Full Paper

Synthesis, Encapsulation and Antitumor Activity of New Betulin Derivatives

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Novel betulin derivatives were prepared and tested for their antitumor activity. Starting from 3-*O*-acetyl- or 3-*O*-methyl-betulinic aldehyde, the synthesis of C-28 ethynyl derivatives was performed; their subsequent transformation with several 1,3-dipolarophiles afforded pyrazoles and 1,2,3-triazoles. Their screening for antitumor activity was performed in a panel of 15 human cancer cell lines by a colorimetric SRB-assay. Thereby, several compounds revealed a higher cytotoxicity than betulinic acid. In addition, the encapsulation of the lead structure **7** into liposomes was investigated. The results from a dye exclusion test and from DNA laddering experiments provided evidence for an apoptotic cell death.

Keywords: Antitumor activity / Betulin / Betulinic acid / 1,3-Dipolar-cycloaddition / Liposomes / SRB assay

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Introduction

Cancer is still one of the leading causes of death. The index of cancer cure is often low and its treatment is still a challenge. Triterpenes represent an important class of natural compounds. In the forests of Europe, Asia and North America betulin and betulinic acid (Fig. 1) [1, 2] are found in the external bark of birches and sycamore trees. These lupane-type triterpenes have recently been investigated for their various pharmacological and medicinal properties, among them antitumor and anti-HIV activity.

Antiviral potency of betulinic acid (BA) derivatives is linked to a particular action by the inhibition of the virus-cell fusion at the gp41-gp120 interface [3, 4] or by altering the process of cell maturation [5, 6] by interfering the CA-SP1 junction in the Gag processing. Interestingly, BA showed in an animal model a selective cytotoxicity for melanoma cells with no acute or chronic side effects to normal cells even at doses of 500 mg/kg [7]. The apoptotic action of BA follows an intrinsic pathway. Thus, a direct interaction between BA and mitochondria leads to an increased permeability and releases cytochrome c and AIF to the cytosol [8, 9], thus, triggering the caspase cascade and nuclear fragmentation. Furthermore, the generation of reactive oxygen species (ROS) [10] was observed, that is associated with an up-regulation [11] of p38 and SAP/JNK kinases.

Herein, we present a synthetic approach to new betulin derived compounds bearing an ethynyl side chain at the C-28 position. In addition, these compounds were used for the synthesis of heterocyclic compounds by 1,3-dipolar cycloadditions with diazoalkanes and azides. The antitumor activity of the compounds was studied using a colorimetric sulphorhodamin B assay (SRB assay) applying 15 different human cancer cell lines.

Results and discussion

The synthesis of acetylenic compounds started from 3-*O*-acetyl- (**1**) or 3-*O*-methyl betulinic aldehyde (**2**) [12, 13] (Scheme 1). Their reaction with ethynyl magnesium bromide [14] afforded the corresponding 28-ethynyl-betulinols as an inseparable mixture of diastereomers; hence, a direct transformation to compounds **3** and **4** was carried out by Jones oxidation [15]. During the reaction of **1** with lithium acetylide [16] cleavage of the acetyl group occurred and led to the formation of **5** in 80% yield. Acetylation of **5** gave the 3,28-di-

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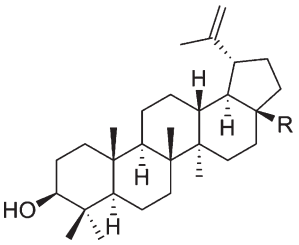


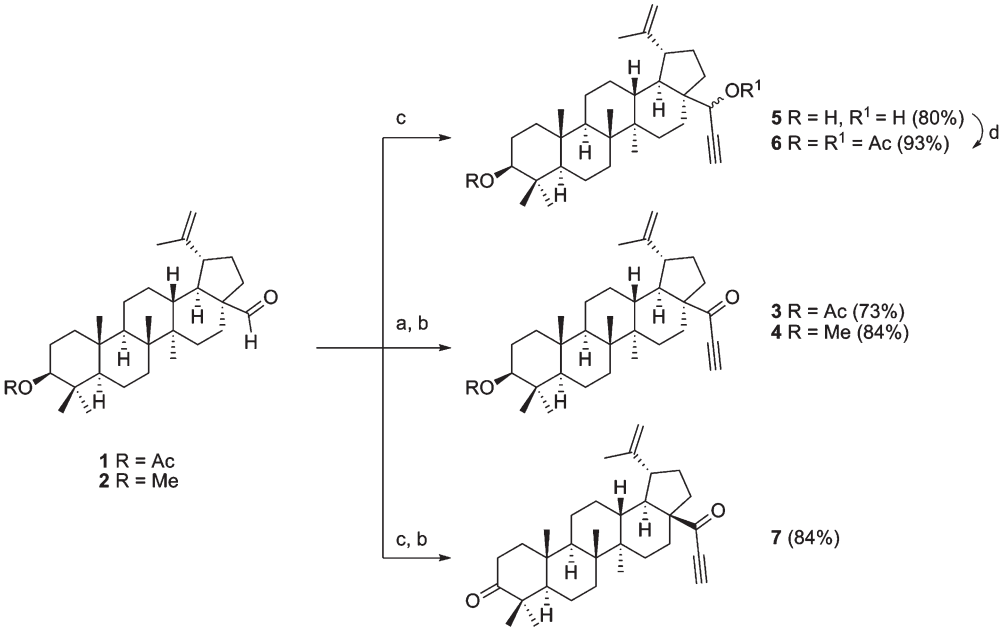
Figure 1. Structure of betulin and betulinic acid.

O-acetyl derivative **6**. Oxidation of **5** yielded the 28-ethynyl-lup-20(29)-en-3,28-dione **7**. This compound was an ideal starting material for further derivatization (Scheme 2). The reaction of **7** with diazomethane [17] or ethyl diazoacetate [18] yielded the corresponding pyrazoles **8** and **9**, respectively; the orientation of this addition follows the rule of von Auwers and Ungemach [19]. Similarly, azides reacted with compound **7** to form triazoles. The 1,3-dipolar cycloadditions of ethyl azidoacetate or 4-azidobenzoic acid with **7** were performed in THF in the presence of catalytic amounts of copper(I) iodide [20] to afford compounds **10** and **11**, respectively.

Likewise, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide or 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl azide [21, 22] gave the triazoles **12** and **13**, respectively. Their treatment with sodium methoxide in methanol yielded deprotected **14** and **15** [23]. In a similar way, **5** gave a 85% yield of **16** whose deacetylation yielded 93% of **17**. Jones oxidation (70%) or Swern oxidation (73%) of **16** again gave **12**. The reaction of compound **7** with DIMCARB (Scheme 3) [24] in ethanol led to the formation of the enamine **18** [25].

All values are derived from dose-response curves obtained by measuring the percentage of viable cells relative to untreated controls after 96 h exposure of the test compounds to the cell line using an SRB-assay for melanoma (518A2), cervic cancer (A431), head and neck tumor (A253, FADU), lung carcinoma (A549), ovarian cancer (A2780), colon cancer (DLD-1, HCT-8, HCT-116, HT-29, SW-480), anaplastic thyroid cancer (8505c, SW-1736), mamma carcinoma (MCF-7) and liposarcoma. Values are the average from at least three independent experiments. Variation was generally ±10%; NA = no inhibition of cell growth at the highest concentration (30 μM).

The compounds were screened for their antitumor activity in a panel of 15 human cancer cell lines in 96 well plates using the colorimetric [26] sulforhodamine B (SRB) protocol. The IC_{50} values of compounds **3–18** are reported in Table 1; they were derived from the corresponding dose-response curves. The 28-ethynyl-betulin derivatives **3**, **4**, and **7** showed a higher cytotoxicity compared to parent betulinic acid. An influence of the substituent at position C-3 can be deduced from these data: Derivatives bearing an acetyl- or methyl-moiety showed lower IC_{50} values than compounds possessing



Scheme 1. Synthesis of ethynyl derivatives: a) ethynyl magnesium bromide, THF, –78°C, 1 h; b) CrO_3, H_2SO_4, acetone, 0°C, 30 min; (c) lithium acetylide ethylenediamine complex, THF, –78°C, 1 h; d) Ac_2O, NEt_3, 24°C, 72 h.

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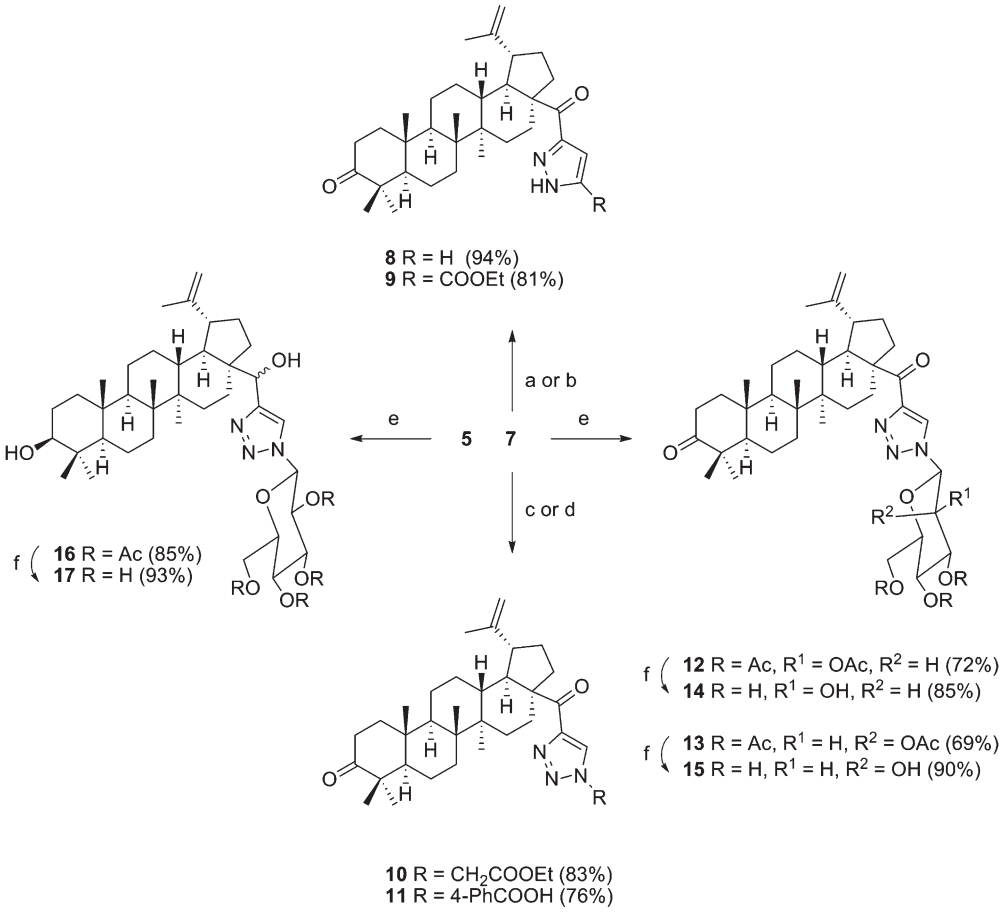
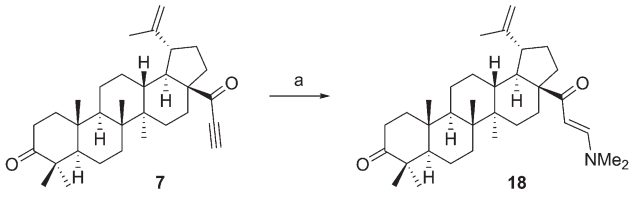


Scheme 2. 1,3-Dipolar cycloadditions: a) CH_2N_2, ether, 0°C, 10 min; b) ethyl diazoacetate, toluene, reflux, 48 h; c) ethyl azidoacetate, CuI, THF, 60°C, 72 h; d) 4-azidobenzoic acid, CuI, THF, 60°C, 72 h; e) 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide (for **12** and **16**) or 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl azide (for **13**), CuI, THF, 60°C, 72 h; f) THF, MeOH, MeONa, 24°C, 12–24 h.

a OH-C(3); the 3-oxo compound **7** is highly cytotoxic (IC_{50}-values 0.16–3.7 μM). For the pyrazole derivative **8** and **9** a noteworthy activity was observed only for the colon cancer cell line SW480 (IC_{50} = 8.6 and 5.2 μM, respectively). Similarly, the triazole **10** showed a low IC_{50} of 3.6 μM only for mamma carcinoma cells (MCF-7). In contrast, the triazole derivative **11**, bearing a 4-carboxyphenyl substituent, showed quite promising results for all cell lines. The presence of a sugar moiety seems to decrease activity; no inhibition of cell growth was observed for compounds **12–17** even at the highest administrated concentration of 30 μM. Compound **18** shows IC_{50} values = 2.7–5.8 μM, hence being more cytotoxic than betulinic acid.



Scheme 3. Synthesis of **18**: (a) DIMCARB, EtOH, 24°C, 24 h, 97%.

Betulinic acid and related compounds possess only a poor solubility in water, hence making drug administration *in vivo* difficult. To overcome this limitation, we studied the possibility of encapsulation applying commercially available liposome formulations. In addition, liposomes are an effective delivering system to mitochondria [27] playing a key role in betulinic acid induced apoptosis. Good encapsulation was achieved for the lead structure **7** using soybean lecithin (Lipoid S75). Subsequent extrusion through a polycarbonate membrane [28] with a pore size of 100 nm by a LiposoFast system gave liposomes with a hydrodynamic diameter of 60–120 nm (*Z*-average 102.0 nm), as determined by dynamic light scattering. The encapsulation efficiency was about 60% as determined by HPLC and the physical stability of the liposomes exceeded several weeks. Evaluation of the

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Table 1. Cytoxicity of the compounds in a panel of various human cancer cell lines.

Cell line	BA	3	4	7	8	9	10	11	12–17	18
518A2	11.9	2.9	2.6	0.17	20.4	23.1	9.2	9.6	NA	4.3
A431	15.4	7.5	7.6	0.91	16.2	12.3	12.7	3.9	NA	4.7
A253	11.1	1.7	1.4	0.16	14.8	14.0	10.3	9.2	NA	4.2
FADU	10.4	12.8	20.5	2.7	19.8	15.9	25.0	3.8	NA	3.0
A549	14.9	2.7	3.8	0.35	20.5	16.1	22.9	6.9	NA	5.8
A2780	11.0	0.55	0.58	0.18	12.5	6.6	14.5	6.7	NA	2.7
DLD-1	17.5	7.2	10.0	1.9	26.4	24.1	12.4	4.7	NA	3.8
HCT-8	17.8	12.5	16.5	3.7	15.0	12.8	10.0	2.5	NA	4.8
HCT-116	13.3	11.8	18.7	1.2	20.1	15.2	18.8	3.6	NA	3.4
HT-29	16.1	1.4	1.3	0.23	15.7	12.7	21.5	5.4	NA	3.6
SW480	6.4	6.9	7.9	0.78	8.6	5.2	12.9	10.4	NA	4.8
8505C	6.7	5.3	5.3	1.7	14.1	14.3	18.0	9.6	NA	4.4
SW1736	11.6	0.62	0.54	0.16	19.3	11.4	29.6	8.5	NA	2.9
MCF-7	14.9	13.7	18.5	3.3	13.4	9.8	3.6	5.5	NA	3.8
Lipo	9.7	6.8	10.3	1.7	18.6	19.2	19.5	10.9	NA	4.2

liposomal formulation of compound **7** in the SRB assay revealed the beneficial effect of encapsulation for several cell lines (Table 2).

Comparison of the calculated IC_{50}-values derived from dose-response curves of compound **7** (solution in DMSO) and as aqueous liposomal formulation.

Additional experiments were performed to proof apoptosis. Thus, the floating cells collected after treatment with the compounds **7** or **18** (applying IC_{90}-concentrations for 24 h) were analyzed by a trypan-blue dye exclusion test and DNA gel electrophoresis (Fig. 2). Apoptotic cells possess an intact cell membrane and can exclude the dye whereas necrotic cells are stained blue. During the process of apoptosis the action of endonucleases leads to DNA fragmentation. These fragments can be detected in gel electrophoresis as ladders [29–31].

Table 2. IC_{50} values of compound 7.

Cell line	7 in DMSO	7 encapsulated
518A2	0.17	0.87
A431	0.91	1.2
A253	0.16	0.23
FADU	2.7	1.5
A549	0.35	0.28
A2780	0.18	0.26
DLD-1	1.9	1.4
HCT-8	3.7	1.6
HCT-116	1.2	1.2
HT-29	0.23	0.28
SW480	0.78	0.30
8505C	1.7	1.5
SW1736	0.16	0.27
MCF-7	3.3	1.5
Lipo	1.7	1.4

In summary, betulin derivatives bearing an ethynyl side chain were synthesized and shown to possess quite promising antitumor activity by triggering apoptosis. To overcome the drawback of a limited solubility in aqueous systems, these compounds can be administered encapsulated in liposomes.

Experimental

General

Melting points are uncorrected (Leica hot stage microscope), NMR spectra were recorded using the Varian spectrometers Gemini 200, Gemini 2000 or Unity 500 (δ given in ppm, J in Hz, internal Me_4Si), optical rotations were obtained using a Perkin-Elmer 341 polarimeter (1 cm micro cell), IR spectra (film or KBr pellet) on a Perkin-Elmer FT-IR spectrometer Spectrum 1000, MS spectra were taken on a Intectra GmbH AMD 402 (electron impact, 70 eV) or on a Finnigan MAT TSQ 7000 (electrospray, voltage 4.5 kV, sheath gas nitrogen) instrument. TLC was performed on silica gel (Merck 5554). Spots were detected by spraying a solution of ammonium molybdate and cerium(IV) sulfate in sulfuric acid, followed by gently heating. The solvents were dried according to usual procedures.

Cell lines and culture conditions

The cell lines 518A2, 8505C, A253, A2780, A431, A549, DLD-1, FaDu, HCT-116, HCT-8, HT-29, LIPO, MCF-7, SW1736, and SW480 were included in this study. Cultures were maintained as monolayer in RPMI 1640 (PAA Laboratories, Pasching, Germany) supplemented with 10% heat inactivated fetal bovine serum (Biochrom AG, Berlin, Germany) and penicillin/streptomycin (PAA Laboratories) at 37°C in a humidified atmosphere of 5% CO_2/95% air.

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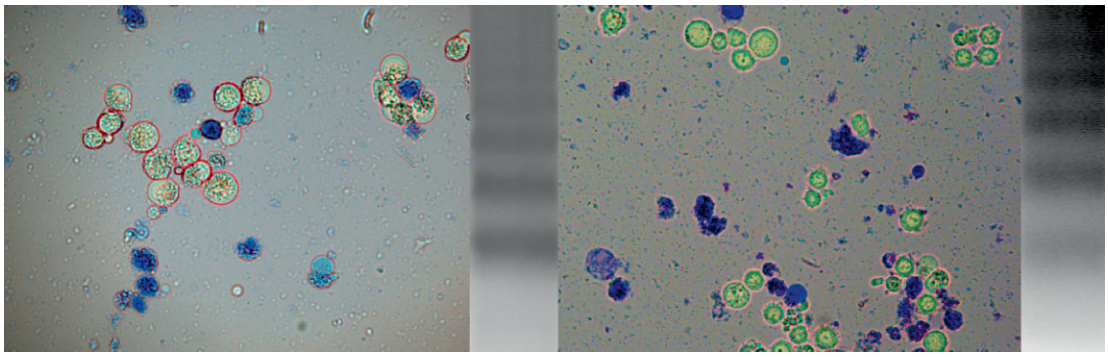

Figure 2. Analysis of floating cells after treatment with IC_{90}-concentrations for 24 h; trypan-blue exclusion test of compound **7** (left) for head and neck tumor cell lineA253 and compound **18** (right) for colon cancer cell line SW480. DNA laddering of compound **7** (left) and compound **18** (right) for colon cancer cell line SW480.

Cytotoxicity assay

The cytotoxicity of the compounds was evaluated using the sulforhodamine-B (SRB) (Sigma Aldrich) microculture colorimetric assay [26]. In short, exponentially growing cells were seeded into 96-well plates on day 0 at the appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24 h, the cells were treated with serial dilutions of the compounds (0–30 μM) for 96 h. The final concentration of DMSO or DMF solvent never exceeded 0.5%, which was non-toxic to the cells. The percentages of surviving cells relative to untreated controls were determined 96 h after the beginning of drug exposure. After a 96 h treatment, the supernatant medium from the 96 well plates was thrown away and the cells were fixed with 10% TCA. For a thorough fixation, the plates were allowed to rest at 4°C. After fixation, the cells were washed in a strip washer. The washing was done four times with water using alternate dispensing and aspiration procedures. The plates were then dyed with 100 μL of 0.4% SRB (sulforhodamine B) for about 20 min. After dying the plates were washed with 1% acetic acid to remove the excess of the dye and allowed to air dry overnight. 100 μL of 10 mM Tris base solution were added to each well and absorbance was measured at 570 nm (using a 96 well plate reader, Tecan Spectra, Crailsheim, Germany). The IC_{50} was estimated from the semi-logarithmic dose-response curves.

Preparation of liposomes

Unilamellar liposomes of approximately 100 nm diameter were obtained by the method of Olson [28] employing a laboratory extruder (LiposoFast, Avestin Inc.). In a typical experiment for preparing 5 mL dispersion of liposomes, 5 mg of the compound were mixed with an excess (125 mg) of phosphatidylcholine formulation (Lipoid S75) in chloroform (5 mL) and evaporated to a film. The lipid film was hydrated with H_2O (5 mL) for 24 h at room temperature. The solution obtained was extruded through a polycarbonate filter of 100 nm pore size. Twenty-one cycles were applied and concentration of the compound was determined by HPLC (RP18, 4.6 × 250 mm, $\lambda = 230$ nm, methanol, 1.3 mL). Liposomes were characterized by DLS.

Apoptosis test: Dye exclusion test

Apoptotic cell death was analyzed by trypan-blue dye (Sigma Aldrich, Germany) on A431 and A2780 cell lines. The cell culture flasks with 70–80% confluence were treated with IC_{90} doses of the compounds for 24 h. The supernatant medium with floating cells was collected after treatment and centrifuged to collect dead and apoptotic cells. This pellet was resuspended in serum free media. Equal amounts of cell suspension and trypan-blue were mixed and analyzed under a microscope. Viable cells exclude the dye and appear colorless whereas cells whose cell membrane is destroyed are stained in blue color. If there are more colorless cells than stained cells, then death of the cells can be characterized as apoptotic.

Apoptosis test: DNA fragmentation assay

Determination of apoptotic cell death was performed by DNA gel electrophoresis. Briefly, cell lines were treated with respective IC_{90} doses of the compounds for 24 h. Floating cells – as induced by drug exposure – were collected, resuspended in HBSS (1 mL) and transferred to 70% ethanol (10 mL). The cells were collected and treated with PCB (40 μL, 96 parts of 0.2 M Na_2HPO_4 and 4 parts of 0.1 M citric acid (pH 7.8)) for 1 h at room temperature. The supernatant was collected and treated with RNAse A (3 μL, 1mg/ml) and Nonide NP40 (3 μL 0.25% in H_2O) at 37°C for 30 min. Then, proteinase K (3 μL, 1 mg/mL) was added and incubated for 30 min at 37°C. DNA laddering was observed by running the samples on 2% agarose gel followed by ethidium bromide (Sigma Aldrich) staining.

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General procedure for the synthesis of substituted 28-ethynyl-betulins (GP1)

A solution of the corresponding betulinic aldehyde (1.03 mmol) was cooled to −78°C, then a solution of ethynylmagnesium bromide (4 mL, 2.0 mmol, 0.5 M in THF) was added. After 1 h at −78°C, the reaction was quenched by the addition of brine (100 mL). The phases were separated and the aq. layer was extracted with ethyl acetate (2 × 50 mL). The combined extracts were dried over Na_2SO_4 and evaporated to dryness. The residue was subjected to column chromatography (silica gel, hexane/ethyl acetate, 8:2) to yield the corresponding 28-ethynyl-betulin as a diastereomeric mixture.

General procedure for Jones oxidation (GP2)

To a stirred solution of the corresponding 28-ethinyl-betulin (1.84 mmol) in acetone (10 mL) at 0°C dropwise a solution of chromium(VI) oxide (300 mg) in aq. H_2SO_4 (35%, 1 mL) was added. After TLC revealed the absence of starting material, the excess chromium(VI) oxide was destroyed by the addition of 2-propanol (2 mL). The solution was concentrated *in vacuo* and the residue partitioned between H_2O (30 mL) and CH_2Cl_2 (30 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (10 mL). The combined organic extracts were dried over Na_2SO_4, evaporated to dryness and purified by column chromatography (silica gel, hexane/ethyl acetate, 9:1).

General procedure for the click reaction (GP3)

A solution of compound **7** (0.20 g, 0.43 mmol), the corresponding azide (0.6 mmol) and copper(I) iodide (40 mg, 0.2 mmol) in THF (12 mL) was stirred at 60°C for 72 h. When TLC revealed the absence of starting material, the solution was filtered and concentrated *in vacuo*. The residue was subjected to column chromatography (silica gel, hexane/ethyl acetate, 7:3).

General procedure for deacetylation (GP4)

A solution of compound **12** or **13** (150 mg, 0.18 mmol) in THF (5 mL) and MeOH (5 mL) containing NaOMe (11 mg, 0.2 mmol) was stirred for 12 h, concentrated *in vacuo* and the residue subjected to column chromatography (silica gel, dichloromethane/methanol, 9:1).

3-O-Acetyl-28-ethinyl-28-oxolup-20(29)-en-3-ol ***(3)***

Compound **3** (0.42 g, 73%) was obtained from 3-*O*-acetylbetulinic aldehyde (**1**) (0.50 g, 1.03 mmol) following GP1 and GP2 as a colorless solid; mp 198–200°C; $[\alpha]_D^{20} = 25.4^\circ$ ($c = 3.0$, $CHCl_3$); $R_f = 0.60$ (silica gel, hexane/ethyl acetate, 9:1); IR (KBr): $\nu = 3244$ m, 3068 w, 2952 s, 2871 m, 2085 m, 1724 s, 1677 s, 1640 w, 1452 m, 1393 m, 1376 m, 1317 w, 1250 s, 1105 w, 1087 w, 1064 m, 1028 s cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 4.71$ (d, 1H, $J = 2.1$ Hz, CH_a (30)), 4.58 (dd, 1H, $J = 2.1$, 1.5 Hz, CH_b (30)), 4.44 (dd, 1H, $J = 10.4$, 5.8 Hz, C*H*OAc (3)), 3.06 (s, 1H, *H*C≡C), 2.92 (ddd, 1H, $J = 11.2$, 11.2, 5.8 Hz, C*H* (19)), 2.47 (ddd, 1H, $J = 13.5$, 3.3, 3.3 Hz, CH_a (16)), 2.24 (ddd, 1H, $J = 12.7$, 12.7, 3.8 Hz, C*H* (13)), 2.03 (dd, 1H, $J = 10.7$, 8.5 Hz, CH_a (22)), 2.02 (s, 3H, Ac), 1.81–1.58 (m, 5H, CH_a (21) + CH_a (12) + CH_a (1) + C*H* (18) + CH_a (2)), 1.65 (s, 3H, CH_3 (29)), 1.56–1.17 (m, 12H, CH_b (16) + CH_2 (6) + CH_b (22) + CH_2 (11) + CH_b (21) + CH_2 (15) + CH_2 (7) + C*H* (9)), 0.98–0.88 (m, 2H, CH_b (12) + CH_b (1)), 0.94 (s, 3H, CH_3 (27)), 0.89 (s, 3H, CH_3 (25)), 0.86–0.78 (m, 1H), 0.83 (s, 3H, CH_3 (24)), 0.82 (s, 3H, CH_3 (26)), 0.81 (s, 3H, CH_3 (23)), 0.75 (d, 1H, $J = 9.1$ Hz, C*H* (5)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = 191.6$ (*C*=O), 170.9 (*C*=O), 150.2 (C20, *C*=CH_2), 109.8 (C30, *C*H_2=C), 80.9 (C3, *C*H), 80.5 (C31, *C*≡C), 77.0 (C32, H*C*≡C), 61.8 (C17, $C_{quart.}$), 55.4 (C5, *C*H), 50.5 (C9, *C*H), 48.5 (C18, *C*H), 46.3 (C19, *C*H), 42.4 (C14, $C_{quart.}$), 40.7 (C8, $C_{quart.}$), 38.4 (C1, *C*H_2), 37.8 (C4, $C_{quart.}$), 37.1 (C13, *C*H), 37.0 (C10, $C_{quart.}$), 35.5 (C22, *C*H_2), 34.2 (C7, *C*H_2), 31.6 (C16, *C*H_2), 29.7 (C21, *C*H_2), 29.5 (C15, *C*H_2), 27.9 (C23, *C*H_3), 25.5 (C12, *C*H_2), 23.7 (C2, *C*H_2), 21.3 (Ac), 20.8 (C11, *C*H_2), 19.2 (C29, *C*H_3), 18.1 (C6, *C*H_2), 16.4 (C24, *C*H_3), 16.2 (C26, *C*H_3), 15.9 (C25, *C*H_3), 14.3 (C27, *C*H_3) ppm; MS (ESI, MeOH): $m/z = 529.4$ (30%, $[M + Na]^+$), 1013.8 (100%, $[2\ M + H^+]$); anal. calcd. for $C_{34}H_{50}O_3$ (506.76) C, 80.58; H, 9.94; found: C, 80.49; H, 9.98.

3-O-Methyl-28-ethinyl-28-oxolup-20(29)-en-3-ol ***(4)***

Compound **4** (0.44 g, 84%) was obtained from 3-*O*-methylbetulinic aldehyde (**2**) (0.50 g, 1.10 mmol) following GP1 and GP2 as a colorless solid; mp 176–179°C; $[\alpha]_D^{20} = 26.6^\circ$ ($c = 3.8$, $CHCl_3$); $R_f = 0.76$ (silica gel, hexane/ethyl acetate, 9:1); IR (KBr): $\nu = 3297$ w, 3202 w, 2944 s, 2869 m, 2080 m, 1674 s, 1453 m, 1378 m, 1358 w, 1246 w, 1182 w, 1097 m, 1050 m cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 4.70$ (d, 1H, $J = 2.1$ Hz, CH_a (30)), 4.58 (dd, 1H, $J = 2.1$, 1.5 Hz, CH_b (30)), 3.32 (s, 1H, OCH_3), 3.06 (s, 1H, *H*C≡C), 2.91 (ddd, 1H, $J = 11.2$, 11.2, 5.8 Hz, C*H* (19)), 2.60 (dd, 1H, $J = 11.6$, 4.6 Hz, C*H*OCH$_3$ (3)), 2.45 (ddd, 1H, $J = 13.5$, 3.3, 3.3 Hz, CH_a (16)), 2.24 (ddd, 1H, $J = 12.7$, 12.7, 3.8 Hz, C*H* (13)), 2.02 (dd, 1H, $J = 10.7$, 8.5 Hz, CH_a (22)), 1.82–1.60 (m, 5H, CH_a (21) + CH_a (2) + CH_a (12) + CH_a (1) + C*H* (18)), 1.65 (s, 3H, CH_3 (29)), 1.56–1.30 (m, 13H, CH_b (16) + CH_2 (6) + CH_b (22) + CH_a (11) + CH_b (21) + CH_a (15) + CH_b (2) + CH_2 (7)), 1.27–1.17 (m, 13H, CH_b (15) + CH_b (11) + C*H* (9)), 0.98–0.88 (m, 1H, CH_b (12)), 0.94 (s, 3H, CH_3 (27)), 0.92 (s, 3H, CH_3 (23)), 0.89 (s, 3H, CH_3 (25)), 0.86–0.78 (m, 1H, CH_b (1)), 0.80 (s, 3H, CH_3 (24)), 0.70 (s, 3H, CH_3 (26)), 0.65 (d, 1H, $J = 9.1$ Hz, C*H* (5)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = 191.6$ (*C*=O), 150.3 (C20, *C*=CH_2), 109.7 (C30, *C*H_2=C), 88.6 (C3, *C*HOCH$_3$), 80.5 (C31, *C*≡C), 77.0 (C32, H*C*≡C), 61.8 (C17, $C_{quart.}$), 57.4 (OCH_3), 55.9 (C5, *C*H), 50.6 (C9, *C*H), 48.2 (C18, *C*H), 46.3 (C19, *C*H), 42.4 (C14, $C_{quart.}$), 40.8 (C8, $C_{quart.}$), 38.8 (C1, *C*H_2), 38.6 (C4, $C_{quart.}$), 37.2 (C10, $C_{quart.}$), 37.1 (C13, *C*H), 35.5 (C22,

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*C*H$_2$), 34.3 (C7, *C*H$_2$), 31.2 (C21, *C*H$_2$), 40.0 (C16, *C*H$_2$), 29.5 (C15, *C*H$_2$), 28.0 (C23, *C*H$_3$), 25.6 (C12, *C*H$_2$), 22.2 (C2, *C*H$_2$), 20.8 (C11, *C*H$_2$), 19.2 (C29, *C*H$_3$), 18.2 (C6, *C*H$_2$), 16.1 (C24, *C*H$_3$), 16.1 (C26, *C*H$_3$), 15.9 (C25, *C*H$_3$), 14.4 (C27, *C*H$_3$) ppm; MS (ESI, MeOH): *m/z* = 501.0 (50%, [M + Na]$^+$), 957.2 (100%, [2 M + H]$^+$); anal. calcd. for $C_{33}H_{50}O_2$ (478.75): C, 82.79; H, 10.53; found: C, 81.76; H, 10.57.

28-Ethinyl-betulin ***(5)***

In analogy to GP1, from 3-*O*-acetylbetulinic aldehyde (**1**) (2 g, 4.1 mmol) and the lithium acetylide ethylenediamine complex (1.5 g, 16.4 mmol) compound **13** (1.52 g, 80%) was obtained as a colorless solid after purification by column chromatography (silica gel, $CHCl_3/Et_2O$, 95:5); mp 173–178°C; $[\alpha]_D^{20} = -14.6^\circ$ (*c* = 5.9, $CHCl_3$); $R_f = 0.32$ (silica gel, $CHCl_3/Et_2O$, 95:5); IR (KBr): ν = 3421 s, 2941 s, 2871 s, 2120 m, 1757 s, 1639 m, 1454 s, 1375 s, 1225 s, 1040 s cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 4.88 (m, 1H, C*H*OH (28)), 4.70 (d, 1H, *J* = 2.02 Hz, C*H*$_a$ (30)), 4.55 (dd, 1H, *J* = 2.0, 1.4 Hz, C*H*$_b$ (30)), 3.17 (dd, 1H, *J* = 5.0, 11.1 Hz, C*H*OH (3)), 2.87 (ddd, 1H, *J* = 6.2, 11.0, 11.0 Hz, C*H* (19)), 2.47 (d, 1H, *J* = 2.0 Hz, C ≡ C*H* (32)), 2.01 (m, 1H, C*H*$_a$ (21)), 2.08 (ddd, 1H, *J* = 1.2, 9.1, 11.1 Hz, C*H*$_a$ (16)), 1.98 (m, 1H, C*H*$_a$ (22)), 1.94 (dd, 1H, *J* = 3.9, 8.2 Hz, C*H* (13)), 1.74 (m, 1H, C*H*$_a$ (12)), 1.66 (s, 3H, C*H*$_3$ (29)), 1.64–1.49 (m, 7H, C*H*$_a$ (6) + C*H*$_a$ (11) + C*H*$_b$ (12) + C*H*$_2$ (2) + C*H*$_2$ (13)), 1.45–1.30 (m, 6H, C*H*$_b$ (6) + C*H*$_b$ (22) + C*H*$_2$ (7) + C*H* (18) + C*H* (9)), 1.29–1.10 (m, 5H, C*H*$_b$ (11) + C*H*$_b$ (16) + C*H*$_2$ (15), C*H*$_b$ (21)), 1.02 (s, 3H, C*H*$_3$ (27)), 0.99 (s, 3H, C*H*$_3$ (25)), 0.89 (ddd, 1H, *J* = 4.5, 12.6, 12.6 Hz, C*H*$_b$ (1)), 0.95 (s, 3H, C*H*$_3$ (24)), 0.81 (s, 3H, C*H*$_3$ (26)), 0.74 (s, 3H, C*H*$_3$ (23)), 0.67 (d, 1H, *J* = 9.2 Hz, (5)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 151.0 (C20, *C*=CH$_2$), 109.6 (C30, *C*H$_2$=C), 84.7 (C31, *C*≡CH) 78.9 (C3, *C*HOH), 74.2 (C32, H*C*≡C), 66.0 (C28, *C*HOH), 55.3 (C5, *C*H), 50.6 (C17, C_{quart}), 50.3 (C9, *C*H), 49.0 (C18, *C*H), 48.6 (C19, *C*H), 43.0 (C14, C_{quart}), 40.9 (C8, C_{quart}), 38.9 (C4, C_{quart}), 38.7 (C1, *C*H$_2$), 37.3 (C13, *C*H), 37.1 (C10, $C_{quart.}$), 34.3 (C7, *C*H$_2$), 34.2 (C21, *C*H$_2$), 33.9 (C16, *C*H$_2$), 32.4 (C22, *C*H$_2$), 28.0 (C23, *C*H$_3$), 27.9 (C15, *C*H$_2$), 27.4 (C2, *C*H$_2$), 25.1 (C12, *C*H$_2$), 20.8 (C11, *C*H$_2$), 18.8 (C29, *C*H$_3$), 18.3 (C6, *C*H$_2$), 16.1 (C24, *C*H$_3$), 16.1 (C26, *C*H$_3$), 15.3 (C25, *C*H$_3$), 15.1 (C27, *C*H$_3$) ppm; MS (ESI, MeOH): *m/z* = 467.4 (60%, [M + H]$^+$); anal. calcd. for $C_{32}H_{50}O_2$ (466.74): C, 82.35; H, 10.78; found: C, 82.04; H, 10.99.

3,28-O-Diacetyl-28-ethinyl-betulin ***(6)***

Acetylation of compound **5** (120 mg, 0.25 mmol with Ac_2O and triethylamine (100 μL, 0.72 mmol) for 72 h at 24°C followed by usual aqueous work-up and chromatography (silica gel, $CHCl_3/Et_2O$, 95:5) gave **6** (128 mg, 93%) as a colorless solid; mp 108–113 °C; $[\alpha]_D^{20} = 1.9^\circ$ (*c* = 5.4, $CHCl_3$); $R_f = 0.93$ (silica gel, $CHCl_3/Et_2O$, 95/5); IR (KBr): ν = 3448 m, 3309 m, 2962 s, 1734 s, 1640 m, 1456 w, 1375 m, 1261 s, 1106 m, 1021 m cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.88 (m, 1H, C*H*OH (28)), 4.61 (d, 1H, *J* = 2.02 Hz, C*H*$_a$ (30)), 4.56 (dd, 1H, *J* = 2.0, 1.4 Hz, C*H*$_b$ (30)), 3.17 (dd, 1H, *J* = 5.0, 11.1 Hz, C*H*OH (3)), 2.87 (ddd, 1H, *J* = 6.2, 11.0, 11.0 Hz, C*H* (19)), 2.47 (d, 1H, *J* = 2.0 Hz, C ≡ C*H* (32)), 2.01 (m, 1H, C*H*$_a$ (21)), 2.08 (ddd, 1H, *J* = 1.2, 9.1, 11.1 Hz, C*H*$_a$ (16)), 1.98 (m, 1H, C*H*$_a$ (22)), 1.94 (dd, 1H, *J* = 3.9, 8.2 Hz, C*H* (13)), 1.74 (m, 1H, C*H*$_a$ (12)), 1.66 (s, 3H, C*H*$_3$ (29)), 1.64–1.49 (m, 7H, C*H*$_a$ (6) + C*H*$_a$ (11) + C*H*$_b$ (12) + C*H*$_2$ (2) + C*H*$_2$ (13)), 1.45–1.30 (m, 6H, C*H*$_b$ (6) + C*H*$_b$ (22) + C*H*$_2$ (7) + C*H* (18) + C*H* (9)), 1.29–1.10 (m, 5H, C*H*$_b$ (11) + C*H*$_b$ (16) + C*H*$_2$ (15), C*H*$_b$ (21)), 1.02 (s, 3H, C*H*$_3$ (27)), 0.99 (s, 3H, C*H*$_3$ (25)), 0.89 (ddd, 1H, *J* = 4.5, 12.6, 12.6 Hz, C*H*$_b$ (1)), 0.95 (s, 3H, C*H*$_3$ (24)), 0.81 (s, 3H, C*H*$_3$ (26)), 0.74 (s, 3H, C*H*$_3$ (23)), 0.67 (d, 1H, *J* = 9.2 Hz, (5)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 170.9 (C=O), 169.6 (C=O), 150.4 (C20, *C*=CH$_2$), 109.8 (C30, *C*H$_2$=C), 81.0 (C31, *C*≡CH) 76.7 (C3, *C*H), 74.2 (C32, H*C*≡C), 66.7 (C28, *C*H), 55.5 (C5, *C*H), 50.3 (C17, C_{quart}), 50.2 (C9, *C*H), 49.2 (C18, *C*H),48.8 (C19, *C*H), 43.1 (C14, C_{quart}), 41.0 (C8, C_{quart}), 38.5 (C4, C_{quart}), 37.9 (C1, *C*H$_2$), 37.5 (C13, *C*H), 37.2 (C10, $C_{quart.}$), 34.7 (C7, *C*H$_2$), 34.3 (C21, *C*H$_2$), 34.1 (C16, *C*H$_2$), 32.4 (C22, *C*H$_2$), 28.0 (C23, *C*H$_3$), 27.8 (C15, *C*H$_2$), 25.2 (C12, *C*H$_2$), 23.8 (C2, *C*H$_2$), 21.6 (Ac), 21.5 (Ac), 20.9 (C11, *C*H$_2$), 18.8 (C29, *C*H$_3$), 18.2 (C6, *C*H$_2$), 16.5 (C24, *C*H$_3$), 16.2 (C26 + C25, 2 *C*H$_3$), 15.1 (C27, *C*H$_3$) ppm; MS (ESI, MeOH): *m/z* = 551.2 (10%, [M + H]$^+$), 573.3 (30%, [M + Na]$^+$), 1123.2 (100%, [2 M + Na]$^+$); anal. calcd. for $C_{36}H_{54}O_4$ (550.81): C, 78.50; H, 9.88; found: C, 78.37; H, 10.02.

28-Ethinyl-lup-20(29)-en-3,28-dione ***(7)***

Compound **7** (0.44 g, 84%) was obtained from 3-*O*-acetylbetulinic aldehyde (**1**) (0.50 g, 1.03 mmol) and lithium acetylide ethylenediamine complex (0.38 g, 4.12 mmol) following GP1 and GP2 as a colorless solid; mp 214°C; $[\alpha]_D^{20} = 45.8^\circ$ (*c* = 5.0, $CHCl_3$); $R_f = 0.38$ (silica gel, hexane/ethyl acetate, 9:1); IR (KBr): ν = 3206 m, 3070 w, 2945 s, 2868 m, 2085 m, 1702 s, 1670 s, 1641 m, 1455 m, 1390 m, 1378 m, 1349 w, 1137 w, 1107 w, 1084 w, 1062 m, 1027 m, 1004 w cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 4.59 (d, 1H, *J* = 2.1 Hz, C*H*$_a$ (30)), 4.46 (dd, 1H, *J* = 2.1, 1.5 Hz, C*H*$_b$ (30)), 3.18 (s, 1H, *H*C≡C), 2.79 (ddd, 1H, *J* = 11.2, 11.0, 4.9 Hz, C*H* (19)), 2.40–2.30 (m, 2H, C*H*$_a$ (16) + C*H*$_a$ (2)), 2.28–2.22 (m, 1H, C*H*$_b$ (2)), 2.16 (ddd, 1H, *J* = 12.8, 11.6, 3.8 Hz, C*H* (13)), 1.93 (dd, 1H, *J* = 12.9, 8.4 Hz, C*H*$_a$ (22)), 1.76 (ddd, 1H, *J* = 13.1, 7.6, 4.5 Hz, C*H* (1)), 1.69–1.50 (m, 3H, C*H*$_a$ (21) + C*H*$_a$ (12) + C*H* (18)), 1.54 (s, 3H, C*H*$_3$ (29)), 1.42 (ddd, 1H, *J* = 13.7, 13.7, 3.8 Hz, C*H*$_b$ (16)), 1.37–1.08 (m, 12H, C*H*$_2$ (6) + C*H*$_b$ (22) + C*H*$_2$ (7) + C*H*$_b$ (21) + C*H*$_2$ (11) + C*H*$_b$ (1) + C*H* (9) + C*H*$_a$ (15) + C*H* (5)), 0.95–0.83 (m, 1H, C*H*$_b$ (12)), 0.92 (s, 3H, C*H*$_3$ (23)), 0.88 (s, 3H, C*H*$_3$ (24)), 0.83 (s, 3H, C*H*$_3$ (27)), 0.81 (s, 3H, C*H*$_3$ (25)), 0.79 (s, 3H, C*H*$_3$ (26)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 217.6 (*C*=O), 191.4 (*C*=O), 149.8 (C20, *C*=CH$_2$), 109.5 (C30, *C*H$_2$=C), 80.1 (C31, *C*≡C), 77.3 (C32, H*C*≡C), 61.4 (C17, $C_{quart.}$), 54.6 (C5, *C*H), 49.6 (C9, *C*H), 48.1 (C18, *C*H), 46.9 (C4, $C_{quart.}$), 46.0

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(C19, *C*H), 42.4 (C14, $C_{quart.}$), 40.3 (C8, $C_{quart.}$), 39.3 (C1, CH_2), 36.9 (C13, *C*H), 36.5 (C10, $C_{quart.}$), 35.1 (C22, CH_2), 33.8 (C2, CH_2), 33.2 (C7, CH_2), 31.2 (C16, CH_2), 29.7 (C21, CH_2), 29.1 (C15, CH_2), 26.3 (C23, CH_3), 25.2 (C12, CH_2), 21.1 (C11, CH_2), 20.7 (C24, CH_3), 19.3 (C6, CH_2), 18.9 (C29, CH_3), 15.6 (C26, CH_3), 15.4 (C25, CH_3), 14.0 (C27, CH_3) ppm; MS (ESI, MeOH): m/z = 463.3 (100%, $[M + H]^+$); anal. calcd. for $C_{32}H_{46}O_2$ (462.71): C, 83.06; H, 10.02; found: C, 83.24; H, 10.06.

28-(Pyrazol-3-yl)-3,28-dioxo-28-ethinyllup-20(29)-en ***(8)***

An ethereal solution of diazomethane was added at 0°C to a solution of compound **7** (0.20 g, 0.43 mmol) in Et_2O (10 mL) until no further consumption of the diazomethane was observed. After stirring for additional 10 min, the excess of diazomethane was destroyed with acetic acid (5%) and the reaction mixture was concentrated *in vacuo*. After column chromatography compound **8** (0.20 g, 94%) was obtained as a colorless solid; mp 165–167°C; $[\alpha]_D^{20} = 25.1^\circ$ (c = 4.2, $CHCl_3$); R_f = 0.29 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr): ν = 3262 s, 3147 w, 2938 s, 2865 s, 1698 s, 1653 s, 1460 m, 1416 m, 1378 m, 1349 w, 1319 m, 1281 s, 1258 w, 1116 m, 1059 m, 1023 w cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.63 (br s, 1H, C*H* (pyrazole)), 6.79 (br s, 1H, C*H* (pyrazole)), 4.75 (d, 1H, J = 2.1 Hz, CH_a (30)), 4.60 (br s, 1H, CH_b (30)), 2.98 (ddd, 1H, J = 11.0, 11.0, 4.6 Hz, C*H* (19)), 2.74–2.65 (m, 2H, CH_a (16) + C*H* (13)), 2.47 (dd, 1H, J = 9.6, 7.6 Hz, CH_a (2)), 2.40 (dd, 1H, J = 7.6, 4.5 Hz, CH_b (2)), 2.33 (dd, 1H, J = 12.2, 7.1 Hz, CH_a (22)), 1.89 (ddd, 1H, J = 12.3, 7.6, 4.5 Hz, C*H* (1)), 1.81–1.59 (m, 5H, CH_a (12) + CH_b (16) + CH_a (21) + C*H* (18) + CH_b (22)), 1.69 (s, 3H, CH_3 (29)), 1.45–1.22 (m, 10H, CH_2 (11) + CH_2 (6) + CH_b (21) + CH_b (1) + CH_2 (7) + C*H* (9) + C*H* (5)), 1.22–1.08 (m, 2H, CH_2 (15)), 1.05–0.95 (m, 1H, CH_b (12)), 1.04 (s, 3H, CH_3 (23)), 0.99 (s, 3H, CH_3 (24)), 0.98 (s, 3H, CH_3 (27)), 0.91 (s, 6H, CH_3 (25) + CH_3 (26)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 218.2 (*C*=O), 197.4 (*C*=O), 150.6 (C20, *C*=CH_2), 142.3 (pyrazole, $C_{quart.}$), 136.4 (pyrazole, *C*H), 109.6 (C30, *C*H_2=C), 108.1 (pyrazole, *C*H), 60.1 (C17, $C_{quart.}$), 55.0 (C5, *C*H), 50.2 (C18, *C*H), 50.0 (C9, *C*H), 47.3 (C4, $C_{quart.}$), 45.6 (C19, *C*H), 42.4 (C14, $C_{quart.}$), 40.6 (C8, $C_{quart.}$), 39.6 (C1, CH_2), 37.0 (C22, CH_2), 36.9 (C13, *C*H), 36.9 (C10, $C_{quart.}$), 34.1 (C2, CH_2), 33.5 (C7, CH_2), 33.0 (C16, CH_2), 30.6 (C21, CH_2), 29.6 (C15, CH_2), 26.6 (C23, CH_3), 25.6 (C12, CH_2), 21.5 (C11, CH_2), 21.0 (C24, CH_3), 19.6 (C6, CH_2), 19.3 (C29, CH_3), 16.0 (C26, CH_3), 15.8 (C25, CH_3), 14.5 (C27, CH_3) ppm; MS (ESI, MeOH): m/z = 505.4 (90%, $[M + H]^+$), 1009.3 (100%, $[2\ M + H]^+$); anal. calcd. for $C_{33}H_{48}N_2O_2$ (504.75): C, 78.53; H, 9.59; N, 5.55; found: C, 78.47; H, 9.59; N, 5.32.

28-(5-(Ethylcarboxy)-pyrazol-3-yl)-3,28-dioxo-28-ethinyllup-20(29)-en ***(9)***

A solution of compound **3** (0.20 g, 0.43 mmol) and ethyl diazoacetate (0.12 g, 1.0 mmol) in toluene (10 mL) was heated under reflux for 24 h. After TLC revealed the absence of starting material, the reaction mixture was concentrated *in vacuo* and the residue subjected to column chromatography (silica gel, hexane/ethyl acetate, 8:2). Compound **9** (0.20 g, 81%) was obtained as a colorless solid; mp 154–157°C; $[\alpha]_D^{20} = 32.3^\circ$ (c = 4.5, $CHCl_3$); R_f = 0.35 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr): ν = 3274 br, 3072 w, 2945 s, 2868 m, 1706 s, 1672 s, 1642 m, 1560 w, 1460 m, 1383 m, 1307 m, 1226 s, 1116 m, 1025 m cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 11.56 (br s, 1H, N*H*), 7.27 (br s, 1H, C*H* (pyrazole)), 4.73 (d, 1H, J = 2.1 Hz, CH_a (30)), 4.60 (br s, 1H, CH_b (30)), 4.40 (q, 2H, J = 7.0 Hz, OCH_2), 2.94 (ddd, 1H, J = 11.0, 11.0, 4.6 Hz, C*H* (19)), 2.62 (ddd, 1H, J = 12.7, 12.7, 5.0 Hz, C*H* (13)), 2.70 (ddd, 1H, J = 13.8, 3.3, 3.3 Hz, CH_a (16)), 2.44 (dd, 1H, J = 9.6, 7.6 Hz, CH_a (2)), 2.40 (dd, 1H, J = 7.6, 4.5 Hz, CH_b (2)), 2.52-2.26 (m, 3H, CH_2 (2) + CH_a (22)), 1.88 (ddd, 1H, J = 12.3, 7.6, 4.5 Hz, C*H* (1)), 1.80–1.58 (m, 5H, CH_a (12) + CH_b (16) + CH_a (21) + C*H* (18) + CH_b (22)), 1.69 (s, 3H, CH_3 (29)), 1.45–1.23 (m, 10H, CH_2 (11) + CH_2 (6) + CH_b (21) + CH_b (1) + CH_2 (7) + C*H* (9) + C*H* (5)), 1.39 (t, 3H, J = 7.0 Hz, CH_3), 1.22–1.08 (m, 2H, CH_2 (15)), 1.05–0.95 (m, 1H, CH_b (12)), 1.03 (s, 3H, CH_3 (23)), 0.98 (s, 3H, CH_3 (24)), 0.97 (s, 3H, CH_3 (27)), 0.90 (s, 3H, CH_3 (25)), 0.89 (s, 3H, CH_3 (26)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 218.1 (*C*=O), 197.1 (*C*=O), 161.0 (C=O), 150.3 (C20, *C*=CH_2), 110.6 (pyrazole, *C*H), 109.7 (C30, *C*H_2=C), 61.1 (OCH_2), 60.2 (C17, $C_{quart.}$), 55.0 (C5, *C*H), 50.2 (C18, *C*H), 50.0 (C9, *C*H), 47.3 (C4, $C_{quart.}$), 45.6 (C19, *C*H), 42.4 (C14, $C_{quart.}$), 40.7 (C8, $C_{quart.}$), 39.6 (C1, CH_2), 36.9 (C13, *C*H), 36.9 (C10, $C_{quart.}$), 36.8 (C22, CH_2), 34.1 (C2, CH_2), 33.5 (C7, CH_2), 32.8 (C16, CH_2), 30.6 (C21, CH_2), 29.6 (C15, CH_2), 26.5 (C23, CH_3), 25.6 (C12, CH_2), 21.5 (C11, CH_2), 21.0 (C24, CH_3), 19.6 (C6, CH_2), 19.3 (C29, CH_3), 15.9 (C26, CH_3), 15.8 (C25, CH_3), 14.2 (C27, CH_3), 14.4 (CH_3) ppm; MS (ESI, MeOH): m/z = 577.3 (60%, $[M + H]^+$), 1175.1 (100%, $[2\ M + Na]^+$); anal. calcd. for $C_{36}H_{52}N_2O_4$ (576.81): C, 74.96; H, 9.09; N, 4.86; found: C, 74.56; H, 8.81; N, 4.75.

28-(1-(Ethylcarboxymethyl)-1H-1,2,3-triazol-4-yl)-3,28-dioxo-28-ethinyllup-20(29)-en ***(10)***

Compound **10** (0.21 g, 83%) was obtained from compound **7** (0.20 g, 0.43 mmol) and ethyl azidoacetate (78 mg, 0.60 mmol) following GP3 as a colorless solid; mp 203–205 °C; $[\alpha]_D^{20} = 22.0^\circ$ (c = 5.0, $CHCl_3$); R_f = 0.23 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr): ν = 3147 w, 3071 w, 2946 s, 2869 s, 1757 s, 1704 s, 1672 s, 1642 w, 1518 m, 1461 s, 1376 s, 1214 s, 1176 s, 1139 w, 1024 s cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.17 (s, 1H, C*H* (triazole)), 5.16 (s, 2H, NCH_2), 4.73 (d, 1H, J = 2.1 Hz, CH_a (30)), 4.58 (dd, 1H, J = 2.1, 1.5 Hz, CH_b (30)), 4.28 (q, 2H, J = 7.0 Hz, OCH_2), 3.13 (ddd, 1H, J = 13.8, 3.3, 3.3 Hz, CH_a (16)), 2.95 (ddd, 1H, J = 11.0, 11.0, 4.6 Hz, C*H* (19)), 2.63 (ddd, 1H, J = 12.7,

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12.7, 5.0 Hz, C*H* (13)), 2.56 (dd, 1H, J = 12.7, 7.1 Hz, CH_a (22)), 2.44 (dd, 1H, J = 9.6, 7.6 Hz, CH_a (2)), 2.40 (dd, 1H, J = 7.6, 4.5 Hz, CH_b (2)), 1.88 (ddd, 1H, J = 12.3, 7.6, 4.5 Hz, C*H* (1)), 1.82–1.55 (m, 5H, CH_a (12) + CH_b (16) + CH_a (21) + C*H* (18) + CH_b (22)), 1.69 (s, 3H, CH_3 (29)), 1.45–1.25 (m, 10H, CH_2 (11) + CH_2 (6) + CH_b (21) + CH_2 (7) + C*H* (9) + CH_b (1) + C*H* (5)), 1.19–1.09 (m, 2H, CH_2 (15)), 1.05–0.95 (m, 1H, CH_b (12)), 1.03 (s, 3H, CH_3 (23)), 0.98 (s, 6H, CH_3 (24) + CH_3 (27)), 0.91 (s, 3H, CH_3 (25)), 0.90 (s, 3H, CH_3 (26)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 218.0 (*C*=O), 198.5 (*C*=O), 165.6 (*C*=O), 150.8 (C20, *C*=CH_2), 147.8 (triazole, $C_{quart.}$), 129.0 (triazole, *C*H), 109.4 (C30, CH_2=*C*), 62.6 (OCH_2), 60.6 (C17, $C_{quart.}$), 54.9 (C5, *C*H), 50.8 (NCH_2), 50.2 (C18, *C*H), 50.0 (C9, *C*H), 47.2 (C4, $C_{quart.}$), 45.9 (C19, *C*H), 42.4 (C14, $C_{quart.}$), 40.7 (C8, $C_{quart.}$), 39.6 (C1, CH_2), 37.0 (C13, *C*H), 36.9 (C10, $C_{quart.}$), 36.1 (C22, CH_2), 34.0 (C2, CH_2), 33.5 (C7, CH_2), 31.7 (C16, CH_2), 30.6 (C21, CH_2), 29.6 (C15, CH_2), 26.6 (C23, CH_3), 25.6 (C12, CH_2), 21.5 (C11, CH_2), 20.9 (C24, CH_3), 19.6 (C6, CH_2), 19.3 (C29, CH_3), 16.0 (C26, CH_3), 15.8 (C25, CH_3), 14.7 (C27, CH_3), 14.0 (Et, CH_3) ppm; MS (ESI, MeOH): m/z = 592.4 (100%, $[M + H]^+$), 614.3 (90%, $[M + Na]^+$), 1205.1 (80% $[2\ M + Na]^+$); anal. calcd. for $C_{36}H_{53}N_3O_4$ (591.82): C, 73.06; H, 9.03, N, 7.10; found: C, 72.74; H, 9.14, N, 7.72.

28-[1-(4-Carboxyphenyl)-1H-1,2,3-triazol-4-yl]-lup-20(29)-en-3,28-dione ***(11)***

Compound **11** (0.20 g, 76%) was obtained from compound **7** (0.20 g, 0.43 mmol) and 4-azidobenzoic acid (98 mg, 0.60 mmol) following GP3 as a colorless solid; mp >250°C; $[\alpha]_D^{20}$ = 9.4° (c = 3.3, $CHCl_3$); R_f = 0.19 (silica gel, chloroform/diethyl ether, 8:2); IR (KBr): ν = 2944 s, 2868 m, 2361 w, 1702 s, 1608 m, 1519 m, 1450 m, 1376 w, 1288 w, 1196 w, 1115 w, 1013 m cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.58 (s, 1H, C*H* (triazole)), 8.29 (d, 2H, J = 8.7 Hz, Ph), 7.92 (d, 2H, J = 8.7 Hz, Ph), 7.92 (d, 2H, J = 8.7 Hz, Ph), 4.76 (d, 1H, J = 2.1 Hz, CH_a (30)), 4.61 (dd, 1H, J = 2.1, 1.5 Hz, CH_b (30)), 3.17 (ddd, 1H, J = 13.8, 3.3, 3.3 Hz, CH_a (16)), 2.97 (ddd, 1H, J = 11.0, 11.0, 4.6 Hz, C*H* (19)), 2.70–2.56 (m, 2H, C*H* (13) + CH_a (22)), 2.53–2.35 (m, 2H, CH_2 (2)), 1.90 (ddd, 1H, J = 12.3, 7.6, 4.5 Hz, C*H* (1)), 1.84–1.63 (m, 5H, CH_a (12) + CH_b (16) + CH_a (21) + C*H* (18) + CH_b (22)), 1.71 (s, 3H, CH_3 (29)), 1.47–1.28 (m, 10H, CH_2 (11) + CH_2 (6) + CH_b (21) + CH_2 (7) + CH_b (1) + C*H* (9) + C*H* (5)), 1.24–1.14 (m, 2H, CH_2 (15)), 1.08–0.97 (m, 1H, CH_b (12)), 1.04 (s, 3H, CH_3 (23)), 1.00 (s, 3H, CH_3 (27)), 0.99 (s, 3H, CH_3 (24)), 0.92 (s, 3H, CH_3 (25)), 0.91 (s, 3H, CH_3 (26)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 218.5 (*C*=O), 198.6 (*C*=O), 169.8 (*C*=O), 150.7 (C20, *C*=CH_2), 148.4 (triazole, $C_{quart.}$), 140.0 (Ph, $C_{quart.}$), 132.1 (Ph, *C*H), 129.9 (Ph, $C_{quart.}$), 125.5 (triazole, *C*H), 120.2 (Ph, *C*H), 109.5 (C30, CH_2=*C*), 60.4 (C17, $C_{quart.}$), 54.9 (C5, *C*H), 50.2 (C18, *C*H), 50.0 (C9, *C*H), 47.3 (C4, $C_{quart.}$), 45.9 (C19, *C*H), 42.5 (C14, $C_{quart.}$), 40.7 (C8, $C_{quart.}$), 39.6 (C1, CH_2), 37.0 (C13, *C*H), 36.9 (C10, $C_{quart.}$), 36.1 (C22, CH_2), 34.1 (C2, CH_2), 33.6 (C7, CH_2), 31.7 (C16, CH_2), 30.6 (C21, CH_2), 29.7 (C15, CH_2), 26.6 (C23, CH_3), 25.6 (C12, CH_2), 21.5 (C11, CH_2), 21.0 (C24, CH_3), 19.6 (C6, CH_2), 19.3 (C29, CH_3), 16.0 (C26, CH_3), 15.8 (C25, CH_3), 14.1 (C27, CH_3) ppm; MS (ESI, MeOH): m/z = 624.7 (100%, $[M - H]^-$); anal. calcd. for $C_{39}H_{51}N_3O_4$ (625.84): C, 74.85; H, 8.21; N, 6.71; found: C, 74.54; H, 8.43; N, 6.56.

28-[1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-1H-1,2,3-triazol-4-yl]-lup-20(29)-en-3,28-dione ***(12)***

From **7**: Compound **12** (0.26 g, 72%) was obtained from compound **7** (0.20 g, 0.43 mmol) and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide (0.16 g, 0.43 mmol) following GP3 as a colorless solid.

From **16**: Compound **12** (70 mg, 70%) was obtained from compound **16** (100 mg, 0.2 mmol) by Jones oxidation following GP2.

From **16**: Compound **12** (290 mg, 73%) was obtained from compound **16** (400 mg, 0.5 mmol) by Swern oxidation applying DMSO (165 μL, 2 mmol), oxalyl chloride (172 μL, 2 mmol) and triethylamine (700 μL, 5 mmol) in dry dichloromethane (5 mL) for 2 h at −40°C and 12 h at 24°C followed by chromatography (silica gel, $CHCl_3$/Et_2O, 95:5); mp 174–177°C; $[\alpha]_D^{20}$ = −15.1° (c = 4.4, $CHCl_3$); R_f = 0.75 (silica gel, hexane/ethyl acetate, 5:5); IR (KBr): ν = 2946 m, 2871 w, 1760 s, 1706 w, 1676 m, 1521 w, 1460 m, 1376 m, 1221 s, 1108 m, 1066 m, 1036 m cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.25 (s, 1H, C*H* (triazole)), 5.87 (d, 1H, J = 8.9 Hz, Glc C*H* (1)), 5.44–5.33 (m, 2H, Glc C*H* (3) + C*H* (2)), 5.22 (dd, 1H, J = 10.1, 9.2 Hz, Glc C*H* (4)), 4.71 (d, 1H, J = 2.1 Hz, CH_a (30)), 4.56 (dd, 1H, J = 2.1, 1.5 Hz, CH_b (30)), 4.29 (dd, 1H, J = 12.7, 4.8 Hz, Glc CH_a (6)), 4.15 (dd, 1H, J = 12.7, 2.0 Hz, Glc CH_b (6)), 4.00 (ddd, 1H, J = 10.1, 4.8, 2.0 Hz, Glc C*H* (5)), 3.04 (ddd, 1H, J = 13.8, 3.3, 3.3 Hz, CH_a (16)), 2.90 (ddd, 1H, J = 11.0, 11.0, 4.6 Hz, C*H* (19)), 2.63 (ddd, 1H, J = 12.7, 12.7, 5.0 Hz, C*H* (13)), 2.50–2.29 (m, 3H, CH_a (22) + CH_2 (2)), 2.06 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.88 (ddd, 1H, J = 12.3, 7.6, 4.5 Hz, C*H* (1)), 1.85 (s, 3H, Ac), 1.77–1.53 (m, 5H, CH_a (12) + CH_b (16) + CH_a (21) + C*H* (18) + CH_b (22)), 1.66 (s, 3H, CH_3 (29)), 1.43–1.25 (m, 10H, CH_2 (11) + CH_2 (6) + CH_2 (7) + C*H* (9) + CH_b (1) + CH_b (21) + C*H* (5)), 1.17–1.06 (m, 2H, CH_2 (15)), 1.03–0.89 (m, 1H, CH_b (12)), 1.00 (s, 3H, CH_3 (23)), 0.96 (s, 6H, CH_3 (24) + CH_3 (27)), 0.89 (s, 3H, CH_3 (25)), 0.88 (s, 3H, CH_3 (26)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 218.3 (*C*=O), 198.1 (*C*=O), 170.4 (*C*=O), 169.9 (*C*=O), 169.2 (*C*=O), 168.7 (*C*=O), 150.7 (C20, *C*=CH_2), 147.8 (triazole, $C_{quart.}$), 126.2 (triazole, *C*H), 109.5 (C30, CH_2=*C*), 85.7 (Glc (1), *C*H), 75.2 (Glc (5), *C*H), 72.4 (Glc (3), *C*H), 70.5 (Glc (2), *C*H), 67.5 (Glc (4), *C*H), 61.4 (Glc (6), CH_2), 60.6 (C17, $C_{quart.}$), 54.9 (C5, *C*H), 50.1 (C18, *C*H), 50.0 (C9, *C*H), 47.2 (C4, $C_{quart.}$), 45.7 (C19, *C*H), 42.4 (C14, $C_{quart.}$), 40.6 (C8, $C_{quart.}$), 39.6 (C1, CH_2), 36.9 (C10, $C_{quart.}$), 36.8 (C13, *C*H), 36.1 (C22, CH_2), 34.0 (C2, CH_2), 33.5 (C7, CH_2), 31.5 (C16, CH_2), 30.5 (C21, CH_2), 29.6 (C15, CH_2), 26.6 (C23, CH_3), 25.6 (C12, CH_2), 21.5 (C11, CH_2), 20.9 (C24, CH_3), 20.6 (Ac),

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20.4 (2 Ac), 20.2 (Ac), 19.6 (C6, *C*H_2), 19.3 (C29, *C*H_3), 15.9 (C26, *C*H_3), 15.8 (C25, *C*H_3), 14.3 (C27, *C*H_3) ppm; MS (ESI, MeOH): m/z = 836.0 (20%, $[M + H]^+$), 858.3 (100%, $[M + Na]^+$), 1693.2 (50%, $[2\ M + Na]^+$); anal. calcd. for $C_{46}H_{65}N_3O_{11}$ (836.02): C, 66.09; H, 7.84; N, 5.03; found: C, 65.74; H, 7.73; N, 4.83.

28-[1-(2,3,4,6-Tetra-O-acetyl-β-D-mannopyranosyl)-1H-1,2,3-triazol-4-yl]-lup-20(29)-en-3,28-dione ***(13)***

Compound **13** (0.25 g, 69%) was obtained from compound **7** (0.20 g, 0.43 mmol) and 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl azide (0.16 g, 0.43 mmol) following GP3 as a colorless solid; mp 243–245°C; $[\alpha]_D^{20} = -26.6^\circ$ (c = 4.3, $CHCl_3$); R_f = 0.17 (silica gel, $CHCl_3$/Et_2O, 9:1); IR (KBr): ν = 2950 s, 2871 m, 1760 s, 1706 m, 1675 m, 1514 w, 1460 m, 1370 m, 1222 s, 1166 w, 1061 m cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.22 (s, 1H, C*H* (triazole)), 6.16 (s, 1H, Man C*H* (1)), 5.67 (d, 1H, J = 2.1 Hz, Man C*H* (2)), 5.32 (dd, 1H, J = 10.0, 10.0 Hz, Man C*H* (4)), 5.25 (dd, 1H, J = 10.0, 3.0 Hz, Man C*H* (3)), 4.69 (d, 1H, J = 2.1 Hz, CH_a (30)), 4.54 (dd, 1H, J = 2.1, 1.5 Hz, CH_b (30)), 4.27 (dd, 1H, J = 12.6, 5.5 Hz, Man CH_a (6)), 4.19 (dd, 1H, J = 12.6, 1.7 Hz, Man CH_b (6)), 4.00 (ddd, 1H, J = 10.0, 5.5, 1.7 Hz, Man C*H* (5)), 3.01 (ddd, 1H, J = 13.8, 3.3, 3.3 Hz, CH_a (16)), 2.90 (ddd, 1H, J = 11.0, 11.0, 4.6 Hz, C*H* (19)), 2.56 (ddd, 1H, J = 12.7, 12.7, 5.0 Hz, C*H* (13)), 2.51–2.30 (m, 3H, CH_a (22) + CH_2 (2)), 2.06 (s, 3H, Ac), 2.03 (s, 6H, 2Ac), 1.94 (s, 3H, Ac), 1.84 (ddd, 1H, J = 12.3, 7.6, 4.5 Hz, C*H* (1)), 1.72–1.52 (m, 5H, CH_a (12) + CH_b (16) + CH_a (21) + C*H* (18) + CH_b (22)), 1.64 (s, 3H, CH_3 (29)), 1.40–1.24 (m, 10H, CH_2 (11) + CH_2 (6) + CH_b (21) + CH_b (1) + CH_2 (7) + C*H* (9) + C*H* (5)), 1.17–1.04 (m, 2H, CH_2 (15)), 0.98–0.87 (m, 1H, CH_b (12)), 0.98 (s, 3H, CH_3 (23)), 0.93 (s, 6H, CH_3 (24) + CH_3 (27)), 0.87 (s, 3H, CH_3 (25)), 0.86 (s, 3H, CH_3 (26)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 218.1 (*C*=O), 198.5 (*C*=O), 170.4 (*C*=O), 169.7 (*C*=O), 169.4 (*C*=O), 169.0 (*C*=O), 150.7 (C20, *C*=CH_2), 147.5 (triazole, $C_{quart.}$), 126.8 (triazole, *C*H), 109.5 (C30, *C*H_2=C), 84.6 (Man (1), *C*H), 75.7 (Man (5), *C*H), 70.6 (Man (3), *C*H), 68.6 (Man (2), *C*H), 64.7 (Man (4), *C*H), 62.0 (Man (6), CH_2), 60.7 (C17, $C_{quart.}$), 54.9 (C5, *C*H), 50.1 (C18, *C*H), 50.0 (C9, *C*H), 47.2 (C4, $C_{quart.}$), 45.8 (C19, *C*H), 42.5 (C14, $C_{quart.}$), 40.6 (C8, $C_{quart.}$), 39.6 (C1, *C*H_2), 37.0 (C13, *C*H), 36.9 (C10, $C_{quart.}$), 36.0 (C22, *C*H_2), 34.1 (C2, *C*H_2), 33.5 (C7, *C*H_2), 31.7 (C16, *C*H_2), 30.6 (C21, *C*H_2), 29.6 (C15, *C*H_2), 26.6 (C23, *C*H_3), 25.6 (C12, *C*H_2), 21.5 (C11, *C*H_2), 20.9 (C24, *C*H_3), 20.7 (Ac), 20.6 (Ac), 20.5 (Ac), 20.4 (Ac), 19.6 (C6, *C*H_2), 19.3 (C29, *C*H_3), 15.9 (C26, *C*H_3), 15.8 (C25, *C*H_3), 14.4 (C27, *C*H_3) ppm; MS (ESI, MeOH): m/z = 836.0 (20%, $[M + H]^+$), 858.3 (100%, $[M + Na]^+$), 1693.2 (30%, $[2\ M + Na]^+$); anal. calcd. for $C_{46}H_{65}N_3O_{11}$ (836.02): C, 66.09; H, 7.84; N, 5.03; found: C, 62.95; H, 7.46; N, 4.89.

28-[1-(β-D-Glucopyranosyl)-1H-1,2,3-triazol-4-yl]-lup-20(29)-en-3,28-dione ***(14)***

Compound **14** (0.11 g, 85%) was obtained from compound **12** (0.15 g, 0.18 mmol) following GP4 as a colorless solid; mp 228–230°C; $[\alpha]_D^{20} = 2.4^\circ$ (c = 4.2, MeOH); R_f = 0.55 (silica gel, CH_2Cl_2/MeOH, 9:1); IR (KBr): ν = 2941 s, 2868 m, 1697 m, 1674 m, 1596 m, 1517 w, 1460 m, 1375 m, 1098 m, 1024 m cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6): δ = 8.87 (s, 1H, C*H* (triazole)), 5.56 (d, 1H, J = 9.2 Hz, Glc C*H* (1)), 4.68 (d, 1H, J = 2.1 Hz, CH_a (30)), 4.54 (dd, 1H, J = 2.1, 1.5 Hz, CH_b (30)), 3.81 (dd, 1H, J = 9.2, 9.1 Hz, Glc C*H* (2)), 3.65 (dd, 1H, J = 10.3, 4.6 Hz, Glc CH_a (6)), 3.42–3.33 (m, 3H, Glc CH_b (6) + C*H* (5) + C*H* (3)), 3.24 (dd, 1H, J = 8.8, 8.8 Hz, Glc C*H* (4)), 2.93 (ddd, 1H, J = 13.8, 3.3, 3.3 Hz, CH_a (16)), 2.85 (ddd, 1H, J = 11.0, 11.0, 4.6 Hz, C*H* (19)), 2.58 (ddd, 1H, J = 12.7, 12.7, 5.0 Hz, C*H* (13)), 2.46–2.28 (m, 3H, CH_a (22) + CH_2 (2)), 1.77 (ddd, 1H, J = 12.3, 7.6, 4.5 Hz, C*H* (1)), 1.64–1.47 (m, 5H, CH_a (12) + CH_b (16) + C*H* (18) + CH_b (22) + CH_a (21)), 1.64 (s, 3H, CH_3 (29)), 1.38–1.14 (m, 10H, CH_2 (11) + CH_2 (6) + C*H* (9) + CH_b (1) + CH_b (21) + CH_2 (7) + C*H* (5)), 1.10–0.98 (m, 2H, CH_2 (15)), 0.94–0.82 (m, 1H, CH_b (12)), 0.94 (s, 3H, CH_3 (23)), 0.93 (s, 3H, CH_3 (27)), 0.88 (s, 3H, CH_3 (24)), 0.82 (s, 6H, CH_3 (25) + CH_3 (26)) ppm; ^{13}C-NMR (125 MHz, DMSO-d_6): δ = 216.6 (*C*=O), 197.9 (*C*=O), 150.5 (C20, *C*=CH_2), 146.0 (triazole, $C_{quart.}$), 126.5 (triazole, *C*H), 109.7 (C30, *C*H_2=C), 87.8 (Glc (1), *C*H), 80.2 (Glc (5), *C*H), 76.8 (Glc (3), *C*H), 71.9 (Glc (2), *C*H), 69.4 (Glc (4), *C*H), 60.7 (Glc (6), CH_2), 60.0 (C17, $C_{quart.}$), 53.9 (C5, *C*H), 49.6 (C18, *C*H), 49.2 (C9, *C*H), 46.5 (C4, $C_{quart.}$), 45.6 (C19, *C*H), 42.1 (C14, $C_{quart.}$), 40.2 (C8, $C_{quart.}$), 38.9 (C1, *C*H_2), 36.4 (C13, *C*H), 36.4 (C10, $C_{quart.}$), 35.6 (C22, *C*H_2), 33.6 (C2, *C*H_2), 33.0 (C7, *C*H_2), 31.3 (C16, *C*H_2), 30.1 (C21, *C*H_2), 29.2 (C15, *C*H_2), 26.4 (C23, *C*H_3), 25.3 (C12, *C*H_2), 21.2 (C11, *C*H_2), 20.7 (C24, *C*H_3), 19.2 (C6, *C*H_2), 18.9 (C29, *C*H_3), 15.7 (C26, *C*H_3), 15.5 (C25, *C*H_3), 14.2 (C27, *C*H_3) ppm; MS (ESI, MeOH): m/z = 668.2 (10%, $[M + H]^+$), 690.4 (100% $[M + Na]^+$); anal. calcd. for $C_{38}H_{57}N_3O_7$ (667.88): C, 68.34; H, 8.60; N, 6.29; found: C, 67.99; H, 8.33; N, 5.83.

28-[1-(β-D-Mannopyranosyl)-1H-1,2,3-triazol-4-yl]-lup-20(29)-en-3,28-dione ***(15)***

Compound **15** (0.12 g, 90%) was obtained from compound **13** (0.15 g, 0.18 mmol) following GP4 as a colorless solid; mp 267–270°C; $[\alpha]_D^{20} = 12.3^\circ$ (c = 4.2, MeOH); R_f = 0.55 (silica gel, CH_2Cl_2/MeOH, 9:1); IR (KBr): ν = 2941 s, 2869 m, 1674 m, 1512 m, 1455 m, 1376 w, 1204 m, 1094 m, 1026 m cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6): δ = 8.58 (s, 1H, C*H* (triazole)), 6.04 (s, 1H, Man C*H* (1)), 4.68 (d, 1H, J = 2.1 Hz, CH_a (30)), 4.54 (dd, 1H, J = 2.1, 1.5 Hz, CH_b (30)), 3.88 (d, 1H, J = 2.1 Hz, Man C*H* (2)), 3.70 (dd, 1H, J = 12.6, 5.5 Hz, Man CH_a (6)), 3.57 (dd, 1H, J = 10.0, 3.0 Hz, Man C*H* (3)), 3.52–3.42 (m, 2H, Man C*H* (4) + CH_b (6)), 3.26–3.14 (m, 1H, Man C*H* (5)), 2.93 (ddd, 1H, J = 13.8, 3.3, 3.3 Hz, CH_a (16)), 2.87 (ddd, 1H, J = 11.0, 11.0, 4.6 Hz, C*H* (19)), 2.57 (ddd, 1H, J = 12.7, 12.7, 5.0 Hz, C*H* (13)), 2.46–2.26 (m, 3H, CH_a (22) + CH_2 (2)), 1.76 (ddd, 1H, J = 12.3, 7.6, 4.5 Hz, C*H* (1)), 1.72–1.48 (m, 5H, CH_a (12) + CH_b (16) + CH_a (21) + C*H* (18) + CH_b (22)), 1.64 (s, 3H, CH_3 (29)),

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1.40–1.24 (m, 10H, CH_2 (11) + CH_2 (6) + CH_b (21) + CH_b (1) + CH_2 (7) + *CH* (9) + *CH* (5)), 1.08–0.99 (m, 2H, CH_2 (15)), 0.93–0.85 (m, 1H, CH_b (12)), 0.93 (s, 6H, CH_3 (23) + CH_3 (27)), 0.88 (s, 3H, CH_3 (24)), 0.81 (s, 6H, CH_3 (25) + CH_3 (26)) ppm; ^{13}C-NMR (125 MHz, DMSO-d_6): δ = 216.6 (*C*=O), 198.0 (*C*=O), 150.5 (C20, *C*=CH_2), 145.7 (triazole, $C_{quart.}$), 127.8 (triazole, *C*H), 109.7 (C30, CH_2=C), 86.1 (Man (1), *C*H), 80.2 (Man (5), *C*H), 72.9 (Man (3), *C*H), 70.2 (Man (2), *C*H), 65.9 (Man (4), *C*H), 60.9 (Man (6), CH_2), 60.0 (C17, $C_{quart.}$), 53.9 (C5, *C*H), 49.6 (C18, *C*H), 49.2 (C9, *C*H), 46.5 (C4, $C_{quart.}$), 45.6 (C19, *C*H), 42.1 (C14, $C_{quart.}$), 40.2 (C8, $C_{quart.}$), 39.8 (C1, CH_2), 36.4 (C13, *C*H), 36.4 (C10, $C_{quart.}$), 35.5 (C22, CH_2), 33.6 (C2, CH_2), 33.0 (C7, CH_2), 31.3 (C16, CH_2), 30.1 (C21, CH_2), 29.1 (C15, CH_2), 26.4 (C23, CH_3), 25.2 (C12, CH_2), 21.1 (C11, CH_2), 20.7 (C24, CH_3), 19.2 (C6, CH_2), 18.8 (C29, CH_3), 15.7 (C26, CH_3), 15.5 (C25, CH_3), 14.2 (C27, CH_3) ppm; MS (ESI, MeOH): *m/z* = 668.3 (20%, $[M + H]^+$), 690.4 (100%, $[M + Na]^+$), 1357.3 (30%, $[2\ M + Na]^+$); anal. calcd. for $C_{38}H_{57}N_3O_7$ (667.88): C, 68.34; H, 8.60; N, 6.29; found: C, 67.89; H, 8.66; N, 5.89.

28-[1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-1H-1,2,3-triazol-4-yl]-betulin ***(16)***

Compound **16** (1.5 g, 85%) was obtained from compound **5** (1.0 g, 2.1mmol) and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide (1.5 g, 4 mmol) following GP3 as a colorless solid; mp 222–226°C; $[\alpha]_D^{20}$ = 4° (*c* = 5.4, $CHCl_3$); R_f = 0.16 (silica gel, $CHCl_3$/Et_2O, 95/5) IR (KBr): ν = 3589 m, 3403 s, 3295 s, 2950 s, 1454 m, 1390 m, 1374 m, 1256 w, 1183 w, 1139 w, 1032 m cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ= 7.76 (s, 1H, *CH* (triazol)), 5.85 (d, 1H, *J* = 9.0 Hz, *CH* Glc (1)), 5.50–5.35 (m, 3H, *CH* Glc (2) + *CH* Glc (3) + *CH*OH (28)), 5.25–5.19 (m, 1H, *CH* Glc (4)), 4.54 (d, 1H, *J* = 1.3 Hz, CH_a (30)), 4.48–4.50 (m, 1H, CH_b (30)), 4.29 (dd, 1H, *J* = 5.2, 12.5 Hz, CH_a Glc (6)), 4.18–4.14 (m, 1H, CH_b Glc (6)), 3.98 (ddd, 1H, *J* = 1.9, 4.7, 10.0 Hz, *CH* Glc (5)), 3.19 (dd, 1H, *J* = 4.9, 11.3 Hz, *CH*OH (3)), 3.00 (ddd, 1H, *J* = 5.8, 11.0, 11.0 Hz, *CH* (19)), 2.20–2.07 (m, 2H, CH_a (21) + *CH* (13)), 2.06 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 1.99 (s, 3H, CH_3), 1.95–183 (m, 2H, CH_a (16) + CH_b (21)), 1.81 (s, 3H, CH_3), 1.79–1.70 (m, 2H, CH_2 (12)), 1.68 (s, 3H, CH_3 (29)), 1.67–1.29 (m, 14H, CH_b (16) + CH_a (1) + CH_2 (2) + CH_2 (11) + CH_2 (22) + CH_2 (7) + CH_2 (6) + *CH* (18) + *CH* (9)), 1.18–1.14 (m, 2H, CH_2 (15)), 1.12 (s, 3H, CH_3 (27)), 1.09–1.01 (m, 1H, CH_b (16)), 0.99 (s, 3H, CH_3 (25)), 0.95 (s, 3H, CH_3 (24)), 0.91–0.85 (m, 1H, CH_b (1)), 0.82 (s, 3H, CH_3 (26)), 0.74 (s, 3H, CH_3 (23)), 0.67 (d, 1H, *J* = 9.0 Hz, *CH* (5)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 171.0 (*C*=O), 170.4 (*C*=O), 169.8 (*C*=O), 168.7 (*C*=O), 151.4 (C20, *C*=CH_2),151.3 (triazole, C_{quart}), 119.7 (triazole, *C*H), 109.5 (C30, CH_2=C), 85.8 (Glc (1), *C*H), 79.0 (C3, *C*H), 75.2 (Glc (5), *C*H), 72.7 (Glc (3), *C*H), 70.4 (Glc (2), *C*H), 69.1 (C28, *C*HOH), 67.9 (Glc (4), *C*H), 61.6 (Glc (6), CH_2), 55.4 (C5, *C*H), 50.4 (C9, *C*H), 50.3 (C17, C_{quart}), 50.2 (C18, *C*H), 48.8 (C19, *C*H), 43.2 (C14, C_{quart}), 41.1 (C8, C_{quart}), 38.9 (C4, C_{quart}), 38.8 (C1, CH_2), 37.3 (C13, *C*H), 37.2 (C10, $C_{quart.}$), 34.3 (C7, CH_2), 33.9 (C21, CH_2), 33.2 (C16, CH_2), 32.8 (C22, CH_2), 28.1 (C23, CH_3), 27.9 (C15, CH_2), 27.5 (C2, CH_2), 25.3 (C12, CH_2), 21.0 (C11, CH_2), 20.7 (Ac), 20.6 (Ac), 20.5 (Ac), 20.1(Ac), 18.4 (6, CH_2), 16.3 (C24, CH_3), 16.1 (C26, CH_3), 15.4 (C25, CH_3), 15.4 (C27, CH_3) ppm; MS (ESI, MeOH): *m/z* = 840.4 (20%, $[M + H]^+$), 826.5 (100%, $[M + Na]^+$), 1702.3 (30%, $[2\ M + Na]^+$); anal. calcd. for $C_{46}H_{67}N_3O_{11}$ (838.06): C, 65.93; H, 8.06; N, 5.01; found: C, 65.72; H, 8.31; N, 4.88.

28-[1-(β-D-Glucopyranosyl)-1H-1,2,3-triazol-4-yl]-betulin ***(17)***

Compound **17** (149 mg, 93%) was obtained from compound **16** (200 mg, 0.24 mmol) following GP4 as a colorless solid; mp 206–209°C; $[\alpha]_D^{20}$ = −3.21° (*c* = 5.7, MeOH); IR (KBr): ν = 3417 br, 2941 s, 2870 s, 1725 w, 2639 m, 1455 s, 1376 m, 1251 m, 1042 s cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.09 (s, 1H, *CH* triazole), 5.47 (d, 1H, *J* = 9.3 Hz, *CH*OH Glc (1)), 5.30–5.25 (m, 2H, CHO*H* Glc (2) + *CH*OH (28)), 5.19 (d, 1H, *J* = 4.9 Hz, CHO*H* Glc (3)), 5.09 (d, 1H, *J* = 5.5 Hz, CHO*H* Glc (4)), 5.03 (d, 1H, *J* = 5.0 Hz, CHO*H* (28)), 4.6 (d, 1H, *J* = 2.2 Hz, CH_a (30)), 4.59 (t, 1H, *J* = 5.5 Hz, CHO*H* Glc (6)), 4.54–4.51 (m, 1H, CH_b (30)), 4.24 (d, 1H, *J* = 5.1 Hz, CHO*H* (3)), 3.79 (ddd, 1H, *J* = 6.2, 9.1, 9.1 Hz, *CH*OH Glc (2)), 3.69 (dd, 1H, *J* = 5.4, 10.0 Hz, CH_a Glc (6)), 3.48–3.32 (m, 3H, CH_b Glc (6) + *CH*OH Glc (5) + *CH*OH Glc (3)), 3.26–3.19 (m, 1H, *CH*OH Glc (4)), 3.07 (ddd, 1H, *J* = 6.2, 11.0, 11.0 Hz, *CH* (19)), 2.97 (ddd, 1H, *J* = 5.6, 5.6, 9.9 Hz, *CH*OH (3)), 2.20–2.05 (m, 2H, *CH* (13) + CH_a (21)), 1.95–1.90 (m, 2H, CH_a (22)), 1.77–1.66 (m, 2H, *CH* (18) + CH_a (2)), 1.65 (s, 3H, CH_3 (29)), 1.60–1.54 (m, 1H, CH_a (1)), 1.50–1.14 (m, 14H, *CH* (9) + CH_2 (6) + CH_b (21) + CH_2 (7) + CH_2 (15) + CH_2 (11) + CH_a (16) + CH_b (2) + CH_2 (12)), 1.11 (s, 3H, CH_3 (27)), 1.09–1.00 (m, 1H, CH_b (16)), 0.97 (s, 3H, CH_3 (25)), 0.95–0.89 (m, 1H, CH_b (22)), 0.87 (s, 3H, CH_3 (24)), 085–0.83 (m, 1H, CH_b (1)), 0.80 (s, 3H, CH_3 (26)), 0.66 (s, 3H, CH_3 (23)), 0.65–0.60 (m, 1H, *CH* (5)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 151.8 (C20, *C*=CH_2),151.3 (triazole, C_{quart}), 122.3 (triazole, *C*H), 109.7 (C30, CH_2=C), 87.8 (Glc (1), *C*H), 80.3 (Glc (5), *C*H), 77.6 (Glc (3), *C*H), 77.3 (C3, *C*HOH), 72.4 (Glc (2), *C*H), 70.0 (Glc (4), *C*H), 67.6 (C28, *C*HOH), 61.2 (Glc (6), *C*H), 55.4 (C5, *C*H), 50.3 (C9, *C*H), 50.1 (C18, *C*H), 50.0 (C17, $C_{quart.}$), 48.6 (C19, *C*H), 43.0 (C14, C_{quart}), 41.0 (C8, C_{quart}), 39.5 (C4, C_{quart}), 38.7 (C1, CH_2), 37.2 (C10, $C_{quart.}$), 36.7 (C13, *C*H), 34.3 (C7, CH_2), 34.0 (C16, CH_2), 33.6 (C22, CH_2), 32.8 (C21, CH_2), 28.6 (C23, CH_3), 27.9 (C15, CH_2), 27.6 (C2, CH_2), 25.4 (C12, CH_2), 20.9 (C11, CH_2), 18.4 (C6, CH_2), 16.4 (C24, CH_3), 16.3 (C26, CH_3), 16.2 (C25, CH_3), 15.5 (C27, CH_3) ppm; MS (ESI, MeOH): *m/z* = 672.5 (17%, $[M + H]^+$), 694.5 (40%, $[M + Na]^+$), 1365.5 (100%, $2\ M + Na]^+$); anal. calcd. for $C_{38}H_{59}N_3O_7$ (669.89): C, 68.13; H, 8.88; N, 6.27; found: C, 67.86; H, 9.05; N, 6.05.

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*28-(2-(Dimethylamino)ethenyl)lup-20(29)-en-3,28-dione- **(18)***

A solution of compound **7** (0.20 g, 0.40 mmol) and DIMCARB (0.13 g, 1.00 mmol) in EtOH (10 mL) was stirred for 24 h at room temperature. The solution was concentrated *in vacuo* and the residue was subjected to column chromatography (silica gel, hexane/ethyl acetate, 8:2). Compound **18** (0.2 g, 97%) was obtained as a colorless solid; mp 249–250°C; $[\alpha]_D^{20} = 7.0^\circ$ ($c = 3.6$, $CHCl_3$); $R_f = 0.27$ (silica gel, hexane/ethyl acetate, 8:2); IR (KBr): $\nu = 3067$ w, 2945 s, 2864 m, 1700 s, 1659 s, 1574 s, 1457 m, 1432 m, 1354 m, 1300 w, 1221 w, 1138 w, 1101 w, 1055 s cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 7.52$ (d, 1H, $J = 12.5$ Hz, C*H* (32)), 5.22 (d, 1H, $J = 12.5$ Hz, C*H* (31)), 4.71 (d, 1H, $J = 2.1$ Hz, CH_a (30)), 4.54 (dd, 1H, $J = 2.1, 1.5$ Hz, CH_b (30)), 3.07 (ddd, 1H, $J = 11.2, 11.0, 4.9$ Hz, C*H* (19)), 2.89 (br s, 6H, NMe), 2.68 (ddd, 1H, $J = 12.8, 11.6, 3.8$ Hz, C*H* (13)), 2.52–2.32 (m, 2H, CH_2 (2)), 2.28–2.22 (ddd, 1H, $J = 12.8, 3.1, 3.1$ Hz, CH_a (16)), 1.92–1.68 (m, 4H, CH_a (1) + CH_a (22) + CH_a (21) + CH_a (12)), 1.66 (s, 3H, CH_3 (29)), 1.51–1.22 (m, 14H, C*H* (18) + CH_b (16) + CH_2 (6) + CH_2 (11) + CH_a (15) + CH_2 (7) + CH_b (1) + C*H* (9) + CH_b (22) + CH_b (21) + C*H* (5)), 1.09 (ddd, 1H, $J = 13.0, 3.6, 3.0$ Hz, CH_b (15)), 0.95–0.87 (m, 1H, CH_b (12)), 1.04 (s, 3H, CH_3 (23)), 1.00 (s, 3H, CH_3 (24)), 0.95 (s, 6H, CH_3 (25) + CH_3 (27)), 0.90 (s, 3H, CH_3 (26)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = 218.3$ (*C*=O), 201.9 (*C*=O), 152.2 (C32, H*C*), 151.7 (C20, *C*=CH_2), 108.9 (C30, *C*H_2=C), 92.4 (C31, H*C*), 58.9 (C17, $C_{quart.}$), 55.0 (C5, *C*H), 50.1 (C9, *C*H), 49.7 (C18, *C*H), 47.3 (C4, $C_{quart.}$), 46.4 (C19, *C*H), 42.6 (C14, $C_{quart.}$), 40.7 (C8, $C_{quart.}$), 39.6 (C1, *C*H_2), 37.8 (C22, *C*H_2), 37.0 (C13, *C*H), 36.9 (C10, $C_{quart.}$), 34.2 (C2, *C*H_2), 33.7 (C7, *C*H_2), 30.7 (C16, *C*H_2), 29.6 (C21, *C*H_2), 29.6 (C15, *C*H_2), 26.6 (C23, *C*H_3), 25.8 (C12, *C*H_2), 21.6 (C11, *C*H_2), 21.0 (C24, *C*H_3), 19.7 (C6, *C*H_2), 19.4 (C29, *C*H_3), 16.1 (C26, *C*H_3), 16.0 (C25, *C*H_3), 14.4 (C27, *C*H_3) ppm; MS (ESI, MeOH): $m/z = 508.8$ (100%, $[M + H]^+$); anal. calcd. for $C_{34}H_{53}NO_2$ (507.79): C, 80.42; H, 10.52; N, 2.76; found: C, 80.33; H, 10.65; N, 2.66.

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