



β -Elimination as major side reaction in periodate-oxidation of cellulosic model mono- and disaccharides

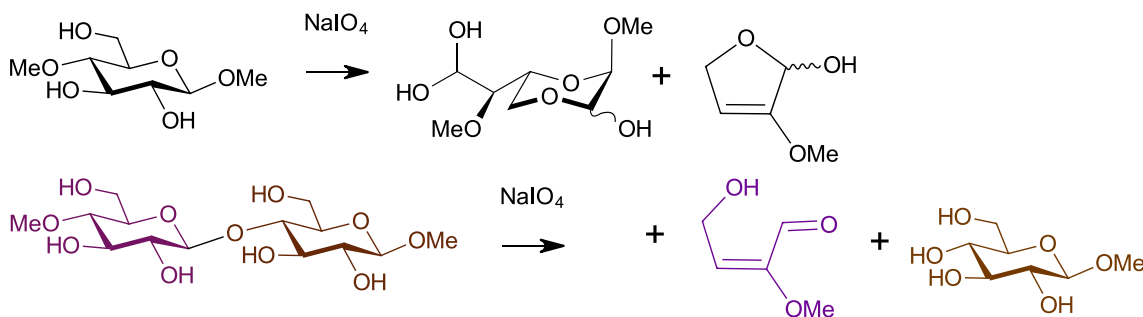
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Abstract

To structurally characterize periodate-oxidized cellulosic substrates, methyl 4-*O*-methyl β -D-glucopyranoside and methyl 4'-*O*-methyl-cellobioside were subjected to periodate treatment at pH 4.8–5.0. Oxidation of the monosaccharide using two molar equivalents of oxidant produced 3-methoxy-2,5-dihydro-2-furanol as main product. To confirm its structure and mode of formation, 6-*O*-bisdeuteromethyl 4-*O*-methyl- β -D-glucopyranoside and methyl 4-*O*-trisdeuteromethyl- β -D-glucopyranoside were synthesized and oxidized to generate 3-methoxy-5-deutero-2-hydro-2-furanol in the former case and 3-trisdeuteromethoxy-2,5-dihydro-2-furanol in the latter case. Oxidation using one molar equivalent of periodate led to preferential formation of hemialdal products and (*E*)-4-hydroxy-2-methoxy-2-butenal. The latter product was also formed upon end-wise oxidation of methyl 4'-*O*-methyl-cellobioside, wherein the reducing unit was released as non-oxidized methyl β -D-glucopyranoside. This data indicate that periodate oxidation of cellulosic model substrates might be accompanied by peeling reactions and formation of β -elimination products even under slightly acidic conditions.

Graphical abstract



Keywords Furanol · Cellulose · β -Elimination · Glycoside · Hydrolysis

Introduction

Since the early findings of Malaprade [1] and Criegee [2] to cleave 1,2-diols using the strong oxidants NaIO_4 and $\text{Pb}(\text{OAc})_4$, respectively, that lead to the formation of dialdehydes, these reactions have had a profound impact both in the structural analysis of polysaccharides (*e.g.*, Smith

degradation) as well as in the field of polysaccharide modification leading to advanced materials with numerous industrial applications [3, 4]. As examples, polysaccharides such as alginates, chitosans, xylans, starch, and cellulose in particular have been subjected to periodate oxidation resulting in a reduction of viscosity of polysaccharide solutions, enhanced biodegradability and providing reactive functional groups for further derivatization and crosslinking [5–16].

Despite several mechanistic studies, a detailed structural characterization of the “dialdehyde” units (Fig. 1) has not been fully accomplished. Since free carbonyl groups have

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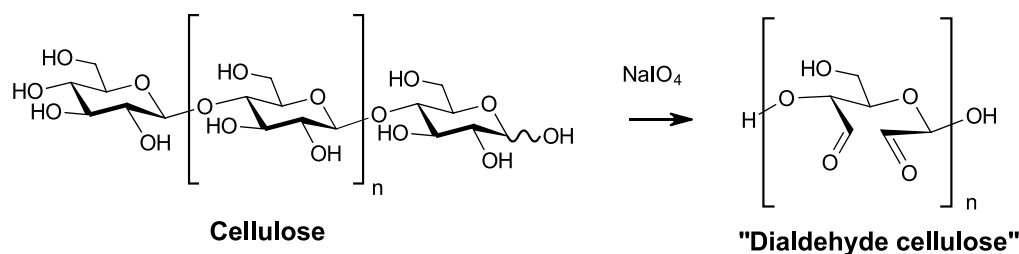


Fig. 1 General scheme for periodate oxidation of cellulose

not been detected by solid state NMR, infrared or Resonance Raman spectroscopy, the general understanding features the dialdehyde units as hydrated moieties or engaged in hemiacetal or hemialdal structures [17–22]. The structural characterization of the oxidized polysaccharides, however, is challenging due to solubility issues, high viscosity, and severe overlap of signals in their respective NMR spectra.

Recently, a thorough analytical study has been published on periodate oxidized xylans using deuterated DMSO as solvent for solution NMR experiments [23]. The authors could indeed show that the dialdehyde xylan units were present as inter-residue 1,4-dioxane-type hemialdals as inferred from low-field shifted ^{13}C NMR signals of C-3 and C-2 at 86 and 91 ppm, respectively. Very recently, a detailed NMR investigation of cellulose and oxidized celluloses solubilized in the ionic liquid ($[\text{P}_{4444}][\text{OAc}]$): DMSO- d_6 has been reported [24]. For the periodate-oxidized sample, substantial degradation was found and diffusion-edited proton NMR data indicated the presence of small mono-, di-, and oligosaccharide fragments with polymeric cellulose still remaining. In the HSQC spectrum, signals of several hemiacetal groups could be detected.

Previously, periodate oxidation of the known model compound 4-*O*-methyl β -D-glucopyranoside (**1**) representing a shortened version of the β -(1→4)-linked glucan has been studied by Thiem, albeit without experimental details of the oxidation and work-up steps [25]. The mechanism of oxidation involves the diol unit of **1** to undergo oxidation via a cyclic periodic acid diester followed by reaction of the primary alcohol at C-6 to the C-2 aldehyde group to eventually give the hemialdal reaction product **2** (Scheme 1). This

product was obtained as a mixture of *trans*- and *cis*-isomers and was fully characterized by ^1H and ^{13}C NMR spectroscopic data.

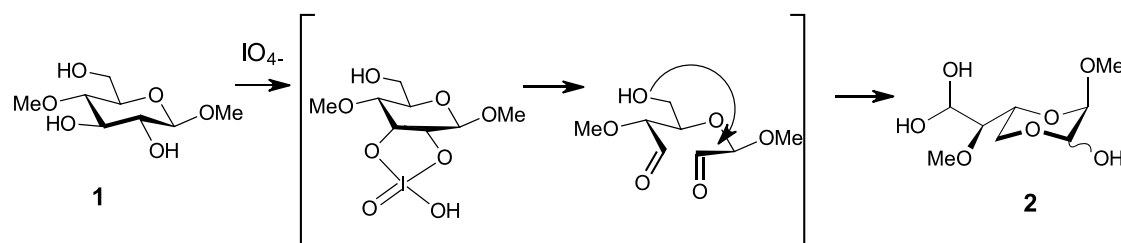
In order to obtain insight into additional potential reaction products, we have now set out to extend these model studies by using cellulosic mono- and disaccharide derivatives for the periodate oxidation under defined reaction and work-up conditions.

Results and discussion

Periodate oxidation of methyl 4-*O*-methyl- β -D-glucopyranoside (**1**) was performed using standard protocols with one or two molar equivalents of sodium metaperiodate in a sodium acetate buffer (pH 4.8–5.0) for 17 h at 0 °C temperature in the dark (Scheme 2). The reaction was then quenched by addition of ethylene glycol. The solution was concentrated and subjected to Gel-chromatography on Bio-Gel G-10. Fractions were pooled according to TLC and staining with anisaldehyde / H_2SO_4 reagent (Fig. S1).

NMR spectra of the first eluting carbohydrate-containing pool revealed a complex composition which was not further analysed (Figs. S1, S2). The later-eluting fractions (fractions 11–23, see Fig. S1), however, showed the presence of a major component of low polarity among other products. The 600 MHz ^1H NMR spectrum of **3** (Fig. 2) in CDCl_3 showed a slightly broadened triplet at 5.77 ppm coupled to the signal of an OH-group at 2.76 ppm ($J = 7.1$ Hz). A second upfield-shifted proton signal with small homonuclear coupling constants was observed at 4.89 ppm in addition to signals of

Scheme 1



Scheme 2

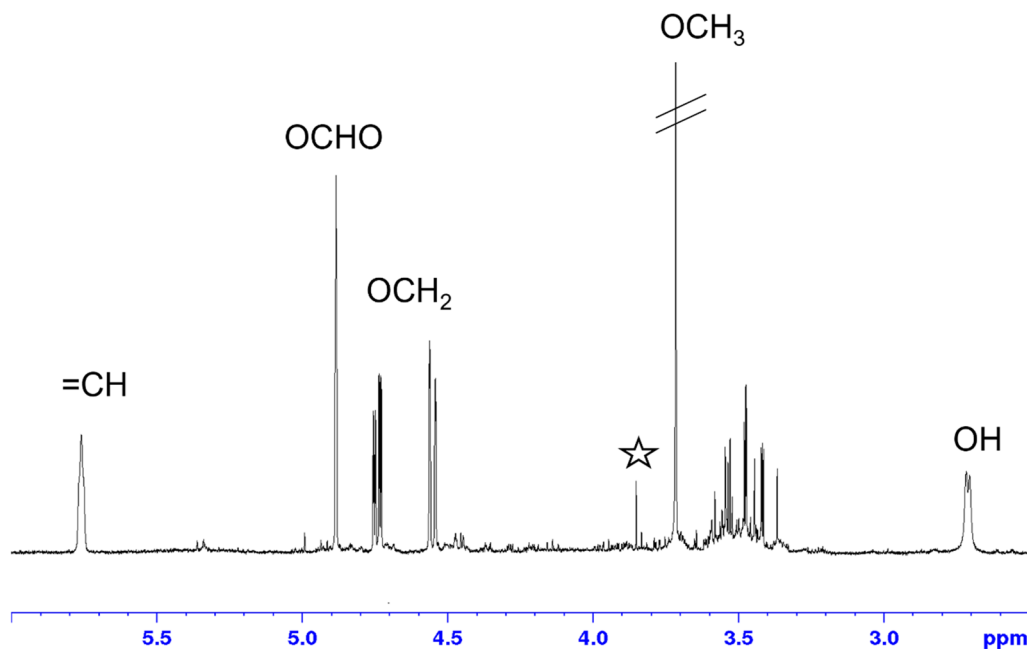
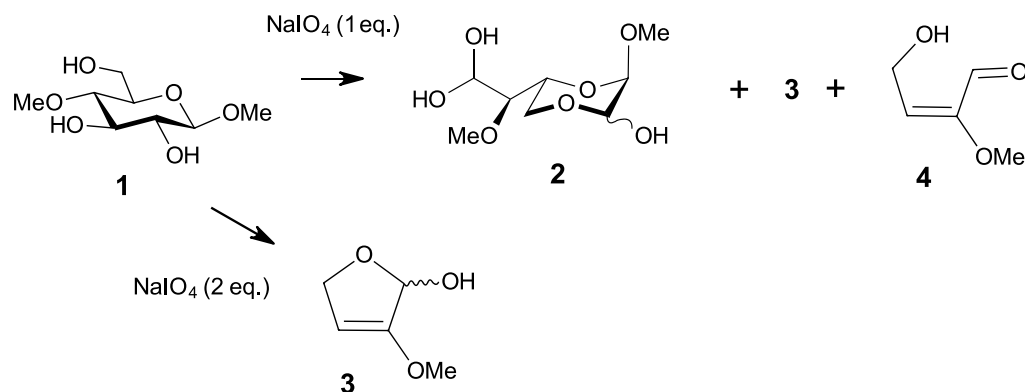


Fig. 2 Expansion plot of the 600 MHz ^1H NMR spectrum of **3** recorded in CDCl_3 . The asterisk denotes residual glycolic acid

geminal protons at 4.76 and 4.56 ppm, respectively, and a signal of a methoxy group at 3.72 ppm. The geminal protons had HSQC correlations to a ^{13}C NMR signal at 71.73 ppm, indicating that this carbon was present in an ether-type linkage. The low-field shifted proton at 5.77 ppm was correlated to a ^{13}C NMR signal at 98.86 ppm and the proton signal at 4.89 ppm to a ^{13}C NMR signal at 93.60 ppm, respectively. A quaternary carbon signal was detected at 155.9 ppm being indicative of an olefinic carbon. The structure of product was **3** was eventually assigned on the basis of HMBC correlations. The olefinic carbon at 155.9 ppm gave correlations to the OCH₂ and methoxy protons. The OCH₂ protons had additional HMBC correlations to the hemiacetal carbon at 98.86 ppm and to the carbon signal at 93.60 ppm, identifying the latter one as the second olefinic carbon. The combined

spectral evidence of **3** indicates that the structure contains an enol ether and corresponds to 3-methoxy-2,5-dihydro-2-furanol. Similar NMR features with slight differences in chemical shifts were also observed for spectra recorded in D_2O . In that case, the upfield shifted geminal proton was only partially visible due to the suppression of the HOD signal (data not shown). The structural assignments were eventually corroborated by using the CSEARCH software [26].

Additional components were also observed in these NMR spectra. A singlet at 3.85/62.50 ppm corresponded to a CH₂-group as identified from a multiplicity-edited HSQC spectrum and was found to correlate to a quaternary carbon at 180.6 ppm, being characteristic for a carboxylic acid carbon. Thus, this spectral data would be consistent with the presence of a tiny amount of glycolic acid,

probably arising from hydrolysis of the second cleavage fragment—methyl glyoxal and a subsequent Cannizzaro-type reaction (Scheme S1).

We then decided to use deuterated analogues to support the structural assignments of the furanol product **3** and the fragmentation pathway. For the synthesis of the 6-bisdeuterated methyl glucoside **11**, the known [27, 28] 6-*O* *p*-methoxybenzyl derivative **5** was methylated with NaH/methyl iodide in DMF in 91% yield followed by oxidative removal of the MBn group by treatment with DDQ to give the primary alcohol **7** in 76% yield (Scheme 3) [28]. Oxidation of **7** with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and subsequent treatment with NaOCl afforded the crude acid **8**, which was isolated as methyl ester **9** after reaction with Cs₂CO₃/CH₃I in DMF in 81% yield. Next, the ester was reduced with NaBD₄ in dry THF to give the bisdeuterated alcohol **10** in 49% yield. Hydrogenation of **10** with 10% Pd-C in MeOH afforded the deuterated model compound **11** in near theoretical yield (Scheme 3).

In addition, the 4-*O*-trisdeuteromethyl ether derivative **12** was prepared in 90% yield from alcohol **5** by reaction with NaH/CD₃I in DMF [29]. Deprotection of **12** was achieved in two steps by DDQ-promoted removal of the 6-*O*-linked *p*-methoxybenzyl group to furnish the primary alcohol **13** followed by hydrogenolysis of the benzyl ether

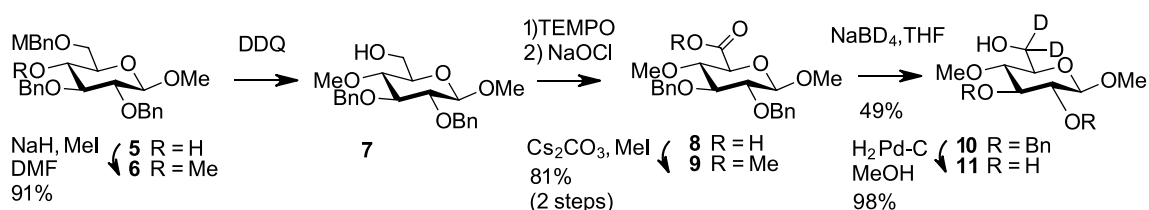
groups to give the deuterated model compound **14** in near quantitative yield (Scheme 4).

Periodate oxidation of **11** was carried out as described for **1** using two equivalents of NaIO₄ at pH 5 (Scheme 5). The NMR spectra of the pooled fractions showed the presence of **15** in a complex product mixture.

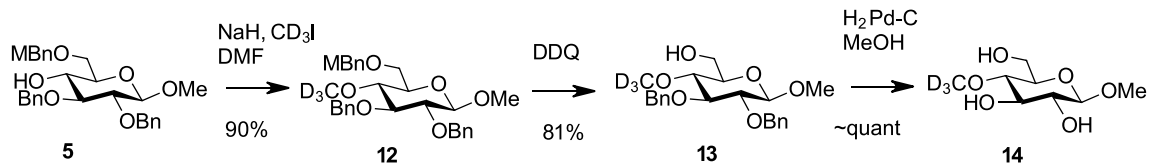
HSQC spectra—recorded in CDCl₃—showed ¹H/¹³C signals at 5.77/98.8, 4.89/93.4 and the methoxyl group at 3.72/57.7 ppm, whereas the signals of the geminal deuterons were absent in the ¹H NMR spectrum. This data confirms the loss of the carbons at the former position 1 and 2 of the glucoside. To cross-check this finding, the 4-deuteromethoxylated derivative **14** was also subjected to periodate oxidation. In the NMR spectrum of **16** the geminal protons were observed at identical shifts as in **3**, but the deuteromethoxyl group was absent in the ¹H NMR spectrum (Fig. 3).

When oxidation of **1** was performed with one molar equivalent of periodate only, the fraction containing the yellow staining product of higher TLC mobility (Fig. S3) again contained mainly the dihydrofuranol **3**, whereas the major fraction showed a complex composition. Partial assignment of the highly overlapped ¹H NMR spectrum was supported by the HSQC spectrum of the mixture allowing for the identification of the *cis*- and *trans*-isomers of hemialdal **2** as based on literature data [25] and the presence of unreacted **1** by using an authentic sample

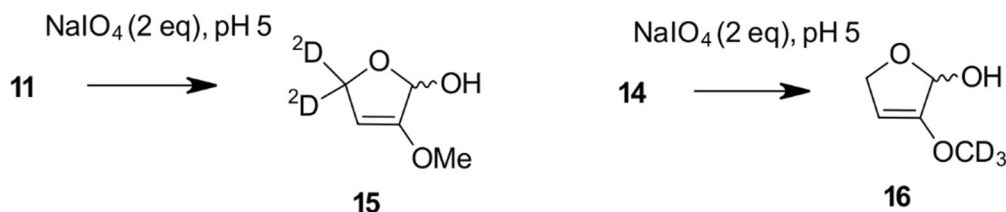
Scheme 3



Scheme 4



Scheme 5



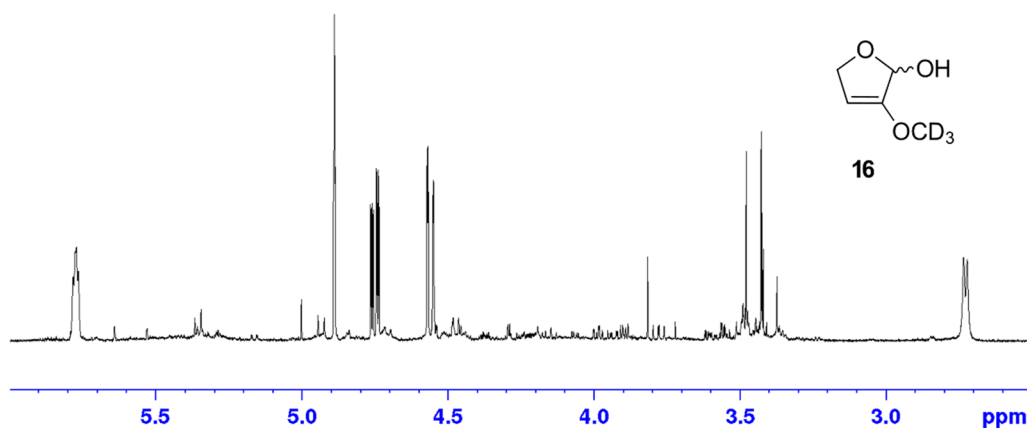


Fig. 3 Expansion plot of the 600 MHz ^1H NMR spectrum of **16** recorded in CDCl_3

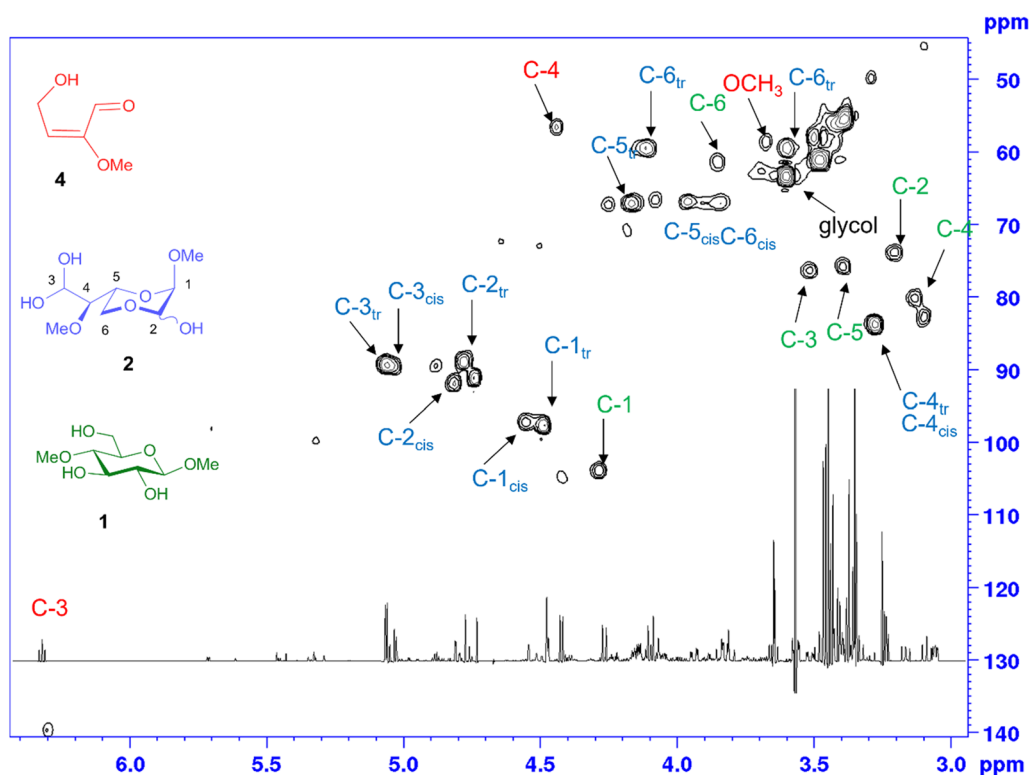


Fig. 4 Expansion plot of the multiplicity-edited HSQC spectrum of the major pool (fractions 14–21, see Fig. S3) obtained by oxidation of **1** using one molar equivalent of periodate. Signals for **1** are labeled in green, for **2** in blue and **4** in red (color figure online)

(Fig. 4). In addition, the α,β -unsaturated aldehyde **4** could be identified. The connectivity of its spin system could be tracked starting from a downfield shifted proton triplet/carbon at 6.32/139.66 ppm and a COSY correlation to a neighboring CH_2 -group at 4.43/56.78 ppm. HMBC connectivities of the proton triplet were observed to an aldehyde proton/carbon at 9.18/193.25 ppm and a second quaternary olefinic carbon at 154.7 ppm, which in turn was

correlated to a methoxy group at 3.70/58.63 ppm (Fig. 5), thereby confirming the presence of the “open-chain” product **4**. Thus, the main components of the mixture were the *cis*- and *trans*-isomers of the hemiacetal product **2** as described by Thiem as well as unreacted starting material **1** and methoxy-butenal **4** in an approximate 1: 0.3: 0.2 ratio. A singlet signal at 4.74/91.3 ppm could not be assigned but could correspond to another hemiacetal

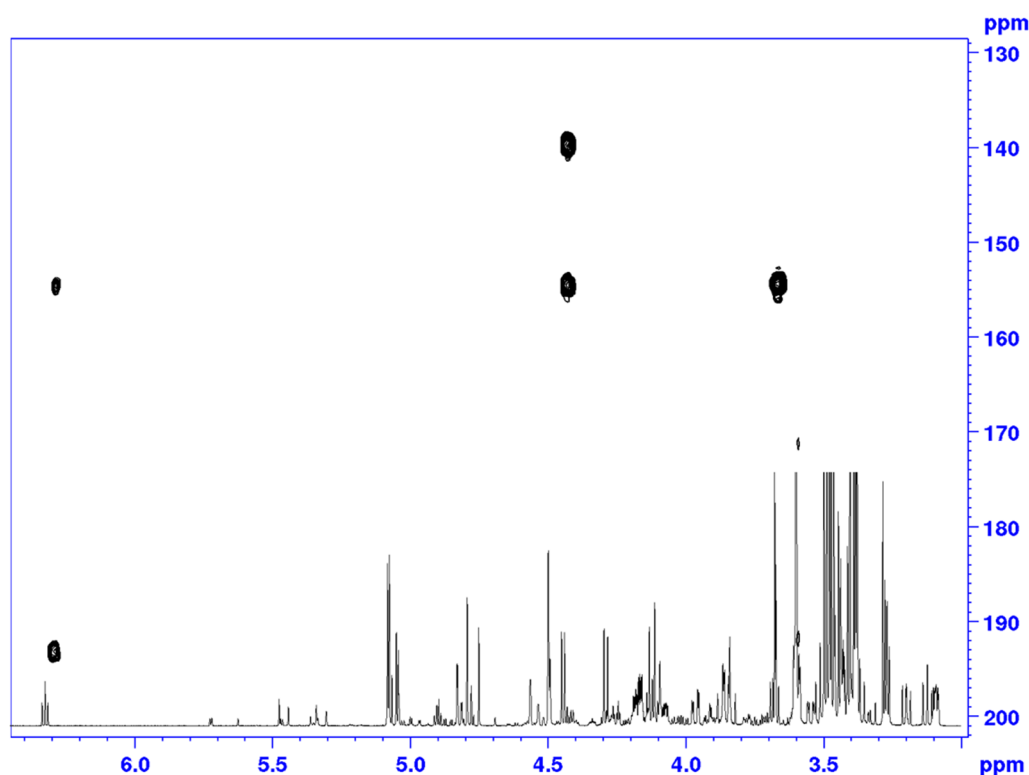


Fig. 5 Expansion plot of the HMBC spectrum displaying the connectivities of **4** in the down-field ^{13}C NMR chemical shift range

group. A putative reaction sequence for the formation of **3** and **4** is shown in the supporting information (Scheme S1).

Having established the structural characterization of the oxidation products of the monosaccharide **1**, we next moved to the known [29] model disaccharide methyl 4'-*O*-methylcellobioside **17**, expecting to obtain highly heterogeneous product mixtures. Oxidation reactions were performed using 1, 2, and 4 equivalents of NaIO_4 , respectively (Scheme 6).

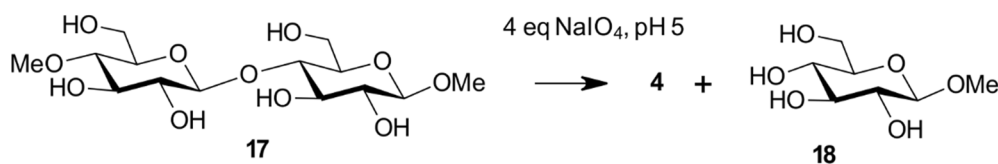
NMR spectra of the fractions obtained upon size-exclusion chromatography on Bio-Gel P2 showed a comparable composition. For one major fraction (Fig. S4), homonuclear *J*-coupling interactions in the ^1H NMR spectra showed three *trans*-oriented proton signals as expected for a β -D-glucopyranoside (Fig. 6). Eventually, an HSQC experiment identified a methyl group at 3.53/59.9 ppm and $^1\text{H}/^{13}\text{C}$ signals at 4.43/104.0, 3.88/61.6, 3.67/61.6, 3.42/76.6, 3.41/76.7, 3.33/70.5, and 3.22/73.9 ppm, respectively as H-1, H-6a, H-6b, H-3, H-5, H-4, and H-2 of methyl

β -D-glucopyranoside **18**, which was confirmed by comparison with an authentic sample. This data again indicated that bond scission had occurred at the anomeric center of the 4-*O*-methylated unit.

The second major compound was identified as 2-methoxybutenal **4** as already characterized in the reaction mixture of the oxidized monosaccharide **1** when one equivalent of NaIO_4 had been used.

When using 1 and 2 molar equivalents of oxidant, respectively, for the treatment of **17**, complex mixtures were obtained (Figs. S5, S6). In both cases (Fig. 7b, c) the main fraction again contained the methyl glucoside **18** (Fig. 7a), which was confirmed by the respective HSQC data. A small fraction obtained upon oxidation of **17** with 1 equivalent of NaIO_4 corresponded to the butenal **4** as seen from the olefinic proton at 6.32 ppm and the aldehyde signal at 9.15 ppm. No further attempt to assign the remaining highly overlapping signals was undertaken at this point.

Scheme 6



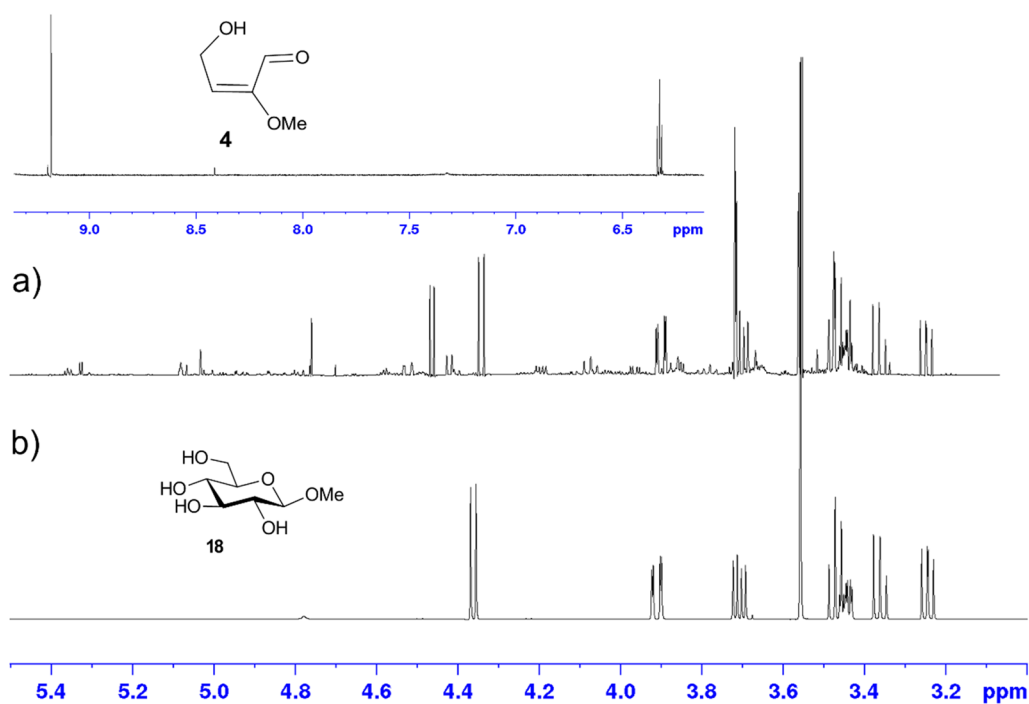


Fig. 6 Expansion plot of the 600 MHz ^1H NMR spectrum of the mixture of **18** and **4** obtained upon oxidation of methyl 4'-*O*-methyl-cellobioside using 4 molar equivalents of NaIO_4 (a) and of authen-

tic methyl 4-*O*-methyl- β -D-glucopyranoside **18** (b). The insert shows downfield-shifted signals of **4** (color figure online)

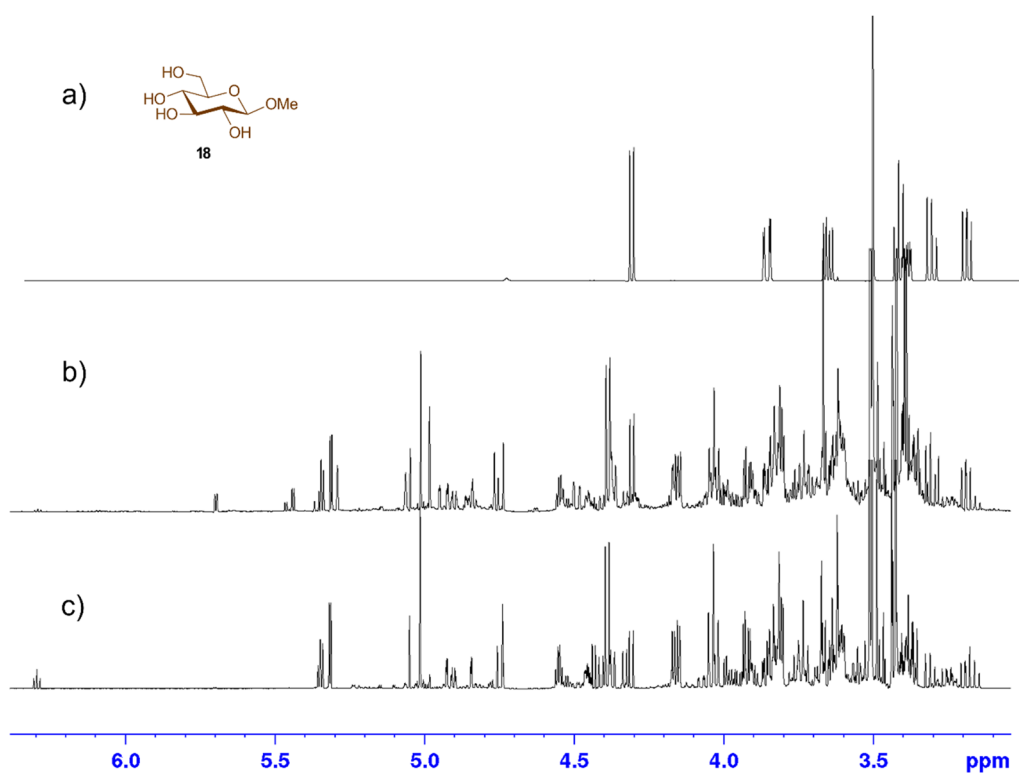


Fig. 7 600 MHz ^1H NMR expansion plots of monosaccharide **18** (a) and the main fractions obtained by oxidation of **17** with 2 equivalents of NaIO_4 (b) and 1 equivalent of oxidant (c)

Recently, it has been shown that quenching of periodate-oxidation reactions by glycol liberates copious amounts of formaldehyde [30]. We have not been able to unambiguously detect formaldehyde hydrate and oligomers [31, 32], which could be due to the workup procedure using concentration on a rotary evaporator of the Bio-Gel separated fractions.

Conclusions

In conclusion, the NMR analysis of fractions obtained upon periodate oxidation of methyl 4-*O*-methyl- β -D-glucopyranoside and methyl 4'-*O*-methyl cellobioside using varying amounts of oxidant indicated pronounced fragmentation starting from the non-reducing terminus leading to the formation of 3-*O*-methyl dihydrofuranol derivatives as products. The presence of intact methyl glucopyranoside observed in the oxidation reaction of the disaccharide model compound was an unexpected finding and indicates a preferential peeling reaction from the 4-*O*-methylated model compound. The possibility that the electronic effects of the 4-*O*-methyl ether group are responsible for this degradation, however, cannot be ruled out. Synthesis and periodate oxidation of model compounds containing an electron-withdrawing substituent at position 4 mimicking the effect of a glycosidic linkage is therefore needed. Of note, the finding that non-oxidized fragments have also been obtained would be in agreement with the findings of periodate oxidation of native celluloses [33, 34].

Experimental

Solvents and reagents were purchased from commercial suppliers and used as provided without further purification unless stated otherwise. THF and DMF were dried over activated 4 Å molecular sieves. Concentration of organic solutions was performed under reduced pressure at < 40 °C. Optical rotation was measured with an Anton Paar MCP100 Polarimeter at 20 °C and values are given in °cm² g⁻¹. Reactions were followed by thin layer chromatography using Merck plates: 5 × 10 cm, layer thickness 0.25 mm, Silica Gel 60F₂₅₄; alternatively, on HPTLC plates with 2.5 cm concentration zone (Merck). Spots were visualized with UV (254 nm) and/or anisaldehyde-H₂SO₄ staining and charring. NMR spectra were recorded with a Bruker Avance III 600 instrument (¹H at 600 MHz, ¹³C at 151 MHz) using standard Bruker NMR software. Chemical shifts are given in ppm down-field from SiMe₄ using the residual peak of CDCl₃ (7.26 for ¹H and 77.00 for ¹³C), or D₂O (0.00 for ¹H, external calibration to 2,2-dimethyl-2-silapentane-5-sulfonic acid) and 67.40 ppm for ¹³C (external calibration to 1,4-dioxane

in D₂O). HRMS ESI-TOF data were obtained on a Waters Micromass Q-TOF Ultima Global instrument.

General protocol for periodate oxidation

Periodate oxidation was performed using 1, 2, or 4 molar equivalents of sodium metaperiodate in a sodium acetate buffer (pH 4.8–5.0) for 17 h at 0 °C in the dark. The reaction was quenched by addition of ethylene glycol. The solution was then concentrated and subjected to Gel-chromatography on Sephadex G-10 or Bio-Gel P2 (column dimensions 30 × 1 cm, H₂O-EtOH 95:5). Fractions (1 cm³) were pooled and concentrated according to TLC staining with anisaldehyde / H₂SO₄.

Oxidation of 1 with 2 equivalents of NaIO₄

A solution of aq. 0.1 M NaIO₄ (4.8 cm³) was added a solution of **1** (50 mg, 0.24 mmol) in aq. sodium acetate buffer (6 cm³) for 17 h at 0 °C in the dark. The reaction was quenched by addition of 3% aq. ethylene glycol (0.5 cm³) and processed as described above to give a brownish-staining fraction (26.6 mg) and a yellow-staining fraction as an oil (8.2 mg) containing mainly **3**. ¹H NMR (CDCl₃): δ = 5.77 (br dd, 1H, *J* = 7.1 Hz, 4.1 Hz, H-2), 4.89 (br t, 1H, *J* = 1.7 Hz, H-4), 4.76 (ddd, 1H, *J* = 1.7 Hz, 4.1 Hz, 11.7 Hz, H-5a), 4.56 (ddd, 1H, *J* = 1.0 Hz, 1.7 Hz, 11.7 Hz, H-5b), 3.72 (s, 3H, OCH₃), 2.76 (br d, 1H, *J* = 7.1 Hz, OH) ppm; ¹³C NMR (CDCl₃): δ = 155.9 (C-3, observed by HMBC), 98.86 (C-2), 93.60 (C-4), 71.73 (C-5), 57.82 (OCH₃) ppm.

Oxidation of 1 with 1 equivalent of NaIO₄

The reaction was carried out as described above using **1** (30 mg, 0.144 mmol), sodium acetate buffer (1.5 cm³), and 0.1 M aq. NaIO₄ (1.44 cm³). Purification on Sephadex G-10 afforded two fractions (3.5 and 8.9 mg, respectively).

Methyl 2,3-di-*O*-benzyl-6-*O*-(*p*-methoxybenzyl)-4-*O*-methyl- β -D-glucopyranoside (6**, C₃₀H₃₆O₇)** NaH (660 mg, 1.67 mmol, 60%) was slowly added to a solution of **5** (412 mg, 0.833 mmol) in dry DMF (6.0 cm³) followed by addition of CH₃I (0.104 cm³, 1.67 mmol). The solution was stirred for 20 h at rt when MeOH (0.6 cm³) was added and stirring continued for 1 h. A satd. aq. solution of NH₄Cl was added and the mixture was extracted with EtOAc and water. The organic layer was dried with Na₂SO₄, concentrated and the residue was purified by flash column chromatography (toluene-EtOAc 6: 1) to give **6** (385 mg, 91%) as syrup. *R*_f = 0.5 (toluene-EtOAc 6: 1); [α]_D²¹ = +46.8 (*c* = 0.8, CHCl₃); NMR data agree with Ref. [28].

Methyl 2,3-di-O-benzyl-4-O-methyl- β -D-glucopyranoside (7, C₂₂H₂₈O₆) DDQ (581 mg, 2.56 mmol) was added to a solution of **6** (1.18 g, 2.33 mmol) in 18:1 DCM-H₂O (2.5 cm³) and the solution was stirred for 17 h at rt. The solution was concentrated and the residue was purified by flash column chromatography (toluene-EtOAc 1: 4) to give **7** (688 mg, 76%) as syrup. R_f =0.6 (toluene-EtOAc 1: 4); $[\alpha]^{23}_D = +36.4$ ($c=1.0$, CHCl₃); ¹H NMR (CDCl₃): δ =7.35–7.25 (m, 10H, Ph), 4.88 (d, 2H, $J=11.0$ Hz, CH₂Ph), 4.79 (d, 1H, $J=10.9$ Hz, CH₂Ph), 4.70 (d, 1H, $J=11.0$ Hz, CH₂Ph), 4.33 (d, 1H, $J_{1,2}=7.8$ Hz, H-1), 3.90 (ddd, 1H, $J_{5,6eq}=1.7$ Hz, $J_{6a,OH}=5.4$ Hz, $J_{6a,6b}=11.5$ Hz, H-6a), 3.75 (ddd, 1H, $J_{5,6b}=3.8$ Hz, $J_{6b,OH}=7.9$ Hz, $J_{6a,6b}=11.7$ Hz, H-6b), 3.57 (s, 3H, OMe), 3.56 (s, 3H, OMe), 3.56–3.53 (m, 1H, H-3), 3.35 (dd, 1H, $J_{1,2}=7.9$ Hz, $J_{2,3}=9.1$ Hz, H-2), 3.30–3.25 (m, 2H, H-4, H-5) ppm; ¹³C NMR (CDCl₃): δ =138.59, 138.48, 128.36, 128.05, 127.92, 127.67, 127.64 (Ph), 104.78 (C-1), 84.32 (C-3), 82.18 (C-2), 79.67 (C-4), 75.58 (CH₂Ph), 75.04 (C-5), 74.85 (CH₂Ph), 62.06 (C-6), 60.80 (OMe), 57.30 (OMe) ppm; HRMS: m/z calcd for C₂₂H₂₈O₆ 388.1886, found 388.1892.

Methyl methyl (2,3-di-O-benzyl-4-O-methyl- β -D-glucopyranosid)uronate (9, C₂₃H₂₈O₇) A solution of TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl (24.1 mg, 0.155 mmol) in DCM (3 cm³) was added to **7** (300 mg, 0.772 mmol) in a solution of KBr (18.3 mg, 0.154 mmol) and Bu₄N⁺Br⁻ (49.7 mg, 0.772 mmol) in satd. aq. NaHCO₃ (3 cm³). After stirring for 10 min, 13% aq. NaOCl (1.8 cm³, 3.09 mmol) was added to the reaction solution and stirring continued for 7 h at 0 °C. The reaction was quenched by addition of Na₂S₂O₃ (128 mg) followed by 2 M HCl aq. to adjust pH to 3.0. The solution was extracted with DCM and concentrated in vacuo to give the crude acid **8**. The residue (345 mg, 0.857 mmol) was dissolved in dry DMF (10 cm³) followed by addition of Cs₂CO₃ (167 mg, 0.514 mmol) and CH₃I (0.064 cm³, 1.028 mmol). The mixture was stirred overnight at rt and MeOH (2 cm³) was added. The reaction mixture was extracted with DCM, dried (Na₂SO₄), concentrated and the residue was purified by flash column chromatography (toluene-EtOAc 6: 1) to give **9** (290 mg, 81%) as syrup. R_f =0.5 (toluene-EtOAc 6: 1); $[\alpha]^{23}_D = +6.9$ ($c=0.86$, CHCl₃); ¹H NMR (CDCl₃): δ =7.35–7.25 (m, 10H, Ph), 4.86 (dd, 2H, $J=11.0$ Hz, CH₂Ph), 4.78 (d, 1H, $J=11.0$ Hz, CH₂Ph), 4.68 (d, 1H, $J=11.0$ Hz, CH₂Ph), 4.33 (d, 1H, $J_{1,2}=7.6$ Hz, H-1), 3.82 (s, 3H, OMe), 3.80 (d, 1H, $J_{4,5}=9.3$ Hz, H-5), 3.57–3.51 (m, 2H, H-3, H-4), 3.55 (s, 3H, OMe), 3.43 (dd, 1H, $J_{1,2}=7.7$ Hz, $J_{2,3}=8.8$ Hz, H-2) ppm; HRMS: m/z calcd for C₃₀H₃₆O₇ 416.1835, found 416.1853.

Methyl 2,3-di-O-benzyl-6,6-bisdeutero-4-O-methyl- β -D-glucopyranoside (10, C₂₂H₂₆D₂O₆) NaBD₄ (9.6 mg, 0.255 mmol) was added to a solution of **9** (106 mg, 0.255 mmol) in

dry THF (4.0 cm³) at rt. The solution was then stirred under reflux for 17 h, cooled to rt, before 2 M HCl was added. The solution was then coevaporated three times with MeOH and concentrated. The crude was purified by flash column chromatography (toluene-EtOAc 1: 1) to give **10** (48.8 mg, 49%) as syrup. R_f =0.5 (toluene-EtOAc 1: 1); $[\alpha]^{23}_D = +20$ ($c=0.7$, CHCl₃); ¹H NMR (CDCl₃): δ =7.39–7.24 (m, 10H, Ph), 4.90 (d, 2H, $J=11.0$ Hz, CH₂Ph), 4.75 (dd, 2H, $J=10.9$ Hz, CH₂Ph), 4.34 (d, 1H, $J_{1,2}=7.8$ Hz, H-1), 3.60–3.50 (m, 1H, H-3), 3.58 (s, 3H, OMe), 3.57 (s, 3H, OMe), 3.36 (t, 1H, $J_{1,2}=J_{2,3}=8$ Hz, H-2), 3.29 (d, 2H, $J_{3,4}=J_{4,5}=4.4$ Hz, H-4, H-5) ppm; HRMS: m/z calcd for C₃₀H₃₆O₇ 390.2011, found 390.2027.

Methyl 6,6-bisdeutero-4-O-methyl- β -D-glucopyranoside (11, C₈H₁₄D₂O₆) A suspension of **10** (48.8 mg, 0.126 mmol) and 10% Pd-carbon in dry MeOH (3 cm³) was hydrogenated at atmospheric pressure for 30 min. The suspension was filtered over Celite© and the filtrate concentrated to give **11** (26.5 mg, 98%) as syrup. $[\alpha]^{23}_D = -11.9$ ($c=1.06$, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ =4.22 (d, 1H, $J_{1,2}=7.6$ Hz, H-1), 3.65 (t, 1H, $J_{2,3}=J_{3,4}=8.8$ Hz, H-3), 3.60 (s, 3H, OMe), 3.57 (s, 3H, OMe), 3.36 (dd, 1H, H-2), 3.31 (d, 1H, H-5), 3.24 (br t, 1H, $J_{5,4}=9.4$ Hz, H-4) ppm; ¹³C NMR (75.47 MHz, CDCl₃): δ =103.49 (C-1), 79.07 (C-4), 76.35 (C-3), 75.14 (C-5), 73.97 (C-2), 60.80, 57.37 (2×OMe) ppm; HRMS: m/z calcd for C₃₀H₃₆O₇ 210.1072, found 210.1074.

Methyl 2,3-di-O-benzyl-4-O-trisdeuteromethyl-6-O-(*p*-methoxybenzyl)- β -D-glucopyranoside (12, C₃₀H₃₃D₃O₇) NaH (60%, 200 mg, 0.500 mmol) and deuterated methyl iodide (0.032 cm³, 0.500 mmol) were successively added to a solution of **5** (123.6 mg, 0.250 mmol) in dry DMF (4 cm³) and the solution was stirred for 20 h at rt. MeOH (0.5 cm³) was added and stirring continued for 1 h. Satd aq. NH₄Cl (1.0 cm³) was added and the mixture was extracted with EtOAc. The organic layer was dried with Na₂SO₄, concentrated and the remainder was purified by flash column chromatography (toluene-EtOAc 6: 1) to give **12** (114 mg, 90%) as syrup. R_f =0.5 (toluene-EtOAc 6: 1); $[\alpha]^{21}_D = +28.4$ ($c=1.0$, CHCl₃); ¹H NMR (CDCl₃): δ =7.40–7.25 (m, 12H, Ph), 6.85–6.90 (m, 2H, Ph), 4.90 (dd, 2H, $J=10.9$ Hz, 11.1 Hz, CH₂Ph), 4.74 (dd, 2H, $J=11.0$ Hz, 11.1 Hz, CH₂Ph), 4.55 (dd, 2H, $J=11.7$ Hz, CH₂Ph), 4.28 (d, 1H, H-1), 3.81 (s, 3H, PhOMe), 3.74 (dd, 1H, $J_{5,6a}=2.0$ Hz, $J_{6a,6b}=10.7$ Hz, H-6a), 3.68 (dd, 1H, $J_{5,6b}=4.6$ Hz, $J_{6a,6b}=10.8$ Hz, H-6b), 3.57 (s, 3H, 1-OMe), 3.53 (t, 1H, $J_{2,3}=J_{3,4}=8.9$ Hz, H-3), 3.39 (dd, 1H, $J_{1,2}=7.8$ Hz, $J_{2,3}=9.3$ Hz, H-2), 3.40–3.35 (m, 1H, H-5), 3.28 (t, 1H, $J_{3,4}=J_{4,5}=9.2$ Hz, H-4) ppm; ¹³C NMR (CDCl₃): δ =138.70, 138.69, 129.34, 128.33, 128.08, 127.92, 127.60, 127.58, 113.73 (Ph), 104.67 (C-1), 84.59 (C-3), 82.16 (C-2), 79.75 (C-4), 75.56 (CH₂Ph),

74.99 (C-5), 74.74 (CH₂Ph), 73.17 (CH₂Ph), 68.77 (C-6), 57.07 (1-OMe), 55.28 (PhOMe) ppm; HRMS: *m/z* calcd for C₃₀H₃₆O₇ 511.2649, found 511.2663.

Methyl 2,3-di-O-benzyl-4-O-trisdeuteromethyl-β-D-glucopyranoside (13, C₂₂H₂₅D₃O₆) DDQ (33.4 mg, 0.147 mmol) was added to a solution of **12** (62.8 mg, 0.123 mmol) in DCM-H₂O 18:1 (2 cm³) and the solution was stirred for 17 h at rt. The solution was then concentrated and the residue was purified by flash column chromatography (toluene-EtOAc 2:1) to give **13** (39.1 mg, 81%) as syrup. *R_f*=0.35 (toluene-EtOAc 2:1); [α]_D²¹ = +40.4 (*c*=0.55, CHCl₃); ¹H NMR (CDCl₃): δ=7.40–7.25 (m, 10H, Ph), 4.90 (d, 2H, *J*=10.9 Hz, CH₂Ph), 4.79 (d, 1H, *J*=11.2 Hz, CH₂Ph), 4.70 (d, 1H, *J*=11.0 Hz, CH₂Ph), 4.34 (d, 1H, *J*=7.7 Hz, H-1), 3.91 (dd, 1H, *J*_{5,6a}=4.95 Hz, *J*_{6a,6b}=11.2 Hz, H-6a), 3.80–3.70 (m, 1H, H-6b), 3.58 (s, 3H, 1-OMe), 3.60–3.50 (m, 1H, H-3), 3.36 (t, 1H, *J*_{1,2}=7.7 Hz, *J*_{2,3}=8.1 Hz, H-2), 3.32–3.25 (m, 2H, H-4, H-5) ppm; ¹³C NMR (CDCl₃): δ=138.60, 138.48, 128.36, 128.05, 127.92, 127.67, 127.64 (Ph), 104.79 (C-1), 84.33 (C-3), 82.19 (C-2), 79.56 (C-4), 75.58 (CH₂Ph), 75.04 (C-5), 74.84 (CH₂Ph), 62.06 (C-6), 57.30 (1-OMe) ppm; HRMS: *m/z* calcd for C₂₂H₂₅D₃O₆ 391.2074, found 391.2087.

Methyl 4-O-trisdeuteromethyl-β-D-glucopyranoside (14, C₈H₁₃D₃O₆) A suspension of **13** (26.9 mg, 0.0687 mmol) in dry MeOH (2 cm³) was hydrogenated in the presence of 10% Pd–C at atmospheric pressure for 30 min. The suspension was filtered over Celite and the filtrate concentrated to give **14** (0.0144 g, 99%) as syrup. *R_f*=0.05 (chloroform-MeOH 9:1); [α]_D²³ = -14.3 (*c*=1.6, CHCl₃); ¹H NMR (CDCl₃): δ=4.23 (d, 1H, *J*_{1,2}=7.9 Hz, H-1), 3.97–3.87 (m, 1H, H-6a), 3.77 (ddd, 1H, *J*_{5,6b}=3.75 Hz, *J*_{6b,OH}=7.9 Hz, *J*_{6a,6b}=11.8 Hz, H-6b), 3.65 (t, 1H, *J*_{2,3}=*J*_{3,4}=8.8 Hz, H-3), 3.57 (s, 3H, 1-OMe), 3.40–3.30 (m, 2H, H-2, H-5), 3.24 (t, 1H, *J*_{3,4}=*J*_{4,5}=9.1 Hz, H-4), 2.68 (s, 1H, OH), 2.53 (s, 1H, OH), 1.99 (dd, 1H, *J*_{6,OH}=5.6 Hz, *J*_{6,OH}=7.9 Hz, OH) ppm; ¹³C NMR (CDCl₃): δ=103.49 (C-1), 78.98 (C-4), 76.35 (C-3), 75.26 (C-5), 73.97 (C-2), 61.97 (C-6), 57.37 (1-OMe) ppm; HRMS: *m/z* calcd for C₈H₁₃D₃O₆ 234.1033, found 234.20 (LCMS).

Oxidation of 11 with 2 equivalents of NaIO₄

The reaction was carried out as described above using **11** (13.8 mg, 0.056 mmol), sodium acetate buffer (2 cm³), and 0.1 M aq. NaIO₄ (1.31 cm³). Purification on Sephadex G-10 afforded two fractions (5.2 and 3.0 mg, respectively). Selected NMR data of **15** in fraction 2: ¹H NMR (CDCl₃): δ=5.77 (br d, 1H, *J*=5.9 Hz, H-2), 4.89 (br s, 1H, H-4), 3.70 (s, 3H, OCH₃), 2.76 (br d, 1H, *J*=5.6 Hz, OH) ppm.

Oxidation of 14 with 2 equivalents of NaIO₄

The reaction was carried out as described above using **14** (15 mg, 0.071 mmol), sodium acetate buffer (2 cm³), and 0.1 M aq. NaIO₄ (1.42 cm³). Purification on Sephadex G-10 afforded two fractions (9.3 and 5.4 mg, respectively). ¹H NMR of **16** in fraction 2 (CDCl₃): δ=5.77 (br dd, 1H, *J*=7.0 Hz, 4.1 Hz, H-2), 4.89 (ddt, 1H, *J*=2×1.6 Hz, 0.7 Hz, H-4), 4.75 (ddd, 1H, *J*=1.6 Hz, 4.1 Hz, 11.7 Hz, H-5a), 4.56 (ddd, 1H, *J*=1.0 Hz, 1.6 Hz, 11.7 Hz, H-5b), 3.72 (s, 3H, OCH₃), 2.74 (br d, 1H, *J*=7.1 Hz, OH) ppm; ¹³C NMR (CDCl₃): δ=155.8 (C-3, observed by HMBC), 98.9 (C-2), 93.6 (C-4), 71.7 (C-5) ppm.

Oxidation of 17 with 4 equivalents of NaIO₄

The reaction was carried out as described above using **17** (30 mg, 0.082 mmol), sodium acetate buffer (1 cm³), and 0.1 M aq. NaIO₄ (3.2 cm³). The reaction was quenched by addition of 3% aq. ethanediol (0.33 cm³). Purification on Bio-Gel P2 afforded two fractions (26.2 and 5.6 mg, respectively). ¹H NMR of **4** in fraction 1 (D₂O): δ=9.18 (s, 1H, H-1), 6.32 (t, 1H, *J*=6.3 Hz, H-2), 4.46 (dd, 2H, *J*=6.3 Hz, H-4a, H-4b), 3.70 (s, 3H, OCH₃) ppm; ¹³C NMR of **4** (D₂O): δ=195.8 (CH=O), 154.7 [C(OCH₃)=], 139.8 (CH=), 60.9 (OCH₃), 58.4 (CH₂O) ppm. ¹H NMR of **18** in fraction 1 (D₂O): δ=4.43 (d, 1H, *J*=8.0 Hz, H-1), 3.88 (dd, 1H, *J*=2.3 Hz, *J*=12.3 Hz, H-6a), 3.67 (dd, 1H, *J*=4.9 Hz, 12.3 Hz, H-6b), 3.53 (s, 3H, OCH₃), 3.42–3.41 (m, 2H, H-3, H-5), 3.33 (t, 1H, *J*=9.5 Hz, H-4), 3.22 (dd, 1H, *J*=8.1 Hz, 9.5 Hz, H-2) ppm; ¹³C NMR of **18** in fraction 1 (D₂O): δ=104.04 (C-1), 76.72 (C-5), 76.58 (C-3), 73.91 (C-2), 70.47 (C-4), 61.57 (C-6), 57.98 (OCH₃) ppm.

Oxidation of 17 with 2 equivalents of NaIO₄

The reaction was carried out as described above using **17** (50 mg, 0.135 mmol), sodium acetate buffer (5 cm³), and 0.1 M aq. NaIO₄ (2.7 cm³). The reaction was quenched by addition of 3% aq. ethanediol (0.33 cm³). Purification on Bio-Gel P2 and pooling afforded 45.3 mg of the main fraction as a solid.

Oxidation of 17 with 1 equivalent of NaIO₄

The reaction was carried out as described above using **17** (50 mg, 0.135 mmol), sodium acetate buffer (1.5 cm³), and 0.1 M aq. NaIO₄ (1.35 cm³). The reaction was quenched by addition of 3% aq. ethanediol (0.33 cm³). Purification on

Bio-Gel P2, pooling and concentration afforded 32.1 mg of the main fraction as a solid.

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Data availability Spectral data are available from the corresponding author upon request.

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