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Efficient Access to 2-Isobetulinic Acid, 2-Isooleanolic Acid, and 2-Isoursolic Acid

Jia Hao, Pu Zhang, Xiaoan Wen, and Hongbin Sun*

Center for Drug Discovery, College of Pharmacy, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China

hbsun2000@yahoo.com

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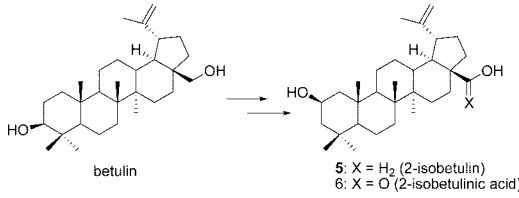

An efficient access to 2-isobetulin, 2-isobetulinic acid, 2-isooleanolic acid, and 2-isoursolic acid has been developed. The key step is a novel one-pot conversion of 2,3-dihydroxy triterpenes to 3-deoxy-2-oxo triterpenes. This method provides a new access to 3-deoxy-2-substituted pentacyclic triterpenes as potential therapeutic agents against metabolic diseases, tumors, and HIV infection.

Pentacyclic triterpenes have recently attracted much attention due to their great potential as multitarget therapeutics.[1] Betulinic acid (**1**), oleanolic acid (**2**), and ursolic acid (**3**) (Figure 1) are three representative members of the pentacyclic triterpene family that are widely distributed throughout the plant kingdom. A variety of biological properties have been ascribed to pentacyclic triterpenes.[1] Triterpenes **2** and **3** are among the major effective components of some well-known Traditional Chinese Medicines (TCM) such as Rehmannia Six Formula (Liu Wei Di Huang Wan), which is one of the most commonly used Chinese herb formulas in the world. On the other hand, pentacyclic triterpenes have also been clinically used in the form of single components, for example, **2** has been used as a nonprescription antihepatitis drug for more than 20 years in China.[2] Betulinic acid (**1**) is currently undergoing phase II clinical trial for treatment of melanoma.[3] Furthermore, synthetic pentacyclic triterpene derivatives also find potential clinical utilization, as evidenced by PA-457 (a synthetic derivative of **1**), which is being tested in a phase II clinical trial as a first-in-class anti-HIV agent.[3] Recently, we first reported that **1**, **2**, **3**, betulin (**4**), and other naturally occurring pentacyclic triterpenes represented a novel class of

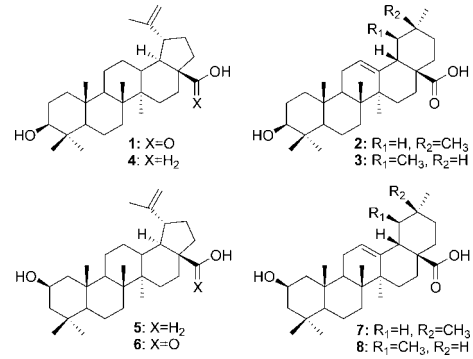

FIGURE 1. Natural triterpenes and their A-ring regioisomers.

inhibitors of glycogen phosphorylases (GP),[4] which has been regarded as a therapeutic approach to type 2 diabetes and its complications.[5] On the other hand, structural modifications based on natural triterpenes have been extensively explored to find more potent pentacyclic triterpenes as preventive and therapeutic agents.[1,6,7] Studies on structure−activity relationships (SAR) of pentacyclic triterpenes showed that A-ring functions had a significant impact on biological activities.[4,6,7] In this regard, it should be interesting and of biological importance to see how the 2β-hydroxy-3-deoxy function would affect biological activities in contrast to naturally occurring pentacyclic triterpenes with 3β-hydroxy function. Herein, we report an efficient access to 2-isobetulin (**5**), 2-isobetulinic acid (**6**), 2-isooleanolic acid (**7**), and 2-isoursolic acid (**8**) (Figure 1), which are A-ring regioisomers of betulin, betulinic acid, oleanolic acid, and ursolic acid, respectively. To the best of our knowledge, this is the first report of syntheses of the title compounds.

For the synthesis of 2-isobetulinic acid (**6**), 2β,3β-diol **12** was prepared as a key intermediate (Scheme 1). Treatment of betulin (**4**) with tritylchloride in the presence of 4-(dimethylamino)pyridine (DMAP), followed by oxidation with pyridinium chlorochromate (PCC) furnished ketone **10**.[8] Treatment of **10** with a large excess of potassium *tert*-butoxide under air led to formation of diketone **11**,[9] which existed in the form of α,β-unsaturated ketone. Reduction of **11** with sodium borohydride gave diol **12** (68% from **10**).

Interestingly, reaction of 2β,3β-diol **12** with *p*-toluenesulfonyl chloride in pyridine at slightly elevated temperature did not give

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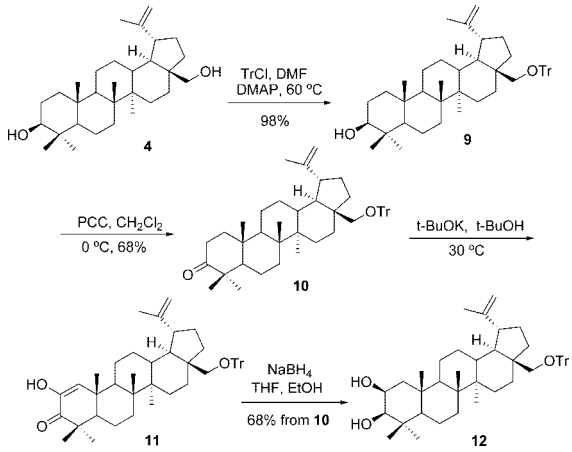
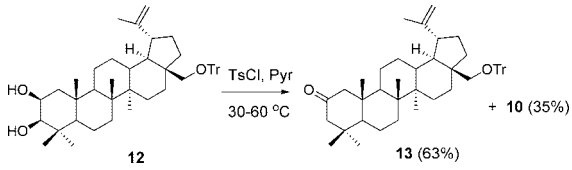
SCHEME 1



SCHEME 2



the anticipated tosyl esters as the major products, but instead gave ketone **13** in 63% yield, together with ketone **10** (35%) as a minor product (Scheme 2). The structure of **13** was confirmed by spectroscopic means. This reaction was intriguing since it might open a new access to the 3-deoxy-2-substituted triterpene scaffold[10] that could be valuable in the synthesis of new biologically active pentacyclic triterpenes. Detailed investigation of this process led to a proposed mechanism for the formation of **13** and **10** (Figure 2).

As shown in Figure 2, 2β,3β-diol **12** might be first converted to tosylates **12a** and **12b**, respectively. It was found that tosylation reactions proceeded very slowly at temperatures below 30 °C. Both tosylates **12a** and **12b** seemed to be unstable under reaction conditions, and were subject to elimination reactions upon raising the temperature to about 60 °C, resulting in **10** and **13**, respectively. The fact that **13** was produced as the major product indicated that formation of tosylate **12b** should be more favored over that of tosylate **12a**. This assumption seems to conflict with our observation[11] that 2β-OH is likely less sterically hindered than 3β-OH since when treating 2β,3β-diols such as **12** with *tert*-butyldimethylsilyl chloride (TBDMSCl) in the presence of imidazole in DMF, 2-*O*-silylated products would be obtained as the major products (>70%) together with 3-*O*-silylated products as the minor products. A reasonable explanation for this conflict is that although formation of tosylate **12a** might be kinetically more favored over that of tosylate **12b** due to a smaller steric hindrance at C-2 than at C-3, the overall reaction should be thermodynamically controlled since tosylate **12a** might be extremely unstable due to very strong 1,3-interactions of the 2-tosylate group with the methyl groups at C-24 and C-25. Thereby, once tosylate **12a** is formed, it would be either quickly subject to elimination to furnish **10** or converted to tosylate **12b**

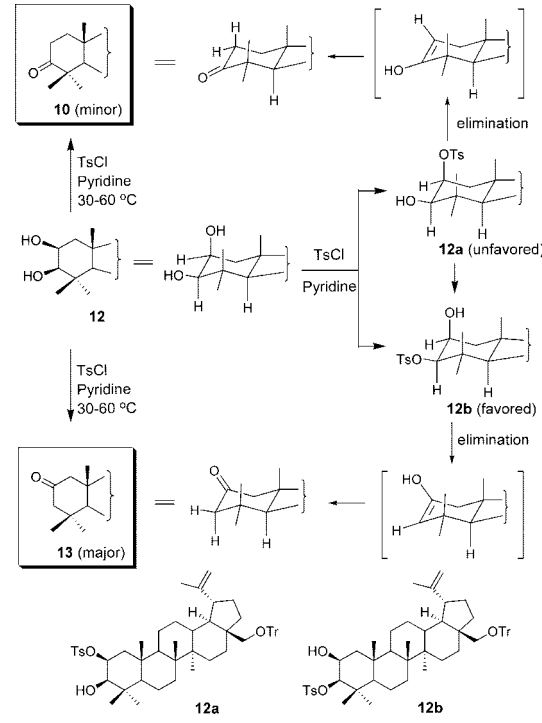
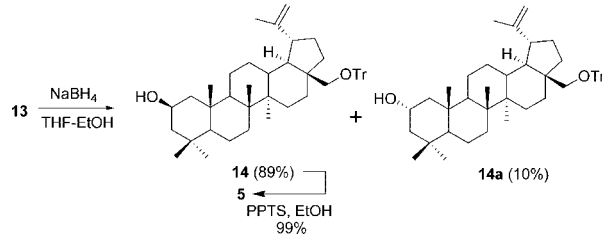


FIGURE 2. Proposed mechanism for the formation of **13** and **10**.

SCHEME 3



via an intramolecular transesterification. In fact, tosylate **12a** could not be isolated and identified even at low temperature probably due to this instability. On the other hand, if the reaction was terminated before its completion, tosylate **12b** could be isolated and its structure was determined by spectroscopic data (see the Supporting Information).

Stereoselective reduction of **13** with sodium borohydride produced 2β-hydroxy triterpene **14** in 89% yield, together with 2α-hydroxy isomer **14a** (10%) (Scheme 3). The stereochemical structures of **14** and **14a** were determined by NOE spectroscopic data (see the Supporting Information). Deprotection of **14** with PPTS afforded 2-isobetulin (**5**) in quantitative yield.

Acetylation of **14** with acetic anhydride in the presence of 4-(dimethylamino)pyridine (DMAP) in anhydrous pyridine, followed by deprotection with PPTS afforded alcohol **15** in 80% yield for two steps (Scheme 4). Oxidation of **15** with pyridinium chlorochromate (PCC) gave aldehyde **16** (92%), which was further oxidized with sodium chlorite ($NaClO_2$) and sodium dihydrogenophosphate in a mixture of *t*-BuOH/THF/2-methyl-2-butene[12] to furnish triterpene acid **17** (86%). Hydrolysis of **17** with aqueous sodium hydroxide in methanol gave 2-isobetulinic acid (**6**) in 96% yield.

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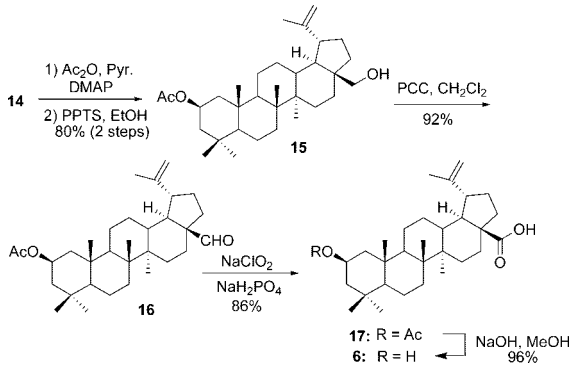
SCHEME 4



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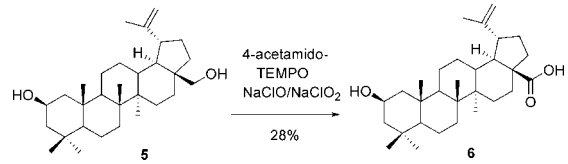

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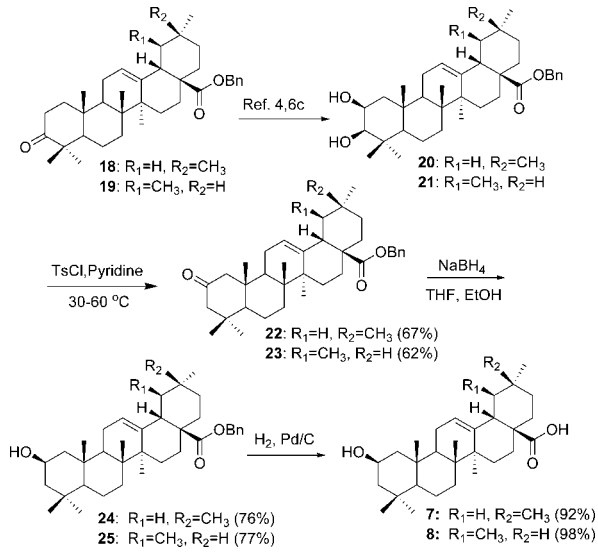

Alternatively, 2-isobetulinic acid (**6**) could also be prepared via 4-acetamido-TEMPO-catalyzed oxidation[13] of **5** without affecting its secondary 2β-hydroxyl group (Scheme 5). This reaction, however, was quite a tricky reaction, requiring strictly controlled conditions. After much experimentation, **6** was obtained in 28% yield (see the Supporting Information).

For the synthesis of 2-isooleanolic acid (**7**) and 2-isoursolic acid (**8**), the same methodology for preparation of **13** was employed as depicted in Scheme 6. Therefore, treatment of diol **20**[4,6c] with *p*-toluenesulfonyl chloride in pyridine with mild heating up to about 60 °C afforded ketone **22** (67%), together with ketone **18** as a minor product. Reduction of **22** with sodium borohydride gave alcohol **24** (76%), together with a small amount of 2α-isomer **24a** (5%). Hydrogenolysis of **24** over palladium/carbon in THF furnished 2-isooleanolic acid (**7**) in 92% yield. In the same fashion, 2-isoursolic acid (**8**) was synthesized starting from ketone **19** (Scheme 6) (see the Supporting Information).

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In conclusion, we have established an efficient method for the syntheses of 2-isobetulin, 2-isobetulinic acid, 2-isooleanolic acid, and 2-isoursolic acid. The key step is a novel one-pot conversion of 2β,3β-dihydroxy triterpenes to 3-deoxy-2-oxo triterpenes. This methodology provides a new access to 3-deoxy-2-substituted pentacyclic triterpenes that may find potential utilities as therapeutic agents against metabolic diseases, tumors, and HIV infection. Biological evaluation of the title compounds and further derivatization of this triterpene scaffold are currently in progress and will be reported in due course.

Experimental Section

General Procedure for the Preparation of Diols 12, 20, and 21. To a solution of 28-*O*-tritylup-20(29)-en-28-ol-3-one (**10**) (1.14 g, 1.67 mmol) in *tert*-butyl alcohol (80 mL) was added potassium *tert*-butoxide (2.07 g, 18.4 mmol). The reaction mixture was stirred under air at 30 °C for 10 h. After the solvent was removed in vacuo, 1 N HCl (15 mL) was added. The mixture was extracted with ethyl acetate (3 × 35 mL). The combined extract was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to give crude diketone **11**. Crude diketone **11** was used for the next reaction without further purification. To a solution of the above diketone **11** in tetrahydrofuran (25 mL) and ethanol (5 mL) was added sodium borohydride (176 mg, 4.65 mmol) at 0 °C. The reaction mixture was then warmed to room temperature and stirred for 1 h. The reaction was quenched with 1 N HCl (25 mL), and then the organic solvents were evaporated. The residue 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 purified by column chromatography (petroleum ether−ethyl acetate, 8:1) to give diol **12** as a white solid (798 mg, 68% from **10**). Mp 199−201 °C; IR (KBr) 3592, 3440, 3062, 2948, 2871, 1709, 1643, 1483, 1364, 1374, 1314, 1218, 1061, 992, 888, 638 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.53 (s, 3 H), 0.88 (s, 3 H), 0.96 (s, 6 H), 1.07 (s, 3 H), 1.63 (s, 3 H), 2.04−2.23 (m, 6 H), 2.90 and 3.13 (d, J = 8.8 Hz, each 1 H), 3.15 (d, J = 3.4 Hz, 1 H), 4.02−4.03 (m, 1 H), 4.51 and 4.58 (d, J = 2.0 Hz, each 1 H), 7.46−7.50 (m, 6 H), 7.26−7.32 (m, 6 H), 7.21−7.24 (m, 3 H); ^{13}C NMR ($CDCl_3$) δ 14.7, 15.9, 17.0, 17.1, 18.1, 19.1, 20.9, 25.2, 26.8, 29.4, 29.6, 29.7, 29.9, 30.2, 34.1, 35.2, 36.8, 37.2, 38.1, 40.7, 42.6, 44.4, 47.6, 47.7, 48.9, 50.8, 55.2, 59.6, 71.2, 85.9, 109.3, 126.8, 127.7, 128.8, 144.5, 150.8; ESI-MS *m*/*z* 723.5 [M + Na]$^+$. Anal. Calcd for $C_{49}H_{64}O_3$: C 83.95, H 9.20. Found: C 83.50, H 9.10.

General Procedure for the Preparation of Ketones 13, 22, and 23. To a solution of diol **12** (500 mg, 0.713 mmol) in pyridine (3 mL) was added portionwise *p*-toluenesulfonyl chloride (190 mg, 1 mmol). The reaction mixture was stirred at 30 °C for 12 h. After the diol **12** was consumed completely (monitored by TLC), the reaction temperature was raised to 60 °C, and the reaction was kept at this temperature for another 12 h. After cooling to room temperature, 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 purified by column chromatography (petroleum ether−ethyl acetate, 80:1) to give **13** as a white solid (308 mg, 63%), together with **10** (171 mg, 35%) as a minor product. For **13**: mp 228−230 °C; IR (KBr) 3397, 3062, 2950, 1707, 1644, 1600, 1484, 1452, 1379, 1268, 1215, 1157, 1062, 994, 890, 761, 701, 638, 552, 477 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.50 (s, 3 H), 0.76 (s, 3 H), 0.85 (s, 3 H), 0.93 (s, 3 H), 1.03 (s, 3 H), 1.63 (s, 3 H), 1.82−1.87 (m, 1 H), 2.09−2.32 (m, 6 H), 2.90 and 3.11 (d, J = 8.8 Hz, each 1 H), 4.52 and 4.58 (d, J = 2.0 Hz, each 1 H), 7.46−7.49 (m, 6 H), 7.25−7.32 (m, 6 H), 7.19−7.24

(m, 3 H); ^{13}C NMR ($CDCl_3$) δ 14.7, 15.5, 17.1, 19.0, 19.1, 20.9, 23.1, 25.0, 26.9, 29.6, 29.7, 29.9, 30.1, 33.3, 33.7, 35.2, 37.2, 39.0, 40.0, 42.6, 42.9, 47.6, 47.7, 48.8, 50.0, 55.6, 56.0, 56.5, 59.6, 85.9, 109.5, 126.8, 127.7, 128.8, 144.5, 150.6, 212.2; ESI-MS *m*/*z* 721.4 $[M + K]^+$. Anal. Calcd for $C_{49}H_{62}O_2 \cdot 0.75H_2O$: C 84.50, H 9.18. Found: C 84.52, H 9.15.

General Procedure for the Preparation of 2*β*-Hydroxy Triterpenes 14, 24, and 25. To a solution of ketone **13** (1 g, 1.46 mmol) in tetrahydrofuran (15 mL) and ethanol (3 mL) was added sodium borohydride (0.08 g, 2.11 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 1 h. The reaction was quenched with 1 N HCl (25 mL), and the organic solvents were evaporated in vacuo. The residue 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 purified by column chromatography (petroleum ether−ethyl acetate, 25:1) to give 2*β*-hydroxy isomer **14** (885 mg, 89%) and 2α-hydroxy isomer **14a** (100 mg, 10%). For **14**: white solid, mp 143−146 °C; IR (KBr) 3417, 3063, 2945, 2870, 1708, 1645, 1455, 1378, 1213, 1157, 1064, 768, 703 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.52 (s, 3 H), 0.89 (s, 3 H), 0.90 (s, 3 H), 0.98 (s, 3 H), 1.01 (s, 3 H), 1.64 (s, 3 H), 1.82−1.87 (m, 1 H), 2.17−2.20 (m, 3 H), 2.90 and 3.14 (d, J = 8.8 Hz, each 1 H), 3.99−4.02 (m, 1 H), 4.52 and 4.58 (d, J = 2.1 Hz, each 1 H), 7.47−7.49 (m, 6 H), 7.25−7.32 (m, 6 H), 7.22−7.24 (m, 3 H); ^{13}C NMR ($CDCl_3$) δ 14.7, 15.7, 19.1, 19.7, 21.2, 24.8, 25.3, 26.8, 29.9, 30.1, 32.5, 32.8, 33.6, 35.2, 37.4, 38.2, 40.8, 42.5, 46.3, 47.6, 47.7, 48.2, 48.9, 50.8, 52.7, 59.6, 67.5, 85.8, 109.3, 126.8, 127.7, 128.8, 144.5, 150.8; ESI-MS *m*/*z* 723.4 $[M + K]^+$. Anal. Calcd for $C_{49}H_{64}O_2 \cdot 0.5H_2O$: C 84.79, H 9.44. Found: C 84.71, H 9.69.

Preparation of 2*β*-*O*-Acetyllup-20(29)-en-28-oic Acid (17). To a solution of aldehyde **16** (60 mg, 0.124 mmol) (see the Supporting Information) in *t*-BuOH (5 mL), THF (1 mL), and 2-methyl-2-betene (1.5 mL) was slowly added 3 mL of a freshly prepared aqueous solution of NaH_2PO_4/$NaClO_2$ (0.15 g/0.15 g) at 0 °C, and the resulting mixture was kept at this temperature for 15 min. The mixture was then warmed to room temperature and stirred for 1 h. The mixture was poured into a saturated solution of NH_4Cl (5 mL) 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, 12:1) to give **17** as a white solid (53.3 mg, 86%). ^{1}H NMR ($CDCl_3$) δ 0.91 (s, 3 H), 0.94 (s, 6 H), 0.98 (s, 3 H), 1.05 (s, 3 H), 1.69 (s, 3 H), 2.00 (s, 3 H), 2.13−2.29 (m, 2 H), 2.98−3.01 (m, 1 H), 4.61, 4.74 (s, each 1 H), 5.04−5.07 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.7, 16.0, 18.5, 18.8, 19.4, 21.2, 21.6, 23.9, 25.6, 29.6, 30.6, 32.2, 32.7, 32.9, 33.9, 37.1, 37.8, 38.6, 40.9, 42.6, 43.0, 44.2, 47.0, 49.3, 51.0, 53.8, 56.4, 70.7, 109.7, 150.3, 170.5, 181.6; ESI-MS *m*/*z* 497.3 $[M - H]^-$. Anal. Calcd for $C_{32}H_{50}O_4 \cdot 0.2H_2O$: C 76.51, H 10.11. Found: C 76.3, H 9.96.

2-Isobetulinic Acid (6). Compound **17** (50 mg, 0.1 mmol) was dissolved in 5 mL of MeOH, then 1 mL of 4 N NaOH aq solution was added dropwise. The reaction mixture was stirred at 40 °C for 2 h. The mixture was diluted in CH_2Cl_2 and washed with 10% HCl and brine. The organic layer was 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 2-isobetulinic acid (**6**) as a white solid (44 mg, 96%). Mp 259−261 °C; ^{1}H NMR (C_5D_5N) δ 0.94 (s, 3 H), 1.05 (s, 3 H), 1.06 (s, 3 H), 1.13 (s, 3 H), 1.25 (s, 3 H), 1.77 (s, 3 H), 2.00−2.22 (m, 2 H), 2.59−2.72 (m, 2 H), 3.49−3.54 (m,1 H), 4.33−4.36 (m, 1 H), 4.76 and 4.93 (s, each 1 H); ^{13}C NMR (C_5D_5N) δ 14.9, 16.4, 19.4, 19.5, 21.7, 24.9, 26.3, 30.2, 31.3, 32.9, 31.3, 33.0, 33.1, 33.3, 34.5, 37.6, 38.4, 38.8, 41.4, 43.0, 47.5, 47.8, 48.5, 49.8, 51.56, 54.1, 56.7, 66.7, 109.9, 151.4, 178.8; ESI-MS *m*/*z* 479.3 $[M + Na]^+$. Anal. Calcd for $C_{30}H_{48}O_3 \cdot 0.75H_2O$: C 76.63, H 10.61. Found: C 76.94, H 10.24.

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Supporting Information Available: Experimental procedures and spectroscopic data for compounds **5**−**8**, **12**−**17**, **22**−**25**, **12b**, **14a**, and **24a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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