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Synthesis and Immunomodulating Activity of New Glycopeptides of Glycyrrhizic Acid Containing Residues of *L*-Glutamic Acid

R. M. Kondratenko[a], L. A. Baltina, Jr.[b], L. A. Baltina[b,1] N. Zh. Baschenko[b], and G. A. Tolstikov[c]

[a] *Bashkortostan State Medical University, Ufa, Bashkortostan, Russia*
[b] *Institute of Organic Chemistry, Bashkir Research Center, pr. Oktyabrya 71, Ufa, 450054 Bashkortostan, Russia*
[c] *Vorozhtsov Institute of Organic Chemistry, Siberian Division, Russian Academy of Sciences, Novosibirsk, Russia*

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Abstract—New glycopeptides of glycyrrhizic acid (GA) containing Glu residues and their α-methyl esters, γ-methyl esters, and α,γ-dimethyl esters were synthesized using *N,N'*-dicyclohexylcarbodiimide in the presence of *N*-hydroxybenzotriazole or *N*-hydroxysuccinimide. Formation of amide bonds was observed on all the three COOH groups of GA, or selectively on the COOH groups of the GA carbohydrate part in dependence on the ratio of reagents and the reaction conditions. The GA glycopeptide with three residues of Glu(OH)-OMe at a dose of 2 mg/kg stimulated the production of antibody-forming cells in mouse spleen in comparison with the control. The GA glycopeptide containing Glu residues only in the GA carbohydrate part turned out to be an immunosuppressor. The glycopeptide of the 30-methyl ester of GA with residues of free Glu in its carbohydrate part increased the hemagglutinine titer at oral doses of 2 and 10 mg/kg. All the studied compounds had practically no effect on the delayed-type hypersensitivity in mice.

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Key words: glutamic acid, glycopeptides, glycyrrhizic acid, immunomodulating activity

INTRODUCTION

Derivatives of glycyrrhizic acid, a triterpene glycoside from basic saponin of liquorice (*Glycyrrhiza glabra* L. and Uralian *Glycyrrhiza uralensis* Fisher) containing residues of amino acids or peptides are promising immunomodulators with the properties interesting for medicine [1–5].[2]

In this paper, we describe synthesis of new GA glycopeptides (**II**)–(**IX**) containing the residues of glutamic acid or its esters.

RESULTS AND DISCUSSION

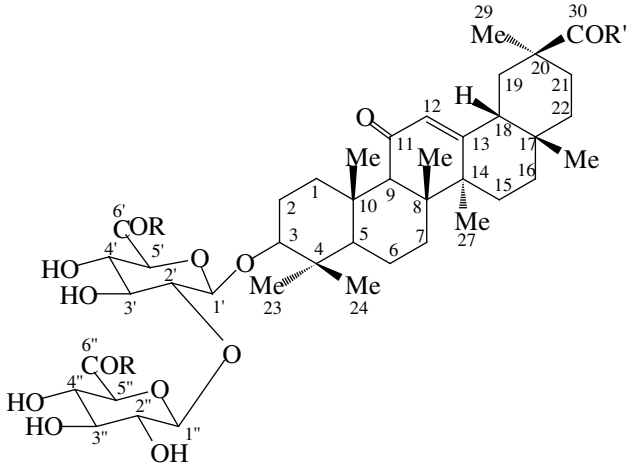
Glycopeptide (**II**) was prepared in 72% yield by coupling of *L*-glutamic acid α-methyl, γ-benzyl ester hydrochloride (4 mmol) with tris-*N*-hydroxybenzotriazole ester of GA, formed by activation of the GA (**I**) carboxyl groups by DCC in the presence of HOBt at 1 : 4 : 3 GA–HOBt–DCC molar ratio, in THF at 0–+5°C in the presence of triethylamine (7 mmol) at 20–22°C.

Hydrogenolysis of (**II**) in 75% acetic acid in the presence of 10% Pd/C gave glycopeptide (**III**) containing three residues of α-methyl ester of glutamic acid. The crude product was purified by column chromatography on silica gel and was finally obtained in 62% yield.

Glycopeptides (**IV**), (**VI**), (**VII**), (**VIII**), and (**IX**) containing the residues of amino acid esters only in the GA carbohydrate part were predominantly formed after activation of the GA carboxyl groups by DCC/HONSu in THF or dioxane at 0–+5°C and 1 : 4–5 : 2.5–3.0 GA–HONSu–DCC molar ratio [6, 7]. The compounds containing free C30-COOH group in the triterpene part of the molecule were separated to homogeneous compounds (TLC) by the column chromatography on silica gel in 50–55% yields. The selective formation of CONH bonds from the carboxyl groups of the GA carbohydrate part was confirmed by preservation of the value of chemical shift of the resonance from C30 aglycone atom in the ^{13}C NMR spectra of these compounds (the same chemical shift (180 ppm) was observed for the GA molecule) [8, 9]. GA derivative (**V**) with two Glu(OH)-OMe residues was prepared by the selective hydrogenolytic deprotection of γ-COOH in (**IV**) in 75% acetic acid in the presence of 10% Pd/C and by the subsequent hydrolysis of glycopeptide (**VI**) with TFA.

[1] Corresponding author; phone: (3472) 35-5288; e-mail: baltina@anrb.ru.

[2] Abbreviations: AFC, antibody-forming cells; DTH, delayed-type hypersensitivity; CC, column chromatography; GA, glycyrrhizic acid; and ShE, sheep erythrocytes.



(**I**) R' = R = OH
(**II**) R' = R = Glu(OBzl)-OMe
(**III**) R' = R = Glu(OH)-OMe
(**IV**) R' = OH; R = Glu(OBzl)-OMe
(**V**) R' = OH; R = Glu(OH)-OMe
(**VI**) R' = OH; R = Glu(OBu^t)-OMe
(**VII**) R' = OH; R = Glu(OMe)-OMe
(**VIII**) R' = OH; R = Glu(OMe)-OH
(**IX**) R' = OH; R = Glu(OBu^t)-OH
(**X**) R' = OH; R = Glu(OH)-OH
(**XI**) R' = OMe; R = Glu(OBzl)-OBzl
(**XII**) R' = OMe; R = Glu(OH)-OH

The removal of *tert*-Bu group by the treatment of (**IX**) with TFA gave glycopeptide (**X**) with free carboxyls of the *L*-Glu residues. Glycopeptide (**XII**) was synthesized by DCC/HOBt coupling of C30-methyl ester of GA [5] with *L*-glutamic acid dibenzyl ester hydrochloride, followed by hydrogenolysis and column chromatography on silica gel.

The structures of all the synthesized compounds were confirmed by spectral methods (IR, UV, ^{1}H NMR, and ^{13}C NMR spectroscopy) and elemental analysis (Tables 1, 2). The assignment of C8 and C14 resonances atoms in the ^{13}C NMR spectra of the new GA glycopeptides was based on a high-resolution ^{13}C NMR spectra of GA and its esters [9].

The residues of Glu and its esters were identified in the ^{13}C NMR spectra by the additional resonances of the carbon atoms in low field (172–176 ppm) (COOH, COOR). Methoxycarbonyl groups and α-carbon atoms of Glu formed a group of resonances in the area of 52–53 ppm.

The effects of glycopeptides (**III**) and (**X**) on the production of AFC to ShE in sterile mice were studied. The examined substances were tested by a single intraperitoneal injection to mice at a dose of 2.0 mg/kg one day after their immunization. The AFC number was determined for the whole spleen calculated per 10^6 splenocytes. Isotonic solution was injected to control animals.

Glycopeptide (**III**) stimulated the AFC production in comparison with control (Table 3). Glycopeptide (**X**) containing Glu residues only in the carbohydrate part proved to be an immunosuppressor (the relative AFC number was three times lower in the mice spleen in comparison with control).

The effect of GA derivatives (**III**) and (**X**) on the functional capacity of T-effector cells was studied in the DTH model. The intensity of DTH was determined as a difference in the masses of the control and experimental legs of mice and expressed in percents. The examined compounds exerted practically no effect on the cell-mediated immunity, although (**III**) slightly inhibited the DTH reaction.

The immunotropic activity of glycopeptide (**XII**) was studied at two doses: 10 mg/kg administration for 7 days and 2 mg/kg for 14 days, as earlier described [7]. The glycopeptide was orally introduced. The response of immune system was evaluated according to the value of $\log_2$ titers of hemagglutinine antibodies (Table 4).

Compound (**XII**) exerted a statistically reliable increase in the hemagglutinine level after 7 days (see Table 4). In the case of 14-days introduction, the agglutinin titer twofold increased in control. The titer of experimental group somewhat exceeded that in control and was reliably higher than that obtained after the 7-day introduction of (**XII**) despite the use of its lower dose. This GA derivative had no influence on the cell-mediated immunity (Table 5).

Thus, the effect of GA glycopeptides containing Glu residues and their methyl esters on humoral immunity depends on the structure of molecules and the way of their introduction.

EXPERIMENTAL

Melting points were determined on a Boetius microplate. IR spectra were recorded on a Specord M80 in a paste with Vaseline oil. UV spectra were recorded on a Specord M400 in methanol. The optical rotation was measured on a Perkin-Elmer 214 MC polarimeter in a tube of 1 dm in length. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-300 spectrometer (300 and 75.5 MHz) in CD_3OD in δ-scale with tetramethylsilane as an internal standard; chemical shifts are given in ppm and spin-coupling constants, in Hz.

TLC was carried out on Silufol plates (Czech Republic) or Sorbfil plates (ZAO Sorbpolimer) in the chromatographic system 45 : 10 : 1 chloroform–methanol–water. The substances were detected by the treatment with 20% solution of phosphotungstic acid in ethanol with the subsequent heating at 110–120°C for 2–3 min.

Table 1. Parameters of the ^{13}C NMR spectra of GA glycopeptides (aglycone and carbohydrate parts) (CD_3OD, δ, ppm, 25°C, 75.5 MHz)

Number of carbon atom	(**III**)*	(**IV**)	(**V**)	(**VII**)	(**VIII**)	(**X**)	(**XII**)
1	39.42	40.38	40.33	40.38	40.16	40.33	40.57
2	26.50	27.67	27.62	27.62	27.47	27.35	27.49
3	89.13	90.60	90.59	90.71	90.96	90.90	90.45
4	39.64	40.70	40.67	40.68	40.50	40.78	40.75
5	55.22	56.55	56.39	56.51	56.34	56.79	56.45
6	18.70	18.51	18.45	18.45	18.29	18.42	18.38
7	32.81	33.90	33.83	33.82	33.68	33.82	33.60
8	45.37	46.78	46.68	46.76	46.72	46.79	46.76
9	61.82	63.18	63.06	63.16	63.04	63.18	63.14
10	36.89	38.13	38.01	38.08	37.90	38.13	38.06
11	200.00	202.34	202.42	202.48	202.20	202.58	202.50
12	128.51	129.03	129.10	129.24	128.69	128.97	129.03
13	171.48	171.45	172.47	171.86	172.41	171.74	171.19
14	43.18	44.59	44.54	44.89	44.54	44.64	44.57
15	27.47	27.75	27.87	27.88	27.47	27.61	27.64
16	27.68	28.22	28.42	28.21	28.22	28.13	28.22
17	31.88	33.01	32.87	32.97	32.88	33.10	32.90
18	47.36	–	–	–	–	–	–
19	41.81	42.52	42.48	42.46	42.24	42.47	42.65
20	43.66	44.92	44.80	45.08	44.90	44.94	44.64
21	31.43	32.21	32.01	32.14	31.89	32.04	32.06
22	37.62	38.50	38.69	39.02	38.88	39.05	38.79
23	28.45	28.54	28.63	28.48	28.22	28.80	28.22
24	16.46	17.02	17.09	17.05	16.99	16.54	16.98
25	17.50	17.22	17.31	17.30	17.23	17.06	17.12
26	19.38	19.45	19.38	19.37	19.25	19.35	19.36
27	23.31	23.91	23.86	23.93	23.80	23.68	23.76
28	29.36	28.87	29.18	28.85	28.77	28.80	29.26
29	29.87	29.23	29.32	29.21	29.17	29.23	29.43
30	175.88	180.31	178.97	180.35	180.18	180.39	177.72
31							51.50
1'	104.00	105.05	105.03	105.02	105.00	105.28	105.62
2'	80.80	82.15	82.24	81.72	83.07	84.17	84.08
3'	76.02	76.59	76.20	76.25	76.98	77.32	77.13
4'	72.90	73.31	73.22	73.29	72.98	72.99	72.84
5'	76.25	77.63	77.62	77.66	77.49	77.70	77.54
6'	170.22	171.57	171.37	171.61	171.46	169.84	168.33
1"	104.46	105.32	105.11	105.08	105.58	106.17	106.29
2"	75.09	76.03	75.92	75.94	75.79	73.57	74.64
3"	74.07	76.09	75.92	76.18	76.03	75.82	76.28
4"	72.98	73.88	73.42	73.51	73.42	72.47	73.01
5"	77.57	77.32	77.09	77.18	77.18	77.38	77.37
6"	170.67	171.89	171.57	171.50	171.46	170.08	170.72

* $CDCl_3 + C_5D_5N$.

Table 2. Chemical shifts of C atom signals of the amino acid moieties in the ^{13}C NMR spectra of GA glycopeptides (CD_3OD, δ, ppm, 25°C, 75.5 MHz)

Amino acid fragments in the compounds	C1	C2	C3	C4	C5	C6
$HOOC^6CH_2^5CH_2^4CH^3(NH—)COO^2CH_3^1$ (III)	51.63 51.52 51.16	172.72 172.61 171.74	52.47 52.31 52.02	27.62 27.45 26.57	31.25 31.11 30.76	174.86 174.73 173.47
$HOOC^6CH_2^5CH_2^4CH^3(NH—)COO^2CH_3^1$ (V)	52.43 52.37	173.73 173.08	53.13 52.86	27.84 27.63	31.25 31.11	174.95 174.52
$CH_3^6OOC^5CH_2^4CH_2^3CH^2(NH—)COO^1CH_3$ (VII)*	52.99 52.82	173.32 173.19	53.11 53.02	27.28 26.30	31.29 30.79	176.02 174.77
$CH_3^6OOC^5CH_2^4CH_2^3CH^2(NH—)COOH^1$ (VIII)	173.42 172.97	52.32 52.50	53.26 53.10	27.23 26.89	31.31 31.15	176.77 175.25
$HOOC^5CH_2^4CH_2^3CH^2(NH—)COOH^1$ (X)	173.16 172.90	51.85 51.70	27.07 26.63	37.59 36.22	174.98 174.76	
$HOOC^5CH_2^4CH_2^3CH^2(NH—)COOH^1$ (XII)	172.59 172.30	52.70 52.50	26.21 26.44	33.88 33.73	174.97 174.42	

* C7 (52.59; 52.40).

Triethylamine and *N*-ethylmorpholine were kept for one day over KOH and distilled. THF and dioxane were purified by the procedure [10].

GA was prepared by the procedure [8] and had the purity of approximately 95%. DCC (Aldrich), *L*-glutamic acid γ-*tert*-butyl ester, γ-methyl ester, and γ-benzyl ester (Reanal, Hungary) were used in this study. 30-Monomethyl ester of GA and *L*-glutamic acid dibenzyl ester hydrochloride were prepared according to the procedures [5] and [11], respectively.

***L*-Glutamic acid α-methyl, γ-benzyl diester hydrochloride.** A freshly distilled thionyl chloride (10 ml) was added to a solution of *L*-glutamic acid γ-benzyl ester (5 g) in anhydrous methanol (300 ml) at 0 to +5°C. The reaction mixture was stirred under cooling until the formation of a solution and for 6 h at 20–22°C. The solvent and thionyl chloride excess were removed in a vacuum. The syrup-like residue was triturated with ether. The obtained powder was filtered and two times recrystallized from a mixture of methanol–diethyl ether to get the title compound; yield 5.6 g (92%); mp 187–189°C. $[\alpha]_D^{20}$ +21.9° (*c* 0.05, ethanol); IR spectrum (ν, cm^{-1}); 3150 (NH), 1730 (COOR), 1680 (C=O), 1610 (C_6H_5), 1580 (NH_3^+), 1510 (C_6H_5). Found, %: C 54.28, H 6.74, N 4.76. Calc. for $C_{13}H_{18}O_4NCl$, %: C 54.17, H 6.29, N 4.79.

Table 3. Effect of GA glycopeptides (**III**) and (**X**) on the AFC production and DTH reaction in mice

Compound	AFC number in the whole spleen	AFC number per 10^6 splenocytes	DTH (%) of edema of the mouse paw
(**III**)	17393 ± 3423	2299 ± 448*	10.4 ± 2.0
(**X**)	3640 ± 605	443 ± 109*	17.0 ± 4.4
Control	15039 ± 2635	1392 ± 301	16.7 ± 4.0

* Reliable in comparison with control ($p < 0.05$).

***L*-Glutamic acid α-methyl, γ-*tert*-butyl diester.** A solution of diazomethane in ether was added to a solution of γ-*tert*-butyl ester of *L*-glutamic acid (5.0 g) in methanol (100 ml) at 0 to +5°C to a stable yellow color. The reaction mixture was kept for 6 h at 20–22°C, and several drops of glacial acetic acid were added. The reaction mixture was evaporated in a vacuum at 30–35°C to dryness. The residue was dissolved in ethyl acetate (50 ml) and washed with 1 N $NaHCO_3$ (3 × 50 ml) and water. The organic phase was dried with $MgSO_4$ and evaporated. The residue was recrystallized from an ethyl acetate–hexane mixture; yield 91%; mp 135–136°C; $[\alpha]_D^{20}$ +17° (*c* 0.03, methanol) (lit. mp 135–136.5°C and $[\alpha]_D^{20}$ +21.7° (*c* 2%, methanol) [11]).

3-*O*-{2-*O*-[*N*-(β-*D*-Glucopyranosyluronoyl)-*L*-glutamic acid α-methyl ester]-*N*-(β-*D*-glucopyranosyluronoyl)-*L*-glutamic acid α-methyl ester]-(3β, 20β)-11-oxo-30-norolean-12-en-30-oyl-(*L*-glutamic acid α-methyl ester) (III). 1. HOBt (0.54 g, 4 mmol) and DCC (0.70 g, 3.2 mmol) were added to a solution of GA (0.82 g, 1 mmol) in dioxane (20 ml) at 0 to +5°C. The reaction mixture was stirred for 1 h at 0 to +5°C and for 6 h at 20–22°C. The precipitate of *N,N'*-dicyclohexylurea was filtered off. The filtrate was cooled on ice bath, and hydrochloride of *L*-Glu(OBzl)-OMe (1.10 g, 4 mmol) and triethylamine (1.0 ml, 7.2 mmol) were added. The reaction mixture was kept under occasional stirring at 20–22°C for 24 h, diluted with cool water, and acidified to pH 4 with citric acid. The precipitated glycopeptide (**II**) was filtered, washed with water, and dried. Recrystallization from an acetone–hexane mixture gave 1.10 g (72%) of product; R_f 0.73; mp 145–148°C; IR spectrum, (ν, cm^{-1}): 3600–3200 (OH, NH), 1740 (COOR), 1650 (C11=O), 1550 (CONH), 1500 (Ph); UV spectrum, [λ_{max}, nm, ($\log\varepsilon$)]: 249 (4.20). Found, %: N 2.79. Calc. for $C_{81}H_{107}O_{25}N_3$, %: N 2.76.

2. The protected glycopeptide (**II**) (0.90 g, 0.6 mmol) was subjected to hydrogenolysis in 75% acetic acid (50 ml) in the presence of 10% Pd/C for 24 h. The catalyst was filtered off; the filtrate was evaporated; and the residue was fractionated on a silica gel column eluted with a $CHCl_3$–MeOH–H_2O mixture at the following volume ratios: 100 : 10 : 1, 50 : 10 : 1, and 25 : 10 : 1. The homogenous product (**III**) was eluted with a 50 : 10 : 1 $CHCl_3$–CH_3OH–H_2O mixture; yield 0.46 g (62%); R_f 0.5; $[\alpha]_D^{20}$ +35° (*c* 0.02, methanol); IR spectrum, (ν, cm^{-1}): 3600–3200 (OH, NH), 1740 (COOMe), 1660 (C11=O), 1540 (CONH); UV spectrum, [λ_{max}, nm, ($\log\varepsilon$)]: 249 (4.02); ^{1}H NMR (CD_3OD, δ, ppm): 0.74, 1.06, 1.08, 1.15, 1.18, 1.44 (21 H, 7 CH_3), 3.74, 3.64 (6 H, both s, OCH_3), 3.30 (3 H, s, OCH_3), 4.56, 4.72 (2 H, H1', H1"), 5.56 (1 H, s, 12-H), 7.90 (1 H, broadened s, NH); ^{13}C NMR spectrum is given in Tables 1, 2. Found, %: N 3.53. Calc. for $C_{60}H_{89}O_{25}N_3$, %: N 3.36.

3-*O*-{2-*O*-[*N*-(β-*D*-Glucopyranosyluronoyl)-*L*-glutamic acid α-methyl ester]-*N*-(β-*D*-glucopyranosyluronoyl)-*L*-glutamic acid α-methyl ester}-(3β,20β)-11-oxo-30-norolean-12-en-30-acid (V). 1. HONSu (0.60 g, 5.2 mmol) and DCC (0.65 g, 3 mmol) were added to a solution of GA (0.82 g, 1 mmol) in THF (20 ml) at 0 to +5°C. The reaction mixture was stirred for 2 h at 0 to +5°C and kept overnight in a fridge. The precipitated *N,N'*-dicyclohexylurea was filtered off. Hydrochloride of *L*-Glu(OBzl)-OMe (0.82 g, 3 mmol) and triethylamine (0.7 ml, 5 mmol) were added to the filtrate at 0 to +5°C. The reaction mixture was stirred under cooling for 1 h and at 20–22°C for 20 h, diluted with cool water, and acidified with citric acid to pH 4. The precipitate was filtered, washed with water, and dried. The product was fractionated on a silica gel column eluted with $CHCl_3$–MeOH–H_2O mixtures at the following volume ratios: 200 : 10 : 1, 100 : 10 : 1, and 50 : 10 : 1. The fractions homogenous according to TLC were combined and evaporated. Yield of (**IV**) 0.69 g (53%); $[\alpha]_D^{20}$ +60° (*c* 0.02, methanol); IR spectrum (ν, cm^{-1}): 3600–3200 (OH, NH), 1740 (COOR), 1650 (C11=O), 1550 (CONH), 1500 (Ph); UV spectrum, [λ_{max}, nm, ($\log\varepsilon$)]: 248 (4.2); ^{1}H NMR (CD_3OD, δ, ppm): 0.74, 1.04, 1.04, 1.10, 1.12, 1.16, 1.44 (21 H, 7CH_3), 3.64, 3.74 (6 H, both s, 2OCH_3), 5.70 (1 H, s, C12-H). Found, %: N 2.19. Calc. for $C_{70}H_{94}O_{22}N_2$, %: N 2.12. The following impurities were isolated and identified by TLC by a comparison with the known samples: (**II**) (20%) and the starting GA (approximately 10%).

2. Glycopeptide (**VI**) was prepared a described for (**IV**) from GA (0.82 g, 1 mmol), HONSu (0.60 g, 5.2 mmol), DCC (0.65 g, 3 mmol), *L*-Glu(OBu^t)-OMe hydrochloride (0.52 g, 3 mmol), and *N*-ethylmorpholine (0.6 ml, 5 mmol) in THF (20 ml). The crude product (0.90 g) was purified by precipitation from methanol with ether; yield 0.68 g (55%); IR spectrum (ν, cm^{-1}): 3600–3200 (OH, NH), 1740 (COOR), 1670 (C11=O), 1540 (CONH). Found, %: N 2.35. Calc. for $C_{62}H_{96}O_{22}N_2$, %: N 2.29.

Table 4. Effect of GA glycopeptide (**XII**) on the hemagglutinin titer of the sensitized mice

Compound	After 7 days (10 mg/kg)	After 14 days (2 mg/kg)
(**XII**)	1.0 ± 0.2*	1.5 ± 0.1*
Control	0.4 ± 0.2	0.8 ± 0.2

* Reliable in comparison with the control ($p < 0.05$).

Table 5. Effect of GA glycopeptide (**XII**) on the DTH reaction in mice sensitized for 7 and 14 days

Compound	Seven days (10 mg/kg)		Forteen days (2 mg/kg)	
	difference in the masses of paws, mg	increase in the paw volume, %	difference in the masses of paws, mg	increase in the paw volume, %
(**XII**)	8.40 ± 1.05	7.56 ± 1.05	9.60 ± 1.21	8.24 ± 1.00
Control	8.14 ± 0.45	7.59 ± 0.73	8.14 ± 0.45	7.59 ± 0.73

3a. The protected glycopeptide (**IV**) (0.65 g, 0.5 mmol) was hydrogenolyzed in 75% acetic acid (30 ml) in the presence of 10% Pd/C for 24 h. The catalyst was filtered off, and the filtrate was evaporated. The residue was fractionated on a silica gel column eluted with $CHCl_3$–MeOH–H_2O mixtures at the following volume ratios: 100 : 10 : 1, 50 : 10 : 1, and 25 : 10 : 1. The homogenous (TLC) product (**V**) was eluted with 50 : 10 : 1 $CHCl_3$–MeOH–H_2O mixture; yield 0.45 g (73%); R_f 0.45. $[\alpha]_D^{20}$ +47° (*c* 0.02, methanol); IR spectrum (ν, cm^{-1}): 3600–3200 (OH, NH), 1740 (COOMe), 1660 (C11=O), 1540 (CONH); UV spectrum, [λ_{max}, nm, ($\log\varepsilon$)]: 249 (4.0); ^{13}C NMR spectrum is given in Tables 1, 2. Found, %: N 2.55. Calc. for $C_{54}H_{80}O_{22}N_2$, %: N 2.52.

3b. The protected glycopeptide (**VI**) (0.60 g, 0.5 mmol) was dissolved in TFA (5 ml), kept for 30 min at 20–22°C, and evaporated to dryness with anhydrous benzene (3 × 10 ml). The residue was purified on a column as described above; yield of (**V**) was 0.38 g (69%); $[\alpha]_D^{20}$ +45° (*c* 0.02, methanol). This sample was identical to that described above.

Compound (VII) was prepared as described for (**V**) from GA (1 mmol), HONSu (5 mmol), DCC (2.5 mmol), *L*-Glu(OMe)-OMe hydrochloride (2.5 mmol), and triethylamine (5 mmol) in THF (20 ml) yield of 0.62 g (55%); R_f 0.5. $[\alpha]_D^{20}$ +43 ± 2° (*c* 0.03, methanol); IR spectrum (ν, cm^{-1}): 3600–3200 (OH, NH), 1740 (COOMe), 1660 (C11=O), 1550 (CONH); UV spectrum, [λ_{max}, nm, ($\log\varepsilon$)]: 249 (4.0); ^{13}C NMR spectrum is given in Tables 1 and 2. Found, %: N 2.55. Calc. for $C_{56}H_{84}O_{22}N_2$, %: N 2.46.

Compound (VIII) was prepared as described for (**V**) from GA (1 mmol), HONSu (3 mmol), DCC (2.5 mmol), and *L*-Glu(OMe)-OH (3 mmol) in dioxane (20 ml) in the presence of triethylamine (5 mmol) in yield of 0.54 g (49%). Glycopeptide (**VIII**) was twice purified by the column chromatography on silica gel and was homogeneous by TLC; R_f 0.42. $[\alpha]_D^{20}$ +62° (*c* 0.04, methanol); IR spectrum (ν, cm^{-1}): 3600–3200 (OH, NH), 1740 (COOMe), 1660 (C11=O), 1530 (CONH); UV spectrum, [λ_{max}, nm, ($\log\varepsilon$)]: 248 (3.9); ^{13}C NMR spectrum is given in Tables 1, 2. Found, %: N 2.60. Calc. for $C_{54}H_{80}O_{22}N_2$, %: N 2.52.

Compound (X). 1. Compound (**IX**) was synthesized as described for (**V**) from GA (1 mmol), HONSu (4 mmol), DCC (2.5 mmol), *L*-Glu(OBu^t)-OH (3 mmol), and triethylamine (5 mmol) in THF (20 ml) in yield of 0.60 g (51.5%); IR spectrum (ν, cm^{-1}): 3600–3200 (OH, NH), 1740 (COOBu^t), 1660 (C11=O), 1540 (CONH); UV spectrum, [λ_{max}, nm, ($\log\varepsilon$)]: 249 (3.9). Found, %: N 2.42. Calc. for $C_{60}H_{82}O_{22}N$, %: N 2.37.

2. Glycopeptide (**IX**) (0.56 g, 0.5 mmol) was treated with TFA (10 ml) as described for (**V**) and (**X**) was purified by column chromatography to homogenous state according to TLC; yield of 0.36 g (63%); **X**). R_f 0.4. $[\alpha]_D^{20}$ +49° (*c* 0.02, methanol); IR spectrum (ν, cm^{-1}): 3600–3200 (OH, NH), 1710 (COOH), 1660 (C11=O), 1530 (CONH); ^{13}C NMR spectrum is given in Tables 1, 2.

Found, %: N 2.62. Calc. for $C_{52}H_{76}O_{22}N_2$, %: N 2.59.

Compound (XII) was prepared as described for (**II**) from C30-methyl ester of GA (1 mmol), HOBt (3 mmol), DCC (2.5 mmol), and *L*-Glu(OBzl)-OBzl hydrochloride in dioxane (20 ml) in the presence of triethylamine (5 mmol). The crude product (0.78 g) was hydrogenolyzed in 75% acetic acid (30 ml) in the presence of Pd/C for 12 h. The solvent was evaporated. The residue was twice purified on a column as described above for (**VI**); yield of (**XII**) was 0.50 g (37%); R_f 0.42. $[\alpha]_D^{20}$ +38° (*c* 0.028, methanol); IR spectrum (ν, cm^{-1}): 3600–3200 (OH, NH), 1705 (COOH), 1650 (C11=O), 1530 (CONH); UV spectrum, [λ_{max}, nm, ($\log\varepsilon$)]: 248 (3.85); ^{13}C NMR spectrum is given in Tables 1, 2.

Found, %: N 3.07. Calc. for $C_{53}H_{78}O_{22}N_2$, %: N 2.56.

Effects of the GA glycopeptides on immune system of white outbred mice was evaluated according to a change in the humoral immune response (production of AFC) by the method of Jerne and Nordin [12] modified by Cunningham [13] and according to a change in the cell-mediated immunity on the DTH model with the use of ShE as test antigens. The examined substances were intraperitoneally injected to the animals one time one day after the immunization at the dose of 2 mg/kg. The number of AFC was determined on the seventh day visually in the whole spleen according to the number of hemolysis zones and calculated per 10^6 splenocytes.

The influence of glycopeptide (**XII**) on the primary immune response was evaluated according to the level of hemagglutinines in the mice blood by the method [14]. An examined compound was introduced to the animals per os beginning from the first day of sensitization of ShE at doses of 10 mg/kg (the introduction for 7 days) and 2 mg/kg (the introduction for 14 days). The isotonic solution was introduced to control animals. On the 7th and 14th days of the sensitization, the resolving dose of ShE at a concentration of 10^8 was subplantary injected into the right paw of the mice of each group ($n = 7$). Another paw remained intact. One day later, the animals were decapitated, their blood was taken, and hemagglutinines were determined in the blood serum. The reaction was evaluated according the value of $\log_2$ antibody titers.

Effect of compounds (**III**), (**X**), and (**XII**) on the cell-mediated immunity was studied in the DTH reaction to ShE. The mice were sensitized by the intravenous injection of ShE at the stimulating dose (2×10^6) and by the subplantary injection at the resolving dose (2×10^8) of ShE [15]. One day after the last injection,

the intensity of DTH reaction was determined according to an increase in the mass and % volume of the mouse paw. The experimental results are given in Tables 3–5.

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