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Solubilization of Peptides in Water and Hexane: Synthesis of Peptide-Terminated Poly(*tert*-butyl acrylate) and Poly(acrylic acid) via Living Anionic Polymerization

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ABSTRACT: A general method for solubilizing peptides in either organic or aqueous solution is presented. The peptide is coupled to a hydrophobic polymer (oil soluble), which can be readily cleaved to a hydrophilic (water soluble) polymer. Poly(*tert*-butyl acrylate) (PtBA) was synthesized via a living anionic polymerization of *tert*-butyl acrylate (tBA) by LiCl-complexed (diphenylhexyl)lithium in tetrahydrofuran (THF) at −78 °C and terminated by carbon dioxide or succinic anhydride. The carboxyl-terminal PtBA was coupled to alanine ethyl ester in only 40% yield by a conventional peptide synthesis method using 1,3-dicyclohexylcarbodiimide (DCC) as the coupling reagent. However, PtBA terminated by succinic anhydride could be coupled to alanine ethyl ester in 94% yield, to alanylalanylalanine methyl ester in 83% yield, and to N^{im}-tosylhistidine methyl ester in 81% yield. The tosyl protecting group on the latter was completely removed to give histidine-terminated PtBA. These alanine- and histidine-terminated PtBAs were quantitatively hydrolyzed to poly(acrylic acid) (PA). These peptides were soluble in solvents ranging from nonpolar hexane to water depending on the nature of the attached polymer chain.

Introduction

Amino acids and peptides are widely distributed components of the natural world and a very active field of research with applications in pharmacology, biology, microbiology, and drug delivery systems. A general approach to control the solubility of these molecules would be of great interest for further research and development. This could be accomplished by linking these peptides either to a hydrophilic or to a hydrophobic polymer. Linking a peptide to a hydrophilic polymer would also be very interesting because of the possibility of placing these peptides at phase boundaries.

Linking peptides to hydrophobic polymers such as polystyrene or polybutadiene has been reported in the literature by several research groups.[1−5] Anionic polymerization of styrene or butadiene leads to a macromolecular carbanion whose living end is converted to a primary amine which initiates the polymerization of *N*-carboxyanhydride monomers (NCA). However, this approach lacks flexibility. Only hydrophobic polymer chains can be linked to the peptide, and the polymerization of NCAs necessarily leads also to the homopolymer of the amino acid under study.

We were interested in a more general approach to be able to link both hydrophobic and hydrophilic polymer chains to peptides and to have the capability of attaching previously assembled peptides to a polymer chain. The monomer of choice is *tert*-butyl acrylate (tBA), which can be homopolymerized via anionic living polymerization. The anionic polymerization of tBA has been studied in great detail, and high control of molecular weight has been achieved.[6,7] With tBA, backbiting and termination reactions can be avoided by using a ligated organolithium initiator.[6] In addition, the *tert*-butyl esters of poly(*tert*-butyl acrylate) (PtBA) can be easily hydrolyzed to form the hydrophilic water-soluble poly(acrylic acid) (PA) analog. Anionic polymerization offers great flexibility and control of the end group in order to introduce appropriate functionalities for linking the polymer to an amino acid or peptide.[8] The anionic living end of PtBA can be transformed either to an amine end group for the reaction with the carboxylic acid function of an *N*-protected peptide or to a carboxylic acid end group for the reaction with an amino acid alkyl ester. Preliminary experiments in this laboratory showed that the latter route would be more appropriate.[9]

In this paper we use the controlled living anionic polymerization of tBA in the presence of lithium chloride to synthesize low molecular weight PtBA and derivatize this polymer to incorporate a carboxylic acid end group. The carboxylated polymer will be used to couple to an amino acid or peptide. The coupling reactions with alanine and histidine derivatives will be described. The solubility of the peptide linked to PtBA will be compared with that of the hydrolyzed polymer.

Experimental Section

Materials. Tetrahydrofuran (THF) was purified by double distillation over sodium and potassium under nitrogen using benzophenone as an indicator for dryness. The monomer, *tert*-butyl acrylate (tBA), and 1,1-diphenylethylene were distilled under reduced pressure prior to use. Butyllithium was purchased from Aldrich and used as received. Lithium chloride was dried in an oven at 110 °C for a week prior to use. L-Alanine ethyl ester hydrochloride (Ala-OEt·HCl) and L-ananyl-L-alanyl-L-alanine methyl ester acetate (Ala^3-OMe-AcOH) were purchased from Aldrich. L-Histidine methyl ester dihydrochloride (His-OMe·2HCl) was purchased from BACHEM California. 1,3-Dicyclohexylcarbodiimide (DCC), succinic anhydride, *N*-hydroxysuccininic anhydride, tosyl chloride (Ts-Cl), and (2-nitrophenyl)sulfenyl chloride (Nps-Cl) were purchased

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from Aldrich and used without further purification. Triethylamine was distilled from KOH.

Instrumentation. ^{1}H NMR spectra were recorded with a Bruker AM-250 nuclear magnetic resonance spectrometer using tetramethylsilane as reference. Size-exclusion chromatography (SEC) was carried out with a set of Phenomenex columns using THF as eluent and polystyrenes as standards. Elemental analyses were performed by Desert Analytics, Tucson, AZ. Melting points were measured with a Thomas-Hoover capillary melting point apparatus and are corrected.

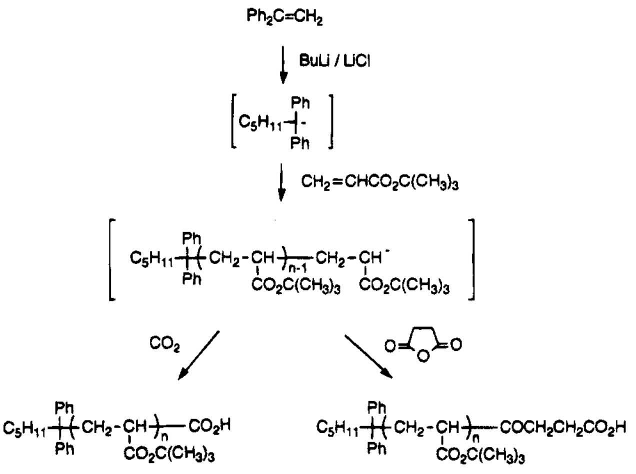
PtBA Terminated by Carbon Dioxide (PtBA(I)). The preparation of PtBA carbanion was carried out according to the modified method of Varshney et al.[7] To dry THF (100 mL) containing 0.7 g of LiCl was added butyllithium (2.35 M, 2.2 mmol) using an airtight syringe. 1,1-Diphenylethylene (0.44 g, 2.4 mmol) was added using an airtight syringe, resulting in a deep red color. After the reaction mixture was cooled to −78 °C, *tert*–butyl acrylate (tBA) (2.87 g, 22.4 mmol) in 20 mL of THF was added dropwise using an airtight syringe. The color of the reaction mixture changed from deep red to light yellow during the addition of the monomer. After stirring for 15 min, gaseous anhydrous carbon dioxide was introduced for 15 min, followed by acidification with aqueous hydrogen chloride (10 vol %). The reaction mixture was poured into a mixture of water and methanol (1:1 by volume) to precipitate the polymer, which was dried under reduced pressure to give 3.33 g (97%) of PtBA(I) as a colorless sticky material: ^{1}H NMR (CDCl$_3$, δ) 1.4 (s, *tert*-butyl), 1.6−2.1 and 2.3 (broad, CH and CH$_2$), 7.1−7.3 (m, phenyls).

PtBA Terminated by Succinic Anhydride (PtBA(II)). PtBA carbanion was prepared according to the above-mentioned method. After stirring for 15 min, succinic anhydride (0.369 g, 3.6 mmol) in 10 mL of THF was added to the living PtBA solution, and the reaction mixture was allowed to warm to room temperature. The reaction mixture was poured into aqueous hydrochloric acid (150 mL) and extracted with 150 mL of dichloromethane. The organic layer was dried with anhydrous sodium sulfate and placed under reduced pressure to give a colorless viscous material. It was charged on a silica gel column using carbon tetrachloride as the eluent. After the first band was collected to remove the excess 1,1-diphenylethylene, the eluent was changed to ethyl acetate and the second band was collected to give 3.55 g (98%) of PtBA(II) as a colorless sticky material: ^{1}H NMR (CDCl$_3$, δ) 1.4 (s, *tert*-butyl), 1.6−2.2 and 2.3 (broad, CH and CH$_2$), 2.6 (m, CH$_2$), 7.1−7.3 (m, phenyls).

PtBA(I)-Ala-OEt. A mixture of PtBA(I) (M_n = 1600) (1.60 g, 1.0 mmol), 1-hydroxybenzotriazole (HOBT) (0.15 g, 1.0 mmol), alanine ethyl ester hydrochloride (Ala-OEt-HCl) (0.31 g, 2.0 mmol), 1,3-dicyclohexylcarbodiimide (DCC) (0.21 g, 1.0 mmol), triethylamine (0.20 g, 2.0 mmol), and 2 mL of dichloromethane was stirred at room temperature under nitrogen for 48 h. Dichloromethane was evaporated under reduced pressure. Carbon tetrachloride was added to the residue and filtered. The filtrate was washed successively with 1 N sulfuric acid, water, 5% aqueous sodium bicarbonate, and water. The filtrate was placed under reduced pressure to give 1.54 g of PtBA(I)-Ala-OEt as a colorless sticky material: ^{1}H NMR (CDCl$_3$, δ) 1.28 (t, CH$_3$), 1.4 (s, *tert*-butyl), 1.6-2.1 and 2.3 (broad, CH and CH$_2$), 4.18 (q, OCH$_2$), 4.5 (m, CH), 6.2 (broad, NH), 7.1−7.3 (m, phenyls).

PtBA(II)-Ala-OEt. A mixture of PtBA(II) (M_n = 1600) (1.6 g, 1.0 mmol), HOBT (0.15 g, 1.0 mmol), Ala-OEt·HCl (0.31 g, 2.0 mmol), DCC (0.21 g, 1.0 mmol), triethylamine (0.20 g, 2.0 mmol), and 2 mL of dichloromethane was stirred at room temperature under nitrogen for 48 h. Dichloromethane was evaporated under reduced pressure. Carbon tetrachloride was added to the residue and filtered. The filtrate was washed successively with 1 N sulfuric acid, water, 5% aqueous sodium bicarbonate, and water. It was dried over anhydrous sodium sulfate and placed under reduced pressure to give 1.50 g of the coupled product as a colorless sticky material: ^{1}H NMR (CDCl$_3$, δ) 1.28 (t, CH$_3$), 1.4 (s, *tert*-butyl), 1.6−2.1 and 2.3 (broad, CH and CH$_2$), 2.5 (m, CH$_2$), 4.18 (q, OCH$_2$), 4.5 (m, CH), 6.2 (broad, NH), 7.1−7.3 (m, phenyls).

Scheme 1



PtBA(I) PtBA(II)

PA(II)-Ala-OEt. PtBA(II)-Ala-OEt was dissolved in formic acid, and the solution was stirred at 60 °C for 20 h. The reaction mixture was placed under reduced pressure to remove volatile materials, and the residue was dissolved in a small amount of THF and poured into excess heptane to precipitate PA(II)-Ala-OEt as a white powder: ^{1}H NMR (DMSO-d_6, δ) 1.1−1.3 (m, CH$_3$), 1.4−2.6 (broad, CH and CH$_2$), 4.0−4.2 (m, CH and OCH$_2$), 7.0−7.3 (m, phenyls).

PtBA(II)-Ala^3-OMe. Dry HCl gas was bubbled into a solution of L-alanyl-L-alanyl-L-alanine methyl ester acetate (Ala^3-OMe·AcOH) (0.31 g, 1.0 mmol) in 5 mL of methanol for 10 min, and the reaction mixture was placed under reduced pressure to remove volatile materials to obtain L-alanyl-L-alanyl-L-alanine methyl ester hydrochloride (Ala^3-OMe·HCl). PtBA(II) (M_n = 1300) (0.65 g, 0.5 mmol), DCC (0.10 g, 0.5 mmol), HOBT (0.077 g, 0.5 mmol), triethylamine (0.10 g, 1.0 mmol), and 3 mL of dichloromethane were added, and the mixture was stirred at room temperature for 48 h. Dichloromethane was evaporated under reduced pressure. Carbon tetrachloride was added to the residue and the mixture was filtered. The filtrate was washed successively with 1 N sulfuric acid, water, 5% aqueous sodium bicarbonate, and water. It was dried over anhydrous sodium sulfate and placed under reduced pressure to give 0.72 g of PtBA(II)-Ala^3-OMe as a colorless sticky material: ^{1}H NMR (CDCl$_3$, δ) 1.1−1.3 (broad, CH$_3$), 1.4 (s, *tert*-butyl), 1.6−2.1 and 2.3 (broad, CH and CH$_2$), 2.5 (m, CH$_2$), 3.74 (s, OCH$_3$), 6.9 (broad, NH), 7.1−7.3 (m, phenyls).

PA(II)-Ala^3-OMe. The PtBA(II)-Ala^3-OMe was dissolved in formic acid, and the solution was stirred at 60 °C for 20 h. The reaction mixture was placed under reduced pressure to remove volatile materials, and the residue was dissolved in a small amount of THF and poured into excess heptane to precipitate PA(II)-Ala^3-OMe as a white powder: ^{1}H NMR (DMSO-d_6, δ) 1.1−1.3 (m, CH$_3$), 1.4−2.4 (broad, CH and CH$_2$), 2.6 (m, CH$_2$), 3.63 (s, OCH$_3$), 7.0−7.3 (m, phenyls), 7.9−8.2 (m, NH).

N^{α}-((2-Nitrophenyl)sulfenyl)histidine Methyl Ester (Nps-His-OMe). Nps-His-OMe was prepared according to the procedure of Phocas et al.[10] Histidine methyl ester dihydrochloride (His-OMe·2HCl) (4.29 g, 18 mmol) was mixed with triethylamine (7.25 g, 72 mmol) in 55 mL of methanol. (2-Nitrophenyl)sulfenyl chloride (Nps-Cl) (6.78 g, 36 mmol) was added dropwise over 30 min at 5−10 °C under nitrogen. After 2 h, water (180 mL) was added to the reaction mixture and the solution was acidified to pH 2 with 1 N sulfuric acid. The mixture was washed with three 70-mL portions of ethyl acetate to remove excess Nps-Cl. Then excess sodium bicarbonate was added to the water layer to separate an oily material which was extracted with ethyl acetate. The extract was washed with water, dried over anhydrous sodium sulfate, and placed under reduced pressure to give 3.58 g (63%) of Nps-His-OMe as a yellow solid: mp 132−135 °C (lit.[10] mp 134−136 °C).

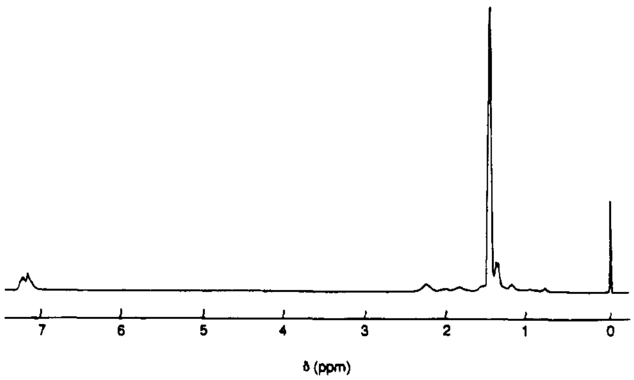


Figure 1. 270-MHz ^{1}H NMR spectrum of PtBA(I).

N^{α}-((2-Nitrophenyl)sulfenyl)-N^{im}-tosylhistidine Methyl Ester (Nps-His(Tos)-OMe). Nps-His(Tos)-OMe was prepared according to the procedure of Fujii et al.[11] Into a solution of Nps-His-OMe (3.58 g, 11 mmol) and triethylamine (1.81 g, 18 mmol) in 36 mL of dioxane was added tosyl chloride (2.51 g, 13 mmol) in 9 mL of dioxane over 15 min at 5−10 °C under nitrogen. The reaction mixture was diluted with water (90 mL), and the separated oil was extracted with ethyl acetate. the extract was washed successively with 1 N sulfuric acid, water, aqueous 5% sodium bicarbonate, and water. It was dried over anhydrous sodium sulfate and placed under reduced pressure to give a yellow solid which was recrystallized from a mixture of ethyl acetate and hexane to obtain 4.72 g (89%) of Nps-His(Tos)-OMe as yellow needles: mp 99 °C (lit.[11] mp 101−102 °C).

N^{im}-Tosylhistidine Methyl Ester (His(Tos)-OMe). Nps-His(Tos)-OMe (0.24 g, 0.5 mmol) was added to trifluoroacetic acid (0.6 mL), and the mixture was stirred under nitrogen at 10 °C for 30 min. Excess trifluoroacetic acid was evaporated under reduced pressure. The residue was charged on a silica gel column using ethyl acetate as eluent. After the first band was collected, the eluent was changed to methanol and the second band was collected to give His(Tos)-OMe·CF_3COOH as a colorless oil. The oil was dissolved into dichloromethane and washed with sodium bicarbonate solution. The organic layer was dried over sodium sulfate and placed under reduced pressure to give 0.15 g (94%) of His(Tos)-OMe as a colorless oil which was used for the next coupling reaction without further purification: ^{1}H NMR ($CDCl_3$, δ) 2.48 (s, 3H), 3.0−3.2 (m, 2H), 3.66 (s, 3H), 4.8−4.9 (m, 1 H), 7.08 (s, 1H), 7.39 (d, J = 8.0 Hz, 2H), 7.81 (d, J = 8.0 Hz, 2H), 8.1−8.2 (m, 2H).

PtBA(II)-His(Tos)-OMe. A mixture of PtBA(II) (M_n = 1600) (1.28 g, 0.8 mmol), freshly prepared His(Tos)-OMe (0.52 g, 1.6 mmol), DCC (0.165 g, 0.8 mmol), N-hydroxysuccinic anhydride (0.092 g, 0.8 mmol), and 3 mL of dichloromethane was stirred at room temperature under nitrogen for 48 h. The reaction mixture was diluted with dichloromethane and filtered. The filtrate was washed successively with 1 N sulfuric acid, water, aqueous 5% sodium bicarbonate, and water. It was dried over anhydrous sodium sulfate and placed under reduced pressure. The residue was charged on a silica gel column using ethyl acetate as the eluent. The first band was collected to give 1.28 g of PtBA(II)-His(Tos)-OMe as a colorless sticky material: ^{1}H NMR ($CDCl_3$, δ) 1.4 (s, *tert*-butyl), 1.6−2.1 and 2.3 (broad, CH and CH_2), 2.45 (s, CH_3), 2.6 (m, CH_2), 3.62 (s, OCH_3), 4.8 (m, CH), 6.7 (m, NH), 7.37 (d, tosyl), 7.82 (d, tosyl), 7.94 (s, CH), 7.1−7.3 (m, phenyls and CH).

PtBA(II)-His-OMe. A mixture of PtBA(II)-His(Tos)-OMe (0.70 g, 0.4 mmol), methanol (7 mL), and potassium hydroxide (0.15 g, 2.5 mmol) was stirred for 30 min at room temperature. The reaction mixture was diluted with 100 mL of dichloromethane and washed with 70 mL of brine. The organic layer was dried with sodium sulfate and placed under reduced pressure. The residue was charged on a silica gel column using ethyl acetate as the eluent. After the first band was collected, the eluent was changed to methanol and the second band was collected to give 0.59 g of PtBA(II)-His-OMe as a colorless sticky material: ^{1}H NMR ($CDCl_3$, δ) 1.4 (s, *tert*-butyl), 1.6−2.1 and 2.3 (broad, CH and CH_2), 2.6 (m, CH_2), 3.62 (s, OCH_3), 4.8 (m, CH), 6.7 (m, NH), 7.94 (s, CH), 7.1−7.3 (m, phenyls and CH).

PA(II)-His-OMe. PtBA(II)-His-OMe was dissolved in formic acid, and the solution was stirred at 60 °C for 20 h. The reaction mixture was placed under reduced pressure to remove volatile materials, and the residue was dissolved in a small amount of THF and poured into excess heptane to precipitate PA(II)-His-OMe as a white powder: ^{1}H NMR (DMSO-d_6, δ) 1.1−2.7 (broad, CH and CH_2), 3.60 (s, OCH_3), 3.7−3.8 (m, CH_2), 4.5 (m, CH_2), 7.0−7.3 (m, phenyls and CH), 7.95 (s, CH).

Model Coupling Reaction. A mixture of given amounts of propionic acid, His(Tos)-OMe, DCC as a coupling reagent, dichloromethane as a solvent, triethylamine, and HOBT or N-hydroxysuccinic anhydride (HOSu) as an additive was stirred under nitrogen at room temperature for 24 h. The reaction mixture was diluted with dichloromethane and

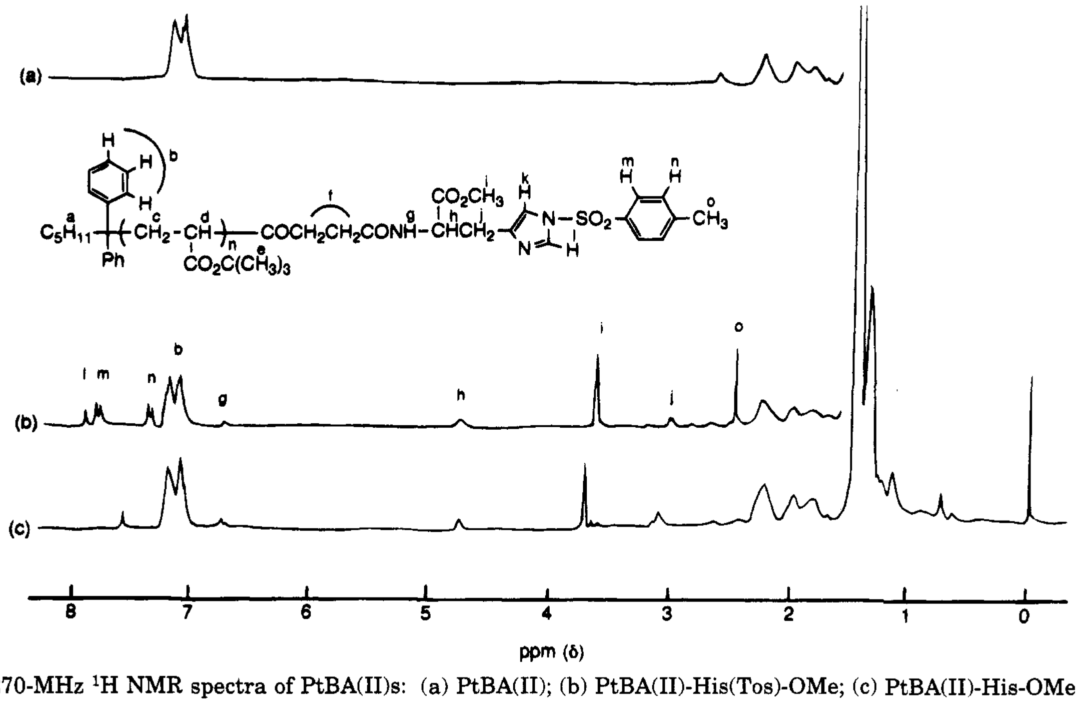


Figure 2. 270-MHz ^{1}H NMR spectra of PtBA(II)s: (a) PtBA(II); (b) PtBA(II)-His(Tos)-OMe; (c) PtBA(II)-His-OMe.

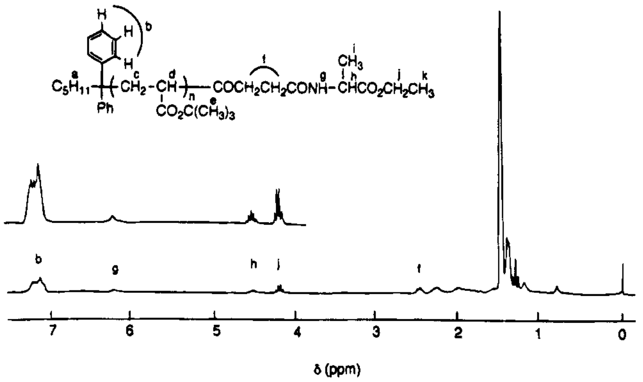
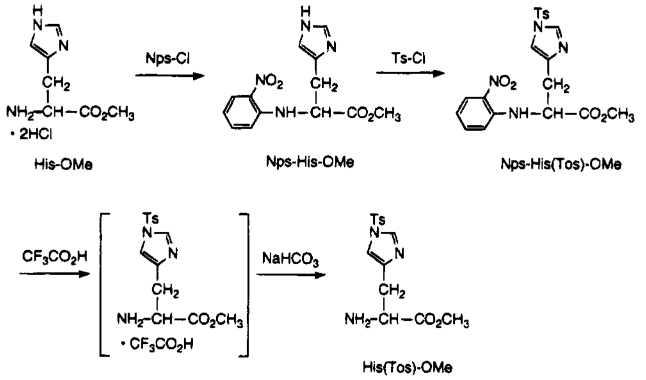


Figure 3. 270-MHz ^{1}H NMR spectrum of PtBA(II)-Ala-OEt.

Scheme 2



filtered. The filtrate was washed with diluted aqueous sulfuric acid solution, aqueous sodium bicarbonate solution, and water. It was dried over anhydrous sodium sulfate and placed under reduced pressure to remove the solvent. The residue was charged on a silica gel column using ethyl acetate as an eluent. The first band was collected to give N^{α}-propanoyl-N^{im}-tosylhistidine methyl ester as white needles: mp 135–136 °C; ^{1}H NMR ($CDCl_3$, δ) 1.12 (t, $J = 7.2$ Hz, 3H), 2.22 (q, $J = 7.2$ Hz, 2 H), 2.44 (s, 3H), 3.03 (m, 2H), 3.65 (s, 3H), 4.83 (m, 1H), 6.70 (m, 1H), 7.06 (s, 1H), 7.36 (d, $J = 9.2$ Hz, 2H), 7.80 (d, $J = 9.2$ Hz, 2H), 7.91 (s, 1H). Anal. Calcd for $C_{17}H_{21}N_3O_5S$: C, 53.81; H, 5.58; N, 11.07; S, 8.45. Found: C, 53.98; H, 5.46; N, 11.22; S, 8.73.

Results and Discussion

Living Anionic Polymerization of tBA. Many studies have achieved living polymerization of acrylic monomers including group-transfer reaction using silylketene acetals,[12] LiCl-modified organolithium-initiated polymerization,[13] immortal polymerization using aluminum porphyrin compounds,[14] and organolanthanide(III) complex-initiated polymerization.[15] We used LiCl-complexed (diphenylhexyl)lithium as initiator in THF at −78 °C. Since we were interested in linking a polyacrylate to a peptide, the molecular weight of the polymer was designed to be less than 2000. These short polymer chains should be sufficient to control the solubility of the attached peptide. Moreover, the low molecular weight simplifies the ^{1}H NMR analysis for the resulting structure.

Synthesis of PtBA(I). To obtain a carboxylic acid-terminated polymer we first investigated the reaction of the living PtBA carbanion with carbon dioxide. Direct termination of a living anionic polymerization with gaseous carbon dioxide has been reported for polystyrene and leads to 60% carboxylated polymer, the remainder being the dimer ketone (30%) and the trimer carbinol (10%).[16] The yield of the carboxylated polymer was increased to 80% when the living polymer was poured over solid carbon dioxide. Recently, Quirk and Yin reported that quantitative carboxylation can be achieved by adding sufficient quantities of Lewis bases such as THF or *N,N,N′,N′*-tetramethylenediamine (TMEDA) prior to addition of gaseous carbon dioxide.[17]

Chart 1

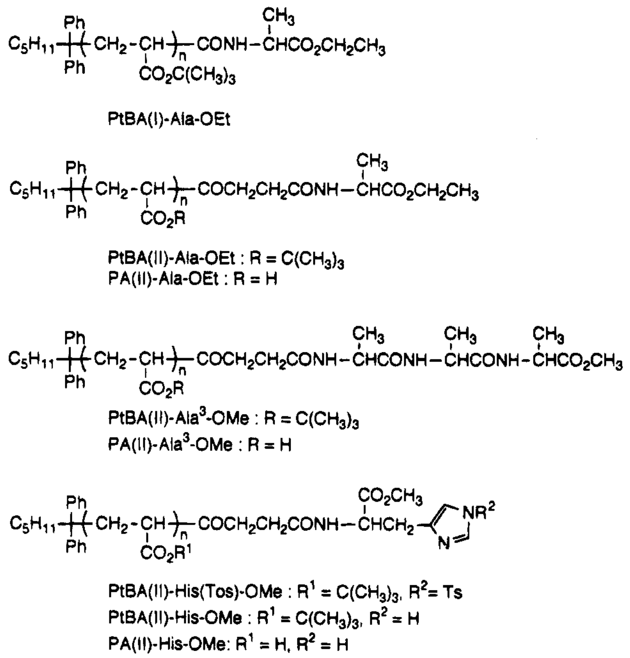


Table 1. Model Coupling Reaction of Propionic Acid and His(Tos)-OMe[a]

run	propionic acid, mmol	His(Tos)-OMe, mmol	DCC, mmol	additive, mmol	yield,[b] %
1	1.5	1.5	1.5	none	29
2	1.5	1.5	1.5	HOBT,[c] 1.5	*d*
3	1.5	1.5	1.5	HOSu,[e] 1.5	39
4	1.5	3.0	1.5	HOSu, 1.5	80

[a] In dichloromethane at 25 °C for 24 h. [b] Based on propionic acid. [c] 1-Hydroxybenzotriazole. [d] Complex mixtures. [e] *N*-Hydroxysuccinic anhydride.

In our experiments the carboxylated PtBA was synthesized using a modified procedure of Fetters et al.[18] Gaseous carbon dioxide was introduced rapidly into the reaction mixture with vigorous stirring. The number-average molecular weight (M_n) of PtBA(I) calculated using polystyrene standards was 1820 and the molecular weight distribution was 1.10, showing that no coupling to dimeric polyketone occurred. The ^{1}H NMR of the product is shown in Figure 1 and the molecular weight of the obtained polymer was also calculated to be 1620 using the peak area ratio between the aromatic and aliphatic protons. The calculated value obtained from the ratio between monomer and initiator concentrations was 1585. Also, titration of the carboxylic acid was accomplished using a procedure developed by Kiljunen et al.[19] We found that more than 90% carboxylation was achieved. It was concluded that living PtBA was effectively terminated by gaseous carbon dioxide in our reaction conditions to give carboxylic acid-terminated polymer with a narrow molecular weight distribution in more than 90% yield.

Synthesis of PtBA(II). In order to reduce the steric hindrance between the bulky *tert*-butyl ester moiety and the incoming peptide, and ethylene spacer was introduced by termination of living anionic PtBA with

Table 2. Solubilities[a] of Peptide-Terminated Polymers

	solvent					
polymer	hexane	chloroform	ether	methanol	water	alkaline water
PtBA(II)-Ala-OEt	+	+	+	+	−	−
PtBA(II)-Ala^3-OMe	+	+	+	+	−	−
PtBA(II)-His-OMe	+	+	+	+	−	−
PA(II)-Ala-OEt	−	−	−	+	+	+
PA(II)-Ala^3-OMe	−	−	−	+	±	+
PA(II)-His-OMe	−	−	−	+	±	+

[a] +, soluble; ±, partially soluble; −, insoluble.

succinic anhydride according to the method of Rempp et al.[20] The ^{1}H NMR spectrum of the PtBA(II) is shown in Figure 2a. The peak at 2.6 ppm is assigned to the methylene protons of the ethylene spacer. The conversion to the carboxylic acid was calculated from the peak area and was more than 95%.

Coupling Reactions of PtBA(I) and PTBA(II) with Alanine Ethyl Ester. The coupling reaction of PtBA(I) with alanine ethyl ester (Ala-OEt) was carried out by a conventional peptide synthesis method using DCC as the coupling reagent. From ^{1}H NMR analysis it was found that only 40% coupling was achieved.

The lack of complete coupling indicates that the carboxylic acid group might be too crowded to effectively attack the peptide. The low coupling yield (40%) led us to introduce the ethylene spacer by using succinic anhydride. The coupling reaction of PtBA(II) with Ala-OEt was carried out under the same coupling conditions. The ^{1}H NMR spectrum of this coupling product is shown in Figure 3. The coupling yield of this polymer was calculated from the peak area to be 94%, indicating that the ethylene spacer was effective in reducing the steric hindrance. The coupling reaction of PtBA(II) was also carried out with alanylalanylalanine methyl ester (Ala^3-OMe). For this reaction commercially available Ala^3-OMe acetate was first converted to Ala^3-OMe hydrochloride in advance to prevent the acetylation of the amino function of Ala^3-OMe during the coupling reaction. The coupling yield of Ala^3-OMe·AcOH to PtBA(II) was 83% as determined from the NMR spectrum.

Coupling Reaction of PtBA(II) with Histidine Derivative. Histidine has an imidazole moiety, which is highly reactive. The imidazole moiety in the histidine side chain can add to carbodiimides to produce substituted guanidines.[21] Several protecting groups for the imidazole moiety have been reported such as benzyl,[22] benzyloxycarbonyl,[23] trityl,[24] adamantyloxycarbonyl,[25] 2,2,2-trifluoro-1-(acylamino)ethyl,[26] and tosyl groups.[11] Among these, the tosyl group can be most easily removed by base treatment. Under these basic conditions the *tert*-butyl ester group is expected to be stable. Therefore, we prepared the tosylated histidine derivative (His(Tos)-OMe) as shown in Scheme 2 by first protecting and then deprotecting the amine functionality. His(Tos)-OMe was used to couple to the carboxyl-terminated PtBA.

Before carrying out the coupling reaction of PtBA(II) with His(Tos)-OMe, the coupling reaction between propionic acid and His(Tos)-OMe was investigated as a model reaction. The results are summarized in Table 1. The addition of 1-hydroxybenzotriazole (HOBT) was not effective. The product was a complex mixture (run 2). Fujii et al. reported the deprotection of tosylated histidine derivative by HOBT;[11] therefore these reaction conditions regenerate the reactive imidazole moiety. On the other hand, *N*-hydroxysuccinimide (HOSu) was rather effective (run 3). The yield of the product was increased to 80% by using 2 molar equiv of amine (His(Tos)-OMe) (run 4).

The coupling reaction between PtBA(II) and His(Tos)-OMe was carried out under similar experimental conditions. The ^{1}H NMR spectrum of the product is shown in Figure 2b, with the absorption peak due to the amide proton at 6.7 ppm. The coupling yield was 81%.

Removal of the Tosyl Group. The deprotection of the imidazole unit in histidine as carried out by alkaline hydrolysis of the tosyl group. The ^{1}H NMR spectrum of the deprotected product is shown in Figure 2c. The peaks at 7.8, 7.3, and 2.5 ppm, assigned to the tosyl group, disappeared completely, while the peak at 3.7 ppm due to the methyl group remained unchanged, indicating that only the tosyl group underwent hydrolysis.

Hydrolysis of the *tert*-Butyl Ester Group. It was found that not only the *tert*-butyl group but also the amide group was hydrolyzed when the peptide-terminated polymer was heated at 80 °C in dioxane in the presence of hydrochloric or *p*-toluenesulfonic acid. The hydrolysis reaction was successfully carried out in formic acid at 60 °C. Conversion of PtBA into polyacid was ascertained by ^{1}H NMR. The *tert*-butyl peak at 1.4 ppm disappeared completely. The peaks due to alanine or histidine derivatives remained unchanged.

Solubility. The solubility properties of peptide-terminated polymers are summarized in Table 2. The poly(*tert*-butyl acrylate)s coupled with alanine, trialanine, and histidine are completely and freely soluble in chloroform, ether, and even hexane. The poly(acrylic acid)-coupled compounds are completely soluble in dilute alkaline water (1 M NaOH). In water they are partially soluble.

Conclusions

Amino acids were successfully linked to either hydrophobic or hydrophilic polymer. In this way the solubility of peptides can be completely controlled. This is a general method to accomplish these diverse solubilities and is currently being extended to other systems.

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