Preparation of $6^1,6^n$-Di-*O*-(*tert*-butyldimethylsilyl)-cyclomalto-octaoses

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Four positional isomers of $6^1,6^n$-di-*O*-(*tert*-butyldimethylsilyl)-cyclomalto-octaose ($n=2$—5) were prepared by reaction of cyclomalto-octaose (1, cG_8) with *tert*-butyldimethylsilyl chloride in pyridine, and were isolated by high-performance liquid chromatography. The regiochemical determination of those positional isomers was performed by comparison with authentic compounds, prepared from $6^1,6^n$-di-*O*-trityl-cG_8s ($n=2$—5).

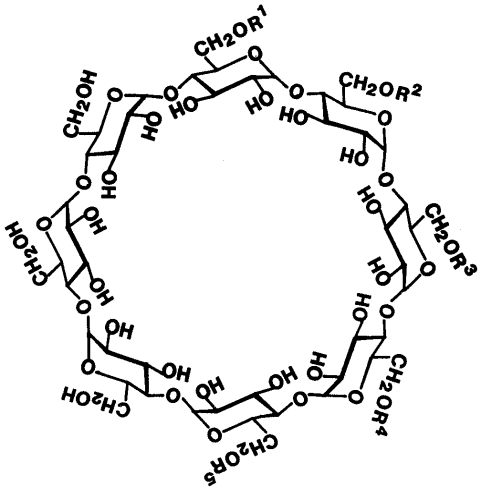
Keywords $6^1,6^n$-di-*O*-(*tert*-butyldimethylsilyl)-cyclomalto-octaose; HPLC; positional isomer; ^{13}C-NMR

Recently, to improve the solubility of the conventional cyclomalto-oligosaccharides (cG_ns), branched cG_ns have been synthesized by enzymatic processes.[1–5] However, such processes give a mixture of mono-, di- and multibranched cG_ns, and it is difficult to isolate and characterize the positional isomers of di- and multibranched cG_ns.

We have already synthesized and isolated four positional isomers of $6^1,6^n$-di-*O*-triphenylmethyl (trityl)-cG_8 derivatives ($n=2$—5) which can be used as intermediates for chemical syntheses of positional isomers of dibranched cG_8.[6] However, the yield of $6^1,6^2$-di-*O*-trityl-cG_8 (**6**) was very low because of the steric hindrance between two bulky trityl groups attached to two neighboring D-glucose units. Further, complete separation of **6** from $6^1,6^3$-di-*O*-substituted isomer (**7**) was not attained even by high-performance liquid chromatography (HPLC) (see Fig. 3). Therefore, sufficient **6** could not be obtained for use as a synthetic intermediate.

In order to solve this problem, we have tried to synthesize $6^1,6^n$-di-*O*-(*tert*-butyldimethylsilyl)-cG_8s ($n=2$—5), since these derivatives can also be used as intermediates for chemical syntheses of dibranched cG_8s. The regiochemical determination of their positional isomers was performed by comparison with authentic compounds, prepared from di-*O*-trityl-cG_8s.

Preparation and Isolation of $6^1,6^n$-Di-*O*-(*tert*-butyldimethylsilyl)-cG_8s (2—5) Selective silylation of dried cG_8 (**1**) was carried out with 3 mol eq of *tert*-butyldimethylsilyl (*tert*-$BuMe_2Si$) chloride in pyridine for 1 h at room temperature.[7,8] The silylation was shown to proceed very rapidly by monitoring the progress of the reaction by thin-layer chromatography (TLC), with chloroform–methanol–water (7 : 4 : 1). To prevent desilyla-



	R^1	R^2	R^3	R^4	R^5
1	H	H	H	H	H
2	*tert*-$BuMe_2Si$	*tert*-$BuMe_2Si$	H	H	H
3	*tert*-$BuMe_2Si$	H	*tert*-$BuMe_2Si$	H	H
4	*tert*-$BuMe_2Si$	H	H	*tert*-$BuMe_2Si$	H
5	*tert*-$BuMe_2Si$	H	H	H	*tert*-$BuMe_2Si$
6	Tr	Tr	H	H	H
7	Tr	H	Tr	H	H
8	Tr	H	H	Tr	H
9	Tr	H	H	H	Tr

Chart 1

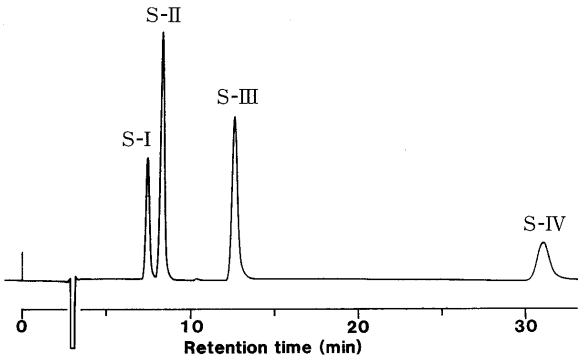


Fig. 1. Elution Profile of Four Positional Isomers of Di-*O*-(*tert*-butyldimethylsilyl)-cyclomalto-octaose from a Daisopak SP-120-5-ODS Column (150 × 6 mm i.d.) with Methanol–Water (70 : 30) at a Flow Rate of 1.0 ml/min

tion during work-up procedures, hydrochloric acid formed in the reaction mixture and included in the cG_8 molecule was completely removed with Amberlite IRA-410 (OH^-). The powdery mixture, thus obtained, contained mono-, di-, and over-silylated compounds which were separated by semi-preparative HPLC on a C_{18}-bonded silica (octadecyl silica (ODS)) column (250 × 20 mm i.d., 7 μm) with methanol–water (73 : 27) as the eluent to give a mixture of **2**—**5** (20—25%). Figure 1 shows the elution profile of the regioisomeric mixture of $6^1,6^n$-di-*O*-(*tert*-$BuMe_2Si$)-cG_8s. The relative ratios of S-I, S-II, S-III and S-IV, calculated from the peak areas in the chromatogram, were approximately 1 : 2 : 2 : 1. Each disilylate was isolated by repeated rechromatography.

Characterization of the Four Positional Isomers In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of S-I—S-IV in pyridine-d_5, signals due to the silyl-substituted C-6s (δ 62—64) were shifted downfield by 2 ppm, compared with those due to other C-6s. The ratio of relative intensities of signals due to C-1 at δ 103—104, the silyl-substituted C-6s at δ 62—64, and methyl groups of *tert*-$BuMe_2Si$ at δ −5 was 8 : 2 : 4. The assignments of the two kinds of C-6 signals were confirmed by the distortionless enhancement by polarization transfer (DEPT) method.[9] These results proved that all four compounds were di-*O*-(*tert*-$BuMe_2Si$)-substituted derivatives. Of these compounds, S-I showed a rather simple spectrum. Namely, the signals due to C-4s consisted of four lines, indicating that the two silyl groups in S-I are symmetrically situated. Thus, S-I was assigned as $6^1,6^5$-disubstituted cG_8 **5**. The substituted positions of other isomers could not be determined from the ^{13}C-NMR spectra.

Next, the regiochemical determination of these positional isomers was performed by utilizing four di-*O*-trityl-cG_8s, the regiochemistry of which had been established. Acetylation of each $6^1,6^n$-di-*O*-trityl-cG_8 derivative (n=2—5, **6**—**9**) followed by *O*-detritylation[10] gave bis(2,3-di-*O*-acetyl)hexakis(2,3,6-tri-*O*-acetyl)-cG_8s (**6**-OH—**9**-OH). Silylation of **6**-OH—**9**-OH with *tert*-$BuMe_2SiCl$ in pyridine[7,8] was unsuccessful. However, treatment of dried **6**-OH—**9**-OH with *tert*-$BuMe_2SiCl$ in *N*,*N*-dimethylformamide in the presence of imidazole[11-14] for 1 h at 45 °C afforded bis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)hexakis(2,3,6-tri-*O*-acetyl)-cG_8 (**6′**Ac—**9′**Ac). *O*-Deacetylation of **6′**Ac—**9′**Ac provided the desired compounds, **6′**—**9′**.

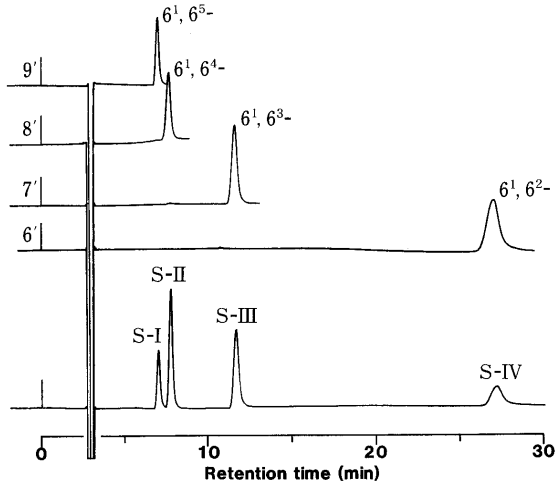


Fig. 2. Elution Profiles of Di-*O*-(*tert*-butyldimethylsilyl)-cyclomalto-octaoses (**6′**—**9′**) Obtained from Di-*O*-trityl-cyclomalto-octaoses (**6**—**9**) and Di-*O*-(*tert*-butyldimethylsilyl)-cyclomalto-octaoses (S-I—S-IV)

Chromatographic conditions were as in Fig. 1.

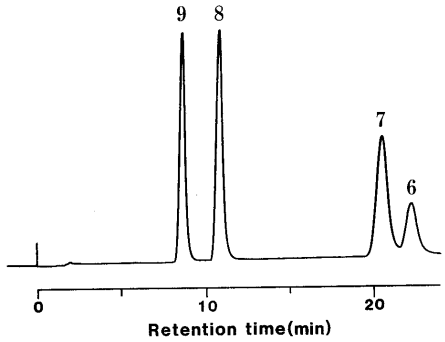


Fig. 3. Elution Profile of Di-*O*-trityl-cyclomalto-octaoses (**6**—**9**)

Chromatographic conditions: eluent, methanol–water (75 : 25); wavelength, 240 nm; other conditions as in Fig. 1.

Figure 2 show HPLC chromatograms of $6^1,6^n$-di-*O*-(*tert*-$BuMe_2Si$)-cG_8s (**6′**—**9′**) obtained from $6^1,6^n$-di-*O*-trityl-cG_8s. From a comparison of the retention time of each peak (**6′**—**9′**) with those of the four regioisomers (S-I—S-IV), it is apparent that S-I, S-II, S-III and S-IV are **5**, **4**, **3** and **2**, respectively.

The elution profile of four positional isomers of $6^1,6^n$-di-*O*-(*tert*-$BuMe_2Si$)-cG_8s from the ODS column has been compared to that of $6^1,6^n$-di-*O*-trityl-cG_8s. The retention order, $6^1,6^5$-, $6^1,6^4$-, $6^1,6^3$- and $6^1,6^2$-, is the same, but the elution pattern is rather different (Fig. 3). It is easier to separate $6^1,6^2$- and $6^1,6^3$-di-*O*-(*tert*-$BuMe_2Si$)-cG_8s than the corresponding trityl derivatives.

In conclusion, as intermediates for chemical syntheses of $6^1,6^n$-di-*O*-(D-glycosyl)-cG_8s, silyl compounds (for $6^1,6^2$- and $6^1,6^3$-derivatives) and trityl compounds (for $6^1,6^4$- and $6^1,6^5$-derivatives) are effective.

Experimental

General Methods Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations

TABLE I. Physico-Chemical Data for Di-*O*-(*tert*-butyldimethylsilyl)-cyclomalto-octaoses

Compound	$[\alpha]_D$ (in CH_3OH)			^{13}C-NMR δ (C_5D_5N)	
	(°)	*c*	Temp. (°C)	Si $(CH_3)_2$	C-6[a]
2	+145.0	1.0	27	−4.88, −4.89 −5.01, −5.06	62.76, 62.88
3	+132.0	1.0	28	−5.00, −5.03 −5.11, −5.13	63.09, 63.13
4	+144.9	1.1	26	−5.00, −5.12	63.08
5	+136.4	0.6	26	−4.98, −5.12	63.03

a) *tert*-$BuMe_2Si$-substituted carbon.

were determined with a JASCO digital polarimeter, model DIP 360. TLC was performed on silica gel (5721, Merck) with detection by charring with sulfuric acid. Centrifugal chromatography was performed with a Harrison Centrifugal thin layer chromatotron, model 7924. HPLC was conducted with a Tri rotar SR-1 or 880-PU pump (JASCO), a U6K universal injector (Waters), and an SE-61 or -71 refractive index monitor (Showa Denko). The columns used were YMC-Pack SH-343-7 ODS (250 × 20 mm i.d.) SH-343-5 ODS (250 × 20 mm i.d.), and Daisopak SP-120-5-ODS (150 × 6 mm i.d.). A Shimadzu Chromatopac C-R3A digital integrator was used for quantitative analyses. ^{13}C-NMR spectra were recorded with a JEOL GSX-500 (125.65 MHz) spectrometer in C_5D_5N (internal Me_4Si).

$6^1,6^2$-, $6^1,6^3$-, $6^1,6^4$-, and $6^1,6^5$-Di-*O*-(*tert*-butyldimethylsilyl)-cyclomalto-octaoses (2—5) *tert*-Butyldimethylsilyl chloride (1.22 g, 3.5 mol eq) was added at 5 °C to a solution of **1** (3.0 g, dried over molecular sieves under reduced pressure for 2 d at 100 °C) in dry pyridine (60 ml).[7,8] The mixture was stirred for 1 h at room temperature, and treated with Amberlite IRA-410 (OH^-) to remove the resulting acid in the solution, then the filtrate was evaporated under reduced pressure. The residue was stirred in a mixture of ice-water (100 ml) and chloroform (100 ml), and the precipitate that was deposited between the two phases was collected by filtration through a 1 μm membrane filter and washed successively with water and chloroform, to give 2.8—3.5 g of powdery silylated cG_8 mixture.

The disilylated compounds were separated from monosilylated and over-silylated ones by semi-preparative HPLC on a YMC-Pack SH-343-7 ODS column (250 × 20 mm i.d.) with methanol–water (73 : 27) as the eluent, to give a mixture of **2**—**5** (25%). Further, each regioisomer was repeatedly separated by HPLC on a YMC-Pack SH-343-5 ODS column (250 × 20 mm i.d.) with a mixture of methanol–water, 80 : 20 for **2**, 78 : 22 for **3**, and 70 : 30 for **4** and **5**. Of those compounds, only **3** could be crystallized from methanol and water, mp 274 °C (dec.). *Anal.* Calcd for $C_{60}H_{108}O_{40}Si_2 \cdot 3H_2O$: C, 45.62; H, 7.27. Found: C, 45.62; H, 7.48. Other physico-chemical data of these compounds are listed in Table I.

Characterization of the Four Positional Isomers Each of the four positional isomers of $6^1,6^n$-di-*O*-trityl-cG_8s (**6**—**9**) was converted into the corresponding $6^1,6^n$-di-*O*-(*tert*-$BuMe_2Si$)-cG_8 (**6′**—**9′**).

Acetylation of a solution of each one, **6** (17 mg), **7** (22 mg), **8** (42 mg), and **9** (18 mg), in anhydrous pyridine (2—3 ml) was performed with acetic anhydride (1—2 ml) for 5 h at 100 °C and the mixture was concentrated. A solution of the residue in chloroform was washed with water, aqueous sodium carbonate, and water, then dried, and evaporated to a syrup. *O*-Detritylation[10] of each residue by stirring in 70% acetic acid (15 ml) for 1 h at 70—80 °C, followed by centrifugal chromatography (1 : 1, hexane–acetone) gave **6**-OH (9 mg, 40.0%), **7**-OH (10 mg, 36.4%), **8**-OH (15 mg, 28.6%), and **9**-OH (5 mg, 22.3%). To a stirred mixture of dried **6**-OH, **7**-OH, **8**-OH, or **9**-OH and imidazole (10—30 mg) in dry *N*,*N*-dimethylformamide (1—2 ml) was added a solution of *tert*-$BuMe_2SiCl$ (30—50 mg) in anhydrous *N*,*N*-dimethylformamide (3—5 ml).[11-14] The mixture was stirred for 1—2 h at 45 °C. Work-up of the mixture as described for acetylation, followed by centrifugal chromatography (3 : 2, hexane–acetone), afforded **6′**Ac—**9′**Ac. The residue was treated with methanolic 0.05 N sodium methoxide for 1 h at room temperature, and the solution was neutralized with Amberlite IR-120B (H^+) resin, filtered, and concentrated. The residue (**6′**—**9′**) was directly analyzed by HPLC.

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