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Cytotoxic betulin-derived hydroxypropargylamines trigger apoptosis

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ABSTRACT

Several novel betulin derivatives were prepared and evaluated for their antitumor activity. Among others, 3-*O*-acetylbetulinic aldehyde served as an ideal starting material for the synthesis of 28-acetylenic derivatives that were further transformed into *Mannich* bases. These hydroxypropargylamines were screened for their antitumor activity in a panel of nine human cancer cell lines in a sulforhodamine B (SRB) assay. Several compounds showed a noteworthy antitumor activity. The results from acridine orange/propidium iodide staining and annexinV-FITC assays as well as DNA laddering experiments provided evidence for an apoptotic cell death.

1. Introduction

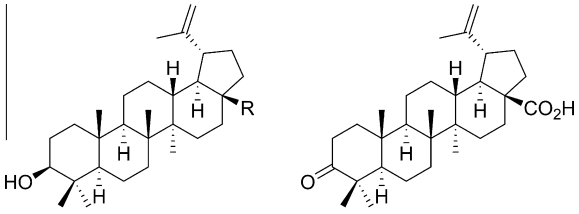
Betulin (**1**, Fig. 1) is a pentacyclic triterpene showing a lupane skeleton. It can be obtained in huge amounts from birch bark waste by extraction. The healing properties of birch bark and birch bark extracts have been known longtime in folk medicine. Betulin **1** itself is inactive against many tumor cell lines. The opposite is true for several of its derivatives, especially for betulinic acid (**2**), betulonic acid (**3**) and their derivatives.

Several reports[1,2] revealed the great potency of these compounds; especially betulinic acid derivatives have been suggested for the therapy of human melanoma tumors;[3–6] no toxic side effects were noted in doses up to 500 mg/kg bodyweight, and their way of action works by an induction of apoptosis.

A major drawback for their application is their low solubility in water.[7,8] Therefore, derivatives of betulin or betulinic acid holding extra polar groups, and showing an increased solubility are needed. Alkynyl substituted derivatives[9–14] have scarcely been prepared so far and their synthetic and antitumor potential remains yet to be explored. Some of these compounds[15,16] showed an increased cytotoxicity towards many human tumor cells compared to parent betulin. So, we decided to combine the higher cytotoxic activity of alkynyl substituted betulin derivatives with improved water solubility by attaching aminogroup-bearing functionalities using *Mannich* reactions.

Mannich reactions are known for exactly 100 years[17] and their significance for the synthesis of fine chemicals, pharmaceuticals and polymers is beyond any doubt.[17–20] *Mannich* reactions using alkynes[21] have been in the focus of synthetic organic chemists since 1933—but moderate yields because of harsh reaction conditions have limited a wider application.[22] A breakthrough, however, was using copper catalysts in these reactions.[23]

Terpenoids have scarcely been subject of aminomethylation reactions.[24,25] There, the reactive site was usually an alkyl ketone group. There are even fewer examples for a successful *Mannich* reaction of alkyne-modified terpenes so far because *Mannich* reactions are often accompanied by changes in the substrate structure due to rearrangement reactions.[26–28]



1 betulin, R = CH_2OH
2 betulinic acid, R = CO_2H

3 betulonic acid

Figure 1. Structure of important lupane-type triterpenes.

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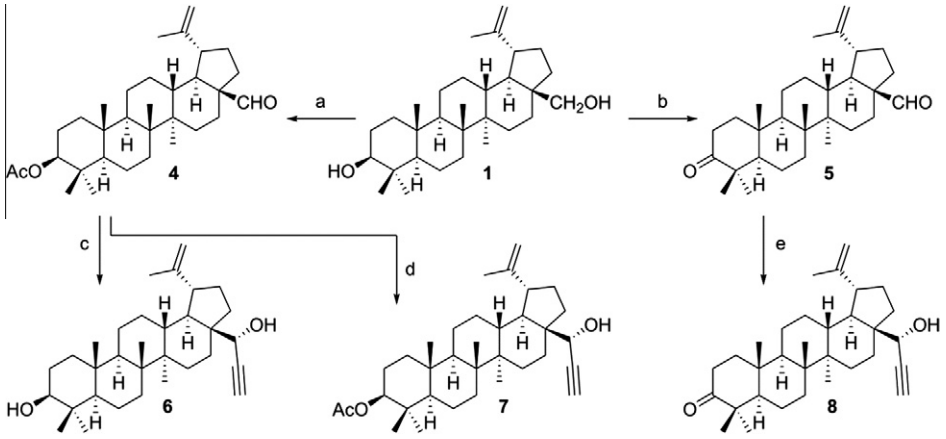
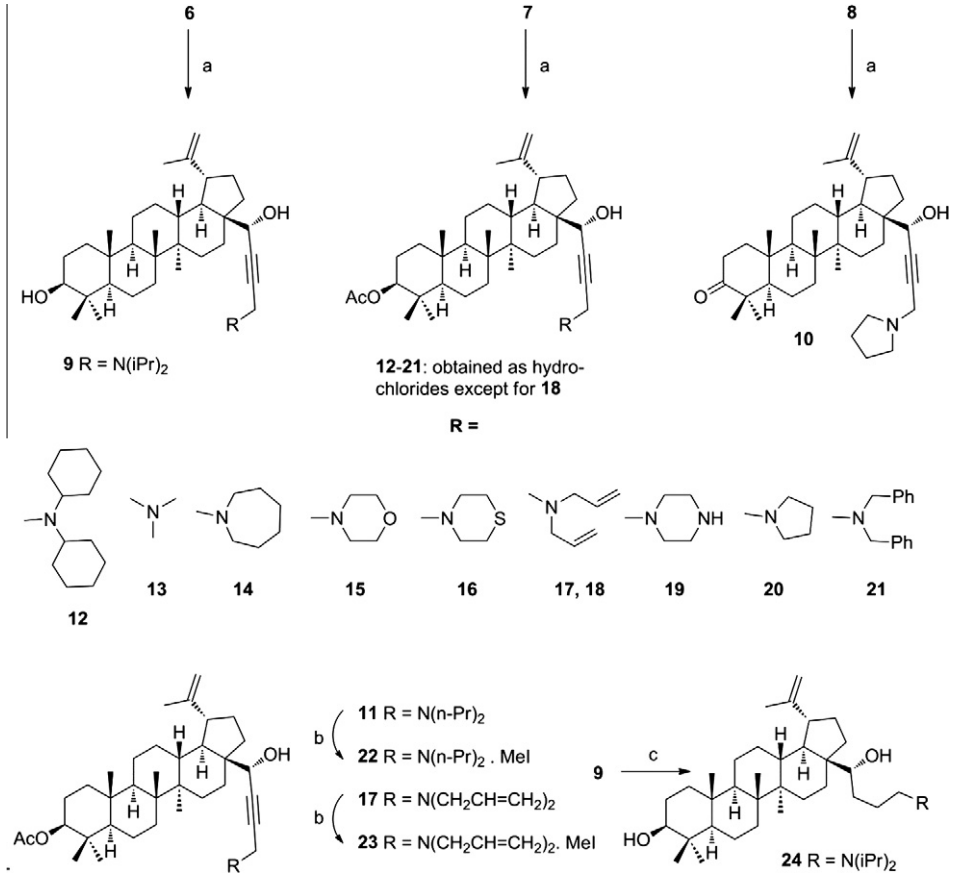
2. Chemistry

Mannich reactions using alkynes, aldehydes and amines (so-called A^3 reactions[29]) yield propargylamines. Suitable starting materials for the synthesis of alkynic terpenes can be obtained easily from naturally occurring betulin (**1**, Scheme 1). Thus, **1** was transformed by known procedures into aldehydes **4**[30–36] and **5**.[33–35,37–46] Reaction of **4** with lithium acetylide[15,16] in THF gave **6**, from the *Grignard* reaction of **4** or **5** alkynic **7** and **8** were obtained.

The *Grignard* reaction of **5** advanced in THF in a chemoselective way, and **8** with an intact carbonyl group at C(3) was obtained. Reactions in diethylether, however, produced a mixture of several compounds. Compound **8** is characterized in its IR spectrum by a strong vibration for the carbonyl group at $\nu = 1702\ cm^{-1}$. In the ^{13}C NMR spectrum the carbonyl group was detected at



Scheme 1. Reagents and conditions: Synthesis of 28-acetylenic betulins: (a) [29–31]; (b) [32, 39, 43]; (c) [29–31]; (d) ethynylmagnesium bromide, 25 °C, 10 h, 75%; (e) ethynylmagnesium bromide, 25 °C, 12 h, 63%.



Scheme 2. Reagents and conditions: (a) Secondary amine, formalin, CuI (cat.), DMSO (or THF), 40 °C, 1–6 d, 40–90%; (b) MeI in Et_2O, 5 d (87% for **22**; 81% for **23**); (c) catalyst, MeOH, H_2 (6 bar), 45%.

Table 1
Cytotxicity of the Mannich compounds **6–24** (SRB assay, IC_{50} values given in μM) and betulinic acid (**BA**)

Cell line	BA	6	7	8	9	10	11	13	14	15	16	17	18	19	20	21	22	23	24
SW1736	11.6 ± 1.0	13.0 ± 3.0	23.0 ± 7.8	18.4 ± 0.7	4.0 ± 1.0	6.0 ± 0.4	7.5 ± 1.1	6.5 ± 2.6	32.3 ± 1.8	8.9 ± 1.1	3.7 ± 1.2	48.0 ± 1.6	30.2 ± 2.2	7.7 ± 0.8	20.0 ± 4.5	>70	3.2 ± 0.2	3.5 ± 0.5	1.7 ± 0.8
MCF-7	14.8 ± 1.2	11.9 ± 2.4	20.5 ± 10.2	17.1 ± 1.1	8.2 ± 1.1	3.1 ± 0.3	16.5 ± 1.3	3.7 ± 0.3	13.5 ± 4.7	7.7 ± 1.0	3.8 ± 0.1	31.1 ± 2.8	15.7 ± 1.5	2.5 ± 0.4	7.7 ± 0.8	27.2 ± 1.1	3.2 ± 0.9	4.5 ± 1.5	0.7 ± 0.1
LIPO	9.7 ± 0.3	12.9 ± 0.2	28.9 ± 0.8	21.7 ± 1.3	10.3 ± 0.1	5.6 ± 1.5	24.2 ± 2.6	8.6 ± 0.2	>30	2.4 ± 0.9	3.5 ± 0.8	17.0 ± 2.0	21.8 ± 0.8	2.7 ± 0.2	11.6 ± 0.7	>70	4.3 ± 1.4	5.3 ± 1.3	2.3 ± 0.7
DLD-1	17.5 ± 1.8	21.9 ± 2.2	>30	19.0 ± 1.3	9.1 ± 0.2	2.6 ± 0.5	20.7 ± 1.5	12.9 ± 0.9	40.8 ± 2.9	5.0 ± 0.7	19.0 ± 1.5	57.6 ± 3.3	44.2 ± 2.2	5.2 ± 0.2	18.8 ± 3.1	>70	4.8 ± 1.5	15.6 ± 2.0	2.3 ± 0.7
A549	14.9 ± 1.5	20.4 ± 1.8	42.2 ± 5.0	21.2 ± 0.8	8.3 ± 2.0	3.5 ± 1.4	25.9 ± 0.4	11.1 ± 1.2	47.0 ± 6.4	7.6 ± 0.5	16.3 ± 1.6	>30	>60	6.5 ± 1.1	12.0 ± 1.9	>70	4.5 ± 0.1	5.3 ± 1.1	2.2 ± 0.1
A2780	11.0 ± 1.0	9.7 ± 1.2	9.3 ± 1.3	9.8 ± 1.9	5.4 ± 0.9	3.6 ± 1.4	12.0 ± 1.3	4.9 ± 0.5	14.4 ± 0.6	4.1 ± 1.5	4.3 ± 1.8	18.2 ± 1.8	12.4 ± 2.6	2.7 ± 0.2	8.3 ± 0.9	>70	2.3 ± 0.6	2.5 ± 0.8	1.6 ± 0.7
A253	11.1 ± 2.2	14.1 ± 3.6	16.5 ± 2.5	13.4 ± 1.3	8.2 ± 1.1	3.4 ± 0.4	15.2 ± 1.0	6.4 ± 0.9	20.1 ± 3.0	5.2 ± 0.2	7.5 ± 1.5	43.1 ± 2.1	32.8 ± 0.5	4.6 ± 1.0	10.4 ± 0.8	>70	3.5 ± 1.2	4.2 ± 0.1	1.8 ± 0.05
8505C	6.7 ± 0–9	16.1 ± 1.1	22.9 ± 2.3	16.7 ± 1.5	5.8 ± 0.1	3.4 ± 0.2	28.0 ± 3.1	6.7 ± 0.6	32.3 ± 2.9	7.2 ± 1.1	2.0 ± 0.1	>60	47.9 ± 4.8	6.9 ± 1.5	11.1 ± 0.8	>30	4.1 ± 0.1	3.2 ± 0.7	2.3 ± 0.5
518A2	11.9 ± 0.9	15.1 ± 0.9	32.5 ± 4.6	22.4 ± 1.5	6.4 ± 0.8	5.6 ± 1.3	21.0 ± 2.0	7.2 ± 1.5	22.4 ± 3.0	7.0 ± 0.5	9.3 ± 1.1	>60	52.0 ± 5.9	5.0 ± 1.1	12.1 ± 0.4	>30	4.6 ± 1.7	4.5 ± 0.4	2.1 ± 0.5
NiH 3T3	10.0 ± 2.1	10.5 ± 1.5	32.3 ± 3.2	>35	6.2 ± 0.6	6.4 ± 2.5	>30	5.8 ± 0.2	17.0 ± 0.1	7.2 ± 0.8	8.8 ± 1.5	>60	>60	4.0 ± 0.2	14.5 ± 1.9	>70	6.8 ± 0.8	5.8 ± 1.2	1.7 ± 0.2

Values are derived from dose–response curves obtained by measuring the percentage of viable cell relative to untreated controls after 96 h expose of the test compounds to the cell line using an SRB assay for the different human cancer cell lines and for comparison a mouse fibroblastic cell line (NiH 3T3). The values were calculated applying the two-parametric Hill slope equation.

δ = 218.0 ppm, the carbons of the alkyne moiety were found at δ = 84.6 and 74.2 ppm, respectively.

Mannich reaction of **6** (Scheme 2) with diisopropylamine and formalin in DMSO (or THF) in the presence of catalytic amounts of CuI[23] gave the *Mannich* base **9** in 90% yield. Similarly, from **6** with *N*-methyl-piperazine compound **13** was obtained. Yields dropped slightly for the reaction of **7** with several secondary amines; the products **11**–**21** were obtained in fair yields.

From the betulone derivative **8** under the same conditions **10** was obtained in 47% yield. For comparison, compounds **11** and **17** were transformed[47,48] into their methiodides **22** and **23**, respectively. Hydrogenation of **11** in the presence of *Lindlar* catalyst gave **24** leaving the isopropenyl group of the lupane skeleton unaffected.

3. Biology

Many structural variations have been applied to the betulin/betulinic acid skeleton to preserve or to increase antitumor activity. Of special interest have been the carboxylic group at position C-28 in ring E and the hydroxy group at position C-3 in ring A.[30–32,49–57]

In general, it seems that esterification of C-28 using small to medium length alcohols (or benzylic alcohol) led to a decrease in activity as well as introducing hydrophilic carbohydrate-derived moieties. Introduction of a bidesmosidic moiety, however, increased activity.[58,59] Increase in cytotoxic activity has been remarked for derivatives varied at C-28 and C-3. Thus, introduction of an extra N-substituent led to an increase in cytotoxicity.[60,61] It can be assumed that this increase of cytotoxicity is due either to an increased solubility of these compounds and/or an improved transport into the tumor cells. The results from in vivo studies suggested that betulinic acid accumulates in regions of lowered pH values.[62,63] Non-sufficient solubility of betulinic acid and many of its derivatives is a notorious problem in gaining good antitumor activity. The problem of solubility is crucial for an application because an aqueous medium is the preferred formulation for injection. To improve the solubility of triterpenes either the use of liposomes, microemulsions[64] or the addition of organic solvents might be indicated. Raising of the pH value of the aqueous reaction medium also increases solubility.[7] To get a first insight into this problem, solubility data for betulinic acid[7,8] and several of our derivatives have been measured in a water/DMSO (95:5, v/v) mixture; for betulinic acid a solubility of 166 μg/ml was found. The *Mannich* bases showed better solubility. Thus, for compound **10** 182 μg/ml, for **19** 178 μg/ml, for **15** 192 μg/ml and for **13** even 312 μg/ml were determined. For the dicyclohexylamine **12** a rather low solubility of 100 μg/ml was found, thus giving a possible explanation for the rather high IC_{50} values (IC_{50} > 30 μM for all cell lines) for this derivative.

Although betulinic acid and most of its derivatives act by triggering apoptosis, a recent study of Fulda and co-workers using B10, a glycosylated betulinic acid derivative, pointed out autophagy as an (extra) reason for cell death.[65] During autophagy a fusion of lysosomes and the macrophagosome takes place, therefore, introducing a proton-accepting group might led to an increased formation of autophagosomes.

Our compounds were tested for their cytotoxic activity (Table 1, Fig. 2) in a colorimetric SRB cell assay.[66–68] In general, 3-*O*-acetyl derivatives showed a higher activity than their parent deacetylated analogs (cf. Fig. 2); in alkynyl substituted C-28 oxo compounds activity is retained. This parallels by and large previous findings of Kim et al.[69,70] whereas all derivatives with C-28 decarbonylated or decarboxylated were shown to be inactive.[31,71]

The results from the SRB assay also revealed (Table 1, Fig. 2) that *Mannich* products having been obtained from small aliphatic

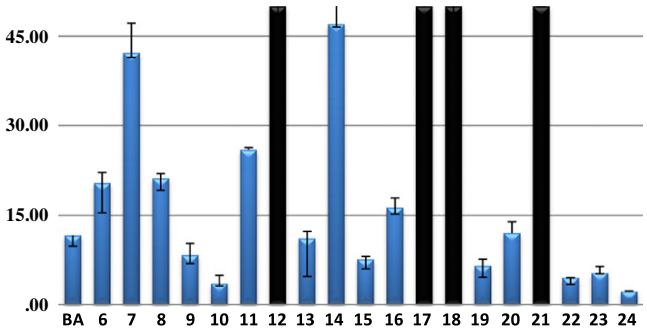


Figure 2. Comparison of IC_{50} values (in μM from SRB assay) for compounds **6–24** and standard betulinic acid (**BA**) for lung carcinoma cell line A549; black bars indicate inactive compounds.

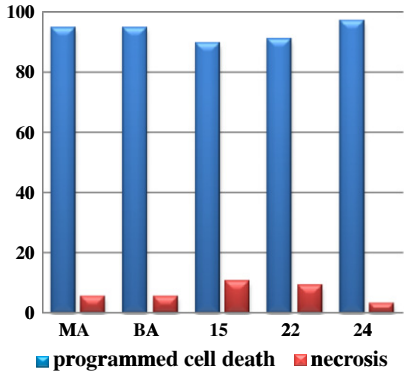


Figure 3. Summary of the FACS based annexin V/propidium iodide assays for A549 cells and compounds (x-axis) **15** (5 μM), **22** (10 μM) or **24** (10 μM) compared to standards betulinic acid (**BA**, 30 μM) and maslinic acid (**MA**, 30 μM); percentage (y-axis) of cells died by necrosis (in red) or by a programmed cell death (blue) after an incubation of the cells for a period of 6 h and concentration as indicated.

amines show the highest cytotoxicity. Steric hindrance seems crucial since the dicyclohexyl derivative **12** as well as the dibenzyl compound **21** displayed decreased activity. Formation of methiodides **22** and **23** gave a 2.5- to 5.8-fold increase in activity compared to their parent compounds. Hydrogenation of the triple bond in **9** gave **24**; the latter compound showed an even better cytotoxicity. In summary, the *Mannich* products holding a C-28 alkynic skeleton show an improved cytotoxicity compared to their parent compounds—as long as the terminal amine substituent is small (or a medium sized cyclic amine containing an extra tertiary amino group). This parallels previous results of Salvador, who found that the introduction of N-heterocyclic moieties at C-28 increased cytotoxicity. Also, a loss or a decrease of activity for compounds acetylated at C-3 is in excellent agreement with previous findings.[50–52]

Betulinic acid and derivatives act by triggering apoptosis[63,72,73]—a programmed cell death wherein cells that are harmful to an organism are disposed of in a neat and orderly manner. SRB tests do not allow conclusions for an apoptotic cell death. Hence, selected compounds were chosen for further studies. These experiments included a dye exclusion test (acridine orange/propidium iode, AO/PI) as well as an annexin V assay.

An indicator for an early stage of apoptosis was shown to be the transfer of phosphatidylserine from the inner membrane to the outer,[74] a finding that can be determined using a FACS based annexin V/propidium iodide assay. Evaluation of the results for compounds **15**, **22** and **24** showed that for > 90% of all cells phosphatidylserine has been translocated to the outer cell membrane (Fig. 3).

An extra AO/PI staining experiment (Fig. 4, left and right part) using A541 cells (after having been treated with compounds **15**, **22** or **24**, respectively) showed an almost complete exclusion of PI by the cells (hence resulting in a green fluorescence during microscopy because of an incorporation of AO into double stranded nucleic acids). DNA laddering, that is, the cleavage of DNA by endonucleases into fragments of 180 bp lengths (detected by gel electrophoresis), is regarded as a late stage indicator of apoptosis. The results from such an experiment are depicted in Figure 4 (middle), and they clearly show the presence of these fragments and hence apoptosis.

Besides staining the cells during the AO/PI dyeing experiments, morphological changes can be found for cells undergoing the process of apoptosis. As marked by small white arrows in Figure 4, blebbing of the cell membrane, condensation of the chromatin as well as a shrinking of the cells can be noted. In Figure 4 (right part) a mitotic cell has been marked by a red arrow. This can be interpreted as an indicator for a G2/M arrest of the cell induced by treating the cells with compound **24** hence paralleling previous findings of Salvador[50] and Yang et al.[75]

In this staining experiment lysosomes or autophagosomes are colored in red—indicating that maybe besides apoptosis autophagy is taking place.[65]

4. Conclusion

In summary, alkynyl Mannich bases of betulin are promising antitumor agents. The results from acridine orange/propidium iodide staining and annexinV-FITC assays as well as DNA laddering experiments provided evidence for an apoptotic cell death. Some

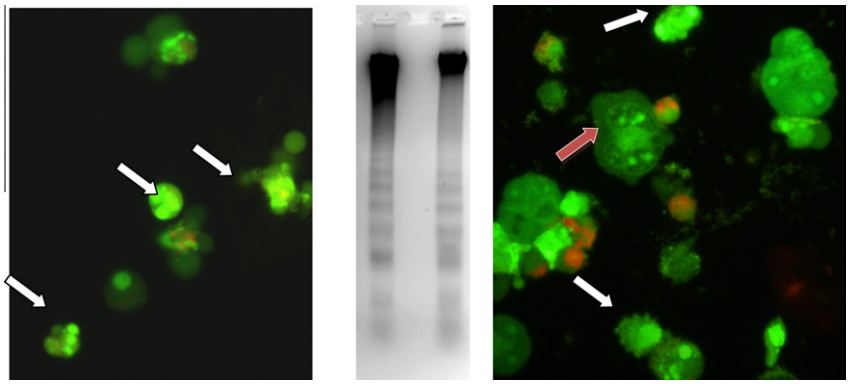


Figure 4. AO/PI staining of A541 human cancer cells (left: with compound **22**; right with compound **24**); in the middle: DNA laddering (A541 cells) after having been treated with compounds **22** or **24**.

of them are strong regulators of tumor cells proliferation and induce a cell cycle arrest. An extra process of autophagy leading to cell death, however, cannot be ruled out. The biological activities make these compounds interesting candidates for further biological evaluation.

5. Experimental

5.1. General

Instrumentation, cell lines and culture conditions, cytotoxicity assay, dye exclusion tests, the annexin V assay and DNA fragmentation was performed as previously described.[76] Solubility was determined in water/DMSO (95:5, v/v) mixtures (HPLC, UV/vis) according to Ref. [8]

5.2. General procedure for the Mannich reactions

A mixture of alkynol (1.0 equiv), secondary amine (1.05–3.5 equiv), formalin (37%, 10–15 equiv), copper iodide (0.01–0.06 equiv) and DMSO (3–6 ml) was stirred at 40 °C for 1–5 days. After the reaction was completed (as indicated by TLC), a solution of aqueous ammonia (30%, 5 ml) in water (5 ml) was added. The mixture was extracted with ethyl acetate (5 × 10 ml), and the solvents were evaporated under reduced pressure. The crude residue dissolved in ethyl acetate (100 ml), the solvent was stripped off, and the residue re-dissolved in diethylether (100 ml). Insoluble material was filtered off, and at 0 °C gaseous hydrogen chloride was passed through the solution until the precipitation of salts had ceased. Crystallization was completed by standing at 4 °C for 12. The product was collected and washed with water and diethylether. Optionally, re-crystallization from methanol (5 ml) and hydrochloric acid (10%, 5 ml) gave an analytical product.

5.3. (3*S*) 28-Oxolup-20(29)en-3-yl acetate (4)

This compound was prepared according to Refs. 30–32

5.4. 3-Oxolup-20(29)en-28-al (5)

This compound was prepared according to Refs. 33,40,44

5.5. (3*S*, 28*S*) 28-Ethynyl-betulin (6)

This compound was prepared according to Refs. 30–32

5.6. (3*S*, 28*S*) 3-*O*-Acetyl-28-ethynyllup-20(29)-en-3,28-diol (7)

A solution of **4** (1.22 g, 2.53 mmol) in THF (50 ml) containing a solution of ethynylmagnesium bromide (0.5 m, 12 ml, 7.2 mmol) was stirred at 25 °C for 10 h; the reaction was quenched with water (10 ml) and extracted with dichloromethane (5 × 100 ml). The dried organic phase (Na_2SO_4) was evaporated, and the residue was subjected to chromatography (silica gel, *n*-hexane/ethyl acetate, 8/2) to yield **7** (0.91 g, 75%) as a colorless solid; mp 201–204 °C; $[\alpha]_D$ +15.6° (*c* 3.80, $CHCl_3$); R_f = 0.63 (*n*-hexane/ethyl acetate, 8/2); IR (KBr): ν = 3440s, 3298m, 3260s, 3073m, 2939s, 2867s, 2529m, 1708s, 1639m, 1452s, 1377s, 1315m, 1269s, 1194m, 1131w, 1108m, 1035s cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 4.86 (m, 1H, C*H* (28)), 4.68 (m, 1H, CH_a (29)), 4.56 (m, 1H, CH_b (29)), 4.45 (dd, 1H, *J* = 10.7, 5.8 Hz C*H* (3)), 2.88 (ddd, 1H, *J* = 11.2, 11.2, 6.3 Hz, C*H* (19)), 2.48 (d, 1H, *J* = 2.1 Hz, C*H* (34)), 2.10–1.90 (m, 3H, CH_a (22) + CH_a (21) + CH_a (16)), 2.02 (s, 3H, CH_3 (32)), 1.93 (ddd, 1H, *J* = 12.2, 11.8, 3.6 Hz, C*H* (13)), 1.74 (dd, 1H, *J* = 12.2, 12.2 Hz, C*H* (18)), 1.71–1.47 (m, 5 H, CH_a (2) + CH_a (15) + CH_b (2) + CH_a (1) + CH_a (12)), 1.66 (s, 3H, CH_3 (30)), 1.44–1.09 (m, 10H, C*H* (9) + CH_a (6) + CH_b (22) + CH_a (7) + CH_b (7) + CH_b (16) + CH_b (21) + CH_a (11) + CH_b (11) + CH_b (6)), 1.03–0.92 (m, 3H, CH_b (15) + CH_b (12) + CH_b (1)), 1.02 (s, 3H, CH_3 (23)), 0.98 (s, 3H, CH_3 (27)), 0.83–0.81 (m, 9H, CH_3 (24) + CH_3 (25) + CH_3 (26)), 0.77 (d, 1H, *J* = 9.5 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.0 (C31, *C*O), 150.9 (C20, *C*=CH_2), 109.7 (C29, C=CH_2), 84.7 (C33, *C*≡CH), 80.9 (C3, *C*H), 74.2 (C34, C≡*C*H), 66.0 (C28, *C*HOH), 55.4 (C5, *C*H), 50.6 (C17, $C_{quart.}$), 50.2 (C9, *C*H), 48.9 (C18, *C*H), 48.7 (C19, *C*H), 43.0 (C14, $C_{quart.}$), 40.9 (C8, $C_{quart.}$), 38.4 (C1, CH_2), 37.8 (C4, $C_{quart.}$), 37.3 (C13, *C*H), 37.1 (C10, $C_{quart.}$), 34.3 (C7, CH_2), 34.1 (C22, CH_2), 33.9 (C21, CH_2), 32.4 (C16, CH_2), 27.9 (C24, CH_3), 27.8 (C15, CH_2), 25.1 (C12, CH_2), 23.7 (C2, CH_2), 21.3 (C32, CH_3), 20.8 (C11, CH_2), 18.8 (C30, CH_3), 18.1 (C6, CH_2), 17.5 (C23, CH_3), 16.5 (C25, CH_3), 16.1 (C26, CH_3), 15.1 (C27, CH_3) ppm; MS (ESI, MeOH): *m*/*z* = 1039.3 (100%, $[2\ M+Na]^+$), 563.2 (25%, [M+Na + MeOH]$^+$), 531.5 (6% $[M+Na]^+$), 509.3 (2%, $[M+H]^+$); Anal. for $C_{34}H_{52}O_3$ (508.77): C 80.26; H, 10.30. Found: C 80.04, H 10.56.

5.7. (28*S*) 28-Ethynyl-28-hydroxylup-20(29)-en-3-on (8)

Following the procedure given for **6**, from the reaction of **23** (3.0 g, 6.46 mmol) with ethynylmagnesium bromide (0.5 M, 20 ml, 10 mmol) for 12 h and chromatography (silica gel, *n*-hexane/ethyl acetate, 8/2) **8** (1.90 g, 63%) was obtained as a colorless solid; mp 110–115 °C; $[\alpha]_D$ +35.6° (*c* 5.60, $CHCl_3$); R_f = 0.52 (*n*-hexane/ethyl acetate 8/2); IR (KBr): ν = 3442s, 3308m, 2943s, 2868s, 1702s, 1639m, 1458m, 1378m, 1248m, 1111m, 1046m, 961m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 4.87 (m, 1H, C*H* (28)), 4.68 (m, 1H, CH_a (29)), 4.56 (m, 1H, CH_b (29)), 2.88 (ddd, 1H, *J* = 10.7, 10.7, 6.4 Hz, C*H* (19)), 2.50–2.43 (m, 1H, CH_a (2)), 2.40–2.34 (m, 1H, CH_b (2)), 2.48 (d, 1H, *J* = 2.1 Hz, C*H* (32)), 2.11–1.93 (m, 4H, CH_a (16) + CH_a (21) + CH_a (22) + C*H* (13)), 1.94 (ddd, 1H, *J* = 12.6, 5.5, 4.3 Hz, CH_a (1)), 1.75 (dd, 1H, *J* = 11.9, 11.9 Hz, C*H* (18)), 1.77–1.65 (m, 2H, CH_a (15) + CH_a (12)), 1.66 (s, 3H, CH_3 (30)), 1.56 (ddd, 1H, *J* = 13.8, 13.8, 4.1 Hz, CH_a (6)), 1.50–1.27 (m, 10H, C*H* (9) + CH_b (22) + CH_a (7) + CH_b (7) + CH_b (16) + CH_b (21) + CH_a (11) + CH_b (11) + CH_b (6) + CH_b (1)), 1.23 (d, 1H, *J* = 8.6 Hz, C*H* (5)), 1.16 (ddd, 1H, *J* = 13.4, 12.4, 4.8 Hz, CH_b (15)), 1.02–0.95 (m, 1H, CH_b (12)), 1.06 (s, 3H, CH_3 (25)), 1.05 (s, 3H, CH_3 (24)), 1.00 (s, 3H, CH_3 (23)), 0.99 (s, 3H, CH_3 (27)), 0.91 (s, 3H, CH_3 (26)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 218.0 (C3, *C*O), 150.9 (C20, *C* = CH_2), 109.7 (C29, C=CH_2), 84.6 (C31, *C*≡CH), 74.2 (C32, C≡*C*H), 65.9 (C28, *C*HOH), 54.9 (C5, *C*H), 50.6 (C17, $C_{quart.}$), 49.6 (C9, *C*H), 48.8 (C18, *C*H), 48.6 (C19, *C*H), 47.3 (C4, $C_{quart.}$), 43.1 (C14, $C_{quart.}$), 40.8 (C8, $C_{quart.}$), 39.5 (C1, CH_2), 37.3 (C13, *C*H), 36.8 (C10, $C_{quart.}$), 34.2 (C22, CH_2), 34.1 (C2, CH_2), 33.9 (C7, CH_2), 33.5 (C21, CH_2), 32.3 (C16, CH_2), 27.8 (C15, CH_2), 26.6 (C24, CH_3), 25.1 (C12, CH_2), 21.3 (C11, CH_2), 21.0 (C23, CH_3), 19.6 (C6, CH_2), 18.8 (C30, CH_3), 15.9 (C25, CH_3), 15.8 (C26, CH_3), 15.0 (C27, CH_3) ppm; MS (ESI, MeOH): *m*/*z* = 951.3 (35% $[2\ M+Na]^+$), 519.1 (56% [M+Na + MeOH]$^+$), 487.5 (5% $[M+Na]^+$), 465.3 (11% $[M+H]^+$); Anal. for $C_{32}H_{48}O_2$ (464.72): C, 82.70; H, 10.41. Found: C, 82.56; H, 10.62.

5.8. (3*S*, 28*S*) 28-[3-(Diisopropylamino)prop-1-yn-1-yl]lup-20(29)-en-3,28-diol hydrochloride (9)

Compound **9** was prepared following the general procedure from **6** (327 mg, 0.7 mmol), diisopropylamine (0.12 ml, 0.85 mmol), formalin (37%, 0.3 ml, 3.7 mmol), and catalytical amounts of CuI in DMSO. The solution was stirred for 20 h at 40 °C to yield **9** (388 mg, 90%) as a colorless solid; mp 235; $[\alpha]_D$ +2.6° (*c* 4.30, MeOH); IR (KBr): ν = 3419m, 2943s, 2503m, 1640m, 1467m, 1397m, 1046m, 877m cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ = 4.95 (m, 1H, C*H* (28)), 4.66 (m, 1H, CH_a (29)), 4.56 (m, 1H, CH_b (29)), 4.18 (d, 2H,

J = 1.6 Hz, CH_2 (33)), 3.90 (*sept.*, 2H, *J* = 6.6, 2.6 Hz, 2 × C*H* (34) + (37)), 3.13 (dd, 1H, *J* = 11.4, 4.9 Hz, C*H* (3)), 2.96 (m, 1H, C*H* (19)), 2.12–1.93 (m, 4H, CH_a (22) + CH_a (21) + CH_a (16) + C*H* (13)), 1.79 (dd, 1H, *J* = 11.8, 11.8 Hz, C*H* (18)), 1.77–1.50 (m, 5 H, CH_a (2) + CH_b (2) + CH_a (15) + CH_a (1) + CH_a (12)), 1.67 (s, 3H, CH_3 (30)), 1.48–1.14 (m, 11H, CH_a (6) + CH_b (6) + C*H* (9) + CH_b (12) + CH_b (22) + CH_a (7) + CH_b (7) + CH_b (16) + CH_b (21) + CH_a (11) + CH_b (11)), 1.45 (dd, 12H, *J* = 6.6 Hz, 1.8 Hz, 4 × CH_3 (35) + (36) + (38) + (39)), 1.07–0.90 (m, 2H, CH_b (15) + CH_b (1)), 1.08 (s, 3H, CH_3 (23)), 1.04 (s, 3H, CH_3 (27)), 0.95 (s, 3H, CH_3 (24)), 0.87 (s, 3H, CH_3 (26)), 0.75 (s, 3H, CH_3 (25)), 0.72 (d, 1H, *J* = 11.0 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ = 151.9 (C20, *C*=CH_2), 108.8 (C29, C=CH_2), 91.3 (C31, *C*≡CH), 78.2 (C3, *C*HOH), 74.8 (C32, C≡*C*H), 64.7 (C28, *C*HOH), 55.3 (C5, *C*H), 55.0 (C34 + C37, 2 × *C*H), 50.6 (C17, $C_{quart.}$), 50.3 (C9, *C*H), 48.9 (C18, *C*H), 48.7 (C19, *C*H), 42.7 (C14, $C_{quart.}$), 40.7 (C8, $C_{quart.}$), 38.6 (C1, *C*H_2), 38.6 (C4, $C_{quart.}$), 37.2 (C13, *C*H), 36.8 (C10, $C_{quart.}$), 36.0 (C33, *C*H_2), 34.1 (C7, *C*H_2), 34.0 (C22, *C*H_2), 31.9 (C21, *C*H_2), 30.1 (C16, *C*H_2), 27.6 (C15, *C*H_2), 27.2 (C24, *C*H_3), 26.6 (C2, *C*H_2), 25.1 (C12, *C*H_2), 20.6 (C11, *C*H_2), 18.2 (C30, *C*H_3), 18.0 (C6, *C*H_2), 17.7 (C35 + C36 + C38 + C39, 4 × *C*H_3), 15.3 (C23, *C*H_3), 15.2 (C25, *C*H_3), 14.7 (C26, *C*H_3), 14.1 (C27, *C*H_3); MS (ESI, MeOH): *m*/*z* = 580.5 (100%, $[M-Cl]^+$); Anal. for $C_{39}H_{66}ClNO_2$ (616.40): C, 75.99; H, 10.79; N, 2.27. Found: C, 75.88; H, 10.96; N, 2.15.

5.9. (3*S*, 28*S*) 28-Hydroxy-28-(3-pyrrolidin-1-yl-prop-1-yn-1-yl)lup-20(29)-en-3-on hydrochloride (10)

Compound **10** was prepared following the general procedure from **8** (466 mg, 1.00 mmol), pyrrolidine (0.1 ml, 1.2 mmol), formalin (37%, 0.4 ml, 4.9 mmol) and CuI (4 mg, 0.02 mmol) in DMSO (4 ml). The solution was stirred 20 h at 40 °C to yield after re-crystallization **10** (215 mg, 37%) as a colorless solid; mp 180 °C; $[\alpha]_D$ +29.8° (*c* 5.50, MeOH); IR (KBr): *ν* = 3374s, 3067s, 2941s, 2584s, 2479s, 1703s, 1639s, 1457s, 1379s, 1201m, 1138s, 1046s, 1021s, 967m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.95 (m, 1H, C*H* (28)), 4.67 (m, 1H, CH_a (29)), 4.56 (m, 1H, CH_b (29)), 4.19 (m, 2H, CH_2 (33)), 3.65 (m, 2H, CH_a (34) + CH_a (37)), 3.24 (m, 2H, CH_b (34) + CH_b (37)), 2.96 (ddd, 1H, *J* = 10.9, 10.9, 5.9 Hz, C*H* (19)), 2.18 (m, 2H, CH_a (35) + CH_a (36)), 2.11–1.87 (m, 9H, CH_a (2) + CH_b (2) + C*Ha* (16) + CH_a (21) + CH_a (22) + C*H* (13) + CH_b (35) + CH_b (36) + CH_a (1)), 1.82 (dd, 1H, *J* = 11.9, 11.9 Hz, C*H* (18)), 1.78–1.63 (m, 2H, CH_a (15) + CH_a (12)), 1.67 (s, 3H, CH_3 (30)), 1.63–1.57 (m, 1H, CH_a (6)), 1.55–1.03 (m, 12H, C*H* (9) + CH_b (22) + CH_a (7) + CH_b (7) + CH_b (16) + CH_b (21) + CH_a (11) + CH_b (11) + CH_b (6) + CH_b (1) + C*H* (5) + CH_b (15)), 1.12 (s, 3H, CH_3 (25)), 1.02–0.93 (m, 1H, CH_b (12)), 1.06 (m, 6 H, CH_3 (24) + CH_3 (27)), 1.02 (s, 3H, CH_3 (23)), 0.95 (s, 3H, CH_3 (26)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 219.4 (C3, *C*O), 150.9 (C20, *C*=CH_2), 108.8 (C29, C=CH_2), 90.7 (C31, *C*≡CH), 74.0 (C32, C≡CH), 64.6 (C28, *C*HOH), 54.6 (C5, *C*H), 53.2 (C34 + C37, 2 × *C*H$_2$), 50.5 (C17, $C_{quart.}$), 49.5 (C9, *C*H), 48.8 (C18, *C*H), 48.6 (C19, *C*H), 47.0 (C4, $C_{quart.}$), 43.2 (C14, $C_{quart.}$), 42.8 (C33, *C*H_2), 40.6 (C8, $C_{quart.}$), 39.1 (C1, *C*H_2), 37.3 (C13, *C*H), 36.6 (C10, $C_{quart.}$), 34.1 (C22, *C*H_2), 34.0 (C2, *C*H_2), 34.0 (C7, *C*H_2), 33.3 (C21, *C*H_2), 31.9 (C16, *C*H_2), 27.6 (C15, *C*H_2), 25.7 (C24, *C*H_3), 25.1 (C12, *C*H_2), 23.1 (C35 + C36, 2 × *C*H_2), 21.1 (C11, *C*H_2), 21.0 (C23, *C*H_3), 19.3 (C6, *C*H_2), 17.8 (C30, *C*H_3), 15.1 (C25, *C*H_3), 15.0 (C26, *C*H_3), 14.1 (C27, *C*H_3) ppm; MS (ESI, MeOH): *m*/*z* = 548.5 (100%, $[M-Cl]^+$); Anal. for $C_{37}H_{58}ClNO_2$ (584.31): C, 76.05; H, 10.00; N, 2.40. Found: C, 75.86; H, 10.24; N, 2.11.

5.10. (3*S*, 28*S*) 3-*O*-Acetyl-28-[3-(dipropylamino)prop-1-yn-1-yl]lup-20(29)-en-3,28-diol hydrochloride (11)

Compound **11** was prepared as described in the general procedure from **7** (433 mg, 0.85 mmol), dipropylamine (0.14 ml, 1.02 mmol), formalin (37%, 0.35 ml, 4.3 mmol) and CuI (4 mg, 0.02 mmol) in DMSO (5 ml). The solution was stirred for 5 d at 40 °C to yield after re-crystallization **11** (297 mg, 53%) as a colorless solid; mp 205 °C; $[\alpha]_D$ +3.9° (*c* 4.70, MeOH); IR (KBr): *ν* = 3319s, 2942s, 2876s, 2636s, 2515s, 1734s, 1639m, 1542m, 1457s, 1376s, 1248s, 1196m, 1108m, 1073m, 1048s, 979s cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.97 (m, 1H, C*H* (28)), 4.67 (m, 1H, CH_a (29)), 4.57 (m, 1H, CH_b (29)), 4.44 (dd, 1H, *J* = 11.3, 5.2 Hz, C*H* (3)), 4.18 (d, 2H, *J* = 1.7 Hz, CH_2 (35)), 3.20 (m, 4H, 2 × CH_a (36) + (39) + 2 × CH_b (36) + (39)), 2.95 (m, 1H, C*H* (19)), 2.09–1.94 (m, 4H, CH_a (22) + CH_a (21) + CH_a (16) + C*H* (13)), 2.01 (s, 3H, CH_3 (32)), 1.83–1.52 (m, 10 H, C*H* (18) + CH_a (2) + CH_a (15) + CH_b (2) + CH_a (1) + CH_a (12) + 2 × CH_2 (37) + (40)), 1.68 (s, 3H, CH_3 (30)), 1.50–1.12 (m, 10 H, C*H* (9) + CH_b (22) + CH_a (7) + CH_b (7) + CH_b (16) + CH_b (21) + CH_a (11) + CH_b (11) + CH_a (6) + CH_b (6)), 1.09 (s, 3H, CH_3 (25)), 1.06–0.99 (m, 12H, CH_b (15) + CH_b (12) + CH_b (1) + CH_3 (27) + 2 × CH_3 (38) + (41)), 0.95 (s, 3H, CH_3 (24)), 0.87 (s, 3H, CH_3 (23)), 0.75 (s, 3H, CH_3 (26)), 0.72 (d, 1H, *J* = 10.1 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.4 (C31, *C*O), 150.9 (C20, *C*=CH_2), 108.8 (C29, C=CH_2), 92.0 (C33, *C*≡CH), 81.0 (C3, *C*H), 72.6 (C34, C≡*C*H), 64.6 (C28, *C*H), 55.3 (C5, *C*H), 54.8 (C36 + C39, 2 × *C*H$_2$), 50.6 (C17, $C_{quart.}$), 50.3 (C9, *C*H), 48.8 (C18, *C*H), 48.7 (C19, *C*H), 42.7 (C14, $C_{quart.}$), 42.0 (C35, *C*H_2), 40.7 (C8, $C_{quart.}$), 38.5 (C1, *C*H_2), 37.4 (C4, $C_{quart.}$), 37.2 (C13, *C*H), 36.8 (C10, $C_{quart.}$), 34.2 (C7, *C*H_2), 34.1 (C22, *C*H_2), 34.0 (C21, *C*H_2), 31.9 (C16, *C*H_2), 27.6 (C15, *C*H_2), 27.2 (C24, *C*H_3), 26.6 (C12, *C*H_2), 25.0 (C2, *C*H_2), 20.6 (C11, *C*H_2), 19.7 (C32, *C*H_3), 18.0 (C6, *C*H_2), 17.8 (C30, *C*H_3), 17.3 (C37 + C40, 2 × *C*H_2), 15.3 (C23, *C*H_3), 15.2 (C25, *C*H_3), 14.7 (C26, *C*H_3), 14.1 (C27, *C*H_3), 9.8 (C38 + C41, 2 × *C*H_3) ppm; MS (ESI, MeOH): *m*/*z* = 622.6 (100%, $[M-Cl]^+$); Anal. for $C_{41}H_{68}ClNO_3$ (658.43): C, 74.79; H, 10.41; N, 2.13. Found: C, 74.49; H, 10.67; N, 2.00.

5.11. (3*S*, 28*S*) 3-*O*-Acetyl-28-[3-(dicyclohexylamino)prop-1-yn-1-yl]lup-20(29)-en-3,28-diol hydrochloride (12)

Compound **12** was prepared as described in the general procedure from **7** (204 mg, 0.4 mmol), dicyclohexylamine (0.1 ml, 0.61 mmol), formalin (37%, 0.2 ml, 2.5 mmol) and CuI (2 mg, 0.01 mmol) in DMSO (5 ml). The solution was stirred for three days at 40 °C to yield after re-crystallization **12** (157 mg, 53%) as a colorless solid; mp 220 °C; $[\alpha]_D$ +11.2° (*c* 2.30, MeOH); IR (KBr): *ν* = 3265s, 2938s, 2862s, 2427s, 1737s, 1646m, 1456s, 1372s, 1244s, 1132m, 1108m, 977m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.97 (m, 1H, C*H* (28)), 4.67 (m, 1H, CH_a (29)), 4.56 (m, 1H, CH_b (29)), 4.44 (dd, 1H, *J* = 11.1, 5.2 Hz, C*H* (3)), 4.20 (m, 2H, CH_2 (35)), 3.56 (m, 2H, 2 × C*H* (36) + (42)), 2.96 (m, 1H, C*H* (19)), 2.13–1.90 (m, 4H, CH_a (22) + CH_a (21) + CH_a (16) + C*H* (13)), 2.02 (s, 3H, CH_3 (32)), 1.80 (dd, 1H, *J* = 11.8, 11.8 Hz, C*H* (18)), 1.77–1.52 (m, 15H, CH_a (2) + CH_a (15) + CH_b (2) + CH_a (1) + CH_a (12) + 4 × CH_a (37) + (41) + (43) + (47) + 4 × CH_a (38) + (40) + (44) + (46) + 2 × CH_a (39) + (45)), 1.68 (s, 3H, CH_3 (30)), 1.51–1.15 (m, 20 H, C*H* (9) + CH_b (22) + CH_a (7) + CH_b (7) + CH_b (16) + CH_b (21) + CH_a (11) + CH_b (11) + CH_a (6) + CH_b (6) + 4 × CH_b (37) + (41) + (43) + (47) + 4 × CH_b (38) + (40) + (44) + (46) + 2 × CH_b (39) + (45)), 1.10 (s, 3H, CH_3 (25)), 1.07–0.97 (m, 3H, CH_b (15) + CH_b (12) + CH_b (1)), 1.06 (s, 3H, CH_3 (27)), 0.91 (s, 3H, CH_3 (26)), 0.87 (s, 3H, CH_3 (23)), 0.86 (s, 3H, CH_3 (24)), 0.82 (m, 1H, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.4 (C31, *C*O), 150.8 (C20, *C*=CH_2), 108.8 (C29, C=CH_2), 92.6 (C33, *C*≡CH), 81.0 (C3, *C*H), 73.2 (C34, C≡*C*H), 64.6 (C28, *C*HOH), 61.8 (C36 + C42, 2 × *C*H), 55.3 (C5, *C*H), 50.6 (C17, $C_{quart.}$), 50.2 (C9, *C*H), 48.9 (C18, *C*H), 48.7 (C19, *C*H), 42.8 (C14, $C_{quart.}$), 40.7 (C8, $C_{quart.}$), 38.1 (C1, *C*H_2), 37.4 (C4, $C_{quart.}$), 37.2 (C13, *C*H), 36.8 (C10, $C_{quart.}$), 36.5 (C35, *C*H_2), 34.1 (C7, *C*H_2), 34.0 (C22, *C*H_2), 31.9 (C21, *C*H_2), 29.1 (C16, *C*H_2), 27.7 (C15, *C*H_2), 27.0

(C24, CH_3), 25.0 (C12, CH_2), 24.8 (C37 + C41 + C43 + C47, 4 × CH_2), 24.6 (C38 + C40 + C44 + C46, 4 × CH_2), 24.0 (C2, CH_2), 23.2 (C39 + C45, 2 × CH_2), 20.6 (C11, CH_2), 19.7 (C32, CH_3), 19.0 (C30, CH_3), 17.8 (C6, CH_2), 15.5 (C23, CH_3), 15.3 (C25, CH_3), 15.2 (C26, CH_3), 14.1 (C27, CH_3) ppm; MS (ESI, MeOH): m/z = 702.5 (100%, $[M-Cl]^+$); Anal. for $C_{47}H_{76}ClNO_3$ (738.56): C, 76.43; H, 10.37; N, 1.90. Found: C, 76.19; H, 10.57; N, 1.65.

5.12. (3*S*, 28*S*) 3-*O*-Acetyl-28-[3-(dimethylamino)prop-1-yn-1-yl]lup-20(29)-en-3,28-diol hydrochloride (13)

Compound **13** was prepared as described in the general procedure from **7** (500 mg, 1 mmol), dimethylamine solution (40%, 0.25 ml, 2 mmol), formalin (37%, 0.4 ml, 5 mmol) and CuI (2 mg, 0.01 mmol) in DMSO (5 ml). The solution was stirred for 24 h at 40 °C to yield **13** (318 mg, 53%) as a colorless solid; mp 189 °C; $[\alpha]_D$ +11.3° (*c* 4.9, MeOH); IR (KBr): ν = 3374s, 2944s, 2873s, 2604s, 2462s, 1734s, 1640m, 1456s, 1375s, 1317m, 1248s, 1132m, 1108m, 1024s, 979s cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 10.9 (br, 1H, N*H*), 4.78 (s, 1H, C*H* (28)), 4.61 (d, 1H, J = 2.1 Hz, CH_a (29)), 4.51 (s, 1H, CH_b (29)), 4.35 (dd, 1H, J = 4.6, 11.5 Hz, C*H* (3)), 4.07 (s, 2H, CH_2 (35)), 2.94–2.86 (m, 1H, C*H* (19)), 2.72 (s, 3H, CH_3 (36)), 2.51 (s, 3H, CH_3 (37)), 2.38–2.33 (m, 1H, CH_a (22)), 2.10–2.01 (m, 1H, CH_a (21)), 1.97 (s, 3H, CH_3 (32)), 1.95–1.85 (m, 2H, CH_a (16) + C*H* (13)), 1.70–1.63 (m, 1H, C*H* (18)), 1.61 (s, 1H, CH_3 (30)), 1.60–1.04 (m, 16 H, CH_2 (12) + CH_2 (11) + CH_2 (6) + CH_2 (15) + CH_2 (7) + CH_a (1) + C*H* (9) + CH_2 (2) + CH_b (22) + CH_b (21)), 0.99 (s, 3H, CH_3 (27)), 0.96 (s, 3H, CH_3 (25)), 0.93–0.84 (m, 2H, CH_b (1) + CH_b (16)), 0.80 (s, 3H, CH_3 (26)), 0.77 (m, 6 H, 2 × CH_3 (24 + 23)), 0.65 (d, 1H, J = 5.8 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 170.1 (C31, *C*O), 150.8 (C20, *C*=CH_2), 109.4 (C29, C=CH_2), 91.6 (C33, *C*≡CH), 79.8 (C3, *C*H), 73.7 (C34, C≡*C*H), 63.8 (C28, *C*HOH), 54.5 (C5, *C*H), 50.1 (C17, $C_{quart.}$), 49.4 (C9, *C*H), 48.4 (C18, *C*H), 48.1 (C19, *C*H), 45.8 (C14, $C_{quart.}$), 42.5 (C8, $C_{quart.}$), 41.7 (C35, CH_3); 40.4 (C36 + C37, 2 × CH_3), 38.9 (C13, *C*H), 37.7 (C1, CH_2), 37.4 (C4, $C_{quart.}$), 36.5 (C10, $C_{quart.}$), 36.5 (C35, CH_2), 34.0 (C7, CH_2), 33.8 (C22, CH_2), 31.7 (C21, CH_2), 29.1 (C16, CH_2), 27.7 (C15, CH_2), 27.0 (C24, CH_3), 24.7 (C12, CH_2), 23.3 (C2, CH_2), 20.9 (C32, CH_3), 20.4 (C11, CH_2), 18.6 (C30, CH_3), 17.7 (C6, CH_2), 16.4 (C25 + C23, 2 × CH_3), 15.8 (C26, CH_3), 14.7 (C27, CH_3) ppm; MS (ESI, MeOH): m/z = 566 (100%, $[M-Cl]^+$); Anal. for $C_{37}H_{60}NO_3Cl$ (602.33): C, 73.78; H, 10.04; N, 2.33. Found: C, 73.59; H, 10.16; N, 2.20.

5.13. (3*S*, 28*S*) 3-*O*-Acetyl-28-[3-azepan-prop-1-yn-1-yl]lup-20(29)-en-3,28-diol hydrochloride (14)

Compound **14** was prepared as described in the general procedure from **7** (500 mg, 1 mmol), azepane (113 µl, 1.15 mmol), formalin (37%, 0.4 ml, 5 mmol) and CuI (2 mg, 0.01 mmol) in DMSO (5 ml). The solution was stirred for 24 h at 40 °C to yield after recrystallization **14** (366 mg, 56%) as a colorless solid; mp 210 °C; $[\alpha]_D$ +21.5° (*c* 6.3, $CHCl_3$); IR (KBr): ν = 3346s, 2943s, 2871s, 2450m, 1734s, 1640m, 1455s, 1375s, 1247s, 1196m, 1125s, 1075m, 1028s, 978s cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 4.96 (s, 1H, C*H* (28)), 4.67 (d, 1H, J = 1.8 Hz, CH_a (29)), 4.56 (m, 1H, CH_b (29)), 4.44 (dd, 1H, J = 5.1, 11.2 Hz, C*H* (3)), 4.15 (s, 2H, CH_2 (35)), 3.62–3.53 (m, 2H, CH_a (36) + CH_a (41)), 3.31–3.29 (m, 2H, CH_b (36) + CH_b (41)), 2.99–2.92 (m, 1H, C*H* (19)), 2.10–203 (m, 3H, CH_a (22) + CH_a (21) + CH_a (16)), 2.01 (s, 3H, CH_3 (32)), 2.00–1.86 (m, 5 H, + CH_2 (37) + CH_2 (40) + CH_a (22)), 1.84–1.69 (m, 8H, +CH_a (1) CH_2 (38) + CH_2 (39) + CH_2 (12) + C*H* (13)), 1.68 (s, 3H, CH_3 (30)), 1.65–1.15 (m, 13H, CH_2 (11) + CH_2 (6) + CH_2 (15) + CH_2 (7) + C*H* (9) + CH_2 (2) + CH_b (21) + C*H* (18)), 1.10 (s, 3H, CH_3 (27)), 1.05 (s, 3H, CH_3 (25)), 1.03–0.96 (m, 2H, CH_b (1) + CH_b (16)), 0.91 (s, 3H, CH_3 (26)), 0.87–0.82 (m, 7H, 2 × CH_3 (24 + 23) + C*H* (5)) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.4 (C31, *C*O), 150.8 (C20, *C* = CH_2), 108.8 (C29, C = *C*H$_2$), 91.4 (C33, *C*≡CH), 81.0 (C3, *C*H), 73.7 (C34, C≡*C*H), 64.6 (C28, *C*HOH), 55.3 (C5, *C*H), 54.4 (C36 + C41, 2 × CH_2), 50.5 (C17, $C_{quart.}$), 50.2 (C9, *C*H), 48.8 (C18, *C*H), 48.1 (C19, *C*H), 47.8 (C14, $C_{quart.}$), 46.8 (C35, CH_2), 42.7 (C8, $C_{quart.}$), 40.7 (C1, CH_2), 38.1 (C10, $C_{quart.}$), 37.4 (C4, $C_{quart.}$), 37.2 (C13, *C*H), 36.8 (C22, CH_2), 34.0 (C7, CH_2), 33.9 (C16, CH_2), 31.9 (C21, CH_2), 27.6 (C15, CH_2), 27.0 (C24, CH_3), 25.6 (C37 + C40, 2 × CH_3), 25.0 (C12, CH_2), 23.7 (C37 + C39, CH_2), 23.2 (C2, CH_2), 20.6 (C32, CH_3), 19.7 (C11, CH_2), 17.8 (C30, CH_3), 17.7 (C6, CH_2), 15.5 (C25, CH_3),, 15.2 (C26 + C23, 2 × CH_3), 14.1 (C27, CH_3) ppm; MS (ESI, MeOH): m/z = 620.7 (100%, $[M-Cl]^+$); Anal. for $C_{41}H_{66}NO_3Cl$ (656.42): C, 75.02; H, 10.13; N, 2.13. Found: C, 74.87; H, 10.02; N, 2.01.

5.14. (3*S*, 28*S*) 3-*O*-Acetyl-28-[3-morpholin-4-yl-prop-1-yn-1-yl]lup-20(29)-en-3,28-diol hydrochloride (15)

Compound **15** was prepared as described in the general procedure from **7** (500 mg, 1 mmol), morpholine (100 µl, 1.15 mmol), formalin (37%, 0.4 ml, 5 mmol) and CuI (2 mg, 0.01 mmol) in DMSO (5 ml). The solution was stirred for 24 h at 40 °C to yield after recrystallization **15** (238 mg, 37%) as a colorless solid; mp 206 °C; $[\alpha]_D$ +5.0° (*c* 6.5, MeOH); IR (KBr): ν = 3346s, 2943s, 2871s, 2450m, 1734s, 1640m, 1455s, 1375s, 1247s, 1196m, 1125s, 1075m, 1028s, 978s cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 11.80 (s, 1H, N*H*), 4.76 (s, 1H, C*H* (28)), 4.59 (d, 1H, J = 2.1 Hz, CH_a (29)), 4.49 (m, 1H, CH_b (29)), 4.33 (dd, 1H, J = 4.8, 11.8 Hz, C*H* (3)), 4.10 (s, 2H, CH_2 (35)), 4.00–3.70 (m, 6 H, CH_2 (36) + CH_2 (39) + CH_a (37) + CH_a (38)), 3.13–2.96 (m, 2H, CH_b (37) + CH_b (38)), 2.93–2.83 (m, 1H, C*H* (19)), 2.00–1.96 (m, 2H, CH_a (22) + CH_a (21), 1.95 (s, 3H, CH_3 (32)), 1.93–1.83 (m, 2H, CH_a (16) + C*H* (13)), 1.68–1.61 (m, 1H, C*H* (18)), 1.60 (s, 3H, CH_3 (30)), 1.58–1.08 (m, 16 H, CH_2 (12) + CH_2 (11) + CH_2 (6) + CH_2 (15) + CH_2 (7) + CH_a (1) + C*H* (9) + CH_2 (2) + CH_b (22) + CH_b (21)), 0.97 (s, 3H, CH_3 (27)), 0.94 (s, 3H, CH_3 (25)), 0.92–0.85 (m, 2H, CH_b (1)+ CH_b (16)), 0.79 (s, 3H, CH_3 (26)), 0.76 (m, 6 H, 2 × CH_3 (24 + 23)), 0.72–0.70 (m, 1H, C*H* (5)) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 169.7 (C31, *C*O), 150.5 (C20, *C*=CH_2), 109.1 (C29, C=CH_2), 91.9 (C33, *C*≡CH), 79.7 (C3, *C*H), 73.1 (C34, C≡*C*H), 64.7 (C36 + 39, 2 × CH_2), 63.7 (C28, *C*HOH), 62.9 (C37 + 38, 2 × CH_2), 54.5 (C5, *C*H), 50.0 (C17, $C_{quart.}$), 49.3 (C9, *C*H), 48.4 (C18, *C*H), 48.0 (C19, *C*H), 44.9 (C35, CH_2), 44.2 (C14, $C_{quart.}$), 42.5 (C8, $C_{quart.}$), 38.9 (C13, *C*H), 37.7 (C1, CH_2), 37.3 (C4, $C_{quart.}$), 36.5 (C10, $C_{quart.}$) 34.0 (C7, CH_2), 33.7 (C22, CH_2), 33.6 (C16, CH_2), 31.7 (C21, CH_2), 27.6 (C15, CH_2), 27.4 (C24, CH_3), 24.7 (C12, CH_2), 23.3 (C2, CH_2), 20.9 (C32, CH_3), 20.4 (C11, CH_2), 18.5 (C30, CH_3), 17.6 (C6, CH_2), 15.8 (C25 + C23, 2 × CH_3), 15.1 (C26, CH_3), 14.7 (C27, CH_3) ppm; MS (ESI, MeOH): m/z = 608.5 (100%, $[M-Cl]^+$); Anal. for $C_{39}H_{62}NO_4Cl$ (644.37): C, 72.69; H, 9.70; N, 2.17. Found: C, 72.51; H, 9.93; N, 2.11.

5.15. (3*S*, 28*S*) 3-*O*-Acetyl-28-[3-thiomorpholin-4-yl-prop-1-yn-1-yl]lup-20(29)-en-3,28-diol hydrochloride (16)

Compound **16** was prepared as described in the general procedure from **7** (500 mg, 1 mmol), thiomorpholine (109 µl, 1.15 mmol), formalin (37%, 0.4 ml, 5 mmol) and CuI (2 mg, 0.01 mmol) in DMSO (5 ml). The solution was stirred for 24 h at 40 °C to yield after re-crystallization **16** (284 mg, 43%) as a colorless solid; mp 208–211 °C; $[\alpha]_D$ +6.0° (*c* 5.5, MeOH); IR (KBr): ν = 3385s, 3068m, 2943s, 2873s, 2363m, 1732s, 1639m, 1454s, 1375s, 1318m, 1248s, 1133m, 1108m, 1028s, 980s cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 4.78 (s, 1H, C*H* (28)), 4.61 (d, 1H, J = 2.0 Hz, CH_a (29)), 4.51 (m, 1H, CH_b (29)), 4.35 (dd, 1H, J = 4.5, 11.5 Hz, C*H* (3)), 4.14 (s, 2H, CH_2 (35)), 3.75–3.65 (m, 2H, CH_a

(36) + CH_a (39)), 3.20–3.05 (m, 4H, CH_b (36) + CH_b (39) + CH_a (37) + CH_a (38)), 2.95–2.80 (m, 3H, CH (19) + CH_b (37) + CH_b (38)), 2.10–2.03 (m, 1H, CH_a (22)), 2.00–1.85 (m, 5 H, CH_3 (32) + CH_a (21) + CH_a (16)), 1.75–1.63 (m, 2H, CH (13) + CH (18)), 1.61 (s, 3H, CH_3 (30)), 1.59–1.01 (m, 16 H, CH_2 (12) + CH_2 (11) + CH_2 (6) + CH_2 (15) + CH_2 (7) + CH_a (1) + CH (9) + CH_2 (2) + CH_b (22) + CH_b (21)), 0.99 (s, 3H, CH_3 (27)), 0.96 (s, 3H, CH_3 (25)), 0.94–0.89 (m, 2H, CH_b (1)+ CH_b (16)), 0.81 (s, 3H, CH_3 (26)), 0.78 (m, 6 H, 2 × CH_3 (24 + 23)), 0.67–0.64 (m, 1H, CH (5)) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 170.1 (C31, CO), 150.8 (C20, C=CH_2), 109.4 (C29, C=CH_2), 91.7 (C33, C≡CH), 79.9 (C3, CH), 73.3 (C34, C≡CH), 63.8 (C28, CHOH), 54.5 (C5, CH), 52.2 (C36 + C39, 2 × CH_2), 50.1 (C17, $C_{quart.}$), 49.4 (C9, CH), 48.5 (C18, CH), 48.1 (C19, CH), 45.7 (C14, $C_{quart.}$), 42.5 (C8, $C_{quart.}$), 40.1 (C36, CH_3), 38.9 (C13, CH), 37.7 (C1, CH_2), 37.3 (C4, $C_{quart.}$), 36.5 (C10, $C_{quart.}$), 36.4 (C35, CH_2), 34.1 (C7, CH_2), 33.8 (C22, CH_2), 33.6 (C16, CH_2), 31.7 (C21, CH_2), 27.6 (C15, CH_2), 27.4 (C24, CH_3), 24.7 (C12, CH_2), 23.9 (C37 + C38, CH_2), 22.3 (C2, CH_2), 20.9 (C32, CH_3), 20.4 (C11, CH_2), 18.5 (C30, CH_3), 17.7 (C6, CH_2), 16.4 (C25 + C23, 2 × CH_3), 15.8 (C26, CH_3), 14.7 (C27, CH_3) ppm; MS (ESI, MeOH): m/z = 624.6 (100%, [M−Cl]$^+$); Anal. for $C_{39}H_{62}NO_3SCl$ (660.43): C, 70.93; H, 9.46; N, 2.12; S, 4.86. Found: C, 70.76; H, 9.64; N, 2.04; S, 4.63.

5.16. (3*S*, 28*S*) 3-*O*-Acetyl-28-[(diallylamino)-1-yl-prop-1-yn-1-yl]lup-20(29)-en-3,28-diol (17)

Compound **17** was prepared as described in the general procedure from **7** (500 mg, 1 mmol), diallylamine (109 μl, 1.15 mmol), formalin (37%, 0.4 ml, 5 mmol) and CuI (2 mg, 0.01 mmol) in THF (5 ml). The crude residue was purified by column chromatography (silica gel, *n*-hexane/ethyl acetate, 8/2) to yield **17** (487 mg, 79%) as a colorless solid; mp 75–80 °C $[\alpha]_D$ +142.9° (*c* 0.6, $CHCl_3$); R_f = 0.26 (*n*-hexane/ethyl acetate, 8/2); IR (KBr): ν = 3457br, 3075m, 2943s, 1735s, 1642s, 1454s, 1376s, 1326s, 1245s, 1028s, 1008s cm^{-1}; ^{1}H NMR (400 MHz, $CHCl_3$): δ = 5.84–5.70 (m, 2H, 2 × CH (37)), 5.21–5.05 (m, 4H, 2 × CH_2 (38)), 4.84 (s, 1H, CH (28)), 4.64 (s, 1H, CH_a (29)), 4.51 (s, 1H, CH_b (29)), 4.40 (dd, 1H, J = 6.4, 9.7 Hz, CH (3)), 3.35 (s, 2H, CH_2 (35)), 3.10–3.00 (m, 4H, 2 × CH_2 (36)), 2.91–2.78 (m, 1H, CH (19)), 2.21–2.10 (m, 2H, CH_a (22) + CH_a (16)), 1.97 (s, 3H, CH_3 (32)), 1.96–1.85 (m, 2H, CH_a (21) + CH (13)), 1.74–1.65 (m, 3H, CH (18) + CH_a (1) + CH_a (12)), 1.62 (s, 3H, CH_3 (30)), 1.69–1.08 (m, 14H, CH_2 (11) + CH_2 (6) + CH_2 (15) + CH_2 (7) + CH (9) + CH_2 (2) + CH_b (22) + CH_b (21) + CH_b (16)), 0.98 (s, 3H, CH_3 (27)), 0.93 (s, 3H, CH_3 (25)), 0.92–0.81 (m, 2H, CH_b (12) + CH_b (1)), 0.80–0.75 (m, 9H, 3 × CH_3 (26) + (24) + (23)), 0.73 (d, 1H, J = 10.5 Hz, CH (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.0 (C31, C=O), 151.0 (C20, C=CH_2), 135.0 (C38, 2 × CH_2=CH), 118.2 (C37, 2 × CH=CH_2), 109.6 (C29, CH_2=C), 85.9 (C33, C≡C), 80.9 (C3, CH), 80.8 (C34, C≡C), 66.1 (C28, CH), 56.3 (C35, CH_2), 55.4 (C5, CH), 50.8 (C17, $C_{quart.}$), 50.2 (C9, CH), 49.0 (C18, CH), 48.7 (C19, CH), 43.0 (C14, $C_{quart.}$), 41.7 (C36, 2 × CH_2), 40.9 (C8, $C_{quart.}$), 38.4 (C4, $C_{quart.}$), 37.8 (C1, CH_2), 37.2 (C13, CH), 37.0 (C10, $C_{quart.}$), 34.7 (C7, CH_2), 34.1 (C21, CH_2), 34.0 (C16, CH_2), 32.4 (C22, CH_2), 28.0 (C23, CH_3), 27.8 (C15, CH_2), 25.1 (C12, CH_2), 23.7 (C2, CH_2), 21.3 (C32, CH_3), 20.9 (C11, CH_2), 18.8 (C30, CH_3), 18.1 (C6, CH_2), 16.5 (C24 + C26, 2 × CH_3), 16.1 (C25, CH_3), 15.0 (C27, CH_3) ppm; MS (ESI, MeOH): m/z = 618.6 (100%, [M+H]$^+$); Anal. for $C_{41}H_{63}NO_3$ (617.94): C, 79.69; H, 10.28; N, 2.27. Found: C, 79.52; H, 10.48; N, 2.14.

5.17. (3*S*, 28*S*) 3-*O*-Acetyl-28-[(diallylamino)-1-yl-prop-1-yn-1-yl]lup-20(29)-en-3,28-diol hydrochloride (18)

Compound **18** was prepared by dissolving compound **17** (150 mg, 0.24 mmol) in diethylether and treating this solution with dry HCl_g at 0 °C for 1 h. Precipitation was completed by standing for 12 h at 4 °C; the product was filtered off, washed with water and diethyl ether and dried to yield **18** (143 mg, 91%) as a colorless solid; mp 226–228 °C; $[\alpha]_D$ +16.6° (*c* 4.05, $CHCl_3$); IR (KBr): ν = 3301m, 2944s, 2477m, 1732s, 1643w, 1455m, 1370m, 1247s, 1132w, 1027m cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.70 (br, 1H, NH), 6.05–5.95 (m, 2H, 2 × CH (37)), 5.60–5.48 (m, 4H, 2 × CH_2 (38)), 4.80 (br, 1H, CH (28)), 4.62 (d, 1H, J = 1.8 Hz, CH_a (29)), 4.52 (s, 1H, CH_b (29)), 4.36 (dd, 1H, J = 4.6, 11.5 Hz, CH (3)), 3.97 (s br, 2H, CH_2 (35)), 3.80–3.65 (m, 4H, 2 × CH_2 (36)), 2.95–2.85 (m, 1H, CH (19)), 2.05–2.00 (m, 1H, CH_a (22)), 1.98 (s, 3H, CH_3 (32)), 1.93–1.81 (m, 3H, CH_a (21) + CH (13) + CH_a (16)), 1.70–1.65 (m, 1H, CH (18)), 1.63 (s, 3H, CH_3 (30)), 1.69–1.08 (m, 15H, CH_2 (11) + CH_2 (6) + CH_2 (15) + CH_2 (7) + CH_a (1) + CH (9) + CH_2 (2) + CH_b (22) + CH_b (21) + CH_b (16)), 1.02–0.89 (m, 8H, CH_2 (12) + 2 × CH_3 (27) + (25)), 0.86–0.83 (m, 1H, CH_b (1)), 0.82–0.75 (m, 9H, 3 × CH_3 (26) + (24) + (23)), 0.65 (d, 1H, J = 8.5 Hz, CH (5)) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 170.0 (C31, C=O), 150.8 (C20, C=CH_2), 125.0 (C37, CH_2=CH), 117.0 (C38, CH=CH_2), 109.4 (C29, CH_2=C), 91.9 (C33, C≡C), 79.8 (C3, CH), 74.3 (C34, C≡C), 66.7 (C28, CH), 55.5 (C5, CH), 50.3 (C17, $C_{quart.}$), 50.6 (C35, CH_2), 50.2 (C9, CH), 49.2 (C18, CH), 48.8 (C19, CH), 43.1 (C14, $C_{quart.}$), 41.0 (C8, $C_{quart.}$), 40.9 (C36, 2 × CH_2), 38.5 (C4, $C_{quart.}$), 37.9 (C1, CH_2), 37.5 (C13, CH), 37.2 (C10, $C_{quart.}$), 34.7 (C7, CH_2), 34.3 (C21, CH_2), 34.1 (C16, CH_2), 32.4 (C22, CH_2), 28.0 (C23, CH_3), 27.8 (C15, CH_2), 25.2 (C12, CH_2), 23.8 (C2, CH_2), 21.6 (C32, CH_3), 20.9 (C11, CH_2), 18.8 (C30, CH_3), 18.2 (C6, CH_2), 16.5 (C24, CH_3), 16.2 (C26 + C25, 2 × CH_3), 15.1 (C27, CH_3) ppm; MS (ESI, MeOH): m/z = 618.8 (100%, [M+H]$^+$); Anal. for $C_{41}H_{64}NO_3Cl$ (654.40): C, 75.25; H, 9.86; N, 2.14. Found: C, 75.13; H, 9.99; N, 1.97.

5.18. (3*S*, 28*S*) 3-*O*-Acetyl-28-[piperidine-1-yl-prop-1-yn-1-yl]lup-20(29)-en-3,28-diol hydrochloride (19)

Compound **19** was prepared as described in the general procedure from **7** (300 mg, 0.59 mmol), piperidine (114 μl, 1.15 mmol), formalin (37%, 0.4 ml, 5 mmol) and CuI (20 mg, 0.1 mmol) in THF (5 ml); **19** (225 mg, 67%) was obtained as a colorless solid (255 mg, 67%); mp 212–215 °C; $[\alpha]_D$ +74.0° (*c* 0.6, $CHCl_3$); IR (KBr) ν = 3405br, 2943s, 2870s, 2641m, 2534m, 1733s, 1638m, 1456s, 1384s, 1247s, 1197w, 1133w, 1107w, 1031w cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): δ = 4.90–4.85 (m, 1H, CH (28)), 4.57 (d, 1H, J = 1.8 Hz, CH_a (29)), 4.49–4.45 (m, 1H, CH_b (29)), 4.34 (dd, 1H, J = 5.4, 10.8 Hz, CH (3)), 4.00 (s br, 2H, CH_2 (35)), 3.62–3.42 (m, 2H, CH_a (36) + CH_a (40)), 3.09–2.80 (m, 4H, CH (19) + CH_b (36) + CH_b (40)), 2.22–1.84 (m, 9H, CH_a (22) + CH_a (16) + CH_3 (32) + CH_a (21) + CH (13) + CH_a (37) + CH_a (39)), 1.74–1.05 (m, 24H, CH_b (37) + CH_b (39) + CH_2 (38) + CH (18) + CH_a (1) + CH_2 (12) + CH_3 (30) + CH_2 (11) + CH_2 (6) + CH_a (15) + CH_2 (7) + CH (9) + CH_2 (2) + CH_b (22) + CH_b (21) + CH_b (16)), 1.01 (s, 3H, CH_3 (27)), 0.96 (s, 3H, CH_3 (25)), 0.94–0.88 (m, 2H, CH_b (15) + CH_b (1)), 0.86 (s, 3H, CH_3 (26)), 0.81 (s, 3H, CH_3 (24)), 0.77 (s, 3H, CH_3 (23)), 0.75–0.71 (m, 1H, CH (5)) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 171.4 (C31, C=O), 150.9 (C20, C=CH_2), 108.8 (C29, CH_2=C), 92.0 (C33, C≡C), 81.0 (C3, CH), 73.1 (C34, C≡C), 64.7 (C28, CH), 55.4 (C5, CH), 52.3 (C36 + C40, 2 × CH_2), 50.5 (C17, $C_{quart.}$), 50.3 (C9, CH), 48.9 (C18, CH),48.7 (C19, CH), 45.9 (C35, CH_2), 42.8 (C14, $C_{quart.}$), 40.7 (C8, $C_{quart.}$), 38.6 (C4, $C_{quart.}$), 38.5 (C1, CH_2), 37.4 (C10, $C_{quart.}$), 37.2 (C13, CH), 36.9 (C16, CH_2), 36.8 (C22, CH_2), 34.1 (C7, CH_2), 31.9 (C21, CH_2), 27.6 (C15, CH_2), 27.0 (C23, CH_3), 25.0 (C12, CH_2), 23.3 (C2, CH_2), 22.8 (C37 + C39, 2 × CH_2), 21.1 (C38, CH_2), 20.6 (C11, CH_2), 19.7 (C32, CH_3), 18.0 (C6, CH_2), 17.7 (C30, CH_3), 15.5 (C24, CH_3), 15.3 (C26, CH_3), 15.2 (C25, CH_3), 14.2 (C27, CH_3) ppm; MS (ESI, MeOH): m/z = 606.7 (100%, [M+H]$^+$); Anal. for $C_{39}H_{63}N_2O_3Cl$ (643.38): C, 72.81; H, 9.87; N, 4.35. Found: C, 72.69; H, 10.03; N, 4.18.

5.19. (3*S*, 28*S*) 3-*O*-Acetyl-28-[3-pyrrolidine-1-yl-prop-1-yn-1-yl]-lup-20(29)-en-3,28-diol hydrochloride (20)

Compound **20** was prepared as described in the general procedure from **7** (200 mg, 0.39 mmol), pyrrolidine (94 μl, 1.15 mmol), formalin (37%, 0.4 ml, 5 mmol) and CuI (20 mg, 0.1 mmol) in THF (5 ml); **20** (164 mg, 67%) was obtained as a colorless solid; mp 206–207 °C; $[\alpha]_D$ +21.1° (*c* 2.49, $CHCl_3$); IR (KBr): ν = 3442br, 2943s, 1705m, 1638s, 1442m, 1384s, 1047w cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ = 4.96–4.91 (m, 1H, C*H* (28)), 4.66 (d, 1H, *J* = 2.2 Hz, CH_a (29)), 4.55 (dd, 1H, *J* = 2.4, 1.3 Hz, CH_b (29)), 4.43 (dd, 1H, *J* = 5.4, 10.9 Hz, C*H* (3)), 4.09–4.02 (s br, 2H, CH_2 (35)), 3.35–3.25 (m, 4H, 2 × CH_2 (36)), 2.99–2.90 (m, 1H, C*H* (19)), 2.10–1.90 (m, 11H, CH_a (22) + CH_a (16) + CH_3 (32) + CH_a (21) + C*H* (13) + 2 × CH_2 (37)), 1.82–1.68 (m, 3H, C*H* (18) + CH_a (1) + CH_a (15)), 1.67 (s, 3H, CH_3 (30)), 1.66–1.13 (m, 14H, CH_2 (12) + CH_2 (11) + CH_2 (6) + CH_2 (7) + C*H* (9) + CH_2 (2) + CH_b (22) + CH_b (21) + CH_b (16)), 1.08 (s, 3H, CH_3 (25)), 1.04 (s, 3H, CH_3 (27)), 1.02–0.93 (m, 2H, CH_b (15) + CH_b (1)), 0.90 (s, 3H, CH_3 (26)), 0.86 (s, 3H, CH_3 (24)), 0.85 (s, 3H, CH_3 (23)), 0.84–0.77 (m, 1H, C*H* (5)) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 173.2 (C31, *C*=O), 152.5 (C20, *C*=CH_2), 110.3 (C29, *C*H_2=C), 91.3 (C33, *C*≡C), 82.7 (C3, *C*H), 76.6 (C34, *C*≡C), 66.2 (C28, *C*H), 56.9 (C5, *C*H), 54.6 (C36, 2 × *C*H_2), 52.1 (C17, $C_{quart.}$), 51.8 (C9, *C*H), 50.4 (C18, *C*H), 50.2 (C19, *C*H), 44.7 (C35, *C*H_2), 44.3 (C14, $C_{quart.}$), 42.3 (C8, $C_{quart.}$), 39.7 (C4, $C_{quart.}$), 39.0 (C1, *C*H_2), 38.7 (C13, *C*H), 38.4 (C10, $C_{quart.}$), 35.7 (C16, *C*H_2), 35.5 (C22, *C*H_2), 35.0 (C7, *C*H_2), 33.5 (C21, *C*H_2), 29.2 (C15, *C*H_2), 28.6 (C23, *C*H_3), 24.8 (C12, *C*H_2), 24.7 (C37, 2 × *C*H_2), 24.6 (C2, *C*H_2), 22.2 (C11, *C*H_2), 21.3 (C32, *C*H_3), 19.4 (C6, *C*H_2), 19.3 (C30, *C*H_3), 17.1 (C24, *C*H_3), 16.8 (C26, *C*H_3), 16.7 (C25, *C*H_3), 15.7 (C27, *C*H_3) ppm; MS (ESI, MeOH): *m*/*z* = 592.6 (100%, $[M+H]^+$); Anal. for $C_{39}H_{62}NO_3Cl$ (628.37): C, 74.55; H, 9.95; N, 2.23. Found: C, 74.33; H, 10.12; N, 2.03.

5.20. (3*S*, 28*S*) 3-*O*-Acetyl-28-[dibenzylamine-1-yl-prop-1-yn-1-yl]-lup-20(29)-en-3,28-diol hydrochloride (21)

Compound **21** was prepared as described in the general procedure from **7** (500 mg, 1 mmol), dibenzylamine (227 μl, 1.15 mmol), formalin (37%, 0.4 ml, 5 mmol) and CuI (20 mg, 0.1 mmol) in THF (5 ml); compound **21** (291 mg, 38%) was obtained as a colorless solid; mp 161–163 °C; $[\alpha]_D$ +7.5° (*c* 4.1, $CHCl_3$); IR (KBr): ν = 3287br, 2943s, 2452br, 1736s, 1641w, 1499w, 1457s, 1376m, 1248s, 1132w, 1028m cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 12.80–12.75 (m, 1H, N*H*), 7.75–7.60 (m, 4H, C*H* (38)), 7.40–7.30 (m, 6 H, CH_3 (39) + (40)), 5.02–4.96 (br, 1H, C*H* (28)), 4.63 (d, 1H, *J* = 1.9 Hz, CH_a (29)), 4.54–4.50 (m, 1H, CH_b (29)), 4.40 (dd, 1H, *J* = 5.7, 10.3 Hz, C*H* (3)), 4.37–4.09 (m, 4H, 2 × CH_2 (36)), 3.65–3.55 (m, 2H, CH_2 (35)), 2.94–2.85 (m, 1H, C*H* (19)), 2.15–2.00 (m, 2H, CH_a (16) + CH_a (21)), 1.97 (s, 3H, CH_3 (32)), 1.95–1.86 (m, 2H, CH_a (22) + C*H* (13)), 1.77–1.64 (m, 3H, C*H* (18) + CH_a (1) + CH_a (12)), 1.63 (s, 3H, CH_3 (30)), 1.60–1.00 (m, 14H, CH_2 (11) + CH_2 (6) + CH_a (15) + CH_2 (7) + C*H* (9) + CH_2 (2) + CH_b (22) + CH_b (21) + CH_b (16) + CH_b (12)), 0.98 (s, 3H, CH_3 (27)), 0.98–0.96 (m, 1H, CH_b (15)), 0.95 (s, 3H, CH_3 (25)), 0.94–0.93 (m, 1H, CH_b (1)), 0.78 (s, 3H, CH_3 (26)), 0.77 (s, 3H, CH_3 (24)), 0.69 (s, 3H, CH_3 (23)), 0.62 (d, 1H, *J* = 9.0 Hz, C*H* (5)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.0 (C31, *C*=O), 150.7 (C20, *C*=CH_2), 131.5 (C39, 4 × *C*H), 130.1 (C40, 2 × *C*H), 129.2 (C38, 4 × *C*H), 128.6 (C37, 2 × *C*H), 109.8 (C29, *C*H_2=C), 91.8 (C33, *C*≡C), 80.8 (C3, *C*H), 73.4 (C34, *C*≡C), 65.7 (C28, *C*H), 56.5 (C36, 2 × *C*H_2), 55.3 (C5, *C*H), 51.6 (C17, $C_{quart.}$), 50.2 (C9, *C*H), 48.9 (C18, *C*H), 48.7 (C19, *C*H), 43.0 (C14, $C_{quart.}$), 40.9 (C35, *C*H_2), 40.8 (C8, $C_{quart.}$), 38.8 (C4, $C_{quart.}$), 38.3 (C1, *C*H_2), 37.7 (C10, $C_{quart.}$), 37.3 (C13, *C*H), 34.5 (C16 + C22, 2 × *C*H_2), 34.2 (C7, *C*H_2), 32.3 (C21, *C*H_2), 27.9 (C23, *C*H_3), 27.9 (C15, *C*H_2), 25.0 (C12, *C*H_2), 23.7 (C2, *C*H_2), 21.3 (C32, *C*H_3), 20.8 (C11, *C*H_2), 18.8 (C30, *C*H_3), 18.1 (C6, *C*H_2), 16.5 (C24, *C*H_3), 16.3 (C26, *C*H_3), 16.1 (C25, *C*H_3), 15.0 (C27, *C*H_3) ppm; MS (ESI, MeOH): *m*/*z* = 718.6 (100%, $[M+H]^+$); Anal. for $C_{49}H_{68}NO_3Cl$ (754.52): C, 78.00; H, 9.08; N, 1.86. Found: C, 77.87; H, 9.17; N, 1.64.

5.21. (3*S*, 28*S*) 3-*O*-Acetyl-28-[3-(methyldipropylaminium)-prop-1-in-1-yl]lup-20(29)-en-3,28-diol iodide (22)

A solution of compound **11** (200 mg, 0.3 mmol) in methanol (5 ml) was treated with an aq. solution of KOH (satd., 0.5 ml) for 10 min. After extraction with ethyl acetate (3 × 100 ml), the organic layers were dried (Na_2SO_4), the solvents were evaporated, and the remaining solid was treated in diethylether (10 ml) with methyl iodide (2 ml, 32.0 mmol) for 5 days under argon. The precipitate was washed with ether to yield **22** (202 mg, 87%) as an off-white solid; mp 170 °C; $[\alpha]_D$ +9.7° (*c* 4.55, MeOH); IR (KBr): ν = 3275s, 3078m, 2965s, 2877s, 1732s, 1648m, 1534m, 1456s, 1392s, 1371s, 1318m, 1246s, 1155m, 1119s, 1077m, 1024s, 977s cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.00 (m, 1H, C*H* (28)), 4.67 (m, 1H, CH_a (29)), 4.57 (m, 1H, CH_b (29)), 4.44 (dd, 1H, *J* = 11.1, 5.2 Hz, C*H* (3)), 4.36 (d, 2H, *J* = 1.7 Hz, CH_2 (35)), 3.37 (m, 4H, 2 × CH_a (36) + (39) + 2 × CH_b (36) + (39)), 3.13 (s, 3H, CH_3 (42)), 2.96 (m, 1H, C*H* (19)), 2.10–1.91 (m, 4H, CH_a (22) + CH_a (21) + CH_a (16) + C*H* (13)), 2.02 (s, 3H, CH_3 (32)), 1.85–1.52 (m, 6 H, C*H* (18) + CH_a (2) + CH_a (15) + CH_b (2) + CH_a (1) + CH_a (12)), 1.81 (*q*, 4H, *J* = 7.3 Hz, 2 × CH_2 (37) + (40)), 1.68 (s, 3H, CH_3 (30)), 1.51–1.14 (m, 10H, CH_a (6) + CH_b (6) + C*H* (9) + CH_b (22) + CH_a (7) + CH_b (7) + CH_b (16) + CH_b (21) + CH_a (11) + CH_b (11)), 1.10 (s, 3H, CH_3 (25)), 1.07–1.00 (m, 3H, CH_b (15) + CH_b (12) + CH_b (1)), 1.06 (s, 3H, CH_3 (27)), 1.03 (*t*, 6 H, *J* = 7.3 Hz, 2 × CH_3 (38) + (41)), 0.91 (s, 3H, CH_3 (24)), 0.87 (s, 3H, CH_3 (23)), 0.86 (s, 3H, CH_3 (26)), 0.83 (d, 1H, J = 11.6 Hz, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.8 (C31, *C*O), 152.2 (C20, *C*=CH_2), 110.3 (C29, C=*C*H_2), 94.9 (C33, *C*≡CH), 82.4 (C3, *C*H), 73.7 (C34, C≡*C*H), 66.1 (C28, *C*HOH), 64.6 (C36 + C39, 2 × *C*H_2), 56.7 (C5, *C*H), 53.3 (C17, $C_{quart.}$), 52.0 (C35, *C*H_2), 51.6 (C9, *C*H), 50.3 (C18, *C*H), 50.1 (C19, *C*H), 48.9 (C42, *C*H_3), 44.2 (C14, $C_{quart.}$), 42.1 (C8, $C_{quart.}$), 39.5 (C1, *C*H_2), 38.8 (C4, $C_{quart.}$), 38.6 (C13, *C*H), 38.2 (C10, $C_{quart.}$), 35.6 (C7, *C*H_2), 35.4 (C22, *C*H_2), 35.3 (C21, *C*H_2), 33.3 (C16, *C*H_2), 29.0 (C15, *C*H_2), 28.4 (C24, *C*H_3), 27.0 (C12, *C*H_2), 26.4 (C2, *C*H_2), 22.0 (C11, *C*H_2), 21.1 (C32, *C*H_3), 19.2 (C6, *C*H_2), 19.2 (C30, *C*H_3), 17.0 (C37 + C40, 2 × *C*H_2), 16.7 (C23, *C*H_3), 16.6 (C25, *C*H_3), 15.6 (C26, *C*H_3), 15.5 (C27, *C*H_3), 10.9 (C38 + C41, 2 × *C*H_3) ppm; MS (ESI, MeOH): *m*/*z* = 636.5 (100%, $[M-Cl]^+$); Anal. for $C_{42}H_{70}INO_3$ (763.91): C, 66.03; H, 9.24; N, 1.83. Found: C 65.86, H 9.43, N 1.72.

5.22. (3*S*, 28*S*) 3-*O*-Acetyl-28-[diallylmethylaminium-prop-1-yn-1-yl]lup-20(29)-en-3,28-diol iodide (23)

Compound **23** was obtained by treating compound **17** (200 mg, 0.23 mmol) with methyl iodide (19 μl, 0.3 mmol) in abs. diethyl ether (10 ml) for 5 days as described above. Compound **23** (141 - mg, 81%) was obtained as an off-white solid; mp 170–173 °C; $[\alpha]_D$ −42.4° (*c* 3.15, MeOH); IR (KBr): ν = 3405br, 2943s, 1732m, 1639w, 1456m, 1376s, 1384s, 1248s, 1113w, 1029m cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ = 6.08–5.92 (m, 2H, C*H* (38 + 39)), 5.7-4–5.63 (m, 4H, 2 × CH_2 (41 + 40)), 4.92 (br, 1H, C*H* (28)), 4.58 (d, 1H, *J* = 1.9 Hz, CH_a (29)), 4.50–4.45 (m, 1H, CH_b (29)), 4.35 (dd, 1-H, *J* = 5.4, 10.8 Hz, C*H* (3)), 4.20 (s, 2H, CH_2 (35)), 4.00–3.93 (m, 4-H, 2 × CH_2 (36 + 37)), 3.00 (s, 3H, CH_3 (42)), 2.93–2.88 (m, 1H, C*H* (19)), 2.05–1.94 (m, 1H, CH_a (22) + CH_a (21)), 1.92 (s, 3H, CH_3 (32)), 1.91–1.82 (m, 2H, CH_a (16) + C*H* (13)), 1.76–1.61 (m, 3H, C*H* (18) + CH_a (12) + CH_a (1)), 1.59 (s, 3H, CH_3 (30)), 1.58–1.08 (m, 14H, CH_2 (11) + CH_2 (6) + CH_a (15) + CH_2 (7) + C*H* (9) + CH_2

(2) + CH_b (22) + CH_b (12) + CH_b (16) + CH_b (21)), 1.01 (s, 3H, CH_3 (27)), 0.97 (s, 3H, CH_3 (25)), 0.95–0.89 (m, 2H, CH_b (1) + CH_b (15)-), 0.82 (s, 3H, CH_3 (26)), 0.79–0.75 (m, 6 H, 2 × CH_3 (24) + (23)), 0.74–0.72 (m, 1H, CH (5)) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 171.5 (C31, C=O), 150.8 (C20, C=CH_2), 128.6 (C40 + C41, CH_2=CH), 124.3 (C38 + C39, CH=CH_2), 108.9 (C29, CH_2=C), 94.1 (C33, C≡C), 81.0 (C3, CH), 72.4 (C34, C≡C), 64.7 (C28, CH), 63.6 (C36 + C37, 2 × CH_2), 55.3 (C5, CH), 51.2 (C17, $C_{quart.}$), 50.5 (C35, CH_2), 50.2 (C9, CH), 48.9 (C18, CH), 48.7 (C19, CH), 46.5 (C42, CH_3), 42.8 (C14, $C_{quart.}$), 40.7 (C8, $C_{quart.}$), 38.1 (C4, $C_{quart.}$), 37.4 (C1, CH_2), 37.2 (C13, CH), 36.8 (C10, $C_{quart.}$), 34.1 (C7, CH_2), 34.0 (C21, CH_2), 33.9 (C16, CH_2), 31.9 (C22, CH_2), 27.6 (C15, CH_2), 27.0 (C23, CH_3), 25.0 (C12, CH_2), 23.2 (C2, CH_2), 20.6 (C11, CH_2), 19.7 (C32, CH_3), 17.8 (C6, CH_2), 17.6 (C30, CH_3), 15.5 (C24, CH_3), 15.3 (C26, CH_3), 15.2 (C25, CH_3), 14.1 (C27, CH_3) ppm; MS (ESI, MeOH): m/z = 632.7 (100%, $[M]^+$), 127.1 (100%, $[I]^-$) ; Anal. for $C_{42}H_{66}NO_3I$ (759.88): C, 66.39; H, 8.75; N, 1.84. Found: C, 66.23; H, 8.95; N, 1.77.

5.23. (3*S*, 28*S*)-28-[3-(Diisopropylamino)propyl]lup-20(29)-en-3,28-diol hydro-chloride (24)

Hydrogenation (6 bar) of **9** (300 mg, 0.49 mmol) in MeOH (5 ml) in the presence of Lindlar's catalyst (1.1 g, 0.5 mmol, 5% Pd auf $CaCO_3$ treated with Pb) followed by usual work-up gave **24** (135 mg, 45%) as a colorless solid; mp 245 °C; $[\alpha]_D$ +6.7° (*c* 4.15, MeOH); IR (KBr): ν = 3417br, 2942s, 2870s, 2660m, 1638m, 1465m, 1386m, 1172w, 1134m, 1089m, 1045m, 982m cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ = 4.68 (m, 1H, CH_a (29)), 4.56 (m, 1H, CH_b (29)), 4.05 (d, 1H, *J* = 10.0 Hz, CH (28)), 3.74 (*sept.*, 2H, *J* = 6.5, 2.6 Hz, 2 × CH (34) + (37)), 3.30 (m, 3H, CH_2 (33) + CH (3)), 2.97 (ddd, 1H, *J* = 11.1, 11.1, 6.1 Hz, CH (19)), 2.16 (ddd, 1H, *J* = 12.3, 12.3, 3.3 Hz, CH (13)), 2.11–1.91 (m, 3H, CH_a (22) + CH_a (21) + CH_a (16)), 1.87–1.51 (m, 10 H, CH (18) + CH_a (2) + CH_b (2) + CH_a (15) + CH_a (1) + CH_a (12) + CH_a (7) + CH_b (7) + CH_2 (32)), 1.68 (s, 3H, CH_3 (30)), 1.49–1.13 (m, 11H, CH (9) + CH_a (31) + CH_b (31) + CH_b (12) + CH_b (22) + CH_b (16) + CH_b (21) + CH_a (11) + CH_b (11) + CH_a (6) + CH_b (6)), 1.40 (dd, 12H, *J* = 6.5 Hz, 1.7 Hz, 4 × CH_3 (35) + (36) + (38) + (39)), 1.11–0.84 (m, 2H, CH_b (15) + CH_b (1)), 1.10 (s, 3H, CH_3 (23)), 1.04 (s, 3H, CH_3 (27)), 0.95 (s, 3H, CH_3 (24)), 0.87 (s, 3H, CH_3 (26)), 0.75 (s, 3H, CH_3 (25)), 0.71 (d, 1H, *J* = 10.4 Hz, CH (5)) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ = 151.3 (C20, C=CH_2), 108.5 (C29, C=CH_2), 78.2 (C3, CH), 70.8 (C28, CHOH), 55.4 (C5, CH), 54.9 (C34 + C37, 2 × CH), 50.4 (C9, CH), 50.0 (C17, $C_{quart.}$), 47.0 (C33, CH_2), 49.9 (C18, CH), 48.7 (C19, CH), 42.6 (C14, $C_{quart.}$), 40.9 (C8, $C_{quart.}$), 38.6 (C1, CH_2), 38.6 (C4, $C_{quart.}$), 36.9 (C10, $C_{quart.}$), 36.6 (C13, CH), 34.1 (C7, CH_2), 33.0 (C22, CH_2), 32.8 (C21, CH_2), 32.2 (C31, CH_2), 28.7 (C16, CH_2), 27.6 (C15, CH_2), 27.2 (C24, CH_3), 26.6 (C2, CH_2), 25.2 (C12, CH_2), 24.9 (C32 CH_2), 20.7 (C11, CH_2), 19.0 (C30, CH_3), 18.0 (C6, CH_2), 17.5 (C35 + C36 + C38 + C39, 4 × CH_3), 16.0 (C23, CH_3), 15.4 (C25, CH_3), 14.7 (C26, CH_3), 14.3 (C27, CH_3) ppm; MS (ESI, MeOH): m/z = 584.5 (100% $[M-Cl]^+$); Anal. for $C_{39}H_{70}ClNO_2$ (620.43): C, 75.50; H, 11.37; N, 2.26. Found: C, 75.32, H, 11.58, N, 2.03.

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