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Original article

Synthesis and antitumor activity of ring A modified glycyrrhetinic acid derivatives

René Csuk*, Stefan Schwarz, Bianka Siewert, Ralph Kluge, Dieter Ströhl

Bereich Organische Chemie, Martin-Luther-Universität Halle-Wittenberg, Kurt-Mothes-Str. 2, D-06120 Halle (Saale), Germany

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ABSTRACT

Triterpenoic acids show many pharmacological effects, among them an antiinflammatory or an antitumor activity. One of these, glycyrrhetinic acid (**1**) is of interest because of its antitumor profile. Glycyrrhetinic acid is not only cytotoxic but also triggers apoptosis in various human tumor cell lines. To improve the cytotoxicity of parent **1** we set out to synthesize new derivatives of it – differing in structure and lipophilicity. These compounds were tested in a sulforhodamine B assay for cytotoxicity, and screened for their ability to induce apoptosis using an acridine orange/ethidium bromide assay and trypan blue staining. The most active compound, **34**, a benzyl glycyrrhetinate holding an extra 3-*N*-(3-aminopropyl)glycyl substituent showed IC_{50} between 1.96 and 5.14 μm for five human cancer cell lines and triggers apoptosis in 80% of the cells.

1. Introduction

While betulinic acid and oleanoic acid and their derivatives have already been studied extensively, there are still some gaps in the knowledge about glycyrrhetinic acid (**1**, Fig. 1). Compound **1** shows a modest cytotoxicity but is able to trigger apoptosis in tumor cells [1–4]. Also, **1** can be accessed easily from the roots of licorice in high yields of up to 24% [5,6]. Nevertheless, the cytotoxicity of **1** needs to be improved: in a previous study we were able to determine the IC_{50} value of **1** for 15 different human tumor cells lines, and for all tumor cell lines $IC_{50} > 80$ μM [7] were measured; this is poor compared to betulinic acid ($IC_{50} = 7–14$ μM [8]).

To improve the cytotoxicity of **1** it seems necessary to change the molecule at various positions. Our focus in this study was on ring A, especially positions O-3 and C-2 seemed of interest; this should lead to compounds displaying an altered pattern of lipophilicity compared to parent **1**. IC_{50} values were determined in sulforhodamine B assays as a measure of the cytotoxicity using different human tumor cell lines. Additional trypan blue staining experiments as well as acridine orange/ethidium bromide assays on human alveolar basal epithelial cells A549 were used to determine their ability of inducing apoptosis in this cancer cell line.

2. Chemistry

To determine whether a configurational inversion at position C-3 has an influence on the cytotoxicity of **1**, the corresponding α-epimer was prepared in a two-step synthesis [9] as depicted in Scheme 1. Thus, oxidation of **1** using CrO_3/acetone provided ketone **2** [3] whose stereoselective reduction [9] with L-selectride [10] at −75 °C gave the 3-*epi* derivative **3**.

To alter the lipophilicity of the molecule and to improve bioavailability, the carboxylic acid at position C-30 was transformed into different esters either by alkylation with alkyl iodides [7,11] in the presence of finely grounded K_2CO_3 in DMF (→**4** [12]) or via a DCC mediated coupling in DCM (→**6**). The hydrogen succinate **5** was obtained from **1** using succinic anhydride in pyridine [13], and the ether **7** was obtained from **4** with sodium hydride/methyl iodide in THF [11]. The tosylate **8** [14] of methyl glycyrrhetinate (**4**) was accessed by tosylation of **4**.

To improve cytotoxicity, we considered the synthesis of derivatives possessing an extra nitrogen-containing moiety. Thus, compound **4** was oxidized at O-3 by a Jones oxidation [9] to yield **11** (Scheme 3). A derivative possessing an anellated pyrazine ring at positions C-2 and C-3 (of ring A) was obtained from the reaction of ketone **11** with ethylenediamine and sulfur in morpholine at 130 °C [15]. The corresponding 3-amino epimers resulted from a reductive amination of **11** with ammonium acetate in ethanol [16] followed by reducing the imine using sodium cyanoborohydride [17] in a one-pot reaction. Both epimers (**13** [18] and **14** [19]) were obtained from this reduction with the β-epimer **14** being the major product under these conditions.

In addition, compound **14** served as a starting material for several derivatives: Steglich esterification of **14** with Boc-L-Ala furnished **15** whose deprotection with trifluoroacetic acid in DCM yielded amine **16**. The synthesis of the oximes **17**–**19** started from **11**. Thus, reaction of **11** with hydroxylamine hydrochloride in

* Corresponding author. Tel.: +49 (0) 345 55 25660; fax: +49 (0) 345 55 27030.
E-mail address: rene.csuk@chemie.uni-halle.de (R. Csuk).

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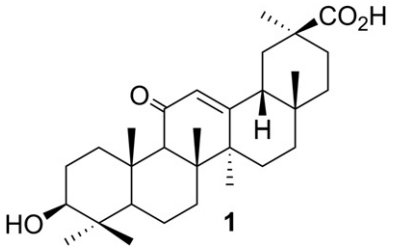


Fig. 1. Structure of glycyrrhetinic acid (**1**).

pyridine followed by an acid work-up furnished **17**; the corresponding alkylated products **18**–**20** were obtained from the alkylation of **17** with an alkyl halide in THF. Compound **20** was obtained from **17**; its deprotection did not result in the formation of a free amine but a seconitrile, hence paralleling previous findings of Askam and Bradley for an analogous tosylate [20].

Another option to modify the lipophilicity and/or polarity pattern was accomplished by the incorporation of a thiol moiety. While a direct sulfurization at position C-11 with Lawesson's reagent [21] failed under a variety of different conditions, dimeric compound **22** was obtained from **4** by a DCC mediated coupling reaction of a bis-Boc protected cysteine followed by the removal of the Boc group with trifluoroacetic acid in DCM. A selective deprotection of the amino group in **23** was achieved in 86% resulting in compound **24** possessing a free amino group as well as a protected side chain.

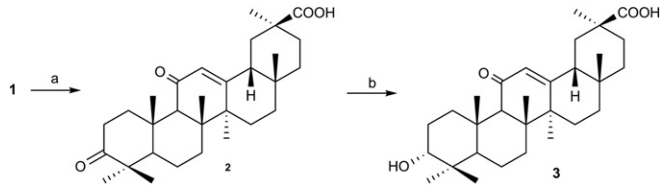
Ring opening reactions concerning ring A (Scheme 5) started from compound **25** [22] (obtained from **9**), and ring A was expanded by an insertion of oxygen using a Baeyer–Villiger oxidation [23] to yield **26**. Ring opening under basic conditions (KOH/EtOH) yielded the hydroxyl-substituted acid **27** whereas an elimination reaction occurred when **26** was treated with hydrochloric acid in EtOH, and **28** was obtained. Prolonged heating under reflux led to the formation of ethyl ester **29**.

Fluorine's special properties (small size, high electronegativity) contribute to its importance in medicinal chemistry. The effects of fluorine substitution on the biological behavior of biologically active molecules have been used effectively in the development of new drugs. To introduce a fluorine substituent on ring A, epoxide **31** [24,25] was reacted with Olah's reagent [26] and **32** was obtained. Nucleophilic ring opening of the epoxide occurred at the less hindered carbon C-2 and followed Fürst–Plattner's [27] rule. Compound **32** is characterized in its ^{1}H NMR spectrum by a chemical shift of H-2 at $\delta = 4.64$ ppm showing $J_{H\text{-}1,\ H\text{-}2} = 5.8$ Hz, $J_{H\text{-}2,\ H\text{-}3} = 6.4$ Hz and $J_{H\text{-}2,\ F} = 49.7$ Hz.

Reaction of **1** with benzyl chloride in the presence of finely grounded potassium carbonate gave benzylester 33 [7,11] that was transformed into the aminopropyl derivative **34**.

3. Results

Screening of the compounds **1**–**3** (Scheme 1) in an SRB assay using 11 different human cancer cell lines (Table 1) revealed that no improvement of the antitumor activity is connected with these structural changes.



Scheme 1. Inversion of configuration at C-3: reagents and conditions: a) CrO_3, acetone, 25 °C, 1 h, 78%; b) L-selectride, THF, −75 °C, 2 h, 61%.

Transforming the carboxylic acid into an ester (Table 2, Scheme 2), however, did not improve cytotoxicity. Except for compounds **6** ($IC_{50} = 28.59$ μM on A253 cells) and **7** ($IC_{50} = 28.99$ μM in 518A2 cells), all IC_{50} values were above 30 μM. For the methyl ether **7** an IC_{50} could not be determined because of its insolubility in solvents usually used in biological tests (DMSO, DMF or MeOH).

Whereas the pyrazine derivative **12** gave $IC_{50} > 30$ μM (Table 3, Scheme 3), the amines **13** and **14** showed an excellent cytotoxicity for the tumor cell lines being about 10 times higher than the activity of parent compound **4**.

For the oxime **17** IC_{50} values of 12–19 μM were determined; for the human ovarian tumor cell line A2780 a low $IC_{50} = 6.18$ μM was determined. Incorporation of alkyl chains in oxime **17**, however, led to a decrease of cytotoxicity.

The cytotoxicity of compounds **21**–**24** (Table 4, Scheme 4) was determined, and their activity was similar to **4**, except for compounds **22** and **24** being about twice as active as their parent compound **4**.

As far as products with an opened ring A are concerned, except for derivative **29**, none of these compounds showed increased cytotoxicity (Table 5, Scheme 5). The IC_{50} values of all compounds were higher than that of parent compound **9**. Compounds **26** and **29**, however, exhibited lower IC_{50} values for A2780 cells ($IC_{50} = 15.00$ μM for **26** and $IC_{50} = 5.89$ μM for **29**) as well as for 518A2 cells ($IC_{50} = 9.28$ μM for **29**).

Fluorine substitution, however, did not lead to compounds of higher cytotoxicity (Table 6, Scheme 6) that parent compound **9**.

Better results were obtained when benzylester **33** was transformed into the aminopropyl derivative **34**. The IC_{50} values of compound **34** (Table 7, Scheme 7) were about 3–5 times better than those of its parent compound **33**.

Glycyrrhetinic acid is known for its ability to trigger apoptosis. To determine the extent of apoptosis, trypan blue staining and counting experiments were performed the results of which are summarized in Table 8. For parent glycyrrhetinic acid an extent of ca. 74% was determined. Most of the compounds gave a positive result in these experiments except for compounds **17** and **22**.

Apoptotic effect [in %] of derivatives of **1** on A549 cells (+/− standard error, 6 experiments each); cells were treated with **1** (90 μM), **13** (4 μM), **14** (4 μM), **16** (8 μM), **17** (20 μM), **22** (20 μM), **24** (20 μM), **32** (60 μM), and **34** (8 μM), respectively.

Additional AO/EB tests support these results. In this test, green fluorescent cells were found, hence indicating an apoptotic behavior of the compounds. On principle, an AO/EB assay does not allow quantification of the extent of apoptosis but confirms the results from the trypan blue staining experiments (Fig. 2).

4. Conclusions

Herein we synthesized 25 derivatives of **1** differing in lipophilicity and structure at ring A, and screened them for their cytotoxicity. Neither configurational inversion at position C-2 nor structural modifications at position C-3 led to higher cytotoxicity. The introduction of at least one nitrogen-containing substituent, however, was quite promising. Thus, derivatives possessing a primary amino group (**13**–**15**) showed the highest activities whereas substitution (**12**, **17**–**20**) decreased cytotoxicity. A similar behavior was established for compounds **21**–**24**. In addition, introduction of sulfur decreased cytotoxicity.

Structural modification of ring A, i.e., expanding (→**26**) or ring opening (→**27**–**29**) by and large decreased cytotoxicity, except for **29** on A2780 cells. It seems that the presence of an intact ring A is essential for cytotoxicity.

Table 1
Cytotoxicity (IC_{50} values in μmol) for **1**–**3** in a panel of various cancer cell lines.

	518A2	A253	A431	A549	DLD-1	HCT-116	HCT-8	HT-29	Lipo	MCF-7	SW 1736
1*	83.92	80.78	79.58	82.76	81.21	78.33	78.85	80.09	81.44	84.70	76.93
2	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30
3	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30

From SRB assays after 96 h of treatment; the values are averaged from at least 3 independent experiments; variation ± 5–7%; * data from a previous study [7].

Attachment of a diaminoalkyl moiety at position 3 of a lipophilic ester of glycyrrhetinic acid, has an increasing effect on the cytotoxicity and was most rewarding: compound **34** exhibits IC_{50} values between 1.96 and 5.14 μM. Hence, compound **34** was the most active compound of this study, thus indicating a feasible route for the development of glycyrrhetinic acid derivatives showing promising cytotoxicity.

5. Experimental

5.1. General

Melting points are uncorrected (*Leica* hot stage microscope), NMR spectra were recorded using the Varian spectrometers Gemini 200, Gemini 2000 or Unity 500 (δ given in ppm, J in Hz, internal Me_4Si), IR spectra (film or KBr pellet) on a Perkin–Elmer FT-IR spectrometer Spectrum 1000, MS spectra were taken on an Intectra GmbH AMD 402 (electron impact, 70 eV) or on a Finnigan MAT TSQ 7000 (electrospray, voltage 4.5 kV, sheath gas nitrogen) instrument. TLC was performed on silica gel (Merck 5554, detection by UV absorption). The solvents were dried according to usual procedures.

5.2. Cell lines and culture conditions

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. Cytotoxicity assay [28]

The cytotoxic activities of our compounds were evaluated using the sulforhodamine B (SRB) [21] (Sigma Aldrich) microculture colorimetric assay. In short, exponentially growing cells were seeded into 96-well plates on day 0 at the appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24 h, the cells were treated with serial dilutions of the compounds (0–30 μM) for 96 h. The final concentration of DMSO or DMF as a solvent never exceeded 0.5%, which was shown to be non-toxic to the cells. The percentages of surviving cells compared to untreated controls were determined 96 h after the beginning of drug exposure. After 96 h of treatment, the supernatant medium from the 96-well plates was discarded and the cells were fixed with 10% TCA. For a thorough fixation, the plates were allowed to stand at 4 °C. After fixation, the cells were washed in a strip washer for four times with water using alternate dispensing and aspiration procedures. The plates were dyed with 100 μl of 0.4% SRB (sulforhodamine B) for about 20 min. After dying, the plates were washed with 1% acetic acid to remove the dye and allowed to air dry overnight. Then 100 μl of 10 mM Tris base solution were added to each well and absorbance was measured at 570 nm using a 96-well plate reader (Tecan Spectra, Crailsheim, Germany). The IC 50 was calculated from the semi-logarithmic dose–response curves.

Table 2
Cytotoxicity (IC_{50} values in μmol) for **4-10** in a panel of various cancer cell lines.

	518A2	8505C	A253	A549	DLD-1	Lipo
4*	27.54	26.07	19.42	23.50	26.12	20.47
5	>30	>30	>30	>30	>30	>30
6	>30	>30	28.59	>30	>30	>30
8	>30	>30	>30	>30	>30	>30
9*	25.23	24.58	25.04	22.74	28.14	27.66
10	28.99	>30	>30	>30	>30	>30

From SRB assays after 96 h of treatment; the values are averaged from at least 3 independent experiments; variation ± 5–7%; * data from a previous study [7].

5.4. Apoptosis tests

5.4.1. Acridine orange/ethidium bromide (AO/EB) [29]

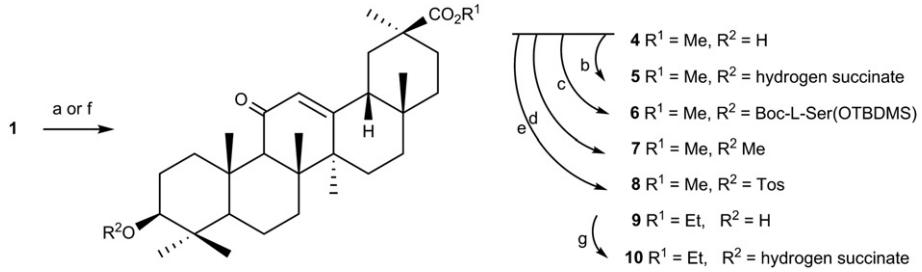
Apoptotic cell death was analyzed by acridine orange/ethidium bromide dye using fluorescence microscopy on A549 cells. Therefore, approx. 500,000 cells were seeded in cell culture flasks and were allowed to grow for 24 h. The medium was removed and the substance loaded medium was added. After 24–48 h, the supernatant medium was collected and centrifuged; the pellet was suspended in phosphate–buffer saline (PBS) and centrifuged again. The liquid was removed, and the pellet was suspended in PBS. After mixing the suspension with a solution of AO/EB, analysis was performed under a fluorescence microscope. While a green fluorescence shows apoptosis, a red colored nucleus indicates necrotic cells.

5.4.2. Trypan blue cell counting

Approx. 500,000 cells (A549) were seeded in cell culture flasks and were allowed to grow for 1 day. After removing of the medium, the substance loaded medium was introduced and the flasks were incubated for about 24–48 h. The supernatant medium was collected and centrifuged; cell pellet was suspended in PBS and centrifuged again. Equal amounts of Trypan blue solution (0.4% in phosphate-buffer saline, pH 7.2) and suspension of the pellet in PBS were mixed and put on chamber slides (invitrogen™). Automatic cell counter (invitrogen™ countess® automated cell counter) was used for counting the cells, differing between cells with an intact cell membrane and cells without.

5.5. (3α)-3-Hydroxy-11-oxoolean-12-en-30-oic acid (**3**) [9]

To a solution of **2** (450 mg, 0.96 mmol) in dry THF (30 ml) at −75 °C, L-Selectride (1 M in THF, 10 ml, 10 mmol) was added, and the mixture was stirred at −75 °C for 2 h. After warming to room temperature, hydrochloric acid (1 M) was added until the pH = 2. The aqueous layer was extracted with chloroform (3 × 15 ml), the combined extracts were washed with water (20 ml), dried (Na_2SO_4) and evaporated. Re-crystallization from methanol afforded **3** (170 mg, 38%) as colorless crystals; mp 308–310 °C (lit. >325 °C [24]); R_f = 0.32 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = +114.66° (c 0.31, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 250 nm (3.95); IR (KBr): ν = 3424*br*, 2960*s*, 1717*m*, 1645*s*, 1458*w*, 1386*m*, 1328*w*, 1253*w*, 1208*w*, 1159*m*, 1088*w*, 1062*w*, 1028*w*, 1003*w* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 12.11 (*s*, 1H, COOH), 5.38 (*s*, 1H, H-12),



Scheme 2. Derivatization of **1**; reagents and conditions: a) K_2CO_3/MeI, DMF, 25 °C, 2 h [7,11]; b) succinic anhydride, pyridine, 85 °C, 48 h, 64%; c) Boc-L-Ser(OTBTMS)–OH, DCC, DMAP, DCM, 25 °C, 20 h, 22%; d) NaH (60% in mineral oil), MeI, THF, reflux, 30 min, 34%; e) *p*-TsCl, pyridine, 0 °C, 4 h, 50%; K_2CO_3/EtI, DMF, 25 °C, 2 h [7,11]; g) succinic anhydride, pyridine, 85 °C, 48 h, 86%.

4.17 (*d*, 1H, OH, $J = 4.3$ Hz), 3.15 (*d*, 1H, H-3, $J = 3.5$ Hz), 2.37 (*s*, 1H, H-9), 2.27 (*ddd*, 1H, H-1, $J = 13.1, 3.5, 3.5$ Hz), 2.08 (*m*, 1H, H-15), 2.05 (*m*, 1H, H-18), 1.85 (*m*, 1H, H-2), 1.78 (*m*, 1H, H-21), 1.71 (*m*, 1H, H-16), 1.64 (*m*, 1H, H-19), 1.62 (*m*, 1H, H-7), 1.37 (*m*, 1H, H-6), 1.35 (*s*, 3H, H-27), 1.34 (*m*, 1H, H-21′), 1.32 (*m*, 1H, H-22), 1.31 (*m*, 1H, H-2′), 1.30 (*m*, 1H, H-7′), 1.29 (*m*, 1H, H-1′), 1.27 (*m*, 1H, H-6′), 1.25 (*m*, 1H, H-22′), 1.18 (*m*, 1H, H-5), 1.13 (*m*, 1H, H-16′), 1.08 (*s*, 3H, H-29), 1.02 (*s*, 6H, H-26 and H-25), 0.94 (*m*, 1H, H-15′), 0.82 (*s*, 3H, H-28), 0.75 (*s*, 3H, H-24), 0.74 (*s*, 3H, H-23) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 199.1$ (C11), 177.6 (C30), 169.5 (C13), 127.3 (C12), 73.5 (C3), 61.1 (C9), 48.0 (C18), 47.5 (C5), 45.0 (C8), 43.0 (C20), 42.9 (C14), 40.6 (C19), 37.5 (C22), 37.1 (C10), 36.7 (C4), 33.0 (C1), 32.1 (C7), 31.5 (C17), 30.3 (C21), 28.7 (C28), 28.3 (C23), 27.8 (C29), 25.9 (C16), 25.8 (C15), 25.1 (C2), 23.1 (C27), 22.2 (C24), 18.3 (C26), 16.9 (C6), 16.1 (C25) ppm; MS (ESI): *m*/*z* (%) = 471.5 ([M + H]$^+$, 70), 493.5 ([M + Na]$^+$, 25), 525.1 ([M + MeOH + Na]$^+$, 100).

5.6. Methyl (3β) 3-hydroxy-11-oxo-olean-12-en-30-oate (**4**)

Compound **4** was prepared according to [12].

5.7. 4-{[(3β)-30-Methoxy-11,30-dioxoolean-12-en-3-yl]oxy}4-oxobutanoic acid (**5**) [13]

To a solution of **4** (960 mg, 1.98 mmol) in dry pyridine (20 ml), succinic anhydride (430 mg, 4.3 mmol) was added, and the mixture was stirred at 85 °C for 2 days. The solvent was removed, DCM (30 ml) added, and the reaction was washed with hydrochloric acid (1 M, 20 ml), extracted with DCM (3 × 20 ml), and the combined organic layers were dried (Na_2SO_4), filtered and evaporated. The residue was subjected to chromatography (silica gel, hexane/ethyl acetate 1:1) to afford **5** (740 mg, 64%) as a colorless powder; mp 260–263 °C (lit. 262–264 °C [13]); $R_f = 0.14$ (hexane/ethyl acetate 1:1); $[\alpha]_D = +154.55°$ (*c* 0.21, $CHCl_3$) (lit. $+156 \pm 2°$ (*c* 0.05, $CHCl_3$) [13]); UV–vis (methanol): λ_{max} (log ε) = 249 nm (4.09); IR (KBr): ν = 3433*br*, 2952*s*, 2875*m*, 1731*s*, 1654*s*, 1465*m*, 1388*m*, 1363*m*, 1329*w*, 1280*m*, 1217*s*, 1167*s*, 1087*w*, 1049*w*, 1021*w*, 989*m* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 5.67$ (*s*, 1H, H-12), 4.55 (*dd*, 1H, H-3, $J = 11.6, 4.8$ Hz), 3.69 (*s*, 3H, CH_3), 2.80 (*ddd*, 1H, H-1, $J = 13.7, 3.3, 3.3$ Hz), 2.69 (*m*, 2H, chain-γ-CH_2), 2.64 (*m*, 2H, chain-β-CH_2), 2.36 (*s*, 1H, H-9), 2.08 (*dd*, 1H, H-18, $J = 13.9, 3.5$ Hz), 2.03 (*m*, 1H, H-15), 1.99 (*m*, 1H, H-21), 1.93 (*ddd*, 1H, H-19, $J = 13.5, 3.8, 2.6$ Hz), 1.82 (*ddd*, 1H, H-16, $J = 13.3, 13.3, 4.2$ Hz), 1.71 (*m*, 1H, H-2), 1.66 (*m*, 1H, H-7), 1.62 (*m*, 1H, H-2′), 1.61 (*dd*, 1H, H-19′, $J = 13.6, 13.6$ Hz), 1.58 (*m*, 1H, H-6), 1.48 (*m*, 1H, H-6′), 1.42 (*m*, 1H, H-7′), 1.40 (*m*, 1H, H-22), 1.36 (*s*, 3H, H-27), 1.31 (*m*, 1H, H-22′), 1.31 (*m*, 1H, H-21′), 1.18 (*m*, 1H, H-16′), 1.16 (*s*, 3H, H-25), 1.15 (*s*, 3H, H-29), 1.13 (*s*, 3H, H-26), 1.08 (*m*, 1H, H-1′), 1.02 (*m*, 1H, H-15′), 0.88 (*s*, 3H, H-24), 0.87 (*s*, 3H, H-23), 0.81 (*s*, 3H, H-28), 0.79 (*m*, 1H, H-5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 200.1$ (C-11), 177.1 (chain-δ-COOH), 176.9 (C-30), 171.8 (chain-α-COO), 169.3 (C-13), 128.5 (C-12), 81.2 (C-3), 61.7 (C-9), 55.0 (C-5), 51.7 (OCH_3), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 29.3 (chain-γ-CH_2), 28.9 (chain-β-CH_2), 28.5 (C-28), 28.3 (C-29), 28.0 (C-23), 26.4 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 18.7 (C-26), 17.3 (C-6), 16.7 (C-24), 16.4 (C-25) ppm; MS (ESI): *m*/*z* (%) = 585.4 ([M + H]$^+$, 12), 607.5 ([M + Na]$^+$, 54), 899.4 ([3M+2Na]$^{2+}$, 88), 1169.2 ([2M + H]$^+$, 18), 1191.6 ([2M + Na]$^+$, 100), 583.2 ([M − H]$^-$, 32), 629.0 ([M + HCO_2]$^-$, 96), 1167.0 ([2M − H]$^-$, 100).

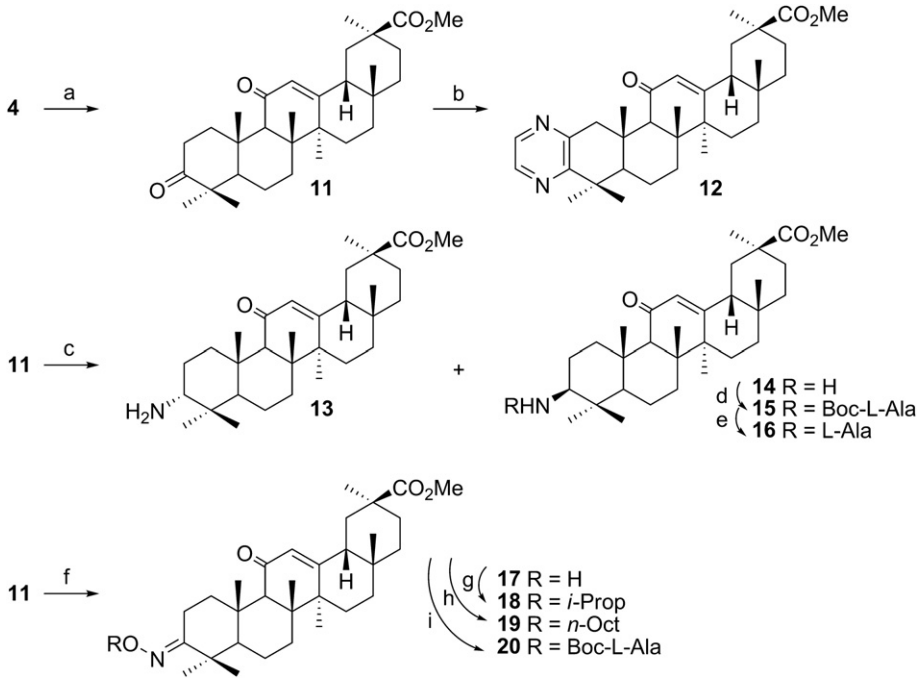
5.8. Methyl (3β)-3-({N-(tert-butoxycarbonyl9)-O-[tert-butyl(dimethyl)silyl]-L-seryl}oxy)-11-oxoolean-12-en-30-oate (**6**)

Compound **4** (410 mg, 0.85 mmol), Boc-L-Ser(OTBDMS)–OH (320 mg, 1.00 mmol) and DMAP (30 mg, 0.25 mmol) were dissolved in dry DCM (25 ml). DCC (210 mg, 1.02 mmol) was added and the solution was stirred at 25 °C for 20 h. The precipitate was filtered off and the filtrate evaporated. Purification via chromatography (silica gel, $CHCl_3$/ether 9:1) yielded **6** (150 mg, 22%) as a colorless powder;

Table 3
Cytotoxicity (IC_{50} values in μmol) for **4**, **11**–**20** in a panel of various cancer cell lines.

	518A2	8505C	A253	A2780	A549	DLD-1	Lipo	MCF-7	SW1736
4*	27.54	26.07	19.42	25.54	23.50	26.12	20.47	22.14	>30
11	>30	>30	>30	>30	>30	>30	>30	>30	29.09
12	>30	>30	>30	>30	>30	>30	>30	>30	>30
13	2.74	2.33	2.44	3.42	3.33	3.21	3.30	2.55	2.84
14	2.52	2.45	2.36	3.32	2.51	2.56	2.74	2.47	2.62
16	5.55	4.46	4.46	5.39	5.38	6.44	5.82	5.08	5.25
17	18.93	17.18	13.56	6.18	17.40	10.46	18.34	15.95	13.00
18	>30	>30	>30	>30	>30	>30	>30	>30	>30
19	>30	>30	>30	19.73	>30	>30	>30	26.35	17.77
20	16.49	15.47	14.12	12.03	15.83	19.10	18.99	15.17	14.61

From SRB assays after 96 h of treatment; the values are averaged from at least 3 independent experiments; variation ± 5–7%; * data from a previous study [7].



Scheme 3. Derivatization of **4** or **11**; reagents and conditions: a) CrO_3, acetone, 25 °C, 20 min [9]; b) S, ethylendiamine, morpholine, 130 °C, 4 h, 39%; c) $H_3CCOONH_4$, MeOH, 25 °C, 10 min followed by $NaBH_3CN$, MeOH, 25 °C, 24 h yielding **13** (20%) and **14** (52%); d) Boc-L-Ala, DCC, DMAP, DCM, 25 °C, 16 h, 57%; F_3CO_2H, DCM, 25 °C, 1 h, 60%; f) $H_2NOH.HCl$, pyridine, 60 °C, 3 h, 80%; *i*-PropI, KOH, THF, reflux, 24 h, 37%; Oct-Br, KOH, THF, reflux, 24 h, 29%; Boc-L-Ala, DCC, DMAP, DCM, 25 °C, 16 h, 48%.

mp 120–123 °C (decomp.); $R_f = 0.68$ (hexane/ethyl acetate 7:3); $[\alpha]_D = +74.12°$ (*c* 0.57, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 249 nm (4.05); IR (KBr): ν = 3448*br*, 2952*s*, 2858*s*, 1732*s*, 1662*s*, 1498*s*, 1465*m*, 1389*m*, 1367*m*, 1257*m*, 1216*s*, 1167*s*, 1115*s*, 1062*m*, 986*m*, 837*w*, 779*m*, 723*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.66 (*s*, 1H, H-12), 5.34 (*d*, 1H, NH, *J* = 8.1 Hz), 4.59 (*dd*, 1H, H-3, *J* = 11.7, 4.8 Hz), 4.30 (*m*, 1H, Ser-CH), 4.07 (*dd*, 1H, Ser-C*H*H′, *J* = 10.0, 2.1 Hz), 3.87 (*dd*, 1H, Ser-CH*H*′, *J* = 10.0, 2.1 Hz), 3.69 (*s*, 3H, OCH_3), 2.81 (*ddd*, 1H, H-1, *J* = 13.6, 3.5, 3.5 Hz), 2.36 (*s*, 1H, H-9), 2.08 (*dd*, 1H, H-18, *J* = 13.3, 3.7 Hz), 2.03 (*m*, 1H, H-15), 1.99 (*m*, 1H, H-21), 1.93 (*ddd*, 1H, H-19, *J* = 13.5, 4.2, 2.7 Hz), 1.82 (*ddd*, 1H, H-16, *J* = 13.7, 13.7, 4.4 Hz), 1.73 (*m*, 1H, H-2), 1.66 (*m*, 1H, H-7), 1.63 (*m*, 1H, H-2′), 1.61 (*dd*, 1H, H-19′, *J* = 13.6, 13.6 Hz), 1.57 (*m*, 1H, H-6), 1.48 (*m*, 1H, H-6′), 1.45 (*s*, 9H, Boc-CH_3), 1.43 (*m*, 1H, H-7′), 1.40 (*m*, 1H, H-22), 1.36 (*s*, 3H, H-27), 1.31 (*m*, 1H, H-22′), 1.31 (*m*, 1H, H-21′), 1.18 (*m*, 1H, H-16′), 1.16 (*s*, 3H, H-25), 1.15 (*s*, 3H, H-29), 1.13 (*s*, 3H, H-26), 1.06 (*m*, 1H, H-1′), 1.01 (*m*, 1H, H-15′), 0.90 (*s*, 3H, H-24), 0.89 (*s*, 3H, H-23), 0.86 (*s*, 9H, TBDMS-CH_3), 0.80 (*s*, 3H, H-28), 0.80 (*m*, 1H, H-5), 0.04 (*s*, 3H, Si–CH_3), 0.02 (*s*, 3H, Si–CH_3) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.9 (C-30), 170.4 (Ser-COO), 169.2 (C-13), 155.2 (Boc-COO), 128.5 (C-12), 81.8 (C-3), 79.6 (Boc-*quart.*–C), 63.8 (Ser-CH_2), 61.7 (C-9), 55.9 (Ser-CH), 55.0 (C-5), 51.7 (OCH_3), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.2 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.4 (Boc-CH_3), 28.4 (C-29), 28.0 (C-23), 26.5 (C-16), 26.4 (C-15), 25.8 (TBDMS-CH_3), 23.6 (C-2), 23.3 (C-27), 18.7 (C-26), 18.2 (TBDMS-*quart.*–C), 17.3 (C-6), 16.7 (C-24), 16.3 (C-25), −5.5 (Si–CH_3), −5.6 (Si–CH_3) ppm; ^{29}Si NMR (100 MHz, $CDCl_3$): δ = 21.4 (TBDMS-Si) ppm; MS (ESI): *m*/*z* (%) = 786.2 ($[M + H]^+$, 11), 808.4 ($[M + Na]^+$, 100), 824.3 ($[M + K]^+$, 6), 1201.1 ($[3M+2Na]^{2+}$, 8), 1593.1 ($[2M + Na]^+$, 16); analysis for $C_{45}H_{75}NO_8Si$ (786.2): C, 68.75; H, 9.62, N, 1.78; found; C, 68.65; H, 9.81; N, 1.63.

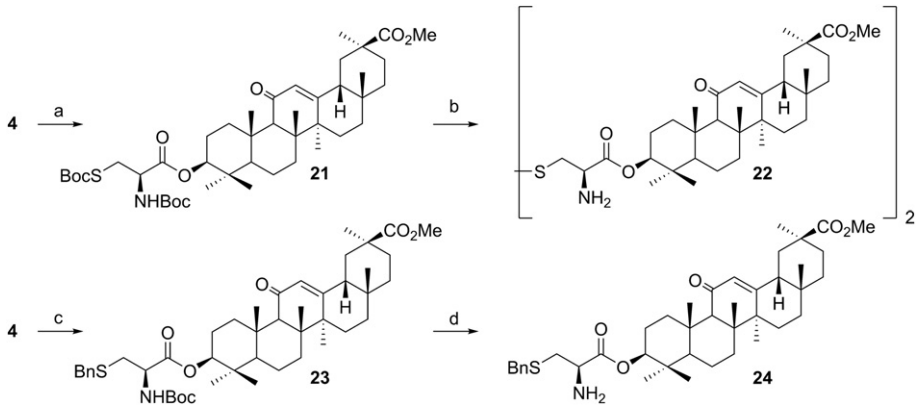
Table 4
Cytotoxicity (IC_{50} values in μmol) for **4**, **21**–**24** in a panel of various cancer cell lines.

	518A2	8505C	A253	A549	DLD-1	Lipo	SW1736
4*	27.54	26.07	19.42	23.50	26.12	20.47	>30
21	>30	>30	>30	>30	>30	>30	>30
22	24.54	15.10	19.07	18.75	>30	22.60	16.82
23	>30	>30	>30	>30	>30	>30	>30
24	16.78	15.45	15.84	17.90	>30	16.72	15.69

From SRB assays after 96 h of treatment; the values are averaged from at least 3 independent experiments; variation ± 5–7%; * data from a previous study [7].

5.9. Methyl (3β)-3-(methoxy)-11-oxo-olean-12-en-30-oate (**7**)

To a solution of **4** (200 mg, 0.41 mmol) in dry THF (10 ml), sodium hydride (60% in mineral oil, 30 mg, 0.75 mmol) was added, and the mixture was refluxed for 30 min. Methyl iodide was added and refluxing was continued for another 30 min. After cooling to 25 °C, water (10 ml) was added dropwise, and the mixture was extracted with DCM (3 × 10 ml). The organic layers were washed with brine (20 ml), dried (Na_2SO_4), filtered and evaporated. The residue was subjected to chromatography (silica gel, hexane/ethyl acetate 9:1) to afford **7** (70 mg, 34%) as a colorless powder; mp >300 °C (lit. >300 °C [3]); $R_f = 0.75$ (hexane/ethyl acetate 7:3); $[\alpha]_D = +160.78°$ (*c* 0.63, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 250 nm (4.02); IR (KBr): ν = 3442*br*, 2930*s*, 2872*s*, 2856*m*, 2816*m*, 1732*s*, 1654*s*, 1616*w*, 1463*m*, 1388*m*, 1358*m*, 1324*m*, 1279*w*, 1264*w*, 1246*w*, 1220*s*, 1184*m*, 1156*s*, 1101*s*, 1087*m*, 1048*w*, 1027*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.65 (*s*, 1H, H-12), 3.67 (*s*, 3H, OCH_3), 3.34 (*s*, 3H, CH_3O), 2.81 (*ddd*, 1H, H-1, *J* = 13.2, 3.7, 3.7 Hz), 2.65 (*dd*, 1H, H-3, *J* = 11.6, 4.4 Hz), 2.31 (*s*, 1H, H-9), 2.06 (*dd*, 1H, H-18, *J* = 14.8, 3.9 Hz), 2.01 (*m*, 1H, H-15), 1.98 (*m*, 1H, H-21), 1.90 (*ddd*, 1H, H-19, *J* = 13.7, 3.9, 2.5 Hz), 1.81 (*m*, 1H, H-16), 1.78 (*m*, 1H, H-2), 1.62 (*m*, 1H, H-7), 1.59 (*dd*, 1H, H-19′, *J* = 13.5, 13.5 Hz), 1.55 (*m*, 1H, H-6), 1.49 (*m*, 1H, H-2′), 1.42 (*m*, 1H, H-6′), 1.38 (*m*, 1H, H-7′), 1.37 (*m*, 1H, H-22), 1.34 (*s*, 3H, H-27), 1.30 (*m*, 1H, H-22′), 1.30 (*m*, 1H, H-21′), 1.16 (*m*, 1H, H-16′), 1.13 (*s*, 3H, H-25), 1.13 (*s*, 3H, H-29), 1.11 (*s*, 3H, H-26), 1.00 (*m*, 1H, H-15′), 0.97 (*s*, 3H,



Scheme 4. Derivatization of **4** leading to sulfur-containing derivatives **22**–**24**; reagents and conditions: a) Boc-L-Cys(SBoc)–OH, DCC, DMAP, DCM, 25 °C, 16 h, 93%; b) F_3CCO2H, DCM, 25 °C, 24 h, 43 and 86%; c) Boc-L-Cys(SBn)–OH, DCC, DMAP, DCM, 25 °C, 16 h, 57%; F_3CCO_2H, DCM, 25 °C, 24 h, 86%.

H-23), 0.88 (*m*, 1H, H-1′), 0.79 (*s*, 3H, H-28), 0.77 (*s*, 3H, H-24), 0.67 (*m*, 1H, H-5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.3 (C-11), 176.9 (C-30), 169.1 (C-13), 128.6 (C-12), 88.3 (C-3), 61.8 (C-9), 57.4 (CH_3O), 55.5 (C-5), 51.7 (OCH_3), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 39.0 (C-1), 39.0 (C-4), 37.7 (C-22), 37.1 (C-10), 32.8 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.1 (C-23), 26.5 (C-16), 26.4 (C-15), 23.3 (C-27), 22.0 (C-2), 18.7 (C-26), 17.4 (C-6), 16.3 (C-24), 16.3 (C-25) ppm; MS (ESI): *m*/*z* (%) = 499.5 ($[M + H]^+$, 56), 521.5 ($[M + Na]^+$, 6), 539.1 ($[M + Na + H_2O]^+$, 11), 552.9 ($[M + Na + MeOH]^+$, 100), 997.2 ($[2M + H]^+$, 20), 1019.3 ($[2M + Na]^+$, 42).

5.10. Methyl (3β)-3-{[(4-methylphenyl)sulfonyl]oxy}-11-oxolean-12-en-30-oate (**8**) [14]

Compound **4** (300 mg, 0.62 mmol) was dissolved in dry pyridine (10 ml) and cooled to 0 °C. 4-Toluenesulfonyl chloride (160 mg, 0.81 mmol) was added, and the solution was stirred for 4 h. After usual aqueous work-up, extraction with ethyl acetate (3 × 10 ml) and chromatography (silica gel, hexane/ethyl acetate 7:3) **8** (200 mg, 50%) was obtained as a colorless powder; mp 205–207 °C; R_f = 0.57 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = +106.7° (*c* 0.53, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 196 (4.58), 228 (4.16), 249 nm (4.02); IR (KBr): ν = 3439*br*, 2950*s*, 2869*s*, 1920*w*, 1731*s*, 1659*s*, 1621*m*, 1598*w*, 1466*s*, 1386*m*, 1338*s*, 1293*m*, 1279*m*, 1261*m*, 1246*m*, 1216*s*, 1189*s*, 1169*s*, 1098*m*, 1088*m*, 1048*w*, 1030*w*, 1018*w*, 983*m*, 943*s*, 930*s*, 913*s*, 881*s*, 840*m*, 808*m*, 794*m*, 768*w*, 714*w*, 703*w*, 690*s*, 629*w*, 610*w*, 582*w*, 559*m*, 547*m*, 538*m*, 475*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.76 (*d*, 2H, aromatic-H2, *J* = 8.1 Hz), 7.28 (*d*, 2H, aromatic-H3, *J* = 8.0 Hz), 5.60 (*s*, 1H, H-12), 4.23 (*dd*, 1H, H-3, *J* = 12.1, 4.6 Hz), 3.64 (*s*, 3H, CH_3), 2.73 (*ddd*, 1H, H-1, *J* = 13.6, 3.8, 3.8 Hz), 2.40 (*s*, 3H, aromatic-CH_3), 2.26 (*s*, 1H, H-9), 2.04 (*dd*, 1H, H-18, *J* = 13.2, 3.3 Hz), 2.00 (*m*, 1H, H-15), 1.95 (*m*, 1H, H-21), 1.87 (*m*, 1H, H-19), 1.78 (*m*, 1H, H-2), 1.77 (*m*, 1H, H-16), 1.61 (*m*, 1H, H-7), 1.58 (*m*, 1H, H-2′), 1.56 (*dd*, 1H, H-19′, *J* = 13.4, 13.4 Hz), 1.54 (*m*, 1H, H-6), 1.41 (*m*, 1H, H-6′), 1.39 (*m*, 1H, H-7′), 1.36 (*m*, 1H, H-22), 1.31 (*s*, 3H, H-27), 1.26 (*m*, 2H, H22′ and H21′), 1.14 (*m*, 1H, H-16′), 1.11 (*s*, 3H, H-29), 1.06 (*s*, 3H, H-25), 1.05 (*s*, 3H, H-26), 0.97 (*m*, 1H, H-15′), 0.90 (*ddd*, 1H, H-1′, *J* = 13.7, 13.7, 2.9 Hz), 0.84 (*s*, 3H, H-23), 0.8 (*s*, 3H, H-24), 0.76 (*s*, 3H, H-28), 0.70 (*d*, 1H, H-5, *J* = 11.5, 1.5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.7 (C11), 176.8 (C30), 169.4 (C13), 144.2 (aromatic-C4), 135.0 (aromatic-C1), 129.6 (aromatic-C2), 128.3 (C12), 127.5 (aromatic-C3), 90.6 (C3), 61.5 (C9), 55.2 (C5), 51.7 (OCH_3), 48.3 (C18), 45.3 (C8), 44.0 (C20), 43.1 (C14), 41.1 (C19), 38.8 (C1), 37.7 (C22), 36.7 (C10), 32.6 (C7), 31.8 (C17), 31.1 (C21), 28.5 (C28), 28.3 (C29), 27.9 (C28), 26.4 (C16), 26.3 (C15), 24.5 (C2), 23.3 (C27), 21.6 (aromatic-CH_3), 18.6 (C26), 17.5 (C6), 16.3 (C24), 16.3 (C25) ppm; MS (ESI): *m*/*z* (%) = 639.4 ($[M + H]^+$, 20), 661.1 ($[M + Na]^+$, 100), 677.1 ($[M + K]^+$, 8), 980.8 ($[3M+2Na]^{2+}$, 8), 1299.0 ($[2M + Na]^+$, 8).

5.11. Ethyl (3β)-3-hydroxy-11-oxoolean-12-en-30-oate (**9**)

Compound **9** was prepared according to [7].

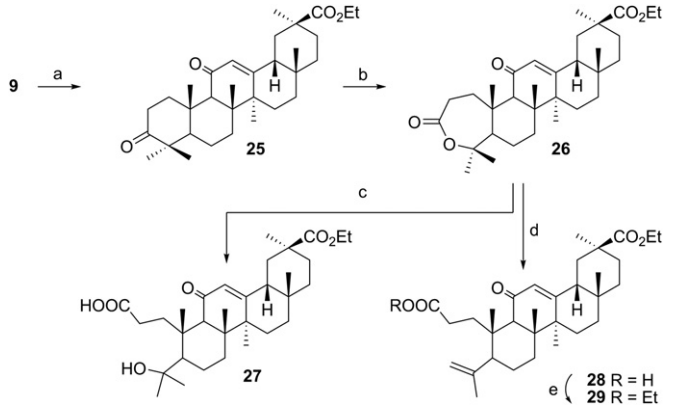
5.12. 4-{(3β)-30-Ethoxy-11,30-dioxo-olean-12-en-3-yl]oxy}-4-oxobutanoic acid (**10**)

Following the procedure given for **5**, from **9** (310 mg, 0.62 mmol), succinic anhydride (60 mg, 0.62 mmol) and dry pyridine (10 ml), **10** (320 mg, 86%) was obtained as colorless solid; mp 221–224 °C; R_f = 0.08 (hexane/ethyl acetate 1:1); $[\alpha]_D$ = +119.54° (*c* 0.31, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 249 nm (4.08); IR (KBr): ν = 3430*br*, 2953*s*, 1728*s*, 1657*s*, 1466*s*, 1387*s*, 1315*m*, 1291*m*, 1215*s*, 1176*s*, 1086*m*, 1022*m*, 987*m*, 958*m* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.64 (*s*, 1H, H-12), 4.55 (*dd*, 1H, H-3, *J* = 11.6, 4.8 Hz), 4.19 (*dt*, 1H, Et-C*H*H′, *J* = 10.8, 7.1 Hz), 4.12 (*dt*, 1H, Et-CH*H*′, *J* = 10.8, 7.1 Hz), 2.80 (*ddd*, 1H, H-1, *J* = 13.7, 3.3, 3.3 Hz), 2.69 (*m*, 2H, chain-γ-CH_2), 2.64 (*m*,

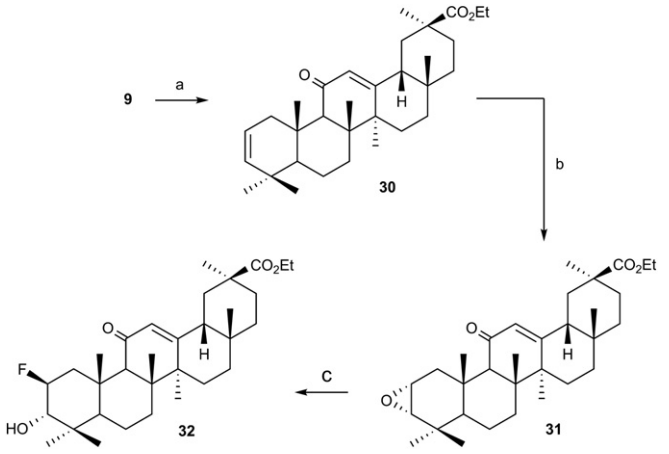
Table 5
Cytotoxicity (IC_{50} values in μmol) for **9**, **25**–**29** in a panel of various cancer cell lines.

	518A2	8505C	A253	A2780	A549	DLD-1	Lipo	MCF-7	SW1736
9*	25.23	24.58	25.04	26.96	22.74	28.14	27.66	18.61	13.37
25	>30	>30	>30	>30	>30	>30	>30	>30	>30
26	>30	>30	28.73	15.00	>30	>30	>30	20.58	12.71
27	>30	>30	>30	>30	>30	>30	>30	>30	>30
28	>30	>30	>30	>30	>30	>30	>30	>30	>30
29	9.28	>30	>30	5.89	>30	>30	>30	25.42	26.74

From SRB assays after 96 h of treatment; the values are averaged from at least 3 independent experiments; variation ± 5–7%; * data from a previous study [7].



Scheme 5. Ring A modifications; reactions and conditions: a) CrO_3, acetone, 25 °C, 20 min [9]; b) *m*-CPBA, $CHCl_3$, 55 °C, 24 h, 81%; c) NaOH, EtOH, 25 °C, 24 h, 20%; d) HCl, EtOH, 25 °C, 5 min, 96%; e) HCl, EtOH, reflux, 30 min, 95%.



Scheme 6. Fluorination at position C-2; reactions and conditions: a) PPh_3, 3,3-dimethyl glutarimide, DEAD,THF, 25 °C, 24 h, 82%; b) *m*-CPBA, CH_2Cl_2, 25 °C, 20 h, 77%; c) Olah's reagent, 25 °C, 5 h, 19%.

2H, chain-β-CH_2), 2.36 (*s*, 1H, H-9), 2.10 (*dd*, 1H, H-18, J = 13.5, 3.9 Hz), 2.03 (*ddd*, 1H, H-15, J = 13.5, 13.5, 4.2 Hz), 1.99 (*m*, 1H, H-21), 1.93 (*ddd*, 1H, H-19, J = 13.6, 4.0, 2.8 Hz), 1.82 (*ddd*, 1H, H-16, J = 13.6, 13.6, 4.3 Hz), 1.71 (*m*, 1H, H-2), 1.66 (*m*, 1H, H-7), 1.63 (*m*, 1H, H-2′), 1.61 (*dd*, 1H, H-19′, J = 13.5, 13.5 Hz), 1.58 (*m*, 1H, H-6), 1.48 (*m*, 1H, H-6′), 1.43 (*m*, 1H, H-7′), 1.39 (*m*, 1H, H-22), 1.36 (*s*, 3H, H-27), 1.33 (*m*, 1H, H-22′), 1.33 (*m*, 1H, H-21′), 1.26 (*t*, 3H, Et-CH_3, J = 7.1 Hz), 1.18 (*m*, 1H, H-16′), 1.16 (*s*, 3H, H-25), 1.14 (*s*, 3H, H-29), 1.12 (*s*, 3H, H-26), 1.04 (*ddd*, 1H, H-1′, J = 13.8, 13.8, 3.6 Hz), 1.02 (*m*, 1H, H-15′), 0.88 (*s*, 3H, H-24), 0.87 (*s*, 3H, H-23), 0.80 (*s*, 3H, H-28), 0.79 (*m*, 1H, H-5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.1 (C-11), 177.1 (chain-δ-COOH), 176.4 (C-30), 171.8 (chain-α-COO), 169.5 (C-13), 128.4 (C-12), 81.2 (C-3), 61.7 (C-9), 60.3 (Et-CH_2), 55.0 (C-5), 48.4 (C-18), 45.4 (C-8), 43.8 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 29.3 (chain-γ-CH_2), 28.9 (chain-β-CH_2), 28.6 (C-28), 28.3 (C-29), 28.0 (C-23), 26.5 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 18.7 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25), 14.3 (Et-CH_3) ppm; MS (ESI): m/z (%) = 599.5 ($[M + H]^+$, 20), 621.4 ($[M + Na]^+$, 68), 921.0 ($[3M+2Na]^{2+}$, 58), 1197.3 ($[2M + H]^+$, 22), 1219.4 ($[2M + Na]^+$, 100); analysis for $C_{36}H_{54}O_7$ (598.8): C, 72.21; H, 9.09; found: C, 72.03; H, 9.24.

5.13. Methyl 3,11-dioxoolean-12-en-30-oate (**11**)

Compound **11** was prepared according to [9].

5.14. Methyl (2S,4aS,6aS,6bR,14aS,16bR)-2,4a,6a,6b,9,9,14a-heptamethyl-15-oxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,14,14a,14b,15,16b-octadecahydrochryseno[1,2-g]quinoxaline-2-carboxylate (**12**)

Compound **11** (0.5 g, 1.00 mmol) was dissolved in morpholine (20 ml), sulfur (powder, 150 mg, 2.50 mmol) and ethylenediamine (15 ml, 2.20 mmol) was added. The mixture was stirred at 130 °C for 4 h. After cooling, the mixture was poured into ice water (50 ml), the aqueous layer was extracted with $CHCl_3$ (3 × 50 ml). The combined organic extracts were dried (Na_2SO_4), filtered and evaporated. Chromatographic purification (silica gel, chloroform/ether 7:3) gave **12** (200 mg, 39%) as an off-white powder; mp 315 °C; R_f = 0.16 (chloroform/ether 7:3); $[\alpha]_D$ = +191.8° (*c* 0.36, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 226 (3.97), 253 (4.14) nm; IR (KBr): ν = 3432*br*, 2941*s*, 1731*s*, 1654*s*, 1451*m*, 1400*m*, 1387*w*, 1361*w*, 1323*w*, 1280*m*, 1209*m*, 1186*s*, 1153*w*, 1120*m*, 1108*m*, 1085*w*, 1063*w*, 1028*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.38 (*d*, 1H, pyrazine-H, J = 2.2 Hz), 8.30 (*d*, 1H, pyrazine-H, J = 2.2 Hz), 5.75 (*s*, 1H, H-12), 4.15 (*d*, 1H, H-1, J = 17.0 Hz), 3.67 (*s*, 3H, CH_3), 2.54 (*s*, 1H, H-9), 2.54 (*d*, 1H, H-1′, J = 17.0 Hz), 2.10 (*dd*, 1H, H-18, J = 13.3, 3.1 Hz), 2.03 (*ddd*, 1H, H-15, J = 13.6, 13.6, 4.3 Hz), 1.96 (*m*, 1H, H-21), 1.93 (*m*, 1H, H-19), 1.85 (*ddd*, 1H, H-16, J = 13.7, 13.7, 4.1 Hz), 1.75 (*m*, 1H, H-7), 1.67 (*m*, 1H, H-6), 1.59 (*dd*, 1H, H-19′, J = 13.5, 13.5 Hz), 1.52 (*ddd*, 1H, H-7′, J = 12.6, 2.8, 2.8 Hz), 1.43 (*dd*, 1H, H-5, J = 11.3, 2.1 Hz), 1.41 (*m*, 1H, H-22), 1.38 (*m*, 2H, H-22′ and H-21′), 1.38 (*s*, 3H, H-24), 1.31 (*s*, 3H, H-27), 1.29 (*s*, 3H, H-23), 1.22 (*m*, 1H, H-16′), 1.18 (*s*, 3H, H-26), 1.12 (*s*, 3H, H-29), 1.10 (*s*, 3H, H-25), 1.01 (*m*, 1H, H-15′), 0.80 (*s*, 3H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 198.9 (C11), 176.8 (C30), 169.4 (C13), 158.8 (C3), 150.7 (C2), 142.1 (pyrazine), 141.7 (pyrazine), 128.6 (C12), 59.6 (C9), 53.0 (C5), 51.7 (OCH_3), 48.7 (C1), 48.4 (C18), 45.0 (C8), 44.0 (C20), 43.3 (C14), 41.2 (C19), 39.2 (C10), 37.7 (C22), 36.5 (C4), 31.8 (C23), 31.7 (C17), 31.7 (C7), 31.1 (C21), 28.5 (C28), 28.3 (C29), 26.5 (C16), 26.4 (C15), 24.2 (C27), 23.3 (C24), 19.3 (C6), 18.2 (C26), 15.8 (C25) ppm; MS (ESI): m/z (%) = 519.5 ($[M + H]^+$, 89), 572.9 ($[M + MeOH + Na]^+$, 100), 1037.2 ($[2M + H]^+$, 39); analysis for $C_{33}H_{46}N_2O_3$ (518.7): C, 76.41; H, 8.94; N, 5.40; found: C, 76.27; H, 9.11; N, 5. 32.

5.15. Methyl (3α)-3-amino-11-oxo-olean-12-en-30-oate (**13**) and methyl (3β)-3-amino-11-oxo-olean-12-en-30-oate (**14**)

To a solution of **11** (1.00 g, 2.06 mmol) in dry methanol (60 ml), ammonium acetate (1.60 g, 20.6 mmol) was added, and the mixture

Table 6
Cytotoxicity (IC_{50} values in μmol) for **9**, **30**–**32** in a panel of various cancer cell lines.

	518A2	A253	A431	A549	DLD-1	HCT-116	HT-29	Lipo	MCF-7
9*	25.23	25.04	23.45	22.74	28.14	21.58	22.14	27.66	18.61
30	>30	>30	>30	>30	>30	>30	>30	>30	>30
31	>30	>30	>30	>30	>30	>30	>30	>30	>30
32	>30	>30	>30	>30	>30	>30	24.72	>30	>30

From SRB assays after 96 h of treatment; the values are averaged from at least 3 independent experiments; variation ± 5–7%; * data from a previous study [7].

Table 7
Cytotoxicity (IC_{50} values in μmol) for **33** and **34** in a panel of various cancer cell lines.

	518A2	8505C	A253	A549	DLD-1	Lipo
33*	18.19	8.10	10.67	6.15	22.69	11.54
34	5.14	2.07	1.96	4.74	4.96	2.99

From SRB assays after 96 h of treatment; the values are averaged from at least 3 independent experiments; variation ± 5–7%; * data from a previous study [7].

was stirred at 25 °C for 10 min. Sodium cyanoborohydride (130 mg, 2.06 mmol) was added, and stirring was continued for 24 h. The mixture was concentrated to 20 ml and acidified with conc. hydrochloric acid. The precipitate was filtered off and washed with water. Purification by chromatography (silica gel, chloroform/methanol 9:1) gave **13** (200 mg, 20%) and **14** (520 mg, 52%) each as a colorless powder.

Data for **13**: mp 212–215 °C; $R_f = 0.71$ (chloroform/methanol 8:2); $[\alpha]_D = +114.3°$ (*c* 0.35, $CDCl_3$); UV–vis (methanol): λ_{max} (log ε) = 249 (4.02) nm; IR (KBr): ν = 3422*br*, 2950*s*, 2361*w*, 1732*s*, 1660*s*, 1618*m*, 1519*m*, 1463*m*, 1385*m*, 1315*w*, 1280*w*, 1259*w*, 1216*m*, 1191*m*, 1155*m*, 1133*w*, 1084*w*, 1063*w*, 1031*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.66 (*s*, 1H, H-12), 3.69 (*s*, 3H, CH_3), 3.03 (*m*, 1H, H-3), 2.73 (*s*, 1H, H-9), 2.60 (*ddd*, 1H, H-1, J = 15.3, 3.4, 3.4 Hz), 2.07 (*m*, 1H, H-18), 2.04 (*m*, 1H, H-6), 2.02 (*m*, 1H, H-15), 1.99 (*m*, 1H, H-21), 1.92 (*m*, 1H, H-19), 1.83 (*m*, 1H, H-6′), 1.80 (*m*, 1H, H-16), 1.78 (*m*, 1H, H-7), 1.63 (*dd*, 1H, H-19′, J = 13.8, 13.8 Hz), 1.49 (*m*, 1H, H-5), 1.44 (*m*, 1H, H-2), 1.43 (*s*, 3H, H-27), 1.38 (*m*, 1H, H-2′), 1.37 (*m*, 1H, H-1′), 1.36 (*m*, 1H, H-22), 1.35 (*m*, 1H, H-7′), 1.31 (*m*, 2H, H-22′ and H-21′), 1.17 (*m*, 1H, H-16′), 1.14 (*s*, 3H, H-29), 1.13 (*s*, 3H, H-25), 1.09 (*s*, 3H, H-26), 1.07 (*s*, 3H, H-29), 1.01 (*m*, 1H, H-15), 0.95 (*s*, 3H, H-24), 0.80 (*s*, 3H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C11), 177.0 (C30), 169.1 (C13), 128.3 (C12), 60.7 (C9), 58.0 (C3), 51.8 (OCH_3), 48.4 (C18), 47.4 (C5), 45.5 (C8), 44.0 (C20), 43.5 (C14), 41.1 (C19), 37.7 (C22), 36.9 (C4), 35.7 (C10), 32.7 (C1), 32.4 (C7), 31.8 (C17), 31.2 (C21), 28.8 (C23), 28.5 (C28), 28.3 (C29), 26.5 (C16), 26.4 (C15), 24.3 (C27), 22.7 (C24), 22.1 (C6), 18.6 (C26), 17.4 (C2), 16.5 (C25) ppm; MS (ESI): *m*/*z* (%) = 484.3 ($[M + H]^+$, 100).

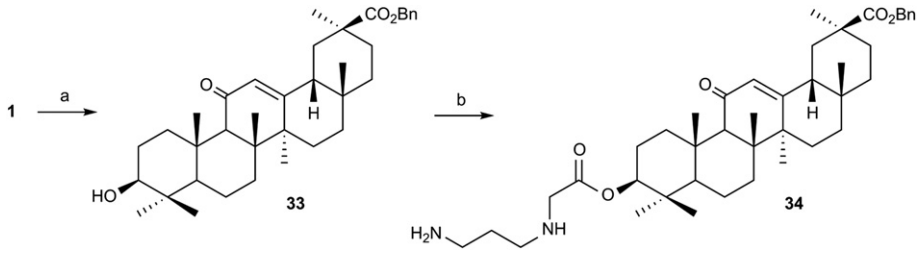
Data for **14**: mp 206 °C; $R_f = 0.64$ (chloroform/methanol 8:2); $[\alpha]_D = +121.8°$ (*c* 0.57, $CDCl_3$); UV–vis (methanol): λ_{max} (log ε) = 249 (4.02) nm; IR (KBr): ν = 3431*br*, 2950*s*, 1731*s*, 1659*s*, 1551*s*, 1465*s*, 1387*s*, 1328*m*, 1278*m*, 1249*m*, 1217*s*, 1190*m*, 1154*s*, 1088*m*, 1014*m*, 993*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.65 (*s*, 1H, H-12), 3.68 (*s*, 3H, CH_3), 2.84 (*ddd*, 1H, H-1, J = 13.0, 2.9, 2.9 Hz), 2.74 (*m*, 1H, H-3), 2.33 (*s*, 1H, H-9), 2.07 (*dd*, 1H, H-18, J = 13.4, 4.3 Hz), 2.01 (*ddd*, 1H, H-15, J = 9.8, 9.8, 3.8 Hz), 1.99 (*m*, 1H, H-21), 1.92 (*ddd*, 1H, H-19, J = 13.5, 4.3, 2.8 Hz), 1.90 (*m*, 1H, H-2), 1.81 (*m*, 1H, H-2′), 1.78 (*m*, 1H, H-16), 1.64 (*m*, 1H, H-7), 1.62 (*m*, 1H, H-6), 1.60 (*dd*, 1H, H-19′, J = 13.5, 13.5 Hz), 1.44 (*m*, 1H, H-6′), 1.41 (*m*, 1H, H-7′), 1.38 (*m*, 1H, H-22), 1.35 (*s*, 3H, H-27), 1.28 (*m*, 2H, H-22′ and H-21′), 1.17 (*m*, 1H, H-16′), 1.14 (*s*, 6H, H-25 and H-29), 1.12 (*s*, 3H, H-24), 1.10 (*s*, 3H, H-23), 0.99 (*m*, 1H, H-15′), 0.96 (*m*, 1H, H-1′), 0.94 (*s*, 3H, H-26), 0.80 (*s*, 3H, H-28), 0.74 (*dd*, 1H, H-5, J = 11.9, 1.3 Hz) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.6 (C11), 176.7 (C30), 169.0 (C13), 128.3 (C12), 61.3 (C9), 59.6 (C3), 55.1 (C5), 51.5 (OCH_3), 48.2 (C18), 45.1 (C8), 43.8 (C20), 43.0 (C14), 41.0 (C19), 38.9 (C4), 37.6 (C1), 37.0 (C10), 36.7 (C22), 32.4 (C7), 31.6 (C17), 31.0 (C21), 28.3 (C29), 28.1 (C28), 28.1 (C23), 26.4 (C16), 26.4 (C15), 24.2 (C2), 23.1 (C27), 18.5 (C24), 17.3 (C6), 16.0 (C26), 15.8 (C25) ppm; MS (ESI): *m*/*z* (%) = 484.3 ($[M + H]^+$, 100).

5.16. Methyl (3β) 3-{[N-(tert-butoxycarbonyl)-L-alanyl]amino}-11-oxoolean-12-en-30-oate (**15**)

Following the procedure given for **20**, compound **15** was obtained from **14** (160 mg, 0.32 mmol), DCC (80 mg, 0.39 mmol), DMAP (10 mg, 0.08 mmol) and Boc-L-alanine (80 mg, 0.42 mmol). Purification by chromatography (silica gel, hexane/ethyl acetate 3:7) and recrystallization from ethyl acetate/hexane gave **15** (120 mg, 57%) as colorless crystals; mp 198–203 °C; R_f = 0.1 (hexane/ethyl acetate 7:3); $[\alpha]_D = +49.6°$ (*c* 0.50, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 249 (3.92) nm; IR (KBr): ν = 3328*br*, 2930*s*, 2852*s*, 1731*s*, 1662*s*, 1626*s*, 1575*m*, 1534*m*, 1456*m*, 1388*m*, 1367*m*, 1313*m*, 1245*m*, 1218*m*, 1166*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 6.02 (*d*, 1H, NH), 5.59 (*s*, 1H, H-12), 4.88 (*m*, 1H, NH), 3.62 (*s*, 3H, CH_3), 3.59 (*dd*, 1H, H-3, J = 11.0, 4.4 Hz), 3.47 (*m*, 1H, Ala-CH), 2.70 (*ddd*, 1H, H-1, J = 13.2, 2.8, 2.8 Hz), 2.32 (*s*, 1H, H-9), 2.01 (*dd*, 1H, H-18, J = 13.7, 3.5 Hz), 1.95 (*ddd*, 1H, H-15, J = 13.5, 13.5, 4.1 Hz), 1.93 (*m*, 1H, H-21), 1.84 (*m*, 1H, H-19), 1.75 (*ddd*, 1H, H-16, J = 13.7, 13.7, 4.3 Hz), 1.62 (*m*, 1H, H-7), 1.55 (*dd*, 1H, H-19′, J = 13.6, 13.6 Hz), 1.58 (*m*, 1H, H-6), 1.56 (*m*, 1H, H-2), 1.46 (*m*, 1H, H-2′), 1.43 (*m*, 1H, H-6′), 1.38 (*s*, 9H, Boc-CH_3), 1.37 (*m*, 1H, H-7′), 1.34 (*m*, 1H, H-22), 1.30 (*s*, 3H, H-27), 1.28 (*s*, 3H, Ala-CH_3), 1.25 (*m*, 2H, H-22′ and H-21′), 1.10 (*m*, 1H, H-16′), 1.08 (*s*, 3H, H-29), 1.07 (*s*, 3H, H-25), 1.05 (*s*, 3H, H-26), 1.00 (*m*, 1H, H-1′), 0.95 (*m*, 1H, H-15′), 0.83 (*s*, 3H, H-23), 0.81 (*m*, 1H, H-5), 0.73 (*s*, 3H, H-28), 0.72 (*s*, 3H, H-24) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.2 (C11), 177.0 (C30), 172.0 (Ala-COO), 169.2 (C13), 156.7 (Boc-COO), 128.5 (C12), 80.1 (Boc-*quart.*–C), 60.7 (C9), 58.0 (C3), 51.8 (OCH_3), 48.4 (C18), 47.4 (C5), 45.4 (C8), 44.0 (C20), 43.2 (C14), 41.1 (C19), 39.6 (C1), 38.2 (C4), 37.7 (C22), 36.9 (C10), 32.7 (C7), 31.8 (C17), 31.1 (C21), 28.5 (C28), 28.4 (C29), 28.3 (C23), 28.3 (Boc-CH_3), 26.4 (C16), 26.4 (C15), 25.6 (C2), 23.3 (C27), 18.6 (C26), 17.9 (Ala-CH_3), 17.7 (C6), 16.5 (C24), 16.2 (C25) ppm; MS (ESI): *m*/*z* (%) = 655.3 ($[M + H]^+$, 32), 677.4 ($[M + Na]^+$, 100), 1004.82 ($[3M+2Na]^{2+}$, 12), 1332.2 ($[2M + Na]^+$, 25); analysis for $C_{39}H_{62}N_2O_6$ (654.9): C, 71.52; H, 9.54; N, 4.28; found: C, 71.41; H, 9.73; N, 4.13.

5.17. Methyl (3β) 3-(L-alanylamino)-11-oxo-olean-12-en-30-oate (**16**)

Compound **15** (100 mg, 0.15 mmol) was dissolved in DCM and trifluoroacetic acid (2 ml; 25.96 mmol) was added. After 1 h of continuous stirring, a saturated solution of sodium



Scheme 7. Synthesis of compound **34**; reactions and conditions: a) benzyl chloride, K_2CO_3, DMF, 25 °C, 2 h [7,11]; b) chloroacetyl chloride, DCM, 25 °C, 24 h, then diaminopropane, K_2CO_3, DMF, 25 °C, 2 h, 82%.

Table 8
Apoptotic effect [in %] of derivatives of **1** on A549 cells.

Compound	**1**	**13**	**14**	**16**	**17**	**20**	**22**	**24**	**32**	**34**
Apoptosis [%]	73.73 ± 1.40	64.88 ± 3.60	74.21 ± 1.85	56.77 ± 4.56	32.43 ± 3.40	68.54 ± 2.40	40.91 ± 3.43	60.64 ± 3.16	80.32 ± 1.68	80.57 ± 3.23

hydrogencarbonate (10 ml) was added, and the organic layer was washed with water (20 ml) afterward. The solvent was removed, and the residue was subjected to chromatography (silica gel, chloroform/methanol 8:2) to yield **16** (50 mg, 60%) as a colorless powder; mp 183–185 °C; R_f = 0.50 (chloroform/methanol 8:2); UV–vis (methanol): λ_{max} (log ε) = 248 (3.57) nm; IR (KBr): ν = 3330*br*, 2930*s*, 2852*m*, 1730*s*, 1659*s*, 1539*s*, 1451*m*, 1385*m*, 1314*w*, 1246*m*, 1218*m*, 1154*w*, 1088*w*, 1030*w* cm^{-1}; ^1H NMR (500 MHz, methanol-d_4): δ = 5.48 (*s*, 1H, H-12), 3.60 (*s*, 3H, CH_3), 3.50 (*dd*, 1H, H-3, *J* = 12.6, 4.0 Hz), 3.39 (*m*, 1H, Ala-CH), 2.67 (*ddd*, 1H, H-1, *J* = 13.0, 3.2, 3.2 Hz), 2.42 (*s*, 1H, H-9), 2.07 (*dd*, 1H, H-18, *J* = 13.9, 4.3 Hz), 2.03 (*ddd*, 1H, H-15, *J* = 13.8, 4.1, 4.1 Hz), 1.87 (*ddd*, 1H, H-21, *J* = 13.6, 5.2, 3.0 Hz), 1.79 (*m*, 1H, H-16), 1.77 (*m*, 1H, H-19), 1.70 (*m*, 1H, H-2), 1.68 (*m*, 1H, H-7), 1.65 (*dd*, 1H, H-19′, *J* = 13.5, 13.5 Hz), 1.62 (*ddd*, 1H, H-2′, *J* = 13.9, 9.1, 3.2 Hz), 1.60 (*m*, 1H, H-6), 1.44 (*m*, 1H, H-6′), 1.43 (*m*, 1H, H-7′), 1.38 (*m*, 1H, H-22), 1.34 (*s*, 3H, H-27), 1.31 (*m*, 2H, H-22′ and H-21′), 1.18 (*d*, 3H, Ala-CH_3, *J* = 7.0 Hz), 1.15 (*m*, 1H, H-16′), 1.06 (*s*, 3H, H-25), 1.05 (*s*, 3H, H-26), 1.05 (*s*, 3H, H-29), 1.00 (*m*, 1H, H-1′), 0.95 (*m*, 1H, H-15′), 0.83 (*dd*, 1H, H-5, *J* = 12.1, 1.2 Hz), 0.77 (*s*, 3H, 23), 0.75 (*s*, 3H, H-24), 0.73 (*s*, 3H, H-28) ppm; ^{13}C NMR (125 Hz, methanol-d_4): δ = 202.2 (C11), 178.4 (C30), 177.1 (Ala-COO), 172.4 (C13), 128.8 (C12), 63.0 (C9), 57.9 (C3), 56.7 (C5), 52.3 (Ala-CH), 51.4 (OCH_3), 49.9 (C18), 46.7 (C8), 45.3 (C20), 44.7 (C14), 42.4 (C19), 40.9 (C1), 39.6 (C4), 39.0 (C22, CH_2), 38.4 (C10), 33.7 (C7), 33.0 (C17), 32.0 (C21), 29.2 (C28), 29.2 (C23), 28.5 (C29), 27.6 (C16), 27.4 (C15), 26.0 (C2), 23.9 (C27), 21.8 (Ala-CH_3), 19.3 (C26), 18.9 (C6), 17.2 (C24), 16.9 (C25) ppm; MS (ESI): *m*/*z* (%) = 555.4 ($[M + H]^+$, 100); analysis for $C_{34}H_{54}N_2O_4$ (554.8): C, 73.61; H, 9.81; N, 5.05; found: C, 73.49; H, 9.99; N, 4.88.

5.18. Methyl 3-(hydroxyimino)-11-oxo-olean-12-en-30-oate (*17*)

To a solution of **11** (1.00 g, 2.10 mmol) in dry pyridine (10 ml), hydroxylamine hydrochloride (348 mg, 4.00 mmol) was added. The mixture was stirred at 60 °C for 3 h and acidified with hydrochloric acid (1 M, 10 ml). Usual aq. work-up followed by chromatography (silica gel, hexane/ethyl acetate 7:3) gave **17** (835 mg, 80%) as a colorless powder; mp 283 °C (lit. 289–290 °C [20]); R_f = 0.66 (hexane/ethyl acetate 8:2); $[\alpha]_D$ = +101.5° (*c* 0.68, $CHCl_3$) (lit. $[\alpha]_D$ = +106.7° (*c* 2.0, $CHCl_3$) [20]); UV–vis (methanol): λ_{max} (log ε) = 249 nm (4.11); IR (KBr): ν = 3278*br*, 2932*s*, 2870*m*, 1729*s*, 1655*s*, 1618*w*, 1464*w*, 1387*s*, 1366*m*, 1320*s*, 1281*m*, 1248*w*, 1218*w*, 1190*w*, 1153*w*, 1087*w*, 1030*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.61 (*s*, 1H, H-12), 3.62 (*s*, 3H, CH3), 2.99 (*ddd*, 1H, H-2, *J* = 15.5, 4.2, 4.2 Hz), 2.81 (*ddd*, 1H, H-1, *J* = 13.2, 5.3, 3.7 Hz), 2.31 (*s*, 1H, H-9), 2.22 (*ddd*, 1H, H-2′, *J* = 15.6, 12.9, 5.7 Hz), 2.02 (*dd*, 1H, H-18, *J* = 13.2, 2.8 Hz), 1.95 (*m*, 1H, H-15), 1.92 (*m*, 1H, H-21), 1.84 (*ddd*, 1H, H-19, *J* = 13.5, 3.7, 2.8 Hz), 1.77 (*ddd*, 1H, H-16, *J* = 13.4, 13.3, 4.2 Hz), 1.60 (*ddd*, 1H, H-7, *J* = 12.6, 12.6, 3.5 Hz), 1.54 (*dd*, 1H, H-19′, *J* = 13.7, 13.7 Hz), 1.54 (*m*, 1H, H-6), 1.45 (*ddd*, 1H, H-6′, *J* = 12.5, 12.5, 2.5 Hz), 1.37 (*ddd*, 1H, H-7′, *J* = 12.5, 2.9, 2.9 Hz), 1.32 (*ddd*, 1H, H-22, *J* = 10.0, 10.0, 3.0 Hz), 1.28 (*s*, 3H, H-29), 1.25 (*m*, 2H, H-21′ and H-22′), 1.19 (*s*, 3H, H-25), 1.12 (*s*, 3H, H-23), 1.12 (*m*, 1H, H-16′), 1.09 (*s*, 3H, H-26), 1.08 (*s*, 3H, H-28), 1.03 (*s*, 3H, H-24), 0.98 (*m*, 1H, H-1′), 0.94 (*m*, 1H, H-15′), 0.91 (*m*, 1H, H-5), 0.74 (*s*, 3H, H-27) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.6 (C11), 176.9 (C30), 169.4 (C13), 167.2 (C3), 128.5 (C12), 61.3 (C9), 55.6 (C5), 51.7 (OCH_3), 48.4 (C18), 45.3 (C8), 44.0 (C20), 43.2 (C14), 41.1 (C19), 40.5 (C4), 39.0 (C1), 37.7 (C22), 37.0 (C10), 32.4 (C7), 31.8 (C17), 31.1 (C21), 28.5 (C28), 28.3 (C29), 27.1 (C23), 26.5 (C16), 26.4 (C15), 23.3 (C27), 23.2 (C24), 18.6 (C26), 18.2 (C6), 17.4 (C2), 15.7 (C25) ppm; MS (ESI): *m*/*z* (%) = 498.5 ($[M + H]^+$, 58), 520.3 ($[M + Na]^+$, 6), 552.0 ($[M + MeOH + Na]^+$, 100), 995.0 ($[2M + H]^+$, 42), 1017.3 ($[2M + H]^+$, 43).

5.19. Methyl 3-(i-propoxyimino)-11-oxo-olean-12-en-30-oate (*18*)

To a solution of **17** (500 mg, 0.60 mmol) in dry THF (20 ml), potassium hydroxide (0.16 g, 3.00 mmol) and *i*-propyl iodide (340 mg, 2.00 mmol) was added. After refluxing for 24 h, the mixture was cooled and poured into water (40 ml). The aqueous layer was extracted with chloroform (3 × 50 ml), the extracts were

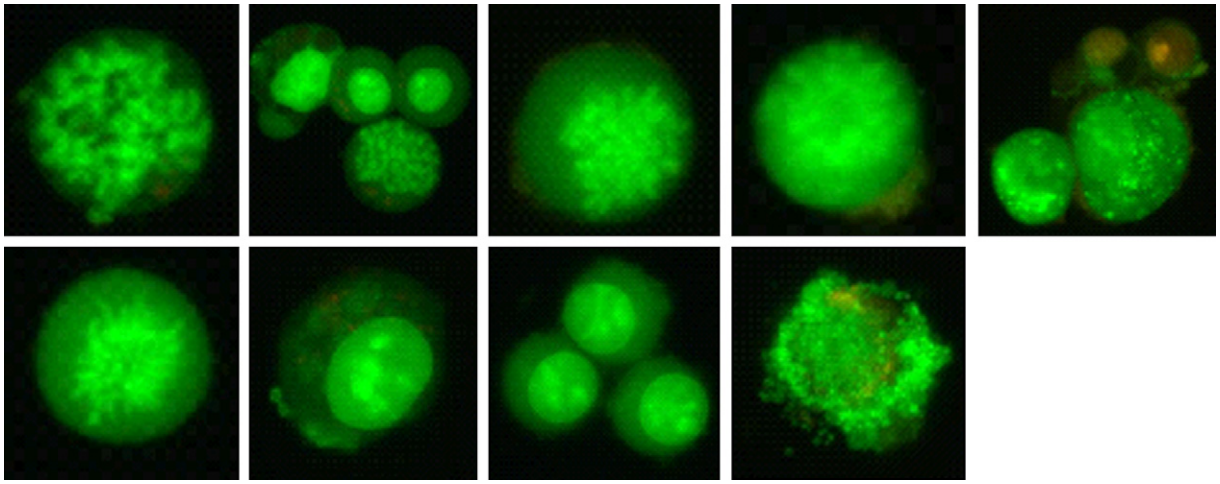


Fig. 2. Results from the AO/EB tests: A549 cells treated with (left to right, upper row then lower row): **13** (4 μM), **14** (4 μM), **16** (8 μM), **17** (20 μM), **20** (20 μM), **22** (20 μM), **24** (20 μM), **32** (60 μM), **34** (8 μM).

dried (Na_2SO_4), filtered and evaporated. Purification by chromatography (silica gel, chloroform/ether 8:2) afforded **18** (120 mg, 37%) as a colorless powder; mp 145 °C; R_f = 0.77 (chloroform/ether 9:1); $[\alpha]_D = 105.42°$ (*c* 0.32, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 257 nm (4.26), IR (KBr): ν = 3446*s*, 2972*s*, 2870*s*, 1732*s*, 1662*s*, 1622*w*, 1458*m*, 1386*m*, 1366*w*, 1325*w*, 1281*w*, 1262*w*, 1218*m*, 1189*m*, 1153*m*, 1088*w*, 1028*w*, 963*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.66 (*s*, 1H, H-12), 4.26 (*qq*, 1H, chain-CH, *J* = 6.2, 6.2 Hz), 3.67 (*s*, 3H, CH_3), 2.89 (*ddd*, 1H, H-2, *J* = 15.7, 5.0, 4.0 Hz), 2.76 (*ddd*, 1H, H-1, *J* = 13.3, 5.7, 4.0 Hz), 2.36 (*s*, 1H, H-9), 2.24 (*ddd*, 1H, H-2′, *J* = 15.6, 12.2, 5.9 Hz), 2.06 (*dd*, 1H, H-18, *J* = 13.5, 3.3 Hz), 1.99 (*m*, 1H, H-15), 1.97 (*m*, 1H, H-21), 1.90 (*ddd*, 1H, H-19, *J* = 13.5, 3.6, 2.7 Hz), 1.82 (*ddd*, 1H, H-16, *J* = 13.6, 13.6, 4.4 Hz), 1.69 (*m*, 1H, H-7), 1.59 (*dd*, 1H, H-19′, *J* = 13.5, 13.5 Hz), 1.56 (*m*, 1H, H-6), 1.52 (*m*, 1H, H-6′), 1.44 (*m*, 1H, H-7′), 1.39 (*m*, 1H, H-22), 1.33 (*s*, 3H, H-27), 1.30 (*m*, 2H, H-22′ and H-21′), 1.21 (*s*, 3H, H-25), 1.20 (*m*, 1H, H-16′), 1.19 (*m*, 6H, chain-CH_3), 1.16 (*s*, 3H, H-23), 1.13 (*s*, 6H, H-26 and H-29), 1.04 (*s*, 3H, H-24), 1.03 (*m*, 1H, H-1′), 1.01 (*m*, 1H, H-5), 1.00 (*m*, 1H, H-15′), 0.78 (*s*, 3H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.6 (C11), 176.7 (C30), 169.0 (C13), 164.5 (C3), 128.6 (C12), 74.3 (chain-CH), 61.5 (C9), 55.6 (C5), 51.8 (OCH_3), 48.6 (C18), 45.5 (C8), 44.2 (C20), 43.4 (C14), 41.3 (C19), 40.3 (C4), 39.1 (C1), 37.9 (C22), 37.1 (C10), 32.6 (C7), 32.0 (C17), 31.3 (C21), 28.7 (C28), 28.5 (C29), 27.8 (C23), 26.7 (C16), 26.7 (C15), 23.7 (C24), 23.5 (C27), 21.8 (chain-CH_3), 18.8 (C26), 18.5 (C6), 18.2 (C2), 15.8 (C25) ppm; MS (ESI): *m*/*z* (%) = 540.5 ($[M + H]^+$, 84), 593.5 ($[M + MeOH + Na]^+$, 100), 1101.3 ($[2M + Na]^+$, 40); analysis for $C_{34}H_{53}NO_4$ (539.8): C, 75.65; H, 9.90; N, 2.59; found: C, 75.47; H, 10.02; N, 2.37.

5.20. Methyl 3-[(octyloxy)imino]-11-oxo-olean-12-en-30-oate (**19**)

To a solution of **17** (300 mg, 0.60 mmol) in dry THF (20 ml), potassium hydroxide (0.1 g, 1.8 mmol) and *n*-bromooctane (230 mg, 1.20 mmol) was added. After refluxing for 24 h, the mixture was cooled and filtered. The solvent was removed and the residue was subjected to chromatography (silica gel, chloroform/ether 9:1) to yield **19** (106 mg, 29%) as a colorless powder; mp 92–96 °C; R_f = 0.81 (hexane/ethyl acetate 95:5); $[\alpha]_D = +81.6°$ (*c* 0.44, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 200 (4.04), 249 (4.03) nm; IR (KBr): ν = 3440*br*, 2927*m*, 2858*s*, 1731*s*, 1655*s*, 1464*s*, 1386*m*, 1363*m*, 1321*w*, 1280*w*, 1262*w*, 1217*w*, 1189*w*, 1155*m*, 1088*m*, 1050*m*, 1028*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.64 (*s*, 1H, CH (12)), 3.95 (*t*, 2H, chain-1, *J* = 6.7 Hz), 3.65 (*s*, 3H, CH_3), 2.91 (*ddd*, 1H, H-2, *J* = 15.7, 5.2, 3.8 Hz), 2.76 (*ddd*, 1H, H-1, *J* = 13.3, 5.8, 3.8 Hz), 2.34 (*s*, 1H, H-9), 2.21 (*ddd*, 1H, H-2′, *J* = 15.7, 12.3, 5.8 Hz), 2.08 (*dd*, 1H, H-18, *J* = 13.5, 3.5 Hz), 1.98 (*m*, 1H, H-15), 1.94 (*m*, 1H, H-21), 1.87 (*ddd*, 1H, H-19, *J* = 13.6, 4.0, 2.6 Hz), 1.82 (*m*, 2H, H-16 and H-7), 1.58 (*dd*, 1H, H-19′, *J* = 13.5, 13.5 Hz), 1.63 (*m*, 2H, chain-4), 1.57 (*m*, 2H, chain-2), 1.41 (*m*, 1H, H-7′), 1.36 (*m*, 1H, H-22), 1.31 (*s*, 3H, H-24), 1.30–1.28 (*m*, 9H, H-22′, H-21′, chain-6, chain-5, chain-3, H-6), 1.19 (*s*, 3H, H-25), 1.13 (*s*, 3H, H-23), 1.11 (*s*, 3H, H-29), 1.10 (*s*, 3H, H-26), 1.02 (*s*, 3H, H-27), 1.02–0.97 (*m*, 3H, H-1′, H-16′, H-15′), 0.84 (*m*, 3H, chain-8), 0.77 (*s*, 3H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.7 (C11), 176.8 (C30), 169.1 (C13), 165.1 (C3), 128.5 (C12), 73.3 (chain-1), 61.3 (C9), 55.5 (C5), 51.7 (OCH_3), 48.3 (C18), 45.2 (C8), 44.0 (C20), 43.2 (C14), 41.1 (C19), 40.1 (C4), 39.0 (C1), 37.7 (C22), 36.9 (C10), 32.8 (C7), 32.4 (C17), 31.8 (C21), 31.1 (chain-6), 29.4 (chain-2), 29.2 (chain-5), 29.1 (chain-4), 28.5 (C28), 28.2 (C29), 28.1 (chain-3), 27.4 (C23), 26.4 (C15), 26.3 (C16), 23.4 (C27), 23.3 (C24), 22.6 (C2), 18.6 (C26), 18.2 (chain-7), 17.8 (C6), 15.6 (C25), 14.1 (chain-8) ppm; MS (ESI): *m*/*z* (%) = 610.5 ($[M + H]^+$, 88), 632.5 ($[M + Na]^+$, 100), 1219.2 ($[2M + H]^+$, 20), 1241.4 ($[2M + Na]^+$, 44); analysis for $C_{39}H_{63}NO_4$ (609.9): C, 76.80; H, 10.41; N, 2.30; found: C, 76.54; H, 10.69; N, 2.11.

5.21. Methyl 3-[6-{(2L)-2-[tert-butoxycarbonylamino]propanoyl}-oxyimino]-11-oxo-olean-12-en-30-oate (**20**)

To a solution of **17** (250 mg, 0.50 mmol) and DMAP (10 mg, 0.08 mmol) in dry DCM (20 ml), Boc-L-alanine (120 mg, 0.63 mmol) and DCC (120 mg, 0.60 mmol) were added. The mixture was stirred at 25 °C for 16 h. The solvent was removed under reduced pressure, the residue purified by chromatography (silica gel, hexane/ethyl acetate 7:3) to afford **20** (160 mg, 48%) as a colorless powder; mp 116–118 °C; R_f = 0.65 (hexane/ethylacetate 7:3); $[\alpha]_D = +95.5°$ (*c* 0.40, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 249 (4.11) nm; IR (KBr): ν = 3385*br*, 2979*s*, 2872*m*, 2361*w*, 1763*m*, 1727*s*, 1660*s*, 1619*w*, 1509*m*, 1456*s*, 1389*m*, 1367*m*, 1345*m*, 1249*m*, 1218*m*, 1161*m*, 1109*s*, 1064*w*, 1026*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.67 (*s*, 1H, H-12), 5.09 (*d*, 1H, Ala-NH, *J* = 7.0 Hz), 4.47 (*m*, 1H, Ala-CH), 3.67 (*s*, 3H, CH_3), 2.88 (*m*, 1H, H-2), 2.85 (*m*, 1H, H-1), 2.41 (*m*, 1H, H-2′), 2.37 (*s*, 1H, H-9), 2.08 (*dd*, 1H, H-18, *J* = 13.2, 3.3 Hz), 2.03 (*m*, 1H, H-15), 1.98 (*m*, 1H, H-21), 1.90 (*ddd*, 1H, H-19, *J* = 13.9, 4.0, 2.7 Hz), 1.82 (*ddd*, 1H, H-16, *J* = 13.5, 13.5, 4.5 Hz), 1.69 (*m*, 1H, H-7), 1.64 (*m*, 1H, H-6), 1.58 (*dd*, 1H, H-19′, *J* = 13.8, 13.8 Hz), 1.51 (*m*, 1H, H-6′), 1.42 (*m*, 1H, H-7′), 1.41 (*s*, 12H, Boc-CH_3 and Ala-CH_3), 1.39 (*m*, 1H, H-22), 1.33 (*s*, 3H, H-27), 1.29 (*m*, 2H, H-22′ and H-21′), 1.26 (*s*, 3H, H-23), 1.25 (*s*, 3H, H-25), 1.18 (*m*, 1H, H-16′), 1.14 (*s*, 3H, H-24), 1.14 (*m*, 1H, H-1′), 1.13 (*m*, 1H, H-5), 1.12 (*s*, 6H, H-26 and H-29), 1.01 (*m*, 1H, H-15′), 0.79 (*s*, 3H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.2 (C11), 176.7 (C30), 175.8 (Ala-COO), 171.4 (C3), 169.5 (C13), 154.9 (Boc-COO), 128.4 (C12), 79.9 (Boc-*quart.*–C), 61.4 (C9), 55.6 (C5), 51.7 (OCH_3), 48.6 (C18), 47.8 (Ala-CH), 45.4 (C8) 44.2 (C20), 43.5 (C14), 41.7 (C4), 41.3 (C19), 39.4 (C1), 37.9 (C22), 37.1 (C10), 32.5 (C7), 32.0 (C17), 31.3 (C21), 28.7 (C28), 28.3 (Boc-CH_3), 28.4 (C29), 27.4 (C23), 26.7 (C16), 26.6 (C15), 23.5 (C27), 23.2 (C24), 19.9 (C2), 19.0 (Ala-CH_3), 18.8 (C26), 18.4 (C6), 16.1 (C25) ppm; MS (ESI): *m*/*z* (%) = 669.1 ($[M + H]^+$, 5), 686.2 ($[M + NH_4]^+$, 5), 691.3 ($[M + Na]^+$, 100), 1359.1 ($[2M + Na]^+$, 24); analysis for $C_{39}H_{60}N_2O_7$ (668.9): C, 70.03; H, 9.04; N, 4.19; found: C, 69.85; H, 9.25; N, 3.98.

5.22. Methyl (3β)-3-({[(2L)-(tert-butoxycarbonyl)amino][(tert-butoxycarbonyl)thio] acetyl}oxy)-11-oxoolean-12-en-30-oate (**21**)

Following the procedure for **20**, **21** (740 mg, 93%) was obtained from **4** (490 mg, 1.01 mmol), Boc-L-Cys(SBoc)–OH (380 mg, 1.18 mmol), DCC (380 mg, 1.84 mmol) and DMAP (30 mg, 0.25 mmol) after purification by chromatography (silica gel, chloroform/ether 9:1) as a colorless powder; mp 130–133 °C; R_f = 0.70 (hexane/ethyl acetate 7:3); $[\alpha]_D = +78.66°$ (*c* 0.41, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 250 nm (4.00); IR (KBr): ν = 3439*br*, 2978*s*, 2875*m*, 1727*s*, 1661*s*, 1500*m*, 1457*m*, 1392*m*, 1369*s*, 1249*m*, 1216*s*, 1168*s*, 1128*s*, 1087*m*, 1050*m*, 1021*m*, 986*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.67 (*s*, 1H, H-12), 5.30 (*d*, 1H, NH, *J* = 7.6 Hz), 4.58 (*dd*, 1H, H-3, *J* = 11.8, 4.8 Hz), 4.54 (*m*, 1H, Cys-CH), 3.69 (*s*, 3H, OCH_3), 3.40 (*dd*, 1H, Cys-C*H*H′, *J* = 14.0, 4.0 Hz), 3.19 (*dd*, 1H, Cys-CH*H*′, *J* = 14.0, 6.6 Hz), 2.82 (*ddd*, 1H, H-1, *J* = 13.7, 3.2, 3.2 Hz), 2.35 (*s*, 1H, H-9), 2.08 (*dd*, 1H, H-18, *J* = 13.0, 3.4 Hz), 2.03 (*ddd*, 1H, H-15, *J* = 13.4, 13.4, 4.3 Hz), 1.99 (*m*, 1H, H-21), 1.92 (*ddd*, 1H, H-19, *J* = 13.6, 3.5, 2.9 Hz), 1.82 (*ddd*, 1H, H-16, *J* = 13.7, 13.7, 4.5 Hz), 1.74 (*m*, 1H, H-2), 1.66 (*m*, 1H, H-7), 1.62 (*m*, 1H, H-2′), 1.61 (*dd*, 1H, H-19′, *J* = 13.5, 13.5 Hz), 1.59 (*m*, 1H, H-6), 1.46 (*s*, 9H, S-Boc-CH_3), 1.45 (*m*, 1H, H-6), 1.44 (*s*, 9H, O-Boc-CH_3), 1.43 (*m*, 1H, H-7′), 1.39 (*m*, 1H, H-22), 1.36 (*s*, 3H, H-27), 1.31 (*m*, 1H, H-22′), 1.31 (*m*, 1H, H-21′), 1.18 (*m*, 1H, H-16′), 1.16 (*s*, 3H, H-25), 1.15 (*s*, 3H, H-29), 1.13 (*s*, 3H, H-26), 1.04 (*m*, 1H, H-1′), 1.01 (*m*, 1H, H-15′), 0.89 (*s*, 3H, H-23), 0.89 (*s*, 3H, H-24), 0.80 (*s*, 3H, H-28), 0.79 (*m*, 1H, H-5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.9 (C-11), 176.9 (C-30), 170.2 (Cys-COO), 169.2 (C-13), 168.4 (S–Boc–COO), 155.0 (O–Boc–COO), 128.5 (C-12), 85.4 (S-Boc-*quart.*–C), 82.5 (C-3), 79.8 (Boc-*quart.*–C), 61.7 (C-9), 55.0 (C-5),

53.9 (Cys-CH), 51.8 (OCH_3), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.2 (C-4), 37.7 (C-22), 36.9 (C-10), 33.0 (Cys-CH_2), 32.7 (C-7), 31.8 (C-17), 31.2 (C-21), 28.5 (C-28), 28.3 (3× O-Boc-CH_3), 28.3 (C-29), 28.1 (3× S–Boc–CH_3), 28.1 (C-23), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.4 (C-27), 18.7 (C-26), 17.4 (C-6), 16.8 (C-24), 16.4 (C-25) ppm; MS (ESI): *m/z* (%) = 788.2 ($[M + H]^+$, 10), 810.3 ($[M + Na]^+$, 100), 1204.5 ($[3M+2Na]^{2+}$, 6), 1597.2 ($[2M + H]^+$, 12); analysis for $C_{44}H_{69}NO_9S$ (788.1): C, 67.06; H, 8.82; N, 1.78; S, 4.07; found: C, 66.88; H, 9.01; N, 1.64; S, 3.86.

5.23. Dimethyl (3β,3′β)-3,3′-(dithiobis{[(2S)-2-amino-1-oxoethane-2,1-diyl]oxy}) bis(11-oxoolean-12-en-30-oate) (**22**)

To a solution of **21** (640 mg, 0.81 mmol) in dry DCM (15 ml), trifluoroacetic acid (4 ml, 51.92 mmol) was added, and the mixture was stirred at room temperature for 1 day. A saturated solution of $NaHCO_3$ (10 ml) was slowly added, and the mixture was extracted with DCM (3 × 10 ml). The organic layers were washed with water (20 ml) and brine (20 ml), dried (Na_2SO_4) and filtered. The solvent was removed and **22** (410 mg, 43%) was obtained as a slightly yellowish powder; mp 220–224 °C; R_f = 0.95 (chloroform/methanol 9:1); $[\alpha]_D$ = +103.37° (*c* 0.49, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 249 nm (4.34); IR (KBr): ν = 3432*br*, 2950*s*, 1732*s*, 1660*s*, 1465*m*, 1388*m*, 1324*w*, 1216*s*, 1087*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.67 (*s*, 1H, H-12), 4.59 (*dd*, 1H, H-3, *J* = 11.7, 4.6 Hz), 3.87 (*m*, 1H, Cys-CH), 3.68 (*s*, 3H, OCH_3), 3.22 (*m*, 1H, Cys-C*H*H′), 2.97 (*m*, 1H, Cys-CH*H*′), 2.82 (*ddd*, 1H, H-1, *J* = 13.7, 3.5, 3.5 Hz), 2.36 (*s*, 1H, H-9), 2.22 (*m*, 2H, NH_2), 2.08 (*dd*, 1H, H-18, *J* = 13.3, 3.5 Hz), 2.02 (*ddd*, 1H, H-15, *J* = 13.6, 13.6, 4.2 Hz), 1.99 (*m*, 1H, H-21), 1.92 (*ddd*, 1H, H-19, *J* = 13.7, 3.6, 2.5 Hz), 1.82 (*ddd*, 1H, H-16, *J* = 13.5, 13.5, 4.4 Hz), 1.73 (*m*, 1H, H-2), 1.68 (*m*, 1H, H-7), 1.65 (*m*, 1H, H-2′), 1.61 (*dd*, 1H, H-19′, *J* = 13.6, 13.6 Hz), 1.57 (*m*, 1H, H-6), 1.45 (*m*, 1H, H-6′), 1.40 (*m*, 1H, H-7′), 1.38 (*m*, 1H, H-22), 1.36 (*s*, 3H, H-27), 1.31 (*m*, 1H, H-22′), 1.31 (*m*, 1H, H-21′), 1.18 (*m*, 1H, H-16′), 1.16 (*s*, 3H, H-25), 1.15 (*s*, 3H, H-29), 1.13 (*s*, 3H, H-26), 1.05 (*m*, 1H, H-1′), 1.01 (*m*, 1H, H-15′), 0.90 (*s*, 3H, H-23), 0.90 (*s*, 3H, H-24), 0.81 (*m*, 1H, H-5), 0.80 (*s*, 3H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.9 (C-11), 176.9 (C-30), 172.9 (Cys-COO), 169.3 (C-13), 128.5 (C-12), 82.2 (C-3), 61.7 (C-9), 55.0 (C-5), 54.0 (Cys-CH), 51.8 (OCH_3), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 44.0 (Cys-CH_2), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.3 (C-23), 26.5 (C-16), 26.4 (C-15), 23.6 (C-2), 23.4 (C-27), 18.7 (C-26), 17.4 (C-6), 16.9 (C-24), 16.4 (C-25) ppm; MS (ESI): *m/z* (%) = 587.4 ($[M+2H]^{2+}$, 100), 1173.3 ($[2M+2H]^{2+}$, 38); analysis for $C_{68}H_{104}N_2O_{10}S_2$ (1173.1): C, 69.59; H, 8.93; N, 2.39; S, 5.46; found: C, 69.37; H, 9.04; N, 2.21; S, 5.41.

5.24. Methyl (3β)-3-({(benzylthio)[(tert-butoxycarbonyl)-(2L)-amino]acetyl}oxy)-11-oxoolean-12-en-30-oate (**23**)

Following the procedure given for **20**, from **4** (730 mg, 1.51 mmol), Boc-L-Cys(SBn)–OH (720 mg, 2.32 mmol), DCC (390 mg, 1.89 mmol) and DMAP (30 mg, 0.25 mmol), followed by chromatography (silica gel, hexane/ethyl acetate 95:5) **23** (670 mg, 57%) was obtained as a colorless powder; mp 112–115 °C; R_f = 0.64 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = +57.94° (*c* 0.43, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 248 nm (4.01); IR (KBr): ν = 3329*br*, 2930*s*, 2852*m*, 1719*s*, 1654*s*, 1627*s*, 1576*m*, 1508*m*, 1455*m*, 1390*m*, 1367*m*, 1341*m*, 1246*m*, 1216*s*, 1167*s*, 1088*w*, 1063*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.32–7.20 (*m*, 5H, H–Ar), 5.67 (*s*, 1H, H-12), 5.29 (*d*, 1H, NH, *J* = 7.9 Hz), 4.57 (*dd*, 1H, H-3, *J* = 11.7, 4.8 Hz), 4.51 (*m*, 1H, Cys-CH), 3.75 (*s*, 2H, Bn-CH_2), 3.69 (*s*, 3H, OCH_3), 2.88 (*m*, 1H, Cys-C*H*H′), 2.84 (*m*, 1H, Cys-CH*H*′), 2.82 (*m*, 1H, H-1), 2.35 (*s*, 1H, H-9), 2.09 (*dd*, 1H, H-18, *J* = 13.5, 3.5 Hz), 2.03 (*m*, 1H, H-15), 1.99 (*m*, 1H, H-21), 1.93 (*m*, 1H, H-19), 1.83 (*ddd*, 1H, H-16, *J* = 13.7, 13.7, 3.7 Hz), 1.74 (*m*, 1H, H-2), 1.72 (*m*, 1H, H-7), 1.66 (*m*, 1H, H-6), 1.64 (*m*, 1H, H-2′), 1.61 (*dd*, 1H, H-19′, *J* = 13.6, 13.6 Hz), 1.58 (*m*, 1H, H-6′), 1.46 (*s*, 9H, Boc-CH_3), 1.43 (*m*, 1H, H-7′), 1.38 (*m*, 1H, H-22), 1.36 (*s*, 3H, H-27), 1.31 (*m*, 1H, H-22′), 1.31 (*m*, 1H, H-21′), 1.18 (*m*, 1H, H-16′), 1.16 (*s*, 3H, H-25), 1.15 (*s*, 3H, H-29), 1.13 (*s*, 3H, H-26), 1.08 (*m*, 1H, H-15′), 1.02 (*m*, 1H, H-1′), 0.87 (*s*, 3H, H-23), 0.85 (*s*, 3H, H-24), 0.81 (*s*, 3H, H-28), 0.79 (*m*, 1H, H-5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.9 (C-30), 170.8 (Cys-COO), 169.3 (C-13), 157.6 (Boc-COO), 137.7 (C_{ar}), 130.1 (C_{ar}), 128.9 (C_{ar}), 128.6 (C_{ar}), 128.5 (C-12), 128.4 (C_{ar}), 127.1 (C_{ar}), 82.4 (C-3), 79.7 (Boc-*quart.*–C), 61.6 (C-9), 55.0 (C-5), 53.5 (Cys-CH), 51.7 (OCH_3), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 36.8 (Bn-CH_2), 33.7 (Cys-CH_2), 32.6 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (3 x Boc-CH_3), 28.3 (C-29), 28.1 (C-23), 26.4 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 18.7 (C-26), 17.3 (C-6), 16.7 (C-24), 16.3 (C-25) ppm; MS (ESI): *m/z* (%) = 778.1 ($[M + H]^+$, 10), 795.3 ($[M + NH_4]^+$, 11), 800.4 ($[M + Na]^+$, 100), 816.3 ($[M + K]^+$, 20), 832.7 ($[M + Na + MeOH]^+$, 13), 1189.7 ($[3M+2Na]^{2+}$, 12), 1578.3 ($[2M + H + Na]^+$, 20); analysis for $C_{46}H_{67}NO_7S$ (778.1): C, 71.01; H, 8.68; N, 1.80; S, 4.12; found: C, 70.88; H, 8.81; N, 1.55; S, 4.00.

5.25. Methyl (3β)-3-{[(2S)-2-amino-2-(benzylthio)acetyl]oxy}-11-oxoolean-12-en-30-oate (**24**)

Following the procedure given for **22**, from **23** (190 mg, 0.24 mmol) and trifluoroacetic acid (1 ml, 12.98 mmol) **24** (140 mg, 86%) was obtained as a colorless powder; mp 128–131 °C; R_f = 0.66 (chloroform/methanol 9:1); $[\alpha]_D$ = +62.38° (*c* 0.43, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 249 nm (4.31); IR (KBr): ν = 3406*br*, 2929*s*, 1732*s*, 1660*s*, 1570*w*, 1454*m*, 1387*m*, 1324*w*, 1217*s*, 1155*m*, 1087*w*, 1028*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.36–7.20 (*m*, 5H, H–Ar), 5.66 (*s*, 1H, H-12), 4.58 (*dd*, 1H, H-3, *J* = 11.8, 4.7 Hz), 3.93 (*m*, 1H, Cys-CH), 3.82 (*s*, 2H, Bn-CH_2), 3.69 (*s*, 3H, OCH_3), 3.02 (*dd*, 1H, Cys-C*H*H′, *J* = 13.9, 4.4 Hz), 2.91 (*dd*, 1H, Cys-CH*H*′, *J* = 13.9, 7.1 Hz), 2.81 (*m*, 1H, H-1), 2.34 (*s*, 1H, H-9), 2.09 (*dd*, 1H, H-18, *J* = 13.5, 3.7 Hz), 2.03 (*m*, 1H, H-15), 1.99 (*m*, 1H, H-21), 1.93 (*m*, 1H, H-19), 1.82 (*ddd*, 1H, H-16, *J* = 13.5, 13.5, 3.9 Hz), 1.71 (*m*, 1H, H-2), 1.68 (*m*, 1H, H-7), 1.66 (*m*, 1H, H-6), 1.63 (*m*, 1H, H-2′), 1.61 (*dd*, 1H, H-19′, *J* = 13.5, 13.5 Hz), 1.56 (*m*, 1H, H-6′), 1.42 (*m*, 1H, H-7′), 1.38 (*m*, 1H, H-22), 1.36 (*s*, 3H, H-27), 1.31 (*m*, 1H, H-22′), 1.31 (*m*, 1H, H-21′), 1.18 (*m*, 1H, H-16′), 1.15 (*s*, 3H, H-25), 1.15 (*s*, 3H, H-29), 1.12 (*s*, 3H, H-26), 1.09 (*m*, 1H, H-15′), 1.02 (*m*, 1H, H-1′), 0.85 (*s*, 3H, H-23), 0.83 (*s*, 3H, H-24), 0.81 (*s*, 3H, H-28), 0.77 (*m*, 1H, H-5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.8 (C-11), 176.9 (C-30), 169.2 (Cys-COO), 169.2 (C-13), 137.6 (C_{ar}), 129.0 (C_{ar}), 129.0 (C_{ar}), 128.6 (C_{ar}), 128.5 (C-12), 128.5 (C_{ar}), 127.2 (C_{ar}), 83.1 (C-3), 61.6 (C-9), 55.0 (C-5), 51.7 (OCH_3), 49.2 (Cys-CH), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.6 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 36.7 (Bn-CH_2), 33.9 (Cys-CH_2), 32.6 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.2 (C-23), 26.4 (C-16), 26.4 (C-15), 23.5 (C-2), 23.4 (C-27), 18.7 (C-26), 17.3 (C-6), 16.8 (C-24), 16.3 (C-25) ppm; MS (ESI): *m/z* (%) = 678.3 ($[M + H]^+$, 100), 1017.2 ($[3M+2H]^{2+}$, 6), 1355.6 ($[2M + H]^+$, 2); analysis for $C_{41}H_{59}NO_5S$ (678.0): C, 72.63; H, 8.77; N, 2.07; S, 4.73; found: C, 72.46; H, 8.88; N, 1.97; S, 4.62.

5.26. Ethyl 3,11-dioxo-olean-12-en-30-oate (**25**)

Compound **25** was prepared according to [9,22].

5.27. Ethyl (7aR,7bS,9aS,12S,13aR,15bS)-5,5,7a,7b,9a,12,15b-heptamethyl-3,15-dioxo9a,10,11,12,13,13a,15,15a,15b-icosahydrochryseno[2,1-c]oxepine-12-carboxylate (**26**)

To a solution of **25** (2.56 g, 5.20 mmol) in chloroform (25 ml), 4-chloroperbenzoic acid (2.63 g, 15.40 mmol) was added. The mixture

was stirred at 55 °C for 24 h, followed by the addition of saturated solution of $NaHCO_3$ (30 ml). The aqueous layer was extracted with DCM (3 × 30 ml), the combined extracts were dried (Na_2SO_4), filtered and evaporated. The residue was purified by chromatography (silica gel, chloroform/ether 9:1) to yield **26** (2.15 g, 81%) as a colorless powder; mp 181–185 °C (lit. 166–171 °C [30]); $R_f = 0.42$ (hexane/ethyl acetate 7:3); $[\alpha]_D = +159.73°$ (*c* 0.23, $CHCl_3$) (lit. $[\alpha]_D = +189°$ (*c* 0.1, $CHCl_3$) [30]); UV–vis (methanol): λ_{max} (log ε) = 250 nm (4.07); IR (KBr): ν = 3432*br*, 2964*s*, 2934*s*, 2866*m*, 1728*s*, 1651*s*, 1618*w*, 1460*m*, 1386*m*, 1329*m*, 1314*m*, 1281*m*, 1251*m*, 1219*m*, 1172*s*, 1152*s*, 1115*s*, 1087*m*, 1017*m*, 980*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.66 (*s*, 1H, H-12), 4.17 (*dq*, 1H, Et-C*H*H′, *J* = 10.8, 7.1 Hz), 4.10 (*dq*, 1H, Et-CH*H*′, *J* = 10.8, 7.2 Hz), 2.69 (*m*, 1H, H-1), 2.59 (*m*, 1H, H-2), 2.53 (*s*, 1H, H-9), 2.11 (*dd*, 1H, H-18, *J* = 13.3, 3.3 Hz), 2.01 (*ddd*, 1H, H-15, *J* = 13.7, 13.7, 4.6 Hz), 1.97 (*m*, 1H, H-21), 1.90 (*ddd*, 1H, H-19, *J* = 13.7, 4.2, 2.5 Hz), 1.81 (*ddd*, 1H, H-16, *J* = 13.7, 13.7, 4.6 Hz), 1.69 (*m*, 1H, H-7), 1.63 (*m*, 1H, H-6), 1.58 (*dd*, 1H, H-19′, *J* = 13.7, 13.7 Hz), 1.57 (*m*, 1H, H-5), 1.53 (*m*, 1H, H-6′), 1.51 (*m*, 1H, H-1′), 1.46 (*s*, 3H, H-23), 1.44 (*m*, 1H, H-2′), 1.43 (*s*, 3H, H-24), 1.41 (*m*, 1H, H-7′), 1.38 (*m*, 1H, H-22), 1.36 (*s*, 6H, H-25 and H-27), 1.32 (*m*, 1H, H-22′), 1.29 (*m*, 1H, H-21′), 1.24 (*t*, 3H, Et-CH_3, *J* = 7.1 Hz), 1.18 (*m*, 1H, H-16′), 1.14 (*s*, 3H, H-26), 1.12 (*s*, 3H, H-29), 1.01 (*m*, 1H, H-15′), 0.79 (*s*, 3H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 198.8 (C11), 176.3 (C30), 175.5 (C3), 169.4 (C13), 128.6 (C12), 85.6 (C4), 61.3 (C9), 60.3 (Et-CH_2), 54.5 (C5), 48.3 (C18), 45.3 (C8), 43.8 (C20), 43.4 (C14), 41.1 (C19), 39.6 (C10), 38.7 (C1), 37.7 (C22), 32.3 (C2), 32.2 (C23), 32.0 (C7), 31.8 (C17), 31.1 (C22), 28.6 (C28), 28.3 (C29), 26.4 (C16), 26.4 (C15), 25.9 (C24), 23.1 (C27), 22.1 (C6), 18.2 (C26), 17.5 (C25), 14.3 (Et-CH_3) ppm; MS (ESI): *m*/*z* (%) = 513.4 ($[M + H]^+$, 100), 535.7 ($[M + Na]^+$, 7), 567.0 ($[M + MeOH + Na]^+$, 46).

5.28. 3-[(1S,4aR,4bS,6aS,9S,10aR)-9-(Ethoxycarbonyl)-2-(1-hydroxy-1-methylethyl)-1,4a,4b,6a,9-pentamethyl-12-oxo-1,2,3,4,4a,4b,5,6,6a,7,8,9,10,10a,12,12a-hexadecahydrochrysen-1-yl]propanoic acid (**27**)

Compound **26** (2.15 g, 4.19 mmol) was dissolved in ethanol (20 ml), potassium hydroxide (1.14 g, 20.32 mmol in 5 ml water) was added. The mixture was stirred at room temperature for 24 h, the pH was adjusted to 7 by adding diluted hydrochloric acid, and the solvents were removed under diminished pressure. Water (20 ml) and ethyl acetate (20 ml) were added, the aqueous layer was extracted with ethyl acetate (3 × 20 ml), the combined organic layers were dried (Na_2SO_4), filtered and evaporated to yield **27** (453 mg, 20%) as colorless crystals; mp 115–119 °C; R_f = 0.16 (hexane/ethyl acetate 7:3); $[\alpha]_D = +99.01°$ (*c* 0.79, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 249 nm (4.01); IR (KBr): ν = 3432*br*, 2976*s*, 1727*s*, 1660*s*, 1560*w*, 1457*m*, 1385*s*, 1313*m*, 1247*w*, 1216*s*, 1175*s*, 1086*m*, 1030*w* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 5.36 (*s*, 1H, H-12), 4.11 (*dq*, 1H, Et-C*H*H′, *J* = 10.8, 7.1 Hz), 4.03 (*m*, 1H, Et-CH*H*′), 2.69 (*s*, 1H, H-9), 2.32 (*m*, 2H, H-2), 2.22 (*m*, 1H, H-1), 2.04 (*ddd*, 1H, H-15, *J* = 13.7, 13.7, 4.2 Hz), 1.95 (*dd*, 1H, H-18, *J* = 12.0, 5.4 Hz), 1.87 (*m*, 1H, H-1′), 1.79 (*m*, 1H, H-21), 1.72 (*m*, 1H, H-19), 1.70 (*m*, 1H, H-19′), 1.67 (*m*, 1H, H- 16), 1.55 (*m*, 1H, H-7), 1.37 (*m*, 1H, H-21′), 1.35 (*m*, 2H, H-6 and H-6′), 1.33 (*s*, 3H, H-27), 1.31 (*m*, 1H, H-22), 1.72 (*m*, 1H, H-7′), 1.26 (*s*, 3H, H-25), 1.25 (*m*, 1H, H-5), 1.20 (*m*, 1H, H-22′), 1.16 (*t*, 3H, Et-CH_3, *J* = 7.1 Hz), 1.13 (*m*, 1H, H-16′), 1.12 (*s*, 3H, H-23), 1.08 (*s*, 3H, H-29), 1.06 (*s*, 3H, H-24), 1.01 (*s*, 3H, H-26), 0.92 (*m*, 1H, H-15′), 0.71 (*s*, 3H, H-28) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 198.8 (C11), 176.3 (C30), 175.6 (C3), 168.1 (C13), 127.8 (C12), 73.6 (C4), 59.8 (Et-CH_2), 52.5 (C9), 49.9 (C5), 48.0 (C18), 44.8 (C8), 43.4 (C20), 43.3 (C14), 40.8 (C10), 40.4 (C19), 37.3 (C22), 34.8 (C2), 33.0 (C23), 31.6 (C17), 31.6 (C7), 31.3 (C1), 30.3 (C21), 28.3 (C28), 27.8 (C29), 27.6 (C24), 26.0 (C16), 25.7 (C15), 22.5 (C27), 21.2 (C6), 19.3 (C25), 18.1 (C26), 14.1 (Et-CH_3) ppm; MS (ESI): *m*/*z* (%) = 531.3 ($[M + H]^+$, 100), 553.5 ($[M + Na]^+$, 73); analysis for $C_{32}H_{50}O_6$ (530.7): C, 72.42; H, 9.50; found: C, 72.35; H, 9.71.

5.29. 3-[(1S,4aR,4bS,6aS,9S,10aR)-9-(Ethoxycarbonyl)-2-isopropenyl-1,4a,4b,6a,9-pentamethyl-12-oxo-1,2,3,4,4a,4b,5,6,6a,7,8,9,10,10a,12,12a-hexadecahydrochrysen-1-yl]propanoic acid (**28**)

The pH of a solution of **27** (357 mg, 0.67 mmol) in ethanol (20 ml) was adjusted to 2 by adding diluted hydrochloric. The mixture was stirred at room temperature for 5 min, followed by extraction with DCM (3 × 15 ml); the organic layer was dried (Na_2SO_4), filtered and evaporated to afford **28** (331 mg, 96%) as a colorless powder; mp 256–260 °C (decomp.); R_f = 0.38 (hexane/ethyl acetate 7:3); $[\alpha]_D = +99.91°$ (*c* 0.41, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 249 nm (3.99); IR (KBr): ν = 3432*br*, 2976*s*, 1727*s*, 1657*s*, 1561*s*, 1455*m*, 1386*s*, 1314*m*, 1218*m*, 1175*w*, 1087*w*, 1032*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.60 (*s*, 1H, H-12), 4.84 (*s*, 1H, H-23), 4.68 (*s*, 1H, H-23′), 4.18 (*dq*, 1H, Et-C*H*H′, *J* = 10.8, 7.1 Hz), 4.11 (*dq*, 1H, Et-CH*H*′, *J* = 10.8, 7.1 Hz), 2.72 (*s*, 1H, H-9), 2.44 (*m*, 1H, H-1), 2.15 (*m*, 1H, H-2), 2.10 (*m*, 1H, H-18), 2.01 (*ddd*, 1H, H-15, *J* = 13.7, 13.7, 3.7 Hz), 1.97 (*m*, 1H, H-2), 1.94 (*m*, 1H, H-21), 1.92 (*m*, 1H, H-5), 1.88 (*m*, 1H, H-19), 1.79 (*m*, 1H, H-6), 1.76 (*m*, 1H, H-16), 1.73 (*s*, 3H, H-24), 1.67 (*m*, 1H, H-1′), 1.62 (*m*, 1H, H-7), 1.58 (*dd*, 1H, H-19′, *J* = 13.7, 13.7 Hz), 1.39 (*m*, 1H, H-6′), 1.37 (*m*, 1H, H-22), 1.34 (*m*, 1H, 7′), 1.29 (*m*, 2H, H-22′ and H-21′), 1.37 (*s*, 3H, H-27), 1.25 (*t*, 3H, Et-CH_3, *J* = 7.1 Hz), 1.18 (*m*, 1H, H-16′), 1.13 (*s*, 3H, H-26), 1.12 (*s*, 3H, H-25), 1.08 (*s*, 3H, H-29), 0.98 (*m*, 1H, H-15′), 0.78 (*s*, 3H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.8 (C11), 181.5 (C3), 176.5 (C30), 169.8 (C13), 147.0 (C4), 128.6 (C12), 113.9 (C23), 60.4 (Et-CH_2), 52.8 (C9), 50.6 (C5), 48.2 (C18), 45.1 (C8), 43.9 (C20), 43.7 (C14), 41.0 (C19), 39.0 (C10), 37.8 (C22), 36.2 (C1), 31.8 (C17), 31.8 (C2), 31.4 (C7), 31.2 (C21), 28.6 (C28), 28.3 (C29), 26.6 (C16), 26.5 (C15), 24.0 (C6), 23.8 (C24), 23.3 (C27), 19.9 (C25), 18.8 (C26), 14.4 (Et-CH_3) ppm; MS (ESI): *m*/*z* (%) = 511.5 ($[M - H]^-$, 100), 557.2 ($[M + HCO_2]^-$, 40); analysis for $C_{32}H_{48}O_5$ (512.7): C, 74.96; H, 9.44; found: C, 74.77; H, 9.51.

5.30. Ethyl (3S,4aR,7S,10aR,10bS,12aS)-7-(3-ethoxy-3-oxopropyl)-8-isopropenyl-3,7,10a,10b,12a-pentamethyl-6-oxo-1,2,3,4,4a,6,6a,7,8,9,10,10a,10b,11,12,12a-hexadecahydrochrysene-3-carboxylate (**29**)

Compound **27** (543 mg, 1.02 mmol) was dissolved in ethanol (50 ml), hydrochloric acid (conc., 2 ml) was added. After refluxing for 30 min, the solvent was removed under reduced pressure, and the residue was subjected to chromatography (silica gel, hexane/ethyl acetate 8:2) to afford **29** (526 mg, 95%) as a colorless powder; mp 101–104 °C; R_f = 0.79 (hexane/ethyl acetate 7:3); $[\alpha]_D = +125.70°$ (*c* 0.44, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 250 nm (4.14); IR (KBr): ν = 3424*br*, 2977*s*, 2942*s*, 1724*s*, 1645*s*, 1611*m*, 1460*m*, 1386*m*, 1364*w*, 1330*m*, 1310*w*, 1278*m*, 1260*m*, 1213*m*, 1171*s*, 1115*w*, 1089*w*, 1064*w*, 1024*w* cm^{-1}; ^1H NMR (500 MHz, CDCl3): δ = 5.64 (*s*, 1H, H-12), 4.87 (*dd*, 1H, H-23, *J* = 1.3, 1.3 Hz), 4.67 (*d*, 1H, H-23′, *J* = 1.2 Hz), 4.17 (*dq*, 1H, C^{30}-C*H*H′, *J* = 10.8, 7.1 Hz), 4.10 (dq, 1H, C^{30}-CH*H*′, *J* = 10.8, 7.1 Hz), 4.05 (*q*, 2H, C^3–CH_2, *J* = 7.1 Hz), 2.59 (*ddd*, 1H, H-1, *J* = 14.1, 12.0, 6.2 Hz), 2.58 (*s*, 1H, H-9), 2.27 (*ddd*, 1H, H-2, *J* = 14.1, 12.0, 4.2 Hz), 2.09 (*m*, 1H, H-18), 2.03 (*m*,1H, H-2′), 1.99 (*m*, 1H, H-15), 1.97 (*m*, 1H, H-21), 1.94 (*dd*, 1H, H-5, *J* = 12.0, 2.5 Hz), 1.91 (*m*, 1H, H-19), 1.83 (*m*, 1H, H-6), 1.80 (*m*, 1H, H-16), 1.74 (*s*, 3H, H-24), 1.72 (*m*, 1H, H-1′), 1.68 (*m*, 1H, H-7), 1.59 (*dd*, 1H, H-19′, *J* = 13.3, 13.3 Hz), 1.42 (*m*, 1H, H-6′), 1.37 (*m*, 1H, H-22), 1.35 (*m*, 1H, H-7′), 1.32 (*m*, 1H, H-22′), 1.28 (*m*, 1H, H-21′), 1.37 (*s*, 3H, H-27), 1.25 (*t*, 3H, C^{30}–CH_3, *J* = 7.1 Hz), 1.21 (*m*, 1H, H-16′), 1.20 (*t*, 3H, C^3–CH_3, *J* = 7.1 Hz), 1.15 (*s*, 3H, H-26), 1.15 (*s*, 3H, H-25), 1.12 (*s*, 3H, H-29), 1.00 (*m*, 1H, H-15′), 0.79 (*s*, 3H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.5 (C11), 176.3 (C30), 173.8 (C3), 169.4 (C13), 146.6

(C4), 128.4 (C12), 114.1 (C23), 60.3 (C^{30}–CH_2), 60.2 (C^3–CH_2), 52.8 (C9), 50.8 (C5), 48.3 (C18), 45.1 (C8), 43.8 (C20), 43.7 (C14), 41.2 (C19), 38.8 (C10), 37.7 (C22), 34.4 (C1), 31.8 (C17), 31.4 (C7), 31.1 (C21), 29.4 (C2), 28.6 (C28), 28.3 (C29), 26.5 (C16), 26.4 (C15), 23.8 (C6), 23.5 (C24), 23.3 (C27), 19.5 (C25), 18.6 (C26), 14.3 (C^{30}–CH_3), 14.2 (C^3–CH_3) ppm; MS (ESI): *m/z* (%) = 541.5 ($[M + H]^+$, 100), 558.3 ($[M + NH_4]^+$, 13), 563.6 ($[M + Na]^+$, 16); analysis for $C_{34}H_{52}O_5$ (540.8): C, 75.51; H, 9.69; found: C, 75.41; H, 9.82.

5.31. Ethyl 11-oxo-olean-2,12-dien-30-oate (**30**)

A mixture of **9** (1.31 g, 2.6 mmol), triphenyl phosphane (2.78 g, 10.6 mmol) and 3,3-dimethyl glutarimide (1.49 g, 10.6 mmol) in dry THF (25 ml) was cooled to 0 °C. Under continuous stirring, DEAD (1.65 ml, 10.4 mmol) was added dropwise, and stirring was continued at 25 °C for 24 h. After concentration to dryness, the residue was subjected to chromatography (silica gel, chloroform/ether 9:1) to yield **30** (1.02 g, 82%) as colorless crystals; mp 138–142 °C; R_f = 0.87 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = 216.97° (*c* 0.33, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 249 nm (4.02); IR (KBr): ν = 3422*br*, 2960*s*, 2872*s*, 1723*s*, 1648*s*, 1612*w*, 1458*m*, 1386*m*, 1360*w*, 1348*w*, 1328*w*, 1310*w*, 1277*w*, 1256*m*, 1210*m*, 1169*s*, 1134*m*, 1088*w*, 1062*w*, 1031*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.67 (*s*, 1H, 12-H), 5.43 (*ddd*, 1H, H-2, *J* = 10.1, 6.1, 1.7 Hz), 5.37 (*dd*, 1H, H-3, *J* = 10.1, 2.3 Hz), 4.19 (*dq*, 1H, Et-C*H*H′, *J* = 10.8, 7.2 Hz), 4.12 (*dq*, 1H, Et-CH*H*′, *J* = 10.8, 7.2 Hz), 3.04 (*dd*, 1H, H-1, *J* = 17.5, 6.0 Hz), 2.41 (*s*, 1H, H-9), 2.11 (*dd*, 1H, H-18, *J* = 12.8, 4.3 Hz), 2.03 (*ddd*, 1H, H-15, *J* = 13.4, 13.4, 4.7 Hz), 1.99 (*m*, 1H, H-21), 1.92 (*ddd*, 1H, H-19, *J* = 13.9, 4.1, 2.9 Hz), 1.83 (*ddd*, 1H, H-16, *J* = 13.6, 13.6, 4.3 Hz), 1.70 (*m*, 1H, H-7), 1.65 (*m*, 1H, H-1′), 1.61 (*dd*, 1H, H-19′, *J* = 13.5, 13.5 Hz), 1.56 (*m*, 1H, H-6), 1.48 (*ddd*, 1H, H-6′, *J* = 12.5, 12.5, 3.2 Hz), 1.43 (*m*, 1H, H-7′), 1.39 (*m*, 1H, H-22), 1.33 (*m*, 1H, H-21′), 1.30 (*m*, 1H, H-22′), 1.36 (*s*, 3H, H-27), 1.26 (*t*, 3H, Me, *J* = 7.2 Hz), 1.21 (*ddd*, 1H, H-16′, *J* = 13.9, 4.4, 2.4 Hz), 1.16 (*s*, 3H, H-25), 1.16 (*s*, 3H, H-26), 1.14 (*s*, 3H, H-29), 1.12 (*m*, 1H, H-5), 1.02 (*m*, 1H, H-15′), 0.96 (*s*, 3H, H-23), 0.91 (*s*, 3H, H-24), 0.82 (s, 3H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.1 (C11), 176.4 (C30), 169.4 (C13), 137.0 (C3), 128.6 (C12), 121.9 (C2), 60.5 (C9), 60.3 (Et-CH_2), 51.8 (C5), 48.4 (C18), 45.3 (C14), 43.8 (C20), 43.3 (C8), 41.5 (C1), 41.2 (C19), 37.7 (C22), 36.2 (C4), 34.3 (C10), 31.9 (C7), 31.9 (C23), 31.8 (C17), 31.1 (C21), 28.6 (C28), 28.3 (C29), 26.5 (C16), 26.5 (C15), 23.3 (C27), 23.0 (C24), 18.7 (C6), 18.3 (C26), 16.1 (C25), 14.3 (Me) ppm; MS (ESI): *m/z* (%) = 481.5 ($[M + H]^+$, 100), 503.3 ($[M + Na]^+$, 7), 534.9 ($[M + MeOH + Na]^+$, 50), 961.3 ($[2M + H]^+$, 66), 983.4 ($[2M + Na]^+$, 54), 999.2 ($[2M + K]^+$, 4); analysis for $C_{32}H_{48}O_3$ (480.72): C, 79.95; H, 10.06; found: C, 79.68; H, 10.18.

5.32. Ethyl (2α,3α)-2,3-epoxy-11-oxo-olean-12-en-31-oate (**31**)

Compound **30** (1.01 g, 2.1 mmol) was dissolved in dry dichloromethane (20 ml), *m*-CPBA (1.14 g, 4.68 mmol) was added, and the mixture was stirred at room temperature for 20 h. An aq. solution of potassium hydrogensulfate (satd., 10 ml) was added, the aqueous layer extracted with dichloromethane (3 × 15 ml), and the combined organic layers were dried (Na_2SO_4), filtrated and evaporated to dryness. Chromatographic purification (silica gel, chloroform/ether 9:1) afforded **31** (806 mg, 77%) as a colorless powder; mp 191–193 °C; R_f = 0.71 (hexane/ethyl acetate 7:3); $[\alpha]_D$ = 143.65° (*c* 0.48, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 251 nm (4.09); IR (KBr): ν = 3416*br*, 2978*s*, 2955*s*, 1736*s*, 1718*s*, 1645*s*, 1614*w*, 1458*m*, 1385*m*, 1314*m*, 1301*m*, 1285*m*, 1260*m*, 1222*s*, 1163*s*, 1113*m*, 1091*m*, 1039*m*, 1014*w*; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.63 (*s*, 1H, H-12), 4.16 (*dq*, 1H, Et-C*H*H′, *J* = 10.8, 7.1 Hz), 4.10 (*dq*, 1H, Et-CH*H*′, *J* = 10.8, 7.1 Hz), 3.19 (*dd*, 1H, H-2, *J* = 6.6, 3.7 Hz), 3.13 (*dd*, 1H, H-1, *J* = 14.9, 6.6 Hz), 2.79 (*d*, 1H, H-3, *J* = 3.7 Hz), 2.29 (*s*, 1H, H-9), 2.09 (*dd*, 1H, H-18, *J* = 13.3, 4.2 Hz), 1.99 (*ddd*, 1H, H-15, *J* = 13.3, 13.3, 4.6 Hz), 1.96 (*m*, 1H, H-21), 1.90 (*ddd*, 1H, H-19, *J* = 13.7, 4.2, 2.9 Hz), 1.78 (*ddd*, 1H, H-16, *J* = 13.7, 13.7, 5.0 Hz), 1.61 (*m*, 1H, H-7), 1.57 (*dd*, 1H, H-19′, *J* = 13.7, 13.7 Hz), 1.48 (*m*, 1H, H-6), 1.39 (*m*, 1H, H-21), 1.37 (*m*, 1H, H-6′), 1.35 (*m*, 1H, H-22), 1.33 (*m*, 1H, H-1′), 1.30 (*s*, 3H, H-27), 1.29 (*m*, 1H, H-22′), 1.27 (*m*, 1H, H-21′), 1.24 (*t*, 1H, *J* = 7.1 Hz, Me), 1.17 (*m*, 1H, H-16′), 1.13 (*s*, 3H, H-26), 1.11 (*s*, 3H, H-28), 1.09 (*s*, 3H, H-23), 1.07 (*s*, 3H, H-25), 1.02 (*s*, 3H, H-24), 0.93 (*m*, 1H, H-15′), 0.92 (*dd*, 1H, H-5, *J* = 11.6, 2.9 Hz), 0.78 (*s*, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.7 (C11), 176.3 (C30), 169.7 (C13), 128.5 (C12), 61.3 (C3), 60.4 (C9), 60.3 (Et-CH_2), 52.6 (C2), 48.4 (C18), 46.6 (C5), 45.1 (C8), 43.8 (C20), 43.3 (C14), 41.1 (C19), 40.6 (C1), 37.7 (C22), 35.9 (C4), 32.6 (C10), 31.9 (C7), 31.8 (C17), 31.1 (C21), 28.6 (C28), 28.3 (C29), 28.2 (C23), 26.4 (C16), 26.4 (C15), 23.2 (C27), 22.0 (C24), 18.3 (C26), 17.9 (C6), 17.9 (C25), 14.3 (Me); MS (ESI): *m/z* (%) = 497.6 ($[M + H]^+$, 92), 519.4 ($[M + Na]^+$, 10), 551.0 ($[M + MeOH + Na]^+$, 62), 767.4 ($3M+2Na]^{2+}$, 6), 993.3 ($[2M + H]^+$, 94), 1015.4 ($[2M + Na]^+$, 100), 1031.3 ($[2M + K]^+$, 12); analysis for $C_{32}H_{48}O_4$ (496.72): C, 77.38; H, 9.74; found: C, 77.26; H, 9.92.

5.33. Ethyl (2β,3α) 2-fluoro-3-hydroxy-11-oxo-olean-12-en-30-oate (**32**)

Compound **31** (619 mg, 1.25 mmol) was dissolved in dry DCM (10 ml) and Olah's-reagent [26] (1 ml, 4.35 mmol) was slowly added. The mixture was stirred at room temperature for 5 h, and crushed ice was added. The aqueous layer was extracted with DCM (3 × 20 ml), the extracts were dried (Na_2SO_4), filtered and evaporated. The residue was subjected to chromatography (silica gel, chloroform/ether 9:1) to afford **32** (120 mg, 19%) as a colorless powder; mp 152–155 °C; R_f = 0.48 (hexane/ethyl acetate = 7:3); $[\alpha]_D$ = +113.77° (*c* 0.64, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 249 nm (3.98); IR (KBr): ν = 3448*br*, 2959*s*, 2873*s*, 1727*s*, 1659*s*, 1456*m*, 1389*s*, 1365*m*, 1314*m*, 1280*m*, 1246*m*, 1218*s*, 1174*s*, 1121*m*, 1089*m*, 1048*m*, 1021*s* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.67 (*s*, 1H, H-12), 4.64 (*ddd*, 1H, H-2, *J* = 49.7, 6.4, 5.6 Hz), 4.18 (*dq*, 1H, Et-C*H*H′, *J* = 10.8, 7.1 Hz), 4.12 (*dq*, 1H, Et-CH*H*′, *J* = 10.8, 7.1 Hz), 3.73 (*dd*, 1H, H-3, *J* = 8.6, 6.4 Hz), 2.91 (*ddd*, 1H, H-1, *J* = 15.4, 15.4, 5.5 Hz), 2.43 (*s*, 1H, H-9), 2.11 (*ddd*, 1H, H-18, *J* = 13.5, 4.2, 1.2 Hz), 2.02 (*ddd*, 1H, H-15, *J* = 13.6, 13.6, 4.6 Hz), 1.98 (*m*, 1H, H-21), 1.91 (*ddd*, 1H, H-19, *J* = 13.6, 4.3, 2.8 Hz), 1.82 (*ddd*, 1H, H-16, *J* = 13.6, 13.6, 4.3 Hz), 1.68 (*ddd*, 1H, H-1′, *J* = 15.4, 15.4, 5.7 Hz), 1.64 (*m*, 1H, H-7), 1.59 (*dd*, 1H, H-19′, *J* = 13.5, 13.5 Hz), 1.53 (*m*, 1H, H-6), 1.50 (*m*, 1H, H-6′), 1.40 (*m*, 1H, H-7′), 1.38 (*m*, 1H, H-22), 1.35 (*s*, 3H, H-27), 1.33 (*m*, 2H, H-22′ and H-21′), 1.26 (*t*, 3H, Et-CH_3, *J* = 7.1 Hz), 1.20 (*m*, 1H, H-16′), 1.15 (*m*, 1H, H-5), 1.13 (*s*, 3H, H-29), 1.13 (*s*, 3H, H-25), 1.11 (*s*, 3H, H-26), 1.04 (*s*, 3H, H-24), 1.03 (*m*, 1H, H-15′), 0.98 (*s*, 3H, H-23), 0.80 (*s*, 3H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.5 (C11), 176.4 (C30), 169.9 (C13), 128.3 (C12), 92.8 (*d*, C2, *J* = 172 Hz), 75.2 (*d*, C3, *J* = 21 Hz), 62.5 (C9), 60.3 (Et-CH_2), 49.1 (C5), 48.3 (C18), 45.3 (C8), 43.8 (C20), 42.5 (*d*, C1, *J* = 18 Hz), 42.2 (C14), 41.2 (C19), 37.7 (C22), 36.9 (C4), 36.8 (C10), 32.1 (C7), 31.8 (C17), 31.1 (C21), 28.6 (C28), 28.3 (C29), 26.6 (C23), 26.4 (C16), 26.4 (C15), 23.5 (C27), 22.1 (C24), 19.5 (C26), 18.4 (C25), 18.0 (C6), 14.3 (Et-CH_3) ppm; ^{19}F NMR (190 MHz, $CDCl_3$): δ = −180.9 (*m*, 1F, F-2) ppm; MS (ESI): *m/z* (%) = 517.5 ($[M + H]^+$, 100), 539.5 ($[M + Na]^+$, 52), 571.1 ($[M + MeOH + Na]^+$, 22); analysis for $C_{32}H_{49}FO_4$ (516.7): C, 74.38; H, 9.56; found: C, 74.21; H, 9.74.

5.34. Benzyl (3α) 3-hydroxy-11-oxo-olean-12-en-30-oate (**33**)

Compound **33** was prepared according to [7,11].

*5.35. Benzyl 3β-3-([N-(3-aminopropyl)glycyl]oxy)-11-oxo-olean-12-en-30-oate (**34**)*

To a solution of **33** (290 mg, 0.51 mmol) in dry DCM (15 ml), chloroacetyl chloride (50 μl, 0.61 mmol) was added. After stirring at 25 °C for 24 h and usual aqueous work-up, the crude product was dissolved in dry DMF (10 ml), finely grounded K_2CO_3 (700 mg, 5.07 mmol) and 1,3-diaminopropane (0.5 ml, 5.95 mmol) were added, and the mixture was stirred at 25 °C for 2 h. The solvent was removed under reduced pressure, and water (30 ml) was added. After the extraction with DCM (3 × 20 ml), the combined organic layers were washed with water (20 ml) and brine (20 ml), dried (Na_2SO_4), filtered, and the solvent was evaporated. Purification by chromatography (silica gel, load with methanol, unload with methanol/diethylamine 9:1) gave **34** (270 mg, 82%) as a slight yellowish powder; mp 154–157 °C; R_f = 0.02 (chloroform/methanol 9:1); $[\alpha]_D = +100.62°$ (*c* 0.49, $CHCl_3$); UV–vis (methanol): λ_{max} (log ε) = 249 nm (3.99); IR (KBr): ν = 3433*br*, 2945*s*, 1728*s*, 1660*s*, 1464*m*, 1387*m*, 1306*w*, 1279*m*, 1248*m*, 1213*s*, 1174*m*, 1145*s*, 1083*m*, 1023*w*, 985*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.38–7.29 (*m*, 5H, H–Ar), 5.52 (*s*, 1H, H-12), 5.18 (*d*, 1H, Bn-C*H*H′, *J* = 12.3 Hz), 5.07 (*d*, 1H, Bn-CH*H*′, *J* = 12.3 Hz), 4.55 (*m*, 1H, H-3), 3.37 (*s*, 2H, CH_2COO), 2.78 (*m*, 1H, H-1), 2.77 (*t*, 2H, chain-3, *J* = 6.8 Hz), 2.67 (*t*, 2H, chain-1, *J* = 6.8 Hz), 2.32 (*s*, 1H, H-9), 2.02 (*m*, 1H, H-18), 1.99 (*m*, 1H, H-15), 1.97 (*m*, 1H, H-21), 1.92 (*m*, 1H, H-19), 1.78 (*m*, 1H, H-16), 1.73 (*m*, 1H, H-2), 1.67 (*m*, 1H, H-7), 1.63 (*m*, 2H, chain-2), 1.62 (*m*, 1H, H-2′), 1.61 (*dd*, 1H, H-19′, *J* = 13.9, 13.9 Hz), 1.55 (*m*, 1H, H-6), 1.46 (*m*, 1H, H-6′), 1.40 (*m*, 1H, H-7′), 1.36 (*m*, 1H, H-22), 1.33 (*s*, 3H, H-27), 1.29 (*m*, 1H, H-22′), 1.29 (*m*, 1H, H-21′), 1.16 (*m*, 1H, H-16′), 1.14 (*s*, 6H, H-25 & H-29), 1.09 (*s*, 3H, H-26), 1.03 (*m*, 1H, H-1′), 0.97 (*m*, 1H, H-15′), 0.87 (*s*, 3H, H-24), 0.86 (*s*, 3H, H-23), 0.79 (*m*, 1H, H-5), 0.71 (*s*, 3H, H-28) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.9 (C-11), 176.2 (C-30), 172.4 (CH_2COO), 169.0 (C-13), 136.1 (C_{ar}), 128.5 (C_{ar}), 128.5 (C_{ar}), 128.4 (C-12), 128.3 (C_{ar}), 128.2 (C_{ar}), 127.8 (C_{ar}), 81.2 (C-3), 66.2 (Bn-CH_2), 61.6 (C-9), 55.0 (C-5), 51.2 (CH_2COO), 48.2 (C-18), 47.4 (chain-1), 45.3 (C-8), 44.0 (C-20), 43.2 (C-14), 41.0 (C-19), 40.3 (chain-3), 38.7 (C-1), 38.1 (C-4), 37.6 (C-22), 36.9 (C-10), 33.4 (chain-2), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.4 (C-28), 28.2 (C-29), 28.1 (C-23), 26.4 (C-16), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27),18.6 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25) ppm; MS (ESI): *m*/*z* (%) = 675.5 ($[M + H]^+$, 4), 701.5 ($[M + Na]^+$, 100); analysis for $C_{42}H_{62}N_2O_5$ (675.0): C, 74.74; H, 9.26; N, 4.15; found: C, 74.57; H, 9.41; N, 4.00.

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