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Chemical synthesis of an amylose-like polysaccharide by polymerization of partially benzylated phenyl 1-thio-β-maltooctaoside derived from γ-cyclodextrin

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Abstract

An amylose-like α-(1,4)-glucan was synthesized by polycondensation and subsequent deprotection of a partially benzylated phenyl 1-thio-β-maltooctaoside having a sole hydroxyl group at the non-reducing end. The key octasaccharide monomer **6** was prepared by means of a single-site acetolytic reaction of fully acetylated γ-cyclodextrin and several subsequent chemical manipulations at its reducing and non-reducing ends. On activation with methyl triflate, the polycondensation of **6** was found to proceed in diethyl ether through intermolecular glycosidation. The molecular weight of the product obtained by preparative GPC on Sephadex LH-60 was 10 000–18 000. Removal of the *O*-benzyl groups under Birch reaction gave α-(1,4)-glucan, the stereoregularity of which was confirmed by ^{1}H and ^{13}C NMR spectroscopy.

Keywords: Amylose; Cyclodextrin; Glycosidation; Maltooctaoside; Polymerization; Polysaccharides

1. Introduction

Chemical synthesis of polysaccharides with well-defined structures (Kochetkov, 1987) constitutes a critical step in carbohydrate research in that, among other things, it permits systematic investigation of the relationship between structure and biological function as well as the analysis of physicochemical properties of natural polysaccharides. Although a few methods were established to synthesize such polysaccharides (e.g., the ring-opening polymerization of 1,6-anhydrosugars (Schuerch, 1973) or cyclic orthoesters (Nakatsubo et al., 1996) for synthesizing homo-polysaccharides having α-(1,6)- or β-(1,4)-linkages, synthesis of other types of polysaccharides were hampered by the difficulty of controlling the regio- and stereo-chemistry of the glycosidic linkages during the polymerization reaction. Moreover, the polycondensation strategy was regarded as an extension of ordinary glycosidation reactions in that its only application is the synthesis of low-molecular-weight derivatives.

Recently, novel glycosidation reactions was developed and used to synthesize many complex oligosaccharides (Paulsen, 1990). A distinctive feature of this approach is the use of stable glycosyl donors such as trichloroacetimidate (Haines, 1976), glycosyl fluoride (Horton and Hutson, 1963), and thioglycosides (Pougny et al., 1977). Using this methodology, we succeeded in synthesizing novel cyclodextrin analogues through cyclization of linear maltooligosaccharides (Sakairi et al., 1995). In this process, we found that the maltooligosaccharides functioning as both glycosyl donor and acceptor acted as crucial intermediates for the cyclization, and that the yield of the cycloglycans was dependent on the chain length of the intermediates. The yields of cyclo-hexaose and heptaose were 40–50% and 10%, respectively. We deduced that intermolecular glycosidation was a major side effect of this process and we undertook to investigate this reaction in detail.

In the present paper, we describe the results of our attempts at polysaccharide synthesis using a macromonomer as the starting reagent. The studies were carried out using partially benzylated phenyl maltooligosaccharide **6** as a model substrate.

2. Experimental

2.1. Materials

All reagents were purchased from Wako Pure Chemicals

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Co. Ltd. (Osaka, Japan) and used as received. (Phenylthio)-trimethylsilane was prepared according to the literature. Diethyl ether and tertrahydrofuran were distilled from lithium aluminum hydride before use. Molecular sieves were activated at 160°C under reduced pressure immediately before use. Hexacosa-*O*-acetylmaltooctaose **2** was prepared by acetolysis of fully acetylated γ-cyclodextrin **1** with acetic anhydride-concentrated sulfuric acid (50:1 v/v) at 50–60°C.

2.2. General methods

^{1}H and ^{13}C NMR spectra were recorded with a Bruker ASX-300 spectrometer at 300.13 MHz and 75.48 MHz, respectively, for solutions in $CDCl_3$ with tetramethylsilane as an internal standard unless otherwise noted. HPLC was performed with a Waters LC module I equipped with a Hitachi L-7490 refractive index detector. Samples were loaded on columns of Shodex KF-802.5 and Shodex Asahipak GS-220 HQ (Showa–denko Co. Ltd., Japan), and eluted with tetrahydrofuran and water at a rate of 1 mL/min, respectively. Preparative gel permeation chromatography was carried out on a column (27 × 1500 mm) of Sephadex LH-60 (50 g) with use of chloroform–methanol (6:4 v/v) as the eluent. Silica gel column chromatography was conducted on Wako gel C-300.

2.3. Preparation of Phenyl 2^1, 3^1, 6^1, 2^2, 3^2, 6^2, 2^3, 3^3, 6^3, 2^4, 3^4, 6^4, 2^5, 3^5, 6^5, 2^6, 3^6, 6^6, 2^7, 3^7, 6^7, 2^8, 3^8, 4^8, 6^8-pentacosa-O-acetyl-1^1-thio-β-maltooctaoside **3**

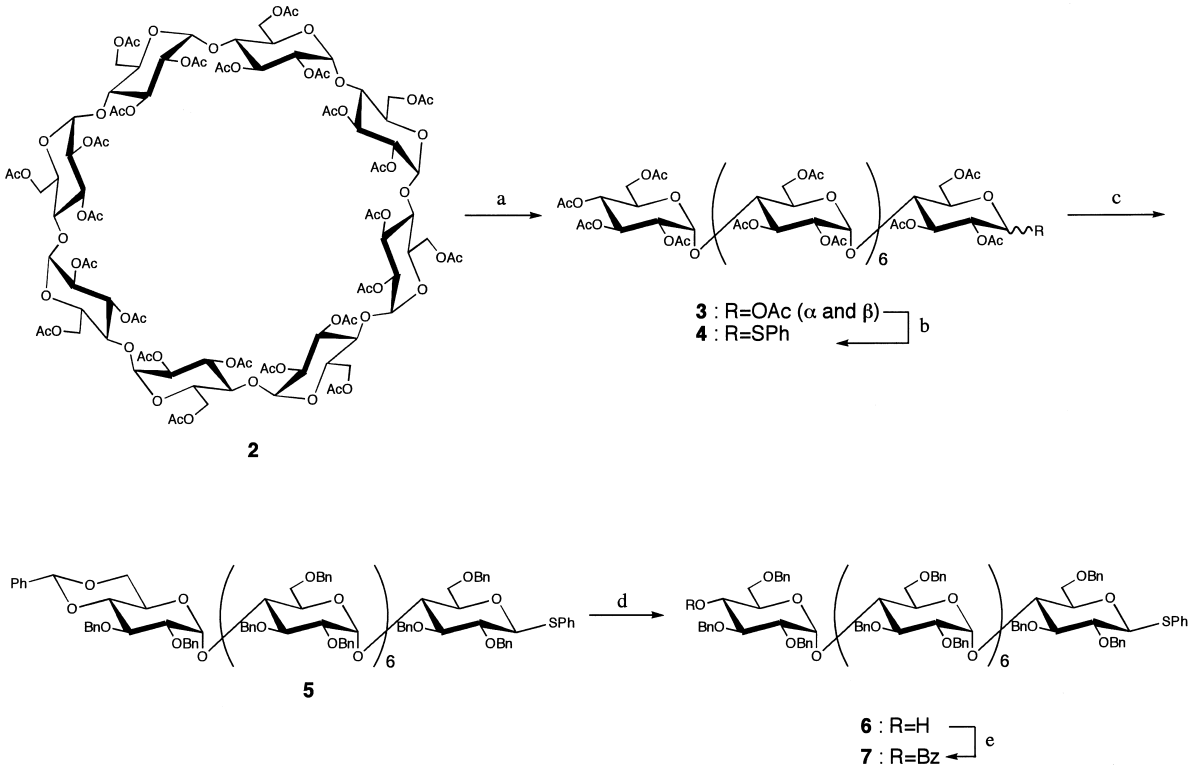
Fully acetylated maltooctaose **2** (4.55 g, 1.89 mmol) was dissolved in anhydrous 1,2-dichloroethane (50 mL). Zinc iodide (2.4 g, 7.5 mmol) and (phenylthio)trimethylsilane (1.4 g, 7.9 mmol) were successively added to the solution with stirring at 0°C. The suspension was stirred at room temperature for 40 h, filtered through a Celite pad and washed with toluene. The filtrate and washings were combined and concentrated. The residue was chromatographed on a column of silica gel with toluene-ethyl acetate (1:2 v/v) as the eluent, giving the thioglycoside **3** (3.60 g, 78%); $[\alpha]_D^{27}$ + 131° (c = 0.82, chloroform); ^{1}H NMR: δ_H 5.24–5.42 (14 H, m, 7 × 1-, 3-H), 5.07 (1 H, t, J = 9.9 Hz, 4^8-H), 4.85 (1 H, dd, J = 4.0 and 10.5 Hz, 2-H), 4.70–4.81 (7 H, m, 7 × 2-H), 3.71–3.76 (1 H, m, 5-H), 2.20, 2.19, 2.18, 2.16, 2.15, 2.09, 2.05, 2.02, 2.01, 2.00, 1.99, 1.98, 1.96 (75 H, 16 s, 25 X OAc): δ_C 20.54, 20.85, 61.42, 62.44, 62.87, 68.02, 68.49, 68.99, 69.40, 70.08, 70.47, 70.78, 71.76, 72.47, 73.34, 73.52, 76.19, 84.90, 95.69, 128.45, 128.88, 131.12, 133.58, 169.41, 169.51, 169.70, 170.03, 170.34, 170.50, 170.63, 170.70. Anal. Calcd. for $C_{104}H_{136}O_{65}S$: C, 50.81; H, 5.58; S, 1.30. Found: C, 50.53; H, 5.54; S, 1.40.

2.4. Preparation of Phenyl 2^1, 3^1, 6^1, 2^2, 3^2, 6^2, 2^3, 3^3, 6^3, 2^4, 3^4, 6^4, 2^5, 3^5, 6^5, 2^6, 3^6, 6^6, 2^7, 3^7, 6^7, 2^8, 3^8-tricosa-O-benzyl-4^8, 6^8-O-benzylidene-1^1-thio-β-maltooctaoside **5**

To a suspension of the thioglycoside **3** (1.92 g, 0.781 mmol) in methanol (30 mL) was added 1 M methanolic sodium methoxide (0.5 mL); the mixture was stirred at room temperature 18 h. Water was added to dissolve the precipitate, and the solution was neutralized with Amberlite IR-120B (H^+ form). The resulting mixture was evaporated to give a quantitative yield of the unprotected thioglycoside **4**, which was dissolved in *N*,*N*-dimethylformamide (10 mL). α,α-Dimethoxytoluene (0.24 g, 1.6 mmol) was added to the solution of **4** and the pH was adjusted to 3.0 with (+)-camphor sulfonic acid. The mixture was stirred at 60°C under reduced pressure (2.5–3 kPa) for 15 h, and sodium hydride-oil dispersion (60%; 1.3 g, 32.5 mmol) was then added to the solution with stirring at 0°C. The suspension was stirred at 0°C for 1 h, and benzyl bromide (5.29 g, 30.4 mmol) was added dropwise to the mixture. The resulting suspension was stirred at room temperature for 20 h, quenched by successive addition of methanol (5 mL) and 25% ammonia water (100 mL), and extracted with ethyl acetate. The extract was successively washed with 1 M hydrochloric acid, aqueous saturated sodium bicarbonate and brine, dried with sodium sulfate and evaporated. The residue was subjected to column chromatography on silica gel with toluene–ethyl acetate (30:1 v/v) as the eluent to give the benzylidene derivative **5** (1.7 g, 72%); $[\alpha]_D^{27}$ + 92° (c = 0.68, chloroform); ^{1}H NMR: δ_H 5.66 (4H, m, 1-H), 5.60 (1H, d, J = 3.4, 1-H), 5.55 (1H, d, J = 3.5 Hz, 1-H), 5.52 (1H, br. s, CHPh) and 5.47 (1H, d, J = 3.5 Hz, 1-H). Anal. Calcd. for $C_{222}H_{228}O_{40}S$: C, 74.73; H, 6.44; S, 0.90. Found: C, 75.00; H, 6.65; S, 0.91.

2.5. Preparation of Phenyl 2^1, 3^1, 6^1, 2^2, 3^2, 6^2, 2^3, 3^3, 6^3, 2^4, 3^4, 6^4, 2^5, 3^5, 6^5, 2^6, 3^6, 6^6, 2^7, 3^7, 6^7, 2^8, 3^8, 6^8-tetracosa-O-benzyl-1^1-thio-β-maltooctaoside **6**

To a solution of the benzylidene derivative **5** (0.68 g, 0.19 mmol) in tetrahydrofuran (10 mL) was added activated molecular sieves 4A (1.0 g), borane-trimethylamine complex (138 mg, 1.90 mmol), and aluminum chloride (234 mg, 1.75 mmol). The suspension was stirred at room temperature for 2 d, filtered through a Celite pad, and washed with ethyl acetate. The combined filtrate and washings were successively washed with 1 M hydrochloric acid, aqueous saturated sodium bicarbonate, and brine, dried with sodium sulfate and evaporated. The residue was subjected to column chromatography on silica gel with toluene–ethyl acetate (30:1 v/v), and gave the 4^8-hydroxyl derivative **6** (400 mg, 59%); $[\alpha]_D^{27}$ + 71° (c = 0.36, chloroform); ^{1}H NMR: δ_H 7.06-7.00 (130 H, m, aromatic), 5.66 (4H, m, 1-H), 5.60 (1H, d, J = 3.4 Hz, 1-H), 5.55 (1H, d, J = 3.4 Hz, 1-H), 5.47 (1H, d, J = 3.6 Hz, 1-H); ^{13}C NMR: δ_C 68.70, 69.13, 69.86, 70.40, 70.86, 71.62, 72.36, 72.79, 73.09,



Scheme 1. Chemical synthesis of α-(1,4)-glucan from γ-cyclodextrin. Reagents and conditions: (a) Acetic anhydride-conc. Sulfuric acid (50:1 v/v), 50–60°C, 24 h; (b) $PhSSiMe_3$-ZnI_2, 1,2-dichloroethane, RT, 40 h; (c) NaOMe/MeOH; $PhCH(OMe)_2$/CSA, DMF, 60°C,15 h; BnBr/NaH, DMF, RT, 20 h; (d) BH_3?Me_3N-$AlCl_3$, THF, 2 d; (e) BzCl, pridine, RT.

73.30, 73.57, 73.75, 74.04, 74.29, 74.56, 75.19, 77.20, 78.93, 79.05, 79.23, 79.53, 79.67, 80.81, 81.39, 81.67, 86.51, 87.31, 96.15, 96.49, 96.73, 97.02. Anal. Calcd. for $C_{222}H_{230}O_{40}S$: C, 74.68; H, 6.49; S, 0.90. Found: C, 74.68; H, 6.70; S, 0.76.

*2.6. Preparation of Phenyl 4^8-O-benzoyl-2^1, 3^1, 6^1, 2^2, 3^2, 6^2, 2^3, 3^3, 6^3, 2^4, 3^4, 6^4, 2^5, 3^5, 6^5, 2^6, 3^6, 6^6, 2^7, 3^7, 6^7, 2^8, 3^8, 6^8-tetracosa-O-benzyl-1^1-thio-β-maltooctaoside **7***

Benzoyl chloride (16 μL, 140 μmol) was added dropwise to a pyridine solution (1 mL) of the 4^8-hydroxyl derivative **6** (100 mg, 28 μmol). The solution was stirred at room temperature for 4 d, quenched with ice water, and extracted with chloroform. The extract was successively washed with 1 M hydrochloric acid, aqueous saturated sodium bicarbonate, and brine, dried with sodium sulfate and evaporated. The residue was subjected to column chromatography on silica gel with toluene–ethyl acetate (30:1 v/v) to give the monobenzoate **7** (80 mg, 78%); $[\alpha]_D^{27}$ + 59° (c = 0.58, chloroform); ^{1}H NMR: δ_H 7.89 (2 H, d, J = 7.8 Hz, PhCO), 7.38 (2 H, t, J 7.9 Hz, PhCO), 7.60–6.82 (126 H, m, aromatic), 5.67 (4H, m, 1-H), 5.62 (1H, br. d, J = 3.7 Hz, 1-H), 5.54 (1H, br. d, J = 3.4 Hz, 1-H), 5.48 (1H, d, J = 3.5 Hz, 1-H), 5.36 (1H, t, J = 9.8 Hz, 4^8-H). FAB-Mass (NBA): $C_{229}H_{234}O_{41}S$ (M) 3671.60; found: m/e 3595 (M-Ph).

*2.7. Glycosidation of the macromonomer **6***

The thioglycoside **6** (100 mg, 28 μmol) was co-evaporated with dried toluene several times and dried over phosphorous pentoxide at 60°C for 24 h. To a solution of the resulting substrate **6** in dried diethyl ether (1 mL) we added activated molecular sieves 4Å (50 mg) and methyltriflate (30 μL). The suspension was stirred in a sealed tube at room temperature for 20 d, quenched with water (2 drops) and pyridine (0.5 mL), and filtered through a Celite pad. The filtrate was diluted with chloroform, successively washed with 1 M hydrochloric acid, aqueous saturated sodium bicarbonate and brine**,** dried with sodium sulfate, and evaporated. The residue was subjected to preparative gel permeation chromatography on a column of Sephadex LH-60 with chloroform–methanol (6:4, v/v) to give the perbenzylated polysaccharide **8** (19 mg); ^{1}H NMR δ_H: 6.8–7.5 (15H, m, aromatic), 5.64 (1H, br. s, H-1); ^{13}C NMR: δ_C 69.00, 71.09, 72.80, 73.42, 79.68, 81.70, 96.10, 96.92, 126.68, 126.96, 127.66, 128.18, 138.11, 138.56, 139.02.

*2.8. Deprotection of the Fully Benzylated Polysaccharide **8***

A solution of the polymer **8** fraction separated (10.4 mg) in tetrahydrofuran (5 mL) was cooled at − 80°C and ammonia gas was passed through the flask. Sodium was added by

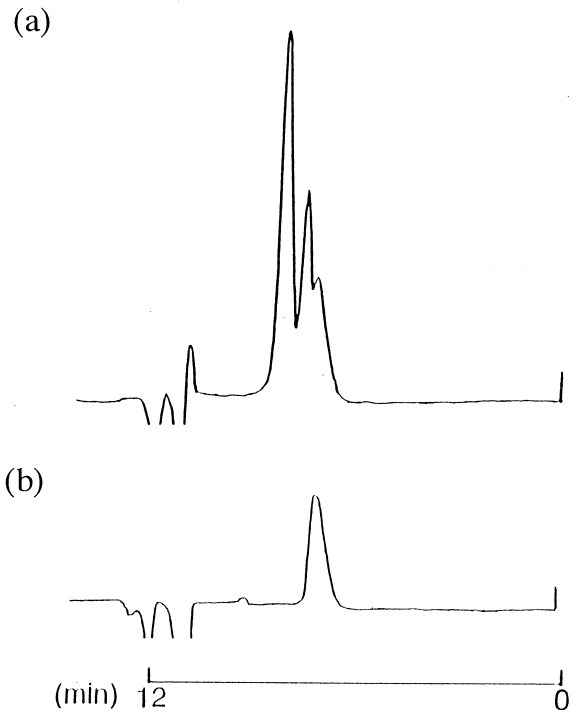


Fig. 1. Gel permeation chromatogram of reaction mixture of glycosidation (a) and purified **8** (b).

small portions to the liquid ammonia solution until the color of the solution turned blue. The mixture was stirred under reflux for 2 h, quenched by addition of ammonium chloride, and allowed to remain at room temperature overnight. The residue was dissolved in water and subjected to gel permeation chromatography on a column of Sephadex G-15 to give the unprotected polysaccharide **9** (1.5 mg); $[\alpha]_D^{26}$ + 178° (c 0.06, water); ^{1}H NMR δ_H (D_2O, at 50°C): δ_H 5.59 (1 H, br. s, H-1), 4.29–3.83 (6H, m, H-2,3,4,5,6); ^{13}C NMR (D_2O at 50°C): δ_C 61.30, 72.02, 72.31, 74.04, 77.91, 100.37; lit. (Gorin, 1981) 72.6 (C-5), 73.8 (C-2), 75.4 (C-3).

3. Results and discussion

Based on our previous findings, we chose phenyl 1-thio-β-maltooctaoside derivative **6** having a sole hydroxyl group at the C-4 position of the non-reducing end as the key monomer for the polycondensation. The acyclic octasaccharide **6** was synthesized from γ-cyclodextrin. As depicted in Scheme 1, γ-cyclodextrin was treated with acetic anhydride-pyridine to give peracetylated γ-cyclodextrin **1**, which was converted into acyclic oligosaccharide **2** by restricted acetolysis in acetic anhydride-concentrated sulfuric acid (50:1, v/v) at 50°C. The peracetate **2** was purified by silica gel column chromatography, and subjected to Lewis–acid catalyzed thioglycosidation using (phenylthio)trimethylsilane and zinc iodide to give the thioglycoside **3**,

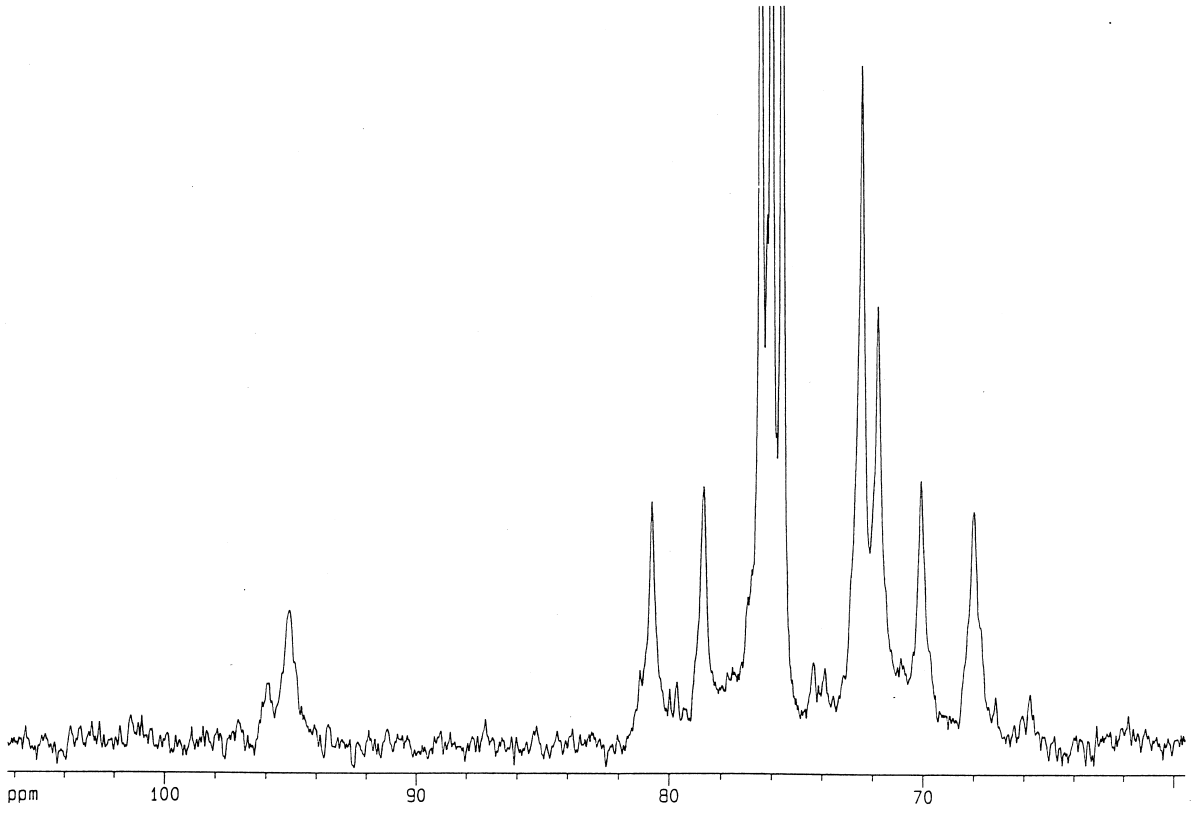


Fig. 2. 75 MHz ^{13}C NMR spectrum of benzylated polysaccharide **8** in $CDCl_3$.

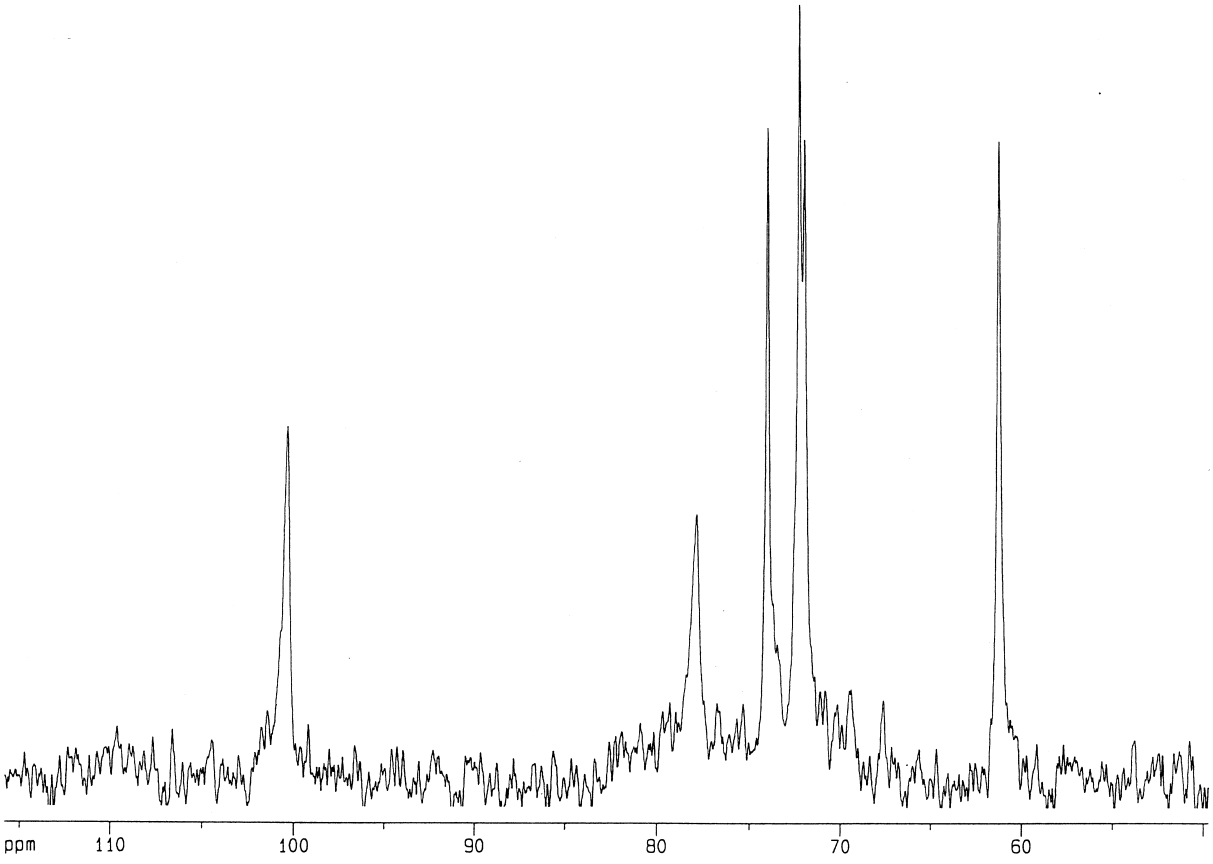


Fig. 3. 75 MHz ^{13}C NMR spectrum of synthesized α-(1,4)-glucan **9** in D_2O at 50°C.

which was found to be useful as a glycosyl donor at a later stage. To introduce a glycosyl acceptor function, **3** was then modified at the non-reducing end in four-step reactions. Removal of all acetyl groups with methanolic sodium methoxide gave a quantitative yield of the unprotected thioglycoside **4**. A D-glucopyranose moiety at the non-reducing end in **4** was discerned by its ability to form cyclic benzylidene acetal. Accordingly, **4** was treated with 1.3 mol equiv. α,α-dimethoxytoluene in *N*,*N*-dimethylformamide in the presence of (+)-camphor sulfonic acid as a catalyst to give the mono-benzylidene acetal. Without isolation, the remaining hydroxyl groups were benzylated with sodium hydride-benzyl bromide. The major product isolated by silica gel column chromatography was the 4^8,6^8-*O*-benzylidene derivative **5**, which was finally subjected to a reductive opening reaction with borane trimethylamine complex-aluminum chloride in tetrahydrofuran to give the desired 4^8-hydroxyl derivative **6**. The overall yield from **3** was 59%. The structure of **6** was confirmed following its transformation into the corresponding mono-benzoate **7**. The ^{1}H NMR spectrum of **7** showed the presence of a triplet signal attributable to H-4^8 at $\delta = 5.36$ ppm with a coupling constant of 9.8 Hz, which indicated that the benzoyl group was located at this position. We can assume, therefore, that the alcohol **6** had the expected structure of the substrate for the polycondensation reaction.

With the key intermediate **6** in our hands, we focused our attention on its reactivity for glycosidation. The thioglycoside was activated with methyl triflate (MeOTf) in the presence of molecular sieves 4Å in diethyl ether. TLC analysis of the reaction mixture showed the presence of several products and gel permeation chromatography (GPC) using polystyrene standards revealed that the molecular weights were higher than 10 000 (see Fig. 1.) The reaction mixture was worked up in the usual manner and the products were fractionated by column chromatography on Sephadex LH-60 employing chloroform-MeOH as the eluent. The fractions containing products with higher molecular weights were pooled and concentrated to give benzylated polysaccharide **8** in 10% yield. The ^{13}C NMR spectrum of **8** shows a simple and monosaccharide-like pattern suggesting its regularity (Fig. 2) and, in addition to signals of aromatic carbons (δ 126–129 ppm), C-1, C-4, and C-6 were observed at δ 96.10, 81.70, and 69.00 ppm, respectively. Therefore, the product was concluded to be the polymer of 2,3,6-tri-*O*-benzyl-α-D-glucopyranose **8**.

To convert compound **8** into free polysaccharide, removal of the *O*-benzyl groups in **8** was examined by use of Birch reduction. A solution of **8** in liquid ammonia was treated with sodium at reflux temperature ($-$ 33°C) and the product was purified by column chromatography on Sephadex G-10. The molecular weight of the isolated polysaccharide **9** was

4000–5000, which was determined by GPC using pullulan standards. The product **9** was highly water-soluble and the value of the specific rotation of in water was large and positive ($[\alpha]_D^{26}$ + 178°, c 0.06). In the ^{13}C NMR spectrum of **9** in D_2O, a signal was observed at δ 100.37 ppm, attributable to the anomeric carbon. The spectrum showed a simple pattern compatible with a regular monosaccharide repeat unit (Fig. 3), and it closely resembled that reported for amylose. Accordingly, we concluded that compound **9** is an α-(1,4)-glucan.

4. Conclusion

An amylose-like α-(1,4)-glucan was, for the first time, chemically synthesized by polymerization of a maltooctaose derivative. The use of an oligosaccharide as a macromonomer overcomes the low degree of polymerization associated with simple polycondensation methodology and thus opens the way for the synthesis of various bioactive polysaccharides with defined structures.

Acknowledgements

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