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Synthesis of Neu5Ac- and Neu5Gc-α-(2→6′)-lactosamine 3-aminopropyl glycosides

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Abstract

In order to prepare 3-aminopropyl glycosides of Neu5Ac-α-(2→6′)-lactosamine trisaccharide **1**, and its *N*-glycolyl containing analogue Neu5Gc-α-(2→6′)-lactosamine **2**, a series of lactosamine acceptors with two, three, and four free OH groups in the galactose residue was studied in glycosylations with a conventional sialyl donor phenyl [methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α- and β-D-*galacto*-2-nonulopyranosid]onates (**3**) and a new donor phenyl [methyl 4,7,8,9-tetra-*O*-acetyl-5-(*N*-*tert*-butoxycarbonylacetamido)-3,5-dideoxy-2-thio-D-*glycero*-α- and β-D-*galacto*-2-nonulopyranosid]onates (**4**), respectively. The lactosamine 4′,6′-diol acceptor was found to be the most efficient in glycosylation with both **3** and **4**, while imide-type donor **4** gave slightly higher yields with all acceptors, and isolation of the reaction products was more convenient. In the trisaccharides, obtained by glycosylation with donor **4**, the 5-(*N*-*tert*-butoxycarbonylacetamido) moiety in the neuraminic acid could be efficiently transformed into the desired *N*-glycolyl fragment, indicating that such protected oligosaccharide derivatives are valuable precursors of sialo-oligosaccharides containing *N*-modified analogues of Neu5Ac.

Keywords: *N*-Acetylneuraminic acid; *N*-Glycolylneuraminic acid; Sialyllactosamine

1. Introduction

Sialo-oligosaccharides play an important role in the process of cell recognition and act as receptors of various lectins.[1] In this paper we describe the synthesis of 5-*N*-acetyl-sialyl-α-(2→6′)-lactosamine 3-aminopropyl glycoside (**1**) and its *N*-glycolyl analogue **2** to be further used as models for the investigations of carbohydrate specificity of sialyl-binding lectins — siglecs. Synthesis of a sialyl-α-(2→6′)-lactosamine derivative, but with 5-amino-3-oxapentyl spacer, has been described recently.[2]

The reported syntheses of sialo-oligosaccharides containing *N*-modified analogues of Neu5Ac used neuraminic acid donors in which the desired *N*-acyl fragment was already present.[3–5] Alternatively, Ogawa et al.[6] used the temporary N-Boc protected sialyl-donor **5** which allowed varying the acyl moiety at the nitrogen atom of the neuraminic acid residue after assembling the oligosaccharide backbone.

Recently it was reported[7,8] that bis *N*-acetylated imide-type sialyl-donor **6** was significantly more reactive and gave higher yields in glycosylations in comparison with its amide-

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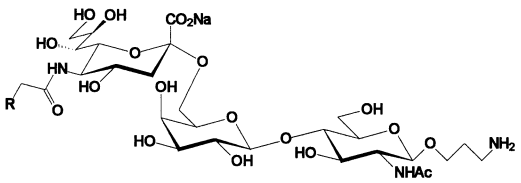
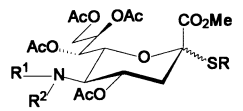
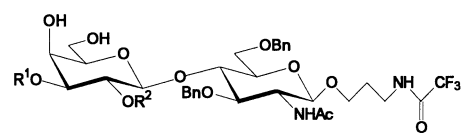
type counterpart **7**. Furthermore, no formation of glycal **12** was observed during glycosylation with the imide donor **6**, thus the reaction could be performed with a 1:1 donor–acceptor ratio. This is in sharp contrast to the traditionally used conditions by Hasegawa et al.[3,9] in which an approximately twofold excess of amide donor **3** or **7** is employed and glycal **11** is a permanent byproduct.



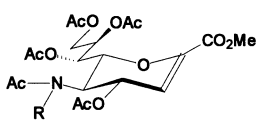
1 R=H (Neu5Ac)
2 R=OH (Neu5Gc)



3 R=Ph, R^1=Ac, R^2=H
4 R=Ph, R^1=Ac, R^2=Boc
5 R=Me, R^1=Boc, R^2=H
6 R=Me, R^1=R^2=Ac,
7 R=Me, R^1=Ac, R^2=H



8 R^1=Bz, R^2=Ac
9 R^1=Bz, R^2=H
10 R^1=R^2=H



11 R=H
12 R=Ac
13 R=Boc

This finding prompted us to investigate a new imide-type sialyl donor **4**, carrying Boc and Ac moieties at the neuraminic acid nitrogen. The donor **4** was chosen because its application opens ready access to Neu5acyl-modified analogues of 6′-sialyl-lactosamine via selective N-deacylation[10] of the mixed Ac–N–Boc imide into the corresponding H–N–Boc carbamate, subsequent Boc group removal, and acylation. Similar methodology has already been applied for the synthesis of (1→5)-amide-linked amino acid dimers derived from sialic acids.[11] It is worth noting that this approach seems particularly attractive because it allows varying the acyl substitutent after assembling the oligosaccharide backbone.

The second aspect which was specially investigated in this work was evaluation of lactosamine acceptor properties in glycosylation with donors **3** and **4**. A systematic study of the influence of acceptor structure on the efficiency and stereoselectivity of its sialylation is currently available for monosaccharide galactose derivatives only: according to Hasegawa et al.[3] sialylation with methylthio donor **7** of 2-trimethylsilylethyl 3-*O*-benzoyl-β-D-galactopyranoside afforded 70% yield of α-(2→6)-linked disaccharide solely, while glycosylation of its 3-*O*-benzyl counterpart gave approximately the same yield of α/β mixtures in the ratio from 3:1 with DMTST as promoter to 2:1 with NIS–TfOH. We employed diol **8**, triol **9** and tetraol **10** in the present study, and the 3′-*O*-benzoyl protection in compounds **8** and **9** was chosen based on the above mentioned assumption that it would contribute to α-selectivity.

2. Results and discussion

Bis *N*-acylated donor **4** was prepared in 85% yield by treatment of **3**[12] with $(Boc)_2O$ and DMAP in refluxing THF.[13] Other conditions tested[10,13] led to incomplete conversion of the starting material **3** and afforded **4** in significantly lower yield. It is worth noting that the reaction product **4** could not be separated from the dark-colored non-carbohydrate impurities by silica gel column chromatography, and thus additional purification by gel-permeation chromatography on the Bio-Beads SX-3 in toluene was required. Compound **4** was formed as an inseparable 4:1 mixture of isomers at the nitrogen atom (NMR data) and their ratio remained unchanged during the

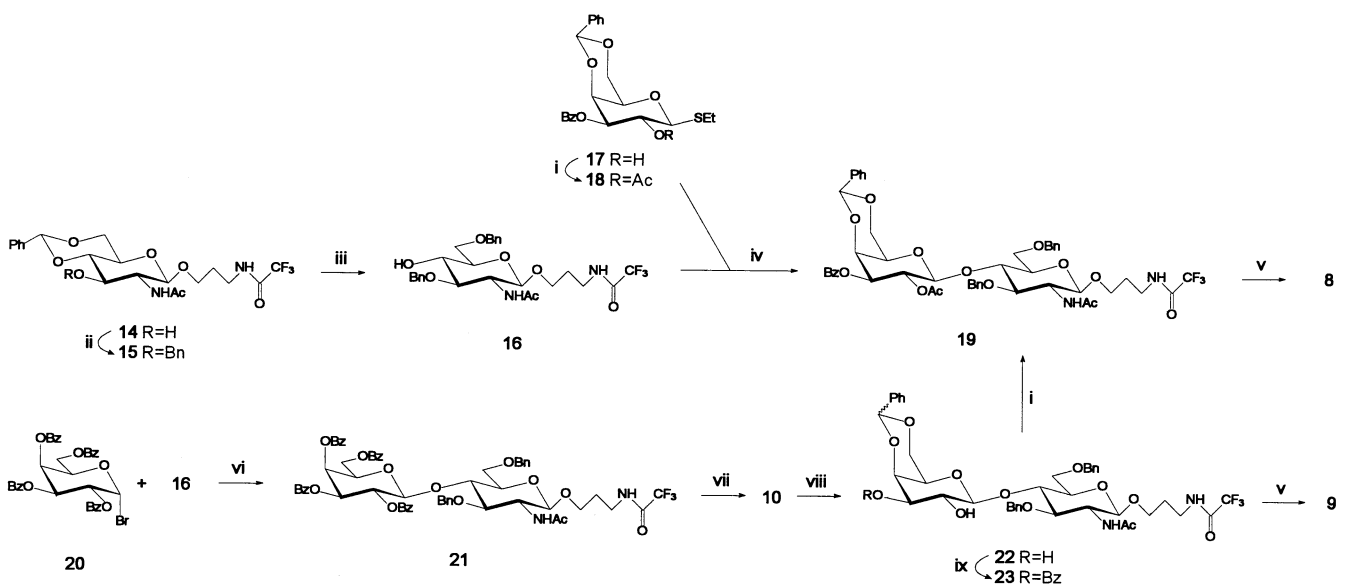
storage at room temperature in either $CDCl_3$ solution or in solid state.

The synthesis of lactosamine acceptors **8**, **9**, and **10** was initiated (Scheme 1) from benzylation of the known spacer-armed glucosamine derivative **14**.[14] In this reaction the excess of NaH was used in order to convert the $HNC(O)CF_3$ functionality into amide anion $NaNC(O)CF_3$ and thereby protect the base-liable trifluoroacetamido group. The trianion formed upon treatment of **14** with excess of NaH was regioselectively *O*-alkylated with benzyl bromide at -15 °C and then protonated with acetic acid to give **15** in 90% yield. The use of only a small excess of benzyl bromide was essential in order to avoid further undesired N-alkylation.

At the next stage the benzylidene acetal ring in compound **15** was subjected to reductive opening by treatment with $Me_3N{\cdot}BH_3$ and anhydrous $AlCl_3$ in dry THF under Ar at room temperature, i.e. following the known protocol.[15] However, after 24 h the unchanged starting material **15** and only traces ($\sim 10\%$) of target **16** were present in the reaction mixture and no further conversion was observed in another 24 h. The use of large (~ 20-fold) excess of the reagents did not improve the yield of **16**, while degradation took place upon heating. Subsequently we found that in the presence of water, mild reductive ring opening occurred. Thus treatment of **15** with $Me_3N{\cdot}BH_3$ (4 equiv), $AlCl_3$ (6 equiv), and water (2 equiv) afforded within hours the 6-*O*-Bn derivative **16** in 87% yield. Location of the benzyloxy group at C-6 in **16** was confirmed by the downfield shift of this carbon atom (70.9 ppm) and shielding of the C-4 signal from 77.6 in **15** to 72.2 ppm. We suppose that the protic acid formed from $AlCl_3$ and water is a much more powerful reagent rather than the equivalent Lewis acid. Other examples of benzylidene ring opening under such conditions will be published elsewhere.

Initially we designed compound **19** as the key intermediate for the preparation of lactosamine sialyl acceptors **8**, **9**, and **10**. Application of **19** allows ready access to diol **8** via debenzylidenation, tetraol **10** via further O-deacylation of **8**, and triol **9** by treatment of **19** with methanolic hydrogen chloride under the conditions of acid-catalyzed deacetylation.[16]

Condensation of **16** with thiogalactoside **18**, prepared by acetylation of **17**, promoted with $CuBr_2$–AgOTf–Bu_4NBr in $MeNO_2$[17] gave lactosamine **8**, isolated after debenzylidenation of **19**, in 37% yield (Scheme 1). The β configuration of the Gal residue in **8** was deduced from the value of $J_{1',2'}$ coupling constant 8 Hz, and no α anomer was formed. The presence of the AcO group at C-2′ position and BzO group at



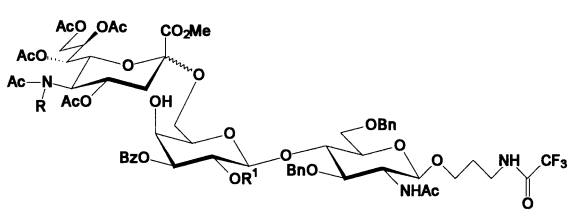
Scheme 1. Reagents and conditions: (i) Ac_2O, Py; (ii) NaH (5 equiv), BnBr (1.2 equiv), DMF, -15 °C, then AcOH; (iii) anhyd $AlCl_3$ (6 equiv), $Me_3N{\cdot}BH_3$ (4 equiv), water (2 equiv), dry THF, rt; (iv) $CuBr_2$, AgOTf, Bu_4NBr, $MeNO_2$, MS-4A, rt; (v) 90% aq CF_3CO_2H, CH_2Cl_2; (vi) AgOTf, CH_2Cl_2, MS-4A, rt; (vii) MeONa, MeOH; (viii) $PhCH(OMe)_2$, TsOH, DMF; (ix) BzCN, Py.

Table 1
Glycosylation of acceptors **8**, **9**, and **10** with donors **3** and **4**

Entry	Donor	Acceptor	Product	Yield (%)	α/β ratio
1	**3**	**8**	**24**	79	1.3:1
2	**3**	**9**	**25**	59	1.2:1
3	**4**	**8**	**26**	84	1.3:1
4	**4**	**9**	**27**	60	1:1
5	**3**	**10**	complex mixture	n.d.	n.d.

C-3′ was confirmed by the downfield location of H-2′ ($\delta_{H\text{-}2'}$ 5.33 ppm) and of H-3′ ($\delta_{H\text{-}3'}$ 4.79 ppm). Glycosylation of **16** with **18** under MeOTf promotion was not effective, whereas with NIS–TfOH gave orthoesters in low yield as the only coupling product. Similar moderate efficiency of glycosylation with 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-α-D-galactopyranosyl trichloroacetimidate or bromide of 5-azido-3-oxapentyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glycopyranoside has been described recently.[2]

Preparative synthesis (Scheme 1) of lactosamines **8**, **9**, and **10** was performed by the reaction sequence involved glycosylation of **16** with benzobromogalactose **20** in the presence of AgOTf to give **21**, NaOMe-catalyzed debenzoylation into **10**, 4′,6′-benzylidenation to produce **22**, regiospecific 3′-O-benzoylation with BzCN[18] to afford **23**, and hydrolytic removal of benzylidene acetal to give **9**. For the preparation of diol **8**, compound **23** was 2′-*O*-acetylated into **19** prior to hydrolysis into **8**. The presence of the Bz group in the 3′ position in compound **9** was confirmed by the downfield chemical shift of H-3′ ($\delta_{H\text{-}3'}$ 4.89 ppm), and neither 2′-O- nor 2′,3′-di-O-benzoylation was observed.



24 R=H, R^1=Ac
25 R=R^1=H
26 R=Boc, R^1=Ac
27 R=Boc, R^1=H

At the next stage of the synthesis of trisaccharides **1** and **2**, the glycosylation of acceptors **8**, **9**, and **10** with sialyl donors **3** and **4** was investigated. All the reactions were performed with 2 equiv of either donor **3** or **4** in MeCN at −35 °C in the presence of NIS, TfOH, and molecular sieves MS-3 Å, i.e., under the conditions recommended by Hasegawa et al.[9] The trisaccharides **24**–**27**, obtained after silica gel column chromatography were used for further synthetic steps as anomeric mixtures and separated into individual isomers by C18 reverse-phase column chromatography after complete removal of protecting groups. Therefore the anomeric ratios obtained in glycosylation reactions were estimated from NMR spectra of purified α/β mixtures **24**–**27** and proved after final separation of deblocked oligosaccharides.

The results of glycosylations (Table 1) indicate that better yields of sialyl-lactosamines were obtained with diol acceptor **8** (Entries 1 and 3), while triol acceptor **9** was less effective (Entries 2 and 4). Sialylation of tetraol **10** with **3** (Entry 5) afforded a complex mixture of at least five trisaccharide products without significant prevalence of any of them, thus indicating the absence of regioselectivity of the reaction. With both acceptors **8** and **9**, however, imide-type donor **4** afforded slightly higher yields, while the stereoselectivity of the glycosylation was found to be independent of both donor and acceptor structure.

During glycosylation with the imide-type donor **4**, formation of glycal **13** was observed in approximately the same quantity as in sialylation with amide-type donor **3**. Therefore a twofold excess of donor **4** was required, and about one half of unreacted acceptor was recovered when glycosylation with a 1:1 donor–acceptor ratio was attempted. It was found that Boc group introduction decreases the reactivity of the sialyl-donor: while **3** reacted within 4 h at −35 °C, **4** was completely

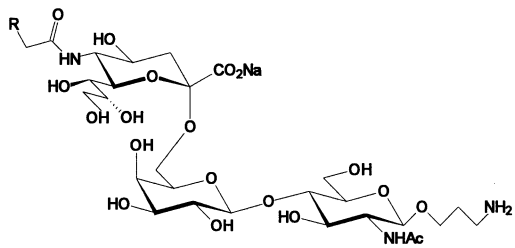
Table 2
Chemical shifts (δ, ppm) and coupling constants (J, Hz) for carbohydrate ring protons in ^{1}H NMR spectra of compounds **1**, **2**, **4** [a], **8**, **9**, **15**, **16**, **21**, **22**, **28**, **29** in the solvent specified

Compound (solvent)	Residue	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3$_{eq}$ ($J_{3eq,4}$)	H-3$_{ax}$ ($J_{3ax,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6a}$)	H-6a ($J_{6a,6b}$)	H-6b ($J_{6b,5}$)	H-7 ($J_{7,8}$)	H-8 ($J_{8,9a}$)	H-9a ($J_{9a,9b}$)	H-9b ($J_{9b,8}$)
1 (D_2O)	GN	4.53 (8.2)	3.76		3.77	3.63	3.62	3.99	3.83				
	Gal	4.45 (7.9)	3.51		3.64 (3.2)	3.92 (0)	3.82	3.97	3.53				
	Neu			2.67 [b] (4.5)	1.7 (12.3)	3.62	3.81	3.69		3.54	3.89	3.88	3.65
2 (D_2O)	GN	4.52 (8)	3.73		3.73	3.59	3.59	3.97	3.79				
	Gal	4.45 (7.7)	3.50		3.63	3.89	3.77	3.96	3.52				
	Neu			2.65 [c] (4.7)	1.69 (12.1)	3.74	3.86	3.79		3.52	3.86	3.84	3.61
4 [a] ($CDCl_3$)				2.73 [d] (4.8)	2.12 (11.2)	5.76 (10)	4.82 (10)	5.42 [e]		5.32 (1.7)	5.07 (2)	4.45 (12.3)	4.0 (8.1)
8 (1:10 CD_3OD–$CDCl_3$)	GN	4.34 (7.9)	3.63		3.56 (9.2)	3.88 (9.2)	3.33	3.69	3.62				
	Gal	4.48 (8)	5.33 (10.1)		4.79 (3)	4.09 (0)	3.34	3.68 (11.9)	3.50 (3.6)				
9 (CD_3OD)	GN	4.47 (8.1)	3.82		3.69 (9.5)	4.04 (9.5)	3.57 (3.4)	4.02 (12)	3.86				
	Gal	4.54 (7.8)	3.98 (10.2)		4.89 (3.4)	4.11 (0)	3.44	3.74	3.61				
15 (1:2 CD_3OD–$CDCl_3$)		4.44 (8.2)	3.60		3.55	3.70	3.31 (5)	4.16 (10.5)	3.65				
16 (acetone-d_6)		4.59 (8.9)	3.79 (10.2)		3.66 (10.2)	3.58	3.49 (1.6)	3.85 (10.8)	3.70 (5.8)				
21 ($CDCl_3$)	GN	4.53 (7.4)	3.65 (7.2)		3.87 (7.2)	4.15 (7.2)	3.44	3.72	3.68				
	Gal	4.83 (8.2)	5.73 (10)		5.47 (2.5)	5.91 (0)	3.99 (6.5)	4.51 (11.3)	4.31 (6.9)				
22 (2:1 CD_3OD–$CDCl_3$)	GN	4.44 (8.3)	3.71		3.68	3.90	3.44	3.93	3.81				
	Gal	4.43 (7.8)	3.59 (9.3)		3.42 (3.1)	4.15 (0)	3.09 (0)	4.17 (12.2)	3.88 (0)				
28 (D_2O)	GN	4.52 (8.1)	3.77		3.75	3.63	3.65	4.02	3.85				
	Gal	4.47 (7.9)	3.55		3.66 (3.3)	3.93 (0)	3.82	3.73	3.52				
	Neu			2.38 [f] (5)	1.62 (12.9)	4.07	3.87	3.78		3.54	3.85	3.85	3.67
29 (D_2O)	GN	4.51 (8)	3.77		3.81	3.61	3.60	3.99	3.82				
	Gal	4.49 (7.7)	3.53		3.62	3.92	3.78	3.71	3.53				
	Neu			2.35 [g] (5)	1.58 (12.1)	4.09	3.91	3.87		3.51	3.84	3.83	3.65

[a] β anomer, major isomer at the nitrogen atom.
[b] $J_{3eq,3ax}$ 12.3 Hz.
[c] $J_{3eq,3ax}$ 12.2 Hz.
[d] $J_{3eq,3ax}$ 13.7 Hz.
[e] $J_{6,7}$ 1.7 Hz.
[f] $J_{3eq,3ax}$ 12.9 Hz.
[g] $J_{3eq,3ax}$ 12.1 Hz.

consumed only after overnight glycosylation at −35 °C. It is also worth noting that **4** has a much more hydrophobic nature in comparison with **3** which simplifies greatly chromatographic purification of glycosylation products.

Trisaccharides **24** and **25** were deprotected by hydrogenolysis followed by saponification to give after gel-permeation chromatography, the anomeric mixture **1** + **28** in 87% overall yield. Subsequent separation of the mixture by reverse-phase C18 chromatography afforded **1** (49%) and its β isomer **28** (38%). The structure of **1** was determined by NMR spectroscopy (Tables 2 and 3). In particular, the chemical shifts of H-3_{eq}Neu (δ 2.67 ppm), H-3_{ax}Neu (δ 1.7 ppm), H-4Neu (δ 3.62 ppm), together with C-1Neu (δ 174.6 ppm) and C-6Neu (δ 73.7 ppm) indicated the anomeric configuration of the neuraminic acid to be α,[19] while the value of C-6Gal (δ 64.5 ppm) proved 6-O-glycosylation position. In the similar fashion, the values of H-3_{eq}Neu (δ 2.38 ppm), H-3_{ax}Neu (δ 1.62 ppm), H-4Neu (δ 4.07 ppm), together with C-1Neu (δ 176.0 ppm) and C-6Neu (δ 71.5 ppm) showed β-Neu linkage in compound **28**.[19]

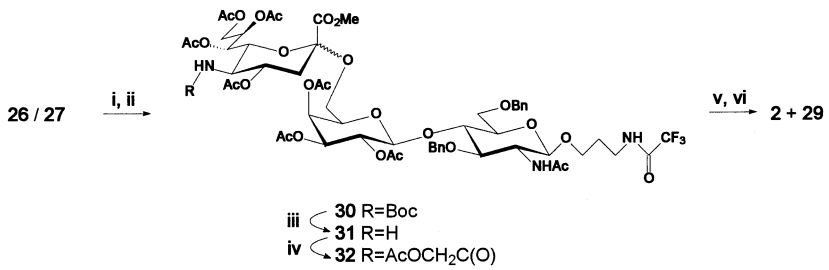


28 R=H (Neu5Ac)
29 R=OH (Neu5Gc)

At the next stage of the synthesis of **2**, selective deblocking of the nitrogen atom in the neuraminic acid residue was performed (Scheme 2). Treatment of compounds **26** and **27** with MeONa in dry MeOH followed by acetylation of the crude reaction mixture afforded carbamate **30** in 78% yield. Removal of the Boc group in **30** with trifluoroacetic acid in CH_2Cl_2 afforded amine **31**, which was immediately acylated with acetoxyacetyl chloride and Et_3N to give *N*-glycolyl derivative **32** in 70% overall yield. The intermediate amine **31** was highly prone to O→N acetyl group migration, which occurred quantitatively even on a TLC plate when eluted with neutral solvent system. Therefore elution with acetic acid containing mixtures was essential for TLC analysis of amine **31**.

Table 3
Chemical shifts (δ, ppm) for carbohydrate ring carbons in ^{13}C NMR spectra of compounds **1**, **2**, **4**, **8**, **15**, **16**, **21**, **28**, **29** in the solvent specified.

Compound (solvent)	Residue	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9
1 (D_2O)	GN	102.2	56.0	73.4	81.8	75.6	61.5			
	Gal	104.6	71.9	73.6	69.5	74.8	64.5			
	Neu	174.6	101.3	41.2	69.4	53.0	73.7	69.6	72.8	63.8
2 (D_2O)	GN	102.2	56.1	73.5	81.8	75.7	61.6			
	Gal	104.6	71.9	73.7	69.6	73.4	64.6			
	Neu	174.7	101.4	41.4	69.2	52.8	74.9	69.6	73.0	63.9
4 ($CDCl_3$)		173.7	89.5	38.9	66.3	52.8	72.2	68.6	72.8	62.4
8 (1:10 CD_3OD–$CDCl_3$)	GN	100.8	54.3	79.5	75.5	74.4	67.5			
	Gal	99.7	69.8	74.2	66.9	75.4	61.5			
15 (1:2 CD_3OD–$CDCl_3$)		101.3	55.4	81.8	77.6	65.8	68.3			
16 (acetone-d_6)		102.4	55.8	83.8	72.2	76.8	70.9			
21 ($CDCl_3$)	GN	101.2	55.0	78.2	75.5	74.9	68.3			
	Gal	99.8	70.3	71.3	68.0	71.3	61.7			
28 (D_2O)	GN	102.3	56.0	73.6	81.2	75.7	61.5			
	Gal	104.4	72.0	73.8	69.9	74.9	63.5			
	Neu	176.0	101.4	40.8	67.9	53.3	71.5	69.4	71.3	64.6
29 (D_2O)	GN	102.4	56.1	75.1	81.4	75.7	61.6			
	Gal	104.5	72.1	73.9	69.9	73.7	63.6			
	Neu	175.9	101.6	40.9	67.8	53.1	71.4	69.4	71.4	64.7



Scheme 2. Reagents and conditions: (i) MeONa, MeOH; (ii) Ac_2O, Py; (iii) CF_3CO_2H, CH_2Cl_2; (iv) $AcOCH_2C(O)Cl$, Et_3N, CH_2Cl_2, 0 °C; (v) H_2, Pd–C, MeOH; (vi) NaOH, MeOH–water.

The trisaccharide **32** was deprotected through the reaction sequence involved catalytic hydrogenolysis followed by alkaline hydrolysis of acyl protections. The anomeric mixture **2** + **29** obtained after gel-permeation chromatography in 62% overall yield was further separated into individual anomers using reverse-phase C18 column chromatography to give **2** (31%) and **29** (31%). The presence of *N*-glycolyl substituent was evident from ^1H and ^{13}C NMR spectra, where it appeared as a two-proton singlet (δ 4.15 ppm) and a signal at 62.3 ppm ($HOCH_2C(O)N$), respectively. In compound **2**, the chemical shifts (Tables 2 and 3) of H-3_{eq}Neu (δ 2.65 ppm), H-3_{ax}Neu (δ 1.69 ppm), H-4Neu (δ 3.74 ppm) together with C-1Neu (δ 174.7 ppm) and C-6Neu (δ 74.9 ppm) indicated α configuration of the neuraminic acid,[19] while β linkage in **29** was deduced from the values of H-3_{eq}Neu (δ 2.35 ppm), H-3_{ax}Neu (δ 1.58 ppm), H-4Neu (δ 4.09 ppm), together with C-1Neu (δ 175.9 ppm) and C-6Neu (δ 71.4 ppm).

In conclusion, 3-aminopropyl glycosides of trisaccharides Neu5Acα-(2→6′)-lactosamine and Neu5Gcα-(2→6′)-lactosamine were synthesized. Examination of sialylation of di-, tri-, and tetraol acceptors with both **3** and **4** did not show any influence on stereoselectivity, but glycosylation with imide-type donor **4** was slightly more effective, and isolation of reaction products was more convenient. Application of imide-type donor **4** opens ready access to oligosaccharides containing modified *N*-acyl analogues of Neu5Ac. Preparation of neoglycoconjugates from compounds **1** and **2** and their use in biological assays will be published elsewhere.

3. Experimental

General methods.—The reagents were purchased from Fluka and Merck, all of the highest grade available. Compound **17**[20] was obtained from Syntesome GmbH, Munich. Molecular sieves were activated by heating (180 °C) under vacuum (0.1 mmHg) for 5–8 h. THF was stored over sodium wire and was freshly distilled from sodium benzophenone ketyl under Ar prior to use. CH_2Cl_2 was washed with 96% H_2SO_4, water, saturated aq $NaHCO_3$, dried ($MgSO_4$), distilled twice from P_2O_5 under Ar, and stored over MS-4 Å. DMF was distilled twice from CaH_2 and stored over molecular sieve UOP Type 13 X (Fluka). Nitromethane was always freshly distilled from CaH_2 under Ar. The catalyst used for hydrogenolysis was 10% Pd–C, oxide form, (Merck-Schuchardt). In glycosylation reactions, molecular sieve Union Carbide type 4 Å (Fluka), or molecular sieve UOP type 3 Å (Fluka) were used. Column chromatography was performed on silica gel 60 (Fluka, 70–230 mesh). TLC was performed on silica gel 60 F_{254} (E. Merck, Darmstadt, Germany) with detection by dipping the chromatograms into 10% H_3PO_4 in EtOH followed by heating at 150 °C. Amines were also visualized by dipping the chromatograms into 0.017 M ninhydrin solution in *n*-butanol–AcOH (33:1), followed by heating. For TLC analysis of deblocked oligosaccharides, solvent systems 1:2:1 *n*-butanol–*n*-propanol–0.1 M aq HCl (BPHCl), 1:1:1 MeCN–MeOH–water (AMW), and their combination were used. Optical rotation was measured with JASCO DIP-360 digital polarimeter at 26–30 °C. NMR spectra were recorded at 27 °C with Bruker DRX-500 instrument (500 MHz for ^1H

and 125 MHz for ^{13}C), assignments were aided by APT, COSY, TOCSY, and $^{1}H-^{13}C$ correlation spectroscopy. *tert*-Butyl alcohol was used as an internal standard for D_2O solutions (1.24 ppm (^{1}H) and 30.29 ppm (^{13}C)) and Me_4Si for other ones. The following notation was used to define the NMR signals: GN for glucosamine unit, Gal for galactose unit, Neu for neuraminic acid unit, and Sp for spacer unit. Numeration of atoms in the spacer is Sug-1-2-3. Mass spectra were recorded using matrix assisted laser desorption ionization (MALDI)-time of flight (TOF) on Vision 2000 mass spectrometer. Melting points were determined with a Kofler apparatus and are uncorrected.

Phenyl [methyl 4,7,8,9-tetra-O-*acetyl-5-(*N-tert-*butoxycarbonylacetamido)-3,5-dideoxy-2-thio*-D-glycero-α- *and* β-D-galacto-*2-nonulopyranosid]onate* (**4**).—A solution of **3** (500 mg, 0.86 mmol, 1:2.3 α/β), Boc_2O (500 mg, 2.3 mmol) and 4-dimethylaminopyridine (DMAP) (70 mg, 0.57 mmol) in dry THF (12 mL) was refluxed under Ar and new portions of Boc_2O (250 mg, 1.15 mmol) and DMAP (35 mg, 0.028 mmol) were added every 6 h. After a total of 24 h, the reaction mixture was cooled to rt, diluted with CH_2Cl_2 (50 mL), washed with 0.5 M aq H_2SO_4, water, saturated aq $NaHCO_3$, dried and concentrated. Chromatography (petroleum ether → 1:1 petroleum ether–EtOAc) on a column of silica gel (50 g) gave the starting **3** (40 mg, 8%) and 600 mg of impure **4** as a yellow syrup. The syrup was subjected to gel-permeation chromatography on a 3×70 cm column of Bio-Beads SX3 (Bio-Rad Laboratories) in toluene to afford **4** (500 mg, 85%) as white foam: R_f 0.8 (3:4 petroleum ether–EtOAc); NMR spectra showed a 4.6:1 ratio of isomers at the nitrogen atom; NMR data for β anomer, major isomer at the nitrogen atom ($CDCl_3$): ^{1}H, see Table 2 for carbohydrate ring protons; δ 7.55–7.25 (m, 5 H, Ph), 3.53 (s, 3 H, OMe), 2.35 (s, 3 H, N–Ac), 2.03 (s, 6 H, 2 OAc), 1.94 (s, 6 H, 2 OAc), 1.71 (s, 9 H, $(CH_3)_3C$); ^{13}C, see Table 3 for carbohydrate ring carbons; δ 170.4, 170.3, 170.1, 169.7, 168.1, 151.9 (6 C=O), 136.1, 129.6, 129.0 (Ph), 85.3 $(CH_3)_3CO$), 52.4 (OCH_3), 28.2 $(CH_3)_3CO$), 26.6 (N–C(O)CH_3), 20.9, 20.8, 20.7 (OC(O)CH_3). Anal. Calcd for $C_{31}H_{41}NO_{14}S$: C, 54.46; H, 6.04; N, 2.05; S, 4.69. Found: C, 54.20; H, 6.12; N, 2.15; S, 4.79.

*3-Trifluoroacetamidopropyl 2-acetamido-4,6-*O-*benzylidene-3-*O-*benzyl-2-deoxy-β-*D-*glucopyranoside* (**15**).—NaH (60% suspension in mineral oil, 2.4 g, 60 mmol) was added portionwise to abs DMF (60 mL) at −20 °C under Ar (the solvent should be redistilled if any gas evolution is observed at this stage). The mixture was stirred until the homogeneous suspension was formed and solid **14** (4.62 g, 10 mmol) was added portionwise for 15 min. The stirring was continued until gas evolution ceased (ca. 1 h at −15 °C), BnBr (1.3 mL, 11 mmol) was added, and the reaction mixture was stirred at −20/−15 °C for ca. 1.5 h, then cooled to −40 °C and glacial AcOH (15 mL, 250 mmol) was carefully added dropwise. The reaction mixture was allowed to attain rt and after dilution with EtOAc (400 mL) the precipitation of **15** started. This biphasic mixture was washed with water (100 mL), saturated aq $NaHCO_3$, and the first crop of **15** was filtered off, washed thoroughly with MeOH, and dried to give **15** (1 g, 18%). The combined mother liquor and washings were concentrated and crystallized from MeOH to afford an additional lot of **15** (4 g, 72%) as fine powder: R_f 0.7 (EtOAc); mp (dec) 279–281 °C; $[\alpha]_D$ −46° (*c* 1, DMF); NMR (1:2 CD_3OD–$CDCl_3$): ^{1}H, see Table 2 for carbohydrate ring protons; δ 7.35–7.1 (m, 10 H, Ph), 5.43 (s, 1 H, PhCH), 4.68 (d, 1 H, *J* 11.8 Hz, $PhCH_2$), 4.49 (d, 1 H, $PhCH_2$), 3.73 (m, H-1aSp), 3.37 (m, 1 H, H-1bSp), 3.29 (m, H-3aSp), 3.09 (m, 1 H, H-3bSp), 1.89 (s, 3 H, N–Ac), 1.65 (m, 2 H, H-2aSp, H-2bSp); ^{13}C, see Table 3 for carbohydrate ring carbons; δ 136.9 (ipso Ph), 128.6–125.6 (Ph), 100.9 (PhCH), 73.8 ($PhCH_2$), 66.5 (C-1Sp), 36.5 (C-3Sp), 28.2 (C-2Sp), 22.2 (NC(O)CH_3). Anal. Calcd for $C_{27}H_{31}F_3N_2O_7$: C, 58.69; H, 5.65; N, 5.07. Found: C, 58.73; H, 5.62; N, 4.99.

*3-Trifluoroacetamidopropyl 2-acetamido-3,6-di-*O-*benzyl-2-deoxy-β-*D-*glucopyranoside* (**16**).—(a) Compound **15** (570 mg, 1.03 mmol) was refluxed in anhydrous THF (25 mL) under Ar until complete dissolution and then the mixture was allowed to attain rt. $Me_3N{\cdot}BH_3$ (301 mg, 4.13 mmol) was added under stirring,

followed by anhyd $AlCl_3$ (822 mg, 6.18 mmol). After the reagents had dissolved, water (0.036 mL, 2 mmol) was added dropwise and stirring was continued for 6 h until the complete conversion of the starting **15** into the product **16** (TLC control in 3:2 toluene–acetone). The reaction mixture was quenched by addition of water (10 mL) followed by 1 M aq HCl (10 mL) and extracted with EtOAc (2 × 100 mL), the extracts were washed with brine, dried, and concentrated. The residue was dissolved in the minimum quantity of EtOAc, the volume was doubled carefully with toluene, and the solution was applied to a column of silica gel (50 g). Gradient elution (toluene → 1:1 toluene–acetone) afforded amorphous **16** (498 mg, 87%): R_f 0.3 (3:2 toluene–acetone); $[\alpha]_D$ −12° (*c* 1, acetone); NMR (acetone-d_6): ^{1}H, see Table 2 for carbohydrate ring protons; δ 7.35–7.18 (m, 10 H, Ph), 4.83 (d, 1 H, *J* 11.6 Hz, $PhCH_2$), 4.72 (d, 1 H, $PhCH_2$), 4.59 (m, 4 H, H-1GN, 2 $PhCH_2$, N–H Sp), 3.87 (m, 2 H, H-1aSp, H-6aGN), 3.58 (m, 2 H, H-4GN, H-1bSp), 3.48 (m, 2 H, H-5GN, H-3aSp), 3.32 (m, 1 H, H-3bSp), 1.89 (s, 3 H, N–Ac), 1.81 (m, 2 H, H-2aSp, H-2bSp); ^{13}C, see Table 3 for carbohydrate ring carbons; δ 170.8 (N–*C*(O)CH_3), 140.5, 140.0 (2 ipso Bn), 129.2–128.1 (Ph), 74.9, 73.9 (2 $PhCH_2$), 66.9 (C-1Sp), 37.9 (C-3Sp), 29.6 (C-2Sp), 23.5 (N–C(O)*C*H_3); Anal. Calcd for $C_{27}H_{33}F_3N_2O_7$: C, 58.48; H, 6.00; N, 5.05. Found: C, 58.33; H, 5.92; N, 4.88.

(b) Performing of the same experiment without addition of water gave unchanged **15** and less then 10% of **16** was observed by TLC. Stirring of the reaction mixture for an additional 48 h at rt did not result in further conversion of **15**.

*3-Trifluoroacetamidopropyl (2-*O*-acetyl-3-*O*-benzoyl-β-*D*-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**8**).—(a) Acetylation of **17** (4.16 g, 10 mmol) with Ac_2O (5 mL) in Py (10 mL) and subsequent column chromatography gave **18** (4.53 g, 99%). A solution of **16** (140 mg, 0.25 mmol), **18** (198 mg, 0.43 mmol), and Bu_4NBr (22 mg, 0.07 mmol) in anhyd $MeNO_2$ (12 mL) was stirred with activated MS-4 Å (3 g) for 1 h under Ar, and AgOTf (167 mg, 0.65 mmol) was added followed by $CuBr_2$ (145 mg, 0.65 mmol). Stirring was continued overnight at rt, and the reaction mixture was filtered through Celite, the filtrate was diluted with EtOAc, washed with saturated aq $NaHCO_3$, 3 M aq $Na_2S_2O_3$, dried, and concentrated. Chromatography (toluene → 3:2 toluene–acetone) on a column of silica gel (50 g) afforded a mixture (187 mg) of **19** (R_f 0.31 in 2:1 toluene–acetone) and unreacted acceptor **16**. This mixture was dissolved in CH_2Cl_2 (5 mL) and treated with 90% aq CF_3CO_2H (0.5 mL) for 30 min at rt, then coevaporated with toluene (10 mL) and water (2 mL). Chromatography (1:1 toluene–EtOAc → EtOAc) on a column of silica gel (50 g) afforded amorphous **8** (80 mg, 37%): R_f 0.5 (double elution in EtOAc); $[\alpha]_D$ +4° (*c* 1, EtOAc); NMR (1:10 CD_3OD–$CDCl_3$): ^{1}H, see Table 2 for carbohydrate ring protons; δ 7.92 (d 2 H, *J* 7.6 Hz, ortho-protons of Bz), 7.45–7.12 (m, 13 H, Ph), 4.88 (d, 1 H, *J* 11.1 Hz, $PhCH_2$), 4.61 (d, 1 H, *J* 12 Hz, $PhCH_2$), 4.51 (d, 1 H, $PhCH_2$), 4.39 (d, 1 H, $PhCH_2$), 3.77 (m, 1 H, H-1aSp), 3.38 (m, H-1bSp, H-3aSp), 3.14 (m, 1 H, H-3bSp), 1.80 (s, 3 H, Ac), 1.79 (s, 3 H, Ac), 1.68 (m, 2 H, H-2aSp, H-2bSp); ^{13}C, see Table 3 for carbohydrate ring carbons; δ 171.4, 169.9, 165.7 (3 C=O), 137.6, 137.2 (2 ipso Bn), 133.2–127.6 (Ph), 74.9 ($PhCH_2$), 73.3 ($PhCH_2$), 66.2 (C-1Sp), 36.5 (C-3Sp), 28.1 (C-2Sp), 22.4 (N–C(O)CH_3), 20.3 (O–C(O)-CH_3). Anal. Calcd for $C_{42}H_{49}F_3N_2O_{14}$: C, 58.46; H, 5.72; N, 3.25. Found: C, 58.14; H, 5.77; N, 3.12.

(b) Conventional acetylation with Ac_2O in pyridine of compound **23** (91 mg, 0.1 mmol) followed by debenzylidenation as described in (a) afforded **8** (75 mg, 87%): identical in all respect to the material described above.

*3-Trifluoroacetamidopropyl (2,3,4,6-tetra-*O*-benzoyl-β-*D*-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**21**).—Acceptor **16** (628 mg, 1.13 mmol) was refluxed in anhyd CH_2Cl_2 (20 mL) under Ar until complete dissolution and then the mixture was allowed to attain rt. Benzobromogalactose **20** (942 mg, 1.43 mmol) was added under Ar, followed by MS-4 Å (4.5 g). After stirring for 15 min, powdered AgOTf (514 mg, 2 mmol) was added, and stirring was continued overnight at rt. Triethylamine (0.1

mL) was added, the mixture was filtered through a pad of Celite, diluted with CH_2Cl_2 (200 mL), washed with saturated aq $NaHCO_3$, 3 M aq $Na_2S_2O_3$, dried, and concentrated. Chromatography (toluene → 1:2 toluene–EtOAc) on a column of silica gel (30 g) afforded **21** (906 mg, 70%) as white foam: R_f 0.4 (1:2 toluene–EtOAc); $[\alpha]_D$ +16° (*c* 1, CH_2Cl_2); NMR ($CDCl_3$): ^{1}H, see Table 2 for carbohydrate ring protons; δ 8.10–7.75 (m, 8 H, ortho-protons of 4 Bz), 7.62–7.15 (m, 22 H, Ph), 5.92 (d, 1 H, $J_{N\text{-}H,2}$ 10 Hz, N–H GN), 4.95 (d, 1 H, *J* 11.8 Hz, $PhCH_2$), 4.75 (d, 1 H, $PhCH_2$), 4.67 (d, 1 H, *J* 11.9 Hz, $PhCH_2$), 4.39 (d, 1 H, $PhCH_2$), 3.77 (m, H-1aSp, H-3aSp), 3.28 (m, 2 H, H-1bSp, H-3bSp), 1.99 (s, 3 H, N–Ac), 1.72 (m, 2 H, H-2aSp, H-2bSp); ^{13}C, see Table 3 for carbohydrate ring carbons; δ 170.8 (N–C(O)CH_3), 165.5 (PhC(O)O), 138.5, 137.8 (2 ipso Bn), 133.7–127.9 (Ph), 73.6 ($PhCH_2$), 67.3 (C-1Sp), 37.6 (C-3Sp), 28.1 (C-2Sp), 23.4 (N–C(O)CH_3). Anal. Calcd for $C_{61}H_{59}F_3N_2O_{16}$: C, 64.66; H, 5.25; N, 2.47. Found: C, 64.64; H, 5.25; N, 2.37.

*3-Trifluoroacetamidopropyl (4,6-*O*-benzylidene-β-*D*-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**22**).—A 0.2 M solution of MeONa in MeOH (0.4 mL, 0.08 mmol) was added to the solution of **21** (900 mg, 0.79 mmol) in anhyd MeOH (10 mL), and after stirring overnight at rt, the reaction mixture was neutralized with KU-2(H^+) cation exchange resin, filtered, concentrated, and dried in vacuo to give the crude **10**, R_f 0.15 (10:1 EtOAc–MeOH), which was used without further purification. A mixture of all obtained **10**, $PhCH(OMe)_2$ (0.2 mL, 1.4 mmol), and TsOH (10 mg) in DMF (4 mL) was stirred at 50 °C for 2 h, neutralized with Et_3N (0.1 mL), and coevaporated with toluene (4 × 20 mL). Chromatography in EtOAc of the residue on a column of silica gel (50 g) afforded **22** (561 mg, 88%) as ca. 4:1 mixture of isomers at the benzylidene acetal center, R_f 0.47 (major) and 0.53 (minor) in 10:1 EtOAc–MeOH. Crystallization of the mixture from MeOH gave individual major isomer: mp 216–218 °C; $[\alpha]_D$ −41° (*c* 2, MeOH); NMR (2:1 CD_3OD–$CDCl_3$): ^{1}H, see Table 2 for carbohydrate ring protons; δ 7.53–7.15 (m, 15 H, Ph), 5.53 (s, 1 H, PhCH), 5.18 (d, 1 H, *J* 11.3 Hz, $PhCH_2$), 4.68 (d, 2 H, 2 $PhCH_2$), 4.58 (d, 1 H, *J* 11.9 Hz, $PhCH_2$), 3.79 (m, H-1aSp), 3.48 (m, H-1bSp), 3.42 (m, 1 H, H-3aSp), 3.38 (m, 1 H, H-3bSp), 1.91 (s, 3 H, N–C(O)CH_3), 1.82 (m, 2 H, H-2aSp, H-2bSp); ^{13}C, see Table 3 for carbohydrate ring carbons; δ 172.8 (N–*C*(O)CH_3), 139.7, 139.2, 139.1 (3 ipso Bn), 104.1, 102.1 (2 C-1), 101.9 (PhCH), 81.7 (C-3GN), 75.3, 74.1 (2 $PhCH_2$), 69.6, 69.3 (2 C-6), 67.4 (C-1Sp), 56.1 (C-2GN), 37.7 (C-3Sp), 29.4 (C-2Sp), 23.1 (N–C(O)*C*H_3). Anal. Calcd for $C_{40}H_{47}F_3N_2O_{12}$: C, 59.70; H, 5.89; N, 3.48. Found: C, 59.40; H, 5.84; N, 3.20.

*3-Trifluoroacetamidopropyl (3-*O*-benzoyl-β-*D*-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**9**).—To a solution of **22** (100 mg, 0.12 mmol) in Py (3 mL), BzCN (150 mg, 1.2 mmol) was added portionwise at rt until TLC indicated complete consumption of the starting material. Methanol (1 mL) was added, and after 30 min the reaction mixture was concentrated to give crude **23**, R_f 0.54 (EtOAc), which was debenzylidenated as described for the preparation of **8**. Chromatography (EtOAc → 10:1 EtOAc–MeOH) of the residue on a column of silica gel (20 g) afforded amorphous **9** (82 mg, 80% overall): R_f 0.27 (EtOAc); $[\alpha]_D$ −3° (*c* 2, MeOH); NMR (CD_3OD): ^{1}H, see Table 2 for carbohydrate ring protons; δ 8.12 (d, 2 H, *J* 7.7 Hz, ortho-protons of Bz), 7.65–7.18 (m, 13 H, Ph), 5.11 (d, 1 H, *J* 10.8 Hz, $PhCH_2$), 4.65 (d, 1 H, *J* 11.9 Hz, $PhCH_2$), 4.61 (d, 1 H, *J* 10.8 Hz, $PhCH_2$), 4.55 (d, 1 H, *J* 11.9 Hz, $PhCH_2$), 3.87 (m, H-1aSp), 3.53 (m, H-1bSp), 3.43 (m, H-3aSp), 3.29 (m, 1 H, H-3bSp), 1.95 (s, 3 H, N–C(O)CH_3), 1.82 (m, 2 H, H-2aSp, H-2bSp); ^{13}C, δ 173.3 (N–*C*(O)CH_3), 167.7 (O*C*(O)Ph), 139.6, 139.5 (2 ipso Bn), 134.3–128.8 (Ph), 104.2, 102.6 (2 C-1), 82.3 (C-3GN), 77.8, 77.5, 77.1, 76.6, 76.1, 74.2, 70.5, 69.2, 67.9, 67.7, 62.7 (C-6Gal), 56.2 (C-2GN), 37.9 (C-3Sp), 29.8 (C-2Sp), 23.0 (N–C(O)-*C*H_3). Anal. Calcd for $C_{40}H_{47}F_3N_2O_{13}$: C, 58.53; H, 5.77; N, 3.41. Found: C, 58.36; H, 5.84; N, 3.30.

*3-Trifluoroacetamidopropyl [methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-α-and β-*D*-galacto-2-nonulopyranosyl)onate]-(2→6)-(2-*O*-acetyl-3-*O*-benzoyl-*

β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (**24**).—A mixture of donor **3** (163 mg, 0.28 mmol), acceptor **8** (120 mg, 0.14 mmol), and anhyd MeCN (3 mL) was stirred with freshly activated MS-3 Å (1 g) during 1 h at rt under Ar, NIS (126 mg, 0.56 mmol) was added, and after it had dissolved, the reaction mixture was cooled to −35 °C and TfOH (0.005 mL) was added. After being stirred for 4 h at −35 °C, the reaction was terminated with Et_3N (0.1 mL), the mixture was filtered through Celite, diluted with CH_2Cl_2, washed with saturated aq $NaHCO_3$, 3 M aq $Na_2S_2O_3$, dried, and concentrated. Chromatography ($CH_2Cl_2 \rightarrow$ MeCN) of the residue on a column of silica gel (30 g) afforded the anomeric mixture **24** (149 mg, 79%) as white foam: R_f 0.39 (β anomer) and 0.33 (α anomer) in EtOAc (double elution); integration of the intensities of H-4Gal in ^{1}H NMR spectrum showed 1.3:1 α/β anomeric ratio; NMR ($CDCl_3$): ^{1}H, δ 8.12–8.01 (2 d, 2 H, ortho-protons of Bz), 7.19–7.22 (m, 13 H, Ph), 5.49 (dd, 1 H, $J_{2,1}$ 8, $J_{2,3}$ 10.2 Hz, H-2Gal), 5.39–5.21 (m, H-8Neu, H-7Neu, H-4βNeu), 5.03 (dd, 1 H, $J_{3,4}$ 3.1 Hz, H-3Gal), 4.89–4.49 (m, 4 $PhCH_2$, H-4αNeu, H-1GN, H-1Gal), 4.41 (dd, 1 H, $J_{9a,9b}$ 12.5, $J_{9a,8}$ 3.5 Hz, H-9aNeu), 4.37 (d, H-4Gal of β anomer), 4.25 (d, H-4Gal of α anomer), 2.51 (broaden dd, 1 H, $J_{3eq,4}$ 5, $J_{3eq,3ax}$ 12.7 Hz, H-3_{eq}Neu), 2.1–1.7 (m, 14 Ac, H-3_{ax}Neu, H-2aSp, H-2bSp). Anal. Calcd for $C_{62}H_{76}F_3N_3O_{26}$: C, 55.73; H, 5.73; N, 3.14. Found: C, 55.70; H, 5.68; N, 3.10.

3-Trifluoroacetamidopropyl [methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α- and β-D-galacto-2-nonulopyranosyl)onate]-(2→6)-(3-O-benzoyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (**25**).—Glycosylation of acceptor **9** (106 mg, 0.13 mmol) with donor **3** (163 mg, 0.28 mmol) in anhyd MeCN (3 mL) promoted by NIS (126 mg, 0.56 mmol), TfOH (0.005 mL), and MS-3 Å (1 g) for 24 h at −35 °C as described for the preparation of **24** and subsequent chromatography ($CH_2Cl_2 \rightarrow$ MeCN) of the reaction products on a column of silica gel (50 g) afforded the anomeric mixture **25** (98 mg, 59%) as transparent glass: R_f 0.34 (β anomer) and 0.31 (α anomer) in EtOAc (double elution); integration of the intensities of C-1Neu in ^{13}C NMR spectrum showed 1.2:1 α/β anomeric ratio; NMR (1:2 CD_3OD–$CDCl_3$): ^{1}H, δ 8.05–7.95 (2 d, 2 H, J 7.8 Hz, ortho-protons of Bz), 7.49–7.12 (m, 13 H, Ph), 3.61 and 3.59 (2 s, OMe), 2.33 (broaden dd, 1 H, $J_{3eq,4}$ 5.1, $J_{3eq,3ax}$ 12.5 Hz, H-3_{eq}Neu), 2.1–1.7 (m, 12 Ac, H-3_{ax}Neu, H-2aSp, H-2bSp); ^{13}C, δ 138.2, 137.4 (ipso Bn), 129.5–127.3 (Ph), 103.0 (C-1GN), 100.8 and 100.6 (C-1Gal), 98.6 and 98.1 (C-2Neu), 79.8 and 79.6 (C-3GN), 68.4 and 68.2 (C-6GN), 66.2 (C-1Sp), 54.7 and 54.4 (C-2GN), 52.5 (OCH_3), 36.9 (C-3Neu), 36.5 (C-3Neu, C-3Sp), 28.1 (C-2Sp), 22.4 and 22.1 (N–C(O)CH_3), 20.1 (O–C(O)CH_3). Anal. Calcd for $C_{60}H_{74}F_3N_3O_{25}$: C, 55.68; H, 5.76; N, 3.25. Found: C, 55.35; H, 5.84; N, 3.02.

3-Trifluoroacetamidopropyl {methyl [4,7,8,9-tetra-O-acetyl-5-(N-tert-butoxycarbonylacetamido)-3,5-dideoxy-D-glycero-α- and β-D-galacto-2-nonulopyranosyl]onate}-(2→6)-(2-O-acetyl-3-O-benzoyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (**26**).—Glycosylation of acceptor **8** (35 mg, 0.04 mmol) with donor **4** (55 mg, 0.08 mmol) in anhyd MeCN (3 mL) promoted by NIS (36 mg, 0.16 mmol), TfOH (0.006 mL), and MS-3 Å (1 g) for 24 h at −35 °C as described for the preparation of **24** and subsequent chromatography (toluene → 1:1 toluene–acetone) of the residue on a column of silica gel (30 g) afforded glycal **13** (22 mg, 50%) and the anomeric mixture **26** (48 mg, 84%) as syrup.

Compound **13**: R_f 0.88 in EtOAc; NMR data for the main isomer at the nitrogen atom ($CDCl_3$): ^{1}H, δ 6.02 (broaden d, 1 H, $J_{3,4}$ 9 Hz, H-3), 5.89 (broaden d 1 H, $J_{4,3}$ 2.8 Hz, H-4), 5.28 (m, 3 H, H-6, H-7, H-8), 4.91 (broaden m, H-5), 4.61 (broaden d, H-9a), 4.17 (dd, 1 H, $J_{9b,9a}$ 12.5, $J_{9b,8}$ 6.2 Hz, H-9b), 3.79 (s, 3 H, OMe), 2.39 (s, 3 H, N–C(O)CH_3), 2.1–1.9 (4 s, 12 H, OC(O)CH_3), 1.55 (s, 9 H $OC(CH_3)_3$); ^{13}C, δ 146.3 (C-2), 109.1 (C-3), 85.2 ($OC(CH_3)_3$), 55.5 (OMe), 27.9 ($OC(CH_3)_3$), 27.1 (N–C(O)CH_3), 20.8 (O–C(O)CH_3). Anal. Calcd for $C_{25}H_{35}NO_{14}$: C, 52.35; H, 6.15; N, 2.44. Found: C, 52.12; H, 5.98; N, 2.18.

Compound **26**: R_f 0.65 in EtOAc; integration of the intensities of C-1Neu in ^{13}C NMR spectrum showed 1.3:1 α/β anomeric ratio; NMR ($CDCl_3$): ^1H, δ 8.17–8.06 (m, 2 H, ortho-protons of Bz), 7.61–7.14 (m, 13 H, Ph), 5.51 (dd, 1 H, $J_{2,1}$ 8, $J_{2,3}$ 10 Hz, H-2Gal), 5.02 (dd, 1 H, $J_{3,4}$ 3 Hz, H-3Gal), 2.61 (m, 1 H, H-3_{eq}Neu), 2.49–1.75 (m, 24 H, 5 OAc, 2 NAc, H-3_{ax}Neu, H-2aSp, H2bSp), 1.59–1.48 (3 s, 9 H, $OC(CH_3)_3$); ^{13}C, δ 138.3, 137.8 (2 ipso Bn), 133.4–127.9 (Ph), 101.1, 100.2 (C-1 GN, C-1Gal), 99.2 and 98.3 (C-2Neu), 85.3 and 85.0 and 84.7 ($OC(CH_3)_3$), 77.8 and 77.5 (C-3GN), 56.3 and 56.1 (C-2GN), 38.3 (C-3Neu), 37.6 (C-3Sp, C-3Neu), 37.1 (C-3Neu), 29.6 (C-2Sp), 27.8 ($OC(CH_3)_3$), 27.6 and 27.2 and 26.7 (Boc–N–C(O)CH_3), 23.4 and 23.3 (N–Ac), 21.1, 20.8, 20.4 (O–Ac). Anal. Calcd for $C_{67}H_{84}F_3N_3O_{28}$: C, 56.02; H, 5.89; N, 2.93. Found: C, 55.98; H, 5.98; N, 2.84.

*3-Trifluoroacetamidopropyl {methyl [4,7,8,9-tetra-*O*-acetyl-5-(*N*-tert-butoxycarbonylacetamido)-3,5-dideoxy-*D*-glycero-α- and β-*D*-galacto-2-nonulopyranosyl]onate} - (2→6)-(3-*O*-benzoyl-β-*D*-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**27**).—Glycosylation of acceptor **9** (58 mg, 0.07 mmol) with donor **4** (96 mg, 0.14 mmol) in anhyd MeCN (5 mL) promoted by NIS (63 mg, 0.16 mmol), TfOH (0.01 mL), and MS-3 Å (1.2 g) for 24 h at −35 °C as described for the preparation of **24** and subsequent chromatography (toluene→1:2 toluene–acetone) of the residue on a column of silica gel (30 g) afforded glycal **13** (48 mg, 50%) and the anomeric mixture **27** (59 mg, 60%) as foam: R_f 0.3 in 3:2 toluene–acetone; integration of the intensities of C-1Neu in ^{13}C NMR spectrum showed 1:1 α/β anomeric ratio; NMR ($CDCl_3$): ^1H, δ 8.25–8.09 (m, 2 H, ortho-protons of Bz), 7.65–7.16 (m, 13 H, Ph), 5.91 (d, 1 H, $J_{N\text{-}H,2}$ 8 Hz, N–H GN), 5.65 (m, H-4βNeu), 5.35 (m, H-4αNeu), 4.95 (m, H-3Gal), 2.51 (m, 1 H, H-3_{eq}Neu), 2.49–1.75 (m, 21 H, 4 OAc, 2 NAc, H-3_{ax}Neu, H-2aSp, H2bSp), 1.59–1.40 (3 s, 9 H, $OC(CH_3)_3$); ^{13}C, δ 138.5, 137.4 (2 ipso Bn), 133.1–127.9 (Ph), 103.3 (C-1GN), 100.7 (C-1Gal), 98.9 and 98.1 (C-2Neu), 85.1 and 84.9 and 84.6 ($OC(CH_3)_3$), 79.2 and 78.9 (C-3GN), 55.9 and 55.8 (C-2GN), 38.2 (C-3Neu), 37.5 (C-3Sp, C-3Neu) and 37.1 (C-3Neu), 28.2 (C-2Sp), 27.7 ($OC(CH_3)_3$), 27.5 and 27.0 and 26.6 (Boc–N–C(O)CH_3), 23.3 (N–Ac), 20.9, 20.7, 20.6, 20.4 (O–Ac). Anal. Calcd for $C_{65}H_{82}F_3N_3O_{27}$: C, 55.99; H, 5.93; N, 3.01. Found: C, 56.27; H, 5.79; N, 2.87.

*3-Aminopropyl [sodium (5-acetamido-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosyl)onate]-(2→6)-(β-*D*-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**1**) *and 3-aminopropyl [sodium (5-acetamido-3,5-dideoxy-*D*-glycero-β-*D*-galacto-2-nonulopyranosyl)onate]-(2→6)-(β-*D*-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**28**).—To a solution of **24** (123 mg, 0.09 mmol) in MeOH (6 mL), Pd–C (20 mg) was added, the mixture was degassed under vacuum with stirring, refilled with hydrogen, and stirred for 2 h under H_2 at rt. The reaction mixture was filtered through Celite, the pad was washed thoroughly with MeOH, and the filtrate was concentrated. A solution of the residue in MeOH (4 mL) was treated with solid MeONa (118 mg, 2.2 mmol), and after 1 h at rt, water (1 mL) was added, and the reaction mixture was stirred overnight. The pH of the solution was carefully made neutral by addition of AcOH, and the reaction mixture was concentrated. Chromatography of the residue on a 1.5 × 100 cm column of TSK HW-40S gel (TOYO SODA Manufacturing Co.) by elution with 0.1 M aq AcOH and subsequent lyophilization afforded anomeric mixture of compounds **1** and **28** (60 mg, 87%). This mixture was subjected to reverse-phase column chromatography on a RP C18 (5 micron particle size) 1 × 25 cm stainless steel column (IBM Instruments, Inc) by elution with water to give (in order of elution) β anomer **28** (26 mg, 38%) and α anomer **1** (34 mg, 49%).

Compound **28**: R_f 0.34 in 1:1 BPHCl–AMW; $[\alpha]_D$ −25° (*c* 1, water); NMR (D_2O): ^1H, see Table 2 for carbohydrate ring protons; δ 4.04 (m, H-1aSp), 3.73 (m, H-1bSp), 3.09 (t, 2 H, *J* 7.2 Hz, H-3aSp, H-3bSp), 2.04 (s, 6 H, 2 N–Ac), 1.95 (m, 2 H, H-2aSp, H-2bSp); ^{13}C, see Table 3 for carbohydrate ring carbons; δ 175.9 (2 N–*C*(O)CH_3), 69.0 (C-1Sp), 38.8 (C-3Sp), 27.8 (C-2Sp), 23.3 (2 N–C(O)*C*H_3); MALDI-TOF-MS: Calcd for $[C_{28}H_{48}N_3NaO_{19} + H]^+$: 754.3. Found 755.1.

Compound **1**: R_f 0.4 in 1:1 BPHCl–AMW; $[\alpha]_D$ −21° (*c* 0.86, water); NMR (D_2O): ^1H, see Table 2 for carbohydrate ring protons; δ 4.02 (m, H-1Sp), 3.68 (m, H-1bSp), 3.09 (t, 2 H, *J* 7 Hz, H-3aSp, H-3bSp), 2.07 (s, 3 H, N-Ac), 2.03 (s, 3 H, N-Ac), 1.95 (m, 2 H, H-2aSp, H-2bSp); ^{13}C, see Table 3 for carbohydrate ring carbons; δ 176.1, 175.9 (2 N–*C*(O)CH_3), 69.1 (C-1Sp), 38.8 (C-3Sp), 27.8 (C-2Sp), 23.4, 23.1 (2 N–C(O)*C*H_3); MALDI-TOF-MS: Calcd for $[C_{28}H_{48}N_3NaO_{19}+Na]^+$: 776.3. Found 777.0.

3-Trifluoroacetamidopropyl {methyl [4,7,8,9-tetra - O - *acetyl* - *5* - *(*N - tert - *butoxycarbonyl-amino)-3,5-dideoxy*-D-glycero-α- *and* β-D-galacto - *2* - *nonulopyranosyl]onate}* - *(2→6)-(2,3,4* - *tri* - O - *acetyl* - β - D - *galactopyranosyl)-(1→4)-2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranoside* (**30**).—A 0.16 M solution of MeONa in MeOH (0.5 mL, 0.08 mmol) was added to the solution of **27** (50 mg, 0.036 mmol) in anhyd MeOH (1 mL), and after stirring for 1 h at rt under Ar TLC (4:1 EtOAc–BPHCl) indicated deacylation to be complete. Acetic acid (0.06 mL) was added, and the reaction mixture was concentrated and dried in vacuo. The residue was dissolved in pyridine (2 mL) and Ac_2O (0.5 mL) was added. After stirring overnight at rt, MeOH (1 mL) was added, and the reaction mixture was concentrated with toluene. Chromatography (toluene → 3:2 toluene–acetone) of the residue on a column of silica gel (30 g) followed by gel-permeation chromatography on a 1.5 × 45 cm column of Bio-Beads SX3 in toluene afforded **30** (38.5 mg, 78%) as white glass: R_f 0.65 (EtOAc); NMR ($CDCl_3$): ^1H, δ 7.51 (broaden s, N–HSp), 7.41–7.25 (m, 10 H, Ph), 5.15 (m, H-4βNeu), 4.99 (m, 1 H, H-3Gal), 4.81 (m, H-4αNeu), 3.88 (m, H-1aSp), 3.51 (m, H-1bSp), 3.44 (H-3aSp), 3.35 (H-3bSp), 2.51-2.49 (m, 1 H, H-3_{eq}Neu), 2.1–1.9 (m, 7 OAc, 1 N-Ac, H-3_{ax}Neu, H-2aSp, H-2bSp), 1.38 (s, 9 H, $OC(CH_3)_3$); ^{13}C, δ 176.9 (N–C(O)CH_3), 170.6, 169.9, 169.7 (C-1Neu, O-C(O)CH_3) 155.8 (N–C(O)Ot–Bu), 138.2 and 137.8 (2 ipso Bn), 128.9–125.3 (Ph), 101.1 and 100.8 (2 C-1GN), 99.8 and 99.6 (2C-1Gal), 98.9 and 98.7 (2 C-2Neu), 38.0 and 37.2 (2 C-3 Neu), 37.6 (C-3Sp), 29.5 (C-2Sp), 28.2 ($OC(CH_3)_3$), 23.3 (N–C(O)CH_3), 21.4–20.6 (O–C(O)CH_3); Anal. Calcd for $C_{62}H_{82}F_3N_3O_{28}$: C, 54.19; H, 6.01; N, 3.06. Found: C, 54.10; H, 6.08; N, 3.15.

3-Aminopropyl [sodium (3,5-dideoxy-5-hydroxyacetamido - D - glycero - α - D - galacto -*2-nonulopyranosyl)onate]- (2→6)- (β* - D - *galacto-pyranosyl)- (1→4)- 2-acetamido- 2-deoxy- β* - D-*glucopyranoside* (**2**) *and 3-aminopropyl (sodium [3,5-dideoxy-5-hydroxyacetamido*-D-glycero-*β*-D - galacto - *2* - *nonulopyranosyl]onate) - (2→6)-(β* - D - *galactopyranosyl)- (1→4)- 2 - acetamido-2-deoxy-β*-D-*glucopyranoside* (**29**).—To a solution of **30** (31 mg, 0.023 mmol) in dry CH_2Cl_2 (1 mL), CF_3CO_2H (0.3 mL) was added. After 30 min at rt the reaction mixture was coevaporated with toluene (10 mL) at the bath temperature ≥ 30 °C, and dried in vacuo to give amine **31** (R_f 0.5 in 20:1:1 CH_2Cl_2–MeOH–AcOH). The residue was dissolved in CH_2Cl_2 (2 mL), cooled to 0 °C, and acetoxyacetyl chloride (0.025 mL, 0.22 mmol) was added, followed by Et_3N (0.05 mL, 0.36 mmol). After stirring for 1 h the reaction was quenched with saturated aq $NaHCO_3$ (1 mL), diluted with CH_2Cl_2 (30 mL), washed with saturated aq $NaHCO_3$, dried, and concentrated. Chromatography (3:1 EtOAc–toluene → EtOAc → 10:1 EtOAc–MeOH) on a column of silica gel (20 g) afforded **32** (22 mg, 70%), as white glass, R_f 0.39 (EtOAc). Deprotection of compound **32** (20 mg, 0.014 mmol) and separation of α and β anomers were performed as described for the preparation of **1** and **28** to give (in order of elution) β anomer **29** (3.5 mg, 31%) and α anomer **2** (3.5 mg, 31%).

Compound **29**: R_f 0.25 in 1:1 BPHCl–AMW; $[\alpha]_D$ −26° (*c* 0.25, water); NMR (D_2O): ^1H, see Table 2 for carbohydrate ring protons; δ 4.15 (s, HO*C*H_2C(O)N), 4.03 (m, H-1aSp), 3.70 (m, H-1bSp), 3.08 (t, 2 H, *J* 6.8 Hz, H-3aSp, H-3bSp), 2.03 (s, 3 H, N-Ac), 1.91 (m, 2 H, H-2aSp, H-2bSp); ^{13}C, see Table 3 for carbohydrate ring carbons; δ 176.7 (N–*C*(O)CH_2OH), 175.9 (N–*C*(O)CH_3), 69.1 (C-1Sp), 62.3 (N–C(O)*C*H_2OH), 38.9 (C-3Sp), 27.9 (C-2Sp), 23.4 (N–C(O)*C*H_3); MALDI-TOF-MS: Calcd for $[C_{28}H_{48}N_3NaO_{20}+H]^+$: 769.3. Found 770.0.

Compound **2**: R_f 0.35 in 1:1 BPHCl–AMW; $[\alpha]_D$ −28° (*c* 0.2, water); NMR (D_2O): ^1H, see Table 2 for carbohydrate ring protons; δ 4.15

(s, 2 H, HOCH_2C(O)N), 3.99 (m, H-1aSp), 3.68 (m, H-1bSp), 3.06 (t, 2 H, J 6.8 Hz, H-3aSp, H-3bSp), 2.06 (s, 3 H, N-Ac), 1.87 (m, 2 H, H-2aSp, H-2bSp); ^{13}C, see Table 3 for carbohydrate ring carbons; δ 176.9 (N–C(O)CH_2OH), 175.9 (N–C(O)CH_3), 69.2 (C-1Sp and C-4Neu), 62.2 (N–C(O)CH$_2$OH), 38.9 (C-3Sp), 27.9 (C-2Sp), 23.5 (N–C(O)CH$_3$); MALDI-TOF-MS: Calcd for $[C_{28}H_{48}N_3NaO_{20} + H]^+$: 769.3. Found 769.5.

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