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Synthesis and Antitumor Activity of Ring A-modified Glycyrrhetinic Acid Derivatives

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The pentacyclic triterpene glycyrrhetinic acid is an interesting natural product exhibiting various biological activities. Especially its ability to induce apoptosis in tumor cells is of high scientific interest. In this study we altered the lipophilicity in ring A by derivatization at positions C-2 and C-3. The consequences of these variations on the cytotoxicity were investigated applying a colorimetric sulforhodamine B assay using 8 different human tumor cell lines. An acridine orange/ethidium bromide (AO/EB) test and a trypan blue test were used to determine the extent of apoptotic activity of some of these compounds.

Key words: Glycyrrhetinic Acid, Antitumor Activity, Apoptosis

Introduction

Glycyrrhetinic acid (**1**, Fig. 1) belongs to the group of triterpenoic acids; this group of natural products shows some interesting biological activities. Most prominent members are betulinic and oleanoic acid. Both compounds were shown to be cytotoxic towards tumor cell lines in *in-vitro* [1 – 3] as well as in *in vivo* tests [4, 5]. Although **1** has a lower cytotoxicity than betulinic acid, **1** shows a similar apoptotic behavior [6 – 9]. Another advantage of **1** is its occurrence in well accessible plants; **1** can be isolated in high yields from the roots of licorice [10, 11].

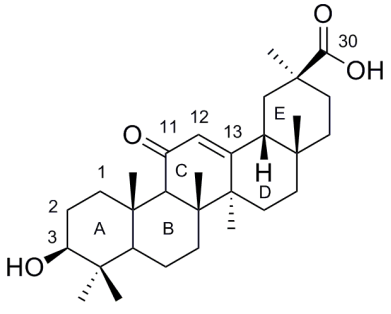


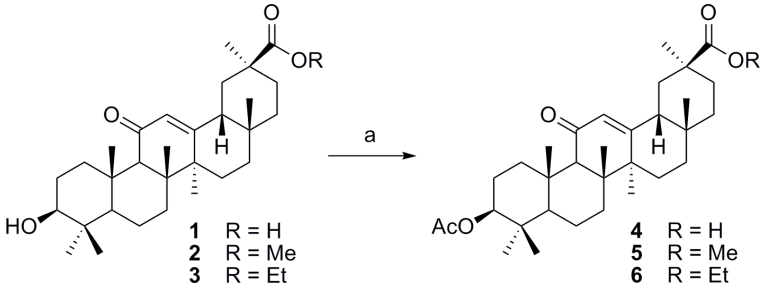
Fig. 1. Structure of glycyrrhetinic acid (**1**).

The parent structure of **1** displays three functionalities best suited for modifications: a carboxyl group in ring E at C-30, an α,β-unsaturated carbonyl function located in ring C and a hydroxyl group in ring A at position C-3. In this study several functional modifications were performed at C-2 and/or C-3 in ring A. The principal aim was to alter the lipophilicity of the molecule by these modifications and to investigate them by a sulforhodamine B assay to determine their IC_{50} values for 8 human tumor cell lines. To affirm an assumed apoptotic way of action, additional trypan blue and acridine orange/ethidium bromide (AO/EB) tests were performed.

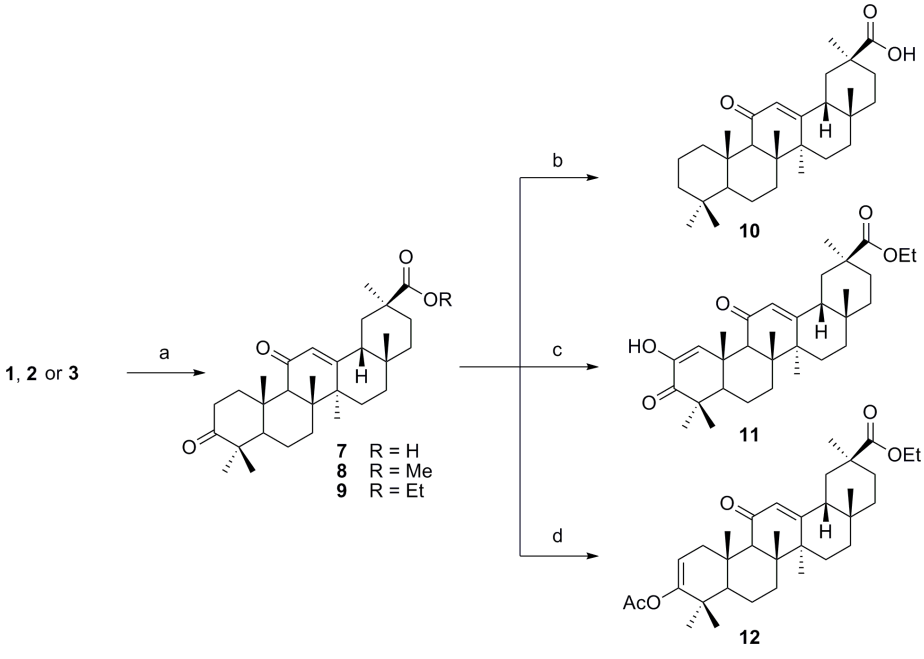
Results and Discussion

The methyl and ethyl esters of **1** (**2** and **3**, Scheme 1) were synthesized by known procedures [9, 12]; compounds **4** – **6**, being acetylated in position O-3, were synthesized in high yields by acetylating **1** or the esters **2** and **3** with acetyl chloride in the presence of pyridine.

Jones oxidation [13] of **1**, **2** or **3** at C-2 (Scheme 2) gave ketones **7** – **9**. Compound **9** is characterized in its ^{13}C NMR spectrum by a signal at δ = 216.1 ppm (C=O). The reaction of **9** with periodic acid in DMSO [14] afforded the 3-keto-2-enol **11** as the main product; in its ^{1}H NMR spectrum 1-H is detected at δ = 7.16 ppm. In the corresponding ^{13}C NMR spectrum carbons C-1, C-2, and C-3 are found at δ = 130.9, 143.3 and 198.8 ppm, respectively. While the reaction of **9** with glacial acetic acid in the presence of *p*-tolu-



Scheme 1. a) AcCl, pyridine, CH_2Cl_2, 25 °C, 2 h, 86 – 91 %.



Scheme 2. a) Jones reagent, 25 °C, 20 – 60 min, 78 – 86 %; b) KOH, hydrazine, ethylene glycol, 200 °C, 24 h, 45 %; c) periodic acid, DMSO, 50 °C, 3 d, 15 %; d) HOAc, *p*-TsOH, 80 °C, 24 h, 59 %.

enesulfonic acid gave **12**, the reaction of **9** applying Wolff-Kishner conditions [15] afforded **10**.

The reaction of **1**, **2** or **3** (Scheme 3) with methanesulfonyl chloride in dry pyridine or triethylamine gave the corresponding mesylates **13** – **15** in excellent yields.

An elimination reaction, however, occurred upon heating of **3** or of the mesylates **13** or **14** in dry DMF in the presence of a base, and products **16** – **18** were obtained although the yields of these reactions never exceeded 50 %. Applying Mitsunobu conditions [16], however, gave **18** in 90 % isolated yield. The 2,3-epoxide **19** was obtained from **18** by reaction with *m*-CPBA in dichloromethane. Reaction of **2** with 1,1′-thiocarbonyl-diimidazole [13] gave **20** whose reaction with AIBN gave **21** in 18 % yield.

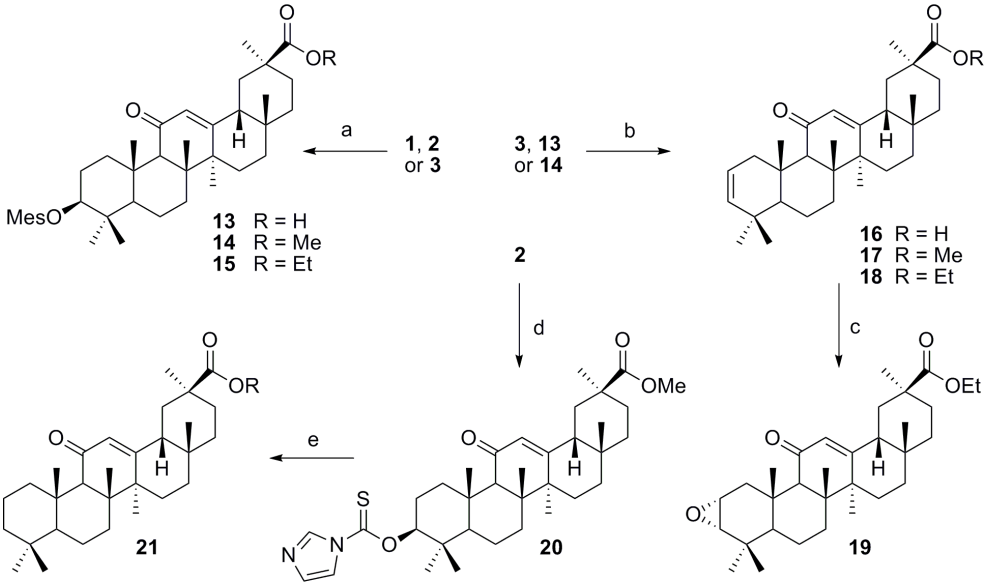
Thus, these variations in ring A afforded compounds differing significantly in lipophilicity compared to parent **1**. Although most of these compounds showed higher IC_{50} values than **1** in the corresponding SRB assays, a few of them gave IC_{50} values $< 30\ \mu$M. All acetates **4** – **6** and oxidized compounds **7** – **9** did not show any significant antitumor activity. With a few exceptions (*e. g.* **17** on A2780 cells), the mesylates **13** – **15** and deoxygenated compounds **16** – **18**, showed a similar behavior. Deoxidized **10** and **21** gave comparable results in this assay; for A2780 ovarian cancer cells, however, **21** showed a significantly higher activity than **10**. The derivatives **12** and **19** are almost inactive within the limits of our test (30 μM) whereas the cytotoxicity of **11** is comparable to that of **3**. Interestingly enough, **12** is cytotoxic especially against SW1736 human thyroid cancer cells (Table 1).

Two of these derivatives (**11** and **15**) were selected as suitable candidates to investigate their ability to induce apoptosis in A549 lung carcinoma cells using an

	518A2	8505C	A2780	A549	DLD-1	Lipo	MCF7	SW1736
1[a]	83.92	86.50	74.57	82.76	81.21	81.44	84.70	76.93
2[a]	27.54	26.07	25.54	23.50	26.12	20.47	22.14	34.82
3[a]	25.23	24.58	26.96	22.74	28.14	27.66	18.61	13.37
4 – 9	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30
10	18.33	19.28	28.83	> 30	> 30	28.74	21.87	16.56
11	29.82	27.69	14.84	26.62	29.56	24.80	28.68	27.00
12	> 30	> 30	> 30	> 30	> 30	> 30	> 30	13.24
13	> 30	29.42	> 30	> 30	> 30	> 30	> 30	29.40
14 – 16	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30
17	> 30	> 30	14.95	> 30	> 30	> 30	> 30	19.14
18, 19	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30
21	23.69	24.30	10.39	> 30	> 30	25.52	> 30	16.98

Table 1. Biological activity (IC_{50} in μM) of derivatives of **1** (error: ±5 %).

[a] Data from previous studies: see ref. [9].



Scheme 3. a) $MeSO_2Cl$, pyridine (or Et_3N for **15**), 25 °C, 1 – 70 h, 94 – 99 %; b) for **16**: K_2CO_3, DMF, 120 °C, 24 h, 44 %; for **17**: Bu_4NF, DMF, 102 °C, 4 d, 51 %; for **18**: PPh_3, 3,3-dimethyl glutarimide, DEAD, THF, 25 °C, 24 h, 82 %; c) *m*-CPBA, CH_2Cl_2, 25 °C, 20 h, 77 %; d) 1,1′-thiocarbonyl-diimidazole, 1,2-dichloroethane, 100 °C, 70 h, 70 %; e) Bu_3SnH, AIBN (cat.), toluene, 115 °C, 40 h, 18 %.

acridine orange/ethidium bromide (AO/EB) and a colorimetric trypan blue test. A quantitative trypan blue test gave evidence that 82 % of the dead cells previously treated with compound **11** (35 μM) had undergone apoptosis, compared to only 47 % of the cells treated with **15** (40 μM).

In this study we were able to synthesize a series of glycyrrhetinic acid derivatives differing in ring A. In summary, acetylated derivatives exhibited a lower cytotoxicity than the parent compound. This is in excellent agreement with previous findings for the corresponding betulinic or ursolic acid derivatives [17 – 19]. A similar trend was observed for oxidized derivatives **7 – 9** when compared to the corresponding analogs in the ursolic or oleanoic acid series [19], but a different behavior was established [20, 21] for betulinic acid derivatives.

Usually esters of triterpenoic acids are more cytotoxic than the corresponding acids [9] but this seems to be not necessarily true for all derivatives of **1**. Apoptotic behavior, however, is retained by-and-large in this series of compounds despite the changes in the substitution pattern in ring A.

Experimental Section

General methods

Reagents were bought from commercial suppliers and used without any further purification. NMR spectra were measured on Varian Gemini 200, Gemini 2000 or Unity 500

spectrometers at 27 °C with tetramethylsilane as an internal standard, δ values are given in ppm and J in Hz. Mass spectra were taken on a Finnigan MAT TSQ 7000 instrument (electron spray, voltage 4.5 kV, sheath gas nitrogen). IR spectra were recorded on a Perkin-Elmer FT-IR spectrometer Spectrum 1000, optical rotations on a Perkin-Elmer 341 polarimeter (1 cm micro cell, 20 °C), and UV/Vis spectra on a Perkin-Elmer unit, Lambda 14. Melting points were measured with a Leica hot stage microscope and are uncorrected. Elemental analysis was done on a Foss-Heraeus Vario EL unit. TLC was performed on silica gel (Merck 5554, detection by UV absorption). Solvents were dried before use according to usual procedures. Derivatives **2** and **3** were synthesized by known procedures [9, 12].

Cell lines and culture conditions

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

Cytotoxicity assay [22]

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 – 100 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 discarded, 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 was added to each well, and the absorbance was measured at 570 nm (using a 96 well plate reader, Tecan Spectra, Crailsheim, Germany). The IC_{50} was estimated by linear regression between the value before and after the 50 % line is crossed in a dose-response curve.

Apoptosis test – acridine orange/ethidium bromide (AO/EB)

Apoptotic cell death was analyzed by acridine orange/ethidium bromide dye using fluorescence microscopy on A549 cells. Approx. 500 000 cells were seeded in cell culture flasks and were allowed to grow for 24 h. The medium was removed, and the loaded medium was added. After 24 – 48 h, the supernatant medium was collected and centrifuged; the pellet was suspended in phosphate-buffer saline (PBS) and centrifuged again. The liquid was removed, and the pellet was suspended in PBS. After mixing the suspension with a solution of AO/EB, analysis was performed under a fluorescence microscope. While a green fluorescence shows apoptosis, a red colored nucleus indicates necrotic cells.

Apoptosis test – trypan blue cell counting

Approx. 500 000 cells (A549) were seeded in cell culture flasks and allowed to grow for 1 d. After removing the medium, the loaded medium was introduced, and the flasks were incubated for about 24 – 48 h. The supernatant medium was collected and centrifuged, and the cell pellet was suspended in PBS and centrifuged again. Equal amounts of a Trypan blue solution (0.4 % in phosphate buffer saline, pH = 7.2) and a suspension of the pellet in PBS were mixed and put on chamber slides (invitrogen™). An automatic cell counter (invitrogen™ countess® automated cell counter) was used for counting the cells, differentiating between cells with and without an intact cell membrane.

*(3β)-3-(Acetyloxy)-11-oxo-olean-12-en-30-oic acid (**4**)*

Compound **1** (1.01 g, 2.2 mmol) was dissolved in dry dichloromethane (50 mL) containing dry pyridine (5 mL). Acetyl chloride (0.28 mL, 3.94 mmol) was added, and the mixture was stirred at r. t. for 40 h. Aqueous workup and extraction with $CHCl_3$ (3 × 30 mL) gave crude **4** whose recrystallization from methanol yielded **4** (1.01 g, 91 %) as colorless crystals. M. p. 171 – 174 °C (lit.: 175 – 177 °C [23], 310 – 313 °C [24]). – R_f = 0.50 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 143.67° (c = 0.44, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log \varepsilon)$ = 250 nm (4.10). – IR (KBr): ν = 3424br, 2958m, 1870m, 1729s, 1706s, 1646s, 1451w, 1383m, 1329w, 1276m, 1259m, 1210w, 1144m, 1090w, 1031w cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.69 (s, 1 H, 12-H), 4.49 (dd, 1 H, J = 11.6, 4.6 Hz, 3-H), 2.77 (ddd, 1 H, J = 13.7, 3.3, 3.3 Hz, 1-H), 2.34 (s, 1 H, 9-H), 2.17 (dd, 1 H, J = 14.1, 3.7 Hz, 18-H), 2.02 (s, 3 H, Ac-Me), 2.01 (m, 1 H, 15-H), 1.97 (m, 1 H, 21-H), 1.91 (m, 1 H, 19-H), 1.81 (ddd, 1 H, J = 13.7, 13.7, 4.2 Hz, 16-H), 1.69 (ddd, 1 H, J = 13.3, 13.3, 3.3 Hz, 2-H), 1.67 (m, 1 H, 7-H), 1.64 (m, 1 H, 2′-H), 1.61 (m, 1 H, 19′-H), 1.59 (m, 1 H, 6-H), 1.43 (m, 1 H, 6′-H), 1.41 (m, 1 H, 22-H), 1.39 (m, 1 H, 22′-H), 1.36 (m, 1 H, 7′-H), 1.34 (s, 3 H, 27-H), 1.30 (m, 1 H, 21′-H), 1.20 (s,

3 H, 29-H), 1.18 (m, 1 H, 16′-H), 1.14 (s, 3 H, 25-H), 1.10 (s, 3 H, 26-H), 1.04 (m, 1 H, 1′-H), 1.00 (m, 1 H, 15′-H), 0.85 (s, 6 H, 23-H and 24-H), 0.81 (s, 3 H, 28-H), 0.78 (m, 1 H, 5-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.3 (C-11), 181.8 (C-30), 171.0 (Ac-COO), 169.4 (C-13), 128.4 (C-12), 80.6 (C-3), 61.7 (C-9), 55.0 (C-5), 48.2 (C-18), 45.4 (C-8), 43.8 (C-20), 43.2 (C-14), 40.8 (C-19), 38.8 (C-1), 38.0 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 30.9 (C-21), 28.5 (C-28), 28.4 (C-29), 28.0 (C-23), 26.5 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 21.3 (Ac-Me), 18.7 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25). – MS (ESI): m/z (%) = 513.5 (100) $[M+H]^+$, 535.5 (60) $[M+Na]^+$, 567.0 (69) $[M+MeOH+Na]^+$.

*Methyl (3β)-3-(acetyloxy)-11-oxo-olean-12-en-30-oate (**5**)*

Compound **2** (360 mg, 0.75 mmol) was dissolved in a mixture of dry dichloromethane (30 mL) and dry pyridine (2 mL), and acetylchloride (1.1 ml, 1.5 mmol) was added. After 2 h of stirring at r. t., the mixture was washed with water (30 mL). The aqueous layer was extracted with $CHCl_3$ (3 × 20 mL), and the extracts were dried (Na_2SO_4), filtered and evaporated to dryness. Recrystallization from methanol yielded **5** (340 mg, 86 %) as colorless crystals. M. p. 297 – 300 °C (lit.: 282 – 284 °C [24]). – R_f = 0.73 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 145.37° (c = 0.50, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 249 nm (4.07). – IR (KBr): ν = 3448br, 2951s, 2875 m, 2361w, 1734s, 1654s, 1618w, 1465m, 1390m, 1366m, 1318w, 1247s, 1216m, 1190m, 1155m, 1087w, 1028m, 987m cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.66 (s, 1 H, 12-H), 4.52 (dd, 1 H, J = 11.6, 4.8 Hz, 3-H), 3.69 (s, 3 H, Me), 2.80 (ddd, 1 H, J = 13.7, 3.6, 3.6 Hz, 1-H), 2.36 (s, 1 H, 9-H), 2.08 (m, 1 H, 18-H), 2.05 (s, 3 H, Ac-Me), 2.03 (m 1 H, 15-H), 1.99 (m, 1 H, 21-H), 1.93 (ddd, 1 H, J = 13.4, 3.8, 2.6 Hz, 19-H), 1.82 (ddd, 1 H, J = 13.7, 13.7, 4.7 Hz, 16-H), 1.71 (m, 1 H, 2-H), 1.68 (m, 1 H, 7-H), 1.63 (m, 1 H, 2′-H), 1.61 (dd, 1 H, J = 13.4, 13.4 Hz, 19′-H), 1.58 (m, 1 H, 6-H), 1.46 (m, 1 H, 6′-H), 1.43 (m, 1 H, 7′-H), 1.39 (m, 1 H, 22-H), 1.36 (s, 3 H, 27-H), 1.31 (m, 2 H, 22′-H and 21′-H), 1.18 (m, 1 H, 16′-H), 1.16 (s, 3 H, 25-H), 1.15 (s, 3 H, 29-H), 1.13 (s, 3 H, 26-H), 1.06 (m, 1 H, 1′-H), 1.01 (m, 1 H, 15′-H), 0.88 (s, 6 H, 24-H and 23-H), 0.81 (s, 3 H, 28-H), 0.80 (m, 1 H, 5-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.9 (C-30), 171.0 (Ac-COO), 169.2 (C-13), 128.5 (C-12), 80.6 (C-3), 61.7 (C-9), 55.0 (C-5), 51.8 (Me), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.8 (C-1), 38.0 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.0 (C-23), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 21.3 (Ac-Me), 18.7 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25). – MS (ESI): m/z (%) = 527.5 (24) $[M+H]^+$, 549.5 (20) $[M+Na]^+$, 581.0 (100) $[M+MeOH+Na]^+$, 812.3 (22) $[3\ M+2Na]^{2+}$, 1053.1 (12) $[2M+H]^+$, 1075.3 (44) $[2M+Na]^+$.

*Ethyl (3β)-3-(acetyloxy)-11-oxo-olean-12-en-30-oate (**6**)*

To a solution of **3** (215 mg, 0.43 mmol) in dry dichloromethane (50 mL) and dry pyridine (5 mL), acetyl chloride (0.8 mL, 1.1 mmol) was added and the mixture stirred for 2 h at r. t. Aqueous workup followed by extraction with $CHCl_3$ (3 × 20 mL), and recrystallization from methanol gave **6** (210 mg, 90 %) as colorless crystals. M. p. 217 – 220 °C; R_f = 0.70 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 135.14° (c = 0.40, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 250 nm (4.10). – IR (KBr): ν = 3442br, 2958s, 2874m, 1731s, 1654s, 1466w, 1390m, 1365m, 1316w, 1246s, 1215m, 1174m, 1152m, 1086w, 1027m, 1000w cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.64 (s, 1 H, 12-H), 4.51 (dd, 1 H, J = 11.6, 4.6 Hz, 3-H), 4.18 (dq, 1 H, J = 10.7, 7.2 Hz, Et-C*H*H′), 4.11 (dq, 1 H, J = 10.7, 7.2 Hz, Et-CH*H*′), 2.79 (ddd, 1 H, J = 13.5, 3.4, 3.4 Hz, 1-H), 2.35 (s, 1 H, 9-H), 2.09 (ddd, 1 H, J = 13.2, 4.1, 1.0 Hz, 18-H), 2.04 (s, 3 H, Ac-Me), 2.02 (ddd, 1 H, J = 13.5, 13.5, 4.7 Hz, 15-H), 1.98 (m, 1 H, 21-H), 1.92 (ddd, 1 H, J = 13.5, 4.1, 2.8 Hz, 19-H), 1.82 (ddd, 1 H, J = 13.5, 13.5, 4.7 Hz, 16-H), 1.71 (m, 1 H, 2-H), 1.68 (m, 1 H, 7-H), 1.62 (m, 1 H, 2′-H), 1.60 (dd, 1 H, J = 13.5, 13.5 Hz, 19′-H), 1.58 (m, 1 H, 6-H), 1.45 (m, 1 H, 6′-H), 1.40 (m, 1 H, 7′-H), 1.38 (m, 1 H, 22-H), 1.35 (s, 3 H, 27-H), 1.32 (m, 1 H, 22′-H), 1.31 (m, 1 H, 21′-H), 1.25 (t, 3 H, J = 7.2 Hz, Me), 1.17 (m, 1 H, 16′-H), 1.15 (s, 3 H, 25-H), 1.13 (s, 3 H, 29-H), 1.12 (s, 3 H, 26-H), 1.05 (ddd, 1 H, J = 13.5, 13.5, 3.8 Hz, 1-H′), 1.01 (m, 1 H, 15′-H), 0.87 (s, 6 H, 24-H and 23-H), 0.79 (s, 3 H, 28-H), 0.79 (m, 1 H, 5-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.4 (C-30), 171.0 (Ac-COO), 169.3 (C-13), 128.4 (C-12), 80.6 (C-3), 61.7 (C-9), 60.3 (Et-CH_2), 55.0 (C-5), 48.4 (C-18), 45.4 (C-8), 43.8 (C-20), 43.2 (C-14), 41.0 (C-19), 38.8 (C-1), 38.0 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.0 (C-23), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 21.3 (Ac-Me), 18.7 (C-26), 17.4 (C-6), 16.6 (C-24), 16.4 (C-25), 14.3 (Me). – MS (ESI): m/z (%) = 541.6 (100) $[M+H]^+$, 563.5 (27) $[M+Na]^+$, 595.1 (59) $[M+MeOH+Na]^+$, 1081.4 (72) ($[2M+H]^+$, 1103.4 (76) $[2M+Na]^+$, 1119.2 (56) $[2M+K]^+$. – $C_{34}H_{52}O_5$ (540.77): calcd. C 75.51, H 9.69; found C 75.27, H 9.81.

*3,11-Dioxo-olean-12-en-30-oic acid (**7**)*

To a solution of **1** (600 mg, 1.3 mmol) in acetone (100 mL), CrO_3 (150 mg, 1.5 mmol) in sulfuric acid (1 M, 15 mL) was added. The mixture was stirred at r. t. for 1 h, followed by the addition of ethanol (50 mL). The precipitate was filtered off, and the filtrate was evaporated to dryness. Recrystallization from methanol afforded **7** (470 mg, 78 %) as colorless crystals. M. p. 308 – 311 °C (lit.: > 310 °C [13], 311 – 313 °C [24], 308 – 310 °C [25]). – R_f = 0.48 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 172.84° (c = 0.49, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ =

250 nm (4.09). – IR (KBr): ν = 3435br, 2965s, 1728s, 1683s, 1645s, 1457w, 1386m, 1367w, 1347w, 1328w, 1279w, 1250w, 1206m, 1144s, 1110w, 1087m, 1028w cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.72 (s, 1 H, 12-H), 2.94 (ddd, 1 H, J = 13.3, 7.1, 4.2 Hz, 1-H), 2.61 (ddd, 1 H, J = 15.8, 7.1, 4.2 Hz, 2-H), 2.42 (s, 1 H, 9-H), 2.34 (ddd, 1 H, J = 15.8, 6.2, 4.2 Hz, 2′-H), 2.20 (dd, 1 H, J = 13.7, 3.3 Hz, 18-H), 2.02 (ddd, 1 H, J = 14.1, 14.1, 4.6 Hz, 15-H), 1.99 (m, 1 H, 21-H), 1.92 (ddd, 1 H, J = 13.7, 4.2, 2.5 Hz, 19-H), 1.84 (ddd, 1 H, J = 4.2, 13.7, 13.7 Hz, 16-H), 1.68 (ddd, 1 H, J = 11.5, 11.5, 5.5 Hz, 7-H), 1.60 (dd, 1 H, J = 13.7, 13.7 Hz, 19′-H), 1.55 (m, 1 H, 6-H), 1.51 (m, 1 H, 6′-H), 1.44 (m, 1 H, 7′-H), 1.42 (m, 1 H, 22-H), 1.41 (m, 1 H, 1′-H), 1.39 (m, 1 H, 22′-H), 1.36 (s, 3 H, 27-H), 1.35 (m, 1 H, 21′-H), 1.30 (m, 1 H, 5-H), 1.25 (s, 3 H, 25-H), 1.21 (s, 3 H, 29-H), 1.19 (m, 1 H, 16′-H), 1.15 (s, 3 H, 26-H), 1.08 (s, 3 H, 23-H), 1.04 (s, 3 H, 24-H), 1.02 (m, 1 H, 15′-H), 0.83 (s, 3 H, 28-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 217.2 (C-3), 199.6 (C-11), 181.9 (C-30), 169.8 (C-13), 128.4 (C-12), 61.0 (C-9), 55.4 (C-5), 48.2 (C-18), 47.7 (C-4), 45.3 (C-8), 43.8 (C-20), 43.3 (C-14), 40.9 (C-19), 39.7 (C-1), 37.7 (C-22), 36.7 (C-10), 34.2 (C-2), 32.1 (C-7), 31.9 (C-17), 30.9 (C-21), 28.5 (C-28), 28.4 (C-29), 26.5 (C-15), 26.4 (C-23), 26.4 (C-16), 23.3 (C-27), 21.4 (C-24), 18.8 (C-6), 18.5 (C-26), 15.6 (C-25). – MS (ESI): m/z (%) = 469.5 (40) $[M+H]^+$, 491.5 (3) $[M+Na]^+$, 522.9 (44) $[M+MeOH+Na]^+$, 937.3 (38) $[2M+H]^+$, 959.3 (100) $[2M+Na]^+$.

*Methyl 3,11-dioxo-olean-12-en-30-oate (**8**)*

A mixture of **7** (1.80 g, 3.84 mmol) and potassium carbonate (4.30 g, 31.1 mmol) in dry DMF (80 mL) was stirred at r. t. for 20 min. Iodomethane (3 mL, 48.2 mmol) was added, and stirring was continued for an additional 24 h. The solvent was removed under reduced pressure and the residue suspended in water (40 mL). The suspension was extracted with dichloromethane (3 × 30 mL), and the combined extracts were washed with brine (20 mL), dried (Na_2SO_4), filtered and evaporated. Recrystallization from methanol gave **8** (1.60 g, 86 %) as colorless crystals. M. p. 244 – 246 °C (lit.: 242 – 243 °C [24], 248 – 250 °C [25]). – R_f = 0.60 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 172.95° (c = 0.31, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log \varepsilon)$ = 249 nm (4.00). – IR (KBr): ν = 3428br, 2962s, 2874s, 1725s, 1706s, 1655s, 1616m, 1540w, 1457m, 1427m, 1386m, 1366m, 1318m, 1280m, 1245m, 1220s, 1181s, 1153s, 1110m, 1087m, 1048w, 1029m, 997m, 986m cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.70 (s, 1 H, 12-H), 3.69 (s, 3 H, Me), 2.97 (ddd, 1 H, J = 13.6, 7.1, 4.1 Hz, 1-H), 2.64 (ddd, 1 H, J = 18.3, 11.2, 7.1 Hz, 2-H), 2.44 (s, 1 H, 9-H), 2.36 (ddd, 1 H, J = 15.8, 6.5, 4.0 Hz, 2′-H), 2.11 (dd, 1 H, J = 13.4, 3.8 Hz, 18-H), 2.04 (ddd, 1 H, J = 13.7, 13.7, 4.3 Hz, 15-H), 2.00 (m, 1 H, 21-H), 1.93 (m, 1 H, J = 13.5, 4.2, 2.7 Hz, 19-H), 1.85 (ddd, 1 H, J = 13.4, 13.4, 4.1 Hz, 16-H), 1.68 (m, 1 H, 7-H), 1.61 (dd, 1 H, J = 13.5, 13.5 Hz, 19′-H), 1.58 (m, 1 H, 6-H), 1.55 (m, 1 H, 6′-H), 1.46 (m, 1 H, J = 12.7, 3.1, 3.1 Hz, 7′-H), 1.43 (m, 1 H, 1′-H), 1.39 (m, 1 H, 22-H), 1.37 (s, 3 H, 27-H), 1.32 (m, 2 H, 21′-H and 22′-H), 1.28 (m, 1 H, 5-H), 1.27 (s, 3 H, 25-H), 1.21 (m, 1 H, 16′-H), 1.17 (s, 3 H, 26-H), 1.15 (s, 3 H, 29-H), 1.11 (s, 3 H, 23-H), 1.07 (s, 3 H, 24-H), 1.03 (m, 1 H, 15′-H), 0.82 (s, 3 H, 28-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 217.0 (C-3), 199.3 (C-11), 176.8 (C-30), 169.6 (C-13), 128.5 (C-12), 61.1 (C-9), 55.5 (C-5), 51.8 (Me), 48.5 (C-18), 47.8 (C-4), 45.3 (C-8), 43.8 (C-20), 43.4 (C-14), 41.3 (C-19), 39.8 (C-1), 37.8 (C-22), 36.8 (C-10), 34.3 (C-2), 32.2 (C-7), 31.9 (C-17), 31.1 (C-21), 28.6 (C-28), 28.4 (C-29), 26.6 (C-23), 26.5 (C-15), 26.5 (C-16), 23.4 (C-27), 21.5 (C-24), 18.9 (C-6), 18.6 (C-26), 15.7 (C-25). – MS (ESI): m/z (%) = 483.5 (66) $[M+H]^+$, 505.4 (6) $[M+Na]^+$, 523.2 (16) $[M+Na+H_2O]^+$, 537.1 (98) $[M+Na+MeOH]^+$, 746.4 (26) $[3M+2Na]^{2+}$, 965.3 (44) $[2M+H]^+$, 987.3 (100) $[2M+Na]^+$.

*Ethyl 3,11-dioxo-olean-12-en-30-oate (**9**)*

Compound **3** (1.03 g, 2.1 mmol) was dissolved in acetone (180 mL), followed by the addition of CrO_3 (227 mg, 2.3 mmol) in diluted sulfuric acid (15 mL). The solution was stirred at r. t. for 70 min. Ethanol (50 mL) was added, and the precipitate was filtered off. The filtrate was evaporated to dryness, and recrystallization from methanol gave **9** (820 mg, 80 %) as colorless crystals. M. p. 138 – 142 °C; R_f = 0.63 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 165.96° (c = 0.52, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log \varepsilon)$ = 249 nm (4.11). – IR (KBr): ν = 3427br, 2974s, 2945s, 2866m, 1723s, 1703s, 1654s, 1616w, 1466m, 1387m, 1365w, 1324w, 1280w, 1258w, 1219m, 1175s, 1155m, 1110w, 1087m, 1030w cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.66 (s, 1 H, 12-H), 4.16 (dq, 1 H, J = 10.8, 7.1 Hz, Et-C*H*H′), 4.10 (dq, 1 H, J = 10.8, 7.1 Hz, Et-CH*H*′), 2.94 (ddd, 1 H, J = 13.7, 6.6, 4.2 Hz, 1-H), 2.61 (ddd, 1 H, J = 15.8, 11.2, 7.1 Hz, 2-H), 2.41 (s, 1 H, 9-H), 2.33 (ddd, 1 H, J = 15.8, 6.6, 4.2 Hz, 2′-H), 2.11 (dd, 1 H, J = 13.3, 3.7 Hz, 18-H), 2.01 (ddd, 1 H, J = 13.7, 13.7, 4.2 Hz, 15-H), 1.97 (m, 1 H, 21-H), 1.90 (m, 1 H, 19-H), 1.82 (ddd, 1 H, J = 13.7, 13.7, 4.2 Hz, 16-H), 1.65 (m, 1 H, 7-H), 1.58 (dd, 1 H, J = 13.3, 13.3 Hz, 19′-H), 1.53 (m, 1 H, 6-H), 1.51 (m, 1 H, 6′-H), 1.44 (m, 1 H, 7′-H), 1.39 (m, 1 H, 1′-H), 1.36 (m, 1 H, 22-H), 1.35 (s, 3 H, 27-H), 1.32 (m, 1 H, 22′-H), 1.30 (m, 1 H, 21′-H), 1.26 (m, 1 H, 5-H), 1.24 (s, 3 H, 25-H), 1.18 (m, 1 H, 16′-H), 1.14 (s, 3 H, 26-H), 1.12 (s, 3 H, 29-H), 1.08 (s, 3 H, 23-H), 1.04 (s, 3 H, 24-H), 1.01 (m, 1 H, 15′-H), 0.79 (s, 3 H, 28-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 216.1 (C-3), 199.4 (C-11), 176.3 (C-30), 169.8 (C-13), 128.4 (C-12), 61.1 (C-9), 60.3 (Et-CH_2), 55.4 (C-5), 48.4 (C-18), 47.8 (C-4), 45.2 (C-8), 43.8 (C-20), 43.3 (C-14), 41.1 (C-19), 39.7 (C-1), 37.7 (C-22), 36.7 (C-10), 34.2 (C-2), 32.1 (C-7), 31.8 (C-17), 31.1 (C-21), 28.6 (C-28), 28.3 (C-29), 26.5

(C-23), 26.4 (C-15), 26.4 (C-16), 23.3 (C-27), 21.4 (C-24), 18.8 (C-6), 18.5 (C-26), 15.6 (C-25), 14.3 (Et-Me). – MS (ESI): m/z (%) = 497.5 (100) $[M+H]^+$, 519.4 (3) $[M+Na]^+$, 551.0 (45) $[M+MeOH+Na]^+$, 767.9 (6) $[3M+2Na+H]^{2+}$, 993.3 (74) $[2M+H]^+$, 1015.3 (52) $[2M+Na]^+$. – $C_{30}H_{44}O_4$ (468.67): calcd. C 76.88, H 9.46; found C 76.66, H 9.63.

*11-Oxo-olean-12-en-30-oic acid (**10**)*

To a mixture of **9** (797 mg, 1.6 mmol) and potassium hydroxide (778 mg, 13.9 mmol) in dry diethylene glycol (20 mL), hydrazine hydrate (80 %, 1.2 mL, 18.4 mmol) was added dropwise. After 24 h of stirring at 200 °C the mixture was cooled, water (50 mL) was added, and the mixture was extracted with dichloromethane (3 × 20 mL). The combined organic layers were dried (Na_2SO_4), filtered and evaporated to dryness. Purification by chromatography (silica gel, dichloromethane / methanol / ammonia 90 : 10 : 1) gave **10** (330 mg, 45 %) as a colorless powder. M. p. 288 – 292 °C (lit.: 298 – 300 °C [13]). – R_f = 0.60 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 99.56° (c = 0.47, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 248 nm (4.00). – IR (KBr): ν = 3425br, 2947s, 1729m, 1701s, 1662s, 1459m, 1387m, 1366w, 1216w, 1113w, 1035w cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.58 (d, 1 H, J = 1.7 Hz, 12-H), 2.60 (m, 1 H, 1-H), 2.30 (s, 1 H, 9-H), 2.23 (ddd, 1 H, J = 12.5, 2.9, 2.1 Hz, 18-H), 1.96 (m, 2 H, 15-H and 16-H), 1.70 (m, 1 H, 21-H), 1.65 (m, 1 H, 7-H), 1.58 (m, 1 H, 21′-H), 1.50 (m, 1 H, 22-H), 1.48 (m, 1 H, 6-H), 1.47 (m, 1 H, 16′-H), 1.46 (m, 1 H, 6′-H), 1.45 (m, 1 H, 19-H), 1.44 (m, 1 H, 7′-H), 1.43 (m, 1 H, 22′-H), 1.39 (m, 1 H, 3-H), 1.38 (m, 1 H, 19′-H), 1.36 (m, 1 H, 2-H), 1.34 (s, 3 H, 29-H), 1.32 (m, 1 H, 2′-H), 1.25 (m, 1 H, 15′-H), 1.25 (s, 3 H, 27-H), 1.19 (s, 3 H, 25-H), 1.15 (m, 1 H, 3-H′), 1.13 (s, 3 H, 26-H), 0.85 (s, 3 H, 23-H), 0.82 (s, 3 H, 28-H), 0.71 (s, 3 H, 24-H), 0.70 (m, 1 H, 5-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.6 (C-11), 183.1 (C-30), 165.6 (C-13), 124.1 (C-12), 60.8 (C-9), 55.8 (C-5), 45.0 (C-8), 44.1 (C-20), 42.3 (C-14), 42.0 (C-3), 40.9 (C-1), 40.4 (C-18), 37.6 (C-22), 37.1 (C-10), 35.9 (C-19), 35.5 (C-4), 33.8 (C-7), 33.5 (C-23), 33.3 (C-17), 31.6 (C-21), 28.3 (C-16), 26.6 (C-15), 21.9 (C-28), 20.7 (C-29), 20.5 (C-27), 18.6 (C-26), 18.4 (C-2), 17.8 (C-6), 16.4 (C-25), 16.0 (C-24). – MS (ESI): m/z (%) = 455.5 (16) ($[M+H]^+$, 495.2 (6) $[M+H_2O+Na]^+$, 509.1 (10) $[M+MeOH+Na]^+$, 909.4 (100) $[2M+H]^+$, 931.4 (40) $[2M+Na]^+$, 947.3 (6) $[2M+K]^+$.

*Ethyl 2-hydroxy-3,11-dioxo-olean-1,12-dien-30-oate (**11**)*

To a solution of **9** (1.07 g, 2.10 mmol) in dry DMSO (25 mL), periodic acid (580 mg, 3.3 mmol) was added. The mixture was stirred at 50 °C for 3 d and then poured into water (30 mL). The aqueous layer was extracted with dichloromethane (3 × 20 mL), and the combined extracts were dried (Na_2SO_4), filtered and evaporated. Purification by chromatography (silica gel, chloroform / ether 9 : 1) gave **11** (160 mg, 15 %) as a slightly yellow powder. M. p. 140 – 144 °C (decomp.). – R_f = 0.23 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 193.38° (c = 0.30, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 253 nm (4.12). – IR (KBr): ν = 3438br, 2977s, 2361w, 1726s, 1660s, 1465m, 1387m, 1314w, 1282w, 1217m, 1153m, 1087w, 1058m, 1030w, 879m, 756s cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 7.16 (s, 1 H, 1-H), 5.73 (s, 1 H, 12-H), 4.19 (dq, 1 H, J = 10.8, 7.2 Hz, Et-C*H*H′), 4.15 (dq, 1 H, J = 10.8, 7.2 Hz, Et-CH*H*′), 2.73 (s, 1 H, 9-H), 2.15 (dd, 1 H, J = 13.0, 4.1 Hz, 18-H), 2.05 (ddd, 1 H, J = 13.4, 13.4, 4.1 Hz, 15-H), 2.01 (m, 1 H, 21-H), 1.93 (ddd, 1 H, J = 13.5, 4.0, 2.7 Hz, 19-H), 1.85 (ddd, 1 H, J = 13.5, 13.5, 4.5 Hz, 16-H), 1.73 (m, 1 H, 7-H), 1.61 (dd, 1 H, J = 13.5, 13.5 Hz, 19′-H), 1.60 (m, 1 H, 6-H), 1.59 (m, 1 H, 5-H), 1.49 (m, 1 H, 6′-H), 1.48 (s, 3 H, 26-H), 1.43 (m, 1 H, 7′-H), 1.39 (m, 1 H, 22-H), 1.38 (s, 3 H, 27-H), 1.34 (m, 2 H, 22′-H and 21′-H), 1.28 (t, 3 H, J = 7.1 Hz, Me), 1.24 (s, 3 H, 29-H), 1.19 (m, 1 H, 16′-H), 1.18 (s, 3 H, 25-H), 1.15 (s, 3 H, 23-H), 1.15 (s, 3 H, 24-H), 1.04 (m, 1 H, 15′-H), 0.83 (s, 3 H, 28-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.4 (C-11), 198.8 (C-3), 176.3 (C-30), 170.3 (C-13), 143.3 (C-2), 130.9 (C-1), 128.1 (C-12), 60.3 (Et-CH_2), 56.9 (C-9), 53.2 (C-5), 48.5 (C-18), 44.0 (C-4), 45.5 (C-8), 43.8 (C-20), 43.5 (C-14), 41.1 (C-19), 37.8 (C-22), 37.7 (C-10), 32.2 (C-7), 31.8 (C-17), 31.1 (C-21), 28.6 (C-28), 28.3 (C-23), 26.9 (C-29), 26.5 (C-15), 26.3 (C-16), 23.4 (C-27), 21.7 (C-24), 20.8 (C-26), 19.0 (C-25), 18.0 (C-6), 14.3 (Et-Me). – MS (ESI): m/z (%) = 511.3 (100) $[M+H]^+$, 533.3 (18) $[M+Na]^+$, 564.7 (46) $[M+MeOH+Na]^+$. – $C_{32}H_{46}O_5$ (510.70): calcd. C 75.26, H 9.08; found C 75.98, H 9.24.

*Ethyl (3β)-3-(acetyloxy)-11-oxo-olean-2,12-dien-30-oate (**12**)*

Compound **9** (990 mg, 1.99 mmol) was dissolved in glacial acetic acid (15 mL) and heated to 80 °C. *p*-TsOH (40 mg, 0.23 mmol) was added, and stirring was continued for an additional 24 h. The mixture was cooled and diluted with dichloromethane (30 mL). After washing with water (20 mL), the aqueous layer was extracted with dichloromethane (3 × 30 mL), and the extracts were dried (Na_2SO_4), filtered and evaporated to dryness. Chromatographic purification (silica gel, chloroform / ether 9 : 1) afforded **12** (630 mg, 59 %) as a colorless powder. M. p. 225 – 228 °C; R_f = 0.73 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 191.71° (c = 0.36, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 249 nm (4.08). – IR (KBr): ν = 3431br, 2977s, 2874s, 2840m, 1751s, 1725s, 1657s, 1623m, 1457m, 1388s, 1364s, 1325m, 1281m, 1259s, 1217s, 1160s, 1101m, 1083s, 1030m, 980m cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.67 (s, 1 H, 12-H), 5.16 (dd, 1 H, J = 6.8, 1.9 Hz, 2-H), 4.17 (dq, 1 H, J = 10.8, 7.1 Hz, Et-C*H*H′), 4.13 (dq, 1 H, J = 10.8, 7.1 Hz, Et-CH*H*′), 3.20 (dd, 1 H, J = 17.4, 6.8 Hz, 1-H), 2.42 (s, 1 H, 9-H), 2.14 (s, 3 H, Ac-Me), 2.12 (m, 1 H,

18-H), 2.03 (ddd, 1 H, J = 13.6, 13.6, 4.7 Hz, 15-H), 1.98 (m, 1 H, 21-H), 1.92 (ddd, 1 H, J = 13.6, 4.2, 2.5 Hz, 19-H), 1.85 (m, 1 H, 1′-H), 1.83 (ddd, 1 H, J = 13.0, 13.0, 5.0 Hz, 16-H), 1.67 (m, 1 H, 7-H), 1.60 (dd, 1 H, J = 13.5, 13.5 Hz, 19′-H), 1.53 (m, 1 H, 6-H), 1.47 (m, 1 H, 6′-H), 1.44 (m, 1 H, 7′-H), 1.37 (m, 1 H, 22-H), 1.35 (s, 3 H, 27-H), 1.32 (m, 2 H, 21′-H and 22′-H), 1.26 (t, 3 H, J = 7.1 Hz, Me), 1.22 (s, 3 H, 25-H), 1.21 (m, 1 H, 16′-H), 1.15 (s, 3 H, 26-H), 1.13 (s, 3 H, 29-H), 1.03 (s, 3 H, 23-H), 1.01 (m, 1 H, 15′-H), 0.95 (s, 3 H, 23-H), 0.81 (s, 3 H, 28-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.6 (C-11), 176.3 (C-30), 169.8 (Ac-COO), 169.6 (C-13), 151.3 (C-3), 128.5 (C-12), 112.4 (C-2), 60.3 (C-9), 60.3 (Et-CH_2), 52.5 (C-5), 48.4 (C-18), 45.0 (C-8), 43.8 (C-20), 43.3 (C-14), 41.2 (C-19), 40.1 (C-1), 37.7 (C-22), 37.4 (C-4), 36.1 (C-10), 31.8 (C-7), 31.8 (C-17), 31.1 (C-21), 28.6 (C-28), 28.3 (C-29), 27.8 (C-23), 26.5 (C-16), 26.4 (C-15), 23.2 (C-27), 21.0 (Ac-Me), 19.8 (C-24), 18.6 (C-6), 18.2 (C-26), 16.0 (C-25), 14.3 (Et-Me). – MS (ESI): m/z (%) = 539.5 (88) $[M+H]^+$, 593.0 (100) $[M+Na+MeOH]^+$, 831.5 (10) $[3M+2Na+H]^+$, 1099.2 (42) $[2M+Na]^+$. – $C_{34}H_{50}O_5$ (538.76): calcd. C 75.80, H 9.35; found C 75.62, H 9.55.

*(3β)-3[(Methylsulfonyl)oxy]-11-oxo-olean-12-en-30-oic acid (**13**)*

Compound **1** (527 mg, 1.2 mmol) was dissolved in dry pyridine (15 mL), methanesulfonyl chloride (120 μL, 1.6 mmol) was added, and the mixture was stirred at r. t. for 70 h. Usual workup gave **13** (600 mg, 99 %) as a slightly yellow powder. M. p. 156 – 158 °C; R_f = 0.37 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 118.89° (c = 0.48, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 250 nm (4.13). – IR (KBr): ν = 3427br, 2955s, 1792w, 1707m, 1653s, 1467m, 1389m, 1334s, 1263w, 1209m, 1172s, 1088w, 1016w, 984w, 932m, 914s, 881m, 836w, 753m cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.69 (s, 1 H, 12-H), 4.36 (dd, 1 H, J = 11.9, 4.9 Hz, 3-H), 3.00 (s, 3 H, Mes-Me), 2.85 (ddd, 1 H, J = 13.9, 3.2, 3.2 Hz, 1-H), 2.32 (s, 1 H, 9-H), 2.18 (dd, 1 H, J = 13.6, 3.5 Hz, 18-H), 2.03 (ddd, 1 H, J = 13.4, 13.4, 4.3 Hz, 15-H), 1.99 (m, 1 H, 21-H), 1.94 (m, 1 H, 19-H), 1.87 (m, 1 H, 2-H), 1.82 (ddd, 1 H, J = 13.3, 13.3, 4.0 Hz, 16-H), 1.65 (m, 1 H, 2-H), 1.64 (m, 1 H, 7-H), 1.60 (dd, 1 H, J = 13.6, 13.6, 19′-H), 1.59 (m, 1 H, 2′-H), 1.48 (m, 1 H, 22-H), 1.45 (m, 1 H, 6-H), 1.41 (m, 1 H, 6′-H), 1.40 (m, 1 H, 22′-H), 1.35 (s, 3 H, 27-H), 1.20 (m, 1 H, 7′-H), 1.19 (m, 1 H, 21′-H), 1.15 (s, 3 H, 29-H), 1.14 (m, 1 H, 16′-H), 1.11 (s, 3 H, 25-H), 1.03 (s, 3 H, 26-H), 1.02 (m, 2 H, 1′-H and 15′-H), 0.87 (s, 6 H, 23-H and 24-H), 0.82 (s, 3 H, 28-H), 0.79 (m, 1 H, 5-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 181.7 (C-30), 169.7 (C-13), 128.3 (C-12), 90.2 (C-3), 61.5 (C-9), 55.2 (C-5), 48.2 (C-18), 45.4 (C-8), 43.8 (C-20), 43.2 (C-14), 40.8 (C-19), 39.0 (C-4), 39.0 (C-1), 38.8 (Mes-Me), 37.7 (C-22), 36.7 (C-10), 32.6 (C-7), 31.8 (C-17), 30.9 (C-21), 28.5 (C-28), 28.4 (C-29), 28.2 (C-23), 26.4 (C-16), 26.3 (C-15), 25.0 (C-2), 23.3 (C-27), 18.6 (C-26), 17.5 (C-6), 16.4 (C-24), 16.3 (C-25). – MS (ESI): m/z (%) = 549.5 (20) $[M+H]^+$, 571.2 (100) $[M+Na]^+$, 845.9 (24) $[3M+2Na]^{2+}$, 1097.2 (38) $[2M+H]^+$, 1119.7 (62) $[2M+Na]^+$. – $C_{32}H_{50}O_6S$ (562.80): calcd. C 68.29, H 8.95; found C 69.99, H 9.01.

*Methyl (3β)-3[(methylsulfonyl)oxy]-11-oxo-olean-12-en-30-oate (**14**)*

Following the procedure given for the preparation of **13**, from **2** (522 mg, 1.1 mmol), dry pyridine (15 mL) and methanesulfonyl chloride (126 μL, 1.1 mmol) **14** (623 mg, 96 %) was obtained as a colorless powder. M. p. 165 – 168 °C (lit.: 169 – 170 °C [26]). – R_f = 0.50 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 123.20° (c = 0.57, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 250 nm (4.06). – IR (KBr): ν = 3428br, 2953s, 2874m, 1724s, 1651s, 1618w, 1467m, 1413w, 1389m, 1358s, 1325m, 1277w, 1264w, 1247w, 1222m, 1192m, 1174s, 1089w, 1030w, 931m, 879s, 838m cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.66 (s, 1 H, 12-H), 4.35 (dd, 1 H, J = 11.8, 5.0 Hz, 3-H), 3.67 (s, 3 H, Me) 3.00 (s, 3 H, Mes-Me), 2.85 (ddd, 1 H, J = 13.9, 3.2, 3.2 Hz, 1-H), 2.33 (s, 1 H, 9-H), 2.06 (dd, 1 H, J = 13.6, 3.5 Hz, 18-H), 2.02 (ddd, 1 H, J = 13.4, 13.4, 4.3 Hz, 15-H), 1.99 (m, 1 H, 21-H), 1.94 (m, 1 H, 19-H), 1.87 (m, 1 H, 2-H), 1.82 (ddd, 1 H, J = 13.3, 13.3, 4.0 Hz, 16-H), 1.65 (m, 1 H, 2-H), 1.64 (m, 1 H, 7-H), 1.60 (dd, 1 H, J = 13.6, 13.6 Hz, 19′-H), 1.59 (m, 1 H, 2′-H), 1.48 (m, 1 H, 22-H), 1.46 (m, 1 H, 6-H), 1.43 (m, 1 H, 6′-H), 1.40 (m, 1 H, 22′-H), 1.34 (s, 3 H, 27-H), 1.20 (m, 1 H, 7′-H), 1.19 (m, 1 H, 21′-H), 1.15 (s, 3 H, 29-H), 1.13 (m, 1 H, 16′-H), 1.11 (s, 3 H, 25-H), 1.03 (s, 3 H, 26-H), 1.02 (m, 2 H, 1′-H and 15-H), 0.87 (s, 6 H, 23-H and 24-H), 0.79 (s, 3 H, 28-H), 0.79 (m, 1 H, 5-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.5 (C-11), 176.7 (C-30), 169.2 (C-13), 128.4 (C-12), 90.2 (C-3), 61.7 (C-9), 55.5 (C-5), 51.8 (Me), 48.6 (C-18), 45.5 (C-8), 44.2 (C-20), 43.4 (C-14), 41.3 (C-19), 39.2 (Mes-Me), 39.0 (C-4), 39.0 (C-1), 37.9 (C-22), 37.0 (C-10), 32.8 (C-7), 32.0 (C-17), 31.3 (C-21), 28.7 (C-28), 28.4 (C-29), 28.4 (C-23), 26.7 (C-16), 26.6 (C-15), 25.2 (C-2), 23.5 (C-27), 18.9 (C-26), 17.8 (C-6), 16.5 (C-24), 16.5 (C-25). – MS (ESI): m/z (%) = 563.5 (38) $[M+H]^+$, 585.1 (100) $[M+Na]^+$, 601.1 (7) $[M+K]^+$, 863.8 (6) $[3M+K+2H]^{2+}$, 866.3 (14) $[3M+2Na]^{2+}$, 1125.1 (32) $[2M+H]^+$, 1147.1 (68) $[2M+Na]^+$, 1162.9 (10) $[2M+K]^+$.

*Ethyl (3β)-3[(methylsulfonyl)oxy]-11-oxo-olean-12-en-30-oate (**15**)*

Following the procedure given for the preparation of **13**, from **3** (1.41 g, 2.8 mmol), triethylamine (1.5 mL, 10.8 mmol) and methanesulfonyl chloride (340 μL, 4.4 mmol) **15** (1.50 g, 94 %) was obtained as a colorless powder. M. p. 172 – 175 °C; R_f = 0.51 (hexane / ethyl ac-

etate 7 : 3). – $[\alpha]_D$ = 129.76° (c = 0.48, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 250 nm (4.04). – IR (KBr): ν = 3422br, 2958s, 2875m, 1718s, 1652s, 1466w, 1389m, 1361s, 1276w, 1247w, 1219m, 1175s, 1155m, 1088w, 1031w, 931m, 913s, 880m cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.64 (s, 1 H, 12-H), 4.37 (dd, 1 H, J = 11.8, 4.9 Hz, 3-H), 4.18 (dq, 1 H, J = 10.8, 7.1 Hz, Et-C*H*H′), 4.11 (dq, 1 H, J = 10.8, 7.1 Hz, Et-CH*H*'), 3.01 (s, 3 H, Mes-Me), 2.86 (ddd, 1 H, J = 13.9, 3.7, 3.7 Hz, 1-H), 2.33 (s, 1 H, 9-H), 2.10 (ddd, 1 H, J = 13.7, 4.2, 1.1 Hz, 18-H), 2.02 (ddd, 1 H, J = 13.9, 13.9, 4.2 Hz, 15-H), 1.99 (m, 1 H, 21-H), 1.97 (m, 1 H, 22-H), 1.92 (m, 1 H, 19-H), 1.89 (m, 1 H, 22′-H), 1.82 (ddd, 1 H, J = 13.9, 13.9, 4.9 Hz, 16-H), 1.66 (m, 1 H, 7-H), 1.60 (dd, 1 H, J = 13.5, 13.5 Hz, 19′-H), 1.60 (m, 1 H, 6-H), 1.47 (m, 1 H, 6′-H), 1.41 (ddd, 1 H, J = 12.8, 2.7, 2.7 Hz, 7′-H), 1.38 (m, 1 H, 22-H), 1.35 (s, 3 H, 27-H), 1.32 (m, 1 H, 22′-H), 1.31 (m, 1 H, 21′-H), 1.25 (t, 3 H, J = 7.1 Hz, Me), 1.17 (m, 1 H, 16′-H), 1.16 (s, 3 H, 25-H), 1.13 (s, 3 H, 29-H), 1.12 (s, 3 H, 26-H), 1.04 (s, 3 H, 23-H), 1.03 (m, 1 H, 1′-H), 0.97 (m, 1 H, 15′-H), 0.88 (s, 3 H, 24-H), 0.80 (m, 1 H, 5-H), 0.80 (s, 3 H, 28-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.7 (C-11), 176.3 (C-30), 169.5 (C-13), 128.4 (C-12), 90.1 (C-3), 61.6 (C-9), 60.3 (Et-CH_2), 55.3 (C-5), 48.4 (C-18), 45.3 (C-8), 43.8 (C-20), 43.2 (C-14), 41.1 (C-19), 39.0 (Mes-Me), 38.8 (C-1 and C-4), 37.7 (C-22), 36.7 (C-10), 32.6 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.2 (C-23), 26.5 (C-16), 26.4 (C-15), 25.0 (C-2), 23.3 (C-27), 18.6 (C-26), 17.6 (C-6), 16.3 (C-24), 16.3 (C-25), 14.3 (Me). – MS (ESI): m/z (%) = 577.4 (50) $[M+H]^+$, 599.1 (100) $[M+Na]^+$, 615.1 (4) $[M+K]^+$, 887.8 (6) $[3\,M+2Na]^{2+}$, 1153.1 (18) $[2M+H]^+$, 1176.0 (17) $[2M+Na]^+$, 1191.1 (2) $[2M+K]^+$. – $C_{33}H_{52}O_6S$ (576.83): calcd. C 75.26, H 9.08; found C 75.06, H 9.23.

*11-Oxo-olean-2,12-dien-30-oic acid (**16**)*

A mixture of **13** (450 mg, 1.0 mmol) and potassium carbonate (152 mg, 1.1 mmol) in dry DMF (10 mL) was stirred at 120 °C for 24 h. After removal of the solvent, aqueous work-up followed by extraction with $CHCl_3$ (3 × 15 mL) and chromatography (silica gel, hexane / ethyl acetate 8 : 2), **16** (200 mg, 44 %) was obtained as colorless crystals. M. p. 292 – 295 °C (lit.: 290 – 296 °C [13]). – R_f = 0.50 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 209.79° (c = 0.66, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 250 nm (4.08). – IR (KBr): ν = 3432br, 2950s, 2362w, 1699s, 1656s, 1458m, 1386m, 1361w, 1328m, 1282w, 1260m, 1232m, 1209m, 1176m, 1088w, 1020w cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.74 (s, 1 H, 12-H), 5.42 (ddd, 1 H, J = 9.8, 6.1, 1.7, 2-H), 5.36 (dd, 1 H, J = 9.9, 2.1 Hz, 3-H), 3.04 (dd, 1 H, J = 17.5, 6.1 Hz, 1-H), 2.42 (s, 1 H, 9-H), 2.20 (dd, 1 H, J = 12.8, 3.5 Hz, 18-H), 2.03 (m, 1 H, 15-H), 2.00 (m, 1 H, 21-H), 1.94 (ddd, 1 H, J = 13.4, 4.1, 2.6 Hz, 19-H), 1.84 (ddd, 1 H, J = 13.7, 13.7, 4.1 Hz, 16-H), 1.68 (ddd, H, J = 12.7, 3.7, 3.7 Hz, 7-H), 1.62 (dd, 1 H, J = 13.4, 13.4 Hz, 19′-H), 1.56 (m, 1 H, 6-H), 1.49 (m, 1 H, 6′-H), 1.44 (m, 1 H, 22-H), 1.42 (m, 1 H, 7′-H), 1.40 (m, 1 H, 22′-H), 1.36 (s, 3 H, 27-H), 1.22 (s, 3 H, 29-H), 1.22 (m, 1 H, 16′-H), 1.22 (m, 1 H, 21′-H), 1.16 (s, 3 H, 25-H), 1.16 (s, 3 H, 26-H), 1.11 (dd, 1 H, J = 11.9, 2.4 Hz, 5-H), 1.03 (m, 1 H, 15′-H), 0.96 (s, 3 H, 23-H), 0.91 (s, 3 H, 24-H), 0.85 (s, 3 H, 28-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.4 (C-11), 181.9 (C-30), 169.6 (C-13), 137.0 (C-3), 128.6 (C-12), 121.9 (C-2), 60.5 (C-9), 51.8 (C-5), 48.2 (C-18), 45.4 (C-14), 43.8 (C-20), 43.3 (C-8), 41.5 (C-1), 40.9 (C-19), 37.7 (C-22), 36.2 (C-4), 34.3 (C-17), 31.9 (C-7), 31.9 (C-23), 31.8 (C-10), 30.9 (C-21), 28.6 (C-28), 28.4 (C-29), 26.5 (C-15), 26.4 (C-16), 23.3 (C-27), 23.0 (C-24), 18.7 (C-6), 18.3 (C-26), 16.1 (C-25). – MS (ESI): m/z (%) = 453.5 (35) $[M+H]^+$, 475.4 (8) $[M+Na]^+$, 507.0 (100) $[M+MeOH+Na]^+$, 905.3 (18) $[2M+H]^+$, 927.3 (40) $[2M+Na]^+$.

*Methyl 11-oxo-olean-2,12-dien-30-oate (**17**)*

To a solution of **14** (219 mg, 0.39 mmol) in dry DMF (10 mL), tetrabutyl ammonium fluoride trihydrate (163 mg, 0.39 mmol) was added. After 4 d of stirring at between 100 – 105 °C, the solvent was removed under reduced pressure, and the residue was subjected to chromatography (silica gel, hexane / ethyl acetate 8 : 2) to yield **16** (92 mg, 51 %) as a colorless powder. M. p. 220 – 222 °C (lit.: 224 – 228 °C [13]). – R_f = 0.81 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 209.85° (c = 0.33, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 248 nm (4.06). – IR (KBr): ν = 3438br, 2956s, 1728s, 1655s, 1617w, 1465m, 1385m, 1360w, 1328w, 1279w, 1259w, 1217m, 1156s, 1089w, 1029w cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.67 (s, 1 H, 12-H), 5.42 (ddd, 1 H, J = 10.0, 5.8, 1.7 Hz, 2-H), 5.35 (dd, 1 H, J = 10.0, 2.1 Hz, 3-H), 3.67 (s, 1 H, Me), 3.03 (dd, 1 H, J = 17.4, 5.8 Hz, 1-H), 2.40 (s, 1 H, 9-H), 2.08 (ddd, 1 H, J = 13.7, 3.7, 1.3 Hz, 18-H), 2.01 (ddd, 1 H, J = 13.7, 13.7, 4.2 Hz, 15-H), 1.98 (m, 1 H, 21-H), 1.91 (ddd, 1 H, J = 13.7, 4.2, 2.5 Hz, 19-H), 1.82 (ddd, 1 H, J = 13.3, 13.3, 4.2 Hz, 16-H), 1.68 (ddd, 1 H, J = 12.0, 12.0, 3.7 Hz, 7-H), 1.64 (m, 1 H, 1′-H), 1.60 (dd, 1 H, J = 13.7, 13.7 Hz, 19′-H), 1.53 (m, 1 H, 6-H), 1.43 (m, 1 H, 6′-H), 1.41 (m, 1 H, 7′-H), 1.38 (m, 1 H, 22-H), 1.35 (s, 3 H, 27-H), 1.30 (m, 2 H, 22′-H and 21′-H), 1.22 (m, 1 H, 16′-H), 1.15 (s, 3 H, 25-H), 1.14 (s, 3 H, 26-H), 1.13 (s, 3 H, 29-H), 1.10 (m, 1 H, 5-H), 1.00 (m, 1 H, 15′-H), 0.95 (s, 3 H, 23-H), 0.90 (s, 3 H, 24-H), 0.80 (s, 3 H, 28-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.8 (C-11), 176.8 (C-30), 169.1 (C-13), 137.0 (C-3), 128.7 (C-12), 122.0 (C-2), 60.6 (C-9), 52.0 (Me), 51.8 (C-5), 48.6 (C-18), 45.5 (C-14), 44.2 (C-20), 43.4 (C-8), 41.7 (C-1), 41.4 (C-19), 38.0 (C-22), 36.4 (C-9), 34.5 (C-10), 32.1 (C-7), 32.0 (C-23), 32.0 (C-17), 31.3 (C-21), 28.7 (C-28), 28.5 (C-29), 26.7 (C-16), 26.7 (C-15), 23.5 (C-27), 23.2 (C-24), 18.9 (C-6), 18.54 (C-26), 16.3 (C-25). – MS (ESI): m/z (%) =

467.5 (100) $[M+H]^+$, 521.0 (73) $[M+MeOH+Na]^+$, 933.2 (86) $[2M+H]^+$, 955.3 (48) $[2M+Na]^+$.

*Ethyl 11-oxo-olean-2,12-dien-30-oate (**18**)*

A mixture of **3** (1.31 g, 2.6 mmol), triphenyl phosphane (2.78 g, 10.6 mmol) and 3,3-dimethyl glutarimide (1.49 g, 10.6 mmol) in dry THF (25 mL) was cooled to 0 °C. Under continuous stirring, DEAD (1.65 ml, 10.4 mmol) was added dropwise, and stirring was continued at r. t. for 24 h. After concentration to dryness, the residue was subjected to chromatography (silica gel, chloroform / ether 9 : 1) to yield **18** (1.02 g, 82 %) as colorless crystals. M. p. 138 – 142 °C; R_f = 0.87 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 216.97° (c = 0.33, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 249 nm (4.02). – IR (KBr): ν = 3422br, 2960s, 2872s, 1723s, 1648s, 1612w, 1458m, 1386m, 1360w, 1348w, 1328w, 1310w, 1277w, 1256m, 1210m, 1169s, 1134m, 1088w, 1062w, 1031m cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.67 (s, 1 H, 12-H), 5.43 (ddd, 1 H, J = 10.1, 6.1, 1.7 Hz, 2-H), 5.37 (dd, 1 H, J = 10.1, 2.3, 3-H), 4.19 (dq, 1 H, J = 10.8, 7.2 Hz, Et-C*H*H′), 4.12 (dq, 1 H, J = 10.8, 7.2 Hz, Et-CH*H*′), 3.04 (dd, 1 H, J = 17.5, 6.0 Hz, 1-H), 2.41 (s, 1 H, 9-H), 2.11 (dd, 1 H, J = 12.8, 4.3 Hz, 18-H), 2.03 (ddd, 1 H, J = 13.4, 13.4, 4.7 Hz, 15-H), 1.99 (m, 1 H, 21-H), 1.92 (ddd, 1 H, J = 13.9, 4.1, 2.9 Hz, 19-H), 1.83 (ddd, 1 H, J = 13.6, 13.6, 4.3 Hz, 16-H), 1.70 (m, 1 H, 7-H), 1.65 (m, 1 H, 1′-H), 1.61 (dd, 1 H, J = 13.5, 13.5 Hz, 19′-H), 1.56 (m, 1 H, 6-H), 1.48 (ddd, 1 H, J = 12.5, 12.5, 3.2 Hz, 6′-H), 1.43 (m, 1 H, 7′-H), 1.39 (m, 1 H, 22-H), 1.33 (m, 1 H, 21′-H), 1.30 (m, 1 H, 22′-H), 1.36 (s, 3 H, 27-H), 1.26 (t, 3 H, J = 7.2 Hz, Me), 1.21 (ddd, 1 H, J = 13.9, 4.4, 2.4 Hz, 16-H′), 1.16 (s, 3 H, 25-H), 1.16 (s, 3 H, 26-H), 1.14 (s, 3 H, 29-H), 1.12 (m, 1 H, 5-H), 1.02 (m, 1 H, 15′-H), 0.96 (s, 3 H, 23-H), 0.91 (s, 3 H, 24-H), 0.82 (s, 3 H, 28-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.1 (C-11), 176.4 (C-30), 169.4 (C-13), 137.0 (C-3), 128.6 (C-12), 121.9 (C-2), 60.5 (C-9), 60.3 (Et-CH_2), 51.8 (C-5), 48.4 (C-18), 45.3 (C-14), 43.8 (C-20), 43.3 (C-8), 41.5 (C-1), 41.2 (C-19), 37.7 (C-22), 36.2 (C-4), 34.3 (C-10), 31.9 (C-7), 31.9 (C-23), 31.8 (C-17), 31.1 (C-21), 28.6 (C-28), 28.3 (C-29), 26.5 (C-16), 26.5 (C-15), 23.3 (C-27), 23.0 (C-24), 18.7 (C-6), 18.3 (C-26), 16.1 (C-25), 14.3 (Me). – MS (ESI): m/z (%) = 481.5 (100) $[M+H]^+$, 503.3 (7) $[M+Na]^+$, 534.9 (50) $[M+MeOH+Na]^+$, 961.3 (66) $[2M+H]^+$, 983.4 (54) $[2M+Na]^+$, 999.2 (4) $[2M+K]^+$. – $C_{32}H_{48}O_3$ (480.72): calcd. C 79.95, H 10.06; found C 79.68, H 10.18.

*Ethyl (2α,3α)-2,3-epoxy-11-oxo-olean-12-en-30-oate (**19**)*

Compound **18** (1.01 g, 2.1 mmol) was dissolved in dry dichloromethane (20 mL), *m*-CPBA (1.14 g, 4.68 mmol) was added, and the mixture was stirred at r. t. for 20 h. An aq. solution of potassium hydrogensulfate (satd., 10 mL) was added, the aqueous layer was extracted with dichloromethane (3 × 15 mL), and the combined organic layers were dried (Na_2SO_4), filtered and evaporated to dryness. Chromatographic purification (silica gel, chloroform / ether 9 : 1) afforded **19** (806 mg, 77 %) as a colorless powder. M. p. 191 – 193 °C; R_f = 0.71 (hexane / ethyl acetate 7 : 3). – $[\alpha]_D$ = 143.65° (c = 0.48, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 251 nm (4.09). – IR (KBr): ν = 3416br, 2978s, 2955s, 1736s, 1718s, 1645s, 1614w, 1458m, 1385m, 1314m, 1301m, 1285m, 1260m, 1222s, 1163s, 1113m, 1091m, 1039m, 1014w cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.63 (s, 1 H, 12-H), 4.16 (dq, 1 H, J = 10.8, 7.1 Hz, Et-C*H*H′), 4.10 (dq, 1 H, J = 10.8, 7.1 Hz, Et-CH*H*′), 3.19 (dd, 1 H, J = 6.6, 3.7 Hz, 2-H), 3.13 (dd, 1 H, J = 14.9, 6.6 Hz, 1-H), 2.79 (d, 1 H, J = 3.7 Hz, 3-H), 2.29 (s, 1 H, 9-H), 2.09 (dd, 1 H, J = 13.3, 4.2 Hz, 18-H), 1.99 (ddd, 1 H, J = 13.3, 13.3, 4.6 Hz, 15-H), 1.96 (m, 1 H, 21-H), 1.90 (ddd, 1 H, J = 13.7, 4.2, 2.9 Hz, 19-H), 1.78 (ddd, 1 H, J = 13.7, 13.7, 5.0 Hz, 16-H), 1.61 (m, 1 H, 7-H), 1.57 (dd, 1 H, J = 13.7, 13.7 Hz, 19′-H), 1.48 (m, 1 H, 6-H), 1.39 (m, 1 H, 21-H), 1.37 (m, 1 H, 6′-H), 1.35 (m, 1 H, 22-H), 1.33 (m, 1 H, 1′-H), 1.30 (s, 3 H, 27-H), 1.29 (m, 1 H, 22′-H), 1.27 (m, 1 H, 21′-H), 1.24 (t, 1 H, J = 7.1 Hz, Me), 1.17 (m, 1 H, 16′-H), 1.13 (s, 3 H, 26-H), 1.11 (s, 3 H, 28-H), 1.09 (s, 3 H, 23-H), 1.07 (s, 3 H, 25-H), 1.02 (s, 3 H, 24-H), 0.93 (m, 1 H, 15′-H), 0.92 (dd, 1 H, J = 11.6, 2.9 Hz, 5-H), 0.78 (s, 3 H, 29-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.7 (C-11), 176.3 (C-30), 169.7 (C-13), 128.5 (C-12), 61.3 (C-3), 60.4 (C-9), 60.3 (Et-CH_2), 52.6 (C-2), 48.4 (C-18), 46.6 (C-5), 45.1 (C-8), 43.8 (C-20), 43.3 (C-14), 41.1 (C-19), 40.6 (C-1), 37.7 (C-22), 35.9 (C-4), 32.6 (C-10), 31.9 (C-7), 31.8 (C-17), 31.1 (C-21), 28.6 (C-28), 28.3 (C-29), 28.2 (C-23), 26.4 (C-16), 26.4 (C-15), 23.2 (C-27), 22.0 (C-24), 18.3 (C-26), 17.9 (C-6), 17.9 (C-25), 14.3 (Me). – MS (ESI): m/z (%) = 497.6 (92) $[M+H]^+$, 519.4 (10) $[M+Na]^+$, 551.0 (62) $[M+MeOH+Na]^+$, 767.4 (6) $[3M+2Na]^{2+}$, 993.3 (94) $[2M+H]^+$, 1015.4 (100) $[2M+Na]^+$, 1031.3 (12) $[2M+K]^+$. – $C_{32}H_{48}O_4$ (496.72): calcd. C 77.38, H 9.74; found C 77.26, H 9.92.

*Methyl (3β)-3[(1H-imidazol-1-yl)carbonothioyl)oxy]-11-oxo-olean-12-en-30-oate (**20**)*

Compound **2** (230 mg, 0.48 mmol) and 1,1′-thiocarbonyl-diimidazole (90 %, 235 mg, 1.06 mmol) were dissolved in dry 1,2-dichloroethane (3.5 mL). The mixture was stirred at 100 °C under Ar for 70 h. After addition of cold hydrochloric acid (1 M, 10 mL), the aqueous layer was extracted with dichloromethane (3 × 10 mL), the extracts were washed with an aq. solution of sodium hydrogencarbonate, water and brine (10 mL each), dried (Na_2SO_4) and evaporated to dryness yielding **20** (200 mg, 70 %) as a colorless powder. M. p. 245 – 247 °C (lit.: 242 – 243 °C [13]). – R_f = 0.46 (hexane / ethyl acetate = 7 : 3). – $[\alpha]_D$ = 142.33° (c = 0.35, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 208 nm (4.38),

252 nm (4.26). – IR (KBr): ν = 3439br, 3155w, 3113w, 2945s, 2863m, 1728s, 1659s, 1622m, 1531m, 1500m, 1464s, 1387s, 1349s, 1327s, 1292s, 1234s, 1153s, 1110s, 1088m, 1038m, 1008m, 974s, 923m, 896m, 828m, 750m, cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 8.35 (s, 1 H, NCHN), 7.62 (m, 1 H, CSNC*H*CHN), 7.04 (s, 1 H, CSNCHC*H*N), 5.68 (s, 1 H, 12-H), 5.25 (dd, 1 H, *J* = 11.8, 4.9, 3-H), 3.68 (s, 3 H, Me), 2.92 (ddd, 1 H, *J* = 13.7, 3.4, 3.4 Hz, 1-H), 2.39 (s, 1 H, 9-H), 2.09 (dd, 1 H, *J* = 13.3, 3.8 Hz, 18-H), 2.02 (ddd, 1 H, *J* = 13.7, 13.7, 4.6 Hz, 15-H), 1.99 (m, 1 H, 21-H), 1.95 (m, 1 H, 2-H), 1.92 (m, 1 H, 19-H), 1.84 (m, 1 H, 16-H), 1.82 (m, 1 H, 2′-H), 1.68 (m, 1 H, 7-H), 1.64 (m, 1 H, 6-H), 1.61 (dd, 1 H, *J* = 13.3, 13.3 Hz, 19′-H), 1.60 (m, 1 H, 6′-H), 1.49 (m, 1 H, 7′), 1.44 (m, 1 H, 22-H), 1.38 (m, 1 H, 22′-H), 1.37 (s, 3 H, 27-H), 1.35 (m, 1 H, 21′-H), 1.20 (s, 3 H, 25-H), 1.19 (m, 1 H, 16-H), 1.14 (s, 3 H, 29-H), 1.14 (s, 3 H, 26-H), 1.11 (ddd, 1 H, *J* = 13.7, 13.7, 3.4 Hz, 1′-H), 1.03 (s, 3 H, 24-H), 1.02 (m, 1 H, 16′-H), 0.97 (s, 3 H, 23-H), 0.90 (dd, 1 H, *J* = 11.8, 1.5 Hz, 5-H), 0.80 (s, 3 H, 28-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.7 (C-11), 183.8 (CS), 176.9 (C-30), 169.4 (C-13), 130.3 (NCHN), 128.4 (C-12), 124.2 (CSNCH*C*HN), 117.8 (CSN*C*HCHN), 91.4 (C-3), 61.5 (C-9), 55.0 (C-5), 51.8 (Me), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.8 (C-4), 38.5 (C-1), 37.7 (C-22), 36.9 (C-10), 32.6 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.1 (C-23), 26.5 (C-16), 26.4 (C-15), 23.4 (C-27), 22.3 (C-2), 18.7 (C-26), 17.5 (C-24), 17.3 (C-6), 16.4 (C-25). – MS (ESI): m/z = 595.1 (100) $[M+H]^+$, 617.2 (28) $[M+Na]^+$, 892.7 (14) $[3M+2H]^{2+}$, 903.6 (10) $[3M+Na+H]^{2+}$, 914.7 (7) $[3M+2Na]^{2+}$, 1189.0 (14) $[2M+H]^+$, 1200.4 (10) $[4M+Na+H]^{2+}$.

*Methyl 11-oxo-olean-12-en-30-oate (**21**)*

To a solution of **20** (200 mg, 0.3 mmol) in dry toluene (15 mL), tributyltin hydride (0.45 ml, 2.2 mmol) and catalytic amounts of AIBN were added. The mixture was stirred at 115 °C for 40 h and concentrated under reduced pressure. The residue was subjected to chromatography (silica gel, hexane / ethyl acetate 9 : 1) to afford **21** (30 mg, 18 %) as a colorless powder. M. p. 215 – 218 °C (lit.: 222 – 223 °C [13]). – R_f = 0.80 (hexane / ethyl acetate = 7 : 3). – $[\alpha]_D$ = 103.98° (*c* = 0.28, $CHCl_3$). – UV/Vis (methanol): $\lambda_{max}(\log\varepsilon)$ = 249 nm (4.02). – IR (KBr): ν = 3435br, 2955s, 2284w, 1728s, 1655s, 1618w, 1465m, 1432w, 1386m, 1317w, 1278m, 1217m, 1188m, 1153m, 1087w, 1074w, 1030w cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.58 (s, 1 H, 12-H), 3.62 (s, 3 H, Me), 2.65 (ddd, 1 H, *J* = 13.0, 4.7, 3.0 Hz, 1-H), 2.32 (s, 1 H, 9-H), 2.00 (m, 1 H, 18-H), 1.97 (m, 1 H, 15-H), 1.94 (m, 1 H, 7-H), 1.85 (ddd, 1 H, *J* = 13.6, 4.2, 2.7 Hz, 19-H), 1.75 (ddd, 1 H, *J* = 13.4, 13.4, 4.3 Hz, 16-H), 1.62 (m, 1 H, 6-H), 1.59 (m, 1 H, 21-H), 1.55 (dd, 1 H, *J* = 13.6, 13.6 Hz, 19′-H), 1.50 (m, 2 H, 2-H and 2′-H), 1.34 (m, 1 H, 21-H), 1.32 (m, 1 H, 22-H), 1.30 (m, 1 H, 3-H), 1.29 (m, 1 H, 6′-H), 1.28 (m, 1 H, 7′-H), 1.27 (m, 1 H, 22′-H), 1.31 (s, 3 H, 27-H), 1.11 (m, 1 H, 16′-H), 1.09 (m, 1 H, 3-H′), 1.08 (s, 6 H, 25-H and 29-H), 1.06 (s, 3 H, 26-H), 0.94 (m, 1 H, 15′-H), 0.80 (s, 3 H, 23-H), 0.77 (s, 3 H, 24-H), 0.77 (m, 1 H, 1′-H), 0.74 (s, 3 H, 28-H), 0.66 (dd, 1 H, *J* = 12.3, 2.2 Hz, 5-H). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.6 (C-11), 176.9 (C-30), 168.8 (C-13), 128.6 (C-12), 61.9 (C-9), 55.7 (C-5), 51.7 (Me), 48.4 (C-18), 45.6 (C-8), 44.0 (C-20), 43.2 (C-14), 42.0 (C-3), 41.1 (C-19), 40.9 (C-1), 37.8 (C-22), 37.4 (C-10), 33.5 (C-23), 33.4 (C-4), 32.8 (C-7), 31.8 (C-17), 31.2 (C-21), 28.5 (C-28), 28.3 (C-29), 26.5 (C-16), 26.4 (C-15), 23.4 (C-27), 21.8 (C-24), 18.8 (C-26), 18.4 (C-2), 17.7 (C-6), 16.3 (C-25). – MS (ESI): m/z (%) = 469.5 (72) $[M+H]^+$, 522.9 (98) $[M+MeOH+Na]^+$, 585.5 (20) $[M+MeOH+Na]^+$, 937.3 (46) $[2M+H]^+$, 959.3 (100) $[2M+Na]^+$, 975.2 (4) $[2M+K]^+$.

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[1] K. Sheth, S. Jolad, R. Wiedhopf, J. R. Cole, *J. Pharm. Sci.* **1972**, *61*, 1819.
[2] D. H. Miles, U. Kokpol, L. H. Zalkow, S. J. Steindel, J. B. Nabors, *J. Pharm. Sci.* **1974**, *63*, 613 – 615.
[3] M. L. Schmidt, K. L. Kuzmanoff, L. Ling-Indeck, J. M. Pezzuto, *Eur. J, Cancer* **1997**, *33*, 2007 – 2010.
[4] P. Rajendran, M. Jaggi, M. Singh, R. Mukherjee, A. Burman, *Invest. New Drugs* **2008**, *26*, 25 – 34.
[5] D. Huang, Y. Ding, Y. Li, W. Zhang, W. Fang, X. Chen, *Cancer Lett.* **2006**, *233*, 289 – 296.
[6] H. Hibasami, H. Iwase, K. Yoshioka, H. Takahashi, *Int. J. Mol. Med.* **2006**, *17*, 215 – 219.
[7] D. Liu, D. Song, G. Guo, R. Wang, J. Lv, Y. Jing, L. Zhao, *Bioorg. Med. Chem.* **2007**, *15*, 5432 – 5439.
[8] C. S. Lee, Y. J. Kim, M. S. Lee, E. S. Han, S. J. Lee, *Life Sci.* **2008**, *83*, 481 – 489.
[9] S. Schwarz, R. Csuk, *Bioorg. Med. Chem.* **2010**, *18*, 7458 – 7474.
[10] D. R. Lauren, D. J. Jensen, J. A. Douglas, J. M. Follet, *Phytochem. Anal.* **2001**, *12*, 332 – 333.
[11] L. A. Baltina, *Curr. Med. Chem.* **2003**, *10*, 155 – 171.
[12] X. Su, H. Lawrence, D. Ganeshapillai, A. Cruttenden, A. Purohit, M. J. Reed, N. Vicker, B. V. L. Potter, *Bioorg. Med. Chem.* **2004**, *12*, 4439 – 4457.

[13] T. Teresawa, T. Okada, T. Hara, K. Itoh, *Eur. J. Med. Chem.* **1992**, *27*, 345 – 351.
[14] X. Wen, P. Zhang, J. Liu, L. Zhang, X. Wu, P. Ni, H. Sun, H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 722 – 726.
[15] M. Renoud-Grappin, C. Vanucci, G. Lhommert, *J. Org. Chem.* **1994**, *59*, 3902 – 3905.
[16] I.-C. Sun, H.-K. Wang, Y. Kashiwada, J.-K. Shen, L. M. Cosentino, C.-H. Chen, L.-M. Yang, K.-H. Lee, *J. Med. Chem.* **1998**, *41*, 4648 – 4657.
[17] F. B. H. Ahmad, M. G. Moghaddam, M. Basri, M. B. A. Rahman, *Biosci. Biotechn. Biochem.* **2010**, *74*, 1025 – 1029.
[18] H. Kommera, G. N. Kaluđerović, J. Kalbitz, R. Paschke, *Arch. Pharm. Chem. Life Sci.* **2010**, *8*, 449 – 457.
[19] L. D. Vechia, S. C. B. Gnoatto, G. Gosmann, *Quim. Nova* **2009**, *32*, 1245 – 1252.
[20] M. Urban, J. Sarek, J. Klinot, G. Korinkova, M. Hajduch, *J. Nat. Prod.* **2004**, *67*, 1100 – 1105.
[21] M. Urban, J. Sarek, I. Tislerova, P. Dzubak, M. Hajduch, *Bioorg. Med. Chem.* **2005**, *13*, 5527 – 5535.
[22] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Nat. Cancer Inst.* **1990**, *82*, 1107 – 1112.
[23] D. Yu, Y. Sakurai, C.-H. Chen, F.-G. Chang, L. Hunag, Y. Kashiwada, K.-H. Lee, *J. Med. Chem.* **2006**, *49*, 5462 – 5469.
[24] I. Beseda, L. Czollner, P. S. Shah, R. Khunt, R. Gaware, P. Kosma, C. Stanetty, M. C. Ruiz-Ruiz, H. Amer, K. Mereiter, T. D. Cunha, A. Odermatt, D. Claßen-Houben, U. Jordis, *Bioorg. Med. Chem.* **2010**, *1*, 433 – 454.
[25] G. S. R. Subba Rao, P. Kondaiah, S. K. Singh, P. Ravanan, M. B. Sporn, *Tetrahedron* **2008**, *64*, 11541 – 11548.
[26] M. H. A. Elgamal, M. B. E. Fayez, *Tetrahedron* **1967**, *23*, 1633 – 1640.