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Synthesis and biological evaluation of antitumour-active betulin derivatives

René Csuk [a,*], Alexander Barthel [a], Ralph Kluge [a], Dieter Ströhl [a], Harish Kommera [b], Reinhard Paschke [b]

[a] Bereich Organische Chemie, Martin-Luther-Universität Halle-Wittenberg, Kurt-Mothes-Str. 2, D-06120 Halle, Saale, Germany
[b] BioSolutions GmbH, Weinbergweg 22, D-06120 Halle, Saale, Germany

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ABSTRACT

The reaction of betulinic aldehydes with various carbon nucleophiles gave a series of new betulin derivatives, among them epoxides, glycidic derivatives and β-hydroxy carbonyl compounds. Subsequent transformations of the β-hydroxy carbonyls lead to 1,3-diketo- and α,β-unsaturated betulin derivatives. These compounds were assayed for cytotoxicity using 15 human cancer cell lines and a colorimetric SRB-assay. Several compounds revealed significant antitumour activity.

1. Introduction

Betulin **I** and betulinic acid (BA) **II** are triterpenes; they are widely spread in plant kingdom and possess a lupane-skeleton (see Fig. 1).

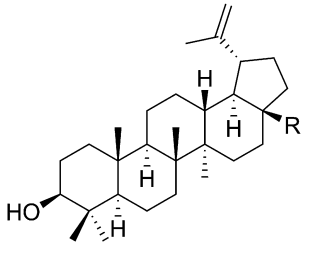
They aroused interest by their versatile pharmacological properties. Firstly recognized were BA and several derivatives for inhibiting HIV-1 propagation in lymphocyte cells.[1] Extending studies revealed two fundamental mechanisms depending on the derivatized position. Thus, BA compounds varied at the hydroxy group interrupts CA-SP1 junction in Gag processing which alters the cell maturation.[2,3] In contrast, amide compounds of BA, for example, RPR103611 affect the interface between gp120 and gp41 resulting in the inhibition of virus–cell fusion.[4,5] Also, betulin derivatives[6] revealed their great potential for cancer therapy because of their high selectivity for cancer cells. They trigger apoptosis[7] by causing a loss of transmembrane potential in mitochondria, and they release soluble apoptotic factors[8], for example, cytochrome *c* and AIF to the cytoplasm. Other studies revealed also the activation of caspases and the formation of reactive oxygen species (ROS) as essential steps of the cell death.[9] Also a partial involvement of the p38 pathway in apoptosis was suggested due to the observation[10] of phosphorylated proapoptotic mitogen-activated protein kinases (MAPKs).

Because of the high selectivity for cancer cells; the synthesis of betulin derivatives has become an appealing research field for cancer therapy. So far only little attention has been paid to betulinic aldehydes as starting materials for further transformations. We examined carbanionic reactions for the synthesis of epoxides, glycidic derivatives and β-hydroxy esters. Their antitumour potency was examined by a sulforhodamine-B (SRB) assay for 15 cancer cell lines.

2. Chemistry

Several[11,12] of betulinic aldehydes were allowed to react with different carbon nucleophiles (Scheme 1).

The synthesis of epoxide compounds was achieved either by the application of sulfonium ylids or *Darzens* reaction.[13] However, the use of the trimethylsulfoxonium iodide/sodium hydride[14] leads to the formation of the two diastereomeric epoxides **1** and **2** in a ratio of 3:1. Noticeable amounts of the pure isomers could only be



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

Figure 1. Structure of betulin and betulinic acid.

* Corresponding author. Tel.: +49 345 55 25660; fax: +49 345 55 27030.
E-mail address: rene.csuk@chemie.uni-halle.de (R. Csuk).

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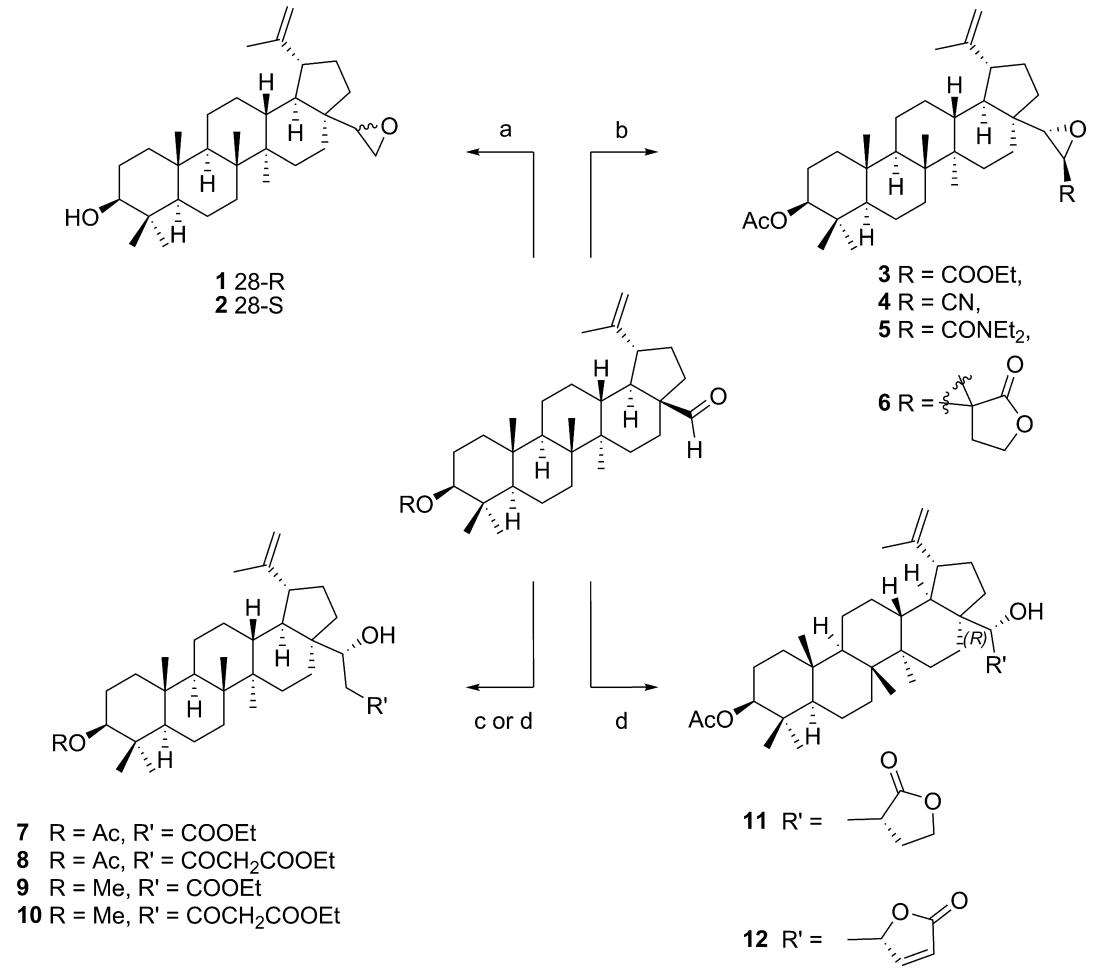


Scheme 1. Addition of C-nucleophiles to betulinic aldehydes. Reagents and conditions: (a) trimethylsulfoxonium iodide, NaH, DMSO; (b) DIA, *n*-BuLi, BF_3OEt_2, dry THF, −78 °C→25 °C, 1 h; (c) DIA, *n*-BuLi, dry THF, −78 °C→25 °C, 1 h; (d) silyl enol ether, BF_3OEt_2, dry THF, −78 °C→25 °C, 2 h.

earned by laborious preparative HPLC. Therefore we refrained from the synthesis of further derivatives and turned towards *Darzens* reaction[15] to produce glycidic derivatives **3**–**6**. This reaction was performed using the corresponding α-halogen carbonyl compound and LDA. A high stereoselectivity was observed yielding only the shown trans-isomer. Structure elucidation was performed by comparison of the observed NOE-correlations with the calculated structure (Fig. 2).

The addition of ester enolates[16] proceeds smoothly under Lewis acid catalysis. Alternatively the reaction could be carried out with the corresponding silyl enol ethers.[17] The conversion with ethyl acetate gave besides the β-hydroxy esters **7** and **9** also β-keto esters **8** and **10**. The β-hydroxy-γ-butyrolactones **11** and **12** were obtained by addition of the corresponding silyl enol ethers. Thereby only the anti-product was formed. However, lithium enolates or silyl enol ethers of ketones or aldehydes gave no reaction at all. Further derivatizations were carried out with β-hydroxy esters **7** and **9.** However, their reactivity was limited. The oxidation under *Swern*[18] or *Jones*[19] conditions gave β-keto-esters **13** and **14**. The synthesis of α,β-unsaturated esters **15** and **16** was achieved by mesylation and subsequently elimination.[20] Similar to the open chained derivatives the γ-butyrolactone compound **11** was transformed to the β-keto-derivative **17** by *Jones* oxidation and to the α-methylene-γ-butyrolactone **18.** Treating the β-hydroxy compounds **7** and **8** with trifluoroacetic acid resulted in a rearrangement[21] of the isopropylidene moiety thus resulting in the formation of the allobetulin derivatives **19** and **20**, respectively (see Scheme 2).

3. Results and discussion

The compounds **1**–**19** were tested for their cytotoxicity against 15 human cancer cell lines by using the SRB-protocol.[22] The summarized IC_{50}-values in Table 1 were obtained from the corresponding dose–response curves. The epoxide compounds **1** and **2** showed no cytotoxicity for several cell lines at the highest tested concentration, but IC_{50}-values below 20 μM were observed for colon cancer cell lines HT-29 and SW480. Glycidic derivative **4** and **6** revealed a lack of activity.

Betulinic acid has three positions (C-3, C-20 and C-28) where modifications can be performed to yield derivatives for structure–activity relationship studies. In this work, compound **5** showed a high antitumour potency with an averaged IC_{50}-value of 5.5 μM. In general, the β-hydroxy esters **7** and **9** are less active then the matching β-keto esters **8** and **10**. Interestingly, oxidizing the β-hydroxy group of compounds **7** and **9** increases the activity, whereas the elimination products **15** and **16** are not cytotoxic even at the highest tested concentration. Similar results were observed for analogue γ-butyrolactone compounds. The β-keto-derivative **17** showed an averaged IC_{50}-value of 8.2 μM whereas the α-methylene-γ-butyrolactone **18** possesses no cytotoxicity in many cell lines at the highest tested concentration of 30 μM. These results support assumptions from other groups, the keto-group at C-28 position is important for antitumour potency.[23,24] Rearranging compound **8** to the allobetulin derivative **20** leads to an higher activity with an averaged IC_{50}-value of 8.5 μM. Our investigations demonstrate that simple modifications of the

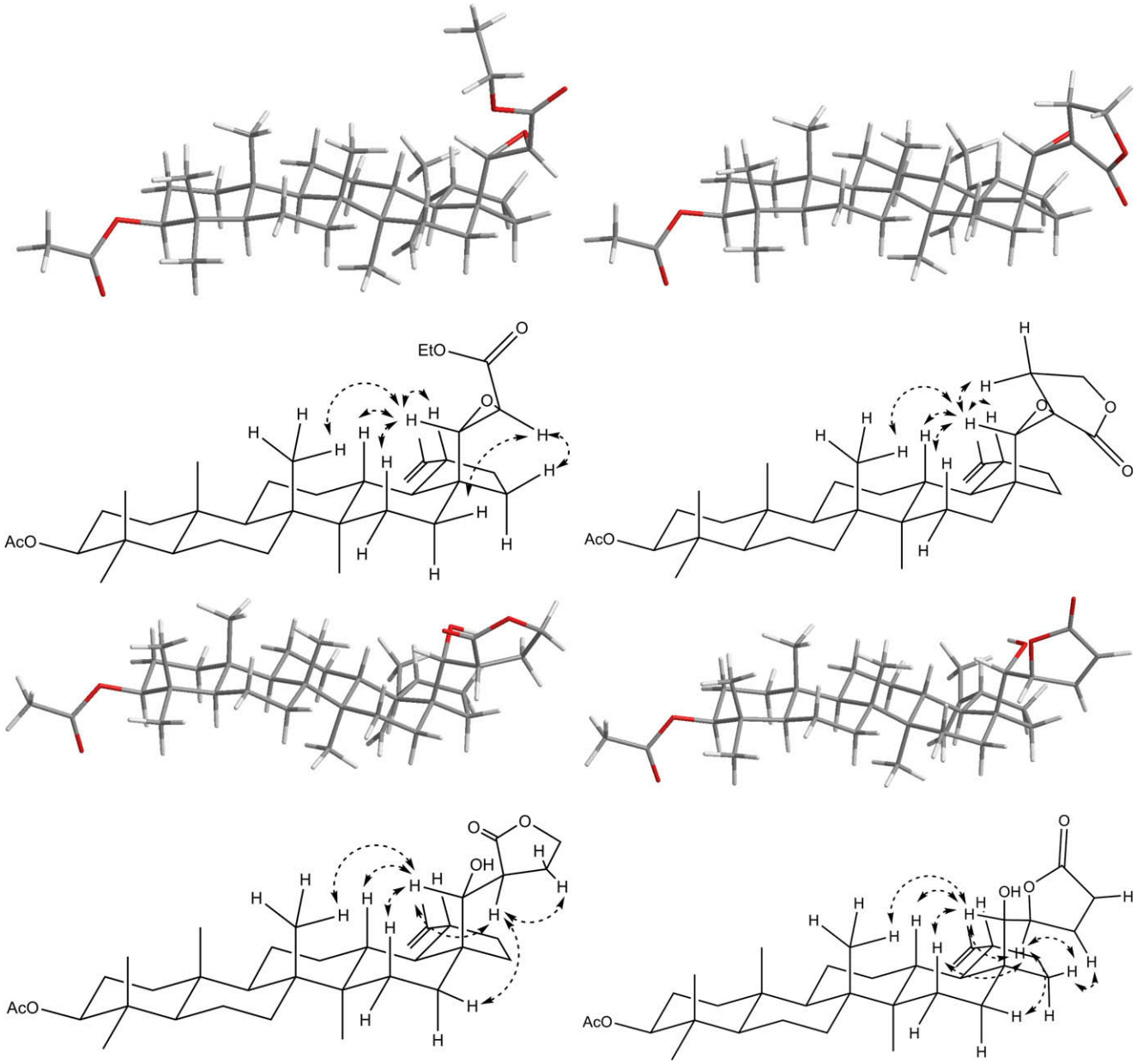


Figure 2. Calculated structures (PM3) and observed characteristic NOE-correlations of **3** (upper left), **6** (upper right), **11** (lower left) and **12** (lower right).

parent structure of betulinic acid can provide potentially active derivatives.

To control whether the compounds induce cell death by triggering apoptosis further investigations were performed for selected compounds **5**, **13** and **19** (Fig. 3). The trypan-blue dye exclusion test is a qualitative test to distinguish between apoptotic cells with an intact cell membrane and necrotic cells that have a lysed cell membrane. The intact cells can exclude the blue dye whereas necrotic cells are stained blue. A characteristically appearance in apoptosis is the fragmentation of the DNA into smaller pieces of ca. 180 bp which can be noted in gel electrophoresis as ladders.[25,26]

4. Conclusion

The synthesis of new betulin derivatives with potent pharmacological properties can be achieved starting from substituted betulinic aldehydes by reaction with carbon nucleophiles.

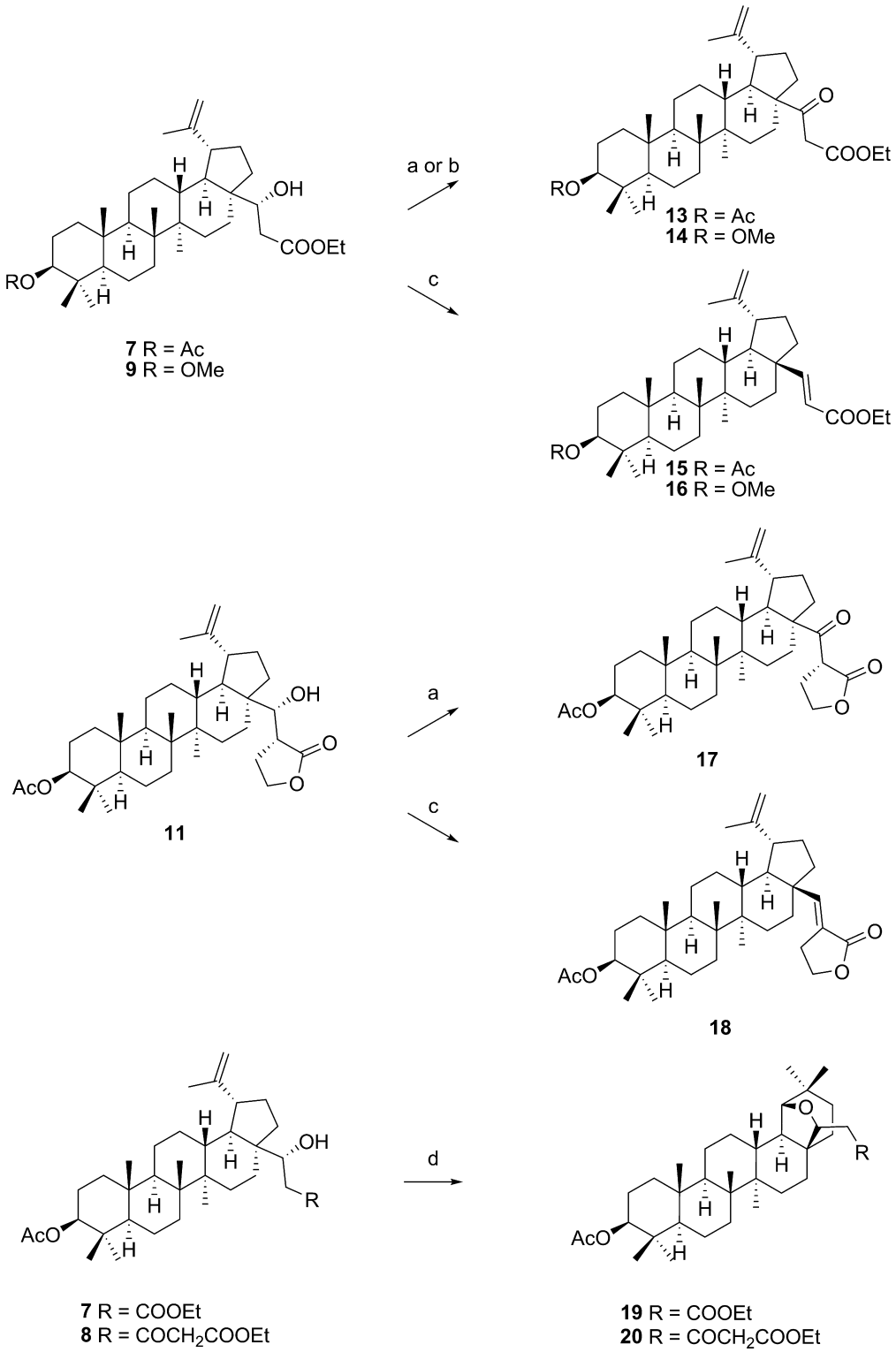
These additions proceed stereoselectively for lithium enolates and silyl enol ethers. Several compounds hold a higher cytotoxicity in comparison to betulinic acid. The best compounds in this screening are glycidic amide 5 with an average IC_{50}-value of 5.5 μM and β-keto-esters **13** and **14** with intermediate IC_{50}-value of 3.4 and 5.7 μM, respectively. However, a greater number of derivatives is needed for establishing a reliable structure–activity relationship study to design a more active betulinic acid derived antitumour agent.

5. Experimental

5.1. Chemistry

5.1.1. 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 microcell), IR spectra (film or KBr pellet) on a Perkin–Elmer FT-IR spectrometer Spectrum 1000, MS spectra were



Scheme 2. Reactions of β-hydroxy esters and β-hydroxy ketones. Reagents and conditions: (a) CrO_3, H_2SO_4, acetone, 0 °C, 30 min; (b) $(CO)_2Cl_2$, DMSO, TEA, dry CH_2Cl_2, −78 °C→25 °C, 1 h; (c) i—methanesulfonyl chloride, TEA, dry CH_2Cl_2, 0 °C→25 °C, 3 h; ii—K_2CO_3, DMF, 60 °C, 24 h; (d) TFA, CH_2Cl_2, 24 h.

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.

5.1.2. General procedure for *Darzens* reactions (GP1)

To a solution of diisopropylamine (417 mg, 4.12 mmol) in dry THF under argon was added at 0 °C butyllithium (1.6 M in hexane, 2.6 ml, 4.12 mmol) and then cooled to −78 °C. Subsequently, a solution of the corresponding 2-halo ester (4.12 mmol) was added and stirring was continued for 20 min at −78 °C. Then a solution of 3-acetylbetulinic aldehyde (0.50 g, 1.03 mmol) and BF_3OEt_2

Table 1
Cytoxicity 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**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
518A2	11.9	26.8	NA	NA	NA	7.0	NA	NA	11.6	NA	19.4	17.9	16.5	2.5	6.3	NA	NA	9.5	NA	NA	10.4
A431	15.4	20.1	23.5	NA	NA	4.3	NA	18.7	16.1	NA	20.7	11.7	8.9	2.6	3.9	NA	NA	7.3	19.4	22.6	9.8
A253	11.1	18.8	20.1	NA	NA	3.5	NA	NA	10.0	NA	17.0	16.8	15.7	2.2	7.4	NA	NA	7.0	NA	25.1	10.9
FADU	10.4	NA	NA	NA	NA	5.9	NA	NA	10.5	NA	16.2	17.5	12.3	3.0	6.5	NA	NA	5.8	NA	24.7	7.0
A549	14.9	NA	NA	NA	NA	5.6	NA	28.2	16.8	NA	14.6	21.1	17.0	2.2	5.3	NA	NA	5.5	NA	21.9	5.2
A2780	11.0	28.2	NA	12.3	NA	4.7	NA	21.0	9.1	NA	12.1	12.4	8.8	1.8	4.3	NA	NA	6.4	NA	11.9	4.7
DLD-1	17.5	NA	NA	NA	NA	7.9	NA	NA	9.3	NA	14.0	17.4	16.3	3.9	7.9	NA	NA	9.5	NA	NA	7.2
HCT-8	17.8	20.6	24.6	NA	NA	2.7	NA	16.5	11.3	NA	12.5	8.3	14.7	3.0	4.1	NA	NA	7.8	NA	12.0	6.1
HCT-116	13.3	19.9	20.3	18.1	NA	2.3	NA	13.0	13.0	NA	14.6	10.7	8.5	2.4	3.6	NA	NA	6.2	13.9	9.3	7.0
HT-29	16.1	15.4	18.2	NA	NA	9.6	NA	21.8	18.5	NA	16.8	NA	29.9	12.0	4.1	NA	NA	14.8	NA	16.7	13.2
SW480	6.4	16.2	16.8	NA	NA	6.0	NA	NA	13.0	NA	15.3	16.4	12.8	2.2	5.9	NA	NA	9.6	NA	17.3	5.8
8505C	6.7	22.3	24.3	NA	NA	8.3	NA	NA	9.6	NA	17.6	22.2	17.0	2.3	7.9	NA	NA	9.2	NA	NA	8.9
SW1736	11.6	NA	NA	17.6	NA	2.7	NA	23.5	10.6	NA	13.8	18.5	14.7	2.3	7.2	NA	NA	6.2	18.8	25.9	11.3
MCF-7	14.9	23.6	28.6	NA	NA	4.6	NA	27.3	13.9	NA	20.7	11.7	12.5	6.8	4.7	NA	NA	7.7	NA	27.1	9.2
Lipo	9.7	22.4	24.7	NA	NA	7.7	NA	NA	9.0	NA	17.8	17.4	15.9	2.2	6.4	NA	NA	10.0	NA	NA	10.5

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 tumour (A253, FADU), lung carcinoma (A549), ovarian cancer (A2780), colon cancer (DLD-1, HCT-8, HCT-116, HT-29, SW480), anaplastic thyroid cancer (8505c, SW-1736), mamma carcinoma (MCF-7) and liposarcoma. Values are the average from at least three independent experiments. Variation was ±10%. NA = no inhibition of cell growth at the highest concentration (30 μM).

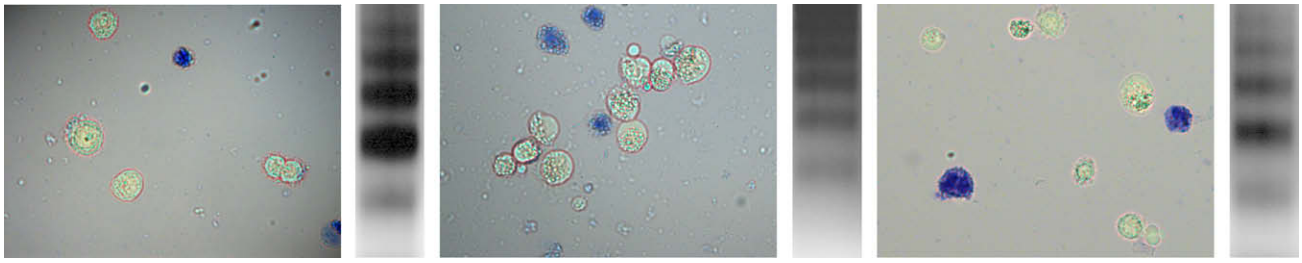


Figure 3. (from left to right): Trypan-blue exclusion test of compounds **5** for colon cancer cell line HCT-116, **13** for head and neck tumour A253 and **19** for lung carcinoma A549 and DNA laddering of compounds **5** for colon cancer cell line SW480, **13** for colon cancer cell line HCT-8 and **19** for colon cancer cell line SW480 after treatment with IC_{90}-concentrations for 24 h.

(0.1 ml) was added. The reaction mixture was warmed to room temperature over 2 h and stirred over night. The solution was diluted with brine (20 ml), the phases were separated and the aqueous layer was extracted with diethyl ether (2 × 20 ml). The combined organic extracts were dried over Na_2SO_4, concentrated in vacuo and the residue purified by column chromatography (silica gel, hexane/ethyl acetate 8:2).

5.1.3. General procedure for the addition of lithium enolates (GP2)

To a solution of diisopropylamine (417 mg, 4.12 mmol) in dry THF under argon was added at 0 °C butyllithium (1.6 M in hexane, 2.6 ml, 4.12 mmol) and then cooled to −78 °C. Subsequently, a solution of the corresponding ester (4.12 mmol) was added and stirring was continued for 20 min at −78 °C. Then a solution of 3-*O*-acetylbetulinic aldehyde (0.50 g, 1.03 mmol) and BF_3OEt_2 (0.1 ml) was added. The reaction mixture was warmed to room temperature over 2 h and stirred over night. The solution was diluted with brine (20 ml), the phases were separated and the aqueous layer extracted with diethyl ether (2 × 20 ml). The combined organic extracts were dried over Na_2SO_4, concentrated in vacuo and the residue purified by column chromatography (silica gel, hexane/ethyl acetate 8:2).

5.1.4. General procedure for the addition of silyl enol ether (GP3)

A mixture of 3-*O*-acetylbetulinic aldehyde (0.50 g, 1.03 mmol) and the corresponding silyl enol ether (3 mmol) in THF (20 ml) under argon was cooled to −78 C, then BF_3OEt_2 (0.1 ml) was added. The reaction mixture was warmed to room temperature and stirred over night. Subsequently, the solution was diluted with brine (20 ml), the phases were separated and the aqueous layer extracted with diethyl ether (2 × 20 ml). The combined organic extracts were dried over Na_2SO_4, concentrated in vacuo and the residue purified by column chromatography (silica gel, hexane/ethyl acetate 8:2).

5.1.5. General procedure for the *Swern* oxidation (GP4)

To a solution of oxalyl chloride (0.6 g, 4.7 mmol) in dry dichloromethane (CH_2Cl_2) (10 ml) at −78 °C a solution of dry DMSO (0.78 ml) in dry dichloromethane (CH_2Cl_2) (10 ml) was slowly added and stirring at −78 °C was continued for another 30 min. Maintaining this temperature a solution of the β-hydroxy-carbonyl compound (4.26 mmol) in dry CH_2Cl_2 (5 ml) was slowly added and stirring at this temperature was continued for another 2 h. Then dry TEA (1.4 ml) was added and then the reaction mixture was allowed to warm to room temperature. Diluted aq HCl (10%, 100 ml) was added under vigorous stirring, the phases were separated, the organic layer was washed with aq Na_2CO_3 (2 × 10 ml), water (2 × 10 ml), and brine (2 × 10 ml), the solvents were removed, and the residue was subjected to chromatography (silica gel, hexane/ethyl acetate 9:1).

5.1.6. General procedure for the *Jones* oxidation (GP5)

Chromium(VI) oxide (300 mg) was dissolved in aq H_2SO_4 (35%, 1 ml) and added dropwise to a stirred solution of the β-hydroxy-carbonyl compound (1.84 mmol) in acetone (10 ml). After TLC revealed the absence of starting material, the excess chromium(VI)

oxide 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).

5.1.7. General procedure for the eliminations (GP6)

To a solution of β-hydroxy-carbonyl compound (0.35 mmol), DMAP (24 mg, 0.2 mmol) and TEA (0.35 g, 3.5 mmol) in dry dichloromethane (CH_2Cl_2) (10 ml) was added methanesulfonyl chloride (0.4 g, 3.5 mmol) and stirring over night. The reaction was quenched by the addition of saturated Na_2CO_3 solution (20 ml). The phases were separated and the aq layer was extracted with CH_2Cl_2 (2 × 10 ml). The combined extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude mesylate and K_2CO_3 (0.48 g, 3.5 mmol) were dissolved in DMF (10 ml) and heated to 70 °C for 5 h. Then the mixture was concentrated in vacuo and treated with water (20 ml) and CH_2Cl_2 (20 ml). The phases were separated and the aq layer was extracted with CH_2Cl_2 (2 × 10 ml). The combined organic layers were dried over Na_2SO_4, evaporated to dryness and purified by column chromatography (silica gel, hexane/ethyl acetate 9:1).

5.1.8. General procedure for allobetulin rearrangement (GP7)

To a solution of the corresponding β-hydroxy carbonyl compound (0.35 mmol) in dichloromethane (CH_2Cl_2) (20 ml) was added TFA (1 ml) and stirred over night. The solution was concentrated in vacuo and the residue purified by column chromatography (silica gel, hexane/ethyl acetate 8:2).

5.1.9. 3β-Hydroxy-17β-[(2*R*)-2-oxiranyl]-28-norlup-20(29)-*en* (1) and 3β-hydroxy-17β-[(2*S*)-2-oxiranyl]-28-norlup-20(29)-*en* (2)

To a mixture of trimethylsulfoxonium iodide (2.7 mmol) in DMSO (10 ml), NaH (60% suspension in mineral oil, 0.11 g, 2.7 mmol) was added and stirred for 15 min. Then betulinic aldehyde (0.6 g, 1.35 mmol) was added and stirring was continued for 24 h. The reaction mixture was diluted with H_2O (100 ml) and extracted with ethyl acetate (2 × 100 ml). The combined organic extracts were dried over Na_2SO_4, concentrated in vacuo and the residue purified by column chromatography (silica gel, hexane/ethyl acetate 8:2). The obtained mixture of the diastereomeric epoxides (0.58 g, 95%, 28*R*/28*S* ratio: 3:1) was separated by semi-preparative HPLC (RP 18, 25 × 250 mm, 10 ml/min, CH_3CN/H_2O, 86:14, λ = 210 nm).

Data for **1**. Mp 236 °C; $[\alpha_D^{20}]$ 26.5 (*c* 4.3, $CHCl_3$); R_F 0.48 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr): *v* 2942s, 2870m, 1639w, 1561w, 1454w, 1376w, 1275w, 1191w, 1106w, 1043w cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 4.64 (d, 1H, *J* = 2.6 Hz, CH_a (30)), 4.48 (s, 1H, CH_b (30)), 3.07 (dd, 1H, *J* = 11.5, 4.7 Hz, C*H*OH (3)), 3.05 (dd, 1H, *J* = 4.4, 2.9 Hz, C*H* (28)), 2.60 (dd, 1H, *J* = 4.8, 4.4 Hz, CH_a (31)), 2.46 (ddd, 1H, *J* = 11.5, 11.0, 6.8 Hz, C*H* (19)), 2.41 (dd, 1H, *J* = 4.8, 2.9 Hz, CH_b (31)), 1.96–1.88 (m, 1H, C*H* (13)), 1.82–1.76 (m, 1H, CH_a (21)), 1.74–1.68 (m, 1H, CH_a (12)), 1.56 (s, 3H, CH_3 (29)), 1.55–1.42 (m, 6H, CH_a (1)+CH_2 (2)+C*H* (18)+CH_a (22)+CH_a (6)), 1.35–1.15 (m, 9H, CH_2 (11)+CH_b (6)+CH_b (22)+CH_2 (7)+CH_a (16)+C*H* (9)+CH_b (21)), 1.05–0.90 (m, 3H, CH_2 (15)+CH_b (12)), 0.89 (s, 3H, CH_3 (27)), 0.88 (s, 3H, CH_3 (25)), 0.85 (s, 3H, CH_3 (23)), 0.87–0.74 (m, 2H, CH_b (16)+CH_b (1)), 0.71 (s, 3H, CH_3 (26)), 0.64 (s, 3H, CH_3 (24)), 0.56 (d, 1H, *J* = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 150.1 (C20, *C*=CH_2), 110.0 (C30, CH_2=*C*), 78.9 (C3, *C*HOH), 55.3 (C5, *C*H), 52.9 (C28, *C*H), 50.4 (C9, *C*H), 48.9 (C19, *C*H), 48.8 (C18, *C*H), 45.6 (C17, $C_{quart.}$), 43.6 (C31, *C*H_2), 42.4 (C14, $C_{quart.}$), 40.9 (C8, $C_{quart.}$), 38.8 (C4, $C_{quart.}$), 38.7 (C1, *C*H_2), 37.3 (C13, *C*H), 37.1 (C10, $C_{quart.}$), 34.2 (C7, *C*H_2), 33.0 (C22, *C*H_2), 32.0 (C16, *C*H_2), 30.8 (C21, *C*H_2), 28.1 (C15, *C*H_2), 27.9 (C23, *C*H_3), 27.4 (C2, *C*H_2), 24.9 (C12, *C*H_2), 20.9 (C11, *C*H_2), 18.7 (C29, *C*H), 18.2 (C6, *C*H_2), 16.1 (C26, *C*H_3), 15.9 (C25, *C*H_3), 15.3 (C24, *C*H_3), 14.5 (C27, *C*H_3) ppm; MS (e.i., 70 eV): *m*/*z* (%) 454 (34), 423 (29), 411 (51), 246 (27), 217 (63), 207 (88), 201 (57), 189 (92), 175 (45), 159 (57), 145 (64), 135 (79), 119 (82), 107 (95), 95 (82), 81 (100); Anal. Calcd for $C_{31}H_{50}O_2$ (454.73): C, 81.88; H, 11.08. Found: C, 81.53; H, 10.80.

Data for **2** *Mp* 217 °C; $[\alpha_D^{20}]$ 23.6 (*c* 5.0, $CHCl_3$); R_F 0.48 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr): *v* 2941s, 2870m, 1642w, 1453w, 1376w, 1293w, 1181w, 1105w, 1082w, 1045w cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 4.60 (d, 1H, *J* = 2.3 Hz, CH_a (30)), 4.49–4.46 (m, 1H, CH_b (30)), 3.07 (dd, 1H, *J* = 11.5, 4.7 Hz, C*H*OH (3)), 3.02 (dd, 1H, *J* = 4.2, 2.9 Hz, C*H* (28)), 2.63 (dd, 1H, *J* = 4.6, 4.2 Hz, CH_a (31)), 2.41 (dd, 1H, *J* = 4.6, 2.9 Hz, CH_b (31)), 2.36 (ddd, 1H, *J* = 11.5, 11.0, 6.8 Hz, C*H* (19)), 1.90 (ddd, 1H, *J* = 12.2, 12.2, 3.8 Hz, C*H* (13)), 1.82 (ddd, 1H, *J* = 13.4, 13.9, 4.4 Hz, CH_a (15)), 1.68–1.56 (m, 2H, CH_a (16)+CH_a (12)), 1.56 (s, 3H, CH_3 (29)), 1.55–1.36 (m, 6H, CH_a (1)+CH_2 (2)+C*H* (18)+CH_a (21)+CH_a (6)), 1.34–1.17 (m, 9H, CH_2 (11)+CH_b (6)+CH_b (16)+CH_2 (7)+C*H* (9)+CH_a (22)+CH_b (21)), 1.06–0.92 (m, 3H, CH_b (22)+CH_b (15)+CH_b (12)), 0.93 (s, 3H, CH_3 (27)), 0.88 (s, 3H, CH_3 (25)), 0.85 (s, 3H, CH_3 (23)), 0.80 (ddd, 1H, *J* = 13.3, 13.3, 3.7 Hz, CH_b (1)), 0.71 (s, 3H, CH_3 (26)), 0.64 (s, 3H, CH_3 (24)), 0.57 (d, 1H, *J* = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 149.8 (C20, *C*=CH_2), 110.3 (C30, CH_2=*C*), 78.9 (C3, *C*HOH), 55.3 (C5, *C*H), 52.8 (C28, *C*H), 50.4 (C9, *C*H), 48.7 (C19, *C*H), 48.6 (C18, *C*H), 46.1 (C31, *C*H_2), 45.5 (C17, $C_{quart.}$), 42.6 (C14, $C_{quart.}$), 41.0 (C8, $C_{quart.}$), 38.8 (C4, $C_{quart.}$), 38.7 (C1, *C*H_2), 37.5 (C13, *C*H), 37.1 (C10, $C_{quart.}$), 34.2 (C7, *C*H_2), 32.7 (C22, *C*H_2), 31.2 (C16, *C*H_2), 30.5 (C21, *C*H_2), 27.9 (C23, *C*H_3), 27.7 (C15, *C*H_2), 27.4 (C2, *C*H_2), 25.0 (C12, *C*H_2), 20.8 (C11, *C*H_2), 18.6 (C29, *C*H), 18.3 (C6, *C*H_2), 16.1 (C26, *C*H_3), 15.9 (C25, *C*H_3), 15.3 (C24, *C*H_3), 14.7 (C27, *C*H_3) ppm; MS (e.i., 70 eV): *m*/*z* (%) 454 (34), 423 (18), 246 (20), 215 (47), 207 (68), 201 (57), 189 (100), 173 (44), 159 (53), 145 (63), 135 (74), 119 (68), 107 (86), 93 (82), 81 (78); Anal. Calcd for $C_{31}H_{50}O_2$ (454.73): C, 81.88; H, 11.08. Found: C, 81.50; H, 10.75.

5.1.10. (3*S*,2*R*)-3-[3β-Acetoxy-28-norlup-20(29)-*en*-17β-yl]-oxirane-2-carboxylic acid methyl ester (3)

Compound **3** (0.25 g, 45%) was obtained from 3-*O*-acetylbetulinic aldehyde (0.50 g, 1.03 mmol) and methyl 2-chloroacetate (0.46 g, 4.12 mmol) following GP1 as a colourless solid; mp 189 °C; $[\alpha_D^{20}]$ 28.0 (*c* 4.7, $CHCl_3$); R_F 0.35 (silica gel, hexane/ethyl acetate, 9:1); IR (KBr): *v* 3424br, 2952s, 2869m, 1743s, 1721s, 1641m, 1455m, 1392m, 1378m, 1246s, 1159w, 1131w, 1105w, 1081w, 1016m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 4.73 (br s, 1H, CH_a (30)), 4.59 (br s, 1H, CH_b (30)), 4.44 (dd, 1H, *J* = 11.4, 4.8 Hz, C*H*OAc (3)), 3.78 (s, 3H, OCH_3), 3.33–3.27 (m, 2H, C*H* (28)+C*H* (31)), 2.58 (ddd, 1H, *J* = 11.2, 10.4, 7.0 Hz, C*H* (19)), 2.02 (s, 3H, Ac), 1.99–1.85 (m, 2H, C*H* (13)+CH_a (21)), 1.83–1.74 (m, 1H, CH_a (12)), 1.66 (s, 3H, CH_3 (29)), 1.70–1.51 (m, 7H, C*H* (18)+CH_a (1)+CH_a (16)+CH_2 (2)+CH_a (6)+CH_a (15)), 1.50–1.13 (m, 9H, CH_a (22)+CH_2 (11)+CH_b (6)+CH_b (16)+CH_2 (7)+C*H* (9)+CH_b (21)), 1.12–0.86 (m, 4H, CH_b (22)+CH_b (15)+CH_b (12)+CH_b (1)), 0.96 (s, 3H, CH_3 (25)), 0.94 (s, 3H, CH_3 (27)), 0.83 (s, 6H, CH_3 (23)+CH_3 (26)), 0.81 (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 170.9 (*C*=O), 170.2 (*C*=O), 149.6 (C20, *C*=CH_2), 110.3 (C30, CH_2=*C*), 80.6 (C3, *C*HOAc), 60.0 (C28, *C*H), 55.3 (C5, *C*H), 52.4 (OCH_3), 50.6 (C31, *C*H), 50.3 (C9, *C*H), 48.8 (C18, *C*H), 48.3 (C19, *C*H), 46.0 (C17, $C_{quart.}$), 42.8 (C14, $C_{quart.}$), 40.9 (C8, $C_{quart.}$), 38.4 (C1, *C*H_2), 37.8 (C4, $C_{quart.}$), 37.5 (C13, *C*H), 37.0 (C10, $C_{quart.}$), 34.1 (C7, *C*H_2), 32.8 (C22, *C*H_2), 32.5 (C16, *C*H_2), 30.3 (C21, *C*H_2), 28.0 (C15, *C*H_2), 27.9 (C23, *C*H_3), 24.9 (C12, *C*H_2), 23.6 (C2, *C*H_2), 21.2 (Ac, *C*H_3), 20.8 (C11, *C*H_2), 18.8 (C29, *C*H_3),

18.1 (C6, CH_2), 16.4 (C24, CH_3), 16.2 (C25, CH_3), 15.7 (C26, CH_3), 14.4 (C27, CH_3) ppm; MS (e.i., 70 eV): *m*/*z* (%) 554 (16), 494 (16), 465 (21), 451 (46), 215 (40), 201 (47), 189 (100) 159 (39), 145 (52), 135 (95), 121 (81), 107 (76), 95 (68), 81 (60); Anal. Calcd for $C_{35}H_{54}O_5$ (554.80): C, 75.77; H, 9.81. Found: C, 75.60; H, 9.69.

5.1.11. (3*S*, 2*R*)-3-[3β-Acetoxy-28-norlup-20(29)-*en*-17β-yl]-oxirane-2-carbonitril (4)

Compound **4** (0.6 g, 89%) was obtained from 3-*O*-acetylbetulinic aldehyde (0.50 g, 1.03 mmol) and 2-chloroacetonitrile (0.62 g, 4.12 mmol) following GP1 as a colourless solid; mp 243 °C; $[\alpha_D^{20}]$ 39.3 (*c* 4.2, $CHCl_3$); R_F 0.63 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr): *ν* 2950s, 2869m, 2242w, 1718s, 1642m, 1465m, 1392m, 1377s, 1320w, 1257s, 1198m, 1148w, 1105w, 1079w, 1027m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 4.74 (d, 1H, *J* = 2.1 Hz, CH_a (30)), 4.58 (dd, 1H, *J* = 2.1, 1.2 Hz, CH_b (30)), 4.45 (dd, 1H, *J* = 10.4, 5.8 Hz, C*H*OAc (3)), 3.53 (d, 1H, *J* = 2.1 Hz, C*H* (28)), 3.20 (d, 1H, *J* = 2.1 Hz, C*H* (31)), 2.48 (ddd, 1H, *J* = 11.2, 10.4, 7.0 Hz, C*H* (19)), 2.02 (s, 3H, Ac), 1.98 (ddd, 1H, *J* = 12.2, 12.2, 3.8 Hz, C*H* (13)), 1.86–1.78 (m, 2H, CH_a (21)+CH_a (12)), 1.66 (s, 3H, CH_3 (29)), 1.66–1.40 (m, 8H, C*H* (18)+CH_a (1)+CH_a (15)+CH_2 (2)+CH_a (16)+CH_a (6)+CH_a(11)), 1.40–1.08 (m, 10H, CH_b (6)+CH_2 (7)+CH_b (21)+C*H* (9)+CH_a (22)+CH_b(11)+CH_b (16)+CH_b (15)+CH_b (12)), 1.03–0.94 (m, 2H, CH_b (22)+CH_b (1)), 1.03 (s, 3H, CH_3 (25)), 0.98 (s, 3H, CH_3 (27)), 0.85 (s, 3H, CH_3 (26)), 0.83 (s, 3H, CH_3 (23)), 0.82 (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* 170.9 (*C*═O), 149.0 (C20, *C*═CH_2), 117.0 (*C*≡N), 110.7 (C30, CH_2═C), 80.8 (C3, *C*HOAc), 60.8 (C28, *C*H), 55.4 (C5, *C*H), 50.3 (C9, *C*H), 48.7 (C18, *C*H), 48.5 (C19, *C*H), 46.0 (C17, $C_{quart.}$), 42.4 (C14, $C_{quart.}$), 40.9 (C8, $C_{quart.}$), 38.7 (C31, *C*H), 38.4 (C1, CH_2), 37.8 (C4, $C_{quart.}$), 37.2 (C13, *C*H), 37.0 (C10, $C_{quart.}$), 34.1 (C7, CH_2), 32.6 (C16, CH_2), 32.0 (C22, CH_2), 30.2 (C21, CH_2), 28.0 (C15, CH_2), 27.9 (C23, CH_3), 24.8 (C12, CH_2), 23.7 (C2, CH_2), 21.3 (Ac, CH_3), 20.8 (C11, CH_2), 18.6 (C29, CH_3), 18.1 (C6, CH_2), 16.5 (C24, CH_3), 16.2 (C25, CH_3), 16.0 (C26, CH_3), 14.5 (C27, CH_3) ppm; MS (ESI, MeOH): *m*/*z* 447.3 (100%, $[M+H]^+$), 469.4 (80%, $[M+Na]^+$); Anal. Calcd for $C_{34}H_{51}NO_3$ (521.77): C, 78.26; H, 9.85; N, 2.68. Found: C, 78.00; H, 9.55; N, 2.31.

5.1.12. (3*S*, 2*R*)-3-[3β-Acetoxy-28-norlup-20(29)-*en*-17β-yl]-oxirane-2-carboxylic acid diethylamide (5)

Compound **5** (0.6 g, 89%) was obtained from 3-*O*-acetylbetulinic aldehyde (0.50 g, 1.03 mmol) and chloro-*N*,*N*-diethylacetamide (0.62 g, 4.12 mmol) following GP1 as a colourless solid; mp 106 °C; $[\alpha_D^{20}]$ 27.8 (*c* 5.0, $CHCl_3$); R_F 0.30 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr): *ν* 2944s, 2873m, 1733s, 1654s, 1464m, 1378m, 1246s, 1149w, 1106w, 1028w cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 4.74 (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)), 3.49–3.37 (m, 4H, C*H* (28)+NCH_2), 3.33 (d, 1H, *J* = 2.1 Hz, C*H* (31)), 2.62 (ddd, 1H, *J* = 11.2, 10.4, 7.0 Hz, C*H* (192.08 (ddd, 1H, *J* = 12.2, 12.2, 3.8 Hz, C*H* (13)))), 2.02 (s, 3H, Ac), 1.97–1.88 (m, 1H, CH_a (21)), 1.84–1.74 (m, 1H, CH_a (12)), 1.66 (s, 3H, CH_3 (29)), 1.66–1.50 (m, 7H, C*H* (18)+CH_a (1)+CH_a (16)+CH_2 (2)+CH_a (6)+CH_a (15)), 1.50–1.19 (m, 12H, CH_a (22)+CH_2 (11)+CH_b (6)+CH_b (16)+CH_2 (7)+C*H* (9)+CH_b (21), 1.24 (*t*, 3H, *J* = 7.1 Hz, CH_2CH_3), 1.18 (*t*, 3H, *J* = 7.1 Hz, CH_2CH_3), 1.13–0.92 (m, 4H, CH_b (22)+CH_b (15)+CH_b (12)+CH_b (1)), 0.98 (s, 3H, CH_3 (25)), 0.97 (s, 3H, CH_3 (27)), 0.82 (s, 6H, CH_3 (23)+CH_3 (26)), 0.81 (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* 170.9 (*C*═O), 167.0 (*C*═O), 149.6 (C20, *C*═CH_2), 110.3 (C30, CH_2═C), 80.9 (C3, *C*HOAc), 59.5 (C28, *C*H), 55.4 (C5, *C*H), 50.4 (C31, *C*H), 50.3 (C9, *C*H), 48.9 (C18, *C*H), 48.5 (C19, *C*H), 45.8 (C17, $C_{quart.}$), 42.5 (C14, $C_{quart.}$), 41.2 (NCH_2), 40.9 (C8, $C_{quart.}$), 40.5 (NCH_2), 38.4 (C1, CH_2), 37.8 (C4, $C_{quart.}$), 37.2 (C13, *C*H), 37.1 (C10, $C_{quart.}$), 34.1 (C7, CH_2), 33.6 (C22, CH_2), 32.4 (C16, CH_2), 30.6 (C21, CH_2), 28.0 (C15, CH_2), 27.9 (C23, CH_3), 24.9 (C12, CH_2), 23.7 (C2, CH_2), 21.3 (Ac, CH_3), 20.9 (C11, CH_2), 18.7 (C29, CH_3), 18.1 (C6, CH_2), 16.4 (C24, CH_3), 16.2 (C25, CH_3), 15.9 (C26, CH_3), 14.8 (NCH_2CH_3), 14.5 (C27, CH_3), 12.8 (NCH_2CH_3) ppm; MS (ESI, MeOH): *m*/*z* 596.4 (100%, $[M+H]^+$), 618.5 (80%, $[M+Na]^+$); Anal. Calcd for $C_{38}H_{61}NO_4$ (595.90): C, 76.59; H, 10.32; N, 2.35. Found: C, 76.10; H, 9.88; N, 2.32.

5.1.13. (2S, 3*S*)-2-[3β-Acetoxy-28-norlup-20(29)-*en*-17β-yl]-1,5-dioxa-spiro[2.4]heptan-4-one (5)

Compound **6** (0.6 g, 89%) was obtained from 3-*O*-acetylbetulinic aldehyde (0.50 g, 1.03 mmol) and 2-bromo-γ-butyrolactone (0.68 g, 4.12 mmol) following GP1 as a colourless solid; mp 249 °C; $[\alpha_D^{20}]$ 41.0 (*c* 4.1, $CHCl_3$); R_F 0.16 (silica gel, hexane/ethyl acetate, 9:1); IR (KBr): *ν* 2944s, 2875m, 1798s, 1724s, 1640w, 1447m, 1392m, 1376m, 1244s, 1115m, 1084w, 1024m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 4.72 (d, 1H, *J* = 2.1 Hz, CH_a (30)), 4.58 (br s, 1H, CH_b (30)), 4.52 (ddd, 1H, *J* = 9.5, 9.5, 2.1 Hz, CH_a (33)), 4.44 (dd, 1H, *J* = 10.8, 5.4 Hz, C*H*OAc (3)), 4.37 (ddd, 1H, *J* = 9.9, 9.5, 6.6 Hz, CH_b (33)), 3.41 (s, 1H, C*H* (28)), 2.61 (ddd, 1H, *J* = 13.3, 9.9, 9.5 Hz, CH_a (32)), 2.43 (ddd, 1H, *J* = 11.2, 10.4, 7.0 Hz, C*H* (19)), 2.34–2.30 (m, 1H, CH_a (16)), 2.23 (ddd, 1H, *J* = 13.3, 6.6, 2.1 Hz, CH_b (32)), 2.02 (s, 3H, Ac), 1.98–1.82 (m, 3H, C*H* (13)+CH_a (21)+CH_a (12)), 1.67 (s, 3H, CH_3 (29)), 1.69–1.54 (m, 6H, CH_a (1)+CH_a (22)+C*H* (18)+CH_2 (2)+CH_a (6)), 1.50–1.08 (m, 11H, CH_b (6)+CH_2 (11)+CH_2 (15)+CH_b (22)+CH_b (16)+CH_2 (7)+C*H* (9)+CH_b (21)), 1.05–0.88 (m, 2H, CH_b (12)+CH_b (1)), 0.98 (s, 3H, CH_3 (27)), 0.97 (s, 3H, CH_3 (24)), 0.84 (s, 6H, CH_3 (25)), 0.83 (s, 6H, CH_3 (23)), 0.81 (s, 3H, CH_3 (26)), 0.77 (d, 1H, *J* = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): *δ* 172.4 (*C*═O), 170.9 (*C*═O), 149.7 (C20, *C*═CH_2), 110.1 (C30, CH_2═C), 80.7 (C3, *C*HOAc), 69.0 (C28, *C*H), 64.4 (C33, CH_2), 59.0 (C31, $C_{quart.}$), 55.3 (C5, *C*H), 50.4 (C9, *C*H), 49.9 (C18, *C*H), 48.6 (C19, *C*H), 45.6 (C17, $C_{quart.}$), 42.0 (C14, $C_{quart.}$), 40.8 (C8, $C_{quart.}$), 38.3 (C1, CH_2), 37.7 (C4, $C_{quart.}$), 37.6 (C13, *C*H), 37.0 (C10, $C_{quart.}$), 34.1 (C7, CH_2), 33.6 (C22, CH_2), 32.5 (C16, CH_2), 30.3 (C21, CH_2), 29.6 (C32, CH_2), 28.4 (C15, CH_2), 27.8 (C23, CH_3), 24.7 (C12, CH_2), 23.6 (C2, CH_2), 21.2 (Ac, CH_3), 20.9 (C11, CH_2), 18.6 (C29, CH_3), 18.1 (C6, CH_2), 16.4 (C24, CH_3), 16.2 (C25, CH_3), 15.8 (C26, CH_3), 14.5 (C27, CH_3) ppm; MS (ESI, MeOH): *m*/*z* 567.1 (40%, $[M+H]^+$), 589.4 (100%, $[M+Na]^+$); Anal. Calcd for $C_{36}H_{54}O_5$ (566.81): C, 76.28; H, 9.60. Found: C, 76.10; H, 9.88.

5.1.14. Ethyl-(3*R*)-3-[3β-acetoxy-28-norlup-20(29)-*en*-17β-yl]-3-hydroxy-propionate (7)

Compound **7** (0.43 g, 73%) was obtained as major product from 3-*O*-acetylbetulinic aldehyde (0.50 g, 1.03 mmol) and ethyl acetate (0.68 g, 4.12 mmol) following GP2 or by reaction with ketene ethyl trimethylsilyl acetal (0.66 g, 4.12 mmol) following GP3 in 65% yield as a colourless solid; mp 226 °C; $[\alpha_D^{20}]$ 28.0 (*c* 9.2, $CHCl_3$); R_F 0.66 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr): *ν* 2939s, 2864m, 1737s, 1707s, 1643w, 1446m, 1373m, 1339w, 1267s, 1176m, 1107w, 1079w, 1027s cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): *δ* = 4.69 (d, 1H, *J* = 2.5 Hz, CH_a (30)), 4.56 (dd, 1H, *J* = 1.2, 2.5 Hz, CH_b (30)), 4.52 (d, 1H, *J* = 10.0, Hz, C*H* (28)), 4.47 (dd, 1H, *J* = 10.4, 5.8 Hz, C*H*OAc (3)), 4.18 (*q*, 2H, *J* = 7.0 Hz, OCH_2), 2.94 (ddd, 1H, *J* = 11.2, 11.0, 5.4 Hz, C*H* (19)), 2.49 (dd, 1H, *J* = 16.2, 2.1 Hz, CH_a (31)), 2.40 (dd, 1H, *J* = 16.2, 10.0 Hz, CH_b (31)), 2.09 (ddd, 1H, *J* = 12.2, 12.2, 3.7 Hz, C*H* (13)), 2.05–1.97 (m, 1H, CH_a (21)), 2.02 (s, 3H, Ac), 1.80 (ddd, 1H, *J* = 12.8, 9.2, 1.5 Hz, CH_a (16)), 1.71–1.49 (m, 8H, CH_a (12)+C*H* (18)+CH_a (1)+CH_2 (2)+CH_a (22)+CH_a (15)+CH_a (6)), 1.66 (s, 3H, CH_3 (29)), 1.48–1.20 (m, 8H, CH_2 (11)+CH_2 (7)+CH_b (6)+CH_b (22)+CH_b (21)+C*H* (9)), 1.27 (*t*, 3H, *J* = 7.0 Hz, CH_2CH_3), 1.19–1.08 (m, 2H, CH_b (12)+CH_b (16)), 1.02–0.91 (m, 2H, CH_b (1)+CH_b (15)), 1.05 (s, 3H, CH_3 (25)), 0.99 (s, 3H, CH_3 (27)), 0.85 (s, 3H, CH_3 (24)), 0.83 (s, 3H, CH_3 (26)), 0.82 (s, 3H, CH_3 (23)), 0.79 (d, 1H, *J* = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz,

$CDCl_3$): δ 173.9 (*C*=O), 170.9 (*C*=O), 151.3 (C20, *C*=CH_2), 109.3 (C30, CH_2=C), 80.9 (C3, *C*HOAc), 68.8 (C28, *C*H), 60.7 (OCH_2), 55.4 (C5, *C*H), 50.3 (C18, *C*H), 50.2 (C9, *C*H), 49.2 (C17, $C_{quart.}$), 48.7 (C19, *C*H), 42.9 (C14, $C_{quart.}$), 40.9 (C8, $C_{quart.}$), 38.3 (C1, CH_2), 37.8 (C4, $C_{quart.}$), 37.4 (C31, CH_2), 37.0 (C10, $C_{quart.}$), 36.7 (C13, *C*H), 34.1 (C7, CH_2), 34.0 (C22, CH_2), 33.2 (C16, CH_2), 32.6 (C21, CH_2), 27.9 (C23, CH_3), 27.7 (C15, CH_2), 25.1 (C12, CH_2), 23.7 (C2, CH_2), 21.3 (Ac, CH_3), 20.9 (C11, CH_2), 19.0 (C29, CH_3), 18.1 (C6, CH_2), 16.5 (C24, CH_3), 16.1 (C25+C26, 2 × CH_3), 15.1 (C27, CH_3), 14.7 (CH_2CH_3) ppm; MS (ESI, MeOH): *m*/*z* 571.3 (20%, $[M+H]^+$), 593.4 (100%, $[M+Na]^+$); Anal. Calcd for $C_{36}H_{58}O_5$ (570.84): C, 75.75; H, 10.24. Found: C, 75.40; H, 9.97.

5.1.15. Ethyl-(5*R*)-5-[3β-acetoxy-28-norlup-20(29)-*en*-17β-yl]-5-hydroxy-3-oxo-valerate (8)

Compound **8** (0.13 g, 20%) was obtained as minor product from 3-*O*-acetylbetulinic aldehyde (0.50 g, 1.03 mmol) and ethyl acetate (0.68 g, 4.12 mmol) following GP2 or by reaction with ketene ethyl trimethylsilyl acetal (0.66 g, 4.12 mmol) following GP3 in 17% yield as a colourless solid; mp 134 °C; $[\alpha_D^{20}]$ 28.7 (*c* 5.2, $CHCl_3$); R_F 0.14 (silica gel, hexane/ethyl acetate, 9:1); IR (KBr): *ν* 2941s, 2868m, 1739s, 1707s, 1643w, 1446m, 1378m, 1326m, 1269s, 1157w, 1083w, 1039m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 4.62 (d, 1H, *J* = 2.5 Hz, CH_a (30)), 4.56 (d, 1H, *J* = 7.6, 4.3 Hz, C*H* (28)), 4.50 (dd, 1H, *J* = 1.2, 2.5 Hz, CH_b (30)), 4.40 (dd, 1H, *J* = 10.3, 6.6 Hz, C*H*OAc (3)), 4.14 (*q*, 2H, *J* = 7.0 Hz, OCH_2), 3.43 (s, 2H, CH_2 (33)), 2.86 (ddd, 1H, *J* = 11.1, 11.1, 5.9 Hz, C*H* (19)), 2.65–2.63 (m, 2H, CH_2 (31)), 2.01 (ddd, 1H, *J* = 12.2, 12.2, 3.7 Hz, C*H* (13)), 1.98–1.91 (m, 1H, CH_a (21)), 1.97 (s, 3H, Ac), 1.71 (ddd, 1H, *J* = 12.3, 9.0, 1.5 Hz, CH_a (16)), 1.71–1.48 (m, 5H, CH_a (12)+C*H* (18)+CH_a (1)+CH_2 (2)), 1.61 (s, 3H, CH_3 (29)), 1.48–1.17 (m, 8H, CH_2 (22)+CH_a (15)+CH_a (6)+CH_2 (11)+CH_2 (7)+CH_b (6)+CH_b (21)+C*H* (9)), 1.22 (*t*, 3H, *J* = 7.0 Hz, CH_2CH_3), 1.15–1.01 (m, 2H, CH_b (12)+CH_b (16)), 0.94–0.85 (m, 2H, CH_b (1)+CH_b (15)), 0.99 (s, 3H, CH_3 (25)), 0.93 (s, 3H, CH_3 (27)), 0.79 (s, 3H, CH_3 (26)), 0.78 (s, 3H, CH_3 (24)), 0.77 (s, 6H, CH_3 (23)), 0.72 (d, 1H, *J* = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 204.4 (*C*=O), 170.9 (*C*=O), 167.7 (*C*=O), 151.3 (C20, *C*=CH_2), 109.3 (C30, CH_2=C), 80.9 (C3, *C*HOAc), 68.2 (C28, *C*H), 61.5 (OCH_2), 55.4 (C5, *C*H), 50.3 (C18, *C*H), 50.2 (C9, *C*H), 50.0 (C33, CH_2), 49.1 (C17, $C_{quart.}$), 48.7 (C19, *C*H), 45.8 (C31, CH_2), 42.9 (C14, $C_{quart.}$), 40.9 (C8, $C_{quart.}$), 38.3 (C1, CH_2), 37.8 (C4, $C_{quart.}$), 37.0 (C10, $C_{quart.}$), 36.7 (C13, *C*H), 34.1 (C7, CH_2), 34.0 (C22, CH_2), 33.5 (C16, CH_2), 32.6 (C21, CH_2), 27.9 (C23, CH_3), 27.7 (C15, CH_2), 25.1 (C12, CH_2), 23.7 (C2, CH_2), 21.3 (Ac, CH_3), 20.9 (C11, CH_2), 19.0 (C29, CH_3), 18.1 (C6, CH_2), 16.4 (C24, CH_3), 16.1 (C25, CH_3), 16.1 (C26, CH_3), 15.1 (C27, CH_3), 14.1 (CH_2CH_3) ppm; MS (ESI, MeOH): *m*/*z* 613.3 (20%, $[M+H]^+$), 635.4 (100%, $[M+Na]^+$); Anal. Calcd for $C_{38}H_{60}O_6$ (612.87): C, 74.47; H, 9.87. Found: C, 74.40; H, 9.65.

5.1.16. Ethyl-(3*R*)-3-[3β-methoxy-28-norlup-20(29)-*en*-17β-yl]-3-hydroxy-propionate (9)

Compound **9** (0.42 g, 75%) was obtained as major product from 3-*O*-methylbetulinic aldehyde (0.470 g, 1.03 mmol) and ethyl acetate (0.68 g, 4.12 mmol) following GP2 or by reaction with ketene ethyl trimethylsilyl acetal (0.66 g, 4.12 mmol) following GP3 in 65% yield as a colourless solid; mp 198–200 °C; $[\alpha_D^{20}]$ 29.3 (*c* 4.0, $CHCl_3$); R_F 0.38 (silica gel, hexane/ethyl acetate, 9:1); IR (KBr): *ν* 2936s, 2840m, 1718s, 1642w, 1453w, 1392w, 1374m, 1192m, 1100m, 1023w cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 4.69 (d, 1H, *J* = 2.5 Hz, CH_a (30)), 4.56 (dd, 1H, *J* = 1.2, 2.5 Hz, CH_b (30)), 4.51 (d, 1H, *J* = 10.0, Hz, C*H* (28)), 4.17 (*q*, 2H, *J* = 7.0 Hz, OCH_2), 3.33 (s, 3H, OMe), 2.93 (ddd, 1H, *J* = 11.2, 11.0, 5.4 Hz, C*H* (19)), 2.62 (dd, 1H, *J* = 11.6, 4.1 Hz, C*H*OMe (3)), 2.49 (dd, 1H, *J* = 16.1, 2.4 Hz, CH_a (31)), 2.40 (dd, 1H, *J* = 16.1, 10.0 Hz, CH_b (31)), 2.13–1.95 (m, 2H, C*H* (13)+CH_a (21)), 1.80–1.63 (m, 5H, CH_a (2)+CH_a (16)+CH_a (1)+C*H* (18)+CH_a (12)), 1.66 (s, 3H, CH_3 (29)), 1.61–1.22 (m, 12H, CH_2 (22)+CH_2 (11)+CH_2 (7)+CH_2 (6)+CH_a (15)+CH_b (2)+C*H* (9)+CH_b (21)), 1.27 (*t*, 3H, *J* = 7.0 Hz, CH_2CH_3), 1.20–1.04 (m, 2H, CH_b (12)+CH_b (16)), 1.05 (s, 3H, CH_3 (25)), 1.01–0.91 (m, 1H, CH_b (15)), 0.99 (s, 3H, CH_3 (27)), 0.93 (s, 3H, CH_3 (23)), 0.86–0.78 (m, 1H, CH_b (1)), 0.82 (s, 3H, CH_3 (24)), 0.73 (s, 3H, CH_3 (26)), 0.65 (d, 1H, *J* = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.9 (*C*=O), 151.4 (C20, *C*=CH_2), 109.3 (C30, CH_2=C), 88.6 (C3, *C*HOMe), 68.8 (C28, *C*H), 60.7 (OCH_2), 57.4 (OMe), 55.9 (C5, *C*H), 50.4 (C18+C9, *C*H), 49.2 (C17, $C_{quart.}$), 48.7 (C19, *C*H), 42.9 (C14, $C_{quart.}$), 41.0 (C8, $C_{quart.}$), 38.8 (C1, CH_2), 38.6 (C4, $C_{quart.}$), 37.4 (C31, CH_2), 37.2 (C10, $C_{quart.}$), 36.8 (C13, *C*H), 34.2 (C7, CH_2), 34.1 (C22, CH_2), 33.3 (C16, CH_2), 32.7 (C21, CH_2), 28.0 (C23, CH_3), 27.8 (C15, CH_2), 25.2 (C12, CH_2), 22.2 (C2, CH_2), 20.9 (C11, CH_2), 19.1 (C29, CH_3), 18.1 (C6, CH_2), 16.2 (C24+C25, 2 × CH_3), 16.1 (C26, CH_3), 14.8 (C27, CH_3), 14.2 (CH_2CH_3) ppm; MS (ESI, MeOH): *m*/*z* 543.3 (20%, $[M+H]^+$), 565.5 (100%, $[M+Na]^+$); Anal. Calcd for $C_{35}H_{58}O_4$ (542.83): C, 77.44; H, 10.77. Found: C, 75.45; H, 10.68.

5.1.17. Ethyl-(5*R*)-5-[3β-methoxy-28-norlup-20(29)-*en*-17β-yl]-5-hydroxy-3-oxo-valerate (10)

Compound **10** (0.09 g, 15%) was obtained as minor product from 3-*O*-methylbetulinic aldehyde (0.50 g, 1.03 mmol) and ethyl acetate (0.68 g, 4.12 mmol) following GP2 or by reaction with ketene ethyl trimethylsilyl acetal (0.66 g, 4.12 mmol) following GP3 in 18% yield as a colourless foam; $[\alpha_D^{20}]$ 27.9 (*c* 4.0, $CHCl_3$); R_F 0.16 (silica gel, hexane/ethyl acetate, 9:1); IR (KBr): *ν* 2943s, 1730m, 1706m, 1646w, 1465w, 1370w, 1318w, 1249w, 1103w, 1025w cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 4.68 (d, 1H, *J* = 2.5 Hz, CH_a (30)), 4.61 (d, 1H, *J* = 7.9, 3.5 Hz, C*H* (28)), 4.55 (dd, 1H, *J* = 1.2, 2.5 Hz, CH_b (30)), 4.14 (*q*, 2H, *J* = 7.0 Hz, OCH_2), 3.48 (s, 2H, CH_2 (33)), 3.33 (s, 3H, OMe), 2.90 (ddd, 1H, *J* = 11.1, 11.1, 5.9 Hz, C*H* (19)), 2.71–2.66 (m, 2H, CH_2 (31)), 2.61 (dd, 1H, *J* = 11.7, 4.3 Hz, C*H*OMe (3)), 2.03 (ddd, 1H, *J* = 12.2, 12.2, 3.7 Hz, C*H* (13)), 1.94–1.83 (m, 1H, CH_a (21)), 1.80–1.42 (m, 8H, CH_a (2)+CH_a (16)+CH_a (12)+CH_a (1)+C*H* (18)+CH_a (22)+CH_a (15)+CH_a (6)), 1.66 (s, 3H, CH_3 (29)), 1.41–1.17 (m, 9H, CH_2 (11)+CH_b (2)+CH_b (6)+CH_b (22)+CH_2 (7)+CH_b (21)+C*H* (9)), 1.27 (*t*, 3H, *J* = 7.0 Hz, CH_2CH_3), 1.16–1.03 (m, 2H, CH_b (12)+CH_b (16)), 1.01–0.93 (m, 1H, CH_b (15)), 1.03 (s, 3H, CH_3 (25)), 0.98 (s, 3H, CH_3 (27)), 0.93 (s, 3H, CH_3 (23)), 0.82 (s, 3H, CH_3 (24)), 0.89–0.77 (m, 1H, CH_b (1)), 0.73 (s, 3H, CH_3 (26)), 0.66 (d, 1H, *J* = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 204.4 (*C*=O), 167.0 (*C*=O), 151.4 (C20, *C*=CH_2), 109.2 (C30, CH_2=C), 88.7 (C3, *C*HOMe), 68.2 (C28, *C*H), 61.5 (OCH_2), 57.5 (OMe), 55.9 (C5, *C*H), 50.4 (C9, *C*H), 50.3 (C18, *C*H), 50.0 (C33, CH_2), 49.1 (C17, $C_{quart.}$), 48.6 (C19, *C*H), 45.8 (C31, CH_2), 42.9 (C14, $C_{quart.}$), 41.0 (C8, $C_{quart.}$), 38.8 (C1, CH_2), 38.5 (C4, $C_{quart.}$), 37.2 (C10, $C_{quart.}$), 36.8 (C13, *C*H), 34.2 (C7, CH_2), 34.1 (C22, CH_2), 33.5 (C16, CH_2), 32.6 (C21, CH_2), 27.9 (C23, CH_3), 27.7 (C15, CH_2), 25.2 (C12, CH_2), 22.2 (C2, CH_2), 20.9 (C11, CH_2), 19.0 (C29, CH_3), 18.1 (C6, CH_2), 16.2 (C24, CH_3), 16.1 (C25, CH_3), 16.0 (C26, CH_3), 15.1 (C27, CH_3), 14.1 (CH_2CH_3) ppm; MS (ESI, MeOH): *m*/*z* 607.4 (20%, $[M+Na]^+$), 1191.3 (100%, $[2M+Na]^+$); Anal. Calcd for $C_{37}H_{60}O_5$ (584.87): C, 75.98; H, 10.34. Found: C, 75.88; H, 9.98.

5.1.18. (*R*)-2-((*R*)-[3β-Acetoxy-28-norlup-20(29)-*en*-17β-yl]hydroxymethyl)-γ-butyro-lactone (11)

Compound **11** (0.6 g, 89%) was obtained from 3-*O*-acetylbetulinic aldehyde (0.50 g, 1.03 mmol) and (4,5-dihydrofuran-2-yloxy)trimethylsilane (0.65 g, 4.12 mmol) following GP3 as a colourless solid; mp 232 °C; $[\alpha_D^{20}]$ 15.9 (*c* 5.8, $CHCl_3$); R_F 0.40 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr): *ν* 2947s, 2873m, 1735s, 1640w, 1453m, 1375s, 1316w, 1247s, 1217m, 1169m, 1109w, 1027s cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 4.78 (d, 1H, *J* = 4.1 Hz, C*H* (28)), 4.67 (d, 1H, *J* = 2.5 Hz, CH_a (30)), 4.56 (dd, 1H, *J* = 1.2, 2.5 Hz, CH_b (30)), 4.44 (dd, 1H, *J* = 11.4, 4.8 Hz, C*H*OAc (3)), 4.36

(ddd, 1H, J = 9.1, 8.7, 1.7 Hz, CH_a (33)), 4.19–4.10 (m, 1H, CH_b (33)), 2.78 (ddd, 1H, J = 11.2, 10.4, 6.2 Hz, CH (19)), 2.75–2.69 (m, 1H, CH (31)), 2.66–2.56 (m, 1H, CH_a (32)), 2.26–2.11 (m, 1H, CH_b (32)), 2.11–2.02 (m, 2H, CH_a (21)+CH (13)), 2.02 (s, 3H, Ac), 1.80 (ddd, 1H, J = 11.2, 8.7, 1.7 Hz, CH_a (16)), 1.75–1.45 (m, 8H, CH_a (12)+CH (18)+CH_a (1)+CH_a (22)+CH_2 (2)+CH_a (15)+CH_a (6)), 1.66 (s, 3H, CH_3 (29)), 1.45–1.20 (m, 8H, CH_2 (11)+CH_b (22)+CH_2 (7)+CH_b (6)+CH_b (21)+CH (9)), 1.19–1.08 (m, 2H, CH_b (12)+CH_b (16)), 1.02–0.91 (m, 2H, CH_b (1)+CH_b (15)), 1.08 (s, 3H, CH_3 (27)), 1.00 (s, 3H, CH_3 (25)), 0.85 (s, 3H, CH_3 (24)), 0.82 (s, 6H, CH_3 (23)+CH_3 (26)), 0.77 (d, 1H, J = 9.5 Hz, CH (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 179.7 (*C*=O), 170.9 (*C*=O), 151.0 (C20, *C*=CH$_2$), 109.6 (C30, *C*H$_2$=C), 80.9 (C3, *C*HOAc), 69.2 (C28, *C*H), 66.9 (C33, *C*H$_2$), 55.3 (C5, *C*H), 50.8 (C18, *C*H), 50.3 (C9, *C*H), 49.1 (C17, $C_{quart.}$), 49.1 (C19, *C*H), 43.2 (C31, *C*H), 42.9 (C14, $C_{quart.}$), 40.9 (C8, $C_{quart.}$), 38.3 (C1, *C*H$_2$), 37.8 (C4, $C_{quart.}$), 37.1 (C13, *C*H), 37.0 (C10, $C_{quart.}$), 34.7 (C22, *C*H$_2$), 34.1 (C7, *C*H$_2$), 33.8 (C16, *C*H$_2$), 32.6 (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$_2$), 22.8 (C32, *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+C26, 2 × *C*H$_3$), 15.1 (C27, *C*H$_3$) ppm; MS (ESI, MeOH): m/z 568.3 (100%, [M+H]$^+$), 591.3 (100%, [M+Na]$^+$); Anal. Calcd for $C_{36}H_{56}O_5$ (568.82): C, 76.01; H, 9.92. Found: C, 75.77; H, 9.60.

5.1.19. (*R*)-4-((*S*)-[3β-Acetoxy-28-norlup-20(29)-*en*-17β-yl]hydroxymethyl)-2-buten-4-olide (12)

Compound **12** (0.43 g, 73%) was obtained from 3-*O*-acetylbetulinic aldehyde (0.50 g, 1.03 mmol) and 2-trimethylsilyloxyfuran (0.48 g, 3.00 mmol) following GP3 as a colourless solid; mp 251 °C; [α_D^{20}] 51.6 (*c* 5.0, $CHCl_3$); R_F 0.20 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr): ν 3502m, 3066w, 2942s, 2875m, 1761s, 1715s, 1637w, 1453w, 1375w, 1335w, 1265s, 1165w, 1100w, 1039w cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.58 (dd, 1H, J = 5.8, 1.2 Hz, CH (32)), 6.17 (dd, 1H, J = 5.8, 2.1 Hz, CH (33)), 5.20 (ddd, 1H, J = 3.3, 2.1, 1.2 Hz, CH (31)), 4.67 (d, 1H, J = 2.5 Hz, CH_a (30)), 4.56 (dd, 1H, J = 1.2, 2.5 Hz, CH_b (30)), 4.53 (dd, 1H, J = 3.3, 3.8 Hz, CH (28)), 4.45 (dd, 1H, J = 11.4, 4.8 Hz, CHOAc (3)), 2.79 (ddd, 1H, J = 11.2, 10.4, 6.2 Hz, CH (19)), 2.02 (s, 3H, Ac), 2.01–1.86 (m, 2H, CH_a (21)+CH (13)), 1.82–1.52 (m, 9H, CH_a (22)+CH_a (16)+CH_a (12)+CH (18)+CH_a (1)+CH_2 (2)+CH_a (15)+CH_a (6)), 1.66 (s, 3H, CH_3 (29)), 1.51–1.14 (m, 10H, CH_b (22)+CH_2 (11)+CH_2 (7)+CH_b (6)+CH (9)+CH_b (21)+CH_b (16)+CH_b (12)), 1.09–1.04 (m, 1H, CH_b (15)), 1.00–0.91 (m, 1H, CH_b (1)), 1.00 (s, 6H, CH_3 (25)+CH_3 (27)), 0.84 (s, 3H, CH_3 (23)), 0.82 (s, 3H, CH_3 (26)), 0.81 (s, 3H, CH_3 (24)), 0.77 (d, 1H, J = 9.5 Hz, CH (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.7 (*C*=O), 170.9 (*C*=O), 153.5 (C33, *C*H=), 150.5 (C20, *C*=CH$_2$), 122.8 (C32, *C*H=), 109.9 (C30, *C*H$_2$=C), 84.6 (C31, *C*H), 80.8 (C3, *C*HOH), 71.6 (C28, *C*H), 55.3 (C5, *C*H), 51.0 (C18, *C*H), 50.2 (C9, *C*H), 48.6 (C19, *C*H), 48.5 (C17, $C_{quart.}$), 42.7 (C14, $C_{quart.}$), 40.9 (C8, $C_{quart.}$), 38.3 (C1, *C*H$_2$), 37.8 (C4, $C_{quart.}$), 37.2 (C13, *C*H), 37.0 (C10, $C_{quart.}$), 34.1 (C7, *C*H$_2$), 34.0 (C16, *C*H$_2$), 33.5 (C22, *C*H$_2$), 32.4 (C21, *C*H$_2$), 27.9 (C23, *C*H$_3$), 28.1 (C15, *C*H$_2$), 24.8 (C12, *C*H$_2$), 23.6 (C2, *C*H$_2$), 21.3 (Ac, *C*H$_3$), 20.9 (C11, *C*H$_2$), 18.7 (C29, *C*H$_3$), 18.1 (C6, *C*H$_2$), 16.4 (C24, *C*H$_3$), 16.1 (C25+C26, 2 × *C*H$_3$), 15.2 (C27, *C*H$_3$) ppm; MS (ESI, MeOH): m/z 568.3 (80%, [M+H]$^+$), 591.3 (100%, [M+Na]$^+$); Anal. Calcd for $C_{36}H_{54}O_5$ (566.81): C, 76.28; H, 9.60. Found: C, 75.77; H, 9.60.

5.1.20. Ethyl-3-[3β-acetoxy-28-norlup-20(29)-*en*-17β-yl]-3-oxo-propionate (13)

Compound **13** (0.49 g, 97%) was obtained from compound **7** (0.50 g, 0.88 mmol) following GP4 or GP5 as a colourless solid; mp 170 °C; [α_D^{20}] 15.0 (*c* 4.6, $CHCl_3$); R_F 0.28 (silica gel, hexane/ethyl acetate, 9:1); IR (KBr): ν 2940s, 2870m, 1748s, 1726s, 1706m, 1645w, 1448m, 1392m, 1365m, 1305m, 1286m, 1246s, 1165w, 1029m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.70 (d, 1H, J = 2.4 Hz, CH_a (30)), 4.56 (dd, 1H, J = 2.4, 1.5 Hz, CH_b (30)), 4.45 (dd, 1H, J = 10.4, 5.8 Hz, CHOAc (3)), 4.20–4.15 (m, 2H, OCH_2), 3.50 (d, 1H, J = 15.1 Hz, CH_a (31)), 3.44 (dd, 1H, J = 15.1 Hz, CH_b (31)), 2.94 (ddd, 1H, J = 11.2, 11.2, 4.4 Hz, CH (19)), 2.32 (ddd, 1H, J = 12.7, 12.2, 3.7 Hz, CH (13)), 1.97 (ddd, 1H, J = 14.2, 3.4, 2.9 Hz, CH_a (16)), 2.03 (s, 3H, Ac), 1.85 (ddd, 1H, J = 12.7, 8.3 Hz, CH_a (22)), 1.78–1.52 (m, 7H, CH_a (21)+CH_a (12)+CH_a (1)+CH (18)+CH_2 (2)+CH_b (16)), 1.66 (s, 3H, CH_3 (29)), 1.50–1.16 (m, 11H, CH_b (22)+CH_2 (7)+CH_2 (6)+CH_b (21)+CH_2 (15)+CH_2 (11)+CH (9)), 1.26 (*t*, 3H, J = 7.0 Hz, CH$_2$CH_3), 1.02–0.92 (m, 2H, CH_b (12)+CH_b (1)), 0.94 (s, 3H, CH_3 (27)), 0.89 (s, 3H, CH_3 (25)), 0.83 (s, 3H, CH_3 (24)), 0.82 (s, 3H, CH_3 (26)), 0.81 (s, 6H, CH_3 (23)), 0.77 (d, 1H, J = 9.5 Hz, CH (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 206.4 (*C*=O), 170.9 (*C*=O), 167.7 (*C*=O), 150.3 (C20, *C*=CH$_2$), 109.6 (C30, *C*H$_2$=C), 80.9 (C3, *C*HOAc), 62.2 (OCH$_2$), 61.1 (C17, $C_{quart.}$), 55.4 (C5, *C*H), 50.5 (C9, *C*H), 49.4 (C18, *C*H), 45.9 (C19, *C*H), 44.7 (C31, *C*H$_2$), 42.5 (C14, $C_{quart.}$), 40.7 (C8, $C_{quart.}$), 38.4 (C1, *C*H$_2$), 37.8 (C4, $C_{quart.}$), 37.1 (C10, $C_{quart.}$), 37.1 (C13, *C*H), 35.0 (C22, *C*H$_2$), 34.2 (C7, *C*H$_2$), 31.4 (C21, *C*H$_2$), 30.2 (C16, *C*H$_2$), 29.6 (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, *C*H$_3$), 20.9 (C11, *C*H$_2$), 19.3 (C29, *C*H$_3$), 18.1 (C6, *C*H$_2$), 16.4 (C24, *C*H$_3$), 16.2 (C26, *C*H$_3$), 16.0 (C25, *C*H$_3$), 14.4 (C27, *C*H$_3$), 14.1 (CH$_2$*C*H$_3$) ppm; MS (ESI, MeOH): m/z 569.7 (10%, [M+H]$^+$), 591.6 (20%, [M+Na]$^+$), 1159.4 (100%, [2M+Na]$^+$); Anal. Calcd for $C_{36}H_{56}O_5$ (568.83): C, 76.01; H, 9.92. Found: C, 75.77; H, 9.63.

5.1.21. Ethyl-3-[3β-methoxy-28-norlup-20(29)-*en*-17β-yl]-3-oxo-propionate (14)

Compound **14** (0.49 g, 97%) was obtained from compound **9** (0.50 g, 0.92 mmol) following GP4 or GP5 as a colourless solid; mp 151 °C; [α_D^{20}] 14.4 (*c* 4.1, $CHCl_3$); R_F 0.55 (silica gel, hexane/ethyl acetate, 9:1); IR (KBr): ν 3448m, 3076w, 2941s, 2873m, 2837m, 1754m, 1709m, 1643w, 1465m, 1391m, 1376w, 1364m, 1322w, 1300m, 1250m, 1183m, 1156w, 1104m, 1033m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.69 (d, 1H, J = 2.4 Hz, CH_a (30)), 4.56 (dd, 1H, J = 2.4, 1.5 Hz, CH_b (30)), 4.20–4.15 (m, 2H, OCH_2), 3.54 (d, 1H, J = 15.1 Hz, CH_a (31)), 3.41 (dd, 1H, J = 15.1 Hz, CH_b (31)), 3.32 (s, 3H, OMe), 2.93 (ddd, 1H, J = 11.2, 11.2, 4.4 Hz, CH (19)), 2.53 (dd, 1H, J = 11.7, 4.2 Hz, CHOMe (3)), 2.32 (ddd, 1H, J = 12.7, 12.2, 3.7 Hz, CH (13)), 2.08 (ddd, 1H, J = 14.2, 3.4, 2.9 Hz, CH_a (16)), 1.84 (ddd, 1H, J = 12.7, 8.3 Hz, CH_a (22)), 1.77–1.50 (m, 4H, CH_a (21)+CH_a (2)+CH_a (12)+CH_a (1)), 1.66 (s, 3H, CH_3 (29)), 1.50–1.13 (m, 14H, CH (18)+CH_b (16)+CH_2 (6)+CH_2 (11)+CH_b (22)+CH_b (2)+CH_b (21)+CH_2 (7)+CH_2 (15)+CH (9)), 1.24 (*t*, 3H, J = 7.0 Hz, CH$_2$CH_3), 1.02–0.93 (m, 2H, CH_b (12)), 0.93 (s, 3H, CH_3 (27)), 0.92 (s, 3H, CH_3 (23)), 0.88 (s, 3H, CH_3 (24)), 0.79 (s, 3H, CH_3 (26)), 0.81–0.72 (m, 1H, CH_b (1)), 0.71 (s, 3H, CH_3 (25)), 0.64 (d, 1H, J = 9.5 Hz, CH (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 206.3 (*C*=O), 167.8 (*C*=O), 150.4 (C20, *C*=CH$_2$), 109.5 (C30, *C*H$_2$=C), 88.6 (C3, *C*HOMe), 62.1 (C17, $C_{quart.}$), 61.2 (OCH$_2$), 57.4 (OMe), 55.9 (C5, *C*H), 50.6 (C9, *C*H), 49.4 (C18, *C*H), 45.9 (C19, *C*H), 44.7 (C31, *C*H$_2$), 42.5 (C14, $C_{quart.}$), 40.8 (C8, $C_{quart.}$), 38.7 (C4, $C_{quart.}$), 38.6 (C1, *C*H$_2$), 37.2 (C10, $C_{quart.}$), 37.1 (C13, *C*H), 35.0 (C22, *C*H$_2$), 34.3 (C7, *C*H$_2$), 31.4 (C16, *C*H$_2$), 30.2 (C21, *C*H$_2$), 29.6 (C15, *C*H$_2$), 28.0 (C23, *C*H$_3$), 25.6 (C12, *C*H$_2$), 22.2 (C2, *C*H$_2$), 20.9 (C11, *C*H$_2$), 19.3 (C29, *C*H$_3$), 18.1 (C6, *C*H$_2$), 16.1 (C24+C26, 2 × *C*H$_3$), 16.0 (C25, *C*H$_3$), 14.4 (C27, *C*H$_3$), 14.1 (CH$_2$*C*H$_3$) ppm; MS (ESI, MeOH): m/z 541.3 (15%, [M+H]$^+$), 563.4 (20%, [M+Na]$^+$), 1103.4 (100%, [2M+Na]$^+$); Anal. Calcd for $C_{35}H_{56}O_4$ (540.81): C, 77.73; H, 10.44. Found: C, 77.45; H, 10.29.

5.1.22. Ethyl-3-[3β-acetoxy-28-norlup-20(29)-*en*-17β-yl]-acrylate (15)

Compound **15** (0.31 g, 65%) was obtained from compound **7** (0.50 g, 0.88 mmol) and following GP6 as a colourless solid; mp 207–209 °C; [α_D^{20}] −7.7 (*c* 4.0, $CHCl_3$); R_F 0.73 (silica gel, hexane/

ethyl acetate, 9:1); IR (KBr): *ν* 2938s, 2873m, 1728s, 1643w, 1468m, 1450m, 1392m, 1366s, 1249s, 1178m, 1032m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): *δ* 7.23 (d, 1H, *J* = 16.1 Hz, C*H* (28)), 5.87 (d, 1H, *J* = 16.1 Hz, C*H* (31)), 4.70 (d, 1H, *J* = 2.5 Hz, CH_a (30)), 4.58 (dd, 1H, *J* = 1.2, 2.5 Hz, CH_b (30)), 4.44 (dd, 1H, *J* = 10.4, 5.8 Hz, C*H*OAc (3)), 4.18 (d*q*, 2H, *J* = 7.0, 1.0 Hz, OCH_2), 2.49 (ddd, 1H, *J* = 11.2, 11.0, 5.4 Hz, C*H* (19)), 2.01 (s, 3H, Ac), 1.91–1.81 (m, 2H, CH_a (21)+CH_a (16)), 1.72 (ddd, 1H, *J* = 12.7, 12.2, 3.7 Hz, C*H* (13)), 1.67 (s, 3H, CH_3 (29)), 1.67–1.51 (m, 8H, CH_a (12)+CH_a (1)+CH_a (22)+CH_a (15)+C*H* (18)+CH_2 (2)+CH_b (21)), 1.50–1.43 (m, 1H, CH_a (6)), 1.41–1.17 (m, 7H, CH_a (11)+CH_b (22)+CH_b (16)+CH_2 (7)+CH_b (6)+C*H* (9)), 1.29 (*t*, 3H, *J* = 7.0 Hz, CH_2CH_3), 1.22–1.14 (m, 1H, CH_b (11)), 1.11–1.02 (m, 1H, CH_b (12)), 1.03–0.89 (m, 2H, CH_b (15)+CH_b (1)), 0.95 (s, 3H, CH_3 (27)), 0.93 (s, 3H, CH_3 (25)), 0.82 (s, 6H, CH_3 (23)+CH_3 (24)), 0.81 (s, 3H, CH_3 (26)), 0.75 (d, 1H, *J* = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): *δ* 170.9 (*C*=O), 167.0 (*C*=O), 153.6 (C28, *C*H=), 149.7 (C20, *C*=CH_2), 120.3 (C31, *C*H=), 110.0 (C30, *C*H_2=C), 80.9 (C3, *C*HOAc), 60.3 (OCH_2), 55.4 (C5, *C*H), 50.3 (C9, *C*H), 49.9 (C17, $C_{quart.}$), 49.7 (C18, *C*H), 47.7 (C19, *C*H), 42.7 (C14, $C_{quart.}$), 40.8(C8, $C_{quart.}$), 38.7 (C13, *C*H), 38.6 (C22, CH_2), 38.3 (C1, CH_2), 37.7 (C4, $C_{quart.}$), 37.0 (C10, $C_{quart.}$), 34.2 (C7, CH_2), 33.4 (C21, CH_2), 29.8 (C16, CH_2), 27.9 (C23, CH_3), 27.7 (C15, CH_2), 25.2 (C12, CH_2), 23.6 (C2, CH_2), 21.3 (Ac, CH_3), 20.7 (C11, CH_2), 19.2 (C29, CH_3), 18.1 (C6, CH_2), 16.5 (C24, CH_3), 16.1 (C25, CH_3), 16.0 (C26, CH_3), 14.6 (C27, CH_3), 14.3 (CH_2CH_3) ppm; MS (ESI, MeOH): *m*/*z* 553.1 (10%, $[M+H]^+$), 575.3 (20%, $[M+Na]^+$), 1127.2 (100%, $[2M+Na]^+$); Anal. Calcd for $C_{36}H_{56}O_4$ (552.83): C, 78.21; H, 10.21. Found: C, 78.00; H, 10.18.

5.1.23. Ethyl-3-[3β-methoxy-28-norlup-20(29)-*en*-17β-yl]-acrylate (16)

Compound **16** (0.34 g, 71%) was obtained from compound **9** (0.50 g, 0.92 mmol) and following GP6 as a colourless solid; mp 185–190 °C; $[\alpha_D^{20}]$ −6.3 (*c* 3.9, $CHCl_3$); R_F 0.50 (silica gel, hexane/ethyl acetate, 9:1); IR (KBr): *ν* 2938s, 2837m, 1723s, 1646m, 1451m, 1392m, 1375m, 1364m, 1313s, 1248m, 1180s, 1100s, 1034m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): *δ* = 7.22 (d, 1H, *J* = 16.1 Hz, C*H* (28)), 5.87 (d, 1H, *J* = 16.1 Hz, C*H* (31)), 4.72 (d, 1H, *J* = 2.5 Hz, CH_a (30)), 4.58 (dd, 1H, *J* = 1.2, 2.5 Hz, CH_b (30)), 4.20 (*q*, 2H, *J* = 7.0 Hz, OCH_2), 3.33 (s, 3H, OMe), 2.61 (dd, 1H, *J* = 11.7, 4.3 Hz, C*H*OMe (3)), 2.50 (ddd, 1H, *J* = 11.2, 11.0, 5.1 Hz, C*H* (19)), 1.92–1.84 (m, 2H, CH_a (21)+CH_a (16)), 1.77–1.53 (m, 7H, C*H* (13)+CH_a (2)+CH_2 (22)+C*H* (18)+CH_a (12)+CH_a (15)), 1.68 (s, 3H, CH_3 (29)), 1.53–1.17 (m, 11H, CH_b (21)+CH_2 (6)+CH_2 (11)+CH_b (2)+CH_b (16)+CH_a (1)+CH_2 (7)+C*H* (9)), 1.30 (*t*, 3H, *J* = 7.0 Hz, CH_2CH_3), 1.13–1.05 (m, 2H, CH_b (12)+CH_b (15)), 0.96 (s, 3H, CH_3 (25)), 0.94 (s, 3H, CH_3 (27)), 0.93 (s, 3H, CH_3 (23)), 0.86–0.78 (m, 1H, CH_b (1)), 0.81 (s, 3H, CH_3 (24)), 0.73 (s, 3H, CH_3 (26)), 0.66 (d, 1H, *J* = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): *δ* 167.1 (*C*=O), 153.6 (C28, *C*H=), 151.4 (C20, *C*=CH_2), 120.3 (C31, *C*H=), 110.0 (C30, *C*H_2=C), 88.6 (C3, *C*HOMe), 60.2 (OCH_2), 57.4 (OMe), 55.8 (C5, *C*H), 50.4 (C9, *C*H), 50.0 (C17, $C_{quart.}$), 49.7 (C18, *C*H), 47.6 (C19, *C*H), 42.8 (C14, $C_{quart.}$), 40.8 (C8, $C_{quart.}$), 38.8 (C13, *C*H), 38.7 (C1, CH_2), 38.7 (C22, CH_2), 38.6 (C4, $C_{quart.}$), 37.2 (C10, $C_{quart.}$), 34.3 (C7, CH_2), 33.4 (C21, CH_2), 29.7 (C16, CH_2), 28.0 (C23, CH_3), 27.7 (C15, CH_2), 25.2 (C12, CH_2), 22.2 (C2, CH_2), 20.8 (C11, CH_2), 19.2 (C29, CH_3), 18.1 (C6, CH_2), 16.1 (C24, CH_3), 16.0 (C25, CH_3), 15.9 (C26, CH_3), 14.7 (C27, CH_3), 14.3 (CH_2CH_3) ppm; MS (ESI, MeOH): *m*/*z* 525.1 (20%, $[M+H]^+$), 547.4 (20%, $[M+Na]^+$), 1071.1 (100%, $[2M+Na]^+$); Anal. Calcd for $C_{36}H_{56}O_4$ (552.83): C, 80.10; H, 10.76. Found: C, 80.05; H, 10.8.

5.1.24. 2-((*RS*)-[3β-Acetoxy-28-norlup-20(29)-*en*-17β-yl]-3-oxomethyl)-γ-butyrolactone (17)

Compound **17** (50 mg, 11%) was obtained as a colourless solid from compound **11** (0.50 g, 0.88 mmol) following GP5. The following NMR data represent chemical shifts of the major isomer; mp 256–259 °C; $[\alpha_D^{20}]$ 16.3 (*c* 6.8, $CHCl_3$); R_F 0.33 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr): *ν* 2943s, 2874m, 1781s, 1728s, 1705m, 1642w, 1452m, 1392m, 1372m, 1247s, 1219m, 1155m, 1024m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): *δ* 4.69 (br s, 1H, CH_a (30)), 4.57 (br s, 1H, CH_b (30)), 4.55–4.45 (m, 1H, CH_a (33)), 4.44 (dd, 1H, *J* = 11.4, 4.8 Hz, C*H*OAc (3)), 4.30 (ddd, 1H, *J* = 8.7, 7.1 Hz, CH_b (33)), 4.04 (dd, 1H, *J* = 8.2, 8.0 Hz, C*H* (31)), 2.87 (ddd, 1H, *J* = 11.2, 10.4, 6.2 Hz, C*H* (19)), 2.46–2.23 (m, 4H, CH_2 (32)+C*H* (13)+CH_a (22)), 2.10 (ddd, 1H, *J* = 14.1, 3.0, 3.0 Hz, CH_a (21)), 2.01 (s, 3H, Ac), 1.87 (ddd, 1H, *J* = 11.2, 8.7, 1.7 Hz, CH_a (16)), 1.71–1.51 (m, 6H, CH_a (12)+C*H* (18)+CH_a (1)+CH_b (21)+CH_2 (2)), 1.65 (s, 3H, CH_3 (29)), 1.50–1.14 (m, 11H, CH_2 (6)+CH_2 (11)+CH_b (16)+CH_b (22)+CH_2 (7)+CH_2 (15)+C*H* (9)), 1.00–0.88 (m, 2H, CH_b (12)+CH_b (1)), 0.94 (s, 3H, CH_3 (27)), 0.86 (s, 3H, CH_3 (25)), 0.82 (s, 3H, CH_3 (24)), 0.81 (s, 3H, CH_3 (26)), 0.80 (s, 3H, CH_3 (23)), 0.75 (d, 1H, *J* = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): *δ* 209.4 (*C*=O), 174.2 (*C*=O), 170.9 (*C*=O), 150.3 (C20, *C*=CH_2), 109.5 (C30, CH_2=C), 80.8 (C3, *C*HOAc), 67.5 (C33, CH_2), 62.8 (C17, $C_{quart.}$), 55.4 (C5, *C*H), 50.5 (C9, *C*H), 49.3 (C18, *C*H), 47.3 (C31, *C*H), 46.0 (C19, *C*H), 42.6 (C14, $C_{quart.}$), 40.7 (C8, $C_{quart.}$), 38.4 (C1, CH_2), 37.8 (C4, $C_{quart.}$), 37.1 (C10, $C_{quart.}$), 37.0 (C13, *C*H), 34.3 (C7, CH_2), 33.6 (C22, CH_2), 31.2 (C21, CH_2), 30.2 (C16, CH_2), 29.4 (C15, CH_2), 28.8 (C32, CH_2), 27.9 (C23, CH_3), 25.5 (C12, CH_2), 23.7 (C2, CH_2), 21.3 (Ac, CH_3), 20.9 (C11, CH_2), 19.4 (C29, CH_3), 18.1 (C6, CH_2), 16.5 (C24, CH_3), 16.2 (C25, CH_3), 16.0 (C26, CH_3), 14.4 (C27, CH_3) ppm; MS (ESI, MeOH): *m*/*z* 565.7 (100%, $[M-H]^-$); Anal. Calcd for $C_{36}H_{54}O_4$ (550.81): C, 76.28; H, 9.60. Found: C, 76.15; H, 9.35.

5.1.25. 2-((*R*)-[3β-Acetoxy-28-norlup-20(29)-*en*-17β-yl]hydroxymethyl)-γ-butyrolactone (18)

Compound **18** (50 mg, 11%) was obtained as a colourless solid from compound **11** (0.50 g, 0.88 mmol) following GP6 with an extended reaction time of 120 h for the mesylation reaction; mp 295–299 °C; $[\alpha_D^{20}]$ −28.4 (*c* 3.2, $CHCl_3$); R_F 0.48 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr): *ν* 2945s, 1753s, 1736s, 1662w, 1639w, 1448w, 1383m, 1244s, 1189m, 1156w, 1030m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): *δ* 6.95 (s, 1H, C*H* (28)), 4.68 (d, 1H, *J* = 2.5 Hz, CH_a (30)), 4.55 (dd, 1H, *J* = 1.2, 2.5 Hz, CH_b (30)), 4.39 (dd, 1H, *J* = 11.4, 4.8 Hz, C*H*OAc (3)), 4.35–4.23 (m, 2H, CH_2 (33)), 2.95–2.80 (m, 2H, CH_2 (32)), 2.52 (ddd, 1H, *J* = 11.0, 10.8, 4.6 Hz, C*H* (19)), 2.02 (s, 3H, Ac), 1.97 (ddd, 1H, *J* = 10.2, 3.6, 2.8 Hz, CH_a (16)), 1.77 (ddd, 1H, *J* = 12.5, 12.5, 3.3 Hz, C*H* (13)), 1.69–1.46 (m, 7H, CH_a (21)+CH_a (22)+CH_a (12)+CH_a (1)+CH_2 (2)+C*H* (18)), 1.63 (s, 3H, CH_3 (29)), 1.45–1.11 (m, 8H, CH_2 (6)+CH_b (16)+CH_2 (11)+CH_b (21)+CH_a (15)+CH_b (22)+CH_2 (7)+C*H* (9)), 1.07–0.90 (m, 3H, CH_b (12)+CH_b (15)+CH_b (1)), 0.99 (s, 3H, CH_3 (27)), 0.83 (s, 3H, CH_3 (25)), 0.77 (s, 3H, CH_3 (24)), 0.77 (s, 6H, CH_3 (26)), 0.76 (s, 6H, CH_3 (23)), 0.71 (d, 1H, *J* = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): *δ* 172.2 (*C*=O), 170.9 (*C*=O), 149.5 (C20, *C*=CH_2), 146.1 (C28, *C*H=C), 124.1 (C31, C=*C*H), 110.1 (C30, *C*H_2=C), 80.9 (C3, *C*HOAc), 65.1 (C33, CH_2), 55.4 (C5, *C*H), 50.4 (C9, *C*H), 50.1 (C18, *C*H), 50.0 (C17, $C_{quart.}$), 47.3 (C19, *C*H), 42.2 (C14, $C_{quart.}$), 40.7 (C8, $C_{quart.}$), 38.9 (C13, *C*H), 38.4 (C1, CH_2), 37.8 (C4, $C_{quart.}$), 37.1 (C10, $C_{quart.}$), 37.0 (C22, CH_2), 34.2 (C7, CH_2), 31.3 (C16, CH_2), 30.1 (C21, CH_2), 27.9 (C23, CH_3), 28.8 (C15, CH_2), 25.9 (C32, CH_2), 25.3 (C12, CH_2), 23.7 (C2, CH_2), 21.2 (Ac, CH_3), 20.8 (C11, CH_2), 19.3 (C29, CH_3), 18.1 (C6, CH_2), 16.4 (C24, CH_3), 16.1 (C25, CH_3), 16.0 (C26, CH_3), 14.4 (C27, CH_3) ppm; MS (ESI, MeOH): *m*/*z* 550.3 (30%, $[M+H]^+$), 1123 (100%, $[2M+Na]^+$); Anal. Calcd for $C_{36}H_{54}O_4$ (550.81): C, 78.50; H, 9.88. Found: C, 78.37; H, 9.60.

5.1.26. (28 *R*)-3-Acetyl-28-(2-ethoxy-2-oxoethyl)allobetulin (19)

Compound **19** (0.20 g, 97%) was obtained from compound **7** (0.20 g, 0.35 mmol) following GP7 as a colourless solid; mp

210 °C; $[\alpha_D^{20}]$ 46.4 (*c* 4.9, $CHCl_3$); R_F 0.57 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr): *ν* 2944s, 2861m, 1727s, 1643w, 1450w, 1368m, 1250s, 1178w, 1140w, 1073w, 1026m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): *δ* 4.51 (dd, 1H, *J* = 8.7, 4.1 Hz, C*H* (28)), 4.46 (dd, 1H, *J* = 11.5, 4.7 Hz, C*H*OAc (3)), 4.20–4.10 (m, 2H, OCH_2), 3.51 (s, 1H, C*H* (19)), 2.63 (dd, 1H, *J* = 14.9, 8.7 Hz, CH_a (31)), 2.35 (dd, 1H, *J* = 14.9, 4.1 Hz, CH_b (31)), 2.03 (s, 3H, Ac), 1.73–1.42 (m, 3H, CH_a (1)+CH_a (21)+CH_a (12)+C*H* (18)+CH_2 (2)+CH_a (6)), 1.44–1.17 (m, 7H, CH_2 (22)+CH_a (15)+CH_a (11)+CH_b (21)+C*H* (13)+CH_2 (7)+CH_2 (16)+CH_b (6)+C*H* (9)), 1.26 (*t*, 3H, *J* = 7.0 Hz, CH_3), 1.08–0.91 (m, 4H, CH_b (11)+CH_b (15)+CH_b (1)+CH_b (12)), 0.98 (s, 3H, CH_3 (25)), 0.89 (s, 6H, CH_3 (27)+CH_3 (30)), 0.86 (s, 3H, CH_3 (24)), 0.83 (s, 3H, CH_3 (26)), 0.82 (s, 6H, CH_3 (23)), 0.80 (s, 3H, CH_3 (29)), 0.80 (d, 1H, *J* = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): *δ* 172.4 (*C*=O), 171.0 (*C*=O), 86.5 (C19, *C*H), 80.9 (C3, *C*HOAc), 75.4 (C28, *C*H), 62.4 (OCH_2), 55.5(C5, *C*H), 50.9 (C9, *C*H), 47.6 (C18, *C*H), 42.3 (C17, $C_{quart.}$), 41.1 (C14, $C_{quart.}$), 40.5 (C8, $C_{quart.}$), 38.6 (C1, CH_2), 37.8 (C4, $C_{quart.}$), 37.2 (C10, $C_{quart.}$), 35.7 (C20, $C_{quart.}$), 34.3 (C31, CH_2), 33.7 (C7, CH_2), 33.6 (C21, CH_2), 33.3 (C13, *C*H), 32.9 (C16, CH_2), 28.7 (C30, CH_3), 27.9 (C23, CH_3), 26.3 (C22, CH_2), 26.2 (C12, CH_2), 26.1 (C15, CH_2), 23.6 (C2, CH_2), 25.3 (C29, CH_3), 21.3 (Ac), 20.8 (C11, CH_2), 18.1 (C6, CH_2), 16.4 (C24+C26, 2 × CH_3), 15.7 (C25, CH_3), 14.2 (CH_2CH_3), 13.5 (C27, CH_3) ppm; MS (ESI, MeOH): *m*/*z* 571.4 (70%, $[M+H]^+$), 1163.3 (100%, $[2M+Na]^+$); Anal. Calcd for $C_{34}H_{56}O_4$ (528.80): C, 77.22; H, 10.67. Found: C, 77.10; H, 10.33.

5.1.27. (28*R*)-3-acetyl-28-(4-ethoxy-2,4-dioxobut-1-yl)allobetulin (20)

Compound **20** (0.20 g, 95%) was obtained from compound **8** (0.21 g, 0.35 mmol) following GP7 as a colourless solid; mp 184 °C; $[\alpha_D^{20}]$ 66.2 (*c* 3.0, $CHCl_3$); R_F 0.33 (silica gel, hexane/ethyl acetate, 9:1); IR (KBr):

5.2. Biology

5.2.1. 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 foetal bovine serum (Biochrom AG, Berlin, Germany) and penicillin/streptomycin (PAA Laboratories) at 37 °C in a humidified atmosphere of 5% CO_2/95% air.

5.2.2. Cytotoxicity assay

The cytotoxicity of the compounds was evaluated using the sulforhodamine-B (SRB) (Sigma–Aldrich) microculture colorimetric assay. 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.

5.2.3. Apoptosis test—dye exclusion test

Apoptotic cell death was analysed by trypan-blue dye (Sigma–Aldrich, Germany) on A431 and A2780 cell lines. The cell culture flasks with 70% to 80% confluence were treated with IC_{90} does 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 re-suspended in serum free media. Equal amounts of cell suspension and trypan-blue were mixed and analysed under a microscope. Viable cells exclude the dye and appear colourless whereas cells whose cell membrane is destroyed are stained in blue colour. If there are more colourless cells than stained cells, then death of the cells can be characterised as apoptotic.

5.2.4. Apoptosis test—DNA fragmentation assay

Determination of apoptotic cell death was performed by DNA gel electrophoresis. Briefly, cell lines A431 and A2780 were treated with respective IC_{90} doses of the compounds for 24 h. Floating cells—as induced by drug exposure—were collected, re-suspended 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 RT. The supernatant was collected and treated with RNAse A (3 μl, 1 mg/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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