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Syntheses of linear tetra-, hexa-, and octa-saccharide fragments of the i-blood group active poly-(*N*-acetyllactosamine) series. Blockwise methods for the synthesis of repetitive oligosaccharide sequences

Jocelyne Alais and Alain Veyrières*
Institut de Chimie Moléculaire d'Orsay, U.R.A. du C.N.R.S. D.0462, Université Paris-Sud, Bât. 420, F-91405 Orsay (France)

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ABSTRACT

N-Phthaloylation of lactosamine gave various glycosyl donors (β-chloride, β-trichloroacetimidate) and glycosyl acceptors (3',4'-diol). Coupling of the chloride with a methyl β-D-glycoside led to the tetrasaccharide fragment, β-D-Gal*p*-(1→4)-β-D-Glc*p*Nac-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAcOMe. Acetolysis of the protected tetrasaccharide, followed by treatment with hydrogen chloride, gave a tetrasaccharide chloride which was coupled with the methyl β-glycoside of lactosamine. A hexasaccharide fragment, [β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)]$_2$-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAcOMe, was thus obtained by this ("n+1") method. A more efficient ("n+n") method was applied for the synthesis of an octasaccharide fragment, [β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)]$_3$-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAcOMe (**38**), where di- and tetra-saccharide intermediates having a 3,4-*O*-isopropylidene-β-D-galactopyranosyl nonreducing terminal group and a benzyl β-D-glycoside group were precursors, either as glycosyl donors (β-trichloroacetimidates) or glycosyl acceptors (3,4-diols as nonreducing terminal groups). Thus, doubling the length of the repetitive oligosaccharide sequence could be efficiently accomplished at each glycosylation step.

INTRODUCTION

Poly(lactosaminoglycans) are complex oligosaccharide structures linked to proteins and lipids of the human erythrocyte membrane and of various other tissues[1]. These compounds are known to be the precursors of the blood-group antigens A, B, O, Lewis, and P_1, and carry the developmental antigens i and I. On fetal and cord erythrocytes, the poly(lactosaminoglycans) are formed by linear sequences of *N*-acetyllactosamine units linked β-D-(1→3) to each other (i antigen). A glycolipid isolated from bovine erythrocyte membranes, lacto-*N*-*nor*-hexaosylceramide [β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)]$_2$-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer], was shown to display i blood-group activity[2]. On adult human erythrocytes, branched structures are preponderant with additional lactosamine units linked β-D-(1→6) to a linear structure (I antigen). The need of well defined oligosaccharides, tested for their haptenic activity or as enzyme substrates, prompted us to develop efficient chemical syntheses of various fragments of the poly-(*N*-acetyllactosamine) series [β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)]$_n$.

* To whom correspondence should be addressed. Present address: Laboratoire de Chimie, École Normale Supérieure, 24 rue Lhomond, F-75231 Paris 05, France.

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We reported previously[3] the synthesis of a trisaccharide, β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-D-Gal, which was found to inhibit two human anti-i-antibodies, though much less efficiently than lacto-*N-nor*-hexaosylceramide[4]. Another synthetic tetrasaccharide[5], β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-GlcNAc, was 9–40 times more active than the aforementioned trisaccharide[6]. However, its ^{1}H-n.m.r. spectrum indicated a mixture (in a 7:3 ratio) of α- and β-D anomers at the reducing terminal *N*-acetylglucosamine unit when the mutarotation equilibrium in water was reached. Since mutarotation always favors the α-D configuration in such oligosaccharides[7], it seems more appropriate to test, in immunochemical assays, oligosaccharides having a permanent β-D configuration at the reducing end. The compounds reported herein were all synthesized as the methyl β-D-glycosides.

The synthesis of repetitive oligosaccharide sequences, such as [β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)]$_n$, can be achieved by elongation of a smaller fragment either from the reducing end or from the nonreducing end. Starting from a tetrasaccharide fragment (n = 2), it could thus be considered either to activate it, then couple it with the initial disaccharide block (n = 1), usually available in large amounts, or to transform it into a glycosyl acceptor (by unmasking an hydroxyl group at the nonreducing endgroup), which will be coupled with the activated disaccharide precursor. The choice between the two strategies will depend on the compared easiness of activation and hydroxyl deprotection steps. This ("n + 1") procedure is exemplified by the preparation[8] of hexasaccharide **33**.

Synthesis of longer sequences (n ≥ 4) requires more rapid procedures, such as doubling the length of the sequence at each glycosylation step. This means that activation and deprotection steps have to be performed on the same block, giving rise to two blocks of the same size which have to be coupled together. This "n + n" procedure was applied to the synthesis[9] of octasaccharide **38**.

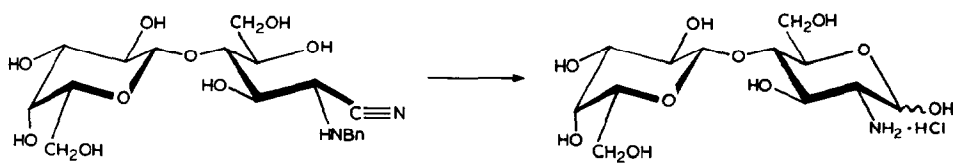
Although activation of a lactosamine block through an oxazoline compound allowed the preparation of the β-D-(1→3) dimer of lactosamine[5], this method of glycosylation is not convenient for longer sequences. In the presence of catalytic amounts of sulfonic acids, oxazolines are not very reactive compounds, and typical glycosylations require heating at temperatures > 60° for several hours[3]. Such prolonged heating can result in partial anomerization of the glycosidic bonds. However, one valuable advantage of this procedure is to provide a direct access to the natural *N*-acetylamino sugar unit.

2-Deoxy-2-phthalimido sugars as the bromide[10], chloride[11], or (more recently) trichloroacetimidate derivative[12], have a much higher reactivity and a complete neighboring group assistance, leading to 1,2-*trans*-glycosides with total stereocontrol. In the preparation of poly(lactosamine) fragments, glycosylations involve OH-3 of a nonreducing, terminal D-galactopyranosyl group. In homogeneous phase with silver triflate as catalyst, this group has a much higher reactivity towards such glycosylating reagents as phthalimido halides, than the neighboring, axial OH-4 group, and thus β-(1→3) linkages are obtained[13] regioselectively from a 3,4-diol. Therefore a 3,4-*O*-isopropylidene group seems to offer the most convenient protection for that diol function, and

glycosylating donors at various stages of the elongation process should contain this acetal group at their terminal nonreducing galactosyl group. Unfortunately, glycosyl halides having an acetal protective group are not easily handled. In contrast, trichloroacetimidate derivatives containing various, acid-labile protecting groups are easily available[14], and their activation by Lewis acids at low temperature is compatible with such groups[15].

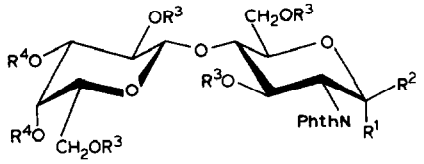
RESULTS AND DISCUSSION

The convenient synthesis of *N*-acetyllactosamine[16] from commercial 3-*O*-β-D-galactopyranosyl-D-arabinose was easily modified to produce derivatives of *N*-phthaloyllactosamine. Catalytic hydrogenation of the intermediate 2-benzylamino-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucononitrile[16,17] (**1**) in acidic medium afforded an aqueous solution of crude lactosamine hydrochloride (**2**) and one equivalent amount of ammonium chloride. Reaction of phthalic anhydride with unprotected 2-amino-2-deoxy sugars is usually performed[10] in methanol in the presence of triethylamine to give 2-(2-carboxybenzamido) sugars ("phthalamic acids"). We found that this reaction can also be done in aqueous acetone with sodium hydrogencarbonate, or in aqueous 1,4-dioxane with triethylamine, as was described[18] for amino acids. The crude *N*-(2-carboxybenzoyl)lactosamine was then treated with acetic anhydride and pyridine to achieve full *O*-acetylation and cyclisation to give a phthalimido derivative, obtained as a 2:1 mixture of the β- (**4**) and α-D anomer (**3**). Pure crystalline β-D anomer **4** was isolated in 32% yield by column chromatography on silica gel. The unresolved mixture of α- and β-D anomers (28%) was treated with a catalytic amount of perchloric acid in acetic anhydride to anomerize the α- into the more stable β-D anomer. The overall yield of isolated **4** was 50%. The formation of the α-D-acetate **3** can be attributed to an acetylation of the anomeric hydroxyl group preceeding the cyclisation of the 2-carboxybenzamido group, since the α side of the pyranose ring becomes well sheltered as soon as the phthalimido group is formed. The highest β:α ratios were always obtained when the acetylating mixture was first heated at 100° for a few minutes, and then kept at room temperature for several hours. The β-D-acetate **4** was treated with dry hydrogen chloride in acetic acid and acetic anhydride to give a nearly quantitative yield of the β-D-chloride **5**, which had been first prepared from the lactal hexaacetate derivative through the nitroso chloride route[11]. Under the same conditions, the α-D-acetate **3** reacted sluggishly with an appreciable acetolysis of the interglycosidic bond. The β-D-acetate **4** also could be smoothly *O*-deacetylated at O-1 by hydrazine acetate in *N,N* dimethylformamide[19] to

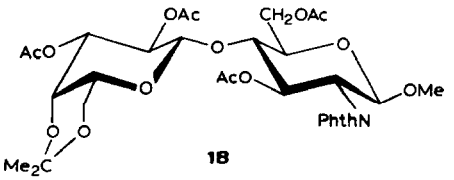


give the hemiacetal **6**. Lemieux *et al.*[20] have described the conversion of **6** into **5** by use of the Vilsmeyer reagent.

Synthesis of tetrasaccharide **22** *and hexasaccharide* **23** *by the "n+1" procedure.* — The β-D-chloride **5** was converted into the methyl β-D-glycoside **7** in 86% yield by reaction with methanol catalyzed with $HgCl_2$–HgO. Treatment of **7** with 0.02M sodium methoxide in 5:1 methanol–1,4-dioxane for 6 h at room temperature removed the *O*-acetyl groups without opening of the phthalimido ring to afford **8** in 84% yield. Treatment of **8** with acetone and a catalytic amount of 4-toluenesulfonic acid gave the 3′,4′-*O*-isopropylidene derivative **9** in 65% yield. Its structure was established by examination of the ^{1}H-n.m.r. spectrum of its *O*-acetylated derivative **10**; signals for H-3′ and H-4′ were absent in the δ 4.8–5.4 region and H-2′ appeared as a triplet at δ 4.87 with a coupling constant $J_{2',3'}$ 7.5 Hz reflecting a distorsion of the pyranose chain by the *cis*-fused dioxolane ring. A minor compound was also isolated in 10% yield from the acetonation mixture; the ^{1}H-n.m.r. spectrum of its *O*-acetylated derivative **18** corresponds to a 4′,6′-*O*-isopropylidene isomer.



3 R^1 = OAc, R^2 = H, R^3 = R^4 = Ac
4 R^1 = H, R^2 = OAc, R^3 = R^4 = Ac
5 R^1 = H, R^2 = Cl, R^3 = R^4 = Ac
6 R^1 = H, R^2 = OH, R^3 = R^4 = Ac
7 R^1 = H, R^2 = OMe, R^3 = R^4 = Ac
8 R^1 = R^3 = R^4 = H, R^2 = OMe
9 R^1 = R^3 = H, R^2 = OMe; R^4, R^4 = Me_2C
10 R^1 = H, R^2 = OMe, R^3 = Ac; R^4, R^4 = Me_2C
11 R^1 = R^4 = H, R^2 = OMe, R^3 = Ac
12 R^1 = H, = R^2 = OBn, R^3 = R^4 = Ac
13 R^1 = R^3 = R^4 = H, R^2 = OBn
14 R^1 = H, R^2 = OBn, R^3 = Ac; R^4, R^4 = Me_2C
15 R^1 = H, R^2 = OH, R^3 = Ac; R^4, R^4 = Me_2C
16 R^1 = H, R^2 = OCNHCCl3, R^3 = Ac; R^4, R^4 = Me_2C
17 R^1 = R^4 = H, R^2 = OBn, R^3 = Ac



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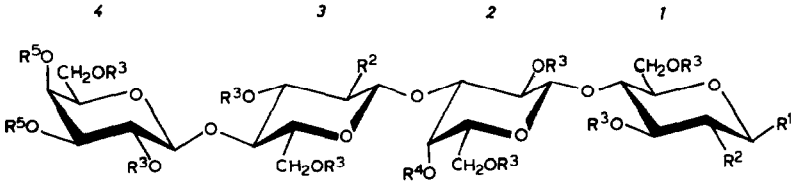
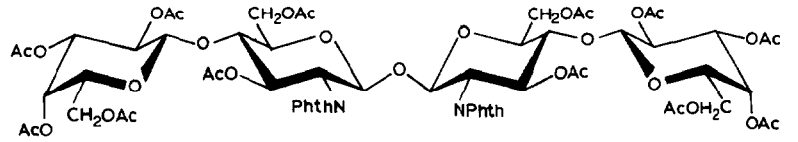
Acid hydrolysis of compound **10** led to a crystalline 3',4'-diol **11**. The two disaccharide blocks, β-D-chloride **5** and diol **11**, were then coupled together with silver triflate–2,4,6-trimethylpyridine as promoter[10] to give the protected tetrasaccharide **19** in 64% yield. Its purification was made difficult by a contaminant having nearly the same chromatographic mobility. This impurity had a ^{1}H-n.m.r. spectrum corresponding to a β,β-nonreducing tetrasaccharide **30** arising from the hydrolysis of the β-D-chloride **5** and subsequent coupling of **6** with **5**. *O*-Acetylation of **19** gave compound **20** showing, in its ^{1}H-n.m.r. spectrum, a signal at δ 5.29 ($J_{3,4}$ 3.5 Hz) attributed to equatorial H-4 of the internal D-galactose unit, and a close signal at δ 5.32 ($J_{3,4}$ 3.5 Hz) already present in the spectrum of **19** and, thus, attributed to equatorial H-4 of the terminal D-galactosyl group. Therefore, glycosylation had occurred as predicted at the more reactive OH-3' of the diol **11**. No trace of an isomeric tetrasaccharide with a newly formed (1→4) linkage or a branched hexasaccharide could be detected in the reaction mixture. Compounds **19** and **20** showed doublets ($J_{1,2}$ 8.5 Hz) at δ 5.42 and 5.35, respectively, corresponding to H-1 of the internal 2-deoxy-2-phthalimido-D-glucose unit. These signals, together with H-1 of the terminal 2-amino-2-deoxy-D-glucose unit (δ 5.20 and 5.21 for **19** and **20**, respectively) were easily identified because of the strong deshielding effect of the phthalimido ring. The large coupling constant (8.5 Hz) demonstrated the β-D configuration of the new (1→3) linkage.

Tetrasaccharide **19** was sequentially treated with sodium methoxide in methanol, hydrazine hydrate, and acetic anhydride–pyridine to give **21** in 72% yield. The ^{1}H-n.m.r. spectrum of **21** showed two signals at δ 5.52 (8.5 Hz) and 5.75 (9.5 Hz) attributed to the two NH groups, and again two signals at δ 5.32 ($J_{3,4}$ 3.0 Hz) and 5.35 ($J_{3,4}$ 2.5 Hz) assigned to H-4 of the two galactose units. *O*-Deacetylation of **21** afforded the unprotected tetrasaccharide **22**, which crystallized as a trihydrate. Its ^{1}H-n.m.r. spectrum in D_2O showed a doublet at δ 4.71 ($J_{1,2}$ 7.5 Hz) corresponding to H-1 of the internal 2-acetamido-2-deoxy-D-glucose unit, thus confirming once more the β-(1→3) linkage obtained in the coupling reaction (δ 4.7–4.8 in other analogous oligosaccharides[3,5]). The three other anomeric protons gave overlapping signals at δ 4.45–4.50 (δ 4.435 for methyl 2-acetamido-2-deoxy-β-D-glucopyranoside[21] and δ 4.49 for *N*-acetyllactosamine[3]). A low-field signal at δ 4.17 ($J_{3,4}$ 3.0 Hz) could be assigned to equatorial H-4 of the internal D-galactose unit [δ 4.16 and 4.22 for 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-α,β-D-galactose[22]].

The protected tetrasaccharide **19** was acetolyzed with sulfuric acid in acetic acid–acetic anhydride without cleavage of any interglycosidic bond, and the crystalline tetrasaccharide β-D-acetate **23** was obtained in nearly quantitative yield. It was converted into the β-D-chloride **24** by treatment with dry hydrogen chloride in acetyl chloride. The reaction was stopped after 24 h, although some starting material was still present, as cleavage products began to appear. The crude β-D-chloride **24** was condensed with diol **11** in the presence of silver triflate–2,3,6-trimethylpyridine as promoter to give the crystalline hexasaccharide **31** in 40% yield. Its ^{1}H-n.m.r. spectrum showed three distinct signals for the anomeric protons of the 2-deoxy-2-phthalimido-D-glucose units (δ 5.18, 5.33, and 5.39; $J_{1,2}$ 8.5 Hz) which are all β-D-linked.



19 R^1 = OMe, R^2 = NPhth, R^3 = R^5 = Ac, R^4 = H
20 R^1 = OMe, R^2 = NPhth, R^3 = R^4 = R^5 = Ac
21 R^1 = OMe, R^2 = NHAc, R^3 = R^4 = R^5 = Ac
22 R^1 = OMe, R^2 = NHAc, R^3 = R^4 = R^5 = H
23 R^1 = OAc, R^2 = NPhth, R^3 = R^4 = R^5 = Ac
24 R^1 = Cl, R^2 = NPhth, R^3 = R^4 = R^5 = Ac
25 R^1 = OBn, R^2 = NPhth, R^3 = Ac, R^4 = H; R^5, R^5 = CMe_2
26 R^1 = OBn, R^2 = NPhth, R^3 = R^4 = Ac; R^5, R^5 = CMe_2
27 R^1 = OH, R^2 = NPhth, R^3 = R^4 = Ac; R^5, R^5 = CMe_2
28 R^1 = $OCNHCCl_3$, R^2 = NPhth, R^3 = R^4 = Ac; R^5, R^5, = CMe_2
29 R^1 = OBn, R^2 = NPhth, R^3 = R^4 = Ac, R^5 = H



30

Sequential *O*-deacetylation, hydrazinolysis, and *N,O*-acetylation gave **32** which showed in its ^{1}H-n.m.r. spectrum three distinct signals for the NH groups (δ 5.48, 5.53, and 5.76) and three signals for the equatorial H-4 of the D-galactose units (δ 5.28, 5.29, and 5.32), which demonstrated that none of the D-galactose units had been glycosylated at OH-4. *O*-Deacetylation of **32** gave the unprotected hexasaccharide **33** which was purified by gel-filtration chromatography. Its ^{1}H-n.m.r. spectrum showed a doublet at δ 4.73 ($J_{1,2}$ 7.5 Hz) assigned to the anomeric protons of the two internal 2-acetamido-2-deoxy-D-glucose units, and several overlapping signals at δ 4.47–4.52 corresponding to the four remaining anomeric protons. The equatorial H-4 of the two internal D-galactose units gave a signal at δ 4.19 ($J_{3,4}$ 3.0 Hz), deshielded by the neighboring (1→3)-glycosidic bond.

Synthesis of octasaccharide **38** *by the ("n+n") procedure.* — Treatment of the disaccharide β-D-acetate **4** with benzyl alcohol and trimethylsilyl triflate gave the known[20] benzyl β-D-glycoside **12** in 91% yield. *O*-Deacetylation with sodium methoxide gave **13** which was treated with acetone as described for **10** to give the 3′,4′-*O*-isopropylidene derivative in 60% yield. Acetylation with acetic anhydride–pyridine led to the crystalline key intermediate **14**.

Catalytic transfer of hydrogen from cyclohexene in boiling ethanol in the pres-

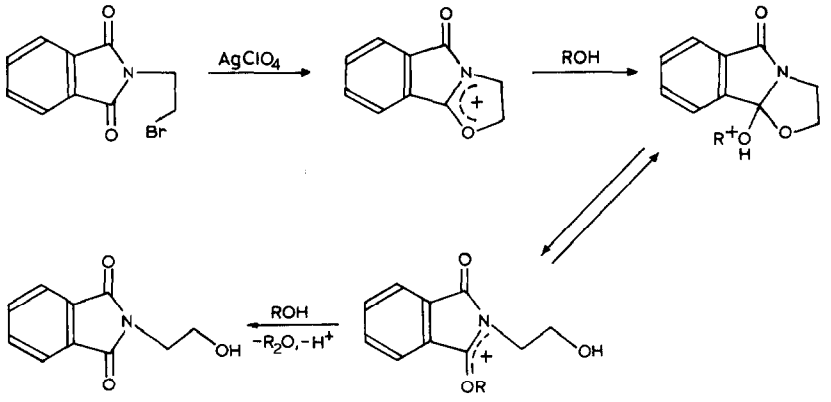
ence of palladium–charcoal[23] converted **14** into the reducing sugar **15** in quantitative yield. Classical hydrogenolysis in ethanol–ethyl acetate at room temperature with Pd–C was sluggish and gave byproducts resulting from the hydrogenation of the phthalimido ring. Treatment of **15** with trichloroacetonitrile and potassium carbonate in dichloromethane gave the crystalline β-D-trichloroacetimidate **16** in 66% yield. Alternatively, **14** was transformed into the crystalline diol **17** by mild acidic hydrolysis (78% yield). The key disaccharide block **14** has thus been converted partly into a glycosyl donor **16** and partly into a glycosyl acceptor **17** in a minimum number of steps and with good yields.

Coupling of **16** with **17** was performed in the presence of trimethylsilyl triflate and gave the crystalline tetrasaccharide **25** in 78% yield. The reaction proceeded within a few minutes and, provided that the reaction mixture was kept at neutral pH at low temperature before the processing, no cleavage of the *O*-isopropylidene group was observed. *O*-Acetylation gave **26** which showed in its ^{1}H-n.m.r. spectrum a characteristic signal for equatorial H-4′ at δ 5.30 ($J_{3,4}$ 3 Hz), which was absent in the spectrum of **25**. Two doublets at δ 5.35 and 5.31 ($J_{1,2}$ 8 Hz) correspond to the anomeric protons of the two 2-deoxy-2-phthalimido-D-glucose units. A β-D-(1→3) linkage had been formed between the two disaccharide blocks with a total regioselectivity since no other tetrasaccharide or branched hexasaccharide could be isolated. As previously observed[15], the trichloroacetimidate procedure allows selective glycosylation at O-3 of a 3,4-diol in D-galactopyranosides.

Tetrasaccharide **26** is a convenient precursor for further block syntheses. Its conversion into the glycosyl donor **28** was done as described for **14**; hydrogenolysis gave a reducing tetrasaccharide **27** which was then converted into the β-D-trichloroacetimidate **28**. Mild acidic hydrolysis of **26** gave the tetrasaccharide diol **29**, the new glycosyl acceptor.

Condensation of the two blocks, **28** and **29**, in the presence of trimethylsilyl triflate gave the crystalline octasaccharide **34** in 50% yield. *O*-Acetylation gave **35** which showed in its ^{1}H-n.m.r. spectrum, in the δ 5.26–5.39 region, four signals corresponding to the anomeric protons of the four β-D-linked 2-deoxy-2-phthalimido-D-glucose units ($J_{1,2}$ 8.5 Hz) and three signals corresponding to the equatorial H-4 of the three internal D-galactose units ($J_{3,4}$ 3 Hz).

Hydrogenolysis of **35** as described for **14** and **26** gave a reducing octasaccharide which could be converted into a β-D-trichloroacetimidate. But all attempts to have this imidate react with methanol in the presence of a Lewis acid gave poor yields of methyl β-D-glycoside; high proportions of hydrolysis product were isolated after working up the reaction mixture. Similar behavior was observed with imidates **16** and **28** and can be explained by an attack of methanol at C-2 of an oxazolinium intermediate generated by participation of one carbonyl group in the phthalimido ring assisting the departure of the anomeric trichloroacetimidate group. Stable oxazolinium salts have been obtained by treatment of *N*-(2-haloethyl)phthalimide with antimonium pentachloride or silver perchlorate[24]. They are attacked at C-2 by nucleophiles, such as water, methanol, ethanol, or cyanide, and give a phthalimidium cation that will be alkylated by an excess of alcohol (when alcohol is the nucleophile) with consecutive formation of a dialkyl ether (Scheme 1).



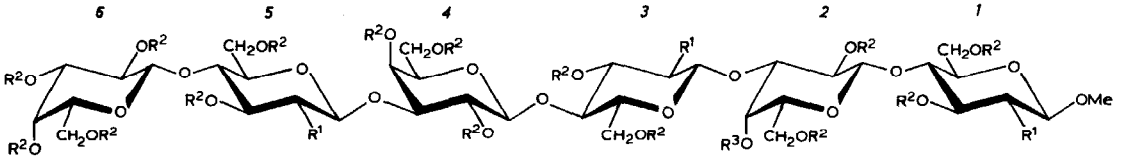
Scheme 1

The octasaccharide methyl β-D-glycoside **36** was best obtained in 65% yield by treatment of the free sugar with methanesulfonic anhydride, 2,4,6-trimethylpyridine, and methanol according to the method of Leroux and Perlin[25]. Sequential treatment with dilute trifluoroacetic acid, sodium methoxide, hydrazine hydrate, and acetic anhydride–pyridine gave the *N,O*-acetylated octasaccharide **37** in 45% yield . Final *O*-deacetylation afforded the unprotected octasaccharide methyl β-D-glycoside **38** which was purified by gel filtration. Its ^{1}H-n.m.r. spectrum showed three doublets at δ 4.732, 4.729, and 4.726 ($J_{1,2}$ 8 Hz) which were assigned to the anomeric protons of the three internal 2-acetamido-2-deoxy-D-glucose units, and several overlapping signals at δ 4.48–4.51 corresponding to the five remaining anomeric protons. The equatorial H-4 of the three internal D-galactose units gave a signal at δ 4.19 ($J_{3,4}$ 3.0 Hz), deshielded by the neighboring (1→3)-glycosidic bond.

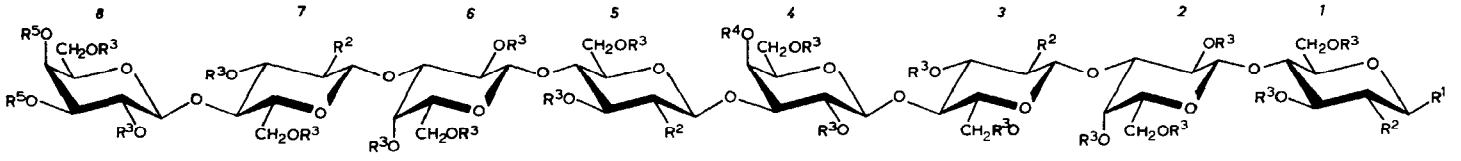
Details of immunochemical tests will be given elsewhere. Briefly, hexasaccharide **33** appears as the most potent synthetic inhibitor known so far, being active in five i-anti-i-systems. Tetrasaccharide **22** and, surprisingly, octasaccharide **38** are five and two times less active, respectively, in the same assays.

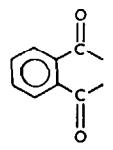
EXPERIMENTAL

General methods. — Melting points were determined with a Büchi apparatus and 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 $60F_{254}$ (Merck) with detection by charring with H_2SO_4. Silica Gel 60 (Merck, 70–230 mesh) was used for column chromatography. ^{1}H-N.m.r. spectra were recorded with a Cameca model STN 250 (250 MHz) spectrometer and a spectrometer (400 MHz) constructed at this University for solutions in $CDCl_3$ (internal Me_4Si) or in D_2O (external 0.2% Me_4Si in $CDCl_3$). Microanalyses were performed by the Laboratoire Central de Microanalyse du C.N.R.S. The purity of **38** was verified by analytical h.p.l.c. (Waters model 590, equipped with a differential refractometer model 410).



31 R^1 = NPhth, R^2 = Ac, R^3 = H
32 R^1 = NHAc, R^2 = R^3 = Ac
33 R^1 = NHAc, R^2 = R^3 = H



Phth = 

34 R^1 = OBn, R^2 = NPhth, R^3 = Ac, R^4 = H; R^5, R^5 = CMe_2
35 R^1 = OBn, R^2 = NPhth, R^3 = R^4 = Ac; R^5, R^5 = CMe_2
36 R^1 = OMe, R^2 = NPhth, R^3 = R^4 = Ac; R^5, R^5 = CMe_2
37 R^1 = OMe, R^2 = NHAc, R^3 = R^4 = R^5 = Ac
38 R^1 = OMe, R^2 = NHAc, R^3 = R^4 = R^5 = H

1,3,6-Tri-O-acetyl-2-deoxy-2-phthalimido-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α- (**3**) *and -β-D-glucopyranose* (**4**). — A solution of 2-benzylamino-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucononitrile[16,17] (**1**; 32.25 g, 75.3 mmol) in 0.5M HCl (350 mL) was hydrogenated at room temperature and atmospheric pressure in the presence of 5% Pd–$BaSO_4$ (11.2 g). After 19 h, the absorption of H_2 had stopped (3080 mL, 85%), and the catalyst was filtered off and washed with water (50 mL). To the filtrate was added $NaHCO_3$ (14.70 g, 175 mmol) and the resulting solution was added dropwise within 15 min to a stirred solution of phthalic anhydride (23.4 g, 158 mmol) in acetone (800 mL). More $NaHCO_3$ (13.27 g, 158 mmol) was then added portionwise within 30 min under stirring. The mixture was kept overnight at room temperature, and then concentrated. The dry residue was treated with acetic anhydride (200 mL) and pyridine (300 mL) at 100°. After a few min, a vigorous reaction ensued, and the mixture was immediately cooled and kept for 24 h at room temperature under stirring. Methanol was added to destroy the excess of anhydride. Evaporation and codistillation with toluene gave a residue which was extracted with dichloromethane (500 mL). The extract was washed successively with M HCl, water, saturated $KHCO_3$, and water, dried, and concentrated. Column chromatography (1:2 ethyl acetate–toluene) of the residue gave three main fractions.

The first-eluted fraction gave pure β-D-acetate (**4**; 18.3 g, 32%) which crystallized from methanol, m.p. 272° (dec), $[\alpha]_D$ +33° (*c* 1.05, chloroform); ^{1}H-N.m.r. ($CDCl_3$): δ 7.90–7.70 (m, 4 H, Phth), 5.83 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 8.0 Hz, H-3), 5.48 (d, 1 H, $J_{1,2}$ 9.0 Hz, H-1), 5.34 (dd, 1 H, $J_{3',4'}$ 3.5 Hz, H-4'), 5.12 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.5 Hz, H-2'), 4.95 (dd, 1 H, H-3'), 4.53 (d, 1 H, H-1'), 4.36 (dd, 1 H, H-2), and 2.20–1.90 (7 s, 21 H, 7 OAc); lit.[11] m.p. 265–266°, $[\alpha]_D^{25}$ +32° (*c* 1.5, chloroform).

Anal. Calc. for $C_{34}H_{39}NO_{19}$: C, 53.33; H, 5.13; N, 1.83; O, 39.70. Found: C, 53.56; H, 5.13; N, 1.75; O, 39.76.

The second fraction contained a mixture of **3** and **4** (16.2 g, 28%).

The third fraction gave pure α-D-acetate (**3**; 1.0 g, 2%) which crystallized from methanol, m.p. 239.5°, $[\alpha]_D$ +69° (*c* 0.975, chloroform); ^{1}H-N.m.r. ($CDCl_3$): δ 7.90–7.70 (m, 4 H, Phth), 6.42 (dd, 1 H, $J_{2,3}$ 11.5, $J_{3,4}$ 9.0 Hz, H-3), 6.23 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.36 (dd, 1 H, $J_{3',4'}$ 3.5 Hz, H-4'), 5.16 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.5 Hz, H-2'), 4.98 (dd, 1 H, H-3'), 4.68 (dd, 1 H, H-2), 4.52 (d, 1 H, H-1'), and 2.20–1.90 (7 s, 21 H, 7 OAc); lit.[11] m.p. 237–238°, $[\alpha]_D^{25}$ +67° (*c* 1.0, chloroform).

Anal. Calc. for $C_{34}H_{39}NO_{19}$: C, 53.33; H, 5.13; N, 1.83; O, 39.70. Found: C, 53.16; H, 5.27; N, 1.72; O, 39.80.

A mixture of **3** and **4** (5.0 g) was dissolved in acetic anhydride (50 mL) containing 70% aq. $HClO_4$ (0.35 mL). After 24 h at room temperature, the dark-brown solution was poured into ice–water, stirred for 1 h, and extracted with dichloromethane. The extract was washed with cold saturated $KHCO_3$ and water, dried, and concentrated. The residue crystallized from methanol to give pure **4** (3.25 g, 65%).

Examination of the mother-liquor of crystallization of **4** by t.l.c. (1:1 toluene–ethyl acetate) showed small amounts of a compound (R_F 0.50) migrating between **3** (R_F 0.47) and **4** (R_F 0.56). This compound could not be purified, but was identified as the

D-*manno* isomer of **3** by examination of the ^{1}H-n.m.r. spectrum of its mixture with **4**: δ 6.19 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.37 (dd, 1 H, $J_{3',4'}$ 3.5 Hz, H-4'), 5.02 (dd, 1 H, $J_{2',3'}$, 10.5 Hz, H-3'), and 4.61 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1').

*3,6-Di-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galacto-pyranosyl)-β-*D*-glucopyranosyl chloride* (**5**). — A solution of **4** (7.65 g, 10 mmol) in acetic acid (75 mL) and acetic anhydride (35 mL) was saturated with dry HCl at 0°, and then kept at room temperature for 48 h. After dilution with dichloromethane (500 mL), the mixture was successively washed with ice–water, cold saturated aq. $KHCO_3$, and water, dried, and concentrated to a small volume. Addition of dry ether gave crystalline **5** (6.80 g, 92%), m.p. 173.5°, $[\alpha]_D$ +30° (*c* 1.0, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.90–7.70 (m, 4 H, Phth), 6.18 (d, 1 H, $J_{1,2}$ 9.5 Hz, H-1), 5.74 (dd, 1 H, $J_{2,3}$ 9.5, $J_{3,4}$ 8.0 Hz, H-3), 5.33 (dd, 1 H, $J_{3',4'}$ 3.0 Hz, H-4'), 5.12 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.5 Hz, H-2'), 4.95 (dd, 1 H, H-3'), 4.54 (d, 1 H, H-1'), 4.40 (dd, 1 H, H-2), and 2.20–1.90 (6 s, 18 H, 6 OAc); lit.[11] m.p. 173–174°, $[\alpha]_D^{25}$ + 29.3° (*c* 1.5, chloroform).

Anal. Calc. for $C_{32}H_{36}ClNO_{17}$: C, 51.79; H, 4.89; Cl, 4.78; N, 1.89; O, 36.65. Found: C, 51.99; H, 4.82; Cl, 5.05; N, 1.82; O, 36.01.

*3,6-Di-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galacto-pyranosyl)-β-*D*-glucopyranose* (**6**). — A solution of **4** (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, and then diluted with dichloromethane (400 mL), washed with 10% aqueous NaCl, and concentrated. The residue crystallized from ethanol–hexane to give **6** (6.15 g, 84%), m.p. 139–140°, $[\alpha]_D$ +34° (*c* 0.99, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.90–7.70 (m, 4 H, Phth), 5.76 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 8.0 Hz, H-3), 5.63 (dd, 1 H, $J_{1,2}$ = $J_{1,OH}$ 7.2 Hz, H-1), 5.31 (dd, 1 H, $J_{3',4'}$ 2.5 Hz, H-4'), 5.08 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.5 Hz, H-2'), 4.94 (dd, 1 H, H-3'), 4.56 (d, 1 H, H-1'), 4.54 (dd, 1 H, H-2), and 2.20–1.90 (6 s, 18 H, 6 OAc); lit.[12] m.p. 138–140°, $[\alpha]_D^{578}$ +39° (*c* 1.0, chloroform).

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

*Methyl 3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**7**). — A mixture of yellow HgO (2.17 g, 10 mmol), $HgCl_2$ (104 mg, 0.38 mmol), and powdered Drierite (5 g) in dichloromethane (200 mL) and methanol (20 mL, 490 mmol) was stirred for 30 min. A solution of **5** (7.42 g, 10 mmol) in dichloromethane (200 mL) was then added dropwise with stirring, and the mixture was stirred overnight at room temperature. After dilution with dichloromethane, the solids were filtered off and washed with dichloromethane; the combined filtrate and washings were successively washed with water, 10% aqueous KI, and water, dried, and concentrated. The residue crystallized from methanol to give **7** (6.34 g, 86%), m.p. 221–222°, $[\alpha]_D$ +18° (*c* 1.08, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.90–7.70 (m, 4 H, Phth), 5.73 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 3.5 Hz, H-3), 5.32 (dd, 1 H, $J_{3',4'}$ 3.5 Hz, H-4'), 5.27 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 5.12 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.5 Hz, H-2'), 4.94 (dd, 1 H, H-3'), 4.54 (d, 1 H, H-1'), 3.42 (s, 3 H, OMe), and 2.20–1.90 (6 s, 18 H, 6 OAc).

Anal. Calc. for $C_{33}H_{39}NO_{18}$: C, 53.73; H, 5.33; N, 1.90; O, 39.04. Found: C, 53.69; H, 5.34; N, 1.88; O, 38.92.

Methyl 2-deoxy-4-O-β-D-galactopyranosyl-2-phthalimido-β-D-glucopyranoside (**8**). — A solution of **7** (7.38 g, 10 mmol) in methanol (700 mL) and 1,4-dioxane (140 mL) was treated with M sodium methoxide in methanol (15 mL) for 6 h at room temperature. Then base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin. The resin was filtered off and washed thoroughly with water. The combined filtrate and washings were concentrated and the residue crystallized from methanol to give **8** (4.08 g, 84%), m.p. 283–285°, $[\alpha]_D$ −67° (*c* 0.508, pyridine).

Anal. Calc. for $C_{21}H_{27}NO_{12}$: C, 51.96; H, 5.60; N, 2.89; O, 39.55. Found: C, 51.73; H, 5.68; N, 2.98; O, 39.60.

Methyl 2-deoxy-4-O-(3,4-O-isopropylidene-β-D-galactopyranosyl)-2-phthalimido-β-D-glucopyranoside (**9**). — A solution of **8** (4.85 g, 10 mmol) in dry acetone (2000 mL) containing 4-toluenesulfonic acid monohydrate (0.70 g, 3.7 mmol) was boiled under reflux for 210 min, and then cooled. The acid was neutralized with Na_2CO_3, and the solutions filtered and concentrated. The residue was chromatographed on a column of silica gel. Elution with 19:1 ether–methanol gave **9** (3.47 g, 65%) which crystallized from ethanol–petroleum ether, m.p. 143–146°, $[\alpha]_D$ +26° (*c* 1.05, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.90–7.70 (m, 4 H, Phth), 5.14 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.58 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1'), 4.44 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 8.0 Hz, H-3), 3.42 (s, 3 H, OMe), 1.50 and 1.32 (2s, 6 H, CMe_2).

Anal. Calc. for $C_{24}H_{31}NO_{12}\cdot 0.5H_2O$: C, 53.93; H, 6.03; N, 2.62; O, 37.42. Found: C, 53.71; H, 6.15; N, 2.78; O, 37.17.

Further elution gave the 4,6-*O*-isopropylidene isomer **18** (0.54 g, 10%) which was *O*-acetylated overnight at room temperature with acetic anhydride and pyridine. After removal of the solvents, the residue was nearly pure **18**; ^{1}H-n.m.r. ($CDCl_3$): δ 7.90–7.70 (m, 4 H, Phth), 5.72 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 8.0 Hz, H-3), 5.26 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 5.18 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.5 Hz, H-2'), 4.78 (dd, 1 H, $J_{3',4'}$ 3.5 Hz, H-3'), 4.42 (d, 1 H, H-1'), 4.27 (dd, 1 H, H-4'), 3.42 (s, 3 H, OMe), 2.20–1.90 (4 s, 12 H, 4 OAc), 1.40 and 1.36 (2 s, 6 H, CMe_2).

Methyl 3,6-di-O-acetyl-2-deoxy-4-O-(2,6-di-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-2-phthalimido-β-D-glucopyranoside (**10**). — A solution of **9** (3.0 g, 5.7 mmol) in pyridine (30 mL) was treated overnight at room temperature with acetic anhydride (30 mL). After removal of the solvents, the residue crystallized from ethanol to give **10** (3.5 g, 90%), m.p. 148–150°, $[\alpha]_D$ +47° (*c* 1.06, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.90–7.70 (m, 4 H, Phth), 5.72 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 8.0 Hz, H-3), 5.27 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.87 (dd, 1 H, $J_{1',2'} = J_{2',3'}$ 7.5 Hz, H-2'), 4.40 (d, 1 H, H-1'), 3.43 (s, 3 H, OMe), 2.20–1.90 (4 s, 12 H, 4 OAc), 1.53 and 1.32 (2 s, 6 H, CMe_2).

Anal. Calc. for $C_{32}H_{29}NO_{16}$: C, 55.41; H, 5.67; N, 2.02; O, 36.90. Found: C, 55.18; H, 5.67; N, 2.20; O, 37.18.

Methyl 3,6-di-O-acetyl-2-deoxy-4-O-(2,6-di-O-acetyl-β-D-galactopyranosyl)-2-phthalimido-β-D-glucopyranoside (**11**). — A solution of **10** (1.7 g, 2.5 mmol) in dichloromethane (45 mL) was treated for 2 h at room temperature with 99% aqueous trifluoroacetic acid (5 mL). The mixture was washed successively with water, saturated aqueous $KHCO_3$, and water, and then concentrated. The residue crystallized from methanol–

ether to give **11** (1.25 g, 77%), m.p. 233–235°, $[\alpha]_D$ −44° (*c* 0.45, pyridine); ^{1}H-n.m.r. ($CDCl_3$): δ 7.90–7.70 (m, 4 H, Phth), 5.74 (dd, 1 H, H-3), 5.29 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.84 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.0 Hz, H-2'), 4.41 (d, 1 H, H-1'), 3.85 (dd, 1 H, $J_{3',4'}$ 3.5 Hz, H-4'), 3.44 (s, 3 H, OMe), 3.33 and 2.94 (bd, 2 H, 2 OH), and 2.20–1.90 (4 s, 12 H, 4 OAc).

Anal. Calc. for $C_{29}H_{35}NO_{16}\cdot 0.5\ H_2O$: C, 52.57; H, 5.48; N, 2.11; O, 39.84. Found: C; 52.72; H, 5.52; N, 2.33; O, 39.17.

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-*(2,6-di*-O-*acetyl-β-*D-*galactopyranosyl)-(1→4)-3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β-*D-*glucopyranoside* (**19**). — A mixture of **11** (663 mg, 1 mmol), 2,4,6-trimethylpyridine (204 mg, 1.7 mmol), silver triflate (408 mg, 1.6 mmol), and powdered molecular sieves 4A (1 g) in dry dichloromethane (30 mL) was stirred for 30 min at room temperature, and then cooled to −30° under N_2. A solution of **5** (1.02 g, 1.4 mmol) in dry dichloromethane (10 mL) was added, and the mixture was stirred for 90 min at −30° under N_2 and then for 16 h at room temperature. After dilution with dichloromethane, the solids were filtered off and washed with dichloromethane. The combined filtrate and washings were successively washed with water, 3% HCl, and water, dried, and concentrated. The residue was chromatographed on silica gel with 1:1 ethyl acetate–toluene as eluent.

The first fractions contained small proportions of **6**, formed by hydrolysis of **5**, followed by amorphous compound **30**, formed by condensation of **5** with **6**; ^{1}H-n.m.r. ($CDCl_3$): δ 7.90–7.70 (m, 4 H, Phth), 5.66 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 8.0 Hz, H-3), 5.51 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 5.27 (dd, 1 H, $J_{3',4'}$ 3.5 Hz, H-4'), 5.02 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.5 Hz, H-2^1), 4.86 (dd, 1 H, H-3'), 4.34 (d, 1 H, H-1'), and 2.20–1.90 (6 s, 18 H, 6 OAc).

The next fractions contained **19**, slightly contaminated by **30**; crystallization from ethanol afforded pure **19** (874 mg, 64%), m.p. 203–205°, $[\alpha]_D$ +20.5° (*c* 0.83, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.90–7.70 (m, 8 H, Phth), 5.64 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 7.5 Hz, H-3^I), 5.58 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 8.0 Hz, H-3^3), 5.42 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1^3), 5.32 (dd, 1 H, $J_{3,4}$ 3.5 Hz, H-4^4), 5.20 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1^I), 5.12 (dd, $J_{1,2}$ 7.8, $J_{2,3}$ 10.5 Hz, H-2^4), 4.94 (dd, 1 H, H-3^4), 4.85 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.5 Hz, H-2^2), 4.56 (d, 1 H, H-1^4), 4.28 (d, 1 H, H-1^2), 4.19 (dd, 1 H, H-2^3), 4.16 (dd, 1 H, H-2^I), 3.38 (s, 3 H, OMe), and 2.20–1.90 (10 s, 30 H, 10 OAc).

Anal. Calc. for $C_{61}H_{70}N_2O_{33}$: C, 53.90; H, 5.19; N, 2.05; O, 38.85. Found: C, 54.03; H, 4.92; N, 2.28; O, 38.85.

The last fractions contained unreacted **11** (130 mg, 20%). A portion of **19** was *O*-acetylated to give **20**; ^{1}H-n.m.r. ($CDCl_3$): δ 7.90–7.70 (m, 8 H, Phth), 5.70 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 8.5 Hz, H-3^1), 5.60 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 8.5 Hz, H-3^3), 5.35 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1^3), 5.32 (dd, 1 H, $J_{3,4}$ 3.5 Hz, H-4^4), 5.29 (dd, 1 H, $J_{3,4}$ 3.5 Hz, H-4^2), 5.21 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1^I), 5.11 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 10.5 Hz, H-2^4), 4.97 (dd, 1 H, H-3^4), 4.78 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.5 Hz, H-2^2), 4.59 (d, 1 H, H-1^4), 4.34 (d, 1 H, H-1^2), 3.38 (s, 3 H, OMe), and 2.20–1.90 (11 s, 33 H, 11 OAc).

Methyl O-*β-*D-*galactopyranosyl-(1→4)*-O-*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1→3)*-O-*β-*D-*galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-*D-*glucopyrano-*

side (**22**). — A solution of **19** (136 mg, 0.1 mmol) in methanol (10 mL) and 1,4-dioxane (3 mL) was treated with M sodium methoxide in methanol (0.1 mL) for 5 h at room temperature. The base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin, the resin was filtered off and washed with methanol, and the combined filtrate and washings were evaporated. The residue was dissolved in ethanol (10 mL) containing hydrazine hydrate (0.2 mL), and the solution was boiled for 6 h under reflux and then evaporated. The dried residue was *N,O*-acetylated overnight at room temperature with acetic anhydride (5 mL) and pyridine (5 mL). The excess of anhydride was destroyed by addition of methanol, the solvents were evaporated off, and the residue was purified by column chromatography (1:1 dichloromethane–acetone) to give the *N,O*-acetylated tetrasaccharide **21** (88 mg, 72%); ^{1}H-n.m.r. ($CDCl_3$): δ 5.75 (d, 1 H, $J_{2,NH}$ 9.5 Hz, NH), 5.52 (d, 1 H, $J_{2,NH}$ 8.5 Hz, NH), 5.35 (dd, 1 H, $J_{3,4}$ 2.5 Hz, H-4^4), 5.32 (dd, 1 H, $J_{3,4}$ 3.0 Hz, H-4^2), 4.97 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-3^4), 4.68 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^3), 4.54 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^4), 4.39 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^1), 4.35 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1^2), 3.46 (s, 3 H, OMe), and 2.20–1.90 (13 s, 39 H, 13 OAc).

A solution of **21** (88 mg) in methanol (5 mL) was treated for 48 h at room temperature with M sodium methoxide in methanol (0.3 mL). After the usual workup, the residue crystallized from methanol to give the free tetrasaccharide **22** (47 mg, 80%), m.p. 259–261°, $[\alpha]_D$ −10° (*c* 1.02, water); ^{1}H-n.m.r. (D_2O): δ 4.71 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1^3), 4.50–4.45 (m, 3 H, H-1^1,1^2,1^4), 4.17 (dd, 1 H, $J_{3,4}$ 3.0 Hz, H-4^2), and 3.52 (s, 3 H, OMe).

Anal. Calc. for $C_{29}H_{50}N_2O_{21}\cdot 3H_2O$: C, 42.64; H, 6.91; N, 3.43; O, 47.01. Found: C, 42.84; H, 7.06; N, 3.37; O, 46.73.

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*(2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)-1,3,6-tri*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranose* (**23**). — To a cold solution of methyl glycoside **19** (680 mg, 0.5 mmol) in acetic anhydride (20 mL) and acetic acid (4 mL) was added cold 1:10 (v/v). conc. H_2SO_4–acetic acid (1.5 mL). The mixture was kept overnight at 0–5°, and then diluted with dichloromethane and poured onto ice with stirring. The dichloromethane extract was washed successively with water, saturated aqueous $KHCO_3$, and water, and evaporated to give a residue (710 mg) which was used for the next step. A portion was chromatographed on silica gel with 2:1 toluene–ethyl acetate as eluent to give the pure *β*-D-acetate **23** which crystallized from ethanol, m.p. 157–158°, $[\alpha]_D$ +21.5° (*c* 0.56, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.90–7.70 (m, 8 H, Phth), 6.42 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1^1), 5.69 (2 dd, 2 H, $J_{2,3}$ 10.5, $J_{3,4}$ 8.0 Hz, H-3^1, 3^3), 5.34 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^3), 5.32 (dd, 1 H, $J_{3,4}$ 3.5 Hz, H-4^4), 5.28 (dd, 1 H, $J_{3,4}$ 3.5 Hz, H-4^2), 5.12 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.5 Hz, H-2^4), 4.96 (dd, 1 H, H-3^4), 4.77 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.5 Hz, H-2^2), 4.58 (d, 1 H, H-1^4), and 4.32 (d, 1 H, H-1^2).

Anal. Calc. for $C_{64}H_{72}N_2O_{35}$: C, 53.78; H, 5.08; N, 1.96; O, 39.18. Found: C, 53.57; H, 5.24; N, 2.15; O, 39.17.

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-*(2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)*-O-*(3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyra-*

nosyl)-(1→3)-O-*(2,6-di*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)-3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranoside* (**31**). — A solution of crude acetate **23** (500 mg, 0.35 mmol) in acetyl chloride (15 mL) was treated with conc. HCl (1.5 mL) for 24 h at room temperature in a sealed tube. After dilution with dichloromethane, t.l.c. (1:2 toluene–ethyl acetate) showed a major compound (R_F 0.55) and some unreacted acetate **23** (R_F 0.48). The solvent was evaporated and the remaining traces of solvent codistilled with toluene. The ^{1}H-n.m.r. spectrum of the crude residue showed a signal at δ 6.10 (d, $J_{1,2}$ 9.0 Hz) assigned to H-1 of the β-D-chloride **24**.

A mixture of diol **11** (332 mg, 0.5 mmol), 2,4,6-trimethylpyridine (103 mg, 0.85 mmol), silver triflate (206 mg, 0.8 mmol), and powdered molecular sieves 4 A (1 g) in dry dichloromethane (30 mL) was stirred at room temperature for 30 min, and then cooled to −35° under N_2. A solution of crude chloride **24** (913 mg, 0.65 mmol) in dry dichloromethane (15 mL) was added, and the mixture was stirred for 90 min at −35°, and then for 16 h at room temperature. T.l.c. (2:1 ethyl acetate–toluene) showed traces of unreacted chloride **24** (R_F 0.55); its contaminant **23** (R_F 0.48), a product arising from hydrolysis of **24** (R_F 0.30); a major new compound (R_F 0.26); and some unreacted diol **11** (R_F 0.12). The mixture was worked up as described for **19**. Column chromatography with 2:1 ethyl acetate–toluene as eluent gave **31** (R_F 0.26; 405 mg, 40%) which crystallized from ethanol, m.p. 182–184°, $[\alpha]_D$ +16° (*c* 0.44, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.90–7.70 (m, 12 H, Phth), 5.67 (dd, 1 H, H-3^I), 5.57 (dd, 1 H, H-3^5), 5.54 (dd, 1 H, H-3^3), 5.39 (d, 1 H, H-1^3), 5.33 (d, 1 H, H-1^5), 5.30 (dd, 1 H, H-4^6), 5.27 (dd, 1 H, H-4^4), 5.18 (d, 1 H, H-1^I), 5.11 (dd, 1 H, H-2^6), 4.95 (dd, 1 H, H-3^6), 4.84 (dd, 1 H, H-2^2), 4.76 (dd, 1 H, H-2^4), 4.56 (d, 1 H, H-1^6), 4.34 (d, 1 H, H-1^4), 4.22 (d, 1 H, H-1^2), and 3.38 (s, 1 H, OMe).

Anal. Calc. for $C_{91}H_{103}N_3O_{49}$: C, 54.03; H, 5.13; N, 2.08; O, 38.76. Found: C, 53.80; H, 5.30; N, 1.88; O, 38.46.

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→3)*-O-*β*-D-*galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β*-D-*glucopyranoside* (**33**). — Compound **31** (405 mg, 0.2 mmol) was *O*-deacetylated, hydrazinolyzed, and *N,O*-acetylated as described for **19** to give **32** which was purified by column chromatography with 2:3 dichloromethane–acetone as eluent (250 mg, 69%); ^{1}H-n.m.r. ($CDCl_3$): δ 5.76, 5.53, and 5.48 (3 d, 3 H, 3 NH), 5.32 (dd, 1 H, H-4^6), 5.29 (dd, 1 H, H-4^4), 5.28 (dd, 1 H, H-4^2), 4.96 (dd, 1 H, H-3^6), 4.67 (d, 1 H, H-1^5), 4.63 (d, 1 H, H-1^3), 4.52 (d, 1 H, H-1^6), 4.38 (d, 1 H, H-1^I), 4.34 (d, 1 H, H-1^2), 4.32 (d, 1 H, H-1^4), 4.14–4.05 (m, 3 H, H-2^I,2^3,2^5), and 3.43 (s, 3 H, OMe).

Compound **32** (250 mg, 0.14 mmol) was O-deacetylated as described for **21**. Solvent removal left a solid which was applied to a Sephadex G-25 column and eluted with water. Freeze-drying gave the free hexasaccharide **33** as a white solid (125 mg, 80%), $[\alpha]_D$ −6° (*c* 0.97, water), ^{1}H-n.m.r. (D_2O): δ 4.73 (2 d, 2 H, H-1^3,1^5), 4.52–4.47 (m, 4 H, H-1^I,1^2,1^4,1^6), 4.19 (2 dd, 2 H, H-4^2,4^4), 3.54 (s, 3 H, OMe), and 2.06 (s, 9 H, 3 NAc).

Anal. Calc. for $C_{43}H_{73}N_3O_{31}\cdot 3\,H_2O$: C, 43.68; H, 6.73; N, 3.55; O, 46.02. Found: C, 43.68; H, 7.03; N, 3.55; O, 45.60.

Benzyl 3,6-di-O-*acetyl-2-deoxy-2-phthalimido-4*-O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-

*galactopyranosyl)-β-*D*-glucopyranoside* (**12**). — A mixture of **4** (7.66 g, 10 mmol), benzyl alcohol (2 mL, 19 mmol), and powdered molecular sieves (4A, 20 g) in dry dichloromethane (200 mL) was stirred under N_2 for 1 h at room temperature, and then cooled to −20°. A 0.5M solution of trimethylsilyl triflate in dichloromethane (20 mL, 10 mmol) was rapidly added. Cooling was discontinued and the mixture was stirred for 2 h at room temperature. The acid was neutralized with triethylamine, and the solution filtered and washed with water. Evaporation gave a syrup which crystallized from ethyl acetate–hexane (7.40 g, 91%), m.p. 185–186°, $[\alpha]_D$ −9° (*c* 2.1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.80–7.68 (m, 4 H, Phth), 7.07 (m, 5 H, Ph), 5.73 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 8.5 Hz, H-3), 5.35 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 5.31 (dd, 1 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 1 Hz, H-4'), 5.09 (dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 10.5 Hz, H-2'), 4.94 (dd, 1 H, H-3'), 4.80 (d, 1 H, *J* 12 Hz, CH_2Ph), 4.52 (d, 1 H, H-1'), and 1.92, 1.98, 2.05, 2.09, 2.15, 2.19 (6 s, 18 H, 6 OAc): lit.[20] m.p. 187–188°, $[\alpha]_D^{25}$ −9.6° (*c* 2.5, chloroform).

*Benzyl 2-deoxy-4-*O*-β-*D*-galactopyranosyl-2-phthalimido-β-*D*-glucopyranoside* (**13**). — To a solution of **12** (8 g, 10 mmol) in methanol (150 mL) and 1,4-dioxane (100 mL) was added a solution of M sodium methoxide in methanol (5 mL). The mixture was stirred for 3 h at room temperature, and the base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin. Filtration and evaporation gave a solid which crystallized from methanol, m.p. 246–248°, $[\alpha]_D$ −66° (*c* 1.04, pyridine).

Anal. Calc. for $C_{27}H_{31}NO_{12}$: C, 57.75; H, 5.56; N, 2.50; O, 34.19. Found C, 57.73; H, 5.54; N, 2.47; O, 33.62.

*Benzyl 3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-*D*-galactopyranosyl)-2-phthalimido-β-*D*-glucopyranoside* (**14**). — A mixture of **13** (5 g, 9 mmol) and 4-toluenesulfonic acid (0.7 g, 3.7 mmol) in acetone (1000 mL) was boiled under reflux for 4 h. T.l.c. (9:1 ether–methanol) showed two new compounds, the 3',4'-*O*-isopropylidene derivative at R_F 0.56 and its 4',6'-*O*-isopropylidene isomer at R_F 0.12. The mixture was cooled and the acid neutralized with solid K_2CO_3. After filtration and evaporation, column chromatography (19:1 ether–methanol) gave the faster migrating product (3:21 g, 60%) which was *O*-acetylated overnight with pyridine (30 mL) and acetic anhydride (30 mL). Removal of the solvents gave a residue which crystallized from ethanol, m.p. 141–143°, $[\alpha]_D$ +9.5° (*c* 1.05, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.78–7.68 (m, 4 H, Phth), 7.08 (m, 5 H, Ph), 5.72 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 8 Hz, H-3), 5.35 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.85 (dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 7.5 Hz, H-2'), 4.80 (d, 1 H, *J* 12 Hz, CH_2Ph), 4.50 (d, 1 H, CH_2Ph), 4.39 (d, 1 H, H-1'), 2.16, 2.12, 2.10, 1.90 (4 s, 12 H, 4 OAc), 1.52 and 1.31 (2 s, 6 H, CMe_2).

Anal. Calc. for $C_{38}H_{43}NO_{16}$: C, 59.29; H, 5.63; N, 1.82; O, 33.26. Found: C, 59.13; H, 5.74; N, 1.88; O, 33.19.

*3,6-Di-*O*-acetyl-2-deoxy-4-*O*-(2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-*D*-galactopyranosyl)-2-phthalimido-β-*D*-glucopyranose* (**15**). — A mixture of **14** (3 g, 3.9 mmol), cyclohexene (3.5 mL, 135 mmol), and 5% Pd–C (3 g) in ethanol (250 mL) was boiled under reflux for 1 h and then cooled, filtered, and evaporated. The residue crystallized from ethanol to give **15** (2.4 g, 90%), m.p. 138–140°, $[\alpha]_D$ +57° (*c* 0.78, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ7.80–7.69 (m, 4 H, Phth), 5.76 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 8 Hz, H-3), 5.65

(t, 1 H, $J_{1,2} = J_{1,OH}$ 7 Hz, H-1), 4.84 (t, 1 H, $J_{1',2'} = J_{2',3'}$ 7 Hz, H-2'), 4.50 (d, 1 H, $J_{6a,6b}$ 12 Hz, H-6a), 4.39 (d, 1 H, H-1'), 3.68 (d, 1 H, OH), 2.14 (s, 6 H, 2 OAc), 2.09, 1.92 (2 s, 6 H, 2 OAc), 1.51 and 1.30 (2 s, 6 H, CMe_2).

Anal. Calc. for $C_{31}H_{37}NO_{16}$: C, 54.78; H, 5.49; N, 2.06; O, 37.67. Found: C, 54.58; H, 5.71; N, 2.12; O, 37.45.

3,6-Di-O-acetyl-2-deoxy-4-O-(2,6-di-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-2-phthalimido-1-O-trichloroacetimidoyl-β-D-glucopyranose (**16**). — A mixture of **15** (1.7 g, 2.5 mmol), anhydrous K_2CO_3 (10 g, 72 mmol), and trichloroacetonitrile (2.5 mL, 25 mmol) in dry dichloromethane (100 mL) was stirred for 4 h at room temperature under N_2, and then filtered over Celite and evaporated. The residue was chromatographed in a short column of silica gel; 17:3 dichloromethane–ethyl acetate eluted **16** which crystallized from ether–hexane (1.36 g, 66%), m.p. 143–145°, $[\alpha]_D$ +58° (*c* 0.55, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 8.63 (s, 1 H, NH), 7.84–7.71 (m, 4 H, Phth), 6.61 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 5.86 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 8.5 Hz, H-3), 4.88 (t, 1 H, $J_{1',2'} = J_{2',3'}$ 7 Hz, H-2'), 4.43 (d, 1 H, H-1'), 2.14, 2.13, 2.09, 1.93 (4 s, 12 H, 4 OAc), 1.52 and 1.31 (2 s, 6 H, CMe_2).

Anal. Calc. for $C_{33}H_{37}Cl_3N_2O_{16}$: C, 48.10; H, 4.52; N, 3.41; O, 31.07. Found: C, 48.30; H, 4.66; N, 3.29; O, 31.32.

Benzyl 3,6-di-O-acetyl-2-deoxy-4-O-(2,6-di-O-acetyl-β-D-galactopyranosyl)-2-phthalimido-β-D-glucopyranoside (**17**). — To a solution of **14** (1.14 g, 1.5 mmol) in dichloromethane (45 mL) was added 99% aqueous trifluoroacetic acid (5 mL), and the mixture was kept for 1 h at room temperature, and then poured onto a cold, saturated aqueous solution of $KHCO_3$. The dichloromethane extract was washed with water, and then evaporated to give a residue which crystallized from ether–methanol (0.84 g, 78%), m.p. 198–200°, $[\alpha]_D$ −13° (*c* 0.91, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.82–7.72 (m, 4 H, Phth), 7.10 (m, 5 H, Ph), 5.76 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 8 Hz, H-3), 5.38 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.90 (dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 10 Hz, H-2'), 4.84 (d, 1 H, *J* 12 Hz, CH_2Ph), 4.52 (d, 1 H, CH_2Ph), 4.39 (d, 1 H, H-1'), 3.20 (d, 1 H, *J* 6 Hz, OH), and 2.16, 2.14, 2.10, 1.88 (4 s, 12 H, 4 OAc).

Anal. Calc. for $C_{35}H_{35}NO_{16}$: C, 57.61; H, 5.39; N, 1.92; O, 35.08. Found: C, 57.77; H, 5.34; N, 1.92; O, 35.14.

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→3)-O-(2,6-di-O-acetyl-β-D-galactopyranosyl)-(1→4)-3,6-di-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**25**). — A solution of **17** (0.73 g, 1 mmol) and **16** (0.99 g, 1.2 mmol) in dry dichloromethane (50 mL) was stirred for 1 h at room temperature under N_2 in the presence of powdered molecular sieves (4A, 5 g), and then cooled to −40°. A 0.5M solution of trimethylsilyl triflate in dichloromethane (3 mL, 1.5 mmol) was added. After 15 min at −40°, t.l.c. (7:3 ethyl acetate–toluene) showed a major compound at R_F 0.61 and traces of unreacted **17** (R_F 0.33). The acid was neutralized with a 10% solution of triethylamine in dichloromethane at −40°, and then brought to room temperature, filtered, and washed with water. After evaporation, the residue was chromatographed on silica gel. Elution with 1:1 ethyl acetate–toluene afforded crystalline **25** (1.11 g, 78%),

m.p. 229–231°, $[\alpha]_D$ +12° (*c* 0.56, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.88–7.69 (m, 8 H, Phth), 7.07 (m, 5 H, Ph), 5.62 (2 dd, 2 H, $J_{2,3}$ 10.5, $J_{3,4}$ 8.5 Hz, H-3^1,3^3), 5.46, 5.29 (2 d, 2 H, $J_{1,2}$ 8 Hz, H-1^1,1^3), 4.87 (2 dd, 2 H, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz, H-2^2,2^4), 4.76 (d, 1 H, *J* 12 Hz, C*H*$_2$Ph), 4.45 (d, 1 H, C*H*$_2$Ph), 4.40 (d, 2 H, H-1^2,1^4), 2.66 (bd, 1 H, OH), 2.13–1.85 (m, 21 H, 7 OAc), 1.52 (s, 3 H, CMe_2), 1.47 (s, 3 H, OAc), and 1.31 (s, 3 H, CMe_2).

Anal. Calc. for $C_{66}H_{74}N_2O_{31}$: C, 56.98; H, 5.36; N, 2.01; O, 35.65. Found: C, 56.70; H, 5.50; N, 2.19; O, 35.53.

Acetylation of **25** with 1:1 acetic anhydride–pyridine for 48 h at room temperature gave **26**, m.p. 143–145°, $[\alpha]_D$ +20° (*c* 0.3, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.80–7.69 (m, 8 H, Phth), 7.05 (m, 5 H, Ph), 5.68, 5.61 (2 dd, 2 H, $J_{2,3}$ 10.5, $J_{3,4}$ 8.5 Hz, H-3^1,3^3), 5.35, 5.31 (2 d, 2 H, $J_{1,2}$ 8 Hz, H-1^1,1^3), 5.30 (dd, 1 H, $J_{3,4}$ 3, $J_{4,5}$ 1 Hz, H-4^2), 4.89 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 6 Hz, H-2^4), 4.79 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz, H-2^2), 4.78 (d, 1 H, *J* 12 Hz, C*H*$_2$Ph), 4.47 (d, 1 H, C*H*$_2$Ph), 4.43 (d, 1 H, H-1^4), 4.33 (d, 1 H, H-1^2), 2.18–1.82 (m, 27 H, 9 OAc), 1.53 and 1.31 (2 s, 6 H, CMe_2).

Anal. Calc. for $C_{68}H_{76}N_2O_{32}$: C, 56.98; H, 5.34; N, 1.95; O, 35.72. Found: C, 56.78; H, 5.48; N, 2.13; O, 35.58.

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→3*)-O-(*2,6-di*-O-*acetyl-β*-D-*galactopyranosyl*)-(*1→4*)-*3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranose* (**27**). — A mixture of **25** (0.6 g, 0.42 mmol), cyclohexene (2 mL, 20 mmol), and 5% Pd–C (0.6 g) in ethanol (150 mL) was boiled for 1 h, and then cooled, filtered, and evaporated. The residue was purified by column chromatography. Elution with 4:1 ethyl acetate–hexane gave **27** (0.5 g, 90%), m.p. 153–156°, $[\alpha]_D$ +35° (*c* 0.45, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.83–7.69 (m, 8 H, Phth), 5.72–5.56 (m, 3 H, H-1^1,3^1,3^3), 5.36 (d, 1 H, $J_{1,2}$ 8 Hz, H-1^3), 5.30 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1 Hz, H-4^2), 4.92–4.75 (m, 3 H, H-2^2,2^4,6^1), 4.44 (d, 1 H, $J_{1,2}$ 8 Hz, H-1^4), 4.35 (d, 1 H, $J_{1,2}$ 8 Hz, H-1^2), 2.18, 2.12, 2.11, 2.10, 2.09, 2.08, 1.88, 1.84, 1.82 (9 s, 27 H, 9 OAc), 1.52 and 1.31 (2 s, 6 H, CMe_2).

Anal. Calc. for $C_{61}H_{70}N_2O_{32}$: C, 54.55; H, 5.25; N, 2.08; O, 38.12. Found: C, 54.18; H, 5.47; N, 1.96; O, 38.30.

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→3*)-O-(*2,6-di*-O-*acetyl-β*-D-*galactopyranosyl*)-(*1→4*)-*3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-1*-O-*trichloroacetimidoyl-β*-D-*glucopyranose* (**28**). — A mixture of **27** (0.7 g, 0.52 mmol), anhydrous K_2CO_3 (3 g, 21 mmol), and trichloroacetonitrile (1 mL, 10 mmol) in dry dichloromethane (100 mL) was stirred for 4 h at room temperature under N_2, and then filtered over Celite and evaporated. The residue was chromatographed on a short column of silica gel; 7:3 dichloromethane–ethyl acetate eluted **28** which crystallized from ether–hexane (0.51 g, 66%), m.p. 141–143°, $[\alpha]_D$ + 56° (*c* 0.53, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 8.63 (s, 1 H, NH), 7.83–7.69 (m, 8 H, Phth), 6.55 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1^1), 5.80–5.64 (m, 2 H, H-3^1,3^3), 5.35 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1^3), 5.30 (dd, 1 H, $J_{3,4}$ 3, $J_{4,5}$ 1 Hz, H-4^2) 4.42 (d, 1 H, $J_{1,2}$ 8 Hz, H-1^2), 4.34 (d, 1 H, $J_{1,2}$ 8 Hz, H-1^2), 2.17–1.81 (m, 27 H, 9 OAc), 1.51 and 1.30 (2 s, 6 H, CMe_2).

Anal. Calc. for $C_{63}H_{70}Cl_3N_3O_{32}$: C, 50.87; H, 4.74; N, 2.82; O, 34.42. Found: C, 50.62; H, 4.74; N, 2.91; O, 34.21.

Benzyl O-*(2,6-di*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)*-O-*(3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)-(1→3)*-O-*(2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)-3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranoside* (**29**). — To a solution of **26** (1 g, 0.7 mmol) in dichloromethane (45 mL) was added 99% aqueous trifluoroacetic acid (5 mL), and the mixture was kept for 30 min at room temperature, and then poured onto a cold, saturated aqueous solution of $KHCO_3$. The dichloromethane extract was washed with water, and then evaporated to give a residue which crystallized from ethanol–hexane (0.82 g, 84%), m.p. 150–152°, $[\alpha]_D$ +4° (*c* 0.71, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.80–7.69 (m, 8 H, Phth), 7.03 (m, 5 H, Ph), 5.72, 5.61 (2 dd, 2 H, H-3^I,3^3), 5.35–5.28 (m, 3 H, H-1^I,1^3,4^2), 4.40 (d, 1 H, $J_{1,2}$ 8 Hz, H-1^4), 4.33 (d, 1 H, $J_{1,2}$ 8 Hz, H-1^2), and 2.16–1.78 (m, 27 H, 9 OAc).

Anal. Calc. for $C_{65}H_{72}N_2O_{32}$: C, 56.03; H, 5.23; N, 2.01; O, 36.75. Found: C, 55.78; H, 5.23; N, 2.05; O, 36.45.

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→3)*-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→3)*-O-*(2,6-di*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)*-O-*(3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)-(1→3)*-O-*(2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)-3,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranoside* (**34**). — A solution of **29** (0.70 g, 0.5 mmol) and **28** (0.89 g, 0.6 mmol) in dry dichloromethane (40 mL) was stirred for 1 h at room temperature under N_2 in the presence of powdered molecular sieves (4A, 4 g), and then cooled to −40°. A 0.5M solution of trimethylsilyl triflate in dichloromethane (1.5 mL, 0.75 mmol) was added. After 30 min at −40°, t.l.c. (2:1 ethyl acetate–toluene) showed a major compound at R_F 0.29 and traces of unreacted **29** (R_F 0.13). The acid was neutralized with a 10% solution of triethylamine in dichloromethane at −40°, brought to room temperature, filtered, and washed with water. After evaporation, the residue was chromatographed on silica gel. Elution with 2:1 ethyl acetate–toluene gave **34** (0.68 g, 50%), m.p. 252–255°, $[\alpha]_D$ −10° (*c* 0.7, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.84–7.70 (m, 16 H, Phth), 7.06 (m, 5 H, Ph), 5.72–5.53 (m, 4 H, H-3^I,3^3,3^5,3^7), 5.42, 5.35, 5.31, 5.25 (4 d, 4 H, $J_{1,2}$ 8 Hz, H-1^I,1^3,1^5,1^7), 5.25 (m, 2 H, H-4^2,4^6), 2.18–1.79 (51 H, 17 OAc), 1.52 (s, 3 H, CMe_2), 1.44 (s, 3 H, OAc), and 1.31 (s, 3 H, CMe_2).

Anal. Calc. for $C_{126}H_{140}N_4O_{63}$: C, 55.67; H, 5.19; N, 2.06; O, 37.08. Found: C, 55.42; H, 5.37; N, 2.06; O, 36.87.

Acetylation with 1:1 (v/v) acetic anhydride–pyridine for 48 h at room temperature gave **35**; ^{1}H-n.m.r. ($CDCl_3$): δ 7.85–7.71 (m, 16 H, Phth), 7.09 (m, 5 H, Ph), 5.73–5.53 (m, 4 H, H-3^I,3^3,3^5,3^7), 5.39–5.26 (m, 7 H, H-1^I,1^3,1^5,1^7,4^2,4^4,4^6), 2.18–1.77 (57 H, 19 OAc), 1.52 and 1.31 (2 s, 6 H, CMe_2).

Methyl 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→3)*-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→3)*-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→3)-(2,4,6-tri*-O-*acetyl-*

β-D-galactopyranosyl)-(1→4)-3,6-di-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**36**). — A mixture of **35** (0.55 g, 0.2 mmol), cyclohexene (1.5 mL, 15 mmol), and 5% Pd–C (0.5 g) in ethanol (150 mL) was boiled for 90 min under reflux, then cooled, filtered, and evaporated. T.l.c. (4:1 ethyl acetate–hexane) showed the product to be pure (R_F 0.21) and free of starting material (R_F 0.44). The residue was dissolved in dry dichloromethane (50 mL), and 2,4,6-trimethylpyridine (0.16 mL, 1.2 mmol), followed by methanesulfonic anhydride (0.11 g, 0.6 mmol) were added, and the mixture was kept for 3 h at room temperature. Methanol (4 mL) was added, the mixture was stirred for 24 h, and then washed with water, evaporated, and chromatographed on silica gel; 2:1 (v/v) ethyl acetate–toluene eluted **36** (0.35 g, 65%), which crystallized from ethanol, m.p. 240–242°, $[\alpha]_D$ +16° (*c* 0.9, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.87–7.72 (m, 16 H, Phth), 5.72–5.53 (m, 4 H, H-3^1,3^3,3^5,3^7), 5.38–5.19 (m, 7 H, H-1^1,1^3,1^5,1^7,4^2,4^4,4^6), 3.38 (s, 3 H, OMe), 2.20–1.81 (57 H, 19 OAc), 1.53, and 1.32 (2 s, 6 H, CMe_2).

Anal. Calc. for $C_{122}H_{138}N_4O_{64}$: C, 54.58; H, 5.18; N, 2.19; O, 38.15. Found: C, 54.44; H, 5.02; N, 2.36; O, 38.20.

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→3)-O-β-D-galactopyranosyl-(1→4)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**38**). — To a solution of **36** (0.3 g, 0.11 mmol) in dichloromethane (54 mL) was added 99% aqueous trifluoroacetic acid (6 mL), and the mixture was kept for 1 h at room temperature, then poured over a cold, saturated aqueous solution of $KHCO_3$. The dichloromethane solution was separated, washed with water, and then evaporated. The residue was dissolved in 2:1 methanol–1,4-dioxane (90 mL), and a M solution of sodium methoxide in methanol (0.9 mL) was added, and the solution was kept for 6 h at room temperature. The base was neutralized with Dowex 50 (H^+) cation-exchange resin, the suspension filtered, and the filtrate evaporated. The residue was dissolved in ethanol (90 mL), hydrazine hydrate (12 mL) was added, and the mixture was heated at reflux overnight. After evaporation, the residue was *N,O*-acetylated in 1:1 (v/v) pyridine–acetic anhydride (60 mL) for 72 h at room temperature. After removal of the solvents, the residue was chromatographed on silica gel with 2:3 dichloromethane–acetone as eluent to give **37** (0.12 g, 45%); ^{1}H-n.m.r. ($CDCl_3$): δ 5.72 (d, 1 H, $J_{2,NH}$ 9.5 Hz, NH), 5.51–5.43 (m, 3 H, 3 NH), 5.36–5.32 (m, 4 H, H-4^2,4^4,4^6,4^8), 3.46 (s, 3 H, OMe), and 2.16–1.89 (m, 75 H, 25 Ac).

Compound **37** (0.12 g, 0.05 mmol) was dissolved into 1:1 1,4-dioxane–methanol (100 mL), and a M solution of sodium methoxide in methanol (1.5 mL) was added. The solution was kept for 5 days at room temperature, the base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin, the solution was concentrated after filtration, and the residue was purified by column chromatography on silica gel with 1:1:1 ethyl acetate–2-propanol–water as eluent. The fractions were analyzed by t.l.c. in the same solvent mixture; those containing pure **38** (R_F 0.53) were combined and evaporated. Gel filtration through Sephadex G-25 removed some soluble silicic acid. The purity of **38** was verified by analytical h.p.l.c. (C-18 Waters) by elution with water

(R_T 31.14 min). Freeze-drying of the desalted fraction gave **38** (50 mg, 67%) as a white solid, $[\alpha]_D$ −21° (*c* 0.37, water); ^{1}H-n.m.r. (D_2O): δ 4.732, 4.729, and 4.726 (3 d, 3 H, $J_{1,2}$ 8.0 Hz, H-1^3,1^5,1^7), 4.511, 4.496, and 4.489 (3 d, 5 H, $J_{1,2}$ 8.0 Hz, H-1^1,1^2,1^4,1^6,1^8), 4.191 (dd, 3 H, $J_{3,4}$ 3.0 Hz, H-4^2,4^4,4^6), 3.964 (dd, 1 H, $J_{3,4}$ 3.0 Hz, H-4^8), 3.534 (s, 3 H, OMe), and 2.064 (s, 12 H, 4 NHAc).

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