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Synthesis and antitumor activity of ring A modified 11-keto-β-boswellic acid derivatives

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ABSTRACT

Beta-boswellic acids are interesting triterpenoic acids that show different biological activities. Their cytotoxic potential, as well as that of their derivates remained unexploited so far. In this study we were able to prepare derivatives of 11-keto-β-boswellic acid that showed lower IC_{50} values as determined by a sulphorhodamine B (SRB) assay using several different human tumour cell lines. Thus, the introduction of an amino group at position C-2 led to a significantly improved cytotoxic activity of amine **18**. An apoptotic effect of compound **18** was determined using DNA laddering and trypan blue staining experiments.

1. Introduction

Cancer is one of the leading causes of death worldwide. This disease can affect any part of the body finally, and there have been 8.2 million cancer associated deaths in 2012 [1]. Cancer seems to be as old [2] as the existence of multi-cell organisms, and the fight against it has lasted for approximately more than two millennia and still continues. Up to now, there has been no panaceum to combat against this deadly disease. However, great strides and progress have been made regarding the therapy of some malignancies that have contributed to positive results, and that have led to increased chances of recovery. Chemotherapy plays an important role in the treatment of cancer (but also after surgery or radiotherapy), and an ideal chemotherapeutic anticancer agent inhibits, delays or even reverts the progress of cancer [3]. However, the complexity of cancer indicates that drugs that affect the malignant cells at various levels have to be developed and to be used in the therapy of cancer. Plant derived triterpenoids represent typical examples of drugs providing antitumor activity through effects on various cellular and metabolic networks [4–8].

A broad variety of different terpenes [9–12] including many triterpenes has been identified from the resins of frankincense [11,12]. Frankincense has been used for approximately 4000 years [13] but the investigation of its active ingredients has started only in 1788 [14–17]. For β-boswellic acids (BA, triterpenoids of the α-amyrin type, i.e. ursane type [17]) many interesting biological properties have been reported [11,12,18–25]. Fig. 1 depicts the most abundant β-BA derivatives, i.e. β-BA (**1**) and its 3-*O*-acetyl derivative β-ABA (**2**), 11-keto-β-boswellic acid (β-KBA, **3**) and its 3-*O*-acetate (β-AKBA, **4**). Recently, we were able to synthesize [26] a highly cytotoxic and apoptotic 1α, 9α-endoperoxide **5** from **3**; this compound showed IC_{50} values (in sulforhodamine B assays employing 15 different human tumor cell lines) as low as 0.4 μM.

Boswellic acids have been isolated from several species of plants of the genus *Boswellia*, trees and shrubs of the Burseraceae family, that are native to the Arabian peninsula, North Africa and parts of India. Thus, *Boswellia papyrifera* Hochst., *Boswellia serrata* Roxb., *Boswellia sacra* Flück and *Boswellia carterii* Birdw. contain β-BA in the highest quantities [11,25,27], while derivatives of BA as well as minor amounts of BAs have also been found in olives, myrrh [28,29], and trace amounts were claimed [30] to be present in untreated old hops. Usually, the resin fraction of frankincense (up to 60% of the incense) contains about 50% of triterpenoic acids;

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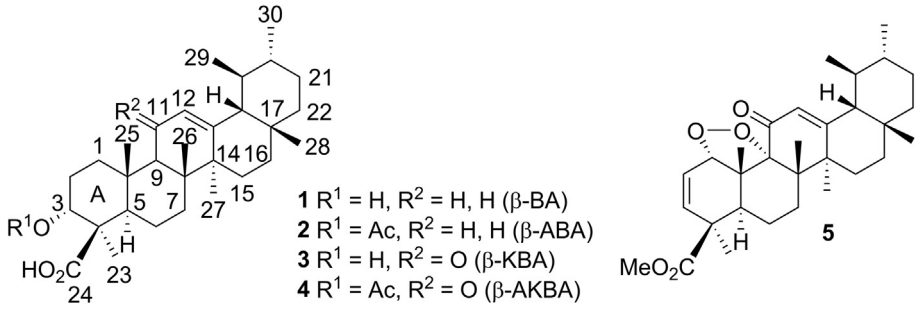


Fig. 1. Structure (and numbering) of main active ingredients of frankincense **1**–**4** and of highly cytotoxic endoperoxide **5**.

boswellic acids are among the major bioactive compounds of the gum resin of *Boswellia* spp., but the amount of boswellic acids and the ratio **1**:**2**:**3**:**4** strongly depends on the species of *Boswellia*, and also to environmental fluctuation [11]. The concentration of the most active boswellic acid, AKBA (**4**) in extracts from *Boswellia* resins was between 0.1 and 3 %, but using the "focussing" approach as described by J. Jauch [28,31] allows the convenient isolation of **4** on a large preparative scale thus avoiding tedious workup and low yields of older preparations [32–35].

Recently, boswellic acids have gained much interest due to their anticancer activity and their ability to induce apoptosis. Although there are several studies dealing with the cytotoxic potential of boswellic acids, the number of reports concerning boswellic acid derivatives remained small – probably because of a limited access to the parent compound. Since the use of boswellic acid to treat cancer has been regarded as an "emerging concept in oncology" [8] we decided to extend our search for cytotoxic triterpenoic acid derivatives, and to investigate boswellic acid derivatives in more detail.

2. Results and discussion

2.1. Chemistry

Frankincense was bought from different local commercial suppliers, and **4** was isolated following Jauch's procedure. Deacetylation of **4** with NaOH in ethanol for 12 h at room temperature (Scheme 1) gave an almost quantitative yield of **6** whose esterification (MeI, Cs_2CO_3, DMF) [36,37] gave the methyl ester **7** in 95% isolated yield.

Reaction of **6** with benzyl bromide/caesium carbonate furnished the benzylester **8**. Interestingly, reduction of **6** with $LiAlH_4$ gave alcohol **9**. In the ^{1}H NMR spectrum of **9** protons H-11 and H-12 were detected as two dubletts centered at δ = 5.61 and 5.43 ppm respectively showing a coupling constant ^3J = 5.8 Hz. In the ^{13}C NMR spectrum C-11 and C-12 were found at δ = 115.5 and 122.9 ppm, respectively. In the IR spectrum the signal of the C=C double bonds were located at ν = 1636 cm^{-1}. While the Jones oxidation [38,39] of **7** furnished only 65% of the methyl 3,11-diketoboswelloate **10**, an almost quantitative yield of **10** was obtained from **7** using Swern conditions [40]. Good yields were obtained for the oxidation of **8** to **11** (91% isolated yield), and the oxidation of **6** to **12** (84% isolated yield).

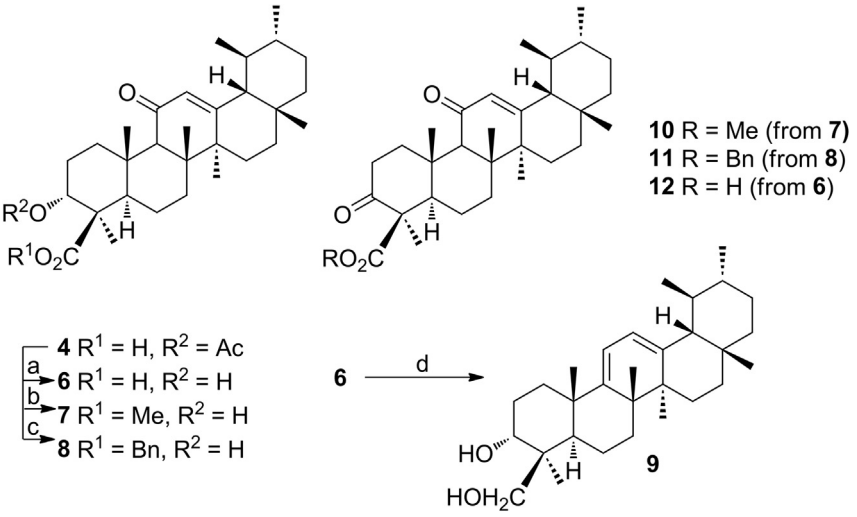
Interestingly enough, from the Jones oxidation of **6** decarboxylated **13** was obtained. This compound was also accessible from **10** by a *syn*-elimination when **10** was heated in an ethanolic solution of sodium hydroxide for 4 h. Thus, **13** results from a decarboxylation reaction proceeding *via* a cyclic transition state. Treatment of **13** with a three-fold molar excess *m*-CPBA furnished lactone **14** in 85% yield.

Oximes **15** and **16** were obtained from the reaction of **10** and **11** respectively with hydroxylamine (Scheme 2) [41]. Reduction of the oxime **15** with $NaBH_3CN$/ammonium acetate/aq. $TiCl_3$ according to Hattori et al. [42] gave the amine **17**. Similar to the known reduction of a 3-keto boswelloate with $NaBH_4$, the reduction of **15** proceeded in a stereoselective manner due to the presence of an axially oriented benzyl carboxylate, resulting only in the formation of product **17** possessing an equatorially oriented amino group [39]. The cleavage of the benzyl ester in **17** failed under a broad variety of conditions, whereas the reduction of **17** with Pd/C/ammonium formate [43–45] in methanol proceeded smoothly to afford **18** in 82% isolated yield.

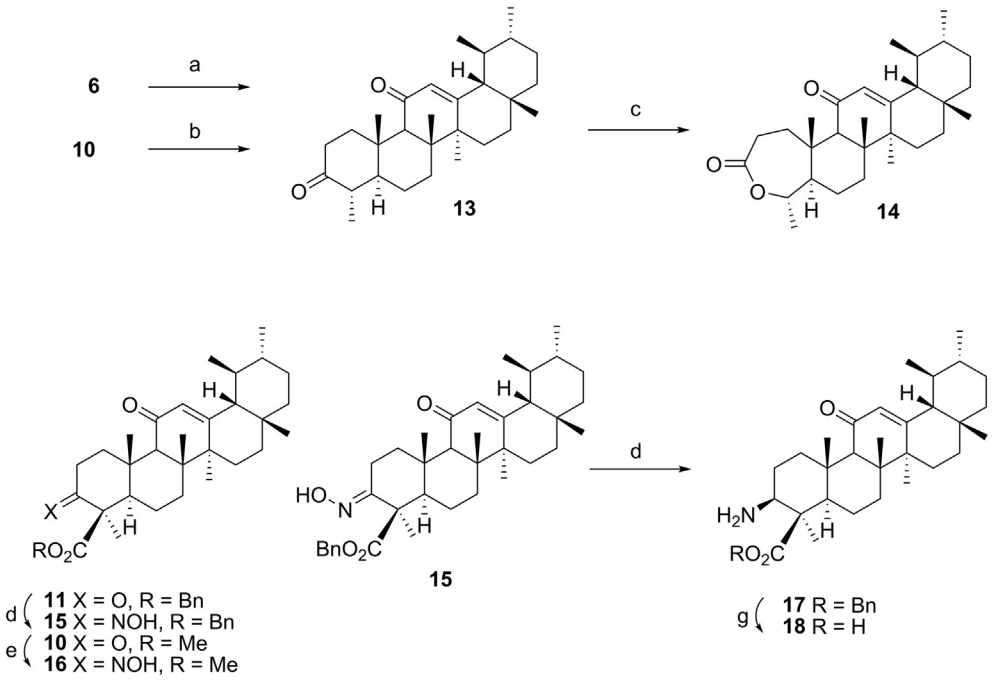
Treatment of **10** (Scheme 3) with bromine in acetic acid [46] proceeded in a rather stereoselective manner and only brominated BA derivative **19** possessing an equatorially-oriented bromine substituent (as confirmed from the ^{1}H NMR spectra) could be isolated from the reaction mixture. Reaction of **19** with potassium carbonate in aqueous acetone gave 32% of a ring contracted *abeo* derivative **20**. The formation of **20** can be explained by a series of transformations taking place, starting with a substitution of the bromine substituent for a hydroxyl group. This intermediate was air-oxidized leading to the formation of a 1,2-dicarbonyl compound. In the presence of an excess of hydroxide ions this intermediate underwent a benzillic acid rearrangement reaction [47–49] to eventually end up with the ring-contracted compound **20**. The absolute configuration at C-3, however, could not be deduced from its NMR spectra.

Reaction of the bromide **19** with sodium azide gave amine **21**, thus paralleling previous findings for the transformation of betulin [50] and maslinic acid derivatives [51]. Acetylation of **21** furnished **22** in moderate yield.

Upon treatment of **6** (Scheme 4) with 3,3-dimethylglutarimide in the presence of DEAD/TPP [52] an elimination reaction occurred to furnish **23** in excellent yield in addition to lactone **24** as aminor product. Interestingly enough, under the same conditions from **6** the lactone **24** was obtained [53–55]. Reaction of **10** with LDA/chlorotrimethylsilane [56–58] followed by a reaction with *m*CPBA furnished a mixture of silylated derivative **25** and alcohol **26**; The later of which was also obtained from **25** by desilylation. As revealed from the ^{1}H NMR spectra, the hydroxyl substituent in **25** is in an axial position. Swern oxidation of **26** furnished enol **27** in 90% isolated yield. In the ^{1}H NMR spectra of **27** H-1 was detected as a singlet at δ = 7.07 ppm; in the ^{13}C NMR spectra C-1 and C-2 were found at δ = 129.9 and 143.5 ppm, respectively. The reaction of **10** with trimethylsulfoxonium iodide [59–62] gave 82% of the spiro-anellated oxirane **28** whose semi-pinacol rearrangement [63–65] furnished aldehyde **29**. Air-oxidation of **29** gave a quantitative yield of acid **30**.



Scheme 1. Synthesis of derivatives **4**–**12**: a) EtOH, NaOH, 25 °C, 12 h, 98%; b) Cs_2CO_3, MeI, 25 °C, 12 h, 95%; c) Cs_2CO_3, BnBr, 25 °C, 12 h, 84.8%; d) $LiAlH_4$, THF, 25 °C, 23 h, 16%; synthesis of **10**–**12**: oxalylic chloride/DMSO/NEt_3, DCM, −78 °C, 15 min: **10** (quant.), **11** (91%), **12** (84%).

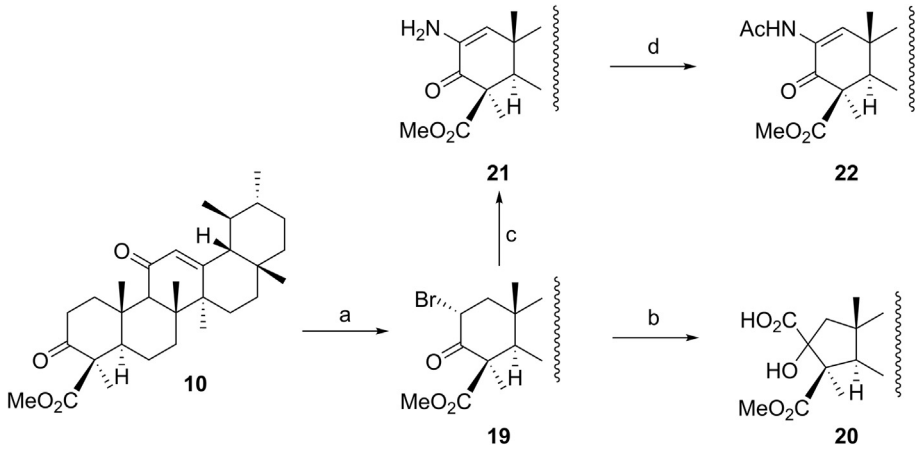


Scheme 2. Synthesis of derivatives **13**–**18**: a) $Na_2Cr_2O_7$. $2H_2O$, conc. H_2SO_4, H_2O, 25 °C, 3 days, 31%; b) EtOH, NaOH, reflux, 4 h, 35%; c) *m*-CPBA, $CHCl_3$, reflux, 12 h, 93%; d) $NH_2OH.HCl$, pyridine, 50 °C, 3 h, 90%; e) $NH_2OH.HCl$, pyridine, 50 °C, 2 h, quant.; f) $NaBH_3CN$, $NH_4^+Ac^-$, $TiCl_3$ (aq.), 25 °C, 12 h, 76.9%; g) Pd/C, NH_4^+ HCO_2^-, MeOH, reflux, 5 h, 95%.

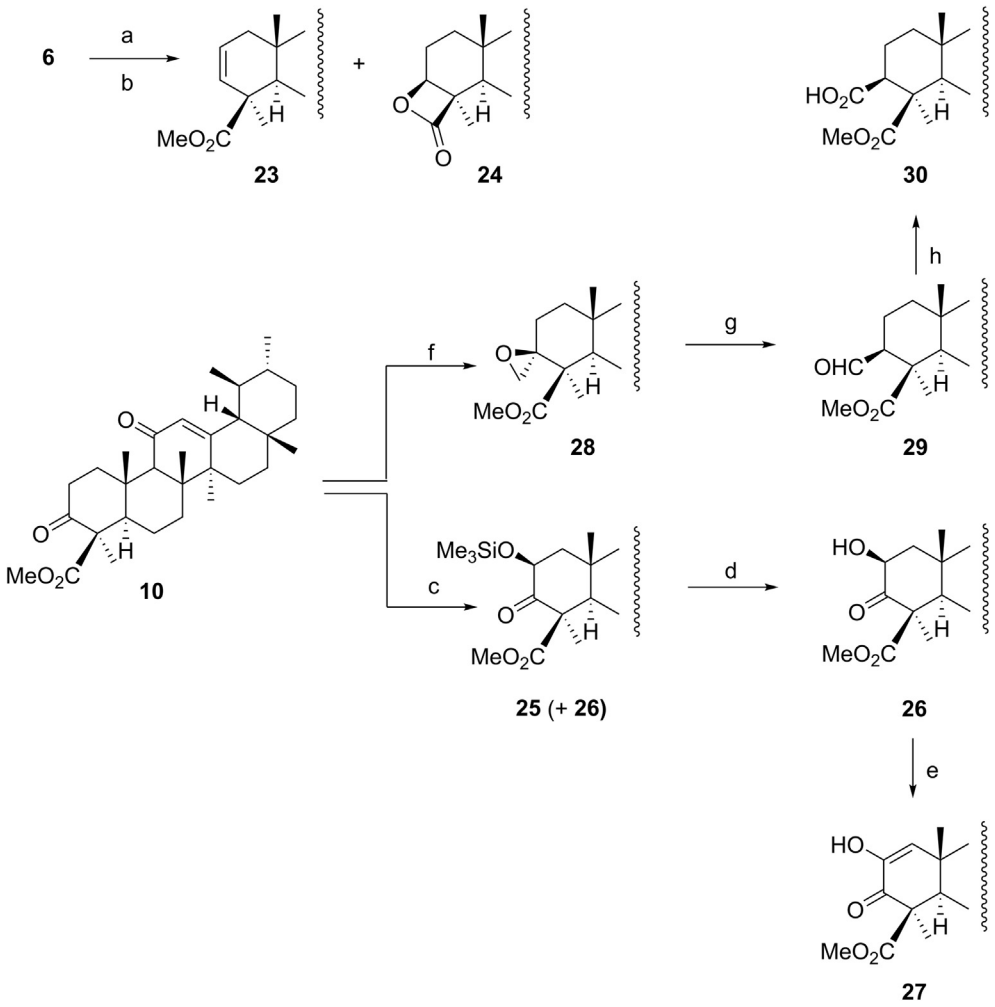
2.2. Biological screening

Many natural occurring triterpenoic acids (*e.g.* of the betulinic, oleanolic, ursolic, glycyrrhetinic and maslinic acid series), and their derivatives are well-known for their anti-cancer activity [5,66], and usually IC_{50} values ranging between 10 and 80 μM have been reported; several of them trigger apoptosis. Some of the boswellic acids prepared herein were screened for their antitumor activity using the well-established photometric sulforhodamine B (SRB) assay [67] employing several human tumor cell lines. This assay is grounded on the proportional binding of a rhodamine dye to surface membrane proteins, and there is a linear relationship between cell density and optical density. For comparison, β-KBA (**3**) and β-AKBA (**4**) were used as standards in these screening experiments, the results of which are summarized in Table 1.

Most of the compounds exhibited a moderate to good IC_{50} values in the SRB tests. Thus, acetylated derivative **4** showed approximately twice the cytotoxicity of β-KBA (**3**). This is in agreement with previous findings for several other triterpenoids exhibiting a higher cytotoxicity in the presence of an acetyl moiety at OH–C(2) [68–73]. While the diene-diol **9** exhibited IC_{50} values for several human tumor cell lines (*e.g.* like DLD-1, A-253 and HT 29) similar to those measured for β-AKBA, only a weak activity was found for A-2780 and HCT-8 cells. Decarboxylated **13** and the ring contracted derivative **20** showed a diminished cytotoxicity which suggests that an intact ring A is necessary for low IC_{50} values. As



Scheme 3. Synthesis of **19**–**22**: a) HOAc, Br_2, 25°, 30 min, 82%; b) K_2CO_3, acetone, reflux, 25 h, 32%; c) NaN_3, DMF, 30 °C, 12 h, quant.; d) NaH, THF, reflux, 15 min, then AcCl, 25 °C, 15 min, 55%.



Scheme 4. Synthesis of **23**–**30**: TPP, DEAD, 3,3-dimethyl-glutarimide, THF, 25 °C, 12 h → **23** (quant.); b) TPP, DEAD, 3,3-dimethyl-glutarimide, THF, 0 °C → 25 °C, 12 h: **23** (56%) and **24** (28%); c) LDA, TMS-Cl, THF, 2 h; mCPBA, 25 °C, 12 h: **25** (56%) and **26** (13%); d) HCl/$CHCl_3$, 25 °C, 2 h, 60%; e) oxalylic chloride, DMSO, NEt_3, DCM, −50 °C, 2 h, 90%; f) NaH, trimethylsulfoxonium iodide, DMSO, 25 °C, 12 h, 52%; g) $BF_3.Et_2O$, DCM, 25 °C, 12 h, 19%; h) air, 25 °C, 72 h, quant.

previously shown for some derivatives of glycyrrhetinic acid, a nitrogen bearing substituent at position 2 seems to lower IC_{50} values [70,74]. Thus, fair IC_{50} values were obtained for the oxime **16**, and improved values for amine **18**. Moreover, the introduction of an additional carboxylic group (as exemplified for **30**) decreased cytotoxicity.

Table 1
Cytotoxicity of β-KBA, β-AKBA and several derivatives (IC_{50} values in μM from SRB assays after 96 h of treatment; the values are averaged from three independent experiments performed each in triplicate; confidence interval CI = 95%). The cell lines are human cancer cell lines: DLD-1 (colorectal adenocarcinoma), A-253 (submandibular carcinoma), HT29 (colorectal adenocarcinoma), A2780 (cis-platin resistant ovarian cancer), HCT-8 (ileocecal adenocarcinoma). The best result is shown in bold.

Compound/Cell line	DLD-1	A-253	HT29	A2780	HCT-8
3 (β-KBA)	57.7 ± 3.5	45.9 ± 3.8	71.9 ± 2.5	37.9 ± 2.1	44.6 ± 2.7
4 (β-AKBA)	20.9 ± 1.7	16.9 ± 1.7	19.4 ± 3.6	14.1 ± 2.0	17.5 ± 3.2
9	19.5 ± 1.9	18.1 ± 1.3	23.9 ± 3.6	43.2 ± 1.9	31.3 ± 1.0
13	57.3 ± 2.1	39.4 ± 1.9	28.2 ± 4.1	23.5 ± 3.4	30.2 ± 2.4
16	13.7 ± 0.9	15.5 ± 3.6	15.4 ± 3.1	18.0 ± 1.5	16.4 ± 1.9
18	9.9 ± 0.5	**5.6±0.3**	8.7 ± 1.1	7.3 ± 0.3	12.5 ± 1.0
20	56.1 ± 3.4	54.9 ± 4.0	51.4 ± 2.6	53.6 ± 3.1	46.9 ± 3.8
23	32.9 ± 2.4	40.2 ± 4.1	30.2 ± 3.2	40.1 ± 2.5	39.4 ± 1.1
27	34.1 ± 2.6	42.3 ± 0.9	29.9 ± 1.3	41.1 ± 3.7	39.7 ± 2.4
30	42.7 ± 3.1	38.2 ± 1.8	34.1 ± 1.7	35.4 ± 3.2	41.7 ± 3.8

As previously shown, several triterpenoids induce apoptosis [66]. In order to evaluate the anti-cancer activity in the synthetically-modified new structures, active compound **18** was tested for an induction of apoptosis using a DNA-laddering assay. During apoptosis DNA is cleaved into smaller fragments by endonucleases. These fragments can be observed by gel electrophoresis as ladders. Thus, the floating cells (obtained after treatment with IC_{90}-concentrations for 24 h) were analyzed by DNA gel electrophoresis and the typical DNA ladders were observed (Fig. 2). In addition, a dye exclusion test was performed. Thus, trypan blue staining indicates the integrity of the cytoplasmatic membrane – hence, a distinction between apoptotic and necrotic cells can be made. As depicted (Fig. 2), the presence of an intact cell membrane in A2780 human ovarian cancer cells in a majority of the cells (having been treated with an IC_{90} concentration of 18 for 24 h) conformed that this compound is able to trigger apoptosis.

3. Conclusion

In this study we were able to synthesize several derivatives of 11-keto-β-boswellic acid; some of these compounds showed an increased cytotoxicity for various human cancer cell lines. The presence of an intact ring A proved mandatory for improved cytotoxicity. Furthermore, lower cytotoxicity was observed with AKBA derivatives that have undergone decarboxylation or ring contraction. A significantly improved cytotoxicity was discovered for analogs bearing an extra nitrogen containing substituent at position 2. Thus, amine **18** exhibited IC_{50} values as low as 5.7 μM. The cytotoxicity of amine **18** is attributed to apoptotic processes as demonstrated by DNA laddering and trypan blue staining experiments.

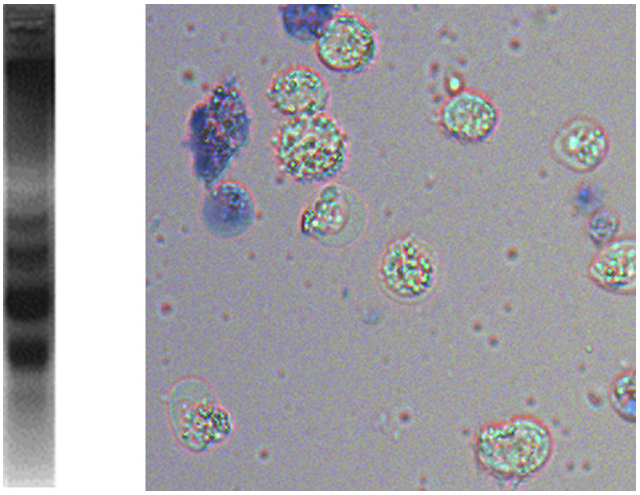


Fig. 2. DNA laddering (left) and trypan blue staining (right) for the ovarian cancer cell line A2780 after treatment with **18** applying IC_{90} concentrations for 24 h.

4. Experimental part

4.1. General – chemistry

Melting points are uncorrected (*Leica* hot stage microscope), NMR spectra were recorded using the Varian spectrometers Gemini 2000 or Unity 500 (δ given in ppm, *J* in Hz, internal Me_4Si), MS spectra were taken on a Finnigan MAT TSQ 7000 (electrospray, voltage 4.5 kV, sheath gas nitrogen) instrument. The optical rotation was measured on a Perkin–Elmer polarimeter at 20 °C; TLC was performed on silica gel (Merck 5554); elemental analyses were performed on a Vario EL (CHNS). The solvents were dried according to usual procedures. The purity of the compounds were determined by HPLC and found to be >98%. Frankincense was obtained from different commercial suppliers in bulk quantities; compound **4** was prepared from frankincense according to Jauch's procedure [31].

4.2. General – biological screening

4.2.1. Cytotoxicity assay [67,73,75–77].

The cytotoxicity of the compounds was evaluated using the sulforhodamine-B (SRB) (Sigma Aldrich) microculture colorimetric assay. In short, exponentially growing cells were seeded into 96-well plates on day 0 at the appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24 h, the cells were treated with serial dilutions of the compounds (0–30 μM) for 96 h. The final concentration of DMSO never exceeded 0.5%, which was non-toxic to the cells. The percentages of surviving cells relative to untreated controls were determined 96 h after the beginning of drug exposure. After a 96 h treatment, the supernatant medium from the 96-well plates was discarded, and the cells were fixed with 10% TCA. For a thorough fixation, the plates were allowed to rest at 4 °C. After fixation, the cells were washed in a strip washer. The washing was done four times with water using alternate dispensing and aspiration procedures. The plates were then dyed with 100 μl of 0.4% SRB for about 20 min. After dying, the plates were washed with 1% acetic acid to remove the excess of the dye and allowed to air-dry overnight. Tris base solution (100 μl, 10 mM) was added to each well and absorbance was measured at λ = 570 nm (using a 96 well plate reader, Tecan Spectra, Crailsheim, Germany). The IC_{50} was estimated by linear regression between the value before and after the 50% line is crossed in a dose–response curve.

4.2.2. Apoptosis test – DNA laddering and trypan blue staining

These assays were performed as described previously [73,76].

4.3. Syntheses

4.3.1. (3α, 4β) 3-hydroxy-11-oxo-urs-12-en-24-oic acid (= 11-keto-β-boswellic acid) (**6**)

AKBA (**4**, 10 g, 19.5 mmol) was dissolved in ethanol (200 mL) and an aq. solution of sodium hydroxide (4 M, 100 mL) was added. After stirring at 25 °C for 12 h, the pH was adjusted (aq. HCl), the product was extracted with $CHCl_3$ (5 × 100 mL), and the crude product was purified by chromatography (silica gel, hexane/ethyl acetate, 98:2) to afford pure **6** in almost quantitative yield (9.18 g, 98%) as a

colorless solid; mp 192–195 °C (lit.: 195–197 °C [78]); $[\alpha]_D = 118.2°$ ($c = 3.72$; $CHCl_3$).

*4.3.2. (3α, 4β) methyl 3-hydroxy-11-oxo-urs-12-en-24-oate (**7**)*

A suspension of **6** (4.73 g, 10.0 mmol) and Cs_2CO_3 (9.8 g, 30 mmol) in THF (50 mL) was stirred at 0 °C for 30 min, then MeI (6.23 mL, 100 mmol) was added, and stirring continued for 12 h. The mixture was diluted with ether (500 mL), washed with water and brine (2 × 50 mL each), dried (Na_2SO_4), and the solvents were evaporated to yield **7** (4.63 g, 95.0%) as an off-white solid (sufficiently pure for the next reactions); mp = 220–225 °C (lit.: 216–219 °C [79]); $[\alpha]_D = 111.2°$ ($c = 4.34$. $CHCl_3$).

*4.3.3. (3α, 4β) benzyl 3-hydroxy-11-oxo-urs-12-en-24-oate (**8**)*

Following the procedure given for **7** from **6** (944 mg, 2 mmol), Cs_2CO_3 (2 g) and benzyl bromide (2.4 mL, 20.2 mmol) **8** (0.95 g, 84.8%) was obtained as a colorless solid; mp 201–204 °C, $[\alpha]_D = 110.7°$ ($c = 0.98$; $CHCl_3$); ^{1}H NMR (400 MHz, acetone-d_6): δ = 7.45–7.30 (*m*, 5H, benzyl), 5.46 (*s*, 1H, H-12), 5.06 and 5.12. (*AB*, *J* = 12.1 Hz, 2H, benzyl), 4.03–4.02 (*m*, 1H, H-3), 2.42 (*s*, 1H, H-9), 2.40–2.36 (*m*, 1H, H-1a), 2.24–2.13 (*m*, 2H, H-2a, H-16a), 1.94–1.80 (*m*, 2H, H-15a, H-6a), 1.74–1.68 (*m*, 2H, H-6b, H-7b), 1.60–1.57 (*m*, 2H, H-5, H-18), 1.52–1.50 (*m*, 2H, H-19, H-22a), 1.47–1.43 (*m*, 3H, H-1b, H-2b, H-21a), 1.40–1.38 (*m*, 1H, H-7b), 1.37 (*bs*, 4H, H-22b, H-27), 1.26 (*s*, 3H, H-23), 1.25–1.22 (*m*, 1H, H-15a), 1.12 (*bs*, 3H, H-26), 1.05–1.01 (*m*, 1H, H-16b), 0.99 (*bs*, 3H, H-25), 0.96 (*bs*, 4H, H-20, H-30), 0.85 (*s*, 3H, H-28), 0.82 (*d*, *J* = 8.1 Hz, 3H, H-29) ppm; ^{13}C NMR (100 MHz, acetone-d_6): δ = 199.9 (C-11), 178.4 (C-24), 165.4 (C-13), 138.2 (C-32), 132.1 (C-12), 130.3 (benzyl), 129.4 (benzyl), 71.4 (C-3), 67.4 (C-31 benzyl), 62.0 (C-9), 60.5 (C-18), 50.3 (C-5), 49.4 (C-4), 46.7 (C-8), 45.5 (C-14), 42.8 (C-22), 41.3 (C-20), 40.8 (C-19), 39.1 (C-10), 35.6 (C-1), 35.6 (C-17), 34.6 (C-7), 32.5 (C-21), 30.7 (C-28), 29.1 (C-16), 28.9 (C-15), 28.1 (C-2), 25.7(C-23), 22.4 (C-30), 21.9 (C-27), 20.9 (C-6), 19.7 (C-26), 18.7 (C-29), 14.7 (C-25) ppm; MS (ESI, methanol) $C_{37}H_{52}O_4$: *m/z* = 561.5 [MH^+, 41%]; analysis calcd for $C_{37}H_{52}O_4$ (560.81): C, 79.24; H, 9.35; found: C, 79.11; H, 9.42.

*4.3.4. (3α, 4β) 3,24-dihydroxy-urs-9,12-diene (**9**)*

A solution of **6** (188 mg, 0.4 mmol) in abs. THF (10 mL) was slowly added to a suspension of $LiAlH_4$ (825 mg, 21.7 mmol) in abs. THF (20 mL), and stirring at 25 °C was continued for 23 h; water (50 mL) and ether (50 mL) were carefully added, and a clear solution was obtained by adding a few drops of conc. sulfuric acid. The aq phase was extracted with ether (50 mL), the combined organic phases were washed with brine (50 mL), the solvent was removed *in vacuo*, and the residue was subjected to column chromatography (silica gel, hexane/ethyl acetate, 1:1) to afford **9** (28 mg, 16%) as a colorless solid; mp = 160–162 °C; $[\alpha]_D = 266.7°$ ($c = 4.58$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.61 (*d*, 1H, H-11, *J* = 5.8 Hz), 5.43 (*d*, 1H, H-12, *J* = 5.8 Hz), 3.85 (*dd*, *J* = 2.5, 3.7 Hz, 1H, H-3), 3.72 (*d*, *J* = 11.2 Hz, 1H, H-24), 3.54 (*d*, *J* = 11.2 Hz, 1H, H-12), 2.00 (*ddd*, *J* = 13.7, 5.0, 13.7 Hz, 1H, H-16a), 1.95 (*m*, 1H, H-2a), 1.88 (*ddd*, *J* = 13.7, 5.0, 13.7 Hz, 1H, H-15a), 1.76 (*m*, 2H, H-1), 1.70 (*m*, 1H, H-15b), 1.58 (*m*, 1H, H-6a), 1.52 (*m*, 1H, H-6b), 1.48 (*m*, 1H, H-22b), 1.47 (*dd*, *J* = 11.2 Hz, 2.1 Hz, 1H), 1.43 (*m*, 1H, H-5), 1.42 (*m*, 2H, H-21), 1.40 (*m*, 2H, H-7), 1.39 (*m*, 1H, H-19), 1.30 (*m*, 1H, H-22a), 1.24 (*m*, 1H, H-2b), 1.19 (*s*, 3H, H-27), 1.14 (*s*, 3H, H-26), 1.10 (*s*, 3H, H-25), 1.02 (*ddd*, *J* = 11.6, 2.1, 2.5 Hz, 1H, H-16b), 0.91 (*s*, 3H, H-23), 0.90 (*s*, 3H, H-30), 0.89 (*m*, 1H, H-20), 0.84 (*s*, 3H, H-28), 0.79 (*d*, *J* = 6.6 Hz, 3H, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 154.5 (C-9), 141.2 (C-13), 122.9 (C-12), 115.5 (C-11), 70.3 (C-3), 66.6 (C-24), 57.3 (C-18), 45.7 (C-5), 4–3.2 (C-4), 43.0 (C-8), 41.3 (C-22), 40.7 (C-14), 39.4 (C-19), 39.0 (C-20), 38.5 (C-10), 33.6 (C-17), 32.3 (C-1), 32.1 (C-7), 31.2 (C-21), 28.7 (C-28), 28.2 (C-2), 26.3 (C-27), 26.1 (C-16), 25.9 (C-15), 21.8 (C-26), 21.5 (C-25 + C-30), 18.4 (C-6), 17.7 (C-29), 17.3 (C-23) ppm; MS (ESI, methanol) $C_{30}H_{48}O_2$: *m/z* = 441.3 ([$MH]^+$, 58%); analysis calcd for $C_{30}H_{48}O_2$ (440.70): C, 81.76; H, 10.98; found: C, 81.52; H, 11.12.

*4.3.5. (4β) methyl 3,11-dioxo-urs-12-en-24-oate (**10**)*

From **7**: To a solution of **7** (194 mg, 0.4 mmol) in ether (40 mL), sodium dichromate dihydrate (120 mg, 0.4 mmol), conc. sulfuric acid (1.2 mL) and water (2.4 mL) were carefully added, and stirring at 25 °C continued for 2 h. Usual work-up followed by chromatography (silica gel, hexane/ethyl acetate, 7:3) yielded **10** (125 mg, 65%) as a colorless solid.

From **7**: Ta a solution of oxalylic chloride (263 mg, 2.06 mmol) in abs. DCM (20 mL) at −78 °C, DMSO (322 mg, 4.12 mmol) in abs. DCM (2 mL) was added and stirred for 10 min, then a solution of **7** (500 mg, 1.03 mmol) in abs. DCM (5 mL) was added and stirring at −60 °C continued for 1 h. Triethylamine (577 μl, 4.12 mmol) was added and stirring continued for 15 min. Usual aq. work-up followed by chromatography (silica gel, chloroform/ether, 9:1) yielded **10** (quant.) as a colorless solid.

*4.3.6. (4β) benzyl 3,11-dioxo-urs-12-en-24-oate (**11**)*

Following the procedure given for **10**, from **8** (0.56 g, 1.0 mmol) **11** (0.51 g, 91%) was obtained as a colorless solid; mp 94–97 °C; $[\alpha]_D = 114.1°$ ($c = 1.0$; CHCl3); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.35 (*m*, 5H, benzyl), 5.57 (*s*, 1H, H-12), 5.09 and 5.13 (*AB*, *J* = 12.0 Hz, 2H, benzyl), 3.04–2.95 (*m*, 2H, H-1a, H-2a), 2.33 (*bs*, 2H, H-2b, H-9), 2.11–2.01 (*dt*, *J* = 12.0, 4.0 Hz, 1H, H-16a), 1.99–1.92 (*m*, 1H, H-6a), 1.92–1.80 (*m*, 2H, H-15a, H-6a), 1.64–1.57 (*m*, 1H, H-7a), 1.54 (*d*, *J* = 12.0 Hz, 1H, H-18), 1.51–1.40 (*m*, 3H, H-7a, H-21a, H-22a), 1.40 (*s*, 3H, H-23), 1.37–1.36 (*m*, 1H, H-19), 1.34–1.28 (*m*, 2H, H-21a, H-22a), 1.25 (*bs*, 4H, H-1b, H-27), 1.20 (*bs*, 1H, H15a), 1.17 (*bs*, 7H, H-5, H-25, H-26), 1.04–1.00 (*m*, 1H, H-16b), 0.94 (*bs*, 4H, H-20, H-30), 0.83 (*s*, 3H, H-28), 0.78 (*d*, *J* = 8.0 Hz, 3H, H-29) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 208.4 (C-3), 198.5 (C-11), 173.1 (C-24), 165.4 (C-13), 134.7 (benzyl), 130.3 (C-12), 128.6, 128.4, 128.5 (each benzyl), 67.0 (benzyl), 60.0 (C-9), 59.0 (C-18), 58.5 (C-5, >CH−), 57.6 (C-4), 44.9 (C-8), 43.9 (C-14), 41.0 (C-1), 40.8 (C-22), 39.3 (C-19, C-20), 37.0 (C-10), 36.7 (C-2), 34.1 (C-17), 32.5 (C-7), 30.9 (C-21), 28.8 (C-28), 27.3 (C-16), 27.3 (C-15), 21.1 (C-23, C-30), 20.5 (C-27), 19.8 (C-6), 18.3(C-26), 17.4 (C-29), 13.2 (C-25) ppm; MS (ESI, methanol): $C_{37}H_{50}O_4$: *m/z* = 559.4 [MH^+, 51%]; analysis calcd for $C_{37}H_{50}O_4$ (558.79): C, 79.53; H, 9.02; found: C, 79.37; H, 10.13.

*4.3.7. (4β) 3,11-dioxo-urs-12-en-24-oic acid (**12**)*

Following the procedure given for **10**, from **6** (0.9 g, 2.0 mmol) **12** (0.8 g, 84%) was obtained as a colorless solid; mp 116–119 °C; $[\alpha]_D = 105.9°$ ($c = 1.2$; $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD): δ = 5.59 (*s*, 1H, H-12), 3.12–3.03 (*m*, 2H, H-2a, H-1a), 2.39–2.33 (*m*, 2H, H-1b, H-9), 2.13–2.04 (*m*, 1H, H-15a), 2.04–2.00 (*m*, 1H, H-6a), 1.94–1.86 (*m*, 1H, H-15a), 1.82–1.78 (*m*, 1H, H-6b), 1.67–1.59 (*m*, 1H, H-7a), 1.58–1.46 (*m*, 4H, H-18, H-7b, H-21a, H-22a), 1.42 (*s*, 3H, H-23), 1.39 (*bs*, 4H, H-25, H-19), 1.34–1.29 (*m*, 3H, H-2b, H-21b, H-22b), 1.28 (*s*, 3H, H-27), 1.23–1.22 (*bs*, 4H, H-5, H-26), 1.18 (*s*, 1H, H-15b), 1.05–1.01 (*m*, 1H, H-16a), 0.96 (*bs*, 4H, H-19, H-30), 0.83 (*s*, 3H, H-28), 0.79 (*d*, *J* = 8.0 Hz, 3H,H-29) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 211.7 (C-3), 198.9 (C-11), 178.7 (C-24), 165.6 (C-13), 130.2 (C-12), 60.1 (C-9), 59.1 (C-18), 58.6 (C-5), 57.4 (C-4), 44.9 (C-8), 43.6 (C-14), 41.0 (C-2), 40.7 (C-22), 39.4 (C-19), 39.1 (C-20), 37.5 (C-10), 36.5 (C-1), 34.1 (C-17), 32.4 (C-7), 30.6 (C-21), 28.8 (C-28), 27.5 (C-16), 27.3 (C-15), 21.0 (C-23), 20.4 (C-6), 19.8 (C-27), 18.5 (C-26), 17.6 (C-29), 13.4 (C-25); MS (ESI, methanol): $C_{30}H_{44}O_4$: *m/z* = 469.4 [MH^+, 53%], 425.4 [M-COOH + H^+, 38%]; analysis calcd for $C_{30}H_{44}O_4$ (468.67): C, 76.88; H, 9.46; found: C, 76.62; H, 9.53.

*4.3.8. (4S) 3,11-dioxo-24-norurs-12-ene (**13**)*

From **6**: To solution of **6** (470 mg, 1.0 mmol) in ether (100 mL), a

mixture of sodium dichromate dihydrate (450 mg, 1.5 mmol), conc. sulfuric acid (4.5 mL)and water (9 mL) was slowly added and stirring at 25 °C was continued for 3 days. The phases were separated, the aq. layer was extracted with ether (2 × 25 mL), the combined organic layers were dried (Na_2SO_4), and the solvent was removed. Chromatography (silica gel, hexane/ethyl acetate, 7:3) gave **13** (130 mg, 31%) as a colorless solid.

From **10**: A solution of **10** (200 mg, 0.42 mmol) in ethanol (30 mL) and aq NaOH (4 M, 15 mL) was heated for 4 h under reflux. After acidification (dil. aq. HCl), aq. work-up, extraction with chloroform, evaporation of the solvent, the residue was subjected to column chromatography (silica gel, hexane/ethyl acetate, 9:1) to afford **13** (62 mg, 35%) as a colorless solid; mp = 170–172 °C; $[\alpha]_D = 133.9°$ ($c = 4.22$. $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.57 (*s*, 1H, H-12), 2.97 (*ddd*, *J* = 13.7, 2.5, 7.1 Hz, 1H, H-2a), 2.54 (*ddd*, *J* = 14.9, 7.1, 14.9 Hz, 1H, H-1a), 2.39 (*s*, 1H, H-9), 2.33 (*m*, 1H, H-4), 2.27 (*ddd*, *J* = 14.9, 2.5, 5.0 Hz, 1H, H-1b), 2.08 (*ddd*, *J* = 13.7, 5.0, 13.7 Hz, 1H, H-16a), 1.88 (*ddd*, *J* = 13.7, 5.0, 13.7 Hz, 1H, H-15a), 1.64 (*ddd*, *J* = 13.7, 3.7, 13.7 Hz, 1H, H-7a), 1.59 (*m*, 1H, H-6b), 1.54 (*dd*, *J* = 11.2, 1.7 Hz, 1H, H-18), 1.48 (*m*, 1H, H-22b), 1.41 (*m*, 1H, H-7b, 2H, H-21), 1.39 (*m*, 1H, H-19), 1.37 (*m*, 1H, H-6a), 1.35 (*s*, 3H, H-25), 1.31 (*m*, 1H, H-2b, 1H, H-22a), 1.27 (*s*, 3H, H-27), 1.20 (*s*, 3H, H-26), 1.19 (*ddd*, *J* = 12.0, 2.1, 2.5 Hz, 1H, H-15b), 1.09 (*ddd*, *J* = 2.5 Hz, 12.0, 12.0 Hz, 1H, H-5), 1.00 (*ddd*, *J* = 13.7, 2.1 Hz, 2.5 Hz, 1H, H-16b), 0.99 (*d*, *J* = 6.6 Hz, 3H, H-23), 0.94 (*m*, 1H, H-20), 0.93 (*s*, 3H, H-30), 0.81 (*s*, 3H, H-28), 0.78 (*d*, *J* = 6.2 Hz, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 213.1 (C-3), 199.1 (C-11), 165.3 (C-13), 130.3 (C-12), 59.1 (C-9), 59.0 (C-18), 53.9 (C-5), 44.8 (C-8), 44.4 (C-4), 43.8 (C-14), 40.9 (C-22), 40.5 (C-2), 39.2 (C-19), 39.2 (C-20), 37.4 (C-1), 36.1 (C-10), 33.9 (C-17), 31.6 (C-7), 30.8 (C-21), 28.8 (C-28), 27.4 (C-16), 27.2 (C-15), 21.1 (C-6), 21.1 (C-30), 20.4 (C-27), 18.4 (C-26), 17.4 (C-29), 13.6 (C-25), 11.6 (C-23) ppm; MS (ESI, methanol) $C_{29}H_{44}O_2$: *m/z* = 425.4 ($[MH]^+$, 100%), 478.9 ($[M + methanol + Na]^+$, 15%); analysis calcd for $C_{29}H_{44}O_2$ (424.66): C, 82.02; H, 10.44; found: C, 81.89; H, 10.57.

4.3.9. (5S,5aR,7aR,7bS,9aR,12R,13S,13aR,15bS)-5,7a,7b,9a,12,13,15b-heptamethyl-1,2,5a,6,7,7a,8,9,9a,10,11,12,13,13a,15a,15b-hexadecahydrochryseno[2,1-c]oxepine-3,15(5H,7bH)-dione (**14**)

To a solution of **13** (0.3 g, 0.71 mmol) in $CHCl_3$ (25 mL) a solution of *m*-CPBA (0.46 g, 2.6 mmol) in $CHCl_3$ (20 mL) was slowly added, and the mixture was heated under reflux for 12 h. Aqeous workup ($Na_2S_2O_3$, $NaHCO_3$) followed by column chromatography (silica gel, hexane/ethyl ether, 1:1) furnished **14** (0.29 g, 93.2%) as a colorless solid; mp 225–288 °C; $[\alpha]_D = 76.3°$ (c = 048, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.55 (*s*, 1H, H-12), 4.57 (*q*, *J* = 5.0 Hz, 1H, H-4), 3.04–2.99 (*dd*, *J* = 15.0, 5.0 Hz, 1H, H-1a), 2.90–2.83 (*dt*, *J* = 15.0, 5.0 1H, H-2a), 2.47 (*s*,1H, H-9), 2.45–2.35 (*dd*, *J* = 15.0, 5.0 Hz, 1H, H-2b), 2.12–2.06 (*dt*, *J* = 10.1, 5.0 Hz, *J* = 1H, H-16a), 1.90–1.84 (*dt*, *J* = 10.1, 5.0 Hz, 1H, H-15a), 1.72–1.69 (*dt*, *J* = 10.1, 5.0 Hz, 1H,H-7a), 1.63–1.59 (*m*, 1H, H-6a), 1.56–1.53 (*dd*, *J* = 10.1, 5.0 Hz, 1H, H-18), 1.48–1.46 (*m*, 2H, H-22a, H-21a), 1.45–1.43 (*m*, 1H, H-7a), 1.40–1.36 (*m*, 2H, H-5a, H-19a), 1.35–1.32 (*m*, 1H, H-22b), 1.30 (*bs*, 5H, H-1b, H-21b, H-26), 1.29–1.28 (*bs*, 7H, H-6b, H-23, H-24), 1.19 (*bs*, 4H, H15b, H-25), 1.04–1.01 (*m*, 1H, H-16b), 0.95 (*bs*, 4H, H-20b, H-29), 0.82 (*s*, 3H, H-27), 0.80–0.78 (*d*, *J* = 5.0 Hz, 3H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.2 (C-11), 176.2 (C-3), 165.3 (C-13), 130.5 (C-12), 74.2 (C-4), 59.0 (C-18), 59.0 (C-9), 52.8 (C-5), 44.6 (C-8), 43.7 (C-14), 40.8 (C-22), 39.3 (C-19,C-20), 37.7 (C-10), 37.3 (C-1), 34.1 (C-17), 31.9 (C-21), 30.8 (C-7), 29.5 (C-2), 28.6 (C-27), 27.5 (C-16), 27.0 (C-15), 21.1 (C-29), 20.3 (C-26), 20.0 (C-6), 19.5 (C-23), 18.2 (C-25), 17.5 (C-28), 14.1 (C-24) ppm; MS (ESI, methanol): $C_{29}H_{44}O_3$: *m/z* = 441.4 ($[MH^+]$, 39%]; analysis calcd for $C_{29}H_{44}O_3$ (440.66): C, 79.04; H, 10.06; found: C, 78.87; H, 10.17.

4.3.10. (4β) benzyl 3-(hydroxyimino)-11-oxo-urs-12-en-24-oate (**15**)

To a solution of **11** (400 mg, 0.72 mmol) in dry pyridine (5 mL), a solution of hydroxylammonium hydrochloride (230 mg, 3.2 mmol) in dry pyridine (5 mL) was added, and the mixture was heated at 50 °C for 3 h. After dilution (CH_2Cl_2, 200 mL), aqueous work-up and chromatography (silica gel, hexane/diethyl ether, 3:1), **15** (370 mg, 90%) was obtained as a colorless solid; mp 234–237 °C; $[\alpha]_D = 58.3°$ (c = 0.85, $CHCl_3$); ^{1}H NMR (400 MHz, CDCl3): δ = 7.36–7.31 (*m*, 5H, benzyl), 5.53 (*s*, 1H, H-12), 5.16 and 5.07 (*AB*, *J* = 12.0 Hz, benzyl), 3.31–3.26 (*m*,1H, H-2a), 2.90–2.83 (*m*, 1H, H-1a), 2.30–2.24 (*m*, 2H, H-9, H-2b), 2.12–2.04 (*dt*, *J* = 4.0 Hz, *J* = 12.0 Hz, 1H, H-16a), 1.90–1.80 (*m*, 3H, H-6a, H-6a, H-15a), 1.59–1.51 (*m*, 2H, H-7a, H-18), 1.49–1.43 (*m*, 6H, H-23, H-22a, H-21a, H-7a), 1.35–1.28 (*m*, 3H, H-19, H-22a, H-21a), 1.26 (*s*, 3H, H-27), 1.20–1.18 (*m*, 1H, H-15a), 1.12 (*s*, 3H, H-26), 1.05 (*s*, 3H, H-25), 1.04–1.00 (*m*, 2H, H-1b, H-16b), 0.95 (*bs*, 4H, H-20, H-30), 0.80 (*s*, 3H, H-28), 0.79 (*d*, *J* = 8.1 Hz, 3H, H-29) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 198.7 (C-11), 174.2 (C-24), 165.3 (C-13), 162.5 (C-3), 135.4 (C-32, benzyl), 130.4 (C-12), 128.6, 128.5, 128.4 (each benzyl), 66.7 (benzyl), 60.1 (C-9), 59.1 (C-18), 58.0 (C-5), 50.7 (C-4), 44.9 (C-8), 43.5 (C-14), 40.7 (C-22), 39.7 (C-1), 39.4 (C-19, C-20), 37.4 (C-10), 33.9 (C-17), 32.9 (C-7), 30.9 (C-21), 28.9 (C-28), 27.4 (C-16), 27.3 (C-15), 22.3 (C-23), 21.0 (C-30), 20.4 (C-27), 19.4 (C-6), 19.0(C-2), 18.4 (C-26), 17.5 (C-29), 13.5 (C-25) ppm; MS (ESI, methanol): $C_{37}H_{51}NO_4$: *m/z* = 574.4 [MH^+, 81%]; analysis calcd for $C_{37}H_{51}NO_4$ (573.81): C, 77.45; H, 8.96; N, 2.44; found: C, 77.33; H, 9.09; N, 2.32.

4.3.11. (4β) methyl 3-(hydroxyimino)-11-oxo-urs-12-en-24-oate (**16**)

A solution of **10** (500 mg, 1.04 mmol) and hydroxylammonium hydrochloride (294 mg, 5.20 mmol) in pyridine (10 mL) was stirred at 50 °C for 2 h, the solvent was removed under reduced pressure, the residue was dissolved in DCM (80 mL) and washed with aq hydrochloric acid (10%, 20 mL) and water (2 × 20 mL). The solvent was removed, and the residue was subjected to column chromatography (silica gel, hexane/ethyl acetate/HOAc, 70:30:1) to afford **16** (quant.) as a colorless solid; mp = 198–201 °C (lit.: 196–200 °C [79]); $[\alpha]_D = 53.4°$ (c = 3.02; $CHCl_3$).

4.3.12. (3β, 4β) benzyl 3-amino-11-oxo-urs-12-en-24-oate (**17**)

To an ice-cold solution of **15** (400 mg, 0.7 mmol) in methanol (10 mL) and DCM (10 mL), ammonium acetate (0.6 g, 7.8 mmol) and a solution of $NaBH_3CN$ (500 mg, 7.8 mmol) in methanol (15 mL) was slowly added; keeping the temperature <5 °C a solution of $TiCl_3$ (15% in 2 N aq. HCl, 1.9 mL) was slowly added; the color of the reaction mixture turned from black to green and discolored after stirring overnight at 25 °C. Aqueous work-up (NaOH, pH = 10), followed by extraction with DCM, and column chromatography (silica gel, hexane/ethyl acetate, 1:9) furnished **17** (0.3 g, 76.9%) as an off-white solid; mp 78–81 °C; $[\alpha]_D = 135.1°$ (c = 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39–7.30 (*m*, 5H, benzyl), 5.52 (*s*, 1H, H-12), 5.15 and 5.08 (*AB*, *J* = 12.0 Hz, 2H, benzyl), 2.79–2.76 (*dd*, *J* = 12.0, 4.0 Hz, 1H, H-1a), 2.51–2.47 (*m*, 1H, H-3), 2.31 (*s*, 1H, H-9), 2.25–2.16 (*dt*, *J* = 12.0, 4.0 Hz, 1H, H-2a), 2.16–2.00 (*dt*, *J* = 12.0, 4.0 Hz, 1H, H-16a), 1.90–1.83 (*m*, 2H, H-15a, H-6a), 1.73–1.56 (*m*, 3H, H-2b, H-6a, H-7a), 1.52–1.50 (*m*, 2H, H-18, H-22a), 1.45 (*s*, 3H, H-23), 1.43–1.37 (*m*, 3H, H-21a, H-7b, H-19), 1.34–1.30 (*m*, 2H, H-21a, H-22b), 1.29 (*s*, 3H, H-27), 1.24–1.16 (*m*, 1H, H-15a), 1.08 (*s*, 3H, H-26), 1.02 (*m*, 1H, H-1b),1.00–0.92 (*bs*, 9H, H-16b, H-20, H-25, H-30, H-5), 0.80 (*s*, 3H, H-28), 0.78 (*d*, *J* = 8.0 Hz, 3H, H-29) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 199.3 (C-11), 178.9 (C-24), 167.0 (C-13), 135.5 (benzyl, 130.5 (C-12), 128.7, 128.6, 128.4 (benzyl each), 66.5 (benzyl), 60.7 (C-9), 59.5 (C-3), 59.2 (C-18), 57.3 (C-5), 48.1 (C-4), 44.8 (C-8), 43.5 (C-14), 41.9 (C-22), 40.2 (C-1), 39.3 (C-19, C-20), 37.1

(C-10), 34.1 (C-17), 33.0 (C-7), 30.8 (C-21), 28.8 (C-28), 27.4 (C-16), 27.3 (C-15), 25.3 (C-2), 24.4 (C-23), 21.2 (C-30), 20.5 (C-27), 19.5 (C-6), 18.0 (C-26), 17.4 (C-29), 13.7 (C-25) ppm; MS (ESI, methanol): $C_{37}H_{53}NO_3$: *m/z* = 560.4 [MH^+, 71%]; analysis calcd for $C_{37}H_{53}NO_3$ (559.82): C, 79.38; H, 9.54; N, 2.50found: C, 79.21; H, 9.77; N, 2.39.

*4.3.13. (3β, 4β) 3-amino-11-oxo-urs-12-en-24-oic acid (**18**)*

A suspension of **17** (120 mg, 0.22 mmol), Pd/C (10%, 120 mg) and ammonium formate (80 mg, 1.3 mmol) in dry methanol (7 mL) was heated under reflux for 5 h. The catalyst was filtered and the solvent was removed to afford **18** (95 mg, 95%) as a colorless solid; mp 295–301 °C; $[\alpha]_D$ = 9.1° (*c* = 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD): δ = 5.53 (*s*, 1H, H-12), 2.85–2.77 (*m*, 2H, H-1a, H-3a), 2.42 (*s*, 1H, H-9a), 2.39–2.27 (*m*, 1H, H-2a), 2.22–2.05 (*m*, 2H, H-16a, H-6a), 2.00–1.90 (*m*, 2H, H-6a, H-15a), 1.71–1.59 (*m*, 3H, H-2b, H-7a, H-18a), 1.55–1.42 (*m*, 4H, H-22a, H-7b, H-21a, H-19a), 1.42–1.40 (*m*, 2H, H-22b, H-21b), 1.35 (*s*, 3H, H-27), 1.31 (*s*, 3H, H-23),1.24 (*s*, 3H, H-25), 1.20 (*s*, 3H, H-26), 1.19–1.10 (*m*, 1H, H-1b), 1.10–1.00 (*m*, 1H, H-16b), 0.99 (*s*, 4H, H-20b, H-30) 0.93–0.88 (*m*, 1H, H-5b), 0.86 (*s*, 3H, H-28), 0.83 (*d*, *J* = 8.0 Hz, 3H, H-29) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ = 201.9 (C-11), 181.5 (C-24), 165.3(C-13), 131.2 (C-12), 61.9 (C-9) 60.3 (C-18), 60.1 (C-3), 57.5 (C-5), 46.8 (C-4), 46.2 (C-8), 45.4 (C-14), 42.0 (C-22), 40.7 (C-19) 40.6 (C-1and C-20), 38.7 (C-10), 35.2 (C-17), 34.3 (C-7), 32.1 (C-21), 29.5 (C-28), 28.7 (C-16), 28.5(C-15), 25.6 (C-2), 25.0 (C-23), 21.4 (C-30), 20.7 (C-27), 20.4 (C-6), 19.1 (C-26), 17.9 (C-29), 14.7 (C-25) ppm; MS (ESI, methanol): $C_{30}H_{47}NO_3$: *m/z* = 470.4 [MH^+, 59%]; analysis calcd for $C_{30}H_{47}NO_3$ (469.70): C, 76.71; H, 10.09; N, 2.98; found: C, 76.59; H, 10.11; N, 2.75.

*4.3.14. (2β, 4β) methyl 2-bromo-3,11-dioxo-urs-12-en-24-oate (**19**)*

To a solution of **10** (230 mg, 0.48 mmol) in glacial acetic acid (20 mL), bromine (77 mg, 0.48 mmol, 1 M in glacial acetic acid) was slowly added and stirring at 25 °C was continued for 30 min. The precipitate was filtered off, and **19** (220 mg, 82%) was obtained as a colorless solid; mp = 254 °C; $[\alpha]_D$ = 78.9° (*c* = 6.08; $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.57 (*s*, 1H, H-12), 5.39 (*dd*, *J* = 6.2, 14.1 Hz, 1H, H-2), 3.71 (*dd*, *J* = 13.3, 6.2 Hz, 1H, H-1b), 3.69 (*s*, 3H, H-31), 2.35 (*m*, 1H, H-9), 2.07 (*ddd*, *J* = 13.7, 5.0, 13.7 Hz, 1H, H-16a), 1.97 (*m*, 1H, H-6b), 1.87 (*ddd*, *J* = 13.7, 5.0, 13.7 Hz, 1H, H-15a), 1.81 (*m*, 1H, H-6a), 1.70 (*dd*, *J* = 13.3, 13.7 Hz, 1H, H-1a), 1.61 (*ddd*, *J* = 12.9, 3.7, 12.9 Hz, 1H, H-7a), 1.54 (*dd*, *J* = 11.2, 1.2 Hz, 1H, H-18), 1.48 (*m*, 1H, H-7b, 1H, H-22b), 1.46 (*s*, 3H, H-23), 1.44 (*m*, 1H, H-21a), 1.39 (*m*, 1H, H-19), 1.32 (*s*, 3H, H-25), 1.30 (*m*, 1H, H-22a), 1.28 (*m*, 1H, H-21b), 1.25 (*s*, 3H, H-27), 1.21 (*m*, 1H, H-5), 1.18 (*s*, 3H, H-26), 1.17 (*m*, 1H, H-15b), 1.01 (*ddd*, *J* = 13.7, 2.5, 2.5 Hz, 1H, H-16b), 0.93 (*m*, 1H, H-20), 0.92 (*s*, 3H, H-30), 0.80 (*s*, 3H, H-28), 0.77 (*d*, *J* = 6.6 Hz, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 198.9 (C-3), 197.9 (C-11), 173.3 (C-24), 165.8 (C-13), 130.1 (C-12), 59.6 (C-9), 59.1 (C-18), 58.4 (C-4), 58.4 (C-5), 53.2 (C-2), 52.9 (C-1), 52.5 (C-31), 44.9 (C-8), 43.9 (C-14), 40.8 (C-22), 39.6 (C-10), 39.3 (C-19), 39.2 (C-20), 34.0 (C-17), 32.5 (C-7), 30.8 (C-21), 28.8 (C-28), 27.4 (C-16), 27.3 (C-15), 21.8 (C-23), 21.1 (C-30), 20.4 (C-27), 19.7 (C-6), 18.4 (C-26), 17.4 (C-29), 13.9 (C-25) ppm; MS (ESI, methanol) $C_{31}H_{45}BrO_4$: *m/z* = 561.4 ([M(^{79}Br)H]$^+$, 95%), 563.4 ([M(^{81}Br)H]$^+$, 88%), 583.3 ([M(^{79}Br)Na]$^+$, 40%), 585.3 ([M(^{81}Br)Na]$^+$, 34%), analysis calcd for $C_{31}H_{45}BrO_4$ (561.59): C, 66.30; H, 8.08; found: C, 66.17; H, 8.21.

*4.3.15. (3R,3aR,5aR,5bS,7aR,10R,11S,11aR,13bS)-2-hydroxy-3-(methoxycarbonyl)-3,5a,5b,7a,10,11,13b-heptamethyl-13-oxo-2,3,3a,4,5,5a,5b,6,7,7a,8,9,10,11,11a,13,13a,13b-octadecahydro-1H-cyclopenta[a]chrysene-2-carboxylic acid (**20**)*

A solution of **19** (476 mg, 0.85 mmol) and potassium carbonate (1.44 g, 10.4 mmol) in acetone (30 mL) and water (15 mL) was heated under reflux for 25 h. The acetone was distilled off, the residue diluted with water (50 mL) and extracted with chloroform (2 × 50 mL). The solvent was removed, and the residue subjected to chromatography (silica gel, hexane/ethyl acetate, 50:50 → 25:75) to yield **20** (140 mg, 32%) as a colorless, amorphous solid; $[\alpha]_D$ = 54.1° (*c* = 5.14; $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.60 (*s*, 1H, H-12), 3.72 (*s*, 3H, H-31), 2.57 (*s*, 1H, H-9), 2.45 (*d*, *J* = 14.5 Hz, 1H, H-1a), 2.29 (*d*, *J* = 14.5 Hz, 1H, H-1b), 2.09 (*ddd*, *J* = 13.7, 5.0, 13.7 Hz, 1H, H-16a), 1.90 (*ddd*, *J* = 13.7, 5.0, 13.7 Hz,1H, H-15a), 1.84 (*m*, 1H, H-6a), 1.72 (*d*, *J* = 12.0 Hz, 1H, H-5), 1.66 (*m*, 1H, C*H*$_2$ (6b), 1H, H-7a), 1.55 (*d*, *J* = 11.2 Hz, 1H, H-18), 1.46 (*m*, 1H, H-22b), 1.42 (*m*, 1H, H-7b, 1H, H-21a), 1.38 (*m*, 1H, H-19), 1.34 (*s*, 3H, H-23), 1.31 (*m*, 1H, H-22a), 1.29 (*s*, 3H, H-27), 1.27 (*m*, 1H, H-21b), 1.19 (*m*, 1H, H-15b), 1.14 (*s*, 3H, H-26), 1.11 (*s*, 3H, H-25), 0.99 (*ddd*, *J* = 13.7, 2.5, 2.5 Hz, 1H, H-16b), 0.92 (*s*, 3H, H-30), 0.90 (*m*, 1H, H-20), 0.81 (*s*, 3H, H-28), 0.76 (*d*, *J* = 6.2 Hz, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 198.9 (C-11), 177.3 (C-3 + C-24), 167.0 (C-13), 129.5 (C-12), 86.8 (C-2), 61.3 (C-9), 59.3 (C-5), 58.8 (C-18), 55.4 (C-1), 53.8 (C-4), 52.1 (C-31), 45.0 (C-8), 43.8 (C-14), 40.9 (C-10), 40.8 (C-22), 39.3 (C-19), 39.2 (C-20), 33.9 (C-17), 32.7 (C-7), 30.8 (C-21), 28.9 (C-28), 27.6 (C-15), 27.5 (C-16), 21.5 (C-23), 21.1 (C-30), 20.6 (C-27), 19.5 (C-6), 18.5 (C-26), 17.8 (C-25) 17.4 (C-29) ppm; MS (ESI, methanol) $C_{31}H_{46}O_6$: *m/z* = 515.5 ([MH]$^+$, 58%), 537.5 ([MNa]$^+$, 68); analysis calcd for $C_{31}H_{46}O_6$ (514.69): C, 72.34; H, 9.01; found: 72.14; H, 9.11.

*4.3.16. (4β) methyl 2-amino-3,11-dioxo-urs-1,12-dien-24-oate (**21**)*

A solution of **19** (300 mg, 0.54 mmol) and sodium azide (90 mg, 1.38 mmol) in DMF (15 mL) was stirred at 30 °C for 12 h. The solvent was removed under reduced pressure, and the residue subjected to column chromatography (silica gel, chloroform/diethyl ether, 4:1) to yield **21** (268 mg, quant.) as a colorless solid; mp = 132 °C; $[\alpha]_D$ = 100.9° (*c* = 4.22; $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.81 (*s*, 1H, H-1), 5.58 (*s*, 1H, H-12), 3.61 (*s*, 3H, H-31), 2.61 (*m*, 1H, H-9), 2.08 (*ddd*, *J* = 13.7, 5.0, 13.7 Hz, 1H, H-16a), 1.88 (*ddd*, *J* = 13.7, 5.0, 13.7 Hz, 1H, H-15a), 1.85 (*m*, 2H, H-6), 1.68 (*ddd*, *J* = 12.5, 4.6, 12.5 Hz, 1H, H-7a), 1.65 (*dd*, *J* = 11.2, 2.5 Hz, 1H, H-5), 1.54 (*dd*, *J* = 11.2, 1.2 Hz, 1H, H-18), 1.50 (*s*, 3H, H-23), 1.46 (*m*, 1H, H-7b, 1H, H-22b), 1.42 (*m*, 1H, H-21a), 1.39 (*m*, 1H, H-19), 1.31 (*s*, 3H, H-25), 1.29 (*m*, 1H, H-21b, 1H, H-22a), 1.27 (*s*, 3H, H-27), 1.19 (*m*, 1H, H-15b), 1.17 (*s*, 3H, H-26), 1.01 (*ddd*, *J* = 13.3, 2.5, 2.5 Hz, 1H, H-16b), 0.94 (*m*, 1H, H-20), 0.93 (*s*, 3H, H-30), 0.81 (*s*, 3H, H-28), 0.77 (*d*, *J* = 6.2 Hz, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 198.8 (C-11), 192.8 (C-3), 173.8 (C-24), 166.2 (C-13), 135.2 (C-1), 130.0 (C-12), 129.8 (C-2), 59.1 (C-18), 56.8 (C-9), 55.1 (C-5), 53.9 (C-4), 52.2 (C-31), 45.1 (C-8), 44.0 (C-14), 40.8 (C-22), 39.2 (C-19), 39.2 (C-20), 37.7 (C-10),, 34.0 (C-17), 32.6 (C-7), 30.8 (C-21), 28.8 (C-28), 27.4 (C-16), 27.2 (C-15), 21.6 (C-23), 21.1 (C-30), 20.4 (C-27), 19.0 (C-6), 18.6 (C-26), 17.3 (C-29), 16.8 (C-25) ppm; MS (ESI, methanol) $C_{31}H_{45}NO_4$: *m/z* = 496.5 ([MH]$^+$, 100%); analysis calcd for $C_{31}H_{45}NO_4$ (495.69): C, 75.11; H, 9.15; N, 2.83; found: C, 75.04; H, 9.26; N, 2.77.

*4.3.17. (4β) methyl N-acetyl-2-amino-3,11-dioxo-urs-1,12-dien-24-oate (**22**)*

A suspension of **21** (150 mg, 0.3 mmol), sodium hydride (60% dispersion in mineral oil, 120 mg, 3.0 mmol) in abs. THF (20 mL) was heated under reflux for 15 min. After cooling to 25 °C, acetyl chloride (118 mg, 1.5 mmol) was added, and the mixture was stirred for 15 min, diluted with DCM (50 mL) and water (20 mL), the pH was adjusted to 3–4 by the addition of conc. HCl, the aq. layer was extracted with DCM (2 × 50 mL), and the organic phases were combined. The solvent was removed and the residue subjected to chromatography (chloroform/ether, 8:2) to yield **22** (88 mg, 55%) as a colorless solid; mp = 133 °C; $[\alpha]_D$ = 43.6° (*c* = 6.52; $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.59 (*s*, 1H, H-1), 7.74 (*s*, 1H, N*H*), 5.61 (*s*, 1H, H-12), 3.61 (*s*, 3H, H-31), 2.66 (*m*, 1H, H-9), 2.07 (*ddd*, *J* = 13.7 Hz, 5.0, 13.7 Hz, 1H, H-16a), 2.06 (*s*, 3H, H-33), 1.88 (*ddd*,

J = 13.7, 5.0, 13.7 Hz, 1H, H-15a), 1.83 (*m*, 2H, H-6), 1.68 (*m*, 1H, H-7a), 1.66 (*dd*, J = 10.4, 2.9 Hz, 1H, H-5), 1.54 (*dd*, J = 11.2, 1.2 Hz, 1H, H-18), 1.51 (*s*, 3H, H-23), 1.48 (*m*, 1H, H-7b), 1.46 (*m*, 1H, H-22b), 1.42 (*m*, 1H, H-21a), 1.36 (*s*, 3H, H-25), 1.35 (*m*, 1H, H-19), 1.32 (*m*, 1H, H-21b), 1.28 (*m*, 1H, H-22a), 1.26 (*s*, 3H, H-27), 1.22 (*m*, 1H, H-15b), 1.19 (*s*, 3H, H-26), 1.00 (*ddd*, J = 13.7, 2.5, 2.5 Hz, 1H, H-16b), 0.93 (*m*, 1H, H-20), 0.92 (*s*, 3H, H-30), 0.80 (*s*, 3H, H-28), 0.78 (*d*, J = 6.2 Hz, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 197.5 (C-11), 191.9 (C-3), 173.2 (C-24), 168.5 (C-32), 165.3 (C-13), 141.7 (C-1), 130.1 (C-12), 128.5 (C-2), 59.1 (C-18), 55.9 (C-9), 54.2 (C-5), 53.8 (C-4), 52.4 (C-31), 45.4 (C-8), 43.9 (C-14), 40.9 (C-22), 39.3 (C-19), 39.3 (C-20), 38.0 (C-10), 33.9 (C-17), 32.4 (C-7), 30.8 (C-21), 28.8 (C-28), 27.4 (C-16), 27.2 (C-15), 24.6 (C-33), 21.6 (C-23), 21.1 (C-30), 20.5 (C-27), 18.9 (C-6), 18.7 (C-26), 17.5 (C-29), 16.6 (C-25) ppm; MS (ESI, methanol) $C_{33}H_{47}NO_5$: m/z = 538.5 ([MH]$^+$, 22%), 560.6 ([MNa]$^+$, 15%); analysis calcd for $C_{33}H_{47}NO_5$ (537.73): C, 73.71; H, 8.81; N, 2.60; found: C, 73.58; H, 9.02; N, 2.55.

4.3.18. (4β) methyl 11-oxo-urs-2,12-dien-24-oate (**23**)

To a solution of **6** (1.55 g, 3.2 mmol), triphenylphosphane (4.2 g, 16.0 mmol) and 3,3-dimethyl-glutarimide (2.3 g, 16.0 mmol) in abs. THF (20 mL) at 0 °C and DEAD (2.8 g, 16.0 mmol) were slowly added, and stirring at 25 °C continued for 12 h. The solvent was removed under reduced pressure, and the residue subjected to column chromatography (silica gel, hexane/ethyl acetate, 7:3) to yield **23** (1.51 g, 100%) as a colorless solid; mp = 185–188 °C; $[\alpha]_D$ = 187.4° (c = 5.32; $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$): δ = 5.58 (*m*, 2H, H-2, H-3), 5.56 (*s*, 1H, H-12), 3.61 (*s*, 3H, H-31), 3.06 (*dd*, J = 17.9, 3.9 Hz,1H, H-1b), 2.39 (*s*, 1H, H-9), 2.07 (*ddd*, J = 13.7, 5.0, 13.7 Hz,1H, H-16a), 1.91 (*m*, 1H, H-6a), 1.88 (*ddd*, J = 13.7, 5.0, 13.7 Hz,1H, H-15a), 1.72 (*m*, 1H, H-6b), 1.67 (*m*, 1H, H-1a), 1.63 (*ddd*, J = 12.9, 2.9, 12.9 Hz,1H, H-7a), 1.53 (*dd*, J = 11.2, 1.7 Hz,1H, H-18), 1.48 (*m*, 1H, H-22b), 1.45 (*m*, 1H, H-7b, 2H, H-21), 1.39 (*m*, 1H, H-19), 1.31 (*m*, 1H, H-22a), 1.28 (*s*, 3H, H-23), 1.27 (*s*, 3H, H-27), 1.24 (*dd*, J = 2.5, 11.6 Hz,1H, H-5), 1.21 (*m*, 1H, H-15b), 1.15 (*s*, 3H, H-26), 1.07 (*s*, 3H, H-25), 0.99 (*ddd*, J = 13.3, 2.5, 2.9 Hz,1H, H-16b), 0.94 (*m*, 1H, H-20), 0.93 (*s*, 3H, H-30), 0.81 (*s*, 3H, H-28), 0.78 (*d*, 3H, H-29, J = 6.6 Hz) ppm; ^{13}CNMR (125 MHz, $CDCl_3$): δ = 199.1 (C-11), 176.2 (C-24), 165.2 (C-13), 130.5 (C-12), 130.2 (C-3), 124.6 (C-2), 59.9 (C-9), 59.1 (C-18), 53.2 (C-5), 51.6 (C-31), 44.8 (C-4), 44.4 (C-8), 43.7 (C-14), 42.3 (C-1), 40.9 (C-22), 39.4 (C-19), 39.2 (C-20), 35.6 (C-10), 34.0 (C-17), 32.2 (C-7), 30.9 (C-21), 28.8 (C-28), 27.9 (C-23), 27.5 (C-16), 27.1 (C-15), 21.1 (C-30), 20.3 (C-27), 19.4 (C-6), 17.7 (C-26), 17.4 (C-29), 14.9 (C-25) ppm; MS (ESI, methanol) $C_{31}H_{46}O_3$: m/z = 467.5 ([MH]$^+$, 90%]), 520.8 ([M + Na + MeOH]$^+$, 26%); analysis calcd for $C_{31}H_{46}O_3$ (466.70): C, 79.78; H, 9.93; found: C, 79.62; H, 10.11.

4.3.19. (3β, 4β) 11-oxo-urs-12-en-24,3-carbolactone-(**24**)

To an ice-cold solution of **6** (200 mg, 0.43 mmol), triphenylphosphane (420 mg, 1.6 mmol) and 3,3-dimethylglutarimide (230 mg, 1.6 mmol) in abs. THF (10 mL) and DEAD (280 mg, 1.6 mmol) were slowly added. Stirring at 25 °C was continued for 12 h, the solvent evaporated, and the residue was subjected to chromatography (silica gel, DCM/methanol, 98:2) to afford **23** (111 mg, 56%) and **24** (50 mg, 28%) as an amorphous solid; data for **24**: $[\alpha]_D$ = 126.3° (c = 3.90; $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.54 (*s*, 1H, H-12), 4.38 (*dd*, J = 2.9, 9.1 Hz, 1H, H-3), 2.78 (*ddd*, J = 13.5, 2.1, 7.1 Hz, 1H, H-1b), 2.33 (*s*, 1H, H-9), 2.28 (*ddd*, J = 2.9, 7.1, 10.0 Hz, 1H, H-2b), 2.15 (*ddd*, J = 2.1, 7.1, 9.1 Hz, 1H, H-2a), 2.07 (*ddd*, J = 13.7, 5.0, 13.7 Hz, 1H, H-16a), 1.87 (*ddd*, J = 13.7, 5.0, 13.7 Hz, 1H, H-15a), 1.71 (*m*, 2H, H-6), 1.63 (*m*, 1H, H-7a), 1.53 (*dd*, J = 11.2, 1.7 Hz, 1H, H-18), 1.48 (*m*, 1H, H-22b), 1.46 (*s*, 3H, H-23), 1.44 (*m*, 1H, H-7b, 2H, H-21), 1.39 (*m*, 1H, H-19), 1.31 (*m*, 1H, H-22a), 1.30 (*s*, 3H, H-25), 1.28 (*s*, 3H, H-27), 1.20 (*s*, 3H, H-26), 1.18 (*m*, 1H, H-15b), 1.00 (*m*, 1H, H-1a, 1H, H-5, 1H, H-16b), 0.93 (*s*, 3H, H-30), 0.92 (*m*, 1H, H-20), 0.81 (*s*, 3H, H-28), 0.78 (*d*, J = 6.2 Hz, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 198.9 (C-11), 175.3 (C-24), 165.3 (C-13), 130.2 (C-12), 77.2 (C-3), 59.0 (C-9), 58.7 (C-18), 54.7 (C-4), 52.8 (C-5), 45.0 (C-8), 43.8 (C-14), 40.8 (C-22), 39.2 (C-19), 39.2 (C-20), 36.1 (C-1), 35.9 (C-10), 33.9 (C-17), 32.2 (C-7), 30.8 (C-21), 28.8 (C-28), 27.4 (C-16), 27.2 (C-15), 23.6 (C-2), 22.9 (C-23), 21.1 (C-30), 20.5 (C-27), 18.8 (C-26), 18.4 (C-6), 17.4 (C-29), 12.8 (C-25) ppm; MS (ESI, methanol) $C_{30}H_{44}O_3$: m/z = 453.3 ([MH$^+$, 100%), 475.2 ([MNa$^+$, 15%); analysis calcd for $C_{30}H_{44}O_3$ (452.67): C, 79.60; H, 9.80; found: C, 79.46; H, 9.96.

4.3.20. (2α, 4β) methyl 3,11-dioxo-2-trimethylsilyloxy-urs-12-en-24-oate (**25**) and (2α, 4β) methyl 3,11-dioxo-2-hydroxy-urs-12-en-24-oate (**26**)

From **10**: Lithium diisopropylamide [prepared from diisopropylamine (532 mg, 5.27 mmol) and *n*-BuLi (1.6 M in hexane, 3.3 mL, 5.27 mmol) in abs. THF (20 mL) at −78 °C] was added to a solution of **10** (1.0 g, 2.07 mmol) in abs. THF (10 mL) containing TMSCl (0.6 g, 5.52 mmol), and the mixture was allowed to warm to 25 °C within 2 h. After dilution with hexane (20 mL), the reaction mixture was washed with sodium hydrogen carbonate (aq., 10%, 50 mL) and water (50 mL). The solvent was removed under reduced pressure, the residue was dissolved in hexane (20 mL), and sodium carbonate (0.41 g) and *m*CPBA (590 mg, 3.42 mmol) were added, and stirring at 25 °C was continued for 12 h. The mixture was diluted with chloroform (50 mL) and washed with potassium disulfite (aq., 10%, 50 mL) and water (50 mL). The solvent was removed, and the residue was subjected to chromatography (silica gel, hexane/ethyl acetate, 8:2) to afford **25** (660 mg, 56%) and **26** (133 mg, 13%).

Compound **26** from **25**: A solution of **84** (150 mg, 0.3 mmol) in chloroform(2 mL) containing conc. aq. hydrochloric acid (0.2 mL) was stirred at 25 °C for 10 min. The solvent was removed and the residue purified by chromatography (silica gel, hexane/ethyl acetate 8:2) to yield **26** (90 mg, 60%).

Data for **25**: white, amorphous solid; $[\alpha]_D$ = 96.0° (c = 4.72; $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.55 (*s*, 1H, H-12), 4.85 (*dd*, J = 7.1, 12.9 Hz,1H, H-2), 3.67 (*s*, 3H, H-31), 3.29 (*dd*, J = 12.9, 7.1 Hz,1H, H-1b), 2.35 (*s*, 1H, H-9), 2.07 (*ddd*, J = 13.7, 5.0, 13.7 Hz, 1H, H-16a), 1.97 (*ddd*, J = 12.5, 3.3, 12.5 Hz,1H, H-6b), 1.87 (*ddd*, J = 13.7, 5.0, 13.7 Hz, 1H, H-15a), 1.80 (*m*, 1H, H-6a), 1.60 (*ddd*, J = 12.9, 3.7, 12.9 Hz, 1H, H-7a), 1.53 (*dd*, J = 11.2, 1.2 Hz, 1H, H-18), 1.47 (*m*, 1H, H-7b, 1H, H-22b), 1.42 (*m*, 2H, H-21), 1.40 (*s*, 3H, H-23), 1.38 (*m*, 1H, H-19), 1.33 (*s*, 3H, H-25), 1.30 (*m*, 1H, H-22a), 1.28 (*m*, 1H, H-1a), 1.22 (*s*, 3H, H-27), 1.18 (*s*, 3H, H-26), 1.17 (*m*, 1H, H-15b), 1.13 (*dd*, J = 1.7, 12.0 Hz,1H, H-5), 0.99 (*ddd*, J = 13.7, 2.5, 2.5 Hz,1H, H-16b), 0.93 (*m*, 1H, H-20), 0.91 (*s*, 3H, H-30), 0.80 (*s*, 3H, H-28), 0.75 (*d*, J = 6.2 Hz,3H, H-29), 0.15 (*s*, 9H, C-32 + C-33 + C-34) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 206.4 (C-3), 198.3 (C-11), 174.0 (C-24), 165.4 (C-13), 130.2 (C-12), 72.2 (C-2), 59.9 (C-9), 59.0 (C-18), 58.4 (C-5), 57.6 (C-4), 52.2 (C-31), 51.0 (C-1), 44.9 (C-8), 43.8 (C-14), 40.8 (C-22), 39.2 (C-19), 39.2 (C-20), 37.6 (C-10),33.9 (C-17), 32.7 (C-7), 30.8 (C-21), 28.9 (C-28), 27.4 (C-16), 27.2 (C-15), 21.1 (C-23), 21.0 (C-30), 20.3 (C-27), 19.8 (C-6), 18.4 (C-26), 17.4 (C-29), 14.6 (C-25), 0.0 (C-32 + C-33 + C-34) ppm; ^{29}Si-NMR (100 MHz, $CDCl_3$): δ = 19.2 (O*Si*$(CH_3)_3$); MS (ESI, methanol) $C_{34}H_{54}O_5Si$: m/z = 571.3 ([MH]$^+$, 26%), 593.5 ([MNa]$^+$, 16%); analysis calcd for $C_{34}H_{54}SiO_5$ (570.88): C, 71.53; H, 9.53; found: C, 71.44; H, 9.74.

Data for **26**: colorless solid; mp = 248–250 °C; $[\alpha]_D$ = 119.4° (c = 4.76; $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.56 (*s*, 1H, H-12), 4.79 (*dd*, J = 7.1, 12.9 Hz,1H, H-2), 3.67 (*s*, 3H, H-31), 3.52 (*dd*, J = 12.9, 7.1 Hz,1H, H-1b), 2.34 (*s*, 1H, H-9), 2.07 (*ddd*, J = 13.7, 5.0, 13.7 Hz, 1H, H-16a), 2.01 (*ddd*, J = 12.9, 3.3, 12.9 Hz, 1H, H-6b), 1.87 (*ddd*, J = 13.7, 5.0, 13.7 Hz, 1H, H-15a), 1.82 (*m*, 1H, H-6a), 1.61 (*ddd*, J = 12.9, 3.7, 12.9 Hz,1H, H-7a), 1.53 (*dd*, J = 11.2, 1.2 Hz, 1H, H-18), 1.50 (*m*, 1H, H-7b), 1.48 (*m*, 1H, H-22b), 1.45 (*s*, 3H, H-23), 1.42 (*m*,

2H, H-21), 1.38 (*m*, 1H, H-19), 1.33 (*s*, 3H, H-25), 1.30 (*m*, 1H, H-22a), 1.24 (*s*, 3H, H-27), 1.19 (*s*, 3H, H-26), 1.16 (*m*, 1H, H-5, 1H, H-15b), 1.06 (*m*, 1H, H-1a), 1.01 (*ddd*, $J = 13.7, 2.5, 2.5$ Hz, 1H, H-16b), 0.93 (*m*, 1H, H-20), 0.92 (*s*, 3H, H-30), 0.81 (*s*, 3H, H-28), 0.76 (*d*, $J = 6.6$ Hz,3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 209.2 (C-3), 198.0 (C-11), 173.1 (C-24), 165.3 (C-13), 130.2 (C-12), 71.1 (C-2), 59.7 (C-9), 59.2 (C-5), 59.0 (C-18), 57.0 (C-4), 52.4 (C-31), 50.3 (C-1), 44.9 (C-8), 43.8 (C-14), 40.8 (C-22), 39.3 (C-19), 39.2 (C-20), 37.5 (C-10), 33.9 (C-17), 32.6 (C-7), 30.8 (C-21), 28.8 (C-28), 27.4 (C-16), 27.2 (C-15), 21.1 (C-23),, 20.9 (C-30), 20.4 (C-27), 19.7 (C-6), 18.4 (C-26), 17.4 (C-29), 14.6 (C-25) ppm; MS (ESI, methanol) $C_{31}H_{46}O_5$: *m/z* = 499.5 ([MH]$^+$, 42%), 521.5 ([MNa]$^+$, 12%); analysis calcd for $C_{31}H_{46}O_5$ (498.69): C, 74.66; H, 9.30; found: C, 74.58; H, 9.42.

4.3.21. (2α, 4β) methyl 3,11-dioxo-2-hydroxy-urs-1,12-dien-24-oate(**27**)

To a solution of oxalylic chloride (107 mg, 0.84 mmol) in abs. dichloromethane (20 mL) at −78 °C, DMSO (131 mg, 1.68 mmol) in abs. dichloromethane (2 mL) was added, and the mixture was stirred for 10 min. A solution of **26** (210 mg, 0.42 mmol) in abs. dichloromethane (5 mL) was added and stirring at −50 °C continued for 2 h; triethylamine (235 μl, 1.68 mmol) was added, stirred for 30 min, and finally the reaction mixture was washed with aq. sodium carbonate (satd., 20 mL) and water (20 mL). The solvent was removed, and the residue subjected to chromatography (silica gel, chloroform/ether 9:1) to afford **27** (187 mg, 90%) as a colorless solid; mp = 220 °C; $[\alpha]_D = 161.6°$ ($c = 4.52$; $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.08 (*s*, 1H, H-1), 5.86 (*s*, 1H, O*H*), 5.59 (*s*, 1H, H-12), 3.62 (*s*, 3H, H-31), 2.63 (*m*, 1H, H-9), 2.07 (*ddd*, $J = 13.7$, 5.0, 13.7 Hz,1H, H-16a), 1.88 (*ddd*, $J = 13.7$, 5.0, 13.7 Hz,1H, H-15a), 1.83 (*m*, 2H, H-6), 1.68 (*m*, 1H, H-7a), 1.65 (*dd*, $J = 10.4$, 3.3 Hz, 1H, H-5), 1.54 (*d*, $J = 11.2$ Hz, 1H, H-18), 1.52 (*s*, 3H, H-23), 1.48 (*m*, 1H, H-7b, 1H, H-22b), 1.44 (*m*, 1H, H-21a), 1.38 (*m*, 1H, H-19), 1.34 (*s*, 3H, H-25), 1.30 (*m*, 1H, H-21b, 1H, H-22a), 1.27 (*s*, 3H, H-27), 1.21 (*m*, 1H, H-15b), 1.18 (*s*, 3H, H-26), 1.01 (*ddd*, $J = 13.7, 2.5, 2.5$ Hz,1H, H-16b), 0.93 (*m*, 1H, H-20), 0.92 (*s*, 3H, H-30), 0.80 (*s*, 3H, H-28), 0.77 (*d*, $J = 6.2$ Hz, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 198.0 (C-11), 193.2 (C-3), 173.0 (C-24), 166.0 (C-13), 143.5 (C-2), 130.3 (C-12), 129.9 (C-1), 59.1 (C-18), 56.3 (C-9), 55.4 (C-5), 53.6 (C-4), 52.4 (C-31), 45.2 (C-8), 44.0 (C-14), 40.8 (C-22), 39.3 (C-19), 39.2 (C-20), 37.9 (C-10), 33.9 (C-17), 32.5 (C-7), 30.8 (C-21), 28.8 (C-28), 27.4 (C-16), 27.2 (C-15), 21.2 (C-23), 21.1 (C-30), 20.4 (C-27), 18.9 (C-6), 18.7 (C-26), 17.3 (C-29), 16.7 (C-25) ppm; MS (ESI, methanol) $C_{31}H_{44}O_5$: *m/z* = 497.5 ([MH]$^+$, 62); analysis calcd for $C_{31}H_{44}O_5$ (496.68): C, 74.96; H, 8.93; found: C, 74.75; H, 9.03.

4.3.22. (3R, 4R) methyl 3-oxiranyl-11-oxo-urs-12-en-24-oate (**28**)

To a solution of sodium hydride (60% in mineral oil, 125 mg, 3.12 mmol) in DMSO (5 mL) trimethyl-sulfoxoniumiodide (688 mg, 3.12 mmol) in DMSO (5 mL)was added, the mixture was stirred for 30 min, and a solution of **10** (270 mg, 0.56 mmol) in DMSO (4 mL) was added and stirring continued for 12 h. After dilution with brine (20 mL) and extraction with chloroform (3 × 30 mL), the combined organic phases were washed with water (20 mL), and the solvents were removed under reduced pressure. The residue was subjected to chromatography (silica gel, chloroform/ether, 98:2) to yield **28** (145 mg, 52%) as an amorphous solid; $[\alpha]_D = 108°$ ($c = 5.36$; $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.52 (*s*, 1H, H-12), 3.66 (*s*, 3H, H-31), 2.90 (*d*, $J = 4.2$ Hz, 1H, H-32a), 2.67 (*m*, 1H, H-1a), 2.58 (*d*, $J = 4.2$ Hz, 1H, H-32b), 2.57 (*m*, 1H, H-2a), 2.43 (*s*, 1H, H-9), 2.07 (*ddd*, $J = 13.7$, 5.0, 13.7 Hz, 1H, H-16a), 1.86 (*ddd*, $J = 13.7$, 5.0, 13.7 Hz,1H, H-15a), 1.81 (*m*, 2H, H-6), 1.65 (*m*, 1H, H-7a), 1.52 (*dd*, $J = 11.2$ Hz, 1.2 Hz,1H, H-18), 1.47 (*m*, 1H, H-22b), 1.43 (*m*, 1H, H-7b, 1H, H-21a), 1.39 (*m*, 1H, H-19), 1.34 (*m*, 1H, H-1b, 1H, H-22a), 1.32 (*dd*, $J = 2.9$, 12.0 Hz, 1H, H-5), 1.30 (*m*, 1H, H-21b), 1.28 (*s*, 3H, H-27), 1.19 (*m*, 1H, H-15b), 1.17 (*s*, 3H, H-26), 1.10 (*s*, 3H, H-25), 1.03 (*m*, 1H, H-2b), 1.02 (*s*, 3H, H-23), 0.99 (*ddd*, $J = 13.3, 2.5, 2.5$ Hz, 1H, H-16b), 0.92 (*s*, 3H, H-30), 0.90 (*m*, 1H, H-20), 0.80 (*s*, 3H, H-28), 0.78 (*d*, $J = 6.6$ Hz,3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.3 (C-11), 176.3 (C-24), 165.0 (C-13), 130.5 (C-12), 60.2 (C-9), 60.0 (C-3), 59.0 (C-18), 54.3 (C-5), 51.8 (C-32), 51.6 (C-31), 47.6 (C-4), 44.8 (C-8), 43.8 (C-14), 40.9 (C-22), 39.3 (C-19, C-20), 37.6 (C-10),, 37.5 (C-1), 34.0 (C-17), 32.8 (C-7), 30.9 (C-21), 28.8 (C-28), 28.4 (C-2), 27.5 (C-16), 27.1 (C-15), 21.1 (C-30), 20.5 (C-27), 19.2 (C-6), 18.7 (C-23), 18.3 (C-26), 17.4 (C-29), 13.2 (C-25) ppm; MS (ESI, methanol) $C_{32}H_{48}O_4$: *m/z* = 497.4 ([MH]$^+$, 59%), 519.3 ([MNa]$^+$, 12%); analysis calcd for $C_{32}H_{48}O_4$ (496.72): C, 77.38; H, 9.74; found: C, 77.27; H, 9.91.

4.3.23. (3β, 4α) methyl 3-formyl-11-oxo-urs-12-en-24-oate (**29**)

To a solution of **28** (350 mg, 0.71 mmol) in abs. dichloromethane (20 mL) $BF_3.Et_2O$ (1.0 mL) was added and stirring continued at 25 °C for 12 h. The solvent was removed, and the residue subjected to chromatography (silica gel, chloroform) to afford **29** (95 mg, 19%) as a colorless, amorphous solid; $[\alpha]_D = 137.8°$ ($c = 4.14$; $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.82 (*s*, 1H, H-32), 5.53 (*s*, 1H, H-12), 3.64 (*s*, 3H, H-31), 2.86 (*ddd*, $J = 13.3, 3.3, 3.3$ Hz,1H, H-1b), 2.33 (*s*, 1H, H-9), 2.13 (*ddd*, $J = 13.7, 4.2, 13.7$ Hz, 1H, H-2a), 2.07 (*ddd*, $J = 13.7, 5.0, 13.7$ Hz, 1H, H-16a), 1.90 (*m*, 2H, H-6), 1.85 (*m*, 1H, H-15a), 1.81 (*m*, 1H, H-2b, 1H, H-5), 1.65 (*m*, 1H, H-7a), 1.52 (*dd*, $J = 11.2$, 1.2 Hz, 1H, H-18), 1.47 (*s*, 3H, H-23), 1.46 (*m*, 1H, H-22b), 1.44 (*m*, 1H, H-7b), 1.42 (*m*, 1H, H-21a), 1.38 (*m*, 1H, H-19), 1.32 (*m*, 1H, H-22a), 1.28 (*s*, 3H, H-27), 1.27 (*m*, 1H, H-21b), 1.19 (*m*, 1H, H-15b), 1.15 (*s*, 3H, H-26), 1.02 (*s*, 3H, H-25), 0.99 (*m*, 1H, H-16b), 0.96 (*m*, 1H, H-1a, 1H, H-3), 0.94 (*m*, 1H, H-20), 0.92 (*s*, 3H, H-30), 0.80 (*s*, 3H, H-28), 0.78 (*d*, $J = 6.2$ Hz, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 206.2 (C-32), 199.0 (C-11), 175.9 (C-24), 164.9 (C-13), 130.5 (C-12), 60.4 (C-9), 59.0 (C-18), 57.5 (C-3), 57.4 (C-5), 51.6 (C-31), 46.6 (C-4), 44.9 (C-8), 43.7 (C-14), 40.9 (C-22), 39.8 (C-1), 39.3 (C-19 + C-20), 37.1 (C-10), 33.9 (C-17), 32.9 (C-7), 30.9 (C-21), 28.8 (C-28), 27.5 (C-16), 27.2 (C-15), 25.5 (C-23), 21.1 (C-30), 20.4 (C-27), 20.0 (C-2), 18.6 (C-6), 18.3 (C-26), 17.4 (C-29), 13.4 (C-25) ppm; MS (ESI, methanol) $C_{32}H_{48}O_4$: *m/z* = 497.4 ([MH]$^+$, 100%); analysis calcd for $C_{32}H_{48}O_4$ (496.72): C, 77.38; H, 9.74; found: C, 77.18; H, 9.90.

4.3.24. (3β, 4α) methyl 3-carboxy-11-oxo-urs-12-en-24-oate (**30**)

Standing of **29** in the open air for 72 h led to the quant. formation of **89** as an amorphous solid; $[\alpha]_D = 102.0°$ ($c = 4.60$; $CHCl_3$);^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.53 (*s*, 1H, H-12), 3.66 (*s*, 3H, H-31), 2.86 (*ddd*, $J = 13.3, 2.9, 3.3$ Hz,1H, H-1b), 2.34 (*ddd*, $J = 13.3, 3.3, 13.3$ Hz, 1H, H-2a), 2.32 (*s*, 1H, H-9), 2.27 (*dd*, $J = 2.9, 12.9$ Hz, 1H, H-3), 2.07 (*ddd*, $J = 13.7, 5.0, 13.7$ Hz, 1H, H-16a), 1.90 (*m*, 1H, H-2b, 2H, H-6), 1.86 (*ddd*, $J = 13.7, 5.0, 13.7$ Hz, 1H, H-15a), 1.62 (*m*, 1H, H-7a), 1.52 (*dd*, $J = 11.2$, 0.8 Hz,1H, H-18), 1.49 (*s*, 3H, H-23), 1.47 (*m*, 1H, H-22b), 1.43 (*m*, 1H, H-7b, 1H, H-21a), 1.37 (*m*, 1H, H-19), 1.31 (*m*, 1H, H-21b), 1.28 (*s*, 3H, H-27), 1.26 (*m*, 1H, H-22a), 1.18 (*m*, 1H, H-15b), 1.14 (*s*, 3H, H-26), 1.03 (*s*, 3H, H-25), 0.99 (*m*, 1H, C*H* (1a), 1H, H-16b), 0.96 (*dd*, $J = 2.1$, 12.0 Hz,1H, H-5), 0.93 (*m*, 1H, H-20), 0.92 (*s*, 3H, H-30), 0.79 (*s*, 3H, H-28), 0.78 (*d*, $J = 6.2$ Hz, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.1 (C-11), 177.3 + 177.0 (C + 24 + C-32), 165.0 (C-13), 130.4 (C-12), 60.8 (C-9), 59.0 (C-18), 57.9 (C-5), 53.5 (C-3), 51.7 (C-31), 46.1 (C-4), 44.9 (C-8), 43.7 (C-14), 40.9 (C-22), 40.5 (C-1), 39.3 (C-19 + C-20), 37.1 (C-10), 34.0 (C-17), 33.0 (C-7), 30.9 (C-21), 28.8 (C-28), 27.5 (C-16), 27.2 (C-15), 26.3 (C-23), 22.2 (C-2), 21.1 (C-30), 20.4 (C-27), 18.9 (C-6), 18.3 (C-26), 17.4 (C-29), 13.9 (C-25) ppm; MS (ESI, methanol) $C_{32}H_{48}O_5$: *m/z* = 511.3 ([M−H]$^-$, 100%), 557.0 ([M + HCO_2]$^-$); analysis calcd for $C_{32}H_{48}O_5$ (512.72): C, 74.96; H, 9.44; found: C, 74.72; H, 9.69.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.01.039.

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