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Synthesis, cytotoxicity and liposome preparation of 28-acetylenic betulin derivatives

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ABSTRACT

Several novel betulin derivatives were prepared and evaluated for their antitumor activity. 3-*O*-Acetyl-betulinic aldehyde served as an ideal starting material for the synthesis of 28-acetylenic derivatives. These compounds were further transformed into pyrazoles and 1,2,3-triazoles. Also, the synthesis of 3-amino substituted butenolides was carried out. The compounds were screened for their antitumor activity in a panel of 15 human cancer cell lines in a sulforhodamine B (SRB) assay. Several compounds showed a noteworthy antitumor activity. In addition, the possibility of encapsulation into liposomes was examined, thereby resulting in an increased cytotoxicity. The results from a trypan-blue test and from DNA laddering provided evidence for an apoptotic cell death.

1. Introduction

Triterpenes of the lupane-series (Fig. 1) are widely spread in the plant kingdom; recently, they came focus of scientific interest because of their promising antitumor and antiviral properties.

Several reports revealed the great potency of betulinic acid (BA) **I** and related compounds as potential therapeutics for HIV therapy. Further studies disclosed their particular action by inhibiting the virus-cell fusion at the gp41–gp120 interface[1,2] or by altering the process of cell maturation by interfering the CA-SP1 junction in Gag processing.[3,4]Also, betulinic acid derivatives have been suggested[5] for the therapy of human melanoma tumors; no toxic side effects were observed in doses up to 500 mg/kg, and their way of action works by an induction of apoptosis.

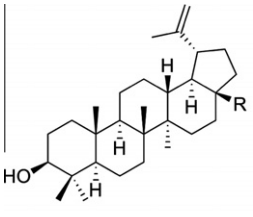
Follow-up studies allowed some insights into this process of apoptosis: Important steps involve the liberation of cytochrome c and AIF by changing the transmembrane potential of mitochondria[6,7] and the generation of reactive oxygen species (ROS).[8] A partial contribution of the p38 pathway was suggested, based on the observation[9] of phosphorylated proapoptotic mitogen-activated protein kinases (MAPKs).

Here, we present a synthetic approach to new betulin-derived compounds bearing an acetylenic side chain at the C-28 position. Also, some of these compounds were used for the synthesis of heterocyclic compounds by 1,3-dipolar cycloaddition. Their antitumor potency was examined by a sulforhodamin B assay (SRB) applying 15 human cancer cell lines.

2. Chemistry

The synthesis of betulin derivatives bearing an acetylenic side chain at C-28 was realized starting from 3-*O*-acetylbetulinic aldehyde[10,11] (Scheme 1). The reaction of methyl propiolate or of 2-propyn-1-ol in the presence of LDA[12] proceeded smoothly. For phenylacetylene butyllithium[13] gave higher yields compared to the use of LDA. The additions advanced stereoselectively; only the (28*S*) isomers could be isolated.

The oxidation of compound **1** (Scheme 2) with *Jones* reagent[14] yielded the 28-keto derivative **4**. Furthermore, the acetylenic



betulinic acid (BA) **I** R = COOH
betulin **II** R = CH_2OH
lupeol **III** R = CH_3

Figure 1. Structure of lupane-type triterpenes.

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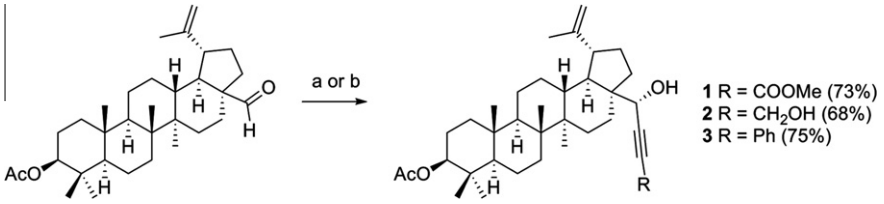
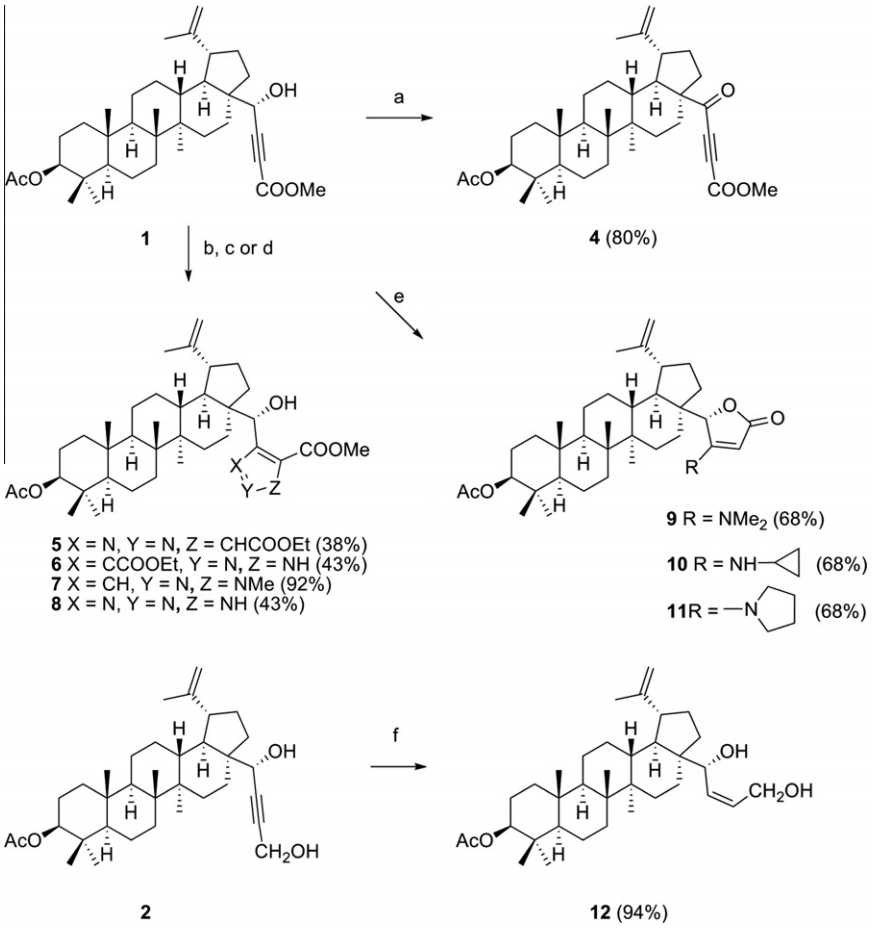


Scheme 1. Synthesis of 28-acetylenic betulins. Reagents and conditions: (a) LDA, dry THF −78 °C, 2 h; (b) *n*-BuLi, dry THF −78 °C, 2 h.



Scheme 2. Transformations of 28-acetylenic betulins. Reagents and conditions: (a) CrO_3, H_2SO_4, acetone 0 °C, 2 h; (b) ethyl diazoacetate, toluene, reflux, 48 h; (c) diazomethane, Et_2O, 0 °C; (d) NaN_3, DMF, reflux, 72 h; (e) amine, EtOH, 70 °C, 12 h; (f) H_2, $Pd/BaSO_4$, quinoline, EtOAc, 30 min.

structure was ideal for the synthesis of the heterocyclic compounds **5**–**8** by 1,3-dipolar cycloaddition reactions. For the reaction of **1** with of ethyl diazoacetate[15] the two isomers **5** and **6** were obtained in a ratio of ca. 1:1. In general, the formation of compound **6** is favored but steric hindrance of the skeletal structure usually leads to an increased formation of compound **5**.

In contrast, adding diazomethane[16] advanced stereoselectively; the direct reaction with an excess of diazomethane, however, gave the matching N-alkylated derivative **7**. The triazole **8** was obtained by reaction of **1** with sodium azide in DMF.[17] In another approach, **1** was allowed to react with primary and secondary amines.[18] Thereby, the Michael addition gave substituted acrylates; intramolecular lactonization afforded the corresponding 3-N-substituted butenolides **9**–**11**. Hydrogenation of **2** in the presence of Lindlar catalyst[19] yielded the matching alkene **12**.

3. Results and discussion

The compounds were tested for their antitumor potential in a panel of 15 human cancer cell lines in 96 well plates by using the colorimetric SRB protocol.[20] The calculated IC_{50} values in Table 1 were obtained from the corresponding dose–response curves. Compound **1** shows IC_{50} values that are considerably lower than those of standard betulinic acid. Changing the carboxymethyl moiety for a hydroxymethyl group led to a decreased activity as observed for compound **2**, whereas the presence of a phenylacetylene moiety is unfavorable. Interestingly, oxidizing the hydroxyl group of **1** led to a significant loss of activity. The pyrazole derivatives **5**–**7** are less active than the parent compound **1**, but compared to betulinic acid for certain cell lines lower IC_{50} values have been observed. For the triazole **8** IC_{50} values between

Table 1
Cytotoxicity of compounds in a panel of various human cancer cell lines

Cell line	IC_{50} values in μM for cancer cell lines												
	BA	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
518A2	11.9	5.9	11.9	NA	NA	21.5	16.4	NA	14.8	4.8	6.2	4.7	20.4
A431	15.4	3.4	10.5	NA	NA	7.6	11.7	12.4	16.6	3.3	2.9	2.1	12.4
A253	11.1	2.7	10.3	NA	NA	12.5	13.2	16.0	10.3	4.9	4.2	4.6	14.8
FADU	10.4	5.0	11.2	NA	NA	9.9	13.9	17.6	15.1	3.8	6.0	6.3	10.7
A549	14.9	3.1	11.9	NA	NA	14.8	18.2	24.5	17.3	6.1	6.6	7.8	12.8
A2780	11.0	3.0	10.4	NA	NA	9.1	13.2	12.7	13.4	2.6	2.5	2.2	12.6
DLD-1	17.5	3.6	NA	NA	NA	NA	NA	NA	15.5	3.6	4.7	5.4	12.3
HCT-8	17.8	6.0	11.6	NA	NA	10.0	13.4	13.4	13.9	1.7	1.9	0.5	10.1
HCT-116	13.3	3.5	11.3	NA	NA	9.8	14.0	20.4	15.6	3.2	3.2	2.1	10.5
HT-29	16.1	5.0	11.6	NA	NA	12.0	17.0	NA	18.2	5.7	5.1	3.9	15.9
SW480	6.4	3.6	6.9	NA	NA	7.7	11.4	7.0	14.7	2.2	8.0	5.7	17.9
8505C	6.7	3.6	8.4	NA	NA	8.9	12.0	10.8	15.4	3.1	6.0	5.3	17.8
SW1736	11.6	2.3	11.5	NA	NA	16.4	19.2	22.8	11.8	2.9	3.9	4.1	9.0
MCF-7	14.9	6.0	9.9	NA	NA	8.0	11.3	12.3	14.8	6.9	4.0	3.7	19.4
Lipo	9.7	4.2	11.4	NA	NA	19.9	14.0	14.1	18.2	5.3	5.8	6.0	18.5

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 cell line to the test compounds using an SRB-assay for melanoma (518A2), zervic 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).

11.8–18.2 μM were measured. The amino-substituted butenolides, however, showed promising cytotoxicity: Best results were observed for the dimethylamino derivative **9** with IC_{50} values between 2.2 and 6.9 μM. Slightly increased IC_{50} values were obtained for the corresponding cyclopropylamine derivative **10** and the pyrrolidinyl derivative **11**. The allyl alcohol **12** displayed only weak activities. Our findings parallel previous results[21] showing that a free C-28 carboxylic acid function is important for the cytotoxicity of these compounds. There are also some exceptions,[21] however, to this general tendency.

Betulinic acid and derivatives often show only poor solubilities in water hence limited in vivo drug administration. To overcome this limit we explored the principal possibility of encapsulation into liposomes. Therefore, compound **1** was introduced into commercial available liposome formulations. Best results were obtained with soybean lecithin (Lipoid S75). Subsequent extrusion through a polycarbonate membrane[22] with a pore size of 100 nm using a LiposoFast system gave liposomes with a hydrodynamic diameter between 70 and 120 nm (Z-average 105.0 nm) as determined by dynamic light scattering. Noteworthy, encapsulation efficiency was nearly 100% as determined by HPLC; their physical stability exceeded 4 weeks. Applicating compound **1** in liposomes in the SRB assay revealed an extra benefit of encapsulation by observing an increased cytotoxicity for most of the cell lines (Table 2).

Table 2
IC_{50} (μM) values of compound **1**

Cell line	**1** in DMSO	**1** Liposome
518A2	5.9	3.7
A431	3.4	2.4
A253	2.7	3.6
FADU	5.0	4.0
A549	3.1	4.2
A2780	3.0	2.0
DLD-1	3.6	3.3
HCT-8	6.0	2.8
HCT-116	3.5	2.3
HT-29	5.0	4.6
SW480	3.6	3.7
8505C	3.6	4.4
SW1736	2.3	4.4
MCF-7	6.0	3.4
Lipo	4.2	4.2

Comparison of the calculated IC_{50} values derived from dose–response curves of compound **1** as solution in DMSO and as aqueous liposomal formulation.

An apoptose-based mechanism seems reasonable. To gain insights in the mode of action of our compounds more tests were performed using **9** and liposomes of compound **1**. Thereby, the floating cells (obtained after treatment with IC_{90}-concentrations for 24 h) were analyzed by a trypan-blue exclusion test and DNA gel electrophoresis (Fig. 2). Apoptotic cells have an intact cell membrane and can exclude the dye whereas necrotic cells are stained blue. During apoptosis DNA is cleaved into smaller fragments by endonucleases. These fragments are observable in gel electrophoresis as ladders.[23–25]

4. Conclusion

We have examined betulin derivatives bearing different acetylenic side chains at C-28. These derivatives showed promising cytoxicity and they are also valuable starting materials for the synthesis of betulin-derived pyrazoles, 1,2,3-triazoles and 3-substituted butenolides. Several of these derivatives revealed a higher cytotoxicity than betulinic acid. Also we looked into the encapsulation of the title compound **1** into liposomes.

5. Experimental section

5.1. General

Instrumentation, cell lines and culture conditions, cytotoxicity assay, dye exclusion test and DNA fragmentation assay as described previously.[11]

5.2. Preparation of liposomes

Unilamellar liposomes of approximately 100 nm diameter were obtained by the method of Olson[22] 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 phosphatidylcholin 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, λ = 230 nm, methanol, 1.3 ml). Liposomes were characterized by DLS.

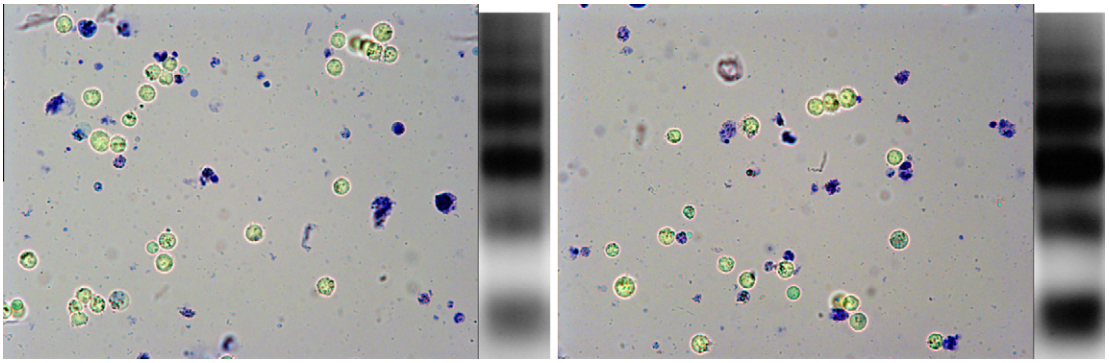


Figure 2. Trypan-blue exclusion test for ovarian cancer cell line A2780 and DNA laddering for colon cancer cell line SW480 of compound **9** (left) and liposomal compound **1** (right) after treatment with IC_{90}-concentrations for 24 h.

5.3. General procedure for the addition of alkynes to 3-*O*-acetylbetulinic aldehyde (GP1)

To a solution of DIA (416 mg, 4.12 mmol) in dry THF (20 ml), *n*-BuLi (2.6 ml, 1.6 M in hexane) was added at −20 °C. After 15 min, the solution was cooled to −78 °C and a solution of the corresponding alkyne (4.12 mmol) in dry THF (2 ml) was added dropwise. Stirring at this temperature was continued for an additional 30 min; then a solution of 3-*O*-acetylbetulinic aldehyde (0.50 g, 1.03 mmol) in dry THF (2 ml) 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).

5.4. General procedure for the synthesis of 3-N-substitued butenolides (GP2)

A solution of compound **1** (0.30 g, 0.53 mmol) and the corresponding amine (2.0 mmol) in ethanol (10 ml) was stirred at 70 °C for 12 h. The solution was concentrated and the residue subjected to column chromatography (silica gel, chloroform/diethyl ether, 9:1).

5.5. Methyl (28*S*)-3-[3β-acetoxy-28-hydroxy-lup-20(29)-en-28-yl]-propiolate (1)

Following GP1, **1** (0.43 g, 73%) was obtained from 3-*O*-acetylbetulinic aldehyde (0.50 g, 1.03 mmol) and methyl propiolate (0.35 g, 4.12 mmol) as a colourless solid; mp 210–212 °C; $[\alpha]_D^{20}$ = +18.5 (*c* 4.5, $CHCl_3$); R_f = 0.55 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr): ν = 3475s, 2955s, 2870m, 2228m, 1713s, 1638w, 1455m, 1431m, 1380m, 1250s, 1155w, 1107w, 1066m, 1049m, 1033m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 4.98 (s, 1H, C*H* (28)), 4.67 (br s, 1H, CH_a (30)), 4.56 (br s, 1H, CH_b (30)), 4.44 (dd, 1H, *J* = 11.4, 4.8 Hz, C*H*OAc (3)), 3.76 (s, 3H, OCH_3), 2.85 (ddd, 1H, *J* = 11.2, 10.4, 7.0 Hz, C*H* (19)), 2.04–1.80 (m, 4H, CH_a (16) + CH_a (21) + CH_a (22) + C*H* (13)), 2.02 (s, 3H, Ac), 1.65 (s, 3H, CH_3 (29)), 1.78–1.45 (m, 8H, C*H* (18) + CH_a (12) + CH_a (1) + CH_2 (2) + CH_a (6) + CH_a (15) + CH_a (11)), 1.45–1.09 (m, 10H, CH_b (6) + CH_b (21) + CH_b (22) + CH_b (16) + CH_2 (7) + C*H* (9) + CH_b (11) + CH_b (12) + CH_b (15)), 1.04–0.86 (m, 1H, CH_b (1)), 1.00 (s, 3H, CH_3 (25)), 0.97 (s, 3H, CH_3 (27)), 0.83 (s, 3H, CH_3 (26)), 0.82 (s, 3H, CH_3 (23)), 0.81 (s, 3H, CH_3 (24)), 0.77 (d, 1H, *J* = 9.5 Hz, C*H* (5)) ppm; ^{13}C (125 MHz, $CDCl_3$): δ = 171.0 (*C*=O), 153.8 (*C*=O), 150.6 (C20, *C*=CH_2), 109.8 (C30, CH_2=C), 88.3 (C31, *C*≡C), 80.9 (C3, *C*HOAc), 77.7 (C32, *C*≡C), 66.0 (C28, *C*H), 55.3 (C5, *C*H), 52.8 (OMe), 51.0 (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, $C_{quart.}$), 38.3 (C1, CH_2), 37.8 (C4, $C_{quart.}$), 37.4 (C13, *C*H), 37.1 (C10, $C_{quart.}$), 34.2 (C7, CH_2), 34.1 (C16, CH_2), 34.0 (C22, CH_2), 32.2 (C21, CH_2), 27.9 (C23, CH_3), 27.8 (C15, CH_2), 25.0 (C12, CH_2), 23.7 (C2, CH_2), 21.3 (Ac, CH_3), 20.8 (C11, CH_2), 18.8 (C29, CH_3), 18.1 (C6, CH_2), 16.5 (C24, CH_3), 16.0 (C25 + C26, 2xCH_3), 15.1 (C27, CH_3) ppm; MS (i.e., 70 eV): *m*/*z* (%) = 566 (12), 453 (100), 393 (61), 203 (19), 189 (24); Anal. for $C_{36}H_{54}O_5$ (566.81): C, 76.28; H, 9.60; found: C, 76.23; H, 9.49.

5.6. (28*S*) 3-*O*-Acetyl-28-(3-hydroxyprop-1-ynyl)-lup-20(29)-en-3,28-diol (2)

Following GP1 but using two equivalents of LDA, compound **2** (0.43 g, 68%) was obtained as a colourless solid from 3-*O*-acetylbetulinic aldehyde (0.50 g, 1.03 mmol) and 2-propyn-1-ol (0.23 g, 4.12 mmol); mp 210–213 °C; $[\alpha]_D^{20}$ = +21.6 (*c* 3.2, $CHCl_3$); R_f = 0.73 (silica gel, hexane/ethyl acetate, 5:5); IR (KBr): ν = 3423br, 2942s, 2866m, 1733m, 1693s, 1455m, 1369m, 1315w, 1273s, 1117w, 1038m, 1015m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 4.91 (s, 1H, C*H* (28)), 4.68 (br s, 1H, CH_a (30)), 4.56 (br s, 1H, CH_b (30)), 4.45 (dd, 1H, *J* = 11.4, 4.8 Hz, C*H*OAc (3)), 4.32 (s, 2H, OCH_2), 2.89 (ddd, 1H, *J* = 11.2, 10.4, 7.0 Hz, C*H* (19)), 2.09–1.90 (m, 4H, CH_a (16) + CH_a (21) + CH_a (22) + C*H* (13)), 2.02 (s, 3H, Ac), 1.66 (s, 3H, CH_3 (29)), 1.76–1.46 (m, 7H, C*H* (18) + CH_a (12) + CH_a (1) + CH_2 (2) + CH_a (15) + CH_a (6)), 1.45–1.08 (m, 9H, CH_2 (11) + CH_b (6) + CH_b (22) + CH_b (21) + CH_2 (7) + C*H* (9) + CH_b (16) + CH_b (12)), 1.04–0.91 (m, 2H, CH_b (15) + CH_b (1)), 1.02 (s, 3H, CH_3 (25)), 0.98 (s, 3H, CH_3 (27)), 0.84 (s, 6H, CH_3 (26)), 0.83 (s, 6H, CH_3 (23)), 0.82 (s, 3H, CH_3 (24)), 0.78 (d, 1H, *J* = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.0 (C=O), 150.9 (C20, *C*=CH_2), 109.7 (C30, CH_2=C), 86.5 (C31, *C*≡C), 84.3 (C32, *C*≡C), 80.9 (C3, *C*HOAc), 66.0 (C28, *C*H), 55.3 (C5, *C*H), 51.1 (C17, $C_{quart.}$), 50.7 (OCH_2), 50.2 (C9, *C*H), 48.9 (C18, *C*H), 48.7 (C19, *C*H), 43.0 (C14, $C_{quart.}$), 40.9 (C8, $C_{quart.}$), 38.3 (C1, CH_2), 37.8 (C4, $C_{quart.}$), 37.2 (C13, *C*H), 37.0 (C10, $C_{quart.}$), 34.3 (C7, CH_2), 34.1 (C16, CH_2), 34.0 (C22, CH_2), 32.3 (C21, CH_2), 27.9 (C23, CH_3), 27.8 (C15, CH_2), 25.0 (C12, CH_2), 23.7 (C2, CH_2), 21.3 (Ac, CH_3), 20.8 (C11, CH_2), 18.8 (C29, CH_3), 18.1 (C6, CH_2), 16.5 (C24, CH_3), 16.1 (C26, CH_3), 16.0 (C25, CH_3), 15.0 (C27, CH_3) ppm; MS (ESI, MeOH): *m*/*z* (%) = 561.5 (40% $[M+Na]^+$), 1099.1 (100% $[2M+Na]^+$); Anal. for $C_{35}H_{54}O_4$ (538.80): C, 78.02; H, 10.10. Found: C, 77.85; H, 10.33.

5.7. (28*S*) 3-*O*-Acetyl-28-(phenylethynyl)-lup-20(29)-en-3,28-diol (3)

To a solution of phenylacetylene (0.31 g, 3.00 mmol) in THF (10 ml) *n*-butyllithium (1.9 ml, 1.6 M in hexane) was added under

argon at −78 C. Stirring at this temperature was continued for an additional 30 min; then, a solution of 3-*O*-acetylbetulinic aldehyde (0.50 g, 1.03 mmol) in dry THF (2 ml) was added. After 1 h at −78 C, the reaction was quenched by the addition of H_2O (15 ml). The phases were separated and the aq layer was extracted with $CHCl_3$ (2 × 20 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 afford compound **3** (0.45 g, 75%) as a white solid; mp 144–146 °C; $[\alpha]_D^{20}$ = +17.3 (*c* 3.0, $CHCl_3$); R_f = 0.68 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr): ν = 2943s, 2872m, 2123w, 1734s, 1638w, 1490m, 1456m, 1376m, 1245m, 1071w, 1028m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.44–7.42 (m, 2H, Ph), 7.31–7.29 (m, 3H, Ph), 5.09 (s, 1H, C*H* (28)), 4.72 (d, 1H, *J* = 2.0 Hz, CH_a (30)), 4.59 (br s, 1H, CH_b (30)), 4.47 (dd, 1H, *J* = 11.4, 4.8 Hz, C*H*OAc (3)), 2.95 (ddd, 1H, *J* = 11.2, 10.4, 7.0 Hz, C*H* (19)), 2.20–2.00 (m, 4H, CH_a (16) + CH_a (22) + CH_a (21) + C*H* (13)), 2.03 (s, 3H, Ac), 1.81–1.58 (m, 6H, C*H* (18) + CH_a (12) + CH_a (1) + CH_a (15) + CH_2 (2)), 1.70 (s, 3H, CH_3 (29)), 1.52–1.13 (m, 11H, CH_2 (6) + CH_2 (11) + CH_b (21) + CH_b (16) + CH_2 (7) + CH_b (22) + C*H* (9) + CH_b (12)), 1.06–0.95 (m, 2H, CH_b (15) + CH_b (1)), 1.06 (s, 3H, CH_3 (25)), 1.01 (s, 3H, CH_3 (27)), 0.86 (s, 3H, CH_3 (26)), 0.84 (s, 3H, CH_3 (23)), 0.83 (s, 3H, CH_3 (24)), 0.80 (d, 1H, *J* = 9.5 Hz, C*H* (5)); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.0 (*C*=O), 151.0 (C20, *C*=CH$_2$), 131.6 (Ph), 128.3 (Ph), 128.2 (Ph), 122.8 (Ph), 109.6 (C30, *C*H$_2$=C), 89.9 (C31, *C*≡C), 86.1 (C32, *C*≡C), 80.9 (C3, *C*HOAc), 66.6 (C28, *C*H), 55.3 (C5, *C*H), 51.1 (C17, $C_{quart.}$), 50.2 (C9, *C*H), 49.0 (C18, *C*H), 48.7 (C19, *C*H), 43.1 (C14, $C_{quart.}$), 40.9 (C8, $C_{quart.}$), 38.4 (C1, *C*H$_2$), 37.8 (C4, $C_{quart.}$), 37.3 (C13, *C*H), 37.1 (C10, $C_{quart.}$), 34.5 (C16, *C*H$_2$), 34.2 (C7, *C*H$_2$), 34.1 (C22, *C*H$_2$), 32.5 (C21, *C*H$_2$), 27.9 (C23, *C*H$_3$), 27.8 (C15, *C*H$_2$), 25.1 (C12, *C*H$_2$), 23.7 (C2, *C*H$_2$), 21.3 (Ac, *C*H$_3$), 20.8 (C11, *C*H$_2$), 18.8 (C29, *C*H$_3$), 18.1 (C6, *C*H$_2$), 16.5 (C24, *C*H$_3$), 16.2 (C25, *C*H$_3$), 16.1 (C26, *C*H$_3$), 15.1 (C27, *C*H$_3$) ppm; MS (ESI, MeOH): *m*/*z* (%) = 1191.1 (100% [2M+Na]$^+$); Anal. for $C_{40}H_{56}O_3$ (584.87): C, 82.14; H, 9.65. Found: C, 82.00; H, 9.85.

5.8. Methyl (28*S*) 3-[3β-acetoxy-28-oxo-lup-20(29)-en-28-yl]-propiolate (4)

A solution of chromium(VI) oxid (300 mg) in aq H_2SO_4 (35%, 1 ml) was slowly added to a stirred solution of compound **1** (1.04 g, 1.84 mmol) in acetone (10 ml) at 0 °C. After TLC revealed the absence of starting material, the excess chromium(VI) oxid was destroyed by the addition of isopropanol (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). Compound **4** (0.40 g, 80%) was obtained as a white solid; mp 103 °C; $[\alpha]_D^{20}$ = +19.2 (*c* 6.0, $CHCl_3$); R_f = 0.65 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr): ν = 2949s, 2871m, 1728s, 1680m, 1643w, 1436m, 1392w, 1368m, 1247s, 1096w, 1024m cm^{-1}; ^{1}H (400 MHz, $CDCl_3$): δ = 4.70 (br s, 1H, CH_a (30)), 4.58 (br s, 1H, CH_b (30)), 4.44 (dd, 1H, *J* = 11.4, 4.8 Hz, C*H*OAc (3)), 3.82 (s, 3H, OCH_3), 2.87 (ddd, 1H, *J* = 11.2, 10.4, 7.0 Hz, C*H* (19)), 2.38 (ddd, 1H, *J* = 13.7, 3.7, 3.3 Hz, CH_a (16)), 2.20 (ddd, 1H, *J* = 12.5, 12.0, 3.7 Hz, C*H* (13)), 2.05–1.99 (m, 1H, CH_a (22)), 2.01 (s, 3H, Ac), 1.76 (ddd, 1H, *J* = 10.8, 7.9, 2.5 Hz, CH_a (21)), 1.65 (s, 3H, CH_3 (29)), 1.70–1.50 (m, 6H, C*H* (18) + CH_a (12) + CH_a (1) + CH_2 (2) + CH_b (16)), 1.49–1.31 (m, 9H, CH_2 (6) + CH_b (22) + CH_b (21) + CH_2 (7) + CH_a (11) + CH_a (15)), 1.25–1.17 (m, 3H, C*H* (9) + CH_b (15) + CH_b (11)), 1.02–0.90 (m, 2H, CH_b (1) + CH_b (12)), 0.93 (s, 3H, CH_3 (27)), 0.88 (s, 3H, CH_3 (25)), 0.82 (s, 6H, CH_3 (26)), 0.81 (s, 6H, CH_3 (23)), 0.80 (s, 3H, CH_3 (24)), 0.77 (d, 1H, *J* = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 190.3 (*C*=O), 170.9 (*C*=O), 152.8 (*C*=O), 149.8 (C20, *C*=CH$_2$), 109.9 (C30, *C*H$_2$=C), 80.3 (C31, *C*≡C), 80.9 (C3, *C*HOAc), 76.9 (C32, *C*≡C), 62.1 (C17, $C_{quart.}$), 55.4 (C5, *C*H), 53.2 (OMe), 50.4 (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, $C_{quart.}$), 37.2 (C13, *C*H), 37.1 (C10, $C_{quart.}$), 35.2 (C22, *C*H$_2$), 34.2 (C7, *C*H$_2$), 31.2 (C16, *C*H$_2$), 29.9 (C21, *C*H$_2$), 29.6 (C15, *C*H$_2$), 27.9 (C23, *C*H$_3$), 25.4 (C12, *C*H$_2$), 23.7 (C2, *C*H$_2$), 21.3 (Ac, *C*H$_3$), 20.8 (C11, *C*H$_2$), 19.1 (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* (%): 587.3 (40% [M+Na]$^+$), 1154.1 (100% [2M+Na]$^+$); Anal. for $C_{36}H_{52}O_5$ (564.80): C, 76.56; H, 9.28. Found: C, 76.28; H, 8.92.

5.9. 28-[3-(Ethylcarboxy)-4-(methylcarboxy)-pyrazol-5-yl]-3, 28-dioxo-28-ethinyllup-20(29)-ene (5) and 28-[3-(ethylcarboxy)-5-(methylcarboxy)-pyrazol-4-yl]-3,28-dioxo-28-ethinyllup-20(29)-ene (6)

A solution of compound **1** (0.30 g, 0.53 mmol) and ethyl diazoacetate (0.23 g, 2 mmol) in toluene (10 ml) was heated under reflux for 48 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).

Data for **5**: Compound **5** (0.14 g, 38%) was obtained as a colourless solid; mp 218–210 °C; $[\alpha]_D^{20}$ = +5.75 (*c* 4.1, $CHCl_3$); R_f = 0.75 (silica gel, hexane/ethyl acetate, 5:5); IR (KBr): ν = 3475s, 2945s, 2873m, 1733s, 1701s, 1455m, 1370m, 1249s, 1159m, 1107m, 1060m, 1028m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.53 (s, 1H, C*H* (28)), 4.71 (d, 1H, *J* = 2.2 Hz, CH_a (30)), 4.58 (br s, 1H, CH_b (30)), 4.46 (dd, 1H, *J* = 11.4, 4.8 Hz, C*H*OAc (3)), 4.36 (*q*, 2H, *J* = 7.0 Hz, OCH_2), 3.85 (s, 3H, OCH_3), 3.08 (ddd, 1H, *J* = 11.2, 10.4, 7.0 Hz, C*H* (19)), 2.23–2.00 (m, 2H, CH_a (21) + C*H* (13)), 2.02 (s, 3H, Ac), 1.87–1.75 (m, 2H, CH_a (16) + C*H* (18)), 1.68 (s, 3H, CH_3 (29)), 1.71–1.56 (m, 5H, CH_a (12) + CH_a (15) + CH_a (1) + CH_2 (2)), 1.53–1.08 (m, 12H, CH_2 (6) + CH_2 (11) + CH_2 (22) + CH_2 (7) + CH_b (21) + C*H* (9) + CH_b (16) + CH_b (12)), 1.36 (*t*, 3H, *J* = 7.0 Hz, CH_3 (Et)), 1.04–0.84 (m, 2H, CH_b (1) + CH_b (15)), 1.05 (s, 3H, CH_3 (26)), 0.98 (s, 3H, CH_3 (27)), 0.84 (s, 3H, CH_3 (25)), 0.82 (s, 3H, CH_3 (23)), 0.81 (s, 3H, CH_3 (24)), 0.79 (d, 1H, *J* = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.0 (*C*=O), 165.3 (*C*=O), 160.7 (*C*=O), 151.2 (C20, *C*=CH$_2$), 150.8 (pyrazole, $C_{quart.}$), 113.0 (pyrazole, $C_{quart.}$), 109.5 (C30, *C*H$_2$=C), 80.9 (C3, *C*HOAc), 68.7 (C28, *C*H), 61.8 (OCH_2), 55.3 (C5, *C*H), 52.6 (OMe), 50.6 (C18, *C*H), 50.5 (C17, $C_{quart.}$), 50.2 (C9, *C*H), 48.2 (C19, *C*H), 43.1 (C14, $C_{quart.}$), 40.9 (C8, $C_{quart.}$), 38.3 (C1, *C*H$_2$), 37.8 (C4, $C_{quart.}$), 37.0 (C10, $C_{quart.}$), 36.8 (C13, *C*H), 34.5 (C16, *C*H$_2$), 34.2 (C7, *C*H$_2$), 33.6 (C22, *C*H$_2$), 32.6 (C21, *C*H$_2$), 27.9 (C23, *C*H$_3$), 27.8 (C15, *C*H$_2$), 25.3 (C12, *C*H$_2$), 23.7 (C2, *C*H$_2$), 21.3 (Ac, *C*H$_3$), 20.8 (C11, *C*H$_2$), 19.0 (C29, *C*H$_3$), 18.2 (C6, *C*H$_2$), 16.5 (C24, *C*H$_3$), 16.1 (C25 + C26, 2 × *C*H$_3$), 15.1 (C27, *C*H$_3$), 14.1 (Et, *C*H$_3$) ppm; MS (ESI, MeOH): *m*/*z* (%) = 681.2 (40% [M+H]$^+$), 703.3 (100% [M+Na]$^+$), 1383.1 (40% [2M+Na]$^+$); Anal. for $C_{40}H_{60}N_2O_7$ (680.91): C, 70.56; H, 8.88; N, 4.11. Found: C, 70.48; H, 8.67; N, 4.02.

Data for **6**: Compound **6** (0.16 g, 43%) was obtained as a colourless solid; mp 210–212 °C; $[\alpha]_D^{20}$ = +12.9 (*c* 4.3, $CHCl_3$); R_f = 0.42 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr): ν = 2947s, 2873m, 1733s, 1640w, 1550w, 1466m, 1378m, 1247s, 1125w, 1074w, 1020m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.77 (s, 1H, C*H* (28)), 4.74 (d, 1H, *J* = 2.2 Hz, CH_a (30)), 4.54 (br s, 1H, CH_b (30)), 4.46–4.36 (m 3H, C*H*OAc (3) + OCH_2), 3.92 (s, 3H, OCH_3), 3.23 (ddd, 1H, *J* = 11.2, 10.4, 7.0 Hz, C*H* (19)), 2.22–2.10 (m, 2H, C*H* (13) + CH_a (21)), 2.01 (s, 3H, Ac), 1.98–1.70 (m, 2H, C*H* (18)), 1.68 (s, 3H, CH_3 (29)), 1.71–1.50 (m, 6H, CH_a (1) + CH_2 (2) + CH_a (16) + CH_a (12) + CH_a (15)), 1.51–1.08 (m, 11H, CH_2 (6) + CH_2 (11) + CH_2 (7) + CH_b (21) + CH_2 (22) + C*H* (9) + CH_b (12)), 1.37 (*t*, 3H, *J* = 7.0 Hz, CH_3 (Et)), 1.03–0.95 (m, 3H, CH_b (1) + CH_b (16) + CH_b

(15)), 1.09 (s, 3H, CH_3 (26)), 0.94 (s, 3H, CH_3 (27)), 0.84 (s, 3H, CH_3 (25)), 0.81 (s, 6H, CH_3 (23) + CH_3 (24)), 0.77 (d, 1H, J = 9.5 Hz, CH (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.0 (*C*=O), 167.7 (*C*=O), 163.7 (*C*=O), 151.4 (C20, *C*=CH_2), 132.4 (pyrazole, $C_{quart.}$), 129.3 (pyrazole, $C_{quart.}$), 109.7 (C30, *C*H_2=C), 81.0 (C3, *C*HOAc), 67.8 (C28, *C*H), 62.4 (O*C*H_2), 55.3 (C5, *C*H), 53.0 (OMe), 52.1 (C17, $C_{quart.}$), 51.5 (C18, *C*H), 50.2 (C9, *C*H), 47.5 (C19, *C*H), 43.1 (C14, $C_{quart.}$), 41.1 (C8, $C_{quart.}$), 38.4 (C1, *C*H_2), 37.8 (C4, $C_{quart.}$), 37.0 (C10, $C_{quart.}$), 36.2 (C13, *C*H), 35.1 (C16, *C*H_2), 34.5 (C22, *C*H_2), 34.2 (C7, *C*H_2), 31.7 (C21, *C*H_2), 27.9 (C23, *C*H_3), 27.8 (C15, *C*H_2), 25.3 (C12, *C*H_2), 23.7 (C2, *C*H_2), 21.3 (Ac, *C*H_3), 20.8 (C11, *C*H_2), 19.0 (C29, *C*H_3), 18.2 (C6, *C*H_2), 16.5 (C24, *C*H_3), 16.2 (C25, 2x*C*H_3), 16.1 (C26, 2 × *C*H_3), 14.9 (C27, *C*H_3), 14.2 (Et, *C*H_3) ppm; MS (ESI, MeOH): *m*/*z* (%) = 681.2 (30% $[M+H]^+$), 703.3 (100% $[M+Na]^+$), 1383.1 (50% $[2M+Na]^+$); UV–vis (Methanol): λ_{max} (log ε) 218 nm (4.41); Anal. for $C_{40}H_{60}N_2O_7$ (680.91): C, 70.56; H, 8.88; N, 4.11. Found: C, 70.32; H, 8.54; N, 3.89.

5.10. 28-[*N*-Methyl-3-(methylcarboxy)-pyrazol-4-yl]-3,28-dioxo-28-ethinyllup-20(29)-ene (7)

An ethereal solution of diazomethane was added to a solution of compound **1** (0.30 g, 0.53 mmol) in Et_2O (10 ml) under cooling with ice until the reaction mixture remained yellow. After stirring for 30 min, the excess diazomethane was destroyed with acetic acid (5%) and the solution was concentrated in vacuo. After column chromatography compound **7** (0.30 g, 92%) was obtained as a colourless solid; mp 213–214 °C; $[\alpha]_D^{20}$ = +16.4 (*c* 7.1, $CHCl_3$); R_f = 0.73 (silica gel, hexane/ethyl acetate, 5:5); IR (KBr): ν = 2949s, 2873m, 1728s, 1638w, 1455m, 1370m, 1246s, 1196w, 1111m, 1018m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.53 (s, 1H, C*H* (pyrazole)), 5.51 (s, 1H, C*H* (28)), 4.73 (d, 1H, J = 2.2 Hz, CH_a (30)), 4.58 (br s, 1H, CH_b (30)), 4.45 (dd, 1H, J = 11.4, 4.8 Hz, C*H*OAc (3)), 4.10 (s, 3H, NCH_3), 3.95 (s, 3H, OCH_3), 3.08 (ddd, 1H, J = 11.2, 10.4, 7.0 Hz, C*H* (19)), 2.21–2.07 (m, 3H, C*H* (13) + CH_a (16) + CH_a (21)), 2.03 (s, 3H, Ac), 1.71 (s, 3H, CH_3 (29)), 1.83–1.72 (m, 2H, C*H* (18) + CH_a (12)), 1.71–1.56 (m, 5H, CH_a (1) + CH_a (15) + CH_a (22) + CH_2 (2)), 1.71–1.34 (m, 5H, CH_2 (6) + CH_a (11) + CH_b (21) + CH_b (16)), 1.33–1.16 (m, 6H, C*H* (9) + CH_b (22) + CH_b (11) + CH_2 (7) + CH_b (12)), 1.06 (s, 3H, CH_3 (26)), 1.04–0.94 (m, 2H, CH_b (1) + CH_b (15)), 1.00 (s, 3H, CH_3 (27)), 0.86 (s, 3H, CH_3 (25)), 0.83 (s, 3H, CH_3 (23)), 0.82 (s, 3H, CH_3 (24)), 0.80 (d, 1H, J = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.0 (*C*=O), 161.3 (*C*=O), 151.5 (C20, *C*=CH_2), 137.7 (pyrazole, *C*H), 130.2 (pyrazole, $C_{quart.}$), 129.0 (pyrazole, $C_{quart.}$), 109.5 (C30, *C*H_2=C), 80.9 (C3, *C*HOAc), 67.2 (C28, *C*H), 55.3 (C5, *C*H), 52.5 (OMe), 50.8 (C18, *C*H), 50.2 (C9, *C*H), 50.2 (C17, $C_{quart.}$), 48.5 (C19, *C*H), 43.0 (C14, $C_{quart.}$), 40.9 (C8, $C_{quart.}$), 40.7 (NMe), 38.3 (C1, *C*H_2), 37.8 (C4, $C_{quart.}$), 37.1 (C10, $C_{quart.}$), 36.9 (C13, *C*H), 34.2 (C7, *C*H_2), 34.7 (C22, *C*H_2), 34.1 (C16, *C*H_2), 32.8 (C21, *C*H_2), 27.9 (C23, *C*H_3), 27.8 (C15, *C*H_2), 25.0 (C12, *C*H_2), 23.7 (C2, *C*H_2), 21.3 (Ac, *C*H_3), 20.9 (C11, *C*H_2), 18.8 (C29, *C*H_3), 18.1 (C6, *C*H_2), 16.5 (C24, *C*H_3), 16.1 (C25, *C*H_3), 16.0 (C26, *C*H_3), 15.1 (C27, *C*H_3) ppm; MS (ESI, MeOH): *m*/*z* (%) = 645.3 (70% $[M+Na]^+$), 1267.2 (100% $[2M+Na]^+$); Anal. for $C_{37}H_{56}N_2O_5$ (608.85): C, 72.99; H, 9.27; N, 4.60. Found: C, 72.68; H, 9.32; N, 4.52.

5.11. 28-(4-(Methylcarboxy)-1*H*-1,2,3-triazol-5-yl)-3,28-dioxo-28-ethinyllup-20(29)-ene (8)

A solution of compound **1** (0.30 g, 0.53 mmol) and sodium azide (0.37 g, 5.0 mmol) in dry DMF (10 ml) was heated under reflux for 72 h. The mixture was concentrated in vacuo and the residue was treated with H_2O (10 ml) and $CHCl_3$ (20 ml). The phases were separated and the aq layer extracted with $CHCl_3$ (20 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). Compound **8** (0.14 g, 43%) was obtained as a colourless solid; mp 196–200 °C; $[\alpha]_D^{20}$ = +13.2 (*c* 4.3, $CHCl_3$); R_f = 0.59 (silica gel, hexane/ethyl acetate, 5:5); IR (KBr): ν = 3440br, 2947s, 2874m, 1734s, 1708s, 1662m, 1456m, 1377m, 1246s, 1117w, 1029m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.64 (d, 1H, J = 9.8, C*H* (28)), 4.79 (d, 1H, J = 9.8, OH), 4.75 (d, 1H, J = 1.9, CH_a (30)), 4.60 (br s, 1H, CH_b (30)), 4.47 (dd, 1H, J = 11.4, 4.8 Hz, C*H*OAc (3)), 4.00 (s, 3H, OCH_3), 3.20 (ddd, 1H, J = 11.3, 11.3, 6.1 Hz, C*H* (19)), 2.25–2.15 (m, 2H, CH_a (21) + C*H* (13)), 2.04 (s, 3H, Ac), 1.95 (ddd, 1H, J = 14.0, 13.8, 3.8 Hz, CH_a (15)), 1.70 (s, 3H, CH_3 (29)), 1.86–1.56 (m, 6H, CH_a (16) + C*H* (18) + CH_a (12) + CH_a (1) + CH_2 (2)), 1.50–1.30 (m, 7H, CH_2 (6) + CH_a (22) + CH_b (21) + CH_a (11) + CH_2 (7)), 1.29–1.08 (m, 5H, C*H* (9) + CH_b (11) + CH_b (22) + CH_b (12) + CH_b (16)), 1.00–0.92 (m, 1H, CH_b (1) + CH_b (15)), 1.10 (s, 3H, CH_3 (26)), 1.00 (s, 3H, CH_3 (27)), 0.85 (s, 3H, CH_3 (25)), 0.83 (s, 3H, CH_3 (23)), 0.82 (s, 3H, CH_3 (24)), 0.80 (d, 1H, J = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.2 (*C*=O), 163.8 (*C*=O), 151.3 (C20, *C*=CH_2), 109.6 (C30, *C*H_2=C), 81.1 (C3, *C*HOAc), 69.4 (C28, *C*H), 55.3 (C5, *C*H), 53.1 (OMe), 51.2 (C17, $C_{quart.}$), 50.9 (C18, *C*H), 50.2 (C9, *C*H), 48.3 (C19, *C*H), 43.0 (C14, $C_{quart.}$), 41.0 (C8, $C_{quart.}$), 38.4 (C1, *C*H_2), 37.8 (C4, $C_{quart.}$), 37.0 (C10, $C_{quart.}$), 36.9 (C13, *C*H), 34.2 (C7, *C*H_2), 34.1 (C16, *C*H_2), 34.0 (C22, *C*H_2), 32.5 (C21, *C*H_2), 27.9 (C23, *C*H_3), 27.8 (C15, *C*H_2), 25.2 (C12, *C*H_2), 23.7 (C2, *C*H_2), 21.3 (Ac, *C*H_3), 20.9 (C11, *C*H_2), 18.9 (C29, *C*H_3), 18.2 (C6, *C*H_2), 16.5 (C24, *C*H_3), 16.2 (C25, *C*H_3), 16.1 (C26, *C*H_3), 15.2 (C27, *C*H_3) ppm; MS (ESI, MeOH): *m*/*z* (%) = 610.3 (40% $[M+H]^+$), 632.5 (100% $[M+Na]^+$); Anal. for $C_{36}H_{55}N_3O_5$ (609.83): C, 70.90; H, 9.09; N, 6.89. Found: C, 70.78; H, 9.24; N, 6.76.

5.12. (4*R*) 3-(Dimethylamino)-4-[3β-hydroxy-28-norlup-20(29)-en-17β-yl]-2-butenolide (9)

Following GP2, compound **9** (0.21 g, 68%) was obtained from **1** (0.30 g, 0.53 mmol) and dimethylammonium dimethyl carbamate (0.27 g, 2.00 mmol) as a colourless solid; mp 175–178 °C; $[\alpha]_D^{20}$ = +135.0 (*c* 3.3, $CHCl_3$); R_f = 0.36 (silica gel, chloroform/diethyl ether, 9:1); IR (KBr): ν = 3456s, 2942s, 1736s, 1642w, 1606s, 1454m, 1377m, 1301w, 1250s, 1167w, 1133m, 1032m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.26 (s, 1H, C*H* (32)), 4.88 (s, 1H, C*H* (28)), 4.74 (d, 1H, J = 1.9 Hz, CH_a (30)), 4.54 (dd, 1H, J = 2.4, 1.4 Hz, CH_b (30)), 4.46 (dd, 1H, J = 11.4, 4.8 Hz, C*H*OAc (3)), 3.04 (ddd, 1H, J = 11.2, 10.4, 7.0 Hz, C*H* (19)), 2.82 (s, 3H, NCH_3), 2.19 (ddd, 1H, J = 12.2, 12.2, 3.6 Hz, C*H* (13)), 2.00–1.95 (m, 1H, CH_a (16)), 2.02 (s, 3H, Ac), 1.83–1.71 (m, 3H, C*H* (18) + CH_b (16) + CH_a (15)), 1.68–1.56 (m, 8H, CH_a (1) + CH_a (12) + CH_2 (2) +), 1.67 (s, 3H, CH_3 (29)), 1.53–1.22 (m, 10H, CH_a (22) + CH_2 (6) + CH_2 (7) + CH_2 (21) + CH_2 (11) + C*H* (9)), 1.17–0.94 (m, 4H, CH_b (15) + CH_b (12) + CH_b (22) + CH_b (1)), 1.06 (s, 3H, CH_3 (26)), 1.04 (s, 3H, CH_3 (27)), 0.85 (s, 3H, CH_3 (23)), 0.84 (s, 3H, CH_3 (25)), 0.83 (s, 3H, CH_3 (24)), 0.79 (d, 1H, J = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 175.6 (*C*=O), 173.6 (C31, *C*=C), 171.0 (*C*=O), 150.8 (C20, *C*=CH_2), 109.6 (C30, *C*H_2=C), 88.3 (C28, *C*H), 80.9 (C3, *C*HOAc), 80.2 (C32, *C*H), 55.4 (C5, *C*H), 51.5 (C17, $C_{quart.}$), 52.0 (C18, *C*H), 50.2 (C9, *C*H), 47.7 (C19, *C*H), 43.0 (C14, $C_{quart.}$), 41.0 (C8, $C_{quart.}$), 42.3 (NMe), 38.3 (C1, *C*H_2), 37.8 (C4, $C_{quart.}$), 37.1 (C10, $C_{quart.}$), 36.5 (C13, *C*H), 34.2 (C7, *C*H_2), 35.1 (C22, *C*H_2), 32.5 (C16, *C*H_2), 32.4 (C21, *C*H_2), 28.0 (C15, *C*H_2), 27.9 (C23, *C*H_3), 25.4 (C12, *C*H_2), 23.7 (C2, *C*H_2), 21.3 (Ac, *C*H_3), 20.7 (C11, *C*H_2), 19.5 (C29, *C*H_3), 18.1 (C6, *C*H_2), 16.5 (C24, *C*H_3), 16.3 (C25, *C*H_3), 16.1 (C26, *C*H_3), 15.4 (C27, *C*H_3) ppm; MS (ESI, MeOH): *m*/*z* (%) = 580.5 (100%

$[M+H]^+$), 602.5 (100% $[M+Na]^+$); Anal. for $C_{37}H_{57}NO_4$ (579.85): C, 76.64; H, 9.91; N, 2.42. Found: C, 76.54; H, 9.74; N, 2.32.

5.13. (4*R*) 3-(Cyclopropylamino)-4-[3β-hydroxy-28-norlup-20(29)-en-17β-yl]-2-butenolide (10)

Following GP2, **10** (0.21 g, 68%) was obtained from **1** (0.30 g, 0.53 mmol) and cyclopropylamine (0.11 g, 2.00 mmol) as an amorphous colourless solid. $[\alpha]_D^{20} = -29.8$ (*c* 3.1, $CHCl_3$); $R_f = 0.50$ (silica gel, chloroform/diethyl ether, 9:1); IR (KBr): $\nu = 2948$s, 2873m, 1735s, 1602s, 1508m, 1455m, 1367m, 1300s, 1245s, 1161m, 1033m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.14$ (s, 1H, C*H* (32)), 5.03 (s, 1H, C*H* (28)), 4.78 (s, 1H, N*H*), 4.73 (d, 1H, *J* = 1.9 Hz, CH_a (30)), 4.58 (dd, 1H, *J* = 2.4, 1.4 Hz, CH_b (30)), 4.46 (dd, 1H, *J* = 11.4, 4.8 Hz, C*H*OAc (3)), 3.00 (ddd, 1H, *J* = 11.2, 10.4, 7.0 Hz, C*H* (19)), 2.54–2.50 (m, 1H, NC*H*), 2.15 (ddd, 1H, *J* = 12.2, 12.2, 3.6 Hz, C*H* (13)), 2.03 (s, 3H, Ac), 1.87–1.46 (m, 11H, CH_a (21) + C*H* (18) + CH_a (16) + CH_a (15) + CH_a (1) + CH_a (12) + CH_2 (2) + CH_a (22) + CH_a (7) + CH_a (6)), 1.67 (s, 3H, CH_3 (29)), 1.44–1.20 (m, 7H, CH_2 (11) + CH_b (7) + CH_b (6) + CH_b (16) + CH_b (21) + C*H* (9)), 1.17–1.08 (m, 3H, CH_b (22) + CH_b (15) + CH_b (12)), 1.00–0.93 (m, 1H, CH_b (1)), 1.03 (s, 3H, CH_3 (26)), 1.01 (s, 3H, CH_3 (27)), 0.85 (s, 3H, CH_3 (25)), 0.83 (s, 3H, CH_3 (24)), 0.82 (s, 3H, CH_3 (23)), 0.82–0.76 (m, 3H, CH_2(cycloprop.) + C*H* (5)), 0.65–0.55 (m, 2H, CH_2(cycloprop.)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 174.0$ (*C*=O), 171.0 (*C*=O), 170.9 (C31, *C*=C), 150.6 (C20, *C*=CH_2), 109.6 (C30, *C*H_2=C), 86.0 (C28, *C*H), 80.8 (C3, *C*HOAc), 79.5 (C32, *C*H), 55.4 (C5, *C*H), 49.8 (C17, $C_{quart.}$), 51.6 (C18, *C*H), 50.2 (C9, *C*H), 47.1 (C19, *C*H), 43.0 (C14, $C_{quart.}$), 41.0 (C8, $C_{quart.}$), 38.3 (C1, *C*H_2), 37.8 (C4, $C_{quart.}$), 37.0 (C10, $C_{quart.}$), 36.6 (C13, *C*H), 34.3 (C22, *C*H_2), 34.2 (C7, *C*H_2), 34.0 (C16, *C*H_2), 32.4 (C21, *C*H_2), 27.9 (C23, *C*H_3), 27.8 (C15, *C*H_2), 26.6 (NCH), 25.3 (C12, *C*H_2), 23.7 (C2, *C*H_2), 21.3 (Ac, *C*H_3), 20.7 (C11, *C*H_2), 19.6 (C29, *C*H_3), 18.1 (C6, *C*H_2), 16.4 (C24, *C*H_3), 16.3 (C25, *C*H_3), 16.1 (C26, *C*H_3), 15.3 (C27, *C*H_3), 7.2 (cycloprop., *C*H_2), 7.0 (cycloprop., *C*H_2) ppm; MS (ESI, MeOH): *m*/*z* (%) = 592.5 (100% $[M+H]^+$), 614.5 (70% $[M+Na]^+$); Anal. for $C_{38}H_{57}NO_4$ (591.86): C, 77.11; H, 9.71; N, 2.37. Found: C, 77.08; H, 9.86; N, 2.18.

5.14. (4*R*) 3-(Pyrrolidyl)-4-[3β-hydroxy-28-norlup-20(29)-en-17β-yl]-2-butenolide (11)

Following GP2, **11** (0.12 g, 38%) was obtained from **1** (0.30 g, 0.53 mmol) and pyrrolidine (0.14 g, 2.00 mmol) as a colourless solid; mp 164–166 °C; $[\alpha]_D^{20} = +2.7$ (*c* 6.3, $CHCl_3$); $R_f = 0.36$ (silica gel, chloroform/diethyl ether, 9:1); IR (KBr): $\nu = 2948$s, 2874m, 1735s, 1641w, 1593s, 1456m, 1392m, 1378m, 1304m, 1246s, 1165m, 1031m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.16$ (s, 1H, C*H* (32)), 4.69 (s, 2H, C*H* (28) + CH_a (30)), 4.51 (dd, 1H, *J* = 2.4, 1.4 Hz, CH_b (30)), 4.41 (dd, 1H, *J* = 11.4, 4.8 Hz, C*H*OAc (3)), 3.24–3.14 (m, 4H, NCH_2), 2.98 (ddd, 1H, *J* = 11.2, 10.4, 7.0 Hz, C*H* (19)), 2.22 (ddd, 1H, *J* = 12.2, 12.2, 3.6 Hz, C*H* (13)), 2.05–1.92 (m, 3H, 2CH_a (pyrrolid.) + CH_a (16)), 1.97 (s, 3H, Ac), 1.88–1.71 (m, 5H, 2CH_a (pyrrolid.) + CH_a (21) + CH_a (15) + C*H* (18)), 1.68–1.52 (m, 5H, CH_a (1) + CH_a (12) + CH_2 (2) + CH_a (22)), 1.61 (s, 3H, CH_3 (29)), 1.47–1.16 (m, 9H, CH_2 (6) + CH_2 (7) + CH_2 (11) + CH_b (16) + C*H* (9) + CH_b (21)), 1.09–0.88 (m, 3H, CH_b (15) + CH_b (22) + CH_b (12) + CH_b (1)), 1.00 (s, 3H, CH_3 (26)), 0.97 (s, 3H, CH_3 (27)), 0.80 (s, 3H, CH_3 (25)), 0.78 (s, 3H, CH_3 (23)), 0.77 (s, 3H, CH_3 (24)), 0.75 (d, 1H, *J* = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 173.6$ (*C*=O), 171.6 (C31, *C*=C), 171.0 (*C*=O), 150.9 (C20, *C*=CH_2), 109.5 (C30, *C*H_2=C), 86.4 (C28, *C*H), 81.4 (C32, *C*H), 80.8 (C3, *C*HOAc), 55.4 (C5, *C*H), 52.4 (C18, *C*H), 51.6 (NCH_2), 51.2 (C17, $C_{quart.}$), 50.2 (C9, *C*H), 47.2 (C19, *C*H), 42.8 (C14, $C_{quart.}$), 41.0 (C8, $C_{quart.}$), 38.3 (C1, *C*H_2), 37.8 (C4, $C_{quart.}$), 37.0 (C10, $C_{quart.}$), 36.5 (C13, *C*H), 35.5 (C22, *C*H_2), 34.3 (C7, *C*H_2), 33.4 (C16, *C*H_2), 32.4 (C21, *C*H_2), 28.0 (C15, *C*H_2), 27.9 (C23, *C*H_3), 25.6 (pyrrolid., *C*H_2),25.4 (C12, *C*H_2), 23.7 (C2, *C*H_2), 21.3 (Ac, *C*H_3), 20.7 (C11, *C*H_2), 19.5 (C29, *C*H_3), 18.1 (C6, *C*H_2), 16.5 (C24, *C*H_3), 16.3 (C25, *C*H_3), 16.1 (C26, *C*H_3), 15.2 (C27, *C*H_3) ppm; MS (ESI, MeOH): *m*/*z* (%) = 606.5 (90% $[M+H]^+$), 628.5 (100% $[M+Na]^+$); Anal. for $C_{39}H_{59}NO_4$ (605.89): C, 77.31; H, 9.82; N, 2.31. Found: C, 77.01; H, 9.98; N, 2.05.

5.15. (28*S*) 3-*O*-Acetyl-28-(3-hydroxyprop-1-enyl)-lup-20(29)-en-3,28-diol (12)

Hydrogen was bubbled for 30 min through a mixture containing **2** (0.2 g, 0.37 mmol), $Pd/BaSO_4$ (20 mg) and quinoline (200 mg) in ethyl acetate (10 ml). After completion of the reaction (as observed by TLC), the reaction was filtered over celite and the solvent removed in vacuo. The residue was subjected to column chromatography (silica gel, hexane/ethyl acetate, 8:2). Compound **12** (0.19 g, 94%) was obtained as a colourless solid; mp 220–222 °C; $[\alpha]_D^{20} = +37.4$ (*c* 3.4, $CHCl_3$); $R_f = 0.68$ (silica gel, hexane/ethyl acetate, 8:2); IR (KBr): $\nu = 3520$br, 2944s, 1701s, 1637m, 1451m, 1379m, 1283m, 1029m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.75–5.72$ (m, 2H, C*H* (31) + C*H* (32)), 4.86 (d, 1H, *J* = 7.3 Hz, C*H* (28)), 4.65 (d, 1H, *J* = 1.9 Hz, CH_a (30)), 4.51 (br s, 1H, CH_b (30)), 4.40 (dd, 1H, *J* = 11.4, 4.8 Hz, C*H*OAc (3)), 4.34 (dd, 1H, *J* = 12.4, 5.6 Hz, C*H* (33)), 4.11 (dd, 1H, *J* = 12.4, 4.0 Hz, C*H* (33)), 2.85 (ddd, 1H, *J* = 11.2, 10.4, 7.0 Hz, C*H* (19)), 2.05 (ddd, 1H, *J* = 12.2, 12.1, 3.4 Hz, C*H* (13)), 1.98–1.85 (m, 2H, CH_a (21) + CH_a (22)), 1.97 (s, 3H, Ac), 1.70–1.47 (m, 6H, CH_a (12) + C*H* (18) + CH_a (1) + CH_2 (2) + CH_a (16)), 1.62 (s, 3H, CH_3 (29)), 1.44–1.02 (m, 12H, CH_2 (6) + CH_a (15) + CH_2 (11) + CH_2 (7) + CH_b (21) + C*H* (9) + CH_b (16) + CH_b (22) + CH_b (12)), 1.00 (s, 3H, CH_3 (26)), 0.95–0.80 (m, 2H, CH_b (15) + CH_b (1)), 0.92 (s, 3H, CH_3 (27)), 0.80 (s, 3H, CH_3 (26)), 0.77 (s, 3H, CH_3 (23)), 0.76 (s, 3H, CH_3 (24)), 0.73 (d, 1H, *J* = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 171.0$ (*C*=O), 151.2 (C20, *C*=CH_2), 133.3 (C31, *C*H), 131.0 (C32, *C*H), 109.6 (C30, *C*H_2=C), 80.9 (C3, *C*HOAc), 68.0 (C28, *C*H), 58.7 (OCH_2), 55.3 (C5, *C*H), 50.3 (C9, *C*H), 49.8 (C18, *C*H), 49.3 (C17, $C_{quart.}$), 48.8 (C19, *C*H), 42.8 (C14, $C_{quart.}$), 40.9 (C8, $C_{quart.}$), 38.4 (C1, *C*H_2), 37.8 (C4, $C_{quart.}$), 37.0 (C10, $C_{quart.}$), 36.9 (C13, *C*H), 34.2 (C7, *C*H_2), 34.1 (C16, *C*H_2), 32.9 (C22, *C*H_2), 32.6 (C21, *C*H_2), 27.9 (C23, *C*H_3), 27.6 (C15, *C*H_2), 25.1 (C12, *C*H_2), 23.7 (C2, *C*H_2), 21.3 (Ac, *C*H_3), 21.0 (C11, *C*H_2), 18.8 (C29, *C*H_3), 18.1 (C6, *C*H_2), 16.5 (C24, *C*H_3), 16.2 (C26, *C*H_3), 16.1 (C25, *C*H_3), 15.2 (C27, *C*H_3) ppm; MS (ESI, MeOH): *m*/*z* (%) = 563.4 (70% $[M+Na]^+$), 1103.2 (100% $[2M+Na]^+$); Anal. for $C_{35}H_{56}O_4$ (540.82): C, 77.73; H, 10.44. Found: C, 77.53; H, 10.66.

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