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Synthesis of the Sd^a determinant and two analogous tetrasaccharides

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Abstract

To contribute to the possibility of studying in greater detail the biological significance of Sd^a-containing glycans as occur in Tamm-Horsfall glycoprotein, the following three spacer-linked tetrasaccharides have been synthesized: the Sd^a determinant α-Neu*p*5Ac-(2 → 3)-[β-D-Gal*p*NAc-(1 → 4)]-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → O)$(CH_2)_5NH_2$ (**1**), the Gal-analogue α-Neu*p*5Ac-(2 → 3)-[β-D-Gal*p*-(1 → 4)]-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → O)$(CH_2)_5NH_2$ (**2**), and the GlcNAc-analogue α-Neu*p*5Ac-(2 → 3)-[β-D-Glc*p*NAc-(1 → 4)]-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → O)$(CH_2)_5NH_2$ (**3**). The general trisaccharide acceptor 5-azidopentyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate)-(2 → 3)-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside was prepared, using methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-non-2-ulopyranosid)-onate as the sialyl donor. For the syntheses of **1**, **2**, and **3** the glycosyl donors 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-α-D-galactopyranosyl bromide, 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide, and 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate, respectively, proved to be the most suitable. © 1997 Elsevier Science Ltd.

Keywords: Sd^a determinant; Tamm-Horsfall glycoprotein; Oligosaccharide, chemical synthesis; Sialylation

1. Introduction

Although the Sd^a determinant α-Neu*p*5Ac-(2 → 3)-[β-D-Gal*p*NAc-(1 → 4)]-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → R [1] was first discovered on human erythrocytes, and therefore termed a blood group antigen, Sd^a activity has also been detected abundantly in stomach, kidney, and colon tissue [2,3]. A structurally related pentasaccharide, the Cad antigen α-Neu*p*5Ac-(2 → 3)-[β-D-Gal*p*NAc-(1 → 4)]-β-D-Gal*p*-(1 → 3)-[α-Neu*p*5Ac-(2 → 6)]-D-Gal*p*NAc, has been isolated in its reduced form from glycophorin A [4]. Moreover, a Sd^a-active ganglioside, carrying the typical Sd^a tetrasaccharide, was isolated from Cad-positive erythrocytes [5]. The Sd^a determinant is typically found as a terminal sequence in N-glycans of human Tamm-Horsfall glycoprotein (TH-gp) [1],

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which was also termed uromodulin [6]. TH-gp is a renal-specific phosphatidylinositol-anchored membrane protein, which is excreted in the urine after cleavage from the membrane (50–200 mg/day) [7]. The structures of sialylated as well as sulfated complex-type carbohydrate chains, originating from TH-gp isolated from the urine of one male donor, were elucidated [8]. In an earlier study [9], the presence of oligomannose-type glycans was also reported.

The physiological function of TH-gp is still unclear, as is the role of its glycans with the Sd^a determinant in particular. However, the binding of TH-gp to neutrophils was reported and a role as a ligand for neutrophil integrins was suggested [10], facilitating neutrophil migration across renal epithelium for example in the case of tubulointerstitial nephritis. TH-gp was also reported to display immunosuppressive properties via its glycans [11]. Therefore, it will be interesting to investigate the extent to which the immunosuppressive properties of TH-gp can be correlated to the Sd^a determinant. Probing for an immunosuppressive activity of Sd^a-active gangliosides is another interesting issue to be investigated, because immunosuppressivity has been reported for gangliosides with the α-Neu*p*5Ac-(2 → 3)-[β-D-Gal*p*NAc-(1 → 4)]-β-D-Gal*p*-(1 → 4) structural element. These glycolipids inhibited T cell proliferative antigen-specific responses in vitro [12] as well as in vivo [13].

TH-gp has been suggested to be involved in the prevention of urinary tract and urinary bladder infections by the inhibitory action of its glycans against the fimbriae-mediated adherence of *Escherichia coli* to uroepithelial cells [14,15]. The protective role of the Sd^a determinant, however, in the process of colonization of pathogenic bacteria and in the adherence of bacterial toxins in the urinary tract, urinary bladder, and intestines is still far from definitive.

To study in greater detail the biological significance of Sd^a-containing glycoconjugates, the tetrasaccharide glycosides **1**, **2**, and **3** with the general formula α-Neu*p*5Ac-(2 → 3)-[β-D-Hex*p*-(1 → 4)]-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → O)$(CH_2)_5NH_2$ (Hex = GalNAc, Gal, or GlcNAc, respectively) were synthesized. These compounds are suitable for conjugation to a carrier by the presence of a 5-aminopentyl spacer, and are therefore useful for application in binding assays. Previously, the corresponding reducing form of **1** has been prepared by a chemo-enzymatic route using β-1,4-*N*-acetylgalactosaminyltransferase [16]. The syntheses of corresponding tetrasaccharide moieties with Gal(β1-3)GalNAc [17] or Gal(β1-4)Glc [18] instead of Gal(β1-4)GlcNAc at the reducing end have also been reported in its protected and free form, respectively. The carbohydrate moiety of **2** was found at the non-reducing end of glycoprotein-derived free oligosaccharides from the unfertilized eggs of *Tribolodon hakonesis* [19].

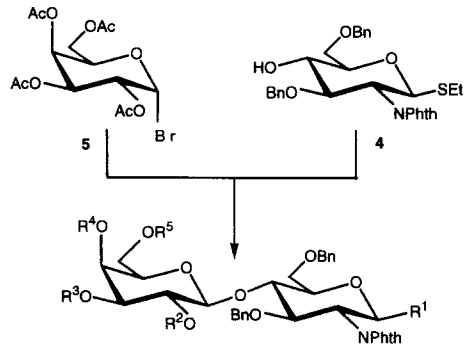
2. Results and discussion

As the general acceptor for the syntheses of the tetrasaccharide glycosides **1**, **2**, and **3**, 5-azidopentyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonate)-(2 → 3)-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**19**) was selected. As already shown in several earlier synthetic studies on gangliosides [20] for Gal-terminated oligosaccharide acceptors with free hydroxyl functions of different reactivity at C-3 and C-4 of Gal, the use of 5-azidopentyl (2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**13**) would allow the regio- as well as the α-stereoselective sialylation with a thio-sialoside donor at HO-3′, followed by the coupling with the respective GalNAc, Gal, and GlcNAc donors at HO-4′.

The synthesis of disaccharide derivative **13** involved the galactosylation of ethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside [21] (**4**) with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**5**) in dichloromethane–toluene at −40 °C via a silver triflate promoted reaction, affording **6** (87%) (Scheme 1). Zemplén deacetylation of **6** (→ **7**), followed by isopropylidenation using 2,2-dimethoxypropane and *p*-toluenesulfonic acid as catalyst, afforded the 6′-*O*-(1-methoxy-1-methylethyl)-3′,4′-*O*-isopropylidene derivative **8**, which was directly converted into the 3′,4′-*O*-isopropylidene derivative **9** by treatment with aqueous trifluoroacetic acid in dichloromethane (→ **9**, 74% from **6**) [22]. The structure of **9** was confirmed by ^1H NMR spectroscopy of the corresponding 2′,6′-di-*O*-acetyl derivative **10**, obtained by *O*-acetylation of **9** with acetic anhydride–pyridine. Benzylation of **9**, applying a standard procedure using benzyl bromide and sodium hydride as a base was unsuccessful, and therefore a milder procedure using benzyl bromide, potassium iodide, and silver(I) oxide [23] in *N*,*N*-dimethylformamide was chosen, giving **11** (96%). Compound **11** has been prepared via an alternative

β-D-Hex-(1→4)
α-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→O)$(CH_2)_5NH_2$

	Hex
1	Gal*p*NAc
2	Gal*p*
3	Glc*p*NAc

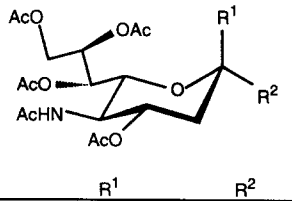


	R1	R2	R3	R4	R5
6	SEt	Ac	Ac	Ac	Ac
7	SEt	H	H	H	H
8	SEt	H	CMe_2		$C(Me)_2OMe$
9	SEt	H	CMe_2		H
10	SEt	Ac	CMe_2		Ac
11	SEt	Bn	CMe_2		Bn
12	$O(CH_2)_5N_3$	Bn	CMe_2		Bn
13	$O(CH_2)_5N_3$	Bn	H	H	Bn

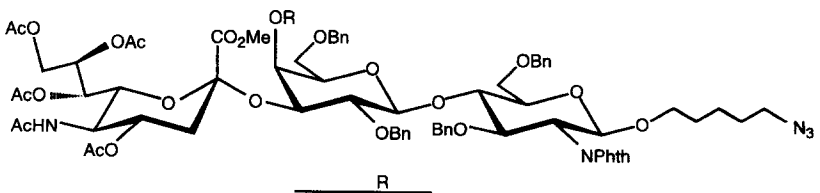
Scheme 1.

route by Spijker et al. [24], however, in a lower overall yield. The preparation of 5-azidopentanol (**14**) was performed by mono-benzoylation of 1,5-pentanediol, followed by reaction with hydrazoic acid in the presence of diethyl azodicarboxylate–triphenylphosphine via the Mitsunobu reaction [25], and debenzoylation (41%; overall). Then, coupling of **11** with 5-azidopentanol (**14**) by activation with *N*-iodosuccinimide (NIS) and a catalytic amount of triflic acid (TfOH) [26] in dichloromethane at 0 °C gave **12** (84%). Finally, de-isopropylidenation of **12** afforded disaccharide derivative **13** (91%).

For the synthesis of the trisaccharide derivative **19**, methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-non-2-ulopyranosid)onate [27] (**15**) was prepared (Scheme 2). The $\alpha:\beta$ ratio of **15**, being 1:8, was estimated from its ^1H NMR spectrum. The sialylation of **13** with **15** in acetonitrile–dichloromethane with NIS–TfOH as promoter system at −45 °C [28], afforded **19** (60%) and 8% of the corresponding β-anomer **19β**. The structure of **19** was confirmed by ^1H NMR spectroscopy of the corresponding 4′-*O*-acetyl derivative **20**. Characteristic ^1H signals for **20** were found at δ 5.350 (dd, 1 H, $J_{6'',7''}$ 2.5 and $J_{7'',8''}$ 8.4 Hz, H-7″), in accordance with the empirical ^1H NMR rule ($\alpha J_{7,8} \gg \beta J_{7,8}$) for the determination of the anomeric configuration of sialic acid derivatives [29], and at δ 5.037 (H-4′), indicating the newly formed glycosidic linkage to be α at O-3′. In parallel experiments two other sialic acid donors were tested. It turned out that the use of methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-non-



	R1	R2
15	SPh, CO_2Me	
16	SMe, CO_2Me	
17	$OP(OEt)_2$	CO_2Me
18	CO_2Me	SC(S)OEt

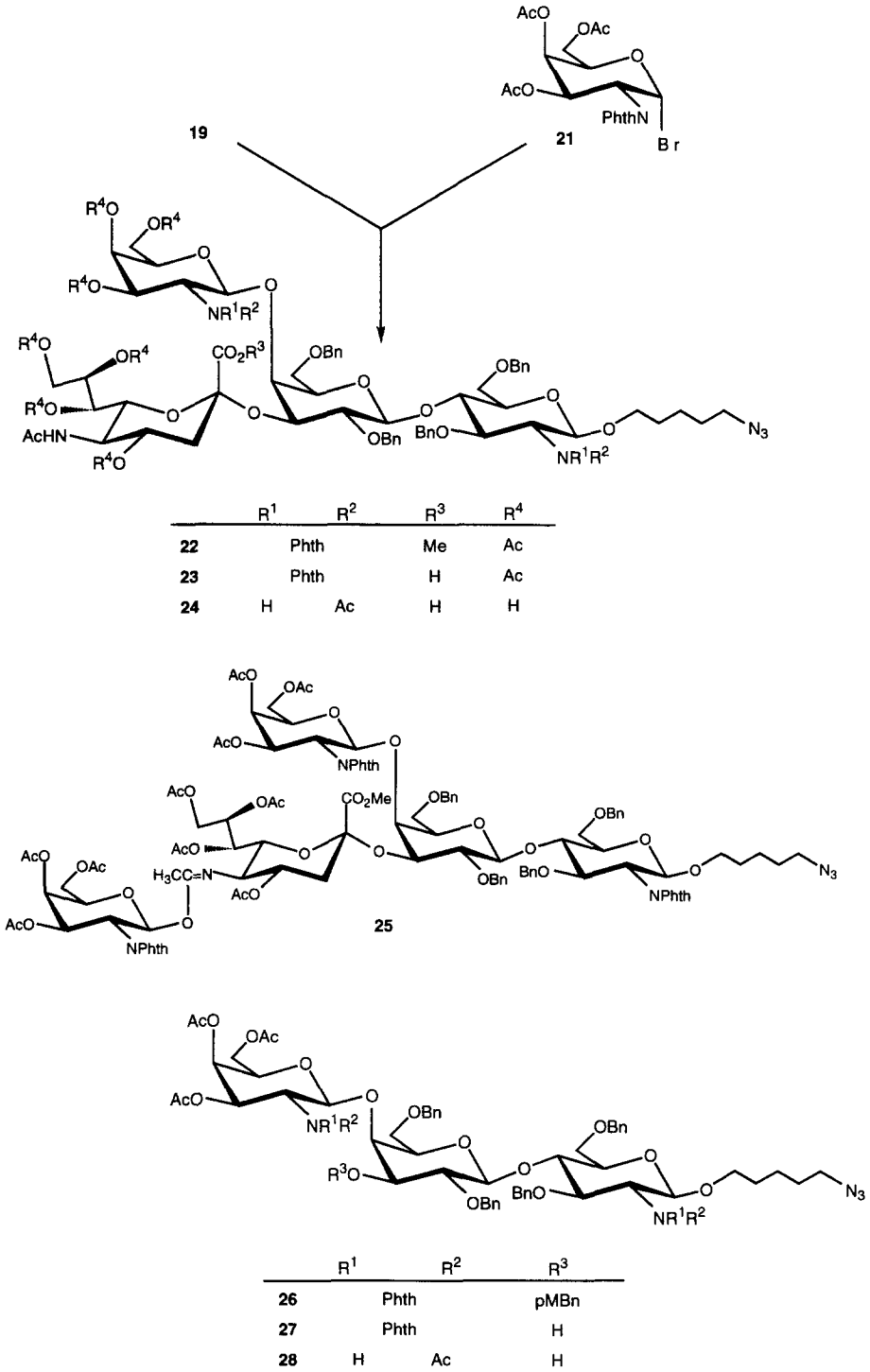


	R
19	H
20	Ac

Scheme 2.

2-ulopyranosid)onate [30] (**16**) in acetonitrile with NIS–TfOH as promoter system at −35 °C, afforded **19** in a yield of 35 and 8% of the corresponding β-anomer **19β**. Furthermore, the condensation of **13** with the sialyl diethylphosphite derivative [31] **17** in acetonitrile at −30 °C in the presence of trimethylsilyl triflate as a catalyst [31] gave **19** in a yield of only 25 and 8% of the corresponding β-anomer **19β**.

The synthesis of tetrasaccharide **1** was performed by condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-α-D-galactopyranosyl bromide [32] (**21**) with **19** applying the inversed addition procedure



	R1	R2	R3	R4
22	Phth		Me	Ac
23	Phth		H	Ac
24	H	Ac	H	H

	R1	R2	R3
26	Phth		pMBn
27	Phth		H
28	H	Ac	H

Scheme 3.

(IAP) [33] in toluene–acetonitrile with $HgBr_2$ as the promoter (Scheme 3). Besides the expected tetrasaccharide derivative **22** (31%), a by-product was obtained, which was identified by FABMS to be the complex imidate **25**. A similar attack of a glycosyl donor on the amide–carbonyl function of a sialyl residue has been described in [17,34,35]. The hydrolysis of the imidate function in **25**, using a catalytic amount of TfOH in dichloromethane–methanol, was unsuccessful. Condensation of **19** with ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside [36], using as a promoter system NIS–TfOH, or 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl trichloroacetimidate [37], using as a catalyst trimethylsilyl triflate, provided the tetrasaccharide derivative **22** in yields of only 16 and 19%, respectively. The conversion of **22** into **24** was performed by demethylation using LiI [38] in pyridine at 115 °C (→ **23**, 75%), followed by dephthaloylation–deacetylation using methylamine in ethanol, and re-*N*-acetylation with acetic anhydride in methanol at 0 °C (→ **24**, 95%). It should be mentioned, that the use of the generally successful dephthaloylating reagents hydrazine monohydrate and 1,2-diaminoethane [39] in this specific case resulted in complex reaction mixtures and in low yields of **24**. Catalytic hydrogenation of **24** over 10% Pd–C in a solution of *tert*-butanol–water, which at the beginning of the incubation contained a small amount of ammonia for a cleaner reduction of the azide function [24], afforded **1** (54%) after lyophilization. As observed also by others [24,40], the behavior of an azide function during catalytic hydrogenolysis is not always clear and can give rise to a moderate yield. Moreover, an inhibitory effect of aliphatic amines on *O*-benzyl hydrogenolysis has been reported [41]. ^1H NMR and positive fast-atom-bombardment collision-induced-decomposition tandem mass-spectrometric (FAB-CID-MS/MS) data of **1** are presented in Tables 1 and 2, respectively.

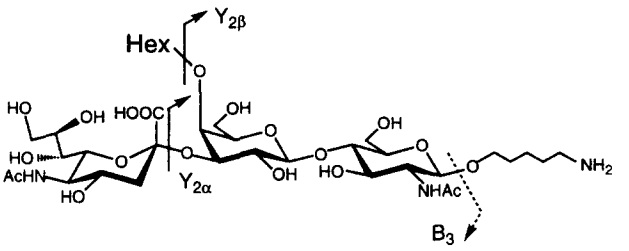
In view of the low yield obtained in the coupling reaction of **21** with **19**, an alternative route for the

Table 1
500-MHz ^1H NMR data of tetrasaccharides **1–3** with the general formula α-Neu*p*5Ac-(2 → 3)-[β-D-Hex-(1 → 4)]-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → O)$(CH_2)_5NH_2$

Residue	Proton (J)	δ (ppm)/J (Hz)		
		1 Hex = Gal*p*NAc	**2** Hex = Gal*p*	**3** Hex = Glc*p*NAc
β-D-Glc*p*NAc	H-1 ($J_{1,2}$)	4.514 (8.1) [a]	4.517 (8.1) [a]	4.513 (8.1) [a]
	H-2 ($J_{2,3}$)	3.71	3.71	3.72
	H-3 ($J_{3,4}$)	n.d. [b]	3.58	3.51
	NAc	2.031	2.029 [c]	2.029 [c]
β-D-Gal*p*	H-1 ($J_{1,2}$)	4.549 (8.0)	4.588 (7.8)	4.544 (8.1)
	H-2 ($J_{2,3}$)	3.356 (9.8)	3.68 (10.0)	3.342 (9.8)
	H-3 ($J_{3,4}$)	4.149 (2.8)	4.216 (3.0)	4.146 (3.0)
	H-4 ($J_{4,5}$)	4.113 (< 1)	4.172 (< 1)	4.094 (< 1)
α-Neu*p*5Ac	H-3eq ($J_{3eq,4}$)	2.661 (4.6)	2.720 (4.9)	2.661 (4.7)
	($J_{3eq,3ax}$)	(−12.5)	(−12.7)	(−12.5)
	H-3ax ($J_{3ax,4}$)	1.908 (n.d.)	1.843 (n.d.)	1.917 (11.3)
	H-4	3.77	3.71	3.79
	NAc	2.031	2.031 [c]	2.033 [c]
Hex	H-1 ($J_{1,2}$)	4.731 (8.6)	4.741 (8.1) [d]	4.795 (8.6)
	H-2 ($J_{2,3}$)	3.91	3.492 (9.8)	3.73
	H-3 ($J_{3,4}$)	3.68	3.673 (3.9)	3.61
	H-4 ($J_{4,5}$)	n.d.	3.908 (< 1)	n.d.
	NAc	2.014	–	2.014
5-Aminopentyl	$O(CH_2)_4CH_2NH_2$	2.951	2.954	2.959

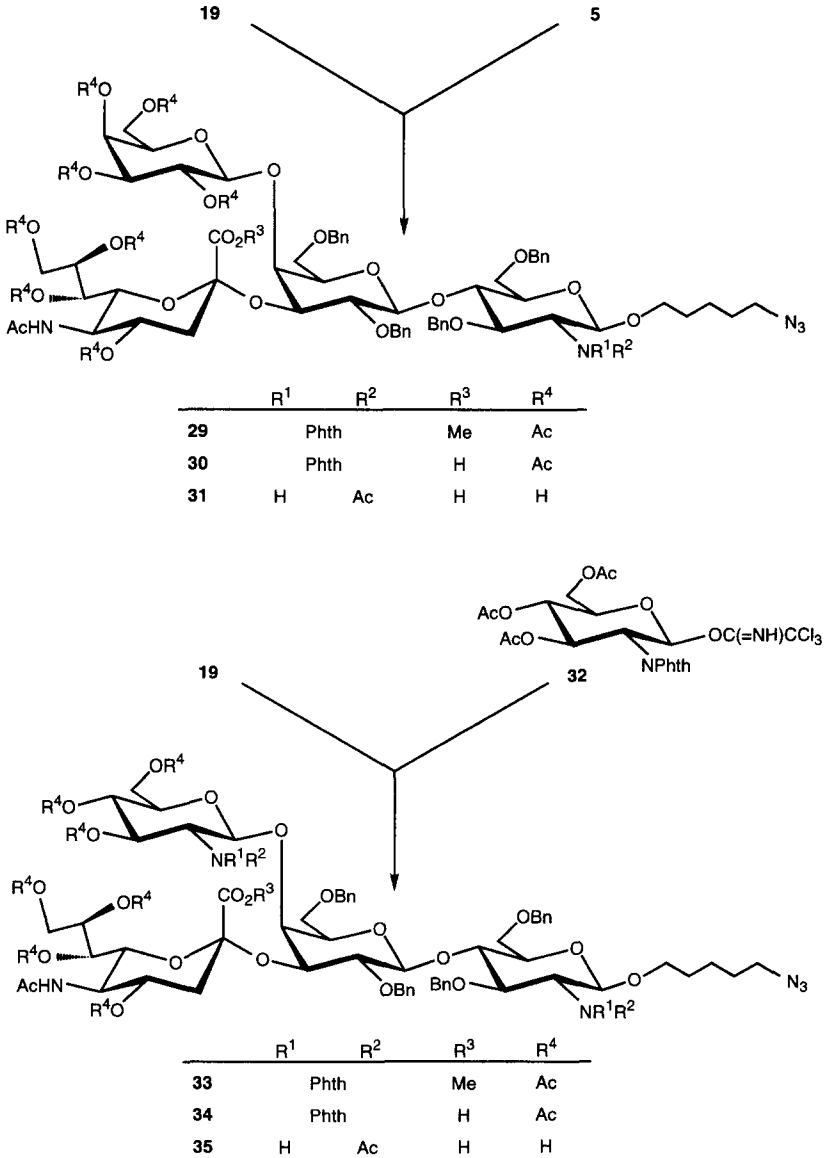
[a] Virtual coupling to H-3.
[b] n.d. = not determined.
[c] Assignments may have to be interchanged.
[d] Measured at 17 °C.

Table 2
Selected positive FAB-CID-MS/MS data for compounds **1**, **2**, and **3**



Compound	Hex	*m/z*			
		$[M+H]^+$	B_3	$Y_{2\beta}$	$Y_{2\alpha}$
1	β-D-Gal*p*NAc	963.6	860.5	760.5	672.4
2	β-D-Gal*p*	922.5	819.4	760.4	631.3
3	β-D-Glc*p*NAc	963.6	860.5	760.5	672.5

construction of **1** was evaluated by studying the coupling of various sialyl donors with potential asialo-trisaccharide acceptors such as 5-azidopentyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-(1 → 4)-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**27**) and 5-azidopentyl (2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-(2,6- di-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside [42] (**28**) (see Scheme 3). To this end, trisaccharide derivative **27** was prepared by de-*p*-methoxybenzylation of 5-azidopentyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-(1 → 4)-(2,6-di-*O*-benzyl-3-*O*-*p*-methoxybenzyl-β-D-galactopyranosyl)-(1



	R^1	R^2	R^3	R^4
29		Phth	Me	Ac
30		Phth	H	Ac
31	H	Ac	H	H

	R^1	R^2	R^3	R^4
33		Phth	Me	Ac
34		Phth	H	Ac
35	H	Ac	H	H

Scheme 4.

→ 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside [42] (**26**), using ammonium cerium(IV) nitrate in acetonitrile–water (→ **27**, 61%). Sialylation of **27** with sialyl donor **15**, using NIS–TfOH as promoter system, in acetonitrile at −25 °C did not lead to any product formation (TLC). Also the use of *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 [27] (**18**) as a donor, in the presence of methylsulfenyl triflate [43] as a promoter, in 9:4 acetonitrile–dichloromethane at −60 °C [28] was not successful. Compound **28**, having two *N*-acetyl groups instead of two phthalimido groups, was expected to cause less steric hindrance during coupling than trisaccharide **27**. However, condensation of sialyl donor **15** with acceptor **28**, applying the same reaction conditions as described above, proved to be unsuccessful.

As concluded from several test reactions with different galactosyl donors, with the aim to synthesize analogue **2**, the most appropriate donor for coupling to acceptor **19** was bromide **5** (Scheme 4). The condensation (IAP) was carried out in toluene–dichloromethane at 0 °C, using silver triflate as a promoter, affording tetrasaccharide derivative **29** (49%). Deprotection of **29** (→ **30**, → **31**, → **2**) was performed in an analogous way as described for **22**, affording, after lyophilization, **2** in an overall yield of 33%. ^{1}H NMR and positive FAB-CID-MS/MS data of **2** are presented in Tables 1 and 2, respectively.

The preparation of analogue **3** involved the coupling of a suitably *N*-phthaloylated glucosaminyl donor with **19**. It turned out that the use of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate [37] (**32**) was superior to that of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose [44], using trimethylsilyl triflate as a promoter, or of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-α/β-D-glucopyranosyl bromide [44], using $HgBr_2$ as a promoter (see Scheme 4). This result is in agreement with an experimental comparison of the coupling efficiency of six standard *N*-phthaloylated glucosaminyl donors with several HO-4 acceptors [45]. When **32** was condensed (IAP) with **19** in dichloromethane in the presence of trimethylsilyl triflate as a catalyst, tetrasaccharide derivative **33** was obtained in a yield of 33%. The formation of the glycal 3,4,6-tri-*O*-acetyl-1,2-dideoxy-2-phthalimido-D-*arabino*-hex-1-enopyranose [44] was observed as the major side reaction. Deblocking of **33** (→ **34**, → **35**, → **3**) was performed in a similar way to those described for both **22** and **29**, to afford **3** in an overall yield of 32%. ^{1}H NMR and positive FAB-CID-MS/MS data of **3** are presented in Tables 1 and 2, respectively.

Comparison of the ^{1}H NMR data of **1–3** showed that the replacement of GalNAc (**1**) by Gal (**2**) has a strong influence on the resonance positions of the Gal residue of the *N*-acetyllactosamine element. Not only Gal H-4 (C-4, attachment site) of this element shows a downfield shift, but also Gal H-1,2,3. Furthermore, a clearly deviating set for Neu5Ac H-3eq,3ax is found. Such remarkable shifts were not observed when replacing GalNAc (**1**) by GlcNAc (**3**). The formation of a hydrogen bond between the HexNAc NH and the Neu5Ac carboxyl group, which are probably in close proximity in the preferred solution conformations of **1** and **3**, could be an explanation of this phenomenon. A similar hydrogen bonding was suggested in a study on the conformational properties of gangliosides [46].

It should be noted that the preparation of two additional analogs of the Sd^a determinant, wherein Neu5Ac has been replaced by a sulfate or a carboxymethyl group, will be published as a separate communication [42]. The evaluation in biochemical recognition assays of these two analogs and compounds **1–3** as inhibitors of microbial adhesion is planned. Additionally, these compounds could be useful for the investigation of their possible immunoregulatory activities and the determination of some of the essential structural features of the Sd^a determinant necessary for recognition by anti-Sd^a antibodies.

3. Experimental

General methods.—Reactions were monitored by TLC on Kieselgel 60 F_{254} (E. Merck), by detection with UV light and then charring with aq 50% H_2SO_4. Column chromatography was performed on Kieselgel 60 (E. Merck, 70–230 mesh). Size-exclusion chromatography was performed on Sephadex LH-20. Solvents were evaporated under reduced pressure at 40 °C (water bath). Optical rotations were measured for solns in $CHCl_3$, unless otherwise stated, at 20 °C with a Perkin–Elmer 241 polarimeter, using a 10-cm 1-mL cell. ^{1}H (300 MHz) and ^{13}C (APT, 75 MHz) NMR spectra were recorded at 27 °C with a Bruker AC 300 spectrometer or a Varian Gemini-300 instrument (^{13}C only). Two-dimensional double-quantum filtered ^{1}H–^{1}H correlation spectra (2D DQF ^{1}H–^{1}H 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. FTIR spectra were recorded on a Mattson Galaxy 5000 spectrometer. Fast-atom-bombardment mass spectrometry (FABMS) and FAB-CID-MS/MS 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).

Ethyl (2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**6**).—A soln of ethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopy-ranoside [21] (**4**; 2.0 g, 3.7 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**5**; 4.16 g, 10.1 mmol) in dry 1:1 CH_2Cl_2–toluene (60 mL), containing powdered 4 Å molecular sieves (1 g), was stirred for 30 min under Ar. Then, a soln of silver triflate (3.83 g, 15.0 mmol) in dry toluene (80 mL) was added dropwise under the exclusion of light in 30 min at −40 °C. After stirring for 2 h, TLC (3:2 hexane–EtOAc) showed the disappearance of **4** (R_f 0.40) and the formation of **6** (R_f 0.20). Pyridine (10 mL) was added, and the mixture was diluted with CH_2Cl_2 (500 mL), filtered through Celite, washed with aq 10% $Na_2S_2O_3$ (3 ×) and water (3 ×), dried (Na_2SO_4), filtered, and concd. Column chromatography (55:45 hexane–EtOAc) of the residue gave **6**, isolated as a colorless syrup (2.80 g, 87%); $[\alpha]_D$ +31° (*c* 1); NMR ($CDCl_3$): ^1H, δ 7.80–6.86 (m, 14 H, 2 Ph and Phth), 5.271 (dd, 1 H, $J_{3',4'}$ 3.5, $J_{4',5'} < 1$ Hz, H-4'), 5.214 (d, 1 H, $J_{1,2}$ 10.1 Hz, H-1), 5.149 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.4 Hz, H-2'), 4.858 (dd, 1 H, H-3'), 4.622 (d, 1 H, H-1'), 2.634 (m, 2 H, CH_3CH_2S), 2.062, 2.024, 2.022, and 1.972 (4 s, each 3 H, 4 Ac), 1.174 (t, 3 H, CH_3CH_2S); ^{13}C, δ 169.9, 169.8, 169.7, and 168.9 (4 $COCH_3$), 167.6 and 167.1 (*C*OPhth), 100.0 (C-1'), 80.8, 78.7, 77.5, 77.3, 70.7, 70.1, 69.2, and 66.6 (C-1,3,4,5,2',3',4',5'), 74.2, 73.3, 67.4, and 60.4 (C-6,6', 2 $PhCH_2O$), 54.4 (C-2), 23.5 (CH_3CH_2S), 14.6 (CH_3CH_2S). Anal. Calcd for $C_{44}H_{49}NO_{15}S$: C, 61.17; H, 5.72. Found: C, 61.06; H, 5.84.

Ethyl β-D-galactopyranosyl-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**7**).—To a soln of **6** (2.80 g, 3.2 mmol) in MeOH (200 mL) was added NaOMe until pH 9. The mixture was stirred overnight, when TLC (8:2 CH_2Cl_2–MeOH) showed a complete conversion of **6** into **7** (R_f 0.55). After neutralization with Dowex-50 (H^+) resin and filtration, the soln was concd to give **7**, isolated as a syrup (2.22 g, quantitatively); $[\alpha]_D$ +49° (*c* 1); NMR ($CDCl_3$): ^1H, δ 7.85–6.86 (m, 14 H, 2 Ph and Phth), 5.225 (d, 1 H, $J_{1,2}$ 10.4 Hz, H-1), 4.515 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1'), 2.630 (m, 2 H, CH_3CH_2S), 1.167 (t, 3 H, CH_3CH_2S); ^{13}C, δ 167.8 and 167.5 (*C*OPhth), 102.8 (C-1'), 80.8, 79.0, 78.6, 77.9, 74.4, 73.5, 72.0, and 69.0 (C-1,3,4,5,2',3',4',5'), 74.8, 73.2, 68.2, and 62.0 (C-6,6', 2 $PhCH_2O$), 54.6 (C-2), 23.6 (CH_3CH_2S), 14.7 (CH_3CH_2S). Anal. Calcd for $C_{36}H_{41}NO_{11}S$: C, 62.15; H, 5.94. Found: C, 62.04; H, 5.91.

Ethyl (3,4-O-isopropylidene-β-D-galactopyranosyl)-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**9**).—To a soln of **7** (1.78 g, 2.6 mmol) in 2,2-dimethoxypropane (75 mL) was added *p*-toluenesulfonic acid (113 mg, 0.65 mmol) (pH 2–3), and the mixture was stirred overnight under Ar at room temperature. TLC analysis (85:15 CH_2Cl_2–acetone) then showed the formation of **8** (R_f 0.81) and the disappearance of **7**. The mixture was neutralized with Et_3N, and co-concd with toluene (3 ×), EtOH (3 ×), and CH_2Cl_2 (3 ×). The residue was dissolved in CH_2Cl_2 (12.5 mL) and aq 50% TFA (0.1 mL) was added. After stirring for 5 min, TLC (85:15 CH_2Cl_2–acetone) showed the disappearance of **8** and the formation of **9** (R_f 0.50). The mixture was neutralized with Et_3N (pH 7–8) and co-concd with toluene (3 ×), EtOH (3 ×), and CH_2Cl_2 (3 ×). Column chromatography (85:15 CH_2Cl_2–acetone) of the residue afforded **9**, isolated as a colorless syrup (1.39 g, 74%); $[\alpha]_D$ +63° (*c* 1); NMR ($CDCl_3$): ^1H, δ 7.85–6.85 (m, 14 H, 2 Ph and Phth), 5.228 (d, 1 H, $J_{1,2}$ 10.4 Hz, H-1), 4.429 (d, 1 H, $J_{1',2'}$ 8.4 Hz, H-1'), 3.002 (d, 1 H, $J_{OH,2'}$ 2.7 Hz, HO-2'), 2.352 (bt, 1 H, HO-6'), 2.643 (m, 2 H, CH_3CH_2S), 1.496 and 1.321 (2 s, each 3 H, Me_2C), 1.176 (t, 3 H, CH_3CH_2S); ^{13}C, δ 110.0 (Me_2C), 101.9 (C-1'), 80.8, 79.3, 78.8, 78.5, 78.1, 74.0, 73.6, and 73.5 (C-1,3,4,5,2',3',4',5'), 74.8, 73.2, 68.2, and 61.9 (C-6,6', 2 $PhCH_2O$), 54.6 (C-2), 27.9 and 26.1 [$(CH_3)_2C$], 23.5 (CH_3CH_2S), 14.7 (CH_3CH_2S). Anal. Calcd for $C_{39}H_{45}NO_{11}S$: C, 63.42; H, 6.08. Found: C, 63.66; H, 6.16.

Compound **9** was further characterized after *O*-acetylation with 1:1 Ac_2O–pyridine, followed by

co-concn with toluene (3 ×), EtOH (3 ×), and CH_2Cl_2 (3 ×), affording **10**; ^1H NMR ($CDCl_3$): δ 7.85–6.90 (m, 14 H, 2 Ph and Phth), 5.209 (d, 1 H, $J_{1,2}$ 9.9 Hz, H-1), 4.963 (dd, 1 H, $J_{1',2'}$ 8.4, $J_{2',3'}$ 7.9 Hz, H-2'), 4.461 (d, 1 H, H-1'), 2.619 (m, 2 H, CH_3CH_2S), 2.08 (bs, 6 H, 2 Ac), 1.520 and 1.308 (2 s, each 3 H, Me_2C), 1.163 (t, 3 H, CH_3CH_2S).

*Ethyl (2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-*D*-galactopyranosyl)-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside* (**11**).—To a stirred soln of **9** (1.39 g, 1.9 mmol) in dry DMF (40 mL) were added silver(I) oxide (5.25 g, 22.7 mmol) and KI (1.57 g, 9.4 mmol) under Ar at 0 °C. Benzyl bromide (2.69 mL, 22.6 mmol) in dry DMF (5 mL) was added dropwise. After 60 min the mixture was allowed to achieve room temperature and after an additional 2 h, TLC (96:4 CH_2Cl_2–acetone) showed a complete conversion of **9** into **11** (R_f 0.72). The mixture was diluted with CH_2Cl_2 (200 mL), filtered through Celite, washed with aq 10% $Na_2S_2O_3$ (2 ×) and aq 5% NaCl (2 ×), dried (Na_2SO_4), filtered, and concd. Column chromatography (96:4 CH_2Cl_2–acetone) of the residue gave **11**, isolated as a colorless syrup (1.66 g, 96%); $[\alpha]_D$ +49° (*c* 1); NMR ($CDCl_3$): ^1H, δ 7.82–6.83 (m, 24 H, 4 Ph and Phth), 5.236 (d, 1 H, $J_{1,2}$ 9.9 Hz, H-1), 4.452 (d, 1 H, $J_{1',2'}$ 8.5 Hz, H-1'), 2.635 (m, 2 H, CH_3CH_2S), 1.370 and 1.329 (2 s, each 3 H, Me_2C), 1.171 (t, 3 H, CH_3CH_2S); ^{13}C, δ 167.6 and 167.0 (*C*OPhth), 109.3 (Me_2C), 101.9 (C-1'), 80.7, 80.2, 79.2, 79.0, 77.8, 77.7, 73.5, and 71.9 (C-1,3,4,5,2',3',4',5'), 74.2, 73.1, 73.0, 72.8, 68.8, and 67.8 (C-6,6', 4 $PhCH_2O$), 54.4 (C-2), 27.6, and 26.0 [$(CH_3)_2C$], 23.4 (CH_3CH_2S), 14.6 (CH_3CH_2S). Anal. Calcd for $C_{53}H_{57}NO_{11}S$: C, 69.49; H, 6.27. Found: C, 69.34; H, 6.33.

5-Azidopentanol (**14**).—To a stirred soln of 1,5-pentanediol (4.5 mL, 42.9 mmol) and 2,4,6-collidine (7.6 mL, 57.2 mmol) in CH_2Cl_2 (75 mL) was added dropwise a soln of benzoyl chloride (6.7 mL, 57.3 mmol) in CH_2Cl_2 (25 mL) at −50 °C under Ar. After stirring for 4 h at −20 °C, TLC (95:5 CH_2Cl_2–acetone) showed the formation of 5-benzoyloxy-pentanol (R_f 0.25), then the mixture was diluted with CH_2Cl_2 (200 mL), washed with M HCl (2 ×) and water (2 ×), dried (Na_2SO_4), filtered, and concd. Column chromatography (95:5 CH_2Cl_2–acetone) of the residue gave 5-benzoyloxy-pentanol, isolated as a colorless oil (4.06 g, 45%); NMR ($CDCl_3$): ^1H, δ 8.05–7.40 (m, 5 H, Ph), 4.334 (t, 2 H, *J* 6.6 Hz, $HO(CH_2)_4CH_2OBz$), 3.672 (t, 2 H, *J* 6.2 Hz, $HOCH_2(CH_2)_4OBz$), 1.86–1.50 (m, 6 H, $HOCH_2(CH_2)_3CH_2OBz$); ^{13}C, δ 165.7 (*C*OPh), 131.9–127.3 (C_6H_5CO), 64.0 [$HO(CH_2)_4CH_2OBz$], 61.1 [$HOCH_2(CH_2)_4OBz$], 31.2, 27.5, and 21.3 [$HOCH_2(CH_2)_3CH_2OBz$].

To a stirred soln of 5-benzoyloxy-pentanol (2.7 g, 13.1 mmol) and triphenylphosphine (3.8 g, 14.5 mmol) in dry THF (60 mL) was added dropwise a 1.2 M hydrazoic acid soln in benzene (16.5 mL) [47], followed by the dropwise addition of diethyl azodicarboxylate (2.3 mL, 14.5 mmol) at 0 °C under Ar. After stirring for 1.5 h at room temperature, TLC (85:15 hexane–EtOAc) showed the formation of 1-azido-5-benzoyloxy-pentane (R_f 0.45), and the mixture was concd. Column chromatography (95:5 CH_2Cl_2–acetone) of the residue gave 1-azido-5-benzoyloxy-pentane, isolated as a colorless oil (2.9 g, 95%); IR (KBr): 2096 cm^{-1}, N_3; NMR ($CDCl_3$): ^1H, δ 8.06–7.41 (m, 5 H, Ph), 4.336 (t, 2 H, *J* 6.6 Hz, $N_3(CH_2)_4CH_2OBz$), 3.306 (t, 2 H, *J* 6.2 Hz, $N_3CH_2(CH_2)_4OBz$), 1.84–1.51 (m, 6 H, $N_3CH_2(CH_2)_3CH_2OBz$); ^{13}C, δ 165.1 (*C*OPh), 131.9–127.4 (C_6H_5CO), 63.6 [$N_3(CH_2)_4CH_2OBz$], 50.2 [$N_3CH_2(CH_2)_4OBz$], 27.5, 27.3, and 22.3 [$N_3CH_2(CH_2)_3CH_2OBz$].

To a soln of 1-azido-5-benzoyloxy-pentane (10.5 g, 45.1 mmol) in MeOH (200 mL) was added NaOMe until pH 10. The mixture was stirred for 48 h, when TLC (1:1 hexane–EtOAc) showed a complete conversion of 1-azido-5-benzoyloxy-pentane into **14** (R_f 0.35). After neutralization with Dowex-50 (H^+) resin, the mixture was filtered, and concd. Column chromatography (1:1 hexane–EtOAc) of the residue gave **14**, isolated as a colorless oil (5.6 g, 97%); IR (KBr): 2094 cm^{-1}, N_3; NMR ($CDCl_3$): ^1H, δ 3.662 (t, 2 H, *J* 6.5 Hz, $N_3(CH_2)_4CH_2OH$), 3.288 (t, 2 H, *J* 6.8 Hz, $N_3CH_2(CH_2)_4OH$), 1.67–1.41 (m, 6 H, $N_3CH_2(CH_2)_3CH_2OH$); ^{13}C, δ 61.5 [$N_3(CH_2)_4CH_2OH$], 50.8 [$N_3CH_2(CH_2)_4OH$], 31.5, 28.0, and 22.4 [$N_3CH_2(CH_2)_3CH_2OH$].

*5-Azidopentyl (2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-*D*-galactopyranosyl)-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**12**).—A soln of **11** (1.23 g, 1.3 mmol) and 5-azidopentanol (**14**) (306 mg, 2.4 mmol) in dry CH_2Cl_2 (20 mL), containing 3 Å molecular sieves (0.5 g), was stirred for 30 min under Ar. Then, the mixture was cooled to 0 °C, and a soln of NIS (362 mg, 1.6 mmol) and TfOH (17.8 μL, 0.2 mmol) in CH_2Cl_2 (30 mL) was added. After 5 min, TLC (95:5 CH_2Cl_2–acetone) showed a complete conversion of **11** (R_f 0.49) into **12** (R_f 0.44). The mixture was neutralized with Et_3N, diluted with CH_2Cl_2 (250 mL), filtered, washed with aq 5% $NaHSO_3$ (2 ×), aq

10% $NaHCO_3$ (2 ×), and water (2 ×), dried (Na_2SO_4), filtered, and concd. Column chromatography (95:5 CH_2Cl_2–acetone) of the residue afforded **12**, isolated as a colorless syrup (1.10 g, 84%); $[\alpha]_D$ +41° (*c* 1); IR (KBr): 2091 cm^{-1}, N_3; NMR ($CDCl_3$): ^1H, δ 7.80–6.83 (m, 24 H, 4 Ph and Phth), 5.100 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.423 (d, 1 H, $J_{1',2'}$ 8.5 Hz, H-1'), 1.376 and 1.330 (2 s, each 3 H, Me_2C); ^{13}C, δ 167.8 and 167.5 (*C*OPhth), 109.5 (Me_2C), 102.2 (C-1'), 98.1 (C-1), 80.4, 79.2, 78.1, 77.0, 75.0, 73.6, and 72.0 (C-3,4,5,2',3',4',5'), 74.2, 73.2, 73.1, 73.0, 68.9, 68.7, and 67.8 (C-6,6', 4 Ph*C*H_2O and O*C*$H_2C_4H_8N_3$), 55.5 (C-2), 50.9, 28.5, 28.1, and 22.8 (OCH_2*C*$_4$$H_8N_3$), 27.7 and 26.2 [(*C*$H_3$)$_2$C]. Anal. Calcd for $C_{56}H_{62}N_4O_{12}$: C, 68.42; H, 6.36. Found: C, 68.35; H, 6.42.

*5-Azidopentyl (2,6-di-*O*-benzyl-β-*D*-galactopyranosyl)-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**13**).—A soln of **12** (0.94 g, 0.96 mmol) in aq 80% HOAc (17 mL) was stirred for 2 h at 80 °C. TLC (95:5 CH_2Cl_2–acetone) then showed a complete conversion of **12** (R_f 0.46) into **13** (R_f 0.29), and the mixture was co-concd with toluene (3 ×), EtOH (3 ×), and CH_2Cl_2 (3 ×). Column chromatography (95:5 CH_2Cl_2–acetone) of the residue afforded **13**, isolated as a colorless syrup (0.83 g, 91%); $[\alpha]_D$ +29° (*c* 1); IR (KBr): 2096 cm^{-1}, N_3; NMR ($CDCl_3$): ^1H, δ 7.80–6.83 (m, 24 H, 4 Ph and Phth), 5.105 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.450 (d, 1 H, H-1'); ^{13}C, δ 167.8 and 167.5 (*C*OPhth), 103.0 (C-1'), 98.2 (C-1), 79.9, 78.2, 77.0, 75.1, 73.4, 72.8, and 69.0 (C-3,4,5,2',3',4',5'), 75.0, 74.4, 73.5, 73.1, 69.1, 69.0, and 67.9 (C-6,6', 4 Ph*C*H_2O and O*C*$H_2C_4H_8N_3$), 55.6 (C-2), 51.0, 28.6, 28.2, and 23.0 (OCH_2*C*$_4$$H_8N_3$). Anal. Calcd for $C_{53}H_{58}N_4O_{12}$: C, 67.50; H, 6.20. Found: C, 67.42; H, 6.40.

*5-Azidopentyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-non-2-ulopyranosylonate)-(2 → 3)-(2,6-di-*O*-benzyl-β-*D*-galactopyranosyl)-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**19**).—A soln of **13** (0.54 g, 0.57 mmol) and methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-*D*-*glycero*-*D*-*galacto*-non-2-ulopyranosid)onate [27] (**15**; 0.62 g, 1.01 mmol) in dry acetonitrile (9 mL)/CH_2Cl_2 (0.5 mL), containing powdered 4 Å molecular sieves (0.4 g), was stirred for 30 min under Ar. The mixture was cooled to −45 °C, and NIS (0.74 g, 3.3 mmol) was added, followed by the dropwise addition of a soln of TfOH (76 μL, 0.86 mmol) in CH_2Cl_2 (1.5 mL). After stirring for 6 h at −45 °C, TLC (6:4 toluene–acetone) showed the complete disappearance of **13** (R_f 0.61) and the formation of **19** (R_f 0.30) and a sialic acid degradation product (R_f 0.24) [48]. Et_3N (150 μL) was added, and the mixture was diluted with CH_2Cl_2 (175 mL), filtered (Celite), washed with aq 5% $Na_2S_2O_3$ (3 ×), aq 10% $NaHCO_3$ (2 ×), and water, dried (Na_2SO_4), filtered, and concd. Column chromatography of the residue was performed sequentially on silica 70–230 mesh (7:3 toluene–acetone) and silica > 230 mesh (8:1 toluene–MeOH) to give **19**, isolated as a colorless syrup (494 mg, 60%), and the corresponding β-anomer **19β** (66 mg, 8%). Compound **19**: $[\alpha]_D$ +12° (*c* 1); R_f 0.16 (8:1 toluene–MeOH); NMR ($CDCl_3$): ^1H, δ 7.80–6.85 (m, 24 H, 4 Ph and Phth), 5.392 (m, 1 H, H-8''), 5.320 (dd, 1 H, $J_{6'',7''}$ 1.9, $J_{7'',8''}$ 7.8 Hz, H-7''), 5.231 (d, 1 H, $J_{NH,4''}$ 9.8 Hz, NH), 5.078 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 3.764 (s, 3 H, OMe), 2.517 (dd, 1 H, $J_{3''eq,3''ax}$ 13.0, $J_{3''eq,4''}$ 4.7 Hz, H-3''eq), 2.081, 2.022, 1.975, 1.947, and 1.878 (5 s, each 3 H, 4 Ac and NAc); ^{13}C, δ 102.5 (C-1'), 98.4 (C-2''), 98.1 (C-1), 75.0, 74.3, 73.3, 73.0, 68.8 (2 C), 67.9, and 62.2 (C-6,6',9'', 4 Ph*C*H_2O and O*C*$H_2C_4H_8N_3$), 51.0, 28.6, 28.2, and 22.9 (OCH_2*C*$_4$$H_8N_3$), 49.1 (O*C*$H_3$), 37.1 (C-3''), 23.0 (NHCO*C*H_3). Anal. Calcd for $C_{74}H_{85}N_5O_{24}$: C, 62.22; H, 6.00. Found: C, 62.06; H, 5.98. Compound **19β**: $[\alpha]_D$ +17° (*c* 1); R_f 0.21 (8:1 toluene–MeOH); NMR ($CDCl_3$): ^1H, δ 7.70–6.81 (m, 24 H, 4 Ph and Phth), 3.671 (s, 3 H, OMe), 2.550 (dd, 1 H, $J_{3''eq,3''ax}$ 13.3, $J_{3''eq,4''}$ 4.5 Hz, H-3''eq), 2.100, 2.089, 1.990, 1.984, and 1.738 (5 s, each 3 H, 4 Ac and NAc). Anal. Calcd for $C_{74}H_{85}N_5O_{24}$: C, 62.22; H, 6.00. Found: C, 62.36; H, 6.10.

O-Acetylation of a small amount of **19** with 1:1 Ac_2O–pyridine for 16 h at 50 °C, followed by co-concn with toluene (3 ×), afforded **20**; NMR ($CDCl_3$): ^1H, δ 7.85–6.80 (m, 24 H, 4 Ph and Phth), 5.595 (m, 1 H, H-8''), 5.350 (dd, 1 H, $J_{6'',7''}$ 2.5, $J_{7'',8''}$ 8.4 Hz, H-7''), 5.037 (dd, 1 H, $J_{3',4'}$ 3.9, $J_{4',5'}$ < 1 Hz, H-4'), 3.827 (s, 3 H, OMe), 2.594 (dd, 1 H, $J_{3''eq,3''ax}$ 12.6, $J_{3''eq,4''}$ 4.7 Hz, H-3''eq), 2.078, 2.015, 1.971, 1.954, 1.862, and 1.834 (6 s, each 3 H, 5 Ac and NAc).

*5-Azidopentyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-non-2-ulopyranosylonate)-(2 → 3)-[(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-galactopyranosyl)-(1 → 4)]-(2,6-di-*O*-benzyl-β-*D*-galactopyranosyl)-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**22**).—A mixture of **19** (69 mg, 48 μmol) and $HgBr_2$ (69 mg, 192 μmol) in dry toluene (2 mL), containing powdered 4 Å molecular sieves (0.1 g),

was stirred for 30 min at room temperature under Ar. A soln of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-α-D-galactopyranosyl bromide [32] (**21**; 48 mg, 96 μmol) in dry acetonitrile (2 mL) and dry toluene (1 mL) was added dropwise in 55 min. After stirring for an additional 2.5 h, TLC (6:4 toluene–acetone) showed the formation of mainly **22** (R_f 0.25) and a minor amount of **25** (R_f 0.21). The mixture was diluted with CH_2Cl_2 (50 mL), filtered through Celite, washed with aq 5% KI (2 ×) and water, dried (Na_2SO_4), filtered, and concd. Size-exclusion chromatography (1:1 CH_2Cl_2–MeOH) of the residue on Sephadex LH-20 resulted in a sequential elution of **25** and **22**, whereby **22** was still contaminated with **25** and **19**. In order to make a separation of **22** and **19** accessible, crude **22** was treated with 1:1:5 trimethylsilylchloride–1,1,1,3,3,3-hexamethyldisilazane–dry pyridine (0.7 mL) for 30 min, affording the conversion (TLC, 6:4 toluene–acetone) of **19** (R_f 0.28) into 4′-*O*-trimethylsilylated **19** (R_f 0.33). After concn, column chromatography (6:4 toluene–acetone) of the residue afforded 4′-*O*-trimethylsilylated **19** (32 mg, 44%), and then **22**, isolated as a colorless glass (28 mg, 31%); $[\alpha]_D$ −4° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.99–6.90 (m, 28 H, 4 Ph and 2 Phth), 6.114 (dd, 1 H, $J_{2''',3'''}$ 11.7, $J_{3''',4'''}$ 3.5 Hz, H-3‴), 5.489 (dd, 1 H, $J_{4''',5'''} < 1$ Hz, H-4‴), 5.379 (d, 1 H, $J_{1''',2'''}$ 8.3 Hz, H-1‴), 5.300 (dd, 1 H, $J_{6'',7''}$ 2.4, $J_{7'',8''}$ 8.9 Hz, H-7″), 3.910 (s, 3 H, OMe), 2.166, 2.080, 2.057, 1.954, 1.939, 1.864, 1.823, and 1.763 (8 s, each 3 H, 7 Ac and NAc); ^{13}C, δ 101.8 (C-1′), 98.3 (C-2″), 97.9 (2 C) (C-1,1″), 51.0, 28.6, 28.2, and 22.9 ($OCH_2C_4H_8N_3$), 49.0 (OCH_3), 36.9 (C-3″), 23.0 ($NHCOCH_3$). FAB$^+$MS ($C_{93}H_{104}N_6O_{33}$): *m/z* 1856 $[M + Na]^+$, 1834 $[M + H]^+$.

Compound **25**; FAB$^+$MS ($C_{113}H_{123}N_7O_{42}$): *m/z* 2273 $[M + Na]^+$, 2251 $[M + H]^+$.

5-Azidopentyl (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-*non-2-ulopyranosylonic acid)-(2 → 3)-[(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1 → 4)]-(2, 6-di-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-2-acetamido-3, 6-di-O-benzyl-2-deoxy-β-D-glucopyranoside* (**24**).—A soln of **22** (43 mg, 23 μmol) and LiI (64 mg, 0.48 mmol) in dry pyridine (2 mL) was stirred overnight in the dark at 115 °C under Ar. Then, TLC (93:7 CH_2Cl_2–MeOH) showed the de-esterification to be complete (**23**; R_f 0.40), and after cooling to room temperature the mixture was co-concd with toluene (2 ×). The residue was dissolved in CH_2Cl_2 (50 mL), washed with M HCl (2 ×) and aq 5% NaCl (3 ×), and the combined aq layers were extracted with CH_2Cl_2 (20 mL). The combined organic phases were dried (Na_2SO_4), filtered, and concd. Column chromatography (93:7 CH_2Cl_2–MeOH) of the residue afforded **23**, isolated as a glass (32 mg, 75%); ^{1}H NMR (CD_3OD): δ 7.80–6.95 (m, 28 H, 4 Ph and 2 Phth), 6.178 (dd, 1 H, $J_{2''',3'''}$ 11.7, $J_{3''',4'''}$ 3.5 Hz, H-3‴), 5.594 (d, 1 H, $J_{1''',2'''}$ 8.4 Hz, H-1‴), 5.532 (m, 1 H, H-8″), 5.486 (dd, 1 H, $J_{4''',5'''} < 1$ Hz, H-4‴), 5.279 (dd, 1 H, $J_{6'',7''}$ 2.5, $J_{7'',8''}$ 8.3 Hz, H-7″), 2.052, 2.046, 2.013, 2.007, 1.866, 1.804, 1.774, and 1.689 (8 s, each 3 H, 7 Ac and NAc).

A soln of **23** (32 mg, 18 μmol) in ethanolic 33% $MeNH_2$ (5 mL) was stirred at room temperature for 10 d, during which time the mixture was repeatedly concd and new reagent (4 × 5 mL) added. After concn, the residue was dissolved in dry MeOH (3 mL), Ac_2O (40 mL) was added at 0 °C, and the mixture was stirred for 2 h. Then, TLC (1:1 CH_2Cl_2–MeOH) showed a complete conversion of **23** into **24** (R_f 0.60), and the soln was concd. Column chromatography (6:4:0.3 CH_2Cl_2–MeOH–water) of the residue afforded **24**, isolated as a colorless glass (23 mg, 95%); $[\alpha]_D$ +11° (*c* 1, MeOH); ^{1}H NMR (CD_3OD): δ 7.51–7.16 (m, 20 H, 4 Ph), 3.256 (t, 2 H, *J* 6.7 Hz, $OC_4H_8CH_2N_3$), 2.796 (dd, 1 H, $J_{3''eq,3''ax}$ 13.0, $J_{3''eq,4''}$ 5.2 Hz, H-3″eq), 2.043, 2.019, and 1.913 (3 s, each 3 H, 3 NAc). FAB$^-$MS ($C_{66}H_{88}N_6O_{24}$): *m/z* 1347 $[M - H]^-$.

5-Aminopentyl (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-*non-2-ulopyranosylonic acid)-(2 → 3)-[(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1 → 4)]-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside* (**1**).—A soln of **24** (17 mg, 12 μmol) in *tert*-butanol (1.8 mL) and water (0.7 mL), adjusted to pH 10–11 with a few drops of aq 25% NH_3, was hydrogenolysed for 1 h in the presence of 10% Pd–C (35 mg). TLC (5:10:4 CH_2Cl_2–MeOH–water) then showed the conversion of **24** into a new compound (R_f 0.75). The soln was flushed with N_2 until pH 7, HOAc was added until pH 4–5, and the hydrogenolysis was continued for 9 h, after which TLC (5:10:4 CH_2Cl_2–MeOH–water) showed the formation of **1** (R_f 0.45). The mixture was filtered through Celite and concd. Column chromatography (5:10:3 CH_2Cl_2–MeOH–water) of the residue, followed by lyophilization from water, afforded **1**, isolated as a white powder (6.4 mg, 54%); $[\alpha]_D$ +2° (*c* 0.25, water); ^{1}H NMR (D_2O): see Table 1. FAB − MS ($C_{38}H_{66}N_4O_{24}$): *m/z* 961 $[M - H]^-$; FAB$^+$MS: *m/z* 963 $[M + H]^+$.

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

benzyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-O-benzyl-2 - deoxy - 2 - phthalimido - β - D - glucopyranoside (**27**).—To a soln of 5-azidopentyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-(1 → 4)-(2,6-di-*O*-benzyl-3-*O*-*p*-methoxybenzyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside [42] (**26**; 100 mg, 68 μmol) in acetonitrile (4.5 mL) and water (0.5 mL) was added ammonium cerium(IV) nitrate (97 mg, 177 μmol), and the mixture was stirred for 2 h at room temperature. Then, TLC (94:6 CH_2Cl_2–acetone) showed the disappearance of **26** and the formation of **27** (R_f 0.24). The mixture was diluted with CH_2Cl_2 (50 mL), washed with aq 10% $NaHCO_3$ (3 ×), and the aq layers were combined and extracted with CH_2Cl_2 (10 mL). The organic layers were combined, dried (Na_2SO_4), filtered, and concd. Column chromatography (94:6 CH_2Cl_2–acetone) of the residue afforded **27**, isolated as a colorless syrup (56 mg, 61%); $[\alpha]_D$ −15° (*c* 1); NMR ($CDCl_3$): ^1H, δ 7.95–6.90 (m, 28 H, 4 Ph and 2 Phth), 6.042 (dd, 1 H, $J_{2'',3''}$ 11.6, $J_{3'',4''}$ 3.5 Hz, H-3''), 5.481 (d, 1 H, H-4''), 5.421 (d, 1 H, $J_{1'',2''}$ 8.4 Hz, H-1''), 5.096 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 2.146, 1.998, and 1.825 (3 s, each 3 H, 3 Ac); ^{13}C, δ 170.2 (2 C), 169.6, 168.0, and 167.2 (3 $COCH_3$ and *C*OPhth), 102.1 (C-1'), 99.5 (C-1''), 98.2 (C-1), 80.8, 77.6, 76.7, 75.8, 75.1, 73.7, 72.9, 70.2, 67.3, and 66.4 (C-3,4,5,2',3',4',5',3'',4'',5''), 55.6 and 51.3 (C-2,2''), 51.0, 28.6, 28.2, and 22.9 ($OCH_2C_4H_8N_3$), 20.6, 20.5, and 20.4 (3 $COCH_3$).

5-Azidopentyl (methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2 → 3)-[(2,3,4,6-tetra-O-β-D-galactopyranosyl)-(1 → 4)]-(2,6-di-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**29**).—A mixture of **19** (69 mg, 48 μmol) and silver triflate (49 mg, 191 μmol) in dry toluene (3 mL), containing powdered 4 Å molecular sieves (0.1 g), was stirred for 10 min at room temperature under Ar. Then, a soln of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**5**; 59 mg, 144 μmol) in dry CH_2Cl_2 (2.5 mL) was added dropwise during 20 min at 0 °C. After stirring for an additional 30 min, TLC (6:4 toluene–acetone) showed the formation of **29** (R_f 0.26) and the almost complete disappearance of **19** (R_f 0.30). The mixture was neutralized with Et_3N, diluted with CH_2Cl_2 (50 mL), filtered through Celite, washed with aq 5% $Na_2S_2O_3$ (2 ×) and water, dried (Na_2SO_4), filtered, and concd. Size-exclusion chromatography (1:1 CH_2Cl_2–MeOH) of the residue on Sephadex LH-20, followed by column chromatography (6:4 toluene–acetone) of the crude product afforded **29**, isolated as a colorless glass (42 mg, 49%); $[\alpha]_D$ 0° (*c* 1); NMR ($CDCl_3$): ^1H, δ 7.90–6.99 (m, 24 H, 4 Ph and Phth), 5.412 (dd, 1 H, $J_{3''',4'''}$ 2.0, $J_{4''',5'''} < 1$ Hz, H-4'''), 3.827 (s, 3 H, OMe), 2.421 (dd, 1 H, $J_{3''eq,3''ax}$ 13.4, $J_{3''eq,4''}$ 4.8 Hz, H-3''eq), 2.139, 2.103, 2.006, 1.997, 1.982, 1.972, 1.947, 1.938, and 1.903 (9 s, each 3 H, 8 Ac and NAc); ^{13}C, δ 102.3 and 101.7 (C-1',1'''), 99.5 (C-2''), 98.2 (C-1), 51.0, 28.6, 28.2, and 22.9 ($OCH_2C_4H_8N_3$), 49.1 (OCH_3), 35.5 (C-3''), 23.0 ($NHCOCH_3$). FAB$^+$MS ($C_{87}H_{103}N_5O_{33}$): *m/z* 1768 $[M + Na]^+$, 1746 $[M + H]^+$.

5-Azidopentyl (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2 → 3)-[β-D-galactopyranosyl-(1 → 4)]-(2,6-di-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (**31**).—A soln of **29** (28 mg, 16 μmol) and LiI (38 mg, 0.28 mmol) in dry pyridine (2 mL) was stirred overnight in the dark at 115 °C under Ar. Then, TLC (93:7 CH_2Cl_2–MeOH) showed the de-esterification to be complete (**30**; R_f 0.22), and after cooling to room temperature the mixture was co-concd with toluene (2 ×). The residue was dissolved in CH_2Cl_2 (50 mL), washed with M HCl (2 ×) and aq 5% NaCl (3 ×), and the combined aq layers were extracted with CH_2Cl_2 (10 mL). The combined organic phases were dried (Na_2SO_4), filtered, and concd. Column chromatography (93:7 CH_2Cl_2–MeOH) of the residue afforded **30**, isolated as a glass (24 mg, 86%); NMR (CD_3OD): ^1H, δ 7.95–6.95 (m, 24 H, 4 Ph and Phth), 5.608 (m, 1 H, H-8''), 2.046, 2.017, 1.985, 1.973, 1.910, 1.879, and 1.823 (7 s, 3,6,3,3,3,3,6 H, 8 Ac and NAc).

A soln of **30** (24 mg, 14 μmol) in ethanolic 33% $MeNH_2$ (6 mL) was stirred for 10 d at room temperature, during which repeatedly the mixture was concd and new reagent (3 × 5 mL) added. After concn, the residue was dissolved in dry MeOH (2 mL), and Ac_2O (30 μL) was added at 0 °C. After stirring for 2 h, TLC (6:4:0.3 CH_2Cl_2–MeOH–water) showed a complete conversion of **30** into **31** (R_f 0.50), and the soln was concd. Column chromatography (6:4:0.3 CH_2Cl_2–MeOH–water) of the residue gave **31**, isolated as a colorless glass (15 mg, 82%); $[\alpha]_D$ +3° (*c* 1, MeOH); ^1H NMR (CD_3OD): δ 7.49–7.18 (m, 20 H, 4 Ph), 3.256 (t, 2 H, *J* 6.8 Hz, $OC_4H_8CH_2N_3$), 2.806 (dd, 1 H, $J_{3''eq,3''ax}$ 12.6, $J_{3''eq,4''}$ 5.1 Hz, H-3''eq), 2.015 and 1.890 (2 s, each 3 H, 2 NAc). FAB$^-$MS ($C_{64}H_{85}N_5O_{24}$): *m/z* 1306 $[M - H]^-$.

5-Aminopentyl (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2 → 3)-[β-

D-*galactopyranosyl-(1 → 4)]-β-*D-*galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-*D-*glucopyranoside* (**2**).—A soln of **31** (12.0 mg, 9.0 μmol) in *tert*-butanol (1.8 mL) and water (0.7 mL), adjusted to pH 10–11 with a few drops of aq 25% NH_3, was hydrogenolysed for 1 h in the presence of 10% Pd–C (30 mg). TLC (5:10:4 CH_2Cl_2–MeOH–water) then showed the disappearance of **31** and the formation of several new compounds. The soln was flushed with N_2 until pH 7, HOAc was added until pH 4–5, and the hydrogenolysis was continued for 4.5 h, after which TLC (5:10:4 CH_2Cl_2–MeOH–water) showed the formation of **2** (R_f 0.51). The mixture was filtered through Celite and concd. Column chromatography (5:10:3 CH_2Cl_2–MeOH–water) of the residue, followed by lyophilization from water, afforded **2**, isolated as a white powder (3.9 mg, 47%); $[\alpha]_D$ +3° (*c* 0.25, water); ^{1}H NMR (D_2O): see Table 1. FAB$^-$MS ($C_{36}H_{63}N_3O_{24}$): *m/z* 920 $[M-H]^-$; FAB$^+$MS: *m/z* 922 $[M+H]^+$.

*5-Azidopentyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D-glycero-α-D-galacto-*non-2-ulopyranosylonate)-(2 → 3)-[(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D-*glucopyranosyl)-(1 → 4)]-(2,6-di-*O*-benzyl-β-*D-*galactopyranosyl)-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D-*glucopyranoside* (**33**).—A soln of **19** (47 mg, 33 μmol) in dry CH_2Cl_2 (1.5 mL), containing 3 Å molecular sieves (0.1 g), was stirred for 30 min at room temperature under Ar. A soln of trimethylsilyl triflate (2.4 μL, 13 μmol) in dry CH_2Cl_2 (100 μL) was added at room temperature, followed by the dropwise addition of a soln of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate [37] (**32**; 49 mg, 85 μmol) in dry CH_2Cl_2 (2.5 mL) during 10 min. Over a period of 4 h, TLC (7:3 toluene–acetone) showed the formation of **33** (R_f 0.19), but a complete conversion of **19** could not be realized. Pyridine (0.1 mL) was added, and the mixture was diluted with CH_2Cl_2 (50 mL), filtered, washed with water (2 ×), dried (Na_2SO_4), filtered, and concd. Column chromatography (7:3 toluene–acetone) of the residue resulted in a sequential elution of the byproduct 3,4,6-tri-*O*-acetyl-1,2-dideoxy-2-phthalimido-D-*arabino*-hex-1-enopyranose [44], **19** (17 mg, 36%), and finally **33**, isolated as a colorless glass (20 mg, 33%); $[\alpha]_D$ +2° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.95–6.80 (m, 28 H, 4 Ph and 2 Phth), 6.053 (dd, 1 H, *J* 9.1, *J* 10.9 Hz, H-3‴), 5.422 (d, 1 H, $J_{1''',2'''}$ 8.4 Hz, H-1‴), 5.384 (ddd, 1 H, $J_{7''',8'''}$ 8.9, $J_{8''',9a'''}$ 2.6, $J_{8''',9b'''}$ 4.9 Hz, H-8‴), 5.286 (dd, 1 H, $J_{6'',7''}$ 2.3 Hz, H-7″), 4.785 (ddd, 1 H, $J_{3''ax,4''}$ 12.3, $J_{3''eq,4''}$ 4.5, $J_{4'',5''}$ 10.7 Hz, H-4″), 3.917 (s, 3 H, OMe), 2.085, 2.044, 2.008, 1.998, 1.945, 1.867, 1.822, and 1.785 (8 s, each 3 H, 7 Ac and NAc); ^{13}C, δ 101.8 (C-1′), 98.6 (C-2″), 97.9 and 97.8 (C-1,1‴), 51.0, 28.6, 28.2, and 22.9 ($OCH_2C_4H_8N_3$), 49.0 (OCH_3), 36.7 (C-3″), 23.1 ($NHCOCH_3$). FAB$^+$MS ($C_{93}H_{104}N_6O_{33}$): *m/z* 1856 $[M+Na]^+$, 1834 $[M+H]^+$.

*5-Azidopentyl (5-acetamido-3,5-dideoxy-*D-glycero-*α-*D-galacto-*non-2-ulopyranosylonic acid)-(2 → 3)-[(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1 → 4)]-(2,6-di-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside* (**35**).—A soln of **33** (57 mg, 31 μmol) and LiI (128 mg, 0.96 mmol) in dry pyridine (2 mL) was stirred overnight in the dark at 115 °C under Ar. Then, TLC (9:1 CH_2Cl_2–MeOH) showed the de-esterification to be complete (**34**; R_f 0.61), and after cooling to room temperature the mixture was co-concd with toluene (2 ×). The residue was dissolved in CH_2Cl_2 (50 mL), washed with M HCl (2 ×) and aq 5% NaCl (3 ×), and the combined aq layers were extracted with CH_2Cl_2 (20 mL). The combined organic phases were dried (Na_2SO_4), filtered, and concd. Column chromatography (93:7 CH_2Cl_2–MeOH) of the residue afforded **34**, isolated as a light yellow glass (45 mg, 80%); ^{1}H NMR (CD_3OD): δ 7.90–6.99 (m, 28 H, 4 Ph and 2 Phth), 6.120 (dd, 1 H, *J* 9.0, *J* 10.8 Hz, H-3‴), 5.682 (d, 1 H, $J_{1''',2'''}$ 8.3 Hz, H-1‴), 5.514 (m, 1 H, H-8″), 5.274 (dd, 1 H, $J_{6'',7''}$ 2.3, $J_{7'',8''}$ 7.9 Hz, H-7″), 2.051, 2.042, 2.018, 1.971, 1.878, 1.812, 1.777, and 1.702 (8 s, each 3 H, 7 Ac and NAc).

A soln of **34** (18 mg, 10 μmol), in ethanolic 33% $MeNH_2$ (4 mL), was stirred for 9 d at room temperature, during which the mixture was repeatedly concd and new reagent (4 × 4 mL) added. After concn, the residue was dissolved in dry MeOH (1.5 mL), Ac_2O (25 mL) was added at 0 °C, and the mixture was stirred for 2 h. Then, TLC (1:1 CH_2Cl_2–MeOH) showed a complete conversion of **34** into **35** (R_f 0.57), and the soln was concd. Column chromatography (6:4:0.2 CH_2Cl_2–MeOH–water) of the residue gave **35**, isolated as a colorless glass (11 mg, 81%); $[\alpha]_D$ +6° (*c* 1, MeOH); NMR (CD_3OD): ^{1}H, δ 7.45–7.18 (m, 20 H, 4 Ph), 3.258 (t, 2 H, *J* 6.7 Hz, $OC_4H_8CH_2N_3$), 2.797 (dd, 1 H, $J_{3''eq,3''ax}$ 12.6, $J_{3''eq,4''}$ 4.8 Hz, H-3″eq), 2.035, 2.016, and 1.928 (3 s, each 3 H, 3 NAc). FAB$^-$MS ($C_{66}H_{88}N_6O_{24}$): *m/z* 1347 $[M-H]^-$.

*5-Aminopentyl (5-acetamido-3,5-dideoxy-*D-glycero-*α-*D-galacto-*non-2-ulopyranosylonic acid)-(2 → 3)-[(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1 → 4)]-β-*D-

*galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**3**).—A soln of **35** (12 mg, 8.7 μmol) in *tert*-butanol (1.8 mL) and water (0.7 mL), adjusted to pH 10–11 with a few drops of aq 25% NH_3, was hydrogenolysed for 1 h in the presence of 10% Pd–C (30 mg). Then, TLC (5:10:4 CH_2Cl_2–MeOH–water) showed the disappearance of **35** and the formation of two new compounds (R_f 0.75 and R_f 0.79). The soln was flushed with N_2 until pH 7, HOAc was added until pH 4–5, and the hydrogenolysis was continued for 5 h, after which TLC (5:10:4 CH_2Cl_2–MeOH–water) showed the formation of **3** (R_f 0.45). The mixture was filtered through Celite and concd. Column chromatography (5:10:3 CH_2Cl_2–MeOH–water) of the residue, followed by lyophilization from water, afforded **3**, isolated as a white powder (4.2 mg, 50%); $[\alpha]_D$ +1° (*c* 0.25, water); NMR (D_2O): ^1H, see Table 1. FAB$^-$MS ($C_{38}H_{66}N_4O_{24}$): *m/z* 961 $[M-H]^-$; FAB$^+$MS: *m/z* 963 $[M+H]^+$.

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References

[1] A.S.R. Donald, A.D. Yates, C.P.C. Soh, W.T.J. Morgan, and W.M. Watkins, *Biochem. Biophys. Res. Commun.*, 115 (1983) 625–631; J. Williams, R.D. Marshall, H. van Halbeek, and J.F.G. Vliegenthart, *Carbohydr. Res.*, 134 (1984) 141–155.

[2] S.I. Macvie, J.A. Morton, and M.M. Pickles, *Vox Sang.*, 13 (1967) 485–492; P.H. Renton, P. Howell, E. Ikin, C.M. Giles, and K.L.G. Goldsmith, ibid, 13 (1967) 493–501.

[3] W.M. Watkins, in J.-P. Cartron and P. Rouger (Eds.), *Blood Cell Biochemistry: Molecular Basis of Major Human Blood Group Antigens*, Vol. 6, Plenum Press, New York, 1995, pp 351–375, and refs. cited therein.

[4] D. Blanchard, J.-P. Cartron, B. Fournet, J. Montreuil, H. van Halbeek, and J.F.G. Vliegenthart, *J. Biol. Chem.*, 258 (1983) 7691–7695.

[5] B.K. Gillard, D. Blanchard, J.-F. Bouhours, J.-P. Cartron, J.A. van Kuik, J.P. Kamerling, J.F.G. Vliegenthart, and D.M. Marcus, *Biochemistry*, 27 (1988) 4601–4606.

[6] A. Muchmore and J.M. Decker, *Science*, 229 (1985) 479–481.

[7] J.S. Hunt, A.R. McGiven, A. Groufsky, K.L. Lynn, and M.C. Taylor, *Biochem. J.*, 227 (1985) 957–963.

[8] K. Härd, G. van Zadelhoff, P. Moonen, J.P. Kamerling, and J.F.G. Vliegenthart, *Eur. J. Biochem.*, 209 (1992) 895–915.

[9] A.-M.M. Afonso, P.A. Charlwood, and R.D. Marshall, *Carbohydr. Res.*, 89 (1981) 309–319.

[10] G. Toma, J.M. Bates, and S. Kumar, *Biochem. Biophys. Res. Commun.*, 200 (1994) 275–282.

[11] N. Sathyamoorthy, J.M. Decker, A.P. Sherblom, and A.V. Muchmore, *Mol. Cell. Biochem.*, 102 (1991) 139–147, and refs. cited therein.

[12] S. Ladisch, R. Li, and E. Olson, *Proc. Natl. Acad. Sci. USA*, 91 (1994) 1974–1978.

[13] R. Li, N. Villacreses, and S. Ladisch, *Cancer Res.*, 55 (1995) 211–214.

[14] J. Parkkinen, R. Virkola, and T.K. Korhonen, *Infect. Immun.*, 56 (1988) 2623–2630.

[15] J.L. Duncan, *J. Infect. Dis.*, 158 (1988) 1379–1382.

[16] C.P.C. Soh, A.S.R. Donald, J. Feeney, W.T.J. Morgan, and W.M. Watkins, *Glycoconjugate J.*, 6 (1989) 319–332.

[17] A. Marra and P. Sinaÿ, *Gazz. Chim. Ital.*, 117 (1987) 563–566.

[18] M. Sugimoto, M. Numata, K. Koike, Y. Nakahara, and T. Ogawa, *Carbohydr. Res.*, 156 (1986) C1–C5.

[19] S. Inoue, M. Iwasaki, K. Ishii, K. Kitajima, and Y. Inoue, *J. Biol. Chem.*, 264 (1989) 18520–18526.

[20] A. Hasegawa and M. Kiso, in H. Ogura, A. Hasegawa, and T. Suami (Eds.), *Carbohydrates*, Kodansha Ltd., Tokyo, 1992, pp 243–266; T. Ogawa, *Chem. Soc. Rev.*, (1994) 397–407; G. Magnusson, *Adv. Drug Del. Rev.*, 13 (1994) 267–284, and refs. cited therein.

[21] J. Kerékgyártó, J.G.M. van der Ven, A. Lipták, J.P. Kamerling, and J.F.G. Vliegenthart, *Carbohydr. Res.*, 238 (1993) 135–145.

[22] G. Catalani, F. Colonni, and A. Marra, *Carbohydr. Res.*, 182 (1988) 297–300.

[23] Y. Ito and T. Ogawa, *Agric. Biol. Chem.*, 50 (1986) 3227–3230.

[24] N.M. Spijker, C.A. Keuning, M. Hooglugt, G.H. Veeneman, and C.A.A. van Boeckel, *Tetrahedron*, 52 (1996) 5945–5960.

[25] O. Mitsunobu, *Synthesis*, (1981) 1–28.

[26] G.H. Veeneman, S.H. van Leeuwen, and J.H. van Boom, *Tetrahedron Lett.*, 31 (1990) 1331–1334; P. Konradsson, U.E. Udodong, and B. Fraser-Reid, *Tetrahedron Lett.*, 31 (1990) 4313–4316.

[27] A. Marra and P. Sinaÿ, *Carbohydr. Res.*, 187 (1989) 35–42.

[28] H. Lönn and K. Stenvall, *Tetrahedron Lett.*, 33 (1992) 115–116.

[29] K. Okamoto, T. Kondo, and T. Goto, *Bull. Chem. Soc. Jpn.*, 60 (1987) 637–643.

[30] A. Hasegawa, H. Ohki, T. Nagahama, H. Ishida, and M. Kiso, *Carbohydr. Res.*, 212 (1991) 277–281.

[31] T.J. Martin and R.R. Schmidt, *Tetrahedron Lett.*, 33 (1992) 6123–6126.

[32] H. Paulsen and A. Bünsch, *Carbohydr. Res.*, 100 (1982) 143–167.
[33] R.R. Schmidt and A. Toepfer, *Tetrahedron Lett.*, 32 (1991) 3353–3356.
[34] J.-C. Jacquinet and H. Paulsen, *Tetrahedron Lett.*, 22 (1981) 1387–1390.
[35] S. Nunomura, Y. Matsuzaki, Y. Ito, and T. Ogawa, *Pure Appl. Chem.*, 66 (1994) 2123–2126.
[36] U. Ellervik and G. Magnusson, *Carbohydr. Res.*, 280 (1996) 251–260.
[37] G. Grundler and R.R. Schmidt, *Carbohydr. Res.*, 135 (1985) 203–218.
[38] F. Elsinger, J. Schreiber, and A. Eschenmoser, *Helv. Chim. Acta*, 43 (1960) 113–118.
[39] O. Kanie, S.C. Crawley, M.M. Palcic, and O. Hindsgaul, *Carbohydr. Res.*, 243 (1993) 139–164.
[40] W. Stahl, U. Sprengard, G. Kretschmar, and H. Kunz, *Angew. Chem. Int. Ed. Engl.*, 33 (1994) 2096–2098.
[41] B.P. Czech and R.A. Bartsch, *J. Org. Chem.*, 49 (1984) 4076–4078.
[42] P.B. van Seeventer, M.A. Corsten, M.P. Sanders, J.P. Kamerling, and J.F.G. Vliegenthart, *Carbohydr. Res.*, 299 (1997) 171–179.
[43] F. Dasgupta and P.J. Garegg, *Carbohydr. Res.*, 177 (1988) C13–C17.
[44] R.U. Lemieux, T. Takeda, and B.Y. Chung, *ACS Symp. Ser.*, 39 (1976) 90–115.
[45] B.A. Silwanis, R.I. El-Sokkay, M.A. Nashed, and H. Paulsen, *J. Carbohydr. Chem.*, 10 (1991) 1067–1078.
[46] S.B. Levery, *Glycoconjugate J.*, 8 (1991) 484–492, and refs cited therein.
[47] H. Wolff, in R. Adams (Ed.), *Organic Reactions*, Vol. 3, Wiley and Sons, New York, 1946, pp 307–336.
[48] D.J.M. van der Vleugel, F.R. Wasserburg, J.W. Zwikker, and J.F.G. Vliegenthart, *Carbohydr. Res.*, 104 (1982) 221–233.