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Synthesis of a peripheral trisaccharide sequence of lutropin, a pituitary glycoprotein hormone; use of chitobiose as a key starting material

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ABSTRACT

A chitobiose derivative, methyl *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside, was derived from the corresponding *N*-acetyl derivative and this was converted into the glycosyl bromide (**5**). Glycosidation reaction between **5** and methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside in the presence of silver trifluoromethanesulfonate gave a β-D-linked trisaccharide derivative. Replacement of the *N,N*-phthaloyl group by acetyl groups resulted in a product that was converted into methyl *O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**11**) by use of a few reaction steps. The 4^3-hydroxyl group of **11** was methanesulfonylated, and the product subjected to S_N2 replacement with acetate anion, to give the D-galactosamine-containing trisaccharide derivative (**12**). After basic hydrolysis of **12**, the 4^3-hydroxyl group was sulfated, and all benzyl groups were removed by hydrogenolysis, giving methyl *O*-(2-acetamido-2-deoxy-4-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-α-D-mannopyranoside monosodium salt, the methyl α-glycoside derivative of the peripheral trisaccharide sequence of the pituitary glycoprotein hormone lutropin.

INTRODUCTION

Recent studies have mainly focused on the preparation of regioselectively protected di- and tri-saccharides, such as those of cellobiose, maltose, and maltotriose[1–3], and on their use as key starting materials for the synthesis of various bioactive substances[4–8]. Attention was then directed to extension of the range of the oligosaccharides to chitobiose, an amino sugar disaccharide; a preparation of chitobiose octaacetate by regiospecific degradation of chitin, and its derivatization for protection against later manipulation, have been described[9]. We now report a synthesis of a unique trisaccharide residue of the biologically important molecule lutropin, using a chitobiose derivative as the starting material.

The structure of the anionic, asparagine-linked carbohydrate chains of lutropin, one of the pituitary glycoprotein hormones, was recently elucidated by Baenziger *et*

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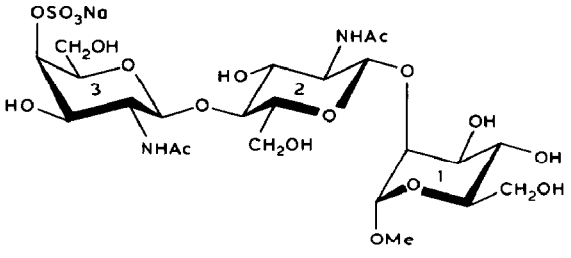
al.[10,11], and found to contain the unusual peripheral sequence $SO_3 \rightarrow 4$-β-GalNAc-(1→4)-β-GlcNAc-(1→2)-α-Man-. To clarify the functional significance of this hormonal glycoprotein, Hindsgaul *et al.*[12] prepared the 8-methoxycarbonyloctyl α-glycoside of this terminally sulfated trisaccharide by repeated glycosidations of monosaccharide units. We, also, completed a synthesis of the methyl α-glycoside (**1**) of this trisaccharide, employing methyl *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**2**) as the key starting-material.

RESULTS AND DISCUSSION

The scheme planned for the synthesis of **1** consisted of three main stages: (*a*) glycosidation reaction between a chitobiose donor and a mannopyranose acceptor, (*b*) inversion of the 4-hydroxyl group of the terminal D-glucosamine residue of the trisaccharide (*i.e.*, configurational change of the terminal constituent from D-*gluco* into D-*galacto*), and (*c*) regioselective mono-*O*-sulfation followed by deblocking. Prior to the glycosidation reaction, the acetyl protective groups of **2**, the precursor to the glycosyl donor, were replaced by the phthaloyl group, because of the advantage of having this (bulky) group at C-2 during formation of the 1,2-*trans*-glycosides. Thus, **2** was converted into methyl *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**3**) in >70% overall yield, after a series of reactions consisting of *O*-deacetylation (sodium methoxide), *N*-deacetylation (hydrazine hydrate), *N*-phthaloylation (phthalic anhydride–triethylamine), and reacetylation. Careful acetolysis of **3** with 100:1 (v/v)[13] acetic anhydride–sulfuric acid resulted in the corresponding β-glycosyl acetate (**4**) as crystals in quantita-



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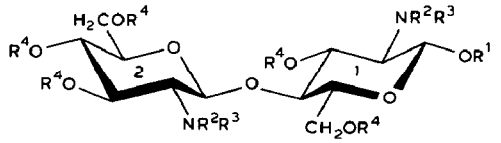
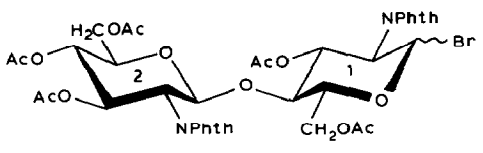


2 $R^1 = CH_3, R^2 = H, R^3 = Ac, R^4 = Ac$
3 $R^1 = CH_3, R^2, R^3 = Phth, R^4 = Ac$
4 $R^1 = Ac, R^2, R^3 = Phth, R^4 = Ac$

tive yield. Compound **4** was treated with titanium tetrabromide to give syrupy glycosyl bromide **5**, which was immediately used, without purification, as the glycosyl donor in the coupling reaction. In parallel with preparation of **5**, the glycosyl acceptor methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**6**) was synthesized according to the literature[14]. Condensation between **5** and **6** was performed in dichloromethane, employing silver trifluoromethanesulfonate, molecular sieves 4A, and 2,4,6-collidine as the catalytic system. The trisaccharide derivative **7** was obtained amorphous in nearly 90% yield (based on the quantity of **4** used). The ^{1}H-n.m.r. spectrum of compound **7** revealed the signal assignable to H-1^2 at δ 5.42 as a doublet with $J_{1^3,2^3}$ 8.5 Hz, indicating that the glycoside bond newly formed had the β configuration. On the basis of the ^{1}H-n.m.r. spectrum and the results of elemental analyses, the glycosidation product was verified to be methyl *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-*O*-(3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**7**). The phthaloyl protecting groups on the N atoms no longer had utility after this stereoselective glycosidation and were disadvantageous because of their lability towards bases; consequently, they were then replaced by acetyl groups. Thus, **7** underwent deacetylation, *N*-dephthaloylation, and reacetylation in one continuous operation, giving the *N*-acetyl trisaccharide derivative **8** in 96% overall yield.

Selective deprotection of the 4^3-hydroxyl group became necessary, in order to carry out its configurational inversion. After Zemplén deacetylation of **8**, the resulting pentahydroxy compound **9** was treated with α,α-dimethoxytoluene in *N*,*N*-dimethylformamide in the presence of a protic acid, and the product was caused to react with benzyl bromide–barium oxide–barium hydroxide, giving methyl *O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-



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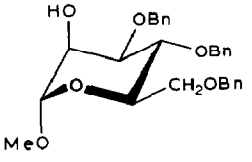

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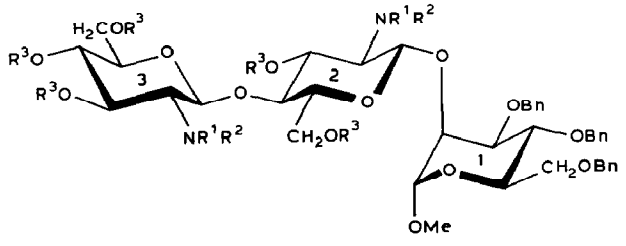
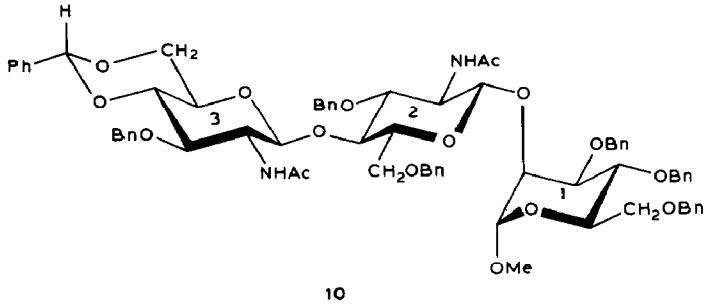


7 R^1, R^2 = Phth, R^3 = Ac
8 R^1 = H, R^2 = Ac, R^3 = Ac
9 R^1 = H, R^2 = Ac, R^3 = H

O-ben-zyl-2-dcoxy-β-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**10**) in almost 90% yield. Selective cleavage of the *O*-benzylidene groups by reduction with borane–trimethylamine–aluminum chloride[15] was successfully performed on **10**, and the expected compound **11** having only one free hydroxyl group (on C-4^3) was obtained. After methanesulfonylation of this group, the resulting 4^3-sulfonate was subjected to S_N2 substitution by treatment with acetate anion in *N,N*-dimethylformamide to give methyl *O*-(2-acetamido-4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**12**). The *galacto* configuration of the nonreducing terminal monosaccharide residue of **12** was confirmed by its giving a ^{1}H-n.m.r. signal, due to H-4^3, appearing at δ 5.56 with $J_{3^3,4^3}$ 2.9 Hz. *O*-Deacetylation of **12** took place on treatment with base, giving the 4^3-hydroxy compound **13** quantitatively.

For sulfation of the 4^3-hydroxyl group, compound **13** was treated with sulfur trioxide–trimethylamine complex[16] in *N,N*-dimethylformamide–pyridine at 40°. After purification by silica gel chromatography, the product was isolated as the monosodium salt (**14**) in >80% yield. The benzyl groups were removed from **14** by hydrogenolysis, using palladium-on-carbon as the catalyst. The product was purified by use of a column of Bio-Gel P-2 and then of an ion-exchange resin, to give in 83% yield a white powder, which was identified as the target compound, methyl *O*-(2-acetamido-2-deoxy-4-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)



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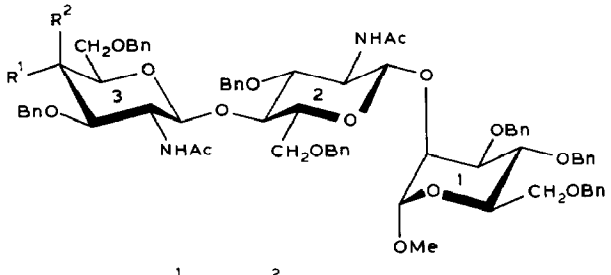

11 R^1 = OH, R^2 = H
12 R^1 = H, R^2 = OAc
13 R^1 = H, R^2 = OH
14 R^1 = H, R^2 = OSO_3Na

-α-D-mnnopyranoside monosodium salt (**1**), from the data provided by its ^{1}H- and ^{13}C-n.m.r. spectra and the satisfactory results of elemental analysis.

In conclusion, one of the trisaccharide structures unique to pituitary glycoprotein hormones has been prepared through use of only one glycosidation reaction, owing to the employment of a chitobiose derivative as a key building-block.

EXPERIMENTAL

General methods. — Melting points were determined with a Yamato micro melting-point apparatus, and are uncorrected. Optical rotations were determined with a Perkin–Elmer Model 241 MC polarimeter and a JASCO DIP-370 digital polarimeter. Reactions were monitored by thin-layer chromatography (t.l.c.) on precoated plates of Silica Gel $60F_{254}$ (0.25 mm; E. Merck, Darmstadt, Germany). Solvents extracts were dried with anhydrous magnesium sulfate, and solutions were evaporated under diminished pressure below 40°. I.r. spectra were recorded with a Shimazu IR-27 spectrophotometer, using potassium bromide disks or thin films on KRS (thallium bromide-iodide). ^{1}H-N.m.r. spectra were recorded at 400 or 500 MHz, with JEOL JNM-GX 400 or 500 spectrometers, using tetramethylsilane as the internal standard and chloroform-*d* as the solvent unless otherwise specified. ^{1}H-N.m.r. spectral data for the sugar CH and CH_2 protons are tabulated in Tables I and II (except the data for **1**). ^{13}C-N.m.r. spectra for solutions in deuterium oxide were recorded at 100 MHz with a JNM-GX 400 spectrometer.

Methyl O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**3**). — To a solution of **2** (9.9 g, 15.3 mmol) in dry methanol (250 mL) was added sodium methoxide (300 mg), and the mixture was stirred for 80 min at room temperature, treated with Dowex 50W X-8 (H^+) ion-exchange resin, and filtered. The filtrate was evaporated to dryness, the residue was dissolved in hydrazine hydrate (130 mL), and the mixture was stirred for 24 h at 120°, diluted with water (100 mL), evaporated to dryness, the residue dissolved in methanol (200 mL), and the solution was treated with phthalic anhydride (2.22 g, 15.3 mmol) and triethylamine (1.54 g, 15.3 mmol) for 3 h at room temperature. Additional aliquots of triethylamine (1.54 g, 15.4 mmol) and phthalic anhydride (2.22 g, 15.4 mmol) were added and stirring was continued for 2 h at 50°. The mixture was made neutral with Dowex 50W X-8 (H^+) resin, filtered, and the filtrate evaporated to dryness. The residue was treated with pyridine (150 mL) and acetic anhydride (100 mL) for 16 h at room temperature, the solution was poured into ice-cold water, and the mixture extracted with chloroform (3 × 100 mL). The extracts were combined, washed successively with M H_2SO_4, aq. $NaHCO_3$, and cold water, dried, and evaporated. The residual syrup was chromatographed on silica gel using 30:1 (v/v) toluene–ethyl acetate as the eluant to give **3**, crystallized from ethanol (9.0 g; 71% from **2**); m.p. 152–153°, $[\alpha]_D^{23}$ +23.5° (*c* 0.163, chloroform); R_F 0.45 (2:1 benzene–ethyl acetate).

Anal. Calc. for $C_{39}H_{40}N_2O_{18}\cdot 0.5\ H_2O$: C, 56.18; H, 4.96; N, 3.36. Found: C, 56.26; H, 4.85; N, 3.39.

TABLE I

^{1}H-N.m.r. data[a]

Atom	*Chemical shift (δ) and multiplicity*							
	3	**4**	**7**	**8**	**10**	**11**	**12**	**13**
H-1^1	5.20d	6.42d	4.43d	4.64d	4.68brs	4.78brd	4.68d	4.69brd
H-2^1	4.14dd	4.32dd	3.70m	4.11brs	4.10brs	4.09brs	4.10brs	4.10brs
H-3^1	5.74dd	5.84t	3.75dd	4.22brd	3.89brd	3.87bd	3.70m	3.87brd
H-4^1	3.97t	4.01t	3.50m	[b]	4.23t	4.19t	[b]	[b]
H-5^1	3.67m	3.85m	3.08dd	3.7m[c]	[b]	3.35m	[b]	3.33m
H-6^{1}a	3.70d	4.32d	3.42d	4.06dd	3.52m	[b]	[b]	3.7[c]
H-6^{1}b	4.34d	3.74dd	3.50m	4.33d	4.13dd	[b]	[b]	3.7[c]
H-1^2	5.46d	5.46d	5.42d	4.93d	4.89d	4.90d	5.02d	5.05d
H-2^2	4.26dd	4.26dd	4.34dd	3.52dd	[b]	3.35m	3.24dd	3.61m[c]
H-3^2	5.74dd	5.73t	5.78t	[b]	3.56	[b]	4.30t	3.95t
H-4^2	5.15t	5.14t	3.98t	[b]	3.76t	[b]	3.93t	4.24t
H-5^2	3.84dt	3.85m	3.70m	3.7m[c]	[b]	[b]	3.57m	3.61m[c]
H-6^{2}a	4.07dd	4.09brd	4.06m	3.90m	[b]	3.9[c]	[b]	3.7[c]
H-6^{2}b	4.44dd	4.43brd	4.0[c]	3.90m	[b]	3.9[c]	[b]	3.7[c]
H-1^3			5.46d	4.72d	4.66d	4.75d	4.75d	4.7[c]
H-2^3			4.27dd	3.75dd	3.52m	[b]	3.50dd	[b]
H-3^3			5.73t	5.28t	[b]	[b]	3.80dd	3.74m
H-4^3			5.14t	5.04t	[b]	[b]	4.56d	4.06brs
H-5^3			3.84m	3.7m[c]	[b]	[b]	[b]	3.42brd
H-6^{3}a			4.5[c]	3.7[c]	[b]	3.9[c]	[b]	3.7[c]
H-6^{3}b			4.5[c]	3.7m[c]	[b]	3.9[c]	[b]	3.7[c]

[a] The data for compound **1** in D_2O are described in the experimental section. [b] Overlapped and not determined. [c] Overlapped.

TABLE II

Coupling constants[a]

Coupled protons	*J-values (Hz)*							
	3	**4**	**7**	**8**	**10**	**11**	**12**	**13**
$1^1,2^1$	8.3	8.8	3.1	1.9	<2	<2	2.0	<2
$2^1,3^1$	10.5	10.7	3.1	[b]	[b]	[b]	[b]	[b]
$3^1,4^1$	9.0	9.8	6.2	[b]	8.0	8.0	[b]	[b]
$4^1,5^1$	9.0	10.1	[b]	[b]	8.0	8.3	[b]	[b]
$5^1,6^1a$	[b]	[b]	[b]	[b]	[b]	[b]	[b]	
$5^1,6^1b$	[b]	4.0	[b]	2.4	4.9	[b]	[b]	[b]
$6^1a.6^1b$	10.4	12.2	11.2	12.4	10.5	[b]	[b]	[b]
$1^2,2^2$	8.3	8.3	8.5	8.3	8.0	8.3	8.3	8.3
$2^2,3^2$	10.6	10.4	10.7	10.3	[b]	[b]	[b]	[b]
$3^2,4^2$	9.0	9.2	9.4	[b]	9.5	[b]	8.1	7.6
$4^2,5^2$	10.0	9.2	9.4	[b]	9.5	[b]	[b]	[b]
$5^2,6^2a$	2.1	[b]	[b]	[b]	[b]	[b]	[b]	[b]
$5^2,6^2b$	4.1	4.3	[b]	[b]	[b]	[b]	[b]	[b]
$6^2a,6^2b$	12.4	12.2	[b]	[b]	[b]	[b]	[b]	[b]
$1^3,2^3$	[b]	[b]	8.2	8.9	8.3	8.0	8.3	[b]
$2^3,3^3$	[b]	[b]	10.7	[b]	10.0	[b]	10.6	[b]
$3^3,4^3$	[b]	[b]	9.7	9.6	[b]	[b]	2.9	<2
$4^3,5^3$	[b]	[b]	9.6	9.6	[b]	[b]	<2	<2
$5^3,6^3a$	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]
$5^3,6^3b$	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]
$6^3a,6^3b$	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]

[a] The data for compound **1** in D_2O are described in the Experimental section. [b] Coupling constant could not be determined.

O-(3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-1,3,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**4**). — A solution of compound **3** (7.3 g, 8.85 mmol) in 100:1 acetic anhydride–sulfuric acid (40 mL) was stirred for 3 h at room temperature, poured into ice-cold water (300 mL), and made neutral by addition of 10% sodium acetate solution. Chloroform extract of the solution was successively washed with aq. $NaHCO_3$ and water, dried, and evaporated to give **4**, which was recrystallized from ethanol (7.2 g, 95%); m.p. 208–210°, $[\alpha]_D^{23}$ +42.2° (*c* 0.096, chloroform).

Anal. Calc. for $C_{40}H_{40}N_2O_{19}$: C, 56.34; H, 4.73; N, 3.29. Found: C, 56.46; H, 4.72; N, 3.47.

Methyl O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-O-(3,6-di-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**7**). — A cold solution of titanium tetrabromide (5.2 g, 14.4 mmol) in ethyl acetate (40 mL) was added to a cold solution of **4** (3.4 g, 4.0 mmol) in dichloromethane (50 mL) at 0°, stirred for 16 h at room temperature, poured into ice-cold water, and extracted with ethyl acetate. The extracts were combined, successively washed with aq. $NaHCO_3$ and water, dried, and evaporated. The residual, syrupy

5 was used without further purification. A mixture of methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**6**, 1.46 g, 3.15 mmol) and molecular sieves 4A (7.5 g) in dichloromethane (20 mL) was stirred for 10 min at room temperature under an argon atmosphere, and then cooled to −15°. Silver trifluoromethanesulfonate (2.22 g, 8.63 mmol), 2,4,6-collidine (1.05 g), and **5** in dichloromethane (20 mL) were added, and the mixture was stirred for 16 h at room temperature, diluted with dichloromethane, and filtered. The filtrate was successively washed with aq. $NaHCO_3$, and water, dried, and evaporated. The residual syrup was chromatographed on silica gel with 2:1 (v/v) hexane–ethyl acetate as the eluant, to give amorphous powdery **7** (3.5 g, 89%); $[\alpha]_D^{23}$ +18.5° (*c* 0.045, chloroform); δ_H: 1.71 (s, 2 H, H_2O), 1.83, 1.88, 1.91, 2.00, and 2.12 (each s, 15 H, 5 $OCOCH_3$), 3.34 (s, 3 H, OCH_3), and 7.08–7.28 (m, 8 H, phthalimido).

Anal. Calc. for $C_{66}H_{68}N_2O_{23}\cdot H_2O$: C, 62.16; H, 5.53; N, 2.20. Found: C, 62.11; H, 5.34; N, 2.25.

Methyl O-*(2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl)-(1→4)*-O-*(2-acetamido-3,6-di*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl)-(1→2)-3,4,6-tri*-O-*benzyl-α*-D-*mannopyranoside* (**8**). — To a solution of **7** (620 mg, 0.49 mmol) in methanol (20 mL) and oxolane (20 mL) was added sodium methoxide (13 mg), and the mixture was stirred for 2 h at room temperature, treated with Dowex 50W X-8 (H^+) resin, and filtered. The filtrate was evaporated, and the residual syrup was treated with hydrazine hydrate (2 mL), ethanol (19 mL), and water (1 mL) for 2 h at 120°, cooled, diluted with water (20 mL), and evaporated. The residue was treated with acetic anhydride (20 mL)–pyridine (30 mL) for 14 h at room temperature, poured into ice-cold water, and extracted with chloroform. The extracts were combined, washed successively with M H_2SO_4, aq. $NaHCO_3$, and water, dried, and evaporated. The residual syrup was chromatographed on silica gel, using 50:1 (v/v) chloroform–methanol as the eluant, to give **8** as a white powder (510 mg, 96%); $[\alpha]_D^{23}$ +16.5° (*c* 0.065, chloroform); R_F 0.5 (10:1 chloroform–methanol); δ_H: 1.70 (s, 2 H, H_2O), 1.81 and 1.94 (each s, 6 H, 2 $NHCOCH_3$), 2.02, 2.03, 2.04, 2.06, and 2.10 (each s, 15 H, 5 $OCOCH_3$), 3.34 (s, 3 H, OCH_3), 5.54 (d, 1 H, NH^2), and 5.86 (d, 1 H, *J* 8.3, NH^3).

Anal. Calc. for $C_{54}H_{68}N_2O_{21}\cdot H_2O$: C, 59.01; H, 6.42; N, 2.55. Found: C, 58.92; H, 6.19; N, 2.96.

Methyl O-*(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1→4)*-O-*(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1→2)-3,4,6-tri*-O-*benzyl-α*-D-*mannopyranoside* (**9**). — To a solution of **8** (2.1 g, 1.95 mmol) in oxolane (20 mL) and methanol (30 mL) was added sodium methoxide (32 mg), and the mixture was stirred for 4 h at room temperature, made neutral with Dowex 50W X-8 (H^+) resin, filtered, and the filtrate evaporated, giving **9** as a white amorphous powder (1.6 g, 95%); $[\alpha]_D^{23}$ +32.8° (*c* 0.082, methanol), R_F 0.25 (65:15:1 chloroform–methanol–water); ν_{max}^{KBr} 3350, 1650, and 1550 cm^{-1}.

Anal. Calc. for $C_{44}H_{58}N_2O_{16}\cdot 1.5\ H_2O$: C, 58.85; H, 6.85; N, 3.12. Found: C, 58.95; H, 6.65; N, 3.35.

Methyl O-*(2-acetamido-3*-O-*benzyl-4,6*-O-*benzylidene-2-deoxy-β*-D-*glucopyranosyl)-(1→4)*-O-*(2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl)-(1→2)-3,4, 6-tri*-O-*benzyl-α*-D-*mannopyranoside* (**10**). — A solution of **9** (1.6 g, 1.85 mmol),

α,α-dimethoxytoluene (0.38 g, 2.5 mmol) and *d*-camphor-10-sulfonic acid (30 mg) in *N*, *N*-dimethylformamide (DMF) (30 mL) was evacuated ~1.3 kPa, rotated for 3 h at 50–60° in a rotary evaporator, diluted with DMF (30 mL), and cooled. Barium oxide (5.6 g), barium hydroxide octahydrate (5.9 g), and benzyl bromide (5.3 g) were added, and the mixture was stirred for 16 h at room temperature, diluted with ethyl acetate (50 mL), and filtered. The filtrate was washed with water, dried, and evaporated to a syrup which was chromatographed on silica gel with 100:10:1 (v/v/v) chloroform–ethyl acetate–methanol as the eluant, to give **10** (2.0 g, 88%); $[\alpha]_D^{23}$ +36.6° (*c* 0.113, chloroform); δ_H: 1.74 and 1.75 (each s, 6 H, 2 NHCOCH_3), 3.31 (s, 3 H, OCH_3), 5.00 (d, 1 H, *J* 7.3 Hz, NH^3), 5.49 (s, 1 H, benzylidene), and 5.85 (d, 1 H, *J* 7.3 Hz, NH^2).

Anal. Calc. for $C_{72}H_{80}N_2O_{16}$: C, 70.34; H, 6.56; N, 2.28. Found: C, 70.25; H, 6.56; N, 2.34.

Methyl O-*(2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl)-(1→4)*-O-*(2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl)-(1→2)-3,4,6-tri*-O-*benzyl-α*-D-*mannopyranoside* (**11**). — A mixture of **10** (800 mg, 0.65 mmol), borane trimethylamine (570 mg), and molecular sieves 4A (3 g) in oxolane (40 mL) was stirred for 30 min at room temperature. Aluminum chloride (1.0 g) was added, and the mixture was stirred for 12 h at room temperature under an argon atmosphere, diluted with ethyl acetate (30 mL), and filtered. The filtrate was treated with Dowex 50W X-8 (H^+) resin, the suspension filtered, and the filtrate washed with brine, dried, and evaporated. The residual syrup was chromatographed on silica gel with 50:1 (v/v) chloroform–methanol as the eluant, to yield **11** (520 mg, 65%); $[\alpha]_D^{23}$ +3.8° (*c* 0.40, chloroform); δ_H: 1.74 and 1.75 (each s, 6 H, 2 NHCOCH_3), 3.00 (d, 1 H, *J* 1.0 Hz, OH-4^3), 4.98 (d, 1 H, *J* 7.5 Hz, NH^3), and 5.83 (d, 1 H, *J* 7.3 Hz, NH^2).

Anal. Calc. for $C_{72}H_{82}N_2O_{16}$: C, 70.34; H, 6.71; N, 2.27. Found: C, 70.34; H, 6.83; N, 2.19.

Methyl O-*(2-acetamido-4*-O-*acetyl-3,6-di*-O-*benzyl-β*-D-*galactopyranosyl)-(1→4)*-O-*(2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl)-(1→2)-3,4,6-tri*-O-*benzyl-α*-D-*mannopyranoside* (**12**). — To a cooled solution of **11** (278 mg, 226 mmol) in pyridine (5 mL) was added methanesulfonyl chloride (0.26 g, 2.26 mmol) at 0–5°, and the mixture was stirred for 5 h at room temperature, poured into ice-cold water, extracted with chloroform, and the extract washed successively with M H_2SO_4, aq. $NaHCO_3$, and water, dried, and evaporated. The residue was chromatographed on silica gel with 50:1 (v/v) chloroform–methanol as the eluant, to give the syrupy 4^3-mesylate (268 mg, 90%); R_F 0.5 (20:1 chloroform–methanol); ν_{max}^{KBr} 1365 and 1170 cm^{-1}; δ_H: 2.77 (s, 3 H, SO_2CH_3).

A mixture of the 4^3-mesylate (100 mg, 76.4 μmol) and potassium acetate (441 mg) in DMF was stirred for 20 h at 120° under an argon atmosphere, cooled, diluted with ethyl acetate, and poured into ice-cold water. The organic layer was washed with brine, dried, and evaporated. The residual syrup was purified by chromatography on silica gel using 50:1 (v/v) chloroform–ethanol as the eluant, to provide **12** as a white powder (90 mg, 93%); $[\alpha]_D^{25}$ +2.7° (*c* 0.55, chloroform); δ_H: 1.74 and 1.78 (each s, 6 H, NHCOCH_3), 2.00 (s, 3 H, OCOCH_3), 3.31 (s, 3 H, OCH_3), 4.97 (d, 1 H, *J* 7.8 Hz, NH^3), and 5.84 (d, 1 H, *J* 7.1 Hz, NH^2).

Anal. Calc. for $C_{74}H_{84}N_2O_{17}$: C, 69.79; H, 6.65; N, 2.20. Found: C, 69.66; H, 6.57; N, 2.08.

Methyl O-*(2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*galactopyranosyl)-(1→4)*-O-*(2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl)-(1→2)-3,4,6-tri*-O-*benzyl-α*-D-*mannopyranoside* (**13**). — To a solution of **12** (100 mg, 78.6 μmol) in methanol (10 mL) was added sodium methoxide (26 mg), and the solution was stirred for 8 h at room temperature, made neutral with Dowex 50W X-8 (H^+) resin, the mixture filtered, and the filtrate evaporated, to give powdery **13** (94 mg, 97%); $[\alpha]_D^{23}$ +5.5° (*c* 0.11, chloroform); δ_H: 1.70 (s, 1 H, H_2O), 1.74 and 1.79 (each s, 6 H, 2 NHCOCH_3), 2.48 (br s, 1 H, O*H*-4[3]), 3.31 (s, 3 H, OCH_3), 5.00 (d, 1 H, *J* 7.3 Hz, N*H*[3]), and 5.85 (d, 1 H, *J* 6.65 Hz, N*H*[2]).

Anal. Calc. for $C_{72}H_{82}N_2O_{16}\cdot 0.5\ H_2O$: C, 69.72; H, 6.74; N, 2.26. Found: C, 69.46; H, 6.67; N, 2.37.

Methyl O-*(2-acetamido-3,6-di*-O-*benzyl-2-deoxy-4*-O-*sulfo-β*-D-*galactopyranosyl)-(1→4)*-O-*(2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl)-(1→2)-3,4,6-tri*-O-*benzyl-α*-D-*mannopyranoside sodium salt* (**14**). — Sulfur trioxide–trimethylamine complex (52 mg, 366 μmol) was added to a cooled solution of **13** (90 mg, 73.1 μmol) in *N,N*-dimethylformamide (1.0 mL) and pyridine (1.0 mL) at 0–5°, and the mixture was stirred for 18 h at 40° under an argon atmosphere, cooled, diluted with methanol (1.5 mL), placed on a column (1.5 × 80 cm) of Sephadex LH-20 pre-equilibrated with 1:1 (v/v) chloroform–methanol, and eluted with the same solvent. The product was chromatographed on a column (1.0 × 20 cm) of Dowex 50W X-8 (Na^+) resin, pre-equilibrated with methanol by using the same solvent to give a crude product which was then chromatographed on a column (2.0 × 10 cm) of silica gel with 9:1 (v/v) chloroform–methanol as the eluant, to give **14** (81 mg, 83%); $[\alpha]_D^{23}$ +6.7° (*c* 0.12, methanol); δ_H: 1.78 and 1.79 (each s, 6 H, 2 NHCOCH_3), and 3.27 (s, 3 H, OCH_3).

Anal. Calc. for $C_{72}H_{81}N_2O_{19}SNa$: C, 64.85; H, 6.12; N, 2.10. Found: C, 64.60; H, 6.02; N, 1.95.

Methyl O-*(2-acetamido-2-deoxy-4*-O-*sulfo-β*-D-*galactopyranosyl)-(1→4)*-O-*(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1→2)-α*-D-*mannopyranoside sodium salt* (**1**). — A solution of **14** (62 mg, 46.5 μmol) in 95% ethanol (5 mL) was stirred under hydrogen in the presence of 10% palladium-on-carbon (50 mg) for 30 h. The catalyst was filtered off, and washed with 95% ethanol. The filtrate and washings were combined, applied to a column of Bio-Gel P-2 (200–400 mesh) (2.5 × 40 cm), and eluted with 10% ethanol. The carbohydrate-containing fractions were concentrated, and chromatographed on a column (1.0 × 10 cm) of Dowex 50W X-8 (Na^+) resin, with water as the eluant. Lyophilization of the eluate gave a hygroscopic white powder (27 mg, 83%); $[\alpha]_D^{22}$ +3.0° (*c* 0.51, water); δ_H (deuterium oxide): 1.99 and 2.01 (each s, 6 H, 2 NHCOCH_3), 3.34 (s, 3 H, OCH_3), 3.41 (t, 1 H, *J* 9.5 Hz, H[1]-4), 3.69 (dd, 1 H, *J* 3.4 and 9.5 Hz, H-3[1]), 3.82 (br d, 1 H, H-3[3]), 3.98 (dd, 1 H, *J* 1.7 and 3.4 Hz, H-2[1]), 4.50 (d, 1 H, *J* 8.7 Hz, H-1[2]), 4.52 (d, 1 H, *J* 8.1 Hz, H-1[3]), 4.63 (d, 1 H, *J* 2.4 Hz, H-4[3]), and 4.71 (br s, 1 H, H-1[1]); δ_C (deuterium oxide): 22.96 and 23.09 (NHCO*C*H$_3$), 53.67 and 55.35 (C-2[2] and C-2[3]), 55.59, 60.88, 61.75, and 62.37 (C-6[1], C-6[2], and C-6[3]), 68.07 (C-4[1]), 70.33 and 70.44

(C-3^1 and C-3^3), 76.23 (C-4^3), 76.92 (C-2^1), 98.67 (C-1^1), 100.11 (C-1^2), 102.56 (C-1^3), and 175.56 ($NHCOCH_3$).

Anal. Calc. for $C_{23}H_{39}N_2NaS \cdot 2.5\,H_2O$: C, 36.92; H, 5.89; N, 3.75. Found: C, 36.83; H, 5.44; N, 3.59.

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