ELSEVIER

Carbohydrate Research 267 (1995) 27–37

CARBOHYDRATE RESEARCH

Preparation of $6^1,6^2$-, $6^1,6^3$-, $6^1,6^4$-, and $6^1,6^5$-di-*O*-(α-D-glucopyranosyl)cyclomalto-octaoses

Toshiko Tanimoto *, Tomoko Sakaki, Kyoko Koizumi

Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 11-68 Koshien Kyuban-cho, Nishinomiya 663, Japan

Received 25 April 1994; accepted 23 August 1994

Abstract

Four positional isomers of $6^1,6^n$-di-*O*-(D-glucopyranosyl)cyclomalto-octaoses (cG_8s) ($n=2$, 3, 4, and 5) were chemically synthesized by using the authentic compounds, $6^1,6^n$-di-*O*-trityl-cG_8s or $6^1,6^n$-di-*O*-(*tert*-butyldimethylsilyl)-cG_8s ($n=2$–5) as the key glucosyl intermediates, and the glucosyl donor, 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl trichloroacetimidate.

Keywords: Cyclomalto-octaose; γ-Cyclodextrin; Di-*O*-glucosylcyclomalto-octaose; Di-*O*-glucosyl-γ-cyclodextrin; Branched cyclomalto-octaose; Branched cyclodextrin

1. Introduction

Branched cyclomalto-oligosaccharides (cG_ns) having mono- or oligo-saccharides linked to hydroxyl groups on C-6 of cG_ns have attracted attention because of their many advantages over the parent cG_ns [1]. In particular, positional isomers of dibranched cG_ns are expected to have characteristic abilities of molecular recognition arising from the differences of the substituted positions. Some monobranched cyclomalto-octaoses (cG_8s) have already been isolated and characterized [2–4], and dibranched cG_8s have also been identified [5]. However the regiochemical determination of four positional isomers of dibranched cG_8 has not been achieved.

We have reported on the synthesis, isolation, and characterization of four positional isomers of $6^1,6^n$-di-*O*-triphenylmethyl (trityl)-cG_8 [6] ($n=2$–5, **2–5**) and $6^1,6^n$-di-*O*-(*tert*-butyldimethylsilyl)-cG_8 [7] ($n=2$–5, **6–9**) that can be used as key glycosyl intermediates for chemical syntheses of positional isomers of dibranched cG_8. Elution profiles of **2–5** and **6–9** in Fig. 1 show that the retention order of $6^1,6^5$-, $6^1,6^4$-, $6^1,6^3$-, and $6^1,6^2$-

* Corresponding author.

0008-6215/95/$09.50 © 1995 Elsevier Science B.V. All rights reserved
SSDI 0008-6215(94)00291-6

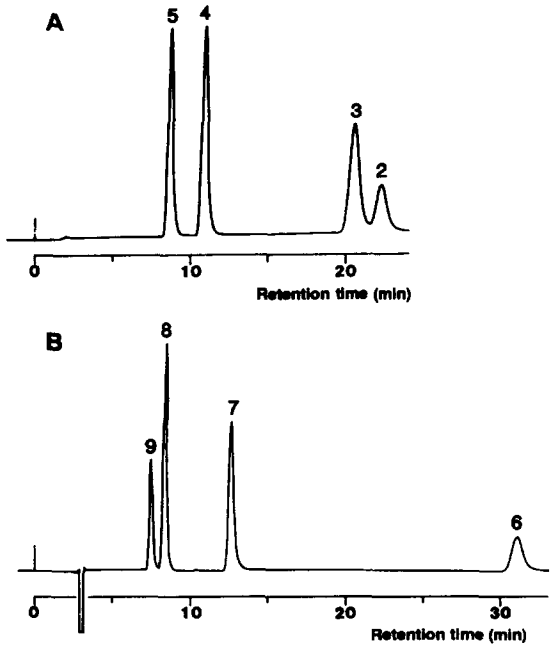


Fig. 1. Elution profiles of each four positional isomers of $6^1,6^n$-di-*O*-trityl-cG_8s ($n=2$–5, **2**–**5**) (A) and $6^1,6^n$-di-*O*-(*tert*-butyldimethylsilyl)-cG_8s ($n=2$–5, **6**–**9**) (B). Chromatographic conditions: column, DAISOPAK SP-120-5-ODS (150×6 mm i.d.); eluent, (A) 75:25 methanol–water, (B) 70:30 methanol–water; flow rate, 1.0 mL/min; detector, (A) UV, wave length, 240 nm, (B) Shodex RI SE-71.

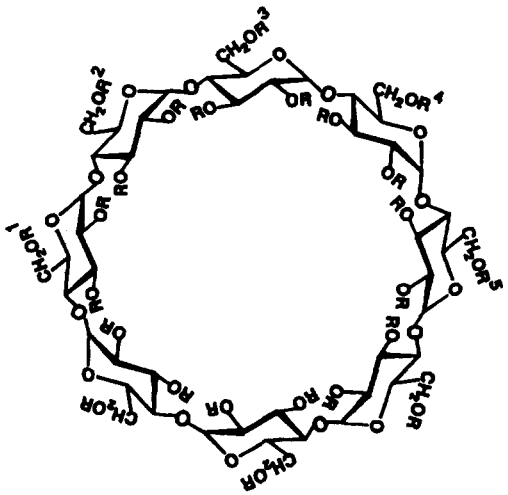
derivatives is the same, but the elution pattern is rather different. It can be seen from Fig. 1 that, as intermediates for chemical syntheses of $6^1,6^n$-di-*O*-(D-glucopyranosyl)-cG_8s, silyl compounds (**6** and **7**) for $6^1,6^2$- and $6^1,6^3$-di-*O*-(D-glucopyranosyl)-cG_8s, and trityl compounds (**4** and **5**) for $6^1,6^4$- and $6^1,6^5$-di-*O*-(D-glucopyranosyl)-cG_8s, are indeed useful. We now describe the synthesis of the diglucosyl-cG_8s as part of studies on the synthesis of diglycosyl-cG_ns (the structures of all compounds are given in Table 1).

2. Results and discussion

Preparation.—Acetylation of **6**, **7**, **4**, or **5** with acetic anhydride–pyridine for 5–6 h at 100°C gave the $6^1,6^2$- and $6^1,6^3$-di-*O*-(*tert*-butyldimethylsilyl)-cG_8 peracetates (**10** and **11**), and $6^1,6^4$- and $6^1,6^5$-di-*O*-trityl-cG_8 peracetates (**12** and **13**) in 68–88% yield after centrifugal chromatography. *O*-Desilylation [8] of **10** and **11** with boron trifluoride etherate in dichloromethane and *O*-detritylation [9] of **12** and **13** with 70% acetic acid afforded the glycosyl acceptor, bis(2,3-di-*O*-acetyl)hexakis(2,3,6-tri-*O*-acetyl)-cG_8s (**14**, **15**, **16**, and **17**), after centrifugal chromatography.

Glucosylation of **14**, **15**, **16**, or **17** with 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl trichloroacetimidate [10,11] in dichloromethane in the presence of trifluoromethanesulfonic acid catalyst [12–14] and molecular sieves for 1 h at −20°C gave $6^1,6^n$-di-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-cG_8 peracetates ($n=2$–5, **26**–**29**) in yields of 75–86%.

Table 1
Structures of compounds **1–25**



	R^1	R^2	R^3	R^4	R^5	R
1	H	H	H	H	H	H
2	Tr	Tr	H	H	H	H
3	Tr	H	Tr	H	H	H
4	Tr	H	H	Tr	H	H
5	Tr	H	H	H	Tr	H
6	X	X	H	H	H	H
7	X	H	X	H	H	H
8	X	H	H	X	H	H
9	X	H	H	H	X	H
10	X	X	Ac	Ac	Ac	Ac
11	X	Ac	X	Ac	Ac	Ac
12	Tr	Ac	Ac	Tr	Ac	Ac
13	Tr	Ac	Ac	Ac	Tr	Ac
14	H	H	Ac	Ac	Ac	Ac
15	H	Ac	H	Ac	Ac	Ac
16	H	Ac	Ac	H	Ac	Ac
17	H	Ac	Ac	Ac	H	Ac
18	Y	Y	H	H	H	H
19	Y	H	Y	H	H	H
20	Y	H	H	Y	H	H
21	Y	H	H	H	Y	H
22	Z	Z	H	H	H	H
23	Z	H	Z	H	H	H
24	Z	H	H	Z	H	H
25	Z	H	H	H	Z	H

X, *tert*-$BuMe_2Si$; Y, D-glucopyranosyl; Z, α-D-glucopyranosyl.

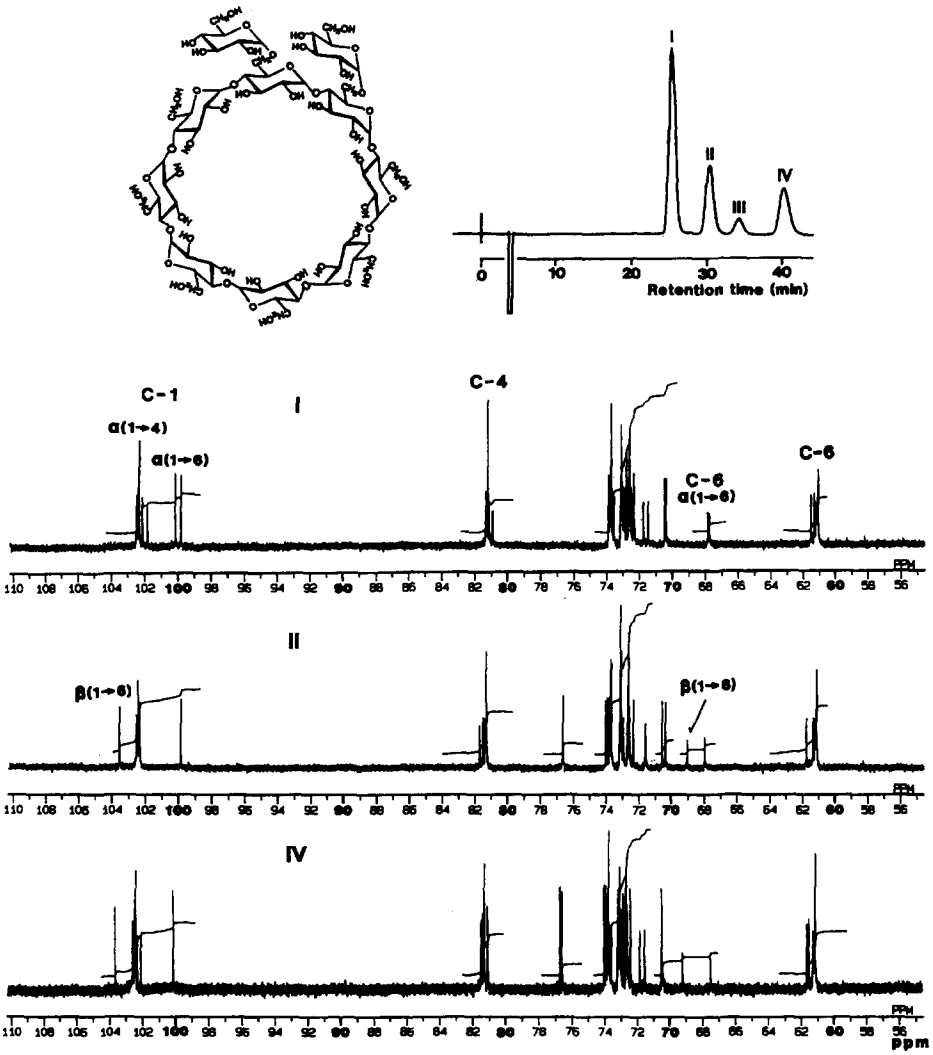


Fig. 2. Chromatogram of $6^1,6^2$-di-*O*-(D-glucopyranosyl)-cG_8 and ^{13}C NMR spectra of components I, II, and IV in D_2O. I = α,α-disubstituted product **22**, II and IV = α,β- or β,α-disubstituted product, III = β,β-disubstituted product. Chromatographic conditions: eluent, 5:95 methanol–water; temperature, 25°C; other conditions as in Fig. 1 (B).

Compounds **26**, **27**, **28**, and **29** were each hydrogenolysed with Pd–C in 1:9 → 2:8 formic acid–methanol for 2–7 h at room temperature, and the products were *O*-deacetylated with methanolic sodium methoxide to give **18**, **19**, **20**, and **21**, each of which was a mixture of configurational isomers containing α-(1 → 6)- and β-(1 → 6)-linkages. The desired compounds having two α-(1 → 6)-linkages, i.e., compounds **22**, **23**, **24**, or **25**, were respectively isolated from **18**, **19**, **20**, or **21** by HPLC.

Separation and characterization of $6^1,6^n$*-di*-O-(*α*-D-*glucopyranosyl*)-cG_8*s* (**22**, **23**, **24**, *and* **25**).—Fig. 2 shows the elution profile of the regioisomeric mixture of $6^1,6^2$-di-*O*-(D-

glucopyranosyl)-cG$_8$ (**18**). This elution pattern is very different from those of 6^1,6^n-di-*O*-(D-glucopyranosyl)-cyclomaltohexaoses [14] and -cyclomaltoheptaoses [13]. In order to confirm the structures, each component was isolated by HPLC on a DAISOPAK SP-120-5 octadecylsilyl-bonded silica (ODS) column, with 5:95 methanol–water. FABMS confirmed that all four components have the same molecular weight, 1620, i.e., that expected for diglucosyl-cG$_8$s.

In the ^{13}C NMR spectra of I, II, and IV in D_2O, signals due to the α-(1→6)-glucosylated C-6 (δ 68) and β-(1→6)-glucosylated C-6 (δ 69–70) shifted downfield by 7 and 9 ppm, respectively, compared with the signals of the other C-6s [13,14]. The assignment of three different kinds of C-6 signals was confirmed by the distortionless enhancement by polarization transfer (DEPT) method [15]. The signals for C-1 of the α-(1→6)-linkage appeared at δ 100, while those of the β-(1→6)-linkage appeared at δ 104 [13,14]. The relative intensities of the signals were also measured. These results indicated that I, II, and IV were indeed diglucosyl-cG$_8$s, and that I was 6^1,6^2-di-*O*-(α-D-glucopyranosyl)-cG$_8$ (**22**), and II and IV were the configurational isomers having one α-(1→6)-linkage and one β-(1→6)-linkage in the molecule. Therefore, the remaining compound, III, is undoubtedly 6^1,6^2-di-*O*-(β-D-glucopyranosyl)-cG$_8$.

Similarly, the elution profiles on HPLC of 6^1,6^3-, 6^1,6^4-, and 6^1,6^5-diglucosyl-cG$_8$s and the ^{13}C NMR spectra of each of their main products are shown in Figs. 3, 4, and 5, respectively. For 6^1,6^3- and 6^1,6^4-diglucosyl-cG$_8$ (Figs. 3 and 4), I was 6^1,6^3-di-*O*-(α-D-glucopyranosyl)-cG$_8$ (**23**) and 6^1,6^4-di-*O*-(α-D-glucopyranosyl)-cG$_8$ (**24**) and II and III were the configurational isomers having one α-(1→6)-linkage and one β-(1→6)-linkage in the molecule. Peak IV, which was observed only in Fig. 4, was determined to have two β-(1→6)-linkages. For 6^1,6^5-diglucosyl-cG$_8$ (Fig. 5), I was 6^1,6^5-di-*O*-(α-D-glucopyranosyl)-cG$_8$ (**25**), and compound II, which was confirmed with ^{13}C NMR spectral data as having one α-(1→6)-linkage and one β-(1→6)-linkage in the molecule. The two glucosyl residues in 6^1,6^5-dibranched cG$_8$ are located at the most remote position from each other, and there is only one compound having one α-(1→6)-linkage and one β-(1→6)-linkage in the molecule. Therefore, III was determined to be the configurational isomer having two β-(1→6)-linkages.

The molar ratios of the configurational isomers in the glucosylation products are summarized in Table 2.

Fig. 6 shows standard chromatograms of the four positional isomers of 6^1,6^n-di-*O*-(α-D-glucopyranosyl)-cG$_8$ (**22**, **23**, **24**, and **25**) on a YMC-Pack SH-312-3 ODS (150×6 mm i.d.) column. The elution order of **23**, **24**, and **25** was unaltered, even if the chromatographic conditions were changed by temperature (25–35°C), the use of other ODS columns, and eluents (2:98→5:95 methanol–water). However, the elution order of **22** was altered by change of temperature; that is, **22** coeluted with **24** at 30°C and was eluted between **24** and **25** at 25°C.

3. Experimental

General.—Optical rotations were determined with a Jasco digital polarimeter, model DIP 360. TLC was performed on Silica Gel 60 plates (E. Merck). Centrifugal chromatography

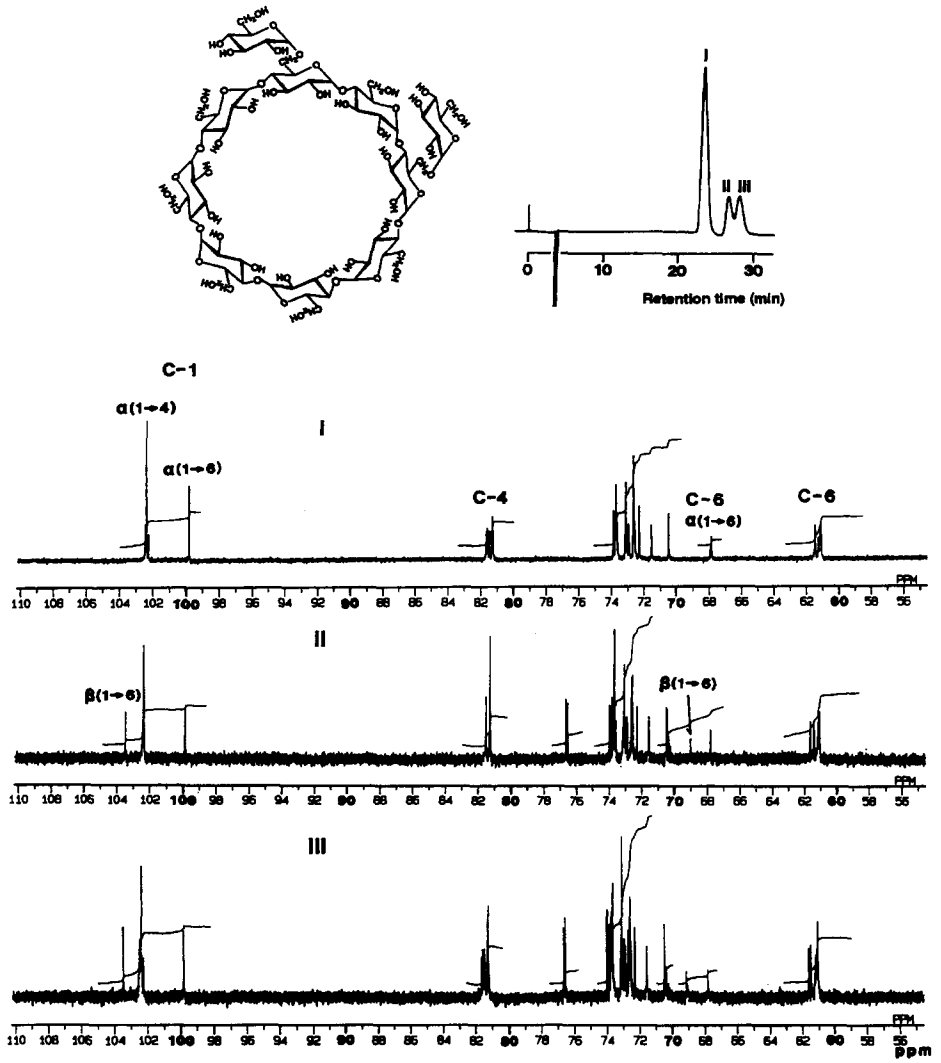


Fig. 3. Chromatogram of $6^1,6^3$-di-*O*-(D-glucopyranosyl)-cG_8 and ^{13}C NMR spectra of components I, II, and III in D_2O. I = α,α-disubstituted product **23**, II and III = α,β- or β,α-disubstituted product. Chromatographic conditions as in Fig. 2.

was performed with a Harrison Centrifugal Thin-Layer Chromatotron, model 7924. HPLC was conducted with a Jasco TRI ROTAR SR-1 or 880-PU pump, a Waters U6K universal injector, a Showa Denko RI SE-71 refractive index monitor, a Jasco UVIDEC-100III variable-wavelength ultraviolet detector, and a Lab-Quatec CO-1093 column oven. The columns used were YMC-Pack SH-343-10 ODS (250×20 mm i.d.), YMC-Pack SH-343-5 ODS (250×20 mm i.d.), YMC-Pack SH-312-3 ODS (150×6 mm i.d.), and DAISOPAK SP-120-5-ODS (150×6 mm i.d.). ^{13}C NMR spectra were recorded with Jeol GSX-500 (125.65 MHz) spectrometer. Chemical shifts are expressed in ppm downfield from the

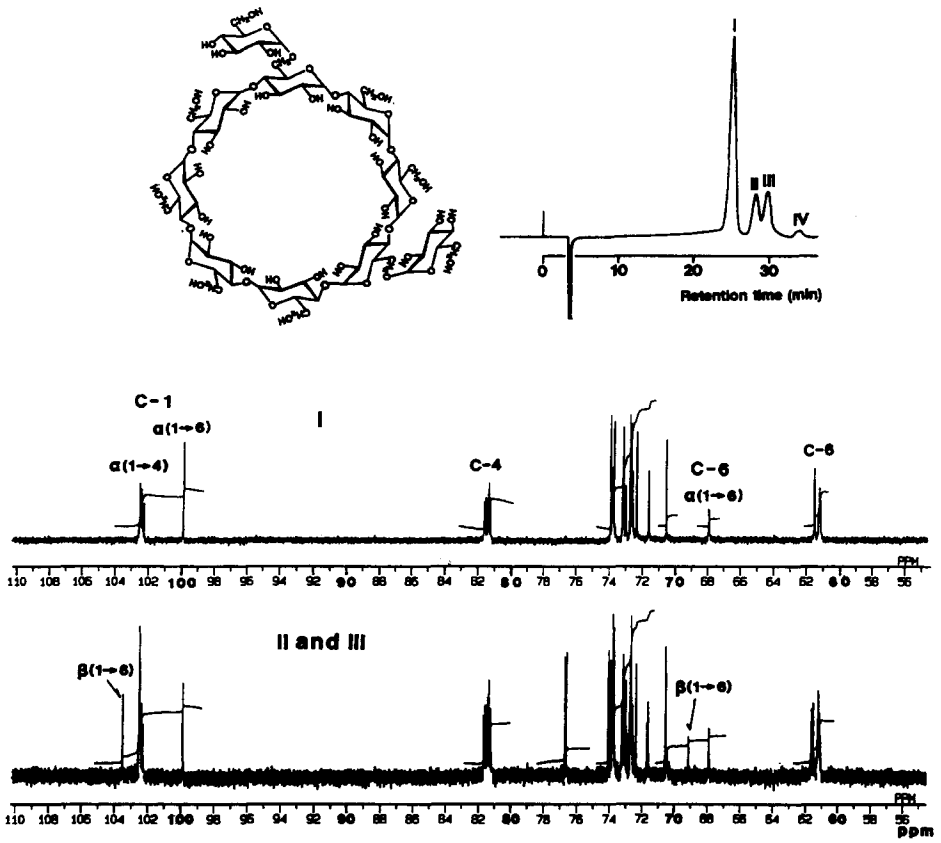


Fig. 4. Chromatogram of $6^1,6^4$-di-*O*-(D-glucopyranosyl)-cG_8 and ^{13}C NMR spectra of components I, and a mixture of II and III in D_2O. I = α,α-disubstituted product **24**, II and III = α,β- or β,α-disubstituted product, IV = β,β-disubstituted product. Chromatographic conditions as in Fig. 2.

signal for internal Me_4Si for solutions in $CDCl_3$, and for solutions in D_2O, in ppm downfield from the signal for Me_4Si, by reference to external dioxane (67.40 ppm).

*$6^1,6^2$- And $6^1,6^3$-di-*O*-(tert-butyldimethylsilyl)cyclomalto-octaose peracetates* (**10** *and* **11**) *and $6^1,6^4$- and $6^1,6^5$-di-*O*-tritylcyclomalto-octaose peracetates* (**12** *and* **13**).—To a solution of **6** (368 mg), **7** (180 mg), **4** (667 mg), or **5** (754 mg) in dry pyridine (8–30 mL) was added Ac_2O (4–15 mL). Each mixture was stirred for 5–6 h at 100°C and then concentrated in vacuo. The residue was extracted with $CHCl_3$, and the extract was washed sequentially with water, aq Na_2CO_3, and water, then dried, and evaporated to a syrup. Centrifugal chromatography (3:1 → 3:2 hexane–acetone) of the residue afforded **10** (402 mg, 68.4%), **11** (234 mg, 81.4%), **12** (890 mg, 87.8%), and **13** (933 mg, 84.9%). The physicochemical data of these compounds are listed in Table 3.

*Bis(2,3-di-*O*-acetyl)hexakis(2,3,6-tri-*O*-acetyl)cyclomalto-octaoses* (**14**, **15**, **16**, *and* **17**).–To a solution of **10** (355 mg) or **11** (195 mg) in dry CH_2Cl_2 (10–20 mL) in an ice–water bath was added 47% boron trifluoride etherate in ether (170–300 μL) with stirring. The stirring was continued at room temperature for 3 h, then the mixture was diluted with CH_2Cl_2, and was poured into ice–water. The organic layer was separated, washed succes-

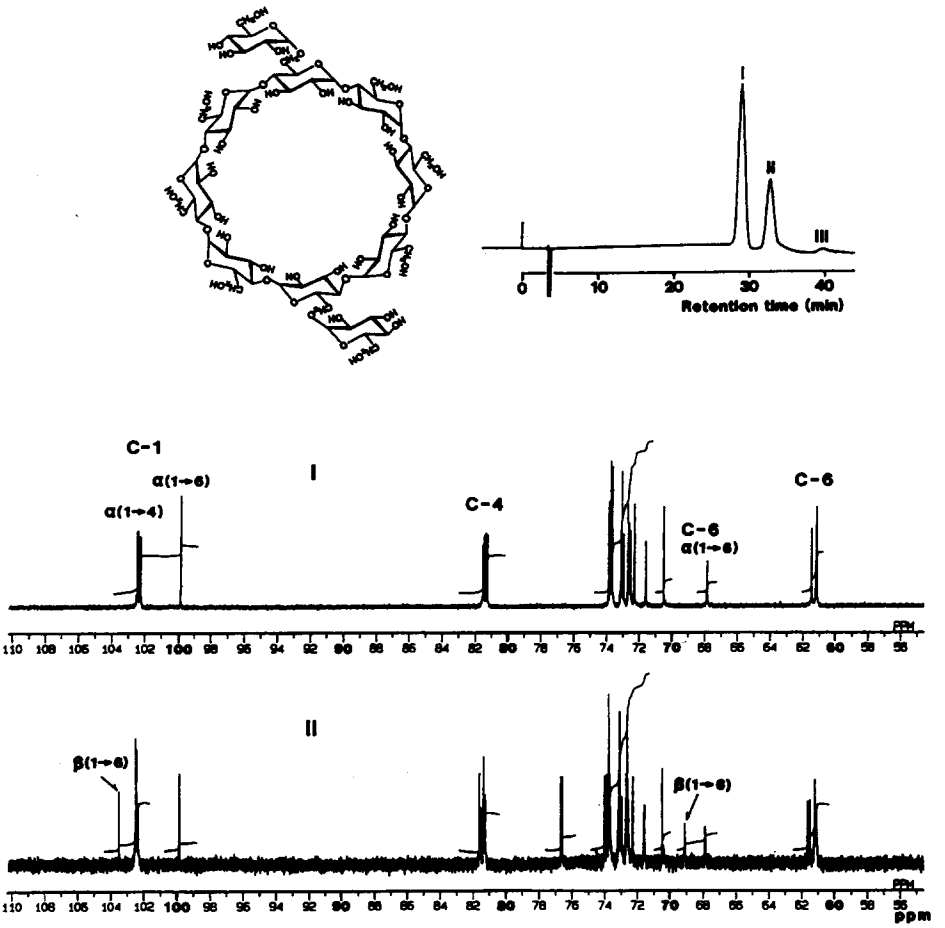


Fig. 5. Chromatogram of $6^1,6^5$-di-*O*-(D-glucopyranosyl)-cG_8 and ^{13}C NMR spectra of components I and II in D_2O. I = α,α-disubstituted product **25**, II = α,β-disubstituted product, III = β,β-disubstituted product. Chromatographic conditions as in Fig. 2.

sively with water, aq $NaHCO_3$, and water, then dried, and concentrated. Centrifugal chromatography (2:1 → 2:3 hexane–acetone) of the residue gave **14** (236 mg, 74.0%) and **15** (138 mg, 78.8%).

Table 2
Ratios of configurational isomers in the glucosylation products

Products	α,α	α,β and β,α	β,β
$6^1,6^2$-di-*O*-(D-glucopyranosyl)-cG_8 (**18**)	53	28	19
$6^1,6^3$-di-*O*-(D-glucopyranosyl)-cG_8 (**19**)	65	35	0
$6^1,6^4$-di-*O*-(D-glucopyranosyl)-cG_8 (**20**)	65	32	3
$6^1,6^5$-di-*O*-(D-glucopyranosyl)-cG_8 (**21**)	69	29	2

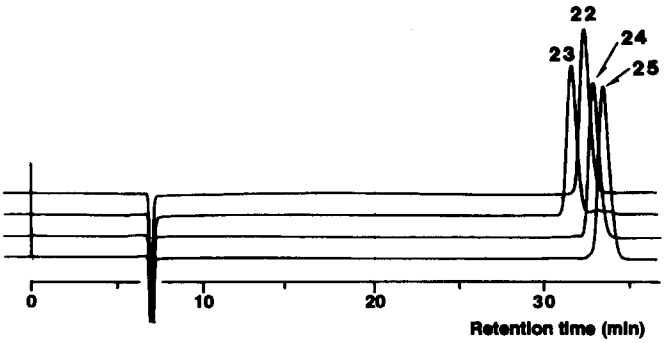


Fig. 6. Elution profile of the four positional isomers of $6^1,6^n$-di-*O*-(α-D-glucopyranosyl)-cG_8s ($n = 2$–5, **22**–**25**). Chromatographic conditions: column, YMC-Pack SH-312-3 ODS (150×6 mm i.d.); eluent, 3:97 methanol–water; flow rate, 0.5 mL/min; temperature, 35°C; other conditions as in Fig. 2.

A solution of **12** (890 mg) or **13** (933 mg) in 70% AcOH (20–25 mL) was stirred for 1 h at 75°C and then evaporated. Workup, as described for **10**–**13**, followed by centrifugal chromatography (2:1 → 2:3 hexane–acetone) gave **16** (292 mg, 40.0%) and **17** (439 mg, 57.3%). The physicochemical data of **14**–**17** are listed in Table 3.

Glucosylation of **14**, **15**, **16**, *and* **17**.—A mixture of **14** (592 mg), **15** (378 mg), **16** (292 mg), or **17** (439 mg) and 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl trichloroacetimidate (1.5–2.6 g) in dry CH_2Cl_2 (20–25 mL) was added under N_2 with stirring and cooling (−20°C) to a solution of trifluoromethanesulfonic acid (50–85 μL) in CH_2Cl_2 (1–2 mL), and dry powdered 4A molecular sieves (4.0 g). After stirring for 1 h at −20°C, Et_3N (1–

Table 3
Physicochemical data for di-*O*-(*tert*-butyldimethylsilyl)-cG_8 peracetates, di-*O*-trityl-cG_8 peracetates, and bis(2,3-di-*O*-acetyl)tetrakis(2,3,6-tri-*O*-acetyl)-cG_8

Compound	$[\alpha]_D$ (in $CHCl_3$)			^{13}C NMR δ, ($CDCl_3$)	
	(°)	c	Temperature (°C)		
				$Si(CH_3)_2$	C-6 [a]
10	+121.3	1.8	18	−5.11, −5.15 −5.29, −5.37	61.43, 61.65
11	+122.7	1.9	17	−5.20, −5.43 −5.45	61.36, 61.43
				CPh_3	C-6 [b]
12	+124.4	2.7	26	86.70, 86.75	61.40
13	+122.2	2.3	25	86.72	61.40
					C-6 [c]
14	+135.5	1.3	23		61.31, 61.45
15	+137.4	2.0	25		61.35, 61.38
16	+129.6	2.5	26		61.28, 61.30
17	+131.3	1.9	26		61.34

[a] *tert*-Butyldimethylsilyloxy-bonded carbon.
[b] *O*-CPh_3-bonded substituted carbon.
[c] Hydroxyl group-bonded carbon.

3 mL) was added to the mixture, which was diluted with CH_2Cl_2, filtered through Celite, washed with M H_2SO_4, aq $NaHCO_3$, and water, then dried, and concentrated in vacuo. Centrifugal chromatography with 5:1 → 1:2 hexane–acetone of the residue afforded chromatographically pure $6^1,6^2$-di-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-cG_8 peracetate (**26**, 715 mg, 82.1%), $6^1,6^3$-di-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-cG_8 peracetate (**27**, 420 mg, 75.6%), $6^1,6^4$-di-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-cG_8 peracetate (**28**, 368 mg, 85.7%), and $6^1,6^5$-di-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-cG_8 peracetate (**29**, 512 mg, 79.3%), respectively.

*$6^1,6^n$-Di-O-(α-D-glucopyranosyl)-cG_8s (n = 2–5, **22**, **23**, **24**, and **25**).—A solution of* **26** (715 mg), **27** (420 mg), **28** (368 mg), or **29** (512 mg) and 10% Pd–C (1.5–3.0 g) in 1:10 → 2:8 formic acid–MeOH (20–50 mL) was stirred under N_2 for 2–7 h at room temperature, then filtered through Celite, and concentrated to give **26′** (517 mg, 92.8%), **27′** (305 mg, 93.2%), **28′** (271 mg, 94.5%), or **29′** (379 mg, 95.0%). These residues were treated with methanolic 0.05 N NaOMe (8–10 mL) for 1 h at room temperature, neutralized with amberlite IR-120B (H^+) resin, filtered, and concentrated, to give **18** (318 mg, 96.5%), **19** (179 mg, 92.2%), **20** (168 mg, 97.4%), or **21** (186 mg, 77.7%).

The desired compounds **22**, **23**, **24**, and **25** were each isolated from **18**, **19**, **20**, and **21**, respectively, by HPLC on a column of DAISOPAK SP-120-5-ODS (150 × 6 mm i.d.) with 7:93 or 5:95 MeOH–water: **22**, $[\alpha]_D^{26}$ +166.8° (*c* 0.67, H_2O); **23**, $[\alpha]_D^{26}$ +176.1° (*c* 0.63, H_2O); **24**, $[\alpha]_D^{26}$ +169.1° (*c* 0.92, H_2O); **25**, $[\alpha]_D^{26}$ +186.5° (*c* 0.70, H_2O).

Acknowledgements

The authors thanks Wacker Chemicals East Asia Ltd., and Mercian Co. Ltd. for a supply of pure cyclomalto-octaose. We also thank Professor W. Kamisako and his staff (Mukogawa Women's University) for recording and measuring the NMR and FABMS spectra.

References

[1] K. Hara and H. Hashimoto, *J. Jpn. Soc. Starch Sci.*, 33 (1986) 152–161.
[2] J. Abe, H. Nagano, and S. Hizukuri, *J. Appl. Biochem.*, 7 (1985) 235–247.
[3] J. Abe, N. Mizowaki, S. Hizukuri, K. Koizumi, and T. Utamura, *Carbohydr. Res.*, 154 (1986) 81–92.
[4] Y. Yoshimura, S. Kitahata, and S. Okada, *Carbohydr. Res.*, 168 (1987) 285–294.
[5] S. Hizukuri, S. Kawano, J. Abe, K. Koizumi, and T. Tanimoto, *Biotechnol. Appl. Biochem.*, 11 (1989) 60–73.
[6] T. Tanimoto, T. Sakaki, and K. Koizumi, *Chem. Pharm. Bull.*, 41 (1993) 866–869.
[7] T. Tanimoto, T. Sakaki, and K. Koizumi, *Chem. Pharm. Bull.*, 42 (1994) 385–387.
[8] K. Takeo, K. Uemura, and H. Mitoh, *J. Carbohydr. Chem.*, 7 (1988) 293–308.
[9] K. Koizumi and T. Utamura, *Chem. Pharm. Bull.*, 29 (1981) 2776–2784.
[10] R.R. Schmidt and J. Michel, *Angew. Chem.*, 92 (1980) 763–767; R.R. Schmidt and M. Stumpp, *Liebigs Ann. Chem.*, (1983) 1249–1256; R.R. Schmidt, *Angew. Chem. Int. Ed. Engl.*, 25 (1986) 212–235.
[11] M. Sugimoto, M. Numata, K. Koike, Y. Nakahara, and T. Ogawa, *Carbohydr. Res.*, 156 (1986) C1–C5; F. Yamazaki, T. Kitajima, T. Nukada, Y. Ito, and T. Ogawa, *Carbohydr. Res.*, 201 (1990) 15–30.
[12] P. Fügedi, *J. Carbohydr. Chem.*, 6 (1987) 377–398; P. Fügedi, P. Nánási, and J. Szejtli, *Carbohydr. Res.*, 175 (1988) 173–181.

[13] K. Koizumi, Y. Okada, N. Nakanishi, T. Tanimoto, Y. Takagi, M. Ishikawa, H. Ishigami, K. Hara, and H. Hashimoto, *J. Carbohydro. Chem.*, 10 (1991) 657–670.
[14] T. Tanimoto, J. Tsujikawa, and K. Koizumi, *Chem. Pharm. Bull.*, 40 (1992) 1125–1129.
[15] D.M. Doddrell and D.T. Pegg, *J. Am. Chem. Soc.*, 102 (1980) 6388–6390.