Preparation of Di-*O*-triphenylmethyl-(trityl-)cyclomalto-octaoses, and Isolation and Characterization by "Hex-5-enose Degradation" of Four Positional Isomers

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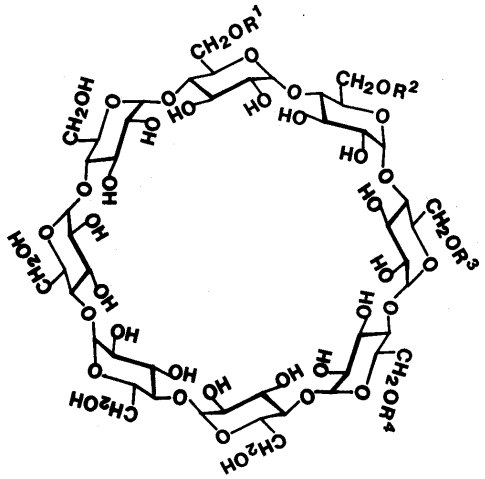
Four regioisomeric ditritylated derivatives of cyclomalto-octaose (1, cG_8), namely, $6^1,6^n$-di-*O*-trityl-cG_8s have been prepared by the reaction of 1 with chlorotriphenylmethane in pyridine and isolated by high-performance liquid chromatography. The regiochemical determination of the four dityrityl-substituted derivatives has been achieved by means of the "hex-5-enose degradation," followed by examination of the products by fast-atom bombardment-mass spectrometry.

Keywords $6^1,6^2$-di-*O*-trityl-cyclomalto-octaose; hex-5-enose degradation; ^{13}C-NMR; HPLC; $6^1,6^3$-di-*O*-trityl-cyclomalto-octaose; $6^1,6^4$-di-*O*-trityl-cyclomalto-octaose

Branched cyclomalto-oligosaccharides (cG_ns) having mono- or oligo-saccharides linked at hydroxyl groups at the 6-position of cG_ns have been the subject of increasing interest in recent years because of their many advantages over the parent cG_ns.[1-4] 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.

We describe herein the synthesis of $6^1,6^n$-di-*O*-trityl-cG_8 derivatives ($n=2,3,4$, and 5) and a method of isolating four positional isomers of dityrityl-cG_8s as intermediates for chemical syntheses of authentic positional isomers of dibranched cG_8s. In this paper we also report the application of the "hex-5-enose degradation"[5] to the regiochemical determination of the dityrityl-substituted cG_8s.



	R^1	R^2	R^3	R^4	R^5
1	H	H	H	H	H
2	Tr	H	H	H	H
3	Tr	Tr	H	H	H
4	Tr	H	Tr	H	H
5	Tr	H	H	Tr	H
6	Tr	H	H	H	Tr

Chart 1

Preparation and Isolation of $6^1,6^n$-Di-*O*-trityl-cG_8s (3—6) Tritylation of **1**, which had been dried by azeotropic distillation with pyridine, with 3 mol eq of chlorotriphenylmethane for 20 h at 45 °C gave, upon work-up, a powdery mixture containing disubstituted compounds as the major products. Dititylates were separated from monotritylated and over-tritylated compounds by semi-preparative high-performance liquid chromatography (HPLC) on an octadecyl silica (ODS) column (250 × 20 mm i.d., 10 μm) eluted with methanol–water (75 : 25), and a mixture of **3**—**6** (34%) was obtained. Figure 1 shows a chromatogram of the regioisomeric mixture of $6^1,6^n$-di-*O*-trityl-cG_8. The relative ratios of I, II, III, and IV as calculated from the chromatogram were approximately 2.0 : 2.5 : 2.3 : 1.0. Each dititylate was isolated by repeated rechromatography on another larger size ODS column packing with an average particle size of 5 μm, with methanol–water (75 : 25) for I and II and methanol–water (78 : 22) for III and IV.

Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR) Spectroscopy In Fig. 2, the ^{13}C-NMR spectra of 1—IV in pyridine-d_5 are compared. The relative intensities of signals due to C-1 at 102—104 ppm and the trityl-substituted C-6s at 64—65 ppm, which were shifted downfield by 2—3 ppm

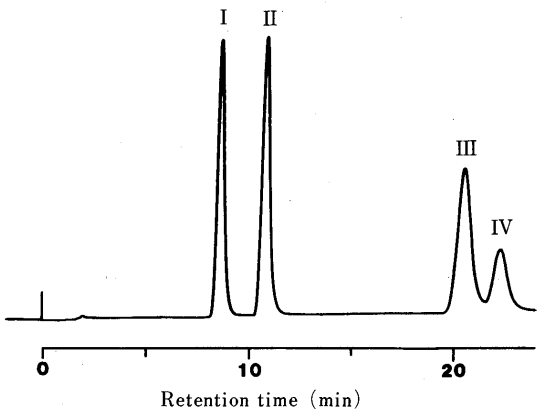


Fig. 1. Elution Profiles of Four Positional Isomers of Di-*O*-trityl-cyclomalto-octaose

Chromatographic conditions: column, Daisopak SP-120-5-ODS (150 × 6 mm i.d.); eluent, methanol–water (70 : 30); flow rate, 1.0 ml/min; detection wavelength, 240 nm.

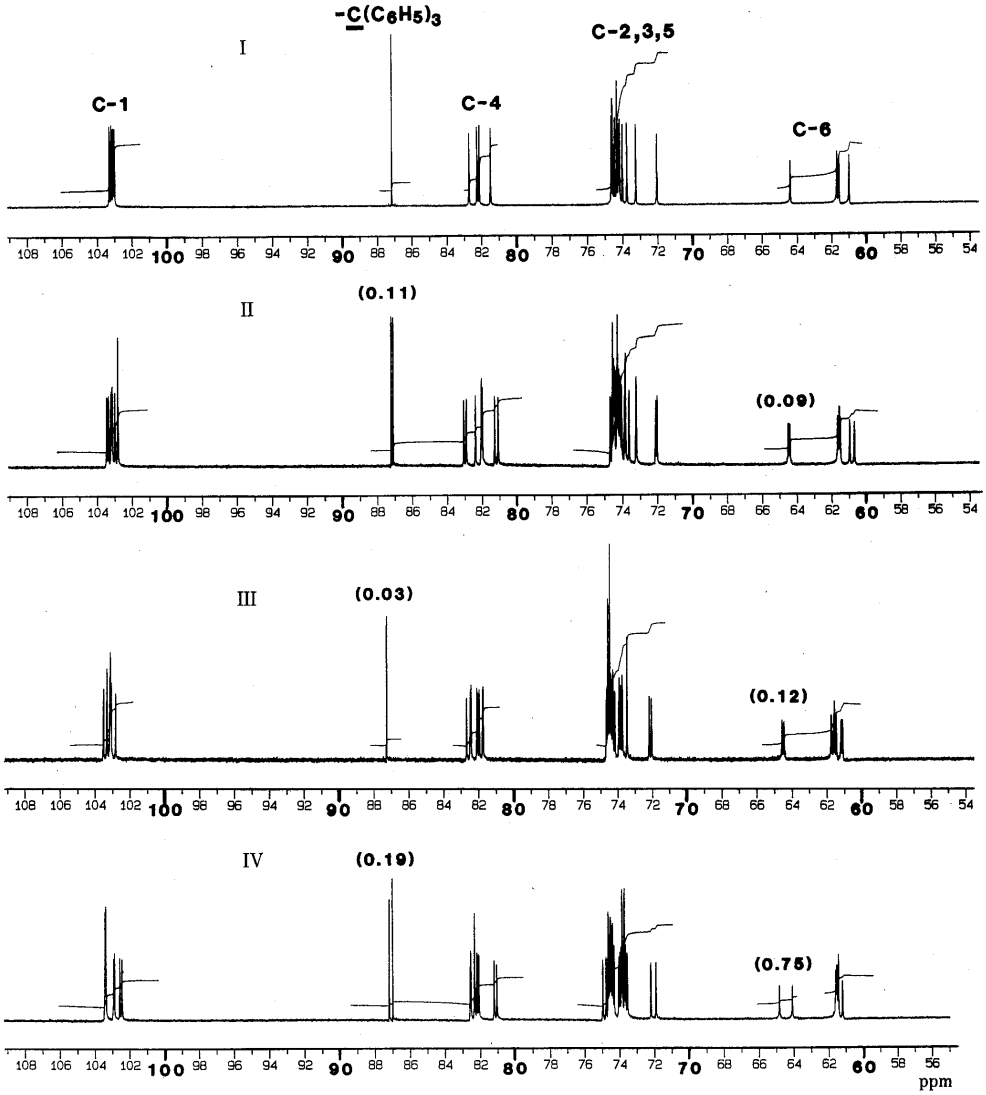
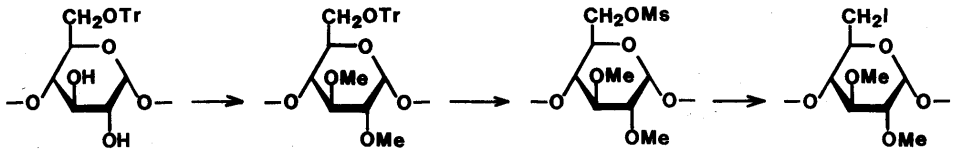


Fig. 2. ^{13}C-NMR Spectra of Isomeric Di-*O*-trityl-cyclomalto-octaoses (I, II, III, and IV) Measured in C_5D_5N at 125.65 MHz
The value in parentheses on the signals is the difference ($\Delta\delta$, ppm) of chemical shifts between the two signals.

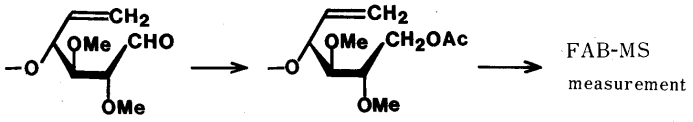
from the other C-6s (60—62 ppm), was 8 : 2, and hence it was clarified that all four compounds were ditrityl-substituted derivatives. The assignments of two kinds of C-6 were confirmed by the distortionless enhancement by polarization transfer (DEPT) method.[6] Each signal for C-1, -4, and -6 in the spectrum of I, in contrast to those in the spectra of other isomers, was split into only four lines. In addition, both the signal of the quarternary carbon of the trityl group and that of the trityl-substituted C-6 were not split. On the contrary, the signals in the spectrum of IV as a whole are complex, and resonances of the quarternary carbon of the trityl group and trityl-substituted C-6 appeared as two signals, with a difference of 0.19 and 0.75 ppm, respectively. A detailed comparison of the spectra of I, II, III, and IV suggests that I is the $6^1,6^5$-di-*O*-trityl-cG_8 **6** and IV, which seemed to have two adjacent bulky trityl groups in the molecule, is the $6^1,6^2$-disubstituted cG_8 **3**. It could not be determined whether II or III is the $6^1,6^3$- or $6^1,6^4$-di-*O*-trityl-cG_8.

Characterization of Four Positional Isomers by the "Hex-5-enose Degradation" The hex-5-enose degradation[5] is suitable for the regiochemical determination of ditrityl-substituted derivatives. We have already applied this procedure for ditrityl-cyclomaltohexaoses and ditrityl-cyclomaltoheptaoses, and were able to determine the structure of each of three regioisomers.[7]

First, methylation[8] of ditrityl derivatives (**3**—**6**→**3A**—**6A**) and detritylation[9] and methylsulfonylation[10] gave $6^1,6^n$-di-*O*-methylsulfonyl-cG_8 per-*O*-methylates (**3B**—**6B**). Nucleophilic displacement of **3B**—**6B** with sodium iodide[10] afforded $6^1,6^n$-dideoxy-$6^1,6^n$-diiodo per-*O*-methyl derivatives (**3C**—**6C**) as the key intermediates. Next, the "hex-5-enose degradation" was applied to the dideoxydiiodo derivatives. Compounds **3C**—**6C** were each treated with freshly prepared activated zinc dust and gave the corresponding two types of 5,6-dideoxy-hex-5-enose-terminated derivatives (D). These two derivatives were conveniently characterized by reduction, followed by acetylation to give two types of partially methylated 1,2-dideoxy-hex-1-enitol acetates (E) in each case. The molecular weight of compound E was measured by FAB-MS. The predictable molecular ions of the degradation products from **3C**—**6C** are summarized in Table I, and Fig. 3 shows the actual spectra of E from I, II, III and IV.



A B C



D E

Chart 2

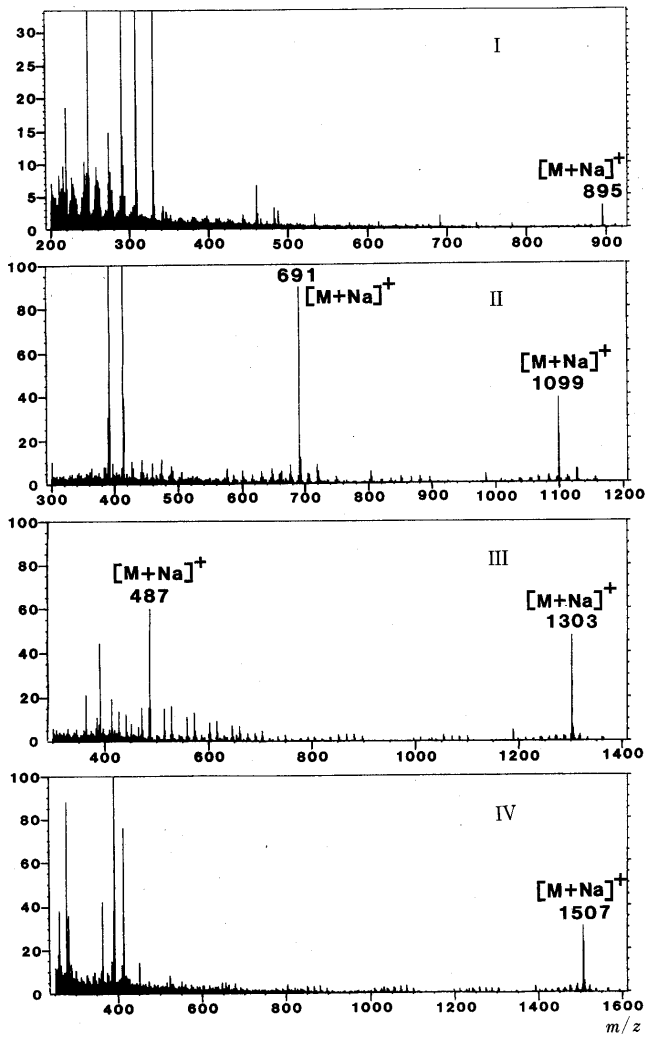
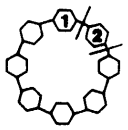
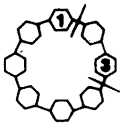
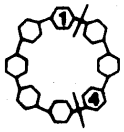
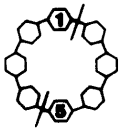


Fig. 3. FAB-MS of Products of the "Hex-5-enose Degradation" of 6^1,6″-Dideoxy-6^1,6″-diiodo-cyclomalto-octaose Permethylates Derived from I, II, III, and IV

From a comparison of the molecular ions shown in Fig. 3 with the predictable molecular ions in Table I, it is apparent that I, II, III, and IV are compounds **6**, **5**, **4**, and **3**, respectively. Thus, the structures of four dityril-substituted

TABLE I. The Molecular Ions of Predictable Products Formed from 6^1,6″-Dideoxy-6^1,6″-diiodo-cG_8 Permethylates (3C—6C) by "Hex-5-enose Degradation"

	$[M+Na]^+$ *m/z*
	283 1507
	487 1303
	691 1099
	895

cG_8 have been unequivocally determined by the "hex-5-enose degradation," followed by FAB-MS measurement.

Experimental

General Methods Melting points were determined 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 (5721, Merck) with detection by charring with sulfuric acid. HPLC was conducted with a TRI ROTAR SR-1 pump (JASCO), a U6K universal injector (Waters), an SE-61 refractive index monitor (Showa Denko), or a Uvidec-100V variable-wavelength detector (JASCO). The columns used were (A) YMC-Pack SH-343-10 ODS (250 × 20 mm i.d.), (B) SH-343-5 AQ ODS (250 × 10 mm i.d.), and (C) Daisopak SP-120-5-ODS (150 × 6 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 JEOL GSX-500 (125.65 MHz) and JEOL JNM-FX 200 (50.10 MHz) spectrometers for solutions in C_5D_5N and $CDCl_3$ (internal Me_4Si). FAB-MS was performed with a JEOL JMS-DX 303 mass

TABLE II. Physico-Chemical and Analytical Data for Mono-*O*-trityl- and Di-*O*-trityl-substituted cG_8s

Compound	mp (°C)[a]	$[\alpha]_D^{26}$ (in CH_3OH)		Elemental analysis Found		^{13}C-NMR δ (C_5D_5N)	
		(°)	*c*	C	H	CPh_3	C-6[b]
2	291—292	+150.6	2.0	49.89	6.33[c]	87.11	64.48
3	293—294	+119.1	1.0	55.88	6.26[d]	87.20, 87.01	64.83, 64.09
4	Syrup	+142.0	1.1			87.31, 87.29	64.60, 64.48
5	291—292	+129.0	1.1	56.74	6.34[e]	87.24, 87.14	64.51, 64.42
6	291—292	+118.2	0.8	56.06	6.11[d]	87.19	64.40

a) Decomposition. *b*) CPh_3 substituted carbon. *c*) *Anal.* Calcd for $C_{67}H_{94}O_{40}\cdot 4H_2O$: C, 49.94; H, 6.38. *d*) *Anal.* Calcd for $C_{86}H_{102}O_{40}\cdot 4H_2O$: C, 55.90; H, 6.00. *e*) *Anal.* Calcd for $C_{86}H_{102}O_{40}\cdot 3H_2O$: C, 56.45; H, 5.95.

spectrometer using xenon atoms having a kinetic energy equivalent to 6 kV at an accelerating voltage of 3 kV. Methanol, *m*-nitrobenzyl alcohol, and sodium chloride were used as the solvent, matrix, and additive, respectively.

6-*O*-Trityl-cyclomalto-octaose (2) and $6^1,6^2$-, $6^1,6^3$-, $6^1,6^4$-, and $6^1,6^5$-Di-*O*-trityl-cyclomalto-octaoses (3, 4, 5, and 6) Compound **1** (3.0 g, dried over phosphorus pentaoxide under reduced pressure for 2 d at 90 °C) was dissolved in dry pyridine (100 ml) and the solvent was distilled at atmospheric pressure until the boiling point of the distillate reached 115 °C. The solution was brought to 80 ml with dry pyridine, and then 1.93 g of chlorotriphenylmethane (3 mol eq) was added and the mixture was stirred for 20 h at 45 °C. The solvent was evaporated, and the residue was poured into a mixture of ice-water (100 ml) and chloroform (100 ml). The precipitate that was deposited between the two phases was collected by filtration and washed successively with water and chloroform. The yield of tritylated cG_8s was 3.5 g. The ditritylated mixture was separated from monotritylated (**2**) and over-tritylated compounds by semi-preparative HPLC on column A eluted with 75 : 25 methanol–water to give a mixture of **3**—**6** (34%). Further, each regioisomer was repeatedly separated on column B with 78 : 22 methanol–water for **3** and **4**, and 75 : 25 methanol–water for **5** and **6**. Compounds **3**, **5**, and **6** were crystallized from methanol–water, 1-propanol–methanol, and methanol, respectively. The physico-chemical and analytical data of these compounds are listed in Table II.

Characterization of Four Positional Isomers Each of the four positional isomers was characterized *via* their permethylates A, methylated dimethylsulfonyl derivatives B, and dideoxydiiodo derivatives C by means of the hex-5-enose degradation.

$6^1,6^n$-Di-*O*-trityl-cG_8 Permethylates (3A—6A) Solutions of **3** (288 mg), **4** (243 mg), **5** (231 mg), or **6** (767 mg) in freshly distilled *N*,*N*-dimethylformamide (30—40 ml) were stirred with sodium hydride (dry, 97%, 250—750 mg). Freshly distilled iodomethane (2.5—8.0 ml) was added dropwise and the mixture was placed under nitrogen, with protection from light, for 4 h at room temperature. The suspension was filtered through a pad of Celite, and the filtrate was concentrated. The residue was dissolved in chloroform, and the solution was successively washed with water, dried, and concentrated. Centrifugal chromatography (3 : 1, hexane–acetone) of the residue gave **3A** (110 mg, 32.5%), **4A** (165 mg, 58.8%), **5A** (212 mg, 79.5%), and **6A** (706 mg, 79.7%). **3A**: $[\alpha]_D^{26}$ +144.5° (*c* = 1.1, $CHCl_3$), ^{13}C-NMR ($CDCl_3$): δ 86.77, 86.68 (CPh_3), 63.30, 62.66 (C-6, CPh_3-substituted). **4A**: $[\alpha]_D^{26}$ +139.1° (*c* = 1.1, $CHCl_3$), ^{13}C-NMR ($CDCl_3$) δ: 86.98, 86.75 (CPh_3), 64.03, 63.71 (C-6, CPh_3-substituted). **5A**: $[\alpha]_D^{26}$ +141.8° (*c* = 1.8, $CHCl_3$), ^{13}C-NMR ($CDCl_3$) δ: 86.79, 86.75 (CPh_3), 63.80, 63.52 (C-6, CPh_3-substituted): **6A**: $[\alpha]_D^{26}$ +140.8° (*c* = 1.5, $CHCl_3$), ^{13}C-NMR ($CDCl_3$): δ 86.53 (2 CPh_3), 63.06 (2 C-6, CPh_3-substituted).

$6^1,6^n$-Di-*O*-methylsulfonyl-cG_8 Permethylates (3B—6B) Solutions of **3A** (110 mg), **4A** (150 mg), **5A** (150 mg), or **6A** (504 mg) in 70% acetic acid (20—30 ml) were each stirred for 1 h at 70—80 °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. The residue in dry pyridine (5—10 ml) was cooled to −10 °C, treated with methylsulfonyl chloride (0.6—1.0 ml), kept overnight at 0 °C, and then concentrated. The residue was dissolved in chloroform, and the solution was washed with water, aqueous sodium hydrogencarbonate, and water, then dried, and concentrated. Centrifugal chromatograpy (3 : 2 hexane–acetone) of the residue afforded **3B** (46 mg, 49.8%), **4B** (77 mg, 55.9%), **5B** (72 mg, 46.8%), and **6B** (250 mg, 59.0%). **3B**: $[\alpha]_D^{29}$ +145.6° (*c* = 1.2, $CHCl_3$), ^{13}C-NMR ($CDCl_3$); δ 37.58, 37.31 (CH_3SO_2). **4B**: $[\alpha]_D^{26}$ +154.4° (*c* = 1.1, $CHCl_3$), ^{13}C-NMR ($CDCl_3$); δ 37.53, 37.50 (CH_3SO_2), **5B**: $[\alpha]_D^{24}$ +152.6° (*c* = 1.2, $CHCl_3$), ^{13}C-NMR ($CDCl_3$): δ 37.48, 37.37 (CH_3SO_2) **6B**: $[\alpha]_D^{26}$ +158.1° (*c* = 1.0, $CHCl_3$), ^{13}C-NMR ($CDCl_3$) δ: 37.49 ($2CH_3SO_2$).

$6^1,6^n$-Dideoxy-$6^1,6^n$-diiodo-cG_8 Permethylates (3C—6C) Sodium iodide (190—600 mg) was added to a solution of **3B** (46 mg), **4B** (77 mg), **5B** (72 mg), or **6B** (200 mg) in *N*,*N*-dimethylformamide (5—12 ml), and the mixture was stirred for 4 h at 100 °C and then concentrated. A solution of the residue in chloroform was washed with water, aqueous sodium thiosulfate, and water, then dried, and concentrated. Centrifugal chromatography (1 : 1, hexane–acetone) of the residue gave **3C** (47 mg, 96.8%), **4C** (64 mg, 78.7%), **5C** (56 mg, 73.7%), and **6C** (150 mg, 71.1%). **3C**: $[\alpha]_D^{26}$ +110.1° (*c* = 1.2, $CHCl_3$), ^{13}C-NMR ($CDCl_3$) δ: 9.77, 8.34 (CH_2I). **4C**: $[\alpha]_D^{28}$ +131.3° (*c* = 1.3, $CHCl_3$), ^{13}C-NMR ($CDCl_3$): 9.18, 8.63 (CH_2I). **5C**: $[\alpha]_D^{28}$ +126.6° (*c* = 1.1, $CHCl_3$), ^{13}C-NMR ($CDCl_3$) δ: 9.14, 8.78 (CH_2I). **6C**: $[\alpha]_D^{28}$ +147.4° (*c* = 2.3, $CHCl_3$), ^{13}C-NMR ($CDCl_3$) δ: 8.53 ($2CH_2I$).

The Hex-5-enose Degradation A solution of **3C**, **4C**, **5C**, or **6C** (30 mg) in 14 : 1, 1-propanol–water (6 ml) was boiled with freshly activated zinc dust (600 mg) under reflux for 1 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated. The residue in 1 : 1, methanol–water (5 ml) was treated with sodium borohydride (300 mg) at room temperature. The reaction mixture was worked up in the usual manner, and the product was acetylated with acetic anhydride in pyridine. The residue was directly analyzed by FAB-MS.

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