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# Synthesis of Antitumor-Active Betulinic Acid-Derived Hydroxypropargylamines by Copper-Catalyzend Mannich Reactions

**René Csuk, Christoph Nitsche, Ronny Sczepek, Stefan Schwarz, and Bianka Siewert**

Bereich Organische Chemie, Martin-Luther-Universität Halle-Wittenberg, Saale, Germany

Several novel betulin derivates were prepared using Mannich reactions as a key step. Starting from 3-ethynyl-3-hydroxy-lup-20(29)-ene derivatives, copper-catalyzed Mannich reactions yielded hydroxypropargyl ammonium hydrochlorides or their corresponding methiodides. All compounds were screened in a sulforhodamine B assay for their antitumor activity using a panel of 9 human cancer cell lines. Some of these compounds showed significant cytotoxicity; they act by triggering apoptotic cell death as shown by additional acridine orange/propidium iodide assays, Trypan blue tests, DNA laddering experiments, and investigations of the cell cycle.

**Keywords:** Antitumor-active compounds / Apoptosis / Betulinic acid / Cell cycle / Mannich reaction

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## Introduction

The natural occurring lupane-type triterpenes betulin and betulinic acid (BA) (Fig. 1) are widely spread in the plant kingdom. These compounds gained interest in medicinal research because of their antiviral [1, 2], antiplasmodial [3], and anti-inflammatorial [1] activity. Of special interest, however, is the ability of BA [4] and many of its derivatives [5–7] to inhibit the growth of human tumor cell lines and *in vivo* even of several tumors by triggering apoptosis [8–10]. In an animal model, BA showed selective cytotoxicity for melanoma cells with no acute or chronic side effects for nonmalignant cells [4].

BA, however, is only slightly soluble in aqueous solvents. Therefore, BA derivatives holding polar groups and hence providing an increased bioavailability are called for. Alkynyl substituted betulin derivatives [11–17] have scarcely been prepared so far; even less their synthetic potential has been explored. Previously, we were able to show [18, 19] that alkynyl derivatives of betulin show an increased cytotoxicity towards human tumor cell lines compared to parent betulin. Here, we report our new strategy to combine the higher cytotoxic activity of alkynyl substituted compounds with improved water solubility by attaching amino group-bearing functionalities employing the alkynyl groups in Mannich reactions.

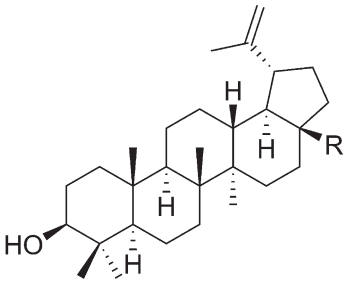
The Mannich reaction [20–23] has been known for exactly 100 years [24]; it has become an important tool for the synthesis of pharmaceutical drugs [23] and more recently in polymer chemistry [25–27]. Although Mannich reactions using alkynes [28] have been reported as early as 1933, rather harsh conditions and moderate yields have limited a wider application. In general, many reaction conditions have been described for Mannich reactions employing alkynes, aldehydes, and amines [29–35]. They differ in solvents, temperatures, pH-values, additives, and catalysts. A first screening of many of these conditions resulted in the decision to use a copper-catalyzed reaction variant that was developed by Reppe [36] as early as 1955 and refined in 2004 by Bieber and da Silva [37].

## Results and discussion

Prerequisites to prepare propargylamines using Mannich reactions are suitable C,H-acidic alkynes. The synthesis of BA derivatives bearing an alkynyl group at carbon C3 was realized by a Grignard reaction using ethynylmagnesium bromide in THF [18, 19], starting from betulonic acid **1** or

**Correspondence:** Prof. Dr. René Csuk, Bereich Organische Chemie, Martin-Luther-Universität Halle-Wittenberg, Kurt-Mothes-Strasse 2, D-06120 Halle, Saale, Germany
**E-mail:** rene.csuk@chemie.uni-halle.de
**Fax:** +49 345 5527030

**Abbreviations: AO/PI,** acridine orange/propidium iodide; **BA,** betulinic acid; **SRB,** sulforhodamine B.

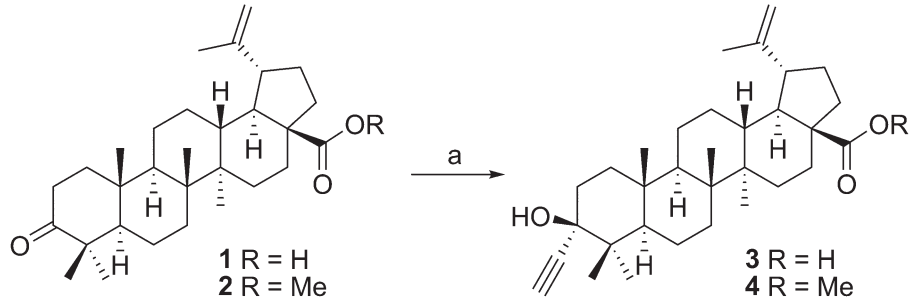



betulinic acid R = COOH
betulin R = $CH_2OH$

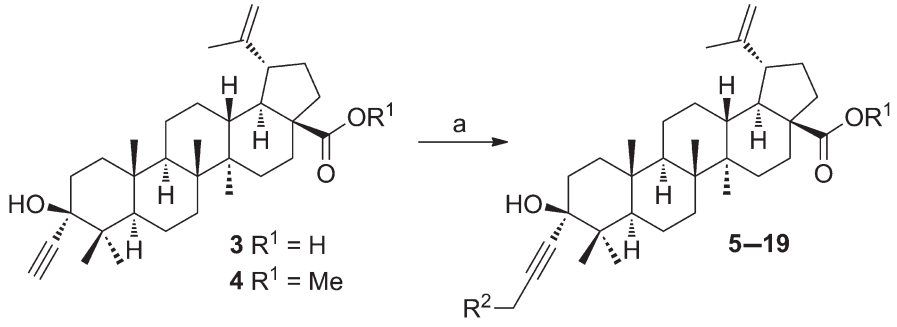
**Figure 1.** Structures of the parent compounds betulinic acid (BA) and betulin.

its methyl ester **2**. This gave alkynic starting materials **3** and **4** (Scheme 1). As shown by exhaustive N.o.e.-NMR experiments, these Grignard reactions advanced in a diastereoselective manner yielding (3 *S*) configurated compounds with an axially oriented α-alkynol moiety in ring A of the triterpenoid skeleton [38]. In diethylether as a solvent, however, invariably mixtures of the diastereomeric compounds were formed. Due to the complexation with the solvent THF, the Grignard reagent is more bulky than in diethylether. The axially oriented methyl group at carbon C4 and the methyl group at carbon C10 hinder an attack of the organometallic reagent along the Bürgi–Dunitz-trajectory [39] from the upper side of the molecule. This parallels quite recent findings of Wagner and coworkers [40, 41].

The Mannich reactions were performed treating alkynols **3** and **4** in DMSO with several secondary amines, aqueous formalin, and CuI as the catalyst for one up to several days (Scheme 2) [37]. During these reactions, the corresponding copper acetylide complexes and methylene-iminium ions are formed, since reactions employing alkynes performed without the copper catalyst did not proceed at all. This is in excellent agreement with early findings of Reppe [36].




**Scheme 1.** Synthesis of 3-ethynylbetulinc acid derivatives **3** and **4**: (a) HC≡CMgBr, THF, 25°C: **3** 77% (from **1**); **4** 68% (from **2**).




**5** $R^1$ = H, $R^2$ = pyyrolidinyl
**6** $R^1$ = H, $R^2$ = piperidinyl
**7** $R^1$ = H, $R^2$ = azepanyl
**8** $R^1$ = H, $R^2$ = diisopropyl
**9** $R^1$ = Me, $R^2$ = pyrrolidinyl
**10** $R^1$ = Me, $R^2$ = piperidinyl
**11** $R^1$ = Me, $R^2$ = azepanyl
**12** $R^1$ = Me, $R^2$ = morpholinyl
**13** $R^1$ = Me, $R^2$ = thiomorpholinyl
**14** $R^1$ = Me, $R^2$ = *N*-methyl-piperidinyl
**15** $R^1$ = Me, $R^2$ = dimethylamino
**16** $R^1$ = Me, $R^2$ = diethylamino
**17** $R^1$ = Me, $R^2$ = diisopropylamino
**18** $R^1$ = Me, $R^2$ = dihexylamino
**19** $R^1$ = Me, $R^2$ = dicyclohexylamino

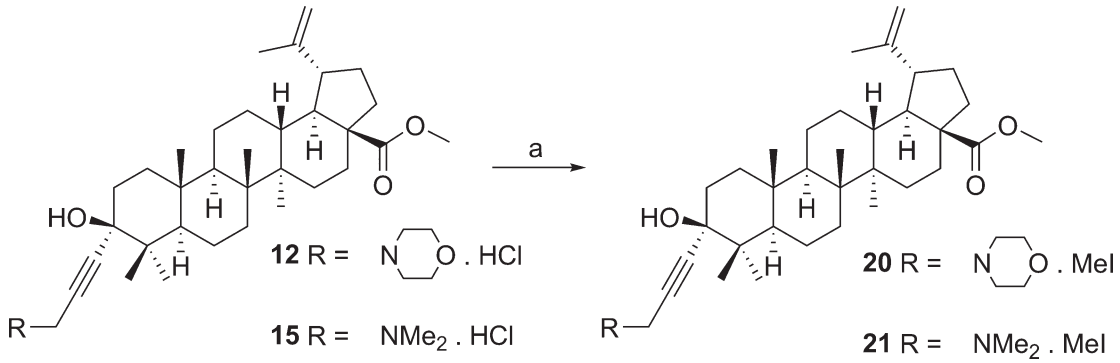
**Scheme 2.** Mannich reactions with alkynol **3** or **4**: (a) secondary amine ($R^2$NH), formalin, CuI (cat.) DMSO, 40°C, 13–64%.

**Table 1.** Cytotoxicity of the propargylamine derivatives measured in SRB assays with different cancer cell lines and non-malignant mouse fibroblasts (NiH 3T3) in comparison to BA

| Cell lines | $IC_{50}$ values in μM of derivatives[a)] | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **BA** |
| *SW1736* | 12.2 ± 0.6 | 20.8 ± 2.1 | 5.7 ± 2.8 | 7.4 ± 0.3 | 18.0 ± 0.7 | 7.6 ± 0.6 | 4.8 ± 1.2 | 7.2 ± 0.7 | 7.3 ± 1.0 | 5.8 ± 0.6 | 11.1 ± 1.7 | 4.4 ± 0.5 | 6.8 ± 0.6 | 8.5 ± 1.0 | 10.6 ± 2.2 | n.a. | 5.0 ± 1.2 | 9.7 ± 0.8 | 9.8 ± 3.0 | 11.6 |
| *MCF-7* | 10.5 ± 0.4 | 27.8 ± 2.8 | 5.5 ± 1.0 | 7.7 ± 2.5 | 21.4 ± 2.4 | 7.3 ± 0.8 | 4.6 ± 1.1 | 7.4 ± 0.7 | 5.1 ± 0.1 | 6.1 ± 1.0 | 12.1 ± 1.5 | 3.4 ± 0.3 | 5.8 ± 2.1 | 7.9 ± 2.9 | 7.7 ± 1.9 | n.a. | 14.5 ± 1.0 | 13.2 ± 1.3 | 10.0 ± 1.5 | 14.9 |
| *LIPO* | 16.8 ± 0.5 | 22.8 ± 2.3 | 9.0 ± 0.4 | 14.4 ± 3.3 | n.a. | 8.0 ± 1.0 | 6.3 ± 0.5 | 8.7 ± 0.9 | 5.6 ± 0.1 | 9.3 ± 0.9 | 14.9 ± 2.0 | 2.5 ± 0.3 | 8.9 ± 0.6 | 10.1 ± 0.4 | 8.8 ± 1.0 | n.a. | 14.8 ± 1.5 | 11.4 ± 1.0 | 14.6 ± 0.7 | 9.7 |
| *DLD-1* | 10.5 ± 1.0 | 16.1 ± 2.5 | 12.1 ± 2.4 | 11.9 ± 0.3 | 28.9 ± 4 | 7.8 ± 0.8 | 5.1 ± 1.7 | 9.6 ± 1.0 | 7.1 ± 0.5 | 12.6 ± 1.3 | 10.1 ± 0.7 | 5.4 ± 0.5 | 7.7 ± 0.6 | 15.4 ± 1.5 | 11.4 ± 0.8 | n.a. | 11.3 ± 1 | n.a. | n.a. | 17.5 |
| *A549* | 13.8 ± 1.6 | 21.8 ± 0.9 | 7.4 ± 0.2 | 9.6 ± 0.1 | 28.7 ± 0.8 | 6.1 ± 1.8 | 4.5 ± 1.3 | 5.8 ± 0.6 | 7.4 ± 1.2 | 7.9 ± 0.8 | 14.4 ± 0.2 | 5.8 ± 0.6 | 7.0 ± 0.6 | 14.3 ± 1.4 | 9.8 ± 2.0 | n.a. | 19.2 ± 0.2 | 23.9 ± 2.4 | 16.6 ± 0.6 | 14.9 |
| *A2780* | 9.8 ± 1.0 | 26.8 ± 1.4 | 9.4 ± 0.5. | 7.3 ± 2.8 | 26.4 ± 2.0 | 7.4 ± 0.4 | 5.0 ± 1.0 | 6.1 ± 0.6 | 6.2 ± 1.0 | 12.4 ± 2.1 | 11.4 ± 2.3 | 4.3 ± 0.4 | 7.6 ± 1.5 | 8.5 ± 3.5 | 8.6 ± 1.0 | n.a. | 6.9 ± 3.0 | 6.7 ± 1.4 | 9.4 ± 1.0 | 11.0 |
| *A253* | 11.7 ± 1.9 | 13.0 ± 0.2 | 6.5 ± 0.3 | 13.4 ± 8.0 | n.a. | 5.3 ± 0.6 | 5.1 ± 2.6 | 7.7 ± 0.8 | 4.0 ± 1.3 | 6.7 ± 1.2 | 8.7 ± 3.9 | 2.6 ± 0.2 | 5.6 ± 2.2 | 7.3 ± 1.4 | 5.6 ± 1.6 | n.a. | 10.0 ± 1.0 | 13.3 ± 1.3 | 12.7 ± 1.2 | 11.1 |
| *8505C* | 15.5 ± 0.5 | 21.9 ± 2.3 | 8.0 ± 0.7 | 13.3 ± 3.0 | 26.3 ± 0.5 | 6.3 ± 0.8 | 4.6 ± 2.5 | 8.8 ± 0.9 | 4.7 ± 0.6 | 7.2 ± 0.5 | 13.4 ± 1.3 | 3.1 ± 0.5 | 5.7 ± 3.2 | 10.1 ± 0.2 | 9.8 ± 0.6 | n.a. | 21.8 ± 2.0 | 9.8 ± 0.2 | 13.9 ± 1.4 | 6.7 |
| *518A2* | 17.1 ± 0.3 | 20.0 ± 1.8 | 5.9 ± 0.5 | 12.9 ± 2.0 | n.a. | 8.1 ± 0.2 | 5.2 ± 1.3 | 9.0 ± 1.0 | 6.7 ± 1.9 | 7.4 ± 0.3 | 13.1 ± 1.1 | 2.5 ± 0.3 | 5.8 ± 3.2 | 9.5 ± 1.3 | 8.6 ± 1.1 | n.a. | 13.1 ± 1.3 | 7.5 ± 3.0 | 12.3 ± 1.1 | 11.9 |
| *NiH 3T3* | 13.5 ± 0.8 | 9.4 ± 0.5 | 6.4 ± 0.1 | 16.5 ± 7.5 | 33.7 ± 3.0 | 8.3 ± 0.1 | 2.6 ± 1.5 | 9.2 ± 0.6 | 3.0 ± 1.1 | 7.5 ± 2.2 | 12.8 ± 1.3 | 4.1 ± 0.5 | 3.3 ± 1.0 | 8.5 ± 0.7 | 5.4 ± 0.5 | n.a. | 21.8 ± 1.9 | 15.9 ± 0.3 | 13.1 ± 0.5 | 10.0 |

[a)] $IC_{50}$ values represent mean values obtained from three independent measurements. The values were obtained from the SRB assays after 96 h of treatment of the cells with the compounds. The values were averaged from at least five independent experiments and calculated applying the two-parametric Hill slope equation; n.a. corresponds to an $IC_{50}$ value >30 μM.




**Scheme 3.** Synthesis of quaternary ammonium iodide salts **20** and **21** from propargylamines **12** and **15**: (a) 1. KOH, 2. MeI, $Et_2O$, 25°C, **20**: 89% (from **12**), **21**: 82% (from **15**).

For the synthesis of the corresponding propargylamines **5**–**19**, acyclic and cyclic secondary amines where used (Table 1). All compounds were isolated as their hydrochlorides, and the copper catalyst was removed by flash chromatography.

The propargylamines **12** and **15** where transformed into their corresponding methiodides **20** and **21**, by reacting the amines with methyl iodide (Scheme 3) [42, 43].

Based on an initial report dealing with the cytotoxic activity of BA [44] quite a huge number of studies dealt with the antineoplastic activity of BA and derivatives [45, 46] *in vivo* and *in vitro*. BA is almost insoluble in water but an aqueous medium is usually the preferred formulation for injection [47]. Thus, solubility data for several of our compounds have been obtained in water/DMSO mixtures (95:5, v/v). Whereas for BA, a solubility of 166 μg/mL was determined, for **5** 175 μg/mL was found. The ester **9** showed a reduced solubility of 82 μg/mL – hence indicating that good solubility is not an ultimate pre-requisite for achieving high cytotoxicity. The derivatives **8** (65 μg/mL) and **12** (61 μg/mL) also exhibited a reduced solubility but still showed high cytotoxicity.

Previous studies concerning modifications performed at C-28 [18, 48–51] emphasized the need of the presence of a carboxylic or carbonylic group at this position for obtaining reasonably high cytotoxicity. Esterification of the C-28 carboxylic acid moiety employing lipophilic alcohols did not result in compounds of improved antitumor activity [45]. Introduction of extra hydrophilic groups in ring A reduced activity whereas derivatives showing aprotic polar functional groups showed lower $IC_{50}$ values [45]. Steric hindrance at position C-3 seems crucial [52].

Testing of our compounds in colorimetric sulforhodamine B (SRB) cell assays [53–55] showed a higher cytotoxicity for the propargylamines compared to their parent alkynic precursors. Propargylamines **5**–**19** mostly even showed an increased cytotoxicity than naturally occurring BA. There is only a limited influence of the secondary amine on the activity (Table 1, Fig. 2). Highest activity was found for the *N*-methylpiperazine derivative **14** with $IC_{50}$-values between 2.5 and 5.8 μM for the different human cancer cell lines. Bulky and more hydrophobic substituents (as in compounds **13** or **19**) resulted in a reduced activity. Compound **18** carrying a dihexylamine substituent showed significantly lower activity than all other derivatives. For the methiodides **20** and **21** a lowered activity was found but these compounds showed a higher selectivity between the different cell lines. The selectivity index ($IC_{50}$ tumor cells vs. NiH 3T3 fibroblasts), however, is low throughout this series of compounds.

Apoptosis is a naturally occurring process by which a cell is directed to programmed cell death. In this process, cells that are detrimental to an organism are disposed of in a neat and orderly manner; this prevents the development of an inflam-

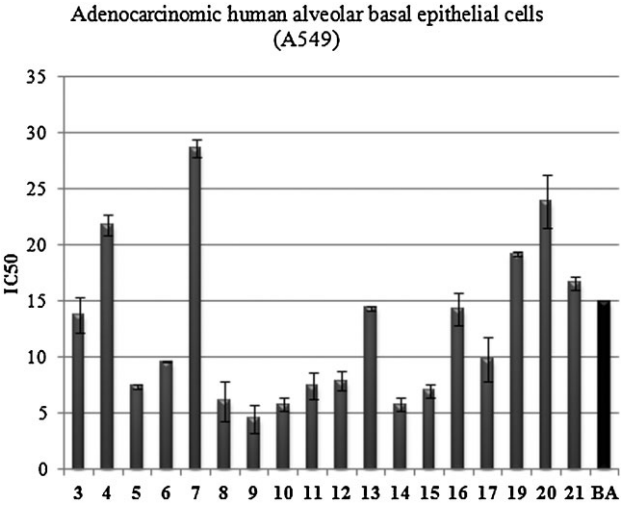



**Figure 2.** Cytotoxicity of the propargylamine derivatives **3**–**20** measured in SRB assays using the A549 cancer cell line in comparison to BA.

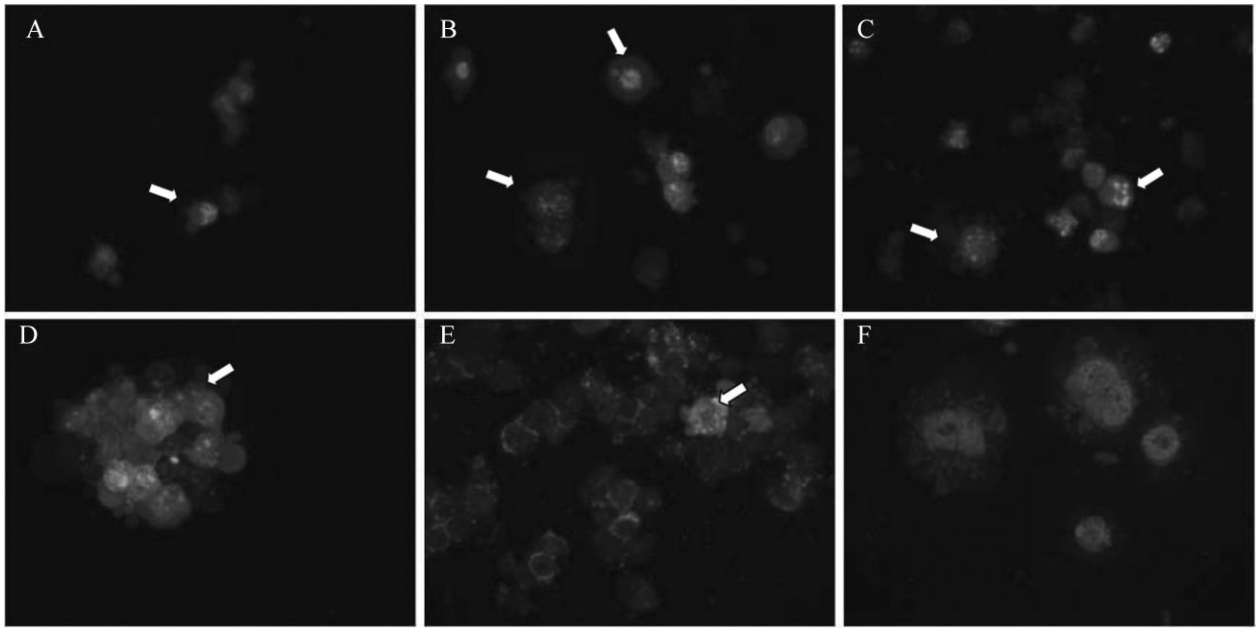



**Figure 3.** AO/PI staining of A549 cancer cells after treatment with selected compounds (fluorescence microscopy): (A) BA (30 μM, 24 h); (B) **5** (15 μM, 48 h); (C) **9** (10 μM, 48 h); (D) **12** (15 μM, 48 h); (E) **14** (10 μM, 48 h); (F) **20** (40 μM, 24 h).

matory response being often associated with necrotic cell death. BA and most of its derivatives induce apoptosis [4, 47–49, 56–60]. Many of our Mannich compounds showed good or moderate antitumor activity. Since SRB tests *a priori* allow no conclusion for an apoptotic cell death, selected compounds were chosen for further studies; these experiments included dye exclusion tests (acridine orange/propidium iodide (AO/PI) and Trypan blue), DNA laddering experiments as well as investigations concerning the cell cycle.

In contrast to necrosis, membranes of cells undergoing apoptosis remain intact until a rather late stage of this process of programmed cell death. The results from the dying of the cells with AO/PI are shown in Fig. 3 for A549 human adenocarcinomic alveolar basal epithelial cells. White arrows point out typical characteristics of an apoptotic process, i.e. blebbing of the membrane and the condensation of the chromatin. Cells treated with compounds **5**, **9**, or **12** (and to a lower extent with compound **14**) revealed also the presence of small cell compartments (probably lysosomes or autophagosomes) exhibiting weak reddish fluorescence. This parallels recent findings of Fulda and coworkers [61] for B10 (a glycosylated derivative of BA) to induce apoptosis as well as to initiate macroautophagy.

Trypan blue staining indicates the integrity of the cytoplasmatic membrane, hence allowing to draw some conclusions for a quantification of necrotic and apoptotic cells. The results of these Trypan blue staining experiments are summarized in Table 2. These results clearly confirm that our compounds are able to trigger apoptosis.

Caspases are a family of cysteine proteases acting in concert in a cascade triggered by apoptosis signaling. The culmination of this cascade is the cleavage of a number of proteins in the cell, followed by cell disassembly, cell death, and, ultimately, the phagocytosis and removal of the cell debris. As a result of the caspase cascade, transcription of endonucleases is increased during apoptosis leading to a cutting of intact DNA into smaller fragments of 180 bp [62]. Experiments using A549 cancer cells and compounds **5**, **9**, **12**, **14**, and **20** (BA was used as an internal reference) revealed the presence of ''DNA ladders'' (Fig. 4) typical of an apoptotic cell death.

Finally, some cell cycle experiments were performed by dyeing the DNA of the A549 cancer cells with DNA-intercalating PI [63–67] followed by measurement of the living cells by flow cytometry after having been incubated with the cyto-

**Table 2.** Results from the Trypan blue staining test of compounds **5**, **9**, **12**, and **20** using A549 cells

| Compound | Apoptotic cell death (%) | Confidence interval |
|---|---|---|
| **5** | 62.8 | 3.9 |
| **9** | 69.7 | 3.6 |
| **12** | 72.5 | 9.3 |
| **20** | 49.1 | 13.6 |

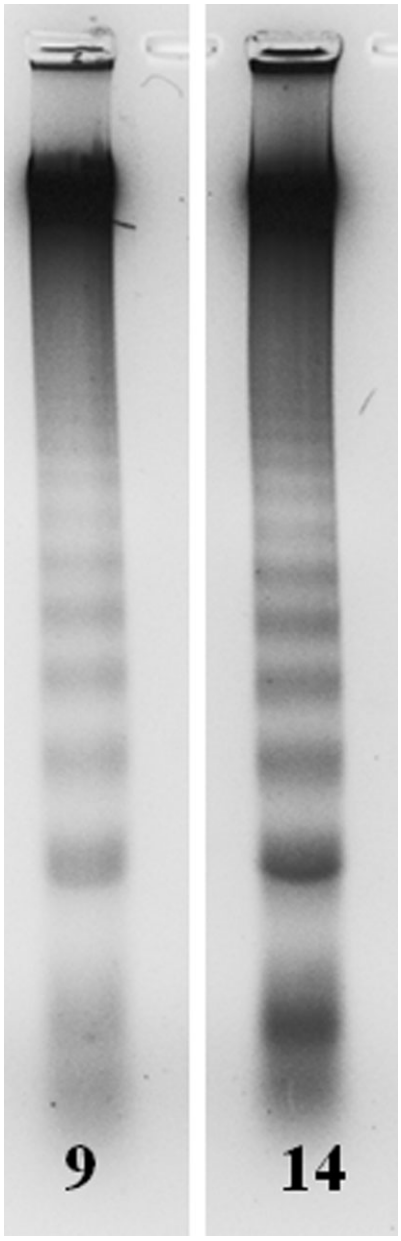



**Figure 4.** DNA ladders in A549 cancer cells that were treated for 24 h with 20 μM of compounds **9** or **14**.

toxic compounds for 24 h. The results from these investigations are depicted in Fig. 5.

Thus, living A549 cells were treated with dye PI and compounds **5**, **9**, **12**, **14**, and **20** for 24 h. The concluding FACS measurements were performed and evaluated using the procedures described by Kallioniemi et al. [67] and Dean et al. [68]. These experiments demonstrate that upon treatment of the cells with the cytotoxic compounds **5**, **12**, **14**, and **20** a degradation of the DNA occurs in the living A549 cells (indicating the start and progressing of an apoptotic process). Evaluation of the PI dyeing showed many cells in SubG1 phase (being quite typical of apoptosis according to Darzynkiewicz et al. [65]). Also, a significant decrease of cells in G2/M phase is observed; in some experiments an arrest of cells in the S phase was observed, hence paralleling previous findings of Santos et al. [62] and Kommera et al. [69] for tumor cells treated with other triterpenoids. In conclusion, the Mannich derivatives of BA have been shown to be excellent inhibitors of cell proliferation leaving only a small number of cells in G2 phase.

In summary, several Mannich bases from BA were prepared and tested for their cytotoxic activity. The results from AO/PI staining and annexinV-FITC assays as well as DNA laddering experiments provided evidence for an apoptotic cell death. Some of them are strong regulators of tumor cell proliferation and induce cell cycle arrest. Thus, evaluation of the PI dyeing showed many cells in SubG1 phase, but also a significant decrease of cells in G2/M phase is observed. The biological activities make these compounds interesting candidates for further biological evaluation.

## Experimental

### General

Melting points are uncorrected (Leica hot stage microscope); NMR spectra were recorded using the Varian spectrometers Gemini 200, Gemini 2000 or Unity 500 ($\delta$ given in ppm, $J$ in Hz, internal Me4Si); optical rotations were obtained using a Perkin–Elmer 341 polarimeter (1 cm microcell, 25°C), IR spectra (film or KBr pellet) on a Perkin–Elmer FT-IR spectrometer Spectrum 1000; MS spectra were taken on an Intectra GmbH AMD 402 (electron impact, 70 eV) or on a Finnigan MAT TSQ 7000 (electrospray, voltage 4.5 kV, sheath gas nitrogen) instrument. TLC was performed on silica gel (Merck 5554, detection by UV absorption). The solvents were dried according to usual procedures. The purity of the compounds was determined by HPLC and found to be >98%. Solubility was determined by HPLC/UV–vis according to [47].

#### *(3β) 3-Ethynyl-3-hydroxylup-20(29)-en-28-acid (**3**)*

To a solution of **1** (5.0 g, 11.0 mmol) in dry THF (150 mL) a solution of ethynylmagnesium bromide (0.5 M in THF, 50 mL, 25 mmol) was slowly added. The reaction mixture was stirred under argon for 4 h at 25°C, quenched by the addition of methanol and water (5 mL each), and the solvents were evaporated under reduced pressure. The residue was subjected to chromatography (silica gel, *n*-hexane/ethyl acetate, 3:1) and **3** (4.06 g, 77%) was obtained as a white solid. Mp 255°C; $[\alpha]_D = +12.5^\circ$ ($c = 5.0$, MeOH); $R_F = 0.42$ (silica gel, *n*-hexane/ethyl acetate, 4:1); IR (KBr): $\nu = 3538s$, $3074m$, $3004s$, $2943s$, $2869s$, $2844s$, $1708s$, $1644m$, $1450s$, $1434m$, $1392m$, $1373m$, $1350m$, $1332m$, $1285m$, $1274m$, $1238m$, $1223m$, $1199s$, $1167s$, $1138m$, $1104m$, $1082m$, $1047s$, $1008m$, $972m$ $cm^{-1}$; $^1H$ NMR (500 MHz, $CD_3OD$): $\delta = 4.71$ ($m$, 1H, $CH_a$ (29)), 4.59 ($m$, 1H, $CH_b$ (29)), 3.02 (*brm*, 1H, *CH* (19)), 2.78 (*s*, 1H, *CH* (32)), 2.46 ($m$, 1H, $CH_a$ (16)), 2.32 ($m$, 1H,

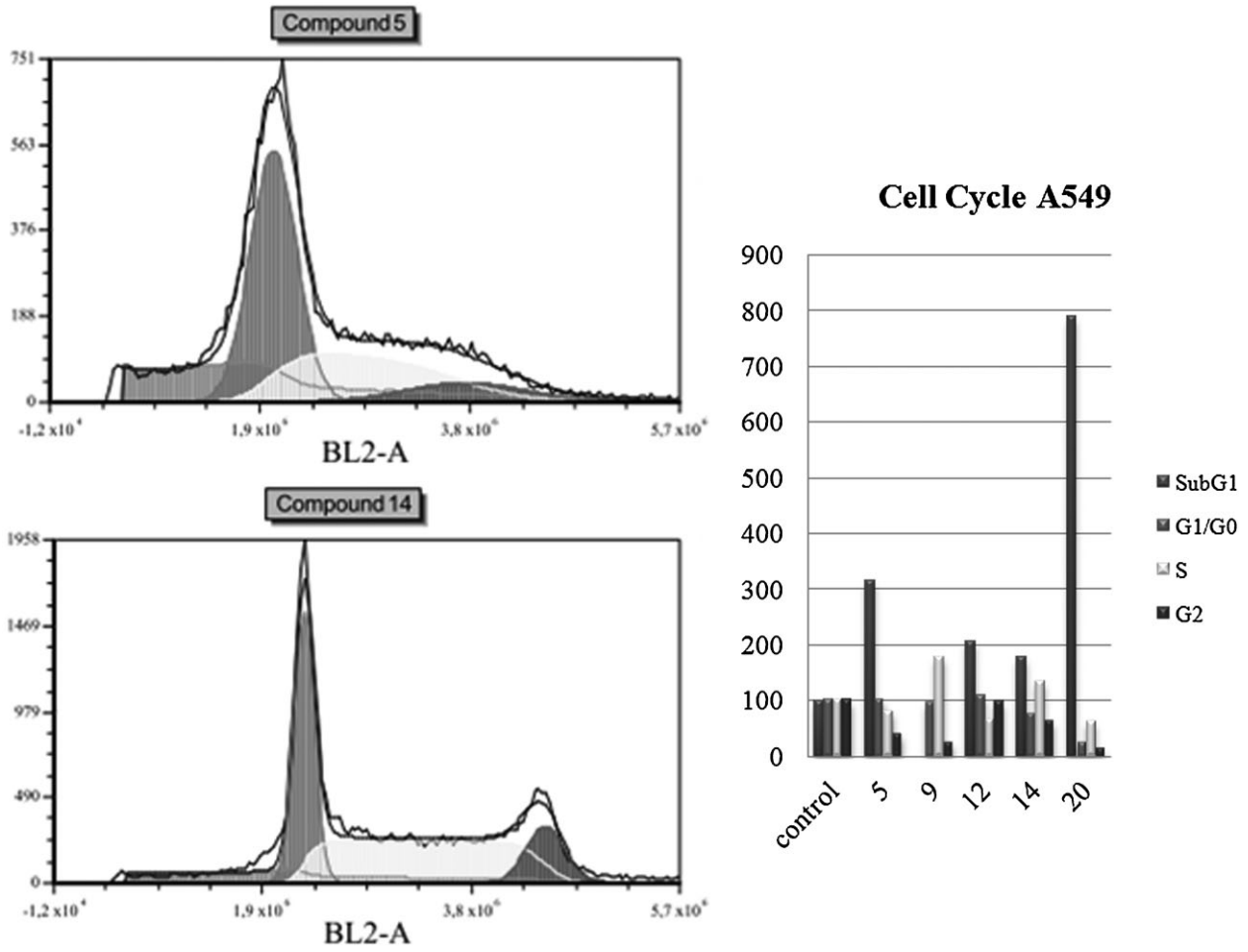



**Figure 5.** FACS measurements of A549 cells after treatment with cytotoxic compounds **5** (15 μM), **9** (10 μM), **12** (15 μM), **14** (10 μM) and **20** (40 μM) for 24 h followed by PI staining.

C*H* (13)), 2.23 (*m*, 1H, C$H_a$ (2)), 1.98−1.87 (*m*, 3H, C$H_a$ (21) + C$H_a$ (22) + C$H_b$ (2)), 1.76−1.21 (*m*, 16H, C*H* (18) + C$H_a$ (1) + C$H_b$ (1) + C$H_a$ (12) + C*H* (9) + C$H_a$ (7) + C$H_b$ (7) + C$H_b$ (16) + C$H_b$ (21) + C$H_b$ (22) + C$H_a$ (15) + C$H_a$ (11) + C$H_b$ (11) + C$H_a$ (6) + C$H_b$ (6) + C*H* (5)), 1.69 (*s*, 3H, C$H_3$ (30)), 1.20−1.14 (*m*, 1H, C$H_b$ (15)), 1.11−1.02 (*m*, 1H, C$H_b$ (12)), 1.04 (*s*, 3H, C$H_3$ (24)), 1.00 (*s*, 3H, C$H_3$ (27)), 0.96 (*s*, 3H, C$H_3$ (26)), 0.86 (*s*, 3H, C$H_3$ (25)), 0.83 (*s*, 3H, C$H_3$ (23)) ppm; $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta$ = 178.5 (C28, *C*O), 150.5 (C20, *C*=$CH_2$), 108.8 (C29, C=*C*$H_2$), 86.8 (C31, *C*≡CH), 74.8 (C32, C≡*C*H), 73.2 (C3, *C*OH), 54.7 (C17, $C_{quart.}$), 53.0 (C5, *C*H), 50.7 (C18, *C*H), 49.8 (C9, *C*H), 49.2 (C19, *C*H), 42.1 (C14, $C_{quart.}$), 41.0 (C4, $C_{quart.}$), 40.5 (C8, $C_{quart.}$), 38.3 (C13, *C*H), 37.6 (C1, *C*$H_2$), 36.9 (C10, $C_{quart.}$), 36.8 (C22, *C*$H_2$), 34.2 (C7, *C*$H_2$), 33.6 (C2, *C*$H_2$), 33.4 (C16, *C*$H_2$), 32.0 (C21, *C*$H_2$), 29.5 (C15, *C*$H_2$), 24.8 (C24, *C*$H_3$), 25.5 (C12, *C*$H_2$), 20.6 (C11, *C*$H_2$), 18.2 (C30, *C*$H_3$), 18.2 (C6, *C*$H_2$), 16.8 (C23, *C*$H_3$), 15.7 (C25, *C*$H_3$), 15.2 (C26, *C*$H_3$), 13.8 (C27, *C*$H_3$) ppm; MS (ESI, MeOH): *m*/*z* = 959.1 (97% [2M−H]$^-$), 525.1 (52% [M+$HCO_2$]$^-$), 479.6 (68% [M−H]$^-$); analysis for $C_{32}H_{48}O_3$ (480.72): C, 79.95; H, 10.06; found: C, 79.75; H, 10.21.

### *Methyl (3β) 3-ethynyl-3-hydroxylup-20(29)-en-28-oate (**4**)*

To a solution of **2** (4.69 g, 10.0 mmol) in dry THF (150 mL), solution of ethynylmagnesium bromide (0.5 M in THF, 50 mL, 25 mmol) was added dropwise. After stirring at 25°C for 4 h under argon, the reaction was quenched by the careful addition of acetic acid (3 mL) and water (400 mL). The mixture was extracted with ethyl acetate (3 × 200 mL), the extracts were washed with an aq. solution of $NaHCO_3$ (150 mL, satd.) and brine (150 mL) and dried with sodium sulfate. The solvents were evaporated, and the residue subjected to chromatography (silica gel, *n*-hexane/ethyl acetate, 4:1) to afford **4** (3.38 g, 68%) as a white solid. Mp 210–215°C; UV–vis (MeOH): $\lambda_{max}$ (nm) (log $\varepsilon$) = 260 (0.08), 220 (0.94); $[\alpha]_D$ = +1.7° (*c* = 4.20, $CHCl_3$); $R_F$ = 0.63 (silica gel, *n*-hexane/ethyl acetate, 4:1); IR (KBr): $\nu$ = 3550*s*, 3476*s*, 3307*m*, 3264*s*, 3072*m*, 2950*s*, 2870*s*, 2107*w*, 1716*s*, 1641*m*, 1461*m*, 1377*s*, 1351*m*, 1317*m*, 1266*m*, 1226*m*, 1190*s*, 1156*s*, 1135*s*, 1108*m*, 1067*m*, 1030*m*, 1008*m*, 984*m*, 949*m*, 914*w* cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 4.66 (*m*, 1H, C$H_a$ (29)), 4.53 (*m*, 1H, C$H_b$ (29)), 3.60 (*s*, 3H, C$H_3$ (31)), 2.92 (*ddd*, 1H, *J* = 11.1, 10.8, 4.8 Hz, C*H* (19)), 2.40 (*s*, 1H, C*H* (33)), 2.18−2.10 (*m*, 2H,

*CH* (13) + *CH*$_a$ (16)), 1.89−1.79 (*m*, 3H, *CH*$_a$ (2) + *CH*$_a$ (21) + *CH*$_a$ (22)), 1.66−1.50 (*m*, 4H, *CH* (18) + *CH*$_a$ (1) + *CH*$_a$ (12) + *CH*$_b$ (2)), 1.61 (*s*, 3H, *CH*$_3$ (30)), 1.44−1.14 (*m*, 12H, *CH* (9) + *CH*$_b$ (1) + *CH*$_b$ (22) + *CH*$_a$ (7) + *CH*$_b$ (7) + *CH*$_b$ (16) + *CH*$_b$ (21) + *CH*$_a$ (15) + *CH*$_a$ (11) + *CH*$_b$ (11) + *CH*$_a$ (6) + *CH*$_b$ (6)), 1.10−1.05 (*m*, 2H, *CH* (5) + *CH*$_b$ (15)), 1.00−0.95 (*m*, 1H, *CH*$_b$ (12)), 0.98 (*s*, 3H, *CH*$_3$ (24)), 0.92 (*s*, 3H, *CH*$_3$ (27)), 0.84 (*s*, 3H, *CH*$_3$ (25)), 0.78 (*s*, 3H, *CH*$_3$ (23)), 0.76 (*s*, 3H, *CH*$_3$ (26)) ppm; $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 176.6 (C28, *CO*), 150.5 (C20, *C*=$CH_2$), 109.5 (C29, C=*CH*$_2$), 87.1 (C32, *C*≡CH), 75.7 (C33, C≡*CH*), 73.5 (C3, *COH*), 56.5 (C17, $C_{quart.}$), 53.1 (C5, *CH*), 51.2 (C31, *CH*$_3$), 50.6 (C18, *CH*), 49.5 (C9, *CH*), 46.9 (C19, *CH*), 42.4 (C14, $C_{quart.}$), 41.3 (C4, $C_{quart.}$), 40.6 (C8, $C_{quart.}$), 38.3 (C13, *CH*), 37.9 (C1, *CH*$_2$), 37.2 (C10, $C_{quart.}$), 36.9 (C22, *CH*$_2$), 34.2 (C7, *CH*$_2$), 32.6 (C2, *CH*$_2$), 32.2 (C16, *CH*$_2$), 30.6 (C21, *CH*$_2$), 29.7 (C15, *CH*$_2$), 25.6 (C24, *CH*$_3$), 25.5 (C12, *CH*$_2$), 20.8 (C11, *CH*$_2$), 19.4 (C30, *CH*$_3$), 18.5 (C6, *CH*$_2$), 17.4 (C23, *CH*$_3$), 16.4 (C25, *CH*$_3$), 15.9 (C26, *CH*$_3$), 14.9 (C27, *CH*$_3$) ppm; MS (ESI, MeOH): *m*/*z* = 1011.1 (27% $[2M+Na]^+$), 549.3 (18% $[M+Na+MeOH]^+$); analysis for $C_{33}H_{50}O_3$ (494.75): C, 80.11; H, 10.19; found: C, 79.98; H, 10.27.

### *General procedure for the synthesis of acids **5**–**8***

A mixture of alkynol **3** (481 mg, 1.0 mmol), secondary amine (2.5–4.0 mmol), formalin (37%, 10–15 mmol), copper iodide (4 mg, 0.02 mmol) and DMSO (5 mL) was stirred at 40°C for 1–5 days. After completion of the reaction (as indicated by TLC), a solution of ammonium chloride (10 g) and aqueous ammonia (30%, 5 mL) in water (30 mL) was added. The mixture was extracted with ethyl acetate (5 × 10 mL), and the solvents was evaporated under reduced pressure. The crude residue was dissolved in diethylether (50 mL), insoluble material was filtered off, and at 0°C gaseous hydrogen chloride was passed through, until the precipitation of salts had ceased. After standing for 12 h at 4°C, the product was filtered off and washed with water and diethylether. Optionally, re-crystallization from methanol (5 mL) and hydrochloric acid (10%, 5 mL) gave an analytical product.

### *(3β) 3-Hydroxy-3-(3-pyrrolidin-1-yl-prop-1-yn-1-yl)lup-20(29)-en-28-acid hydrochloride (**5**)*

Compound **5** was prepared as described in the general procedure from **3** (481 mg, 1.0 mmol), pyrrolidine (0.3 mL, 3.7 mmol), formalin (37%, 1.2 mL, 14.8 mmol) and copper iodide (4 mg, 0.02 mmol) in DMSO (5 mL) (40°C for 5 days), and **5** (262 mg, 44%) was obtained after re-crystallization as a white solid. Mp 222°C; $[\alpha]_D$ = +5.8° (*c* = 4.30, MeOH); IR (KBr): $\nu$ = 3395*s*, 2948*s*, 2869*s*, 2585*s*, 1687*s*, 1640*s*, 1455*s*, 1376*s*, 1319*m*, 1241*s*, 1192*s*, 1135*s*, 1167*m*, 1075*m*, 1038*s*, 983*m*, 948*m* cm$^{-1}$; $^1$H NMR (500 MHz, $CD_3OD$): $\delta$ = 4.71 (*m*, 1H, *CH*$_a$ (29)), 4.59 (*m*, 1H, *CH*$_b$ (29)), 4.22 (*s*, 2H, *CH*$_2$ (33)), 3.66 (*m*, 2H, *CH*$_a$ (34) + *CH*$_a$ (37)), 3.26 (*m*, 2H, *CH*$_b$ (34) + *CH*$_b$ (37)), 3.01 (*ddd*, 1H, *J* = 10.6, 10.6, 4.6 Hz, *CH* (19)), 2.32 (*m*, 1H, *CH* (13)), 2.26−2.14 (*m*, 3H, *CH*$_a$ (16) + *CH*$_a$ (35) + *CH*$_a$ (36)), 2.07 (*m*, 2H, *CH*$_b$ (35) + *CH*$_b$ (36)), 2.00−1.87 (*m*, 3H, *CH*$_a$ (2) + *CH*$_a$ (21) + *CH*$_a$ (22)), 1.76−1.64 (*m*, 3H, *CH*$_a$ (1) + *CH*$_a$ (12) + *CH*$_b$ (2)), 1.69 (*s*, 3H, *CH*$_3$ (30)), 1.62 (*dd*, 1H, *J* = 11.4, 11.4 Hz, *CH* (18)), 1.58−1.22 (*m*, 12H, *CH* (9) + *CH*$_b$ (1) + *CH*$_b$ (22) + *CH*$_a$ (7) + *CH*$_b$ (7) + *CH*$_b$ (16) + *CH*$_b$ (21) + *CH*$_a$ (15) + *CH*$_a$ (11) + *CH*$_b$ (11) + *CH*$_a$ (6) + *CH*$_b$ (6)), 1.19−1.09 (*m*, 2H, *CH* (5) + *CH*$_b$ (15)), 1.07−1.01 (*m*, 1H, *CH*$_b$ (12)), 1.06 (*s*, 3H, *CH*$_3$ (24)), 1.00 (*s*, 3H, *CH*$_3$ (27)), 0.97 (*s*, 3H, *CH*$_3$ (26)), 0.88 (*s*, 3H, *CH*$_3$ (25)), 0.85 (*s*, 3H, *CH*$_3$ (23)) ppm; $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta$ = 178.5 (C28, *CO*), 150.5 (C20, *C* = $CH_2$), 108.7 (C29, C = *CH*$_2$), 93.2 (C31, *C*≡CH), 75.1 (C32, C≡*CH*), 73.7 (C3, *COH*), 56.0 (C17, $C_{quart.}$), 53.8 (C5, *CH*), 53.2 (C34 + C37, 2×*CH*$_2$), 50.9 (C18, *CH*), 49.0 (C9, *CH*), 47.0 (C19, *CH*), 43.1 (C33, *CH*$_2$), 42.2 (C14, $C_{quart.}$), 41.3 (C4, $C_{quart.}$), 40.5 (C8, $C_{quart.}$), 38.2 (C13, *CH*), 37.8 (C1, *CH*$_2$), 37.0 (C10, $C_{quart.}$), 36.7 (C22, *CH*$_2$), 34.2 (C7, *CH*$_2$), 31.9 (C2, *CH*$_2$), 31.8 (C16, *CH*$_2$), 30.3 (C21, *CH*$_2$), 29.4 (C15, *CH*$_2$), 25.5 (C12, *CH*$_2$), 25.1 (C24, *CH*$_3$), 23.1 (C35 + C36, 2×*CH*$_2$), 20.6 (C11, *CH*$_2$), 18.2 (C6, *CH*$_2$), 18.2 (C30, *CH*$_3$), 16.7 (C23, *CH*$_3$), 15.6 (C25, *CH*$_3$), 15.2 (C26, *CH*$_3$), 13.9 (C27, *CH*$_3$) ppm; MS (ESI, MeOH): *m*/*z* = 564.4 (100% $[M+H]^+$); analysis for $C_{37}H_{58}ClNO_3$ (600.31): C, 74.03; H, 9.74; N, 2.33; found: C, 73.89; H, 9.99; N, 2.23.

### *(3β) 3-Hydroxy-3-(3-piperidin-1-yl-prop-1-yn-1-yl)lup-20(29)-en-28-acid hydrochloride (**6**)*

Compound **6** was prepared as described in the general procedure from **3** (481 mg, 1.0 mmol), piperidine (0.35 mL, 3.5 mmol), formalin (37%, 1.2 mL, 14.8 mmol) and copper iodide (4 mg, 0.02 mmol) in DMSO (5 mL) (40°C for 5 days), and **6** (180 mg, 30%) was obtained as a colorless solid. Mp 235°C; $[\alpha]_D$ = +8.4° (*c* = 4.45, MeOH); IR (KBr): $\nu$ = 3346*s*, 2948*s*, 2628*s*, 2519*s*, 1688*s*, 1640*s*, 1455*s*, 1376*s*, 1319*m*, 1319*s*, 1241*s*, 1195*s*, 1135*s*, 1078*s*, 1039*s*, 984*s* cm$^{-1}$; $^1$H NMR (500 MHz, $CD_3OD$): $\delta$ = 4.70 (*m*, 1H, *CH*$_a$ (29)), 4.59 (*m*, 1H, *CH*$_b$ (29)), 4.13 (*s*, 2H, *CH*$_2$ (33)), 3.62 (*m*, 2H, *CH*$_a$ (34) + *CH*$_a$ (38)), 3.10−2.96 (*m*, 3H, *CH*$_b$ (34) + *CH*$_b$ (38) + *CH* (19)), 2.31 (*ddd*, 1H, *J* = 12.9, 11.5, 3.2 Hz, *CH* (13)), 2.23 (*ddd*, 1H, *J* = 12.9, 3.1, 2.7 Hz, *CH*$_a$ (16)), 2.03−1.76 (*m*, 7H, *CH*$_a$ (35) + *CH*$_a$ (37) + *CH*$_b$ (35) + *CH*$_b$ (37) + *CH*$_a$ (2) + *CH*$_a$ (21) + *CH*$_a$ (22)), 1.76−1.65 (*m*, 3H, *CH*$_a$ (1) + *CH*$_a$ (12) + *CH*$_b$ (2)), 1.67 (*s*, 3H, *CH*$_3$ (30)), 1.62 (*dd*, 1H, *J* = 11.4, 11.4 Hz, *CH* (18)), 1.58−1.02 (*m*, 17H, *CH* (9) + *CH*$_b$ (1) + *CH*$_b$ (22) + *CH*$_a$ (7) + *CH*$_b$ (7) + *CH*$_b$ (16) + *CH*$_b$ (21) + *CH*$_a$ (15) + *CH*$_a$ (11) + *CH*$_b$ (11) + *CH*$_a$ (6) + *CH*$_b$ (6) + *CH*$_a$ (36) + *CH*$_b$ (36) + *CH* (5) + *CH*$_b$ (15) + *CH*$_b$ (12)), 1.06 (*s*, 3H, *CH*$_3$ (24)), 1.00 (*s*, 3H, *CH*$_3$ (27)), 0.97 (*s*, 3H, *CH*$_3$ (26)), 0.88 (*s*, 3H, *CH*$_3$ (25)), 0.86 (*s*, 3H, *CH*$_3$ (23)) ppm; $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta$ = 178.4 (C28, *CO*), 150.5 (C20, *C*=$CH_2$), 108.7 (C29, C=*CH*$_2$), 94.5 (C31, *C*≡CH), 75.1 (C32, C≡*CH*), 72.8 (C3, *COH*), 56.0 (C17, $C_{quart.}$), 53.8 (C5, *CH*), 53.3 (C34 + C38, 2×*CH*$_2$), 51.0 (C18, *CH*), 49.0 (C9, *CH*), 47.0 (C19, *CH*), 45.8 (C33, *CH*$_2$), 42.2 (C14, $C_{quart.}$), 41.3 (C4, $C_{quart.}$), 40.5 (C8, $C_{quart.}$), 38.2 (C13, *CH*), 37.8 (C1, *CH*$_2$), 37.0 (C10, $C_{quart.}$), 36.7 (C22, *CH*$_2$), 34.2 (C7, *CH*$_2$), 31.9 (C2, *CH*$_2$), 31.8 (C16, *CH*$_2$), 30.3 (C21, *CH*$_2$), 29.4 (C15, *CH*$_2$), 25.5 (C12, *CH*$_2$), 25.1 (C24, *CH*$_3$), 22.8 (C35 + C37, 2×*CH*$_2$), 21.2 (C36, *CH*$_2$), 20.6 (C11, *CH*$_2$), 18.2 (C6, *CH*$_2$), 18.2 (C30, *CH*$_3$), 16.7 (C23, *CH*$_3$), 15.6 (C25, *CH*$_3$), 15.2 (C26, *CH*$_3$), 14.0 (C27, *CH*$_3$) ppm; MS (ESI, MeOH): *m*/*z* = 578.4 (100% $[M+H]^+$); analysis for $C_{38}H_{60}ClNO_3$ (614.34): C, 74.29; H, 9.84; N, 2.28; found: C, 74.02; H, 10.04; N, 2.16.

### *(3β) 3-(3-Azepan-1-yl-prop-1-yn-1-yl)-3-hydroxylup-20(29)-en-28-acid hydrochloride (**7**)*

Compound **7** was prepared as described in the general procedure from **3** (481 mg, 1.0 mmol), azepane (0.3 mL, 2.6 mmol), formalin (37%, 1.2 mL, 14.8 mmol) and copper iodide (4 mg, 0.02 mmol) in DMSO (5 mL) (40°C for 5 days), and **7** (85 mg, 14%) was obtained after re-crystallization as a colorless solid. Mp 231°C; $[\alpha]_D$ = +1.0° (*c* = 5.10, MeOH); IR (KBr): $\nu$ = 3385*m*, 2941*s*, 2867*s*, 2602*m*, 1718*m*, 1638*m*, 1464*m*, 1376*m*, 1168*m*, 1133*m*, 1038*m*, 1011*m*, 983*m*, 947*w* cm$^{-1}$; $^1$H NMR (500 MHz, $CD_3OD$): $\delta$ = 4.71 (*m*, 1H, *CH*$_a$ (29)), 4.59 (*m*, 1H, *CH*$_b$ (29)), 4.19 (*s*, 2H, *CH*$_2$ (33)), 3.59 (*m*, 2H, *CH*$_a$ (34) + *CH*$_a$ (39)), 3.11 (*m*, 2H,

$CH_b$ (34) + $CH_b$ (39)), 3.01 (*ddd*, 1H, $J$ = 10.8, 10.8, 4.8 Hz, C*H* (19)), 2.32 (*ddd*, 1H, $J$ = 12.8, 11.8, 3.5 Hz, C*H* (13)), 2.24 (*ddd*, 1H, $J$ = 12.8, 3.2, 3.2 Hz, $CH_a$ (16)), 2.06−1.85 (*m*, 5H, $CH_a$ (35) + $CH_a$ (38) + $CH_a$ (2) + $CH_a$ (21) + $CH_a$ (22)), 1.81−1.66 (*m*, 5H, $CH_b$ (35) + $CH_b$ (38) + $CH_a$ (1) + $CH_a$ (12) + $CH_b$ (2)), 1.69 (*s*, 3H, $CH_3$ (30)), 1.62 (*dd*, 1H, $J$ = 11.4, 11.4 Hz, C*H* (18)), 1.57−1.00 (*m*, 19H, C*H* (9) + $CH_b$ (1) + $CH_b$ (22) + $CH_a$ (7) + $CH_b$ (7) + $CH_b$ (16) + $CH_b$ (21) + $CH_a$ (15) + $CH_a$ (11) + $CH_b$ (11) + $CH_a$ (6) + $CH_b$ (6) + $CH_a$ (36) + $CH_b$ (36) + $CH_a$ (37) + $CH_b$ (37) + C*H* (5) + $CH_b$ (15) + $CH_b$ (12)), 1.06 (*s*, 3H, $CH_3$ (24)), 1.00 (*s*, 3H, $CH_3$ (27)), 0.98 (*s*, 3H, $CH_3$ (26)), 0.88 (*s*, 3H, $CH_3$ (25)), 0.86 (*s*, 3H, $CH_3$ (23)) ppm; $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta$ = 178.4 (C28, *C*O), 150.5 (C20, *C*=$CH_2$), 108.7 (C29, C=*C*$H_2$), 94.0 (C31, *C*≡CH), 75.1 (C32, C≡*C*H), 73.4 (C3, *C*OH), 56.0 (C17, $C_{quart.}$), 54.4 (C34 + C39, 2×$CH_2$), 53.9 (C5, *C*H), 51.0 (C18, *C*H), 49.0 (C9, *C*H), 47.0 (C19, *C*H), 46.8 (C33, *C*$H_2$), 42.2 (C14, $C_{quart.}$), 41.3 (C4, $C_{quart.}$), 40.5 (C8, $C_{quart.}$), 38.2 (C13, *C*H), 37.8 (C1, *C*$H_2$), 37.0 (C10, $C_{quart.}$), 36.7 (C22, *C*$H_2$), 34.2 (C7, *C*$H_2$), 31.9 (C2, *C*$H_2$), 31.8 (C16, *C*$H_2$), 30.3 (C21, *C*$H_2$), 29.4 (C15, *C*$H_2$), 25.6 (C35 + C38, 2×*C*$H_2$), 25.5 (C12, *C*$H_2$), 25.1 (C24, *C*$H_3$), 23.8 (C36 + C37, 2×*C*$H_2$), 20.6 (C11, *C*$H_2$), 18.2 (C6, *C*$H_2$), 18.2 (C30, *C*$H_3$), 16.7 (C23, *C*$H_3$), 15.6 (C25, *C*$H_3$), 15.2 (C26, *C*$H_3$), 14.0 (C27, *C*$H_3$) ppm; MS (ESI, MeOH): $m/z$ = 592.5 (100% $[M+H]^+$); $m/z$ = 1181.1 (100% $[2M-H]^-$), 636.1 (95% $[M+HCO_2]^-$), 590.5 (14% $[M-H]^-$); analysis for $C_{39}H_{62}ClNO_3$ (628.37): C, 74.55; H, 9.95; N, 2.23; found: C, 74.32; H, 10.11; N, 2.03.

### *(3β) 3-[3-(Diisopropylamino)prop-1-yn-1-yl]-3-hydroxylup-20(29)-en-28-acid hydrochloride (**8**)*

Compound **8** was prepared as described in the general procedure from **3** (481 mg, 1.0 mmol), diisopropylamine (0.35 mL, 2.5 mmol), formalin (37%, 0.8 mL, 9.9 mmol) and copper iodide (4 mg, 0.02 mmol) in DMSO (5 mL) (40°C for 20 h), and **8** (390 mg, 62%) was obtained after re-crystallization as a colorless solid. Mp 240°C; $[\alpha]_D$ = +1.9° ($c$ = 4.60, MeOH); IR (KBr): $\nu$ = 3172*m*, 2949*s*, 2868*s*, 2539*m*, 2462*m*, 1727*s*, 1644*m*, 1469*m*, 1392*m*, 1376*m*, 1316*m*, 1255*w*, 1167*m*, 1134*s*, 1067*m*, 1037*m*, 1010*m*, 982*w* $cm^{-1}$; $^1$H NMR (500 MHz, $CD_3OD$): $\delta$ = 4.71 (*m*, 1H, $CH_a$ (29)), 4.59 (*m*, 1H, $CH_b$ (29)), 4.23 (*s*, 2H, $CH_2$ (33)), 3.91 (*sept*, 2H, $J$ = 6.6 Hz, 2×*CH* (34) + (37)), 3.01 (*ddd*, 1H, $J$ = 10.8, 10.8, 4.6 Hz, C*H* (19)), 2.32 (*ddd*, 1H, $J$ = 12.8, 11.8, 3.2 Hz, C*H* (13)), 2.24 (*ddd*, 1H, $J$ = 12.8, 3.2, 3.0 Hz, $CH_a$ (16)), 2.01−1.88 (*m*, 3H, $CH_a$ (2) + $CH_a$ (21) + $CH_a$ (22)), 1.77−1.66 (*m*, 3H, $CH_a$ (1) + $CH_a$ (12) + $CH_b$ (2)), 1.69 (*s*, 3H, $CH_3$ (30)), 1.62 (*dd*, 1H, $J$ = 11.4, 11.4 Hz, C*H* (18)), 1.58−1.22 (*m*, 12H, C*H* (9) + $CH_b$ (1) + $CH_b$ (22) + $CH_a$ (7) + $CH_b$ (7) + $CH_b$ (16) + $CH_b$ (21) + $CH_a$ (15) + $CH_a$ (11) + $CH_b$ (11) + $CH_a$ (6) + $CH_b$ (6)), 1.47 (*d*, 12H, $J$ = 6.6 Hz, 4×$CH_3$ (35) + (36) + (38) + (39)), 1.20−1.01 (*m*, 3H, C*H* (5) + $CH_b$ (15) + $CH_b$ (12)), 1.05 (*s*, 3H, $CH_3$ (24)), 1.00 (*s*, 3H, $CH_3$ (27)), 0.97 (*s*, 3H, $CH_3$ (26)), 0.88 (*s*, 3H, $CH_3$ (25)), 0.85 (*s*, 3H, $CH_3$ (23)) ppm; $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta$ = 178.4 (C28, *C*O), 150.5 (C20, *C*=$CH_2$), 108.7 (C29, C=*C*$H_2$), 93.8 (C31, *C*≡CH), 75.2 (C32, C≡*C*H), 74.3 (C3, *C*OH), 56.0 (C17, $C_{quart.}$), 54.5 (C34 + C37, 2×*C*H), 53.9 (C5, *C*H), 51.0 (C18, *C*H), 49.0 (C9, *C*H), 47.4 (C19, *C*H), 42.2 (C14, $C_{quart.}$), 41.4 (C4, $C_{quart.}$), 40.5 (C8, $C_{quart.}$), 38.2 (C13, *C*H), 37.8 (C1, *C*$H_2$), 37.0 (C10, $C_{quart.}$), 36.7 (C22, *C*$H_2$), 35.7 (C33, *C*$H_2$), 34.2 (C7, *C*$H_2$), 31.9 (C2, *C*$H_2$), 31.8 (C16, *C*$H_2$), 30.3 (C21, *C*$H_2$), 29.4 (C15, *C*$H_2$), 25.5 (C12, *C*$H_2$), 25.2 (C24, *C*$H_3$), 20.7 (C11, *C*$H_2$), 18.2 (C6, *C*$H_2$), 18.2 (C30, *C*H3), 17.6 (C35 + C36 + C38 + C39, 4×*C*$H_3$), 16.8 (C23, *C*$H_3$), 15.6 (C25, *C*$H_3$), 15.2 (C26, *C*$H_3$), 14.0 (C27, *C*$H_3$) ppm; MS (ESI, MeOH): $m/z$ = 594.5 (100% $[M+H]^+$); analysis for $C_{39}H_{64}ClNO_3$ (630.38): C, 74.31; H, 10.23; N, 2.22; found: C 74.09, H 10.54, N 2.09.

### *General procedure for the synthesis of the methyl esters **9**–**19***

A mixture of alkynol **4** (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 was 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.

### *Methyl (3β) 3-hydroxy-3-(3-pyrrolidin-1-yl-prop-1-yn-1-yl)lup-20(29)-en-28-oate hydrochloride (**9**)*

Compound **9** was prepared as described in the general procedure from **4** (495 mg, 1.0 mmol), pyrrolidine (0.2 mL, 2.4 mmol), formalin (37%, 0.8 mL, 9.8 mmol) and copper iodide (12 mg, 0.06 mmol) in DMSO (3 mL) (40°C for 4 days), and **9** (220 mg, 38%) was obtained after re-crystallization as a colorless solid. Mp 200°C; $[\alpha]_D$ = −2.6° ($c$ = 5.60, MeOH); UV–vis (MeOH): $\lambda_{max}$ (nm) (log $\varepsilon$) = 219 (0.51); IR (KBr): $\nu$ = 3424*m*, 2949*s*, 2868*m*, 2608*w*, 1126*m*, 1640*w*, 1458*m*, 1377*m*, 1189*m*, 1154*m*, 1137*m*, 1039*w* $cm^{-1}$; $^1$H NMR (500 MHz, $CD_3OD$): $\delta$ = 4.72 (*m*, 1H, $CH_a$ (29)), 4.60 (*m*, 1H, $CH_b$ (29)), 4.22 (*s*, 2H, $CH_2$ (34)), 3.66 (*m*, 2H, $CH_a$ (35) + $CH_a$ (38)), 3.65 (*s*, 3H, $CH_3$ (31)), 3.25 (*m*, 2H, $CH_b$ (35) + $CH_b$ (38)), 2.99 (*ddd*, 1H, $J$ = 10.8, 10.8, 5.0 Hz, C*H* (19)), 2.28−2.15 (*m*, 4H, C*H* (13) + $CH_a$ (16) + $CH_a$ (36) + $CH_a$ (37)), 2.07 (*m*, 2H, $CH_b$ (36) + $CH_b$ (37)), 1.97 (*ddd*, 1H, $J$ = 15.0, 12.6, 3.7 Hz, $CH_a$ (2)),1.90−1.83 (*m*, 2H, $CH_a$ (21) + $CH_a$ (22)), 1.76−1.65 (*m*, 3H, $CH_a$ (1) + $CH_a$ (12) + $CH_b$ (2)), 1.69 (*s*, 3H, $CH_3$ (30)), 1.64 (*dd*, 1H, $J$ = 11.3, 11.3 Hz, C*H* (18)), 1.55−1.25 (*m*, 12H, C*H* (9) + $CH_b$ (1) + $CH_b$ (22) + $CH_a$ (7) + $CH_b$ (7) + $CH_b$ (16) + $CH_b$ (21) + $CH_a$ (15) + $CH_a$ (11) + $CH_b$ (11) + $CH_a$ (6) + $CH_b$ (6)), 1.19−1.09 (*m*, 2H, C*H* (5) + $CH_b$ (15)), 1.08−1.00 (*m*, 1H, $CH_b$ (12)), 1.06 (*s*, 3H, $CH_3$ (24)), 1.00 (*s*, 3H, $CH_3$ (27)), 0.94 (*s*, 3H, $CH_3$ (26)), 0.88 (*s*, 3H, $CH_3$ (25)), 0.85 (*s*, 3H, $CH_3$ (23)) ppm; $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta$ = 178.1 (C28, *C*O), 151.8 (C20, *C*=$CH_2$), 110.3 (C29, C=*C*$H_2$), 94.5 (C32, *C*≡CH), 76.4 (C33, C≡*C*H), 75.2 (C3, *C*OH), 57.9 (C17, $C_{quart.}$), 55.1 (C5, *C*H), 54.5 (C35 + C38, 2×*C*$H_2$), 52.3 (C31, *C*$H_3$), 51.8 (C18, *C*H), 50.2 (C9, *C*H), 48.5 (C19, *C*H), 44.5 (C34, *C*$H_2$), 43.6 (C14, $C_{quart.}$), 42.7 (C4, $C_{quart.}$), 41.9 (C8, $C_{quart.}$), 39.6 (C13, *C*H), 39.2 (C1, *C*$H_2$), 38.4 (C10, $C_{quart.}$), 37.8 (C22, *C*$H_2$), 35.6 (C7, *C*$H_2$), 33.2 (C2, *C*$H_2$), 33.1 (C16, *C*$H_2$), 31.6 (C21, *C*$H_2$), 30.8 (C15, *C*$H_2$), 26.8 (C12, *C*$H_2$), 26.5 (C24, *C*$H_3$), 24.6 (C36 + C37, 2×*C*$H_2$), 22.0 (C11, *C*$H_2$), 19.6 (C6, *C*$H_2$), 19.6 (C30, *C*$H_3$), 18.2 (C23, *C*$H_3$), 17.0 (C25, *C*$H_3$), 16.5 (C26, *C*$H_3$), 15.4 (C27, *C*$H_3$) ppm; MS (ESI, MeOH): $m/z$ = 578.4 (100% $[M+H]^+$); analysis

for $C_{38}H_{60}ClNO_3$ (614.34): C, 74.29; H, 9.84; N, 2.28; found: C, 74.02; H, 9.97; N, 2.12.

### *Methyl (3β) 3-hydroxy-3-(3-piperidin-1-yl-prop-1-yn-1-yl)lup-20(29)-en-28-oate hydrochloride (**10**)*

Compound **10** was prepared as described in the general procedure from **4** (371 mg, 0.75 mmol), piperidine (0.1 mL, 1.0 mmol), formalin (37%, 0.3 mL, 3.7 mmol) and copper iodide (3 mg, 0.016 mmol) in DMSO (4 mL) (40°C for 20 h), and **10** (85 mg, 18%) was obtained as a colorless solid. Mp 215°C; $[\alpha]_D = -5.6^\circ$ ($c = 4.35$, MeOH); UV–vis (MeOH): $\lambda_{max}$ (nm) (log $\varepsilon$) = 220 (1.28), 212 (0.37); IR (KBr): $\nu = 3422m$, 2947*s*, 2869*m*, 2529*m*, 1727*m*, 1641*w*, 1458*m*, 1376*m*, 1188*m*, 1154*m*, 1136*m*, 1039*m*, 883*w* $cm^{-1}$; $^1H$ NMR (500 MHz, $CD_3OD$): $\delta = 4.71$ (*m*, 1H, C$H_a$ (29)), 4.59 (*m*, 1H, C$H_b$ (29)), 4.13 (*s*, 2H, C$H_2$ (34)), 3.65 (*s*, 3H, C$H_3$ (31)), 3.62 (*m*, 2H, C$H_a$ (35) + C$H_a$ (39)), 3.06 (*m*, 2H, C$H_b$ (35) + C$H_b$ (39)), 2.99 (*ddd*, 1H, $J = 10.8$, 10.8, 5.1 Hz, C*H* (19)), 2.29–2.21 (*m*, 2H, C*H* (13) + C$H_a$ (16)), 2.01–1.77 (*m*, 7H, C$H_a$ (36) + C$H_a$ (38) + C$H_b$ (36) + C$H_b$ (38) + C$H_a$ (2) + C$H_a$ (21) + C$H_a$ (22)), 1.76–1.66 (*m*, 3H, C$H_a$ (1) + C$H_a$ (12) + C$H_b$ (2)), 1.69 (*s*, 3H, C$H_3$ (30)), 1.64 (*dd*, 1H, $J = 11.3$, 11.3 Hz, C*H* (18)), 1.58–1.02 (*m*, 17H, C*H* (9) + C$H_b$ (1) + C$H_b$ (22) + C$H_a$ (7) + C$H_b$ (7) + C$H_b$ (16) + C$H_b$ (21) + C$H_a$ (15) + C$H_a$ (11) + C$H_b$ (11) + C$H_a$ (6) + C$H_b$ (6) + C$H_a$ (37) + C$H_b$ (37) + C*H* (5) + C$H_b$ (15) + C$H_b$ (12)), 1.06 (*s*, 3H, C$H_3$ (24)), 0.99 (*s*, 3H, C$H_3$ (27)), 0.94 (*s*, 3H, C$H_3$ (26)), 0.88 (*s*, 3H, C$H_3$ (25)), 0.86 (*s*, 3H, C$H_3$ (23)) ppm; $^{13}C$ NMR (125 MHz, $CD_3OD$): $\delta = 178.1$ (C28, *C*O), 151.8 (C20, *C* = $CH_2$), 110.3 (C29, C = *C*$H_2$), 95.9 (C32, *C*≡CH), 76.6 (C33, C≡*C*H), 74.2 (C3, *C*OH), 57.9 (C17, $C_{quart.}$), 55.2 (C5, *C*H), 53.7 (C35 + C39, 2×*C*$H_2$), 52.4 (C31, *C*$H_3$), 51.8 (C18, *C*H), 50.7 (C9, *C*H), 48.4 (C19, *C*H), 47.2 (C34, *C*$H_2$), 43.5 (C14, $C_{quart.}$), 42.7 (C4, $C_{quart.}$), 41.9 (C8, $C_{quart.}$), 39.7 (C13, *C*H), 39.2 (C1, *C*$H_2$), 38.4 (C10, $C_{quart.}$), 37.8 (C22, *C*$H_2$), 35.6 (C7, *C*$H_2$), 33.2 (C2, *C*$H_2$), 33.1 (C16, *C*$H_2$), 31.6 (C21, *C*$H_2$), 30.8 (C15, *C*$H_2$), 26.8 (C12, *C*$H_2$), 26.5 (C24, *C*$H_3$), 24.3 (C36 + C38, 2×*C*$H_2$), 22.6 (C37, *C*$H_2$), 22.0 (C11, *C*$H_2$), 19.7 (C6, *C*$H_2$), 19.6 (C30, *C*$H_3$), 18.1 (C23, *C*$H_3$), 17.0 (C25, *C*$H_3$), 16.5 (C26, *C*$H_3$), 15.5 (C27, *C*$H_3$) ppm; MS (ESI, MeOH): $m/z = 592.5$ (100% $[M+H]^+$); analysis for $C_{39}H_{62}ClNO_3$ (628.37): C, 74.55; H, 9.95; N, 2.23; found: C, 74.77; H, 10.02; N, 2.14.

### *Methyl (3β) 3-(3-azepan-1-yl-prop-1-yn-1-yl)-3-hydroxylup-20(29)-en-28-oate hydrochloride (**11**)*

Compound **11** was prepared as described in the general procedure from **4** (248 mg, 0.5 mmol), azepane (0.3 mL, 2.6 mmol), formalin (37%, 1.0 mL, 12.3 mmol) and copper iodide (2 mg, 0.01 mmol) in DMSO (6 mL) (40°C for 2 days), and **11** (205 mg, 64%) was obtained after re-crystallization as a colorless solid. Mp 214°C; $[\alpha]_D = +1.2^\circ$ ($c = 5.50$, MeOH); IR (KBr): $\nu = 3406m$, 3073*w*, 1946*s*, 2868*s*, 2601*m*, 1726*s*, 1641*m*, 1457*s*, 1377*m*, 1352*m*, 1317*m*, 1189*m*, 1154*m*, 1136*s*, 1079*w*, 1038*m*, 984*m* $cm^{-1}$; $^1H$ NMR (500 MHz, $CD_3OD$): $\delta = 4.71$ (*m*, 1H, C$H_a$ (29)), 4.60 (*m*, 1H, C$H_b$ (29)), 4.18 (*s*, 2H, C$H_2$ (34)), 3.66 (*s*, 3H, C$H_3$ (31)), 3.63–3.45 (*brm*, 2H, C$H_a$ (35) + C$H_a$ (40)), 3.44–3.28 (*brm*, 2H, C$H_b$ (35) + C$H_b$ (40)), 2.99 (*ddd*, 1H, $J = 10.7$, 10.7, 5.1 Hz, C*H* (19)), 2.28–2.28 (*m*, 2H, C*H* (13) + C$H_a$ (16)), 2.06–1.84 (*m*, 5H, C$H_a$ (36) + C$H_a$ (39) + C$H_a$ (2) + C$H_a$ (21) + C$H_a$ (22)), 1.81–1.66 (*m*, 5H, C$H_b$ (36) + C$H_b$ (39) + C$H_a$ (1) + C$H_a$ (12) + C$H_b$ (2)), 1.69 (*s*, 3H, C$H_3$ (30)), 1.64 (*dd*, 1H, $J = 11.4$, 11.4 Hz, C*H* (18)), 1.55–1.02 (*m*, 19H, C*H* (9) + C$H_b$ (1) + C$H_b$ (22) + C$H_a$ (7) + C$H_b$ (7) + C$H_b$ (16) + C$H_b$ (21) + C$H_a$ (15) + C$H_a$ (11) + C$H_b$ (11) + C$H_a$ (6) + C$H_b$ (6) + C$H_a$ (37) + C$H_b$ (37) + C*H* (5) + C$H_b$ (15) + C$H_b$ (12)), 1.06 (*s*, 3H, C$H_3$ (24)), 1.00 (*s*, 3H, C$H_3$ (27)), 0.95 (*s*, 3H, C$H_3$ (26)), 0.88 (*s*, 3H, C$H_3$ (25)), 0.86 (*s*, 3H, C$H_3$ (23)) ppm; $^{13}C$ NMR (125 MHz, $CD_3OD$): $\delta = 176.6$ (C28, *C*O), 150.3 (C20, *C*=$CH_2$), 108.8 (C29, C=*C*$H_2$), 94.0 (C32, *C*≡CH), 75.1 (C33, C≡*C*H), 73.4 (C3, *C*OH), 56.4 (C17, $C_{quart.}$), 54.4 (C35 + C40, 2×$CH_2$), 53.8 (C5, *C*H), 51.0 (C31, *C*$H_3$), 50.4 (C18, *C*H), 49.2 (C9, *C*H), 47.0 (C19, *C*H), 46.8 (C34, *C*$H_2$), 42.1 (C14, $C_{quart.}$), 41.3 (C4, $C_{quart.}$), 40.5 (C8, $C_{quart.}$), 38.2 (C13, *C*H), 37.8 (C1, *C*$H_2$), 37.0 (C10, $C_{quart.}$), 36.4 (C22, *C*$H_2$), 34.2 (C7, *C*$H_2$), 31.8 (C2, *C*$H_2$), 31.7 (C16, *C*$H_2$), 30.2 (C21, *C*$H_2$), 29.4 (C15, *C*$H_2$), 25.6 (C36 + C39, 2×*C*$H_2$), 25.5 (C12, *C*$H_2$), 25.1 (C24, *C*$H_3$), 23.8 (C37 + C38, 2×*C*$H_2$), 20.6 (C11, *C*$H_2$), 18.2 (C6, *C*$H_2$), 18.2 (C30, *C*$H_3$), 16.7 (C23, *C*$H_3$), 15.6 (C25, *C*$H_3$), 15.1 (C26, *C*$H_3$), 14.1 (C27, *C*$H_3$) ppm; MS (ESI, MeOH): $m/z = 606.5$ (100% $[M+H]^+$); analysis for $C_{40}H_{64}ClNO_3$ (642.39): C, 74.79; H, 10.04; N, 2.18; found: C, 74.59; H, 10.23; N, 2.04.

### *Methyl (3β) 3-hydroxy-3-(3-morpholin-4-yl-prop-1-yn-1-yl)lup-20(29)-en-28-oate hydrochloride (**12**)*

Compound **12** was prepared as described in the general procedure from **4** (347 mg, 0.7 mmol), morpholine (0.07 mL, 0.8 mmol), formalin (37%, 0.35 mL, 4.3 mmol) and copper iodide (4 mg, 0.02 mmol) in DMSO (6 mL) (40°C for 2 days), and **12** (260 mg, 59%) was obtained after re-crystallization as a colorless solid. Mp 207°C; $[\alpha]_D = -4.9^\circ$ ($c = 5.75$, MeOH); UV–vis (MeOH): $\lambda_{max}$ (nm) (log $\varepsilon$) = 220 (1.39), 217 (1.43), 214 (0.70); IR (KBr): $\nu = 3405s$, 2947*s*, 2869*s*, 2543*m*, 2451*m*, 1727*s*, 1642*m*, 1453*s*, 1390*s*, 1376*s*, 1352*m*, 1318*m*, 1265*m*, 1238*m*, 1189*s*, 1155*s*, 1134*s*, 1073*s*, 1038*s*, 1012*m*, 983*m* $cm^{-1}$; $^1H$ NMR (500 MHz, $CD_3OD$): $\delta = 4.71$ (*m*, 1H, C$H_a$ (29)), 4.60 (*m*, 1H, C$H_b$ (29)), 4.22 (*s*, 2H, C$H_2$ (34)), 4.10 (*m*, 2H, C$H_a$ (36) + C$H_a$ (37)), 3.80 (*m*, 2H, C$H_b$ (36) + C$H_b$ (37)), 3.65 (*s*, 3H, C$H_3$ (31)), 3.56 (*m*, 2H, C$H_a$ (35) + C$H_a$ (38)), 3.27 (*m*, 2H C$H_b$ (35) + C$H_b$ (38)), 2.99 (*ddd*, 1H, $J = 10.8$, 10.8, 5.0 Hz, C*H* (19)), 2.29–2.21 (*m*, 2H, C*H* (13) + C$H_a$ (16)), 1.98 (*ddd*, 1H, $J = 14.4$, 13.1, 3.8 Hz, C$H_a$ (2)), 1.90–1.77 (*m*, 2H, C$H_a$ (21) + C$H_a$ (22)), 1.76–1.66 (*m*, 3H, C$H_a$ (1) + C$H_a$ (12) + C$H_b$ (2)), 1.69 (*s*, 3H, C$H_3$ (30)), 1.64 (*dd*, 1H, $J = 11.4$, 11.4 Hz, C*H* (18)), 1.55–1.24 (*m*, 12H, C*H* (9) + C$H_b$ (1) + C$H_b$ (22) + C$H_a$ (7) + C$H_b$ (7) + C$H_b$ (16) + C$H_b$ (21) + C$H_a$ (15) + C$H_a$ (11) + C$H_b$ (11) + C$H_a$ (6) + C$H_b$ (6)), 1.20–1.08 (*m*, 2H, C*H* (5) + C$H_b$ (15)), 1.07–1.00 (*m*, 1H, C$H_b$ (12)), 1.06 (*s*, 3H, C$H_3$ (24)), 0.99 (*s*, 3H, C$H_3$ (27)), 0.94 (*s*, 3H, C$H_3$ (26)), 0.88 (*s*, 3H, C$H_3$ (25)), 0.86 (*s*, 3H, C$H_3$ (23)) ppm; $^{13}C$ NMR (125 MHz, $CD_3OD$): $\delta = 176.7$ (C28, *C*O), 150.4 (C20, *C*=$CH_2$), 108.8 (C29, C=*C*$H_2$), 95.1 (C32, *C*≡CH), 75.2 (C33, C≡*C*H), 72.2 (C3, *C*OH), 63.6 (C36 + C37, 2×$CH_2$), 56.4 (C17, $C_{quart.}$), 53.8 (C5, *C*H), 50.9 (C35 + C38, 2×$CH_2$), 50.8 (C31, *C*$H_3$), 50.4 (C18, *C*H), 49.2 (C9, *C*H), 47.0 (C19, *C*H), 45.9 (C34, *C*$H_2$), 42.1 (C14, $C_{quart.}$), 41.3 (C4, $C_{quart.}$), 40.5 (C8, $C_{quart.}$), 38.2 (C13, *C*H), 37.8 (C1, *C*$H_2$), 37.0 (C10, $C_{quart.}$), 36.4 (C22, *C*$H_2$), 34.1 (C7, *C*$H_2$), 31.8 (C2, *C*$H_2$), 31.7 (C16, *C*$H_2$), 30.2 (C21, *C*$H_2$), 29.4 (C15, *C*$H_2$), 25.4 (C12, *C*$H_2$), 25.1 (C24, *C*$H_3$), 20.6 (C11, *C*$H_2$), 18.2 (C6, *C*$H_2$), 18.1 (C30, *C*$H_3$), 17.0 (C23, *C*$H_3$), 15.5 (C25, *C*$H_3$), 15.1 (C26, *C*$H_3$), 14.0 (C27, *C*$H_3$) ppm; MS (ESI, MeOH): $m/z = 594.4$ (100% $[M+H]^+$); analysis for $C_{38}H_{60}ClNO_4$ (630.34): C, 72.41; H, 9.59; N, 2.22; found: C, 72.39; H, 9.73; N, 2.07.

### *Methyl (3β) 3-hydroxy-3-(3-thiomorpholin-4-yl-prop-1-yn-1-yl)lup-20(29)-en-28-oate hydrochloride (**13**)*

Compound **13** was prepared as described in the general procedure from **4** (248 mg, 0.5 mmol), thiomorpholine (0.06 mL, 0.6 mmol), formalin (37%, 0.2 mL, 2.5 mmol) and copper iodide

(2 mg, 0.01 mmol) in DMSO (5 mL) (40°C for 2 days), and **13** (160 mg, 50%) was obtained after twofold re-crystallization as a colorless solid. Mp 198°C; $[\alpha]_D = -2.3^\circ$ ($c = 3.50$, MeOH); UV–vis (MeOH): $\lambda_{max}$ (nm) (log $\varepsilon$) = 250 (0.15), 208 (1.60); IR (KBr): $\nu$ = 3355*s*, 2946*s*, 2868*s*, 2724*s*, 2656*s*, 2457*s*, 1723*s*, 1641*s*, 1591*s*, 1455*s*, 1390*s*, 1376*s*, 1317*s*, 1266*s*, 1188*s*, 1154*s*, 1135*s*, 1039*s*, 1003*s*, 981*s*, 920*s* $cm^{-1}$; $^1H$ NMR (500 MHz, $CD_3OD$): $\delta$ = 4.71 (*m*, 1H, C$H_a$ (29)), 4.59 (*m*, 1H, C$H_b$ (29)), 4.21 (*s*, 2H, C$H_2$ (34)), 3.95–2.80 (*br m* 8H, C$H_a$ (36) + C$H_a$ (37) + C$H_b$ (36) + C$H_b$ (37) + C$H_a$ (35) + C$H_a$ (38) + C$H_b$ (35) + C$H_b$ (38)), 3.65 (*s*, 3H, C$H_3$ (31)), 2.99 (*ddd*, 1H, $J$ = 11.0, 11.0, 5.1 Hz, C*H* (19)), 2.29–2.20 (*m*, 2H, C*H* (13) + C$H_a$ (16)), 1.97 (*m*, 1H, C$H_a$ (2)), 1.89–1.83 (*m*, 2H, C$H_a$ (21) + C$H_a$ (22)), 1.75–1.68 (*m*, 3H, C$H_a$ (1) + C$H_a$ (12) + C$H_b$ (2)), 1.69 (*s*, 3H, C$H_3$ (30)), 1.64 (*dd*, 1H, $J$ = 11.4, 11.4 Hz, C*H* (18)), 1.55–1.23 (*m*, 12H, C*H* (9) + C$H_b$ (1) + C$H_b$ (22) + C$H_a$ (7) + C$H_b$ (7) + C$H_b$ (16) + C$H_b$ (21) + C$H_a$ (15) + C$H_a$ (11) + C$H_b$ (11) + C$H_a$ (6) + C$H_b$ (6)), 1.18–1.03 (*m*, 3H, C*H* (5) + C$H_b$ (15) + C$H_b$ (12)), 1.07 (*s*, 3H, C$H_3$ (24)), 1.00 (*s*, 3H, C$H_3$*i*(27)), 0.94 (*s*, 3H, C$H_3$ (26)), 0.88 (*s*, 3H, C$H_3$ (25)), 0.86 (*s*, 3H, C$H_3$ (23)) ppm; $^{13}C$ NMR (125 MHz, $CD_3OD$): $\delta$ = 176.7 (C28, *C*O), 150.3 (C20, *C*=CH$_2$), 108.9 (C29, C=*C*H$_2$), 95.2 (C32, *C*≡CH), 75.2 (C33, C≡*C*H), 72.2 (C3, *C*OH), 56.4 (C17, $C_{quart.}$), 53.9 (C5, *C*H), 53.2 (C35 + C38, 2×*C*H$_2$), 50.9 (C31, *C*H$_3$), 50.4 (C18, *C*H), 49.2 (C9, *C*H), 47.0 (C19, *C*H), 46.6 (C34, *C*H$_2$), 42.2 (C14, $C_{quart.}$), 41.3 (C4, $C_{quart.}$), 40.5 (C8, $C_{quart.}$), 38.2 (C13, *C*H), 37.9 (C1, *C*H$_2$), 37.0 (C10, $C_{quart.}$), 36.4 (C22, *C*H$_2$), 34.1 (C7, *C*H$_2$), 31.8 (C2, *C*H$_2$), 31.7 (C16, *C*H$_2$), 30.2 (C21, *C*H$_2$), 29.4 (C15, *C*H$_2$), 25.4 (C12, *C*H$_2$), 25.1 (C24, *C*H$_3$), 24.6 (C36 + C37, 2×*C*H$_2$), 20.6 (C11, *C*H$_2$), 18.2 (C6, *C*H$_2$), 18.2 (C30, *C*H$_3$), 16.7 (C23, *C*H$_3$), 15.6 (C25, *C*H$_3$), 15.1 (C26, *C*H$_3$), 14.1 (C27, *C*H$_3$) ppm; MS (ESI, MeOH): $m/z$ = 610.4 (100% $[M+H]^+$); analysis for $C_{38}H_{60}ClNO_3S$ (646.41): C, 70.61; H, 9.36; N, 2.17; found: C, 70.55; H, 9.42; N, 2.07.

### *Methyl (3β) 3-hydroxy-3-[3-(4-methylpiperazin-1-yl)prop-1-yn-1-yl]lup-20(29)-en-28-oate hydrochloride (**14**)*

Compound **14** was prepared as described in the general procedure from **4** (248 mg, 0.5 mmol), *N*-methylpiperazine (0.09 mL, 0.8 mmol), formalin (37%, 0.3 mL, 3.7 mmol) and copper iodide (2 mg, 0.01 mmol) in DMSO (6 mL) (40°C for 2 days), and **14** (218 mg, 64%) was obtained after re-crystallization as a colorless solid. Mp 238°C; $[\alpha]_D = +1.9^\circ$ ($c$ = 4.40, MeOH); UV–vis (MeOH): $\lambda_{max}$ (nm) (log ε) = 270 (0.10), 210 (1.50); IR (KBr): $\nu$ = 3406*s*, 2943*s*, 2869*s*, 2404*s*, 1715*s*, 1639*m*, 1451*s*, 1377*s*, 1318*m*, 1190*s* $cm^{-1}$; $^1H$ NMR (500 MHz, $CD_3OD$): $\delta$ = 4.71 (*m*, 1H, C$H_a$ (29)), 4.59 (*m*, 1H, C$H_b$ (29)), 4.29 (*s*, 2H, C$H_2$ (34)), 3.91–3.51 (*m*, br 8H, C$H_a$ (36) + C$H_a$ (37) + C$H_b$ (36) + C$H_b$ (37) + C$H_a$ (35) + C$H_a$ (38) + C$H_b$ (35) + C$H_b$ (38)), 3.65 (*s*, 3H, C$H_3$ (31)), 3.05–2.93 (*m*, 4H, C$H_3$ (39) + C*H* (19)), 2.29–2.18 (*m*, 2H, C*H* (13) + C$H_a$ (16)), 1.96 (*m*, 1H, C$H_a$ (2)), 1.90–1.81 (*m*, 2H, C$H_a$ (21) + C$H_a$ (22)), 1.78–1.65 (*m*, 3H, C$H_a$ (1) + C$H_a$ (12) + C$H_b$ (2)), 1.69 (*s*, 3H, C$H_3$ (30)), 1.64 (*dd*, 1H, $J$ = 11.3, 11.3 Hz, C*H* (18)), 1.54–1.21 (*m*, 12H, C*H* (9) + C$H_b$ (1) + C$H_b$ (22) + C$H_a$ (7) + C$H_b$ (7) + C$H_b$ (16) + C$H_b$ (21) + C$H_a$ (15) + C$H_a$ (11) + C$H_b$ (11) + C$H_a$ (6) + C$H_b$ (6)), 1.20–1.00 (*m*, 3H, C*H* (5) + C$H_b$ (15) + C$H_b$ (12)), 1.07 (*s*, 3H, C$H_3$ (24)), 1.01 (*s*, 3H, C$H_3$ (27)), 0.93 (*s*, 3H, C$H_3$ (26)), 0.87 (*s*, 3H, C$H_3$ (25)), 0.85 (*s*, 3H, C$H_3$ (23)) ppm; $^{13}C$ NMR (125 MHz, $CD_3OD$): $\delta$ (ppm) = 178.2 (C28, *C*O), 151.8 (C20, *C*=CH$_2$), 110.3 (C29, C=*C*H$_2$), 96.8 (C32, *C*≡CH), 76.7 (C33, C≡*C*H), 73.7 (C3, *C*OH), 57.9 (C17, $C_{quart.}$), 54.9 (C5, *C*H), 52.0 (C31, *C*H$_3$), 51.8 (C18, *C*H), 51.5 (C36 + C37, 2×*C*H$_2$), 50.6 (C9, *C*H), 48.9 (C35 + C38, 2×*C*H$_2$), 48.5 (C19, *C*H), 46.8 (C34, *C*H$_2$), 43.6 (C14, $C_{quart.}$), 43.3 (C39, *C*H$_3$), 42.7 (C4, $C_{quart.}$), 41.9 (C8, $C_{quart.}$), 39.6 (C13, *C*H), 39.1 (C1, *C*H$_2$), 38.4 (C10, $C_{quart.}$), 37.9 (C22, *C*H$_2$), 35.4 (C7, *C*H$_2$), 33.2 (C2, *C*H$_2$), 33.1 (C16, *C*H$_2$), 31.6 (C21, *C*H$_2$), 30.8 (C15, *C*H$_2$), 26.8 (C12, *C*H$_2$), 26.6 (C24, *C*H$_3$), 22.0 (C11, *C*H$_2$), 19.6 (C6, *C*H$_2$), 19.6 (C30, *C*H$_3$), 18.2 (C23, *C*H$_3$), 17.0 (C25, *C*H$_3$), 16.5 (C26, *C*H$_3$), 15.5 (C27, *C*H$_3$); MS (ESI, MeOH): $m/z$ = 607.4 (100% $[M+H]^+$); analysis for $C_{39}H_{65}Cl_2N_2O_3$ (680.85): C, 68.80; H, 9.62; N, 4.11; found: C, 68.55; H, 9.83; N, 3.98.

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

Compound **15** was prepared as described in the general procedure by using **4** (297 mg, 0.6 mmol), dimethylamine solution (7.9 M in $H_2O$, 0.15 mL, 1.2 mmol), formalin (37%, 0.25 mL, 3.1 mmol) and copper iodide (4 mg, 0.02 mmol) in DMSO (6 mL) at 40°C for 20 h to obtain **15** after recrystallization as a colorless solid (120 mg, 34%). Mp 215°C; $[\alpha]_D = -4.3^\circ$ ($c$ = 5.25, MeOH); UV–vis (MeOH): $\lambda_{max}$ (nm) (log $\varepsilon$) = 221 (1.44), 218 (1.28), 215 (0.64), 213 (0.22); IR (KBr): $\nu$ = 3384*s*, 2947*s*, 2869*s*, 2605*m*, 1726*s*, 1641*m*, 1464*s*, 1376*m*, 1189*m*, 1154*s*, 1135*s*, 1038*m*, 1012*m*, 983*m* $cm^{-1}$; $^1H$ NMR (500 MHz, $CD_3OD$): $\delta$ = 4.71 (*m*, 1H, C$H_a$ (29)), 4.59 (*m*, 1H, C$H_b$ (29)), 4.16 (*s*, 2H, C$H_2$ (34)), 3.65 (*s*, 3H, C$H_3$ (31)), 3.02–2.95 (*m*, 7H, C*H* (19) + 2×C$H_3$ (35) + (36)), 2.29–2.21 (*m*, 2H, C*H* (13) + C$H_a$ (16)), 1.97 (*ddd*, 1H, $J$ = 14.3, 13.1, 3.7 Hz, C$H_a$ (2)), 1.90–1.82 (*m*, 2H, C$H_a$ (21) + C$H_a$ (22)), 1.75–1.65 (*m*, 3H, C$H_a$ (1) + C$H_b$ (2) + C$H_a$ (12)), 1.69 (*s*, 3H, C$H_3$ (30)), 1.63 (*dd*, 1H, $J$ = 11.3, 11.3 Hz, C*H* (18)), 1.55–1.25 (*m*, 12H, C*H* (9) + C$H_b$ (1) + C$H_b$ (22) + C$H_a$ (7) + C$H_b$ (7) + C$H_b$ (16) + C$H_b$ (21) + C$H_a$*i*(15) + C$H_a$ (11) + C$H_b$ (11) + C$H_a$ (6) + C$H_b$ (6)), 1.17–1.09 (*m*, 2H, C*H* (5) + C$H_b$ (15)), 1.09–1.03 (*m*, 1H, C$H_b$ (12)), 1.06 (*s*, 3H, C$H_3$ (24)), 0.99 (*s*, 3H, C$H_3$ (27)), 0.94 (*s*, 3H, C$H_3$ (26)), 0.88 (*s*, 3H, C$H_3$ (25)), 0.85 (*s*, 3H, C$H_3$ (23)) ppm; $^{13}C$ NMR (125 MHz, $CD_3OD$): $\delta$ = 178.0 (C28, *C*O), 151.8 (C20, *C*=CH$_2$), 110.3 (C29, C=*C*H$_2$), 95.9 (C32, *C*≡CH), 76.5 (C33, C≡*C*H), 74.4 (C3, *C*OH), 57.9 (C17, $C_{quart.}$), 55.2 (C5, *C*H), 52.2 (C31, *C*H$_3$), 51.8 (C18, *C*H), 50.6 (C9, *C*H), 48.5 (C19, *C*H), 48.0 (C34, *C*H$_2$), 43.5 (C14, $C_{quart.}$), 42.8 (C35 + C36, 2×*C*H$_3$), 42.7 (C4, $C_{quart.}$), 41.9 (C8, $C_{quart.}$), 39.6 (C13, *C*H), 39.2 (C1, *C*H$_2$), 38.4 (C10, $C_{quart.}$), 37.8 (C22, *C*H$_2$), 35.2 (C7, *C*H$_2$), 33.2 (C2, *C*H$_2$), 33.1 (C16, *C*H$_2$), 31.6 (C21, *C*H$_2$), 30.8 (C15, *C*H$_2$), 26.8 (C12, *C*H$_2$), 26.5 (C24, *C*H$_3$), 22.0 (C11, *C*H$_2$), 19.6 (C6, *C*H$_2$), 19.5 (C30, *C*H$_3$), 18.2 (C23, *C*H$_3$), 17.0 (C25, *C*H$_3$), 16.5 (C26, *C*H$_3$), 15.2 (C27, *C*H$_3$) ppm; MS (ESI, MeOH): $m/z$ = 552.4 (100% $[M+H]^+$); analysis for $C_{36}H_{58}ClNO_3$ (588.30): C, 73.50; H, 9.94; N, 2.38; found: C, 73.22; H, 10.12; N, 2.23.

### *Methyl (3β) 3-[3-(diethylamino)prop-1-yn-1-yl]-3-hydroxylup-20(29)-en-28-oate hydrochloride (**16**)*

Compound **16** was prepared as described in the general procedure from **4** (247 mg, 0.5 mmol), diethylamine (0.06 mL, 0.6 mmol), formalin (37%, 0.2 mL, 2.5 mmol) and copper iodide (2 mg, 0.01 mmol) in DMSO (5 mL) (40°C for 2 days), and **16** (80 mg, 13%) was obtained after re-crystallization as a colorless solid. Mp 231°C; $[\alpha]_D = -5.9^\circ$ ($c$ = 4.80, MeOH); IR (KBr): $\nu$ = 3384*s*, 2947*s*, 2869*s*, 2602*m*, 1726*s*, 1635*m*, 1464*s*, 1378*m*, 1189*m*, 1164*m*, 1136*m*, 1037*m*, 1039*w*, 982*w* $cm^{-1}$; $^1H$ NMR (500 MHz, $CD_3OD$): $\delta$ = 4.71 (*m*, 1H, C$H_a$ (29)), 4.59 (*m*, 1H, C$H_b$ (29)), 4.22 (*s*, 2H, C$H_2$ (34)), 3.65 (*s*, 3H, C$H_3$ (31)), 3.30 (*br m*, 4H, 2×C$H_2$ (35) + (37)), 2.99 (*m*, 1H, C*H* (19)), 2.29–2.19 (*m*, 2H, C*H* (13) + C$H_a$ (16)), 1.97 (*m*, 1H, C$H_a$ (2)), 1.90–1.80 (*m*, 2H, C$H_a$

(21) + C$H_a$ (22)), 1.75−1.59 (*m*, 4H, C$H_a$ (1) + C$H_b$ (2) + C$H_a$ (12) + C*H* (18)), 1.68 (*s*, 3H, C$H_3$ (30)), 1.48−1.01 (*m*, 21H, C*H* (9) + C$H_b$ (1) + C$H_b$ (22) + C$H_a$ (7) + C$H_b$ (7) + C$H_b$ (16) + C$H_b$ (21) + C$H_a$ (15) + C$H_a$ (11) + C$H_b$ (11) + C$H_a$ (6) + C$H_b$ (6) + C*H* (5) + C$H_b$ (15) + C$H_b$ (12) + 2×C$H_3$ (36) + (38)), 1.06 (*s*, 3H, C$H_3$ (24)), 0.99 (*s*, 3H, C$H_3$ (27)), 0.94 (*s*, 3H, C$H_3$ (26)), 0.88 (*s*, 3H, C$H_3$ (25)), 0.86 (*s*, 3H, C$H_3$ (23)) ppm; $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta$ = 176.7 (C28, *C*O), 150.3 (C20, *C*=$CH_2$), 108.8 (C29, C=$CH_2$), 94.4 (C32, *C*≡CH), 75.1 (C33, C≡*C*H), 72.2 (C3, *C*OH), 56.4 (C17, $C_{quart.}$), 53.9 (C5, *C*H), 51.0 (C31, $CH_3$), 50.4 (C18, *C*H), 49.2 (C9, *C*H), 48.1 (C36 + C38, 2×$CH_2$), 47.0 (C19, *C*H), 42.1 (C14, $C_{quart.}$), 41.3 (C4, $C_{quart.}$), 40.6 (C34, $CH_2$), 40.5 (C8, $C_{quart.}$), 38.2 (C13, *C*H), 37.9 (C1, $CH_2$), 37.0 (C10, $C_{quart.}$), 36.4 (C22, $CH_2$), 34.1 (C7, $CH_2$), 31.8 (C2, $CH_2$), 31.7 (C16, $CH_2$), 30.2 (C21, $CH_2$), 29.3 (C15, $CH_2$), 25.4 (C12, $CH_2$), 25.1 (C24, $CH_3$), 20.6 (C11, $CH_2$), 18.2 (C6, $CH_2$), 18.1 (C30, $CH_3$), 16.7 (C23, $CH_3$), 15.5 (C25, $CH_3$), 15.1 (C26, $CH_3$), 14.1 (C27, $CH_3$), 8.3 (C36 + C38, 2×$CH_3$) ppm; MS (ESI, MeOH): *m*/*z* = 580.4 (100% $[M+H]^+$); analysis for $C_{38}H_{62}ClNO_3$ (616.36): C, 74.05; H, 10.14; N, 2.27; found: C, 73.87; H, 10.33; N, 2.11.

### *Methyl (3β) 3-[3-(diisopropylamino)prop-1-yn-1-yl]-3-hydroxylup-20(29)-en-28-oate hydrochloride (**17**)*

Compound **17** was prepared as described in the general procedure from **4** (297 mg, 0.6 mmol), diisopropylamine (0.09 mL, 0.64 mmol), formalin (37%, 0.25 mL, 2.5 mmol) and copper iodide (2 mg, 0.01 mmol) in DMSO (6 mL) (40°C for 20 h), and **17** (155 mg, 48%) was obtained as a colorless solid. Mp 215°C; $[\alpha]_D = -7.7^\circ$ (*c* = 6.15, MeOH); UV–vis (MeOH): $\lambda_{max}$ (nm) (log $\varepsilon$) = 220 (1.49); IR (KBr): $\nu$ = 3252*m*, 2946*s*, 2472*m*, 1724*m*, 1642*w*, 1465*m*, 1389*m*, 1318*w*, 1165*m*, 1136*m*, 1038*m*, 1039*w*, 982*w* $cm^{-1}$; $^1$H NMR (500 MHz, $CD_3OD$): $\delta$ = 4.71 (*m*, 1H, C$H_a$ (29)), 4.60 (*m*, 1H, C$H_b$ (29)), 4.21 (*s*, 2H, C$H_2$ (34)), 3.90 (*sept.* 2H, *J* = 6.7 Hz, 2×C*H* (35) + (38)), 3.65 (*s*, 3H, C$H_3$ (31)), 2.99 (*ddd*, 1H, *J* = 10.6, 10.6, 5.1 Hz, C*H* (19)), 2.29−2.21 (*m*, 2H, C*H* (13) + C$H_a$ (16)), 1.96 (*m*, 1H, C$H_a$ (2)), 1.90−1.82 (*m*, 2H, C$H_a$ (21) + C$H_a$ (22)), 1.76−1.65 (*m*, 3H, C$H_a$ (1) + C$H_a$ (12) + C$H_b$ (2)), 1.69 (*s*, 3H, C$H_3$ (30)), 1.64 (*dd*, 1H, *J* = 11.4, 11.4 Hz, C*H* (18)), 1.54−1.21 (*m*, 12H, C*H* (9) + C$H_b$ (1) + C$H_b$ (22) + C$H_a$ (7) + C$H_b$ (7) + C$H_b$ (16) + C$H_b$ (21) + C$H_a$ (15) + C$H_a$ (11) + C$H_b$ (11) + C$H_a$ (6) + C$H_b$ (6)), 1.47 (*d*, 12H, *J* = 6.7 Hz, 4×C$H_3$ (36) + (37) + (39) + (40)), 1.20−1.14 (*m*, 2H, C*H* (5) + C$H_b$ (15)), 1.10−1.01 (*m*, 1H, C$H_b$ (12)), 1.05 (*s*, 3H, C$H_3$ (24)), 0.99 (*s*, 3H, C$H_3$ (27)), 0.94 (*s*, 3H, C$H_3$ (26)), 0.88 (*s*, 3H, C$H_3$ (25)), 0.85 (*s*, 3H, C$H_3$ (23)) ppm; $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta$ = 178.1 (C28, *C*O), 151.8 (C20, *C*=$CH_2$), 110.3 (C29, C=*C*H$_2$), 94.9 (C32, *C*≡CH), 76.6 (C33, C≡*C*H), 74.4 (C3, *C*OH), 57.9 (C17, $C_{quart.}$), 55.8 (C35 + C38, 2×*C*H), 55.3 (C5, *C*H), 52.3 (C31, $CH_3$), 51.8 (C18, *C*H), 50.7 (C9, *C*H), 48.4 (C19, *C*H), 43.6 (C14, $C_{quart.}$), 42.9 (C4, $C_{quart.}$), 41.9 (C8, $C_{quart.}$), 39.6 (C13, *C*H), 39.2 (C1, $CH_2$), 38.4 (C10, $C_{quart.}$), 37.8 (C22, $CH_2$), 37.1 (C34, $CH_2$), 35.6 (C7, $CH_2$), 33.2 (C2, $CH_2$), 33.1 (C16, $CH_2$), 31.6 (C21, $CH_2$), 30.8 (C15, $CH_2$), 26.9 (C12, $CH_2$), 26.6 (C24, $CH_3$), 22.0 (C11, $CH_2$), 19.6 (C6, $CH_2$), 19.6 (C30, $CH_3$), 18.2 (C23, $CH_3$), 18.2 (C36 + C37 + C39 + C40, 4×$CH_3$), 17.0 (C25, $CH_3$), 16.5 (C26, $CH_3$), 15.4 (C27, $CH_3$) ppm; MS (ESI, MeOH): *m*/*z* = 608.5 (100% $[M+H]^+$); analysis for $C_{40}H_{66}ClNO_3$ (644.41): C, 74.55; H, 10.32; N, 2.17; found: C, 74.36; H, 10.56; N, 2.11.

### *Methyl (3β) 3-[3-(dihexylamino)prop-1-yn-1-yl]-3-hydroxylup-20(29)-en-28-oate hydrochloride (**18**)*

A mixture of **4** (248 mg, 0.5 mmol), dihexylamine (0.14 mL, 0.6 mmol), formalin (37%, 0.2 mL, 2.5 mmol), copper iodide (2 mg, 0.01 mmol) and DMSO (4 mL) was stirred at 40°C for 20 h. After the reaction was completed, 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 residue was subjected to chromatography (silica gel, *n*-hexane/ethyl acetate, 3:1) to afford a pale yellow oil which was dissolved in diethylether (10 mL). At 0°C gaseous hydrogen chloride was passed through until the precipitation of salts had ceased. Crystallization was completed by standing at 4°C for 12 h. The product was filtered off and dried. Compound **17** (54 mg, 15%) was obtained as a colorless solid. Mp 209–211°C; $[\alpha]_D = -6.7^\circ$ (*c* = 3.60, MeOH); UV–vis (MeOH): $\lambda_{max}$ (nm) (log $\varepsilon$) = 217 (0.56), 212 (0.44); IR (KBr): $\nu$ = 3313*m*, 2937*s*, 2869*s*, 2528*m*, 1725*s*, 1640*w*, 1456*m*, 1377*m*, 1317*m*, 1189*m*, 1155*m*, 1134*m*, 1039*m*, 982*w*, 882*m* $cm^{-1}$; $^1$H NMR (500 MHz, $CD_3OD$): $\delta$ = 4.71 (*m*, 1H, C$H_a$ (29)), 4.59 (*m*, 1H, C$H_b$ (29)), 4.21 (*s*, 2H, C$H_2$ (34)), 3.65 (*s*, 3H, C$H_3$ (31)), 3.23 (*m*, 4H, 2×C$H_a$ (35) + (41) + 2×C$H_b$ (35) + (41)), 2.99 (*ddd*, 1H, *J* = 10.8, 10.8, 4.7 Hz, C*H* (19)), 2.30−2.21 (*m*, 2H, C*H* (13) + C$H_a$ (16)), 1.98 (*ddd*, 1H, *J* = 14.3, 13.3, 3.9 Hz, C$H_a$ (2)), 1.90−1.83 (*m*, 2H, C$H_a$ (21) + C$H_a$ (22)), 1.80−1.67 (*m*, 7H, + C$H_a$ (1) + C$H_a$ (12) + C$H_b$ (2) + 2×C$H_2$ (36) + (42)), 1.69 (*s*, 3H, C$H_3$ (30)), 1.63 (*dd*, 1H, *J* = 11.5, 11.5 Hz, C*H* (18)), 1.55−1.02 (*m*, 27H, C*H* (9) + C$H_b$ (1) + C$H_b$ (22) + C$H_a$ (7) + C$H_b$ (7) + C$H_b$ (16) + C$H_b$ (21) + C$H_a$ (15) + C$H_a$ (11) + C$H_b$ (11) + C$H_a$ (6) + C$H_b$ (6), + 2×C$H_2$ (37) + (43) + 2×C$H_2$ (38) + (44) + 2×C$H_2$ (39) + (45) + C*H* (5) + C$H_b$ (15) + C$H_b$ (12)), 1.05 (*s*, 3H, C$H_3$ (24)), 1.00 (*s*, 3H, C$H_3$ (27)), 0.95 (*s*, 3H, C$H_3$ (26)), 0.93 (*t*, 6H, *J* = 7.2 Hz, 2×C$H_3$ (40) + (46)), 0.89 (*s*, 3H, C$H_3$ (25)), 0.86 (*s*, 3H, C$H_3$ (23)) ppm; $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta$ = 178.0 (C28, *C*O), 151.7 (C20, *C*=$CH_2$), 110.4 (C29, C=$CH_2$), 95.9 (C32, *C*≡CH), 76.5 (C33, C≡*C*H), 73.6 (C3, *C*OH), 57.9 (C17, $C_{quart.}$), 55.4 (C5, *C*H), 55.0 (C35 + C41, 2×$CH_2$), 52.4 (C31, $CH_3$), 51.8 (C18, *C*H), 50.7 (C9, *C*H), 48.5 (C19, *C*H), 43.6 (C14, $C_{quart.}$), 42.8 (C34, $CH_2$), 42.8 (C4, $C_{quart.}$), 41.9 (C8, $C_{quart.}$), 39.6 (C13, *C*H), 39.4 (C1, $CH_2$), 38.5 (C10, $C_{quart.}$), 37.9 (C22, $CH_2$), 35.6 (C7, $CH_2$), 33.3 (C2, $CH_2$), 33.1 (C16, $CH_2$), 32.4 (C38 + C44, 2×$CH_2$), 31.6 (C21, $CH_2$), 30.8 (C15, $CH_2$), 27.5 (C36 + C42, 2×$CH_2$), 26.9 (C12, $CH_2$), 26.6 (C24, $CH_3$), 25.3 (C37 + C43, 2×$CH_2$), 23.5 (C38 + C44, 2×$CH_2$), 22.0 (C11, $CH_2$), 19.6 (C6, $CH_2$), 19.6 (C30, $CH_3$), 18.1 (C23, $CH_3$), 17.0 (C25, $CH_3$), 16.5 (C26, $CH_3$), 15.6 (C27, $CH_3$), 14.3 (C40 + C46, 2×$CH_3$) ppm; MS (ESI, MeOH): *m*/*z* = 692.5 (100% $[M+H]^+$); analysis for $C_{46}H_{78}ClNO_3$ (728.57): C, 75.83; H, 10.79; N, 1.92; found: C, 75.67; H, 10.88; N, 1.78.

### *Methyl (3β) 3-[3-(dicyclohexylamino)prop-1-yn-1-yl]-3-hydroxylup-20(29)-en-28-oate hydrochloride (**19**)*

Compound **19** was prepared as described in the general procedure from **4** (247 mg, 0.5 mmol), dicyclohexylamine (0.13 mL, 0.79 mmol), formalin (37%, 0.2 mL, 2.5 mmol) and copper iodide (4 mg, 0.02 mmol) in DMSO (5 mL) (40°C for 3 days), and **19** (153 mg, 42%) was obtained after re-crystallization as a colorless solid. Mp 220°C; $[\alpha]_D = +0.8^\circ$ (*c* = 5.10, MeOH); UV–vis (MeOH): $\lambda_{max}$ (nm) (log $\varepsilon$) = 218 (0.59); IR (KBr): $\nu$ = 3423*m*, 2938*m*, 2862*m*, 2423*m*, 1723*m*, 1639*w*, 1458*m*, 1376*w*, 1136*w*, 1037*w*, 1012*m*, 880*w* $cm^{-1}$; $^1$H NMR (500 MHz, $CD_3OD$): $\delta$ = 4.71 (*m*, 1H, C$H_a$ (29)), 4.60 (*m*, 1H, C$H_b$ (29)), 4.26 (*s*, 2H, C$H_2$ (34)), 3.66 (*s*, 3H, C$H_3$ (31)), 3.59 (*m*, 2H, 2×C*H* (35) + (41)), 2.99 (*ddd*, 1H, *J* = 10.8, 10.8, 4.9 Hz, C*H* (19)), 2.29−2.21 (*m*, 2H, C*H* (13) + C$H_a$ (16)), 2.20−2.04 (*m*, 4H, 4×C$H_a$ (36) + (40) + (42) + (46)), 2.01−1.93 (*m*, 5H, 4×C$H_a$

(37) + (39) + (43) + (45) + C$H_a$ (2)), 1.91−.83 (*m*, 2H, C$H_a$ (21) + C$H_a$ (22)), 1.77−1.62 (*m*, 6H, C$H_a$ (1) + C$H_a$ (12) + C$H_b$ (2) + C*H* (18) + 2×C$H_a$ (38) + (44)), 1.69 (*s*, 3H, C$H_3$ (30)), 1.55−1.03 (*m*, 25H 4×C$H_b$ (36) + (40) + (42) + (46) + 4×C$H_b$ (34) + (39) + (43) + (45) + 2×C$H_b$ (38) + (44) + C*H* (9) + C$H_b$ (1) + C$H_b$ (22) + C$H_a$ (7) + C$H_b$ (7) + C$H_b$ (16) + C$H_b$ (21) + C$H_a$ (15) + C$H_a$ (11) + C$H_b$ (11) + C$H_a$ (6) + C$H_b$ (6) + C*H* (5) + C$H_b$ (15) + C$H_b$ (12)), 1.06 (*s*, 3H, C$H_3$ (24)), 1.01 (*s*, 3H, C$H_3$ (27)), 0.95 (*s*, 3H, C$H_3$ (26)), 0.89 (*s*, 3H, C$H_3$ (25)), 0.86 (*s*, 3H, C$H_3$ (23)) ppm; $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta = 177.8$ (C28, *C*O), 151.5 (C20, *C*=$CH_2$), 109.9 (C29, C=*C*$H_2$), 95.2 (C32, *C*≡CH), 76.5 (C33, C≡*C*H), 75.6 (C3, *C*OH), 62.6 (C35 + C41, 2×*C*H), 57.6 (C17, $C_{quart.}$), 55.2 (C5, *C*H), 52.3 (C31, *C*$H_3$), 52.0 (C18, *C*H), 50.5 (C9, *C*H), 48.2 (C19, *C*H), 43.4 (C14, $C_{quart.}$), 42.7 (C4, $C_{quart.}$), 41.8 (C8, $C_{quart.}$), 39.5 (C13, *C*H), 39.2 (C1, *C*$H_2$), 38.3 (C34, *C*$H_2$), 37.6 (C10, $C_{quart.}$), 37.6 (C22, *C*$H_2$), 35.5 (C7, *C*$H_2$), 33.1 (C2, *C*$H_2$), 32.9 (C16, *C*$H_2$), 31.5 (C21, *C*$H_2$), 30.7 (C15, *C*$H_2$), 26.8 (C12, *C*$H_2$), 26.4 (C24, *C*$H_3$), 26.2 (C36 + C40 + C42 + C46, 4×*C*$H_2$), 25.8 (C37 + C39 + C43 + C45, 4×*C*$H_2$), 25.3 (C38 + C44, 2×*C*$H_2$), 21.9 (C11, *C*$H_2$), 19.5 (C6, *C*$H_2$), 19.5 (C30, *C*$H_3$), 18.0 (C23, *C*$H_3$), 16.8 (C25, *C*$H_3$), 16.3 (C26, *C*$H_3$), 15.6 (C27, *C*$H_3$) ppm; MS (ESI, MeOH): $m/z = 688.6$ (100% $[M+H]^+$); analysis for $C_{46}H_{74}ClNO_3$ (724.54): C, 76.25; H, 10.29; N, 1.93; found: C, 76.11; H, 10.45; N, 1.74.

### *Methyl (3β) 3-[3-(4-methylmorpholin-4-ium-4-yl)prop-1-yn-1-yl]lup20(29)-en-28 oate iodide (**20**)*

Compound **20** was obtained by treating a methanolic solution of **12** (200 mg, 0.32 mmol) in methanol with KOH (satd. aq.), followed by extraction with ethyl acetate (3 × 100 mL). The organic layers were dried over $Na_2SO_4$, the solvent was evaporated, and the remaining solid was treated under argon with diethylether and methyl iodide (2 mL, 32.0 mmol) for 7 days. The product was filtered off and washed with ether to yield **20** (210 mg, 89%) as an off-white solid. Mp 180°C, $[\alpha]_D = -2.5°$ ($c = 5.10$, MeOH), IR (KBr): $\nu$ = 3385*s*, 2947*s*, 2870*s*, 2361*w*, 1727*s*, 1640*m*, 1450*s*, 1377*s*, 1318*m*, 1189*s*, 1154*s*, 1136*s*, 1066*m*, 1037*s*, 983*m*, 948*m*, 896*m* $cm^{-1}$; $^1$H NMR (500 MHz, $CD_3OD$): $\delta = 4.71$ (*m*, 1H, C$H_a$ (29)), 4.61 (*d*, 2H, $J_{N,H} = 2.7$ Hz, C$H_2$ (34)), 4.59 (*m*, 1H, C$H_b$ (29)), 4.09−4.01 (*m*, 4H C$H_a$ (36) + C$H_a$ (37) + C$H_b$ (36) + C$H_b$ (37)), 3.68−3.63 (*m*, 2H C$H_a$ (35) + C$H_a$ (38)), 3.65 (*s*, 3H, C$H_3$ (31)), 3.58−3.52 (*m*, 2H C$H_b$ (35) + C$H_b$ (38)), 3.35 (*s*, 3H, C$H_3$ (39)), 2.99 (*ddd*, 1H, $J$ = 10.8, 10.8, 5.2 Hz, C*H* (19)), 2.29−2.21 (*m*, 2H, C*H* (13) + C$H_a$ (16)), 1.98 (*ddd*, 1H, $J$ = 14.3, 13.2, 3.8 Hz, C$H_a$ (2)), 1.90−1.83 (*m*, 2H, C$H_a$ (21) + C$H_a$ (22)), 1.75−1.67 (*m*, 3H, C$H_a$ (1) + C$H_a$ (12) + C$H_b$ (2)), 1.69 (*s*, 3H, C$H_3$ (30)), 1.64 (*dd*, 1H, $J$ = 11.4, 11.4 Hz, C*H* (18)), 1.55−1.22 (*m*, 12H, C*H* (9) + C$H_b$ (1) + C$H_b$ (22) + C$H_a$ (7) + C$H_b$ (7) + C$H_b$ (16) + C$H_b$ (21) + C$H_a$ (15) + C$H_a$ (11) + C$H_b$ (11) + C$H_a$ (6) + C$H_b$ (6)), 1.20−1.14 (*m*, 2H, C*H* (5) + C$H_b$ (15)), 1.12−1.02 (*m*, 1H, C$H_b$ (12)), 1.07 (*s*, 3H, C$H_3$ (24)), 0.99 (*s*, 3H, C$H_3$ (27)), 0.94 (*s*, 3H, C$H_3$ (26)), 0.88 (*s*, 3H, C$H_3$ (25)), 0.87 (*s*, 3H, C$H_3$ (23)) ppm; $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta = 177.9$ (C28, *C*O), 151.6 (C20, *C*=$CH_2$), 110.1 (C29, C=*C*$H_2$), 98.2 (C32, *C*≡CH), 76.5 (C33, C≡*C*H), 72.8 (C3, *C*OH), 61.5 (C36 + C37, 2×*C*$H_2$), 60.2 (C35 + C38, 2×*C*$H_2$), 57.7 (C17, $C_{quart.}$), 56.1 (C34, *C*$H_2$), 55.3 (C5, *C*H), 52.2 (C18, *C*H), 51.7 (C31, *C*$H_3$), 50.5 (C9, *C*H), 48.8 (C19, *C*H), 48.3 (C39, *C*$H_3$), 43.4 (C14, *C* $C_{quart.}$), 42.6 (C4, $C_{quart.}$), 41.7 (C8, $C_{quart.}$), 39.4 (C13, *C*H), 39.2 (C1, *C*$H_2$), 38.2 (C10, $C_{quart.}$), 37.7 (C22, *C*$H_2$), 35.4 (C7, *C*$H_2$), 33.0 (C2, *C*$H_2$), 32.9 (C16, *C*$H_2$), 31.5 (C21, *C*$H_2$), 30.6 (C15, *C*$H_2$), 26.6 (C12, *C*$H_2$), 26.5 (C24, *C*$H_3$), 21.9 (C11, *C*$H_2$), 19.5 (C6, *C*$H_2$), 19.4 (C30, *C*$H_3$), 17.9 (C23, *C*$H_3$), 16.8 (C25, *C*$H_3$), 16.4 (C26, *C*$H_3$), 15.2 (C27, *C*$H_3$) ppm; MS (ESI, MeOH): 608.5 (100% $[M-Cl]^+$); analysis for $C_{39}H_{62}INO_4$ (735.82): C, 63.66, H, 8.49; N, 1.90, I, 17.25; found: C 63.60, H 8.62, N 2.07.

### *Methyl 3-[(3β) 3-hydroxy-28-methoxy-28-oxolup-20(29)en-3-yl]-N,N,N-trimethylprop-2-yn-1-aminium iodide (**21**)*

Following the procedure given for **20**, compound **21** (180 mg, 82%) from **15** (174 mg, 0.32 mmol) and methyl iodide (2 mL, 32.0 mmol) as an off-white solid. Mp 216°C, $[\alpha]_D = +1.1°$ ($c = 5.80$, MeOH), IR (KBr): $\nu$ = 3385*m*, 2947*s*, 2869*m*, 1726*s*, 1642*m*, 1458*m*, 1377*m*, 1318*m*, 1189*m*, 1154*m*, 1137*m*, 1075*w*, 1038*m*, 1009*m*, 984*m* $cm^{-1}$; $^1$H NMR (500 MHz, $CD_3OD$): $\delta = 4.71$ (*m*, 1H, C$H_a$ (29)), 4.59 (*m*, 1H, C$H_b$ (29)), 4.46 (*d*, 2H, $J_{N,H} = 2.3$ Hz, C$H_2$ (34)), 3.66 (*s*, 3H, C$H_3$ (31)), 3.26 (*s*, 9H, 3×C$H_3$ (35) + (36) + (37)), 2.99 (*ddd*, 1H, $J = 10.8$, 10.8, 5.0 Hz, C*H* (19)), 2.28−2.21 (*m*, 2H, C*H* (13) + C$H_a$ (16)), 1.99 (*ddd*, 1H, $J = 14.3$, 13.2, 3.8 Hz, C$H_a$ (2)), 1.90−1.83 (*m*, 2H, C$H_a$ (21) + C$H_a$ (22)), 1.75−1.68 (*m*, 3H, C$H_a$ (1) + C$H_b$ (2) + C$H_a$ (12)), 1.69 (*s*, 3H, C$H_3$ (30)), 1.63 (*dd*, 1H, $J = 11.4$, 11.4 Hz, C*H* (18)), 1.55−1.23 (*m*, 12H, C*H* (9) + C$H_b$ (1) + C$H_b$ (22) + C$H_a$ (7) + C$H_b$ (7) + C$H_b$ (16) + C$H_b$ (21) + C$H_a$ (15) + C$H_a$ (11) + C$H_b$ (11) + C$H_a$ (6) + C$H_b$ (6)), 1.18−1.04 (*m*, 3H, C*H* (5) + C$H_b$ (15) + C$H_b$ (12)), 1.08 (*s*, 3H, C$H_3$ (24)), 0.98 (*s*, 3H, C$H_3$ (27)), 0.94 (*s*, 3H, C$H_3$ (26)), 0.89 (*s*, 3H, C$H_3$ (25)), 0.87 (*s*, 3H, C$H_3$ (23)) ppm; $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta = 178.1$ (C28, *C*O), 151.7 (C20, *C*=$CH_2$), 110.3 (C29, C=*C*$H_2$), 97.6 (C32, *C*≡CH), 76.6 (C33, C≡*C*H), 74.0 (C3, *C*OH), 57.9 (C17, $C_{quart.}$), 57.7 (C34, *C*$H_2$), 55.4 (C5, *C*H), 53.4 (C35 + C36 + C37, 3×*C*$H_3$), 52.4 (C18, *C*H), 51.8 (C31, *C*$H_3$), 50.6 (C9, *C*H), 48.5 (C19, *C*H), 43.5 (C14, $C_{quart.}$), 42.8 (C4, $C_{quart.}$), 41.9 (C8, $C_{quart.}$), 39.6 (C13, *C*H), 39.3 (C1, *C*$H_2$), 38.4 (C10, $C_{quart.}$), 37.8 (C22, *C*$H_2$), 35.6 (C7, *C*$H_2$), 33.2 (C2, *C*$H_2$), 33.1 (C16, *C*$H_2$), 31.6 (C21, *C*$H_2$), 30.7 (C15, *C*$H_2$), 26.8 (C12, *C*$H_2$), 26.6 (C24, *C*$H_3$), 22.0 (C11, *C*$H_2$), 19.6 (C6, *C*$H_2$), 19.6 (C30, *C*$H_3$), 18.1 (C23, *C*$H_3$), 17.0 (C25, *C*$H_3$), 16.5 (C26, *C*$H_3$), 15.3 (C27, *C*$H_3$) ppm; MS (ESI, MeOH): 566.5 (100% $[M-Cl]^+$); analysis for $C_{37}H_{60}INO_3$ (693.78): C, 64.05; H, 8.72; N, 2.02; found: C 64.00, H 8.89, N 1.85.

## Cell lines and culture conditions

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% $CO_2$/95% air.

## Biological testing

The cytotoxicity assay (SRB), the AO/PI test, the Trypan blue counting, the DNA laddering and the cell cycle analysis experiments were performed as previously described [56, 70, 71].

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