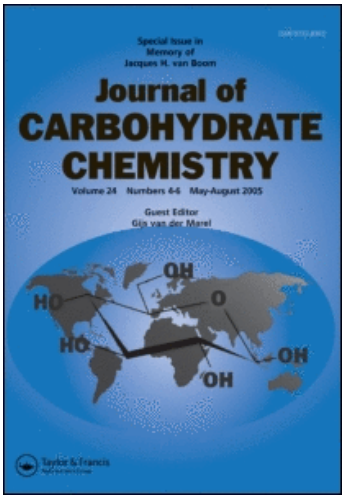

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PREPARATION AND CHARACTERIZATION OF $6^1,6^n$-DI-*O*-(α-D-GALACTOPYRANOSYL)CYCLOMALTOOCTAOSES

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ABSTRACT

Four positional isomers of $6^1,6^n$-di-*O*-(D-galactopyranosyl)cyclomaltooctaoses (cG_8s, γ-cyclodextrins) (n = 2–5) were chemically synthesized using the trichloroacetimidate method. The desired compounds having two α-(1→6)-linkages were isolated from a mixture of configurational isomers by HPLC, and their structures were confirmed by ^{13}C NMR spectroscopy and FAB-high resolution mass spectra (HRMS). The elution behavior of their four positional isomers on an ODS column by HPLC is discussed.

INTRODUCTION

The chemical synthesis of branched cyclomaltooligosaccharides (cG_ns, cyclodextrins) having sugars or sugar chains that were expected to display physiological activities was attempted. As cG_ns can form inclusion complexes, branched cG_ns may be useful as drug carriers in targeting drug delivery systems. In particular, dibranched cG_ns have been considered to be promising in view of the cluster effect. A dibranched

cG_8 has four positional isomers and the distances between the two sugar side chains on the cG_8 ring differ among the four isomers. Therefore, the positional isomers should help elucidate the distance between the two receptors on cells participating in molecular recognition.

In earlier studies, the structures of the positional isomers of $6^1,6^n$-di-*O*-(α-D-glucopyranosyl)-cG_8[1] and $6^1,6^n$-di-*O*-(α-D-mannopyranosyl)-cG_8[2] were regiochemically determined.

We now describe the chemical syntheses of $6^1,6^n$-di-*O*-(D-galactopyranosyl)-cG_8s (**9-12**) using $6^1,6^n$-bis-*O*-(dimethoxytrityl)-cG_8s[3] (**1-4**) as the key glycosyl intermediates, isolation of the desired $6^1,6^n$-di-*O*-(α-D-galactopyranosyl)-cG_8s (**13-16**), and their characterization by ^{13}C NMR spectroscopy and FAB-HRMS.

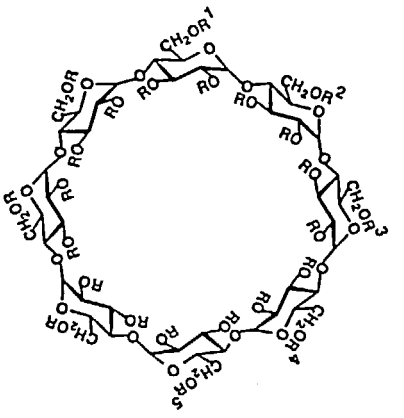
RESULTS AND DISCUSSION

Preparation Acetylation of **1**, **2**, **3**, or **4** with acetic anhydride-pyridine for 5 h at 100 °C, followed by *O*-dedimethoxytritylation[3,4] with boron trifluoride etherate in

Table 1. Structures of compounds **1-16**

	R^1	R^2	R^3	R^4	R^5	R
1	DMTr	DMTr	H	H	H	H
2	DMTr	H	DMTr	H	H	H
3	DMTr	H	H	DMTr	H	H
4	DMTr	H	H	H	DMTr	H
5	H	H	Ac	Ac	Ac	Ac
6	H	Ac	H	Ac	Ac	Ac
7	H	Ac	Ac	H	Ac	Ac
8	H	Ac	Ac	Ac	H	Ac
9	X	X	H	H	H	H
10	X	H	X	H	H	H
11	X	H	H	X	H	H
12	X	H	H	H	X	H
13	Y	Y	H	H	H	H
14	Y	H	Y	H	H	H
15	Y	H	H	Y	H	H
16	Y	H	H	H	Y	H

DMTr, dimethoxytrityl; X, D-galactopyranosyl;
Y, α-D-galactopyranosyl.



dichloromethane afforded the glycosyl acceptor, bis(2,3-di-*O*-acetyl)hexakis(2,3,6-tri-*O*-acetyl)-cG$_8$s (**5**, **6**, **7**, and **8**), after centrifugal chromatography. These compounds were obtained from glycosyl intermediates (**1**-**4**) in 47.2 – 65.5% yield.

Galactosylation of **5**, **6**, **7**, or **8** with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl trichloroacetimidate[5-7] in dichloromethane in the presence of trimethylsilyl trifluoromethanesulfonate[8] and molecular sieves for 1 h at −20 °C gave $6^1,6^n$-di-*O*-(2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl)-cG$_8$ peracetates (n = 2–5, **17**-**20**) in yields of 66.8 – 75.0%. Compounds **17**, **18**, **19**, and **20** were individually hydrogenated with Pd-C / H_2 in methanol for 1 – 1.5 h at room temperature, and the products were *O*-deacetylated with methanolic sodium methoxide to give **9**, **10**, **11**, and **12**, 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 **13**, **14**, **15**, and **16**, were isolated from **9**, **10**, **11**, and **12**, respectively, by HPLC.

Separation and characterization of $6^1,6^n$-di-*O*-(α-D-galactopyranosyl)-cG$_8$s (13, 14, 15, and 16). Fig. 1 shows the elution profile of the configurational isomers of $6^1,6^4$-di-*O*-(D-galactopyranosyl)-cG$_8$s (**11**). In order to confirm the structures, each component was isolated by HPLC on a Daisopak SP-120-5 ODS column, with 3.5 : 96.5 methanol-water. FABMS showed that all four components had the same molecular weight, 1620, expected for digalactosyl-cG$_8$s. In the ^{13}C NMR spectra of II, III, and IV in D_2O, signals due to the α-D-galactosylated C-6 (G'-6, δ 68.0) and β-D-galactosylated C-6 (G'-6, δ 69.2) shifted downfield by 6.7 – 6.9 ppm[9] and 7.9 – 8.1 ppm, respectively, compared with the signals of the C-6s of the six unsubstituted cG$_8$ ring glucoses (G-6, δ 61.1 – 61.3). C-6 signals of two galactosyl side chains (Ga-6) were observed at δ 61.8 – 62.0. The assignments of the C-6 signals were confirmed by distortionless enhancement using the polarization transfer (DEPT) method.[10] The signal for C-1 of the α-(1→6)-linkage (Ga-1) appeared at δ 100.0, while that of the β-(1→6)-linkage (Ga-1) appeared at δ 104.2. The relative intensities of the signals for G-6, Ga-6, and G'-6 were 6 : 2 : 2 and those for the C-1 signals of cG$_8$ ring glucoses (G-1, δ 102.2 – 102.5) and Ga-1 were 8 : 2. These results indicated that II, III, and IV were indeed digalactosyl-cG$_8$s, that IV was $6^1,6^4$-di-*O*-(α-D-galactopyranosyl)-cG$_8$ (**15**), and that II and III were configurational isomers having one α-(1→6)-linkage and one β-(1→6)-

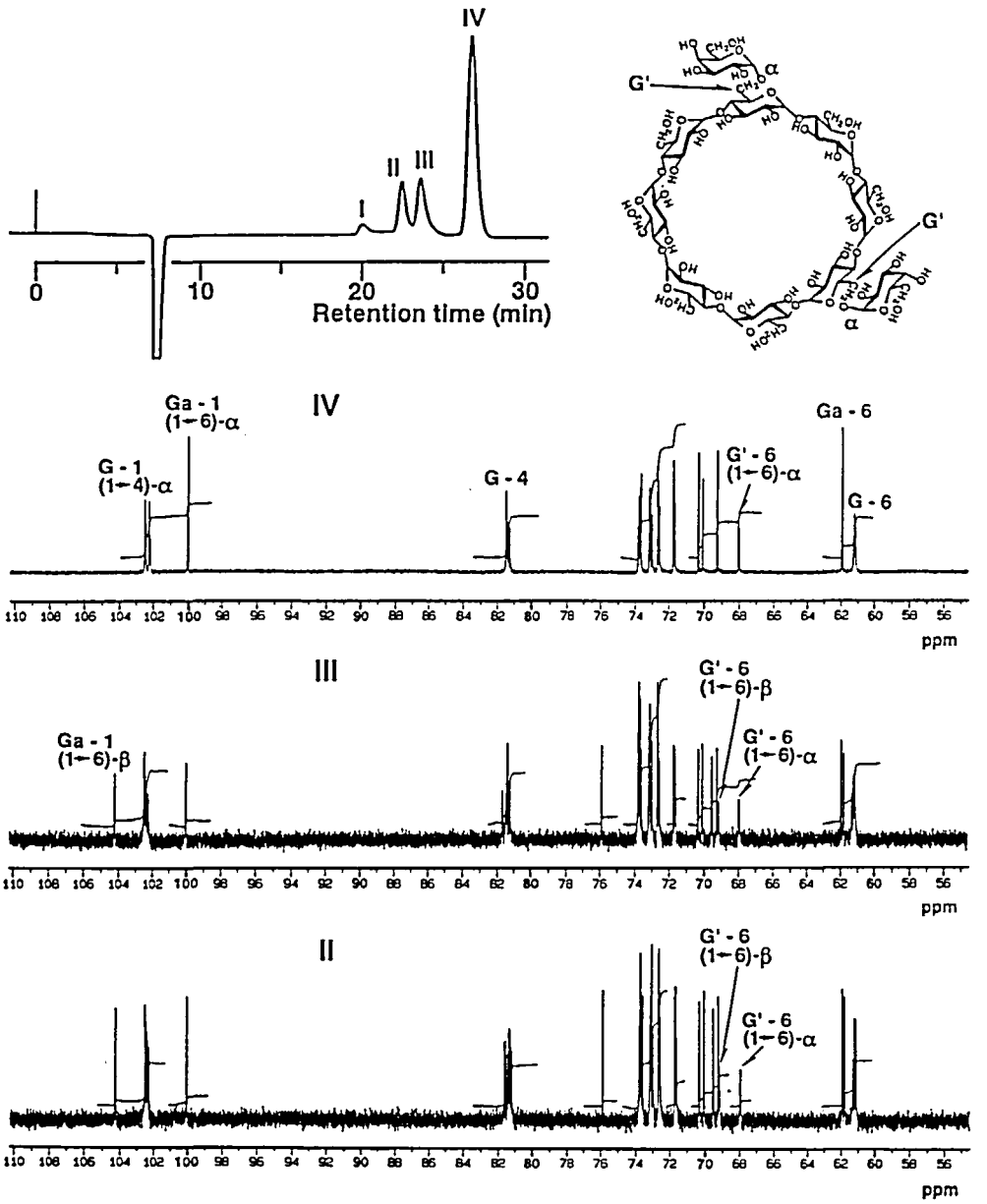


Fig. 1 Chromatogram of a mixture of $6^1,6^4$-di-*O*-(D-galactopyranosyl)-cG$_8$s (**11**) and ^{13}C NMR spectra of components II, III, and IV in D_2O. IV = α,α-disubstituted product **15**, II and III = α,β- or β,α-disubstituted product, I = β,β-disubstituted product. Chromatographic conditions: column, YMC-Pack A-312-3 ODS (150 x 6 mm i.d.); eluent, 3:97 methanol-water; flow rate, 0.5 mL/min; detector, Shodex RI-71; temperature, 30 °C.

linkage in the molecule. Therefore, the remaining compound, I, was considered to be $6^1,6^4$-di-*O*-(β-D-galactopyranosyl)-cG_8.

The elution order of the four configurational isomers of $6^1,6^4$-di-*O*-(D-galactopyranosyl)-cG_8s on an ODS column was ββ-, αβ- or βα-, βα- or αβ-, and αα-isomers (Fig. 1), that is, the retention time of $6^1,6^4$-di-*O*-(D-galactopyranosyl)-cG_8 having two α-(1→6)-linkages was longer than that of digalactosylated cG_8s having a β-(1→6)-linkage. This elution order was the opposite of that of $6^1,6^4$-di-*O*-(D-glucopyranosyl)-cG_8s[1] in which retention of the dibranched cG_8 having two β-(1→6)-linkages was stronger than dibranched cG_8s having an α-(1→6)-linkage.

Fig. 2 shows the elution profiles of the configurational isomers of $6^1,6^2$-, $6^1,6^3$-, and $6^1,6^5$-di-*O*-(D-galactopyranosyl)-cG_8s (**9**, **10**, and **12**). Each peak IV, which was the main product, was isolated by HPLC on ODS column. The structures were determined by ^{13}C NMR spectroscopy (Fig. 2). They were found to be the desired substances, $6^1,6^n$-di-*O*-(α-D-galactopyranosyl)-cG_8s (n = 2, 3, and 5 for **13**, **14**, and **16**, respectively). We inferred by analogy with $6^1,6^4$-digalactosylated cG_8s and $6^1,6^n$-diglucosylated cG_8s[1] that II and III were configurational isomers having one α-(1→6)-linkage and one β-(1→6)-linkage in the molecule, and I was $6^1,6^n$-di-*O*-(β-D-galactopyranosyl)-cG_8.

The molar ratios of the configurational isomers in the galactosylation products are summarized in Table 2. The ratios of configurational isomers of $6^1,6^n$-di-*O*-(D-galactopyranosyl)-cG_8s were almost the same as those of $6^1,6^n$-di-*O*-(D-glucopyranosyl)-cG_8s[1]. As it was more difficult to prepare digalactosylated cG_8s than diglucosylated cG_8s, trimethylsilyl trifluoromethanesulfonate as a mild catalyst was used instead of trifluoromethanesulfonic acid.[1] However, the yields of galactosylation were 66.8 – 75.0% and they did not reach the yields of glucosylation.

The elemental compositions of the resulting compounds, **13**, **14**, **15**, and **16**, were confirmed by FAB-HRMS. It is generally accepted that accurate elemental analysis data can not be obtained for the cG_n derivatives, in particular, as those having many hydroxy groups can form hydrogen bonds and complexes with the solvents.

Fig. 3 shows chromatograms of the four positional isomers of $6^1,6^n$-di-*O*-(α-D-galactopyranosyl)-cG_8 (**13**, **14**, **15**, and **16**) on an ODS column with methanol-water.

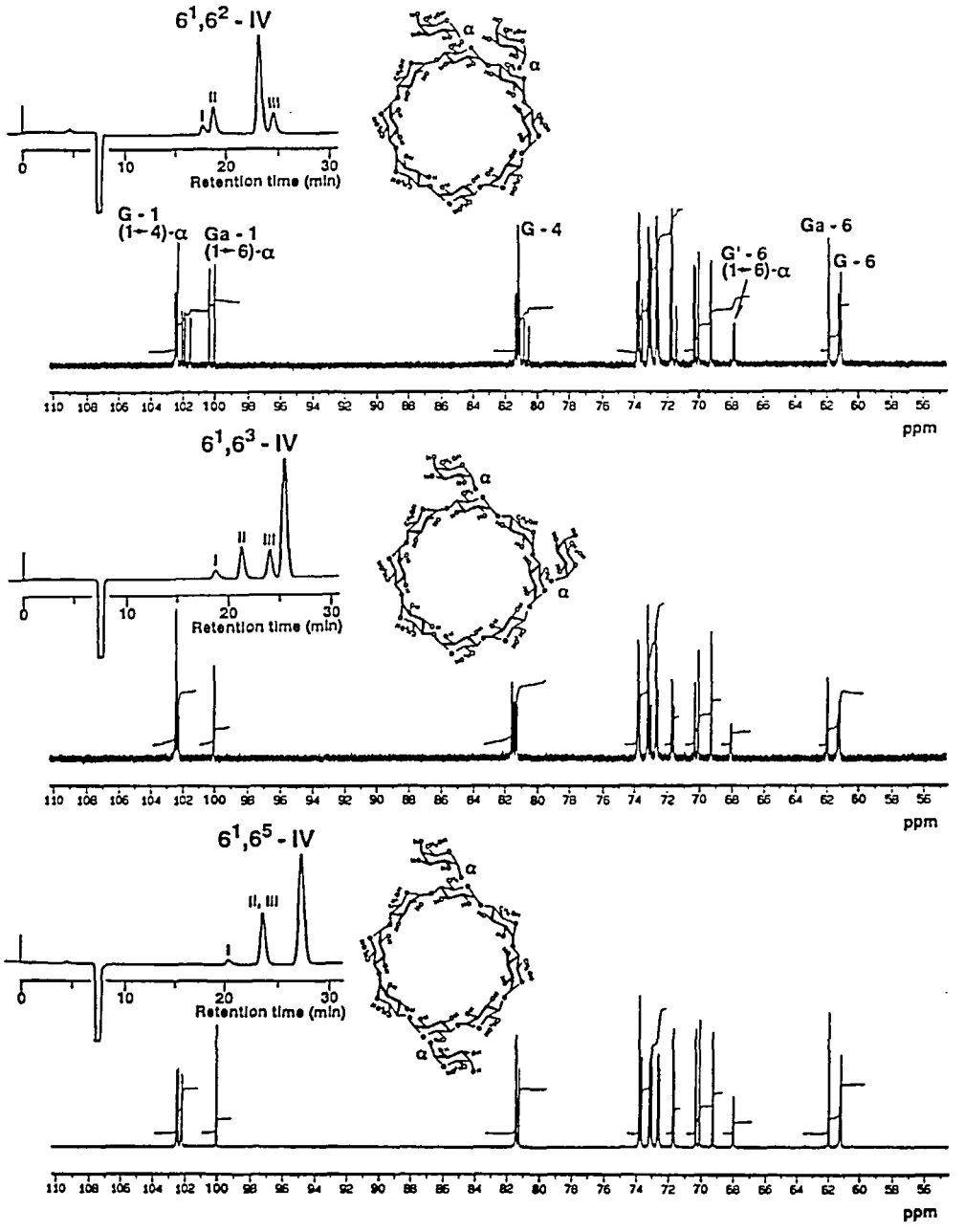


Fig. 2 Chromatograms of $6^1,6^2$-, $6^1,6^3$-, and $6^1,6^5$-di-*O*-(D-galactopyranosyl)-cG$_8$s (9, 10, and 12) and ^{13}C NMR spectra of each component IV in D_2O. IV = α,α-disubstituted product 13, 14 and 16. Chromatographic conditions as in Fig. 1.

Table 2. Ratios of configurational isomers in the galactosylation products

Products	α,α	α,β and β,α	β,β
6¹,6²-di-*O*-(D-galactopyranosyl)-cG$_8$ (9)	64	31	5
6¹,6³-di-*O*-(D-galactopyranosyl)-cG$_8$ (10)	56	39	5
6¹,6⁴-di-*O*-(D-galactopyranosyl)-cG$_8$ (11)	63	33	4
6¹,6⁵-di-*O*-(D-galactopyranosyl)-cG$_8$ (12)	69	28	3

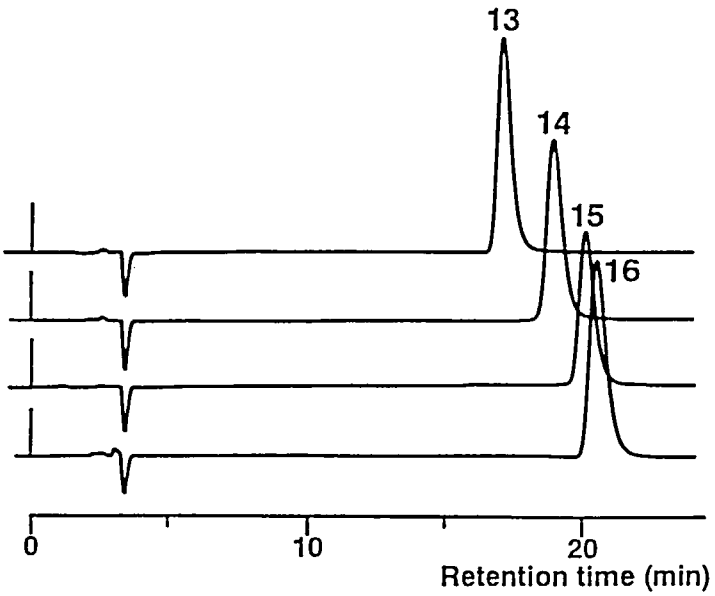


Fig. 3 Elution profiles of the four positional isomers of 6¹,6ⁿ-di-*O*-(α-D-galacto-pyranosyl)-cG$_8$ (n = 2–5, 13-16). Chromatographic conditions: column, Hikarisil C18-4D (150 x 4.6 mm i.d.); eluent, 2:98 methanol-water; flow rate, 0.7 mL/min; detector, Shodex RI-71; temperature, 30 °C.

The retention order was 6¹,6²-, 6¹,6³-, 6¹,6⁴-, and 6¹,6⁵-di-*O*-(α-D-galactopyranosyl)-cG$_8$s, and this order did not vary even if the mobile phase was changed to 0.5 : 99.5 acetonitrile-water and, moreover, a graphitized carbon column[11] with 14 : 86 acetonitrile-water was used.

The correlation between hydrophobic effects and structures of three and four positional isomers of 6¹,6ⁿ-di-*O*-triphenylmethyl- or 6¹,6ⁿ-di-*O*-*tert*-butyldimethylsilyl-

cyclomaltohexaoses (cG_6s, α-cyclodextrin) (n = 2–4), -cyclomaltoheptaoses (cG_7s, β-cyclodextrin) (n = 2–4), and cG_8s (n = 2–5) on an ODS column has been discussed in a recent paper.[12] Cyclodextrins with two hydrophobic-substituted groups bonded to hydroxyl groups tend to show low retention of positional isomers in which the binding positions of the two substituted groups on the cyclodextrin ring are far apart from each other. However, in the present case of two hydrophilic-substituted groups bonded to hydroxyl groups, the farther apart the binding positions of the two galactosyl groups were on the cG_8 ring, the greater the retention tended to be. It is thought that isomers with two galactosyl groups on the cG_8 ring in adjacent binding positions are less subject to a hydrophobic effect due to the intramolecular hydrogen bonding than isomers in which the binding positions of the two galactosyl groups on the cG_8 ring are far apart from each other.

The physiological activities of $6^1,6^n$-di-*O*-(α-D-galactopyranosyl)-cG_8 (**13**, **14**, **15**, and **16**) will be studied in the near future.

EXPERIMENTAL

General methods. Optical rotations were determined with a Jasco digital polarimeter, model P-1020. TLC was performed on Silica Gel 60 plates (E. Merck). Centrifugal chromatography was performed with a Harrison Centrifugal Thin-Layer Chromatotron, model 7924. HPLC was conducted with a Jasco TRI ROTAR SR-1, 880-PU, or 980-PU pump, a Waters U6K universal injector, a Shodex RI SE-61 or RI-71 refractive index monitor, and a Lab-Quatec CO-1093 column oven. The columns used were YMC-Pack SH-343-7 ODS (250 x 20 mm i.d.), Hikarisil C18-4D (150 x 4.6 mm i.d.), Daisopak SP-120-5-ODS (250 x 20 mm i.d.), TSK gel Amide-80 (300 x 7.8 mm i.d.), Daisopak SP-120-5-ODS-BP (250 x 10 mm i.d.), YMC-Pack A-312-3 ODS (150 x 6 mm i.d.), and Hypercarb S (100 x 4.6 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. FABMS was performed with a Jeol JMS-DX 303 mass spectrometer and FAB-HRMS was measured in the

positive-ion mode using a Jeol MS 700 mass spectrometer with xenon atoms, and glycerol was used as the matrix.

Bis(2,3-di-*O*-acetyl)hexakis(2,3,6-tri-*O*-acetyl)-cG$_8$s (5, 6, 7, and 8) Compounds **1** (1029 mg), **2** (722 mg), **3** (932 mg), or **4** (1456 mg) were acetylated with acetic anhydride (16 – 32 mL) in dry pyridine (24 – 47 mL) for 5 h at 100 °C, and the mixtures were concentrated. The residues, dissolved in chloroform, were washed sequentially with water, aqueous sodium hydrogen carbonate and water, then dried, and concentrated. To a solution of each residue in dry dichloromethane (40 – 50 mL) in an ice-water bath was added boron trifluoride diethyl etherate (600 – 1000 μL) with stirring. The stirring was continued for 1 h at room temperature. A solution in chloroform was washed with water, aqueous sodium hydrogen carbonate, and water, then dried, and concentrated. Centrifugal chromatography (2 : 1 → 2 : 3 hexane-acetone) of the residue gave **5** (787 mg, 65.5%), **6** (463 mg, 54.9%), **7** (514 mg, 47.2%), or **8** (942 mg, 55.3%).

Galactosylation of 5, 6, 7, and 8 Mixtures of **5** (787 mg), **6** (463 mg), **7** (514 mg), or **8** (942 mg), 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl trichloroacetimidate (2.5 – 3.9 g), and dry powdered 4Å molecular sieves (2.0 g) in dry dichloromethane (20 – 30 mL) were stirred under nitrogen at −20 °C. A solution of trimethylsilyl trifluoromethanesulfonate (100 – 200 μL) in dichloromethane (1 – 2 mL) was added. After stirring for 1 h at −20 °C, triethylamine (0.3 – 1 mL) was added to the mixture, which was diluted with chloroform, filtered through Celite, washed sequentially with 1 M sulfuric acid, aqueous sodium hydrogen carbonate and water, then dried, and concentrated. Centrifugal chromatography with 4 : 1 → 1 : 1 hexane-acetone of the residue afforded chromatographically pure $6^1,6^2$-di-*O*-(2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl)-cG$_8$ peracetate (**17**, 773 mg, 66.8%), $6^1,6^3$-di-*O*-(2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl)-cG$_8$ peracetate (**18**, 504 mg, 74.0%), $6^1,6^4$-di-*O*-(2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl)-cG$_8$ peracetate (**19**, 567 mg, 75.0%), and $6^1,6^5$-di-*O*-(2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl)-cG$_8$ peracetate (**20**, 986 mg, 71.2%), respectively.

$6^1,6^n$-Di-*O*-(α-D-galactopyranosyl)-cG$_8$s (n = 2–5, 13, 14, 15, and 16) Solutions of **17** (773 mg), **18** (504 mg), **19** (567 mg), or **20** (986 mg) and 10%

Pd-C (0.9 – 2.2 g) in methanol (20 – 25 mL) were stirred under hydrogen for 1 – 1.5 h at room temperature, then filtered, and concentrated, to give **17'** (516 mg, 85.7%), **18'** (365 mg, 92.9%), **19'** (422 mg, 95.5%) or **20'** (704 mg, 91.6%), respectively. These residues were treated with methanolic 0.05 M sodium methoxide (5 – 10 mL) for 1 h at room temperature, neutralized with Amberlite IR-120B (H^+) resin, filtered, and concentrated, to give **9** (318 mg, 96.8%), **10** (230 mg, 98.9%), **11** (267 mg, 99.4%), or **12** (435 mg, 97.0%), respectively.

The desired compounds **13**, **14**, **15**, and **16** were isolated from **9**, **10**, **11**, and **12**, respectively, by HPLC on a column of Daisopak SP-120-5-ODS-BP (250 x 10 mm i.d.) with 3.5 : 96.5 – 5 : 95 methanol–water : **13**, $[\alpha]_D^{26}$ +166.5° (*c* 1.09, H_2O), FAB-HRMS Calcd for $C_{60}H_{101}O_{50}$ (M + H) : 1621.5361. Found : 1621.5331., **14**, $[\alpha]_D^{26}$ +164.1° (*c* 0.64, H_2O), FAB-HRMS Calcd for $C_{60}H_{101}O_{50}$ (M + H) : 1621.5361. Found : 1621.5446., **15**, $[\alpha]_D^{26}$ +179.7° (*c* 1.07, H_2O), FAB-HRMS Calcd for $C_{60}H_{101}O_{50}$ (M + H) : 1621.5361. Found : 1621.5316., and **16**, $[\alpha]_D^{26}$ +167.2° (*c* 1.01, H_2O), FAB-HRMS Calcd for $C_{60}H_{101}O_{50}$ (M + H) : 1621.5361. Found : 1621.5432.

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REFERENCES

1. T. Tanimoto, T. Sakaki, and K. Koizumi, *Carbohydr. Res.*, **267**, 27 (1995).
2. Y. Okada, K. Matsuda, K. Koizumi, K. Hamayasu, H. Hashimoto, and S. Kitahata, *Carbohydr. Res.*, **310**, 229 (1998).
3. T. Tanimoto, A. Ikuta, and K. Koizumi, *J. Carbohydr. Chem.*, **16**, 1445 (1997).
4. K. Takeo, K. Uemura, and H. Mitoh, *J. Carbohydr. Chem.*, **7**, 293 (1988).
5. S. Koto, N. Morishima, Y. Miyata, and S. Zen, *Bull. Chem. Soc. Jpn.*, **49**, 2639 (1976).

6. R. R. Schmidt, J. Michel, and M. Roos, *Liebigs Ann. Chem.*, 1343 (1984).
7. M. Numata, M. Sugimoto, K. Koike, and T. Ogawa, *Carbohydr. Res.*, **163**, 209 (1987).
8. L. Lay, L. Manzoni, and R. R. Schmidt, *Carbohydr. Res.*, **310**, 157 (1998).
9. K. Hara, K. Fujita, N. Kuwahara, T. Tanimoto, H. Hashimoto, K. Koizumi, and S. Kitahata, *Biosci., Biotechnol., Biochem.*, **58**, 652 (1994).
10. D. M. Doddrell and D. T. Pegg, *J. Am. Chem. Soc.*, **102**, 6388 (1980).
11. K. Koizumi, *J. Chromatogr. A*, **720**, 119 (1996).
12. T. Tanimoto, A. Ikuta, K. Koizumi, and K. Kimata, *J. Chromatogr. A*, **825**, 195 (1998).