Doctoral Thesis

Ring-opening polymerization of new branched 1, 6-anhydro di- and trisaccharide monomers and synthesis of branched polysaccharides

新規 1，6-無水 2 糖、3 糖モノマーの 開環重合と分枝糖鎖の合成

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ABBREVIATIONS and ACRONYM

CH_2Cl_2	Dichloromethane
GPC	Gel permeation chromatography
$CHCl_3$	Chloroform
Hex	Hexane
COSY	Correlated spectroscopy
HIV	Human immune deficiency virus
c1	Concentration1%
HMQC	Heteronuclear multiple quantum correlation
EtOAc	Ethyl acetate
KBr	Potassium bromide
Et_3N	Trimethylamine
CCl_3CN	Trichloroacetonitrile
FT-IR	Fourier transform infrared spectroscopy
$Ba(OH)_2$	Barium hydroxide
DMF	Dimethyl formaldehyde
DP	Degree of polymerization
DMSO	Dimethyl sulfoxide
Na	Sodium, lump in kerosene

NaH	Sodium hydride
MeOH	Methanol
NaOH	Sodium hydroxide
Na$_2$SO$_4$	Sodium sulfate
NH$_3$	Ammonia
NMR	Nuclear magnetic resonance
PF$_5$	Phosphorus pentafluoride
BF$_3$Et$_2$O	Boron trifluoride ethyl ether
	Trimethylsilyl
TMAOTf	Trifluoromethanesulfonate
$[\alpha]_D^{25}$	Specific rotation
$\overline{M}_n$	Molecule weight
^{1}H	Proton
^{13}C	Carbon

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CHAPTER 1.
General Introduction

1.1 Polysaccharides

Polysaccharides are polymeric carbohydrate molecules consisting of long chain monosaccharides units linked together by glycosidic linkages and contained in a variety of polymeric materials originated from animal, plant and algar in nature, which are formed with glycosidic linkages of monosaccharides [1]. Polysaccharides have divided two different structures such as linear or branched chain polysaccharides, which were based on the monosaccharide unit in the nature and have so many reactive functional groups in their chemical structure such as hydroxyl, amino, and carboxylic acid groups, indicating the possibility for chemical modification [2.3]. However, the molecular weight of naturally occurring polysaccharides is high and hundreds or thousands of Daltons, furthermore diversity [4].

At present, great attention has been paid to naturally occurring polysaccharides and oligosaccharides because of their great influence on the biological activities. For examples (Fig 1), the natural polysaccharides dextran consisting of 1, 6-α linked poly-D-glucose was first discovered from wine as a microbial product and used as a medical function like an antithrombotic(antiplatelet), anti-blood viscosity, and a volume expander in hypovolaemia [5.6]. A

naturally occurring polysaccharide lentinan has 1, 3-β-D-glucopyranosidic structure in the main chain with a 1, 6-β-D-glucopyranosidic branch in every three glucose units. Lentinan has used as a cancer drug against stomach cancer [9], because of its strong antitumor activity [7.8].

Yoshida reported that the structure having 1, 3-β-D-glucan can inhibit the multiplication of Sarcoma 180 and Lewis lung cancer in rat and produce interleukin-12(IL-12) in vivo [10]. In the natural polymers, Chitin and Chitosan consisting of 2-acetoamide-2-deoxy-β-D-glucose for chitin and 2-amino-2-deoxy-β-D-glucose for chitosan with 1, 4-β glycoside linkage are the most abundant in the earth except for the cellulose, which have so specific biological activities such as effects on granulation tissue in cats [11], on canine polymorphonuclear (PMN) cells [12].

Even though the natural polysaccharides have so many biological activities, it is difficult to clearly explain the relation between their structures and activities owing to their complex structures.
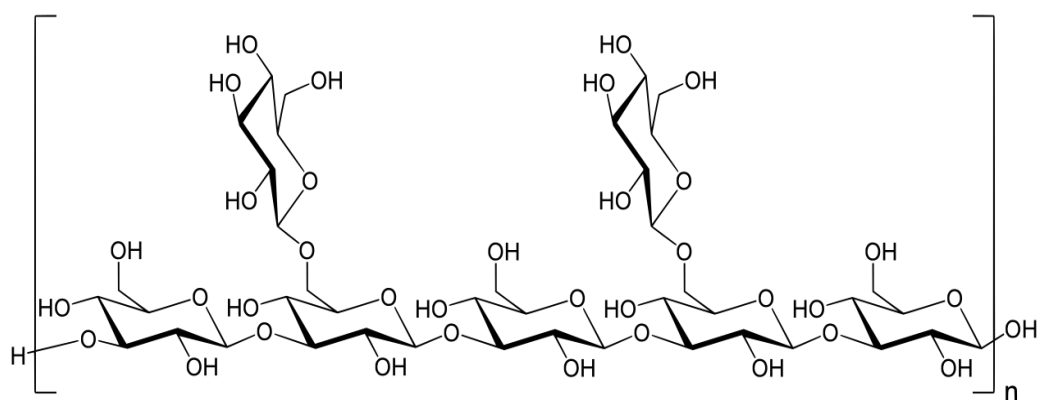
Structure of Lentinan
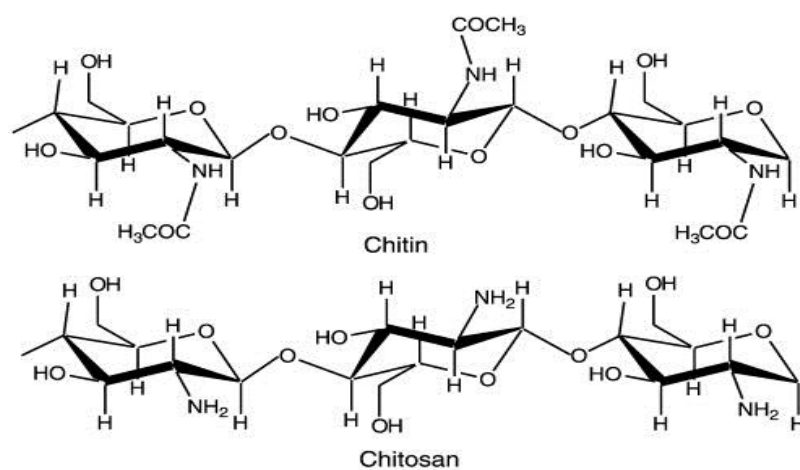
Chitin

Chitosan

Structure of Chitin and Chitosan

α-1,6
+
α-1,3

α-1,6
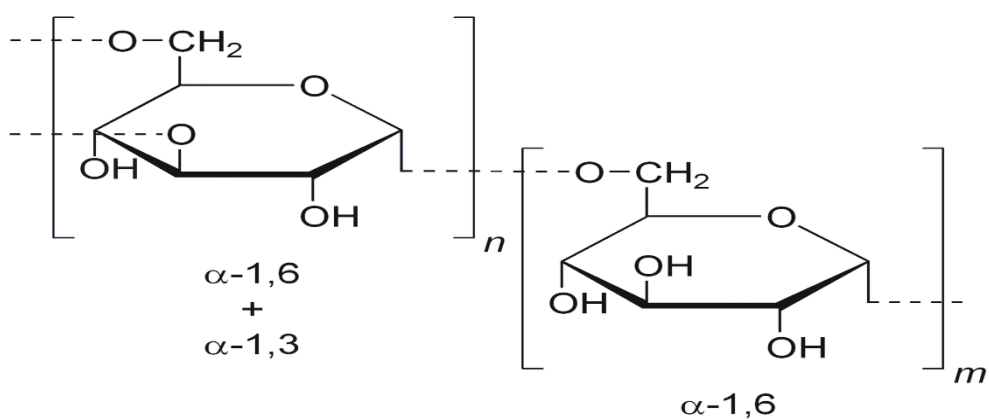
Structure of Dextran

Fig 1 Sucture of some natural polysaccharides

1.2 Ring-Opening of Polymerization

In polymer chemistry, **ring-opening polymerization** (**ROP**), ingeneral is a reaction on a chain-growth form to get polymers. It means that on the terminal end of the polymer chain under the propagating reaction radical, anionic or cationic and acts as reactive center where the cyclic monomers open its ring system and form a
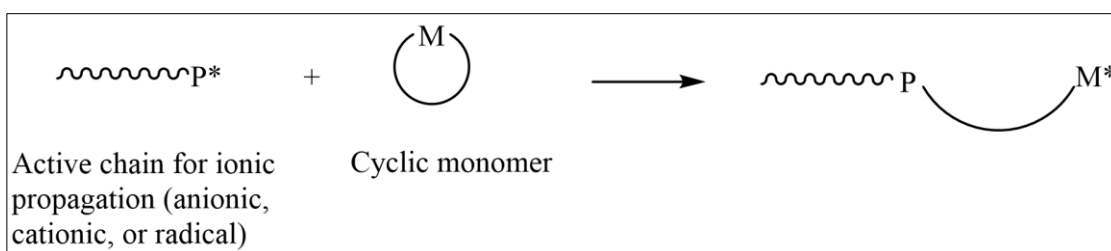
Fig.2 the magnesium of ring-opening polymerization

longer polymer chain (Fig 2).

For obtaining stereoregular polysaccharides with high molecular weights and define their structures by ring-opening polymerization of anhydrosugar monomers is a good method [13]. The resulting polysaccharides become good biomaterials for investigating the relationship between their structures and biological activities, because the natural polysaccharides have complex structures, in general, and it is difficult to know the relationship [14]. In order to investigate the relationship and then to synthesize the new materials having specific biological activities, the synthesis of polysaccharides having defined structures play an important role. In 1966, Ruckel and Schuerch noted for the first time a stereoregular (1→6)-α-D-glucopyranan (dextran) synthesized by ring-opening polymerization of benzylated levoglucosan [15].

1.3 Human Immunodeficiency Virus (HIV)

The human immunodeficiency virus (HIV) is a subgroup of retrovirus called as lentivirus, which results a condition of the acquired immunodeficiency syndrome (AIDS) in humans. HIV is damaged prevent the immune system to progressive and therefore lead to the life is threaten to infections opportunistically and thrived to be cancers [16.17]. HIV is classified as two distinct viruses such as HIV-1 and HIV-2. HIV-1 is considered to be predominant virus, and generally speaking when HIV is not specified the virus type by the people they are referring to HIV-1. It was reported firstly that the field serum samples about HIV infection in humans from Kinshasa Congo Republic are stored in 1959. And in 1981, the cases of AIDS have been officially reported for the first time [18.19].

Currently there is so many treatment on AIDS, but it can't be cured. All treatments are pointed to control or delay progression of disease and improvement of symptoms, improvement life quality.

Therefore, under the urgent condition, there is a need to make or develop some effective drugs for anti-HIV with low side effectiveness. At present, the researchers paid attention to the polysaccharides because of strong biological activities on anti-HIV.
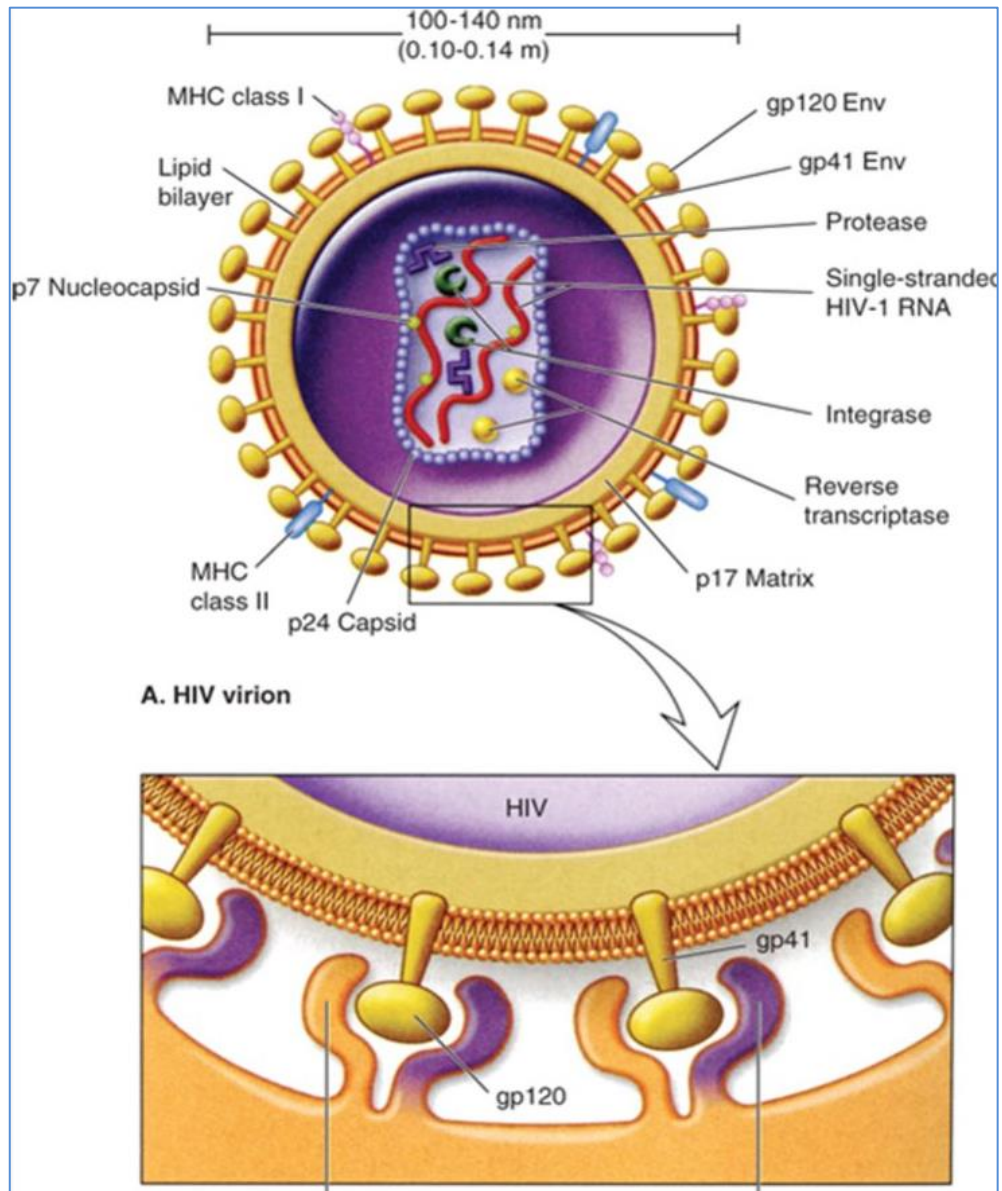
Figure: The structure of HIV-1

(From https://jp.pinterest.com/miarienstra/immunology/)

1.4 Synthetic Sulfated Polysaccharides

Many sulfated polysaccharides extracted from sea alga were found by researchers. Gerber and Sherman found that sulfated polysaccharides extracted from *Gelidium cartilagenium* had biological activities that were protected infection of influenza B and mump virus to embryonic eggs [20]. In 1987, Nakashima and Yamamoto reported firstly that natural sulfonated polysaccharides from sea alga had the effect of human immunodeficiency virus (HIV) [21]. Uryu and Yoshida first reported that synthetic sulfated polysaccharides obtained by ring-opening polymerization had the potent anti-HIV activity [22]. Up to now, it has reported the synthesis of branched polysaccharides by ring-opening polymerization of anhydro disaccharide and trisaccharide monomers to investigate the relationship between structure of polysaccharides and biological activies, such as the anticoagulation and anti-HIV activities. Sulfation of synthetic and naturally polysaccharides was carried out with piperidine-N-sulfonic acid or sulfur trioxide pyridine complex [14] (table 1).

Table 1: Structures of synthetic polysaccharides

Polysaccharides	structure	references
Arabinans ·	α-(1→4)-L-arabinopyranoses	Yoshida *et al*, 1986
Branched dextran ·	α-(1→6)-D-glucose main chain	Hatanaka *et al*, 1989
Glucopyranan	α-(1→4)-D-glucopyranose derivaties	Yoshida,Uryu,1997
Ribofuranan ·	α-(1→4)-D-ribofuranose, α-(1→5)-D-ribofuranose, α-(1→5)-D-xylofuranose ·	Choi and Uryu,1997

Astragalus ·	α-(1→4)-D-glucan·	Liu and Uryu, 2003
Lactopyranans ·	α-(1→6)-D-glucopyranose main chain,	Han,Yoshida,2008
· ·	α-(1→3)-D-galactopyranose branched·	
Glucan	Methylated (1→6)-α-D-glucopyranose	Yoshida and Yoshida, 2008
Ribofuranan	3-O-(β-D-lactosyl)-α-D-ribofuranan	Yoshida and Yoshida, 2009
Poly-mannosamine ☐α-(1→6)-D-mannosamine derivatives		Hattori and Yoshida, 2012
Alkyl glucopyranan ☐	α-(1→6)-D-glucopyranose, 3-O-long alkyl groups	Bai and Yoshida, 2015
Galactomannan ☐	α-(1→6)-D-mannopyranose, ☐ α-(1→4)-D-galactopyranose	Budragchaa and Yoshida, 2015
Glucopyranan	α-(1→6)-D-glucopyranose, α-(1→3)-D-gulcopyranose α-(1→3)-D-mannopyranose α-(1→3)-D-maltopyranose	Bai chaolumen and Yoshida, 2017

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CHAPTER 2.
Synthetic Disaccharide

ABSTRACT

New 3-*O*-branched 1, 6-anhydro glucopyranose disaccharide monomers, 1, 6-anhydro-2,4-di-*O*-benzyl-3-*O*-(2',3',4',6'-tetra-*O*-benzyl-α-D-mannopyranosyl)- (LGM **6**) and -glucopyranosyl)- β-D-glucopyranose (LGG **7**), were synthesized and polymerized. It was found that the 3-*O*-branched1, 6-anhydro disaccharide monomers were polymerized. However, the polymerizability was lower than that of the 4-*O*-branched disaccharide monomers reported previously. Debenzylation of the resulting polymers gave 3-*O*-gluco- and mannopyranosidic (1→6)-β-D-glucopyranans in moderate yields. These results are the first reports of the polymerization of 3-*O*-branched 1, 6-anhydro glucopyranose disaccharide monomers to give 3-*O*-branched polysaccharides.

2.1. INTRODUCTION

Ring-opening polymerization of anhydrosugar monomers is a superior method to afford stereoregular polysaccharides with high molecular weights and define their structures [1]. The resulting polysaccharides become good biomaterials for investigation of the relationship between the structure and biological activity, because

natural polysaccharides have complex structures, in general, and it is difficult to know the relationship [2].

Several reports appeared on the ring-opening polymerization of anhydro disaccharide monomers that were exclusively 4-*O*-branched 1, 6-anhydro gluco and mannopyranose monomers [3-5]. We have reported on the synthesis of stereoregular polysaccharides by the ring-opening polymerization of anhydrosugar monomers [6]. Among them, monosaccharide-branched polysaccharides were also synthesized by the ring-opening polymerization of several anhydro disaccharide monomers. 1, 6- Anhydro-2, 3-di-*O*-benzyl-4-*O*-(2', 3', 4',   6'-tetra-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranose (benzylated 1,6-anhydro lactose (LSHBE)), was synthesized and polymerized by pentafluoro phosphate (PF$_5$) as a catalyst to give a stereoregular (1→ 6)-β-D-lactopyranan with $\overline{M}_n$ =7.3x10^3 in good yield after debenzylation [7]. The copolymerization of LSHBE with benzylated 1, 6-anhydro-β-D-glucopyranose (LGTBE) in several feeds gave the corresponding copolysaccharides, (1→6)-β-D-glucopyranosidic main chain having different proportions of lactose and glucose units, in good yields. After sulfation, sulfated (1→6)-β-D-lactopyranans and copoly(lactose and glucose)s units had potent anti-HIV and blood anticoagulant activities, activities that were found to increase with a decrease in the proportion of lactose units. Sulfated copolysaccharides with lactose and glucose units in the proportions of 15 and 85 mol% had the highest biological activities [7]. Recently, we also reported the synthesis of galactomannans   (4-*O*-galactopyranosidic   (1→6)-β-D-

mannopyranans) by the ring-opening polymerization of a disaccharide monomer, 1, 6-anhydro-2, 3-di-*O*-benzyl-4-*O*-(2', 3', 4', 6'-tetra-*O*-   benzyl-β-D-galactopyranosyl)-β-D-mannopyranose (LMGABE) [8]. Copolymerization with 1, 6-anhydro-2, 3, 4-tri-*O*-benzyl-β-D-mannopyranose (LGMBE) was also carried out to give corresponding stereoregular copolysaccharides consisting of a (1→6)-β-D- mannopyranosidic main chain with various proportions of galactose units. After sulfation, sulfated synthetic galactomannans had potent anti-HIV and blood anticoagulant activities, mechanisms that were elucidated by measuring SPR, DLS, and zeta potential with poly-L-lysine as a model peptide of the HIV surface protein. The sulfated synthetic galactomannans were found to have strong interactions with poly-L-lysine, suggesting that the anti-HIV activity of sulfated synthetic galactomannans is hypothetically due to the interaction of the negatively charged sulfated groups with the positively charged surface proteins of HIV.

The disaccharide monomers that were polymerized so far by ring-opening have been exclusively 4-*O*-branched glucopyranoses. In this paper, we report for the first time the ring-opening polymerization of 3-*O*-branched 1, 6-anhydro glucose disaccharide monomers by PF_5 as a catalyst at -60°C. The polymerizability of the disaccharide monomers was elucidated by comparison with those of 4-*O*-branched disaccharide monomers on the yields, molecular weights, and the proportion of disaccharide units in the main chain of the resulting copolysaccharides.

2.2. Experimental

2.2.1. Materials

1, 6-Anhydro-2, 4-di-*O*-benzyl-β-D-glucopyranose (LGDBE) **2** was synthesized by selective benzylation of 1, 6-anhydro-β-D-glucopyranose (LG) with benzyl bromide by using anhydrous barium hydroxide according to the method of our previous paper [9]. Dry methylene chloride was distilled under a vacuum below 10^{-5} mmHg using a high vacuum line and stored in a glass ampule under the same high vacuum. The amount of phosphorus pentachloride needed was divided in an ampule under the high vacuum and used directly under pressure. A Diaion SK 1B ion exchange resin was used for the neutralization after alkaline deacetylation.

2.2.3 Synthesis of 3-O-branched 1, 6-anhydro glucopyranose disaccharides

To a 2, 3, 4, 6-tetra-O-acety-D-glucopyranose [10] (5 g, 14.4 mmol) was added the dry dichloromethane (25 ml) under the nitrogen and after dissolving the trichloroacetonitrile (2.5 ml) and 6, 8-diazabicycloundecene (0.5 ml) were added then was stirred for 30 min at 0 °C , and then triethylamine was added to stop the reaction. After evaporation of dichloromethane below 25 °C, the sample was purified by a silica gel column chromatography with hexane：ethyl acetate (2:1) in the presence of triethylamine (0.5 vol-%). Yield, 67% (4.8 g).

2, 3, 4, 6-Tetra-*O*-acetyl-1-*O*-trichloroacetimidoyl-β-D-glucopyranose (2.7g, 5.45 mmol) and 1, 6-anhydro-2, 4-di-*O*-benzyl-β-D-glucopyranose **2** were dissolved in dry CH$_2$Cl$_2$ (16 mL) in the presence of molecular sieves (4A, 1.2g). Boron trifluoride etherate (BF$_3$OEt$_2$) (0.14 mL) was added dropwise to the mixture at -60°C. After the mixture was stirred for 20 min, the molecular sieves were removed by filtration and then chloroform (50 mL) was added to the clear filtrate. The filtrate was washed several times with 5% aqueous sodium hydrogen carbonate solution and water, and then dried over anhydrous sodium sulfate. After evaporation, the residue was purified by silica gel column chromatography with a hexane-ethyl acetate (2:1 vol) mixed solvent to give 1, 6- anhydro-2, 4-di-*O*-benzyl-3-*O*-(2', 3', 4', 6'-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranose (1.34 g) in 49.0% yield.

For deacetylation, 1, 6-anhydro-2, 4-di-*O*-benzyl-3-*O*-(2', 3', 4', 6'-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranose (1.34 g) in methanol (10 mL) was stirred with sodium methoxide (40.2 mg, 0.75 mmol) at room temperature. After 4 h, to the mixture was added an ion exchange resin (H$^+$) to neutralize the solution. After filtration of the ion exchange resin, the filtrate was evaporated to give 1, 6-anhydro-2, 4-di-*O*-benzyl-3-*O*-(β-D-glucopyranosyl)-β-D-glucopyranose (0.9 g, 1.8 mmol) in 91.4% yield, which was directly used for the next reaction. 1, 6-anhydro-2, 4-di-*O*-benzyl-3-*O*-(β-D-glucopyranosyl)- β-D-glucopyranose (0.9 g, 1.8 mmol) in dry DMF (5 mL) solution was added dropwise to dry DMF (15 mL) solution with NaH (0.36 g, 15 mmol) and the mixture was stirred for 0.5 h at

room temperature. Benzyl bromide (1.64 mL, 14.4 mmol) was added dropwise to the mixture. After stirring for 3 h at 0°C, the mixture was evaporated to give 1, 6-anhydro-2, 4-di-*O*-benzyl-3-*O*-(2', 3', 4', 6'-tetra-*O*-benzyl-β-D-glucopyranosyl)-β-D-glucopyranose (LGG) **7** (1.30 g) in 84.2% yield after silica gel column chromatography with a hexane-ethyl acetate (3:1 vol) mixed solvent. The specific rotation and elementary analysis were $[\alpha]_D^{25}$=+16.4° (c1, CHCl$_3$) and found: C, 74.89%; H, 6.43% (calcd for C$_{54}$H$_{56}$O$_{10}$: C, 74.98%; H, 6.52%).

Another disaccharide monomers, 1, 6-anhydro-2, 4-di-*O*-benzyl-3-*O*-(2', 3', 4', 6'- tetra-*O*-benzyl-β-D-mannopyranosyl)-β-D-glucopyranoses **6** were prepared by the same method as above from 1, 6- anhydro-2, 4-di-*O*-benzyl-β-D-glucopyranose **2** and 2, 3, 4, 6-tetra-*O*-acetyl-1-*O*- trichloroacetimidoyl-β-D-mannnopyranose **3** in 44.0% yields from **2**. The specific rotation at 25°C and elemental analysis were $[\alpha]_D^{25}$=+27.6° (c1, CHCl$_3$) and found: C, 74.88%; H, 6.47% (calcd for C$_{54}$H$_{56}$O$_{10}$: C, 74.98%; H, 6.52%) for **6**.

2.2.4. Ring-opening polymerization and copolymerization of 3-O-branched 1, 6-anhydro glucopyranose monomers

A typical procedure for the ring-opening copolymerization of the disaccharides monomers is as follows (No. 3 in Table 1). The polymerization was carried out under high vacuum condition below 10^{-5} mmHg at -60°C. The disaccharide monomer LGG (**7**) (0.13 g) and LGTBE (0.07 g) were measured in a glass

polymerization ampoule and then dry CH_2Cl_2 (0.4 mL) was added under pressure reduced below 10^{-5} mmHg to dissolve the monomers. After the polymerization ampoule had been cooled in a liquid N_2 Dewar vessel,
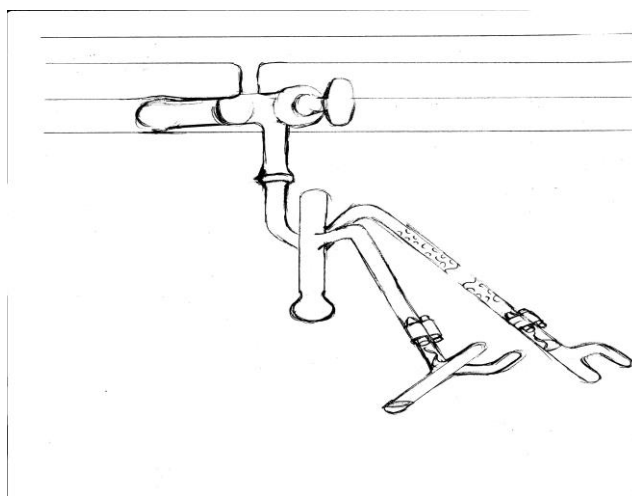
Figure 2.5. Glassware of ring-opening polymerization method

PF_5 (20 mol% to the monomers) was transferred to the polymerization ampoule at the same temperature. The polymerization ampoule was maintained with gentle stirring for 24 h at -60°C and then the polymerization was terminated by addition of a small amount of MeOH to produce a white precipitate of the corresponding copolymer. The copolymer was purified by reprecipitation using $CHCl_3$-MeOH mixed solvent several times to remove unpolymerized monomers and then freeze-dried from benzene to give the copolymer as a white precipitate in 29.5% yield. The copolymer had the molecular weight of $\overline{M}_n = 8.4 \times 10^3$ and specific rotation of $[\alpha]_D^{25} = +74.1°$ (c1, $CHCl_3$). The proportion of the disaccharide unit was 15.3 mol% according to the ^{13}C NMR measurement. Other polymerization and copolymerization were performed by the same procedures as above. The results are shown in Tables 1 and 2.

2.2.5. Debenzylation of benzylated 3-O-branched polymers

Copoly(LGG and LGTBE) (0.30 g) with $\overline{M}_n=4.2 \times 10^3$ and the proportion of LGG unit of 10.0 mol% dissolved in 3 mL of dry CH_2Cl_2 was added dropwise to a solution of Na (0.5 g) in liquid NH_3 (50 mL) at -78°C, and the mixture was stirred for 1 h at -78°C under N_2 atmosphere. After addition of small amounts of NH_4Cl and MeOH, liquid NH_3 was evaporated at room temperature. The residue was washed with ethyl ether and then dissolved in water (30 mL). The aqueous solution was dialyzed with water for 2 d. After filtration, the filtered water was concentrated to 10 mL and then freeze-dried to give 3-*O*-β-D-glucopyranosyl (1→6)-β-D-glucopyranan (0.05 g) in 40.6% yield. The molecular weight was $\overline{M}_n=4.4 \times 10^3$ and the proportion of LGG unit was 7.8 mol%.

3-*O*-β-D-Mannopyranosyl (1-6)-β-D-glucopyranan (0.05 g) was also obtained from copoly(LGM and LGTBE) (0.16 g) by the same method as described above. The results are shown in Table 3.

2.3. Measurements

Elemental analysis of monomers was performed by a CE-440 elemental analyzer and a Mettler TOLEDO XS3DU electronic microbalance. Specific rotations were measured on a JASCO DIP-140 digital polarimeter with a 1 dm cell in chloroform or water at 25°C and a concentration of 1 mg/mL. FT-IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer by a KBr pellet method. ^{1}H and ^{13}C NMR spectra were obtained by a JEOL JMN AEC-600 spectrometer at 25°C or 40°C in $CDCl_3$, DMSO-d_6, or D_2O solvent with tetramethylsilane as an internal standard. The

monomers were purified by an organic phase HPLC system with an RI-8020 detector using a TSK-gel column (Silica-60, 21.5 mmx300 mm) eluted with hexane-ethyl acetate mixed solvent at 40°C. Molecular weights of copolymers or copolysaccharides were estimated by an organic or aqueous phase GPC system using organic phase TSK-gel columns (G2500PW$_{XL}$, G4000PW$_{XL}$, and G5000PW$_{XL}$, 7.6 mmx300 mmx3) eluted with chloroform or aqueous phase columns (G2500H$_{XL}$, G4000H$_{XL}$, and G5000H$_{XL}$ 7.6 mmx300 mmx3) eluted with 66.7 mmol of phosphate buffer at pH=6.68) at 40°C. Polystyrene (Shodex standard SM-105) or dextran (Shodex standard P-82) was used as a molecular weight reference.

2.4. Results and Discussion

2.4.1. Synthesis of new 3-O-branched 1, 6-anhydro glucopyranose disaccharide monomers

The ring-opening polymerization of 4-*O*-branched 1, 6-anhydro disaccharide monomers has been reported to give branched polysaccharides with stereoregular (1→6) - main chain having a monosaccharide branch in each sugar unit. So far, all of the 1, 6-anhydro disaccharide monomers without 1, 4-anhydro ribopyranose monomers were 4-*O*-branched 1, 6-anhdro gluco and mannopyranoses, monomers that should have lower steric hindrance at the 4-*O* position in 1, 6- anhydro glucopyranose for the ring-opening polymerization than that of the 3-*O*-branched 1, 6-anhydro hexopyranose monomers due to the distance between the

C1 and C4 positions. In this work, new 3-*O*-branched 1, 6-anhydro glucopyranose monomers were synthesized for the first time and the polymerizability was compared with that of the 4-*O*-branched hexopyranose monomers previously reported.

Scheme 1 shows the synthesis and ring-opening polymerization of 3-*O*-branched 1, 6-anhydro
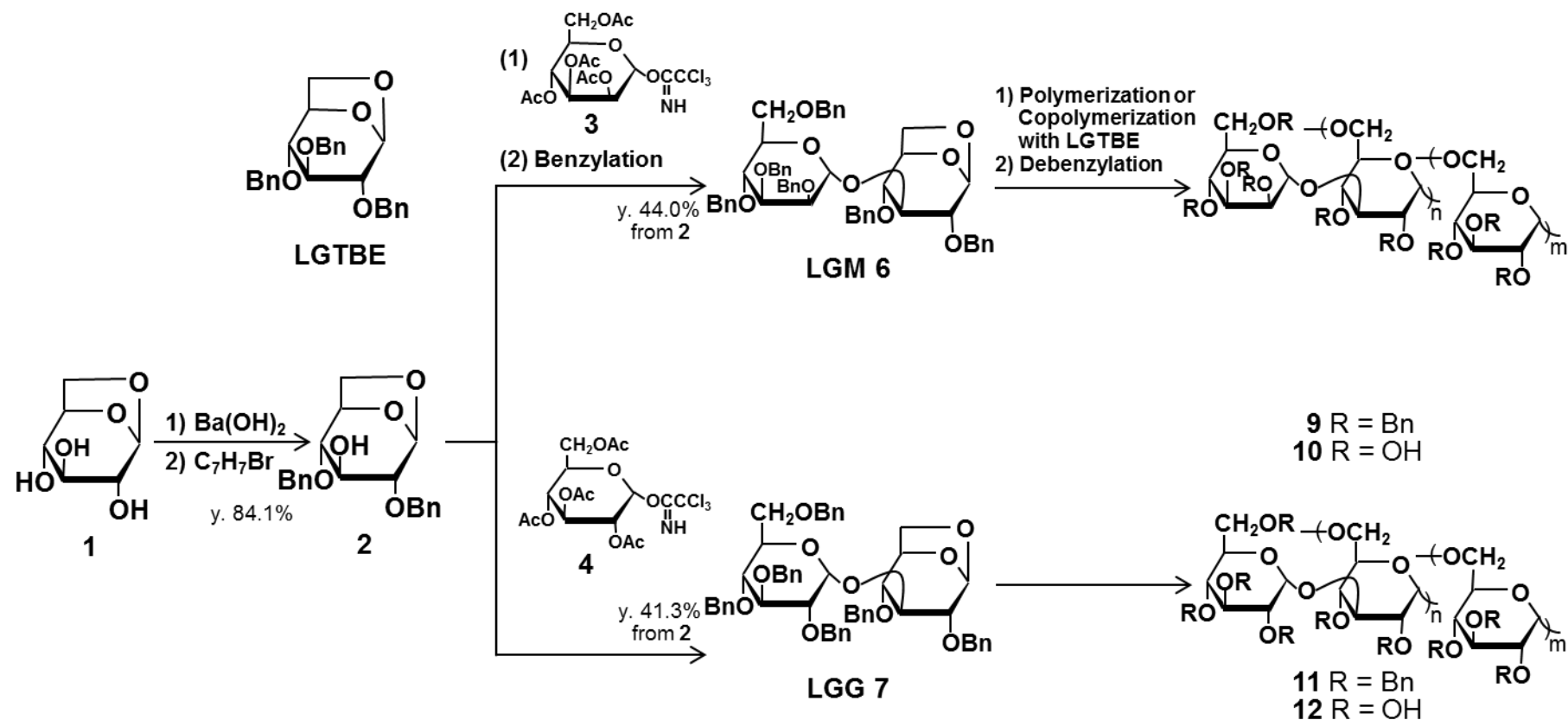
**Scheme 1** Synthesis and ring-opening polymerization of 3-*O*-branched 1,6-anhydro glucose monomers **6** and **7**. The polymerization was carried out with PF_5 as a catalyst at -60°C under high vacuum condition.

glucopyranose monomers. 1, 6-Anhydro-2, 4-di-*O*-benzyl-3-*O*-(2', 3', 4', 6'-tetra-*O*-benzyl-β-D- mannopyranosyl)- β-D-glucopyranose (LGM) (**6**) was prepared by the glycosylation of the 3-*O* position of 1, 6-anhydro-2, 4-di-*O*-benzyl-β-D-glucopyranose **2** with 2, 3, 4, 6-tetra-*O*-acetyl-1-*O*- trichloroacetimidoyl-β-D-mannopyranose **3**, a viscous liquid, in 44.0% yield after deacetylation and subsequent benzylation. In the same manner, other 3-*O*-branched disaccharide and trisaccharide monomers, 1, 6- anhydro-2, 4-di-*O*-benzyl-3-*O*-(2', 3', 4', 6'-tetra-*O*-benzyl-β-D-glucopyranosyl)- β-D-glucopyranose (LGG) (**7**) and 1, 6-anhydro-2, 4-di-*O*-benzyl-3-*O*-(2', 3', 6', 2", 3", 4", 6"- hepta-*O*-benzyl-β-D-maltosyl)- β-D-glucopyranose (LGMAL) (**8**), were synthesized by the glycosylation of **2** with the glucose **4** and maltose imidates **5**, respectively, in 41.3% and 35.7% yields. Before polymerization, the 3-*O*-branched disaccharide and trisaccharide monomers were highly purified by using HPLC. The elemental analysis of the monomers was in good agreement with the calculated ones. The monomers with low purity were not polymerized.

Figure 1 shows the ^{13}C NMR spectra of the monomers. All carbon signals were assigned by the combination of H-H COSY and HMQC 2D NMR measurements. The C1 signals due to 1, 6-anhydro glucopyranose appeared between 100 and 102 ppm, respectively. The C1' signal of the 3-*O*-branched mannose and glucose were absorbed at 98.0 ppm Figure 1A), 103.5 ppm (Figure 1B), respectively. The C1' signal due to the branched mannose in Figure 1A appeared at the relatively higher magnetic field of 98.0 ppm than that of the branched glucose in Figures 1B. The specific rotation of the monomers at 25°C showed positive and moderate values, $[\alpha]_D^{25}$=+27.6° and +16.4°, (c1, CHCl$_3$), moderate and positive values that were attributed to the □-configuration of the 1, 6-anhydro glucopyranose and □-configuration of the branched glucose and mannose moieties. The specific rotation of the monosaccharide monomer, 1, 6-anhydro-2, 3, 4-tri-*O*-benzyl-β-D-glucopyranose (LGTBE), was a large negative

value, $[\alpha]_D^{25}=-30.8°$ (c2.7, CHCl$_3$) due to the configuration [11].
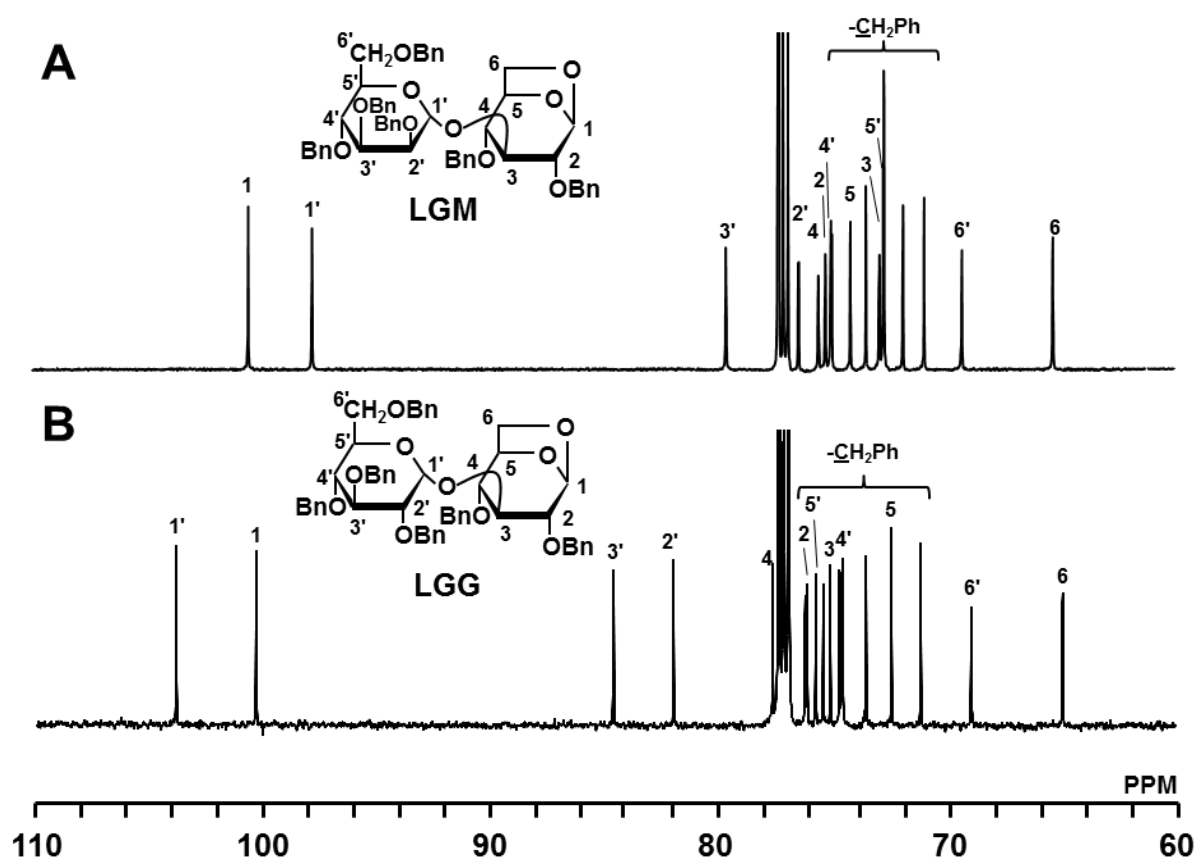
Figure 1. 150 MHz ^{13}C NMR Spectra of 3-O-branched monomers.
(A) 1, 6-Anhydro-2, 4-di-O-benzyl-3-O-(2', 3', 4', 6'-tetra-O-benzyl-α-D-mannopyranosyl)-β-D-glucopyranose (LGM), (B) 1, 6-anhydro-2, 4-di-O-benzyl-3-O-(2', 3', 4', 6'-tetra-O-benzyl-α-D-glucopyranosyl)-β-D-glucopyranose (LGG) in CDCl$_3$ at 25°C.
The specific rotation at 25 C was (A) $[\alpha]_D^{25}$ =+27.6°, (B) +16.4°, respectively. All signals were assigned by H-H COSY and HMQC spectra.

2.4.2. Ring-opening polymerization of 3-O-branched 1, 6-anhydro glucopyranose monomers

These 3-O-branched monomers were highly purified by using a preparative

HPLC and then polymerized by PF_5 as a catalyst under high vacuum condition for 24 h at -60°C. A relatively higher quantity of PF_5 catalyst (20 mol% to the feeds) was used with reference to previous results [7]. Table 1 shows the results for the polymerization of the 3-*O*-branched disaccharide monomer,

Table 1
Ring-opening polymerization and copolymerization of 1, 6-anhydro-2, 4-di-*O*-benzyl-3-*O*-(2', 3', 4', 6'-tetra-*O*-benzyl-α-D-glucopyranosyl)-β-D-glucopyranose (LGG) with 1, 6-anhydro-2, 3, 4-tri-*O*-benzyl-β-D-glucopyranose (LGTBE)[a]

No	Monomer				Polymer			
	LGG		LGTBE		Yield	$[\alpha]_D^{25}$ [b]	$\overline{M}_n$ [c]	LGG
	g	mol%	g	mol%	%	deg	$\times 10^3$	unit in polymer[d] mol%
1	0		0.20	100	79.5	+114.1	13.1	0
2	0.08	25	0.12	75	64.8	+75.9	2.6	0.4
3	0.13	50	0.07	50	29.5	+74.1	8.4	15.3
4	0.17	75	0.03	25	29.6	+66.0	4.8	8.4
5	0.20	100	0	0	12.8	+50.0	1.8	-[e]

a Total monomer weight: 0.2 g, Solvent: CH_2Cl_2; 0.4 ml, Catalyst: PF_5; 20 mol% to feed, Temperature: -60°C, Polymerization time: 24 h.
b Measured in $CHCl_3$ (c1) at 25°C..
c Determined by chloroform GPC.
d Calculated from ^{13}C NMR spectrum.
e The proportion could not be measured due to low yield of the polym

1, 6-anhydro-2, 4-di-*O*-benzyl-3-*O*-(2', 3', 4', 6'-tetra-*O*-benzyl-β-D-glucopyranosyl)- β-D- glucopyranose (LGG) (**7**). It was found that the monomer was polymerized to give the corresponding polymer, but the yield was low. Therefore, copolymerization with the LGTBE monomer was performed in several monomer feeds. The copolymerization of LGG (**7**) with LGTBE in the monomer feeds of 25:75, 50:50, and 75:25 mol% gave the corresponding copolymers in low to moderate yields, 29.5-64.8% yields, respectively, and the yields increased with increasing proportions of LGTBE monomer as shown in Nos. 2-4 in Table 1. In No. 3, although the copolymerization of LGG with LGTBE in the feed of 50:50 mol% afforded the corresponding polymer in 29.5% yield, and proportion of the LGG unit in the

copolymer was 15.3 mol%, which was the highest proportion in the copolymerization of the LGG and LGTBE monomers. These copolymerization results suggest that the 3-*O*-branched 1, 6- anhydro glucopyranose disaccharide monomer gave the corresponding copolymers. However, the disaccharide monomers had lower polymerizability than the 4-*O*-branched disaccharide monomers.

The 3-*O*-Mannopyranosyl 1, 6-anhydro glucopyranose monomer LMG **6** was also copolymerized with LGTBE by the same polymerization conditions as the LGG monomer **7**. The results are represented in Table 2. The LGM monomer also had low polymerizability (No. 4 in Table 2).

Table 2
Ring-opening polymerization and copolymerization of 1, 6-anhydro-2, 4-di-*O*-benzyl-3-*O*-(2', 3', 4', 6'-tetra-*O*-benzyl-α-D-mannopyranosyl)-β-D-glucopyranose (LGM) with 1, 6-anhydro-2, 3, 4-tri-*O*-benzyl-β-D-glucopyranose (LGTBE)[a]

No	Monomer				Polymer			
	LGM		LGTBE		Yield	$[\alpha]_D^{25}$[b]	$\overline{M}_n$[c]	LGM
	g	mol%	g	mol%	%	deg	x10^3	unit in polymer[d] mol%
1	0.08	25	0.12	75	68.5	+86.8	5.7	1.71
2	0.13	50	0.07	50	52.4	+97.4	49.7	16.1
3	0.17	75	0.03	25	51.4	+60.1	4.2	6.8

a Total monomer weight: 0.2 g, Solvent: CH$_2$Cl$_2$; 0.4 ml, Catalyst: PF$_5$; 20 mol% to feed, Temperature: -60°C, Polymerization time: 24 h.
b Measured in CHCl$_3$ (c1) at 25°C..
c Determined by chloroform GPC.
d Calculated from ^{13}C NMR spectrum.

As shown in No. 2, the copolymerization of LGM with LGTBE in the feed of 50:50 mol% was found to give the corresponding polymer in 52.4% yield. The molecular weight was relatively high, $\overline{M}_n$=49.7x10^3, and the proportion of the LGM disaccharide unit in the copolymer was 16.1 mol%. Both disaccharide monomers LGG and LGM in the feed of 50 mol% gave the highest proportion of the disaccharide unit in the copolymer. The reason why the higher

proportion was exclusively obtained when the disaccharide unit was used in 50 mol% feed is under consideration. The yield of the copoly(LGM and LGTBE) increased with increasing feed of the LGTBE monomer having high ring-opening polymerizability [12], and the proportion of the disaccharide unit in the copolymers was lower than that of the feed.

We reported previously that the 4-*O*-branched 1, 6-anhydro hexopyranose monomers, benzylated 1, 6-anhydro-β-D-lactopyranose and benzylated 4-*O*-galactopyranosyl 1, 6-anhydro mannopyranose, had high polymerizability to give the corresponding homopolymers in 68.5% and 46.7% yields, respectively [7.8].The copolymerization with LGTBE gave the corresponding copolymers in 78.0% and 93.5% yields, respectively. The proportion of the disaccharide units in the copolymers was almost the same as that in the feeds. Therefore, the 4-*O*-branched 1, 6-anhydro hexopyranose monomers had higher polymerizability than the 3-*O*-branched 1, 6-anhydro hexopyranose monomers. The low polymerizability of the 3-*O*-branched 1, 6-anhydro glucopyranose monomers was affected by the steric hindrance of the bulky 3-*O*-branched sugar moiety compared with that of the 4-*O*-branched disaccharide monomers. In addition, the distance between a C-C single bond is 0.154 nm [13.14] and the distance between the C1 and C3 positions in the 1, 6-anhydro glucopyranose (0.308 nm) was shorter than that between the C1 and C4 positions (0.462 nm). Therefore, the steric hindrance of the sugar branch at the C3 position was relatively higher than that of the C4 position.

Figure 2 shows the ^{13}C NMR spectra of the copolymers (A)-(C) consisting of LGG and LGTBE Figure 2 units in the proportion of (A) 8.4:91.6 mol%, (B) 15.3:84.7 mol%, and (C) 0.4:99.6 mol%, respectively, and (D) the homopolymer, 2, 4, 6-tri-*O*-benzyl-(1→ 6)- β-D-glucopyranan. All signals were assigned by the combination of H-H COSY and HMQC measurements. In Figure 2B, the carbon signals due to the LGTBE unit appeared as large intense signals. The C1 and C6

signals due to 3-*O*-branched glucopyranose units were absorbed as small signals at 97.0 ppm and 69.2 ppm for the C1 and C6', and 102.8 ppm and 65.8 ppm for the C1" and C6" signals, respectively. The ^{13}C NMR spectra of copoly(LGM and LGTBE) are presented in Figure 3, in which the C1" and C1' Figure 3 signals due to the LGM unit consisting of mannose and glucose appeared at 99.2 ppm and 97.5 ppm as overlapped signal of the C1 signal due to the LGTBE unit, respectively. Taking into account higher and positive specific rotations, copoly(LGG and LGTBE)s and copoly(LGM and LGTBE)s had a

(1-6)-β-D-glucopyranosidic main chain with β-D-glucopyranose and β-D-mannopyranose branches at the C3 position in the main chain, respectively. From the NMR spectra, it was found that both disaccharide monomers were copolymerized with LGTBE to give the corresponding (1→6)-β-D-glucopyranosidic stereoregular copolymers.
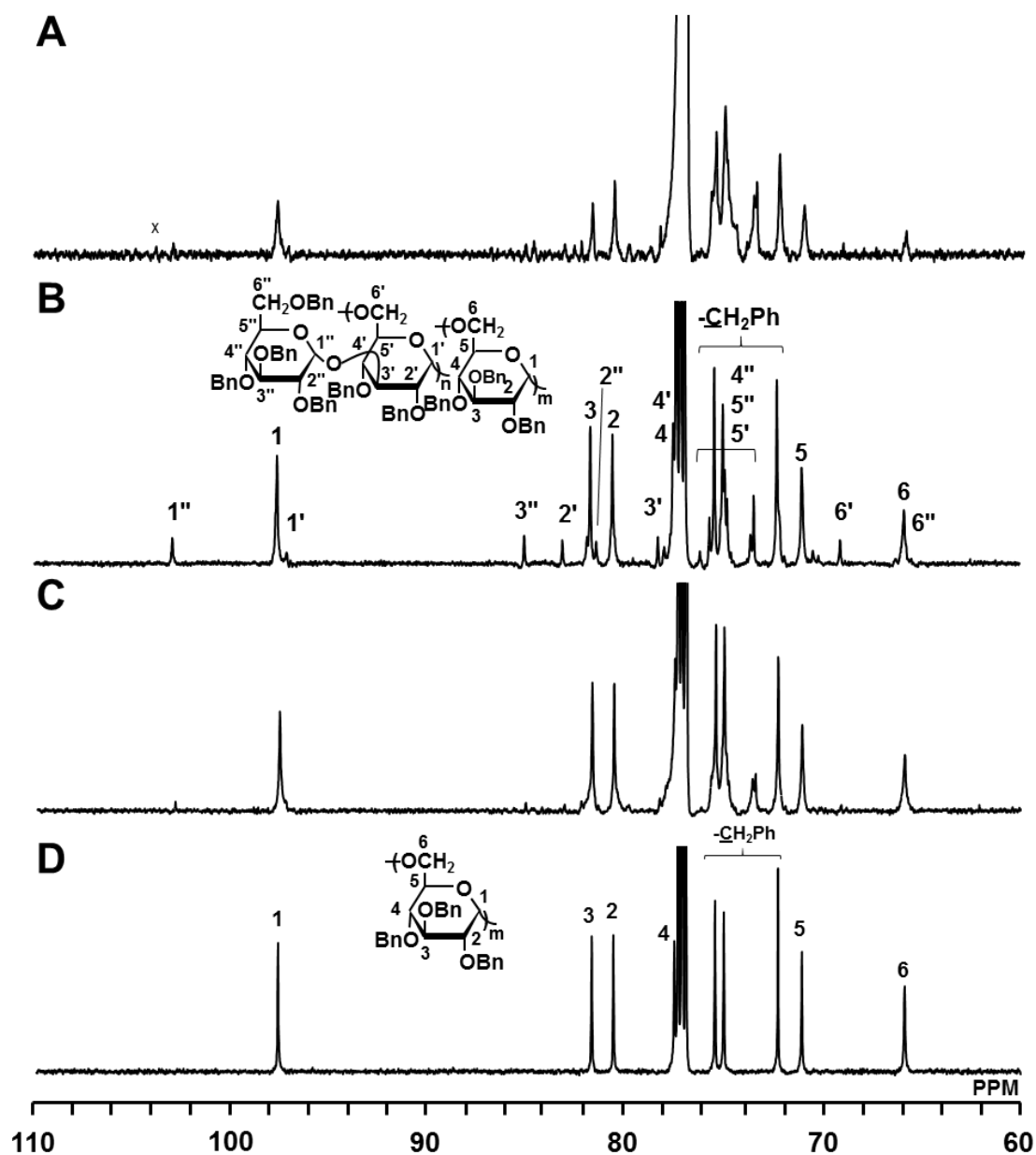
FIGURE 2 150 MHz ^{13}C NMR Spectra of (A)-(C) copoly(LGG and LGTBE)s and (D) poly(LGTBE) in CDCl$_3$ at 25 °C. The proportions of disaccharide units in the copolymers were (A) 8.4 mol%, (B) 15.3 mol%, and (C) 0.4 mol%, respectively. The specific rotation at 25 °C was (A) $[\alpha]_D^{25}$ =+66.0°, (B) +74.1°, (C) +75.9°, and (D) +114.1° (c1, CHCl$_3$), respectively. Signals were assigned by H-H COSY and HMQC spectra.
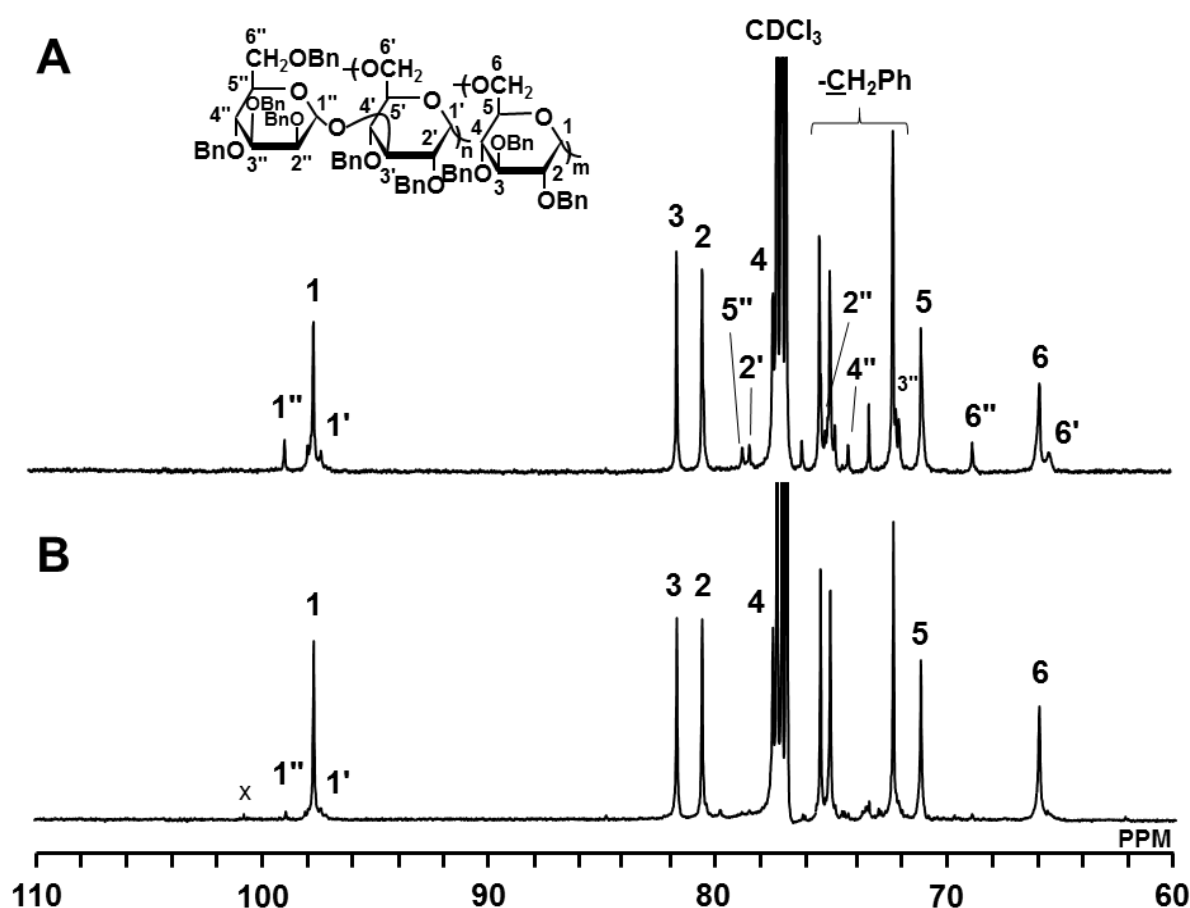
FIGURE 3 150 MHz ^{13}C NMR Spectra of copoly(LGM and LGTBE)s in $CDCl_3$ at 25 °C. The proportions of the disaccharide units were (A) 16.1 mol% and (B) 1.7 mol%, respectively. The specific rotation at 25 °C was (A) $[\alpha]_D^{25}=+97.4^\circ$ and (B) $+86.8^\circ$ (c1, $CHCl_3$).

2.4.3. Debenzylation to 3-O-gluco and mannopyranosyl glucopyranans

The copolymers were debenzylated with Na in liquid ammonia at -78°C to give free copolysaccharides after dialysis with water and then freeze-drying. The dialyzed membrane with a molecular weight elimination limit of 3500 Da in water was used because the molecular weight of the copolymers was low. Table 3 shows the results of debenzylation of copoly(LGG and LGTBE) Table 3 with $\overline{M}_n=4.2\times10^3$, $[\alpha]_D^{25}=+78.1^\circ$ (c1, $CHCl_3$), with the proportion of LGG unit of 10.0 mol%, and copoly(LGM and LGTBE) with $\overline{M}_n=15.5\times10^3$, $[\alpha]_D^{25}=+74.2^\circ$ (c1, $CHCl_3$), with the proportion of LGM unit of 10.3 mol%, respectively. For

copoly(LGG and LGTBE), the debenzylated copolysaccharide with $\overline{M}_n$=4.4x10^3 was obtained in 40.6% yield. The proportion of the disaccharide unit in the copolysaccharide was 7.8 mol%. Copoly(LGM and LGTBE) gave the corresponding copolysaccharide with $\overline{M}_n$=6.1x10^3 in 50.5% yield. The disaccharide unit was 7.1 mol% in the copolysaccharide. The specific rotation of both copolysaccharides increased to high positive values, +132.3° and +134.2° from +78.1° and +74.2° before debenzylation, respectively, suggesting that the copolysaccharides had (1-6)- β-D- glucopyranosidic stereoregularity in the main chain with β-D-glucopyranose and β-D-mannopyranose branches.

Figure 4 shows the ^{13}C NMR spectra of (A) 3-*O*-glucopyranosyl and (B) 3-*O*-mannopyranosyl (1→6)-β-D-glucopyranans with the branched sugar proportions of 7.8 mol% and 7.1 mol%, respectively. After debenzylation, the signals due to benzyl groups disappeared. It was found that the C1 signals due to main chain glucopyranose without 3-*O*-branched sugar units appeared at 100.5 ppm as sharp and single signals, indicating that the copolysaccharides had highly (1→ 6)- β-D- glucopyranosidic stereoregularity. The C1' signals due to the main chain glucopyranose unit with 3-*O*-gluco and mannopyranose branches appeared as overlapped signals at 100.5 ppm, respectively, and the C1" signals of the 3-*O*-branched gluco and mannopyranose were absorbed at 105.6 ppm and 104.0 ppm as small and singlet signals, respectively. The C6" signals appeared around 63.5 ppm as several small absorptions, respectively, probably due to the effect of the random copolymerization of the disaccharide and LGTBE units. The FT-IR spectra before and after debenzylation of copoly(LGG and LGTBE) are shown in Figure 5. Before debenzylation in Figure 5A, several small Figure 5 and large intense signals due to benzyl groups appeared between 1700-2000 cm^{-1}, around 1500 cm^{-1}, and at 750 cm^{-1}. After debenzylation to recover hydroxyl groups in Figure 5B, a large absorption due to hydroxyl groups appeared at 3400 cm^{-1} and the signals due to benzyl groups

disappeared. In addition the medium absorption at 1650 cm^{-1} due to a bending vibration of water appeared.

Table 3 Debenzylation of copoly(LGG and LGTBE) and copoly(LGM and LGTBE)[a]

No		Benzylated copolymer[b]					Free copolysaccharide				
		Proportion[c]			$\overline{M}_n$[d]	$[\alpha]_D^{25}$[e]		Yield	$\overline{M}_n$[f]	Disaccharide unit in main chain[c]	$[\alpha]_D^{25}$[g]
		LGG	LGM	LGTBE							
	g		mol%		x10^3	deg	g	%	x10^3	mol%	deg
1	0.30	10.0	-	90.0	4.2	+78.1	0.05	40.6	4.4	7.8	+132.3
2	0.16	-	10.3	89.7	15.5	+74.2	0.03	50.5	6.1	7.1	+134.2

a Condition: Na; 0.5 g, Solvent: Liq. NH$_3$; 50 mL, Temperature: -78°C, Time: 60 min.
b LGG: 1, 6-anhydro-2, 4-di-*O*-benzyl-3-*O*-(2', 3', 4', 6'-tetra-*O*-benzyl-α-D-glucopyranosyl)-β-D-glucopyranose,
   LGM: 1, 6-anhydro-2, 4-di-*O*-benzyl-3-*O*-(2', 3', 4', 6'-tetra-*O*-benzyl-α-D-mannopyranosyl)-β-D-glucopyranose,
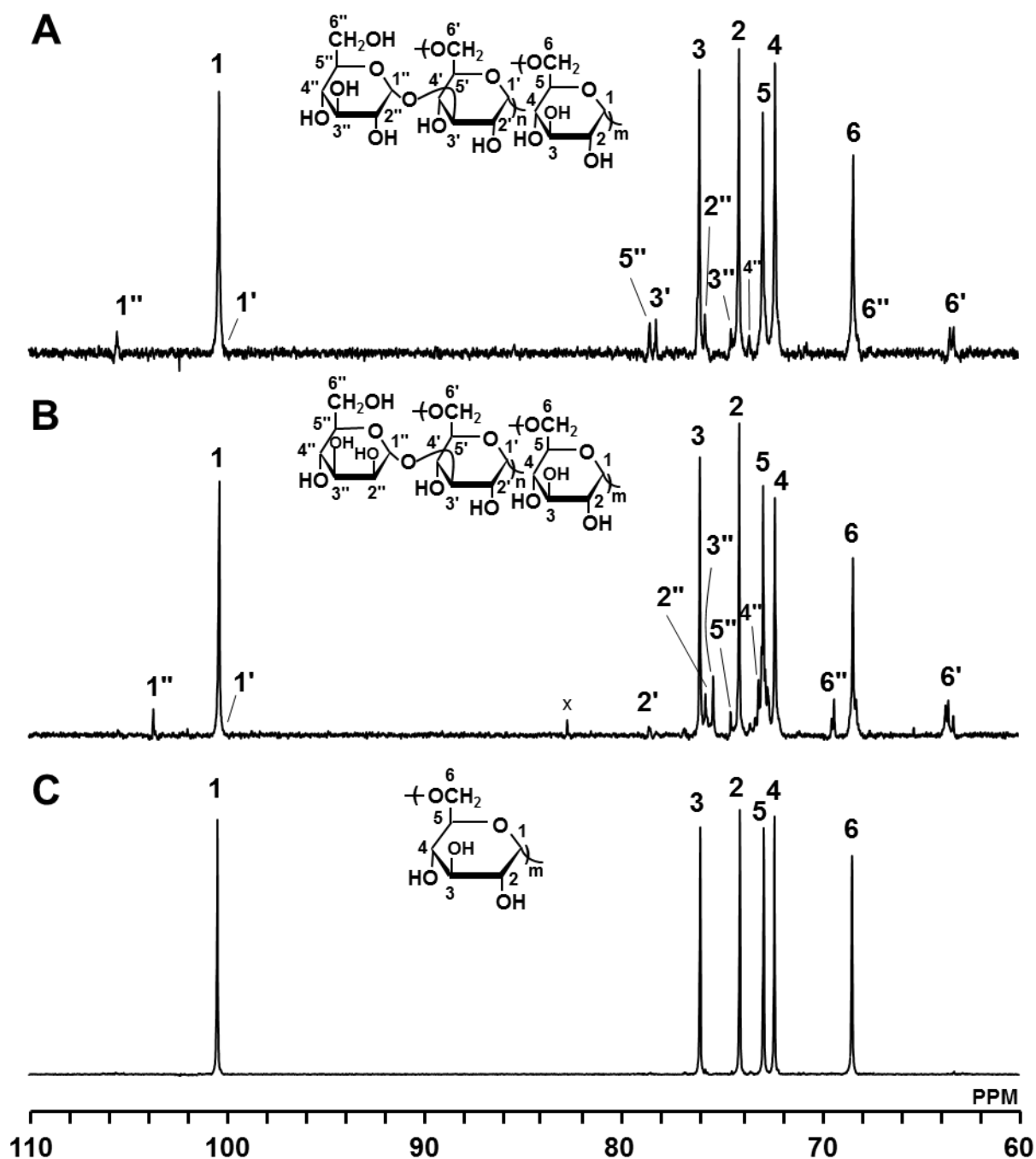   LGTBE: 1, 6-anhydro-2, 3, 4-tri-*O*-benzyl-β-D-glucopyranose.
c Calculated from the intensity of the C1 signal of the ^{13}C NMR spectrum (mol%).
d Determined by chloroform GPC.
e Measured in CDCl$_3$ (c1) at 25°C.
f Determined by aqueous GPC.
g Measured in H$_2$O (c1) at 25°C.

FIGURE 4 150 MHz ^{13}C NMR Spectra of debenzylated polymers.
(A) Copoly(LGG and LGTBE), (B) copoly(LGM and LGTBE), (C) poly(LGTBE) in D$_2$O
at 40 °C. The specific rotation at 25 °C was (A) $[\alpha]_D^{25}$ = +132.3°, (B) +134.2°,
and (C) +139.6°, respectively. The proportions of the disaccharide units were (A) 7.8
mol% and (B) 7.1 mol%, respectively. Signals were assigned by H-H COSY and
HMQC spectra.
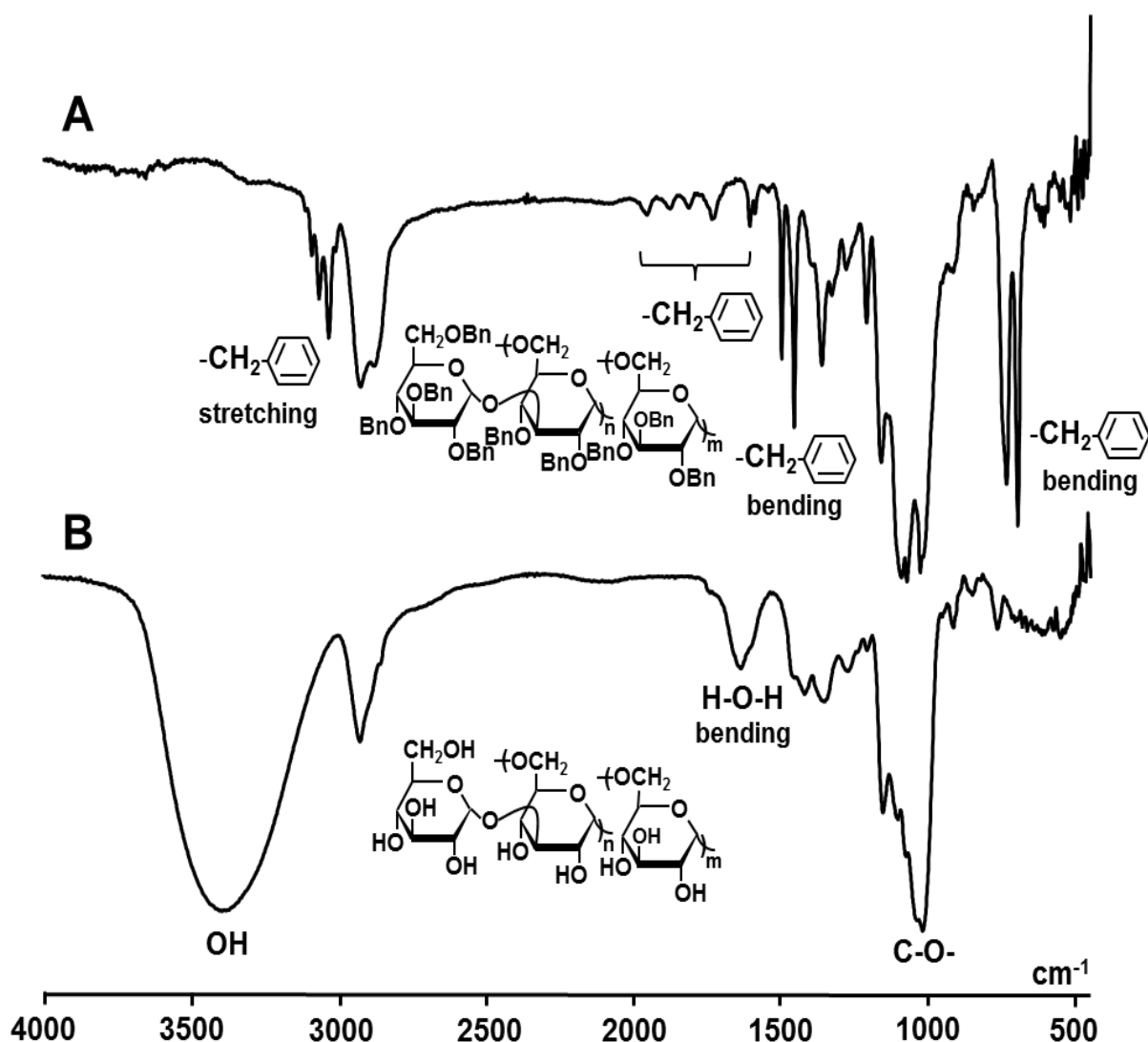
Figure 5. FT-IR spectra (A) before and (B) after debenzylation of copoly(LGG and LGTBE) measured by a KBr method.
The proportions of disaccharide units were (A) 10.0 mol% and (B) 7.8 mol%, respectively.
The specific rotations at 25 °C was (A)$[\alpha]_D^{25}$=+78.1° (c1, CHCl$_3$) and (B) +132.3° (c1, H$_2$O), respectively.

2.5. CONCLUSION

Two new 3-*O*-branched 1, 6-anhdro glucopyranose disaccharide monomers, 1, 6-anhydro-2, 4-di-*O*-benzyl-3-*O*-(2', 3', 4', 6'-tetra-*O*-benzyl-β-D-glucopyranosyl)- and -mannopyranosyl)- β-D- glucopyranose (LGG and LGM),

and a trisaccharide monomer, were synthesized and the ring-opening polymerizability and copolymerizability with 1, 6-anhydro-2, 3, 4- tri-*O*-benzyl-β-D-glucopyranose (LGTBE) were examined for the first time by using PF_5 as a catalyst at -60°C. Although the homopolymerizability of the disaccharide monomers was low, the copolymerizations with LGTBE were found to give the corresponding copolysaccharides, 3-*O*-glucopyranosyl and -mannopyranosyl (1→6)-β-D-glucopyranans, respectively, after deprotection to recover hydroxyl groups. The polymerization yields of the 3-*O*-branched disaccharide monomers were not very high, 12.8-68.5%, but the monomer feeds of 50:50 mol% gave higher molecular weights of $\overline{M}_n$=8.4x10^3 for copoly(LGG and LGTBE) and $\overline{M}_n$ =49.7x10^3 for copoly(LGM and LGTBE), respectively, The proportions of the disaccharide monomer units in the corresponding copolymers were 15.3 mol% and 16.1 mol%, respectively. This is the first report on the ring-opening polymerization of 3-*O*-branched disaccharide monomers. The polymerization of 3-*O*-branched disaccharide monomers was further investigated by examining the polymerization conditions of catalysts, solvents, and temperatures, and the resulting 3-*O*- glucopyranosyl and -mannopyranosyl (1→6)-β -D-glucopyranans were sulfated to give sulfated 3-*O*-branched glucopyranans. In addition, the relationship between structure and antiviral activities is investigated by comparison with that of sulfated 4-*O*-branched glucopyranans.

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CHAPTER 3.

Synthetic Trisaccharide

ABSTRACT

New 3-*O*-branched 1, 6-anhydro glucopyranose trisaccharide monomer, 1, 6-anhydro-2, 4-di-*O*-benzyl-3-*O*-□-(2', 3', 6', 2", 3", 4", 6"-hepta-*O*-benzyl-β-D-maltopyranosyl)- β-D-glucopyranose (LGMAL **8**), New 4-*O*-branched 1, 6-anhydro mannopyranose trisaccharide monomer, 1, 6-anhydro-2, 4-di-*O*-benzyl-4-*O*-□-(2', 3', 6', 2", 3", 4", 6"-hepta-*O*-benzyl-β-D-maltopyranosyl)- β-D-mannopyranose (LMMAL **9**) were synthesized and polymerized. It was found that the trisaccharide monomer LGMAL was not polymerizable, and trisaccharide monomer LMMAL was polymerizable, probably due to the steric hindrance of the branched bulky mono and disaccharide units at the 3-*O* position in 1, 6-anhydro glucopyranose.

3.1. INTRODUCTION

Previously, we reported that a new ribo-disaccharide monomer, 1, 4-anhydro-2-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-β-D-ribopyranose (A2B3GalR), gave a galactose-branched (1- 5)-β-D-ribofuranan with high molecular weights in good yields [1]. The high polymerizability of the 1, 4-anhydro ribose monomer should originate from a high deformation of the anhydro ribose ring. Copolymerization with a 1, 4-anhydro-2, 3-di-*O*-benzyl-β-D-ribopyranose (ADBR) monomer gave galactopyranose-branched ribofuranans with various proportions of galactopyranose branches. Until now, all of the 1, 6-anhydro disaccharide monomers were 1, 6- anhydro hexopyranoses with branches at the 4-*O*-position without a 1, 4-anhydro ribo disaccharide monomer.

For the synthesis and ring-opening polymerization of an anhydro ribo trisaccharide monomer, 1, 4- anhydro-2-*O*-benzyl-3-*O*-(2', 3', 6', 2", 3", 4", 6"-

hepta-*O*-benzyl-β-D-lactosyl)- β-D-ribopyranose (A2B3LR) synthesized from 1, 4-anhydro ribose and D-lactose, was reported and the 1, 4-anhydro ribose trisaccharide monomer (A2B3LR) was expected to have high polymerizability due to a highly polymerized 1, 4-anhydro ribose unit. We found that the A2B3LR monomer was polymerized by BF3OEt2 as a catalyst to give lactose-branched (1→ 5) - β-D-ribofuranan with high molecular weights [2]. This is the first report so far of the ring-opening polymerization of anhydro trisaccharide monomer.

In this paper, we report for the first time the ring-opening polymerization of 3-*O*-branched 1, 6-anhydro glucose trisaccharide monomers and 4-*O*-branched 1, 6-anhydro manno trisaccharide monomers by PF_5 as a catalyst at -60°C. The polymerizability of the trisaccharide monomers was elucidated by comparison with those of 4-*O*-branched trisaccharide monomers on the yields, molecular weights, and the proportion of trisaccharide units in the main chain of the resulting copolysaccharides.

3.2. Experimental

3.2.1. Materials

Dry methylene chloride was distilled under a vacuum below 10^{-5} mmHg using a high vacuum line and stored in a glass ampule under the same high vacuum. The amount of phosphorus pentachloride needed was divided in an ampule under the high vacuum and used directly under pressure. A Diaion SK 1B ion exchange resin was used for the neutralization after alkaline deacetylation.

3.2.2 Synthesis of 3-O-branched 1, 6-anhydro glucopyranose trisaccharides

The peracetylated maltose (5g, 7.4mmol) was dissolved in 16ml DMF and

heated to 55°C with oil bath. And then hydrazine acetate (1.53g, 1.7mmol) was added to the solution. The resulting mixture was stirred for 30min after that checked with TLC. The solution was added ethyl acetate, and washed with water, saturated aqueous $NaHCO_3$ solution, and brine three times respectively and then dried over $MgSO_4$. After evaporation, the residue was purified by a silica chromatography with hexane-ethyl acetate (1:2) to get yield, 53.3% (2.5g).

To a solution of 1-hydroxy-hepta-O-acetyl maltose (1.8g, 2.8mmol) dissolved with dry dichloromethane (9 ml) was added trichloroacetonitrile (0.9 ml) and 6, 8-diazabicycloundecene (0.18 ml) at 0°C with ice bath,which was stirred for 30min after that was checked TLC. And then triethlamine (0.9 ml) was added to stop the reaction. After evaporation of dichloromethane below 20°C, the residue was purified by a silica chromatography with hexane-ethyl acetate (2:1 v/v) in the presence of triethlamine (0.5vol-%) as eluent. Yield, 68.4% (1.5g)

Peracetylated 1-O-trichloroacetimidoyl-α-D-maltose (3.5 g, 4.5mmol) in the dry CH_2Cl_2 (25ml) was added to LGDBE (0.77g, 2.2mmol) in the presence of powdery molecular sieves 4Å (1.71g). The mixture was stirred for 15min at the room temperature. The mixture was cooled to -50℃, and the $BF_3 \cdot OEt_2$ (0.15ml) was added. After the mixture was stirred for a further 1.5h, and then a small amount of $NaHCO_3$ was added to neutralize it. After filtration, the filtrate was washed with saturated $NaHCO_3$ and water several times, dried on anhydrous sodium sulfate, and concentrated. The residue was chromatographed on silica gel eluted with hexane/ethyl acetate (1:2 v/v) to give the acetylated trisaccharide (1.5g) in 42% yield.

For the deacetylation of the acetylated trisaccharide, a small amount of MeONa was added to a MeOH (17ml) solution of the acetylated trisaccharide (1.5g). The mixture was stirred for 1h at room temperature and then an ion exchange resin (H⁺) was added to neutralize the solution. After filtration of the

ion exchange resin, the filtrate was evaporated to give a trisaccharide with free hydroxyl groups (0.92g) in 66% yield.

The free trisaccharide in dry DMF (5ml) was added dropwise to a solution of NaH (0.5g, 21mmol) in DMF (5ml). After stirring for 1h, benzyl bromide in the DMF (5ml) was added dropwise and then allowed to react overnight with stirring at 40 ℃. Methanol was added to deactivate NaH. After removal of DMF and excess benzyl bromide by evaporation under reduced pressure, the residue was extracted with CHCl₃. The CHCl₃ solution was washed with water several times, and then concentrated. The residue was purified by silica gel column chromatography eluted with hexane/ethyl acetate (2:1) to give LGMNBE (1.5g, 1.2mmol) in 85% yield as a colorless oil. For the polymerization, the resulting LGMAL was further purified by using HPLC on silica gel column. The specific rotation was $[\alpha]_D^{25}$=+52.3° (c1, CHCl₃) and found: C, 74.86%; H, 6.47% (calcd for $C_{81}H_{84}O_{15}$: C, 74.98%; H, 6.52%) for **8**.

Another trisaccharide monomers, 4-*O*-branched 1, 6-anhydro mannopyranose trisaccharide monomer, 1, 6-anhydro-2, 4-di-*O*-benzyl-4-*O*-□-(2', 3', 6', 2", 3", 4", 6"-hepta-*O*-benzyl-β-D-maltopyranosyl)- β-D-mannopyranose (LMMAL **9**) was prepared by the same method as above in 41.2% yields from **2**. The specific rotation at 25°C and elemental analysis were $[\alpha]_D^{25}$=+25.4° (c1, CHCl₃) and found: C, 74.87%; H, 6.43% (calcd for $C_{54}H_{56}O_{10}$: C, 74.98%; H, 6.52%).

3.2.3 Ring-opening polymerization of 3-O-branched 1, 6-anhydro glucopyranose trisaccharide monomer

A typical procedure for the ring-opening copolymerization of the trisaccharide monomers is as follows. The polymerization was carried out under high vacuum condition below 10^{-5} mmHg at -60°C. The trisaccharide monomer and LGTBE were measured in a glass polymerization ampoule and

then dry CH_2Cl_2 was added under pressure reduced below 10^{-5} mmHg to dissolve the monomers. After the polymerization ampoule had been cooled in a liquid N_2 Dewar vessel, PF_5 (20 mol% to the monomers) was transferred to the polymerization ampoule at the same temperature. The polymerization ampoule was maintained with gentle stirring for 24 h at -60°C and then the polymerization was terminated by addition of a small amount of MeOH to produce a white precipitate of the corresponding copolymer. The copolymer was purified by reprecipitation using $CHCl_3$-MeOH mixed solvent several times to remove unpolymerized monomers and then freeze-dried from benzene to give the polymer.

3.2.4 Measurements

Elemental analysis of monomers was performed by a CE-440 elemental analyzer and a Mettler TOLEDO XS3DU electronic microbalance. Specific rotations were measured on a JASCO DIP-140 digital polarimeter with a 1 dm cell in chloroform or water at 25°C and a concentration of 1 mg/mL. ^1H and ^{13}C NMR spectra were obtained by a JEOL JMN AEC-600 spectrometer at 25°C or 40°C in $CDCl_3$, DMSO-d_6, or D_2O solvent with tetramethylsilane as an internal standard. The monomers were purified by an organic phase HPLC system with an RI-8020 detector using a TSK-gel column (Silica-60, 21.5 mmx300 mm) eluted with hexane-ethyl acetate mixed solvent at 40°C.

3.3. Results and Discussion

3.3.1. Synthesis of new 3-O-branched 1, 6-anhydro glucopyranose trisaccharide monomers and 4-O-branched 1, 6-anhydro mannopyranose monomers

3-*O*-branched trisaccharide monomers, 1, 6-anhydro-2, 4-di-*O*-benzyl-3-*O*-(2', 3', 6', 2", 3", 4", 6"- hepta-*O*-benzyl-β-D-maltosyl) - β-D-glucopyranose

(LGMAL) (**8**), were synthesized by the glycosylation of **2** with the maltose imidates **5**, in 35.7% yields (Scheme 2). Before polymerization, the 3-*O*-branched trisaccharide monomers were highly purified by using HPLC. The elemental analysis of the monomers was in good agreement with the calculated ones. The monomers with low purity were not polymerized.
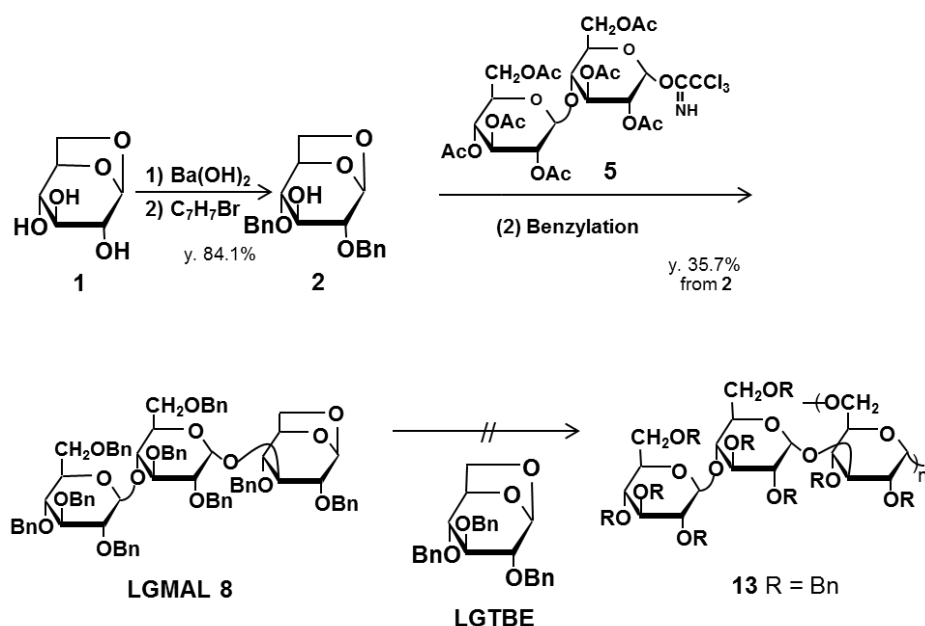
Scheme 2 Synthesis and ring-opening polymerization of 3-*O*-branched 1,6-anhydro glucose monomers **8**. The polymerization was carried out with PF_5 as a catalyst at -60°C under high vacuum condition.

Figure 6 shows the ^{13}C NMR spectra of the monomers. All carbon signals were assigned by the combination of H-H COSY and HMQC 2D NMR measurements. The C1 signals due to 1, 6-anhydro glucopyranose appeared between 100 and 102 ppm, respectively. The C1' signal of the 3-*O*-branched maltose was absorbed at 98.0 ppm 103.3 ppm (Figure 1C), respectively. On the trisaccharide monomer in Figure 1C, the C1" signal due to the terminal glucose moiety was found to appear at the higher magnetic field of 96.5 ppm. Thespecific rotation of the monomers at 25°C showed positive and moderate values, $[\alpha]_D^{25} = +52.3°$ (c1, CHCl$_3$), moderate and positive values that were attributed to the □-configuration of the 1, 6-anhydro glucopyranose and □-

configuration of the branched maltose moieties. The specific rotation of the monosaccharide monomer, 1, 6-anhydro-2, 3, 4-tri-*O*-benzyl-β-D-glucopyranose (LGTBE), was a large negative value, $[\alpha]_D^{25}=-30.8°$ (c2.7, CHCl$_3$) due to the configuration [3]. The FT-IR spectra LGMAL is shown in Figure 7. In the Figure, several small and large intense signals due to benzyl groups appeared between 1700-2000 cm^{-1}, around 1500 cm^{-1}, and at 750 cm^{-1}.
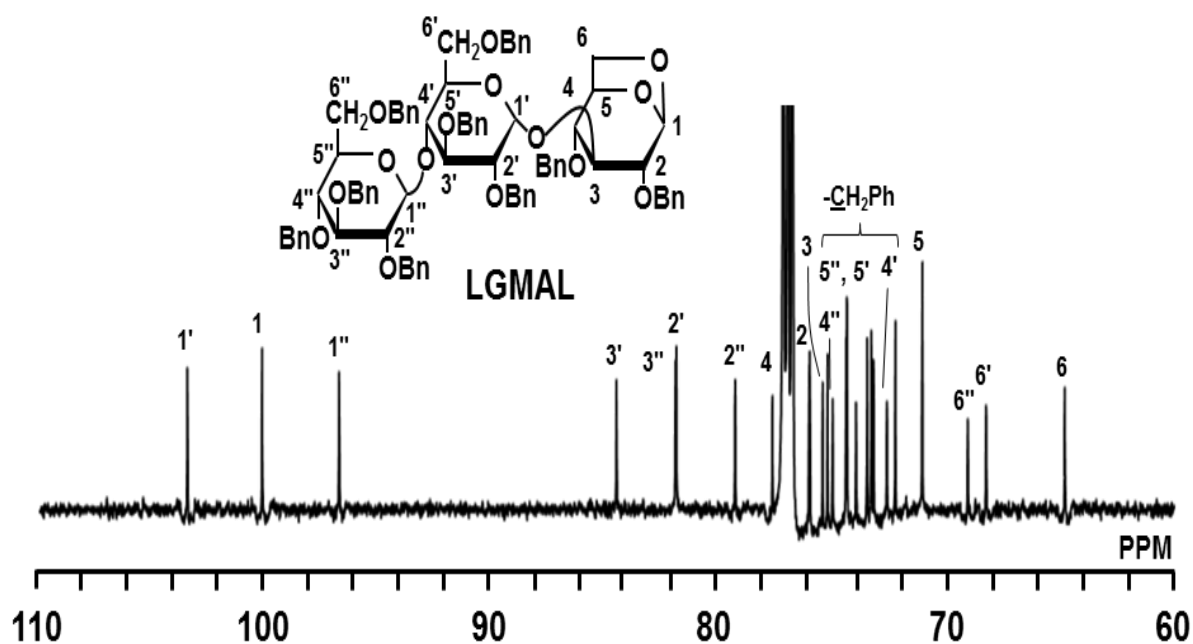
Figure 6. 150 MHz ^{13}C NMR Spectra of 3-*O*-branched monomers.
1, 6-anhydro-2, 4-di-O-benzyl-3-O-(2', 3', 4', 6'-tetra-O-benzyl-α-D-maltosyl)-β-D-glucopyranose (LGMAL) in CDCl$_3$ at 25 °C. The specific rotation at 25 C was (C)$[\alpha]_D^{25}$ = +52.3°(c1,CHCl$_3$), respectively. All signals were assigned by H-H COSY and HMQC spectra.
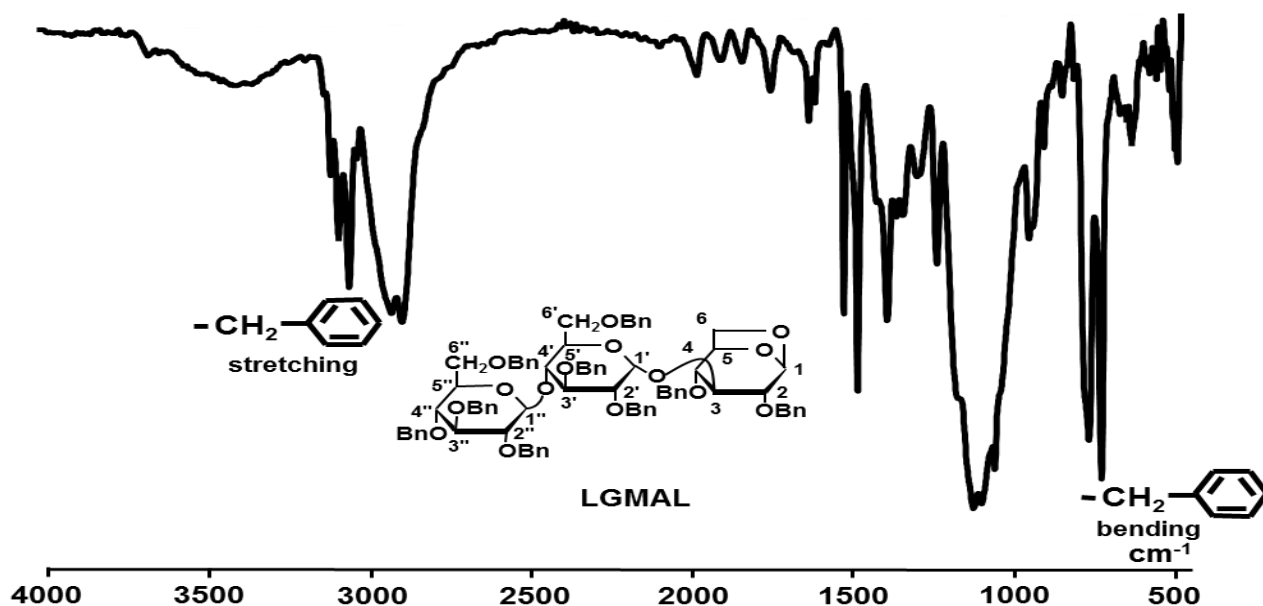
Figure 7. FT-IR spectra 1, 6-anhydro-2, 4-di-*O*-benzyl-3-*O*-(2', 3', 4', 6'-tetra-*O*-benzyl-α-D-maltosyl)-β-D-glucopyranose (LGMAL) measured by a KBr method.
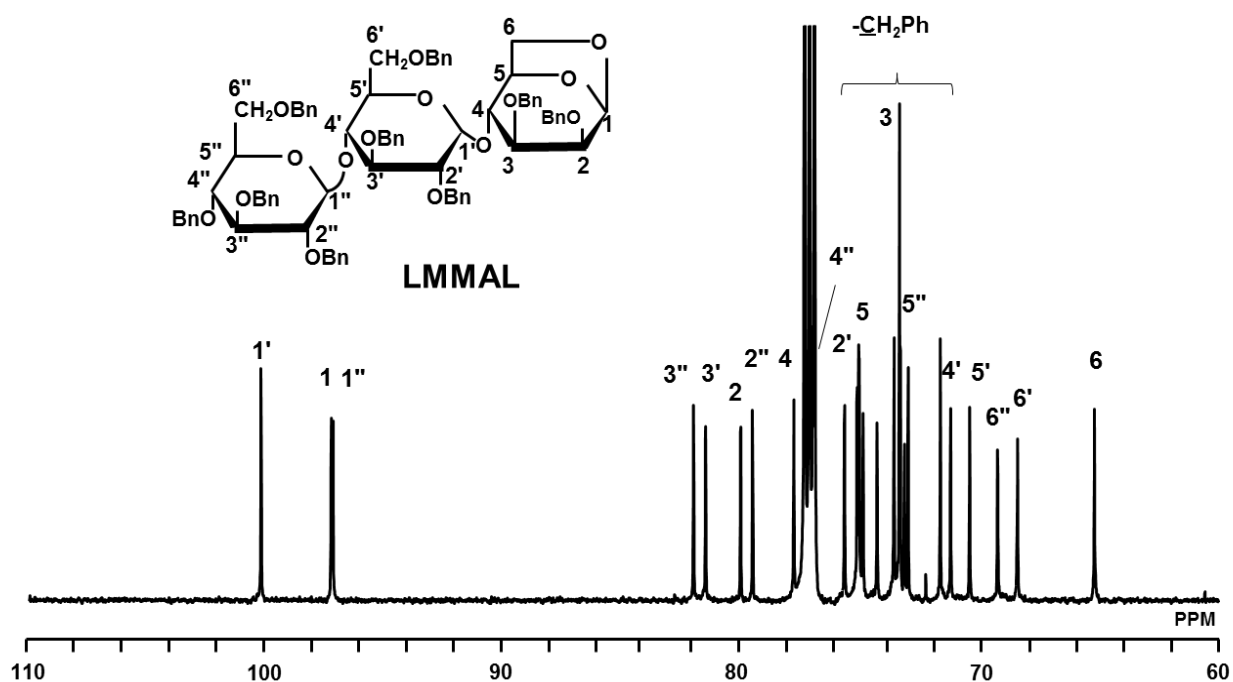The specific rotations at 25°C was $[\alpha]_D^{25}$ =+52.3°(c1, CHCl₃)

4-*O*-branched trisaccharide monomers, 1, 6-anhydro-2, 4-di-*O*-benzyl-4-*O*-(2', 3', 6', 2'', 3'', 4'', 6''- hepta-*O*-benzyl-β-D-maltosyl) - β-D-mannopyranose (LMMAL), was synthesized by the glycosylation of **2** with the maltose imidates **5**, in 39.8% yields (Scheme 3). Before polymerization, the 4-*O*-branched trisaccharide monomers were highly purified by using HPLC. The elemental analysis of the monomers was in good agreement with the calculated ones. The monomers with low purity were not polymerized

Figure 7 shows the ¹³C NMR spectra of the monomers. All carbon signals were assigned by the combination of H-H COSY and HMQC 2D NMR measurements. The C1 signals due to 1, 6-anhydro mannocopyranose appeared between 96.0 and 98.0 ppm, respectively. The C1' signal of the 4-*O*-branched maltose was absorbed at 100.2 ppm (Figure 1C), respectively. On the trisaccharide monomer in Figure 1C, the C1'' signal due to the terminal glucose moiety was found to appear at the higher magnetic field of 96.6 ppm. The

specific rotation of the monomers at 25°C showed positive and moderatevalues, $[\alpha]_D^{25}$=+25.4° (c1, CHCl$_3$), moderate and positive values that were attributed to the □-configuration of the 1, 6-anhydro mannopyranose and □-configuration of the branched maltose moieties. The FT-IR spectra LMMAL is shown in Figure 8. In the Figure, several small and large intense signals due to benzyl groups appeared between 1700-2000 cm^{-1}, around 1500 cm^{-1}, and at 750 cm^{-1}.
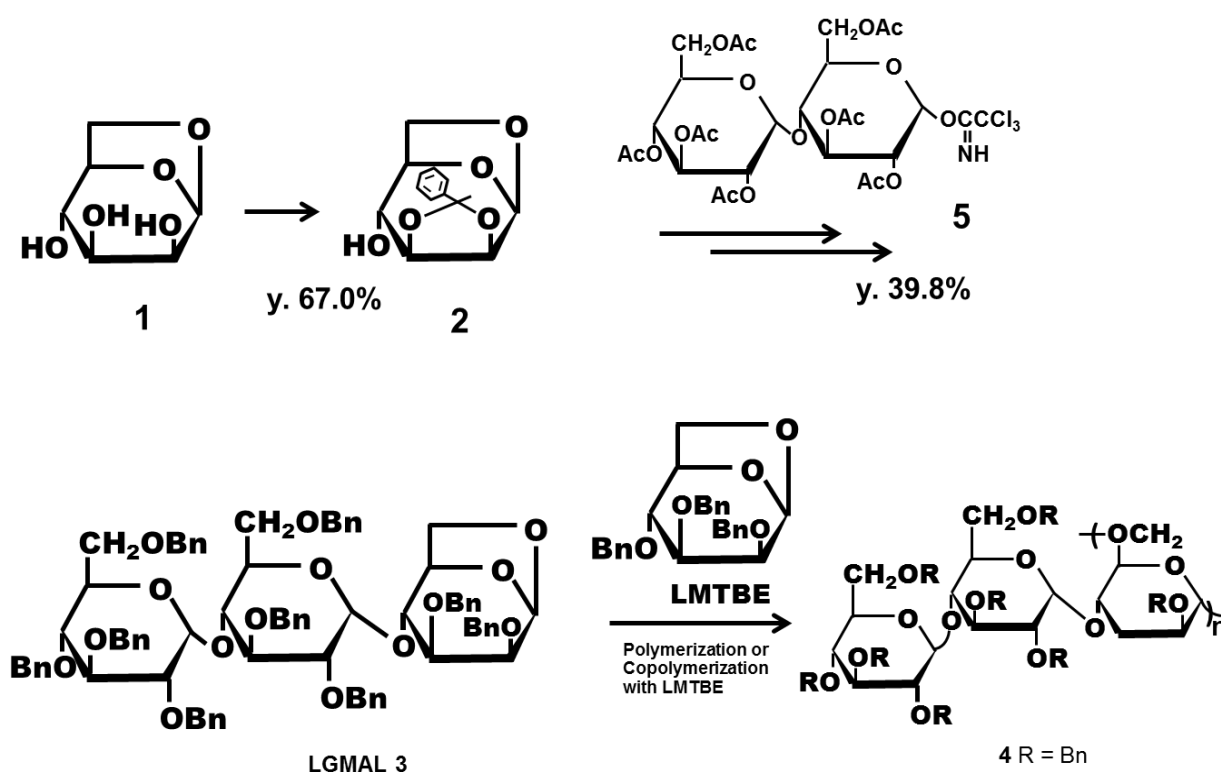
Scheme 3 Synthesis and ring-opening polymerization of 4-O-branched 1,6-anhydro mannose monomers **9**. The polymerization was carried out with PF$_5$ as a catalyst at -60°C under high vacuum condition.

Figure 7. 150 MHz ^{13}C NMR Spectra of 4-*O*-branched monomers.
1, 6-anhydro-2, 4-di-*O*-benzyl-4-*O*-(2', 3', 4', 6'-tetra-*O*-benzyl-α-D-maltosyl)-β-D-mannocopyranose (LMMAL) in CDCl$_3$ at 25 °C. The specific rotation at 25 °C was (C) $[α]_D^{25}$ = +25.4°(c1,CHCl$_3$), respectively. All signals were assigned by H-H COSY and HMQC spectra.
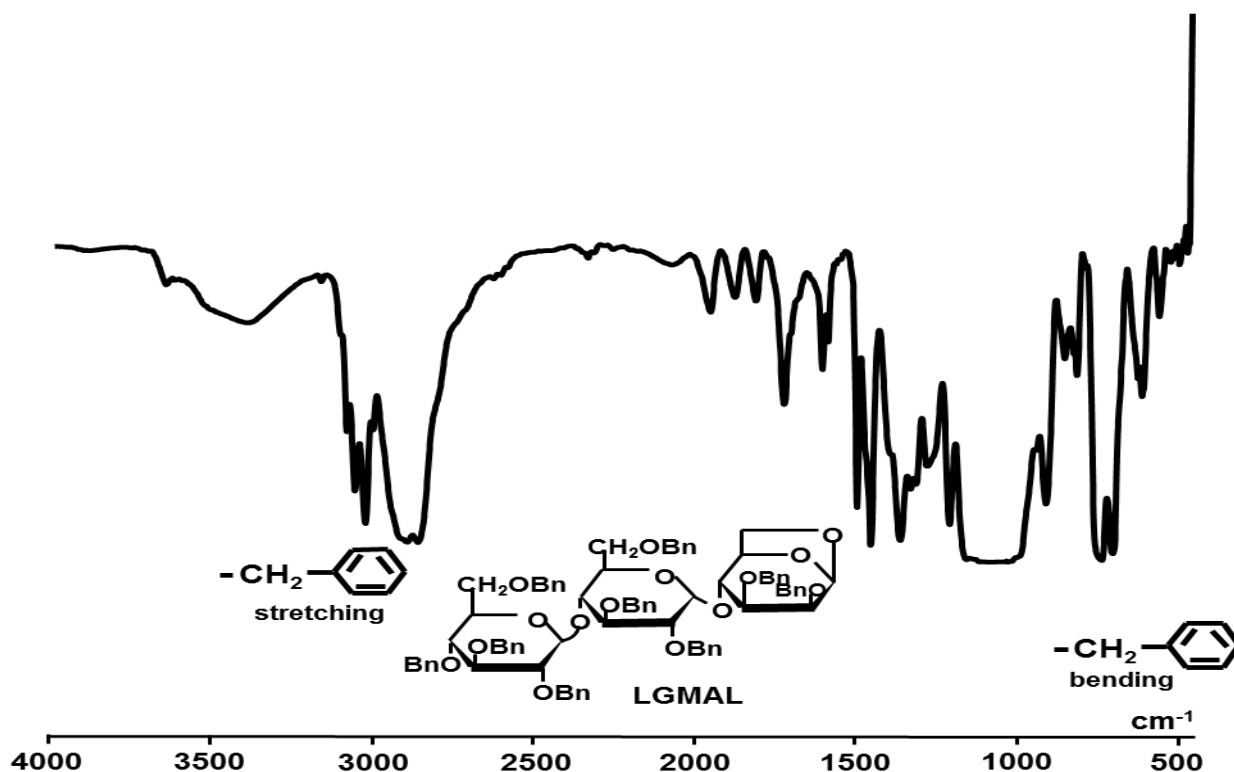
Figure 7. FT-IR spectra 1, 6-anhydro-2, 4-di-*O*-benzyl-4-*O*-(2', 3', 4', 6'-tetra-*O*-benzyl-α-D-maltosyl)-β-D-mannocopyranose (LMMAL) measured by a KBr method.
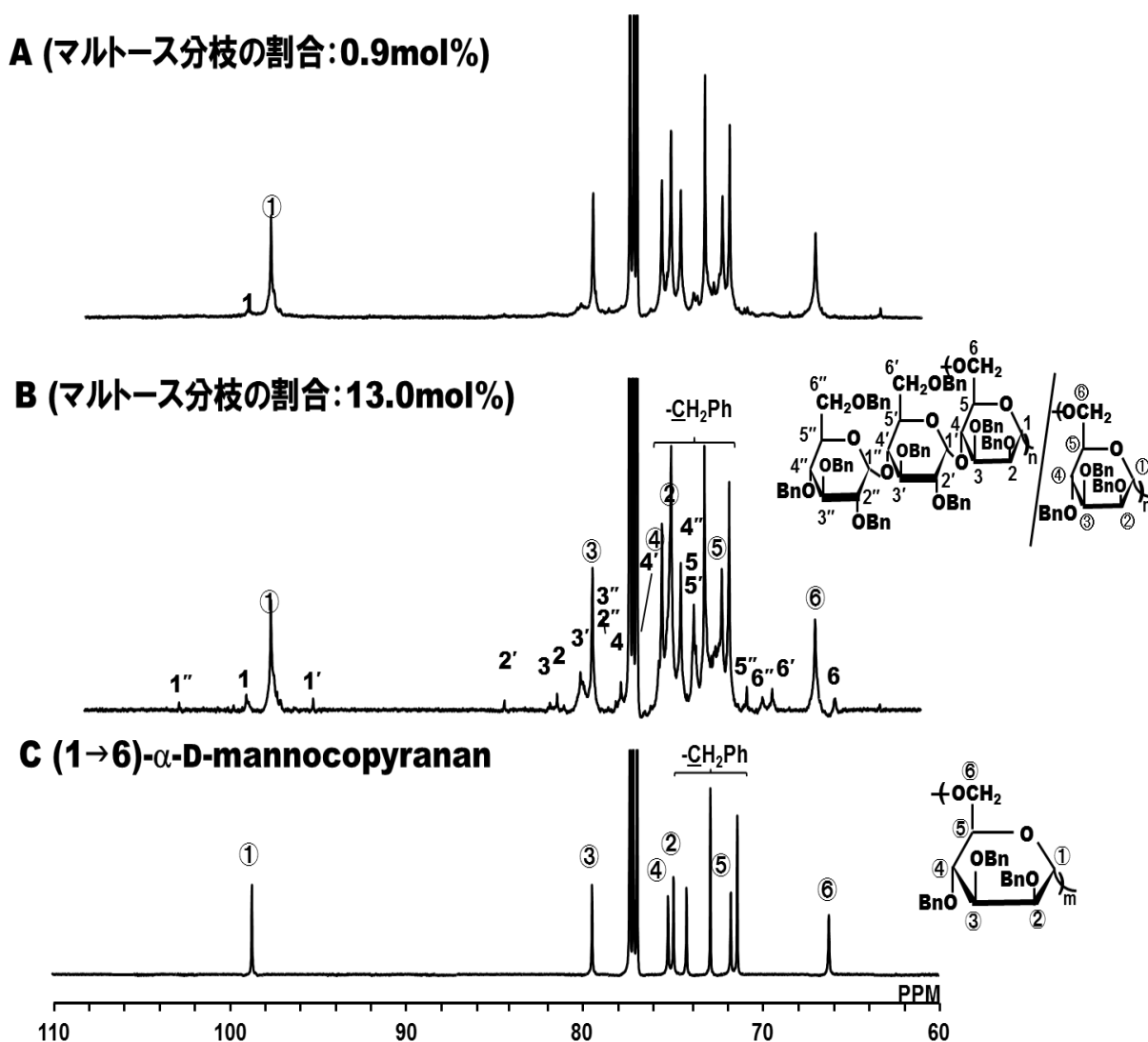The specific rotations at 25°C was $[α]_D^{25}$ =+25.4°(c1, CHCl$_3$)

- 14 -

3.3.2. Ring-opening polymerization of 3-O-branched 1, 6-anhydro glucopyranose monomers and 4-O-branched 1, 6-anhydro mannopyranose monomers

The 3-*O*-branched trisaccharide monomer LGMAL **8** showed no polymerizability under any polymerization conditions, probably because of the steric hindrance of the bulky 3-*O*-maltopyranosyl branch and lowering of polymerizability due to the increasing molecular weight. In general, anhydro trisaccharide monomers decrease the ring-opening polymerizability because of increasing the steric hindrance, molecular weight, and stability of the anhydro ring. We reported previously that a ribo trisaccharide monomer, 1, 4-anhydro-2-*O*-benzyl-3-*O*-(2', 3', 6', 2", 3", 4", 6"-hepta-*O*-benzyl-β-D- lactopyranosyl)- β-D-ribopyranose, had relatively high polymerizability to give the corresponding ribofuranans with a lactose branch in every ribofuranose unit in 45-62% yields. The polymerizability of the 3-*O*-lactopyranosyl ribo trisaccharide monomer may be attributed to the high ring-opening polymerizability of the 1, 4-anhydro ring in ribopyranose moiety. The ring-opening polymerizability of the 1, 4-anhydro ribopyranose ring should be higher than that of the 1, 6-anhydro glucopyranose ring [4].

As shown in No. 5, the copolymerization of LMMAL with LMTBE in the feed of 25:75 mol% was found to give the corresponding polymer in 71.5% yield. The molecular weight was relatively high, $\overline{M}_n$=7.6x10^3, and the proportion of the LMMAL disaccharide unit in the copolymer was 13.0 mol%.

Figure 5 shows the ^{13}C NMR spectra of the copolymers (A)-(C) consisting of LMMAL and LMTBE Figure 2 units in the proportion of (A) 0.9 : 99.1 mol%, (B) 13.0 : 87.0 mol%, and (C) the homopolymer, 2, 4, 6-tri-*O*-benzyl-(1→6)- β-D-mannocopyranan. All signals were assigned by the combination of H-H COSY and HMQC measurements. In Figure 2B, the carbon signals due to the LMTBE unit appeared as large intense signals. The C1 and C6 signals due to 4-*O*-

branched glucopyranose units were absorbed as small signals at 99.8 ppm and 65.9 ppm for the C1 and C6, 94.9 ppm and 69.6 ppm the C1′ and C6′, and 102.3 ppm and 69.8 ppm for the C1″ and C6″ signals, respectively. From the NMR spectra, it was found that bot trisaccharide monomers were copolymerized with LMTBE to give the corresponding (1→6)-β-D-mannopyranosidic stereoregular copolymers.

Figure 7. 150 MHz ^{13}C NMR Spectra of (A)-(B) copoly(LMMAL and LMTBE)s and (C) poly(LMTBE) in CDCl$_3$ at 25 °C. The proportions of trisaccharide units in the copolymers were (A) 0.9 mol%, (B) 13.0 mol%, respectively. The specific rotation at 25 °C was (A) $[\alpha]_D^{25}$ =+44.9°, (B) +41.3°, (C) +58.8°(c1, CHCl$_3$), respectively. Signals were assigned by H-H COSY and HMQC spectra.

Table 4

Ring-opening polymerization and copolymerization of 1, 6-anhydro-2, 4-di-*O*-benzyl-3-*O*-((2', 3', 6', 2", 3", 4", 6"-hepta-*O*-benzyl-β-D-maltosyl)-β-D-glucopyranose (LGMAL) with 1, 6-anhydro-2, 3, 4-tri-*O*-benzyl-β-D-glucopyranose (LGTBE)

No	Monomer				C2H2	PF5	TEM	Yeild
	LGMAL		LGTBE					
	g	mol%	g	mol%	mL	%	C	%
1	0.03	5	0.17	95	0.4	20	-60	nd
2	0.10	25	0.10	75	0.4	20	-60	nd
3	0.15	50	0.05	50	0.4	20	-60	nd
4	0.20	100	0	0	0.4	20	-60	nd

Table 5

Ring-opening polymerization and copolymerization of 1, 6-anhydro-2, 4-di-*O*-benzyl-4-*O*-((2', 3', 6', 2", 3", 4", 6"-hepta-*O*-benzyl-β-D-maltosyl)-β-D-mannopyranose (LMMAL) with 1, 6-anhydro-2, 3, 4-tri-*O*-benzyl-β-D-mannocopyranose (LMTBE)

| No	Monomer				Time	Yield	$\overline{M}_n$	$[\alpha]_D^{25}$	3 糖 unit
	LMMAL		LMTBE						
	g	mol%	g	mol%	h	%	x10^3	deg	in polymer
1	0	0	0.20	100	24	70.0	14.7	+58.8	0
2	0.05	5	0.10	95	24	51.2	7.8	+44.9	0.9
3	0.10	25	0.10	75	24	71.5	7.6	+41.3	13.0

3.4. CONCLUSION

The new 3-*O*-branched 1, 6-anhdro glucopyranose trisaccharide monomers, 1, 6-anhydro-2, 4-di-*O*-benzyl-3- *O*-β-(2', 3', 6', 2", 3", 4", 6"-hepta-*O*-benzyl-β-D-maltosyl)- β-D-glucopyranose (LGMAL), was synthesized and the ring-opening polymerizability and copolymerizability with 1, 6-anhydro-2, 3, 4- tri-*O*-benzyl-β-D-glucopyranose (LGTBE) were examined for the first time by using PF$_5$ as a catalyst at -60°C. Unfortunately, the 1, 6-anhydro trisaccharide monomer LGMAL showed no homopolymerizability and copolymerizability with LGTBE. We reported previously, probably due to the steric hindrance of the bulky 3-*O*-branched sugar moieties. This is the first report on the ring-opening polymerization of 3-*O*-branched trisaccharide monomers.

The new 4-*O*-branched 1, 6-anhdro mannopyranose trisaccharide monomers, 1, 6-anhydro-2, 4-di-*O*-benzyl-4 - *O*-β-(2', 3', 6', 2", 3", 4", 6"-hepta-*O*-benzyl-β-D-maltosyl)- β-D-mannopyranose (LMMAL), was synthesized and the ring-opening polymerizability and copolymerizability with 1, 6-anhydro-2, 3, 4- tri-*O*-benzyl-β-D-mannopyranose (LMTBE) were examined for the first time by using PF$_5$ as a catalyst at -60°C. The 1, 6-anhydro trisaccharide monomer LMMAL showed copolymerizability with LMTBE. This probably the steric hindrance of the bulky 4-O-branched sugar moieties was smaller than the steric hindrance of the bulky 3-*O*-branched sugar moieties. This is the first report on the ring-opening polymerization of 4-*O*-branched trisaccharide monomers. The polymerization of 4-*O*-branched trisaccharide monomers was further investigated by examining the polymerization conditions of catalysts, solvents, and temperatures, and the resulting 4-*O*-mannopyranosyl (1→6)-β - D-mannopyranans was debenzylation and sulfated to give sulfated 4-*O*-branched mannopyranans. In addition, the relationship between structure and antiviral activities is investigated by comparison with that of sulfated 4-*O*-

branched glucopyranans.

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[2] D. Yoshida, S. Han, K. Narita, K. Hattori, T. Yoshida, Synthesis of oligosaccharide-branched ribofuranan by ring-opening polymerization of a new anhydro ribo trisaccharide monomer. Macromol. Biosci. 9 (2009) 687-693.

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(1- 6)-β-D-ribopyranan. Macromol. 14 (1981) 1-9

APPENDIX
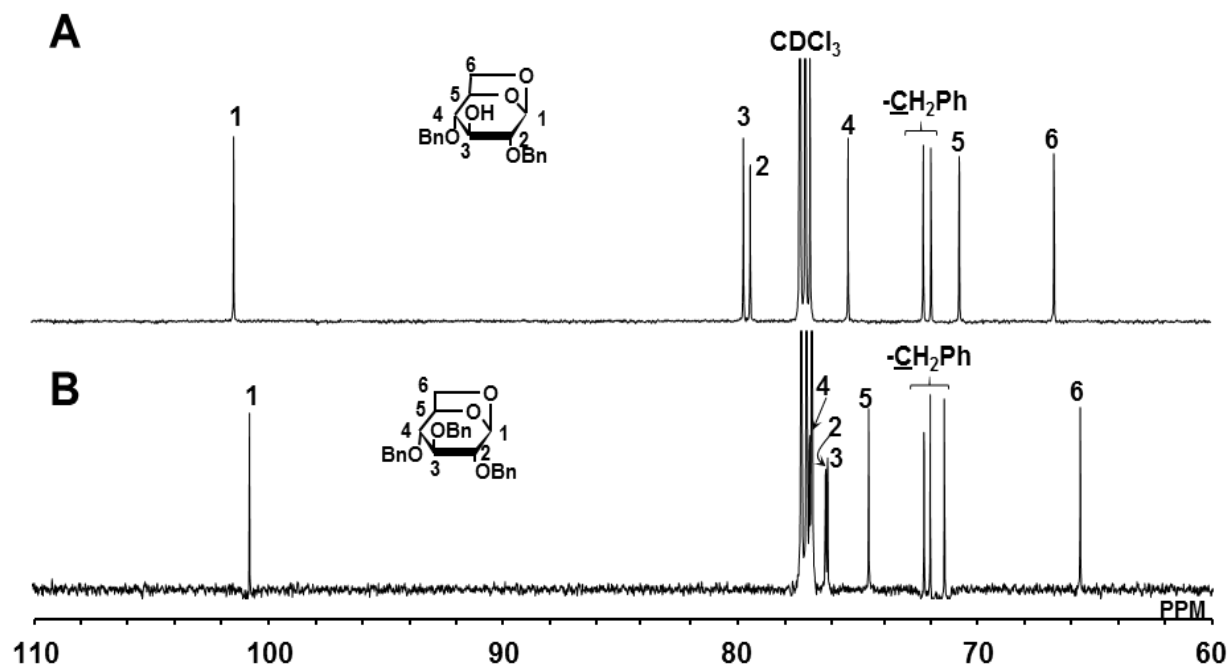
Supplementary Figure 1. 150 MHz ^{13}C NMR Spectra of (A) 1, 6-anhydro-2, 4-di-O-benzyl-β-D-glucopyranose LGDBE **2** and (B) 1, 6-anhydro-2, 3, 4-tri-O-benzyl-β-D-glucopyranose LGTBE in CDCl$_3$ at 25°C.
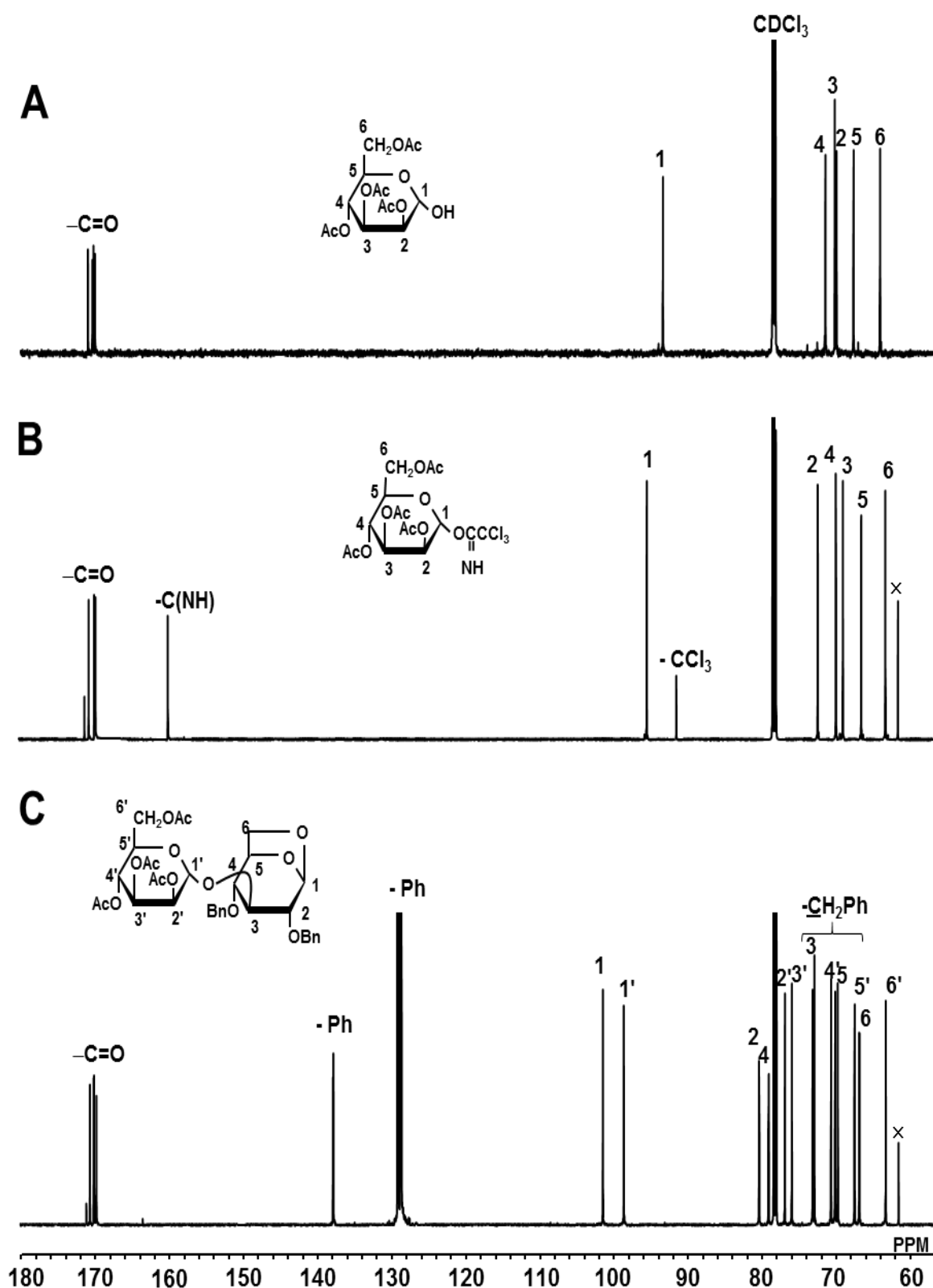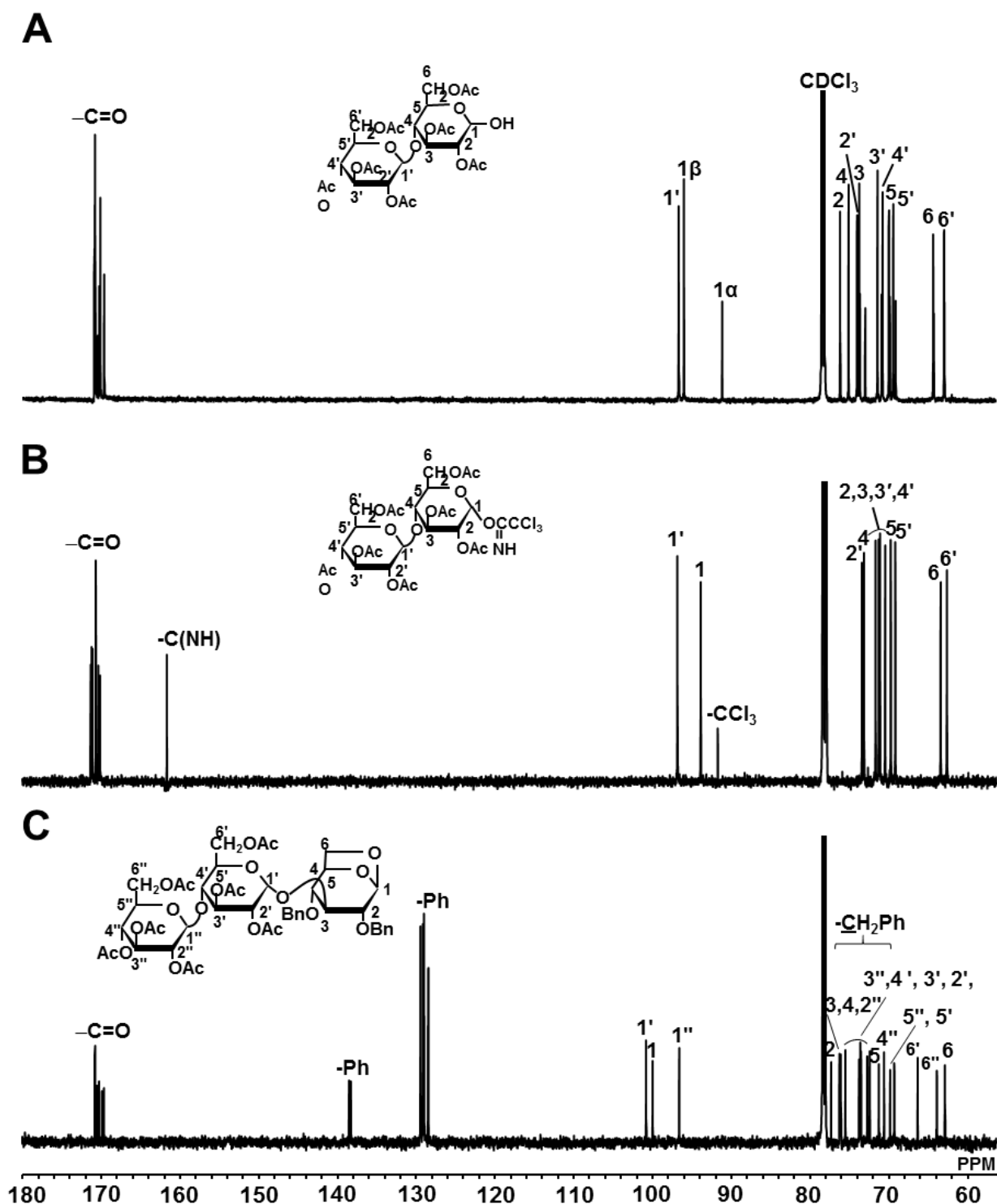
Supplement Figure 2. 150 MHz ^{13}C NMR Spectra of (A) 2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranose, (B) 2, 3, 4, 6-Tetra-*O*-acetyl-1-*O*-trichloroacetimidoyl-α-D-mannopyranose, and (C) 1, 6-anhydro-2, 4-di-*O*-benzyl-3-*O*-(2', 3', 4', 6'-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranose in CDCl$_3$ at 25°C.
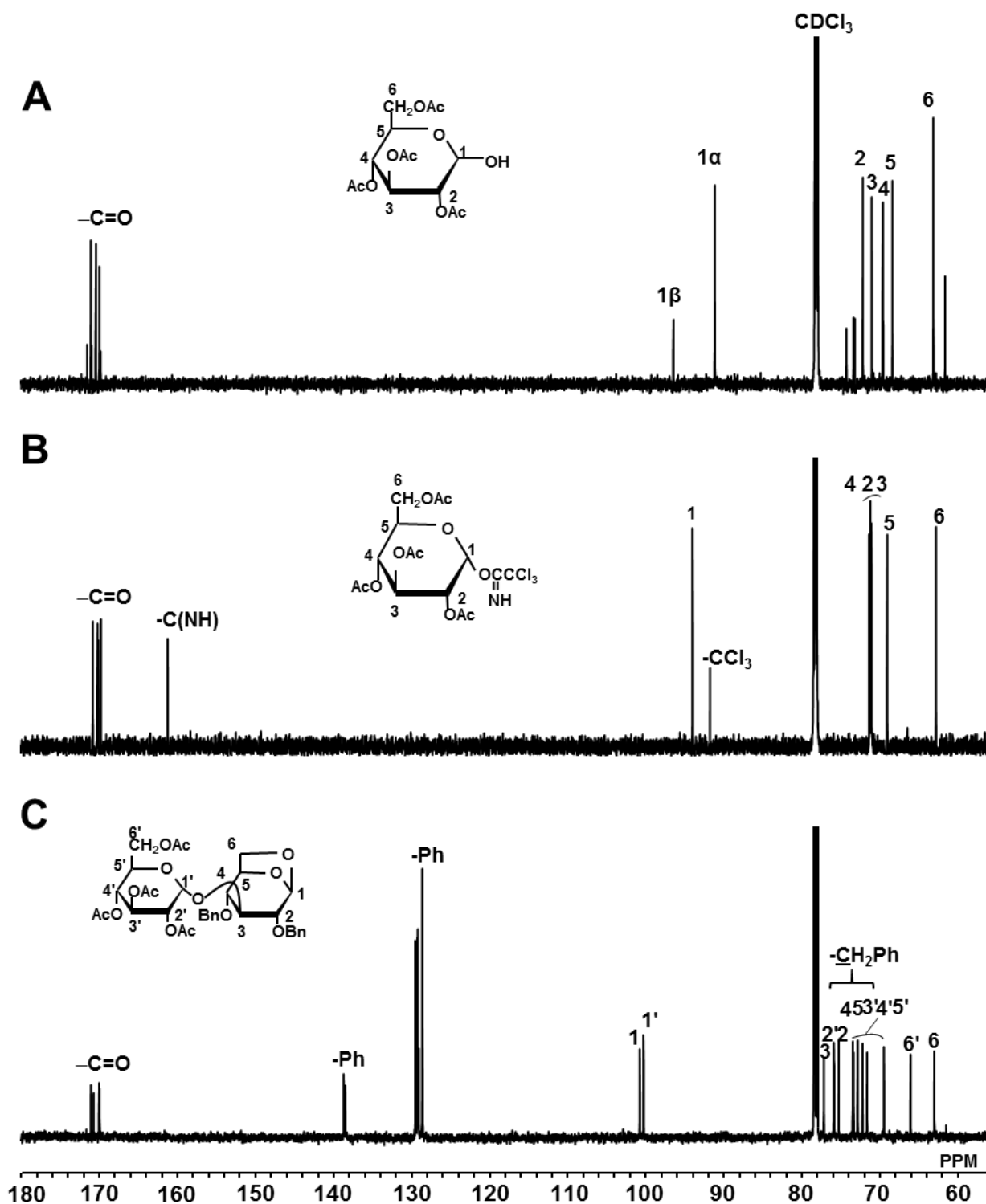
Supplement Figure 3. 150 MHz ^{13}C NMR Spectra of (A) 2, 3, 4, 6-tetra-*O*-acetyl-α-D-glucopyranose, (B) 2, 3, 4, 6-tetra-*O*-acetyl-1-*O*-trichloroacetimidoyl-α-D-glucopyranose, and (C) 1, 6-anhydro-2, 4-di-*O*-benzyl-3-*O*-(2', 3', 4', 6'-tetra-*O*-acetyl-α-D-glucopyranossyl)-β-D-glucopyranose in CDCl$_3$ at 25°C.

Supplement Figure 4. 150 MHz ^{13}C NMR Spectra of (A) 2, 3, 4, 6-tetra-*O*-acetyl-α-D-maltopyranose, (B) 2, 3, 4, 6-Tetra-*O*-acetyl-1-*O*-trichloroacetimidoyl-α-D-maltopyranose, and (C)1, 6-anhydro-2, 4-di-*O*-benzyl-3-*O*-(2', 3', 4', 6'-tetra-*O*-acetyl-α-D-glucopyranosyl)-β-D-glucopyranose in CDCl$_3$ at 25 °C.

SUMMARY

Two new 3-O-branched 1, 6-anhdro glucopyranose disaccharide monomers, 1, 6-anhydro-2, 4-di-O-benzyl-3-O-(2', 3', 4', 6'-tetra-O-benzyl-β-D-glucopyranosyl)- and -mannopyranosyl)- β-D- glucopyranose (LGG and LGM), and a trisaccharide monomer, 1, 6-anhydro-2, 4-di-O-benzyl-3- O-β-(2', 3', 6', 2", 3", 4", 6"-hepta-O-benzyl-β-D-maltosyl)- β-D-glucopyranose (LGMAL), were synthesized and the ring-opening polymerizability and copolymerizability with 1, 6-anhydro-2, 3, 4- tri-O-benzyl-β-D-glucopyranose (LGTBE) were examined for the first time by using PF_5 as a catalyst at -60°C. Unfortunately, the 1, 6-anhydro trisaccharide monomer LGMAL showed no homopolymerizability and copolymerizability with LGTBE. Although the homopolymerizability of the disaccharide monomers was low, the copolymerizations with LGTBE were found to give the corresponding copolysaccharides, 3-O-glucopyranosyl and -mannopyranosyl (1→6)-β-D-glucopyranans, respectively, after deprotection to recover hydroxyl groups. The polymerization yields of the 3-O-branched disaccharide monomers were not very high, 12.8-68.5%, but the monomer feeds of 50:50 mol% gave higher molecular weights of $\overline{M}_n$=8.4x10³ for copoly(LGG and LGTBE) and $\overline{M}_n$ =49.7x10³ for copoly(LGM and LGTBE), respectively, The proportions of the disaccharide monomer units in the corresponding copolymers were 15.3 mol% and 16.1 mol%, respectively. The polymerizability of the 3-O-branched disaccharide monomers was relatively lower than that of the 4-O-branched disaccharide monomers we reported previously, probably due to the steric hindrance of the bulky 3-O-branched sugar moieties. The distance between the C1 and C3 positions in 1, 6-anhydro glucopyranose was shorter than that between the C1 and C4 positions. This is the first report on the ring-opening polymerization of 3-O-branched disaccharide monomers. The polymerization of 3-O-branched disaccharide monomers was further investigated by

examining the polymerization conditions of catalysts, solvents, and temperatures, and the resulting 3-*O*- glucopyranosyl and -mannopyranosyl (1→6)-β -D-glucopyranans were sulfated to give sulfated 3-*O*-branched glucopyranans. In addition, the relationship between structure and antiviral activities is investigated by comparison with that of sulfated 4-*O*-branched glucopyranans.

The new 4-*O*-branched 1, 6-anhdro mannopyranose trisaccharide monomers, 1, 6-anhydro-2, 4-di-*O*-benzyl-4 - *O*-β-(2', 3', 6', 2", 3", 4", 6"-hepta-*O*-benzyl-β-D-maltosyl)- β-D-mannopyranose (LMMAL), was synthesized and the ring-opening polymerizability and copolymerizability with 1, 6-anhydro-2, 3, 4- tri-*O*-benzyl-β-D-mannopyranose (LMTBE) were examined for the first time by using PF$_5$ as a catalyst at -60°C. The 1, 6-anhydro trisaccharide monomer LMMAL showed copolymerizability with LMTBE. This probably the steric hindrance of the bulky 4-O-branched sugar moieties was smaller than the steric hindrance of the bulky 3-*O*-branched sugar moieties. This is the first report on the ring-opening polymerization of 4-*O*-branched trisaccharide monomers. The polymerization of 4-*O*-branched trisaccharide monomers was further investigated by examining the polymerization conditions of catalysts, solvents, and temperatures, and the resulting 4-*O*-mannopyranosyl (1→6)-β -D-mannopyranans was debenzylation and sulfated to give sulfated 4-*O*-branched mannopyranans. In addition, the relationship between structure and antiviral activities is investigated by comparison with that of sulfated 4-*O*-branched glucopyranans.

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