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SYNTHESIS AND ANTI-HIV ACTIVITY OF TRITERPENE 3-*O*-GALACTOPYRANOSIDES, ANALOGS OF GLYCYRRHIZIC ACID

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*A new method for synthesizing triterpene 3-*O*-galactosides, analogs of glycyrrhizic acid (GA) based on 18β-glycyrrhetic acid (GLA) methyl esters and 18,19-dehydro-GLA, using 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide as the glycosyl donor, I–Br promoter, and 4-Å molecular sieves was developed. The method could produce primarily 3-*O*-α-D- or β-D-galactopyranosides depending on the reaction conditions. The 3-*O*-α-D-galactopyranoside of GLA exhibited an index of selectivity (IS) 2.9 times greater than that of GA for inhibition of accumulation of virus-specific protein p24 of HIV-1. β-D-Galactopyranoside of GLA was more cytotoxic for MT-4 cells and exhibited weak anti-HIV-1 activity.*

Keywords: triterpene glycosides, glycyrrhizic acid, glycyrrhetic acid, glycosylation, iodine monobromide, anti-HIV-1 activity.

The synthesis of model triterpene glycosides that are analogs of glycyrrhizic acid (GA) with a modified carbohydrate chain is of great interest for studying the molecular mechanisms of GA biological activity, toxicity, and structure–activity relationships [1–5].

The sugar moiety in most natural triterpene glycosides is bonded to the sapogenin 3-OH through a 1,2-*trans*-glycosidic bond [6]. Therefore, a key step in the synthesis of saponins is 3-*O*-glycosylation of the corresponding triterpene sapogenins by mono- and disaccharide glycosyl donors (GD). Forming such a bond between GD and sterically hindered 3-OH of triterpene alcohols is a rather complicated problem. Glycosylation of natural triterpenoids by readily available GD such as glycosylhalides is usually carried out using compounds of mercury [$Hg(CN)_2$, $HgBr_2$] and silver (Ag_2O, Ag_2CO_3, AgOTf) as promoters [1–5, 7]. Use of mercury salts for practical syntheses of triterpene glycosides is limited by their high toxicity whereas silver salts are expensive. As a rule, both methods produce a mixture of α- and β-*O*-glycosides in 30–70% yields [1–4].

In continuation of our studies of the synthesis of biologically active GA analogs [8], we studied glycosylation of triterpenoids from licorice root using the convenient and inexpensive reagent iodine monobromide (I–Br), which was proposed earlier by Field and Kartha for synthesizing various simple glycosides and disaccharides [9, 10]. However, it has not yet been used to synthesize triterpene glycosides.

We used methyl esters of 18β-glycyrrhetic acid (MeGLA) (**3**) and 18,19-dehydro-GLA (**7**) as alcohol components for glycosylation; 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosylbromide (GalBr), as the GD [11], because glycosylation of sterically hindered triterpene alcohols by galactopyranosyl GD is rather complicated and poorly studied from a chemical viewpoint.

Table 1 gives the results from the glycosylation. Carrying out the reaction in a mixture of anhydrous CH_3CN:CH_2Cl_2 (5:1, v/v) at –20 → –10°C for 18 h with GalBr:MeGLA:I–Br (2:1:2) formed a mixture of α- and β-D-galactopyranosides **4** and **5** in overall yield 35.6% (Scheme 1). The ratio of α- and β-anomers was 6:1 after separation of the reaction products over a column of silica gel (SG), i.e., the major product was the α-anomer **4**, which was easily isolated from the product mixture also by recrystallization from EtOH. MeGLA 3-*O*-acetate **6** was isolated in small quantities as a side product.

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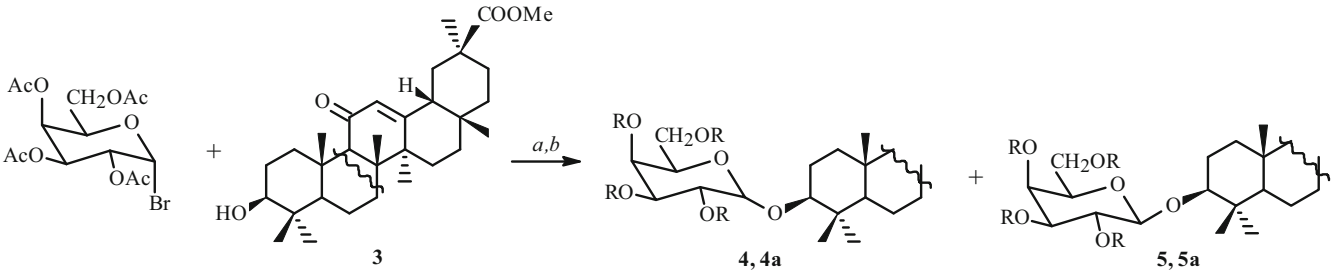
TABLE 1. Glycosylation of Triterpenoids with 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosylbromide (2 mmol) in the Presence of I–Br

Triterpenoid	I–Br, mmol	Solvents, CH_3CN:CH_2Cl_2, %	Reaction temperature, time	Ratio of **4** and **5** anomers	Glycoside yield, %	Yield, g
3	2	5:1	–20 → –10°C, 20 h	α/β, 6:1	35.6	**6**, 0.04
3	2	5:1	–20 → –10°C, 8 h, 20°C – 2 h	α/β, 3:1	26.5	**6**, 0.08
3*	4	5:2	0 → 20°C, 20 h	β	43.2	**6**, 0.08
3*	8	5:4	0 → 20°C, 24 h	β	61.7	**6**, 0.10
7*	8	5:4	0 → 20°C, 22 h	β	56.5	**9**, 0.10

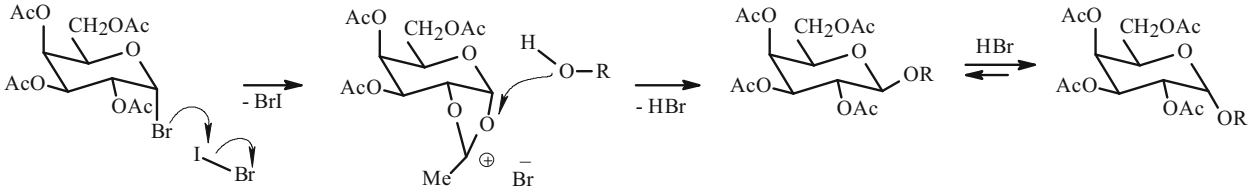
*Reaction performed with 4-Åmolecular sieves.



a. I-Br, CH_3CN:CH_2Cl_2; *b*. 0.1 N NaOMe/MeOH:CH_2Cl_2

Scheme 1

A similar anomerization during the synthesis of complicated glycosides that are produced using I–Br and GD with a C-2 OAc group involved in forming the *O*-glycoside bond probably occurs according to Scheme 2.



Scheme 2

Carrying out the reaction for 10 h decreased the ratio of α/β-anomers to 3:1 as the reaction time at 20 –22°C increased. However, the overall yield of the glycosides was less than 26.5%. Glycosylation of MeGLA (**3**) in the presence of 4-Å molecular sieves at higher temperatures (0 → 20 –22°C) with twice the amount of activator formed only the β-galactoside **5** in 43.2% yield (Table 1) whereas glycosylation at 0 –20°C with GalBr:MeGLA:I–Br = 2:1:8 for 24 h produced the β-glycoside **5** in 61.7% yield. This was comparable with the literature for the synthesis of MeGLA β-glycosides in the presence of silver, mercury, and tin salts [1-3, 8].

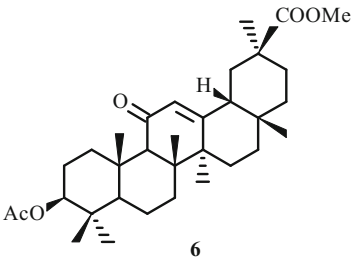
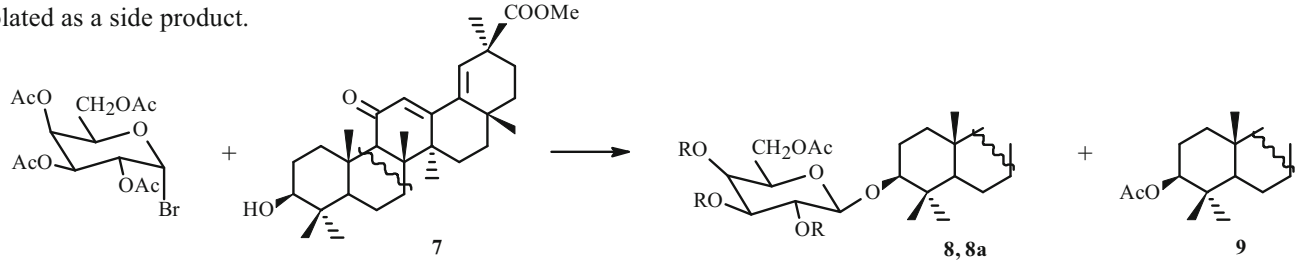


TABLE 2. Chemical Shifts of C Atoms in ^{13}C NMR Spectra (75.5 MHz, $CDCl_3$, δ, ppm, 25°C, TMS internal standard)

C atom	**4**	**8**	**4a**	**5a**	**8a**
1	38.5	38.6	35.5	39.1	39.3
2	26.3	24.6	22.7	26.4	26.3
3	85.7	90.0	85.6	89.6	89.9
4	38.6	38.7	38.7	39.4	39.6
5	55.2	55.0	55.2	55.2	55.6
6	17.3	17.1	17.3	17.3	17.6
7	32.6	33.6	32.6	32.7	34.2
8	45.3	44.0	45.3	43.8	43.7
9	61.7	60.4	61.7	60.6	61.4
10	36.7	36.8	36.7	36.8	36.9
11	200.2	19.5	200.3	200.0	200.4
12	128.5	12.7	128.5	128.4	124.4
13	169.4	16.3	169.4	169.0	162.9
14	43.1	44.8	43.9	45.4	45.4
15	26.3	25.5	26.3	26,0	26.3
16	26.2	36.4	26.3	26.4	36.4
17	31.7	35.8	31.7	31.7	35.1
18	48.3	142.4	48.3	48.3	143.0
19	41.0	129.2	41.0	41.0	129.7
20	43.9	43.1	43.1	43.1	44.6
21	31.0	29.2	31.0	31.1	28.1
22	37.6	34.5	37.6	37.8	35.0
23	28.2	27.3	28.2	28.2	28.2
24	16.2	16.0	16.2	16.4	16.9
25	16.4	16.2	16.4	16.7	17.0
26	18.5	18.0	18.5	18.7	18.7
27	23.2	19.3	23.2	24.4	19.8
28	28.4	24.6	28.4	28.4	24.6
29	28.2	27.8	28.4	29.6	25.3
30	177.0	176.2	177.0	176.8	177.0
31	51.7	51.7	51.7	51.6	52.4
1′	94.1	103.0	94.1	105.7	105.8
2′	68.2	70.0	68.2	71.5	73.8
3′	68.2	70.6	77.0	73.3	74.3
4′	66.6	66.8	68.2	68.4	68.0
5′	67.7	68.9	68.2	73.6	72.0
6′	61.9	61.1	61.9	61.7	61.0
C=O	170.6	169.8			
Ac	170.5	169.8			
	170.4	169.6			
	170.2	168.4			
CH_3	20.7	20.4			
	20.6	20.2			
	20.5	20.1			

Glycosylation of 18,19-dehydro-GLA methyl ester (**7**) by GalBr at 0 –20°C (Scheme 3) gave *β*-galactoside **8** in 56.5% yield. The 3-*O*-acetate **9**, which was identified by TLC with an authentic sample and PMR and ^{13}C NMR spectra, was isolated as a side product.



Scheme 3

8: R = Ac; **8a:** R = H

TABLE 3. Quantitative Characteristics of p24 HIV-1 Inhibition in MT-4 Cell Culture (HIV-1/EVK)*

Compound	CD_{50}, μg/mL	p24 inhibition, μg/mL		IS
		ID_{50}	ID_{90}	
4a	500	17	>100	29.40
5a	100	160	>1000	0.63
GA	1960	125	950	10.30

*ID_{50} is the compound concentration suppressing virus production by 50%; ID_{90}, by 90%; IS, selectivity index as the ratio of toxic dose CD_{50} to effective dose ID_{50}.

Thus, the yield of triterpene 3-*O*-glycosides depended on the amount of I–Br promoter and the reaction temperature and time. Using an excess of promoter and carrying out the glycosylation at 20 –22°C for 22-24 h formed primarily β-glycosides **5** and **8** whereas carrying out the reaction below –10 °C gave primarily the α-glycosides.

The structures of the synthesized glycosides were confirmed by spectral methods (IR, UV, PMR, ^{13}C NMR), melting points, $[\alpha]_D^{20}$, and NMR with authentic samples [8, 12]. ^{13}C NMR spectra of acetylated and deacetylated glycosides **4** and **8** and **4a**, **5a**, and **8a**, respectively, were recorded for the first time and are given in Table 2. The ^{13}C NMR spectrum of the α-anomer of **4** contained a resonance for glycosidic C-1′ with chemical shift (CS) 94.1 ppm. The glycosylation effect was seen as a weak-field shift of the resonance of C-3 by 7 ppm. The resonance of anomeric H-1′ was observed as a singlet at 5.22 ppm. This confirmed that the glycoside center had the α-configuration.

Deacetylation of the synthesized triterpene 3-*O*-glycosides by NaOMe (0.1 N) in MeOH:CH_2Cl_2 at 20–22°C gave in 81–83% yields glycosides **4a**, **5a**, and **8a**, which are D-galactopyranoside analogs of GA [8]. Comparison of the ^{13}C NMR spectra of de-acetylated glycosides and their acetates showed that the C-atom resonances of the aglycon and monosaccharide moieties were related (Table 2).

The anti-HIV-1 activity of **4a** and **5a** was studied using the traditional model of primary HIV-1 infected MT-4 lymph cells (acute HIV-infection model) [13] and strain HIV-1/EVK in comparison with GA. The inhibition effect of the compounds was estimated at four days of cultivation by measuring the amount of viral antigen, virus-specific protein p24. The reference drug was highly purified GA (97%) [14] at a concentration of 100 μg/mL.

Table 3 presents the experimental results for the anti-HIV-1 activity of these glycosides. Changing the GA carbohydrate chain to D-galactopyranose changed the cytotoxicity and anti-HIV-1 activity of the GA analogs. GA 3-*O*-α-D-galactopyranoside (**4a**) had moderate anti-HIV-1 activity and inhibited accumulation of virus-specific protein p24 with a smaller ID_{50} than GA. The index of selectivity (IS) was also 2.9 times greater than that of GA (Table 3). Glycoside **5a** with the β-configuration was more cytotoxic for MT-4 cells and had weak anti-HIV-1 activity.

EXPERIMENTAL

IR spectra were recorded in mineral oil mulls on a Specord M-80 spectrophotometer; UV spectra, in MeOH or EtOH on a UF-400 spectrometer; PMR and ^{13}C NMR spectra, in $CDCl_3$ and Py-d_5 on Bruker AM 300 (300 MHz for ^{1}H; 75.5, ^{13}C) and Bruker Avance 400 (400 MHz for ^{1}H; 100, ^{13}C) spectrometers with broad-band and off-resonance proton decoupling and TMS internal standard. Resonances were assigned using one- and two-dimensional experiments (^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC) and by comparison with the literature for GLA glycosides [8]. Molecular ions were detected in the EPSRC mass spectrometry centre of Swansea University College (Great Britain) and in a Shimadzu LCMS-2010 GC–MS. Specific rotation was determined on a Perkin–Elmer 341 MC polarimeter in a 1-dm tube. Melting points were measured on a Boetius microstage.

TLC was performed on Kizelgel F-60 (Merck, Germany) using toluene:EtOAc (3:1, A). Spots were detected by phosphotungstic acid solution (20%) or H_2SO_4 solution (5%) in EtOH with subsequent heating at 110–120°C for 2–3 min. Column chromatography (CC) was performed over silica gel L (40/100 μm) (Aldrich) and KSK (50/150 μm) (ZAO Sorbpolimer).

GLA (Aldrich, Germany) and pharmacopoeial 18,19-dehydro-GLA were used as the triterpene acids. Methyl esters of 18β-GLA (**3**) and 18,19-dehydro-GLA (**7**) were prepared by refluxing the appropriate triterpenoids (5 g) in MeOH (250–300 mL) for 15–16 h in the presence of conc. H_2SO_4 (10 mL) as before [16] and recrystallizing from EtOH. Methyl ester of 18β-GLA (**3**): yield 82%, mp 254–256°C, $[\alpha]_D^{20}$ +164° (*c* 0.03, $CHCl_3$), lit. [15] mp 252–256°C. Methyl ester of

18,19-dehydro-GLA (**7**): yield 74%, mp 207–209°C, $[\alpha]_D^{20}$ +270° (*c* 0.04, $CHCl_3$), lit. [16] mp 207–209°C, $[\alpha]_D^{20}$ +270° (*c* 0.04, $CHCl_3$). 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosylbromide was prepared by the literature method [11]. Deacetylation of glycosides used the usual method [8] with NaOMe:MeOH (0.1 N). Solvents were purified and dried as usual [17]. Solvents were evaporated in vacuo at 40–50°C. 4-Å molecular sieves were calcined for 3 h at 300–350°C.

Glycosylation of 18β-GLA Methyl Ester. 1. A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosylbromide (2 mmol, 0.84 g) and 18β-GLA methyl ester (**3**, 1 mmol, 0.48 g) in dry CH_3CN (10 mL) at –20°C was treated with I–Br (2 mmol, 2 mL, 1 M in CH_2Cl_2), stirred at –20 → –10°C for 2 h, and held at –10°C for 18 h in a freezer. The mixture was diluted with $CHCl_3$ (20 mL), washed with saturated $Na_2S_2O_3$ solution (2 × 20 mL) and water (2 × 20 mL), dried, and evaporated in vacuo. Column chromatography of the solid (1.24 g) over SG (toluene:EtOAc, 19:1 → 1:1, v/v, stepped gradient) produced three fractions. The first (0.04 g) was identified by TLC with an authentic sample and PMR and ^{13}C NMR spectra as GLA methyl ester 3-*O*-acetate (**6**); the second (0.25 g), α-D-galactopyranoside **4**; the third (0.04 g), β-D-galactopyranoside **5** of GLA methyl ester. The overall yield of the mixture of α- and β-glycosides was 0.29 g (35.6%). Table 1 gives the ratio of stereoisomers.

2. A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosylbromide (2 mmol) and 18β-GLA methyl ester (**3**, 1 mmol) in dry CH_3CN (10 mL) at –20°C was treated with I–Br solution (4 mmol), stirred at –20 → –10°C for 8 h and at 20–22°C for 2 h. The mixture was worked up as described above. The dry solid (1.18 g) was recrystallized from EtOH. The solid was filtered off to afford pure α-D-galactopyranoside **4** (0.33 g). The mother liquor was separated by CC over SG as described above. The overall yield of the mixture of α- and β-glycosides was 0.43 g (26.5%). Table 1 gives the ratio of stereoisomers.

3. A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosylbromide (2 mmol) and 18β-GLA methyl ester (**3**, 1 mmol) in CH_3CN (10 mL) was treated with calcined 4-Å molecular sieves (2 g), stirred for 30 min, cooled in an ice bath, treated with I–Br solution (4 mmol), stirred at 0–5°C for 5 h, left for 15 h at 20–22°C, and worked up as described above. The solid was recrystallized from EtOH to afford pure β-glycoside **5** (0.25 g). The mother liquor was separated by CC over SG. The yield of β-glycoside **5** was 0.35 g (43.2%).

4. A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosylbromide (2 mmol) and 18β-GLA methyl ester (**3**, 1 mmol) in CH_3CH (10 mL) was treated with calcined 4-Å molecular sieves (4 g), stirred for 30 min, cooled in an ice bath, treated with I–Br solution (8 mmol), stirred at 0–5°C for 4 h, left for 20 h at 20–22°C, and worked up as described above and separated by CC over SG. The yield of β-glycoside **5** was 0.50 g (61.7%).

Glycosylation of 18,19-Dehydroglycyrrhetic Acid Methyl Ester. A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosylbromide (2 mmol) and 18,19-dehydro-GLA methyl ester (**7**, 1 mmol, 0.48 g) in CH_3CN (10 mL) was treated with 4-Å molecular sieves (4 g), stirred for 30 min, cooled in an ice bath, treated with I–Br solution (8 mmol), stirred with cooling for 2 h, left for 20 h at 20–22°C, worked up as described above, and separated by CC over SG to afford β-glycoside **8** (0.46 g, 56.5%) and 3-*O*-acetate **9** (0.1 g).

Glycyrrhetic Acid Methyl Ester 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranoside (4). R_f 0.5 (A), mp 262–264°C (EtOH), $[\alpha]_D^{20}$ +100° (*c* 0.12, $CHCl_3$), lit. [8] mp 264–266°C, $[\alpha]_D^{20}$ +95° (*c* 0.06, $CHCl_3$). UV spectrum (MeOH, λ_{max}, nm): 248 (log ε 4.0).

PMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.80 (3H, s, H-28), 0.84 (3H, s, H-24), 1.00 (3H, s, H-23), 1.12 (3H, s, H-26), 1.18 (6H, s, H-29, H-28), 1.37 (3H, s, H-27), 1.97, 2.00, 2.05, 2.12 (12H, all s, 4 Ac), 2.29 (1H, s, H-9), 3.67 (3H, s, OCH_3), 4.05 (2H, m, H-6′a, H-6′e), 4.36 (1H, t, J_1 = 6.3, J_2 = 6.3, H-5′), 5.07 (1H, dd, J_1 = 3.6, J_2 = 10.9, H-3′), 5.21 (1H, d, J = 3.6, H-1′), 5.26 (1H, dd, J_1 = 3.2, J_2 = 10.9, H-2′), 5.46 (1H, d, J = 3.2, H-4′), 5.64 (1H, s, H-12).

Table 2 lists the ^{13}C NMR spectrum. $[M + NH_4]^+$ 832.5, $C_{45}H_{66}O_{13}$, MW = 814.9.

Glycyrrhetic Acid Methyl Ester 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranoside (5). *Rf* 0.45 (A), mp 193–195°C (EtOH), $[\alpha]_D^{20}$ +87° (*c* 0.08, EtOH), lit. [8] mp 195–197°C, $[\alpha]_D^{20}$ +85° (*c* 0.08, MeOH). IR spectrum (ν, cm^{-1}): 1760 (OAc, OMe), 1670 (C_{11}=O). UV spectrum (MeOH, λ_{max}, nm): 247.5 (log ε 4.1). $[M + NH_4]^+$ 832.2, $C_{45}H_{66}O_{13}$, MW = 814.9.

18,19-Dehydroglycyrrhetic Acid Methyl Ester 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranoside (8). Yellow amorphous powder, R_f 0.45 (A), $[\alpha]_D^{20}$ +149° (*c* 0.1, EtOH), lit. [8] $[\alpha]_D^{20}$ +170° (*c* 0.12, EtOH). UV spectrum (MeOH, λ_{max}, nm): 278 (log ε 4.3). Table 2 gives the ^{13}C NMR spectrum. $[M + H]^+$ 813.8, $C_{45}H_{64}O_{13}$, MW = 812.9.

Glycyrrhetic Acid Methyl Ester α-D-Galactopyranoside (4a). Yield 0.20 g (83.3%), mp 266–268°C (EtOH), $[\alpha]_D^{20}$ +185° (*c* 0.06, EtOH), lit. [8] mp 265–267°C. IR spectrum (ν, cm^{-1}): 3600–3200 (OH), 1740 (COOMe), 1660 (C_{11}=O). UV spectrum (MeOH, λ_{max}, nm): 248 (log ε 4.2).

PMR spectrum (300 MHz, $CD_3OD + CDCl_3$, δ, ppm, J/Hz): 0.70 (3H, s, H-28), 0.78 (3H, s, H-24), 0.95 (3H, s, H-23), 1.04 (3H, s, H-26), 1.08 (3H, s, H-29), 1.26 (3H, s, H-27), 2.63 (1H, s, H-9), 3.18 (2H, m, H-6′), 3.62 (H-2′), 3.65 (3H, s, OCH_3), 3.94 (H-4′, H-5′), 4.90 (1H, d, J = 3.1, H-1′), 5.46 (1H, s, H-12).

Table 2 lists the ^{13}C NMR spectrum.

Glycyrrhetic Acid Methyl Ester β-D-Galactopyranoside (5a). Yield 82.5% (amorphous powder), $[\alpha]_D^{20}$ +100° (*c* 1.0, EtOH), lit. [8] $[\alpha]_D^{20}$ +95° (*c* 1.0, $CHCl_3$). UV spectrum (EtOH, λ_{max}, nm): 249 (log ε 4.1). IR spectrum (ν, cm^{-1}): 3400–3200 (OH), 1735 (COOMe), 1660 (C_{11}=O).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm): 0.77 (3H, s, H-28), 0.81 (3H, s, H-24), 1.02 (3H, s, H-23), 1.11 (6H, s, H-26, H-29), 1.22 (3H, s, H-27), 1.33 (3H, s, H-25), 2.72 (1H, s, H-9), 3.16 (2H, m, H-6′), 3.46 (1H, br.s, H-2′), 3.65 (3H, s, OCH_3), 4.05-4.50 (4H, m, H-1′, H-3′, H-4′, H-5′), 5.64 (1H, s, H-12).

Table 2 lists the ^{13}C NMR spectrum.

18,19-Dehydroglycyrrhetic Acid Methyl Ester β-D-Galactopyranoside (8a). Yield 80.3% (yellow amorphous powder), $[\alpha]_D^{20}$ +138° (*c* 0.05, MeOH), lit. [8] $[\alpha]_D^{20}$ +134° (*c* 0.06, EtOH). UV spectrum (MeOH, λ_{max}, nm): 279 (log ε 4.5). Table 2 lists the ^{13}C NMR spectrum.

Anti-HIV-1 Activity. The anti-HIV-1 activity of the compounds was evaluated using MT-4 cells (2×10^6 cells/mL) infected with strain HIV-1/EVK at 0.2–0.5 infection units per cell. After adsorption of virus for 1 h at 37°C, infected and control cells (without virus) were diluted with growth culture medium to an innoculation concentration of 5×10 cells/mL and placed in a 96-well culture plate. Then, solutions of the tested compounds were placed into the appropriate wells (three wells for each dilution). The final concentration of the tested preparations in the cell suspension was from 0.1 to 100 μg/mL.

The cytotoxicity of the tested compounds was evaluated using a culture of transplanted human T-lymphocytes (MT-4 line). The preparations were dissolved in DMSO and placed at the appropriate dilutions into 96-well plates (three for each dilution) during cell sorting. The fraction of living cells in the Goryaev chamber was counted after the incubation by dying with trypan blue. Dose-dependent curves were constructed. The concentration of compound causing death of 50% of the cells, CD_{50} (toxic dose), was determined.

The inhibiting effect of the compounds was estimated on the fourth day of incubation by measuring the amount of virus antigen, virus-specific protein p24, by an immuno-enzyme method. Furthermore, the fraction of living cells was determined after dying with trypan blue by counting in the Goryaev chamber. Dose-dependent curves were constructed based on the experimental results. The quantitative inhibition characteristics were determined. ID_{50} was the compound concentration suppressing by 50% production of virus or providing 50% protection of cells from death as a result of the infection. ID_{90} was the compound concentration that suppressed by 90% production of virus or provided 90% protection of cells from death as a result of infection. IS was the index of selectivity or the ratio of the cytotoxic dose CD_{50} to its effective dose ID_{50}.

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