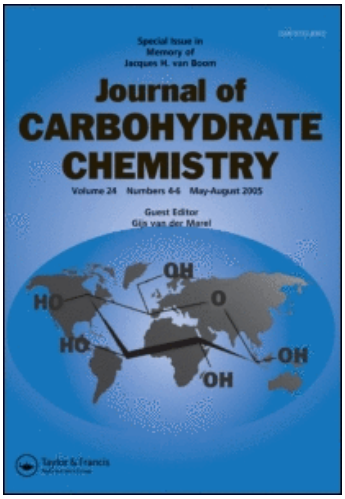

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Synthesis of a Site-Specific Deuterium Substituted Methyl β-D-Glucan Decasaccharide

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SYNTHESIS OF A SITE-SPECIFIC DEUTERIUM SUBSTITUTED METHYL β-D-GLUCAN DECASACCHARIDE

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ABSTRACT

Synthesis of a β-D-glucan decasaccharide, with alternating β-1,3 and β-1,4-linkages, is described. The decasaccharide is fully C-deuterated except for the central cellobiose residue. The main glycosylation methods used were activation of glycosyl bromides with silver triflate or activation of thioglycosides with dimethyl(methylthio)sulfonium triflate (DMTST). In order to establish the anomeric configuration of the various oligosaccharide derivatives, a fully C-protonated decasaccharide was also synthesized by a parallel route.

INTRODUCTION

Isotopic labeling or substitution of biomolecules using *e.g.* ^{2}H, ^{13}C, ^{15}N or ^{19}F greatly facilitates nuclear magnetic resonance studies of these molecules.[1] Deuterium in place of proton at site-specific positions or in specific biomolecule residues reduces the spectral overlap, increases NOE enhancements,[2] eliminates certain relaxation pathways and introduces additional probes for relaxation measurements.

C-Deuterated or tritiated carbohydrates are utilized in a wide variety of chemical and biochemical applications. Deuterium labelling has served specially as a useful NMR probe in molecular organization and dynamics studies. The syntheses of various partially deuterated oligosaccharides have been reported.[3-5] As part of a programme aimed at studying carbohydrate dynamics, synthetic site-specific deuterated oligosaccharides were needed.

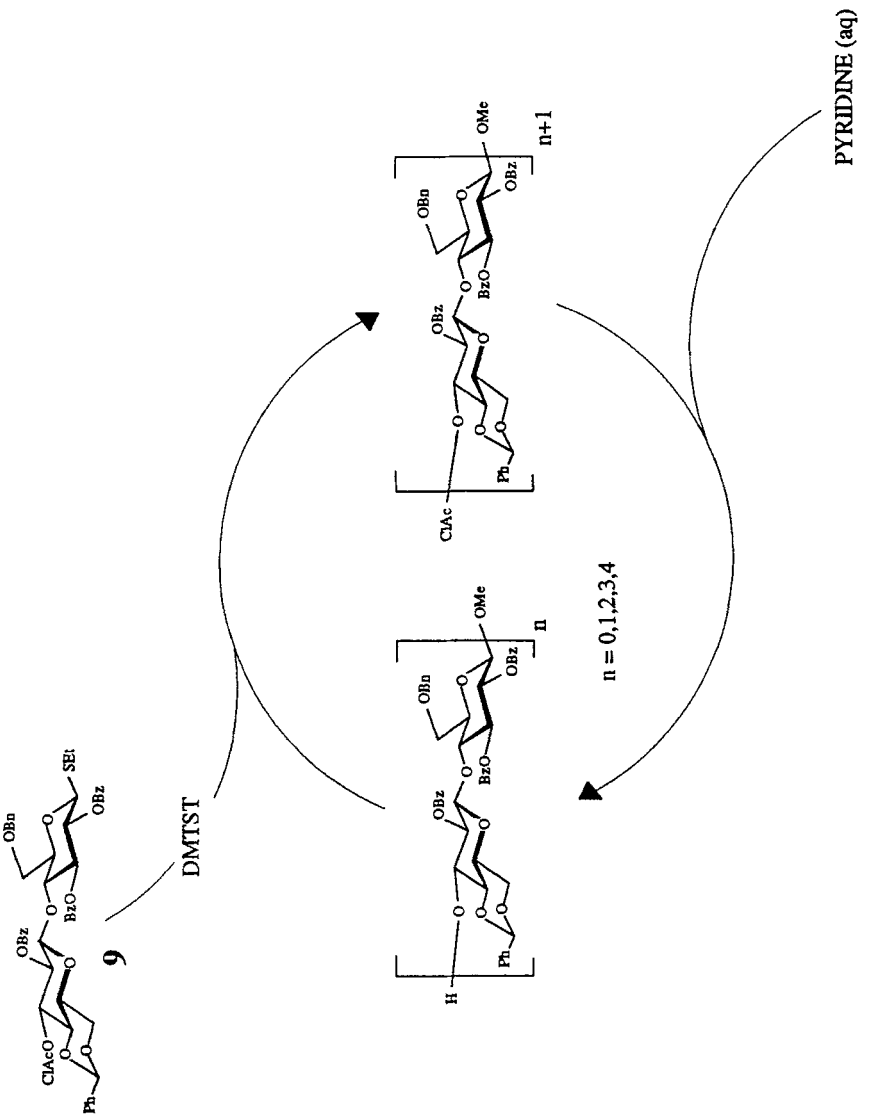
RESULTS AND DISCUSSION

The C-protonated ("cold") decasaccharide glycoside **19** was synthesized *via* a stepwise blocksynthesis using the suitably protected disaccharide building block **9** as the glycosyl donor (Scheme 1). Removal of the chloroacetyl group from the growing oligosaccharide chain, with aqueous pyridine,[6] gives a new glycosyl acceptor suitable for further DMTST-promoted[7, 8] glycosylation with disaccharide derivative **9** as the glycosyl donor. Repeating this cycle four times gives, after deprotection, the target decasaccharide glycoside **19**.

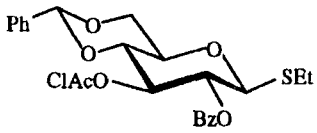
The target disaccharide derivative **9** was synthesized as follows.

The benzylidene group in ethyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside[9] was reductively opened, using sodium cyanoborohydride[10, 11] in acidified tetrahydrofuran (THF), to the corresponding 6-*O*-benzyl derivative **8** (57%) with 38% of unreacted starting material being recovered. Ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside[12] was chloroacetylated and gave **6** (95%). Compound **6** was then treated with bromine[13, 14] to give the corresponding glycosyl bromide which was immediately used in a silver triflate-promoted[15] glycosylation with **8** to produce the target disaccharide **9** (87%).

A DMTST-promoted glycosylation of disaccharide derivative **9** with methanol (Scheme 1, n = 0) gave the corresponding methyl glycoside **10** (92%). Dechloroacetylation, using aqueous pyridine, gave glycosyl acceptor **11** (68%) and subsequent DMTST-promoted glycosylation with disaccharide derivative **9** gave tetrasaccharide derivative **12** (60%). Repeating this cycle, *i.e.*, dechloroacetylation to give glycosyl acceptor **13** (68%) and subsequent DMTST-promoted glycosylation with disaccharide derivative **9**, gave



Scheme 1



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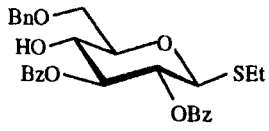

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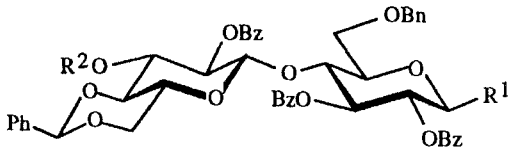


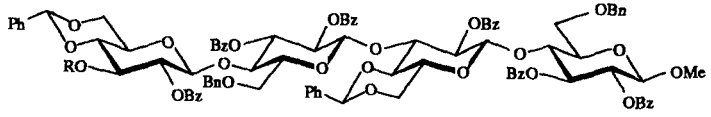
9 R^1 = SEt, R^2 = ClAc
10 R^1 = OMe, R^2 = ClAc
11 R^1 = OMe, R^2 = H

Scheme 2

hexasaccharide derivative **14** (58%). Octasaccharide derivative **16** (83%) was prepared by another cycle of dechloroacetylation to give glycosyl acceptor **15** (83%), which was subjected to DMTST-promoted glycosylation with disaccharide derivative **9**. Repeating this cycle a final time gave glycosyl acceptor **17** (70%) and the fully protected decasaccharide derivative **18** (73%). Deprotection using methanolic sodium methoxide followed by hydrogenolysis gave decasaccharide glycoside **19** (78%).

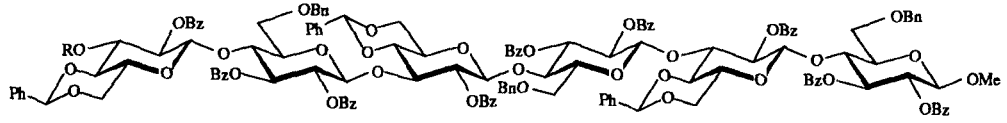
The anomeric configurations were determined either from the homonuclear $J_{H\text{-}1,H\text{-}2}$ values, extracted from 1D-spectra, or, in more complicated structures, by visual examination of the off-diagonal crosspeaks between H-1 and H-2 in H,H COSY-spectra.

Attempts to synthesize the decasaccharide glycoside **19** using a tetrasaccharide building block (with the same protecting groups as used here) and thioglycoside-mediated condensations had to be abandoned, since the tetrasaccharide glycosyl donor used gave no coupling product with hexasaccharide glycosyl acceptor **15**.



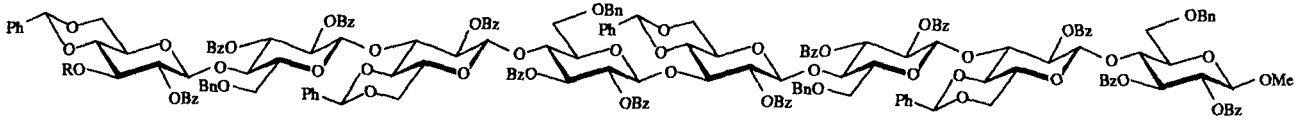
12 R = ClAc

13 R = H

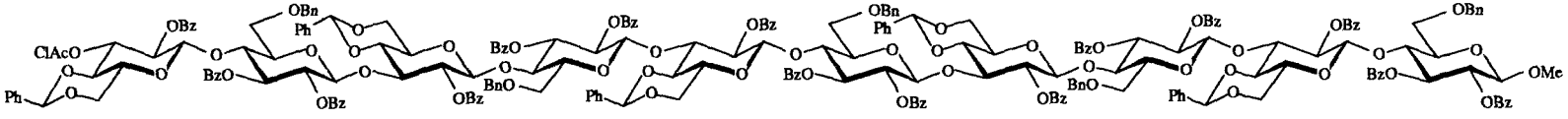


14 R = ClAc

15 R = H



16 R = ClAc
17 R = H



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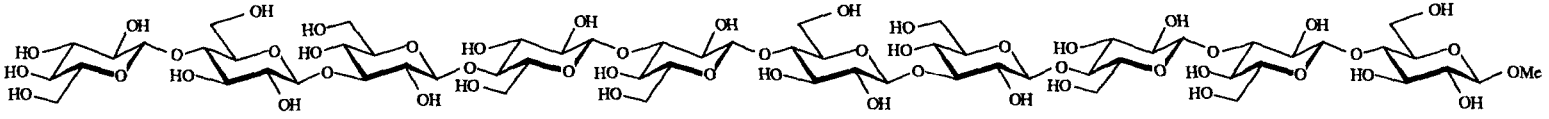


19

Scheme 3

The positions for isotopic substitution of protons with deuterons were chosen to reduce spectral overlap and to simplify NMR analysis by reducing the number of conformations needed to be taken into account in the spectral analysis. The substitution of H-3 and H-5 with deuterons results in a well separated H-4 signal. The two hydroxymethyl group protons are substituted in order to be able to disregard the different conformations of the hydroxymethyl group in the analysis. The use of the same isotopically substituted monosaccharide building block for the disaccharide, greatly simplifies the synthesis while still maintaining an appropriate model system. The site-specific deuterium substitution of the monosaccharide units of the central cellobiose residue in the C-deuterated ("hot") synthesis was performed using deuterated Raney nickel in deuterium oxide at elevated temperature, a method developed by Koch and Stuart.[16-18] 1,2-*O*-Isopropylidene-α-D-glucofuranose[19] was deuterated at C-5 and C-6 as reported earlier[20] and the deuterium exchange was monitored using a sample that was per-benzoylated, using standard methods, and purified by HPLC. The remaining well separated ^{1}H-signals from H-5, H-6 and H-6′ were quantified and refluxing in deuterium oxide was continued until deuterium exchange was >98 atom%. Removal of the isomerization products[18] obtained in the deuterium exchange process could not be satisfactorily performed until the following transformations had been performed: hydrolysis of the 1,2-*O*-isopropylidene group, peracetylation, 1-thioglycosylation, Zemplén deacetylation and benzylidenation giving **20** in 23% overall yield.

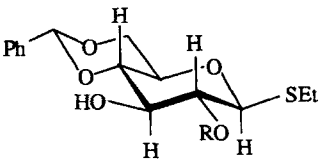
Solid-liquid phase transfer catalyzed benzoylation of **20**[21, 22] gave the 2-*O*-benzoyl derivative **21** (43%) which was oxidized to the corresponding ulose, using dimethyl sulfoxide (DMSO) / acetic anhydride (Albright-Goldman) as the oxidizing agent. The ulose was then directly reduced with sodium borodeuteride to give **22** (56%). The corresponding *allo*-epimer (14%) and the methylthiomethyl ether (5%, Pummerer product[23]) were also isolated. The deuterium content at C-3 was quantified, >98 atom%, using ^{1}H NMR. Chloroacetylation or benzoylation of **22** gave **23** and **24** (88 and 94%, respectively) and subsequent reductive opening of the benzylidene group in **24**, using sodium cyanoborohydride in acidified THF, gave the corresponding 6-*O*-benzyl derivative **25** (86%). Thioglycoside derivative **23** was converted into the corresponding glycosyl bromide upon treatment with bromine and immediately used in a silver triflate-promoted

condensation with **25** to give disaccharide derivative **26** (56%). This disaccharide was later used as the glycosyl donor in the "hot synthesis" for introduction of the central disaccharide residue. Since a reference disaccharide glycoside was needed for NMR purposes, methanol-$^2H_4$ was glycosylated with **26** to give **27** (87%), which, after conventional deblocking gave methyl glycoside **28** (92%). NMR data from the fully C-protonated analogue of this substance was published earlier[24] and confirmed the proposed structure.

Synthesis of the "hot" decasaccharide was performed using the same relative molar proportions and procedures as those described for the synthesis of the "cold" decasaccharide. The various "hot" oligosaccharide derivatives were identified by TLC, optical rotation and FAB-MS (only glycosylation products). Optical rotations were performed on uncrystallized material and references are given to "cold" analogues. The structures of compounds **20-28** and **1d-19d** are shown in Schemes 4 and 5 and all the hexose carbons substituted with the natural abundance of hydrogen are marked with H and all other hexose carbons are C-deuterated. The ^1H-spectra of glycosides **28** and **19d** are shown in Figure 1.

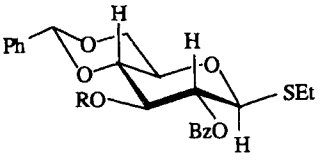
EXPERIMENTAL

General methods. Melting points are corrected. Optical rotations were recorded at room temperature (22-25 °C) using a Perkin-Elmer 241 polarimeter. NMR spectra were recorded at 25 °C for solutions in $CDCl_3$ using a JEOL GSX-270 spectrometer, and chemical shifts are given in ppm downfield from tetramethylsilane, unless otherwise stated. The spectra were invariably in accordance with postulated structures and only selected values are given below. All ^1H assignments are based on 2D experiments and within 0.01 ppm accuracy. The FAB-MS spectra were recorded using a JEOL SX102 instrument. Ions were produced by a beam of xenon atoms (4-6 keV), using a matrix consisting of glycerol, thioglycerol or *m*-nitrobenzyl alcohol. The pseudomolecular ions were identified *via* comparison of the experimental and the simulated ion cluster and all showed good agreement. Concentrations were performed at reduced pressure at a bath temperature not exceeding 40 °C. Toluene used for co-evaporation was previously dried



20 R = H

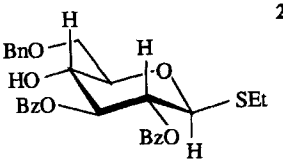
21 R = Bz



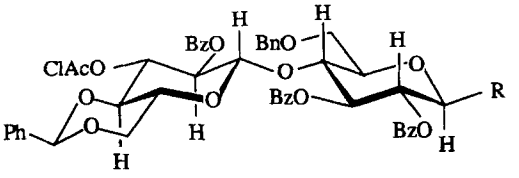
22 R = H

23 R = ClAc

24 R = Bz

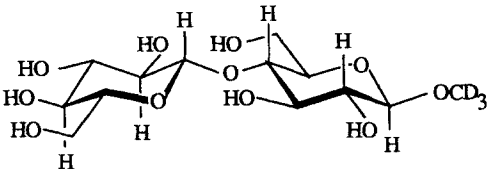


25

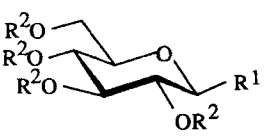


26 R = SEt

27 R = OCD_3



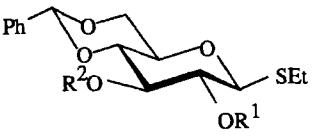
28



1d R^1 = OAc, R^2 = Ac

2d R^1 = SEt, R^2 = Ac

3d R^1 = SEt, R^2 = H



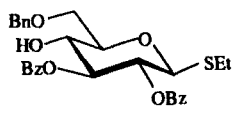
4d $R^1 = R^2$ = H

5d R^1 = Bz, R^2 = H

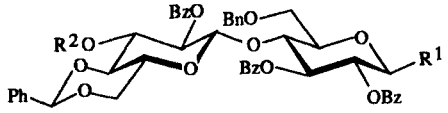
6d R^1 = Bz, R^2 = ClAc

7d R^1 = Bz, R^2 = Bz

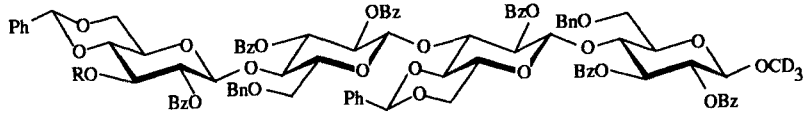
Scheme 4



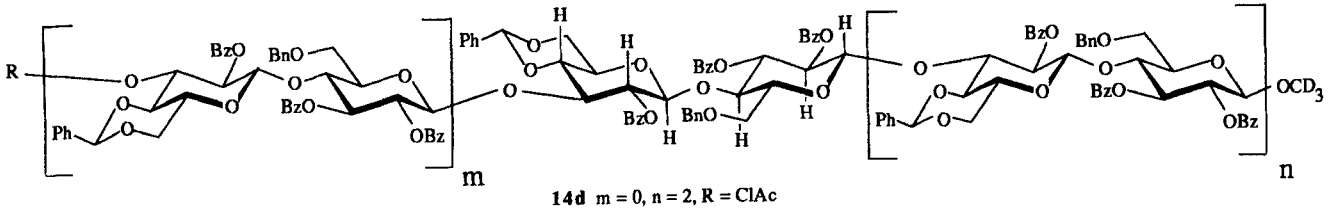
8d



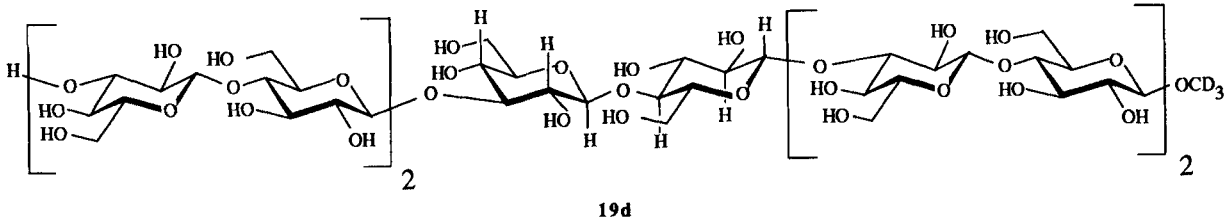
9d R^1 = SEt, R^2 = ClAc
10d R^1 = OCD_3, R^2 = ClAc
11d R^1 = OCD_3, R^2 = H



12d R = ClAc
13d R = H



14d m = 0, n = 2, R = ClAc
15d m = 0, n = 2, R = H
16d m = 1, n = 2, R = ClAc
17d m = 1, n = 2, R = H
18d m = 2, n = 2, R = ClAc



19d

Scheme 5

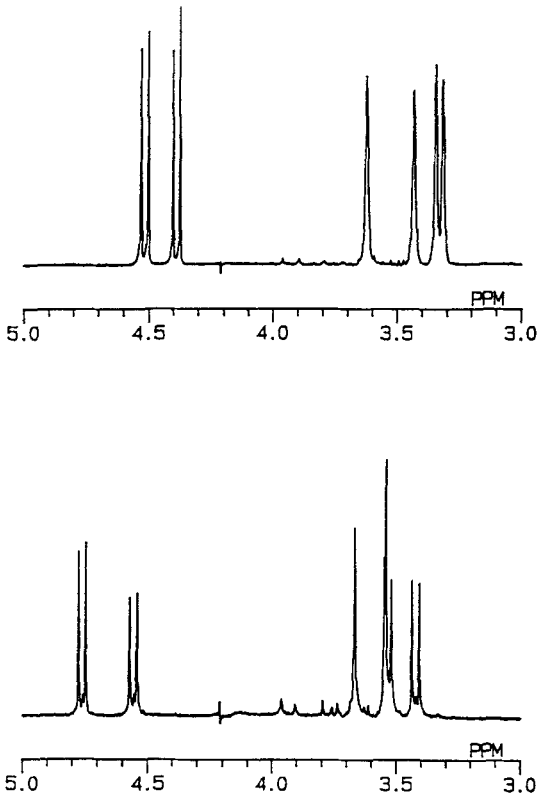


Figure 1. ^1H NMR spectra recorded at 85 °C in D_2O of disaccharide **28** (upper) and decasaccharide **19d** (lower).

over sodium wire. Column chromatography was performed on silica gel (Matrex Silica Si 60A, 35-70 μ, Amicon). Yields were not subjected to optimization procedures. Elemental analyses were performed by Analytische Laboratorien (Engelskirchen, Germany). Glucose-1,2,3,4,5,6,6′-$^2H_7$ (97.4 atom% ^2H) was purchased from MSD ISOTOPES (Montreal, Canada) and methanol-$^2H_4$ (99.95 atom% ^2H) from Dr Glaser AG (Basel, Switzerland).

Ethyl 2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-1-thio-β-D-glucopyranoside (6). Chloroacetyl chloride (370 μL, 4.6 mmol) in dichloromethane (2 mL) was added at 0 °C to a solution of ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (1.74 g, 4.2 mmol) in dichloromethane (50 mL) containing pyridine (1.0 mL). After 5 min,

methanol (1 mL) was added and stirring was continued for 5 min. Saturated aqueous sodium hydrogencarbonate was added and the organic layer was separated, dried ($MgSO_4$), filtered, concentrated to give **6** (1.96 g, 95%): mp 154-155 °C (from ethyl acetate-isooctane); $[\alpha]_{578}$ -26° (*c* 0.5, chloroform); ^{13}C NMR δ 14.9 ($SCH_2\underline{C}H_3$), 24.5 ($S\underline{C}H_2CH_3$), 40.5 ($Cl\underline{C}H_2CO$), 68.5 (C-6), 70.9 (C-5), 70.9 (C-2), 74.6 (C-3), 78.3 (C-4), 84.4 (C-1), 101.6 ($Ph\underline{C}H$), 126.2-136.7 (aromatic C), 165.4, 166.6 (benzoyl C=O, chloroacetyl C=O); ^1H NMR (*inter alia*) δ 1.23 (t, 3H, $SCH_2C\underline{H}_3$), 2.72 (m, 2H, $SC\underline{H}_2CH_3$), 3.67 (ddd, 1H, H-5), 3.81 (dd, 1H, H-6b), 3.82 (dd, 1H, H-4), 4.40 (dd, 1H, H-6a), 4.73 (d, 1H, $J_{1,2}$ = 10.1 Hz, H-1), 5.35 (dd, 1H, H-2), 5.53 (s, 1H, $PhC\underline{H}$), 5.59 (dd, 1H, H-3).

Anal. Calcd for $C_{24}H_{25}ClO_7S$: C, 58.5; H, 5.1. Found: C, 58.4; H, 5.2.

Ethyl 2,3-Di-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-glucopyranoside (8). Diethyl ether, saturated with hydrogen chloride, was added at room temperature to a stirred mixture of ethyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (11.0 g, 21.1 mmol) and sodium cyanoborohydride (2.66 g, 42.2 mmol) in tetrahydrofuran (250 mL) containing ground molecular sieves (3Å) until the mixture was acidic (determined with indicator paper). When TLC (toluene-ethyl acetate, 3:1) indicated complete reaction, triethylamine (25 mL) was added, and stirring was continued for another 5 min. The mixture was filtered through Celite, concentrated and co-evaporated once from toluene. Column chromatography (toluene-ethyl acetate, 9:1) gave **8** (6.23 g, 57%): $[\alpha]_{578}$ +59° (*c* 1.0, chloroform); 4.13 g starting material (38%) was recovered; ^{13}C NMR δ 15.0 ($SCH_2\underline{C}H_3$), 24.2 ($S\underline{C}H_2CH_3$), 70.0 (C-6), 70.4 (C-2), 70.6 (C-4), 73.7 (benzyl), 77.6 (C-3), 79.0 (C-5), 83.5 (C-1), 125.3-137.8 (aromatic C), 165.4, 167.1 (two benzoyl C=O); ^1H NMR (*inter alia*) δ 1.25 (t, 3H, $SCH_2C\underline{H}_3$), 2.74 (m, 2H, $SC\underline{H}_2CH_3$), 3.70 (ddd, 1H, H-5), 3.87 (dd, 2H, H-6), 3.97 (dd, 1H, H-4), 4.70 (d, 1H, $J_{1,2}$ = 9.3 Hz, H-1), 5.44 (dd, 1H, $J_{2,3}$ = 9.8 Hz, H-2), 5.52 (dd, 1H, H-3).

Anal. Calcd for $C_{29}H_{30}O_7S$: C, 66.6; H, 5.8. Found: C, 66.5; H, 5.8.

Ethyl 2,3-Di-*O*-benzoyl-4-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-glucopyranosyl)-6-*O*-benzyl-1-thio-β-D-glucopyranoside (9). Bromine (56 μL, 1.1 mmol) was added at 0 °C to a solution of **6** (540 mg, 1.1 mmol) in dichloromethane (2 mL). After 10 min, the solution was concentrated and the residue was co-evaporated with toluene. The residue in dichloromethane (2 mL) was added to a mixture of **8** (458 mg, 0.88 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) (180 mg, 0.88 mmol) in dichloromethane (25 mL) containing molecular sieves (4Å) and stirred for

10 min. Silver triflate (352 mg, 1.3 mmol) in toluene (1 mL) was added at 0 °C to the mixture above. After 5 min, aqueous sodium thiosulfate was added and the mixture was filtered through a layer of Celite, washed with sodium hydrogencarbonate, dried ($MgSO_4$), and concentrated. Column chromatography (toluene-ethyl acetate, 15:1) gave **9** (727 mg, 87%): mp 224-226 °C (from ethyl acetate-isooctane); $[\alpha]_{578}$ +14° (*c* 0.5, chloroform); ^{13}C NMR δ 14.9 ($SCH_2\underline{C}H_3$), 24.0 ($S\underline{C}H_2CH_3$), 40.4 ($Cl\underline{C}H_2CO$), 65.9 (C-5′), 67.3 (C-6), 67.6 (C-6′), 70.5 (C-2), 72.3 (C-2′), 73.4 (C-3′), 73.5 (benzyl), 74.5 (C-3), 75.8 (C-4), 77.7 (C-4′), 78.6 (C-5), 83.4 (C-1), 100.9 (C-1′), 101.2 ($Ph\underline{C}H$), 125.3-137.9 (aromatic C), 164.8, 165.1, 165.2, 166.5 (three benzoyl C=O, chloroacetyl C=O); ^1H NMR (*inter alia*) δ 3.19 (ddd, 1H, H-5′), 3.45 (dd, 1H, H-4′), 3.46 (dd, 1H, H-5), 3.51 (dd, 1H, H-6b), 3.62 (dd, 1H, H-6a), 3.65 (dd, 1H, H-6′b), 4.20 (dd, 1H, H-4), 4.57 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1), 4.68 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1′), 5.18 (dd, 1H, H-2′), 5.18 (s, 1H, $PhC\underline{H}$), 5.32 (dd, 1H, H-3′), 5.34 (dd, 1H, H-6′a), 5.41 (dd, 1H, H-2), 5.62 (dd, 1H, H-3).

Anal. Calcd for $C_{51}H_{49}ClO_{14}S$: C, 64.2; H, 5.2. Found: C, 64.4; H, 5.2.

Methyl 2,3-Di-*O*-benzoyl-4-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-glucopyranosyl)-6-*O*-benzyl-β-D-glucopyranoside (10). DMTST (145 mg, 0.56 mmol) was added at room temperature to a stirred solution of **9** (448 mg, 0.47 mmol), methanol (38 μL, 0.94 mmol) and DTBMP (77 mg, 0.38 mmol) in dichloromethane (20 mL) containing ground molecular sieves (3Å) under nitrogen. After 40 min, triethylamine (200 μL) was added and the solution was stirred for 5 min. Toluene (5 mL) was added and the solution was concentrated. Column chromatography (toluene-ethyl acetate, 8:1) gave **10** (399 mg, 92%): mp 231-233 °C (from ethyl acetate-isooctane); $[\alpha]_{578}$ +13° (*c* 0.6, chloroform); ^{13}C NMR δ 40.5 ($Cl\underline{C}H_2CO$), 57.0 (OMe), 66.0-77.8 (ring C, benzyl), 100.9, 101.3, 101.9 (two C-1, $Ph\underline{C}H$, $^1J_{C,H}$ = 166 Hz, 164 Hz and 161 Hz, respectively), 126.2-137.9 (aromatic C), 164.9, 165.2, 165.2, 166.5 (three benzoyl C=O, chloroacetyl C=O); ^1H NMR (*inter alia*) δ 4.46 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 4.65 (d, 1H, $J_{1,2}$ = 9.3 Hz, H-1).

Anal. Calcd for $C_{50}H_{47}ClO_{15}$: C, 65.0; H, 5.1. Found: C, 65.0; H, 5.1.

Methyl *O*-(2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside (12). 10 (500 mg, 0.59 mmol) was treated at room temperature with aqueous pyridine (80%, 10 mL) for 16 h

and then the solution was concentrated. Column chromatography (toluene-ethyl acetate, 6:1) gave methyl 2,3-di-*O*-benzoyl-4-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-6-*O*-benzyl-β-D-glucopyranoside **(11)** (338 mg, 68%): $[\alpha]_{578}$ +15° (*c* 1.0, chloroform); ^{13}C NMR δ 57.0 (OMe), 66.0-80.5 (ring C, benzyl), 101.1, 101.6, 101.8 (two C-1, PhCH), 125.3-138.1 (aromatic C), 165.3, 165.3, 165.3 (three benzoyl C=O).

DMTST (95 mg, 0.37 mmol) was added at room temperature to a stirred mixture of the latter compound (230 mg, 0.27 mmol), **9** (284 mg, 0.30 mmol), and DTBMP (45 mg, 0.22 mmol) in dichloromethane (30 mL) containing ground molecular sieves (4Å) under nitrogen. After 20 min the reaction mixture was processed as described for the preparation of **10**. Column chromatography (toluene-ethyl acetate, 5:1) gave **12** (285 mg, 60%): mp 141-143 °C (from ethyl acetate-isooctane); $[\alpha]_{578}$ +26° (*c* 0.4, chloroform); ^{13}C NMR δ 40.3 ($ClCH_2CO$), 56.8 (OMe), 65.9-79.3 (ring C, benzyl), 100.0, 100.9, 100.9, 101.2, 101.4 101.7 (four C-1, two PhCH), 125.3-138.0 (aromatic C), 163.9, 164.6, 164.7, 165.0, 165.0, 165.2, 166.5 (six benzoyl C=O, chloroacetyl C=O); ^1H NMR (*inter alia*) δ 4.39 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.47 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 4.58 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.60 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1).

Anal. Calcd for $C_{97}H_{89}ClO_{28}$: C, 67.0; H, 5.2. Found: C, 67.0; H, 5.2.

Methyl *O*-(2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside (14). **12** (124 mg, 71 μmol) was treated at room temperature with aqueous pyridine (80%, 5 mL) for 16 h and then the solution was concentrated. Column chromatography (toluene-ethyl acetate, 4:1) gave methyl *O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside (**13**) (86 mg, 90%): mp 147-149 °C (from toluene-light petroleum 40-60); $[\alpha]_{578}$ +27° (*c* 1.0, chloroform); ^{13}C NMR δ 56.9 (OMe), 65.7-80.3 (ring C, benzyl), 100.0, 100.8, 101.0, 101.3, 101.5, 101.7 (four C-1, two PhCH), 125.3-138.2 (aromatic C), 163.9, 164.7, 165.0, 165.2, 165.3 (six benzoyl C=O, partially overlapping).

Anal. Calcd for $C_{95}H_{88}O_{27}$: C, 68.7; H, 5.3. Found: C, 68.4; H, 5.3.

DMTST (10 mg, 36 μmol) was added at room temperature to a stirred mixture of the latter compound (25 mg, 15 μmol), **9** (28 mg, 30 μmol), and DTBMP (5 mg, 24 μmol) in dichloromethane (5 mL) containing ground molecular sieves (4Å) under nitrogen. After 20 min the reaction mixture was processed as described for the preparation of **10**. Column chromatography (toluene-ethyl acetate, 5:1) gave **14** (28 mg, 74%): mp >250 °C (from ethyl acetate-isooctane); $[\alpha]_{578}$ +34° (*c* 1.0, chloroform); ^{13}C NMR δ 40.5 (Cl<u>C</u>H_2CO), 57.1 (OMe), 65.9-79.5 (ring C, benzyl), 100.0, 100.1, 100.9, 101.0, 101.4, 101.5, 101.9 (six C-1, three Ph<u>C</u>H, partially overlapping), 126.1-138.3 (aromatic C), 163.9, 164.0, 164.7, 164.8, 164.8, 164.9, 165.1, 165.1, 165.4, 166.6 (nine benzoyl C=O, chloroacetyl C=O); ^{1}H NMR (*inter alia*) δ 4.38 (d, 1H, H-1), 4.43 (d, 1H, H-1), 4.43 (d, 1H, H-1), 4.53 (d, 1H, H-1), 4.56 (d, 1H, H-1), 4.56 (d, 1H, H-1).

Anal. Calcd for $C_{144}H_{131}ClO_{41}$: C, 67.7; H, 5.2. Found: C, 67.5; H, 5.2.

Methyl *O*-(2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside (16). **14** (66 mg, 26 μmol) was treated at room temperature with aqueous pyridine (80%, 5 mL) for 16 h and then the solution was concentrated. Column chromatography (toluene-ethyl acetate, 3:1) gave methyl *O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside (**15**) (53 mg, 83%): $[\alpha]_{578}$ +37° (*c* 1.0, chloroform); ^{13}C NMR δ 57.0 (OMe), 65.7-80.4 (ring C, benzyl), 100.0, 100.1, 101.0, 101.3, 101.4, 101.6, 101.8 (six C-1, three Ph<u>C</u>H, partially overlapping), 125.4-138.2 (aromatic C), 163.9, 163.9, 164.7, 164.8, 164.9, 165.1, 165.3 (nine benzoyl C=O, partially overlapping).

DMTST (60 mg, 46 μmol) was added at room temperature to a stirred mixture of the latter compound (77 mg, 31 μmol), **9** (148 mg, 155 μmol), and DTBMP (26 mg, 124 μmol) in dichloromethane (2 mL) containing ground molecular sieves (4Å) under nitrogen. After 20 min the reaction mixture

was processed as described for the preparation of **10**. Column chromatography (toluene-ethyl acetate, 9:2) followed by precipitation from toluene-light petroleum (40-60) gave **16** (87 mg, 83%): mp 166-169 °C; $[\alpha]_{578}$ +23° (*c* 1.0, chloroform). Positive ion FAB-MS showed an M+H ion at m/z 3368. ^{13}C NMR δ 40.5 ($ClCH_2CO$), 57.0 (OMe), 66.0-79.2 (ring C, benzyl), 100.0, 100.1, 100.9, 101.0, 101.3, 101.4, 101.8, (eight C-1, four PhCH, partially overlapping), 125.4-138.2 (aromatic C), 163.8-166.6 (twelve benzoyl C=O, chloroacetyl C=O, partially overlapping); ^{1}H NMR (400 MHz, *inter alia)* δ 4.37 (d, 1H, H-1), 4.39 (d, 1H, H-1), 4.41 (d, 1H, H-1), 4.43 (d, 1H, H-1), 4.49 (d, 1H, H-1), 4.52 (d, 1H, H-1), 4.56 (d, 1H, H-1), 4.56 (d, 1H, H-1).

Anal. Calcd for $C_{191}H_{173}ClO_{54}$: C, 68.1; H, 5.2. Found: C, 68.2; H, 5.3.

Methyl *O*-(2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside (18). **16** (26 mg, 7.7 μmol) was treated at room temperature with aqueous pyridine (80%, 3 mL) for 16 h and then the solution was concentrated. Column chromatography (toluene-ethyl acetate, 3:1) gave methyl *O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside **(17)** (18 mg, 70%): $[\alpha]_{578}$ +34° (*c* 0.5, chloroform); ^{13}C NMR δ 57.1 (OMe), 65.8-80.5 (ring C, benzyl), 100.1, 100.1, 101.0, 101.4, 101.4, 101.7, 101.9 (eight C-1, four PhCH, partially overlapping), 126.1-138.3 (aromatic C), 163.9-165.4 (twelve benzoyl C=O, partially overlapping).

DMTST (11 mg, 42 μmol) was added at room temperature to a stirred mixture of the latter compound (18 mg, 5.3 μmol), **9** (25 mg, 26 μmol), and DTBMP (4.4 mg, 21 mmol) in dichloromethane (2 mL) containing ground molecular sieves (4Å) under nitrogen. After 20 min the reaction mixture

was processed as described for the preparation of **10**. Column chromatography (toluene-ethyl acetate, 9:2) gave **18** (16 mg, 73%): $[\alpha]_{578}$ +35° (*c* 1.0, chloroform). Positive ion FAB-MS showed an M+H ion at m/z 4183. ^{13}C NMR δ 57.1 (OMe), 65.9-79.5 (ring C, benzyl), 100.0, 100.1, 100.9, 101.0, 101.4, 101.5, 101.9 (ten C-1, five PhCH, partially overlapping), 126.1-138.3 (aromatic C), 163.9, 164.0, 164.7, 164.8, 164.8, 164.9, 165.1, 165.1, 165.4, 166.6 (fifteen benzoyl C=O, chloroacetyl C=O, partially overlapping); ^1H NMR (400 MHz, *inter alia*) δ 4.37 (d, 1H, H-1), 4.37 (d, 1H, H-1), 4.38 (d, 1H, H-1), 4.41 (d, 1H, H-1), 4.44 (d, 1H, H-1), 4.48 (d, 1H, H-1), 4.52 (d, 1H, H-1), 4.55 (d, 1H, H-1), 4.56 (d, 1H, H-1), and one signal superimposed.

No destructive elemental analysis was performed on this precious compound.

Methyl *O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (19). **18** (17 mg, 4.0 μmol) was treated with methanolic sodium methoxide (0.2 M, 10 mL) at room temperature for 2 h. Dowex 50W-X8 (H^+ form) was added and the mixture was stirred for another 5 min. The mixture was filtered and concentrated. The product was hydrogenolyzed in ethyl acetate-ethanol-water (12:3:2, 3 mL) over 10% Pd/C at 400 kPa overnight. The product was filtered through Celite and concentrated. Column chromatography on Bio-Gel P2 (1% aqueous 1-butanol) gave amorphous **19** (5.1 mg, 78%): $[\alpha]_{578}$ -14° (*c* 0.4, water). Negative ion FAB-MS showed an M-H ion at m/z 1652. ^{13}C NMR (D_2O, 70 °C; Me_2CO δ_C at 31.0) δ 57.9 (OMe), 61.1-85.2 (ring C), 103.1, 103.3, 103.9 (ten C-1, partially overlapping).

Ethyl 4,6-*O*-Benzylidene-1-thio-β-D-glucopyranoside-5,6,6′-$^2H_3$ (20). Raney Nickel (10 mL) exchanged with deuterium oxide was added to a stirred solution of 1,2-O-isopropylidene-α-D-glucofuranose (10 g, 45.4 mmol) in deuterium oxide (100 mL) and refluxed for 5 days. The reaction mixture was allowed to attain room temperature, filtered through Celite, and concentrated to give crude 1,2-*O*-isopropylidene-α-D-glucofuranose-5,6,6′-$^2H_3$. Hydrochloric acid (0.2 M, 100 mL) was added and the mixture was heated to 60 °C for 2 h and then cooled to 0 °C. Sodium hydroxide (0.1 M) was added slowly until pH 7 and the solution was concentrated and co-evaporated once with toluene to give crude glucose-5,6,6′-$^2H_3$. Sodium

acetate (5 g) and boiling acetic anhydride (50 mL) were added and the resulting mixture was refluxed for 5 min and then poured onto crushed ice. After several hours, dichloromethane (50 mL) was added and the organic layer was separated, dried ($MgSO_4$), concentrated and co-evaporated several times with toluene to give crude 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose-5,6,6′-$^2H_3$. Boron trifluoride etherate (8 mL, 65 mmol) was added at room temperature to the residue in dichloromethane (50 mL), containing ethanethiol (3.5 mL, 50 mmol) and ground molecular sieves (4Å). When TLC (toluene-ethyl acetate, 1:1) indicated complete reaction, the reaction mixture was filtered through Celite and the filtrate was partitioned between dichloromethane and saturated aqueous sodium hydrogencarbonate. The organic layer was dried ($MgSO_4$), filtered and concentrated. Column chromatography (toluene-ethyl acetate, 9:1) gave crude ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside-5,6,6′-$^2H_3$. The residue was treated with methanolic sodium methoxide (0.2 M, 50 mL) at room temperature for 2 h. Dowex 50W-X8 (H^+ form) was added and the mixture was stirred for another 5 min. The mixture was filtered and concentrated to give crude ethyl 1-thio-β-D-glucopyranoside-5,6,6′-$^2H_3$. *p*-Toluenesulfonic acid (10 mg, cat) was added at 60 °C to a stirred solution of the residue in THF (100 mL), containing benzaldehyde dimethylacetal (4.8 mL, 32 mmol). When TLC (toluene-ethyl acetate, 1:1) indicated complete reaction, triethylamine (1 mL) was added and the mixture was concentrated. Column chromatography (chloroform-acetone, 9:1) gave **20** (3.26 g, 23%): $[\alpha]_{578}$ -47° (*c* 1.0, chloroform); lit.[9] $[\alpha]_D$ -47° (*c* 1, chloroform); ^{13}C NMR δ 15.3 ($SCH_2\underline{C}H_3$), 24.7 ($S\underline{C}H_2CH_3$), 73.3, 74.6, 80.3 (C-2, C-3, C-4), 86.5 (C-1), 101.9 (Ph$\underline{C}$H), 126.4-137.0 (aromatic C).

Ethyl 2-*O*-Benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside-5,6,6′-$^2H_3$ (21). Benzoyl chloride (772 μL, 6.66 mmol) was added at room temperature to a mixture of **20** (1.75 g, 5.55 mmol), potassium carbonate (3.84 g, 27.8 mmol) and tetrabutylammonium iodide (3.10 g, 8.32 mmol) in dichloromethane (100 mL) and stirred for 48 h. Water (100 mL) was added and the organic layer was separated, dried ($MgSO_4$) and concentrated. Column chromatography (toluene-ethyl acetate, 9:1) gave **21** (g, 43%): $[\alpha]_{578}$ -32° (*c* 1.0, chloroform); lit.[12] $[\alpha]_D$ -36.4°; ^{13}C NMR δ 14.9 ($SCH_2\underline{C}H_3$), 24.1 ($S\underline{C}H_2CH_3$), 73.0, 73.4, 80.7 (C-2, C-3, C-4), 84.0 (C-1), 101.8 (Ph$\underline{C}$H), 126.4-137.0 (aromatic C), 165.8 (benzoyl C=O).

Ethyl 2-*O*-Benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside-3,5,6,6′-$^2H_4$ (22). A solution of **21** (1.00 g, 2.38 mmol) in DMSO-acetic

anhydride (30 mL, 2:1) was stirred at room temperature for 16 h. The mixture was lyophilized and the crude product was dissolved in dichloromethane-ethyl acetate (50 mL, 1:1). Sodium borodeuteride (50 mg) was added to the stirred solution at 0 °C and after 5 min saturated aqueous ammonium chloride (20 mL) was added. When the evolution of gas ceased, the organic phase was separated, dried ($MgSO_4$) and concentrated. Column chromatography (toluene-ethyl acetate, 9:1) gave **22** (565 mg, 41%): $[\alpha]_{578}$ -30° (*c* 1.0, chloroform); lit.[12] $[\alpha]_D$ -36.4°; ^{13}C NMR δ 14.8 ($SCH_2\underline{C}H_3$), 23.9 ($S\underline{C}H_2CH_3$), 72.8, 80.3 (C-2, C-4), 83.8 (C-1), 101.5 (Ph$\underline{C}$H), 125.2-137.0 (aromatic C), 165.6 (benzoyl C=O).

Ethyl 2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-1-thio-β-D-glucopyranoside-3,5,6,6′-$^2H_4$ (23). Prepared as described for **6**. Yield: 88%; $[\alpha]_{578}$ -25° (*c* 1.0, chloroform); ^{13}C NMR δ 14.9 ($SCH_2\underline{C}H_3$), 24.5 ($S\underline{C}H_2CH_3$), 40.6 ($Cl\underline{C}H_2CO$), 70.8, 78.1 (C-2, C-4), 84.4 (C-1), 101.6 (Ph$\underline{C}$H), 126.2-136.7 (aromatic C), 165.4, 166.7 (benzoyl C=O, chloroacetyl C=O); ^1H NMR (*inter alia*) δ 1.23 (t, 3H, $SCH_2C\underline{H}_3$), 2.36 (m, 2H, $SC\underline{H}_2CH_3$), 3.81 (s, 1H, H-4), 4.74 (d, 1H, $J_{1,2}$ = 10.1 Hz, H-1), 5.35 (d, 1H, H-2), 5.53 (s, 1H, PhC$\underline{H}$).

Ethyl 2,3-Di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside-3,5,6,6′-$^2H_4$ (24). Benzoyl chloride (1 mL) was added to a stirred solution of **22** (609 mg, 1.45 mmol) in pyridine (50 mL) and heated to 60 °C for 2 h. The solution was allowed to attain room temperature and methanol (1mL) was added and the solution was concentrated and partitioned between dichloromethane and saturated aqueous sodium hydrogencarbonate. The organic layer was dried ($MgSO_4$), filtered and concentrated. Column chromatography (toluene-ethyl acetate, 6:1) gave **24** (714 mg, 94%): $[\alpha]_{578}$ +23° (*c* 0.5, chloroform); lit.[9] $[\alpha]_D$ +16° (*c* 1, chloroform); ^{13}C NMR δ 14.9 ($SCH_2\underline{C}H_3$), 24.5 ($S\underline{C}H_2CH_3$), 71.0, 78.7 (C-2, C-4), 84.5 (C-1), 101.5 (Ph$\underline{C}$H), 126.2-136.9 (aromatic C), 165.4, 165.7 (two benzoyl C=O); ^1H NMR (*inter alia*) δ 3.92 (s, 1H, H-4), 4.81 (d, 1H, $J_{1,2}$ = 10.1 Hz, H-1), 5.52 (d, 1H, H-2), 5.54 (s, 1H, PhC$\underline{H}$).

Ethyl 2,3-Di-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-glucopyranoside-3,5,6,6′-$^2H_4$ (25). Prepared as described for **8**. Yield: 86%; $[\alpha]_{578}$ +62° (*c* 1.0, chloroform); ^{13}C NMR δ 14.9 ($SCH_2\underline{C}H_3$), 24.1 ($S\underline{C}H_2CH_3$), 70.1, 70.3, 73.5 (C-2, C-4, benzyl), 83.3 (C-1), 125.2-137.8 (aromatic C), 165.3, 166.8 (two benzoyl C=O); ^1H NMR (*inter alia*) δ 3.93 (s, 1H, H-4), 4.70 (d, 1H, $J_{1,2}$ = 10.1 Hz, H-1), 5.44 (d, 1H, H-2).

Ethyl 2,3-Di-*O*-benzoyl-4-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-glucopyranosyl-3,5,6,6′-$^2H_4$)-6-*O*-benzyl-1-thio-β-D-glucopyranoside 3,5,6,6′-$^2H_4$ (26). Prepared as described for **9**. Yield: 56%; $[\alpha]_{578}$ +18° (*c* 1.0, chloroform); ^{13}C NMR δ 14.9 ($SCH_2\underline{C}H_3$), 24.1 ($S\underline{C}H_2CH_3$), 40.4 ($Cl\underline{C}H_2CO$), 70.5 (C-2), 72.3 (C-2′), 73.4 (benzyl), 75.7 (C-4), 77.6 (C-4′), 83.4 (C-1), 100.9 (C-1′), 101.2 (Ph$\underline{C}$H) 126.1-137.9 (aromatic C), 164.8, 165.2, 165.3, 166.6 (three benzoyl C=O, chloroacetyl C=O); ^1H NMR (*inter alia*) δ 3.44 (s, 1H, H-4′), 4.19 (s, 1H, H-4), 4.56 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1), 4.68 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1′), 5.17 (s, 1H, PhC$\underline{H}$), 5.20 (d, 1H, H-2′), 5.41 (d, 1H, H-2).

Methyl-$^2H_3$ 2,3-Di-*O*-benzoyl-4-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-glucopyranosyl-3,5,6,6′-$^2H_4$)-6-*O*-benzyl-β-D-glucopyranoside 3,5,6,6′-$^2H_4$ (27). Prepared as described for **10**. Yield: 87%; $[\alpha]_{578}$ +20° (*c* 1.0, chloroform); ^{13}C NMR δ 40.5 ($Cl\underline{C}H_2CO$), 71.8, 72.4, 73.5, 75.9, 77.6 (ring C, benzyl), 101.0, 101.3, 101.8 (C-1, C-1′, Ph$\underline{C}$H) 126.2-138.0 (aromatic C), 164.8, 165.3, 165.3, 166.6 (three benzoyl C=O, chloroacetyl C=O); ^1H NMR (*inter alia*) δ 3.45 (s, 1H, H-4′), 4.17 (s, 1H, H-4), 4.47 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 4.65 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1′), 5.18 (s, 1H, PhC$\underline{H}$), 5.18 (d, 1H, H-2′), 5.36 (d, 1H, H-2).

Methyl-$^2H_3$ 4-*O*-β-D-glucopyranosyl-3,5,6,6′-$^2H_4$-β-D-glucopyranoside 3,5,6,6′-$^2H_4$ (28). Prepared as described for **19**. Yield: 92%; $[\alpha]_{578}$ -15° (*c* 0.5, water). Negative ion FAB-MS showed an M-H ion at m/z 366. ^{13}C NMR (D_2O, 85 °C, Me_2CO, δ_C at 31.0) δ 70.5, 73.8, 74.2, 79.8 (ring C), 103.4, 103.9 (C-1, C-1′); ^1H NMR[24] (D_2O, 85 °C, Me_2CO, δ_H at 2.225) δ 3.33 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-2), 3.33 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-2′), 3.43 (s, 1H, H-4′), 3.62 (s, 1H, H-4), 4.39 (s, 1H, H-1), 4.52 (d, 1H, H-1′).

1,2,3,4,6-Penta-*O*-acetyl-α,β-D-glucopyranose-1,2,3,4,5,6,6′-$^2H_7$ (1d). Prepared according to reference 25. Yield: 100% (as an anomeric mixture with excess of the β-isomer); $[\alpha]_{578}$ +36° (*c* 1.0, chloroform); lit.[25] (for β-isomer) $[\alpha]_D$ +4° (chloroform); lit.[25] (for α-isomer) $[\alpha]_D$ +102° (chloroform).

Ethyl 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranoside-1,2,3,4,5,6,6′-$^2H_7$ (2d). Boron trifluoride etherate (734 μL, 6.0 mmol) was added at room temperature to a solution of crude **1d** (1.58 g, 4.0 mmol) in dichloromethane (50 mL), containing ethanethiol (324 μL, 50 mmol) and ground molecular sieves (4Å). When TLC (toluene-ethyl acetate, 1:1) indicated complete reaction, the reaction mixture was filtered through Celite and the filtrate was partitioned between dichloromethane and saturated aqueous sodium hydrogencarbonate. The organic layer was dried ($MgSO_4$), filtered and

concentrated. Column chromatography (toluene-ethyl acetate, 6:1) gave **2d** (1.34 g, 84%): $[\alpha]_{578}$ -25° (*c* 1.0, chloroform); lit.[26] $[\alpha]_D$ -22.27° (1,1,2,2-tetrachloretan).

Ethyl 1-Thio-β-D-glucopyranoside-1,2,3,4,5,6,6′-$^2H_7$ (3d). **2d** (3.89 g, 9.74 mmol) was treated with methanolic sodium methoxide (0.2 M, 10 mL) at room temperature for 2 h. Dowex 50W-X8 (H^+ form) was added and the mixture was stirred for another 5 min. The mixture was filtered and concentrated to give **3d** (2.25 g, 100%): $[\alpha]_{578}$ -19° (*c* 1.0, methanol); lit.[26] $[\alpha]_D$ -22.3°.

Ethyl 4,6-*O*-Benzylidene-1-thio-β-D-glucopyranoside-1,2,3,4,5,6,6′-$^2H_7$ (4d). *p*-Toluenesulfonic acid (10 mg, cat) was added at 60 °C to a stirred solution of **3d** (2.85 g, 12.3 mmol) in THF (100 mL), containing benzaldehyde dimethylacetal (2.8 mL, 18.5 mmol). When TLC (toluene-ethyl acetate 1:1) indicated complete reaction, triethylamine (1 mL) was added and the mixture was concentrated. Column chromatography (chloroform-acetone, 9:1) gave **4d** (2.98 g, 76%): $[\alpha]_{578}$ -41° (*c* 1.0, chloroform); lit.[9] $[\alpha]_D$ -47° (*c* 1, chloroform).

Ethyl 2-*O*-Benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside-1,2,3,4,5,6,6′-$^2H_7$ (5d). Prepared as described for **21**. Yield: 37%; $[\alpha]_{578}$ -17° (*c* 0.5, chloroform); lit.[12] $[\alpha]_D$ -36.4°.

Ethyl 2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-1-thio-β-D-glucopyranoside-1,2,3,4,5,6,6′-$^2H_7$ (6d). Prepared as described for **6**. Yield: 96%; $[\alpha]_{578}$ -25° (*c* 1.0, chloroform).

Ethyl 2,3-Di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside-1,2,3,4,5,6,6′-$^2H_7$ (7d). Prepared as described for **24**. Yield: 93%; $[\alpha]_{578}$ +16° (*c* 1.0, chloroform); lit.[9] $[\alpha]_D$ +16° (*c* 1, chloroform).

Ethyl 2,3-Di-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-glucopyranoside-1,2,3,4,5,6,6′-$^2H_7$ (8d). Prepared as described for **8**. Yield: 68%; $[\alpha]_{578}$ +62° (*c* 1.0, chloroform).

Ethyl 2,3-Di-*O*-benzoyl-4-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-6-*O*-benzyl-1-thio-β-D-glucopyranoside-1,2,3,4,5,6,6′-$^2H_7$ (9d). Prepared as described for **9**. Yield: 66%; $[\alpha]_{578}$ +18° (*c* 1.0, chloroform). Positive ion FAB-MS showed an M+H ion at m/z 967.

Methyl-$^2H_3$ 2,3-Di-*O*-benzoyl-4-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-6-*O*-benzyl-β-D-gluco-

pyranoside-1,2,3,4,5,6,6′-$^2H_7$ (10d). Prepared as described for **10**. Yield: 67%; $[\alpha]_{578}$ +13° (*c* 1.0, chloroform). Positive ion FAB-MS showed an M+H ion at m/z 940.

Methyl-$^2H_3$ *O*-(2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside-1,2,3,4,5,6,6′-$^2H_7$ (12d). Prepared as described for **12**. Dechloroacetylation gave methyl-$^2H_3$ 2,3-di-*O*-benzoyl-4-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-6-*O*-benzyl-β-D-glucopyranoside-1,2,3,4,5,6,6′-$^2H_7$ (**11d**). Yield: 95%; $[\alpha]_{578}$ +21° (*c* 1.0, chloroform). Subsequent DMTST-mediated glycosylation with **9d** gave **12d**. Yield: 88%; $[\alpha]_{578}$ +26° (*c* 1.0, chloroform). Positive ion FAB-MS showed an M+H ion at m/z 1769.

Methyl-$^2H_3$ *O*-(2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-glucopyranosyl-3,5,6,6′-$^2H_4$)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-3,5,6,6′-$^2H_4$)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside-1,2,3,4,5,6,6′-$^2H_7$ (14d). Prepared as described for **14**. Dechloroacetylation gave methyl-$^2H_3$ *O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside-1,2,3,4,5,6,6′-$^2H_7$ (**13d**). Yield: 93%; $[\alpha]_{578}$ +37° (*c* 1.0, chloroform). Subsequent DMTST-mediated glycosylation with **26** gave **14d**. Yield: 75%; $[\alpha]_{578}$ +32° (*c* 1.0, chloroform). Positive ion FAB-MS showed an M+H ion at m/z 2591.

Methyl-$^2H_3$ *O*-(2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-3,5,6,6′-$^2H_4$)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-3,5,6,6′-$^2H_4$)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-

D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside-1,2,3,4,5,6,6′-$^2H_7$ (16d). Prepared as described for **16**. Dechloroacetylation gave methyl-$^2H_3$ *O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-3,5,6,6′-$^2H_4$)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-3,5,6,6′-$^2H_4$)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl)-1,2,3,4,5,6,6′-$^2H_7$-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside-1,2,3,4,5,6,6′-$^2H_7$ (**15d**). Yield: 92%; $[\alpha]_{578}$ +33° (*c* 1.0, chloroform). Subsequent DMTST-mediated glycosylation with **9d** gave **16d**. Yield: 87%; $[\alpha]_{578}$ +35° (*c* 1.0, chloroform). Positive ion FAB-MS showed an M+H ion at m/z 3420.

Methyl-$^2H_3$ *O*-(2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-3,5,6,6′-$^2H_4$)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-3,5,6,6′-$^2H_4$)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside-1,2,3,4,5,6,6′-$^2H_7$ (18d**).** Prepared as described for **18**. Dechloroacetylation gave methyl-$^2H_3$ *O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-3,5,6,6′-$^2H_4$)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-3,5,6,6′-$^2H_4$)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→4)-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside-1,2,3,4,5,6,6′-$^2H_7$ (**17d**). Yield: 90%; $[\alpha]_{578}$ +43° (*c* 1.0, chloroform). Subsequent DMTST-mediated glycosylation with **9d** gave **18d**. Yield: 76%; $[\alpha]_{578}$ +36° (*c* 1.0, chloroform). Positive ion FAB-MS showed an M+H ion at m/z 4249.

Methyl-$^2H_3$ *O*-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$-(1→4)-*O*-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$-(1→3)-*O*-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$-

(1→4)-*O*-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$-(1→3)-*O*-β-D-glucopyranosyl-3,5,6,6′-$^2H_4$-(1→4)-*O*-β-D-glucopyranosyl-3,5,6,6′-$^2H_4$-(1→3)-*O*-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$-(1→4)-*O*-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$-(1→3)-*O*-β-D-glucopyranosyl-1,2,3,4,5,6,6′-$^2H_7$-(1→4)-β-D-glucopyranoside-1,2,3,4,5,6,6′-$^2H_7$ (19d). Prepared as described for **19**. Yield: 52%; $[\alpha]_{578}$ -23° (*c* 0.5, water). Negative ion FAB-MS showed an M-H ion at m/z 1719. ^{13}C NMR (D_2O, 85 °C, Me_2CO, δ_C at 31.0) δ 69.0, 73.8, 74.2, 79.6 (ring C), 103.1, 103.4, (two C-1); ^1H NMR (D_2O, 85 °C, Me_2CO, δ_H at 2.225) δ 3.42 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-2), 3.53 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-2), 3.54 (s, 1H, H-4), 3.66 (s, 1H, H-4), 4.55 (d, 1H, H-1), 4.76 (d, 1H, H-1).

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