

Synthesis and cytotoxic properties of alkyne triterpenoid Mannich compounds

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Abstract: In summary, we prepared alkyne Mannich bases of betulinic or glycyrrhetic acid; many of these compounds showed an improved cytotoxicity as compared to their parent compounds glycyrrhetic or betulinic acid. The highest cytotoxicity was determined for a Mannich compound derived from glycyrrhetic acid, and EC₅₀ values as low as 3.3 μ M were achieved for this compound employing the human submandibular carcinoma cell line A253. As proven by AO/PI staining as well as by DNA-laddering experiments this compound acts by apoptosis.

Keywords: Betulin; Betulinic acid; Glycyrrhetic acid; Mannich compounds; Antitumor.

Introduction

The therapy of cancer is still a very important medicinal issue. Although there has been an enormous progress in the treatment of cancer, improvements in therapy, prophylaxis and the

development of more selective drugs are still called for and remain a critical topic. Recently, derivatives of betulin **1** and betulinic acid **2** (Fig. 1) have quite successfully passed *in-vitro* tests, and preliminary preclinical *in-vivo* trials gave good results^{1,2}.

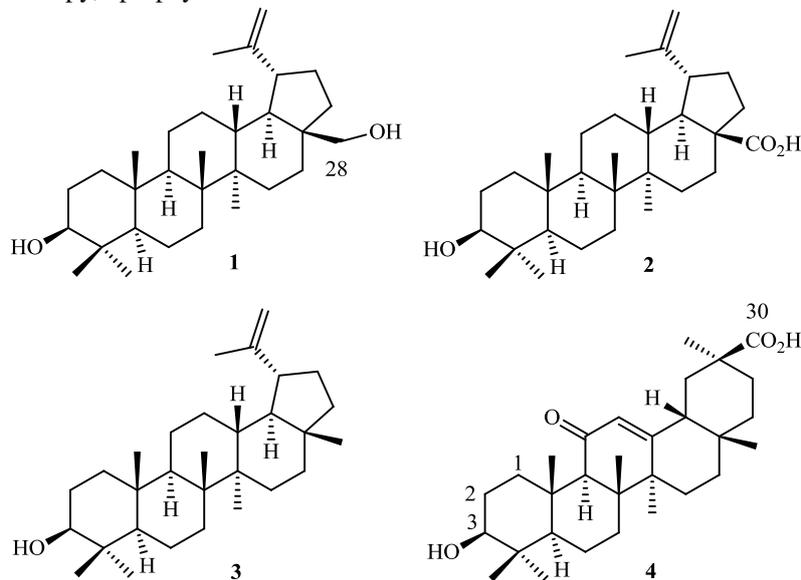


Figure 1. Structure of triterpenoids betulin **1**, betulinic acid **2**, lupeol **3** and glycyrrhetic acid **4**.

Thus, several of these candidates were selected for an extended preclinical testing and clinical

investigations are expected to start soon¹. Triterpenic compounds of the betulin-type are

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usually synthesized from their parent compounds **1** and **2**, respectively. Betulin **1** and to a lesser extent betulinic acid **2** can be extracted from various plant materials^{3,4}. The isolation of **1** from renewable resources seems of special interest. Hence, there are numerous reports and patents on the extraction of **1** from birch bark. The latter is a cheap by-product in wood processing. Several kinds of birch trees have been investigated so far for their content of **1**, and *Betula platyphylla* seems a very promising tree. It can grow very easily, and is abundant in many areas of Eurasia. This makes this tree a very interesting source for the isolation of natural products. There are several reports on improving the yield⁵ of **1** or manipulating⁶ the tree during its growing. Unfortunately, less work has been accomplished to investigate how to extract **1** in an economic, efficient and high yielding manner.

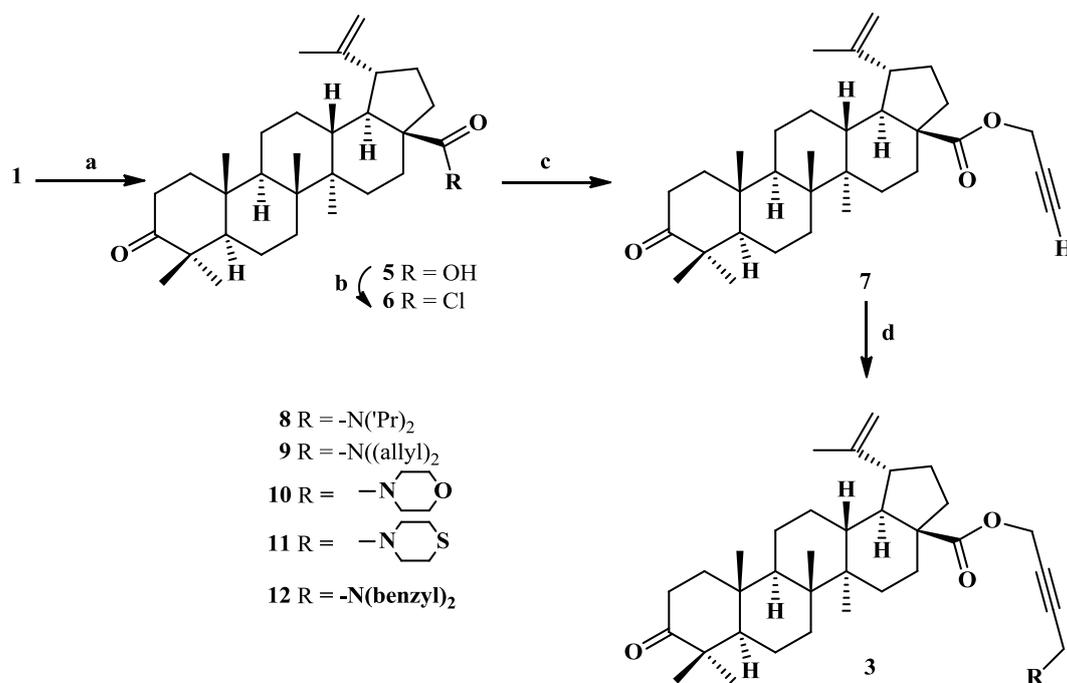
Results and Discussion

As far as cytotoxic betulin derivatives are concerned, two major problems, are to be investigated in more detail: Firstly, we searched for and tried to develop drugs being more selective for cancer cells than to nonmalignant cells, secondly we wanted to develop their economic synthesis. Usually for the extraction of betulin, the plant material is dried, crunched and extracted with solvents like toluene⁷, several water-organic-solvent mixtures⁸, supercritical CO₂^{9, 10} or ionic liquids¹¹. Given the physico-chemical properties of **1** (logP = 9.01 ± 0.39)¹², a comparison between the extraction with

hexanes (according to literature) and diethyl ether was performed. While the extraction of finely powdered dry birch bark with hexanes for 15 h in a Soxhlet apparatus furnished only 10.5% (recovery from the weight of bark submitted to extraction) of an extract containing 47% betulin and 43% of lupeol, the extraction with diethyl ether under the same conditions yielded 27.2% recovery. This extract consisted of 5% lupeol and 81% of betulin that could easily be separated by chromatography. Thus, the extraction of the birch bark with ether is more efficient and rewarding than its extraction with hexane.

Recently we were able to show that alkyne Mannich bases derived from triterpenoids showed promising cytotoxic activity^{13, 14}. In this study, we are interested in a comparison of analogous derivatives of betulin, betulinic acid as well as of glycyrrhetic acid **4**.

Jones oxidation of **1** furnished betulonic acid **5** (Scheme 1)^{15, 16}, whose reaction with oxalyl chloride gave chloride **6**. Reaction of compound **6** with propargylic alcohol yielded alkyne **7** (70% yield). This compound is characterized in its ¹³C NMR spectrum by the presence of signals at δ = 78.1 and 74.3 ppm being assigned to the alkyne moiety. Mannich reaction of **7** with diisopropylamine and formalin in the presence of CuI¹⁷ furnished **8**. In an analogous manner, from the reaction of **7**^{18, 19} with diallylamine, morpholine, thiomorpholine or benzylamine the products **9-12** were obtained in good yields.



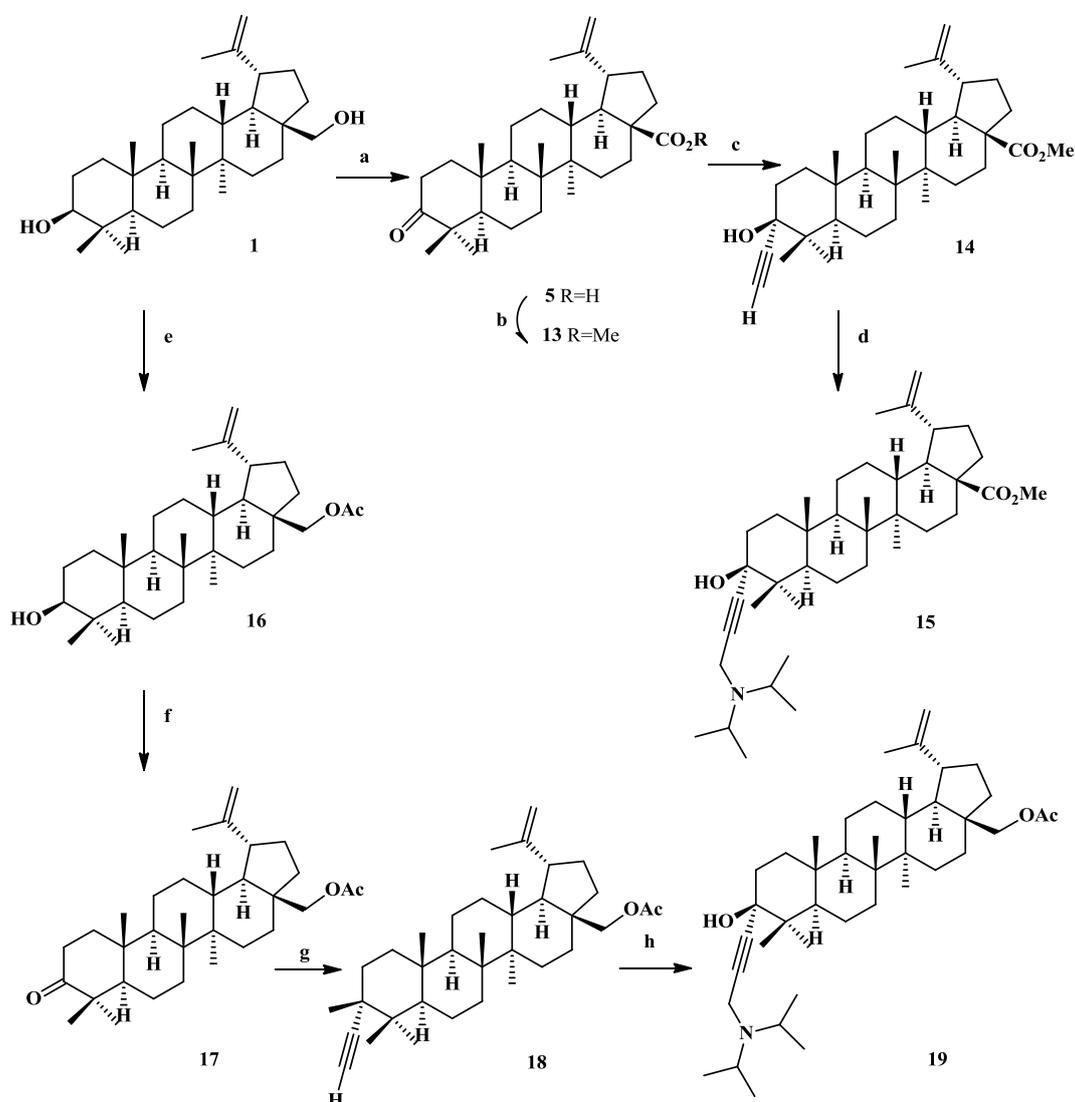
Scheme 1. Synthesis of Mannich bases **8-12**: a) CrO₃, H₂SO₄, acetone, 93% [ref. 15, 16]; b) (COCl)₂, DCM, 25 °C, 1 h (in-situ); c) propargylic alcohol, DCM, 25 °C, 5 h, 70%; d) amine, formalin, CuI, DMSO, 40 °C, 72 h, then dry HCl_g in Et₂O: **8** (75%), **9** (64%), **10** (71%), **11** (61%), **12** (81%).

To evaluate the influence of substitution at positions C-3 vs position C-28, several analogues derived from betulonate were prepared. To access these compounds, **1** was oxidized to betulonic acid **5** (Scheme 2) that was transformed into its methyl ester **13**²⁰. Reaction of **13** with ethynylmagnesium bromide gave alkynol **14**¹⁴. From the CuI catalyzed Mannich reaction^{17,21} of **14** product **15** was obtained. From an acetylation of **1** the mono-acetate **16** was obtained, whose Swern oxidation furnished compound **17**²². This compound was transformed into the alkynol **18** as described above, and subjected to a Mannich reaction with diisopropylamine, formalin and CuI to yield **19** in 74% yield.

For comparison, similar compounds were prepared from glycyrrhetic acid **4** (Scheme 3).

Thus **4** was converted into the methylester **20**²³ and benzylester **21**²⁴. Oxidation of these esters gave the ketones **22**²⁵ and **23**²⁵. The reaction of **22** or **23** with ethynylmagnesium bromide furnished the alkynols **24**²⁶⁻³³ and **25**, respectively. From the reaction of **24** or **25** with diallylamine or diisopropylamine and formalin in the presence of CuI the Mannich bases **26-28** were obtained in fair to good yields.

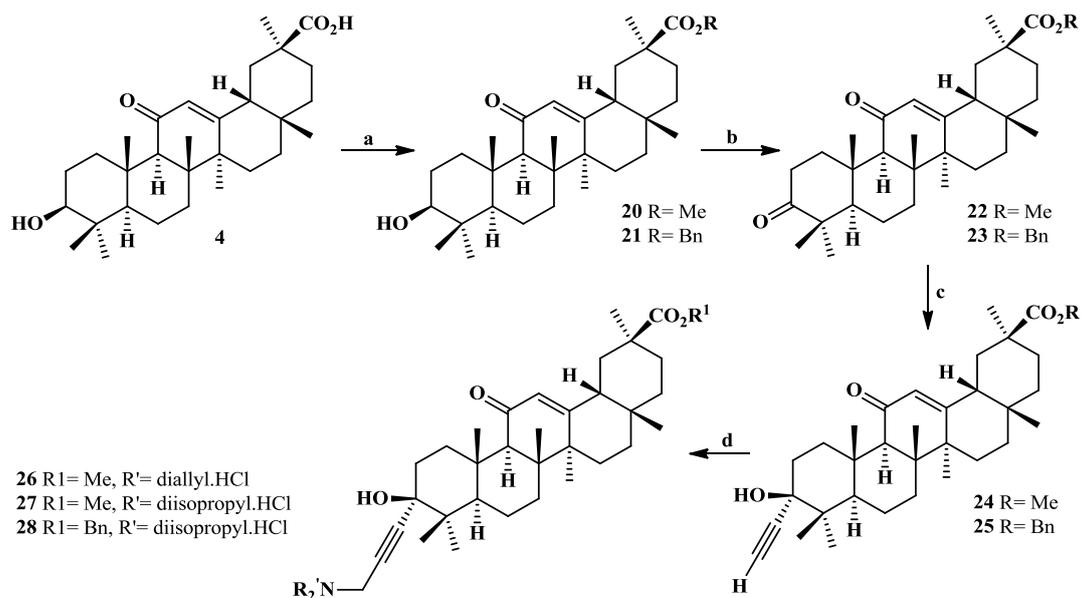
To explore the cytotoxic activity of the compounds, photometric sulforhodamin B assays (SRB)³⁴ were performed, and the EC₅₀ values were determined. The results from these assays employing nine different human cancer cell lines and nonmalignant mouse fibroblasts are compiled in Table 1.



Scheme 2. Synthesis of Mannich bases **15** and **19**: a) CrO₃, H₂SO₄, acetone, 93% [ref. 15, 16], b) (COCl)₂, 25 °C, 1 h, in-situ, then MeOH, 25 °C, 4 h, 86%; c) ethynylmagnesium bromide, THF, 25 °C, 72 h, 68%; d) (iProp)₂NH, formalin, CuI, DMSO, 40 °C, 72 h, 48%; e) Ac₂O, NEt₃, DMAP, DCM, 25 °C, 6 h, 73%; f) (COCl)₂, DMSO, NEt₃, 81%; g) ethynylmagnesium bromide, THF, 25 °C, 72 h, 75%; h) (iProp)₂NH, formalin, CuI, DMSO, 40 °C, 72 h, 74%.

As compared to parent compound betulinic acid **2**, a significantly reduced cytotoxicity was observed for the ester **7** as well as for the Mannich bases derived from this ester (**8-10**). Also, alkynol **14** showed a slightly diminished cytotoxicity while the Mannich base **15** was shown to be approximately twice as cytotoxic as its parent compound **14**. A reduced cytotoxicity was also observed for alkynol **18** and its Mannich base **19**. For glycyrrhetic acid derivatives, the alkynols **24** and **25** gave EC₅₀ values in the same range as betulinic acid but they showed

a significantly improved solubility in aqueous media. The Mannich bases **26-28**, however, exhibited EC₅₀ values approximately half of standard betulinic acid. For the most active compound of this series, glycyrrhetic acid-derived compound **27** some extra assays were performed to gain a deeper insight into its mode of action. Thus, the death of A549 lung epithelial carcinoma cells was investigated by fluorescence microscopy³⁵⁻³⁸ using an AO/PI (acridine orange/propidium iodide) assay.



Scheme 3. Synthesis of Mannich bases **26-28**: a) K₂CO₃, DMF, 25 °C, 12 h, 97% (with MeI for **20**), 75% (with BnBr for **21**); b) (COCl)₂, DMSO, NEt₃, 87% (for **22**), CrO₃, H₂SO₄, acetone 87% (for **23**); c) ethynylmagnesium bromide, THF, 25 °C, 72 h, 86% (for **24**), 75% (for **25**); d) amine, CuI, DMSO, 40 °C, 72 h, then dry HCl_g in Et₂O: 81% (with diallylamine for **26**), 55% (with diisopropylamine for **27**), 63% (with diisopropylamine for **28**).

The cells were treated for 48 h with betulinic acid **2** or compound **27**. The presence of green fluorescent cells in this assay is a hallmark of apoptosis while the presence of orange colored cells indicates that they have died by a process of (secondary) necrosis. Hence, the results from this assay indicate that these cells had died from a controlled cell death: In addition, a DNA laddering experiment was performed. DNA laddering is a feature that is observed when DNA fragments

resulting from an apoptotic DNA fragmentation by caspase-activated DNase are detected after separation by gel electrophoresis. In this process DNA is cleaved by caspase-activated DNase at internucleosomal linker regions, and DNA fragments of multiples of 180-185 base-pairs are formed. The experiments showed the formation of characteristic “DNA ladders” that are also a hallmark for apoptotic cell death.

Table 1: Cytotoxicity (EC₅₀ in μM) of selected compounds measured in SRB-assays with different human cancer cell lines 518A2 (melanoma), 8505C (thyroid carcinoma), A253 (submandibular carcinoma), A549 (lung epithelial carcinoma), A2780 (ovarian carcinoma), DLD-1 (colon adenocarcinoma), LIPO (lipocarcinoma), MCF-7 (breast adenocarcinoma), SW1736 (thyroid carcinoma) and non-malignant mouse fibroblasts (NiH 3T3) in comparison with betulinic acid **2**. The EC₅₀ values represent mean values obtained from three independent measurements each performed in triplicate (cut-off 30 μM). The values were obtained from the SRB assays after 96 h treatment of the cells with all the compounds. The values were averaged from at least 5 independent experiments and calculated applying the two-parametric Hill slope equation.

	518A2	8505C	A253	A549	A2780	DLD-1	LIPO	MCF-7	SW1736	NIH 3T3
2	11.9 ± 0.7	6.7 ± 0.1	11.1 ± 0.3	14.8 ± 0.4	11.0 ± 0.4	17.5 ± 0.6	9.7 ± 0.2	14.9 ± 0.9	11.6 ± 0.7	10.0 ± 0.6
7	19.3 ± 1.0	19.4 ± 1.1	22.3 ± 0.9	28.1 ± 1.2	10.2 ± 1.2	22.3 ± 1.7	> 30	14.9 ± 1.7	11.6 ± 1.3	10.0 ± 1.5
8	22.1 ± 1.6	15.9 ± 2.0	14.7 ± 1.1	> 30	13.1 ± 0.7	27.9 ± 2.3	> 30	23.3 ± 0.9	28.1 ± 1.9	22.2 ± 1.8
9	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30
10	17.1 ± 1.1	14.3 ± 1.0	13.9 ± 2.1	> 30	13.0 ± 0.9	19.0 ± 1.3	> 30	13.3 ± 1.7	22.8 ± 1.8	19.1 ± 2.3
14	20.0 ± 1.8	21.9 ± 2.3	13.0 ± 0.2	21.8 ± 0.9	26.8 ± 1.4	16.1 ± 2.5	22.8 ± 2.3	27.8 ± 2.8	20.8 ± 2.1	9.4 ± 0.8
15	5.7 ± 1.2	5.6 ± 0.8	5.6 ± 0.7	7.2 ± 0.6	7.4 ± 0.9	7.3 ± 0.4	8.5 ± 0.7	5.1 ± 1.0	6.3 ± 0.1	3.7 ± 0.5
16	20.9 ± 1.1	21.0 ± 2.1	19.1 ± 1.9	> 30	16.6 ± 2.3	21.2 ± 1.7	> 30	19.5 ± 2.0	23.9 ± 2.5	27.6 ± 2.1
17	10.8 ± 1.1	11.6 ± 0.9	> 30	13.7 ± 0.5	5.9 ± 0.1	7.3 ± 0.5	9.7 ± 0.4	6.8 ± 0.3	18.8 ± 1.3	10.1 ± 0.9
18	24.0 ± 1.9	15.2 ± 0.9	17.1 ± 2.3	> 30	11.4 ± 2.1	21.3 ± 2.3	> 30	22.4 ± 2.5	22.3 ± 1.7	27.1 ± 2.2
19	17.6 ± 1.6	13.4 ± 0.7	11.3 ± 1.5	> 30	10.2 ± 0.9	15.2 ± 1.5	> 30	15.1 ± 1.8	18.3 ± 0.9	18.9 ± 2.1
24	23.0 ± 1.6	12.7 ± 0.8	13.3 ± 1.1	> 30	6.1 ± 0.7	16.2 ± 1.3	> 30	12.4 ± 1.3	17.3 ± 0.8	20.9 ± 1.9
25	7.8 ± 0.6	9.9 ± 0.5	10.4 ± 1.1	15.4 ± 1.9	14.3 ± 2.0	10.1 ± 1.5	8.3 ± 0.9	10.3 ± 1.6	10.4 ± 1.5	10.9 ± 1.1
26	7.5 ± 1.1	8.4 ± 1.3	8.8 ± 0.9	11.4 ± 1.6	6.8 ± 1.0	8.6 ± 0.8	7.3 ± 0.9	9.1 ± 0.7	9.9 ± 0.9	5.8 ± 1.2
27	5.0 ± 0.3	3.4 ± 0.7	3.3 ± 0.3	7.1 ± 0.4	7.1 ± 0.2	6.3 ± 0.1	> 30	4.9 ± 0.8	7.1 ± 0.6	3.5 ± 0.7
28	6.5 ± 0.9	4.1 ± 0.6	5.1 ± 0.6	8.6 ± 0.5	8.9 ± 1.1	6.2 ± 0.7	> 30	5.9 ± 0.5	8.1 ± 0.4	5.3 ± 0.9

Conclusion

In summary, the formation of Mannich bases of betulinic or glycyrrhetic acid derivatives improved their cytotoxicity. The highest cytotoxicity was determined for the **27**, and EC₅₀ values as low as 3.3 μM were determined for this compound employing the human submandibular carcinoma cell line A253. As proven by AO/PI staining as well as by DNA-laddering experiments this compound acts by apoptosis.

Acknowledgements

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Experimental Section

Melting points are uncorrected (*Leica* hot stage microscope), NMR spectra were recorded using the Varian spectrometers Gemini 2000 or Unity 500 (δ given in ppm, J in Hz, internal Me₄Si; typical experiments: H-H-COSY, HMBC, HMQC, NOESY, DQF-COSY), ESI-MS spectra were taken on a Finnigan MAT LCQ 700 (electrospray, voltage 4.1 kV, sheath gas nitrogen) instrument. The optical rotation was measured on a Perkin-Elmer polarimeter at 20 °C; TLC was performed on silica gel (Merck 5554), column chromatography on silica gel (Merck KG60); elemental analyses were performed on a Vario EL (C-HNS). The solvents were dried according to usual procedures. The purity of the compounds were determined by HPLC and found to be > 98%. Glycyrrhetic acid was obtained from Orgentis Chemicals GmbH (Gatersleben) in bulk quantities.

The cultures of the cell were maintained as monolayer in RPMI 1640 (PAA Laboratories, Pasching, Germany) supplemented with 10% heat inactivated fetal bovine serum (Sigma AG,

Germany) and penicillin/streptomycin (PAA Laboratories) at 37 °C in a humidified atmosphere of 5% CO₂/ 95% air. The cytotoxicity of the compounds was evaluated using the sulforhodamine-B (SRB) (Sigma Aldrich)³⁴ micro-culture colorimetric assay as previously reported³⁵⁻³⁸. EC₅₀ values were calculated from semi logarithmic dose response curves by non-linear regression applying a two parametrical Hill-slope equation. Values are given with a confidence interval CI = 95%.

The Acridine orange/ propidium iodide dye exclusion assay was performed as previously reported³⁵⁻³⁸ employing approx. 500,000 cells (A2780). Visual analysis was performed under a fluorescence microscope. While green fluorescence showed apoptosis, a deep red colored nucleus indicated necrotic cells. The DNA laddering assay has been performed as previously described³⁵⁻³⁸.

Extraction of betulin 1 and lupeol 3

Dry birch bark (*Betula platyphylla*, 90 g) was finely crushed and extracted with hexanes or diethylether for 15 h in a Soxhlet apparatus. The solvent was removed, and impure material was obtained (9.45 g (10.5%) from the hexane extraction and 24.5 g (27.2%) from the diethylether extraction). By chromatographic work up (silica gel, toluene/ethyl acetate/heptane/formic acid, 80/20/10/3) from the hexane extract betulin (4.4 g, 47%) and lupeol (4.06 g, 43%) were obtained, while the diethylether extract consisted of lupeol (1.2 g, 5%) and betulin (19.8 g, 81%).

Betulonic acid 5

Betulonic acid was prepared from betulin 1 (18 g, 0.04 mol) by Jones oxidation as previously described¹⁵; **5** (17.02 g, 93%) was obtained as a colorless solid; mp 243-246 °C (lit.: 245 – 247¹⁵); [α]_D = 36.7° (*c* = 6.20, CHCl₃) (lit.: 45° (*c* = 5.1, CHCl₃)¹⁵; R_F = 0.51 (*n*-hexane/ethyl acetate, 8/2).

Prop-2-yn-1-yl 3-oxolup-20(29)-en-28-oate 7

Oxalyl chloride (1.4 mL, 16.3 mmol) was added to a solution of **5** (3.7 g, 8.13 mmol) in anhydrous DCM (10 mL), and the mixture was stirred at 25 °C for 5 hours. The volatiles were evaporated under reduced pressure, the residue (consisting of acid chloride **6**) was dissolved in anhydrous DCM (10 mL), evaporated (dissolving in DCM and evaporation was repeated 4 x) and finally dissolved in anhydrous THF (20 mL). Prop-2-yn-1-ol (1 mL, 18 mmol) was added, and stirring at 25 °C was continued for another 3 hours. The solvent was removed, and the residue was subjected to column chromatography (SiO₂, hexanes/ ethyl acetate, 8/2) to yield **7**^{18, 19} (2.8 g, 70%) as a colorless solid; mp 162-165 °C; [α]_D = 29.5° (*c* = 5.3, CHCl₃); R_F = 0.64 (*n*-hexane/ethyl acetate, 8/2); ¹H NMR (500 MHz, CDCl₃): δ = 4.77 (*d*, 1H, *J* = 2.1 Hz, H-29a), 4.74 (*dd*, 1H, *J* = 2.4, 15.5 Hz, H-31a), 4.66 (*dd*, 1H,

J = 2.5, 15.5 Hz, H-31b), 4.64 (*dd*, 1H, *J* = 1.4, 2.1 Hz, H-29b), 3.05 (*ddd*, 1H, *J* = 4.8, 10.9, 10.9 Hz, H-19), 2.51 (*ddd*, 1H, *J* = 7.6, 9.8, 15.7, H-2a), 2.46 (*t*, 1H, *J* = 2.4 Hz, H-33), 2.42 (*ddd*, 1H, *J* = 4.4, 7.6, 15.6 Hz, H-2b), 2.33-2.29 (*m*, 1H, H-16), 2.28-2.24 (*m*, 1H, H-13), 1.99-1.89 (*m*, 3H, H-1a + H-21a + H-22a), 1.78-1.72 (*m*, 1H, H-12), 1.71 (*s*, 3H, H-30), 1.65 (*t*, 1H, *J* = 11.4 Hz, H-18), 1.52-1.26 (*m*, 13H, H-9 + H-21b + H-16 + H-1b + H-7 + H-6 + H-5 + H-22b + H-15a + H-11), 1.24-1.20 (*m*, 1H, H-15b), 1.09 (*s*, 3H, H-24), 1.08-1.05 (*m*, 1H, H-12b), 1.04 (*s*, 3H, H-27), 1.01 (*s*, 3H, H-26), 0.99 (*s*, 3H, H-25), 0.95 (*s*, 3H, H-23) ppm; ¹³C NMR (500 MHz, CDCl₃): δ = 218.0 (C-3), 175.1 (C-28), 150.3 (C-20), 109.7 (C-29), 78.1 (C-32, C \equiv CH), 74.3 (C-33, C \equiv CH), 56.5 (C-17), 55.0 (C-5), 51.3 (C-31), 49.9 (C-18), 49.4 (C-9), 47.3 (C-4), 46.8 (C-19), 42.5 (C-8), 40.7 (C-14), 39.6 (C-1), 38.3 (C-13), 36.9 (C-2), 36.7 (C-10), 34.1 (C-22), 33.6 (C-7), 31.8 (C-16), 30.5 (C-21), 29.6 (C-15), 26.6 (C-23), 25.5 (C-12), 21.4 (C-11), 21.0 (C-30), 19.6 (C-6), 19.4 (C-24), 15.9 (C-26), 15.8 (C-25), 14.6 (C-27) ppm; MS (ESI, MeOH): *m/z* = 493.3 (53%, [M+H]⁺), 547.0 (100%, [M+Na+MeOH]⁺), 1007.2 (66%, [2M+Na]⁺); analysis calculated for C₃₃H₄₈O₃ (492.73) C 80.44, H 9.82; found: C 80.31, H 10.02.

3-Diisopropylaminoprop-2-yn-1-yl 3-oxolup-20(29)-en-28-oate hydrochloride 8

Compound **7** (200 mg, 0.41 mmol) was dissolved in THF (10 mL) and allowed to react at 40 °C with diisopropylamine (126 μ L, 0.9 mmol), formalin (37%, 0.16 mL, 2 mmol) and CuI (20 mg, 0.1 mmol) for 72 hours. The volatiles were removed under reduced pressure, the residue was dissolved in DCM (50 mL), washed with ammonium hydroxide (aq., 0.05 M) and water, dried (Na₂SO₄), the solvent was evaporated, and the residue subjected to column chromatography (SiO₂, hexanes/ethyl acetate, 8/2; methanol). Fractions containing the product were collected, the solvent was evaporated, the residue was dissolved in diethyl ether, and dry HCl gas was passed through for several minutes. After standing at 4 °C for 12 hours, the precipitate was filtered off, washed with water and diethyl ether to afford **8** (198 mg, 75%) as a colorless solid; mp 171-173 °C; [α]_D = 46.2° (*c* = 5.6, MeOH); ¹H NMR (400 MHz, CDCl₃): δ = 4.66 (*d*, 1H, *J* = 2.1 Hz, H-29a), 4.63-4.60 (*m*, 2H, H-31), 4.54 (*dd*, 1H, *J* = 1.4, 2.2 Hz, H-29b), 3.45-3.40 (*m*, 2H, H-34), 3.16 (*ddd*, 2H, *J* = 6.5, 6.5, 13.1 Hz, H-35), 2.98-2.88 (*m*, 1H, H-19), 2.42 (*ddd*, 1H, *J* = 7.5, 9.8, 15.8 Hz, H-2a), 2.32 (*ddd*, 1H, *J* = 4.4, 7.5, 15.6 Hz, H-2b), 2.23-2.13 (*m*, 2H, H-13 + H-21a), 1.89-1.77 (*m*, 3H, H-22a + H-16 + H-1a), 1.69-1.62 (*m*, 1H, H-12), 1.62 (*s*, 3H, H-30), 1.54 (*t*, 1H, *J* = 11.4 Hz, H-18), 1.44-1.08 (*m*, 16H, H-9 + H-21b + H-16 + H-1b + H-22b + H-7 + H-6 + H-5 + H-15 + H-11), 1.06 (*s*, 6H, H-36), 1.04 (*s*, 6H, H-36), 1.00 (*s*, 3H, H-24), 0.95 (*s*, 3H, H-27), 0.94-0.93 (*m*, 1H, H-12), 0.90 (*s*, 3H, H-26), 0.89 (*s*, 3H, H-25), 0.85 (*s*, 3H, H-23) ppm; ¹³C

NMR (125 MHz, CDCl₃): δ = 218.0 (C-3), 175.3 (C-28), 150.4 (C-20), 109.7 (C-29), 85.4 (C-32, C \equiv C), 77.9 (C-33, C \equiv C), 56.6 (C-17), 55.0 (C-5), 52.0 (C-31), 49.9 (C-9), 49.4 (C-18), 48.8 (C-35), 47.3 (C-4), 46.9 (C-19), 42.4 (C-8), 40.6 (C-14), 39.6 (C-1), 38.3 (C-13), 36.9 (C-10), 36.8 (C-22), 34.4 (C-34), 34.1 (C-2), 33.6 (C-7), 31.9 (C-21), 30.5 (C-16), 29.6 (C-15), 26.6 (C-23), 25.5 (C-12), 21.4 (C-11), 20.4 (C-36, 4 x CH₃), 19.6 (C-6), 19.3 (C-30), 15.9 (C-24 + C-26), 15.7 (C-25), 14.6 (C-27) ppm; MS (ESI, MeOH): m/z = 606.6 (100%, [M+H]⁺); analysis calculated for C₄₀H₆₄ClNO₃ (642.46): C 74.79, H 10.04, N 2.18; found: C 74.61, H 10.27, N 2.13.

3-Diallylaminoprop-2-yn-1-yl 3-oxolup-20(29)-en-28-oate hydrochloride 9

Following the procedure for the synthesis of **8**, from **7** (200 mg, 0.41 mmol), diallylamine (110 μ L, 0.9 mmol), formalin (37%, 0.16 mL, 2 mmol) and CuI (20 mg, 0.1 mmol) **9** (194 mg (64%) was obtained as a colorless solid; mp 195-198 °C; [α]_D = 36.8° (c = 5.5, MeOH); ¹H NMR (500 MHz, CD₃OD): δ = 6.08-5.96 (*m*, 2H, H-36), 5.75-5.64 (*m*, 4H, H-37), 4.90-4.87 (*m*, 2H, H-31), 4.74 (*d*, 1H, J = 1.6 Hz, H-29a), 4.64 (*dd*, 1H, J = 1.4, 2.2 Hz, H-29b), 4.16-4.14 (*m*, 2H, H-34), 3.96-3.89 (*m*, 4H, H-35), 3.02 (*ddd*, 1H, J = 4.9, 10.9, 10.9 Hz, H-19), 2.35-2.25 (*m*, 2H, H-13 + H-21a), 1.94-1.82 (*m*, 3H, H-22a + H-16 + H-1a), 1.80-1.75 (*m*, 2H, CH₂ (12)), 1.72 (*s*, 3H, H-30), 1.59-1.06 (*m*, 15H, H-9 + H-18 + H-21b + H-16 + H-1b + H-22b + H-7 + H-6 + H-5 + H-15 + H-11), 1.05 (*s*, 3H, H-24), 1.03 (*s*, 3H, H-27), 1.01 (*s*, 3H, H-26), 0.98 (*s*, 3H, H-25), 0.93 (*s*, 3H, H-23) ppm; ¹³C NMR (125 MHz, CDCl₃): δ = 218.0 (C-3), 175.2 (C-28), 150.1 (C-20), 126.6 (C-37), 125.8 (C-36), 109.1 (C-29), 86.1 (C-32, C \equiv C), 73.9 (C-33, C \equiv C), 56.6 (C-17), 54.8 (C-35), 54.6 (C-5), 50.9 (C-31), 49.7 (C-18), 49.2 (C-9), 47.1 (C-19), 46.9 (C-4), 42.3 (C-8), 41.0 (C-34), 40.5 (C-14), 39.2 (C-1), 38.4 (C-13), 36.7 (C-10), 36.3 (C-22), 33.3 (C-7), 31.4 (C-21), 30.2 (C-16), 29.4 (C-15), 25.8 (C-23), 25.4 (C-12), 21.2 (C-11), 19.8 (C-30), 19.4 (C-6), 18.1 (C-24), 15.2 (C-26), 15.1 (C-25), 13.7 (C-27) ppm; MS (ESI, MeOH): m/z = 602.6 (100%, [M+H]⁺); analysis calculated for C₄₀H₆₀ClNO₃ (638.36): C 75.26, H 9.47, N 2.19; found: C 75.13, H 9.65, N 2.11.

3-Morpholin-4-yl-prop-2-yn-1-yl 3-oxolup-20(29)-en-28-oate hydrochloride 10

Following the procedure given for the synthesis of **8**, from **7** (200 mg, 0.41 mmol), morpholine (39 mL, 4.46 mmol), formalin (37%, 0.16 mL, 2 mmol) and CuI (20 mg, 0.1 mmol) **10** (183 mg, 71%) was obtained as a colorless solid; mp 153-155 °C; [α]_D = 18.5° (c = 3.45, MeOH); ¹H NMR (400 MHz, CD₃OD): δ = 4.75-4.72 (*m*, 2H, H-31), 4.62 (*d*, 1H, J = 1.4 Hz, H-29a), 4.54-4.50 (*m*, 1H, H-29b), 4.13-4.09 (*m*, 2H, H-34), 4.06-3.94 (*m*, 2H, H-36a), 3.75-3.62 (*m*, 2H, H-36b), 3.53-3.38 (*m*, 2H, H-35a), 3.31-3.10 (*m*, 2H, H-35b), 2.89 (*ddd*, 1H, J = 4.8,

10.8, 10.8 Hz, H-19), 2.24-2.12 (*m*, 2H, H-13 + H-21a), 1.85-1.71 (*m*, 3H, H-22a + H-16 + H-1a), 1.69-1.57 (*m*, 5H, H-12 + H-30 + H-18), 1.50-0.98 (*m*, 14H, H-9 + H-21b + H-16 + H-1b + H-22b + H-7 + H-6 + H-5 + H-15 + H-11), 0.97 (*s*, 3H, H-24), 0.96-0.94 (*m*, 1H, H-12b), 0.94 (*s*, 3H, H-27), 0.92 (*s*, 3H, H-26), 0.90 (*s*, 3H, H-25), 0.85 (*s*, 3H, H-23) ppm; ¹³C NMR (125 MHz, CDCl₃): δ = 219.5 (C-3), 175.2 (C-28), 150.1 (C-20), 109.1 (C-29), 85.9 (C-32, C \equiv C), 73.5 (C-33, C \equiv C), 63.6 (C-36), 56.6 (C-17), 54.6 (C-5), 51.1 (C-35), 50.8 (C-31), 49.7 (C-9), 49.1 (C-18), 47.0 (C-19), 45.6 (C-4), 42.2 (C-8), 40.5 (C-14), 40.4 (C-34), 39.1 (C-1), 38.4 (C-13), 36.6 (C-10), 36.2 (C-22), 33.3 (C-7), 31.4 (C-21), 30.1 (C-16), 29.4 (C-15), 25.7 (C-23), 25.4 (C-12), 21.2 (C-11), 19.9 (C-30), 19.3 (C-6), 18.0 (C-24), 15.2 (C-26), 15.0 (C-25), 13.6 (C-27) ppm; MS (ESI, MeOH): m/z = 592.6 (100%, [M+H]⁺); analysis calculated for C₃₈H₅₈ClNO₄ (628.32): C 72.64, H 9.30, N 2.23; found: C 72.49, H 9.41, N 2.18.

3-Thiomorpholin-4-yl-prop-2-yn-1-yl 3-oxolup-20(29)-en-28-oate hydrochloride 11

Following the procedure for the synthesis of **8**, from **7** (200 mg, 0.41 mmol), thiomorpholine (116 μ L, 1.15 mmol), formalin (37%, 0.16 mL, 2 mmol) and CuI (20 mg, 0.1 mmol) **11** (147 mg, 61%) was obtained as a colorless solid; mp 173-178 °C; [α]_D = 31.2° (c = 3.1, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 4.67 (*d*, 1H, J = 2.0 Hz, H-29a), 4.64 (*dt*, 2H, J = 1.9, 9.3 Hz, H-31), 4.54 (*dd*, 1H, J = 1.4, 2.1 Hz, H-29b), 3.25 (*t*, 2H, J = 1.9 Hz, H-34), 2.98-2.90 (*m*, 1H, H-19), 2.76-2.61 (*m*, 8H, H-35 + H-36), 2.42 (*ddd*, 1H, J = 7.5, 9.8, 15.8 Hz, H-2a), 2.32 (*ddd*, 1H, J = 4.4, 7.5, 15.7 Hz, H-2b), 2.24-2.14 (*m*, 2H, H-13 + H-21a), 1.89-1.76 (*m*, 3H, H-22a + H-16 + H-1a), 1.69-1.63 (*m*, 1H, H-12), 1.62 (*s*, 3H, H-30), 1.55 (*t*, 1H, J = 11.4 Hz, H-18), 1.45-1.01 (*m*, 14H, H-9 + H-21b + H-16 + H-1b + H-22b + H-7 + H-6 + H-5 + H-15 + H-11), 1.00 (*s*, 3H, H-24), 0.99-0.96 (*m*, 1H, H-12b), 0.95 (*s*, 3H, H-27), 0.91 (*s*, 3H, H-26), 0.90 (*s*, 3H, H-25), 0.85 (*s*, 3H, H-23) ppm; ¹³C NMR (125 MHz, CDCl₃): δ = 218.0 (C-3), 175.2 (C-28), 150.4 (C-20), 109.7 (C-29), 87.1 (C-32, C \equiv C), 79.4 (C-33, C \equiv C), 56.6 (C-17), 55.0 (C-5), 53.8 (C-35), 51.7 (C-31), 49.9 (C-9), 49.4 (C-18), 48.4 (C-34), 47.3 (C-4), 46.9 (C-19), 42.5 (C-8), 40.7 (C-14), 39.6 (C-1), 38.3 (C-13), 36.9 (C-10), 36.8 (C-22), 34.1 (C-2), 33.6 (C-7), 31.9 (C-21), 30.5 (C-16), 29.6 (C-15), 28.0 (C-36), 26.6 (C-23), 25.5 (C-12), 21.4 (C-11), 19.6 (C-6), 19.3 (C-30), 15.9 (C-24 + C-26, 2 x CH₃), 15.8 (C-25), 14.6 (C-27) ppm; MS (ESI, MeOH): m/z = 608.5 (100%, [M+H]⁺); analysis calculated for C₃₈H₅₈ClNO₃S (644.39): C 70.83, H 9.07, N 2.17, S 4.98; found: C 70.68, H 9.24, N 2.03, S 4.77.

[3-(Dibenzylamino)-prop-2-yn-1-yl] 3-oxolup-20(29)-en-28-oate hydrochloride **12**

Following the procedure for the synthesis of **8**, from **7** (200 mg, 0.41 mmol), dibenzylamine (20 μ L, 4.46 mmol), formalin (37%, 0.16 mL, 2 mmol) and CuI (20 mg, 0.1 mmol) **12** (245 mg, 81%) was obtained as a colorless solid; mp 187-192 °C; $[\alpha]_D = 18.6^\circ$ ($c = 3.6$, MeOH); ^1H NMR (400 MHz, CD_3OD): $\delta = 7.62\text{-}7.47$ (*m*, 10H, 10 x CH (Ph)), 4.97 (*d*, 1H, $J = 15.9$ Hz, H-31a), 4.90 (*d*, 1H, $J = 16.0$ Hz, H-31b), 4.69 (*d*, 1H, $J = 0.9$ Hz, H-29a), 4.62-4.58 (*m*, 1H, H-29b), 4.55-4.46 (*m*, 4H, H-35), 3.88-3.84 (*m*, 2H, H-34), 3.03 (*ddd*, 1H, $J = 4.8$, 10.8, 10.8 Hz, H-19), 2.34-2.23 (*m*, 2H, H-13 + H-21a), 1.99-1.89 (*m*, 3H, H-22a + H-16 + H-1a), 1.76-1.78 (*m*, 5H, H-12 + H-30 + H-18), 1.60-1.05 (*m*, 14H, H-9 + H-21b + H-16 + H-1b + H-22b + H-7 + H-6 + H-5 + H-15 + H-11), 1.04 (*s*, 3H, H-24), 1.02 (*s*, 3H, H-27), 1.01-0.99 (*m*, 1H, H-12b), 0.99 (*s*, 3H, H-26), 0.88 (*s*, 3H, H-25), 0.85 (*s*, 3H, H-23) ppm; ^{13}C NMR (125 MHz, CD_3OD): $\delta = 219.5$ (C-3), 175.3 (C-28), 150.1 (C-20), 131.0 (Ph, 4 x CH), 130.2 (Ph, 2 x CH), 129.2 (Ph, 4 x CH), 128.8 (Ph, 2 x C_{quart}), 109.1 (C-29), 87.2 (C-32, C \equiv C), 73.5 (C-33, C \equiv C), 56.9 (C-35), 56.6 (C-17), 54.5 (C-5), 51.1 (C-31), 49.6 (C-9), 49.2 (C-18), 47.1 (C-19), 46.9 (C-4), 42.3 (C-8), 40.6 (C-14), 40.5 (C-34), 39.1 (C-1), 38.4 (C-13), 36.6 (C-10), 36.3 (C-22), 33.3 (C-7), 31.4 (C-21), 30.2 (C-16), 29.4 (C-15), 25.8 (C-23), 25.3 (C-12), 21.2 (C-11), 19.9 (C-30), 19.3 (C-6), 18.1 (C-24), 15.2 (C-26), 15.1 (C-25), 13.6 (C-27) ppm; MS (ESI, MeOH): $m/z = 702.5$ (100%, $[\text{M}-\text{Cl}]^+$); MS (ESI, CD_3OD): $m/z = 703.7$ (46%, $[\text{M}+\text{D}]^+$), 704.6 (100%, $[\text{M}-\text{H}+2\text{D}]^+$), 705.5 (60%, $[\text{M}-2\text{H}+3\text{D}]^+$); analysis calculated for $\text{C}_{48}\text{H}_{64}\text{ClNO}_3$ (738.48): C 78.07, H 8.74, N 1.90; found: C 77.68, H 8.83, N 1.74.

Methyl 3-oxolup-20(29)-en-28-oate **13**

Compound **13** was obtained by esterification of **5** as previously described²⁰.

Methyl (3 β) 3-ethynyl-3-hydroxylup-20(29)-en-28-oate **14**

From **13** (4.69 g, 10.0 mmol) and ethynylmagnesium bromide; compound **4** (3.38 g, 68%) was obtained as a white solid as previously reported¹⁴.

Methyl (3 β) 3-[3-(diisopropylamino)prop-1-yn-1-yl]-3-hydroxylup-20(29)-en-28-oate hydrochloride **15**

Compound **15** was prepared (as described for the synthesis of **24**) from **14** (297 mg, 0.6 mmol), diisopropylamine (0.09 mL, 0.64 mmol), formalin (37%, 0.25 mL, 2.5 mmol) and CuI (2 mg, 0.01 mmol) and **15** (155 mg, 48%) was obtained as a colorless solid; mp 215 °C; $[\alpha]_D = -7.7^\circ$ ($c = 6.15$, MeOH); ^1H NMR (500 MHz, CD_3OD): $\delta = 4.71$ (*m*, 1H, H-29a), 4.60 (*m*, 1H, H-29b), 4.21 (*s*, 2H, H-34), 3.90 (*sept.*, 2H, $J = 6.7$ Hz, H-35 + H-38), 3.65

(*s*, 3H, H-31), 2.99 (*ddd*, 1H, $J = 10.6$, 10.6, 5.1 Hz, H-19), 2.29-2.21 (*m*, 2H, H-13 + H-16), 1.96 (*m*, 1H, H-2a), 1.90-1.82 (*m*, 2H, H-21a + H-22a), 1.76-1.65 (*m*, 3H, H-1a + H-12 + H-2b), 1.69 (*s*, 3H, H-30), 1.64 (*dd*, 1H, $J = 11.4$, 11.4 Hz, H-18), 1.54-1.21 (*m*, 12H, H-9 + H-1b + H-22b + H-7a + H-7b + H-16 + H-21b + H-15a + H-11a + H-11b + H-6a + H-6b), 1.47 (*d*, 12H, $J = 6.7$ Hz, C-36 + C-37 + C-39 + C-40), 1.20-1.14 (*m*, 2H, H-5 + H-15b), 1.10-1.01 (*m*, 1H, H-12b), 1.05 (*s*, 3H, H-24), 0.99 (*s*, 3H, H-27), 0.94 (*s*, 3H, H-26), 0.88 (*s*, 3H, H-25), 0.85 (*s*, 3H, H-23) ppm; ^{13}C NMR (125 MHz, CD_3OD): $\delta = 178.1$ (C-28), 151.8 (C-20), 110.3 (C-29), 94.9 (C-32, C \equiv CH), 76.6 (C-33, C \equiv CH), 74.4 (C-3), 57.9 (C-17), 55.8 (C-35 + C-38), 55.3 (C-5), 52.3 (C-31), 51.8 (C-18), 50.7 (C-9), 48.4 (C-19), 43.6 (C-14), 42.9 (C-4), 41.9 (C-8), 39.6 (C-13), 39.2 (C-1), 38.4 (C-10), 37.8 (C-22), 37.1 (C-34), 35.6 (C-7), 33.2 (C-2), 33.1 (C-16), 31.6 (C-21), 30.8 (C-15), 26.9 (C-12), 26.6 (C-24), 22.0 (C-11), 19.6 (C-6), 19.6 (C-30), 18.2 (C-23), 18.2 (C-36 + C37 + C39 + C40), 17.0 (C-25), 16.5 (C-26), 15.4 (C-27) ppm; MS (ESI, MeOH): $m/z = 608.5$ (100% $[\text{M}+\text{H}]^+$); analysis calculated for $\text{C}_{40}\text{H}_{66}\text{ClNO}_3$ (644.41): C, 74.55; H, 10.32; N, 2.17; found: C, 74.36; H, 10.56; N, 2.11.

(3 β) 3-Hydroxy-lup-20(29)-en-28-yl acetate

16

Betulin **1** (10.0 g, 22 mmol) was acetylated as previously described, and **16** (7.8 g, 73%) was obtained as a colorless solid; mp 210-212 °C (lit.: 210-212 °C³⁹); $[\alpha]_D = 7.4^\circ$ ($c = 5.7$, CHCl_3), 8.5° ($c = 1.58$, CHCl_3)³⁹; $R_F = 0.49$ (*n*-hexane/ethyl acetate, 8/2).

3-Oxolup-20(29)-en-28-yl acetate **17**

Swern oxidation of **16** (10 g, 22 mmol) as described above followed by an extraction with DCM (4 x 50 mL) and column chromatography (SiO_2 , hexanes/ethyl acetate, 8/2) gave **17** (4.98 g, 81%) as a colorless solid; mp 89-91 °C (lit.: 77-79 °C²²); $[\alpha]_D = 36.1^\circ$ ($c = 5.1$, CHCl_3); $R_F = 0.66$ (*n*-hexane/ethyl acetate, 8/2); ^1H NMR (400 MHz, CDCl_3): $\delta = 4.62$ (*d*, 1H, $J = 1.8$ Hz, H-29a), 4.52 (*m*, 1H, H-29b), 4.19 (*dd*, 1H, $J = 1.5$, 11.1 Hz, H-28a), 3.79 (*d*, 1H, $J = 10.9$ Hz, H-28b), 2.47-2.26 (*m*, 1H, H-19 + H-2), 2.00 (*s*, 3H, H-32), 1.95-1.62 (*m*, 5H, H-21a + H-16 + H-22a + H-1a + H-13), 1.62 (*s*, 3H, H-30), 1.60-1.49 (*m*, 3H, H-18 + H-12 + H-15a), 1.43-1.11 (*m*, 11H, H-6 + H-21b + H-7 + H-11 + H-9 + H-16 + H-1b + H-5), 1.07-1.01 (*m*, 2H, H-15b + H-12b), 1.00 (*s*, 6H, H-27 + H-25), 0.99 (*m*, 1H, H-22b), 0.94 (*s*, 3H, H-24), 0.92 (*s*, 3H, H-26), 0.86 (*s*, 3H, H-23) ppm; ^{13}C NMR (125 MHz, CDCl_3): $\delta = 218.1$ (C-3), 171.6 (C-31), 150.0 (C-20), 109.9 (C-29), 62.7 (C-28), 54.9 (C-5), 49.7 (C-9), 48.7 (C-18), 47.6 (C-19), 47.3 (C-4), 46.3 (C-17), 42.7 (C-14), 40.8 (C-8), 39.6 (C-1), 37.6 (C-13), 36.9 (C-10), 34.5 (C-22), 34.1 (C-2), 33.5 (C-7), 29.7 (C-21), 29.6 (C-16), 27.0 (C-12), 26.5 (C-23), 25.2 (C-15), 21.3 (C-11), 21.0 (C-32), 21.0 (C-30),

19.6 (C-6), 15.9 (C-24 + C-26), 15.8 (C-25), 14.7 (C-27) ppm; MS (ESI, MeOH): m/z = 483.3 (32%, $[M+H]^+$), 537.1 (49%, $[M+Na+MeOH]^+$), 746.5 (100%, $[3M+2Na]^+$), 987.3 (67%, $[2M+Na]^+$); analysis calculated for $C_{32}H_{50}O_3$ (482.74): C 79.62, H 10.64; found: C 79.55, H 10.79.

(3 β) 3-Ethynyl-3-hydroxy-lup-20(29)-en-28-yl acetate 18

As described for the synthesis of **14**, reaction of **17** (4.2 g, 8.7 mmol) with ethynylmagnesium bromide (0.5 M, 52 mL, 26 mmol) followed by chromatographic workup (column, SiO₂, hexanes/ethyl acetate, 8/2) yielded **18** (3.35 g, 75%) as a colorless solid; mp 109-110 °C; $[\alpha]_D = 14.3^\circ$ ($c = 3.8$, CHCl₃); $R_F = 0.55$ (*n*-hexane/ethyl acetate, 8/2); ¹H NMR (400 MHz, CDCl₃): $\delta = 4.66$ (*d*, 1H, $J = 1.6$ Hz, H-29a), 4.56 (*d*, 1H, $J = 1.2$ Hz, H-29b), 4.23 (*d*, 1H, $J = 10.9$ Hz, H-28a), 3.84 (*d*, 1H, $J = 11.0$ Hz, H-28b), 2.47-2.36 (*m*, 2H, H-19 + H-34), 2.04 (*s*, 3H, H-32), 2.00-1.85 (*m*, 2H, H-21a + H-2a), 1.85-1.79 (*m*, 1H, H-16), 1.74 (*dd*, 1H, $J = 8.4$, 12.4 Hz, H-22a), 1.71-1.67 (*m*, 1H, H-1a), 1.65 (*s*, 3H, H-30), 1.64-1.52 (*m*, 5H, H-18 + H-2b + H-13 + H-12 + H-15a), 1.51-1.20 (*m*, 10H, H-6 + H-21b + H-1b + H-7 + H-11a + H-12b + H-9 + H-16), 1.19-1.04 (*m*, 4H, H-22b + H-15b + H-11b + H-5), 1.03 (*s*, 3H, H-27), 1.01 (*s*, 3H, H-25), 0.97 (*s*, 3H, H-24), 0.84 (*s*, 3H, H-26), 0.81 (*m*, 3H, H-23) ppm; ¹³C NMR (125 MHz, CDCl₃): $\delta = 171.6$ (C-31), 150.1 (C-20), 109.8 (C-29), 87.2 (C-33, C \equiv CH), 75.7 (C-3), 73.5 (C-34, C \equiv CH), 62.8 (C-28), 53.1 (C-5), 50.4 (C-9), 48.8 (C-18), 47.7 (C-19), 46.3 (C-17), 42.7 (C-14), 41.3 (C-1), 40.9 (C-8), 38.9 (C-4), 37.6 (C-13), 37.1 (C-10), 34.5 (C-7), 34.2 (C-22), 29.8 (C-21), 29.6 (C-16), 27.2 (C-12), 25.6 (C-23), 25.6 (C-15), 25.2 (C-2), 21.0 (C-32), 20.8 (C-11), 19.1 (C-30), 18.5 (C-6), 17.4 (C-24), 16.4 (C-26), 16.0 (C-25, CH₃), 14.9 (C-27) ppm; MS (ESI, MeOH): m/z = 531.5 (22%, $[M+Na]^+$), 563.1 (34%, $[M+MeOH]^+$), 1039.3 (100%, $[2M+H]^+$); analysis calculated for $C_{34}H_{52}O_3$ (508.77): C 80.26, H 10.30; found: C 80.02, H 10.41.

(3 β) 3-[3-(Diisopropylamino)-prop-1-yn-1-yl]-3-hydroxylup-20(29)-en-28-yl acetate 19

Following the procedure given for **8**, from the reaction of **18** (500 mg, 1 mmol), diisopropylamine (160 μ L, 1.15 mmol), formalin (37%, 0.4 mL, 5 mmol) and CuI (20 mg, 0.1 mmol) followed by chromatographic workup (column, SiO₂, hexanes/ethyl acetate, 8/2) **19** (464 mg, 74%) was obtained as a colorless solid; mp 132-135 °C; $[\alpha]_D = 7.3^\circ$ ($c = 3.7$, CHCl₃); $R_F = 0.04$ (*n*-hexane/ethyl acetate, 8/2); ¹H NMR (400 MHz, CDCl₃): $\delta = 4.63$ (*d*, 1H, $J = 1.7$ Hz, H-29a), 4.55-4.51 (*m*, 1H, H-29b), 4.19 (*d*, 1H, $J = 10.4$ Hz, H-28a), 3.91-3.85 (*s br*, 1H, H-33), 3.78 (*d*, 1H, $J = 11.1$ Hz, H-28b), 3.68-3.50 (*m*, 2H, H-34), 2.38 (*ddd*, 1H, $J = 5.9$, 11.1, 11.2 Hz, H-19), 2.00 (*s*, 3H, CH₃ (37)), 1.95-1.63 (*m*, 6H, H-21a + H-16 + H-22a + H-15a + H-13

+ H-2a), 1.62 (*s*, 3H, H-30), 1.61-1.48 (*m*, 4H, H-1a + H-2b + H-18 + H-12), 1.46-0.98 (*m*, 26H, H-35 + H-6 + H-21b + H-1b + H-7 + H-11 + H-15b + H-5 + H-12b + H-22b + H-9 + H-16), 0.96 (*s*, 3H, H-27), 0.95 (*s*, 3H, H-25), 0.89 (*s*, 3H, H-24), 0.80 (*s*, 3H, H-26), 0.77 (*s*, 3H, H-23) ppm; ¹³C NMR (125 MHz, CDCl₃): $\delta = 171.6$ (C-36), 150.1 (C-20), 109.9 (C-29), 90.4 (C-31, C \equiv C), 76.0 (C-3), 75.0 (C-32, C \equiv C), 62.7 (C-28), 53.9 (C-5 + C-34), 50.8 (C-9), 48.8 (C-18), 47.7 (C-19), 46.3 (C-17), 42.7 (C-14), 41.7 (C-4), 40.9 (C-8), 38.1 (C-1), 37.6 (C-13), 37.2 (C-10), 34.5 (C-22 + C33, 2 x CH₂), 34.3 (C-7), 32.6 (C-2), 29.7 (C-21), 29.6 (C-16), 27.0 (C-15), 26.0 (C-23), 25.2 (C-12), 21.0 (C-37), 20.8 (C-11), 19.2 (C-30 + C-35), 18.5 (C-6), 17.5 (C-24), 16.4 (C-26), 16.0 (C-25), 14.9 (C-27) ppm; MS (ESI, MeOH): m/z = 622.6 (100%, $[M+H]^+$); analysis calculated for $C_{41}H_{67}NO_3$ (621.98): C 79.12, H 10.86; found: C 78.98, H 10.92, N 10.61.

Methyl (3 β) 3-hydroxy-11-oxo-olean-12-en-30-oate 20

Esterification of glycyrrhetic acid (**4**, 5.0 g, 10.6 mmol) in DMF (50 mL) with potassium carbonate (2.49 g, 18 mmol) and methyl iodide (806 μ L, 12.9 mmol) as previously described followed by chromatographic workup (column, SiO₂, hexanes/ethyl acetate, 8/2) furnished **20** (4.98 g, 97%) as a colorless solid; mp 254-256 °C (lit.: 254-258 °C); $[\alpha]_D = 141.4^\circ$ ($c = 0.32$, CHCl₃); $R_F = 0.27$ (*n*-hexane/ethyl acetate, 8/2).

Benzyl (3 β) 3-hydroxy-11-oxo-olean-12-en-30-oate 21

Reaction of **4** (5.0 g, 10.6 mmol) in DMF (75 mL) with potassium carbonate (2.49 g, 18 mmol) and benzyl bromide (1.5 mL, 12.9 mmol) followed by chromatographic workup (column, SiO₂, hexanes/ethyl acetate, 8/2) gave **21**²⁴ (4.46 g, 75%) as a colorless solid; mp 137-139 °C; $[\alpha]_D = 137.4^\circ$ ($c = 0.20$, CHCl₃); $R_F = 0.45$ (*n*-hexane/ethyl acetate, 4:1); ¹H NMR (500 MHz, CDCl₃): $\delta = 7.33$ -7.23 (*m*, 5H, phenyl), 5.48 (*s*, 1H, H-12), 5.13 (*d*, 1H, $J = 12.2$ Hz, H-31a), 5.02 (*d*, 1H, $J = 12.2$ Hz, H-31b), 3.15 (*dd*, 1H, $J = 5.5$, 10.8 Hz, H-3), 2.72 (*ddd*, 1H, $J = 3.5$, 3.5, 13.4 Hz, H-1a), 2.25 (*s*, 1H, H-9), 2.00-1.89 (*m*, 3H, H-18 + H-21a + H-16), 1.86 (*ddd*, 1H, $J = 2.7$, 4.1, 13.6 Hz, H-19a), 1.78-1.69 (*m*, 1H, H-15a), 1.65-1.48 (*m*, 5H, CH₂ (2) + H-22a + H-19b + H-6a), 1.47-1.17 (*m*, 8H, H-7 + H-21b + H-22b + H-6b + H-27), 1.13-1.08 (*m*, 4H, H-28 + H-16), 1.06 (*s*, 3H, H-26), 1.04 (*s*, 3H, H-25), 0.96-0.85 (*m*, 5H, H-1b + H-23 + H-15b), 0.73 (*s*, 3H, H-24), 0.66 (*s*, 3H, H-29), 0.62 (*d*, 1H, $J = 10.1$ Hz, H-5) ppm; ¹³C NMR (100 MHz, CDCl₃): $\delta = 200.1$ (C-11), 176.2 (C-30), 168.9 (C-13, C=CH), 136.1 (Ph), 128.6 (C-12, C=CH), 128.6 (Ph), 128.5 (Ph), 128.3 (Ph), 128.2 (Ph), 128.2 (Ph), 78.7 (C-3), 66.2 (C-31), 61.8 (C-9), 54.9 (C-5), 48.2 (C-18), 45.3 (C-14), 43.9 (C-20), 43.1 (C-8), 41.1 (C-19), 39.1 (C-1), 39.1 (C-4), 37.6 (C-22), 37.0 (C-10), 32.7 (C-7), 31.7(C-17), 31.1

(C-21), 28.4 (C-29), 27.3 (C-28), 28.1 (C-23), 27.3 (C-2), 26.4 (C-15 + C16), 23.6 (C-27), 18.6 (C-26), 17.5 (C-6), 16.3 (C-25), 15.5 (C-24) ppm; MS (ESI, MeOH): $m/z = 561.5$ (56%, $[M+H]^+$), 583.4 (26%, $[M+Na]^+$), 863.9 (10%, $[3M+2Na]^{2+}$), 1121.2 (54%, $[2M+H]^+$), 1143.3 (100%, $[2M+Na]^+$); analysis calculated for $C_{37}H_{52}O_4$ (560.81): C 79.24, H 9.35; found: 79.03, H 9.51.

Methyl 3,11-dioxo-olean-12-en-30-oate 22

Swern oxidation of **20** (4.98 g, 10.1 mmol) with DMSO (1.4 mL, 20 mmol), oxalyl chloride (1.7 mL, 20 mmol) and triethylamine (42 mL, 0.3 mol) as described above followed by chromatographic workup (column, SiO₂, hexanes/ethyl acetate, 8/2) gave **22** (3.95 g, 87%) as a colorless solid; mp 242-243 °C; $[\alpha]_D = 187.7^\circ$ ($c = 0.61$, CHCl₃); $R_F = 0.50$ (*n*-hexane/ethyl acetate, 8/2).²⁵

Benzyl 3,11-dioxo-olean-12-en-30-oate 23

Jones oxidation of **21** (2.0 g, 3.5 mmol) as described above followed by extraction with DCM (Soxhlet apparatus, 12 h) and column chromatography (SiO₂, hexanes/ethyl acetate, 8/2) gave **23**²⁵ (1.57 g, 87%) as a colorless solid; mp 136-138 °C; $[\alpha]_D = 185.5^\circ$ ($c = 4.45$, CHCl₃); $R_F = 0.25$ (*n*-hexane/ethyl acetate, 4/1); ¹H NMR (500 MHz, CDCl₃): $\delta = 7.34-7.23$ (*m*, 5H, arom), 5.51 (*s*, 1H, H-12), 5.14 (*d*, 1H, $J = 12.2$ Hz, H-31a), 5.02 (*d*, 1H, $J = 12.2$ Hz, H-31b), 2.89 (*ddd*, 1H, $J = 4.1, 7.1, 13.6$ Hz, H-1a), 2.56 (*ddd*, 1H, $J = 7.1, 11.2, 15.8$ Hz, H-2a), 2.35 (*s*, 1H, H-9), 2.29 (*ddd*, 1H, $J = 4.0, 6.5, 15.8$ Hz, H-2b), 2.00 (*dd*, 1H, $J = 3.9, 9.0$ Hz, H-18), 1.97-1.90 (*m*, 2H, H-21a + H-16), 1.87 (*ddd*, 1H, $J = 2.8, 4.1, 13.6$ Hz, H-19a), 1.81-1.71 (*m*, 1H, H-15a), 1.65-1.43 (*m*, 4H, H-22a + H-19b + H-6), 1.41-1.16 (*m*, 12H, H-5 + H-1b + H-7 + H-21b + H-28 + H-22b + H-27), 1.12-1.06 (*m*, 7H, H-26 + H-25 + H-16), 1.03 (*s*, 3H, H-23), 1.00 (*s*, 3H, H-24), 0.97-0.90 (*m*, 1H, H-15b), 0.68 (*s*, 3H, H-29) ppm; ¹³C NMR (125 MHz, CDCl₃): $\delta = 217.1$ (C-3), 199.3 (C-11), 176.1 (C-30), 169.4 (C-13), 136.1 (Ph), 128.6 (C-12), 128.4 (Ph), 128.3 (Ph), 128.3 (Ph), 128.2 (Ph), 128.2 (Ph), 66.2 (C-31), 61.0 (C-9), 55.4 (C-5), 48.2 (C-18), 47.8 (C-4), 45.2 (C-14), 43.9 (C-20), 43.3 (C-8), 41.1 (C-19), 39.8 (C-1), 37.6 (C-22), 36.7 (C-10), 34.2 (C-2), 32.1 (C-7), 31.8 (C-17), 31.1 (C-21), 28.4 (C-29), 28.3 (C-28), 26.4 (C-15 + C-16), 26.4 (C-23), 23.3 (C-27), 21.4 (C-24), 18.8 (C-6), 18.5 (C-26), 15.6 (C-25) ppm; MS (ESI, MeOH): $m/z = 559.6$ (76%, $[M+H]^+$), 581.5 (20%, $[M+Na]^+$), 866.9 (50%, $[3M+2Na]^{2+}$), 1117.3 (72%, $[2M+H]^+$), 1139.2 (100%, $[2M+Na]^+$); analysis calculated for $C_{37}H_{50}O_4$ (558.79): C 79.53, H 9.02; found: C 79.41, H 9.17.

Methyl (3 β)-3-ethynyl-3-hydroxy-11-oxo-olean-12-en-30-oate 24

Reaction of **22** (500 mg, 1.03 mmol) in anhydrous THF (30 mL) with ethynylmagnesium bromide (0.5 M in THF, 4 mL, 2 mmol) at 25 °C for

72 hours followed by chromatography (SiO₂, hexanes/ethyl acetate, 8/2) yielded **24** (450 mg, 86%) as a colorless solid; mp 231-235 °C (lit.: 247-247.5 °C²⁶); $[\alpha]_D = 112.6^\circ$ ($c = 3.1$, CHCl₃); $R_F = 0.27$ (*n*-hexane/ethyl acetate, 8/2); ¹H NMR (500 MHz, CDCl₃): $\delta = 5.66$ (*s*, 1H, H-12), 3.68 (*s*, 3H, CH₃ (31)), 3.00-2.78 (*ddd*, 1H, $J = 3.4, 3.4, 13.8$ Hz, H-1a), 2.45 (*s*, 1H, H-33), 2.42 (*s*, 1H, H-9), 2.08 (*dd*, 1H, $J = 3.8, 14.1$ Hz, H-18), 2.05-1.97 (*m*, 3H, H-21a + H-2a + H-16), 1.90 (*ddd*, 1H, $J = 2.8, 4.1, 13.6$ Hz, H-19a), 1.83 (*m*, 1H, H-15a), 1.73-1.53 (*m*, 4H, H-7a + H-2b + H-19b + H-6a), 1.47-1.28 (*m*, 9H, H-27 + H-6b + H-7b + H-16 + H-1b + H-22), 1.22-1.15 (*m*, 2H, H-21b + H-5), 1.14 (*s*, 6H, H-28 + H-25), 1.12 (*s*, 3H, H-26), 1.08 (*s*, 3H, H-23), 1.04-0.98 (*m*, 1H, H-15b), 0.90 (*s*, 3H, H-24), 0.80 (*s*, 3H, H-29) ppm; ¹³C NMR (125 MHz, CDCl₃): $\delta = 200.1$ (C-11), 176.9 (C-30), 169.1 (C-13, C=CH), 128.4 (C-12), 87.0 (C-32, C \equiv CH), 75.4 (C-33, C \equiv CH), 73.5 (C-3), 61.9 (C-9), 52.5 (C-5), 51.7 (C-31), 48.3 (C-18), 45.3 (C-14), 44.0 (C-20), 43.1 (C-8), 41.3 (C-19), 41.1 (C-4), 38.1 (C-1), 37.7 (C-22), 37.0 (C-10), 32.6 (C-7), 32.4 (C-2), 31.8 (C-21), 31.1 (C-17), 28.5 (C-29), 28.2 (C-28), 26.4 (C-15 + C-16), 25.7 (C-23), 23.4 (C-27), 18.6 (C-26), 17.6 (C-6), 17.6 (C-25), 16.7 (C-24) ppm; MS (ESI, MeOH): $m/z = 509.6$ (100%, $[M+H]^+$), 1017.3 (94%, $[2M+H]^+$), 1039.3 (51%, $[2M+Na]^+$); analysis calculated for $C_{33}H_{48}O_4$ (508.73): C 77.91, H 9.51; found: C 77.85, H 9.63.

Benzyl (3 β)-3-ethynyl-3-hydroxy-11-oxo-olean-12-en-30-oate 25

From the reaction of **23** (2.0 g, 4 mmol) with ethynylmagnesium bromide (0.5 M in THF, 20 mL, 10 mmol) as described above followed by chromatographic workup (column, SiO₂, hexanes/ethyl acetate, 8/2) gave **25** (1.58 g, 75%) as a colorless solid; mp 176-178 °C; $[\alpha]_D = 1200^\circ$ ($c = 0.56$, CHCl₃); $R_F = 0.33$ (*n*-hexane/ethyl acetate, 8/2); ¹H NMR (500 MHz, CDCl₃): $\delta = 7.34-7.23$ (*m*, 5H, arom), 5.49 (*s*, 1H, H-12), 5.13 (*d*, 1H, $J = 12.3$ Hz, H-31a), 5.02 (*d*, 1H, $J = 12.3$ Hz, H-31b), 2.71 (*ddd*, 1H, $J = 3.5, 3.5, 13.8$ Hz, H-1a), 2.38 (*s*, 1H, H-33), 2.33 (*s*, 1H, H-9), 2.08-1.90 (*m*, 4H, H-18 + H-21a + H-2a + H-15a), 1.86 (*ddd*, 1H, $J = 2.8, 4.3, 13.7$ Hz, H-19a), 1.79-1.69 (*m*, 1H, H-16), 1.66-1.43 (*m*, 4H, H-7a + H-2b + H-19b + H-6a), 1.41-1.32 (*m*, 2H, H-6b + H-1b), 1.31 (*s*, 3H, H-27), 1.30-1.16 (*m*, 4H, H-21b + H-7b + H-22), 1.13-1.05 (*m*, 8H, H-28 + H-25 + H-16 + H-5), 1.04 (*s*, 3H, H-26), 1.01 (*s*, 3H, H-23), 0.96-0.89 (*m*, 1H, H-15b), 0.81 (*s*, 3H, H-24), 0.67 (*s*, 3H, H-29) ppm; ¹³C NMR (125 MHz, CDCl₃): $\delta = 200.1$ (C-11), 176.2 (C-30), 169.0 (C-13), 136.1 (Ph), 128.6 (Ph), 128.5 (C-12), 128.3 (Ph), 128.2 (Ph), 87.1 (C-32, C \equiv CH), 75.4 (C-33, C \equiv CH), 73.5 (C-3), 66.2 (C-31), 61.9 (C-9), 52.6 (C-5), 48.2 (C-18), 45.3 (C-14), 44.0 (C-20), 43.1 (C-8), 41.4 (C-19), 41.1 (C-4), 38.1 (C-1), 37.7 (C-22), 37.0 (C-10), 32.7 (C-7), 32.5 (C-2), 31.8 (C-21), 31.2 (C-17), 28.4 (C-29), 28.3 (C-28), 26.5

(C-15), 26.4 (C-16), 25.8 (C-23), 23.4 (C-27), 18.7 (C-26), 17.7 (C-6), 17.6 (C-25), 16.8 (C-24) ppm; MS (ESI, MeOH): m/z = 585.5 (38%, [M+H]⁺), 607.4 (8%, [M+Na]⁺), 1169.4 (94%, [2M+H]⁺), 1191.1 (100%, [2M+Na]⁺); analysis calculated for C₃₉H₅₂O₄ (584.83x): C 80.09, H 8.96; found: C 79.77, H 9.04.

Methyl (3β) 3-[3-(diallylamino)-prop-1-yn-1-yl]-3-hydroxy-11-oxo-olean-12-en-30-oate hydrochloride 26

Mannich reaction of **24** (1 g, 2 mmol) in DMSO (7 mL) with diallylamine (284 μL, 2.3 mmol), formalin (37%, 0.8 mL, 10 mmol) and CuI (20 mg, 0.1 mmol) as described above followed by chromatographic workup (column, SiO₂, hexanes/ethyl acetate, 8/2) gave **26** (948 mg, 81%) as a colorless solid; mp 244-247 °C; [α]_D = 742° (*c* = 6.0, CHCl₃); R_F = 0.05 (*n*-hexane/ethyl acetate, 8/2); ¹H NMR (500 MHz, CDCl₃): δ = 6.03-5.92 (*m*, 2H, H-36), 5.67 (*s*, 1H, H-12), 5.59-5.49 (*m*, 4H, H-37), 3.93 (*s*, 2H, H-34), 3.73-3.64 (*m*, 7H, H-31 + H-35), 2.92-2.84 (*m*, 1H, H-1a), 2.34 (*s*, 1H, H-9), 2.12-1.96 (*m*, 4H, H-18 + H-21a + H-2a + H-16), 1.93-1.86 (*m*, 1H, H-19a), 1.82 (*ddd*, 1H, *J* = 4.3, 13.6, 13.6 Hz, H-15a), 1.74-1.35 (*m*, 7H, H-7 + H-2b + H-19b + H-22a + H-6), 1.34 (*s*, 3H, H-27), 1.33-1.16 (*m*, 4H, H-16 + H-1b + H-21b + H-22b), 1.15 (*s*, 6H, H-28 + H-25), 1.12 (*s*, 3H, H-26), 1.09 (*s*, 3H, H-23), 1.08-0.99 (*m*, 2H, H-5 + H-15b), 0.92 (*s*, 3H, H-24), 0.80 (*s*, 3H, H-29) ppm; ¹³C NMR (125 MHz, CDCl₃): δ = 199.8 (C-11), 176.9 (C-30), 169.7 (C-13), 128.4 (C-12), 127.0 (C-36), 124.0 (C-37), 83.2 (C-32, C≡C), 75.8 (C-3), 74.0 (C-33, C≡C), 62.0 (C-9), 55.4 (C-35), 53.5 (C-5), 51.8 (C-31), 48.4 (C-18), 45.3 (C-14), 44.2 (C-20), 43.2 (C-8), 41.6 (C-34), 41.6 (C-4), 41.3 (C-19), 38.5 (C-1), 37.7 (C-22), 37.1 (C-10), 32.7 (C-7), 32.5 (C-2), 31.8 (C-21), 31.0 (C-17), 28.5 (C-29), 28.3 (C-28), 26.4 (C-15 + C-16), 26.3 (C-23), 23.4 (C-27), 18.6 (C-26), 17.7 (C-6), 17.7 (C-25), 16.7 (C-24) ppm; MS (ESI, MeOH): m/z = 618.5 (100%, [M+H]⁺); analysis calculated for C₄₀H₅₉NO₄ (617.90): C 77.75, H 9.62, N 2.27; found: C 77.63, H 9.74, N 2.12.

Methyl (3β) 3-[3-(diisopropylamino)-prop-1-yn-1-yl]-3-hydroxy-11-oxo-olean-12-en-30-oate hydrochloride 27

Mannich reaction of **24** (500 mg, 1 mmol) as described above with diisopropylamine (0.52 mL, 3.7 mmol), formalin (37%, 1.8 mL, 14.8 mmol) and CuI (20 mg, 0.1 mmol) followed by chromatographic workup yielded crude **27**. A solution of this material in diethyl ether was treated with dry hydrochloric acid at 0 °C. After standing at 4 °C overnight the precipitate was filtered off and washed with water and diethyl ether to yield **27** (342 mg, 55%) as a colorless solid; mp 256-257 °C; [α]_D = 665° (*c* = 0.52, CHCl₃); ¹H NMR (500 MHz, CD₃OD): δ = 5.62 (*s*, 1H, H-12), 4.24 (*s*, 2H, H-34), 3.92 (*dt*, 2H, *J* = 6.6, 13.3 Hz, H-35), 3.73 (*s*, 3H,

H-31), 2.79 (*ddd*, 1H, *J* = 3.3, 3.3, 10.7 Hz, H-1a), 2.48 (*s*, 1H, H-9), 2.21-2.13 (*m*, 2H, H-18 + H-2a), 2.06 (*ddd*, 1H, *J* = 3.4, 13.7, 13.7 Hz, H-15a), 2.03-1.91 (*m*, 2H, H-16 + H-21a), 1.88 (*ddd*, 1H, *J* = 2.5, 4.1, 13.5 Hz, H-19a), 1.80-1.62 (*m*, 4H, H-2b + H-7a + H-6a + H-19b), 1.58-1.50 (*m*, 2H, H-7b + H-22a), 1.51-1.43 (*m*, 16H, H-36 + H-27 + H-6b), 1.43-1.25 (*m*, 4H, H-21b + H-1b + H-22b + H-16), 1.19-1.04 (*m*, 14H, H-25 + H-23 + H-5 + H-24 + H-28 + H-15b), 0.93 (*s*, 3H, H-26), 0.85 (*s*, 3H, H-29) ppm; ¹³C NMR (125 MHz, CDCl₃): δ = 200.7 (C-11), 177.1 (C-30), 171.3 (C-13), 127.6 (C-12), 93.8 (C-32, C≡C), 75.0 (C-3), 74.6 (C-33, C≡C), 61.9 (C-9), 54.5 (C-35), 53.2 (C-5), 51.0 (C-31, OCH₃), 48.5 (C-18), 45.3 (C-14), 43.9 (C-20), 43.2 (C-8), 41.6 (C-4), 41.0 (C-19), 38.0 (C-1), 37.6 (C-22), 37.0 (C-10), 35.8 (C-34), 32.4 (C-2), 31.6 (C-7), 31.5 (C-17), 30.6 (C-21), 27.7 (C-29), 27.1 (C-28), 26.2 (C-16), 25.9 (C-15), 25.3 (C-24), 22.7 (C-27), 17.8 (C-36), 17.4 (C-6), 17.0 (C-26), 15.9 (C-23), 14.1 (C-25) ppm; MS (ESI, MeOH): m/z = 622.6 (100%, [M-Cl]⁺); analysis calculated for C₄₀H₆₄ClNO₄ (658.39): C 72.97, H 9.80, N 2.13; found: C 72.69, H 9.96, N 2.00.

Benzyl (3β) 3-[3-(diisopropylamino)-prop-1-yn-1-yl]-3-hydroxy-11-oxo-olean-12-en-30-oate hydrochloride 28

Mannich reaction of **25** (250 mg, 0.42 mmol) with diisopropylamine (70 μL, 0.5 mmol), formalin (37%, 0.2 mL, 2.5 mmol) and CuI (20 mg, 0.1 mmol) as described above, column chromatography (SiO₂, hexanes/ethyl acetate 8/2 → methanol) followed by treating the free base with gaseous hydrochloric acid gave **28** (271 mg, 63%) as a colorless solid; mp 253-254 °C; [α]_D = 671° (*c* = 0.52, CHCl₃); ¹H NMR (500 MHz, CD₃OD): δ = 7.34-7.19 (*m*, 5H, arom), 5.36 (*s*, 1H, H-12), 5.14 (*d*, 1H, *J* = 12.0 Hz, H-31a), 5.00 (*d*, 1H, *J* = 12.0 Hz, H-31b), 4.05 (*s br*, 2H, H-34), 3.80-3.67 (*m*, 2H, H-35), 2.70-2.60 (*m*, 1H, H-1a), 2.33 (*s*, 1H, H-9), 2.02 (*ddd*, 1H, *J* = 4.4, 13.8, 14.0 Hz, H-15a), 1.98-1.47 (*m*, 9H, H-6a + H-7 + H-16 + H-18 + H-2a + H-21a + H-19), 1.46-1.34 (*m*, 3H, H-2b + H-21b + H-6b), 1.33-1.27 (*m*, 15H, H-27 + H-36), 1.27-1.09 (*m*, 4H, H-22 + H-16 + H-1b), 1.10-0.97 (*m*, 13H, H-25 + H-23 + H-5 + H-24 + H-28), 0.96-0.88 (*m*, 1H, H-15b), 0.80 (*s*, 3H, H-26), 0.62 (*s*, 3H, H-29); ¹³C NMR (125 MHz, CD₃OD): δ = 200.7 (C-11), 176.4 (C-30), 171.1 (C-13), 136.3 (Ph), 128.3 (Ph), 128.0 (Ph), 127.6 (C-12), 93.8 (C-32, C≡C), 74.9 (C-3), 74.5 (C-33, C≡C), 66.0 (C-31), 61.9 (C-9), 53.7 (C-35), 53.1 (C-5), 48.3 (C-18), 45.2 (C-14), 43.8 (C-20), 43.1 (C-8), 41.5 (C-4), 41.0 (C-19), 38.0 (C-1), 37.4 (C-22), 37.0 (C-10), 35.5 (C-34), 32.4 (C-2), 31.6 (C-7), 31.5 (C-17), 30.6 (C-21), 27.6 (C-29), 27.1 (C-28), 26.2 (C-16), 25.9 (C-15), 25.3 (C-24), 22.7 (C-27), 17.8 (C-36), 17.4 (C-6), 17.0 (C-26), 16.5 (C-23), 15.9 (C-25) ppm; MS (ESI, MeOH): m/z = 698.8 (100%, [M-Cl]⁺); analysis calculated for C₄₆H₆₈ClNO₄

(734.49): C 75.22, H 9.33, N 1.91; found: C 75.04, H 9.51, N 1.81.

References

- 1- R. Csuk, *Expert Opin. Ther. Pat.* **2014**, 24, 913-923.
- 2- R. Csuk, in *Novel Apoptotic Regulators in Carcinogenesis*, ed. by G. G. Chen and P. B. Lai, Springer, Dordrecht, **2012**, pp 267-288.
- 3- R. H. Cichewicz, S. A. Kouzi, *Med. Res. Rev.* **2004**, 24, 90-114.
- 4- M. Co, P. Kostelka, P. Eklund-Aakergren, K. Srinivas, J. W. King, P. J. R. Sjoeborg, C. Turner, *Green Chem.* **2009**, 11, 668-674.
- 5- R. Ferreira, H. Garcia, A. F. Sousa, C. S. R. Freire, A. J. D. Silvestre, W. Kunz, L. P. N. Rebelo, C. S. Pereira, *RSC Advances* **2013**, 3, 21285-21288.
- 6- J. Yin, H. Ma, Y. Gong, J. Xiao, L. Jiang, Y. Zhan, C. Li, C. Ren, Y. Yang, *Am. J. Plant Sci.* **2013**, 4, 7-15.
- 7- V. S. Klimakov, A. V. Zorin, S. S. Vershinin, V. V. Zorin, Russian Patent RU2523545C1, **2014**; Chem. Abs. **2014**, 161 201186.
- 8- F. N. Lugemwa, *Molecules* **2012**, 17, 9274-9282.
- 9- M. S. Yunusov, N. G. Komissarova, N. G. Belenkova, Russian Patent RU2270201C1, **2006**; Chem. Abs. **2006**, 144, 229523.
- 10- Y.-h. Zhang, T. Yu, Y. Wang, *J. For. Res. (Engl. Ed.)* **2003**, 14, 202-204.
- 11- A. K. Ressmann, K. Strassl, P. Gaertner, B. Zhao, L. Greiner, K. Bica, *Green Chem* **2012**, 14, 940-944.
- 12- J. Achrem-Achremowicz, E. Kepczynska, M. Zylewski, Z. Janeczko, *Biomed. Chromatogr.* **2010**, 24, 261-267.
- 13- R. Csuk, R. Sczepek, B. Siewert, C. Nitsche, *Bioorg. Med. Chem.* **2013**, 21, 425-435.
- 14- R. Csuk, C. Nitsche, R. Sczepek, S. Schwarz, B. Siewert, *Arch. Pharm.* **2013**, 346, 232-246.
- 15- A. Barthel, S. Stark, R. Csuk, *Tetrahedron* **2008**, 64, 9225-9229.
- 16- R. Csuk, K. Schmuck, R. Schäfer, *Tetrahedron Lett.* **2006**, 47, 8769-8770.
- 17- L. W. Bieber, M. F. da Silva, *Tetrahedron Lett.* **2004**, 45, 8281-8283.
- 18- L. V. Anikina, D. A. Shemyakina, L. V. Pavlogradskaya, A. N. Nedugov, V. A. Glushkov, *Russ. J. Org. Chem.* **2014**, 50, 1180-1183.
- 19- A. I. Govdi, S. F. Vasilevsky, N. V. Sokolova, I. V. Sorokina, T. G. Tolstikova, V. G. Nenajdenko, *Mendeleev Commun.* **2013**, 23, 260-261.
- 20- R. Csuk, S. Stark, C. Nitsche, A. Barthel, B. Siewert, *Eur. J. Med. Chem.* **2012**, 53, 337-345.
- 21- M. Arend, B. Westermann, N. Risch, *Angew. Chem. Int. Ed.* **1998**, 37, 1045-1070.
- 22- L. Pohjala, S. Alakurtti, T. Ahola, J. Yli-Kauhaluoma, P. Tammela, *J. Nat. Prod.* **2009**, 72, 1917-1926.
- 23- R. Csuk, S. Schwarz, R. Kluge, D. Ströhl, *Eur. J. Med. Chem.* **2010**, 45, 5718-5723.
- 24- S. Schwarz, R. Csuk, *Bioorg. Med. Chem.* **2010**, 18, 7458-7474.
- 25- R. Csuk, S. Schwarz, B. Siewert, R. Kluge, D. Ströhl, *Z. Naturforsch., B: J. Chem. Sci.* **2011**, 66, 521-532.
- 26- G. A. Tolstikov, M. I. Goryaev, L. V. Tolstikova, *Dokl. Akad. Nauk SSSR* **1967**, 173, 1110-1112.
- 27- L. Huang, X. Yang, Q. Li, H. Luo, X. Hao, Chinese Patent CN103923158A, **2014**; Chem. Abs. **2014**, 161, 290984.
- 28- C.-F. Hung, United States Patent US20120129929A1, **2012**; Chem. Abs. **2012**, 157, 2366.
- 29- C.-N. Lin, K.-W. Lin, A. M. Huang, T.-C. Hour, S.-C. Yang, Y.-S. Pu, J.-G. Chang, United States Patent US20130109752A1, **2013**; Chem. Abs. **2013**, 158, 627265.
- 30- K.-W. Lin, A. M. Huang, T.-C. Hour, S.-C. Yang, Y.-S. Pu, C.-N. Lin, *Bioorg. Med. Chem.* **2011**, 19, 4274-4285.
- 31- C. Gao, F.-J. Dai, H.-W. Cui, S.-H. Peng, Y. He, X. Wang, Z.-F. Yi, W.-W. Qiu, *Chem. Biol. Drug Des.* **2014**, 84, 223-233.
- 32- Y.-q. Meng, J.-q. Ding, Y. Liu, H.-h. Nie, S. Guan, C. Zou, N. Zhao, H. Chen, B. Cao, *Chem. Res. Chin. Univ.* **2012**, 28, 214-219.
- 33- Y. Gao, X. Guo, X. Li, D. Liu, D. Song, Y. Xu, Y.; Sun, M.; Jing, Y.; Zhao, L. *Molecules* **2010**, 15, 4439-4449.
- 34- P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Natl. Cancer Inst.* **1990**, 82, 1107-1112.
- 35- B. Siewert, J. Wiemann, A. Köwitsch, R. Csuk, *Eur. J. Med. Chem.* **2014**, 72, 84-101.
- 36- B. Siewert, E. Pianowski, A. Obernauer, R. Csuk, *Bioorg. Med. Chem.* **2014**, 22, 594-615.
- 37- B. Siewert, E. Pianowski, R. Csuk, *Eur. J. Med. Chem.* **2013**, 70, 259-272.
- 38- B. Siewert, R. Csuk, *Eur. J. Med. Chem.* **2014**, 74, 1-6.
- 39- D. Thibeault, C. Gauthier, J. Legault, J. Bouchard, P. Dufour, A. Pichette, A. Bioorg. Med. Chem. **2007**, 15, 6144-6157.