Preparation of Poly(L-serine ester): A Structural Analogue of Conventional Poly(L-serine)

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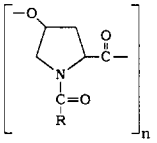
ABSTRACT: The preparation of poly(*N*-acyl-L-serine ester) from derivatives of *N*-acyl-L-serine was investigated in detail. *N*-(Benzyloxycarbonyl)-L-serine was selected as a model monomer since the benzyloxycarbonyl group can be readily removed without degradation of the polymer backbone. The direct polyesterification of *N*-(benzyloxycarbonyl)-L-serine in solution and the melt transesterification of *N*-(benzyloxycarbonyl)-L-serine methyl ester failed to yield polymers of high molecular weight. Best results were obtained by the ring-opening polymerization of *N*-(benzyloxycarbonyl)-L-serine β-lactone. This polymerization was accompanied by a chain-transfer reaction. Under optimized conditions, poly[*N*-(benzyloxycarbonyl)-L-serine ester] with a molecular weight (M_w) of ca. 40 000 (GPC, relative to polystyrene standards) was obtained. Removal of the *N*-benzyloxycarbonyl group by catalytic transfer hydrogenation yielded for the first time optically pure poly(L-serine ester·HCl), a structural analogue of conventional poly(L-serine). Such serine-derived polyesters may be used as model compounds in structural studies and may find applications as biomaterials.

Introduction

Although synthetic poly(amino acids) have been widely investigated, very few practical applications have been identified for these polymers. With few exceptions, poly(amino acids) tend to be insoluble polymers that decompose in the molten state. Most synthetic poly(amino acids) therefore cannot be processed into shaped objects by conventional fabrication techniques.[1] Synthetic poly(amino acids) also tend to be expensive materials, partly because of the need to secure *N*-carboxyanhydrides as the monomeric starting materials. This combination of material properties has clearly limited the range of practical applications for synthetic poly(amino acids).

Recently, we reported that the polymerization of trifunctional α-L-amino acids via the side-chain functional groups results in the incorporation of non-amide linkages into the poly(amino acid) backbone.[2,3] The resulting polymers can be regarded as backbone-modified, "pseudo"-poly(amino acids). Specific examples of such materials include a polyester derived from N-protected 4-*trans*-hydroxy-L-proline (**1**)[3,4] and a series of poly(iminocarbonates) made of derivatives of L-tyrosine (**2**).[3,5] These



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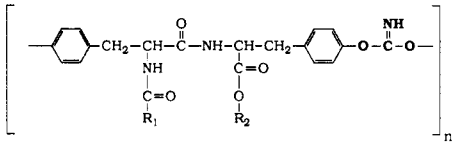

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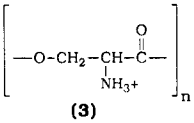
polymers had better material properties (solubility in organic solvents, thermal stability in the molten state, processibility, mechanical strength) than the corresponding conventional poly(amino acids). In addition, poly(*N*-acyl-4-hydroxy-L-proline ester) and the tyrosine-derived poly(iminocarbonates) were found to be biocompatible when subjected to several preliminary toxicological assays.[6,7] Consequently, the incorporation of non-amide linkages into the backbone of poly(amino acids) appears to be a promising approach for the design of new, chiral polymers that may find a variety of medical or industrial applications.

As part of our ongoing exploration of backbone-modified poly(amino acids), we report here on the preparation of poly(L-serine ester·HCl) (**3**), a structural analogue of conventional poly(L-serine) (**4**). Previous efforts



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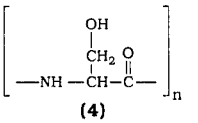

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to prepare polymer (**3**) had failed: Fasman attempted to synthesize poly(D,L-serine ester) from poly(D,L-serine) by means of the N → O acyl shift of serine but obtained only a structurally ill-defined amide–ester copolymer.[8] Later, Jarm and Fles prepared poly[*N*-(phenylsulfonyl)-serine ester] of relatively low molecular weight by the ring-opening polymerization of *N*-(phenylsulfonyl)-serine β-lactone.[9] Since the phenylsulfonyl group cannot be readily removed from the polymer, this reaction scheme cannot be used for the preparation of unprotected poly(serine ester) (**3**). A further disadvantage is the use of the Miyoshi procedure[10] for the lactonization of serine. Since this procedure is reportedly limited to the benzenesulfonamide function as the amino protecting group,[11] the reaction scheme suggested by Jarm and Fles cannot be easily adapted to the preparation of polymers carrying different N-protecting groups.

In order to find a generally applicable synthetic route for the preparation of optically pure poly(L-serine ester), we explored the tendency of N-protected serine derivatives to undergo polyesterification reactions. While the commercially important, terephthalic acid derived polyesters are usually prepared by transesterification reactions,[12] the ring-opening polymerization of lactones is the method of choice for the preparation of several important poly(hydroxy acids) such as poly(lactic acid), poly(glycolic acid), or polycaprolactone.[13] In our studies, three different approaches, the direct esterification of N-protected serine in solution, the melt transesterification of N-protected serine methyl ester, and the ring-

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opening polymerization of N-protected serine β-lactone, were investigated.

Experimental Section

Materials. *N*-(Benzyloxycarbonyl)-L-serine (*N*-Z-L-Ser) and *N*-(*tert*-butyloxycarbonyl)-L-serine (*N*-Boc-L-Ser) (Chemical Dynamics, South Plainfield, NJ) were recrystallized from ethyl acetate, diethyl azodicarboxylate (Aldrich, Milwaukee, WI) was freshly distilled, and triphenylphosphine (Aldrich) was dried over phosphorus pentoxide. Palladium (10%) on activated carbon (Aldrich) was used for all catalytic transfer hydrogenations as supplied. *N*-(Benzyloxycarbonyl)dehydroalanine and *N*-(benzyloxycarbonyl)dehydroalanine methyl ester (Bachem Biosciences, Philadelphia, PA) were used as NMR reference compounds as supplied. Tetrahydrofuran (THF) and acetonitrile (Fisher, Springfield, NJ) were dried by distillation under argon from metallic sodium and phosphorus pentoxide, respectively. All other solvents were of HPLC grade and were used without further purification. Tetraethylammonium benzoate[14] and *N*-(benzyloxycarbonyl)-L-serine methyl ester[15] were prepared according to published procedures.

Analysis. ^1H and ^{13}C NMR spectra were recorded on a VXR-200 spectrometer. IR spectra were recorded on a Mattson Cygnus 100 FTIR spectrophotometer. A Perkin-Elmer DSC-4 system was used for differential scanning calorimetry. The molecular weight was determined by gel permeation chromatography (GPC) on a chromatographic system consisting of a Perkin-Elmer Series 410 LC pump, a Waters Model 410 RI detector, and a Perkin-elmer 3600 data station. Chromatograms were obtained in DMF containing 0.1% (w/v) of LiBr at a flow rate of 1 mL/min using two PL gel columns (17 × 30 mm, 5-μm particle size, 100- and 1000-Å pore size, respectively). Elemental analysis was done by Robertson Laboratories (Madison, NJ).

***N*-Hexadecanoyl-L-serine.** HCl·Ser-OMe (3.887 g, 25 mmol) was dissolved in 50 mL of water and placed into a three-necked flask. Chloroform (125 mL) and sodium bicarbonate (12.5 g) were added. With vigorous stirring, a solution of palmitoyl chloride (6.872 g, 25 mmol) in 50 mL of chloroform was added over a period of 1 h. Stirring was continued for 1 h. The reaction mixture was transferred to a 500-mL separatory funnel, and the phases were separated by the addition of a saturated solution of sodium chloride. The organic phase was washed with 0.1 N HCl (50 mL), dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness under reduced pressure: Yield of *N*-hexadecanoyl-L-serine methyl ester: 8.1 g (94%); mp 79–82 °C. The crude product was twice recrystallized from hexane: mp 84–87.5 °C.

Next the methyl group was removed by basic hydrolysis for 2 h in a solution of lithium hydroxide (8 g) in 600 mL of methanol/water (3:1). The reaction mixture was acidified with 2 N HCl to pH 3, and *N*-palmitoyl-L-serine was extracted into ethyl acetate. The extract was dried with magnesium sulfate and filtered, and the filtrate was evaporated to dryness under reduced pressure. Yield of *N*-hexadecanoyl-L-serine: 6.5 g (85%). The crude product was recrystallized from ethyl acetate: mp 93–105 °C.

Solution Polymerization of *N*-Z-L-Ser. The monomer (5 g) was suspended in 75 mL of toluene and placed into a Batzer polymerization apparatus.[16] *p*-Toluenesulfonic acid (50 mg) was added, and the mixture was refluxed for 24 h. During this time the reaction mixture turned brown. After evaporation of the solvent, a brown residue was obtained. Molecular weight (GPC in DMF/LiBr, relative to polystyrene): M_n = 420, M_w = 1750.

Melt Transesterification of *N*-Z-L-Ser Methyl Ester. Ca. 1 g of monomer was thoroughly mixed with ca. 10 mg (1% (w/w)) of the appropriate catalyst (toluenesulfonic acid, potassium *tert*-butoxide, sodium methoxide, aluminum isopropoxide, or cadmium acetate). The mixture was placed into a polymerization tube, and the transesterification reaction was allowed to proceed for 6 h at 120–160 °C under a pressure of ca. 5×10^{-3} mmHg. The crude reaction products were analyzed by FT-IR, ^1H NMR, and GPC (see Results).

***N*-Z-L-Serine β-Lactone.** The Vederas procedure[11,19] was modified as follows: To a solution of triphenylphosphine (13.12 g, 50 mmol) in 200 mL of a mixture of acetonitrile and THF (85:15) at room temperature was added diethyl azodicarboxylate (8.71 g, 50 mmol) under an atmosphere of argon. After stirring for 30 min, the reaction mixture was cooled to just above its freezing point (ca. −48 to −45 °C). Then *N*-Z-L-Ser (11.91 g, 49.8 mmol) in 200 mL of a mixture of acetonitrile and THF (85:15) was slowly added over a period of 1 h. Thereafter, stirring was continued for an additional 30 min. The reaction mixture was slowly warmed to room temperature over a period of 3 h. After evaporation of the solvents under reduced pressure at a bath temperature of ca. 25 °C, a white solid material was obtained. The crude product was dissolved in 20 mL of methylene chloride/ethyl acetate (85:15). Crystals of 1,2-dicarbethoxyhydrazine precipitated and were removed by filtration. The filtrate was further purified by flash chromatography on silica gel 60, using methylene chloride/ethyl acetate (85:15) as the mobile phase: yield 8.92 g, 81%; mp 131–133 °C. After recrystallization from ethyl acetate/hexane, the melting point increased to 133–134 °C (lit.[19] mp 133–134 °C). IR (KBr, cm^{-1}) ν 3366 (NH), 1835 (lactone C=O), 1688 (urethane C=O), 1532 (C–N), 1267 (C–O–C), 1109, 1019, 884; ^1H NMR ($CDCl_3$) δ 4.42 (2 H, d, CH_2), 5.04 (1 H, m, CH), 5.12 (2 H, s, CH_2Ph), 5.57 (1 H, d, NH), 7.34 (5 H, s, Ph); ^{13}C NMR ($CDCl_3$) δ 168.7, 155.2, 135.4, 128.6, 128.5, 128.4, 128.2, 67.8, 66.3, 59.6. Anal. Calcd for $C_{11}H_{11}NO_4$: C, 59.73; H, 5.01; N, 6.33. Found: C, 59.46; H, 4.95; N, 6.21.

Melt Polymerization of *N*-Z-L-Serine β-Lactone. Ca. 1 g of lactone was placed into a sealed Pyrex polymerization tube under a pressure of 1×10^{-3} mmHg of nitrogen and reacted at 135 °C for 3–6 h.

Ring-Opening Polymerization of *N*-Z-L-Serine β-Lactone. Under argon, Z-L-serine β-lactone (2.7 g, 12.2 mmol) was dissolved in 12 mL of THF. Tetraethylammonium benzoate (15 mg, 0.060 mmol) was added. The mixture was kept at 30 °C for 7 days. At that time, TLC and GPC analysis showed that virtually all Z-L-serine β-lactone had been consumed. The reaction mixture solidified in the course of the polymerization. The polymerization was terminated by the addition of 2 mL of methanol, and the polymer was fully precipitated by the addition of 50 mL of ethyl ether. After filtration and extensive washings with ethyl ether, the polymer was obtained as a white powder: yield 2.46 g, 90%. For purification, the crude polymer was dissolved in DMF and reprecipitated in ethyl ether. ^{13}C NMR (DMSO-d_6), see Figure 1; ^1H NMR (DMSO-d_6), see Figure 5B. Anal. Calcd for $C_{11}H_{11}NO_4$: C, 59.73; H, 5.01; N, 6.33. Found: C, 59.44; H, 5.16; N, 6.21. Molecular weight (GPC in DMF/LiBr, relative to polystyrene): M_n = 29 700, M_w = 38 300, DP ~ 134.

Copolymerization of *N*-Z-Serine β-Lactone and *N*-Boc-Serine β-Lactone. *N*-Boc-Serine β-lactone was prepared as described previously.[19] The procedure for the ring-opening polymerization of *N*-Z-serine β-lactone was followed, using mixtures containing various proportions of *N*-Z-serine β-lactone and *N*-Boc-serine β-lactone in THF. All copolymerizations were conducted at 35 °C, using tetraethylammonium benzoate as the initiator at a monomer to initiator ratio of 200/1.

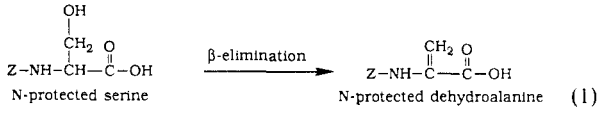
Deprotection of Poly(*N*-Z-L-serine ester). To a solution of poly(Z-serine ester) (300 mg) in DMF (4 mL) was added palladium catalyst (1 g). With vigorous stirring, 98% formic acid (14 mL) was slowly added to the mixture. The evolution of hydrogen was vigorous at first and ceased after ca. 1 h. Stirring was continued at room temperature for 14 h, and then the palladium catalyst was removed by filtration and washed with 20 mL of 1 N HCl. The washings were combined with the filtrate. The combined solution was concentrated to a total volume of 5 mL by partial evaporation under reduced pressure. The concentrated solution was then mixed with 1 N HCl (10 mL) to ensure the complete replacement of the formate salt by hydrochloric acid. Finally, the acidic polymer solution was added dropwise into a large excess of acetone, resulting in the precipitation of poly(L-serine ester hydrochloride) as a colorless powder: yield 150 mg (89%). ^1H NMR (D_2O), see Figure 5C; IR (KBr), see Figure 6B.

Results

Direct Esterification in Solution. Direct esterification of N-protected serine in the presence of a suitable

catalyst represents probably the most straightforward approach to the preparation of poly(*N*-acyl-L-serine ester). In 1951 Batzer et al.[16] reported the successful synthesis of polyesters with molecular weights in excess of 50 000 from mixtures of diacids and dialcohols by azeotropic distillation in the presence of catalytic amounts of various acids. In analogy to Batzer's procedure,[16] we suspended *N*-(benzyloxycarbonyl)-L-serine (*N*-Z-L-Ser) in toluene in a modified Dean–Stark distillation apparatus in the presence of 1% (w/w) of *p*-toluenesulfonic acid. Upon heating to reflux, the reaction mixture turned brown. GPC analysis showed that the reaction product was a mixture of low oligomers and unreacted monomer. Initially, we suspected that the instability of the benzyloxycarbonyl protecting group under acidic conditions interfered with the polymerization. We therefore repeated the above experiment using *N*-hexadecanoyl-L-serine as monomer. Although this monomer carries a stable amino protecting group, no high polymers were obtained.

The formation of dehydroalanine due to a β-elimination reaction at the serine side chain (eq 1) represents



N-protected serine N-protected dehydroalanine (1)

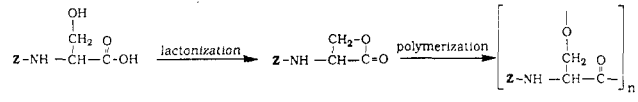
one plausible explanation for the failure of the direct esterification technique to yield polyesters of serine. Formation of dehydroalanine is a well-known side reaction of serine[17] that would lead to the premature termination of the polymerization. In order to test this hypothesis, we obtained an authentic sample of *N*-(benzyloxycarbonyl)-dehydroalanine and compared the ^{1}H NMR spectrum of the authentic sample to the spectrum of the crude product obtained from the polymerization reaction. In deuterated chloroform, *N*-(benzyloxycarbonyl)dehydroalanine exhibits peaks at 5.9 and 6.3 ppm (in DMSO the corresponding absorptions are shifted to 5.6 and 5.8 ppm, respectively; see Figure 5A). These peaks are characteristic of the terminal methylene group (=CH_2) and can serve as markers for the presence of dehydroalanine in the reaction mixture. In some (but not all) of the crude reaction products, very small peaks at ca. 5.9 and 6.3 ppm were indeed observed. Although our results are not conclusive, they indicate that dehydroalanine formation may contribute to the failure of the direct esterification technique to yield serine-derived polyesters of high molecular weight.

Melt Transesterification. Next we investigated the transesterification of *N*-(benzyloxycarbonyl)-L-serine methyl ester (Z-Ser-OMe). Transesterifications have the advantage of being catalyzed by a variety of catalysts such as bases, Lewis acids, and numerous coordination compounds.[18] We therefore attempted the polymerization of Z-Ser-OMe in the presence of several known transesterification catalysts, including *p*-toluenesulfonic acid, potassium *tert*-butoxide, sodium methoxide, aluminum isopropoxide, and cadmium acetate.

In all reaction mixtures, some transesterification occurred, as indicated by the evolution of bubbles of methanol that were collected in a cold trap. The use of strong bases as catalysts (potassium *tert*-butoxide and sodium methoxide) resulted in the rapid formation of a deep brown color, indicative of massive decomposition. Analysis by GPC confirmed that no high polymer was present in the reaction products. *p*-Toluenesulfonic acid, the only strong acid tested, was surprisingly inactive. Even after 6 h of reaction, ^{1}H NMR analysis showed that the reaction mixture consisted predominantly of unreacted starting material. With cadmium acetate, the reaction mixture turned brown within 6 h. Again, no polymeric material was formed.

The most promising results were obtained with aluminum isopropoxide as the catalyst: After 6 h at ca. 120 °C the transesterification of Z-Ser-OMe resulted in the formation of a nearly colorless, viscous melt that rapidly solidified. The number-average molecular weight (M_n) of this material was 600 by GPC. ^{1}H NMR and FT-IR confirmed that the reaction product was oligomeric poly(*N*-Z-serine ester). Apparently, the rapid increase in the viscosity of the reaction mixture prevented the formation of high polymers. Since the reaction mixture turned brown and decomposed above 120 °C, it was not possible to simply increase the reaction temperature in order to bring the polymerization to completion.

Ring-Opening Polymerization. The difficulties observed in the preparation of polymers of high molecular weight by direct esterification or by transesterification prompted us to search for an alternative polymerization technique. The recent development by Vederas et al. of a procedure for the synthesis of serine β-lactones[11,19] made it possible to consider the ring-opening polymerization of N-protected L-serine β-lactones (eq 2). This reaction had heretofore not been studied in detail. We investigated both the thermally initiated melt polymerization and the catalyzed solution polymerization of Z-serine β-lactone.



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A. Thermally Initiated Melt Polymerization. Since Z-serine β-lactone melts at ca. 133–135 °C, we selected 135 °C as the lowest possible reaction temperature. Over a period of 6 h, the thermally initiated polymerization resulted in the formation of oligomers of low molecular weight (as determined by GPC). When the reaction time was prolonged beyond 6 h, the gradual formation of a brown color indicated the occurrence of decomposition reactions.

The reported facile thermal decarboxylation of β-lactones[20] at ca. 140 °C is probably an important chain-terminating reaction during the thermally initiated polymerization of Z-serine β-lactone. In order to lower the polymerization temperature, we added a high-boiling, inert solvent (diphenylmethane) to the monomer. This allowed the polymerization to be carried out at ca. 100 °C. Now the formation of brown color was substantially reduced, but no polymers were formed over a period of 72 h. This observation indicated that the thermally initiated polymerization of Z-serine β-lactone is not feasible, since thermal initiation apparently requires temperatures that are sufficiently high to induce the thermal degradation of the monomer as well.

B. Polymerization in Solution. Polymers of high molecular weight were reportedly obtained when, among others, triethylamine,[21,22] α-trimethylammonium acetate (betaine),[9,21] (tetraphenylporphinato)aluminum chloride,[23] potassium acetate/dicyclohexyl-18-crown ether-6,[24] or tetraethylammonium benzoate (TEAB)[25] was used as initiator of the solution polymerization of propiolactone and its α- or β-substituted derivatives. We therefore tested these compounds as initiators of the ring-opening polymerization of Z-serine β-lactone. These exper-

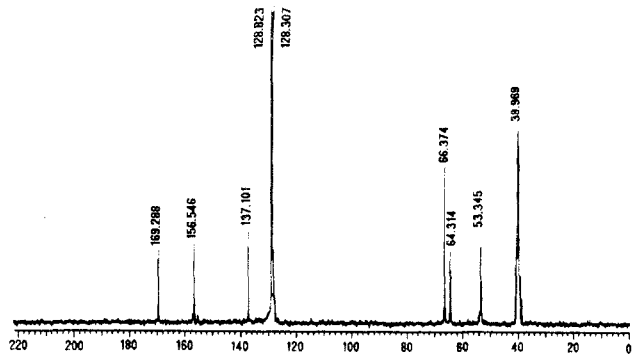


Figure 1. ^{13}C NMR spectrum of poly(Z-serine ester) in deuterated DMSO. Peak assignment (δ): 169.288 (ester carbonyl), 156.546 (urethane carbonyl), 137–128 (phenyl), 66.374 ($PhCH_2$), 64.314 (CH_2O), 53.345 (CH), 39.969 (DMSO).

iments were done in THF at 35 °C employing a constant monomer/initiator ratio of 200/1. Among all tested initiators only potassium acetate/dicyclohexyl-18-crown ether-6 and TEAB reproducibly yielded polymeric products of high molecular weight. In order to avoid the mechanistic complications associated with the use of a phase-transfer catalyst in a heterogeneous reaction mixture, we preferred TEAB over potassium acetate/dicyclohexyl-18-crown ether-6 as the model initiator for our further investigation of the ring-opening polymerization of Z-serine β-lactone.

In correspondence to eq 2, the polymeric product obtained from the reaction of Z-serine β-lactone in the presence of TEAB was assumed to be poly(Z-serine ester). Spectroscopic evidence obtained from ^{13}C NMR (Figure 1), ^1H NMR (Figure 5B), and FT-IR (Figure 6A) was in complete agreement with this structural assignment. Elemental analysis (Calcd for poly(Z-serine ester): C, 59.73; H,5.01; N, 6.33. Found: C, 59.44; H, 5.16; N, 6.21) also concurred with the proposed structure. Furthermore, upon acid hydrolysis of the polymer, followed by amino acid analysis of the hydrolysate, 86% of the theoretical amount of optically pure L-serine was recovered (see below for details). Together, these results represent conclusive evidence for the proposed structure of poly(Z-serine ester).

Next we investigated the effect of several solvents on the polymerization reaction. Due to the insolubility of poly(*N*-Z-serine ester) in nonpolar solvents, we limited our experiments to aprotic, polar solvents such as tetrahydrofuran (THF), dimethylformamide (DMF), *N*-methylpyrrolidone (NMP), and hexamethylphosphoramide (HMPA). Aprotic, polar solvents had previously been reported as solvents for the ring-opening polymerization of α,α-dimethylpropiolactone and other β-lactones.[26]

The ring-opening polymerizations of Z-serine β-lactone were performed with TEAB as the initiator at 40 °C, using a molar ratio of monomer/initiator of 76/1. The molecular weight of the polymeric products was monitored during the polymerization by GPC. Due to the relatively high concentration of initiator, the polymerizations proceeded rapidly. In all tested solvents, the molecular weight reached a maximum value after ca. 9 h and seemed to decline thereafter. The decrease of the molecular weight was accompanied by a significant broadening of the molecular weight distribution. The obtainable maximum molecular weight was clearly related to the reaction solvent and increased in the order HMPA, DMF, NMP, THF (Figure 2). This observation indicated that the use of less polar solvents may be advantageous. Unfortunately, however, the insolubility of poly(Z-serine ester) in solvents less polar than THF made it impossible to test this hypothesis.

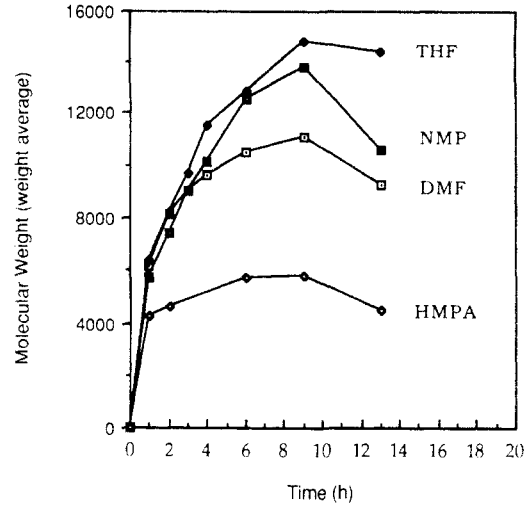


Figure 2. Solvent effect on the ring-opening polymerization of *N*-Z-serine β-lactone. Four solvents were tested by following the polymer molecular weight as a function of time. Best results were obtained for THF.

Table I
Temperature Dependence of the Ring-Opening Polymerization of Z-Serine β-Lactone

reacn temp,[a] °C	approx reacn time for quant conv,[b] h	mol wt[c] M_w	mol wt[c] M_n	M_w/M_n
4	n/a	n/a	n/a	n/a
25	36	10800	5800	1.86
35	18	12000	9100	1.32
40	10	11300	8600	1.31
60	3	9900	4900	2.02

[a] Other reaction conditions are as described in the Experimental Section, except that the ratio of monomer to initiator was 50:1. [b] The reactions were followed by GPC till free monomer could no longer be detected in the reaction mixture. Thus the reactions were essentially allowed to proceed to completion. At 4 °C, the reaction was still incomplete when the experiment was terminated after 7 days (168 h). [c] Molecular weights were determined by GPC in DMF/LiBr relative to polystyrene standards.

In many reports on the ring-opening polymerization of β-lactones, reaction temperatures of ca. 30–80 °C were used.[9,25] Considering the facile thermal decarboxylation of Z-serine β-lactone, we initially assumed that low reaction temperatures would be preferable. Due to the limited solubility of Z-serine β-lactone in cold THF, 4 °C was the lowest practically applicable reaction temperature. We therefore conducted the ring-opening polymerization of Z-serine β-lactone at temperatures ranging from 4 to 60 °C.

Besides the obvious increase in the rate of polymerization with increasing temperature, we found no significant effect of the reaction temperature on the *average* molecular weight of the final polymeric product. A more detailed analysis of the polymers by GPC revealed some broadening of the molecular weight distribution at reaction temperatures below 30 °C and above 40 °C (Table I). Since poly(Z-serine ester) precipitated in the course of the reaction, the reaction kinetics are apparently quite complicated. No attempt was made to explain the observed broadening of the molecular weight distribution. From a practical point of view, the most important conclusion of these experiments was that the polymerization of Z-serine β-lactone is best conducted at temperatures of ca. 30–40 °C.

C. Chain-Transfer Reactions. In order to obtain a preliminary indication whether the ring-opening polymerization of *N*-Z-serine β-lactone could possibly be a living polymerization, we performed the following exper-

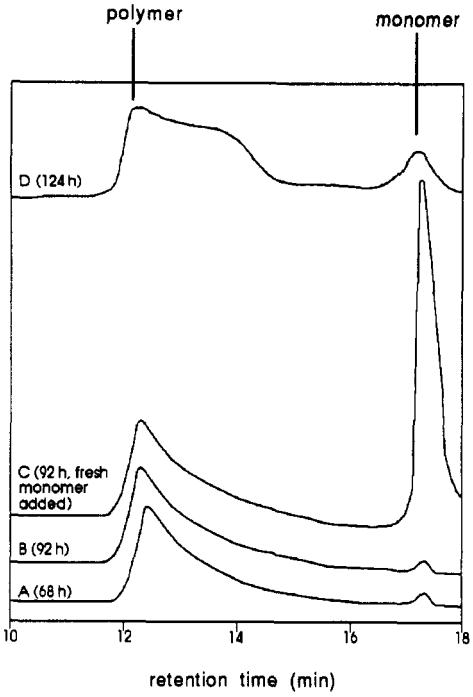


Figure 3. Delayed addition of fresh monomer to a ring-opening polymerization mixture. The reaction mixture was analyzed by GPC at various times (curves A–D). Fresh monomer, added to the reaction mixture 92 h after initiation of the reaction, was converted to polymer, indicating that some reactive species remained in the reaction mixture. However, the broadening of the molecular weight distribution seen in curve D indicates that the polymerization mechanism is not a typical "living polymerization". For details, see text.

iment: 0.3 g of *N*-Z-serine β-lactone was allowed to polymerize under the usual conditions (THF solution, monomer/initiator ratio = 200/1, 30 °C). After 68 h, most of the monomer had been consumed (Figure 3, curve A). The reaction mixture was then kept for an additional 24 h. During this time no further changes in the molecular weight distribution of the reaction products occurred (Figure 3, curve B). Then, a total of 92 h after the initiation of the polymerization reaction, 3.6 mL of a THF solution containing 0.2 g of fresh monomer was added to the polymerization mixture (Figure 3, curve C). In a living polymerization, the addition of monomer would simply result in an increase of the polymer molecular weight. On the other hand, if the polymerization had been terminated, no conversion of the added monomer to polymer would occur.

We followed the reaction by GPC analysis over an additional period of 32 h. The added monomer was indeed converted to polymer; however, the average molecular weight of the polymer did not increase as expected for a living polymerization. Instead, GPC analysis revealed that the low molecular weight fractions of the polymer increased in relative abundance, resulting in an overall broadening of the molecular weight distribution (Figure 3, curve D). This observation indicated that although there were still reactive species in the reaction mixture at the time the fresh monomer was added, the polymerization of Z-serine β-lactone is not a simple living polymerization.

As a further test of the polymerization mechanism, we polymerized *N*-Z-serine β-lactone using five different ratios of monomer/initiator under the usual conditions. A *linear* relationship between the monomer/initiator ratio and the polymer molecular weight is usually indicative of a living polymerization. In our experiments, no linear relationship between the monomer/initiator ratio and the polymer molecular weight was observed (Figure 4). This

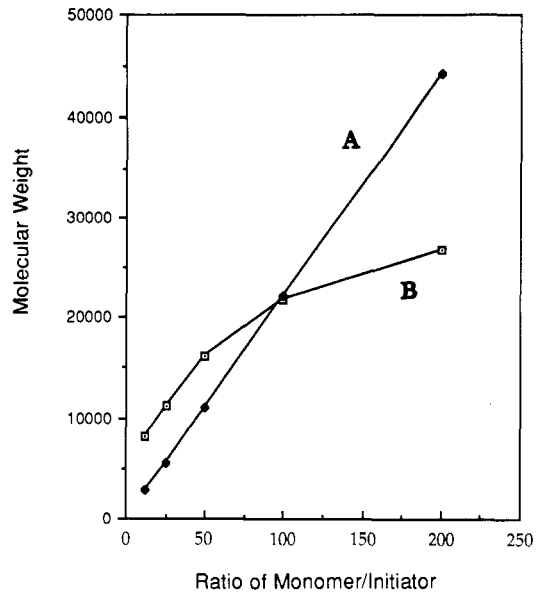
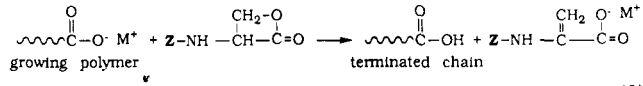


Figure 4. Correlation of the monomer to initiator ratio vs polymer molecular weight (M_w by GPC relative to polystyrene standards) for the ring-opening polymerization of *N*-Z-serine β-lactone. Curve A represents the calculated correlation based on an ideal "living polymerization mechanism" at quantitative monomer conversion. Curve B represents the experimentally determined molecular weights at quantitative monomer conversion under the conditions described in the text.

result was clearly inconsistent with the mechanism of a "living polymerization".

These experimental results indicated that chain-transfer reactions may occur during the anionic polymerization of Z-serine β-lactone. The occurrence of chain-transfer reactions had previously been observed during the polymerization of β-lactones that carry hydrogen atoms at the α position.[27] In our case, the chain-transfer mechanism suggested by Yamashita[27] would lead to the formation of the anion of *N*-(benzyloxycarbonyl)dehydroalanine (eq 3).



(3)

As mentioned before, the characteristic peaks of the terminal methylene group are detectable by ^{1}H NMR. In deuterated DMSO, authentic samples of *N*-(benzyloxycarbonyl)dehydroalanine and *N*-(benzyloxycarbonyl)dehydroalanine methyl ester exhibited singlets at 5.60 and 5.77 ppm (Figure 5A). Although theoretically two doublets would have been expected, the small geminal coupling in alkenes can often not be observed experimentally. When we analyzed samples of poly(*N*-Z-serine ester), the NMR spectra revealed two small but clearly visible peaks at 5.60 and 5.80 ppm (Figure 5B). The relative intensity of these peaks tended to increase as the duration of the polymerization reaction was prolonged. There can be little doubt that these peaks must be assigned to the cis and trans protons of the terminal methylene group of dehydroalanine. The presence of dehydroalanine derivatives in poly(*N*-Z-serine ester) provides strong evidence for the occurrence of the proposed chain-transfer reaction (eq 3).

Properties of Poly(*N*-Z-serine ester). Poly(*N*-Z-serine ester) was obtained as a white powder that was insoluble in water and all common organic solvents, with the exception of highly polar, aprotic solvents such as DMF and DMSO. The polymer started to decompose

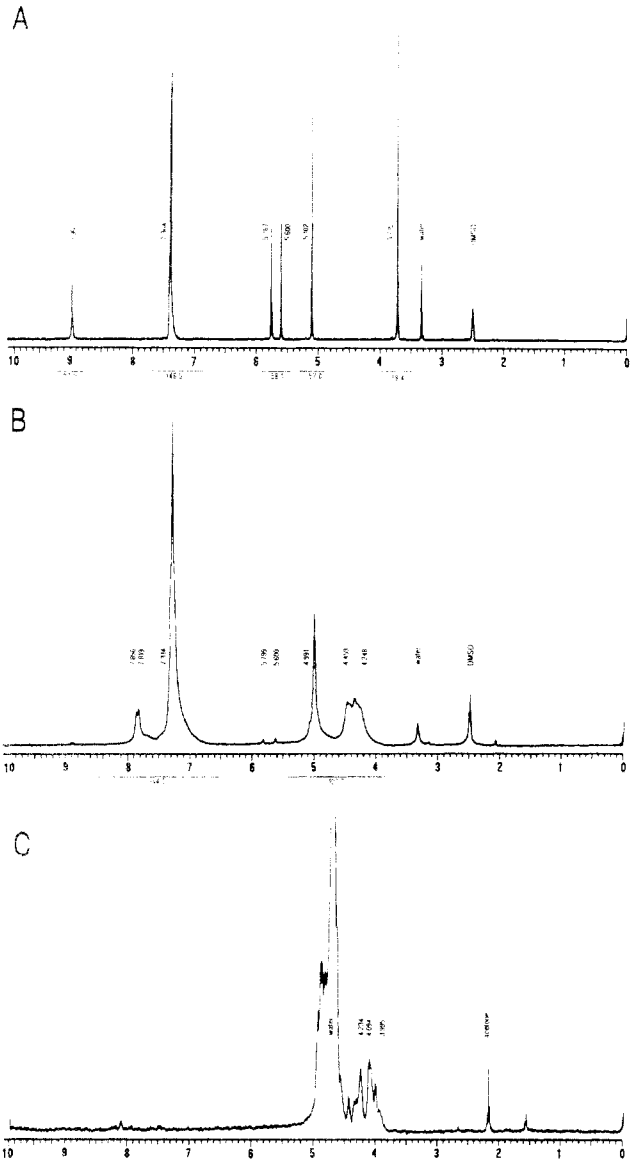


Figure 5. Representative ^1H NMR spectra. (A) ^1H NMR spectrum of an authentic sample of Z-dehydroalanine methyl ester in deuterated DMSO. The spectrum shows two singlets at 5.767 and 5.600 ppm that must be assigned to the terminal methylene protons of the dehydroalanine side chain. The theoretically expected, small geminal coupling of these protons is not observed. (B) ^1H NMR spectrum of poly(Z-serine ester) in deuterated DMSO. Two small peaks at 5.799 and 5.600 ppm may be assigned to the terminal methylene protons of the dehydroalanine side chain. The possible presence of dehydroalanine derivatives in poly(Z-serine ester) provides evidence for the proposed chain-transfer reaction. The major peaks are in agreement with the proposed structure of poly(Z-serine ester): Peak assignment (δ): 4.25–4.46 (3 H, br m, $CHCH_2$), 4.99 (2 H, s, CH_2), 7.31 (5 H, m, Ph), 7.84 (1 H, d, NH). (C) ^1H NMR spectrum of poly(serine ester·HCl) in D_2O. The resonances of the backbone protons and possibly also the resonances of the amino protons were partially overlapped by the strong water absorption at 4.7 ppm. Residual acetone, used in the workup of the polymer, gave rise to the peak at 2.1 ppm. The most significant feature of this spectrum is the absence of the aromatic absorptions at 7.3 ppm, indicating the quantitative removal of the benzyloxycarbonyl protecting group.

at ca. 220 °C, as determined by differential scanning calorimetry. Powder X-ray diffraction analysis showed that poly(*N*-Z-serine ester) is completely amorphous in the solid state. Detailed studies of the physicomechanical properties of poly(*N*-Z-serine ester) are currently in progress.

In order to determine to which extent the optical purity of L-serine was preserved during lactonization and ring-opening polymerization, samples of poly(Z-serine ester) were hydrolyzed for 8 h at 110 °C in constant-boiling HCl. Amino acid analysis of the hydrolysate showed that 86% of the theoretical amount of serine was recovered. Thereafter, the hydrolysate was derivatized with pentafluoropropionic anhydride and analyzed on a chiral GC column according to the procedure of Frank et al.[28] Within the limits of detection of this technique (~0.5%) no D-serine was detected, indicating that lactonization, ring-opening polymerization, and polymer hydrolysis proceeded with virtually complete retention of optical purity.

Table II
Copolymerization of Z-Serine β-Lactone and Boc-Serine β-Lactone

monomer ratio in reacn mixt (Z/Boc)	polymer comp[a] (Z/Boc)	polymer M_w[b]
100/0	100/0	38300
80/20	82/18	55200
60/40	65/35	56500
40/60	48/52	42300
20/80	31/69	33800
0/100	0/100	n/a

[a] Polymer composition was determined by ^1H NMR. See text for details. [b] Weight-average molecular weight was determined by GPC in DMF/LiBr relative to polystyrene standards.

Copolymers of *N*-Boc-L-Serine β-Lactone and *N*-Z-Serine β-Lactone. In order to confirm the general applicability of the above results, *N*-Boc-L-serine β-lactone was prepared and polymerized, using the procedures developed for *N*-Z-serine β-lactone. No significant differences in the behavior of the two monomers were evident. Surprisingly, however, poly(*N*-Boc-serine ester) was insoluble in all common organic solvents, while poly(*N*-Z-serine ester) was soluble in DMF, DMSO, and NMP.

Next we prepared a series of copolymers of *N*-Z-serine β-lactone and *N*-Boc-serine β-lactone, using monomer ratios of 80/20, 60/40, 40/60, and 20/80 in the polymerization mixture. The polymerizations proceeded readily. The resulting copolymers were more soluble in THF than either one of the homopolymers. Consequently, the copolymers did not form gels during the polymerization as readily as the homopolymers. This property may have contributed to the observed tendency of the copolymers to reach higher molecular weights than the homopolymers (Table II).

All copolymerizations were allowed to proceed for 7 days. At that time, GPC analysis of the reaction mixture revealed only small amounts of residual monomer. Our analytical techniques did not allow us to determine the ratio of free *N*-Boc-serine β-lactone to *N*-Z-serine β-lactone in the reaction mixture with accuracy. On the other hand, after workup, the composition of the resulting copolymers could be readily determined by ^1H NMR, using the integration values of the singlet at 7.31 ppm (due to the aromatic ring of the Z group) and the singlet at 1.36 ppm (due to the methyls of the Boc group). Our results (Table II) show that the resulting polymers were slightly richer in *N*-Z-serine β-lactone than the mixture of monomers used as feed. Since the polymerizations were allowed to go to completion, the fact that the polymers were enriched in one of the monomers indicated that over the course of the 7-day reaction period some of the lactones were deactivated, possibly via decarboxylation or β-elimination. Overall, our results show that *N*-Z-serine β-lactone had a slightly higher tendency to participate in the ring-opening polymerization than *N*-Boc-serine β-lactone.

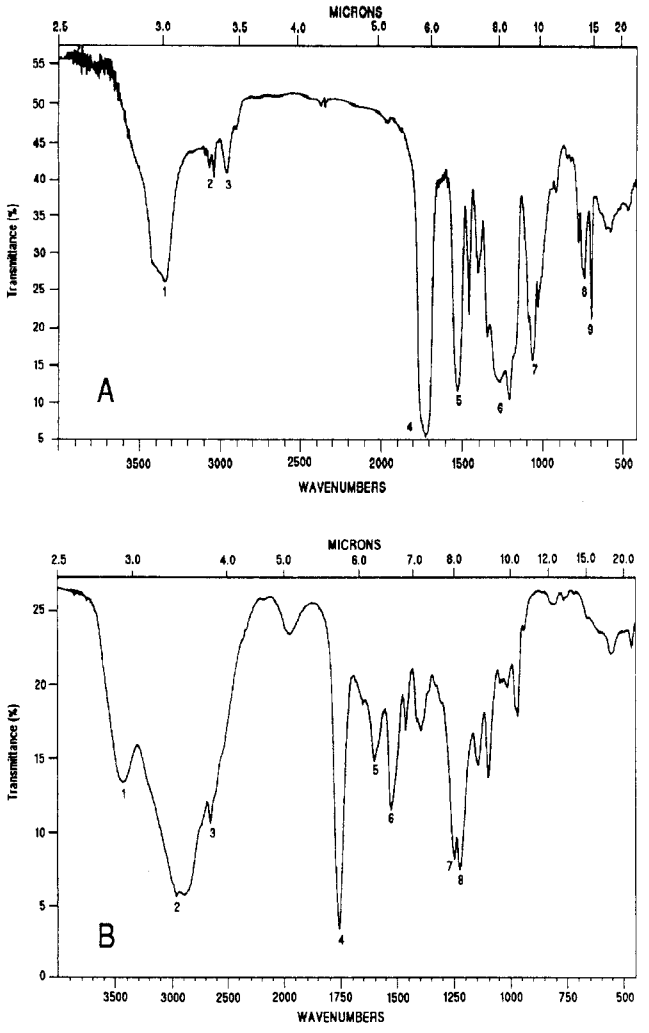


Figure 6. Representative FT-IR spectra. (A) FT-IR spectrum (KBr pellet) of poly(Z-serine ester). Peak assignment (ν, cm^{-1}): peak 1, 3421–3340 (br, NH); peak 2, 3060–3040 (arom CH); peak 3, 2950 (CH); peak 4, 1724 (ester carbonyl); peak 5, 1524 (CN of urethane), peak 6, three overlapping absorptions at 1341, 1267, 1203 (CO for ester and urethane); peak 7, 1060; peaks 8 and 9, 740 and 698 (monosubstituted benzene). (B) FT-IR spectrum (KBr pellet) of poly(serine ester·HCl). The most important features of the spectrum are the strong ammonium band and the lack of the aromatic "out-of-plane bending" absorptions at 740 and 698 cm^{-1}, indicating quantitative deprotection and formation of the proposed free amino group. Peak assignment (ν, cm^{-1}): peak 1, 3447; peak 2, 2960–2900 (ammonium band); peak 3, 2659; peak 4, 1759 (ester carbonyl); peak 5, 1602 (NH_3^+); peak 6, 1530 (NH_3^+); peaks 7 and 8, 1250, 1225 (CO).

Synthesis of Poly(serine ester) by Removal of the Amino Protecting Group. The benzyloxycarbonyl group is usually removed either by acidolysis (HBr/HOAc) or by catalytic hydrogenation.[29] In order to avoid the possible degradation of the polyester backbone, we preferred catalytic hydrogenation over treatment with strong acid. For our experiments, we selected the procedure by ElAmin et al., who used formic acid as the hydrogen donor.[30] After deprotection, we treated the polymer with 1 N HCl followed by evaporation in order to exchange the formic acid salt by the corresponding hydrochloric acid salt. This step eliminated the absorption bands of the formate counterion in the IR and NMR spectra of the deprotected polymer and thus simplified the structural analysis of our reaction products.

Since the unprotected polymer, poly(serine ester·HCl), is only soluble in water, solution NMR spectra could only be obtained in D_2O. Unfortunately, the backbone proton resonances were partially obscured by the water peak (Figure 5C). This reduced the value of ^1H NMR as a tool for the structural analysis of the polymer. On the other hand, the progress of the deprotection reaction could readily be followed by ^1H NMR by monitoring the disappearance of the aromatic proton resonances of the benzyloxycarbonyl group at ca. 7.3 ppm. At room temperature, the quantitative removal of the benzyloxycarbonyl group required ca. 14 h (Figure 5C).

The FT-IR spectrum (KBr pellet) of poly(serine ester·HCl) was in agreement with the assigned structure. Prominent bands at 1759 and 1250–1225 cm^{-1} can be assigned to the ester carbonyl and C–O absorptions. The typical peaks of the R–NH_3^+ group at 2960–2900, 1602, and 1530 cm^{-1} were also observed. Noteworthy is the absence of the aromatic C–H absorptions at 740 and 698 cm^{-1}, confirming the quantitative removal of the benzyloxycarbonyl group (Figure 6B).

Conclusions

Protected poly(*N*-acyl-L-serine esters) with molecular weights of ca. 40 000 (M_w) (as determined in DMF/LiBr relative to polystyrene standards) can be prepared by the ring-opening polymerization of the corresponding N-protected L-serine β-lactones. The polymerization is accompanied by chain-transfer reactions. Attempts to prepare poly(*N*-acyl-L-serine esters) by direct esterification of N-protected L-serine or by melt transesterification of N-protected L-serine methyl esters failed, apparently due to the tendency of the serine side chain to undergo β-elimination reactions.

After removal of the benzyloxycarbonyl protecting group by catalytic transfer hydrogenation, poly(L-serine ester·HCl) was obtained. Poly(L-serine ester·HCl) is a new, chiral polymer that carries one pendent amino group per repeat unit. This rare feature among currently available polyesters should facilitate the attachment of cross-linkers, drug molecules, or various other pendent groups to the polymer backbone. We therefore expect that poly(L-serine ester) may find applications as a degradable "biomaterial".

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Spacer-Modified Polymeric Catalysts Containing Phosphonium Salts for Regioselective Addition Reaction of Epoxy Compounds with Active Esters

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ABSTRACT: The catalytic activity of insoluble polymer-supported catalysts with alkylene spacer chains between polystyrene backbones and quaternary phosphonium salts (catalysts **1**-**19**) was examined for regioselective addition of epoxy compounds (**22a**-**c**) with active esters (**23a**,**b**). The activity was strongly affected by the structure of onium salts, the degree of ring substitution (DRS), the degree of cross-linking (DC), the length of the alkylene spacer chain, and the reaction solvents. The chloride-containing catalysts exhibited higher activity than the bromide-containing catalysts. The catalysts with low DRS had a higher activity than those with high DRS. The catalysts with low DC also had a higher activity than those with high DC. The activity of the spacer-modified catalysts was higher than that of catalysts with no spacer chains. It was found that suitable combination of catalyst and solvent gave a higher activity than low molecular weight catalysts such as tetrabutylammonium bromide (**20**) or chloride (**21**). Furthermore, catalysts with suitable DRS and alkylene spacer can be reused for at least 10 runs.

Introduction

Epoxy compounds with high reactivity are highly useful materials for synthetic organic chemistry and polymer synthesis. It has been reported[1] that quaternary onium halides have a higher catalytic activity than tertiary amines for the addition reaction of epoxy compounds with carboxylic acids. Nishikubo et al.[2] have used some quaternary ammonium and phosphonium halides not only as phase-transfer catalysts for modifications of polymers containing pendant chloromethyl groups but also as catalysts[3] for the addition of pendant epoxide groups of polymers with various carboxylic acids.

About 10 years ago, Funahashi reported[4] on the addition of epoxy compounds with phenyl esters using tertiary amine or potassium *tert*-butoxide as catalysts. However, this reaction was carried out at relatively elevated temperatures. We found recently that the reaction of pendant epoxide groups of polymers with various active esters[5] proceeded smoothly in the presence of quaternary onium halides to give the corresponding adducts under relatively mild reaction conditions. Similar reactions of epoxy compounds with acyl halides,[6] alkyl halides,[7,8] carbon dioxide,[9,10] β-butyrolactone,[11] and diphenyl carbonate[4a,12] have also been conducted by using quaternary onium halides as catalysts.

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