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Synthesis of Amino Acid Conjugates of Glycyrrhizic Acid Using *N*-Hydroxyphthalimide and *N*,*N*′-Dicyclohexylcarbodiimide

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Abstract—Synthesis of amino acid conjugates of glycyrrhizic acid with the use of *N*-hydroxyphthalimide, *N*,*N*′-dicyclohexylcarbodiimide, and *tert*-butyl esters of L-amino acids (valine, isoleucine, phenylalanine, and methionine) was performed followed by deprotection with trifluoroacetic acid. The target amino acid conjugates were isolated by column chromatography on silica gel in 40–45% yield. The structure of the prepared compounds was confirmed by IR and ^{13}C NMR spectroscopy.

Keywords: glycyrrhizic acid, amino acids *tert*-butyl esters, conjugates, *N*-hydroxyphthalimide, *N*,*N*-dicyclohexylcarbodiimide
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Glycyrrhizic acid **1** is the main triterpene glycoside of roots licorice (*Glycyrrhiza glabra L.* and *Glycyrrhiza uralensis Fisher*) that belongs to the natural glycosides with promising properties for medicine for designing new immunomodulators and antiviral agents [1–5]. A number of conjugates of glycyrrhizic acid with amino acids and dipeptides possesses meaningful anti-AIDS-1 activity [6, 7], and is of interest as anti-SARS CoV-agents [8] and inhibitors of Epstein-Barr virus [9]. It was found recently that some amino acid conjugates of glycyrrhizic acid possess significant virus inhibition activity towards pandemic influenza virus A/H1N1/pdm2009 [10, 11]. Earlier we developed methods for the preparation of glycyrrhizic acid conjugates with alkyl and benzyl (4-nitrobenzyl) esters of amino acids with the use of *N*-hydroxybenzotriazole (HOBt), *N*,*N*-dicyclohexylcarbodiimide (DCC), or *N*-hydroxysuccinimide (HOSu) that allowed synthesis of the derivatives of glycyrrhizic acid with two or three amino acid residues or their esters depending on the reaction conditions [4, 5, 12, 13].

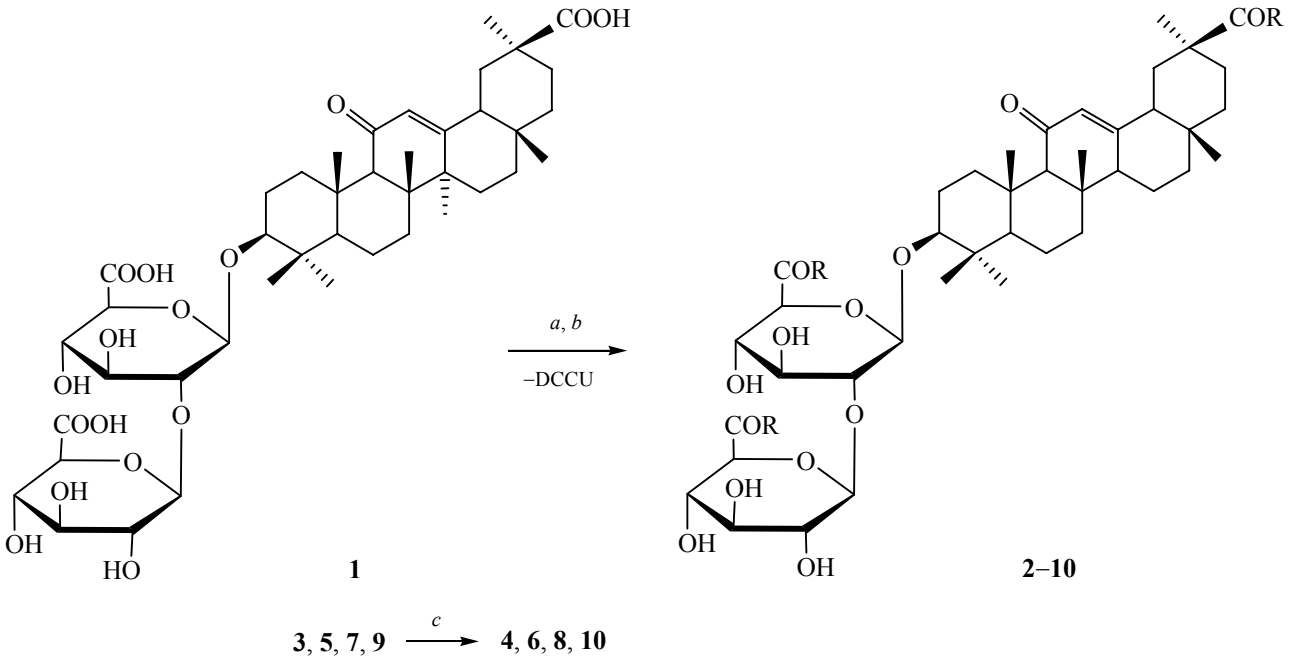
N-Hydroxyphthalimide (HOPt) has been also applied as a nucleophilic reagent at the formation of the amide bond in the carbodiimide mediated synthesis of peptides [14]; but its use in the synthesis of amino acid conjugates of glycyrrhizic acid has not yet been reported.

The goal of the present work was the synthesis of amino acid conjugates of glycyrrhizic acid with the use of HOPt and DCC and *tert*-butyl esters of L-amino acids for activation of carboxy groups of the glycoside molecule.

The condensation of glycyrrhizinic acid **1** with amino acids was performed in two steps by conversion of it to activated ester **2** using HOPt and DCC in dioxane or tetrahydrofuran at the ratio **1** : HOPt : DCC = 1 : (3–3.5) : (3–3.5). After separation of precipitated *N*,*N*-dicyclohexylurea (DCCU), a solution of ester **2** was brought into the reaction with amino components, L-amino acids *tert*-butyl esters hydrochlorides, in the presence of an excess of tertiary amine as a base (triethylamine) at room temperature (20–22°C) for 24 h (Scheme 1). Carboxyl-protected conjugates **3**, **5**, **7**, and **9** were formed in 65–70% yield; their physicochemical characteristics were the same as described before [5].

Deprotection of the products obtained was performed by treating with trifluoroacetic acid in methylene chloride at 20–22°C. Free amino acid con-

Scheme 1.



a, HOPt, DCC; *b*, HA, Et_3N; *c*, CF_3COOH; HA = L-ValOBu^t·HCl, L-IleOBu^t·HCl, L-PheOBu^t·HCl, L-MetOBu^t·HCl. R = 2-oxy-1,3-dioxoisoindolyl (**2**), L-ValOBu^t (**3**), L-ValOH (**4**), L-IleOBu^t (**5**), L-IleOH (**6**), L-PheOBu^t (**7**), L-PheOH (**8**), L-MetOBu^t (**9**), L-MetOH (**10**).

jugates **4**, **6**, **8**, and **10** were isolated by column chromatography on silica gel in 40–45% yields.

The structure of the prepared compounds was confirmed by IR and ^{13}C NMR spectroscopy and by the comparison of their properties with those of the model compounds prepared by the described methods [5, 12, 15]. Thus, in IR spectra of conjugates **4**, **6**, **8**, and **10** the absorption maxima of OH and NH groups (3600–3200 cm^{-1}) and CONH fragment (1530–1550 cm^{-1}) were detected. In ^{13}C NMR spectra of conjugates **4**, **6**, **8**, and **10** an additional signals of the carbon of COOH group of the amino acid moiety in a weak field (173–177 ppm), carbon atoms α-CHNH at 53–59 ppm, and another signals of amino acid residues were observed. The data of elemental analyses matched well the calculated values.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded on a Bruker AMX-300 spectrometer operating at 300 and 75.5 MHz. IR spectra were registered on an IR Prestige-21 Shimadzu spectrophotometer as mulls in mineral oil. Optical rotation angles were measured on a Perkin-Elmer 341 polarimeter (l = 1 dm) at 20–22°C (λ_{Na} = 546 nm). Thin layer chromatography was done on Sorbfil plates (by Plc "Sorbpolimer") with the use of a solvent system $CHCl_3$–MeOH–H_2O, 45 : 10 : 1; spots visualization was done with 5% solution of H_2SO_4 in EtOH with following heating at 110–120°C for 2–3 min. Column chromatography was performed on a silica gel (fraction 50–160 mm).

The solvents were purified by the known methods [16]. Solvents were evaporated in a vacuum at 40–45°C. The used glycyrrhizic acid was isolated from the roots of liquorice ural (*Glycyrrhiza uralensis Fisher*) of the Siberian populations [17]. The following chemicals were applied: *N*-hydroxybenzotriazole and *N*,*N*-dicyclohexylcarbodiimide by Sigma Aldrich, hydrochlorides of *tert*-butyl esters of L-amino acids by Chemapol.

General method for the synthesis of glycyrrhizic acid conjugates. *N*-Hydroxyphthalimide (3.0–3.5 mmol) and *N*,*N*-dicyclohexylcarbodiimide (3.0–3.5 mmol) were added to a solution of glycyrrhizic acid (1.0 mmol) in 20 mL of dioxane or THF at 0–5°C and the mixture was stirred for 1 h at this temperature, then at 20–22°C for 4 h. The precipitated dicyclohexylurea was filtered off, and *tert*-butyl ester of amino acid hydrochloride

(4 mmol) and triethylamine (5–6 mmol) were added to the filtrate. The mixture was kept for 24 h at 20–22°C with occasional stirring. Then the mixture was poured in cold 5% solution of $NaHCO_3$, the precipitate was filtered off, washed with water, dried, and re-precipitated from aqueous ethanol to provide carboxyl-protected conjugates **3**, **5**, **7**, and **9** in 60–65% yield. To remove the ester *tert*-butyl group the obtained products (0.6–0.8 g) were dissolved in a mixture of methylene chloride and trifluorocetic acid (1 : 1, 10 mL). The mixture was kept for 1 h at 20–22°C, then evaporated in a vacuum and purified by column chromatography on silica gel eluting with a mixture chloroform–methanol–water (300 : 10 : 1, 200 : 10 : 1, 100 : 10 : 1, 50 : 10 : 1, vol). The individual fractions (by TLC) were combined and evaporated.

3-*O*-{2-*O*-[*N*-(β-D-Glucopyranosyluronoyl)-L-valyl]-*N*-(β-D-glucopyranosyluronoyl)-L-valyl}-(3β,20β)-11-oxo-30-(*N*-carbonyl-L-valyl)-30-norolean-12-ene (4). Yield 40%, R_f 0.52, $[\alpha]_D^{20}$ 40°±1° (*c* = 0.04, EtOH) {$[\alpha]_D^{20}$ 42.5° (*c* = 0.02, MeOH) [15]}. IR spectrum, ν, cm^{-1}: 3600–3200 (OH, NH), 1710 (COOH), 1660 (C^{11}=O), 1540 (CONH). ^{13}C NMR spectrum (CD_3OD), δ_C, ppm: 40.4 (C^1), 27.9 (C^2), 90.3 (C^3), 40.7 (C^4), 56.5 (C^5), 18.2 (C^6), 33.9 (C^7), 46.8 (C^8), 63.2 (C^9), 38.2 (C^{10}), 202.7 (C^{11}), 129.2 (C^{12}), 171.6 (C^{13}), 44.6 (C^{14}), 27.5 (C^{15}), 28.7 (C^{16}), 33.0 (C^{17}), 48.2 (C^{18}), 42.8 (C^{19}), 44.7 (C^{20}), 32.1 (C^{21}), 38.8 (C^{22}), 28.8 (C^{23}), 17.0 (C^{24}), 17.3 (C^{25}), 19.4 (C^{26}), 23.8 (C^{27}), 29.1 (C^{28}), 29.4 (C^{29}), 178.6 (C^{30}), 104.8 ($C^{1'}$), 81.7 ($C^{2'}$), 76.4 ($C^{3'}$), 73.8 ($C^{4'}$), 77.4 ($C^{5'}$), 171.7 ($C^{6'}$), 105.0 ($C^{1''}$), 75.2 ($C^{2''}$), 76.1 ($C^{3''}$), 73.8 ($C^{4''}$), 77.8 ($C^{5''}$), 172.4 ($C^{6''}$); 174.9, 174.2, 172.8, 59.0, 58.9, 58.4, 32.2, 32.1, 31.8, 19.8, 19.6, 19.5, 18.7, 18.5, 18.0 (Val). Found N, %: 3.5. $C_{57}H_{89}N_3O_{19}$. Calculated N, %: 3.8.

3-*O*-{2-*O*-[*N*-(β-D-Glucopyranosyluronoyl)-L-isoleucinyl]-*N*-(β-D-glucopyranosyluronoyl)-L-isoleucinyl}-(3β,20β)-11-oxo-30-(*N*-carbonyl-L-isoleucinyl)-30-norolean-12-ene (6). Yield 42%, R_f 0.50, $[\alpha]_D^{20}$ 42°±1° (*c* = 0.02, MeOH) {$[\alpha]_D^{20}$ 45° (*c* = 0.02, EtOH) [15]}. IR spectrum, ν, cm^{-1}: 3600–3200 (OH, NH), 1720 (COOH), 1660 (C^{11}=O), 1530 (CONH). ^{13}C NMR spectrum (CD_3OD), δ_C, ppm: 40.3 (C^1), 27.6 (C^2), 90.3 (C^3), 40.7 (C^4), 56.4 (C^5), 18.5 (C^6), 33.8 (C^7), 46.8 (C^8), 63.2 (C^9), 38.1 (C^{10}), 202.7 (C^{11}), 129.6 (C^{12}), 171.3 (C^{13}), 44.6 (C^{14}), 27.4 ($C^{15,16}$), 33.0 (C^{17}), 48.2 (C^{18}), 42.5 (C^{19}), 44.9 (C^{20}), 32.0 (C^{21}), 38.7 (C^{22}), 28.4 (C^{23}), 17.0 (C^{24}), 17.3 (C^{25}), 19.4 (C^{26}), 23.9 (C^{27}), 28.8 (C^{28}), 29.2 (C^{29}), 178.6 (C^{30}), 104.9 ($C^{1'}$), 81.7 ($C^{2'}$), 76.3 ($C^{3'}$), 73.4 ($C^{4'}$), 77.3 ($C^{5'}$), 171.4 ($C^{6'}$), 105.0 ($C^{1''}$), 75.2 ($C^{2''}$), 76.0 ($C^{3''}$), 73.6 ($C^{4''}$), 77.7 ($C^{5''}$), 171.5 ($C^{6''}$); 175.0, 174.5, 172.9, 57.9, 57.8, 57.7, 39.0, 38.9, 38.7, 26.5, 26.2, 26.1, 16.1, 16.0, 12.1, 12.0, 11.8 (Ile). Found N, %: 3.3. $C_{60}H_{95}N_3O_{19}$. Calculated N, %: 3.6.

3-*O*-{2-*O*-[*N*-(β-D-Glucopyranosyluronoyl)-L-phenylalanyl]-*N*-(β-D-glucopyranosyluronoyl)-L-phenylalanyl}-(3β,20β)-11-oxo-30-(*N*-carbonyl-L-phenylalanyl)-30-norolean-12-ene (8). Yield 43%, R_f 0.50, $[\alpha]_D^{20}$ 52°±1° (*c* = 0.02, MeOH) {$[\alpha]_D^{20}$ 57° (*c* = 0.02, EtOH) [5]}. IR spectrum, ν, cm^{-1}: 3600–3200 (OH, NH), 1725 (COOH), 1660 (C^{11}=O), 1545 (CONH), 1500 (Ph). ^{13}C NMR spectrum (CD_3OD), δ_C, ppm: 40.3 (C^1), 27.6 (C^2), 90.6 (C^3), 40.6 (C^4), 56.5 (C^5), 18.5 (C^6), 33.9 (C^7), 46.7 (C^8), 63.1 (C^9), 38.0 (C^{10}), 202.7 (C^{11}), 129.6 (C^{12}), 171.3 (C^{13}), 44.3 (C^{14}), 27.4 ($C^{15,16}$), 32.8 (C^{17}), 48.2 (C^{18}), 42.6 (C^{19}), 44.8 (C^{20}), 32.1 (C^{21}), 38.4 (C^{22}), 28.4 (C^{23}), 17.0 (C^{24}), 17.3 (C^{25}), 19.4 (C^{26}), 23.8 (C^{27}), 29.0 (C^{28}), 29.3 (C^{29}), 178.5 (C^{30}), 104.8 ($C^{1'}$), 81.3 ($C^{2'}$), 75.9 ($C^{3'}$), 73.6 ($C^{4'}$), 77.3 ($C^{5'}$), 171.3 ($C^{6'}$), 104.8 ($C^{1''}$), 75.2 ($C^{2''}$), 76.0 ($C^{3''}$), 73.5 ($C^{4''}$), 77.9 ($C^{5''}$), 172.6 ($C^{6''}$); 175.1, 174.1, 172.6, 138.9, 138.0, 137.7, 130.9, 138.0, 137.7, 128.1, 128.0, 127.8, 54.9, 54.6, 54.4, 37.9, 37.8, 37.2 (Phe). Found N, %: 3.3. $C_{69}H_{89}O_{19}N_3$. Calculated N, %: 3.3.

3-*O*-{2-*O*-[*N*-(β-D-Glucopyranosyluronoyl)-L-methioninyl]-*N*-(β-D-glucopyranosyluronoyl)-L-methioninyl}-(3β,20β)-11-oxo-30-(*N*-carbonyl-L-methyonyl)-30-norolean-12-ene (10). Yield 45%, R_f 0.52, $[\alpha]_D^{20}$ 35°±1° (*c* = 0.02, MeOH) {$[\alpha]_D^{20}$ 34° (*c* = 0.02, MeOH) [15]}. IR spectrum, ν, cm^{-1}: 3600–3200 (OH, NH), 1720 (COOH), 1655 (C^{11}=O), 1550 (CONH). ^{13}C NMR spectrum (CD_3OD), δ_C, ppm: 40.2 (C^1), 27.6 (C^2), 90.6 (C^3), 40.6 (C^4), 56.3 (C^5), 18.3 (C^6), 33.7 (C^7), 46.7 (C^8), 63.0 (C^9), 37.9 (C^{10}), 202.5 (C^{11}), 129.1 (C^{12}), 171.4 (C^{13}), 44.4 (C^{14}), 27.4 (C^{15}), 28.4 (C^{16}), 32.8 (C^{17}), 48.1 (C^{18}), 42.4 (C^{19}), 44.7 (C^{20}), 32.5 (C^{21}), 38.6 (C^{22}), 28.8 (C^{23}), 17.0 (C^{24}), 17.3 (C^{25}), 19.3 (C^{26}), 23.8 (C^{27}), 29.1 (C^{28}), 29.3 (C^{29}), 178.5 (C^{30}), 104.7 ($C^{1'}$), 79.8 ($C^{2'}$), 75.8 ($C^{3'}$), 73.4 ($C^{4'}$), 77.0 ($C^{5'}$), 172.5 ($C^{6'}$), 104.8 ($C^{1''}$), 75.9 ($C^{2''}$), 76.1 ($C^{3''}$), 73.3 ($C^{4''}$), 77.5 ($C^{5''}$), 171.6 ($C^{6''}$); 177.4, 173.1, 173.0, 52.9, 52.7, 52.6, 31.9, 31.7, 31.4, 31.3, 31.0, 30.6, 17.0, 16.9, 15.4 (Met). Found, %: N 3.4; S 7.8. $C_{57}H_{86}O_{19}N_3S_3$. Calculated, %: N 3.5; S 8.0.

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