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The chemical and biological potential of C ring modified triterpenoids

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ABSTRACT

A convenient and elegant route has been developed to separate the natural regioisomers triterpenoids ursolic acid (**UA**) and oleanolic acid (**OA**) as well as derivatives thereof. Eleven unknown derivatives of **OA** were designed, synthesized, and their cytotoxicity was investigated. Further sixteen compounds were prepared to correlate all compounds in a SAR study. It could be shown that C-ring modifications of **OA** and **UA** have only a moderate influence onto the cytotoxic activity of the compounds but a significant impact onto the ability to trigger apoptosis in ovarian cancer cells (cell line A2780).

1. Introduction

Triterpenoids are promising candidates [1] for the development of new drugs. As products of the secondary metabolism they fulfill a function in nature in their own right. But in terms of a future use for medicinal applications, they are far off of being optimized for this use. For these highly complex molecules carrying several stereogenic centers, however, total syntheses are quite challenging but for larger scale synthesis highly uneconomic and libraries of analogs are difficult to obtain. Thus, semi-synthetic strategies are most rewarding; they shorten the time-to-market period tremendously. As a prerequisite, suitable precursors have to be gained from plant material. However, these precursors occur only in small amounts, and, in addition, they are most often part of complex mixtures. Thus, their isolation and/or separation is difficult, sometimes laborious and uneconomic. For their straightforward isolation, extractive steps have to be combined with selective derivatization reactions. The demand for these compounds has increased since clinical trials for NVX-207 [2] or CDDO-Me [3,4] have already begun.

To guarantee the successful commercial exploitation and the development of analogs, effective isolation methods are mandatory and called for. Recently, we were able to present [5] a convenient separation of regioisomeric triterpenoids, ursolic (**UA**) and oleanolic acid (**OA**) on a larger scale. These compounds (Scheme 1) are widely spread in the plant kingdom and known for their broad pharmaceutical activities, *e.g.*, for noteworthy antiviral, antibacterial and anticancer activities [6–8]. However, both molecules occur very often together in leafs or peels of plants [9]. In continuation of our previous work looking for triterpenoids of improved cytotoxic properties, the development of an easy protocol for their separation was still in the focus of our scientific interest.

Several groups were able to show that the introduction of an oxygen substituent into ring C increased the biological activity of the compounds. Due to their ability to act as "reactive oxygen species (ROS) producer", the presence of α,β-unsaturated systems seems promising. For example, for the well-known compound CDDO (2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid) several pharmaceutical activities, *e.g.*, anti-inflammatory, anti-diabetic nephropathy and cytotoxic activities, have been reported [10,11]. Furthermore, clinical trial using ester derivatives of CDDO have been started, thus increasing the demand for this class of compounds [10,12,13]. CDDO esters showed a fast first-pass metabolism; hence, there is still a necessity for improvement [12]. Recently, Leal et al. [14] presented several promising anticancer active lactones possessing an **UA** backbone.

Many data reported for the biological activity of triterpenoids, however, are hardly comparable, and the impact of modifications of the C-ring onto the cytotoxicity of the compounds still remains unclear. Thus, we became interested in studying the structure–activity relationships (SAR) of some of these derivatives combined with a more detailed inspection of their mode of action (MOA).

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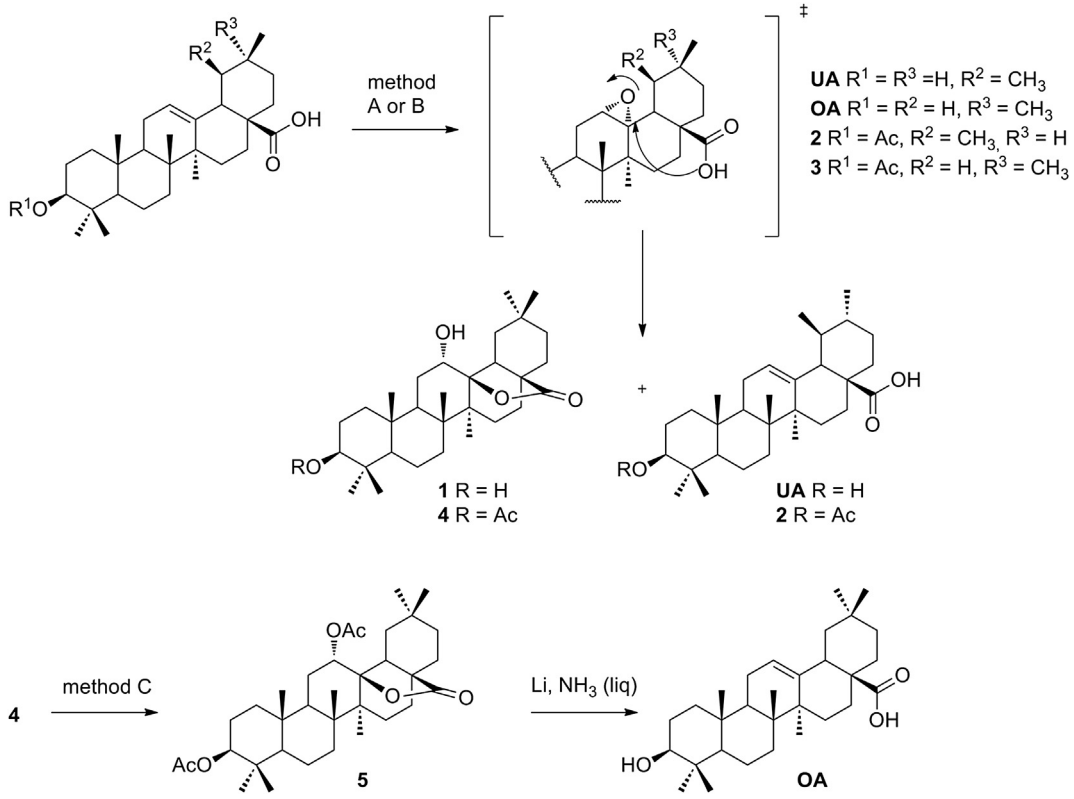


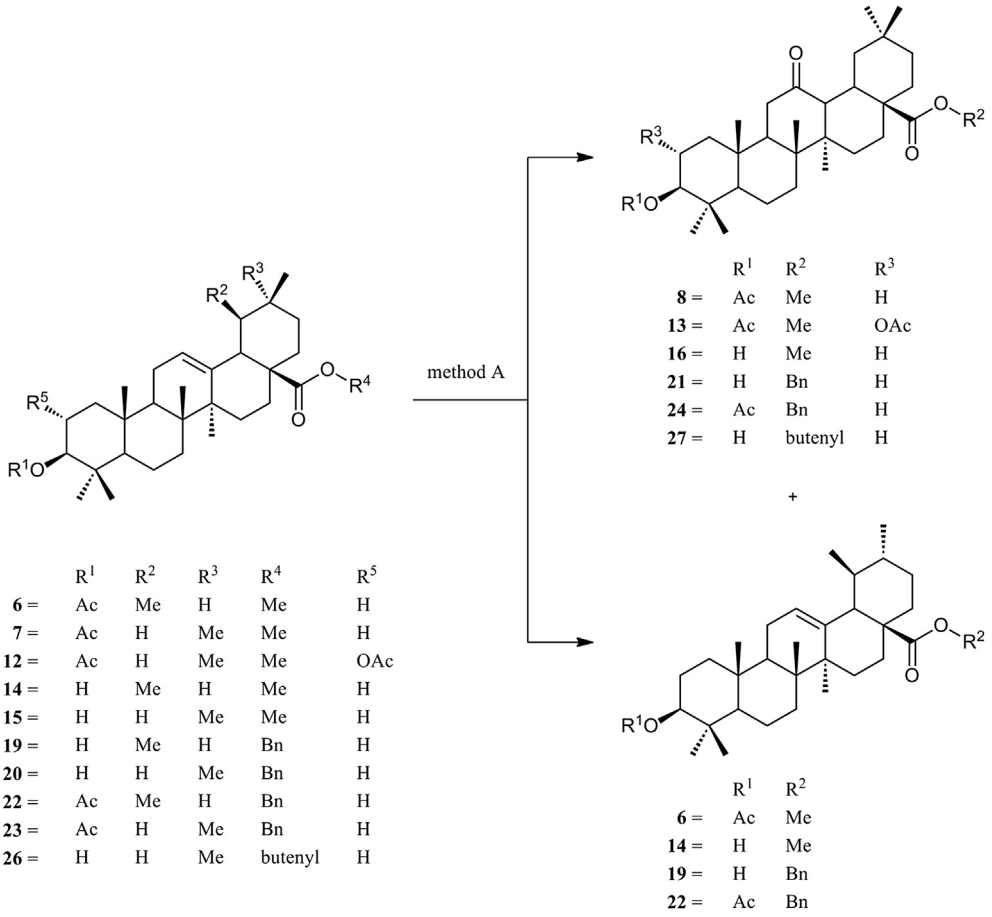
Scheme 1. Separation of a mixture of **OA**/**UA** (and derivatives thereof) and the regeneration of **OA**.

2. Chemistry

Usually the separation of regio- or diastereoisomers can easily be accomplished by chromatography, by recrystallization or by chemo-selective reactions with/without the use of enzymes. Previously, smaller amounts of **UA**/**OA** mixtures have been separated by chromatography [15,16] or by treating these mixtures with HBr [17]. Recently, we presented a new chemo-selective separation of the two constitutional isomers **UA** and **OA** by treating of a **UA**/**OA** mixture with peracids (either performic acid or *m*-chloroperbenzoic acid) [5] under mild conditions. Thus, **OA** is selectively transformed into its corresponding 12-hydroxy-lactone **1** (Scheme 1) while leaving **UA** unchanged. The formation of an epoxide from **UA** is not observed using mild conditions and low temperatures. **UA** is a very valuable starting material [18] for the synthesis of cytotoxic compounds. As early as in 1966, Barton et al. [19] showed that **OA** can be recovered by a reductive elimination (using lithium in ammonia) starting from the 12-acetoxy-lactone **5**. Compound **5** can be prepared either from the 3-hydroxy-lactone **1** by its acetylation with acetic anhydride in pyridine or from the 3-acetoxy-lactone **4**, which was accessed by the separation of a mixture of **2** and **3**. The presence of an additional sharp singulet at $\delta = 2.10$ ppm in the ^{1}H NMR spectrum of **5** indicates – together with an IR vibration band at $\nu = 1766$ cm^{-1} – the formation of **5**. Nevertheless, a low turnover rate of 20% devaluates this separation [19,20]. Consequently, we were looking for a more elegant strategy. Given the potential of C-ring modifications – as exemplified in CDDO-Me – we tried to shorten the synthetic scheme and to optimize the process of extraction and separation.

The treatment of mixtures of ester analogs from **OA**/**UA** mixtures with peracids furnished 12-oxo derivatives from **OA** exclusively but not from **UA**. Thus, **UA** esters remained unchanged under these mild reaction conditions. Moreover, this type of reaction (Scheme 2) could be used for the separation **OA**/**UA** quite universally. Neither the presence of a second double bond in the molecule (as exemplified for **26**) nor the presence of an additional hydroxyl group in ring A (as in **12**) restricted these reactions.

To investigate the influence of C-ring modifications onto the biological activity an oxygen substituent at position 11 (compounds **10** and **17**, Scheme 3) was inserted *via* an α-allylic, chromate-assisted oxidation. For compound **10**, the characteristic IR band for the newly created α,β-unsaturated system was found at $\nu = 1724$ cm^{-1}, in combination with an a signal in the ^{13}C NMR spectrum at $\delta = 200.3$ ppm. Subsequent treatment of **8**, **15** and **24** with selenium dioxide in refluxing 1,4-dioxane resulted in the formation of unknown products. Usually, this type of reaction is known [21–23] to establish a 9,11-ene-12-oxo moiety in steroid-derived structures. However, due to the presence of terminal methyl groups located at positions 26 and 27 in the backbone of the triterpenoids, an elimination of the hydroxyl group at position C-11 is not possible any longer. Hence, a keto group is created instead and rearranged into a thermodynamically more stable enol-system (Scheme 3). For this structural feature, a new signal at $\delta = 142.3$ ppm (C12) is observed in the ^{13}C NMR spectrum. Additional IR vibrations at $\nu = 1652$ and 1614 cm^{-1} confirm the structure [24]. The configuration (Scheme 3) of ring C (compounds **9** and **25**) was ascertained by the shift of the equatorial proton H-1 to higher fields. This shifting (which has already been observed in the past for glycyrrhetinic acid and was also found in compounds **10** and **17**) is caused by an anisotropy tensor of the 11-oxo function [25–27]. Furthermore, cross-peaks in gHMBC NMR experiments between C12 and C18 as well as between C9 and C11 finally confirm this structure which is further ascertained by its ESI-MS spectra ($\Delta m/z = +16$ as compared to the starting material).



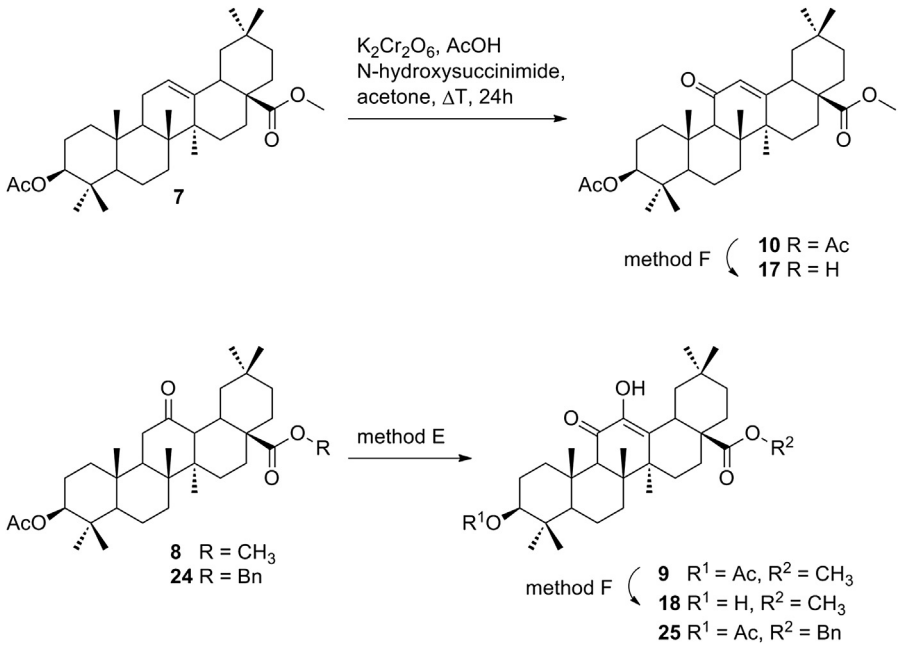
	R^1	R^2	R^3	R^4	R^5
6 =	Ac	Me	H	Me	H
7 =	Ac	H	Me	Me	H
12 =	Ac	H	Me	Me	OAc
14 =	H	Me	H	Me	H
15 =	H	H	Me	Me	H
19 =	H	Me	H	Bn	H
20 =	H	H	Me	Bn	H
22 =	Ac	Me	H	Bn	H
23 =	Ac	H	Me	Bn	H
26 =	H	H	Me	butenyl	H

	R^1	R^2	R^3
8 =	Ac	Me	H
13 =	Ac	Me	OAc
16 =	H	Me	H
21 =	H	Bn	H
24 =	Ac	Bn	H
27 =	H	butenyl	H

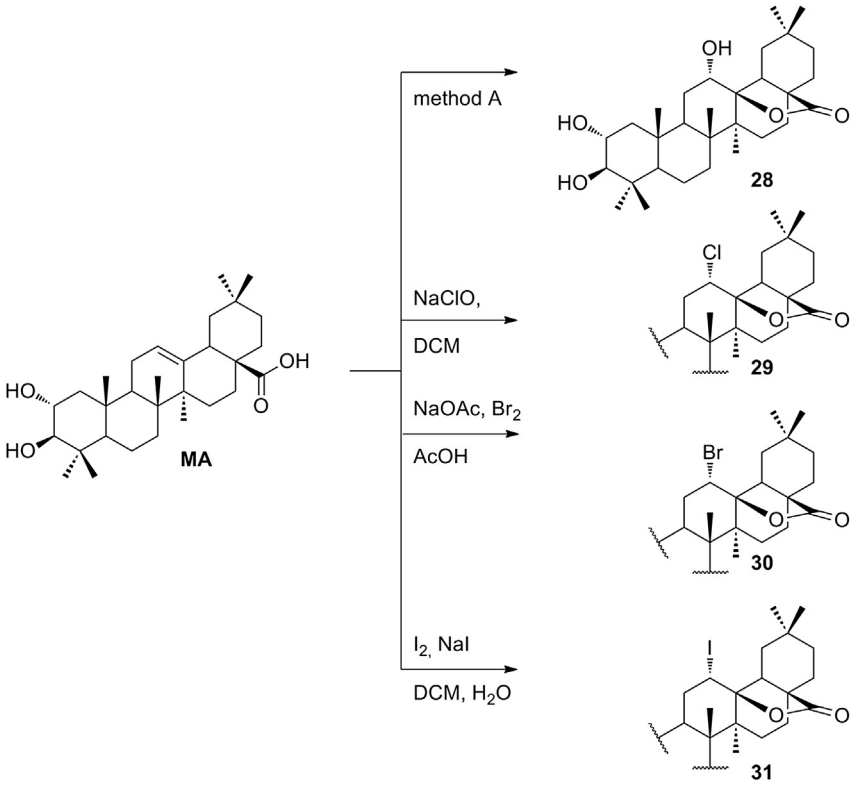
	R^1	R^2
6 =	Ac	Me
14 =	H	Me
19 =	H	Bn
22 =	Ac	Bn

Scheme 2. Separation of mixtures of the **OA**/**UA** ester analogs.

As depicted in Scheme 4, in general, the position of H-12 was assumed to be axial. By comparison with data from literature and by the presence of a cross-peak between H-12 and H-18 in the 2D NMR spectra as well as suiting coupling constants (*e.g.*, $^3J_{H12(ax)-H11(ax)} = 3.9$ Hz, $^3J_{H12(ax)-H11(eq)} = 2.3$ Hz for compound **30**) the configuration of this stereogenic center is confirmed. A characteristic IR absorption at $\nu = 1699\ cm^{-1}$ was detected for compounds **4**, **5** and **28**–**31** being typical for the presence of a lactone moiety.



Scheme 3. Oxidation of the C-28 ester analogs.



Scheme 4. Lactonization of maslinic acid (**MA**).

3. Biological investigations

Many natural occurring triterpenoids are well-known for their cytotoxic activity with IC_{50} values between 10 and 80 μM [8]. Several of them trigger apoptosis, and **OA** as well as **MA** show promising tumor-to-control selectivity. Screening of the compounds to detect their cytotoxic activity is usually performed either by an MTT [28] or a SRB [29] assay; the first of these uses the mitochondrial dependent reduction of a thiazolium salt whereas the latter is based on the proportional binding of a rhodamine dye to surface membrane proteins. Advantages of Skehan's SRB assay are found in the linearity between cell density and the optical density. The comparison of cytotoxicity measured by different assays (using different theoretical assumptions to describe more or less the same cellular feature) remains critical, and is even more complicated by numerous modifications of the original assay. Hence, we decided to include several already known compounds into this study, and to test all compounds using the same protocol and standardized conditions. Several triterpenoids have been shown to exhibit some selectivity towards cancer cells and some derivatives of **OA** are able to induce apoptosis [8]; hence, we set out to investigate the influence of the C ring onto antitumor activity, their tumor-to-control selectivity as well as onto their ability to trigger apoptosis.

Table 1 and Fig. 1 summarize some of the results. Most of the compounds exhibited a moderate to good IC_{50} value in the SRB tests. As far as the cytotoxic activity of the lactones (**4**, **5**, **28**–**32**) is concerned, the presence of a hydroxyl group seems unfavorable. For the halide-substituted compounds activity increased with the atomic radius or was reciprocally proportional to an increasing electronegativity. The importance of an intact C ring for retaining good biological activity has already been demonstrated for glycyrrhetinic acid derivatives. The mode of action of these compounds, however, has been subject to controversial discussions. Thus, on the one side the presence of an α,β-unsaturated moiety was regarded less important for the ability to produce ROS; a comparison of the cytotoxicity of a 12-oxo-9,11-ene and a 11-oxo-12,13-ene revealed no significant differences between these two compounds [30,31]. Contrary to these findings, activity was claimed to depend crucial on the presence of an intact *Michael* acceptor [31], *e.g.*, the presence of a 12-oxo-9,11-ene moiety. The significance of an intact C ring has also been discussed for the explanation of interactions between tyrosine phosphatase 1B and triterpenoids [32]. In addition, several semicarbamates derived from **1** showed an increased cytotoxicity as compared to parent OA [33]. The introduction of lipophilic moieties into ring A seems to result in better cytotoxicity of these compounds [14].

Our own data make a nucleophilic attack onto position 13 as a key step quite improbable. If an attack at position 13 should be important, one would expect an increasing activity with an increasing positive polarization of C-13 – or at least the activity should be proportional to an increasing electronegativity of the adjacent halogen substituent. Quite on the contrary, the iodolactone is the most active compound of this series. This led to the assumption that either a nucleophilic attack at position 12 or a stereoelectronic hindrance by this substituent must be essential. Our results parallel previous findings of Salvador et al. [33] suggesting a nucleophilic attack.

Comparison of the IC_{50} values of the products resulting from the oxidation of the **OA** esters (Fig. 1) indicate that introducing an extra oxygen seems less important for obtaining good cytotoxicity than variations at *e.g.*, position 3. Cytotoxicity of the **OA** derivatives can be raised by these modifications up to ca. 111 fold [33]. Inspection of the IC_{50} values (listed in Table 1) shows oxidation in ring C by and large insignificant for obtaining good cytotoxic effects. These findings are in perfect agreement with results obtained by Chadalapaka

Table 1
Cytotoxicity of **OA**, **MA** and derivatives (IC_{50} values in μM from SRB assays after 96 h of treatment; the values are averaged from at least three independent experiments performed each in triplicate; confidence interval CI = 95%; the individual errors (positive upper and negative lower values are given; n.D. not detected). Cell lines 518A2, 8505C, A2780, A549, HT29 and MCF are human cancer cell lines, NiH 3T3 cells are a non-malignant mouse fibroblasts, STP is stauroporine.

IC_{50}	518A2		8505C		A2780		A549		HT29		MCF7		NiH 3T3	
OA	>60	0.0	>60	0.0	14.0	1.1	72.3	3.5	38.8	5.4	>60	0.0	76.4	1.5
		0.0		0.0		1.1		1.9		4.8		0.0		1.4
MA	13.7	1.9	17.0	2.1	19.5	1.8	23.4	0.6	28.8	1.0	16.6	0.9	21.4	0.7
		1.7		1.9		1.7		0.6		0.9		0.8		0.7
1	>30	0.0	>30	0.0	>30	0.0	>30	0.0	>30	0.0	>30	0.0	>30	0.0
		0.0		0.0		0.0		0.0		0.0		0.0		0.0
		0.0		0.0		0.0		0.0		0.0		0.0		0.0
4	>90		>90		63.2	2.1	>90		>90		>90		85.7	4.5
						2.5								2.3
5	6.8	0.0	7.0	0.0	5.6	0.3	6.6	0.0	14.1	2.3	18.5	0.3	7.2	0.1
		0.0		0.0		0.3		0.0		2.0		0.3		0.1
7	34.1	1.9	20.5	0.8	8.6	1.3	26.1	3.2	26.7	5.9	16.7	1.0	34.3	4.1
		1.8		0.8		1.1		2.8		4.9		1.0		3.7
8	40.8	5.5	31.1	5.8	>30		>30	0.0	46.8	2.9	>30	0.0	28.2	0.3
		4.8		4.9				0.0		2.8		0.0		0.3
9	7.7	0.1	8.4	0.6	4.0	0.7	8.2	0.4	13.5	2.7	9.4	0.4	12.5	1.3
		0.1		0.5		0.6		0.4		2.2		0.4		1.2
10	24.1	2.0	16.2	4.6	40.0	1.7	66.1	0.0	29.4	6.0	0.0	0.0	33.6	2.6
		1.9		3.6		1.6		0.0		5.0				2.4
11	15.6	1.3	14.7	1.3	17.8	4.3	17.8	0.5	12.8	1.5	16.3	0.9	21.4	0.7
		1.2		1.2		3.4		0.4		1.3		0.8		0.7
12	8.6	1.8	13.3	3.0	6.8	2.3	7.8	2.1	26.3	1.1	>30		30.5	1.6
		1.5		2.5		1.7		1.7		0.7				1.5
13	9.6	0.0	10.3	0.1	11.6	0.9	14.1	0.9	17.6	1.2	23.8	1.9	31.7	0.3
		0.0		0.1		0.8		0.8		1.1		1.8		0.3
14	17.7	0.6	20.6	2.2	15.3	3.1	20.2	0.0	25.4	0.4	22.2	2.1		
		0.6		2.0		2.6		0.0		0.4		1.9		
17	15.6	0.1	13.0	1.8	6.6	1.2	15.1	0.2	18.5	0.3	21.3	0.1	24.8	0.2
		0.1		1.6		1.0		0.2		0.3		0.1		0.2
18	21.2	0.2	23.3	0.5	17.5	1.2	25.6	1.3	15.3	1.4	25.8	1.7	25.8	0.0
		0.2		0.5		1.1		1.2		1.3		1.6		0.0
20	28.0	2.1	29.1	1.5	23.0	1.7	28.0	2.6	28.7	2.5	15.8	0.9	22.4	3.0
		2,0		1,7		1,8		2,4		2,3		0,8		2,7
24	73.2	1.6	41.9	6.5	19.8	5.7	69.1	3.9	>90	0.0	>90	0.0	>90	0.0
		1.6		5.6		4.5		3.7		0.0		0.0		0.0
25	>60		16.4	1.4	8.2	3.0	>60		>60	0.0	>60	0.0	>60	0.0
				0.8		2.2				0.0		0.0		0.0
28	78.4	2.5	>90		39.9	1.0	>90		92.8	0.3	>90		22.1	2.3
		3.8				0.9				0.3				3.9
29	n.D.	0.0	18.0		34.2	3.8	33.1		509.7		n.D.	0.0	>90	
		0.0				3.4						0.0		
30	n.D.	0.0	36.5	2.7	25.9	4.3	29.1	2.2	>90		n.D.	0.0	43.1	8.5
		0.0		2.5		3.7		2.7				0.0		7.1
31	n.D.	0.0	72.1	7.0	31.5	3.8	34.4	3.6	>90		n.D.	0.0	80.2	2.1
		0.0		6.4		3.4		3.2				0.0		1.9
32	n.D.	0.0	22.2	0.4	15.5	0.7	16.4	1.9	46.9	3.2	n.D.	0.0	29.2	4.4
		0.0		0.4		0.7		1.7		3.0		0.0		3.8
STP	0.2	0.0	0.2	0.0	0.2	0.0	0.6	0.0	0.2	0.0	0.4	0.0	0.2	0.0
		0.0		0.0		0.0		0.0		0.0		0.0		0.0

et al. [34]; these authors were able to show that the location of an α,β-unsaturated system ($\Delta^{9,11}$ or $\Delta^{12,13}$) is insignificant for obtaining good cytotoxicity.

However, a comparison of IC_{50} values is not sufficient to describe the biological activity of these compounds. To get a deeper insight, some representative molecules were selected and submitted to several MOA investigation assays applying the same concentration (30 μM) for all compounds. Fig. 2 shows some results from cell cycle investigations.

To evaluate whether the activity (as shown in the SRB assay) is caused by a cytostatic effect or rather by a cytotoxic effect, living ovarian cancer cells were investigated after 24 h of treatment with the compounds. A significant G1/G0 arrest (as previously found for parent maslinic acid, unpublished data) or ursolic acid [14], was not detected at all. A weak G0/G1 arrest (<20%) was observed for compounds **8** and **25**. Compounds **4**, **5** or **7**, however, showed practically no influence onto the cell cycle. The treatment of the cells with compounds **1**, **9** or **24** led to a reduction of the G2/M phase; compounds **1** or **24** were the most active compounds of this series.

Obviously at least compounds **4**, **5** and **7** are cytotoxic, and hence investigations of the dead cells seemed highly interesting. Usually an undesired necrotic cell death (happening most accidentally) is described by a loss of the integrity of the cell membrane [35]. The integrity of the cell membrane can be investigated by a microscopic fluorescence dye-exclusion assay (AO/PI; Fig. 3). In this test, acridine orange (AO) as an un-polar green fluorescence dye permeates the cell membrane and stains dead cells possessing an intact membrane [36]. Thus, cells having died by activating a programmed cell death show green fluorescence. Propidium iodide (PI) [37], however, a double positive charged dye, stains only dead cells with a broken cell membrane; hence, a deep red color is observed.

As depicted in Fig. 4, all dead cells had died due to a programmed process. Several death mechanisms are known [38]; for

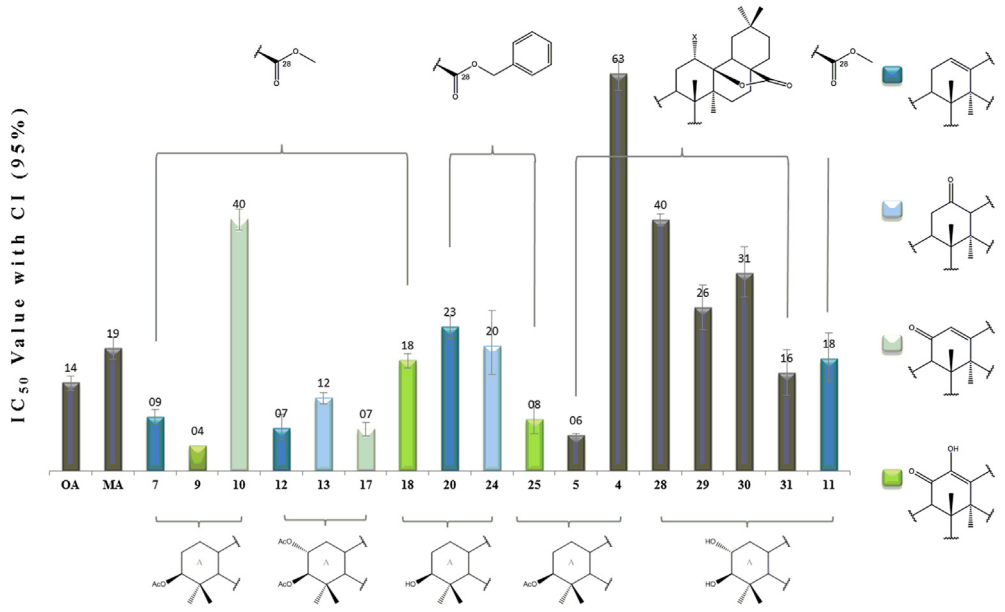


Fig. 1. IC_{50} values (in μM from SRB with a confidence interval (95%) for **OA**, **MA** and derivatives utilizing the human ovarian cancer cell line A2780. The colors of the bars indicate the type of modification at positions C-3 and C-28. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

triterpenoids most commonly apoptosis [8] as well as autophagy [39] was reported.

Microscopic inspection of the dead cells allowed a preliminary evaluation: membrane blebbing and condensed nuclei as well as cell shrinking indicated apoptosis [40]. Staining the cells with AO/PI gave red dots – a sign for at least partial autophagy [41]. Fig. 3 depicts representative microscopic views of the cells, and significant morphologic changes have been marked using different colored arrows. One unique feature of apoptosis is the activation of ICAD [42]; this results in the degradation of DNA into several 178 bp long pieces (Fig. 3). After 24 h of treatment only compounds **5** and **17** showed significant DNA ladders as indicated by electrophoresis and staining of the DNA. In the electrophoretic gels for compounds **1**, **9** and **10** only weak ladders were found.

Membrane integrity and DNA-laddering are typical hallmarks for an apoptotic death; another hallmark of apoptosis is the translocation of phosphatidylserine. This compound is located on the inner side of the cell and translocates to the outer cell membrane during apoptosis thus acting as a signal for macrophages [43]. During apoptosis several executing proteinases, also known as caspases, are activated in a highly controlled manner. During this lethal caspase-cascade process other enzymes get activated and/or essential parts of the cell skeleton are broken down leading to a subsequent exposition of the phosphatidylserine. This process can be measured using an annexin V assay. By double staining and submitting the stained cell population to a FACS measurement the distribution of vital *versus* dead cells can be determined. Fig. 4 shows the result of one of these assays. The experimental data were evaluated by plotting the green fluorescence (belonging to the annexin V-FITC) *versus* the red one (belonging to propidium iodide) and splitting these plots into four quadrants. While PI stained only cells possessing a leak membrane, red labeled cells (upper left) are usually regarded as necrotic cells. Cells carrying both signals (upper right) are assumed to be secondary necrotic; this phenomenon is known to occur for cells held in cell cultures.

As indicated in Fig. 4, the treatment of the cells with **17** or **18** triggered apoptosis after incubating for 24 h in >50% of the cell population. In addition, 3,12-diacetylated **5** as well as compound **9** triggered apoptosis. These results are in excellent agreement to the results from the DNA ladder assays; therein, after treatment of the cells with **5**, **9**, **17** or **18** the typical DNA ladders were found. Furthermore, the ability of **9**, **17** and **18** to trigger apoptosis affirms that the presence of an α,β-unsaturated system in ring C seems necessary for inducing apoptosis very quickly.

The ability to trigger apoptosis is independent from the IC_{50} values; although compound **9** exhibited a lower IC_{50} value in the SRB tests than compound **18**, the latter of which showed a significantly higher ability to trigger apoptosis. The drop down of cytotoxicity found for compounds **24** and **25** might be explained by the low polarity of these compounds. The presence of an increased necrotic population after having treated the cells with **9** might be due to a concentration dependent toxic effect. Hence, **OA** methyl esters possessing a modified ring C are promising compounds possessing the ability to induce programmed cell death very quickly.

4. Conclusion

A convenient method for separating a mixture of regioisomeric triterpenoids **UA**/**OA** was found. This separation technique using per-acids was shown to be quite universal. **UA** could be recovered very easily from the mixtures, and the intermediates were transformed into compounds showing promising antitumor activity. In addition, different MOAs were found for compounds differing only slightly in their structure. As far as the OA derived lactone 1 is concerned, the introduction of an additional hydroxy substituent at position C-2 (as in 28) as well as of a lipophilic moiety at position C-12 (as in 5) decreases the IC50 values significantly. An additional oxygen substituent in ring C increases the cytotoxicity, too. Thus, the highest activity was determined for the previously unknown 11-oxo-12-hydroxy-olean-12-enes **17** and **18**, respectively.

5. Experimental part

5.1. General – chemistry

Reagents were bought from commercial suppliers without any further purification. Melting points were measured with a LEICA hot stage microscope and were not corrected. NMR spectra were recorded on VARIAN Gemini 2000 or Unity 500 spectrometers at

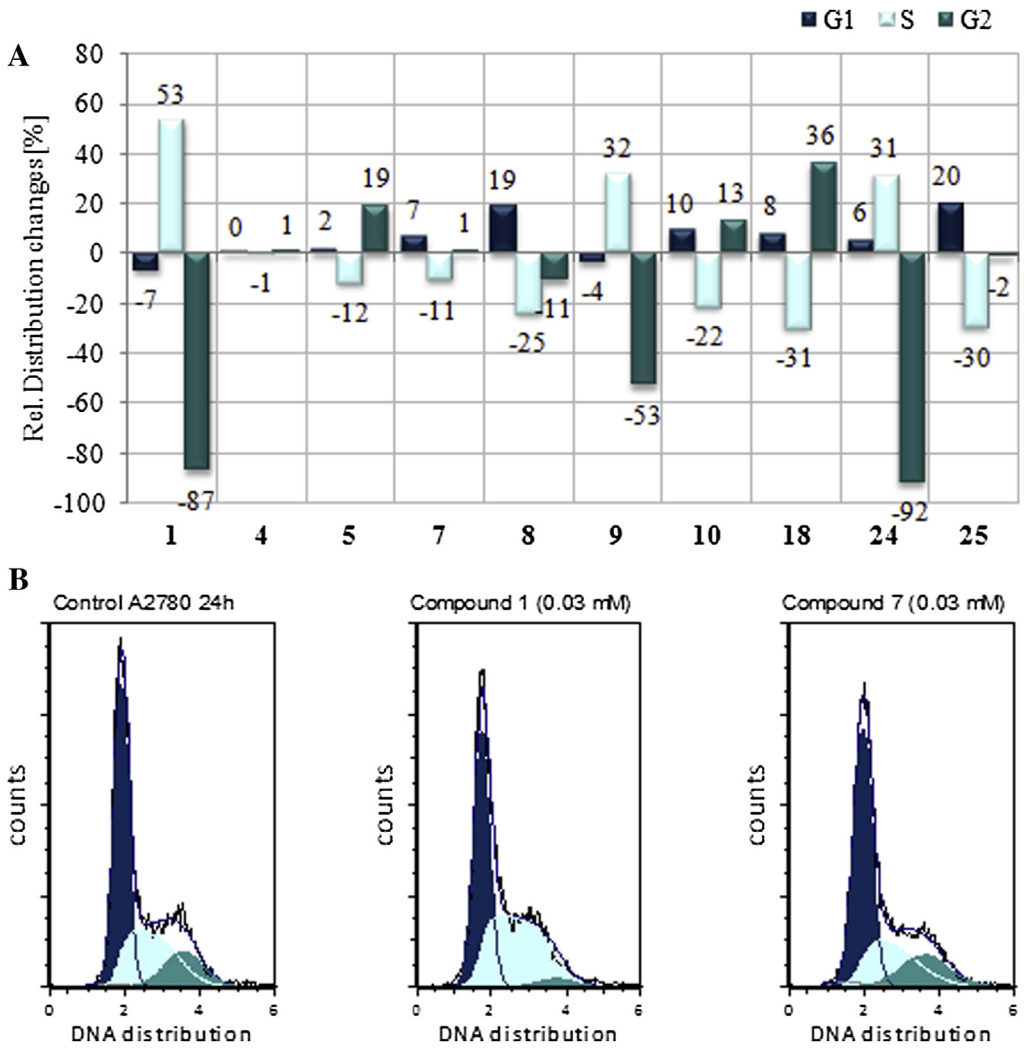


Fig. 2. Cell cycle investigations. Ovarian cancer cells (A2780) were treated for 24 h with a 30 μM solution of compounds **1**, **4**, **5**, **7**–**10**, **18**, **24** or **25**, respectively. The living cells were harvested, fixed, the population was aligned and submitted to a FACS supported measurement. The DNA was dyed with PI after treatment with RNAse. In the upper part of the figure (A) the relative changes to control have been depicted; the lower part (B) shows typical graphs for the cell cycle distribution.

27 °C (chemical shifts δ are given in ppm and J in Hz. Mass spectra were taken on a FINNIGAN MAT TSQ 7000 (electrospray, voltage 4.5 kV, sheath gas nitrogen) instrument. Elemental analyses were measured on a Foss-Heraeus Vario EL unit. IR spectra were recorded on a Perkin Elmer FT-IR spectrometer Spectrum 1000, optical rotations on a Perkin Elmer 341 polarimeter (1 cm micro cell, 25 °C) and UV–vis spectra on a Perkin Elmer unit, Lambda 14. Elemental analysis was performed for all new compounds and correct values (±0.3 for C, H, N) were obtained. TLC was performed on silica gel (Merck 5554, detection with ceriummolybdate spray reagent). Solvents were dried according to usual procedures. The purity of the compounds was checked by HPLC/DAD (Prontosil C18, MeOH/H_2O 95/5, 1% H_3PO_4) and found to be >98% for each compound.

5.2. General procedures

5.2.1. Oxidation with hydrogen peroxide and formic acid (method A)

To a mixture of ursolic acid and oleanolic acid (1 equiv., 2:1) in DCM, formic acid (concd, 2 equiv.)/H_2O_2 (30%, 2 equiv.) was added, and the mixture was stirred at 25 °C for 24 h. After washing with an aq soln. of sodium thiosulfate, and usual work up, the solvents were evaporated under diminished pressure. The residue was subjected to chromatography (silica gel, hexane/ethyl acetate).

5.2.2. Oxidation with mCPBA (method B)

To a mixture of ursolic acid and oleanolic acid (1 equiv., 2:1) in DCM *m*CPBA (1.5 equiv.) was added, and the mixture was stirred at 25 °C for 24 h. After washing with an aq soln. of sodium thiosulfate and usual workup, the solvents were evaporated under diminished pressure. The residue was subjected to chromatography (silica gel, hexane/ethyl acetate).

5.2.3. Acetylation (method C)

Acetylation was performed in dry DCM (100 mL) with acetic anhydride (2 equiv.) and triethylamine (0.3 equiv.) for 12 h at 24 °C and gave, after usual work-up and recrystallization from ethanol, the product.

5.2.4. Esterfication (method D)

The starting material (1 equiv.) was dissolved in dry DMF (5 mL), and finely grounded potassium carbonate (5 equiv.) was added. After 60 min of stirring at room temperature, the alkylbromide (2

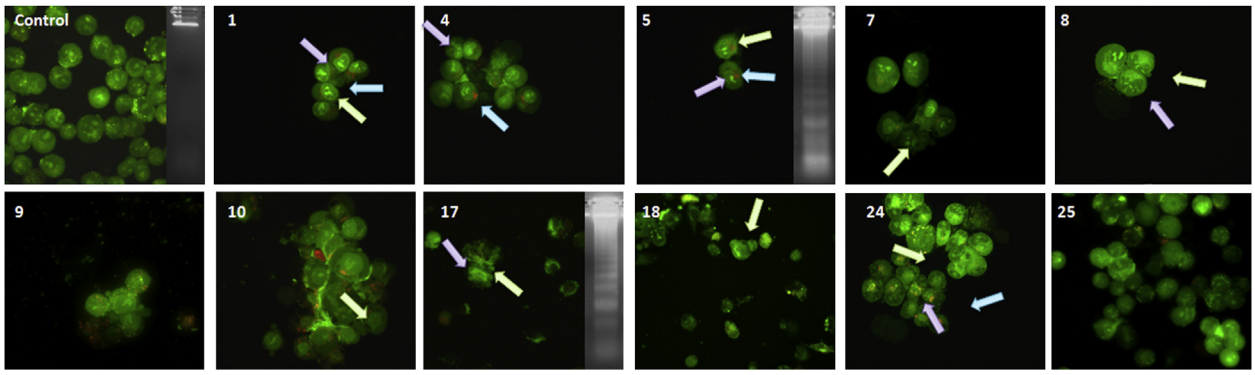


Fig. 3. Dye exclusion (AO/PI) assay and representative DNA laddering experiments. Ovarian cancer cells (A2780) were treated with the compounds **1**, **4**, **5**, **7**–**10**, **17**, **18**, **24** or **25** (30 μM each, 24 h). The floating (dead) cell cells were collected, washed with PBS and submitted to a fluorescence microscopic investigation. Green dyed cells are typical for a programmed, non-necrotic death. Light green colored arrows indicate typical membrane blebbing; light purple arrows indicate the typical membrane blebbing of the nucleus membrane, and the blue arrows point to orange colored dots which could be autophagosomes and indicate an autophagy mediated apoptosis. In addition, the results from the DNA laddering experiment are shown for a control experiment as well as for the experiments using compounds **5** and **17**, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

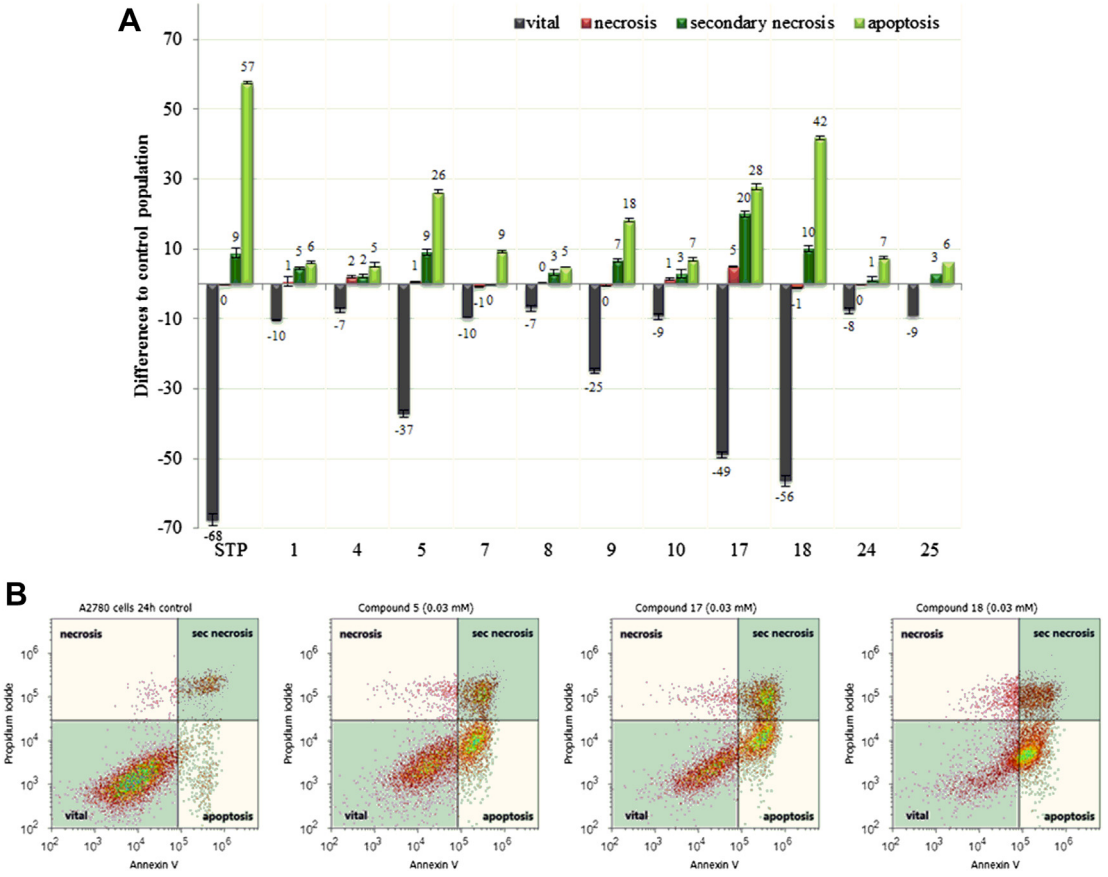


Fig. 4. Annexin V/PI assay: Ovarian cancer cells (A2780) were treated with compounds **1**, **4**, **5**, **7**–**10**, **17**, **18**, **24** or **25** (30 μM each), respectively. After 24 h, all cells were harvested and submitted to a FACS based assay. Diagram A (upper part) shows the relative changes in the distribution compared to control. Measurements were performed as technical replicates. Typical samples of these experiments are depicted in the lower part (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

equiv.) was added, and stirring was continued for an additional 18 h. The mixture was poured into an ice cold solution of aq HCl (3.7%, 50 mL), and the white precipitate was filtered off. Chromatographic purification (silica gel, hexane/ethyl acetate, 7:3) and recrystallization (ethanol) afforded the product.

5.2.5. Oxidation with selenium dioxide (method E)

Equal molar amounts of selenium dioxide and the 12-oxo compound were stirred in refluxing 1,4-dioxane for 24 h. After filtration, an aq solution of sodium thiosulfate was added, and the reaction mixture was extracted with DCM. After following usual

workup, the crude product was subjected to a chromatographic purification followed by recrystallization from ethanol.

5.2.6. Deacetylation (method F)

The acetylated starting material (1 equiv.) was dissolved in methanol. Finally grounded KOH (1.2 equiv.) was added, and the mixture was stirred at room temperature until completed as checked by TLC. The mixture was poured into a cold aq solution of HCl (3.7%), and the precipitate was collected. Usual work up furnished the product.

5.3. Biological material and procedures

5.3.1. Cell lines and culture conditions

The cell lines 518A2, 8505C, A2780, A549, HT29, MCF-7, NiH 3T3 and WW030272 were included in this study. Cultures were maintained as monolayer in RPMI 1640 (PAA Laboratories, Pasching, Germany) supplemented with 10% heat inactivated fetal bovine serum (Biochrom AG, Berlin, Germany) and penicillin/streptomycin (PAA Laboratories) at 37 °C in a humidified atmosphere of 5% CO_2/95% air.

5.3.2. Cytotoxicity assay, dye-exclusion assay, DNA-laddering and apoptosis assay

Instrumentation, cell lines and culture conditions, cytotoxicity assay, dye exclusion tests and DNA fragmentation was performed as previously described [44,45].

5.3.3. Cell cycle investigation

Approximately $1*10^6$ cells (HT29, A2780 or NIH 3T3) were seeded in cell culture flasks (25 cm^2), and the cells were allowed to grow for 24 h. After removing of the used medium, the substance loaded medium was reloaded (or a blank fresh medium as a control). After 24 or 48 h, the living cells were harvested, washed with PBS (with Mg^{2+} and Ca^{2+}) twice and fixed with ethanol (70%, 4 °C, 1 h). After removing of the fixation and permeabilization agent, the cells were washed with PBS buffer (with Mg^{2+} and Ca^{2+}, containing 1% BSA and 0.1% NaN_3, 3 × 1 mL, 1000 rpm) and adjusted to $1*10^5$ million cells. The pellet was gently suspended in staining buffer (PBS buffer containing BSA, RNAase, NaN_3 and PI following the procedure of Darzynkiewicz et al. [41]) and incubated for 30 min at 37 °C. Analyses were performed using the Attune® FACS machine; collecting data from the BL-2A channel. Doublet cells were excluded from the measurements by plotting BL-2A against BL-2H. For each cell cycle distribution 20.000 events were collected. Distribution was calculated by the method of Dean et al. [46].

5.3.4. Annexin V/PI assay

Approx. 500,000 cells (A2780) were seeded in cell culture flasks and were allowed to grow for 1 day. After removing of the medium, the substance loaded medium was added, and the flasks were incubated for about 24 h. All cells were harvested, centrifuged (1200 rpm, 5 min) and washed twice (PBS (w/w). Approx. 100,000 cells were washed with annexin V bounding buffer (BD) and treated with a propidium iodide solution (3 μL, 50 μg/mL) and Annexin V (5 μL, life technologies™) for 15 min at room temperature in the dark. After adding Annexin V bounding buffer (400 μL), the suspension was submitted to a FACS measurement. Calculation was performed as suggested from the supplier (BD Biosciences®).

5.4. Syntheses

*5.4.1. Ursolic acid (**UA**)*

By method **A** or **B**, **UA** was obtained from a mixture of **UA**/**OA** (ca. 2:1); yield: 57%; m.p.: 257–259 °C (lit.: [47]: 258°); R_F = 0.62 (*n*-hexane/ethyl acetate, 5:3); ^{1}H NMR (400 MHz, d_6 – pyridine): δ = 5.45 (*dd*, *J* = 3.5, 3.5 Hz, 1H, C*H* (12)), 3.43 (*dd*, *J* = 9.6, 6.5 Hz, 1H, C*H* (3)), 2.60 (*d*, *J* = 11.2 Hz, 1H, C*H* (18)), 2.29 (*ddd*, *J* = 13.3, 13.3, 4.5 Hz, 1H, CH_a (15)), 2.09 (*ddd*, *J* = 13.3, 13.3, 4.1 Hz, 1H, CH_a (16)), 1.98–1.90 (*m*, 5H, CH_b (16) + CH_2 (7) + CH_2 (11)), 1.88–1.77 (*m*, 2H, CH_2 (2)), 1.62–1.51 (*m*, 4H, C*H* (9) + CH_a (6) + CH_a (22) + CH_b (1)), 1.46–1.39 (*m*, 3H, C*H* (19) + CH_2 (21)), 1.38–1.25 (*m*, 2H, CH_b (6) + CH_b (22)), 1.21 (*s*, 3H, CH_3 (27)), 1.20 (*s*, 3H, CH_3 (23)), 1.23–1.11 (*m*, 1H, CH_b (15)), 1.02 (*s*, 3H, CH_3 (30)), 0.99 (*s*, 3H, CH_3 (25)), 0.97 (*d*, 3H, ^3J = 6.8 Hz, CH_3 (29)), 0.92 (*d*, 3H, ^3J = 6.1 Hz, CH_3 (24)), 0.86 (*s*, 3H, CH_3 (26)), 0.82 (*m*, 1H, C*H* (5)) ppm; ^{13}C NMR (400 MHz, d_6 – pyridine): δ = 180.1 (*C*=O, C28), 139.5 (*C*=CH, C13), 125.9 (*C*H=C, C12), 78.4 (*C*HOH, C3), 56.1 (*C*H, C5), 53.8 (*C*H, C18), 48.3 (*C*H, C9), 48.3 (C_{quart}, C17), 42.7 (C_{quart}, C14), 40.2 (C_{quart}, C8), 39.7 (*C*H, C19), 39.7 (*C*H, C20), 39.6 (C_{quart}, C4), 39.3 (CH_2, C1), 37.7 (CH_2, C22), 37.5 (C_{quart}, C10), 33.8 (CH_2, C7), 31.3 (CH_2, C21), 29.1 (CH_3, C23), 28.9 (CH_2, C15), 28.4 (CH_2, C2), 25.2 (CH_2, C16), 24.2 (CH_3, C27), 23.8 (CH_2, C11), 21.7 (CH_3, C30), 19.0 (CH_2, C6), 17.7 (CH_3, C29), 17.7 (CH_3, C26), 16.8 (CH_3, C24), 15.9 (CH_3, C25) ppm; ESI-MS (MeOH): *m*/*z* = 456.6 (44%, $[M - H]^-$), 501.2 (100%, $[M + HCO_2]^-$), 911.0 (44%, $[2M - H]^-$).

*5.4.2. (3β, 12α) 3,12 Dihydroxy-18β-olean-28-oic acid 28,13-lactone (**1**)*

By using method **A** or **B**, compound **2** was obtained from a mixture of **UA**/**OA** (ca. 2:1); yield: 10%; m.p.: 249–252 °C (lit.: [32]: 247–249 °C); R_F = 0.25 (hexane/ethyl acetate, 5:3); $[\alpha]_D$ = +32.4° (*c* 0.35, $CHCl_3$); IR (KBr): ν = 3668*w*, 3524*s*, 2947*s*, 2867*s*, 1736*s*, 1470*m*, 1396*m*, 1362*m*, 1306*w*, 1255*m*, 1226*m*, 1176*w*, 1146*m*, 1092*w*, 1077*m*, 1060*m*, 1037*m* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.83–3.80 (*dd*, *J* = 2.8, 2.8 Hz, 1H, C*H* (12)), 3.15 (*dd*, *J* = 11.4, 4.4 Hz, 1H, C*H* (3)), 2.07 (*ddd*, *J* = 13.3, 5.8, 5.8 Hz, 1H, CH_a (16)), 2.01–1.88 (*m*, 3H, C*H* (18) + CH_a (19) + CH_a (11)), 1.86–1.75 (*m*, 2H, CH_b (19) + CH_a (15)), 1.66 (*ddd*, *J* = 12.8, 12.8, 3.5 Hz, 1H, CH_a (1)), 1.59–1.51 (*m*, 3H, CH_2 (2) + CH_a (22)), 1.53–1.48 (*m*, 1H, C*H* (9)), 1.49–1.44 (*m*, 2H, CH_a (6) + CH_a (7)), 1.42–1.37 (*m*, 2H, CH_b (11) + CH_b (16)), 1.35–1.30 (*m*, 1H, CH_b (21)), 1.24 (*s*, 3H, CH_3 (27)), 1.22–1.15 (*m*, 4H, CH_2 (15) + CH_b (7) + CH_b (6)), 1.12–1.09 (*m*, 1H, CH_b (22)), 1.08 (s, 3H, CH_3 (26)), 0.92 (*s*, 3H, C*H* (23)), 0.91 (*s*, 3H, C*H* (29)), 0.92–0.87 (*m*, 1H, CH_b (21)), 0.83 (*s*, 3H, C*H* (30)), 0.81 (*s*, 3H, C*H* (25)), 0.71 (*s*, 3H, C*H* (24)), 0.68 (*dd*, *J* = 11.8, 1.9 Hz, 1H, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 179.9 (*C*=O, C28), 90.6 (C_{quart}, C13), 78.9 (*C*HOH, C3), 76.4 (*C*H, C12), 55.2 (*C*H, C5), 51.1 (*C*H, C18), 44.7 (C_{quart}, C17), 44.6 (*C*H, C9), 42.3 (C_{quart}, C14), 42.1 (C_{quart}, C8), 39.4 (CH_2, C19), 38.9 (CH_2, C1), 38.9 (C_{quart}, C4), 36.5 (C_{quart}, C10), 34.2 (CH_2, C21), 34.0 (CH_2, C7), 33.3 (CH_3, C29), 31.6 (C_{quart}, C20), 28.8 (CH_2, C11), 28.1 (CH_3, C23), 28.1 (CH_2, C15), 28.0 (CH_2, C22), 27.5 (CH_2, C2), 23.9 (CH_3, C30), 21.2 (CH_2, C16), 18.6 (CH_3, C27), 18.5 (CH_3, C26), 17.8 (CH_2, C6), 16.3 (CH_3, C25), 15.4 (CH_3, C24) ppm; MS (ESI, MeOH): *m*/*z* = 473.5 (27%, $[M + H]^+$), 495.6 (15%, $[M + Na]^+$), 527.1 (26%, $[M + Na + MeOH]^+$), 967.3 (100%, $[2M + Na]^+$).

*5.4.3. (3β) 3-Acetoxy-urs-11-en-28-acid (**2**)*

Compound **2** was obtained as a colorless solid using method **A** from a mixture of acetylated **2** and **3.** The latter compounds were obtained either using method **C** or directly from **UA** applying method **C**; yield: 88%; m.p.: 257–259 °C (lit.: 258 °C [47]); $[\alpha]_D$ = +69° (*c* 0.19, $CHCl_3$); R_F = 0.64 (*n*-hexane/ethyl acetate, 8:2); UV–Vis (MeOH): λ_{max} (log ε) = 224 nm (3.71); IR (KBr): ν = 3423*br*, 2947*s*, 1736*s*, 1696*s*, 1464*m*, 1366*m*, 1245*s*, 1180*w*, 1148*w*, 1097*w*, 1076*w*, 1028*m*, 1009 *m* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.26 (*m*, 1H, C*H* (12)), 4.48 (*dd*, *J* = 9.4, 7.0 Hz, 1H, C*H* (3)), 2.80 (*dd*, *J* = 3.8, 4.3 Hz, 1H, C*H* (18)), 2.08 (*s*, 3H, Ac), 1.97 (*ddd*, *J* = 13.7, 13.5, 4.0 Hz, 1H, CH_a (16)), 1.90–1.84 (*m*, 2H, CH_2 (11)), 1.76 (*ddd*, *J* = 14.0, 13.8, 4.3 Hz, 1H, CH_a (22)), 1.69 (*ddd*, *J* = 13.8, 13.8, 3.7 Hz, 1H, CH_a (15)),

1.64–1.49 (*m*, 8H, C*H* (9) + C*H*$_a$ (1) + C*H*$_a$ (19) + C*H*$_a$ (6) + C*H*$_b$ (22) + C*H*$_a$ (16) + C*H*$_2$ (2)), 1.45–1.22 (*m*, 5H, C*H*$_b$ (6)+ C*H*$_2$ (21) + C*H*$_2$ (7)), 1.20–1.02 (*m*, 3H, C*H*$_a$ (19) + C*H*$_b$ (1) + C*H*$_b$ (15)), 1.12 (*s*, 3H, C*H*$_3$ (27)), 0.93 (*s*, 3H, C*H*$_3$ (23)), 0.91 (*s*, 3H, C*H*$_3$ (30)), 0.89 (*s*, 3H, C*H*$_3$ (29)), 0.86–0.81 (*m*, 1H, C*H* (5)), 0.85 (*s*, 3H, C*H*$_3$ (25)), 0.84 (*s*, 3H, C*H*$_3$ (26)), 0.74 (*s*, 3H, CH$_3$ (24)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 184.4 (*C*=O, C28), 171.1 (*C*=O, Ac), 143.5 (*C*=CH, C13), 122.5 (*C*H=C, C12), 80.9 (*C*HOH, C3), 55.3 (*C*H, C5), 47.5 (*C*H, C9), 46.5 (C_{quart}, C17), 45.8 (CH_2, C19), 41.5 (C_{quart}, C14), 40.9 (*C*H, C18), 39.3 (C_{quart}, C8), 38.0 (CH_2, C1), 37.6 (C_{quart}, C4), 36.9 (C_{quart}, C10), 33.7 (CH_2, C21), 33.0 (CH_3, C29), 32.5 (CH_2, C7), 32.4 (CH_2, C22), 30.6 (C_{quart}, C20), 28.0 (CH_3, C23), 27.6 (CH_2, C15), 25.9 (CH_3, C27), 23.6 (CH_3, C30), 23.5 (CH_2, C11), 23.4 (CH_2, C2), 22.8 (CH_2, C16), 21.3 (CH_3, Ac), 18.1 (CH_2, C6), 17.0 (CH_3, C24), 16.6 (CH_3, C26), 15.3 (CH_3, C25) ppm; MS (ESI, MeOH): *m*/*z* = 497.7 (100%, $[M-H]^-$), 543.3 (50%, $[M+HCO_2^-]^-$).

*5.4.4. (3β) 3-Acetoxy-olean-11-en-28-acid (**3**)*

Compound **3** was obtained as colorless needles applying method **C** starting from **OA**; yield: 95%; m.p.: 257–261 °C, (lit.: 257–259 °C [48]); $[\alpha]_D$ = +65° (*c* 0.33, $CHCl_3$) [lit.: +74° (*c* 1.0; $CHCl_3$ [49])]; R_F = 0.60 (toluene/ethyl acetate/*n*-heptane/formic acid, 80:20:30:4); IR (KBr): *ν* = 3219*br*, 2942*s*, 1731*s*, 1680*m*, 1466*m*, 1370*m*, 1252*s*, 1179*m*, 1161*m*, 1127*w*, 1026*m*, 1010 *m* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.20 (*dd*, *J* = 3.5, 3.5 Hz, 1H, C*H* (12)), 4.47 (*dd*, *J* = 8.5, 7.3 Hz, 1H, C*H* (3)), 2.80 (*dd*, *J* = 13.7, 4.1 Hz, 1H, C*H* (18)), 2.02 (*s*, 3H, Ac), 1.95 (*ddd*, *J* = 13.4, 13.4, 3.9 Hz, 1H, C*H*$_a$ (16)), 1.90–1.77 (*m*, 2H, C*H*$_2$ (11)), 1.73 (*ddd*, *J* = 14.9, 14.6, 4.4 Hz, 1H, C*H*$_a$ (7)), 1.67 (*ddd*, *J* = 13.3, 13.3, 4.1 Hz, 1H, C*H*$_a$ (15)), 1.54–1.34 (*m*, 8H, C*H*$_a$ (1) + C*H*$_b$ (16) + C*H*$_a$ (19) + C*H* (9) + C*H*$_a$ (6) + C*H*$_a$ (22) + C*H*$_2$ (2)), 1.34–1.11 (*m*, 5H, C*H*$_b$ (7) + C*H*$_b$ (6) + C*H*$_2$ (21) + C*H*$_b$ (22)), 1.10 (*s*, 3H, C*H*$_3$ (27)), 1.08–0.93 (*m*, 3H, C*H*$_b$ (19) + C*H*$_b$ (15) + C*H*$_b$ (1)), 0.92 (*s*, 3H, C*H*$_3$ (25)), 0.90 (*s*, 3H, C*H*$_3$ (30)), 0.88 (*s*, 3H, C*H*$_3$ (29)), 0.84 (*s*, 3H, C*H*$_3$ (23)), 0.86–0.74 (*m*, 1H, C*H* (5)), 0.83 (*s*, 3H, C*H*$_3$ (26)), 0.72 (*s*, 3H, C*H*$_3$ (24)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 184.3 (*C*=O, C28), 171.0 (*C*=*O*, C31), 143.6 (*C*=CH, C13), 122.5 (*C*H=C, C12), 80.9 (CHOAc, C3), 55.3 (*C*H, C5), 47.5 (*C*H, C9), 46.5 (C_{quart}, C17), 45.8 (CH_2, C19), 41.5 (C_{quart}, C14), 40.8 (*C*H, C18), 39.2 (C_{quart}, C8), 38.0 (CH_2, C1), 37.6 (C_{quart}, C4), 36.9 (C_{quart}, C10), 33.7 (CH_2, C21), 33.0 (CH_3, C29), 32.5 (CH_2, C7), 32.4 (CH_2, C22), 30.6 (C_{quart}, C20), 28.0 (CH_3, C23), 27.6 (CH_2, C15), 26.9 (CH_2, C2), 25.8 (CH_3, C27), 23.6 (CH_3, C30), 23.5 (CH_2, C16), 23.3 (CH_2, C11), 21.2 (CH_3, Ac), 18.1 (CH_2, C6), 17.1 (CH_3, C24), 16.6 (CH_3, C26), 15.3 (CH_3, C25) ppm; MS (ESI, MeOH): *m*/*z* = 497.5 (100%, $[M-H]^-$), 543.1 (66%, $[M+HCO_2]^-$), 995.1 (64%, $[2M-H]^-$), 1017.5 (16%, $[2M-2H+Na]^-$).

*5.4.5. (3β, 12α) 3-Acetyl-12-hydroxy-18β-olean-28-oic acid 28,13-lactone (**4**)*

Compound **4** was obtained from a mixture of **2** and **3** or directly from **1** using method **C**; yield: 54.4% (from **1**); m.p.: 295–298 °C (lit.: [50]: 261–263 °C; lit.: [51]: 285–287 °C); R_F = 0.36 (toluene/ethyl acetate/*n*-heptane/formic acid, 80:20:30:4); $[\alpha]_D$ = +44.4° (*c* 0.34, $CHCl_3$), IR (KBr): *ν* = 3528 *brs*, 3448*vs*, 2948*s*, 1736*vs*, 1468*m*, 1384*s*, 1248*s*, 1060*m*, 1028 *m* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.48 (*dd*, *J* = 10.1, 6.2 Hz, 1H, C*H* (3)), 3.87 (*dd*, *J* = 2.7, 2.7 Hz, 1H, C*H* (12)), 2.13 (*ddd*, *J* = 13.3, 13.3, 5.8 Hz, 1H, C*H* (16)), 2.03 (*s*, 3H, C*H*$_3$ (Ac)), 2.06–1.84 (*m*, 4H, C*H* (18) + C*H*$_2$ (19) + C*H*$_a$ (7)), 1.84 (*ddd*, *J* = 13.5, 13.5, 6.1 Hz, 1H, C*H*$_a$ (15)), 1.76–1.66 (*m*, 1H, C*H*$_a$ (1)), 1.69–1.1.52 (m, 6H, C*H* (9) + C*H*$_a$ (22) + C*H*$_2$ (11) + C*H*$_2$ (2)), 1.52–1.40 (m, 3H, C*H*$_2$ (6) + C*H*$_b$ (7)), 1.39–1.32 (*m*, 1H, C*H*$_b$ (22)), 1.30 (*s*, 3H, C*H*$_3$ (27)), 1.29–1.22 (*m*, 3H, C*H*$_b$ (16) + C*H*$_2$ (21)), 1.19–1.12 (*m*, 1H, C*H*$_b$ (15)), 1.14 (*s*, 3H, C*H*$_3$ (26)), 1.13–1.09 (*m*, 1H, C*H*$_b$ (1)), 0.97 (*s*, 3H, C*H*$_3$ (30)), 0.89 (*s*, 6H, C*H*$_3$ (25) + C*H*$_3$ (29)), 0.86 (*s*, 3H, C*H*$_3$ (23)), 0.85 (*s*, 3H, C*H*$_3$ (24)), 0.85–0.82 (*m*, 1H, C*H* (5)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 180.2 (*C*=O, C28), 171.3 (*C*=O, Ac), 90.8 (C_{quart}, C13), 81.0 (CHOH, C3), 76.3 (CHOH, C12), 55.4 (*C*H, C5), 51.2 (*C*H, C18), 44.8 (C_{quart}, C17), 44.6 (*C*H, C9), 42.4 (C_{quart}, C14), 42.2 (C_{quart}, C8), 39.5 (CH_2, C19), 38.6 (CH_2, C1), 38.0 (C_{quart}, C4), 36.5 (C_{quart}, C10), 34.3 (CH_2, C7), 34.1 (CH_2, C21), 33.4 (CH_3, C30), 31.7 (C_{quart}, C20), 28.9 (CH_2, C22), 28.2 (CH_2, C15), 28.3 (CH_3, C23), 27.6 (CH_2, C11), 24.0 (CH_3, C27), 23.7 (CH_2, C2), 21.4 (CH_3, Ac), 21.4 (CH_2, 16), 18.7 (CH_3, C29), 18.7 (CH_3, C26), 17.8 (CH_2, C6), 16.6 (CH_3, C24), 16.5 (CH_3, C25) ppm; MS (ESI): *m*/*z* = 527.3 (100%, $[M+H]^+$), 549.3 (10%, $[M+Na]^+$), 1053.2 (25%, $[2M+H]^+$), 1075.2 (25%, $[2M+Na]^+$).

*5.4.6. (3β, 12α) 3,12-Diacetoxy-olean-28-oic acid 28,13-lactone (**5**)*

From compound **4** following method **C**, **5** was obtained as fine colorless needles (from ethanol); yield: 76%; m.p.: 236–239 °C (lit.: 241–243 °C [50]); R_F = 0.80 (*n*-hexane/ethyl acetate, 6:4); $[\alpha]_D$ = +60.6° (*c* 0.27, $CHCl_3$); IR (KBr): *ν* = 3448*brs*, 2938*s*, 1766*s*, 1746*m*, 1724*m*, 1474*m*, 1466*m*, 1458*m*, 1438*m*, 1394*m*, 1376*m*, 1246*m*, 1232*m*, 1044*m*, 1032*m*, 1012 *m* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.7 (*brs*, 1H, C*H* (12)), 4.48 (*dd*, *J* = 9.8, 6.3 Hz, 1H, C*H* (12)), 2.10–2.03 (*m*, 1H, C*H* (16)), 2.10 (*s*, 3H, C*H*$_3$ (Ac)), 2.04 (*s*, 3H, C*H*$_3$ (Ac)), 1.96–1.86 (*m*, 4H, C*H* (18) + C*H*$_a$ (19) + C*H*$_a$ (7) + C*H*$_a$ (15)), 1.66–1.48 (*m*, 9H, C*H*$_a$ (6) + C*H*$_a$ (1) + C*H*$_a$ (16) + C*H* (9) + C*H*$_a$ (22) + C*H*$_2$ (11) + C*H*$_2$ (2)), 1.45–1.28 (*m*, 6H, C*H*$_b$ (6) + C*H*$_b$ (19) + C*H*$_b$ (7) + C*H*$_2$ (21) + C*H*$_b$ (16)), 1.27 (*s*, 3H, C*H*$_3$ (27)), 1.21 (*dd*, *J* = 14.5, 8.1 Hz, 1H, C*H*$_b$ (22)), 1.16 (*s*, 3H, C*H*$_3$ (26)), 1.19–1.15 (*m*, 2H, C*H*$_b$ (1) + C*H*$_b$ (15)), 0.96 (*s*, 3H, C*H*$_3$ (30)), 0.88 (*s*, 3H, C*H*$_3$ (25)), 0.87 (*s*, 3H, C*H*$_3$ (29)), 0.84 (*s*, 3H, C*H*$_3$ (23)), 0.82 (*s*, 3H, C*H*$_3$ (24)), 0.91–0.88 (*m*, 1H, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 179.0 (*C*=O, C28), 171.1 (*C*=O, Ac), 169.4 (*C*=O, Ac), 89.4 (C_{quart}, C13), 80.7 (CHOH, C3), 55.3 (CH, C5), 50.2 (CH, C18), 45.3 (C_{quart}, C17), 44.6 (CH, C9), 42.3 (C_{quart}, C14), 42.2 (C_{quart}, C8), 39.5 (CH_2, C19), 38.4 (CH_2, C1), 37.8 (C_{quart}, C4), 36.4 (C_{quart}, C10), 33.9 (CH_2, C7), 34.1 (CH_2, C21), 33.3 (CH_3, C29), 31.5 (C_{quart}, C20), 27.9 (CH_2, C22), 27.8 (CH_2, C15), 27.4 (CH_3, C23), 25.2 (CH_2, C11), 23.8 (CH_3, C27), 23.5 (CH_2, C2), 21.4 (CH_3, Ac), 21.3 (CH_3, Ac), 21.1 (CH_2, 16), 18.5 (CH_3, C30), 18.4 (CH_3, C26), 17.6 (CH_2, C6), 16.4 (CH_3, C24), 16.3 (CH_3, C25) ppm; MS (ESI, MeOH): *m*/*z* = 579.3 (100%, $[M+Na]^+$), 1135.3 (100%, $[2M+Na]^+$).

*5.4.7. (3β) Methyl 3-acetoxy-urs-11-en-28-oate (**6**)*

Compound **6** was obtained as colorless needles either by using method **A** from a mixture of the methyl triterpenoates **6** and **7** or directly using method **D** starting from **2**; yield: 91%; m.p.: 243–246 °C (lit.: 243–245 °C [45,52]); R_F = 0.75 (hexane/ethyl acetate, 8:2); $[\alpha]_D$ = +64.7° (*c* 0.30, $CHCl_3$) [lit [45,52].: +61 °C ($CHCl_3$)]; IR (KBr): *ν* = 3433*br*, 2942*s*, 2868*m*, 1734*s*, 1458*m*, 1385*m*, 1371*m*, 1312*w*, 1244*s*, 1202*m*, 1187*m*, 1167*w*, 1150*w*, 1114*w*, 1073*w*, 1027*m*, 1006*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$):δ = 5.23 (*m*, 1H, C*H* (12)), 4.48 (*dd*, *J* = 10.6, 5.5 Hz, 1H, C*H* (3)), 3.59 (*s*, 3H, C*H*$_3$), 2.17 (*d*, *J* = 11.3 Hz, 1H, C*H* (18)), 2.03 (*s*, 3H, Ac), 1.98 (*ddd*, *J* = 13.5, 13.4, 4.3 Hz, 1H, C*H*$_a$ (16)), 1.89 (*dd*, *J* = 8.7, 3.4 Hz, 2H, C*H*$_2$ (11)), 1.85 (*ddd*, *J* = 13.6, 13.5, 4.3 Hz, 1H, C*H*$_a$ (15)), 1.69–1.55 (*m*, 6H, C*H*$_2$ (7) + C*H*$_a$ (1) + C*H*$_b$ (16) + C*H*$_2$ (2)), 1.54–1.43 (*m*, 4H, C*H* (9) + C*H*$_a$ (6) + C*H*$_a$ (21) + C*H*$_a$ (22)), 1.38–1.26 (*m*, 4H, C*H* (19) + C*H*$_b$ (6) + C*H*$_b$ (22) + C*H*$_b$ (21)), 1.10–1.02 (*m*, 2H, C*H*$_b$ (15) + C*H*$_b$ (1)), 1.06 (*s*, 3H, C*H*$_3$ (27)), 0.99–0.95 (*m*, 1H, C*H* (20)), 0.93 (*s*, 3H, C*H*$_3$ (25)), 0.93 (*d*, *J* = 5.0 Hz, 3H, C*H*$_3$ (30)), 0.87–0.83 (*m*, 1H, C*H* (5)), 0.86 (*s*, 3H, C*H*$_3$ (23)), 0.85 (*d*, *J* = 5.4 Hz, 3H, C*H*$_3$ (29)), 0.84 (*s*, 3H, C*H*$_3$ (26)), 0.73 (*s*, 3H, C*H*$_3$ (24)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.0 (*C*=O, C28), 170.9 (*C*=O, Ac), 138.1 (*C*=CH, C13), 125.4 (*C*H=C, C12), 80.9 (CHOH, C3), 55.3 (*C*H, C5), 52.8 (*C*H, C18), 51.4 (CH_3, C31), 48.0 (C_{quart}, C17), 47.5 (*C*H, C9), 41.9 (C_{quart}, C14), 39.5 (C_{quart}, C8), 39.0 (*C*H, C19), 38.8 (*C*H, C20), 38.3 (CH_2, C1), 37.6 (C_{quart}, C4), 36.8 (CH_2, C21), 36.6 (C_{quart}, C10), 32.9 (CH_2, C7), 30.6 (CH_2, C22), 28.0 (CH_3, C23), 28.0 (CH_2, C15), 24.2 (CH_2, C16), 23.5 (CH_3, C27), 23.5 (CH_2,

C2), 23.3 (CH_2, C11), 21.3 (CH_3, Ac), 21.1 (CH_3, C29), 18.2 (CH_2, C6), 17.0 (CH_3, C30), 17.0 (CH_3, C24), 16.8 (CH_3, C26), 15.5 (CH_3, C25) ppm; MS (ESI, MeOH): m/z = 513.2 (40%, $[M + H]^+$), 535.5 (100%, $[M + Na]^+$).

*5.4.8. (3β) Methyl 3-acetoxy-olean-11-en-28-oate (**7**)*

Compound **7** was obtained from **3** as a colorless solid using method **D**; yield: 85%; m.p.: 220–221 °C (lit.: 219–220 °C [53]); $[\alpha]_D$ = +66° (*c* 0.32, $CHCl_3$) [lit.: +69° ($CHCl_3$ [53])]; R_F = 0.70 (toluene/ethyl acetate/*n*-heptane/formic acid, 80:20:30:4); IR (KBr): ν = 3428*br*, 2938*s*, 2861*m*, 1731*s*, 1451*m*, 1364*m*, 1266*m*, 1240*s*, 1162*m*, 1123*w*, 1038*m*, 1022 *m* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.28 (*dd*, *J* = 3.5, 3.5 Hz, 1H, C*H* (12)), 4.49 (*dd*, *J* = 8.9, 7.1 Hz, 1H, C*H* (3)), 3.62 (*s*, 3H, CH_3 (31)), 2.86 (*dd*, *J* = 13.9, 4.1 Hz, 1H, C*H* (18)), 2.04 (*s*, 3H, CH_3 (Ac)), 1.97 (*ddd*, *J* = 14.3, 5.6, 3.8 Hz, 1H, CH_a (16)), 1.92–1.82 (*m*, 2H, CH_a (2) + CH_a (11)), 1.70 (*dd*, *J* = 13.8, 4.5 Hz, 1H, CH_a (19)), 1.67–1.57 (*m*, 6H, CH_a (1) + CH_b (11) + CH_b (2) + CH_b (16) + CH_a (15) + CH_a (7)), 1.57–1.47 (*m*, 2H, CH_a (22) + CH_a (6)), 1.51 (*dd*, *J* = 10.9, 3.8 Hz, 1H, C*H* (9)), 1.47–1.40 (*m*, 2H, CH_b (7) + CH_b (6)), 1.34 (*ddd*, *J* = 14.7, 10.5, 5.9 Hz, 1H, CH_a (21)), 1.28–1.14 (*m*, 3H, CH_b (21) + CH_b (19) + CH_b (22)), 1.12 (*s*, 3H, CH_3 (27)), 1.10–0.94 (*m*, 2H, CH_b (15) + CH_b (1)), 0.93 (*s*, 3H, CH_3 (30)), 0.92 (*s*, 3H, CH_3 (25)), 0.90 (*s*, 3H, CH_3 (29)), 0.86 (*s*, 3H, CH_3 (23)), 0.85 (*s*, 3H, CH_3 (24)), 0.79 (*dd*, *J* = 12.9, 7.2 Hz, 1H, C*H* (5)), 0.72 (*s*, 3H, CH_3 (26)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.3 (*C*=*O*, C28), 170.9 (*C*=*O*, Ac), 143.8 (*C*=CH, C13), 122.2 (*CH*=C, C12), 80.9 (*CHOAc*, C3), 55.3 (*CH*, C5), 51.5 (CH_3, C31), 47.6 (*CH*, C9), 46.7 (C_{quart}, C17), 45.8 (CH_2, C19), 41.6 (C_{quart}, C4), 41.3 (*CH*, C18), 41.2 (C_{quart}, C8), 38.1 (CH_2, C1), 37.7 (C_{quart}, C14), 36.9 (C_{quart}, C10), 33.4 (CH_2, C21), 33.1 (CH_3, C29), 32.6 (CH_2, C7), 32.4 (CH_2, C22), 30.7 (C_{quart}, C20), 28.0 (CH_3, C23), 27.6 (CH_2, C15), 25.9 (CH_3, C27), 23.6 (CH_3, C30), 23.5 (CH_2, C2), 23.4 (CH_2, C16), 23.1 (CH_2, C11), 21.2 (CH_3, Ac), 18.2 (CH_2, C6), 16.8 (CH_3, C26), 16.7 (CH_3, C24), 15.3 (CH_3, C25) ppm; MS (ESI, MeOH): m/z = 535.5 (22%, $[M + Na]^+$), 1047.3 (100%, $[2M + Na]^+$).

*5.4.9. (3β) Methyl 3-acetoxy-12-oxo-olean-28-oate (**8**)*

Compound **7** was treated according to method **A** to yield **8**; yield 75%; R_F = 0.5 (*n*-hexane/ethyl acetate, 8:2); m.p.: 189–191 °C (lit.: 194 °C [54]; $[\alpha]_D$ = −13.3° (*c* 0.54, $CHCl_3$; lit.: −12° (*c* 1.4, $CHCl_3$ [54]); IR (KBr): ν = 3426*br*, 2948*s*, 2866*m*, 1728*vs*, 1700*s*, 1466*m*, 1388*m*, 1368*m*, 1244*vs*, 1192*m*, 1162*m*, 1082*w*, 1030*m*, 1004 *m* cm^{-1}; UV–Vis ($CHCl_3$): λ_{max} (log ε) = 278 nm (4.31); ^1H NMR (500 MHz, $CDCl_3$): δ = 4.47 (*dd*, *J* = 11.5, 4.9 Hz, 1H, C*H* (3)), 3.67 (*s*, 3H, CH_3 (31)), 2.81–2.76 (*ddd*, *J* = 13.4, 4.3, 3.5, 1H, C*H* (18)), 2.61 (*d*, *J* = 4.3 Hz, 1H, C*H* (13)), 2.23 (*dd*, *J* = 16.8, 5.1 Hz, 1H, CH_a (11)), 2.14 (*dd*, *J* = 16.8, 3.5 Hz, 1H, CH_b (11)), 2.04 (*s*, 3H, CH_3 (Ac)), 1.93 (*ddd*, *J* = 13.5, 3.6, 2.5 Hz, 1H, CH_a (19)), 1.89 (*ddd*, *J* = 14.8, 14.8, 4.2 Hz, 1H, CH_a (16)), 1.79 (*ddd*, *J* = 13.7, 13.7, 4.6 Hz, 1H, CH_a (7)), 1.68–1.58 (*m*, 6H, C*H* (18) + CH_a (15) + CH_2 (2) + CH_a (6) + CH_b (16)), 1.55 (*ddd*, *J* = 12.9, 7.5, 3.8 Hz, 1H, CH_a (1)), 1.49–1.43 (*m*, 3H, CH_b (6) + CH_b (7) + CH_a (22)), 1.36–1.28 (*m*, 2H, CH_b (22) + CH_a (21)), 1.27–1.17 (*m*, 2H, CH_b (21) + CH_b (19)), 1.06 (*ddd*, *J* = 13.3, 4.1, 1.8 Hz, 1H, CH_b (15)), 1.04–0.98 (*m*, 1H, CH_b (1)), 0.97 (s, 3H, CH_3 (30)), 0.96 (s, 3H, CH_3 (26)), 0.93 (*s*, 3H, CH_3 (27)), 0.90 (s, 3H, CH_3 (29)), 0.87 (*s*, 3H, CH_3 (25)), 0.86 (*s*, 3H, CH_3 (23)), 0.85 (*s*, 3H, CH_3 (24)), 0.84–0.80 (*m*, 1H, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 211.7 (*C*=O, C12), 178.5 (*C*=O, C28), 171.0 (*C*=O, Ac), 80.6 (*CHOAc*, C3), 55.3 (*CH*, C5), 52.0 (CH_3, C13), 51.9 (*CH*, C31), 49.8 (*CH*, C9), 47.5 (C_{quart}, C17), 42.0 (C_{quart}, C14), 41.4 (C_{quart}, C8), 38.6 (CH_2, C11), 37.9 (CH_2, C1), 37.8 (C_{quart}, C10), 37.0 (C_{quart}, C4), 36.4 (CH_2, C19), 34.6 (CH_2, 21), 33.5 (CH_3, C29), 33.1 (CH_2, C22), 32.1 (*CH*, C18), 31.9 (CH_2, C7), 30.8 (C_{quart}, C20), 28.1 (CH_3, C23), 27.7 (CH_2, C15), 23.6 (CH_2, C2), 23.3 (CH_3, C30), 22.9 (CH_2, C16), 21.4 (CH_3, Ac), 20.7 (CH_3, C27), 18.3 (CH_2, C6), 16.6 (CH_3, C24), 16.3 (CH_3, C26), 15.4 (CH_3, C25) ppm; MS (ESI, MeOH): m/z = 529.1 (20%, $[M + H]^+$), 551.3 (100%, $[M + Na]^+$), 583.2 (17%, $[M + Na + MeOH]^+$).

*5.4.10. (3β) Methyl 3-acetoxy-12-hydroxy-11-oxo-olean-Δ-12,13-en-28-oate (**9**)*

By using method **E** compound **9** was obtained from **8**; yield 83%; R_F = 0.5 (n-hexane/ethyl acetate, 8:2); m.p.: 199–201 °C; $[\alpha]_D$ = +112.7° (*c* 0.34, $CHCl_3$); IR (KBr): ν = 3454*br*, 2948*s*, 2868*m*, 1730*vs*, 1664*m*, 1636*s*, 1466*m*, 1390*m*, 1366*s*, 1306*m*, 1244*vs*, 1202*m*, 1164*m*, 1140*w*, 1120*w*, 1034*m* cm^{-1}; UV–Vis ($CHCl_3$): λ_{max} (log ε) = 280.0 nm (4.51); ^1H NMR (500 MHz, $CDCl_3$): δ = 6.21 (*s*, 1H, *OH*), 4.50 (*dd*, *J* = 11.6, 4.9 Hz, 1H, C*H* (3)), 3.71–3.64 (*ddd*, *J* = 9.2, 9.2, 1.6, 1H, C*H* (18)), 3.63 (*s*, 3H, CH_3 (31)), 2.80 (*ddd*, *J* = 13.6, 13.6, 3.6 Hz, 1H, CH_a (1)), 2.46 (*s*, 1H, C*H* (9)), 2.04 (*ddd*, *J* = 13.8, 13.8, 3.0 Hz, 1H, CH_a (16)), 2.05 (*s*, 3H, CH_3 (Ac)), 1.76 (*ddd*, *J* = 13.9, 13.9, 4.4 Hz, 1H, CH_a (7)), 1.73–1.67 (*m*, 2H, CH_2 (2)), 1.67–1.60 (*m*, 2H, CH_b (7) + CH_b (16)), 1.60–1.55 (*m*, 3H, CH_a (21) + CH_a (15) + CH_a (6)), 1.43–1.36 (*m*, 5H, CH_2 (19) + CH_a (22) + CH_b (21) + CH_b (6)), 1.36 (*s*, 3H, CH_3 (27)), 1.29–1.23 (*m*, 1H, CH_b (22)), 1.20 (*ddd*, *J* = 14.0, 14.0, 3.4 Hz, 1H, CH_b (15)), 1.13 (*s*, 3H, CH_3 (25)), 1.10 (*ddd*, *J* = 13.3, 13.3, 4.1 Hz, CH_b (1)), 1.00 (*s*, 3H, CH_3 (30)), 0.93 (s, 3H, CH_3 (29)), 0.92 (*s*, 3H, CH_3 (26)), 0.87 (*s*, 6H, CH_3 (23) + CH_3 (24)), 0.85–0.77 (*m*, 1H, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 195.5 (*C*=O, C11), 178.0 (*C*=O, C28), 171.1 (*C*=O, Ac), 142.3 (*COH*=C, C12), 136.9 (*C*=CHOH, C13), 80.6 (*CHOH*, C3), 60.5 (*CH*, C9), 55.2 (*CH*, C5), 52.0 (CH_3, C31), 46.2 (C_{quart}, C1), 45.6 (C_{quart}, C17), 41.7 (C_{quart}, C8), 40.4 (CH_2, C19), 38.8 (CH_2, C1), 38.2 (C_{quart}, C4), 37.5 (C_{quart}, C10), 34.2 (CH_2, C22), 33.4 (*CH*, C18), 33.2 (CH_2, C21), 33.0 (CH_3, C29), 32.0 (CH_2, C7), 30.7 (C_{quart}, C20), 28.2 (CH_3, C23), 28.0 (CH_2, C15), 23.7 (CH_3, C27), 23.4 (CH_3, C30), 23.3 (CH_2, C2), 21.4 (CH_3, Ac), 19.0 (CH_3, C26), 17.4 (CH_2, C6), 16.8 (CH_3, C24), 16.5 (CH_3, C25) ppm; MS (ESI, MeOH): m/z = 543.2 (38%, $[M + H]^+$), 565.4.5 (50%, $[M + Na]^+$), 1107.3 (100%, $[2M + Na]^+$).

*5.4.11. (3β) Methyl 3-acetoxy-11-oxo-olean-12-en-28-oate (**10**)*

N-Hydroxysuccinimide (1.2 g, 10.4 mmol) and potassium dichromate (1.1 g, 3.6 mmol) were added to a solution of **7** (0.5 g, 0.94 mmol) in acetone (50 mL) containing glacial acetic acid (5 mL); the mixture was stirred at 40° for 48 h. The reaction was quenched with potassium disulfite solution and after usual work up **10** was obtained as a colorless solid; yield: 65%; m.p.: 216–220 °C (lit.: [55]: 245–247 °C); $[\alpha]_D$ = +82° (*c* 0.14, $CHCl_3$); R_F = 0.62 (hexane/ethyl acetate, 7:3); UV–Vis (MeOH): λ_{max} (log ε) = 269 nm (4.03); IR (KBr): ν = 3339*br*, 2949*s*, 2866*s*, 1724*s*, 1661*s*, 1466*m*, 1387*m*, 1365*w*, 1330*w*, 1304*w*, 1261*m*, 1227*w*, 1209*m*, 1189*m*, 1162*m*, 1125*w*, 1089*w*, 1039*m*, 1013*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.61 (*s*, 1H, C*H* (12)), 3.61 (*s*, 3H, CH_3 (31)), 3.20 (*dd*, *J* = 10.8, 5.5 Hz, 1H, C*H* (3)), 2.98 (*dd*, *J* = 13.8, 3.7 Hz, 1H, C*H* (18)), 2.85–2.75 (*m*, 1H, CH_a (1)), 2.30 (*s*, 1H, C*H* (9)), 2.02 (*ddd*, *J* = 13.8, 13.8, 4.0 Hz, 1H, CH_a (16)), 1.75–1.52 (*m*, 9H, CH_a (19) + CH_b (16) + CH_2 (7) + CH_a (15) + CH_a (22) + CH_2 (2) + CH_a (6)), 1.42–1.13 (*m*, 6H, CH_b (19) + CH_b (22) + CH_2 (21) + CH_b (15) + CH_b (6)), 1.34 (*s*, 3H, CH_3 (27)), 1.08 (*s*, 3H, CH_3 (25)), 1.00–0.95 (*m*, 1H, CH_b (1)), 0.97 (*s*, 3H, CH_3 (23)), 0.92 (*s*, 3H, CH_3 (30)), 0.91 (*s*, 3H, CH_3 (29)), 0.89 (*s*, 3H, CH_3 (26)), 0.78 (*s*, 3H, CH_3 (24)), 0.66 (*brd*, *J* = 11.3 Hz, 1H, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.3 (*C*=O, C11), 177.4 (*C*=O, C28), 168.6 (*C*=CH, C13), 127.9 (*CH*=C, C12), 78.7 (*CHOH*, C3), 61.7 (*CH*, C9), 55.0 (*CH*, C5), 51.8 (CH_3, C31), 46.2 (C_{quart}, C17), 45.0 (C_{quart}, C8), 44.2 (CH_2, C19), 43.4 (C_{quart}, C14), 41.5 (*CH*, C18), 39.1 (CH_2, C1), 38.8 (C_{quart}, C4), 37.2 (C_{quart}, C10), 33.7 (CH_2, C21), 32.9 (CH_2, C7), 32.8 (CH_3, C29), 31.6 (CH_2, C22), 30.6 (C_{quart}, C20), 28.1 (CH_3, C23), 27.7 (CH_2, C15), 27.3 (CH_2, C2), 23.5 (CH_3, C27), 23.4 (CH_3, C30), 22.9 (CH_2, C16), 18.9 (CH_3, C26), 17.4 (CH_2, C6), 16.1 (CH_3, C25), 15.5 (CH_3, C24) ppm; MS (ESI, MeOH): m/z = 485.6 (100%, $[M + H]^+$), 507.5 (35%, $[M + Na]^+$).

*5.4.12. (2α,3β) Methyl 2,3-dihydroxy-olean-12-en-28-oate (**11**)*

Compound **11** [56] was obtained from **MA** (100 mg, 0.21 mmol) using method **D** as fine colorless needles; yield: 88%; m.p. 229–231 °C (214–216 °C [56]); R_F = 0.4 (*n*-hexane/ethyl acetate, 6:4); $[\alpha]_D$ = +60.9° (*c* 0.65, $CHCl_3$); IR (KBr) ν = 3571*br*, 3300*s*, 2947*s*, 1739*s*, 1461*s*, 1386*m*, 1363*m*, 1262*m*, 1229*m*, 1190*s*, 1162*s*, 1124*m*, 1052*s*, 1037*s*, 984*m*, 958*s*, 921*s* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.28 (*dd*, *J* = 3.4, 3.4 Hz, 1H, C*H* (12)), 3.72–3.65 (*m*, 1H, C*H* (2)), 3.61 (*s*, 3H, CH_3 (31)), 3.00 (*d*, *J* = 9.4 Hz, 1H, C*H* (3)), 2.85 (*dd*, *J* = 13.8, 4.1 Hz, 1H, C*H* (18)), 2.21 (*br*, 2H, O*H*), 1.97 (*dd*, *J* = 10.4, 3.9 Hz, 1H, CH_a (1)), 1.96–1.83 (*m*, 3H, CH_a (16) + CH_2 (11)), 1.68 (*ddd*, *J* = 13.9, 13.9, 4.4 Hz, 1H, CH_a (7)), 1.64–1.56 (*m*, 4H, C*H* (9) + CH_a (19) + CH_a (15) + CH_b (16)), 1.55–1.52 (*m*, 1H, CH_a (6)), 1.50 (*ddd*, *J* = 14.0, 3.4, 3.4 Hz, CH_a (22)), 1.43 (*m*, 1H, CH_b (7)), 1.38 (*ddd*, *J* = 12.4, 12.4, 2.5 Hz, CH_b (6)), 1.29 (*ddd*, *J* = 13.8, 9.6, 2.7 Hz, 1H, CH_a (21)), 1.29–1.27 (*m*, 1H, CH_b (22)), 1.16 (*ddd*, *J* = 14.0, 4.2, 4.2 Hz, 1H, CH_b (21)), 1.15–1.11 (*m*, 1H, CH_b (19)), 1.12 (*s*, 3H, CH_3 (C27)), 1.06–1.02 (*m*, 1H, CH_b (15)), 1.02 (*s*, 3H, CH_3 (C23)), 0.97 (*s*, 3H, CH_3 (C25)), 0.92 (*s*, 3H, CH_3 (C30)), 0.92–0.87 (*m*, 1H, CH_b (1)), 0.89 (*s*, 3H, CH_3 (C29)), 0.84–0.82 (*m*, 1H, C*H* (5)), 0.82 (*s*, 3H, CH_3 (C24)), 0.71 (*s*, 3H, CH_3 (C26)) ppm; ^{13}C NMR (125 Hz, $CDCl_3$): δ = 178.2 (*C*=O, C28), 143.8 (*C*=CH, C13), 122.1 (*C*H=C, C12), 84.0 (*C*HOH, C3), 68.9 (*C*HOH, C2), 55.3 (*C*H, C5), 51.5 (CH_3, C31), 47.6 (*C*H, C9), 46.7 (C_{quart}, C17), 46.3 (CH_2, C1), 45.8 (CH_2, C19), 41.6 (C_{quart}, C14), 41.2 (*C*H, C18), 39.3 (C_{quart}, C8), 39.1 (C_{quart}, C4), 38.3 (C_{quart}, C10), 33.8 (CH_2, C21), 33.1 (CH_3, C29), 32.5 (CH_2, C22), 32.3 (CH_2, C7), 30.7 (C_{quart}, C20), 28.6 (CH_3, C23), 27.6 (CH_2, C15), 25.9 (CH_3, C27), 23.6 (CH_3, C30), 23.4 (CH_2, C16), 23.0 (CH_2, C11), 18.3 (CH_2, C6), 16.9 (CH_3, C26), 16.7 (CH_3, C24), 16.6 (CH_3, C25) ppm; MS (ESI, MeOH, source CID): *m/z* = 487.4 (48.8%, $[M + H]^+$), 504.5 (53.4%, $[M + NH_4]^+$), 509.5 (100%, $[M + Na]^+$), 541.2 (22.0%, $[M + Na + MeOH]^+$), 741.5 (14.6%, $[3M + Na + H]^{2+}$), 749.6 (73.2%, $[3M + K + H]^{2+}$), 992.8 (48.8%, $[4M + K + H]^{2+}$).

*5.4.13. (2α,3β) Methyl 2,3-diacetyl-olean-12-en-28-oate (**12**)*

Obtained from **11** using method **C** as a colorless solid; yield: 92%; m.p.: 173–175 °C (lit.: 165–168 °C [57], 176–178 °C [58]; R_F = 0.48 (n-hexane/ethylacetate, 8:2); $[\alpha]_D$ = +25.1° (*c* 0.37, $CHCl_3$); IR (KBr): ν = 3430*br*, 2972*s*, 2949*s*, 2882*m*, 2866*m*, 1745*vs*, 1722*s*, 1460*m*, 1433*m*, 1393*m*, 1369*m*, 1250*vs*, 1190*m*, 1165*m*, 1042*m*, 1034*m* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.28 (*dd*, *J* = 3.6, 3.6 Hz, 1H, C*H* (12)), 5.11 (*ddd*, *J* = 11.7, 10.4, 4.8 Hz, 1H, C*H* (2)), 4.73 (*d*, *J* = 10.4 Hz, 1H, C*H* (3)), 3.60 (*s*, 3H, CH_3 (31)), 2.85 (*dd*, *J* = 13.8, 4.4 Hz, 1H, C*H* (18)), 2.04 + 1.97 (s, 6H, CH_3 (Ac)), 2.08–1.93 (*m*, 3H, CH_a (1) + CH_a (16) + CH_a (11)), 1.93–1.88 (*m*, 1H, CH_b (11)), 1.73–1.68 (*m*, 1H, CH_a (7)), 1.66–1.58 (*m*, 4H, CH_a (15) + CH_a (19) + CH_b (16) + C*H* (9)), 1.55–1.48 (*m*, 2H, CH_a (6) + CH_b (7)), 1.48–1.38 (*m*, 2H, CH_a (22) + CH_b (6)), 1.35–1.28 (*m*, 2H, CH_b (22) + CH_a (21)), 1.20–1.12 (*m*, 2H, CH_b (21) + CH_b (19)), 1.11 (*s*, 3H, CH_3 (27)), 1.10 (*m*, 2H, CH_b (1) + CH_b (15)), 1.06 (*s*, 3H, CH_3 (23)), 1.00–0.92 (*m*, 1H, C*H* (5)), 0.94 (*s*, 3H, CH_3 (25)), 0.90 (s, 3H, CH_3 (30)), 0.89 (*s*, 3H, CH_3 (29)), 0.89 (*s*, 3H, CH_3 (24)), 0.74 (*s*, 3H, CH_3 (26)); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.3 (*C*=O, C28), 170.7 (*C*=O, Ac), 170.3 (*C*=O, Ac), 143.8 (*C*=CH, C13), 122.2 (*C*H=C, C12), 80.6 (*C*HOAc, C3), 70.0 (*C*HOAc, C2), 54.9 (*C*H, C5), 47.6 (*C*H, C9), 46.7 (C_{quart}, C17), 45.9 (CH_2, C19), 43.8 (CH_2, C1), 41.6 (C_{quart}, C14), 41.2 (*C*H, C18), 39.5 (C_{quart}, C4), 39.3 (C_{quart}, C8), 38.3 (C_{quart}, C10), 33.8 (CH_2, C21), 33.0 (CH_3, C29), 32.4 (CH_2, C22), 32.3 (CH_2, C7), 30.6 (C_{quart}, C20), 28.5 (CH_3, C23), 27.6 (CH_2, C15), 25.8 (CH_3, C27), 23.8 (CH_3, C30), 23.4 (CH_2, C11), 23.1 (CH_2, C16), 21.0 (CH_3, Ac), 20.9 (CH_3, Ac), 18.3 (CH_2, C6), 17.7 (CH_3, C26), 16.9 (CH_3, C24), 16.5 (CH_3, C25) ppm; MS (MeOH): *m/z* = 571.1 (41%, $[M + H]^+$), 588.2 (71%, $[M + NH_4]^+$), 593.5 (16%, $[M + Na]^+$, 1163.4 (100%, $[2M + Na]^+$). $C_{35}H_{54}O_6$

*5.4.14. Methyl (2α,3β)2,3–di-O-acetyl-12-oxo-olean-28-oate (**13**)*

Compound **13** obtained as colorless solid from **12** using method **B**; yield: 84%; m.p.: 144–147 °C; R_F = 0.21 (*n*-hexane/ethyl acetate, 8:2); $[\alpha]_D$ = −46.7° (*c* 0.40, $CHCl_3$); IR (KBr): ν = 3588*w*, 3442*s*, 3430*s*, 2949*m*, 2867*w*, 1742*vs*, 1727*s*, 1700*m*, 1472*m*, 1458*m*, 1440*w*, 1395*w*, 1387*m*, 1369*m*, 1250*vs*, 1192*m*, 1160*m*, 1154*m*, 1119*w*, 1086*w*, 1042*m* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.06 (*ddd*, *J* = 11.4, 10.4, 4.7 Hz, 1H, C*H* (2)), 4.74 (*d*, *J* = 10.4 Hz, 1H, C*H* (3)), 3.65 (*s*, 3H, CH_3 (31)), 2.80 (*ddd*, *J* = 13.6, 4.0, 4.0 Hz, 1H, C*H* (18)), 2.60 (*d*, *J* = 4.0 Hz, 1H, C*H* (13)), 2.18 (*m*, 1H, CH_a (11)), 2.16 (*m*, 1H, CH_b (11)), 2.05 + 1.98 (*s*, 6H, 2× CH_3 (Ac)), 2.00–1.88 (*m*, 3H, CH_a (19) + CH_a (1) + CH_a (16)), 1.85–1.78 (*m*, 1H, CH_a (7)), 1.79–1.71 (*m*, 1H, C*H* (9)), 1.70–1.60 (*m*, 3H, CH_a (15) + CH_b (16) + CH_a (6)), 1.52–1.42 (*m*, 3H, CH_b (6) + CH_b (7) + CH_a (22)), 1.39–1.28 (*m*, 2H, CH_b (22) + CH_a (21)), 1.24–1.18 (*m*, 1H, CH_b (19) + CH_b (21)), 1.12–1.08 (*m*, 1H, CH_b (15)), 1.06–0.88 (*m*, 1H, CH_b (1)), 1.02 (*s*, 3H, CH_3 (27)), 0.98 (*s*, 3H, CH_3 (25)), 0.97 (*s*, 3H, CH_3 (30)), 0.96 (*m*, 1H, C*H* (5)), 0.94 (*s*, 3H, CH_3 (23)), 0.90 (*s*, 3H, CH_3 (24)), 0.89 (*s*, 3H, CH_3 (29)), 0.89 (*s*, 3H, CH_3 (26)); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 210.6 (*C*=O, C12), 178.0 (*C*=O, C28), 170.4 (*C*=O, Ac), 170.5 (*C*=O, Ac), 80.1 (*C*HOAc, C3), 69.5 (*C*HOAc, C2), 54.8 (*C*H, C5), 51.6 (*C*H, C13), 49.6 (*C*H, C9), 47.4 (C_{quart}, C17), 43.3 (CH_2, C1), 41.7 (C_{quart}, C14), 41.2 (C_{quart}, C8), 39.0 (C_{quart}, C4), 38.5 (CH_2, C11), 38.1 (C_{quart}, C10), 36.4 (CH_2, C19), 34.5 (CH_2, C21), 33.5 (CH_3, C29), 32.8 (CH_2, C7), 32.0 (*C*H, C18), 31.6 (CH_2, C22), 30.8 (C_{quart}, C20), 28.2 (CH_3, C23), 27.7 (CH_2, C15), 23.0 (CH_3, C30), 22.9 (CH_2, C16), 21.0 (CH_3, Ac), 21.1 (CH_3, Ac), 20.4 (CH_3, C27), 18.3 (CH_2, C6), 17.5 (CH_3, C26), 16.5 (CH_3, C24), 16.0 (CH_3, C25) ppm; MS (ESI, MeOH): *m/z* = 587.3 (37%, $[M + H]^+$), 604.4 (26%, $[M + NH_4]^+$), 609.5 (100%, $[M + Na]^+$), 1190.2 (29%, $[2M + NH_4]^+$), 1195.1 (85%, $[2M + Na]^+$). $C_{35}H_{54}O_7$

*5.4.15. (3β) Methyl 3-hydroxy-urs-11-en-28 oate (**14**)*

Compound **14** was obtained from **UA** as fine colorless needles using method **D**; yield: 95%; m.p.: 165–167 °C (lit [59].: 166–168 °C); R_F = 0.47 (*n*-hexane/ethyl acetate, 8:2); $[\alpha]_D$ = +68° (*c* 0.53; $CHCl_3$) [lit.: 49.8° (*c* 1.0; $CHCl_3$) [52]; UV–Vis (MeOH): λ_{max} (log ε) = 224 nm (3.62); IR (KBr): ν = 3374*br*, 2927*s*, 2871*m*, 1728*s*, 1457*m*, 1386*m*, 1378*m*, 1308*w*, 1270*w*, 1232*m*, 1200*m*, 1187*m*, 1168*w*, 1142*m*, 1113*w*, 1093*w*, 1031m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.22 (*m*, 1H, C*H* (12)), 3.59 (*s*, 3H, CH_3 (31)), 3.20 (*dd*, *J* = 11.0, 4.9 Hz, 1H, C*H* (3)), 2.21 (*d*, *J* = 11.3 Hz, 1H, C*H* (18)), 1.98 (*ddd*, *J* = 13.4, 13.4, 4.6 Hz, 1H, CH_a (2)), 1.90 (*dd*, *J* = 8.9, 3.7 Hz, 2H, CH_2 (11)), 1.85 (*ddd*, *J* = 13.8, 13.8, 4.8 Hz, 1H, CH_a (15)), 1.69–1.56 (*m*, 5H, CH_2 (7) + CH_a (1) + CH_a (16) + CH_b (2)), 1.55–1.44 (*m*, 5H, C*H* (9) + CH_a (6) + CH_a (21) + CH_a (22) + CH_b (16)), 1.36–1.24 (*m*, 4H, C*H* (19) + CH_b (6) + CH_b (22) + CH_b (21)), 1.08–1.03 (*m*, 1H, CH_b (15)), 1.06 (*s*, 3H, CH_3 (27)), 1.01–0.95 (*m*, 2H, C*H* (20) + CH_b (1)), 0.97 (*s*, 3H, CH_3 (23)), 0.93 (*d*, *J* = 6.0 Hz, 3H, CH_3 (30)), 0.91 (*s*, 3H, CH_3 (25)), 0.85 (*d*, *J* = 6.4 Hz, 3H, CH_3 (29)), 0.77 (*s*, 3H, CH_3 (24)), 0.73 (*s*, 3H, CH_3 (26)), 0.71 (*brd*, *J* = 10.4 Hz, 1H, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 177.9 (*C*=O, C28), 138.1 (*C*=CH, C13), 125.5 (HC=C, C12), 79.0 (CHOH, C3), 55.2 (*C*H, C5), 52.9 (*C*H, C18), 51.4 (CH_3, C31), 48.0 (C_{quart}, C17), 47.5 (*C*H, C9), 41.9 (C_{quart}, C14), 39.5 (C_{quart}, C8), 39.0 (*C*H, C19), 38.8 (*C*H, C20), 38.7 (C_{quart}, C4), 38.6 (CH_2, C1), 36.9 (C_{quart}, C10), 36.7 (CH_2, C22), 32.9 (CH_2, C7), 30.6 (CH_2, C21), 28.1 (CH_3, C23), 28.0 (CH_2, C15), 27.2 (CH_2, C16), 24.2 (CH_2, C2), 23.6 (CH_3, C27), 23.3 (CH_2, C11), 21.1 (CH_3, C30), 18.3 (CH_2, C6), 17.0 (CH_3, C29), 16.9 (CH_3, C26), 15.6 (CH_3, C24), 15.4 (CH_3, C25) ppm; MS (ESI, MeOH): *m/z* = 471.2 (80%, $[M + H]^+$), 493.4 (100%, $[M + Na]^+$).

*5.4.16. (3β) Methyl 3-hydroxy-olean-11-en-28 oate (**15**)*

OA (1 equiv.) was treated with iodomethane following method **D.** Compound **16** was obtained as fine colorless needles; yield: 88.3%; m.p.: 198–200 °C (lit [60].: 198–200 °C); R_F = 0.50 (n-

hexane/ethyl acetat, 8:2); $[\alpha]_D$ = +70° (*c* 0.43; $CHCl_3$) [lit [61].: +70° (*c* 1.0; $CHCl_3$)]; IR (KBr): ν = 3334*br*, 2947*s*, 2865*m*, 1725*s*, 1662*w*, 1464*m*, 1386*m*, 1363*m*, 1304*w*, 1263*m*, 1241*m*, 1202*m*, 1190*m*, 1162*m*, 1125*m*, 1094*w*, 1065*w*, 1032*m* cm^{-1}; UV–Vis (MeOH): λ_{max} (log ε) = 223 nm (3.66); ^1H NMR (500 MHz, $CDCl_3$): δ = 5.26 (*brs*, 1H, C*H* (12)), 3.60 (s, 3H, CH_3 (31)), 3.19 (*dd*, *J* = 11.0, 4.4 Hz, 1H, C*H* (3)), 2.84 (*dd*, *J* = 13.9, 4.2 Hz, 1H, C*H* (18)), 1.95 (*ddd*, *J* = 14.5, 14.4, 4.1 Hz, 1H, CH_a (16)), 1.88–1.82 (*m*, 2H, CH_2 (11)), 1.67 (*ddd*, *J* = 13.9, 13.9, 4.4 Hz, 1H, CH_a (7)), 1.63–1.47 (*m*, 9H, C*H* (9) + CH_a (1) + CH_a (19) + CH_a (6) + CH_a (15) + CH_b (7) + CH_b (16) + CH_2 (2)), 1.43–1.22 (*m*, 4H, CH_a (21) + CH_2 (22) + CH_b (6)), 1.19–1.12 (*m*, 2H, CH_b (19) + CH_b (21)), 1.10 (*s*, 3H, CH_3 (27)), 1.08–0.98 (*m*, 1H, CH_b (15)), 0.97–0.92 (*m*, 1H, CH_b (1)), 0.96 (*s*, 3H, CH_3 (23)), 0.90 (*s*, 3H, CH_3 (30)), 0.88 (*s*, 3H, CH_3 (29)), 0.87 (*s*, 3H, CH_3 (25)), 0.76 (*s*, 3H, CH_3 (24)), 0.73–0.68 (*m*, 1H, C*H* (5)), 0.70 (*s*, 3H, CH_3 (26)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.2 (*C*=O, C28), 143.7 (*C*=CH, C13), 122.3 (*C*H=C, C12), 79.0 (C3, *C*HOH), 55.2 (*C*H, C5), 51.5 (CH_3, C31), 47.6 (*C*H, C9), 46.7 (C_{quart}, C17), 45.8 (CH_2, C19), 41.6 (C_{quart}, C14), 41.3 (*C*H, C18), 39.2 (C_{quart}, C8), 38.7 (C_{quart}, C4), 38.4 (CH_2, C1), 37.0 (C_{quart}, C10), 33.8 (CH_2, C21), 33.1 (CH_3, C29), 32.6 (CH_2, C7), 32.3 (CH_2, C22), 30.6 (C_{quart}, C20), 28.1 (CH_3, C23), 27.7 (CH_2, C15), 27.1 (CH_2, C2), 25.9 (CH_3, C27), 23.6 (CH_3, C30), 23.4 (CH_2, C11), 23.0 (CH_2, C16), 18.3 (CH_2, C6), 16.8 (CH_3, C26), 15.5 (CH_3, C24), 15.3 (CH_3, C25) ppm; MS (ESI, MeOH): *m*/*z* = 493.5 (100%, $[M + Na]^+$).

5.4.17. (3β) Methyl 3-hydroxy-12-oxo-olean-28-oate (**16**)

Compound **16** was obtained using method **A**; yield: 42%; R_F = 0.20 (hexane/ethyl acetate, 8:2); m.p.: 194–196 °C (lit [62].: 132–133 °C); $[\alpha]_D$ = −26.0° (*c*0.35; $CHCl_3$); IR (KBr): ν = 3492*vs*, 3446*vs*, 3432*s*, 2994*w*, 2970*m*, 2946*m*, 2926*m*, 2860*w*, 1718*m*, 1688*s*, 1654*w*, 1636*w*, 1628*w*, 1470*w*, 1458*w*, 1438*w*, 1240*m*, 1192*w*, 1178*w*, 1162*w*, 1048*w*, 1038*w*, 994*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 3.67 (*s*, 3H, CH_3 (31)), 3.19 (*dd*, *J* = 11.4, 4.4 Hz, 1H, C*H* (3)), 2.81–2.74 (*m*, 1H, C*H* (18)), 2.61 (*d*, *J* = 3.9 Hz, 1H, C*H* (13)), 2.23 (*dd*, *J* = 16.7, 4.8 Hz, 1H, CH_a (11)), 2.12 (*dd*, *J* = 16.1, 13.8 Hz, 1H, CH_b (11)), 1.94 (*m*, 1H, CH_a (19)), 1.87 (*ddd*, *J* = 14.6, 14.6 Hz, 4.1 Hz, 1H, CH_a (16)), 1.79 (*ddd*, *J* = 13.7, 13.7, 4.3 Hz, 1H, CH_a (7)), 1.69–1.56 (*m*, 4H, C*H* (9) + CH_2 (16) + CH_a (2)), 1.54 (*m*, 1H, CH_a (1)), 1.50–1.39 (*m*, 2H, CH_a (22) + CH_a (21)), 1.35–1.24 (*m*, 2H, CH_b (22) + CH_b (21)), 1.20 (*dd*, *J* = 13.3, 13.3 Hz, 1H, CH_b (19)), 1.07 (*d*, 1H, CH_b (2)), 0.98 (*s*, 3H, CH_3 (30)), 0.98–0.95 (*m*, 1H, CH_b (16)), 0.96 (*s*, 3H, CH_3 (27)), 0.95 (*s*, 3H, CH_3 (23)), 0.93 (*s*, 3H, CH_3 (28)), 0.89 (*s*, 3H, CH_3 (29)), 0.84 (*s*, 3H, CH_3 (26)), 0.77 (*s*, 3H, CH_3 (25)), 0.74–0.67 (*m*, 1H, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 211.7 (*C*=O, C12), 178.2 (*C*=O, C28), 78.6 (*C*HOH, C3), 55.1 (*C*H, C5), 51.8 (*C*H, C13), 51.9 (CH_3, C31), 49.7 (*C*H, C9), 47.3 (C_{quart}, C17), 41.9 (C_{quart}, C14), 41.2 (C_{quart}, C8), 38.8 (CH_2, C11), 38.5 (CH_2, C1), 37.9 (C_{quart}, C10), 36.9 (C_{quart}, C4), 36.2 (CH_2, C19), 34.4 (CH_2, C1), 33.4 (CH_3, C29), 32.9 (CH_2, C7 + C22), 32.0 (CH_2, C21), 31.8 (*C*H, C18), 30.6 (C_{quart}, C20), 27.9 (CH_3, C29), 27.5 (CH_2, C2), 27.04 (CH_3, C23), 23.1 (CH_3, C27), 22.8 (CH_2, C16), 20.5 (CH_3, C30), 18.3 (CH_2, C6), 16.0 (CH_3, C24), 15.3 (CH_3, C26), 15.2 (CH_3, C25) ppm; MS (ESI, MeOH): *m*/*z* = 487.5 (54.8%, $[M + H]^+$), 509.6 (79.6%, $[M + Na]^+$), 995.3 (100%, $[2M + Na]^+$).

5.4.18. (2α, 3β) Methyl 3,12-dihydroxy-11-oxo-olean-12-en-28-oate (**17**)

Compound **17** was obtained using method **E** starting from compound **16** as colorless crystals; yield: 96%; m.p.: 206–210 °C; R_F = 0.43 (*n*-hexane/ethyl acetate, 7:3); $[\alpha]_D$ = +70° (*c* 0.43; $CHCl_3$); IR (KBr):ν = 3530*s*, 3472*m*, 3008*m*, 2986*s*, 2948*s*, 2928*s*, 2864*s*, 1726*vs*, 1652*m*, 1614*s*, 1470*m*, 1454*m*, 1390*s*, 1366*s*, 1306*m*, 1276*m*, 1262*s*, 1242*m*, 1204*m*, 1192*s*, 1162*m*, 1046*s*, 758*s*, 736 *s* cm^{-1}; UV–Vis ($CHCl_3$): λ_{max} (log ε) = 281.2 nm (4.48); ^1H NMR (500 MHz, $CDCl_3$): δ = 6.22 (*s*, 1H, O*H*), 3.66 (*ddd*, *J* = 9.0, 9.0, 1.8 Hz, 1H, C*H* (18)), 3.62 (*s*, 3H, CH_3 (31)), 3.22 (*dd*, *J* = 10.4, 5.9 Hz, 1H, C*H* (3)), 2.79 (*ddd*, *J* = 13.4, 3.4, 3.4 Hz, 1H, CH_a (1)), 2.44 (*s*, 1H, C*H* (9)), 2.04 (*ddd*, *J* = 13.7, 13.7, 3.9 Hz, 1H, CH_a (16)), 1.76 (*ddd*, *J* = 13.9, 13.9, 4.4 Hz, 1H, CH_a (7)), 1.70 (*brd*, *J* = 13.7 Hz, 1H, CH_b (6)), 1.67–1.52 (*m*, 6H, CH_b (7) + CH_a (21) + CH_2 (11) + CH_a (15) + CH_a (6)), 1.38–1.26 (*m*, 5H, CH_2 (19) + CH_b (21) + CH_a (22) + CH_b (6)), 1.36 (*s*, 3H, CH_3 (27)), 1.26–1.22 (*m*, 1H, CH_b (22)), 1.19 (*ddd*, *J* = 14.0, 3.2, 3.2 Hz, 1H, CH_b (15)), 1.10 (*s*, 3H, CH_3 (25)), 1.04–0.95 (*m*, 1H, CH_b (1)), 0.99 (s, 6H, CH_3 (30) + CH_3 (23)), 0.92 (*s*, 3H, CH_3 (29)), 0.91 (*s*, 3H, CH_3 (26)), 0.79 (s, 3H, CH_3 (24)), 0.70 (*brd*, *J* = 11.4 Hz, 1H, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 195.6 (*C*=O, C11), 178.0 (*C*=O, C28), 142.3 (*C*OH=C, C12), 136.8 (*C*=COH, C13), 78.8 (*C*HOH, C3), 60.6 (*C*H, C9), 55.1 (*C*H, C5), 52.0 (CH_3, C31), 46.2 (C_{quart}, C14), 45.6 (C_{quart}, C17), 41.7 (C_{quart}, C8), 40.4 (CH_2, C1), 39.3(CH_2, C19), 39.2 (C_{quart}, C4), 37.6 (C_{quart}, C10), 34.2 (CH_2, C22), 33.4 (*C*H, C18), 33.2 (CH_2, C21), 33.0 (CH_3, C29), 32.0 (CH_2, C7), 30.7 (C_{quart}, C20), 28.2 (CH_3, C23), 28.0 (CH_2, C15), 27.4 (CH_2, C11), 23.5 (CH_3, C27), 23.4 (CH_3, C30), 23.3 (CH_2, C2), 18.9 (CH_3, C26), 17.5 (CH_2, C6), 16.5 (CH_3, C25), 15.7 (CH_3, C24) ppm; MS (ESI): *m*/*z* = 501.3 (100%, $[M + H]^+$), 1023.4 (64%, $[2M + Na]^+$).

5.4.19. (3β) Methyl 3-hydroxy-11-oxo-olean-12-en-28-oate (**18**)

To a mixture of **16** (450 mg, 0.88 mmol) in acetone (50 mL) and glacial acetic acid (5 mL) *N*-hydroxysuccinimide (950 mg, 8.25 mmol) and potassium dichromate (780 mg, 2.64 mmol) were added, and the mixture was stirred at 45 °C for 48 h. The mixture was quenched with an aq potassium disulfide solution and washed with an aq. sodium hydrogen carbonate solution. After extracting with DCM, the organic phases were combined, washed, dried and evaporated. Chromatographic purification gave compound **18**; yield 71%; m.p.: 184–188 °C (lit.: [36]: 181–188 °C); R_F = 0.62 (n-hexane/ethyl acetate, 7:3; $[\alpha]_D$ = +82° (*c* 0.14; $CHCl_3$); UV–Vis (MeOH): λ_{max} (log ε) = 269 nm (4.03); IR (KBr): ν = 3339*br*, 2949*s*, 2866*s*, 1724*s*, 1661*s*, 1466*m*, 1387*m*, 1365*w*, 1330*w*, 1304*w*, 1261*m*, 1227*w*, 1209*m*, 1189*m*, 1162*m*, 1125*w*, 1089*w*, 1039*m*, 1013*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.61 (*s*, 1H, C*H* (12)), 3.61 (*s*, 3H, CH_3 (31)), 3.20 (*dd*, *J* = 10.8, 5.5 Hz, 1H, C*H* (3)), 2.98 (*dd*, *J* = 13.8, 3.7 Hz, 1H, C*H* (18)), 2.80 (*d*, *J* = 13.4 Hz, 1H, CH_a (1)), 2.30 (*s*, 1H, C*H* (9)), 2.02 (*ddd*, *J* = 13.8, 13.8, 4.0 Hz, 1H, CH_a (16)), 1.75–1.52 (*m*, 9H, CH_a (19) + CH_b (16) + CH_a (7) + CH_a (15) + CH_2 (22) + CH_2 (2) + CH_a (6)), 1.42–1.13 (*m*, 6H, CH_b (19) + CH_b (7) + CH_2 (21) + CH_b (15) + CH_b (6)), 1.34 (*s*, 3H, CH_3 (27)), 1.08 (*s*, 3H, CH_3 (23)), 1.00–0.95 (*m*, 1H, CH_b (1)), 0.97 (*s*, 3H, CH_3 (30)), 0.92 (*s*, 3H, CH_3 (29)), 0.91 (*s*, 3H, CH_3 (25)), 0.89 (*s*, 3H, CH_3 (24)), 0.78 (*s*, 3H, CH_3 (26)), 0.66 (*brd*, *J* = 11.3 Hz, 1H, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.3 (*C*=*O*, C11), 177.4 (*C*=*O*, C28), 168.6 (*C* = CH, C13), 127.9 (*C*H = C, C12), 78.7 (*C*HOH, C3), 61.7 (*C*H, C9), 55.0 (*C*H, C5), 51.8 (CH_3, C31), 46.2 (C_{quart}, C17), 45.0 (C_{quart}, C8), 44.2 (CH_2, C19), 43.4 (C_{quart}, C14), 41.5 (*C*H, C18), 39.1 (CH_2, C1), 38.8 (C_{quart}, C4), 37.2 (C_{quart}, C10), 33.7 (CH_2, C21), 32.9 (CH_2, C7), 32.8 (CH_3, C29), 31.6 (CH_2, C22), 30.6 (C_{quart}, C20), 28.1 (CH_3, C23), 27.7 (CH_2, C15), 27.3 (CH_2, C2), 23.5 (CH_3, C27), 23.4 (CH_3, C30), 22.9 (CH_2, C16), 18.9 (CH_3, C26), 17.4 (CH_2, C6), 16.1 (CH_3, C24), 15.5 (CH_3, C25) ppm; MS (ESI, MeOH): *m*/*z* = 485.6 (100%, $[M + H]^+$), 507.5 (35%, $[M + Na]^+$).

5.4.20. (3β) Benzyl 3-hydroxy-urs-12-en-28-oate (**19**)

UA (1 equiv.) was dissolved in DMF and treated with benzylchloride following method **D**. Compound **19** was obtained as a colorless solid; yield: 82%; m.p.: 182 °C (lit.: 180–182 °C [63]); R_F = 0.25 (*n*-hexane/ethyl actetate 8:2); $[\alpha]_D$ = +45.6° (*c* 0.30, $CHCl_3$); IR (KBr): ν = 3518*vs*, 3474*br*, 3266*m*, 3034*w*, 2988*s*, 2970*s*, 2942*s*, 2922*vs*, 2868*s*, 2854*s*, 2836*m*, 2798*w*, 1714*vs*, 1498*w*, 1464*m*, 1452*m*, 1376*m*, 1308*w*, 1288m, 1272*m*, 1248*m*, 1230*m*, 1198*m*, 1180*m*, 1164*m*, 1140*m*, 1114*m*, 1096*w*, 1050*m*, 1028 *m* cm^{-1}; ^1H NMR

(500 MHz, $CDCl_3$): δ = 7.53–7.29 (m, 5H, CH_{aromat}), 5.37 (*br*, 1H, OH), 5.24 (*dd*, *J* = 3.4, 3.4 Hz, 1H, C*H* (12)), 5.10 (*d*, *J* = 12.5 Hz, 1H, CH_a (31)), 4.98 (*d*, *J* = 12.5 Hz, 1H, CH_b (31)), 3.21 (*dd*, *J* = 11.0, 4.5 Hz, 1H, C*H* (3)), 2.27 (*d*, *J* = 11.3 Hz, 1H, C*H* (18)), 2.01 (*ddd*, *J* = 13.3, 13.3, 4.4 Hz, 1H, CH_a (16)), 1.94–1.84 (*m*, 2H, CH_2 (2)), 1.84–1.75 (*m*, 1H, CH_a (15)), 1.75–1.67 (*m*, 1H, CH_b (16)), 1.67–1.57 (*m*, 5H, CH_2 (22) + CH_a (1), CH_2 (11)), 1.52–1.40 (*m*, 4H, CH_a (6) + CH_a (7) + CH_a (21) + C*H* (9)), 1.41–1.20 (*m*, 5H, C*H* (19) + C*H* (20) + CH_b (7) + CH_b (21) + CH_b (6)), 1.07 (*s*, 3H, CH_3 (27)), 1.02–0.95 (*m*, 2H, CH_b (15) + CH_b (1)), 0.99 (*s*, 3H, CH_3 (23)), 0.94 (*d*, *J* = 6.2 Hz, 3H, CH_3 (30)), 0.89 (*s*, 3H, CH_3 (25)), 0.85 (*d*, *J* = 6.4 Hz, 3H, CH_3 (29)), 0.78 (*s*, 3H, CH_3 (24)), 0.71 (*brd*, *J* = 11.7 Hz, 1H, C*H* (5)), 0.65 (*s*, 3H, CH_3 (26)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 177.4 (*C*=O, C28), 138.2 (*C*=CH, C13), 136.5 (C_{aromat}, C32), 128.5 (CH_{aromat}, C33), 128.3 (CH_{aromat}, C34), 128.1 (CH_{aromat}, C35), 125.9 (*C*H=C, C12), 79.2 (*C*H, C3), 66.1 (CH_2, C31), 55.4 (*C*H, C5), 53.0 (*C*H, C18), 48.3 (C_{quart}, C17), 47.7 (*C*H, C9), 42.2 (C_{quart}, C14), 39.7 (C_{quart}, C8), 39.2 (*C*H, C19), 39.0 (*C*H, C20), 38.9 (C_{quart}, C4), 38.8 (CH_2, C1), 37.1 (C_{quart}, C10), 36.8 (CH_2, C22), 33.2 (CH_2, C7), 30.8 (CH_2, C21), 28.3 (CH_3, C23), 28.1 (CH_2, C15), 27.4 (CH_2, C11), 24.4 (CH_2, C16), 23.7 (CH_3, C27), 23.4 (CH_2, C2), 21.3 (CH_3, C30), 18.5 (CH_2, C6), 17.2 (CH_3, C29), 17.1 (CH_3, C26), 15.8 (CH_3, C24), 15.6 (CH_3, C25) ppm; MS (ESI, MeOH): *m*/*z* = 547.3 (10%, $[M + H]^+$), 569.5 (100%, $[M + Na]^+$), 1115.2 (82%, $2M + Na]^+$).

5.4.21. (3β) Benzyl 3-hydroxy-olean-12-en-28-oate (**20**)

OA (1 equiv.) was treated with benzylchloride following method **D**, and **21** was obtained as fine colorless needles; yield: 92%; m.p.: 185–187 °C (lit [64].: 189 °C); $[\alpha]_D$ = +58.4° (*c* 0.45; $CHCl_3$) [lit.: [65]: 50.1° (*c* 2.44; $CHCl_3$)]; R_F = 0.42 (*n*-hexane/ethyl acetate, 8:2); IR (KBr): ν = 3583*s*, 2938*s*, 1726*s*, 1498*w*, 1463*m*, 1386*m*, 1363*w*, 1323*w*, 1305*w*, 1264*w*, 1234*w*, 1201*m*, 1182*m*, 1162*m*, 1129*m*, 1095*w*, 1044*m*, 1016 *m* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.37–7.27 (*m*, 5H, CH_{aromat}), 5.29 (*dd*, *J* = 3.6, 3.6 Hz, 1H, C*H* (12)), 5.09 (*d*, *J* = 12.6 Hz, 1H, CH_a (31)), 5.05 (*d*, *J* = 12.5 Hz, 1H, CH_b (31)), 3.20 (*dd*, *J* = 11.3 Hz, 4.5 Hz, 1H, C*H*OH (3)), 2.91 (*dd*, *J* = 13.8, 4.2 Hz, 1H, C*H* (18)), 1.98 (*ddd*, *J* = 13.4, 13.0, 4.1 Hz, 1H, CH_a (16)), 1.85 (*dd*, *J* = 8.9, 3.6 Hz, 1H, CH_2 (11)), 1.76–1.14 (*m*, 16H, C*H* (9) + CH_2 (19) + CH_a (1) + CH_2 (21)+ CH_2 (7) + CH_2 (22) + CH_a (15)+ CH_2 (2)+ CH_b (16)+ CH_2 (6)), 1.13 (*s*, 3H, CH_3 (27)), 1.09–1.00 (*m*, 2H, CH_b (1) + CH_b (15)), 0.98 (*s*, 3H, CH_3 (23)), 0.92 (*s*, 3H, CH_3 (30)), 0.90 (*s*, 3H, CH_3 (29)), 0.88 (*s*, 3H, CH_3 (25)), 0.77 (*s*, 3H, CH_3 (24)), 0.70 (*brd*, *J* = 11.5 Hz, 1H, C*H* (5)), 0.61 (*s*, 3H, CH_3 (26)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.4 (*C*=O, C28), 143.7 (*C*=CH, C13), 136.4 (C_{quart}, C32), 128.4 (CH_{aromat}, C35), 128.0 (CH_{aromat}, C34), 127.9 (CH_{aromat}, C33), 122.5 (*C*H=C, C12), 80.0 (*C*HOH, C3), 65.9 (CH_2, C31), 55.2 (*C*H, C5), 47.6 (*C*H, C9), 46.7 (C_{quart}, C17), 45.9 (CH_2, C19), 41.7 (C_{quart}, C14), 41.4 (*C*H, C18), 39.3 (C_{quart}, C8), 38.7 (C_{quart}, C4), 38.4 (CH_2, C1), 37.0 (C_{quart}, C10), 33.8 (CH_2, C21), 33.1 (CH_3, C29), 32.7 (CH_2, C7), 32.4 (CH_2, C22), 30.7 (C_{quart}, C20), 28.1 (CH_3, C23), 27.6 (CH_2, C15), 27.2 (CH_2, C2), 25.9 (CH_3, C27), 23.6 (CH_3, C30), 23.4 (CH_2, C11), 23.0 (CH_2, C16), 18.3 (CH_2, C6), 16.9 (CH_3, C26), 15.6 (CH_3, C24), 15.3 (CH_3, C25) ppm; MS (ESI, MeOH): *m*/*z* = 569.5 (100%, $[M + Na]^+$).

5.4.22. (3β) Benzyl 3-hydroxy-12-oxo-olean-28-oate (**21**)

Compound **21** was obtained following method **A** as a colorless solid; yield: 83%; m.p.: 198–201 °C (lit.: 196–198 °C [4]); R_F = 0.74 (silica gel, toluene/ethyl acetate/formic acid/*n*-heptane, 80:20:3:20); $[\alpha]_D$ = +54.1° (*c* 0.5, $CHCl_3$); UV–Vis ($CHCl_3$): λ_{max} (log ε) = 258.17 (1.35) nm; IR (KBr):ν = 2942*s*, 1728*s*, 1711*s*, 1472*m*, 1456*m*, 1387*w*, 1372*m*, 1240*s*, 1204*m*, 1180*m*, 1161*m*, 1140*m*, 1119*m*, 1086*w*, 1037*m*, 1005*w* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36–7.29 (*m*, 5H, C*H* (33) + C*H* (34) + C*H* (35)), 5.19 (*d*, *J* = 11.6 Hz, 1H, CH_a (31)), 5.07 (*d*, *J* = 11.6 Hz, 1H, CH_b (31)), 4.45 (*dd*, *J* = 11.3, 5.0 Hz, 1H, C*H* (3)), 2.83 (*ddd*, *J* = 10.2, 3.3, 3.3 Hz, 1H, C*H* (18)), 2.45 (*d*, *J* = 4.2 Hz, 1H, C*H* (13)), 2.18 (*dd*, *J* = 16.7, 16.7, 4.8 Hz, 1H, CH_a (16)), 2.03 (s, 3H, CH_3 (37)), 2.04–1.98 (*m*, 1H, CH_a (15)), 1.95–1.90 (m, 1H, CH_a (19)), 1.89–1.80 (m, 2H, CH_a (7) + CH_b (16)), 1.69–1.52 (*m*, 5H, CH_b (15) + CH_a (6) + CH_2 (11) + CH_a (2)), 1.51–1.47 (*m*, 3H, C*H* (9) + CH_a (21) + CH_b (7)), 1.44–1.30 (*m*, 3H, CH_a (1) + CH_b (6) + CH_a (22)), 1.29–1.15 (*m*, 3H, CH_b (1) + CH_b (22) + CH_b (19)), 1.05–0.94 (*m*, 1H, CH_b (2)), 0.99 (*s*, 3H, CH_3 (30)), 0.97 (*s*, 3H, CH_3 (23)), 0.90 (*s*, 6H, CH_3 (27) + CH_3 (29)), 0.87–0.84 (*m*, 1H, CH_b (15)), 0.85 (*s*, 3H, CH_3 (25)), 0.81 (*s*, 3H, CH_3 (24)), 0.79 (*m*, 1H, C*H* (5)), 0.61 (*s*, 3H, CH_3 (26)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 211.6 (*C*=O, C12), 177.6 (*C*=O, C28), 171.0 (*C*=O, C36), 136.5 (C_{quart}, C32), 128.7 (C_{aromat}, C33), 128.5 (C_{aromat}, C35), 128.3 (C_{aromat}, C34), 80.6 (*C*H, C3), 66.1 (CH_2, C31), 55.3 (*C*H, C5), 52.0 (*C*H, C13), 49.8 (*C*H, C9), 47.4 (C_{quart}, C17), 42.0 (C_{quart}, C14), 41.3 (C_{quart}, C8), 38.6 (C_{quart}, C4), 37.9 (CH_2, C21), 37.8 (CH_2, C11), 36.9 (C_{quart}, C10), 36.4 (CH_2, C19), 34.6 (CH_2, C7), 33.5 (CH_3, C29), 33.0 (CH_2, C22), 32.2 (*C*H, C18), 31.8 (CH_2, C1), 30.8 (C_{quart}, C20), 28.0 (CH_3, C23), 27.5 (CH_2, C15), 23.5 (CH_2, C2), 23.3 (CH_3, C30), 22.9 (CH_2, C16), 21.4 (CH_3, C37), 20.6 (CH_3, C27), 18.3 (CH_2, C6), 16.6 (CH_3, C26), 15.9 (CH_3, C24), 15.3 (CH_3, C25) ppm; MS (ESI, MeOH): *m*/*z* = 605.1 (56%, $[M + H]^+$), 622.1 (26%, $[M + NH_4]^+$), 585.3 (32%, $[M + Na]^+$), 929.9 (26%, $[3M+2Na]^{2+}$), 1209.2 (26%, $[2M + H]^+$), 1231.3 (100%, $[2M + Na]^+$).

5.4.23. (3β) Benzyl 3-acetoxy-urs-11-en-28-oate (**22**)

Compound **22** [66] was obtained from a mixture of **22** and **23** by using method **A**; yield: 56%; R_F = 0.7 (*n*-hexane/ethyl acetate, 85:15); m.p.: 173–174 °C; $[\alpha]_D$ = +45.1° (*c* 0.66, $CHCl_3$); IR (KBr): ν = 3433*w*, 3069*w*, 2926*m*, 2926*s*, 2874*s*, 1727*s*, 1499*w*, 1465*s*, 1388*m*, 1379*s*, 1039*m*, 1283*m*, 1270*m*, 1237*s*, 1197*s*, 1182*s*, 1140*s*, 1110*s*, 1028*s*, 1005*m*, 986*s*, 970*s*, 697*s* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.37–7.27 (*m*, 5H, C*H* (33) + C*H* (35) + C*H* (36)), 5.23 (*dd*, *J* = 3.5, 3.5 Hz, 1H, C*H* (12)), 5.10 (*d*, *J* = 12.5 Hz, 1H, CH_a (31)), 4.98 (*d*, *J* = 12.5 Hz, 1H, CH_b (31)), 4.49 (*dd*, *J* = 9.7, 6.3 Hz, 1H, C*H* (3)), 2.26 (*d*, *J* = 11.2 Hz, 1H, C*H* (18)), 2.04 (*s*, 3H, CH_3 (37)), 2.00 (*dd*, *J* = 13.2, 13.2, 4.6 Hz, 1H, $CH_á$ (16)), 1.88 (*m*, 2H, CH_a (2)), 1.85 (*ddd*, *J* = 10.5, 10.5, 4.5 Hz, 1H, CH_a (11)), 1.80 (*ddd*, *J* = 13.5, 13.5, 4.6 Hz, 1H, CH_a (15)), 1.69 (*ddd*, 1H, *J* = 10.5, 3.2, 3.2 Hz, CH_b (11)), 1.66–1.60 (*m*, 4H, CH_2 (22) + CH_a (1) + CH_b (2)), 1.52–1.44 (*m*, 4H, C*H* (9) + CH_a (6) + CH_a (21) + CH_b (16)), 1.33–1.30 (*m*, 1H, C*H* (20)), 1.30–1.27 (*m*, 1H, CH_b (21)), 1.28–1.25 (*m*, 1H, CH_b (7)), 1.07 (*s*, 3H, CH_3 (27)), 1.11–1.03 (*m*, 2H, CH_b (1) + CH_b (15)), 0.99 (*dd*, *J* = 11.6, 5.7 Hz, 1H, C*H* (19)), 0.94 (*s*, 3H, CH_3 (30)), 0.91 (*s*, 3H, CH_3 (25)), 0.86 (*s*, 6H, CH_3 (23) + CH_3 (29)), 0.85 (*s*, 3H, CH_3 (24)), 0.87–0.80 (*m*, 1H, C*H* (5)), 0.64 (*s*, 3H, CH_3 (26)) ppm; ^{13}C NMR (500 MHz, $CDCl_3$): δ = 177.2 (*C*=O, C28), 170.9 (*C*=O, C36), 138.1 (*C*=CH, C13), 136.4 (C_{quart}, C32), 128.4 (CH_{aromat}, C34), 128.1 (CH_{aromat}, C33), 127.9 (CH_{aromat}, C35), 125.6 (*C*H=C, C12), 80.9 (*C*HOAc, C3), 65.9 (CH_2, C31), 55.3 (*C*H, C5), 52.9 (*C*H, C18), 48.1 (C_{quart}, C17), 47.5 (*C*H, C9), 42.0 (C_{quart}, C14), 39.5 (C_{quart}, C8), 39.1 (*C*H, C19), 38.8 (C_{quart}, C20), 38.3 (CH_2, C1), 37.7 (C_{quart}, C4), 36.8 (C_{quart}, C10), 36.6 (CH_2, C21), 32.9 (CH_2, C7), 30.6 (CH_2, C22), 28.1 (CH_3, C29), 27.9 (CH_2, C15), 24.2 (CH_2, C16), 23.5 (CH_2, C11), 23.5 (CH_3, C27), 23.2 (CH_2, C2), 21.3 (CH_3, Ac), 21.1 (CH_3, C30), 18.2 (CH_2, C6), 17.0 (CH_3, C26), 17.0 (CH_3, C24), 16.7 (CH_3, C23), 15.5 (CH_3, C25) ppm; MS (ESI, MeOH): *m*/*z* = 589.2 (30%, $[M + H]^+$), 611.5 (30%, $[M + Na]^+$), 1199.2 (100%, $[2M + Na]^+$).

5.4.24. (3β) Benzyl 3-acetoxy-olean-11-en-28-oate (**23**)

Compound **23** [67] **was** obtained using method **D** as a colorless solid; yield: 83%; R_F = 0.7 (*n*-hexane/ethyl acetate, 85:15); m.p.: 228–230 °C; $[\alpha]_D$ = +55.8° (*c* 0.33, $CHCl_3$); IR (KBr): ν = 2940*brs*, 1726*vs*, 1468*w*, 1456*w*, 1388*w*, 1378*w*, 1364*w*, 1264*m*, 1242*s*, 1200*w*, 1178*m*, 1162*m*, 1122*w*, 1030*m*, 1012*m* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.37–7.27 (*m*, 5H, CH_{aromat}), 5.28 (*dd*, *J* = 3.5, 3.5 Hz, 1H,

CH (12)), 5.09 (*d*, *J* = 12.6 Hz, 1H, CH_a (31)), 5.04 (*d*, *J* = 12.5 Hz, 1H, CH_b (31)), 4.48 (*m*, 1H, CH (3)), 2.90 (*dd*, *J* = 13.7, 4.1 Hz, 1H, CH (18)), 2.04 (*s*, 3H, CH_3 (Ac)), 1.98 (*ddd*, *J* = 13.5, 13.5, 4.1 Hz, 1H, CH_a (16)), 1.85 (*dd*, *J* = 8.9, 3.5 Hz, 2H, CH_2 (11)), 1.75–1.68 (*ddd*, *J* = 13.8, 13.8, 4.3 Hz, 1H, CH_a (7)), 1.68–1.58 (*m*, 7H, CH_a (19) + CH_a (1) + CH_b (7) + CH_a (15) + CH_2 (2) + CH_b (16)), 1.60–1.48 (*m*, 1H, CH_a (6)), 1.42 (*ddd*, *J* = 12.6, 12.6, 3.4 Hz, 1H, CH_a (21)), 1.38–1.29 (*m*, 2H, CH_b (6) + CH_b (22)), 1.27–1.20 (*ddd*, *J* = 9.7, 3.5, 2.5 Hz, 1H, CH_b (21)), 1.22–1.16 (*ddd*, *J* = 11.7, 4.1, 2.4 Hz, 1H, CH_b (22)), 1.20–1.13 (*ddd*, *J* = 11.5, 4.5, 2.3 Hz, 1H, CH_b (19)), 1.12 (*s*, 3H, CH_3 (27)), 1.08–0.98 (*m*, 2H, CH_b (1) + CH_b (15)), 0.92 (*s*, 3H, CH_3 (30)), 0.90 (*s*, 3H, CH_3 (25)), 0.90 (*s*, 3H, CH_3 (29)), 0.86 (*s*, 3H, CH_3 (23)), 0.85 (*s*, 3H, CH_3 (24)), 0.84–0.78 (*dd*, *J* = 11.8, 1.7 Hz, 1H, CH (5)), 0.61 (*s*, 3H, CH_3 (26)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 177.6 (*C*=O, C28), 171.1 (*C*=O, Ac), 143.9 (*C*=CH, C13), 136.6 (C_{aromat}, C32), 128.6 (CH_{aromat}, C34), 128.1 (CH_{aromat}, C33), 128.0 (CH_{aromat}, C35), 122.6 (*C*H=C, C12), 81.1 (*C*HOH, C3), 66.1 (CH_2, C31), 55.5 (*C*H, C5), 47.7 (*C*H, C9), 46.9 (C_{quart}, C17), 46.0 (CH_2, C19), 41.8 (C_{quart}, C8), 41.6 (*C*H, C18), 39.5 (C_{quart}, C20), 38.3 (CH_2, C1), 37.8 (C_{quart}, C4), 37.1 (C_{quart}, C14), 34.0 (CH_2, C22), 33.2 (CH_3, C29), 32.8 (CH_2, C21), 32.5 (CH_2, C7), 30.9 (C_{quart}, C10), 28.2 (CH_3, C23), 27.8 (CH_2, C15), 26.0 (CH_3, C27), 23.8 (CH_3, C30), 23.7 (CH_2, C2), 23.6 (CH_2, C11), 23.2 (CH_2, C16), 21.5 (CH_3, Ac), 18.4 (CH_2, C6), 17.0 (CH_3, C26), 16.8 (CH_3, C24), 15.5 (CH_3, C25) ppm; MS (ESI, MeOH): *m*/*z* = 589.2 (42.9%, $[M + H]^+$), 611.3 (82.5%, $[M + Na]^+$), 1199.4 (100%, $[2M + Na]^+$).

5.4.25. (3β) Benzyl 3-acetoxy-12-oxo-olean-28-oate (**24**)

Treatment of **23** following method **A** gave **24** as a colorless solid; yield: 83%; m.p.: 196–198 °C (lit [68].: 196–198 °C); R_F = 0.38 (*n*-hexane/ethyl acetate, 8:2); $[\alpha]_D$ = +5.2° (*c* 0.63, $CHCl_3$); IR (KBr):*ν* = 3404*w*, 2943*s*, 1728*s*, 1711*s*, 1472*m*, 1456*m*, 1420*w*, 1387*m*, 1366*m*, 1332*w*, 1308*w*, 1241*s*, 1204*m*, 1180*m*, 1161*m*, 1140*m*, 1119*m*, 1086*w*, 1038*w*, 1006*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.39–7.27 (*m*, 5H, CH_{aromat}), 5.21 (*d*, *J* = 12.2 Hz, 1H, CH_a (31)), 5.08 (*d*, *J* = 12.2 Hz, 1H, CH_b (31)), 4.47 (*dd*, *J* = 11.5, 4.8 Hz, 1H, CH (3)), 2.85 (*ddd*, *J* = 10.3, 3.2, 3.8 Hz, 1H, CH (18)), 2.46 (*d*, *J* = 4.2 Hz, 1H, CH (13)), 2.19 (*dd*, *J* = 16.6, 4.8 Hz, 1H, CH_a (11)), 2.05 (*s*, 3H, CH_3 (Ac)), 2.02 (*dd*, *J* = 16.5, 3.1 Hz, 1H, CH_b (11)), 1.94 (*ddd*, *J* = 12.8, 2.4, 2.2 Hz, 1H, CH_a (1)), 1.90–1.85 (*m*, 1H, CH_a (16)), 1.88 (*ddd*, *J* = 13.8, 4.4, 4.4 Hz, 1H, CH_a (22)), 1.70–1.64 (*m*, 1H, CH_b (16)), 1.64–1.55 (*m*, 5H, CH (9) + CH_2 (2) + CH_a (21) + CH_a (6)), 1.56–1.48 (*m*, 1H, CH_b (19)), 1.47 (*ddd*, 1H, *J* = 14.0, 3.4, 3.4 Hz, CH_b (7)), 1.41–1.33 (*m*, 3H, CH_b (22) + CH_b (6) + CH_a (15)), 1.33–1.26 (*m*, 3H, CH_b (22) + CH_b (15) + CH_b (1)), 1.06–0.99 (*m*, 1H, CH_b (21)), 1.00 (*s*, 3H, CH_3 (27)), 0.99–0.95 (*m*, 1H, CH_b (19)), 0.91 (*s*, 6H, CH_3 (30) + CH_3 (28)), 0.86 (*s*, 3H, CH_3 (29)), 0.86 (*s*, 3H, CH_3 (24)), 0.83 (*s*, 3H, CH_3 (25)), 0.80 (*dd*, *J* = 11.1, 1.1 Hz, 1H, CH (5)), 0.68 (*s*, 3H, CH_3 (26)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 211.4 (*C*=O, C12), 177.4 (*C*=O, C28), 170.8 (*C*=O, Ac), 136.3 (C_{aromat}, C32), 128.5 (CH_{aromat}, C34), 128.4 (CH_{aromat}, C33), 128.1 (CH_{aromat}, C35), 80.4 (*C*HOH, C3), 65.9 (CH_3, C31), 55.1 (*C*H, C5), 51.8 (*C*H, C13), 49.6 (*C*H, C9), 47.2 (C_{quart}, C17), 41.8 (C_{quart}, C14), 41.2 (C_{quart}, C8), 38.4 (CH_2, C11), 37.7 (C_{quart}, C10), 37.6 (CH_2, C19), 36.8 (C_{quart}, C4), 36.2 (CH_2, C1), 34.5 (CH_2, C21), 33.4 (CH_3, C29), 32.9 (CH_2, C7), 32.1 (*C*H, C18), 31.7 (CH_2, C22), 30.6 (C_{quart}, C20), 27.9 (CH_3, C30), 27.4 (CH_2, C16), 23.4 (CH_2, C2), 23.2 (CH_3, C27), 22.7 (CH_2, C15), 21.2 (CH_3, Ac), 20.5 (CH_3, C23), 18.1 (CH_2, C6), 16.4 (CH_3, C24), 15.7 (CH_3, C26), 15.2 (CH_3, C25) ppm; (ESI, MeOH): *m*/*z* = 605.5 (100%, $[M + H]^+$), 622.4 (54.8%, $[M + NH_4]^+$), 627.5 (32.6%, $[M + Na]^+$), 929.8 (20%, $[3M+2Na]^{2+}$), 1209.2 (27.9%, $[2M + H]^+$), 1231.2 (76.0%, $[2M + Na]^+$).

5.4.26. (3β) Benzyl 3-acetoxy-11-oxo-12-hydroxy-olean-Δ-12,13-en-28-oate (**25**)

Obtained from **24** as a colorless solid following method **E**; yield: 75%; m.p.: 237–240 °C; R_F = 0.69 (toluene/ethyl acetate/formic acid/*n*-heptane, 80:20:3:20); $[\alpha]_D$ = +98.9° (*c* 0.34, $CHCl_3$); IR (KBr): *ν* = 3461*m*, 3033*w*, 2948*s*, 2868*m*, 1720*s*, 1667*m*, 1640*s*, 1498*s*, 1463*m*, 1372*s*, 1322*w*, 1306*m*, 1286*m*, 1263*s*, 1238*m*, 1205*m*, 1182*m*, 1163*s*, 1140*m*, 1118*m*, 1085*m*, 1085*w*, 1070*w*, 1035*s*, 1012*m* cm^{-1}; UV–Vis ($CHCl_3$): λ_{max} (log ε) = 289.93 (4.09) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.41–7.27 (*m*, 5H, CH (33) + CH (34) + CH (35)), 6.23 (*s*, 1H, CO*H*=C (12)), 5.12–5.05 (*m*, 2H, CH_2 (31)), 4.50 (*dd*, *J* = 11.5, 4.9 Hz, 1H, CH (3)), 3.75–3.71 (m, 1H, CH (18)), 2.78 (*ddd*, *J* = 13.4, 3.4, 3.4 Hz, 1H, CH_a (1)), 2.42 (*s*, 1H, CH (9)), 2.09–2.00 (m, 1H, CH_a (16)), 2.04 (*s*, 3H, CH_3 (Ac)), 1.80 (*ddd*, *J* = 13.9, 13.9, 4.5 Hz, 1H, CH_a (7)), 1.76–1.71 (*m*, 3H, CH_b (16) + CH_2 (2)), 1.71–1.62 (*m*, 1H, CH_b (7)), 1.58–1.50 (m, 3H, CH_a (6) + CH_a (15) + CH_a (22)), 1.43–1.34 (*m*, 2H, CH_2 (19) + CH_a (21)), 1.34 (*s*, 3H, CH_3 (27)), 1.33–1.24 (*m*, 3H, CH_b (21) + CH_b (6) + CH_b (22)), 1.18–1.13 (*ddd*, *J* = 14.2, 3.3, 3.3 Hz, 1H, CH_b (15)), 1.07 (*s*, 3H, CH_3 (25)), 1.09–1.03 (*m*, 1H, CH_b (1)), 0.99 (*s*, 3H, CH_3 (30)), 0.92 (*s*, 3H, CH_3 (29)), 0.86 (*s*, 6H, CH_3 (23) + CH_3 (24)), 0.82–0.75 (*m*, 1H, CH (5)), 0.69 (*s*, 3H, CH_3 (26)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 195.4 (*C*=O, C11), 177.1 (*C*=O, C28), 171.1 (*C*=O, CAc), 142.2 (*C*OH=C, C12), 136.7 (*C*=COH, C13), 136.3 (C_{quart}, C32), 128.6 (C_{aromat}, C34), 128.1 (C_{aromat}, C33), 128.1 (C_{aromat}, C35), 80.6 (CH, C3), 66.2 (CH_2, C31), 60.4 (CH, C9), 55.2 (CH, C5), 46.0 (C_{quart}, C17), 45.6 (C_{quart}, C14), 41.7 (C_{quart}, C8), 40.4 (CH_2, C19), 38.8 (CH_2, C1), 38.2 (C_{quart}, C4), 37.4 (C_{quart}, C10), 34.2 (CH_2, C21), 33.4 (CH, C18), 33.2 (CH_2, C7), 33.0 (CH_3, C29), 32.0 (CH_2, C22), 30.7 (C_{quart}, C20), 28.2 (CH_3, C23), 27.9 (CH_2, C15), 23.6 (CH_2, C2), 23.4 (CH_3, C27), 23.3 (CH_3, C30), 23.2 (CH_2, C16), 21.4 (CH_3, C37), 18.8 (CH_3, C26), 17.4 (CH_2, C6), 16.8 (CH_3, C24), 16.5 (CH_3, C25) ppm; MS (ESI, MeOH): *m*/*z* = 619.1 (48%, $[M + H]^+$), 641.3 (29%, $[M + Na]^+$), 1259.3 (100%, $[2M + Na]^+$).

5.4.27. (3β) But-3-enyl 3–hydroxy-olean-12-en-28-oate (**26**)

Compound **26** was prepared according to method **D** and obtained as colorless, fine needles (recrystallization from ethanol); yield 84%; m.p.: 80–81 °C; R_F = 0.52 (*n*-hexane/ethyl acetate, 6:4); $[\alpha]_D$ = +110.8 (*c* 0.31, $CHCl_3$); IR (KBr): *ν* = 3442*br*, 2944*m*, 1636*m*, 1620*w*, 1458*s*, 1446*m*, 1432*s*, 1174*w*, 1106*m*, 1032*m*, 1010*m* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.79 (*dddd*, *J* = 17.0, 10.2, 6.7, 6.7 Hz, 1H, CH (33)), 5.27 (*dd*, *J* = 3.5 Hz, 1H, CH (12)), 5.10 (*ddd*, *J* = 17.2, 3.2, 1.5 Hz, 1H, CH_a (34)), 5.06 (*ddd*, *J* = 10.3, 3.1, 1.6 Hz, 1H, CH_b (34)), 4.12–4.01 (*m*, 2H, CH_2 (31), *AA* 'XX'-system), 3.21 (*dd*, *J* = 11.4, 4.6 Hz, 1H, CH (3)), 2.86 (*dd*, *J* = 13.9, 4.3 Hz, 1H, CH (18)), 2.39–2.32 (*m*, 2H, CH_2 (32), AA'*XX*' system), 1.96 (*ddd*, *J* = 11.6, 11.6, 4.5 Hz, 1H, CH_a (16)), 1.89–1.84 (*m*, 2H, CH_2 (11)), 1.69 (*ddd*, *J* = 13.8, 13.8, 4.4 Hz, 1H, CH_a (7)), 1.66–1.50 (*m*, 9H, CH_a (19) + CH (9) + CH_a (1) + CH_b (7) + CH_2 (2) + CH_a (15) + CH_b (16) + CH_a (6)), 1.50–1.45 (*m*, 1H, CH_a (22)), 1.45–1.38 (*m*, 1H, CH_b (6)), 1.38–1.30 (*m*, 1H, CH_a (21)), 1.31–1.25 (*m*, 1H, CH_b (22)), 1.20–1.10 (*m*, 2H, CH_b (19) + CH_b (21)), 1.12 (*s*, 3H, CH_3 (27)), 1.07–1.00 (*m*, 1H, CH_b (15)), 0.98 (*s*, 3H, CH_3 (23)), 0.97–0.92 (*m*, 1H, CH_b (1)), 0.91 (*s*, 3H, CH_3 (30)), 0.90 (*s*, 3H, CH_3 (25)), 0.89 (*s*, 3H, CH_3 (29)), 0.77 (*s*, 3H, CH_3 (24)), 0.73 (*s*, 3H, CH_3 (26)), 0.72 (*dd*, *J* = 9.6, 1.4 Hz, 1H, CH (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 177.8 (*C*=O, C28), 143.9 (*C*=CH, C13), 134.5 (CH=CH_2, C33), 122.5 (*C*H=C, C12), 117.1 (CH_2=CH, C34), 79.2 (*C*HOH, C3), 63.4 (CH_2, C1), 55.4 (*C*H, C5), 47.8 (*C*H, C9), 46.8 (C_{quart}, C17), 46.1 (CH_2, C19), 41.9 (C_{quart}, C8), 41.4 (*C*H, C18), 39.5 (C_{quart}, C20), 38.9 (C_{quart}, C14), 38.6 (CH_2, C1), 37.2 (C_{quart}, C4), 34.1 (CH_2, C22), 33.3 (CH_3, C29), 33.3 (CH_2, C32), 32.9 (CH_2, C21), 32.6 (CH_2, C7), 30.9 (C_{quart}, C10), 28.3 (CH_3, C23), 27.8 (CH_2, C15), 27.4 (CH_2, C2), 26.0 (CH_3, C27), 23.7 (CH_3, C30), 23.6 (CH_2, C11), 23.2 (CH_2, C16), 18.5 (CH_2, C6), 17.2 (CH_3, C26), 15.7 (CH_3, C24), 15.5 (CH_3, C25) ppm; MS (ESI): *m*/*z* = 511.3 (30%, $[M + H]^+$), 1043.4 (100%, $[2M + Na]^+$).

*5.4.28. (3β) But-3-enyl 3–hydroxy-12-oxo-olean-28-oate (**27**)*

Compound was prepared using method **B** followed by chromatography (silica gel, hexane/ethyl acetate, 7:3) and recrystallization from ethanol as a colorless solid; yield 76%; m.p.: 72–73 °C; R_F = 0.36 (*n*-hexane/ethyl acetate, 6:4); $[\alpha]_D$ = −19.2 (*c* 0.31, $CHCl_3$); IR (KBr): *ν* = 3440*br*, 2972*s*, 2940*s*, 2862*m*, 2362*m*, 2344*m*, 1700*vs*, 1654*m*, 1646*m*, 1636*m*, 1466*m*, 1458*m*, 1438*m*, 1418*m*, 1388*m*, 1304*m*, 1256*m*, 1242*m*, 1188*m*, 1176*m*, 1088*m*, 1048*m*, 1036*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.82–5.73 (*m*, 1H, C*H* (33)), 5.11 (*ddd*, *J* = 17.2, 3.2, 1.5 Hz, 1H, CH_a (34)), 5.06 (*ddd*, *J* = 10.2, 1.6, 1.1 Hz, 1H, CH_b (34)), 4.19–4.08 (*m*, 2H, C*H* (31)), 3.20 (*dd*, *J* = 11.4, 4.7 Hz, 1H, C*H* (3)), 2.79 (*dd*, *J* = 10.1, 3.4, 3.4 Hz, 1H, C*H* (18)), 2.62 (*d*, *J* = 4.3 Hz, 1H, C*H* (13)), 2.38 (*brdd*, *J* = 6.6, 6.6 Hz, 2H, CH_2 (32)), 2.23 (*dd*, *J* = 16.7, 5.0 Hz, 1H, CH_a (11)), 2.13 (*dd*, *J* = 16.6, 13.2 Hz, 1H, CH_b (11)), 1.97–1.90 (*m*, 1H, CH_a (19)), 1.91–1.84 (*m*, 1H, CH_a (16)), 1.79 (*ddd*, *J* = 13.8, 13.8, 4.7 Hz, 1H, CH_a (7)), 1.70–1.51 (*m*, 6H, CH_b (16) + CH_a (6) + CH_2 (2) + C*H* (9) + CH_a (1)), 1.48–1.37 (*m*, 3H, CH_b (7) + CH_b (6) + CH_a (22)), 1.36–1.29 (*m*, 2H, CH_a (21) + CH_b (22)), 1.25–1.18 (*m*, 1H, CH_b (19)), 1.11–1.02 (*m*, 1H, CH_b (15)), 0.99 (*s*, 3H, CH_3 (30)), 0.97 (*s*, 3H, CH_3 (23)), 0.97 (*s*, 3H, CH_3 (25)), 0.94 (*s*, 3H, CH_3 (27)), 0.90 (*s*, 3H, CH_3 (29)), 0.92–0.87 (*m*, 1H, CH_b (1)), 0.85 (*s*, 3H, CH_3 (24)), 0.78 (*s*, 3H, CH_3 (26)), 0.74–0.70 (*m*, 1H, C*H* (5)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 211.9 (*C*═O, C12), 177.9 (*C*═O, C28), 134.2 (*C*H, C13), 117.4 (CH_2═CH, C34), 78.8 (*C*HOH, C3), 63.6 (CH_2, C31), 55.3 (*C*H, C5), 52.0 (*C*H, C13) 49.9 (*C*H, C9), 47.5 (C_{quart}, C17), 42.1 (C_{quart}, C14), 41.5 (C_{quart}, C8), 39.0 (CH_2, C11), 38.7 (C_{quart}, C20), 38.1 (CH_2, C1), 37.1 (C_{quart}, C4), 36.4 (CH_2, C19), 34.7 (CH_2, C32), 33.5 (CH_3, C29), 33.3 (*C*H_2, C21), 33.2 (*C*H_2, C7), 32.1 (*C*H, C18), 32.0 (*C*H_2, C32), 30.8 (C_{quart}, C10), 28.1 (CH_3, C23), 27.7 (CH_2, C15), 27.2 (CH_2, C2), 23.3 (*C*H_3, C30), 22.9 (*C*H_3, C21), 20.7 (*C*H_3, C30), 18.5 (CH_2, C6), 16.4 (*C*H_3, C24), 15.5 (*C*H_3, C26), 15.4 (*C*H_3, C25) ppm; MS (ESI): *m/z* = 527.2 (100%, $[M + H]^+$), 544.2 (20%, $[M + NH_4]^+$), 1053.3 (51%, $[2M + Na]^+$), 1075.3 (42%, $[2M + Na]^+$).

*5.4.29. (2α, 3β, 12α) 2,3,12–Trihydroxy-olean-28-oic acid 28,13-lactone (**28**)*

Compound **28** obtained from **MA** following method **A**; yield: 40%; m.p.: 229–231 °C; R_F = 0.21 (*n*-hexane/ethyl acetate, 1:1); $[\alpha]_D$ = 18.8° (*c* 0.36, $CHCl_3$); IR (KBr): *ν* = 3442*vs*, 3432*vs*, 2950*s*, 2936*s*, 2866*m*, 1754*s*, 1466*m*, 1456*m*, 1386*m*, 1366*m*, 1252*m*, 1222*m*, 1144*m*, 1134*m*, 1084*m*, 1046*m*, 1032*m*, 974*m*, 942*m*, 732*m* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 3.89 (*brs*, 1H, C*H* (12)), 3.85–3.68 (*m*, 1H, C*H* (2)), 3.06 (*d*, *J* = 8.6 Hz, 1H, C*H* (3)), 3.15–2.80 (*brs*, 2H, O*H*), 2.15 (*ddd*, *J* = 13.5, 13.5, 5.6 Hz, 1H, CH_a (16)), 2.10–1.91 (*m*, 5H, C*H* (18) + CH_a (1) + CH_a (19) + CH_2 (11)), 1.90–1.79 (*m*, 1H, CH_a (15)), 1.72–1.59 (*m*, 3H, C*H* (9) + CH_2 (7)), 1.58–1.45 (*m*, 4H, CH_a (22) + CH_2 (6) + CH_b (16)), 1.46–1.34 (*m*, 2H, CH_b (22) + CH_a (21)), 1.31 (*s*, 3H, CH_3 (27)), 1.29–1.20 (*m*, 2H, CH_b (19) + CH_b (21)), 1.15–1.09 (*m*, 1H, CH_b (15)), 1.13 (*s*, 3H, CH_3 (26)), 1.03 (*s*, 3H, CH_3 (23)), 0.98 (*s*, 3H, CH_3 (30)), 1.08–0.89 (*m*, 1H, CH_b (1)), 0.94 (*s*, 3H, CH_3 (25)), 0.90 (*s*, 3H, CH_3 (29)), 0.88–0.83 (*m*, 1H, C*H* (5)), 0.82 (*s*, 3H, CH_3 (24)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 180.2 (*C*═O, C28), 90.8 (*C*–O, C13), 84.0 (*C*HOH, C3), 76.1 (*C*HOH, C12), 69.2 (*C*HOH, C2), 55.4 (*C*H, C5), 51.2 (*C*H, C18), 46.7 (CH_2, C1), 44.9 (C_{quart}, C17), 44.7 (*C*H, C9), 42.5 (C_{quart}, C14), 42.3 (C_{quart}, C8), 39.5 (CH_2, C19), 39.4 (C_{quart}, C4), 37.9 (C_{quart}, C10), 34.3 (CH_2, C21), 34.0 (CH_2, C7), 33.4 (CH_3, C30), 31.7 (C_{quart}, C20), 29.0 (CH_2, C11), 28.6 (CH_3, C23), 28.1 (CH_2, C15), 27.6 (CH_2, C22), 24.0 (CH_3, C29), 21.3 (CH_2, C16), 18.8 (CH_3, C27), 18.7 (CH_3, C26), 17.9 (CH_2, C6), 17.7 (CH_3, C25), 16.7 (CH_3, C24) ppm; MS (ESI, MeOH): *m/z* = 489.2 (60%, $[M + H]^+$), 977.4 (12%, $[2M + H]^+$), 944.4 (100%, $[2M + Na]^+$).

*5.4.30. (2α,3β) 2,3–Diydroxy-12-chloro-olean-28-oic acid 28,13-lactone (**29**)*

MA (0.3 g, 0.6 mmol) was solved in a DCM/water mixture (1:1, 40 mL) and sodium hypochlorite (3 mL; 13% Cl_2) was added. After stirring for 5 h at 25 °C, the mixture was quenched with sodium sulfide and stirred for one additional hour. Usual workup afforded **29** as a colorless solid; yield: 84%; m.p.: 306–309 °C; R_F = 0.40 (*n*-hexane/ethyl acetate, 1:2); $[\alpha]_D$ = +34.5° (*c* 0.5, $CHCl_3$); IR (KBr): *ν* = 3395*m*, 2950*s*, 1767*s*, 1464*m*, 1395*m*, 1361*m*, 1254*m*, 1216*m*, 1134*m*, 1050*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 4.16 (*dd*, *J* = 3.9, 2.3 Hz, 1H, C*H* (12)), 3.72 (*ddd*, *J* = 11.5, 9.7, 4.7 Hz, 1H, C*H* (2)), 3.03 (*d*, *J* = 9.6 Hz, 1H, CH (3)), 2.28 (*ddd*, *J* = 15.0, 12.9, 4.0 Hz, 1H, CH_a (11)), 2.18–2.10 (*m*, 2 H, CH_a (19) + CH_a (16)), 2.08–2.05 (*m*, 1H, CH_a (1)), 2.04–1.87 (*m*, 3H, CH_b (19) + C*H* (18) + CH_a (15)), 1.77–1.71 (*m*, 2H, C*H* (9) + CH_b (11)), 1.68–1.59 (*m*, 2H, CH_2 (7)), 1.57–1.49 (*m*, 2H, CH_a (22) + CH_a (6)), 1.47–1.42 (*m*, 1H, CH_b (6)), 1.37 (*s*, 3H, CH_3 (27)), 1.36–1.26 (*m*, 2H, CH_a (21) + CH_b (22)), 1.27–1.10 (*m*, 3H, CH_a (16) + CH_b (21) + CH_b (15)), 1.15 (*s*, 3H, CH_3 (26)), 1.00 (*s*, 3H, CH_3 (23)), 1.03–0.96 (*m*, 1H, CH_b (1)), 0.99 (*s*, 3H, CH_3 (30)), 0.95 (*s*, 3H, CH_3 (25)), 0.89 (*s*, 3H, CH_3 (29)), 0.90–0.84 (*m*, 1H, C*H* (5)), 0.84 (*s*, 3H, CH_3 (24)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 179.2 (*C*═O, C28), 91.7 (C_{quart}, C13), 83.4 (*C*HOH, C3), 69.1 (*C*HOH, C2), 64.6 (*C*HCl, C12), 55.5 (*C*H, C5), 51.8 (*C*H, C18), 46.6 (*C*H_2, C1), 45.1 (C_{quart}, C17), 44.8 (*C*H, C9), 43.2 (C_{quart}, C14), 42.4 (C_{quart}, C8), 39.6 (CH_2, C19), 39.2 (C_{quart}, C4), 37.9 (C_{quart}, C10), 34.2 (CH_2, C7), 33.7 (CH_2, C21), 33.5 (*C*H_3, C30), 31.7 (C_{quart}, C20), 29.4 (*C*H_2, C11), 29.3 (CH_2, C15), 28.4 (CH_3, C23), 27.3 (CH_2, C22), 23.5 (CH_3, C29), 21.2 (CH_2, C16), 20.2 (CH_3, C27), 18.7 (*C*H_3, C26), 18.1 (CH_3, C25), 17.9 (CH_2, C6), 16.5 (CH_3, C24) ppm; MS (ESI, MeOH): *m/z* = 529.5 (85%, $[M + Na]^+$), 561.1 (37%, $[M + Na + MeOH]^+$), 1035.3 (100%, $[2M + Na]^+$).

*5.4.31. (2α,3β) 2,3–Dihydroxy-12-bromo-olean-28-oic acid 28,13-lactone (**30**)*

MA (300 mg, 0.63 mmol) in acetic acid (20 mL, 90%) was treated with sodium acetate (0.5 g, 6.1 mmol) and bromine (50 μL, 1 mmol). After 10 min stirring at room temperature the reaction was quenched with water and an aq. solution of sodium sulfide. The white precipitate were filtered off and washed with water to yield **30** as a colorless solid; yield: 72%; m.p.: 260–262 °C (lit. 261–263 °C [69]); R_F = 0.44 (*n*-hexane/ethyl acetate, 1:2); $[\alpha]_D$ = +33.0° (*c*0.50, $CHCl_3$) [lit. 31.6° (*c* 3.0, $CHCl_3$ [69])]; IR (KBr): *ν* = 3384*s*, 2950*s*, 1768*s*, 1462*m*, 1394*m*, 1360*m*, 1301*w*, 1246*w*, 1210*m*, 1130*m*, 1051 *m* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 4.26 (*dd*, *J* = 3.7, 2.3, 1H, C*H* (12)), 3.70 (*ddd*, *J* = 11.3, 9.6, 4.6 Hz, 1H, CH_a (2)), 3.05 (*d*, *J* = 9.6 Hz, 1H, C*H* (3)), 2.39 (*ddd*, *J* = 15.0, 12.4, 3.9 Hz, 1H, CH_a (11)), 2.34–2.28 (*m*, 1H, CH_a (19)), 2.18–2.00 (*m*, 2H, CH_a (16) + CH_a (1)), 2.01–1.90 (*m*, 3H, C*H* (18) + CH_b (19) + CH_a (15)), 1.89–1.83 (*m*, 1H, CH_b (11)), 1.82–1.75 (*m*, 1H, C*H* (9)), 1.69–1.52 (*m*, 2H, CH_2 (7)), 1.60–1.50 (*m*, 1H, CH_a (22)), 1.55–1.48 (*m*, 1H, CH_a (6)), 1.47–1.44 (*m*, 1H, CH_b (6)), 1.44 (*s*, 3H, CH_3 (27)), 1.40–1.37 (*m*, 1H, CH_a (21)), 1.33–1.38 (*m*, 1H, CH_b (22)), 1.29–1.20 (*m*, 2H, CH_b (16) + CH_b (21)), 1.17 (*s*, 3H, CH_3 (26)), 1.19–1.10 (*m*, 1H, CH_b (15)), 1.01 (*s*, 3H, CH_3 (23)), 1.03–0.95 (*m*, 1 H, CH_b (1)), 0.99 (*s*, 3H, CH_3 (30)), 0.94 (*s*, 3H, CH_3 (25)), 0.92–0.89 (*m*, 1H, C*H* (5)), 0.89 (*s*, 3H, CH_3 (29)), 0.82 (*s*, 3H, CH_3 (24)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.7 (*C*═O, C28), 91.5 (*C*–O, C13), 83.8 (*C*HOH C3), 69.3 (*C*HOH, C2), 56.0 (*C*HBr, C12), 55.5 (*C*H, C5), 52.1 (*C*H, C18), 46.1 (*C*H_2, C1), 45.6 (*C*H, C9), 45.6 (C_{quart}, C17), 43.7 (C_{quart}, C14), 42.4 (C_{quart}, C8), 39.8 (CH_2, C19), 39.2 (C_{quart}, C4), 37.9 (C_{quart}, C10), 34.3 (CH_2, C7), 33.8 (CH_2, C21), 33.5 (CH_3, C30), 31.7 (C_{quart}, C20), 30.5 (CH_2, C11), 29.3 (*C*H_2, C15), 28.4 (CH_3, C23), 27.4 (*C*H_2, C22), 23.6 (*C*H_3, C29), 21.2 (*C*H_2, C16), 21.1 (CH_3, C27), 19.0 (CH_3, C26), 18.0 (*C*H_3, C25), 17.8 (CH_2, C6), 16.8 (CH_3, C24) ppm; MS (ESI, MeOH): *m/z* = 551.3 (11%, $[M + H]^+$), 568.3 (19%,

$[M + NH_4]^+$), 573.5 (62%, $[M + Na]^+$), 605.1 (20%, $[M + Na + MeOH]^+$), 1123.1 (100%, $[2M + Na]^+$).

*5.4.32. (2α,3β) O,O′-2,3–Diydroxy-12-iodo-olean-28-oic acid 28,13-lactone (**31**)*

MA (0.3 g, 0.6 mmol)was solved in DCM/water (1:1, 40 mL) and treated with sodium hydrocarbonate (250 mg, 3 mmol), potassium iodide (1 g, 6 mmol) and iodine (500 mg, 2 mmol). After 48 h stirring at 25 °C and work up as described above, compound **31** was obtained as a colorless solid; yield: 46%; m.p.: 230–233 °C; R_F = 0.44 (*n*-hexane/ethyl acetate, 1:2); $[\alpha]_D$ = +69.1° (*c* 0.40, $CHCl_3$); IR (KBr): ν = 3424*s*, 2954*s*, 1775*s*, 1637*w*, 1468*m*, 1386*m*, 1368*w*, 1301*w*, 1210*w*, 1198*w*, 1179*m*, 1130*m*, 1103*w*, 1052*m* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 4.45 (*dd*, *J* = 4.5, 2.3 Hz, 1H, C*H* (12)), 3.76 (*ddd*, *J* = 11.7, 9.2, 4.5 Hz, 1H, CH_a (2)), 3.07 (*d*, *J* = 9.2 Hz, 1H, C*H* (3)), 2.60–2.51 (*m*, 1H, CH_a (19)), 2.42–2.36 (*m*, 1H, CH_a (11)), 2.14–2.08 (*m*, 1H, CH_a (16)), 2.10–2.06 (*m*, 1H, CH_a (1)), 1.98–1.83 (*m*, 4H, CH_b (11) + CH_b (15) + CH_b (19) + C*H* (18)), 1.79–1.72 (*m*, 1H, C*H* (9)), 1.68–1.40 (*m*, 4H, CH_2 (22) + CH_a (7) + CH_a (6)), 1.50 (*s*, 3H, CH_3 (27)), 1.50–1.40 (*m*, 1H, CH_b (6)), 1.33–1.20 (*m*, 3H, CH_b (7) + CH_a (21) + CH_b (16)), 1.23 (*s*, 3H, CH_3 (26)), 1.21–1.13 (*m*, 2H, CH_b (21) + CH_a (15)), 1.12–1.08 (*m*, 1H, CH_b (1)), 1.04 (*s*, 3H, CH_3 (23)), 0.99 (*s*, 3H, CH_3 (30)), 0.94 (*s*, 3H, CH_3 (25)), 0.95–0.85 (*m*, 1H, C*H* (5)), 0.88 (*s*, 3H, CH_3 (29)), 0.80 (*s*, 3H, CH_3 (24)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.3 (*C*=*O*, C28), 91.4 (C_{quart}, C13), 83.6 (*C*HOH, C3), 69.2 (*C*HOH, C2), 55.2 (*C*H, C5), 52.7 (*C*H, C18), 47.1 (*C*H, C9), 45.8 (C_{quart}, C17), 45.9 (CH_2, C1), 44.1 (C_{quart}, C14), 42.6 (C_{quart}, C8), 40.0 (CH_2, C19), 39.4 (C_{quart}, C4), 38.0 (C_{quart}, C10), 34.6 (CH_2, C7), 33.7 (CH_2, C21), 33.6 (CH_3, C30), 33.1 (CH_2, C11), 32.0 (*C*HI, C12), 32.1 (C_{quart}, C20), 29.6 (CH_2, C15), 28.3 (CH_3, C23), 27.6 (CH_2, C22), 23.4 (CH_3, C29), 22.9 (CH_3, C27), 21.5 (CH_2, C16), 19.4 (CH_3, C26), 18.5 (CH_3, C25), 17.6 (CH_2, C6), 16.8 (CH_3, C24) ppm; MS (ESI, MeOH): *m*/*z* = 599.1 (16%, $[M + H]^+$), 621.3 (100%, $[M + Na]^+$), 652.9 (49%, $[M + Na + MeOH]^+$), 1218.9 (87%, $[2M + Na]^+$).

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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.2013.11.025.

References

[1] H.J. Böhm, G. Klebe, H. Kubinyi, Wirkstoffdesign: Der Weg zum Arzneimittel, Spektrum-Akademischer Vlg, 2002.
[2] M. Willmann, V. Wacheck, J. Buckley, K. Nagy, J. Thalhammer, R. Paschke, T. Triche, B. Jansen, E. Selzer, Characterization of NVX-207, a novel betulinic acid-derived anti-cancer compound, Eur. J. Clin. Invest. 39 (2009) 384–394.
[3] B. Fernandez Fernandez, U. Elewa, M.D. Sanchez-Nino, J.E. Rojas-Rivera, C. Martin-Cleary, J. Egido, A. Ortiz, 2012 update on diabetic kidney disease: the expanding spectrum, novel pathogenic insights and recent clinical trials, Minerva Med. 103 (2012) 219–234.
[4] M.B. Sporn, K.T. Liby, M.M. Yore, L. Fu, J.M. Lopchuk, G.W. Gribble, New synthetic triterpenoids: potent agents for prevention and treatment of tissue injury caused by inflammatory and oxidative stress, J. Nat. Prod. 74 (2011) 537–545.
[5] R. Csuk, B. Siewert, A convenient separation of ursolic and oleanolic acid, Tetrahedron Lett. 52 (2011) 6616–6618.
[6] J. Liu, Pharmacology of oleanolic acid and ursolic acid, J. Ethnopharmacol. 49 (1995) 57–68.
[7] Z. Ovesna, K. Kozics, D. Slamenova, Protective effects of ursolic acid and oleanolic acid in leukemic cells, Mutat. Res. 600 (2006) 131–137.
[8] J.A.R. Salvador, Pentacyclic Triterpenes as Promising Agents in Cancer, Nova Science Pub Inc, 2010.
[9] S. Jäger, H. Trojan, T. Kopp, M. Laszczyk, A. Scheffler, Pentacyclic triterpene distribution in various plants – rich sources for a new group of multi-potent plant extracts, Molecules 14 (2009) 2016–2031.
[10] K.T. Liby, M.B. Sporn, Synthetic oleanane triterpenoids: multifunctional drugs with a broad range of applications for prevention and treatment of chronic disease, Pharmacol. Rev. 64 (2012) 972–1003.
[11] N. Suh, Y. Wang, T. Honda, G.W. Gribble, E. Dmitrovsky, W.F. Hickey, R.A. Maue, A.E. Place, D.M. Porter, M.J. Spinella, C.R. Williams, G. Wu, A.J. Dannenberg, K.C. Flanders, J.J. Letterio, D.J. Mangelsdorf, C.F. Nathan, L. Nguyen, W.W. Porter, R.F. Ren, A.B. Roberts, N.S. Roche, K. Subbaramaiah, M.B. Sporn, A novel synthetic oleanane triterpenoid, 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid, with potent differentiating, antiproliferative, and anti-inflammatory activity, Cancer Res. 59 (1999) 336–341.
[12] H.E. Abboud, Synthetic oleanane triterpenoids: magic bullets or not? Kidney Int. 83 (2013) 785–787.
[13] G. Speranza, M. Gutierrez, S. Kummar, J. Strong, R. Parker, J. Collins, Y. Yu, L. Cao, A. Murgo, J. Doroshow, A. Chen, Phase I study of the synthetic triterpenoid, 2-cyano-3, 12-dioxoolean-1, 9-dien-28-oic acid (CDDO), in advanced solid tumors, Cancer Chemother. Pharmacol. 69 (2012) 431–438.
[14] A.S. Leal, R. Wang, J.A.R. Salvador, Y. Jing, Semisynthetic ursolic acid fluorolactone derivatives inhibit growth with induction of p21waf1 and induce apoptosis with upregulation of NOXA and downregulation of c-FLIP in cancer cells, ChemMedChem 7 (2012) 1635–1646.
[15] M. Martelanc, I. Vovk, B. Simonovska, Separation and identification of some common isomeric plant triterpenoids by thin-layer chromatography and high-performance chromatography, J. Chromatogr. A 1216 (2009) 6662–6670.
[16] M. Ganbold, J. Barker, R. Ma, L. Jones, M. Carew, Cytotoxicity and bioavailability studies on a decoction of *Oldenlandia diffusa* and its fractions separated by HPLC, J. Ethnopharmacol. 131 (2010) 396–403.
[17] K. Lewis, D. Tucker, The separation of substituted olean-12-en-28-oic acids from the corresponding urs-12-en-oic isomers, Austr. J. Chem. 36 (1983) 2297–2305.
[18] J.A.R. Salvador, V.M. Moreira, B.M.F. Goncalves, A.S. Leal, Y. Jing, Ursane-type pentacyclic triterpenoids as useful platforms to discover anticancer drugs, Nat. Prod. Rep. 29 (2012) 1463–1479.
[19] D.H.R. Barton, P.G. Sammes, M. Silva, Photochemical transformations—XX: a partial synthesis of cincholic acid, Tetrahedron 22 (Suppl. 7) (1966) 57–67.
[20] T. Konoike, K. Takahashi, Y. Araki, I. Horibe, Practical partial synthesis of myriceric acid a, an endothelin receptor antagonist, from oleanolic acid, J. Org. Chem. 62 (1997) 960–966.
[21] E. Schwenk, E. Stahl, Preparation of 3-hydroxy-delta 9,11-12-ketocholenic acid and its lower homologues, Arch. Biochem. 14 (1947) 125–129.
[22] B.F. Mckenzie, V.R. Mattox, L.L. Engel, E.C. Kendall, Steroids derived from bile acids .6. An improved synthesis of methyl 3,9-epoxy-delta-11-cholenate from desoxycholic acid, J. Biol. Chem. 173 (1948) 271–281.
[23] K. Courault, C. Lindig, Partialsynthesen von Cardenoliden und Cardenolid-Analogen. XIII. Synthese substituierter 14,21-Epoxy-5β,14β-card-20(22)-enolide, J. Prakt. Chem. 330 (1988) 445–452.
[24] C.J.W. Brooks, G. Eglinton, L. Hanaineh, Infra-red studies of solvent effects—I. Carbonyl absorptions of cyclohexanones, and of steroid and triterpenoid ketones, Spectrochim. Acta 22 (1966) 131–145.
[25] S. Mahato, A. Nandy, G. Roy, Triterpenoids, Phytochemistry 31 (1992) 2199–2249.
[26] B.G. Bag, P.P. Dey, S.K. Dinda, W.S. Sheldrick, I.M. Oppel, A simple route for renewable nano-sized arjunolic and asiatic acids and self-assembly of arjunabromolactone, Beilstein J. Org. Chem. 4 (2008), http://dx.doi.org/10.3762/Bjoc.4.24.
[27] C.R. Pungitore, M. Garcia, J.C. Gianello, M.E. Sosa, C.E. Tonn, Insecticidal and antifeedant effects of *Junellia aspera* (Verbenaceae) triterpenes and derivatives on *Sitophilus oryzae* (Coleoptera: Curculionidae), J. Stored Prod. Res. 41 (2005) 433–443.
[28] M. Ferrari, M.C. Fornasiero, A.M. Isetta, MTT colorimetric assay for testing macrophage cytotoxic activity in vitro, J. Immunol. Methods 131 (1990) 165–172.
[29] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. Mcmahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, New colorimetric cytotoxicity assay for anticancer-drug screening, J. Natl. Cancer Inst 82 (1990) 1107–1112.
[30] C. Fiore, M. Salvi, M. Palermo, G. Sinigaglia, D. Armanini, A. Toninello, On the mechanism of mitochondrial permeability transition induction by glycyrrhetinic acid, Biochim. Biophys. Acta Bioenerg. 1658 (2004) 195–201.

[31] E.B. Logashenko, O.V. Salomatina, A.V. Markov, D.V. Korchagina, N.F. Salakhutdinov, G.A. Tolstikov, V.V. Vlassov, M.A. Zenkova, Synthesis and pro-apoptotic activity of novel glycyrrhetinic acid derivatives, ChemBioChem 12 (2011) 784–794.
[32] S. Qian, H. Li, Y. Chen, W. Zhang, S. Yang, Y. Wu, Synthesis and biological evaluation of oleanolic acid derivatives as inhibitors of protein tyrosine phosphatase 1B, J. Nat. Prod. 73 (2010) 1743–1750.
[33] A.S. Leal, R. Wang, J.A.R. Salvador, Y. Jing, Synthesis of novel heterocyclic oleanolic acid derivatives with improved antiproliferative activity in solid tumor cells, Org. Biomol. Chem. 11 (2013) 1726–1738.
[34] G. Chadalapaka, I. Jutooru, A. McAlees, T. Stefanac, S. Safe, Structure-dependent inhibition of bladder and pancreatic cancer cell growth by 2-substituted glycyrrhetinic and ursolic acid derivatives, Bioorg. Med. Chem. Lett. 18 (2008) 2633–2639.
[35] D. Wlodkowic, J. Skommer, Z. Darzynkiewicz, Cytometry in cell necrobiology revisited. Recent advances and new vistas, Cytometry Part A 77A (2010) 591–606.
[36] Z. Alexander, in: W.T. Mason (Ed.), Fluorescent and Luminescent Probes for Biological Activity, second ed., Academic Press, london, 1999, pp. 117–135.
[37] D. Baskic, S. Popovic, P. Ristic, N.N. Arsenijevic, Analysis of cycloheximide-induced apoptosis in human leukocytes: fluorescence microscopy using annexin V/propidium iodide versus acridin orange/ethidium bromide, Cell Biol. Int. 30 (2006) 924–932.
[38] D.V.G. Melino, Cell Death, vol. 1, John Wiley & Sons, New York, 2010.
[39] D.M. Wu, D. Zhao, D.Z. Li, D.Y. Xu, W.F. Chu, X.F. Wang, D.M. Wu, D. Zhao, D.Z. Li, D.Y. Xu, W.F. Chu, X.F. Wang, Maslinic acid induces apoptosis in salivary gland adenoid cystic carcinoma cells by Ca^{2+} – evoked p38 signaling pathway, Naunyn Schmiedeberg's Arch. Pharmacol. 383 (2011) 321–330.
[40] Z. Darzynkiewicz, P. Pozarowski, J. Gloria, in: E.C. Julio (Ed.), Cell Biology, Third ed., Academic Press, Burlington, 2006, pp. 279–289.
[41] Z. Darzynkiewicz, H.D. Halicka, H. Zhao, Analysis of cellular DNA content by flow and laser scanning cytometry, Adv. Exp. Med. Biol. 676 (2010) 137–147.
[42] D. Arigoni, H. Bosshard, J. Dreiding, O. Jeger, Zur Kenntnis der Triterpene. 181. Mitteilung. Über Umlagerungen im Ring C der Ursolsäure, Helv. Chim. Acta 37 (1954) 2173–2184.
[43] I. Vermes, C. Haanen, H. Steffens-Nakken, C. Reutellingsperger VI, J. Immunol. Methods 184 (1995) 39–51.
[44] R. Csuk, B. Siewert, C. Dressel, R. Schäfer, Tormentic acid derivatives: synthesis and apoptotic activity, Eur. J. Med. Chem. 56 (2012) 237–245.
[45] A. Niesen, A. Barthel, R. Kluge, A. Köwitsch, D. Ströhl, S. Schwarz, R. Csuk, Antitumoractive endoperoxides from triterpenes, Arch. Pharm. 342 (2009) 569–576.
[46] P.N. Dean, J.H. Jett, Mathematical analysis of DNA distributions derived from flow microfluorometry, J. Cell. Biol. 60 (1974) 523–527.
[47] D.E. White, L.S. Zampatti, The chemistry of western Australian plants. VII. Oleanolic acid acetate from *Eucalyptus calophylla* bark, J. Chem. Soc (1952) 5040.
[48] O. Jeger, J. Norymberski, S. Szpilfogel, V. Prelog, Eine Methode Zur Überfuhrung Von Carbonsäuren in Primäre Alkohole, Helv. Chim. Acta 29 (1946) 684–692.
[49] G. Topcu, E.N. Altiner, S. Gozcu, B. Halfon, Z. Aydogmus, J.M. Pezzuto, B.N. Zhou, D.G.I. Kingston, Studies on di- and triterpenoids from *Salvia staminea* with cytotoxic activity, Planta Med. 69 (2003) 464–467.
[50] A. Garcia-Granados, P.E. Lopez, E. Melguizo, A. Parra, Y. Simeo, Partial synthesis of C-ring derivatives from oleanolic and maslinic acids. Formation of several triene systems by chemical and photochemical isomerization processes, Tetrahedron 60 (2004) 1491–1503.
[51] F. Hichri, J.H. Ben, J. Cheriaa, S. Jegham, Z. Mighri, Antibacterial activities of a few prepared derivatives of oleanolic acid and of other natural triterpenic compounds, C. R. Chim. 6 (2003) 473–483.
[52] M. Miyazawa, Y. Okuno, K. Imanishi, Suppression of the SOS-inducing activity of mutagenic heterocyclic amine, Trp-p-1, by triterpenoid from *Uncaria sinensis* in the *Salmonella typhimurium* TA1535/pSK1002 Umu test, J. Agric. Food Chem. 53 (2005) 2312–2315.
[53] D. Frazier, C.R. Noller, Saponins and sapogenins. XXVI. The conversion of echinocystic acid into oleanolic acid, J. Am. Chem. Soc. 66 (1944) 1267–1268.
[54] P. Dietrich, O. Jeger, Triterpenes. CXLIX. The transformation of betulin and oleanolic acid into isomeric unsaturated hydrocarbons $C_{29}H_{48}$. A hypothesis on the biosynthesis of pentacyclic triterpenes, Helv. Chim. Acta 33 (1950) 711–722.
[55] G.A. Tolstikov, M.I. Goryaev, H.-O. Kim, Triterpenoids. VII. Synthesis of pyrazoles of oleanolic and 11-oxooleanolic acids, Izv. Akad. Nauk Kaz. SSR, Ser. Khim. 16 (1966) 76–80.
[56] K. Majumdar, M. Biswas, U.K. Som, S. Das, Chemical constituents of the bark of *Terminalia myriocarpa*, J. Indian Chem. Soc 82 (2005) 673–674.
[57] A.S.R. Anjaneyulu, A.V.R. Reddy, G.R. Mallavarapu, R.S. Chandrasekhara, 3-Acetylmaslinic acid from the root bark of *Terminalia alata*, Phytochemistry 25 (1986) 2670–2671.
[58] A. Yagi, N. Okamura, Y. Haraguchi, K. Noda, I. Nishioka, Studies on the constituents of *Zizyphi fructus*. II. Structure of new p-coumaroylates of maslinic acid, Chem. Pharm. Bull. 26 (1978) 3075–3079.
[59] T.R. Govindachari, K. Nagarajan, B.R. Pai, S. Rajappa, Chemical investigation of Khet-papra, J. Sci. Ind. Res. 17B (1958) 73–75.
[60] T. Takemoto, K. Kometani, Triterpene glycosides (mubenins) from the seeds of *Stauntonia hexaphylla*, Justus Liebigs Ann. Chem. 685 (1965) 237–246.
[61] R.L. Ramachandra, S.G. Purnananda, R.P.V. Subba, R.M. Gopala, 4-Flavanols, Curr. Sci. 31 (1962) 459–460.
[62] O.B. Kazakova, N.I. Medvedeva, O.S. Kukovinets, G.A. Tolstikov, E.F. Khusnutdinova, L. Zaprutko, B. Bednarczyk-Cwynar, Z. Paryzek, Chemoselective oxidation of oleanolic acid derivatives with ozone, Chem. Nat. Compd. 46 (2010) 397–399.
[63] X. Wen, H. Sun, J. Liu, K. Cheng, P. Zhang, L. Zhang, J. Hao, P. Ni, S.E. Zographos, D.D. Leonidas, K.M. Alexacou, T. Gimisis, J.M. Hayes, N.G. Oikonomakos, Naturally occurring pentacyclic triterpenes as inhibitors of glycogen phosphorylase: synthesis, structure-activity relationships, and X-ray crystallographic studies, J. Med. Chem. 51 (2008) 3540–3554.
[64] J.-Y. Hu, Y.-Q. Xu, Y. Ju, Benzyl oleanolate, Acta Crystallogr. Sect. E Struct. Rep. Online 63 (2007) o4882/4881–o4882/4811.
[65] M.-S. Cheng, M.-C. Yan, Y. Liu, L.-G. Zheng, J. Liu, Synthesis of β-hederin and hederacolchiside A1: triterpenoid saponins bearing a unique cytotoxicity-inducing disaccharide moiety, Carbohydr. Res. 341 (2005) 60–67.
[66] Y. Zhang, Y. Ding, Y. Lai, S. Zhang, Z. Huang, S. Peng, Preparation of pentacyclotriterpene-13,28-lactone compound and medical application as antitumor agents, CN102079772A, CAPLUS AN (2011) 699481.
[67] H. Li, H. Zou, L. Gao, T. Liu, F. Yang, J. Li, J. Li, W.-W. Qiu, J. Tang, Synthesis and biological evaluation of oleanolic acid derivatives as novel inhibitors of protein tyrosine phosphatase 1B, Heterocycles 85 (2012) 1117–1139.
[68] Y. Ding, Z. Huang, J. Yin, Y. Lai, S. Zhang, Z. Zhang, L. Fang, S. Peng, DDQ-promoted dehydrogenation from natural rigid polycyclic acids or flexible alkyl acids to generate lactones by a radical ion mechanism, Chem. Commun. 47 (2011) 9495–9497.
[69] K.T. Potts, S.K. Roy, Triterpenoid constituents of *Backhousia angustifolia* F Muell, Aust. J. Chem. 18 (1965) 767–768.