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SYNTHESIS AND STUDY OF MUTAGENIC PROPERTIES OF LUPANE TRITERPENOIDS CONTAINING 1,2,3-TRIAZOLE FRAGMENTS IN THE C-30 POSITION

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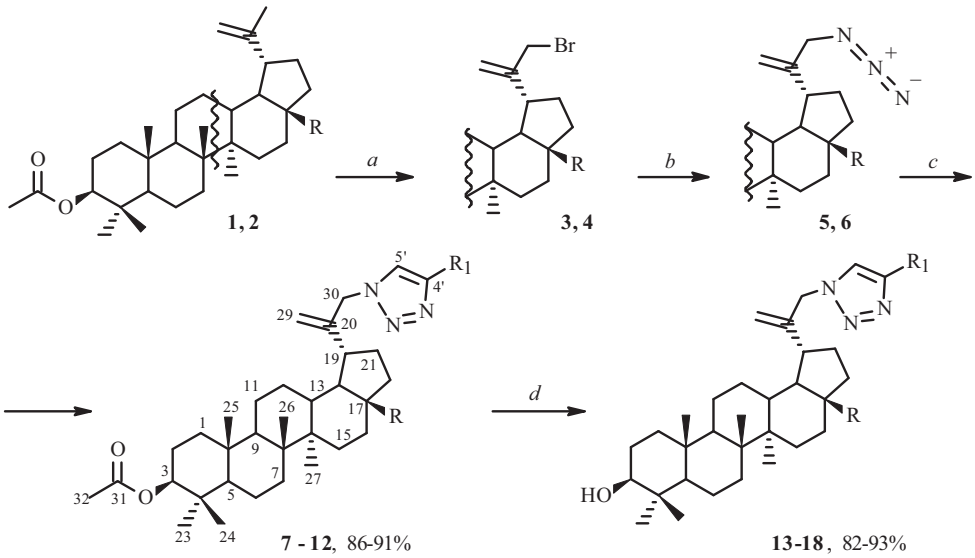
*New derivatives of betulin, betulinic acid methyl ester, and their acetate analogs containing 1,2,3-triazole fragments in the C-30 position were synthesized. It was shown using the Ames test that 30-azidolup-20(29)-enes and 3β,28-diacetoxy-30-(4-*R*-1,2,3-triazol-1-yl)lup-20(29)-enes did not exhibit mutagenic properties.*

Keywords: betulin, betulinic acid, 1,2,3-triazoles, Cu-catalyzed 1,3-dipolar cycloaddition reaction, XSA, Ames test.

Lupane triterpenoids exhibit various types of biological activity and are interesting as starting materials for preparing pharmacologically valuable compounds. Thus, betulin and its derivatives, in particular betulinic and betulonic acids, afforded a series of derivatives containing various substituents in the C-3 and C-28 positions [1, 2]. Significantly less attention has been paid to chemical transformations of lupane triterpenoids at the C-30 position [1–5].

Therefore, it seemed interesting to synthesize and study new derivatives of lupane triterpenoids containing various substituents in the C-30 position, in particular, a 1,2,3-triazole group. Compounds with this structural fragment are known to exhibit antibacterial [6] and antitumor [7] activity. Selective inhibitors of HIV-1 protease [8] and tyrosine kinase [9] were discovered among triazole-substituted compounds.



7: R = CH_2OAc, R_1 = CH_2OH; **8:** R = CH_2OAc, R_1 = Ph; **9:** R = CH_2OAc, R_1 = pyridyn-2-yl
10, 16: R = COOMe, R_1 = CH_2OH; **11, 17:** R = COOMe, R_1 = Ph; **12, 18:** R = COOMe, R_1 = pyridyn-2-yl
13: R = R_1 = CH_2OH; **14:** R = CH_2OH, R_1 = Ph; **15:** R = CH_2OH, R_1 = pyridyn-2-yl

a. NBS, CCl_4, 20°C; *b*. NaN_3, MeCN, reflux; *c*. R_1-C≡CH, $CuSO_4 \cdot 5H_2O$, AscNa, DMF, 50°C; *d*. 4 N NaOH, MeOH, THF, 20°C, 24 h

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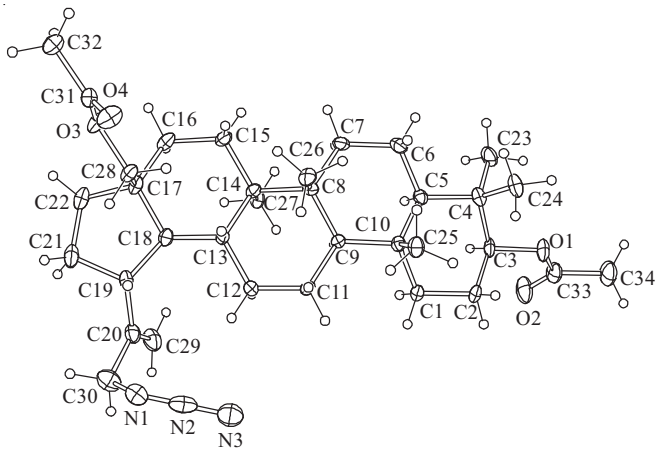

Fig. 1. Molecular structure and atomic numbering of 3β,28-diacetoxy-30-azidolup-20(29)-ene (**5**).

Herein we present results for the synthesis of derivatives of betulin and betulinic acid methyl ester and their acetate analogs containing a 1,2,3-triazole group in the C-30 position and the analysis of the mutagenic properties of several synthesized compounds in two standard histidine-dependent test strains *Salmonella typhimurium* TA98 (hisD3052) and TA102 (hisG428).

Betulin diacetate (**1**) and 3β-acetylbetulinic acid methyl ester (**2**) were used as starting materials for preparing new derivatives containing various 1,2,3-triazoles in the C-30 position. The key 1,2,3-triazole precursors were azides [10]. A method for preparing them was the reaction of the bromo-derivatives with sodium azide [10, 11].

Reaction of 30-bromo-derivatives **3** and **4**, which were synthesized by the literature method [4], with sodium azide produced the corresponding derivatives of betulin diacetate (**5**) and 3β-acetylbetulinic acid methyl ester (**6**) containing a C-30 azide group. The reaction was carried out with refluxing in CH_3CN for 24 h. The products from the reaction of **5** and **6** were isolated by column chromatography over Al_2O_3 in 91 and 87% yield, respectively.

Target 1,2,3-triazole derivatives **7**–**12** were prepared by Cu-catalyzed 1,3-dipolar cycloaddition of azides **5** and **6** to terminal acetylenes. The reaction was carried out in DMF with heating to 50°C in the presence of copper sulfate and sodium ascorbate [10]. The synthesized 1,2,3,-triazoles **7**–**12** were isolated by chromatography over silica gel in 86–91% yield.

Alkaline hydrolysis of **5**–**12** by NaOH solution (4 N) in MeOH and THF at room temperature afforded new triazole derivatives **13**–**18** in good yields.

The compositions and structures of **5**–**18** were confirmed by mass, IR, and NMR spectra. An X-ray crystal structure analysis (XSA) was performed for betulin diacetate azide **5**. Figure 1 shows the space structure of **5**. The bond lengths and ring conformations in the lupane framework of **5** were similar to the literature data for betulin diacetate (**1**) [12]. Thus, the conformation of the five-membered ring was an envelope with C^{17} deviating from the plane of the other atoms by an angle of 41.7°. The conformations of all six-membered rings were chairs. The conformations of the C^3 and C^{17} substituents in **5** also agreed with those in **1** [12]. The torsion angles $C^4C^3O^1C^{33}$ and $C^{16}C^{17}C^{28}O^3$ were –145.1° and 62.3°. The orientation of the C^{19} isopropyl group was slightly different after adding the azide to the C^{30} position. The $C^{18}C^{19}C^{20}C^{29}$ torsion angle in **1** was –47.8° and decreased to –29.6° in **5**. Compound **5** had shortened intramolecular contacts C–H…O, $H^3…O^2$ 2.34, $H^{22A}…O^3$ 2.48, $H^{23B}…O^1$ 2.50, and $H^{16A}…O^3$ 2.51 Å [distances C…O 2.703(4), 2.904(4), 2.896(4), and 2.889(3) Å; and C–H…O angles 100, 105, 104, 102°] and C^{12}–H…N^3 with parameters H…N 2.60, C…N 3.488(5) Å and angle C–H…N 150° (sum of Van-der-Waals radii H…O 2.68, C…O 3.35, H…N 2.74, and C…N 3.41 Å [13]). The shortened intermolecular contact C^{30}–H…O^2 [distances H…O 2.60, C…O 3.443(4) Å and angle C–H…O 144°] could be identified in the three-dimensional crystal structure of **5**.

A characteristic feature of IR spectra of **5** and **6** was a strong absorption band in the range 2098–2110 cm^{-1} that corresponded to azide stretching vibrations. PMR and ^{13}C NMR spectra of **7**–**18** agreed fully with their structures and contained one set of resonances for the lupane framework and the C-30 substituent. This indicated that a single structural isomer had formed. Resonances in NMR spectra of **7**–**18** were assigned based on two-dimensional NMR spectra of **7**, **9**, **12**, and **17** with referral to the literature data for betulin and betulinic acid [14–17]. Formation of the 1,2,3-triazole ring was confirmed by a singlet for H-5′ (7.50–8.18 ppm) in the PMR spectrum and resonances for C-4′ and C-5′ of the triazole ring in the ^{13}C NMR spectrum in the ranges 147.70–149.53 and 119.91–122.67 ppm, respectively.

TABLE 1. Mutagenic Activity of **5**–**9** in the Ames Test

Compound	Concentration, M	Number of colonies in Petri dish		Compound	Concentration, M	Number of colonies in Petri dish	
		TA98	TA102			TA98	TA102
DMSO	–	18 ± 3	343 ± 7	**6**	0.01	19 ± 2	341 ± 8
t-BuO_2H*	10^{-5}	–	528 ± 17**	**7**	0.01	21 ± 3	348 ± 14
NQO*	1.6×10^{-3}	164 ± 11**	–	**8**	0.01	19 ± 2	340 ± 7
5	0.01	17 ± 2	347 ± 9	**9**	0.01	22 ± 3	341 ± 10

*Positive controls, *tret*-butylhydroperoxide (*t*-BuO_2H) and 4-nitroquinoline-1-oxide (NQO); **values statistically significant from the control with probability $P < 0.005$.

TABLE 2. Physicochemical Characteristics of **7**–**18**

Compound	Yield, %	mp, °C	[α],°(*c*, g/100 mL)	Empirical formula	M^+	
					found	calculated
7	91	142–144	+2 (0.75)	$C_{37}H_{57}N_3O_5$	623.4299	623.4297
8	87	125–127	+10 (1.02)	$C_{42}H_{59}N_3O_4$	669.4504	669.4500
9	91	131–133	+5 (1.56)	$C_{41}H_{58}N_4O_4$	670.4400	670.4453
10	86	132–133	+8 (1.02)	$C_{36}H_{55}N_3O_5$	609.4299	609.4297
11	92	115–117	+2 (1.94)	$C_{41}H_{57}N_3O_4$	655.4338	655.4344
12	87	111–113	–2 (1.61)	$C_{40}H_{56}N_4O_4$	656.4294	656.4296
13	82	198–200	+6 (1.32)	$C_{33}H_{53}N_3O_3$	539.4252	539.4297
14	86	203–205	–9 (0.98)	$C_{38}H_{55}N_3O_2$	585.4293	585.4289
15	84	219–220	+3(1.47)	$C_{37}H_{54}N_4O_2$	586.4236	586.4242
16	87	223–225	+2 (1.69)	$C_{34}H_{53}N_3O_4$	567.4256	567.4297
17	93	182–183	–8 (1.40)	$C_{39}H_{55}N_3O_3$	613.4504	613.4500
18	86	196–198	+10 (0.53)	$C_{38}H_{54}N_4O_3$	614.4546	614.4539

An analysis of the 2D ^{13}C–^{1}H (COLOC) NMR spectrum of 1,2,3-triazole derivative **7**, in which cross peaks were observed between H-30 (4.89 ppm) and C-5′ (122.07 ppm), indicated that a 4-substituted 1,2,3-triazole had formed. This was consistent with the regioselectivity characteristic of a wide range of Cu-catalyzed 1,3-dipolar cycloaddition reactions [18]. PMR spectra of **13**–**18** lacked resonances for acetates at 2.00–2.02 ppm and exhibited H-3 resonances that were shifted by 1.3 ppm to strong field. This agreed with the literature data for betulin and its acylated derivatives [14–17].

The Ames test was carried out in order to analyze the mutagenic properties of **5**–**9**. We used the two standard test strains *Salmonella typhimurium* TA98 (hisD3052) and TA102 (hisG428) that contained various point mutations in the histidine operon [19–21] and exhibited increased cell-wall permeability and a compromised DNA-repair system [21].

Addition of **5**–**9** at a concentration of 0.01 M to cells of the two *S. typhimurium* strains did not cause a statistically significant increase of reversion compared with the control (addition of only DMSO) (Table 1). This suggested that these compounds did not possess mutagenic properties.

Thus, Cu(I)-catalyzed 1,3-dipolar cycloaddition of 30-azidolup-20(29)-enes to terminal alkynes was an effective method for modifying lup-20(29)-enes by introducing substituents into the C-30 position. It was shown that 30-azidolupenes and triazole derivatives of betulin diacetate did not exhibit mutagenic properties.

TABLE 3. Chemical Shifts of C Atoms in ^{13}C NMR Spectra of **7**–**18**

C atom	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
1	38.33 t	38.32	38.21	38.25	38.34	38.24	38.33	38.36	38.22	38.54	38.60	38.58
2	23.61 t	23.62	23.52	23.53	23.63	23.55	27.61	27.54	27.85	27.32	27.26	27.27
3	80.80 d	80.81	80.70	80.73	80.82	80.75	78.80	78.81	78.79	78.83	78.80	78.81
4	37.73 s	37.74	37.63	37.65	37.73	37.66	38.63	38.75	38.76	38.74	38.73	38.73
5	55.28 d	55.28	55.17	55.24	55.34	55.25	55.19	55.24	55.21	55.21	55.18	55.18
6	18.08 t	18.08	17.98	18.00	18.10	18.02	18.10	18.15	18.09	18.09	18.14	18.14
7	34.05 t	34.05	33.95	34.08	34.18	34.09	34.15	34.09	34.17	34.16	34.18	34.18
8	40.84 s	40.84	40.74	40.52	40.63	40.54	40.49	40.55	40.58	40.54	40.54	40.53
9	50.11 d	50.12	50.01	50.20	50.31	50.21	50.32	50.45	50.53	50.43	50.47	50.31
10	36.99 s	36.98	36.88	36.95	37.04	36.96	37.04	37.48	37.00	36.96	37.05	37.05
11	20.81 t	20.83	20.70	20.81	20.93	20.81	20.82	20.83	20.82	20.79	20.84	20.81
12	26.90 t	26.85	26.69	26.74	26.86	26.73	26.80	26.85	26.80	26.79	26.78	26.75
13	37.33 d	37.34	37.25	37.98	38.11	38.02	37.12	37.10	37.08	37.14	38.07	38.05
14	42.61 s	42.63	42.53	42.18	42.30	42.21	42.36	42.30	42.28	42.34	42.23	42.23
15	26.80 t	26.92	26.82	26.74	26.86	29.47	26.59	26.49	26.56	26.66	29.49	29.48
16	29.72 t	29.74	29.63	29.44	29.55	31.71	29.63	29.74	29.75	29.74	31.75	31.69
17	46.29 s	46.30	46.18	56.47	56.43	56.33	46.09	46.10	46.08	56.36	56.36	56.33
18	49.94 d	49.96	49.91	50.47	50.56	50.54	49.95	49.94	49.89	50.49	50.33	50.54
19	43.74 d	43.79	43.88	43.16	43.37	43.42	43.75	43.79	43.87	42.17	46.43	43.47
20	148.97 s	149.14	148.73	149.29	147.88	149.16	148.87	149.00	148.99	149.19	149.46	149.15
21	31.19 t	31.28	31.07	31.77	31.88	31.82	30.72	31.04	31.18	31.76	31.82	31.85
22	34.26 t	34.30	34.21	36.39	36.50	36.46	34.27	34.35	34.33	36.42	36.45	36.49
23	27.88 q	27.88	27.79	27.79	27.88	27.80	27.84	27.87	27.90	27.86	27.85	27.85
24	16.09 q	16.10	16.34	16.35	16.44	16.36	15.66	15.54	15.68	15.91	15.98	15.99
25	16.43 q	16.44	15.99	16.04	16.12	16.04	16.09	16.10	16.09	15.82	15.81	15.81
26	15.96 q	15.97	15.87	15.79	15.89	15.81	15.94	15.99	15.98	15.29	15.24	15.26
27	14.66 q	14.69	14.57	14.51	14.62	14.52	14.66	14.56	14.68	14.56	14.58	14.58
28	62.33 t	62.36	62.26	176.15	176.22	176.15	62.28	62.36	62.23	176.17	176.16	176.16
29	111.95 t	112.15	112.12	111.52	111.84	111.76	111.65	112.19	112.05	111.64	111.71	111.62
30	54.45 s	54.65	54.41	54.46	54.60	54.39	54.41	54.47	55.36	54.42	54.38	54.35
31	170.97 s	170.98	170.84	170.91	170.94	170.88						
32	20.96 q	20.97	21.16	21.19	21.27	21.20						
33	171.45 s	171.44	171.31									
34	21.27 q	21.29	20.85									
O<u>C</u>H$_3$				51.29	51.35	51.28				51.34	51.29	51.31
4′	147.99 s	148.00	148.45	147.70	149.53	149.16	148.36	148.07	148.38	149.34	147.83	148.33
5′	122.07 d	119.91	122.37	122.08	120.01	122.57	122.15	119.96	122.42	122.06	119.93	122.67
<u>C</u>H$_2$OH	56.49 t			56.32			56.21			56.12		
<u>Ar</u>												
6′		130.54	150.10		130.59	150.02		130.56	150.01		130.53	149.83
7′		125.69	–		125.70	–		124.97	–		125.63	–
8′		128.79	149.21		128.77	148.98		128.59	149.86		128.69	148.95
9′		128.14	122.74		128.10	122.78		128.10	122.78		128.02	122.88
10′		128.79	136.81		128.77	137.03		128.59	137.20		128.69	137.19
11′		125.69	120.13		125.70	120.24		124.97	120.16		125.63	120.30

EXPERIMENTAL

IR spectra were recorded in KBr pellets on a Vector-22 instrument. Mass spectra were measured in a high-resolution DFS (Double Focusing Sector) mass spectrometer (Thermo Electron Corp.) at ionizing potential 70 eV. Specific rotations ($[\alpha]_D$) were measured on a PolAAr3005 polarimeter in $CHCl_3$ at room temperature (20–25°C). NMR spectra of $CDCl_3$ solutions were recorded on Bruker AV-300 (operating frequencies 300.13 MHz for ^1H and 75.47 MHz for ^{13}C) and AV-400 (operating frequencies 400.13 MHz for ^1H and 100.78 MHz for ^{13}C) spectrometers. Multiplicities of resonances in ^{13}C NMR spectra were determined by standard methods for recording spectra in J-modulation (JMOD) and with off-resonance

proton suppression. 2D NMR ^{1}H–^{1}H (COSY) and ^{13}C–^{1}H (COXH, COLOC) spectra of **7**, **9**, **12**, and **17** were recorded in $CDCl_3$ on a Bruker AV-600 instrument at operating frequency 600.30 MHz for ^{1}H and 150.96 MHz for ^{13}C using standard Bruker programs. Solvent resonances of $CDCl_3$ (δ_C 76.90 ppm) and residual $CHCl_3$ protons (δ_H 7.24 ppm) were used as internal standards. Various types of H–H and C–H correlation spectroscopy (COSY, COXH, COLOC) and literature data [14–17] were used to assign resonances in NMR spectra.

The course of reactions and purity of products were monitored by TLC on Silufol UV-254 plates using $CHCl_3$–CH_3CN (15:1) or $CHCl_3$ as eluents. Spots were detected by spraying with H_2SO_4 (20%) and subsequent heating to 100°C. The syntheses of triterpenoid bromo-derivatives **3** and **4** were reported by us previously [4]. We used commercial agar (Difco, USA), glucose (Alfa Aesar), 4-nitroquinoline-1-oxide and *t*-butylhydroperoxide (Sigma, USA), and ampicillin (AppliChem, Germany). Histidine-dependent stains of *S. typhimurium* TA98 (rfa, ΔuvrB, +R) and TA102 (rfa, +, +R) were supplied by Dr. B. Ames (USA) [19, 21]. Both strains were checked for the principal genetic markers before use. Table 2 presents the physicochemical characteristics of **7**–**18** (yields, constants, mass spectra). Table 3 presents ^{13}C NMR spectra.

Ascorbic Acid Sodium Salt. A mixture of ascorbic acid (1.77 g, 0.01 mol) and $NaHCO_3$ (0.85 g, 0.01 mol) in H_2O (10 mL) was stirred at room temperature for 0.5 h until CO_2 evolution ceased and treated with EtOH–CH_3CN (1:1, 10 mL). The resulting precipitate was filtered off to afford sodium ascorbate (1.75 g, 87%) that was then used without additional purification. $C_6H_7NaO_6$, mp 221°C (dec.).

General Method for Synthesizing 30-azide Derivatives (5, 6). A mixture of **3** or **4** (0.6 mmol) and NaN_3 (0.08 g, 1 mmol) in CH_3CN (20 mL) was refluxed for 24 h and poured into a mixture of ice and HCl. The resulting precipitate was filtered off, washed with H_2O until neutral, and dried in air. The resulting compound was dissolved in CH_2Cl_2 (5 mL) and passed over a layer of Al_2O_3 (Reakhim, TU 6-09-3916-78) with elution by CH_2Cl_2 (product isolation was monitored by TLC using CH_2Cl_2 eluent).

3β,28-Diacetoxy-30-azidolup-20(29)-ene (5). Yield 91%, mp 192–193°C (CH_3CN), $[\alpha]_D^{20}$ +1° (*c* 1.40, $CHCl_3$). Found: *m/z* 567.4030 $(M)^+$. $C_{34}H_{53}N_3O_4$. Calcd 567.4031 $(M)^+$. IR spectrum (KBr, ν, cm^{-1}): 1647 (C=C), 1732 (C=O), 2098 (N_3). ^{1}H NMR spectrum (δ, ppm, J/Hz): 0.76 (1H, d, J = 9.4, H-5), 0.81 (3H, s, Me), 0.82 (6H, s, 2Me), 0.94 (3H, s, Me), 1.00 (3H, s, Me), 2.01 (3H, s, Me-34), 2.04 (3H, s, Me-32), 2.34 (1H, m, H-19), 3.73 (2H, br.s, H-30, 30), 3.80 (1H, d, J = 11.0, H-28), 4.22 (1H, d, J = 11.0, H-28), 4.44 (1H, dd, J_1 = 10.6, J_2 = 6.2, H-3), 4.94 and 4.98 (2H, br.s, H-29, 29) (only characteristic proton resonances are given). ^{13}C NMR spectrum (δ, ppm): 14.68 (q, C-27), 16.00 (q, C-26), 16.13 (q, C-25), 16.46 (q, C-24), 18.12 (t, C-6), 20.88 (t, C-11), 21.01 (q, C-32), 21.30 (q, C-34), 23.64 (t, C-2), 26.76 (t, C-12), 26.96 (t, C-15), 27.91 (q, C-23), 29.72 (t, C-16), 31.16 (t, C-21), 34.10 (t, C-7), 34.29 (t, C-22), 37.02 (s, C-10), 37.36 (d, C-13), 37.76 (s, C-4), 38.35 (t, C-1), 40.88 (s, C-8), 42.62 (s, C-14), 44.09 (d, C-19), 46.32 (s, C-17), 49.78 (d, C-9), 50.17 (d, C-18), 55.31 (d, C-5), 55.36 (s, C-30), 62.42 (t, C-28), 80.84 (d, C-3), 111.65 (t, C-29), 148.48 (s, C-20), 170.99 (s, C-31), 171.55 (s, C-33).

3β-Acetoxy-30-azidolup-20(29)-en-28-oic Acid Methyl Ester (6). Yield 87%, mp 167–168°C (CH_3CN), $[\alpha]_D^{20}$ –3° (*c* 1.91, $CHCl_3$). Mass spectrum, *m/z* 525.3809 $(M - N_2)^+$. $C_{33}H_{51}N_3O_4$. Calcd 553.3874 $(M)^+$, 525.3813 $(M - N_2)^+$. IR spectrum (KBr, ν, cm^{-1}): 1643 (C=C), 1716 (COOMe), 1732 (C=O), 2110 (N_3). ^{1}H NMR spectrum (δ, ppm, J/Hz): 0.76 (1H, d, J = 9.4, H-5), 0.80 (3H, s, Me-24), 0.81 (6H, s, Me-23, Me-25), 0.88 (3H, s, Me-26), 0.94 (3H, s, Me-27), 0.96 (1H, m, H-1), 1.03 (1H, m, H-12), 1.15 (1H, m, H-15), 1.18–1.29 (2H, m, H-9, 11), 1.30–1.43 (8H, m, H-6, 6, 7, 7, 11, 15, 16, 21), 1.44–1.50 (2H, m, H-12, 22), 1.53–1.67 (4H, m, H-1, 2, 2, 18), 1.85 (1H, dd, J_1 = 12.6, J_2 = 7.9, H-22), 1.99 (1H, m, H-16), 2.01 (3H, s, Me-32), 2.15 (1H, m, H-13), 2.24 (1H, m, H-21), 2.92 (1H, td, J_1 = 11.0, J_2 = 4.5, H-19), 3.64 (3H, s, OMe), 3.74 (2H, AB-system, J_{gem} = 14.2, H-30, 30), 4.45 (1H, dd, J_1 = 10.8, J_2 = 5.4, H-3), 4.94 and 5.00 (2H, s, H-29, 29). ^{13}C NMR spectrum (δ, ppm): 14.52 (q, C-27), 15.83 (q, C-26), 16.06 (q, C-25), 16.36 (q, C-24), 18.04 (t, C-6), 20.87 (t, C-11), 21.20 (q, C-32), 23.55 (t, C-2), 26.66 (t, C-12), 27.81(q, C-23), 29.53 (t, C-15), 31.85 (t, C-16), 31.87 (t, C-21), 34.12 (t, C-7), 36.54 (t, C-22), 36.97 (s, C-10), 37.67 (s, C-4), 38.01 (d, C-13), 38.27 (t, C-1), 40.57 (s, C-8), 42.20 (s, C-14), 43.40 (d, C-19), 50.26 (d, C-9), 50.38 (d, C-18), 51.24 (q, OMe), 55.28 (d, C-5), 55.38 (s, C-30), 56.43 (s, C-17), 80.78 (d, C-3), 111.25 (t, C-29), 148.89 (s, C-20), 170.88 (s, C-31), 176.30 (s, C-28).

General Method for Synthesizing 30-(1,2,3-triazol-1-yl)lupanes (7–12). A mixture of betulin diacetate azide (0.308 g, 0.5 mmol) and copper sulfate (0.023 g, 0.092 mmol) in DMF (5 mL) was treated with terminal acetylene (0.5 mmol), stirred for 1 h at room temperature, treated with sodium ascorbate (0.023 g, 0.1 mmol), stirred for 24 h at 50°C, and poured out into a mixture of ice and HCl. The resulting precipitate was filtered off, washed with H_2O, and dried in air to afford a compound that was chromatographed over SiO_2 (Acros, 0.035–0.070 mm) using $CHCl_3$ as eluent. Compounds **7**–**10** were additionally recrystallized from MeOH.

3β,28-Diacetoxy-30-(4-hydroxymethyl-1,2,3-triazol-1-yl)lup-20(29)-ene (7). ^{1}H NMR spectrum (δ, ppm, J/Hz): 0.75 (1H, d, J = 9.4, H-5), 0.80 (3H, s, Me-25), 0.81 (6H, s, Me-23, 24), 0.93 (3H, s, Me-27), 0.96 (1H, m, H-1), 0.98 (3H, s, Me-26), 1.09–1.11 (3H, m, H-12, 15, 22), 1.16 (1H, m, H-11), 1.20–1.29 (4H, m, H-9, 15, 16, 21), 1.32–1.40 (4H, m, H-6, 7, 7, 11), 1.47 (1H, m, H-6), 1.54–1.69 (6H, m, H-1, 2, 2, 12, 13, 18), 1.74 (1H, m, H-22), 1.80 (1H, m, H-16), 1.94 (1H, m, H-21), 2.00 (3H, s, Me-32), 2.02 (3H, s, Me-34), 2.30 (1H, m, H-19), 3.72 (1H, d, J = 11.0, H-28), 4.19 (1H, d, J = 11.0, H-28), 4.43 (1H, dd, J_1 = 10.9, J_2 = 5.3, H-3), 4.60 (1H, s, H-29), 4.76 (2H, s, C$\underline{H_2}$OH), 4.89 (2H, AB-system, J_{gem} = 15.5, H-30, 30), 4.99 (1H, s, H-29), 7.50 (1H, s, H-5′).

3β,28-Diacetoxy-30-(4-phenyl-1,2,3-triazol-1-yl)lup-20(29)-ene (8). ^{1}H NMR spectrum (δ, ppm, J/Hz): 0.75 (1H, d, J = 9.4, H-5), 0.81 (6H, s, 2Me), 0.82 (3H, s, Me), 0.92 (3H, s, Me), 0.99 (3H, s, Me), 1.73 (1H, t, J = 11.1, H-18), 2.02 (3H, s, Me-34), 2.03 (3H, s, Me-32), 2.36 (1H, td, J_1 = 10.5, J_2 = 5.1, H-19), 3.73 (1H, d, J = 11.0, H-28), 4.22 (1H, d, J = 11.0, H-28), 4.44 (1H, dd, J_1 = 11.3, J_2 = 5.6, H-3), 4.71 (1H, s, H-29), 4.97 (2H, AB-system, J_{gem} = 15.5, H-30, 30), 5.04 (1H, s, H-29), 7.31 (1H, t, J = 7.4, H-9′), 7.40 (2H, t, J = 7.8, H-8′, 10′), 7.72 (1H, s, H-5′), 7.81 (2H, d, J = 8.7, H-7′, 11′) (only characteristic proton resonances are given).

3β,28-Diacetoxy-30-(4-pyridyl-1,2,3-triazol-1-yl)lup-20(29)-ene (9). ^{1}H NMR spectrum (δ, ppm, J/Hz): 0.73 (1H, d, J = 9.9, H-5), 0.80 (3H, s, Me-24), 0.81 (6H, s, Me-23, 25), 0.92 (1H, m, H-1), 0.93 (3H, s, Me-27), 0.99 (3H, s, Me-26), 1.02–1.17 (4H, m, H-11, 12, 15, 22), 1.20–1.25 (2H, m, H-9, 16), 1.26–1.44 (6H, m, H-6, 7, 7, 11, 15, 21), 1.46 (1H, m, H-6), 1.53–1.66 (5H, m, H-1, 2, 2, 12, 13), 1.71 (1H, t, J = 11.2, H-18), 1.74 (1H, m, H-22), 1.80 (1H, m, H-16), 1.97 (1H, m, H-21), 2.01 (3H, s, Me-34), 2.02 (3H, s, Me-32), 2.37 (1H, td, J_1 = 10.5, J_2 = 5.2, H-19), 3.73 (1H, d, J = 11.1, H-28), 4.21 (1H, d, J = 11.0, H-28), 4.44 (1H, dd, J_1 = 11.1, J_2 = 6.3, H-3), 4.68 (1H, s, H-29), 4.96 (2H, AB-system, J_{gem} = 15.6, H-30, 30), 5.03 (1H, s, H-29), 7.20 (1H, ddd, J_1 = 7.9, J_2 = 4.8, J_3 = 1.2, H-9′), 7.76 (1H, td, J_1 = 7.7, J_2 = 1.8, H-10′), 8.11 (1H, s, H-5′), 8.16 (1H, dt, J_1 = 8.0, J_2 = 1.0, H-11′), 8.55 (2H, dq, J_1 = 4.82, J_2 = 1.8, J_3 = 1.0, H-8′).

3β-Acetoxy-30-(4-hydroxymethyl-1,2,3-triazol-1-yl)lup-20(29)-en-28-oic Acid Methyl Ester (10). ^{1}H NMR spectrum (δ, ppm, J/Hz): 0.75 (1H, d, J = 8.8, H-5), 0.80 (3H, s, Me), 0.81 (6H, s, 2Me), 0.86 (3H, s, Me), 0.92 (3H, s, Me), 1.66 (1H, t, J = 11.5, H-18), 1.82 (1H, dd, J_1 = 12.0, J_2 = 8.1, H-22), 1.90 (1H, m, H-16), 2.01 (3H, s, Me-32), 2.14 (1H, td, J_1 = 12.7, J_2 = 2.3, H-13), 2.21 (1H, m, H-21), 2.91 (1H, td, J_1 = 11.2, J_2 = 5.1, H-19), 3.63 (3H, s, OMe), 4.44 (1H, dd, J_1 = 10.5, J_2 = 5.9, H-3), 4.58 (1H, s, H-29), 4.78 (2H, s, C$\underline{H_2}$OH), 4.91 (2H, AB-system, J_{gem} = 15.7, H-30, 30), 5.02 (1H, s, H-29), 7.52 (1H, s, H-5′) (only characteristic proton resonances are given).

3β-Acetoxy-30-(4-phenyl-1,2,3-triazol-1-yl)lup-20(29)-en-28-oic Acid Methyl Ester (11). ^{1}H NMR spectrum (δ, ppm, J/Hz): 0.75 (1H, d, J = 8.3, H-5), 0.80 (3H, s, Me), 0.81 (6H, s, 2Me), 0.87 (3H, s, Me), 0.92 (3H, s, Me), 1.70 (1H, t, J = 11.4, H-18), 2.01 (3H, s, Me-32), 2.96 (1H, td, J_1 = 10.8, J_2 = 4.4, H-19), 3.62 (3H, s, OMe), 4.44 (1H, dd, J_1 = 10.0, J_2 = 5.0, H-3), 4.68 (1H, s, H-29), 4.96 (2H, AB-system, J_{gem} = 16.3, H-30, 30), 5.05 (1H, s, H-29), 7.30 (1H, t, J_1 = 7.4, H-9′), 7.40 (2H, t, J_1 = 7.2, H-8′, 10′), 7.73 (1H, s, H-5′), 7.81 (2H, d, J = 7.0, H-7′, 11′).

3β-Acetoxy-30-(4-pyridyl-1,2,3-triazol-1-yl)lup-20(29)-en-28-oic Acid Methyl Ester (12). ^{1}H NMR spectrum (δ, ppm, J/Hz): 0.75 (1H, d, J = 8.9, H-5), 0.80 (3H, s, Me-24), 0.81 (6H, s, Me-23, 25), 0.87 (3H, s, Me-26), 0.92 (3H, s, Me-27), 0.94 (1H, m, H-1), 1.05 (1H, m, H-12), 1.13 (1H, m, H-15), 1.19–1.26 (2H, m, H-9, 11), 1.27–1.36 (5H, m, H-6, 7, 7, 15, 16), 1.39–1.48 (5H, m, H-6, 11, 12, 21, 22), 1.53–1.66 (3H, m, H-1, 2, 2), 1.69 (1H, t, J = 11.3, H-18), 1.83 (1H, dd, J_1 = 12.6, J_2 = 8.1, H-22), 1.92 (1H, m, H-16), 2.02 (3H, s, Me-32), 2.16 (1H, m, H-13), 2.23 (1H, m, H-21), 2.98 (1H, td, J_1 = 11.0, J_2 = 4.6, H-19), 3.63 (3H, s, OMe), 4.44 (1H, dd, J_1 = 10.5, J_2 = 5.1, H-3), 4.64 (1H, s, H-29), 4.98 (2H, AB-system, J_{gem} = 15.9, H-30, 30), 5.05 (1H, s, H-29), 7.22 (1H, ddd, J_1 = 7.5, J_2 = 5.1, J_3 = 0.8, H-9′), 7.78 (1H, td, J_1 = 7.8, J_2 = 1.6, H-10′), 8.16 (1H, s, H-5′), 8.19 (1H, d, J = 7.8, H-11′), 8.55 (1H, dq, J_1 = 4.8, J_2 = 1.6, J_3 = 0.8, H-8′).

General Method for Alkaline Hydrolysis of 30-(1,2,3-triazol-1-yl)lupanes (7–12). A solution of triazole (0.3 mmol) in a mixture of MeOH (10 mL) and THF (5 mL) under Ar at 0°C was treated with NaOH solution (0.37 mL, 4 N), held at room temperature for 24 h, and poured out into a mixture of ice and HCl. The resulting precipitate was filtered off, washed with H_2O, and dried in air. The obtained product was chromatographed over SiO_2 (Acros, 0.035–0.070 mm) using a mixture of $CHCl_3$ and MeOH (20:1) as eluent. Compounds **13**–**18** were obtained as white precipitates; **13**–**16** were additionally recrystallized from MeOH.

3β,28-Dihydroxy-30-(4-hydroxymethyl-1,2,3-triazol-1-yl)lup-20(29)-ene (13). ^{1}H NMR spectrum (δ, ppm, J/Hz): 0.75 (1H, d, J = 8.8, H-5), 0.81 (9H, s, Me-23, 24, 25), 0.87 (3H, s, Me-27), 0.96 (3H, s, Me-26), 2.31 (1H, m, H-19), 3.15 (1H, dd, J_1 = 10.4, J_2 = 4.6, H-3), 3.24 (1H, d, J = 11.1, H-28), 3.73 (1H, d, J = 11.1, H-28), 4.70 (1H, s, H-29), 4.75 (2H, s, C$\underline{H_2}$OH), 4.84 (2H, AB-system, J_{gem} = 15.7, H-30, 30), 5.04 (1H, s, H-29), 7.50 (1H, s, H-5′) (only characteristic proton resonances are given).

3β,28-Dihydroxy-30-(4-phenyl-1,2,3-triazol-1-yl)lup-20(29)-ene (14). ^{1}H NMR spectrum (δ, ppm, J/Hz): 0.74 (1H, d, J = 9.2, H-5), 0.81 (6H, s, 2Me), 0.82 (3H, s, Me), 0.86 (3H, s, Me), 0.97 (3H, s, Me), 1.69 (1H, t, J = 11.6, H-18), 2.30 (1H, td, J_1 = 11.6, J_2 = 5.4, H-19), 3.17 (1H, dd, J_1 = 11.2, J_2 = 4.6, H-3), 3.24 (1H, d, J = 11.0, H-28), 3.73 (1H, d, J = 11.0, H-28), 4.72 (1H, s, H-29), 4.97 (2H, AB-system, J_{gem} = 14.9, H-30, 30), 5.04 (1H, s, H-29), 7.29 (1H, t, J = 7.6, H-9′), 7.41 (2H, t, J = 8.1, H-8′, 10′), 7.71 (1H, s, H-5′), 7.81 (2H, d, J = 8.6, H-7′, 11′) (only characteristic proton resonances are given).

3β,28-Dihydroxy-30-(4-pyridyl-1,2,3-triazol-1-yl)lup-20(29)-ene (15). ^{1}H NMR spectrum (δ, ppm, J/Hz): 0.66 (1H, d, J = 8.9, H-5), 0.74 (3H, s, Me), 0.80 (3H, s, Me), 0.95 (3H, s, Me), 0.97 (3H, s, Me), 0.99 (3H, s, Me), 2.32 (1H, td, J_1 = 11.2, J_2 = 6.1, H-19), 3.18 (1H, dd, J_1 = 10.4, J_2 = 4.6, H-3), 3.25 (1H, d, J = 11.2, H-28), 3.75 (1H, d, J = 11.2, H-28), 4.68 (1H, s, H-29), 4.98 (2H, AB-system, J_{gem} = 15.4, H-30, 30), 5.03 (1H, s, H-29), 7.22 (1H, m, H-9′), 7.77 (1H, td, J_1 = 8.3, J_2 = 2.1, H-10′), 8.13 (2H, m, H-5′, 11′), 8.56 (1H, d, J = 4.8, H-8′) (only characteristic proton resonances are given).

3β-Hydroxy-30-(4-hydroxymethyl-1,2,3-triazol-1-yl)lup-20(29)-en-28-oic Acid Methyl Ester (16). ^{1}H NMR spectrum (δ, ppm, J/Hz): 0.76 (1H, d, J = 9.2, H-5), 0.81 (3H, s, Me), 0.83 (6H, s, 2Me), 0.88 (3H, s, Me), 0.96 (3H, s, Me), 1.67 (1H, t, J = 11.2, H-18), 1.84 (1H, dd, J_1 = 12.4, J_2 = 8.6, H-22), 1.92 (1H, m, H-16), 2.14 (1H, m, H-13), 2.21 (1H, m, H-21), 2.91 (1H, td, J_1 = 11.2, J_2 = 5.1, H-19), 3.19 (1H, dd, J_1 = 11.4, J_2 = 6.2, H-3), 3.63 (3H, s, OMe), 4.59 (1H, s, H-29), 4.76 (2H, s, CH_2OH), 4.90 (2H, AB-system, J_{gem} = 15.5, H-30, 30), 5.01 (1H, s, H-29), 7.50 (1H, s, H-5′) (only characteristic proton resonances are given).

3β-Hydroxy-30-(4-phenyl-1,2,3-triazol-1-yl)lup-20(29)-en-28-oic Acid Methyl Ester (17). ^{1}H NMR spectrum (δ, ppm, J/Hz): 0.65 (1H, d, J = 9.3, H-5), 0.73 (3H, s, Me), 0.79 (3H, s, Me), 0.87 (3H, s, Me-27), 0.93 (3H, s, Me), 0.94 (3H, s, Me-26), 1.70 (1H, t, J = 11.3, H-18), 1.83 (1H, dd, J_1 = 12.3, J_2 = 8.1, H-22), 1.93 (1H, m, H-16), 2.16 (1H, td, J_1 = 12.9, J_2 = 3.1, H-13), 2.24 (1H, m, H-21), 2.98 (1H, td, J_1 = 10.9, J_2 = 4.4, H-19), 3.16 (1H, dd, J_1 = 11.6, J_2 = 4.7, H-3), 3.63 (3H, s, OMe), 4.66 (1H, s, H-29), 4.97 (2H, AB-system, J_{gem} = 15.7, H-30, 30), 5.05 (1H, s, H-29), 7.31 (1H, t, J_1 = 7.5, H-9′), 7.40 (2H, t, J = 7.6, H-8′, 10′), 7.73 (1H, s, H-5′), 7.82 (2H, d, J = 7.5, H-7′, 11′) (only characteristic proton resonances are given).

3β-Hydroxy-30-(4-pyridyl-1,2,3-triazol-1-yl)lup-20(29)-en-28-oic Acid Methyl Ester (18). ^{1}H NMR spectrum (δ, ppm, J/Hz): 0.65 (1H, d, J = 9.2, H-5), 0.73 (3H, s, Me), 0.79 (3H, s, Me), 0.87 (3H, s, Me), 0.94 (6H, s, 2 Me), 1.70 (1H, t, J = 11.4, H-18), 1.84 (1H, dd, J_1 = 12.6, J_2 = 8.2, H-22), 1.94 (1H, m, H-16), 2.17 (1H, td, J_1 = 12.6, J_2 = 3.2, H-13), 2.22 (1H, m, H-21), 2.98 (1H, td, J_1 = 11.1, J_2 = 4.7, H-19), 3.15 (1H, dd, J_1 = 11.3, J_2 = 4.5, H-3), 3.63 (3H, s, OMe), 4.61 (1H, s, H-29), 4.98 (2H, AB-system, J_{gem} = 15.7, H-30, 30), 5.05 (1H, s, H-29), 7.24 (1H, m, H-9′), 7.79 (1H, t, J = 7.3, H-10′), 8.18 (2H, m, H-5′, 11′), 8.56 (H, d, J = 4.3, H-8′) (only characteristic proton resonances are given).

X-ray Crystal Structure Analysis (XSA). Single crystals of **5** were grown from CH_3CN. The XSA was performed on a Bruker Kappa Apex II diffractometer at 150(2) K (Mo Kα-radiation, graphite monochromator, CCD detector, 2θ = 52.0° maximum). Crystals of **5** were orthorhombic, *a* = 12.7283(7), *b* = 15.6463(9), *c* = 15.6495(9) Å, V = 3116.6(3) Å^3, space group $P2_12_12_1$, *Z* = 4, $C_{34}H_{53}N_3O_4$, d_{calcd} = 1.210 g/cm^3, μ = 0.079 mm^{-1}, crystal size 0.02 × 0.06 × 0.40 mm. Intensities of 6019 independent reflections were measured. Absorption corrections were applied using the SADABS program (transmission 0.8505–0.9703). The structure was solved by direct method using the SHELXS-97 program and refined anisotropically and isotropically (for H) using the SHELXL-97 program. Positions of H atoms were calculated geometrically. Parameters of H atoms were refined isotropically using a rider model. The final refinement parameters were wR_2 = 0.1577 and S = 1.03. The number of refined parameters was 378 (R = 0.0526 for 4120 F > 4σ). Atomic coordinates and bond lengths and angles were deposited in the Cambridge Crystallographic Data Centre (CCDC 864133).

The Ames test was carried out by the standard method [21]. *S. typhimurium* was cultivated to optical density 0.45 OD_{550} (2×10^8 cells/mL) at 37°C. Then, upper agar (2 mL, 0.6%) in a tube was treated with 1-d culture (100 μL) and test compound (10 μL, 0.01 M) dissolved in DMSO. The mixture was thoroughly stirred by hand rotation and poured out into Petri dishes with the minimal amount of glucose agar. The dishes were incubated in a thermostat at 37°C for 48 h and then counted.

The experiment was carried out twice independently in triplicate. The final result was presented as the average number of colonies per Petri dish.

The Ames test results were analyzed statistically by the literature method [22]. Student *t*-criteria were used to evaluate statistically significant differences between averages. Measurement error was determined as the dispersion of the average for independent measurements of a single sample [23].

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