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Synthesis of α-D-galactopyranosyl-linked oligosaccharides containing the α-Gal → β-Gal → GlcNAc sequence employing methyl-2,3,4,6-tetra-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside as an efficient glycosyl donor ☆

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Abstract

Synthesis of two trisaccharides and a tetrasaccharide, namely, α-Gal-(1→3)-β-Gal-(1→3)-GlcNAc-β-OBn (**6**), α-Gal-(1→3)-β-Gal-(1→4)-GlcNAc-β-OBn (**9**) and α-Gal-(1→3)-β-Gal-(1→4)-GlcNAc-β-(1→6)-GalNAc-α-OBn (**19**) was accomplished through development and utilization of a key α-galactosyl donor, methyl 2,3,4,6-tetra-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside (**1**).

Keywords: Glycosyl donor; Synthesis of α-D-galactopyranosyl-linked oligosaccharides

1. Introduction

The primary receptor for sperm in the mouse is a zona pellucida (ZP) glycoprotein called mZP_3 which consists of 3 or 4 complex-type *N*-linked oligosaccharides and an, as yet, undetermined number of *O*-linked carbohydrate chains [1]. The precise structure of this sperm receptor still remains unresolved and has become a subject of controversy [2,3].

☆ Synthetic Studies in Carbohydrates, Part 91. For part 90 see Ref [17].

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Wassarman et al. [1] have reported that an α-D-galactopyranosyl residue on the nonreducing terminus of the *O*-linked oligosaccharide is essential for sperm receptor binding. Based upon this observation we became interested in the synthesis of the α-Gal → β-Gal → GlcNAc carbohydrate residue which is a part of the *O*-linked oligosaccharide structure of the ZP_3 glycoprotein.

Our interest in the study of α-L-fucosyltransferases in human and various other tissues and cell lines [4,5] has prompted us to develop the chemical synthesis of oligosaccharides containing the α-Gal → β-Gal → GlcNAc sequence. Recently, α-Gal-(1 → 3)-β-Gal-(1 → 4)-GlcNAc-β-R was utilized as an acceptor for α-(1 → 3/4)-fucosyltransferase activities by Van den Eijnden and co-workers [6].

In addition to this interest, the fact that α-Gal-(1 → 3)-Gal residues are reported to be expressed at the cell surface of malignant human cancer cells [7] further encourages our efforts to synthesize this class of compounds.

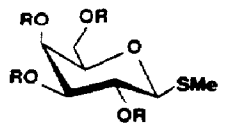
The major emphasis of our synthetic efforts has been directed toward obtaining an oligosaccharide structures possessing an anomeric benzyl group. These derivatives are very useful in our glycosyltransferase investigations as they permit the utilization of a convenient and rapid Sep-pack C_{18} cartridge methodology. This report describes a much facilitated procedure towards the production of these very important and useful class of carbohydrates.

2. Results and discussion

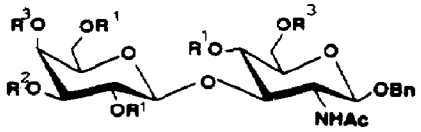
Among the methods in the literature for the stereoselective synthesis of α-linked oligosaccharides the following glycosyl donors have shown to be the most satisfactory: the in situ anomerization (of per-*O*-benzylated α-glycosyl bromides) procedure of Lemieux et al. [8][a–c], *n*-pentenyl [8][d–e], β-thiocyano [8][f], α-fluoro [8][g], alkyl [8][h–k], aryl [8][l–o], and hetero aryl thioglycosyl [8][p–s] per-*O*-benzylated donors. We herewith report a novel and efficient glycosyl donor, methyl 2,3,4,6-tetra-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside (**1**), for the synthesis of α-linked galactosyl oligosaccharides which avoids the need for hydrogenolysis to remove protecting groups and thus allows for the convenient production of moieties incorporating anomeric benzyl and nitrophenyl groups. We have reported the synthesis and glycosylating capability of methyl 2,6-di-*O*-(4-methoxybenzyl)-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside [9] and its employment in the synthesis of various disaccharides. Its utility for the synthesis of higher oligosaccharides, however, was not realized in our hands. We found that donor **1** offered a more satisfactory route toward the synthesis of the title series of compounds.

Methyl 1-thio-β-D-galactopyranoside [10] was reacted with 4-methoxybenzyl chloride, sodium hydride, and tetrabutyl ammonium bromide to afford methyl 2,3,4,6-tetra-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside (**1**) in 80% yield after purification through a silica gel column.

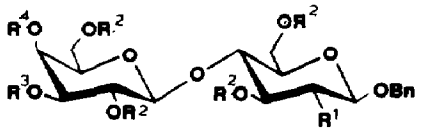
α-Galactosylation reaction of **2** [11] with **1** under $CuBr_2$–Bu_4NBr [12] in 5:1 dichloroethane–DMF yielded the fully protected trisaccharide, benzyl *O*-[2,3,4,6-tetra-*O*-(4-methoxybenzyl)-α-D-galactopyranosyl]-(1 → 3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**5**) in 65% yield.



1 R=4-Methoxybenzyl

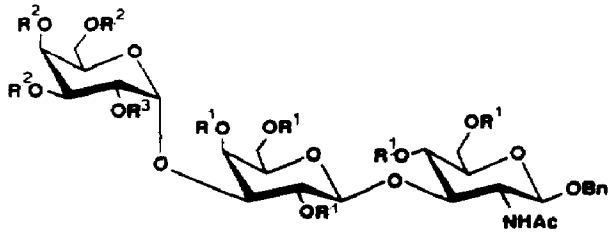


2 $R^1=R^3=Ac$; $R^2=H$



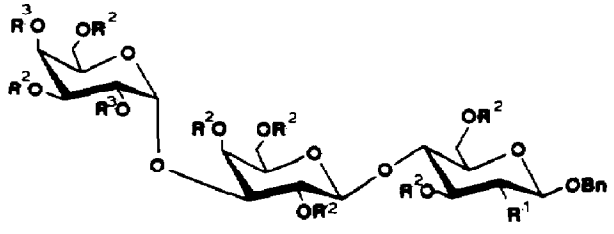
3 R^1=NPhth; R^2=Ac; $R^3=R^4=H$

4 R^1=NPhth; $R^2=R^4$=Ac; $R^3=H$



5 R^1=Ac; R^2=4-Methoxybenzyl

6 $R^1=R^2=H$



7 R^1=NPhth; R^2=Ac; R^3=4-Methoxybenzyl

8 R^1=NHAc; $R^2=R^3$=Ac

O-Demethoxybenzylation [13] of **5** with ceric ammonium nitrate in 9:1 acetonitrile–H_2O afforded the intermediate derivative which was extracted with 50% ethyl acetate in *n*-butanol, due to its solubility in water. Purification through a silica gel column followed by *O*-deacetylation afforded benzyl *O*-(α-D-galactopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (**6**) in 38% overall yield from

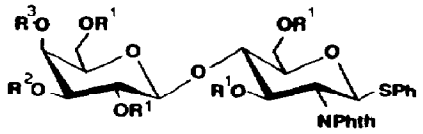
5. An attempt for the complete removal of 4-methoxybenzyl group from compound **5** with DDQ–CH_2Cl_2 or $CHCl_3$–CF_3CO_2H–H_2O was not successful.

The ^1H NMR spectrum of **6** showed an H-1″ doublet at δ 5.14 with a coupling constant of 3.8 Hz which was evidence of the newly introduced α-galactosyl moiety. Its ^{13}C NMR spectrum showed three anomeric carbons at δ 102.3, 98.6, and 94.5 ppm which accounts for the trisaccharide structure.

For the synthesis of the trisaccharide benzyl *O*-(α-D-galactopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**9**), the glycosyl acceptor,benzyl *O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**4**) was the key intermediate. Compound **4** was prepared in 95% overall yield from benzyl *O*-(2,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**3**) [14] by sequential 3′,4′-orthoester formation followed by regioselective opening of the ortho ester. The 3,4-diol **3** was synthesized in 55% yield following a published procedure [14] with the exception that stannic chloride was utilized as the catalyst [15] during the reaction of the fully acetylated compound with benzyl alcohol.

The condensation of **4** with **1** under similar conditions as described for the preparation of compound **5** afforded the trisaccharide, benzyl *O*-[2,3,4,6-tetra-*O*-(4-methoxybenzyl)-α-D-galactopyranosyl]-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**7**) in 56% yield. The conversion of **7** into **8** was carried out in 3 steps: (1) ceric ammonium nitrate in acetonitrile (removal of 4-methoxybenzyl group), (2) NH_2–$NH_2 \cdot H_2O$-EtOH (phthalimido group removal), and (3) pyridine–acetic anhydride (*N*- and *O*-acetylation). *O*-Deacetylation of **8** afforded **9** in 84% yield. The ^1H NMR spectrum of trisaccharide **9** showed a doublet at 4.99 with a spacing of 3.83 Hz for H-1″. Other signals were consistent with the structure assigned. The ^{13}C NMR spectrum of this compound showed three anomeric carbons at 101.8, 98.8, and 94.4 ppm.

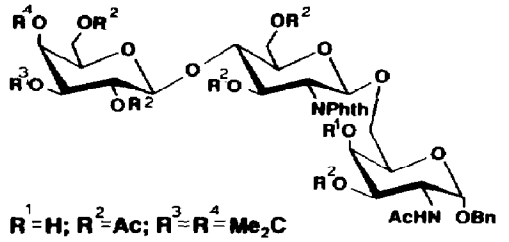
Synthesis of benzyl O-*(α-D-galactopyranosyl)-(1→3)*-O-*(β-D-galactopyranosyl)-(1→4)*-O-*(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-2-acetamido-2-deoxy-α-galactopyranoside* (**19**).—The key glycosyl donor, phenyl-(2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**12**) was prepared from phenyl-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**10**) [16] by *O*-deacetylation, 3′,4′-*O*-isopropylidination, and *O*-acetylation in 91% overall yield. Regioselective glycosidation with benzyl 2-acetamido-3-*O*-acetyl-2-deoxy-α-D-galactopyranoside through utilization of **12** afforded trisaccharide **13** in 70% yield. The axial HO-4 in trisaccharide **13** was acetylated to afford the fully protected trisaccharide, benzyl *O*-(2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)-2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-galactopyranoside (**14**). The utility and advantage of employing glycosyl donor **12** is realized in the subsequent conversion of **14** into **16** performed by convenient sequential reactions of *O*-deisopropylidenation, 3,4-*O*-ortho ester formation, and selective opening of the ortho ester affording product **16** in 74% overall yield. The ^1H NMR spectrum of **16** showed four characteristic signals in the region δ 5.2–



10 $R^1=R^2=R^3=Ac$

11 $R^1=R^2=R^3=H$

12 $R^1=Ac$; R^2, $R^3=Me_2C$

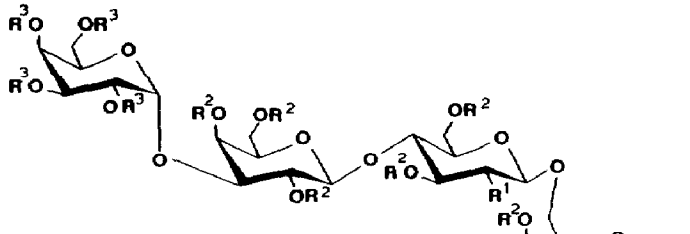


13 $R^1=H$; $R^2=Ac$; $R^3=R^4=Me_2C$

14 $R^1=R^2=Ac$; $R^3=R^4=Me_2C$

15 $R^1=R^2=Ac$; $R^3=R^4=H$

16 $R^1=R^2=R^3=Ac$; $R^4=H$



17 R^1=NPhth; R^2=Ac; R^3=4-Methoxybenzyl

18 R^1=NHAc; $R^2=R^3$=Ac

19 R^1=NHAc; $R^2=R^3$=H

5.5 for –NH–, H-1′, H-1, and H-4″. A downfield shift of 0.64 ppm was observed for the H-4″ signal relative to the same signal in diol **15**.

The coupling of trisaccharide acceptor **16** with **1** was performed in the presence of copper bromide and tetrabutylammonium bromide to furnish the protected tetrasaccharide **17** in 60% yield. The ^{1}H NMR spectrum of **17** exhibited characteristic signals for –NH–, H-1′, H-1, and a doublet at δ 5.38 ($J_{1,2}$ 2.8 Hz), which confirmed the α-linkage for the newly incorporated galactopyranosyl moiety. Sequential *O*-demethoxybenzylation, *N*-dephthalimidation followed by *N*,*O*-acetylation of **17** gave the fully acetylated derivative of α-Gal-(1→3)-β-Gal-(1→4)-β-GlcNAc-(1→6)-GalNAc-α-OBn (**18**) in 46% overall yield. The ^{1}H NMR spectrum of **18** was consistent with the assigned structure. *O*-Deacetylation of **18** gave the final tetrasaccharide in 60% yield. The ^{13}CNMR spectrum of **19** exhibited four anomeric carbons at 101.8, 100.4, 95.2, and 94.4 ppm.

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. The silica gel used for

column chromatography was Baker Analyzed (60–200 mesh). NMR spectra were recorded at ~25°C; ^{1}H NMR spectra with a Varian EM-390 at 90 MHz and with a Bruker AM-400 at 400 MHz, and ^{13}C NMR spectra with a Bruker AM-400 at 100.6 MHz. All chemical shifts are referenced to tetramethylsilane. Solutions in organic solvents were generally dried with anhyd Na_2SO_4. Dichloromethane, DMF, 1,2-dichloroethane, benzene, and 2,2-dimethoxypropane were kept dry over 4A molecular sieves. Elemental analyses were performed by the Robertson Laboratory, Madison, NJ, USA.

*Methyl 2,3,4,6-tetra-*O*-(4-methoxybenzyl)-1-thio-β-*D*-galactopyranoside* (**1**).—To a solution of methyl-1-thio-β-D-galactopyranoside (5 g, 23.8 mmol) in dry dimethylformamide (40 mL) was added NaH (60% in oil; 5.7 g, 142.8 mmol). The mixture was stirred at room temperature for 15 min. 4-Methoxybenzyl chloride (16.14 mL, 119 mmol) was then added slowly, followed by 0.5 g of Bu_4NBr and stirring was continued for 5 h at room temperature. TLC of the mixture in 1:1 hexane–EtOAc showed the disappearance of starting material (R_f 0.0) and the appearance of a single product (R_f 0.5). The reaction was cooled to 0°C and MeOH (20 mL) was added to decompose excess NaH. Solvents were removed and the resultant syrup was purified by silica gel column chromatography using 3:2 hexane–EtOAc containing a few drops of Et_3N as the eluent to yield the title compound (13 g) in 80% yield; $[\alpha]_D$ +4.3 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.3–7.1 and 6.87–6.81 (m, 16 H, arom.), 4.28 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.8–3.78 (4 s, 12 H, 4×OMe), 2.17 (s, 3 H, SMe). Anal. Calcd for $C_{39}H_{46}O_9S$: C, 71.09; H, 7.05. Found: C, 70.89; H, 7.18.

Benzyl O-*(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**4**).—To a solution of benzyl *O*-(2,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**3**; 0.475 g, 0.65 mmol) in dry benzene (25 mL) were added triethylorthoacetate (5 mL) and 4-toluenesulfonic acid monohydrate (0.06 g). The solution was stirred for 2 h at room temperature. Triethylamine was added to neutralize acid and the mixture concentrated to dryness under diminished pressure to give the 3,4-orthoester in quantitative yield.

This compound was dissolved in aq 80% AcOH (50 mL) and the solution was stirred at room temperature for 1.5 h. TLC analysis of the mixture showed the formation of a faster moving spot (R_f 0.4, in 2:1 $CHCl_3$–acetone) from compound **3** (R_f 0.2). Solvents were evaporated under diminished pressure, the last traces of AcOH being removed by coevaporation with toluene. The compound obtained was purified by column chromatography using 25% acetone in $CHCl_3$ as eluent to give compound **4** (0.470 g, 95%); $[\alpha]_D$ +49.3 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.78–7.73 (m, 4 H, Phth), 7.26–7.09 (m, 5 H, Ph), 5.74 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 8.1 Hz, H-3), 5.37 (d, 1 H, $J_{1,2}$ 8.31 Hz, H-1), 5.28 (d, 1 H, *J* 2.9 Hz, H-4′), 4.85 (dd, 1 H, $J_{1',2'}$ 8.3, $J_{2',3'}$ 10.3 Hz, H-2′), 4.47 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1′), 2.10–1.88 (3 S, 15 H, 5×OAc). Anal. Calcd for $C_{37}H_{41}NO_{17}$: C, 57.59; H, 5.32. Found: C, 57.45; H, 5.25.

Benzyl O-*[(2,3,4,6-tetra-*O*-(4-methoxybenzyl)-α-*D*-galacto-pyranosyl)]-(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* (**5**).—A mixture of methyl 2,3,4,6-tetra-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside (**1**; 0.69 g, 1.17 mmol) and benzyl *O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**2**; 0.4 g, 0.59 mmol) in dry 5:1 $ClCH_2$–CH_2Cl–DMF (24 mL) containing 4A molecular sieves (4 g) was stirred at room temperature under N_2 for 20 min. Tetrabu-

tylammonium bromide (0.39 g, 1.2 mmol) followed by copper bromide (0.28 g, 1.2 mmol) were added, and stirring was continued at room temperature under N_2 for 30 h. TLC assay (2:1 $CHCl_3$–acetone) indicated a major faster moving spot (R_f 0.5) than compound **2** (R_f 0.1).

The mixture was diluted with $CHCl_3$ (50 mL) and filtered through a Celite bed. The residue was washed with more $CHCl_3$. The filtrate and washings were transferred to a separating funnel and washed with 3 × 100 mL of satd cold aq sodium bicarbonate, followed by 1 × 100 mL of H_2O. The organic layer was dried over anhyd sodium sulfate, filtered, and evaporated to dryness (a few drops of Et_3N were added before evaporating the solvents). The compound obtained was purified by silica gel column chromatography using 25% acetone in $CHCl_3$ as eluent to give **5** (0.5 g, 65%); $[\alpha]_D$ +41.2 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.5–6.7 (m, 21 H, Ph), 5.52 (d, 1 H, $J_{2,NH}$ 7.4 Hz, NH), 5.39 (bs, 1 H, H-1″), 4.05 (d, 1 H, $J_{1,2}$ 6.3 Hz, H-1′), 3.90–3.7 (4 s, 12 H, 4 × OMe), 2.08–1.85 (5 s, 18 H, 5 × OAc, NHAc). Anal. Calcd for $C_{69}H_{83}NO_{25}$: C, 62.47; H, 6.25. Found: C, 62.01; H, 6.35.

Benzyl O-*(α-*D*-galactopyranosyl)-(1 → 3)-*O-*(β-*D*-galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**6**).—To a solution of compound **5** (0.33 g, 0.26 mmol) in 9:1 MeCN-H_2O (15 mL) was added ceric ammonium nitrate (1.25 g, 2.3 mmol). The solution was stirred at room temperature for 6 h. TLC assay (4:1 $CHCl_3$–MeOH) indicated the disappearance of starting material and the appearance of a single slower moving spot (R_f 0.25). Sodium bicarbonate (0.5 g) was then added to the mixture and the solvents were removed. The resultant residue was taken up in EtOAc containing 20% *n*-butanol (150 mL) and washed with aq satd sodium carbonate solution (1 × 50 mL) and H_2O (2 × 50 mL). The organic phase was evaporated to dryness and applied to a silica gel column for purification using 9:1 $CHCl_3$–MeOH as eluent. The pure compound obtained was deacetylated using 0.005 N NaOMe in MeOH (30 mL). TLC analysis (6:4:1 $CHCl_3$–MeOH–H_2O) showed a single slower moving spot (R_f 0.3). The mixture was neutralized with IR 120 H^+ resin, filtered, and evaporated to dryness. The compound was further purified by silica gel column chromatography using 13:6:1 $CHCl_3$–MeOH–H_2O to afford compound **6** (0.061 g, 38.6%); $[\alpha]_D$ +42.4 (*c* 1, H_2O); ^1H NMR (D_2O): δ 7.4–7.3 (m, 5 H, Ph), 5.14 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1″), 4.61 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.46 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1′), 1.94 (s, 3 H, NHAc). Anal. Calcd for $C_{27}H_{41}NO_{16}$: C, 51.02; H, 6.46. Found: C, 50.89; H, 6.32.

Benzyl O-*[(2,3,4,6-tetra-*O*-(4-methoxybenzyl)-α-*D*-galacto-pyranosyl)]-(1 → 3)-*O-*(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1 → 4)-3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**7**).—Compound **1** (0.68 g, 1.17 mmol), compound **4** (0.45 g, 0.58 mmol), and molecular sieves (4 g) in dry 5:1 $ClCH_2$–CH_2Cl–DMF were stirred under N_2 at room temperature. Tetrabutylammonium bromide (0.45 g, 1.4 mmol) and copper bromide (0.33 g, 1.4 mmol) were added and stirring was continued for 36 h. TLC assay (1:1 toluene–EtOAc) revealed a new product (R_f 0.45). The mixture was processed as described for compound **5**. Silica gel column purification employing 1:1 hexane–EtOAc containing 0.05% Et_3N yielded compound **7** (0.46 g, 56%); $[\alpha]_D$ +32.5 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.77–6.77 (m, 25 H, Ph), 5.39 (bs, 1 H, H-1″), 5.37 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.38 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1′), 3.81, 3.80, 3.78, 3.74 (4 s, 12 H, 4 × OMe),

2.12–1.85 (4 s, 15 H, 5×OAc). Anal. Calcd for $C_{75}H_{83}NO_{26}$: C, 63.67; H, 5.86. Found: C, 62.89; H, 5.92.

Benzyl O-(α-D-galactopyranosyl)-(1→3)-O-(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**9**).—Compound **7** (0.3 g, 0.21 mmol) was dissolved in 9:1 MeCN–H_2O (15 mL), ceric ammonium nitrate (0.83 g, 1.7 mmol) was added, and the mixture stirred at room temperature for 6 h. TLC examination in 9:1 $CHCl_3$–MeOH revealed the disappearance of **7** (R_f 0.9) and the appearance of a slower moving product (R_f 0.3). The mixture was diluted with $CHCl_3$ (200 mL) and washed with satd aq sodium bicarbonate solution (2×50 mL), brine solution (2×50 mL), and finally with H_2O (50 mL). The organic layer was dried, filtered, and evaporated. Column purification afforded a pure compound (0.12 g; 57%). This compound was dissolved in EtOH (50 mL) and hydrazine hydrate (5 mL) and the mixture was stirred at 70°C for 16 h. After cooling to room temperature the solvents were removed and the resultant material was acetylated. Purification by silica gel column chromatography using 4:1 $CHCl_3$–acetone afforded compound **8** (0.08 g, 62%).

Compound **8** (0.08 g) was deacetylated using 0.005 N NaOMe in MeOH (20 mL) at room temperature for 36 h and neutralized with IR 120 H^+ resin to give compound **9** (0.03 g, 84% yield); $[\alpha]_D$ +49.9 (*c* 1, H_2O); ^1H NMR (D_2O): δ 7.3–7.2 (m, 5 H, Ph), 4.99 (d, 1 H, $J_{1,2}$ 3.83 Hz, H-1″), 4.4 (d, 1 H, *J* 6.5 Hz, H-1), 4.38 (d, 1 H, $J_{1,2}$ 6.1 Hz, H-1′), 1.77 (s, 3 H, NHAc). Anal. Calcd for $C_{27}H_{41}NO_{16}\cdot H_2O$: C, 49.60; H, 6.64. Found: C, 49.76; H, 6.52.

Phenyl O-(2,6-di-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-(1→4)-3,6-di-O-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**12**).—A solution of compound **10** (5 g) in 4:1 MeOH–THF (100 mL) was treated with dropwise addition of 0.1 N NaOMe in MeOH (1.5 mL) and was then stirred at room temperature for 2 h. When TLC assay (9:1 $CHCl_3$–MeOH) showed a single slower moving spot (R_f 0.05) it was neutralized with (H^+) resin. The mixture was filtered, the residue washed with MeOH, and the filtrates combined, evaporated, and co-distilled thrice with toluene to give compound **11** (3.3 g, 95%).

To a solution of compound **11** (2.5 g) in 2,2′-dimethoxypropane (100 mL) was added camphor sulfonic acid (0.13 g), and the mixture was stirred at room temperature for 2 days. TLC assay (9:1 $CHCl_3$–MeOH) indicated the disappearance of compound **11**. The mixture was neutralized with Et_3N and evaporated to dryness. The resultant residue was refluxed with 10:1 MeOH–H_2O for 16 h. TLC assay (9:1 $CHCl_3$–MeOH) revealed a single product (R_f 0.5). Removal of solvents and drying under high vacuum yielded the 3,4-*O*-isopropylidenated compound. This compound was acetylated using 3:2 pyridine–Ac_2O (50 mL) at room temperature for 16 h. TLC assay (1:1 hexane–EtOAc) indicated a faster moving single productt (R_f 0.4). The mixture was cooled to 0°C, MeOH (20 mL) was added, and then stirred for 0.5 h. Solvent evaporation, co-distillation with several added portions of toluene followed by silica gel column purification using 3:2 hexane–EtOAc yielded compound **12** (3 g, 88%); ^1H NMR ($CDCl_3$); $[\alpha]_D$ +49.3 (*c* 1, $CHCl_3$); 7.85–7.73 (m, 4 H, Phth), 7.40–7.24 (m, 5 H, Ph), 5.75 (dd, 1 H, $J_{2,3}$ 9.7, $J_{3,4}$ 9.0 Hz, H-3), 5.69 (d, 1 H, $J_{1,2}$ Hz, 10.6, H-1), 2.16–1.89 (4 s, 12 H, 4×OAc), 1.50, 1.30 (2 s, 6 H, CMe_2). Anal. Calcd for $C_{37}H_{41}NO_{15}S$: C, 57.58; H, 5.32. Found: C, 57.32; H, 5.35.

Benzyl O-(2,6-di-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-(1→4)-O-(3,6-di-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)-2-acetamido-3,4-di-O-acetyl-2-deoxy-α-D-galactopyranoside (**14**).—Compound **12** (2 g, 2.6 mmol), benzyl 2-acetamido-3-*O*-acetyl-2-deoxy-α-D-galactopyranoside (0.96 g, 2.6 mmol), and *N*-iodosuccinimide (1.75 g, 7.7 mmol) were dissolved in dry CH_2Cl_2 (150 mL), and activated 4A molecular sieves (10 g) were added. The mixture was cooled to −20°C under N_2 and a homogenous solution of triflic acid (50 μl) in dry CH_2Cl_2 (10 mL) was added dropwise. The mixture was stirred for 45 min at −10°C. TLC examination in 9:1 $CHCl_3$–MeOH revealed a new product (R_f 0.3) in between the donor (R_f 0.9) and acceptor (R_f 0.1). The mixture was diluted with CH_2Cl_2 (100 mL) and filtered through Celite into cold aq satd sodium bicarbonate. The filtrate was washed with more bicarbonate solution (2×100 mL), 10% sodium thiosulfate (2×100 mL) and H_2O, dried over sodium sulfate, and concentrated. The resultant material was purified by silica gel column chromatography using 97:3 $CHCl_3$–MeOH containing a few drops of pyridine to yield compound **13** (1.95 g, 70%), which was acetylated to afford the title compound **14** (2 g) in quantitative yield; $[\alpha]_D$ +66.9 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.75–7.65 (m, 4 H, Phth), 7.26–7.15 (m, 5 H, Ph), 5.45 (d, 1 H, $J_{1,NH}$ 9.5 Hz, NH), 5.36 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1′), 5.06 (d, *J* 8.5 Hz, H-2), 4.64 (d, 1 H, $J_{3,4}$ 3.1 Hz, H-4), 4.36 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1″), 2.13–1.89 (5 s, 18 H, 6 OAc), 1.84 (s, 3 H, NHAc), 1.51, 1.30 (2 s, 6 H, CMe_2). Anal. Calcd for $C_{50}H_{61}N_2O_{24}\cdot H_2O$: C, 54.98; H, 5.82. Found: C, 54.89; H, 5.84.

Benzyl O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(3,6-di-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)-2-acetamido-3,4-di-O-acetyl-2-deoxy-α-D-galactopyranoside (**16**).—Compound (**14**, 1.2 g) was treated with 80% aq AcOH at 65°C for 2 h. TLC of the mixture in 3:2 $CHCl_3$–acetone (R_f 0.3) indicated that the reaction was complete. The mixture was cooled, the solvents evaporated under diminished pressure, and the residue co-distilled with toluene until the last traces of AcOH were removed. This compound (**15**) was crystallized from EtOH (0.9 g, 81%); $[\alpha]_D$ +54.3 (*c* 1, $CHCl_3$).

To a solution of compound **15** (0.8 g) in dry benzene (30 mL) triethyl orthoacetate (6 mL) and *p*-toluenesulfonic acid (0.05 g) were added, and the mixture stirred at room temperature for 3 h. TLC assay (2:1 $CHCl_3$–acetone) revealed the formation of the orthoester (R_f 0.6). The mixture was neutralized with Et_3N and evaporated to dryness. The resultant residue was treated with 80% aq AcOH (50 mL) at room temperature for 3 h, and the solvents were evaporated under reduced pressure. Silica gel column purification of this material using 4:1 $CHCl_3$–acetone as eluent yielded compound **16** (0.56 g, 68%); $[\alpha]_D$ +55.4 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.82–7.67 (m, 4 H, Phth), 7.28–7.15 (m, 5 H, Ph), 5.46 (d, 1 H, $J_{1,NH}$ 9.6 Hz, NH), 5.36 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1′), 5.28 (d, 1 H, *J* 2.6 Hz, H-4″), 5.20 (bs, 1 H, H-1), 5.04 (d, 1 H, *J* 8.6 Hz, H-2), 4.45 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1″), 2.14–1.87 (4×s, 21 H, 7×OAc), 1.84 (s, 3 H, NHAc). Anal. Calcd for $C_{49}H_{59}N_2O_{24}$: C, 55.50; H, 5.57. Found: C, 55.47; H, 5.50.

Benzyl O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-2-acetamido-3,4-di-O-acetyl-2-deoxy-α-D-galactopyranoside (**18**).—A mixture of compound **1** (0.36 g, 0.52 mmol) and compound **16** (0.26 g, 0.25 mmol) in dry 5:1 1,2-dichloroethane–DMF (12 mL) with 4A molecular sieves (3 g) was stirred at room temperature under N_2 for 20 min. Tetra *n*-butylammonium bromide (0.17, 0.52

mmol) and copper bromide (0.12 g, 0.52 mmol) were then added, and stirring was continued (36 h) until TLC (3:3 $CHCl_3$–acetone) showed the absence of donor (R_f 0.8) and acceptor (R_f 0.2), and the presence of a new product (R_f 0.4). The mixture was worked up as described for compound **5**. Silica gel column purification using 5:1 $CHCl_3$–acetone containing a few drops of pyridine yielded compound **17** (0.25 g, 60% yield); $[\alpha]_D$ +55.6 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.8–7.6 (m, 4 H, Phth), 7.26–6.60 (m, 21 H, Ph), 5.45 (d, 1 H, NH), 5.38 (d, 1 H, $J_{1,2}$ 2.8 Hz, H-1‴), 5.36 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1″), 5.2 (bs, 1 H, H-1), 2.1–1.84 (5 s, 18 H, 6 OAc), 1.61 (s, 3 H, NHAc).

Compound **17** (0.23 g, 0.13 mmol) was treated with ceric ammonium nitrate (0.72 g, 1.32 mmol) in 9:1 MeCN–H_2O at room temperature for 6 h. TLC assay (3:2 $CHCl_3$–acetone) showed a single slower moving product (R_f 0.3). The mixture was diluted with $CHCl_3$ (50 mL) and washed with H_2O (3×20 mL), evaporated, dried, and purified by silica gel column chromatography using 2:1 $CHCl_3$–acetone as eluent. The residue was dissolved in EtOH (10 mL) and hydrazine hydrate (0.6 mL), heated at 70°C for 16 h, cooled, and evaporated to dryness. Treatment of the residue with pyridine (18 mL) and Ac_2O (12 mL) for 10 h gave the acetylated product which was processed as described for compound **12**. Silica gel column purification using 3:1 $CHCl_3$–acetone as eluent yielded the title compound **18** (0.08 g, 46%); $[\alpha]_D$ +56.7 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.28–7.16 (m, 5 H, Ph), 5.46 and 5.40 (each d, 1 H, 2×NH), 5.27 (d, 1 H, $J_{3,4}$ 2.5 Hz, H-4‴), 5.25 (d, 1 H, $J_{3,4}$ 2.6 Hz, H-4‴), 2.15–1.88 (6 s, 33 H, 11×OAc), 1.84 and 1.82, (each s, 2×NHAc).

Benzyl O-*(α-*D-*galactopyranosyl)-(1→3)*-O-*(β-*D-*galactopyranosyl)-(1→4)*-O-*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1→6)-2-acetamido-2-deoxy-α-*D-*galactopyranoside* (**19**).—Compound **18** (0.07 g) was deacetylated using MeOH (20 mL) and 0.1 N NaOMe in MeOH (0.2 mL) at room temperature for 3 days. TLC monitoring with 5:4:1 $CHCl_3$–MeOH–H_2O showed a single product (R_f 0.25). The base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin, the solution was concentrated after filtration, and the residue was purified by silica gel column chromatography using 13:6:1 $CHCl_3$–MeOH–H_2O as eluent. Fractions containing **19** were combined and evaporated, and the residue was further purified on a column of Bio-Gel P2 with pyridinium acetate buffer (100 mM, pH 5.5) as eluent. Freeze-drying of the fractions containing **19** gave an amorphous solid (0.027 g, 60%); $[\alpha]_D$ +75.6 (*c* 0.9; H_2O); ^1H NMR (D_2O): δ 7.46 (m, 5 H, Ph), 5.18 (d, 1 H, *J* 3.2 Hz, H-1‴), 5.01 (d, 1 H, *J* 3.5 Hz, H-1), 1.98 (s, 6 H, 2 NHAc); ^{13}C NMR (D_2O): 173.5, 173.2 (2 $NHCOH_3$) 135.9–127.6 (Ph), 101.8 (C-1″), 100.4 (C-1′), 95.2 (C-1), 94.4 (C-1‴), 21.2, 20.85 (2 NAc). Anal. Calcd for $C_{35}H_{54}N_2O_{21}\cdot H_2O$: C, 49.05; H, 6.60; N, 3.26. Found: C, 48.98, H, 6.43; N, 3.29.

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