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Note

Synthesis of 4-methylumbelliferyl-β-D-glucan oligosaccharides as specific chromophoric substrates of (1 → 3),(1 → 4)-β-D-glucan 4-glucanohydrolases

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Among the glycosidases, (1 → 3),(1 → 4)-β-D-glucan 4-glucanohydrolases are endo-depolymerases that catalyze the regio- and stereo-specific hydrolysis of β-D-glucans containing mixed β-(1 → 3) and β-(1 → 4) linkages. The enzymic hydrolysis of barley β-D-glucan yields the trisaccharide *O*-β-D-glucopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 3)-D-glucopyranose (**1**) and the tetrasaccharide *O*-β-D-glucopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 3)-D-glucopyranose (**2**), thus defining the specificity of cleavage at β-(1 → 4)-D-glucosidic linkages on 3-β-*O*-substituted glucopyranose residues [1–3]. Because of its importance in carbohydrate technology [4,5] the enzyme is being currently studied at the molecular level. Recent progress on the structure–function relationships involves identification of the catalytic residues by site-directed mutagenesis [6,7] and chemical modification with active site-directed inhibitors [8,9], determination of the tertiary structure by X-ray crystallography [10,11], and characterization of the stereochemical course of the enzymic cleavage of the natural substrates by ^{1}H NMR spectroscopy [12].

The methods for determination of (1 → 3),(1 → 4)-β-D-glucanase activity are based on the use of natural polymeric substrates, namely, cereal β-D-glucans, lichenan, and the

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reduced SIII pneumococcal polysaccharide, a regular polymer containing alternant (1 → 3)- and (1 → 4)-β-D-glucopyranosyl residues [3]. Detection of enzymic cleavage of the polysaccharide backbone is usually performed using viscosimetric methods [13], by determination of the amount of reducing sugars released during hydrolysis [8,14,15], or by specific complexation of high molecular weight β-D-glucans with Congo Red dye [16]. A chromogenic (dye-labeled) carboxymethylated low molecular weight β-D-glucan has also been introduced to simplify the methods of analysis for routine uses [17]. Nevertheless, a detailed kinetic and thermodynamic analysis of the mode of action of these enzymes has not been reported so far since it would require the use of low molecular weight oligosaccharides of well-defined structure.

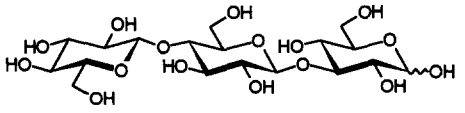
Synthetic chromophoric oligosaccharides are of widespread use as substrates and ligands to study the kinetic properties of several glycosidases, and among them the *p*-nitrophenyl, 3,4-dinitrophenyl, and 4-methylumbelliferyl glycosides have proved to be valuable tools in the studies of these enzymes. Since van Tilbeurgh et al. introduced the 4-methylumbelliferyl derivatives of cellooligosaccharides for the detection and differentiation of the components of cellulase systems [18,19], similar chromophoric oligosaccharides have been prepared and used as substrates for cellobiohydrolases (EC 3.2.1.91) [20,21], endo-(1 → 4)-β-D-glucanases (EC 3.2.1.4) [22–24], alpha-amylases (EC 3.2.1.1) [25], α-D-glucosidases (EC 3.2.1.20) [26], β-D-glucosidases (EC 3.2.1.21) [27], and xylanases (EC 3.2.1.8) [28]. There is no previous report on the synthesis of chromophoric derivatives of oligosacharides having mixed β-(1 → 3)- and β-(1 → 4)-D-glucosidic linkages, potential chromophoric substrates for (1 → 3),(1 → 4)-β-D-glucanases.

Here we describe the synthesis of a new family of low molecular weight β-D-glucan oligosaccharides of general structure [β-D-Glc*p*-(1 → 4)]$_n$-β-D-Glc*p*-(1 → 3)-β-D-Glc*p*-MeUmb where $n = 0–3$, and MeUmb = 4-methylumbelliferyl (**3a–d**). Preliminary enzymology with a *Bacillus licheniformis* β-D-glucanase [29] has shown that all these glycosides are cleaved only at one glycosidic bond, with release of the chromophoric aglycon. Thus the use of **3a–d** provides a useful, specific, and sensitive assay for (1 → 3),(1 → 4)-β-D-glucanases. Details concerning the application of these substrates in the enzymology of these enzymes will be published elsewhere.

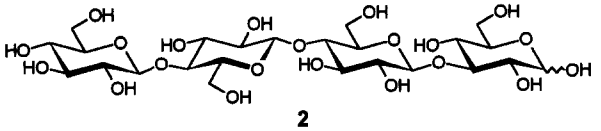
Design.—The rationale for choosing the target compounds **3a–d** as specific substrates of (1 → 3),(1 → 4)-β-D-glucanases is based on the minimum structural requirements of natural substrates to be hydrolyzed by the enzyme. They are deduced from the structure of the products of hydrolysis of barley β-D-glucan, lichenan, and reduced SIII pneumococcal polysaccharide [8], together with the lack of reaction on cellulose derivatives [β-(1 → 4)] and laminarin [β-(1 → 3) polysaccharides]. So, the scissile glycosidic bond should be adjacent to a β-(1 → 3) linkage that must be preceded by a β-(1 → 4) bond on the non-reducing end: —G(3/4)*G4G3G*—.

The simplest family of substrates according to these considerations is **3a–d**, which fulfils the following criteria: (*a*) minimum structural requirements common to all the family members, (*b*) a single scissile glycosidic bond, and (*c*) release of a chromophore upon enzymic hydrolysis.

Synthesis.—The general synthesis of **3a–d** was based on a Koenigs–Knorr type β-(1 → 3)-glycosidation, between a common monosaccharide acceptor bearing the chro-



1



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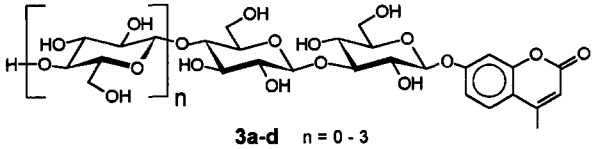
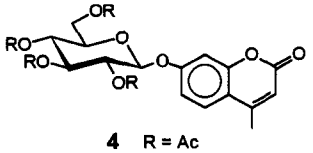


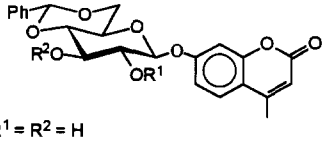
3a-d n = 0 - 3

mophoric aglycon, and activated, fully protected cellooligosaccharides from D-glucose to cellotetraose. As glycosyl acceptors, the 2-*O*-benzoyl and the 2-*O*-benzyl derivatives **7** and **10** were tested, and as glycosyl donors either the β-phenylthio derivatives **15a,b** or the α-glycosyl bromides of peracetylated cellooligosaccharides **14a–d** were used.

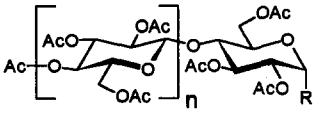
4-Methylumbelliferyl β-D-glucopyranoside (**5**) was the starting material for the preparation of glycosyl acceptors. It was synthesized from tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**14a**) and 7-hydroxy-4-methylcoumarin (4-methylumbelliferone) according to van Tilbeurgh et al. [24]. The chromophoric monosaccharides **7** and **10** having an unprotected 3-hydroxy group and, respectively, benzoyl and benzyl groups at the 2-*O* position were synthesized in two steps from the parent chromophoric glucoside **5** using standard protecting group strategies. Thus, acid-catalyzed transacetalation [30] of



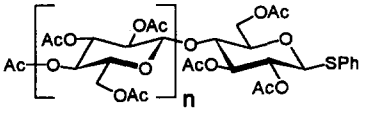
4 R = Ac
5 R = H



6 $R^1 = R^2 = H$
7 $R^1 = PhCO$, $R^2 = H$
8 $R^1 = H$, $R^2 = PhCO$
9 $R^1 = R^2 = PhCO$
10 $R^1 = PhCH_2$, $R^2 = H$
11 $R^1 = H$, $R^2 = PhCH_2$
12 $R^1 = R^2 = PhCH_2$



13a-d n = 0 - 3 R = OAc
14a-d n = 0 - 3 R = Br



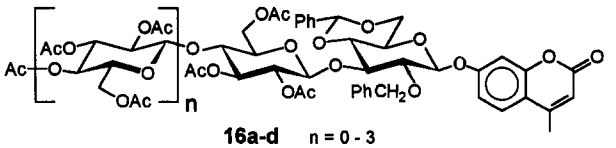
15a,b n = 0 - 1

5 with α,α-dimethoxytoluene in anhydrous benzene afforded the 4,6-benzylidene acetal **6** in 95% yield. Partial benzoylation of **6** with *N*-benzoylimidazole [31] in refluxing acetonitrile gave after chromatographic separation the 2-*O*-benzoyl (25%), 3-*O*-benzoyl (30%), and 2,3-di-*O*-benzoyl (39%) derivatives **7**, **8**, and **9**. The observed regioselectivity in the reaction was similar to that described by Hönig and Weidmann [32] in the partial benzoylation of methyl 4,6-*O*-benzylidene-β-D-glucopyranoside. On the other hand, partial benzylation of the acetal **6** under phase-transfer conditions [33], using benzyl bromide in dichloromethane, aq 5% sodium hydroxide, and tetrabutylammonium hydrogen sulfate as catalyst, was far more regioselective than the benzoylation, affording 4-methylumbelliferyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**10**) in 47% yield. The 3-*O*-benzyl and 2,3-di-*O*-benzyl derivatives **11** and **12** were also isolated in 11% and 8% yields.

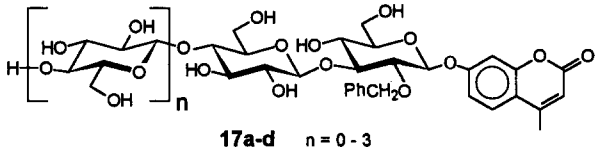
The α-glycosyl bromides **14a–d** used as glycosyl donors were synthesized in almost quantitative yield from the corresponding peracetates **13a–d** by treatment with 33% HBr in acetic acid [34] at room temperature during 30–60 min. α-D-Glucose and α-cellobiose peracetates (**13a,b**) were commercial products, while α-cellotriose and α-cellotetraose peracetates (**13c,d**) were obtained by chromatographic separation of a cellulose acetolyzate according to Wolfrom and Thompson [35,36]. A typical run afforded 9.24 g of α-cellotriose undecaacetate (**13c**) and 6.19 g of α-cellotetraose tetradecaacetate (**13d**) from 100 g of cellulose powder. Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**15a**) and phenyl 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-1-thio-β-cellobioside (**15b**) also used as glycosyl donors were prepared from α-D-glucose and α-cellobiose peracetates (**13a,b**) by treatment with thiophenol and tin tetrachloride in anhydrous benzene [37].

First attempts at glycosidation of 2,3,6,2′,3′,4′,6′,-hepta-*O*-acetyl-α-cellobiosyl bromide (**14b**) with the 2-*O*-benzoylated glycosyl acceptor **7**, using either $Hg(CN)_2$, $Hg(CN)_2/HgBr_2$, or silver triflate in dichloromethane, failed, the acceptor being recovered unchanged from the reaction mixtures in all cases. The extremely low reactivity of the glycosyl acceptor **7** was unexpected since similar glycosidation conditions have been reported by Rodriguez and Stick [38] in the successful coupling of methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside with bromide **14b**. The long-distance electron-withdrawing effect of the coumarin ring appears to be decisive for the lower nucleophilicity of the 3-hydroxy group of compound **7** with respect to the parent methyl α-D-glucopyranoside.

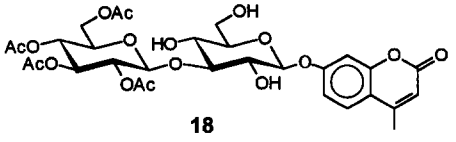
Changing the substituent at the 2-*O* position of the acceptor by a benzyl group (**10**) increased the reactivity of the vicinal 3-hydroxy group by enough to allow glycosidation. Standard Helferich [39] or methyl triflate [40] conditions were satisfactory for the successful coupling of **10** with the corresponding donors. Thus, glycosidation of **10** with the α-glycosyl bromides **14a–d**, using an equimolar mixture of $HgBr_2$ and $Hg(CN)_2$ in dichloromethane at room temperature in the presence of 4Å powdered molecular sieves, afforded the corresponding chromophoric glycosides in 71% (**16a**), 59% (**16b**), 42% (**16c**), and 33% (**16d**) yields after column chromatography. Similarly, methyl triflate glycosidation of **10** with the phenyl thioglycosides **15a,b** in dichloromethane in the presence of 4Å molecular sieves afforded the di- and tri-saccharides **16a,b** in 42% and 34% yields after chromatographic purification. Because of the slightly lower yields



16a-d n = 0 - 3



17a-d n = 0 - 3



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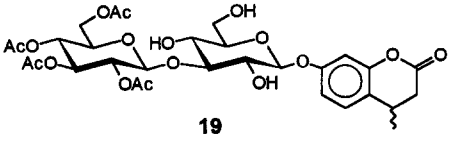

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obtained using phenyl thioglycosides as donors compared with the Helferich method using glycosyl bromides, the latter method was used for the synthesis of the higher members of the family (**16c,d**).

Two-step strategies were assayed at first for the removal of the different protecting groups of **16a–d**. Attempts at simultaneous deblocking of the 4,6-benzylidene acetal and the 2-*O*-benzyl group of **16b** by catalytic hydrogenolysis using 10% Pd–C in acetic acid gave the desired product **18** in a disappointing 38% yield, the 3,4-dihydro derivative **19** being isolated in 21% yield. Unsatisfactory results were also obtained using the sequence Zemplén transesterification–hydrogenolysis of the 4,6-benzylidene acetal and 2-benzyl ether, the starting material being recovered unchanged from the hydrogenolysis step in most cases. Finally, deprotection of the chromophoric oligosaccharides **16a–d** was performed in a satisfactory and general way using a three-step sequence: (*a*) acid hydrolysis of the 4,6-benzylidene acetal, (*b*) Zemplén transesterification, and (*c*) hydrogenolysis of the 2-benzyl ether. Hydrolysis of the 4,6-benzylidene acetal using refluxing 60% acetic acid followed by deacetylation with methanolic sodium methoxide afforded the 2-*O*-benzyl derivatives **17a–d**, which crystallized directly from the reaction mixtures as essentially pure materials in 72%–88% yields. The hydrogenolysis conditions with regard to solvent composition and temperature were adjusted to avoid reduction of the 3,4-double bond of the 4-methylumbelliferyl chromophore. Thus, hydrogenolysis of the 2-benzyl ether using 10% Pd–C in a mixture of ethanol–water–tetrahydrofuran (1:1:1; except for **17c**, 2:1:2.2) at 30°C afforded the final chromophoric oligosaccharides **3a–d** in 66–71% yields after one crystallization from ethanol–water.

The structures of the final products, their purity, and the anomeric configuration of the newly formed glycosidic bonds were confirmed by NMR data. The anomeric region of the ^{1}H NMR spectra of **3b–d** in Me_2SO-d_6 showed a highly deshielded signal at δ 5.14 (J 8 Hz) for H-1A (δ 5.17 for **3a**), a characteristic doublet for H-1B at δ 4.47 (δ

4.37 for **3a**) with J 8 Hz, thus confirming the β configuration of the (1 → 3) bond formed in the glycosidation step, and a set of doublets at δ 4.23–4.32 ($J \approx 8$ Hz) for the anomeric (1 → 4) protons of **3c–d**. The ^{13}C NMR spectra of **3a–d** in Me_2SO-d_6 showed a set of signals corresponding to the anomeric carbon atoms at δ 99.8 ± 0.1 (C-1A) and δ 103.6 ± 0.5 (C-1B,C,D), and deshielded signals for C-3A of **3a–d** (δ 86.9 ± 0.2), C-4B of **3b–d** (δ 80.3 ± 0.2), C-4C of **3c–d** (δ 80.3 ± 0.1), and C-4D of **3d** (δ 80.3). All ^1H and ^{13}C resonances were assigned on the basis of the 1D ^1H NMR and ^{13}C NMR and 2D COSY or DQF-COSY, TOCSY, and HMQC spectra. Figs. 1a and 1b show the partial 500-MHz COSY and TOCSY spectra of **3b** in the δ 2.8–5.3 region. Coherence transfer in the 2D TOCSY experiment between the Glc$p$ protons was achieved by isotropic mixing using a mixing time of 70 ms. Under these conditions the TOCSY subspectra through the anomeric regions showed clear connectivities to H-2,3,4,5. The assignment of each cross-peak was based also on the information obtained from COSY, DQF-COSY, and HMQC experiments. Fig. 2 shows the HMQC spectrum in the δ 2.8–4.0 (^1H region) and δ 55–90 (^{13}C region) for compound **3b**. The most relevant cross-peaks showing ^1H–^1H and ^1H–^{13}C connectivities have been labelled. The complete list of ^1H and ^{13}C NMR data for compounds **3a–d** are summarized in Table 1 and Table 2. Assignment of the quaternary ^{13}C-atoms of the coumarin ring was according to Cussans and Huckerby [41].

1. Experimental

General methods.—Optical rotations were measured at 20°C with a Perkin–Elmer 241 instrument, and elemental analyses determined with a CHNS-O Carlo Erba EA1108 analyzer. NMR spectra were recorded on Bruker Ac-80, Varian Gemini 200, Varian Gemini 300, and Varian VXR 500 spectrometers. Proton chemical shifts were referenced to internal Me_4Si for solutions in $CDCl_3$ and to internal sodium 3-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$ for solutions in Me_2SO-d_6 or D_2O; ^{13}C chemical shifts were referenced to the solvent signal. The DQF-COSY and TOCSY experiments were carried out in the phase-sensitive mode. The pure absorption one-bond proton–carbon correlation experiments and the multiple-bond proton–carbon correlation experiments were carried out in the ^1H-detection modes using HMQC and HMBC pulse sequences and a reverse probe. TLC was performed on precoated Silica Gel 60 F_{254} plates with detection by UV light or by charring with 50% H_2SO_4 and heating at 125°C for 15 min. The ^1H and ^{13}C NMR data for compounds **3a–d** are listed in Tables 1 and 2.

Peracetylated α-cellooligosaccharides **13a–d**.—α-D-Glucopyranose pentaacetate (**13a**) and α-cellobiose octaacetate (**13b**) were from Fluka. α-Cellotriose undecaacetate (**13c**) and α-cellotetraose tetradecaacetate (**13d**) were prepared by controlled acid-catalyzed acetolysis of cellullose according to Wolfrom and Thompson [35,36]. Summarizing, cellulose powder (117 g, 20-micron powder, Aldrich) was dissolved in a mixture of Ac_2O (450 mL), AcOH (450 mL), and H_2SO_4 (47 mL), and the mixture was stirred at 35°C for 26 h. The crude was poured on to ice–water (3 L) and the solid that precipitated was filtered off, washed with water, and dried in vacuo over KOH. The solid (245 g) was fractionated by resuspending it in acetone (540 mL), filtering, and

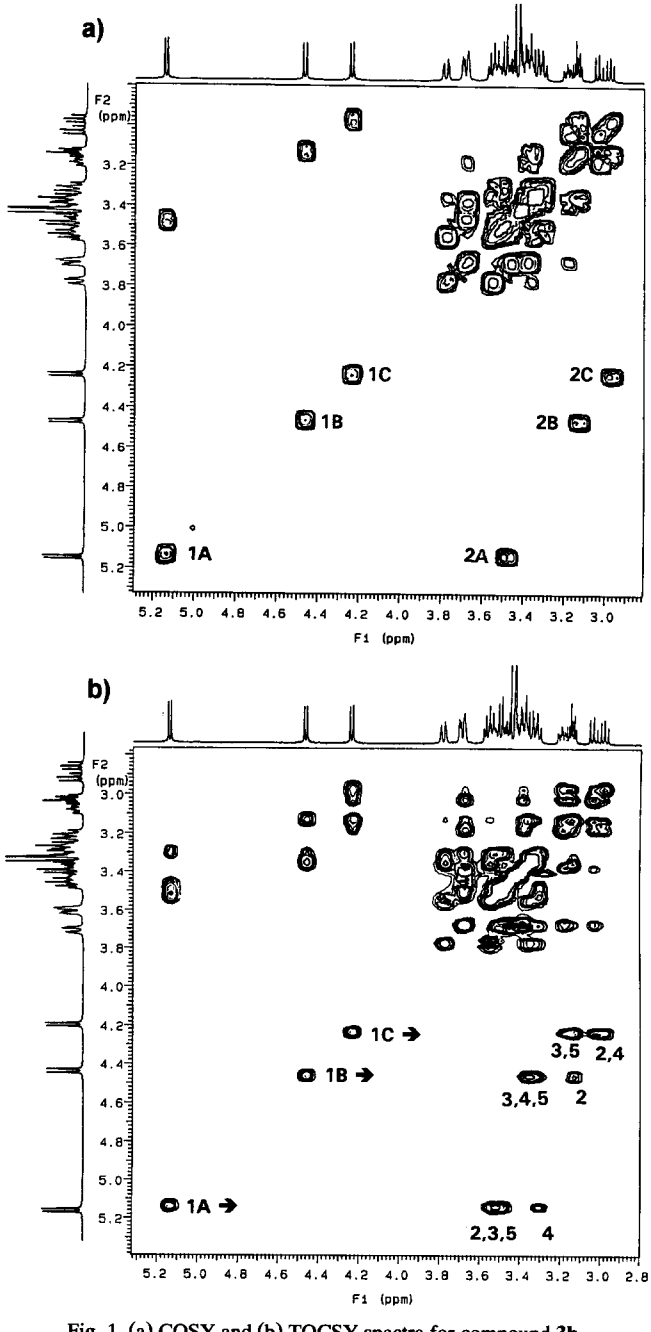

Fig. 1. (a) COSY and (b) TOCSY spectra for compound **3b**.

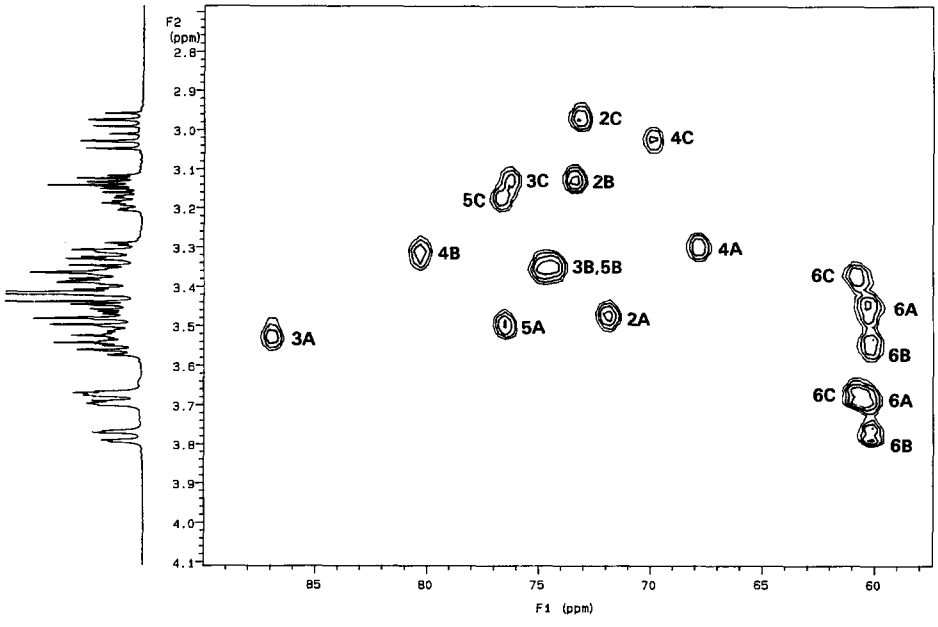

Fig. 2. HMQC experiment for compound **3b** showing the connectivities between carbon atoms and their directly bonded protons.

Table 1
^{1}H-NMR [a] chemical shifts (δ) for compounds **5** and **3a–d**

		H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
G-MeUmb (**5**)								
β-D-Glc*p*	A	5.01	3.25	3.28	3.14	3.42	3.42	3.68
G3G-MeUmb (**3a**)								
β-D-Glc*p*-(1 → 3)	B	4.37	3.08	3.19	3.04	3.20	3.39	3.69
β-D-Glc*p*	A	5.17	3.49	3.52	3.30	3.52	3.47	3.68
G4G3GMeUmb (**3b**)								
β-D-Glc*p*-(1 → 4)	C	4.24	2.98	3.14	3.03	3.18	3.38	3.68
β-D-Glc*p*-(1 → 3)	B	4.47	3.13	3.35	3.31	3.35	3.54	3.78
β-D-Glc*p*	A	5.14	3.48	3.52	3.30	3.50	3.44	3.68
G4G4G3G-MeUmb (**3c**)								
β-D-Glc*p*-(1 → 4)	D	4.23	2.98	3.14	3.03	3.18	3.37	3.68
β-D-Glc*p*-(1 → 4)	C	4.31	3.04	3.32	3.31	3.35	3.54	3.78
β-D-Glc*p*-(1 → 3)	B	4.47	3.14	3.36	3.31	3.35	3.54	3.78
β-D-Glc*p*	A	5.14	3.48	3.52	3.30	3.50	3.44	3.68
G4G4G4G3G-MeUmb (**3d**)								
β-D-Glc*p*-(1 → 4)	E	4.23	2.97	3.14	3.03	3.18	3.37	3.68
β-D-Glc*p*-(1 → 4)	D	4.31	3.04	3.32	3.32	3.35	3.54	3.78
β-D-Glc*p*-(1 → 4)	C	4.32	3.04	3.32	3.32	3.35	3.54	3.78
β-D-Glc*p*-(1 → 3)	B	4.47	3.14	3.36	3.32	3.35	3.54	3.78
β-D-Glc*p*	A	5.14	3.48	3.52	3.30	3.50	3.44	3.68
-MeUmb	H-3	H-5	H-6	H-8	Me			
	6.24	7.70	7.03	7.05	2.39			

[a] Me_2SO-d_6 + D_2O, 25°C, 500 MHz, reference Me_4Si $\delta = 0$.

Table 2
^{13}C-NMR [a] chemical shifts (δ) for compounds **5** and **3a–d**

				C-1	C-2	C-3	C-4	C-5	C-6	
G-MeUmb (**5**)										
β-D-Glc*p*			A	100.3	73.3	76.7	69.9	77.3	60.9	
G3G-MeUmb (**3a**)										
β-D-Glc*p*-(1 → 3)			B	104.1	74.1	76.3	70.4	77.2	61.4	
β-D-Glc*p*			A	99.8	72.4	87.1	68.4	76.9	60.8	
G4G3GMeUmb (**3b**)										
β-D-Glc*p*-(1 → 4)			C	103.5	73.6	76.5	70.3	77.1	61.3	
β-D-Glc*p*-(1 → 3)			B	103.7	73.8	74.7	80.5	75.3	60.5	
β-D-Glc*p*			A	99.7	72.4	86.9	68.3	76.8	60.7	
G4G4G3G-MeUmb (**3c**)										
β-D-Glc*p*-(1 → 4)			D	103.5	73.6	76.6	70.3	77.1	61.3	
β-D-Glc*p*-(1 → 4)			C	103.1	73.3	75.0	80.4	75.4	60.5	
β-D-Glc*p*-(1 → 3)			B	103.7	73.8	74.7	80.4	75.3	60.5	
β-D-Glc*p*			A	99.7	72.5	86.9	68.3	76.9	60.8	
G4G4G4G3G-MeUmb (**3d**)										
β-D-Glc*p*-(1 → 4)			E	103.6	73.8	76.6	70.4	77.2	61.4	
β-D-Glc*p*-(1 → 4)			D	103.2	73.4	75.1	80.3	75.4	60.6	
β-D-Glc*p*-(1 → 4)			C	103.2	73.4	75.1	80.3	75.4	60.6	
β-D-Glc*p*-(1 → 3)			B	103.9	73.9	74.8	80.2	75.5	60.6	
β-D-Glc*p*			A	99.9	72.6	86.7	68.5	76.9	60.9	
-MeUmb	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-1a	C-4a	Me
	161.0	112.2	154.3	127.1	114.1	160.3	103.7	154.8	114.8	18.7

[a] Me_2SO-d_6, 25°C, 75 MHz, reference Me_2SO-d_6 δ 39.5.

evaporating the solvent. Cellooligosaccharides in the soluble fraction (138 g) were separated by column chromatography over silica gel using a gradient of CH_2Cl_2–EtOAc. Fractions containing cellotriose (25.21 g) and cellotetraose (25.91 g) peracetates were purified by flash column chromatography using 1:1 toluene–EtOAc and 1:1.5 toluene–EtOAc, respectively. Crystallization from 95% EtOH afforded α-cellotriose undecaacetate (**13c**, 12.47 g), mp 220–222°C, $[\alpha]_D^{20}$ +20.7° (*c* 0.555, $CHCl_3$); lit. [36] mp 223–224°C, $[\alpha]_D^{20}$ +22.6° (*c* 5, $CHCl_3$); and α-cellotetraose tetradecaacetate (**13d**, 8.35 g), mp 228–230°, $[\alpha]_D^{20}$ +11.2° (*c* 0.851, $CHCl_3$); lit. [36] mp 230–234°, $[\alpha]_D^{20}$ +13.4° (*c* 5, $CHCl_3$).

General synthesis of glucopyranosyl bromides **14a–d**.—A suspension of the peracetate of the corresponding α-cellooligosaccharide in 4.1 M HBr in AcOH (3 mL per mmol) was stirred at room temperature under anhydrous conditions until all the solid was dissolved (30–60 min). The resulting solution was poured into ice–water (8 g per mL of HBr solution), the white solid that precipitated was filtered off and extracted with $CHCl_3$, and the solution washed with saturated aq $NaHCO_3$ until neutral, followed by water. After drying over $MgSO_4$ and filtering, evaporation of the solvent afforded the corresponding bromides as white amorphous solids in 93–97% yields. The products were used immediately in the next glycosidation step without further purification. The purity of the crude products was controlled by TLC and ^{1}H NMR ($CDCl_3$): all bromides showed δ 6.5 (d, 1 H, $J_{1,2}$ 4 Hz, H-1A).

Phenyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (**15a**) [37].—Tin(IV) chloride (21 mL, 0.179 mol) was added to a solution of α-D-glucopyranose pentaacetate (**13a**; 100 g, 0.256 mol) and thiophenol (32 mL, 0.308 mol) in anhydrous benzene (1 L). The solution was stirred at room temperature under Ar and with rigorous exclusion of moisture for 9 h. The resulting yellow solution was neutralized with aq 5% $NaHCO_3$ and the organic layer was washed with water, dried, and evaporated to dryness. The crude product (112.8 g) was crystallized from diisopropyl ether–light petroleum, affording **15a** as colorless needles (78.3 g, 72%); mp 116–118°C, $[\alpha]_D^{20}$ − 42.0° (*c* 1.02, toluene); lit. [42] mp 118°, $[\alpha]_D^{20}$ − 40.1°; NMR data: ^1H (200 MHz, $CDCl_3$), δ 1.93–2.25 (4 s, 12 H, CH_3CO), 3.72 (m, 1 H, H-5), 4.13–4.41 (m, H, H-6a,6b), 4.72 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1), 4.98 (dd, 1 H, $J_{1,2}$ 10.0, $J_{2,3}$ 9.0 Hz, H-2), 5.04 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.0$ Hz, H-4), 5.24 (dd, 1 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-3), 7.25–7.58 (m, 5 H, Ph); ^{13}C (50 MHz, $CDCl_3$), δ 20.4–20.5 (CH_3CO), 62.0 (C-6), 68.1, 69.8, 73.8, 75.7 (C-2,3,4,5), 85.6 (C-1), 128.5, 129.0, 133.2, 131.7 (Ph), 169.5, 169.6, 170.4, 170.8 (CO).

Phenyl 2,3,6,2',3',4',6'-hepta-O-acetyl-1-thio-β-cellobioside (**15**) [37].—Tin(IV) chloride (0.6 mL, 5.1 mmol) was added to a solution of α-cellobiose octaacetate (10 g, 14.7 mol) and thiophenol (2 mL, 19.6 mmol) in anhydrous benzene (150 mL). The solution was stirred at 60°C under Ar and with rigorous exclusion of moisture for 9 h. Standard workup as described above and crystallization from EtOAc–diisopropyl ether afforded **15b** (2.85 g, 27%); mp 223–225°C, $[\alpha]_D^{20}$ − 8.6° (*c* 0.335, $CHCl_3$); NMR data: ^1H (500 MHz, $CDCl_3$), δ 1.96–2.09 (7 s, 21 H, CH_3CO), 3.59–3.64 (m, 2 H, H-5A,5B), 3.70 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.3$ Hz, H-4A), 4.00 (dd, 1 H, $J_{5,6}$ 2.8, $J_{6a,6b}$ 12.5 Hz, H-6aA or 6aB), 4.07 (dd, 1 H, $J_{5,6b}$ 5.5, $J_{6a,6b}$ 12.0 Hz, H-6bA or 6bB), 4.35 (dd, 1 H, $J_{5,6a}$ 4.6, $J_{6a,6b}$ 12.5 Hz, H-6bB or 6bA), 4.47 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1B), 4.54 (dd, 1 H, $J_{5,6a}$ 2.0, $J_{6a,6b}$ 12.0 Hz, H-6aB or 6aA), 4.64 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1A), 4.83 (dd,1 H, $J_{1,2}$ 10.0, $J_{2,3}$ 9.3 Hz, H-2A), 4.90 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.5 Hz, H-2B), 5.04 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4B), 5.11 (dd, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3B), 5.17 (dd, 1 H, $J_{2,3} = J_{3,4} = 9.3$ Hz, H-3A), 7.24–7.46 (m, 5 H, Ph); ^{13}C (50 MHz, $CDCl_3$), δ 20.5–20.8 (CH_3CO), 61.5, 61.9 (C-6A,6B), 67.7, 70.1, 71.5, 71.9, 72.9, 73.5, 76.4, 76.7 (C-2A,2B,3A,3B,4A,4B,5A,5B), 85.5 (C-1A), 100.7 (C-1B), 128.3, 128.9, 131.7, 133.0 (Ph), 169.0, 169.3, 169.5, 169.7, 170.2, 170.5 (CO).

4-Methylumbelliferyl β-D-glucopyranoside (**5**).—This was either from FLUKA or synthesized on the multigram scale according to De Boeck et al. [19]. Summarizing, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**14a**; 200 g, 0.486 mol) was dissolved in acetone (600 mL) and a freshly prepared solution of 4-methylumbelliferone monohydrate (114 g, 0.587 mol) and NaOH (23.3 g, 0.583 mol) in water (300 mL) was added. The mixture was stirred in the dark for 7 h at room temperature. After evaporation of the solvent, $CHCl_3$ (1 L) was added, and the insoluble solid (4-methylumbelliferone, 68 g) was filtered off. The organic layer was extracted with 1M NaOH (250 mL) and water (250 mL). After drying over $MgSO_4$ the resulting solution was concentrated to ca. 400 mL, heated under reflux, and crystallized by addition of diisopropyl ether to yield **4** (107 g, 43%). The product was deacetylated by treating with 6 mM methanolic NaOMe (1.7 L) during 5 h at room temperature. The white solid that precipitated (41.3 g) was filtered off, washed with MeOH, and dried in vacuo. The

filtrate and washes were treated with excess of Amberlite IR-120 resin, concentrated to ca. 700 mL, and allowed to crystallize. This second crop of product (21.5 g) proved to be chromatographically identical (TLC) with the first precipatate (88% overall yield); mp 201–207°C, $[\alpha]_D^{20}$ − 101° (*c* 0.262, water); commercial specifications (from FLUKA), mp 210–210°C, $[\alpha]_D^{20}$ − 98 ± 2° (*c* 0.3, water).

4-Methylumbelliferyl 4,6-O-benzylidene-β-D-glucopyranoside (**6**).—A mixture of **5** (13.0 g, 38.4 mmol), α,α-dimethoxytoluene (17.5 mL, 117 mmol), (±)-10-camphorsulfonic acid (0.525 g, 2.26 mmol), and anhydrous benzene (700 mL) was heated under reflux for 12 h. Most of the solvent was evaporated under reduced pressure and the resulting suspension was filtered. The solid was washed with hexane, resuspended in aq 1% $NaHCO_3$ (250 mL) at 0°C for 30 min, filtered, and washed with distilled water. After drying in vacuo over KOH, **6** was obtained as an amorphous solid (15.6 g, 95%); $[\alpha]_D^{20}$ − 31.6° (*c* 0.155, 4:1 MeCN–EtOAc); NMR data: ^{1}H (200 MHz, $CDCl_3$ + CD_3OD), δ 2.45 (d, 3 H, $J_{3',Me}$ 1.1 Hz, Me), 3.55–3.89 (m, 5 H, H-2,3,4,5,6*ax*), 4.40 (dd, 1 H, $J_{5,6eq}$ 4.5, J_{gem} 8.0 Hz, H-6*eq*), 5.14 (d, 1 H, $J_{1,2}$ 7.4 Hz, H-1), 5.59 (s, 1 H, PhC*H*), 6.21 (q, 1 H, $J_{3',Me}$ 1.1 Hz, H-3′), 6.95–7.10 (m, 2 H, H-6′, H-8′), 7.35–7.61 (m, 6 H, Ph, H-5′); ^{13}C (50 MHz, $CDCl_3$ + CD_3OD), δ 18.5 (Me), 68.3 (C-6), 66.4, 72.9, 73.8, 80.1 (C-2,3,4,5), 100.7 (C-1), 101.8 (PhCH), 104.1 (C-8′), 112.4 (C-3′), 113.6 (C-6′), 115.0 (C-4a′), 125.7, 126.1, 128.1, 129.1, 136.7 (Ph, C-5′), 153.0, 154.5 (C-4′,1a′), 159.7 (C-7′), 161.6 (C-2′). Anal. Calcd for $C_{23}H_{22}O_8$:C, 64.8; H, 5.20. Found: C, 64.6; H, 5.22.

Benzoylation of **6**: *4-Methylumbelliferyl 2-O-benzoyl-4,6-O-benzylidene-β-D-glucopyranoside* (**7**).—A mixture of **6** (1.50 g, 3.52 mmol) and freshly prepared *N*-benzoylimidazole [13] (970 mg, 5.63 mmol) in MeCN (20 mL) was heated under reflux for 12 h. The mixture was cooled to room temperature and water (1 mL) was added. After stirring for 30 min, the solvent was distilled off at reduced pressure and the residue was poured on to ice–water. The precipitate was filtered off, washed with water, and redissolved in CH_2Cl_2. The solution was extracted with 5% HCl, water, and aq $NaHCO_3$. After drying and evaporation of the solvent, the crude product (2.01 g) was purified by flash column chromatography using cyclohexane–EtOAc (2:1, 3:2, and 1:1). Three main fractions were obtained.

Eluted first was 4-methylumbelliferyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**9**; 871 mg, 39%); NMR data: ^{1}H (80 MHz, $CDCl_3$), δ 2.35 (d, 3 H, $J_{3',Me}$ 1.2 Hz, Me), 3.70–4.54 (m, 4 H, H-4,5,6*ax*,6*eq*), 5.48 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 5.58 (s, 1 H, PhC*H*), 5.65–6.00 (m, 2 H, H-2,3), 6.16 (q, 1 H, $J_{3',Me}$ 1.2 Hz, H-3′), 6.80–7.00 (m, 2 H, H-6′, H-8′), 7.15–7.6 (m, 12 H, Ph, H-5′), 7.89–8.02 (m, 4 H, H*ortho* PhCO)

Eluted second was **7** (467 mg, 25%); $[\alpha]_D^{20}$ +21.1° (*c* 0.204, $CHCl_3$); NMR data: ^{1}H (200 MHz, $CDCl_3$), δ 2.36 (d, 3 H $J_{3',Me}$ 1.1 Hz, Me), 3.05 (d, 1 H, $J_{3,OH}$ 2.8 Hz, OH), 3.62–3.92 (m, 3 H, H-4,5,6*ax*), 4.20 (ddd, 1 H, $J_{3,2} = J_{3,4} = 8.0$, $J_{3,OH}$ 2.8 Hz, H-3), 4.46 (dd, 1 H, $J_{5,6eq}$ 4.3, J_{gem} 10.0 Hz, H-6*eq*), 5.34 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 5.51 (dd, 1 H, $J_{2,1} = J_{2,3} = 8.0$ Hz, H-2), 5.60 (s, 1 H, PhC*H*), 6.16 (q, 1 H, $J_{3',Me}$ 1.1 Hz, H-3′), 6.89 (dd, 1 H, $J_{5',6'}$ 8.7, $J_{6',8'}$ 2.4 Hz, H-6′), 6.96 (d, 1 H, $J_{6',8'}$ 2.4 Hz, H-8′), 7.12–7.68 (m, 9 H, Ph, H-5′), 8.05 (d, 2 H, J_{ortho} 7.9 Hz, H*ortho* PhCO); ^{13}C (50 MHz, $CDCl_3$), δ 18.5 (Me), 68.4 (C-6), 66.6, 72.2, 74.2, 80.4 (C-2,3,4,5), 99.2, 102.0

(C-1, PhCH), 104.1 (C-8′), 113.0 (C-3′), 114.1 (C-6′), 115.5 (C-4a′), 125.7–136.7 (Ph, C-5′), 152.2, 154.7 (C-4′,1a′), 159.3 (C-7′), 160.9 (C-2′), 165.7 (PhCO). Anal. Calcd for $C_{30}H_{26}O_9$: C, 67.9; H, 4.94. Found: C, 67.6; H, 5.00.

Eluted third was 4-methylumbelliferyl 3-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**8**; 560 mg, 30%); NMR data: ^{1}H (80 MHz, $CDCl_3$), δ 2.39 (d, 3 H, $J_{3',Me}$ 1.2 Hz, Me), 3.75–4.48 (m, 6 H, H-2,4,5,6*ax*,6*eq*, OH), 5.26 (d, 1 H, $J_{1,2}$ 7.4 Hz, H-1), 5.55 (dd, 1 H, $J_{2,3} = J_{3,4} = 9.9$ Hz, H-3), 5.55 (s, 1 H, PhC*H*), 6.18 (q. 1 H, $J_{3',Me}$ 1.2 Hz, H-3′), 7.01 (dd, 1 H, $J_{5',6'}$ 9.5, $J_{6',8'}$ 2.4 Hz, H-6′), 7.02 (d, 1 H, $J_{6',8'}$ 2.4 Hz, H-8′), 7.26–7.61 (m, 9 H, Ph, H-5′), 8.08 (m, 2 H, H*ortho* PhCO).

Benzylation of **6**: *4-Methylumbelliferyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside* (**10**).—A mixture of **6** (5.0 g, 11.7 mmol), tetrabutylammonium hydrogen sulfate (0.81 g, 2.4 mmol), benzyl bromide (2.4 mL, 20.0 mmol), CH_2Cl_2 (200 mL), and aq 5% NaOH (17 mL) was heated under reflux for 24 h. After cooling, the organic layer was separated, washed with water (2 × 100 mL), dried over $MgSO_4$, filtered, and evaporated to dryness. The solid thus obtained (5.24 g) was purified by flash column chromatography using 6:1 toluene–EtOAc and EtOAc. Four main fractions were obtained.

Eluted first was 4-methylumbelliferyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**12**; 580 mg, 8%); $[\alpha]_D^{20}$ −33.3° (*c* 0.397, $CHCl_3$); NMR data: ^{1}H (200 MHz, $CDCl_3$), δ 2.40 (d, 3 H, $J_{3',Me}$ 1.1 Hz, Me), 3.60 (m, 1 H, H-5), 3.73–3.94 (m, 4 H, H-2,3,4,6*ax*), 4.41 (dd, 1 H, $J_{5,6eq}$ 4.8, J_{gem} 10.3 Hz, H-6*eq*), 4.80–5.00 (m, 4 H, $PhCH_2$), 5.19 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 5.60 (s, 1 H, PhC*H*), 6.19 (q, 1 H, $J_{3',Me}$ 1.1 Hz, H-3′), 6.95–7.10 (m, 2 H, H-6′, H-8′), 7.35–7.61 (m, 16 H, Ph, H-5′); ^{13}C (50 MHz, $CDCl_3$), δ 18.7 (Me), 68.5 (C-6), 75.2, 75.6 ($PhCH_2$), 66.3, 80.7, 81.1, 81.4 (C-2,3,4,5), 101.2 (C-1, PhCH), 104.2 (C-8′), 113.0 (C-3′), 113.4 (C-6′), 115.3 (C-4a′), 125.7, 126.0, 127.8 127.9, 128.0, 128.1, 128.3, 128.9, 129.0, 137.0, 137.8, 138.2 (Ph, C-5′), 152.2, 154.8 (C-4′,1a′), 159.5 (C-7′), 160.9 (C-2′). Anal. Calcd for $C_{37}H_{34}O_8$: C, 73.3; H, 5.65. Found: C, 73.0; H, 5.63.

Eluted second was **10** (2.85 g, 47%); mp 198–200°C (from $CHCl_3$–light petroleum), $[\alpha]_D^{20}$ −24.5° (*c* 0.229, $CHCl_3$); NMR data: ^{1}H (500 MHz, $CDCl_3$), δ 2.40 (d, 3 H, $J_{3',Me}$ 1.1 Hz, Me), 2.62 (s, 1 H, OH), 3.58 (m, 1 H, H-5), 3.62 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.0$ Hz, H-4), 3.67 (dd, 1 H, $J_{1,2} = J_{2,3} = 9.0$ Hz, H-2), 3.78 (dd, 1 H, $J_{5,6ax} = J_{gem} = 9.0$ Hz, H-6*ax*), 3.96 (dd, 1 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-3), 4.38 (dd, 1 H, $J_{5,6eq}$ 4.5, J_{gem} 9.0 Hz, H-6*eq*), 4.85 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2), 4.96 (d, 1 H, J_{gem} 11.0 Hz, PhCH_2), 5.17 (d, 1 H, $J_{1,2}$ 9.0 Hz, H-1), 5.53 (s, 1 H, PhC*H*), 6.18 (q, 1 H, $J_{3',Me}$ 1.1 Hz, H-3′), 6.94 (dd, 1 H, $J_{5',6'}$ 9.0, $J_{6',8'}$ 2.5 Hz, H-6′), 6.98 (d, 1 H, $J_{6',8'}$ 2.5 Hz, H-8′), 6.93–7.52 (m, 11 H, Ph, H-5′); ^{13}C (50 MHz, $CDCl_3$), δ 18.7 (Me), 68.4 (C-6), 75.2 ($PhCH_2$), 66.3, 73.3, 80.0, 81.3 (C-2,3,4,5), 100.8, 101.8 (C-1, PhCH), 104.2 (C-8′), 113.0 (C-3′), 113.4 (C-6′), 115.3 (C-4a′), 125.7, 126.3, 128.1, 128.3, 128.6, 129.3, 136.7, 137.7 (Ph, C-5′), 152.2, 154.8, (C-4′,1a′), 159.4 (C-7′), 160.9 (C-2′). Anal. Calcd for $C_{30}H_{28}O_8$: C, 69.8; H, 5.46. Found: C, 69.5; H, 5.70.

Eluted third was 4-methylumbelliferyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**11**; 651 mg, 11%); $[\alpha]_D^{20}$ −17.7° (*c* 0.423, $CHCl_3$); NMR data: ^{1}H (200 MHz, $CDCl_3$), δ 2.43 (d, 3 H, $J_{3',Me}$ 1.2 Hz, Me), 3.64 (m, 1 H, H-5), 3.72–3.95 (m, 4 H, H-2,3,4,6*ax*), 4.43 (dd, 1 H, $J_{5,6eq}$ 4.5, J_{gem}10.2 Hz, H-6*eq*), 4.83 (d, 1 H, J_{gem}

11.6 Hz, PhCH_2), 5.05 (d, 1 H, J_{gem} 11.6 Hz, PhCH_2, 5.13 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1), 5.63 (s, 1 H, PhCH), 6.21 (q, 1 H, $J_{3',Me}$ 1.2 Hz, H-3'), 6.98–7.04 (m, 2 H, H-6',8'), 7.28–7.58 (m, 11 H, Ph, H-5'); ^{13}C (50 MHz, $CDCl_3$), δ 18.7 (Me), 68.5 (C-6), 74.8 (PhCH_2), 66.7, 73.7, 80.1, 81.0 (C-2,3,4,5), 100.6, 101.4 (C-1, PhCH), 104.3 (C-8'), 113.0 (C-3'), 113.7 (C-6'), 125.7, 126.0, 128.1, 128.2, 128.4, 128.6, 129.2 (Ph, C-5'). Anal. Calcd for $C_{30}H_{28}O_8$: C, 69.8; H, 5.46. Found: C, 69.7; H, 5.61.

Starting material (**6**) was recovered in 4% yield (210 mg) as the fourth fraction.

4-Methylumbelliferyl O-*(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-(1 → 3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-*D*-glucopyranoside* (**16a**).—*Method a.* Compound **10** (6.85 g, 13.3 mmol), $HgBr_2$ (3.96 g, 11.0 mmol), $Hg(CN)_2$ (2.78 g, 11.0 mmol), powdered 4Å molecular sieves (8.5 g), and CH_2Cl_2 (35 mL) were mixed and stirred in the dark under Ar for 2 h. Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**14a**; 6.81 g, 16.6 mmol) in CH_2Cl_2 (10 mL) was added and the mixture was stirred for 17 h with rigorous exclusion of light and moisture, then filtered over Celite, extracted with CH_2Cl_2, and washed with saturated aq $NaHCO_3$ and water. After drying and evaporating the solvent, the residue (13.48 g) was purified by flash chromatography over silica gel (gradient 20:1 CH_2Cl_2–EtOAc to EtOAc), to yield **16a** (7.99, 71%); $[\alpha]_D^{20}$ −48.5° (*c* 0.310, $CHCL_3$); NMR data: ^{1}H (500 MHz, $CDCl_3$), δ 1.82–1.98 (4 s, 12 H, CH_3CO), 2.39 (d, 3 H, $J_{3',Me}$ 1.0 Hz, Me), 3.36 (m, 1 H, H-5A), 3.57 (m, 1 H, H-5B), 3.72–3.90 (m, 3 H, H-2A,4A,6A*ax*), 4.17 (dd, 1 H, $J_{5,6eq}$ 2.0, J_{gem} 12.0 Hz, H-6A*eq*), 4.76 (d, 1 H, J_{gem} 10.5 Hz, PhCH_2), 4.85 (d, 1 H, J_{gem} 10.5 Hz, PhCH_2), 4.87 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1B), 5.03–5.13 (m, 6 H, H-2B,3A,3B,4B,6aB,6bB), 5.16 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1A), 5.55 (s, 1 H, PhCH), 6.18 (q, 1 H, $J_{3',Me}$ 1.1 Hz, H-3'), 6.88 (dd, 1 H, $J_{5',6'}$ 9.0, $J_{6',8'}$ 2.5 Hz, H-6'), 6.94 (d, 1 H, $J_{6',8'}$ 2.5 Hz, H-8'), 7.24–7.47 (m, 10 H, Ph), 7.51 (d, 1 H, $J_{5',6'}$ 8.5 Hz, H-5'); ^{13}C (50 MHz, $CDCl_3$), δ 18.7 (Me), 20.6 (CH_3CO), 61.6 (C-6B), 68.5 (C-6A), 75.7 (PhCH_2), 60.4, 66.3, 67.9, 71.8, 73.0, 79.3, 80.6, 80.7 (C-2A,2B,3A,3B,4A,4B,5A,5B), 100.6, 101.0, 101.5 (C-1A,1B, PhCH), 104.1 (C-8'), 113.1 (C-3'), 113.4 (C-6'), 115.4 (C-4a'), 125.8, 126.0, 128.0, 128.1, 128.4, 128.5, 129.4, 136.8, 137.5 (Ph, C-5'), 152.2, 154.9 (C-4',1a'), 159.3 (C-7'), 160.9 (C-2'), 169.3, 169.4, 170.3, 170.6 (CO). Anal. Calcd for $C_{44}H_{46}O_{17}$: C, 62.4; H, 5.48. Found: C, 62.5; H, 5.50.

Method b. Compound **10** (7.16 g, 13.9 mmol), phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**15a**; 9.38 g, 21.3 mmol), powdered 4 Å molecular sieves (10 g), and CH_2Cl_2 (230 mL) were mixed and stirred under Ar for 2 h. Methyl trifluoromethanesulfonate (4.8 mL, 43.8 mmol) was added and the mixture was stirred for 44 h. Triethylamine (19 mL) was added and the mixture was shaken for an additional 3 h. After filtration and evaporation the crude product was purified by flash column chromatography (2:1 and 1:1 cyclohexane–EtOAc), to yield **16a** (4.88g, 42%).

4-Methylumbelliferyl O-*(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-acetyl-β-*D*-glucopyranosyl)-(1 → 3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-*D*-glucopyranoside* (**16b**).—*Method a.* Compound **10** (7.28 g, 14.1 mmol), $HgBr_2$ (6.34 g, 17.6 mmol), $Hg(CN)_2$ (4.45 g, 17.6 mmol), powdered 4 Å molecular sieves (9.2 g), and CH_2Cl_2 (50 mL) were stirred under Ar for 1 h. 2,3,6,2',3',4',6'-Hepta-*O*-acetyl-α-cellobiosyl bromide (**14b**; 18.17 g, 26.0 mmol) in CH_2Cl_2 (30 mL) was added and the mixture was stirred at room temperature for 21 h. Standard workup as described for **16a** and chromatographic purification (3:2 $CHCl_3$–EtOAc and EtOAc) yielded the title

product (9.4 g, 59%); $[\alpha]_D^{20}$ −43.2° (*c* 0.405, $CHCl_3$); NMR data: ^1H (500 MHz, $CDCl_3$), δ 1.75–2.00 (7 s, 21 H, CH_3CO), 2.33 (d, 3 H, $J_{3',Me}$ 1.0 Hz, Me), 3.14 (m, 1 H, H-5A), 3.48, 3.60 (2 m, 2 H, H-5B,5C), 3.66–3.76 (m, 3 H, H-2A,4A,6A*ax*), 4.17 (dd, 1 H, $J_{5,6eq}$ 2.0, J_{gem} 12.0 Hz, H-6A*eq*), 3.90–4.15, 4.25–4.31, 4.68–4.79, 4.91–5.01 (m, 14 H, PhCH_2, H-1B,1C,2B,2C,3B,3C,4B,4C,6aB,6aC,6bB,6bC), 5.11 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1A), 5.51 (s, 1 H, PhC*H*), 6.12 (q, 1 H, $J_{3',Me}$ 1.1 Hz, H-3′), 6.83 (dd, 1 H, $J_{5',6'}$ 9.0, $J_{6',8'}$ 2.5 Hz, H-6′), 6.88 (d, 1 H, $J_{6',8'}$ 2.5 Hz, H-8′), 7.24–7.42 (m, 10 H, Ph), 7.45 (d, 1 H, $J_{5',6'}$ 8.5 Hz, H-5′); ^{13}C (125 MHz, $CDCl_3$), δ 18.7 (Me), 20.5–20.9 (CH_3CO), 61.4, 61.5 (C-6B,6C), 68.6 (C-6A), 75.6 ($PhCH_2$), 66.3, 67.7, 71.4, 71.8, 72.0, 72.5, 72.8, 76.0, 79.4, 80.4, 80.8 (C-2A–C,3A–C,4A–C,5A–C), 100.3, 100.8, 100.9, 101.7 (C-1A–C, PhCH), 104.1 (C-8′), 113.1 (C-3′), 113.4 (C-6′), 115.4 (C-4a′), 125.8, 126.2, 128.1, 128.4, 128.5, 129.4, 136.9, 137.5 (Ph, C-5′), 152.2, 154.9 (C-4′,1a′), 159.3 (C-7′), 160.9 (C-2′), 168.9, 169.3, 169.7, 169.8, 170.2, 170.5 (CO). Anal. Calcd for $C_{56}H_{62}O_{25}$: C, 59.26; H, 5.51. Found: C, 59.10; H, 5.74.

Method b. Compound **10** (103 mg, 0.20 mmol), phenyl 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-1-thio-β-cellobioside (**15b**; 171 mg, 0.24 mmol), powdered 4 Å molecular sieves (1 g), and CH_2Cl_2 (6 mL) were mixed and stirred under Ar for 1 h. Methyl trifluoromethanesulfonate (0.11 mL, 1.0 mmol) was added and the mixture was stirred for 44 h. Workup as described for **16a** and purification by column chromatography (1:1 cyclohexane–EtOAc) yielded **16b** (81 mg, 34%).

4-Methylumbelliferyl O-*(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-acetyl-β-*D*-glucopyranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-acetyl-β-*D*-glucopyranosyl)-(1 → 3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-*D*-glucopyranoside* (**16c**).—Compound **10** (2.86 g, 5.53 mmol), $HgBr_2$ (1.14 g, 3.17 mmol), $Hg(CN)_2$ (0.80 g, 3.17 mmol), powdered 4 Å molecular sieves (3 g), and CH_2Cl_2 (11 mL) were stirred under Ar for 1 h. Deca-*O*-acetyl-α-cellotriosyl bromide (**14c**; 5.47 g, 5.55 mmol) in CH_2Cl_2 (5 mL) was added and the mixture stirred at room temperature for 25 h. Standard workup and chromatographic purification (1.2:1 toluene–EtOAc and EtOAc) yielded the title product (3.31 g, 42%); $[\alpha]_D^{20}$ −39.3° (*c* 0.303, $CHCl_3$); NMR data: ^1H (500 MHz, $CDCl_3$), δ 1.80–2.10 (10 s, 30 H, CH_3CO), 2.39 (d, 3 H, $J_{3',Me}$ 1.0 Hz, Me), 3.17 (m, 1 H, H-5A), 3.48, 3.56, 3.61 (3 m, 3 H, H-5B–D), 3.69–3.82 (m, 3 H, H-2A,4A,6A*ax*), 4.23 (dd, 1 H, $J_{5,6eq}$ 2.0, $J_{6ax,6eq}$ 12.0 Hz, H-6A*eq*), 4.00–4.08, 4.29–4.45, 4.73–5.11 (m, 21 H, PhCH_2, H-1B–D,2B–D,3A–D,4B–D,6aB–D,6bB–D), 5.16 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1A), 5.57 (s, 1 H, PhC*H*), 6.18 (q, 1 H, $J_{3',Me}$ 1.1 Hz, H-3′), 6.88 (dd, 1 H, $J_{5',6'}$ 9.0, $J_{6',8'}$ 2.5 Hz, H-6′), 6.93 (d, 1 H, $J_{6',8'}$ 2.5 Hz, H-8′), 7.29–7.48 (m, 10 H, Ph), 7.51 (d, 1 H,$J_{5',6'}$ 8.5 Hz, H-5′); ^{13}C (125 MHz, $CDCl_3$), δ 18.6 (Me), 20.4–20.9 (CH_3CO), 61.3, 61.4, 62.1 (C-6B–D), 68.5 (C-6A), 75.6 ($PhCH_2$), 66.3, 67.7, 71.5, 71.6, 72.0, 72.1, 72.5, 72.6, 72.8, 76.0, 76.1, 79.4, 80.3, 80.8 (C-2A–D,3A–D,4A–D,5A–D), 100.2, 100.6, 100.8, 100.9, 101.7 (C-1A–D, PhCH), 104.1 (C-8′), 113.1, (C-3′), 113.4 (C-6′), 115.4 (C-4a′), 125.8–137.5 (Ph, C-5′), 152.1, 154.8, (C-4′,1a′), 159.3 (C-7′), 160.8 (C-2′), 169.1–170.4 (CO). Anal. Calcd for $C_{68}H_{78}O_{33}$: C, 57.4; H, 5.52. Found: C, 57.2; H, 5.50.

4-Methylumbelliferyl O-*(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-acetyl-β-*D*-glucopyranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-acetyl-β-*D*-glucopyranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-acetyl-β-*D*-glucopyranosyl)-(1 → 3)-2-*O*-benzyl-4,6-*O*-ben-*

zyldene-β-D-glucopyranoside (**16d**).—Compound **10** (4.00 g, 7.74 mmol), $HgBr_2$ (1.55 g, 4.30 mmol), $Hg(CN)_2$ (1.06 g, 4.20 mmol), powdered 4 Å molecular sieves (5 g), and CH_2Cl_2 (15 mL) were stirred under Ar for 1 h. Trideca-*O*-acetyl-α-cellotetraosyl bromide (**14d**; 8.04 g, 6.30 mmol) in CH_2Cl_2 (12 mL) was added and the mixture stirred at room temperature for 25 h. Standard workup and chromatographic purification (gradient 4:1 to 1:1 CH_2Cl_2–EtOAc) yielded the title product (3.57 g, 33%); $[\alpha]_D^{20}$ −34.3° (*c* 0.300, $CHCl_3$); NMR data: ^1H (500 MHz, $CDCl_3$), δ 1.80–2.12 (13 s, 39 H, CH_3CO), 2.39 (d, 3 H, $J_{3',Me}$ 1.0 Hz, Me), 3.17 (m, 1 H, H-5A), 3.47, 3.53, 3.57, 3.60 (4 m, 4 H, H-5B–E), 3.67–3.82 (m, 3 H, H-2A,4A,6A*ax*), 4.22 (dd, 1 H, $J_{5,6eq}$ 2.0, $J_{6ax,6eq}$ 12.0 Hz, H-6A*eq*), 3.99–4.12, 4.27–4.45, 4.71–5.11 (m, 27 H, PhCH_2, H-1B–E,2B–E,3A–E,4B–E,6aB–E,6bB–E), 5.16 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1A), 5.57 (s, 1 H, PhC*H*), 6.18 (q, 1 H, $J_{3',Me}$ 1.1 Hz, H-3′), 6.88 (dd, 1 H, $J_{5',6'}$ 9.0, $J_{6',8'}$ 2.5 Hz, H-6′), 6.93 (d, 1 H, $J_{6',8'}$ 2.5 Hz, H-8′), 7.29–7.48 (m, 10 H, Ph), 7.51 (d, 1 H, $J_{5',6'}$ 8.5 Hz, H-5′); ^{13}C (75 MHz, $CDCl_3$), δ 18.6 (Me), 20.4–21.0 (CH_3CO), 60.3, 61.3, 61.4, 62.0 (C-6B–E), 68.5 (C-6A), 75.6 ($PhCH_2$), 66.3, 67.7, 71.5, 71.7, 72.0, 72.1, 72.6, 72.7, 72.8, 76.1, 76.2, 79.4, 80.3, 80.8 (C-2A–E,3A–E,4-E,5-E), 100.1, 100.5, 100.6, 100.8, 100.9, 101.7 (C-1A–E, PhCH), 104.1 (C-8′), 113.1, (C-3′), 113.4 (C-6′), 115.4 (C-4a′), 125.8–137.5 (Ph, C-5′), 152.1, 154.8 (C-4′,1a′), 159.3 (C-7′), 160.8 (C-2′), 169.0–170.4 (CO). Anal. Calcd for $C_{80}H_{94}O_{41}$: C, 56.1; H, 5.54. Found: C, 55.9; H, 5.28.

4-Methylumbelliferyl 3-O-β-D-glucopyranosyl-β-D-glucopyranoside (**3a**).—*Method a.* Compound **16a** (7.98 g) was dissolved in 60% AcOH (250 mL) and heated under reflux for 45 min. The resulting solution was evaporated under reduced pressure, and the residue was dried in vacuo and then deacetylated using 20 mM methanolic NaOMe (150 mL) at room temperature (15 h). The solid that precipitated (**17a**) was collected by filtration, washed with MeOH, and dried in vacuo (3.42 g, 61%). An additional fraction of product was obtained from the mother liquors by flash column chromatography (0.75 g, 75% overall yield). Debenzylation of the first fraction of **17a** was performed by catalytic hydrogenolysis using 10% Pd–C (3.40 g) in water–EtOH–THF (1:1:1, 250 mL) at 30°C for 3 h. After filtration, the solution was concentrated to ca. 20 mL and EtOH was added until incipient turbidity, to give **3a** as a white powder (1.90 g, 66%); mp 253–255°C (dec.), $[\alpha]_D^{20}$ −60.3° (*c* 0.335, water). Anal. Calcd for $C_{22}H_{28}O_{13}$: C, 52.8; H, 5.64. Found: C, 52.5; H, 5.52.

Method b. Compound **16a** (4.67 g) was dissolved in glacial AcOH (250 mL) and stirred under H_2 at room temperature using 10% Pd–C (1.53 g) as catalyst for 90 h. Filtration of the solvent and evaporation yielded 3.98 g of crude material that was purified by flash column chromatography (3:1 EtOAc–$CHCl_3$). Eluted first was 3,4-dihydro-4-methylumbelliferyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1 → 3)-β-D-glucopyranoside (**19**; 770 mg, 21%); NMR data: ^1H (80 MHz, CD_3COCD_3), δ 1.28 (d, 3 H $J_{4'Me}$ 8 Hz, Me), 1.95–2.10 (4 s, 12 H, CH_3CO), 2.58–2.81 (m, 3 H, H-3′,4′), 3.00–5.41 (m, 17 H, Glc*p* and OH protons), 6.72–6.91 (m, 2 H, H-6′,8′), 7.23 (d, 1 H, $J_{5',6'}$ 9.9 Hz, H-5′).

Eluted second was 4-methylumbelliferyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1 → 3)-β-D-glucopyranoside (**18**; 1.39 g, 38%); NMR data: ^1H (80 MHz, CD_3COCD_3), δ 1.95–2.08 (4 s, 12 H, CH_3CO), 2.44 (d, 3 H, $J_{3'Me}$ 1.2 Hz, Me),

3.40–5.43 (m, 17 H, Glc*p* and OH protons), 6.16 (d, 1 H, $J_{3'Me}$ 1.2 Hz, H-3′), 6.97–7.07 (m, 2 H, H-6′,8′), 7.68 (d, 1 H, $J_{5',6'}$ 9.5 Hz, H-5′). Deacetylation of **18** using 6 mM methanolic NaOMe (85 mL) at room temperature for 2.5 h afforded **3a** (714 mg) which crystallized directly from the reaction mixture. An additional fraction of **3a** was obtained from the mother liquors after neutralization with Amberlite IR-120(H^+) resin, evaporation of the solvent, and recrystallization of the residue from MeOH (178 mg, 86% overall yield).

4-Methylumbelliferyl O-*β*-D-*glucopyranosyl-(1 → 4)*-O-*β*-D-*glucopyranosyl-(1 → 3)-β*-D-*glucopyranoside* (**3b**).—Sequential deprotection of **16b** (9.95 g), as described for **16a**, using refluxing 60% AcOH (120 mL, 35 min) and 20 mM methanolic NaOMe (225 mL, 8 h), afforded 4-methylumbelliferyl *O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-glucopyranosyl-(1 → 3)-2-*O*-benzyl-*β*-D-glucopyranoside (**17b**) in 72% yield (4.75 g). Hydrogenolysis using 10% Pd–C in 1:1:1 water–EtOH–THF at 30°C for 1.5 h afforded **3b** as a white powder (2.80 g, 67%). After one recrystallization from EtOH–water, **3b** had mp 252–254°C (dec.), $[\alpha]_D^{20}$ −47.2° (*c* 0.106, water). Anal. Calcd for $C_{28}H_{38}O_{18}$: C, 50.8; H, 5.78. Found: C, 50.9; H, 6.00.

4-Methylumbelliferyl O-*β*-D-*glucopyranosyl-(1 → 4)*-O-*β*-D-*glucopyranosyl-(1 → 4)*-O-*β*-D-*glucopyranosyl-(1 → 3)-β*-D-*glucopyranoside* (**3c**).—Sequential treatment of **16c** (1.91 g, 1.34 mmol) with refluxing 60% AcOH (65 mL, 1 h) and 20 mM methanolic NaOMe (85 mL) afforded 4-methylumbelliferyl *O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-glucopyranosyl-(1 → 3)-2-*O*-benzyl-*β*-D-glucopyranoside (**17c**) in 74% yield (903 mg). Hydrogenolysis using 10% Pd–C (900 mg) in 2:1:2.2 water–EtOH–THF at 30°C for 1.5 h afforded **3c** as a white powder (552 mg, 68%). After one recrystallization from EtOH–water, **3c** had mp 236–238°C (dec.), $[\alpha]_D^{20}$ −28.9° (*c* 0.107, water). Anal. Calcd for $C_{34}H_{48}O_{23}$: C, 49.5; H, 5.87. Found: C, 49.3; H, 5.65.

4-Methylumbelliferyl O-*β*-D-*glucopyranosyl-(1 → 4)*-O-*β*-D-*glucopyranosyl-(1 → 4)*-O-*β*-D-*glucopyranosyl-(1 → 3)-β*-D-*glucopyranoside* (**3d**).—Sequential treatment of **16d** (2.89 g) with refluxing 60% AcOH (100 mL, 1 h) and 20 mM methanolic NaOMe (100 mL, 30°C, 14 h) afforded 4-methylumbelliferyl *O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-*β*-D-glucopyranosyl-(1 → 3)-2-*O*-benzyl-*β*-D-glucopyranoside (**17d**) which crystallized directly from the reaction mixture (1.60 g, 88%). Catalytic hydrogenolysis using 10% Pd–C (1.65 g) in 1:1:1 water–EtOH–THF at 30°C for 1 h 45 min and crystallization of the crude product from water–EtOH afforded **3d** as a white solid (1.03 g, 71%); mp 282–284°C (dec.), $[\alpha]_D^{20}$ − 25.8° (*c* 0.178, water). Anal. Calcd for $C_{40}H_{58}O_{28}$: C, 48.7; H, 5.92. Found: C, 48.4; H, 5.85.

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