INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

U·M·I

University Microfilms International
A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
313/761-4700 800/521-0600

Order Number 1356784

Terminal-functionalized poly-t-butyl acrylates via living anionic polymerization

Mollberg, William, M.S.

The University of Arizona, 1993

U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

TERMINAL-FUNCTIONALIZED POLY-t-BUTYL ACRYLATES VIA LIVING ANIONIC POLYMERIZATION

by

WILLIAM CARL MOLLBERG

A Thesis Submitted to the Faculty of the

DEPARTMENT OF CHEMISTRY

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE
WITH A MAJOR IN CHEMISTRY

In the Graduate College

THE UNIVERSITY OF ARIZONA

1993

STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: William C Mallory

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

H.K. Hall J.r.

H.K. Hall
Professor of Chemistry

12/9/93

Date

ACKNOWLEDGMENTS

The author wishes to thank Prof. H.K. Hall, Jr. for his guidance, encouragement, support and understanding during the course of this research. I am also indebted to Dr. Anne Padias and Dr. Calvert for their help regarding scientific discussions. The United States Army Research Office is acknowledged for their financial support.

TABLE OF CONTENTS

LIST OF TABLES

ABSTRACT

Block copolymers were made containing peptides coupled to a hydrophobic regions that could be cleaved to obtain a hydrophilic region, similar to globular proteins. These polymers were synthesized by anionic polymerization of t-butyl acrylate followed by coupling with polypeptides. Anionic polymerization allowed control of molecular weight, polydispersity as well as end groups of the polymer. The polymer was terminated with a carboxylic acid or a primary amine, the desired end groups needed to couple this polymer to peptides by conventional peptide coupling reactions. Upon hydrolysis of the esters, the resulting hydrophilic-hydrophobic polymer may be used to act as specific channels through lipid membranes, to increase the solubility of peptides in hydrophilic and hydrophobic solvents or to modify particle growth of inorganic salts.

CHAPTER ONE

INTRODUCTION AND RESEARCH PLAN

The synthesis of block copolymers containing hydrophilic and hydrophobic sequences mimicking bilayers has great interest, but the technology has not yet been available. One would envision these copolymers could be used to form a controllable ion channel in a lipid bilayer or to help promote the nucleation and growth of inorganic particles. Several barriers must be overcome, including monodispersity and control of degree of polymerization of the hydrophilic region, functionalization of these polymers to allow for coupling with a polypeptide to achieve a polymer mimicking such a bilayer.

Recently, monodisperse derivatives of poly(α,l-glutamic acid) were synthesized by Tirrell et al. showing the ability to produce complex biological macromolecular architectures.[1] A synthetic bilayer however, requires a hydrophobic core, amino acids, surrounded by hydrophilic residues with ionizable acid or base groups that ensure water solubility.

The synthetic approach with the most promise of achieving such hydrophilic moieties that are monodisperse and with a control of molecular weight, is the use of acrylic monomers. Anionic polymerization will allow us to polymerize these acrylates with great control of macromolecular architecture which after hydrolysis will result in a polymer mimicking the ends of a protein bilayer in both molecular size and physical properties. When these monomers are polymerized, the ends of the polymers will be functionalized so that they may be coupled to amino acids which will form the hydrophobic core of the bilayer.

Controlled Polymer Synthesis

Very few methods are available for the controlled polymerization of macromolecules. In the Merrifield synthesis peptides were formed by sequential addition of amino acids to a chain attached to a surface.[2] The reliability of producing polymers of more than ten units long is limited, due to reduction in the probability of amino acids attacking one another as the polymer grows. This method however, provides us with the means of coupling the hydrophilic polymer synthesized from the acrylates to a polypeptide.

The most widely used method of controlled synthetic polymerization is via a "living" polymerization. Szwarc showed that "living" polymerization of styrenes and dienes could be achieved using anionic methods.[3] Anionic polymerizations are initiated by an anion, such as n-butyl lithium, and carried out in a stable polar solvent such as tetrahydrofuran. The advantages of living polymerization conditions over conventional methods are that the monomers add stepwise to each chain at the same rate since there is no termination or chain transfer, resulting in a monodisperse polyanion which can be functionalized. One also has control over the degree of polymerization by decreasing or increasing the amount of initiator used with respect to the monomer, and in certain cases may manipulate stereochemistry, polymer architecture and sequence distribution. In this work we used t-butyl acrylate under anionic polymerization conditions in the presence of lithium chloride. The advantage of t-butyl acrylate is that backbiting and termination may be avoided and the t-butyl esters may be hydrolyzed to form the hydrophilic section of bilayers. Lithium chloride is used to stabilize the living anion. Methyl acrylate has also

been polymerized with the above properties using group transfer polymerization, but is technically more difficult to accomplish.

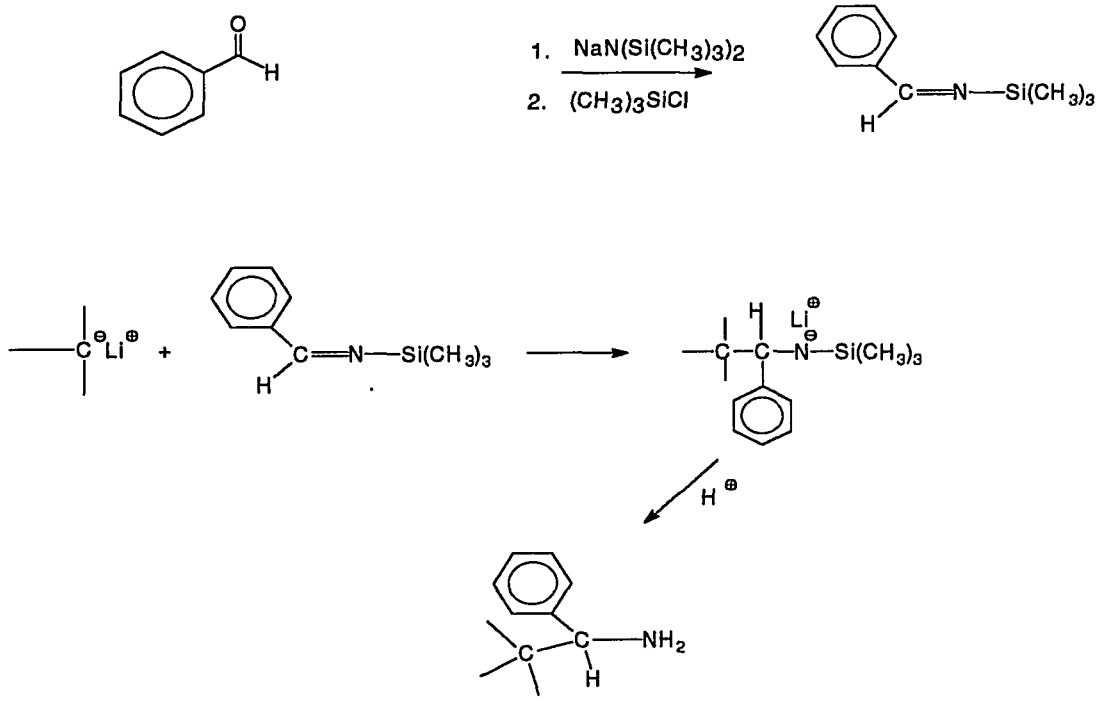
Functionalization

There are two different end group functionalities desired for coupling, the first one containing a carboxylic acid that may then be coupled to a free amine on a peptide, or the other functional end group would be a primary amine that may be coupled a peptide containing a free carboxylic acid while having a protected amine. The synthesis of a carboxylated polyisoprene has been reported in the literature by Fetters et al.[4] They found that gaseous carbon dioxide addition to a "living" anionic polymerization of isoprene resulted in a carboxylated product. The procedure used avoided the formation of coupled by-products known to form in aliphatic hydrocarbon solvents. In THF, a polar solvent as well as aromatic solvents, the living end group should have a small equilibrium constant for the dissociated state, avoiding coupling. Wyman et al. however found that direct reaction of living polymer(polystyrene) in benzene with carbon dioxide gas resulted in 60% carboxylate polymer, the remainder being the dimer ketone (30%), and the trimer carbinol (10%) as shown below[5]

$$PLi + CO_2 \xrightarrow{} \xrightarrow{H_3O^+} PCO_2H + P_2CO + P_3COH$$

where P represents a polymeric chain. The yield of the carboxylate polymer increased to 80% if the living polymer was poured over solid carbon dioxide. Yin et al. recently reported that quantitative carboxylation can be achieved by adding sufficient quantities of Lewis bases such as tetrahydrofuran or N,N,N',N'-tetramethylethylenediamine prior to addition of gaseous carbon dioxide.[6]

The synthesis of primary amine terminated polymers obtained from living anionic polymerization has just recently been reported by Hattori using polyisoprene with a protected aminating reagent: N-(benzylidene)trimethylsilylamine.[7,8]

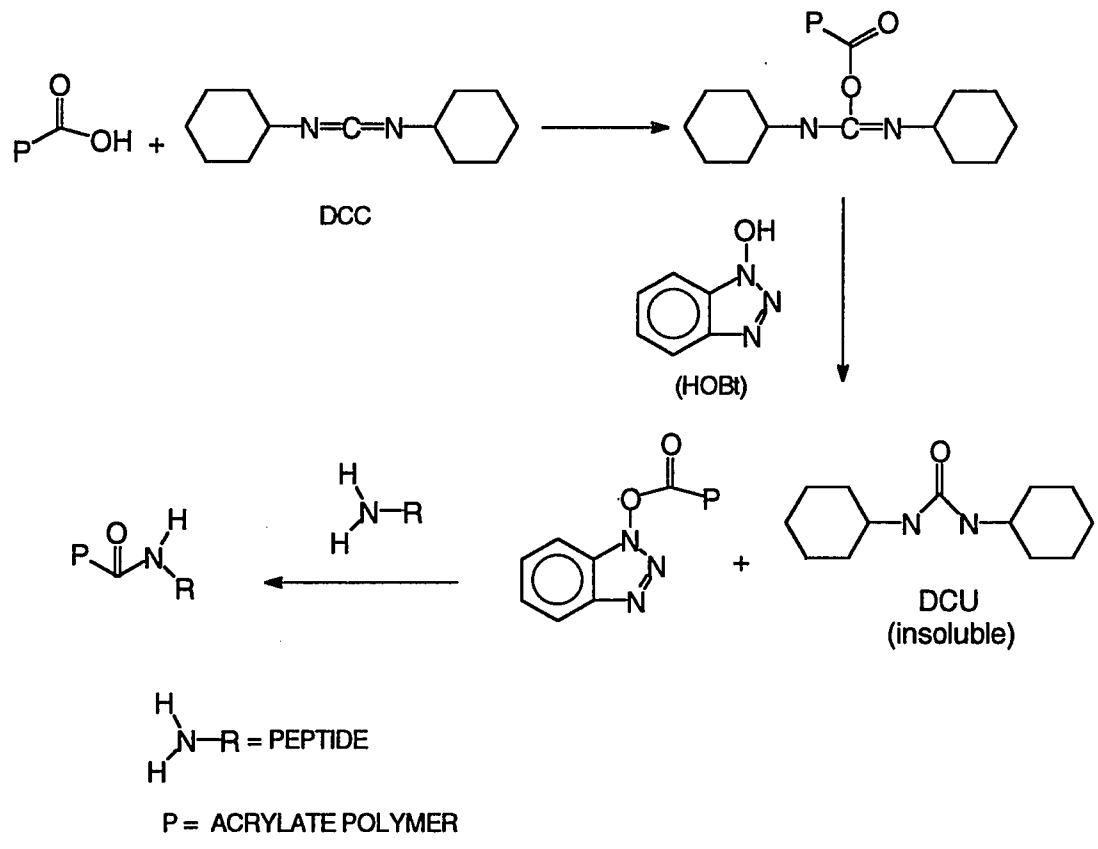


With lithium as a gegen ion the yield was reported quantitative. The use of sodium or potassium naphthalides as gegen ions was reported to result in lower yields due to the more reactive counter ions attacking the N-Si bond. The authors also reacted ethyl bromide with sodium bis(trimethylsilyl)amide and found that the terminal silylated primary amine was only 60% efficient.[7,8] Thus, we will use our polyacrylate terminated with the aldimine derivative, to avoid side reactions such as elimination and substitution.

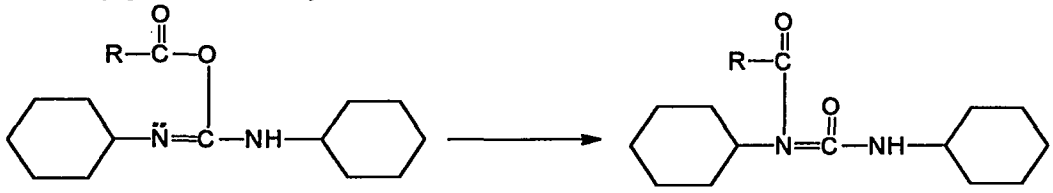
Coupling

The resulting functionalized polyanion meets the requirements set forth for a monodisperse polymer of predetermined molecular weight, that may then be coupled to a polypeptide to form a synthetic bilayer. Coupling of the carboxylic acid and the primary amine terminated polymers to a polypeptide to form a peptide bond will be attempted using dicyclohexylcarbodiimide (Scheme One) in the presence of hydroxy benzotriazole.

SCHEME ONE

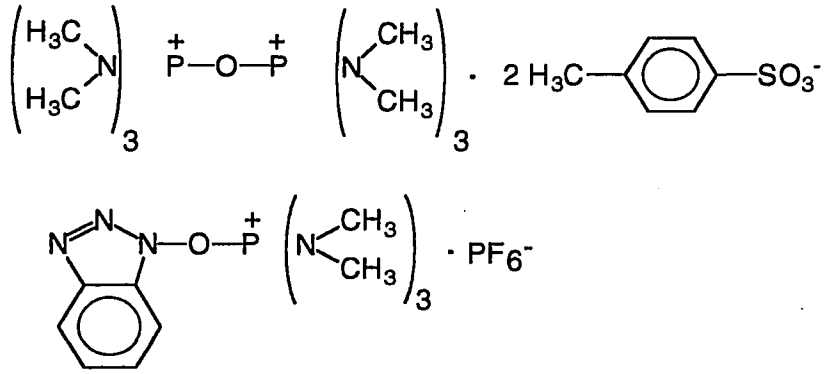


The use of a carbodiimide allows the carboxylate and the amine component to couple quantitatively via an active ester. The advantage of using the above method is that the coupling reagents can be added to the mixture of the carboxyl component and the amine component, allowing coupling to proceed in one reaction pot. The O-acyl-isourea, the intermediate formed in the addition of carboxylic acid to the carbodiimides, provides the activation required through the N=C bond for coupling to occur. The simplicity of this method for peptide bond formation has its problems when chiral purity is desired. The activated intermediate is very reactive causing side reactions to occur. Also the nucleophilic center on the O-acylisoureas competes with the amine component leading to unreactive by-products, N-acylureas:



Both racemization and N-acylurea formation are suppressed by adding 1-hydroxybenzotriazole (HOBt), an auxiliary nucleophile, which yields an O-acyl-1-hydroxybenzotriazole. This nucleophile helps to decrease the concentration and the lifetime of the O-acylisourea and thus diminishes racemization and oxygen-nitrogen acyl migration.

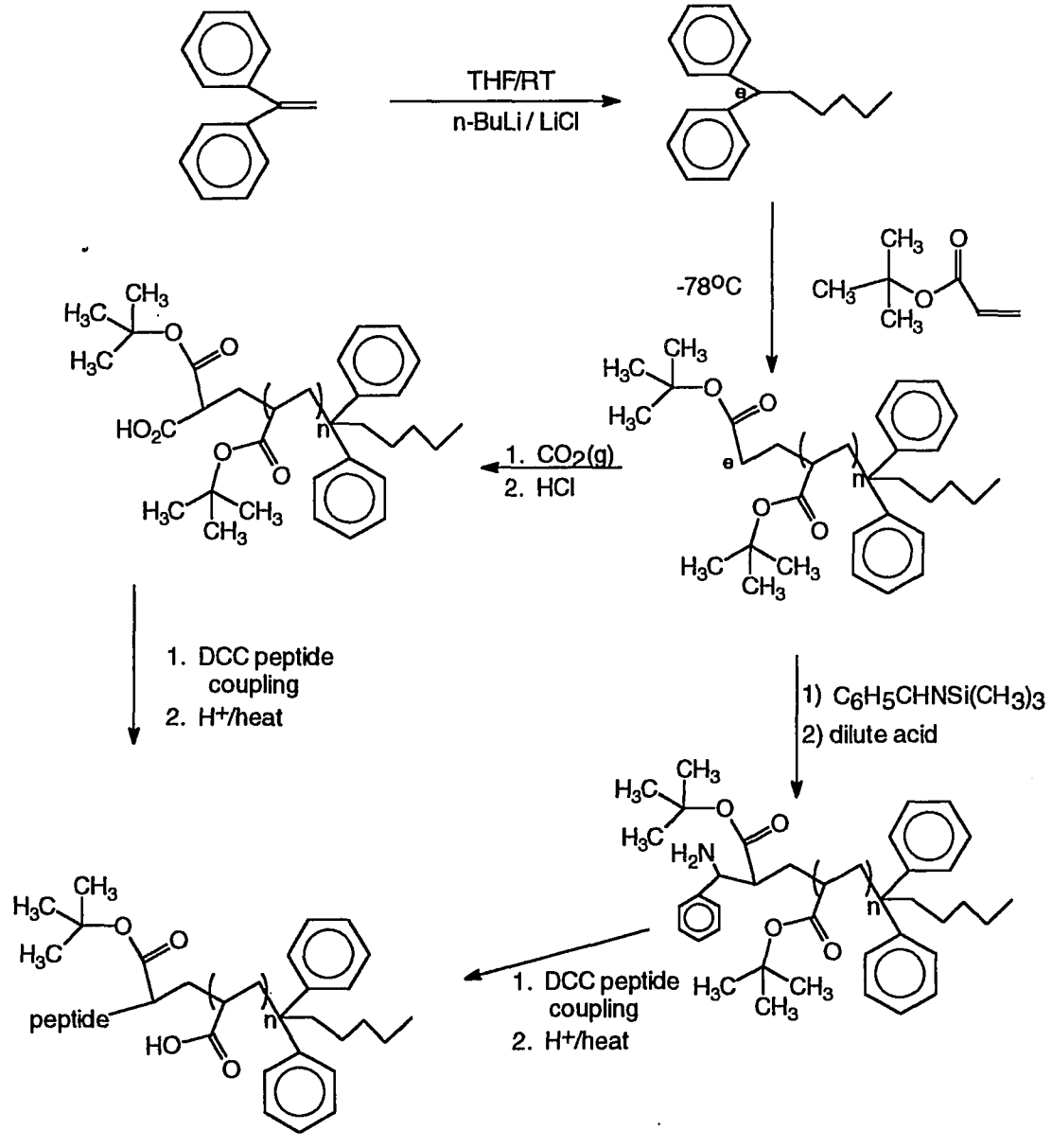
Other methods used for peptide bond formation include phosphonium derivatives or the BOP reagent, benzotriazolyl N-oxytri-dimethylamino-phosphonium hexafluorophosphate, but DCC still remains the most popular method.[9]



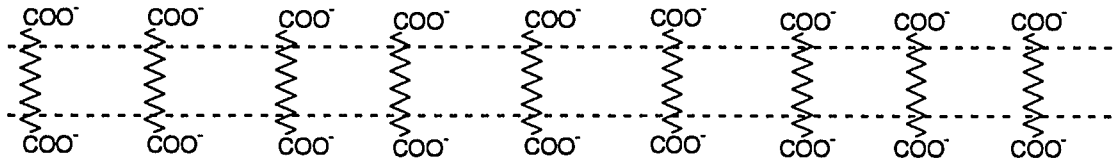
Hydrolysis of Esters

When coupled to a polypeptide, approximately the thickness of a lipid bilayer, one may hydrolyze the esters off the acrylic hydrophobic polymer, resulting in a hydrophilic polymer coupled to a hydrophobic polypeptide (Scheme Two).

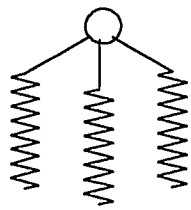
SCHEME TWO



The hydrophilic-hydrophobic block copolymers can then be coupled into groups of several chains or into clusters to make channels.



Polymer structure mimicking bilayer membrane



Cluster

In the future the resulting copolymer can then be tested to show that it possesses similar activity to that of a globular protein. This can be accomplished by showing that the hydrophobic end groups are water soluble. Future applications include incorporating specific amino acids in the hydrophobic region that would produce particle growth and design clusters by adding ion binding groups to helices. This would be similar to the biological mineralization by proteins.[10]

CHAPTER TWO

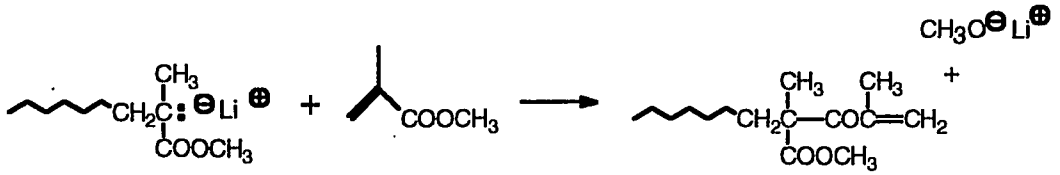
RESULTS

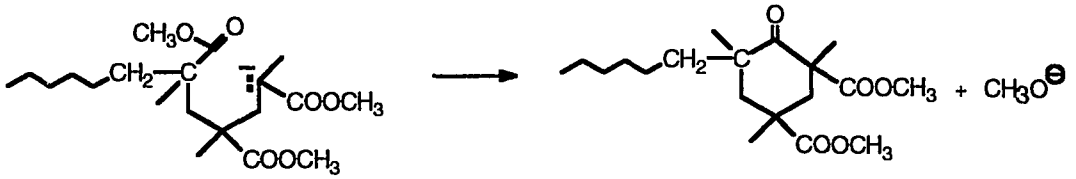
Living Anionic Polymerization

The living anionic polymerization was attempted with t-butyl acrylate since the t-butyl esters are easily hydrolyzable by acid. Anionic polymerization of t-butyl acrylate requires scrupulous purification of all monomers and solvent. One can obtain pure alkyl acrylate monomers by using trialkylaluminum compounds.[11] The trialkylaluminum reacts with alcohols and moisture, resulting in anionic polymerization grade t-butyl acrylate monomer. Purification of the monomer with triethylaluminum and calcium hydride was monitored by molecular weight and polydispersity determination using size exclusion chromatography(SEC) of the polymer obtained. For molecular weights under 5,000 the polydispersities remained the same for t-butyl acrylate purified with calcium hydride only or by the triethylaluminum method, so the simpler calcium hydride procedure was used. If monodisperse high molecular weight acrylates were desired, then the triethylaluminum would be superior. Calcium hydride is incapable of fully drying the somewhat hydrophilic acrylates according to McGrath et al.[9] Also, the commercial synthesis of acrylates involves the transesterification with an alcohol. Calcium hydride is not capable of removing the alcoholic terminating impurities. This requires the use of the trialkylaluminum, which forms a yellow complex when the monomer is dry.

The anionic polymerization was carried out at -78^{o}C in tetrahydrofuran(THF). Tetrahydrofuran was purified by multiple distillations over sodium and potassium under argon atmosphere and was transferred by gas tight syringes. Problems with nucleophilic

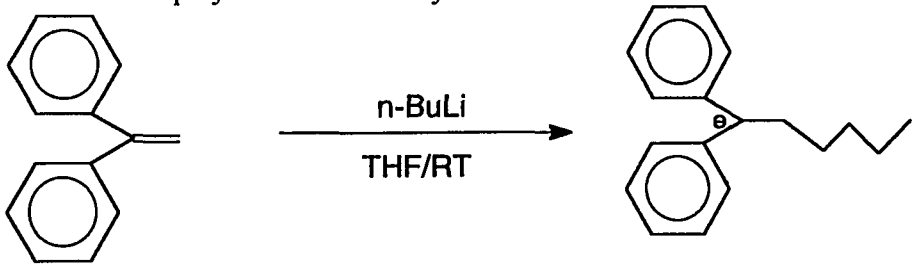
side reactions with the carbonyl are well known for methyl esters. Since only low molecular weight poly-t-butyl acrylate was desired, approximately 1000-1500, the monomer was purified by refluxing over calcium hydride for three hours, followed by distillation under inert atmosphere.





The use of t-butylacrylate, low temperatures and polar solvents helps to avoid nucleophilic and backbiting side reactions reported for methyl esters.[16]

Alkyl lithium initiators based on 1,1-diphenylethylene have been shown to be useful for the anionic polymerization of acrylates.[12,13]



The resulting anion is more hindered and less basic than butyl lithium, preventing nucleophilic attack on the carbonyl. Diphenylethylene is incapable of homopolymerization due to steric hindrance. An attempt using α-methyl styrene instead of 1,1-

diphenylethylene resulted in higher bimodal polydispersities and molecular weights than the 1,300 expected following a procedure similar to Hirai et al.[14]

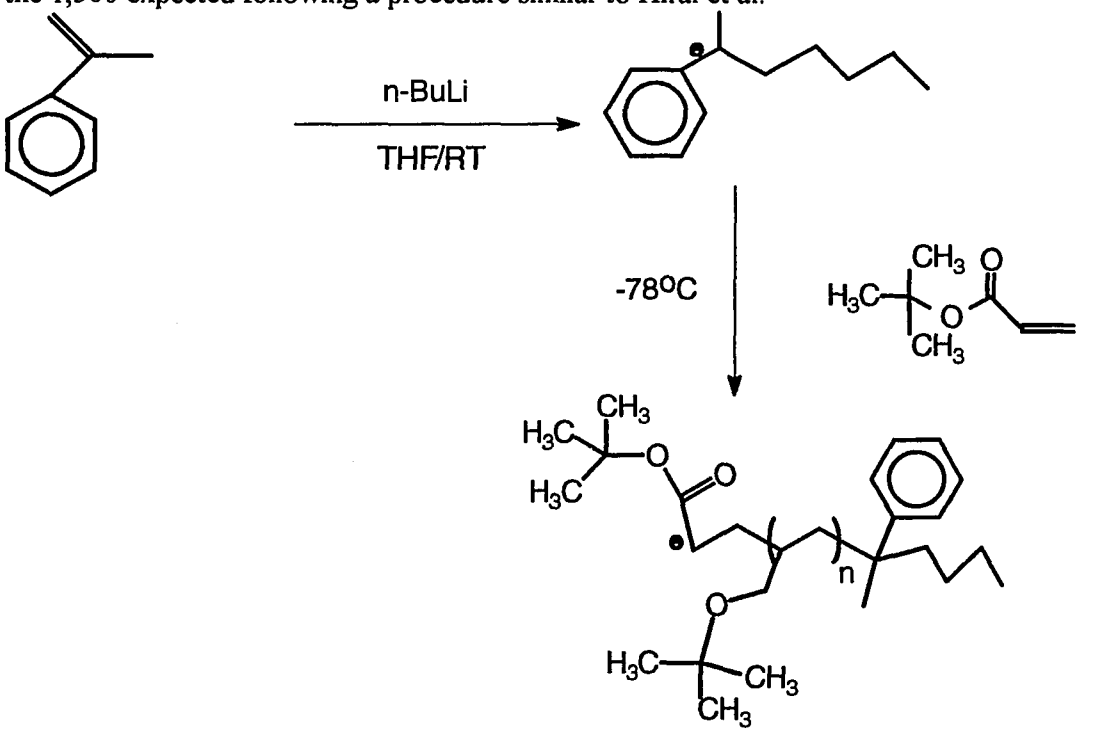


TABLE ONE

MOLECULAR WEIGHTS OF POLY-t-BUTYL ACRYLATE

INITIATED BY LiCl / α-METHYL STYRENE

	Mn	Mw	PDI
Peak 1	117,000	266,000	2.28
Peak 2	9,300	9,900	1.07

Munk et al. also found that the styryl anion, unlike 1,1-diphenylethylene, was "too energetic" resulting in nucleophilic attack at the ester.[15] Proton nmr, confirmed possible homopolymerization of the α-methylstyrene which would account for the the higher than expected molecular weight and polydispersities SEC data obtained (Table One). The carbanion was stabilized by adding 1.1 equivalents of lithium chloride with respect to the

initiator. Subsequent polymerizations indicate that a five fold excess of lithium chloride is needed, to obtain a monodisperse po2lymer. This data suggests that lithium chloride forms a complex in solution, requiring the excess equivalents to stabilize the living anion. Teyssie et al. showed that when the carbanion is complexed with excess lithium chloride, attack was decreased.[16,17] Teyssie also found that when no lithium chloride was used the anionic polymerization of t-butyl acrylate resulted in a broad multimodal molecular weight distribution (3.60) similar to our data.[18]

The addition of t-butyl acrylate to the diphenyl anion complexed with lithium chloride, formed by the addition of n-butyl lithium to diphenylethylene in dry tetrahydrofuran, resulted in monodisperse polyacrylates with control of molecular weight providing conditions were carefully controlled. The addition of t-butyl acrylate was carried out at -78°C using a 10 wt % solution in tetrahydrofuran to allow a slow stepwise addition to occur. After ten minutes the reaction was terminated with methanol, carbon dioxide or n-(benzylidene)trimethylsilylamine depending on the desired end group.

TABLE TWO

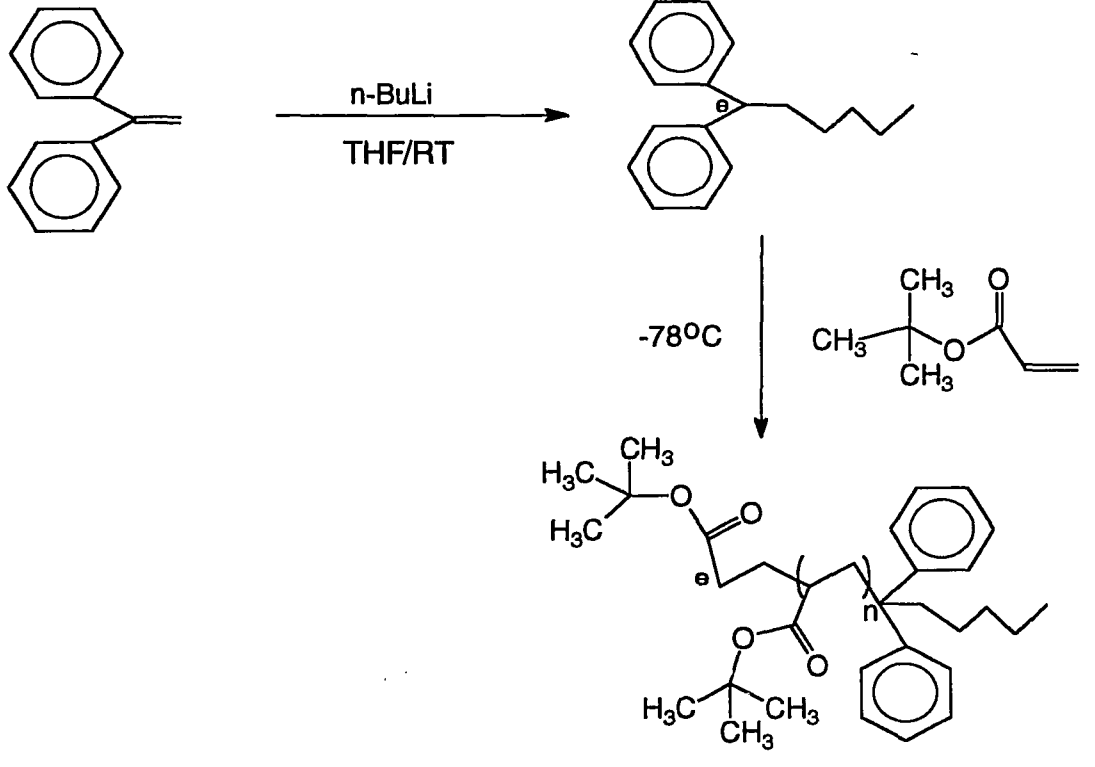
MW DATA FOR POLY-t-BUTYL ACYLATE

DETERMINED BY SEC

Poly-t-Bu-Acrylate*	M_w	M_n (^1H)	PD	Theoretical
H	1100		1.04	
CO_2H	2800	1400	1.07	1514

*End Group

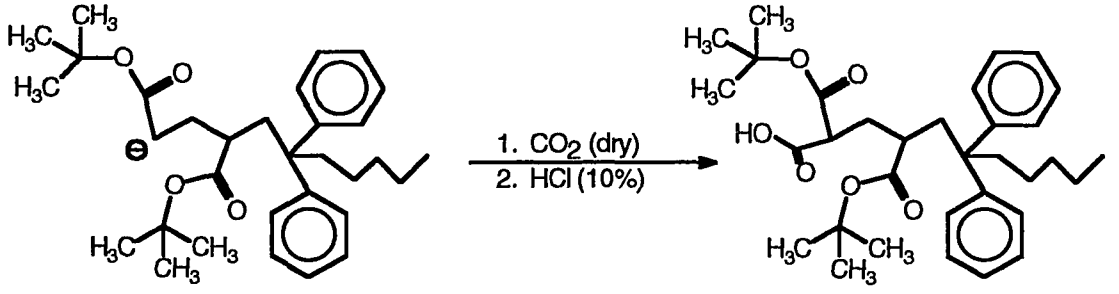
Upon addition of terminating agent at -78°C, the reaction was concentrated and the resulting product was precipitated in a 50/50 methanol water solution.



The aromatic protons of 1,1-diphenylethylene were used to determine the degree of polymerization(M_n) by ^1H nmr. Reliable estimates were obtained when the protons of the aromatic peaks were compared to the integrated intensity of the t-butyl ester. Size exclusion chromatography(SEC) analysis resulted in higher degrees of polymerization data perhaps due to the use of polystyrene standards instead of acrylates. Size exclusion chromatography was still valuable in showing that when lithium chloride and 1,1-diphenylethylene were used monodisperse poly-t-butyl acrylate was obtained.

Functionalization

The carbanion obtained from the living anionic polymerization of t-butyl acrylate was used to functionalize the polymer. The desired end groups are either carboxylic acid or primary amine allowing coupling to a polypeptide. When gaseous carbon dioxide was introduced rapidly into our reaction vessel while vigorously stirring, it was found that a carboxylate polyacrylate was obtained in good yield, in contrast to that reported by Goethals.[7]



The results supported the data obtained from Fetters and Yin using the polar THF as the solvent, avoiding coupling.[4,6]

Titration of the carboxylic acid was accomplished using a procedure developed by Kiljunen et al. We found that 90% carboxylation was achieved.[19]

TABLE THREE

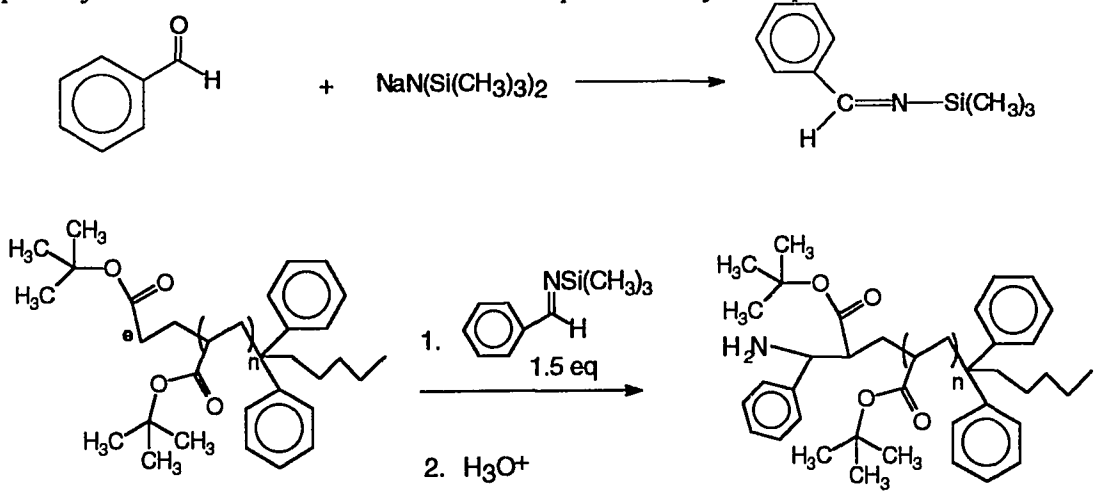
CALCULATED VS. INTEGRATED MOLECULAR WEIGHTS

Theoretical	741 g/mole
Proton NMR	852 g/mole

Carbon 13 NMR analysis of the carboxylate polymer also showed the presence of a carboxylic acid carbonyl at 196 ppm.

The efficiency of preparing carboxylated polymers using gaseous carbon dioxide was found to be dependent upon the experimental conditions, stirring and the rate of addition of carbon dioxide. If experimental conditions were not anhydrous one found that coupling occurred, seen in the doubling of molecular weight (SEC). The disadvantages reported in the literature, the inability to obtain quantitative coupling with peptides (see below) and the inadequate selection of commercial polypeptides with the carboxylate protected resulted in another method being sought for functionalizing the carbanion.

The other useful functional end group, a primary amine, was synthesized so it may be coupled to a carboxylic acid terminated polypeptide. Primary amine-terminated polymers, have eluded anionic synthesis because the amine hydrogens kill living polymer chains.[20] The use of a protected amine, n-(benzylidene)trimethylsilylamine, prevented side reactions associated with other methods from occurring, and resulted in 93% yield of primary amine. The results are similar to the quantitative yield reported in the literature.[19]



When the N-(benzylidene)trimethylsilylamine was added to the polymer, a primary amine terminated poly-t-butyl acrylate was obtained after acid treatment. Integration of the aromatic region as well as the trimethylsilyl protons, resulted in consistent measurements of the degree of polymerization.

TABLE FOUR

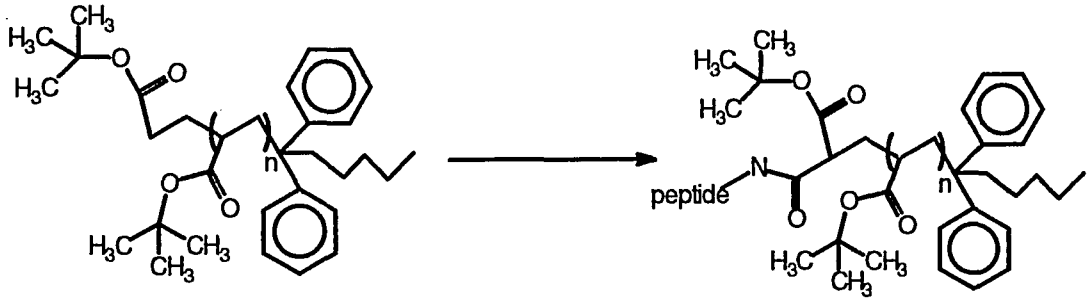
DEGREE OF POLYMERIZATION USING ^1H INTEGRATED PHENYLS

Polymer	DP
$Si(CH_3)_3$*	12.0
NH_2	12.8
$Si(CH_3)_3$	11.1

* using $Si(CH_3)_3$ integration

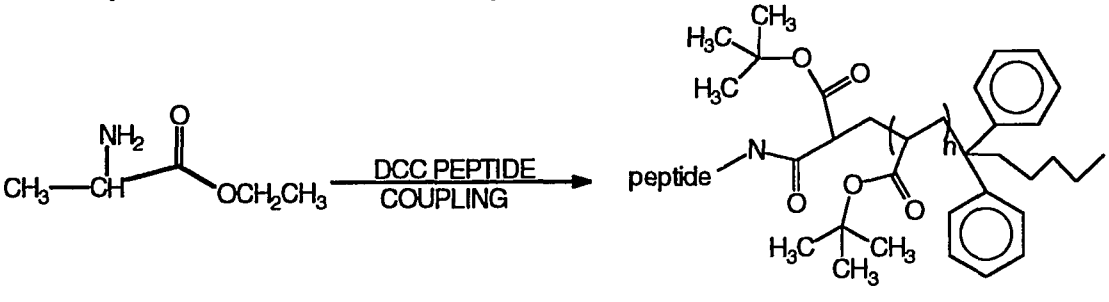
Peptide Coupling

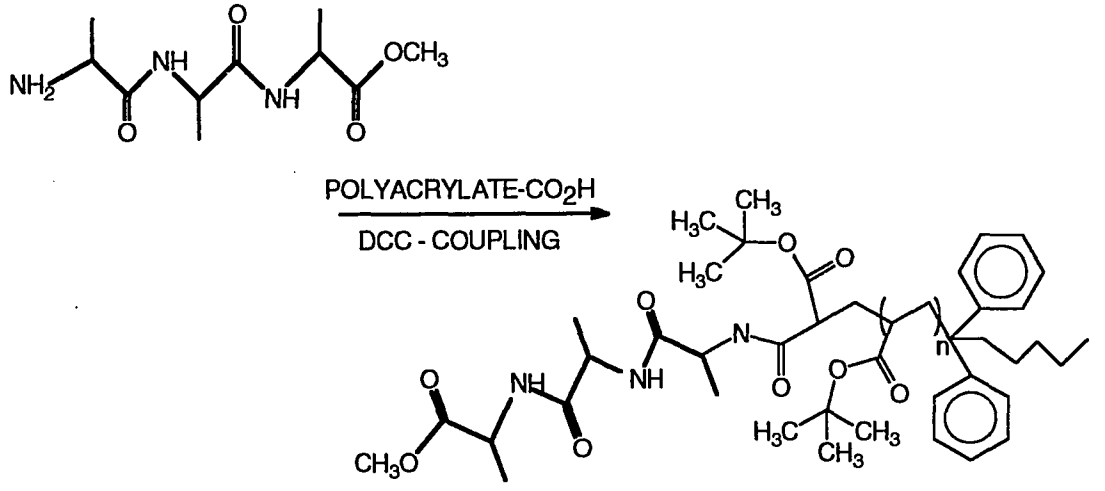
The poly-t-butyl acrylate terminated with either a carboxylic acid or a primary amine was then coupled to amino acids or polypeptides using dicyclohexyl carbodiimide (DCC) peptide procedure. Coupling succeeded with polybutadiene carboxylate with p-methoxyaniline and 1,6-hexanediamine, confirming that the dicyclohexylcarbodiimide peptide coupling reaction was quantitative.



Dicylohexycarbodiimide was found to proceeded quantitative also with l-alanine ethyl ester. The reaction was allowed to proceed for one day in dichloromethane or DMF at room temperature. Dichloromethane was preferred due to its easy purification. Workup included many extractions with brine and aqueous hydrochloric acid to ensure that all of the dicyclohexyl urea and unreacted amine were removed. Proton nuclear magnetic resonance showed that coupling occurred with the carboxylic acid end group on the polymer.

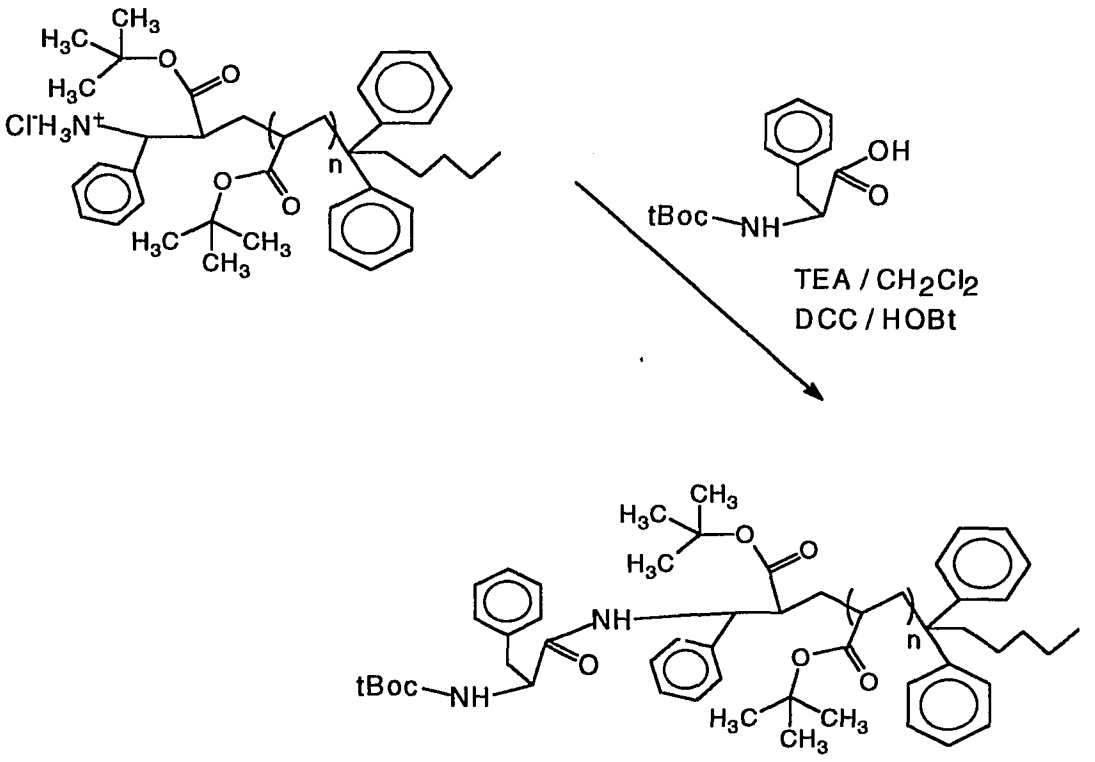
Attempted coupling with l-alanyl-l-alanyl-l-alanyl methyl ester, however, was only 40 percent complete according to proton nmr. The lack of complete coupling may be caused by steric hindrance of the carboxylic acid.





Titration and ^{13}C nmr showed the presence of a carboxylic acid end group prior to peptide coupling. The results indicate that the carboxylic acid group might be too crowded to effectively attach polypeptides or the polymer three dimensional structure is globular in solution preventing coupling with the carboxylic acids inside the coil.

The lack of quantitative yield and the small choice of commercial protected carboxylic amino acids led us to investigate coupling the polymer with the primary amine-terminated poly-t-butyl acrylate with amine blocked peptides.



The polymer was coupled with N-(tert-butoxycarbonyl)-l-phenylalanine and was identified using proton and carbon 13 NMR analysis.

TABLE FIVE

Carbonyl Analysis of Coupled Amino Poly-t-Butyl Acrylate

ppm	Functional Group
174.0	t-Butyl esters
171.0	t-Boc
168.5	Amide (peptide bond)

Carbon NMR analysis also showed extra peaks in the aromatic region for the phenyl on the n-(benzylidene)trimethylsilylamine moiety as well as a greater integration for the phenyl region in the proton nmr.

CHAPTER THREE

EXPERIMENTAL

Instrumentation

All proton and carbon nmr were taken on a Bruker WM250 nuclear magnetic resonance spectrometer at 250 MHz using TMS as an internal reference. Number average molecular weights and polydispersities were measured on Phenomenex Phenogel $10\text{-}10^{-3}$ A and a $10\text{-}10^{-4}$ A columns calibrated with polystyrene standards using tetrahydrofuran as the mobile phase and a RI detector.

Solvents

The polymerization solvent, tetrahydrofuran(Aldrich), was purified by double distillation over sodium and potassium under argon, using benzophenone as an indicator for dryness.

Reactants

The monomers, t-butyl acrylate and 1,1-diphenylethylene(DPE), were purchased from Aldrich, distilled from calcium hydride and stored under argon at 0^{o}C. The reagents were re-distilled prior to use. The initiators, sec-butyl lithium and n-butyl lithium, purchased from Aldrich, as solutions in hexane, were used as received. The initiators were titrated following a procedure of Kiljunen et al. using 1-pyreneacetic acid.[18] Lithium

chloride purchased from Aldrich was dried in an oven at 110 ^{o}C for a week, prior to being used.

Synthesis of Poly-t-butyl acrylate Carbanion

All glassware involved in the polymerization reactor were thoroughly cleaned in a sulfuric acid bath, followed by a potassium hydroxide bath and dried overnight at 110^{o}C. The glassware was then assembled, dried lithium chloride was added (0.7g, 0.0165 moles) and the resulting apparatus was flame-dried under vacuum. The vacuum-flame-argon purge cycle was repeated twice to ensure dryness. The argon was dried by passing through a drying column containing sodium sulfate and P_2O_5.

Dried tetrahydrofuran (100 ml) was added to the reaction flask through a rubber septum and a stop-cock using a cannula. The initiator, n-butyl lithium (2.28M, 0.00285 moles) was added using an air tight syringe. 1,1-Diphenylethylene (0.43 ml, 0.00246 moles) was then added, resulting in a deep red diphenyl anion. After cooling the reaction vessel to -78^{o}C the monomer, t-butyl acrylate (3.28 ml, 0.0224 moles), was added dropwise after diluting with 25 ml of THF. The red color disappeared immediately during the addition of the monomer, indicating fast initiation. The addition of the monomer was carried out for two minutes to avoid exotherms that could cause side reactions with the acrylate carbanion. The ratio of initiator and monomer used were calculated to obtain a molecular weight of 1403.

Functionalization

Once the carbanion was formed, the polymerization was terminated by end-capping the polymer with a carboxylic acid or a primary amine, the desired functionalities for peptide coupling.

The carboxylated poly-t-butyl acrylate was synthesized using a modified procedure of Fetters et al.[4] To the polymerization reactor gaseous anhydrous carbon dioxide was introduced for a period of fifteen minutes, followed by acidification with aqueous HCl (10 vol %). The product, a sticky goo, was precipitated from 50/50 methanol-water to remove the lithium chloride, and then thoroughly dried under vacuum.

The extent of carboxylation was determined by titration with 1-pyreneacetic acid. Equal molar amounts of polymer and acid were added to a flame dried flask. n-Butyl lithium of known concentration was slowly added. The intense red dianion of the pyrene acetic acid appeared at the end point, indicating all of the carboxylic acids were converted into the lithium salts.

^{1}H NMR ($CDCl_3$) : δ 1.46 (s, t-butyl), 1.56 and 2.17 (broad, CH_2, and CH), 7.33 (s, phenyls) ppm.

^{13}C NMR ($CDCl_3$) : δ. 13,19, 80, 114, 126, 128, 131, 150, 174 and 176 (esters), 196 (acid) ppm.

SEC : MW = 2800, PDI = 1.07

N-(benzylidene)trimethylsilylamine was prepared using a modified procedure of Hirao.[21,22] To a flamed dried flask containing 100 ml of freshly distilled benzene was added sodium bistrimethylsilyl amide (36g, 0.2 moles) at 50°C. Benzaldehyde (21.2g, 0.2 moles) diluted with 25 ml of benzene was then added, and the resulting solution was

heated at 70°C for three hours. Trimethylsilyl chloride (21.6g, 0.2moles) in 25 ml of benzene was then added. The reaction was refluxed for three hours, cooled, filtered, concentrated, distilled using a Vigreux column (0.8mm Hg, 55°C). N-(Benzylidene)trimethylsilylamine was obtained in 62% yield. All of the reagents used in its preparation were purchased from Aldrich.

NMR ($CDCl_3$): δ 0.25 ($Si(CH_3)_3$, 9H), 7.4 and 7.8 (phenyl, 5H), 9.0 (CH, 1H) ppm.

Termination of the telechelic t-butyl acrylate carbanion with the protected amine was accomplished by adding 2.0 equivalents of the Schiff's base to the "living" polymerization at room temperature. The reaction was allowed to proceed for three hours before quenching with methanol / water. The reaction was then concentrated, resulting in an orange oil.

^1H NMR ($CDCl_3$) : δ 0.1 ($Si(CH_3)_3$),.1.4 (t-butyl), 1.8 and 2.1 (CH_2 and CH polymer), 3.7, 7.3 (phenyls).

Hydrolysis of the trimethylsilyl substituent was accomplished by dissolving the polymer in dicloromethane followed by the addition of 5 vol. % HCl. The resulting polymer was precipitated in 50/50 methanol/water, resulting in a gooey sticky white polymer floating on the surface.

^1H NMR ($CDCl_3$) : δ 1.4 (t-butyl), 1.8 and 2.2 (CH_2 and CH polymer), 7.1-7.3 (phenyls).

^{13}C NMR ($CDCl_3$) : δ .27.8, 35.0, 41.3, 41.6, 42.0, 80.1, 80.2, 125 127, and 128 (phenyls), 173 (carbonyl esters).

Peptide Coupling

The amino acid component (1 eq.), N,N-diisopropylethyl amine (1 eq), 1-hydroxybenzotriazole (2 eq.) and dicyclcohexylcarbodiimide (1.1 eq) were added sequentially to a solution of the carboxylated or amino poly-t-butyl acrylate dissolved in freshly distilled dichloromethane. The reaction was stirred at room temperature under a positive pressure of argon for 48 hours. The N,N-dicyclohexylurea was filtered off followed by concentrating. The resulting solid was dissolved in ethyl acetate and extracted consecutively with brine, 5% sodium bicarbonate, 5 vol % HCl and brine. The organic phase was dried with magnesium sulfate, filtered, concentrated to dryness and placed under vacuum overnight before analysis.

Coupling of carboxylated poly-t-butyl acrylate with ethyl ester alanine:

^{1}H NMR ($CDCl_3$) : δ . 1.5 (t-butyl), 2.2, 3.45 (CH, amino acid), 4.2 (CH_2 of amino ester), 7.38 (phenyls) ppm.

Coupling of carboxylated poly-t-butyl acrylate with l-alanyl-l-alanyl-l-alanyl methyl ester:

^{1}H NMR ($CDCl_3$): δ..1.4 (t-butyl), 1.8-1.9 and 2.2 (CH and CH_2 broad), 3.3 (CH amino acid), 3.7 (methoxy), 4.2 (quartet), 7.1-7.5 (phenyls, broad) ppm.

Coupling of amino poly-t-butyl acrylate with t-butoxycarbonyl phenyl alanine.

^{1}H NMR ($CDCl_3$): δ 1.4 (t-butyl), 1.8 and 2.2 (CH and CH_2 broad), 3.3 (CH amino acid), 7.1-8.0 (phenyls) ppm

^{13}C NMR ($CDCl_3$) : δ 14.0, 24.7,27.8, 27.9, 28.0, 33.5, 41.9, 127.6, 127.7, 129.2, 168.5 (amide carbonyl), 171.0 (t-boc carbonyl), 174.0 (esters carbonyls) ppm.

CHAPTER FOUR

CONCLUSIONS

The synthesis of a telechelic polyacrylate, using anionic initiators, mimicking a bilayer was shown to be feasible. The ability to control molecular weight as well as polydispersity was accomplished using lithium chloride. The synthesis of telechelic acrylate polymers containing either a primary amine or a carboxylic acid was also accomplished allowing coupling with amino acids and polypeptides using well established peptide coupling reactions.

The synthesis of monodisperse poly-t-butyl acrylates was found to be dependent upon the amount of lithium chloride used. The use of one equivalent of lithium chloride with respect to the initiator, resulted in a bimodal distribution. However, when five equivalents was used a monodisperse poly-t-butyl acrylate was obtained. The influence of lithium chloride seems to stabilize the living anionic center by forming lithium complexes, requiring the excess equivalents.

The resulting carbanion was then functionalized with a carboxylic acid or a primary amine to allow peptide coupling to be accomplished. Coupling of amino acids occurred quantitatively, however yields dropped when a polypeptide was used to couple with a carboxylic acid. This is probably caused by the carboxylic acid functionality being buried in a globular structure, preventing coupling with the polypeptide. Coupling with other amino acids and polypeptides would be desired to study the feasibility of the peptide coupling with amino and carboxylated poly-t-butyl acrylate. This would enable one to better determine the reactivity of the polymers towards coupling. If it is discovered that coupling is hindered by the t-butyl ester, the carboxylic acid or the primary amine could be extended away from the t-butyl esters to increase the reactivity. Another desired

experiment would be to determine if the polymer is globular or extended in solution, which could have an effect on the reactivity of the polymer during coupling.

The results achieved have met our goal to show that a synthetic bilayer may be achieved by coupling a monodisperse poly-t-butyl acrylate with a boc-amino acid, followed by removal of the tert-butoxy carbonyl protection. The resulting polymer with a free primary amine may then be coupled to a monodisperse carboxylated poly-t-butyl acrylate followed by hydrolysis of the esters, yielding a hydrophilic-hydrophobic-hydrophilic polymer, mimicking a bilayer.

LIST OF REFERENCES

[1]Zhang, G.; Fournier, M.J.; Mason, T.L.; and Tirrell, D.A., Macromolecules, **25**, 3601-3603 (1992).

[2]Merrifield, R.B.; J. Amer. Chem. Soc., **85**, 2140 (1963).

[3]Szwarc, M., "Carbanions, Living Polymers and Electron Trasfer Processes" Wiley, 1968.

[4]Fetters, L.J.; Graessley, W.; Hadjichiistidis, N.; Kiss, A.D.; Pearson, D.S. ; and Younghouse, L.B.; Macromolecules, **21**, 1644-1653 (1988).

[5]Wyman, D.P.; Allen, V.R.; and Alterres, T.; J. Polym. Sci. (Part A), **2**, 4545 (1964).

[6]Quirk, R.P.; and Yin, J.; J. Polym. Sci: Part A, **30**, 2349-2355 (1992).

[7]Goethals, E.J.; "Telechelic Polymers Synthesis and Application," CRC Press, p. 44 (1989).

[8]Hattori, I.; Hirao, A., Yamaguchi, K.; Nakahama, S.; and Yamazaki; N.; Makromol. Chem., **184**, 1355-1362 (1983).

[9]Bodanszky, Miklos; Peptide Chemistry, Springer-Verlag Berlin Heidelberg, 1988.

[10]Berman, A.; Addadi, L.; Kuick, A.; Leiserowitz, L.; Nelson, M.; and Weiner, S.; Science, **250**, 664(1990).

[11]Allen, R. D.; Long, T.E., and McGrath, J. E.; Polymer Bulletin, **15**, 127-134 (1986).

[12]Freyss, D., Rempp, P.; and Benoit, H.; J. Polym. Sci. Polym Lett. Ed., **2**, 217 (1964).

[13]Willes, D.M.; and Bywater, S.; Trans. Farad. Soc., **61**, 150 (1965).

[14]Hirai, A.; Horri, F.; and Kitamaru, R.; Macromolecules, **20**, 1442-1444 (1987).

[15]Ramireddy, C.; Tuzar, Z.; Prochazka, K.; Webber, S.E.; and Munk, P.; Macromolecules, **25**, 2541-2545 (1992).

[16]Fayt, R.; Forte, R.; Jacobs, C.; Jerome, R.; Ouhadi, T.; Teyssie, Ph.; Macromolecules, **20**, 1442 (1987).

[17]Teyssie, Ph.; Fayt. R.; Jacobs, C.; Jerome, R.; Leemans, J.; and Varshney, S.; Polymer Prepr., **29**(2), 52 (1988).

[18]Teyssie, Ph.; Fayt, R.; Hautedeer, J.P.; Jacobs, C.; Jerome, R.; Leemans, L.; and Varshney, S.K.; Makromol. Chem., Macromol. Symp., **32**, 61 (1990).

[19]Kiljunen, H.; and Hase, T.A.; J. Org. Chem., **56**, 6950 (1991).

[20]Schulz, D.;Halasa, A.; Journal Poly. Sci. Polym. Chem., **15**, 2401 (1977).

[21]Hirao, A.; Hattori, I.; Sasagawa, T.; Yamaguchi, K.; Nakahama, S.; and Yamazaki, N.; Makromol. Chem., Rapid Commun., **3**, 59 (1982).

[22]Hirao, A.; Hattori, I; Yamaguchi, K.; Nakahama, S.; and Yamazaki, N.; Synthesis, **461** (1992).