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SYNTHESIS OF GLYCYRRHIZIC ACID CONJUGATES CONTAINING L-LYSINE

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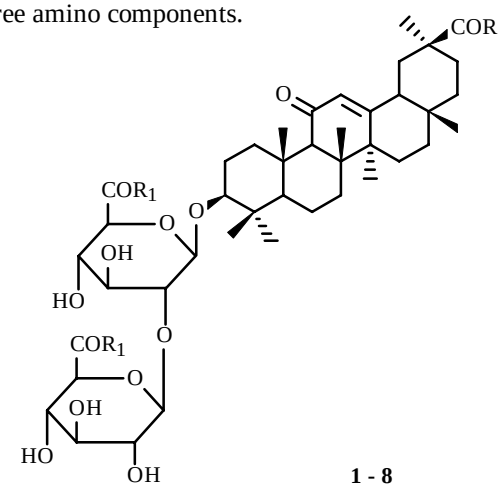
Activated esters and N-*hydroxybenzotriazole*-N,N′-*dicyclohexylcarbodiimide (DCC) or* N-*hydroxy-succinimide-DCC were used to synthesize conjugates of glycyrrhizic acid (GA) with* N^{ε}*-carbobenzyloxy-L-lysine [Lys(*Z*)-OH] and its esters containing two or three amino components. It was shown that the conjugate of GA 30-methyl ester with Lys(*Z*)-OH possessed anti-HIV-1 activity.*

Key words: glycyrrhizic acid, L-lysine.

Chemical modification of glycyrrhizic acid (GA, **1**), the principal triterpene glycoside in the extract of licorice root (*Glycyrrhiza glabra* L. and *G. uralensis* Fisher), is a promising method for preparing new medically valuable and biologically active anti-inflammatory, antiulcer, immunomodulating, and antiviral compounds [1, 2].

We have previously synthesized glucopeptide conjugates (GC) with various amino acids and dipeptides, among which were found stimulators of the primary immune response [3-5] and anti-inflammatory, antiulcer, and anti-HIV agents [6-8].

In continuation of our research on the synthesis of new biologically active GA derivatives, we synthesized new conjugates (**2**-**8**) with an amide bond formed through the α-NH_2 group of N^{ε}-carbobenzyloxy-L-lysine or its esters containing two or three amino components.



1 - 8

1: R = R_1 = OH
2: R = R_1 = Lyz(Z)-OMe
3: R = OH, R_1 = Lys(Z)-OMe
4: R = OH, R_1 = Lys(Z)-OBu^t
5: R = OMe, R_1 = Lys(Z)-OMe
6: R = OMe, R_1 = Lys(Z)OBu^t
7: R = OH, R_1 = Lys(Z)-OH
8: R = OMe, R_1 = Lys(Z)-OH,
Lys(Z)-OH = $C_6H_5CH_2OCONH^5CH_2{}^4CH_2{}^3CH_2{}^2CH_2{}^1CH(COOH)NH$-

Conjugate **2** was synthesized by condensation of GA with the trifluoroacetate of N^{ε}-carbobenzyloxy-L-lysine methyl ester [Lys(*Z*)-OMe·CF_3COOH] using *N*-hydroxybenzotriazole (HOBt)-*N,N′*-dicyclohexylcarbodiimide (DCC) in the presence of triethyloamine (Et_3N) as described previously [9]. The yield of **2** after purification by column chromatography (CC) over silica gel (SG) was 52%.

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TABLE 1. ^{13}C NMR Spectra of the Aglycon of Conjugates **2-8** (75.5 MHz, δ, ppm, 25°C)

C atom	**2** (CD_3OD)	**3** (CD_3OD)	**4** (CD_3OD)	**5** (CD_3OD+$CDCl_3$)	**6** (acetone-d_6)	**7** (DMF-d_7)	**8** (acetone-d_6)
1	40.6	40.2	39.8	38.7	39.9	40.1	39.3
2	27.4	27.1	26.8	27.4	25.8	26.3	26.2
3	90.9	90.8	88.7	90.6	88.3	88.9	88.5
4	40.9	40.7	40.7	39.0	40.6	40.2	40.3
5	56.7	56.4	54.5	56.4	54.5	56.4	54.6
6	18.8	18.6	16.6	18.5	16.9	17.7	17.1
7	34.0	33.8	31.2	33.8	32.1	31.7	32.4
8	47.0	46.8	46.2	46.7	-	44.8	45.1
9	63.4	63.1	61.2	63.1	61.2	61.0	61.5
10	38.1	38.1	38.6	38.1	37.3	36.9	37.5
11	202.8	202.7	200.7	202.5	198.4	199.0	199.3
12	129.2	128.5	127.7	129.5	129.9	128.1	128.9
13	171.7	171.3	171.3	171.4	170.1	169.3	170.5
14	44.9	44.7	43.2	44.6	44.8	43.5	43.0
15	27.6	27.5	26.9	27.4	26.9	27.6	27.3
16	27.9	27.9	27.4	27.6	26.9	27.8	27.9
17	33.2	33.0	32.0	33.0	31.5	-	31.5
18	-	-	-	-	47.8	48.1	48.2
19	41.8	42.5	42.7	42.5	42.4	42.8	43.1
20	44.9	44.5	44.9	44.9	43.4	43.5	43.9
21	32.8	32.5	31.1	32.5	31.3	31.2	31.3
22	38.4	39.0	38.9	38.1	38.6	38.9	38.8
23	28.7	28.5	26.9	28.8	-	-	-
24	17.4	17.1	15.5	17.1	15.6	15.7	15.9
25	17.7	17.4	15.7	17.4	16.0	16.9	16.2
26	19.7	19.4	17.8	19.4	17.7	17.9	18.2
27	23.9	23.4	22.4	24.1	22.6	22.5	-
28	29.1	28.8	27.6	28.5	-	-	-
29	29.5	29.3	28.7	29.2	29.4	25.5	-
30	174.0	180.5	179.1	176.3	175.9	180.0	176.3
31	-	-	-	51.2	50.9	-	51.0

TABLE 2. ^{13}C NMR Spectra of the Carbohydrate Part of Conjugates **2-8** (75.5 MHz, δ, ppm, 25°C)

C atom	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1′	105.2	104.9	102.9	105.0	103.1	106.4	103.2
2′	81.4	81.6	81.7	81.8	81.2	79.9	81.2
3′	76.2	76.4	73.7	76.3	74.2	75.9	74.6
4′	73.6	73.1	71.6	73.3	72.1	72.0	72.2
5′	78.0	77.7	75.7	77.8	75.7	76.6	76.0
6′	171.6	171.3	169.4	171.3	168.6	168.8	169.6
1″	105.3	105.3	103.0	105.2	103.2	108.7	103.7
2″	76.1	76.1	73.3	76.0	73.0	75.0	73.1
3″	76.5	76.5	73.9	76.4	73.5	75.9	74.3
4″	73.8	73.6	71.8	73.5	72.1	72.0	72.2
5″	77.5	77.4	75.1	77.3	75.3	76.2	75.5
6″	171.7	171.6	169.6	171.4	169.5	169.0	169.5

Compounds **3** and **4** with two Lys(*Z*)-OMe or Lys(*Z*)-O-*t*-Bu units only in the carbohydrate part of the molecule were prepared by activation of the GA carboxyls with *N*-hydroxysuccinimide (HOSu)-DCC at a mole ratio GA:HOSu:DCC:Lys(*Z*)-OR 1:4:2.3-2.5:3 mmol in dioxane. The free COOH group in the aglycon part of **3** and **4** was confirmed by retention of the chemical shift (CS) of C-30 at about 180 ppm in ^{13}C NMR spectra of these compounds, like in the spectrum of GA [10, 11].

TABLE 3. Chemical Shifts of Amino Acids [Lys(*Z*)-] in ^{13}C NMR Spectra of Conjugates **2-8** (75.5 MHz, δ, ppm, 25°C)

Compound	C-1	C-2	C-3	C-4	C-5
2	174.0	53.8	30.8	24.3	68.8
	173.3	53.5	30.5	24.1	67.7
	173.2	53.2	29.5	23.9	67.0
3	174.7	53.6	32.1	24.1	68.6
	172.9	53.0	30.6	23.9	67.4
4	170.9	52.0	30.4	22.2	65.7
	170.4	51.9	30.5	21.7	65.5
5	174.7	53.8	32.7	24.3	64.7
	173.9	53.5	32.3	23.9	64.3
6	171.0	52.3	30.9	22.2	65.1
	170.2	51.9	30.3	21.9	65.1
7	176.4	52.0	31.9	22.2	65.0
	173.4	51.0	31.4	21.8	65.0
8	172.8	51.6	30.9	22.8	65.7
	172.6	51.4	30.5	22.2	65.7

Other signals for **2**: 138.6, 138.1, 129.9, 129.7, 129.5, 129.0, 128.9, 128.2 (C_6H_5), 53.0, 53.2 ($COOCH_3$).
3: 129.7, 129.5, 129.3, 128.8, 128.7, 128.0 (C_6H_5), 52.8, 52.7 ($COOCH_3$).
4: 137.2, 127.9, 127.6, 127.3 (C_6H_5), 27.9, 26.9 (*t*-Bu).
5: 138.6, 138.0, 129.8, 129.7, 129.5, 129.2, 129.0, 128.9 (C_6H_5), 53.2, 53.0 ($COOCH_3$).
6: 156.0, 137.2, 127.9, 127.6, 127.3 (C_6H_5), 27.93, 26.93 (*t*-Bu).
7: 139.7, 134.0, 131.9, 129.2, 128.1, 127.5, 113.3 (C_6H_5).
8: 158.4, 156.7, 137.2, 127.9, 127.8, 117.4 (C_6H_5).

30-Methyl esters **5** and **6** were prepared by treating **3** and **4** with an ether solution of diazomethane in MeOH. The C-30 signal in the ^{13}C NMR spectrum of **5** shifted to 177.3 ppm, like in the spectrum of methylglycyrrhetate [12].

The *t*-butyl esters in **4** and **6** were removed with CF_3COOH in CH_2Cl_2 to produce **7** and **8**, respectively, in 79-81% yields. The structures of the new GA derivatives were confirmed by elemental analyses and IR, UV, PMR, and ^{13}C NMR spectra (Tables 1-3). Signals of C atoms in the ^{13}C NMR spectra of **2-7** were assigned using literature data for analogous GA derivatives [4, 9-12].

Introducing Lys(*Z*)-OH or its esters causes the appearance in the ^{13}C NMR spectra of additional signals at 171-174 ppm (COOH); 52-54 and 64-68 ppm (Lys); and aromatic C atoms of the C_6H_5 group.

The cytotoxicity and anti-HIV-1 activities of **8** were studied using a model for primary infection of HIV-1 in MT-4 lymphoid cells and were compared with GA as described before [7, 8]. Inhibition of HIV-1 reproduction in the cells was determined from the decrease in accumulation of viral antigen p24 (by immunoenzyme analysis) in culture medium on the fourth day of cultivation by comparison with a control (without added preparation). It was found that **8** possessed moderate anti-HIV-1 activity in MT-4 cell culture. The 50% inhibition dose (ID_{50}) was 200 μg/mL; 50% toxic dose (CD_{50}) causing 50% cell death, 2000 μg/mL; index of selectivity (IS) (ratio of CD_{50} to ID_{50}) for p24 inhibition, 10. Thus, **8** exhibited 50% inhibition of HIV-1 at a lower concentration [ID_{50} GA (97%) = 250 μM]. The IS was comparable with that of GA (IS GA = 9.6) [13]. It is also important to note that **8** exhibited no noticeable protective effect against the cytopathogenic activity of the virus.

EXPERIMENTAL

Column chromatography was performed over silica gel L (Chemapol, Czech Rep.) using $CHCl_3$:CH_3OH:H_2O mixtures (300:10:1, 200:10:1, 100:10:1, v/v, stepwise gradient). TLC used Silufol plates (Chemapol, Czech Rep.) and $CHCl_3$:CH_3OH:H_2O (45:10:1). Spots were developed using phosphotungstic acid solution (20%) in ethanol (96%) and heating to 100-120°C for 2-3 min. Optical activity was measured on a Perkin—Elmer 241 MC polarimeter in a 1-dm tube with CH_3OH.

IR spectra were recorded on a Specord M80 spectrophotometer in mineral-oil mulls; UV spectra, on a Shimadzu UV-365 spectrophotometer in CH_3OH; PMR and ^{13}C NMR, on a Bruker AM-300 spectrometer at 300 and 75.5 MHz working frequency in CD_3OD, $CDCl_3$+CD_3OD, or DMF-d_7 with TMS internal standard.

Et_3N, DMF, and dioxane were purified by literature methods [14]. Solvents were evaporated in vacuo at 45-50°C. We used GA (92 ± 2%) prepared as before [15], DCC (Ferak, Germany), N^{α}-Boc-N^{ε}-*Z*-L-Lys and N^{ε}-*Z*-L-Lys-O-*t*-Bu·HCl (Reanal, Hungary).

N^{α}-*t*-Butyloxycarbonyl-N^{ε}-carbobenzyloxy-L-lysine Methyl Ester. A solution of N^{α}-Boc-N^{ε}-Z-Lys (13.4 g, 74 mmol) in CH_3OH (180 mL) was cooled on an ice bath, treated with an ether solution of diazomethane until a yellow color was stable, stirred for 15-20 min, treated with glacial acetic acid (2 drops) to decompose the excess of diazomethane, and evaporated in vacuo. The solid was dissolved in ethylacetate (150 mL) and washed with $NaHCO_3$ solution (1 N) and water. The organic phase was dried over $MgSO_4$ and evaporated. The dry solid was recrystallized from ethylacetate:hexane. Yield 10.0 g (72%), R_f 0.71 (benzene:alcohol, 10:1), mp 115-118°C, $[\alpha]_D^{20}$ +13° (*c* 0.02, EtOH); lit. [16] mp 117°C, $[\alpha]_D^{20}$ 16.7°C (2%, CH_3OH); lit. [17] mp 115.5-116.56°C, $[\alpha]_D^{20}$ +12.2° (2%, 0.1 N HCl).

3-*O*-{2-*O*-[*N*-(β-D-Glucopyranosyluronoyl)-N^{ε}-(*Z*)-L-lysine methyl ester]-*N*-(β-D-glucopyranosyluronoyl-N^{ε}-(*Z*)-L-lysine methyl ester}-(3β,20β)-11-oxo-30-(*N*-carbonyl-N^{ε}-(*Z*)-L-lysine methyl ester)-olean-12-en (2).

1. N^{ε}-Carbobenzyloxy-L-lysine methyl ester trifluoracetate. N^{α}-Boc-Lys(*Z*)-OMe (10 g) was dissolved in CF_3COOH (20 mL), held for 30 min at 20-22°C, diluted with dry CH_2Cl_2 (20 mL), and evaporated in vacuo. The solid was dried with dry benzene (3 × 15 mL) to produce Lys-(*Z*)-OMe·CF_3COOH (5.8 g) that was reprecipitated from ethylacetate with hexane and condensed with GA without further purification. $C_{17}H_{23}N_6O_6F_3$.

2. A solution of GA (0.82 g, 1 mmol) in dioxane (20 mL) at 0-5°C was treated with HOBt (0.47 g, 3.5 mmol) and DCC (0.72 g, 3.5 mmol), stirred with cooling for 1 h and at 20-22°C for 6 h, left overnight in a refrigerator, and filtered to remove solid *N*,*N*′-dicyclohexylurea. The filtrate was treated with Lys-(*Z*)-OMe·CF_3COOH (1.86 g, 4 mmol), DMF (10 mL), and Et_3N (0.7 mL, 5.1 mmol). The mixture was periodically stirred at 20-22°C for 24 h, diluted with cold water, and acidified with citric acid until the pH was about 3. The solid was filtered off, washed with water, and dried to produce **2** (1.04 g) that was chromatographed over a column of SG (100/160 μm) with elution by $CHCl_3$:CH_3OH:H_2O (300:10:1, 200:10:1, 100:10:1, 50:10:1, v/v). The 100:10:1 mixture eluted **2** (0.86 g, 52%) that was homogeneous by TLC as an amorphous yellow compound, R_f 0.58, $[\alpha]_D^{20}$ +35° (*c* 0.03). IR spectrum (ν, cm^{-1}): 3600-3200 (OH, NH); 1730 (COOR); 1660 (C_{11}=O); 1540 (CONH); 1520 (C_6H_5). UV spectrum (λ_{max}, nm): 249 (log ε 4.38). $C_{87}H_{122}N_6O_{25}$.

PMR spectrum (300 MHz, CD_3OD, δ, ppm): 0.80, 0.82, 1.05, 1.10, 1.14, 1.24, 1.28 (21H, all s, $7CH_3$), 2.85 (1H, s, H-9), 3.70, 3.72 (9H, s, $3COOCH_3$), 5.05 (4NH), 5.56 (1H, s, H-12), 7.34 (C_6H_5). Tables 1-3 give the ^{13}C NMR spectra.

3-*O*-{2-*O*-[*N*-(β-D-Glucopyranosyluronoyl)-N^{ε}-(*Z*)-L-lysine methyl ester]-*N*-(β-D-glucopyranosyluronoyl)-N^{ε}-(*Z*)-L-lysine methyl ester}-(3β,20β)-11-oxo-olean-12-en-30-oic Acid (3). A solution of GA (0.82 g, 1 mmol) in dioxane (15 mL) at 0-5°C was treated with HOSu (0.46 g, 4 mmol) and DCC (0.55 g, 2.5 mmol), stirred at this temperature for 2 h, stored in a refrigerator overnight, and filtered to remove solid *N*,*N*′-dicyclohexylurea. The filtrate was cooled to 0-5°C; treated with CF_3COOH·Lys-(*Z*)-OMe (1.39 g, 3 mmol), DMF (10 mL), and Et_3N (0.7 mL, 5.1 mmol); held at 20-22°C with periodic stirring for 24 h, diluted with cold water, and acidified with citric acid until the pH was about 3. The solid was filtered off, washed with water, and dried to produce crude product (1.0 g) that was chromatographed over a column of SG as described above. Yield 0.62 g (45%), R_f 0.6, $[\alpha]_D^{20}$ +60° (*c* 0.02). IR spectrum (ν, cm^{-1}): 3600-3200 (OH, NH); 1740 (COOR); 1665 (C_{11}=O); 1540 (CONH). UV spectrum (λ_{max}, nm): 248 (log ε 4.22). $C_{72}H_{102}O_{22}N_4$.

PMR spectrum (300 MHz, CD_3OD, δ, ppm): 0.80, 1.04, 1.08, 1.10, 1.14, 1.16, 1.40 (21H, all s, $7CH_3$), 3.30, 3.35 (6H, both s, $2COOCH_3$), 5.56 (1H, s, H-12), 7.34 ($2C_6H_5$). Tables 1-3 give the ^{13}C NMR spectra.

Conjugate **2** (10%) and starting GA (20%) were isolated from the reaction products as impurities and were identified by TLC with markers.

3-*O*-{2-*O*-[*N*-(β-D-Glucopyranosyluronoyl)-N^{ε}-(*Z*)-L-lysine-*t*-butyl ester]-*N*-(β-D-glucopyranosyluronoyl)-N^{ε}-(*Z*)-L-lysine-*t*-butyl ester}-(3β,20β)-11-oxo-olean-12-en-30-carboxylic Acid (4). A solution of GA (0.82 g, 1 mmol) in dioxane (30 mL) at 0-5°C was treated with HOSu (0.6 g, 5.2 mmol) and DCC (0.65 g, 3 mmol), stirred and cooled for 2 h, and stored in a refrigerator overnight. Solid *N*,*N*′-dicyclohexylurea was filtered off. The filtrate was cooled in an ice bath, treated with Lys-(*Z*)-*O*-*t*-Bu·HCl (1.12 g, 1 mmol) and Et_3N (0.6 mL, 4 mmol), held at 20-22°C with periodic stirring for 24 h, diluted with cold water, and acidified with citric acid until the pH was about 3. The solid was filtered off, washed with water and dried. The resulting product (1.07 g) was reprecipitated from $CHCl_3$:CH_3OH with ether and chromatographed over a column of SG

as described above. Yield 0.68 g (48%), R_f 0.50, $[\alpha]_D^{20}$ +55° (*c* 0.02). IR spectrum (ν, cm^{-1}): 3600-3200 (OH, NH); 1740 (COOR); 1660 (C_{11}=O); 1540 (CONH). UV spectrum (λ_{max}, nm): 248.8 (log ε 4.20). $C_{76}H_{114}O_{22}N_4$.

PMR spectrum (300 MHz, CD_3OD, δ, ppm, J/Hz): 0.74, 0.86, 0.90, 0.97, 1.04, 1.07, 1.14, 1.30, 1.37, 1.39 (39H, all s, 13CH_3), 2.58 (1H, s, H-9), 3.05 (1H, d, J = 6.0, H-3), 4.30 (1H, d, J = 6.9, H-5′), 4.48 (1H, d, J = 7.0, H-1′), 4.98 (4H, s, NH), 5.54 (1H, s, H-12), 6.24, 7.25 (2C_6H_5). Tables 1-3 give the ^{13}C NMR spectra.

General Method for Methylating 3 and 4. A solution of **3** or **4** (0.5 g) in CH_3OH (20 mL) at 0-5°C was treated with an ether solution of diazomethane until the yellow color was stable. The solutions were filtered and evaporated. The solid was chromatographed over a column of SG as described above.

30-Methyl Ester of 5. Yield 82%, $[\alpha]_D^{20}$ +35° (*c* 0.02). IR spectrum (ν, cm^{-1}): 3600-3200 (OH, NH); 1750-1730 (COOMe); 1600 (C_{11}=O); 1540 (CONH). UV spectrum (λ_{max}, nm): 249 (log ε 4.10). $C_{73}H_{104}O_{22}N_4$. Tables 1-3 give the ^{13}C NMR spectra.

30-Methyl Ester of 6. Yield 0.43 g (86%), $[\alpha]_D^{20}$ +35° (*c* 0.02). IR spectrum (ν, cm^{-1}): 3600-3200 (OH, NH); 1740 (COOR); 1660 (C_{11}=O); 1540 (CONH). UV spectrum (λ_{max}, nm): 247 (log ε 4.31). $C_{76}H_{117}O_{22}N_4$.

PMR spectrum (300 MHz, acetone-d_6, δ, ppm, J/Hz): 0.78, 0.86, 1.10, 1.14, 1.44, 1.50 (42H, all s, 14CH_3), 2.65 (1H, s, H-9), 3.14 (1H, d, J = 5.4, H-3), 3.65 (3H, s, $COOCH_3$), 4.40 (1H, d, J = 7.5, H′), 4.75 (1H, d, J = 7.5, H″), 4.90 (1H, H-2′), 5.10 (4H, s, NH), 5.55 (1H, s, H-12), 7.30, 7.35 (2C_6H_5).

General Method for Deblocking 4 and 6. Conjugate **4** or **6** (0.5 g) was dissolved in a mixture of CF_3COOH (5 mL) and CH_2Cl_2 (5 mL) and held at 20-22°C for 1 h. Solvent and CF_3COOH were evaporated with dry benzene (3 × 5 mL) in vacuo at about 50°C. The solid was chromatographed over SG with elution by $CHCl_3$:CH_3OH:H_2O (200:10:1, 100:10:1, 50:10:1, v/v). Fractions that were homogeneous by TLC were combined and evaporated.

3-*O*-{2-*O*-[*N*-(β-D-glucopyranosyluronoyl)-N^{ε}-(*Z*)-L-lysine]-*N*-(β-D-glucopyranosyluronoyl)-N^{ε}-(*Z*)-L-lysine}-(3β,20β)-11-oxo-olean-12-en-30-oic Acid (7). Yield 0.38 g (80.8%), R_f 0.4, $[\alpha]_D^{20}$ +55° (*c* 0.02). IR spectrum (ν, cm^{-1}): 3600-3200 (OH, NH); 1670 (C=O); 1530 (CONH); 1450 (C_6H_5). UV spectrum (λ_{max}, nm): 248 (log ε 4.2). $C_{70}H_{98}O_{22}N_4$.

PMR spectrum (300 MHz, DMF-d_7, δ, ppm): 0.50, 0.70, 0.75, 0.82, 0.86, 1.00, 1.20 (21H, all s, 7CH_3), 4.80 (4H, NH), 5.25 (1H, s, H-12), 7.10, 7.25 (2C_6H_5). Tables 1-3 give the ^{13}C NMR spectra.

Methyl Ester of 3-*O*-{2-*O*-[*N*-(β-D-Glucopyranosyluronoyl)-N^{ε}-(*Z*)-L-lysine]-*N*-(β-D-glucopyranosyluronoyl)-N^{ε}-(*Z*)-L-lysine}-(3β,20β)-11-oxo-olean-12-en-30-oic Acid (8). Yield 0.37 g (78.7%), R_f 0.45, $[\alpha]_D^{20}$ +25° (*c* 0.02). IR spectrum (ν, cm^{-1}): 3600-3200 (OH, NH); 1740 (COOMe); 1700 (COOH); 1660 (C_{11}=O); 1540 (CONH). UV spectrum (λ_{max}, nm): 248 (log ε 4.1). $C_{70}H_{98}O_{22}N_4$.

PMR spectrum (300 MHz, acetone-d_6, δ, ppm, J/Hz): 0.80, 0.86, 0.94, 1.10, 1.40 (21H, all s, 7CH_3), 3.64 (3H, s, $COOCH_3$), 4.54 (1H, d, J = 7.5, H-1″), 5.05 (1H, d, J = 7.0, H-1′), 5.56 (1H, s, H-12), 6.50 (4H, NH), 7.65 (C_6H_5). Tables 1-3 give the ^{13}C NMR spectra.

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