Synthesis and Cytotoxic Activity of Ursolic Acid Derivatives

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Fourteen ursolic acid derivatives, among them four novel compounds, were synthesized by modification either at the C-3, C-28 or both positions. The cytotoxic activity of the obtained derivatives was evaluated against the four human cancer cell lines KB (human mouth epidermal carcinoma), HepG2 (human hepatocellular carcinoma), MCF7 (human breast carcinoma) and Lu (human lung carcinoma). As the result, compounds 7 and 8 were from two to three times more active than ursolic acid on all four tested cell lines. This is the first report on cytotoxic effects of the synthetized ursolic acid derivatives 4, 8, and 10–15.

Key words: Ursolic Acid Derivatives, Cytotoxic Activity

Introduction

The genus Eriobotrya (Rosaceae) contains about 26 species. Only one of these species, Eriobotrya japonica (Thunb.) Lindl. (loquat) was hitherto intensively studied and contains many interesting chemical constituents with biological activities. Our phytochemical investigation of leaves of Eriobotrya poilanei J. E. Vid. growing in Vietnam showed the presence of ursolic acid in large amount (0.32% of dry leaves weight). Ursolic acid has been reported to possess a series of biological activities such as antitumor, antimicrobial, anti-inflammatory, hepatoprotective, and cardioprotective properties [1]. A series of ursolic acid derivatives and their biological activities have been previously published [2, 3]. This article describes the isolation of ursolic acid from Eriobotrya poilanei’s leaves, the synthesis of its derivatives, among them four are new compounds (8, 10, 14, 15), and their cytotoxic activity against the human cancer cell lines KB, HepG2, MCF7, and Lu. The cytotoxic activity test results show that ten derivatives exhibited activity on all four tested cancer cell lines, two of them, compounds 7 and 8, are two to three times more active than ursolic acid itself.

Results and Discussion

Scheme 1 outlines the synthesis of ursolic acid derivatives 2–15. The hydroxyl group at C-3 of ursolic acid (1) was acylated with acetic, succinic and phthalic anhydrides to afford esters 2 (90%), 3 (60%) and 4 (60%), respectively. Jones oxidation of ursolic acid yielded ketone 5 as the main product with a yield of 65%. With the aim to introduce a nitrogen function to the ursolic acid skeleton, ketone 5 was transformed into ketoxime 6 and then to its acetyl product 7 with good yield (62% for 6; 87% for 7). For the synthesis of derivatives with a nitrogen function at C-18 of ursolic acid, the 3-hydroxy group was acetylated and the acetyl product 2 reacted with oxalyl chloride, then with the corresponding amines giving the amides 8, 9 and 11 (after hydrolysis of 9). The correlations between the amide proton at $\delta_H = 6.00$ ppm and the carbonyl carbon ($\delta_C = 178.5$ ppm) and the methylene carbon ($\delta_C = 39.71$ ppm) in the HMBC spectrum as well as the correlation between this proton and two methylene protons at $\delta_H = 2.99$ and 3.31 ppm (each 1 H, m) in the $^1H$-$^1H$ COSY spectrum of compound 8 confirmed that the amidation took place at the primary amine group. If an aqueous ammonia solution
Scheme 1. Synthesis of ursolic acid derivatives.

was used instead of the amines, the amide 10 was obtained (85% yield) which was hydrolyzed to amide 13 and then to the acid amides 14 and 15 with the corresponding diacid anhydrides. Interestingly, in the reaction of amide 10 with acetyl chloride after 2 hours at room temperature the dehydration product 12 was obtained (76%) instead of an expected diacetyl product.

Reagents and reaction conditions

(a): (CH₃CO)₂O, pyridine, r. t., 24 h
(b): (CH₂CO)₂O, DMAP, pyridine, r. t., 24 h
(c): C₆H₄(CO)₂O, DMAP, pyridine, r. t., 24 h
(d): CrO₃, H₂SO₄, acetone, r. t., 2 h
(e): NH₂OH.HCl, 60 °C, 4 h
(f): (COCl)₂, CH₂Cl₂, 25 °C, 48 h
(g): R¹NH₂, TEA/CH₂Cl₂, r. t., 24 h
(h): NH₃·H₂O, 0 °C; r. t., 12 h
(i): NaOH 4N, THF/MeOH (1/1), r. t., 12 h
(k): CH₃COCI, CH₂Cl₂, r. t., 2 h
Table 1. The cytotoxic activity of ursolic acid (1) and the synthesized derivatives.

<table>
<thead>
<tr>
<th>Compound</th>
<th>KB</th>
<th>HepG2</th>
<th>MCF-7</th>
<th>LU</th>
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<tbody>
<tr>
<td>I (Ursolic acid)</td>
<td>10.23</td>
<td>11.75</td>
<td>8.0</td>
<td>12.23</td>
</tr>
<tr>
<td>2</td>
<td>8.00</td>
<td>4.73</td>
<td>27.50</td>
<td>34.79</td>
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<td>3</td>
<td>19.60</td>
<td>6.17</td>
<td>21.07</td>
<td>24.35</td>
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<tr>
<td>4</td>
<td>17.05</td>
<td>8.00</td>
<td>19.73</td>
<td>40.72</td>
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<tr>
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<td>3.51</td>
<td>4.36</td>
<td>25.36</td>
<td>19.79</td>
</tr>
<tr>
<td>7</td>
<td>4.32</td>
<td>4.31</td>
<td>3.43</td>
<td>5.44</td>
</tr>
<tr>
<td>8</td>
<td>4.90</td>
<td>5.03</td>
<td>4.78</td>
<td>4.96</td>
</tr>
<tr>
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<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
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<tr>
<td>11</td>
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<td>12</td>
<td>&gt;128</td>
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<td>13</td>
<td>62.92</td>
<td>53.46</td>
<td>43.44</td>
<td>71.23</td>
</tr>
<tr>
<td>14</td>
<td>26.0</td>
<td>9.14</td>
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<tr>
<td>15</td>
<td>6.39</td>
<td>8.00</td>
<td>44.62</td>
<td>49.31</td>
</tr>
<tr>
<td>Ellipticin</td>
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<td>0.79</td>
<td>0.72</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Ursolic acid (1) and its twelve synthesized derivatives have been tested against four human cancer cell lines: human mouth epidermal carcinoma (KB), human hepatocellular carcinoma (Hep-G2), human breast carcinoma (MCF-7), and human lung carcinoma (LU). The results show that, except compounds 10 and 12, all other compounds were active against all 4 tested cancer cell lines with different IC₅₀ values (Table 1). Especially compounds 7 and 8, where the nitrogen function has been introduced at the position C-3, the cytotoxicity was from 2 to 3 times higher than that of ursolic acid itself against all tested cell lines. Besides these two derivatives (7 and 8), there are some other good active ones, for example compound 2 against Hep-G2 (IC₅₀ = 4.73 µg mL⁻¹) and 5 against KB and Hep-G2 (IC₅₀ = 5.31 and 4.36 µg mL⁻¹, respectively).

It has been reported that 3-oxo-ursolic acid (5) and 3β-O-acetylursolic acid (2) possess cytotoxic activity against HONE-1 (human nasopharyngeal carcinoma), KB, and HT29 (colorectal carcinoma) cancer cell lines [4]. Among the components isolated from the dichloromethane extract of the dried fruits of Chaenomeles speciosa (Sweet) Nakai (Rosaceae), 3β-O-acetyl ursolic acid showed the highest activity against both prostaglandin-H-synthase isoenzymes [5]. 3β-Succinoyl-urs-12-18-28-oic acid (3) exhibited cytotoxicity against NTUB1 (human bladder cancer cell line) with IC₅₀ = 8.65 µM [6]. This is the first report about the cytotoxicity against MCF7 and LU cancer cell lines of 3β-O-acetylursolic acid (2) and 3-acetoximino-urs-12-en-28-oic acid (7). Until now there were no investigations on the cytotoxicity of the remaining synthetized derivatives. Our results indicate again a potential for the study of the structure-activity relationship of ursolic acid derivatives.

Experimental Section

General


Isolation of ursolic acid (1)

The leaves of Eriobotrya poilanei were collected in March 2009 in Bi Dup National Park, Nui Ba, Lam Dong province of Vietnam, and identified by Dr. Nguyen Tien Hiep, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. A voucher specimen (VH4212) is deposited at the Herbarium of this Institute.

Dried leaves (1800 g) were extracted exhaustively with methanol-water = 85 : 15 at room temperature. The organic solvent was evaporated under reduced pressure, and the aqeous solution was successively extracted with n-hexane, ethyl acetate and n-butanol. Evaporation of these extracts yielded 9.4, 50 and 130 g of a residue, respectively. The ethyl acetate extract (50 g) was purified on a silica gel column eluting with increasing polarity of n-hexane/ethyl acetate and then ethyl acetate/methanol to furnish 5.8 g of 1 (0.32% of the dried plant material). The spectral data of 1 are in good agreement with those reported for ursolic acid [7].

Bioactivity assays

Bioactivity assays were carried out in the Institute of Chemistry, Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam. Cytotoxic assays were performed according to Likhiwitayawuid et al. [8] and Skehan et al. [9] at different concentrations in 96-well plates. The HEP-G2, KB and MCF-7 cell lines were maintained in the RPMI-1640 culture medium with 10% fetal bovine serum (FBS). The LU cell line was maintained in DMEM culture medium with 10% fetal bovine serum (FBS).
3β-Succinoyl-urs-12-en-28-oic acid (3) and 3β-phthaloyl-urs-12-en-28-oic acid (4)

A mixture of ursolic acid (1) (0.1 mmol), succinic acid (0.5 mmol) or phthalic anhydrides (0.5 mmol) and 4-dimethylaminopyridine (DMAP, 0.3 mmol) in pyridine (5 mL) was stirred at 60 °C. After stirring for 8 h, water was added, and the mixture was extracted with EtOAc. The organic phase was neutralized with 1N HCl solution, washed with water, dried, evaporated and chromatographed on a silica gel column (CH₂Cl₂-MeOH = 95:5) to furnish 32 mg of 3 or 35 mg of 4 as colorless solids (60%).

**Compound 3**: colorless powder. – MS ((+)-ESI): m/z = 555 [M-H]⁻, 579 [M+Na]⁺ (C₃₈H₅₂O₁₅). – ¹H NMR (500 MHz, CDCl₃ + CD₃OD): δ = 0.80 (3H, s, CH₃), 0.82 (3H, d, 6.2 Hz, CH₃), 0.86 (3H, s, CH₃), 0.87 (3H, s, CH₃), 1.09 (3H, s, CH₃), 1.63 (3H, s, CH₃), 1.75 (3H, s, CH₃).

**Compound 4**: colorless powder. – MS ((+)-ESI): m/z = 603 [M–H]⁻, 627 [M+Na]⁺ (C₃₆H₄₅O₁₄). – ¹H NMR (500 MHz, CDCl₃ + CD₃OD): δ = 0.82 (3H, s, CH₃), 0.87 (3H, d, 6.0 Hz, CH₃), 0.92 (3H, s, CH₃), 0.94 (3H, d, 8.0 Hz, CH₃), 1.11 (3H, s, CH₃), 1.26 (3H, s, CH₃), 1.41 (3H, s, CH₃), 1.91 (3H, d, 7.3 Hz, H-3), 5.25 (1H, t-like, 12-H), 7.50–7.72 ppm (4H, m). – ¹³C NMR (125 MHz, CDCl₃ + CD₃OD): δ = 15.32q, 16.70q, 16.90q, 18.09q, 21.03q, 23.19q, 24.08q, 27.90q, 28.02q, 29.56q, 30.56q, 32.84q, 36.70q, 37.71q, 38.20q, 38.79q, 38.97q, 39.38q, 41.96q, 47.37q, 47.70q, 52.69q, 55.35q, 82.61q, 125.26q, 128.45q, 130.05q, 130.83q, 131.92q, 138.13q, 168.21q (× 2), 180.66q ppm.

**Oxo-urs-12-en-28-oic acid (5)**

To a solution of ursolic acid (1) (46 mg, 0.1 mmol) in acetone (10 mL) were added CrO₃ (100 mg, 1.0 mmol) and SO₃·H₂O (20%, 10 mL). The reaction mixture was stirred at r.t. for 4 h, then concentrated in vacuo and extracted with ethyl acetate (50 mL). The organic phase was washed with water (2 × 20 mL), dried over Na₂SO₄, filtered and concentrated in vacuo, then purified by silica gel column chromatography (n-ethyl-acetone acetate = 7:1) to give 30 mg (65%) of 5 as a colorless solid. – IR (KBr): ν = 3432, 2314, 2932, 2967, 1460, 1387, 1275, 1030 cm⁻¹.

**3-Hydroxyimino-urs-12-en-28-oic acid (6)**

A mixture of 5 (46 mg, 0.1 mmol) and NH₄OH-HCl (40% in water, 1 mL) in pyridine/ethanol (1/1.2 mL) was heated at 60 °C for 4 h. After cooling to r.t., the reaction mixture was evaporated and extracted with ethyl acetate (3 times). The organic phase was washed with water, dried over Na₂SO₄, filtered, concentrated and chromatographed on a silica gel column (dichloromethane-methanol = 98:2) to furnish 29 mg (62%) of 6 as a colorless powder. The NMR spectroscopic data of compounds 5 and 6 were identical with published data [3].

**3-Acetoxymino-urs-12-en-28-oic acid (7)**

A mixture of 6 (30 mg, 0.064 mmol) and acetic anhydride (0.1 mL) in pyridine (1 mL) was stirred at r.t. After 2 h, the reaction mixture was concentrated in vacuo to dryness and then purified using a silica gel column, eluted with n-hexane-ethyl acetate = 4:1 to furnish 28 mg (87%) of 7 as a colorless amorphous powder. – IR (KBr): ν = 2947, 2869, 1774, 1692, 1623, 1459, 1371, 1211 cm⁻¹. – ¹H NMR (500 MHz, CDCl₃): δ = 0.80 (3H, s, CH₃), 0.86 (3H, d, 6.3 Hz, CH₃), 0.94 (3H, d, 6.3 Hz, CH₃), 1.02 (3H, s, CH₃), 1.07 (3H, s, CH₃), 1.12 (3H, s, CH₃), 2.18 (3H, s, -COCH₂). – ¹³C NMR (125 MHz, CDCl₃): δ = 52.04, 59.71, 77.82, 122.89, 122.90 ppm.

Compounds 8, 9 and 10 were synthesized as following: To a solution of 2 (49 mg, 0.1 mmol) in CH₂Cl₂ (3 mL) was added oxalyl chloride (0.1 mL) at r.t. After 48 h, the mixture was evaporated in vacuo to yield 2a.

N-3β-Acetoxy-urs-12-en-28-oyl)-2,6-diaminohexane (8)

A solution of 2a in CH₂Cl₂ (5 mL) was added to a mixture of NH₂(CH₂)₃CH(CH₃)NH₂ (58 mg, 0.5 mmol), triethylamine (15 mg) and CH₂Cl₂ (10 mL) at r.t. After stirring overnight, the reaction mixture was diluted with CH₂Cl₂ (30 mL) and washed with water. The organic layer was dried over Na₂SO₄, evaporated, purified by silica gel column chromatography using dichloromethane-methanol = 100:5 as eluent to give 42 mg of 8 as a colorless powder, 70%. – MS ((+)-ESI): m/z = 595 [M-H]⁻. – HRMS ((+)-ESI): m/z = 597.49961 (calcd. 597.49952 for C₃₆H₅₁N₂O₁, [M+H]⁺). – ¹H NMR (500 MHz, CDCl₃): δ = 0.77 (3H, s, CH₃), 0.86 (3H, d, 5.0 Hz, CH₃), 0.87 (3H, s, CH₃), 0.88 (3H, s, CH₃), 0.95 (3H, s, CH₃), 0.96 (3H, d, 6.2 Hz, CH₃), 0.96 (3H, s, CH₃), 1.09 (3H, s, CH₃), 2.05 (3H, s, OCOCH₃), 2.71 [1H, m, -CH(CH₃)₂-NH₂]. – ¹³C NMR (125 MHz, CDCl₃): δ = 15.58q, 16.74q, 16.98q, 17.30q, 17.51q, 18.17q, 21.25q, 21.32q, 23.23q, 23.43q, 23.55q, 24.89q, 26.70q, 27.85q, 28.08q, 30.91q, 31.48q, 32.70q, 36.86q, 37.26q, 37.70q, 38.33t, 39.09d, 39.41d, 39.58s, 39.71q, 39.79q, 42.52q, 47.09s, 47.48q, 47.68d, 53.87d, 55.24d, 80.85d (C-3), 125.49d (C-12), 140.05s (C-13), 171.02s (OCOCH₃), 178.5s ppm (NH-C=O).
N-(3β-Acetoxy-urs-12-en-28-oyl)-11-amino deucanooic acid methyl ester (9)

Compound 2a (51 mg) in CH₂Cl₂ (5 mL) and 50 L of triethylamine were dropped slowly into a solution of 22 mg (0.1 mmol) of NH₂(CH₂)₉(OOCCH₃)CH₂Cl₂ (3 mL) and stirred at r.t. for 12 h. The mixture was then diluted with CH₂Cl₂ (30 mL), washed with water (20 mL), dried over Na₂SO₄, evaporated in vacuo and chromatographed over a silica gel column (n-hexane-ethyl acetate = 2:1) to give 60 mg of 9 as a colorless powder. – IR (KBr): υ = 3405, 2923, 2874, 1717, 1632, 1530, 1459, 1383, 1263, 1034 cm⁻¹. – ¹H NMR (500 MHz, CDCl₃): δ = 0.84 (3H, s, CH₃), 0.88 (3H, d, 6.4 Hz, CH₃), 0.95 (3H, s, CH₃), 1.05 (3H, d, 5.6 Hz, CH₃), 1.06 (3H, s, CH₃), 1.09 (3H, s, CH₃), 1.13 (3H, s, CH₃), 2.30 (2H, t, 7.5 Hz, -CH₂-COOCH₃), 2.56, 3.03 (each 1H, m, -CONH-CH₂), 3.28 (1H, m, 3-H), 3.66 (3H, s, -COOCH₃), 5.33 (1H, br s, 12-H), 5.88 ppm (1H, t, JV = 5.5 Hz, -CONH-).

3β-Acetoxy-urs-12-en-28-carboxamide (10)

A solution of 2a (94 mg) in THF (10 mL) was slowly added to aqueous NH₃ (25%, 10 mL) at 0°C. The reaction mixture was stirred at r.t. overnight and then concentrated. After addition of ethyl acetate, the solution was washed with 1 N HCl, neutralized with NaHCO₃, washed with water, dried, and evaporated in vacuo. The residue was chromatographed on a silica gel column (n-hexane-ethyl acetate = 3:1) to afford 77 mg (85%) of compound 10 as a colorless powder. – IR (KBr): υ = 3477, 3173, 2947, 2869, 1730, 1674, 1607, 1458, 1377, 1251, 1038 cm⁻¹. – HRMS (±ESI): m/z = 498.39461 (calcd. 498.39472 for C₃₂H₅₁NO₃·Na·H₂O). – ¹H NMR (500 MHz, CDCl₃): δ = 0.85 (3H, s, CH₃), 0.86 (3H, d, 5.5 Hz, CH₃), 0.87 (3H, s, CH₃), 0.88 (3H, d, 6.8 Hz, CH₃), 0.95 (3H, s, CH₃), 0.96 (3H, s, CH₃), 1.06 (3H, s, CH₃), 1.10 (3H, s, CH₃), 2.05 (3H, s, OCOC₂H₅), 4.50 (1H, dd, 5.5, 10.0 Hz, -3-H), 5.30 (1H, t,-like, 12-H), 5.90 ppm (2H, s, NH₂). – ¹³C NMR (125 MHz, CDCl₃): δ = 15.53q, 16.68q, 17.11q, 17.23q, 18.12q, 21.18q, 21.26q, 23.18q, 23.35t, 23.50t, 24.81t, 27.84t, 28.03q, 30.84t, 32.70t, 36.83t, 37.11t, 37.65s, 38.28t, 39.04q, 39.38s, 39.71d, 42.43s, 47.44d, 47.91s, 54.21d, 55.22d, 80.81d (C-3), 125.62d (C-12), 139.74s (C-13), 170.95s (OCOCH₃), 181.36s ppm (C-28).

N-(3β-Hydroxy-urs-12-en-28-oyl)-11-amino deucanooic acid (11) and 3β-hydroxy-urs-12-en-28-carboxamide (13)

Compounds 11 and 13 were obtained through the hydrolysis of 9 and 10, respectively. A 4 N NaOH solution (0.5 mL) was added to a mixture of 9 or 10 (0.05 mmol in 5 mL of THF/CH₂Cl₂ = 1/1) at r.t. After stirring for 15 h, the reaction mixture was evaporated in vacuo. The residue was dissolved in water, neutralized with 2 N HCl to pH = 7 and extracted with CH₂Cl₂ (50 mL). The organic layer was washed with water, dried over Na₂SO₄, filtered and evaporated in vacuo. Purification of the crude product on a silica gel column (n-hexane-ethyl acetate = 2:1) furnished 30 mg of 11, while chloroform-methanol = 4:1 yielded 35 mg of 13.

Compound 11: colorless powder, yield 95%. – IR (KBr): υ = 3405, 2923, 2874, 1717, 1632, 1530, 1459, 1383, 1263, 1034 cm⁻¹. – ¹H NMR (500 MHz, CDCl₃): δ = 0.78 (3H, s, CH₃), 0.87 (3H, d, 6.5 Hz, CH₃), 0.93 (3H, d, 7.0 Hz, CH₃), 0.99 (6H, s, CH₃), 1.07 (3H, s, CH₃), 1.26 (3H, s, CH₃), 2.32 (2H, t, 7.5 Hz, -CH₂-COOH), 3.02 (1H, br s), 3.22 (1H, m), 3.28 (1H, m, 3-H), 5.31 (1H, br s, 12-H), 5.93 ppm (1H, br s, -CONH-).

Compound 13: colorless powder, yield 76%. – MS (±ESI): m/z = 502 [M+Na]+. – HRMS (±ESI): m/z = 502.36555 (calcd. 502.36610 for C₃₂H₅₀NO₂Na·[M+Na]+). – ¹H NMR (500 MHz, CDCl₃): δ = 0.84 (3H, d, 6.5 Hz, CH₃), 0.87 (3H, s, CH₃), 0.88 (3H, s, CH₃), 0.94 (3H, d, 7.5 Hz, CH₃), 0.99 (3H, s, CH₃), 1.08 (6H, s, 2 × CH₃), 2.05 (3H, s, OCOC₂H₅), 4.50 (1H, dd, 7.0, 9.3 Hz), 5.36 ppm (1H, t-like, 12-H). – ¹³C NMR (125 MHz, CDCl₃): δ = 15.64q, 16.74q, 16.93q, 17.40q, 18.19q, 20.85q, 21.28q, 23.03q, 23.34t, 23.51t, 25.42t, 28.09q, 28.54t, 29.99q, 33.31q, 36.45s, 36.88s, 37.69s, 38.42t, 38.68t, 39.68d, 39.78d, 42.30s, 47.52s, 47.52d, 55.32d, 55.36d, 80.85d (C-3), 125.00s (CN), 127.32d (C-12), 136.75s (C-13), 170.99s ppm (OCOCH₃).

3β-Succinoyl-urs-12-en-28-carboxamide (14) and 3β-phthaloyl-urs-12-en-28-carboxamide (15)

Compound 13 was converted into 14 and 15 by the same manner and with the same reagents as for 9 and 4, respectively.

Compound 14: colorless powder, yield 60%. – IR (KBr): υ = 3458, 3201, 2922, 2865, 1735, 1713, 1641, 1577, 1454, 1385, 1267, 1213, 1000 cm⁻¹. – MS (±ESI): m/z = 556 [M+H]+; 554 [M–H]+. – HRMS (±ESI):
ESI): \( m/z = 556.40019 \) (calcd. 556.40020 for \( C_{34}H_{54}NO_5 \), \([M+H]^+\)). – \( ^{1}H \) NMR (300 MHz, CDCl\(_3\)): \( \delta = 0.81 \) (3H, s, CH\(_3\)), 0.85 (3H, d, 8.5 Hz, CH\(_3\)), 0.89 (3H, s, CH\(_3\)), 0.92 (3H, d, 6.2 Hz, CH\(_3\)), 0.95 (3H, s, CH\(_3\)), 0.96 (3H, s, CH\(_3\)), 1.10 (3H, s, CH\(_3\)), 2.64 (4H, br s, H-2' and H-3'), 4.52 (1H, dd, 6.0, 10.0 Hz, 3-H), 5.30 (1H, br s, 12-H), 5.93, 6.78 ppm (each 1H, br s, NH\(_2\)). – \( ^{13}C \) NMR (75 MHz, CDCl\(_3\)): \( \delta = 15.71q, 16.90q, 17.16q, 17.23q, 18.19t, 21.19q, 23.24q, 23.35t (\times 2), 24.61t, 27.81t, 28.11q, 29.24t, 29.67t, 30.74t, 32.52t, 36.83s, 37.11t, 37.72s, 38.04t, 39.00d, 39.33s, 39.66d, 42.28s, 47.20d, 47.79s, 53.94d, 55.09d, 81.25d, 125.73d, 139.50s, 171.84s, 176.32s, 182.81s ppm.

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