Synthesis of a New 2-Amino-Glycan, Poly-(1→6)-α-D-mannosamine, by Ring-Opening Polymerization of 1,6-Anhydro-mannosamine Derivatives

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ABSTRACT: A stereoregular 2-amino-glycan composed of a mannosamine residue was prepared by ring-opening polymerization of anhydro sugars. Two different monomers, 1,6-anhydro-2-azido-mannose derivative (**3**) and 1,6-anhydro-2-(*N, N*-dibenzylamino)-mannose derivative (**6**), were synthesized and polymerized. Although **3** gave merely oligomers, **6** was promptly polymerized into high polymers of the number-average molecular weight (M_n) of 2.3×10^4 to 2.9×10^4 with 1,6-α stereoregularity. The differences of polymerizability of **3** and **6** from those of the corresponding glucose homologs were discussed. It was found that an *N*-benzyl group is exceedingly suitable for protecting an amino group in the polymerization of anhydro sugars of a mannosamine type. The simultaneous removal of *O*- and *N*-benzyl groups of the resulting polymers was achieved by using sodium in liquid ammonia to produce the first 2-amino-glycan, poly-(1→6)-α-D-mannosamine, having high molecular weight through ring-opening polymerization of anhydro sugars.© 2012 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 000: 000–000, 2012

KEYWORDS: 2-amino-glycan; anhydro sugar; cationic polymerization; polysaccahrides; ring-opening polymerization

INTRODUCTION The 2-amino-glycans composed of 2-amino-2-deoxy-hexose (hexosamine) are frequently derived from natural sources and exhibit various biological activities. Poly-(1→4)-β-D-glucosamine (chitosan), usually obtained by de-*N*-acetylation of chitin, is the most well-known and possesses numerous functions such as antibacterial activity,[1,2] antitumor activity,[3,4] wound-healing effect,[5,6] nontoxicity,[6,7] and biocompatibility.[5,7] Poly-(1→4)-α-D-galactosamine was first discovered in the fluid portion of the culture of fungus *Aspergillus parasiticus*,[8] and it is also produced by the microorganism *Paecilomyces* sp. I-1 strain.[9] This 2-amino-glycan shows significantly higher antitumor effects *in vivo* compared to chitosan.[10] Its action mechanism is believed to be similar to that of chitosan and is ascribed to the activation of macrophage lineage cells and/or interferon produced from T lymphocytes.[11] However, it is unclear whether the higher activity originates from the structural differences of the repeating hexosamine unit, glycosidic linkage, and molecular weight or a difference in the essential action mechanism. Interestingly, among three biologically important hexosamines corresponding to glucosamine, galactosamine, and mannosamine, only the polymer of mannosamine has not yet been found. On the other hand, a unique linear 2-amino-glycan composed of *N*- and partially *O*-acetylated D-mannosamines linked via a (1→6)-α phosphodiester bond occurs in the capsule of meningococcal,[12] which plays a role in the infection of meningitis in humans. Although, such phosphoglycans are generally not classified as "polysaccharides, " they are attractive for some different biological properties from those of normal polysaccharides.[13] The amino or acetylated amino group of the sugar moiety is responsible for all the aforementioned biological functions. However, the structure of natural 2-amino-glycans is not quite homogeneous. The existence of a few branches and/or partial modifications of amino and hydroxyl groups greatly affects the biological activities and complicates structure–activity relationship studies. Therefore, the preparation of 2-amino-glycans having a definite repeating unit and glycosidic linkage, and high molecular weights is desired.

Several reports have been published on the chemical synthesis of 2-amino-glycans. Repeating the stepwise or blockwise coupling of monosaccharides or oligosaccharides is suitable for the elongation of multicomponent sugars.[14–17] Recently, a (1→6)-β-D-glucosamine undecamer[18] and *N*-Ac-(1→4)-β-D-glucosamine tetramer[19] were synthesized using this methodology. Apparently, this method is not appropriate for preparing large glycans with a single repeating unit, because a long complicated process is involved for obtaining high molecular weights. Kadokawa et al. devised the novel synthetic method of 2-amino-glycans by polyaddition of the oxazoline derivatives of *N*-Ac-D-glucosamine in the presence of acid catalysts to give poly-*N*-Ac-(1→4)-β-D-glucosamine (chitin)[20,21] and

poly-*N*-Ac-(1→6)-*β*-D-glucosamine[21] that have M_n of 2.8 × 10^3 and 5.6 × 10^3, respectively. Unfortunately, this method cannot provide glycans with 1,2-*cis* glycosidic linkages. Kobayashi et al.[22,23] synthesized the first artificial chitin from a chitobiose oxazoline derivative making use of the reverse reaction of hydrolase of chitin. In this strategy, high molecular weight glycans can be obtained in high yields without protecting the hydroxyl groups of the repeating sugar units, however, it has inherent limitations in the formation of only the natural type of glycosidic linkage due to the dependence on enzymes.

Cationic ring-opening polymerization of anhydro sugars is one of the most powerful methods for the chemical synthesis of polysaccharides.[24–26] To prepare amino-glycans by this technique, Uryu et al.[27] first used 1,6-anhydro-glucose derivatives bearing an azido group, which is convertible into an amino group. An amino group prevents cationic polymerization. However, the 2-azido derivative possessed low polymerizability, as the maximum degree of polymerization (DP) of the products was 6. Others have reported the polymerization of 1,6-anhydro-glucosamine derivatives with a phthaloyl-protected amino group to afford (1→6)-*β* linked oligomers with DP of only 3–7.[28,29] We also examined the use of three amino protecting groups such as a benzyl, cyclic alkyl, and cyclic silyl groups toward the synthesis of poly-(D-glucosamine) with a different type of glycosidic linkage from that of chitosan by polymerization of 1,6-anhydro-glucosamine derivatives.[30] All products were still oligomers having (1→6)-α linkages with DP of up to 8. Thus, stereoregular 2-amino-glycans of high molecular weights have not yet been obtained through ring-opening polymerization of anhydro sugars. Among several factors that affect the polymerizability,[31] we proposed that the reason for the low polymerizability of 1,6-anhydro-glucosamine derivatives is mainly the steric hindrance of the bulky substituted axial amino group against the elongation of the sugar unit. Hence, a 1,6-anhydro-mannosamine derivative, which has less steric hindrance than the glucosamine homolog, is more likely to be polymerized. This article describes the synthesis and ring-opening polymerization of 1,6-anhydro-2-azido- and -2-(protected amino)-mannose derivatives, and the synthesis of a new 2-amino-glycan, poly-(1→6)-α-D-mannosamine, with a high molecular weight.

EXPERIMENTAL

General

All chemicals were reagent grade and used without purification unless specified. Sodium hydride (Kishida Chemical, Japan) was used after the coating of mineral oil was washed with hexane. Cation-exchange resin (Dowex® 50WX8, H^+ form, 50-100 mesh) was washed with methanol prior to use. *p*-Chlorobenzenediazonium hexafluorophosphate was obtained from Tokyo Chemical Industry, Japan, and purified by recrystallization from water twice. Dichloromethane (Sigma–Aldrich, USA) and 1,2-dimethoxyethane (Kishida Chemical) were dried over CaH_2 and distilled just before use. The dialysis membrane was a Cellulose Dialyzer Tubing VT-803, exclusive $M_w < 8000$, purchased from Nacalai Tesque.

Melting points were determined by a Yamato MP-21 apparatus. Specific rotations were measured on a JASCO P-1020 polarimeter in a water-jacketed 1-dm cell. IR spectra were obtained with a Perkin Elmer Spectrum One using a KBr pellet for solid samples or a NaCl disk for liquid samples. ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-ECX400 spectrometer at 400 and 100 MHz, respectively, in $CDCl_3$ with tetramethylsilane or in D_2O with sodium 3-trimethylsilyl-1-propanesulfonate as an internal standard. Two-dimensional spectra were measured with pulsed field gradients. Elemental analyses were conducted on a J-Science Micro Corder JM10. Preparative high-performance liquid chromatography (HPLC) was executed through a Wakosil 5SIL silica gel column (10 × 300 mm^2, Wako Pure Chemical Industries, Japan) for purification of liquid samples. Molecular weights and distribution of polymers were evaluated by means of gel permeation chromatography (GPC) calibrated with standard polystyrenes (Shodex SM-105; Showa Denko, Japan) in $CHCl_3$ or standard pullulans (Shodex P-82; Showa Denko) in 66.7 mM of phosphate buffer (pH 6.86). The columns were Tosoh TSK gel $G3000H_{XL}$, $G4000H_{XL}$, and $G5000H_{XL}$ for the $CHCl_3$ eluent and TSK gel $G2500PW_{XL}$, $G3000PW_{XL}$, and $G4000PW_{XL}$ for the buffer solution, connected in series.

Synthesis of 3,4-Di-*O*-acetyl-1,6-anhydro-2-azido-2-deoxy-*β*-D-mannopyranose (2)

Sodium methoxide (0.80 g, 15 mmol) was added to a solution of 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranose (**1**)[32] (5.22 g, 14.0 mmol) in tetrahydrofuran (THF; 20 mL) and methanol (30 mL) with stirring. After 4 h at room temperature, cation-exchange resin was added until the pH was 7. The resin was filtered off, and the filtrate was evaporated *in vacuo*. To a solution of the resulting yellowish solid (3.05 g) in pyridine (20 mL), a solution of *p*-toluenesulfonyl chloride (3.0 g, 16 mmol) in pyridine (5 mL) was added dropwise at 0°C under nitrogen. The mixture was stirred at room temperature for 3 h, quenched by a small portion of methanol and then concentrated *in vacuo*. To a solution of the residue in ethanol (20 mL), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (4.5 mL, 30 mmol) was added dropwise at room temperature. The solution was stirred for 12 h and then concentrated *in vacuo* to give a brown syrup. Acetic anhydride (10 mL) was added to a solution of the syrup in pyridine (20 mL) with stirring. After 6 h at room temperature, the mixture was concentrated *in vacuo* and diluted with chloroform and water. The chloroform portion was washed with water twice, dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The crude product was purified by silica gel chromatography (hexane/ethyl acetate 2/1 as eluent) to afford 2.94 g of **2** as colorless crystals. The yield was 78% from **1**.

mp: 115–117°C. $[\alpha]_D^{25}$: +7.8 (c = 1.0, $CHCl_3$). Anal. Calcd for $C_{10}H_{13}N_3O_6$ (271.2): C, 44.28; H, 4.83; N, 15.49. Found: C, 44.15; H, 4.81; N, 15.41. IR (KBr, cm^{-1}): 2114 (s, N_3), 1756 and 1748 (s, C=O).

^{1}H NMR ($CDCl_3$, ppm): δ 2.15 (s, 3H, O4—CO—CH_3), 2.20 (s, 3H, O3—CO—CH_3), 3.34 (d, 1H, H2, $J_{2,3}$ = 4.76 Hz), 3.88 (dd, 1H, H6$_{exo,}$ $J_{5,6exo}$ = 5.86 Hz, $J_{6exo,6endo}$ = 7.87 Hz), 4.23 (d, 1H, H6$_{endo,}$ $J_{6exo,6endo}$ = 7.87 Hz), 4.64 (d, 1H, H5, $J_{5,6exo}$ = 5.86 Hz), 4.77 (s, 1H, H4), 5.29 (d, 1H, H3, $J_{2,3}$ = 4.76), 5.59 (s, 1H, H1).

^{13}C NMR ($CDCl_3$, ppm): δ 20.8 (O3—CO—*C*H$_3$), 20.9 (O4—CO—*C*H$_3$), 56.7 (C2), 65.4 (C6), 69.1 (C3), 71.2 (C4), 73.5 (C5), 100.7 (C1), 169.4 (O3—*C*O—CH$_3$), 169.5 (O4—*C*O—CH$_3$).

Synthesis of 1,6-Anhydro-2-azido-3,4-di-*O*-benzyl-2-deoxy-*β*-D-mannopyranose (3)

To a solution of **2** (2.72 g, 10.0 mmol) in *N, N*-dimethyl formamide (DMF) (30 mL), benzyl bromide (3.6 mL, 30 mmol) was added dropwise in the presence of $Ba(OH)_2$ (3.4 g, 20 mmol) and BaO (7.6 g, 50 mmol) at room temperature. After 12 h of stirring, a small amount of methanol was added, and the mixture was subject to vacuum filtration using a membrane with a pore size of 0.45 μm (Millipore®). The filtrate was concentrated *in vacuo* and diluted with chloroform. The solution was washed with water three times, dried over anhydrous Na_2SO_4, and then evaporated *in vacuo*. The residue was purified through silica gel chromatography and subsequent HPLC (hexane/ethyl acetate 2/1 as both eluents) to give 2.78 g (75%) of **3** as a colorless syrup. $[\alpha]_D^{25}$: +13.1 (c = 1.0, $CHCl_3$). Anal. Calcd for $C_{20}H_{21}N_3O_4$ (367.4): C, 65.38; H, 5.76; N, 11.44. Found: C, 65.20; H, 5.79; N, 11.48. IR (NaCl, cm^{-1}): 2102 (s, N_3), 1605, 739, and 698 cm^{-1} (w, aromatic).

^{1}H NMR ($CDCl_3$, ppm): δ 3.14 (dd, 1H, H2, $J_{2,3}$ = 5.49 Hz, $J_{2,4}$ = 1.65 Hz), 3.46 (ddd, 1H, H4, $J_{2,4}$ = $J_{3,4}$ = 1.65 Hz, $J_{4,5}$ = 1.83 Hz), 3.75 (dd, 1H, H6$_{exo,}$ $J_{5,6exo}$ = 6.04 Hz, $J_{6exo,6endo}$ = 7.32 Hz), 3.86 (ddd, 1H, H3, $J_{2,3}$ = 5.49 Hz, $J_{3,4}$ = 1.65 Hz, $J_{3,5}$ = 3.11 Hz), 4.25 (d, 1H, H6$_{endo,}$ $J_{6exo,6endo}$ = 7.32 Hz), 4.44 and 4.49 (d, 2H, O4—CH_2Ph, J = 12.26 Hz), 4.48 and 4.68 (d, 2H, O3—CH_2Ph, J = 11.90 Hz), 4.54 (ddd, 1H, H5, $J_{3,5}$ = 3.11 Hz, $J_{4,5}$ = 1.83 Hz, $J_{5,6exo}$ = 6.04 Hz), 5.55 (s, 1H, H1), 7.24–7.39 (m, 10H, phenyl × 2).

^{13}C NMR ($CDCl_3$, ppm): δ 57.5 (C2), 65.1 (C6), 71.4 (O4—*C*H$_2$Ph), 73.7 (O3—*C*H$_2$Ph), 74.0 (C5), 75.8 (C4), 76.7 (C3), 101.0 (C1), 127.8–137.3 (phenyl).

Synthesis of 2-Amino-1,6-anhydro-3,4-di-*O*-benzyl-2-deoxy-*β*-D-mannopyranose (5)

Sodium tetrahydroborate (0.19 g, 5.0 mmol) was added to a solution of **3** (1.84 g, 5.00 mmol) in methanol (30 mL) at room temperature. The solution was stirred at 60°C for 2 h, quenched by the addition of acetone, and concentrated *in vacuo*. The residue was dissolved in chloroform and water. The chloroform layer was washed with water twice, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The crude product was chromatographed on silica gel (ethyl acetate/methanol 5/1 as eluent), giving 1.57 g (92%) of purified colorless syrupy **5**. $[\alpha]_D^{25}$: +27.9 (c = 1.0, $CHCl_3$). Anal. Calcd for $C_{20}H_{23}NO_4$ (341.4): C, 70.36; H, 6.79; N, 4.10. Found: C, 70.21; H, 6.81; N, 4.09. IR (NaCl, cm^{-1}): 3382, 3314, and 1586 (m, NH), 1601, 740, and 699 (m, aromatic).

^{1}H NMR ($CDCl_3$, ppm): δ 1.57 (s, 2H, NH_2), 2.98 (d, 1H, H2, $J_{2,3}$ = 5.49 Hz), 3.44 (s, 1H, H4), 3.63–3.67 (m, 2H, H3 and H6$_{exo}$), 4.08 (d, 1H, H6$_{endo,}$ $J_{6exo,6endo}$ = 6.96 Hz), 4.48 (s, 2H, O3—CH_2Ph), 4.50–4.56 (m, 3H, H5 and O4—CH_2Ph), 5.23 (s, 1H, H1), 7.25–7.36 (m, 10H, phenyl × 2).

^{13}C NMR ($CDCl_3$, ppm): δ 50.7 (C2), 64.4 (C6), 71.1 (O4–*C*H$_2$Ph), 73.2 (O3–*C*H$_2$Ph), 73.5 (C5), 75.0 (C4), 76.8 (C3), 103.6 (C1), 127.6–137.6 (phenyl).

Synthesis of 1,6-Anhydro-3,4-di-*O*-benzyl-2-(*N, N*-dibenzylamino)-2-deoxy-*β*-D-mannopyranose (6)

To a solution of **5** (1.71 g, 5.00 mmol) in DMF (10 mL), dried NaH (0.30 g, 13 mmol) and benzyl bromide (1.5 mL, 13 mmol) were added with stirring at room temperature. After 12 h, methanol was added to the solution until bubbles were no longer produced, and the mixture was concentrated under reduced pressure. The resultant syrup was dissolved in chloroform and water. The separated chloroform portion was washed with water twice, dried over anhydrous Na_2SO_4, and then evaporated prior to column chromatography on silica gel. Elution from the column with hexane/ethyl acetate 3/1 afforded syrupy **6**, which was further purified by means of HPLC (the same eluent), affording 2.4 g (92%) of the desired product without any detectable impurity in the ^{1}H NMR spectrum. $[\alpha]_D^{25}$: +18.3 (c = 1.0, $CHCl_3$). Anal. Calcd for $C_{34}H_{35}NO_4$ (521.6): C, 78.28; H, 6.76; N, 2.69. Found: C, 78.18; H, 6.75; N, 2.65. IR (NaCl, cm^{-1}): 1603, 734, and 698 (m, aromatic).

^{1}H NMR ($CDCl_3$, ppm): δ 3.09 (dd, 1H, H2, $J_{2,3}$ = 4.85 Hz, $J_{2,4}$ = 0.92 Hz), 3.40 (dd, 1H, H4, $J_{2,4}$ = 0.92 Hz, $J_{3,4}$ = 1.60 Hz), 3.67 (dd, 1H, H6$_{exo,}$ $J_{5,6exo}$ = $J_{6exo,6endo}$ = 6.50 Hz), 3.83 (ddd, 1H, H3, $J_{2,3}$ = 4.85 Hz, $J_{3,4}$ = 1.60 Hz, $J_{3,5}$ = 3.10 Hz), 3.99 (s, 4H, *N*-CH_2Ph × 2), 4.18 (dd, 1H, H6$_{endo,}$ $J_{5,6exo}$ = 0.73 Hz, $J_{6exo,6endo}$ = 6.50 Hz), 4.44 and 4.51 (d, 2H, O3—CH_2Ph, J = 10.43 Hz), 4.45 (m, 3H, H5 and O4—CH_2Ph), 5.63 (s, 1H, H1), 7.16–7.35 (m, 10H, phenyl × 2).

^{13}C NMR ($CDCl_3$, ppm): δ 55.7 (*N*-*C*H$_2$Ph × 2), 57.6 (C2), 64.7 (C6), 71.2 (O4—*C*H$_2$Ph), 72.8 (O3—*C*H$_2$Ph), 74.7 (C5), 75.4 (C4), 78.3 (C3), 102.3 (C1), 126.6–140.6 (phenyl).

Polymerization

Ring-opening polymerization was performed in dichloromethane under high vacuum conditions (~10^{-5} mmHg) at −60 and 0°C as reported previously.[31] The polymers produced were purified by reprecipitation with chloroform–petroleum benzine or chloroform–methanol three times and freeze-dried from benzene. The supernatants after reprecipitation were confirmed by GPC whether polymeric products still remained or not. The spectral features of polymers **4** and **7** are as follows.

2-Azido-3,4-di-O-benzyl-2-deoxy-(1→6)-α-D-mannopyranan (4)

IR (KBr, cm^{-1}): 2104 (s, N_3), 1602, 736, and 696 (m, aromatic).

^{1}H NMR ($CDCl_3$, ppm): δ 3.22 (br s, 1H, H2), 3.43 (br s, 1H, H4), 3.78 (br s, 1H, H5), 3.97 (br s, 1H, H3), 3.4–4.2 (m, 2H, H6, 6′), 4.47 and 4.97 (d, 2H, O3—CH_2Ph, J = 12.1 Hz), 4.83 (s, 2H, O4—CH_2Ph), 5.07 (s, 1H, H1), 7.2–7.5 (m, 10H, phenyl × 2).

^{13}C NMR ($CDCl_3$, ppm): δ 64.8 (C2), 67.1 (C6), 70.8 (C5), 73.3 (O3—*C*H_2Ph), 74.5 (O4—CH_2Ph), 77.3 (C4), 81.7 (C3), 95.2 (C1), 126.1–138.2 (phenyl).

***3,4-Di*-O-*benzyl*-2-(*N*, *N*-dibenzylamino)-2-deoxy-(1→6)-α-D-*mannopyranan* (7)**

IR (KBr, cm^{-1}): 1601, 740, and 697 (m, aromatic).

^{1}H NMR ($CDCl_3$, ppm): δ 3.35 (s, 1H, H4), 3.42 (s, 1H, H2), 3.74 (br s, 1H, H6), 3.93 (s, 1H, H3), 4.05 (s, 4H, *N*-CH_2Ph × 2), 4.21 (d, 1H, H6′, J = 6.59 Hz), 4.46 and 4.57 (dd, 2H, O4—CH_2Ph, J = 12.3 Hz), 4.56 (s, 1H, H5), 4.52 and 4.71 (dd, 2H, O3—CH_2Ph, J = 11.8 Hz), 5.73 (s, 1H, H1).

^{13}C NMR ($CDCl_3$, ppm): δ 56.2 (*N*-*C*H_2Ph × 2), 60.5 (C2), 63.2 (C6), 69.9 (C5), 71.5 (C4), 72.3 (O3—*C*H_2Ph), 74.9 (O4—*C*H_2Ph), 77.0 (C3), 97.7 (C1), 126.2–138.4 (phenyl).

Debenzylation

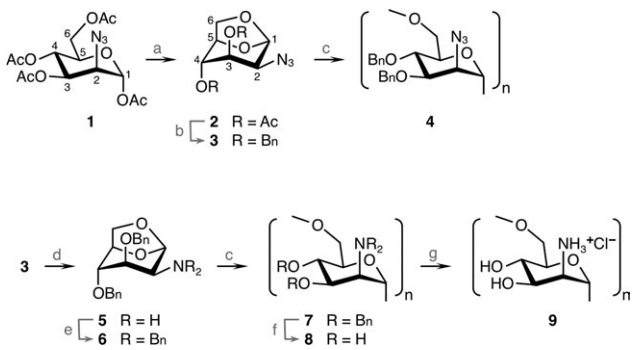
The polymer **7** (100 mg, $M_n = 2.5 \times 10^4$, $M_w/M_n = 2.4$) was treated by the same procedure as in the literature[31] to give 28 mg (91%) of 2-amino-2-deoxy-(1→6)-α-D-mannopyranan (**8**). After IR analysis, **8** was dissolved in dilute HCl (3%) due to its insolubility in water. Another purification by dialysis and subsequent freeze-drying of the solution provided water-soluble solid **9** as the HCl salt of **8** (34 mg). The following data are based on the HCl salt **9**. $M_n = 1.3 \times 10^4$, $M_w/M_n = 2.0$. $[\alpha]_D^{25}$: +65.2 (c = 1.0, H_2O). IR (KBr, cm^{-1}): 3000−3500 (br, OH and NH), 1620 (br, NH).

^{13}C NMR (D_2O, ppm): δ 57.3 (C2), 66.2 (C6), 70.3 (C4), 71.2 (C3), 73.0 (C5), 96.8 (C1).

RESULTS AND DISCUSSION

Synthesis of Monomers

First, we focused on the azido derivative **3** shown in Scheme 1 as a monomer for the synthesis of 2-amino-mannans through ring-opening polymerization. The improved large-scale preparation of the 2-azido-mannose acetate **1** from D-glucose that was reported recently[32] facilitated this work. The conversion of **1** into **2** was accomplished via a four-step sequence without any isolation and purification of the intermediates as follows. The treatment of **1** with sodium methoxide in methanol and subsequent cation-exchange using Dowex® resin achieved the deacetylation almost quantitatively. The formation of the 1,6-anhydro ring followed the method of Boullanger and coworkers.[33] At a low temperature, the use of 1.1 equivalents of *p*-toluenesulfonyl chloride relative to the 2-azido-mannose allowed selective tosylation on C6, and then the addition of DBU accomplished the cyclization between C1 and C6 involving detosylation. The main product at this point, assumed to be 1,6-anhydro-2-azido-2-deoxy-β-D-mannopyranose, was difficult to separate from the salts coproduced, and thus it was isolated as the acetate form **2** by chromatography after



SCHEME 1 Synthesis and ring-opening polymerization of **3** and **6**. The numbers denote the position of each carbon. *Reagents and conditons:* (a) i—NaOMe, MeOH/THF, rt, 4 h; ii—*p*-TsCl, Py, 0°C then rt, 3 h; iii—DBU, EtOH, rt, 12 h; iv—Ac_2O, Py, rt, 6 h, 78%; (b) BnBr, $Ba(OH)_2$, BaO, DMF, rt, 12 h, 75%; (c) PF_5, CH_2Cl_2, −60°C, under high vacuum; (d) $NaBH_4$, MeOH, 60°C, 2 h, 92%; (e) BnBr, NaH, DMF, rt, 12 h, 92%; (f) Na, liquid NH_3, −78°C, 1 h, 91%; (g) 3% HCl, quantitative.

treatment with acetic anhydride and pyridine. The IR spectrum and elemental analysis indicated that the azido group was not damaged during cyclization. Deacetylation and subsequent benzylation of **2** were performed in a one-pot reaction. A large amount of $Ba(OH)_2$ and BaO removed the acetyl groups in addition to catalyzing the subsequent benzylation to afford **3** without significant side reactions. IR and NMR analyses of **3**, after purification by preparative HPLC, proved the existence of an azido group and two benzyl groups as shown in Figure 1(A, B).

As will be described earlier, polymer **4** obtained by polymerization of **3** could not be converted to the corresponding 2-amino-mannan, and therefore another monomer **6** was designed. The azido group of **3** was reduced with sodium tetrahydroborate, and then the amino group of the resulting 1,6-anhydro-mannosamine derivative **5** was protected with benzyl groups using sodium hydride and benzyl bromide. In this case, both amino protons must be substituted not to prevent cationic polymerization. The resultant syrup was carefully purified by preparative HPLC and analyzed by NMR spectroscopy. Two *N*-benzyl groups were observed in the ^{1}H NMR spectrum shown in Figure 1(C), indicating that the desired monomer **6** was obtained.

Polymerization of 3 and 6

The ring-opening polymerization of **3** and **6** was accomplished at −60 and 0°C in dichloromethane using PF_5 as an initiator. The experimental results are summarized in Table 1. In the polymerization of **3**, ~1 mol % of the initiator, a typical amount for obtaining high molecular weights of polysaccharides,[34] did not afford any products; only unreacted monomer was recovered after 24 h. Increasing the concentration of PF_5 to 3 and 5 mol % gave oligomeric products in about 10% yields (Entries 1 and 2), which were partly soluble in methanol and isolated by reprecipitation with petroleum benzine from the chloroform solution. Their M_ns were calculated by GPC to be 1.5×10^3 and 1.8×10^3,

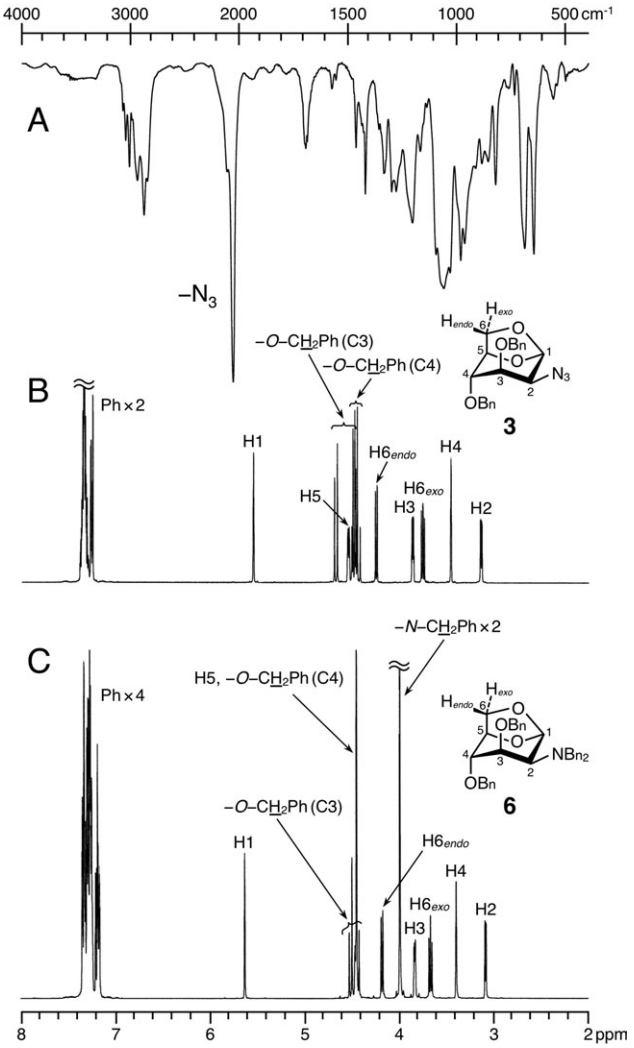


FIGURE 1 IR spectrum of **3** (A) and 400 MHz ^{1}H NMR spectra of **3** (B) and **6** (C) in $CDCl_3$.

corresponding to DPs of 4 and 5, respectively. The use of 10 mol % of PF_5 and 24 h of polymerization time slightly improved M_n up to 4.6×10^3 (DP = 13), though the yield hardly changed (Entry 3). The increment of the molecular weight was likely due to the higher concentration of the initiator, not longer polymerization time, because further extension of the time did not result in a significant difference in either molecular weight or yield (Entry 4).

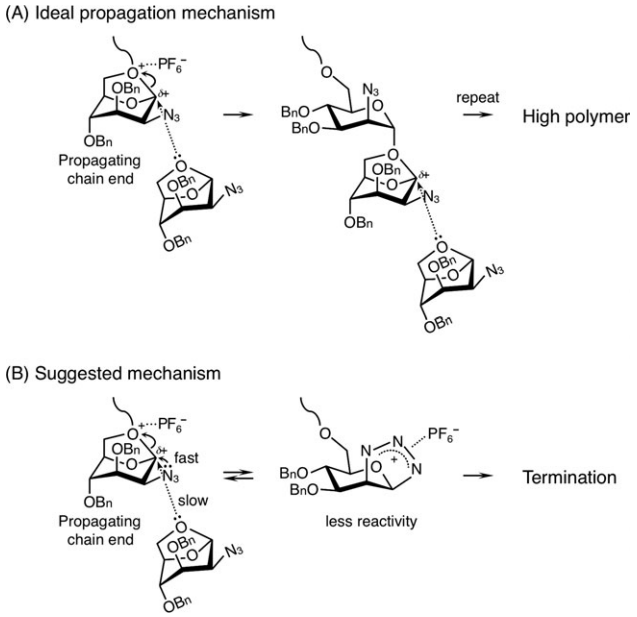
Thus, the polymerizability of a 1,6-anhydro-2-azido-mannose derivative was extremely low compared to that of the usual 1,6-anhydro-mannose homologs without an azido group.[24,25,35] This behavior resembled that of a 1,6-anhydro-2-azido-glucose derivative.[27] It seems that such low polymerizability of 2-azido anhydro sugars is derived not only from the existence of an azido group but also some regiospecific factor that interferes with polymerization, because 1,6-anhydro-3-azido-glucose[27] and -allose[36] derivatives can be converted to high polymers. One reasonable explanation is that, as illustrated in Scheme 2(B), a nonlocalized electron pair of the azido group attacks the electron-deficient C1 at the propagating end, and the resultant species composed of a 1,2,3-triazole cation and counter anion PF_6^- is less reactive. As a result, chain propagation would be terminated. This mechanism can be applied to only the 2-azido derivatives whose azido group is adjacent to the C1 position and is independent of the configuration of the azido group. Similar neighboring group participation of an azido group was reported in several articles.[37,38] An ideal propagation mechanism for 1,6-anhydro sugars is established as shown in Scheme 2(A).[39]

The structure of polymer **4** was characterized by NMR spectroscopy and optical rotations. Figure 2 shows the 100 MHz ^{13}C NMR spectra of monomer **3** (A) and polymer **4** (B) expanded from 50 to 110 ppm, in $CDCl_3$ at room temperature. The peak assignment was accomplished by heteronuclear single quantum coherence (HSQC) and heteronuclear multiple-bond correlation (HMBC) spectra. The ^{13}C NMR spectra of the monomer and polymer are quite different

TABLE 1 Ring-Opening Polymerization of **3** and **6**.

Entry[a]	Monomer	PF_5 (mol %)	Temp. (°C)	Time (h)	Yield[b] (%)	M_n[c] ($\times 10^3$)	M_w/M_n	$[\alpha]_D^{25}$[d] (°)
1	**3**	3	–60	16	8	1.8	1.9	+78.3
2	**3**	5	–60	16	10	1.5	2.2	+73.6
3	**3**	10	–60	24	11	4.6	2.2	+75.0
4	**3**	10	–60	72	9	3.5	2.4	+79.9
5	**6**	3	–60	12	20	26.6	1.9	+46.9
6	**6**	5	–60	4	29	28.7	2.1	+45.3
7	**6**	5	–60	8	38	26.3	1.9	+46.1
8	**6**	10	–60	8	35	22.9	2.0	+45.9
9	**6**	10	0	8	0	–	–	–
10	**6**	20	0	24	0	–	–	–

[a] Monomer: 200 mg, dichloromethane: 0.20 mL.
[b] Petroleum benzine-insoluble part.
[c] Determined by GPC.
[d] Measured in $CHCl_3$ ($c = 1.0$).



SCHEME 2 Proposed propagation mechanism in ring-opening polymerization of **3**.

from each other, probably due to the conformational change of the pyranose ring from $^1C_4$ to $^4C_1$. In Figure 2(B), each peak, particularly the C1 peak at 95.2 ppm, was single, indicating that the repeating unit of **4** has one stereoselective

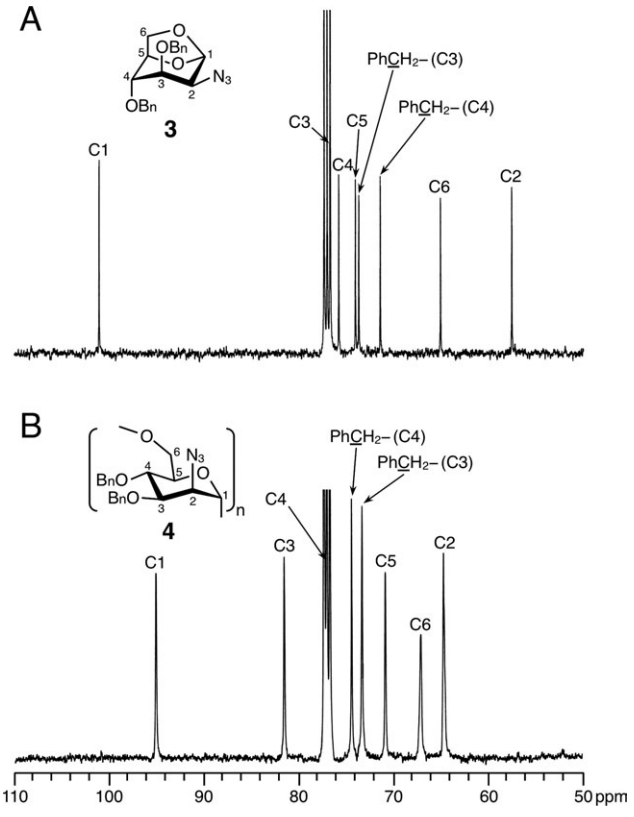


FIGURE 2 100 MHz ^{13}C NMR spectra of **3** (A) and **4** (B) in $CDCl_3$.

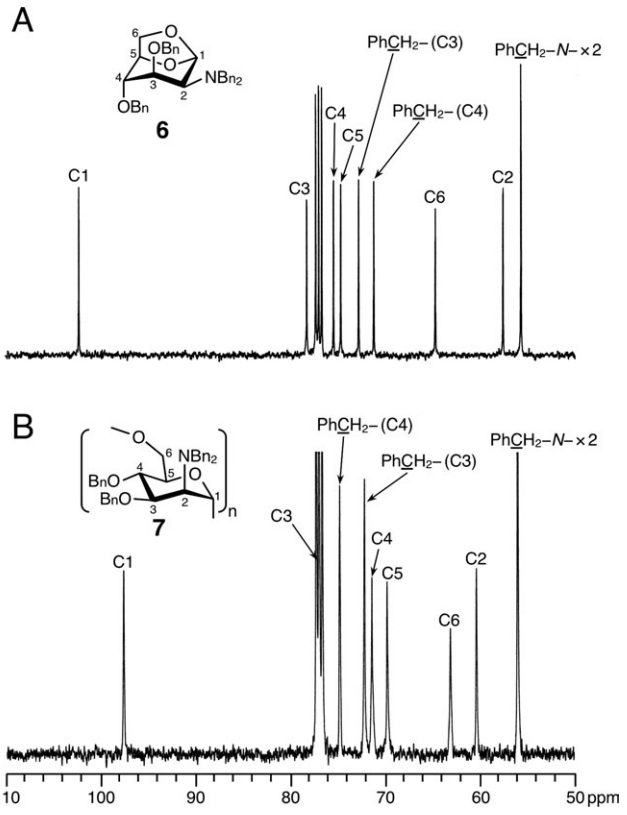


FIGURE 3 100 MHz ^{13}C NMR spectra of **6** (A) and **7** (B) in $CDCl_3$.

glycosidic linkage. The specific rotations of **4** were large and positive from +74 to +80°, compared with that of **3** at +13.1°. Such a remarkable difference between monomer **3** and polymer **4** is ascribed to the inversion of configuration at C1, according to the typical results of polymerization of 1,6-anhydro sugars.[25] Hence, every glycosidic linkage of **4** was determined to adopt an α anomeric configuration.

The yields and molecular weights of polymer **4** were not enough to produce a 2-amino-glycan. Therefore, another monomer **6** was investigated. The concentration of PF_5 from 3 to 5 mol % relative to **6** afforded polymers with high molecular weights of M_n of 2.6×10^4 to 2.8×10^4 (DP = 50–55) in ~30% yield (Entries 5–7 in Table 1). Chromatographic analysis revealed no oligomer, but partially unreacted **6** was recovered in the supernatant after reprecipitation of the polymer. At higher concentrations of PF_5 of more than 5 mol %, a decline in M_n was observed, though the same polymerization conditions were used (Entry 8). The polymerization time of 8 h gave favorable yield and M_n, and the shorter time of 4 h caused low conversion (Entries 6 and 7).

Similarly, we previously studied ring-opening polymerization of a 1,6-anhydro-glucosamine derivative having an *N, N*-dibenzyl protecting group on C2.[30] However, it was little polymerized under the same conditions as this study. These results strongly suggest that the higher polymerizability of **6** more than that of the corresponding glucosamine derivative

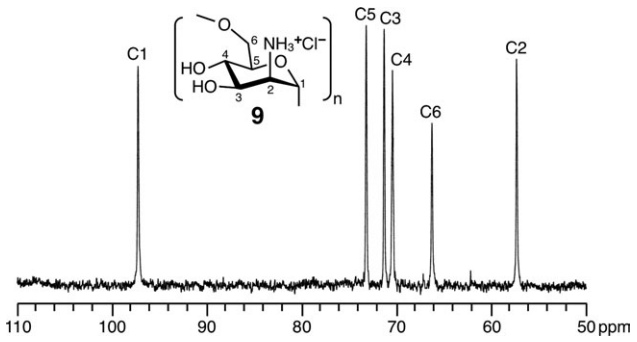


FIGURE 4 100 MHz ^{13}C NMR spectrum of **9** in D_2O.

is responsible for the configuration of a substituent on C2. Such a bulky axial substituent on C2 as an *N, N*-dibenzylamino group interferes with monomers attacking from the α side. As the configuration of the substituent on C2 of **6** that is a mannosamine-type is equatorial, the *N, N*-dibenzylamino group would not reduce the polymerizability.

Figure 3 shows the 100 MHz ^{13}C NMR spectra of monomer **6** (A) and polymer **7** (B) in the region from 50 to 110 ppm in $CDCl_3$ at room temperature. Each peak was assigned by HSQC and HMBC measurements. As in the case of **4**, it was found that **7** has only (1→6)-α glycosidic linkages from the single C1 absorption at 97.7 ppm and large positive specific rotations of $+45$ to $+46^\circ$.

Kanno et al.[28] reported that a 1,6-anhydro-glucosamine derivative whose amino group was protected with a phthaloyl group was polymerized at a higher temperature of 0°C to obtain oligomers with (1→6)-β stereoregularity, though the conversion was low. However, at the same temperature, polymerization of **6** did not proceed at all (Entries 9 and 10). The reason is deduced as follows: in the polymerization of 1,6-anhydro sugars using PF_5, the trialkyloxonium ion of the propagating species is stable at temperatures as low as -60°C, as Schuerch et al. pointed out.[24,39] At approximately -40°C or higher, chain transfer and/or other side reactions are frequently involved to interfere with the formation of high polymers.[24] As for the monomer in Kanno's work, chain growth would proceed via an oxazoline intermediate by neighboring group participation, which is generally induced at high temperatures,[40] of a phthalimide group, though this was not mentioned in their report. As the 1,2-oxazoline of glucosamine is known to give the corresponding β-glycoside, the β-oligomer was produced only at high temperatures. The present mannosamine-type monomer **6** is not subjected to neighboring group participation on C2, and large steric hindrance of the equatorial *N, N*-dibenzylamino group prevents an attack from the β-side. Therefore, it is suggested that the (1→6)-β polymer was not obtained under any polymerization conditions.

Debenzylation of 7

There are no reports on the removal of *N*-benzyl protecting groups of amino-polysaccharides. De-*O*- and -*N*-benzylation of polymer **7** were simultaneously achieved by the Birch reduction, using sodium in liquid NH_3 at -78°C.[31] The reaction was terminated in 1 h, and subsequent conventional work-up procedure was carried out to give a clear aqueous solution, resulting in the amino-glycan **8** dissolved in water. However, **8** became insoluble in water when subjected to lyophilization. A similar phenomenon was also observed in the synthesis of 3-amino-glucan.[27] This was attributed to the intermolecular and intramolecular hydrogen bonds between the glycan chains formed by the elimination of water during drying. The dried **8** was soluble in dilute HCl, but insoluble in NaOH aqueous solution and even in high polar solvents such as DMF and dimethyl sulfoxide. Figure 4 shows the ^{13}C NMR spectrum of **9**, obtained from dialysis of the HCl salt of **8**, in D_2O at 40°C. Resonances of six carbons appeared, whereas there were no signals derived from benzyl groups, indicating that every repeating unit of **9** was homogeneous and the *O*- and *N*-benzyl groups were completely removed. The absorption of C2 possessing an amino group was shifted to the most upfield position of 57.3 ppm. The M_n and molecular weight distribution of **9** were estimated to be 1.3×10^4 and 2.0, respectively. The narrowed distribution of **9** compared with that of the starting **7** was explained as some low molecular weight components were removed by dialysis.

CONCLUSIONS

In this study, two different monomers, 1,6-anhydro-2-azido-mannose, and 1,6-anhydro-2-(*N, N*-dibenzylamino)-mannose derivatives were synthesized and polymerized to prepare 2-amino-glycans with high molecular weights by ring-opening polymerization. The polymerizability of both monomers was higher than that of the corresponding glucose homologs. However, the 2-azido derivative still did not give sufficiently high polymers to produce a free glycan because of insufficient yield and molecular weight. It was concluded that the 2-azido group of hexoses is involved in neighboring group participation with the positively charged C1 at the propagating chain end and consequently prevents polymerization. On the other hand, the *N, N*-dibenzylated mannosamine monomer yielded high polymers with $M_n = 2.3 \times 10^4$ to 2.9×10^4. Both *O*- and *N*-benzyl groups in the polymer were removed simultaneously by the Birch reduction. It was found that an *N*-benzyl group is exceedingly suitable for protecting an amino group in the polymerization of anhydro sugars of a mannosamine type. Here, a synthetic 2-amino-glycan, (1→6)-α-D-mannosamine, having high molecular weight and a completely homogeneous repeating unit including the glycosidic linkage was obtained. The stability of such non-natural amino-glycans against enzymes and microorganisms capable of hydrolyzing hexosamine residues are advantage over natural ones. This artificial amino-polysaccharide will be a helpful tool for investigating various biological activities.

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