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Dedicated to Full Member of the Russian Academy of Sciences N.S. Zefirov on His 70th Anniversary

Synthesis of Aminoethyl Glycosides of Type 2 Chain A Tetrasaccharide and Related Trisaccharides

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Abstract—β-Aminoethyl glycosides of type 2 chain A tetrasaccharide and the corresponding trisaccharides were synthesized from selectively protected L-fucose, D-galactose, D-glucosamine, and D-galactosamine derivatives.

A-Antigens are expressed on the surface of erythrocytes and epithelium and endothelium cells as both glycosphyngolipids and *N*-bonded glycoproteins. In 1965 [1], the structure of one carbohydrate fragment was determined as the GalNAcα(1→3)[Fucα(1→2)]-Galβ(1→4)GlcNAc tetrasaccharide including trisaccharide epitope A and related to type 2 structures containing the disaccharide lactosamine fragment Galβ(1→4)GlcNAc [2]. The above tetrasaccharide may be a terminal fragment of oligosaccharides typical of blood subgroup A2 [3], or it may contain trisaccharide epitope A at the O^3 atom in the GalNAc residue as in type 3 chain A antigen of blood subgroup A1 erythrocytes [4]. The syntheses of individual type 2 chain A tetrasaccharide [5] and its spacered derivative [6] have been reported.

The present article describes the synthesis of β-aminoethyl glycosides of type 2 chain A tetrasaccharide **I** and trisaccharides **II** and **III** (which are structural fragments of **I**) from selectively protected L-fucose, D-galactose, D-glucosamine, and D-galactosamine derivatives. The target oligosaccharides were obtained in the spacered form for the subsequent conjugation with carriers and labels.

GalNAcα(1→3)[Fucα(1→2)Gal]β(1→4)GlcNAcβ-$OCH_2CH_2NH_2$

I

Fucα(1→2)Galβ(1→4)GlcNAcβ-$OCH_2CH_2NH_2$

II

GalNAcα(1→3)Galβ(1→4)GlcNAcβ-$OCH_2CH_2NH_2$

III

All structures **I**–**III** comprise an *N*-acetyllactosamine fragment, Galβ(1→4)GlcNAc; therefore, in their synthesis we used selectively protected lactosamine derivatives which were prepared by galactosylation of a common spacered precursor having a free hydroxy group on C^4. For this purpose, *N*-(2-hydroxyethyl)trifluoroacetamide (**V**) [7] was brought into reaction with α-glucosaminyl chloride **IV** [8] in the presence of $CdCO_3$ to obtain 95% of glycoside **VI** (Scheme 1). β-Configuration of the glycoside bond in **VI** follows from the characteristic spin–spin coupling constant $J_{1,2}$ = 8 Hz in the ^{1}H NMR spectrum (Table 1). Deacetylation of glycoside **VI** afforded triol **VII** which was protected at the 4-OH and 6-OH groups via transacetalization with benzaldehyde dimethyl acetal, and alcohol **VIII** thus obtained was benzoylated at 3-O. Reductive deacetalization of **IX** gave 3,6-*O*-dibenzyl derivative **X** (Scheme 1). The position of the free hydroxy group in **X** was unambiguously confirmed by comparing its ^{1}H NMR spectrum with that of the corresponding *O*-acetyl derivative which was prepared by treatment of **X** with a mixture of acetic anhydride and pyridine (2:3). The 4-H signal in the spectrum of the acetylated product appeared in a considerably weaker field (δ 5.01 ppm) than that of hydroxy derivative **X** (δ 3.73 ppm), indicating the presence of OH group on C^4 in **X**.

Target compounds **I** and **II** contain a fucopyranose residue on the galactose 2-O atom. Therefore, we required a protected precursor with a free galactose 2-OH group. It was synthesized by glycosylation of monosaccharide **X** with ethyl-2,3-di-*O*-acetyl-4,6-*O*-

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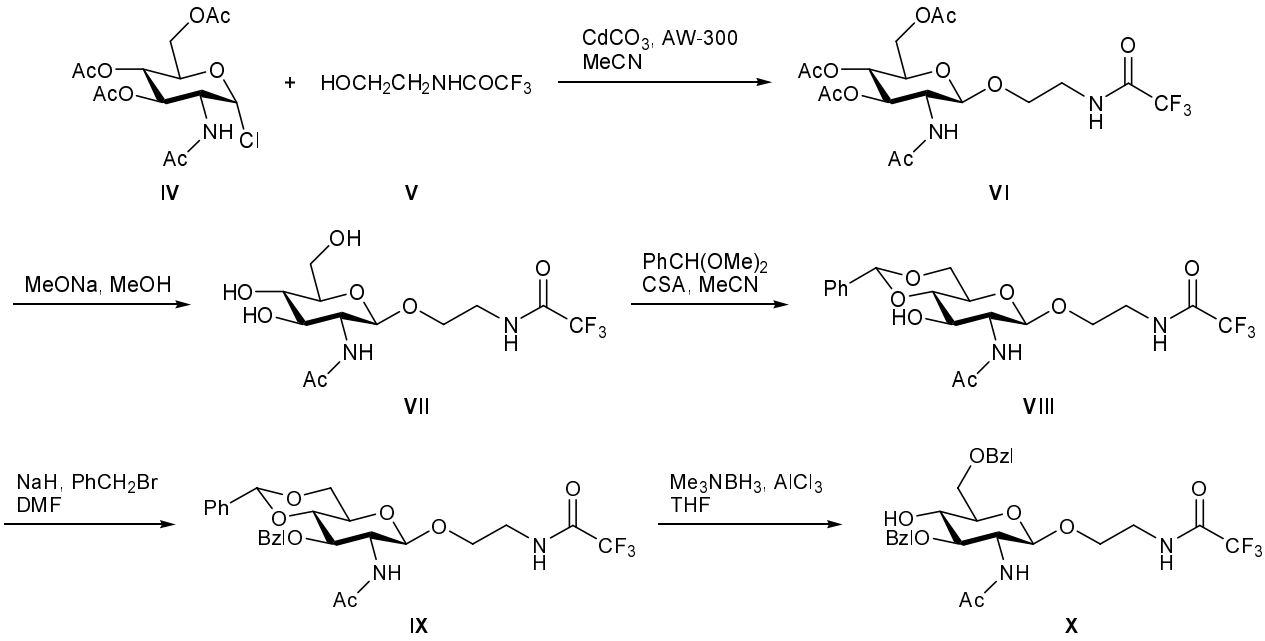
Scheme 1.



benzylidene-1-thiogalactoside (**XI**) [9] in the presence of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (Scheme 2). The glycosylation was stereoselective, and it afforded disaccharide **XII** in 83% yield. β-Configuration of the newly formed glycoside bond in **XII** unambiguously followed from the coupling constant $J_{1,2}$ = 8.0 Hz in the ^{1}H NMR spectrum. Compound **XII** was subjected to deacetylation to obtain diol **XIII**. Regioselective acetylation of the latter at the 3-OH group gave 91% of disaccharide **XIV** with a free hydroxy group on the galactose C^2 atom. The location of the acetyl group in **XIV** was confirmed by the downfield position of the 3-H signal (δ 4.55 ppm) relative to 2-H (δ 3.63 ppm) in the ^{1}H NMR spectrum.

Fucosylation of monoacetoxy derivative **XIV** with ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-fucopyranoside (**XV**) [10] in the presence of methyl trifluoromethanesulfonate was stereoselective, and protected trisaccharide **XVI** was obtained in 76% yield (Scheme 3). α-Configuration of the newly formed glycoside bond unambiguously followed from the coupling constant $J_{1,2}$ = 2.2 Hz (Table 1) in the fucopyranose fragment.

In order to obtain tetrasaccharide **I**, protected trisaccharide **XVI** was deacetylated by treatment with sodium methoxide in methanol (Scheme 3), and

Scheme 2.

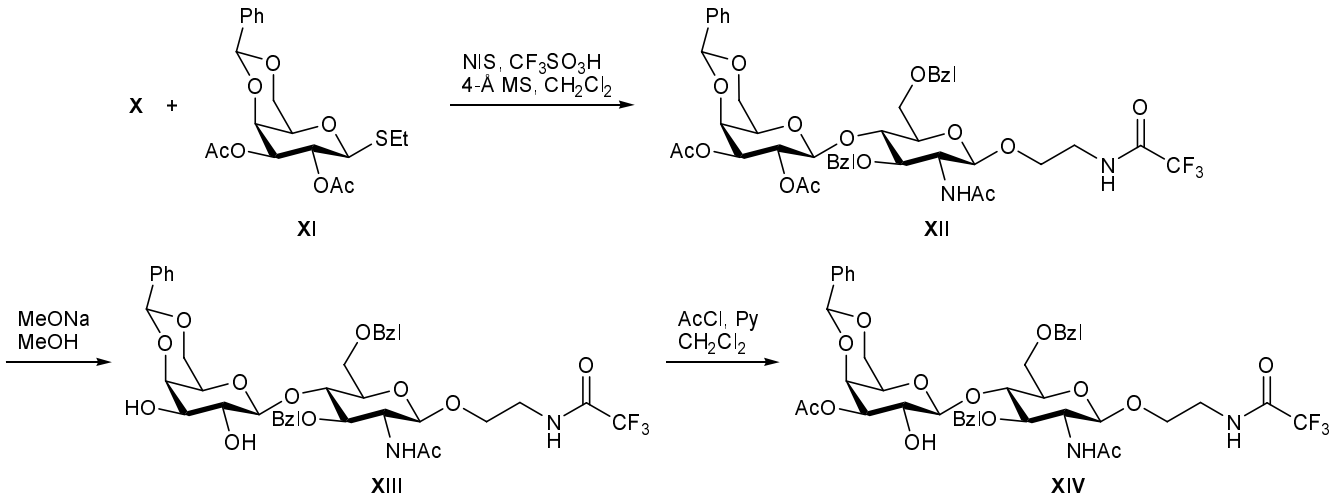
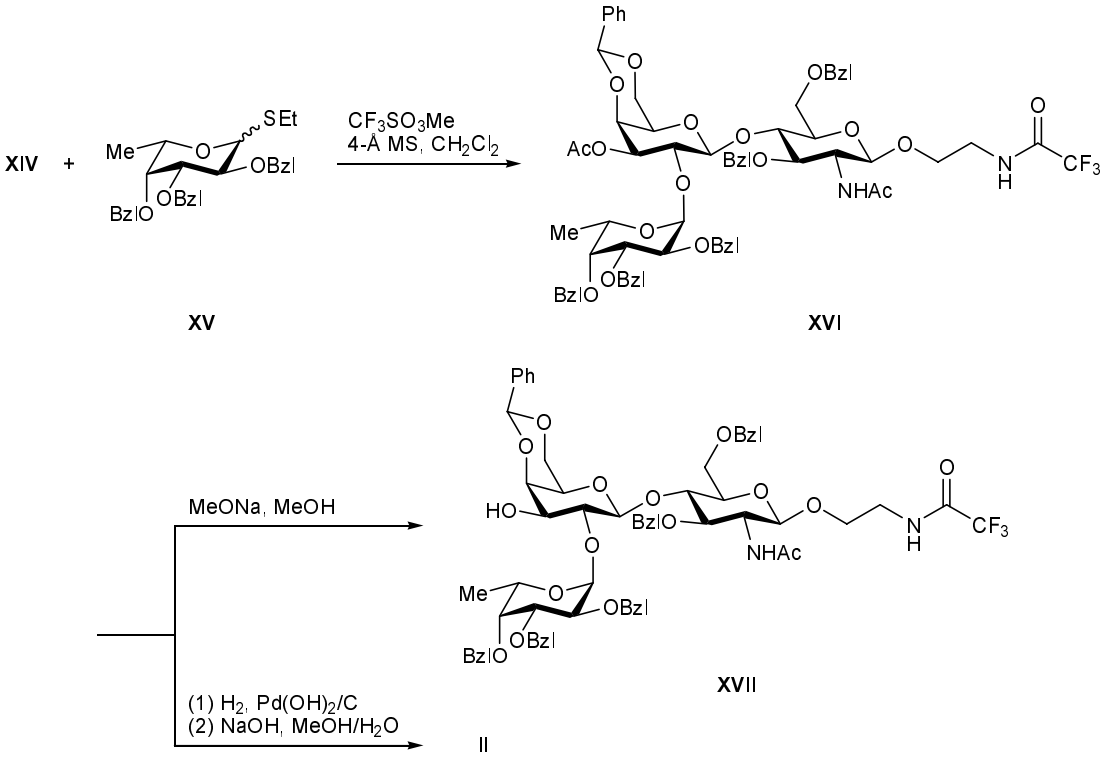


Table 1. ^{1}H NMR spectra (chemical shifts δ, ppm, and coupling constants *J*, Hz) of compounds **I**–**III** in D_2O, of **VIII** and **XIII** in CD_3OD), and of **VI**, **X**, **XII**, and **XIV**–**XXIV** in $CDCl_3$,

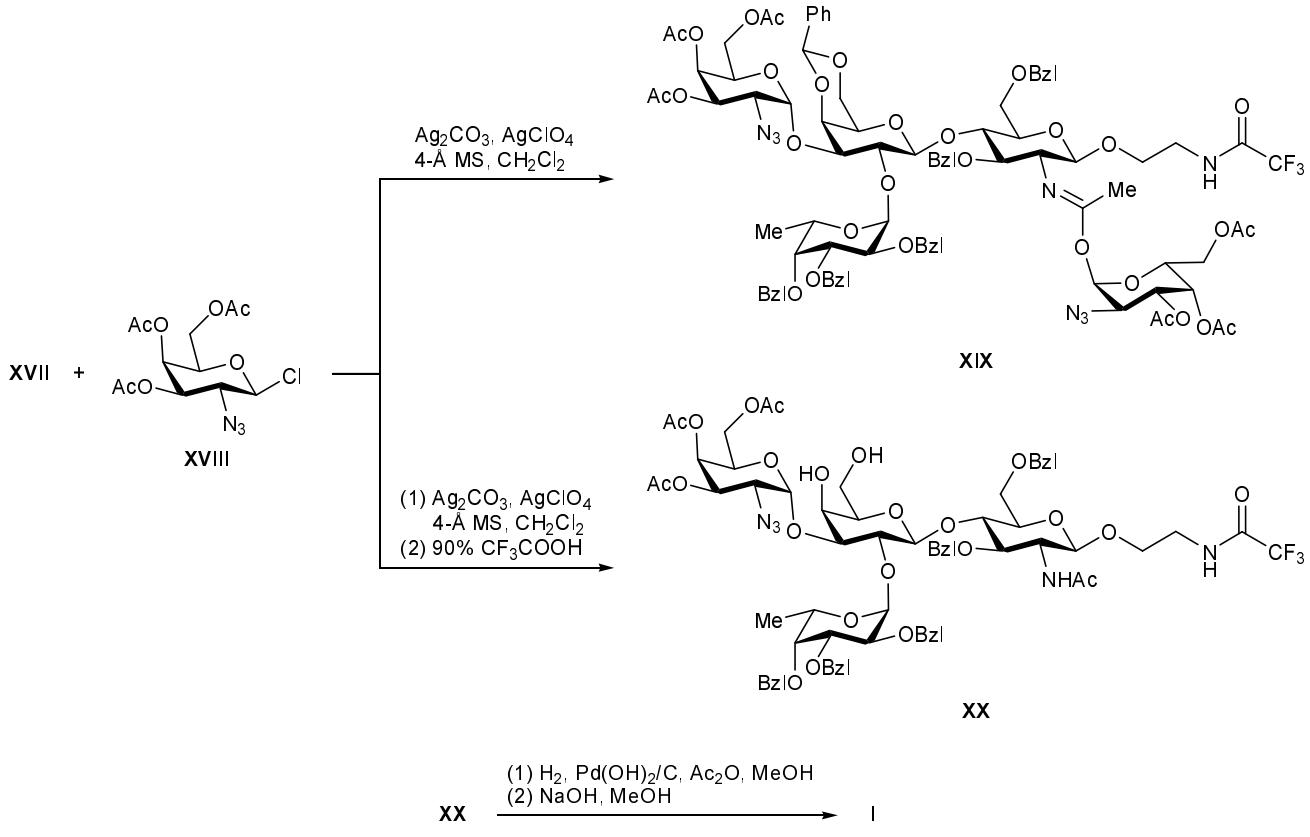
Comp. no.	Monosaccharide unit	1-H	2-H	3-H	4-H	5-H	6-H_A	6-H_B	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6A}$	$J_{5,6B}$	$J_{6A,6B}$
I[a]	GlcNAc	4.57	3.82	3.74	3.82	3.48	3.82	4.00	8.5						
	Gal	4.61	3.92	4.98	4.23	3.67	3.65–3.90		8.5						
	Fuc	5.36	3.81	3.72	3.83	4.32	1.25		4.0				6.5		
	GalNAc	5.18	4.25	3.90	4.01	4.22	3.65–3.90		3.7						
II[a]	GlcNAc	4.57	3.83	3.69	3.80	3.50	3.83	4.00	8.1	10.9	10.9			<1	12.2
	Gal	4.50	3.68	3.87	3.90	3.71	3.74	3.77	8.2			<1	10.8	<1	12.5
	Fuc	5.31	3.82	3.79	3.82	4.22	1.24		2.7			<1	6.4		
III[a]	GlcNAc	4.59	3.80	3.76	3.74	3.63	3.85	4.01	8.2					5.2	12.3
	Gal	4.54	3.60	3.77	4.11	3.69	3.75–3.80		7.9	9.7	3.0	<1	3.8	8.2	
	GalNAc	5.09	4.23	4.00	4.04	4.19	3.75		3.1	10.9	2.8	<1	6.2		
VI	GlcNAc	4.63	3.97	5.18	5.08	3.72	4.16	4.24	8.0	9.5	9.5	9.5	4.8	2.3	12.4
VIII	GlcNAc	4.54	3.77	3.50	3.44	3.71	3.72–3.84		7.8						
X	GlcNAc	4.56	3.38	3.84	3.73	3.57	3.40–3.60		7.7	9.6	9.6	9.6			
XII[b]	GlcNAc	4.61	3.82	3.78	4.01	3.58	3.75	3.80	6.3	4.5	6.7	6.7			
	Gal	4.52	5.34	4.84	4.31	3.25	3.96	4.22	8.0	10.5	3.4	<1	<1	<1	12.4
XIII	GlcNAc	4.36	3.67	3.62	3.88	3.44	3.73	3.87	7.8	8.3	8.3				9.7
	Gal	4.31	3.53	3.36	3.75	3.00	3.80	4.02	7.7		3.4	<1	<1	<1	12.5
XIV	GlcNAc	4.35	3.64	3.60	3.87	3.40	3.70	3.83			7.5	7.5	3.9	<1	11.8
	Gal	4.37	3.63	4.55	4.12	3.00	3.55	3.97		10.6	3.6				
XVI	GlcNAc	4.77	3.58	4.76	4.06	3.53	3.63	3.66	7.8				<1	<1	12.5
	Gal	4.45	4.12	4.88	4.28	3.14	3.96	4.22							
	Fuc	5.20	4.10	3.84	3.69	3.90	1.19		2.2						
XVII	GlcNAc	4.68	3.67	3.66	4.06	3.55	3.65	3.89			7.5	7.5			
	Gal	4.20	3.80	3.67	4.17	3.18	4.00	4.27	8.1	8.1	2.7		<1	<1	12.5
	Fuc	5.00	4.09	3.91	3.70	3.92	1.13		2.9	9.9					
XIX	GlcNAc	4.33	3.32–3.33		4.15	3.26	3.66	3.92	6.8				<1	9.1	
	Gal	4.44	4.16	3.70	4.28	3.09	4.05	4.42	7.5			<1	<1	<1	12.4
	Fuc	5.50	4.17	3.90	3.80	4.23	1.20								
	$GalN_3$	5.28	3.56	5.25	5.20	4.24	3.87	3.40		10.5					
	$GalN_3$[c]	6.61	3.62	5.47	5.55	4.25	4.05	4.15		10.9	<1	<1			
XX[b]	GlcNAc	4.77	3.56	3.73	4.05	3.47	3.89	3.70	7.4	7.3	7.3				
	Gal	4.44	4.11	3.70	4.31	3.12	4.25	4.00	7.8	9.0	3.5	<1	<1	<1	11.8
	Fuc	5.46	4.13	3.88	3.77	4.25	1.25		3.5	10.0		<1	6.5		
	$GalN_3$	5.28	3.56	5.27	5.22	4.26	3.87	3.45	3.4	7.6	3.1	<1		10.8	3.8
XXIII	GlcNAc	4.25	3.82	3.64	4.07	3.38	3.54	3.67	7.6	7.6	7.6	7.6	10.3	<1	12.5
	Gal	4.62	5.30	3.73	4.18	3.22	3.97	4.26	8.1	9.9	3.6	<1	<1	<1	11.3
XXIV[c]	GlcNAc	4.23	3.93	3.70	4.11	3.50		3.68	7.0		7.8	7.8		4.3	
	Gal	4.64	5.60	3.93	4.34	3.23	4.30	4.04	8.0		2.9	<1	<1	<1	12.5
	$GalN_3$	5.14	3.60	5.20	5.18	4.10	3.88	3.77	3.4					5.7	

[a] δ, ppm: 4.06 ($OCH_2CH_2NH_2\cdot AcOH$), 3.23–3.89 ($OCH_2CH_2NH_2\cdot AcOH$), 1.9 (GlcNHCOC**H**$_3$), 2.05 (GalNHCOC**H**$_3$).
[b] δ, ppm: 3.35–3.92 ($OCH_2CH_2NHCOCF_3$), 3.18–3.59 ($OCH_2CH_2NHCOCF_3$), 1.30–1.82 (GlcNHCOC**H**$_3$).
[c] Other signals, δ, ppm: 5.50–5.58 (PhC**H**), 4.25–5.25 (PhC**H**$_2$), 5.65 (NH).

Scheme 3.



Scheme 4.



trisaccharide block **XVII** thus formed was glycosylated with chloride **XVIII** [11] in the presence of silver carbonate and silver perchlorate (Scheme 4). However, the major product (yield 49%) was not the expected tetrasaccharide; the product was characterized by a considerably higher chromatographic mobility than might be anticipated for protected tetrasaccharide. Analysis of its ^{1}H NMR and mass spectra showed that its molecule contains two 2-azido-2-deoxygalactose residues, one of which is linked by a normal glycoside bond to the galactose 3-O atom while the other is attached to the carbonyl oxygen atom of the acetylamino group in the *N*-acetylglucosamine moiety; i.e., it has the structure of imido ester **XIX** (Scheme 4).

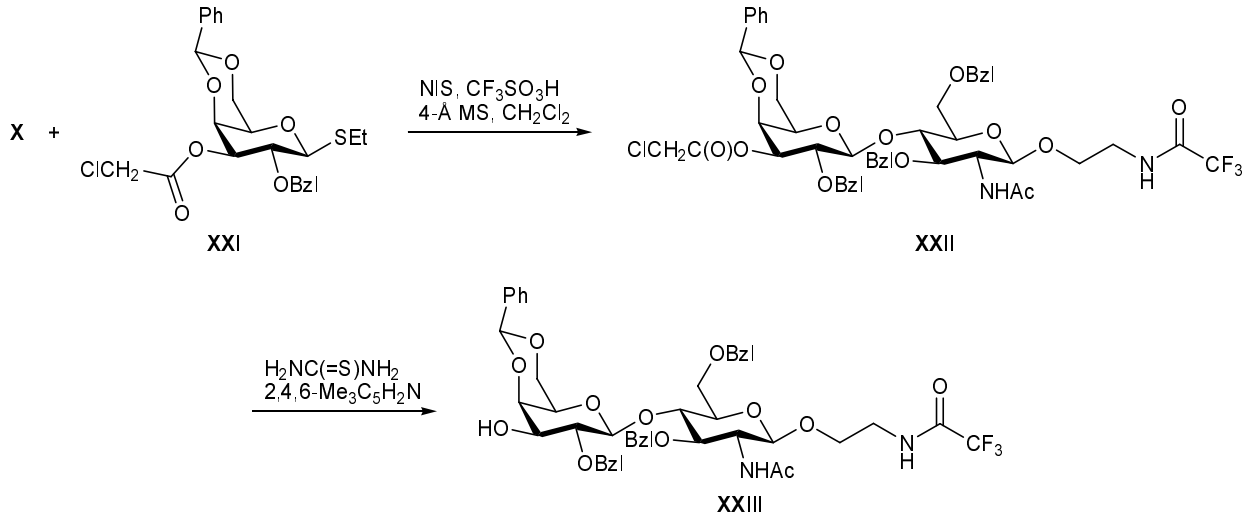
The formation of glycosyl imidoates in the glycosylation of *N*-acetylglucosamine derivatives was reported previously [12]. The presence in molecule **XIX** of a readily hydrolyzable (under acidic conditions) imidoate moiety was confirmed by facile elimination of one 2-azido-2-deoxygalactose residue on treatment with an acid under mild conditions; as a result, a mixture of tetrasaccharide **XX** and 2-azido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranose was formed. Therefore, the reaction mixture was additionally treated with 90% trifluoroacetic acid to isolate diol **XX** in 52% yield. The coupling constant $J_{1,2}$ = 3.4 Hz (Table 1) for the azidogalactose residue in the ^{1}H NMR spectrum of **XX** unambiguously indicated α-configuration of the newly formed glycoside bond.

The azido group in diol **XX** was subjected to catalytic hydrogenation over $Pd(OH)_2$/C in the presence of acetic anhydride. Under these conditions, the reaction was accompanied by acetylation of the emerging amino group. The subsequent debenzylation and removal of the *N*-trifluoroacetyl group by alkaline hydrolysis afforded 71% of target tetrasaccharide **I** which was isolated as a salt with acetic acid by gel chromatography in 0.1 M aqueous acetic acid. By catalytic hydrogenation of compound **XVI**, alkaline hydrolysis, and separation by gel chromatography in 0.1 M aqueous acetic acid we isolated 85% of trisaccharide **II** as a salt with acetic acid (Scheme 4).

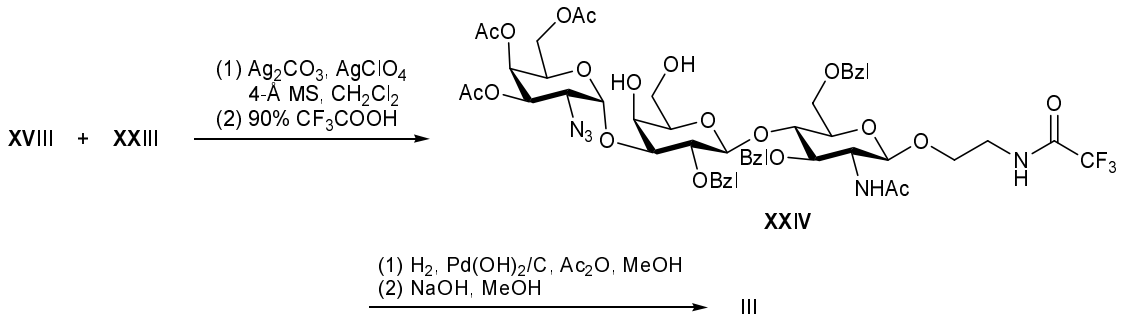
Trisaccharide **III** was synthesized from spacered lactosamine precursor **XXIII** with a free OH group in position *3* of the galactose residue. It was prepared as follows. Di-*O*-benzyl glucosamine derivative **X** was glycosylated with thiogalactoside **XXI** [13] having a chloroacetyl protecting group on 3-O in the presence of *N*-iodosuccinimide and trifluoromethanesulfonic acid (Scheme 5). This reaction afforded 87% of disaccharide **XXII** which was treated with thiourea in the presence of 2,4,6-trimethylpyridine to remove chloroacetyl protection. As a result, disaccharide block **XXIII** was isolated in 87% yield. The location of the free hydroxy group on C^3 in the galactose residue was confirmed by the upfield position of the 3-H signal (δ 3.73 ppm) in the ^{1}H NMR spectrum of **XXIII**, and the coupling constant $J_{1,2}$ = 8.1 Hz (Table 1) for the galactose residue unambiguously indicated β-configuration of the newly formed glycoside bond.

Hydroxy derivative **XXIII** was then glycosylated with chloride **XVIII** in the presence of silver carbonate and silver perchlorate (Scheme 6). As in the glycosylation of trisaccharide acceptor **XVII**, a compound with high chromatographic mobility (presumably imidic acid ester) was formed (TLC); therefore, the primary

Scheme 5.



Scheme 6.



product was treated with 90% trifluoroacetic acid to isolate diol **XXIV** in 80% yield. The newly formed glycoside bond in **XXIV** had α-configuration, as followed from the coupling constant $J_{1,2}$ = 3.4 Hz for the azidogalactose residue. The azido group in trisaccharide **XXIV** was subjected to catalytic hydrogenation over $Pd(OH)_2/C$ in the presence of acetic anhydride. The process was accompanied by acetylation of the emerging amino group and removal of the benzyl protecting groups. Alkaline treatment and subsequent gel chromatography with 0.1 M aqueous acetic acid afforded 81% of target trisaccharide **III** as a salt with acetic acid.

Signals in the ^{1}H NMR spectra of free oligosaccharides **I**–**III** were assigned using two-dimensional ^{1}H–^{1}H COSY and TOCSY techniques, and in the ^{13}C NMR spectra, using ^{1}H–^{13}C HSQC heteronuclear two-dimensional experiments. The anomeric configuration of each monosaccharide unit was rigorously confirmed by the corresponding coupling constants $J_{1,2}$ which were equal to ~3.5 Hz for the ending α-fucose and α-galactosamine moieties and ~7.5 Hz for the β-galactose and β-glucosamine units constituting the lactosamine disaccharide fragment. Analysis of the ^{13}C chemical shifts (Table 2) of compound **I** confirmed substitution in the GlcNAc residue at 4-O (the signal from C^4 in GlcNAc characteristically appeared at δ_C 77.3 ppm) and in the Gal residue at 2-O and 3-O (the C^2 and C^3 signals were located at δ_C 73.6 and 76.9 ppm, respectively). The ^{13}C NMR data indicated the presence of 1→4 bond between Gal and GlcNAc (GlcNAc: δ_{C^4} 77.2 ppm) and 1→2 bond between the fucose and galactose units (Gal: δ_{C^2} 77.7 ppm) in trisaccharide **II**; likewise, 1→4 bond between Gal and GlcNAc (GlcNAc: δ_{C^4} 79.9 ppm) and 1→3 bond between the galactosamine and galactose units (δ_{C^3} 78.0 ppm) in **III** were identified.

The chemical shifts of C^4 in the GlcNAc unit and of C^1 in the Gal unit in the ^{13}C NMR spectra of compounds **I** **II** differ from the corresponding parameters of trisaccharide **III** as a result of 1,2-pseudobranching in the galactose unit of **I** and **II** (Table 1). The addition of α-fucopyranosyl moiety to galactose unit through the 1→2 bond also changes conformation of the

Table 2. ^{13}C NMR spectra of compounds **I**–**III** in D_2O, δ_C, ppm

Compound no.	Monosaccharide unit	C^1	C^2	C^3	C^4	C^5	C^6
I	GlcNAc	102.2	56.3	73.4	77.3	76.4	61.2
	Gal	101.3	73.6	76.9	64.2	76.4	62.4
	Fuc	99.8	68.8	71.2	72.9	68.0	16.8
	GalNAc	92.5	50.7	69.0	69.7	72.3	62.5
II	GlcNAc	102.2	56.3	73.3	77.2	76.4	61.3
	Gal	101.5	77.7	74.7	70.3	76.5	62.3
	Fuc	100.6	69.4	70.9	72.9	68.1	16.9
III[a]	GlcNAc	102.1	56.2	73.6	79.9	75.9	61.3
	Gal	104.0	70.8	78.0	66.0	76.5	62.2
	GalNAc	95.2	50.9	68.8	69.6	72.1	62.2

[a] Other signals, δ_C, ppm: ~67 (O**C**$H_2CH_2NH_2$), ~41 (OCH_2**C**H_2NH_2), ~23.5 (NHCO**C**H_3), ~176 (NH**C**OCH_3).

Galβ(1→4)GlcNAc disaccharide fragment, which is reflected in the spectral data [14]. An analogous effect is observed for the vicinal 2,3-branching in tetrasaccharide **I**, which essentially restricts conformational mobility of the Fuc(1→2)Gal disaccharide fragment. Therefore, there is a considerable difference between the chemical shifts of C^2 in Gal and C^1 in Fuc [15] in the ^{13}C NMR spectra of **I** and **II** (Table 2).

Thus we have synthesized β-aminoethyl glycosides of type 2 chain A tetrasaccharide and its trisaccharide fragments and studied spectral effects of glycosylation in their synthesis.

EXPERIMENTAL

β-Aminoethanol, benzaldehyde dimethyl acetal, camphorsulfonic acid, triethylamine, benzyl bromide, $AlCl_3$, and $Me_3N \cdot BH_3$ were commercial reagents (Fluka). Cadmium carbonate was purchased from *REAKhIM*. Methylene chloride was distilled twice over P_2O_5 and once over CaH_2 under argon. Acetonitrile was distilled thrice over P_2O_5 under argon. Molecular sieves AW-300 and MS 4 Å were activated by heating at 180°C under reduced pressure (oil pump). The ^1H and ^{13}C NMR spectra were recorded on Bruker DRX-500 and Bruker AM-300 spectrometers at 25°C. The mass spectra (electrospray ionization) were obtained on a Finigann LCQ spectrometer. The melting points were determined on a Kofler apparatus. The optical rotations were measured on a PU-07 digital polarimeter (GNIITsNP) at 18–25°C. Thin-layer chromatography was performed on Kieselgel-60 silica gel plates (Merck); spots were detected by treatment with a 10 vol % solution of orthophosphoric acid in ethanol or (for amines) with a solution of ninhydrin (c = 3 g/l) in butanol–acetic acid (30:1), followed by heating at ~150°C. Silica gel 60 (0.040–0.063 mm, Merck) was used for column chromatography. Gel chromatography was performed on a column charged with TSK-HW40(S) gel (1.5×90 cm) using 0.1 M aqueous acetic acid as eluent (flow rate 1 ml/min). Reversed-phase HPLC was performed using a 250×10-mm Chrompack semipreparative column charged with Chromspher C-18 (5 μm) and a Knauer 98.00 refractometer as detector. Hydrogenation was carried out over 20% $Pd(OH)_2/C$ (Aldrich) under atmospheric pressure. All glycosylation reactions were carried out in anhydrous methylene chloride or anhydrous acetonitrile in argon atmosphere.

2-(Trifluoroacetylamino)ethyl 2-acetylamino-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (VI). A solution of 1.7 g (4.67 mmol) of chloride **IV** and 5.8 g (37 mmol) of *N*-(2-hydroxyethyl)trifluoroacetamide (**V**) in 30 ml acetonitrile containing 2.6 g of activated AW-300 molecular sieves and 1 g of cadmium carbonate was heated for 2 h under reflux. The mixture was filtered through a layer of celite, 100 ml of chloroform was added to the filtrate, and the mixture was washed with a 1 M aqueous solution of KBr (2×30 ml) and water (50 ml). The organic phase was concentrated and subjected to chromatography (gradient elution with chloroform–methanol) to isolate 2.0 g (95%) of glycoside **VI** as a white foam-like material, R_f 0.30 (chloroform–methanol, 12:1), $[\alpha]_D$ = –10.7° (c = 1, chloroform). The ^1H NMR data are given in Table 1. Found, %: C 44.54; H 5.02; N 5.49. Calculated, %: C 44.45; H 5.18; N 5.76.

2-(Trifluoroacetylamino)ethyl 2-acetylamino-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (VIII). A solution of 1.3 g (2.9 mmol) of compound **VI** in 22 ml of 0.01 M sodium methoxide in methanol was kept for 1 h at room temperature, KU-2 (H^+) cation exchanger was added to neutral reaction, the mixture was filtered, and the filtrate was evaporated. The residue was dried under reduced pressure (oil pump) and dissolved in 40 ml of anhydrous acetonitrile, 0.8 ml of benzaldehyde dimethyl acetal and 33 mg of camphorsulfonic acid were added, the mixture was heated for 2 h at 75°C and cooled, and 0.5 ml of triethylamine was added. The mixture was kept for 20 h at 0°C, and the crystals were filtered off, washed with cold ethyl acetate, and dried under reduced pressure (oil pump). Yield of alcohol **VIII** 742 mg (56%), R_f 0.20 (chloroform–methanol, 12:1), mp 256°C, $[\alpha]_D$ = –64° (c = 0.5, methanol). The ^1H NMR data are given in Table 1. Found, %: C 50.90; H 5.19; N 6.09. Calculated, %: C 50.89; H 5.17; N 6.25.

2-(Trifluoroacetylamino)ethyl 2-acetylamino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (X). A mixture of 38 ml of anhydrous DMF and 760 mg of a 60% suspension of sodium hydride in mineral oil was stirred for 1 h at –20°C, 3 g (6.7 mmol) of compound **VIII** was added in portions, the mixture was stirred for 1 h at –20°C, 0.9 ml of benzyl bromide was added, and the mixture was stirred for an additional 1 h at –20°C. It was then cooled to –40°C, 2 ml of acetic acid and 800 ml of chloroform were added, and the mixture was washed with a saturated solution of $NaHCO_3$ (2×100 ml) and water (100 ml). The organic phase was evaporated, and the residue was ground with 10 ml of ethyl acetate. The crystals thus formed were separated and dried under reduced pressure (oil

pump). Yield of **IX** 3.06 g (85%), R_f 0.41 (chloroform–methanol, 12:1). The product was dissolved in 200 ml of anhydrous THF, 1.66 g (23 mmol) of $Me_3N \cdot BH_3$ was added, 4.55 g (34 mmol) of $AlCl_3$ was added in portions under stirring, the mixture was stirred for 30 min at room temperature, and 0.205 ml of water was added dropwise. The mixture was stirred for 15 h, 100 ml of 1 M hydrochloric acid was added, and the mixture was diluted with 400 ml of chloroform. The organic phase was separated, washed with water (200 ml), a saturated solution of $NaHCO_3$ (100 ml), and water again, and evaporated. The residue was subjected to column chromatography on silica gel (gradient elution with chloroform–methanol) to isolate 2.78 g (90%) of dibenzyl derivative **X** as a syrupy material, R_f 0.32 (chloroform–methanol, 12:1), $[\alpha]_D$ = 3.2° (*c* = 1, chloroform). The ^{1}H NMR data for compound **X** are given in Table 1.

2-(Trifluoroacetylamino)ethyl 2-acetylamino-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (XII). A mixture of 1.18 g (2.19 mmol) of alcohol **X** and 1.4 g (3.5 mmol, 1.6 equiv) of thioglycoside **XI** in 88 ml of methylene chloride and 6.6 g of AW-300 activated molecular sieves was stirred for 2 h at room temperature. The mixture was cooled to –10°C, 832 mg of *N*-iodosuccinimide was added, the mixture was stirred for 30 min and cooled to –30°C, and 395 μl of trifluoromethanesulfonic acid was added dropwise. The mixture was stirred for 2 h at –30 to –20°C, 0.5 ml of pyridine was added, the mixture was filtered through a layer of celite, and the filtrate was diluted with 100 ml of chloroform and washed with 50 ml of a 1 M solution of sodium thiosulfate. The organic phase was evaporated, and the residue was subjected to chromatography (gradient elution with chloroform–methanol). Yield 1.6 g (83%), white foam, R_f 0.4 (chloroform–methanol, 12:1), $[\alpha]_D$ = +13° (*c* = 1.3, chloroform). The ^{1}H NMR data for compound **XII** are given in Table 1.

2-(Trifluoroacetylamino)ethyl 2-acetylamino-3,6-di-*O*-benzyl-4-*O*-(4,6-*O*-benzylidene-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside (XIII). A solution of 1.35 g (1.5 mmol) of diacetate **XII** in a mixture of 9 ml of 0.01 M methanolic sodium methoxide and 6 ml of anhydrous methylene chloride was kept for 1 h at room temperature, 1 ml of acetic acid was added, the mixture was evaporated, and the residue was subjected to chromatography (gradient elution with chloroform–methanol). Yield 1.11 g (94%), R_f 0.23 (chloroform–methanol, 12:1), mp 208°C, $[\alpha]_D$ = –7° (*c* = 1, chloroform). For ^{1}H NMR data, see Table 1. Found, %: C 59.35; H 5.79; N 3.49. Calculated, %: C 59.24; H 5.74; N 3.54.

2-(Trifluoroacetylamino)ethyl 2-acetylamino-4-*O*-(3-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (XIV). A solution of 0.061 ml of acetyl chloride in 1 ml of anhydrous methylene chloride was added under stirring at –25°C to a solution of 382 mg (0.484 mmol) of diol **XIII** in a mixture of 12 ml of anhydrous methylene chloride and 0.76 ml of pyridine. The mixture was stirred for 5 min, 0.5 ml of methanol and 50 ml of chloroform were added in succession, and the mixture was washed with 30 ml of water. The organic phase was concentrated, and the residue was subjected to chromatography (gradient elution with chloroform–methanol). Yield 367 mg (91%), white foam, R_f 0.26 (chloroform–methanol, 12:1), $[\alpha]_D$ = –36° (*c* = 1, chloroform). The ^{1}H NMR data are given in Table 1. Found, %: C 59.09; H 5.56; N 3.15. Calculated, %: C 59.13; H 5.69; N 3.36.

2-(Trifluoroacetylamino)ethyl (2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-(3-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2-acetylamino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (XVI). A mixture of 693 mg (0.832 mmol) of compound **XIV**, 419 mg (0.848 mmol) of ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-fucopyranoside, and 2.5 g of activated MS 4-Å molecular sieves in 30 ml of methylene chloride was stirred for 2 h, 0.480 ml of methyl trifluoromethanesulfonate was added, and the mixture was stirred for 40 min. The mixture was then diluted with 70 ml of chloroform and washed with 50 ml of water. The organic phase was concentrated, and the residue was subjected to chromatography (gradient elution with chloroform–methanol) to isolate 800 mg (76%) of compound **XIV** as a white foam-like material, R_f 0.54 (chloroform–methanol, 12:1), $[\alpha]_D$ = –7° (*c* = 2, chloroform). The ^{1}H NMR data are given in Table 1. Found, %: C 66.61; H 5.97; N 2.20. Calculated, %: C 66.44; H 6.22; N 2.25.

2-(Trifluoroacetylamino)ethyl (2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-(4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2-acetylamino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (XVII). A solution of 78 mg (0.062 mmol) of compound **XVI** in 2 ml of 0.01 M methanolic sodium methoxide was kept for 2 h, 1 ml of acetic acid was added, and the mixture was concentrated. The residue was subjected to chromatography (gradient elution with chloroform–

methanol) to isolate 69 mg (92%) of compound **XVI** as a white foam-like material, R_f 0.52 (chloroform–methanol, 12:1), $[\alpha]_D$ = –29° (*c* = 1, chloroform). The ^{1}H NMR data are given in Table 1. Found, %: C 66.54; H 6.16; N 2.10. Calculated, %: C 66.76; H 6.27; N 2.32.

2-(Trifluoroacetylamino)ethyl (2-azido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-4,6-*O*-benzylidene-β-D-galactopyranosyl]-(1→4)-2-[1-(2-azido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyloxy)ethylideneamino]-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (XIX). A mixture of 272 mg (0.223 mmol) of trisaccharide **XVII**, 171 mg (0.49 mmol) of chloride **XVIII**, 686 mg of silver carbonate, and activated MS 4-Å molecular sieves in 6 ml of methylene chloride was stirred for 2 h, 12 mg of silver perchlorate was added, and the mixture was stirred for 20 h. It was then filtered through a layer of celite, the filtrate was diluted with 100 ml of chloroform, washed with a 1 M solution of sodium thiosulfate (40 ml) and water (40 ml), and evaporated, and the residue was subjected to chromatography using toluene–acetone (6:1) as eluent. Yield 205 mg (49%), white foam, R_f 0.12 (toluene–acetone, 12:1), $[\alpha]_D$ = +27° (*c* = 1, chloroform). Mass spectrum, *m*/*z* (I_{rel}, %): 1855.6 (100) $[M + Na]^+$, 1872.6 (60) $[M + K]^+$. The ^{1}H NMR data are given in Table 1.

2-(Trifluoroacetylamino)ethyl (2-azido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-β-D-galactopyranosyl]-(1→4)-2-acetylamino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (XX). A mixture of 76 mg (0.062 mmol) of trisaccharide **XVII**, 48 mg of chloride **XVIII**, 191 mg of silver carbonate, and 80 mg of MS 4-Å molecular sieves in 1.6 ml of methylene chloride was stirred for 2 h, 10 mg of silver perchlorate was added, and the mixture was stirred for 48 h. It was then filtered through a layer of silica gel (2 g), the sorbent was washed with ethyl acetate (40 ml), and the filtrate was combined with the washings and concentrated. The residue was dissolved in 3 ml of chloroform, 0.3 ml of 90% trifluoroacetic acid was added, and the mixture was kept for 15 min. Toluene, 20 ml, was added, and the mixture was evaporated; this procedure was repeated three times until the odor of trifluoroacetic acid disappeared. The residue was subjected to chromatography to isolate 49 mg (52%) of tetrasaccharide **XX** as a white foam-like material, R_f 0.42 (chloroform–methanol, 12:1), $[\alpha]_D$ = +13° (*c* = 1, chloroform). The ^{1}H NMR data are given in Table 1. Found, %: C 66.14; H 6.16; N 5.10. Calculated, %: C 60.45; H 6.06; N 4.90.

2-(Trifluoroacetylamino)ethyl 2-acetylamino-3,6-di-*O*-benzyl-4-*O*-(4,6-*O*-benzylidene-2-*O*-benzoyl-3-*O*-chloroacetyl-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside (XXII). A solution of 156 mg (0.29 mmol) of alcohol **X** and 242 mg (0.50 mmol) of thioglycoside **XXI** in 12 ml of methylene chloride containing 1.0 g of activated AW-300 molecular sieves was stirred for 2 h, the mixture was cooled to –10°C, 188 mg of *N*-iodosuccinimide was added, the mixture was stirred for 30 min and cooled to –35°C, and 0.087 ml of trifluoromethanesulfonic acid was added. The mixture was stirred for 2 h at –35 to –40°C, 0.5 ml of pyridine was added, the mixture was filtered through a layer of celite, and the filtrate was diluted with chloroform (50 ml) and washed with a 1 M solution of sodium thiosulfate (20 ml). The organic phase was evaporated, and the residue was subjected to chromatography (gradient elution with chloroform–methanol) to isolate 228 mg (81%) of disaccharide **XXII** as a white foam-like material, R_f 0.60 (chloroform–methanol, 12:1), $[\alpha]_D$ = +10° (*c* = 1, chloroform). The ^{1}H NMR data are given in Table 1. Found, %: C 58.88; H 5.04; N 2.65. Calculated, %: C 58.97; H 5.05; N 2.93.

2-(Trifluoroacetylamino)ethyl 2-acetylamino-3,6-di-*O*-benzyl-4-*O*-(4,6-*O*-benzylidene-2-*O*-benzoyl-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside (XXIII). A solution of 210 mg (0.22 mmol) of disaccharide **XXII**, 83 mg of thiourea, and 0.030 ml of 2,4,6-trimethylpyridine in a mixture of 3.5 ml of methanol and 2.4 ml of methylene chloride was kept for 24 h at room temperature, diluted with 80 ml of chloroform, and washed with 50 ml of water. By chromatography using gradient elution with chloroform–methanol we isolated 172 mg (87%) of disaccharide **XXIII** as a white foam-like material, R_f 0.52 (chloroform–methanol, 12:1), $[\alpha]_D$ = –13° (*c* = 1, $CHCl_3$). The ^{1}H NMR data are given in Table 1.

2-(Trifluoroacetylamino)ethyl (3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→3)-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2-acetylamino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (XXIV) was synthesized from 52 mg (0.058 mmol) of disaccharide **XXIII** and 44 mg (0.126 mmol, 2.2 equiv) of chloride **XVIII**, following the procedure described above for tetrasaccharide **XX**. Yield 51 mg (80%), white foam-like material, R_f 0.26 (chloroform–methanol, 12:1), $[\alpha]_D$ = +44° (*c* = 1,

chloroform). The ^{1}H NMR data are given in Table 1. Found, %: C 54.72; H 5.51; N 6.33. Calculated, %: C 54.69; H 5.40; N 6.25.

2-Aminoethyl (2-acetylamino-2-deoxy-α-D-galactopyranosyl)-(1→3)-[α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl]-(1→4)-2-acetylamino-2-deoxy-β-D-glucopyranoside (I). A solution of 22 mg (0.015 mmol) of protected tetrasaccharide **XX** in a mixture of 3 ml of methanol, 0.7 ml of ethyl acetate, and 0.3 ml of acetic anhydride was stirred for 2 h in a hydrogen atmosphere over 60 mg of $Pd(OH)_2/C$. The mixture was filtered through a layer of celite, the filtrate was evaporated, the residue was dissolved in 1 ml of water, 0.2 ml of pyridine was added to the solution, and the mixture was kept for 20 min to decompose the remaining acetic anhydride. To the resulting solution we added 0.2 ml of 1 N aqueous sodium hydroxide, the mixture was kept for 20 h, 1 ml of acetic acid was added, the mixture was concentrated, and the residue was subjected first to gel chromatography and then to HPLC using 0.05 M aqueous ammonium hydrogen carbonate as eluent. Lyophilization from water gave 9 mg (71%) of tetrasaccharide **I** as a salt with acetic acid, $[\alpha]_D$ = +12° (c = 0.2, water). The ^{1}H NMR data are given in Table 1.

2-Aminoethyl α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-2-acetylamino-2-deoxy-β-D-glucopyranoside (II). A solution of 25 mg (0.021 mmol) of protected trisaccharide **XVI** in 2 ml of methanol was stirred over 50 mg of $Pd(OH)_2/C$ under hydrogen. The mixture was filtered through a layer of celite, the filtrate was evaporated, the residue was dissolved in 3 ml of methanol, 0.2 ml of 1 N aqueous sodium hydroxide was added, and the mixture was kept for 20 h. Acetic acid, 1 ml was then added, the mixture was concentrated, and the residue was subjected to gel chromatography. Lyophilization from water gave 11 mg (85%) of amine **II** as a salt with acetic acid, $[\alpha]_D$ = +185° (c = 0.2, water). The ^{1}H NMR data are given in Table 1.

2-Aminoethyl (2-acetylamino-2-deoxy-α-D-galactopyranosyl)-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetylamino-2-deoxy-β-D-glucopyranoside (III) was synthesized from 26 mg (0.023 mmol) of protected trisaccharide **XXIV**, following the procedure described above for tetrasaccharide **I**. Compound **III** was isolated as a salt with acetic acid. Yield 13 mg (81%), $[\alpha]_D$ = +52° (c = 1, water). The ^{1}H NMR data are given in Table 1.

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