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GLYCOSYLATION OF LACTOSE: SYNTHESIS OF BRANCHED OLIGOSACCHARIDES INVOLVED IN THE BIOSYNTHESIS OF GLYCOLIPIDS HAVING BLOOD-GROUP I ACTIVITY*

AURELIO MARANDUBA[§] AND ALAIN VEYRIÈRES[†]

Laboratoire de Chimie Organique Multifonctionnelle, Bt. 420, Université de Paris-Sud, F-91405 Orsay (France)

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ABSTRACT

Methyl 4-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (**2**) was prepared in four steps from methyl β-lactoside. Crystalline **2** was a convenient substrate for the synthesis of branched oligosaccharides derived from lactose. High-yield glycosylations were performed first at the 3′-position and then, after removal of the benzylidene group, at the 6′-position, using trichloroacetimidates of *N*-phthaloylamino sugars. 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate was thus used in two consecutive glycosylations, and also in a simultaneous disubstitution of the triol **9**, leading in each sequence to the branched tetrasaccharide, β-D-Glc*p*NAc-(1→3)-[β-D-Glc*p*NAc-(1→6)]-β-D-Gal*p*-(1→4)-β-D-GlcOMe. Similar glycosylations performed with 3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl trichloroacetimidate afforded the branched hexasaccharide β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-[β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6)]-β-D-Gal*p*-(1→4)-β-D-GlcOMe, which corresponds to the human-milk oligosaccharide lacto-*N*-*neo*hexaose and has a strong blood-group I activity.

INTRODUCTION

Piller *et al.*[1] have reported the presence in hog gastric mucosa of a (1→6)-2-acetamido-2-deoxy-β-D-glucosyltransferase which synthesises branch points in branched lactosaminoglycans. A synthetic trisaccharide-glycoside[2], β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-GlcOMe, was used as a monospecific acceptor, and the product of the transferase reaction was identified as the branched tetrasaccharide β-D-Glc*p*NAc-(1→3)-[β-D-Glc*p*NAc-(1→6)]-β-D-Gal*p*-(1→4)-β-D-GlcOMe

*Dedicated to Roger W. Jeanloz.

[§]Fellow of the Conselho Nacional de Desenvolvimento Cientifico e Tecnologico–CNPq, Brazil.

[†]To whom correspondence should be addressed.

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(**12**). In turn, **12** was a good acceptor for a (1→4)-β-D-galactosyltransferase from bovine milk, to give the branched lacto-*N*-*neo*hexaose sequence[3] β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-[β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6)]-β-D-Gal*p*-(1→4)-β-D-GlcOMe (**20**). As expected[4], **20** was able to inhibit the hemagglutination of two specific anti-I antisera. Consequently, these two enzymic glycosylations are probably involved in the biosynthesis of structures having blood-group I activity.

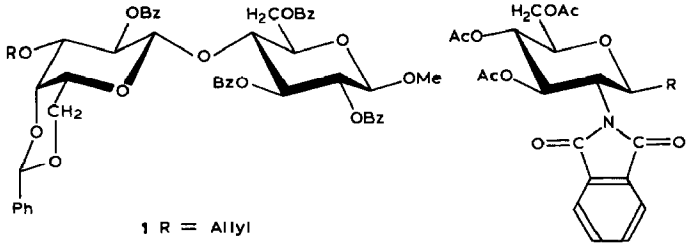
As part of our studies of the glycosylation of lactose, we now report the synthesis of **12** and **20**.

RESULTS AND DISCUSSION

The free oligosaccharides corresponding to the sequences of **12** and **20** have already been obtained by diglycosylation of 1,6-anhydro-2,3,2′,4′-tetra-*O*-benzyl-β-lactose with an oxazoline of the appropriate amino sugar[5]. This derivative of lactose is an excellent substrate for the synthesis of branched oligosaccharides, but its preparation requires many steps, one of them being the selective 3′-tosylation of 1,6-anhydro-4′,6′-*O*-benzylidene-β-lactose[6].

We have reported a one-step preparation of methyl 3′-*O*-allyl-β-lactoside, in good yield and without chromatography, by regioselective mono-allylation of the dibutylstannylene complex of methyl β-lactoside[7]. This crystalline compound was easily modified to allow glycosylation at O-3 of its D-galactopyranosyl group[2].

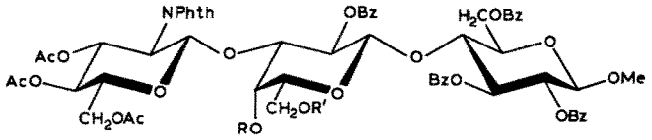
By successive benzylidenation and benzoylation, methyl 3′-*O*-allyl-β-lactoside was converted into 70% of **1**. Treatment of **1** with chlorotris(triphenylphosphine)rhodium, followed by cleavage of the intermediate prop-1-enyl ether by $HgCl_2$–HgO in acetone–water, afforded 79% of the crystalline alcohol **2**. In both **1** and **2**, H-5′ gave an n.m.r. signal at high field (δ 2.83 and 2.96, respectively) with small coupling constants ($J_{5,6a}$ ~1, $J_{5,6b}$ ~2 Hz, respectively).



3 R = OH
4 R = Cl
5 R = $OCNHCCl_3$

Glycosylation of **2** with the phthalimidochloride[8] **4** in the presence of silver trifluoromethanesulfonate and 2,4,6-trimethylpyridine[9] gave 44% of the trisaccharide derivative **6**. As often found in this type of condensation, the reaction was not

complete even with a large excess of chloride and silver salt. The amount of base used seems to be critical, and the literature contains examples of such glycosylations conducted under base-deficient conditions[10,11]. The benzylidene group of **6** was then removed to give the crystalline diol **7**.



6 R, R′ = CHPh

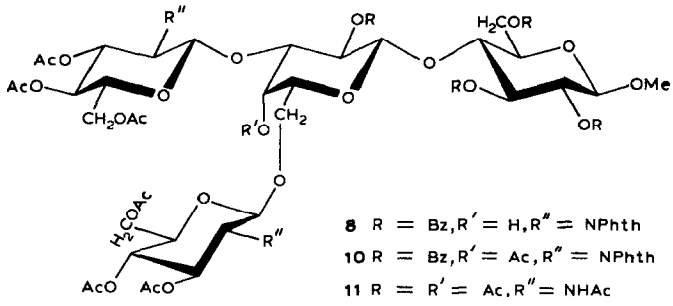
7 R = R′ = H

Grundler and Schmidt[12] have reported an efficient procedure for the synthesis of 1,2-*trans*-glycosides of 2-amino-2-deoxy sugars. The amino group is protected by *N*-phthaloylation, and activation is effected through a 1,2-*trans*-trichloroacetimidate. In the presence of a Lewis acid, such a compound reacts with an alcohol to give exclusively the 1,2-*trans*-glycoside, usually rapidly and in high yield. Imidate **5** was prepared[12] from alcohol **3** (conveniently obtained by treatment of the corresponding β-acetate[9] with hydrazine acetate in *N*,*N*-dimethylformamide[13]). The α-acetate is more slowly cleaved and gives polar by-products resulting from the opening of the phthalimido ring. Compound **3** crystallised in the β-form[14].

Glycosylation of **2** by imidate **5** at 0° in the presence of boron trifluoride etherate rapidly gave the trisaccharide derivative **6** together with the diol **7**, presumably formed by debenzylidenation of **6**. Acid hydrolysis of the mixture converted the remaining **6** into **7**, which was readily isolated by column chromatography. When the glycosylation of **2** was performed at −20° with rigorous exclusion of moisture, the benzylidene group was retained and 71% of **7** was obtained.

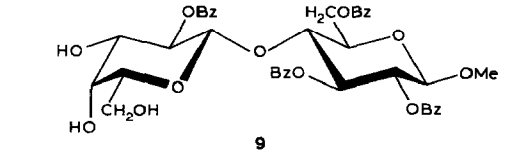
Glycosylation of **7** with **5** (20% excess) and trimethylsilyl trifluoromethanesulfonate as promoter at 0° was complete in <15 min (the use of boron trifluoride etherate resulted in slower and incomplete reaction) and 68% of the branched tetrasaccharide derivative **8** was isolated. Glycosylation at the 4′-position could not be detected. The trichloroacetimidate procedure also allows selective glycosylation at O-3 of a 3,4-diol in D-galactopyranosides[15]. Therefore, the synthesis of 3,6-branched structures built on D-galactopyranose residues could be simplified by glycosylating directly a 3,4,6-triol derivative.

Acid hydrolysis of the benzylidene group in **1**, followed by removal of the allyl group from the product with 5% Pd/C and toluene-*p*-sulfonic acid in methanol–water[16], gave 76% of the triol **9**. Benzaldehyde had to be completely removed after the first step since it inhibits the isomerisation of the allyl ether. Diglycosylation of **9** with 3 mol of imidate **5** at 0° in the presence of trimethylsilyl trifluoromethanesulfonate followed by acetylation of the products gave the major product **10** (75%) which was identical with the 4′-acetate of **8**. Compound **10** gave three high-field ^{1}H-n.m.r. signals (δ 2.89 and 3.34 for H-6′,6′ and 3.78 for H-3′)



and one at low field (δ 5.19 for H-4') which clearly demonstrated the presence of (1→6) and (1→3) linkages.

Compound **8** (or **10**) was treated with hydrazine hydrate in boiling ethanol, and the product was treated with acetic anhydride–pyridine to give **11**, methanolysis of which afforded the desired tetrasaccharide-glycoside **12**. The ^{1}H-n.m.r. spectrum of **12** contained two sets of signals for anomeric protons. One set at lower field corresponded to the 2-acetamido-2-deoxy-β-D-glucopyranosyl groups [δ 4.68, $J_{1,2}$ 8.4 Hz, for H-1 of the (1→3)-linked group; and δ 4.61, $J_{1,2}$ 8.4 Hz, for H-1 of the (1→6)-linked group]. The two other signals at δ 4.43 and 4.41 ($J_{1,2}$ 8 Hz) can be attributed to H-1' and H-1, respectively, by comparison with the values for methyl β-lactoside[17] (δ 4.43 for H-1' and 4.39 for H-1). As in other oligosaccharides having the sequence β-D-Glc*p*NAc-(1→3)-β-D-Gal-, the equatorial H-4' of the galactosyl residue gives a characteristic signal at δ 4.15, shifted downfield when compared to that of H-4' of methyl β-lactoside[17] (δ 3.91).



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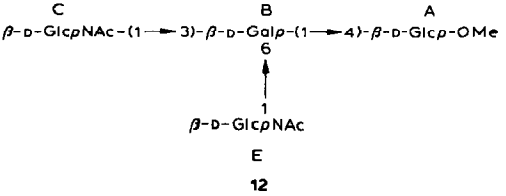
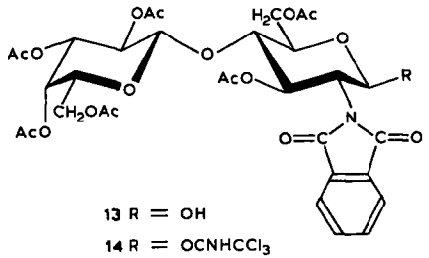


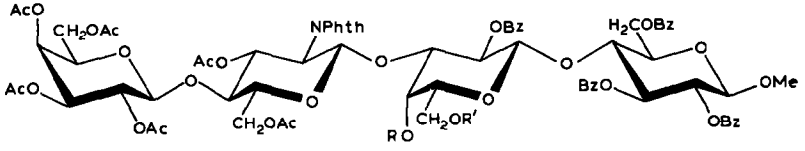
12

The disaccharide-imidate **14**, derived from lactosamine, has been used[12] in the block synthesis of oligosaccharides. Its precursor **13** can be easily obtained as the crystalline β-form from the corresponding β-acetate[8] by the method described above for the preparation of **3**.

Compound **2** was glycosylated with **14** at −20° in the presence of boron trifluoride etherate, to give 75% of the tetrasaccharide derivative **16**. The sequence in



13 R = OH
14 R = $OCNHCCl_3$
15 R = F



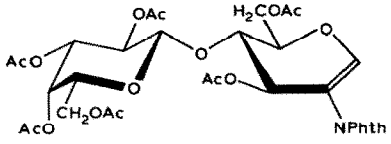
16 R, R' = CHPh
17 R = R' = H

16 corresponds to that in lacto-*N*-*neo*tetraose[18] which is thus obtainable by an efficient new synthesis[2,8,19]. Among the by-products derived from imidate **14** was a crystalline β-phthalimido fluoride, produced by reaction with the hydrogen fluoride present in the catalyst.

The benzylidene group of **16** was removed by acid hydrolysis to afford **17**, which was glycosylated with **14** at −20° in the presence of trimethylsilyl trifluoromethanesulfonate to give 80% of the hexasaccharide derivative **19**. An unsaturated compound **18**, probably formed by elimination of trichloroacetamide in **14**, was also obtained. Lemieux *et al.*[9] have reported the formation of a glycoseen in glycosylations performed with a phthalimido bromide activated by silver trifluoromethanesulfonate–2,4,6-trimethylpyridine.

Treatment of **19** with hydrazine hydrate in boiling ethanol followed by *N*-acetylation with acetic anhydride in methanol gave the hexasaccharide-glycoside **20**. The ^{1}H-n.m.r. spectrum of **20** contained two signals at low field for the anomeric protons of the 2-acetamido-2-deoxy-β-D-glucopyranosyl residues [δ 4.71, $J_{1,2}$ 7.5 Hz, for H-1 of the (1→3)-linked residue; and 4.64, $J_{1,2}$ 7.5 Hz, for H-1 of the (1→6)-linked residue]. The equatorial H-4 of the internal D-galactopyranosyl residue gave a characteristic signal at δ 4.15.

The ^{13}C-n.m.r. data of **12, 20,** methyl β-lactoside, and two oligosaccharides previously synthesised[2], **21** and **22**, are given in Table I. The proposed assignments accord with the data compiled for oligosaccharides[20,21]. The methyl β-D-glucopyranoside residue A in each of the five oligosaccharides has comparable carbon resonances. In the internal β-D-galactopyranosyl residue B, the linkage through O-3 shifts the C-3 resonance downfield by 9.0–9.2 p.p.m., the C-2 resonance upfield by 1.0–1.1 p.p.m., and the C-4 resonance upfield by only 0.3 p.p.m. As noticed by



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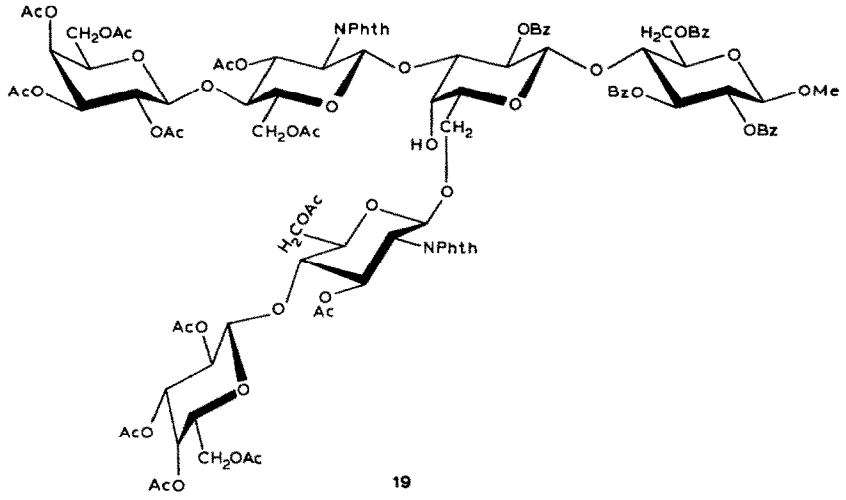

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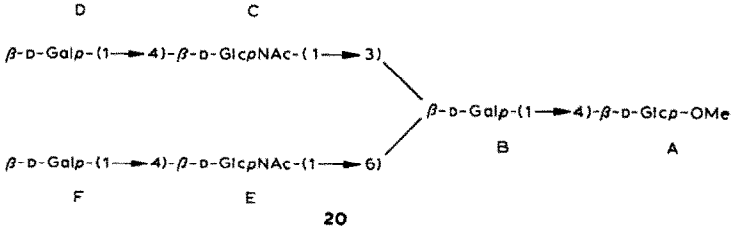

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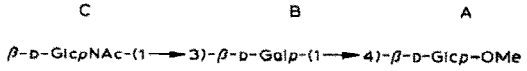

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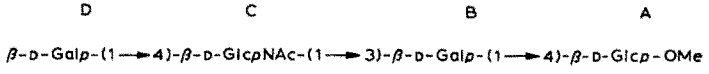

22

Messer *et al.*[22], the resonance of a β-carbon atom having an axial hydroxyl group can show a very small upfield shift. The linkage through O-6 of the galactosyl residue B in **12** and **20** shifts the C-6 resonance downfield by 7.6–7.9 p.p.m., and the C-5 resonance upfield by 1.2–1.4 p.p.m.

TABLE I

^{13}C-N.M.R. DATA[a] FOR FREE OLIGOSACCHARIDES

Compound	*Unit*[b]	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*NCOCH$_3$*	*NCOCH$_3$*	*OMe*
Methyl β-lactoside[20]	A	103.2	73.0	74.9	78.9	74.7	60.5			57.3
	B	103.1	71.2	73.0	68.9	75.5	61.2			
21	A	103.33	73.05	75.03	78.82	74.68	60.44			57.43
	B	103.22	70.06	82.20	68.59	75.14	61.18			
	C	102.97	55.98	73.89	70.29	75.96	60.85	22.46	175.17	
22	A	103.30	73.03	74.83	78.86	74.67	60.31			57.40
	B	103.17	70.23	82.24	68.55	75.10	61.17			
	C	102.77	55.51	72.45	78.80	75.00	60.46	22.48	175.03	
	D	103.17	71.24	72.85	68.82	75.57	61.20			
12	A	103.41	73.20	75.04	79.37	74.59	60.43			57.53
	B	103.41	70.09	82.02	68.56	73.94	69.01			
	C	103.11	56.04	73.86	70.25	76.04	60.91	22.49	175.00	
	E	101.43	55.86	74.23	70.25	76.23	61.07	22.71	175.37	
20	A	103.26	73.07	74.81	79.24	74.68	60.33			57.43
	B	103.26	70.08	82.01	68.61	73.70	68.81			
	C	102.86	55.47	72.43	78.77	74.93	60.33	22.45	174.72	
	D	103.13	71.21	72.79	68.81	75.58	61.24			
	E	101.20	55.27	72.68	78.56	74.97	60.33	22.66	175.09	
	F	103.13	71.21	72.79	68.81	75.58	61.24			

[a]Solution in D_2O (internal 1,4-dioxane, δ 67.40). [b]A–F refer to the monosaccharide units as indicated in formulae **12, 20, 21,** and **22.**

The 2-acetamido-2-deoxy-β-D-glucose residues C and E in **12** and **20** give signals at similar values (±0.3 p.p.m.) for all their carbon atoms, except for C-1 which gives a signal at higher field ($\Delta\delta$ ~1.7 p.p.m.) for the (1→6)-linked residue. Finally, the two terminal galactosyl groups, D and F, in the branched hexasaccharide **20** have exactly the same signals for all their carbon atoms.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Optical rotations were measured at 20° with a Roussel–Jouan electronic, digital micropolarimeter. All reactions were monitored by t.l.c. on Silica gel 60 F_{254} (Merck) with detection by charring with sulfuric acid. Silica gel 60 (Merck, 70–230 mesh) was used for column chromatography. ^{1}H-N.m.r. spectra (400 MHz) were recorded for solutions in $CDCl_3$ (internal Me_4Si) or in D_2O (external 0.2% Me_4Si in $CDCl_3$). ^{13}C-N.m.r. spectra (62.9 MHz) were recorded with a Bruker AM-250 spectrometer. Microanalyses were performed by the Laboratoire Central de Micro-Analyse du C.N.R.S.

Methyl 4-O-(3-O-allyl-2-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranosyl)-2,3,6-tri-O-benzoyl-β-D-glucopyranoside (**1**). — A mixture of methyl 4-*O*-(3-*O*-allyl-β-D-galactopyranosyl)-β-D-glucopyranoside[7] (3.96 g, 10 mmol), anhydrous zinc chloride (3.2 g, 23 mmol), and benzaldehyde (60 mL) was stirred for 24 h at room temperature. Water (50 mL) and methanol (5 mL) were added, and the solution was extracted with hexane (4 × 10 mL). The aqueous layer was then stirred for 1 h with sodium carbonate (3.2 g). The inorganic material was collected and washed with methanol, and the combined filtrate and washings were concentrated. The residue was extracted with acetone. T.l.c. (3:2:1 ethyl acetate–2-propanol–water) of the extract revealed a nearly pure compound (R_F 0.61).

The extract was concentrated, and the residue was treated with benzoyl chloride (5.8 mL, 50 mmol) and 4-dimethylaminopyridine (10 mg) in pyridine (16 mL) for 1 h at 0°, and then for 24 h at room temperature. The solution was diluted with dichloromethane, washed with saturated aqueous sodium hydrogencarbonate, dried, and concentrated. The residue crystallised from ether to give **1** (6.33 g, 70%), m.p. 216°, $[\alpha]_D^{20}$ +86° (*c* 0.9, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 2.83 (s, 1 H, H-5′), 3.42 (s, 3 H, OMe), 3.57 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 3.5 Hz, H-3′), 3.59 (dd, 1 H, $J_{6a,6b}$ 12, $J_{5,6a}$ 2 Hz, H-6′a), 3.75 (dd, 1 H, $J_{6a,6b}$ 12, $J_{5,6b}$ 1 Hz, H-6′b), 3.86 (m, 1 H, H-5), 3.94–4.08 (m, 2 H, $OCH_2CH{=}CH_2$), 4.06 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4′), 4.19 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 4.43 (dd, 1 H, $J_{6a,6b}$ 12, $J_{5,6a}$ 4.5 Hz, H-6a), 4.61 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.63 (dd, 1 H, $J_{6a,6b}$ 12, $J_{5,6b}$ 2 Hz, H-6b), 4.73 (d, 1 H, $J_{1,2}$ 8 Hz, H-1′), 5.04–5.17 (m, 2 H, -CH=CH_2), 5.32 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 9.5 Hz, H-2), 5.33 (s, 1 H, C*H*Ph), 5.52 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz, H-2′), 5.67–5.77 (m, 1 H, -C*H*=CH_2), 5.82 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), and 7.25–8.02 (m, 25 H, 5 Ph).

Anal. Calc. for $C_{51}H_{48}O_{15}$: C, 67.99; H, 5.37; O, 26.64. Found: C, 67.45; H, 5.59; O, 26.62.

*Methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-*D*-galacto-pyranosyl)-β-*D*-glucopyranoside* (**2**). — A solution of **1** (1.80 g, 2 mmol) in 9:5:1 ethanol–1,2-dichloroethane–water (30 mL) was boiled under reflux for 24 h with chlorotris(triphenylphosphine)rhodium (130 mg). T.l.c. (4:1 toluene–ethyl acetate) then showed complete conversion of **1** (R_F 0.34) into a prop-1-enyl ether (R_F 0.39) and traces of **2** (R_F 0.21). The solution was cooled and concentrated, and the residue was treated with mercury(II) chloride (0.54 g, 2 mmol) and mercury(II) oxide (0.43 g, 2 mmol) in 10:1 acetone–water (80 mL) for 3 h at room temperature. The solution was filtered and concentrated, the residue was extracted with dichloromethane, and the extract was washed successively with saturated aqueous potassium iodide and water, dried, and concentrated. Column chromatography (17:3 toluene–ethyl acetate) of the crude product gave **2** (1.36 g, 79%) which crystallised from ethanol; m.p. 241–243°, $[\alpha]_D^{20}$ +44° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 2.43 (d, 1 H, $J_{3,OH}$ 12 Hz, OH), 2.96 (s, 1 H, H-5′), 3.43 (s, 3 H, OMe), 3.59 (dd, 1 H, $J_{6a,6b}$ 12, $J_{5,6a}$ 2 Hz, H-6′a), 3.67 (dd, 1 H, $J_{6a,6b}$ 12, $J_{5,6b}$ 1 Hz, H-6′b), 3.72 (ddd, 1 H, $J_{3,OH}$ 12, $J_{2,3}$ 9.5, $J_{3,4}$ 4 Hz, H-3′), 3.85 (m, 1 H, H-5), 4.00 (d, 1 H, $J_{3,4}$ 4 Hz, H-4′), 4.19 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 4.49 (dd, 1 H, $J_{6a,6b}$ 12, $J_{5,6a}$ 4.5 Hz, H-6a), 4.61 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.63 (dd, 1 H, $J_{6a,6b}$ 12, $J_{5,6b}$ 2 Hz, H-6b), 4.68 (d, 1 H, $J_{1,2}$ 8 Hz, H-1′), 5.32 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz, H-2), 5.34 (s, 1 H, C*H*Ph), 5.36 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 9.5 Hz, H-2′), 5.81 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), and 7.29–8.06 (m, 25 H, 5 Ph).

Anal. Calc. for $C_{48}H_{44}O_{15}$: C, 66.97; H, 5.15; O, 27.88. Found: C, 66.75; H, 5.18; O, 27.68.

*Methyl O-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-*D*-glucopyranoside* (**6**). — A solution of **2** (1.72 g, 2 mmol) in dichloromethane (50 mL) was stirred for 1 h at room temperature under nitrogen in the presence of 2,4,6-trimethylpyridine (0.34 g, 2.8 mmol) and powdered molecular sieves Type 4A (6 g). Silver trifluoromethanesulfonate (0.67 g, 2.6 mmol) was added, the mixture was cooled to 0°, and a solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl chloride[9] (**4**; 1.8 g, 2.6 mmol) in dichloromethane (50 mL) was added dropwise. The mixture was stirred for 48 h at room temperature, more **4** (0.64 g, 1.4 mmol), silver trifluoromethanesulfonate (0.36 g, 1.4 mmol), and 2,4,6-trimethylpyridine (0.18 g, 1.5 mmol) being added after 24 h. T.l.c. (19:1 dichloromethane–acetone) then revealed a new product (R_F 0.24), **2** (R_F 0.37), and **3** (R_F 0.11) produced by hydrolysis of **4**. The mixture was diluted with dichloromethane, filtered, washed with water, and concentrated. Column chromatography (97:3 dichloromethane–acetone) of the residue gave **6** (1.12 g, 44%) which crystallised from ethanol; m.p. 279–281°, $[\alpha]_D^{20}$ +61° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.74, 1.97, and 2.00 (3 s, each 3 H, 3 Ac), 2.88 (s, 1 H, H-5′), 3.37 (s, 3 H, OMe), 3.64 (dd, 1 H, $J_{6a,6b}$ 11, $J_{5,6a}$ 2 Hz, H-6′a), 3.68 (m, 1 H, H-5″), 3.80–3.85 (m, 3 H, H-5,3′,6′b), 4.08 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 4.14–4.22 (m, 3 H, H-4′,6″a,6″b), 4.31–4.36 (m, 2 H, H-6a,2″), 4.43 (dd,

1 H, $J_{6a,6b}$ 12, $J_{5,6b}$ 2 Hz, H-6b), 4.54 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.64 (d, 1 H, $J_{1,2}$ 8 Hz, H-1'), 5.15 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4''), 5.28 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 9.5 Hz, H-2), 5.37 (s, 1 H, C*H*Ph), 5.39 (dd, 1 H, H-2'), 5.57 (d, 1 H, $J_{1,2}$ 8 Hz, H-1''), 5.65 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 9.5 Hz, H-3''), 5.76 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), and 7.11–7.99 (m, 29 H, aromatic protons).

Anal. Calc. for $C_{68}H_{63}NO_{24}$: C, 63.90; H, 4.97; N, 1.09; O, 30.04. Found: C, 63.95; H, 4.94; N, 1.11; O, 30.26.

Methyl O-*(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-*D*-glucopyranoside* (**7**). — (*a*) A solution of **6** (1.00 g) in aqueous 80% acetic acid (60 mL) was heated for 40 min at 100°. T.l.c. (21:4 dichloromethane–acetone) then showed complete hydrolysis of **6** (R_F 0.77) into **7** (R_F 0.26). The solution was cooled and concentrated, and the residue was crystallised from methanol to give **7** (0.84 g, 90%), m.p. 283°, $[\alpha]_D^{20}$ +56° (*c* 0.9, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.75, 2.00, and 2.05 (3 s, each 3 H, 3 Ac), 2.53 (s, 1 H, OH), 3.12–3.31 (m, 4 H, OH, H-5',6'a,6'b), 3.37 (s, 3 H, OMe), 3.62 (m, 1 H, H-5''), 3.71 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 3.5 Hz, H-3'), 3.82 (m, 1 H, H-5), 3.97 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4'), 4.07 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 4.13 (dd, 1 H, $J_{6a,6b}$ 12, $J_{5,6a}$ 5 Hz, H-6''a), 4.21 (dd, 1 H, $J_{6a,6b}$ 11, $J_{5,6a}$ 5 Hz, H-6a), 4.24 (dd, 1 H, $J_{6a,6b}$ 12, $J_{5,6b}$ 2.5 Hz, H-6''b), 4.30 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 11 Hz, H-2''), 4.38 (dd, 1 H, $J_{6a,6b}$ 11, $J_{5,6b}$ 2 Hz, H-6b), 4.50 and 4.51 (2 d, 2 H, $J_{1,2}$ 8 Hz, H-1,1'), 5.08 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4''), 5.29 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz, H-2), 5.35 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz, H-2'), 5.50 (d, 1 H, $J_{1,2}$ 8 Hz, H-1''), 5.60 (t, 1 H, $J_{2,3} = J_{3,4} = 10$ Hz, H-3), 5.63 (dd, 1 H, $J_{2,3}$ 11, $J_{3,4}$ 9.5 Hz, H-3''), and 7.05–7.99 (m, 24 H, aromatic protons).

Anal. Calc. for $C_{61}H_{59}NO_{24}$: C, 61.56; H, 5.00; N, 1.18; O, 32.26. Found: C, 61.11; H, 5.07; N, 1.24; O, 32.53.

(*b*) A solution of boron trifluoride etherate (0.43 g, 3 mmol) in dichloromethane (10 mL) was added dropwise to a solution of **2** (1.72 g, 2 mmol) and **5** (2.32 g, 4 mmol) in dichloromethane (50 mL) at 0° under nitrogen. The mixture was stirred for 90 min at 0°; t.l.c. (17:3 dichloromethane–acetone) then showed two major (R_F 0.30 and 0.69) and two minor compounds (R_F 0.42 and 0.52) formed, respectively, by hydrolysis of **5** and condensation of **5** with its hydrolysis product; **2** also had R_F 0.69, but its absence was verified by t.l.c. in 19:1 dichloromethane–acetone. The mixture was poured into saturated aqueous sodium hydrogencarbonate, and the dichloromethane layer was decanted, washed with water, and concentrated. The residue was treated with aqueous 80% acetic acid (60 mL) for 40 min at 100°. T.l.c. (17:3 dichloromethane–acetone) then showed complete hydrolysis of the benzylidene compound (R_F 0.69) into the diol (R_F 0.30). The solution was cooled and concentrated, and the residue was subjected to column chromatography (19:1 dichloromethane–acetone) to give **7** (1.69 g, 71%).

Methyl O-*(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1→3)-*O*-[(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1→6)]-*O*-(2-*O*-benzoyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-*D*-glucopyra-*

noside (**8**). — A solution of trimethylsilyl trifluoromethanesulfonate (0.27 g, 1.2 mmol) in dichloromethane (2 mL) was added dropwise to a solution of **7** (1.19 g, 1 mmol) and **5** (0.70 g, 1.2 mmol) in dichloromethane (25 mL) at 0° under nitrogen. The mixture was stirred for 15 min at 0°. T.l.c. (17:3 dichloromethane–acetone) then showed a major product (R_F 0.55), minor compounds derived from **5** (R_F 0.42 and 0.52), and traces of **7** (R_F 0.30). The mixture was worked-up as described above to give **8** (1.09 g, 68%), which crystallised from ethanol; m.p. 162–164°, $[\alpha]_D^{20}$ +32° (*c* 1.4, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.73, 1.86, 2.00, 2.05, and 2.18 (5 s, 18 H, 6 Ac), 2.58 (d, 1 H, OH), 3.00 (dd, 1 H, $J_{6a,6b}$ 10, $J_{5,6a}$ 5 Hz, H-6′a), 3.19 (m, 1 H, H-5′), 3.37 (dd, 1 H, $J_{6a,6b}$ 10, $J_{5,6b}$ 8 Hz, H-6′b), 3.39 (s, 3 H, OMe), 3.47 (m, 1 H, H-5‴), 3.55 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 3.5 Hz, H-3′), 3.61 (m, 1 H, H-5), 3.81–3.86 (m, 2 H, H-4′,5″), 3.91 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9 Hz, H-4), 4.06 (dd, 1 H, $J_{6a,6b}$ 12, $J_{5,6a}$ 2 Hz, H-6‴a), 4.19 (dd, 1 H, $J_{6a,6b}$ 11, $J_{5,6a}$ 2 Hz, H-6″a), 4.23–4.38 (m, 6 H, H-6a,6b,2″,6″b,2‴,6‴b), 4.43 (d, 1 H, $J_{1,2}$ 8 Hz, H-1′), 4.51 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 5.04 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4‴), 5.16 (d, 1 H, $J_{1,2}$ 8 Hz, H-1‴), 5.18 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4″), 5.26 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz, H-2′), 5.29 (d, 1 H, $J_{1,2}$ 8 Hz, H-1″), 5.30 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz, H-2), 5.58 (m, 2 H, H-3,3‴), 5.79 (dd, 1 H, $J_{2,3}$ 11, $J_{3,4}$ 9.5 Hz, H-3″), and 6.99–7.94 (m, 28 H, aromatic protons).

Anal. Calc. for $C_{81}H_{78}N_2O_{33}$: C, 60.52; H, 4.90; N, 1.74; O, 32.84. Found: C, 60.29; H, 4.99; N, 1.80; O, 32.77.

*Methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2-*O*-benzoyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**9**). — A solution of **1** (3.00 g) in aqueous 80% acetic acid (120 mL) was heated for 30 min at 100°. T.l.c. (17:3 dichloromethane–acetone) then showed the hydrolysis to be complete. The solution was cooled and concentrated to dryness, and a solution of the residue in 21:4 methanol–water (150 mL) was boiled under reflux with 5% Pd/C (140 mg) and toluene-*p*-sulfonic acid (140 mg) until t.l.c. (7:3 ethyl acetate–toluene) showed nearly complete removal of the allyl group (2 h). The mixture was filtered, the methanol was evaporated, and the aqueous residue was extracted with dichloromethane. The extract was washed with saturated aqueous sodium chloride and then concentrated. Column chromatography (1:1 toluene–ethyl acetate) of the residue gave **9** (1.97 g, 75%), which crystallised from methanol; m.p. 210–212°, $[\alpha]_D^{20}$ +44° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 2.05 (bd, 2 H, 2 OH), 3.05 (dd, 1 H, $J_{6a,6b}$ 11, $J_{5,6a}$ 5 Hz, H-6′a), 3.15–3.24 (m, 3 H, OH, H-5′,6′b), 3.43 (s, 3 H, OMe), 3.63 (dd, 1 H, $J_{2,3}$ 9.5, $J_{3,4}$ 3 Hz, H-3′), 3.84 (m, 1 H, H-5), 3.88 (d, 1 H, $J_{3,4}$ 3 Hz, H-4′), 4.11 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9 Hz, H-4), 4.48 (dd, 1 H, $J_{6a,6b}$ 12, $J_{5,6a}$ 5 Hz, H-6a), 4.58–4.61 (3 H, H-1,6b,1′), 5.28 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz, H-2), 5.41 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz, H-2′), 5.68 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 7.28–8.05 (m, 20 H, 4 Ph).

Anal. Calc. for $C_{41}H_{40}O_{15} \cdot H_2O$: C, 62.27; H, 5.35; O, 32.37. Found: C, 62.69; H, 5.39; O, 31.73.

*Methyl O-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1→3)-*O*-[(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1→6)]-*O*-(4-*O*-acetyl-2-*O*-benzoyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-*D*-*

glucopyranoside (**10**). — A solution of trimethylsilyl trifluoromethanesulfonate (0.67 g, 3 mmol) in dichloromethane (5 mL) was added dropwise to a solution of **9** (0.79 g, 1 mmol) and **5** (1.74 g, 3 mmol) in dichloromethane (50 mL) at 0° under nitrogen. The mixture was stirred for 15 min at 0°. T.l.c. (17:3 dichloromethane–acetone) then showed a major compound (R_F 0.55) identical to **8**. The mixture was worked-up as described above and the product was acetylated overnight in 2:1 pyridine–acetic anhydride (15 mL) in the presence of a catalytic amount of 4-dimethylaminopyridine. Column chromatography (7:2 toluene–ethyl acetate) of the crude product gave **10** (1.24 g, 75%), which crystallised from ethanol; m.p. 154–155°, $[\alpha]_D^{20}$ +27° (*c* 1.1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.73, 1.80, 1.88, 1.96, 2.07, and 2.12 (6 s, 21 H, 7 Ac), 2.89 (dd, 1 H, $J_{6a,6b}$ 11, $J_{5,6a}$ 8 Hz, H-6'a), 3.34 (dd, 1 H, $J_{6a,6b}$ 11, $J_{5,6b}$ 4 Hz, H-6'b), 3.41 (s, 3 H, OMe), 3.46 (m, 1 H, H-5'), 3.53 (m, 1 H, H-5‴), 3.69 (m, 1 H, H-5), 3.78 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 3.5 Hz, H-3'), 3.85 (m, 1 H, H-5″), 3.99 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9 Hz, H-4), 4.09–4.38 (m, 8 H, H-6a,6b,2″,6″a,6″b,2‴,6‴a,6‴b), 4.45 (d, 1 H, $J_{1,2}$ 8 Hz, H-1'), 4.51 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 5.07 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz, H-2'), 5.08 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4‴), 5.17 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4″), 5.19 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4'), 5.29 (d, 1 H, $J_{1,2}$ 8 Hz, H-1‴), 5.30 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz, H-2), 5.33 (d, 1 H, $J_{1,2}$ 8 Hz, H-1″), 5.53 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9 Hz, H-3), 5.58 (dd, 1 H, $J_{2,3}$ 11, $J_{3,4}$ 9.5 Hz, H-3‴), 5.82 (dd, 1 H, $J_{2,3}$ 11, $J_{3,4}$ 9.5 Hz, H-3″), and 7.15–8.01 (m, 28 H, aromatic protons).

Anal. Calc. for $C_{83}H_{80}N_2O_{34}$: C, 60.43; H, 4.89; N, 1.70; O, 32.98. Found: C, 60.04; H, 4.91; N, 1.99; O, 33.06.

Methyl O-*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1→3)*-O-[*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1→6)*]-O-*β-*D-*galactopyranosyl-(1→4)-β-*D-*glucopyranoside* (**12**). — A solution of **8** or **10** (1.00 g) in ethanol (80 mL) and aqueous 85% hydrazine hydrate (12 mL) was boiled under reflux for 6 h. T.l.c. (1:3:2 dichloromethane–methanol–conc. NH_3) then showed the reaction to be complete. The mixture was cooled and concentrated, and the residue was acetylated for 24 h at room temperature in 2:1 pyridine–acetic anhydride (10 mL). After conventional processing, column chromatography (4:1 ethyl acetate–toluene) of the product gave **11** (0.52 g, 70%), $[\alpha]_D^{20}$ +5° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.92–2.13 (39 H, 13 Ac), 3.48 (s, 3 H, OMe), 5.70 and 6.65 (2 d, 2 H, $J_{2,NH}$ 9 Hz, 2 NH).

A solution of **11** (0.52 g) in methanol (40 mL) was treated with methanolic M sodium methoxide (2 mL). After 24 h at room temperature, the solution was neutralised with Amberlite IR-120 (H^+) resin, filtered, and concentrated. The residue (0.32 g) was homogeneous **12**, as revealed by t.l.c. (3:3:1 ethyl acetate–2-propanol–water and 4:5:3 1-butanol–acetone–water; R_F 0.53), and had $[\alpha]_D^{20}$ −13° (*c* 0.4, water). ^{1}H-N.m.r. data (D_2O): δ 2.01 and 2.04 (2 s, 6 H, 2 Ac), 3.57 (s, 3 H, OMe), 4.15 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4'), 4.41 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.43 (d, 1 H, $J_{1,2}$ 8 Hz, H-1'), 4.61 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1‴), and 4.68 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1″).

*3,6-Di-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galac-*

*topyranosyl)-β-*D*-glucopyranose* (**13**). — A solution of 1,3,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranose[8] (7.66 g, 10 mmol) in *N,N*-dimethylformamide (75 mL) was treated with hydrazine acetate (1.01 g, 11 mmol) for 4 h at room temperature. Crystallisation of the product from ethanol gave **13** (6.15 g, 84%), m.p. 138–140°, $[\alpha]_D^{20}$ +34° (*c* 1, chloroform); lit.[12,23] m.p. 132–140°; $[\alpha]_{578}^{25}$ +39° (chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.91, 1.96, 2.04, 2.06, and 2.14 (5 s, 18 H, 6 Ac), 4.56 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1′), 4.94 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 3.2 Hz, H-3′), 5.08 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 10.5 Hz, H-2′), 5.31 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4′), 5.63 (t, 1 H, $J_{1,2} = J_{1,OH}$ = 7.3 Hz, H-1), 5.76 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 8 Hz, H-3), and 7.67–7.83 (m, 4 H, aromatic protons).

Anal. Calc. for $C_{32}H_{37}NO_{18} \cdot 0.5\ H_2O$: C, 52.45; H, 5.23; N, 1.91; O, 40.40. Found: C, 52.63; H, 5.12; N, 1.87; O, 40.36.

*3,6-Di-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl fluoride* (**15**) *and methyl* O-*(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-*D*-glucopyranoside* (**16**). — A solution of boron trifluoride etherate (0.43 g, 3 mmol) in dichloromethane (10 mL) was added dropwise to a solution of **2** (1.72 g, 2 mmol) and **14** (3.47 g, 4 mmol) in dichloromethane (50 mL) at −20° under nitrogen, and the mixture was stirred for 30 min at −20°. T.l.c. (1:1 toluene–ethyl acetate) then showed a major product (R_F 0.33), traces of **2** (R_F 0.51), and products (R_F 0.16 and 0.09) derived from the imidate **14**. Excess of solid sodium hydrogencarbonate was added, the mixture was allowed to attain 0°, water was added, and the aqueous layer was extracted with dichloromethane. The extract was washed with water and concentrated. Column chromatography (3:1 toluene–ethyl acetate) of the residue gave **15**, and then **16** (R_F 0.37; 0.14 g, 5% based on **14**), m.p. 225–226° $[\alpha]_D^{20}$ +30° (*c* 0.7, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.94, 1.98, 2.07, 2.09, 2.16, and 2.18 (6 s, 18 H, 6 Ac), 4.35 (m, 1 H, H-2), 4.56 (d, 1 H, $J_{1,2}$ 7 Hz, H-1′), 4.98 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 3.5 Hz, H-3′), 5.15 (dd, 1 H, $J_{1,2}$ 7, $J_{2,3}$ 10 Hz, H-2′), 5.36 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4′), 5.80 (m, 1 H, H-3), 6.12 (dd, 1 H, $J_{1,2}$ 7.5, $J_{1,F}$ 52 Hz, H-1), and 7.79–7.91 (m, 4 H, aromatic protons).

Anal. Calc. for $C_{32}H_{36}FNO_{17}$: C, 52.97; H, 5.00; F, 2.62; N, 1.93. Found: C, 52.72; H, 5.03; F, 2.37; N, 2.09.

Further elution gave **16** (2.35 g, 75% based on **2**), which crystallised from ethanol; m.p. 281–283°, $[\alpha]_D^{20}$ +37° (*c* 0.75, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.80, 1.97, 1.98, 2.02, 2.07, and 2.14 (6 s, 18 H, 6 Ac), 2.88 (s, 1 H, H-5′), 3.38 (s, 3 H, OMe), 4.44 (dd, 1 H, $J_{6a,6b}$ 12, $J_{5,6b}$ 2 Hz, H-6b), 4.54 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.56 (d, 1 H, $J_{1,2}$ 8 Hz, H-1‴), 4.64 (d, 1 H, $J_{1,2}$ 8 Hz, H-1′), 4.79 (dd, 1 H, $J_{6a,6b}$ 12, $J_{5,6b}$ 2 Hz, H-6″b), 4.99 (dd, 1 H, $J_{2,3}$ 11, $J_{3,4}$ 3 Hz, H-3‴), 5.17 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 11 Hz, H-2‴), 5.28 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 9.5 Hz, H-2), 5.34 (d, 1 H, $J_{3,4}$ 3 Hz, H-4‴), 5.38 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz, H-2′), 5.40 (s, 1 H, C*H*Ph), 5.55 (d, 1 H, $J_{1,2}$ 8 Hz, H-1″), 5.61 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 9.5 Hz, H-3″), 5.77 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz), 7.15–8.07 (m, 29 H, aromatic protons).

Anal. Calc. for $C_{80}H_{79}NO_{32}$: C, 61.33; H, 5.08; N, 0.90; O, 32.69. Found: C, 61.07; H, 5.04; N, 0.83; O, 32.68.

Methyl O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)*-O-*(3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)-(1→3)*-O-*(2*-O-*benzoyl-β*-D-*galactopyranosyl)-(1→4)-2,3,6-tri*-O-*benzoyl-β*-D-*glucopyranoside* (**17**). — A solution of **16** (2.00 g) in aqueous 80% acetic acid (80 mL) was heated for 25 min at 100°, cooled, and concentrated. Column chromatography (3:2 toluene–ethyl acetate) of the residue gave **17** (1.70 g, 90%), m.p. 244–245°, $[\alpha]_D^{20}$ +52° (*c* 1.1, chloroform).

Anal. Calc. for $C_{73}H_{75}NO_{32}$: C, 59.31; H, 5.11; N, 0.95; O, 34.63. Found: C, 59.04; H, 5.09; N, 0.93; O, 34.69.

Methyl O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)*-O-*(3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)-(1→3)*-O-[*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)*-O-*(3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)-(1→6)*]-O-*(2*-O-*benzoyl-β*-D-*galactopyranosyl)-(1→4)-2,3,6-tri*-O-*benzoyl-β*-D-*glucopyranoside* (**19**). — A solution of trimethylsilyl trifluoromethanesulfonate (0.27 g, 1.2 mmol) in dichloromethane (3 mL) was added dropwise to a solution of **17** (1.48 g, 1 mmol) and **14** (1.04 g, 1.2 mmol) in dichloromethane (30 mL) at −20° under nitrogen. The mixture was stirred at −20° for 15 min; t.l.c. (7:3 ethyl acetate–toluene) then showed the absence of **17** (R_F 0.26) and the formation of a product (R_F 0.35). The mixture was processed as described for the preparation of **16**. Column chromatography (4:1 toluene–ethyl acetate) of the crude product gave amorphous **18**, and then **19** (0.11 g, 13% based on **14**), R_F 0.37. ^{1}H-N.m.r. data ($CDCl_3$): δ 1.96, 2.01, 2.03, 2.13, 2.22, and 2.24 (6 s, 18 H, 6 Ac), 3.92 (ddd, 1 H, $J_{4,5}$ 1, $J_{5,6a}$ 6, $J_{5,6b}$ 7 Hz, H-5'), 4.05–4.35 (m, 4 H, H-4,6a,6'a,6'b), 4.45 (m, 1 H, H-5), 4.56 (dd, 1 H, $J_{5,6}$ 2.5, $J_{6a,6b}$ 12 Hz, H-6b), 4.72 (d, 1 H, $J_{1,2}$ 8 Hz, H-1'), 5.02 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 3.5 Hz, H-3'), 5.26 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz, H-2'), 5.38 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1 Hz, H-4'), 5.82 (d, 1 H, $J_{3,4}$ 4 Hz, H-3), 6.74 (s, 1 H, H-1), and 7.77–7.93 (m, 4 H, aromatic protons).

Further elution gave **19** (1.75 g, 80% based on **17**), which crystallised from ethanol; m.p. 144–147°, $[\alpha]_D^{20}$ +29.5° (*c* 1.2, chloroform).

Anal. Calc. for $C_{105}H_{110}N_2O_{49}$: C, 57.74; H, 5.08; N, 1.28; O, 35.90. Found: C, 57.49; H, 5.14; N, 1.53; O, 35.62.

Methyl O-*β*-D-*galactopyranosyl-(1→4)*-O-*(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1→3)*-O-[*β*-D-*galactopyranosyl-(1→4)*-O-*(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1→6)*]-O-*β*-D-*galactopyranosyl-(1→4)-β*-D-*glucopyranoside* (**20**). — A solution of **19** (1.0 g) in ethanol (100 mL) and aqueous 85% hydrazine hydrate (10 mL) was boiled under reflux for 6 h, then cooled, and concentrated. To a solution of the residue in methanol (50 mL) was added acetic anhydride (5 mL). The solution was left at room temperature overnight. T.l.c. (4:5:3 1-butanol–acetone–water) then showed a major product (R_F 0.34). Column chromatography (4:5:1 1-butanol–acetone–water) gave **20** (0.32 g, 64%), $[\alpha]_D^{20}$ −15° (*c* 0.6, water). ^{1}H-N.m.r. data (D_2O): δ 2.02 and 2.05 (2 s, 6 H, 2 Ac), 3.57 (s, 3 H, OMe), 4.15 (d,

1 H, $J_{3,4}$ 3 Hz, H-4B), 4.42 (d, 1 H, $J_{1,2}$ 8 Hz, H-1A), 4.44 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1B), 4.48 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1F), 4.49 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1D), 4.64 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1E), and 4.71 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1C).

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