Supporting Information

for

Efficient synthesis of piperazinyl amides of 18β-glycyrrhetinic acid

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*Beilstein J. Org. Chem.* **2020**, *16*, 798–808. doi:10.3762/bjoc.16.73

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Experimental

Materials and methods

Unless otherwise required, all reagents used in the experiment were purchased as commercial analytical grade and used without further purification. Melting points were obtained in open capillary tubes with a WRS-1B melting point apparatus and were uncorrected (Shen Guang Electric Appliances Co., Ltd., Shanghai, CHN). The structures of the synthetic compounds were confirmed by $^1$H NMR and $^{13}$C NMR spectra on 400/54Premium Shielded NMR Magnet System (Agilent Technologies, Santa Clara, CA, USA) with tetramethylsilane (TMS) as an internal standard. HRMS spectral data were collected from an Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System B.05.01. (B5125) and Thermo Scientific LTQ-Orbitrap XL in positive ion modes (Agilent Technologies, Santa Clara, CA, USA). X-ray single-crystal structure determinations were carried out on a Bruker SMART APEX II CCD diffractometer (Bruker AXS GMBH, Karlsruhe, GER).

Synthesis of 18β–GA analogs

$3\beta$-Acetoxy-11-oxo-18β-olean-12-en-30-oic acid (2)

18β-Glycyrrhetinic acid (0.47 g, 1.0 mmol) was heated at 130 °C with acetic anhydride (2.04 g, 20 mmol) for 1 h. Then, H$_2$O was added to the cool solution. The product was filtered off and washed with cold H$_2$O.
A white solid; yield, 99.2%; m.p. 304.4-306.1 °C (literature [1]: 312.0-313.0 °C); \(^1\text{H}\) NMR (400 MHz, Chloroform-\(d\)) \(\delta\) 5.69 (d, \(J = 1.7\) Hz, 1H, CH-12), 4.50 (dt, \(J = 11.7, 2.8\) Hz, 1H, CH-3), 2.77 (dd, \(J = 14.0, 4.0\) Hz, 1H, CH-1), 2.35 (s, 1H, CH-9), 2.03 (s, 2H, acetyloxy CH\(_3\)), 1.35 (m, 3H, CH\(_3\)-27), 1.21 (s, 3H, CH\(_3\)-25), 1.14 (s, 3H, CH\(_3\)-26), 1.10 (s, 3H, CH\(_3\)-29), 0.86 (s, 6H, CH\(_3\)-23/24), 0.85 (s, 3H, CH\(_3\)-28), 0.77 (m, 1H, CH-5); \(^{13}\text{C}\) NMR (101 MHz, Chloroform-\(d\)) \(\delta\) 200.41 (C11), 181.77 (30), 171.08 (acetyloxy C=O), 169.55 (C13), 128.37 (C12), 80.61 (C3), 61.66 (C9), 54.97 (C5), 48.21 (C18), 45.44 (C14), 43.79 (C20), 43.17 (C8), 40.78 (C19), 38.73 (C1), 38.02 (C4), 37.67 (C22), 36.89 (C10), 32.66 (C7), 31.84 (C17), 30.86 (C21), 28.52 (C29), 28.44 (C28), 28.02 (C23), 26.43 (C2), 26.35 (C15), 23.54 (C16), 23.32 (C27), 21.32 (acetyloxy CH\(_3\)), 18.64 (C26), 17.33 (C6), 16.67 (C25), 16.39 (C24). HRMS (m/z): [M + H\(^+\)] calcd. for C\(_{32}\)H\(_{49}\)O\(_5\): 513.3580, found: 513.3580.

General procedure for the preparation of compounds (4) and (5)

The compound 3 (0.44 g, 0.90 mmol) was dissolved in CH\(_2\)Cl\(_2\) (30 mL) at 0 °C under stirring, then triethylamine (0.3 g, 3.00 mmol) and anhydrous piperazine (0.23 g, 2.70 mmol) were added. The reaction was stirred at 0 °C for 30 min. After reaction, the mixture was removed, and the residue was subjected to column chromatography (silica gel, CH\(_2\)Cl\(_2\)-methanol, 5:1) to yield compounds (4) and (5).

\(3\beta\)-Acetoxy-11-oxo-18\(\beta\)-olean-12-en-30-carbonyl piperazine (4) A white solid; yield, 36.1%; m.p. 237.2-239.0 °C (literature [2]: 160 °C-decomp.); \(^1\text{H}\) NMR (400 MHz, Chloroform-\(d\)) \(\delta\) 5.69 (d, \(J = 1.7\) Hz, 1H, CH-12), 4.50 (dt, \(J = 11.7, 2.8\) Hz, 1H, CH-3), 2.77 (dd, \(J = 14.0, 4.0\) Hz, 1H, CH-1), 2.35 (s, 1H, CH-9), 2.03 (s, 2H, acetyloxy CH\(_3\)), 1.35 (m, 3H, CH\(_3\)-27), 1.21 (s, 3H, CH\(_3\)-25), 1.14 (s, 3H, CH\(_3\)-26), 1.10 (s, 3H, CH\(_3\)-29), 0.86 (s, 6H, CH\(_3\)-23/24), 0.85 (s, 3H, CH\(_3\)-28), 0.77 (m, 1H, CH-5); \(^{13}\text{C}\) NMR (101 MHz, Chloroform-\(d\)) \(\delta\) 200.41 (C11), 181.77 (30), 171.08 (acetyloxy C=O), 169.55 (C13), 128.37 (C12), 80.61 (C3), 61.66 (C9), 54.97 (C5), 48.21 (C18), 45.44 (C14), 43.79 (C20), 43.17 (C8), 40.78 (C19), 38.73 (C1), 38.02 (C4), 37.67 (C22), 36.89 (C10), 32.66 (C7), 31.84 (C17), 30.86 (C21), 28.52 (C29), 28.44 (C28), 28.02 (C23), 26.43 (C2), 26.35 (C15), 23.54 (C16), 23.32 (C27), 21.32 (acetyloxy CH\(_3\)), 18.64 (C26), 17.33 (C6), 16.67 (C25), 16.39 (C24). HRMS (m/z): [M + H\(^+\)] calcd. for C\(_{32}\)H\(_{49}\)O\(_5\): 513.3580, found: 513.3580.
MHz, Chloroform-\textit{d}) \delta 5.67 (s, 1H, CH-12), 4.49 (dd, J = 11.7, 4.8 Hz, 1H, CH-3), 3.61 (q, J = 4.7 Hz, 4H, piperazine CH$_2$×2), 2.85 (t, J = 5.0 Hz, 4H, piperazine CH$_2$×2), 2.77 (dt, J = 13.7, 3.6 Hz, 1H, CH-1), 2.33 (s, 1H, CH-9), 2.29 – 2.23 (m, 1H, CH-16), 2.16 (s, 3H, acetyloxy CH$_3$), 1.33 (s, 3H, CH$_3$-27), 1.20 (s, 3H, CH$_3$-25), 1.13 (s, 3H, CH$_3$-26), 1.10 (s, 3H, CH$_3$-29), 0.85 (s, 6H, CH$_3$-23/24), 0.79 (s, 3H, CH$_3$-28), 0.76 (m, 1H, CH-5); $^{13}$C NMR (101 MHz, Chloroform-\textit{d}) \delta 200.01 (C11), 173.86 (C30), 171.03 (acetyloxy C=O), 169.67 (C13), 128.46 (C12), 80.59 (C3), 61.67 (C9), 54.99 (C5), 48.21 (C18), 46.13 (piperazine C×2), 45.27 (C14), 43.77 (C20), 43.71 (C8), 43.26 (piperazine C×2), 38.78 (C19), 38.01 (C1/4), 37.73 (C22), 36.91 (C10), 33.38 (C7), 32.72 (C17), 31.76 (C21), 28.41 (C29), 28.02 (C28), 27.02 (C23), 26.69 (C2), 26.41 (C15), 23.54 (C16), 23.07 (C27), 21.32 (acetyloxy CH$_3$), 18.65 (C26), 17.35 (C6), 16.66 (C25), 16.41 (C24); HRMS (\textit{m/z}): [M + H]$^+$ calcd. for C$_{36}$H$_{57}$N$_2$O$_4$: 581.4318, found: 581.4316.

\textit{Bisamide} (5) A white solid; yield, 64.6%; m.p. 211.4-212.0 °C. HRMS (\textit{m/z}): [M + Na]$^+$ calcd. for C$_{68}$H$_{102}$N$_2$NaO$_8$: 1097.7534, found: 1097.7535.

3$\beta$-Acetyloxy -11-oxo-18$\beta$-olean-12-en-30-carbonyl piperazine (4)

Compound 8 (0.68 g, 1.0 mmol) was dissolved in CH$_2$Cl$_2$ (10 mL) at 0 °C under stirring. Trifluoroacetic acid (5 mL) was added, and the reaction was stirred at 0 °C for 3 h. After reaction, the mixture was made basic with a saturated Na$_2$CO$_3$ solution. This mixture was extracted with CH$_2$Cl$_2$, dried over Na$_2$SO$_4$, and concentrated to give the desired product.
A white solid; yield, 94.1%; the chemical structures were characterized as above.

1H-Benzod[1,2,3]triazol-1-yl-3β-acetoxy-11-oxoolean-12-en-30-oate (6)

Compound 2 (0.51 g, 1.0 mmol) was dissolved in acetonitrile (20 mL), then EDCI (0.23 g, 1.2 mmol), triethylamine (0.13 g, 1.2 mmol) and HOBt (0.16 g, 1.2 mmol) were added. The mixture was stirred at room temperature for 1 h. The solvent was removed under vacuum to give a residue which was treated with a mixture of ethanol and water. The solution was stirred at room temperature for 30 min, and a solid was obtained by filtration while washing with H2O.

A white solid; yield, 97.8%; m.p. 208.7 °C - decomp. (literature [3]: 192-195 °C, decomp.); 1H NMR (400 MHz, Chloroform-d) δ 8.08 (d, J = 8.4 Hz, 1H, phenyl), 7.56 (t, J = 7.6 Hz, 1H, phenyl), 7.44 (t, J = 7.7 Hz, 1H, phenyl), 7.34 (d, J = 8.3 Hz, 1H, phenyl), 5.71 (s, 1H, CH12), 5.22 (dd, J = 10.7, 5.5 Hz, 1H, OH-3), 2.77 (dt, J = 13.5, 3.6 Hz, 1H, CH-1), 2.39 – 2.23 (m, 2H, CH9/16), 1.41 (s, 3H, CH3-27), 1.15 (s, 3H, CH3-25), 1.13 (s, 3H, CH3-26), 1.00 (s, 3H, CH3-29), 0.93 (s, 3H, CH3-23), 0.80 (s, 3H, CH3-24), 0.72 (d, J = 11.6 Hz, 1H, CH-5); 13C NMR (101 MHz, Chloroform-d) δ 199.91 (C11), 172.51 (C30), 167.60 (C13), 143.54 (phenyl), 129.02 (phenyl), 128.83 (phenyl), 128.54 (C12), 124.82 (phenyl), 120.66, (phenyl) 107.81 (phenyl), 78.70 (C3), 61.83 (C9), 54.89 (C5), 48.20 (C18), 45.36 (C20), 44.36(C8), 43.15(C19), 40.85 (C1), 39.11 (C4), 37.75 (C22), 37.06 (C10), 32.72 (C7), 31.97 (C17),
31.16 (C21), 28.54 (C29), 28.08 (C28), 28.02 (C23), 27.25 (C2), 26.34 (C15/16), 23.48 (C27), 18.67 (C26), 17.45 (C6), 16.34 (C25), 15.57 (C24);

HRMS (m/z): [M + H]^+ calcd. for C_{36}H_{50}N_{3}O_{4}: 588.3801, found: 588.3801.

1H-Benz[d][1,2,3]triazol-1-yl-3β-hydroxy-11-oxoolean-12-en-30-oate (7)

18β-Glycyrrhetinic acid (0.47 g, 1.0 mmol) was dissolved in acetonitrile (20 mL), then EDCI (0.23 g, 1.2 mmol), triethylamine (0.13 g, 1.2 mmol) and HOBt (0.16 g, 1.2 mmol) were added. The mixture was stirred at room temperature for 1 h. The solvent was removed under vacuum to give a residue which was treated with a mixture of ethanol and water. The solution was stirred at room temperature for 30 min, and a solid was obtained by filtration while washing with H_{2}O.

A white solid; yield, 97.2%; m.p. 263.4-264.4 °C; ^1H NMR (400 MHz, Chloroform-d) δ 8.09 (d, J = 8.4 Hz, 1H, phenyl), 7.57 (t, J = 7.6 Hz, 1H, phenyl), 7.44 (t, J = 7.7 Hz, 1H, phenyl), 7.35 (d, J = 8.3 Hz, 1H, phenyl), 5.72 (s, 1H, CH-12), 4.52 (dd, J = 11.6, 4.8 Hz, 1H, CH-3), 2.78 (dt, J = 13.8, 3.7 Hz, 1H, CH-1), 2.37 (s, 1H, CH-9), 2.05 (s, 3H, acetyloxy CH_{3}), 1.41 (s, 3H, CH_{3}-27), 1.21 (s, 3H, CH_{3}-25), 1.16 (s, 6H, CH_{3}-26/29), 0.94 (s, 3H, CH_{3}-23), 0.88 (s, 6H, CH_{3}-24/28), 0.80 (m, 1H, CH-5); ^13C NMR (101 MHz, Chloroform-d) δ 199.76 (C11), 172.51 (30), 171.01 (acetyloxy C=O), 167.60 (C13), 143.55 (phenyl), 128.99 (phenyl), 128.81 (phenyl), 128.54 (C12), 124.80 (phenyl), 120.67 (phenyl), 107.80 (phenyl), 80.53 (C3), 61.74 (C9), 54.98 (C5), 48.19 (C18), 45.37 (C14), 44.35 (C20), 43.14 (C8), 40.84 (C19), 38.74 (C1), 38.02
(C4), 37.74 (C22), 36.91 (C10), 32.67 (C7), 31.97 (C17), 31.17 (C21), 28.53
(C29), 28.02 (C28/23), 26.33 (C2/15), 23.52 (C16), 23.42 (C27), 21.32
(acetyloxy CH3), 18.67 (C26), 17.34 (C6), 16.66 (C25), 16.38 (C24); HRMS
(m/z): (M + H+) calcd. for C38H52N3O5: 630.3907, found: 630.3904.

**tert-Butyl 4-(3β-acetoxy-11-oxo-18β-olean-12-en-30-carbonyl)piperazine-1-
carboxylate (8)**

Method A: Compound 2 (0.51 g, 1.0 mmol) was dissolved in acetonitrile (20
mL), then EDCl (0.23 g, 1.2 mmol), triethylamine (0.13 g, 1.2 mmol) and HOBt
(0.16 g, 1.2 mmol) were added. The mixture was stirred at room temperature for
20 min. The 1-Boc-piperazine (0.46 g, 2.5 mmol) was added, and the mixture
was stirred under reflux for 10 h. The solvent was removed under vacuum to
give a residue which was treated with a mixture of ethanol and water. The
solution was stirred at room temperature for 30 min, and a solid was obtained
by filtration while washing with H2O.

Method B: Compound 9 (0.64 g, 1.0 mmol) was heated at 130 °C with acetic
anhydride (2.04 g, 20 mmol) for 1 h. Then, H2O was added to the cool solution.
The product was filtered off and washed with cold H2O.

A white solid; yield, 95.7%; m.p. 221.6-223.0 °C; 1H NMR (400 MHz, Chloroform-
d) δ 5.66 (s, 1H, CH-12), 4.50 (dd, J = 11.7, 4.7 Hz, 1H, CH-3), 3.66 – 3.50 (m,
4H, piperazine CH2×2), 3.40 (d, J = 5.1 Hz, 4H, piperazine CH2×2), 2.79-2.74
(m, 1H, CH-1), 2.33 (s, 1H, CH-9), 2.30 – 2.22 (m, 1H, CH-16), 2.03 (s, 3H,
acetyloxy CH3), 1.45 (s, 9H, tert-butyl CH3×3), 1.33 (s, 3H, CH3-27), 1.20 (s, 3H,
CH₃-25), 1.14 (s, 3H, CH₃-26), 1.09 (s, 3H, CH₃-29), 0.85 (s, 6H, CH₃-23/24), 0.79 (s, 3H, CH₃-28), 0.76 (m, 1H, CH-5); ¹³C NMR (101 MHz, Chloroform-d) δ 199.95 (C11), 174.12 (C30), 171.03 (acetyloxy C=O), 169.46 (C13), 154.52 (Boc C=O), 128.50 (C12), 80.58 (tert-butyl C), 80.24 (C3), 61.67 (C9), 54.99 (C5), 48.12 (C18), 45.27 (C14), 43.87 (piperazine C×2), 43.75 (C20), 43.25 (C8/19), 38.77 (C1/C4), 38.01 (piperazine C×2), 37.70 (C22), 36.90 (C10), 33.22 (C7), 32.72 (C17), 31.75 (C21), 28.40 (C29), 28.35 (tert-butyl CH₃×3), 28.02 (C28), 27.05 (C23), 26.68 (C2), 26.38 (C15), 23.54 (C16), 23.08 (C27), 21.31 (acetyloxy CH₃), 18.65 (C26), 17.35 (C6), 16.66 (C25), 16.40 (C24); HRMS (m/z): [M + H]+ calcd. for C₄₁H₆₅N₂O₆: 681.4843, found: 681.4841.

tert-Butyl 4-(3β-hydroxyl-11-oxo-18β-olean-12-en-30-carbonyl)piperazine-1-carboxylate (9)

18β-Glycyrrheticin acid (0.47 g, 1.0 mmol) was dissolved in acetonitrile (20 mL), then EDCI (0.23 g, 1.2 mmol), triethylamine (0.13 g, 1.2 mmol) and HOBt (0.16 g, 1.2 mmol) were added. The mixture was stirred at room temperature for 20 min. The 1-Boc-piperazine (0.46 g, 2.5 mmol) was added, and the mixture was stirred under reflux for 10 h. The solvent was removed under vacuum to give a residue which was treated with a mixture of ethanol and water. The solution was stirred at room temperature for 30 min, and a solid was obtained by filtration while washing with H₂O.

A white solid; yield, 94.3%; m.p. 224.3-225.7 °C; ¹H NMR (400 MHz, Chloroform-d) δ 5.66 (s, 1H, CH-12), 3.63-3.52 (m, 4H, piperazine CH₂×2), 3.39 (t, J = 5.2
Hz, 4H, piperazine CH$_2$×2), 3.22-3.18 (m, 1H, OH-3), 2.79-2.74 (m, 1H, CH-1), 2.31 (s, 1H, CH-9), 2.30-2.23 (m, 1H, CH-16), 1.45 (s, 9H, tert-butyl CH$_3$×3), 1.34 (s, 3H, CH$_3$-27), 1.20 (s, 3H, CH$_3$-25), 1.11 (s, 3H, CH$_3$-26), 1.10 (s, 3H, CH$_3$-29), 0.98 (s, 3H, CH$_3$-23), 0.79 (s, 3H, CH$_3$-24), 0.78 (s, 3H, CH$_3$-28), 0.68 (d, $J = 11.6$ Hz, 1H, CH-5); $^{13}$C NMR (101 MHz, Chloroform-$d$) $\delta$ 200.10 (C11), 174.13 (C30), 169.40 (C13), 154.53 (Boc C=O), 128.56 (C12), 80.25 (C3), 78.75 (tert-butyl C), 61.77 (C9), 54.92 (C5), 48.08 (C18), 45.26 (C14), 43.88 (C20), 43.82 (piperazine C×2), 43.26 (C8/C19), 39.12 (C1/C4), 39.10 (piperazine C×2), 37.70 (C22), 37.06 (C10), 33.16 (C7), 32.79 (C17), 31.75 (C21), 28.40 (C29), 28.36 (tert-butyl CH$_3$×3), 28.07 (C28), 27.28 (C23), 27.05 (C2), 26.69 (C15), 26.39 (C16), 23.14 (C27), 18.66 (C26), 17.46 (C6), 16.36 (C25), 15.56 (C24); HRMS (m/z): [M + H]$^+$ calcd. For C$_{39}$H$_{63}$N$_2$O$_5$: 639.4737, found: 639.4736.

$N$-(2-chloroacetyl) piperazinyl 3β-(2-chloroacetoxy)-11-oxo-18β-olean-12-en-29-amide (11)

Compound 9 (0.64 g, 1.0 mmol) was heated at 130 °C with chloroacetic anhydride (3.42 g, 20 mmol) for 1 h. Then, H$_2$O was added to the cool solution. The product was filtered, washed with cold H$_2$O and dried.

A white solid; yield, 99%; m.p. 177.4-175.7 °C; $^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ 5.67 (s, 1H, CH-12), 4.60 (dd, $J = 11.8$, 4.7 Hz, 1H, CH-3), 4.14 – 3.99 (m, 4H, piperazine CH$_2$×2), 3.73 – 3.66 (m, 4H, piperazine CH$_2$×2), 3.66 – 3.55 (m, 2H, CH$_2$-Cl), 3.53 (d, $J = 5.4$ Hz, 2H, CH$_2$-Cl), 2.81 (dt, $J = 13.8$, 3.7 Hz, 1H, CH-1), 2.35 (s, 1H, CH-9), 2.30 (dd, $J = 13.4$, 3.8 Hz,
1H, CH-16), 1.35 (m, 3H, CH₃-27), 1.23 (s, 3H, CH₃-25), 1.15 (s, 3H, CH₃-26), 1.11 (s, 3H, CH₃-29), 0.89 (s, 6H, CH₃-23/24), 0.81 (s, 3H, CH₃-28), 0.79 (m, 1H, CH-5); ¹³C NMR (101 MHz, chloroform-d) δ 199.81 (C11), 174.25 (C30), 169.44 (C13), 167.12 (chloroacetoxy C=O), 165.32 (chloroacetoxy C=O), 128.50 (C12), 82.97 (C3), 54.93 (C5), 48.04 (C18), 46.27 (C14), 45.25 (piperazine C), 43.92 (piperazine C), 43.83 (C20), 43.26 (C8), 42.20 (C19), 41.24 (C1), 40.67 (C-Cl), 38.66 (C4), 38.21 (piperazine C×2), 37.67 (C22), 36.86 (C10), 32.98 (C7), 32.66 (C17), 31.75 (C21), 28.39 (C29), 28.00 (C28), 27.02 (C23), 26.63 (C2), 26.34 (C15), 23.39 (C16), 23.10 (C27), 22.63, 18.64 (C26), 17.30 (C6), 16.60 (C25), 16.40 (C24); HRMS (m/z): [M + H]^+ calcd. for C₃₈H₅₇Cl₂N₂O₅: 691.3645, found: 691.3641.

3β-(2-Chloroacetoxy)-11-oxo-18β-olean-12-en-30-oic acid (12)

18β-Glycyrrhetic acid (0.47 g, 1.0 mmol) was heated at 130 °C with chloroacetic anhydride (3.42 g, 20 mmol) for 1 h. Then, H₂O was added to the cool solution. The product was filtered off and washed with cold H₂O. A white solid; yield, 98.0%; m.p. 259.0 °C - decomp. (literature [1]: 260.8–261.8 °C); ¹H NMR (400 MHz, Chloroform-d) δ 5.70 (s, 1H, CH-12), 4.59 (dd, J = 11.8, 4.8 Hz, 1H, CH-3), 4.05 (d, J = 2.3 Hz, 2H, CH₂-Cl), 2.81 (m, 1H, CH-1), 2.35 (s, 1H, CH-9), 2.17 (dd, J = 13.6, 4.1 Hz, 1H, CH-16), 1.36 (m, 3H, CH₃-27), 1.21 (s, 3H, CH₃-25), 1.15 (s, 3H, CH₃-26), 1.11 (s, 3H, CH₃-29), 0.88 (s, 6H, CH₃-23/24), 0.82 (s, 3H, CH₃-28), 0.78 (m, 1H, CH-5); ¹³C NMR (101 MHz, Chloroform-d) δ 200.26 (C11), 181.51(C30), 169.59 (C13), 167.12 (acetyloxy...
C=O), 128.37 (C12), 83.00 (C3), 61.60 (C9), 54.94 (C5), 48.21 (C18), 45.43 (C14), 43.78 (C20), 43.18 (C8), 41.24 (C19), 40.80 (C-Cl), 38.64 (C1), 38.23 (C4), 37.67 (C10), 32.62 (C7), 31.84 (C17), 30.87 (C21), 28.52 (C29), 28.43 (C28), 28.00 (C23), 26.43 (C2), 26.34 (C15), 23.41 (C16), 23.35 (C27), 18.64 (C26), 17.30 (C6), 16.61 (C25), 16.39 (C24); HRMS (m/z): [M + H]
+ calcd. for C₃₂H₄₈ClO₅: 547.3190, found: 547.3188.

3β-(2-Morpholinoacetoxy)-11-oxo-olean-12-ene-30-oic acid (13)

Compound 12 (0.55 g, 1.0 mmol), morpholine (0.13 g, 1.5 mmol), K₂CO₃ (0.69 g, 5.0 mmol) and a catalytic amount of I₂ in absolute ethanol (15 mL) was stirred under reflux for 12 h. The reaction mixture was evaporated under reduced pressure and the residue was dissolved in ethanol/H₂O mixture and the white precipitate was collected by filtration.

A white solid; yield, 92.0%; m.p.275.3-276.4 °C; ¹H NMR (400 MHz, Chloroform-d) δ 5.72 (t, J = 3.0 Hz, 1H, CH-12), 4.60 (dd, J = 11.2, 5.5 Hz, 1H, CH-3), 3.76 (d, J = 4.9 Hz, 4H, morpholine), 3.26 – 3.20 (m, 2H, CH₂), 2.80 (d, J = 12.9 Hz, 1H, CH-1), 2.63 (s, 4H, morpholine), 2.37 (t, J = 3.0 Hz, 1H, CH-9), 2.19 (d, J = 13.5 Hz, 1H, CH-16), 2.05 – 1.01 (m, 17H), 1.37 (s, 3H, CH₃-27), 1.27 (s, 3H, CH₃-25), 1.16 (s, 3H, CH₃-26), 1.13 (s, 3H, CH₃-29), 0.87 (s, 3H, CH₃-23/24), 0.83 (s, 3H, CH₃-28), 0.80 (s, 1H, CH-5); ¹³C NMR (101 MHz, Chloroform-d) δ 200.25 (C11), 181.03 (C30), 169.70 (C13), 169.51 (acetoxyl, C=O), 128.40 (C12), 81.09 (C3), 66.67 (morpholine C×2), 61.64 (C9), 59.53 (acetoxyl, CH₂), 54.93 (C5), 53.14 (morpholine C×2), 48.25 (C18), 45.43 (C14), 43.75 (C20), 43.19
(C8), 40.90 (C19), 38.68 (C1), 38.07 (C4), 37.70 (C22), 36.89 (C10), 32.65 (C7),
31.86 (C17), 30.93 (C21), 28.55 (C29), 28.44 (C28), 28.13 (C23), 26.46 (C2),
26.37 (C15), 23.66 (C16), 23.36 (C27), 18.66 (C26), 17.36 (C6), 16.78 (C25),
16.42 (C24); HRMS (m/z): [M + Na]^+ calcd. For C_{36}H_{56}NO_{6}: 598.4108, found:
598.4150.

tert-Butyl 4-(3β-(2-morpholinoacetoxy)-11-oxo-18β-olean-12-en-30-carbonyl)-
piperazine-1-carboxylate (14)

Compound 13 (0.60 g, 1.0 mmol) was dissolved in acetonitrile (20 mL), then
EDCl (0.23 g, 1.2 mmol), triethylamine (0.13 g, 1.2 mmol) and HOBt (0.16 g, 1.2
mmol) were added. The mixture was stirred at room temperature for 20 min. The
1-Boc-piperazine (0.46 g, 2.5 mmol) was added, and the mixture was stirred
under reflux for 10 h. The solvent was removed under vacuum to give a residue
which was treated with a mixture of ethanol and water. The solution was stirred
at room temperature for 30 min, and a solid was obtained by filtration while
washing with H_2O.

A white solid; yield, 93.9%; m.p. 204.3-205.3 °C; ^1H NMR (400 MHz,
Chloroform-d) δ 5.72 (t, J = 3.4 Hz, 1H, CH-12), 4.69 – 4.58 (m, 1H, CH-3),
3.79 (dt, J = 6.5, 3.3 Hz, 4H, morpholine CH_2×2), 3.72 – 3.59 (m, 4H,
piperazine CH_2×2), 3.45 (d, J = 5.4 Hz, 4H, pipерazine CH_2×2), 3.25 (q, J = 2.2
Hz, 2H, Morpholinoacetoxy CH_2), 2.84 (d, J = 13.6 Hz, 1H, CH-1), 2.64 (q, J =
5.1, 4.6 Hz, 4H, morpholine CH_2×2), 2.39 (d, J = 3.3 Hz, 1H, CH-9), 2.33 (d, J
= 13.5 Hz, 1H, CH-16), 1.56 (s, 9H, tert-butyl CH_3×3), 1.36 (s, 3H, CH_3-27),
1.27 (s, 3H, CH₃-25), 1.19 (s, 3H, CH₃-26), 1.15 (s, 3H, CH₃-29), 0.91 (s, 6H, CH₃-23/24), 0.84 (s, 3H, CH₃-28), 0.82 (m, 1H, CH-5); ¹³C NMR (101 MHz, Chloroform-d) δ 199.88 (C11), 174.10 (C30), 169.96 (acetyloxy C=O), 169.49 (C13), 154.51 (Boc C=O), 128.49 (C12), 81.01 (tert-butyl C), 80.23 (C3), 66.80 (morpholine C×2), 61.63 (C9), 59.75 (acetoxy CH₂), 54.93 (C5), 53.27 (morpholine C×2), 48.10 (C18), 45.26 (C14), 43.86 (piperazine C×2), 43.78 (C20), 43.26 (C8/19), 38.71 (C1/C4), 38.04 (piperazine C×2), 37.70 (C22), 36.88 (C10), 33.18 (C7), 32.70 (C17), 31.75 (C21), 28.40 (C29), 28.35 (tert-butyl CH₃×3), 28.12 (C28), 27.05 (C23), 26.67 (C2), 26.38 (C15), 23.65 (C16), 23.09 (C27), 18.65 (C26), 17.35 (C6), 16.75 (C25), 16.40 (C24); HRMS (m/z): [M + H]+ calcd. for C₄₅H₇₂N₃O₇: 766.5370, found: 766.5301.

4-(3β-(2-Chloroacetoxy)-11-oxo-18β-olean-12-en-30-carbonyl)piperazine-1-carboxylic acid (15)

Chloroacetic anhydride (1.37 g, 8.0 mmol) and K₂CO₃ (2.76 g, 20.0 mmol) was heated at 130 ºC in toluene for 20 min with a constant water separator. Then, compound 9 (0.64 g, 1.0 mmol) was added to the hot stirred suspension and the reaction mixture was stirred for another 1 h at 130 ºC. On removal of the toluene, the H₂O (50 ml) was added to the cool residue, After stirring for 1 h at room temperature, the white product was filtered, washed with cold H₂O and dried. A white solid; yield, 96.3%; m.p.205.7.0-206.6 ºC; ¹H NMR (400 MHz, Chloroform-d) δ 5.66 (s, 1H, CH-12), 4.59 (dd, J = 11.8, 4.7 Hz, 1H, CH-3), 4.15 – 3.97 (m, 4H, piperazine CH₂×2), 3.65 (s, 4H, piperazine CH₂×2), 3.52 (s, 2H,
CH$_2$-Cl), 2.85 – 2.75 (m, 1H, CH-1), 2.34 (s, 1H, CH-9), 2.28 (d, $J = 12.1$ Hz, 1H, CH-16), 1.34 (m, 3H, CH$_3$-27), 1.22 (s, 3H, CH$_3$-25), 1.14 (s, 3H, CH$_3$-26), 1.10 (s, 3H, CH$_3$-29), 0.88 (s, 6H, CH$_3$-23/24), 0.80 (s, 3H, CH$_3$-28), 0.78 (m, 1H, CH-5); $^{13}$C NMR (101 MHz, chloroform-$d$) $\delta$ 199.83 (C11), 174.27 (C30), 169.43 (C13), 167.13 (chloroacetoxy C=O), 165.34 (-COOH), 128.51 (C12), 82.99 (C3), 61.62 (C9), 54.95 (C5), 48.06 (C18), 46.28 (C14), 45.26 (piperazine C), 43.93 (piperazine C), 43.83 (C20), 43.27 (C8), 42.21 (C19), 41.23 (C1), 40.65 (C-Cl), 38.67(C4), 38.22 (piperazine C×2), 37.67(C22), 36.88 (C10), 33.01 (C7), 32.68 (C17), 31.76 (C21), 28.39 (C29), 28.00 (C28), 27.03 (C23), 26.65 (C2), 26.35 (C15), 23.40 (C16), 23.11 (C27), 18.65 (C26), 17.31 (C6), 16.60 (C25), 16.40 (C24); HRMS ($m/z$): [M + H]$^+$ calcd. for C$_{37}$H$_{56}$ClN$_2$O$_6$: 659.3827, found: 659.3873.

4-(3β-(2-Morpholinoacetoxy)-11-oxo-18β-olean-12-en-30-carbonyl)piperazine-1-carboxylic acid (16)

Compound 15 (0.66 g, 1.0 mmol), morpholine (0.13 g, 1.5 mmol), K$_2$CO$_3$ (0.69 g, 5.0 mmol) and a catalytic amount of I$_2$ in absolute ethanol (15 mL) was stirred under reflux for 12 h. The reaction mixture was evaporated under reduced pressure and the residue was dissolved in ethanol/H$_2$O mixture and the white precipitate was collected by filtration.

A white solid; yield, 91.2%; m.p. 196.0-196.9 °C; $^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ 5.66 (d, $J = 2.9$ Hz, 1H, CH-12), 4.58 (dd, $J = 11.7$, 4.3 Hz, 1H, CH-3), 3.70 (t, $J = 4.5$ Hz, 4H, morpholine CH$_2$×2), 3.60 (s, 8H, piperazine CH$_2$×4 ), 3.26 –
3.16 (m, 2H, morpholinoacetoxy CH\(_2\)), 2.86 – 2.74 (m, 1H, CH-1), 2.52 (d, \(J = 5.2\) Hz, 4H, morpholine CH\(_2\)×2), 2.33 (s, 1H, CH-9), 2.27 (d, \(J = 13.2\) Hz, 1H, CH-16), 1.34 (s, 3H, CH\(_3\)-27), 1.21 (s, 3H, CH\(_3\)-25), 1.14 (s, 3H, CH\(_3\)-26), 1.09 (s, 3H, CH\(_3\)-29), 0.87 (s, 6H, CH\(_3\)-23/24), 0.79 (s, 3H, CH\(_3\)-28), 0.77 (m, 1H, CH-5); \(^{13}\)C NMR (101 MHz, Chloroform-\(d\)) δ 199.84 (C11), 174.27 (C30), 169.52 (C13), 167.71 (acetyloxy C=O), 167.13 (COOH), 128.48 (C12), 82.98 (C3), 66.76 (morpholine C×2), 61.62 (C9), 61.45 (acetoxy CH\(_2\)), 54.95 (C5), 53.44 (morpholine C×2), 48.10 (C18), 45.26 (C14), 43.91 (piperazine C×2), 43.79 (C20), 43.27 (C8), 41.86 (19), 41.23 (C1), 38.66 (C4), 38.22 (piperazine C×2), 37.68 (C22), 36.87 (C10), 33.09 (C7), 32.67 (C17), 31.76 (C21), 28.40 (C29), 28.00 (C28), 27.04 (C23), 26.66 (C2), 26.36 (C15), 23.40 (C16), 23.11 (C27), 18.64 (C26), 17.31 (C6), 16.60 (C25), 16.40 (C24); HRMS (Methanol as solvent, \(m/z\)): [M\(_{\text{Methyl ester}} + H\)]\(^+\) calcd. for C\(_{42}\)H\(_{66}\)N\(_3\)O\(_7\): 724.4901, found: 724.9726.

3\(\beta\)-(2-Morpholinoacetoxy)-11-oxo-18\(\beta\)-olean-12-en-30-carbonyl piperazine (17)

Compound 14 (0.77g, 1.0 mmol) or compound 16 (0.71 g, 1.0 mmol) was dissolved in CH\(_2\)Cl\(_2\) (10 mL) at 0 °C under stirring. Trifluoroacetic acid (5 mL) was added, and the reaction was stirred at 0 °C for 3 h. After reaction, the mixture was made basic with a saturated Na\(_2\)CO\(_3\) solution. This mixture was extracted with CH\(_2\)Cl\(_2\), dried over Na\(_2\)SO\(_4\), and concentrated to give the desired product. A white solid; yield, 91.7%; m.p. 215.9-216.7 °C; \(^1\)H NMR (400 MHz, Chloroform-\(d\)) δ 5.70 (t, \(J = 3.2\) Hz, 1H, CH-12), 4.60 (dt, \(J = 11.2, 4.7\) Hz, 1H, CH-3), 3.76 (dt, \(J = 6.7, 3.3\) Hz, 4H, morpholine CH\(_2\)×2), 3.62 (t, \(J = 5.5\) Hz, 4H, piperazine
CH₂×2), 3.21 (dd, J = 4.5, 2.4 Hz, 2H, morpholinoacetoxy CH₂), 2.91 – 2.84 (m, 4H, piperazine CH₂×2), 2.81 (d, J = 13.0 Hz, 1H, CH-1), 2.60 (t, J = 5.2 Hz, 4H, morpholine CH₂×2), 2.36 (t, J = 3.3 Hz, 1H, CH-9), 2.30 (d, J = 13.5 Hz, 1H, CH-16), 1.36 (s, 3H, CH₃-27), 1.23 (s, 3H, CH₃-25), 1.16 (s, 3H, CH₃-26), 1.12 (s, 3H, CH₃-29), 0.88 (s, 6H, CH₃-23/24), 0.82 (s, 3H, CH₃-28), 0.79 (s, 1H, CH-5);

¹³C NMR (101 MHz, cdcl₃) δ 199.95 (C11), 173.84 (C30), 170.01 (acetyloxy C=O), 169.73 (C13), 128.47 (C12), 81.03 (C3), 66.83 (morpholine C×2), 61.65 (C9), 59.78 (morpholinoacetoxy CH₂), 54.95 (C5), 53.30 (morpholine C×2), 48.23(C18), 46.30 (piperazine C×2), 45.28 (C14), 43.79 (C20), 43.75 (C8), 43.28 (piperazine C×2), 38.75 (C19), 38.06 (C1/4), 37.75 (C22), 36.91 (C10), 33.40 (C7), 32.71 (C17), 31.77 (C21), 28.43 (C29), 28.13 (C28), 27.05 (C23), 26.71 (C2), 26.44 (C15), 23.66 (C16), 23.11 (C27), 18.67 (C26), 17.37 (C6), 16.77 (C25), 16.42 (C24) ; HRMS (m/z): [M + H]⁺ calcd. for C40H₆₄N₃O₅: 666.4846, found: 666.4795.

3β-Acetoxy-30-(4-(3-fluorobenzoyl)-1-piperazinyl)-olean-12-ene-11,30-dione (18)

Compound 4 (0.58 g, 1.0 mmol) and triethylamine (0.13 g, 1.2 mmol) were dissolved in CH₂Cl₂ (20 mL) at 0 °C under stirring. 3-fluorobenzoyl chloride (0.158 g, 1.0 mmol) was added, and the reaction was stirred at room temperature for 3 h. After reaction, the mixture was washed twice with water. The organic layer was dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The crude product was then chromatographed on silica
(DCM-methanol, 20:1).

A white solid; yield, 88.2%; m.p. 234.1-225.9 °C; $^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ 7.41 (td, $J = 7.8$, 5.6 Hz, 1H, phenyl), 7.23 – 7.09 (m, 3H, phenyl), 5.68 (s, 1H, CH-12), 4.52 (dd, $J = 11.6$, 4.7 Hz, 1H, CH-3), 3.71 – 3.45 (m, 8H, morpholine CH$_2$$\times$4), 2.79 (dt, $J = 13.5$, 3.6 Hz, 1H, CH-1), 2.35 (s, 1H, CH-9), 2.32 – 2.23 (m, 1H, CH-16), 2.05 (s, 3H, acetyloxy CH$_3$), 1.35 (s, 3H, CH$_3$-27), 1.25 (s, 3H, CH$_3$-25), 1.24 (s, 3H, CH$_3$-26), 1.16 (s, 3H, CH$_3$-29), 1.12 (s, 3H, CH$_3$-23), 0.88 (s, 3H, CH$_3$-24), 0.82 (s, 3H, CH$_3$-28), 0.79 (m, 1H, CH-5); $^{13}$C NMR (101 MHz, Chloroform-$d$) $\delta$ 199.92 (C11), 174.25 (C30) – acetyloxy C=O), 169.31 (C13), 169.06 (benzoyl), 169.04 (benzoyl), 163.78 (C-F), 161.31 (C-F), 137.17 (phenyl), 137.10 (phenyl), 130.52 (phenyl), 130.44 (phenyl), 128.53 (C12), 122.73 (phenyl), 122.70 (phenyl), 117.21 (phenyl), 117.01 (phenyl), 114.56 (phenyl), 114.33 (phenyl), 80.56 (C3), 61.69 (C9), 55.00 (C5), – (C18), 45.28 (C14), 43.91 (C20), 43.70 (morpholine), 43.26 (C8), 38.78 (C19), 38.02 (C1/4), 37.67 (C22), 36.91 (C10) – (C7), 32.72 (C17), 31.78 (C21), 28.39 (C29), 28.02 (C28), 27.04 (C23), 26.66 (C2), 26.37 (C15), 23.54 (C16), 23.08 (C27), 21.32 (acetyloxy CH$_3$), 18.65 (C26), 17.35 (C6), 16.67 (C25), 16.41 (C24); HRMS (m/z): [M + H]$^+$ calcd. for C$_{43}$H$_{60}$FN$_2$O$_5$: 703.4486, found: 703.4486.
Crystal structure analysis of compound (18)

The single crystal X-ray diffraction data of compound 18 was collected on a Bruker SMART APEX II CCD detector employing graphite-monochromated Cu Kα radiation (λ = 1.54178 Å) at 273 (2) K. The structures were solved by direct methods using SHELXL-97 and refined using full-matrix least-squares calculation on F2 using SHELXL-97. All non-hydrogen atoms were refined anisotropically. The hydrogen atom positions were geometrically idealized and allowed to ride on their parent atoms. Crystallographic data for compound 18 has been deposited at the Cambridge Crystallographic Data Centre. CCDC 1904891. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
$3\beta$-Acetoxy-11-oxo-18$\beta$-olean-12-en-30-oic acid (2)
**tert-Butyl 4-(3β-acetoxy-11-oxo-18β-olean-12-en-30-carbonyl)piperazine-1-carboxylate (8)**
3β-(2-Chloroacetoxy)-11-oxo-18β-olean-12-en-30-oic acid (12)
1H-Benzo[d][1,2,3]triazol-1-yl-3\(\beta\)-acetoxy-11-oxoolean-12-en-30-oate (6)
$1H$-Benzo[d][1,2,3]triazol-1-yl-$3\beta$-hydroxy-11-oxo-olean-12-en-30-oate (7)
tert-Butyl 4-(3β-hydroxyl-11-oxo-18β-olean-12-en-30-carbonyl)piperazine-1-carboxylate (9)
tert-Butyl 4-(3β-(2-morpholinoacetoxy)-11-oxo-18β-olean-12-en-30-carbonyl)piperazine-1-carboxylate (14)
N-(2-Chloroacetyl)piperazinyl 3β-(2-chloroacetoxyl)-11-oxo-18β-olean-12-en-30-amide (11)
GA2 #211 RT: 1.75 AV: 1 NL: 1.06E7
F: FTMS + p ESI Full ms [100.00-1000.00]
4-(3β-(2-Chloroacetoxy)-11-oxo-18β-olean-12-en-30-carbonylpiperazine-1-carboxylic acid (15)
$3\beta$-(2-Morpholinoacetoxy)-11-oxo-olean-12-ene-30-oic acid (13)
4-(3β-(2-Morpholinoacetoxy)-11-oxo-18β-olean-12-en-30-carbonyl)piperazine-1-carboxylic acid (16)
3β-Acetoxy-11-oxo-18β-olean-12-en-30-carbonyl piperazine (4)
Bisamide product

*ESI Scan (13.258-14.005 min, 36 Scans) Frag=135.0V DA.d Subtract

Counts (%) vs. Mass-to-Charge (m/z)

Counts:

1097.753474
$3\beta$-(2-Morpholinoacetoxy)-11-oxo-18$\beta$-olean-12-en-30-carbonyl piperazine (17)
3β-Acetoxy-30-(4-(3-fluorobenzoyl)-1-piperazinyl)-olean-12-ene-11,30-dione (18)
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