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A convenient synthesis of lacto-*N*-biose I [β-D-Gal*p*-(1 → 3)-β-D-Glc*p*NAc] linked oligosaccharides from phenyl *O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside *

Rakesh K. Jain, Robert D. Locke and Khushi L. Matta

Department of Gynecologic Oncology, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263 (USA)

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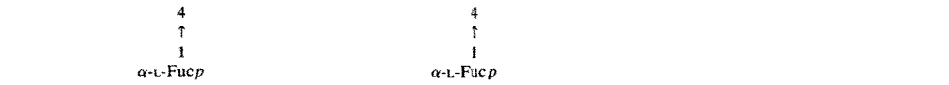
ABSTRACT

The synthesis of two tetrasaccharides and one trisaccharide containing lacto-*N*-biose I (β-D-Gal*p*-(1 → 3)-β-D-Glc*p*NAc) as their terminal unit was accomplished through development and utilization of a key glycosyl donor, namely, phenyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside.

INTRODUCTION

The lacto-*N*-biose I [β-D-Gal*p*-(1 → 3)-β-D-Glc*p*NAc] unit was first isolated through partial hydrolysis of lacto-*N*-tetrose[2]. This moiety has been shown to be a prominent feature in the carbohydrate structure of many glyconjugates, including tumor-associated antigens[3,4]. It is also an important determinant structure for establishing lectin activities[5,6]. Hakomori et al. have recently reported the expression of the following extended type 1 chains,



in glycoconjugate structures associated with gastrointestinal, breast, lung, liver and pancreatic cancers. It is also a part of lactotetraosyl ceramide (Lc4 antigen) which

Correspondence to: Dr. K.L. Matta, Dept. of Gynecologic Oncology, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263, USA.

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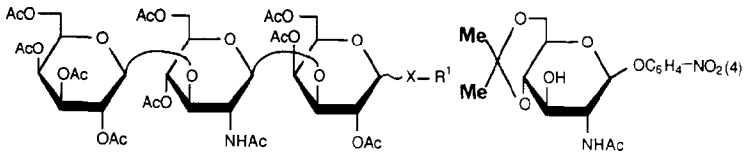
is expressed in endometrial cancers[7]. This moiety is a basic unit in the antigenic determinants for Lewis a and b activities[8,9]. Therefore, we have become interested in the preparation of 4-nitrophenyl oligosaccharides containing the type 1 chain. These compounds, upon reduction of the nitro group to an amino group, can be covalently linked to a protein carrier and employed as synthetic or artificial antigens. The generation of a series of antibodies raised towards different type I oligosaccharide structures can provide specific probes for the detection of these antigens in biological sources. This could result in the development of highly sensitive tumor indicators. These synthetic type 1 chain compounds can act as acceptors for α-L-(1 → 4)-fucosyltransferase, and thus they may provide a facile enzymic method for the preparation of α-(1 → 4)-linked fucosyl oligoscaccharides.

In addition to their application as immunogens, these type 1 oligosaccharides provide us with valuable substrates for the investigation and establishment of endoglycosidase activities that are needed for the structural elucidation of glycoconjugates. This paper describes our successful effort at devising a more efficient method for the synthesis of compounds incorporating the lacto-*N*-biose I unit. Syntheses of 2-acetamido-2-deoxy-β-D-glucopyranosyl-linked oligosaccharides have been reported employing glycosyl oxazolines[10] and bromides[11] or chlorides[12,13] of 2-deoxy-2-phthalimido sugars as glycosyl donors. More recently, the glycosyl trichloroacetimidate[14], fluoride[15], and phenylthio[16] derivatives have been utilized for this purpose. Methods for the construction of lacto-*N*-biose I containing oligosaccharides have been reported using a glycosyl chloride[17] and an oxazoline[18]. More recently, the syntheses of *O*-linked oligosaccharides enlisting thioglycoside donors have been accomplished using various thiophillic reagents as promoters[19]. We have initiated a program to design a convenient synthesis of type I containing oligosaccharides. In this regard, our approach now involves the preparation and use of phenyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**14**) as an effective glycosylating reagent for the desired compounds.

RESULTS AND DISCUSSION

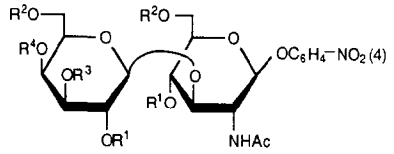
The disaccharide donor **14** was synthesized in three steps from known phenyl 4,6-*O*-benzylidine-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[16]. The glycosidation of this acetal with tetra-*O*-acetyl-α-D-galactopyranosyl bromide under catalysis by silver triflate–2,4,6-trimethylpyridine afforded the β-linked disaccharide **12** in 43% yield. The ^{1}H NMR spectrum contained signals in support of the overall structure expected.

The debenzylidenation of **12** in 80% aqueous acetic acid, followed by acetylation with pyridine–acetic anhydride, furnished the glycosyl donor **14** in 86% yield. The ^{1}H NMR spectrum of **14** displayed two doublets at δ 5.42 and 4.73 with spacings of ~ 10.4 Hz and 10.1 Hz, respectively, confirming a β-orientation for the anomeric carbon as well as for the newly introduced glycosidic linkage. The

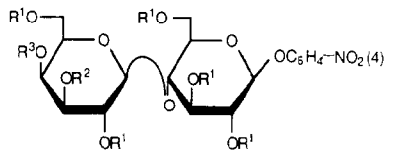


1. X = O, R' = Ac
2. X = S, R' = Ph

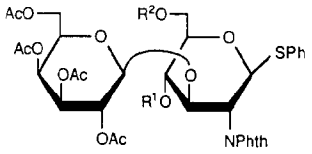
3



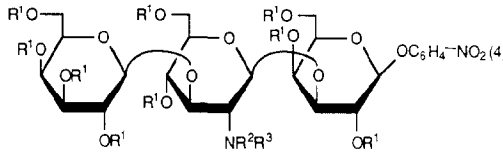
4. R' = R³ = R⁴ = H; R² = Bu' Ph_2Si
5. R' = R² = H; R³, R⁴ = CMe_2
6. R' = R² = Ac; R³ = R⁴ = H
7. R' = R² = R⁴ = Ac; R³ = H



8. R' = H; R², R³ = CMe_2
9. R' = Ac; R², R³ = CMe_2
10. R' = Ac; R² = R³ = H
11. R' = R³ = Ac; R² = H

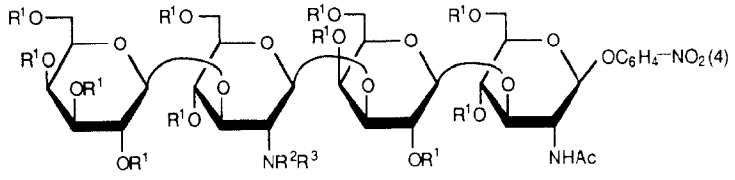


12. R', R² = PhCH
13. R' = R² = H
14. R' = R² = Ac

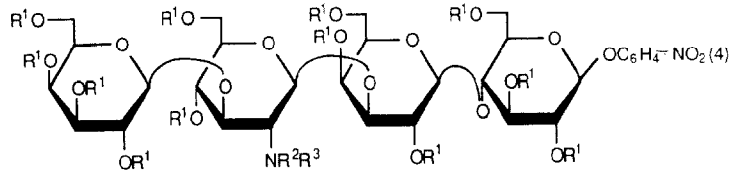


15. R' = Ac; R², R³ = Phth
16. R' = H; R², R³ = H, Ac

disaccharide acceptor **7** was prepared in six steps from the known disaccharide[20], 4-nitrophenyl *O*-(β-D-galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-β-D-glucopyranoside, which upon treatment with *tert*-butylchlorodiphenylsilane, followed by acetalation with 2,2-dimethoxypropane–acetone in the presence of 4-toluenesulfonic acid, then the removal of the *tert*-butyldiphenylsilyl group with fluoride ion, provided the 3',4'-*O*-isopropylidene compound **5** in 63% yield. Acetylation of compound **5** with pyridine–acetic anhydride, followed by cleavage of the 3',4'-*O*-isopropylidene group with chloroform–trifluoroacetic acid–water, furnished the diol **6** in 66% yield. The diol **6** was converted into its 3,4-(ethyl orthoacetate), which was hydrolyzed to give a key intermediate, 4-nitrophenyl *O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**7**), in 67% yield. The ^1H NMR spectrum of **7** exhibited a low-field chemical shift at δ 4.55, confirming that diol **6** had been acetylated at O-4 to give **7**. A similar reaction sequence was employed for the synthesis of **11** from **8**, as that



17. R^1 = Ac; R^2, R^3 = Phth
18. R^1 = H; R^2, R^3 = H, Ac



19. R^1 = Ac; R^2, R^3 = Phth
20. R^1 = H; R^2, R^3 = H, Ac

described for the preparation of **7** from **5**. The ^{1}H NMR spectrum of **11** was in accordance with the structure assigned.

Glycosylation of 4-nitrophenyl 2,4,6-tri-*O*-acetyl-β-D-galactopyranoside[21] with **14** in dichloromethane in the presence of *N*-iodosuccinimide–triflic acid[22] afforded, in 50% yield, the protected trisaccharide derivative **15** after silica gel column chromatography. The ^{1}H NMR spectrum of this product was in accord with the expected structure. The conversion of **15** into the known trisaccharide[23] **16** was then carried out in 4 steps: (*1*) 0.02 M sodium methoxide–methanol (*O*-deacetylation, to facilitate TLC monitoring of step *2*); (*2*) NH_2NH_2–H_2O–EtOH (phthalimido group removal); (*3*) pyridine–acetic anhydride *N*- and *O*-acetylation; and (*4*) 0.02 M sodium methoxide–methanol (*O*-deacetylation). The structure of **16** was confirmed by ^{13}C NMR spectroscopy (see Experimental).

For the synthesis of tetrasaccharide **18**, two procedures appeared equally feasible. In the first, we attempted the condensation of compound **2** [which was obtained by the treatment of fully acetylated trisaccharide[23] (**1**) with (phenylthio)-trimethylsilane and trimethylsilyl triflate[24] in dichloromethane] with 4-nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (**3**) at −35 to −40°C. However, after the removal of protecting groups from this intermediate, compound **18** was obtained in very low yield (15%). The low yield may be due to rapid hydrolysis of glycosyl donor **3** under these conditions. The reaction of **2** with 4-nitrophenyl 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside under the NIS–triflic acid procedure to give fully protected tetrasaccharide was not successful.

TABLE I

^{13}C NMR DATA[a]

Residue	Chemical shifts						
	C-1	C-2	C-3	C-4	C-5	C-6	NAc
Compound **16**							
β-D-Gal-(1 → 3)	106.26	73.49	75.28	71.33	78.05	63.81	
β-D-GlcNAc-(1 → 3)	105.34	57.56	84.91	71.33	78.21	63.41	25.05
β-D-Gal-(1 → O)-$C_6H_4NO_2$-(4)	102.90	72.29	84.49	70.93	78.05	63.36	
Compound **18**							
β-D-Gal-(1 → 3)	106.27	73.50	75.30	71.21	78.03	63.81	
β-D-GlcNAc-(1 → 3)	105.18	57.53	84.96	71.33	78.59	63.36	25.06
β-D-Gal-(1 → 3)	106.17	72.67	84.36	71.12	77.63	63.75	
β-D-GlcNAc-(1 → O)-$C_6H_4NO_2$-(4)	101.21	57.15	84.53	71.33	78.08	63.28	24.99
Compound **20**							
β-D-Gal-(1 → 3)	106.27	73.49	75.24	71.29	78.01	63.81	
β-D-GlcNAc-(1 → 3)	105.32	57.51	84.94	71.33	78.08	63.34	25.04
β-D-Gal-(1 → 4)	105.75	72.82	84.79	71.12	77.91	63.76	
β-D-Glc-(1 → O)-$C_6H_4NO_2$-(4)	102.05	75.30	76.87	80.76	77.72	62.62	

[a] For solutions in D_2O with Me_4Si as the external standard. Assignments, for the most part, are intuitive and not rigorously proven by ^1H–^{13}C coupling techniques.

Alternatively, compound **18** was obtained through condensation of donor **14** with acceptor **7**. Conventional transformation in four steps as just described for the preparation of **16** from **15** provided the target compound **18** in fair yield. A similar *N*-iodosuccinimide–triflic acid catalyzed glycosylation of 4-nitrophenyl *O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (**11**) with glycosyl donor **14**, and processing in a manner analogous to that described for the preparation of **16** (from **15**), afforded compound **20** in good yield. The ^{13}C NMR spectra of **18** and **20** were consistent with the structure assigned (see Table I).

In the ^{13}C NMR spectrum of **12**, the resonances for C-1 were observed at δ 84.35 (C-1) and 100.43 (C-1′), a fact which accounts for the β-D-configuration of the 2-deoxy-2-phthalimido-β-D-glucopyranose, as well as of the newly introduced β-D-galactopyranosyl moiety in this compound. In the ^{13}C NMR spectra of compounds **16**, **18**, and **20**, resonances for the C-1 of 2-acetamido-2-deoxy-β-D-glucopyranosyl moiety were observed at δ 105.18–105.34, which is a clear indication of the β-D-configuration for the newly introduced lacto-*N*-biose I moiety in these compounds. Similarly, the resonances for C-3 of the β-D-galactopyranose unit (acceptor) displayed a downfield shift (δ 84.36–84.79) in these compounds, confirming the site of glycosylation.

EXPERIMENTAL

General methods.—Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured at ~25°C with a

Perkin–Elmer 241 Polarimeter. TLC was conducted on glass plates, precoated with 0.25-mm layers 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. 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 ^{13}C-spectra with a Bruker AM 400 instrument at 100.6 MHz. All chemical shifts are referenced to Me_4Si. Solutions in organic solvents were generally dried with anhyd Na_2SO_4. Dichloromethane, *N*,*N*-dimethylformamide, benzene, and 2,2-dimethoxypropane were dried over 4A molecular sieves. Elemental analyses were performed by the Robertson Laboratory, Madison, New Jersey, USA.

Phenyl O-*(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1 → 3)-*O*-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1 → 3)-2,4,6-tri-*O*-acetyl-1-thio-α,β-*D*-galactopyranoside* (**2**).—To a stirred solution of *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-*O*-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-1,2,4,6-tetra-*O*-acetyl-α,β-D-galactopyranose[23] (1.1 g, 1.1 mmol) in CH_2Cl_2 (40 mL) was added (phenylthio)trimethylsilane (1.0 mL, 5.1 mmol) and trimethylsilyl triflate (0.25 mL, 1.04 mmol). After 16 h, an equal amount of (phenylthio)trimethylsilane was added and the stirring continued for a total of 2 days. The mixture was washed with cold water, satd aq $NaHCO_3$, dried, and concentrated. The residue was applied to a column of silica gel and eluted with a solvent gradient consisting of 15–20% acetone in $CHCl_3$ to give **2** (1.0 g, 86.4%) as an amorphous solid: $[\alpha]_D$ + 83° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.52–7.27 (m, 4 H, Ar), 5.06 (d, 1 H, *J* 8.0 Hz, H-1′), 4.61 (d, 1 H, *J* 9.9 Hz, H-1″), 2.14–1.88 (clusters of s, 27 H, 9 × OAc), and 1.71 (s, 3 H, NAc). Anal. Calcd for $C_{44}H_{57}NO_{24}S$: C, 52.01; H, 5.66; N, 1.38. Found: C, 51.86; H, 5.54; N, 1.26.

4-Nitrophenyl O-*(3,4-*O*-isopropylidene-β-*D*-galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**5**).—To a cold (0°C bath), stirred solution of 4-nitrophenyl (*O*-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-β-D-glucopyranoside[20] (1.9 g, 3.8 mmol) in anhyd DMF (30 mL) containing imidazole (1.7 g, 25.0 mmol) was added *tert*-butylchlorodiphenylsilane (3.3. mL, 12 mmol), and the stirring was continued for 1 h at ~0°C. It was then brought to room temperature and stirred for another 1 h, after which time the mixture was poured into ice–water and extracted with $CHCl_3$. The solution was washed with water, dried, and concentrated to give an intermediate **4** (2.5 g), which was used as such for the next step.

To a solution of **4** in dry acetone (40 mL) were added 2,2-dimethoxypropane (40 mL) and 4-toluenesulfonic acid monohydrate (0.4 g). The mixture was stirred for 1 h at room temperature, made neutral by the addition of triethylamine, and evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ and washed with water, dried, and concentrated. A stirred solution of this crude compound in anhyd oxolane (100 mL) was treated with a molar solution of tetrabutylammonium fluoride (10 mL), and the stirring was continued for 2 h at

room temperature. The mixture was evaporated to dryness, and the residue was purified on a column of silica gel with 1:9 MeOH–$CHCl_3$ as eluent to give **5** (1. 3 g, 63.4%) as an amorphous solid: $[\alpha]_D$ + 10.5° (*c* 0.6, MeOH); ^{1}H NMR (CD_3OD): δ 8.23 (d, 2 H, *J* 8.6 Hz, Ar), 7.20 (d, 2 H, *J* 7.8 Hz, Ar), 5.35 (d, 1 H, *J* 7.8 Hz, H-1), 4.57 (d, 1 H, *J* 9.5 Hz, NH), 4.33 (d, 1 H, *J* 7.7 Hz, H-1′), 1.99 (s, 3 H, NAc), 1.51 and 1.36 (each s, 3 H, CMe). Anal. Calcd for $C_{23}H_{32}N_2O_{13}$: C, 50.73; H, 5.92; N, 5.15. Found: C, 50.61; H, 6.18; N, 5.02.

4-Nitrophenyl O-(2,6-di-O-acetyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-4,6-di-O-acetyl-2-deoxy-β-D-glucopyranoside (**6**).—A solution of **5** (1.1 g, 2 mmol) in 1:2 acetic anhydride–pyridine (75 mL) was stirred for 16 h at room temperature. The pyridine and acetic anhydride were evaporated under diminished pressure, the last traces being removed by coevaporation with several portions of toluene. To a solution of this residue in $CHCl_3$ (100 mL) saturated with water, trifluoroacetic acid (6.0 mL) was added. After stirring for 1 h at room temperature, the solution was evaporated to dryness. Residual acid was removed by several coevaporations with toluene. The residue was purified on a column of silica gel with 5% MeOH in $CHCl_3$ as the eluant to afford **6** (0.9 g, 66.2%) as a solid on trituration with MeOH: $[\alpha]_D$ −22° [*c* 0.4, 1:1 $CHCl_3$–MeOH]; ^{1}H NMR ($CDCl_3$ + CD_3OD): δ 8.21 (d, 2 H, *J* 8.9 Hz, Ar), 7.13 (d, 2 H, *J* 9.1 Hz, Ar), 4.55 (d, 1 H, *J* 7.0 Hz , H-1), 4.27 (d, 1 H, *J* 4.2 Hz, H-4′), 4.17 (d, 1 H, *J* 8.0 Hz, H-1′), and 2.15–2.02 (cluster of s, 15 H, 4 × OAc and NAc). Anal. Calcd for $C_{28}H_{36}N_2O_{17}$: C, 50.00; H, 5.40; N, 4.16. Found: C, 50.07; H, 5.21; N, 4.00.

4-Nitrophenyl O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-4,6-di-O-acetyl-2-deoxy-β-D-glucopyranoside (**7**).—To a solution of **6** (0.9 g, 1.3 mmol) in dry benzene (40 mL) were added triethyl orthoacetate (10 mL) and 4-toluenesulfonic acid monohydrate (0.03 g). The solution was stirred for 2 h at room temperature. Triethylamine was added to neutralize the acid, and the solution was washed with cold water, dried, and concentrated under diminished pressure to give the 3,4-orthoester in quantitative yield. This was dissolved in 80% aq acetic acid (50 mL), and the solution was stirred for 1.5 h at room temperature. Acetic acid was evaporated under diminished pressure, the last traces being removed by coevaporation with several portions of toluene. The crude product was applied to a column of silica gel and eluted with 5% MeOH in $CHCl_3$. Concentration of the fractions corresponding to the product gave **7** (0.5 g, 67.2%): $[\alpha]_D$ −18° (*c* 0.9 $CHCl_3$); ^{1}H NMR ($CDCl_3$ + CD_3OD): δ 8.17 (d, 2 H, *J* 8.9 Hz, Ar), 7.07 (d, 2 H, *J* 9.0 Hz, Ar), 5.70 (d, 1 H, *J* 7.2 Hz, NH), 5.03 (dd, 1 H, *J* 9.4 and 8.6 Hz, H-4), 4.91 (dd, 1 H, *J* 8.1 and 9.5 Hz, H-2′), 4.59 (d, 1 H, *J* 7.8 Hz, H-1), 4.55 (d, 1 H, *J* 3.7 Hz, H-4′), 4.50 (dd, 1 H, *J* 9.1 and 8.5 Hz, H-3), 4.15 (d, 1 H, *J* 9.1 Hz, H-1′), and 2.02–2.18 (cluster of s, 18 H, 5 × OAc and NAc). Anal. Calcd for $C_{30}H_{38}N_2O_{18}$: C, 50.42; H, 5.36; N, 3.92. Found: C, 50.23; H, 5.09; N, 3.63.

4-Nitrophenyl O-(3,4-O-isopropylidene-β-D-galactopyranosyl)-(1 → 4)-β-D-glucopyranoside (**8**).—A mixture of 4-nitrophenyl *O*-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranoside (7 g, 15.1 mmol), 2,2-dimethoxypropane (7.0 mL) and 4-

toluenesulfonic acid monohydrate (0.15 g) in DMF (100 mL) was heated at 80–85°C for 15 min. The mixture was cooled, the acid was neutralized by the addition of triethylamine, and the solution was concentrated to dryness. The residue was dissolved in a solution of 3:2 EtOH–CH_2Cl_2 (25 mL). Addition of hexane furnished **8** (5.85 g, 77%) as a white amorphous solid: $[\alpha]_D$ −35.7° (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD + $CDCl_3$): δ 8.20 (d, 2 H, *J* 9.1 Hz, Ar), 7.22 (d, 2 H, *J* 9.2 Hz, Ar), 1.51 and 1.35 (each s, 3 H, CMe). Anal. Calcd for $C_{21}H_{29}NO_{13}$: C, 50.10; H, 5.81; N, 2.78. Found: C, 50.21; H, 5.76; N, 2.51.

4-Nitrophenyl O-*(2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-*D*-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranoside* (**9**).—Compound **8** (4.7 g, 9. 3 mmol) was added to a stirred mixture of 2:1 pyridine–acetic anhydride (150 mL). This was allowed to stir at room temperature for 24 h. The mixture was concentrated and coevaporated with toluene to remove the traces of pyridine. This residue was crystallized from EtOH to give **9** (5.9 g, 88%): mp 123–124°C; $[\alpha]_D$ −7.1° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.20 (d, 2 H, *J* 9.1 Hz, Ar), 7.05 (d, 2 H, *J* 9.1 Hz, Ar), 5.23 (d, 1 H, *J* 7.1 Hz, H-1), 2.13–2.06 (cluster of s, 15 H, 5 × OAc), 1.54 and 1.32 (each s, 3 H, CMe). Anal. Calcd for $C_{31}H_{39}NO_{18}$: C, 52.17; H, 5.51; N, 1.96. Found: C, 51.98; H, 5.63; N, 1.72.

4-Nitrophenyl O-*(2,6-di-*O*-acetyl-β-*D*-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranoside* (**10**).—Compound **9** (4.2 g, 5.9 mmol) in 80% aq acetic acid (100 mL) was heated for 1 h at ~ 70°C. Acetic acid was evaporated under diminished pressure, the last traces being removed by coevaporation with several portions of toluene. The residue was purified in a column of silica gel employing 8:1 $CHCl_3$–acetone as the eluent to give **10** (3.4 g, 86%): $[\alpha]_D$ −24.1° (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.20 (d, 2 H, *J* 9.0 Hz, Ar), 7.06 (d, 2 H, *J* 9.1 Hz, Ar), 5.24 (*d*, 1 H, *J* 7.5 Hz, H-1), and 2.21–2.05 (cluster of s, 15 H, 5 × OAc). Anal. Calcd for $C_{28}H_{35}NO_{18}$: C, 49.92; H, 5.24; N, 2.08. Found: C, 50.13; H, 5.17; N, 1.98.

4-Nitrophenyl O-*(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranoside)-(1 → 4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranoside* (**11**).—This compound was obtained from **10** (6.0 g, 8.9 mmol) by the same reaction sequence as described for the preparation of **7** (from **6**); amorphous solid (6.1 g, 96%): $[\alpha]_D$ −27.7° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$ + CD_3OD): δ 8.22 (d, 2 H, *J* 9.1 Hz, Ar), 7.15 (d, 2 H, *J* 9.2 Hz, Ar), 5.41 (d, 1 H, *J* 7.4 Hz, H-1), 5.32 (d, 1 H, *J* 2.7 Hz, H-4′), 5.22 (dd, 1 H, $J_{3,4}$ 10.2, $J_{2,3}$ 9.1 Hz, H-3), 4.94 (dd, 1 H $J_{2,3}$ 8.0, $J_{1,2}$ 10.2 Hz, H-2′), 4.81 (dd, 1 H, $J_{3,4}$, $J_{2,3}$ 6.3 Hz, H-3′), 4.52 (d, 1 H, *J* 7.7 Hz, H-1′), and 2.17–2.07 (cluster of s, 18 H, 6 × OAc). Anal. Calcd for $C_{30}H_{37}NO_{19}$: C, 50.35; H, 5.21; N, 1.96. Found: C, 50.57; H, 5.11; N, 2.02.

Phenyl O-*(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1 → 3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside* (**12**).—A mixture of phenyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[16] (3.9 g, 8.0 mmol), 2,3,4,-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (8.2 g, 20 mmol) and powdered 4A molecular sieves (12 g) in toluene (40 mL), protected from light

and moisture, was stirred at −20°C for 30 min under N_2. A solution of silver trifluoromethanesulfonate (8.2 g, 32 mmol) and 2,4,6-trimethylpyridine (2.98 mL, 24.8 mmol) in 3:2 CH_2Cl_2–toluene (40 mL) was then added dropwise over 20 min, and stirring was continued for an additional 30 min. Aqueous 10% sodium thiosulfate (90 mL) and toluene (180 mL) were added to the mixture, which was then filtered through a bed of Celite. The organic layer was washed successively with ice-cold water, cold satd $NaHCO_3$ and water, dried, and concentrated. The residue was purified on a column of silica gel using 4:1 hexane–EtOAc as the eluant to give **12** (4.27 g, 43%): $[\alpha]_D$ +24.5° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.78–7.22 (m, 4 H, Ar), 5.57 (s, 1 H, PhC*H*), 5.56 (d, 1 H, *J* 10.6 Hz, H-1), 5.18 (d, 1 H, *J* 3.3 Hz, H-4′), 4.96 (dd, 1 H, $J_{2,3}$ 8, $J_{1,2}$ 8 Hz, H-2′), 4.75 (dd, 1 H, $J_{2,3}$ 9.8, $J_{3,4}$ 3.2 Hz, H-3′), 4.54 (d, 1 H, *J* 7.6 Hz, H-1′), 4.40 (dd, 1 H, $J_{3,4}$ 10.2, $J_{2,3}$ 6.5 Hz, H-3), 2.06, 1.90, 1.83, and 1.53 (each s, 3 H, 4 × OAc); ^{13}C NMR ($CDCl_3$): δ 101.56 (PhCH), 100.43 (C-1′), 84.35 (C-1), 80.82 (C-3), and 54.39 (C-2). Anal. Calcd for $C_{41}H_{41}NO_{15}S$: C, 60.06; H, 5.04; N, 1.71. Found: C, 59.85; H, 5.16; N, 1.63.

Phenyl O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1 → 3)-1-thio-β*-D-*glucopyranoside* (**14**).—Compound **12** (4.3 g, 5.25 mmol) in 80% aq acetic acid was stirred for 30–45 min at ~70°C. Acetic acid was evaporated under diminished pressure, the last traces being removed by coevaporation with several portions of toluene. This crude compound **13** was stirred for 3 h at room temperature in 2:1 pyridine–acetic anhydride (120 mL). Pyridine and acetic anhydride were evaporated under reduced pressure with the aid of several portions of toluene to give a residue that was purified on a column of silica gel using 7:3 hexane–EtOAc as the eluant to give **14** (3.66 g, 86%): $[\alpha]_D$ +21.8° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.88–7.23 (m, 9 H, Ar), 5.42 (d, 1 H, *J* 10.4 Hz, H-1), 5.22 (d, 1 H, *J*, 3.0 Hz, H-4′), 5.05 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 4.92 (dd, 1 H, $J_{2,3}$ 10.2, $J_{1,2}$ 8.2 Hz, H-2′), 4.71 (dd, 1 H, H-3′), 4.62 (d, 1 H, *J* 7.2 Hz, H-1′), and 2.09–1.57 (cluster of s, 18 H, 6 × OAc). Anal. Calcd for $C_{38}H_{41}NO_{17}S$: C, 55.94; H, 5.02; N, 1.72. Found: C, 55.72; H, 4.91; N, 1.68.

General procedure for glycosidation.—A solution of donor **14** (0.94 g, 1.2 mmol), acceptor (1 mmol), and *N*-iodosuccinimide (0.55 g, 2.5 mmol) in CH_2Cl_2 (25 mL) was stirred for 0.5 h with 4A molecular sieves (5 g) under an Ar atmosphere at ~0°C. Then a dilute solution of trifluoromethanesulfonic acid (0.1 mL in 15 mL CH_2Cl_2) was added dropwise. Stirring was continued at the same temperature for another 1 h, after which time the acid was neutralized with a few drops of triethylamine. The mixture was filtered through Celite and the solids were thoroughly washed with $CHCl_3$. The filtrate and washings were combined, successively washed with water, satd $NaHCO_3$, 10% aq sodium thiosulfate, dried, and then concentrated in vaccuo.

4-Nitrophenyl O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1 → 3)*-O-*(4,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)-(1 → 3)-2,4,6-tri*-O-*acetyl-β*-D-*galactopyranoside* (**15**).—Glycosidation of 4-nitrophenyl 2,4,6-tri-*O*-acetyl-β-D-galactopyranoside[21] (0.75 g, 1.7 mmol) afforded **15** (1.0 g, 50.3%) after silica gel

column chromatography (10–15% acetone in $CHCl_3$): $[\alpha]_D$ −2.0° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.19–6.96 (m, 8 H, Ar), 5.20 (d, 1 H, *J* 8.8 Hz, H-1′), 4.95 (d, 1 H, *J* 8.6 Hz, H-1), 4.20 (d, 1 H, *J* 8.0, H-1″), and 2.16–1.84 (cluster of s, 27 H, 9 × OAc). Anal. Calcd for $C_{62}H_{73}N_3O_{35}$: C, 53.00; H, 4.98; N, 2.47. Found: C, 52.80; H, 4.76; N, 2.41.

4-Nitrophenyl O-(*β*-D-*galactopyranosyl*)-(*1 → 3*)-O-(*2-acetamido-2-deoxy-β*-D-*glucopyranosyl*)-(*1 → 3*)-*β*-D-*galactopyranoside* (**16**).—A solution of compound **15** (0.6 g) in 0.02 M NaOMe in MeOH (30 mL) was stirred for 2 h at room temperature. The base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin, the resin suspension was filtered, and the filtrate was concentrated. The solid so obtained was heated under reflux for 16 h in a mixture of EtOH (200 mL) and hydrazine hydrate (4.0 mL). The liquids were then evaporated to give a residue which was dissolved in pyridine (45 mL) and acetic anhydride (30 mL), then stirred overnight at room temperature. Solvent and reagent were removed under reduced pressure, and the residue was applied to a column of silica gel and eluted with a solvent gradient consisting of 30–40% acetone in $CHCl_3$. TLC (3:2 $CHCl_3$–acetone) of the fractions corresponding to the product revealed some impurities (noncarbohydrate, UV detection). However, the material was subjected to *O*-deacetylation without further purification.

To achieve this it was suspended in 0.02 M NaOMe (30 mL) and stirred overnight at room temperature. The base was neutralized by the addition of a few drops of glacial acetic acid, and the solid material was filtered and thoroughly washed with EtOH. The solid was dissolved in water and treated with Amberlite IR-120 (H^+) cation-exchange resin. Filtration and lyophilization afforded the known compound **16** (ref. 23; 0.18 g, 51%): $[\alpha]_D$ −8.0° (*c* 0.5, Me_2SO); for ^{13}C NMR data, see Table I.

4-Nitrophenyl O-(*2,3,4,6-tetra*-O-*acetyl*-*β*-D-*galactopyranosyl*)-(*1 → 3*)-O-(*4,6-di*-O-*acetyl-2-deoxy-2-phthalimido*-*β*-D-*glucopyranosyl*)-(*1 → 3*)-O-(*2,4,6-tri*-O-*acetyl*-*β*-D-*galactopyranosyl*)-(*1 → 3*)-*2-acetamido-4,6-di*-O-*acetyl-2-deoxy*-*β*-D-*glucopyranoside* (**17**).—Glycosidation of **7** (0.55 g, 0.77 mmol) with donor **14** (0.76 g, 0.86 mmol) gave **17** (0.58 g, 55%) after silica gel column chromatography (solvent gradient consisting of 20–25% acetone in $CHCl_3$): $[\alpha]_D$ −11.2° (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.16 (d, 2 H, *J* 8.7 Hz, Ar), 7.85–7.27 (m, 4 H, Ar), 7.02 (d, 2 H, *J* 8.9 Hz, Ar), 4.95 (d, 1 H, *J* 8.3 Hz, H-1), 4.51 (d, 1 H, *J* 7.7 Hz, H-1′), 4.15 (d, 1 H, *J* 8.5 Hz, H-1″), and 2.16–1.64 (cluster of s, 36 H, 11 × OAc and NAc). Anal. Calcd for $C_{62}H_{73}N_3O_{35}$: C, 52.43; H, 5.18; N, 2.96. Found: C, 52.43; H, 5.09; N, 2.70.

4-Nitrophenyl O-(*β*-D-*galactopyranosyl*)-(*1 → 3*)-O-(*2-acetamido-2-deoxy-β*-D-*glucopyranosyl*)-(*1 → 3*)-O-(*β*-D-*galactopyranosyl*)-(*1 → 3*)-*2-acetyl-2-deoxy-β*-D-*glucopyranoside* (**18**).—*(A) From* **17**. Compound 18 (from **17**; 0.3 g, 0.2 mmol) was obtained by the same reaction sequence as described for the preparation of **16** (from **15**); amorphous solid (0.135 g, 73.5%): $[\alpha]_D$ +8.4° (*c* 0.3, H_2O); ^1H NMR (D_2O): δ 8.25 (d, 2 H, *J* 9.3 Hz, Ar), 7.26 (d, 2 H, *J* 9.3 Hz, Ar), 5.29 (d, 1 H, *J*

8.6 Hz, H-1), 4.75 (d, 1 H, *J* 8.3 Hz, H-1″), 4.63 (d, 1 H, *J* 7.5 Hz, H-1‴), 4.45 (d, 1 H, *J* 7.6 Hz, H-1′), 2.06 and 2.03 (each s, 3 H, 2 × NAc); for ^{13}C NMR data, see Table I. Anal. Calcd for $C_{31}H_{51}N_3O_{22} \cdot 2H_2O$: C, 45.08; H, 6.12; N, 4.63. Found: C, 44.77; H, 5.85; N, 4.35.

(B) From donor **2**. Glycosidation of 4-nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (**3**) (0.09 g, 0.24 mmol) with donor **2** (0.2 g, 0.2 mmol) using *N*-iodosuccinimide (0.11 g) and triflic acid (0.025 mL in 15 mL CH_2Cl_2) at −35 to −40°C for 1.5 h, followed by removal of all protecting groups, afforded compound **18** (0.025 g, 14.5%), which was identical in all respects to compound **18** obtained from **17**.

4-Nitrophenyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 3)-O-(4,6-di-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**19**).—Glycosidation of compound **11** (0.7 g, 0.98 mmol) gave compound **19** (0.72 g, 50.3%) after silica gel column chromatography (solvent gradient consisting of 10–15% acetone in $CHCl_3$): $[\alpha]_D$ −6.0° (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.18 (d, 2 H, *J* 8.9 Hz, Ar), 7.83–7.82 (m, 4 H, Ar), 7.15 (d, 2 H, *J* 8.7 Hz, Ar), 5.19 (d, 1 H, *J* 7.9 Hz, H-1″), 5.07 (d, 1 H, *J* 9.5 Hz, H-1), 4.27 (d, 1 H, *J* 8.4 Hz, H-1′), 4.14 (d, 1 H, *J* 7.4 Hz, H-1‴), 2.21–1.59 (cluster of s, 36 H, 12 × OAc). *Anal. Calcd for $C_{66}H_{72}N_2O_{36}$: C, 52.39; H, 5.11; N, 1.97. Found: C, 52.53; H, 5.18; N, 1.85.*

4-Nitrophenyl O-(β-D-galactopyranosyl)-(1 → 3)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-O-(β-D-galactopyranosyl)-(1 → 4)-β-D-glucopyranoside (**20**). —This compound was furnished by the same sequence of reactions as described for the preparation of **16** (from **15**); amorphous solid (0.09 g, 58%): $[\alpha]_D$ −38.2° (*c* 0.3, H_2O); ^1H NMR (D_2O): δ 8.28 (d 2 H, *J* 9.3 Hz, Ar), 7.26 (d, 2 H, *J* 9.3 Hz, Ar), 5.32 (d, 1 H, *J* 7.6 Hz, H-1), 4.83 (d, 1 H, *J* 9.8 Hz, H-1″), 4.48 (d, 1 H, *J* 7.8 Hz, H-1‴), 4.45 (d, 1 H, *J* 7.6 Hz, H-1′), and 2.04 (s, 3 H NAc); for ^{13}C NMR data, see Table I. Anal. Calcd for $C_{31}H_{50}N_2O_{23} \cdot H_2O$: C, 44.49; H, 6.14; N, 3.34. Found: C, 44.58; H, 5.96; N, 3.60.

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