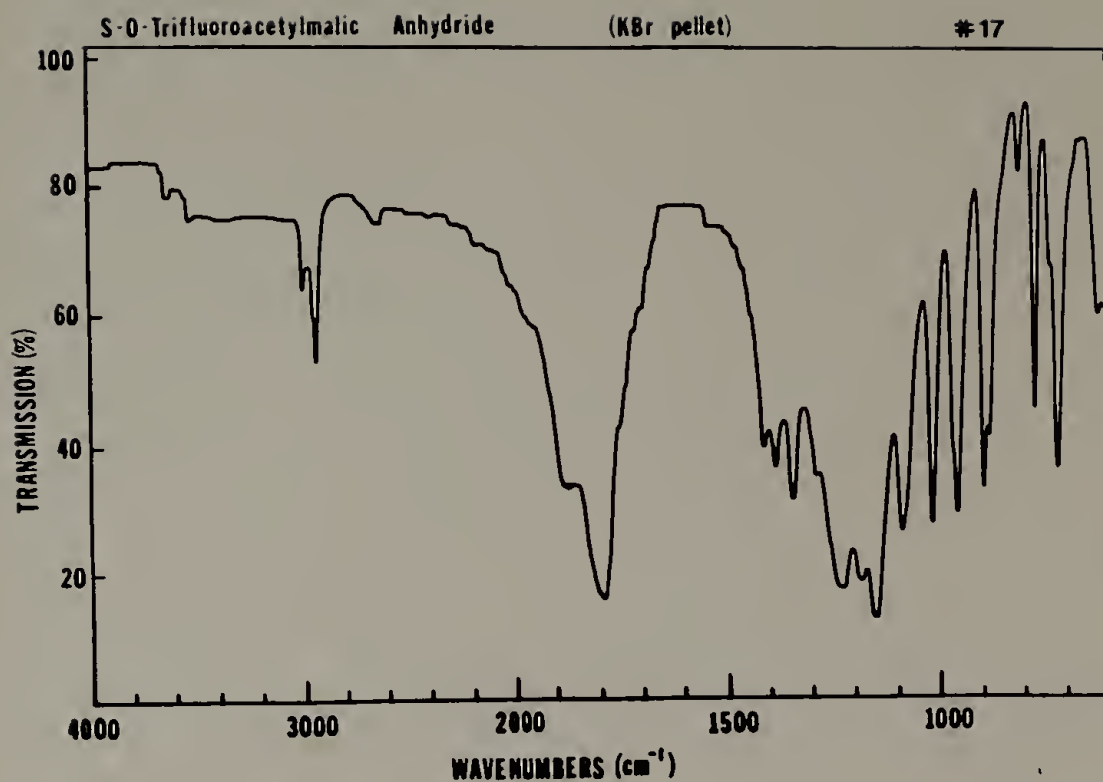


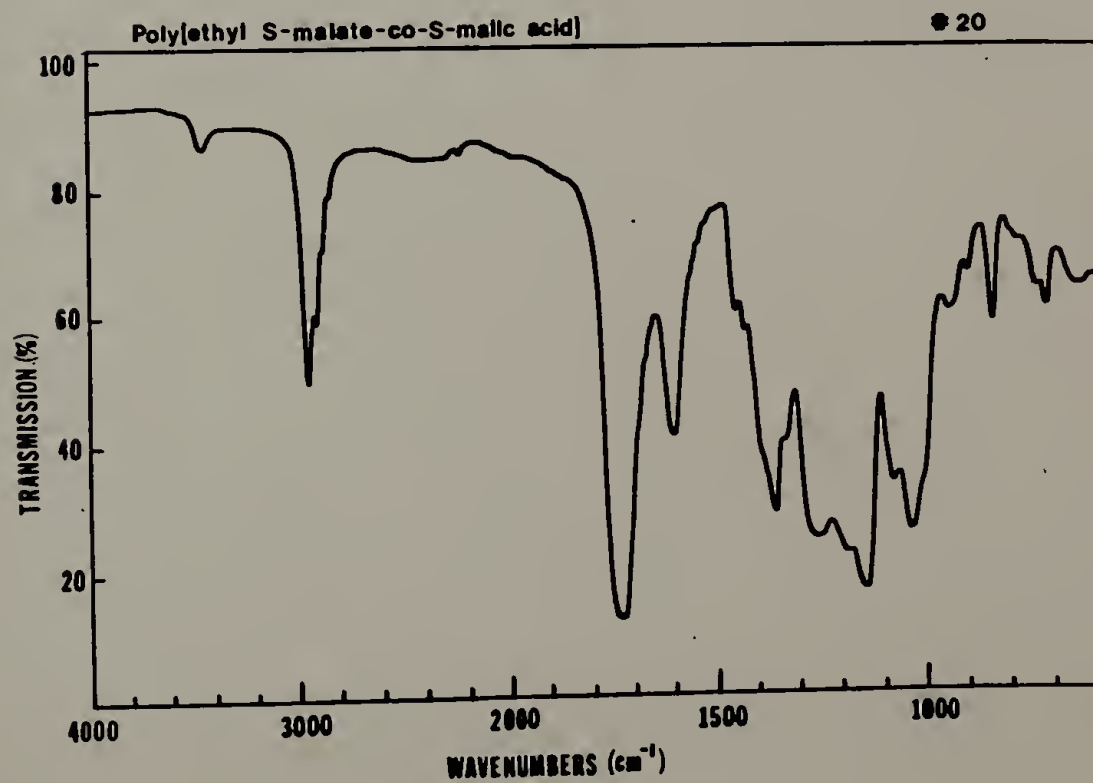
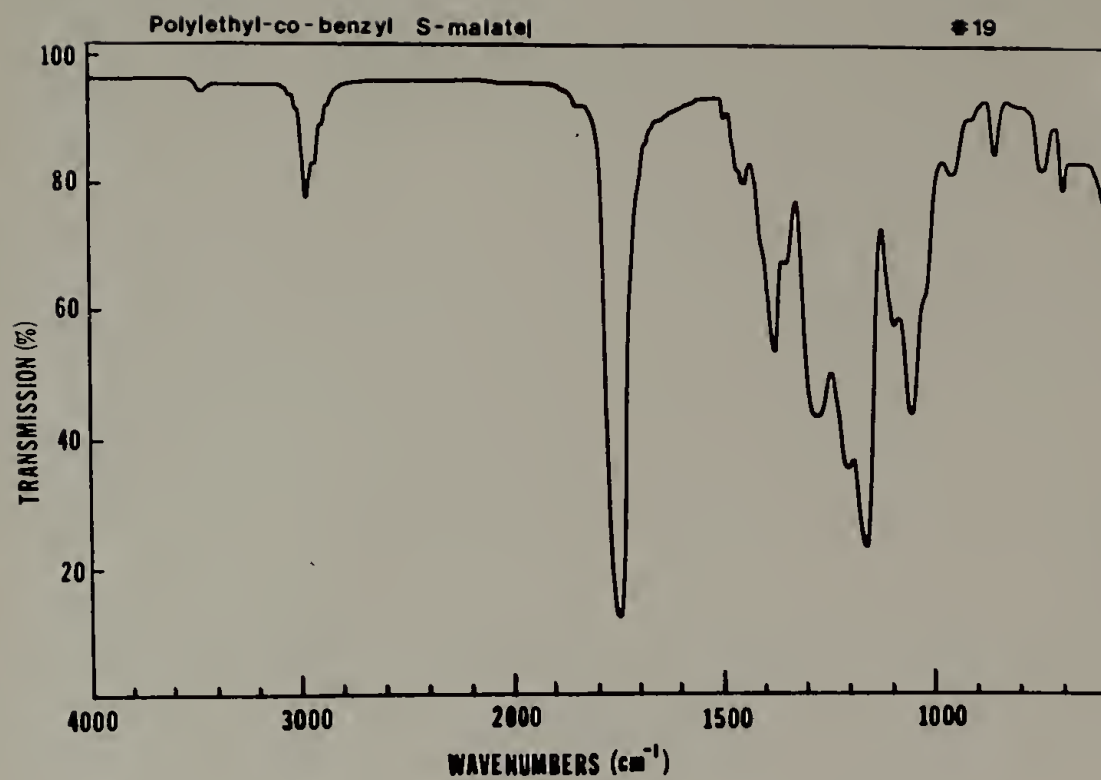












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