Chemoenzymic Approaches to the Preparation of 5-*C*-(Hydroxymethyl)hexoses

Adam W. Mazur* and George D. Hiler, II

Procter & Gamble Pharmaceuticals, Health Care Research Center, P.O. Box 8006, Mason, Ohio 45040-8006

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Synthesis of 5-*C*-(hydroxymethyl)hexoses, carbohydrates resistant to metabolism, is described. These compounds are obtained in the reaction of hexose 6-aldehydes with formaldehyde. 5-*C*-(Hydroxymethyl)-L-*arabino*-hexopyranoses can be efficiently obtained from D-galactosides by a two-step chemoenzymic synthesis using galactose oxidase for the preparation of required hexose 6-aldehydes. This method is an example of carbohydrate synthesis without use of protecting groups. Other 5-*C*-(hydroxymethyl)hexoses are prepared by a typical chemical methodology requiring specific protection of the hexose hydroxyl groups.

Introduction

5-*C*-(Hydroxymethyl)hexoses constitute an interesting group of sugars that resist metabolism by mammalian enzymes and by intestinal anaerobic bacteria. The additional hydroxymethyl group present at the hexose C-5 appears to not only prevent degradation of the hexose backbone but also inhibit cleavage of the glycosidic linkages in simple glycosides and in disaccharides.[1] Due to these properties, 5-*C*-(hydroxymethyl)hexose derivatives have been proposed as components of foods[2] and pharmaceuticals.[3]

This report describes a methodology used to introduce the hydroxymethyl group at the 5-carbon in hexoses. A general scheme for hydroxymethylation of carbohydrates at the penultimate carbon is shown in Figure 1. The primary alcohol group in hexose is converted first into the aldehyde and then subjected to an aldol-type condensation with formaldehyde. The hydroxymethyl sugar aldehyde, initially formed in condensation, undergoes a spontaneous Canizzaro reduction with the excess of formaldehyde to give the (hydroxymethyl)hexose. The hydroxymethylation procedure was first applied to carbohydrate transformations by by Schaeffer[4] and later used to make a number of 2-*C*- and 4-*C*-(hydroxymethyl)pentose and -hexose derivatives in the syntheses of natural products such as D- and L-apioses[5] and D-hamamelose.[6] Hydroxymethylation of C-2 in D-mannose derivatives produced branched alditols proposed as artificial sweeteners.[7] The hydroxymethylation of pentose-1,5-dialdoses led to the preparation of 4-*C*-(hydroxymethyl)pentose-based aminosugars[8] and nucleosides.[9−11] Interestingly, syntheses of 5-*C*-(hydroxymethyl)hexoses have not been reported in the literature prior to our research.

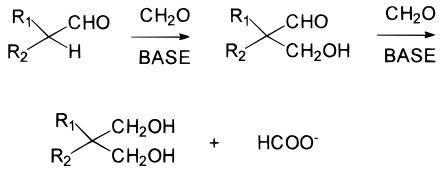

Figure 1. Hydroxymethylation of the α-carbon in aldehydes.

The availability of hexose 6-aldehydes is an important factor in the synthesis of 5-*C*-(hydroxymethyl)hexoses. In general, these aldehydes can be obtained by oxidation of the hexose primary alcohol group[12] or azide[13] or periodate oxidation of heptosides.[14] Also, a reduction of glucuronic acid ester to *gluco*-1,5-dialdohexose via organoboron intermediates has been reported.[15] We will describe two approaches to the synthesis of 5-*C*-(hydroxymethyl)hexoses. One method is based on a traditional chemical synthesis and requires the use of protecting groups to selectively oxidize the hexose 6-hydroxyl to the corresponding aldehyde. The second method greatly simplifies synthesis of the title compounds. It utilizes enzyme galactose oxidase, which allows for carrying out this oxidation as well as subsequent hydroxymethylation on the unprotected carbohydrate in the aqueous solution. However, the latter method is currently limited to the galactose-containing carbohydrates due to the enzyme specificity for this sugar structure.

Results and Discussion

For the chemical oxidations of the primary hydroxyl group in hexoses having D-gluco, D-manno, and D-fructo configurations, we have chosen pirydinium dichromate[16] (Figure 2) and the Swern reagent[17] (Figure 3). Since these oxidations do not discriminate between the unprotected primary vs secondary hydroxyl groups, the hexose

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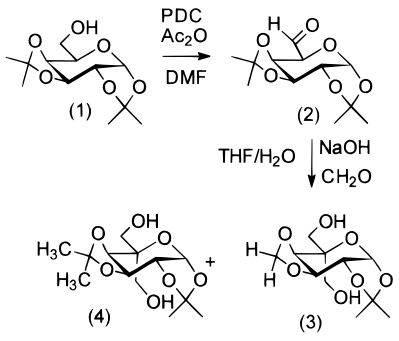

Figure 2. Chemical synthesis of 5-*C*-(hydroxymethyl)-L-*arabino*-hexopyranose derivatives.

substrates had to be selectively protected. Consequently, we have prepared protected hexose 6-aldehydes from 1,2:3,4-di-*O*-isopropylidene-α-D-galactose (**1**), methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**5**), 1,2,3,4-tetra-*O*-benzyl-α-D-mannopyranoside (**6**), and methyl 1,3,4-tri-*O*-benzyl-α-D-fructofuranoside (**13**). Compounds **5** and **6** were obtained in three steps from D-glucose and benzyl α-D-mannoside, respectively, by known procedures.[18] The synthesis of **13** consisted of seven steps from sucrose by a method analogous to one used by Szarek et al.[19]

Condensation of 1,2:3,4-diisopropylidene-α-D-*galacto*-hexodialdo-1,5-pyranose (**2**) with formaldehyde (Figure 2) is accompanied by replacement of the 3,4-isopropylidene with the methylene group. The exchange mechanism is believed to involve a retro-Michael elimination of the acetone molecule from the aldehyde followed by a Michael addition of formaldehyde to the resulting α,*β*-unsaturated aldehyde (Figure 4). The major product of this reaction was 5-*C*-(hydroxymethyl)-1,2-isopropylidene-3,4-methylene-5-*C*-(hydroxymethyl)-*β*-L-*arabino*-hexopyranose (**3**), while 1,2:3,4-di-*O*-isopropylidene-5-*C*-(hydroxymethyl)-*β*-L-*arabino*-hexopyranose (**4**) was only a minor product with a 10:1 ratio of **3** to **4**. The presence of a 3,4-methylene group in **3** was confirmed by a characteristic carbon-13 resonance at 94.4 ppm, while the signals from the isopropylidene acetal carbons in **4** were observed at 108 ppm. Also, proton NMR of **3** showed two signals at 4.95 and 4.68 ppm, each corresponding to one geminal proton from the methylene group. A similar exchange of acetal groups has been noticed by others during the reaction of 2,3-*O*-isopropylidene-*β*-D-*ribo*-pentodialdofuranose,[9] although the reported ratio of 2,3-*O*-methylene vs 2,3-*O*-isopropylidene derivatives was only 1:9. The difference in the ratios of products in these two reactions may reflect a different degree of competition between the retro-Michael reactions of hexodialdopyranose and pentodioaldofuranose and their condensations with formaldehyde. In our case, the complete conversion of **2** requires about 4 h, which seems to be a relatively slow process compared to 30 min reported for the hydroxymethylation of 2,3-*O*-isopropylidene-*β*-D-*ribo*-pentodialdofuranose.[10] Clearly, the slower condensation increases the probability of the acetal exchange.

In the case of unprotected D-galactose, the primary hydroxyl group can be efficiently oxidized to the corresponding aldehyde by air in the presence of enzyme galactose oxidase (GOase).[20] This is the only known enzyme allowing for conversion of the hexose C-6 carbon bearing the primary hydroxyl group into the aldehyde functionality. Galactose oxidase has been known for over 30 years[21] and used extensively in biochemical analysis.[20] Yet, attempts to apply GOase-catalyzed oxidation in the carbohydrate synthesis were in the past only partially successful, and consequently, GOase was not used in practical synthesis. Low levels of substrate conversion[22] and simultaneous formation of galacturonic acid[23−25] were reported to be the key limitations for the synthetic applications of the enzyme. These results were construed as indicating that the synthetic utility of GOase might be limited by the intrinsic catalytical properties of the protein. In contrast, we have found that GOase allows for efficient oxidation of galactose-containing carbohydrates to the corresponding 6-aldehydes if the enzyme is properly purified.[20,26] In our hands, the oxidation of D-galactose-based carbohydrates has been almost quantitative (>95% conversion) and practically has not created by products. In particular, we have not detected (by C-13 NMR) formation of galacturonic acid. Obtained aldehydes (1,5-dialdoses) have been identified by ^{13}C NMR of lyophilized samples from crude oxidation mixtures. Purification of galactose 6-aldehydes is difficult because they have a tendency, particularly upon drying, to form impurities that are probably intermolecular acetal structures.[27] In order to minimize these side reactions, the crude solution of the oxidation product was used directly for the condensation with formaldehyde (Figure 5). In contrast with the hydroxymethylation of **2**, we have not detected the retro-Michael elimination during the condensation of the unprotected hexodialdo-1,5-pyranoses. This result is not surprising since the unprotected aldehydes do not contain a good leaving group at the *β*-carbon. On the other hand, there was a possibility that the hydroxyl group at the *β*-carbon might epimerize. This phenomenon was detected in the hydroxymethylation of pentofuranose 5-aldehydes[11] as a result of a reversed aldol cleavage of the bond between the aldehyde α- and *β*-carbon atoms in the initial carbohydrate aldehyde−formaldehyde adduct. A careful examination of a model condensation leading to **24** (Figure 5) revealed the presence of only 1% of the C-4 epimer. This result indicates that the stereochemistry at C-4 is highly conserved in this reaction compared to hydroxymethylation of pentofuranose 5-aldehydes.

All the transformations of protected sugars, that is oxidation and subsequent condensation of aldehydes to 5-*C*-(hydroxymethyl) derivatives, can be conveniently followed by TLC since the substrates and the products have distinctive R_f values on the normal-phase silica plates. In enzymic oxidation of unprotected sugars, however, we found TLC techniques to be less reliable

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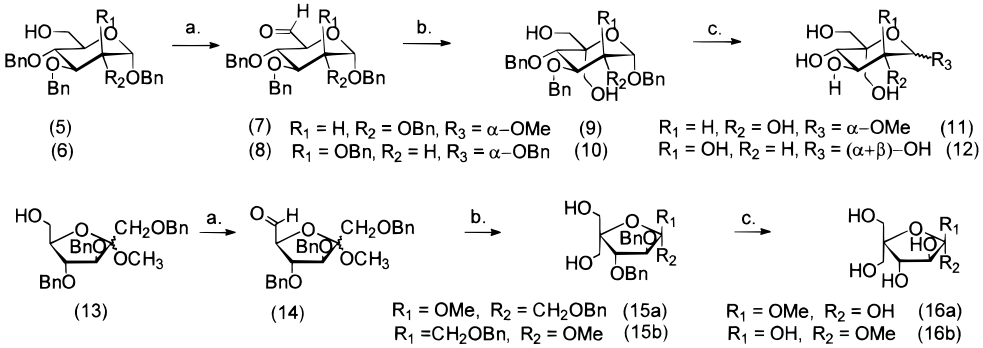


Figure 3. Chemical synthesis of 5-*C*-(hydroxymethyl)-D-*xylo*-hexose, -D-*lyxo*-hexose, and -D-*erythro*-hexulose derivatives. Key: (a) $(COCl)_2$, DMSO, TEA; (b) CH_2O, NaOH; (c) $Pd(OH)_2$, H_2; Ac_2O/pyridine for **12**.

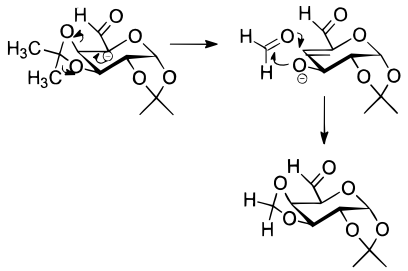
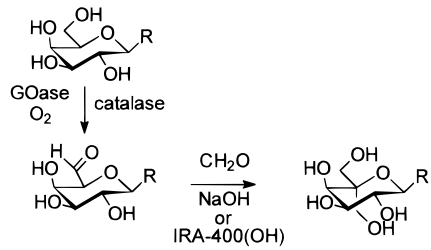


Figure 4. Mechanism of isopropylidene/methylene exchange during hydroxymethylation of **2**.



R	R
OMe (17)	OMe (21)
OCH_2Ph (18)	OCH_2Ph (22)
(19)	(23)
(20)	(24)

Figure 5. Chemoenzymic synthesis of 5-*C*-(hydroxymethyl)-L-*arabino*-hexopyranose derivatives.

mostly due to a low resolution of aldehydes from other reaction components (e.g., Whatman KF5 plates). An effective method to quantitate the formation of aldehyde during the enzymic oxidation proved to be the 2,3,5-triphenyltetrazolium assay[28] adapted for this reaction. Alternatively, HPLC was used to measure the disappearance of the carbohydrate substrate in the oxidation and formation of 5-*C*-(hydroxymethyl)hexose during the subsequent condensation with formaldehyde. Unfortunately, we have not found an HPLC method to simultaneously detect the substrate and the product, during the oxidation, using the same column.

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The sugar 6-aldehydes and 5-*C*-(hydroxymethyl)hexoses are easy to identify by carbon-13 NMR. Formation of the aldehyde during oxidation of the protected hexoses is evidenced by appearance of the carbonyl signal at about 200 ppm with simultaneous disappearance of the primary alcohol carbon resonance at about 62 ppm. On the other hand, the carbonyl signal in unprotected aldehydes emerges at about 88 ppm and no signal is detected at 200 ppm. This clearly indicates a presence of the hydrated aldehyde group. A transient formation of the nonhydrated aldehyde **17** was detected by proton NMR when the enzymic reaction was run in deterium oxide. Conversion of the aldehyde group into 5-*C*-(hydroxymethyl)hexose was confirmed by the absence of the resonances corresponding to the carbonyl carbon and the appearance of the signals at 57−61 ppm of two hydroxymethyl groups formed in the reaction between sugar 6-aldehyde and formaldehyde.

Finally, a word on the catalysis of hydroxymethylation reaction. Both stages of this process (Figure 1), that is the initial aldehyde condensation and the following Canizzarro reaction, reqiure basic catalysts. In fact, it is necessary to use a base in amounts well exceeding the catalytic requirement because formic acid created in the Canizzarro reaction neutralizes the catalyst. If an alkali metal hydroxide serves as the catalyst in the condensation of unprotected carbohydrates, the soluble salts present at the end of reaction are difficult to separate from the carbohydrate product. A remedy for this situation proved to be the catalysis provided by the ion-exchange resin IRA-400(HO^-). The resin serves not only as the catalyst but also simultaneously removes formic acid produced in the reaction.

Summary

We have shown that 5-*C*-(hydroxymethyl)hexoses can be obtained by condensation of hexose 6-aldehydes with formaldehyde. Preparation of 5-*C*-(hydroxymethyl)-L-*arabino*-hexopyranose mono- and oligosaccharides is simple since one can easily obtain the required hexose 6-aldehyde by enzymic oxidation of the galactose primary hydroxyl group. The entire synthesis in this case including oxidation and condensation is conducted in water. It does not involve protecting groups and can be conveniently applied on a kg scale. The condensation of unprotected hexodialdo-1,5-pyranoses produces highly pure 5-*C*-(hydroxymethyl)hexoses without substantial epimerization at C-4 or retro-Michael elimination from the carbohydrate aldehyde. In contrast, the synthesis of 5-*C*-(hydroxymethyl)hexoses from carbohydrates that do not have the "*galacto*" configuration at C-4 and C-5

in the hexose molecule generally require elaborate chemical transformations.

Experimental Section

Galactose oxidase was isolated from the fermentation medium of Dactylium dendroides according to the method of Tressel and Kosman[29] or by our procedure described below. Activity of enzyme was measured by a coupled horseradish peroxidase assay,[30] and protein content was determined by a standard Bio-Rad assay. The HPLC measurements of the substrate concentration during the GOase catalyzed oxidation were done on a Supelco-NH_2 column with acetonitrile−water 85:15 at a flow rate 1.25 mL/min. NMR spectra were measured with QE-300 (General Electric) and JEOL FX-270 spectrometers. Molecular weights of products were confirmed by mass spectroscopy, chemical ionization, or FAB methods using HP5985B (Hewlett-Packard) or ZAB2F (VG-Fisons) spectrometers.

Preparation of Galactose Oxidase. Fermentation of Dactylium dendroides (ATCC 46032, NRRL 2903) was done according to the literature procedure.[29] The fermentation broth (24 L, 6.5 units/mL) was filtered through a nylon bag to remove mycelia. The supernatant containing copper sulfate (5 mM) and histidine (10 mM) at pH 6−7 was concentrated by ultrafiltration to about 5% of the initial volume. The retentate was equilibrated with the buffer containing 20 mM NaH_2PO_4, 10 mM histidine, and 5 mM $CuSO_4$ at pH 5.3. The Millipore Pellicon system equipped with a 10 000 MWCO membrane was used for concentration and equilibration. The final volume of the equlibrated crude enzyme concentrate was 1120 mL and the activity 136 units/mL. The enzyme (960 mL, 125 000 units) was then purified on a carboxysulfone column 21.4 mm × 35 cm (J. T. Baker Co.) equilibrated with the same buffer (4 L). The superntant was loaded in 4 × 200 mL and 1 × 120 mL portions on the column. Each load was followed by elution of the void volume with the fresh equlibration buffer (200 mL). Following the last void volume, the column was washed with the buffer containing 100 mM NaH_2PO_4, 10 mM histidine, and 5 mM $CuSO_4$ at pH 7.0 (250 mL). The final elution was done with a buffer made of 300 mM NaH_2PO_4, 10 mM histidine, and 5 mM $CuSO_4$ at pH 7.0. The enzyme was collected in the fractions between 130 and 250 mL. The activity of the recovered enzyme in 120 mL of eluant was 932 u/mL giving a total of 111 900 units (89% yield of chromatographic purification) with a specific activity of 1435 u/mg.

1,2:3,4-Di-*O*-isopropylidene-α-D-*galacto*-hexodialdo-l,5-pyranose (2). 1,2:3,4-Di-*O*-isopropy1idene-α-D-galactose[31] (**1**) (100 g, 0.38 mol) was added to a solution of pyridinium dichromate (PDC) (72 g, 0.19 mol), acetic anhydride (124 mL, 1.31 mol), and *N*,*N*-dimethylformamide (240 mL, 3.1 mol) in dichloromethane (850 mL). The reaction mixture was refluxed for approximately 3−4 h until TLC showed complete conversion of the carbohydrate substrate. The dark green solution was then cooled to room temperature. Most of the chromium salts were precipitated by diluting the reaction mixture with toluene (6000 mL). To remove these salts thoroughly, the toluene mixture was filtered twice: through a Celite pad (24 × 7.5 cm) and silica gel (35−70 pm) pad (24 × 7.5 cm). Silica gel was finally washed with toluene (2000 mL) followed by ethyl acetate (1000 mL). The slightly green solution was evaporated at reduced pressure to a viscous oil, which after distillation (0.06 mmHg, 125−135 °C) gave 58.0 g (58%) of the product (**2**): TLC (Analtech GF) R_f = 0.50, AcOEt:hexanes = 45:55; ^{13}C NMR ($CDCl_3$, 67.8 MHz) 199.72 (C=O) 109.60, 108.60 ($(CH_3)_2C$), 95.89 (C-1), 72.8 (C-5), 71.87, 71.35, 70.09 (C-2, C-3, C-4), 25.63, 25.45, 24.48, 23.90 ($(CH_3)_2C$).

5-*C*-(Hydroxymethyl)-l,2-*O*-isopropylidene-3,4-*O*-methylene-*β*-L-*arabino*-hexopyranose (3). A homogeneous solution of l ,2:3,4-di-*O*-isopropylidene-α-D-*galacto*-hexodialdo-l,5-pyranose (**2**) (60.0 g, 0.225 mol) in 36% formaldehyde (700 mL) and tetrahydrofuran (1750 mL) was treated with 1 N sodium hydroxide (700 mL) and stirred at room temperature for 18−24 h until TLC showed completion of the reaction. After neutralization to pH 6−7 with 44% formic acid and concentration under reduced pressure to 500 mL (removal of THF and formaldehyde), the reaction mixture was diluted to 1200 mL with distilled water. The product was extracted with ethyl acetate (5 × 300 mL), washed with distilled water (2 × 200 mL), and dried with sodium sulfate (anhydrous). Evaporation of the solvent under reduced pressure produced a viscous oil that contained substantial amounts of formaldehyde polymers. These impurities are usually not seen on TLC but can easily be detected by proton NMR and can be removed by heating and stirring of the impure product for 2 h at 120 °C under vacuum. The final purification was accomplished by column chromatography (silica gel, e.g., Matrex 60A) or HPLC (gives better yield) with solvent toluene:acetone = 4:1. Three products were eluted in the following order: l,2:3,4-di-*0*-isopropylidene-α-D-galactopyranose (**1**), l,2:3,4-di-*0*-isopropylidene-5-*C*-(hydroxymethyl)-*β*-L-*arabino*-hexopyranose (**4**), and 5-*C*-(hydroxymethyl)-l,2-isopropylidene-3,4-methylene-*β*-L-*arabino*-hexopyranose (**3**) in a ratio of approximately 8:8:84. The yield of **3** was 35.0 g (59%). For **3**: TLC (Analtech GF), R_f = 0.22, AcOEt:hexanes = 45:55; ^{13}C NMR ($CDCl_3$, 67.8 MHz) d 94.8 (C-1), 70.8 (C-2), 72.3 (C-3), 72.2 (C-4), 76.5 (C-5), 62.1 (C-6), 61.7 (C-7), 108.6, (CH_3CHCH_3), 94.4 CH_2, 25.9, 24.5 (CH_3); ^{1}H NMR ($CDC1_3$, 300 MHz): d 5.22 (d, 1H, $J_{1,2}$ = 5.1 Hz, H-1), 4.72 (s, 1H, CH_2), 4.40 (s, 1H, CH_2), 4.05 (d, 1H, $J_{3,4}$ = 7.3 Hz, H-4), 3.88 (dd, 1H, $J_{1,2}$ = 5.1 Hz, H-2), 3.63 (d, 1H, $J_{3,4}$ = 7.3, H-3), 3.30−3.50 (m, 4H, H-6, H-7). For **4**: TLC (Analtech GF) R_f = 0.45, $CHCl_3$:MeOH = 9:1; ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 94.5 (C-1), 70.3 (C-2), 71.8 (C-3), 72.8 (C-4), 76.5 (C-5), 62.0 (C-6), 61.4 (C-7), 108.0, 107.9 (CH_3CHCH_3), 26.0, 26.7 (CH_3); ^{1}H NMR ($CDC1_3$, 300 MHz) δ 5.65 (d, 1H, $J_{1,2}$ = 5.2 Hz, H-1), 4.60 (dd, 1H, $J_{3,4}$ = 7.4 Hz, H-3), 4.24 (dd, 1H, $J_{1,2}$ = 5.2 Hz, $J_{2,3}$ = 1.0 Hz, H-2), 4.09 (d, 1H, $J_{3,4}$ = 7.4 Hz, H-4), 3.58−3.86 (m, 4H, H-6, H-7), 2.84 (m, 1H, OH), 2.48 (m, 1H, OH), 1.56 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.35 (s, 3H, CH_3). Anal. Calcd for $C_{11}H_{18}O_7$: C, 50.38; H, 6.92. Found: C, 50.65; H, 6.92

Methyl 2,3,4-Tri-*O*-benzyl-5-*C*-(hydroxymethyl)-α-D-*xylo*-hexopyranoside (9). An oxidizing mixture[17] was made by a careful addition at −70 °C of a dry solution of DMSO (3.84 mL, 53.7 mmol) and CH_2Cl_2 (30 mL) into a solution of oxalyl chloride (2.16 mL, 24.7 mmol) in dry CH_2Cl_2 (110 mL) and stirring the resulting solution for 10 min. While the temperature was maintained −70 °C, a solution of methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**5**)[18] (12.0 g, 26 mmol) in dry CH_2Cl_2 was added over a period of 10 min, and stirring was continued for additional 45 min. After subsequent addition of dry Et_3N (18.1 mL, 130 mmol), the reaction mixture was allowed to warm to room temperature. The product solution was washed with water, 1 N HCl, $NaHCO_3$, and H_2O. After evaporation of the solvent, the crude residue was dissolved in dioxane (225 mL) and stirred with 37% aqueous formaldehyde (40 mL) and 1 M NaOH (40 mL) at room temperature for 20 h. The product solution was neutralized with 1 N HCl and evaporated to dryness. The crude product was purified on a silica column using a solution of hexane and ethyl acetate 45/55. Two components were collected: a minor, faster moving starting material (**5**) (2 g) and a major, the product **6** (5.75 g, 45%): TLC (Analtech GF) R_f = 0.62, AcOEt: hexanes = 45:55; ^{13}C NMR ($CDCl_3$, 75 MHz) d 100.40 (C-1), 80.77 (C-2), 80.40 (C-3) 79.43 (C-4), 80.98 (C-5), 65.08 (C-6), 65.01 (C-7) 139.35, 138.71, 138.61, 129.19, 129.08, 128.68, 128.55, 128.32 (Ph), 74.13, 76.36, 76.75 ($PhCH_2$) 57.12 (CH_3); ^{1}H NMR ($CDC1_3$, 300 MHz) δ 7.50−7.20 (m, 15H, Ph's), 5.02 (d, 1H, $J_{a,b}$ = 10,8 Hz, CH_2 Ph), 4.94 (d, 1H, $J_{a,b}$ = 10.9 Hz, CH_2 Ph), 4.85 (d, 1H, $J_{a,b}$ = 10.8 Hz, CH_2 Ph), 4.77 (d, 1H, $J_{a,b}$ = 11.9 Hz, CH_2 Ph), 4.60−4.50 (m, 3H, CH_2 Ph, H-1), 4.24 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.7 Hz, H-3), 3.88 (d, 1H, $J_{3,4}$ = 9.7 Hz, H-4), 3.80−3.60 (m, 4H, H-6, H-7), 3.51 (dd, 1H, $J_{1,2}$ = 3.9 Hz, $J_{2,3}$ = 9.7 Hz H-2), 3.42 (S, 3H, CH_3).

Benzyl 2,3,4-tri-*0*-benzyl-5-*C*-(hydroxymethyl)-α-L-*lyxo*-hexopyranoside (10) was prepared from benzyl 2,3,4-tri-*O*-benzyl-α-D-mannoside (**6**)[18] (23.7 g, 44 mmol) analogously to (**9**). The final product was purified on a silica column with a solution of hexane and ethyl acetate 65/35. The yield of **10** was 9 g (36%): ^{13}C NMR ($CDCl_3$, 75 MHz) δ 98.45 (C-1), 75.22 (C-2), 76.83 (C-3), 76.74 (C-4), 80.51 (C-5), 65.06 (C-6), 65.02

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(C-7) 138.29, 138.13, 138.04, 137.02, 128.59, 128.47, 128.03, 127.75 (Ph) 70.31, 72.43, 72.90, 76.22 ($PhCH_2$).

Methyl 5-*C*-(hydroxymethyl-α-D-*xylo*-hexopyranoside (11) was obtained by hydrogenolysis of methyl 2,3,4-tri-*O*-benzyl-5-*C*-(hydroxymethyl)-α-D-*xylo*-hexopyranoside (**10**) (10 mmol) in a solution of water (50 mL), methanol (50 mL), and acetic acid (15 mL) containing a catalyst, 20% $Pd(OH)_2$ on charcoal (3.0 g), under normal conditions for 16 h. The reaction mixture was filtered through Celite, the filtrate was evaporated, and the residue was dissolved in water (50 mL), deionized with IRA-400(OH^-), and evaporated. The yield of the product (**11**) was in excess of 90%: TLC (Whatman K5F) $R_f = 0.34$, MeCN:H_2O = 75:25; ^{13}C NMR ($CDCl_3$, 75 MHz) d 101.03 (C-1), 70.62, 71.21 (C-2,C-3) 69.24 (C-4), 80.35 (C-5), 62.47 (C-6), 61.90 (C-7), 57.0 (CH_3).

5-*C*-(Hydroxymethyl)-(α+*β*)-D-*1yxo*-hexopyranose (12) was obtained analogously to **11** from benzyl 2,3,4-tri-*O*-benzyl-5-*C*-(hydroxymethyl)-*β*-D-*lyxo*-hexopyranoside (**10**). The product **12**, TLC (Whatman K5F) $R_f = 0.26$, MeCN:H_2O = 4:1, was identified as the peracetylated derivative, which was made by acetylation of the crude product with Ac_2O in pyridine. The acetyl derivative was extracted with CH_2Cl_2 after a treatment of the acetylation mixture with water and purified on HPLC using a Whatman Particil 5 column with EtOAc:hexanes = 1:1. 5-*C*-(Acetoxyxymethyl)-1,2,3,4-tetraacetyl-(α+*β*)-D-*lyxo*-hexopyranose (**12**): ^{13}C NMR ($CDCl_3$, 75 MHz) *δ* 87.50, 90.18 (C-1), 67.85, 66.43 (C-2), 67.47, 68.23 (C-3), 66.82, 66.03 (C-4), 76.62, 77.08 (C-5), 64.71, 63.86 (C-6), 61.87, 62.94 (C-7). Resonance peaks from the acetyl groups are at about 20 ppm (CH_3) and 168−170 ppm (C═O).

Methyl 5-*C*-(hydroxymethyl)-1,3,4-tri-*O*-benzyl-α-D-*erythro*-hexulofuranoside (15a) and methyl 5-*C*-(hydroxymethyl)-1,3,4-tri-*O*-benzyl-*β*-D-*erythro*-hexulofuranoside (15b) were prepared analogously to **9** from methyl 1,3,4-tri-*O*-benzyl-(α+*β*)-fructofuranosides[19] (**13**) (13.5 g, 29 mmol). The crude product containing **15a** and **15b** was fractionated on a silica column using a mixture of toluene: acetone 10:1. The yields of the α-somer **15a** was 2.25 g (15.7%): ^{13}C NMR ($CDCl_3$, 75 MHz) *δ* 137.89, 137.73, 137.45, 129.14, 128.54, 128.35, 128.16, 127.86 (phenyl rings), 107.01 (C-2), 86.94 (C-3), 85.35 (C-5), 83.52 (C-4), 73.71, 73.07, 72.85 (CH_2-Ph), 67.74 (C-1), 64.54, 64.16 (C-6, C-7), 49.56 (CH_3). The yield of the *β*-isomer **15b** was 1.75 g (12.2%): ^{13}C NMR ($CDCl_3$, 75 MHz) *δ* 137.07, 137.87, 137.23, 128.48, 128.33, 128.23, 127.95 (phenyl rings), 102.31 (C-2), 84.35 (C-3), 83.57 (C-4), 83.13 (C-5), 73.56, 73.51, 73.12 (CH_2Ph), 68.60 (C-1), 65.66, 63.43 (C-7, C-6), 49.51 (CH_3); TLC (Analtech GF), R_f (**15a**) = 0.17, R_f (**15b**) = 0.11, toluene:Me_2CO = 2:1.

Methyl 5-*C*-(Hydroxymethyl)-α-D-*erythro*-hexulofuranoside (16a) and Methyl 5-*C*-(Hydroxymethyl)-*β*-D-*erythro*-hexulofuranoside (16b). These products were obtained from methyl 5-*C*-(hydroxymethyl)-1,3,4-tri-*O*-benzyl-α-D-*erythro*-hexulofuranoside (**15a**) (2.25 g, 4.5 mmol) and methyl 5-*C*-(hydroxymethyl)-1,3,4-tri-*O*-benzyl-*β*-D-*erythro*-hexulofuranoside (**15b**) (1.75 g, 2.5 mmol) by hydrogenolysis under normal conditions. The hydrogenation was carried out in methanol (50 mL), in the presence of 10% $Pd(OH)_2$ on charcoal (3.0 g), during 20 h. The reaction mixture was filtered through Celite, the cake was washed with hot water, and the filtrate was evaporated to dryness. The yields of the products were in excess of 90%. Methyl 5-*C*-(hydroxymethyl)-α-D-*erythro*-hexulofuranoside (**16a**): ^{13}C NMR ($CDCl_3$, 67.8 MHz) *δ* 108.34 (C-2), 86.92 (C-5), 80.24 (C-3), 77.25 (C-4), 64.99 (C-6), 61.99 (C-7), 59.98 (C-1), 49.09 (CH_3). Methyl 5-*C*-(hydroxymethyl)-*β*-D-*erythro*-hexulofuranoside (**16b**): ^{13}C NMR ($CDCl_3$, 67.8 MHz) *δ* 102.87 (C-2), 83.70 (C-5), 77.94 (C-3), 77.25 (C-4), 64.99 (C-6), 61.99 (C-7), 59.98 (C-1), 49.09 (CH_3).

Preparation of 5-*C*-(Hydroxymethyl)hexoses from Unprotected Carbohydrates. **A. Oxidation with Galactose Oxidase (GOase)**. To a solution of carbohydrate substrate (250 mM) in 100 mM sodium phosphate buffer at pH 7 (400 mL) containing $CuSO_4$ (5 mM) and histidine (10 mM) at 4 °C were added catalase (Sigma C-40, 83 000 units) and galactose oxidase (8300 units). The reaction mixture was stirred and aerated at this temperature until completion (14−24 h). Galactose oxidase was recovered in the retentate by ultrafiltration of the reaction mixture using an Amicon ultrafiltration stired cell equipped with 10 000 or 30 000 MWCO membrane. The retantate was washed with the fresh buffer to remove the remaining aldehyde. About 60−85% of the galactose oxidase activity was usually recovered after each run. A sample of the crude oxidation mixture was lyophilized for ^{13}C NMR determination of the product.

Methyl *β*-D-*galacto*-hexodialdo-1,5-pyranoside (17): ^{13}C NMR ($CDCl_3$, 75 MHz) *δ* 104.31 (C-1), 88.71 (C-6), 77.13 (C-5), 73.16 (C-3), 70.97 (C-2) 68.67 (C-4), 57.73 (CH_3).

Benzyl *β*-D-*galacto*-hexodialdo-1,5-pyranoside (18): ^{13}C NMR ($CDCl_3$, 75 MHz) *δ* 136.67, 128.71, 128.44 (Ph), 101.95 (C-1), 88.30 (C-6), 76.82 (C-5), 72.71 (C-3), 71.45 (C-2), 70.59 (CH_2), 68.18 (C-4).

2′,3′-Isopropylidene-(D + L)-glyceryl *β*-D-*galacto*-hexodialdo-1,5-pyranoside (19): ^{13}C NMR ($CDCl_3$, 75 MHz) *δ* 110.59 ($C(CH_3)_2$), 103.74, 103.51 (C-1) 88.71 (C-6), 77.19 (C-5), 74.77 (C-2′), 73.04 (C-3), 71.03, 70.45 (C-2, C-1″, 68.61 (C-4), 65.90 (C-3′), 26.05, 24.67 (CH_3).

4-*O*-(*β*-D-*galacto*-Hexodialdo-1,5-pyranosyl)-(1→4)-*β*-D-sorbitol (20): ^{13}C NMR ($CDCl_3$, 75 MHz) *δ* 103.51 (C-1), 88.65 (C-6′), 79.78 (C-4′), 77.19 (C-5), 71.32, 72.64, 72.87 (C-2, C-2′, C-3, C-5′), 69.76 (C-3′), 68.67 (C-4), 63.00, 62.45 (C-1′, C-6′).

B. Condensation with Formaldehyde. The filtrate of the crude aldehyde solution obtained in **A** was combined with 37% aqueous formaldehyde solution (10 mL) and 50% NaOH (144 mL). A cooling bath was applied during addition of NaOH since an exothermic reaction ensued upon hydroxide addition. Alternatively, the anion exchange resin IRA-400(OH^-) (180 mL) can be used instead of NaOH. With the resin, the exothermic reaction has not been detected. The reaction mixture was stirred at room temperature for 8−16 h, heated to 55 °C, and deionized using sequentially ion-exchange columns (2.5 cm × 100 cm) containing resins IR-120(H^+) and IRA-400(OH^-) followed by IRA-400(HSO_3^-) to remove formaldehyde. The product was obtained by evaporation of the solvent followed by drying the residue over P_2O_5 under vacuum.

Methyl 5-*C*-(hydroxymethyl)-α-L-*arabino*hexopyranoside (21): yield 60%; TLC (Whatman K5F) $R_f = 0.54$, MeCN:H_2O = 80:20; $[\alpha]_D = -37.44$ (c, 2.94, H_2O, 23.0 °C); ^{13}C NMR ($CDCl_3$, 75 MHz) 100.40 (C-1), 79.63 (C-5), 70.70, 70.55 (C-2, C-3), 68.40 (C-4), 60.82 (C-6), 57.86 (C-7), 57.14 (CH_3).

Benzyl 5-*C*-(hydroxymethyl)-α-L-*arabino*hexopyranoside (22): yield 57%; TLC (Whatman K5F) $R_f = 0.44$, MeCN:H_2O = 80:20; $[\alpha]_D = -56.1$ (c, 1.60 H_2O, 23.O °C); ^{13}C NMR ($CDCl_3$, 75 MHz) 137.26, 129.08, 128.79 (phenyl), 98.90 (C-1), 80.01 (C-5), 71.26, 71.08 (C-2, C-3, CH_2), 68.90 (C-4), 61.64 (C-6), 58.59 (C-7).

(D + L)-Glyceryl 5-*C*-(hydroxymethyl)-α-L-*arabino*-hexopyranoside (23) was prepared from 2′,3′-isopropylidene-(D + L)-glyceryl *β*-D-*galacto*-hexodialdo-1,5-pyranoside (**19**). The isopropylidene group was cleaved during the workup of the condensation product with IR-120(H^+): yield 36%; TLC (Whatman K5F) $R_f = 0.19$, MeCN:H_2O = 75:25; $[\alpha]_D = -31.39$ (c, 1.77, H_2O, 23.0 °C); ^{13}C NMR ($CDCl_3$, 75 MHz) 99.97, 99.75 (C-1), 70.94, 70.80, 70.68 (C-2, C-3, C-4, C-2′), 68.34 (C-1′), 62.49, 62.34, 60.80, 57.80 (C-6, C-7, C-3′).

4-*O*-[5′-*C*-(Hydroxymethyl)-α-L-*arabino*-hexopyranosyl]-D-sorbitol (24): yield 62%; TLC (Whatman K5F) $R_f = 0.40$, MeCN:H_2O = 70:30; $[\alpha]_D = +20.48$ (c, 0.53, H_2O, 23.0 °C); ^{13}C NMR ($CDCl_3$, 75 MHz) 99.94 (C-1), 80.24 (C-5′), 79.61 (C-4), 72.52, 71.37, 70.68, 69.53 (C-2, C-2′, C-3, C-3′, C-5), 68.84 (C-4), 62.91 (C-6′), 62.39 (C-6), 61.30 (C-1), 58.19 (C-7).

Benzyl 4-*O*-[5′-*C*-(hydroxymethyl)-α-L-*arabino*-hexopyranosyl]-(1→4)-*β*-D-glucopyranoside (25): yield 44%; TLC (Analtech GF) $R_f = 0.11$, $CHCl_3$:CH_3OH = 8:2; $[\alpha]_D = -15.61$ (c, 6.45, H_2O, 23.4 °C); ^{13}C NMR ($CDCl_3$, 67.8 MHz) 136.97, 129.08, 128.79 (Ph), 101.43 (C-1′), 100.22 (C-1′), 80.59 (C-5′), 79.03 (C-4), 75.06, 74.82, 73.16, 71.16, 71.32, 70.74 (C-5, C-3, C-3′, C-2, C-2′, CH_2), 68.72 (C-4′), 61.29, 60.55 (C-6, C-6′), 58.85 (C-7′).

Supporting Information Available: NMR spectra for **2**−**4**, **8**−**12**, **14**, **15a,b**, **16a,b**, **18**−**21**, **21a**, **22**, **23**, and **25** (32 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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