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Chemical and chemo-enzymatic synthesis of the α-Neu*p*5Ac-(2 → 6)-β-D-Gal*p*NAc-(1 → 4)-β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p* element that is part of N-linked carbohydrate chains of human lutropin

Paul B. van Seeventer [a], János Kerékgyártó [a,b], Johannes A.L.M. van Dorst [a], Koen M. Halkes [a], Johannis P. Kamerling [a], Johannes F.G. Vliegenthart [a,*]

[a] *Bijvoet Center, Department of Bio-Organic Chemistry, Utrecht University, P.O. Box 80.075, NL-3508 TB Utrecht, The Netherlands*

[b] *Institute of Biochemistry, L. Kossuth University, P.O. Box 55, H-4010 Debrecen, Hungary*

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Abstract

In the framework of a project aimed at the elucidation of the nature of the functional importance of the N-glycosylation of the α-subunit of the glycoprotein hormones human lutropin and human chorionic gonadotropin, the structural element α-Neu*p*5Ac-(2 → 6)-β-D-Gal*p*NAc-(1 → 4)-β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*, which is part of the carbohydrate chains of human lutropin, has been prepared by chemical and chemo-enzymatic synthesis in the form of its propyl glycoside. Condensation of 4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-α/β-D-glucopyranosyl trichloroacetimidate with allyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside gave after deacetylation allyl (3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside. Ethyl 3-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside was converted into the *galacto*-derivative ethyl 4,6-di-*O*-acetyl-3-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside via an oxidation-reduction route, as well as via S_N2-type substitution with acetate. The use of this *galacto* thioglycoside, after its conversion into the corresponding bromide, as GalN donor for condensation with the mentioned disaccharide derivative yielded after deacetylation allyl (3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-(1 → 4)-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside. Methylsulfenyl bromide–silver triflate promoted sialylation of this trisaccharide derivative with *O*-ethyl *S*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosyl)onate] dithiocarbonate and subsequent deprotection resulted into the aimed tetrasaccharide structural element. Alternatively, this compound was prepared via a block synthesis, which, however, was not superior to the linear strategy. Finally, a stereose-

* Corresponding author.

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lective sialylation of synthetically prepared β-D-Gal*p*NAc-(1 → 4)-β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-(1 → O)$CH_2CH_2CH_3$ with CMP-Neu5Ac and rat liver α-2,6-sialyltransferase was accomplished affording the same tetrasaccharide structural element. © 1997 Elsevier Science Ltd.

Keywords: Human lutropin; *N*-Acetyl-D-galactosamine; Chemical sialylation; Enzymatic sialylation; Oligosaccharide synthesis

1. Introduction

The glycoprotein hormones human lutropin (hLH) and human chorionic gonadotropin (hCG) are synthesized in the pituitary gland and the placenta, respectively. They are vital components of the reproductive process [1], both showing almost identical physiological action, and interaction with the same receptor [2]. The α-subunits of these heterodimeric hormones have an almost identical amino acid sequence, and are N-glycosylated at Asn-52 and Asn-78. The β-subunit of hCG is N-glycosylated at Asn-13 and Asn-30, while the β-subunit of hLH contains only one N-glycan at Asn-30. The effects of glycosylation on the functioning of these hormones have been subject of numerous investigations. Their carbohydrate moieties are essential for subunit association, correct folding of the protein, secretion, and metabolic clearance [3]. Deglycosylation of hCG, either by chemical procedures or site-directed mutagenesis, leads generally to an almost complete loss of bioactivity [4]. Furthermore, it has been shown [5] that glycopeptides and oligosaccharides prepared from hCG can inhibit the binding of hCG to the LH/CG-receptor. Additionally, the glycans of its α-subunit, especially those on Asn-52, are involved in eliciting the hormone response i.e. the activation of a G protein which in turn stimulates the adenylate cyclase system [3]. The structural differences in the Asn-linked oligosaccharides of hLH and hCG are confined to peripheral sequences. The N-glycans of hCG comprise mainly monosialylated monoantennary and disialylated diantennary *N*-acetyllactosamine-type, and monosialylated hybrid-type structures [6]. In contrast, hLH was found to contain predominantly diantennary *N*-acetyllactosamine- and *N,N'*-diacetyllactosediamine-type structures, and only insignificant amounts of hybrid-type and monoantennary chains occur [7]. These oligosaccharide chains terminate in general with sialic acid, although the terminal GalNAc residue of *N,N'*-diacetyllactosediamine-type sequences can be sulfated or sialylated at O-4 or O-6, respectively [7].

Neither the 4-*O*-sulfated nor the α-(2 → 6)-sialylated *N,N'*-diacetyllactosediamine-type structures have been observed in the N-glycans of hCG. Therefore, the question can be raised if the mentioned structural differences for the N-glycans of hLH and hCG affect intracellular transport, receptor binding, subsequent signal transduction, and more in general modulate the hormone action. To contribute to the study of the subunit- and site-specific functions of the N-glycans of hLH and hCG, we here report on the synthesis of α-Neu*p*5Ac-(2 → 6)-β-D-Gal*p*NAc-(1 → 4)-β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-(1 → O)-$CH_2CH_2CH_3$ (**2**). Both a chemical and a chemo-enzymatic synthesis, using α-2,6-sialyltransferase (EC 2.4.99.1) [8,9], were examined for the preparation of tetrasaccharide **2**. The synthesis of tetrasaccharide **2** is part of a project, wherein the synthesis is planned of larger oligosaccharides as well, containing the $Man_3GlcNAc_2$ core structure. Thereby, a further study of the biochemical and conformational properties of these interesting oligosaccharide structures will be enabled. It has to be noted that the chemical synthesis of β-D-Gal*p*NAc-(1 → 4)-β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-(1 → O)$(CH_2)_8COOCH_3$ and its 3″- and 4″-*O*-sulfate esters have been reported earlier [10].

2. Results and discussion

Key intermediate allyl (3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**6**) was chosen to prepare α-Neu*p*5Ac-(2 → 6)-β-D-Gal*p*NAc-(1 → 4)-β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-(1 → O)$CH_2CH_2CH_3$ (**2**) along three different routes. In the first route, GalN and Neu5Ac donors were coupled sequentially to **6**. The second route involved the coupling of α-Neu5Ac-(2 → 6)GalN donor to acceptor **6**. The third route comprised the coupling of a GalN donor to **6**, followed by the enzymatic α-(2 → 6)-sialylation with CMP-Neu5Ac of the generated β-D-Gal*p*NAc-(1 → 4)-β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-(1 → O)$CH_2CH_2CH_3$ (**1**).

The key acceptor **6** was prepared by coupling of 4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-α/β-D-glucopyranosyl trichloroacetimidate (**3**) [11] with allyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**4**) [12], using boron trifluoride diethyletherate in dichloromethane as a catalyst at −30 → 0 °C (→ **5**, 86%), and subsequent Zemplén deacetylation (→ **6**, 68%) (Scheme 1).

Linear strategy.—The galactosaminyl donor ethyl 4,6-di-*O*-acetyl-3-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside (**12**) was synthesized along two different routes, both starting from diol **7**, which was obtained by hydrolysis of the benzylidene ring of ethyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside [13] with acetic acid–water (→ **7**, 78%). The first route involved an oxidation-reduction sequence. After 6-*O*-tritylation of **7** (→ **8**), the oxidation of HO-4, using dimethylsulfoxide–*N,N'*-dicyclohexylcarbodiimide–pyridinium trifluoroacetate [14], resulted in the formation of the 4-ulose derivative **9**. Then, compound **9** was reduced with tetrabutylammonium borohydride in tetrahydrofuran to give the *gluco*- (**8**) and *galacto*- (**10**) epimers in a 1:10 ratio (TLC). After separation of **8** and **10** by column chromatography, detritylation of **10** (→ **11**) and subsequent *O*-acetylation resulted in the formation of **12** (**7** → **12**; 55% overall yield).

Alternatively, the galactosaminyl donor **12** was prepared via a S_N2 displacement reaction of *O*-triflate by *O*-acetate [15]. To this end, the 4,6-di-*O*-triflate derivative **13** was prepared from **7** using trifluoromethanesulfonic anhydride in dichloromethane in the presence of pyridine and a catalytic amount of 4-dimethylaminopyridine at 0 °C. After the formation of **13**, tetrabutylammonium acetate was directly added to the mixture, resulting in a displacement of the triflate group at O-6 by the acetate group without prior displacement by nucleophilic pyridine as side-reaction [16]. Then, *N,N*-dimethylformamide and an additional amount of tetrabutylammonium acetate were added, resulting in the substitution of the equatorial triflate group at O-4 by an axial acetate group, giving **12** (**7** → **12**; 67% overall yield). This shorter route afforded **12** in a higher yield than the oxidation-reduction route described above.

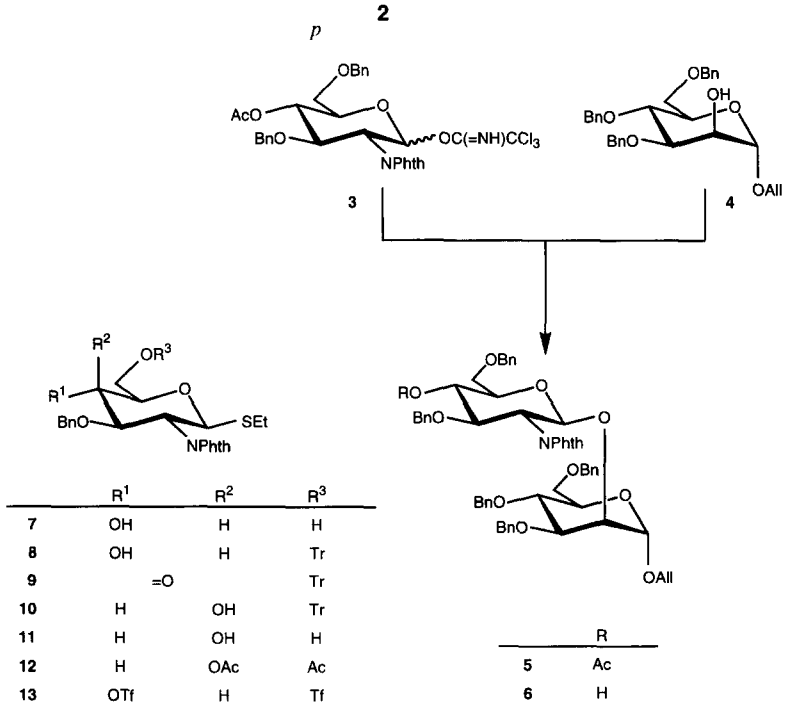
For the preparation of trisaccharide derivative **16**, donor **12** was converted into bromide **14** by treatment with bromine in dichloromethane at 0 °C [17]. Then,

β-D-Gal*p*NAc-(1→4)-β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→O)$CH_2CH_2CH_3$

1

α-Neu*p*5Ac-(2→6)-β-D-Gal*p*NAc-(1→4)-β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→O)$CH_2CH_2CH_3$

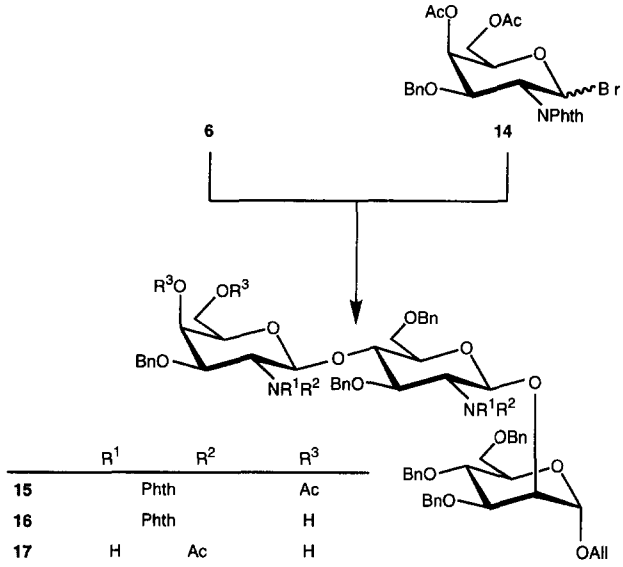
2



	R^1	R^2	R^3
7	OH	H	H
8	OH	H	Tr
9	=O		Tr
10	H	OH	Tr
11	H	OH	H
12	H	OAc	Ac
13	OTf	H	Tf

	R
5	Ac
6	H

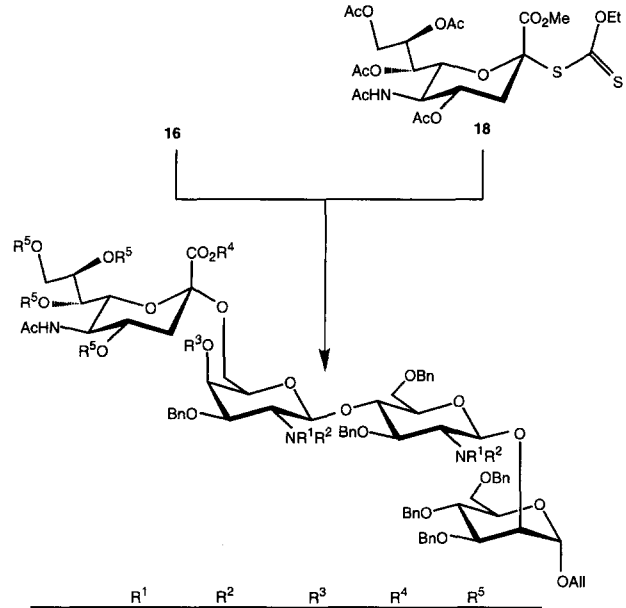
Scheme 1.



Scheme 2.

14 was activated by silver triflate and coupled with **6** in 4:11 dichloromethane–toluene at −40 °C (→ **15**), followed by deacetylation for purificational purposes to give trisaccharide derivative **16** in an overall yield of 75% (Scheme 2). Alternatively, condensation of donor **12** with **6**, using *N*-iodosuccinimide (NIS) and a catalytic amount of triflic acid (TfOH) [18], in 15:1 dichloromethane–acetonitrile as solvent system at 0 °C, followed by deacetylation, afforded trisaccharide derivative **16** in an overall yield of 50%. The synthesis of tetrasaccharide derivative **20** was carried out by condensation of the Neu5Ac donor *O*-ethyl *S*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosyl)onate] dithiocarbonate [19] (**18**) with **16** using methylsulfenyl bromide in the presence of silver triflate [20] in 2:1 acetonitrile–dichloromethane at −60 °C [21], followed by acetylation with acetic anhydride–pyridine (→ **19**, → **20**; 59%) (Scheme 3). Additionally, the corresponding β-anomer **20**β (15%) was found. A characteristic signal in the ^{1}H NMR spectrum of **20** was found for H-7‴ at δ 5.318 with $J_{6''',7'''} < 1$ and $J_{7''',8'''}$ 9.6 Hz (**20**β: $J_{7''',8'''}$ 2.8 Hz), in accordance with the empirical ^{1}H NMR rule (α $J_{7,8} \gg \beta$ $J_{7,8}$) for the determination of the anomeric configuration of sialic acid derivatives [22]. Taken into account the signal for H-4″ at δ 5.562 (acetylated O-4″), these data indicated that in compound **20** α-Neu5Ac is linked to O-6″. Deprotection of **20** using lithium iodide [23] in pyridine at 115 °C, followed by treatment with 1,2-diaminoethane [24] in *n*-butanol at 90 °C, re-*N*,*O*-acetylation with acetic anhydride–pyridine, de-*O*-acetylation (→ **21**, → **22**, → **23**; 59%), and finally catalytic hydrogenolysis using palladium on carbon, gave tetrasaccharide **2** (66%). For ^{1}H NMR data, see Table 1.

Block synthesis.—The second route involved the coupling of the α-Neu5Ac-(2 → 6)-GalN donor **31** with acceptor **6** (see Scheme 4). For this purpose, compound **27** was selected as synthon. The conversion of allyl 3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**24**) [11] into **26** (68%) was performed using the triflate-displacement reaction, described above for the preparation of **12**. Zemplén deacetylation of **26** afforded acceptor **27** (83%). Coupling of **18** with **27** in 3:1 acetonitrile–dichloromethane at −60 °C, using methylsulfenyl triflate as a promoter, followed by acetylation with acetic anhydride–pyridine, gave the disaccharide derivative **29** in a good yield (→ **28**, → **29**; 73%) and the corresponding β-anomer **29**β (11%). Characteristic signals in the ^{1}H NMR spectrum of **29** were found for H-7′ at δ 5.340 with $J_{6',7'}$ 1.6 and $J_{7',8'}$ 8.5 Hz (**29**β: $J_{7',8'}$ 4.9 Hz), in accordance with the empirical ^{1}H NMR rule (α $J_{7,8} \gg \beta$ $J_{7,8}$) for the determination of the anomeric configuration of sialic acid derivatives [22], and for H-4 at δ 5.622 (acetylated O-4), which indicated that in compound **29** α-Neu5Ac is linked to O-6. Deallylation of **29**



Scheme 3.

Table 1
500-MHz ^{1}H NMR data of trisaccharide **1** and tetrasaccharide **2**

Residue	Proton (J)	δ (ppm) (J, Hz)	
		1	**2**
α-D-Man*p*	H-1 ($J_{1,2}$)	4.861 (1.0)	4.887 (0.9)
	H-2 ($J_{2,3}$)	4.041 (3.4)	4.053 (3.4)
	H-3 ($J_{3,4}$)	n.d. [a]	3.80
β-D-Glc*p*NAc	H-1 ($J_{1,2}$)	4.561 (8.2) [b]	4.586 (8.3)
	H-2 ($J_{2,3}$)	n.d.	3.74
	NAc	2.068 [c]	2.071 [c]
β-D-Gal*p*NAc	H-1 ($J_{1,2}$)	4.518 (8.5)	4.501 (8.4)
	H-2 ($J_{2,3}$)	n.d.	3.94
	NAc	2.044 [c]	2.068 [c]
α-Neu*p*5Ac	H-3eq ($J_{3eq,4}$) ($J_{3eq,3ax}$)	–	2.662 (4.9) (−12.5)
	H-3ax ($J_{3ax,4}$)	–	1.717 (n.d.)
	H-4	–	3.66
	NAc	–	2.030
Propyl	$O(CH_2)_2CH_3$	0.915	0.918

[a] n.d. = Not determined.
[b] Virtual coupling to H-3.
[c] Assignments may have to be interchanged.

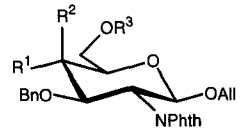
using the Wilkinson's catalyst in the presence of 1,4-diazabicyclo[2.2.2]octane [25] followed by iodine induced hydrolysis [26], gave **30** (predominantly β, 63%). When trichloroacetimidate **31**, obtained from **30** via treatment with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene [27] (→ **31**, predominantly β, 83%), was used as the donor, condensation with **6** in dichloromethane, using boron trifluoride etherate as a catalyst at −25 → 0 °C, gave only 26% of the desired tetrasaccharide derivative **20** (data not shown).

Chemo - enzymatic synthesis.—The trisaccharide acceptor β-D-Gal*p*NAc-(1 → 4)-β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-(1 → O)$CH_2CH_2CH_3$ (**1**), needed for the enzymatic α-(2 → 6)-sialylation, was prepared from **16** by dephthaloylation using 1,2-diaminoethane, followed by re-*N*-acetylation with acetic anhydride in methanol (→ **17**, 79%), and finally hydrogenolysis using palladium on carbon (→ **1**, 86%). For ^{1}H NMR data, see Table 1. In a preparative experiment [8,9], compound **1** was incubated with CMP-Neu5Ac and rat liver α-2,6-sialyltransferase during five days, then the absence of acceptor was shown by TLC. Tetrasaccharide **2** was isolated from the incubation mixture by fractionation on Sephadex LH-20 and column chromatography in a yield of 90%.

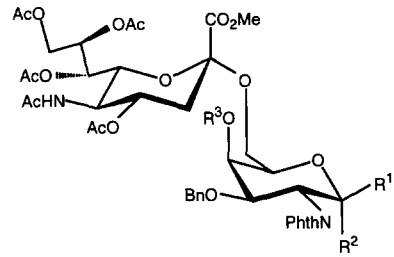
Concluding remarks.—Comparing the linear strategy with the block synthesis, it is clear that, in this case, the linear strategy has the advantage, that it can be integrated with a chemo-enzymatic approach. Apart from that, the linear strategy afforded a higher overall yield. The potency of rat liver β-D-galactoside α-2,6-sialyltransferase to transfer α-Neu5Ac in α-(2 → 6)-linkage to GalNAc, was used successfully for the complete α-stereoselective sialylation of trisaccharide **1** and offers a useful alternative for the chemical introduction of the sialic acid residue. The availability of tetrasaccharide derivative **20** will allow the conversion of **20** into a useful donor for the syntheses of larger structures of the complex-type, e.g. containing the common $Man_3GlcNAc_2$ core structure.

3. Experimental

General.—Reactions were monitored by TLC on Kieselgel 60 F_{254} (E. Merck) by detection with UV



	R1	R2	R3
24	OH	H	H
25	OTf	H	Tf
26	H	OAc	Ac
27	H	OH	H



	R1	R2	R3
28	OAll	H	H
29	OAll	H	Ac
30	OH, H		Ac
31	$OC(=NH)CCl_3$, H		Ac

Scheme 4.

light and then charring with aq 50% H_2SO_4. Column chromatography was performed on Kieselgel 60 (E. Merck, 70–230 mesh) and size exclusion chromatography on Sephadex LH-20. Solvents were evaporated under reduced pressure at 40 °C (bath). Optical rotations were measured at 20 °C for solns in $CHCl_3$ unless otherwise stated, with a Perkin–Elmer 241 polarimeter, using a 10-cm 1-mL cell. The ^1H (300 or 200 MHz) and ^{13}C (APT, 75 MHz) NMR spectra were recorded at 27 °C with a Bruker AC 300, a Bruker WP-200 SY, or a Varian Gemini-300 spectrometer. Two-dimensional double-quantum filtered ^1H–^1H correlation spectra (2D DQF ^1H–^1H COSY) were recorded using a Bruker AMX 500 apparatus (500 MHz) at 27 °C. Chemical shifts (δ) are given in ppm relative to the signal for internal Me_4Si (δ 0) for solns in $CDCl_3$, indirectly to CD_3OD (δ 3.30) for solns in CD_3OD, or by reference to acetone (δ 2.225) for solns in D_2O (pH ~ 8, pH meter reading has not been corrected for D isotope effect), for ^1H, and indirectly to $CDCl_3$ (δ 76.9) for solns in $CDCl_3$ or indirectly to CD_3OD (δ 49.0) for solns in CD_3OD, for ^{13}C. Fast-atom-bombardment mass spectrometry (FABMS) was performed on a JEOL JMS SX/SX 102A four-sector mass spectrometer, operated at 10 kV accelerating voltage, equipped with a JEOL MS-FAB 10 D FAB gun, operated at 10 mA emission current, producing a beam of 6-keV Xenon atoms. Elemental analyses were carried out by H. Kolbe Mikroanalytisches Laboratorium (Mülheim an der Ruhr, Germany). CMP-Neu5Ac: Gal-β-(1 → 4)-GlcNAc α-2,6-sialyltransferase from rat liver (EC 2.4.99.1) and calf intestinal alkaline phosphatase (CIAP) were purchased from Sigma. CMP-Neu5Ac was a gift from Dr. U. Kragl, Forschungszentrum Jülich GmbH, Germany.

*Allyl (3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1 → 2)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside* (**6**).—A soln of allyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside [12] (**4**; 3.2 g, 6.5 mmol) and 4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-α/β-D-glucopyranosyl trichloroacetimidate [11] (**3**; 5.6 g, 8.3 mmol) in dry CH_2Cl_2 (25 mL), containing 3 Å molecular sieves (7.5 g), was stirred for 30 min under Ar at −30 °C. Then $BF_3 \cdot Et_2O$ (2 M in CH_2Cl_2, 1.0 mL) was added, and the mixture was stirred for 1.5 h (−30 → 0 °C), after which TLC (3:1 toluene–EtOAc) showed the complete disappearance of **4** (R_f 0.42) and the formation of **5** (R_f 0.68). Triethylamine (0.3 mL) was added and the mixture was diluted with CH_2Cl_2 (350 mL), filtered through Celite, washed with water (3 ×), dried ($MgSO_4$), and concd. Column chromatography (5:2 hexane–EtOAc) of the residue gave **5**, isolated as a colourless syrup (5.6 g, 86%); $[\alpha]_D$ +8° (*c* 1); R_f 0.32 (5:2 hexane–EtOAc); ^1H NMR ($CDCl_3$): δ 7.65–6.86 (m, 29 H, 5 Ph and Phth), 5.748 (m, 1 H, $H_2C{=}CHCH_2O$), 5.276 (d, 1 H, $J_{1',2'}$ 8.6 Hz, H-1'), 5.164 and 5.095 (2 m, each 1 H, $H_2C{=}CHCH_2O$), 5.115 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 1.947 (s, 3 H, Ac); ^{13}C: δ 117.1 ($H_2C{=}CHCH_2O$), 96.8 and 96.0 (C-1,1'), 77.5, 76.5, 74.4, 73.5, 73.3, 72.4, and 71.6 (C-2,3,4,5,3'4',5'), 74.7, 73.4 (2 C), 72.6, 70.6, 70.0, 69.8, and 67.7 (C-6,6', $H_2C{=}CHCH_2O$, and 5 $PhCH_2O$), 55.2 (C-2'), 20.7 ($COCH_3$).

To a soln of **5** (3.0 g, 3.0 mmol) in MeOH (50 mL) was added NaOMe (pH 9–10). The soln was stirred for 16 h, when TLC (5:1 toluene–EtOAc) showed the deacetylation to be complete, then neutralized with Dowex-50W (H^+) resin, filtered, and concd. Column chromatography (5:1 toluene–EtOAc) of the residue gave **6**, isolated as a colourless syrup (1.95 g, 68%); $[\alpha]_D$ +3° (*c* 1); R_f 0.40 (5:1 toluene–EtOAc); NMR ($CDCl_3$): ^1H, δ 7.65–6.94 (m, 29 H, 5 Ph and Phth), 5.755 (m, 1 H, $H_2C{=}CHCH_2O$), 5.266 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 5.122 and 5.075 (2 m, each 1 H, $H_2C{=}CHCH_2O$); ^{13}C, δ 117.1 ($H_2C{=}CHCH_2O$), 96.8 and 96.0 (C-1,1'), 55.0 (C-2'). Anal. Calcd for $C_{58}H_{59}NO_{12}$: C, 72.41; H, 6.18. Found: C, 72.61; H, 6.10.

*Ethyl 3-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside* (**7**).—A soln of ethyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside [13] (1.22 g, 2.3 mmol) in 6:4 HOAc–water (20 mL) was kept for 2 h at 80 °C, then co-concd with toluene (5 ×). Crystallization of the residue from EtOAc–hexane gave **7**, isolated as needles (0.80 g, 78%); mp 147 °C; $[\alpha]_D$ +54° (*c* 0.7); ^1H NMR ($CDCl_3$): δ 7.90–7.60 and 7.08–6.90 (m, 9 H, Ph and Phth), 5.32 (d, 1 H, $J_{1,2}$ 10 Hz, H-1), 4.76 and 4.53 (2 d, each 1 H, $PhCH_2O$), 3.09 and 2.40 (2 bs, each 1 H, 2 OH), 2.66 (m, 2 H, CH_3CH_2S), 1.15 (t, 3 H, CH_3CH_2S). Anal. Calcd for $C_{23}H_{25}NO_6S$: C, 62.28; H, 5.68. Found: C, 62.05; H, 5.71.

*Ethyl 3-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-6-*O*-trityl-β-*D*-glucopyranoside* (**8**).—To a stirred soln of **7** (665 mg, 1.5 mmol) in pyridine (25 mL) was added trityl chloride (976 mg, 3.5 mmol). After stirring overnight, the mixture was concd, diluted with CH_2Cl_2, washed with aq 5% $NaHCO_3$ and water, dried (Na_2SO_4), filtered, and concd. Column chromatography (6:4 hexane–EtOAc) of the residue af-

forded **8**, isolated as a colourless syrup (964 mg, 94%); $[\alpha]_D$ +29° (*c* 0.3); R_f 0.63 (6:4 hexane–EtOAc); ^{1}H NMR ($CDCl_3$): δ 7.80–6.90 (m, 24 H, Tr, Ph, and Phth), 5.27 (d, 1 H, $J_{1,2}$ 9.5 Hz, H-1), 4.76 and 4.52 (2 d, each 1 H, PhCH_2O), 3.85 (m, after deuteration bt, 1 H, $J_{4,5} \approx J_{3,4}$ 8.5 Hz, H-4), 3.63 (m, 1 H, H-5), 2.70 (d, 1 H, $J_{HO,4}$ 2.4 Hz, HO-4), 2.66 (m, 2 H, CH_3CH_2S), 1.20 (t, 3 H, CH_3CH_2S). Anal. Calcd for $C_{42}H_{39}NO_6S$: C, 73.55; H, 5.73. Found: C, 73.19; H, 5.76.

*Ethyl 3-O-benzyl-2-deoxy-2-phthalimido-1-thio-6-O-trityl-β-*D-xylo*-hexopyranosid-4-ulose* (**9**).—Compound **8** (600 mg, 0.88 mmol) was dissolved in 1:1 anhyd Me_2SO–CH_2Cl_2 (6 mL), and pyridine (70 μL) and CF_3COOH (35 μL) were added. After the addition of DCC (543 mg), the mixture was stirred overnight at room temperature, then diluted with CH_2Cl_2, filtered through Celite, washed with water (3 ×), dried (Na_2SO_4), filtered, and concd. Column chromatography (98:2 CH_2Cl_2–EtOAc) of the residue afforded **9**, isolated as a white solid (516 mg, 86%); mp 194 °C (EtOAc–hexane); $[\alpha]_D$ +101° (*c* 0.5); R_f 0.71 (98:2 CH_2Cl_2–EtOAc); ^{1}H NMR ($CDCl_3$): δ 7.80–6.90 (m, 24 H, Tr, Ph, and Phth), 5.60 (d, 1 H, $J_{1,2}$ 10 Hz, H-1), 4.82 and 4.32 (2 d, each 1 H, PhCH_2O), 4.72 (d, 1 H, $J_{2,3}$ 10 Hz, H-3), 4.60 (t, 1 H, H-2), 4.25 (m, 1 H, H-5), 3.60 and 3.51 (2 dd, each 1 H, $J_{6a,6b}$ 11 Hz, H-6a and H-6b), 2.72 (m, 2 H, CH_3CH_2S), 1.29 (t, 3 H, CH_3CH_2S). Anal. Calcd for $C_{42}H_{37}NO_6S$: C, 73.77; H, 5.45. Found: C, 74.20; H, 5.42.

*Ethyl 3-O-benzyl-2-deoxy-2-phthalimido-1-thio-6-O-trityl-β-*D*-galactopyranoside* (**10**).—To a stirred soln of **9** (300 mg, 0.44 mmol) in dry THF (8 mL) was added Bu_4NBH_4 (225 mg, 0.88 mmol) at 0 °C. After 10 min, when TLC (75:25 hexane–EtOAc) showed the absence of **9** and the presence of the *gluco*- (**8**, R_f 0.36) and *galacto*- (**10**, R_f 0.33) derivatives in the ratio of 1:10, the mixture was diluted with CH_2Cl_2, washed with water (3 ×), dried (Na_2SO_4), filtered, and concd. Column chromatography (75:25 hexane–EtOAc) of the residue gave **10**, isolated as a solid (247 mg, 82%); mp 118–120 °C (EtOAc–hexane); $[\alpha]_D$ +42° (*c* 0.2); ^{1}H NMR ($CDCl_3$): δ 7.90–6.95 (m, 24 H, Tr, Ph, and Phth), 5.21 (d, 1 H, $J_{1,2}$ 9.5 Hz, H-1), 4.62 and 4.35 (2 d, each 1 H, PhCH_2O), 4.56 (t, 1 H, $J_{2,3}$ 9.5 Hz, H-2), 4.29 (dd, 1 H, $J_{3,4}$ 2.9 Hz, H-3), 4.16 (m, 1 H, H-4), 2.69 (m, 2 H, CH_3CH_2S), 2.49 (bs, 1 H, HO-4), 1.20 (t, 3 H, CH_3CH_2S). Anal. Calcd for $C_{42}H_{39}NO_6S$: C, 73.55; H, 5.73. Found: C, 73.71; H, 5.72.

*Ethyl 3-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-*D*-galactopyranoside* (**11**).—A soln of **10** (200 mg, 0.29 mmol) in 8:2 HOAc–water (10 mL) was stirred for 1 h at 80 °C, then concd, and toluene (5 ×) was evaporated from the residue. Column chromatography (95:5 CH_2Cl_2–MeOH) of the crude product gave **11**, isolated as a colourless syrup (117 mg, 91%); $[\alpha]_D$ +70° (*c* 0.5); R_f 0.48 (95:5 CH_2Cl_2–MeOH); ^{1}H NMR ($CDCl_3$): δ 7.90–6.90 (m, 9 H, Ph and Phth), 5.25 (d, 1 H, $J_{1,2}$ 10.5 Hz, H-1), 4.64 and 4.35 (2 d, each 1 H, PhCH_2O), 2.91 and 2.40 (2 bs, each 1 H, 2 OH), 2.70 (m, 2 H, CH_3CH_2S), 1.18 (t, 3 H, CH_3CH_2S). Anal. Calcd for $C_{23}H_{25}NO_6S$: C, 62.28; H, 5.68. Found: C, 62.40; H, 5.71.

*Ethyl 4,6-di-*O*-acetyl-3-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-*D*-galactopyranoside* (**12**).—Procedure A: Compound **11** (80 mg, 0.18 mmol) was conventionally acetylated in 1:1 pyridine–Ac_2O (6 mL) to give after co-concn with toluene (3 ×), EtOH (3 ×), and CH_2Cl_2 (3 ×) **12** (84 mg, 91%).

Procedure B (via triflate displacement): To a soln of **7** (50 mg, 0.11 mmol) in dry CH_2Cl_2 (2 mL), containing dry pyridine (46 μL) and a catalytic amount of 4-dimethylaminopyridine, was added triflic anhydride (57 μL, 0.44 mmol) under Ar at 0 °C. The mixture was stirred for 3 h at 0 °C, when TLC (97.5:2.5 CH_2Cl_2–EtOAc) showed the complete conversion into a new product (R_f 0.90). Then Bu_4NOAc (170 mg, 0.56 mmol) was added and after stirring for 1 h at 0 °C an additional amount of Bu_4NOAc (100 mg, 0.34 mmol) in dry DMF (1 mL) was added. The stirring was continued for 3.5 h at room temperature. Then, TLC (97.5:2.5 CH_2Cl_2–EtOAc) showed the reaction to be complete and the mixture was diluted with CH_2Cl_2 (50 mL), and washed with aq 5% $NaHCO_3$ (2 ×) and water. The combined aq layers were re-extracted with CH_2Cl_2 (2 × 20 mL) and the combined organic layers were dried ($MgSO_4$), filtered, and concd. Column chromatography (96:4 CH_2Cl_2–EtOAc) of the residue gave **12**, isolated as a colourless syrup (40 mg, 67%); mp 106 °C (EtOAc–hexane); $[\alpha]_D$ +92° (*c* 1); R_f 0.21 (97.5:2.5 CH_2Cl_2–EtOAc); NMR ($CDCl_3$): ^{1}H, δ 7.90–7.60 and 7.08–6.90 (m, 9 H, Ph and Phth), 5.618 (dd, 1 H, $J_{3,4}$ 3.4, $J_{4,5}$ < 1 Hz, H-4), 5.295 (d, 1 H, $J_{1,2}$ 10.5 Hz, H-1), 4.582 and 4.248 (2 d, each 1 H, PhCH_2O), 4.480 (dd, 1 H, $J_{2,3}$ 10.7 Hz, H-2), 4.340 (dd, 1 H, H-3), 4.013 (m, 1 H, H-5), 2.661 (m, 2 H, CH_3CH_2S), 2.210 and 2.093 (2 s, each 3 H, 2 Ac), 1.198 (t, 3 H, CH_3CH_2S); ^{13}C, δ 81.5 (C-1), 74.6, 73.3, and 65.7 (C-3,4,5), 71.0 and 62.2 (C-6 and PhCH_2O), 51.3

(C-2), 24.2 (CH_3CH_2S), 20.8 and 20.6 (2 $COCH_3$), 14.8 (CH_3CH_2S). Anal. Calcd for $C_{27}H_{29}NO_8S$: C, 61.46; H, 5.54. Found: C, 61.61; H, 5.56.

Allyl (3-O-benzyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-(1 → 4)-(3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**16**).—To a soln of **12** (100 mg, 0.19 mmol) in dry CH_2Cl_2 (4 mL) was added a soln of bromine (19 μL, 0.38 mmol) in dry CH_2Cl_2 (2 mL) under Ar at 0 °C. After stirring for 30 min at room temperature TLC (1:1 EtOAc–hexane) showed the complete disappearance of **12** (R_f 0.54) and the formation of two new compounds (R_f 0.65 and R_f 0.50). The mixture was co-concd with dry toluene (2 ×). To the residue was added a soln of **6** (101 mg, 0.11 mmol) in 1:1 dry CH_2Cl_2–toluene (4 mL). Powdered 4 Å molecular sieves (0.2 g) were added and the mixture was stirred for 30 min under Ar at −40 °C. Then silver triflate (81 mg, 0.32 mmol) in dry toluene (3.5 mL) was added dropwise, while keeping the soln protected from light. After stirring for 1 h at −40 °C TLC (55:45 hexane–EtOAc) showed the complete disappearance of **6** (R_f 0.52) and the formation of a new product (R_f 0.45). Pyridine (0.5 mL) was added and the mixture was diluted with CH_2Cl_2 (125 mL), filtered through Celite, washed with aq 10% $Na_2S_2O_3$ (3 ×) and water, dried ($MgSO_4$), filtered, and concd. The crude product was treated with NaOMe in 6:1 MeOH–CH_2Cl_2 (7 mL) (pH 9) for 16 h, when TLC (85:15 CH_2Cl_2–acetone) showed the deacetylation to be complete. After neutralization with Dowex-50W (H^+) resin, filtration, and concn, column chromatography (85:15 CH_2Cl_2–acetone) of the residue gave **16**, isolated as a colourless syrup (106 mg, 75%); $[\alpha]_D$ −3° (*c* 1); R_f 0.41 (85:15 CH_2Cl_2–acetone); NMR ($CDCl_3$): ^1H, δ 7.91–6.81 (m, 38 H, 6 Ph and 2 Phth), 5.681 (m, 1 H, $H_2C{=}CHCH_2O$), 5.266 (d, 1 H, $J_{1',2'}$ 8.4 Hz, H-1'), 5.125 (d, 1 H, $J_{1'',2''}$ 7.9 Hz, H-1''), 5.061 and 5.015 (2 m, each 1 H, $H_2C{=}CHCH_2O$); ^{13}C, δ 117.1 ($H_2C{=}CHCH_2O$), 97.1, 96.5, and 95.9 (C-1,1',1''), 55.2 and 52.7 (C-2',2''). FAB$^+$MS ($C_{79}H_{78}N_2O_{18}$): *m/z* 1366 $[M + Na]^+$.

Allyl (2-acetamido-3-O-benzyl-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-(2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**17**).—A soln of **16** (71 mg, 53 μmol) in 1,2-diaminoethane (0.53 mL) and *n*-butanol (5 mL) was stirred for 16 h under Ar at 75 °C. After cooling to room temperature and co-concn with toluene (3 ×), TLC (95:5 CH_2Cl_2–MeOH) showed a complete conversion of **16** into a new product (R_f 0.30). The residue was *N*-acetylated in 1:5 Ac_2O–MeOH (6 mL) for 2 h at room temperature, after which the mixture was co-concd with toluene (3 ×). Sequential column chromatography of the residue on silica (95:5 CH_2Cl_2–MeOH) and Sephadex LH-20 (1:1 CH_2Cl_2–MeOH) yielded **17**, isolated as a colourless glass (49 mg, 79%); $[\alpha]_D$ +21° (*c* 1); R_f 0.35 (95:5 CH_2Cl_2–MeOH); NMR ($CDCl_3$): ^1H, δ 7.40–7.14 (m, 30 H, 6 Ph), 1.785 and 1.711 (2 s, each 3 H, 2 NAc); ^{13}C: δ 171.1 and 170.5 (2 $NHCOCH_3$), 117.0 ($H_2C{=}CHCH_2O$), 99.1, 97.8, and 96.9 (C-1,1',1''), 78.1, 77.5, 76.6, 75.8, 74.5, 74.4, 74.0, 73.2, 71.5, and 65.4 (C-2,3,4,5,3',4',5',3'',4'',5''), 74.9, 73.7, 73.1 (2 C), 70.9, 70.8, 69.2, 69.0, 68.0, and 61.8 (C-6,6',6'', $H_2C{=}CHCH_2O$, and 6 $PhCH_2O$), 56.4 and 53.3 (C-2',2''), 23.4 and 23.2 (2 $NHCOCH_3$). FAB$^+$MS ($C_{67}H_{78}N_2O_{16}$): *m/z* 1168 $[M + H]^+$.

Propyl (2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-α-D-mannopyranoside (**1**).—To a soln of **17** (26 mg, 22 μmol) in 14:2:1 2-propanol–water–HOAc (2.5 mL) was added 10% Pd–C (30 mg). Hydrogenolysis was performed at atmospheric pressure for 4 h, when TLC (5:5:1 CH_2Cl_2–MeOH–water) showed the formation of one product (R_f 0.60), and the mixture was filtered through Celite, and concd. Column chromatography (8:8:1 CH_2Cl_2–MeOH–water) of the residue, followed by lyophilization from water, gave **1**, isolated as a white amorphous solid (12 mg, 86%); $[\alpha]_D$ +2° (*c* 1; MeOH); ^1H NMR (D_2O), see Table 1. FAB$^+$MS ($C_{25}H_{44}N_2O_{16}$): *m/z* 629 $[M + H]^+$.

Allyl (methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2 → 6)-(4-O-acetyl-3-O-benzyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-(1 → 4)-(3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**20**).—A mixture of **16** (55 mg, 41 μmol) and *O*-ethyl *S*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl- 3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyrano-syl)onate] dithiocarbonate [19] (**18**; 48 mg, 80 μmol) in 2:1 dry acetonitrile–CH_2Cl_2 (4.5 mL), containing powdered 4 Å molecular sieves (0.2 g), was stirred for 30 min under Ar. Silver triflate (25 mg, 98 μmol) was added and the mixture was cooled to −60 °C and kept protected from light. Then, methylsulfenyl bromide (75 μL, 1 M in 1,2-dichloroethane, 75 μmol) was added [20], and after stirring for 1 h at −60 °C TLC (7:3 CH_2Cl_2–acetone)

showed the formation of a new product (R_f 0.50) and the disappearance of **16** (R_f 0.75). Diisopropylamine (50 μL) was added and the stirring was continued for 30 min at −40 °C. The mixture was diluted with CH_2Cl_2 (75 mL), filtered through Celite, and concd. The residue was fractionated on Sephadex LH-20 (1:1 CH_2Cl_2–MeOH) and the crude product was acetylated in 1:1 Ac_2O–pyridine (4 mL). After stirring for 16 h the mixture was co-concd with toluene (3 ×). Column chromatography (80:15 CH_2Cl_2–THF) of the residue gave **20**, isolated as a colourless syrup (45 mg, 59%), and the corresponding β-anomer (12 mg, 15%). Compound **20**: $[\alpha]_D$ +4° (*c* 1); R_f 0.35 (80:15 CH_2Cl_2–THF); NMR ($CDCl_3$): ^{1}H, δ 7.91–6.85 (m, 38 H, 6 Ph and 2 Phth), 5.680 (m, 1 H, H_2C=C*H*CH_2O), 5.562 (dd, 1 H, $J_{3'',4''}$ 4.2, $J_{4'',5''}$ < 1 Hz, H-4″), 5.446 and 5.096 (2 d, each 1 H, *J* 8.2, *J* 8.0 Hz, H-1′ and H-1″), 5.318 (dd, 1 H, $J_{6''',7'''}$ < 1, $J_{7''',8'''}$ 9.6 Hz, H-7‴), 5.047 and 5.001 (2 m, each 1 H, H_2C=CHCH_2O), 4.858 (m, 1 H, H-4‴), 3.785 (s, 3 H, OMe), 2.507 (dd, 1 H, $J_{3'''eq,3'''ax}$ 12.9, $J_{3'''eq,4'''}$ 4.8 Hz, H-3‴eq), 2.147, 2.121, 2.113, 2.047, 1.993, and 1.889 (6 s, each 3 H, 5 Ac and NAc); ^{13}C, δ 117.1 (H_2C=CHCH_2O), 98.8 (C-2‴), 97.6, 96.6, and 95.9 (C-1,1′,1″), 55.4 and 52.8 (C-2′,2″), 49.3 (OCH_3), 37.4 (C-3‴), 23.0 ($NHCOCH_3$). Anal. Calcd for $C_{101}H_{107}N_3O_{31}$: C, 65.26; H, 5.80. Found: C, 65.34; H, 5.85.

Compound **20**β: R_f 0.48 (80:15 CH_2Cl_2–THF); ^{1}H NMR ($CDCl_3$): δ 7.90–6.75 (m, 38 H, 6 Ph and 2 Phth), 5.920 (d, 1 H, $J_{NH,5'''}$ 10.1 Hz, NH), 5.776 (dd, 1 H, $J_{3'',4''}$ 3.1, $J_{4'',5''}$ < 1 Hz, H-4″), 5.661 (m, 1 H, H_2C=C*H*CH_2O), 5.396 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1′), 5.318 (t, 1 H, $J_{6''',7'''} = J_{7''',8'''}$ = 2.8 Hz, H-7‴), 5.045 (d, 1 H, $J_{1'',2''}$ 6.9 Hz, H-1″), 3.810 (s, 3 H, OMe), 2.513 (dd, 1 H, $J_{3'''eq,3'''ax}$ 12.8, $J_{3'''eq,4'''}$ 4.9 Hz, H-3‴eq), 2.292, 2.148, 2.022, 2.018, 1.887, and 1.860 (6 s, each 3 H, 5 Ac and NAc).

Allyl (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-*non-2-ulopyranosylonic acid)-(2 → 6)-(2-acetamido-3*-O-*benzyl-2-deoxy-β*-D-*galactopyranosyl)-(1 → 4)-(2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl)-(1 → 2)-3,4,6-tri*-O-*benzyl-α*-D-*mannopyranoside* (**23**).—A soln of **20** (15 mg, 8 μmol) and LiI (21 mg, 157 μmol) in dry pyridine (1 mL) was kept at 115 °C in the dark for 18 h under Ar. TLC (9:1 CH_2Cl_2–MeOH) then showed a complete conversion of **20** into a new product (R_f 0.41) and after cooling to room temperature the mixture was co-concd with toluene (2 ×). The residue was dissolved in CH_2Cl_2 (50 mL), and the soln was washed with M HCl (2 ×) and water (2 ×), dried ($MgSO_4$), filtered, and concd. Column chromatography (9:1 CH_2Cl_2–MeOH) of the residue afforded a yellowish glass (10 mg), which was treated with 1,2-diaminoethane (0.25 mL) and *n*-butanol (1 mL) for 16 h under Ar at 90 °C. After cooling to room temperature and co-concn with toluene (2 ×), TLC (6:4 CH_2Cl_2–MeOH) showed a complete conversion of the product with R_f 0.41 into a new product (R_f 0.75). The residue was acetylated with 1:1 Ac_2O–pyridine (4 mL) for 16 h, when TLC (9:1 CH_2Cl_2–MeOH) showed the formation of one new product (R_f 0.35). The mixture was co-concd with toluene (3 ×) and to a soln of the residue in 4:10 CH_2Cl_2–MeOH (1.4 mL) was added NaOMe until pH 10, and the mixture was stirred for 3 h, during which the deacetylation did not go to completion (TLC). Therefore, water (0.2 mL) was added and stirring was continued for 16 h. Then, TLC (75:25 CH_2Cl_2–acetone) showed the deacetylation to be complete. The mixture was neutralized with Dowex-50W (H^+) resin, filtered, and concd. The residue was fractionated on Sephadex LH-20 (1:1 CH_2Cl_2–MeOH) and **23** was isolated as a colourless glass (7.0 mg, 59%); $[\alpha]_D$ −0.4° (*c* 1, MeOH); R_f 0.42 (75:25 CH_2Cl_2–MeOH); ^{1}H NMR (CD_3OD): δ 7.40–7.08 (m, 30 H, 6 Ph), 5.921 (m, 1 H, H_2C=C*H*CH_2O), 2.813 (dd, 1 H, $J_{3'''eq,3'''ax}$ 12.0, $J_{3'''eq,4'''}$ 3.9 Hz, H-3‴eq), 2.008, 1.878, and 1.832 (3 s, each 3 H, 3 NAc).

Propyl (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-*non-2-ulopyranosylonic acid)-(2 → 6)-(2-acetamido-2-deoxy-β*-D-*galactopyranosyl)-(1 → 4)-(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1 → 2)-α*-D-*mannopyranoside* (**2**).—To a soln of **23** (7.0 mg, 4.7 μmol) in 14:2:1 2-propanol–water–HOAc (2 mL) was added 10% Pd–C (10 mg). Hydrogenolysis was performed at atmospheric pressure for 16 h, then the mixture was filtered through Celite, and concd. Column chromatography (7:5:1 CH_2Cl_2–MeOH–water) of the residue, followed by lyophilization from water, afforded **2**, isolated as a white amorphous solid (2.9 mg, 66%); $[\alpha]_D$ −28° (*c* 0.5, water); R_f 0.35 (7:5:1 CH_2Cl_2–MeOH–water); ^{1}H NMR (D_2O), see Table 1. FAB$^-$MS ($C_{36}H_{61}N_3O_{24}$): *m/z* 918 $[M-H]^-$; FAB$^+$MS: *m/z* 920 $[M+H]^+$.

Enzymatic synthesis of propyl (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-*non-2-ulopyranosylonic acid)-(2 → 6)-(2-acetamido-2-deoxy-β*-D-*galactopyranosyl)-(1 → 4)-(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1 → 2)-α*-D-*mannopyranoside sodium salt* (**2**).—Compound **1** (3.2 mg, 5.1 μmol) and CMP-Neu5Ac (4.7 mg, 7.2 μmol) were dissolved in 50 mM sodium cacodylate buffer (pH 6.5, 1

mL) containing Triton X-100 (0.5%), bovine serum albumin (1 mg), and alkaline phosphatase (9 U). Rat liver Gal-β-(1 → 4)-GlcNAc α-2,6-sialyltransferase (5 mU) was added and the mixture was incubated at 37 °C. After 36 h, 60 h, and 96 h additional equal portions of CMP-Neu5Ac (totally 12.3 mg, 18.7 μmol) were added and after the last addition the incubation was proceeded for another 24 h. Then, TLC (7:6:1 CH_2Cl_2–MeOH–water) showed a complete conversion of **1** (R_f 0.43) into **2** (R_f 0.20), and water (2 mL) was added. After lyophilization, the residue was fractionated on Sephadex LH-20 (5:5:1 CH_2Cl_2–MeOH–water), and the product-containing fractions were treated with Dowex-50 (Na^+), filtered, and concd. Column chromatography (6:6:1 CH_2Cl_2–MeOH–water) of the residue, followed by lyophilization from water afforded **2**, isolated as a white powder (4.3 mg, 90%); ^1H NMR (D_2O), see Table 1.

Allyl 4,6-di-O-acetyl-3-O-benzyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (**26**).—To a soln of allyl 3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside [11] (**24**; 600 mg, 1.37 mmol) in dry pyridine (0.55 mL) and dry CH_2Cl_2 (25 mL), containing a catalytic amount of 4-dimethylaminopyridine, was added triflic anhydride (685 μL, 4.11 mmol) under Ar at 0 °C. The mixture was stirred for 1.5 h at 0 °C, when TLC (95:5 CH_2Cl_2–acetone) showed the complete conversion of **24** into a new product (R_f 0.90). Then Bu_4NOAc (2.1 g, 7.0 mmol) was added and after stirring for 2 h at room temperature an additional amount of Bu_4NOAc (2.0 g, 6.9 mmol) in dry DMF (10 mL) was added. Stirring was continued for 16 h, when TLC (95:5 CH_2Cl_2–EtOAc) showed the formation of **26**. The mixture was diluted with CH_2Cl_2 (250 mL) and the soln was washed with M HCl (3 ×), aq 5% $NaHCO_3$ (2 ×), and water, dried ($MgSO_4$), filtered, and concd. Column chromatography (95:5 CH_2Cl_2–EtOAc) of the residue gave **26**, isolated as a colourless syrup (486 mg, 68%); $[\alpha]_D$ +72° (*c* 1); R_f 0.31 (95:5 CH_2Cl_2–EtOAc); NMR ($CDCl_3$): ^1H, δ 7.90–7.58 and 7.10–6.88 (m, 9 H, Ph and Phth), 5.689 (m, 1 H, H_2C=C*H*CH_2O), 5.579 (dd, 1 H, $J_{3,4}$ 3.4, $J_{4,5}$ 1.2 Hz, H-4), 5.184 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.444 (dd, 1 H, $J_{2,3}$ 11.1 Hz, H-2), 4.313 (dd, 1 H, H-3), 4.588 and 4.240 (2 d, each 1 H, PhCH_2O), 2.207 and 2.103 (2 s, each 3 H, 2 Ac); ^{13}C, δ 170.0 (2 C), 167.8, and 167.2 (2 $COCH_3$ and *C*O Phth), 117.1 (H_2C=CHCH_2O), 97.1 (C-1), 72.5, 70.6, and 65.3 (C-3,4,5), 70.8, 69.4, and 61.7 (C-6, H_2C=CHCH_2O, and PhCH_2O), 52.4 (C-2), 20.4 and 20.3 (2 CO*C*H_3). Anal. Calcd for $C_{28}H_{29}NO_9$: C, 64.24; H, 5.58. Found: C, 64.50; H, 5.47.

Allyl 3-O-benzyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (**27**).—To a soln of **26** (101 mg, 0.19 mmol) in MeOH (5 mL) was added NaOMe until pH 9. The soln was stirred for 3 h, when TLC (95:5 CH_2Cl_2–MeOH) showed the deacetylation to be complete, then neutralized with Dowex 50W-(H^+) resin, filtered, and concd. Column chromatography (95:5 CH_2Cl_2–MeOH) of the residue gave **27**, isolated as a colourless syrup (70 mg, 83%); $[\alpha]_D$ +44° (*c* 1); R_f 0.33 (95:5 CH_2Cl_2–MeOH); NMR ($CDCl_3$): ^1H, δ 7.92–7.60 and 7.10–6.80 (m, 9 H, Ph and Phth), 5.686 (m, 1 H, H_2C=C*H*CH_2O), 5.184 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 4.650 and 4.345 (2 d, each 1 H, PhCH_2O), 4.520 (dd, 1 H, $J_{2,3}$ 10.8 Hz, H-2); ^{13}C, δ 117.0 (H_2C=CHCH_2O), 97.4 (C-1), 74.8, 74.2, and 65.7 (C-3,4,5), 71.0, 69.5, and 62.0 (C-6, H_2C=CHCH_2O, and PhCH_2O), 52.2 (C-2). Anal. Calcd for $C_{24}H_{25}NO_7$: C, 65.59; H, 5.73. Found: C, 65.89; H, 5.68.

Allyl (methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2 → 6)-4-O-acetyl-3-O-benzyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (**29**).—A soln of **27** (37 mg, 84 μmol) and *O*-ethyl *S*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosyl)onate] dithiocarbonate [19] (**18**; 112 mg, 188 μmol) in 3:1 dry acetonitrile–CH_2Cl_2 (4 mL), containing powdered 4 Å molecular sieves (0.2 g), was stirred for 30 min under Ar. Silver triflate (50 mg, 196 μmol) was added, and the mixture was cooled to −60 °C and kept in the dark. Methylsulfenyl bromide (170 μL, 1 M in 1,2-dichloroethane, 170 μmol) was added. After stirring for 1 h at −60 °C, TLC (9:1 EtOAc–hexane) showed the complete disappearance of **27** (R_f 0.50) and the formation of a new product (R_f 0.25). Diisopropylamine (110 μL) was added and the stirring was continued for 40 min at −30 °C. Then the mixture was diluted with CH_2Cl_2 (75 mL), filtered through Celite, and concd. The residue was fractionated on Sephadex LH-20 (1:1 CH_2Cl_2–MeOH) and the isolated crude product was acetylated for 16 h in 1:1 Ac_2O–pyridine (4 mL). Column chromatography (85:18:1 CH_2Cl_2–THF–Et_3N) of the residue, obtained by co-concn with toluene (3 ×), gave **29**, isolated as a colourless syrup (60 mg, 73%), and the corresponding β-anomer (9 mg, 11%). Compound **29**: $[\alpha]_D$ +24° (*c* 1); R_f 0.35 (85:18:1 CH_2Cl_2–THF–Et_3N); NMR ($CDCl_3$): ^1H, δ 7.90–7.55 and 7.05–6.85 (m, 9 H, Ph and Phth), 5.690 (m,

1 H, H_2C=CHCH_2O), 5.622 (dd, 1 H, $J_{3,4}$ 3.0, $J_{4,5}$ < 1 Hz, H-4), 5.398 (ddd, 1 H, $J_{7',8'}$ 8.5, $J_{8',9a'}$ 2.5, $J_{8',9b'}$ 5.1 Hz, H-8'), 5.340 (dd, 1 H, $J_{6',7'}$ 1.6 Hz, H-7'), 5.189 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.884 (ddd, 1 H, $J_{4',3eq'}$ 4.7, $J_{4',3ax'}$ 12.1, $J_{4',5'}$ 9.8 Hz, H-4'), 3.822 (s, 3 H, OMe), 2.614 (dd, 1 H, $J_{3'eq,3'ax}$ 12.9 Hz, H-3'eq), 2.195, 2.162, 2.140, 2.041, 2.031, and 1.893 (6 s, each 3 H, 5 Ac and NAc); ^{13}C: δ 117.3 (H_2C=CHCH_2O), 98.6 (C-2'), 97.4 (C-1), 52.8 (C-2), 49.3 (OCH_3), 37.9 (C-3'), 23.0 (NHCOCH_3). Anal. Calcd for $C_{46}H_{54}N_2O_{20}$: C, 57.86; H, 5.70. Found: C, 57.94; H, 5.78.

Compound **29**β: R_f 0.40 (85:18:1 CH_2Cl_2–THF–Et_3N); ^{1}H NMR ($CDCl_3$): δ 7.90–7.60 and 7.05–6.85 (m, 9 H, Ph and Phth), 5.923 (d, 1 H, $J_{NH,5'}$ 10.2 Hz, NH), 5.817 (dd, 1 H, $J_{3,4}$ 3.0, $J_{4,5}$ < 1 Hz, H-4), 5.657 (m, 1 H, H_2C=CHCH_2O), 5.386 (dd, 1 H, $J_{6',7'}$ 2.6, $J_{7',8'}$ 4.9 Hz, H-7'), 5.198 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 5.147 (m, 1 H, H-4'), 3.841 (s, 3 H, OMe), 2.506 (dd, 1 H, $J_{3'eq,3'ax}$ 12.9, $J_{3'eq,4'}$ 5.0 Hz, H-3'eq), 2.377, 2.169, 2.130, 2.052, 2.034, and 1.932 (6 s, each 3 H, 5 Ac and NAc).

*(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-di-deoxy-*D*-glycero-*α*-*D*-galacto-non-2-ulopyranosyl-onate)-(2 → 6)-4-*O*-acetyl-3-*O*-benzyl-2-deoxy-2-phthalimido-α / β-*D*-galactopyranose* (**30**).—A soln of **29** (46 mg, 47 μmol), tris(triphenylphosphine)-rhodium(I) chloride (18 mg, 19 μmol), and 1,4-diazabicyclo[2.2.2]octane (9 mg, 80 μmol) in EtOH (2.5 mL) was boiled under reflux for 2 h. TLC (7:3 CH_2Cl_2–acetone) then showed the formation of a new compound (R_f 0.56), and the mixture was cooled and concd. A soln of the residue in CH_2Cl_2 (75 mL) was washed with aq 10% NaCl (3 ×) and water (2 ×), dried ($MgSO_4$), filtered, and concd. The residue was dissolved in THF (2.5 mL), and NIS (32 mg, 142 μmol) and some drops of water were added. After stirring for 1.5 h at room temperature, TLC (7:3 CH_2Cl_2–acetone) showed a complete conversion into **30** (R_f 0.38). The soln was diluted with CH_2Cl_2 (75 mL), washed with aq 5% $NaHSO_3$ (3 ×) and aq 10% NaCl, dried ($MgSO_4$), filtered, and concd. Column chromatography (7:3 CH_2Cl_2–acetone) of the residue gave **30**, isolated as a colourless glass (28 mg, 63%); ^{1}H NMR ($CDCl_3$): δ 7.85–7.45 and 7.10–6.90 (m, 9 H, Ph and Phth), 5.78 (bd, 0.2 H, $J_{3,4}$ 3, $J_{4,5}$ < 1 Hz, H-4α), 5.609 (dd, 0.8 H, $J_{3,4}$ 3.0, $J_{4,5}$ < 1 Hz, H-4β), 5.388 (m, 0.8 H, H-8'β), 5.301 (dd, 0.8 H, $J_{6',7'}$ 1.8, $J_{7',8'}$ 7.5 Hz, H-7'β), 4.913 (m, 0.8 H, H-4'β), 3.819 (s, 0.6 H, OMeα), 3.812 (s, 2.4 H, OMeβ), 2.587 (dd, 0.8 H, $J_{3'eq,3'ax}$ 13.3, $J_{3'eq,4'}$ 5.1 Hz, H-3'eq β), 2.213, 2.206, 2.123, 2.037, 2.032, and 1.889 (6 s, each 3 × 0.8 H, 5 Acβ and NAcβ). FAB$^+$MS ($C_{43}H_{50}N_2O_{20}$): *m/z* 915 $(M + H)^+$.

*(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-di-deoxy-*D*-glycero-α-*D*-galacto-non-2-ulopyranosyl-onate)-(2 → 6)-4-*O*-acetyl-3-*O*-benzyl-2-deoxy-2-phthalimido-α / β-*D*-galactopyranosyl trichloroacetimidate* (**31**).—To a soln of **30** (28 mg, 30 μmol) in dry CH_2Cl_2 (1.5 mL) and trichloroacetonitrile (0.1 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (1 μL) at 0 °C. After stirring for 3 h at 0 °C, column chromatography (7:3 CH_2Cl_2–acetone) of the mixture gave **31**, isolated as an almost colourless foam (27 mg, 83%); ^{1}H NMR ($CDCl_3$): δ 8.554 [s, 1 H, C(NH)CCl_3], 7.85–7.40 and 7.10–6.85 (m, 9 H, Ph and Phth), 6.479 (d, 0.1 H, $J_{1,2}$ 3.5 Hz, H-1α), 6.446 (d, 0.9 H, $J_{1,2}$ 8.9 Hz, H-1β), 5.687 (dd, 0.9 H, $J_{3,4}$ 2.9, $J_{4,5}$ < 1 Hz, H-4β), 5.324 (dd, 0.9 H, $J_{6',7'}$ 1.6, $J_{7',8'}$ 8.3 Hz, H-7'β), 3.836 (s, 3 × 0.1 H, OMeα), 3.812 (s, 3 × 0.9 H, OMeβ), 2.231, 2.164, 2.132, 2.042, 2.034, and 1.892 (6 s, each 3 × 0.9 H, 5 Acβ and NAcβ).

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