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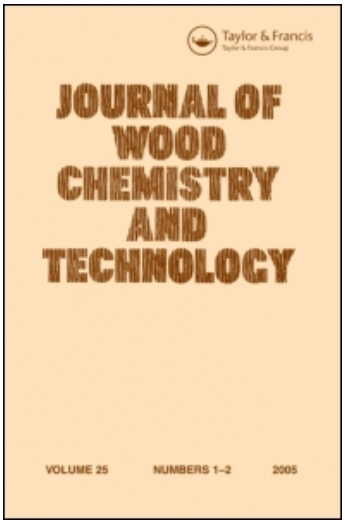

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Non-Chlorine Bleaching of Kraft Pulp II. Ozonation of Methyl 4-*O*-Ethyl-β-D-glucopyranoside (1) Preparation of Authentic Carbonyl Sugars and Their Analysis by Gas Chromatography and Mass Spectrometry

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JOURNAL OF WOOD CHEMISTRY AND TECHNOLOGY, 15(4), 453–471 (1995)

NON-CHLORINE BLEACHING OF KRAFT PULP II. OZONATION OF METHYL 4-*O*-ETHYL-β-D-GLUCOPYRANOSIDE (1) PREPARATION OF AUTHENTIC CARBONYL SUGARS AND THEIR ANALYSIS BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

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ABSTRACT

Methyl 4-*O*-ethyl-β-D-glucopyranoside (**1**) was prepared as a model compound for cellulose to investigate the reactions of ozone with polysaccharides during ozone bleaching of kraft pulp. The model compound was converted into authentic carbonyl sugars, methyl 3,6-di-*O*-acetyl-4-*O*-ethyl-β-D-*arabino*-hexopyranosidulose (**2**), methyl 2,6-di-*O*-acetyl-4-*O*-ethyl-β-D-*ribo*-hexopyranoside-3-ulose (**3**), methyl 2,3-di-*O*-acetyl-4-*O*-ethyl-β-D-*gluco*-hexodialdo-1,5-pyranoside (**4**). These carbonyl sugars were converted into *O*-methyloximes and analyzed by gas chromatography and mass spectrometry.

INTRODUCTION

Ozone has attracted increasing attention as a non-chlorine bleaching agent in recent years. However, there remains a problem that

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ozone reacts not only with lignin, but also with polysaccharides, impairing pulp viscosity and fiber strength. To elucidate the degradation mechanism of cellulose during ozone bleaching, low molecular weight model compounds, such as glucose,[1] methyl α-D-glucopyranoside,[2] methyl β-D-glucopyranoside,[3] and cellobiose[1] have been used, because they are commercially available and easily analyzed by gas chromatography. A 1,4-substituted glucose derivative is more suitable for this purpose, because it has a protective group at 4- -position. The reactivity of the inner glucose unit of cellulose can be elucidated by use of this derivative.

Oxidation of polysaccharide is one of the predominant reactions which cause degradation of polysaccharide and viscosity drop during bleaching, because oxidizing reagents such as chlorine and oxygen are generally used in usual bleaching sequences. In most case alcohol groups are oxidized to produce carbonyl groups in the molecule. Oxidation of alcohol groups of cellulose during ozone bleaching have also been reported.[2] Acetylated carbonyl sugars derived from the 1,4-substituted model compound are suitable samples for the analysis of the ozonated model compound by gas chromatography.

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In this paper, we report a synthetic route for methyl 4-*O*-ethyl-β-D-glucopyranoside (**1**) as a model compound for cellulose to investigate the reaction of ozone with polysaccharides, and the preparation of the acetylated carbonyl sugars (**2-4**) from the model compound. The carbonyl sugars were converted into *O*-methyloximes and analyzed by gas chromatography and mass spectrometry.

RESULTS AND DISCUSSION

Synthesis of Methyl 4-*O*-Ethyl-β-D-Glucopyranoside (1)

A model compound, methyl 4-*O*-methyl-β-D-glucopyranoside has been synthesized by the methylation of the 2,3,4-tri-*O*-acetylated derivative *via* acyl migration[4] or the methylation of the 2,3-di-*O*-benzylated 6-*O*-tritylated derivative.[5] These synthetic routes give low

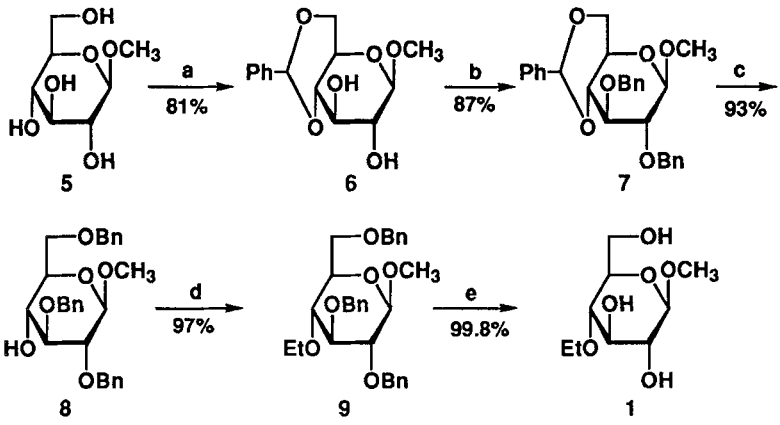

FIGURE 1. Synthetic route for methyl 4-*O*-ethyl β-D-glucoside.

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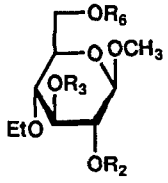
yields or are rather tedious, because the yield of the direct methylation of the acetylated derivative in alkaline conditions is 45 %, and many reaction steps are needed for the tritylated and benzylated derivative. We developed a synthetic route for the 1,4-substituted model compound, methyl 4-*O*-ethyl-β-D-glucopyranoside (**1**) as shown in **FIGURE 1**. This route is simple and gives high yields. Here, ethyl group was chosen for the substituent at 4-*O*-position, because the extents of elimination of protecting group at 1-*O*- or 4-*O*-position by ozone-oxidation can be elucidated separately by gas chromatography.

Commercially available methyl β-Dglucopyranoside (**5**) was converted into methyl 4,6-*O*-benzylidene-β-D-glucopyranoside (**6**) in a 81 % yield, and then benzylated to afford methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**7**) in a 87 % yield. Reductive cleavage of the 4,6-*O*-benzylidene acetal derivative (**7**) with sodium cyanoborohydride and trimethylsilyl chloride in acetonitrile afforded methyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**8**) in a 93 % yield.[6]



10: R_2= R_3=R_6=Ac
11: R_2=H, R_3=R_6=Ac
12: R_2=Ac, R_3=H, R_6=Ac
13: R_2=R_3=Ac, R_6=H
14: R_2=R_3=Ac, R_6=Tr

Tr=$C(C_6H_5)_3$

FIGURE 2. Regiospecifically acetylated model compounds.

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Ethylation of compound **8** with ethyl iodide and sodium hydride afforded methyl 2,3,6-tri-*O*-benzyl-4-*O*-ethyl-β-D-glucopyranoside (**9**) in a 97 % yield. The benzyl groups were removed with palladium carbon under H_2 to afford methyl 4-*O*-ethyl-β-D-glucopyranoside (**1**) in an almost quantitative yield. The overall yield of compound **1** from compound **5** was 63 %.

Synthesis of Carbonyl Sugars by Oxidation of Regiospecifically Acetylated Model Compounds

Regioselective acetylation of model compound 1

It is difficult to oxidize 1,2-diols such as compound **1** to α-hydroxy ketones, because both hydroxyl groups are oxidized to α-diketones, or carbon-carbon cleavages take place. In fact, methyl 4-*O*-methyl-β-D-glucopyranoside has been oxidized directly with chromic acid to afford 2-keto, 3-keto, and 6-aldehyde derivatives[7] only in low yields: 0.2, 2.6, and 3.1 %, respectively. Therefor, we tried the oxidation of partially acetylated model compounds as shown in **FIGURE 2.**

Regioselective acetylation of compound **1** was performed with acetyl chloride (8.5eq) and 2,6-lutidine (8.5eq) in ethyl acetate under reflux for 12h,[8] to afford methyl 2,3,6-tri-*O*-acetyl-4-*O*-ethyl-β-D-

glucopyranoside (**10**), methyl 3,6-di-*O*-acetyl-4-*O*-ethyl-β-D-glucopyranoside (**11**), and methyl 2,6-di-*O*-acetyl-4-*O*-ethyl-β-D-glucopyranoside (**12**) in 30, 20 and 50 % yields, respectively. The bulky salt of acetyl chloride and 2,6-lutidine is more accessible to 2-*O*-position than 3-*O*-position, because 4-*O*-position is substituted for more bulky ethyl group. Methyl 2,3-di-*O*-acetyl-4-*O*-ethyl-β-D-glucopyranoside (**13**) was prepared from methyl 2,3-di-*O*-acetyl-4-*O*-ethyl-6-*O*-trityl-β-D-glucopyranoside (**14**) by the detritylation with *p*-toluenesulfonic acid.

Preparation of carbonyl sugars

Methyl 3,6-di-*O*-acetyl-4-*O*-ethyl-β-D-*arabino*-hexopyranosidulose (**2**), methyl 2,6-di-*O*-acetyl-4-*O*-ethyl-β-D-*ribo*-hexopyranoside-3-ulose (**3**), and methyl 2,3-di-*O*-acetyl-4-*O*-ethyl-β-D-*gluco*-hexodialdo-1,5-pyranoside (**4**) were prepared from the corresponding partially acetylated derivatives (**11-13**) by oxidation with pyridinium chlorochromate (PCC). These carbonyl sugars are shown in **FIGURE 3**. The ^{1}H-and ^{13}C-NMR spectral data of acetylated carbonyl sugars are shown in **TABLE 1**.

The 2-hydroxyl derivative (**11**) was treated with PCC for 31 h. After removing the unreacted starting material **11** (Rf: 0.24) by preparative thin layer chromatography (PTLC) (1/1 ethyl acetate/*n*-hexane), the expected compound **2** was obtained as a syrup in a 64 % yield. The tailing of chromatographic spot of the product with Rf value (0.15) suggested that the product was a hydrate form as reported by Baker et al,[9] because carbonyl compound has usually larger Rf value than that of the corresponding alcohol on silica-gel TLC plate. The ^{13}C-NMR signal assigned to ketone carbon (C2) was observed at 192.2 ppm. The C1 signal was observed at 100.2 ppm. The ^{1}H-NMR signals for C1-, C3-, and C4-protons were observed at 4.82 ppm (singlet), 5.38 ppm (doublet, $J_{3,4}$=9.5), 3.74 ppm (triplet, $J_{4,5}$=9.5). The ^{1}H-NMR and ^{13}C-NMR spectra were consistent with the 2-keto derivative (**2**).

The 3-hydroxyl derivative (**12**) was treated with PCC for 35 h to afford 3-keto derivative (**3**) in 79 % yield. The ^{13}C-NMR signal attributed to ketone carbon (C3) was observed at 198.3 ppm, and the

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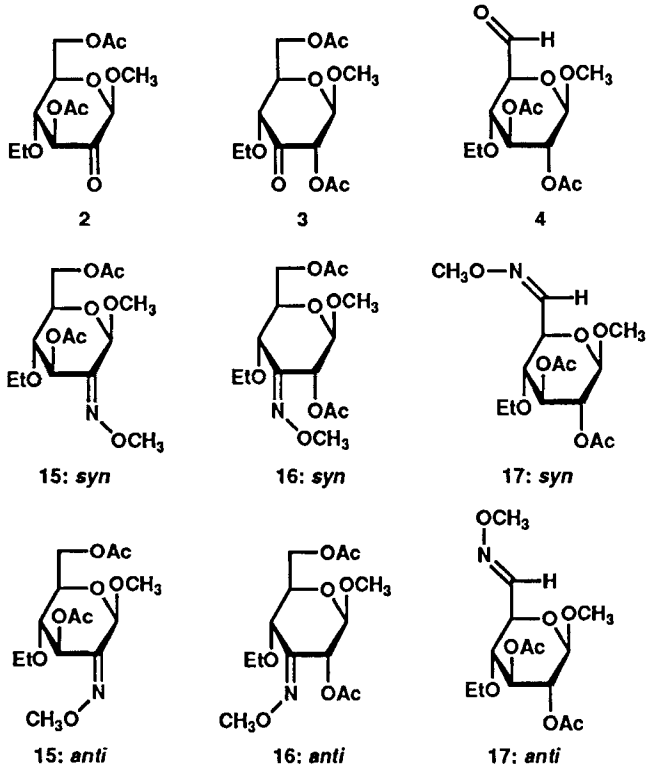

FIGURE 3. Synthesized acetylated carbonyl sugars and their *O*-methyloximes.

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C1 signal was observed at 102.4 ppm. The ^{1}H-NMR signals for C1-, C2- and C4-protons were observed at 4.52 ppm (doublet, $J_{1,2}$=8.0), 5.12 ppm (double doublet, $J_{2,4}$=1.5), and 4.00 ppm (double doublet, $J_{4,5}$=10), respectively. The ^{1}H-NMR and ^{13}C-NMR spectra were consistent with the 3-keto derivative (**3**).

The 6-hydroxyl derivative (**13**) was easily oxidized by PCC at room temperature within 2.5 h to afford 6-aldehyde derivative (**4**) in a 62 % yield under optimum reaction conditions. When the treatment was prolonged, side reaction occurred and the expected product was not obtained. The ^{13}C-NMR signal attributed to aldehyde carbon (C6) was

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TABLE 1

^{1}H- and ^{13}C-NMR Spectral Data of Acetylated Carbonyl Sugars.

Compound	2	3	4
		^{1}H-NMR	
		Chemical Shift (ppm)	
H-1	4.82s	4.52d	4.54d
H-2	-	5.12dd	4.90t
H-3	5.38d	-	5.22t
H-4	3.74t	4.00dd	
H-5	3.93m	3.63m	3.96d
H-6a	4.31dd	4.35dd	9.74s
H-6b	4.48dd	4.46dd	-
C-OCH_3	3.58s	3.61s	3.56s
-OCH_2CH_3	1.16t	1.25t	1.16t
-OCH_2CH_3		3.46m	
		3.89m	
-$OCOCH_3$	2.10s	2.16s	2.08s
	2.22s	2.24s	
		Coupling Constants (Hz)	
$J_{1,2}$		8.0	8.0
$J_{2,3}$	-	-	8.0
$J_{3,4}$	9.5	-	8.0
$J_{4,5}$	9.5	10.0	9.0
$J_{5,6a}$	5.0	4.5	-
$J_{5,6b}$	2.0	2.5	-
$J_{6a,6b}$	12.0	12.0	-
$J_{2,4}$	-	1.5	-
		^{13}C-NMR	
		Chemical Shift (ppm)	
C-1	100.2	102.4	101.6
C-2	192.2		
C-3		198.3	
C-6			196.3

observed at 196.3 ppm, and the C1 signal was observed at 101.6 ppm. The ^{1}H-NMR signal for aldehyde proton (C6-proton) was observed at 9.74 ppm, and the signal for C5-proton was observed at 3.96 ppm (doublet, $J_{4,5}$=9.0). The ^{1}H-NMR and ^{13}C-NMR spectra were consistent with the 6-aldehyde derivative (**4**).

Preparation of *O*-Methyloximes of Carbonyl Sugars (2-4) and Analysis by Gas Chromatography and Mass Spectrometry

It is difficult to analyze directly the mixtures of carbonyl sugars obtained by oxidation of glycoside, because they are extremely sensitive under alkaline conditions at room temperature or under neutral and slightly acidic conditions at elevated temperature.[10] Larm investigated the stabilization and quantitative analysis of the carbonyl sugars obtained by bromine oxidation of methyl β-D-glucoside as trimethylsilylated *O*-methyloxime derivatives.[11] The *O*-methyloxime derivatives of steroid ketones also have been used for analytical and structural studies by gas chromatographic and mass spectrometric techniques.[12] Oxime or *O*-methyloxime derivatives of aldoses[13] and ketoses[14] have been used for quantitative analysis by gas chromatography. The oxime or *O*-methyloxime derivatives of carbonyl sugars are stable to alkali and enolization does not occur in contrast to the behavior often observed for ketones.

The acetylated carbonyl sugars (**2-4**) were treated with methoxylamine hydrochloride and pyridine to afford *O*-methyloximes (**15-17**), respectively. The geometric isomers of the *syn* and *anti* types were formed during the reaction of methoxylamine with the carbonyl sugars (**2-4**), as shown in **FIGURE 3**. The ^{1}H-NMR spectral data of *O*-methyloximes **15-17** are shown in **TABLE 2**. The configurational assignment of *syn* and *anti* forms of oximes based on ^{1}H-NMR spectral data has been reported.[15] The deshielding of a vicinal hydrogen by the oxime hydroxyl allows an assignment of configuration. For example, the chemical shift of C1-proton of the *syn*-*O*-methyloxime **15** is 0.51 ppm lower than that of the *anti*-*O*-methyloxime **15**. On the other hand, the chemical shift of C3-proton of the *syn*-*O*-methyloxime **15** is 0.51 ppm

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TABLE 2

^{1}H-NMR Spectral Data of *O*-Methyloximes **15-17**

Compound	**15**		**16**		**17***	
	syn	*anti*	*syn*	*anti*	*syn*	*anti*
H-1	5.51s	5.00s	4.84d	4.84d		4.43d
H-2	-	-	5.87dd	5.24dd		4.88dd
H-3	5.49d	6.00d	-	-	5.17t	5.16t
H-4	3.67dd	3.98dd	3.94d	4.70d	3.38t	3.45t
H-5	3.74-3.84m	3.82m	4.22m	(4.11-4.24m)	4.72dd	3.96dd
H-6a	4.33dd	4.28dd	4.12dd		6.72d	7.35d
H-6b	4.38dd	4.40dd	4.27dd		-	-
C-OCH_3	3.53s	3.50s	3.47s	3.46s		3.49s
N-OCH_3	3.94s	3.91s	3.93s	3.94s	3.92s	3.90s
-OCH_2CH_3	1.19t	1.15t	1.22t	1.21t		1.10t
-OCH_2CH_3	3.57m-3.79m	3.52-3.69m	3.42m-3.62m	3.43-3.53m		3.51-3.66m
-$OCOCH_3$	2.10s	2.10s	2.08s	2.08s		2.04s
	2.12s	2.12s	2.09s	2.12s		2.06s
			Coupling Constants (Hz)			
$J_{1,2}$	-	-	4.4	2.2		7.8
$J_{2,3}$	-	-	-	-		9.7
$J_{3,4}$	4.5	7.0	-	-	9.1	9.4
$J_{4,5}$	6.5	8.4	2.3	1.6	9.4	9.5
$J_{5,6a}$	6.4	5.1	5.2		6.7	6.9
$J_{5,6b}$	4.9	3.6	4.7		-	-
$J_{6a,6b}$	11.6	11.8	10.0		-	-
$J_{2,4}$	-	-	0.6	0.9	-	-

*The chemicl shifts and the coupling constants were read from the *syn* and *anti* mixture.

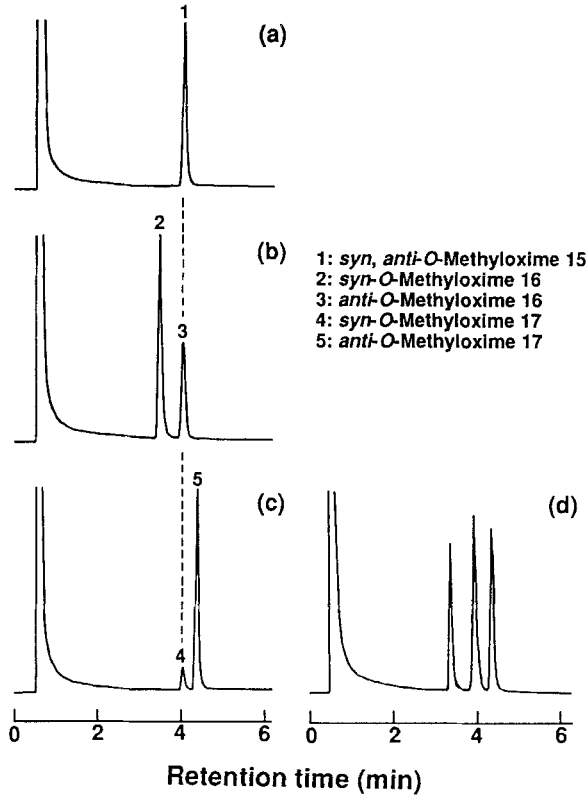

FIGURE 4. Gas chromatograms of *O*-methyloximes, (a): *O*-methyloxime **15**, (b): *O*-methyloxime **16**, (c): *O*-methyloxime **17**, and (d): mixture of *O*-methyloximes **15-17**.

higher than that of *anti-O*-methyloxime **15**. The configuration of *syn* and *anti* forms of *O*-methyloximes **16** and **17** also can be assigned by ^{1}H-NMR spectral data.

The approximate *syn/anti* ratios of *O*-methyloximes **15-17** also can be determined by ^{1}H-NMR analysis of mixtures of the isomers; by the ratios of the peak areas of $NOCH_3$ protons for *O*-methyloxime **15**, C2-protons for *O*-methyloxime **16**, and C6-protons for *O*-methyloxime **17**. The *syn/anti* ratios of the *O*-methyloximes **15**, **16** and **17** were 1.3, 2.0 and 0.1, respectively.

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The gas chromatograms of *O*-methyloximes **15-17** are shown in **FIGURE 4**. The *syn* and *anti* forms of *O*-methyloxime **15** were inseparable by gas chromatography. However, the *syn* and *anti* forms of *O*-methyloximes **16** and **17** were separable. The *syn/anti* ratios of *O*-methyloximes **16** and **17** measured by gas chromatography are in good agreement with the results by ^{1}H-NMR analysis. The peak of *O*-methyloxime **15** overlapped with *anti-O*-methyloxime **16** and *syn-O*-methyloxime **17** under our analytical condition. The *syn/anti* ratios were variable under slightly acidic conditions at room temperature.

Mass spectra (CI-MS) of the *O*-methyloxime **15-17** are shown in **FIGURES 5-7**, respectively. The mass spectra of *syn* and *anti* forms of *O*-methyloximes **15-17** were similar to each other. The mass spectra of *O*-methyloximes **15-17** did not show molecular ion peak at m/e 333, but showed distinct peak at m/e 302 attributed to fragment (M^+-OCH_3). The mass spectra of *O*-methyloxime **17** showed a characteristic ion peak at m/e 242 (M^+-OCH_3-HOAC).

Thus, it was found that the ulose derivatives can be analyzed by GC-MS after *O*-methyloximation and subsequent acetylation. We reported that the viscosity drop caused by GC- (glycosidic bond cleavage) reaction was greater than that by OX- (oxidation) reaction during ozone bleaching of kraft pulp.[16] These reactions of ozone with cellulose can be elucidated in more detail by analyzing the ulose derivatives from ozonation of methyl 4-*O*-ethyl-β-D-glucoside as a model. We will report the reactions of ozone with ring carbons of the model compound in the next paper. The present method may be also applied to the investigations of the oxidation mechanisms with various reagents such as chlorine, oxygen and Fenton's reagent.

EXPERIMENTAL

General. —— All melting points (m.p.) are uncorrected. ^{1}H-NMR spectra and ^{13}C-NMR spectra were recorded with a Varian XL-200 FT-NMR (200MHz) spectrometer and a JEOL FX-90 FT-NMR (22.5MHz), respectively, in chloroform-*d* with tetramethylsilane (TMS) as an internal standard. Chemical sifts (δ) and coupling constants (*J*) are given in δ-values (ppm) and Hz, respectively.

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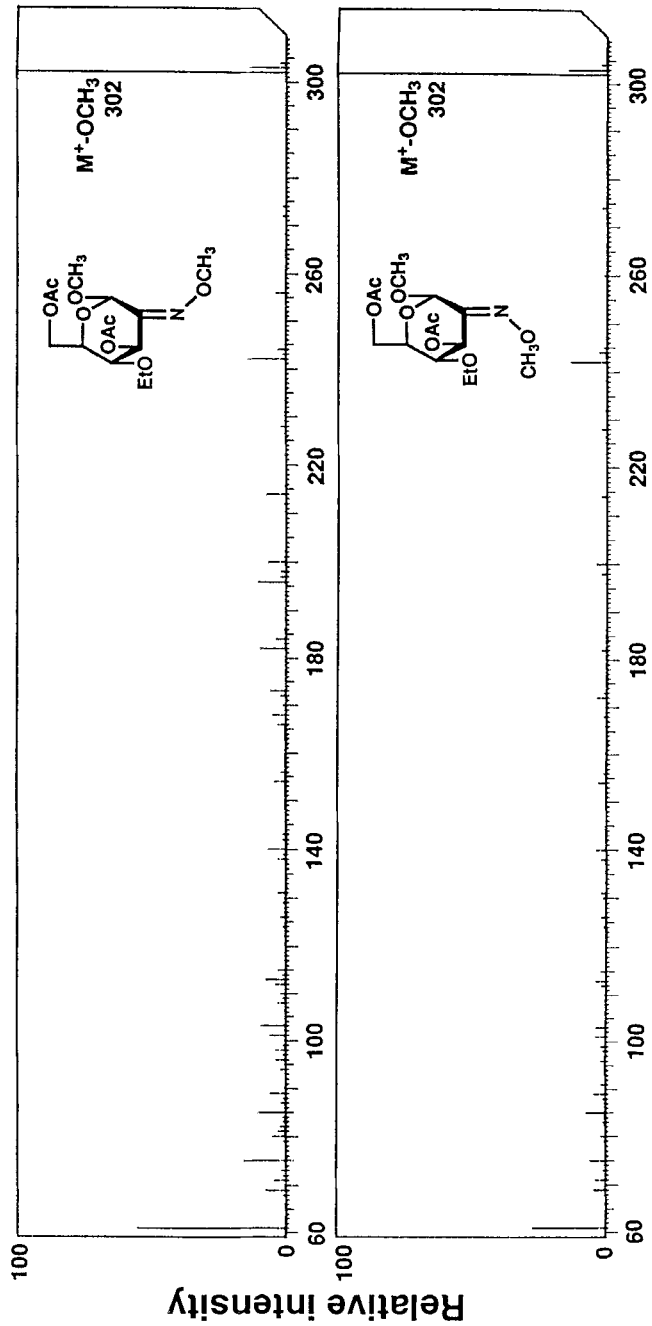

FIGURE 5. Mass spectra of *O*-methyloxime **15**.

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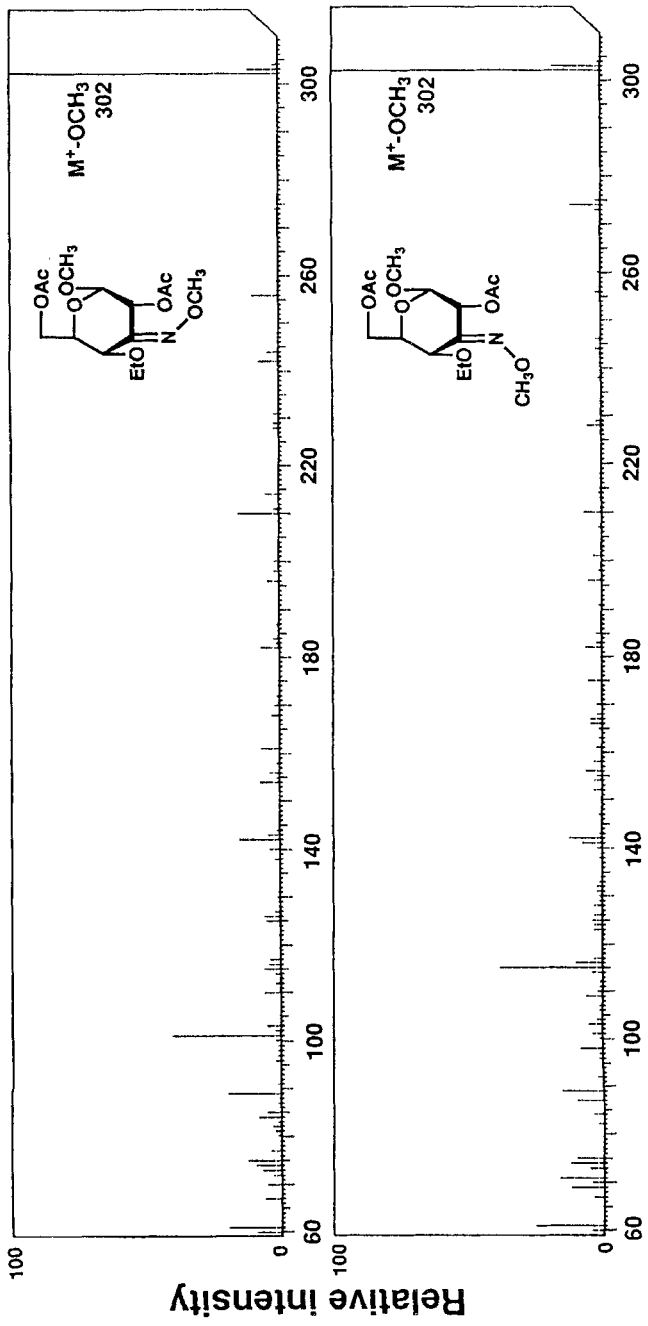

FIGURE 6. Mass spectra of *O*-methyloxime **16**.

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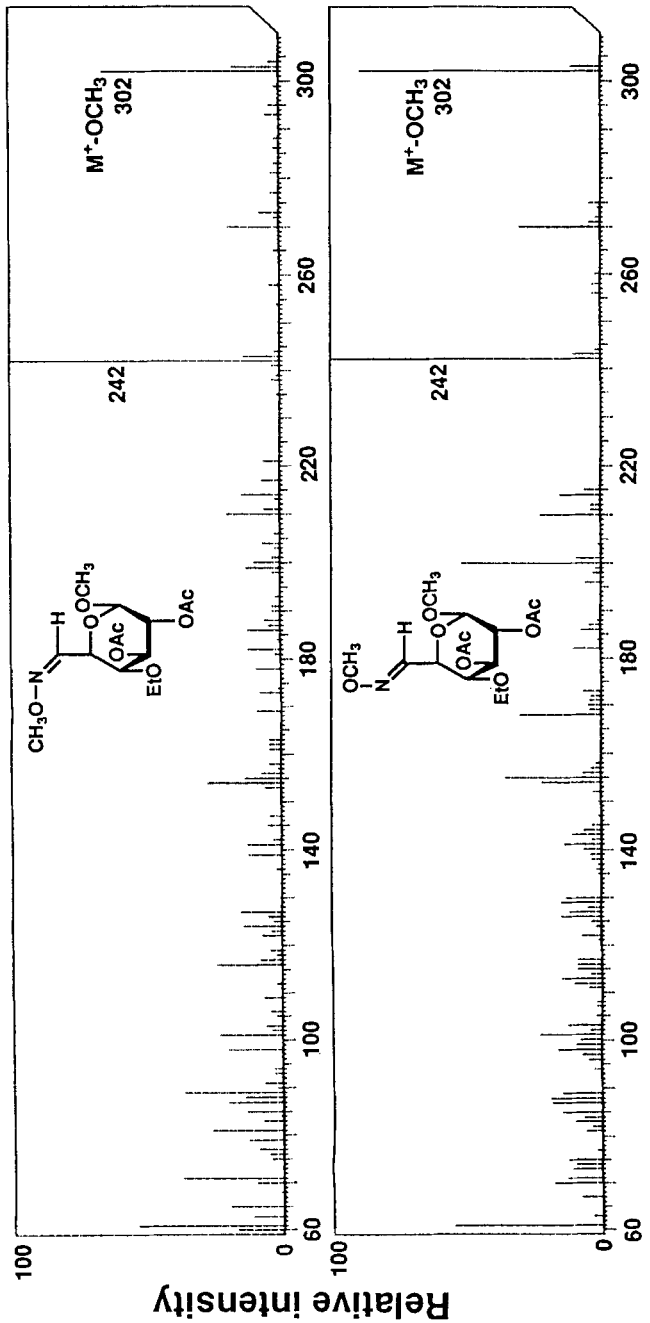

FIGURE 7. Mass spectra of *O*-methyloxime **17**.

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Some chemical sift assignments were made by using a decoupling method; others were made by analogy with model compounds. Optical rotations were measured using a JASCO Dip-4 digital polarimeter. Preparative thin layer chromatography (PTLC) was performed on silica-gel plates (Kieselgel 60 F254, Merck). The standard work-up procedure included diluting with an ethyl acetate, washing with aq. $NaHCO_3$, and a brine, drying over Na_2SO_4, and evaporating in *vacuo.* Analysis was carried out with Shimadzu gas chromatograph GC 14A equipped with hydrogen flame detector and sp2330 capillary column (0.15mm x 15m). The temperature program was 180-220° at 2 °/min. The injection zone temperature was 240° and the detector bath was at 250°. The typical gas pressures were: helium (carrier gas) 1.0 kg/cm^2; nitrogen 5.0 kg/cm^2; hydrogen 0.6 kg/cm^2; and air 0.5 kg/cm^2. Mass spectrometry (GC-MS) was performed with Shimadzu GC MS QP-1000 mass spectrometer, using chemical ionization method.

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Methyl 4,6-*O*-benzylidene-β-D-glucopyranoside (**6**) —— To a solution of methyl β-D-glucopyranoside (12.77 g, 65.8 mmol) in *N*,*N*-dimethylformamide (DMF) (90 ml), benzaldehyde dimethylacetal (19.8 ml, 0.131 mol) and *p*-toluenesulfonic acid (1.7 g, 9.87 mmol) were added. The solution was kept at 50 °C under 15 mmHg for 30 min. Solid $NaHCO_3$ (1.7 g) was added to the mixture. The solution was concentrated to a syrup. The syrup was worked-up by the standard method, and then triturated from *n*-hexane to a colorless crystals (15.0 g, 81 %), m.p. 202-205°, $[\alpha]_D$ +65.2° (c 0.66, $CHCl_3$), ^{1}H-NMR ($CDCl_3$): δ 3.58 (s (singlet), 3H (protons), -OCH_3), 4.33 (d (doublet), 1H, $J_{1,2}$=7.5, C_1-H), 5.54 (s, 1H, CHC_6H_5).

Anal. Calc. for $C_{14}H_{18}O_6$: C, 59.56; H, 6.43. Found: C, 59.30; H, 6.41.

Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**7**) —— To a stirred solution of compound **6** (14.88 g, 52.8 mmol) in DMF (100 ml), sodium hydride (6.32 g, 0.158 mol, 60 % in mineral oil), tetra-*n*-butyl ammonium iodide (1.95 g, 5.28 mmol), and benzyl bromide (18.8 ml, 0.158 mol) were added at 0 °C. The reaction mixture was kept at room temperature for 1.5 h, and then methanol (10 ml) was added for the decomposition of excess benzyl bromide. The reaction mixture was worked-up by the standard method to afford a colorless syrup. The syrup was triturated from *n*-hexane to afford a colorless crystals (21.2 g, 87 %), m.p. 120-122° $[\alpha]_D$ -32.4° (c 1.02, $CHCl_3$), ^{1}H-NMR ($CDCl_3$): δ 3.58 (s, 3H, -OCH_3), 4.42 (d, 1H, $J_{1,2}$=7.5, C_1-H), 4.75, 4.79, 4.87, 4.91 (d, 4H, $CH_2C_6H_5$), 5.57 (s, 1H, -CHC_6H_5).

Anal. Calc. for $C_{28}H_{30}O_6$: C, 72.71; H, 6.54. Found: C, 72.85; H, 6.52.

Methyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**8**) —— To a stirred solution of compound **7** (0.924 g, 2 mmol) in acetonitrile (10 ml), powdered Molecular Sieves 4A (MS4A) (1 g) and sodium cyanoborohydride (0.529 g, 8 mmol) were added. Trimethylchlorosilane (2.5 ml, 19.7 mmol) was added dropwise over a period of 2 h to the reaction mixture at room temperature. The reaction mixture was filtered by the use of Celite 535 and the residue was washed with ethyl acetate. The combined filtrate and washing was worked-up by the standard method to afford a syrup. The product was purified on a silica gel column (Wacogel C-200) with ethyl acetate/*n*-hexane (1/4, *v/v*) to give colorless crystals (867 mg, 93 %), m.p. 69-70° $[\alpha]_D$ -14.0° (c 0.93, $CHCl_3$), ^{1}H-NMR ($CDCl_3$): δ 3.59 (s, 3H, $-OCH_3$), 4.32 (d, 1H, $J_{1,2}$=7.0, C_1-H), 4.58 (s, 2H, $CH_2C_6H_5$), 4.71, 4.93 (dd (double doublet), 4H, $-CH_2C_6H_5$).

Anal. Calc. for $C_{28}H_{32}O_6$: C, 72.39; H, 6.94. Found: C, 72.61; H, 6.85.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-ethyl-β-D-glucopyranoside (**9**) —— To a stirred solution of compound **8** (7.3 g, 15.7 mmol) in DMF (70 ml), sodium hydride (1.26 g, 31.4 mmol) and ethyl iodide (2.52 ml, 31.4 mmol) were added. The reaction mixture was kept at room temperature for 1 h, and then worked-up by the standard method to afford a colorless syrup. The syrup was triturated from *n*-hexane to afford a colorless crystals (7.6 g, 97 %), m.p. 61-62°, $[\alpha]_D$ +30.0° (c 0.80, $CHCl_3$), ^{1}H-NMR ($CDCl_3$): δ 1.27 (t (triplet), 3H, *J*=7.0, $-OCH_2\underline{CH_3}$), 3.76 (s, 3H, $-OCH_3$), 4.46 (d, 1H, $J_{1,2}$=7.5, C_1-H), 4.73, 4.82, 4.85, 4.93, 5.04, 5.06 (d, 6H, $-CH_2C_6H_5$).

Anal. Calc. for $C_{30}H_{36}O_6$: C, 73.14; H, 7.37. Found: C, 73.12; H, 7.43.

Methyl 4-*O*-ethyl-β-D-glucopyranoside (**1**) —— A stirred solution of compound **9** (1.979 g, 4.02 mmol) in ethanol / acetic acid (1/1, *v/v*) (20 ml) was treated with 10 % palladium carbon (2 g) under H_2 at 50 °C for 4 h. The reaction mixture was filtered and concentrated. The product was purified on silica gel column with methanol/dichloromethane (1/9, *v/v*) to afford a colorless crystals (891 mg, 99.8 %). For ozonation compound **1** was recrystallized from ethanol (737 mg, 83 %), m.p. 138-139°, $[\alpha]_D$ -10.1° (c 1.09, CH_3OH).

Anal. Calc. for $C_9H_{18}O_6$: C, 48.64; H, 8.16. Found: C, 48.37; H, 8.06.

Methyl 2,3,6-tri-*O*-acetyl-4-*O*-ethyl-β-D-glucopyranoside (**10**), methyl 3,6-di-*O*-acetyl-4-*O*-ethyl-β-D-glucopyranoside (**11**), and methyl 2,6-di-*O*-acetyl-4-*O*-ethyl-β-D-glucopyranoside (**12**) —— To a stirred solution of compound **1** (0.5 g,

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2.25 mmol) in ethyl acetate (40 ml), acetyl chloride (1.36 ml, 19.2 mmol) and 2,6-lutidine (2.24 ml, 19.2 mmol) were added. The reaction mixture was kept under reflux for 12 h, and then worked-up by the standard method to afford a colorless syrup. The products were purified on a silica gel column with ethyl acetate/*n*-hexane (1/2, *v/v*) to afford **10** (231 mg, 30 %), **11** (145 mg, 20 %), and **12** (341 mg, 50 %), respectively.

10: m.p. 92-94°, $[\alpha]_D$ -22.4° (c 0.49, $CHCl_3$), IR ν_{max} cm^{-1} (KBr): 1747, 1754 (ester C=O), ^{1}H-NMR ($CDCl_3$): δ1.16 (t, 3H, *J*=7, $-OCH_2C\underline{H}_3$), 2.08, 2.09, 2.14 (s, 9H, $-OCOCH_3$), 3.52 (s, 3H, $-OCH_3$), 4.24 (dd, 1H, J_{gem}=12, $J_{6a,5}$=4.5, C_6-H_a), 4.38 (d, 1H, $J_{1,2}$=7.5, C_1-H), 4.39 (dd, 1H, $J_{6b,5}$=2, C_6-H_b), 4.87 (dd, 1H, $J_{2,3}$=9.5, C_2-H), 5.16 (dd, 1H, $J_{3,4}$=8.5, C_3-H).

Anal. Calc. for $C_{15}H_{24}O_9$: C, 51.72; H, 6.94. Found: C, 51.22; H, 6.89.

11: m.p. 85-87°, $[\alpha]_D$ +5.1° (c 0.59, $CHCl_3$), IR ν_{max} cm^{-1} (KBr): 1721, 1753 (ester C=O), ^{1}H-NMR ($CDCl_3$): δ 1.17 (t, 3H, *J*=7, $-OCH_2C\underline{H}_3$), 2.14, 2.18 (s, 6H, $-OCOCH_3$), 3.39 (t, 1H, $J_{4,5}$=9.5, C_4-H), 3.44 (dd, 1H, $J_{2,3}$=9.5, C_2-H), 3.59 (s, 3H, $-OCH_3$), 4.24 (d, 1H, $J_{1,2}$=7.5, C_1-H), 4.25 (dd, 1H, J_{gem}=12, $J_{6a,5}$=4.5, C_6-H_a), 4.37 (dd, 1H, $J_{6b,5}$=2.5, C_6-H_b), 5.06 (t, 1H, $J_{3,4}$=9.5, C_3-H).

Anal. Calc. for $C_{13}H_{22}O_8$: C, 50.97; H, 7.24. Found: C, 50.71; H, 7.35.

12: syrup, $[\alpha]_D$ -29.4° (c 1.36, $CHCl_3$), IR ν_{max} cm^{-1} (KBr): 1747 (ester C=O), ^{1}H-NMR ($CDCl_3$): δ 1.11 (t, 3H, *J*=7, $-OCH_2C\underline{H}_3$), 2.05, 2.08 (s, 6H, $-OCOCH_3$), 3.31 (t, 1H, $J_{4,5}$=9.5, C_4-H), 3.46 (m (multiplet), 1H, C_5-H), 3.47 (s, 3H, $-OCH_3$), 3.63, 3.86 (m, 2H, $-OC\underline{H}_2CH_3$), 3.69 (t, 1H, $J_{3,4}$=9.5, C_3-H), 4.23 (dd, 1H, J_{gem}=12, $J_{6a,5}$=5.0, C_6-H_a), 4.32 (d, 1H, $J_{1,2}$=7.5, C_1-H), 4.39 (dd, 1H, $J_{6b,5}$=2.0, C_6-H_b), 4.77 (dd, 1H, $J_{2,3}$=9.5, C_2-H).

Anal. Calc. for $C_{13}H_{22}O_8$: C, 50.97; H, 7.24. Found: C, 50.45; H, 7.48.

Methyl 2,3-di-*O*-acetyl-4-*O*-ethyl-β-D-glucopyranoside (**13**) —— To a stirred solution of compound **14** (165 mg, 0.23 mmol) in CH_3OH/CH_2Cl_2 (3 ml, 1:4, *v/v*), *p*-toluenesulfonic acid (62 mg, 0.361 mmol) was added. The reaction mixture was kept at room temperature for 2 h, and then worked-up by the standard method to afford a syrup. The products were purified on a silica gel column with CH_2Cl_2 to afford a colorless crystals (78 mg, 85 %), $[\alpha]_D$ -39.5° (c 1.57, $CHCl_3$), IR ν_{max} cm^{-1} (KBr): 1754 (ester C=O), ^{1}H-NMR ($CDCl_3$): δ 1.16 (t, 3H, *J*=7, $-OCH_2C\underline{H}_3$), 2.07, 2.08 (s, 6H, $-OCOCH_3$), 3.39 (m, 1H, C_5-H), 3.52 (s, 3H, $-OCH_3$), 3.53 (t, $J_{4,5}$=9, C_4-H), 3.64 (q (quartet), 2H, $-OC\underline{H}_2CH_3$), 3.76 (dd,

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1H, J_{gem}=12, $J_{6a,5}$=4, C_6-H_a), 3.93 (dd, 1H, $J_{6b,5}$=2, C_6-H_b), 4.42 (d, 1H, $J_{1,2}$=8, C_1-H), 4.85 (dd, 1H, $J_{2,3}$=10, C_2-H), 5.16 (dd, 1H, $J_{3,4}$=9, C_3-H).

Anal. Calc. for $C_{13}H_{22}O_8$: C, 50.97; H, 7.24. Found: C, 50.80; H, 7.33.

Methyl 3,6-di-*O*-acetyl-4-*O*-ethyl-β-D-*arabino*-hexopyranosidulose (**2**) To a stirred solution of compound **11** (21.9 mg, 0.072 mmol) in CH_2Cl_2 (2 ml), MS4A (160 mg) and PCC (157 mg, 0.72 mmol) were added. The reaction mixture was kept at room temperature for 32 h. Ether was added, the mixture was filtered by the use of Celite 535, and the residue was washed with ether. The combined filtrate and washing was concentrated. The unreacted starting material was removed by PTLC to afford a syrup (14.0 mg, 64 %).

Methyl 2,6-di-*O*-acetyl-4-*O*-ethyl-β-D-*ribo*-hexopyranoside-3-ulose (**3**) To a stirred solution of compound **12** (21.3 mg, 0.070 mmol) in CH_2Cl_2 (4 ml), MS4A (100 mg) and PCC (92 mg, 0.42 mmol) were added. The reaction mixture was kept at room temperature for 35 h, and then filtered and concentrated. The products were purified by PTLC to afford a colorless syrup (16.7 mg, 79 %).

Methyl 2,3-di-*O*-acetyl-4-*O*-ethyl-β-D-*gluco*-hexodialdo-1,5-pyranoside (**4**) To a stirred solution of compound **13** (16.2 mg, 0.053 mmol) in CH_2Cl_2 (2 ml), MS4A (100 mg) and PCC (35 mg, 0.16 mmol) were added. The reaction mixture was kept at room temperature for 2.5 h, and then filtered and concentrated. The products were purified by PTLC (CH_3OH/CH_2Cl_2, 2/98, *v/v*) to afford a colorless syrup (10.0 mg, 62 %).

The ^1H- and ^{13}C-NMR spectral data of carbonyl sugars (**2-4**) are shown in **TABLE 1**.

O-Methyloximation procedure —— In typical experiment, to a stirred solution of compound **3** (16.0 mg, 0.052 mmol) in methanol (2 ml), methoxylamine hydrochloride (13.2 mg, 0.16 mmol) and pyridine (21 μl, 0.26 mmol) were added. The reaction mixture was kept at room temperature for 2.5 h, and then worked-up by the standard method. The products were purified by PTLC (1:2, *v/v* ethyl acetate / *n*-hexane) to afford *O*-methyloxime **16** (*syn* and *anti* mixture) as a syrup (15.2 mg, 87 %). The ^1H-NMR spectral data of *O*-methyloximes (**15-17**) are shown in **TABLE 2**.

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