SYNTHESIS OF *N*-GLYCOCONJUGATES OF GLYCYRRHETIC ACID

S. R. Mustafina,[1] L. A. Baltina, Jr.,[1,2] R. M. Kondratenko,[1,2] L. A. Baltina,[1] F. Z. Galin,[1] and G. A. Tolstikov[3]

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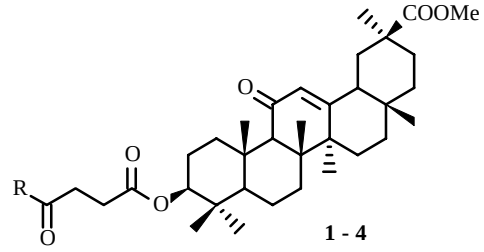
New N*-glycoconjugates that are analogs of glycyrrhizic acid were synthesized by condensation of* α*-L-rhamnopyranosylamine and* β*-D-lactosylamine with acid succinate and phthalate of glycyrrhetic acid methyl ester using* N,N′*-dicyclohexylcarbodiimide (DCC) or DCC-*N*-hydroxybenzotriazole.*

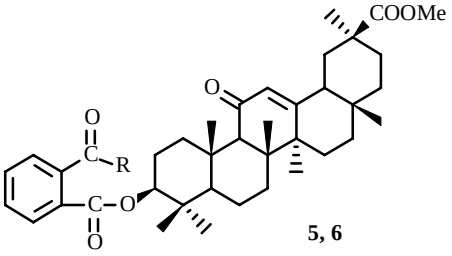
Key words: glycosylamines, *N*-glycoconjugates, glycyrrhetic acid.

Synthetic analogs of bioactive natural triterpene glycosides are of interest in studies of structure—activity relationships. We synthesized previously triterpene conjugates of D-glucoseamine that were modified analogs of glycyrrhizic acid (GA), the principal component of licorice roots (*Glycyrrhiza glabra* L. and *G. uralensis* Fisher) that possesses a broad spectrum of pharmacological activity (anti-inflammatory, antiulcer, antiviral, antiallergic, antidotal, antioxidant, etc.) [1-3].

In continuation of this research we synthesized for the first time *N*-glycoconjugates with spacers (**1**, **3**, and **4**) using unsubstituted glycosylamines (α-L-rhamnopyranosylamine and β-D-lactosylamine) as the amino components. The triterpene components were glycyrrhetic acid methyl ester 3-*O*-hemisuccinate and -phthalate, which were prepared previously [4]. These were condensed with the glycosylamines using *N*,*N*′-dicyclohexylcarbodiimide (DCC) in dimethylformamide (DMF):pyridine (Py).

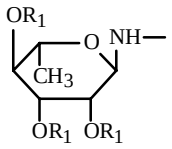


1 - 4

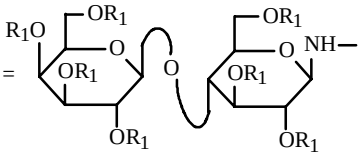


5, 6

1, 2: R =



3, 4: R =



5: R = LacN
6: R = LacNAc

1: R_1 = H; **2:** R_1 = Ac

3: R_1 = H (LacN); **4:** R_1 = Ac (LacAc)

The target compounds **1** and **3** were isolated by column chromatography (CC) over silica gel (SG) or Al_2O_3 and purified further by acetylation using Ac_2O:Py. The low yield (49%) of **3** was explained by losses during chromatography and formation of *N*-acylurea as a side product [5]. The starting material methylglycyrrhetate 3-*O*-hemisuccinate was isolated as an impurity (30%) during chromatography and identified by TLC and ^{13}C NMR spectroscopy [4]. The yield of *N*-glycoconjugate **5** was higher (62%) if methylglycyrrhetate acid phthalate was acylated with β-D-lactosylamine using DCC in the presence of a nucleophile, *N*-hydroxybenzotriazole (HOBt).

1) Institute of Organic Chemistry, Ufa Scientific Center, Russian Academy of Sciences, 450054, Ufa, fax (3472) 35 60 66, e-mail: baltina@anrb.ru; 2) Bashkir State Medical University, 450023, Ufa, Lenina, 3, fax (3472) 72 37 51; 3) N. N. Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Division, Russian Academy of Sciences, 630090, Novosibirsk, pr. Akad. Lavrent′eva, 9, fax (3832) 34 47 52. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 54-56, January-February, 2006. Original article submitted December 15, 2005.

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The formation of the CONH bonds in **1**, **3**, and **5** was confirmed by the appearance of a strong peak at 1540-1530 cm^{-1} in the IR spectra. The ^{13}C NMR spectrum of **1** contained signals for C atoms of the α-L-rhamnopyranosylamine at 68-88 ppm. The PMR spectrum of acetylated *N*-glycoconjugate **2** contained three singlets for the acetyls at 2.0-2.1 ppm. H1′ of the anomeric center of α-L-rhamnopyranosylamine resonated as a broad singlet at 5.5 ppm.

The C1′ atom had a chemical shift (CS) of 88.0 ppm in the ^{13}C NMR spectrum of **2**, of 95.9 and 91.2 ppm in those of the β-D-lactosylamines **3** and **6** (β-configuration), respectively. The signal for C1′ of the first sugar monomer (GlcN) in the ^{13}C NMR spectrum of **6** was shifted to stronger field because of shielding by the aromatic spacer. The PMR spectrum of peracetate **4** showed seven acetyls in the range 2.0-2.1 ppm, the succinic CH_2 at 2.3 ppm, and the NH proton at 7.2 ppm. The triterpene parts of the ^{13}C NMR spectra of **2**, **3**, and **6** were identical to those of the starting 3-*O*-acylates [4]. Signals in the ^{13}C NMR spectra of newly synthesized *N*-glycoconjugates were assigned based on literature data for glycyrrhetic acid derivatives [4, 5] and starting β-glycosylamines [6-8]. The CSs of C8 and C14 were defined more accurately using our published ^1H and ^{13}C high-resolution spectra (600 and 125 MHz) for glycyrrhizic acid and its derivatives [9].

EXPERIMENTAL

IR spectra were recorded on a Specord M80 spectrophotometer as vaseline oil mulls; PMR and ^{13}C NMR spectra, on a Bruker AM 300 instrument at working frequency 300 and 75.5 MHz in $CDCl_3$ with TMS internal standard. Electronic absorption spectra were recorded on a Specord UF-400 instrument in MeOH. Specific rotation was determined on a Perkin—Elmer 241 MC polarimeter in a 1-dm tube. Melting points were measured on a Boetius microstage.

TLC was performed on Silufol (Czech Rep.) plates using benzene:methanol (5:1, A) and benzene:ethanol (10:1, B); CC, over silica gel L (40/100 μm, Czech Rep.) or Al_2O_3 (Brockmann neutral). Spots were developed by phosphotungstic acid solution (20%) with subsequent heating at 110-120°C for 2-3 min.

DMF and Py were purified as before [10]. DCC (Aldrich) was purchased. α-L-Rhamnopyranosylamine and β-D-lactosylamine were prepared by the literature methods [6, 8] in 80-82% purity and were used without further purification. Acid succinate and phthalate of methyl-18β-glycyrrhetate were synthesized as before [4].

Methyl Ester of 3-*O*-[3-*N*-1-Deoxy-2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-1-yl)-carbamoyl]propionyl-18β-glycyrrhetic Acid (2).

1. A solution of methyl glycyrrhetate hemisuccinate (0.58 g, 1 mmol) in DMF (10 mL) and Py (5 mL) at 0-5°C was treated with α-L-rhamnopyranosylamine (0.6 g) and DCC (0.24 g, 1.2 mmol) and stirred at 0-5°C for 1 h and at 20-22°C for 18-20 h. The solid *N*,*N*′-dicyclohexylurea was filtered off. The filtrate was diluted with cold water (50 mL) and acidified with HCl (5%) until the pH was about 4. The solid was filtered off, washed with water, dried, and chromatographed over a column of SG or Al_2O_3 with elution by ethylacetate:MeOH (200:1, 100:1, 50:1, v/v, stepwise gradient). Fractions that were homogeneous by TLC with R_f 0.3 were combined and evaporated. Yield 0.47 g (58.8%).

IR spectrum (ν, cm^{-1}): 3600-3200 (NH), 1730 ($COOCH_3$), 1660 (C^{11}=O), 1530 (CONH). A more mobile fraction with R_f 0.5 (A) turned out to be starting 3-*O*-acylate (0.2 g).

2. *N*-Glycoconjugate (**1**, 0.4 g) was acetylated by Ac_2O:Py (1:1, 10 mL) at 20-22°C for 48 h. The mixture was diluted with cold water. The solid was filtered off and recrystallized from aqueous EtOH. Yield 0.48 g (92.3%), R_f 0.22 (B), mp 128-130°C, $[\alpha]_D^{20}$ +135° (*c* 0.02, MeOH).

IR spectrum (ν, cm^{-1}): 3400-3200 (NH), 1740-1720 (OAc, $COOCH_3$), 1600 (C^{11}=O), 1540 (CONH).

UV spectrum (MeOH, λ_{max}, nm, log ε): 248 (3.95).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.80, 0.86, 1.02, 1.10, 1.12, 1.24, 1.28 (all s, 21H, $7CH_3$), 1.45 (s, 1H, CH_3 Rha), 1.55-1.80 (m, CH, CH_2 of aglycon), 2.00, 2.04, 2.08 (all s, 9H, 3Ac), 2.32 (s, 1H, H9), 2.72 (s, 4H, $2CH_2$), 3.66 (s, 3H, OCH_3), 4.16 (m, 1H, H5′), 4.25 (d, 1H, H2′, $J_{2',3'}$ = 4.5), 4.50 (dd, 1H, H3′, $J_{3',2'}$ = 4.9, $J_{3',4'}$ = 10.3), 4.95 (d, 1H, H4′, $J_{4',3'}$ = 9.1, $J_{4',5'}$ = 9.1), 5.46 (br.s, 1H, H1′), 5.62 (s, 1H, H12), 5.75 (s, 1H, NH).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 38.9 (C1), 23.6 (C2), 80.7 (C3), 38.1 (C4), 55.1 (C5), 17.4 (C6), 33.9 (C7), 45.4 (C8), 61.8 (C9), 37.0 (C10), 200.1 (C11), 128.5 (C12), 171.1 (C13), 43.4 (C14), 26.5 (C15), 25.6 (C16), 32.0 (C17), 49.2. (C18), 42.1 (C19), 44.1 (C20), 31.6 (C21), 37.5 (C22), 28.1 (C23), 16.4 (C24), 16.7 (C25), 18.8 (C26), 23.6 (C27), 28.1 (C28), 28.6 (C29), 177.0 (C30), 51.8 (C31), 169.4 (C32), 32.8 (C33), 33.2 (C34), 174.7 (C35), 88.0 (C1′), 72.7 (C2′), 70.7 (C3′), 74.1 (C4′), 68.0 (C5′), 23.3 (C6′), 169.3, 169.2, 169.0 (C=O, Ac), 21.3, 20.6 (CH_3 Ac). Found, %: N 1.52. $C_{47}H_{62}O_{13}N$. Calc., %: N 1.65.

Methyl Ester of 3-*O*-{[3-*N*-1-Deoxy-2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl-1-yl]-carbamoyl}propionyl-18β-glycyrrhetic Acid (4).

1. Analogously to **1.** above, methylglycyrrhetate acid succinate (0.58 g), β-D-lactopyranosylamine (0.8 g), and DCC (0.24 g) produced crude *N*-glycoconjugate **3** (1.1 g) that was chromatographed twice over a SG column with elution by CH_2Cl_2:MeOH (200:1, 100:1, 50:1, v/v, stepwise gradient). Fractions that were homogeneous by TLC were combined. Yield 0.45 g (49.5%).

IR spectrum (ν, cm^{-1}): 3600-3200 (OH, NH), 1730 ($COOCH_3$), 1660 (C^{11}=O), 1540 (CONH).

UV spectrum (MeOH, λ_{max}, nm, log ε): 250 (4.10).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 39.2 (C1), 23.4 (C2), 81.4 (C3), 37.8 (C4), 55.1 (C5), 17.6 (C6), 33.8 (C7), 45.5 (C8), 61.8 (C9), 37.1 (C10), 200.2 (C11), 128.6 (C12), 169.5 (C13), 43.3 (C14), 26.3 (C15), 25.6 (C16), 31.9 (C17), 48.5 (C18), 41.2 (C19), 44.1 (C20), 31.2 (C21), 37.8 (C22), 28.2 (C23), 15.7 (C24), 16.4 (C25), 18.8 (C26), 23.6 (C27), 26.3 (C28), 26.5 (C29), 177.0 (C30), 51.9 (C31), 169.4 (C32), 28.6 (C33), 28.4 (C34), 173.4 (C35), 95.9 (C1′), 68.9 (C2′), 71.5 (C3′), 83.9 (C4′), 78.9 (C5′, C5″), 61.9 (C6′), 97.5 (C1″), 65.9 (C2″), 71.2 (C3″), 68.0 (C4″), 61.8 (C6″).

2. 3 (0.40 g) was acetylated by Ac_2O:Py as described above. Yield of **4**, 0.44 g (84%), $[\alpha]_D^{20}$ +85° (*c* 0.02, MeOH).

IR spectrum (ν, cm^{-1}): 3400-3200 (NH), 1740 (OAc), 1720 ($COOCH_3$), 1660 (C^{11}=O), 1540 (CONH).

UV spectrum (MeOH, λ_{max}, nm, log ε): 250 (4.13).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.71, 0.81, 1.13, 1.15, 1.15, 1.26, 1.37 (all s, 21H, $7CH_3$), 2.04, 2.06, 2.12, 2.15, 2.17, 2.19 (all s, 21H, 7Ac), 2.34 (s, 4H, $2CH_2$), 3.70 (s, 3H, OCH_3), 4.50 (d, 1H, H1″ Gal, J = 7.9), 4.56 (d, H1′ GlcN, J = 7.9), 5.30 (s, 1H, H4″), 3.60-5.10 (m, H2′, H2″, H3′, H3″, H4′, H4″, H5′, H5″), 5.58 (s, 1H, H12), 7.20 (s, 1H, NH). Found, %: N 1.00. $C_{61}H_{87}O_{23}N$. Calc., %: N 1.16.

Methyl Ester of 3-*O*-{[3-*N*-β-D-1-Deoxy-2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)glucopyranosyl-1-yl]-carbamoyl}phthalyl-18β-glycyrrhetic Acid (6).

1. A solution of methylglycyrrhetate acid phthalate (0.62 g, 1 mmol) in DMF (10 mL) and Py (2 mL) at 0-5°C was treated with β-D-lactosylamine (0.8 g), DCC (0.24 g), and HOBt (0.2 g). The mixture was stirred at 0°C for 1 h and at 20-22°C for 20 h. The reaction mixture was worked up as above for **2**. CC over Al_2O_3 with elution by ethylacetate:MeOH (200:1, 100:1, 50:1, v/v, stepwise gradient) produced **5** (0.76 g, 62%).

IR spectrum (ν, cm^{-1}): 3400-3200 (OH, NH), 1740 ($COOCH_3$), 1660 (C^{11}=O), 1540 (CONH).

2. 5 (0.7 g) was acetylated by Ac_2O:Py (1:1, 20 mL). Yield of acetate 0.87 g (86%), mp 135-137°C (EtOH), $[\alpha]_D^{20}$ +92° (*c* 0.04, MeOH).

IR spectrum (ν, cm^{-1}): 3400-3200 (NH), 1750-1730 (OAc, $COOCH_3$), 1660 (C^{11}=O), 1540 (CONH), 1510 (Ph).

UV spectrum (MeOH, λ_{max}, nm, log ε): 249 (4.3).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.80, 0.95, 1.10, 1.14, 1.19, 1.32, 1.43 (all s, 21H, $7CH_3$), 1.95-2.15 (all s, 21H, 7Ac), 2.36 (s, 1H, C9), 2.75 (s, 1H, H18), 3.10 (s, 1H, H3), 3.68 (s, 3H, OCH_3), 4.10-5.10 (m, H2′, H2″, H3″, H4′, H4″, H5′, H5″, H6′, H6″), 4.48 (d, 1H, H1″, J = 7.8 Gal), 4.52 (d, H1, H1′, J = 7.4 GlcN), 5.34 (s, 1H, H4″), 7.40-7.54, 7.80-7.90 (m, H_{arom}).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 38.4 (C1), 23.2 (C2), 82.9 (C3), 38.0 (C4), 54.8 (C5), 17.0 (C6), 33.6 (C7), 45.1 (C8), 61.3 (C9), 36.6 (C10), 199.6 (C11), 128.7 (C12), 170.6 (C13), 43.7 (C14), 26.1 (C15), 25.7 (C16), 31.5 (C17), 48.0 (C18), 40.7 (C19), 42.7 (C20), 31.5 (C21), 37.4 (C22), 27.8 (C23), 16.1 (C24), 16.5 (C25), 18.3 (C26), 23.0 (C27), 28.0 (C29), 28.2 (C28), 176.6 (C30), 51.4 (C31), 166.2 (C32), 139.4, 134.6, 132.2, 131.3, 130.7, 129.6 (C33-C37), 153.4 (C38), 91.2 (C1′ GlcN), 70.6 (C2′), 73.0 (C3′), 82.5 (C4′), 75.3 (C5′), 60.7 (C6′), 100.8 (C1″ Gal), 66.3 (C2″), 70.2 (C3″), 68.8 (C4″), 75.3 (C5″), 60.5 (C6″), 170.0, 169.9, 169.8, 169.7, 169.0, 168.8, 168.6 (7C=O Ac), 20.7, 20.6, 20.5, 20.3, 20.2 ($7CH_3$ Ac).

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