ELSEVIER

Carbohydrate Research 337 (2002) 451–457

CARBOHYDRATE RESEARCH

www.elsevier.com/locate/carres

Note

Preparative route to *N*-glycolylneuraminic acid phenyl 2-thioglycoside donor and synthesis of Neu5Gc-α-(2→3′)-lactosamine 3-aminopropyl glycoside

Andrei A. Sherman, Olga N. Yudina, Alexander S. Shashkov, Vladimir M. Menshov, Nikolay E. Nifantiev*

N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky Prospect 47, 119991 Moscow B-334, Russia

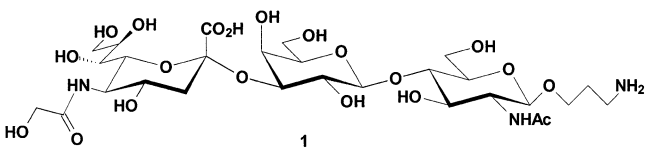
Received 28 August 2001; accepted 20 December 2001

Abstract

The spacer-armed trisaccharide, Neu5Gc-α-(2→3′)-lactosamine 3-aminopropyl glycoside, was synthesized by regio- and stereoselective sialylation of the suitably protected triol acceptor, 3-trifluoroacetamidopropyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(6-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside, with the donor methyl [phenyl 5-acetoxyacetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α,β-D-*galacto*-2-nonulopyranosid]onate. The donor was obtained, in turn, from methyl [phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α,β-D-*galacto*-2-nonulopyranosid]onate by *N*-*tert*-butoxycarbonylation of the acetamido group followed by total *N*- and *O*-deacetylation, per-*O*-acetylation, subsequent Boc group removal, and *N*-acetoxyacetylation.

Keywords: Sialylation; Sialyllactosamine; *N*-Deacetylation

Sialo-oligosaccharides play an important role in the process of cell adhesion and act as receptors of various lectins.[1] In order to study the carbohydrate specificity of the sialyloligosaccharides-binding lectins—selectins and siglecs—we have synthesized various spacer-armed oligosaccharides and neoglycoconjugates thereof, including sialyl Lewis A and sialyl Lewis X,[2] sialyl-lacto-*N*-neotetraose and sialyl-lacto-*N*-tetraose,[3] and 5-*N*-glycolyl-neuraminyl-α-(2→6′)-lactosamine.[4] Extending this research work, we report herein the synthesis of 5-*N*-glycolyl-neuraminyl-α-(2→3′)-lactosamine 3-aminopropyl glycoside (**1**).



1

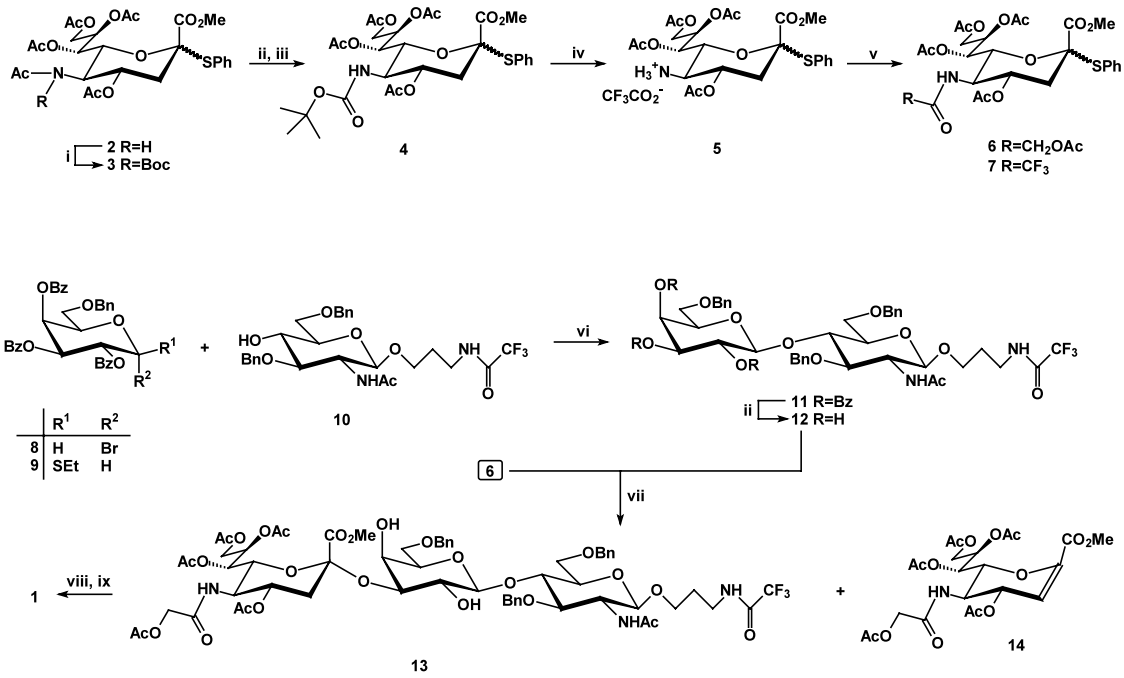
The known syntheses of the sialyl donors of *N*-modified analogues of *N*-acetylneuraminic acid used harsh conditions for the *N*-deacetylation of Neu5Ac derivatives, such as heating with Me_4NOH,[5,6] hydrazine hydrate,[7,8] $Ba(OH)_2$,[9] or methanesulfonic acid.[10,11] A few syntheses[12,13] of the Neu5Gc containing oligosaccharides started from *N*-glycolylneuraminic acid, which can be obtained by enzymatic methods[14,15] but still is poorly available.

We have shown[4] that the sialyl-α-(2→6′)-lactosamine trisaccharide derivative that had the neuraminic acid acetamido group *N*-*tert*-butoxycarbonylated could be efficiently transformed into the desired 5-*N*-glycolyl-sialyl oligosaccharide by mild *N*,*O*-deacetylation, per-*O*-acetylation, Boc group removal, and *N*-glycolylation. In the present work, the same reaction sequence was applied for the preparation of *N*-glycolyl sialyl donor **6**, which was then used for the construction of the trisaccharide **1**.

Previous applications of such a methodology include amide-to-carbamate transformation of amino acid

* Corresponding author. Fax: +7-095-1358784.
E-mail address: nen@ioc.ac.ru (N.E. Nifantiev).

0008-6215/02/$ - see front matter
PII: S0008-6215(02)00003-4



Scheme 1. Reagents and conditions: (i) Boc_2O, DMAP, THF, reflux, see Ref. 4 for details; (ii) MeONa, MeOH; (iii) Ac_2O, Py; (iv) 90% aq CF_3CO_2H, CH_2Cl_2; (v) $AcOCH_2C(O)Cl$, Et_3N, CH_2Cl_2, 0 °C; (vi) AgOTf, MS-4 Å, CH_2Cl_2, rt; (vii) NIS, TfOH, MS-3 Å, MeCN, 18 h, −20 °C; (viii) H_2, Pd–C, MeOH; (ix) KOH, H_2O.

derivatives,[16] cleavage of lactams,[17] and *N*-deacetylation of methyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranoside[18] and benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enosonate.[19]

The mixed imide **3** was obtained by *N*-*tert*-butoxycarbonylation of the thioglycoside **2** as described,[4] but the thorough purification of **3** by gel-permeation chromatography was found unnecessary for further steps, and the crude **3** could be used equally effective (Scheme 1).

As expected,[4,17] treatment of **3** with methanolic sodium methoxide resulted in rapid removal of the *N*-acetyl group with concomitant *O*-deacetylation. Reacetylation with acetic anhydride in pyridine afforded carbamate **4** in near quantitative overall yield.

Deprotection of the amino group in **4** with 90% aqueous trifluoroacetic acid in dichloromethane gave the salt **5**, which was then immediately acylated with acetoxyacetyl chloride and triethylamine into the *N*-glycolyl derivative **6** in 74% overall yield. Stability of the thioglycoside function in the former reaction is worth noting, because it is known from peptide chemistry that Boc group removal from sulfur-containing derivatives, e.g., those of methionine or cysteine, may be problematic without a scavenger.[20] In contrast, deprotection of **4** with both 2 M HCl in diethyl ether and 4 M HCl in 1,4-dioxane gave a significantly less clean reaction. The presence of acetoxyacetyl moiety in **6** was evident from the ^{1}H NMR spectrum, where its methylene protons appeared as two doublets (J 15.4 Hz) at δ 4.62 and 4.31 ppm, and the methyl protons as a singlet at δ 2.04 ppm.

In addition to the *N*-glycolyl derivative **6**, the trifluoroacetamide **7** byproduct was formed in 25% yield during acylation of the salt **5** with acetoxyacetyl chloride. The structure of **7** was determined by NMR spectroscopy. Firstly, the ^{1}H NMR spectrum of **7** contained four Ac groups, and the downfield location of the signals of H-4, H-7, H-8, H-9a, and H-9b (see Table 1) indicated that all these positions were acylated. Secondly, a single peak at −75.9 ppm was observed in the ^{19}F NMR spectrum of **7**, while its ^{13}C NMR spectrum showed two large-constant quartets at 157.7 and 115.4 ppm, and all these signals are characteristic for the trifluoroacetic acid amides and esters. Thirdly, the value of δ_{N-H} 6.92 ppm, which is too downfield for the AcN–H proton[†] but fit for $CF_3C(O)N$–H one, excluded any 5-acetamido-tri-*O*-acetyl-*O*-trifluoroacetyl structure. The formation of trifluoroacetamide byproduct during acylation of the ammonium salts that have the trifluoroacetate as a counter-ion has been rationalized to proceed via the highly reactive mixed anhydride intermediate.[20] Compounds **6**[13] and **7**[21] have already been prepared by different routes, but not fully characterized.

For the synthesis of the sialyl acceptor **12**, the known spacer-armed glucosamine derivative **10**[4] was glycosylated with galactosyl bromide **8**[22] in the presence of AgOTf to afford lactosamine **11** in 73% yield. In contrast, NIS-TfOH promoted the coupling of **10** and

[†] E.g., for compound **2** δ_{N-H} is normally about 5.2 ppm.

Table 1
^{1}H NMR data (δ, ppm and J, Hz) for the carbohydrate ring protons in compound **1**, **4** (β anomer), **6** (β anomer), **7** (β anomer), **11**, and **14** in the solvent specified

Compound (solvent)	Unit	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3eq ($J_{3eq,4}$)	H-3ax ($J_{3ax,4}$)	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	GN	4.51	3.75		3.67	3.72	3.60	4.01	3.85				
(D_2O)		(7.9)					(3.5)	(10.7)					
	Gal	4.54	3.57		4.11	3.96	3.72	3.76–3.69					
		(7.9)	(9.9)		(3.2)	(0)							
	Neu			2.77 [a]	1.80	3.73	3.84	3.71		3.62	3.87	3.89	3.65
				(4.6)	(12)								
4				2.69 [b]	2.05	5.31	3.77	4.58 [c]		5.54	4.98	4.50	3.99
($CDCl_3$)				(4.8)	(13.5)	(11.2)	(11.5)			(>1)	(1.6)	(12.1)	(9.5)
6				2.69 [d]	2.18	5.44	4.17	4.70 [e]		5.44	4.99	4.50	4.01
($CDCl_3$)				(4.8)	(13)	(10.4)	(10.4)			(0)	(1.9)	(12)	(8.1)
7				2.74 [f]	2.16	5.54	4.14	4.87 [g]		5.52	5.04	4.55	4.07
($CDCl_3$)				(4.7)	(14.5)	(10.3)	(10.3)			(0)	(1)	(12.3)	(8.3)
11	GN	4.57	3.68		3.88	4.15	3.44	3.70	3.58				
($CDCl_3$)		(7.4)	(7.6)		(7.6)	(7.3)	(3.6)	(10.3)					
	Gal	4.87	5.72		5.49	5.95	3.96	3.57	3.59				
		(8)	(10.4)		(3.1)	(0)	(6.4)	(10.1)	(6.4)				
12	GN	4.21	3.53										
(1:1 $CDCl_3$–CD_3OD)		(7.7)											
	Gal	4.12	3.28		3.13	3.57							
		(8.6)	(9.5)		(3.3)	(0)							
13	GN	4.75											
($CDCl_3$)		(7.5)											
	Gal	4.62	3.73		4.03	3.75							
		(8.2)	(10.0)		(3.5)	(>1)							
	Neu			2.75 [h]	2.05	5.06	4.00	4.20		5.28 [i]	5.46	4.30	4.05
				(4.5)	(11.5)	(11.5)	(10.8)			(8.6)	(2.3)	(12.6)	(6)
14				5.98		5.64	4.39	4.42 [j]		5.43	5.34	4.63	4.19
($CDCl_3$)				(2.9)		(7.5)				(3)	(3)	(12.4)	(7)

[a] $J_{3eq,3ax}$ 12.5 Hz.
[b] $J_{3eq,3ax}$ 13.8 Hz.
[c] $J_{6,7}$ 2.6 Hz.
[d] $J_{3eq,3ax}$ 13.5 Hz.
[e] $J_{6,7}$ 2.2 Hz.
[f] $J_{3eq,3ax}$ 14 Hz.
[g] $J_{6,7}$ 1.8 Hz.
[h] $J_{3eq,3ax}$ 13 Hz.
[i] $J_{6,7}>1$ Hz.
[j] $J_{6,7}$ 4.6 Hz.

Table 2
^{13}C NMR data (δ, ppm) for carbohydrate ring carbons in compound **1**, **4** (β anomer), **6** (β anomer), **7** (β anomer), **11**, and **12** in the solvent specified

Compound (solvent)	Unit	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9
1 (D_2O)	GN	102.4	56.2	73.4	79.7	75.9	61.2			
	Gal	103.8	70.6	76.7	68.7	76.4	62.2			
	Neu	175.0	101.1	40.9	69.2	52.6	73.8	69.2	73.0	63.8
4 ($CDCl_3$)		168.3	88.9	37.7	69.0	50.8	72.9	69.4	73.2	62.8
6 ($CDCl_3$)		167.7	89.0	37.6	68.2	49.4	73.1	68.8	73.1	62.7
7 ($CDCl_3$)		167.7	89.1	37.5	68.4	50.2	72.1	68.6	73.1	62.5
11 ($CDCl_3$)	GN	101.0	54.9	78.4	75.4	75.0	68.3			
	Gal	99.9	70.5	71.5	68.2	72.6	67.3			
12 (1:1 CD_3OD–$CDCl_3$)	GN	100.9	54.5	79.8	76.2	74.6	68.2			
	Gal	102.9	71.5	73.1	68.5	73.3	68.7			
13 ($CDCl_3$)	GN		55.6	78.7	77.3					
	Gal									
	Neu		97.7	37.6		49.7				

thiogalactoside **9**[23] was less effective, and gave **11** in 40% yield only (details are not included in the Experimental). The β-Gal linkage in **11** was confirmed (Table 1) by the value of $J_{1,2}$ coupling constant 8 Hz, and no α isomer was formed. Debenzoylation of **11** with methanolic sodium methoxide gave **12** in high yield.

Sialylation of **12** with **6** (1.5 equiv) in MeCN at −20 °C,‡ promoted by NIS, TfOH, and MS-3 Å proceeded slowly (18 h) but regio- and stereoselectively, and gave the α-linked trisaccharide **13** in 40% yield. Unreacted acceptor **12** (43%) and glycal **14** (61%) were also isolated from the reaction mixture. Analysis of the ^1H NMR spectrum of **13**, in particular the values of $\delta_{H\text{-}3eqNeu}$ (2.75 ppm) and $\delta_{H\text{-}4Neu}$ (5.06 ppm), supported α configuration of the neuraminic acid residue.[24]

Deprotection of **13** by catalytic hydrogenolysis followed by saponification with aqueous KOH afforded the target trisaccharide **1**, the structure of which was completely confirmed by NMR spectroscopy. In particular, the presence of *N*-glycolyl moiety in **1** was evident from ^1H and ^{13}C NMR spectra, where it appeared as a two-proton singlet (δ 4.12 ppm) and a signal at 62.2 ppm (HO*C*H_2C(O)N), respectively. The chemical shifts (Tables 1 and 2) of H-3_{eq}Neu (δ 2.77 ppm), H-3_{ax}Neu (δ 1.80 ppm), H-4Neu (δ 3.73 ppm) together with C-1Neu (δ 175 ppm) and C-6Neu (δ 73.8 ppm) indicated the neuraminic acid linkage to be α.[24]

In conclusion, the Neu5Gc donor **6** was efficiently prepared from the readily available Neu5Ac phenylthioglycoside (**2**) and employed for the synthesis of the spacer-armed trisaccharide Neu5Gc-α-(2→3′)-lactosamine 3-aminopropyl glycoside (**1**). The use of the neoglycoconjugates obtained from **1** in biological studies will be published elsewhere.

‡ Below this temperature, virtually no activation of the donor took place (TLC data).

1. Experimental

General methods.—Molecular sieve Union Carbide type 4 Å (Fluka), or molecular sieve UOP type 3 Å (Fluka) were activated by heating (180 °C) under vacuum (0.1 mmHg) for 8 h. The catalyst used for hydrogenolysis was 10% Pd–C, oxide form (E. Merck–Schuchardt). TLC was performed on Silica Gel 60 F_{254} (E. Merck, Darmstadt, Germany) and for the elution 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 ^1H–^{13}C correlation spectroscopy. *tert*-Butyl alcohol was used as an internal standard for D_2O solutions (1.24 ppm (^1H) and 30.29 ppm (^{13}C)) and Me_4Si for other ones. ^{19}F NMR spectrum was recorded using $CFCl_3$ (δ 0.0 ppm) as an external standard. The following notation was used to define the NMR signals: GN for the glucosamine unit, Gal for the galactose unit, Neu for the neuraminic acid unit, and Sp for the spacer unit. Numeration of the atoms in spacer is Sug-1-2-3. Mass spectrum was recorded using matrix-assisted laser desorption ionization (MALDI)-time of flight (TOF) on a Vision 2000 mass spectrometer.

Methyl [phenyl 4,7,8,9-tetra-O-*acetyl-5-(*N-tert-*butoxycarbonylamino)-3,5-dideoxy-2-thio*-D-glycero-α,β-D-galacto-*non-2-ulopyranosid]onate* (**4**).—MeONa (1 M) in MeOH (0.1 mL, 0.1 mmol) was added to a solution of **3**[4] (211 mg, 0.31 mmol, α:β 1:2.3) in abs MeOH (5 mL), and after stirring for 1 h at rt under Ar, TLC (EtOAc) indicated the deacetylation to be com-

plete. Acetic acid (0.05 mL) was added, and the reaction mixture was concentrated and dried in vacuo. The residue was dissolved in pyridine (3 mL) and Ac_2O (2 mL) was added. After stirring overnight at rt MeOH (2 mL) was added, and the reaction mixture was concentrated with toluene. Chromatography (toluene → 3:2 toluene–EtOAc) of the residue on a column of silica gel (10 g) afforded **4** (196 mg, 98%) as white foam: R_f 0.65 (1:1 petroleum ether–EtOAc); NMR ($CDCl_3$, data for β anomer): ^{1}H, see Table 1 for carbohydrate ring protons; δ 7.52–7.12 (m, 5 H, Ph), 4.69 (d, 1 H, $J_{N-H,5}$ 10.6 Hz, N–H), 3.55 (s, 3 H, OMe), 2.05, 2.03, 1.99, 1.91 (4 s, 3 H each, 4 Ac), 1.37 (s, 9 H, $C(CH_3)_3$); ^{13}C, see Table 2 for carbohydrate ring carbons; δ 170.9, 170.5, 170.3, 169.7 (4 O*C*(O)CH_3), 155.3 (N*C*(O)O*C*-$(CH_3)_3$), 136.1 (Ph), 129.6 (Ph), 129.0 (Ph), 80.0 (O*C*$(CH_3)_3$), 52.5 (OCH_3), 28.2 (OC(*C*$H_3)_3$), 21.1–20.7 (4 OC(O)*C*H_3). Anal. Calcd for $C_{29}H_{39}NO_{13}S$ (641.68): C, 54.28; H, 6.13; N, 2.18. Found: C, 53.91; H, 5.99; N, 2.04.

Methyl [phenyl 5-acetoxyacetamido-4,7,8,9-tetra-O-*acetyl-3,5-dideoxy-2-thio*-D-glycero-α,β-D-galacto-*non-2-ulopyranosid]onate* (**6**) *and methyl [phenyl 4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-2-thio-5-trifluoroacetamido*-D-glycero-α,β-D-galacto-*non-2-ulopyranosid]onate* (**7**).—To a solution of **4** (200 mg, 0.31 mmol) in CH_2Cl_2 (3 mL), 90% aq CF_3CO_2H (1 mL) was added, and after stirring for 1 h at rt, the reaction mixture was coevaporated with toluene (6 × 5 mL) with the bath temperature below 30 °C, and dried extensively in vacuo to give the expected ammonium salt **5**, R_f 0.24 (20:1:1 CH_2Cl_2–MeOH–AcOH). To a solution of **5** in abs CH_2Cl_2 (5 mL), $AcOCH_2C(O)Cl$ (0.045 mL, 0.41 mmol) was added at 0 °C, followed by Et_3N (0.11 mL, 0.81 mmol). After stirring for 20 min at 0 °C, the reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with satd aq $NaHCO_3$, dried, and coevaporated with toluene. Chromatography (toluene → 2:1 toluene–acetone) of the residue on a column of silica gel (20 g) afforded (in order of elution) trifluoroacetamide **7** (50 mg, 25%) and acetoxyacetamide **6** (149 mg, 74%).

Data for **6**: white foam; R_f 0.37 (2:1 toluene–acetone); NMR ($CDCl_3$, data for β anomer): ^{1}H, see Table 1 for carbohydrate ring protons; δ 7.55–7.12 (m, 5 H, Ph), 6.16 (d, 1 H, $J_{N-H,5}$ 10.3 Hz, N–H), 4.62 and 4.31 (2 d, 1 H each, *J* 15.4 Hz, $AcOCH_2C(O)N$), 3.59 (s, 3 H, OMe), 2.21, 2.11, 2.09, 2.04, 1.93 (5 s, 3 H each, 5 Ac); ^{13}C, see Table 2 for carbohydrate ring carbons; δ 171.1, 171.0 (2 O*C*(O)CH_3), 170.2 (2 O*C*(O)CH_3), 169.7 (O*C*(O)CH_3), 168.1 (N*C*(O)CH_2OAc), 136.2 (Ph), 129.8 (Ph), 129.1 (Ph), 52.6 (OCH_3), 62.7 (NC(O)*C*H_2OAc), 21.5-20.8 (5 OC(O)*C*H_3). Anal. Calcd for $C_{28}H_{35}NO_{14}S$ (641.64): C, 52.41; H, 5.50; N, 2.18. Found: C, 52.65; H, 5.61; N, 2.01.

Data for **7**: white foam; R_f 0.6 (2:1 toluene–acetone); NMR ($CDCl_3$, data for β anomer): ^{1}H, see Table 1 for carbohydrate ring protons; δ 7.51–7.29 (m, 5 H, Ph), 6.92 (d, 1 H, $J_{N-H,5}$ 9.6 Hz, N–H), 3.57 (s, 3 H, OMe), 2.12 (s, 6 H, 2 Ac), 2.07 (s, 3 H, Ac), 1.96 (s, 3 H, Ac); ^{13}C, see Table 2 for carbohydrate ring carbons; δ 171.7, 170.9, 170.2, 169.8 (4 O*C*(O)CH_3), 157.7 (q, $^2J_{C,F}$ 38 Hz, $F_3CC(O)N$), 136.4 (Ph), 129.8 (Ph), 128.8 (Ph), 115.4 (q, $^1J_{C,F}$ 275 Hz, $F_3CC(O)N$), 52.5 (OCH_3), 20.9 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3), 20.5 (2 OC(O)*C*H_3); ^{19}F, δ −75.9 (CF_3). Anal. Calcd for $C_{26}H_{30}F_3NO_{12}S$ (637.58): C, 48.98; H, 4.74; F, 8.94; N, 2.20; S, 5.03. Found: C, 48.81; H, 4.86; F, 9.06; N, 2.01; S, 5.27.

3-Trifluoroacetamidopropyl O-*(2,3,4-tri*-O-*benzoyl-6*-O-*benzyl-β*-D-*galactopyranosyl)-(1→4)-2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranoside* (**11**).—Acceptor **10**[4] (250 mg, 0.45 mmol) was refluxed in abs CH_2Cl_2 (15 mL) under Ar until dissolution, then the mixture was allowed to attain rt, and **8**[23] (376 mg, 0.58 mmol) was added, followed by MS-4 Å (1.5 g). After stirring for 15 min, powdered AgOTf (231 mg, 0.9 mmol) was added, and stirring was continued for 18 h at rt. Et_3N (0.5 mL) was added, and the mixture was filtered through a pad of Celite, diluted with CH_2Cl_2 (150 mL), washed with satd aq $NaHCO_3$, 3 M aq $Na_2S_2O_3$, dried, and concentrated. Chromatography (toluene → 2:1 toluene–acetone) on a column of silica gel (50 g) afforded recovered acceptor **10** (44 mg, 17%) and lactosamine **11** (368 mg, 73%) as a white foam: R_f 0.51 (3:2 toluene–acetone); $[\alpha]_D$ 22° (*c* 0.6, CH_2Cl_2); NMR ($CDCl_3$): ^{1}H, see Table 1 for carbohydrate ring protons; δ 7.96, 7.91, 7.81 (3 d, 2 H each, *J* 7.4 Hz, ortho protons of 3 Bz), 7.59–7.14 (m, 24 H, Ph), 5.92 (d, 1 H, $J_{N-H,2}$ 8 Hz, N–H GN), 4.98 and 4.77 (2 d, 1 H each, *J* 11.7 Hz, $PhCH_2$), 4.69 and 4.43 (2 d, 1 H each, *J* 12 Hz, $PhCH_2$), 4.50 and 4.36 (2 d, 1 H each, *J* 11.9 Hz, $PhCH_2$), 3.78 (m, 1 H, H-1aSp), 3.47 (m, 1 H, H-3aSp), 3.32 (m, 2 H, H-1bSp, H-3bSp) 1.96 (s, 3 H, Ac), 1.75 (m, 2 H, H-2aSp, H-2bSp); ^{13}C, see Table 2 for carbohydrate ring carbons; δ 170.5 (N*C*(O)CH_3), 165.4 (O*C*(O)Ph), 157.1 ($F_3CC(O)N$), 138.5, 137.7, 137.5 (3 ipso Bn), 133.5–127.7 (Ph), 114.8 ($F_3CC(O)N$), 73.6 (3 $PhCH_2$), 67.1 (C-1Sp), 37.5 (C-3Sp), 28.1 (C-2Sp), 23.3 (NC(O)*C*H_3). Anal. Calcd for $C_{61}H_{61}F_3N_2O_{15}$ (1119.2): C, 65.47; H, 5.49; N, 2.50. Found: C, 65.42; H, 5.30; N, 2.56.

3-Trifluoroacetamidopropyl O-*(2,3,4-tri*-O-*benzoyl-6*-O-*benzyl-β*-D-*galactopyranosyl)-(1→4)-2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranoside* (**12**).—MeONa (1 M) in MeOH (0.03 mL, 0.03 mmol) was added to a solution of **11** (328 mg, 0.29 mmol) in abs MeOH (1.5 mL). After stirring overnight at rt, the reaction mixture was neutralized with KU-2 (H^+) cation-exchange resign, and the resign was filtered off, and the filtrate was concentrated. Chromatography (EtOAc → 9:1 EtOAc–MeOH) of the residue on a column of silica gel (10 g) gave **12** (213 mg, 90%) as amorphous mass: R_f 0.48 (1:2 toluene–acetone); $[\alpha]_D$

−6° (*c* 0.5, MeOH); NMR (1:1 CD_3OD–$CDCl_3$): ^1H, see Table 1 for carbohydrate ring protons; δ 7.15–6.92 (m, 15 H, Ph), 4.66 (d, 1 H, *J* 11.3 Hz, $PhCH_2$), 4.40–4.07 (m, 5 H, $PhCH_2$), 1.61 (s, 3 H, Ac), 1.56 (m, 2 H, H-2aSp, H-2bSp); ^{13}C, see Table 2 for carbohydrate ring carbons; δ 171.6 ($NC(O)CH_3$), 138.5 (ipso Bn), 137.6 (2 ipso Bn), 128.0–127.0 (Ph), 73.1 (2 $PhCH_2$), 73.0 ($PhCH_2$), 66.2 (C-1Sp), 36.5 (C-3Sp), 28.2 (C-2Sp), 22.2 ($NC(O)CH_3$). Anal. Calcd for $C_{40}H_{49}F_3N_2O_{12}$ (806.8): C, 59.55; H, 6.12; N, 3.47. Found: C, 59.41; H, 6.11; N, 3.15.

3-Trifluoroacetamidopropyl O-*[methyl (5-acetoxyacetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate]-(2→3)-(6*-O-*benzyl-β*-D-*galactopyranosyl)-(1→4)-2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranoside* (**13**).—A mixture of donor **6** (78 mg, 0.12 mmol), acceptor **12** (62 mg, 0.077 mmol), and abs MeCN (8 mL) was stirred with freshly activated MS-3 Å (800 mg) for 5 h, then cooled to −30 °C and NIS (54 mg, 0.24 mmol) was added, followed by TfOH (0.005 mL, 0.06 mmol). After stirring for 18 h at −20 °C, the reaction was terminated by the addition of Et_3N (0.1 mL), the mixture was filtered through a pad of Celite, diluted with CH_2Cl_2, washed with satd aq $NaHCO_3$, 3 M aq $Na_2S_2O_3$, dried, and concentrated. Chromatography (toluene→2:3 toluene–acetone) of the residue on a column of silica gel afforded, in order of elution, glycal **14** (39 mg, 61%), trisaccharide **13** (41 mg, 40%), and recovered acceptor **12** (27 mg, 43%).

Data for **13**: white foam; R_f 0.25 (1:1 toluene–acetone); $[\alpha]_D$ −6° (*c* 1, CH_2Cl_2); NMR ($CDCl_3$): ^1H, see Table 1 for carbohydrate ring protons; δ 7.57 (N–H Sp), 7.33–7.15 (m, 15 H, Ph), 5.62 (d, 1 H, *J* 7.1 Hz, N–H GN), 4.90 (d, 1 H, *J* 11.7 Hz, $PhCH_2$), 4.65–4.56 (m, 5 H, 3 $PhCH_2$, $AcOCH_2C(O)O$, H-1Gal), 4.46 (broad s, 2 H, $PhCH_2$), 4.33 (d, 1 H, *J* 15.6 Hz, $AcOCH_2C(O)O$), 3.81 (s, 3 H, OMe), 3.47 and 3.36 (2 m, 1 H each, H-3aSp, H-3bSp), 3.01 (s, 1 H, HO-2Gal), 2.62 (s, 1 H, HO-4Gal), 2.20, 2.17, 2.15 2.04, 2.00, 1.82 (6s, 3 H each, 6 Ac), 1.80 (m, 2 H, H-2aSp, H-2bSp); ^{13}C, see Table 2 for carbohydrate ring carbons; δ 74.0, 73.4, 73.0 (3 $PhCH_2$), 53.1 (OMe), 37.6 (C-3Sp), 28.2 (C-2Sp), 23.4 ($NC(O)CH_3$). Anal. Calcd for $C_{62}H_{78}F_3N_2O_{26}$ (1338.3): C, 55.64; H, 5.87; N, 3.14. Found: C, 55.24; H, 6.02; N, 2.85.

Data for **14**: white foam; R_f 0.61 (1:1 toluene–acetone); $[\alpha]_D$ 33° (*c* 1, EtOAc); NMR ($CDCl_3$): ^1H, see Table 1 for carbohydrate ring protons; δ 6.34 (d, 1 H, $J_{N-H,5}$ 9 Hz, N-H), 4.56 and 4.37 (2 d, 1 H each, *J* 15.3 Hz, $AcOCH_2(O)O$), 3.81 (s, 3 H, OMe), 2.15, 2.12 (2 s, 3 H each, 2 Ac), 2.06 (s, 6 H, 2 Ac), 2.03 (s, 3 H, Ac); ^{13}C, δ 170.8–169.6 ($CH_3C(O)O$), 161.4 (C-1), 144.9 (C-2), 108.1 (C-3), 76.6, 70.8, 67.7, 67.5, 63.7 ($AcOCH_2(O)O$), 61.8 (C-9), 52.4 (OMe), 46.3 (C-5), 20.6–20.4 ($CH_3C(O)O$). Anal. Calcd for $C_{22}H_{29}N_2O_{14}$ (531.5): C, 49.72; H, 5.50; N, 2.64. Found: C, 49.90; H, 5.48; N, 2.81.

3-Aminopropyl O-*(3,5-dideoxy-5-hydroxyacetamido*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)-(2→3)*-O-*(β*-D-*galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β*-D-*glucopyranoside* (**1**).—To a solution of **13** (56.5 mg, 0.042 mmol) in MeOH (5 mL), Pd–C (20 mg) was added, and the mixture was degassed under vacuum with stirring, and then refilled with hydrogen, and stirred under H_2 overnight at rt. The reaction mixture was filtered through Celite, the pad was washed thoroughly with MeOH, and the filtrate was concentrated. The residue was treated with 0.3 M aq KOH (2 mL) and after stirring for 1 h at rt, the pH of the solution was carefully made neutral by the 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 by elution with 0.1 M aq AcOH and subsequent lipohilization afforded compound **1** (26 mg, 82%). According to ^1H NMR, the material contained >5% of contamination, which appeared as a doublet of doublets at δ 2.48 ppm (*J* 4.7 and *J* 12 Hz) and a triplet (*J* 12 Hz) at δ 1.71 ppm and perhaps might be tentatively assigned to be the β-Neu linked isomer of **1**.[24] Purification by reverse-phase column chromatography on a RP C18 (5 micron particle size) 1 × 25 cm column (IBM Instruments, Inc.) by elution with water gave (in order of elution) the mixed fraction (3.6 mg) and the α anomer **1** (22 mg, 70%), HPLC analysis of which indicated 99% purity: hygroscopic amorphous mass; R_f 0.45 in 1:1 BPHCl–AMW; $[\alpha]_D$ −12° (*c* 0.5, water); NMR (D_2O): ^1H, see Table 1 for carbohydrate ring protons; δ 4.12 (NC(O)–CH_2–OH), 4.01 (m, 2 H, H-6aGN, H-1aSp), 3.71 (m, H-1bSp), 3.04 (t, 2 H, *J* 7 Hz, H-3aSp, H-3bSp), 2.02 (s, 1 H, Ac), 1.92 (m, 2 H, H-2aSp, H-2bSp); ^{13}C, see Table 2 for carbohydrate ring carbons; δ 177.0 ($NC(O)CH_3$), 175.8 ($NC(O)$–CH_2–OH), 69.2 (C-1Sp), 62.2 (NC(O)–CH_2–OH), 38.8 (C-3Sp), 28.2 (C-2Sp), 23.3 ($NC(O)CH_3$). MALDI-TOF–MS: Calcd for $[C_{28}H_{49}N_3O_{20}$ (747.29) + Na$]^+$: 770.3. Found 770.1.

Acknowledgements

This work was supported by GlycoSense AG (Jena, Germany).

References

1. Varki, A. *FASEB J.* **1997**, *11*, 248–255.
2. Nifant'ev, N. E.; Tsvetkov, Y. E.; Shashkov, A. S.; Kononov, L. O.; Menshov, V. M.; Tuzikov, A. B.; Bovin, N. V. *J. Carbohydr. Chem.* **1996**, *15*, 939–953.

3. Sherman, A. A.; Yudina, O. N.; Mironov, Y. V.; Sukhova, E. V.; Shashkov, A. S.; Menshov, V. M.; Nifantiev, N. E. *Carbohydr. Res.* **2001**, *336*, 13–46.
4. Sherman, A. A.; Yudina, O. N.; Shashkov, A. S.; Menshov, V. M.; Nifantiev, N. E. *Carbohydr. Res.* **2001**, *330*, 445–458.
5. Roy, R; Laferriere, C. A. *Can. J. Chem.* **1990**, *68*, 2045–2054.
6. Schmid, W; Avila, L. Z.; Williams, K. W.; Whitesides, G. M. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 747–752.
7. Simeoni, L. A.; Bairamova, N. E.; Bovin, N. V. *Bioorgan. Khim.* **1996**, *22*, 599–605.
8. Schreiner, E.; Zbiral, E.; Kleineidam, R. G.; Schauer, R. *Carbohydr. Res.* **1991**, *216*, 61–66.
9. Fujita, S.; Numata, M.; Sugimoto, M.; Tomita, K.; Ogawa, T. *Carbohydr. Res.* **1992**, *228*, 347–370.
10. Sugata, T.; Higuchi, R. *Tetrahedron Lett.* **1996**, *37*, 2613–2614.
11. Tanahashi, E.; Fukunaga, K.; Ozawa, Y.; Toyoda, T.; Ishida, H.; Kiso, M. *J. Carbohydr. Chem.* **2000**, *19*, 747–768.
12. Numata, M.; Sugimoto, M.; Shibayama, S.; Ogawa, T. *Carbohydr. Res.* **1988**, *174*, 73–85.
13. Hasegawa, A.; Uchimura, A.; Ishida, H.; Kiso, M. *Biosci. Biotech. Biochem.* **1995**, *59*, 1091–1094.
14. Kuboki, A.; Okazaki, H.; Sugai, T.; Ohta, H. *Tetrahedron* **1997**, *53*, 2387–2400.
15. Lubineau, A.; Augé, C.; Gautheron-Le Narvor, C.; Ginet, J.-C. *BioMed. Chem.* **1994**, *2*, 669–674.
16. Burk, M. J.; Allen, J. G. *J. Org. Chem.* **1997**, *62*, 7054–7057.
17. Flynn, D. L.; Zelle, R. E.; Grieco, P. A. *J. Org. Chem.* **1983**, *48*, 2424–2426.
18. Henry, C.; Joly, J. P.; Chapleur, Y. *J. Carbohydr. Chem.* **1999**, *18*, 689–695.
19. Gervay, J.; Flaherty, T. M.; Nguyen, C. *Tetrahedron Lett.* **1997**, *38*, 1493–1496.
20. Bodanszky, M. *Principles of Peptide Chemistry*; Springer-Verlag: New York, 1984.
21. Komba, S.; Galustian, C.; Ishida, H.; Feizi, T.; Kannagi, R.; Kiso, M. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 1131–1133.
22. Ehara, T.; Kameyama, A.; Yamada, Y.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1996**, *281*, 237–252.
23. Nilsson, S.; Lönn, H.; Norberg, T. *Glycoconjugate J.* **1991**, *8*, 9–15.
24. Vliegenthart, J. F. G.; Dorland, L.; van Halbeek, H.; Haverkamp, J. In *Cell Biology Monographs*; Schauer, R., Ed. NMR spectroscopy of Sialic Acids; Springer-Verlag: New York, 1982; Vol. 10, pp. 127–172 and references cited therein.