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Synthesis of the Polymer of 1 {3-*O*[(R)-1 (*L*-Alanyl-*D*-Isoglutamyl Carbonyl) Ethyl]-α-*D*-Glucopyranos-6-*O*-Carbonycarbonyl}Ethylene

Tatsuro Ouchi[a]; Toshiyuki Emura[a]; Takashi Taniguchi[a]; Itsuo Maeda[a]
[a] Department of Applied Chemistry, Faculty of Engineering Kansai University Suita, Osaka, Japan

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SYNTHESIS OF THE POLYMER OF 1-{3-*O*-[(R)-1(*L*-ALANYL-*D*-ISOGLUTAMYL CARBONYL) ETHYL]-α-*D*-GLUCOPYRANOS-6-*O*-CARBONYL}ETHYLENE

TATSURO OUCHI, TOSHIYUKI UEMURA, TAKASHI TANIGUCHI, and ITSUO MAEDA

Department of Applied Chemistry
Faculty of Engineering
Kansai University
Suita, Osaka 564, Japan

ABSTRACT

The polymer of 1- 3-*O*-[(*R*)-1-(*L*-alanyl-*D*-isoglutamyl carbonyl)ethyl]-α-*D*-glucopyranos-6-*O*-carbonyl ethylene was synthesized as a acryloyl type polymer by fixing the *D*-glucose analog (GADP) of *N*-acetylmuramyl-*L*-alanyl-*D*-isoglutamine (MDP), which is the minimum required structure responsible for the immunoadjuvant activity of bacterial cell-wall peptidoglycan. *N*-[2-(1,2-*O*-Isopropylidene-6-*O*-acryloyl-α-*D*-glucofuranos-3-*O*-yl)-(*R*)-propionyl]-*L*-alanyl-*D*-isoglutamine benzyl ester (**6**) was prepared as a key monomer in the synthesis. The homopolymerization of **6** and the copolymerization of **6** with hydrophobic acryloyl monomers were carried out in benzene at 60°C by using 2,2′-azobisisobutyronitrile as a radical initiator to give homopolymer **7** and copolymer **10**, respectively. Removal of isopropylidene and benzyl protecting groups from **7**, **10** and **8**, **11** was carried out by acid treatment with trifluoroacetic acid/water (6:1 v/v) and by catalytic hydrogenolysis with palladium carbon, respectively, to afford the homopolymer **9** and the copolymer **12**.

INTRODUCTION

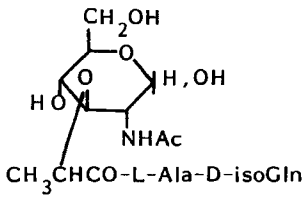
Since Adam et al. [1, 2] and Shiba et al. [3, 4] elucidated independently that *N*-acetylmuramyl-*L*-alanyl-*D*-isoglutamine (MDP) is the minimum required structure responsible for the immunoadjuvant activity attributable to peptidoglycan of the bacterial cell wall, MDP has been of interest from the standpoint of tumor immunotherapy. However, MDP itself has no effect in suppressing the growth of a tumor [5]. On the other hand, mycobacterium bovis bacille de Calmette-Guerin (BCG) cell wall is well known to be highly effective in tumor immunotherapy.

One of the remarkable differences between such a cell wall and MDP is seen in the lack of lipophilicity in the latter, since the BCG cell wall is covered with mycolic acid (one of fatty acid) on the surface [6]. Actually, it is clear by chemical modification of the primary hydroxy group of MDP that the antitumor effect of the cell wall might be attributed to the lipophilic character of the mycolic acid moiety; it was reported that a unique immunopotentiating activity including an antitumor effect can be generated by introduction of a lipophilic mycolic acid [7], quinonyl acid [8], or branched long-chain fatty acid group [9] into the hydroxy group at the 6′ position of MDP via an ester bond.

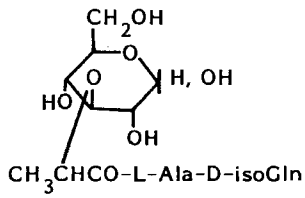
Another marked difference of MDP from the cell wall might be recognized in the lack of polymeric character in the former because the main chain of the cell wall consists of such polymeric materials as peptidoglycan [6]. Moreover, it was demonstrated that a polymeric molecule of MDP exhibits a significantly higher activity than MDP itself [10].

Tabata and Ikada reported recently on the activation of the antitumor of macrophase by capsules consisting of a copolymer of glycolic acid and lactic acid in which the MDP derivative was included [11].

On the other hand, Kiso et al. found that the immunoadjuvant activity of the carbohydrate analog of MDP (GADP) was higher than that of MDP itself [12].

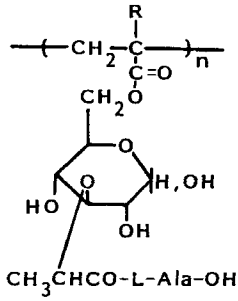


MDP



GADP

As the previous stage of the synthesis of a variety of polymeric and lipophilic analogs of GADP, we have already prepared lipophilic methacryloyl-type (PGMA) [13] and acryloyl-type (PGA) [14] polymers with the pendent simple monoamino acid analog of GADP, *N*-[2-(3-*O*-*D*-glucopyranosyl)propionyl]-*L*-alanine, instead of GADP itself.



PGA(R=H), PGMA(R=CH_3)

Thus, the present paper deals with the synthesis of poly-1-{3-*O*-[(*R*)-1-(*L*-alanyl-*D*-isoglutamyl carbonyl)ethyl]-α-*D*-glucopyranos-6-*O*-carbonyl}ethylene (**9**), in which GADP units and polyethylene main chain are combined through ester bonds.

Moreover, in order to heighten the antitumor activity, the introduction of hydrophobic groups to the polymer was carried out. Four kinds of copolymer **12** of 1-{3-*O*-[(*R*)-1-(*L*-alanyl-*D*-isoglutamyl carbonyl)ethyl]-α-*D*-glucopyranos-6-*O*-carbonyl}ethylene with lauryl, stearyl acrylates, and two kinds of acrylate-containing fluoro groups were prepared.

EXPERIMENTAL

Materials

Benzyl alcohol, triethyl amine (TEA), acrylic acid, and thionyl chloride were purified by distillation. Lauryl and stearyl acrylates, and acrylates (Viscoat 3F and Viscoat 17F) containing some types of fluoro group supplied by Osaka Yuki Co. were purified by distillation. 2,2′-Azobisisobutyronitrile (AIBN) was recrystallized three times from methanol. Acrylic anhydride was prepared according to the method of Brotherton et al. [15].

D-Glucose, sodium hydride (fine powder dispersed in mineral oil at 60% concentration), *L*-α-alanine, Boc-*L*-α-alanine, *D*-glutamic acid, dicyclohexyl-

carbodiimide (DCC), *N*-hydroxysuccinimide (HONSu), *O*-*t*-butyl *S*-(4,6-dimethyl-2-pyrimidinyl) thiocarbonate, dicyclohexylamine (DCHA), *p*-nitrophenol, trifluoroacetic acid (TFA), and palladium black were of reagent commercial grade and used without further purification.

Other reagents were commercially supplied and purified by the usual methods.

Chromatography

The progress of the reactions was monitored by thin-layer chromatography (TLC) with Merck F_{254} silica gel plates.

The silica gel used for column chromatography was Wakogel C-300 prepared by Wako Pure Chemical Industries Co., while the silica gel used for medium-pressure column chromatography was Kieselgel 60 made by Merck Co.

Spectroscopic Measurements

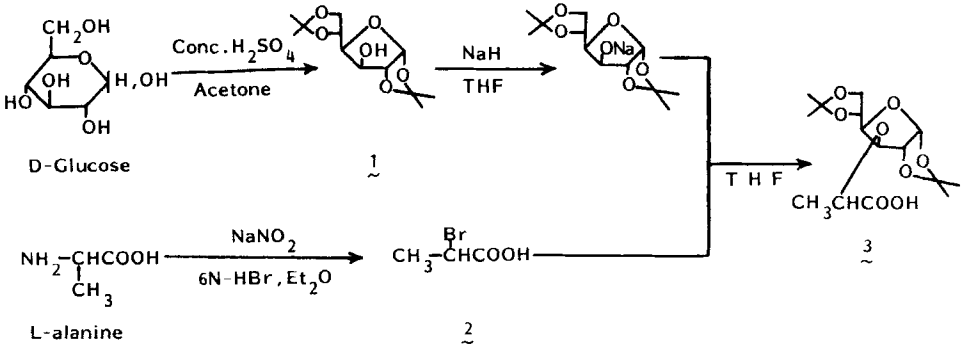
Optical rotation was determined with a Union Digital PM-101 polarimeter. IR spectra were recorded on a JASCO A-202 spectrophotometer. ^{1}H-NMR spectra were measured with a JEOL JNM-PMX-60 or a JEOL JNM-GSX-400 spectrometer using TMS as the internal reference. Mass spectra were obtained on a JEOL JMS-01SG double-focusing mass spectrometer at 75 eV. The molecular weights of the polymers obtained were measured by the GPC method (column: TKS GEL (G3000H8 + G4000H6 + G5000H6); eluent: THF; detector: UV_{270}) or by light-scattering photometry with a Union LS-601 light-scattering photometer.

1,2:5,6-Di-*O*-isopropylidene-3-*O*-[1-(*R*)-carboxyethyl]-α-*D*-glucofuranose (3)

1,2:5,6-Di-*O*-isopropylidene-3-*O*-[1-(*R*)-carboxyethyl]-α-*D*-glucofuranose (**3**) was prepared from *D*-glucose and *L*-alanine through the reaction steps shown in Scheme 1.

1,2:5,6-Di-*O*-isopropylidene-α-*D*-glucofuranose (**1**) was prepared by the sulfuric acid-catalyzed condensation of *D*-glucose with acetone according to Schmidt's method [16], mp 109.5-110.5°C (Ref. 16, mp 110°C).

L-α-Bromopropionic acid (**2**) was prepared according to the method of Fu et al. [17], 74%, bp 83-85°C/5 torr, $[\alpha]_D^{25}$ -24.2°, d^{25} 1.6894 (Ref. 17, 65%, 78°C/4 torr, $[\alpha]_D^{25}$ -27.2°, d^{25} 1.6915). First, **1** (22.7 g, 87 mmol) was dissolved in dry tetrahydrofuran (THF; 300 mL) at room temperature with stirring. Sodium hydride (10.4 g, 60% in oil) was then added to the



SCHEME 1

solution under dry nitrogen. After the initial vigorous reaction had subsided, the mixture was refluxed for 30 min with stirring. After **2** (40 g, 260 mmol) was added to the solution cooled to room temperature, the reaction mixture was again refluxed for 8 h. Water was then added carefully to the reaction mixture with cooling, and THF was evaporated under reduced pressure. The residue was dissolved in water (100 mL), and then the solution was extracted with chloroform (50 mL). The aqueous phase was adjusted to pH 2 with 1 *N* hydrochloric acid with cooling, and the precipitate was extracted with chloroform (100 mL × 3). The combined organic layer was washed with water/*n*-hexane, and the aqueous layer was extracted with chloroform (100 mL). A layer of chloroform was dried over anhydrous sodium sulfate. After the drying agent was filtered off and the filtrate was evaporated under reduced pressure, the residue was subjected to column chromatography on Wakogel C-300. Elution with chloroform/methanol (10:1 v/v) afforded **3** as a syrup, 13.7 g (49%), $[\alpha]_D^{25}$ 5.6° (*c* 1.0, $CHCl_3$).

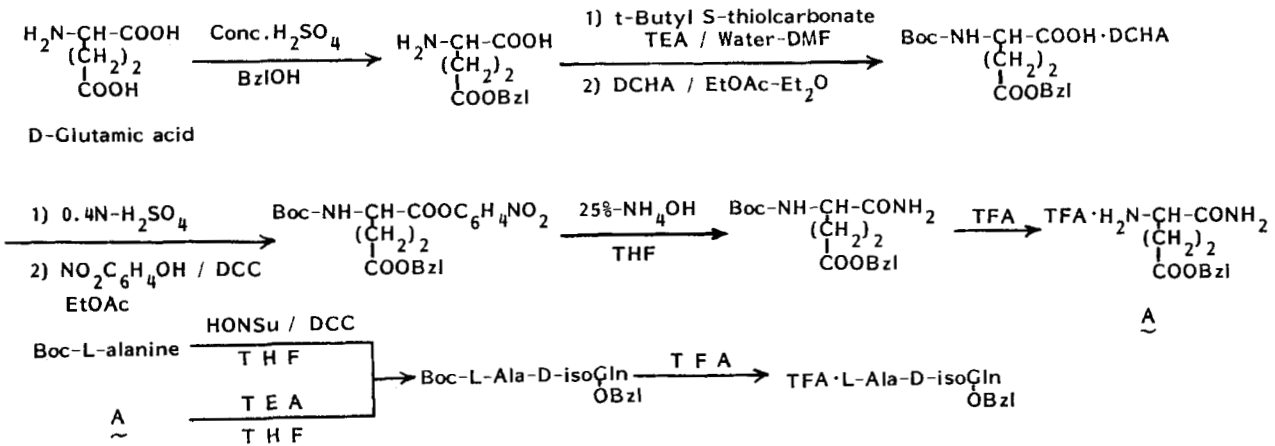
IR (neat NaCl): absorptions at 2630 (COOH), 1740 (C=O), 850 cm^{-1} ($(CH_3)_2C$).

^1H NMR ($CDCl_3$): δ1.31-1.50 (m, $5CH_3$, 15H), 4.80 (d, H-2, 1H), 5.91 (d, H-1, 1H), and 9.19 ppm (s, COOH, 1H).

Analysis: Calculated for $C_{15}H_{24}O_8$: C, 54.21; H, 7.28%. Found: C, 54.10; H, 7.35%.

Dipeptide Unit

The synthesis of the dipeptide unit was performed through seven reaction steps as shown in Scheme 2.



SCHEME 2

D-Glutamic Acid γ-Benzyl Ester. This compound was synthesized according to the method of Guttman et al. [18], 68%, mp 178°C, $[\alpha]_D^{25}$ -19.0° (*c* 3.2, AcOH).

t-Butoxycarbonyl-D-Glutamic Acid γ-Benzyl Ester DCHA Salt. This compound was synthesized by the coupling reaction of *D*-glutamic acid γ-benzyl ester with *t*-butyl *S*-(4,6-dimethyl-2-pyrimidimyl)thiol carbonate according to the method of Nagasawa et al. [19], 71%, mp 141-143°C, $[\alpha]_D^{25}$ -14.0° (*c* 0.9, MeOH).

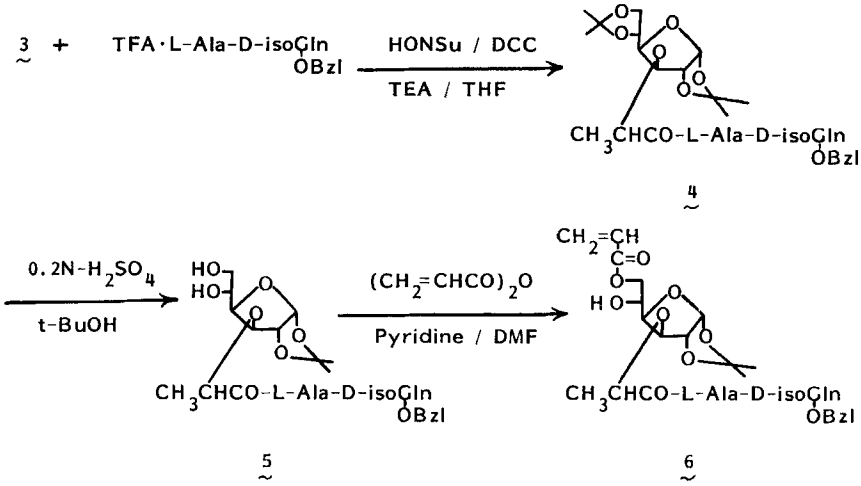
t-Butoxycarbonyl-D-isoglutamine Benzyl Ester. This compound was synthesized from *t*-butoxycarbonyl-*D*-glutamic acid γ-benzyl ester DCHA salt through *t*-butoxycarbonyl-*D*-glutamic acid-α-*p*-nitrophenyl-γ-benzyl diester (92%) according to the method of Kobayashi et al. [20], 95%, mp 122.4-123.2°C, $[\alpha]_D^{25}$ -2.4° (*c* 0.5, MeOH).

t-Butoxycarbonyl-L-alanyl-D-isoglutamine Benzyl Ester. This compound was synthesized by coupling *t*-butoxycarbonyl-*D*-isoglutamine benzyl ester with *t*-butoxycarbonyl-*L*-alanine by the active ester method [4], 93%, mp 137-139°C, $[\alpha]_D^{25}$ -9.6° (*c* 1.0, MeOH).

L-Alanyl-D-isoglutamine Benzyl Ester Trifluoroacetate. *t*-Butoxycarbonyl-*L*-alanyl-*D*-isoglutamine benzyl ester was treated with trifluoroacetic acid in THF to give a THF solution of the object compound readily.

1,2:5,6-Di-*O*-isopropylidene-3-*O*-[(*R*)-1-(*L*-alanyl-*D*-isoglutamyl Benzyl Ester) Carbonyl Ethyl]-α-*D*-glucofuranose (4)

This compound was synthesized according to the method of Kiso et al. [12], as shown in Scheme 3. DCC (0.763 g, 3.7 mmol) and HONSu (0.426 g, 3.7 mmol) were added to an ice-cooled solution of **3** (1.19 g, 3.7 mmol) in THF (30 mL). The reaction mixture was stirred at room temperature for 1 h, and then the *N,N'*-dicyclohexylurea formed was filtered off and washed with THF. Moreover, the THF solution of *L*-alanyl-*D*-isoglutamine benzyl ester trifluoroacetate (1.56 g, 3.7 mmol) obtained above and triethylamine (0.374 g, 3.7 mmol) were added to the combined filtrate and the washings cooled in an ice bath. The mixture was stirred at room temperature for 24 h. The insoluble materials were filtered off, and the solvent was evaporated under reduced pressure. The residual syrup was extracted with ethyl acetate (50 mL) and the insoluble material was decanted off. After evaporation of the ethyl



SCHEME 3

acetate layer, the syrupy residue was subjected to medium-pressure column chromatography on Kieselgel 60. Elution with chloroform/methanol (100:1 v/v) afforded the product as a syrup, 2.2 g (95%) $[\alpha]_D^{25}$ -5.7° (*c* 1.6, $CHCl_3$).

IR (neat NaCl): absorptions at 3280 (NH), 1720 and 1250 (ester), 1650 and 1510 (amide), 840 ($(CH_3)_2C$), and 750 and 690 cm^{-1} (Ph).

^1H NMR ($CDCl_3$): δ1.26-1.45 (m, $6CH_3$, 18H), 4.47 (d, H-2, 1H), 5.07 (s, $C\underline{H}_2Ph$, 2H), 5.86 (d, H-1, 1H), and 7.26 ppm (s, Ph, 5H).

Analysis: Calculated for $C_{30}H_{43}N_3O_{11}$: C, 57.96; H, 6.97; N, 6.76%. Found: C, 57.80; H, 7.18; N, 6.52%.

N-[2-(1,2-*O*-Isopropylidene-α-*D*-glucofuranos-3-*O*-yl)-(*R*)-propionyl]-*L*-alanyl-*D*-isoglutamine Benzyl Ester (5)

0.2 *N* Sulfuric acid (100 mL) was added with stirring to **4** (9.82 g, 16 mmol) in *t*-butanol (200 mL), and then the mixture was stirred at 55°C for 4 h. The reaction mixture was neutralized with barium carbonate for 12 h. After the salts were filtered off, the filtrate was evaporated under reduced pressure. The residue was subjected to medium-pressure column chromatography on Kieselgel 60, using chloroform/methanol (100:1 v/v) as the developing solvent to afford **5** as a syrup, 8.3 g (88%), λ_{max}^{MeOH} 257 nm, ϵ_{max} 2650, $[\alpha]_D^{25}$ 5.2° (*c* 1.0, MeOH).

N-[2-(1,2-*O*-Isopropylidene-6-*O*-acryloyl-α-*D*-glucofuranos-3-*O*-yl)-(*R*)-propionyl]-*L*-alanyl-*D*-isoglutamine Benzyl Ester (6)

Acrylic anhydride (6.9 g, 57 mmol) was added to a solution of **5** (6.75 g, 11 mmol) in dry *N,N*-dimethylformamide (DMF) (100 mL) containing pyridine (4.5 g, 57 mmol), cooled -15°C in an ice-salt bath, and stirred at the same temperature for 1 h. The reaction mixture was allowed to warm to room temperature and then was stirred for a further 10 h. After a large amount of water was added to the mixture to decompose the excess acrylic anhydride, it was extracted with chloroform and then the chloroform extract was washed thoroughly with water, 0.8% aqueous sulfuric acid, saturated sodium hydrogen carbonate solution, and water, and dried over anhydrous sodium sulfate. After the drying agent was filtered off, the filtrate was evaporated under reduced pressure in the presence of CuCl as an inhibitor to polymerization. The residue was subjected to medium-pressure column chromatography on Kieselgel 60. Elution with chloroform/methanol (10:1 v/v) as a developing solvent afforded the product as a syrup. Further purification of the syrup was carried out by medium-pressure column chromatography. The fractions containing the object monoacrylate **6** and the by-product diacrylate were collected and evaporated under reduced pressure to afford two kinds of colorless syrup, 5.7 g (77%) and 1.2 g (15%), respectively. The identification data for **6** were as follows.

IR (neat NaCl): absorptions at 3350 (NH), 1760 (ester), 1670 (amide), and 760 cm^{-1} (Ph).

^1H NMR ($CDCl_3$): δ1.26-1.50 (m, $(CH_3)_2C$, $2CH_3CH$, 12H), 2.00, 2.17 (m, $(CH_2)_2$, 4H), 2.44-2.59 (m, 3CH, 3H), 4.15-4.57 (m, H-2-6 of furan, 5H), 5.12 (s, CH_2Ph, 2H), 5.86 (d, H-1 of furan, 1H), 5.89 (d, CH_2=CH, 2H), 6.42 (t, CH_2=CH, 1H), 7.09, 7.20 (2CONH, 2H), 7.34 (s, Ph, 5H), and 7.55 ppm (NH_2, 2H).

^{13}C NMR ($CDCl_3$): δ17.4, 17.6 ($2CH_3CH$), 26.3, 26.7 ($(CH_3)_2C$), 26.9, 30.5 ($2CH_2$ of glutamine unit), 48.9, 52.6, 55.2 (3CHCO of glutamine, alanine, and propionic acid units), 66.8, 74.7, 79.9, 80.0, 82.2, 105.1 (C-6-1 of furan), 67.6 (CH_2Ph), 112.1 ($(CH_3)_2C$), 128.1 (CH_2=CH), 128.3, 128.4, 128.6, 135.5 (Ph), 130.9 (CH_2=CH), 166.1 (CH_2=CHCOO), 172.9 ($COOCH_2Ph$), and 173.5, 173.5, 173.8 ppm (3CONH).

R_f for TLC monitored with UV_{254} and I_2 (developing solvent: chloroform/methanol of 5:1 v/v) was 0.61.

MS: *m/e* 620(M^+-15); $[\alpha]_D^{25}$ -16.0° (*c* 0.5, $CHCl_3$).

Analysis: Calculated for $C_{30}H_{41}N_3O_{12}$: C, 56.69; H, 6.50; N, 6.61%. Found: C, 56.53; H, 6.29; N, 6.43%.

R_f for TLC (as above) for the diacrylate of **5** obtained as a by-product was 0.71.

Poly-1-{1,2-*O*-Isopropylidene-3-*O*-[(*R*)-1-*L*-alanyl-*D*-isoglutamyl Benzyl Ester Carbonyl]-1-ethyl}-α-*D*-glucofuranos-6-*O*-carbonyl -1-ethylene (7)

The vinyl monomer **6** was polymerized in benzene with AIBN as initiator. A solution of **6** (0.43 g, 1.67 mmol) in benzene (10 mL) and AIBN (11.0 mg, 67 μmol) were mixed in a glass tube. The tube was sealed *in vacuo* after thawing with nitrogen and kept at 60°C in a water bath. After 5 h the contents of the tube were poured into a large amount of *n*-hexane to precipitate the polymer. The polymer, obtained as a white powder, was purified by reprecipitation from chloroform solution with *n*-hexane, 0.072 g (17%).

IR (neat NaCl): absorptions at 3600-3200 (OH), 3350 (CONH), 1660 (ester C=O), 740 cm^{-1} (Ph).

^1H NMR ($CDCl_3$): δ1.2-1.5 (m, $3CH_3$, 9H), 1.5-2.0 (m, $4CH_2$, 8H), 4.0-4.8 (m, H-2-6 of furan), 5.1 (s, CH_2Ph, 2H), 5.9 (d, H-1 of furan, 1H), and 7.3 ppm (s, Ph, 5H).

The molecular weights $\bar{M}_w$ and $\bar{M}_n$ of polymer **7** measured in THF by GPC were 3040 and 2230, respectively.

$[\alpha]_D^{25}$ 7.5° (*c* 0.4, $CHCl_3$).

Analysis: Calculated for $(C_{30}H_{41}N_3O_{12})_n$: C, 56.69; H, 6.50; N, 6.61%. Found: C, 56.40; H, 6.36; N, 6.21%.

Poly-1-{3-*O*-[(*R*)-1-(*L*-Alanyl-*D*-isoglutamyl Benzyl Ester Carbonyl)ethyl] -α-*D*-glucopyranol-6-*O*-carbonyl}ethylene (8)

Polymer **7** (0.19 g, 0.29 mmol of repeat unit) was dissolved in TFA-water (6:1 v/v) (4 mL) with stirring. After being stirred at room temperature for 30 min, TFA and water were removed *in vacuo* with ice cooling. The resulting syrup, dissolved in a small amount of acetone, was poured into a large amount of benzene to precipitate the polymer **8** as a white powder. The purification of the polymer was carried out by reprecipitation from DMF with benzene.

IR (KBr): absorptions at 3400 (OH, NH), 3000 (CONH), 1730 (ester), 1660 (amide), and 700 cm^{-1} (Ph).

^1H NMR (DMSO-d6): δ0.73-1.60 (m, $2CH_3$, 6H), 4.13-4.19 (m, H-2-6 of pyran), 4.60 (d, H-1 of pyran, 1H), 5.06 (s, CH_2Ph, 2H), and 7.32 ppm (s, Ph, 5H). In the ^1H-NMR spectrum, the signal assigned to the H-1 proton

in the glucofuranoid structure at 5.90 ppm disappeared and the signal assigned to the H-1 proton in the glucopyranoid structure appeared at 4.96 ppm.

$[\alpha]_D^{25}$ 51.8° (*c* 0.5, DMF).

Analysis: Calculated for $(C_{27}H_{37}N_3O_{12})_n$: C, 54.45; H, 6.26; N, 7.06%. Found: C, 54.08; H, 5.99; N, 6.35%.

Poly-1-{3-*O*-[(*R*)-1-*L*-Alanyl-*D*-isoglutamyl Carbonyl)ethyl]-α-*D*-glucopyranos-6-*O*-carbonyl}ethylene (9)

A solution of polymer **8** (0.10 g, 0.16 mmol of repeat unit) in DMF of 10 cm^3 was hydrogenolyzed in the presence of palladium black (0.01 g) at room temperature for 30 h. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure and poured into a large amount of benzene to precipitate the object homopolymer **9** as a white powder.

IR (KBr): absorptions at 3430 (OH, NH), 1740 (ester), and 1678 cm^{-1} (amide). The absorptions at 740-695 cm^{-1} assigned to Ph disappeared.

^1H NMR (DMSO-d6): δ1.03-1.50 (m, $2CH_3$, 6H) and 4.05-4.68 ppm (m, H-1-6 of pyran). The peak at δ7.30 (s, 5H, Ph) disappeared.

$[\alpha]_D^{25}$ 26.0° (*c* 0.2, DMF).

Analysis: Calculated for $(C_{20}H_{31}N_3O_{12})_n$: C, 47.52; H, 6.18; N, 8.31%. Found: C, 47.35; H, 6.29; N, 8.02%.

Copolymerization of 6 with Hydrophobic Acrylates

Keeping the concentrations of **6**, the comonomer, and AIBN at 73, 183, and 22 mmol/L in benzene, respectively, the copolymerization of **6** with lauryl acrylate, stearyl acrylate, Viscoat 3F, or Viscoat 17F was carried out at 60°C for 5 h by the sealed method. The copolymerization of **6** with lauryl or stearyl acrylate proceeded homogeneously, while that of **6** with Viscoat 3F or 17F was heterogeneous. After reaction, the contents were poured into a large amount of methanol to precipitate the polymer. The composition of copolymer **10** was determined by the nitrogen content obtained by elemental analysis. The $\bar{M}_w$ and $\bar{M}_n$ of copolymers **6** with lauryl or stearyl acrylate were measured in THF by the GPC method, while the $\bar{M}_w$ of the copolymers of **6** with Viscoat 3F or 17F was measured in benzotrifluoride by light-scattering photometry.

Removal of the Isopropylidene Groups in Copolymer 10 and Splitting off of the Benzyl Groups in Copolymer 11

The acidic removal of the isopropylidene groups in copolymer **10** and the splitting off of the benzyl groups in copolymer **11** were performed by

the same procedures as those applied to homopolymers **7** and **8**. DMF and benzotrifluoride-THF (1:1 v/v) were used as the solvents in the reactions of fluorine-free **10** and **11** and of fluorine-containing **10** and **11**, respectively.

The reconfirmation of these reactions was carried out by measurement of the ^{1}H-NMR spectra of the resulting **11** and **12** copolymers.

RESULTS AND DISCUSSION

We had previously prepared 1,2:5,6-di-*O*-isopropylidene-3-*O*-[1-(carboxy ethyl)]-α-*D*-glucofuranose through 1,2:5,6-di-*O*-isopropylidene-3-*O*-[1-(ethyl carbonyl) ethyl]-α-*D*-glucofuranose [13, 14] as model units for MDP or GADP. However, the α-position of the propionic acid unit of MDP or GADP had been found to have to be of the *D* type in order to achieve the immunoadjuvant activity [3, 4, 12].

The synthesis of compound **3** was therefore performed by the direct coupling reaction of the separately prepared *L*-α-bromopropionic acid **2** with the sodium salt of 1,2:5,6-di-*O*-isopropylidene-α-*D*-glucofuranose, as shown in Scheme 1.

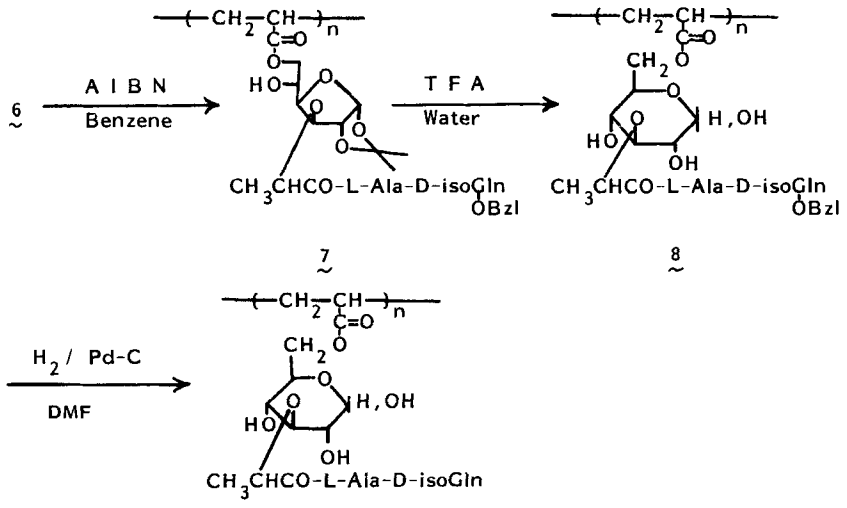
L-Alanyl-*D*-isoglutamine benzyl ester trifluoroacetate, as the dipeptide unit of GADP, was synthesized via seven reaction steps, as shown in Scheme 2, according to the method of Kusumoto et al. [4].

The new key monomer **6** was synthesized by the three reaction steps shown in Scheme 3.

The coupling reaction of **3** with *L*-alanyl-*D*-isoglutamine benzyl ester trifluoroacetate was conducted by the active-ester method, with HONSu as an activating agent. The 1-succinimidyl ester of **3**, as the intermediate, was allowed to react with *L*-alanyl-*D*-isoglutamine benzyl ester to give **4** in good yield. Its structure was confirmed by IR and ^{1}H-NMR spectra in which the signals assigned to *L*-alanyl-*D*-isoglutamine benzyl ester and the glucose residue were clearly observed.

The acid removal of the 5,6-isopropylidene group in **4** was carried out with 0.2 *N* sulfuric acid in *t*-butanol at room temperature to afford **5** in good yield.

Acrylation of **5** was carried out by reaction with acrylic anhydride in DMF at -15°C up to room temperature. Acryloylation of the primary hydroxyl group at the 6-position of **5** with acrylic anhydride occurred almost exclusively, so that 6-*O*-monoacrylate **6** was isolated by a chromatographic technique in 77% yield as a colorless heavy syrup, which was freely soluble in many organic solvents, such as chloroform, benzene, methanol, and DMF, but insoluble in water. Its composition was identified by IR and ^{1}H-NMR spectra, TLC, and elemental analysis.



SCHEME 4

The synthesis of the object homopolymer **9** was performed by the reaction steps shown in Scheme 4.

The homopolymerization of monomer **6** was carried out in benzene at 60°C for 5 h, with AIBN as initiator, to give the white polymer **7**. The signals at δ5.89-6.42 ppm, assigned to two kinds of protons of the vinyl group which were recognized in the ^{1}H-NMR spectrum of monomer **6**, disappeared in polymer **7**. However, yield and molecular weight were very small owing to the steric hindrance of the pendent bulky group of monomer **6**.

The acid removal of the isopropylidene groups in homopolymer **7** was performed by treatment with TFA-water (6:1, v/v) to convert the glucofuranose structure into the glucopyranose structure. The sulfonic acid method was not applicable because of degradation at the pendent moiety.

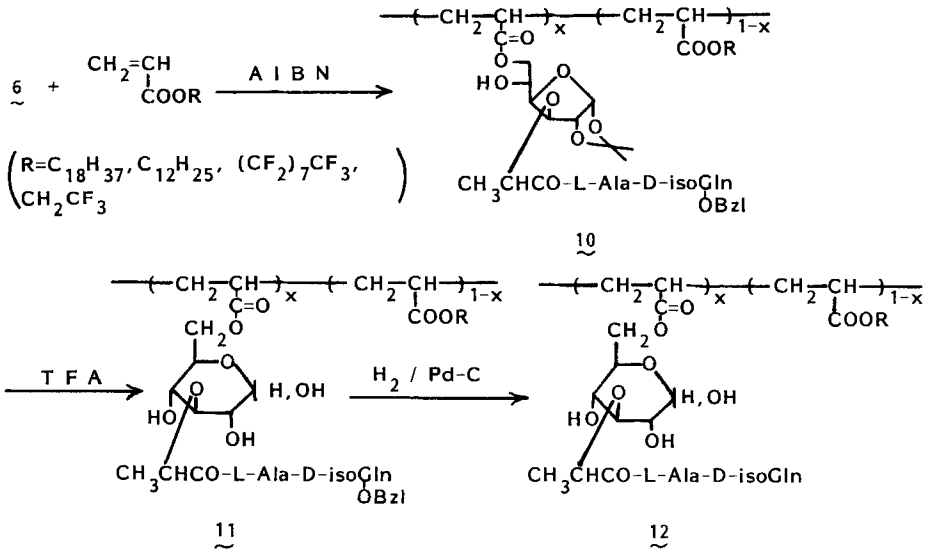
Splitting off of the benzyl groups in polymer **8** was attempted by hydrogenation with palladium black as the catalyst in DMF. It was clear from the ^{1}H-NMR data of polymer **9** that the elimination of the protecting benzyl groups recognized in polymer **8** was complete.

In order to obtain high molecular weight polymer containing the hydrophobic units, the copolymerization technique shown in Scheme 5 was applied. The results of copolymerization of **6** with four kinds of hydrophobic acryloyl monomer are summarized in Table 1. This table shows that copoly-

TABLE 1. Results of Copolymerization of **6** with Acryloyl Monomer[a]

			Copolymer			
6, mol/L	Comonomer, mol/L	Conversion, %	Mole fraction of **6**	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
7.3×10^{-2}	$CH_2{=}CHCOOC_{18}H_{37}$ 1.83×10^{-1}	44	0.125	1.12×10^4 [b]	9.10×10^3 [b]	1.23[b]
7.3×10^{-2}	$CH_2{=}CHCOOC_{12}H_{25}$ 1.83×10^{-1}	52	0.109	1.51×10^4 [b]	9.93×10^3 [b]	1.52[b]
7.3×10^{-2}	$CH_2{=}CHCOO(CF_2)_7CF_3$ 1.83×10^{-1}	75	0.114	4.52×10^4 [c]	–	–
7.3×10^{-2}	$CH_2{=}CHCOOCH_2CF_3$ 1.83×10^{-1}	89	0.102	5.17×10^4 [c]	–	–

[a]AIBN 22 mmol/L in benzene; 60°C, 5 h.
[b]Measured in THF by GPC.
[c]Measured in benzotrifluoride by light-scattering photometry.



SCHEME 5

mer **10** of relatively high molecular weight can be obtained by the copolymerization technique.

The removal of isopropylidene and benzyl protecting groups from copolymers **10** and **11** was performed by the same methods as were applied to homopolymers **7** and **8**.

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