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Influence of esterification and modification of A-ring in a group of lupane acids on their cytotoxicity

Milan Urban,[a] Jan Sarek,[a,*] Iva Tislerova,[a] Petr Dzubak[b] and Marian Hajduch[b]

[a]*Department of Organic and Nuclear Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 43 Prague 2, Czech Republic*
[b]*Laboratory of Experimental Medicine, Departments of Pediatrics and Oncology, Faculty of Medicine, Palacky University in Olomouc, Puskinova 6, 775 20 Olomouc, Czech Republic*

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Abstract—The aim of this work was to find an optimal ester group for preparation of lupane derivatives connecting high cytotoxicity with good chemical and pharmacological properties. Activities of methyl-, pivaloyloxymethyl- (Pom-), and acetoxymethyl- (Acm-) esters were compared with the activity of free acids. Although the methyl- and Pom-esters were generally less active than free acids, some Acm-esters had cytotoxicity similar to or even better than the starting compounds. Cytotoxic activity was measured in five cancer cell lines.

1. Introduction

Betulinic acid (**1a**) is a pentacyclic triterpene, which occurs, for example, in the bark of *Platanus hispanica*. Betulinic acid (**1a**) is a compound with anti-HIV[1] activity, cytotoxicity,[2] and anti-tumor[3] properties. The cytotoxic activity on human melanoma (MEL-2)[4] and lung carcinoma[5] (A549) cell lines was the first to be reported and subsequent research showed that this activity is not limited to those cell lines, since similar results were obtained on malignant cells of different histogenetic origin, though with a certain selectivity against neuroectodermal tumors. In more recent studies,[6–10] the cytotoxic activity of certain betulinic acid derivatives has been investigated. Their activity was usually associated with a free carboxylic or a carbonylic group in position C-28.[8] In contrast, all alkyl esters were found to be inactive.[6,7] Moreover, hydrogenation of the 20(29) double bond caused a significant increase in the cytotoxicity to HeLa and OVCAR-3 cell lines.[7] Analogous oxidation of the 3β-hydroxy group of **1a** afforded derivatives with higher activity and substitution with an amino group gave derivatives with properties similar to **1a**. In contrast, acylation of the 3β-hydroxy group caused a decrease in cytotoxicity.

Although the alkyl esters of betulinic acid (**1a**) were found to be inactive, they dispose of better chemical and pharmacological properties. Esters are usually more soluble than free acids in all organic solvents, which is a significant advantage for both the chemical preparation and purification. The main aim of this work was to find an ester group that has optimal properties and does not cause a decrease in cytotoxic activity. This can be performed using esters, which are easily cleavable by non-specific intracellular esterases. Moreover, our study was performed using Pom- and Acm-esteric groups, which were previously shown to better penetrate cellular membranes and to release free compounds after intracellular enzymatic deprotection, as demonstrated on penicillins.[11,12] Methyl ester and free acids were used as standards for comparison of the biological activity.

2. Chemistry

Betulinic acid **1a** was extracted from the bark of a plane tree, *P. hispanica* (collected in the Czech Republic).

Thirty-two compounds (**1b–1h**, **2a–2d**, **3a–3f**, **4a–4g**, **5a–5e**, and **6a–6c**), betulinic acid analogues, were prepared from **1a** as shown in Schemes 1 and 2. Esters **1b–1d** were

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* Corresponding author. Tel./fax: +420 221 951 332; e-mail: jan.sarek@volny.cz

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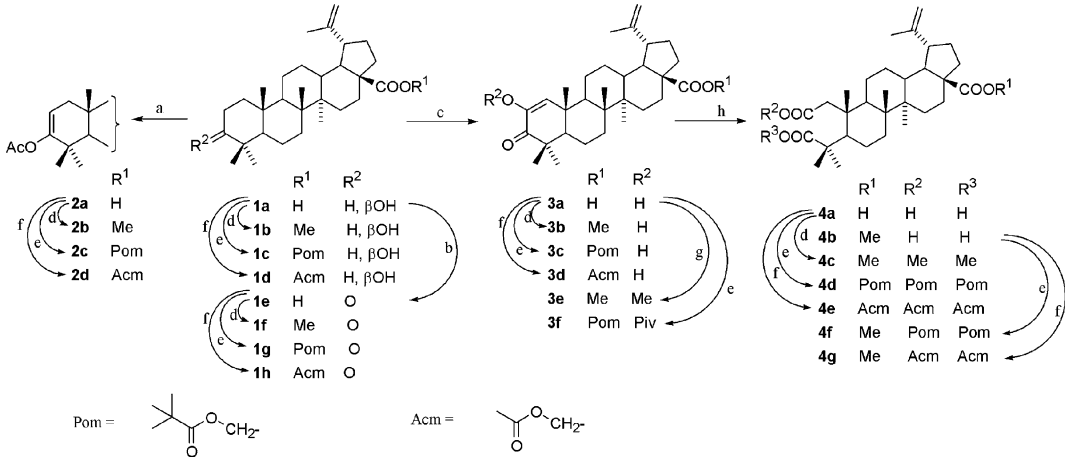
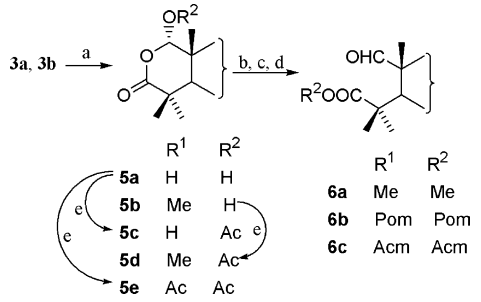


Scheme 1. Derivatization of betulinic acid **1a**, betulonic acid **1e**, and diosphenol **3a**. Reagents and conditions: (a) Isopropenyl acetate, TsCl, reflux; (b) CrO_3, DMF, rt; (c) O_2, *t*-BuOK, *t*-BuOK, 400 °C, 40 min; (d) CH_2N_2, ether, rt; (e) PomCl, MeCN, CH_2Cl_2, DBU, rt; (f) AcmBr, MeCN, CH_2Cl_2, DBU, rt; (g) Me_2SO_4, KOH, dioxane, reflux; (h) H_2O_2, KOH, MeOH, refl.



Scheme 2. Derivatization of oxidized derivatives of betulinic acid **1a**. Reagents and conditions: (a) O_2, *t*-BuOK, *t*-BuOK, 400 °C, 48 h; (b) CH_2N_2, ether, rt; (c) PomCl, MeCN, CH_2Cl_2, DBU, rt; (d) AcmBr, MeCN, CH_2Cl_2, DBU, rt; (e) Ac_2O, pyridine rt.

obtained by alcylation of **1a** (**1b**: CH_2N_2 in diethyl ether/$CHCl_3$; **1c**: PomCl with DBU in CH_2Cl_2 and MeCN; **1d**: AcmBr with DBU in CH_2Cl_2 and MeCN). Previously published procedure[9] was used for the preparation of oxidized compounds. Oxidation of acid **1a** with CrO_3 in DMF afforded ketone **1e**. Esters **1f**–**1h** were prepared in the same manner as described for compounds **1b**–**1d**. Enol acetate **2a** was prepared by treatment of **1e** with isopropenyl acetate in presence of TsOH and than alcylated in same conditions as described above to give esters **2b**–**2d**. Further oxidation of **1e** and **1f** was performed by introducing air 40 min into the *tert*-butyl alcohol solution of each ketone in presence of potassium 2-methyl-2-propoxide.[9,13]

Two types of products resulted from the reaction, the major products were diosphenols **3a** and **3b**. Compounds **5a** and **5b** were obtained as by-products, when the reaction time was longer than 1.5 h. Pure compounds **5a** and **5b** were obtained by further oxidation of diosphenols **3a** and **3b** in the same conditions as they were prepared, when the reaction time was 48 h.[9] After the alcylation of **3a** in usual conditions, esters **3c** and **3d** were obtained. In contrast to all previous alcylation, double-alcylated product **3f** was also obtained (35% yield). Subsequent treatment of diosphenol **3a** with dimethyl sulfate in the presence of KOH afforded methyl ether **3e**. A reaction of **5a** and **5c** with acetic anhydride in pyridine gave compounds **5b**, **5d**, and **5e**. Diosphenols **3a** and **3b** were then cleaved with a solution of hydrogen peroxide and KOH in refluxing MeOH.[9] The reactions afforded cleaved secoderivatives **4a** and **4b**. Methyl esters **4c** and **6a** were prepared by the reaction of acids **4a** and **5a** with diazomethane in diethylether and $CHCl_3$. Pom esters **4d**, **4f**, and **6b** were prepared by alcylation of free acids **4a**, **4e**, and **5a** with PomCl and DBU in $CHCl_2$ and MeCN. Acm-esters **4e**, **4g**, and **6c** were prepared by alcylation of free acids **4a**, **4b**, and **5a** with AcmBr and DBU in $CHCl_2$ and MeCN.

3. Results and discussion

Eight lupane derivatives **1a**–**1h**, four of them new (**1c**, **1d**, **1g**, and **1h**), four new enol acetates (**2a**–**2d**), six diosphenols **3a**–**3f**, three of them new (**3c**, **3d**, and **3f**), seven secoderivatives **4a**–**4g**, four of them new (**4d**–**4g**), five lactols (**5a**–**5e**), three of them new (**5c**–**5e**), and three new aldehydes (**6a**–**6c**) were synthesized from **1a**. All new compounds were characterized using spectral and physical data.

Methyl-, Pom-, and Acm-esters were synthesized from terpenic acids to compare their structure–activity relationships. Also, acetates were prepared in derivatives **5**.

Our results correspond to previously published lit.[8] showing that esterification of the C-28 carboxyl group with alkyl ester (methyl) decreases in vitro the cytotoxicity majority of the synthesized derivatives. Similar effect was obtained with Pom esters in our study (Table 1). Compounds **1b**, **1c**, **1f**, **1g**, **2b**, **2c**, **5b**, and **5d** are less active than corresponding free acids **1a**, **1e**, **2a**, and **5a**. Also activity of acetate **5d** was even lower than free lactol **5a** and methyl ester**5b**.

Acm esters **1d**, **1h**, **2d**, and **6c**, however, showed higher activity than both of the previously mentioned ester

Table 1. Cytotoxic activity of compounds **1a**–**6c** against A 549, DU 145, MCF 7, K562, and K562-Tax cell lines

Compound	IC_{50}[a] (μmol/L)				
	A 549	DU 145	MCF 7	K562	K562-Tax
1a	146	196	143	56	112
1b	184	110	120	71	65
1c	>250	>250	>250	>250	>250
1d	83	71	96	83	34
1e	15	36	29	6	17
1f	>250	>250	>250	239	>250
1g	>250	>250	>250	250	>250
1h	19	12	24	15	29
2a	40	79	100	49	34
2b	>250	>250	>250	>250	>250
2c	>250	>250	>250	>250	>250
2d	112	153	225	80	95
3a	10	23	14	4	11
3b	11	9	6	60	25
3c	230	178	203	48	36
3d	69	50	47	35	140
3e	23	18	12	10	10
3f	>250	>250	>250	>250	240
4a	>250	>250	>250	145	>250
4b	99	85	71	77	75
4c	184	113	107	104	76
4d	>250	>250	>250	>250	>250
4e	110	97	88	75	47
4f	110	66	74	41	45
4g	149	119	119	110	67
5a	93	107	126	107	121
5b	115	124	130	250	91
5c	20	17	21	32	82
5d	>250	206	243	178	>250
5e	197	41	107	135	186
6a	>250	206	243	>250	248
6b	207	210	>250	188	155
6c	167	179	215	108	76

Value >250 μmol/L means that compound is not active at maximum tested concentration.

[a] The concentration lethal to 50% of tumor cells. mean value from 3 to 4 independent experiments with standard deviation <20% of the average.

types. Cytotoxicity was similar to free acids, and moreover, in the case of compound **1d**, even higher than the corresponding acid **1a**.

In contrast with these facts, general structure–activity relationship was not confirmed in groups of derivatives **3a**–**3f**, **4a**–**4g**, and **6a**–**6c**. This is probably due to wider modification in the A-ring which affects mutual interaction and accessability of ester groups to esterases, and their activity can be caused by this modification. The most interesting is the activity of diosphenols **3b** and **3e** which are active as methyl esters themselves.

4. Conclusion

The cytotoxicity of a large group of triterpenoid esters was studied in five cancer cell lines. It was found that methyl and Pom esters are inactive; however, Acm esters have activity similar to the starting acids (Table 1). Acm esters can be used as a suitable ester group for protection of free acids, preserving their biological activities. Although the cytotoxic activity of some of the derivatives presented here was in the high micromolar ranges, several compounds recruited from the group of diosphenols **3** showed interesting anti-cancer activity in vitro, and deserve further investigation. An influence of A-ring modification on the cytotoxicity of derivatives prepared here was also studied. The most active compounds were found among diosphenols **3a**–**3f** with a conjugated dicarbonylic system on the A-ring, which seems to be a more efficient modification than esterification. Among more oxidized compounds, the secoacids and esters **4a**–**4g** were less active than starting material. Also, the lactols **5a**–**5e** and aldehydes **6a**–**6c** are not very promising anti-cancer agents. Protecting the 3-oxogroup in betulinic acid **1e** was not successful either.

5. Materials and methods

5.1. Chemicals

Potassium *tert*-butoxide, *tert*-butyl alcohol, dimethyl sulphate, isopropenyl acetate, DBU, PomCl, and AcmBr were purchased from Sigma–Aldrich company.

5.2. Cell lines

Cell lines A 549, DU 145, MCF 7, and K562 were purchased from the American Tissue Culture Collection (ATTC). Paclitaxel-resistant subline of K562 cells (K-562-Tax) was prepared and characterized in our laboratories.[17] The cells were maintained in Nunc/Corning 80 cm^2 plastic tissue culture flasks and cultured in cell culture medium (DMEM/RPMI 1640 with 5 g/L glucose, 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 10% fetal calf serum, and $NaHCO_3$).

5.3. Cytotoxic MTT assay

Cell suspensions were prepared and diluted according to the particular cell type and the expected target cell density (2500–30,000 cells/well based on cell growth characteristics). Cells were added by pipette (80 μL) into 96-well microtiter plates. Inoculates were allowed a pre-incubation period of 24 h at 37 °C and 5% CO_2 for stabilization. Fourfold dilutions, in 20-μL aliquots, of the intended test concentration were added at time zero to the microtiter plate wells. All test compound (dissolved in 10 μL of 10% DMSO) concentrations were examined in duplicate. Incubation of the cells with the test compounds lasted for 72 h at 37 °C, in a 5% CO_2 atmosphere at 100% humidity. At the end of the incubation period, the cells were assayed using MTT. Aliquots (10 μL) of the MTT stock solution were pipetted into each well and incubated for a further 1–4 h. After this incubation period formazan produced was dissolved by the addition of 100 μL/well of 10% aq SDS (pH 5.5), followed by a further incubation at 37 °C overnight. The optical density (OD) was measured at 540 nm with a Labsystem iEMS Reader MF. Tumor cell survival (TCS) was calculated using the following equation: TCS = (OD of drug-exposed well/OD of control

wells) × 100%. The TCS_{50} value, the drug concentration (in μmol/L) lethal to 50% of the tumor cells, was calculated from appropriate dose–response curves.

6. Experimental

6.1. General experimental procedures

Melting points were determined using a Kofler block and are uncorrected. Optical rotations were measured using $CHCl_3$ solutions (unless otherwise stated) on an Autopol III (Rudolph Research, Flanders, NJ) polarimeter. NMR spectra were recorded on a Varian UNITY INOVA 400 instrument (^1H NMR spectra at 399.95 MHz) using $CDCl_3$ solutions (unless otherwise stated), with $SiMe_4$ as an internal standard. EIMS were recorded on an INCOS 50 (Finigan MAT) spectrometer at 70 eV and an ion source temperature of 150 °C. The samples were introduced from a direct exposure probe at a heating rate of 10 mA/s. Relative abundances stated are related to the most abundant ion in the region of $m/z > 50$. TLC was carried out using silica gel 60 F_{254}, detection was by spraying with 10% aq H_2SO_4 and heating to 150–200 °C. Column chromatography was performed using silica gel 60 (Merck 7734). The HPLC system consisted of a High Pressure Pump Gilson (model 361), and Rheodyne Injections Valve, preparative column (25 × 250 mm) with silica gel fillings (Biospher 7 μm), Differential-Refractometrical Detector (Laboratorni pristroje, Praha, CZ) connected with PC (software Chromulan) and Automatic Fraction Collector Gilson (model 246). A mixture of ethyl acetate and hexane was used as the mobile phase, its composition specified for each experiment separately.

Work-up refers to pouring the reaction mixture into H_2O, extraction of the product with Et_2O, and washing the organic layer successively with H_2O, dilute aq HCl, H_2O, saturated aq $NaHCO_3$, and again H_2O, followed by drying over $MgSO_4$, filtration, and evaporation of the filtrate under reduced pressure. Analytical samples were dried over P_2O_5 under diminished pressure.

6.1.1. General procedure for the preparation of methyl esters. Each acid was dissolved in chloroform and CH_2N_2 in diethyl ether was added since the development of N_2 gas stopped. Methyl ester was than purified over silica gel eluted with toluene and crystallized from methanol.

6.1.2. General procedure for a preparation of Pom esters. 0.50 mL (3.33 mmol) of PomCl was added to a solution of 0.52 mL (3.42 mmol) of DBU and 3.2 mmol of each acid in 9 mL CH_2Cl_2 and 3 mL MeCN. The reaction mixture was worked-up after 10–12 h. Crude product was than purified by chromatography on silica gel (30 g) eluted with toluene and crystallized from methanol.

6.1.3. General procedure for a preparation of Acm esters. 0.51 mL (3.33 mmol) of AcmBr was added to a solution of 0.52 mL (3.42 mmol) of DBU and 3.2 mmol of each acid in 9 mL CH_2Cl_2 and 3 mL MeCN. The reaction mixture was worked-up after 10–12 h. Crude product was than purified by chromatography on silica gel (30 g) eluted with toluene/diethyl ether (5:1) and crystallized from methanol.

6.1.4. Betulinic acid (1a). Bark of the plane tree *P. hispanica* (5 kg) was extracted with MeOH (15 L, twice). Collected extracts were evaporated and the residual crude betulinic acid (**1**) (150 g) was then purified by several crystallizations from MeOH (70 mL/g) to give white needles (50 g, 1% yield): mp 304–305 °C. $[\alpha]_D$ +8° (*c* 0.37). IR *v* ($CHCl_3$) cm^{-1}: 1720vb, 1643.[14]

6.1.5. Methyl 3β-hydroxylup-20(29)-en-28-oate (1b). Diazomethane (50 mmol) in diethylether was added to a solution of betulinic acid (**1**) (5 g, 10.9 mmol) in $CHCl_3$ (400 mL). Organic solvents were evaporated in vacuum and crude product was chromatographed over silica gel (50 g) eluted with toluene and then crystallized from methanol to afford methyl ester **2** (4.5 g, 87% yield): mp 224–228 °C. $[\alpha]_D$ +3° (*c* 0.36). IR *v* ($CHCl_3$) cm^{-1}: 1720vb, 1643.[14]

6.1.6. Pivaloyloxymethyl 3β-hydroxylup-20(29)-en-28-oate (1c). 1.5 g (3.3 mmol) of **1a** was treated with PomCl in the general manner to give white needles of **1c** (1.4 g, 75% yield): mp 155–158 °C (methanol). $[\alpha]_D$ +6° (*c* 0.34). IR *v* ($CHCl_3$) cm^{-1}: 1748, 1721 sh, 1642. ^1H NMR δ 0.76, 0.82, 0.92, 0.96 (15H, each s, 5 × CH_3), 1.22 (9H, s, 3 × CH_3 Pom), 1.68 (3H, m, H-30), 1.80–1.93 (2H, m), 2.20–2.28 (2H, m), 2.97 (1H, td, *J*(H-19β, H-18α) = 11.0 Hz, *J*(H-19β, H-21α) = 11.0 Hz, *J*(H-19β, H-21β) = 4.8 Hz, H-19β), 3.18 (1H, dd, *J*(H-3α, H-2β) = 11.2 Hz, *J*(H-3α, H-2α) = 5.0 Hz, H-3α), 4.60 (1H, m, H-29 *pro-E*), 4.73 (1H, d, *J* = 2.3 Hz, H-29 *pro-Z*), 5.75 (1H, d, *J*(H-31a, H-31b) = 5.43 Hz, H-31a), 5.79 (1H, d, *J*(H-31b, H-31a) = 5.5 Hz, H-31b). MS *m/z* (relative intensity): 570 (6, M^+), 552 (1), 540 (0.1), 455 (8), 438 (15), 423 (4), 410 (50), 395 (9), 362 (11), 332 (3), 320 (4), 271 (4), 247 (8), 234 (10), 230 (10), 220 (24), 207 (67), 189 (100). Anal. Calcd for $C_{36}H_{58}O_5$: C, 75.75%; H, 10.24%. Found C, 75.73%; H, 10.77%.

6.1.7. Acetoxymethyl 3β-hydroxylup-20(29)-en-28-oate (1d). 1.6 g (3.5 mmol) of acid **1a** was treated with AcmBr in the general manner to give needles of **1d** (1.4 g, 75% yield): mp 186–189 °C (methanol). $[\alpha]_D$ +12° (*c* 0.35). IR *v* ($CHCl_3$) cm^{-1}: 1752b, 1642. ^1H NMR δ 0.75, 0.82, 0.92, 0.96, (15H, all s, 5 × CH_3), 1.68 (3H, s, H-30), 1.85–1.95 (2H, m), 2.10 (3H, s, CH_3 Acm), 2.18 (1H, td, J_1 = 13.1 Hz, J_2 = 13.1 Hz, J_3 = 3.8 Hz), 2.26 (1H, m), 3.00 (1H, td, *J*(H-19β, H-18α) = 11.4 Hz), *J*(H-19β, H-21α) = 11.4 Hz, *J*(H-19β, H-21β) = 5.1 Hz, (H-19β), 3.18 (1H, m, Σ*J* = 16.3 Hz, (H-3α), 4.61 (1H, m, H-29 *pro-E*), 4.47 (1H, bd, *J* = 2.5 Hz, H-29 *pro-Z*), 5.71 (1H, d, *J*(H-31a, H-31b) = 6.8 Hz, H-31a), 5.80 (1H, d, *J*(H-31b, H-31a) = 6.8 Hz, H-31a). MS *m/z* (relative intensity): 528 (19, M^+), 510 (5), 456 (5), 438 (9), 423 (4), 410 (35), 395 (8), 320 (32), 306 (6), 248 (9), 235 (14), 220 (26), 207 (68), 189 (100). Anal. Calcd for $C_{33}H_{52}O_5$: C, 74.96%; H, 9.91%. Found C, 74.95%; H, 10.00%.

6.1.8. 3-Oxolup-20(29)-en-28-oic acid (1e). Chromium (VI) oxide (15.0 g, 150.1 mmol) and sulfuric acid (1 mL, 98%) was added to a solution of acid **1a** (15.0 g, 32.8 mmol) in DMF (300 mL). The reaction mixture was stirred for 12 h at room temperature. The product was precipitated by pouring into vigorously stirred copious water, filtered, and washed with water. A column chromatography of crude ketone **7** (14.1 g) over silica gel (300 g) eluted with chloroform afforded ketone **7** (10.7 g, 71% yield): mp 250–254 °C (methanol). $[\alpha]_D$ +32° (*c* 0.37).[15]

6.1.9. Methyl 3-oxolup-20(29)-en-28-oate (1f). Sodium dichromate (37.0 g, 124.2 mmol) and sodium acetate (8.0 g) was added to a vigorously stirred solution of crude methyl ester 1b (33.0 g, 70.2 mmol) in a mixture of dioxane (750 mL), glacial acetic acid (250 mL), and acetic anhydride (100 mL). The solution was then stirred for 28 h at room temperature and worked-up in the usual manner. Column chromatography of crude product **1f** (30.5 g) over silica gel (300 g) eluted with toluene afforded ketoester **1f** (16.1 g, 49% yield): R_f 0.57. mp 161–165 °C (methanol). $[\alpha]_D$ +28° (*c* 0.41).[16]

6.1.10. Pivaloyloxymethyl 3-oxolup-20(29)-en-28-oate (1g). 1.5 g (3.3 mmol) of acid **1e** was treated with PomCl in the general manner to give needles of **1g** (1.04 g, 56% yield): mp. 133–138 °C (methanol). $[\alpha]_D$ +25° (*c* 0.40). IR *v* ($CHCl_3$) cm^{-1}: 1748, 1700, 1643. ^1H NMR δ 0.93, 0.97, 1.02 1.07 (15H, all s, $3 \times CH_3$), 1.22 (9H, s, CH_3-Pom), 1.68 (3H, s, H-30), 1.82–1.94 (2H), 2.23–2.32 (2H, m), 2.35–2.54 (2H, m), 2.97 (1H, td, *J*(H-19β, H-18α) = 11.1 Hz, *J*(H-19β, H-21α) = 11.1 Hz, *J*(H-19β, H-21β) = 4.7 Hz, H-19β), 4.61 (1H, m, H-29 *pro-E*), 4.73 (1H, bd, *J* = 2.3 Hz, H-29 *pro-Z*), 5.76 (1H, d, *J*(H-31a, H-31b) = 5.3 Hz, H-31a), 5.80 (1H, d, *J*(H-31b, H-31a) = 5.5 Hz, H-31b). MS *m/z* (relative intensity): 568 (9, M^+), 538 (4), 454 (12), 436 (17), 421 (2), 408 (100), 393 (8), 245 (10), 230 (7), 218 (23), 205 (37), 189 (50). Anal. Calcd for $C_{36}H_{56}O_5$: C, 76.01%; H, 9.92%. Found C, 75.83%; H, 9.77%.

6.1.11. Acetoxymethyl 3-oxolup-20(29)-en-28-oate (1h). Two gram (4.4 mmol) of **1e** was treated with AcmBr in the general manner to give white needles of **1h** (1.54 g, 66%): mp 86–91 °C (methanol). $[\alpha]_D$ +31° (*c* 0.32). IR *v* ($CHCl_3$) cm^{-1}: 1750b, 1699, 1643. ^1H NMR δ 0.93, 0.96, 0.98, 1.02, 1.07, (15H, all s, $5 \times CH_3$), 1.68 (3H, s, H-30), 1.84–1.96 (3H, m), 2.10 (3H, s, CH_3 Acm), 2.18–2.31 (2H, m), 2.35–2.55 (2H, m), 3.00 (1H, td, *J*(H-19β, H-18α) = 11.3 Hz, *J*(H-19β, H-21α) = 11.3 Hz, *J*(H-19β, H-21β) = 4.7 Hz, H-19β), 4.61 (1H, m, H-29 *pro-E*), 4.74 (1H, m, H-29 *pro-Z*), 5.72 (1H, d, *J*(H-31a, H-31b) = 5.5 Hz, H-31a), 5.81 (1H, d, *J*(H-31b, H-31a) = 5.5 Hz, H-31b). MS *m/z* (relative intensity): 526 (23, M^+), 511 (2), 496 (3), 483 (0.5), 466 (0.5), 454 (21), 446 (10), 436 (23), 408 (100), 393 (11), 365 (8), 354 (10), 320 (45), 307 (8), 259 (10), 248 (19), 235 (26), 218 (31), 205 (63), 189 (87). Anal. Calcd for $C_{33}H_{50}O_5$: C, 75.25%; H, 9.57%. Found C, 75.20%; H, 9.44%.

6.1.12. 3β-Acetoxylup-2,20(29)-ene-28-oic acid (2a). TsOH (0.5 g) was added to a solution of betulonic acid **1e** (3 g, 6.6 mmol) in isopropenyl acetate (150 mL). The solution was refluxed for 9 h and 50 mL of solvents was distilled off during that time. Then the reaction mixture was worked-up and crude product was purified by chromatography over silica gel (50 g) eluted with (toluene/diethylether 20:1) to afford **2a** (1.8 g, 55% yield): mp 261–264 °C (methanol). $[\alpha]_D$ +30° (*c* 0.30). IR *v* ($CHCl_3$) cm^{-1}: 1752 sh, 1740, 1695, 1642. ^1H NMR δ 0.89, 0.94, 0.97, 0.98, 1.00 (15H, all s, $5 \times CH_3$), 1.70 (3H, m, H-30), 2.14 (3H, s, OAc), 3.02 (1H, td, *J*(H-19β, H-18α) = 10.7 Hz, *J*(H-19β, H-21α) = 10.7 Hz, *J*(H-19β, H-21β) = 4.8 Hz, H-19β), 4.62 (1H, m, H-29 *pro-E*), 4.75 (1H, bd, *J* = 2.3 Hz, H-29 *pro-Z*), 5.13 (1H, dd, *J*(H-2, H-1α) = 6.7 Hz, *J*(H-2, H-1β) = 2.0 Hz, H-2). MS *m/z* (relative intensity): 496 (7, M^+), 481 (1), 454 (100), 439 (5), 408 (8), 393 (3), 248 (6), 235 (6), 218 (8), 205 (36), 189 (24), 175 (16), 161 (10), 147 (16), 135 (26), 119 (38), 105 (38). Anal. Calcd for $C_{32}H_{48}O_4$: C, 77.38%; H, 9.74%. Found C, 77.73%; H, 9.55%.

6.1.13. Methyl 3β-acetoxylup-2,20(29)-ene-28-oate (2b). One hundred milligram (0.2 mmol) of **2a** was treated with CH_2N_2 using general procedure which yielded crystals of **2b** (70 mg, 68%): mp 234–238 °C (methanol). $[\alpha]_D$ +35° (*c* 0.35). IR *v* ($CHCl_3$) cm^{-1}: 1753, 1721, 1689, 1642. ^1H NMR δ 0.90, 0.93, 0.95, 0.96, 1.00 (15H, all s, $5 \times CH_3$), 1.69 (3H, m, H-30), 2.14 (3H, s, OAc), 3.00 (1H, td, *J*(H-19β, H-18α) = 11.1 Hz, *J*(H-19β, H-21α) = 11.1 Hz, *J*(H-19β, H-21β) = 4.6 Hz, H-19β), 3.67 (3H, s, OCH_3), 4.60 (1H, m, H-29 *pro-E*), 4.74 (1H, bd, *J* = 2.3 Hz, H-29 *pro-Z*), 5.13 (1H, dd, *J*(H-2, H-1α) = 6.7 Hz, *J*(H-2, H-1β) = 2.0 Hz, H-2). MS *m/z* (relative intensity): 510 (25, M^+), 495 (4), 468 (100), 450 (7), 435 (5), 409 (13), 354 (5), 262 (32), 249 (32), 233 (12), 218 (25), 205 (82), 189 (88), 175 (46), 175 (46), 161 (21), 147 (30), 133 (46), 119 (73), 105 (70). Anal. Calcd for $C_{33}H_{50}O_4$: C, 77.60%; H, 9.87%. Found C, 77.63%; H, 10.03%.

6.1.14. Pivaloyloxymethyl 3β-acetoxylup-2,20(29)-ene-28-oate (2c). Compound **2c** was prepared from **2a** (100 mg, 0.2 mmol) using general procedure, which yielded crystals of **2c** (70 mg, 57%): mp 180–183 °C (methanol). $[\alpha]_D$ +31° (*c* 0.34). IR *v* ($CHCl_3$) cm^{-1}: 1749b, 1692, 1642. ^1H NMR δ 0.90, 0.93, 0.96, 1.00 (15H, all s, $5 \times CH_3$), 1.22 (9H, s, CH_3-Pom), 1.68 (3H, s, H-30), 2.14 (3H, s, OAc), 2.98 (1H, td, *J*(H-19β, H-18α) = 11.0 Hz, *J*(H-19β, H-21α) = 11.0 Hz, *J*(H-19b, H-21β) = 4.6 Hz, H-19β), 4.60 (1H, m, H-29 *pro-E*), 4.74 (1H, m, H-29 *pro-Z*), 5.13 (1H, dd, *J*(H-2, H-1α) = 6.7 Hz, *J*(H-2, H-1β) = 2.0 Hz, H-2), 5.76 (1H, d, *J*(H-31a, H-31b) = 5.5 Hz, H-31a), 5.80 (1H, d, *J*(H-31b, H-31a) = 5.4 Hz, H-31b). MS *m/z* (relative intensity): 610 (10, M^+), 568 (86), 495 (7), 478 (6), 453 (100), 436 (16), 408 (67), 393 (10), 355 (6), 311 (14), 247 (13), 231 (13), 218 (18), 205 (58), 189 (34), 175 (36), 161 (29), 147 (29), 135 (56), 119 (76), 105 (80). Anal. Calcd for $C_{38}H_{58}O_6$: C, 74.71%; H, 9.57%. Found C, 74.55%; H, 9.63%.

6.1.15. Acetoxymethyl 3β-acetoxylup-2,20(29)-ene-28-oate (2d). Compound **2d** was prepared from **2a** (500 mg, 1 mmol) using general procedure, which yielded crystals of **2c** (360 mg, 63%): mp 141–144 °C (methanol). $[\alpha]_D$

+38° (*c* 0.31). IR *v* ($CHCl_3$) cm^{-1}: 1753b, 1690, 1642. ^1H NMR δ 0.90, 0.93, 0.95, 0.97, 1.00 (15H, all s, $5 \times CH_3$), 1.69 (3H, s, H-30), 2.10 (3H, s, CH_3 Acm), 3.01 (1H, m, ΣJ = 26.9 Hz, H-19β), 4.61 (1H, m, H-29 *pro-E*), 4.74 (1H, dm, H-29 *pro-Z*), 5.13 (1H, dd, *J*(H-2, H-1α) = 6.6 Hz, *J*(H-2, H-1β) = 2.0 Hz, H-2), 5.72 (1H, d, *J*(H-31a, H-31b) = 5.5 Hz, H-31a), 5.80 (1H, d, *J*(H-31b, H-31a) = 5.6 Hz, H-31b). MS *m*/*z* (relative intensity): 568 (14, M^+), 553 (0.5), 538 (0.2), 526 (100), 496 (7), 478 (4), 453 (50), 436 (12), 408 (49), 393 (8), 356 (5), 320 (16), 247 (14), 235 (23), 218 (24), 205 (71), 189 (71), 175 (42), 161 (29), 147 (39), 135 (57), 119 (89), 105 (92). Anal. Calcd for $C_{35}H_{52}O_6$: C, 73.91%; H, 9.21%. Found C, 74.02%; H, 9.17%.

6.1.16. 2-Hydroxy-3-oxolupa-1,20(29)-dien-28-oic acid (3a). This product was obtained by oxidation of **1e** (10 g, 22 mmol) with air in a mixture of *tert*-butyl alcohol (900 mL) and *tert*-butoxide (10 g, 88 mmol) for 40 min according to lit.[9] This yielded **3a** (4.1 g, 82%): mp 204–205 °C (diethylether). $[\alpha]_D$ +12° (*c* 0.56). IR *v* ($CHCl_3$) cm^{-1}: 1730 sh, 1696, 1669, 1643.[9]

6.1.17. Methyl 2-hydroxy-3-oxolupa-1,20(29)-dien-28-oate (3b). This product was obtained by oxidation of **1f** (6 g, 13 mmol) with air in a mixture of *tert*-butyl alcohol (450 mL) and *tert*-butoxide (6 g, 53 mmol) for 40 min according to lit.[9] This yielded **3b** (4.5 g, 75%): mp 119–122 °C (diethylether). $[\alpha]_D$ +3° (*c* 0.64). IR *v* ($CHCl_3$) cm^{-1}: 1720 sh, 1643.[9]

6.1.18. Pivaloyloxymethyl 2-hydroxy-3-oxolupa-1,20(29)-dien-28-oate (3c). 0.3 g (0.7 mmol) of **3a** was treated with PomCl in the general manner to give white crystals of **3c** (0.2 g, 52% yield): mp 108–111 °C (methanol). $[\alpha]_D$ +37° (*c* 0.65). IR *v* ($CHCl_3$) cm^{-1}: 1796, 1747vb, 1668, 1644. ^1H NMR δ 0.96, 0.99, 1.10, 1.12, 1.20 (15H, all s, $5 \times CH_3$), 1.22 (9H, s, CH_3-Pom), 1.68 (3H, m, H-30), 2.99 (1H, td, *J*(H-19β, H-18α) = 11.0 Hz, *J*(H-19β, H-21α) = 11.0 Hz, *J*(H-19β, H-21β) = 4.9 Hz, H-19β), 4.63 (1H, m, H-29 *pro-E*), 4.74 (1H, bd, *J* = 2.0 Hz, H-29 *pro-Z*), 5.76 (1H, d, *J*(H-31a, H-31b) = 5.6 Hz, H-31a), 5.80 (1H, d, *J*(H-31b, H-31a) = 5.4 Hz, H-31b), 5.90 (1H, s, OH), 6.44 (1H, s, H-1). MS *m*/*z* (relative intensity): 582 (3, M^+), 552 (7), 468 (14), 450 (6), 423 (41), 407 (2), 340 (9), 238 (61), 215 (100). Anal. Calcd for $C_{36}H_{54}O_6$: C, 74.19%; H, 9.34%. Found C, 74.01%; H, 9.22%.

6.1.19. Acetoxymethyl 2-hydroxy-3-oxolupa-1,20(29)-dien-28-oate (3d). 0.3 g (0.7 mmol) of **3a** was treated with AcmBr in the general manner to give white oil of **3d** (0.17 g, 48% yield): mp 65–68 °C (methanol). $[\alpha]_D$ +13° (*c* 0.42). IR *v* ($CHCl_3$) cm^{-1}: 1748, 1644. ^1H NMR δ 0.96, 0.99, 1.10, 1.12, 1.20 (15H, all s, $5 \times CH_3$), 1.69 (3H, m, H-30), 2.11 (3H, s, CH_3 Acm), 2.17–2.30 (2H, m), 3.01 (1H, td, *J*(H-19β, H-18α) = 11.0 Hz, *J*(H-19β, H-21α) = 11.0 Hz, *J*(H-19β, H-21β) = 5.0 Hz, H-19 β), 4.63 (1H, m, H-29 *pro-E*), 4.75 (1H, bd, *J* = 2.3 Hz, H-29 *pro-Z*), 5.73 (1H, d, *J*(H-31a, H-31b) = 5.5 Hz, H-31a), 5.81 (1H, d, *J*(H-31b, H-31a) = 5.5 Hz, H-31b), 5.90 (1H, bs, OH), 6.44 (1H, s, H-1). MS *m*/*z* (relative intensity): 540 (25, M^+), 526 (5), 480 (14), 468 (100), 450 (27), 422 (79), 408 (26), 340 (37), 320 (29), 269 (19). Anal. Calcd for $C_{33}H_{48}O_6$: C, 73.30%; H, 8.95%. Found C, 73.37%; H, 9.06%.

6.1.20. Methyl 2-methoxy-3-oxolupa-1,20(29)-dien-28-oate (3e). KOH (0.5 g, 8.8 mmol) in water (10 mL) was added to a mixture of diosphenol **3a** (1 g, 2.1 mmol) in dioxan (20 mL) and dimethylsulfate (2 g, 16 mmol). The mixture was then refluxed for 1 h and worked-up. Crude product was crystallized from methanol to give compound **3e** (0.95 g, 87% yield): mp 105–107 °C (methanol). $[\alpha]_D$ +46° (*c* 0.57). IR *v* ($CHCl_3$) cm^{-1}: 1713, 1674, 1642, 1621.[9]

6.1.21. Pivaloyloxymethyl 2-pivaloyloxy-3-oxolupa-1,20(29)-dien-28-oate (3f). 0.3 g (0.7 mmol) of **3a** was treated with PomCl in the general manner to give white oil of **3c** (0.2 g, 52% yield): mp 103–105 °C (methanol). $[\alpha]_D$ +34° (*c* 0.80). IR *v* ($CHCl_3$) cm^{-1}: 1747, 1682, 1644. ^1H NMR δ 0.97, 1.01, 1.12, 1.15, 1.19 (15H, all s, $5 \times CH_3$), 1.22 (9H, s, $3 \times CH_3$ Pom), 1.29 (9H, s, $3 \times CH_3$ Piv-2), 1.68 (3H, m, H-30), 1.76–1.95 (3H, m), 2.34–2.36 (2H, m), 2.98 (1H, td, *J*(H-19β, H-18α) = 11.2 Hz, *J*(H-19β, H-21α) = 11.2 Hz, *J*(H-19β, H-21β) = 4.8 Hz, H-19 β), 4.61 (1H, m, H-29 *pro-E*), 4.74 (1H, bd, *J* = 2.3 Hz, H-29 *pro-Z*), 5.76 (1H, d, *J*(H-31a, H-31b) = 5.4 Hz, H-31a), 5.80 (1H, d, *J*(H-31b, H-31a) = 5.4 Hz, H-31b), 6.71 (1H, s, H-1). MS *m*/*z* (relative intensity): 666 (2, M^+), 636 (1), 565 (3), 553 (5), 535 (2), 507 (31), 469 (13), 451 (5), 421 (16), 238 (100). Anal. Calcd for $C_{41}H_{62}O_7$: C, 73.89%; H, 9.37%. Found C, 73.75%; H, 9.25%.

6.1.22. Preparation of 2,3 secoderivatives 4a and 4b. A solution of each diosphenol 9, 10 (4.5 mmol) in a mixture of KOH (6.5 g) and methanol (350 mL) was heated under reflux and hydrogen peroxide (35 mL, 30%) was added during 100 min. The solution was then worked-up and chromatographed over silica gel (200 g), eluted with chloroform/ethyl acetate/acetic acid (100:15:1) and crystallized to give secoacids 16 and 17 in 35%, respectively, 80% yields.

6.1.23. 2,3-Secolup-20(29)en-2,3,28-trioic acid (4a). Mp 276–277 °C (methanol/chloroform). $[\alpha]_D$ +10° (*c* 0.31). IR *v* ($CHCl_3$) cm^{-1}: 2600-3400, 1725, 1643.[9]

6.1.24. 28-Methyl ester of 2,3-secolup-20(29)en-2,3,28-trioic acid (4b). Mp 132–135 °C (hexane/diethylether). $[\alpha]_D$ +23° (*c* 0.27). IR *v* ($CHCl_3$) cm^{-1}: 2400–3400, 1718, 1690, 1642.[9]

6.1.25. Trimethyl 2,3-secolup-20(29)en-2,3,28 trioate (4c). Two hundred milligram (0.4 mmol) of **4a** was treated with CH_2N_2 using general procedure which yielded crystals of **4c** (150 mg, 69% yield): mp 188–190 °C (methanol). $[\alpha]_D$ −8° (*c* 0.44). IR *v* ($CHCl_3$) cm^{-1}: 1720vb, 1641.[9]

6.1.26. Tripivaloyloxymethyl 2,3-secolup-20(29)en-2,3,28 trioate (4d). One hundred fifty milligram (0.3 mmol) of **4a** was treated with PomCl using general procedure which yielded crystalls of **4d** (140 mg, 56% yield): mp

80–82 °C (methanol). $[\alpha]_D$ +15° (*c* 0.26). IR *v* ($CHCl_3$) cm^{-1}: 1749, 1643. ^1H NMR δ 0.91, 0.92, 0.98 (9H, all s, $3\times CH_3$), 1.20 (9H, s, $3\times CH_3$ Pom-2), 1.20 (9H, s, $3\times CH_3$ Pom-3), 1.21 (9H, s, $3\times CH_3$ Pom-28), 1.23, 1.25 (6H, all s, $2\times CH_3$), 1.66 (3H, s, H-30), 1.78–1.95 (2H, m), 2.18–2.30 (2H, m), 2.33–2.41 (2H. m), 2.41 (1H, d, *J*(H-1α, H-1β) = 18.5 Hz, H-1a), 2.50 (1H, d, *J*(H-1β, H-1α) = 18.5 Hz, H-1β), 2.97 (1H, td, *J*(H-19β, H-18α) = 11.1 Hz, *J*(H-19β, H-21α) = 11.1 Hz, *J*(H-19β, H-21β) = 4.6 Hz, H-19β), 4.58 (1H, m, H-29 *pro-E*), 4.72 (1H, bd, *J* = 2.3 Hz, H-29 *pro-Z*), 5.62 (1H, d, *J* = 5.4 Hz), 5.70 (1H, d, *J* = 5.5 Hz), 5.75 (1H, d, *J* = 5.3 Hz), 5.78 (1H, d, *J* = 5.5 Hz), 5.79 (1H, d, *J* = 5.5 Hz), 5.82 (1H, d, *J* = 5.5 Hz, $3\times CH_2$ Pom). MS *m/z* (relative intensity): 844 (1, M^+), 799 (0.9), 712 (9.4), 684 (40), 670 (10), 653 (16), 642 (6), 599 (6), 571 (16), 553 (20), 523 (15), 511 (28), 483 (100), 469 (32), 439 (48), 397 (41), 351 (62), 309 (34), 215 (24), 189 (41), 172 (33), 163 (21), 145 (21), 133 (27), 107 (38). Anal. Calcd for $C_{48}H_{76}O_{12}$: C, 68.22%; H, 9.06%. Found C, 69.99%; H, 8.97%.

6.1.27. Triacetoxymethyl 2,3-secolup-20(29)en-2,3,28 trioate (4e). Two hundred milligram (0.4 mmol) of **4a** was treated with PomCl using general procedure and chromatographed on HPLC (phase 25) to give oil of **4e** (130 mg, 49% yield): mp < 20 °C. $[\alpha]_D$ +12° (*c* 0.59). IR *v* ($CHCl_3$) cm^{-1}: 1761b, 1642. ^1H NMR δ 0.92, 0.92, 0.99, 1.25, 1.25, (15H, all s, $5\times CH_3$), 1.67 (3H, m, H-30), 1.82-1.94 (2H, m), 2.09 (3H, s, Ac), 2.10 (3H, s, Ac), 2.11 (3H, s, Ac), 2.38 (1H, d, *J* = 18.2 Hz, H-1α), 2.53 (1H, d, *J* = 17.9 Hz, H-1β), 3.00 (1H, td, *J*(H-19β, H-18α) = 11.1 Hz, *J*(H-19β, H-21α) = 11.1 Hz, *J*(H-19β, H-21β) = 4.8 Hz, H-19β), 4.59 (1H, m, H-29 *pro-E*), 4.73 (1H, bd, *J* = 2.3 Hz, H-29 *pro-Z*), 5.66 (1H, d, *J* = 5.5 Hz), 5.71 (1H, d, *J* = 5.5 Hz), 5.72 (1H, d, *J* = 5.7 Hz), 5.75 (1H, d, *J* = 5.6 Hz), 5.76 (1H, d, *J* = 5.7 Hz), 5.80 (1H, d, *J* = 5.5 Hz, $3\times CH_2$ Acm). MS *m/z* (relative intensity): 718 (not found, M^+), 628 (9), 600 (36), 586 (16), 558 (9), 529 (19), 511 (12), 469 (19), 441 (7), 427 (41), 411 (15), 397 (40), 369 (19), 351 (37), 331 (20), 309 (18), 189 (35). Anal. Calcd for $C_{39}H_{58}O_{12}$: C, 65.16%; H, 8.13%. Found C, 65.53%; H, 8.27%.

6.1.28. 28-Methyl 2,3-dipivaloyloxymethyl 2,3-secolup-20(29)en-2,3,28 trioate (4f). Two hundred milligram (0.4 mmol) of **4b** was treated with PomCl using general procedure and chromatographed on HPLC (phase 10) to give crystals of **4e** (150 mg, 51% yield): mp 113–115 °C. $[\alpha]_D$ +3° (*c* 0.35). IR *v* ($CHCl_3$) cm^{-1}: 1749, 1723sh, 1642. ^1H NMR δ 0.91, 0.91, 0.98, (9H, all s, $3\times CH_3$), 1.20 (9H, s, $3\times CH_3$ Pom), 1.21 (9H, s, $3\times CH_3$ Pom), 1.23, 1.25, (6H, all s, $2\times CH_3$), 1.67 (3H, m, H-30), 2.41 (1H, d, *J* = 18.5 Hz, H-1α), 2.55 (1H, d, *J* = 18.5 Hz, H-1β), 3.00 (1H, m, H-19β), 4.57 (1H, m, H-29 *pro-E*), 4.72 (1H, bd, *J* = 2.4 Hz, H-29 *pro-Z*), 5.62 (1H, d, *J* = 5.3 Hz), 5.71 (1H, d, *J* = 5.5 Hz), 5.78 (1H, d, *J* = 5.7 Hz), 5.82 (1H, d, *J* = 5.2 Hz, $2\times CH_2$ Pom), MS *m/z* (relative intensity): 744 (1, M^+), 744 (1), 729 (0.5), 714 (0.6), 684 (3), 630 (1), 612 (1), 583 (84), 570 (18), 542 (23), 483 (18), 471 (19), 439 (13), 411 (39), 383 (15), 369 (100), 351 (46), 273 (29), 247 (19), 239 (29), 215 (13), 201 (32), 189 (34), 175 (34). Anal. Calcd for $C_{43}H_{68}O_{10}$: C, 69.32%; H, 9.20%. Found C, 69.30%; H, 9.17%.

6.1.29. 28-Methyl 2,3-diacetoxymethyl 2,3-secolup-20(29)en-2,3,28 trioate (4g). Three hundred milligram (0.6 mmol) of **4b** was treated with PomCl using general procedure to give crystals of **4g** (200 mg, 52% yield): mp 126–127 °C, $[\alpha]_D$ +6.0° (*c* 0.34). IR *v* ($CHCl_3$) cm^{-1}: 1761b, 1722, 1642. ^1H NMR δ 0.92, 0.98, 1.25, (15H, all s, $5\times CH_3$), 1.68 (3H, m, H-30), 1.83-1.93 (2H, m), 2.09, 2.11 (6H, all s, $2\times CH_3$-Acm), 2.19-2.30 (3H, m), 2.37 (1H, m), 2.39 (1H, d, *J*(H-1a, H-1b) = 18.2 Hz, H-1a), 2.53 (1H, d, *J* = 18.0 Hz, H-1b), 2.99 (1H, m, H-19β), 3.66 (3H, s, OCH_3), 4.58 (1H, m, H-29 *pro-E*), 4.73 (1H, bd, *J* = 2.3 Hz, H-29 *pro-Z*), 5.65 (1H, d, *J* = 5.5 Hz), 5.72 (1H, d, *J* = 5.7 Hz), 5.74 (1H, d, *J* = 5.8 Hz), 5.76 (1H, d, *J* = 5.5 Hz, $2\times CH_2$ Acm). MS *m/z* (relative intensity): 660 (2, M^+), 600 (5), 528 (20), 500 (31), 471 (27), 441 (48), 411 (48), 383 (19), 369 (100), 351 (67), 309 (19), 273 (43), 253 (43), 227 (70), 201 (57), 187 (80), 176 (77), 135 (46), 121 (75). Anal. Calcd for $C_{37}H_{56}O_{10}$: C, 67.25%; H, 8.54%. Found C, 67.36%; H, 8.63%.

6.1.30. 1β-Hydroxy-2-oxa-3-oxolup-20(29)en-28-oic acid (5a). This product was obtained by oxidation of **3a** (1 g, 2.2 mmol) with air in a mixture of *tert*-butyl alcohol (100 mL) and *tert*-butoxide (6 g, 53 mmol) for 48 h according to lit.[9] This yielded **5a** (0.5 g, 50% yield): mp 275–276 °C (methanol). $[\alpha]_D$ −116° (*c* 0.02). IR *v* ($CHCl_3$) cm^{-1}: 3280 vb, 1693, 1643.[9]

6.1.31. Methyl 1β-hydroxy-2-oxa-2-oxolup-20(29)en-28-oate (5b). This product was obtained by oxidation of **3b** (1 g, 2.1 mmol) with air in a mixture of *tert*-butyl alcohol (100 mL) and *tert*-butoxide (6 g, 53 mmol) for 48 h according to lit.[9] This yielded **5b** (0.7 g, 70% yield): mp 144–145 °C (methanol). $[\alpha]_D$ +14° (*c* 0.56). IR *v* ($CHCl_3$) cm−1: 3200–3550, 1720, 1642.[9]

6.1.32. Reaction of lactol 5a with acetic anhydride in pyridine. Ten milliliter of acetic anhydride was added to a solution of both: **5a** (300 mg, 0.6 mmol) in pyridine (10 mL), and **5b** (300 mg, 0.6 mmol) in pyridine (10 mL). The mixture was stirred at room temperature for 3 days and worked up. Crude products were than purified by HPLC in phase 10 to give **5c** and **5e** (from **5a**) and **5d** (from **5b**).

6.1.33. 1β-Acetoxy-2-oxa-3-oxolup-20(29)en-28-oic acid (5c). It was obtained 150 mg (46% yield): mp 258–259 °C (methanol). $[\alpha]_D$ +51° (*c* 0.36). IR *v* ($CHCl_3$) cm^{-1}: 1773, 1739, 1695, 1643. ^1H NMR δ 0.98, 0.99, 1.06, 1.22, 1.30 (15H, all s, $5\times CH_3$), 1.68 (3H, m, H-30), 1.95–2.04 (2H, m), 2.16 (3H, s, OAc), 2.22 (1H, ddd, J_1 = 13.0 Hz, J_2 = 12.0 Hz, J_3 = 3.7 Hz), 2.29 (1H, dt, J_1 = 12.5 Hz, J_2 = 3.0 Hz, J_3 = 3.0 Hz), 2.97 (1H, td, *J*(H-19β, H-18α) = 11.2 Hz, *J*(H-19β, H-21α) = 11.2 Hz, *J*(H-19β, H-21β) = 4.6 Hz, H-19β), 4.63 (1H, m, H-29 *pro-E*), 4.73 (1H, bd, *J* = 2.3 Hz, H-29 *pro-Z*), 6.20 (1H, s, H-1α). MS *m/z* (relative intensity): 514 (not found, M^+),

479 (1), 455 (20), 439 (10), 426 (8), 408 (28), 393 (14), 382 (16), 367 (18), 356 (12), 340 (23), 321 (11), 311 (8), 259 (15), 241 (41), 220 (38), 201 (35), 187 (100). Anal. Calcd for $C_{31}H_{46}O_6$: C, 72.34%; H, 9.01%. Found C, 72.21%; H, 9.17%.

6.1.34. Acetyl 1β-acetoxy-2-oxa-3-oxolup-20(29)en-28-oate (5e). It was obtained 90 mg (25% yield): mp 258–259 °C (not crystallized). $[\alpha]_D$ +48° (*c* 0.21). IR *v* ($CHCl_3$) cm^{-1}: 1812, 1772, 1741, 1697, 1643. ^1H NMR δ 0.97, 1.02, 1.07, 1.23, 1.29 (15H, all s, $5 \times CH_3$), 1.67 (3H, m, H-30), 1.92–2.04 (2H, m), 2.15 (3H, s, 1-OAc), 2.23 (3H, s, 28-OAc), 2.23 (1H, m), 2.31 (1H, ddd, J_1 = 13.1 Hz, J_2 = 11.7 Hz, J_3 = 3.7 Hz), 2.94 (1H, td, *J*(H-19β, H-18α) = 10.4 Hz, *J*(H-19β, H-21α) = 10.4 Hz, *J*(H-19β, H-21β) = 4.6 Hz, H-19β), 4.64 (1H, m, H-29 *pro-E*), 4.73 (1H, bd, *J* = 2.1 Hz, H-29 *pro-Z*), 6.20 (1H, s, H-1α). MS *m*/*z* (relative intensity): 556 (not found, M^+), 515 (1), 497 (1), 469 (2), 455 (4), 439 (1), 408 (10), 393 (3), 382 (3), 367 (3), 356 (1), 340 (2), 321 (1), 311 (3), 241 (17), 220 (19), 201 (23), 187 (42), 175 (38), 107 (100). Anal. Calcd for $C_{33}H_{48}O_7$: C, 71.19%; H, 8.69%. Found C, 71.27%; H, 8.77%.

6.1.35. Methyl 1β-acetoxy-3-oxo-2-oxalup-20(29)en-28-oate (5d). It was obtained **5d** (150 mg, 45% yield): mp 201–203 °C (methanol). $[\alpha]_D$ +35° (*c* 0.29). IR *v* ($CHCl_3$) cm^{-1}: 1770sh, 1738, 1723, 1643. ^1H NMR δ 0.96, 0.97, 1.06, 1.23, 1.29 (15H, all s, $5 \times CH_3$), 1.67 (3H, m, H-30), 2.15 (3H, s, OAc), 2.20–2.28 (3H, m), 2.96 (1H, td, *J*(H-19β, H-18α) = 10.8 Hz, *J*(H-19β, H-21α) = 10.8 Hz, *J*(H-19β, H-21β) = 4.4 Hz, H-19β), 3.67 (3H, s, OCH_3), 4.61 (1H, m, H-29 *pro-E*), 4.72 (1H, bd, *J* = 2.3 Hz, H-29 *pro-Z*), 6.20 (1H, s, H-1α). MS *m*/*z* (relative intensity): 528 (2, M^+), 468 (99), 453 (14), 446 (6), 436 (12), 425 (9), 409 (46), 396 (27), 381 (23), 365 (12), 354 (45), 339 (9), 321 (18), 305 (10), 273 (12), 255 (13), 247 (24), 241 (27), 220 (39), 201 (37), 187 (100), 175 (62), 149 (49), 133 (57), 119 (70), 107 (70). Anal. Calcd for $C_{32}H_{48}O_6$: C, 72.69%; H, 9.15%. Found C, 72.59%; H, 8.97%.

6.1.36. Dimethyl 1-oxo-1,3-seco-2-norlup-20(29)en-3,28-dioate (6a). Three hundred milligram (0.6 mmol) of **5a** was treated with CH_2N_2 using general procedure and chromatographed on HPLC in phase 10 to give crystals of **6a** (100 mg, 31% yield): mp 175–177 °C (methanol). $[\alpha]_D$ −26° (*c* 0.48). IR *v* ($CHCl_3$) cm^{-1}: 1743, 1719, 1643. ^1H NMR δ 0.95, 1.03, 1.04, 1.07, 1.17 (15H, all s, $5 \times CH_3$), 1.61 (1H, t, J_1 = 11.5 Hz, J_2 = 11.5 Hz, H-18α), 1.66 (3H, m, H-30), 2.96 (1H, td, *J*(H-19β, H-18α) = 11.1 Hz, *J*(H-19β, H-21α) = 11.1 Hz, *J*(H-19β, H-21β) = 4.6 Hz, H-19β), 3.59 (3H, s, OCH_3), 3.66 (3H, s, OCH_3), 4.58 (1H, m, H-29 *pro-E*), 4.71 (1H, bd, *J* = 2.0 Hz, H-29 *pro-Z*), 9.13 (1H, s, H-1). MS *m*/*z* (relative intensity): 500 (6, M^+), 457 (1), 441 (3), 411 (5), 175 (45), 102 (70). Anal. Calcd for $C_{31}H_{48}O_5$: C, 74.36%; H, 9.66%. Found C, 74.55%; H, 9.71%.

6.1.37. Dipivaloyloxymethyl 1-oxo-1,3-seco-2-norlup-20(29)en-3,28-dioate (6b). Three hundred milligram (0.6 mmol) of **5a** was treated with PomCl using general procedure and chromatographed on HPLC in phase 15 to give crystals of **6b** (185 mg, 42% yield): mp 107–112 °C (methanol). $[\alpha]_D$ −14° (*c* 0.68). IR *v* ($CHCl_3$) cm^{-1}: 1746sh, 1705, 1643. ^1H NMR δ 0.96, 1.02, 1.05, 1.07, 1.15 (15H, all s, $5 \times CH_3$), 1.21 (9H, s, $3 \times CH_3$ Pom), 1.21 (9H, s, $3 \times CH_3$ Pom), 1.66 (3H, m, H-30), 1.79 (1H, dd, J_1 = 12.7 Hz, J_2 = 3.1 Hz), 1.82–1.95 (2H, m), 2.10 (1H, dd, J_1 = 12.5 Hz, J_2 = 2.1 Hz), 2.20–2.34 (2H, m), 2.94 (1H, td, *J*(H-19β, H-18α) = 11.1 Hz, *J*(H-19β, H-21α) = 11.1 Hz, *J*(H-19β, H-21β) = 4.5 Hz, H-19β), 4.58 (1H, m, H-29 *pro-E*), 4.70 (1H, bd, *J* = 2.1 Hz, H-29 *pro-Z*), 5.66 (1H, d, *J* = 5.4 Hz), 5.75 (1H, d, *J* = 5.3 Hz), 5.75 (1H, d, *J* = 5.5 Hz), 5.78 (1H, d, *J* = 5.3 Hz, $2 \times CH_2$ Pom), 9.22 (1H, s, H-1). MS *m*/*z* (relative intensity): 700 (not found, M^+), 673 (1), 643 (1), 601 (1), 573 (1), 543 (2), 513 (5), 485 (3), 458 (6), 440 (13), 413 (35), 259 (10), 213 (12), 187 (85), 139 (79), 109 (100). Anal. Calcd for $C_{41}H_{64}O_9$: C, 70.25%; H, 9.20%. Found C, 70.41%; H, 9.33%.

6.1.38. Diacetoxymethyl 1-oxo-1,3-seco-2-norlup-20(29)en-3,28-dioate (6c). Three hundred milligram (0.6 mmol) of **5a** was treated with AcmBr using general procedure and chromatographed on HPLC in phase 25 to give crystals of **6c** (145 mg, 37% yield): mp 40–41 °C (methanol/pentane). $[\alpha]_D$ −14° (*c* 0.36). IR *v* ($CHCl_3$) cm^{-1}: 1754sh, 1643. ^1H NMR δ 0.96, 1.03, 1.05, 1.08, 1.17 (15H, all s, $5 \times CH_3$), 1.66 (3H, m, H-30), 1.79 (1H, dd, J_1 = 12.9 Hz, J_2 = 3.1 Hz), 1.84–1.94 (2H, m), 2.10, 2.11 (3H, all s, $2 \times CH_3$ Acm) 2.20–2.29 (2H, m), 2.97 (1H, td, *J*(H-19β, H-18α) = 11.2 Hz, *J*(H-19β, H-21α) = 11.2 Hz, *J*(H-19β, H-21β) = 4.8 Hz, H-19β), 4.59 (1H, m, H-29 *pro-E*), 4.70 (1H, bd, *J* = 2.2 Hz, H-29 *pro-Z*), 5.65 (1H, d, *J* = 5.6 Hz), 5.71 (2H, d, *J* = 5.7 Hz), 5.79 (1H, d, *J* = 5.5 Hz, $2 \times CH_2$ Acm), 9.16 (1H, s, H-1). MS *m*/*z* (relative intensity): 616 (not found, M^+), 588 (1), 516 (2), 500 (4), 471 (6), 427 (2), 409 (5), 339 (47), 259 (58), 213 (49), 201 (60), 187 (100). Anal. Calcd for $C_{35}H_{52}O_9$: C, 68.16%; H, 8.50%. Found C, 68.19%; H, 8.44%.

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