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The Synthesis and Antiviral Activity of Glycyrrhizic Acid Conjugates with α-*D*-Glucosamine and Some Glycosylamines

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Abstract—Glycyrrhizic acid and its 30-methyl ester were conjugated with 2-amino-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-*D*-glucopyranose, 2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl amine, 2,3,4-tri-*O*-acetyl-α-*L*-arabinopyranosyl amine, 2-acetamido-2-deoxy-β-*D*-glucopyranosyl amine, and β-*D*-galactopyranosyl amine using *N*,*N*'-dicyclohexylcarbodiimide and its mixtures with *N*-hydroxybenzotriazole. Structures of the conjugates were confirmed by IR, UV, ^{1}H, and ^{13}C NMR spectroscopy. The glycoconjugate with the residues of 2-acetamido-2-deoxy-β-*D*-glucopyranosyl amine in the carbohydrate part of its molecule exhibited antiviral activity (ID_{50} 4 μg/ml) toward the herpes simplex type 1 virus (HSV-1) in the VERO cell culture. Two compounds demonstrated anti-HIV-1 activity (50–70% inhibition of p24) in a culture of MT-4 cells at concentrations of 0.5–20 μg/ml.

Key words: antiviral activity, glycyrrhizic acid conjugates, *D*-glucosamine, β-glycosylamines

INTRODUCTION

Glycyrrhizic acid is the major glycoside of liquorice (dried roots of *Glycyrrhiza glabra* L. or *G. uralensis* Fisher), which is known due to its high and various biological activities (antiphlogistic, antiulcer, immunomodulating, antioxidant, antiallergic, etc.) [1].[2] The antiviral activity of GA attracts a particular interest of researchers. GA and its salts inhibit the replication of DNA- and RNA-containing viruses (such as Vaccinia, Newcastle disease, Vesicular stomatitis, and Herpes simplex) *in vitro* [2].

Moreover, GA completely inhibits HIV-1 in the MT-4 cell culture at the concentrations 0.5–1.0 mg/ml [3]. The preliminary clinical trials demonstrated that the administration of GA to the patient suffering from AIDS increases the level of T4 lymphocytes and decreases the level of viral antigen [4]. A number of chemically modified GA derivatives exhibited a more clearly expressed inhibiting effect toward the HIV-1 replication 1 *in vitro* [3, 5–8]. The injections of monoammonium salt of GA are used in Japan for curing viral hepatitis B and C [9]. GA and its derivatives are the first group of substances that inhibit the replication of the Marburg virus [10].

RESULTS AND DISCUSSION

We modified GA molecule by the introduction into its glycoside chain the residues of α-*D*-glucosamine and β-*D*-glycosylamines for the first time. Our goal was the increase in the hydrophilicity and coordinating capacity of GA. It is known that *D*-glucosamine is a main component of various glycoconjugates [11]. This residue is usually *N*-acetylated and has the β-configuration of glycoside bond. No α-*D*-glucosamine conjugates in position C2 are described in literature.

We synthesized the GA and its 30-methyl ester conjugates (**III**)–(**IX**) for the first time. Each of them contained two aminosugar residues in carbohydrate chain linked by amide bonds with the diglucuronide chain of GA (scheme). We used the following amino components: 2-amino-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-*D*-glucopyranose hydrochloride (**X**), 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-*D*-glucopyranosyl amine hydrochloride (**XI**), 2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl amine (**XII**), 2,3,4-tri-*O*-acetyl-α-*L*-arabinopyranosyl amine (**XIII**), 2-acetamido-2-deoxy-β-*D*-glucopyranosyl amine (**XIV**), and β-*D*-galactopyranosyl amine (**XV**). The acylation of ((**X**)–(**XIII**) with GA 30-methyl ester (**II**) was carried out by means of

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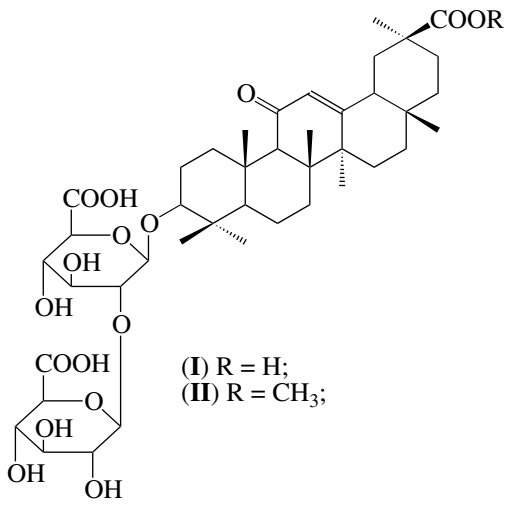
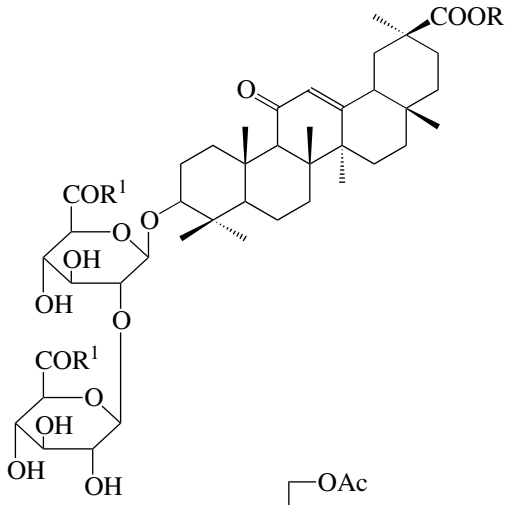
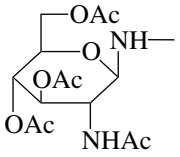
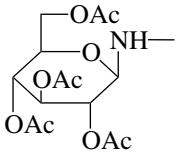
[2] Abbreviations: CD_{50}, 50% cytotoxic dose; GA, glycyrrhizic acid; HOBt, *N*-hydroxybenzotriazole; HOSu, *N*-hydroxysuccinimide; HSV-1 and HSV-2, herpes simplex type 1 and type 2 viruses; ID_{50}, the concentration of compound that provides 50% protection of cells from death or 50% inhibition of the virus replication in cells; $MNTD_{50}$, maximal nontoxic dose that caused the death of no more than 50% cells in comparison with control.

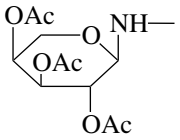


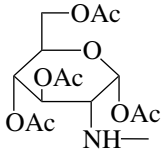
(**I**) R = H;
(**II**) R = CH_3;

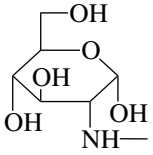


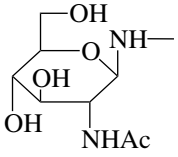
(**III**) R = CH_3; R^1 =  ; R^1H (**XI**)

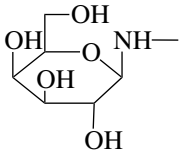
(**IV**) R = CH_3; R^1 =  ; R^1H (**XII**)

(**V**) R = CH_3; R^1 =  ; R^1H (**XIII**)

(**VI**) R = H; R^1 =  ; R^1H (**X**)

(**VII**) R = H; R^1 = 

(**VIII**) R = H; R^1 =  ; R^1H (**XIV**)

(**IX**) R = H; R^1 =  ; R^1H (**XV**)

DCC in a DMF–pyridine mixture. The resulting conjugates were isolated in pure state by column chromatography on silica gel in 40–50% yields. The coupling reaction was complicated by the formation of a side product, GA *N*-acylurea, which was formed owing to the rearrangement of an active intermediate, GA *O*-acylisourea [12]. These side reactions and the chromatography losses may account for the relatively low yields of the target conjugates (**III**)–(**V**).

A selective synthesis of (**VI**), a GA (**I**) conjugate with (**X**), was performed using DCC at the (**I**)–(**X**)–DCC molar ratio of 1 : 2–2.5 : 2 under mild conditions. The target product (**VI**) was obtained in homogeneous (TLC) state by column chromatography on silica gel in 40% yield. The starting GA (30%) was also isolated at the chromatography as a more polar fraction; it was identified according to TLC and ^{13}C NMR. We failed to isolate other reaction products in pure state.

The signals of Ac groups of the α-*D*-glucosamine residues in conjugate (**VI**) resonated in its ^{1}H NMR spectrum at 1.9–2.0 ppm, anomeric protons as broadened singlets (α-configuration) at 4.95 and 4.99 ppm, and protons of NH groups at 7.80–7.92 ppm. In ^{13}C NMR spectrum of (**VI**), C2 atoms of the α-*D*-glucosamine residues at the CONH-bond exhibit chemical

Table 1. The cytotoxicity and antiviral activity of GA conjugates (**VII**)–(**IX**) in the VERO cell culture

Compound	Cytotoxic activity $MNTD_{50}$, μg/ml	Antiviral activity ID_{50}, μg/ml	
		HSV-1	HSV-2
(**VII**)	250	125	>250
(VIII)	500	4	500
(IX)	32	16	>32

shifts at 54.3 and 53.3 ppm and anomeric C1 atoms at 90.4 and 90.3 ppm as in the spectrum of starting (**X**).

The deacetylation of (**VI**) was achieved with 0.5% KOH solution in a methanol–dichloromethane mixture; it led to the unprotected conjugate (**VII**) containing two residues of α-*D*-glucosamine in its carbohydrate moiety. In its ^{13}C NMR spectrum, C1 atoms of α-*D*-glucosamines resonate at 91.9 and 91.8 ppm and atoms C2 at 55.5 and 55.1 ppm as in the spectrum of α-*D*-GlcNAc [13, 14].

We assigned signals in the spectra of the GA conjugates on the basis of comparison with the literature data for the GA derivatives containing amide bonds [12, 15, 16] and glycosides and *N*-acyl derivatives of α-*D*-glucosamine and other glycopyranosyl amines [13, 14, 17–25].

The use of per-*O*-acetates of α-*D*-glucosamine and β-glycopyranosyl amines as amino components in the synthesis of conjugates requires an additional stage of deacetylation. Therefore, for the conjugation with GA, we decided to use β-*D*-glycopyranosyl amines (**XIV**) and (**XV**) that do not contain *O*-acetyl groups. They were prepared by the known procedures [17–20] and were coupled with GA by the DCC-method. The yields of the corresponding free glycoconjugates (**VIII**) and (**IX**) were 42–45% after the separation of reaction products by column chromatography on silica gel. Two singlet signals of AcNH groups at 2.10 and 2.15 ppm were found in the ^{1}H NMR spectrum of (**VIII**). In its ^{13}C NMR spectrum, carbon atoms at *N*-glycosyl centers resonated at 92.5 and 91.8 ppm (β-*D*-GlcNβ) and C2 atoms (C–NHAc) at 55.6 and 55.2 ppm. The chemical shifts of free 30-carboxyl groups of aglycone in the ^{13}C NMR spectra of (**VIII**) and (**IX**) were 178.2 and 179.1 ppm, respectively. We also isolated starting GA (25–30%) at the chromatographic separation of reaction products.

It is known in peptide chemistry that the presence of nucleophiles HOSu and HOBt in the reaction mixture inhibits side reactions at the formation of amide bond [26]. We tried to increase the yields of target conjugates of GA at the formation of amide bonds between the carboxy groups of the GA carbohydrate moiety and the amino groups of sugars (**X**), (**XIV**), and (**XV**) using DCC method with the addition of HOBt. The GA carboxyls were activated at the GA–DCC–HOBt molar ratio of 1 : 2–2.5 : 2–2.5 in the presence of a sugar excess in DMF. Pyridine was used as a tertiary base. The target conjugates (**VI**), (**VIII**), and (**IX**) were isolated by column chromatography on silica gel in 60–62% yields. Therefore, the use of DCC–HOBt method for conjugating GA with the monosaccharide derivatives containing amino groups allowed us to increase the yields of target glycoconjugates by ~20%.

We studied the cytotoxicity and antiviral activity of the GA conjugates (**VII**)–(**IX**) toward HSV-1 and HSV-2 *in vitro*. The cytotoxicity was determined in the VERO cell culture as $MNTD_{50}$. The antiviral activity was determined as the degree of protection from death of the cells infected by viruses (cytopathic action of viruses) as ID_{50}. The results of these experiments are given in Table 1.

We established that (**VIII**) has a low cytotoxicity in the VERO cell culture (500 μg/ml) and exhibits a pronounced antiviral activity (ID_{50} 4 μg/ml) toward HSV-1. The cytotoxicities of (**VIII**) and (**IX**) dissolved in DMSO was determined in the culture of transferable human T lymphocytes (MT-4 line). Their CD_{50} were 34 (29.69 μM) and 32.5 (28.38 μM), respectively.

The anti-HIV activities of (**VIII**) and (**IX**) were studied at nontoxic concentrations on a conventional model of the MT-4 cell culture primarily infected with HIV-1/EVK strain. The antiviral action was estimated according to the accumulation of p24 protein. In addition, the anti-HIV activity was determined from the degree of protection of infected cells from death resulting from the viral infection. The anti-HIV preparation AZT (azidothymidine) was used as a standard (Table 2). As a result of the experiments, we showed that the two compounds under study practically did not protected the infected cells from death but, at the same time, exhibit a mean anti-HIV activity by providing 50–70%

Table 2. The quantitative characteristics of anti-HIV-1 activity of GA conjugates (**VIII**) and (**IX**) in the MT-4 cell culture

Compound	CD_{50}, μM	ID_{50}, μM	IS
(**VIII**)	29.69	0.35	84.83
(**IX**)	28.38	0.09	315.33
Azidothymidine	40	0.014	2857.14

inhibition of p24: ID_{50} were 0.35 for (**VIII**) and 0.09 μM for (**IX**); the therapeutic index was higher for (**IX**) (IS 315.33).

EXPERIMENTAL

TLC was carried out on Silufol (Czech Republic) and Kieselgel F-60 (Merck, Germany) plates. Spots of substances were detected by spraying with 20% solution of phosphowolframic acid in ethanol followed by heating at 110–120°C for 2–3 min. Silica gel L (40/100 μm) was used for column chromatography. IR spectra (ν, cm^{-1}) were obtained on a Specord M-80 spectrometer in Vaseline oil paste. UV spectra were recorded on a Specord UF-400 spectrophotometer in methanol or ethanol. ^1H and ^{13}C NMR spectra (δ, ppm, *J*, Hz) were measured in deuterochloroform (unless otherwise specified) on a Bruker AM-300 spectrometer with the working frequency of 300 and 75.5 MHz, respectively. A wideband and off-resonance of proton noise decoupling were used. Tetramethylsilane was an internal standard. Optical activity was measured on a Perkin-Elmer 241MC polarimeter in a tube with the length of 1 dm. Melting points were determined on a Boetius micro plate.

DCC was from Aldrich (United States). DMF and pyridine were kept over KOH for a day and distilled over BaO. Other solvents were purified according to procedures in [27].

Solvents were evaporated in a vacuum at 50–60°C. GA was prepared by the procedure [28] and had 92 ± 2% content. GA methyl ester (**II**) was obtained by the method [29].

2-Amino-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-*D*-glucopyranose hydrochloride (X) was obtained by the procedure [30] from acetochloroglucosamine [obtained as described in [31], mp 126–127°C, $[\alpha]_D^{20}$ +115 ± 2° (*c* 0.05, chloroform; lit. [31] mp 133–134°C, $[\alpha]_D^{20}$ +118° (*c* 1, chloroform)] and recrystallized from glacial acetic acid; white needles; yield 54.8%; mp (decomposition) 182–183°C; $[\alpha]_D^{20}$ +138 ± 2° (*c* 0.04, water)(lit. [30] $[\alpha]_D^{20}$ +141° (*c* 1, water); IR spectrum: 1770 and 1750 (OAc), 1610 and 1600 (NH_3 + Cl), 1540 (NH); ^1H NMR: 1.96, 2.06, 2.08, 2.10 (12 H, 4 s, 4 Ac), 4.00–4.15 (2 H, m, $H6_a$, $H6_b$), 4.26–5.22 (m, H3, H4, H5), 5.68 (2 H, t, NH_2), 6.18 (1 H, d, *J* 3.5, H1); ^{13}C NMR: 171.69, 170.62, 169.20, 168.57 (CO of Ac), 90.73 (C1), 71.05 (C5), 69.78 (C4), 67.64 (C3), 61.62 (C6), 51.15 (C2), 22.99, 20.86, 20.65, 20.52 (4 CH_3 of Ac).

2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-*D*-glucopyranosyl amine (XI). Sodium azide (9.0 g) was added to a solution of acetochloroglucosamine (3.7 g, 10 mmol) in dry DMF (60 ml), and the mixture was heated at 70–75°C for 24 h. The mixture was diluted with cold water (200 ml) and extracted with chloroform (3 × 50 ml). The extract was washed with water, dried with $MgSO_4$, and evaporated in a vacuum. The residue (2.7 g) was recrystallized from ethanol to give 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-*D*-glucopyranosyl azide; yield 66.5%; mp 165–167°C; $[\alpha]_D^{20}$ –45 ± 2° (*c* 0.04; $CHCl_3$); IR: 2130 (N_3), 1740 (OAc), 1660 (C=O); ^{13}C NMR: 87.58 (C1), 57.48 (C2), 72.47 (C3), 68.05 (C4), 74.18 (C5), 61.56 (C6). Lit. [32]: mp 166–168°C, $[\alpha]_D^{24}$ –50° (*c* 0.83, $CHCl_3$); lit. [33]: mp 169–170°C, $[\alpha]_D^{20}$ –43.8° (*c* 2.0, $CHCl_3$); [34]: mp 166–168°C; $[\alpha]_D^{20}$ –60°C (*c* 2.0; $CHCl_3$).

Freshly prepared Raney Ni catalyst (3.0 g) was added to a solution of 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-*D*-glucopyranosyl azide (2.0 g, 5.4 mmol) in ethyl acetate (40 ml), and the mixture was hydrogenated at 20–22°C and atmospheric pressure for 72 h. The catalyst was filtered off, filtrate was evaporated at 20–22°C, and the residue was recrystallized from methanol to give (**XI**); yield 76.2%; mp 150–152°C(decomp.); $[\alpha]_D^{20}$ –15 ± 2° (*c* 0.02; $CHCl_3$) [lit. [32]: mp 152–153°C (decomp.), $[\alpha]_D^{20}$ –13° (*c* 1.0, $CHCl_3$); lit. [34]: mp 150°C (decomp.), $[\alpha]_D^{23}$ –25.5° (*c* 2.0, $CHCl_3$)]; IR: 1760 and 1740 (Ac), 1670 (C=O), and 1570 (NH); ^{13}C NMR: 85.05 (C1), 52.32 (C2), 72.80 (C3), 69.02 (C4), 73.45 (C5), and 62.56 (C6).

2,3,4,6-Tetra-*O*-acetyl-β-*D*-glucopyranosyl amine (XII). A mixture of 2,3,4,6-tetra-*O*-acetyl- α-*D*-glucopyranosyl bromide (3.4 g, 8 mmol) and NaN_3 (5.0 g) was stirred in DMF (20 ml) at 70–75°C for 3 days, diluted with cold water (100 ml), and extracted with chloroform (3 × 50 ml). The extract was washed with water, dried with $MgSO_4$, and evaporated. The residue was recrystallized from chloroform–ether mixture to give 2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl azide; yield 67.8%, mp 128–130°C; IR: 2140 (N_3); ^{13}C NMR: 87.98 (C1), 70.90 (C2), 72.75 (C3), 68.10 (C4), 7416 (C5), and 61.75 (C6). Found, %: N 11.05. $C_{14}H_{19}N_3O_9$. Calculated, %: N 11.20.

Raney Ni catalyst (3.0 g) was added to a solution of 2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl azide (2.0 g) in ethyl acetate (20 ml), and the mixture was hydrogenated for 72 h at 20–22°C. The catalyst was filtered off, filtrate was evaporated, and the residue was recrystallized from ethanol to give (**XII**); yield 1.27 g (68.5%), mp 138–140°C; $[\alpha]_D^{20}$ +28° (*c* 0.04, $CHCl_3$) [lit. [32]: $[\alpha]_D^{20}$ +26.7° ($CHCl_3$)]; ^{13}C NMR: 84.96 (C1), 72.25 (C2), 72.77 (C3), 69.10 (C4), 73.38 (C5), and 62.48 (C6).

2,3,4-Tri-*O*-acetyl-α-*L*-arabinopyranosyl amine (XIII). A mixture of 2,3,4-tri-*O*-acetyl-β-*L*-arabinopyranosyl bromide (5.4 g) and NaN_3 (11 g) was stirred in

dry DMF (40 ml) at 70–75°C for 72 h, diluted with water, and extracted with chloroform. The extract was washed with water, dried with $MgSO_4$, and evaporated. The residue was recrystallized from methanol to give 2,3,4-tri-*O*-acetyl-α-*L*-glucopyranosyl azide; yield 58.5%, mp 84–86°C; $[\alpha]_D^{20}$ –5° (c 0.04; $CHCl_3$). IR: 2130 (N_3); 1750 (Ac). Found, %: N 13.46. $C_{11}H_{15}O_7N_3$. Calculated, %: N 13.95.

Raney Ni catalyst (3.0 g) was added to a solution of 2,3,4-tri-*O*-acetyl-α-*L*-arabinopyranosyl azide (1.0 g) in ethanol (20 ml), and the mixture was hydrogenated for 72 h at 20–22°C. The catalyst was filtered off, the filtrate was evaporated, and the residue of crude (**XIII**) (0.74 g, 81%) was further used without additional purification; IR: 3400–3200 (NH_2), 1760 (Ac), 1680 (C=O), 1560 (NH).

2-Acetamido-2-deoxy-β-*D*-glucopyranosyl amine (XIV) was prepared according to procedures [17, 20] as an amorphous substance; yield 56%; $[\alpha]_D^{20}$ –4.5° (*c* 0.5, water); lit. [17]: $[\alpha]_D^{20}$ –4.7° (*c* 1.7, water).

β-*D*-Galactopyranosyl amine (XV) was obtained by the procedures [17, 18, 20]; its content was ~80%, and it was further used without additional purification.

General procedure of the synthesis of conjugates of GA 30-methyl ester (III)–(V). Sugar (**XI**)–(**XIII**) (1.4 mmol) and DCC (0.24 g, 1 mmol) were added to a solution of GA 30-methyl ester (**II**) (0.45 g, 0.5 mmol) in a mixture of DMF (10 ml) and pyridine (2 ml) at 0–+5°C. The reaction mixture was stirred for 1 h at this temperature, kept for 20 h at 20–22°C, and filtered from the *N*,*N*'-dicyclohexylurea precipitate. The filtrate was diluted with cold water, acidified with citric acid to pH ~3, the residue was filtered, dried, and chromatographed on a silica gel column eluted with 300 : 10 : 1, 200 : 10 : 1, and 100 : 10 : 1 chloroform–methanol–water mixtures. The fractions containing the target products were combined and evaporated.

Glycoconjugate of GA 30-methyl ester with 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-*D*-glucopyranosyl amine (III) yield 40%; R_f 0.22 (10 : 1 $CHCl_3$–EtOH); UV (MeOH): λ_{max} 248 nm (log ε 4.04); IR: 1760 (OAc), 1670 (11-C=O), 1560 (CONH); ^{1}H NMR (CD_3OD): 0.70–1.30 (21 H, 7 CH_3), 1.80–2.00 (36 H, 12 Ac), 3.65 (3 H, s, OCH_3), 5.60 (1 H, s H12), 7.20 (4 NH); ^{13}C NMR (CD_3OD): 39.31 (C1), 92.55 (C3), 55.10 (C5), 61.4 (C9), 200.3 (C11), 128.71 (C12), 171.32 (C13), 48.07 (C18), 44.14 (C20), 175.20 (C30), 51.82 (C31), 104.00 (C1''), 103.00 (C1'), 84.50 (C2'), 73.20 (C3'), 72.64 (C4'), 73.31 (C5'), 71.17 (C2''), 72.65 (C4''), 170.83 (C6'), 170.20 (C6''), 91.76 and 91.00 (C1 of GlcN), 52.75 and 52.38 (C2 of GlcN), 61.88 and 61.73 (C6 of GlcN), Ac: 170.05, 169.76, and 169.22, 168.58 (C=O), 20.95, 20.68, 20.63, and 20.20 (CH_3). Found, %: N 3.53. $C_{71}H_{104}N_4O_{30}$. Calculated, %: N 3.22.

Glycoconjugate of GA 30-methyl ester with 2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl amine (IV): yield 50%; mp 102–104°C (aqueous ethanol); R_f 0.53 (10 : 1 $CHCl_3$–EtOH); IR: 3600–3200 (OH, NH), 1760 (OAc), 1670 (11-CO), 1550 (CONH); ^{1}H NMR: 0.80, 0.86, 1.00, 1.12, 1.12, 1.24, and 1.36 (21 H, all s, 7 CH_3), 1.95–2.10 (24 H, 8 OAc), 2.85 (1 H, s, H18), 3.60 (3 H, s, OCH_3), 5.70 (1 H, s H12); ^{13}C NMR: 39.15 (C1), 90.09 (C3), 54.98 (C5), 61.85 (C9), 200.37 (C11), 128.74 (C12), 169.74 (C13), 48.13 (C18), 42.88 (C20), 174.50 (C30), 52.55 (C31), 104.00 (C1', C1''), 171.68 and 171.25 (C6', C6''), 90.47 and 89.97 (C1 of Glc), 62.10 and 62.55 (C6 of Glc), Ac: 168.83, 169.29, 169.72, 168.97, 170.25, and 170.85, 170.92 (C=O), 20.61, 20.66, and 20.73 (CH_3). Found, %: N 2.32. $C_{70}H_{102}N_2O_{25}$. Calculated, %: N 2.03.

Glycoconjugate of GA 30-methyl ester with 2,3,4-tri-*O*-acetyl-α-*L*-arabinopyranosyl amine (V): yield 46%; IR: 3600–3200 (OH, NH), 1740 (OAc), 1670 (11-C=O), 1540 (CONH); ^{1}H NMR: 0.76, 0.84, 0.96, 1.05, 1.20, 1.34, and 1.36 (21 H, all s, 7 CH_3), 1.96–2.10 (18 H, 6 Ac), 2.80 (1 H, s, H18), 3.70 (1 H, s, OCH_3), 5.00–5.40 (m, H3', H3'', H1', H1'', H1 of AraN), 5.68 (1 H, s, H12); ^{13}C NMR: 39.12 (C1), 90.21 (C3), 61.85 (C9), 200.32 (C11), 128.71 (C12), 169.68 (C13), 177.86 (C30), 101.82 (C1', C1''), 170.65 and 170.42 (C6', C6''), 93.36 and 92.84 (C1 of Ara).

General procedures of preparing the GA conjugates (VI), (VIII), and (IX). 1. A sugar (**X**), (**XIV**), or (**XV**) (2.1–2.5 mmol) and DCC (0.46 g, 2 mmol) were added to a solution of GA (0.82 g, 1 mmol) in a mixture of DMF (20 ml) and pyridine (10 ml) at 0 to +5°C. The reaction mixture was stirred for 1 h at this temperature and kept for 20 h at 20–22°C. The precipitate of *N*,*N*'-dicyclohexylurea was filtered off, the filtrate was diluted with cold water, and acidified with citric acid to pH ~3. The resulting precipitate was filtered, washed with water, and dried. The product was twice chromatographed on silica gel columns successively eluted with 200 : 10 : 1, 100 : 10 : 1, and 50 : 10 : 1 chloroform–methanol–water mixtures.

2. A sugar (**X**), (**XIV**), or (**XV**) (1.20–1.25 mmol), DCC (1.00–1.20 mmol), and HOBt (0.14–0.17 g, 1–1.25 mmol) were added to a solution of GA (0.41 g, 0.5 mmol) in DMF (10 ml) and pyridine (5 ml). The mixture was stirred for 12 h at 20–22°C, the *N*,*N*'-dicyclohexylurea precipitate was filtered off, the filtrate was evaporated, and the residue was chromatographed on a silica gel column successively eluted with 200 : 10 : 1, 100 : 10 : 1, and 50 : 10 : 1 chloroform–methanol–water mixtures.

The GA conjugate with 2-amino-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-*D*-glucopyranose (VI): yield 40.5% (method 1) and 60.5% (method 2); white powder; R_f 0.5 (45 : 10 : 1 chloroform–methanol–water); $[\alpha]_D^{20}$ +45 ± 2° (*c* 0.04; MeOH); UV (MeOH): λ_{max}

246 nm ($\log\varepsilon$ 4.25); IR: 3600–3200 (OH, NH), 1750 (OAc), 1670 (11-CO), 1550 ($CONH_2$); ^{1}H NMR: 1.00, 1.08, 1.10, 1.12, 1.15, 1.24, and 1.38 (21 H, all s, 7 CH_3), 1.90–2.00 (24 H, 8 OAc), 2.38 (1 H, s, H29), 3.25–3.60 (4 H, m, H4 and H5 of GlcN), 3.64–3.74 (4 H, m, H3 and H6 of GlcN), 3.90–4.08 (2 H, m, H5'and H6 of GlcN), 4.16–4.26 (2 H, m, H5" and H6 of GlcN), 4.95 and 4.99 (2 H, two br. s, H1 of GlcN), 5.14 (1 H, s, H3"), 5.28 (1 H, dd, *J* 9.2 and 9.6, H3'), 5.54 (1 H, s, H12), 7.80 and 7.12 (2 H, two s, NH); ^{13}C NMR ($CDCl_3$ + CD_3OD): 38.45 (C1), 88.67 (C3), 56.19 (C5), 44.57 (C8), 35.82 (C10), 208.55 (C11), 126.79 (C12), 170.34 (C13), 178.22 (C30), 103.05 (C1"), 102.55 (C1'), 71.73 (C4'), 70.76 (C4"), 75.08 (C5"), 75.05 (C5'), 168.93 (C6'), 169.24 (C6"), 90.41 and 90.13 (C1 of GlcN), 68.31 and 67.97 (C4 of GlcN), 61.39 and 60.91 (C6 of GlcN), 54.26 and 53.27 (C2 of GlcN), Ac: 170.63, 170.34, and 170.00, 169.50 (C=O). Found, %: N 2.00. $C_{70}H_{100}N_2O_{32}$. Calculated, %: N 1.89.

The starting GA was also isolated at the chromatography of reaction product as a more polar fraction (30%); it was identified by TLC (R_f 0.3) and ^{13}C NMR [35].

The GA conjugate with 2-amino-2-deoxy-α-*D*-glucopyranose (VII). A mixture of (**VI**) (0.5 g), dichloromethane (5 ml), and 1% KOH in methanol (5 ml) was stirred for 1 h at 20–22°C and evaporated. The residue was dissolved in methanol (20 ml) and treated with cation exchanger KU-2-8 (H^+) to pH ~5. The resin was filtered off, washed with methanol, and the combined filtrate was evaporated. The residue was reprecipitated from dry methanol with ether. The yield of (**VII**) was 84%; $[\alpha]_D^{20}$ +50 ± 2° (*c* 0.02; EtOH); UV (EtOH): λ_{max} 251 nm ($\log\varepsilon$ 3.96); IR: 3600–3200 (OH, NH), 1710 (COOH), 1660 (11-C=O), 1540 (CONH); ^{13}C NMR (DMF-d_7): 39.93 (C1), 89.29 (C3), 54.98 (C5), 43.74 (C8), 199.86 (C11), 128.20 (C12), 49.02 (C18), 44.03 (C20), 178.70 (C30), 104.97 (C1"), 104.28 (C1'), 82.00 (C2'), 74.25 and 73.07 (C3" and C3'), 72.40 and 72.98 (C4'and C4"), 77.20 and 76.79 (C5" and C5'), 170.57 (C6' and C6"), 91.89 and 91.78 (C1 of GlcN), 55.47 and 55.13 (C2 of GlcN), 75.91 and 75.48 (C3 of GlcN), 72.23 and 72.06 (C4 of GlcN), 62.42 and 62.13 (C6 of GlcN). Found, %: N 2.32. $C_{54}H_{104}N_2O_{24}$. Calculated, %: N 2.40.

The GA glycoconjugate with 2-acetamido-2-deoxy-β-*D*-glucopyranosyl amine (VIII): yield 42% (method 1) and 60% (method 2); R_f 0.36 (3 : 1 chloroform–ethanol); $[\alpha]_D^{20}$ +80 ± 2° (*c* 0.06; MeOH); UV (MeOH): λ_{max} 249 nm ($\log\varepsilon$ 4.20); IR: 3600–3200 (OH, NH), 1660 (11-CO), 1550 (CONH); ^{1}H NMR (DMF-d_7): 0.70–1.40 (21 H, 7 CH_3), 2.10–2.15 (6 H, two s, 2 Ac), 2.56 (1 H, s, H18), 5.50 (1 H, s, H12); ^{13}C NMR (DMF-d_7): 39.45 (C1), 89.70 (C3), 56.19 (C5), 200.03 (C11), 128.20 (C12), 172.69 (C13), 45.79 (C20), 178.51 (C30), 105.00 (C1"), 104.34 (C1'), 74.80 (C3'), 75.04 (C3"), 72.88 (C4'), 71.50 (C4"), 77.82 (C5"), 77.11 (C5'), 170.66 (C6' and C6"), 92.50 and 91.90 (C1 of GlcN), 55.56 and 55.21 (C2 of GlcN), 72.60, 72.10, 69.36, and 69.00 (C3–C4 of GlcN), 76.00 and 75.50 (C5 of GlcN), 62.60 and 62.40 (C6 of GlcN). Found, %: N 4.42. $C_{58}H_{86}N_4O_{24}$. Calculated, %: N 4.58.

The starting GA was also isolated at chromatography of the reaction product as a more polar fraction (28%); it was identified by TLC (R_f 0.3) and ^{13}C NMR [35].

The GA glycoconjugate with β-*D*-galactopyranosyl amine (IX): yield 45% (method 1) and 62% (method 2); R_f 0.30 (3 : 1 chloroform–ethanol); $[\alpha]_D^{20}$ +55 ± 2° (*c* 0.04; MeOH); UV (MeOH): λ_{max} 249 nm ($\log\varepsilon$ 4.20); IR: 3600–3200 (OH, NH), 1710 (COOH), 1660 (11-CO), 1550 (CONH); ^{13}C NMR (pyridine-d_5): 39.56 (C1), 89.50 (C3), 55.50 (C5), 62.02 (C9), 199.64 (C11), 128.49 (C12), 170.00 (C13), 48.62 (C18), 45.45 (C20), 179.12 (C30), 104.68 (C6"), 104.59 (C6'), 84.30 (C2'), 75.76 (C3'), 76.79 (C3"), 72.03 (C4'), 71.01 (C4"), 77.55 (C5" and C5'), 168.31 (C6' and C6"), 98.50 and 97.90 (C1 of Gal), 75.50, 74.50, 74.20, 73.50,72.70, 71.80, and 70.50 (C2–C5 of Gal), 62.50 and 62.70 (C6 of Gal). Found, %: N 2.10. $C_{54}H_{84}N_2O_{24}$. Calculated, %: N 2.44.

The starting GA was also isolated at chromatography of the reaction product as a more polar fraction (25%); it was identified by TLC.

Cytotoxicity and antiviral activity of GA conjugates (**VII**)–(**IX**) was determined on a VERO cell culture (a kidney cell line of green monkeys, ICN). The experiments were carried out in duplicated chambers, with each of them containing 5×10^4 cells. The cytotoxicity was determined after 48-h incubation in atmosphere with 5% CO_2 at 37°C. The maximal nontoxic dose ($MNTD_{50}$) was defined as the dose of compound that caused the death of 50% cells in comparison with control.

The antiviral activity was determined toward HSV-1 and HSV-2. The antiviral effect was evaluated in a monolayer of cells that were infected two times by the dose of one viral particle per 10^3 cells in the presence of the substance under study at concentrations from 100 to 0.1 μg/ml. The antiviral action was determined from the degree of protection of the infected cells from death as a result of viral infection (cytopathic action of viruses) in a cell monolayer. It was counted with the help of microscope in comparison with the control (untreated cells) as the inhibiting dose (ID_{50}); i.e., the concentration of a substance that provides 50% protection of cells or inhibits the replication of virus by 50% in cells after 48-h incubation at 37°C in 5% CO_2 according to the procedure [36]. The results of the experiments are given in Table 1.

The anti-HIV-1 activity and cytotoxicity of (**VIII**) and (**IX**) were studied on the transferable cell line of human leucocytes MT-4 as described in [5, 6]. The cytotoxicity was estimated when the compounds dissolved in DMSO were introduced at the corresponding dilutions into the wells of 96-well plates (three wells per each dilution) when the MT-4 cells were inoculated in the wells at the concentration of 0.5×10^6 cells/ml. The cells were cultured in 96-well plates from Costar (United States) on the nutrient medium RPMI-1640 supplemented with 10% calf serum, 0.06% *L*-glutamine, and 100 μg/ml of gentamycin at 37°C and 5% CO_2 for 4 days. After the incubation was completed, the proportion of viable cells was counted in the Goryaev chamber after staining with Trypan Blue. The dose-dependent curve was then plotted, and the CD_{50} (the concentration of a compound that causes the death of 50% of cells) was determined.

The anti-HIV-1 activity of the compounds under study was determined by the use of the MT-4 cells (at the concentration of 2×10^6 cells/ml) infected with HIV-1 strain EVK with the infection multiplicity of 0.2–0.5 infection units per cell. The virus was absorbed for 1 h at 37°C and then the infected and the control (without virus) cells were diluted with the growing cultural medium up to the inoculum concentration of 5×10^5 cells /ml and introduced into the wells of 96-well culture plates. The compounds under study were then placed in the corresponding wells (three wells per each dilution), and the plates were cultured as described above. Azidothymidine, a known anti-HIV agent [37], was used as a reference preparation. The final concentrations of the preparations under study in the cell culture were from 0.1 to 20 μg/ml. The inhibiting effect was determined after 4 days of culturing by measuring the quantity of the viral antigen p24 by EIA [38]. The curves of dose–p24 dependence served for the determination of compound concentrations inhibiting by 50% the accumulation of p24 (ID_{50}), and the selectivity index (IS) was calculated as the ratio of the cytotoxic to the effective dose. The results are given in Table 2.

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