Glycoconjugate Journal 20, 427–433, 2004
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SHORT COMMUNICATION

Synthesis, protein-binding ability and phytoalexin-elicitor activity of epoxyalkyl (1→3)-β-D-oligoglucosides

Gang-Liang Huang[1], Man-Xi Liu[1], Xin-Ya Mei[2] and Yuan-Cheng Cao[1]

[1] *School of Life Science and Technology, Huazhong University of Science and Technology (East Campus), 1037 Luoyu Lu, Wuhan, 430074, China,* [2] *School of Chemistry, Central China Normal University, 100 Luoyu Lu, Wuhan, 430079, China*

We describe a approach for the synthesis of (1→3)-β-D-oligosaccharide derivatives 10–18. 1–9 were synthesized by treating peracetylated (1→3)-β-D-oligosaccharides with the corresponding alkenyl alcohols and Lewis acid ($SnCl_4$) catalyst. Epoxidation of the corresponding alkenyl oligoglucosides took place by m-CPBA. NaOMe in dry methanol was used for the deacetylation of the blocked derivatives, to give 10–18 in an overall yields of 25–32%. In subsequent glucan-binding protein of soybean assays, we found that 16 was most active, with an IC_{50} value of 9 mM. However, the activities of 17, 18, 13, 14, 15, 10, 11, and 12 were gradually decreased. At the same time, we found 16 was most active as compared to the other (1→3)-β-D- oligoglucoside derivatives in eliciting phytoalexin accumulation in soybean cotyledon tissue, and 16 was kept longer time than (1→3)-β-D-glucohexaose, which indicated 16 is much more stable than (1→3)-β-D-glucohexaose. ***Published in 2004.***

Keywords: **(1→3)-β-D-oligoglucosides, synthesis, protein-binding ability, phytoalexin-elicitor activity**

Introduction

Higher plants have the ability to initiate various defence reactions such as the production of phytoalexins, antimicrobial proteins, reactive oxygen species, and reiforcement of cell wall when they are infected by pathogens such as fungi, bacteria and viruses. If these reactions occur in a timely manner, the infection will not proceed further. However, if the defence reactions occur too late or are suppressed, the infection process will proceed successfully [1]. Thus, it is critically important for plant to detect infecting pathogens effectively and deliver such information intracellularly/intercellularly to activate their defence machinery.

It is believed that the detection of pathogens is mediated by chemical substances secreted/generated by the pathogens. Various types of such compounds (elicitor molecules) including oligosaccharides, (glyco)proteins, (glyco)peptides and lipids, have been shown to induce defence responses in plant cells and their involvement in the detection of (potential) pathogens in plant has been discussed [2–4]. Oligosaccharides derived from fungal and plant cell wall polysaccharides are one class of well characterized elicitors that, in some cases, can induce defence responses at a very low concentration e.g. nM. At the same time, the elicitor activity of oligosaccharides is dependent on the structural properties of oligosaccharide molecules, such as the number of saccharide units, the length of the aglycon chain and the molecule configuration [5]. However, the elicitor-active oligosaccharides can be hydrolysed by endo- and exohydrolases from higher plants, and give elicitor-inactive oligosaccharide fragments [6]. Therefor, improving the stability of the elicitor-active oligosaccharides is the key to developing the biological pesticide of oligosaccharides.

The use of epoxyalkyl glycosides as active-site-directed inhibitors has been invaluable in delineating the mechanism of action for a variety of hydrolases, *e.g.* β-D-glucan endo- and exohydrolases [7,8]. The epoxyalkyl glycoside moiety targets the inhibitor to the substrate-binding site and if the length of the alkyl chain is correct, the epoxide group is brought into the vicinity of the catalytic amino acids. Protonation of the epoxide oxygen opens the epoxide ring and results in the formation of a stable ester linkage between the inhibitor and the catalytic nucleophile. It has been well demonstrated [9] that the chain length of aglycon in the mechanism-based epoxide-bearing inhibitors have a significant effect on their activity.

To whom correspondence should be addressed: Gang-Liang Huang, Tel.: 86-27-87464580; E-mail: huanghgl226.student@sina.com

With the aim of improving the stability of elicitor-active oligosaccharides and the study of their protein-binding ability to glucan-binding protein of soybean and phytoalexin-elicitor activity in soybean cotyledon tissue, herein we have synthesized epoxyalkyl (1→3)-β-D-oligoglucosides having 4–6 glucosidic residues. These are analogues of oligosaccharides, where the epoxyalkyl of various chain-lengths have been introduced at the reducing ends of the glycons.

Results and discussion

One method [10] may be used to prepare epoxyalkyl (1→3)-β-D-oligoglucosides, that is to say, epoxyalkyl (1→3)-β-D-oligoglucosides are prepared by a modified Koenigs-Knorr condensation of peracetylated (1→3)-α-D-oligoglucopyranosyl bromides with the appropriate unsaturated alcohol (4-penten-1-ol, 3-buten-1-ol, and 2-propen-1-ol). The double bonds are oxidized to epoxides with m-chloroperoxybenzoic acid. Lastly, NaOMe in dry methanol at room temperature is used for the deacetylation of the blocked derivatives, to give the corresponding epoxyalkyl (1→3)-β-D-oligoglucosides. We think that the above route is very tedious and low yielding (about 14% [10]). Here, we wish to report another procedure for preparation of epoxyalkyl (1→3)-β-D-oligoglucosides, and its route shown in Scheme 1, which was one step less than that of [10].

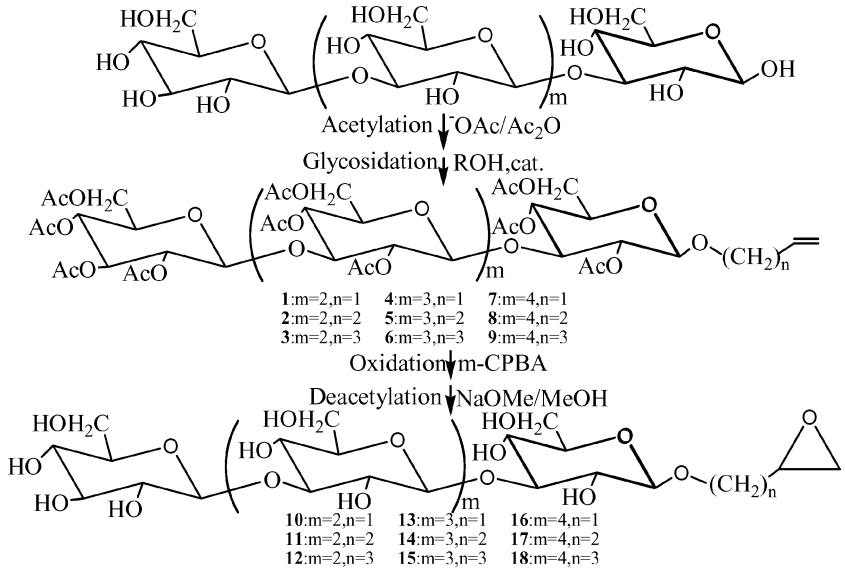
(1→3)-β-D-oligosaccharides were acetylated with potassium acetate-acetic anhydride to maximize the yield of peracetylated (1→3)-β-D-oligosaccharides. (1→3)-β-D-oligosaccharides (tetraose through hexaose) were individually acetylated to afford the respective peracetylated (1→3)-β-D-oligosaccharides in high (87–96%) yields.

To examine the effect of epoxyalkyl chain-length on elicitor activity, the peracetylated (1→3)-β-D-oligosaccharides were treated with linear alkenyl alcohols of various chain-lengths and stanntic chloride as a Lewis acid catalyst. The yields of **1**–**9** were in the range of 38–61%, with no clear dependence on the number of glucosidic residues or the alkenyl chain-length. The ^{13}C NMR spectra showed signals corresponding to the unsaturated moiety incorporated into the sugar molecule in the glycosidation reaction.

The reaction of peracetylated alkenyl (1→3)-β-D-oligoglucosides with *m*-chloroperoxybenzoic acid (*m*-CPBA) in dichloromethane at room temperature gave the corresponding oxiranes. NaOMe in dry methanol at room temperature was used for the deacetylation of the blocked derivative, to give the corresponding epoxyalkyl (1→3)-β-D-oligoglucosides **10**–**18** in an overall yields of 25–32%. Epoxidation of the alkenyl chains of peracetylated alkenyl (1→3)-β-D-oligoglucosides introduced new chiral centers at C-2,3,4 of the aglycons, respectively. In all cases, the major isomers were isolated and purified by column chromatography on silica gel. The ^{1}H and ^{13}C NMR spectra showed signals of the oxirane system for each of the compounds.

The ability of soybean β-glucan-binding sites to bind the epoxyalkyl (1→3)-β-D-oligoglucos-ides was analyzed in competition experiments with the ^{125}I-labeled hepta-β-glucoside (HG-APEA).

An hexa-β-glucoside is the biologically active motif in the hepta-β-glucoside elicitor described by Cheong et al. [11]. Affinity measurements at the β-glucan-binding sites gave apparent K_d values of about 1 to 3 nM for the hepta-β-glucoside [12,13]. Competition for binding of HG-APEA by increasing concentrations of **10**–**18** demonstrated progressive inhibition of binding of radioiodinated HG-APEA. The concentrations of the different epoxyalkyl (1→3)-β-D-oligoglucosides required to inhibit binding of the radioligand at the 50% level (IC_{50}



Scheme 1. Synthetic route for epoxyalkyl (1 → 3)-β-D-oligoglucosides.

Table 1. Binding and phytoalexin elicitor activity of (1→3)-β-D-oligosaccharides and their epoxyalkyl derivatives

Compound	*Ligand competition (IC_{50}) (mM)*	*Biological activity (EC_{50}) (mM)*		
		22 h	*44 h*	*66 h*
Control (H_2O)	Infinity	Infinity	Infinity	Infinity
Tetraose	18	80.2	500	800
Pentaose	13	55	500	800
Hexaose	10.6	22.1	500	800
10	15	80	100	200
11	15.2	80	102	200
12	15.9	81	100	202
13	11.4	55	80	157
14	11.7	54.7	80	161
15	12	55	84	161
16	9	22	30	100
17	9.5	22.4	38	130
18	9.8	22.9	43	136

values) were shown in Table 1. We found that **16** was most active, with an IC_{50} value of 9 mM. **16** was, however, about three orders of magnitude less active as competitors than the natural hepta-*O*-glucoside [12,13]. The activities of **17**, **18**, **13**, **14**, **15**, **10**, **11**, and **12** were gradually decreased.

The ability of each of **10**–**18** to induce phytoalexin accumulation in soybean cotyledon tissue was determined. The results of these bioassays, shown in Table 1, demonstrated that **10**–**18** were equally biologically active as compared to the corresponding (1→3)-β-D-oligoglucosides at early time points, but were more active than the corresponding (1→3)-β-D-oligoglucosides at late time points which was possible because the oligosaccharide-hydrolase complexes were unstable, so the oligosaccharides would have been hydrolyzed gradually by hydrolases. It indicated the reducing end of the β-glucoside was not important for maximum biological activity, and **10**–**18** were much more stable than the corresponding (1→3)-β-D-oligoglucosides. Again, we found **16** was most active among **10**–**18**, which indicate those oligoglucosides having a high elicitor activity were efficient competitors of the radiolabeled ligand, whereas biologically less active oligoglucosides were less efficient. Higher stability and activity suggest that **16** might be a useful candidate for inducing phytoalexin accumulation in soybean cotyledon tissue.

Experimental section

General

Optical rotations were determined with a Perkin-Elmer Model 241 MC polarimeter at ambient temperature (25°C). All ^1H and ^{13}C NMR spectra were recorded on a varian XL-300 spectrometer using $CDCl_3$ or D_2O. Broad-band decoupling NMR technique is used for the ^{13}C-NMR peak assignments in compounds 10–18. ^1H (300 MHz) and ^{13}C (75 MHz) chemical shifts are reported relative to TMS as internal standard. IR spectra were recorded with FT-IR apparatus, and wave numbers are reported in cm^{-1}. Negative-ion FAB-MS were measured on a VG Autospec 3000 mass spectrometer (Micromass, Manchester, UK). Column chromatography was carried out with silica gel (70–230 mesh). Kieselgel $60F_{254}$ (E. Merck) was used for TLC. (1→3)-β-D-tetraglucoside, (1→3)-β-D-pentaglucoside, and (1→3)-β-D-hexaglucoside were purchased from Sigma Aldrich Chemical Company. Solvents dichloromethane and 1,2-dichloroethane were distilled from P_2O_5.

Plant material and chemicals

Soybean (*Glycine* max L. cv. Canton) seeds were obtained from Asgrow (Yunan province, China). The hepta-(1→3)-(1→6)-β-glucoside was from Biocarb (Guizhou province, China).

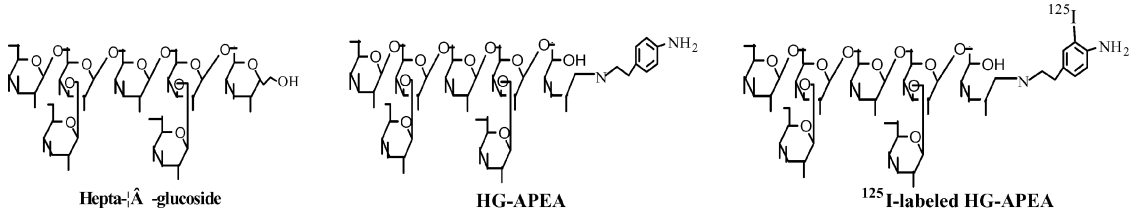


Figure 1. Stuctures of hepta-β-glucoside, HG-APEA and ^{125}I-labeled HG-APEA.

Binding assays

The 2-(4-aminophenyl) ethylamine conjugate of the hepta-β-glucoside (HG-APEA) was prepared and radioiodinated as described previously [14,15]. The average specific radioactivity of the radioligand was 10 TBq $mmol^{-1}$. Binding assays were carried out using a standardized glucan- binding assay [12]. Inflection points (IC_{50} values) were obtained from ligand competition experiments using increasing concentrations of **10**–**18** as competitors. Protein content was measured according to Bradford [16].

Biological activity assays

Detached cotyledons from 5-d-old greenhouse seedlings of the soybean cultivar Canton were cut and aliquots of β-glucooligosaccharide solutions (60 mL) were placed on wounded areas. The cotyledons were incubated for 22 h, 44 h, and 66 h at 27°C on moist filter paper in Petri dishes in the dark, respectively. Phytoalexin accumulation in the wound-droplet solutions was determined by measuring the absorbance (A) at 285 nm.

General procedure for preparing **1**–**9**

To boiling Ac_2O (20 mL) in a three-necked flask, 200 mg of KOAc was added. Then, 200 mg of pentaose was gradually added under vigorous stirring. The solution was kept for 1 h at 140°C, and then cooled to room temperature. The peracetate was purified by column chromatography on silica gel. A portion of this material (350 mg) and 30 mg of 3-buten-1-ol were added to 21 mL of dry 1,2-dichloroethane at 45°C, followed by the addition of 58 mg of $SnCl_4$. The mixture was stirred for 5 h. After standard processing work-up, 190 mg of peracetylated 3-butenyl (1→3)-β-D-pentaoside **5** were obtained. Other oligoglucosides were synthesized similarly.

*2-Propenyl (2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1→3)-bis[(2,4,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→3)]-2,4,6-tri-O-acetyl-β-D-glucopyranoside (**1**)*

53% yield; ^{13}C NMR (75 MHz, $CDCl_3$): 170.7–164.3 (13C=O), 133.3 ($OCH_2\underline{C}H{=}CH_2$), 117.7 ($OCH_2CH{=}\underline{C}H_2$), 101.5, 101.0, 100.8, 100.5 (C-1^1, 1^2, 1^3, 1^4), 78.9, 78.3, 78.2 (C-3^1, 3^2, 3^3), 62.2, 62.1, 61.95, 61.7 (C-6^1, 6^2, 6^3, 6^4); Anal. Calcd. for $C_{53}H_{72}O_{34}$: C, 50.80; H, 5.75. Found: C, 50.72; H, 5.88.

*3-Butenyl (2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1→3)-bis[(2,4,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→3)]-2,4,6-tri-O-acetyl-β-D-glucopyranoside (**2**)*

56% yield; ^{13}C NMR (75 MHz, $CDCl_3$): 170.7–169.3 (13C=O), 134.4 ($OCH_2CH_2\underline{C}H{=}CH_2$), 116.8 ($OCH_2CH_2CH{=}\underline{C}H_2$), 101.5, 101.0, 100.8, 100.5 (C-1^1, 1^2, 1^3, 1^4), 78.9, 78.3, 78.2 (C-3^1, 3^2, 3^3), 62.2, 62.1, 61.95, 61.7 (C-6^1, 6^2, 6^3, 6^4); Anal. Calcd. for $C_{54}H_{74}O_{34}$: C, 51.18; H, 5.85. Found: C, 51.33; H, 5.59.

*4-Pentenyl (2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1→3)-bis[(2,4,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→3)]-2,4,6-tri-O-acetyl-β-D-glucopyranoside (**3**)*

65% yield; ^{13}C NMR (75 MHz, $CDCl_3$): 170.7–169.3 (13C=O), 137.8 ($OCH_2CH_2CH_2\underline{C}H{=}CH_2$), 115.1 ($OCH_2CH_2CH_2CH{=}\underline{C}H_2$), 101.5, 101.0, 100.8, 100.5 (C-1^1, 1^2, 1^3, 1^4), 78.9, 78.3, 78.2 (C-3^1, 3^2, 3^3), 62.2, 62.1, 61.95, 61.7 (C-6^1, 6^2, 6^3, 6^4); Anal. Calcd. for $C_{55}H_{76}O_{34}$: C, 51.56; H, 5.94. Found: C, 51.70; H, 5.81.

*2-Propenyl (2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1→3)-tris[(2,4,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→3)]-2,4,6-tri-O-acetyl-β-D-glucopyranoside (**4**)*

50% yield; ^{13}C NMR (75 MHz, $CDCl_3$): 170.8–164.7 (16C=O), 133.6 ($OCH_2\underline{C}H{=}CH_2$), 117.7 ($OCH_2CH{=}\underline{C}H_2$), 101.5, 101.0, 100.8, 100.6, 100.5 (C-1^1, 1^2, 1^3, 1^4, 1^5), 78.95, 78.3, 78.2 (2C) (C-3^1, 3^2, 3^3, 3^4), 62.2, 62.1, 62.05, 61.9, 61.7 (C-6^1,6^2, 6^3, 6^4, 6^5); Anal. Calcd. for $C_{65}H_{88}O_{42}$: C, 50.65; H, 5.71. Found: C, 50.77; H, 5.87.

*3-Butenyl (2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1→3)-tris[(2,4, 6-tri-O-acetyl-β-D-glucopyranosyl)-(1→3)]-2,4,6-tri-O-acetyl-β-D-glucopyranoside (**5**)*

57% yield; ^{13}C NMR (75 MHz, $CDCl_3$): 170.8–164.7 (16C=O), 135.0 ($OCH_2CH_2\underline{C}H{=}CH_2$), 116.8 ($OCH_2CH_2CH{=}\underline{C}H_2$), 101.5, 101.0, 100.8, 100.6, 100.5 (C-1^1, 1^2, 1^3, 1^4, 1^5), 78.95, 78.3, 78.2 (2C) (C-3^1, 3^2, 3^3, 3^4), 62.2, 62.1, 62.05, 61.9, 61.7 (C-6^1, 6^2, 6^3, 6^4, 6^5); Anal. Calcd. for $C_{66}H_{90}O_{42}$: C, 50.97; H, 5.79. Found: C, 51.06; H, 6.00.

*4-Pentenyl (2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1→3)-tris[(2,4,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→3)]-2,4,6-tri-O-acetyl-β-D-glucopyranoside (**6**)*

68% yield; ^{13}C NMR (75 MHz, $CDCl_3$): 170.8–164.7 (16C=O), 138.0 ($OCH_2CH_2CH_2\underline{C}H{=}CH_2$), 115.1 ($OCH_2CH_2CH_2CH_2CH{=}\underline{C}H_2$), 101.5, 101.0, 100.8, 100.6, 100.5 (C-1^1, 1^2, 1^3, 1^4, 1^5), 78.95, 78.3, 78.2 (2C) (C-3^1, 3^2, 3^3, 3^4), 62.2, 62.1, 62.05, 61.9, 61.7 (C-6^1, 6^2, 6^3, 6^4, 6^5); Anal. Calcd. for $C_{67}H_{92}O_{42}$: C, 51.28; H, 5.87. Found: C, 51.46; H, 5.70.

*2-Propenyl (2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1→3)-tetrakis[(2,4,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→3)]-2,4,6-tri-O-acetyl-β-D-glucopyranoside (**7**)*

58% yield; ^{13}C NMR (75 MHz, $CDCl_3$): 171.0–165.1 (19C=O), 134.0 ($OCH_2\underline{C}H{=}CH_2$), 118.1 ($OCH_2CH{=}\underline{C}H_2$), 101.5, 101.0, 100.8, 100.7 (2C), 100.5 (C-1^1, 1^2, 1^3, 1^4, 1^5, 1^6), 79.0, 78.35 (2C), 78.3, 78.25 (C-3^1, 3^2, 3^3, 3^4, 3^5), 62.2, 62.1, 62.0 (2C), 61.9, 61.7 (C-6^1, 6^2, 6^3, 6^4, 6^5, 6^6); Anal. Calcd. for $C_{77}H_{104}O_{50}$: C, 50.55; H, 5.69. Found: C, 50.39; H, 5.79.

*3-Butenyl (2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1→3)-tetrakis[(2,4,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→3)]-2,4,6-tri-O-acetyl-β-D-glucopyranoside (**8**)*

55% yield; ^{13}C NMR (75 MHz, $CDCl_3$): 171.0–165.1 (19C=O), 135.5 ($OCH_2CH_2\underline{C}H{=}CH_2$), 116.9 ($OCH_2CH_2CH{=}\underline{C}H_2$), 101.5, 101.0, 100.8, 100.7 (2C), 100.5 (C-1^1, 1^2, 1^3, 1^4, 1^5, 1^6), 79.0, 78.35 (2C), 78.3, 78.25 (C-3^1, 3^2, 3^3, 3^4, 3^5), 62.2, 62.1, 62.0 (2C), 61.9, 61.7 (C-6^1, 6^2, 6^3, 6^4, 6^5, 6^6); Anal. Calcd. for $C_{78}H_{106}O_{50}$: C, 50.81; H, 5.75. Found: C, 50.90; H, 5.53.

*4-Pentenyl (2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1→3)-tetrakis[(2,4,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→3)]-2,4,6-tri-O-acetyl-β-D-glucopyranoside (**9**)*

69% yield; ^{13}C NMR (75 MHz, $CDCl_3$): 171.0–165.1 (19C=O), 138.1 ($OCH_2CH_2CH_2\underline{C}H{=}CH_2$), 115.1 ($OCH_2CH_2CH_2CH{=}\underline{C}H_2$), 101.5, 101.0, 100.8, 100.7 (2C), 100.5 (C-1^1, 1^2, 1^3, 1^4, 1^5, 1^6), 79.0, 78.35 (2C), 78.3, 78.25 (C-3^1, 3^2, 3^3, 3^4, 3^5), 62.2, 62.1, 62.0 (2C), 61.9, 61.7 (C-6^1, 6^2, 6^3, 6^4, 6^5, 6^6); Anal. Calcd. for $C_{79}H_{108}O_{50}$: C,51.08; H, 5.82. Found: C, 51.25; H, 5.91.

General Procedure for Synthesis of **10**–**18**

To a solution of **1**–**9** (0.38 mmol) in dichloromethane (7 mL), *m*-chloroperoxybenzoic acid (1.0 mmol) was added and the suspension was stirred. When TLC shown that all starting compound had been consumed, the reaction mixture was washed successively with a 5% aqueous solution of sodium hydroxide and water, dried ($MgSO_4$), filtered, and the filtrate evaporated to dryness. The solids obtained were purified by recrystallization from ethanol or by column chromatography on silica gel. The obtained materials were stored in the dark at 4°C. Prior to use, they were suspended in anhydrous MeOH to a concentration of 100 mg/mL and deacetylated with an equal volume of 1M NaOMe at room temperature for 60 min with continuous mixing. The sample was then desalted and neutralized by addition of Amberlite IRC-50 (H^+) ion-exchange resin (40 mg/mg inhibitor) previously washed in MeOH. After 30 min of vigorous shaking, the deacetylated inhibitor was recovered and the IRC-50 ion exchanger washed with MeOH. Then, the combined washings were evaporated to dryness under reduced pressure at 45°C.

*2,3-Epoxypropyl β-D-glucopyranosyl-(1→3)-bis[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (**10**)*

51% yield, amorphous solid; $[\alpha]_D$-27° (c 1.5, H_2O); ^{1}HNMR (300 MHz, D_2O): δ = 2.61–2.82[m, 2H, $OCH_2CH(O)C\underline{H}_2$], 3.23 (m, 12H, H-2, 4, 5), 3.25[m, 1H, $OCH_2C\underline{H}(O)CH_2$], 3.48 (m, 7H, H-3, 6), 3.70 (m, 4H, H-6), 4.40 (m, 4H, H-1), 4.52 (br. s, 8H, OH-4, 6), 5.01 (br. s, 4H, OH-2), 5.39 (br. s, 1H, OH-3); ^{13}C NMR (75 MHz, D_2O): 103.8, 103.6 (C-1^1, 1^4), 103.4 (2C) (C-1^2, 1^3), 85.4 (C-3^1), 85.1 (C-3^2), 84.95 (C-3^3), 76.8 (C-5^4), 76.4 (4C) (C-3^4, 5^1, 5^2, 5^3), 74.3 (C-2^4), 74.1, 74.05 (C-2^2, 2^3), 73.6 (C-2^1), 70.4 (C-4^4), 69.0, 68.95, 68.9 (C-4^1, 4^2, 4^3), 61.5 (4C) (C-6^1, 6^2, 6^3, 6^4), 50.4 [$OCH_2\underline{C}H(O)CH_2$], 44.2,44.1 [$OCH_2CH(O)\underline{C}H_2$]; IR (solid): 3375, 2920, 1370, 1270, 1080, 890, 839; Negative-ion FAB-MS (*m/z*): 721[M-H]$^-$; Anal. Calcd. for $C_{27}H_{46}O_{22}$: C, 44.88; H, 6.37. Found: C, 44.77; H, 6.45.

*3,4-Epoxybutyl β-D-glucopyranosyl-(1→3)-bis[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (**11**)*

68% yield, amorphous solid; $[\alpha]_D$-27° (c 1.5, H_2O); ^{1}H NMR (300 MHz, D_2O): δ = 1.60 [m, 2H, $OCH_2C\underline{H}_2CH(O)CH_2$], 2.56–2.81 [m, 2H, $OCH_2CH_2CH(O)C\underline{H}_2$], 3.06 [m, 1H, $OCH_2CH_2C\underline{H}(O)CH_2$], 3.23 (m, 12H, H-2, 4, 5), 3.48 (m, 7H, H-3, 6), 3.70 (m, 4H, H-6), 4.05 [m, 2H, $OC\underline{H}_2CH_2CH(O)CH_2$], 4.45 (m, 4H, H-1), 4.52 (br. s, 8H, OH-4, 6), 5.01 (br. s, 4H, OH-2), 5.39 (br. s, 1H, OH-3); ^{13}C NMR (75MHz, D_2O): 103.8, 103.6 (C-1^1, 1^4), 103.4 (2C) (C-1^2, 1^3), 85.4 (C-3^1), 85.1 (C-3^2), 84.95 (C-3^3), 76.8 (C-5^4), 76.4 (4C) (C-3^4, 5^1, 5^2, 5^3), 74.3 (C-2^4), 74.1, 74.05 (C-2^2, 2^3), 73.6 (C-2^1), 70.4 (C-4^4), 69.0, 68.95, 68.9 (C-4^1, 4^2, 4^3), 61.5 (4C) (C-6^1, 6^2, 6^3, 6^4), 50.0[$OCH_2CH_2\underline{C}H(O)CH_2$], 47.3, 47.0[$OCH_2CH_2CH(O)\underline{C}H_2$]; IR (solid): 3375, 2920, 1370, 1269, 1080, 890, 839; Negative-ion FAB-MS (*m/z*): 735 [M-H]$^-$; Anal. Calcd. for $C_{28}H_{48}O_{22}$: C, 45.65; H, 6.52. Found: C, 45.57; H, 6.68.

*4,5-Epoxypentyl β-D-glucopyranosyl-(1→3)-bis[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (**12**)*

70% yield, amorphous solid; $[\alpha]_D$-27° (c 1.5, H_2O); ^{1}H NMR (300 MHz, D_2O): δ = 1.42 [m, 2H, $OCH_2CH_2C\underline{H}_2CH(O)CH_2$], 1.53 [m, 2H, $OCH_2C\underline{H}_2CH_2CH(O)CH_2$], 2.50–2.75 [m, 2H, $OCH_2CH_2CH_2CH(O)C\underline{H}_2$], 3.00 [m, 1H, $OCH_2CH_2CH_2C\underline{H}(O)CH_2$], 3.23 (m, 12H, H-2, 4, 5), 3.48 (m, 7H, H-3, 6), 3.70 (m, 4H, H-6), 3.99 [m, 2H, $OC\underline{H}_2CH_2CH_2CH(O)CH_2$], 4.45 (m, 4H, H-1), 4.52 (br. s, 8H, OH-4, 6), 5.01 (br. s, 4H, OH-2), 5.39 (br. s, 1H, OH-3); ^{13}C NMR (75 MHz, D_2O): 103.8, 103.6 (C-1^1, 1^4), 103.4 (2C) (C-1^2, 1^3), 5.4 (C-3^1), 85.1 (C-3^2), 84.95 (C-3^3), 76.8 (C-5^4), 6.4 (4C) (C-3^4, 5^1, 5^2, 5^3), 74.3 (C-2^4), 74.1, 74.05 (C-2^2, 2^3), 73.6 (C-2^1), 0.4 (C-4^4), 69.0, 68.95, 68.9 (C-4^1, 4^2,4^3), 61.5 (4C) (C-6^1, 6^2, 6^3, 6^4), 54.5[$OCH_2CH_2CH_2\underline{C}H(O)CH_2$], 48.9, 48.2[$OCH_2CH_2CH_2CH(O)\underline{C}H_2$]; IR (solid): 3375, 2920, 1370, 1265, 1080, 890, 837; Negative-ion FAB-MS (*m/z*): 749[M-H]$^-$; Anal. Calcd. for $C_{29}H_{50}O_{22}$: C, 46.40; H, 6.67. Found: C,4 6.29; H, 6.77.

*2,3-Epoxypropyl β-D-glucopyranosyl-(1→3)-tris[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (**13**)*

49% yield, amorphous solid; $[\alpha]_D$-26° (c 1.0, H_2O); ^{1}HNMR (300 MHz, D_2O): δ = 2.61–2.82 [m, 2H, $OCH_2CH(O)C\underline{H}_2$], 3.23 (m, 12H, H-2, 4, 5), 3.25 [m, 1H, $OCH_2C\underline{H}(O)CH_2$], 3.49 (m, 10H, H-3, 6), 3.70 (m, 5H, H-6), 4.49 (m, 5H, H-1), 4.54 (br. s, 10H, OH-4, 6), 5.05 (br. s, 5H, OH-2), 5.39

(br. s, 1H, OH-3); ^{13}C NMR (75 MHz, D_2O): 103.8, 103.65 (C-1^1, 1^5), 103.4 (3C)(C-1^2, 1^3, 1^4), 85.4 (C-3^1), 85.2 (C-3^2), 85.0 (2C) (C-3^3, 3^4), 6.8 (C-5^5), 76.45, 76.4 (5C) (C-3^5, 5^1, 5^2, 5^3, 5^4), 74.3 (C-2^5), 74.1 (3C) (C-2^2, 2^3, 2^4), 73.6 (C-2^1), 70.4 (C-4^5), 69.0, 68.9 (4C) (C-4^1, 4^2, 4^3, 4^4), 61.55 (5C) (C-6^1, 6^2, 6^3, 6^4, 6^5), 50.4[$OCH_2\underline{C}H(O)CH_2$], 44.2, 44.1[$OCH_2CH(O)\underline{C}H_2$]; IR (solid): 3369, 2920, 1370, 1270, 1080, 890, 839; Negative-ion FAB-MS (*m/z*): 883[M-H]$^-$; Anal. Calcd. for $C_{33}H_{56}O_{27}$: C, 44.80; H, 6.33. Found: C, 4.68; H, 6.45.

*3,4-Epoxybutyl β-D-glucopyranosyl-(1→3)-tris[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (**14**)*

68% yield, amorphous solid; $[\alpha]_D$-26° (c 1.0, H_2O); ^{1}HNMR (300 MHz, D_2O): δ = 1.60 [m, 2H, $OCH_2\underline{CH_2}CH(O)CH_2$], 2.56–2.81 [m, 2H, $OCH_2CH_2CH(O)\underline{CH_2}$], 3.06 [m, 1H, $OCH_2CH_2\underline{C}H(O)CH_2$], 3.23 (m, 15H, H-2, 4, 5), 3.48 (m, 10H, H-3, 6), 3.70 (m, 5H, H-6), 4.05 [m, 2H, $O\underline{CH_2}CH_2CH(O)CH_2$], 4.48 (m, 5H, H-1), 4.52 (br. s, 10H, OH-4, 6), 5.01 (br. s, 5H, OH-2), 5.39 (br. s, 1H, OH-3); ^{13}C NMR (75 MHz, D_2O): 103.8, 103.65 (C-1^1, 1^5), 103.4 (3C) (C-1^2, 1^3, 1^4), 85.4 (C-3^1), 85.2 (C-3^2), 85.0 (2C) (C-3^3, 3^4), 76.8 (C-5^5), 76.45, 6.4 (5C) (C-3^5, 5^1, 5^2, 5^3, 5^4), 74.3 (C-2^5), 74.1 (3C) (C-2^2, 2^3, 2^4), 73.6 (C-2^1), 70.4 (C-4^5), 69.0, 68.9 (4C) (C-4^1, 4^2, 4^3, 4^4), 61.55 (5C) (C-6^1, 6^2, 6^3, 6^4, 6^5), 50.0 [$OCH_2CH_2\underline{C}H(O)CH_2$], 47.3, 47.0 [$OCH_2CH_2CH(O)\underline{C}H_2$]; IR (solid): 3369, 2920, 1370, 1269, 1080, 890, 839; Negative-ion FAB-MS (*m/z*): 897[M-H]$^-$; Anal. Calcd. for $C_{34}H_{58}O_{27}$: C, 45.43; H, 6.46. Found: C, 45.60; H, 6.29.

*4,5-Epoxypentyl β-D-glucopyranosyl-(1→3)-tris[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (**15**)*

76% yield, amorphous solid; $[\alpha]_D$-26° (c 1.0, H_2O); ^{1}H NMR (300 MHz, D_2O): δ = 1.42 [m, 2H, $OCH_2CH_2\underline{CH_2}$ $CH(O)CH_2$], 1.53 [m, 2H, $OCH_2\underline{CH_2}CH_2CH(O)CH_2$], 2.50-2.75 [m, 2H, $OCH_2CH_2CH_2CH(O)\underline{CH_2}$], 3.00 [m, 1H, $OCH_2CH_2CH_2\underline{C}H(O)CH_2$], 3.23 (m, 15H, H-2, 4, 5), 3.48 (m, 10H, H-3, 6), 3.70 (m, 5H, H-6), 3.99 [m, 2H, $O\underline{CH_2}CH_2CH_2CH(O)CH_2$], 4.45 (m, 5H, H-1), 4.52 (br. s, 10H, OH-4, 6), 5.01 (br. s, 5H, OH-2), 5.39 (br. s, 1H, OH-3); ^{13}C NMR (75 MHz, D_2O): 103.8, 103.65 (C-1^1, 1^5), 103.4 (3C) (C-1^2, 1^3, 1^4), 85.4 (C-3^1), 5.2 (C-3^2), 85.0 (2C) (C-3^3, 3^4), 76.8 (C-5^5), 76.45, 76.4 (5C) (C-3^5, 5^1, 5^2, 5^3, 5^4), 74.3 (C-2^5), 74.1 (3C) (C-2^2, 2^3, 2^4), 73.6 (C-2^1), 70.4 (C-4^5), 69.0, 68.9 (4C) (C-4^1, 4^2, 4^3, 4^4), 61.55 (5C) (C-6^1, 6^2, 6^3, ^{4}, 6^5), 54.5[$OCH_2CH_2CH_2\underline{C}H(O)CH_2$], 48.9, 48.2[$OCH_2CH_2CH_2CH(O)\underline{C}H_2$]; IR (solid): 3369, 2920, 1370, 1265, 1080, 890, 837; Negative-ion FAB-MS (*m/z*): 911[M-H]$^-$; Anal. Calcd. for $C_{35}H_{60}O_{27}$: C, 46.05; H, 6.58. Found: C, 46.90; H, 6.68.

*2,3-Epoxypropyl β-D-glucopyranosyl-(1→3)-tetrakis[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (**16**)*

55% yield, amorphous solid; $[\alpha]_D$-25° (c 1.2, H_2O); ^{1}HNMR (300 MHz, D_2O): δ = 2.61–2.82[m, 2H, OCH_2-$CH(O)C\underline{H_2}$], 3.23 (m, 18H, H-2, 4, 5), 3.25[m, 1H, $OCH_2C\underline{H}(O)CH_2$], 3.50 (m, 12H, H-3, 6), 3.72 (m, 6H, H-6), 4.58 (m, 6H, H-1), 4.61 (br. s, 12H, OH-4, 6), 5.15 (br. s, 6H, OH-2), 5.40 (br. s, 1H, OH-3); ^{13}C NMR (75 MHz, D_2O): 103.8, 103.65 (C-1^1, 1^6), 103.4 (4C) (C-1^2, 1^3, 1^4, 1^5), 85.4 (C-3^1), 85.2 (C-3^2), 5.0 (3C) (C-3^3, 3^4, 3^5), 76.8 (C-5^6), 76.45, 76.4 (6C) (C-3^6, 5^1, 5^2, 5^3, 5^4, 5^5), 74.3 (C-2^6), 74.1 (4C) (C-2^2, 2^3, 2^4, ^{5}), 73.6 (C-2^1), 70.4 (C-4^6), 69.0, 68.9 (5C) (C-4^1, 4^2, 4^3, 4^4, 4^5), 61.5 (6C) (C-6^1, 6^2, 6^3, 6^4, 6^5, 6^6), 50.4 [$OCH_2\underline{C}H(O)CH_2$], 44.2, 44.1 [$OCH_2CH(O)\underline{C}H_2$]; IR (solid): 3358, 2920, 1370, 1270, 1080, 890, 839; Negative-ion FAB-MS (*m/z*): 1045[M-H]$^-$; Anal. Calcd. for $C_{39}H_{66}O_{32}$: C, 50.98; H, 7.19. Found: C, 50.90; H, 7.35.

*3,4-Epoxybutyl β-D-glucopyranosyl-(1→3)-tetrakis[β-D-glucopyranosyl-(1→3)] -β-D-glucopyranoside (**17**)*

78% yield, amorphous solid; $[\alpha]_D$-25° (c 1.2, H_2O); ^{1}HNMR (300 MHz, D_2O): δ = 1.60 [m, 2H, $OCH_2\underline{CH_2}$ $CH(O)CH_2$], 2.56–2.81 [m, 2H, $OCH_2CH_2CH(O)\underline{CH_2}$], 3.06 [m, 1H, $OCH_2CH_2\underline{C}H(O)CH_2$], 3.23 (m, 18H, H-2, 4, 5), 3.48 (m, 12H, H-3, 6), 3.70 (m, 6H, H-6), 4.05 [m, 2H, $O\underline{CH_2}CH_2CH(O)CH_2$], 4.50 (m, 6H, H-1), 4.53 (br. s, 12H, OH-4, 6), 5.07 (br. s, 6H, OH-2), 5.39 (br. s, 1H, OH-3); ^{13}C NMR (75 MHz, D_2O): 103.8, 103.65 (C-1^1, 1^6), 103.4 (4C) (C-1^2, 1^3, 1^4, 1^5), 85.4 (C-3^1), 85.2 (C-3^2), 85.0 (3C) (C-3^3, 3^4, 3^5), 76.8 (C-5^6), 76.45, 76.4 (6C) (C-3^6, 5^1, 5^2, 5^3, 5^4, 5^5), 74.3 (C-2^6), 74.1 (4C) (C-2^2, 2^3, 2^4, 2^5), 73.6 (C-2^1), 70.4 (C-4^6), 69.0, 68.9 (5C) (C-4^1, 4^2, 4^3, 4^4, 4^5), 61.5 (6C) (C-6^1, 6^2, 6^3, 6^4, 6^5, 6^6), 50.0[$OCH_2CH_2\underline{C}H(O)CH_2$], 47.3, 47.0[$OCH_2CH_2CH(O)\underline{C}H_2$]; IR (solid): 3358, 2920, 1370, 1269, 1080, 890, 839; Negative-ion FAB-MS (*m/z*): 1059[M-H]$^-$; Anal. Calcd. for $C_{40}H_{68}O_{32}$: C, 51.50; H, 7.30. Found: C, 51.31; H, 7.41.

*4,5-Epoxypentyl β-D-glucopyranosyl-(1→3)-tetrakis[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (**18**)*

77% yield, amorphous solid; $[\alpha]_D$-25° (c 1.2, H_2O); ^{1}HNMR (300 MHz, D_2O): δ = 1.42 [m, 2H, $OCH_2CH_2C\underline{H_2}CH(O)CH_2$], 1.53 [m, 2H, $OCH_2C\underline{H_2}CH_2CH(O)CH_2$], 2.50–2.75 [m, 2H, $OCH_2CH_2CH_2CH(O)C\underline{H_2}$], 3.00 [m, 1H, $OCH_2CH_2CH_2C\underline{H}(O)CH_2$], 3.27 (m, 18H, H-2, 4, 5), 3.49 (m, 12H, H-3, 6), 3.70 (m, 6H, H-6), 3.99 [m, 2H, $O\underline{CH_2}CH_2CH_2CH(O)CH_2$], 4.50 (m, 6H, H-1), 4.57 (br. s, 12H, OH-4, 6), 5.01 (br. s, 6H, OH-2), 5.41 (br. s, 1H, OH-3); ^{13}C NMR (75 MHz, D_2O): 103.8, 103.65 (C-1^1, 1^6), 103.4 (4C) (C-1^2, 1^3, 1^4, 1^5), 85.4 (C-3^1), 85.2 (C-3^2), 85.0 (3C) (C-3^3, 3^4, 3^5), 76.8 (C-5^6), 76.45, 76.4 (6C) (C-3^6, 5^1, 5^2, 5^3, 5^4, 5^5), 74.3 (C-2^6), 74.1 (4C) (C-2^2, 2^3, 2^4, 2^5), 73.6 (C-2^1), 70.4 (C-4^6), 69.0, 68.9 (5C) (C-4^1, 4^2, 4^3, 4^4, 4^5), 61.5 (6C) (C-6^1, 6^2, 6^3, 6^4, 6^5, 6^6), 54.5[$OCH_2CH_2CH_2\underline{C}H(O)CH_2$], 48.9, 48.2[$OCH_2CH_2CH_2CH(O)\underline{C}H_2$]; IR (solid): 3358, 2920, 1370, 1265, 1080, 890, 837; Negative-ion FAB-MS (*m/z*):

1073 $[M\text{-}H]^-$; Anal. Calcd. for $C_{41}H_{70}O_{32}$: C, 52.01; H, 7.40. Found: C, 52.20; H, 7.35.

Acknowledgments

The project was financially supported by "The Scaling the Height Program" of the State Science and Technology Committee of China.

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Received 19 December 2003; revised 25 March 2004;
accepted 26 April 2004