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Synthesis of oligosaccharides containing the X-antigenic trisaccharide (α-L-Fuc*p*-(1→3)-[β-D-Gal*p*-(1→4)]-β-D-Glc*p*NAc) at their nonreducing ends*

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ABSTRACT

The "armed" methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside was reacted with "disarmed" phenyl *O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside in the presence of $CuBr_2$–Bu_4NBr complex to give phenyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**6**) as a novel glycosyl donor. The glycosylating capability of **6** was further examined using *N*-iodosuccinimide–triflic acid as a reagent. This led to the synthesis of a tetrasaccharide and a pentasaccharide incorporating the X-antigenic structure represented by **6**.

INTRODUCTION

A number of glycoconjugates incorporating the terminal structure α-L-Fuc*p*-(1→3)-[β-D-Gal*p*-(1→4)]-β-D-Glc*p*NAc have been isolated and characterized from ovarian cyst fluid[2], blood group substances of glycoprotein origin[3–5], human milk[6], human adenocarcinomas[7], and hog gastric mucosa[8].

We have initiated a program on the chemical synthesis of glycoconjugates having the foregoing X-antigenic trisaccharide unit at their nonreducing ends. Such compounds are expected to be useful in specificity studies of an antibody raised against a related, synthetic antigen that we are currently investigating. In continuation of these efforts, we describe herein the synthesis of a tetrasaccharide (**9**) and a pentasaccharide (**12**).

RESULTS AND DISCUSSION

Syntheses of oligosaccharides containing an α-L-Fuc*p*-(1→3)-[β-D-Gal*p*-(1→4)]-β-D-Glc*p*NAc cluster at their nonreducing ends, employing the trisaccharide ethyl thioglycoside, trichloroacetimidate, and fluoride as glycosyl donors, have been reported

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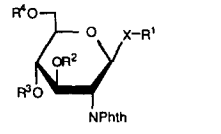
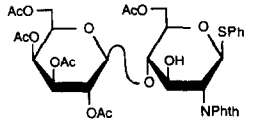
in the literature[9–13]. In our continuous effort to seek out new and more economical reaction schemes we have been successful in the development of a new glycosyl donor, phenyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**6**), which provides a more efficient and high yielding route to the title oligosaccharides. This thioglycoside was prepared in six steps from 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**1**).

In the first step β-acetate **1**, on treatment with trimethyl(phenylthio)silane and trimethylsilyl triflate[14] in dichloromethane, furnished phenylthio compound **2** in 69.5% yield. *O*-Deacetylation of **2** with methanolic sodium methoxide, followed by treatment with benzaldehyde dimethyl acetal and 4-toluenesulfonic acid monohydrate in *N,N*-dimethylformamide, afforded a good yield of 4,6-*O*-benzylidene compound **3**. Treatment of **3** in acidic medium in the presence of sodium cyanoborohydride[15] then gave, in 85% yield, phenyl 6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**4**). The ^{1}H-n.m.r. spectra of both **3** and **4** included signals characteristic of the expected structures. Thus, whereas the spectrum of **3** contained a one-proton resonance at δ 5.51, attributable to the benzylidene methine proton, that of **4** was devoid of such a resonance. However, in both spectra, the ratio of aromatic to other protons remained the same, a clear indication that the benzylidene acetal group of **3** was not completely removed, but rather had undergone reductive cleavage. In the ^{13}C-n.m.r. spectrum of **4**, the noticeable downfield shift of the resonance of C-6 (δ 70.96) was indicative of O-6 as the site of benzylation.



	X	R^1	R^2	R^3	R^4
1	O	Ac	Ac	Ac	Ac
2	S	Ph	Ac	Ac	Ac
3	S	Ph	H	⌐Bzd⌐	
4	S	Ph	H	H	Bn



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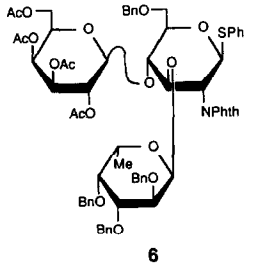

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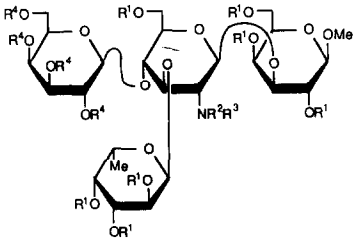


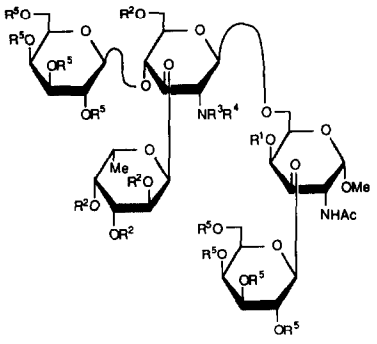
	R^1	R^2	R^3	R^4
7	Bn	⌐Phth⌐		Ac
8	Bn	H, Ac		H
9	H	H, Ac		H

Glycosylation of diol **4** with 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl fluoride[16] under Mukaiyama's conditions[17] ($SnCl_2$–AgOTf) in 5:1 (v/v) dichloromethane–toluene in the presence of 4A molecular sieves afforded a major β-(1→4)-linked disaccharide **5** in 64% yield, along with some minor products.

Methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside has been utilized for α-L-fucopyranosylation in the presence of $CuBr_2$–Bu_4NBr complex (*in situ* bromide formation). We became interested in examining the coupling reaction of a methylthio ("armed") donor with the 2-deoxy-2-phthalimido phenylthio compound **5** ("disarmed" acceptor) in the presence of $CuBr_2$–Bu_4NBr complex. This reaction proceeded with α-stereospecificity to afford the key glycosyl donor **6** in high yield. The ^{1}H-n.m.r. spectrum of **6** showed a doublet at δ 5.18 with spacing of ~ 3 Hz, confirming the newly introduced glycosidic bond as an α-linkage. Recently, the coupling of perbenzylated thioglycosides ("armed" donors) with partially benzoylated thioglycosides ("disarmed" acceptors) to give only the cross-product, with no evidence of self coupling, has been reported by Van Boom *et al.*[18] We then examined the glycosylating capability of **6**, with methyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside as acceptor, in the presence of *N*-iodosuccinimide–triflic acid[20] in dichloromethane at ice-bath temperature. To our surprise, t.l.c. of the reaction mixture in 3:2 hexane–ethyl acetate revealed two major products that were slower migrating than the starting materials. The products were tentatively identified as the disaccharide and trisaccharide resulting from the loss of the α-L-fucopyranosyl residue from the decomposed donor and from the initially formed products, respectively, as indicated by their ^{1}H-n.m.r. spectra.

The behavior of **6** with methyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside in dichloromethane at $-50°$ to $-60°$ was more encouraging. The reaction was very fast. After 10 min., t.l.c. in 1:1 hexane–ethyl acetate showed the disappearance of phenylthio compound **6**, and the formation of one major product moving just below **6**. The ^{1}H-n.m.r. spectrum of this product (**7**, 83% yield) was in conformity with the expected structure. The conversion of **7** into the tetrasaccharide **9** was then carried out in 5 steps: (*1*) 0.02M sodium methoxide–methanol (*O*-deacetylation, to facilitate t.l.c. monitoring of step 2); (*2*) $NH_2NH_2 \cdot H_2O$/EtOH (phthalimido group removal); (*3*) pyridine–acetic anhydride (*N*- and *O*-acetylation); (*4*) 0.02M sodium methoxide–methanol (*O*-deacetylation); and (*5*) 10% Pd–C/H_2 (hydrogenolysis for the removal of *O*-benzyl groups). The structure of **9** was confirmed by ^{13}C-n.m.r. and f.a.b.-mass spectroscopy (see Experimental section).

Similarly, *N*-iodosuccinimide–triflic acid-catalyzed glycosylation of methyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside with donor **6** afforded, in 71% yield, the protected pentasaccharide derivative **10**. Conventional transformation of **10** into the pentasaccharide **12** was then achieved in 5 steps as just described for the preparation of **9** from **7**. The ^{13}C-n.m.r. spectrum of **12** was also consistent with the structure assigned (see Table I). To the best of our knowledge, this is the first report describing the employment of methyl 1-thio-β-L-fucopyranoside as the glycosyl donor in reaction with a phenyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside derivative (**5**) as acceptor to provide compound **6**. In the



	R^1	R^2	R^3	R^4	R^5
10	H	Bn	⌞Phth⌟		Ac
11	Ac	Bn	H,Ac		Ac
12	H	H	H,Ac		H

TABLE I

^{13}C-N.m.r. data[a] (proposed assignments)

Residue	*Chemical shifts*							
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*NAc*	*OMe*
Compound **9**								
Gal-β-(1→4)	105.17	73.86	75.30	71.13	77.54	63.70	–	–
Fuc-α-(1→3)	101.34	70.52	72.01	74.72	69.47	18.09	–	–
GlcNAc-β-(1→3)	104.57	59.99	77.70	77.96	75.92	64.26	25.08	–
Gal-β-OMe	106.71	72.50	85.12	71.13	77.47	62.48	–	58.84
Compound **12**								
Gal-β-(1→4)	104.63	72.89	75.28	71.38	77.75	63.76	–	–
Fuc-α-(1→3)	101.43	71.14	72.03	74.72	70.51	18.09	–	–
GlcNAc-β-(1→6)	104.15	58.51	79.87	78.15	77.70	64.23	25.07	–
Gal-β-(1→3)	107.45	73.42	75.34	71.97	77.75	62.58	–	–
GalNAc-α-OMe	101.00	51.31	76.22	71.74	73.85	69.49	24.81	57.72

[a] For solutions in D_2O with Me_4Si as the external standard.

presence of *N*-iodosuccinimide–triflic acid, **6** can be directly utilized for the synthesis of oligosaccharides containing the X-antigenic trisaccharide structure at their nonreducing ends.

^{13}C-N.m.r. assignment. — In the ^{13}C-n.m.r. spectrum of **5** a downfield shift of 7.44 p.p.m. was observed in the resonance for C-4, along with an upfield shift of 1.53 p.p.m. for C-3, in comparison to the spectrum of the parent compound **4**, evidencing O-4 as the site of glycosylation. On the other hand, the resonance for C-1′ was observed at δ 102.00, a clear indication of the β-configuration of the newly introduced D-galactopyranosyl moiety. In the ^{13}C spectra of compounds **9** and **12**, the resonances for C-1 of the GlcNAc

and Gal residues were all in the region characteristic of β-glycosidic linkages. Similarly, the resonances for C-1 of L-fucose in these compounds were observed at δ 101.34 and 101.43, which accords with an α configuration. The resonances for C-3 and C-4 of the 2-acetamido-2-deoxy-β-D-glucopyranose residue suffered a downfield shift, confirming that O-3 and O-4 were the sites of glycosylation. In the spectrum of **9**, an analogous downfield shift was observed for the C-3 resonance (δ 85.12) of the β-D-Gal-*O*-Me residue, indicating the site of linkage of the trisaccharide to this unit. In the ^{13}C spectrum of **12**, the resonance for C-6 of α-D-GalNAc-*O*-Me displayed a downfield shift (δ 69.49), confirming this position as 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° with a Perkin–Elmer 241 Polarimeter. T.l.c. was conducted on glass plates, precoated with 0.25 mm layers of silica gel 60F-254 (Analtech GHLF uniplates); the components were located by exposure to u.v. light or by spraying with 5% H_2SO_4 in EtOH and charring, or by both techniques. The silica gel used for column chromatography was Baker Analyzed (60–200 mesh). N.m.r. spectra were recorded at ~ 25°, ^1H spectra with a Varian EM-390 and ^{13}C spectra with a Bruker AM-400 instrument, at 90 and 100.6 MHz, respectively. All chemical shifts are referenced to tetramethylsilane. Solutions in organic solvents were generally dried with anhydr. Na_2SO_4. Dichloroethane, dichloromethane, and *N,N*-dimethylformamide were dried over 4A molecular sieves. Elemental analyses were performed by the Robertson Laboratory, Madison, New Jersey, U.S.A.

Phenyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**2**). — To a stirred solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**1**; 20 g) in CH_2Cl_2 (250 mL) was added trimethyl(phenylthio) silane (25 mL) and trimethylsilyl triflate (21 mL). Stirring was continued for 72 h at room temperature. After neutralization with triethylamine the reaction mixture was diluted with $CHCl_3$, washed with water, dried, and concentrated. The residue was applied to a column of silica gel and eluted with a 30–40% gradient of EtOAc in hexane. Evaporation gave an amorphous solid which on crystallization from MeOH furnished **2** in 69.5% yield, $[\alpha]_D$ + 53.0° (*c* 1.4, $CHCl_3$); m.p. 145–146°, ^1H-n.m.r. ($CDCl_3$): δ 7.95–7.15 (m, 9 H, arom.), 5.73 (d, *J* ~ 10 Hz, 1 H, H-1), 2.20, 2.13, and 1.93 (3 s, 9 H, OAc).

Anal. Calc. for $C_{26}H_{25}NO_9S$: C, 59.19; H, 4.78; N, 2.66. Found: C, 59.11; H, 4.85; N, 2.55.

Phenyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**3**). — A solution of **2** (11 g) in 0.02M sodium methoxide (200 mL) was stirred for 3 h at room temperature. The base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin, the resin suspension was filtered, and the filtrate concentrated to give a solid residue. To a stirred solution of this solid in *N,N*-dimethylformamide (75 mL) were then added 4-toluenesulfonic acid monohydrate (0.2 g) and α,α-dimethoxytoluene (15 mL). The stirring was continued for 16 h at room temperature. The acid was neutralized with

a little triethylamine and the solution concentrated under reduced pressure. The residue was purified on a column of silica gel using a 25–30% gradient of EtOAc in hexane to furnish **3** (7.5 g, 73.5%); $[\alpha]_D$ + 34.2° (*c* 1.3, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 7.97–7.13 (m, 14 H, arom.), 5.69 (d, $J \sim 10$ Hz, 1 H, H-1), and 5.51 (s, 1 H, PhC*H*).

Anal. Calc. for $C_{27}H_{23}NO_6S$: C, 66.24; H, 4.73; N, 2.86. Found: C, 66.24; H, 5.11; N, 2.58.

*Phenyl 6-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside* (**4**). — To a cold (0°, bath), stirred mixture of **3** (7.5 g), sodium cyanoborohydride (10.5 g), and powdered 3A molecular sieves (10 g) in dry oxolane (75 mL) was added, dropwise, a saturated solution of HCl in ether (45 mL), and stirring was continued for 20 min. T.l.c. (4:1 $CHCl_3$–acetone) revealed the disappearance of **3** and the presence of a major product migrating more slowly than **3**. A trace of a still slower-migrating contaminant (presumably from the complete cleavage of the acetal group of **3**) was also evident. The mixture was diluted with $CHCl_3$, and the solids were filtered through Celite and washed with $CHCl_3$. The combined filtrate and washings were washed with cold water, cold sat. $NaHCO_3$, and water, dried, and concentrated under reduced pressure. The residue was purified on a column of silica gel eluted with 1:1 hexane–EtOAc. The fractions corresponding to the product **4** were concentrated to give an amorphous solid (6.4 g, 84.9%), $[\alpha]_D$ + 25.2° (*c* 1.3, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 7.92–7.12 (m, 14 H, arom.) and 5.57 (d, $J \sim 10$ Hz, 1 H, H-1); ^{13}C-n.m.r. (CD_3OD): δ 85.15 (C-1), 81.53 (C-5), 73.85 (C-3), 72.47 (C-4), 70.96 (C-6), and 57.65 (C-2).

Anal. Calc. for $C_{27}H_{25}NO_6S$: C, 65.97; H, 5.13; N, 2.85. Found: C, 65.95; H, 5.15; N, 2.63.

*Phenyl O-(2,3,4,6-tetra-O-acetyl-β-*D*-galactopyranosyl)-(1→4)-6-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside* (**5**). — A solution of **4** (4.2 g, 8.5 mmol) and 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl fluoride (5.3 g, 15.1 mmol) in 5:1 CH_2Cl_2–toluene (84 mL) was stirred for 0.5 h at − 15° with 4A molecular sieves (8 g), protected from light and moisture under an argon atmosphere. A mixture of stannous chloride (1.75 g, 9.3 mmol) and silver trifluoromethanesulfonate (2.45 g, 9.6 mmol) was then added, and the reaction mixture was allowed to gradually warm to room temperature, with stirring continued for an additional 2 h. Examination by t.l.c. (1:1 hexane–EtOAc) showed the presence of one major spot migrating faster than the starting material, along with minor products. The mixture was filtered through Celite and the solids were thoroughly washed with $CHCl_3$. The combined filtrate and washings were washed with sat. aq. $NaHCO_3$, filtered through Celite to remove precipitated inorganic material, washed with water, dried, and concentrated under reduced pressure. The residue was applied to a column of silica gel and eluted with a 30–40% gradient of EtOAc in hexane. On concentration the fractions corresponding to **5** (4.5 g, 64%) gave an amorphous solid, $[\alpha]_D$ + 26.6° (*c* 1.3, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 7.93–7.10 (m, 14 H, arom.), 5.57 (d, $J \sim 10$ Hz, 1 H, H-1), and 2.18–1.77 (cluster of s, 12 H, 4 OAc); ^{13}C-n.m.r. (CD_3OD): δ 102.00 (C-1′), 84.90 (C-1), 79.91 (C-4), 72.32 (C-3), and 57.06 (C-2).

Anal. Calc. for $C_{41}H_{43}NO_{15}S$: C, 59.92; H, 5.27; N, 1.70. Found: C, 59.69; H, 5.39; N, 1.46.

Phenyl O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)*-O-*[(2,3,4-tri*-O-*benzyl-α*-L-*fucopyranosyl)-(1→3)]*-6-O-*benzyl-2-deoxy-2-phthalimido-1-thio-β*-D-*glucopyranoside* (**6**). — A solution of compound **5** (3.0 g, 3.6 mmol) and methyl 2,3,4-tri-*O*-benzyl-1-thio-*β*-L-fucopyranoside (2.1 g, 4.5 mmol) in 5:1 dichloroethane–*N,N*-dimethylformamide (120 mL) was stirred for 0.5 h with 4A molecular sieves (12 g) under protection from light and moisture. Tetrabutylammonium bromide (2.25 g, 7.0 mmol) and $CuBr_2$(1.63 g, 7.0 mmol) were then added and the mixture was stirred for 2 days at room temperature. Further amounts of thiofucopyranoside (1.05 g), tetrabutylammonium bromide (1.25 g) and $CuBr_2$ (0.8 g) were added, and the stirring was continued for a total of 4 days; t.l.c. (1:1 hexane–EtOAc) then showed the presence of one major spot migrating faster than the starting material. The mixture was filtered through Celite, the solids were thoroughly washed with $CHCl_3$, and the combined filtrate and washings were washed with aq. $NaHCO_3$ and water, dried, and concentrated to a small volume. The concentrate was applied to a column of silica gel and eluted with a 30–40% gradient of EtOAc in hexane. The earlier fractions contained the faster-migrating compound **6**. On concentration, these fractions afforded a solid, which was crystallized from ether (2.5 g; 90% on the basis of **5** reacted); $[\alpha]_D$ +9.7° (*c* 1.3, $CHCl_3$); m.p. 202–204°; ^{1}H-n.m.r. ($CDCl_3$): δ 7.66–7.08 (m, 29 H, arom.), 5.44 (d, $J \sim 10$ Hz, 1 H, H-1), 5.18 (d, $J \sim 3$ Hz, 1 H, H-1″), 1.97 (s, 6 H, 2 OAc), 1.91 and 1.81 (2 s, 6 H, 2 OAc), and 1.17 (d, $J \sim 7$ Hz, 3 H, CMe).

Anal. Calc. for $C_{68}H_{71}NO_{19}S$: C, 65.95; H, 5.78; N, 1.13. Found: C, 65.82; H, 5.81; N, 1.01.

The later fractions contained the pure unreacted compound **5** (0.8 g).

Methyl O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)*-O-*[(2,3,4-tri*-O-*benzyl-α*-L-*fucopyranosyl)-(1→3)]*-O-*(6*-O-*benzyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)-(1→3)-2,4,6-tri*-O-*benzyl-β*-D-*galactopyranoside* (**7**). — A solution of compound **6** (0.6 g, 0.5 mmol), methyl 2,4,6-tri-*O*-benzyl-*β*-D-galactopyranoside[21] (0.49 g, 0.86 mmol), and *N*-iodosuccinimide (0.4 g, 1.8 mmol) in CH_2Cl_2 (40 mL) was stirred with 4A molecular sieves (6.0 g) for 0.5 h at −50° to −60° under an argon atmosphere. Then, a dilute solution of trifluoromethanesulfonic acid (0.024 mL in 20 mL of CH_2Cl_2) was added dropwise. After 10 min at −50° to −60° t.l.c. in 1:1 hexane–EtOAc showed the disappearance of the phenylthio donor **6** and the formation of one major product migrating just below this donor. The acid was then neutralized with a few drops of triethylamine. The mixture was filtered through Celite, the solids were thoroughly washed with $CHCl_3$, and the combined filtrate and washings were washed successively with water, sat. $NaHCO_3$ solution, and 10% $Na_2S_2O_3$, dried, and concentrated *in vacuo*. The residue was purified on a column of silica gel with a 30–40% gradient of EtOAc in hexane to provide **7** (0.65 g, 83%); $[\alpha]_D$ −20.4° (*c* 0.5, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 7.40–7.09 (m, 39 H, arom.), 5.14 (d, $J \sim 3$ Hz, 1 H, H-1″), 3.42 (s, 3 H, OMe), 1.91 (s, 6 H, 2 OAc), 1.86 and 1.75 (2 s, 6 H, 2 OAc), and 1.13 (d, $J \sim 7$ Hz, 3 H, CMe).

Anal. Calc. for $C_{90}H_{97}NO_{26}$: C, 67.19; H, 6.08; N, 0.87. Found: C, 67.37; H, 6.20; N, 0.73.

Methyl O-*β*-D-*galactopyranosyl-(1→4)*-O-*[(2,3,4-tri*-O-*benzyl-α*-L-*fucopyrano-*

syl)-(1→3)]-O-*(2-acetamido-6-O-benzyl-2-deoxy-β-*D-*glucopyranosyl)-(1→3)-2,4,6-tri-O-benzyl-β-*D-*galactopyranoside* (**8**). — A solution of compound **7** (0.65 g) in 0.02M sodium methoxide in MeOH (20 mL) was stirred for 4 h at room temperature. The base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin, the resin suspension was filtered, and the filtrate concentrated. The solid so obtained was heated under reflux for 16 h in a mixture of EtOH (70 mL) and hydrazine hydrate (5.0 mL). The liquids were then evaporated to give a residue, which was dissolved in pyridine (40 mL) and acetic anhydride (20 mL) and stirred overnight at room temperature. Solvent and reagent were removed under reduced pressure, then the residue was applied to a column of silica gel and eluted with 19:1 $CHCl_3$–acetone. On t.l.c. (9:1 $CHCl_3$–acetone) of the fractions corresponding to the product some impurities (non-carbohydrate) were revealed by u.v. light. However, the material was subjected to *O*-deacetylation without further purification.

To accomplish this it was suspended in 0.02M sodium methoxide (20 mL) and stirred overnight at room temperature. The base was neutralized by IR-120 (H^+) cation-exchange resin. The resin was filtered off and thoroughly washed with MeOH, and the filtrate and washings were combined and concentrated. The residue was purified on a silica gel column using 49:1 MeOH–$CHCl_3$ as the eluent to give **8** (0.41 g, 76%); $[\alpha]_D$ −40° (*c* 1.0, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 7.50–7.03 (m, 35 H, arom.), 3.42 (s, 3 H, OMe), 1.38 (s, 3 H, NAc), and 1.10 (d, $J \sim 7$ Hz, 3 H, CMe).

Anal. Calc. for $C_{76}H_{89}NO_{20}$: C, 68.30; H, 6.71; N, 1.05. Found: C, 68.02; H, 6.80; N, 1.06.

Methyl O-*β-*D-*galactopyranosyl-(1→4)*-O-*[α-*L-*fucopyranosyl-(1→3)]*-O-*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1→3)-β-*D-*galactopyranoside* (**9**). — A mixture of **8** (0.3 g) and 10% Pd–C (0.5 g) in glacial acetic acid (30 mL) was shaken under hydrogen at ~ 345 kPa for 2 days at room temperature. The suspension was filtered through a bed of Celite, the solids were thoroughly washed with glacial acetic acid, and the combined filtrate and washings were then concentrated under reduced pressure. The crude product was applied to a column of silica gel and eluted with 5:4:1 $CHCl_3$–MeOH–water. The fractions corresponding to **9** were concentrated and lyophilized to give an amorphous solid (0.09 g, 57%); $[\alpha]_D$ −48.2° (*c* 1.0, H_2O); *m/z*: 706.4 [M + 1]$^+$, 704.1 [M − 1]$^-$; for ^{13}C-n.m.r. data see Table I.

Anal. Calc. for $C_{27}H_{47}NO_{20}$: C, 45.95; H, 6.71; N, 1.98. Found: C, 45.67; H, 6.49; N, 1.81.

Methyl O-*(2,3,4,6-tetra-*O-*acetyl-β-*D-*galactopyranosyl)-(1→4)*-O-*[(2,3,4-tri-*O-*benzyl-α-*L-*fucopyranosyl)-(1→3)]*-O-*(6-*O-*benzyl-2-deoxy-2-phthalimido-β-*D-*glucopyranosyl)-(1→6)*-O-*[(2,3,4,6-tetra-*O-*acetyl-β-*D-*galactopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α-*D-*galactopyranoside* (**10**). — Compound **6** (0.6 g, 0.48 mmol) was reacted with methyl *O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside[22] (0.34 g, 0.6 mmol) in CH_2Cl_2 (40 mL) in the presence of *N*-iodosuccinimide (0.28 g, 1.23 mmol), trifluoromethanesulfonic acid (0.034 mL in 20 mL CH_2Cl_2), and 4A molecular sieves (6.0 g) in a manner analogous to that described for the preparation of **7**. After processing as for **7** the crude product was

applied to a column of silica gel and eluted with a 20–30% gradient of acetone in $CHCl_3$. Evaporation of the fractions corresponding to the product yielded **10** (0.58 g, 71%), amorphous, $[\alpha]_D$ +18.8° (*c* 1.0, $CHCl_3$), ^{1}H-n.m.r. ($CDCl_3$): δ 7.60–7.10 (m, 24 H, arom.), 2.07–1.77 (cluster of s, 27 H, 9 OAc and NAc), 1.13 (d, $J \sim 7$ Hz, 3 H, CMe).

Anal. Calc. for $C_{85}H_{100}N_2O_{34}$: C, 60.27; H, 5.95; N, 1.65. Found: C, 59.99; H, 5.92; N, 1.45.

Methyl O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)*-O-*[(2,3,4-tri*-O-*benzyl-α*-L-*fucopyranosyl)-(1→3)]*-O-*(2-acetamido-6*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl)-(1→6)*-O-*[(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1→3)]-2-acetamido-4*-O-*acetyl-2-deoxy-α*-D-*galactopyranoside* (**11**). — Compound **10** (0.55 g) was treated with 0.02M sodium methoxide in MeOH and stirred for 4 h at room temperature. After processing as described for the initial *O*-deacetylation of **7** the product was treated with hydrazine hydrate–EtOH (see **7→8**) and then stirred with 2:1 pyridine–acetic anhydride (60 mL) overnight at room temperature. The residue from this treatment was purified on a column of silica gel by elution with a 30–40% gradient of acetone in $CHCl_3$. Upon concentration the fractions corresponding to the product gave compound **11** as a solid (0.41 g, 77%, $[\alpha]_D$ −5° (*c* 0.8, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 7.40–7.10 (m, 20 H, arom.), 3.26 (s, 3 H, OMe), 2.08–1.75 (cluster of s, 33 H, 11 OAc and NAc), and 1.12 (d, $J \sim 7$ Hz, 3 H, CMe).

Anal. Calc. for $C_{81}H_{102}N_2O_{34}$: C, 59.04; H, 6.24; N, 1.70. Found: C, 58.78; H, 6.30; N, 1.85.

Methyl O-*β*-D-*galactopyranosyl-(1→4)*-O-*[α*-L-*fucopyranosyl-(1→3)]*-O-*(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1→6)-O-[β*-D-*galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-α*-D-*galactopyranoside* (**12**). — Compound **11** (0.36 g) was stirred in 0.05M methanolic sodium methoxide (50 mL) for 16 h at room temperature. The solution was deionized with Amberlite IR-120 (H^+) cation-exchange resin, filtered, and concentrated under reduced pressure. The residue was dissolved in glacial acetic acid (30 mL) and shaken with 10% Pd–C (0.6 g) under hydrogen at ~345 kPa exactly as described for the preparation of compound **9**. After purification over a silica gel column with 4:5:1 $CHCl_3$–MeOH–water as the eluent, **12** (0.15 g, 70%) was obtained as an amorphous solid, $[\alpha]_D$ −6.5° (*c* 0.6, H_2O); *m/z:* 909.5 $[M + 1]^+$, 907.2 $[M - 1]^-$; for ^{13}C-n.m.r. data see Table I.

Anal. Calc. for $C_{35}H_{60}N_2O_{25}$: C, 46.25; H, 6.65; N, 3.08. Found: C, 45.97; H, 6.49; N, 2.94.

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