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Synthesis of precursors for the dimeric 3-*O*-SO_3Na Lewis X and Lewis A structures

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Abstract

Stereoselective syntheses of 3-*O*-SO_3Na-β-Gal-(1 → 4)-β-GlcNAc-(1 → 3)-β-Gal-(1 → 4)-GlcNAc-β-OBn (**15**) and 3-*O*-SO_3Na-β-Gal-(1 → 3)-β-GlcNAc-(1 → 3)-β-Gal-(1 → 3)-β-GlcNAc-(1 → 3)-β-Gal-(1 → 4)-Glc-β-OBn (**25**) were accomplished through the use of two novel glycosyl donors, namely, ethyl *O*-(2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1 → 4)-3-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-6-*O*-trimethylacetyl-β-D-glucopyranoside (**8**) and ethyl *O*-(2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1 → 3)-4-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-6-*O*-trimethylacetyl-β-D-glucopyranoside (**18**).

Keywords: 3-*O*-Sialyl Lewis^x; 3-*O*-Sialyl Lewis^a; 3-*O*-Sulfo Lewis^x; 3-*O*-Sulfo Lewis^a; Sialyltransferases; Sulfotransferases; Fucosyltransferases

1. Introduction

The dimeric sialyl Lewis^x and sialyl Lewis^a structures have both been reported to form a part of the carbohydrate moiety in various tumor-associated glycoconjugates [1,2]. The well recognized biosynthetic pathway shown in Fig. 1 for dimeric sialyl Le^x involves the building of repeating units of *N*-acetyllactosamine (dimeric *N*-acetyllactosamine) which are then α-(2 → 3)-sialylated to give α-NeuAc-(2 → 3)-β-Gal-(1 → 4)-β-GlcNAc-(1 → 3)-β-Gal-(1 → 4)GlcNAc. Further branching by α-L-fucosyltransferase then occurs to give dimeric sialyl Lewis^x [2]a[3]. It is known that α-(2 → 3)-

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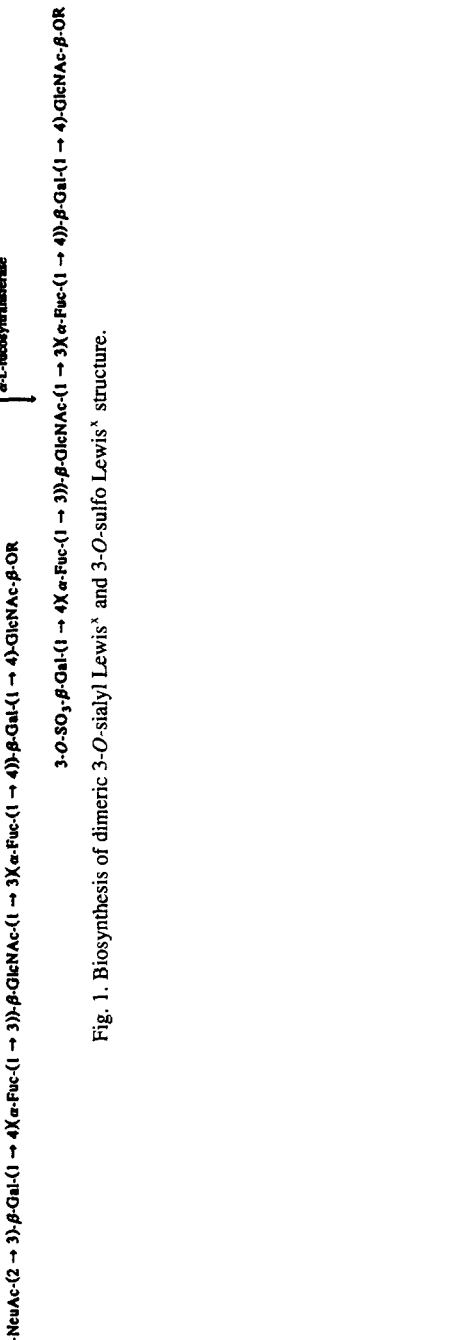
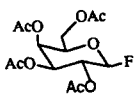


Fig. 1. Biosynthesis of dimeric 3-*O*-sialyl Lewis^x and 3-*O*-sulfo Lewis^x structure.

sialyltransferase and 3-*O*-sulfotransferase can each compete for the C-3 position of galactose in β-Gal-(1 → 4)-GlcNAc.



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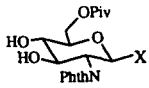
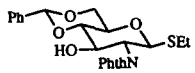


2 . X=SEt

3 . X=OBn



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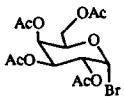

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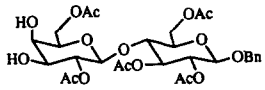

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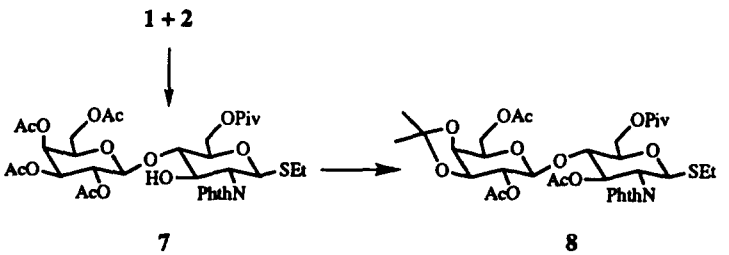
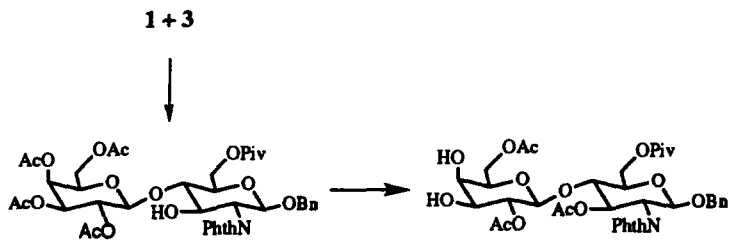
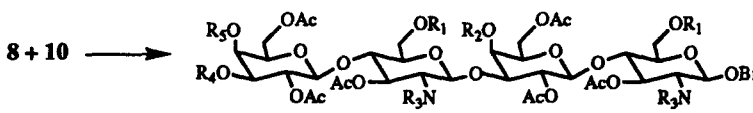
This suggests that we can expect the expression of 3-*O*-SO_3-β-Gal-(1 → 4)-β-GlcNAc-(1 → 3)-β-Gal-(1 → 4)GlcNAc. Additionally, if aberrant fucosylation is a characteristic of the source tissue, this, along with aberrant sulfation, will give sulfated dimeric Le^x structures. Similar pathways are followed for the biosynthesis of 3-*O*-sulfo or 3-*O*-sialyl Le^a structures. The novel aspects of this synthesis are utilization of regioselective glycosidation procedures which in turn provide short and efficient approaches to the target molecules **15** and **25** in good yields. This paper also demonstrates the introduction of new glycosyl donors such as **8** and **18** to construct repeating units of lactosamine and Lacto-*N*-biose. Compounds **7** and **17** are potential intermediates for the synthesis of $Lewis^x$ and $Lewis^a$ trisaccharide donors, respectively.

2. Results and discussion

Synthesis of 3-O-SO_3Na-β-Gal-(1 → 4)-β-GlcNAc-(1 → 3)-β-Gal-(1 → 4)-GlcNAc-β-OBn (**15**).—Compound **15** was acquired through the utilization of a key glycosyl donor, ethyl *O*-(2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1 → 4)-3-*O*-acetyl-2-deoxy-2-phthalimido-6-*O*-pivaloyl-1-thio-β-D-glucopyranoside (**8**). This reagent, after glycosylation followed by isopropylidene removal and selective acetylation at O′-4, provided a moiety having the desired C-3′ hydroxyl available for further glycosylation or sulfation. Treatment of ethyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside [4] as well as benzyl 2-deoxy-2-phthalimido-β-D-glucopyranoside with pivaloyl chloride in pyridine afforded the corresponding 6-*O*-pivaloyl compounds **2** (80%) and **3** (65%), respectively. Glycosylation of **2** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl fluoride [5] (**1**) under Mukaiyama's condition [6] ($SnCl_2$–AgOTf) afforded a major β-(1 → 4)-linked compound **7** in 60% yield along with some minor products (Scheme 1). The ^1H NMR spectrum of **7** displayed characteristic signals for H-1 and H-1′ at δ 5.37 (d, *J* 10.5 Hz) and 4.56 (d, *J* 8.12 Hz), which confirmed a β-linkage for



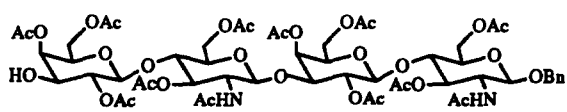




11 . R_1=Piv,R_2=H,R_3=Phth,R_4,R_5=Me_2C

12 . R_1=R_2=Ac,R_3=HAc,R_4,R_5=Me_2C

13 . R_1=R_2=Ac,R_3=HAc,R_4,R_5=H



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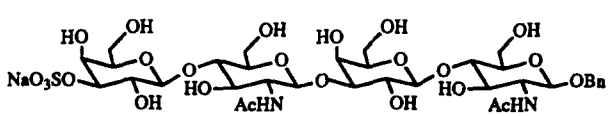

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Scheme 1.

the newly incorporated galactopyranosyl residue. In the ^{13}C NMR spectrum, the resonance for C-4 displayed a downfield shift (δ 80.98) as compared to compound **2** (δ 71.72), confirming this position as the site of glycosylation. Selective de-*O*-acetylation of **7** in the presence of an *O*-pivaloyl group followed by isopropylidenation [7] and acetylation afforded the key donor **8** in 80% yield. Similarly, glycosylation of **3** with **1** provided the major β-(1 → 4)-linked disaccharide **9** in 56% yield. A similar reaction sequence was enlisted for the synthesis of **10** from **9** as was described for the preparation of **8** from **7**, followed by removal of the isopropylidene group with 80% aq acetic acid at 80 °C. Regioselective glycosylation of **8** with **10** in CH_2Cl_2 in the presence of NIS-triflic acid [8] at −10 °C afforded the β-(1 → 3)-linked tetrasaccharide **11** in 70% yield. The utility and advantage of employing glycosyl donor **8** is realized in the subsequent conversion of **11** into the key intermediate **14** in 4 steps: (1) NH_2–$NH_2 \cdot H_2O$/EtOH (phthalimido and acyl groups removal), (2) pyridine–acetic anhydride (*N*- and *O*-acetylation to give **12**), (3) 80% aq acetic acid (hydrolysis of isopropylidene group to afford diol **13**), (4) triethyl orthoacetate/80% aq acetic acid (to convert diol **13** into the 3-hydroxy compound **14**). The ^1H NMR spectrum of **12** exhibited one low field chemical shift at δ 5.29 (d, *J* 3.2 Hz, H-4′), confirming that compound **10** had been glycosylated at O-3′. Similarly, the ^1H NMR spectrum of **14** displayed two low field chemical shifts at δ 5.31 (d, *J* 2.7 Hz, H-4‴) and 5.28 (d, *J* 3.1 Hz, H-4′), confirming that compound **14** had been acetylated at O-3‴. Sulfation of **14** with SO_3–pyridine complex in DMF followed by de-*O*-acetylation with methanolic sodium methoxide and passage through IR-120 (Na^+) resin gave the title compound **15** as an amorphous sodium salt. The structure of **15** was confirmed by ^{13}C NMR spectroscopy (see Table 1).

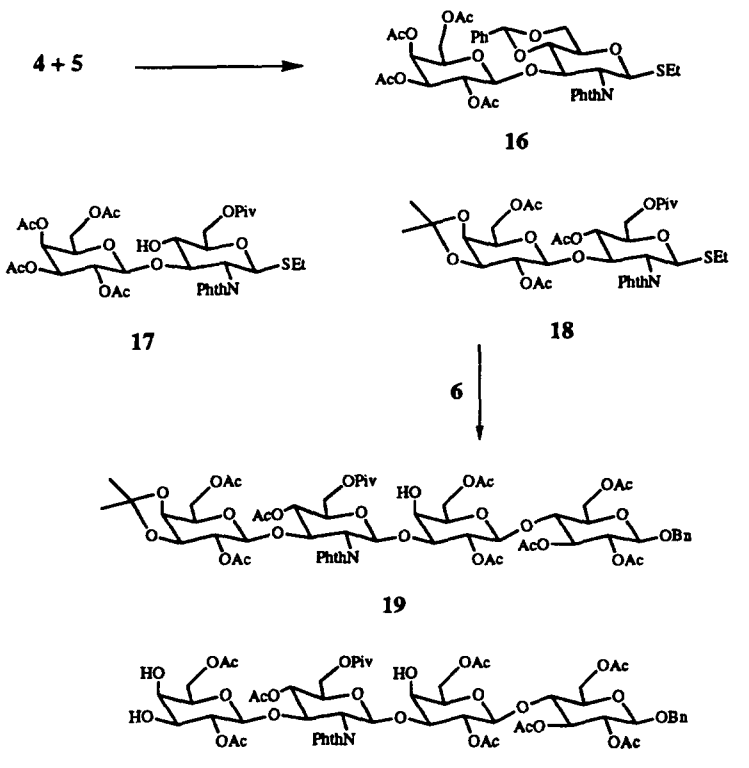
Table 1
^{13}C NMR data [a] (proposed assignments)

Residue	Compound No.	C-1	C-2	C-3	C-4	C-5	C-6	NAc
β-Gal-(1 → 4)	[b]	101.86	70.46	71.17	68.93	73.77	59.98	
β-GlcNAc-(1 → 3)		101.68	54.18	71.50	77.50	74.32	59.10	21.16
β-Gal-(1 → 4)		101.86	69.94	81.02	67.53	73.53	59.89	
GlcNAc-β-OBn		98.80	53.99	71.31	77.23	73.84	58.88	21.10
3-*O*-SO_3Na-β-Gal-(1 → 4)	**15**	101.87	68.94	79.01	65.83	73.77	59.90	
β-GlcNAc-(1 → 3)		101.45	54.18	71.31	77.49	73.85	59.10	21.16
β-Gal-(1 → 4)		101.71	70.46	81.03	67.27	73.51	59.90	
GlcNAc-β-OBn		98.81	53.99	71.16	77.16	73.77	58.85	21.10
3-*O*-SO_3Na-β-Gal-(1 → 4)	**25**	102.34	67.56	80.68	65.72	73.85	59.91	
β-GlcNAc-(1 → 3)		101.51	53.70	70.51	79.13	73.85	59.55	21.26
β-Gal-(1 → 4)		102.15	68.97	81.44	67.29	73.79	59.91	
β-GlcNAc-(1 → 3)		101.32	53.58	70.51	77.44	73.85	59.55	21.26
β-Gal-(1 → 4)		101.92	68.84	80.97	67.23	73.79	59.91	
Glc-β-OBn		100.02	68.98	71.80	74.18	73.41	59.12	

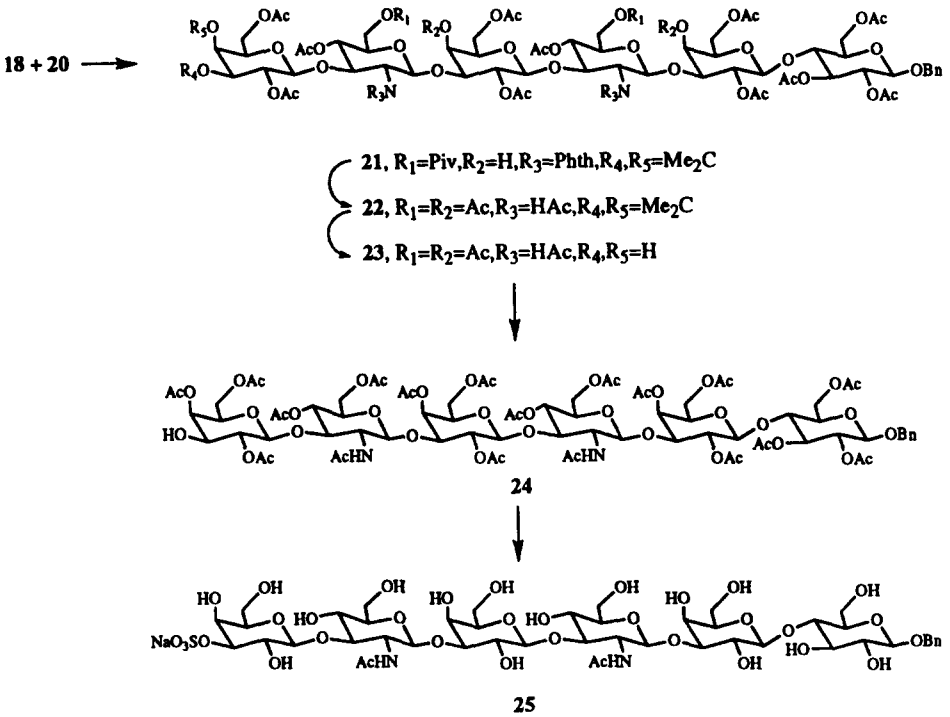
[a] Solutions in D_2O with Me_4Si as the external standard.
[b] β-Gal-(1 → 4)β-GlcNAc-(1 → 3)-β-Gal-(1 → 4)GlcNAc-β-OBn. The chemical shifts for this compound are included for comparison purposes.

Synthesis of 3-O-SO_3Na-β-Gal-(1 → 3)-β-GlcNAc-(1 → 3)-β-Gal-(1 → 3)-β-GlcNAc-(1 → 3)-β-Gal-(1 → 4)-Glc-β-OBn (**25**).—The synthesis of the hexasaccharide **25** involved the utilization of glycosyl donor **18**. Reaction of ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**4**) [4] with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**5**) in the presence of silver triflate and 2,6-di-*O*-(*tert*-butyl)-4-methylpyridine [9] followed by hydrolysis of the benzylidene group from **16** and treatment with pivaloyl chloride in pyridine gave **17** in 85% yield. A reaction sequence similar to that described for the preparation of **8** from **7** was performed for the synthesis of **18** from **17** in 80% yield. The introduction of a pivaloyl group at O-6 of the GlcNPhth moiety avoids the formation of the 4,6-*O*-isopropylidene acetal in the β-(1 → 3)-linked oligosaccharide during the synthesis of **18** (Scheme 2).

The condensation of benzyl *O*-(2,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (**6**) [10] with **18** under similar NIS-triflic acid conditions provided the protected tetrasaccharide **19** in 52% yield. Removal of the isopropylidene group from **19** provided the diol **20** in 80% yield which on condensation with **18** gave the protected hexasaccharide **21** in 52% yield (Scheme 3). Compound **21** was converted to **24** employing the same reaction sequence described for the preparation of



Scheme 2.



Scheme 3

14 from **11**; yield 75%. The ^{1}H NMR spectrum of **22** exhibited two low field chemical shifts at δ 5.17 (d, J 2.7 Hz, H-4‴) and 5.14 (bs, H-4′) confirming that compounds **6** and **20** had been glycosylated at O-3. The ^{1}H NMR spectrum of **24** displayed characteristic signals at δ 5.32 (d, J 1.2 Hz, H-4⁗), 5.27 (bs, H-4‴) and 5.17 (d, J 1.1 Hz, H-4′), confirming that compound **24** had been acetylated at O-3⁗. Sulfation of **24** with SO_3–pyridine complex followed by de-*O*-acetylation afforded the 3-*O*-sulfate hexasaccharide **25**. The ^{13}C NMR spectrum of **25** was also consistent with the structure assigned (see Table 1).

In summary, a facile regio- and stereoselective synthesis of precursors for the dimeric 3-*O*-SO_3Na Lewis^x and Lewis^a **15** and **25** was accomplished by rationally designed glycosyl donors **8** and **18**. Synthetic applications of this procedure could be further elaborated to make lactosamine and Lacto-*N*-biose repeating units in a given molecule.

^{13}C NMR assignments.—In the ^{13}C NMR spectra of **15** and **25** the resonance for C-4 of the GlcNAc residue displayed a downfield shift (δ 77.16–79.13), confirming the site of glycosylation in these compounds. Similarly, the resonance of C-3 of the internal Gal residue in **15** and **25** was observed at δ 80.97–81.44, confirming that O-3 was the site of glycosylation. Similarly, the resonance of C-3 of terminal Gal residue in compounds **15** and **25** displayed a downfield shift (δ 79.01 and 80.68) compared to the unsulfated tetrasaccharide (δ 71.17), confirming this position as the site of sulfation.

3. Experimental

General methods.—Optical rotations were measured at ~ 25 °C with a Perkin–Elmer 241 Polarimeter. TLC was conducted on glass plates precoated with a 0.25 mm layer of silica gel 60F-254 (Analtech GHLF uniplates). The compounds were located by exposure to UV light and (or) by spraying with 5% H_2SO_4 in EtOH and charring on a hot plate. The silica gel used for column chromatography was Baker Analyzed (60–200 mesh). NMR spectra were recorded at ~ 25 °C; ^1H spectra with a Varian EM-390 at 90 MHz and with a Bruker AM-400 at 400 MHz, and ^{13}C spectra with a Bruker AM-400 at 100.6 MHz. All chemical shifts were referenced to tetramethylsilane. Solutions in organic solvents were generally dried with anhydrous sodium sulfate. Dichloromethane, *N,N*-dimethylformamide, 1,2-dichloroethane, benzene and 2,2-dimethoxypropane were kept dried over 4 Å molecular sieves. Elemental analyses were performed by Robertson Laboratory, Madison, NJ, USA.

*Ethyl 2-deoxy-2-phthalimido-1-thio-6-*O*-trimethylacetyl-β-*D*-glucopyranoside* (**2**).—To a solution of ethyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (10 g; 28 mmol) in dry pyridine (150 mL) was added trimethylacetyl chloride (4.1 mL, 33.6 mmol) over a period of 30 min at 0 °C. Stirring was continued at room temperature for 36 h. TLC of the reaction mixture in 9:1 chloroform:methanol showed a faster moving spot (R_f 0.5). The mixture was cooled to 0 °C and ethanol (30 mL) was added to stop the reaction. Solvents were evaporated and the resultant syrup was purified by column chromatography using 3% methanol in chloroform as an eluent to yield the title compound **2** (9.8 g, 80%). $[\alpha]_D$ −20.7 (*c* 1, $CHCl_3$): ^1H NMR ($CDCl_3$); δ 7.80–7.20 (m, 4 H, arom.), 5.33 (d, 1 H, *J* 10.41, H-1), 4.54 (dd, 1 H, *J* 4.4, *J* 12.23, H-6), 4.42 (dd, 1 H, *J* 8.8, H-2), 4.37 (dd, 1 H, *J* 12.6, H-6′), 4.32 (t, 1 H, *J* 8.19, H-3), 3.64 (m, 1 H, H-5), 3.36 (dd, 1 H, *J* 9.46, H-4), 2.7–2.5 (m, 2 H, S–CH_2–CH_3), 1.24 (s, 9 H, –$C(CH_3)_3$), 1.23 (t, 3 H, S–CH_2–CH_3). ^{13}C NMR ($CDCl_3$): 179.81, 134.18, 131.69–123.5 (arom.), 81.13 (C-1), 78.2 (C-5), 72.29 (C-3), 71.72 (C-4), 63.37 (C-6), 55.37 (C-2), 27.23 [$C(CH_3)$], 23.92, 15.02 (CH_2, CH_3). Anal. Calcd for $C_{21}H_{27}NO_7S$: C, 57.65; H, 6.23. Found: C, 57.36; H, 6.08.

*Benzyl 2-deoxy-2-phthalimido-6-*O*-trimethylacetyl-β-*D*-glucopyranoside* (**3**).—Benzyl 2-deoxy-2-phthalimido-β-D-glucopyranoside (8 g, 20 mmol) was regiospecifically esterified with trimethylacetyl chloride (2.95 mL, 24 mmol) in pyridine (120 mL) as described for compound **2**. TLC examination in 9:1 chloroform:methanol revealed a new product spot (R_f 0.55). Column fractionation of the reaction mixture after workup using 2:3 hexane:ethyl acetate afforded pure compound **3** (6.3 g) in 65% yield. $[\alpha]_D$ −104.9 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.77–7.06 (m, 9 H, arom.), 5.18 (d, 1 H, *J* 8.36, H-1), 4.80 and 4.49 (each d, *J* 12.1 Hz, 4 H, 2 × OCH_2), 4.14 (t, *J* 8.5 Hz, 1 H, H-2), 1.24 [s, 9 H, $C(CH_3)_3$]. Anal. Calcd for $C_{26}H_{29}NO_8$: C, 64.57; H, 6.05. Found: C, 64.42; H, 6.09.

*Ethyl (2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1 → 4)-2-deoxy-2-phthalimido-1-thio-6-*O*-trimethylacetyl-β-*D*-glucopyranoside* (**7**).—A mixture of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl fluoride (**1**, 3 g, 8.5 mmol) and compound **2** (2.9 g, 6.58 mmol) in dry dichloromethane:toluene (5:1) (100 mL) containing 4 Å molecular sieves (5 g) was stirred at room temperature under a nitrogen atmosphere for 20 min. The

mixture was cooled to −15 °C and tin(II) chloride (1.25 g, 6.58 mmol) was added followed by silver triflate (1.7 g, 6.58 mmol). Stirring was continued for 4 h at room temperature with protection from light. TLC monitoring using 2:3 hexane:ethyl acetate solvent revealed a new spot (R_f 0.60). The reaction mixture was diluted with dichloromethane (100 mL) containing sodium bicarbonate (5 g). Solids were filtered off and the filtrate was washed successively with saturated aq sodium bicarbonate solution and brine solution. The organic layer was dried over sodium sulfate (anhydrous), filtered and evaporated. Purification of the resulting substance by column chromatography using 3:2 hexane:ethyl acetate as an eluent afforded pure compound **7** (3 g) in 60% yield. $[\alpha]_D$ +23.7 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): 7.8–7.7 (m, 4 H, arom.), 5.37 (d, 1 H, *J* 10.5, H-1), 5.35 (bs, 1 H, H-4′), 4.56 (d, 1 H, *J* 8.12, H-1′), 2.72–2.58 (m, 2 H, S–CH_2CH_3), 2.13, 2.08, 1.97, 1.87 (4 s, 12 H, 4 × OAc), 1.24 [s, 9 H, $C(CH_3)_3$], 1.21 (t, 3 H, S–CH_2–CH_3). ^{13}C NMR ($CDCl_3$): δ 178.04, 170.32, 169.96, 169.89, 168.06, 168.06, 167.62 (–CO–), 134.12–123.29 (arom.), 102.12 (C-1′), 83.64 (C-1), 80.98 (C-4), 71.45 (C-3), 62.92, 61.62 (C-6 and C-6′), 54.97 (C-2), 27.27 [$C(CH_3)_3$], 24.03, 20.56, 20.45 (OAc), 20.3, 15.07 (S–CH_2–CH_3). Anal. Calcd for $C_{35}H_{45}NO_{16}S$: C, 54.75; H, 5.92. Found: C, 54.71; H, 5.89.

*Ethyl (2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-*D*-galactopyranosyl)-(1 → 4)-3-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-6-*O*-trimethylacetyl-β-*D*-glucopyranoside* (**8**).—Compound **7** (4 g), dissolved in 1:1 methanol:dichloromethane (100 mL), received a dropwise addition of 0.1 N sodium methoxide in methanol (1 mL). After warming to 40 °C for 2 min, the solution was allowed to stir at room temperature for 1 h. When TLC (9:1 chloroform:methanol) showed a single slower moving spot (R_f 0.20), the solution was neutralized with IR 120(H^+) resin. The mixture was filtered, residues were washed with methanol, filtrates were combined and evaporated, then co-distilled three times from toluene to give the deacetylated compound in 95% yield (2.95 g).

To a solution of the above compound (2.9 g) in 2,2′-dimethoxypropane (100 mL) was added camphorsulfonic acid (0.13 g), and the mixture was stirred at room temperature for 3 days. TLC (3:1 chloroform:acetone) indicated disappearance of the starting material and appearance of a faster moving spot (R_f 0.5). The reaction mixture was neutralized with triethylamine and evaporated to dryness. The resultant residue was refluxed with 10:1 methanol:water for 16 h. TLC using the above solvent system showed a single product (R_f 0.2). Removal of solvents and drying under high vacuum yielded the isopropylidenated compound. This compound was acetylated with 3:2 pyridine:acetic anhydride (50 mL) at room temperature for 16 h. TLC monitoring (1:1 hexane:ethyl acetate) indicated a faster moving spot (R_f 0.5). After cooling to 0 °C methanol (20 mL) was added and the mixture stirred for 0.5 h. Solvent evaporation, co-distillation with several added portions of toluene followed by column chromatography using 3:2 hexane:ethyl acetate as an eluent yielded compound **8** (2.2 g) in 75% yield; $[\alpha]_D$ +43.6 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.87–7.72 (m, 4 H, arom.), 5.47 (d, 1 H, *J* 10.57, H-1), 4.38 (d, 1 H, *J* 7.52, H-1′), 2.12, 2.09, 1.90 (3 s, 9 H, 3 × OAc), 1.53, 1.30 [2 s, 6 H, $(CH_3)_2$], 1.24 [s, 9 H, $C(CH_3)_3$], 1.20 (t, 3 H, S–CH_2–CH_3). Anal. Calcd for $C_{36}H_{47}NO_{15}S$: C, 56.45; H, 6.19. Found: C, 56.41; H, 6.12.

Benzyl O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1 → 4)-2-deoxy-2-phthalimido-6-*O*-trimethylacetyl-β-*D*-glucopyranoside* (**9**).—Compounds **1** (3 g, 8.5

mmol) and **3** (2.9 g, 6.5 mmol) were condensed in 5:1 dichloromethane:toluene (100 mL) in the presence of tin(II) chloride (1.25 g, 6.5 mmol), silver triflate (1.7 g, 6.5 mmol) and molecular sieves (5 g) as described above for **7**. The usual workup and purification by column chromatography (3:2, hexane:ethylacetate) afforded **9** (3 g, 56%). $[\alpha]_D$ −16.1 (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.8–7.7 (m, 4 H, arom.), 7.25–7.2 (m, 5 H, arom.), 5.30 (d, H 10.2 Hz, 1 H, H-1), 4.58 (d, *J* 7.8 Hz, 1 H, H-1′), 2.20–1.80 (4 s, 12 H, 4 × OAc), 1.24 [s, 9 H, $C(CH_3)_3$]. Anal. Calcd for $C_{40}H_{47}NO_{17}$: C, 59.08; H, 5.83. Found: C, 59.01; H, 5.80.

Benzyl O-(2,6-di-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-3-O-acetyl-2-deoxy-2-phthalimido-6-O-trimethylacetyl-β-D-glucopyranoside (**10**).—Compound **9** (4 g) was selectively deacetylated in the presence of the trimethylacetyl group and subsequently isopropylidenated with 2,2′-dimethoxypropane (100 mL) and camphorsulfonic acid (0.13 g). Acetylation with 3:2 pyridine:acetic anhydride (50 mL) followed by the hydrolysis using 80% aq acetic acid (100 mL) at 80 °C for 2 h and subsequent purification over a silica gel column using 2:3 hexane:ethylacetate as solvent afforded **10** (3.0 g, 80%); $[\alpha]_D$ −8.5 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.90–7.70 (m, 4 H, arom. Phth), 7.15–7.00 (m, 5 H, arom.), 5.35 (d, 1 H, H-1), 4.36 (d, 1 H, H-1′), 2.20–1.85 (3 s, 9 H, OAc), 1.26 [s, 9 H, $C(CH_3)_3$]. Anal. Calcd for $C_{38}H_{45}NO_{16}$: C, 59.12; H, 5.88. Found: C, 59.09; H, 5.82.

Benzyl O-(2,6-di-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-(1 → 4)-(3-O-acetyl-2-deoxy-2-phthalimido-6-O-trimethylacetyl-β-D-glucopyranosyl)-(1 → 3)-(2,6-di-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-3-O-acetyl-2-deoxy-2-phthalimido-6-O-trimethylacetyl-β-D-glucopyranoside (**11**).—A solution of **10** (0.52 g, 0.67 mmol) and **8** (0.56 g, 0.73 mmol) in dry dichloromethane (50 mL) containing molecular sieves (5 g) was stirred at room temperature under nitrogen for 20 min. The mixture was then cooled to −25 °C and *N*-iodosuccinimide (0.25 g, 1.12 mmol) was added followed by a solution of trifluoromethanesulfonic acid in dichloromethane (0.2 mL in 30 mL CH_2Cl_2). Stirring was continued at −25 °C to −10 °C for 30 min. TLC examination (1:2 hexane:ethyl acetate) revealed a new spot (R_f 0.6) between the donor (R_f 0.8) and the acceptor (R_f 0.25). The mixture was diluted with dichloromethane and filtered through Celite into cold, aq saturated sodium bicarbonate. The filtrate was washed with more bicarbonate solution (2 × 100 mL), 10% sodium thiosulfate (2 × 100 mL) and water, dried over sodium sulfate and concentrated. Column chromatography using 3:2 hexane:ethyl acetate as an eluent afforded the pure title compound (**11**, 0.69 g, 70%). $[\alpha]_D$ +12.4 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.85–7.04 (m, 13 H, arom.), 5.59 (d, 1 H, *J* 8.71, H-1), 5.57 (d, 1 H, *J* 8.69, H-1″), 4.86 (d, 1 H, *J* 7.85, H-1‴), 4.42 (d, 1 H, *J* 8.15, H-1′), 2.10–1.53 (6 s, 18 H, 6 × OAc), 1.51, 1.30 [2 s, 6 H, $C(CH_3)_2$], 1.242, 1.240 [2 s, 18 H, $C(CH_3)_3$]. Anal. Calcd for $C_{72}H_{86}N_2O_{31}$: C, 58.56; H, 5.95. Found: C, 58.79; H, 5.74.

Benzyl O-(2,6-di-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-(1 → 4)-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranoside (**12**).—A solution of **11** (0.55 g, 0.37 mmol) in methanol (50 mL) was partially deacetylated (room temperature, 2 h) using 0.1 N sodium methoxide in methanol (1 mL). This solution was neutralized with IR H^+ resin, filtered and evaporated to dryness.

The resultant substance was dissolved in ethanol (50 mL), hydrazine hydrate (12 mL) was added, and the contents stirred at 100 °C for 16 h. After cooling, the solvents were evaporated, followed by co-distillation with toluene (3 × 50 mL) and thorough drying under high vacuum. The resultant substance was acetylated at room temperature for 10 h in 2:1 pyridine:acetic anhydride (60 mL). TLC (9:1 chloroform:methanol) revealed a single spot product (R_f 0.9). The reaction mixture was cooled to 0 °C and methanol (10 mL) was added. Evaporation of solvents and purification by column chromatography using 3:1 chloroform:acetone as an eluent afforded **12** (0.4 g, 85%). $[\alpha]_D$ −0.9 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.24 (m, 5 H, arom.), 5.45 (d, 1 H, *J* 9.47, H-1), 5.35 (d, 1 H, *J* 8.42, H-1″), 5.29 (d, *J* 3.2 Hz, 1 H, H-4′), 4.61 (d, 1 H, *J* 7.62, H-1‴), 4.40 (d, 1 H, *J* 7.35, H-1′), 2.10–2.0 (m, OAc), 1.90–1.87 (2 s, 6 H, NHAc), 1.50, 1.29 [2 s, 6 H, $C(CH_3)_2$]. Anal. Calcd for $C_{56}H_{76}N_2O_{30}$: C, 53.49; H, 6.10. Found: C, 53.59; H, 5.99.

Benzyl O-*(2,6-di-*O*-acetyl-β-*D*-galactopyranosyl)-(1 → 4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1 → 4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranoside* (**13**).—A solution of **12** (0.35 g, 0.28 mmol) in 80% aq acetic acid (60 mL) was stirred at 80 °C for 2 h. TLC (9:1 chloroform:methanol) revealed a single slower moving spot (R_f 0.15). Cooling to room temperature, solvent evaporation and co-evaporation with toluene yielded a solid substance which was further purified through a short column using 9:1 chloroform:methanol as the eluent to afford the title compound **13** (0.27 g, 80%); $[\alpha]_D$ −7.6 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.26–7.24 (m, 5 H, arom.), 5.87 (d, 1 H, *J* 9.2, H-1), 5.48 (d, 1 H, *J* 9.26, H-1″), 4.60 (d, 1 H, *J* 7.95, H-1‴), 4.41 (d, 1 H, *J* 7.11, H-1′), 2.13–1.88 (m, OAc and NAc). Anal. Calcd for $C_{53}H_{72}N_2O_{30}$: C, 53.29; H, 5.97. Found: C, 53.27; H, 5.93.

Benzyl O-*(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1 → 4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1 → 4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranoside* (**14**).—To a solution of **13** (0.25 g, 0.21 mmol) in benzene (50 mL) and triethylorthoacetate (8 mL) was added *p*-toluenesulfonic acid (0.1 g) and stirring was continued at room temperature for 3 h. TLC monitoring (9:1 chloroform:methanol) revealed the appearance of a faster moving spot (R_f 0.9). Triethylamine was added to neutralize the acid and the mixture was concentrated to dryness under diminished pressure to give the 3,4-orthoester in quantitative yield. This compound was dissolved in 80% aq acetic acid (50 mL) and the solution was stirred at room temperature for 6 h. TLC (9:1 chloroform:methanol) revealed the formation of a product spot (R_f 0.25) moving faster than **13** (R_f 0.15). Solvents were removed under reduced pressure, and the last traces of acetic acid were removed by several co-evaporations with toluene. The solid substance obtained was purified through a short column using 9:1 chloroform:methanol as an eluent to give **14** (0.20 g, 75%). $[\alpha]_D$ −8.1 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.28–7.25 (m, 5 H, arom.), 5.45 (d, 1 H, *J* 9.4, H-1), 5.38 (d, 1 H, *J* 8.64, H-1″), 5.31 (d, *J* 2.7 Hz, 1 H, H-4‴), 5.28 (d, *J* 3.1 Hz, 1 H, H-4′), 4.64 (d, 1 H, *J* 7.4, H-1‴), 4.44 (d, 1 H, *J* 7.95, H-1′), 2.12–1.86 (m, OAc and NAc). Anal. Calcd for $C_{55}H_{74}N_2O_{31}$: C, 52.45; H, 5.93. Found: C, 52.46; H, 6.00.

Benzyl O-*(3-*O*-sulfo-β-*D*-galactopyranosyl sodium salt)-(1 → 4)-(2-acetamido-2-*

deoxy-β-D-galactopyranosyl)-(1 → 3)-(β-D-galactopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**15**).—To a solution of **14** (0.14 g, 0.11 mmol) in dry *N*,*N*-dimethylformamide (20 mL) was added sulfur–trioxide–pyridine complex (0.044 g, 0.27 mmol). Contents were stirred at room temperature under a nitrogen atmosphere until all of the starting material was consumed. A slower moving spot (R_f 0.2) was revealed by TLC (4:1 chloroform:methanol). The reaction mixture was then cooled to 0 °C and a few drops of methanol were added followed by a few drops of pyridine and stirring for 20 min. Solvents were evaporated and the product purified through a small silica gel column using 4:1 chloroform:methanol as an eluent.

The pure material so obtained (0.076 g, 48%) was then dissolved in dry methanol (10 mL), 0.1 N sodium methoxide in methanol (1 mL) was added, and the contents were stirred at room temperature for 3 d. TLC (4:5:1 chloroform:methanol:water) showed a single slower moving product spot (R_f 0.45). The reaction mixture was neutralized with acetic acid (0.5 mL) at 0 °C, and diluted with water (10 mL). Careful removal of solvents at diminished pressure and < 30 °C gave a solid material which was subsequently purified on a short silica gel column using 5:4:1 chloroform:methanol:water as solvent. Additional purification was performed on a BioGel P2 column to eliminate soluble silicates. Pure fractions were combined and evaporated, and the resultant substance was dissolved in distilled water (20 mL) and passed through a 2 × 15 cm bed of thoroughly washed Na^+ resin. The pure material obtained in the form of a sodium salt was freeze-dried to afford the title compound **15** (0.024 g) in 80% yield. $[\alpha]_D$ −12.2 (*c* 0.5, H_2O); ^1H NMR (D_2O): δ 7.5–7.41 (m, 5 H, arom.), 4.64 (d, 1 H, *J* 7.84, H-1‴), 4.59 (d, 1 H, *J* 8.34, H-1″), 4.50 (d, 1 H, *J* 7.87, H-1′), 2.0, 1.97 (2 s, 6 H, NHAc). For ^{13}C NMR see Table 1. Anal. Calcd for $C_{35}H_{53}N_2O_{24}SNa \cdot H_2O$: C, 43.83; H, 5.58. Found: C, 43.42; H, 5.53.

Ethyl-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 3)-2-deoxy-2-phthalimido-6-O-trimethylacetyl-1-thio-β-D-glucopyranoside (**17**).—To a solution of ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**4**, 6 g, 13.6 mmol) and acetobromo-galactose (**5**, 8.4 g, 20.4 mmol) in dry dichloromethane (200 mL) containing molecular sieves (4 Å, 20 g), was added 2,6-di-*tert*-butyl-4-methylpyridine (2.8 g, 13.6 mmol). The mixture was stirred at −30 °C for 30 min. A solution of silver triflate (5.2 g, 20.4 mmol) in dry toluene (50 mL) was then added to above stirred solution under inert atmosphere. Stirring was continued (2 h) at −10 °C until TLC (1:1 hexane:ethyl acetate) showed that all of the acceptor was consumed. The new product spot (R_f 0.5) moved more slowly than both acceptor (R_f 0.75) and donor (R_f 0.8). The reaction was stopped by adding collidene (6 mL) and 10% aq sodium thiosulfate (50 mL), and stirring was continued until the mixture reached room temperature. The mixture was diluted with dichloromethane (200 mL), then filtered through a Celite bed. The filtrate was successively washed with 2 × 100 mL aq sodium bicarbonate solution and 2 × 100 mL brine solution. The organic layer was dried over anhyd sodium sulfate and evaporated to dryness. Column purification using 2:1 hexane:ethyl acetate as an eluent afforded fully protected disaccharide **16** (7.3 g, 70%) which was utilized directly in the next step.

A solution of **16** (5.8 g, 7.5 mmol) in 3:2 acetic acid:water (100 mL) was stirred at 60 °C for 0.5 h. TLC (3:1 chloroform:acetone) revealed a slower moving product spot (R_f 0.55). The reaction was brought to room temperature and solvents were evaporated

under diminished pressure. Co-distillation with toluene followed by drying gave a solid material which was directly used in the next step.

The diol (5.1 g, 7.5 mmol) was dissolved in pyridine (120 mL) and trimethylacetyl chloride (1 mL, 8.3 mmol) was added slowly at 0 °C. Stirring was continued for 16 h at room temperature. Workup as described for compound **2** and column purification using 3:2 hexane:ethyl acetate afforded the title compound (**17**, 4.9 g) in 85% yield. $[\alpha]_D$ +38.6 (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.9–7.2 (m, 4 H, arom.), 5.30 (dd, 1 H, *J* 2.6, H-4′), 5.15 (d, 1 H, *J* 10.4, H-1), 4.40 (d, 1 H, *J* 8.05, H-1′), 2.69–2.54 (m, 2 H, S–CH_2–CH_3), 2.13, 2.07, 1.88, 1.51 (4 s, 12 H, OAc), 1.23 [s, 9 H, –$C(CH_3)_3$], 1.18 (t, 3 H, –SCH_2CH_3). ^{13}C NMR ($CDCl_3$): δ 178.19–168.81 (CO), 134.53–123.69 (arom.), 101.07 (C-1′), 82.81 (C-1), 78.00–66.83 (ring carbons), 63.43, 61.49 (C-6 and C-6′), 53.86 (C-2), 27.24 [–$C(CH_3)_3$], 23.91–20.38 (OAc), 19.84, 14.99 (S–CH_2, CH_3). Anal. Calcd for $C_{35}H_{45}NO_{16}S$: C, 54.74; H, 5.91. Found: C, 54.78; H, 5.89.

*Ethyl (2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-*D*-galactopyranosyl)-(1 → 3)-4-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-6-*O*-trimethylacetyl-β-*D*-glucopyranoside* (**18**).— Acetyl groups in compound **17** (4 g, 5.2 mmol) were selectively removed in the presence of the trimethylacetyl group by a procedure similar to that described for **8**. Subsequently, the resultant material was isopropylidenated and acetylated to give the title compound **18** (3.11 g) in 78% overall yield; $[\alpha]_D$ +36.0 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.89–7.26 (m, 4 H, arom.), 5.15 (d, 1 H, *J* 10.41, H-1), 4.12 (d, 1 H, *J* 7.82, H-1′), 2.68–2.59 (m, SCH_2CH_3), 2.14, 2.06, 1.91 (3 s, 9 H, OAc), 1.47, 1.20 [2 s, 6 H, $C(CH_3)_2$], 1.23 [s, 9 H, –$C(CH_3)_2$], 1.18 (t, 3 H, SCH_2CH_3). ^{13}C NMR ($CDCl_3$): δ 134.0–123.63 (arom.), 110.70 [$C(CH_3)_2$], 100.14 (C-1′), 80.98 (C-1), 77.32–69.27 (C-ring), 62.98, 62.18 (C-6, C-6′), 54.67 (C-2), 27.13 [–$C(CH_3)_3$], 20.85–20.73 (OAc), 20.5, 14.9 (SCH_2CH_3). Anal. Calcd for $C_{36}H_{47}NO_{15}S$: C, 56.45; H, 6.19. Found: C, 56.39, H, 6.11.

Benzyl O*-(2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-*D*-galactopyranosyl)-(1 → 3)-(4-*O*-acetyl-2-deoxy-2-phthalimido-6-*O*-trimethylacetyl-β-*D*-glucopyranosyl)-(1 → 3)-(2,6-di-*O*-acetyl-β-*D*-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranoside* (**19**). —To a solution of benzyl *O*-(2,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (**6**, 1.43 g, 2.23 mmol) and **18** (1.9 g, 2.48 mmol) in dry dichloromethane (50 mL) containing molecular sieves (4 Å, 5 g) was added *N*-iodosuccinimide (1.68 g, 7.45 mmol). The mixture was stirred for 30 min under an inert atmosphere. The reaction mixture was then cooled to −10 °C and triflic acid (50 μL) was added. Stirring was continued for 40 min at this temperature when TLC (4:1 chloroform:acetone) showed that the acceptor was consumed and a new spot had appeared between the acceptor and donor (R_f 0.55). Workup and purification was essentially the same as described for **11** affording pure compound **19** (1.56 g, 52%); $[\alpha]_D$ −5.1 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.84–7.22 (m, 9 H, arom.), 5.13 (d, 1 H, *J* 8.38, H-1″), 4.92 (d, 1 H, *J* 7.81, H-1), 4.90 (d, 1 H, *J* 7.88, H-1′), 4.43 (d, 1 H, *J* 7.79, H-1‴), 2.13–1.74 (7 s, 24 H, 8 × OAc), 1.62, 1.46 [2 s, 6 H, $C(CH_3)_2$], 1.23 [s, 9 H, –$C(CH_3)_3$]. ^{13}C NMR ($CDCl_3$): δ 178.01–168.36 (–C–), 136.68–123.52 (arom.), 110.69 [$C(CH_3)_2$], 100.28, 100.06, 99.15, 98.47 (C-1‴,1′,1″,1), 79.18–68.05 (c-ring), 62.89, 62.52, 62.13, 61.96 (C-6‴,6′,6″,6), 55.44 (C-2″), 27.13 [–$C(CH_3)_3$], 27.25, 26.09 [$C(CH_3)_2$], 20.58–20.12 (OAc). Anal. Calcd for $C_{63}H_{79}NO_{31}$: C, 56.19; H, 5.92.

Found: C, 56.22; H, 5.62.

Benzyl O-(2,6-di-O-acetyl-β-D-galactopyranosyl-(1 → 3)-(4-O-acetyl-2-deoxy-2-phthalimido-6-O-trimethylacetyl-β-D-glucopyranosyl)-(1 → 3)-(2,6-di-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**20**).—A solution of **19** (1.2 g, 0.89 mmol) in 4:1 acetic acid:water was stirred at 80 °C for 2 h. TLC (3:1 chloroform:acetone) revealed a single slower moving product spot (R_f 0.15) which was then isolated as a pure material (0.93 g, 80%) after removal of solvents and purification through a column using 3:1 chloroform:acetone as an eluent; $[\alpha]_D$ −13.8 (*c* 1, $CHCl_3$); ^{1}H NMR: δ 7.25–7.22 (m, 9 H, arom.), 5.13 (d, 1 H, *J* 8.35, H-1″), 4.99 (d, 1 H, *J* 9.16, H-1), 4.40 (d, 1 H, *J* 7.69, H-1′), 4.01 (d, 1 H, *J* 7.67, H-1‴), 2.2–1.60 (m, OAc), 1.23 [s, 9 H, $-C(CH_3)_3$]. ^{13}C NMR ($CDCl_3$): δ 134.55–123.51 (arom.), 100.31, 100.06, 99.19, 98.45 (C-1‴,1′,1″,1), 79.26–68.08 (c-ring), 55.47 (C-2^3), 27.17 [$-C(CH_3)_3$], 20.79–20.61 (OAc). Anal. Calcd for $C_{60}H_{75}NO_{31}$: C, 55.16; H, 5.79. Found: C, 55.12; H, 5.63.

Benzyl O-(2,6-di-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-(1 → 3)-(4-O-acetyl-2-deoxy-2-phthalimido-6-O-trimethylacetyl-β-D-glucopyranosyl)-(1 → 3)-(2,6-di-O-acetyl-β-D-galactopyranosyl)-(1 → 3)-(4-O-acetyl-2-deoxy-2-phthalimido-6-O-trimethylacetyl-β-D-glucopyranosyl)-(1 → 3)-(2,6-di-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**21**).—To a solution of **20** (0.49 g, 0.37 mmol) and **18** (0.38 g, 0.49 mmol) in dry dichloromethane (25 mL) containing dry molecular sieves (4 Å, 3 g), was added *N*-iodosuccinimide (0.25 g, 1.2 mmol). The mixture was stirred at −10 °C under an inert atmosphere for 30 min. Triflic acid (25 mL) in dry dichloromethane (10 mL) was then added to the above solution at −10 °C. Stirring was continued at this temperature for 45 min. TLC monitoring (3:1 chloroform:acetone) showed a middle spot (R_f 0.45). The reaction mixture was diluted with dichloromethane (100 mL) and filtered through Celite into a cold satd sodium bicarbonate solution (50 mL). The filtrate was washed successively with 2 × 50 mL sodium bicarbonate solution, 2 × 50 mL 10% aq $Na_2S_2O_7$ solution, and 1 × 50 mL brine solution. The organic layer was dried over sodium sulfate (anhyd) and evaporated. Column purification using 3:1 → 10:1 ethyl acetate:hexane containing 1% methanol as an eluent afforded pure **21** (0.39 g, 50% yield); $[\alpha]_D$ −13.1 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): 7.86–7.38 (m, 14 H, arom.), 5.14, 5.09 (2 d, 2 H, *J* 8.23, 9.47, H-1⁗ and H-1″), 4.47 (d, 1 H, *J* 7.78, H-1⁗⁗), 2.16–1.65 (m, OAc), 1.52, 1.30 [2 s, 6 H, $C(CH_3)_2$], 1.24, 1.22 [2 s, 18 H, 2 × $-C(CH_3)_3$]. ^{13}C NMR ($CDCl_3$): δ 134.39–123.5 (arom.), 100.29 (C-1⁗⁗ and C-1‴), 100.07 (C-1′), 99.18 (C-1″ and C-1⁗), 98.39 (C-1), 79.05–68.05 (c-ring), 27.12 [$-C(CH_3)_3$], 20.79–19.94 (OAc). Anal. Calcd for $C_{94}H_{119}N_2O_{46}$: C, 56.07; H, 5.97. Found: C, 56.26; H, 5.83.

Benzyl O-(2,6-di-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-(1 → 3)-(2-acetamido-4,6-O-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**22**).—To a solution of **21** (0.32 g, 0.16 mmol) in dry ethanol (30 mL) was added 0.1 N sodium methoxide in methanol (0.1 mL) and the solution was stirred for 2 h. TLC showed partial deacetylation. The solution was then neutralized with IR 120 H^+ resin, filtered and dried. The resultant residue was dissolved in ethanol (50 mL)

containing hydrazine hydrate (12 mL), and the solution stirred at 100 °C for 16 h. A similar processing and subsequent acetylation with 2:1 pyridine:acetic anhydride (50 mL) as described for **12**, afforded **22** (0.224 g, 78%). $[\alpha]_D$ +24 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.30 (m, 5 H, arom.), 6.37, 6.25 (2 d, 2 H, 2 × NHAc), 5.17 (d, *J* 2.7 Hz, 1 H, H-4‴), 5.14 (bs, 1 H, H-4′), 5.12, 5.04, 4.58, (3 d, H-1‴, H-1″, H-1⁗), 2.13–1.95 (m, OAc and NAc), 1.51, 1.30 [2 s, 6 H, $C(CH_3)_2$]. ^{13}C NMR ($CDCl_3$): δ 128.3–127.59 (arom.), 110.8 [$-C(CH_3)_2$], 100.46 (C-1⁗), 100.27 (C-1‴), 100.15 (C-1′), 98.95 (C-1⁗ and C-1″), 98.75 (C-1). Anal. Calcd for $C_{78}H_{105}N_2O_{46}$: C, 51.84; H, 5.87. Found: C, 51.88; H, 5.60.

Benzyl O-*(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1 → 3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1 → 3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranoside* (**24**).—The isopropylidene group in **22** (0.21 g, 0.12 mmol) was hydrolyzed with 80% aq acetic acid (50 mL) at 80 °C for 3 h following the procedure described for **13** to give compound **23** (0.204 g, 78%); $[\alpha]_D$ +8.2 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): 7.33 (m, arom.), 5.16 (bs, 1 H, H-4‴), 5.09 (bs, 1 H, H-4′), 5.12 (d, 1 H, *J* 9.61, H-1⁗), 4.96 (d, 1 H, *J* 8.25, H-1), 4.94 (d, 1 H, *J* 9.0, H-1″), 4.50 (d, 1 H, *J* 8.15, H-1⁗), 2.15–1.95 (OAc). ^{13}C NMR ($CDCl_3$): δ 128.46–127.7 (arom.), 100.67 (C-1⁗), 100.42 (C-1‴), 100.26 (C-1′), 99.12 (C-1⁗), 98.93 (C-1″), 98.64 (C-1), 21.09–20.75 (OAc). Anal. Calcd for $C_{78}H_{105}N_2O_{46}$: C, 50.98; H, 5.77. Found: C, 50.88; H, 5.67.

To a solution of **23** (0.15 g, 0.085 mmol) in benzene (25 mL) was added triethylorthoacetate (3 mL) followed by *p*-toluenesulfonic acid (0.025 g) and the solution was stirred at room temperature for 3 h. TLC (9:1 chloroform:methanol) revealed a faster moving product spot (R_f 0.8). After processing, purification and subsequent regioselective ring opening of the orthoester with 4:1 acetic acid:water (20 mL) following a procedure similar to that described for compound **14**, the title compound **24** was obtained (0.10 g, 65%); $[\alpha]_D$ +10.4 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.25 (m, 5 H, arom.), 5.32 (d, *J* 1.2 Hz, 1 H, H-4⁗), 5.27 (bs, 1 H, H-4‴), 5.17 (d, *J* 1.1 Hz, 1 H, H-4′), 5.12 (d, 1 H, *J* 9.6, H-1‴), 4.97 (d, 1 H, *J* 8.3, H-1), 4.90 (d, 1 H, *J* 8.9, H-1″), 4.50 (d, 1 H, *J* 8.16, H-1⁗), 2.16–1.99 (m, OAc). Anal. Calcd for $C_{80}H_{107}N_2O_{47}$: C, 51.96; H, 5.84. Found: C, 51.80; H, 5.71.

Benzyl O-*(3-*O*-sulfo-β-*D*-galactopyranosyl sodium salt)-(1 → 3)-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1 → 3)-(β-*D*-galactopyranosyl)-(1 → 3)-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1 → 3)-(β-*D*-galactopyranosyl)-(1 → 4)-β-*D*-glucopyranoside* (**25**).—A solution of **24** (0.06 g, 0.032 mmol) in dry *N*,*N*-dimethylformamide (10 mL) was sulfated using sulfur trioxide–pyridine complex (0.014 g, 0.085 mmol) at room temperature for 1 h to give in 48% yield the sulfated compound (0.031 g) which was subsequently *O*-deacetylated as described for **15**. After similar processing, purification and freeze drying, **25** was obtained as a white solid (0.016 g, 80%). $[\alpha]_D$ −0.8 (*c* 0.5, H_2O); ^1H NMR (D_2O): δ 7.52–7.48 (m, 5 H, arom.), 4.61 (d, 1 H, *J* 7.78, H-1⁗), 4.49 (d, 1 H, *J* 7.77, H-1‴), 2.07–1.96 (2 s, NHAc); *m/z* found for $C_{47}H_{73}N_2O_{34}SNa$ $(M + H)^+$ 1265.7. Anal. Calcd for $C_{47}H_{73}N_2O_{34}SNa.H_2O$: C, 43.98; H, 5.74, N, 2.18. Found: C, 43.94; H, 5.61, N, 2.00.

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