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Enzymatic synthesis of disaccharides using *Agrobacterium* sp. β-glucosidase

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Abstract

The synthesis of several oligosaccharides, in yields up to 65%, has been achieved by utilizing the transglycosylation activity of *Agrobacterium* sp. β-glucosidase (EC 3.2.1.21). The regioselectivity of the glycosylation reaction was investigated using *p*-nitrophenyl β-D-galactopyranoside, β-D-mannopyranosyl fluoride and D-glucal as glycosyl donors and a series of phenyl 1-thio-, benzyl 1-thio-, and benzyl β-D-glycopyranosides as glycosyl acceptors. The isolated products were determined to contain a mixture of β-(1 → 3) and/or β-(1 → 4) glycosidic linkages for all glycosyl acceptors, except for β-D-*galacto*-configured acceptors where a mixture of β-(1 → 3) and β-(1 → 6) linkages was observed. The wide range of glycosyl donors and acceptors utilized by the enzyme in transglycosylation experiments demonstrates the versatility of this system for the aqueous-phase synthesis of oligosaccharides. © 1998 Elsevier Science Ltd.

Keywords: Transglycosylation; Enzymatic oligosaccharide synthesis; *Agrobacterium* sp. β-glucosidase

1. Introduction

Although there has been remarkable progress in chemical oligosaccharide synthesis in recent years [1–4], the formation of glycosidic linkages still remains a challenging task [5,6]. The potential of enzymes to assist in oligosaccharide synthesis has been the focus of much research in the last 15 years. Glycosyltransferases have demonstrated their usefulness as an alternative to the multistep reaction sequences and intense protecting group strategies that are characteristic of chemical synthesis [7–11]. However, due to the limited availability of glycosyl transferases, research has also focused on the large family of glycosyl hydrolases (glycosidases) [12,13]. Although glycosidases normally hydrolyze glycosidic linkages, under certain conditions, they are able to catalyze the stereospecific formation of glycosidic linkages. Thus, by exploiting either their reverse hydrolysis activity (thermodynamically controlled approach) or their transglycosylation potential (kinetically controlled approach), the synthesis of a variety of oligosaccharides has been achieved [14–23]. The main drawbacks of glycosidases are the low product yields due to product hydrolysis and the relaxed regioselectivity of transfer, giving rise to several products. In several cases, greater control over the

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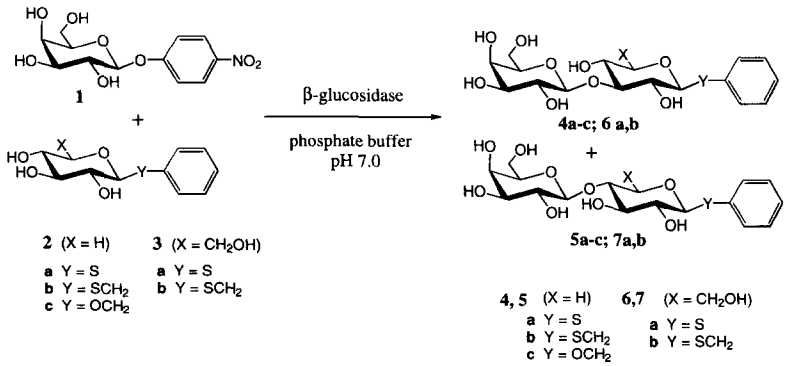
regioselectivity has been achieved by altering the configuration and substitution of the anomeric centre of the glycosyl acceptor [24–26]. These results indicate the importance of the binding interactions between the glycosyl acceptor and the enzyme for control of the transfer reaction. In order to understand these interactions better, we have exploited the synthetic potential of a well-studied enzyme, the *Agrobacterium* sp. (formerly known as *Agrobacterium faecalis*) β-glucosidase (Abg) [27–29]. This enzyme has been subjected to a wide range of kinetic studies, including detailed studies of its substrate specificity [30–32]. While the enzyme shows a strong preference for the exo-glucosidic cleavage of cello-oligomers, it has been shown to have a relatively broad specificity. We here report on its potential in oligosaccharide synthesis.

2. Results and discussion

The range of glycosyl donors acceptable to *Agrobacterium* sp. β-glucosidase was explored by testing the enzyme's capacity for glycosyl transfer using *p*-nitrophenyl β-D-galactopyranoside (PNPGal), β-D-mannosyl fluoride (ManF) and D-glucal. These donors were studied since transfer of such sugars by Abg yields a product that is itself a poor acceptor; thus, a single glycosyl transfer occurs, yielding a disaccharide. In contrast, transfer of a glucosyl moiety yields a product that is an excellent acceptor; thus, further glucosyl transfers occur, yielding trisaccharides and higher oligosaccharides. The glycosyl acceptor specificity was similarly explored using a range of nonhydrolyzable sugar derivatives, primarily thiophenyl and thiobenzyl glycosides. The reason for using thioglycosides as acceptors was their stability against the hydrolysis activity of the enzyme as well as the option of a subsequent chemical activation of the anomeric position for further glycosylation reactions [33]. Thioglycosides have proven to be powerful glycosylating agents in chemical synthesis by activation with specific thiophilic reagents [2,34,35]. Characterization of the glycosylation reaction products was performed on the per-*O*-acetylated sugars.

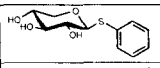
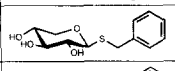
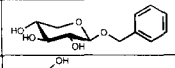
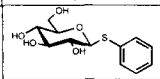
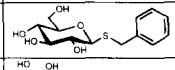
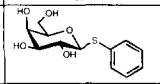
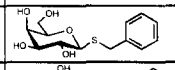
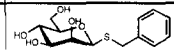
Experiments involving PNPGal as a glycosyl donor and a variety of aryl glycosides as acceptors are shown in Schemes 1 and 2 and are summarized in Table 1. Early experiments performed involved the use of very high initial concentrations of PNPGal to drive the reaction. Unfortunately, this resulted in a substantial amount of transfer of PNPGal to another molecule of PNPGal, yielding *p*-nitrophenyl β-D-galactopyranosyl-(1 → 6)-β-D-galactopyranoside. To circumvent this transfer, the *p*-nitrophenyl β-D-galactopyranoside was therefore added in small portions over the course of 2–3 h. Under these conditions, such 'self transfer' was minimized.

The xylosides (**2a–c**) and glucosides (**3a,b**) turned out to be the most effective acceptors in terms of yields. Even at relatively low concentrations of acceptor (50–125 mM), and despite competing hydrolysis of the donor by water, good yields could be achieved, indicating a relatively tight binding of these acceptors in the aglycone-binding subsite of the enzyme. An exceptional yield of 67% was observed for the galactosylation of benzyl 1-thio-β-D-glucopyranoside. The only coupling products that could be isolated from the reaction mixture were disaccharides having either β-(1 → 3) or β-(1 → 4) glycosidic link-



Scheme 1. Transglycosylation reactions carried out with PNPGal and aryl β-D-xylopyranosides or β-D-glucopyranosides.

Table 1
Transglycosylation reactions of *Agrobacterium* sp. β-glucosidase with *p*-nitrophenyl β-D-galactopyranoside (donor) and aryl glycosides (acceptors)

#	ACCEPTOR	PRODUCTS (% YIELD)		
		β-(1→3)-linked	β-(1→4)-linked	Total Yield† ratio (1→3, 1→4)
2a		**4a** (37%)	**5a** (9%)	**46%** (4 : 1)
2b		**4b** (38%)	**5b** (11%)	**49%** (3.6 : 1)
2c		**4c** (39%)	**5c** (11%)	**50%** (4 : 1)
3a		**6a** (38%)	**7a** (20%)	**58%** (1.9 : 1)
3b		**6b** (50.5%)	**7b** (16.5%)	**67%** (3 : 1)
8a		**9a** (9.5%)	-	**19%** ‡ (1 : 1)
8b		**9b** (8.8%)	-	**14%** ‡ (3.6 : 1)
11		-	**12** (24%)	**24%**

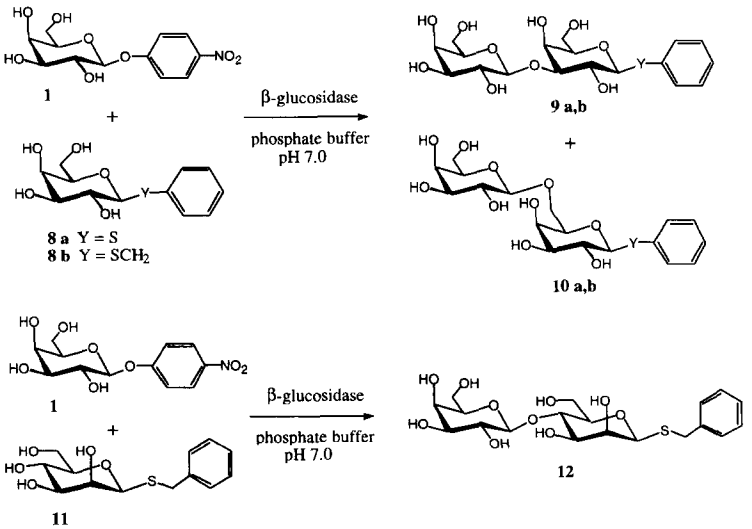
[a]Yields are based on isolated products. [b]The remainder of the yield and ratio refer to a second isolated regioisomer which is β-(1 → 6)-configured.

ages. Interestingly, the β-(1 → 3)-linked regioisomers were always obtained in higher yields than those of the β-(1 → 4)-linked products, despite the fact that the latter are the natural substrates of the enzyme.

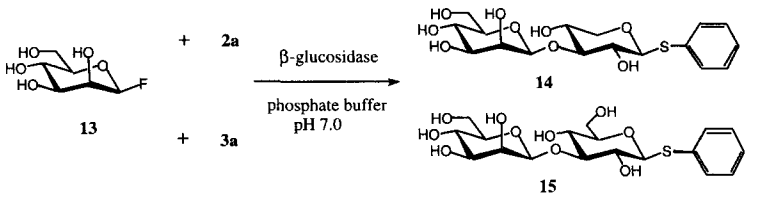
The reactions using *galacto-* or *manno*-configured aryl 1-thioglycosides as acceptors are outlined in Scheme 2 and again are summarized in Table 1. The aryl 1-thiogalactosides **8a** and **8b** clearly function as poor acceptors. This result is perhaps expected in the case of transfer to the axial 4-position of the sugar. The reduced yield for transfer to the 3-position could be due to inherently lower rates of transfer to that position or could arise if the axial 4-hydroxyl group greatly reduced the binding affinity between the acceptor and the enzyme. The only other linkage observed besides the β-(1 → 3) regioisomer is that of the β-(1 → 6) regioisomer (NMR data of peracetylated **10a** are in good agreement with those published [33]). No trace of any of the β-(1 → 4) regioisomer could be detected at any point during the course of reaction. The β-(1 → 6) linkage was not observed for any of the other sugar acceptors.

The transglycosylation experiments using benzyl 1-thio-β-D-mannopyranoside **11** as the acceptor resulted in only a single product, the β-(1 → 4)-linked disaccharide **12** in 24% yield. Thus, altering the configuration of the 2-hydroxyl from equatorial to axial results in complete loss of transfer to the 3-position. This result once again demonstrates the effect of the sugar configuration upon the linkage type observed.

The identity and stereochemistry of the anomeric substituent of the acceptor has been shown previously to be an important factor in controlling the regiochemical outcome of the reaction [24,36]. This can also be seen in the case of aryl glucoside and aryl galactoside acceptors, but not with aryl xylosides (see Table 1) where the addition of a methylene group to



Scheme 2. Transglycosylation reactions carried out with PNPGal and aryl 1-thio-β-D-galactopyranosides or -β-D-mannopyranoside.



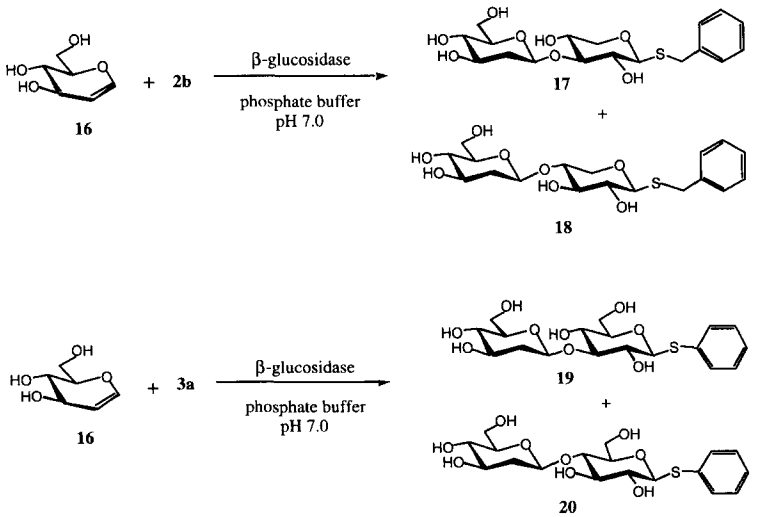
Scheme 3. Transglycosylation reactions carried out with β-D-mannopyranosyl fluoride and aryl 1-thio-β-D-xylopyranosides or -β-D-glucopyranosides.

the substituent results, in the best case, in a 4-fold increase in regioselectivity. A similar observation was reported for *E. coli* β-galactosidase in the galactosylation of aryl xylosides [26].

The potential of Abg for synthesis of 'difficult' glycosidic bonds was explored, as these cases might be the most attractive targets for enzymatic synthesis. As is shown in Scheme 3, β-glucosidase is indeed also capable of transferring β-D-mannopyranosyl fluoride to different acceptors. The yields dropped dramatically to below 10%, which is consistent with the relatively low k_{cat} value of Abg for β-mannopyranosides [27] and the limited stability of β-D-mannopyranosyl fluoride to hydrolysis. However, this is still considered to be a noteworthy result since the chemical synthesis of β-mannopyranosides usually requires a multistep synthesis [37–39]. The only regioisomer isolated was the β-(1 → 3)-linked product.

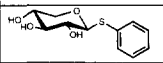
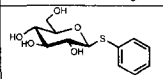
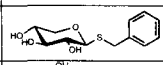
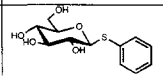
In order to synthesize 2-deoxy-D-glucopyranosides, D-glucal was used as the glycosyl donor, as demonstrated earlier, for enzymatic transglycosylations employing glycosidases (Scheme 4) [40]. Initial experiments with D-glucal at the enzyme concentration used for the other donors resulted in no observable products over the same time period. However, when the enzyme was used at high concentrations (0.7 mg/mL), Abg proved to be suitable for synthesizing 2-deoxyglycosides. Benzyl 1-thio-β-D-xylopyranoside and phenyl 1-thio-β-D-glucopyranoside were used successfully as acceptors, yielding the appropriate 2-deoxydisaccharides in moderate yields (27–32%, not optimized). Almost no regioselectivity could be observed in the formation of the two regioisomers (β-(1 → 3) and β-(1 → 4)) as indicated by the ratios in Table 2.

These results, therefore, demonstrate the versatility of *Agrobacterium* sp. β-glucosidase for the synthesis of oligosaccharides, particularly attractive features being its relatively broad specificity and the high yields obtainable. These findings also offer further insight into acceptor/enzyme interactions. The use of thioglycosides in chemoenzymatic synthesis may also



Scheme 4. Transglycosylation reactions carried out with D-glucal and aryl 1-thio-β-D-xylopyranosides or -β-D-glucopyranosides.

Table 2
Transglycosylation reactions of *Agrobacterium* sp. β-glucosidase with β-D-mannopyranosyl fluoride and D-glucal as donors and aryl glycosides as acceptors

DONOR	#	ACCEPTOR	PRODUCTS (% YIELD)		
			β-(1→3)-linked	β-(1→4)-linked	Total Yield[a]
 13	2 a		14 (9%)	-	9%
	3 a		15 (7%)	-	7%
 16	2 b		17 (16%)	18 (16%)	32%
	3 a		19 (15%)	20 (12%)	27%

[a]Yields are based on isolated products.

prove valuable since the sulfur provides both an enzymatically inert linkage on the acceptor and a chemically activatable moiety for further chemical couplings.

3. Experimental

General.—Buffer chemicals and other reagents were obtained from Sigma Chemical unless otherwise noted. Melting points (mp) were determined on a Laboratory Devices Mel-Temp II melting point apparatus and are uncorrected. NMR spectra were recorded on a Bruker WH 400, a Bruker AMX 500 or a Varian XL 300 instrument and are referenced to the solvent peak. Where required, the interpretations were supported by COSY or APT NMR experiments. Mass spectra were obtained by desorption chemical-ionization (DCI) on a Delsi Nermag R10-10C mass spectrometer with ammonia as the reactive gas. Microanalyses were performed by Mr. Peter Borda in the Microanalytical Laboratory of the University of British Columbia. Reactions were monitored by thin-layer chromatography (TLC) using E. Merck Kieselgel 60 F_{254} aluminium-backed sheets. Compounds were detected (where possible) under UV light or by charring with 10% H_2SO_4 in MeOH or with 10% ammonium molybdate in 2 M H_2SO_4. Separation of the mixtures of regioisomers [β-(1 → 3) and β-(1 → 4) linked disaccharides] was achieved using both flash chromatography and HPLC. Flash chromatography was performed on short columns (10–15 cm) of E. Merck Kieselgel 60 (230–400 mesh) with specified eluants. HPLC was performed on a Waters HPLC using a Dynamax column and 70:30 acetonitrile–water as the eluant (flow rate 7.0 mL/min).

Synthesis of the aryl 1-thio-β-glycosides.—All β-thioglycosides were prepared by minor variations of published methods. Benzyl 1-thio-β-D-glycosides were synthesized via the isothiouronium derivatives that were subsequently hydrolyzed to the thiols and then reacted with benzyl bromide [41,42]. The phenyl 1-thio-β-D-glycosides were obtained by reacting the β-peracetates with thiophenol and boron trifluoride etherate as a catalyst [26]. The physical data of all the obtained aryl 1-thio-β-D-glycosides were in accordance with those in the literature.

Purification of Agrobacterium sp. β-glucosidase.—Abg was expressed as previously described [30,43]. The enzyme was purified according to the protocol of Kempton and Withers [30] with the following modifications. The cell paste (~ 60 g) was suspended in 50 mM sodium phosphate, 2 mM EDTA, 10 mM β-mercaptoethanol, pH 7.0 buffer (ratio of 1–2 mL buffer/g of cell paste) and French pressed twice using a SLM Aminco French Pressure Cell Press with a 20-K cell. The cellular debris was removed via centrifugation (10,000 g, 30 min at 4 °C). Nucleic acids were removed from the crude extract by precipitation with 1.5% streptomycin sulfate (4 h at 4 °C), followed by centrifugation (10,000 g, 30 min at 4 °C). The extract was loaded onto a DEAE-Sepharose (Pharmacia) column (5.0 cm × 50 cm) previously equilibrated with 50 mM sodium phosphate, 2 mM EDTA, pH 7.0 buffer, and eluted with a 2 × 2 L linear gradient of 0–1 M NaCl in the starting buffer. The fractions containing Abg were pooled, concentrated using Amicon Centiprep 30 centrifuge ultrafiltration devices, and then dialyzed against the starting buffer. The protein was loaded onto a Source Q (Pharmacia) column (1.6 cm × 10 cm) previously equilibrated with 50 mM sodium phosphate, 2 mM EDTA, pH 7.0 buffer and eluted with a gradient of 0–40% of 1 M NaCl in the starting buffer over 30-column volumes. The fractions containing Abg were pooled, concentrated (40–50 mg/mL) and loaded onto a Superdex 200 or Superose 12 (Pharmacia) column (1.6 cm × 60 cm) previously equilibrated with 50 mM sodium phosphate, 2 mM EDTA, pH 7.0. The enzyme was judged to be homogeneous by SDS-PAGE silver stain. Protein concentration was determined using the absorbance value of $\varepsilon^{0.1\%} = 2.18\ cm^{-1}$ at 280 nm. Enzyme activity during purification was determined by assaying with *p*-nitrophenyl-β-D-glucopyranoside (PNPGlc). A spe-

cific activity of 200 μmol mg^{-1} min^{-1} at 37 °C with 1.0 mM PNPGlc, 0.1% BSA, 50 mM sodium phosphate, pH 7.0 was determined for the purified enzyme. All columns were performed at 4 °C.

General procedure for transglycosylation reactions using Agrobacterium sp. β-glucosidase.—The reactions were usually set up with 1.1–1.3 equivalents of the acceptor relative to the donor. Typical reactions were performed on a scale of 75–800 mg of donor and 100–1000 mg of acceptor.

p-Nitrophenyl β-D-galactopyranoside as the donor. In a typical experiment, the acceptor was dissolved in 50 mM sodium phosphate buffer, pH 7.0 at room temperature to give a concentration of 50–125 mM. In some cases, the concentration was limited by the solubility of the acceptor (e.g., max. conc. for **2b**, ~ 56 mM). Subsequently, the donor *p*-nitrophenyl β-D-galactopyranoside was added, typically starting with the addition of approximately 25% of the calculated required mass, followed by the addition of *Agrobacterium* sp. β-glucosidase (0.02–0.05 mg/mL). The balance of the donor was added in 3 equal batches with 1 h delay each time. The reaction was followed by TLC. (solvent system 7:2:1 EtOAc–MeOH–water) and stopped by freeze-drying the whole sample when the complete consumption of the donor was indicated (4–8 h). Separation of the regioisomers was achieved by using HPLC and/or silica gel chromatography on both the unprotected (gradient 40:2:1–20:2:1 EtOAc–MeOH–water) and the acetylated (3:2 petroleum ether–EtOAc) sugars.

β-D-Mannopyranosyl fluoride as the donor. 2,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranosyl fluoride [44] was deprotected using Zemplén and Pascu conditions [45] just before it was used for the reaction. The reaction was performed on small scale (approx. 50 mg) and worked up by neutralization with Dowex-50 × 8 resin with removal of the solvent under vacuum. The deprotected β-fluoride was used immediately without further purification (^{1}H NMR indicated that it contained less than 10% of hydrolyzed product) by dissolving in 250 mM sodium phosphate buffer, pH 7.0 to give a 1-M stock solution. The required amount of β-glycosyl fluoride, 1.2 equiv. based on the acceptor, was added in 4 portions over a period of 4 h to a buffer solution (250 mM sodium phosphate, pH 7.0) containing the acceptor (80–100 mM) and the enzyme (0.25–0.35 mg/mL). Slower reaction rates with this donor and its limited stability necessitated use of high enzyme concentrations. The reaction was stopped by freeze-drying the whole sample when the complete consumption of the donor was indicated (8–12 h). Purification was performed as described above.

D-Glucal as the donor. In a typical experiment, the acceptor was dissolved in 50 mM sodium phosphate buffer, pH 7.0 to give a concentration of 40–55 mM. Subsequently, 1 equiv. of D-glucal was added, followed by the enzyme. The final enzyme concentrations used were between 0.77 and 0.85 mg/mL. The reaction was allowed to stand overnight at room temperature and was stopped by freeze-drying the whole sample after 18 h. Separation of the regioisomers was achieved by using silica gel chromatography on both the unprotected (gradient 40:2:1–20:2:1 EtOAc–MeOH–water) and the acetylated (3:2 petroleum ether–EtOAc) disaccharides.

Acetylation of the unprotected oligosaccharides was carried out using Ac_2O–pyridine 2:3 (v/v) at room temperature. The mixtures were allowed to stand overnight (12 h), and then the solvent was removed by evaporation in vacuo.

Phenyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1 → 3)-2,4-di-O-acetyl-1-thio-β-D-xylopyranoside (per-O-acetylated **4a**).—^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43 (m, 2 H, Ph), 7.25 (m, 3 H, Ph), 5.37 (dd, 1 H, $J_{4',5'}$ 0.6 Hz, H-4'), 5.18 (dd, 1 H, $J_{2',3'}$ 10.4 Hz, H-2'), 4.99 (dd, 1 H, $J_{3',4'}$ 2.7 Hz, H-3'), 4.98 (m, 2 H, H-1, H-2), 4.88 (ddd, 1 H, $J_{4,5a}$ 3.8 Hz, H-4), 4.63 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.38 (dd, 1 H, $J_{5a,5b}$ 12.4 Hz, H-5eq), 4.18 (dd, 1 H, $J_{5',6'a}$ 6.4 Hz, H-6'a), 4.06 (dd, 1 H, $J_{6'a,6'b}$ 11.2 Hz, H-6'b), 3.92 (ddd, 1 H, $J_{5',6'b}$ 7.2 Hz, H-5'), 3.90 (dd, 1 H, $J_{3,4}$ 5.5 Hz, H-3), 3.50 (dd, 1 H, $J_{4,5b}$ 6.1 Hz, H-5ax), 2.14, 2.12, 2.08, 2.05, 2.04, 1.97 (6 s, 18 H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4, 170.2 (2 ×), 169.6, 169.4, 169.3 (Ac), 134.1, 131.5, 129.0, 127.6 (Ph), 101.1 (C-1'), 86.0 (C-1), 75.5 (C-3), 71.1 (C-2), 70.7 (C-5'), 70.5 (C-3'), 68.5 (C-2'), 68.4 (C-4), 66.9 (C-4'), 62.8 (C-5), 61.0 (C-6'), 21.0, 20.9 (2 ×), 20.7, 20.6, 20.5. MS (DCI) *m/z* 674 (M + 18). Anal. Calcd. for $C_{29}H_{36}O_{15}S$ (656.66): C, 53.04; H, 5.53; S, 4.88. Found: C, 53.25; H, 5.43; S, 4.66.

Phenyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1 → 4)-2,3-di-O-acetyl-1-thio-β-D-xylopyranoside (per-O-acetylated **5a**).—^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44 (m, 2 H, Ph), 7.25 (m, 3 H, Ph), 5.34 (dd, 1 H, $J_{4',5'}$ 0.6 Hz, H-4'), 5.14 (dd, 1 H, $J_{3,4}$ 7.8 Hz, H-3), 5.08 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-2'), 4.96 (dd, 1 H, $J_{3',4'}$ 3.5 Hz, H-3'), 4.89 (dd, 1 H, $J_{2,3}$ 8.1 Hz, H-2), 4.79 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.48 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.13 (dd, 1 H, $J_{5a,5b}$ 12.0 Hz, H-5eq), 4.08 (m, 2 H, H-6'a, H-6'b), 3.88 (ddd, 1 H, $J_{5',6'a}$ 6.5 Hz, $J_{5',6'b}$ 7.2 Hz, H-5'), 3.78 (ddd, 1 H, $J_{4,5a}$ 4.8 Hz, H-4), 3.38 (dd, 1 H, $J_{4,5b}$ 8.7 Hz, H-5ax), 2.15, 2.11,

2.09, 2.08, 2.05, 1.97 (6 s, 18 H, Ac). MS (DCI) *m/z* 674 (M + 18). Anal. Calcd. for $C_{29}H_{36}O_{15}S$ (656.66): C, 53.04; H, 5.53; S, 4.88. Found: C, 53.14; H, 5.77; S, 4.59.

*Benzyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 3)-2,4-di-*O*-acetyl-1-thio-β-*D*-xylopyranoside (per-*O*-acetylated* **4b**).—^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25 (m, 5 H, Ph), 5.34 (dd, 1 H, $J_{4',5'}$ 0.9 Hz, H-4'), 5.13 (dd, 1 H, $J_{2',3'}$ 10.4 Hz, H-2'), 4.93 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3'), 4.90 (m, 1 H, H-4), 4.87 (dd, 1 H, $J_{2,3}$ 9.0 Hz, H-2), 4.58 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.48 (d, 1 H, $J_{1,2}$ 6.4 Hz, H-1), 4.30 (dd, 1 H, $J_{4,5a}$ 4.2 Hz, $J_{5a,5b}$ 12.2 Hz, H-5eq), 4.17 (dd, 1 H, $J_{5',6'a}$ 6.3 Hz, H-6'a), 4.05 (dd, 1 H, $J_{6'a,6'b}$ 11.2 Hz, H-6'b), 3.85 (ddd, 1 H, $J_{5',6'b}$ 7.2 Hz, H-5'), 3.82 (dd, 1 H, $J_{3,4}$ 7.5 Hz, H-3), 3.83, 3.77 (2 d, 2 H, SCH_2), 3.38 (dd, 1 H, $J_{4,5b}$ 6.1 Hz, H-5ax), 2.12, 2.07, 2.03, 2.02, 2.00, 1.96 (6 s, 18 H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4, 170.2, 170.1, 169.6, 169.4, 169.3 (Ac), 137.1, 129.1, 128.5, 127.2 (Ph), 101.1 (C-1'), 81.6 (C-1), 76.2 (C-3), 71.0 (C-5'), 70.6 (C-3'), 70.2 (C-2), 68.5 (2 ×, C-4, C-2'), 66.8 (C-4'), 63.3 (C-5), 60.9 (C-6'), 34.1 (SCH_2), 20.9, 20.8, 20.7 (2 ×), 20.6, 20.5 (Ac). MS (DCI) *m/z* 688 (M + 18). Anal. Calcd. for $C_{30}H_{38}O_{15}S$ (670.68): C, 53.73; H, 5.71; S, 4.78. Found: C, 53.44; H, 5.65; S, 4.73.

*Benzyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 4)-2,3-di-*O*-acetyl-1-thio-β-*D*-xylopyranoside (per-*O*-acetylated* **5b**).—^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28 (m, 5 H, Ph), 5.32 (dd, 1 H, $J_{4',5'}$ 1.0 Hz, H-4'), 5.08 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-2'), 5.06 (dd, 1 H, $J_{3,4}$ 8.2 Hz, H-3), 4.96 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3'), 4.88 (dd, 1 H, $J_{2,3}$ 8.1 Hz, H-2), 4.49 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.38 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 4.11 (dd, 1 H, $J_{5a,5b}$ 11.8 Hz, H-5eq), 4.08 (m, 2 H, H-6'a, H-6'b), 3.88 (ddd, 1 H, $J_{5',6'a}$ 6.4 Hz, $J_{5',6'b}$ 7.2 Hz, H-5'), 3.84, 3.78 (2 d, 2 H, SCH_2), 3.77 (ddd, 1 H, $J_{4,5a}$ 4.8 Hz, H-4), 3.38 (dd, 1 H, $J_{4,5b}$ 8.9 Hz, H-5ax), 2.11, 2.02, 2.01 (2 ×), 1.99, 1.94 (6 s, 18 H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4, 170.1, 170.05 (2 ×), 169.6, 169.5, 168.5 (Ac), 136.9, 128.9, 128.6, 127.3 (Ph), 101.1 (C-1'), 82.4 (C-1), 75.5 (C-4), 72.1 (C-3), 70.8 (C-5'), 70.75 (C-3'), 69.7 (C-2), 68.9 1 (C-2'), 66.8 (C-4'), 65.5 (C-4), 61.1 (C-6'), 34.2 (SCH_2), 20.7, 20.65, 20.6 (3 ×), 20.5 (Ac). MS (DCI) *m/z* 688 (M + 18). Anal. Calcd. for $C_{30}H_{38}O_{15}S$ (670.68): C, 53.73; H, 5.71; S, 4.73. Found: C, 53.38; H, 5.92; S, 4.77.

*Benzyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 3)-2,4-di-*O*-acetyl-β-*D*-xylopyranoside (per-*O*-acetylated* **4c**).—^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30 (m, 5 H, Ph), 5.33 (dd, 1 H, $J_{4',5'}$ 0.9 Hz, H-4'), 5.12 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-2'), 4.94 (dd, 1 H, $J_{3',4'}$ 3.5 Hz, H-3'), 4.90 (dd, 1 H, $J_{2,3}$ 6.2 Hz, H-2), 4.89 (m, 1 H, H-4), 4.78, 4.54 (2 d, 2 H, OCH_2), 4.57 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.48 (d, 1 H, $J_{1,2}$ 5.6 Hz, H-1), 4.18 (dd, 1 H, $J_{5',6'a}$ 6.3 Hz, H-6'a), 4.00 (dd, 1 H, $J_{4,5a}$ 5.0 Hz, $J_{5a,5b}$ 12.1 Hz, H-5eq), 4.05 (dd, 1 H, $J_{6'a,6'b}$ 11.2 Hz, H-6'b), 3.86 (ddd, 1 H, $J_{5',6'b}$ 7.2 Hz, H-5'), 3.83 (dd, 1 H, $J_{3,4}$ 7.1 Hz, H-3), 3.36 (dd, 1 H, $J_{4,5b}$ 6.1 Hz, H-5ax), 2.12, 2.06, 2.05, 2.04, 1.97, 1.96 (6 s, 18 H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4, 170.2 (2 ×), 169.7, 169.3, 169.1, (Ac), 136.9, 128.4, 127.8, 127.7 (Ph), 101.1 (C-1'), 98.5 (C-1), 76.3 (C-3), 71.1 (C-5'), 70.7 (C-3'), 70.5 (C-2), 69.6 (OCH_2), 68.9 (C-4), 68.5 (C-2'), 66.8 (C-4'), 61.1 (C-5), 60.9 (C-6'), 20.9 (2 ×), 20.6 (2 ×), 20.5 (2 ×), (Ac). Anal. Calcd. for $C_{30}H_{38}O_{16}$ (654.62): C, 55.04; H, 5.85. Found: C. 54.72; H, 5.68.

*Benzyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 4)-2,3-di-*O*-acetyl-β-*D*-xylopyranoside (per-*O*-acetylated* **5c**).—^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30 (m, 5 H, Ph), 5.33 (dd, 1 H, $J_{4',5'}$ 0.8 Hz, H-4'), 5.09 (dd, 1 H, $J_{3,4}$ 8.4 Hz, H-3), 5.08 (dd, 1 H, $J_{2',3'}$ 10.0 Hz, H-2'), 4.97 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3'), 4.89 (dd, 1 H, $J_{2,3}$ 8.5 Hz, H-2), 4.82, 4.56 (2 d, 2 H, OCH_2), 4.51 (d, 1 H, $J_{1,2}$ 6.5 Hz, H-1), 4.48 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.09 (m, 2 H, H-6'a, H-6'b), 3.98 (dd, 1 H, $J_{5a,5b}$ 11.9 Hz, H-5eq), 3.88 (ddd, 1 H, $J_{5',6'a}$ 6.5 Hz, $J_{5',6'b}$ 7.2 Hz, H-5'), 3.82 (ddd, 1 H, $J_{4,5a}$ 4.9 Hz, H-4), 3.32 (dd, 1 H, $J_{4,5b}$ 8.9 Hz, H-5ax), 2.12, 2.03, 2.02 (2 ×), 2.00, 1.97 (6 s, 18 H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4, 170.2, 170.1, 169.9, 169.7, 169.0, (Ac), 136.9, 128.4, 127.9, 127.6 (Ph), 101.1 (C-1'), 98.4 (C-1), 75.7 (C-4), 71.7, (C-3), 70.9 (2 ×, C-5', C-3'), 70.7 (OCH_2), 70.4 (C-2), 68.9 (C-2'), 66.8 (C-4'), 62.5 (C-5), 61.1 (C-6'), 20.8, 20.7 (2 ×), 20.6 (2 ×), 20.5 (Ac). Anal. Calcd. for $C_{30}H_{38}O_{16}$ (654.62): C, 55.04; H, 5.85. Found: C, 54.79; H, 5.76.

*Phenyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-1-thio-β-*D*-glucopyranoside (per-*O*-acetylated* **6a**).—^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47 (m, 2 H, Ph), 7.28 (m, 3 H, Ph), 5.31 (dd, 1 H, $J_{4',5'}$ 0.7 Hz, H-4'), 5.04 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-2'), 5.01 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-2), 4.92 (dd, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 4.91 (dd, 1 H, $J_{3',4'}$ 3.5 Hz, H-3'), 4.58 (d, 1 H, $J_{1,2}$ 10.1 Hz, H-1), 4.53 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.19 (dd, 1 H, $J_{5,6a}$ 2.6 Hz, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.15 (m, 2 H, H-6b, H-6'a), 4.03 (dd, 1 H, $J_{5',6'a}$ 7.2 Hz, $J_{6'a,6'b}$ 11.1 Hz, H-6'b), 3.90 (dd, 1 H, $J_{3,4}$ 9.3 Hz, H-3), 3.85 (ddd, 1 H, $J_{5',6'b}$ 6.5 Hz,

H-5′), 3.68 (ddd, 1 H, $J_{5,6b}$ 5.2 Hz, H-5), 2.14, 2.12, 2.05, 2.03, 2.01, 2.00, 1.93 (7 s, 21 H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4, 170.3, 170.2, 170.1, 169.4, 169.2, 168.9 (Ac), 133.0, 132.3, 128.9, 128.1 (Ph), 101.2 (C-1′), 86.2 (C-1), 79.7 (C-3), 75.8 (C-5), 71.5 (C-2), 71.0 (C-5′), 70.4 (C-3′), 68.6 (C-2′), 68.2 (C-4), 66.7 (C-4′), 62.4 (C-6′), 60.9 (C-6), 21.0, 20.9, 20.8, 20.7, 20.7, 20.6, 20.5 (Ac). Anal. Calcd. for $C_{32}H_{40}O_{17}S$ (728.72): C, 52.74; H, 5.53; S, 4.40. Found: C, 52.57; H, 5.48; S, 4.25.

*Phenyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-1-thio-β-*D*-glucopyranoside (per-O-acetylated* **7a**).—^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48 (m, 2 H, Ph), 7.28 (m, 3 H, Ph), 5.31 (dd, 1 H, $J_{4',5'}$ 0.7 Hz, H-4′), 5.19 (dd, 1 H, $J_{3,4}$ 9.1 Hz, H-3), 5.07 (dd, 1 H, $J_{2',3'}$ 10.4 Hz, H-2′), 4.92 (dd, 1 H, $J_{3',4'}$ 2.9 Hz, H-3′), 4.87 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 4.65 (d, 1 H, $J_{1,2}$ 10.1 Hz, H-1), 4.50 (dd, 1 H, $J_{5',6'a}$ 1.8 Hz, $J_{6'a,6'b}$ 11.8 Hz, H-6′a), 4.44 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.13–4.03 (m, 3 H, H-6a, H-6b, H-6′b), 3.84 (ddd, 1 H, $J_{5',6'b}$ 6.5 Hz, H-5′), 3.71 (dd, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 3.61 (ddd, 1 H, $J_{5,6a}$ 1.8 Hz, $J_{5,6b}$ 5.5 Hz, H-5), 2.10, 2.06, 2.04, 2.01, 2.00, 1.98, 1.92 (7 s, 21 H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3, 170.2, 170.1, 170.0, 169.7, 169.5, 169.0 (Ac), 134.1, 131.7, 128.9, 128.3 (Ph), 101.0 (C-1′), 85.5 (C-1), 76.7 (C-4), 76.1 (C-5), 73.8 (C-3), 70.9 (C-5′), 70.7 (C-3′), 70.3 (C-2), 69.1 (C-2′), 66.6 (C-4′), 62.1 (C-6′), 60.8 (C-6), 20.8, 20.7, 20.6, 20.6, 20.6, 20.5, 20.5 (Ac). Anal. Calcd. for $C_{32}H_{40}O_{17}S$ (728.72): C, 52.74; H, 5.53; S, 4.40. Found: C, 52.82; H, 5.43; S, 4.33.

*Benzyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-1-thio-β-*D*-glucopyranoside (per-O-acetylated* **6b**).—^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25 (m, 5 H, Ph), 5.31 (dd, 1 H, $J_{4',5'}$ 0.8 Hz, H-4′), 5.06 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 5.04 (dd, 1 H, $J_{2',3'}$ 10.4 Hz, H-2′), 4.93 (dd, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 4.89 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3′), 4.50 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.16 (dd, 1 H, $J_{6a,6b}$ 12.4 Hz, H-6a), 4.15 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.10 (m, 2 H, H-6b, H-6′b), 4.01 (dd, 1 H, $J_{5',6'a}$ 7.4 Hz, $J_{6'a,6'b}$ 11.0 Hz, H-6′a), 3.89 (d, 1 H, SCH_2), 3.82 (m, 1 H, H-5′), 3.81 (dd, 1 H, $J_{3,4}$ 9.4 Hz, H-3), 3.78 (d, 1 H, SCH_2), 3.55 (ddd, 1 H, $J_{5,6a}$ 3.8 Hz, $J_{5,6b}$ 7.6 Hz, H-5), 2.11, 2.09, 2.07, 2.03, 2.00, 1.99, 1.93 (7 s, 18 H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.7, 170.4, 170.3, 170.2, 169.3, 169.2, 169.1 (Ac), 136.9, 129.1, 128.5, 127.3 (Ph), 101.1 (C-1′), 81.7 (C-1), 79.7 (C-3), 75.9 (C-5), 71.3 (C-2), 71.0 (C-5′), 70.4 (C-3′), 68.5 (C-2′), 68.3 (C-4), 66.7 (C-4′), 62.5 (C-6), 60.8 (C-6′), 33.4 (SCH_2), 20.9, 20.8, 20.7 (2 ×), 20.6, 20.5, 20.4 (Ac). Anal. Calcd. for $C_{33}H_{42}O_{17}S$ (742.75): C, 53.36; H, 5.70; S, 4.32. Found: C, 53.24; H, 5.60; S, 4.34.

*Benzyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-1-thio-β-*D*-glucopyranoside (per-O-acetylated* **7b**).—^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25 (m, 5 H, Ph), 5.31 (dd, 1 H, $J_{4',5'}$ 0.8 Hz, H-4′), 5.09 (dd, 1 H, $J_{3,4}$ 9.1 Hz, H-3), 5.06 (dd, 1 H, $J_{2',3'}$ 10.4 Hz, H-2′), 4.94 (dd, 1 H, $J_{2,3}$ 9.7 Hz, H-2), 4.92 (dd, 1 H, $J_{3',4'}$ 3.8 Hz, H-3′), 4.45 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.44 (m, 1 H, H-6a), 4.24 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1), 4.07 (m, 3 H, H-6b, H-6′a, H-6′b), 3.88 (d, 1 H, SCH_2), 3.83 (ddd, 1 H, $J_{5',6'a}$ 7.0 Hz, $J_{5',6'b}$ 7.0 Hz, H-5′), 3.78 (d, 1 H, SCH_2), 3.74 (dd, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 3.48 (ddd, 1 H, $J_{5,6a}$ 1.8 Hz, $J_{5,6b}$ 5.5 Hz, H-5), 2.14 (2 ×), 2.04 (2 ×), 2.0, 1.98, 1.92 (7 s, 21 H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3 (2 ×), 170.1, 170.0 (2 ×), 169.6, 169.1, 169.0 (Ac), 136.8, 129.0, 128.6, 127.4 (Ph), 101.0 (C-1′), 81.6 (C-1), 76.6 (C-4), 76.5 (C-5), 73.8 (C-3), 71.0 (C-5′), 70.7 (C-3′), 70.2 (C-2), 69.1 (C-2′), 66.6 (C-4′), 62.3 (C-6), 60.8 (C-6′), 33.8 (SCH_2), 20.9, 20.8 (2 ×), 20.6 (2 ×), 20.5 (Ac). Anal. Calcd. for $C_{19}H_{28}O_{10}S$ (448.48; unprotected) C, 50.88; H, 6.29; S, 7.15. Found: C, 50.61; H, 5.93; S, 7.30.

*Phenyl 2,3,4,6-tetra-*O*-acetyl-galactopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-1-thio-β-*D*-galactopyranoside (per-*O*-acetylated* **9a**).—^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48 (m, 2 H, Ph), 7.27 (m, 3 H, Ph), 5.38 (dd, 1 H, $J_{4',5'}$ 0.6 Hz, H-4), 5.32 (dd, 1 H, $J_{4',5'}$ 1.0 Hz, H-4′), 5.23 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2), 5.07 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-2′), 4.91 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3′), 4.61 (d, 1 H, $J_{1,2}$ 10.1 Hz, H-1), 4.55 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.09 (m, 4 H, H-6a, H-6b, H-6′a, H-6′b), 3.87 (dd, 1 H, $J_{3,4}$ 3.4 Hz, H-3), 3.84 (m, 2 H, H-5, H-5′), 2.13, 2.11, 2.09, 2.03, 2.02, 2.00, 1.95 (7 s, 21 H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4, 170.3 (2 ×), 170.1, 170.0, 169.1, 169.0 (Ac), 133.2, 132.2, 128.58, 127.9 (Ph), 101.2 (C-1′), 86.7 (C-1), 77.1 (C-3), 70.9 (C-5′), 70.7 (C-3′), 69.4, 69.2 (2 ×, C-2, C-4), 68.7 (C-2′), 66.8 (C-4′), 62.5 (C-6), 61.1 (C-6′), 20.9, 20.8, 20.7, 20.6 (2 ×), 20.5 (2 ×), (Ac). Anal. Calcd. for $C_{32}H_{40}O_{17}S$ (728.72): C, 52.74; H, 5.53; S, 4.40. Found: C, 52.47; H, 5.45; S, 4.35.

*Benzyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl-(1 → 6)-2,3,4-tri-*O*-acetyl-1-thio-β-*D*-galactopyranoside (per-*O*-acetylated* **10b**).—^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30 (m, 5 H, Ph), 5.37 (br s, 2 H, H-4, H-4′), 5.24 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 5.19 (dd, 1 H, $J_{2',3'}$ 10.4 Hz, H-2′), 4.99 (dd, 1 H, $J_{3',4'}$ 3.4 Hz,

H-3), 4.89 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3'), 4.48 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1'), 4.26 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1), 4.13 (m, 2 H, H-6'a, H-6'b), 3.94 (d, 1 H, SCH_2), 3.88 (m, 1 H, H-5), 3.82 (d, 1 H, SCH_2), 3.78 (m, 2 H, H-6a, H-5'), 3.70 (dd, 1 H, $J_{5,6b}$ 8.3 Hz, $J_{6a,6b}$ 12.0 Hz, H-6b), 2.12 (2 ×), 2.04, 2.02, 1.98, 1.94, 1.93 (7 s, 21 H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.5, 170.4, 170.2 (2 ×), 170.0, 169.2, 169.1 (Ac), 137.1, 129.2, 128.6, 127.3 (Ph), 100.9 (C-1'), 82.4 (C-1), 71.9 (C-5), 70.9 (C-5'), 70.6 (C-3'), 68.5 (C-2'), 67.9, 67.4, 67.1, 66.9 (C-2, C-3, C-4, C-6), 66.8 (C-4'), 61.2 (C-6'), 33.7 (SCH_2), 20.8, 20.7 (3 ×), 20.6 (2 ×), 20.5 (Ac). Anal. Calcd. for $C_{33}H_{42}O_{17}S$ (742.75): C, 53.36; H, 5.70; S, 4.32. Found: C, 53.34; H, 5.79; S, 4.30.

Benzyl 2,3,4,6-tetra-O-*acetyl*-β-D-*galactopyranosyl*-(*1 → 3*)-*2,4,6-tri*-O-*acetyl-1-thio*-β-D-*galactopyranoside* (*per*-O-*acetylated* **9b**).—^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30 (m, 5 H, Ph), 5.38 (dd, 1 H, $J_{4',5'}$ 0.6 Hz, H-4), 5.32 (dd, 1 H, $J_{4',5'}$ 0.7 Hz, H-4'), 5.23 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2), 5.03 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-2'), 4.89 (dd, 1 H, $J_{3',4'}$ 3.5 Hz, H-3'), 4.52 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.13 (d, 1 H, $J_{1,2}$ 10.1 Hz, H-1), 4.08 (m, 4 H, H-6a, H-6b, H-6'a, H-6'b), 3.92 (d, 1 H, SCH_2), 3.79 (m, 2 H, SCH_2, H-5'), 3.77 (dd, 1 H, $J_{3,4}$ 3.5 Hz, H-3), 3.73 (m, 1 H, H-5), 2.13, 2.11, 2.08, 2.02, 2.02, 1.98, 1.94 (7 s, 21 H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.5, 170.4 (2 ×), 170.2, 170.0, 169.2, 169.1 (Ac), 137.1, 129.1, 128.5, 127.3 (Ph), 101.1 (C-1'), 81.9 (C-1), 76.6 (C-3), 75.2 (C-5), 70.7 (C-5'), 70.6 (C-3'), 69.2 (2 ×, C-2, C-4), 68.5 (C-2'), 66.6 (C-4'), 62.5 (C-6), 60.9 (C-6'), 33.5 (SCH_2), 20.9, 20.8, 20.7 (2 ×), 20.6, 20.5, 20.4 (Ac). Anal. Calcd. for $C_{33}H_{42}O_{17}S$ (742.75): C, 53.36; H, 5.70; S, 4.32. Found: C, 53.32; H, 5.77; S, 4.36.

Benzyl 2,3,4,6-tetra-O-*acetyl*-β-D-*galactopyranosyl*-(*1 → 4*)-*2,3,6-tri*-O-*acetyl-1-thio*-β-D-*mannopyranoside* (*per*-O-*acetylated* **12**).—^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25 (m, 5 H, Ph), 5.32 (dd, 1 H, $J_{4',5'}$ 0.9 Hz, H-4'), 5.27, (dd, 1 H, $J_{3,4}$ 8.5 Hz, H-3), 5.25 (dd, 1 H, $J_{2,3}$ 3.6 Hz, H-2), 4.94 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3'), 4.51 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.28 (m, 2 H, H-5, H-6a), 4.17 (dd, 1 H, $J_{5,6b}$ 5.7 Hz, $J_{6a,6b}$ 11.8 Hz, H-6b), 4.13 (dd, 1 H, $J_{5',6'a}$ 6.1 Hz, H-6'a), 4.01 (dd, 1 H, $J_{6'a,6'b}$ 11.1 Hz, H-6'b), 3.90 (dd, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 3.84 (ddd, 1 H, $J_{5',6'b}$ 7.6 Hz, H-5'), 3.74, 3.68 (2 d, 2 H, SCH_2), 2.13, 2.11, 2.08, 2.06, 2.02, 1.98, 1.96 (7 s, 21 H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4 (2 ×), 170.1 (2 ×), 170.0, 169.6, 169.2, 169.1 (Ac), 136.8, 129.0, 128.7, 127.3 (Ph), 101.1 (C-1'), 80.8 (C-1), 74.5 (C-4), 71.0 (C-5'), 70.7 (C-3'), 70.4, 70.0, 69.4 (C-2, C-3, C-5), 69.1 (C-2'), 66.5 (C-4'), 62.5 (C-6), 60.8 (C-6'), 34.2 (SCH_2), 20.9, 20.8, 20.7, 20.6 (3 ×), 20.5 (Ac). Anal. Calcd. for $C_{33}H_{42}O_{17}S$ (742.75): C, 53.36; H, 5.70; S, 4.32. Found: C, 53.32; H, 5.60; S, 4.20.

Phenyl 2,3,4,6-tetra-O-*acetyl*-β-D-*mannopyranosyl*-(*1 → 3*)-*2,4-di*-O-*acetyl-1-thio*-β-D-*xylopyranoside* (*per*-O-*acetylated* **14**).—^{1}H NMR (500 MHz, $CDCl_3$): δ 7.42 (m, 2 H, Ph), 7.25 (m, 3 H, Ph), 5.47 (dd, 1 H, $J_{2',3'}$ 3.3 Hz, H-2'), 5.21 (dd, 1 H, $J_{4',5'}$ 10.0 Hz, H-4'), 5.02 (dd, 1 H, $J_{3',4'}$ 10.0 Hz, H-3'), 5.00 (d, 1 H, $J_{1,2}$ 5.5 Hz, H-1), 4.98 (ddd, 1 H, $J_{4,5b}$ 2.6 Hz, H-4), 4.94 (dd, 1 H, $J_{2,3}$ 5.5 Hz, H-2), 4.80 (d, 1 H, $J_{1',2'}$ 1.1 Hz, H-1'), 4.40 (dd, 1 H, $J_{4,5a}$ 3.7 Hz, H-5eq), 4.29 (dd, 1 H, $J_{5',6'a}$ 5.6 Hz, H-6'a), 4.10 (dd, 1 H, $J_{6'a,6'b}$ 9.5 Hz, H-6'b), 3.94 (dd, 1 H, $J_{3,4}$ 5.8 Hz, H-3), 3.65 (ddd, 1 H, $J_{5',6'b}$ 2.6 Hz, H-5'), 3.50 (dd, 1 H, $J_{5a,5b}$ 12.4 Hz, H-5ax), 2.17, 2.10, 2.085, 2.08, 2.03, 1.98 (6 s, 18 H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.1, 170.6, 170.2, 170.0, 169.5, 169.3 (Ac), 134.2, 131.5, 129.0, 127.6 (Ph), 98.6 (C-1'), 85.8 (C-1), 76.1 (C-3), 72.6 (C-5'), 70.9 (C-3'), 70.1 (C-2), 68.7 (C-4), 68.5 (C-2'), 65.9 (C-4'), 62.5 (C-6'), 53.3 (C-5), 21.0, 20.9, 20.8, 20.7, 20.6, 20.5 (Ac). Anal. Calcd. for $C_{29}H_{36}O_{15}S$ (656.66): C, 53.04; H, 5.53; S, 4.88. Found: C, 53.24; H, 5.28; S, 4.67.

Phenyl 2,3,4,6-tetra-O-*acetyl*-β-D-*mannopyranosyl*-(*1 → 3*)-*2,4,6-tri*-O-*acetyl-1-thio*-β-D-*glucopyranoside* (*per*-O-*acetylated* **15**).—^{1}H NMR (500 MHz, $CDCl_3$): δ 7.48 (m, 2 H, Ph), 7.28 (m, 3 H, Ph), 5.28 (dd, 1 H, $J_{2',3'}$ 3.0 Hz, H-2'), 5.18 (dd, 1 H, $J_{4',5'}$ 10.0 Hz, H-4'), 5.00 (dd, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 4.98 (dd, 1 H, $J_{2,3}$ 9.4 Hz, H-2), 4.96 (dd, 1 H, $J_{3',4'}$ 10.0 Hz, H-3'), 4.64 (d, 1 H, $J_{1',2'}$ 0.7 Hz, H-1'), 4.59 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1), 4.31 (dd, 1 H, $J_{5',6'a}$ 4.9 Hz, H-6'a), 4.15 (m, 2 H, H-6a, H-6b), 4.07 (dd, 1 H, $J_{6'a,6'b}$ 12.3 Hz, H-6'a), 3.91 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3), 3.62 (ddd, 1 H, $J_{5',6'b}$ 2.3 Hz, H-5'), 3.58 (ddd, 1 H, $J_{5,6a}$ 2.5 Hz, $J_{5,6b}$ 2.5 Hz, $J_{6a,6b}$ 10.1 Hz, H-5), 2.14, 2.11, 2.06, 2.05, 2.04, 2.00, 1.96 (7 s, 21 H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6, 170.4, 170.1, 170.0, 169.5, 169.1, 168.8 (Ac), 132.5, 128.9, 128.1 (Ph), 98.5 (C-1'), 86.3 (C-1), 80.2 (C-3), 76.0 (C-5'), 72.5 (C-5), 71.4, 70.9, 68.1 (C-2, C-3', C-4), 68.3 (C-2'), 65.8 (C-4'), 62.4 (C-6), 62.3 (C-6'), 21.0, 20.7, 20.6, 20.6, 20.6, 20.5, 20.5 (Ac). Anal. Calcd. for $C_{32}H_{40}O_{17}S$ (728.72): C, 52.74; H, 5.53; S, 4.40. Found: C, 52.61; H, 5.55; S, 4.41.

Benzyl 3,4,6-tri-O-*acetyl-2-deoxy*-β-D-arabino-*hexopyranosyl*-(*1 → 3*)-*2,4-di*-O-*acetyl-1-thio*-β-D-*xylopyranoside* (*per*-O-*acetylated* **17**).—^{1}H NMR

(400 MHz, $CDCl_3$): δ 7.29 (m, 5 H, Ph), 4.94 (m, 3 H, H-4, H-4′, H-3′), 4.85 (dd, 1 H, $J_{2,3}$ 5.8 Hz, H-2), 4.64 (dd, 1 H, $J_{1',2'ax}$ 9.7 Hz, $J_{1',2'eq}$ 2.0 Hz, H-1′), 4.57 (d, 1 H, $J_{1,2}$ 5.7 Hz, H-1), 4.32 (dd, 1 H, $J_{5a,4}$ 3.8 Hz, $J_{5a,5b}$ 12.2 Hz, H-5a), 4.28 (dd, 1 H, $J_{6'a,5'}$ 4.6 Hz, H-6′a), 4.05 (dd, 1 H, $J_{6'b,6'b}$ 12.3 Hz, H-6′b), 3.84 (dd, 1 H, $J_{3,4}$ 6.2 Hz, H-3), 3.82, 3.74 (2 d, 2 H, SCH_2) 3.56 (ddd, 1 H, $J_{5',4'}$ 9.5 Hz, $J_{5',6'b}$ 2.4 Hz, H-5′), 3.43 (ddd, 1 H, $J_{5b,4}$ 6.2 Hz, H-5b), 2.24 (m, 1 H, H-2′eq), 2.07, 2.05 (2 ×), 2.04, 2.03, 2.01 (5 s, 15 H, Ac), 1.72 (m, 1 H, H-2′ax); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6, 170.2, 169.7 (2 ×), 169.3 (Ac), 137.3, 129.1, 128.5, 127.2 (Ph), 99.5 (C-1′), 81.6 (C-1), 75.8 (C-3), 72.1 (C-5′), 70.4 (2 ×, C-2, C-4′), 68.9 (C-3′), 68.5 (C-4), 62.7 (C-5), 62.3 (C-6′), 36.1 (C-2′), 34.5 (SCH_2), 20.9, 20.8 (2 ×), 20.67 (2 ×), (Ac). MS (DCI) *m/z* 630 (M + 18) $C_{28}H_{36}O_{13}S$ (612.65).

*Benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-arabino-hexopyranosyl-(1 → 4)-2,3-di-*O*-acetyl-1-thio-β-*D*-xylopyranoside (per-*O*-acetylated* **18**).—^1H NMR (400 MHz, $CDCl_3$): δ 7.29 (m, 5 H, Ph), 5.08 (dd, 1 H, $J_{3,4}$ 8.3 Hz, H-3) 4.95 (m, 2 H, H-3′, H-4′), 4.91 (dd, 1 H, $J_{2,3}$ 8.3 Hz, H-2), 4.59 (dd, 1 H, $J_{1',2'a}$ 9.6 Hz, $J_{1',2'b}$ 2.0 Hz, H-1′), 4.37 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.28 (dd, 1 H, $J_{6'a,5'}$ 4.9 Hz, $J_{6'a,6'b}$ 12.3 Hz, H-6′a), 4.13 (dd, 1 H, $J_{5a,4}$ 4.9 Hz, $J_{5a,5b}$ 11.8 Hz, H-5a), 4.03 (dd, 1 H, $J_{6'b,5'}$ 2.3 Hz, H-6′b), 3.86 (ddd, 1 H, H-4), 3.86, 3.79 (2 d, 2 H, SCH_2) 3.58 (ddd, 1 H, $J_{5',4'}$ 9.3 Hz, H-5′), 3.32 (ddd, 1 H, $J_{5b,4}$ 9.1 Hz, H-5b), 2.24 (m, 1 H, H-2′eq), 2.07, 2.04, 2.02, 2.01, 2.00 (5 s, 15 H, Ac), 1.68 (m, 1 H, H-2′ax); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6, 170.2, 169.9, 169.7, 169.5 (Ac), 137.0, 129.1 (2 ×), 128.6 (2 ×), 127.3 (Ph), 98.3 (C-1′), 82.5 (C-1), 73.8 (C-4), 72.1 (C-3, C-5′), 70.5 (C-4′), 69.7 (C-2), 68.8 (C-3′), 66.0 (C-5), 62.3 (C-6′), 36.2 (C-2′), 34.2 (SCH_2), 20.8, 20.7, 20.67 (3 ×), (Ac). MS (DCI) *m/z* 630 (M + 18) $C_{28}H_{36}O_{13}S$ (612.65).

*Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-arabino-hexopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-1-thio-β-*D*-glucopyranoside (per-*O*-acetylated* **19**).—^1H NMR (400 MHz, $CDCl_3$): δ 7.48 (m, 2 H, Ph), 7.28 (m, 3 H, Ph), 4.98 (dd, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 4.94 (dd, 1 H, $J_{2,3}$ 9.8 Hz, H-2), 4.91 (m, 2 H, H-3′, H-4′), 4.59 (d, 1 H, $J_{1,2}$ 10.1 Hz, H-1), 4.55 (dd, 1 H, $J_{1',2'ax}$ 9.7 Hz, $J_{1',2'eq}$ 1.8 Hz, H-1′), 4.31 (dd, 1 H, $J_{5',6'a}$ 4.4 Hz, H-6′a), 4.18 (br d, 2 H, H-6a, H-6b), 4.01 (dd, 1 H, $J_{6'a,6'b}$ 12.3 Hz, H-6′b), 3.87 (dd, 1 H, $J_{3,4}$ 9.3 Hz, H-3), 3.67 (ddd, 1 H, $J_{5,6b}$ 3.9 Hz, $J_{5,6b}$ 4.0 Hz, H-5), 3.53 (ddd, 1 H, $J_{5',6'b}$ 2.3 Hz, H-5′), 2.08 (m, 1 H, H-2′eq), 2.14, 2.05, 2.04, 2.02, 2.00, 1.99 (6 s, 18 H, Ac), 1.65 (ddd, 1 H, $J_{2'ax,3'}$ 10.5 Hz, $J_{2'eq,2'ax}$ 12.5 Hz, H-2′ax); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6 (2 ×), 170.3, 169.7, 169.4, 168.9 (Ac), 132.7, 128.9, 128.2 (Ph), 100.2 (C-1′), 85.9 (C-1), 80.9 (C-3), 75.9 (C-5), 72.0 (C-5′), 71.7, 68.6, 68.0 (C-3′, C-4′, C-4), 70.3 (C-2), 62.4 (C-6), 62.1 (C-6′), 36.3 (C-2′), 20.9 (2 ×), 20.8 (2 ×), 20.7 (2 ×), (Ac). Anal. Calcd. for $C_{30}H_{38}O_{15}S$ (670.68): C, 53.73; H, 5.53; S, 4.78. Found: C, 53.65; H, 5.44; S, 4.58.

*Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-arabino-hexopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-1-thio-β-*D*-glucopyranoside (per-*O*-acetylated phenyl 2′-deoxy-1-thio-β-*D*-cellobioside* **20**).—^1H NMR (400 MHz, $CDCl_3$): δ 7.48 (m, 2 H, Ph), 7.28 (m, 3 H, Ph), 5.17 (dd, 1 H, $J_{3,4}$ 9.3 Hz, H-3), 4.96 (ddd, 1 H, $J_{2'eq,3'}$ 4.9 Hz, $J_{3',4'}$ 9.3 Hz, H-3′), 4.90 (dd, 1 H, $J_{2,3}$ 9.8 Hz, H-2), 4.89 (dd, 1 H, $J_{4',5'}$ 9.4 Hz, H-4′), 4.64 (d, 1 H, $J_{1,2}$ 10.1 Hz, H-1), 4.52 (dd, 1 H, $J_{1',2'ax}$ 9.7 Hz, $J_{1',2'eq}$ 1.9 Hz, H-1′), 4.42 (dd, 1 H, $J_{5,6a}$ 2.2 Hz, H-6a), 4.32 (dd, 1 H, $J_{5',6'a}$ 4.7 Hz, H-6′a), 4.13 (dd, 1 H, $J_{6a,6b}$ 12.0 Hz, H-6b), 4.00 (dd, 1 H, $J_{6'a,6'b}$ 12.3 Hz, H-6′b), 3.74 (dd, 1 H, $J_{4,5}$ 9.4 Hz, H-4), 3.62 (ddd, 1 H, $J_{5,6b}$ 5.2 Hz, H-5), 3.52 (ddd, 1 H, $J_{5',6'b}$ 2.2 Hz, H-5′), 2.22 (ddd, 1 H, $J_{2'eq,2'ax}$ 12.5 Hz, H-2′eq), 2.09, 2.08, 2.02, 2.01, 2.00, 1.99 (6 s, 18 H, Ac), 1.65 (ddd, 1 H, $J_{2'ax,3'}$ 10.7 Hz, H-2′ax); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.5, 170.3, 170.2, 170.1, 169.7, 169.5 (Ac), 133.1, 128.9, 128.3 (Ph), 99.1 (C-1′), 85.6 (C-1), 76.9 (C-4), 75.3 (C-5), 73.6 (C-3), 72.1 (C-5′), 70.2 (C-4′), 70.1 (C-2), 68.7 (C-3′), 62.5 (C-6), 62.1 (C-6′), 36.2 (C-2′), 20.8, 20.7 (2 ×), 20.65, 20.6, 20.6 (Ac). Anal. Calcd. for $C_{30}H_{38}O_{15}S$ (670.68): C, 53.73; H, 5.53; S, 4.78. Found: C, 53.61; H, 5.66; S, 4.51.

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