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SYNTHESIS OF BETULONIC ACID DERIVATIVES CONTAINING AMINO-ACID FRAGMENTS

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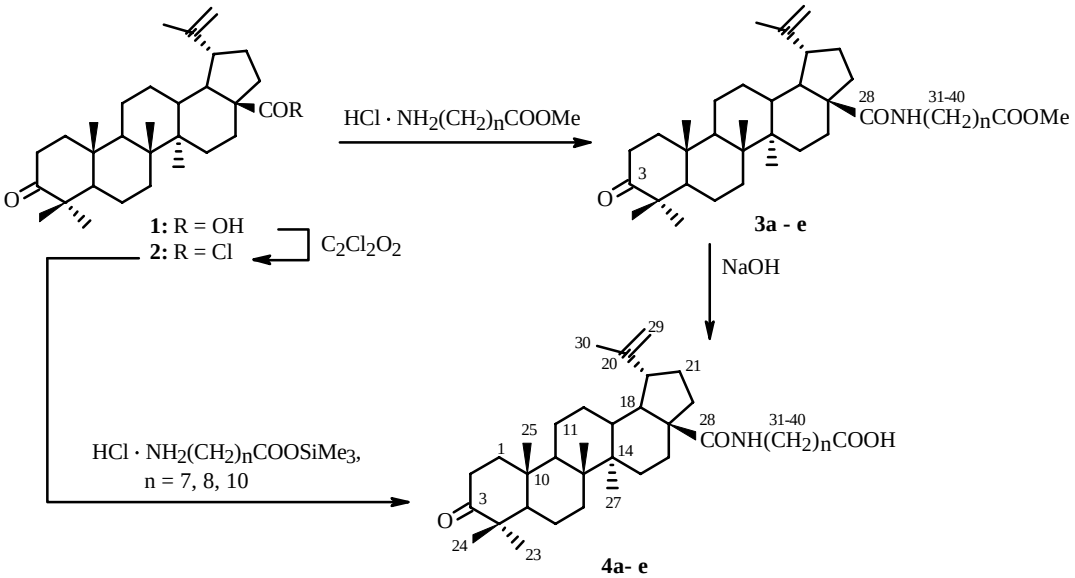
New derivatives of betulonic acid containing on C-28 fragments of amino acids or their methyl esters were prepared as potential biologically active agents.

Key words: betulinic and betulonic acids, amino acids, dipeptides, condensation, NMR spectra.

Betulinic acid suppresses the growth of melanoma and other cancerous cells of neuroectodermal origin [1-3]. Further research has showed that it has a wide spectrum of biological activity, exhibiting antimalarial [4], antitumor [5-8], anti-inflammatory [9], and antiviral [10] activity, the same as its derivatives [8, 11-20]. Derivatives of betulinic acid with fragments of long-chain ω-amino acids in their structure are especially interesting because they are active anti-HIV agents [13, 18-20].

The most accessible derivative of betulinic acid is betulonic acid (**1**), which possesses antitumor [3, 8, 17], antiherpes [21, 22], and immunostimulating [22] activity and is easily prepared from betulin. In order to seek new biologically active compounds, we synthesized new derivatives of betulonic acid with various amino-acid fragments or their methyl esters on C-28.

The reaction of acid chloride **2** with the methyl esters of glycine, β-alanine, and long-chain 8-, 9-, and 11-ω-aminocarboxylic acids in the form of their hydrochlorides produced derivatives **3a-e** with fragments of the corresponding methyl esters of amino acids on C-28 (Scheme 1). The condensation was carried out at room temperature in anhydrous CH_2Cl_2 in the presence of Et_3N. The yields were 90-96%.



Scheme 1.

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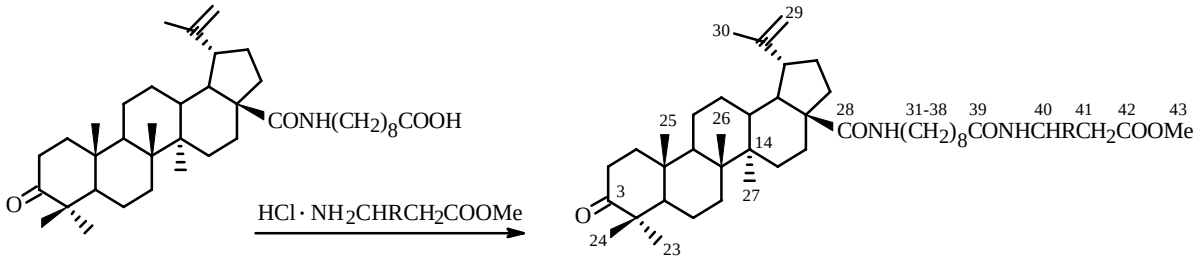
TABLE 1. Physicochemical Properties of **3-8**

Compound	Yield, %	mp, °C	[α], deg (*c*)	Empirical formula	M^+	
					Found	Calc.
3a	96	179-181 (CH_3CN)	+26 (4.39)	$C_{33}H_{51}NO_4$	525.38179	525.38178
3b	95	A	+34 (7.43)	$C_{34}H_{53}NO_4$	539.40137	539.39743
3c	93	A	+26 (4.96)	$C_{39}H_{63}NO_4$	609.47567	609.47568
3d	93	A	+28 (3.49)	$C_{40}H_{65}NO_4$	623.49035	623.49133
3e	90	A	+27 (4.95)	$C_{42}H_{69}NO_4$	651.52584	651.52263
4a	98	187-189 (CH_3CN)	+33 (3.94)	$C_{32}H_{49}NO_4$	511.36557	511.36614
4b	97	214-216 (CH_3CN)	+31 (3.04)	$C_{33}H_{51}NO_4$	525.38371	525.38178
4c	97	A	+26 (4.17)	$C_{38}H_{61}NO_4$	595.46088	595.46003
4d	96	A	+27 (4.16)	$C_{39}H_{63}NO_4$	609.47507	609.47568
4e	95	A	+22 (1.81)	$C_{41}H_{67}NO_4$	637.50871	637.50698
5a	89	A	+20 (2.45)	$C_{43}H_{70}N_2O_5$	694	694
5b	95	A	+21 (4.50)	$C_{49}H_{74}N_2O_5$	770	770
6	98	A	-16 (3.94)	$C_{40}H_{66}N_2O_4$	638.50330	638.50223
7	93	A	+3 (5.74)	$C_{40}H_{67}NO_4$	625.40872	625.50295
8	93	A	+7 (1.52)	$C_{42}H_{69}NO_5$	667.51956	667.51754

A: Compound isolated as an amorphous powder.



4d **5:** R = H (**a**), Ph (**b**)

Scheme 2.

Alkaline hydrolysis of **3a-e** proceeds smoothly to give the corresponding amino-acid derivatives **4a-e**. The long-chain amino-acid derivatives **4c-e** were prepared from **2** via reaction with trimethylsilyl esters of the corresponding amino acids prepared in situ (Scheme 1). Condensation of **2** with the trimethylsilyl esters of the amino acids occurs less smoothyl than with the corresponding methyl esters. The yields were 55-65%.

Two dipeptides of betulonic acid, **5a** and **b**, with 9-aminopelargonic acid in combination with the methyl ester of *β*-alanine or *β*-phenyl-*β*-alanine were also synthesized. Compound **4d** was condensed with hydrochlorides of amino-acid methyl esters using N,N-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole (Scheme 2). The reaction was carried out in CH_2Cl_2 in the presence of Et_3N. The yields were about 90%.

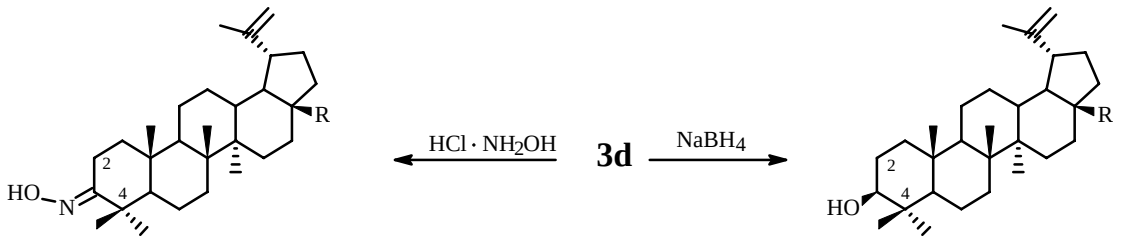
The compositions and structures of **3-5** were proved using elemental analysis and spectral methods. Thus, the IR spectra of the synthesized compounds exhibit bands for C=O stretching vibrations of the amide and ester or carboxyl groups in the ranges 1668-1672, 1736-1750, and ~1709 cm^{-1}, respectively. The PMR spectra of the amino-acid derivatives have characteristic signals for the two protons of the terminal double bond of the triterpene skeleton and the protons of the CONH–, CONH$\underline{CH_2}$–, and $\underline{CH_2}$COO$\underline{Me}$ (or $\underline{CH_2}$CO$\underline{OH}$) groups. Signals of C atoms in the ^{13}C NMR spectra of **3-5** were assigned based on 2D NMR spectra of **2**, **3a**, **3d**, and **5b** and literature data for betulonic acid [23, 24]. Signals of C atoms in the CH_2-groups of the amino-acid part were assigned based on ^{13}C NMR data for amino-acid derivatives of glycyrrhetic acid [25].

TABLE 2. Chemical Shifts of C Atoms in ^{13}C NMR Spectra of **3**, **4**, **6-8** (δ, ppm)

Atom	**3a**	**3b**	**3c**	**3d**	**3e**	**4a**	**4b**	**4c**	**4d**	**4e**	**6**	**7**	**8**
C-1	39.40 t	39.38	39.38	39.27	39.35	39.39	39.37	39.38	39.38	39.36	38.58	38.54	38.18
C-2	33.91 t	33.86	33.83	33.75	33.81	33.89	33.84	33.86	33.85	33.78	16.93	27.11	23.44
C-3	217.95 s	217.70	217.55	217.77	217.57	218.82	218.74	218.23	218.28	218.18	166.59	78.44d	80.66d
C-4	47.08 s	47.03	46.99	46.90	46.97	47.13	47.08	47.06	47.06	47.01	40.13	38.54	37.52
C-5	54.75 d	54.75	54.78	54.59	54.74	54.74	54.69	54.75	54.74	54.74	55.44	55.17	55.22
C-6	19.41 t	19.41	19.40	19.27	19.37	19.43	19.40	19.38	19.38	19.39	18.77	18.00	17.92
C-7	33.41 t	33.30	33.48	33.34	33.45	33.42	33.40	33.46	33.46	33.46	33.79	34.16	34.11
C-8	40.44 s	40.44	40.47	40.31	40.44	40.45	40.43	40.45	40.44	40.45	40.53	40.47	40.51
C-9	49.75 d	49.74	49.91[a]	49.63	49.74[a]	49.74	49.66[a]	49.74[a]	49.74[a]	49.74[a]	50.00	50.37	50.31
C10	36.67 s	36.66	36.66	36.53	36.63	36.67	36.64	36.65	36.65	36.64	37.02	36.90	36.86
C-11	21.20 t	21.33	21.25	21.13	21.23	21.22	21.23	21.23	21.23	21.23	20.97	20.85	20.71
C-12	25.37 t	25.39	25.41	25.27	25.38	25.39	25.37	25.39	25.38	25.40	25.38	25.36	25.34
C-13	37.47 d	37.55	37.49	37.31	37.45	37.55	37.57	37.50	37.50	37.49	37.45	37.38	37.40
C-14	42.29 s	42.26	42.26	42.11	42.23	42.31	42.26	42.26	42.26	42.25	42.23	42.14	42.19
C-15	29.10 t	29.04	29.16	29.04	29.17	29.13	29.08	29.15	29.14	29.16	29.14	29.14	29.42
C-16	33.28 t	33.30	33.48	33.29	33.45	33.26	33.27	33.46	33.46	33.46	33.53	33.46	33.52
C-17	55.44 s	55.36	55.28	55.13	55.25	55.52	55.43	55.31	55.32	55.30	55.30	55.26	55.29
C-18	49.75 d	49.74	49.77[a]	49.75	49.88[a]	49.74	49.69[a]	49.84[a]	49.83[a]	49.86[a]	49.87	49.90	49.92
C-19	46.35 d	46.46	46.41	46.29	46.39	46.38	46.43	46.41	46.41	46.39	46.47	46.39	46.50
C-20	150.54 s	150.52	150.63	150.57	150.61	150.43	150.38	150.62	150.63	150.56	150.74	150.66	150.72
C-21	30.51 t	30.56	30.64	30.50	30.61	30.51	30.53	30.61	30.60	30.61	30.63	30.61	30.64
C-22	38.00 t	38.00	38.15	38.06	38.14	38.04	38.04	38.19	38.20	38.17	38.23	38.13	38.18
C-23	26.38 q	26.38	26.38	26.26	26.34	26.42	26.40	26.37	26.36	26.37	26.93	27.73	27.68
C-24	20.78 q	20.75	20.72	20.62	20.69	20.80	20.75	20.75	20.75	20.71	22.62	15.11	16.22
C-25	15.71 q	15.66	15.71[b]	15.59	15.69[b]	15.72[a]	15.70[b]	15.68	15.69	15.65	15.55	15.86	15.90
C-26	15.64 q	15.66	15.65[b]	15.61	15.62[b]	15.58[a]	15.57[b]	15.68	15.69	15.65	15.89	15.86	15.90
C-27	14.30 q	14.29	14.28	14.17	14.25	14.32	14.29	14.29	14.29	14.27	14.27	14.32	14.34
C-28	176.42 s	175.98	175.69	175.61	175.69	177.07	176.55	175.99	176.00	175.96	175.74	175.69	175.71
C-29	109.18 t	109.13	109.00	108.92	108.98	109.30	109.27	109.09	109.10	109.04	109.08	108.91	109.05
C-30	19.25 q	19.22	19.24	19.13	19.22	19.21	19.19	19.24	19.24	19.23	19.25	19.21	19.22
C-31	40.84 t	34.55	38.88	38.77	38.91 t	41.05	34.48	38.94	38.98	38.99	38.90	38.84	38.88
C-32		33.53	29.51	29.47	29.54 t		33.62	29.46	29.50	29.48	2960	29.51	29.59
C-33			26.51	26.55	26.69 t			26.50	26.64	26.68	26.68	26.60	26.67
C-34			28.75[c]	28.81[a]	29.00[c] t			28.72[b]	28.88[b]	29.16[b]	28.94[a]	28.83[a]	29.18[a]
C-35			28.61[c]	28.72[a]	28.95[c] t			28.62[b]	28.79[b]	28.99[b]	28.84[a]	28.76[a]	28.97[a]
C-36			24.53	28.66[a]	28.91[c] t			24.35	28.70[b]	28.91[b]	28.79[a]	28.70	28.80[a]
C-37			33.72	24.52	28.91[c] t			33.77	24.43	28.91[b]	24.64	24.56	24.64
C-38				33.63	28.80[c] t				33.85	28.75[b]	33.79	33.69	33.74
C-39					24.63 t					24.45			
C-40					33.75 t					33.78			
$\underline{C}OOCH_3$	170.56 s	172.91	173.77	173.88	173.80						174.01	173.85	173.92
$COO\underline{C}H_3$	51.96 q	51.41	51.06	51.01	51.03						51.21	51.05	51.15
$\underline{C}OOH$						173.16s	176.22	178.52	178.75	178.38			
$\underline{C}H_3CO$													21.03q
$CH_3\underline{C}O$													170.67s

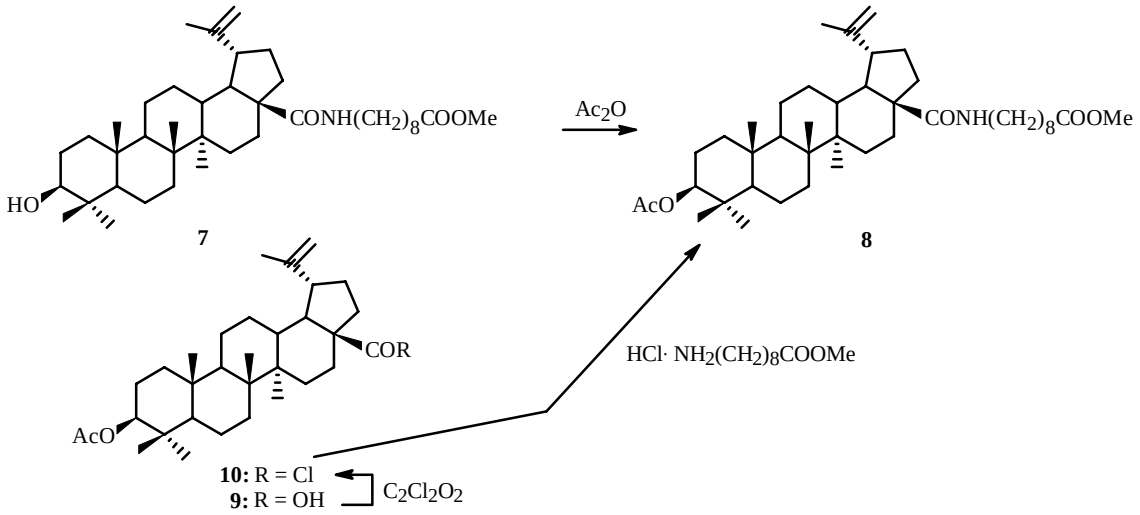
Chemical shifts denoted by identical letters may perhaps be switched within the same column.

Oxime **6** and the 3-hydroxyderivative **7** were prepared from **3d**, which contains the methyl ester of 9-aminopelargonic acid (Scheme 3). The NMR spectra indicate that **6** is formed as one geometric isomer. Thus, its PMR spectrum exhibits one 1H signal at 9.63 ppm, which corresponds to the oxime proton. The ^{13}C NMR spectrum also has one set of signals for the C atoms, which is consistent with the formation of one isomer. Steric considerations and a comparison of the chemical shifts of C-2 (16.93 ppm) and C-4 (40.13 ppm) in the ^{13}C NMR spectrum of **6** with those of starting **3d** (33.75 and 46.90 ppm) indicate that the isomer of **6** shown in Scheme 3 was formed.



6: R = $CONH(CH_2)_8COOMe$ **7:** R = $CONH(CH_2)_8COOMe$

Scheme 3.



Scheme 4.

Reduction of **3d** by $NaBH_4$ in THF produced the 3-hydroxy derivative **7** in 93% yield (Scheme 3). According to the NMR spectra, the reduction is highly stereoselective. The reaction occurs quantitatively with formation of a single epimer (see [26-28]). The orientation of the substituents situated in the 3-position is known to determine the magnitude of the SSCC of the methine proton (H-3) [29]. Unfortunately, this signal in the PMR of **7** is overlapped by those of the CH_2 protons of the amino-acid fragment. The H-3 signal in the 3-acetyl derivatives appears at weaker field [23, 28, 29]. Therefore, we prepared **8** (Scheme 4). The signal for H-3 in the PMR spectrum of **8** appears at 4.38 ppm. The magnitude of the SSCC (J_1 = 10.2 Hz, J_2 = 6.6 Hz) is consistent with the α(axial)-orientation of H-3 and the β(equatorial)-orientation of the AcO group and, therefore, the OH group in **7** (see [30, 31]). The stereochemistry of the C-3 acetate was also confirmed by direct synthesis of **8** from 3β-acetyl-betulinic acid **9**, a compound known to have an acetate with the β-orientation (Scheme 4).

The acid chloride of betulonic acid (**2**) was synthesized in 89% yield by reacting **1** with oxalyl chloride in anhydrous CH_2Cl_2 (Scheme 1). The physicochemical properties of **2** have not been reported although its preparation has [13]. Betulonic acid (**1**) was prepared by oxidation of betulin using modified methods [26, 27].

EXPERIMENTAL

IR spectra were recorded on a Vector-22 instrument in CCl_4; NMR spectra, on Bruker AC-200 and Bruker AM-400 instruments at working frequencies 200.13 and 400.13 MHz for ^1H and 50.32 and 100.61 MHz for ^{13}C in $CDCl_3$. The multiplicity of signals in the ^{13}C NMR spectra was determined by standard methods for obtaining spectra using J-modulation (JMOD) and with off-resonance saturation of protons. 2D ^1H—^1H (COSY) and ^{13}C—^1H(COSY 125 Hz, COLOC 7 Hz) NMR

spectra of **2**, **3a**, **3d**, **5b**, **6**, and **8** were recorded on a Bruker DRX-500 instrument at working frequency 500.13 MHz for ^1H and 125.76 MHz for ^{13}C in $CDCl_3$ using standard Bruker programs. Solvent signals ($CDCl_3$, δ_C = 76.90 ppm) and the signal of residual H in $CDCl_3$ (δ_H = 7.24 ppm) were used as internal standards. Mass spectra were measured in a high-resolution Finnigan MAT-8200 mass spectrometer at 70 eV ionization potential. Specific rotations ($[\alpha]_{580}$) were measured on a Polamat A polarimeter in $CHCl_3$ at room temperature (20-25°C); melting points, on a Kofler micro-heating stage. Elemental analyses were performed on a Carlo Erba 1106 CHN analyzer.

The course of reactions and purity of products were monitored by TLC on Silufol UV-254 plates using the solvent systems $CHCl_3$—CH_3CN (15:1) for **3a-e** and **5-8** and $CHCl_3$—EtOH (10:1) for **4a-e**. Spots were developed by spraying with H_2SO_4 (20%) and heating to 100°C. Column chromatography was performed over aluminum oxide (activity II, CH_2Cl_2 eluent) and silica gel [KSK, 70-140 μ, eluent $CHCl_3$ containing CH_3CN (5-10%)].

We used glycine, β-alanine, 11-aminoundecanoic acid, oxalyl chloride, N,N-dicyclohexylcarbodiimide, and Me_3SiCl (Reanal and Aldrich) without further purification. 1-Hydroxybenzotriazole (Lancaster) was recrystallized from water and dried in vacuum at 80°C. Hydrochlorides of 8-aminocaprylic and 9-aminopelargonic acids [25] and β-phenyl-β-alanine [32] were synthesized as before.

Table 1 lists the physicochemical properties of **3-8** (yields, constants, mass-spectral data). Table 2 contains the ^{13}C NMR spectra of **3**, **4**, and **6-8**. Elemental analyses of the compounds agreed with those calculated.

3-Oxo-20(29)-lupen-28-oic Acid (1). A mixture of betulin (44.2 g) obtained by hot extraction with trichloroethylene of dried bark of white birch and acetic anhydride (150 mL) was boiled for 1 h and cooled to room temperature. The precipitate was filtered off, washed with water, and dried in air. The diacetate of betulin was dissolved in CH_2Cl_2 (50 mL) and passed over a layer of Al_2O_3 (35×8 cm) with elution by CH_2Cl_2. The filtrate was evaporated. The solid was dissolved in MeOH—THF (410—600 mL), cooled to 0°C, treated with NaOH solution (77 mL, 4 N), held at room temperature for one week, and poured onto a mixture of ice and dilute HCl. The precipitate was filtered off, washed with water, and dried in air and then a vacuum desiccator over P_2O_5 to give betulin (~35 g) containing lupeol (~5% according to PMR). Recrystallization twice from benzene gave pure betulin, mp 259-261°C, lit. [33] mp 261°C.

A suspension of betulin (10 g) containing lupeol in acetone (400 mL, distilled over $KMnO_4$) under Ar at 0°C was treated dropwise with Jones reagent (26 mL) in acetone (100 mL) over 0.5 h. The reaction mixture was stirred at 0°C for 7 h and treated with MeOH until it turned dark green. The reaction mixture was poured onto a mixture of ice and dilute HCl. The precipitate was filtered off, washed with water, and dissolved in Et_2O. The organic layer was washed successively with HCl (10%), H_2O, $NaHCO_3$ (5%), saturated $NaHCO_3$, and H_2O. Most of the Et_2O was distilled off. The remainder was treated with NaOH solution (12 mL, 2 N). The resulting precipitate of the sodium salt of betulonic acid was filtered off, washed successively with Et_2O, saturated NaCl solution, and cold H_2O, and dried over P_2O_5. The dried sodium salt of betulonic acid was dissolved with heating in MeOH and treated with CH_3CO_2H (2 mL). The product was precipitated by warm H_2O. The precipitate was filtered off, washed with H_2O, and dried in a vacuum desiccator over P_2O_5 to give **1** (4.8 g, 47%), mp 230-233°C. After repeated purification through the sodium or ammonium salt, mp 246-248°C, [α] +43° (*c* 4.05); lit. [27] mp 247-249°C; [34] mp 261-264°C, [α] +37.4° (*c* 0.83). ^{13}C NMR spectra are identical to those published [23, 24].

3-Oxo-20(29)-lupen-28-oic acid chloride (2). Betulonic acid (2.82 g, 6.2 mmole) in anhydrous CH_2Cl_2 (70 mL) under Ar was treated with oxalyl chloride (1.1 mL, 12.6 mmole). The reaction mixture was stirred at room temperature for 6 h. Solvent was removed in a rotary evaporator at temperatures <30°C. The solid was treated with anhydrous CH_2Cl_2. Solvent was again removed. This procedure was repeated several times. Then, the solid was washed with anhydrous Et_2O to give **2** (2.60 g, 89%), mp 209-212°C. Mass spectrum, *m*/*z*: 472.31425 $[M]^+$, $C_{30}H_{45}ClO_2$. Calc. 472.31079 ($[M]^+$).

IR spectrum (KBr, ν, cm^{-1}): 1644 (C=C), 1705 (C=O), 1804 (COCl).

PMR spectrum (δ, ppm, J/Hz): 0.87 (3H, s, Me-25), 0.92 (6H, s, Me-26,27), 0.94 (1H, m, H-12), 0.95 (3H, s, Me-24), 1.00 (3H, s, Me-23), 1.20-1.54 (14H, m, H-1,5-7,9,11,15,16,21,22), 1.61 (3H, s, Me-30), 1.63 (1H, m, H-12), 1.70 (1H, t, J = 11.3, H-18), 1.83 (2H, m, H-1,21), 2.16 (2H, m, H-13,22), 2.34 (1H, m, H-2), 2.40 (2H, m, H-2,16), 2.72 (1H, td, J_1 = 11.3, J_2 = 4.3, H-19), 4.56 and 4.66 (2H, both br.s, $=CH_2$).

^{13}C NMR spectrum (δ, ppm): 14.45 (q, C-27), 15.45 (q, C-26), 15.72 (q, C-25), 19.08 (q, C-30), 19.34 (t, C-6), 20.77 (q, C-24), 21.08 (t, C-11), 25.07 (t, C-12), 26.34 (q, C-23), 29.29 (t, C-15), 29.56 (t, C-21), 31.89 (t, C-16), 33.30 (t, C-7), 33.82 (t, C-2), 35.88 (t, C-22), 36.64 (s, C-10), 37.51 (d, C-13), 39.37 (t, C-1), 40.39 (s, C-8), 42.22 (s, C-14), 45.70 (d, C-19), 47.03 (s, C-4), 49.32 (d, C-18), 49.66 (d, C-9), 54.68 (d, C-5), 67.48 (s, C-17), 110.11 (t, C-29), 148.89 (s, C-20), 177.00 (s, C-28), 217.47 (s, C-3).

General Synthesis of Methyl Esters of N-[3-Oxo-20(29)-lupen-28-oyl]-amino Acids (3a-e). A suspension of methyl ester of aminocarboxylic acid hydrochloride (2.6 mmole) in anhydrous CH_2Cl_2 (40 mL) under Ar was treated with distilled Et_3N (0.73 mL, 5.2 mmole) and **2** acid chloride (0.95 g, 2 mmole). The reaction mixture was held at room temperature with periodic stirring for one day, diluted with CH_2Cl_2, washed with HCl in H_2O (10%), dried over anhydrous $MgSO_4$, and evaporated to dryness. Analytically pure samples of **3a-e** were chromatographed over Al_2O_3 and dried in vacuum at 60°C over P_2O_5.

IR spectrum of **3a** (ν, cm^{-1}): 1643 (C=C), 1672 (CONH), 1707 (C=O), 1750 (COOMe).

PMR spectrum (δ, ppm, J/Hz): 0.84 (3H, s, Me-25), 0.90 (6H, s, Me-26,27), 0.94 (3H, s, Me-24), 0.95 (1H, m, H-12), 0.99 (3H, s, Me-23), 1.11 (1H, m, H-15), 1.20-1.45 (11H, m, H-1,5-7,9,11,21,22), 1.49-1.55 (3H, m, H-15,16,18), 1.61 (3H, s, Me-30), 1.64 (1H, m, H-12), 1.75-2.00 (4H, m, H-1,16,21,22), 2.32 (1H, m, H-2), 2.37-2.46 (2H, m, H-2,13), 3.03 (1H, td, J_1 = 11.0, J_2 = 4.6, H-19), 3.67 (3H, s, OMe), 3.91 and 3.94 (2H, AB-system, J_1 = 18.0, J_2 = 5.5, CONH$\underline{CH_2}$), 4.51 and 4.66 (2H, both br.s, $=CH_2$), 6.21 (1H, t, J = 5.5, CONH).

IR spectrum of **3d** (ν, cm^{-1}): 1644 (C=C), 1668 (CONH), 1707 (C=O), 1742 (COOMe).

PMR spectrum (δ, ppm, J/Hz): 0.79 (3H, s, Me-25), 0.84 (6H, s, Me-26,27), 0.88 (3H, s, Me-24), 0.89 (1H, m, H-12), 0.93 (3H, s, Me-23), 1.02 (1H, m, H-15), 1.15-1.39 (23H, m, H-1,5-7,9,11,15,16,21,22,32-36), 1.42 (1H, t, J = 11.5, H-18), 1.47 (2H, m, H-37), 1.54 (3H, s, Me-30), 1.58 (1H, m, H-12), 1.63 (1H, m, H-22), 1.73-1.88 (3H, m, H-1,16,21), 2.16 (2H, t, J = 7.5, $\underline{CH_2}$COOMe), 2.27 and 2.34 (2H, both m, H-2), 2.42 (1H, ddd, J_1 = 12.9, J_2 = 11.5, J_3 = 3.5, H-13), 3.02 (2H, m, H-19, CONH$\underline{CH_2}$), 3.16 (1H, m, CONH$\underline{CH_2}$), 3.52 (3H, s, OMe), 4.44 and 4.59 (2H, both br.s, $=CH_2$), 5.88 (1H, t, J = 5.5, CONH).

General Synthesis of N-[3-Oxo-20(29)-lupen-28-oyl]-amino Acids (4a-e).

A. A solution of **3a-e** (1 mmole) in a mixture of MeOH (10 mL) and THF (5 mL) under Ar at 0°C was treated with NaOH solution (2 mL, 8 mmole, 4 N). The reaction mixture was held at room temperature for one day and poured onto a mixture of ice and dilute HCl. The precipitate was filtered off, washed with H_2O, and dried over P_2O_5. Analytically pure samples of **4a-e** were obtained by chromatography over silica gel and drying at 60°C over P_2O_5.

IR spectrum of **4d** (ν, cm^{-1}): 1645 (C=C), 1668 (CONH), 1709 (C=O, COOH).

PMR spectrum (δ, ppm, J/Hz): 0.84 (3H, s, Me-25), 0.90 (6H, s, Me-26,27), 0.94 (3H, s, Me-24), 0.95 (1H, m, H-12), 0.98 (3H, s, Me-23), 1.10 (1H, m, H-15), 1.12-1.57 (26H, m, H-1,5-7,9,11,15,16,18,21,22,32-37), 1.60 (3H, s, Me-30), 1.65 (2H, m, H-12,22), 1.84 (3H, m, H-1,16,21), 2.24 (2H, t, J = 7.5, $\underline{CH_2}$COOMe), 2.26-2.46 (3H, m, H-2,13), 3.05 (2H, m, H-19, CONH$\underline{CH_2}$), 3.22 (1H, m, CONH$\underline{CH_2}$), 4.50 and 4.65 (2H, both br.s, $=CH_2$), 5.80 (1H, t, J = 5.5, CONH), 8.26 (1H, br.s, COOH).

B. A mixture of amino-acid hydrochloride (2.2 mmole), freshly distilled Me_3SiCl (0.56 mL, 4.4 mmole), and distilled Et_3N (0.31 mL, 2.2 mmole) in anhydrous CH_2Cl_2 (100 mL) was boiled for 4 h under Ar, cooled to 0-5°C, treated with **2** acid chloride (0.95 g, 2 mmole) and distilled Et_3N (0.91 mL, 6.5 mmole), held at room temperature with periodic stirring for one day, diluted with CH_2Cl_2, washed successively with HCl (10%), H_2O, $NaHCO_3$ (5%), and H_2O, dried over anhydrous $MgSO_4$, and evaporated. The solid was chromatographed over silica gel and dried at 60°C over P_2O_5 to give **4c-e** in 65, 61, and 55% yields, respectively.

N′-{N-[3-Oxo-20(29)-lupen-28-oyl]-9-aminononanoyl}-3-aminopropionic Acid (5a). A solution of **4d** (1.41 g, 2.3 mmole) in anhydrous CH_2Cl_2 (150 mL) at 0°C under Ar was stirred and treated with 1-hydroxybenzotriazole (0.3 g, 2.5 mmole) and N,N-dicyclohexylcarbodiimide (0.52 g, 2.5 mmole). The reaction mixture was stirred for 0.5 h at 0°C and 5 h at room temperature, cooled to 0°C, treated with the hydrochloride of β-alanine methyl ester (0.42 g, 3 mmole) and Et_3N (0.42 mL, 3 mmole), held with periodic stirring at room temperature for one day, and cooled to 0°C. The precipitate of dicyclohexylurea was filtered off and washed with cooled CH_2Cl_2. The combined filtrates were washed successively with HCl (10%), H_2O, $NaHCO_3$ (5%), and H_2O, dried over anhydrous $MgSO_4$, and evaporated. The solid was dissolved in a small volume of CH_2Cl_2 (~5 mL) and cooled to -10°C. The precipitate of dicylcohexylurea was filtered off and washed with cooled CH_2Cl_2. The filtrate was evaporated. The procedure was repeated twice to give **5a** as an amorphous powder.

IR spectrum (ν, cm^{-1}): 1668 (CONH), 1706 (C=O), 1736 (COOMe).

PMR spectrum (δ, ppm, J/Hz): 0.80 (3H, s, Me-25), 0.86 (6H, s, Me-26,27), 0.89 (3H, s, Me-24), 0.90 (1H, m, H-12), 0.94 (3H, s, Me-23), 1.04 (1H, m, H-15), 1.11-1.40 (23H, m, H-1,5-7,9,11,15,16,21,22,32-36), 1.45 (1H, t, J = 11.2, H-18), 1.47 (2H, m, H-37), 1.54 (3H, s, Me-30), 1.62 (2H, m, H-12,22), 1.72-1.90 (3H, m, H-1,16,21), 2.00 (1H, t, J = 7.7, $\underline{CH_2}$CONH), 2.21-2.47 (3H, m, H-2,13), 2.40 (2H, t, J = 6.3, $\underline{CH_2}$COOMe), 3.01 (2H, m, H-19, CONHCH_2), 3.13 (1H, m, CONH$\underline{CH_2}$), 3.37 (2H, AB-system, J_1 = 6.3, J_2 = 5.8, $\underline{CH_2}CH_2$COOMe), 3.55 (3H, s, OMe), 4.44 and 4.58 (2H, both br.s, $=CH_2$), 5.86 (1H, t, J = 5.5 Hz, CONH), 6.32 (1H, t, J = 5.8, CONH).

^{13}C NMR spectrum (δ, ppm): 14.18 (q, C-27), 15.57 (q, C-25,26), 19.13 (q, C-30), 19.30 (t, C-6), 20.63 (q, C-24), 21.16 (t, C-11), 25.20 (t, C-37), 25.31 (t, C-12), 26.29 (q, C-23), 26.50 (t, C-33), 28.78, 28.70, 28.64 (t, C-34-36), 29.06 (t, C-15), 29.40 (t, C-32), 30.54 (t, C-21), 33.34 (t, C-7,16), 33.58 (t, C-41), 33.74 (t, C-2), 34.54 (t, C-40), 36.16 (t, C-38), 36.56 (s, C-10), 37.38 (d, C-13), 38.05 (t, C-22), 38.78 (t, C-31), 39.28 (t, C-1), 40.36 (s, C-8), 42.16 (s, C-14), 46.33 (d, C-19), 46.90 (s, C-4), 49.66 (d, C-9 or C-18), 49.80 (d, C-18 or C-9), 51.27 (q, C-43), 54.86 (d, C-5), 55.18 (s, C-17), 108.92 (t, C-29), 150.56 (s, C-20), 172.44 (s, C-42), 172.62 (s, C-39), 175.70 (s, C-28), 217.60 (s, C-3).

N′-{N-[3-Oxo-20(29)-lupen-28-oyl]-9-aminononanoyl}-3-amino-3-phenylpropionic Acid (5b). The reaction was performed analogously using **4d** and β-alanine. An analytically pure sample of **5b** was obtained by chromatography over Al_2O_3 and drying in vacuum at 60°C over P_2O_5.

IR spectrum (ν, cm^{-1}): 1669 (CONH), 1706 (C=O), 1739 (COOMe).

PMR spectrum (δ, ppm, J/Hz): 0.84 (3H, s, Me-25), 0.90 (6H, s, Me-26,27), 0.93 (3H, s, Me-24), 0.94 (1H, m, H-12), 0.98 (3H, s, Me-23), 1.08 (1H, m, H-15), 1.18-1.45 (23H, m, H-1,5-7,9,11,15,16,21,22,32-36), 1.48 (1H, t, J = 11.3, H-18), 1.54 (2H, m, H-37), 1.60 (3H, s, Me-30), 1.64 (1H, m, H-12), 1.67 (1H, m, H-22), 1.78-1.91 (3H, m, H-1,16,21), 2.12 (1H, t, J = 7.4, $\underline{CH_2}$CONH), 2.31 and 2.39 (2H, both m, H-2), 2.47 (1H, ddd, J_1 = 13.0, J_2 = 11.3, J_3 = 3.6, H-13), 2.74 and 2.83 (2H, ABX-system with J_1 = 15.6, J_2 = 6.1 and J_1 = 15.6, J_2 = 6.4, $\underline{CH_2}$COOMe), 3.06 (2H, m, H-19, CONH$\underline{CH_2}$), 3.17 (1H, m, CONH$\underline{CH_2}$), 3.52 (3H, s, OMe), 4.51 and 4.65 (2H, both br.s, =CH_2), 5.36 (1H, dt, J_1 = 8.5, J_2 = 6.1, C$\underline{H}$Ph), 5.79 (1H, t, J = 5.5, CONH), 6.75 (1H, d, J = 8.5, CONH), 7.14-7.25 (5H, m, Ph).

^{13}C NMR spectrum (δ, ppm): 14.27 (q, C-27), 15.65 (q, C-25 or C-26), 15.70 (q, C-26 or C-25), 19.21 (q, C-30), 19.38 (t, C-6), 20.73 (q, C-24), 21.22 (t, C-11), 25.29 (t, C-37), 25.38 (t, C-12), 26.35 (q, C-23), 26.58 (t, C-33), 28.76, 28.79, 28.89 (t, C-34-36), 29.13 (t, C-15), 29.49 (t, C-32), 30.61 (t, C-21), 33.44 (t, C-7,16), 33.84 (t, C-2), 36.33 (t, C-38), 36.63 (s, C-10), 37.47 (d, C-13), 38.15 (t, C-22), 38.86 (t, C-31), 39.35 (t, C-1), 39.68 (t, C-41), 40.43 (s, C-8), 42.24 (s, C-14), 46.42 (d, C-19), 47.01 (s, C-4), 49.18 (d, C-40), 49.73 (d, C-9), 49.85 (d, C-18), 51.43 (q, C-43), 54.73 (d, C-5), 55.25 (s, C-17), 109.03 (t, C-29), 125.98 (2C, d, Ph), 127.22 (d, Ph), 128.33 (2C, d, Ph), 140.53 (s, Ph), 150.64 (s, C-20), 171.27 (s, C-42), 172.09 (s, C-39), 175.73 (s, C-28), 217.81 (s, C-3).

Methyl Ester of N-[3-Hydroxyimino-20(29)-lupen-28-oyl]-9-amino Acid (6). A. A solution of **3d** (0.20 g, 0.32 mmole) in alcohol (6.5 mL) was treated with hydroxylamine hydrochloride (0.05 g, 0.71 mmole) in pyridine (1.3 mL). The reaction mixture was held at room temperature with periodic stirring for one day and poured onto a mixture of ice and HCl. The precipitate was filtered off, washed with H_2O, and dried in a desiccator over P_2O_5.

IR spectrum (ν, cm^{-1}): 1500, 1639, 1668, 1742 (COOMe).

PMR spectrum (δ, ppm, J/Hz): 0.85 (3H, s, Me-25), 0.87 (3H, s, Me-27), 0.89 (3H, s, Me-26), 0.91 (1H, m, H-12), 0.95 (1H, m, H-5), 0.96 (3H, s, Me-24), 1.06 (3H, s, Me-23), 1.07 (1H, m, H-15), 1.18-1.46 (21H, m, H-6,7,9,11,15,16,21,22,32-36), 1.47 (1H, t, J = 11.2, H-18), 1.54 (2H, m, H-37), 1.60 (3H, s, Me-30), 1.63 (1H, m, H-12), 1.68 (1H, m, H-22), 1.72 (1H, m, H-1), 1.87 (2H, m, H-16,21), 2.15 (1H, m, H-2), 2.23 (1H, t, J = 7.6, $\underline{CH_2}$COOMe), 2.42 (1H, ddd, J_1 = 12.8, J_2 = 11.6, J_3 = 3.4, H-13), 2.92 (1H, dt, J_1 = 15.2, J_2 = 4.6, H-2), 3.08 (2H, m, H-19, CONH$\underline{CH_2}$), 3.22 (1H, m, CONH$\underline{CH_2}$), 3.59 (3H, s, OMe), 4.51 and 4.66 (2H, both br.s, =CH_2), 5.70 (1H, t, J = 5.6, CONH), 9.63 (1H, br.s, =NOH).

B. Compound **3d** (0.62 g, 1 mmole) in alcohol (20 mL) was treated with hydroxylamine hydrochloride (0.11 g, 1.57 mmole) and CH_3CO_2Na (0.21 g, 2.56 mmole). The reaction mixture was boiled for 7 h, cooled to room temperature, and worked up as described above. The yield of **6** was 96%.

Methyl Ester of N-[3β-Hydroxy-20(29)-lupen-28-oyl]-9-amino Acid (7). A solution of **3d** (0.50 g, 0.8 mmole) in anhydrous THF (25 mL) at 0°C was vigorously stirred and treated in small portions with $NaBH_4$ (0.5 g, 13.2 mmole). The reaction mixture was held at room temperature for 5 h and poured onto a mixure of ice and dilute HCl. The precipitate was filtered off, washed with H_2O, and dried in a vacuum desiccator over P_2O_5. An analytically pure sample of **7** was obtained by chromatography over Al_2O_3 and drying in vacuum at 60°C over P_2O_5.

IR spectrum (ν, cm^{-1}): 1645 (C=C), 1667 (CONH), 1741 (COOMe), 3467 (OH).

PMR spectrum (δ, ppm, J/Hz): 0.82 (3H, s, Me-24), 0.85 (3H, s, Me-25), 0.92 (3H, s, Me-26), 0.93 (6H, s, Me-23,27), 1.65 (3H, s, Me-30), 2.18 (2H, t, J = 7.6, $\underline{CH_2}$COOMe), 2.43 (1H, ddd, J_1 = 12.7, J_2 = 11.5, J_3 = 3.6, H-13), 3.27-3.02 (4H, m, H-3,19, CONH$\underline{CH_2}$), 3.54 (3H, s, OMe), 4.46 and 4.61 (2H, both br.s, =CH_2), 5.78 (1H, t, J = 5.7, CONH) (only characteristic signals are given).

3β-Acetoxy-20(29)-lupen-28-oic Acid (9). A mixture of pure betulin (4.42 g, 10 mmole) and acetic anhydride (15 mL) was boiled for 1 h and cooled to room temperature. The precipitate was filtered off, washed with H_2O, and dried in

air. The resulting betulin diacetate (5.04 g, 9.6 mmole, 96%) was dissolved in THF (160 mL), cooled, treated with KOH (0.56 g, 10.0 mmole) in MeOH (90 mL), held at room temperature for 12 h, and poured onto ice and dilute HCl. The precipitate was filtered off, washed with H_2O, dried in air, and chromatographed over Al_2O_3 to give 3β-acetylbetulin (2.97 g, 64%), which was oxidized by Jones reagent analogously to betulin to give 3β-acetylbetulinic acid (**9**), yield 45%, mp 280-282°C, lit. [27] mp 288-290°C, [6] mp 283-285°C. ^{13}C NMR spectra are identical with those previously reported [27].

3β-Acetoxy-20(29)-lupen-28-oic acid chloride (10) was synthesized from 3β-acetylbetulinic acid (**9**) analogously to the acid chloride of **2** in 85% yield, mp 219-222°C, lit. [35] mp 232-233°C.

Mass spectrum, *m*/*z*: 516 $[M]^+$. $C_{32}H_{49}ClO_3$. Calc.: 516 $[M]^+$.

IR spectrum (KBr, ν, cm^{-1}): 1644 (C=C), 1736 (AcO), 1803 (COCl).

N-[3β-Acetoxy-20(29)-lupen-28-oyl]-9-amino Acid Methyl Ester (8). A. A suspension of the methyl ester of 9-aminopelargonic acid hydrochloride (0.22 g, 0.98 mmole) in anhydrous CH_2Cl_2 (17 mL) under Ar was treated with distilled Et_3N (0.3 mL, 2.1 mmole) and **10** (0.42 g, 0.81 mmole). The reaction mixture was held at room temperature with periodic stirring for one day, diluted with CH_2Cl_2, washed with HCl (10%) and H_2O, dried over anhydrous $MgSO_4$, and evaporated to dryness. An analytically pure sample of **8** was obtained by chromatography over Al_2O_3 and drying in vacuum at 40°C over P_2O_5.

IR spectrum (ν, cm^{-1}): 1643 (C=C), 1668 (CONH), 1737 (COOMe, AcO).

PMR spectrum (δ, ppm, J/Hz): 0.71 (1H, m, H-5), 0.76 (9H, s, Me-23-25), 0.86 (3H, s, Me-26), 0.88 (3H, s, Me-27), 0.95 (1H, m, H-12), 1.05 (1H, m, H-15), 1.16-1.59 (28H, m, H-1,2,6,7,9,11,15,16,18,21,22,32-37), 1.60 (3H, s, Me-30), 1.65 (2H, m, H-12,22), 1.87 (2H, m, H-16,21), 1.95 (3H, s, AcO), 2.21 (2H, t, J = 7.5, $\underline{CH_2}$COOMe), 2.40 (1H, ddd, J_1 = 12.8, J_2 = 11.5, J_3 = 3.8, H-13), 3.07 (2H, m, H-19, CONH$\underline{CH_2}$), 3.21 (1H, m, CONH$\underline{CH_2}$), 3.58 (3H, s, OMe), 4.38 (1H, dd, J_1 = 10.2, J_2 = 6.6, H-3), 4.50 and 4.65 (2H, both br.s, =CH_2), 5.72 (1H, t, J = 5.6, CONH).

B. A mixture of **7** (0.1 g, 0.16 mmole) and acetic anhydride (1 mL) in anhydrous pyridine (5 mL) was held at room temperature for two days and poured onto a mixture of ice and dilute HCl. The precipitate was filtered off, washed with H_2O, and dried in a desiccator over P_2O_5 to give a compound (0.12 g, 95%), the physicochemical properties of which were identical to those of **8**.

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