

Powder-to-powder polycondensation of natural saccharides. Facile preparation of highly branched polysaccharides

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Solid-state polycondensation of a natural saccharide was found to take place in the presence of H_3PO_4 (5 mol%) at 110 °C under a N_2 flow, giving a highly branched polysaccharide (conv. 11–84%, $M_w = 1400$ –19 000, $M_n = 1200$ –3700); the reaction mixture was powdery throughout the polymerization. Interestingly, α - and β -anomers showed different polymerization behaviour; the former was polymerized more slowly, however, they gave comparable molecular weight polymers. The polysaccharide product was per-*O*-methylated and subjected to structure analyses. The acid-hydrolysis products, the partially *O*-methylated monosaccharides, suggested that the polysaccharide products have highly branched structures. MALDI-TOF mass analysis revealed that intramolecular glycosylation and acetal exchange reactions are involved in the polymerization mechanism.

Introduction

There are numerous studies on the synthesis of oligo- and polysaccharides by chemical^{1–8} and enzymatic methods⁹ since artificial polysaccharides are expected to have specific properties that are not found in natural polysaccharides. Recently, novel preparation methods for highly branched polysaccharides have been widely noticed because they have potential uses in functional food additives, biocompatible and medical materials, and so on.^{6,10} Highly branched polysaccharides have been prepared by a ring-opening polyaddition of an anhydrosugar² and by melt or suspension polycondensation of a natural saccharide with the aid of acid catalysts.^{3–7} Solid-state polycondensation of a natural saccharide should also be an attractive way to synthesize highly branched polysaccharides because it has several advantages as compared with the other methods. This is green chemistry with a simple process and easy handling. The polysaccharide products could be expected to have specific structures that arise from the molecular arrangement in a monomer crystal. Additionally, undesirable side reactions will be suppressed due to the lower reaction temperature compared to the melt polymerization. Old literature has already reported the solid-state polycondensation of natural saccharide in the presence of dry HCl gas, metaphoric acid, and an ion exchange resin.⁷ However, there is insufficient knowledge on the details of the polymer structure and the reaction mechanism.

In this article, we have further investigated the synthesis and characterization of highly branched polysaccharides by use of the solid-state polycondensation of natural saccharide, where the reaction mixture is powdery from beginning to end. The α - and β -anomers, which readily undergo interconversion in solution, were separately crystallized in order to disclose their difference in reactivity. The polysaccharide products were analyzed by MALDI-TOF mass spectrometry, which was informative for the structure of the repeating and terminal units as well as for the reaction mechanism. The branched structures were validated by analyzing the hydrolysis products from per-*O*-methylated polysaccharide by means of ¹H NMR spectroscopy, ESI-MS spectrometry, and HPLC.

Results and discussion

Thermogravimetry and differential thermal analysis (TG-DTA)

The efficient acid catalyst can be optimised by TG-DTA, which shows weight-loss and endo- or exothermic peaks due to the

dehydration reaction. The dotted lines in Fig. 1 show TG-DTA profiles of the ground mixture of β -Glc with a catalytic amount (5 mol%) of H_3PO_4 (85 wt% solution in water). Observed simultaneously are the weight-loss and the endothermic peaks starting (T_{onset} : 134 °C) below the melting point of β -Glc (mp: 153 °C (the solid line of DTA)), suggesting that the solid-state polymerization could occur in the presence of H_3PO_4 . In a similar manner, the efficiency of other acid catalysts was evaluated by TG-DTA. Camphorsulfonic acid, *p*-toluenesulfonic acid, phosphotungstic acid, and sulfated zirconia, which are stronger acids than H_3PO_4 , were found to show similar TG-DTA profiles to that given by H_3PO_4 . On the other hand, using either H_3BO_3 or citric acid, which are weaker acids than H_3PO_4 , the weight-loss in TGA was not observed below the mp of β -Glc. In this study, H_3PO_4 was adopted as the acid catalyst, since the undesirable side reactions could be suppressed due to the weakest acidity of H_3PO_4 among the efficient catalysts. Each TG-DTA profile of other monomers such as α -Glc, α -Gal, β -Gal, and β -Mal (Chart 1) with H_3PO_4 similarly showed weight-loss below their mp along with the endothermic peak, indicating that H_3PO_4 is an effective catalyst also for the polymerization of these natural saccharides.

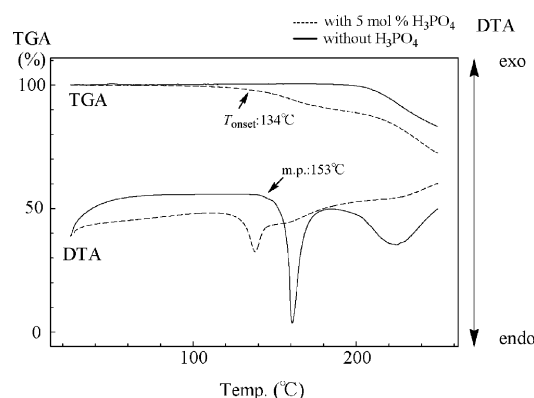


Fig. 1 TG-DTA profiles of β -Glc with and without H_3PO_4 (5 mol%).

Polymerization

The natural saccharides shown in Chart 1 were ground with H_3PO_4 (5 mol%) in an agate mortar and subjected to polymerization in a test tube under a N_2 flow at 110 °C (Table 1). In all cases, the product polysaccharides were a slightly brown

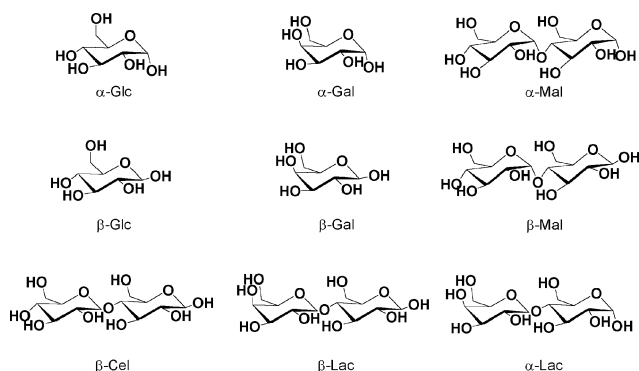


Chart 1 Natural saccharides used as monomers.

and water-soluble powder. WAXD analysis of the reaction mixture (run 2–2) revealed that the resultant polysaccharide was amorphous and that the crystals of unreacted β -Glc were contained. The monomer conversion and the molecular weight of the product polymer were traced by GPC (eluent: 2M NaNO₃ aq., calibration: pullulan standards). Although the molecular weights evaluated here were not exact because of the highly branched structure of the resultant polysaccharide, they can be used as the relative values to follow the polymerization. The good reproducibility of the obtained polysaccharides was confirmed by repeating the experiments several times.

The α - and β -forms of natural saccharides were isolated by recrystallization and then polymerized respectively. The lines (a) and (c) in Fig. 2(A) show the time–conversion relationship of the solid-state polycondensation of β -Glc with and without grinding the reaction mixture at 5, 10, and 24 h during the polymerization, respectively. This grinding process was found to accelerate the polymerization by assisting the diffusion of the saccharide, the product polysaccharide, and H₃PO₄. Thus, all of the reactions in Table 1 were carried out with occasionally grinding the reaction mixtures. As shown in Fig. 2 and Table 1, β -Glc was found to react faster than α -Glc, however, they produced comparable molecular weight polysaccharides. A similar tendency was also observed in the polymerization of α - and β -Gals (Table 1, runs 5 and 6).

When the reactions of α - and β -Glc were carried out at 120 °C, the powdery mixtures gradually melted and yielded a dark brown and water-soluble lump of polysaccharide (Table 1, runs 3 and 4). In contrast with the polymerization at 110 °C, no differences were observed between α - and β -Glc in the monomer conversion and the molecular weight of the resultant polysaccharide.

Accordingly, we believe that the crystal structures of natural saccharides could cause the above difference in their polymerization at 110 °C, although their crystal structures gradually disappear with the progress of the polymerization. It is noteworthy that the higher molecular weight polysaccharide was obtained at 110 °C rather than at 120 °C. This finding should be

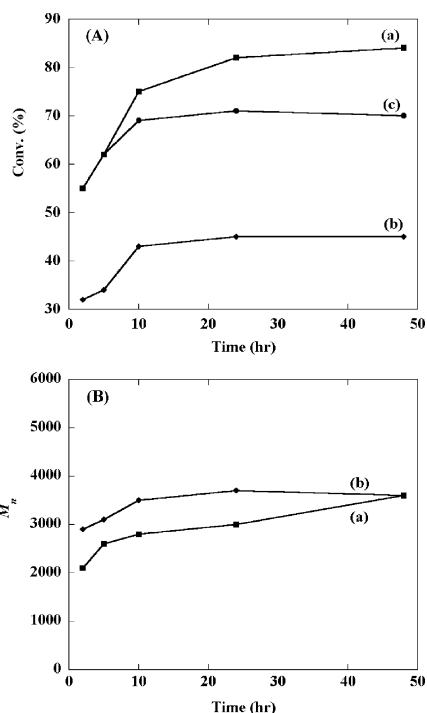


Fig. 2 Solid-state polycondensation of α - and β -Glc in the presence of H₃PO₄ (5 mol%) at 110 °C under a N₂ flow. The provisional M_n values of the product polymer and the conversions of Glc were evaluated by GPC (eluent: 2M NaNO₃ aq., calibration: pullulan standards). Plot lines (a) and (b) are for α - and β -Glc, respectively with grinding the reaction mixture at 5, 10, and 24 h. Plot line (c) is for β -Glc without the grinding.

ascribable to the following reason: the generated water could be more easily removed from the powdery mixture at 110 °C due to the larger surface area as compared with the melted–resolidified mixture at 120 °C.

Melt polycondensations of natural saccharides were reported in some patents, in which they are heated above their melting points in the presence of acid catalysts to produce branched polysaccharides.⁶ In the wake of these patents, we performed the melt polycondensation of α - (mp: 155 °C) and β -Glc (mp: 153 °C) at 155 °C with 5 mol% of H₃PO₄ under a N₂ flow. The reaction mixtures became dark brown within 30 min and the products contained water-insoluble parts. Some undesirable side reaction should be involved due to the higher reaction temperature; dehydration forming C=C bonds and subsequent C–O–C bond formation most likely take place at various positions, producing cross-linked polysaccharides.

An appropriate melting point of the monomer was found to be required for this polymerization, suggested by some attempts to polymerize the natural disaccharides shown in Chart 1. Although β -Mal with the lowest melting point (117 °C) was

Table 1 Polymerization of natural saccharides in the presence of H₃PO₄ (5 mol%) under a N₂ flow

Run	Saccharide	Mp/°C ^a	Temp/°C	Time/h	Conv. (%) ^b	M_w^b	M_n^b
1–1	α -Glc	155	110	5	34	6700	3100
1–2	α -Glc	—	110	48	45	10 000	3700
2–1	β -Glc	153	110	5	65	4700	2600
2–2	β -Glc	—	110	48	84	10 000	3600
3	α -Glc	—	120	5	94	3000	2000
4	β -Glc	—	120	5	93	2300	1700
5	α -Gal	162	110	24	11	19 000	3100
6	β -Gal	149	110	24	61	12 000	3400
7	α -Mal	191	110	24	0	—	—
8	β -Mal	117	110	24	78	1400	1200

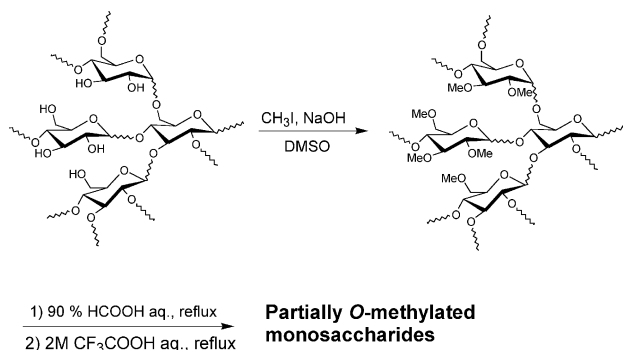
^a Evaluated by differential thermal analysis (heating rate: 10 °C min⁻¹, under N₂ flow). ^b Evaluated by GPC (eluent: 2M NaNO₃ aq., calibration: pullulan standards).

polymerized at 110 °C in a 78% monomer conversion, the smaller molecular weight polysaccharide ($M_w = 1400$, $M_n = 1200$) was obtained compared to the polymerization of other monosaccharides. This might be caused by the smaller surface area of the particles in the reaction mixture, which comes from microscopic fusion due to the lower mp of β -Mal. In contrast, other disaccharides such as α -Mal, β -Cel, α -Lac, and β -Lac were quite inert below 150 °C, which is ascribable to their high melting points (191–224 °C). The forced reactions of these disaccharides above 150 °C produced water-insoluble and black polysaccharides.

Polymer structure

^1H and ^{13}C NMR spectra were not informative to analyze the structure of the obtained polysaccharide due to broadening of signals. In order to validate the highly branched structure, the product polymer from β -Glc was analyzed representatively. The polysaccharide was per-*O*-methylated, isolated, and then hydrolyzed by successive treatment with 90% HCOOH aq. and 2M CF_3COOH aq. (Scheme 1), producing a mixture of partially *O*-methylated monosaccharides. The polymer structure can be evaluated by characterizing these partially *O*-methylated monosaccharides since their free OH groups originate from the glycosidic bonds forming the main polymer chain. Chart 2 shows the products of the partially methylated monosaccharides, which are classified into five groups (A)–(E) by the number of OH groups. A compound of group (A), 2,3,4,6-tetra-*O*-methylglucopyranose, originates from the non-reducing terminal unit of the polysaccharide. Similarly, diols of group (B) are from the linear repeating units or the reducing terminal units, and tri-, tetra-, and pentaols of groups (C), (D), and (E) are from the branched repeating units and/or the reducing terminal units.

When the hydrolysis products from the per-*O*-methylated polysaccharide (run 2–2 in Table 1) were analyzed by HPLC



Scheme 1 Per-*O*-methylation and subsequent hydrolysis of the polysaccharide product.

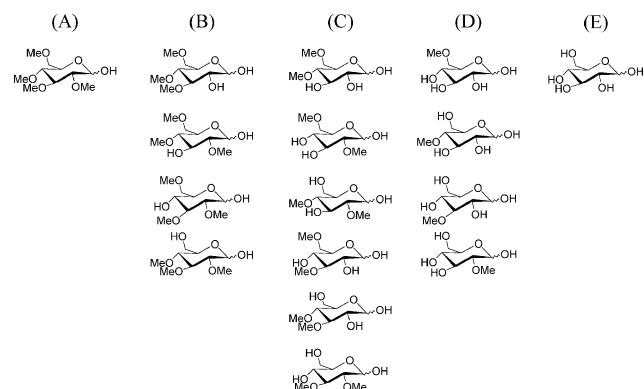


Chart 2 The hydrolysis products from the highly branched per-*O*-methylated polysaccharide (run 2–2, Table 1). (A) A non-reducing terminal unit; (B) reducing terminal and linear units; (C), (D), and (E) reducing terminal and branched units.

(acetone–hexane–MeOH = 1 : 1 : 0.025), several peaks were observed (Fig. 3). Compounds showing these peaks were separated into three fractions by column chromatography and then assigned by ESI-MS spectrometry. Consequently, these fractions were found to be identical to groups (A), (B), and (C) in Chart 2. In addition, the hydrolysis mixtures were acetylated and analyzed by ESI-MS spectrometry. The ion peaks that were assignable to groups (A), (B), (C), and (D) were observed, while a peak due to group (E) was not detected. Although quantitative discussion is impossible for the ESI-MS spectrometry, the amount of the compound of group (E) is probably very small.

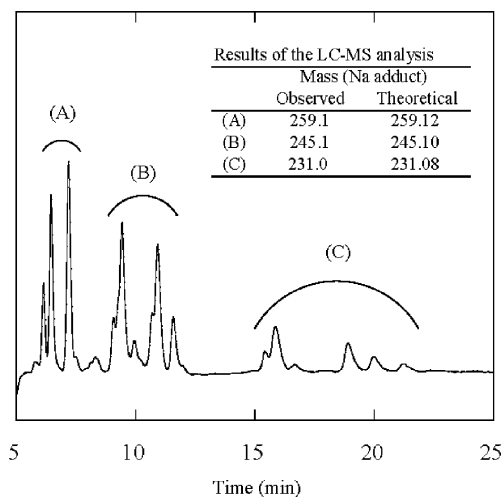


Fig. 3 HPLC profiles (acetone–hexane–MeOH = 1 : 1 : 0.025, RI detector) of the hydrolysis products from the per-*O*-methylated polysaccharide obtained at run 2–2 in Table 1.

Representatively, the acetylated products of the fractionation of peak group (B) were analyzed by ^1H NMR spectroscopy. As indicated by Fig. 4, the 1,2-, 1,3-, 1,4- and 1,6-diacetylated derivatives were contained in the product mixture. In summary, these findings suggest that the polysaccharide product has a highly branched structure, which is probably composed of the glucopyranose units with all possible glycosidic linkages.

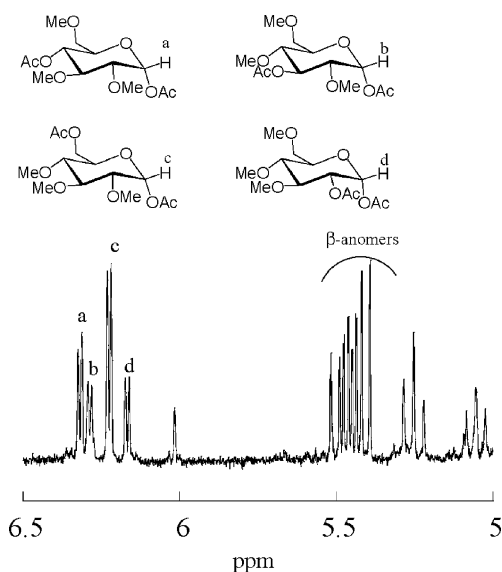


Fig. 4 ^1H NMR spectrum (300 MHz, CDCl_3) of the acetylated products from fraction (B) in Fig. 3.

MALDI-TOF mass analyses

In order to get more information about the polymer structure, the per-*O*-methylated polysaccharide (run 2–2, Table 1) was

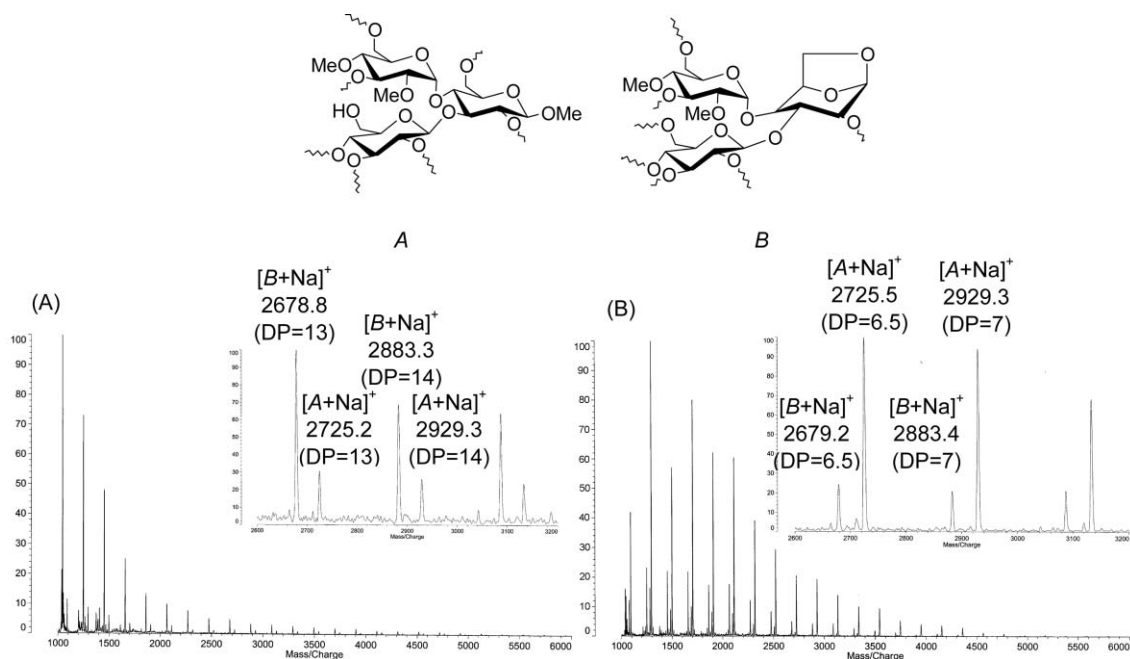
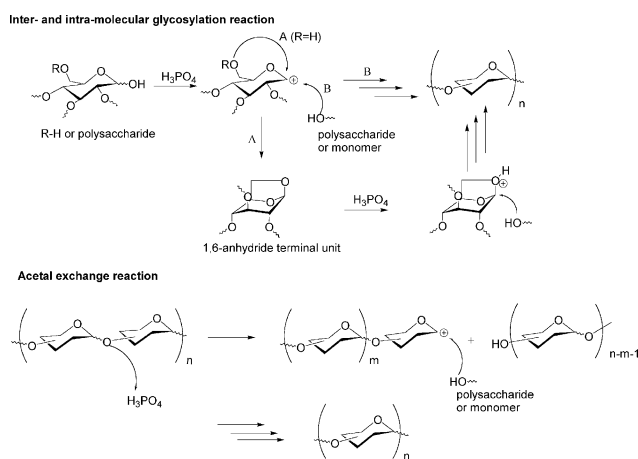


Fig. 5 MALDI-TOF mass spectrum (matrix: 2,5-dihydroxybenzoic acid) of the per-*O*-methylated polysaccharide produced at (A) run 2–2 in Table 1: yield = 51%, $M_w = 8000$, $M_n = 4300$ (hexane-insoluble part, GPC (eluent: CHCl_3 , calibration: Pst standards)) and (B) run 8 in Table 1: yield: 13%, $M_w = 7500$, $M_n = 6000$ (hexane-insoluble part, GPC (eluent: CHCl_3 , calibration: Pst standards)).

subjected to MALDI-TOF mass analysis. In Fig. 5(A),¹¹ there are observed peaks positioned with regular intervals (204 u), which are consistent with the mass value of the repeating unit, showing the differences in the degree of polymerization. Additionally, it should be noted that there are two kinds of peak series $[A + \text{Na}]^+$ and $[B + \text{Na}]^+$, where $+\text{Na}$ means that the peaks are detected as the adducts with Na^+ . When the mass values of the polymers are calculated for structure A shown in Fig. 5, the peaks of series $[A + \text{Na}]^+$ showed good agreement with them. Therefore, peak series $[B + \text{Na}]^+$ is assignable to the polymers with a different terminal structure. On each couple of peaks $[A + \text{Na}]^+$ and $[B + \text{Na}]^+$, the latter shows the smaller mass value by 46 u, which is equal to the formula mass of $\text{C}_2\text{H}_6\text{O}$ ($= 2 \times \text{CH}_3 + \text{O}$). This means that two more hydroxyl groups intramolecularly form an ether linkage in the original polysaccharides. Accordingly, there are two possibilities for the terminal structure of the $[B + \text{Na}]^+$ series of polymers. One is a macrocyclic structure that is formed by the glycosylation between the reducing terminal unit and the inner unit. However, this possibility was excluded because $[B + \text{Na}]^+$ series peaks were detected even for the tri- and tetra-saccharides that would be too small to give the macrocyclic polysaccharide. Thus, the other possibility should be correct; an anhydride structure, the most stable 1,6-anhydride, should be included in the reducing terminal unit. An article published in 1998 reported that the identical terminal structure was detected in the suspension oligomerization of glucose.⁴

Accordingly, the polymerization takes place through two routes as shown in Scheme 2. An anomeric hydroxyl group is activated by phosphoric acid to generate the carbocation, which undergoes intramolecular (A) or intermolecular (B) addition of a hydroxyl group, producing the 1,6-anhydride terminal unit or the polymer chain, respectively. The 1,6-anhydride terminal unit participates in further polymerization in the presence of an acid catalyst.

The MALDI-TOF mass spectrum of per-*O*-methylated polysaccharide produced from β -Mal gave additional information on the solid-state polycondensation. In Fig. 5(B), the 204 u interval of peaks, which is consistent with the molecular weight of the glucopyranosyl repeating unit, was observed, suggesting that the repeating unit of the polysaccharide product was not



Scheme 2 Reaction mechanisms of the solid-state polycondensation of natural saccharides.

the disaccharide but the monosaccharide. This means that the acetal exchange reaction takes place during the polymerization (Scheme 2).

It is noteworthy that the Lewis acid-catalyzed polyaddition of 1,6-anhydrosugar in solution to produce hyper-branched polysaccharides has been independently reported by old and recent literatures.² As compared with them, the solid-state polymerization presented here is a more facile method to prepare highly branched polysaccharide. Our research project is underway to demonstrate how solid-state polymerization is widely applicable to a variety of saccharides such as aldopentose. In addition, the solid-state polymerization has the potential to be applied to syntheses of not only polysaccharides but also other “artificial” biopolymers like nucleotides and peptides.¹²

Experimental

Materials

Commercially available α -Glc, α -Gal, β -Mal, and α -Lac were dried at 80 °C under vacuum before use. The crystals of β -Glc, β -Gal, α -Mal, and β -Lac were obtained by anomerization of

Table 2 Purity of the saccharides used as monomers

Saccharide	α : β^a	Saccharide	α : β^a
α -Glc	96 : 4	α -Mal	93 : 7
β -Glc	4 : 96	β -Mal	5 : 95
α -Gal	97 : 3	α -Lac	95 : 5
β -Gal	7 : 93	β -Lac	0 : 100
β -Cel	10 : 90		

^a The contents of the α - and β -anomers were determined by ¹H NMR spectroscopy in DMSO-d₆.

the α - or β -isomers according to the literatures.^{13–15} β -Cel was obtained by the recrystallization of the commercially available D-cellobiose from 75% EtOH aq.¹³ The contents of the α - and β -anomers were determined by ¹H NMR spectroscopy in DMSO-d₆ (Table 2). DMSO was fractionally distilled under vacuum and dried over 4A molecular sieves. Powdery NaOH was prepared by the adequate hydrolysis of NaH in dry THF. Other reagents of H₃PO₄, CH₃I, NaNO₃, HCOOH, and CF₃COOH were used as received.

Measurements

Thermogravimetry and differential thermal analyses (TG-DTA) were performed with a Simadzu DTG-60 apparatus (heating rate: 10 °C min⁻¹, under a N₂ flow). ¹H NMR spectra were recorded with a Bruker DPX300 spectrometer (solvent: CDCl₃, internal standard: Me₄Si). ESI mass spectra were obtained by using a Shimadzu LCMS-QP 8000A system (solvent: MeOH). MALDI-TOF MS spectra were recorded with a KRATOS AXIMA-CFR-S spectrometer (matrix: 2,5-dihydroxybenzoic acid). Gel permeation chromatography was conducted on a TOSOH TSK-GEL ALFA-3000 column (eluent: 2M NaNO₃ aq. calibration: pullulan standards) or a Shodex K-804L column (eluent: CHCl₃, calibration: polystyrene standards). HPLC analyses were performed on a GL Science Sil-100A (eluent: acetone-*n*-hexane-MeOH = 1 : 1 : 0.025). Silica gel 60N and 60 F₂₅₄ (Merk) were used for column chromatography and TLC, respectively.

Solid-state polymerization

Natural saccharide (300 mg) was ground well with H₃PO₄ (85 wt% solution in water, 5 mol% for monomer) in an agate mortar and pestle. The mixture in a test tube with a N₂ inlet was heated at 110 °C on an aluminium block (Eyela dry thermo bath MG-2000) under a N₂ flow. In some cases, the reaction mixture was ground at 5, 10, and 24 h.

In the case of α - or β -Glc, a small portion of the reaction mixture was sampled and subjected to GPC analysis after 2, 5, 10, 24, and 48 h (eluent: 2M NaNO₃ aq., calibration: pullulan standards) in order to get the time-conversion and molecular weight curves.

Methylation of the resultant polysaccharide

Into a dry DMSO solution (9 ml) of the above reaction mixture (ca. 300 mg, run 2-2) was suspended powdery NaOH (1.2 g, 0.03 mol) and the mixture was stirred for 1 h. CH₃I (1.2 ml,

2.7 g, 0.02 mol) was added to this mixture and stirred for another 24 h. The resultant mixture was poured into water (20 ml) and extracted with CH₂Cl₂ (20 ml). The organic phase was dried with MgSO₄ and concentrated. The residue underwent the same methylation process twice. Then, the obtained material was dissolved in AcOEt and poured into hexane to give per-*O*-methylated polysaccharide as a yellow powder.

Hydrolysis of the methylated polysaccharide

The per-*O*-methylated polysaccharide (100 mg) was refluxed for 2 h in 90% HCOOH aq. (1 ml). To the reaction mixture was added 2M CF₃COOH aq. (1 ml) and the mixture was refluxed for another 12 h. The solvent was removed under reduced pressure, giving a brown oil.

Acetylation of compounds of group (B)

Compounds of groups (A), (B), and (C) were fractionated by column chromatography (acetone-hexane = 2 : 1). Into a pyridine (1 ml) solution of fraction (B) (10 mg) was added acetic anhydride (1 ml) and the mixture was stirred at rt for 24 h. The solvent was removed under reduced pressure. The residue was dissolved into CH₂Cl₂ (20 ml) and successively washed with 1M HCl aq. (20 ml), saturated NaHCO₃ aq. (20 ml), and saturated NaCl aq. (20 ml). The organic layer was dried over MgSO₄ and concentrated under reduced pressure to obtain the acetylated products of fraction (B).

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