ELSEVIER

Carbohydrate Research 337 (2002) 691–700

CARBOHYDRATE RESEARCH

www.elsevier.com/locate/carres

Synthesis of oxidized methyl 4-*O*-methyl-β-D-glucopyranoside and methyl β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside derivatives as substrates for fluorescence labeling reactions

Jürgen Röhrling, Antje Potthast, Thomas Lange, Thomas Rosenau, Immanuel Adorjan, Andreas Hofinger, Paul Kosma*

Christian-Doppler-Laboratory, Institute of Chemistry, University of Agricultural Sciences, Muthgasse 18, A-1190 Vienna, Austria

Received 28 November 2001; accepted 4 February 2002

Abstract

The synthetic cellulose model compounds methyl 4-*O*-methyl-β-D-glucopyranoside and methyl 4-*O*-methyl-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside and related 6-*O*-protected intermediates were oxidized in good to fair yields using Swern-conditions or bromine/bis(tributyltin) oxide, respectively, to afford compounds containing 6-aldehyde, 3-keto, and 2,3-diketo groups. Cellobiose and oxidized monosaccharides were then labeled with the carbonyl-selective fluorescence marker 9-(7-amino-1,4,7-trioxaheptyl)-9*H*-carbazolecarboxamide (CCOA). The labeled derivatives serve as model compounds for the determination of minute amounts of carbonyl groups in cellulosic polysaccharides.

Keywords: Oxidation; Cellulose; Cellobioside; Fluorescence labelling

1. Introduction

The reliable and accurate determination of oxidized groups in cellulosic materials still represents a largely unsolved, but fundamental problem in the pulp and paper industry. Carbonyl and carboxylic acid groups, which are introduced into cellulosic fibers by a number of bleaching procedures, constitute 'hot spots' along the cellulosic chain. Carbonyl groups are implicated in various detrimental effects such as loss in fiber strength due to peeling reactions and β-elimination, as well as decrease of brightness in cellulosic products induced by thermal or photochemical yellowing reactions.[1]

The determination and quantitation of carbonyl groups (aldehyde groups, reducing-end groups, 2-keto-, 3-keto-, and 2,3-diketo groups) has to meet a number of experimental shortcomings. Traditional methods rely on heterogeneous derivatization of carbonyls with hydroxylamine or cyanide, which suffer from limited reproducibility and insufficient yields due to restrained accessibility of the polymeric substrate.[2,3] The problem of accurate quantitation becomes even more serious taking into account the small amount of carbonyls usually found in pulps, which is in the range of a few μmols per gram.

We have previously reported on the synthesis of a fluorescence label, 9-(7-amino-1,4,7-trioxaheptyl)-9*H*-carbazolecarboxamide (CCOA), which permits a homogeneous derivatization reaction of carbonyl groups in the common cellulose solvent system *N*,*N*-dimethylacetamide–LiCl.[4] The precolumn derivatization with the fluorescence label is followed by a subsequent chromatographic separation of the labeled polysaccharide and the simultaneous determination of the molecular mass using multi-angle laser-light scattering (MALLS) detection. Hence, the label to be used must not interfere with the spectral characteristics of the laser light system (at 488 or 633 nm for the commercial GPC-MALLS equipment). The fluorescence label was attached to a spacer group to provide similar spectral characteristics of the labeled material independently of the type and position of the carbonyl groups. In order to develop the method into a reliable procedure for analytical use,

* Corresponding author. Tel.: +43-1-360066055; fax: +43-1-360066059.

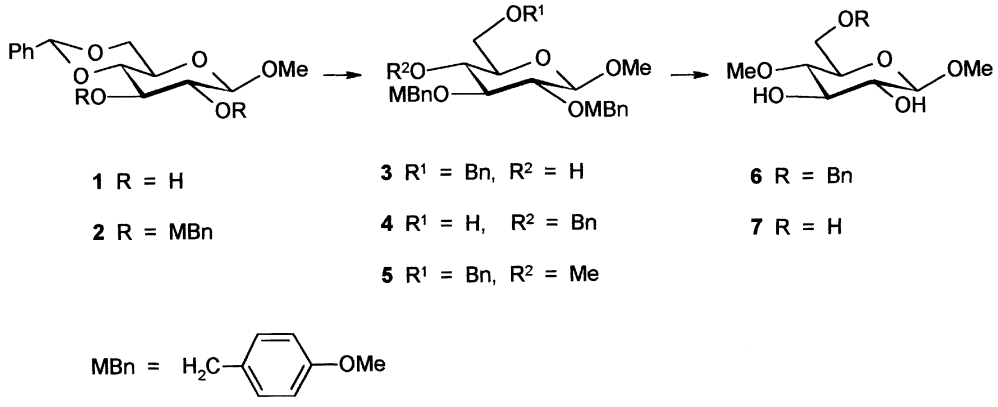
E-mail address: pkosma@edv2.boku.ac.at (P. Kosma).

0008-6215/02/$ - see front matter
PII: S0008-6215(02)00048-4

model compounds were needed as reference materials. Related oxidized 4-*O*-ethyl derivatives of methyl β-D-glucopyranoside have been used as *O*-methyloxime derivatives for GC-MS analysis.[5] Herein we report on the chemical synthesis and subsequent fluorescence labeling of oxidized 4-*O*-methyl glucopyranoside derivatives as soluble model compounds for similar structural entities occurring in oxidized cellulosic substrates. Validation of the method and application for the determination of carbonyl group profiles in oxidized pulps will be published elsewhere.

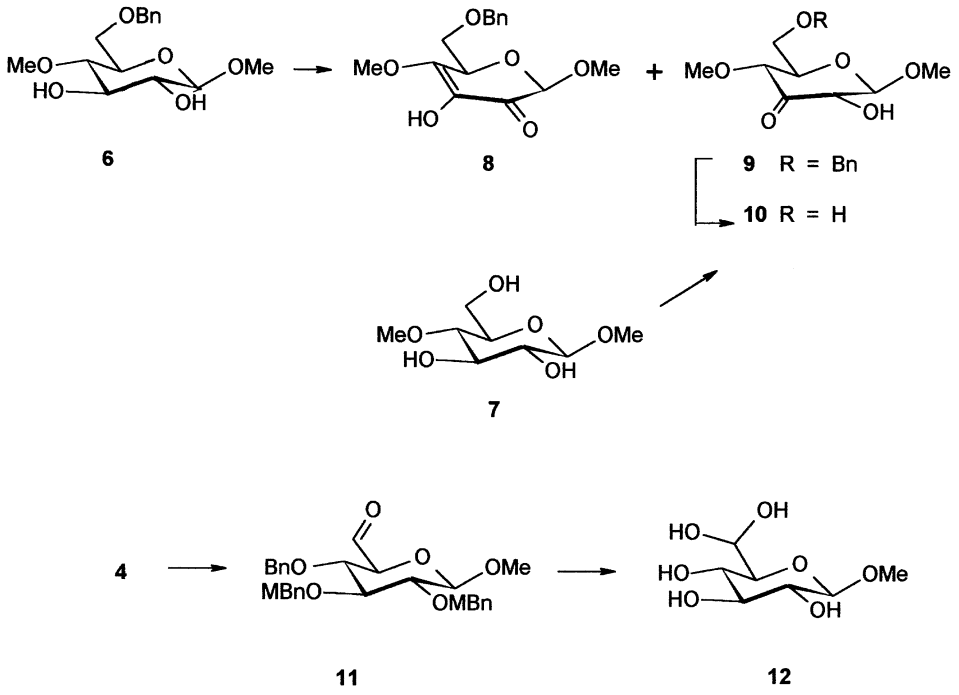
2. Results and discussion

For the synthesis of the hemi-protected derivatives **6** and **7**, known methyl 4,6-*O*-benzylidene-β-D-glucopyranoside (**1**)[6] was converted into the 2,3-di-*O*-methoxybenzyl derivative **2** in 76% yield by reaction with *p*-methoxybenzyl chloride–NaH in DMF.[7] Subsequent reductive ring opening with HCl–$NaCNBH_3$ in THF–diethyl ether[8] afforded preferentially the 6-*O*-benzyl ether derivative **3** in 56% yield, together with a small proportion of the 4-*O*-benzyl ether derivative **4** (11%), which were separated by column chromatography. Subsequent *O*-methylation of **3** with MeI–NaH in THF produced the 4-*O*-methyl derivative **5** in 96% yield. For selective oxidation at the C-2 and C-3 positions, the *p*-methoxybenzyl groups were removed by DDQ oxidation in 82% yield to furnish **6**. Further deblocking of the 6-*O*-benzyl group was achieved by hydrogenolysis in the presence of 10% Pd–carbon in methanol in 92% yield to give known methyl 4-*O*-methyl-β-D-glucopyranoside (**7**).[9] Swern oxidation of **6** with Me_2SO–trifluoroacetic anhydride[10,11] was best performed using three equivalents of oxidant, which allowed recovery of educt **6** (56%) and furthermore, provided a better chromatographic separation of the oxidized products. Thus, the enol form of the labile 2,3-diketo derivative **8** was obtained (34%) in addition to a small amount of the 3-oxo derivative **9** (3%). The presence of the α,β-unsaturated carbonyl moiety was assigned on the basis of the ^{13}C NMR data observed for C-2, C-3, and C-4 at 186.27, 138.14, and 151.01 ppm, respectively, whereas the ^{13}C NMR signal of C-3 in **9** was measured at 205.18 ppm. Assignments were confirmed by HMQC and HMBC measurements. While **8** was too unstable for subsequent deprotection, the methyl hex-3-ulopyranoside derivative **9** was subjected to hydrogenolysis in MeOH which afforded known methyl 4-*O*-methyl-β-D-*ribo*-hex-3-ulopyranoside (**10**) in 98% yield. The compound had previously been obtained in 2.6% yield upon chromium trioxide treatment of **7**.[12] Alternatively, **10** was much more efficiently prepared by reaction of methyl 4-*O*-methyl glucopyranoside (**7**) with bis(tri-*n*-butyltin) oxide followed by oxidation with bromine in $CHCl_3$,[13] which gave a nearly quantitative yield (98%) of **10**. In aqueous solution, **10** occurred as a mixture of the keto- and hydrate form in a 5:3 ratio, which was deduced from the integration values of the anomeric protons, which were correlated—based on HMBC measurements—to the ^{13}C NMR signals at C-3 at 206.81 and 95.78 ppm, respectively.



Swern oxidation of **4** afforded a good yield (82%) of the 6-aldehydo derivative **11**, which was deprotected by hydrogenolysis with 10% Pd–C to furnish known[14] methyl β-D-*gluco*-hexodialdo-1,5-pyranoside (**12**) in 27% yield after chromatography. Optical rotation values were not in agreement with published values; NMR-data indicated that the aldehyde group at C-6 was fully hydrated.[14]

In addition to the monosaccharide derivatives, the previously described[15] cellulose model disaccharide derivative **13** was subjected to oxidative modification. Tritylation using trityl chloride–pyridine at 100 °C afforded the 6,6′-bis-*O*-trityl ether derivative **14** in 74% yield. Swern oxidation with Me_2SO–trifluoroacetic acid anhydride at −60 °C produced the fully oxidized disaccharide derivative **18** as the major product in 50% yield containing keto groups at C-2 and C-3′, a hydrated keto group at C-2′, and a keto group at C-3 being present in the enol form. The structural assignments



and differentiation between the two units were achieved following HMBC assignment of the two methoxyl groups linked to C-1 and C-4′, respectively, which then provided the distinction between the C-4 and C-4′ as well as C-1 and C-1′ signals, respectively. As a minor byproduct, the 3′-*O*-methylthiomethyl ether derivative **15** was isolated in 12% yield,[16,17] and the disaccharide derivative **16** was obtained in 7% yield. Similar to **18**, the dicarbonyl group in the reducing unit of disaccharide **16** occurred as the conjugated enone, the terminal residue, however, had only one hydrated keto group at C-2′. Removal of the trityl ether groups of **16** and **18** was accomplished by hydrogenolysis with 10% Pd–C in methanol, which furnished the disaccharide model compounds **17** and **19** in 30% and 68% yield, respectively. All assignments were confirmed by HMQC and HMBC measurements. Thus, the HMBC spectrum (Fig. 1) of **19** showed the connectivity of the *O*-methyl group signal at 3.63 ppm to the carbon signal at 78.73 ppm (C-4′), whereas the signal of the glycosidic *O*-methyl group was correlated to the anomeric carbon at 99.91 ppm (C-1), which in turn displayed the connectivity to the H-5 signal at 4.91 ppm. The signal of H-5 gave correlations with C-1, C-6 and the olefinic carbons at 127.58 (C-3) and 152.14 ppm (C-4), respectively. The latter signals are comparable with the shifts observed for **8** as holds true for the carbonyl signal of C-2 at 186.06 ppm (compound **8**: C-2 at 186.27 ppm). The remaining carbonyl groups of the nonreducing unit at 95.19 and 92.74 ppm are consistent with the presence of hydrated keto groups. The compounds will be used for the development of other fluorescence labels which might differentiate between mono- and dicarbonyl moieties.

Derivatization with CCOA **20** was tested using the 6-*O*-benzyl derivative **9** and compound **10**, which gave the corresponding oxime derivatives **21** and **22** as mix-

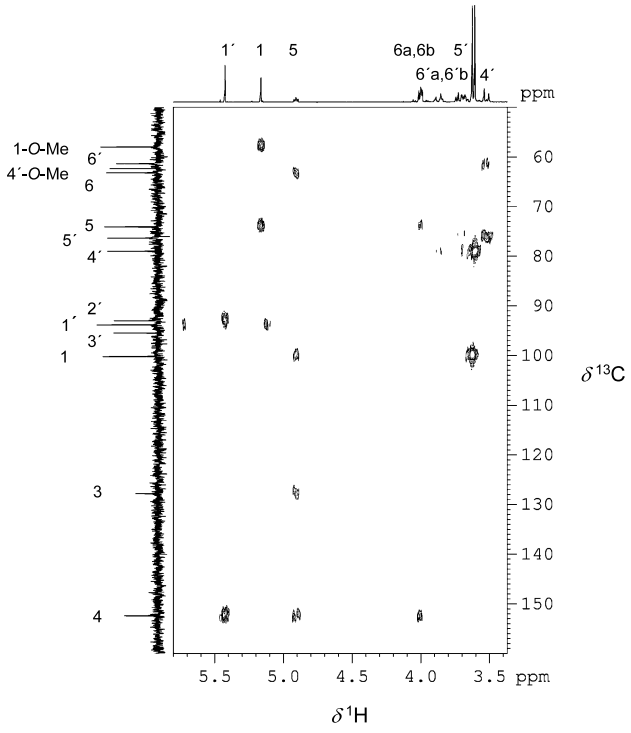
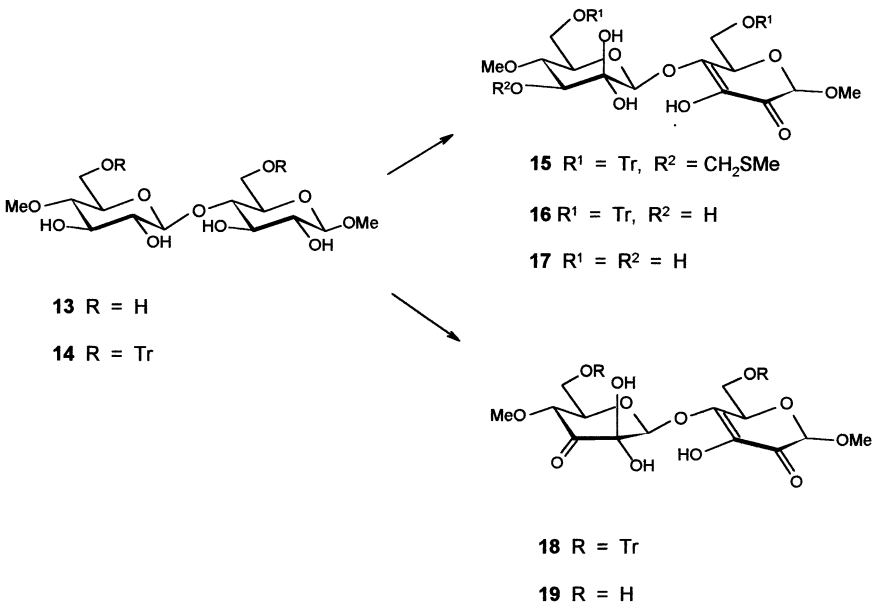


Fig. 1. Two-dimensional plot of the HMBC-spectrum of **19** in D_2O (hydrated form).



13 R = H

14 R = Tr

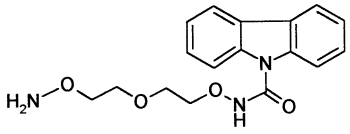
15 R^1 = Tr, R^2 = CH_2SMe

16 R^1 = Tr, R^2 = H

17 R^1 = R^2 = H

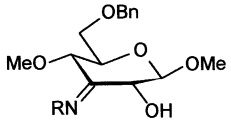
18 R = Tr

19 R = H



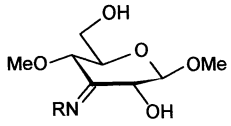
20

9 →



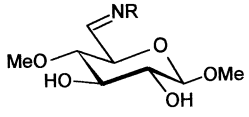
21

10 →

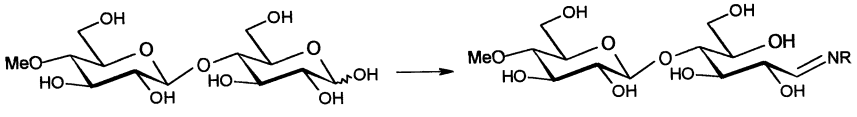


22

12 →

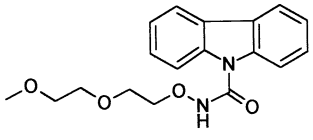


23



24 **25**

R =



tures of *E*/*Z* isomers in good yields and with a slight preference for the formation of the *Z*-isomers. To ensure that the steric influence of the *E*/*Z*—arrangement does not translate into different spectral properties of the fluorescence label, the isomers were separated by chromatography. Excitation and emission wavelengths of the isomers proved to be identical in the cellulose solvent system *N*,*N*-dimethylacetamide–LiCl. The same held true for the labeled aldehyde derivative **23**, which was obtained as the *E*-configured isomer in 93% yield together with a small proportion of the *Z*-derivative. Finally, reducing end groups also reacted smoothly with the oxyamine **20** as shown for cellobiose **24**, which afforded derivative **25** in 75% yield, although the reaction time was significantly longer compared to the monosaccharide model compounds.

Further optimization of the labeling reaction and application for the carbonyl quantification in oxidized pulps will be presented in a forthcoming publication.

3. Experimental

General methods.—Melting points were determined with a hot stage and are uncorrected. Optical rotations were measured with a Perkin–Elmer 243 B polarimeter. ^{1}H NMR spectra were recorded at 297 K with a Bruker Avance DPX instrument operating at 300 MHz for ^{1}H using $CDCl_3$ as solvent and tetramethylsilane as internal standard, unless stated otherwise. Coupling constants are given in Hz (first order values). ^{13}C NMR spectra were measured at 75.47 MHz and referenced to 1,4-dioxane (δ 67.40). Homo- and heteronuclear 2D NMR spectroscopy was performed with Bruker standard software. TLC was performed on E. Merck precoated plates (5 × 10 cm, layer thickness 0.25 mm, Silica Gel 60F_{254}); detection was effected by spraying with anisaldehyde–H_2SO_4. For column chromatography, silica gel (0.040–0.063 mm) was used. Concentration of solutions was performed at reduced pressure and < 40 °C. Elemental analyses were provided by Dr J. Theiner, Mikroanalytisches Laboratorium, Institut für Physikalische Chemie, University of Vienna. MALDI-TOF mass spectra were obtained on a Dynamo (Thermo BioAnalysis) instrument in the positive ion mode using 2% 2,5-dihydroxybenzoic acid as matrix, by Dr F. Altmann, Institut für Chemie, University of Agricultural Sciences, Vienna. LC-MS measurements were performed by Dr E. Rosenberg, Institut für Analytische Chemie, TU Vienna, using HP1100 HPLC/MSD with APCI interface.

*Methyl 4,6-*O*-benzylidene-2,3-di-*O*-(4-methoxybenzyl)-β-*D*-glucopyranoside* (**2**).—Powdered NaH (2.38 g, 99.2 mmol) was added gradually to a solution of methyl 4,6-*O*-benzylidene-β-D-glucopyranoside (7.00 g, 24.8 mmol) in dry DMF (100 mL). The suspension was stirred at rt for 1 h. Then 4-methoxybenzyl chloride (7.40 mL, 54.6 mmol) was added dropwise during 10 min. The mixture was kept at rt for 60 h. Another portion of 4-methoxybenzyl chloride (5.00 mL, 36.8 mmol) was added and the mixture was stirred for 20 h. After addition of MeOH (5 mL) and stirring for 1 h, the mixture was poured onto satd aq NH_4Cl solution. After extraction with EtOAc, the organic layer was dried ($MgSO_4$) and concentrated. The crude product was chromatographed (toluene → 9:1 toluene–EtOAc) to give **2** as colorless crystals (9.89 g, 76%): mp 110–112 °C (MeOH); $[\alpha]_D^{20}$ −24° (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.53–7.22 (m, 9 H, Ph), 6.90–6.79 (m, 4 H, Ph), 5.56 (s, 1 H, C*H*Ph), 4.85–4.65 (m, 4 H, 4 CH_2Ph), 4.39 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.36 (dd, 1 H, $J_{5,6b}$ 4.9, $J_{6a,6b}$ 11.2 Hz, H-6b), 3.80 (s, 3 H, OMe), 3.79 (s, 3 H, OMe), 3.76 (dd, 1 H, $J_{5,6a}$ 1.7 Hz, H-6a), 3.71 (dd, 1 H, $J_{2,3}$ 9.2, $J_{3,4}$ 9.2 Hz, H-3), 3.65 (dd, 1 H, $J_{4,5}$ 9.2 Hz, H-4), 3.58 (s, 3 H, 1-OMe), and 3.43–3.34 (m, 2 H, H-2, H-5). Anal. Calcd for $C_{30}H_{34}O_8$: C, 68.95; H 6.56. Found: C, 68.71; H, 6.50.

*Methyl 6-*O*-benzyl-2,3-di-*O*-(4-methoxybenzyl)-β-*D*-glucopyranoside* (**3**) *and methyl 4-*O*-benzyl-2,3-di-*O*-(4-methoxybenzyl)-β-*D*-glucopyranoside* (**4**).—A solution of HCl in Et_2O (2 M, 2 mL) was added dropwise to a suspension of **2** (115 mg, 0.22 mmol), $NaCNBH_3$ (207 mg, 3.3 mmol), and powdered molecular sieves (0.2 g, 3 Å) in dry THF (15 mL) at −20 °C until the evolution of gas ceased. The solution was stirred for 1 h at −20 °C and for 30 min at 5 °C. For complete conversion, $NaCNBH_3$ (207 g, 3.3 mmol) and then a solution of HCl in Et_2O (2 M, 2 mL) were added dropwise at 5 °C until the evolution of gas ceased. The mixture was stirred for 15 min, Et_3N (3 mL) was added and the mixture was poured onto water–EtOAc (50 mL). Molecular sieves were removed by filtration. After extraction of the filtrate with EtOAc, the organic layer was dried ($MgSO_4$) and concentrated. The crude material was chromatographed (60:30:1 *n*-hexane–EtOAc–Et_3N) to give **3** as colorless crystals (65 mg, 56%): mp 60–61 °C (MeOH); $[\alpha]_D^{20}$ −9° (*c* 0.9, $CHCl_3$); R_f 0.31 (3:1 *n*-hexane–EtOAc); ^{1}H NMR ($CDCl_3$): δ 7.34–7.22 (m, 9 H, Ph), 6.90–6.80 (m, 4 H, Ph), 4.89–4.55 (m, 6 H, 6 CH_2Ph), 4.31 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 3.80 (m, 6 H, 2 OMe), 3.78 (dd, 1 H, $J_{5,6b}$ 3.2, $J_{6a,6b}$ 10.1 Hz, H-6b), 3.69 (dd, 1 H, $J_{5,6a}$ 4.5 Hz, H-6a), 3.60–3.50 (m, 4 H, 1-OMe, H-4), 3.45–3.30 (m, 3 H, H-2, H-5, H-3), and 2.44 (br. s, 1 H, OH). Anal. Calcd for $C_{30}H_{36}O_8$: C, 68.69; H 6.92. Found: C, 68.47; H, 6.84.

Further elution gave **4** as colorless crystals (13.2 mg, 11%): mp 79–81 °C (MeOH); $[\alpha]_D^{20}$ +15° (*c* 0.3, $CHCl_3$); R_f 0.14 (3:1 *n*-hexane–EtOAc); ^{1}H NMR ($CDCl_3$): δ 7.35–7.18 (m, 9 H, Ph), 6.88–6.80 (m, 4 H, Ph), 4.88–4.58 (m, 6 H, 6 CH_2Ph), 4.32 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.86 (dd, 1 H, $J_{5,6b}$ 2.8, $J_{6a,6b}$ 12.1 Hz, H-6b), 3.79 (m, 3 H, OMe), 3.78 (m, 3 H, OMe), 3.70 (dd, 1 H,

$J_{5,6a}$ 4.8 Hz, H-6a), 3.62 (dd, 1 H, $J_{2,3}$ 9.0, $J_{3,4}$ 9.0 Hz, H-3), 3.57 (s, 3 H, 1-OMe), 3.52 (dd, 1 H, $J_{4,5}$ 9.0 Hz, H-4), 3.39–3.30 (m, 2 H, H-2, H-5), and 1.70 (br. s, 1 H, OH). Anal. Calcd for $C_{30}H_{36}O_8$: C, 68.69; H 6.92. Found: C, 68.41; H, 6.93.

*Methyl 6-*O*-benzyl-2,3-di-*O*-(4-methoxybenzyl)-4-*O*-methyl-β-*D*-glucopyranoside* (**5**).—Powdered NaH (175 mg, 7.31 mmol) was added gradually to a solution of **3** (1.92 g, 3.66 mmol) in dry THF (50 mL). The suspension was stirred at rt for 1 h. Then methyl iodide (455 μL, 7.31 mmol) was added. After 20 h MeOH (5 mL) was added and the mixture was stirred for 1 h. Satd aq NH_4Cl was added and the mixture was extracted with EtOAc (199 mL). The organic layer was dried ($MgSO_4$) and concentrated to give pure **5** as colorless crystals (1.89 g, 96%): mp 61–62 °C (MeOH); $[\alpha]_D^{20}$ +44° (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.48–7.24 (m, 9 H, Ph), 6.90–6.85 (m, 4 H, Ph), 4.87–4.55 (m, 6 H, 6 CH_2Ph), 4.28 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.83 (m, 6 H, 2 OMe), 3.78 (dd, 1 H, $J_{5,6b}$ 2.0, $J_{6a,6b}$ 11.7 Hz, H-6b), 3.70 (dd, 1 H, $J_{5,6a}$ 4.5 Hz, H-6a), 3.60 (s, 3 H, 4-OMe), 3.52–3.48 (m, 4 H, H-3, 1-OMe), 3.42–3.33 (m, 2 H, H-2, H-5), and 3.27 (dd, 1 H, $J_{3,4}$ 9.6, $J_{4,5}$ 9.6 Hz, H-4). Anal. Calcd for $C_{31}H_{38}O_8$: C, 69.13; H 7.11. Found: C, 69.10; H, 7.13.

*Methyl 6-*O*-benzyl-4-*O*-methyl-β-*D*-glucopyranoside* (**6**).—A solution of **5** (1.75 g, 3.25 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (2.21 g, 9.75 mmol) in 18:1 CH_2Cl_2–water (30 mL) was stirred at rt for 40 min. The mixture was concentrated and chromatographed (1:5 toluene–EtOAc) to give **5** as colorless crystals (793 mg, 82%): mp 76–78 °C (EtOAc); $[\alpha]_D^{20}$ −18° (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.37–7.25 (m, 5 H, Ph), 4.66 (d, 1 H, *J* 12.2 Hz, CH_2Ph), 4.57 (d, 1 H, CH_2Ph), 4.16 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 3.77 (dd, 1 H, $J_{5,6b}$ 2.2, $J_{6a,6b}$ 10.9 Hz, H-6b), 3.70 (dd, 1 H, $J_{5,6a}$ 6.0 Hz, H-6a), 3.62 (dd, 1 H, $J_{2,3}$ 9.0, $J_{3,4}$ 9.0 Hz, H-3), 3.56 (s, 3 H, OMe), 3.52 (s, 3 H, OMe), 3.43–3.35 (m, 2 H, H-2, H-5), 3.26 (dd, 1 H, $J_{4,5}$ 9.0 Hz, H-4), 2.60 (br. s, 1 H, OH), and 2.46 (br. s, 1 H, OH). Anal. Calcd for $C_{15}H_{22}O_6$: C, 60.39; H 7.43. Found: C, 60.53; H, 7.38.

*Methyl 4-*O*-methyl-β-*D*-glucopyranoside* (**7**).—Compound **6** (70.0 mg, 0.235 mmol) was hydrogenolyzed in the presence of 10% Pd–C (12.0 mg) in dry MeOH (20 mL) at atmospheric pressure for 15 h at rt. The catalyst was filtered off, and the mixture was concentrated to give **7** as a colorless solid (45.0 mg, 92%): $[\alpha]_D^{20}$ −18° (*c* 0.7, water), lit. −18° (*c* 1.0, water);[9] ^{1}H NMR (D_2O): δ 4.34 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.90 (dd, 1 H, $J_{5,6b}$ 2.3, $J_{6a,6b}$ 12.3 Hz, H-6b), 3.73 (dd, 1 H, $J_{5,6a}$ 5.5 Hz, H-6a), 3.56 (dd, 1 H, $J_{2,3}$ 9.3, $J_{3,4}$ 9.3 Hz, H-3), 3.54 (s, 3 H, OMe), 3.53 (s, 3 H, OMe), 3.43 (ddd, 1 H, $J_{4,5}$ 9.3 Hz, H-5), 3.24 (dd, 1 H, H-2), and 3.17 (dd, 1 H, $J_{4,5}$ 9.3 Hz, H-4); ^{13}C NMR (D_2O): δ 103.97 (C-1), 80.14 (C-4), 76.25 (C-3), 75.74 (C-5), 73.90 (C-2), 61.20 (C-6), 60.87 (4-OMe), and 58.00 (1-OMe).

*Methyl 6-*O*-benzyl-4-*O*-methyl-β-*D*-erythro-hex-3-eno-2-ulopyranoside* (**8**) *and methyl 6-*O*-benzyl-4-*O*-methyl-β-*D*-ribo-hex-3-ulopyranoside* (**9**).—Dry Me_2SO (476 μL, 6.70 mmol) in dry CH_2Cl_2 (50 mL) was treated with trifluoroacetic anhydride (703 μL, 5.03 mmol) at −60 °C. The solution was stirred for 10 min, then a solution of **6** (500 mg, 1.68 mmol) in dry CH_2Cl_2 (5 mL) was added. The mixture was stirred at −60 °C for 1.5 h. After the addition of Et_3N (1.39 mL, 10.0 mmol) in dry CH_2Cl_2 (5 mL), the reaction mixture was stirred at −60 °C for a further 1.5 h. The solution was allowed to warm up to −40 °C. After addition of 19:1 MeOH–water (3 mL), the solution was evaporated and the residue was chromatographed (34:15:1 $CHCl_3$–*n*-hexane–MeOH) to afford **8** as a colorless syrup (169 mg, 34%): $[\alpha]_D^{20}$ −139° (*c* 0.2, $CHCl_3$); R_f 0.76 (49:1 CH_2Cl_2–MeOH); IR (KBr): 1747 (C=O), and 1694 (C=C); ^{1}H NMR ($CDCl_3$): δ 7.40–7.26 (m, 5 H, Ph), 5.57 (s, 1 H, OH), 4.95 (s, 1 H, H-1), 4.66 (dd, 1 H, $J_{5,6a}$ 3.3, $J_{5,6b}$ 8.5 Hz, H-5), 4.63 (s, 2 H, CH_2Ph), 4.16 (s, 3 H, 4-OMe), 3.96 (dd, 1 H, $J_{6a,6b}$ 9.0 Hz, H-6a), 3.77 (dd, 1 H, H-6b), and 3.57 (s, 1 H, 1-OMe); ^{13}C NMR ($CDCl_3$): δ 186.27 (C-2), 151.01 (C-4), 138.14 (C-3), 129.64–128.30 (Ph), 98.80 (C-1), 74.37 (C-5), 73.87 (CH_2Ph), 73.06 (C-6), 60.77 (4-OMe), and 57.01 (1-OMe); MALDI-TOF MS: *m*/*z* 317.67 $[M + Na]^+$.

Further elution gave **9** as a colorless solid (13.2 mg, 3%): $[\alpha]_D^{20}$ +30° (*c* 0.5, $CHCl_3$); R_f 0.45 (49:1 CH_2Cl_2–MeOH); IR (KBr): *v* 1726 (C=O); ^{1}H NMR ($CDCl_3$): δ 7.38–7.26 (m, 5 H, Ph), 4.72 (d, 1 H, *J* 12.2 Hz, CH_2Ph), 4.59 (d, 1 H, CH_2Ph), 4.19 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.12 (d, 1 H, H-2), 4.08 (d, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 3.85 (dd, 1 H, $J_{5,6b}$ 2.0, $J_{6a,6b}$ 11.3 Hz, H-6b), 3.78 (dd, 1 H, $J_{5,6a}$ 3.7 Hz, H-6a), 3.62 (s, 3 H, 1-OMe), 3.57 (br. s, 1 H, OH), and 3.54–3.47 (m, 4 H, H-5, 4-OMe); ^{13}C NMR ($CDCl_3$): δ 205.18 (C-3), 137.89 (Ph), 128.40–127.76 (Ph), 105.49 (C-1), 80.51 (C-4), 77.30 (C-2), 75.00 (C-5), 73.66 (CH_2Ph), 68.29 (C-6), 59.52 (4-OMe), and 57.31 (1-OMe). Anal. Calcd for $C_{15}H_{20}O_6$: C, 60.80; H 6.80. Found: C, 60.99; H, 6.81. Finally, starting material **6** (282 mg, 56%) was isolated.

*Methyl 4-*O*-methyl-β-*D*-ribo-hex-3-ulopyranoside* (**10**).—Hydrogenolysis of **9** (15.0 mg, 0.05 mmol) was performed as described for **7**. The crude product was chromatographed (19:1 CH_2Cl_2–MeOH) to give **10** as a colorless solid (10.1 mg, 98%): mp 149–152 °C (MeOH), lit. 152–153 °C (PrOH);[12] $[\alpha]_D^{23}$ +9° (*c* 0.3, water), lit. +11° (*c* 0.5, water);[12] IR (KBr): *v* 1731 (C=O); ^{1}H NMR (D_2O): δ keto form: 4.41 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.20 (dd, 1 H, $J_{2,4}$ 1.6 Hz, H-2), 4.12 (dd, 1 H, $J_{4,5}$ 10.1 Hz, H-4), 3.92 (dd, 1 H, $J_{5,6b}$ 2.2, $J_{6a,6b}$ 12.8 Hz, H-6b), 3.76 (dd, 1 H, $J_{5,6a}$ 4.6 Hz, H-6a), 3.56 (s, 3 H, 1-OMe), 3.53–3.43 (m, 4 H, 4-OMe, H-5); hydrate form: 4.35 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.92 (dd, 1 H, $J_{5,6b}$ 2.2, $J_{6a,6b}$ 12.8 Hz, H-6b), 3.67 (dd, 1 H, $J_{5,6a}$ 5.4 Hz, H-6a), and 3.53–3.43 (m, 7 H, 1-OMe, 4-OMe, H-5);

^{13}C NMR (D_2O): δ keto form: 206.81 (C-3), 105.19 (C-1), 81.45 (C-4), 77.64 (C-2), 75.73 (C-5), 61.16 (C-6), 60.22 (4-OMe), and 58.20 (1-OMe); hydrate form: 103.08 (C-1), 95.78 (C-3), 80.64 (C-4), 75.29 (C-5), 74.68 (C-2), 62.03 (4-OMe), 61.45 (C-6), and 57.86 (1-OMe); 5:3 ketone–hydrate.

Alternative synthesis of **10**.—A suspension of **7** (300 mg, 1.44 mmol), (n-Bu_3Sn)$_2$O (1.48 mL, 2.90 mmol) and molecular sieves 3 Å (300 mg) in $CHCl_3$ (25 mL) were heated under reflux for 3 h. The suspension was cooled to 0 °C and bromine (170 μL, 3.32 mmol) was added until a faint coloration was observed. The suspension was applied onto a column of silica gel and washed with $CHCl_3$. Elution with 19:1 $CHCl_3$–MeOH furnished **10** as a solid. Yield: 290 mg (98%).

*Methyl 4-*O*-benzyl-2,3-di-*O*-(4-methoxybenzyl)-β-*D*-gluco-hexodialdo-1,5-pyranoside* (**11**).—Dry Me_2SO (14.9 μL, 0.210 mmol) in dry CH_2Cl_2 (10 mL) was treated with trifluoroacetic anhydride (26.6 μL, 0.191 mmol) at −60 °C. The solution was stirred for 10 min and then **4** (20 mg, 0.0381 mmol) in dry CH_2Cl_2 (5 mL) was added. The mixture was stirred at −60 °C for 1.5 h. After addition of Et_3N (52.8 μL, 0.379 mmol) in dry CH_2Cl_2 (5 mL), the reaction mixture was stirred at −60 °C for 1.5 h. The solution was allowed to warm up to −40 °C. A solution of 19:1 MeOH–water (3 mL) was added, the solution was concentrated and the residue was chromatographed (3:1 toluene–EtOAc) to afford **11** as a colorless solid (16.5 mg, 82%): $[\alpha]_D^{20}$ +7° (c 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 9.61 (d, 1 H, $J_{5,6}$ 1.0 Hz, H-6), 7.38–7.15 (m, 9 H, Ph), 6.90–6.80 (m, 4 H, Ph), 4.86–4.57 (m, 6 H, 6 CH_2Ph), 4.40 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 3.85–3.77 (m, 7 H, H-5, 2 OMe), 3.73–3.63 (m, 2 H, H-3, H-4), 3.58 (s, 3 H, 1-OMe), and 3.40 (dd, 1 H, $J_{2,3}$ 9.0 Hz, H-2); ^{13}C NMR ($CDCl_3$): δ 197.13 (C-6), 159.47 (Ph), 159.44 (Ph), 137.59 (Ph), 128.19–130.57 (Ph), 113.98 (Ph), 104.76 (C-1), 83.53 (C-3), 81.52 (C-2), 78.39 (C-5), 77.32 (C-4), 75.40 (CH_2Ph), 74.96 (CH_2Ph), 74.47 (CH_2Ph), 57.47 (1-OMe), and 55.42 (2 OMe); MALDI-TOF MS: m/z 545.12 $[M+Na]^+$.

*Methyl β-*D*-gluco-hexodialdo-1,5-pyranoside-6-hydrate* (**12**).—Hydrogenolysis of **11** (300 mg, 0.574 mmol) was performed as described for **7**. The crude product was chromatographed (4:1 CH_2Cl_2–MeOH) to give **12** as a colorless solid (33.0 mg, 27%): $[\alpha]_D^{20}$ −47° (c 0.3, water), lit. $[\alpha]_D^{22}$ −71° (c 0.5, water);[14] ^{1}H NMR (D_2O): δ 5.25 (d, 1 H, $J_{5,6}$ 2.3 Hz, H-6), 4.37 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 3.57 (s, 3 H, 1-OMe), 3.52–3.47 (m, 2 H, H-3, H-4), 3.40 (m, 1 H, H-5), and 3.26 (dd, 1 H, $J_{2,3}$ 9.7 Hz, H-2); ^{13}C NMR (D_2O): δ 104.23 (C-1), 88.74 (C-6), 77.30 (C-5), 76.43 (C-3), 73.77 (C-2), 70.94 (C-4), and 58.06 (1-OMe).

*Methyl 4-*O*-methyl-6-*O*-triphenylmethyl-β-*D*-gluco-pyranosyl-(1→4)-6-*O*-triphenylmethyl-β-*D*-glucopyranoside* (**14**).—Methyl 4-*O*-methyl-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (400 mg, 1.08 mmol) and triphenylchloromethane (723 mg, 2.59 mmol) were dissolved in dry pyridine (10 mL) and stirred at 100 °C for 2 h. For complete conversion, another portion of triphenylchloromethane (300 mg, 1.07 mmol) was added and the mixture was kept at 100 °C for a further 2 h. The suspension was concentrated and the residue was chromatographed (1:5 toluene–EtOAc→EtOAc) to afford **14** as a colorless solid (674 mg, 74%): $[\alpha]_D^{20}$ +2° (c 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.50–7.15 (m, 30 H, Ph), 4.33 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 4.27 (d, 1 H, $J_{3,OH}$ 1.7 Hz, 3-OH), 4.17 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 3.75–3.45 (m, 9 H, H-4, 1-OMe, H-3, H-5, H-6b, H-6b, H-2), 3.37–3.10 (m, 9 H, H-3′, H-5′, H-6a, 4′-OMe, H-4′, H-2′, H-6a′), 2.49 (d, 1 H, $J_{3',OH}$ 2.2 Hz, 3′-OH), 2.47 (d, 1 H, $J_{2,OH}$ 1.4 Hz, 2-OH), and 1.54 (br. s, 1 H, 2′-OH). Anal. Calcd for $C_{52}H_{54}O_{11}\cdot H_2O$: C, 71.54; H 6.47. Found: C, 71.61; H, 6.52.

*Methyl 4-*O*-methyl-3-*O*-methylthiomethyl-6-*O*-triphenylmethyl-β-*D*-arabino-hex-2-ulopyranosyl-2-hydrate-(1→4)-6-*O*-triphenylmethyl-β-*D*-erythro-hex-3-eno-2-ulopyranoside* (**15**), *methyl 6-*O*-triphenylmethyl-4-*O*-methyl-β-*D*-arabino-hex-2-ulopyranosyl-2-hydrate-(1→4)-6-*O*-triphenylmethyl-β-*D*-erythro-hex-3-eno-2-ulopyranoside* (**16**), *and methyl 4-*O*-methyl-6-*O*-triphenylmethyl-β-*D*-erythro-hex-2,3-diulopyranosyl-2-hydrate-(1→4)-6-*O*-triphenylmethyl-β-*D*-erythro-hex-3-eno-2-ulopyranoside* (**18**).—Dry Me_2SO (329 μL, 4.63 mmol) in dry CH_2Cl_2 (50 mL) was treated with trifluoroacetic anhydride (588 μL, 4.21 mmol) at −60 °C. The solution was stirred for 10 min and then **14** (600 mg, 0.702 mmol) in dry CH_2Cl_2 (5 mL) was added. The mixture was stirred at −60 °C for 1.5 h. After the addition of Et_3N (1.28 mL, 9.26 mmol) in dry CH_2Cl_2 (5 mL), the reaction mixture was stirred at −60 °C for 1.5 h. The solution was allowed to warm up to −40 °C. After addition of MeOH (5 mL) and water (0.5 mL), the solution was concentrated and the residue was chromatographed (34:15:1 $CHCl_3$–n-hexane–MeOH) to afford first **15** as a colorless solid (77.8 mg, 12%): $[\alpha]_D^{20}$ −12° (c 0.4, $CHCl_3$); R_f 0.90 (19:1 $CHCl_3$–MeOH); ^{1}H NMR ($CDCl_3$): δ 7.50–7.05 (m, 30 H, 6 Ph), 5.10 (s, 1 H, H-1′), 4.87 (d, 1 H, J 11.0 Hz, OCH_2S), 4.86 (s, 1 H, H-1), 4.78 (d, 1 H, $J_{3',4'}$ 3.4 Hz, H-3′), 4.72 (d, 1 H, J 11.0 Hz, OCH_2S), 4.58 (dd, 1 H, $J_{5,6a}$ 4.3, $J_{5,6b}$ 4.3 Hz, H-5), 3.85–3.75 (m, 2 H, H-5′, H-4′), 3.65–3.55 (m, 2 H, H-6b, H-6a), 3.46 (s, 3 H, 1-OMe), 3.41–3.37 (m, 2 H, H-6b′, H-6a′), 3.36 (s, 3 H, 4′-OMe), and 2.16 (s, 3 H, SCH_3); ^{13}C NMR ($CDCl_3$): δ 183.19 (C-2), 151.60 (C-4), 143.91 (Ph), 129.17–127.46 (Ph, C-3), 99.52 (C-1), 98.17 (C-2′), 95.37 (C-1′), 87.49 (2 CPh_3), 84.53 (C-3′), 79.13 (C-4′), 78.07 (C-5′), 72.41 (C-5), 70.01 (SCH_2O), 63.79 (C-6), 62.76 (C-6′), 58.95 (4′-OMe), 56.80 (1-OMe), and 14.99 (SCH_3); LC-MS: m/z 243.1 $[Ph_3C]^+$.

Subsequent elution gave **16** as a colorless solid (46.7 mg, 7%): $[\alpha]_D^{20}$ −4° (c 0.2, $CHCl_3$); R_f 0.55 (19:1

$CHCl_3$–MeOH); ^1H NMR ($CDCl_3$): δ 7.60–7.05 (m, 30 H, Ph), 5.15 (s, 1 H, H-1′), 4.93 (s, 1 H, H-1), 4.85 (dd, 1 H, $J_{5,6a}$ 4.3, $J_{5,6b}$ 8.0 Hz, H-5), 3.85–3.79 (m, 2 H, H-6b, H-6a), 3.76–3.32 (m, 6 H, H-6b′, H-6a′, H-4′, H-3′, H-5, H-5′), 3.31 (s, 3 H, 1-OMe), 3.25 (s, 3 H, 4′-OMe), and 3.14 (dd, 1 H, $J_{5',6a'}$ 3.5, $J_{6a',6b'}$ 10.0 Hz, H-6a′); ^{13}C NMR ($CDCl_3$): δ 184.19 (C-2), 149.97 (C-4), 144.09 (Ph), 143.91 (Ph), 127.28–129.29 (Ph), 126.19 (C-3), 100.29 (C-1), 93.90 (C-1′), 91.77 (C-2′), 87.63 (CPh_3), 86.61 (CPh_3), 78.39 (C-4′), 75.84 (C-3′ or C-5′), 73.46 (C-5), 72.59 (C-5′ or C-3′), 66.53 (C-6), 61.52 (C-6′), 61.12 (4′-OMe), and 57.02 (1-OMe). Anal. Calcd for $C_{52}H_{50}O_{12}\cdot 1.3\ H_2O$: C, 70.15; H 5.95. Found: C, 70.19; H, 5.71.

Finally, **18** was obtained as a colorless solid (302 mg, 50%): $[\alpha]_D^{20}$ −12° (*c* 0.8, $CHCl_3$); R_f 0.45 (19:1 $CHCl_3$–MeOH); ^1H NMR ($CDCl_3$): δ 7.48–7.09 (m, 30 H, Ph), 5.02 (s, 1 H, H-1′), 4.89 (s, 1 H, H-1), 4.77 (dd, 1 H, $J_{5,6a}$ 4.6, $J_{5,6b}$ 7.1 Hz, H-5), 4.69 (d, 1 H, $J_{4',5'}$ 9.5 Hz, H-4′), 3.80–3.72 (m, 2 H, H-6b, H-6a), 3.65–3.49 (m, 2 H, H-6b′, H-5′), 3.27 (s, 3 H, 1-OMe), 3.25 (s, 3 H, 4′-OMe), and 3.16 (dd, 1 H, $J_{5',6a'}$ 3.6, $J_{6a',6b'}$ 11.0 Hz, H-6a′); ^{13}C NMR ($CDCl_3$): δ 199.20 (C-3′), 182.77 (C-2), 149.20 (C-4), 143.92 (Ph), 143.85 (Ph), 129.15–127.44 (Ph), 126.41 (C-3), 100.40 (C-1), 95.01 (C-1′), 91.43 (C-2′), 87.73 (CPh_3), 86.81 (CPh_3), 79.38 (C-4′), 75.64 (C-5′), 73.27 (C-5), 66.43 (C-6), 61.23 (C-6′), 60.28 (4′-OMe), and 57.12 (1-OMe). Anal. Calcd for $C_{52}H_{48}O_{12}\cdot 1.3\ H_2O$: C, 70.25; H 5.75. Found: C, 70.26; H, 5.86.

Methyl 4-O-*methyl*-β-D-arabino-*hex*-2-*ulopyranosyl*-2-*hydrate*-(1→4)-β-D-erythro-*hex*-3-*eno*-2-*ulopyranoside* (**17**).—A suspension of **16** (67.0 mg, 0.078 mmol) and 10% Pd–C (12.0 mg) in dry MeOH (20 mL) was hydrogenolyzed at atmospheric pressure for 15 h at rt. The catalyst was filtered off, and the mixture was concentrated. The residue was chromatographed (9:1 CH_2Cl_2–MeOH) to give **17** as a colorless solid (8.8 mg, 30%): $[\alpha]_D^{20}$ +39° (*c* 0.3, water); UV: λ_{max} 274 nm (ε 9.0×10^4 dm^2/mol, MeOH); IR (KBr): *v* 1686 (C=O), and 1652 (C=C); ^1H NMR (D_2O): δ 5.37 (s, 1 H, H-1′), 5.16 (s, 1 H, H-1), 4.91 (dd, 1 H, $J_{5,6a}$ 4.8, $J_{5,6b}$ 4.8 Hz, H-5), 4.05–3.95 (m, 2 H, H-6b, H-6a), 3.88 (dd, 1 H, $J_{5',6b'}$ 2.1, $J_{6a',6b'}$ 12.4 Hz, H-6b′), 3.85 (d, 1 H, $J_{3',4'}$ 9.3 Hz, H-3′), 3.74 (dd, 1 H, $J_{5',6a'}$ 5.3 Hz, H-6a′), 3.63–3.58 (m, 7 H, H-5′, 1-OMe, 4′-OMe), and 3.42 (dd, 1 H, $J_{3',4'}$ 9.3, $J_{4',5'}$ 9.3 Hz, H-4′); ^{13}C NMR (D_2O): δ 186.28 (C-2), 152.14 (C-4), 127.61 (C-3), 99.94 (C-1), 94.21 (C-1′), 92.41 (C-2′), 78.44 (C-4′), 76.64 (C-5′), 76.41 (C-3′), 73.79 (C-5), 62.91 (C-6), 61.23 (4′-OMe), 60.89 (C-6′), and 57.72 (1-OMe); LC-MS: *m*/*z* 364.1 [M−water]$^-$. Anal. Calcd for $C_{14}H_{22}O_{12}$: C, 43.98; H 5.80. Found: C, 44.16; H, 5.60.

Methyl 4-O-*methyl*-β-D-erythro-*hex*-2,3-*diulopyranosyl*-2-*hydrate*-(1→4)-β-D-erythro-*hex*-3-*eno*-2-*ulopyranoside* (**19**).—Compound **18** (120.0 mg, 0.139 mmol) was treated as described for **7** to give **19** as a colorless solid (35.9 mg, 68%): $[\alpha]_D^{20}$ +31° (*c* 0.2, water); UV: λ_{max} 274 nm (ε 1.0×10^5 dm^2/mol, MeOH); IR (KBr): *v* 1755 and 1691 (C=O), and 1651 (C=C); ^1H NMR (D_2O): δ 5.43 (s, 1 H, H-1′), 5.18 (s, 1 H, H-1), 4.91 (dd, 1 H, $J_{5,6a}$ 5.5, $J_{5,6b}$ 3.8 Hz, H-5), 4.10–3.95 (m, 2 H, H-6b, H-6a), 3.91–3.83 (m, 1 H, H-6b′), 3.76–3.60 (m, 2 H, H-5′, H-6a′), 3.63 (s, 3 H, 1-OMe), 3.61 (s, 3 H, 4′-OMe), and 3.52 (d, 1 H, $J_{4',5'}$ 9.7 Hz, H-4′); ^{13}C NMR (D_2O): δ 186.06 (C-2), 152.14 (C-4), 127.58 (C-3), 99.91 (C-1), 95.19 (C-3′ or C-2′), 93.55 (C-1′), 92.74 (C-2′ or C-3′), 78.73 (C-4′), 76.07 (C-5′), 74.79 (C-5), 62.90 (C-6), 62.08 (4′-OMe), 61.07 (C-6′), and 57.69 (1-OMe); LC-MS: *m*/*z* 379.1 [M−H]$^-$. Anal. Calcd for $C_{14}H_{20}O_{12}$: C, 44.22; H 5.30. Found: C, 44.09; H, 5.12.

General procedure for labeling of keto sugars.—The keto compound (0.03–0.05 mmol) and a fivefold molar excess of **20** were dissolved in water (1 mL) and stirred overnight at rt. The solution was treated with satd aq $NaHCO_3$, and extracted with EtOAc (50 mL). The organic layer was dried ($MgSO_4$) and concentrated. Column chromatography of the residue afforded the labeled compounds (*E*- and *Z*-isomer) and unreacted **20**. In case of highly polar products, the extraction step was omitted and the reaction mixture was freeze-dried prior to chromatography.

Methyl 6-O-*benzyl*-*4*-O-*methyl*-β-D-ribo-*hex*-3-*ulopyranoside {7*-*[(9*H*-9*-*carbazolylcarbonyl)*-*amino]*-*1,4,7*-*trioxaheptyl}imine* (**21**).—Reaction of **9** (10.0 mg, 0.034 mmol) and **20** (89 mg, 0.24 mmol) was performed according to the general procedure. Final purification by chromatography (1:1→1:2 *n*-hexane–EtOAc) gave **21** (*E*-isomer) as a colorless oil (7.3 mg, 36%): $[\alpha]_D^{20}$ −20° (*c* 0.6, $CHCl_3$); R_f 0.35 (1:1 *n*-hexane–EtOAc); UV: λ_{max} 230 nm (ε 5.6×10^5 dm^2/mol, MeOH); Fluorescence: λ_{exc} 290 nm, λ_{em} 346 nm [DMAc/LiCl 0.9% (m/v)]; IR (KBr): *v* 1715 (C=N); ^1H NMR ($CDCl_3$): δ 8.81 (br. s, 1 H, NH), 8.11 (d, 2 H, $J_{4,5}$ 8.3 Hz, H-5), 8.00 (d, 2 H, $J_{2,3}$ 7.7 Hz, H-2), 7.48 (ddd, 2 H, $J_{2,4}$ 1.0, $J_{3,4}$ 7.9 Hz, H-4), 7.35 (dd, 2 H, H-3), 7.30–7.20 (m, 5 H, Ph), 4.75 (m, 1 H, H-4′), 4.65 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1′), 4.44 (s, 2 H, CH_2Ph), 4.33 (t, 2 H, *J* 4.6 Hz, CH_2ON), 4.25 (t, 2 H, *J* 4.4 Hz, CH_2ON), 4.14 (m, 1 H, H-5′), 3.90–3.75 (m, 5 H, CH_2OCH_2, H-2′), 3.52–3.42 (m, 2 H, H-6a′, H-6b′), 3.35 (s, 3 H, 4′-OMe), and 3.34 (s, 3 H, 1′-OMe); ^{13}C NMR ($CDCl_3$): δ 154.16 (C=O), 149.44 (C-3′), 138.30, 138.16 (C-1, Ph), 128.77 (Ph), 128.14 (Ph), 127.98 (Ph), 127.54 (C-4), 125.83 (C-6), 123.25 (C-3), 120.44 (C-2), 114.90 (C-5), 104.73 (C-1′), 76.35 (C-5′), 76.11 (CH_2ON), 73.73 (CH_2ON, CH_2Ph), 71.62 (C-4′), 71.27 (C-2′), 70.83 (CH_2OCH_2), 70.35 (CH_2OCH_2), 70.11 (C-6′) 58.06 (4′-OMe), and 56.75 (1′-OMe); MALDI-TOF MS: *m*/*z* 631.05 [M + Na]$^+$.

Further elution gave **21** (*Z*-isomer) as a colorless oil (9.6 mg, 47%): $[\alpha]_D^{20}$ −44° (*c* 1.0, $CHCl_3$); R_f 0.25 (1:1

n-hexane–EtOAc); UV: λ_{max} 230 nm (ε 5.8×10^5 dm^2/mol, MeOH); Fluorescence: λ_{exc} 290 nm, λ_{em} 344 nm [DMAc/LiCl 0.9% (m/v)]; IR (KBr): ν 1724 (C=N); ^{1}H NMR ($CDCl_3$): δ 9.17 (br. s, 1 H, NH), 8.13 (d, 2 H, $J_{4,5}$ 8.3 Hz, H-5), 8.00 (d, 2 H, $J_{2,3}$ 7.7 Hz, H-2), 7.49 (ddd, 2 H, $J_{2,4}$ 1.2, $J_{3,4}$ 7.9, $J_{4,5}$ 8.3 Hz, H-4), 7.34 (dd, 2 H, $J_{3,5}$ 0.9 Hz, H-3), 7.30–7.20 (m, 5 H, Ph), 4.52–4.42 (m, 4 H, H-1′, H-2′, CH_2Ph), 4.36–4.27 (m, 4 H, 2 CH_2ON), 4.13 (ddd, 1 H, $J_{4',5'}$ 1.5, $J_{5',6a'}$ 8.4, $J_{5',6b'}$ 4.8 Hz, H-5′), 3.90–3.69 (m, 6 H, CH_2OCH_2, H-4′), 3.51 (dd, 1 H, $J_{6a',6b'}$ 9.9 Hz, H-6b′), 3.42 (dd, 1 H, H-6b′), 3.32 (s, 3 H, 4′-OMe), and 3.14 (s, 3 H, 1′-OMe); ^{13}C NMR ($CDCl_3$): δ 153.99, 153.76 (C=O, C-3), 137.94, 137.84 (C-1, Ph), 128.36 (Ph), 127.69 (Ph), 127.60 (Ph), 127.12 (C-4), 125.38 (C-6), 122.73 (C-3), 119.95 (C-2), 114.55 (C-5), 101.81 (C-1′), 77.56 (C-4′), 77.16 (C-5′), 75.85 (CH_2ON), 73.35 (CH_2Ph), 72.89 (CH_2ON), 69.99 (CH_2OCH_2), 69.70, 69.60 (C-6′, CH_2OCH_2), 65.53 (C-2′) 56.54 (4′-OMe), and 56.38 (1′-OMe); MALDI-TOF MS: m/z 631.54 $[M + Na]^+$. Anal. Calcd for $C_{32}H_{39}N_3O_9$: C, 63.04; H, 6.45; N, 6.89. Found: C, 63.20; H, 6.29; N, 6.68.

*Methyl 4-*O*-methyl-β-*D*-*ribo*-hex-3-ulopyranoside {7-[(9*H*-9-carbazolylcarbonyl)-amino]-1,4,7-trioxaheptyl}-imine* (**22**).—Compound **10** (7.0 mg, 0.034 mmol) and **20** (62.0 mg, 0.17 mmol) were reacted according to the general procedure. Final purification by chromatography (19:1 CH_2Cl_2–MeOH) gave **23** (*E*-isomer) as a colorless oil (4.8 mg, 27%): $[\alpha]_D^{20}$ −25° (*c* 0.4, MeOH); R_f 0.35 (19:1 CH_2Cl_2–MeOH); UV: λ_{max} 230 nm (ε 5.9×10^5 dm^2/mol, MeOH); Fluorescence: λ_{exc} 290 nm, λ_{em} 346 nm [DMAc/LiCl 0.9% (m/v)]; IR (KBr): ν 1701 (C=N); ^{1}H NMR (CD_3OD): δ 8.10–8.04 (m, 4 H, H-2, H-5), 7.49 (ddd, 2 H, $J_{3,4}$ 7.9, $J_{4,5}$ 8.3, $J_{2,4}$ 1.2 Hz, H-4), 7.34 (ddd, 2 H, $J_{2,3}$ 7.7, $J_{2,4}$ 1.0 Hz, H-3), 4.73 (dd, 1 H, H-4′), 4.71 (d, 1 H, $J_{1',2'}$ 2.9 Hz, H-1′), 4.35–4.29 (m, 2 H, CH_2ON), 4.26–4.21 (m, 2 H, CH_2ON), 3.96–3.80 (m, 6 H, CH_2OCH_2, H-2′, H-5′), 3.53 (d, 2 H, $J_{5',6'}$ 6.3 Hz, H-6′), 3.41 (s, 3 H, 1′-OMe), and 3.38 (s, 3 H, 4′-OMe); ^{13}C NMR (CD_3OD): δ 156.09 (C=O), 153.31 (C-3′), 139.47 (C-1), 127.94 (C-4), 126.46 (C-6), 123.61 (C-3), 120.96 (C-2), 115.36 (C-5), 105.34 (C-1′), 79.30 (C-5′), 76.94 (CH_2ON), 74.68 (CH_2ON), 72.04 (C-2′), 71.15 (C-4′), 70.77 (CH_2OCH_2), 63.12 (C-6′), 57.93 (4′-OMe), and 56.76 (1′-OMe); MALDI-TOF MS: m/z 540.73 $[M + Na]^+$.

Further elution gave **22** (*Z*-isomer) as a colorless oil (8.6 mg, 49%): $[\alpha]_D^{20}$ +8° (*c* 0.4, MeOH); R_f 0.29 (19:1 CH_2Cl_2–MeOH); UV: λ_{max} 230 nm (ε 5.9×10^5 dm^2/mol, MeOH); Fluorescence: λ_{exc} 290 nm, λ_{em} 346nm [DMAc/LiCl 0.9% (m/v)]; IR (KBr): ν 1706 (C=N); ^{1}H NMR (CD_3OD): δ 8.10–8.04 (m, 4 H, H-5, H-2), 7.48 (ddd, 2 H, $J_{3,4}$ 7.4, $J_{4,5}$ 7.4, $J_{2,4}$ 1.2 Hz, H-4), 7.35 (ddd, 2 H, $J_{2,3}$ 8.0, $J_{3,5}$ 1.0 Hz, H-3), 4.59 (d, 1 H, $J_{1',2'}$ 6.1 Hz, H-1′), 4.47 (d, 1 H, H-2′), 4.34 (m, 2 H, CH_2ON), 4.25 (m, 2 H, CH_2ON), 3.95–3.79 (m, 6 H, CH_2OCH_2, H-5′, H-4′), 3.57 (dd, 1 H, $J_{5',6b'}$ 5.3, $J_{6a',6b'}$ 11.4 Hz, H-6b′), 3.46 (dd, 1 H, $J_{5',6a'}$ 7.0, H-6a′), 3.42 (s, 3 H, 1′-OMe), and 3.29 (s, 3 H, 4′-OMe); ^{13}C NMR (CD_3OD): δ 156.09 (C=O), 155.03 (C-3′), 139.39 (C-1), 127.94 (C-4), 126.47 (C-6), 123.64 (C-3), 120.96 (C-2), 115.37 (C-5), 103.80 (C-1), 80.75 (C-5′), 79.04 (C-4′), 76.92 (CH_2ON), 74.79 (CH_2ON), 70.77 (CH_2OCH_2), 70.71 (CH_2OCH_2), 66.51 (C-2′), 63.10 (C-6′), 57.10 (4′-OMe), and 56.52 (1-OMe); MALDI-TOF MS: m/z 540.25 $[M + Na]^+$. Anal. Calcd for $C_{25}H_{31}N_3O_9$: C, 58.02; H, 6.04; N, 8.12. Found: C, 57.72; H, 5.93; N, 7.88.

*Methyl β-*D*-gluco-hexodialdo-1,5-pyranoside {7-[(9*H*-9-carbazolylcarbonyl)amino]-1,4,7-trioxaheptyl}-imine* (**23**).—Compound **12** (10.0 mg, 0.048 mmol) and **20** (87.0 mg, 0.24 mmol) were reacted according to the general procedure. Final purification by chromatography (93:7 CH_2Cl_2–MeOH) gave **23** (*E*-isomer) as colorless crystals (22.2 mg, 93%): mp 170–172 °C (MeOH); $[\alpha]_D^{20}$ −29° (*c* 0.3, MeOH); R_f 0.27 (93:7 CH_2Cl_2–MeOH); UV: λ_{max} 231 nm (ε 5.0×10^5 dm^2/mol, MeOH); Fluorescence: λ_{exc} 290 nm, λ_{em} 346 nm [DMAc/LiCl 0.9% (m/v)]; IR (KBr): ν 1674 (C=N); ^{1}H NMR (d_6-Me_2SO): δ 11.50 (br. s, 1 H, NH), 8.18 (d, 2 H, $J_{2,3}$ 7.7 Hz, H-2), 8.00 (d, 2 H, $J_{4,5}$ 8.4 Hz, H-5), 7.51 (dd, 2 H, $J_{3,4}$ 8.4 Hz, H-4), 7.36 (dd, 2 H, H-3), 7.36 (d, 1 H, $J_{5',6'}$ 7.3 Hz, H-6′), 5.14 (d, 2 H, *J* 4.9 Hz, 2 OH), 5.08 (d, 1 H, *J* 4.1 Hz, OH), 4.19–4.13 (m, 5 H, H-1′, 2 CH_2ON), 3.80–3.66 (m, 5 H, CH_2OCH_2, H-5′), 3.36 (s, 3 H, OMe), 3.23–3.10 (m, 2 H, H-3′, H-4′), and 3.05–2.94 (m, 1 H, H-2′); ^{13}C NMR (d_6-Me_2SO): δ 152.82 (C=O), 148.71 (C-6′), 137.66 (C-1), 126.76 (C-4), 124.13 (C-6), 122.06 (C-3), 120.18 (C-2), 114.09 (C-5), 104.03 (C-1′), 75.94 (C-3′), 75.01 (CH_2ON), 73.18 (C-5′, C-2′), 72.73 (CH_2ON), 71.57 (C-4′), 68.75 (CH_2OCH_2), 68.60 (CH_2OCH_2), and 56.18 (1′-OMe); MALDI-TOF MS: m/z 527.69 $[M + Na]^+$.

Further elution gave **23** (*Z*-isomer) as a colorless solid: (0.9 mg, 4%): R_f 0.20 (93:7 CH_2Cl_2–MeOH); UV: λ_{max} 231 nm (ε 4.5×10^5 dm^2/mol, MeOH); ^{1}H NMR (CD_3OD): δ 8.08 (d, 2 H, $J_{4,5}$ 8.5 Hz, H-5), 8.07 (d, 2 H, $J_{2,3}$ 7.9 Hz, H-2), 7.48 (dd, 2 H, $J_{3,4}$ 8.5 Hz, H-4), 7.33 (dd, 2 H, H-3), 6.70 (d, 1 H, $J_{5',6'}$ 6.8 Hz, H-6′), 4.55 (dd, 1 H, $J_{4',5'}$ 9.2 Hz, H-5′), 4.28 (t, 2 H, *J* 4.5 Hz, CH_2ON), 4.23 (t, 2 H, *J* 4.5 Hz, CH_2ON), 4.14 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 3.89 (t, 2 H, CH_2OCH_2), 3.83 (t, 2 H, CH_2OCH_2), 3.46 (s, 3 H, OMe), 3.34–3.29 (m, 1 H, H-3′), 3.24 (dd, 1 H, $J_{3',4'}$ 9.2 Hz, H-4′), and 3.12 (dd, 1 H, $J_{2',3'}$ 8.1 Hz, H-2′); ^{13}C NMR (CD_3OD): δ 153.25 (C=O), 149.33 (C-6′), 127.78 (C-4), 126.23 (C-6), 123.18 (C-3), 120.90 (C-2), 115.24 (C-5), 105.48 (C-1), 77.45 (C-3′), 76.32 (CH_2ON), 74.93 (C-2′), 74.69 (CH_2ON), 73.79 (C-4′), 70.88 (CH_2OCH_2), 70.71 (CH_2OCH_2), 69.06 (C-5′), and 57.47 (1-OMe); MALDI-TOF MS: m/z 527.53 $[M + Na]^+$. Further elution (17:3 CH_2Cl_2–MeOH) gave unchanged **20** (50 mg, 57%).

β-D-*Glucopyranosyl-(1→4)-β*-D-*glucose {7-[(9*H*-9-carbazolylcarbonyl)amino]-1,4,7-trioxaheptyl}imine* (**25**). —Cellobiose (20.0 mg, 0.058 mmol) and **20** (107 mg, 0.29 mmol) were dissolved in water (3 mL) and stirred for 7 days at rt. A satd aq $NaHCO_3$ solution (0.1 M, 0.5 mL) was added and the solution was freeze-dried. The solid residue was purified by chromatography (17:3 CH_2Cl_2–MeOH → MeOH) to give **25** as a colorless solid (28.5 mg, 75%): $[\alpha]_D^{20}$ −3° (*c* 0.4, MeOH); UV: λ_{max} 230 nm (ε 5.8×10^5 dm^2/mol, MeOH); Fluorescence: λ_{exc} 290 nm, λ_{em} 344 nm [DMAc/LiCl 0.9% (m/v)]; IR (KBr): *ν* 1696 (C=N); ^{1}H NMR (CD_3OD): δ 8.18–8.06 (m, 6.9 H, H-2, H-5), 7.64 (d, 1 H, $J_{1,2}$ 5.6 Hz, H-1′, *E*-isomer), 7.59–7.48 (m, 3.5 H, H-4), 7.44–7.36 (m, 3.5 H, H-3), 6.95 (d, 0.2 H, $J_{1,2}$ 5.5 Hz, H-1′, *Z*-isomer), 5.03 (dd, 0.3 H, $J_{1,2}$ 5.5, $J_{2,3}$ 8.0 Hz, H-2′, *Z*-isomer), 4.64–4.54 (m, 2.4 H), 4.43–4.27 (m, 7.0 H), 4.10–3.58 (m, 20.4 H), and 3.50–3.22 (m, 8.4 H); MALDI-TOF MS: *m*/*z* 676.73 $[M + Na]^+$.

Acknowledgements

Technical assistance by Maria Hobel is gratefully acknowledged. Financial support by the Christian-Doppler Research Society and Lenzing AG is gratefully acknowledged. Thomas Lange has been supported by the European Commission within the Programme 'Improving Human Research Potential and the Socio-economic base' MC-fellowship HPMF-CT-2000-00531. The authors also thank Dr. Michael Puchberger for recording NMR spectra.

References

1. Klemm, D.; Philipp, B.; Heinze, T.; Heinze, U.; Wagenknecht, W. *Comprehensive Cellulose Chemistry*; Wiley-VCH: Weinheim, Germany, 1998; pp. 302–314.
2. Green, J. W. In *Methods in Carbohydrate Chemistry*; Whistler, R. L.; Green, J. W.; Bemiller, J. N.; Wolfrom, M. L., Eds.; Academic Press: New York, 1963; Vol. 3, pp. 49–54.
3. Lewin, M.; Epstein, J. A. *J. Polym. Sci.* **1962**, *58*, 1023–1037.
4. Röhrling, J.; Potthast, A.; Rosenau, T.; Lange, T.; Borgards, A.; Sixta, H.; Kosma, P. *Synlett* **2001**, *5*, 682–684.
5. Kishimoto, T.; Nakatsubo, F.; Murakami, K. *J. Wood Chem. Technol.* **1995**, *15*, 453–471.
6. Selke, R.; Ohff, M.; Riepe, A. *Tetrahedron* **1996**, *52*, 15079–15102.
7. Marco, J. L.; Hueso-Rodriquez, J. A. *Tetrahedron Lett.* **1988**, *29*, 2459–2462.
8. Garegg, P. J.; Hultberg, J.; Wallin, S. *Carbohydr. Res.* **1982**, *108*, 97–101.
9. Bouveng, H. O.; Lindberg, B.; Theander, O. *Acta Chem. Scand.* **1957**, *11*, 1788–1789.
10. Amon, C. A.; Banwell, M. G.; Gravatt, G. L. *J. Org. Chem.* **1987**, *52*, 4851–4855.
11. Wright, M. W.; Welker, M. E. *J. Org. Chem.* **1996**, *61*, 133–141.
12. Assarsson, A.; Theander, O. *Acta Chem. Scand.* **1964**, *18*, 727–731.
13. Tsuda, Y.; Hanajima, M.; Matsuhira, N.; Okuno, Y.; Kanemitsu, K. *Chem. Pharm. Bull.* **1989**, *37*, 2344–2350.
14. Singh, S.; Nambiar, S.; Porter, R. A.; Sander, T. L.; Taylor, K. G.; Doyle, R. J. *J. Org. Chem.* **1989**, *54*, 2300–2307.
15. Mackie, I.; Röhrling, J.; Gould, R.; Pauli, J.; Jäger, C.; Walkinshaw, M.; Potthast, A.; Rosenau, T.; Kosma, P. *Carbohydr. Res.* **2002**, *337*, 161–166.
16. (a) Mancuso, A.; Swern, D. *Synthesis* **1981**, 165–185; (b) Tidwell, T. *Synthesis* **1990**, 857–870.
17. Snyder, S. L.; Vigo, T. L.; Welch, C. M. *Carbohydr. Res.* **1974**, *34*, 91–98.