Synthesis and Radical Polymerization of A Styrene Derivative Bearing an Alanyl Ester

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Abstract

Novel styrene monomer bearing alanyl ester with or without free amino terminus was synthesized and polymerized with AIBN at 80 ℃ via radical pathway. A Boc-protected 4'-vinylbenzyl-alanyl ester was synthesized by a reaction of 4'-vinylbenzyl chloride with Boc-alanine in the presence of triethylamine and ethanol at 80 ℃. This condition is similar to one of a synthetic method introducing N-Boc-protected peptides to the polystyrene resin in the Merrifield technique. However, this coupling reaction led to the formation of some by-products, and alternatively, treatment of them with an inorganic base at 60 ℃ in DMF solution containing small amount of water afforded successful formation of the N-Boc-protected alanyl ester in good yield. Deprotection of the Boc-group from the ester gave the monomer with free amino moiety. The styrene derivatives were polymerized with AIBN at 80 ℃ to yield polystyrenes with high molecular-weight.

1. INTRODUCTION

In recent years, highly enantiomerically pure amino acids and their derivatives becoming inexpensive have opened the way to development of various amino acids or peptides-containing functional molecules, which can perform important functions as biomaterials. Particularly, polyolefins bearing amino acids or peptides in the side chain of the monomer unit have been studied by several scientists [1]. These polyolefins have been prepared aiming at the following objects; (1) mimicking the corresponding poly(amino acid)s [2], such as antibiotic polyleucine and polymethioninesulfone which has oxygen permeability [3], (2) aggregating functions of the residues of amino acids on the polymer main chain [4], (3) polyelectrolytes or efficient metal-ion absorbents [5], (4) stereo-regulated polymers in the main chain, of which the chirality is induced by chiral amino acids and their derivatives [6-8].

These amino acid or peptide-substituted monomers were able to be facilely prepared as acrylic or methacrylic acid derivatives [2], and polymerized via radical pathway. Thus, the significant feature of the structures of these polymers is that the terminal amino group of the amino acid or the peptide forms amide bond with the monomer unit; whereas the carboxylic group at the opposite side, which can be anionic in the presence of base, is free [9]. It is of interest that polyolefins containing free amino groups of amino acids or peptides might show the similar activity to

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that of poly(amino acid)s bearing free cationic ammonium residues, such as poly-lysine and poly-ornithine, which perform significant bacteriostatic actions towards bacteria and fungus absorbing to the cell walls of them with the ammonium residues [10]. However, polyolefines bearing such amino groups in the polymer side chain have never been synthesized. These prompted us to prepare new styrene monomers having free amino termini of amino acids or peptides and to polymerize them via radical pathway preparing *amino-acid-functionalized polystyrenes*.

Polystyrene has generally used as synthetic resin to combine with amino acids and peptides in the Merrifield technique [11]. However, synthesis of a styrene monomer binding to an amino acid or a peptide-side chain is rarely known. We describe herein an efficient synthesis of an (L)-alanine-combined styrene monomer by reaction of 4'-vinylbenzyl chloride with a Boc-protected alanine and subsequent deprotection of the Boc group, polymerization of this monomer via radical pathway, and preliminary investigation of their bacteriostatic activity.

2. EXPERIMENTAL

2-1. General

Benzene and THF (tetrahydrofuran) used for radical polymerization were distilled over sodium benzophenone ketyl under reduced pressure just before use. Ethyl acetate was distilled before use. Other solvents, DMF(dimethylformamide) and ethanol were used as purchased as otherwise noted. Boc-alanine was prepared according to the literature [12]. Other reagents, 4'-vinylbenzyl chloride and AIBN were used as purchased from Wako. Polymerization was carried out in a glass ampule sealed under reduced pressure. NMR spectra were taken with a VARIAN Mercury Y plus 400MHz spectrometer. Chemical shifts were recorded in ppm from the internal standard (^{1}H, ^{13}C: solvent). IR spectra were recorded in cm^{-1} on a PERKIN ELMER Spectrum One spectrometer equipped with a universal diamond ATR. Size exclusion chromatography (SEC) analysis of the polymers was carried out using a JASCO HPLC system (Column: Shodex KF-804L+KF-804L at 40℃) in THF(1.0mL/min) calibrated by standard samples of polystyrene. Column chromatography was performed on a silica gel 60N (neutral) (KANTO Chemicals). Melting points were recorded on YANACO Micro Melting Point Apparatus MP-SU using glass capilary. The optical rotations were measured with a JASCO DIP-140 digital polarimeter in acetic acid at 25 ℃. The concentration was at 1g/dL. The path length was 0.1cm and the light source was sodium D line. The circular dichroism (CD) spectra were recorded on a JASCO J-600 spectropolarimeter at 25℃ over the range of 195 to 260nm using a quartz cell of 0.1cm path length at a sample concentration of 1×10^{-3}M. The data were expressed in terms of the molar ellipticity. The elemental analysis was carried out with YANACO CHN Corder MT-5, AUTO-SAMPLER. Thin Layer Chromatography (TLC) was carried out on the Merck 25 TLC aluminum sheet 20×20cm silica gel $60F_{254}$ with the following solvent system: R_f^1, dichloromethane, R_f^2, acetic acid-methanol=(1∶1, v/v), R_f^3, 1-butanol-acetic acid-pyridine-water (4∶1∶1∶2, v/v). The minimum inhibitory concentration (MIC) was determined by the standard agar dilution method using trypticase soy agar as described by Okonogi et al. [13].

2-2. Preparation of Boc-Ala-Styrene (1) ; part 1

To a 100mL round bottom flask equipped with a reflux condenser, Boc-alanine (1.42g, 7.50mmol), 4'-vinylbenzyl chloride (0.71mL, 5.00mmol), triethylamine (0.77mL, 5.50mmol), and ethanol (25.0mL) were added at room

temperature. The mixture was stirred at 80 ℃ for 10hours, and then volatiles were evaporated under reduced pressure. The residue was washed with water and extracted three times with ethyl acetate using a separating funnel. After removal of the solvent in vacuo, the residue was purified by column chromatography (eluent; hexane and dichloromethane (1 : 1)). Recrystallization from methanol and hexane at −35℃ gave **1** (0.48g, 1.60 mmol, yield 32%). m.p. (℃): 28～29, $[\alpha]_D^{20}$ = 25.4 ± 0.3 (c = 1.00, CH_3COOH). Anal. Calcd. for $C_{17}H_{23}NO_4$; C: 66.86, H: 7.59, N: 4.59. Found; C: 66.91, H: 7.47, N: 4.69. ^{1}H NMR (400.4MHz, $CDCl_3$) δ 7.41 (d, J = 8.0Hz, 2H, Ph), 7.31 (d, J = 8.0Hz, 2H, Ph), 6.71 (dd, J = 10.8Hz, 1H, -CH=), 5.78 (t, J = 17.6Hz, 1H, =CH_2), 5.28 (d, J = 6.40Hz, 1H, =CH_2), 5.17 (AB pattern, 2H, CH_2), 5.10 (t, J = 21.6Hz, 1H, NH), 4.35 (quint, J = 6.80Hz, 1H, CH), 1.40 (d, J = 16.4Hz, 3H, CH_3), 1.44 (s, 9H, tBu).
^{13}C NMR (100.6MHz, $CDCl_3$) δ 17.4, 27.2, 48.1, 65.5, 78.5, 113.1, 125.0, 127.0, 133.5, 134.8, 136.3, 153.7, 171.7. IR (ν, cm^{-1}) 3384 (s), 2981 (s), 2936 (sh), 1748 (sh), 1719 (vs), 1630 (w), 1516 (s), 1455 (m), 1368 (m), 1254 (m), 1167 (s), 1071 (m), 830 (m), 588 (br).

2-3. Preparation of Boc-Ala-Styrene (1) ; part 2

To a 50mL round bottom flask, Boc-alanine (0.48g, 2.52mmol), 4'-vinylbenzyl chloride (0.30mL, 2.1mmol), sodium hydrogen carbonate (0.11g, 1.05mmol), and dimethylformamide (9.26mL) containing 1% water was added and stirred at 60℃ for 7hours. Complete consumption of the starting materials was confirmed by means of the ^{1}H NMR measurement of the crude mixture in $CDCl_3$. After removal of the solvent, the residue was washed and extracted from water and ethyl acetate. Purification of the resulted mixture (containing a little amount of 4'-vinylbenzyl chloride and DMF) by column chromatography gave white solid **1** (0.49g, 1.60mmol, yield 76%).

2-4. Deprotection of the Boc group from 1 ; Preparation of Ala-Styrene (2)

To a 500mL flask, Boc-alanyl styrene **1** (2.50g, 8.18mmol) and trifluoroacetic acid solution in toluene (25%, 245mL) were added and stirred at room temperature for 1 or 2 hours. The solution was concentrated with N_2 gas bubbling, and then evaporated under reduced pressure. The resulted yellow oil was dissolved in a small amount of water, and aqueous sodium hydrogen carbonate (4%) was added to the solution at 0℃ to raise its pH up to 7～8. After extraction with ethyl acetate and salting-out from aqueous solution, the ethyl acetate solution was dried over sodium sulfate anhydride and evaporation of the solvent gave a pale-yellow solid **2** (1.19g, yield 71%). m.p. (℃): 47-52. ^{1}H NMR (400.4MHz, $CDCl_3$); δ 7.37 (d, J = 8.41Hz, 2H, Ar), 7.25 (d, J = 8.41Hz, 2H, Ar), 6.68 (dd, J = 16.0Hz, 1H, -CH=), 5.74 (d, J = 17.6Hz, 1H, CH=), 5.27 (d, J = 11.2Hz, 1H, CH=), 5.13 (dd, J = 32.4Hz, 2H, CH_2), 4.04 (q, J = 21.6Hz, 1H, CH), 1.56 (d, J = 7.21Hz, 3H, CH_3).
IR (ν, cm^{-1}) 3359, 2973, 2930, 2873, 1732, 1629, 1513, 1454, 1407, 1368, 1177, 1071, 1013, 903, 822.

2-5. General Procedure for the Radical Polymerization of 1 and 2

The general procedure for the polymerization of **1** and **2** is as follows. To a 10mL glass ampule, a solution of monomer **1** (0.15 g, 0.49mmol) in benzene (0.15mL, 3mol/L) and AIBN (0.74mg, 0.45 μmol) were added and degassed. The ampule was sealed under reduced pressure and stirred at 80℃ for 24 hours. The solvent was removed in vacuo, and extracted with a small amount of toluene.

The solution was dropwised to the stirring hexane (30mL) to form white precipitate (**3**: 0.040g, yield 27%). The polymer **4** was also obtainable from **2** in 63% yield. Because the polymer **4** was hardly soluble in chloroform-*d*, the NMR measurement was carried out in D_2O dissolved in the presence of excess amount of formic acid.

(**3**): M_n(SEC) = 25000, M_w(SEC) = 83000, M_w/M_n = 3.3. ^{1}H NMR (400.4MHz, $CDCl_3$): δ 6.97, 6.42, 5.41, 5.07, 4.34, 1.43, 1.36. ^{13}C NMR (100.6MHz, $CDCl_3$): δ 18.8, 28.7, 40.6, 49.5, 66.8, 79.9, 127.7, 33.1, 146.5, 155.5, 173.3. IR (ν, cm^{-1}); 3393 (brs), 2981 (s), 2934 (s), 1748 (sh), 1719 (vs), 1514 (s), 1457 (m), 1368 (m), 1254 (m), 1167 (s), 1071 (m), 820 (m), 604 (br).

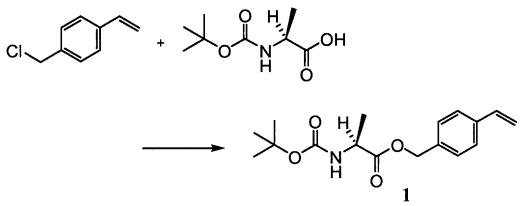
(**4**): M_n(SEC) = 7200, M_w(SEC) = 9400, M_w/M_n = 1.3. ^{1}H NMR (400.4 MHz, D_2O); δ 6.05, 5.54, 4.14, 3.21, 0.50. ^{13}C NMR (100.6 MHz, D_2O); δ 169.8, 165.4, 131.2, 127.3, 114.2, 67.4, 48.4. IR(ν, cm^{-1}); 2921(brs), 1738 (s), 1610 (br), 1512 (m), 1455 (m), 1197 (m), 1111 (m), 812 (m), 410 (w).

3. RESULTS AND DISCUSSION

3-1. Preparation of a Styrene Derivative Bearing an Alanyl Ester

Among several methods to introduce amino acids to the aromatic ring, we chose the benzyl ester formation from the reaction of an N-protected amino acid with a benzyl chloride to prepare the styrene monomer bearing amino acid as shown in Table 1. Because benzyl esters can be hydrolyzed in the basic condition, N-Boc protecting group, which is hardly deprotected in the basic condition, was adopted for this coupling reaction [11]. The reaction of Boc-alanine with 4'-vinylbenzyl chloride in the presence of triethylamine in ethanol at 80℃ for 10hours formed a monomer, 4'-vinylbenzyl-alanyl ester (**1**). After washing the crude mixture with water,

Table 1 Synthesis of 4'-Vinylbenzyl-Alanyl (**1**).



1

entry	base	solvent	temp / °C	time / h	yield / %
1	Et_3N	EtOH	80	10	40
2	$NaHCO_3$	DMF*	40	90	0
3	$NaHCO_3$	DMF/H_2O**	60	7	76

* DMF: dimethylformamide. ** H_2O: >1 wt % in DMF

purification of the residual oil by column chromatography gave **1** in 40% yield (Table 1, entry 1). The ^{1}H NMR observation of the crude mixture in $CDCl_3$ showed the formation of **1** in 50-60% yield and some by-products derived from 4'-vinylbenzyl chloride in 40-50% yield, suggesting that the selectivity in the generation of **1** should be improved by changing the reaction condition.

For this purpose, we investigated what the by-products are in this coupling reaction. The ^{1}H NMR spectra of the purified two by-products by column chromatography clarified that they are 4'-vinylbenzyl ethyl ether and 4'-vinylbenzyl triethyl ammonium chloride yielded by nucleophilic substitution reactions of 4'-vinylbenzyl chloride with ethanol and triethylamine, respectively, which were added in larger quantity than Boc-alanine. When this coupling reaction was monitored by ^{1}H NMR spectroscopy measured at 5, 10, 22, and 27 hours, these by-products formed slowly with almost the same rate and also increased even after 10hours, whereas the compound **1** generated in 56 % in 5 hours and the ratio of **1** did not changed after 10hours. And the ratios of the by-products reached to 18 and 22%, respectively. These results suggested that the choice of the solvent and the base is quite important for the successful high-yield preparation of **1**.

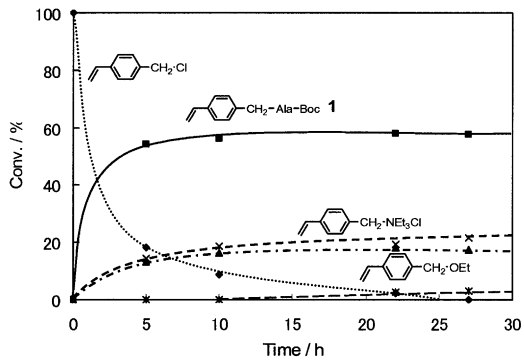
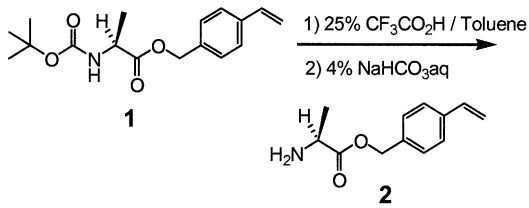


Figure 1. Time vs. Conversion Plots of the Coupling Reaction of 4'-Vinylbenzyl Chloride with Alanine.

Thus, in order to improve the reaction condition, we alternatively used less nucleophilic solvent and base. Finally, a combination of wet DMF and sodium hydrogen carbonate is the best choice as the solvent and the base, respectively. When commercially available 99.8% DMF (water<0.15%) was used as purchased, no reaction occurred in this condition (Table 1, entry 2) [14]. Careful observation of the reaction mixture at room temperature revealed that white solid was existed at the bottom of the flask in all the reaction time. Probably, this is sodium hydrogen carbonate which is insoluble in this grade of DMF. Using DMF containing a little amount of water (~1wt%) in order to dissolve sodium hydrogen carbonate, the coupling reaction at 60℃ for 7hours resulted in high-yield formation of **1** with no by-product, and after purification, the compound **1** was successfully obtainable in 76% yield (Table 1, entry 3).

In the next stage of the synthesis of the styrene monomer, deprotection of N-Boc-protected alanine derivative **1** by hydrolysis was carried out to form the amino-free alanylstyrene derivative **2** as shown in Scheme 1. Unfortunately, deprotection using aqueous solutions of some carboxylic acids, which are the useful reagents for deprotection of N-Boc-protected peptides in the solution-phase peptide synthesis [15], was in fail, because the N-Boc-alanylstyrene **1** was hardly soluble in water. Among some acids in non-polar solvents, 25% trifluoroacetic acid in toluene is suitable for the deprotection of **1** to successfully form an ammonium cation of **2** [16], which was subsequently neutralized with 4% sodium hydrogen carbonate in water (pH: 6 ~7) to give the 4'-vinylbenzyl alanyl ester **2** in high yield (71%).



Scheme 1

The compounds **1** and **2** were fully characterized on the basis of usual spectroscopic methods. Especially, formation of the benzyl alanyl ester in **1** and **2** was evidenced in the ^{1}H NMR spectra, which showed characteristic signals with AB coupling pattern at δ 5.17 and 5.14 derived from the geminal benzyl protons of **1** and **2**, respectively, because they were diastereotopic with each other due to the chirality of the alanyl moiety, whereas the signal due to the benzyl protons of the starting material, 4'-vinylbenzyl chloride, appeared as a singlet peak at δ 4.48. Typical sets of three signals due to the three vinyl protons in **1** and **2** appeared at δ 5.28, 5.78, and 6.71, and δ 5.27, 5.74, and 6.68, respectively. These chemical shifts were quite similar to those of the resonances due to the vinyl protons of 4'-vinylbenzyl chloride at δ 5.65, 5.17, and 6.62. Successful deprotection of the N-Boc group in **1** was confirmed by disappearance of the signal derived from *tert*-butyl moiety at δ 1.45 in the ^{1}H NMR spectrum of **2**. As for the stereochemistry, the optical rotation angles of the compound **1** synthesized with the method as listed in Table 1, entry 1

was -25.4 ± 0.3.

3-2. Radical Polymerization of the Styrene Derivatives 1 and 2

Generally, styrenes are known as versatile monomers polymerized via anionic, cationic, or radical pathway to form poly(styrene)s. Since the styrenes containing alanyl esters, **1** and **2**, could be hydrolyzed both in anionic and cationic conditions, radical polymerization in the neutral condition is appropriate to yield the *amino-acid-functionalized polystyrenes* [17]. In this study, we adopted the usual radical polymerization method with AIBN as a radical initiator in the non-polar solvent to polymerize **1** and **2**. The results of polymerization studies using **1** and **2** were listed in Table 2. They are polymerized in benzene or THF in the presence of AIBN at 80℃ to form the corresponding poly(styrene derivative)s, **3** and **4**, respectively. The polymer **3** with N-Boc protected amino termini in each monomer unit was obtained from hexane as white precipitate in 27% yield, whereas the polymer **4**, which has much low solubility toward the solvent, THF, was given in 63% yield by washing with THF and drying. The molecular weight of the polymers, Mn of **3** and **4** was 25,000 and 7,200, respectively, determined by SEC analysis calibrating with a polystyrene standard sample. And the ^{1}H NMR and IR resonances due to the vinyl groups (^{1}H NMR: δ 5~6; IR: $\nu_{(C=C)}$ 1620 cm^{-1}) of **1** and **2** were all disappeared in polymers **3** and **4**, being compatible with consuming of all of the monomers in these polymerization reactions. The melting points of **3** and **4** (**3**: 80-90, **4**: 94-125℃) roughly measured by a melting point apparatus showed no specific stereoregularity induced in the polymer main chain [18]. Further, an analysis about the optical property was done. The specific rotation of compounds **3** was $-12.9°$. This suggested that the optical purity of the amino-acid side chains may not decrease in the polymerization processes.

Table 2 Polymerization of **1** and **2**[a]

monomer	yield / %	m.p. / °C	Mn[d]	Mw/Mn[d]
1[b]	27	80-90	25000	3.3
2[c]	63	94-125	7200	1.3

[a] Polymerization conditions; monomer : AIBN = 100 : 1, at 80 °C, 24 h. [b] In benzene. [c] In THF. [d] Determined by means of SEC. Calibration was carried out by using polystyrene standards.

The antibiotic activity of the synthetic polymer **4** bearing free-amino termini in the side chain was preliminary examined using poly(α-lysine) having antistatic activity toward Gram-positive bacteria as control. The results did not show the activity of the polymer **4** against both Gram-positive and -negative bacteria.

4. SUMMARY

In this text, a styrene monomer bearing alanyl ester with or without free amino (NH_2) terminus was synthesized and polymerized with AIBN at 80℃ via radical pathway. A N-Boc-protected 4'-vinylbenzyl alanyl ester was synthesized in high yield in the reaction of 4'-vinylbenzyl chloride with Boc-alanine in the presence of less nucleophilic base and solvent. Deprotection of the Boc-group from the ester **1** by adding 25% trifluoroacetic acid solution in toluene gave the targeting monomer **2** in good yield. These established synthetic methods of monomers did not allow decreasing their optical purity. The styrene derivatives polymerized with AIBN at 80℃ to give poly(styrene derivative)s successfully with high molecular weight. This is a first example preparing poly(styrene)s bearing N-free chiral amino termini in the side chain targeting the *chiral-amino-acid-functionalized polystyrenes*. The preparative method of the styrene derivatives may be applicable to the other amino-acid

containing styrenes, and further studies of synthesis and properties and antibiotic examination of amino acid functionalized poly(styrene)s are underway.

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