Synthetic Carbohydrate Polymers Containing Trehalose Residues in the Main Chain: Preparation and Characteristic Properties

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ABSTRACT: Synthesis of 6,6′-diamino-6,6′-dideoxy-α,α-D-trehalose, the polyaddition with diisocyanates, and the characteristics of the resulting synthetic carbohydrate polymers have been disclosed. Among four preparative approaches to efficient preparation of diaminotrehalose starting from trehalose, that involving first protection of the C-6,6′ hydroxyl groups with trityl groups, acetylation of the remaining hydroxyl groups, detritylation, tosylation, azidation, deacetylation, and finally catalytic reduction proved to be superior to the others, and the overall yield was as high as 26%. The resulting diaminotrehalose was subjected to polyaddition with diisocyanates to give polyureas containing trehalose residues in the main chain. The polyureas showed high solubility in organic solvents. The derived membranes were evaluated in dialysis with urea and vitamin B_{12} and exhibited marked permeability. The polyureas were also susceptible to trehalase and α-amylase, suggesting the high potential as a novel type of biodegradable synthetic carbohydrate polymers.

Introduction

Polysaccharides, cellulose and chitin in particular, are attracting much attention because of the easy accessibility as well as structural characteristics associated with the high potential to develop sophisticated molecular design leading to advanced functions. They have, however, difficulties in modification reactions owing to the multifunctionality and limited solubility. In view of improving processability of polysaccharides while retaining their characteristic properties, incorporation of carbohydrate residues into the synthetic polymer main chain would be a solution. Synthesis of polymers containing carbohydrate units in the main chain is therefore considered of interest as a novel type of polymeric material composed of both natural and synthetic building blocks. They are expected to show various characteristics of polysaccharides and synthetic polymers.

Protected cellulose or amylose oligomer mixtures having terminal free hydroxyl groups were coupled with diisocyanates or other coupling agents to give block-type copolymers.[1–6] They are interesting as biodegradable polymers. Recently, sucrose was utilized as a monomer for chemoenzymatic synthesis of linear polyesters.[7,8] We have been interested in mono- and disaccharides as monomers to prepare carbohydrate polymers containing short sugar residues by direct polymerization with diisocyanates or dicarboxylic acid chlorides. As sugar monomers, glucosamine,[9–11] cellobiose,[12–14] and trehalose[15] have been subjected to polymerization to give synthetic carbohydrate polymers. The polymerizations were, however, sometimes difficult to give reproducible results since the reaction selectivity between primary and secondary hydroxyl groups is not always enough. Diamino sugars were hence considered to be superior as monomers, and diaminogulose was used for polymerization with diisocyanates. The resulting polyureas, however, exhibited only poor solubility due to the relatively high content of urea linkages forming strong intermolecular hydrogen bonding.[16] This suggests that diaminodisaccharides would be more suitable to improve the reproducibility in polymerization leading to high molecular weight linear polymers as well as to enhance the solubility of the carbohydrate polymers. 6,6′-Diamino-6,6′-dideoxy-α,α-D-trehalose has thus been evaluated as a potentially useful diamine monomer for polyaddition with diisocyanates to give polyureas, and the characteristic properties of the polyureas including solubility, membrane permeability, and biodegradability have been examined.

Experimental Section

General Procedures. Tosyl chloride was recrystallized from hexane. Diisocyanates were purified by distillation under reduced pressure. α,α-D-Trehalose dihydrate and other reagents were of reagent grade and used without purification. Solvents were dried and distilled in usual manners and stored over 3-Å molecular sieves.

IR and UV spectra were taken with a Jasco IR-700 or JEOL JIR-3510 and Ubest-30, respectively. ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-GX270 at 270 and 67.8 MHz, tetramethylsilane being used as the internal reference. X-ray diffraction diagrams were obtained by the powder method using nickel-filtered Cu Kα radiation with a Rigaku RAD-IA diffractometer. Thermal analysis was carried out with a Seiko SSC5200. Elemental analysis was performed with a Yanaco MT-3 CHNcorder.

Preparation of Diaminotrehalose (Method 1). To a solution of 10.00 g (26.5 mmol) of α,α-D-trehalose (1) dihydrate in 100 mL of dry pyridine was added 17 g of Drielite. The mixture was left standing at room temperature overnight, and a solution of 12.20 g (64.0 mmol) of tosyl chloride in 34 mL of chloroform was added at 0 °C with stirring. The mixture was kept in a refrigerator for 2 days and filtered. Water was added to the filtrate, and the mixture was washed with diluted sulfuric acid several times to remove the pyridine completely. It was finally washed with water and dried with sodium sulfate. Evaporation of the solvent under reduced pressure gave a white solid. It was dissolved in 20 mL of pyridine and treated with 20 mL of acetic anhydride at room temperature overnight. The solution was poured into water, and the

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isolated white precipitate was recrystallized from ethanol to give 3.50 g (15%) of the hexaacetyl–ditosyl derivative (**2**) as colorless needles; mp 169–170 °C (lit.[17] mp 169–172 °C); IR (KBr) ν 1750 cm^{-1} (C=O).

2 (0.30 g, 0.33 mmol) was dissolved in 25 mL of dimethyl sulfoxide (DMSO), and 0.16 g (2.5 mmol) of sodium azide was added. The mixture was stirred at 100 °C for 2 h under nitrogen. After cooling, the solution was poured into water, and the precipitate was collected by filtration. It was recrystallized from ethanol to give 0.20 g (93%) of the hexaacetyl–diazido derivative (**3**) as colorless needles; mp 119–120 °C; IR (KBr) ν 2106 (N_3) and 1755 cm^{-1} (C=O).

To a solution of 2.10 g (3.3 mmol) of **3** in 60 mL of dry methanol was added 16 mg (0.30 mmol) of sodium methoxide, and the solution was stirred at room temperature overnight under nitrogen. The solution was neutralized with a cation-exchange resin (DOWEX 50w X-8, H^+ form) and evaporated under reduced pressure. The resulting white solid was recrystallized from a mixture of methanol and 2-butanone to give 1.09 g (85%) of the deacetylated diazido derivative (**4**): mp 190–192 °C (dec); IR (KBr) ν 3408 (OH), 2106 cm^{-1} (N_3).

A solution of 0.26 g (0.66 mmol) of **4** in 30 mL of dry methanol was purged with nitrogen, and a small amount of 5% Pd/C was added. The mixture was stirred under hydrogen at 1.1 kg/cm^2 at room temperature for 5 h. A small amount of the catalyst was added again, and the mixture was stirred under hydrogen overnight. It was filtered, and the filtrate was evaporated under reduced pressure to give a white solid, which was dissolved in a mixture of 3% aqueous ammonia and methanol (3:1). Ethanol was then added, and the solution was kept in a refrigerator. Diaminotrehalose (**5**) dihydrate crystallized out as colorless plates: yield 0.19 g (76%); mp 200 °C (dec); IR (KBr) ν 3350 (OH and NH), 1639 (NH), 1150–1000 cm^{-1} (pyranose).

Anal. Calcd for $C_{12}H_{24}N_2O_9 \cdot 2H_2O$: C, 38.30; H, 7.50; N, 7.44. Found: C, 38.40; H, 7.58; N, 7.34.

Preparation of Diaminotrehalose (Method 2). To a solution of 8.80 g (23.3 mmol) of **1** dihydrate in 80 mL of dry pyridine was added 21.40 g (76.9 mmol) of triphenylmethyl (trityl) chloride, and the solution was heated at 90 °C for 7 h. It was cooled to room temperature, and 42 mL of acetic anhydride was added. After stirring at room temperature overnight, 20 mL of toluene was added and the solution was evaporated under reduced pressure. Ice water (100 mL) was added to the residue, and the mixture was extracted with chloroform. The extract was washed consecutively with diluted sulfuric acid, water, aqueous sodium hydrogen carbonate, aqueous sodium chloride, and water and dried over magnesium sulfate. After filtration, the filtrate was concentrated under reduced pressure to give a syrup, which was chromatographed on silicic acid in toluene/ethyl acetate/triethylamine (100:1:0.01). The product was recrystallized from ethanol to give 23.10 g (92%) of the hexaacetyl–ditrityl derivative (**6**) as fine colorless crystals: mp 233–234 °C; $[\alpha]^{25}_D$ +104.5° (*c* 0.30, $CHCl_3$); IR (KBr) ν 1750 (C=O), 750 and 690 cm^{-1} (phenyl); ^1H NMR ($CDCl_3$) δ 1.75 (s, 6H, $COCH_3$), 1.80 (s, 6H, $COCH_3$), 1.99 (s, 6H, $COCH_3$), 3.12 (d, J = 3.7 Hz, 4H, H-6a,6b,6a′,6b′), 4.11–4.15 (m, 2H, H-5,5′), 5.11–5.50 (m, 8H, H-1,1′,2,2′,3,3′,4,4′), 7.22–7.43 (m, 30H, phenyl).

Anal. Calcd for $C_{62}H_{62}O_{17}$: C, 69.01; H, 5.79. Found: C, 68.87; H, 5.88.

6 (1.00 g, 0.93 mmol) was dissolved in 3 mL of chloroform, and 5 mL of dichloroacetic acid was added. The solution was stirred at room temperature for 30 min and poured into ice water. The mixture was extracted with chloroform, and the extract was washed with saturated sodium hydrogen carbonate, dried over magnesium sulfate, concentrated under reduced pressure, and chromatographed on silicic acid in chloroform/methanol (30:1). The product was recrystallized from ethanol to give 0.54 g (98%) of the detritylated hexaacetyl derivative (**7**) as colorless needles: mp 94–94.5 °C; $[\alpha]^{25}_D$ +130.4° (*c* 0.30, $CHCl_3$); IR (KBr) ν 3478 (OH), 1748 cm^{-1} (C=O).

Anal. Calcd for $C_{24}H_{34}O_{17} \cdot H_2O$: C, 47.06; H, 5.92. Found: C, 46.67; H, 5.70.

7 (0.50 g, 0.82 mmol) was dissolved in 5 mL of pyridine, and a solution of 2.18 g (11.4 mmol) of tosyl chloride in 5 mL of a 1:1 mixture of pyridine and chloroform was added dropwise over a period of 30 min. The mixture was stirred at room temperature overnight and poured into ice water. The mixture was extracted with chloroform, and the extract was washed with diluted sulfuric acid, water, saturated sodium hydrogen carbonate, saturated sodium chloride, and water. It was dried over magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure to give a white syrup, which was purified by chromatography on silicic acid in toluene/ethyl acetate (50:1) and recrystallized from ethanol to give 0.35 g (48%) of **2** as colorless needles: mp 168–170 °C (lit.[17] mp 169–172 °C).

The resulting **2** was used for the preparation of **5** in the same manner as that described in method 1 above.

Preparation of Diaminotrehalose (Method 3). **6** (4.20 g, 3.9 mmol) was dissolved in a mixture of 100 mL of dry methanol and 30 mL of tetrahydrofuran (THF) with heating, and 21 mg (0.39 mmol) of sodium methoxide was added. The solution was stirred at room temperature under nitrogen overnight and neutralized with a cation-exchange resin (DOWEX 50w X-8, H^+ form). The mixture was filtered, and the filtrate was concentrated under reduced pressure to give a white solid. It was recrystallized from ethanol to give 3.20 g (99%) of the ditrityl derivative (**8**) as colorless needles: mp 168–169 °C; $[\alpha]^{25}_D$ +50.68° (*c* 0.30, *N*,*N*-dimethylformamide (DMF)); IR (KBr) ν 3396 (OH), 3100–2900 (CH), 1150–1000 (pyranose), 746 and 703 cm^{-1} (phenyl).

To a solution of 3.20 g of **8** (5.5 mmol) obtained above in 50 mL of DMF were added 9 mL (13.0 g, 76 mmol) of benzyl bromide and 2.2 g (92 mmol) of sodium hydride (55% oil dispersion type), and the mixture was stirred at room temperature under nitrogen in the dark overnight. It was diluted with 30 mL of diethyl ether and 40 mL of ethyl acetate and poured into 100 mL of ice water. Ethyl acetate (100 mL) was added, and the mixture was shaken in a separatory funnel. The organic layer was separated, washed with saturated sodium chloride, and dried over magnesium sulfate. After filtration, the filtrate was concentrated under reduced pressure to give a syrup, which was chromatographed on silicic acid in hexane/ethyl acetate (50:1). The product was recrystallized from ethanol to give 4.80 g (91%) of the hexabenzyl–ditrityl derivative (**9**) as colorless needles: mp 82–83 °C; $[\alpha]^{25}_D$ +51.25° (*c* 0.30, $CHCl_3$); IR (KBr) ν 3090–2880 (CH), 1150–1000 (pyranose), 755 and 700 cm^{-1} (phenyl).

Anal. Calcd for $C_{92}H_{86}O_{11} \cdot H_2O$: C, 79.74; H, 6.40. Found: C, 79.83; H, 6.26.

9 (2.00 g, 1.4 mmol) was dissolved in 50 mL of dichloroacetic acid, and the solution was stirred at room temperature under nitrogen for 3 h. It was poured into 100 mL of ice water, and the mixture was extracted with chloroform. The extract was washed with saturated sodium hydrogen carbonate and water and dried over magnesium sulfate. Filtration and evaporation of the filtrate gave a syrup, which was purified by chromatography on silicic acid in hexane/ethyl acetate (50:1). The solution was evaporated to give 1.10 g (86%) of the detritylated hexabenzyl derivative (**10**) as a colorless syrup: IR (neat) ν 3480 (OH), 3100–2900 (CH), 750 and 700 cm^{-1} (phenyl).

To 1.50 g (1.7 mmol) of **10** in 20 mL of pyridine was added dropwise a solution of 6.50 g (34.1 mmol) of tosyl chloride in 20 mL of a mixture of pyridine and chloroform (1:1), and the mixture was stirred at room temperature for 24 h. It was poured into 100 mL of ice water and extracted with chloroform. The chloroform extract was washed with saturated sodium hydrogen carbonate and water and dried over magnesium sulfate. Filtration and evaporation of the filtrate gave a syrup. It was purified by chromatography on silicic acid in hexane/ethyl acetate (20:1) to give 1.80 g (89%) of the hexabenzyl–ditosyl derivative (**11**) as a colorless syrup: IR (neat) ν 3090–2890 (CH), 1598 (*p*-phenylene), 1178 (SO_2), 814 (*p*-phenylene), 735 and 697 cm^{-1} (phenyl); ^1H NMR ($CDCl_3$) δ 2.30 (s, 6H, CH_3), 3.45–4.99 (m, 26H, CH and CH_2), 7.11–7.32 (m, 34H, aromatic), 7.68 (d, J = 8.3 Hz, 4H, tosyl).

To a solution of 1.60 g (1.34 mmol) of **11** in 30 mL of dry DMSO was added 0.81 g (12.5 mmol) of sodium azide, and the solution was heated at 100 °C under nitrogen for 2 h. After cooling to room temperature, 100 mL of water was added to precipitate white crystals. Recrystallization from ethanol gave

1.13 g (90%) of the diazido–hexabenzyl derivative (**12**) as colorless needles: IR (KBr) ν 3090–2890 (CH), 2100 (N_3), 736 and 698 cm^{-1} (phenyl).

12 (1.00 g, 1.1 mmol) was dissolved in 60 mL of dry methanol, and the solution was purged with nitrogen. A small amount of 5% Pd/C was added, and the mixture was stirred under hydrogen at 1.1 kg/cm^2 at room temperature for 6 h. The catalyst was added again, and the mixture was stirred overnight under the same conditions. The mixture was filtered, and the filtrate was concentrated under reduced pressure to give the crude amine as a syrup. It was crystallized from aqueous ammonia/methanol/ethanol to give 0.18 g (44%) of **5** dihydrate.

Preparation of Diaminotrehalose (Method 4). To 1.60 g (2.7 mmol) of **7** was added a solution of 1.41 g (8.1 mmol) of diethyl azodicarboxylate in 8 mL of THF dropwise. A solution of 1.20 g (8.2 mmol) of phthalimide and 2.2 g (8.4 mmol) of triphenylphosphine in 20 mL of THF was added dropwise, and the solution was stirred at room temperature for 48 h. The solvent was removed under reduced pressure to give a syrup, which was chromatographed on silicic acid in toluene/ethyl acetate (50:1). The product was recrystallized from ethanol to give 1.40 g (60%) of the hexacetyl–diphthalimido derivative (**13**) as colorless needles: mp 162–163 °C; $[\alpha]^{25}_D$ +145.4° (*c* 0.30, $CHCl_3$); IR (KBr) ν 1755 (acetyl C=O), 1717 cm^{-1} (imide C=O); ^1H NMR ($CDCl_3$) δ 2.00 (s, 6H, $COCH_3$), 2.08 (s, 6H, $COCH_3$), 2.20 (s, 6H, $COCH_3$), 3.57–3.61 (m, 2H, H-6a,6a′), 3.80–3.89 (m, 2H, H-6b,6b′), 4.06 (t, J = 7.1 Hz, 2H, H-5,5′), 4.86–4.95 (m, 6H, H-1,1′,2,2′,4,4′), 5.46 (t, J = 9.7 Hz, 2H, H-3,3′), 7.73–7.86 (m, 8H, aromatic).

Anal. Calcd for $C_{40}H_{40}N_2O_{19}\cdot 0.5H_2O$: C, 55.75; H, 4.80; N, 3.25. Found: C, 55.68; H, 4.73; N, 3.31.

13 (4.70 g, 5.5 mmol) was dissolved in 100 mL of dry methanol, and 30 mg (0.56 mmol) of sodium methoxide was added. The solution was stirred at room temperature under nitrogen for 2 h and neutralized with a cation-exchange resin (DOWEX 50w X-8, H^+ form). After filtration, the filtrate was concentrated under reduced pressure to give the diphthalimido derivative (**14**) as a syrup: IR (neat) ν 3360 (OH), 1711 cm^{-1} (C=O).

To **14** obtained above were added 2.14 mL (2.2 g, 44 mmol) of hydrazine hydrate and 50 mL of 95% ethanol. The solution was stirred at 90 °C under nitrogen for 5 h and then at room temperature overnight. It was evaporated under reduced pressure. Chloroform was added to the residue, and the mixture was extracted with water. The aqueous extract was passed through a column of an anion-exchange resin (AMBERLITE IR-400, Type 2) and concentrated under reduced pressure to give 1.51 g (73% from **13**) of crude **5**. It was recrystallized five times from ammonia/methanol/ethanol to give 0.30 g (15% from **13**) of **5** dihydrate.

Deprotection was also possible with butylamine as follows. **13** (2.00 g, 2.3 mmol) was dissolved in 25 mL of ethanol with heating at 50 °C, and 3.42 g (46.8 mmol) of butylamine was added. The solution was stirred at 50 °C for 2 h and at room temperature overnight and evaporated to give the hexaacetyl–diamino derivative (**15**) as a syrup. It was dissolved in 20 mL of methanol, and 37 mg (0.69 mmol) of sodium methoxide was added. The resting solution was stirred at room temperature for 2 h and neutralized with a cation-exchange resin (DOWEX 50w X-8, H^+ form). Evaporation of the solvent gave a syrup, which was dissolved in 5 mL of deionized water and passed through a column of an anion-exchange resin (DOWEX 1-X 8, OH^- form). The solution was concentrated under reduced pressure to give 0.44 g (50% from **13**) of crude **5**. It was recrystallized from ammonia/methanol/ethanol. Five recrystallizations gave 0.20 g (23% from **13**) of **5** dihydrate.

Polyaddition of Diaminotrehalose with Diisocyanates. To a solution of 0.151 g (0.40 mmol) of **5** dihydrate, pulverized and dried thoroughly with phosphorus pentoxide, in 3 mL of DMSO was added 0.100 g (0.40 mmol) of diphenylmethane diisocyanate (MDI). The mixture was stirred at 20 °C under nitrogen for 24 h to give a viscous solution. It was diluted with 3 mL of DMSO and poured into chloroform to precipitate the polymer. The polymer was collected by filtration, washed thoroughly with chloroform, and dried to give 0.224 g (95%) of the polyurea (**16a**). The inherent viscosity was 0.50 dL/g as determined in *N,N*-dimethylacetamide (DMAc) at a concentration of 0.25 g/dL at 25 °C; IR (KBr) ν 1655 (C=O), 1600 (*p*-phenylene), 1150–1000 cm^{-1} (pyranose); ^1H NMR (DMSO-d_6) δ 3.1–3.8 (m, H-5,5′,6,6′ and OH), 4.55, 4.67, 4.83, and 4.96 (each m, 8H, H-1,1′,2,2′,3,3′,4,4′), 5.96 (t, 2H, NH-trehalose), 7.03 (d, J = 8.4 Hz, 4H, aromatic), 7.26 (d, J = 8.4 Hz, aromatic), 8.45 (s, 2H, NH-aromatic); ^{13}C NMR (DMSO-d_6) δ 70.69, 71.56, 71.63, and 72.52 (C-2,2′,3,3′,4,4′,5,5′), 93.40 (C-1,1′), 117.82 and 128.66 (aromatic), 134.19 (*N*-aromatic), 138.28 (*O*-aromatic), 155.47 (C=O).

Anal. Calcd for $(C_{27}H_{34}N_4O_{11}\cdot 1.5H_2O)_n$: C, 52.51; H, 6.04; N, 9.07. Found: C, 52.44; H, 6.19; N, 8.67.

Permeation through the Polyurea Membranes. Polyureas derived from MDI (**16a**) and diphenyl ether diisocyanate (**16b**) were dissolved in DMF, and the solutions were cast on glass plates. The solvent was removed thoroughly under reduced pressure. The resulting membranes of 5×10^{-3}–1×10^{-2} mm thick were kept in water overnight and placed between two cells with a window of 1.77 cm^2, one of which contained 25 mL of a 3.0×10^{-2} mol/L urea solution and the other of which contained the same amount of distilled water. Each cell was stirred with a magnetic stirrer, and the apparatus was kept at 25 °C. After a given interval, 1 mL of aliquot was taken, and 0.5 mL of 0.2 mol/L citrate buffer (pH 5.0) and 1.2 mL of a KCN–ninhydrin solution were added. The mixture was heated in boiling water for 15 min and diluted with 3 mL of 60% aqueous ethanol. The absorbance at 570 nm was measured and compared with a calibration curve to determine the permeated urea.

In the permeation of vitamin B_{12}, an aqueous solution of concentration 7.0×10^{-5} mol/L was used, and the permeation was followed with the absorbance at 361 nm.

Permeation constants, P_{urea} and $P_{B_{12}}$, were calculated according to the following generally used equation, where C_0 = initial concentration of the feed solution, C_t = concentration of solute transported at time t, A = membrane area, δ = membrane thickness, and V = solution volume.

$$P = -\frac{V\delta}{2At} \ln \frac{C_0 - 2C_t}{C_0}$$

Biodegradation of the Polyureas. Polyurea **16a** (30 mg, η_{inh} = 0.50 dL/g) was pulverized and dispersed in 12 mL of acetate buffer (pH 5.7). To the mixture was added 1/4 unit of a trehalase (from porcine kidney, Sigma; optimum conditions, pH 5.7 at 37 °C), and the mixture was shaken (65 strokes/min) at 37 °C. At a prescribed time, the turbidity was evaluated with the absorbance at 660 nm.

After 96 h, the suspended polyurea was filtered, washed with saturated sodium chloride solution and water thoroughly, and dried to recover 20 mg of the polyurea. The inherent viscosity was 0.23 dL/g as determined in DMAc at a concentration of 0.25 g/dL at 25 °C.

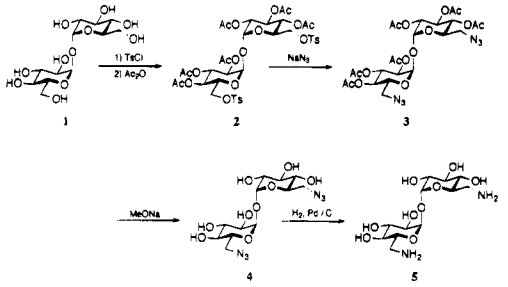
Biodegradation with an α-amylase (from *Bacillus subtilis*, Sigma; optimum conditions, pH 6.0 at 40 °C) was conducted in a similar manner in acetate buffer (pH 6.0) at 40 °C. After 96 h of reaction, 20 mg of the polyurea was recovered, whose inherent viscosity was 0.25 dL/g as determined in DMAc at a concentration of 0.25 g/dL at 25 °C.

Release of *p*-Nitrophenol from the Polyurea Conjugate. To a solution of 30 mg of polyurea **16a** in 1 mL of DMF was added 10 mg of *p*-nitrophenol. Part of the solution, 0.2 mL, was cast on a glass plate, and the solvent was evaporated. The resulting membrane was pulverized, washed with methanol several times, and dispersed in 12 mL of acetate buffer (pH 5.7). To this was added 1/4 unit of the trehalase or α-amylase, and the mixture was shaken at 37 °C. The released *p*-aminophenol was determined with the absorbance at 311 nm.

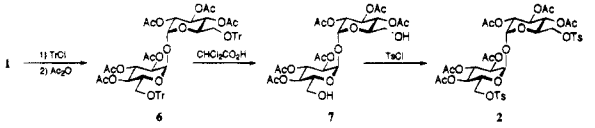
Results and Discussion

Synthesis of Diaminotrehalose. Four approaches have been examined to establish efficient transforma-

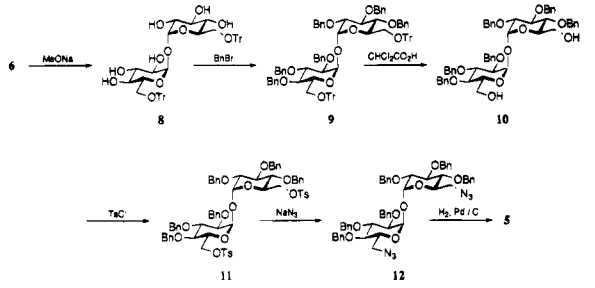
Scheme 1



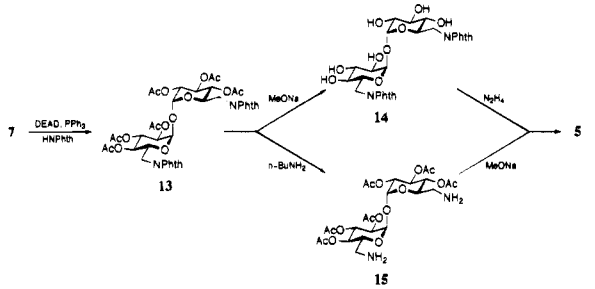
Scheme 2



Scheme 3



Scheme 4



tion of α,α-D-trehalose (**1**) to diaminotrehalose, 6,6′-diamino-6,6′-dideoxy-α,α-D-trehalose (**5**).

It was first synthesized by a series of reactions involving direct tosylation at C-6,6′, acetylation of the remaining hydroxyl groups, azidation at C-6,6′, deacetylation, and catalytic hydrogenation (method 1; Scheme 1). Since tosylation at the primary hydroxyl groups of trehalose was not completely selective, it was difficult to isolate the ditosyl derivative. The product was thus isolated after acetylating the secondary hydroxyl groups, and the yield of the hexaacetyl–ditosyl derivative (**2**) was as low as 15% at best. The subsequent reactions, however, resulted in rather high yields, and the overall yield from **1** to **5** was 9%.

When trityl groups were used for discriminating the primary and secondary hydroxyl groups, tosylation could be attained regioselectively (method 2; Scheme 2). **1** was first ditritylated to protect the C-6,6′ hydroxyl groups, acetylated (**6**), and then detritylated to give the derivative **7** having free hydroxyl groups at the C-6,6′ positions. Detritylation is effected only slowly with acetic acid to give **7** in low yield but facilely in quantitative yield with dichloroacetic acid. Subsequent tosylation afforded **2**. Although this approach required two more reaction steps than the direct tosylation method mentioned above, the overall yield from **1** to **2** was enhanced to 43%.

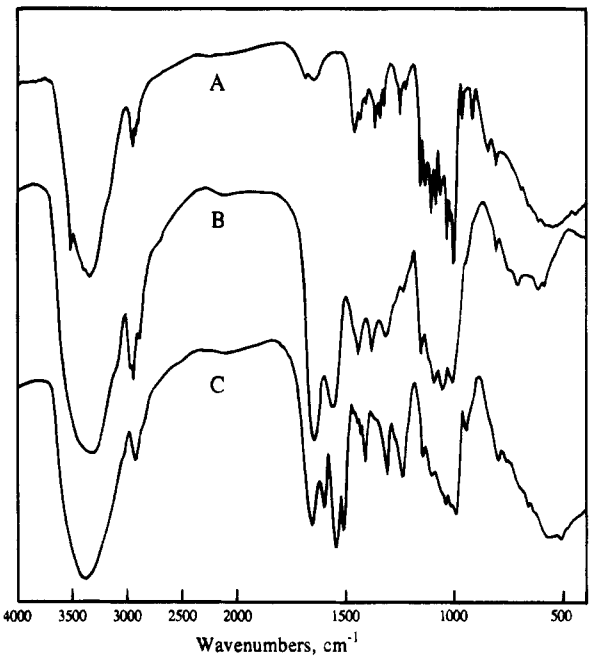


Figure 1. IR spectra (KBr method) of (A) trehalose, (B) diaminotrehalose, and (C) polyurea **16a**.

Protection of the secondary hydroxyl groups was also conducted with benzyl groups instead of acetyl groups to avoid possible acetyl migration (method 3; Scheme 3). Ditrityl–trehalose (**8**) was prepared by deacetylation of **6** and benzylated with benzyl bromide and sodium hydride. The resulting benzylated derivative (**9**) was detritylated (**10**) with dichloroacetic acid and then tosylated to give hexabenzyl–ditosyl–trehalose (**11**). **11** was then converted into the corresponding diazide (**12**), which was treated with hydrogen in the presence of Pd/C to give **5** in one step. Although the overall yield from **9** to **5** (30%) is only a little higher than that from **6** by method 2 (27%), the resulting **5** was easier to purify.

The Mitsunobu reaction is another possibility for introducing amino groups into **1** (method 4; Scheme 4). **7** was thus subjected to the reaction with diethyl azodicarboxylate (DEAD), phthalimide, and triphenylphosphine to give hexaacetyl–diphthalimido–trehalose (**13**) in moderate yields. It was deacetylated with methoxide (**14**) and dephthaloylated with hydrazine to afford **5**. **13** was also first dephthaloylated with butylamine to give the corresponding diamine (**15**) and subsequently deacetylated with methoxide, resulting in the formation of **5**. The diamine prepared by these procedures was, however, laborious to purify, and the overall yields after several recrystallizations were not high.

5 was reported to be isolated as dihydrochloride,[17] but it could be isolated in the form of a free diamine that is much more suitable for polymerization. Recrystallization of **5** was accomplished by first dissolving in aqueous ammonia and methanol, followed by diluting with ethanol and keeping the solution in a refrigerator to give colorless plates. Elemental analysis of the diaminotrehalose indicated that it was isolated as a dihydrate. The IR spectrum of **5** in Figure 1 shows a characteristic band due to free amino groups at 1639 cm^{-1}. Thermogravimetry showed a two-step weight loss, the first corresponding to dehydration. As shown in Figure 2, the 9.6% weight decrease in the range of 128–150 °C is exactly the same weight percentage of 2 equiv of water. Attempts were made to dehydrate with phosphorus

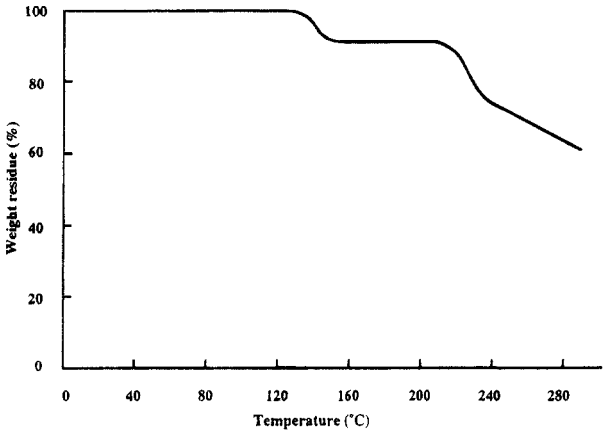


Figure 2. Thermogravimetric curve for diaminotrehalose dihydrate at a heating rate of 3 °C/min in air.

Scheme 5

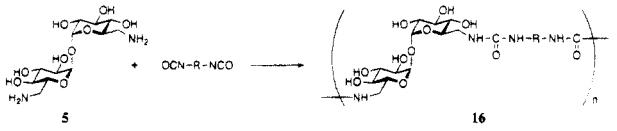


Table 1. Polyaddition of Diaminotrehalose with Diisocyanates[a]

polymer	diisocyanate	solvent[b]	temp and time	yield, %	η_{inh},[c] dL/g
16a	diphenylmethane	DMAc	5 °C, 4 h → 20 °C, 20 h	84	0.24
16a	diphenylmethane	DMSO	20 °C, 24 h	94	0.56
16a	diphenylmethane	HMPA	5 °C, 4 h → 15 °C, 20 h	98	0.32
16a	diphenylmethane	HMPA	5 °C, 4 h → 20 °C, 20 h	95	0.48
16b	diphenyl ether	DMSO	20 °C, 24 h	95	0.36
16c	*p*-phenylene	DMSO	20 °C, 24 h	95	0.27
16d	tetramethylene	DMSO	20 °C, 24 h	89	0.49*

[a] Monomers, 0.4 mmol each; solvent, 3 mL. [b] DMAc, *N,N*-dimethylacetamide; DMSO, dimethyl sulfoxide; HMPA, hexamethylphosphoramide. [c] In DMAc (*dichloroacetic acid), c = 0.25 g/dL, 25 °C.

pentoxide at elevated temperatures, but they were unsuccessful. **5** dihydrate was thus used for the polyaddition with diisocyanates.

Synthesis of Polyureas. The resulting **5** was subjected to polyaddition with diisocyanates to synthesize polyureas having trehalose residues in the main chain (Scheme 5). The polyaddition was first examined with MDI in polar solvents including DMAc, DMSO, and hexamethylphosphoramide (HMPA). The reaction was carried out at 5 °C first and then at 15 or 20 °C in DMAc and HMPA and at 20 °C in DMSO. The resulting polyurea (**16a**) was isolated by pouring the viscous solution into chloroform. As listed in Table 1, DMSO appeared to be suitable for polymerization, judging from the high inherent viscosity. Polyadditions with other diisocyanates such as diphenyl ether, *p*-phenylene, and tetramethylene diisocyanates to give polyureas **16b**, **16c**, and **16d** were thus conducted in DMSO at 20 °C, and the results are included in Table 1.

The polyureas were obtained as white fibrous materials. The IR spectra of the polyureas showed a characteristic absorption band at 1655 cm^{-1} due to the urea linkages and bands at 1000−1150 cm^{-1} due to pyranose units. A typical spectrum is included in Figure 1. The structures of the polymers were unambiguously supported by ^{13}C NMR spectroscopy, and the spectrum of

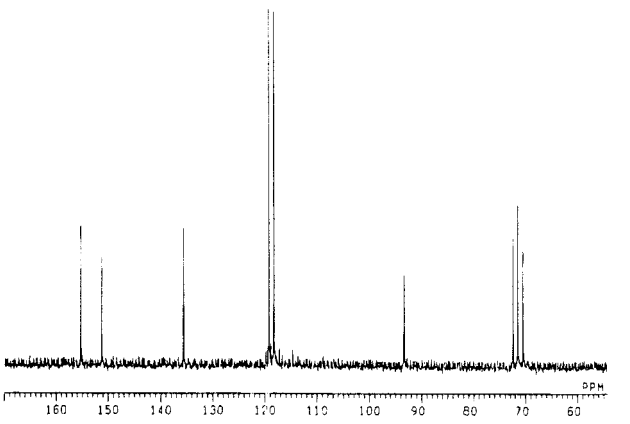


Figure 3. ^{13}C NMR spectrum of polyurea **16b** in DMSO-d_6.

Table 2. Solubility of the Polyureas[a]

	solubility						
polymer	DMAc	HMPA	DMSO	*m*-cresol	HCO_2H	DCA	H_2O
16a	+	+	+	±	+	+	−
16b	+	±	+	±	+	+	−
16c	+	±	+	±	+	+	−
16d	±	±	+	±	+	+	−

[a] DCA, dichloroacetic acid; +, soluble; ±, partially soluble or swelled; −, insoluble.

polyurea **16b** is given in Figure 3 as a typical example. It was taken in DMSO-d_6, and thus the peak due to the C-6,6′ is superimposed with the solvent peaks around 40 ppm. Four peaks due to C-2,2′,3,3′,4,4′,5,5′ of trehalose are observed at 70.47−72.40 ppm, and a peak due to C-1,1′ is observed at 93.36 ppm. Four aromatic carbon peaks appear in the range of 118.20−151.22 ppm, and a carbonyl peak appears at 155.28 ppm. All the polyureas were found to be amorphous, judging from the X-ray diffraction diagrams obtained by the powder method.

Qualitative solubility of the polyureas was examined in various solvents, and, as summarized in Table 2, all the polyureas showed high solubility in sharp contrast to the limited solubility of common polyureas and the polyureas containing gulose residues in the main chain.[16] This high solubility is ascribable to the low density of urea linkages and high density of bulky sugar units. Compared with the other polyureas **16a**−**c** from aromatic diisocyanates, **16d** turned out to be less soluble.

Permeability of the Polyurea Membranes. Films were cast from DMAc solutions of **16a** and **16b** to evaluate as dialysis membranes. In order to improve the membrane strength, an equal amount of polyacrylonitrile was added, and blend membranes were also cast in the same manner. As permeates, urea and vitamin B_{12} were chosen, and the permeation rates were determined by UV spectroscopy.

As evident in Table 3, polyurea membranes showed fairly high permeation for both urea and vitamin B_{12}, but the permeations decreased on blending with polyacrylonitrile. Incorporation of trehalose residues in synthetic polymers has thus proved to enhance the permeability markedly. The permeations were, however, lower than that of a cellulose membrane. This may be associated with the membrane water content. The water contents of the membranes from **16a** and **16b** were 37% and 39% under dialysis conditions, respectively, while that of the cellulose membrane was 55%.

Biodegradability of the Polyureas. The polyureas prepared here are considered to be interesting because

Table 3. Permeability Constants for Urea and Vitamin B_{12}[a]

membrane	$P_{urea} \times 10^7$, cm²/s	$P_{B12} \times 10^7$, cm²/s
16a	0.93	0.15
16b	0.84	0.15
16a + PAN	0.29	0.073
16b + PAN	0.29	0.093
PAN	~0	~0
cellulose	1.49	0.31

[a] Membrane thickness was about 5×10^{-3}–1×10^{-2} mm, except for PAN (polyacrylonitrile, 2×10^{-2} mm) and cellulose (Visking tube, 2×10^{-2} mm).

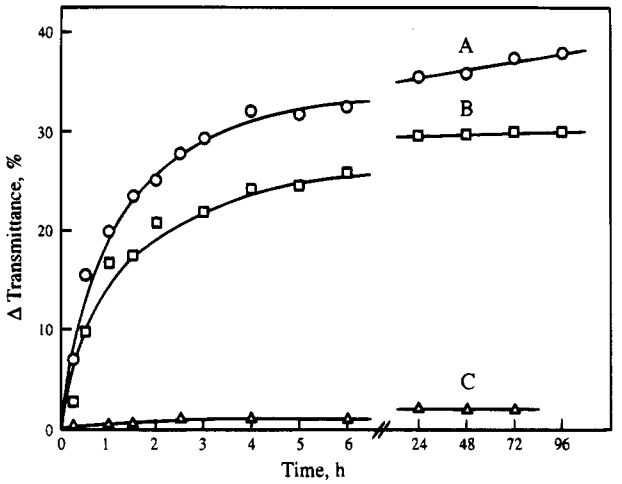


Figure 4. Degradation of polyurea **16a**: (A) with trehalase; (B) with α-amylase; (C) control.

Table 4. Viscosity Change of Suspended Polyurea 16a

enzyme	η_{inh},[a] dL/g initial	η_{inh},[a] dL/g enzyme-treated[b]
trehalase	0.50	0.23 (4 days)
α-amylase	0.50	0.25 (4 days)
none	0.50	0.34 (120 days)

[a] In DMSO, c = 0.25 g/dL, 25 °C. [b] Portion of the polyurea remained undissolved.

of the presence of trehalose units which would be biodegraded. Susceptibility of **16a** to a trehalase was thus examined in an acetate buffer of pH 5.7 at 37 °C. The enzymatic hydrolysis was followed by the change in turbidity of the suspension. As shown in Figure 4, the turbidity decreased rapidly in the presence of the enzyme. The polyurea was also degraded even by an α-amylase, though less efficiently. It is noteworthy that the polyurea prepared here is highly biodegradable not only with a trehalase but with a much more common enzyme, an α-amylase. These results indicate that the polyurea is a highly biodegradable polymeric material.

In the course of the enzyme treatment, the mixture became apparently less turbid. After 4 days, the suspended polyurea was isolated, and the viscosity was determined. As shown in Table 4, the initial viscosity of 0.50 dL/g decreased to 0.23 dL/g with the trehalase and to 0.25 dL/g with the α-amylase. Moreover, the isolated polyureas exhibited much more enhanced solubility than the original polyurea, indicating partial degradation. As a control, the polyurea was allowed to stand without enzymes, but no appreciable decrease in viscosity was observed in 4 days. Even after 120 days, the resulting polyurea had a viscosity of 0.34 dL/g.

These results suggest a high potential of the polyureas in drug delivery systems, and the drug-release behavior was examined with *p*-nitrophenol. A conjugate was

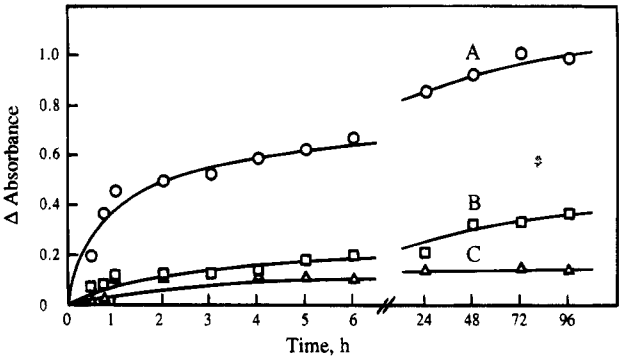


Figure 5. Release of *p*-aminophenol from the conjugate with polyurea **16a**: (A) with trehalase; (B) with α-amylase; (C) control.

prepared from *p*-nitrophenol and **16a** as a model. It was suspended in an acetate buffer, and the release of *p*-nitrophenol was followed by UV spectroscopy. Owing to the presence of trehalase units, *p*-nitrophenol was released quite efficiently as evident in Figure 5. Although the release was much less facile with α-amylase, it was more rapid than the control without any enzymes.

Conclusions

Efficient preparative procedures for free diaminotrehalose were established. Polyaddition of diaminotrehalose with diisocyanates afforded polyureas having trehalose residues in the main chain. The resulting polyureas showed favorable properties characteristic of both synthetic polymers and polysaccharides including solubility, membrane permeability, and biodegradability. These synthetic carbohydrate polymers may be useful as a new type of biodegradable materials with a high potential of developing advanced functions.

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