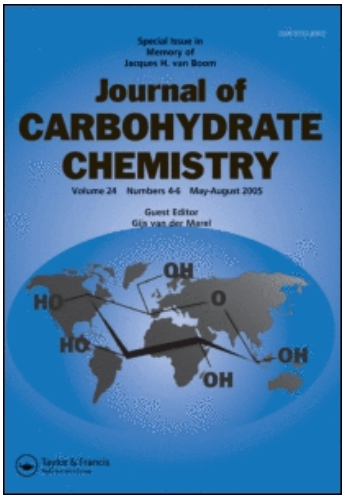

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Selectin Receptors 4: Synthesis of Tetrasaccharides Sialyl Lewis A and Sialyl Lewis X Containing A Spacer Group[1,2]

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SELECTIN RECEPTORS 4: SYNTHESIS OF TETRASACCHARIDES SIALYL LEWIS A AND SIALYL LEWIS X CONTAINING A SPACER GROUP[1,2]

Nikolay E. Nifant'ev,[a]* Yury E. Tsvetkov,[a] Alexander S. Shashkov,[a] Leonid O. Kononov,[a] Vladimir M. Menshov,[a] Alexander B. Tuzikov,[b] and Nicolai V. Bovin[b]

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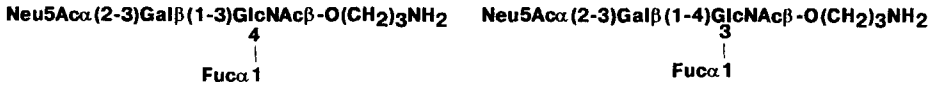
ABSTRACT

Synthesis of two isomeric tetrasaccharides, namely Neu5Acα(2→3)Galβ(1→3)[Fucα(1→4)]GlcNAcβ (sLe^a) and Neu5Acα(2→3)Galβ(1→4)[Fucα(1→3)]GlcNAcβ (sLe^x) as 3-aminopropyl glycosides is described. Preparation of these compounds was performed by sialylation of selectively protected trisaccharides Le^a and Le^x which contain three unsubstituted OH groups at positions 2, 3 and 4 of Gal residue. Glycosylation of Le^x trisaccharide with ethylthio sialoside under promotion by NIS and TfOH in acetonitrile was effective and regio- and stereoselective to give sLe^x derivative in 81% yield. In contrast, sialylation of the Le^a acceptor was accompanied by a variety of undesirable by-processes, namely, *N*-thioethylation of the GlcNAc residue, β-sialylation, and lactonisation. In order to improve the yield of sLe^a tetrasaccharide the glycosylation of Le^a acceptor by sialyl donors of ethyl and phenyl thioglycoside (promoted by NIS-TfOH or NBS-Bu_4NBr), xanthate (promotion by NIS-TfOH mixture or MeOTf) and phosphite (promoted by TMSOTf) types was also studied. Among the reactions investigated the glycosylation by phenyl thioglycoside sialoside promoted by NIS-TfOH gives the best yield (39%) of sLe^a tetrasaccharide product.

INTRODUCTION

sLe^a and sLe^x tetrasaccharides were shown to be weak affinity ligands for selectins and some other cell-adhesion molecules.[3-6] Several syntheses of these tetrasaccharides and larger oligosaccharides which contain them as terminal fragments have been published.[7-15]

In this paper we describe the synthesis of sLe^a and sLe^x tetrasaccharides as their 3-aminopropyl glycosides (**1**) and (**2**) suitable for further conjugation with polymeric carriers in order to produce a variety of glycoconjugates for glycobiology research (see ref. 16-20).

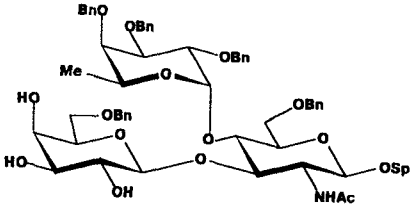


1 **2**

RESULTS AND DISCUSSION

The synthesis of target compounds **1** and **2** was performed by sialylation of the selectively substituted spacergroup containing Le^a (**3**)[16] and Le^x (**4**, preparation will be described elsewhere) trisaccharide derivatives, which contain free OH groups at positions 2,3, and 4 of the Gal residues. These compounds were chosen taking into account high effectiveness of such triolic sialyl acceptors, which was shown previously.[21]

Aiming at the synthesis of sLe^x tetrasaccharide (**8**) containing a suitable spacer group we carried out the glycosylation of acceptor (**3**) by ethyl [methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranoside)onate][22] (**9**, α:β ~1:1) promoted by NIS and TfOH.[21] This reaction was highly effective, stereo- and regiospecific and gave tetrasaccharide **8** in 81% yield. The latter was then subjected to hydrogenolysis and saponification to give target spacer group containing oligosaccharide **2** (92%), whose structure was determined using ^{1}H and ^{13}C NMR spectroscopy (Table 1 and 2). In particular, the α-configuration of neuraminic acid residue was confirmed on the basis of characteristic[23] values of H-3*eq* chemical shift (δ 2.78) and spin-spin coupling constant $J_{C\text{-}1,H\text{-}3ax}$ (5.9 Hz). Substitution of the Gal unit at position 3 was followed from the low-field location (δ 77.0) of the signal of C-3Gal. ^{1}H and ^{13}C NMR spectra of **2** are in good agreement with those of sLe^x tetrasaccharide ethyl glycoside which were published previously.[13]



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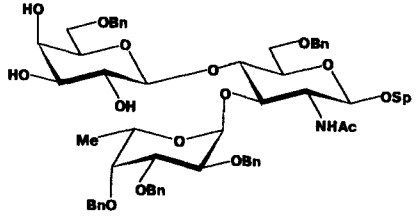

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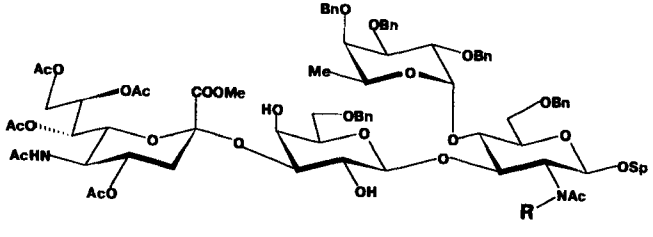
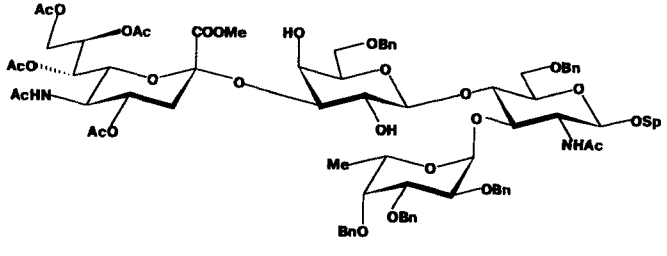


5 R = H
6 R = SEt
7 R = SPh



8

Sp = $CF_3CONH(CH_2)_3$-

Table 1. ^{1}H NMR data (δ in ppm, *J* in Hz) for **1**, **2**, **14**, and **15** in D_2O and for lactone 13 in $CDCl_3$.
a). Chemical shifts (δ in ppm)

Compound	*Residue*	*H-1*	*H-2*	*H-3$_{ax}$*	*H-3$_{eq}$*	*H-4*	*H-5*	*H-6a*	*H-6b*	*H-7*	*H-8*	*H-9a*	*H-9b*	*COCH$_3$*	*OCH$_2$—CH$_2$—CH$_2$N*		
1	GlcNAc	4.45	3.82	3.99		3.64	3.48	3.75	3.90					1.95[a]	3.63;	1.86	2.99
	Gal	4.46	3.41	3.95		3.83	3.45	3.62	3.62						3.92		
	Fuc	4.92	3.71	3.81		3.70	4.75	1.09									
	Neu			1.68	2.69	3.59	3.75	3.55		3.54	3.77	3.57	3.73	1.97[a]			
2	GlcNAc	4.53												2.01	3.71;	1.93	3.06
	Gal	4.51	3.52	4.08											4.06		
	Fuc	5.10					4.79	1.27									
	Neu			1.78	2.78									2.04			
13	GlcNAc	4.47	3.94	4.03		3.73	3.53	3.84	3.99					b	n.d.	n.d.	n.d.
	Gal	4.93	3.85	4.80		4.14											
	Fuc	4.95	3.77	3.84		3.78	4.78	1.15									
	Neu			2.10	2.62	5.22	4.15	4.34		5.25	5.38	4.30	4.48	b			
14	GlcNAc	4.58	4.03	4.22		3.86	3.64	3.87	3.98					1.97[a]	3.72;	1.96	3.10
	Gal	4.65	3.78	3.71		3.88	3.62	3.73	3.73						4.03		
	Fuc	5.01	3.82	3.86		3.78	4.61	1.18									
	Neu			1.70	2.44	3.96	3.94	4.08		3.60	3.95	3.77	3.92	2.11[a]			
15	GlcNAc	4.60	3.88	4.09		3.76	3.63	3.87	4.01					1.99[a]	3.75;	1.97	3.12
	Gal	4.52	3.57	3.64		4.20	3.54	3.72	3.72						4.03		
	Fuc	5.02	3.82	3.91		3.81	4.83	1.19									
	Neu			1.71	2.50	4.23	3.93	3.98		3.53	3.82	3.70	3.87	2.06[a]			

a. Assignments may be interchanged. b. 6x3H, 1.98, 2.05, 2.07, 2.12, 2.13, 2.14.

Table 1. (continued)
b). Coupling constants (J in Hz)

Compound	*Residue*	$J_{1,2}$	$J_{2,3}$	$J_{3ax,4}$	$J_{3eq,4}$	$J_{3ax,3eq}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$	$J_{6,7}$	$J_{7,8}$	$J_{8,9a}$	$J_{8,9b}$	$J_{9a,9b}$
1	GlcNAc	8.2	10.0	9.5			9.5	4.0	2.0	12.5					
	Gal	7.6	9.6	3.1			<1	6.0	6.0	0					
	Fuc	3.8		2.8			<1	6.5							
	Neu			12.0	4.5	12.0	10.0	10.0						3.0	10.8
2	GlcNAc	7.5													
	Gal	7.8	9.0	2.8											
	Fuc	3.9					<1	6.1							
	Neu			12.0	4.1	12.0									
13	GlcNAc	7.5					9.1	4.8	2.2	12.0					
	Gal	7.5	9.4	3.1			<1								
	Fuc	3.0						6.4							
	Neu			11.0	5.5	12.8	10.0	10.0			2.0	4.2	6.5	4.5	12.0
14	GlcNAc	8.0	8.0	8.0			8.0								
	Gal	7.1	8.2	3.9			<1	5.7	5.7						
	Fuc	3.8	8.2	3.0			<1	6.5							
	Neu				3.5	12.6		10.6			1.2	8.5			12.1
15	GlcNAc	8.0	7.6	7.6			7.6	3.6	2.2	9.6					
	Gal	7.2	9.6	3.0			<1	5.0	5.0						
	Fuc	3.7					<1	6.6							
	Neu			10.0	4.0	10.7	9.7				7.0		3.0	2.2	10.0

Table 2. ^{13}C NMR data (δ in ppm) for **1**, **2**, **14**, and **15** in D_2O.

Compound	*Residue*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-7*	*C-8*	*C-9*	*$CO\underline{C}H_3$*	*$\underline{C}OCH_3$*	*OCH_2-CH_2-CH_2N*			*$J_{C\text{-}1,H\text{-}3ax}$*
1	GlcNAc	102.2	56.9	77.3	73.6	76.7	61.0				23.3[a]	175.6[b]	69.2	28.1	39.0	
	Gal	104.0	70.1	77.0	68.2	76.0	62.9									
	Fuc	99.3	69.1	70.4	73.2	68.1	16.6									
	Neu	175.1	100.7	41.3	69.4	53.0	74.1	69.6	73.1	63.7	23.7[a]	176.3[b]				5.6
2	GlcNAc	102.4	57.1	76.2[c]	74.6[c]	76.5[c]	60.9				23.4[a]	175.8[b]	69.4	28.2	38.9	
	Gal	102.9	70.5	77.0	69.0[c]	76.0[c]	62.8									
	Fuc	99.9	69.0[c]	70.5	73.2	68.0	16.6									
	Neu	175.3	101.0	41.1	69.4[c]	53.0	74.2[c]	69.6[c]	73.2	63.9	23.5[a]	176.4[b]				5.9
14	GlcNAc	102.9	57.1	77.0	74.2	77.7	62.6				23.6[a]	175.3[b]	69.5	28.2	39.1	
	Gal	103.2	76.0	74.9	70.5	76.2	63.5									
	Fuc	99.3	69.3	70.6	73.3	68.3	17.1									
	Neu	176.7	102.7	42.4	67.8	53.6	74.1	71.8	72.4	64.9	23.6[a]	175.8[b]				<1
15	GlcNAc	102.1	57.2	76.9	73.8	77.8	61.5				23.5[a]	175.6[b]	69.2	28.0	39.2	
	Gal	104.6	70.2	79.1	69.3	76.1	62.5									
	Fuc	99.2	69.4	70.7	73.4	68.1	16.7									
	Neu	176.4	104.5	42.1	67.5	53.7	72.9	70.4	71.5	64.9	23.8[a]	176.0[b]				<1

a,b. Assignments may be interchanged. c. Tentative assignment based on the data of ref. 13.

Comparing the reaction between **4** and **9**, the sialylation of Le^a trisaccharide **3** by **9** under the similar conditions (Table 3, run 1) was less effective and gave the target sLe^a tetrasaccharide **5** in a yield of only 19%. Removal of the blocking groups in **5** gave quantitatively the target aminopropyl glycoside of sLe^a tetrasaccharide **1**. Its ^1H and ^{13}C NMR data (Tables 1 and 2) unambiguously confirmed α-configuration of Neu residue and (2→3)-linkage between Neu and Gal units.

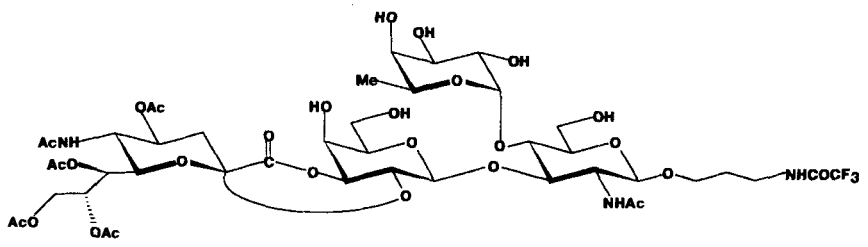
In addition to **5** two types of side products were obtained. The faster moving compound (see Experimental) was obtained in a yield of 29%, and is, presumably, the product of *N*-thioethylation of the NHAc group of the GlcNAc residue (**6**). It is unstable and decomposed during storage at room temperature with formation of **5** and expression of a strong mercaptan smell. Catalytic hydrogenolysis and subsequent saponification of **6** gave compound **1** in 77% yield.

The structure of **6** was deduced based on its ^1H and ^{13}C NMR spectral data. Its ^1H NMR spectrum contains signals of CH_3CH_2 group (δ 1.20, m, 3H; 2.92, m, 2H; see Experimental). To determine the location of this fragment the ^{13}C NMR spectra of **5** and **6** (see Experimental) were compared and it was shown that they have several characteristic differences and the most informative one is the absence in the spectrum of **6** of the C-2GlcNAc signal in the typical area at δ 50-60. At the same time, the ^{13}C NMR spectrum of **6** contains a signal at δ 49.5 that is typical for the signal of C5Neu. These facts together with observed formation of **1** and **5** from **6** provides evidence that the most probable structure for compound **6** contains an EtS substituent which is connected to the acetamido group of GlcNAc residue.

In the reaction of **3** with **9** a broad fraction of compounds with interstitial chromatographic mobility (as compared to that of **5** and **6**) was also isolated. They are unstable during column and thin-layer chromatography. ^1H and ^{13}C NMR spectra of the whole fraction shows it contains several components and none of them has a MeO-group. These data allowed us to conclude that a mixture of isomeric tetrasaccharide lactones was formed during glycosylation of **3** by **9**. One of the components of the mixture was isolated, after removal of benzyl groups, in individual form as compound **13**. It was surprising that after catalytic hydrogenolysis and subsequent saponification of the entire lactonic fraction, only two tetrasaccharides, namely compounds **14** and **15**, were obtained. Their structures were determined on the basis of ^1H and ^{13}C NMR spectra. In particular, β-configuration of Neu-residues was confirmed on the basis of characteristic[23] values of H-3eq chemical shift in ^1H NMR spectra of **14** and **15** (δ 2.44 and 2.50, respectively, Table 1) and spin-coupling constant $J_{C\text{-}1,H\text{-}3ax}$ (<1 Hz) in ^{13}C NMR spectra (Table 2). Substitution of the Gal unit at O-2 in **14** and at O-3 in **15** was followed from

the low-field location of the signal of C-2Gal (δ 76.0) in the ^{13}C NMR spectrum of **14** and of the signal of C-3Gal (δ 79.1) in the ^{13}C NMR spectrum of **15** (Table 2).

The monosaccharide sequence in **13** was deduced taking into account the structure of tetrasaccharide **14** which was obtained by saponification of **13**. The location of the lactone ring in **13** was confirmed from the low-field location of the H-3Gal signal in the ^{1}H NMR spectrum (δ 4.80, Table 1) that may be caused only by acylation by the carboxy function of the Neu residue. The formation of oligosaccharide lactones during glycosylation by sialyl donors and above self-lactonization of neuraminic acid containing oligosaccharides is well documented.[15,24-26]



13

Neu5Acβ(2-2)Galβ(1-3)GlcNAcβ-O(CH$_2$)$_3$NH$_2$
4
|
Fucα1

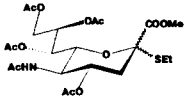
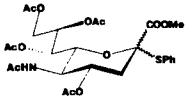
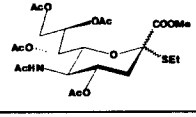
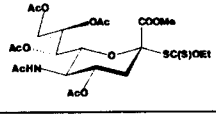
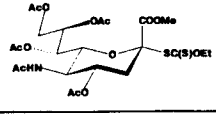
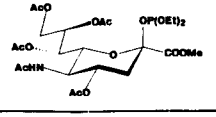
14

Neu5Acβ(2-3)Galβ(1-3)GlcNAcβ-O(CH$_2$)$_3$NH$_2$
4
|
Fucα1

15

In order to improve the yield of the substituted SiLe^a tetrasaccharide **5**, glycosylation of trisaccharide triol **3** under modified reaction conditions has been also studied (Table 3, *runs 2-6*). In particular, glycosylation of **3** by phenyl thioglycoside **10** also promoted by NIS-TfOH (Table 3, *run 2*) was investigated and as was recently shown[27] has higher effectiveness when compared with that of ethyl thioglycoside **9**. Glycosylation by **10** gave a better yield of target tetrasaccharide **5** (39%; 42% based on consumed **5**) and no formation of the undesirable side product of *N*-thiophenylation (**8**) was observed. At the same time, the reaction of **5** with **10** was also characterized by the formation of a large lactonic fraction (35%) which was higher than in *run 1* (28%). Lactones were deprotected and the resulted tetrasaccharides **1**, **14**, and **15** were separated by HPLC in the yields of 13, 37, and 36%, respectively (see Experimental). Thus, on glycosylation by phenyl thioglycoside **10**, which needs longer reaction time, the

Table 3. Glycosylation of selectively substituted spacered Lewis A trisaccharide **3** by sialyl donors **9-12**

Run	Glycosyl Donor	Donor/ Acceptor	Promoter	Reaction Conditions	Products	Yield	Recovery of Acceptor
1	 **9**	**1.7 : 1**	**NIS-TfOH**	**MeCN, -40°C**	**5 6 Lactones**	**19% 29% 28%**	–
2	 **10**	**1.7 : 1**	**NIS-TfOH**	**MeCN, -40°C**	**5 7 Lactones**	**39% No 35%***	**8%**
3	 **9**	**2.8 : 1**	**NBS-Bu_4NOTf**	**EtCN, 0°C**	**5 Lactones**	**11% n.d.**	**54%**
4	 **11**	**2 : 1**	**NIS-TfOH**	**MeCN, -40°C**	**5 Lactones**	**27% 23%**	**36%**
5	 **11**	**2 : 1**	**MeSOTf**	**MeCN-CH_2Cl_2 -78°C**	**5 Lactones**	**21% n.d.**	**28%**
6	 **12**	**1.8 : 1**	**TMSOTf**	**MeCN, -40°C**	**5 Lactones**	**17% 37%**	**33%**

*Contains 13% (5% as calculated for **3**) of compound(s) with Sialyl Lewis A backbone (see Experimental).

lactonization of both α- (in small part) and β-sialylated products takes place. This shows the lactonization of β-isomers is faster.

The sialylation reactions of trisaccharide triol **3** by ethyl thioglycoside **9** under promotion with NBS-Bu_4NOTf mixture[28] (*run 3*), by sialyl xanthate **11**[22] under promotion with NIS-TfOH[29] (*run 4*) and MeSOTf[15,30,31] (*run 5*), and by sialyl phosphite **12**[29,32,33] catalyzed by TMSOTf (*run 6*) were markedly less effective. In all these reactions lactonic fractions were isolated but were not analyzed separately. By deprotection of lactones from *runs 3-6* the isomeric tetrasaccharides **14** and **15** were obtained in a similar ratio (1.1:1) to that in the case of *runs 1,2* and no tetrasaccharide **1** was indicated.

In conclusion, the sialylation of Le^a trisaccharide triol **5** by the different sialyl donors was most effective with the use of phenyl thioglycoside donor **10**. It is noteworthy that on sialylation of compound **5** three types of side reactions, namely formation of β-sialosides, lactonization and *N*-thioethylation, were observed. These reactions are acceptor structure dependent and do not take place in the glycosylation of the similar trisaccharide triol but with the Le^x backbone.

EXPERIMENTAL

General methods. TLC was performed on Silica Gel 60 F_{254} (E. Merck, Darmstadt, Germany) with benzene-acetone (3:2) and with detection by charring with H_3PO_4. Medium pressure liquid chromatography was performed on Silica Gel L 40-100 μm (C.S.F.R.) by gradient elution with benzene-acetone. Optical rotations for substituted compounds **12-23** and **29** were determined on solutions in $CHCl_3$, and for oligosaccharides **2-6** and **9** in water with a Jasco DIP-360 digital polarimeter at 26-30 °C.

^1H and ^{13}C NMR spectra were recorded at 303 °K on a Bruker AMX 300 (300.13 MHz for ^1H and 75.47 MHz for ^{13}C); $CDCl_3$ was used as the solvent for substituted derivatives and D_2O for unsubstituted tetrasaccharides **1**, **2**, **14**, and **15**. Assignments of ^1H spectra (Table 2) were made using a combination of COSY and RCT 2D experiments (standard Bruker softwares for ASPECT-3000). The assignments of the ^{13}C NMR spectra (Table 3) were made by using of 2D ^1H -^{13}C correlated spectroscopy.

Glycosylation reactions were carried out under argon with freshly distilled absolute solvents.

Sialylation of Le^a triol 3 (Table 3).

Run 1. A mixture of triol **3**[16] (215 mg, 0.19 mmol), thiosialoside **9**[22] (170 mg, 0.32 mmol), powdered molecular sieves 3A, and MeCN (7 mL) was stirred for 2 h at 20

°C under Ar. The mixture was cooled to -30 - -40 °C and NIS (181 mg, 0.80 mmol) together with TfOH (0.08 mmol, 7.1 μL) were added. The stirring was continued for 1 h at -30 - -40 °C, Py (0.2 mL) was added, the mixture was filtered through Celite, and washed with $CHCl_3$ (20 mL). The filtrate was washed with water (10 mL), aq $Na_2S_2O_3$ (2x10mL) and water (10mL), filtered through cotton wool and concentrated. The residue was subjected to column chromatography to give 3-(trifluoroacetamido)propyl *O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-*O*-(6-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→4)-2-(*N*-ethylthio)acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**6**) (92 mg, 29%), fraction with R_F 0.20-0.24 (solvent A) of lactones (83 mg, 28%), and 3-(trifluoroacetamido)propyl *O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)onate]-(2→3)-*O*-(6-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→4)-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**5**) (58 mg, 19%).

Compound **5**: $[\alpha]_D$ -28° (*c* 1, $CHCl_3$), R_F 0.12; selected ^{13}C NMR data: δ_C 16.6 (C6Fuc), 20.6-21.0 ($4COC\underline{H}_3$), 23.1 and 23.3 ($2NHCOC\underline{H}_3$), 27.7 ($OCH_2\underline{C}H_2CH_2N$), 36.6 (3Neu), 37.1 ($OCH_2CH_2\underline{C}H_2N$), 49.3 (C5Neu), 53.0 ($OCH_3$), 54.6 (C2GlcN), 62.6 (C9Neu), 97.5 (C1Fuc), 98.7 (C2Neu), 100.1 and 100.5 (C1 of GlcN and C1Gal).

Anal. Calcd for compound **5**; $C_{80}H_{98}N_3O_{28}F_3$ (1606.66): C, 59.81; H, 6.15; N, 2.62. Found: C, 59.49; H 6.38; N 2.40.

Compound **6**: $[\alpha]_D$ -26° (*c* 1, $CHCl_3$), R_F 0.31; selected ^1H NMR data: δ_H 1.20 (m, 3H, $SCH_2C\underline{H}_3$), 2.92 (m, 2H, $SC\underline{H}_2CH_3$); selected ^{13}C NMR data: δ_C 11.4 ($SCH_2\underline{C}H_3$), 16.8 (C6Fuc), 20.7-21.2 ($4COC\underline{H}_3$), 23.2 ($NHCOC\underline{H}_3$), 28.4 ($OCH_2\underline{C}H_2CH_2N$), 37.4 (3Neu), 37.6 ($OCH_2CH_2\underline{C}H_2N$), 49.5 (C5Neu), 53.1 ($OCH_3$), 62.1 (C9Neu), 97.8 (C1Fuc), 97.9 (C2Neu), 99.4 and 100.1 (C1 of GlcN and C1Gal).

Run 2. Glycosylation of trisaccharide **3** (215 mg, 0.19 mmol) by phenylthio sialoside **10**[22] (187 mg, 0.32 mmol) was performed as in *run* 1, but with 3.5 h duration of the glycosylation step, to give tetrasaccharide **5** (119 mg, 39%), starting triol **4** (17 mg, 8%), and lactonic fraction (105 mg, 35%).

Run 3. A mixture of ethylthio sialoside **9** (101 mg, 0.19 mmol), trisaccharide **3** (75 mg, 0.07 mmol), powdered molecular sieves 3A, and EtCN (1 mL) was stirred for 3 h at 20 °C under Ar. The mixture was cooled to -78 °C and the solution of *N*-bromosuccinimide (34.3 mg, 0.19 mmol) and Bu_4NOTf (25.1 mg, 0.07 mmol) in EtCN (0.6 mL) was added. The stirring was continued for 7 h at -78 °C, then for 12 h at -40 - -50 °C, and finally for 48 h at room temperature. The mixture was diluted with CH_2Cl_2 (20 mL), filtered through Celite, and washed with $CHCl_3$ (30 mL), the filtrate was washed with aq. $NaHCO_3$ (2x15 mL) and water (20 mL), filtered through cotton wool

and concentrated. The residue was subjected to column chromatography to give tetrasaccharide **5** (12 mg, 11%), and starting triol **3** (41 mg, 54%); lactones were not separated in this experiment due to their marked destruction during workup of the reaction mixture.

Run 4. Glycosylation of trisaccharide **3** (140 mg, 0.12 mmol) by xanthate **11**[22] (142 mg, 0.24 mmol) in the presence of NIS (110 mg, 0.48 mmol) and TfOH (5 μL, 0.06 mmol) was performed like in *run* 1 to give tetrasaccharide **5** (53 mg, 27%), starting triol **3** (68 mg, 36%), and lactonic fraction (32 mg, 23%).

Run 5. A mixture of triol **3** (80 mg, 0.07 mmol), xanthate **11** (84 mg, 0.14 mmol), powdered molecular sieves 3A, CH_2Cl_2 (0.75 mL) and MeCN (0.3 mL) was stirred for 30 min at 20 °C under Ar. AgOTf (38 mg, 0.15 mmol) was added, the mixture was cooled to -78 °C and stirred 10 min, and then 4.4 M solution of MeSBr (0.035 mL) in 1,2-dichloroethane. The stirring was continued for 2 h at -78 °C, iso-Pr_2NH (0.2 mL) was added, the mixture was stirred 2 h, diluted with $CHCl_3$, filtered through Celite, and washed with $CHCl_3$ (30 mL).The filtrate was washed with aq $NaHCO_3$ (2x15 mL) and water (20 mL), filtered through cotton wool and concentrated. The residue was subjected to column chromatography to give tetrasaccharide **5** (24 mg, 21%) and starting triol **4** (22 mg, 28%); lactones were not separated in this experiment due to their marked destruction during workup of the reaction mixture.

Run 6. A mixture of triol **3** (200 mg, 0.18 mmol), sialosyl phosphite **12**[29,32] (200 mg, 0.32 mmol), powdered molecular sieves 3A, and MeCN (4 mL) was stirred for 30 min at 20 °C under Ar. The mixture was cooled to -30 - -40 °C and 0.1 M solution of TMSOTf (0.3 mL) in MeCN was added. The stirring was continued for 1 h at -30 - -40 °C, Et_3N (0.2 mL) was added, the mixture was filtered through Celite, and concentrated. The residue was subjected to column chromatography to give **5** (50 mg, 17%), starting triol **4** (60 mg, 33%), and lactonic fraction (105 mg, 37%).

3-Aminopropyl *O*-[Potassium (5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-*O*-(β-D-galactopyranosyl)-(1→3)-*O*-[(α-L-fucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (1). *a).* Compound **5** (120 mg, 0.075 mmol) was subjected to catalytic hydrogenolysis in EtOH-EtOAc (1:2, 12 mL) under 10% Pd-C at 41 °C and atm. pressure for 4 h [R_F →0.15 (solvent B)]. The mixture was filtered and the solvent was evaporated *in vacuo*, a solution of the residue in aqueous 0.15M KOH (4.4 mL) was kept for 2 h at 20 °C. A mixture was neutralized with AcOH and concentrated to dryness. HPLC of the residue on the column (250x25 mm) with Silasorb C-18 (5 μm) with elution by pure water gave **1** (61 mg, 89%), $[\alpha]_D$ -57° (*c* 1, H_2O). The ^1H and ^{13}C NMR data are presented in Tables 1 and 2.

Anal. Calcd for compound **1**; $C_{34}H_{58}N_3O_{23}K$ (955.04): C, 44.59; H, 6.38; N, 4.59. Found: C, 44.31; H 6.10; N 4.71.

b). Compound **6** (44 mg, 0.03 mmol) was subjected to catalytic hydrogenolysis and subsequent saponification as described above (see *run a*) to give **1** (19 mg, 77%).

Deprotection of lactonic products from run 1. *a).* Lactonic products (145 mg, 0.092 mmol) were subjected to catalytic hydrogenolysis and subsequent saponification as described for the preparation of **1** to give 3-aminopropyl *O*-[potassium (5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl)onate]-(2→2)-*O*-(β-D-galactopyranosyl)-(1→3)-*O*-[(α-L-fucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside {**14**, 23 mg, 45%, $[\alpha]_D$ -65° (*c* 2, H_2O)} and 3-aminopropyl *O*-[potassium (5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl) onate]-(2→3)-*O*-(β-D-galactopyranosyl)-(1→3)-*O*-[(α-L-fucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside {**15**, 19 mg, 38%, $[\alpha]_D$ -64° (*c* 2, H_2O)}. The ^1H and ^{13}C data for **14** and **15** are presented in Tables 1 and 2.

Anal. Calcd for compound **14**; $C_{34}H_{58}N_3O_{23}K$ (955.04): C, 44.59; H, 6.38; N, 4.59. Found: C, 44.87; H 6.51; N 4.73.

Anal. Calcd for compound **15**; $C_{34}H_{58}N_3O_{23}K$ (955.04): C, 44.59; H, 6.38; N, 4.59. Found: C, 44.30; H 6.24; N 4.74.

b). Lactonic products (70 mg, 0.045 mmol) were subjected to catalytic hydrogenolysis as described for preparation of **1**, from the resulting mixture the (1→3)-lactone **13** was separated {25 mg, $[\alpha]_D$ -15° (*c* 2, H_2O)} in individual form by HPLC on the column (250x25 mm) with Silasorb C-18 (5 μm) with elution by MeOH-water (9:11). Saponification of **13** gave **14** (20 mg, 90%). The ^1H data for **13** are presented in Table 1.

Deprotection of lactonic products from run 2. Lactonic products (105 mg, 0.067 mmol) were subjected to catalytic hydrogenolysis and subsequent saponification as described for preparation of **1** to give isomeric tetrasaccharides **1** (8 mg, 13%; 5% calculated to starting trisaccharide **3**), **14** (23 mg, 37%) and **15** (22 mg, 36%).

3-(Trifluoroacetamido)propyl *O*-[Methyl (5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-*O*-(6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (8). Glycosylation of trisaccharide **4** (synthesis of **4** will be described elsewhere) (124 mg, 0.11 mmol) by thiosialoside **9** (99 mg, 0.19 mmol) as described in *run 1* (see above) to give amorphous **8** (143 mg, 81%), $[\alpha]_D$ -43° (*c* 1, $CHCl_3$), R_F 0.26 (solvent A).

Anal. Calcd for compound **8**; $C_{80}H_{98}N_3O_{28}F_3$ (1606.66): C, 59.81; H, 6.15; N, 2.62. Found: C, 59.64; H 6.43; N 2.51.

3-Aminopropyl *O*-[Potassium (5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-[(α-L-fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (2). Compound **8** (115 mg, 0.072 mmol) was subjected to catalytic hydrogenolysis and subsequent saponification as described for the preparation of **1** to give **2** (76 mg, 92%), $[\alpha]_D$ -42° (*c* 2, H_2O). The ^1H and ^{13}C NMR data are presented in Tables 1 and 2.

Anal. Calcd for compound **1**; $C_{34}H_{58}N_3O_{23}K$ (955.04): C, 44.59; H, 6.38; N, 4.59. Found: C, 44.36; H 6.24; N 4.38.

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