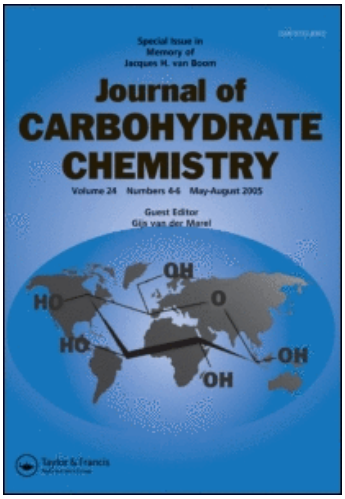

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Preparation, Isolation and Characterization of all of the Regioisomeric 6^1, 6^N-Bis-*O*-(Monomethoxytrityl) and-(Dimethoxytrityl) Derivatives of Cyclomalto-Olgosaccharides

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PREPARATION, ISOLATION AND CHARACTERIZATION OF ALL OF THE REGIOISOMERIC 6^1, 6^n-BIS-*O*-(MONOMETHOXYTRITYL) AND -(DIMETHOXYTRITYL) DERIVATIVES OF CYCLOMALTO-OLIGOSACCHARIDES

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ABSTRACT

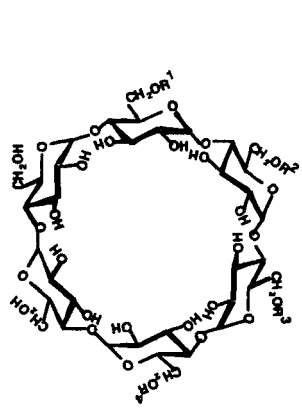
Regioisomeric 6^1,6^n-bis-*O*-(monomethoxytrityl) or 6^1,6^n-bis-*O*-(dimethoxytrityl) cyclomaltohexaose, -cyclomaltoheptaose (n = 2-4), and -cyclomaltooctaose derivatives (n = 2-5) were prepared by the reaction of cyclomaltohexaose (**1**, cG_6, αCD), cyclomalto-heptaose (**11**, cG_7, βCD) or cyclomaltooctaose (**21**, cG_8, γCD) and 4-monomethoxytrityl chloride or 4,4'-dimethoxytrityl chloride in pyridine. Products were isolated by HPLC. The regiochemical determination of these positional isomers was done by converting these compounds to the respective 6^1, 6^n-bis-*O*-(*tert*-butyldimethylsilyl) derivatives[1] whose structures have been already established.

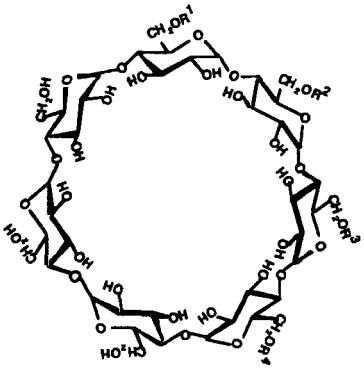
INTRODUCTION

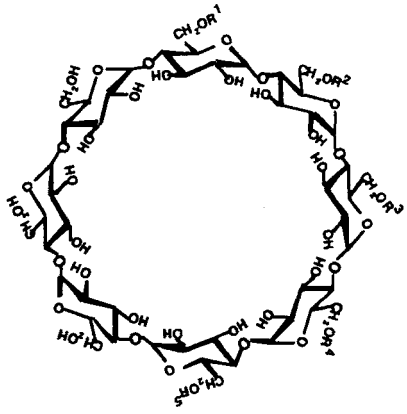
As intermediates for chemical syntheses of positional isomers of dibranched cyclomalto-oligosaccharides (cG_ns, CDs),[2-4] we have already synthesized and isolated the positional isomers of 6^1, 6^n-di-*O*-triphenylmethyl (trityl)- or 6^1, 6^n-bis-*O*-(*tert*-butyldimethylsilyl) (*tert*-$BuMe_2Si$)-cyclomaltohexaoses (cG_6s, αCDs),[5,6] -cyclomalto-heptaoses (cG_7s, βCDs) (n = 2-4),[5,6] and -cyclomaltooctaoses (cG_8s, γCDs) (n = 2-5).[1,7] The requirements for these derivatives as intermediates are easy separation of the positional isomers by HPLC and as high yields of all of them as possible. Although the *tert*-$BuMe_2Si$ derivatives of cG_6 and cG_7 fulfill both requirements, they cannot be

detected on HPLC by UV, which is the commonly used procedure. In the cases of trityl derivatives, the yields of 6^1, 6^2-di-*O*-trityl-cG$_n$s are generally very low, because of the steric hindrance between the two bulky trityl groups attached to the two neighboring D-glucose units.[4-6] Moreover, for preparative chromatography of the four positional isomers of cG$_8$ derivatives, it is difficult to separate 6^1, 6^4-bis-*O*-(*tert*-BuMe$_2$Si)cG$_8$ (**24**) from 6^1, 6^5-bis-*O*-(*tert*-BuMe$_2$Si)cG$_8$ (**25**), and 6^1, 6^2-di-*O*-trityl-cG$_8$ (**26**) from 6^1, 6^3-di-*O*-trityl-cG$_8$ (**27**).[1,7]

In this study we undertook the synthesis of bis-*O*-(monomethoxytrityl) and bis-*O*-(dimethoxytrityl)cG$_n$s having bulkier substituted groups than cG$_n$s with trityl groups in order to evaluate the stereospecificity of the reactions and isolate positional isomers.







	R^1	R^2	R^3	R^4
1	H	H	H	H
2	Tr	Tr	H	H
3	Tr	H	Tr	H
4	Tr	H	H	Tr
5	MTr	MTr	H	H
6	MTr	H	MTr	H
7	MTr	H	H	MTr
8	DTr	DTr	H	H
9	DTr	H	DTr	H
10	DTr	H	H	DTr

	R^1	R^2	R^3	R^4
11	H	H	H	H
12	Tr	Tr	H	H
13	Tr	H	Tr	H
14	Tr	H	H	Tr
15	MTr	MTr	H	H
16	MTr	H	MTr	H
17	MTr	H	H	MTr
18	DTr	DTr	H	H
19	DTr	H	DTr	H
20	DTr	H	H	DTr

	R^1	R^2	R^3	R^4	R^5
21	H	H	H	H	H
22	Si	Si	H	H	H
23	Si	H	Si	H	H
24	Si	H	H	Si	H
25	Si	H	H	H	Si
26	Tr	Tr	H	H	H
27	Tr	H	Tr	H	H
28	Tr	H	H	Tr	H
29	Tr	H	H	H	Tr
30	MTr	MTr	H	H	H
31	MTr	H	MTr	H	H
32	MTr	H	H	MTr	H
33	MTr	H	H	H	MTr
34	DTr	DTr	H	H	H
35	DTr	H	DTr	H	H
36	DTr	H	H	DTr	H
37	DTr	H	H	H	DTr

Tr, trityl; MTr, monomethoxytrityl; DTr, dimethoxytrityl;
Si, *tert*-BuMe$_2$Si

We also describe their regiochemical determination and compare their chromatographic behavior with those of di-*O*-trityl-cG$_n$s for which the regiochemistry has been already established.

RESULTS AND DISCUSSION

Preparation and Isolation of $6^1,6^n$-Bis-*O*-(monomethoxytrityl)cG$_6$s (5-7), -cG$_7$s (15-17), and -cG$_8$s (30-33), and $6^1,6^n$-Bis-*O*-(dimethoxytrityl)cG$_6$s (8-10), -cG$_7$s (18-20), and -cG$_8$s (34-37). Regioselective monomethoxytritylation and dimethoxytritylation of **1**, **11**, or **21**, which had been dried by azeotropic distillation with pyridine, with 2.3 mol equiv of 4-monomethoxytrityl chloride or 4,4'-dimethoxytrityl chloride for 5-6 h at 45 °C gave, upon work-up, a powdery mixture containing disubstituted compounds as the major products. Bis-*O*-monomethoxytritylates or bis-*O*-dimethoxytritylates were separated from mono-substituted and over-substituted compounds by HPLC on an octadecyl silyl silica (ODS) column eluted with methanol-water. Each mixture of **5** - **7** (59%), **8** - **10** (51%), **15** - **17** (48%), **18** - **20** (47%), **30** - **33** (28%), and **34** - **37** (25%) was obtained. Figures 1 and 2 show chromatograms of each of the three positional isomers of bis-*O*-(monomethoxytrityl) and bis-*O*-(dimethoxytrityl)cG$_6$s and cG$_7$s, together with those of trityl disubstituted derivatives for reference on ODS column with methanol-water (A) and acetonitrile-water (B). Similarly, chromatograms of each of the four positional isomers of di-*O*-trityl-, bis-*O*-(monomethoxytrityl), and bis-*O*-(dimethoxytrityl)cG$_8$s are shown in Fig. 3. Though the peaks of αM-I and αM-II and of βD-II and βD-III could not be separated with methanol-water, these two isomers were isolated using acetonitrile-water with baseline separation. The elution orders of three or four positional isomers of bis-*O*-(monomethoxytrityl) and bis-*O*-(dimethoxytrityl)cG$_7$s (Fig. 2) and cG$_8$s (Fig. 3) with two eluents, A and B, were almost the same, while those of cG$_6$ derivatives (Fig. 1) were markedly different. Moreover, retention times of cG$_6$ derivatives on the C$_{18}$ bonded silica column were significantly long compared with those of cG$_7$ and cG$_8$ derivatives. This phenomenon is considered to be due to a higher hydrophobic effect of the two trityl groups occupying a larger portion of the molecules of cG$_6$ derivatives having the smallest ring structure. The elution order of αM-II and αD-I, which are confirmed to be $6^1,6^2$-substituted derivatives as described later, is different with two eluents, A and B. Retention of $6^1,6^2$-substituted derivatives, especially αM-II was particularly influenced by temperature (Fig. 4). The mechanism of retention onto bonded-phase chromatography has not been clarified.

Each ditritylate was isolated by rechromatography on several kinds of ODS columns with methanol-water and acetonitrile-water as described in detail in the experimental section.

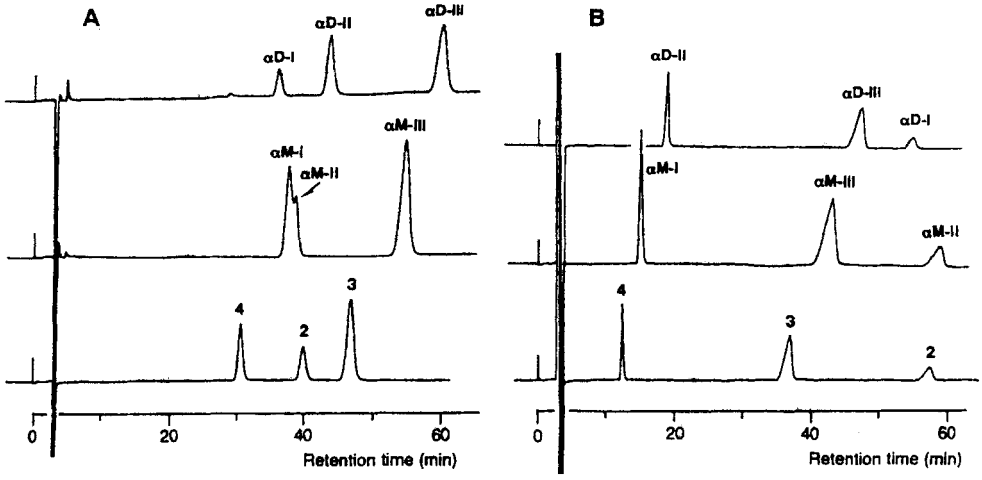


Fig. 1 Elution profiles of three positional isomers of bis-*O*-(monomethoxytrityl) cG6s (αM- I – αM-III), bis-*O*-(dimethoxytrityl)cG6s (αD-I – αD-III), and di-*O*-trityl-cG6s (**2** - **4**). Chromatographic conditions: column, YMC-Pack A-312 ODS (150 x 6 mm i.d.); eluent, (**A**) methanol-water (75-25), (**B**) acetonitrile-water (42-58); flow rate, 1.0 mL/min; detector, UV, wavelength, 240 nm; temperature, 30 °C.

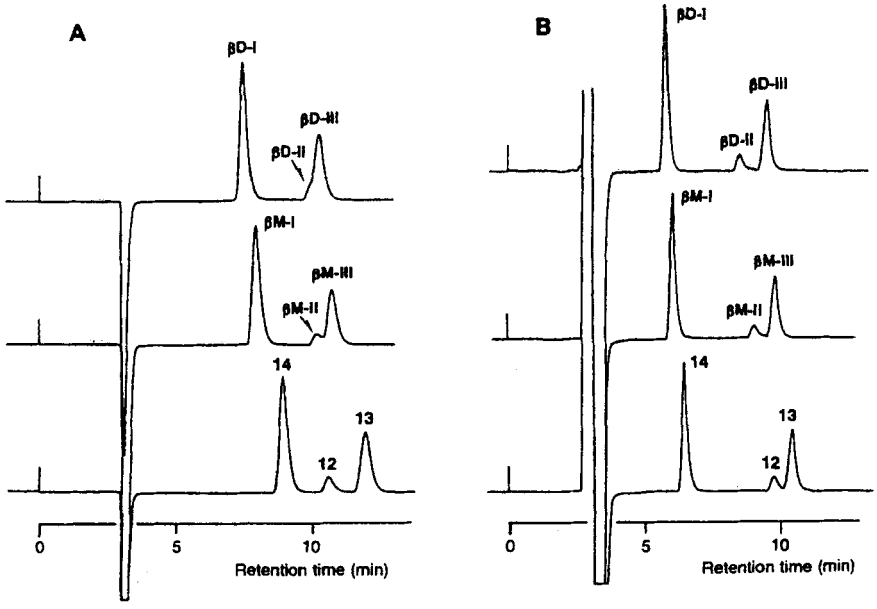


Fig. 2 Elution profiles of three positional isomers of bis-*O*-(monomethoxytrityl)cG7s (βM-I – βM-III), bis-*O*-(dimethoxytrityl)cG7s (βD-I – βD-III), and di-*O*-trityl-cG7s (**12** - **14**). Chromatographic conditions as in Fig. 1.

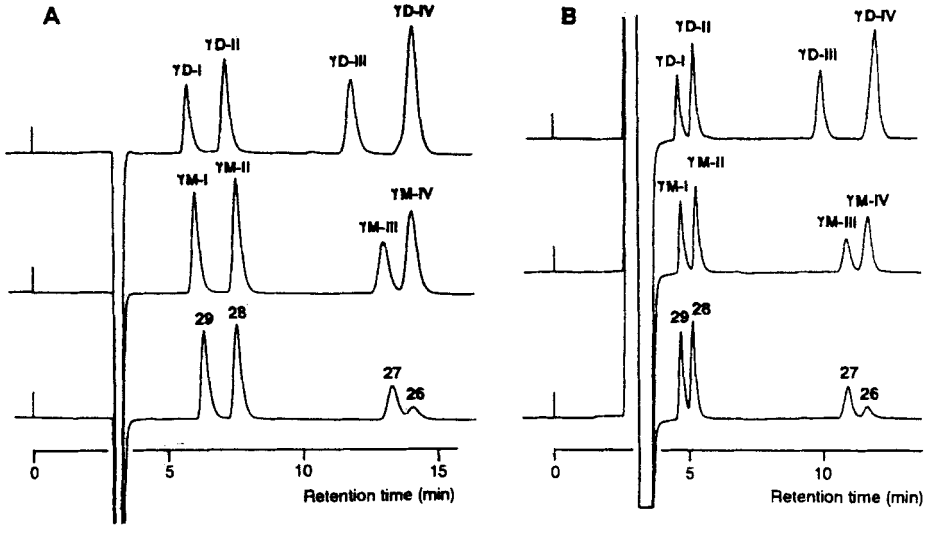


Fig. 3 Elution profiles of four positional isomers of bis-*O*-(monomethoxytrityl) cG8s (γM- I – γM-IV), bis-*O*-(dimethoxytrityl)cG8s (γD-I – γD-IV), and di-*O*-trityl-cG8s (**26** - **29**). Chromatographic conditions as in Fig. 1.

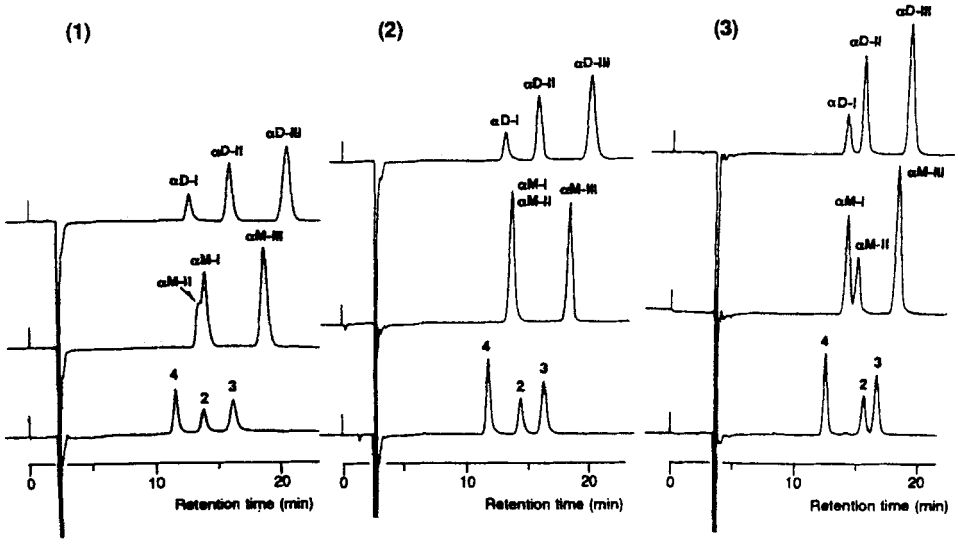


Fig. 4 Elution profiles of three positional isomers of bis-*O*-(monomethoxytrityl) cG6s (αM- I – αM-III), bis-*O*-(dimethoxytrityl)cG6s (αD-I – αD-III), and di-*O*-trityl-cG6s (**2** - **4**) at (1) 25 °C, (2) 30 °C, and (3) 40 °C. Chromatographic conditions: column, Hikarisil C18-4D (150 x 4.6 mm i.d.); eluent, methanol-water (80-20); flow rate, 0.7 mL/min; detector, UV, wavelength, 240 nm.

Characterization of the Positional Isomers In the ^{13}C NMR spectra of all positional isomers of monomethoxytrityl derivatives (αM-I – αM-III, βM-I – βM-III, and γM-I – γM-IV) and dimethoxytrityl derivatives (αD-I – αD-III, βD-I – βD-III, and γD-I – γD-IV) in pyridine-d_5, signals due to the monomethoxytrityl- or dimethoxytrityl-substituted C-6s (d 63.6 - 64.8) were shifted downfield by 2 ppm, compared with those due to other C-6s. The assignment of C-6 signals was confirmed by distortionless enhancement by the polarization transfer (DEPT) method.[8] All the above compounds were disubstituted derivatives from the ratio of relative intensities of signals due to C-1 at δ 103 - 104 and substituted C-6s, for example, 6 : 2 for αM-I – αM-III and αD-I – αD-III. In the spectra of αM-I and αD-II, and γM-I and γD-I, each signal for C-1, -4, and -6 was split into only three and four lines, respectively. In addition, no splitting was observed from both the signals of the quarternary carbon of the trityl group and of the trityl-substituted C-6. cG_6 and cG_8 are made up of even membered glucose units and the two trityl groups in these compounds are symmetrically situated. Thus, αM-I and αD-II were assigned as $6^1,6^4$-disubstituted cG_6, and γM-I and γD-I were assigned as $6^1,6^5$-disubstituted cG_8. On the other hand, in the spectra of αM-II, αD-I, βM-II, βD-II, γM-III, and γD-III, both the signals of the quarternary carbon of the trityl group and of the trityl-substituted C-6 appeared as two signals, with a large difference between them. There seemed to be two adjacent bulky trityl groups in the molecules, that is, $6^1,6^2$-disubstituted cG_6, cG_7, and cG_8. It was confirmed by FABMS that all three or four isomers were disubstituted derivatives, that is, they had the same molecular weight: 1516 for αM-I – αM-III, 1678 for βM-I – βM-III, 1840 for γM-I – γM-IV, 1576 for αD-I – αD-III, 1738 for βD-I – βD-III, and 1900 for γD-I – γD-IV.

Next, the regiochemical determination of each three or four positional isomers was performed by conversion to bis-*O*-(*tert*-$BuMe_2Si$)cG_6s,[6] cG_7s,[6] and cG_8s,[1] of which the regiochemistry had been established. For example, acetylation of γM-II, γM-III, and γM-IV, and *O*-detritylation and silylation with *tert*-$BuMe_2SiCl$ in *N,N*-dimethylformamide in the presence of imidazole[1,9-12] followed by *O*-deacetylation afforded the desired compounds, γM-II-Si, γM-III-Si, and γM-IV-Si. Thus far, *O*-detritylation had been performed with 80% aqueous acetic acid solution at 100 °C for 30 min, but yields were usually low (40-50%).[1,13] Ishido *et al.* reported that *O*-dedimethoxytritylation could be done with 70-80% aqueous acetic acid solution at room temperature for 30 min, followed by concentration of the resulting mixture.[14] Regretably, the yield was not reported. We tried to modify the method of *O*-desilylation.[4,9] A solution in dichloromethane was added to boron trifluoride diethyl etherate, and the mixture was stirred for 1 h at room temperature and then diluted with dichloromethane. The organic layer was washed with water. The yields averaged 60-70% . The advantages of this

method are the short treatment at room temperature and no need for concentration by raising the temperature. Figure 5 shows HPLC chromatograms of silylated compound, γM-II-Si, γM-III-Si, and γM-IV-Si obtained from γM-II, γM-III, and γM-IV, and authentic compounds, $6^1,6^n$-bis-*O*-(*tert*-BuMe$_2$Si)cG$_8$s (n = 2-5) (**22** - **25**). From comparison of the retention time of each compound, it was apparent that γM-II, γM-III, and γM-IV were **32**, **30**, and **31**, respectively, and therefore the remaining γM-I was **33**. In a similar manner, the substituted positions of all positional isomers were established. The results and relative production ratios calculated from the peak areas in the chromatograms are summarized in Table 1.

Initially we expected that the stereospecificity of bulkier substituted trityl groups would give only one or two positional isomers. However there were not marked differences between monomethoxytrityl, dimethoxytrityl, and trityl derivatives. Conveniently in regard to bis-*O*-(dimethoxytrityl)cG$_8$s, the requirements that as intermediates they should be easily separated by HPLC and that all four positional isomers should be prepared in high yields were achieved.

In conclusion, we found that efficient intermediates for chemical syntheses of positional isomers of dibranched cG$_n$s are trityl or dimethoxytrityl derivatives for cG$_6$s, trityl derivatives for cG$_7$s, and dimethoxytrityl derivatives for cG$_8$s.

EXPERIMENTAL

General methods. Unless stated otherwise, the synthesized compounds were the same as described previously.[6] HPLC was conducted with a JASCO TRIROTAR SR-1 or 880-PU pump, a Waters U6K universal injector, a Shodex RI-71 refractive index monitor, a JASCO UVIDEC-100 III variable-wavelength ultraviolet detector, and a Lab-Quatec CO-1093 column oven. The columns used were: (A) YMC-Pack SH-343-7 ODS (250 x 20 mm i.d.), (B) YMC-Pack SH-312-5 ODS (150 x 6 mm i.d.), (C) Daisopak SP-120-5-ODS (250 x 20 mm i.d.), (D) Daisopak SP-120-5-ODS (150 x 6 mm i.d.), (E) Hikarisil C18-2E (250 x 20 mm i.d.), and (F) Hikarisil C18-4D (150 x 4.6 mm i.d.).

$6^1,6^2$-, $6^1,6^3$-, And 6^1, 6^4-bis-*O*-(monomethoxytrityl)cyclomaltohexaoses (5-7), $6^1,6^2$-, $6^1,6^3$-, And 6^1, 6^4-bis-*O*-(monomethoxytrityl)cyclomaltoheptaoses (15-17), and $6^1,6^2$-, $6^1,6^3$-, 6^1, 6^4-, And $6^1,6^5$-bis-*O*-(monomethoxytrityl)cyclomaltooctaoses (30-33) Compound **1**, **11** or **21** (3.0 g, dried over molecular sieves under reduced pressure for 2 d at 100 °C) was dissolved in the dry pyridine (100 mL) and the solvent was distilled at atmospheric pressure until the temperature of the boiling distillate reached 115 °C. The solution was diluted to 80 mL with dry pyridine and then 4-monomethoxytrityl chloride (2.3 mol equiv, 2.20 g for **1**, 1.92 g for **11**, 1.64 g for **21**) was

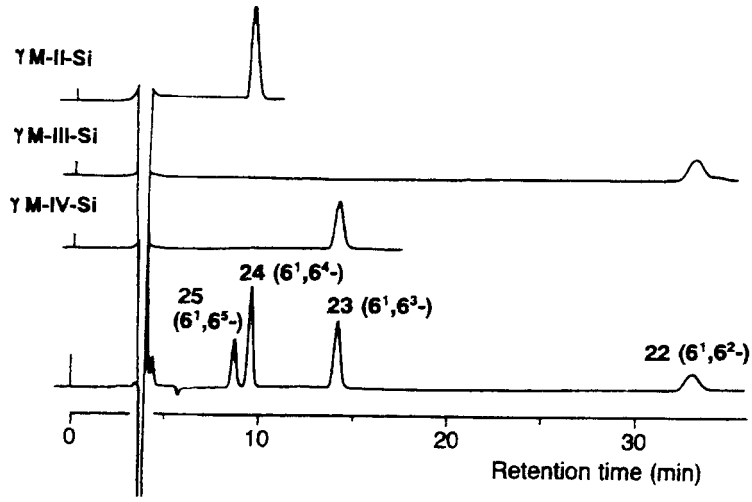


Fig. 5 Elution profiles of bis-*O*-(*tert*-butyldimethylsilyl)cG8s (γM-II-Si – γM-IV-Si) obtained from bis-*O*-(monomethoxy trityl)cG8s (γM-II – γM-IV) and bis-*O*-(*tert*-butyldimethyl silyl)cG8s (**22** - **25**). Chromatographic conditions: column, Daisopak SP-120-5-ODS (150 x 6 mm i.d.); eluent, methanol-water (70-30); flow rate, 0.8 mL/min; detector, Shodex RI-71; temperature, 30 °C.

Table 1. Regiochemical determination of each three or four positional isomers of six kinds of bis-*O*-(monomethoxytrityl) and bis-*O*-(dimethoxytrityl) derivatives and their relative proportions.

	$6^1, 6^2$-	$6^1, 6^3$-	$6^1, 6^4$-	$6^1, 6^5$-
αM-ratio	II (**5**) 1 :	III (**6**) 3 :	I (**7**) 2	
βM-ratio	II (**15**) 1 :	III (**16**) 5 :	I (**17**) 10	
γM-ratio	III (**30**) 6 :	IV (**31**) 11 :	II (**32**) 11 :	I (**33**) 9
αD-ratio	I (**8**) 2 :	III (**9**) 8 :	II (**10**) 5	
βD-ratio	II (**18**) 7	III (**19**) :	I (**20**) 10	
γD-ratio	III (**34**) 4 :	IV (**35**) 8 :	II (**36**) 4 :	I (**37**) 3

added and the reaction mixture stirred at 45 °C. The progress of the reaction was monitored by TLC on silica gel plates with 7:4:1 chloroform-methanol-water. After 5 h the mixture was treated with Amberlite IRA-410 (OH^-) to remove the resulting acid and most of the remaining reagent from the solution, then the filtrate was concentrated under reduced pressure. The residue was stirred in ice-water (100 mL), and the precipitate was collected by filtration through a 1-μm membrane filter and washed with water, to give 4.8 g, 3.9 g, and 3.4 g of powdery monomethoxytritylated cG_6s, cG_7s, and cG_8s mixture, respectively. Each bis-monomethoxytritylated mixture was separated from mono- and over-monomethoxytritylated compounds by semi-preparative HPLC on column A eluted with 85:15 methanol-water to give a mixture of **5** - **7** (59%), with 76:24 methanol-water to give a mixture of **15** - **17** (48%), or with 78:22 methanol-water to give a mixture of **30** - **33** (28%). Each regioisomer was then repeatedly separated on column E with 85:15 methanol-water to obtain a mixture of **5** and **7** plus **6**, with 50:50 acetonitrile-water for **5** and **7**, with 75:25 methanol-water for **17** and a mixture of **15** and **16**, or with 40:60 acetonitrile-water for **15** and **16**, or on column C with 78:22 methanol-water for **30** and **31** or with 72:28 methanol-water for **32** and **33**. Among those compounds, **5**, **15** , **17**, and **33** could be crystallized from methanol and water; **5** mp 284 °C (dec.), **15** mp 286 °C (dec.), **17** mp 288 °C (dec.), and **33** mp 279 °C (dec.). The other physico-chemical data of these compounds are listed in Table 2.

$6^1,6^2$-, $6^1,6^3$-, And 6^1, 6^4-bis-*O*-(dimethoxytrityl)cyclomaltohexaoses (8-10), $6^1,6^2$-, $6^1,6^3$-, And 6^1, 6^4-bis-*O*-(dimethoxytrityl)cyclomaltoheptaoses (18-20), and $6^1,6^2$-, $6^1,6^3$-, 6^1, 6^4-, And $6^1,6^5$-bis-*O*-(dimethoxytrityl)cyclomaltooctaoses (34-37) Dimethoxytritylation of **1**, **11**, or **21** (3.0 g), as described for the preparation of **5** - **7**, **15** - **17**, and **30** - **33**, afforded **8** - **10** (51%), **18** - **20** (47%), and **34** - **37** (25%). Each regioisomer was separated in the same manner as described above derivatives. Among those compounds, only **37** could be crystallized from methanol, mp 280 °C (dec.). The other physico-chemical data of these compounds are listed in Table 2.

Characterization of the positional isomers Each of the positional isomers of $6^1,6^n$-bis-*O*-(monomethoxytrityl) and $6^1,6^n$-bis-*O*-(dimethoxytrityl)cG_ns was converted into the authentic compound, $6^1,6^n$-bis-*O*-(*tert*-$BuMe_2Si$)cG_ns.

Compounds γM-II (28 mg), γM-III (34 mg), and γM-IV (32 mg) were each acetylated with acetic anhydride (1 mL) in anhydrous pyridine (2-3 mL) for 5 h at 100 °C, and the mixture was concentrated. The residue, dissolved in chloroform, was washed sequentially with water, aqueous sodium hydrogencarbonate, and water, then dried, and concentrated. *O*-Demonomethoxytritylation of each residue in dichloromethane (2 mL) was performed with boron trifluoride diethyl etherate (30 μL) for 1 h at room temperature. A solution in chloroform was washed with water, aqueous

Table 2. Physico-chemical data for bis-*O*-(monomethoxytrityl) and bis-*O*-(dimethoxytrityl)cG$_n$s

Compound	$[\alpha]_D$ (in CH_3OH)			^{13}C NMR		
	(°)	*c*	temp. (°C)	δ, (C_5D_5N)		
				CPh_3	C-6[a]	OCH_3
5	+109.1	1.0	28	86.59, 87.14	64.06, 64.78	55.27, 55.29
6	+116.7	0.6	28	86.89(2)	63.75, 63.82	55.22, 55.26
7	+115.0	0.6	28	86.90(2)	63.85(2)	55.21(2)
8	+116.8	1.0	28	86.27, 86.93	63.78, 64.56	55.23, 55.26(2), 55.31
9	+108.8	1.3	28	86.57, 86.60	63.58, 63.64	55.21(2), 55.25(2)
10	+103.6	1.4	28	86.58(2)	63.67(2)	55.20(4)
15	+ 94.9[b]	1.2	24	86.68, 86.98	63.73, 65.06	55.30, 55.32
16	+114.3	1.1	24	87.18, 87.21	64.16, 64.23	55.35, 55.38
17	+103.8[b]	1.3	25	86.96, 87.05	64.26, 64.46	55.19(2)
18	+ 91.1	1.1	24	86.41, 86.79	64.78, 64.84	55.20, 55.25(2), 55.32
19	+ 96.8	1.0	24	86.76, 86.77	63.97, 64.17	55.22(2), 55.27(2)
20	+101.7[b]	1.2	24	86.70, 86.81	64.10, 64.24	55.21(4)
30	+119.5	1.0	27	86.75, 87.02	64.02, 64.78	55.25, 55.29
31	+143.2	1.1	27	87.00(2)	64.32, 64.40	55.18, 55.21
32	+128.1	1.0	28	86.94, 87.03	64.30, 64.38	55.18(2)
33	+117.5	1.0	27	86.95(2)	64.24(2)	55.18(2)
34	+119.3	1.1	26	86.53, 86.88	63.95, 64.67	55.25, 55.26(2), 55.34
35	+129.8	1.3	26	86.77(2)	64.22(2)	55.21(2), 55.25(2)
36	+127.2	1.3	26	86.71, 86.78	64.11, 64.20	55.21(4)
37	+113.7	1.0	26	86.70(2)	64.04(2)	55.22(4)

a. *O*-CPh_3-bonded substituted carbon. b. Solution in 90% methanol. Values in parentheses are numbers of carbons.

sodium hydrogencarbonate, and water, then dried, and concentrated. Centrifugal chromatography (1:1 hexane-acetone) gave γM-II-OH (19 mg, 56%), γM-III-OH (23 mg, 56%), and γM-IV-OH (16 mg, 41%). A solution of dried γM-II-OH, γM-III-OH or γM-IV-OH in *N,N*-dimethylformamide (2 mL) was added to *tert*-$BuMe_2SiCl$ (65-94 mg) and imidazole (32-46 mg), and the mixture was stirred for 1-2 h at 45 °C, concentrated, and

processed as described for acetylation. Centrifugal chromatography (3:2 hexane-acetone) of the product gave γM-II-Si-Ac, γM-III-Si-Ac, or γM-IV-Si-Ac. The residue was treated with methanolic 0.05 M sodium methoxide for 1 h at room temperature, and the solution was neutralized with Amberlite IR-120B (H^+) resin, filtered, and concentrated. The residue (γM-II-Si, γM-III-Si, and γM-IV-Si) was directly analyzed by HPLC.

All other monomethoxytrityl- and dimethoxytrityl-cG_ns were similarly treated and analyzed by HPLC.

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