Preparation of Three Positional Isomers of Diglucosyl-cyclomaltohexaose

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Three positional isomers of diglucosyl-cyclomaltohexaose (diglucosyl-cG$_6$) were chemically synthesized *via* 6^1,6^2-, 6^1,6^3-, and 6^1,6^4-di-*O*-(*tert*-butyldimethylsilyl)-cG$_6$s (1, 2, and 3) prepared regiospecifically. Glucosylation of bis(2,3-di-*O*-acetyl)tetrakis(2,3,6-tri-*O*-acetyl)-cG$_6$s obtained from the three regioisomeric compounds 1, 2, and 3 with 2,3,4,6-tetra-*O*-benzyl-1-*O*-trichloroacetimidoyl-α-D-glucopyranose, followed by debenzylation and then deacetylation, afforded 6^1,6^2-, 6^1,6^3-, and 6^1,6^4-di-*O*-(α-D-glucopyranosyl)-cG$_6$s (10, 11, and 12) together with configurational isomers. The desired compounds 10, 11, and 12 containing two (1→6)-α-linkages were isolated from the mixtures of their configurational isomers by high performance liquid chromatography. The three diglucosyl-cG$_6$s synthesized chemically were used as authentic samples to identify the components in a mixture of diglucosyl-cG$_6$s produced by an enzymatic process.

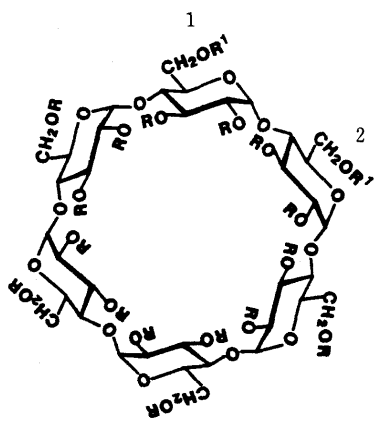
Keywords 6^1,6^2-di-*O*-(α-D-glucopyranosyl)-cyclomaltohexaose; 6^1,6^3-di-*O*-(α-D-glucopyranosyl)-cyclomaltohexaose; 6^1,6^4-di-*O*-(α-D-glucopyranosyl)-cyclomaltohexaose; 2,3,4,6-tetra-*O*-benzyl-1-*O*-trichloroacetimidoyl-α-D-glucopyranose; glucosylation; ^{13}C-NMR; HPLC

In recent years branched cyclomalto-oligosaccharides (cG$_n$s) have attracted much attention.[1] A variety of monobranched cG$_n$s have already been isolated and characterized,[2–7] while multibranched cG$_n$s have also been identified and some have been isolated.[8–10] Furthermore, cyclomaltohexaose (cG$_6$) and cyclomaltoheptaose (cG$_7$) having diglucosyl or dimaltosyl branches are now commercially available as a mixture of positional isomers, individually. In the previous paper we reported on the isolation and characterization of three positional isomers each of dimaltosyl-cG$_7$s[9] and diglucosyl-cG$_7$s[10] which were contained in a mixture of maltosyl-cG$_7$s produced from maltose and cG$_7$ through the reverse action of *Klebsiella pneumoniae* pullulanase and in a mixture of glucosyl-cG$_7$s prepared by glucoamylolysis of a mixture of maltosyl-cG$_7$s, respectively.

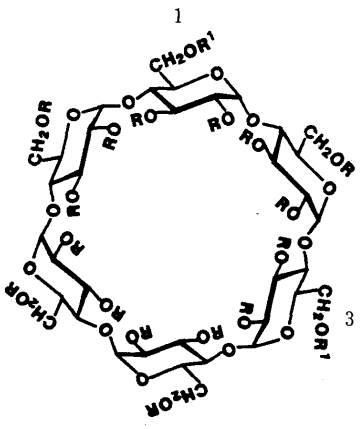
On the other hand, isolation of the three positional isomers of diglucosyl-cG$_6$ from a mixture prepared from maltose and cG$_6$ in the same way as done for the mixture of glucosyl-cG$_7$s described above was very difficult. Therefore, we wished to synthesize authentic positional isomers of diglucosyl-cG$_6$ for use as standard samples in the identification of the components in a mixture of diglucosyl-cG$_6$s prepared by the enzymatic process.

Results and Discussion

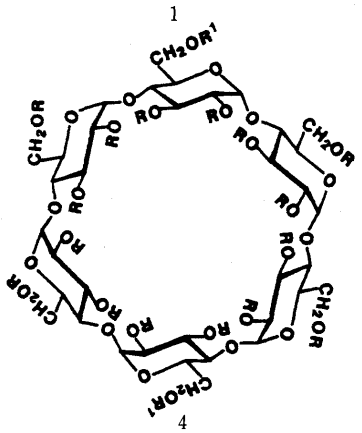
Synthesis Three regioisomers, 6^1,6^n-di-*O*-(*tert*-butyldimethylsilyl)-cG$_6$ (**1**, **2**, and **3**),[11] whose structures had been unambiguously established, were used as the key intermediates for chemical syntheses of 6^1,6^n-di-*O*-(α-D-glucopyranosyl)-cG$_6$s (**10**, **11**, and **12**). Acetylation of **1**, **2**, or **3** with acetic anhydride–pyridine for 5—6 h at 100 °C gave the crystalline 6^1,6^n-di-*O*-(*tert*-butyldimethylsilyl)-cG$_6$ peracetates (**4**, **5**, and **6**) in 80—85% yield after centrifugal chromatography. *O*-Desilylation of **4**, **5**, or **6** with boron trifluoride etherate in dichloromethane produced bis(2,3-di-*O*-acetyl)tetrakis(2,3,6-tri-*O*-acetyl)-cG$_6$s (**7**, **8**, and **9**) in high yields, and all of these compounds were obtained in crystal form after centrifugal chromatography. Glucosylation of **7**, **8**, or **9** was satisfactorily achieved according to the procedure of Fügedi *et al.*[12] by reaction with 2,3,4,6-tetra-*O*-benzyl-1-*O*-trichloroacetimidoyl-α-D-gluco-



	R	R^1
1	H	*tert*-BuMe$_2$Si
4	Ac	*tert*-BuMe$_2$Si
7	Ac	H
10	H	α-D-glucose



	R	R^1
2	H	*tert*-BuMe$_2$Si
5	Ac	*tert*-BuMe$_2$Si
8	Ac	H
11	H	α-D-glucose



	R	R^1
3	H	*tert*-BuMe$_2$Si
6	Ac	*tert*-BuMe$_2$Si
9	Ac	H
12	H	α-D-glucose

Chart 1

pyranose[13] in dichloromethane in the presence of molecular sieves at −20 °C, using trifluoromethanesulfonic acid as a catalyst.[14] An attempt at *O*-debenzylation of each glucosylation product with Pd–C in acetic acid at room temperature overnight was unsuccessful. In contrast, *O*-debenzylation with Pd–C in 10% formic acid–methanol for 2 h at room temperature proceeded smoothly. After deacetylation, a mixture containing diglucosyl-cG$_6$ having two (1→6)-α-linkages, **10**, **11**, or **12**, was obtained in each case.

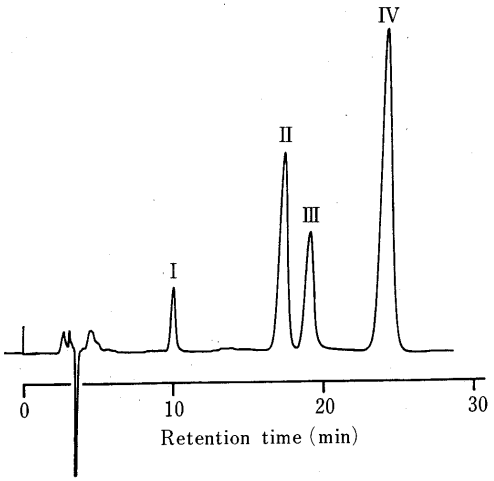


Fig. 1. Chromatogram of 6^1,6^2-Di-*O*-(D-glucopyranosyl)-cG$_6$S

I=β, β substituted product, II, III=α, β or β, α substituted products, IV=α, α substituted product (**10**). Chromatographic conditions: column, YMC-Pack A-312 ODS (150×6 mm i.d.); eluent, methanol–water (5:95); flow rate, 1.0 ml/min; detector, Shodex RI SE-61.

Separation and Characterization The elution profile of 6^1,6^2-di-*O*-(D-glucopyranosyl)-cG$_6$s, products from **7**, is shown in Fig. 1. Each component was isolated by high-performance liquid chromatography (HPLC) on a YMC-Pack A-323-5 octadecyl silica (ODS) column with methanol–water (6:94), and characterized by fast-atom bombardment-mass spectrometry (FAB-MS) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectroscopy.

The molecular weights of the four components confirmed by FAB-MS are all 1296. This result suggested that all four compounds are diglucosyl-cG$_6$.

Figure 2 shows the ^{13}C-NMR spectra of II, III, and IV in D$_2$O. The assignments of three kinds of C-6 signals were confirmed by the distortionless enhancement by polarization transfer (DEPT) method.[15] The large downfield shift of signals for two C-6 indicates that the side-chain D-glucose residues are attached. Usui *et al.*[16] reported that the signal for C-6 involved in the (1→6)-α-linkage of isomaltose appeared at 67.4 ppm and that involved in the (1→6)-β-linkage of gentiobiose was observed at 70.2 ppm. The signals for C-6 in the spectra of II and III appear at 67.68 and 69.68 ppm, and 67.97 and 69.38 ppm, respectively. Similarly, the signals for C-1 at 100.29 and 104.02 ppm in the spectrum of II, and at 99.98 and 103.87 ppm in the spectrum of III were assigned by reference to the report of Usui *et al.*[16] to C-1 involved in (1→6)-α-linkage and (1→6)-β-linkage, respectively. The signals for C-1 at 99.86 and 100.27 ppm and C-6 at 67.88 and 67.97 ppm in the spectrum of the main product, IV, suggested only (1→6)-α-linkage. The relative

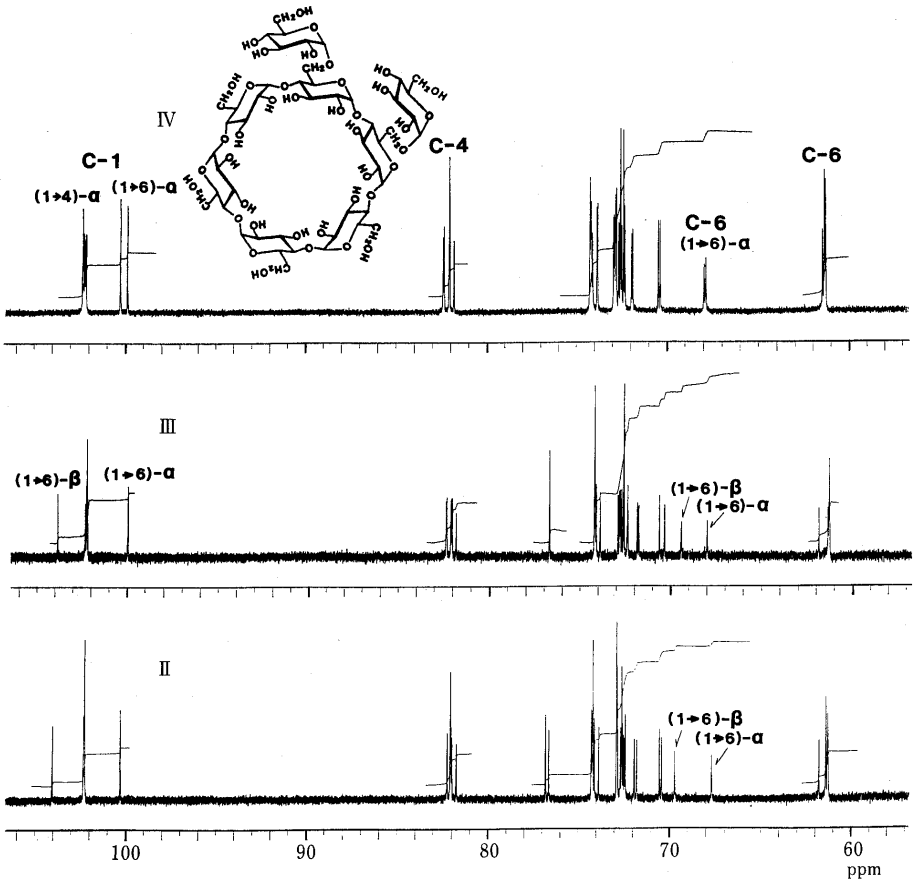


Fig. 2. ^{13}C-NMR Spectra of 6^1,6^2-Di-*O*-(D-glucopyranosyl)-cG$_6$s in D$_2$O

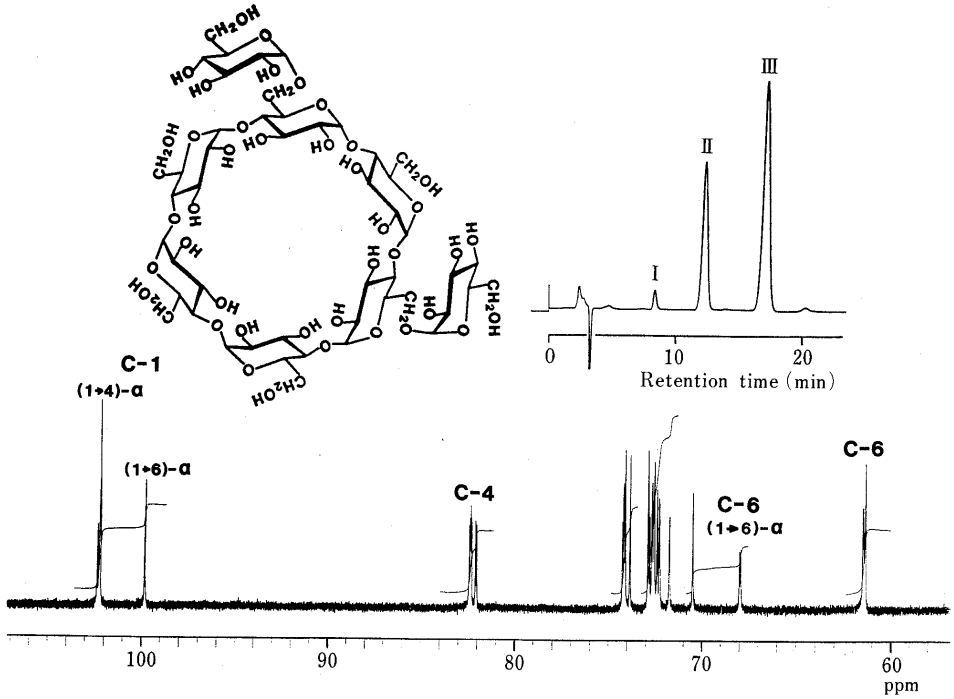


Fig. 3. Chromatogram of $6^1,6^3$-Di-*O*-(D-glucopyranosyl)-cG_6s, and ^{13}C-NMR Spectrum of III in D_2O

III=α, α substituted product (**11**). Chromatographic conditions as in Fig. 1.

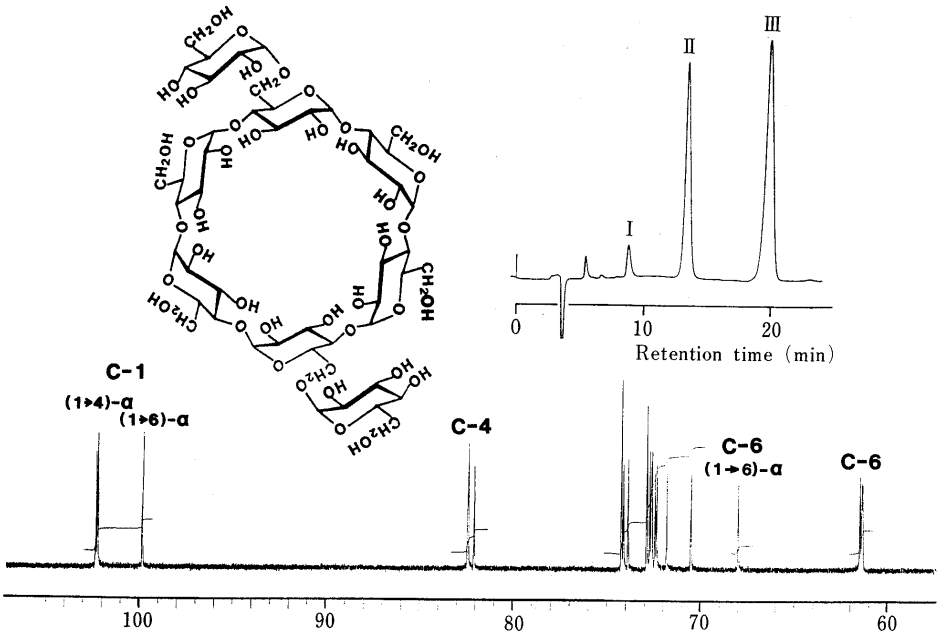


Fig. 4. Chromatogram of $6^1,6^4$-Di-*O*-(D-glucopyranosyl)-cG_6s, and ^{13}C-NMR Spectrum of III in D_2O

III=α, α substituted product (**12**). Chromatographic conditions as in Fig. 1.

intensities of the signals for C-1 and C-6 of the cG_6 ring and branch-point glucose residues in the spectra of II, III and IV were also measured. These results proved that all three compounds were indeed diglucosyl-cG_6s, and that IV was $6^1,6^2$-di-*O*-(α-D-glucosyl)-cG_6, **10**, and II and III were the configurational isomers having one (1→6)-α- and one β-linkage in the molecule. Consequently, the remaining compound, I may be $6^1,6^2$-di-*O*-(β-D-glucopyranosyl)-cG_6, though the amount obtained was too little to allow measurement of the spectrum.

The elution profiles on HPLC of $6^1,6^3$- and $6^1,6^4$-diglucosyl-cG_6s and the ^{13}C-NMR spectrum of each main product shown in Figs. 3 and 4, respectively, are similar to those of $6^1,6^2$-diglucosyl-cG_6s (Figs. 1 and 2). The component corresponding to the last peak in each chromatogram is the desired and main product. The molar ratios of configurational isomers in the glucosylation products are summarized in Table I.

Application as Standard Samples on HPLC Figure 5 shows standard chromatograms of the three positional isomers of diglucosyl-cG_6 and a separation of a commercially available mixture of diglucosyl-cG_6s on a Daiso-

pak ODS column. On the other ODS columns tested the separation of peaks 1 and 2 was more difficult. A graphitized carbon column, Hypercarb, was somewhat more favorable for the distinction of $6^1,6^3$- and $6^1,6^4$-substituted isomers than the ODS column, though their elution order was reversed on the two columns (Fig. 6).

TABLE I. Ratios of Configurational Isomers in the Glucosylation Products

Products	α, α	α, β and β, α	β, β
$6^1,6^2$-Diglucosyl-cG_6	55	40	5
$6^1,6^3$-Diglucosyl-cG_6	65	32	3
$6^1,6^4$-Diglucosyl-cG_6	60	36	4

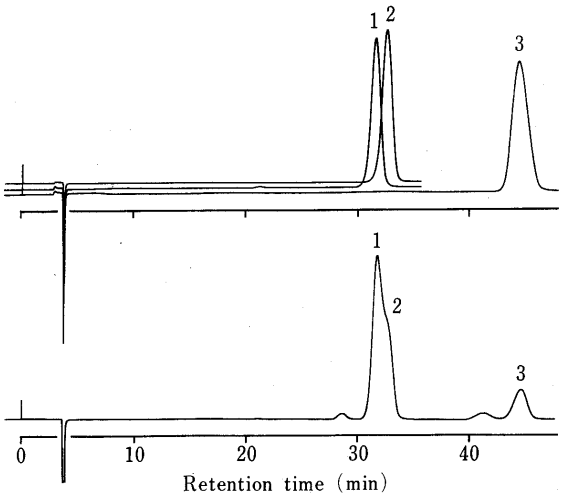


Fig. 5. Elution Profiles of Standard Samples of Three Regioisomeric $6^1,6^n$-Di-*O*-(α-D-glucopyranosyl)-cG_6s and of Components in a Commercially Available Mixture of Diglucosyl cG_6s on an ODS Column

1, 2, and 3 = $6^1,6^3$-, $6^1,6^4$-, and $6^1,6^2$-substituted isomers (**11**, **12**, and **10**) respectively. Chromatographic conditions: column, Daisopak SP-120-5-ODS (150 × 6 mm i.d.); eluent, methanol–water (3 : 97); temperature, 35 °C, other conditions as in Fig. 1.

Experimental

General Methods Melting points were measured with a Yanagimoto micro melting-point apparatus and are uncorrected. Optical rotations were determined with a Jasco digital polarimeter, model DIP 360. Thin-layer chromatography (TLC) was performed on Silica gel 60 TLC plates (E. Merck) with detection by spraying with sulfuric acid. HPLC was conducted with a Jasco tri rotar SR-1 pump, a Waters U6K universal injector, an SE-61 refractive index monitor (Showa Denko), and a column oven SSC-3510C (Senshu). The columns used were YMC-Pack SH-323-5 AQ ODS (250 × 10 mm i.d.), YMC-Pack A-312 ODS (150 × 6 mm i.d.), Daisopak SP-120-5-ODS (150 × 6 mm i.d.) and Hypercarb (100 × 4.7 mm i.d.). A Shimadzu Chromatopac C-R3A digital integrator was used for quantitative analyses. Centrifugal chromatography was performed with a Harrison Centrifugal Thin Layer Chromatotron, model 7924. ^{13}C-NMR spectra were recorded with a JEOL GSX-500 (125.65 MHz) spectrometer for solutions in D_2O and $CDCl_3$ (internal Me_4Si). FAB-MS was performed with a JEOL JMS-DX 303 mass spectrometer.

$6^1,6^n$-Di-*O*-(*tert*-butyldimethylsilyl)-cyclomaltohexaose Peracetates (4, 5, and 6) Compound **1** (325 mg), **2** (565 mg), and **3** (280 mg) were each acetylated with acetic anhydride (10—15 ml) in anhydrous pyridine (13—20 ml) for 4—5 h at 60 °C. Each mixture was further stirred for 5—6 h at 100 °C and then concentrated. The residue was extracted with chloroform, and the extract was washed sequentially with water, aqueous sodium carbonate, and water, then dried, and evaporated to a syrup. Centrifugal chromatography (hexane–acetone 2 : 1) of the residue gave **4**

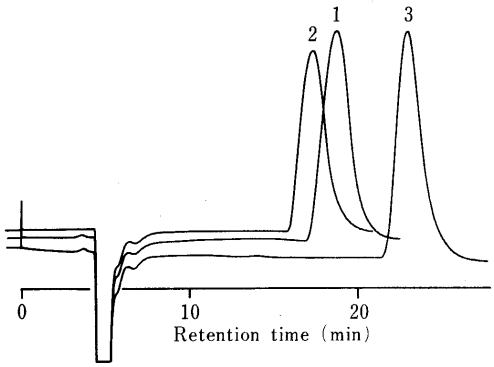


Fig. 6. Elution Profiles of Standard Samples of Three Regioisomeric $6^1,6^n$-Di-*O*-(α-D-glucopyranosyl)-cG_6s on a Graphitized Carbon Column

1, 2, and 3 are the same as in Fig. 5. Chromatographic conditions: column, Hypercarb (100 × 4.7 mm i.d.); eluent, ethanol–water (14 : 86); flow rate, 0.3 ml/min; other conditions as in Fig. 5.

TABLE II. Physico-Chemical and Analytical Data for $6^1,6^n$-Di-*O*-(*tert*-butyldimethylsilyl)-cG_6s Peracetates

Compound	mp (°C)	$[\alpha]_D^{27}$ (in $CHCl_3$)		Elemental analysis Found[a]		^{13}C-NMR δ ($CDCl_3$)			
		(°)	*c*	C	H	C-1	C-6		$-Si(CH_3)_2-$
4	130—132	+98.0	1.0	50.50	6.55	97.02 96.91 96.83 96.67 96.37 96.19	63.39 63.34 63.26 63.19	62.04[b] 61.87[b]	−5.07 −5.32 −5.11 −5.34
5	130—132	+94.0	1.0	50.66	6.51	98.09 97.60 97.42 96.19 96.19 96.00	63.50 63.50 63.32 63.02	61.78[b] 61.68[b]	−5.19 −5.45
6	130—132	+98.0	1.0	50.78	6.62	96.97 96.97 96.46 96.46 96.37 96.37	63.53 63.53 63.36 63.36	61.80[b] 61.80[b]	−5.18 −5.45

a) *Anal.* Calcd for $C_{80}H_{120}O_{46}Si_2 \cdot H_2O$: C, 50.78; H, 6.50. b) *tert*-Butyldimethylsilyloxy group-bonded ring glucosyl carbon.

TABLE III. Physico-Chemical and Analytical Data for Bis(2,3-di-*O*-acetyl)tetrakis(2,3,6-tri-*O*-acetyl)-cG$_6$s

Compound	mp (°C)	$[\alpha]_D^{27}$ (in $CHCl_3$)		Elemental analysis Found[a]		^{13}C-NMR δ ($CDCl_3$)		
		(°)	*c*	C	H	C-1	C-6	
7	148—150	+107.4	1.8	48.85	5.71	96.76 96.71 (2) 96.67 96.43 96.33	63.36 63.28 63.25 63.21	62.25 (2)[b]
8	149—150	+108.2	1.5	48.84	5.89	96.92 96.60 (2) 96.54 96.43 96.37	63.51 63.42 63.37 63.17	62.27[b] 62.18[b]
9	148—150	+111.6	1.6	48.81	5.78	96.77 (2) 96.74 (2) 96.21 (2)	63.48 (2) 63.20 (2)	62.34 (2)[b]

a) *Anal.* Calcd for $C_{68}H_{92}O_{46}\cdot 2H_2O$: C, 48.57; H, 5.75. *b*) Hydroxyl group-bonded ring glucosyl carbon. Values in parentheses are numbers of carbons.

(433 mg, 85.1%), **5** (750 mg, 84.7%), and **6** (355 mg, 80.9%). These compounds were crystallized from hexane–acetone. Their physicochemical and analytical data are listed in Table II.

Bis(2,3-di-*O*-acetyl)tetrakis(2,3,6-tri-*O*-acetyl)-cyclomaltohexaoses (7, 8, and 9) A solution of **4** (100 mg), **5** (100 mg), or **6** (100 mg) in dichloromethane (5 ml) was treated with 47% boron trifluoride etherate in ether (80 μl). The mixture was stirred for 3 h at room temperature, diluted with dichloromethane, and poured into ice-water. The dichloromethane layers was separated, rinsed successively with water, aqueous sodium hydrogencarbonate, and water, then dried, and concentrated. Centrifugal chromatography (benzene–acetone 2 : 1) of the residue afforded **7** (70 mg, 60.1%), **8** (80 mg, 91.5%), or **9** (73 mg, 83.5%). These compounds were crystallized from hexane–ethanol. Their physicochemical and analytical data are listed in Table III.

Glucosylation of 7, 8, and 9 A solution of 2,3,4,6-tetra-*O*-benzyl-1-*O*-trichloroacetimidoyl-α-D-glucopyranose (0.80 g) in anhydrous dichloromethane (5 ml) was added dropwise during 10 min to a stirred solution of a mixture of **7** (195 mg) and powdered 4 Å molecular sieve (1.5 g) in dry dichloromethane (7 ml) under nitrogen at −20 °C with exclusion of moisture, and the mixture was stirred at −20 °C. After 30 min, a solution of trifluoromethanesulfonic acid (22 μl) in dichloromethane (1 ml) was added. After 1 h, triethylamine (1 ml) was added, and the mixture was filtered through a pad of Celite, washed with 1 M sulfuric acid, aqueous sodium hydrogencarbonate, and water, then dried, and concentrated. Centrifugal chromatography with hexane–acetone (5 : 1—2 : 1) of the product afforded chromatographically pure 6^1,6^2-di-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-cG$_6$ peracetate (**13**, 441 mg, 52.7%).

In the same manner as described above, 6^1,6^3-di-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-cG$_6$ peracetate (353 mg, 74.5%) and 6^1,6^4-di-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-cG$_6$ peracetate (548 mg, 66.8%) were obtained from **8** (297 mg) and **9** (500 mg), respectively.

6^1,6^n-Di-*O*-(α-D-glucopyranosyl)-cG$_6$s (10, 11, and 12) A solution of **13** (441 mg) in 10% formic acid–methanol (100 ml) was hydrogenated in the presence of 10% Pd–C (2.5 g) in a nitrogen atmosphere for 2 h at room temperature. The catalyst was filtered off, washed with methanol, and concentrated. The residue (354 mg) was treated with methanolic 0.05 N sodium methoxide (30 ml) for 2 h at room temperature, neutralized with Amberlite IR-120B (H^+) resin, filtered, and concentrated. Compound **10** was isolated from the residue (198 mg, 93.1%) containing four configurational isomers by HPLC on a column of YMC-Pack A-323 ODS with methanol–water (6 : 94), $[\alpha]_D^{27}$ +152.1° (*c*=0.6, H_2O).

Similarly compound **11** (160 mg, 94.1%) or **12** (187 mg, 75.8%) was isolated from the residue containing four configurational isomers in each case by HPLC. **11**, $[\alpha]_D^{27}$ +157.4° (*c*=0.6, H_2O). **12**, $[\alpha]_D^{27}$ +15.1° (*c*=1.1, H_2O).

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