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Efficient access to isomeric 2,3-dihydroxy lupanes: first synthesis of alphitolic acid

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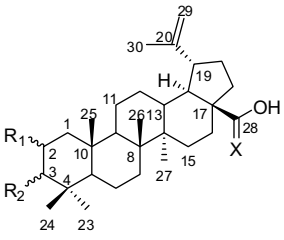
ABSTRACT

Alphitolic acid (**3**) is a naturally occurring lupane type of pentacyclic triterpene, which possesses various pharmacological properties. Efficient synthesis of **3** has been accomplished in 10 steps with an overall yield of 19% starting from the readily available diketone **11**. An alternative approach to the key intermediate **17** has also been developed, and based on this approach, **3** could be obtained in 10 steps with an overall yield of 26% starting from **11**. Moreover, seven other isomeric 2,3-dihydroxy lupanes **4**–**10** have been synthesized. The synthesized triterpenes **3**–**10** were evaluated for their inhibitory activity against rabbit muscle GPa (RMGPa), and some of them exhibited moderate inhibitory activity against RMGPa.

1. Introduction

Betulin (**1**) and betulinic acid (**2**) (Fig. 1), two well-known members of lupane type of pentacyclic triterpenes, have recently attracted much attention due to their anti-cancer and anti-HIV activities.[1] An ointment containing **2** is currently under phase II clinical trial for treatment of dysplastic nevi.[2] Previously, we reported that **1**, **2** and other naturally occurring pentacyclic triterpenes, represented a novel class of inhibitors of glycogen phosphorylases (GP).[3] Alphitolic acid (2α-hydroxybetulinic acid, **3**), which possesses various pharmacological properties such as antibacterial, anti-inflammatory, antiproliferative, antiplasmodial, anti-HIV activities,[4–8] was firstly isolated from the ethereal extract of *Alphitonia whitei* Braid.[9,10] In this study, we developed efficient synthetic approaches to **3** and related isomeric 2,3-dihydroxy lupanes **4**–**10**[11,12] so as to assist further pharmaceutical research on these biologically important molecules. To the best of our knowledge, this is the first synthesis of **3**. Moreover, the synthesized triterpenes **3**–**10** were evaluated for their inhibitory activity against rabbit muscle GPa (RMGPa).



1 R_1= H R_2= α-OH X=H_2
2 R_1= H R_2= α-OH X=O
3 R_1= β-OH R_2= α-OH X=O
4 R_1= β-OH R_2= α-OH X=H_2
5 R_1= β-OH R_2= β-OH X=O
6 R_1= β-OH R_2= β-OH X=H_2
7 R_1= α-OH R_2= β-OH X=O
8 R_1= α-OH R_2= β-OH X=H_2
9 R_1= α-OH R_2= α-OH X=O
10 R_1= α-OH R_2= α-OH X=H_2

Figure 1. Alphitolic acid (**3**) and related triterpenes.

2. Results and discussion

2.1. Chemistry

In previous studies, introduction of the 2α-hydroxy function into oleananes and ursanes could be accomplished by stereoselective hydroxylation of the corresponding 3-ketones with *m*-CPBA catalyzed by H_2SO_4, or by hydroboration–oxidation of the corresponding enol acetates.[13,14] Treatment of lupanes under the similar conditions, however, would affect the *iso*-propylene group to afford 2,30-dihydroxy compounds.[15] When using $Pb(AcO)_4$ as an oxidant to build up the 2α-hydroxy function in betulin derivatives, the *iso*-propylene group was still affected to result in 2,30-diacetyloxy products.[16] The high reactivity of the *iso*-propylene function group in lupanes thereby seemingly made it a difficult task to selectively carry out hydroxylation at C-2. Herein, we report new access to isomeric 2,3-dihydroxy lupanes **3**–**10**.

The synthetic approach to **3** is shown in Scheme 1. Treatment of diketone **11**, which was readily prepared from **1**,[17] with *tert*-butyldimethylsilylchloride (TBDMSCl) and imidazole in DMF gave enol silyl ether **12** in quantitative yield. Reduction of **12** with sodium borohydride afforded 2β-hydroxy-3β-*O*-silylated product **13** as the major product (80%), together with the 2β-*O*-silylated product **14** as a minor product (14%). Obviously, a transsilylation occurred during this reduction reaction. The migration of the TBS group from the C-2 oxygen atom to its C-3 neighbor might have taken place via an enediolate intermediate.[18] The relative configurations of **13** and **14**

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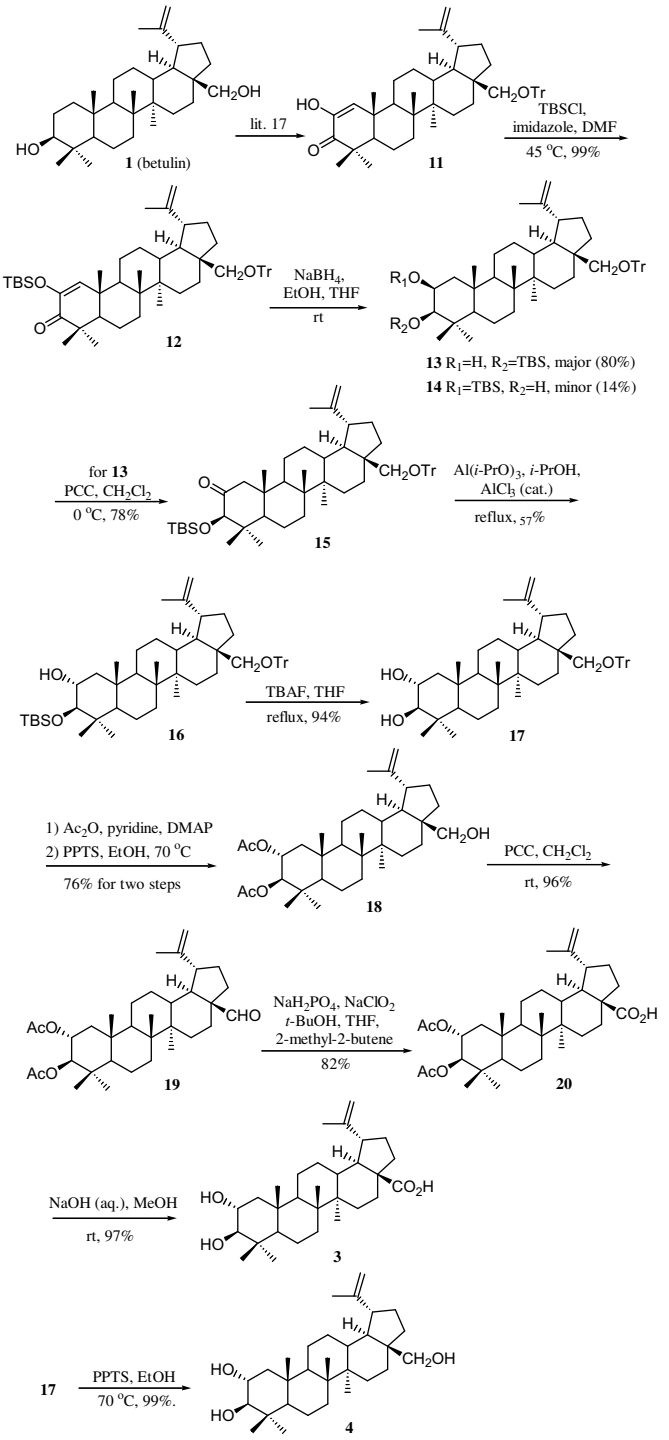
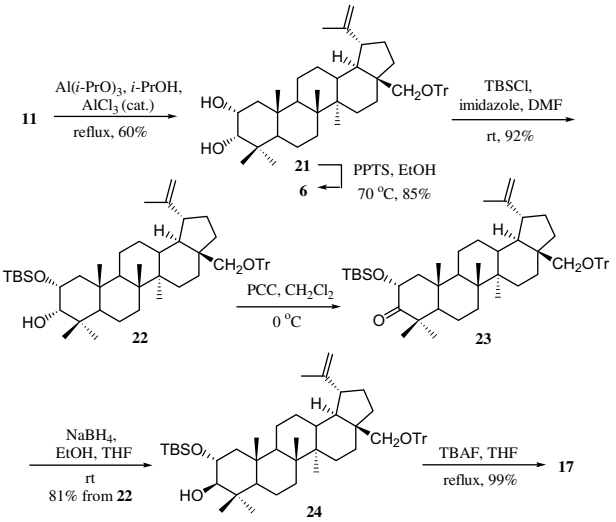


Scheme 1. Synthesis of alphitolic acid (**3**) and 2α,3β,28-lup-20(29)-en-triol (**4**).

have been confirmed by NOE experiments (see Supplementary data). It's interesting that 2β-hydroxy-3β-*O*-silylated product **13** could be obtained in high regioselectivity and stereoselectivity. Our previous studies showed that C2β–OH was likely less steric hindered than C3β–OH[21], and therefore, direct silylation of 2β,3β-diols could provide 2β-hydroxy-3β-*O*-silylated products only as the minor products, together with 2β-*O*-silylated products as the major products (>70%).[21] Oxidation of **13** with pyridinium chlorochromate (PCC) at 0 °C produced 2-ketone **15** in 78% yield. Meerwein–Pondorf reduction[19] of **15** afforded 2α-hydroxy isomer **16** as the major product (57%), together with the 2β-hydroxy isomer **13** as the minor product (38%). Removal of the TBS group in **16** with tetrabutylammonium fluoride (TBAF) in refluxing THF gave 2α,3β-diol **17** (94%). Acetylation of **17** with acetic anhydride in the presence of 4-dimethylaminopyridine (DMAP) in pyridine, followed by deprotection with PPTS afforded alcohol **18** in 76% yield for two steps. Oxidation of **18** with pyridinium chlorochromate (PCC) gave aldehyde **19** (96%), which was further oxidized with sodium chlorite ($NaClO_2$) and sodium dihydrogenophosphate in a mixture of *t*-BuOH/THF/2-methyl-2-butene[20] to furnish triterpene acid **20** (82%). Hydrolysis of **20** with aqueous sodium hydroxide in methanol gave alphitolic acid (**3**) in 97% yield. Deprotection of **17** with PPTS afforded 2α,3β,28-lup-20(29)-en-triol (**4**) in quantitative yield.

On the other hand, an alternative approach to the key intermediate **17** has also been developed. As shown in Scheme 2, Meerwein–Pondorf reduction of **11** gave 2α,3α-diol **21** as the major product (60%), together with **17** as the minor product (22%). Deprotection of **21** with PPTS afforded 2α,3α,28-lup-20(29)-en-triol (**6**) in 85% yield. Selective protection of **21** with *tert*-butyldimethylsilylchloride (TBDMSCl) and imidazole in DMF gave 2-TBS ether **22** in high yield (92%) due to a less steric hindrance at 2α-hydroxy than that at 3α-hydroxy. Oxidation of **22** with pyridinium chlorochromate (PCC) at 0 °C followed by sodium borohydride reduction afforded 3β-alcohol **24** (81%, for two steps). Deprotection of **24** with tetrabutylammonium fluoride (TBAF) in refluxing THF gave **17** (99%).

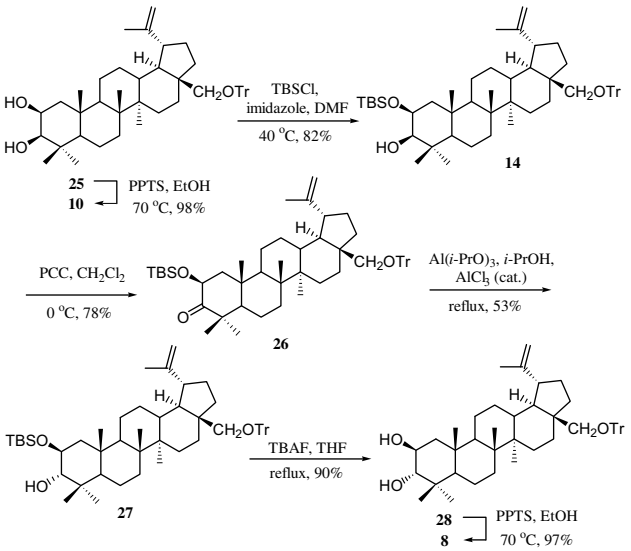


Scheme 2. Synthesis 2α,3α,28-lup-20(29)-en-triol (**6**) and an alternative approach to the key intermediate **17**.

In the above regards, two synthetic approaches to **3** have been established starting from **11**. For the first approach, which employed a reduction–transsilylation as the key step (Scheme 1), **3** was obtained in 10 steps with an overall yield of 19%. Based on the second approach, which started with stereoselective Meerwein–Pondorf reduction of **11** (Scheme 2), **3** was gained in 10 steps with a total yield of 26%.

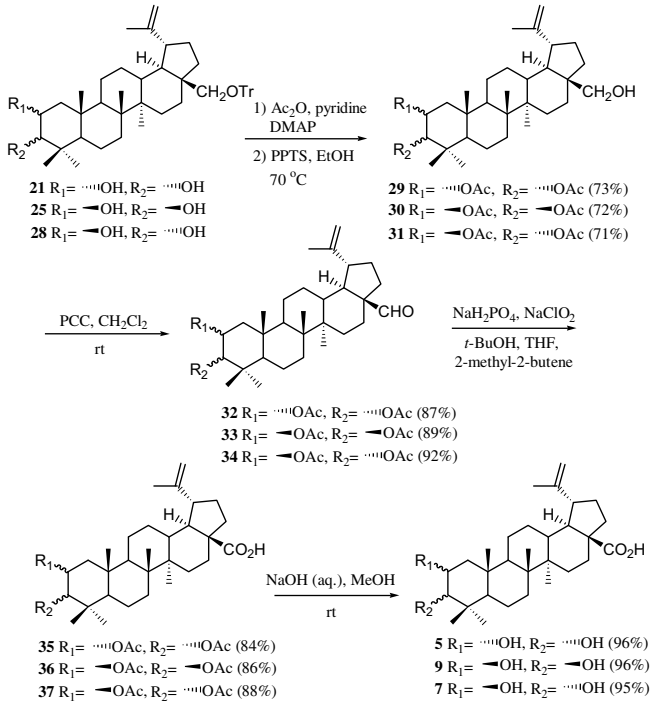
2β,3α,28-Lup-20(29)-en-triol (**8**) was prepared according to the methodology developed for preparation of bredemolic acid (Scheme 3).[21] Therefore, treatment of diol **25**[17] with *tert*-butyldimethylsilylchloride (TBDMSCl) and imidazole in DMF gave the 2-silyl ether **14** as the major product (82%), together with the 3-silyl ether **13** as a minor product (11%). Oxidation of **14** with pyridinium chlorochromate (PCC) at 0 °C followed by Meerwein–Pondorf reduction afforded alcohol **27** (41% for two steps). Deprotection of **27** with tetrabutylammonium fluoride (TBAF) in refluxing THF gave 2β,3α-diol **28** (90%). Deprotection of **28** with PPTS afforded

2β,3α-dihydroxy-lup-20(29)-en-28-ol (**8**) (97%). On the other hand, deprotection of **25** with PPTS afforded 2β,3β-dihydroxy-lup-20(29)-en-28-ol (**10**) (98%).



Scheme 3. Synthesis of 2β,3α,28-lup-20(29)-en-triol (**8**) and 2β,3β-dihydroxy-lup-20(29)-en-28-ol (**10**).

Using the same methodology for the conversion of **17** to **3** (Scheme 1), isomeric 2,3-dihydroxy lupane acids **5**, **7**, and **9** were synthesized starting from **21**, **28**, and **25**, respectively (Scheme 4). Acetylation of **21**, **25**, and **28** with acetic anhydride in the presence of 4-dimethylaminopyridine (DMAP) in anhydrous pyridine, followed by deprotection with PPTS afforded alcohol **29** (73%), **30** (72%), and **31** (71%), respectively. Oxidation of **29**, **30**, and **31** with pyridinium chlorochromate (PCC) gave aldehyde **32** (87%), **33** (89%), and **34** (92%), respectively. Oxidation of **32**, **33**, and **34** with sodium chlorite ($NaClO_2$) and sodium dihydrogenophosphate in a mixture of *t*-BuOH/THF/2-methyl-2-butene furnished triterpene acids **35** (84%), **36** (86%), and **37** (88%), respectively. Hydrolysis of



Scheme 4. Synthesis of isomeric 2,3-dihydroxy lupane acids **5**, **7**, and **9**.

35, **36**, and **37** with aqueous sodium hydroxide in methanol gave **5** (96%), **9** (96%), and **7** (95%), respectively.

2.2. Biological activity

The synthesized 2,3-dihydroxy lupanes **3**–**10** were evaluated for their inhibitory activity against rabbit muscle GPa (RMGPa). The activity of RMGPa was measured by detecting the release of phosphate from glucose-1-phosphate in the direction of glycogen synthesis.[22] The bioassay results (Table 1) showed that most of the 2,3-dihydroxy lupanes exhibited moderate inhibition against RMGPa. Naturally occurring triterpenes **3** (IC_{50} 20.7 μM) and **5** (IC_{50} 13.1 μM) were more potent inhibitors than the other synthetic triterpenes. Further biological evaluation of these triterpenes is warranted to determine their therapeutic potential.

Table 1
IC_{50} values (μM) for the inhibition of rabbit muscle GPa

Compound	GPa IC_{50}[a]
1	41.5
2	20.9
3	20.7
4	98.5
5	13.1
6	499
7	26.9
8	105.5
9	28.4
10	34.2
Caffeine	98.5

[a] Values are means of three experiments.

3. Conclusions

In summary, efficient synthesis of alphitolic acid (**3**) has been accomplished in 10 steps with an overall yield of 19% starting from the readily available diketone **11**. An alternative approach to the key intermediate **17** has also been developed, and based on this approach, **3** could be obtained in 10 steps with an overall yield of 26% starting from the same material. Seven other isomeric 2,3-dihydroxy lupanes **4**–**10** have also been synthesized. The synthesized triterpenes have been biologically evaluated as inhibitors of glycogen phosphorylase, and the result shows that naturally occurring triterpenes **3** (IC_{50} 20.7 μM) and **5** (IC_{50} 13.1 μM) are moderate GP inhibitors.

4. Experimental section

4.1. General

All commercially available solvents and reagents were used without further purification. Melting points are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively. Chemical shifts are reported as values from an internal tetramethylsilane standard. Low- and high-resolution mass spectra (LRMS and HRMS) were given with electron impact mode. Infrared spectra were recorded either on neat samples (KBr disks) or as thin film.

4.2. 28-Trityl-2-(*tert*-butyldimethylsilyloxy)-3-oxo-lup-1,20(29)-dien-28-ol (12)

Compound **11**[17] (1 g, 1.44 mmol) was dissolved in 10 mL of DMF, then imidazole (801 mg, 11.78 mmol) and *tert*-butyl dimethysilyl chloride (887 mg, 5.89 mmol) were added. The reaction mixture was stirred at 45 °C for 2 h. The mixture was cooled to rt, then 10 mL of ice water was added to the reaction mixture, the mixture

was extracted with ethyl acetate (4×50 mL). The combined extract was washed with brine, dried over anhydrous sodium sulfate, filtered, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (petroleum ether–ethyl acetate, 5:1) to give **12** as a white solid (1.15 g, 99%). Mp 247–248 °C; IR (KBr, cm^{-1}) 3068, 2949, 2859, 2352, 1681, 1673, 1620, 1453, 1381, 1254, 1216, 1061, 842, 782, 705, 632, 584; ^1H NMR (C_6D_6N) δ 0.27 (s, 3H), 0.30 (s, 3H), 0.73 (s, 3H), 0.87 (s, 3H), 1.02 (s, 9H), 1.06 (s, 9H), 1.15 (s, 3H), 1.20 (s, 3H), 1.70 (s, 3H), 2.33–2.43 (m, 3H), 3.17 and 3.39 (d, J=8.9 Hz, each 1H), 4.70 and 4.76 (br s, each 1H), 6.61 (s, 1H), 7.30–7.34 (m, 3H), 7.39–7.44 (m, 6H), 7.70–7.73 (m, 6H); ^{13}C NMR (C_6D_6N) δ −4.3, −4.1, 14.8, 16.6, 18.8, 19.3, 19.4, 20.6, 21.3, 21.9, 25.6, 26.0, 27.3, 28.3, 30.4, 30.5, 34.0, 35.6, 37.7, 39.2, 41.8, 43.0, 45.4, 45.6, 47.95, 48.02, 49.1, 53.7, 60.0, 86.5, 110.1, 127.4, 128.3, 129.3, 138.0, 145.1, 145.7, 150.8, 200.9; ESI-MS m/z: 811.4 $[M+H]^+$; HRMS calcd for $C_{55}H_{75}O_3Si$ $[M+H]^+$: 811.5485, found: 811.5480.

4.3. 28-Trityl-3β-(*tert*-butyldimethylsilyloxy)-2β-hydroxy-lup-20(29)-en-28-ol (13)

Compound **12** (240 mg, 0.30 mmol) was dissolved in THF (5 mL) and EtOH (1 mL), then $NaBH_4$ (13.23 mg, 0.35 mmol) was added, and the reaction mixture was stirred for 5 h at rt. The reaction was quenched by 1 N HCl at 0 °C, then organic solvent was removed under reduced pressure, the mixture was extracted with ethyl acetate (4×40 mL). The organic layers were combined and washed with brine, dried over anhydrous sodium sulfate, filtered, and the solvent was removed under reduced pressure, the residue was purified by column chromatography (petroleum ether/ethyl acetate, 160:1) to give 28-trityl-3β-(*tert*-butyldimethylsilyloxy)-2β-hydroxy-lup-20(29)-en-28-ol (**13**) as a white solid (193 mg, 80%). Mp 134–136 °C; IR (film, cm^{-1}) 3571, 2949, 2854, 1640, 1489, 1470, 1448, 1381, 1252, 1108, 1087, 1062, 877, 837, 744, 765, 705, 631; ^1H NMR ($CDCl_3$) δ 0.07 (s, 3H), 0.10 (s, 3H), 0.52 (s, 3H), 0.88 (s, 3H), 0.89 (s, 3H), 0.93 (s, 9H), 0.95 (s, 3H), 1.09 (s, 3H), 1.63 (s, 3H), 2.12–2.24 (m, 5H), 2.48 (br s, 1H), 2.90 and 3.13 (d, J=8.8 Hz, each 1H), 3.21 (d, J=3.9 Hz, 1H), 3.88–3.89 (m, 1H), 4.53 and 4.59 (d, J=2.1 Hz, each 1H), 7.22–7.33 (m, 9H), 7.47–7.50 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 14.7, 15.9, 17.1, 17.6, 18.2, 19.2, 20.9, 25.3, 25.9, 26.9, 29.7, 29.8, 30.0, 30.2, 34.2, 35.2, 36.6, 37.3, 38.6, 40.7, 42.6, 43.3, 47.6, 47.7, 49.0, 50.9, 55.3, 59.6, 71.6, 80.1, 85.9, 109.3, 126.8, 127.7, 128.8, 144.5, 150.8; ESI-MS m/z: 813.4 $[M-H]^-$; HRMS calcd for $C_{55}H_{78}NaO_3Si$ $[M+Na]^+$: 837.5618, found: 837.5612.

4.4. 28-Trityl-3β-(*tert*-butyldimethylsilyloxy)-lup-20(29)-en-28-ol-2-one (15)

To a solution of **13** (280 mg, 0.34 mmol) in dichloromethane was added PCC (88.36 mg, 0.41 mmol), the reaction mixture was stirred at 0 °C for 10 h, the mixture was filtered and concentrated in vacuo, the residue was purified directly by column chromatography (petroleum ether/ethyl acetate, 160:1) to give 28-trityl-3β-(*tert*-butyldimethylsilyloxy)-lup-20(29)-en-28-ol-2-one (**15**) as a white solid (217.8 mg, 78%). Mp 126–128 °C; IR (film, cm^{-1}) 2947, 1724, 1452, 1253, 1152, 1062, 835, 772, 704, 631; ^1H NMR ($CDCl_3$) δ −0.00 (s, 3H), 0.11 (s, 3H), 0.53 (s, 3H), 0.74 (s, 3H), 0.77 (s, 3H), 0.95 (s, 9H), 0.96 (s, 3H), 1.13 (s, 3H), 1.67 (s, 3H), 2.22–2.36 (m, 4H), 2.95 and 3.15 (d, J=8.8 Hz, each 1H), 3.94 (s, 1H), 4.56 and 4.62 (br s, each 1H), 7.23–7.53 (m, 15H); ^{13}C NMR ($CDCl_3$) δ −5.5, -4.2, 14.7, 15.6, 16.6, 16.7, 19.0, 19.2, 21.0, 25.1, 25.9, 27.0, 29.2, 29.7, 30.0, 30.2, 33.9, 35.2, 37.3, 41.0, 42.6, 43.4, 46.0, 46.2, 47.6, 47.7, 48.9, 50.2, 54.4, 55.4, 59.6, 84.6, 85.9, 109.5, 126.8, 127.7, 128.8, 144.5, 150.6, 209.4; ESI-MS m/z: 813.4 $[M+H]^+$; HRMS calcd for $C_{55}H_{77}O_3Si$ $[M+H]^+$: 813.5642, found: 813.5636.

4.5. 28-Trityl-3β-(*tert*-butyldimethylsilyloxy)-2α-hydroxy-lup-20(29)-en-28-ol (16)

To a solution of **15** (660 mg, 0.812 mmol) in dry isopropanol was added 12 mL of fresh prepared aluminum isopropoxide (1.54 mmol/ml) in dry isopropanol and cat. amount of aluminum chloride, the reaction mixture was stirred at 110 °C for 5 days. The reaction mixture was cooled to rt, the solvent was removed in vacuo, and the pH was adjusted to 3.0 by 1 N HCl at 0 °C. The mixture was extracted with ethyl acetate (4×40 mL). The combined extract was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 120:1) to give 28-trityl-3β-(*tert*-butyldimethylsilyloxy)-2α-hydroxy-lup-20(29)-en-28-ol (**16**) as a white solid (376 mg, 57%). Mp 101–103 °C; IR (film, cm^{-1}) 2948, 2858, 1448, 1257, 1109, 1088, 1064, 834, 774, 742, 705, 631; ^1H NMR ($CDCl_3$) δ 0.04 (s, 3H), 0.05 (s, 3H), 0.44 (s, 3H), 0.69 (s, 3H), 0.76 (s, 3H), 0.82 (s, 3H), 0.85 (s, 3H), 0.86 (s, 9H), 1.56 (s, 3H), 1.84–1.89 (m, 1H), 2.10–2.13 (m, 4H), 2.84 and 3.06 (d, J=8.8 Hz, each 1H), 2.91 (d, J=9.1 Hz, 1H), 3.50–3.55 (m, 1H), 4.46 (br s, 1H), 4.51 (d, J=1.6 Hz, 1H), 7.12–7.43 (m, 15H); ^{13}C NMR ($CDCl_3$) δ −3.2, −4.3, 14.7, 16.0, 17.0, 17.3, 18.61, 18.65, 19.1, 20.9, 25.1, 26.2, 26.9, 29.0, 30.0, 30.2, 34.2, 35.2, 37.3, 38.1, 40.2, 40.7, 42.5, 46.5, 47.6, 47.8, 49.0, 50.3, 55.4, 59.6, 69.2, 85.6, 85.9, 109.4, 126.8, 127.7, 128.8, 144.5, 150.7; ESI-MS m/z: 813.9 $[M-H]^-$; HRMS calcd for $C_{55}H_{78}NaO_3Si$ $[M+Na]^+$: 837.5618, found: 837.5612.

4.6. 28-Trityl-2α,3β-dihydroxy-lup-20(29)-en-28-ol (17) (prepared from 16)

Compound **16** (180 mg, 0.22 mmol) was dissolved in THF (12 mL) and two drops of water were added, then 0.56 mL of TBAF solution (1 M) was added to the solution, the reaction mixture was heated to reflux, and maintained for 2 h, then the solvent was removed directly, and the residue was purified directly by column chromatography (petroleum ether/ethyl acetate, 5:1) to give 28-trityl-2α,3β-dihydroxy-lup-20(29)-en-28-ol (**17**) as a white solid (145 mg, 94%). Mp 142–144 °C; IR (film, cm^{-1}) 3400, 3060, 2942, 2867, 1641, 1594, 1489, 1448, 1374, 1264, 1064, 1032, 983, 880, 774, 764, 742, 705, 631; ^1H NMR ($CDCl_3$) δ 0.50 (s, 3H), 0.79 (s, 3H), 0.83 (s, 3H), 0.89 (s, 3H), 1.00 (s, 3H), 1.63 (s, 3H), 1.95–2.00 (m, 1H), 2.12–2.28 (m, 3H), 2.91 and 3.12 (d, J=9.0 Hz, each 1H), 2.95 (d, J=9.5 Hz, 1H), 3.60–3.68 (m, 1H), 4.52 and 4.58 (d, J=2.1 Hz, each 1H), 7.22–7.50 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 14.7, 15.9, 16.5, 17.3, 18.3, 19.1, 20.8, 25.0, 26.9, 28.4, 29.9, 30.1, 34.0, 35.2, 37.2, 38.5, 39.2, 40.7, 42.5, 46.7, 47.6, 47.8, 48.9, 50.2, 55.3, 59.5, 69.2, 83.9, 85.8, 109.4, 126.8, 127.7, 128.8, 144.5, 150.7; ESI-MS m/z: 699.5 $[M-H]^-$; HRMS calcd for $C_{49}H_{64}NaO_3$ $[M+Na]^+$: 723.4753, found: 723.4747.

4.7. 2α,3β-Diacetyloxy-lup-20(29)-en-28-ol (18)

To a solution of **17** (570 mg, 0.81 mmol) in dry pyridine (3 mL) was added Ac_2O (1 mL), the reaction mixture was stirred at rt overnight. After cooling to 0 °C, 1 N HCl (25 mL) was added into the reaction mixture. The mixture was extracted with ethyl acetate (3×30 mL). The combined extract was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude product was used directly without further purification. The crude residue was dissolved in absolute ethanol (10 mL) and was then added PPTS (732 mg, 2.92 mmol). The reaction mixture was stirred at 70 °C for 8 h. After cooling to room temperature, the solvent was evaporated in vacuo. Ice water (15 mL) was added to the residue, and extracted with ethyl acetate (3×20 mL). The combined extract was washed with brine, dried over anhydrous sodium sulfate, filtered, and

concentrated in vacuo. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 10:1) to give 2α,3β-diacetyloxy-lup-20(29)-en-28-ol (**18**) as a white solid (335 mg, 76% from **17**). Mp 142–144 °C; IR (film, cm^{-1}) 3551, 3352, 2944, 2868, 1741, 1641, 1454, 1393, 1369, 1252, 1233, 1037, 882, 737, 704; ^1H NMR ($CDCl_3$) δ 0.88 (s, 3H), 0.89 (s, 3H), 0.97 (s, 6H), 1.02 (s, 3H), 1.67 (s, 3H), 1.96 (s, 3H), 2.05 (s, 3H), 2.34–2.43 (m, 1H), 3.33 and 3.78 (d, *J*=10.7 Hz, each 1H), 4.57 and 4.68 (br s, each 1H), 4.72 (d, *J*=10.4 Hz, 1H), 5.04–5.13 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.7, 16.0, 17.2, 17.4, 18.2, 19.1, 20.9, 21.0, 21.1, 25.1, 27.0, 28.3, 29.2, 29.8, 34.0, 34.1, 37.3, 38.4, 39.4, 41.0, 42.8, 44.3, 47.8, 48.8, 50.4, 55.1, 60.6, 70.3, 80.7, 109.8, 127.7, 150.3, 170.5, 170.8; ESI-MS *m/z*: 560.6 $[M+NH_4]^+$; HRMS calcd for $C_{34}H_{54}NaO_5$ $[M+Na]^+$: 565.3868, found: 565.3863.

4.8. 2α,3β-Diacetyloxy-lup-20(29)-en-28-aldehyde (19)

To a solution of compound **18** (180 mg, 0.33 mmol) in CH_2Cl_2 was added PCC (142 mg, 0.66 mmol), the reaction mixture was stirred at rt for 40 min. Silica gel was poured into the reaction mixture and the solvent was evaporated under reduced pressure to dryness. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate, 19:1) to give 2α,3β-diacetyloxy-lup-20(29)-en-28-aldehyde (**19**) as a white solid (173 mg, 96%). Mp 105–109 °C; IR (film, cm^{-1}) 3435, 2945, 2868, 1741, 1641, 1453, 1369, 1250, 1041, 737; ^1H NMR ($CDCl_3$) δ 0.87 (s, 3H), 0.88 (s, 3H), 0.91 (s, 3H), 0.97 (s, 6H), 1.69 (s, 3H), 1.96 (s, 3H), 2.04 (s, 3H), 2.85–2.87 (m, 1H), 4.62 and 4.75 (br s, each 1H), 4.72 (d, *J*=10.4 Hz, 1H), 5.04–5.13 (m, 1H), 9.67 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.2, 15.9, 17.2, 17.4, 18.2, 19.0, 20.8, 20.9, 21.0, 25.4, 28.3, 28.8, 29.2, 29.9, 33.2, 34.2, 38.4, 39.4, 40.9, 42.6, 44.3, 47.5, 48.1, 50.4, 55.1, 59.3, 70.2, 80.7, 110.2, 149.6, 170.7, 206.4; ESI-MS *m/z*: 563.6 $[M+Na]^+$; HRMS calcd for $C_{34}H_{52}NaO_5$ $[M+Na]^+$: 563.3712, found: 563.3707.

4.9. 2α,3β-Diacetyloxy-lup-20(29)-en-28-oic acid (20)

Compound **19** (130 mg, 0.24 mmol) was dissolved in 7.5 mL of *tert*-butyl alcohol, 1.5 mL of distilled THF, and 2.25 mL of 2-methyl-2-butene. The solution was stirred and cooled in an ice-bath. Then 5.4 mL of freshly prepared aqueous solution of $NaH_2PO_4/NaClO_2$ (378.7 mg/291 mg) was slowly added to the solution and the mixture was stirred for 15 min. The mixture was then raised to rt and stirred for 1 h. Finally, the mixture was poured into 10 mL of saturated solution of NH_4Cl and extracted with CH_2Cl_2. The combined extract was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 93:7) to give 2α,3β-diacetyloxy-lup-20(29)-en-28-oic acid (**20**) as a white solid (110 mg, 82%). Mp 240–243 °C; IR (film, cm^{-1}) 2947, 2869, 1741, 1694, 1454, 1369, 1250, 1041, 882, 738; ^1H NMR ($CDCl_3$) δ 0.88 (s, 6H), 0.94 (s, 3H), 0.97 (s, 6H), 1.74 (s, 3H), 1.97 (s, 3H), 2.05 (s, 3H), 2.12–2.30 (m, 3H), 2.97–3.04 (m, 1H), 4.61 and 4.74 (br s, each 1H), 4.73 (d, *J*=10.2 Hz, 1H), 5.05–5.14 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.6, 16.0, 17.2, 17.4, 18.2, 19.4, 20.9, 21.1, 25.4, 28.3, 29.7, 30.6, 32.2, 34.2, 37.0, 38.4, 39.4, 40.8, 42.5, 44.3, 46.9, 49.3, 50.5, 55.1, 56.3, 70.3, 80.7, 109.8, 150.2, 170.5, 170.8, 180.6; ESI-MS *m/z*: 555.3 $[M-H]^-$; HRMS calcd for $C_{34}H_{52}NaO_6$ $[M+Na]^+$: 579.3661, found: 579.3656.

4.10. 2α,3β-Dihydroxylup-20(29)-en-28-oic acid (3)

Compound **20** (100 mg, 0.18 mmol) was dissolved in 5 mL of MeOH, then 1 mL of 4 N NaOH aq was added dropwise. The reaction mixture was stirred at rt for 2 h. The solvent was removed in vacuo, and the pH was adjusted to 3.0 by 1 N HCl at 0 °C. The mixture was extracted with ethyl acetate (4×20 mL). The combined extract was washed with brine, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 4:1) to give 2α,3β-dihydroxylup-20(29)-en-28-oic acid (**3**) as a white solid (82 mg, 97%). Mp 310–314 °C (lit.[23] mp 275–278 °C); $[\alpha]_D^{20}$ −2.7 (*c* 0.39, pyridine) (lit.[23] $[\alpha]_D^{20}$ −4.0 (*c* 1.0, pyridine)); IR (KBr, cm^{-1}) 3472, 2944, 2871, 2376, 1701, 1685, 1560, 1458, 1375, 1221, 1182, 1076, 1047, 964, 885; ^1H NMR (C_5D_5N) δ 0.92 (s, 3H), 1.06 (s, 9H), 1.26 (s, 3H), 1.79 (s, 3H), 1.87–1.95 (m, 2H), 2.23–2.34 (m, 3H), 2.61–2.78 (m, 2H), 3.39 (d, *J*=9.4 Hz, 1H), 3.48–3.57 (m, 1H), 4.05–4.13 (m, 1H), 4.77 and 4.93 (d, *J*=2.2 Hz, each 1H); ^{13}C NMR (C_5D_5N) δ 14.9, 16.5, 17.4, 17.7, 18.8, 19.5, 21.4, 26.1, 29.2, 30.2, 31.2, 32.9, 34.8, 37.6, 38.6, 38.8, 39.9, 41.2, 42.9, 47.8, 48.2, 49.8, 51.0, 56.1, 56.7, 68.9, 83.8, 109.9, 151.3, 179.1; ESI-MS *m/z*: 471.4 $[M-H]^-$; HRMS calcd for $C_{30}H_{48}NaO_4$ $[M+Na]^+$: 495.3450, found: 495.3445.

4.11. 2α,3β,28-Lup-20(29)-en-triol (4)

To a solution of **17** (80 mg, 0.11 mmol) in absolute ethanol (5 mL) was added PPTS (114 mg, 0.46 mmol). The reaction mixture was stirred at 70 °C for 8 h. After cooling to room temperature, the solvent was evaporated in vacuo. Ice water (20 mL) was added to the residue, and extracted with ethyl acetate (3×25 mL). The combined extract was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 3:1) to give 2α,3β,28-lup-20(29)-en-triol (**4**) as a white solid (52 mg, 99%). Mp 254–256 °C (lit.[12] mp 258–260 °C); $[\alpha]_D^{20}$ +10.5 (*c* 0.24, $CHCl_3$) (lit.[12] $[\alpha]_D^{20}$ +11 (*c* 0.59, $CHCl_3$–MeOH 9:1)); IR (film, cm^{-1}) 2918, 2861, 2398, 2298, 1726, 1641, 1459, 1363, 1260, 1100, 1026, 880, 745, 571; ^1H NMR ($CDCl_3$) δ 0.83 (s, 3H), 0.93 (s, 3H), 1.01 (s, 3H), 1.04 (s, 3H), 1.06 (s, 3H), 1.71 (s, 3H), 1.92–2.07 (m, 4H), 2.40–2.42 (m, 1H), 3.00 (d, *J*=9.5 Hz, 1H), 3.37 (d, *J*=10.8 Hz, 1H), 3.82 (dd, *J*=1.7, 10.8 Hz, 1H), 3.65–3.70 (m, 1H), 4.61 (dd, *J*=1.4, 2.2 Hz, 1H), 4.71 (d, *J*=2.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 14.8, 16.1, 16.5, 17.4, 18.4, 19.1, 21.0, 25.3, 27.2, 28.5, 29.3, 29.9, 34.0, 34.3, 37.4, 39.2, 41.1, 42.9, 46.9, 47.9, 48.9, 50.5, 53.6, 60.7, 69.3, 77.2, 84.0, 109.7, 150.4; ESI-MS *m/z*: 939.8 $[2M+Na]^+$; HRMS calcd for $C_{30}H_{50}O_3$: 458.3760, found: 458.3764.

4.12. 28-Trityl-2α, 3α-dihydroxylup-20(29)-en-28-ol (21)

To a solution of **11** (1.76 g, 2.53 mmol) in dry isopropanol were added 20 mL of fresh prepared aluminum isopropoxide (1.54 mmol/ml) in dry isopropanol and cat. amount of aluminum chloride, the reaction mixture was stirred at 110 °C for 16 h. The reaction mixture was cooled to rt, the solvent was removed in vacuo, and the pH was adjusted to 3.0 by 1 N HCl at 0 °C. The mixture was extracted with ethyl acetate (4×40 mL). The combined extract was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, filtered, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 5:1) to give diol **21** as a white solid (1.06 g, 60%). Mp 179–181 °C; IR (film, cm^{-1}) 3430, 2941, 2868, 2360, 2337, 1555, 1453, 1063, 1036, 759, 704; ^1H NMR ($CDCl_3$) δ 0.52 (s, 3H), 0.82 (s, 3H), 0.84 (s, 3H), 0.92 (s, 3H), 1.00 (s, 3H), 1.64 (s, 3H), 2.18–2.22 (m, 4H), 2.92 and 3.14 (d, *J*=8.8 Hz, each 1H), 3.41 (d, *J*=2.5 Hz, 1H), 3.95 (m, 1H), 4.53 and 4.59 (br s, each 1H), 7.21–7.34 (m, 9H), 7.49–7.51 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 14.8, 15.9, 17.0, 18.0, 19.1, 20.7, 21.6, 25.1, 26.9, 28.5, 29.9, 30.2, 33.9, 35.2, 37.2, 38.3, 38.5, 40.8, 42.1, 42.6, 47.6, 47.8, 48.1, 48.9, 50.0, 59.6, 66.7, 79.0, 85.9, 109.4, 126.8, 127.7, 128.8, 144.5, 150.8; ESI-MS *m/z*: 699.5 $[M-H]^-$; HRMS calcd for $C_{49}H_{64}NaO_3$ $[M+Na]^+$: 723.4753, found: 723.4748.

4.13. 2α,3α,28-Lup-20(29)-en-triol (6)

To a solution of **21** (70 mg, 0.1 mmol) in absolute ethanol (2 mL) was added PPTS (99.8 mg, 0.4 mmol). The reaction mixture was stirred at 70 °C for 8 h. After cooling to room temperature, the solvent was evaporated in vacuo. Ice water (20 mL) was added to the residue, and extracted with ethyl acetate (3×20 mL). The combined extract was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 5:1) to give 2α,3α,28-lup-20(29)-en-triol (**6**) as a white solid (38.8 mg, 85%). Mp 242–244 °C; $[\alpha]_D^{20}$ +14.4 (*c* 0.20, $CHCl_3$); IR (film, cm^{-1}) 3392, 2946, 2870, 1455.9, 1375, 1032, 993.3, 884; ^1H NMR ($CDCl_3$) δ 0.84 (s, 3H), 0.93 (s, 3H), 0.99 (s, 3H), 1.00 (s, 3H), 1.01 (s, 3H), 1.68 (s, 3H), 2.34–2.43 (m, 1H), 3.34 (d, *J*=10.9 Hz, 1H), 3.79 (dd, *J*=10.9, 1.4 Hz, 1H), 3.41 (d, *J*=2.7 Hz, 1H), 3.94–4.00 (m, 1H), 4.58 (dd, *J*=2.0, 1.3 Hz, 1H), 4.68 (d, *J*=2.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 14.8, 16.0, 17.1, 18.0, 19.1, 20.8, 21.6, 25.1, 27.0, 28.4, 29.2, 29.8, 33.96, 34.00, 37.2, 38.3, 38.6, 41.1, 42.2, 42.8, 47.8, 48.2, 48.7, 50.1, 60.6, 66.6, 79.0, 109.7, 150.4; ESI-MS *m*/*z*: 457.4 $[M-H]^-$; HRMS calcd for $C_{30}H_{50}O_3$: 458.3760, found: 458.3761.

4.14. 28-Trityl-2α-(*tert*-butyldimethylsilyloxy)-3α-hydroxy-lup-20(29)-en-28-ol (22)

Compound **21** (300 mg, 0.43 mmol) was dissolved in 8 mL DMF, then imidazole (234.2 mg, 3.44 mmol) and TBSCl (259.1 mg, 1.72 mmol) were added, the reaction mixture was stirred at rt for 3 h, the reaction was quenched by pouring 30 mL ice water to the reaction mixture, then EtOAc extracted four times, the combined organic layer was washed by brine and dried over anhydrous sodium sulfate, the residue was purified by column chromatography (petroleum ether/ethyl acetate, 20:1) to give 28-trityl-2α-(*tert*-butyldimethylsilyloxy)-3α-hydroxy-lup-20(29)-en-28-ol (**22**) as a white solid (320 mg, 92%). Mp 264–265 °C; IR (film, cm^{-1}) 3587, 2948, 2860, 1641, 1591, 1489, 1448, 1375, 1255, 1065, 997, 901, 836, 775, 705, 631, 583; ^1H NMR ($CDCl_3$) δ 0.04 (s, 3H), 0.05 (s, 3H), 0.50 (s, 3H), 0.78 (s, 3H), 0.81 (s, 3H), 0.87 (s, 9H), 0.89 (s, 3H), 1.00 (s, 3H), 1.63 (s, 3H), 2.13–2.22 (m, 3H), 2.45 (br s, 1H), 2.90 and 3.14 (d, *J*=8.9 Hz, each 1H), 3.25 (d, *J*=2.7 Hz, 1H), 3.93–4.00 (m, 1H), 4.51 (br s, 1H), 4.57 (d, *J*=2.3 Hz, 1H), 7.22–7.50 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 14.8, 15.9, 17.2, 17.9, 18.0, 19.0, 20.7, 21.8, 25.1, 25.8, 26.9, 28.4, 29.9, 30.2, 33.9, 35.2, 37.2, 37.8, 38.3, 40.8, 42.2, 42.6, 47.6, 47.8, 48.9, 49.9, 59.6, 68.1, 79.1, 85.9, 109.3, 126.8, 127.7, 128.8, 144.5, 150.9; ESI-MS *m*/*z*: 813.5 $[M-H]^-$; HRMS calcd for $C_{55}H_{79}O_3Si$ $[M+H]^+$: 815.5798, found: 815.5793.

4.15. 28-Trityl-2α-(*tert*-butyldimethylsilyloxy)-lup-20(29)-en-28-ol-3-one (23)

Compound **22** (274 mg, 0.34 mmol) was dissolved in dichloromethane (5 mL) and cooled to 0 °C, then PCC (88 mg, 0.41 mmol) was added. Then reaction mixture was stirred overnight. The reaction mixture was filtered, and the solvent was removed under reduced pressure, the residue was purified by column chromatography (petroleum ether/ethyl acetate, 150:1) to give 28-trityl-2α-(*tert*-butyldimethylsilyloxy)-lup-20(29)-en-28-ol-3-one (**23**) as a white solid (235 mg, 86%). Mp 145–146 °C; IR (film, cm^{-1}) 2948, 2861, 1722, 1489, 1449, 1374, 1250, 1121, 1064, 882, 837, 775, 705, 632; ^1H NMR ($CDCl_3$) δ −0.01 (s, 6H), 0.11 (s, 3H), 0.56 (s, 3H), 0.86 (s, 3H), 0.88 (s, 9H), 1.03 (s, 3H), 1.06 (s, 3H), 1.08 (s, 3H), 1.63 (s, 3H), 2.16–2.24 (m, 4H), 2.92 and 3.13 (d, *J*=9.0 Hz, each 1H), 4.49–4.58 (m, 3H), 7.20–7.50 (m, 15H); ^{13}C NMR ($CDCl_3$) δ −5.5, −4.6, 14.6, 16.1, 16.8, 18.5, 19.0, 19.2, 21.1, 21.6, 25.1, 25.2, 25.8, 26.9, 29.9, 30.2, 34.0, 35.2, 37.3, 37.9, 40.8, 42.6, 47.6, 47.8, 48.3, 48.9, 50.0, 50.4, 56.7, 59.6, 71.3, 109.5, 126.8, 127.7, 128.8, 144.5, 150.7, 214.0; ESI-MS *m*/*z*: 813.4 $[M+H]^+$; HRMS calcd for $C_{55}H_{77}O_3Si$ $[M+H]^+$: 813.5642, found: 813.5636.

4.16. 28-Trityl-2α-(*tert*-butyldimethylsilyloxy)-3β-hydroxy-lup-20(29)-en-28-ol (24)

Compound **23** (222 mg, 0.27 mmol) was dissolved in THF (5 mL) and EtOH (1 mL), the solution was cooled to 0 °C, then $NaBH_4$ (14.4 mg, 0.38 mmol) was added, and the reaction mixture was stirred for 5 h. The reaction was quenched by 1 N HCl at 0 °C, then organic solvent was removed under reduced pressure. The mixture was extracted with ethyl acetate. The organic layers were combined and washed with brine, dried over anhydrous sodium sulfate, filtered, and the solvent was removed under reduced pressure, the residue was purified by column chromatography (petroleum ether/ethyl acetate, 150:1) to give 28-trityl-2α-(*tert*-butyldimethylsilyloxy)-3β-hydroxy-lup-20(29)-en-28-ol (**24**) as a white solid (209 mg, 94%). Mp 250–251 °C; IR (film, cm^{-1}) 3594, 2947, 2859, 1489, 1448, 1252, 1107, 1067, 882, 837, 775, 705, 631; ^1H NMR ($CDCl_3$) δ 0.05 (s, 3H), 0.07 (s, 3H), 0.51 (s, 3H), 0.80 (s, 3H), 0.81 (s, 3H), 0.87 (s, 3H), 0.88 (s, 9H), 1.01 (s, 3H), 1.64 (s, 3H), 2.17–2.24 (m, 3H), 2.91 and 3.14 (d, *J*=9.0 Hz, each 1H), 2.95 (d, *J*=9.3 Hz, 1H), 3.61–3.69 (m, 1H), 4.53 and 4.58 (br s, each 1H), 7.20–7.50 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 14.7, 15.6, 16.0, 16.6, 17.3, 18.0, 18.1, 19.1, 20.9, 25.2, 25.9, 26.9, 28.6, 30.0, 30.2, 34.1, 35.2, 37.2, 38.6, 38.8, 40.7, 42.6, 47.4, 47.6, 47.8, 48.9, 50.1, 55.3, 59.6, 71.0, 83.2, 109.3, 126.8, 127.7, 128.8, 144.5, 150.9; ESI-MS *m*/*z*: 813.4 $[M-H]^-$; HRMS calcd for $C_{51}H_{69}O_3Si$ [M−*t*-Bu]: 757.5016, found: 757.5021.

4.17. 28-Trityl-2α,3β-dihydroxy-lup-20(29)-en-28-ol (17) (prepared from 24)

Compound **24** (110 mg, 0.135 mmol) was dissolved in THF (5 mL) and one drop of water was added, then 0.4 mL of TBAF solution (1 M) was added to the solution, the reaction mixture was heated to refluxing, and maintained for 2 h, then the solvent was removed directly, and the residue was purified directly by column chromatography (petroleum ether/ethyl acetate, 4:1) to give 28-trityl-2α,3β-dihydroxy-lup-20(29)-en-28-ol (**17**) as a white solid (94 mg, 99%).

4.18. 2β,3β,28-Lup-20(29)-en-triol (10)

To a solution of **25**[17] (320 mg, 0.46 mmol) in absolute ethanol (5 mL) was added PPTS (456 mg, 1.82 mmol). The reaction mixture was stirred at 70 °C for 8 h. After cooling to room temperature, the solvent was evaporated in vacuo. Ice water (50 mL) was added to the residue, and extracted with ethyl acetate (3×35 mL). The combined extract was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 3:1) to give 2β,3β,28-lup-20(29)-en-triol (**10**) as a white solid (205 mg, 98%). Mp 194–195 °C; $[\alpha]_D^{20}$ +32.6 (*c* 0.29, $CHCl_3$); IR (film, cm^{-1}) 3353, 2938, 1742, 1609, 1509, 1447, 1364, 1219, 1031, 877, 757; ^1H NMR ($CDCl_3$) δ 0.97 (s, 3H), 0.98 (s, 3H), 0.99 (s, 3H), 1.04 (s, 3H), 1.14 (s, 3H), 1.68 (s, 3H), 1.71–1.96 (m, 3H), 2.04–2.17 (m, 3H), 2.34–2.40 (m, 1H), 3.19 (br s, 1H), 3.33 and 3.80 (d, *J*=10.8 Hz, each 1H), 4.08 (br s, 1H), 4.58 and 4.69 (br s, each 1H); ^{13}C NMR ($CDCl_3$) δ 14.7, 16.0, 17.1, 18.1, 19.1, 21.0, 25.3, 27.0, 29.2, 29.6, 29.8, 34.0, 34.2, 36.9, 37.3, 38.2, 41.1, 42.9, 44.5, 47.8, 48.8, 50.9, 55.3, 60.6, 71.2, 78.5, 109.7, 150.4; ESI-MS *m*/*z*: 481.4 $[M+Na]^+$; HRMS calcd for $C_{30}H_{50}O_3$: 458.3760, found: 458.3762.

4.19. 28-Trityl-2β-(*tert*-butyldimethylsilyloxy)-3β-hydroxy-lup-20(29)-en-28-ol (14)

Compound **25** (1.57 g, 2.2 mmol) was dissolved in 28 mL of DMF, then imidazole (1.20 g, 17.6 mmol) and *tert*-butyldimethysilyl chloride (1.36 g, 9.02 mmol) were added. The reaction mixture was stirred at 40 °C for 2 h. The mixture was cooled to rt, and then 10 mL of ice water was added to the reaction mixture, the mixture was extracted with ethyl acetate (4×30 mL). The combined extract was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, filtered, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 160:1) to give 28-trityl-2β-(*tert*-butyldimethylsilyloxy)-3β-hydroxy-lup-20(29)-en-28-ol (**14**) as a white solid (1.49 g, 82%). Mp 128–130 °C; IR (film, cm^{-1}) 3580, 2949, 2854, 1644, 1489, 1448, 1375, 1254, 1084, 1065, 1035, 976, 897, 836, 775, 765, 706, 631; ^1H NMR ($CDCl_3$) δ 0.05 (s, 3H), 0.07 (s, 3H), 0.49 (s, 3H), 0.88 (s, 9H), 0.89 (s, 3H), 0.92 (s, 3H), 0.96 (s, 3H), 1.03 (s, 3H), 1.62 (s, 3H), 1.96–2.21 (m, 5H), 2.91 and 3.10 (d, J=8.7 Hz, each 1H), 3.01 (br s, 1H), 4.02–4.03 (m, 1H), 4.51 (br s, 1H), 4.57 (d, J=1.8 Hz, 1H), 7.22–7.32 (m, 9H), 7.47–7.50 (m, 6H); ^{13}C NMR ($CDCl_3$) δ −5.2, −3.9, 14.9, 16.2, 17.0, 17.2, 18.3, 19.3, 21.1, 25.4, 26.1, 27.0, 29.8, 30.0, 30.1, 30.4, 34.3, 35.5, 37.1, 37.5, 38.7, 40.9, 42.8, 45.3, 47.8, 48.0, 49.1, 50.8, 55.5, 59.8, 72.4, 78.4, 109.6, 127.1, 128.0, 129.0, 144.8, 151.1; ESI-MS m/z: 813.9 $[M-H]^-$; HRMS calcd for $C_{55}H_{78}NaO_3Si$ $[M+Na]^+$: 837.5617, found: 837.5612.

4.20. 28-Trityl-2β-(*tert*-butyldimethylsilyloxy)-lup-20(29)-en-28-ol-3-one (26)

To a solution of **14** (1.8 g, 2.2 mmol) in dichloromethane was added PCC (0.57 g, 2.65 mol), the reaction mixture was stirred at 0 °C for 12 h, the mixture was filtered and concentrated in vacuo, the residue was purified directly by column chromatography (petroleum ether/ethyl acetate, 150:1) to give 28-trityl-2β-(*tert*-butyldimethylsilyloxy)-lup-20(29)-en-28-ol-3-one (**26**) as a white solid (1.40 g, 78%). Mp 162–164 °C; IR (film, cm^{-1}) 2949, 2863, 1726, 1448, 1385, 1250, 1064, 991, 837, 741, 706, 632; ^1H NMR ($CDCl_3$) δ −0.01 (s, 3H), 0.12 (s, 3H), 0.49 (s, 3H), 0.68 (s, 3H), 0.87 (s, 9H), 0.95 (s, 3H), 1.05 (s, 3H), 1.07 (s, 3H), 1.64 (s, 3H), 2.00–2.07 (m, 1H), 2.14–2.25 (m, 3H), 2.91 and 3.12 (d, J=8.8 Hz, each 1H), 4.52 and 4.58 (br s, each 1H), 4.62–4.69 (m, 1H), 7.19–7.49 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 14.7, 15.2, 18.6, 19.1, 19.4, 20.0, 21.9, 25.4, 25.8, 26.9, 29.7, 29.9, 30.1, 32.8, 35.2, 37.1, 37.6, 40.5, 42.6, 46.2, 47.6, 47.7, 48.9, 49.8, 51.8, 52.0, 59.6, 70.9, 109.4, 126.8, 127.7, 128.8, 144.4, 150.7; ESI-MS m/z: 813.4 $[M+H]^+$; HRMS calcd for $C_{55}H_{77}O_3Si$ $[M+H]^+$: 813.5642, found: 813.5636.

4.21. 28-Trityl-2β-(*tert*-butyldimethylsilyloxy)-3α-hydroxy-lup-20(29)-en-28-ol (27)

To a solution of **26** (600 mg, 0.74 mmol) in dry isopropanol were added 8 mL of fresh prepared aluminum isopropoxide (1.54 mmol/ml) in dry isopropanol and cat. amount of aluminum chloride, the reaction mixture was stirred at 110 °C for 18 h. The reaction mixture was cooled to rt, the solvent was removed in vacuo, and the pH was adjusted to 3.0 by 1 N HCl at 0 °C. The mixture was extracted with ethyl acetate (4×40 mL). The combined extract was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, filtered, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 150:1) to give 28-trityl-2β-(*tert*-butyldimethylsilyloxy)-3α-hydroxy-lup-20(29)-en-28-ol (**27**) as a white solid (318.8 mg, 53%). Mp 136–138 °C; IR (film, cm^{-1}) 3395, 2946, 2861, 1722, 1594, 1449, 1384, 1250, 1087, 1064, 835, 774, 705, 632; ^1H NMR ($CDCl_3$) δ 0.05 (s, 3H), 0.06 (s, 3H), 0.50 (s, 3H), 0.87 (s, 12H), 0.89 (s, 3H), 0.96 (s, 3H), 1.00 (s, 3H), 1.65 (s, 3H), 2.16–2.24 (m, 4H), 2.91 and 3.13 (d, J=8.8 Hz, each 1H), 3.54 (d, J=8.9 Hz, 1H), 3.68–3.73 (m, 1H), 4.54 and 4.59 (d, J=2.1 Hz, each 1H), 7.22–7.50 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 14.7, 15.7, 18.0, 19.2, 19.4, 20.7, 21.4, 23.0, 24.8, 25.4, 25.8, 26.9, 30.0, 30.1, 33.4, 35.2, 37.1, 37.5, 37.7, 40.8, 42.6, 47.4, 47.6, 47.8, 48.9, 50.4, 51.1, 59.6, 71.3, 85.9, 109.3, 126.8, 127.7, 128.8, 144.5, 150.9; ESI-MS m/z: 813.8 $[M-H]^-$; HRMS calcd for $C_{55}H_{78}NaO_3Si$ $[M+Na]^+$: 837.5617, found: 837.5612.

4.22. 28-Trityl-2β,3α-dihydroxy-lup-20(29)-en-28-ol (28)

Compound **27** (1.42 g, 1.74 mmol) was dissolved in THF (95 mL) and 14 drops of water were added, then TBAF (0.68 g, 2.60 mmol) in THF was added to the solution, the reaction mixture was heated to reflux, and maintained for 2 h, then the solvent was removed directly, and the residue was purified directly by column chromatography (petroleum ether/ethyl acetate, 4:1) to give 28-trityl-2β,3α-dihydroxy-lup-20(29)-en-28-ol (**28**) as a white solid (1.10 mg, 90%). Mp 165–167 °C; IR (film, cm^{-1}) 3400, 2939, 2868, 1637, 1489, 1448, 1374, 1264, 1063, 987, 883, 764, 742, 706, 631; ^1H NMR ($CDCl_3$) δ 0.50 (s, 3H), 0.88 (s, 3H), 0.89 (s, 3H), 0.93 (s, 3H), 0.98 (s, 3H), 1.63 (s, 3H), 2.16–2.20 (m, 3H), 2.90 and 3.12 (d, J=8.8 Hz, each 1H), 3.59–3.70 (m, 2H), 4.52 and 4.58 (br s, each 1H), 7.20–7.50 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 14.7, 15.6, 19.1, 19.9, 21.6, 23.2, 23.4, 25.4, 26.9, 30.0, 30.1, 33.3, 35.2, 37.59, 37.63, 40.8, 42.6, 47.6, 47.8, 48.0, 48.9, 51.0, 51.1, 59.6, 69.1, 78.1, 109.4, 126.8, 127.7, 128.8, 129.7, 144.5, 150.7; ESI-MS m/z: 699.5 $[M-H]^-$; HRMS calcd for $C_{49}H_{64}NaO_3$ $[M+Na]^+$: 723.4753, found: 723.4748.

4.23. 2β,3α,28-Lup-20(29)-en-triol (8)

To a solution of **28** (160 mg, 0.23 mmol) in absolute ethanol (5 mL) was added PPTS (228 mg, 0.91 mmol). The reaction mixture was stirred at 70 °C for 8 h. After cooling to room temperature, the solvent was evaporated in vacuo. Ice water (20 mL) was added to the residue, and extracted with ethyl acetate (3×25 mL). The combined extract was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 3:1) to give 2β,3α,28-lup-20(29)-en-triol (**8**) as a white solid (101.6 mg, 97%). Mp 230–232 °C; $[\alpha]_D^{20}$ +46.6 (*c* 0.259, $CHCl_3$); IR (KBr, cm^{-1}) 3373, 2934, 2865, 2391, 2292, 1741, 1723, 1640, 1453, 1410, 1387, 1372, 1240, 1108, 1077, 1043, 1023, 978, 880, 560, 544, 517; ^1H NMR (C_5D_5N) δ 0.98 (s, 3H), 1.00 (s, 3H), 1.18 (s, 3H), 1.23 (s, 3H), 1.26 (s, 3H), 1.75 (s, 3H), 2.00–2.17 (m, 2H), 2.36–2.44 (m, 2H), 2.55–2.64 (m, 1H), 3.64 and 4.07 (d, J=10.8 Hz, each 1H), 3.98 (d, J=8.4 Hz, 1H), 4.24–4.31 (m, 1H), 4.72 (br s, 1H), 4.87 (d, J=2.3 Hz, 1H); ^{13}C NMR (C_5D_5N) δ 14.9, 16.0, 19.3, 19.9, 20.9, 21.8, 23.5, 25.9, 26.2, 27.5, 30.0, 30.4, 34.1, 34.9, 37.8, 37.9, 38.1, 41.4, 43.1, 47.2, 48.3, 48.6, 49.1, 50.9, 51.4, 59.4, 70.3, 78.2, 109.9, 151.2; ESI-MS m/z: 476.4 $[M+NH_4]^+$; HRMS calcd for $C_{30}H_{50}NaO_3$ $[M+Na]^+$: 481.3657, found: 481.3652.

4.24. 2α,3α-Diacetyloxy-lup-20(29)-en-28-ol (29)

To a solution of **21** (500 mg, 0.713 mmol) in dry pyridine (3 mL) was added Ac_2O (1 mL), the reaction mixture was stirred at rt overnight. After cooling to 0 °C, 1 N HCl (25 mL) was added into the reaction mixture. The mixture was extracted with ethyl acetate (3×30 mL). The combined extract was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude product was used directly without further purification. The crude residue was dissolved in absolute ethanol (10 mL) and was then added PPTS (637 mg, 2.55 mmol). The reaction mixture was stirred at 70 °C for 8 h. After cooling to room temperature, the solvent was evaporated

in vacuo. Ice water (15 mL) was added to the residue, and extracted with ethyl acetate (3×20 mL). The combined extract was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 20:1) to give 2α,3α-diacetyloxy-lup-20(29)-en-28-ol (**29**) as a white solid (282 mg, 73%). Mp 233–235 °C; IR (film, cm^{-1}) 3551, 2943, 2870, 1745, 1453, 1374, 1252, 1035, 882, 737; ^1H NMR ($CDCl_3$) δ 0.86 (s, 3H), 0.95 (s, 3H), 0.97 (s, 3H), 1.03 (s, 6H), 1.68 (s, 3H), 1.94 (s, 3H), 2.12 (s, 3H), 2.33–2.43 (m, 1H), 3.34 and 3.80 (d, *J*=11.1 Hz, each 1H), 4.58 (dd, *J*=2.2, 1.4 Hz, 1H), 4.68 (d, *J*=2.2 Hz, 1H), 4.95 (d, *J*=2.3 Hz, 1H), 5.19–5.25 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 15.0, 16.0, 17.0, 17.8, 19.1, 20.8, 21.0, 21.1, 21.4, 25.1, 27.1, 27.7, 29.2, 29.8, 34.0, 37.2, 38.2, 38.7, 39.2, 41.2, 42.9, 47.7, 47.8, 48.8, 49.8, 50.3, 60.6, 68.4, 77.2, 109.8, 150.4, 170.4, 170.6; ESI-MS *m/z*: 565.4 $[M+Na]^+$; HRMS calcd for $C_{34}H_{54}NaO_5$ $[M+Na]^+$: 565.3868, found: 565.3864.

4.25. 2β,3β-Diacetyloxy-lup-20(29)-en-28-ol (30)

Following the procedure described for preparation of **29**, 2β,3β-diacetyloxy-lup-20(29)-en-28-ol (**30**) was synthesized from **25** as a white solid (72%). Mp 241–242 °C; IR (KBr, cm^{-1}) 3506, 2947, 2399, 2284, 1722, 1641, 1462, 1398, 1375, 1367, 1228, 1188, 1031, 981, 895, 879, 663, 605; ^1H NMR ($CDCl_3$) δ 0.88 (s, 3H), 0.97 (s, 3H), 1.02 (s, 3H), 1.04 (s, 3H), 1.10 (s, 3H), 1.68 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.34–2.42 (m, 1H), 3.33 and 3.78 (d, *J*=10.9 Hz, each 1H), 4.58–4.60 (m, 2H), 4.68 (br s, 1H), 5.29–5.33 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.7, 16.1, 16.7, 17.5, 18.0, 19.0, 20.8, 21.0, 21.2, 25.1, 27.0, 29.0, 29.2, 29.7, 34.0, 34.1, 36.9, 37.2, 37.4, 41.0, 42.2, 42.9, 47.8, 48.7, 50.8, 55.2, 60.6, 69.6, 78.0, 109.8, 150.3, 170.2, 170.7; ESI-MS *m/z*: 565.4 $[M+Na]^+$; HRMS calcd for $C_{34}H_{55}O_5$ $[M+H]^+$: 543.4049, found: 543.4044.

4.26. 2β,3α-Diacetyloxy-lup-20(29)-en-28-ol (31)

Following the procedure described for preparation of **29**, 2β,3α-diacetyloxy-lup-20(29)-en-28-ol (**31**) was synthesized from **28** as a white solid (71%). Mp 104–106 °C; IR (film, cm^{-1}) 3466, 2943, 2870, 1742, 1455, 1371, 1249, 1028, 882, 737; ^1H NMR ($CDCl_3$) δ 0.91 (s, 3H), 0.97 (s, 3H), 1.00 (s, 3H), 1.02 (s, 3H), 1.06 (s, 3H), 1.67 (s, 3H), 1.99 (s, 3H), 2.06 (s, 3H), 2.33–2.40 (m, 1H), 3.33 and 3.78 (d, *J*=10.8 Hz, each 1H), 4.57 and 4.67 (br s, each 1H), 4.91–4.98 (m, 1H), 5.08 (d, *J*=8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 14.9, 15.8, 19.0, 19.6, 21.0, 21.2, 21.4, 22.3, 25.3, 26.0, 27.0, 29.2, 29.8, 33.6, 34.0, 37.0, 37.4, 37.5, 41.0, 42.9, 43.0, 47.8, 48.8, 50.7, 50.9, 60.6, 70.6, 76.1, 109.8, 150.3, 170.1, 170.4; ESI-MS *m/z*: 560.6 $[M+NH_4]^+$; HRMS calcd for $C_{34}H_{54}NaO_5$ $[M+Na]^+$: 565.3869, found: 565.3864.

4.27. 2α,3α-Diacetyloxy-lup-20(29)-en-28-aldehyde (32)

To a solution of **29** (200 mg, 0.369 mmol) in CH_2Cl_2 was added PCC (159 mg, 0.738 mmol), the reaction mixture was stirred at rt for 40 min. Silica gel was poured into the reaction mixture and the solvent was evaporated under reduced pressure to dryness. The residue was directly purified by column chromatography on silica gel (petroleum ether/ethyl acetate, 19:1) to give 2α,3α-diacetyloxy-lup-20(29)-en-28-aldehyde (**32**) as a white solid (173 mg, 87%). Mp 112–114 °C; IR (KBr, cm^{-1}) 3437, 2945, 2872, 2376, 1734, 1458, 1375, 1232, 1036, 1157, 1036, 953, 888, 614; ^1H NMR ($CDCl_3$) δ 0.86 (s, 3H), 0.92 (s, 3H), 0.94 (s, 3H), 0.96 (s, 3H), 1.02 (s, 6H), 1.70 (s, 3H), 1.94 (s, 3H), 2.12 (s, 3H), 2.82–2.91 (m, 1H), 4.63 and 4.76 (d, *J*=1.7 Hz, each 1H), 4.95 (d, *J*=2.4 Hz, 1H), 5.19–5.25 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.5, 16.0, 17.0, 17.8, 19.0, 20.8, 20.96, 21.05, 21.4, 25.5, 27.7, 28.8, 29.3, 29.9, 33.2, 34.1, 38.2, 38.6, 38.7, 39.2, 41.1, 42.7, 47.5, 48.1, 49.8, 50.4, 59.3, 68.4, 77.4, 110.2, 149.6, 170.3, 170.6, 206.5; ESI-MS *m/z*: 563.6 $[M+Na]^+$; HRMS calcd for $C_{34}H_{52}NaO_5$ $[M+Na]^+$: 563.3712, found: 563.3707.

4.28. 2β,3β-Diacetyloxy-lup-20(29)-en-28-aldehyde (33)

Following the procedure described for preparation of **32**, 2β,3β-diacetyloxy-lup-20(29)-en-28-aldehyde (**33**) was synthesized from **30** as a white solid (89%). Mp 227–229 °C; IR (film, cm^{-1}) 2944, 2868, 1742, 1641, 1450, 1395, 1367, 1252, 1188, 1031, 883, 737; ^1H NMR ($CDCl_3$) δ 0.88 (s, 3H), 0.93 (s, 3H), 0.96 (s, 3H), 1.02 (s, 3H), 1.10 (s, 3H), 1.70 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.81–2.90 (m, 1H), 4.60 (d, *J*=3.9 Hz, 1H), 4.64 and 4.75 (br s, each 1H), 5.30–5.33 (m, 1H), 9.67 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.2, 16.1, 16.8, 17.5, 18.0, 19.0, 20.8, 20.9, 21.2, 25.5, 28.7, 29.0, 29.2, 29.9, 33.3, 34.2, 37.0, 37.4, 38.7, 41.0, 42.3, 42.8, 47.6, 48.1, 51.0, 55.2, 59.3, 69.6, 78.0, 110.2, 149.6, 170.2, 170.7, 206.5; ESI-MS *m/z*: 558.3 $[M+NH_4]^+$; HRMS calcd for $C_{34}H_{52}O_5$: 540.3815, found: 540.3817.

4.29. 2β,3α-Diacetyloxy-lup-20(29)-en-28-aldehyde (34)

Following the procedure described for preparation of **32**, 2β,3α-diacetyloxy-lup-20(29)-en-28-aldehyde (**34**) was synthesized from **31** as a white solid (92%). Mp 188–190 °C; IR (film, cm^{-1}) 2943, 2869, 1742, 1641, 1452, 1369, 1246, 1027, 884, 736; ^1H NMR ($CDCl_3$) δ 0.91 (s, 6H), 0.96 (s, 3H), 0.99 (s, 3H), 1.06 (s, 3H), 1.69 (s, 3H), 2.00 (s, 3H), 2.06 (s, 3H), 2.81–2.90 (m, 1H), 4.62 and 4.75 (br s, each 1H), 4.91–4.98 (m, 1H), 5.08 (d, *J*=8.0 Hz, 1H), 9.67 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.4, 15.7, 19.0, 19.6, 20.9, 21.2, 21.3, 22.3, 25.6, 26.0, 28.8, 29.2, 29.9, 33.2, 33.6, 37.0, 37.4, 38.9, 40.9, 42.8, 43.0, 47.5, 48.1, 50.7, 51.0, 59.3, 70.5, 76.0, 110.2, 149.5, 170.1, 170.3, 206.5; ESI-MS *m/z*: 558.3 $[M+NH_4]^+$; HRMS calcd for $C_{34}H_{52}NaO_5$ $[M+Na]^+$: 563.3712, found: 563.3707.

4.30. 2α,3α-Diacetyloxy-lup-20(29)-en-28-oic acid (35)

Compound **32** (50 mg, 0.092 mmol) was dissolved in 2.9 mL of *tert*-butyl alcohol, 0.58 mL of distilled THF and 0.87 mL of 2-methyl-2-butene. The solution was stirred and cooled in an ice-bath. Then 2.8 mL of freshly prepared aqueous solution of NaH_2PO_4/$NaClO_2$ (146.2 mg/112.14 mg) was slowly added to the solution and the mixture was stirred for 15 min. The mixture was then raised to rt and stirred for 1 h. Finally, the mixture was poured into 5 mL of a saturated solution of NH_4Cl and extracted with CH_2Cl_2. The combined extract was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 93:7) to give 2α,3α-diacetyloxy-lup-20(29)-en-28-oic acid (**35**) as a white solid (42 mg, 84%). Mp 182–183 °C; IR (film, cm^{-1}) 2946, 2870, 1744, 1695, 1452, 1374, 1251, 1234, 1155, 1036, 885, 737, 609; ^1H NMR ($CDCl_3$) δ 0.86 (s, 3H), 0.94 (s, 6H), 0.96 (s, 3H), 1.03 (s, 3H), 1.69 (s, 3H), 1.94 (s, 3H), 2.12 (s, 3H), 2.97–3.04 (m, 1H), 4.61 and 4.74 (br s, each 1H), 4.95 (d, *J*=2.4 Hz, 1H), 5.19–5.25 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.9, 16.1, 17.0, 17.8, 19.4, 20.8, 20.96, 21.05, 21.4, 25.4, 27.7, 29.7, 30.6, 32.2, 34.1, 37.0, 38.2, 38.3, 38.7, 39.2, 41.0, 42.6, 46.9, 49.3, 49.8, 50.4, 56.3, 68.4, 77.4, 109.7, 150.3, 170.4, 170.6, 180.5; ESI-MS *m/z*: 574.6 $[M+NH_4]^+$. Anal. Calcd for $C_{34}H_{53}O_6$: C, 73.21; H, 9.58. Found: C, 73.21; H, 9.625.

4.31. 2β,3β-Diacetyloxy-lup-20(29)-en-28-oic acid (36)

Following the procedure described for preparation of **35**, 2β,3β-diacetyloxy-lup-20(29)-en-28-oic acid (**36**) was synthesized from **33** as a white solid (86%). Mp 246–248 °C; IR (film, cm^{-1}) 2945, 2870, 1743, 1693, 1451, 1369, 1252, 1188, 1055, 1031, 982, 885, 738; ^1H NMR ($CDCl_3$) δ 0.88 (s, 3H), 0.96 (s, 3H), 0.97 (s, 3H), 1.02 (s, 3H),

1.10 (s, 3H), 1.69 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.17–2.30 (m, 3H), 2.97–3.03 (m, 1H), 4.60 and 4.74 (d, J=1.9 Hz, each 1H), 4.61 (s, 1H), 5.30–5.34 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.6, 16.2, 16.8, 17.5, 18.0, 19.4, 20.8, 21.0, 21.2, 25.4, 29.0, 29.6, 29.7, 30.6, 32.2, 34.2, 37.1, 37.4, 38.4, 40.9, 42.2, 42.6, 46.9, 49.3, 51.0, 55.3, 56.3, 69.7, 78.0, 109.8, 150.2, 170.2, 170.7, 180.6; ESI-MS m/z: 555.3 $[M-H]^-$; HRMS calcd for $C_{34}H_{52}O_6$: 556.3764, found: 556.3763.

4.32. 2β,3α-Diacetyloxy-lup-20(29)-en-28-oic acid (37)

Following the procedure described for preparation of **35**, 2β,3α-diacetyloxy-lup-20(29)-en-28-oic acid (**37**) was synthesized from **34** as a white solid (88%). Mp 267–269 °C; IR (film, cm^{-1}) 2944, 2870, 1742, 1694, 1641, 1455, 1370, 1247, 1028, 880, 737; ^{1}H NMR ($CDCl_3$) δ 0.91 (s, 3H), 0.93 (s, 3H), 0.96 (s, 3H), 1.00 (s, 3H), 1.06 (s, 3H), 1.68 (s, 3H), 2.00 (s, 3H), 2.06 (s, 3H), 2.16–2.30 (m, 2H), 2.96–3.04 (m, 1H), 4.61 and 4.74 (br s, each 1H), 4.92–4.98 (m, 1H), 5.08 (d, J=8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 14.8, 15.8, 19.0, 19.3, 19.6, 20.9, 21.2, 21.4, 22.3, 25.5, 26.0, 29.6, 30.6, 32.2, 33.6, 36.9, 37.0, 37.4, 38.6, 40.8, 42.6, 43.0, 46.9, 49.3, 50.7, 51.0, 56.3, 70.6, 76.6, 109.8, 150.2, 170.1, 170.3; ESI-MS m/z: 555.3 $[M-H]^-$; HRMS calcd for $C_{34}H_{52}NaO_6$ $[M+Na]^+$: 579.3662, found: 579.3656.

4.33. 2α,3α-Dihydroxylup-20(29)-en-28-oic acid (5)

Compound **35** (25 mg, 0.04 mmol) was dissolved in 5 mL of MeOH, then 1 mL of 4 N NaOH aq was added dropwise. The reaction mixture was stirred at rt for 2 h. The solvent was removed in vacuo, and the pH was adjusted to 3.0 by 1 N HCl at 0 °C. The mixture was extracted with ethyl acetate (4×10 mL). The combined extract was washed with brine, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 4:1) to give 2α,3α-dihydroxylup-20(29)-en-28-oic acid (**5**) as a white solid (20 mg, 96%). Mp 257–259 °C (lit.[11] mp 298–300 °C); $[\alpha]_D^{20}$ +18.7 (c 0.227, pyridine); IR (film, cm^{-1}) 3517, 3387, 2943, 2870, 1733, 1694, 1641, 1455, 1376, 1153, 1125, 1034, 881; ^{1}H NMR (C_5D_5N) δ 0.86 (s, 6H), 0.92 (s, 3H), 1.03 (s, 3H), 1.23 (s, 3H), 1.74 (s, 3H), 1.87–1.99 (m, 3H), 2.19–2.25 (m, 2H), 2.56–2.73 (m, 2H), 3.47–3.54 (m, 1H), 3.74 (d, J=2.6 Hz, 1H), 4.26–4.30 (m, 1H), 4.74 (br s, 1H), 4.90 (d, J=2.1 Hz, 1H); ^{13}C NMR (C_5D_5N) δ 14.9, 16.5, 17.5, 18.5, 19.5, 21.2, 22.1, 26.1, 29.4, 30.0, 30.3, 31.2, 32.9, 34.8, 37.6, 38.6, 38.9, 41.4, 42.9, 43.3, 47.8, 48.8, 49.8, 50.8, 56.6, 66.3, 79.4, 109.9, 151.3, 178.8; ESI-MS m/z: 471.3 $[M-H]^-$; HRMS calcd for $C_{30}H_{48}NaO_4$: 472.3553, found: 472.3559.

4.34. 2β,3α-Dihydroxylup-20(29)-en-28-oic acid (7)

Following the procedure described for preparation of **5**, 2β,3α-dihydroxylup-20(29)-en-28-oic acid (**7**) was synthesized from **37** as a white solid (95%). Mp 278–280 °C; $[\alpha]_D^{20}$ +37.3 (c 0.239, pyridine); IR (KBr, cm^{-1}) 3412, 2942, 2868, 1696, 1641, 1453, 1374, 1206, 1044, 1024, 979, 885; ^{1}H NMR (C_5D_5N) δ 0.94 (s, 3H), 0.99 (s, 3H), 1.09 (s, 3H), 1.17 (s, 6H), 1.71 (s, 3H), 1.93–2.00 (m, 1H), 2.13–2.21 (m, 2H), 2.52–2.71 (m, 2H), 3.42–3.50 (m, 1H), 3.91 (d, J=8.4 Hz, 1H), 4.18–4.25 (m, 1H), 4.69 (dd, J=2.3, 1.4 Hz, 1H), 4.86 (d, J=2.3 Hz, 1H); ^{13}C NMR (C_5D_5N) δ 14.9, 16.2, 19.5, 20.0, 21.9, 23.5, 26.1, 26.3, 30.2, 31.2, 32.9, 34.3, 37.6, 38.0, 38.1, 38.9, 41.3, 43.0, 47.4, 47.8, 49.8, 51.0, 51.7, 56.7, 70.3, 78.2, 109.9, 151.3, 178.8; ESI-MS m/z: 471.4 $[M-H]^-$; HRMS calcd for $C_{30}H_{48}NaO_4$ $[M+Na]^+$: 495.3450, found: 495.3445.

4.35. 2β,3β-Dihydroxylup-20(29)-en-28-oic acid (9)

Following the procedure described for preparation of **5**, 2β,3β-dihydroxylup-20(29)-en-28-oic acid (**9**) was synthesized from **36** as a white solid (96%). Mp 273–276 °C; $[\alpha]_D^{20}$ +31.1 (c 0.262, pyridine); IR (KBr, cm^{-1}) 3523, 2941, 2391, 2291, 1709, 1458, 1375, 1176, 1158, 1133, 1057, 1029, 875, 756; ^{1}H NMR (C_5D_5N) δ 1.09 (s, 3H), 1.10 (s, 3H), 1.24 (s, 3H), 1.34 (s, 3H), 1.43 (s, 3H), 1.80 (s, 3H), 2.18–2.29 (m, 2H), 2.35–2.40 (m, 1H), 2.62–2.79 (m, 2H), 3.44 (d, J=3.9 Hz, 1H), 3.53–3.58 (m, 1H), 4.43–4.44 (m, 1H), 4.77 and 4.95 (br s, each 1H); ^{13}C NMR (C_5D_5N) δ 15.0, 16.5, 17.5, 18.0, 18.7, 19.5, 21.5, 26.2, 30.1, 30.2, 31.3, 33.0, 34.9, 37.5, 37.6, 38.7, 38.9, 41.3, 43.0, 45.5, 47.8, 49.9, 51.5, 56.0, 56.7, 71.5, 78.4, 109.9, 151.4, 178.8; ESI-MS m/z: 471.4 $[M-H]^-$; HRMS calcd for $C_{34}H_{48}O_4$: 472.3553, found: 472.3554.

5. Enzyme assay

The inhibitory activity of the test compounds against rabbit muscle glycogen phosphorylase a (GPa) was monitored using microplate reader (BIO-RAD) based on the published method. In brief, GPa activity was measured in the direction of glycogen synthesis by the release of phosphate from glucose-1-phosphate. Each test compound was dissolved in DMSO and diluted at different concentrations for IC_{50} determination. The enzyme was added into 100 L of buffer containing 50 mM Hepes (pH=7.2), 100 mM KCl, 2.5 mM $MgCl_2$, 0.5 mM glucose-1-phosphate, 1 mg/mL glycogen and the test compound in 96-well microplates (Costar). After the addition of 150 L of 1 M HCl containing 10 mg/mL ammonium molybdate and 0.38 mg/mL malachite green, reactions were run at 22 °C for 25 min, and then the phosphate absorbance was measured at 655 nm. The IC_{50} values were estimated by fitting the inhibition data to a dose-dependent curve using a logistic derivative equation.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.07.047.

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