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Synthesis and Cytotoxicity of Monomethylated Betulinic Acid 3-*O*-α-L-Rhamnopyranosides

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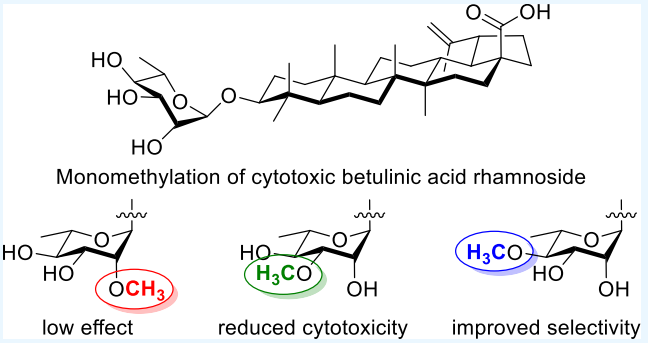
ABSTRACT: Three original derivatives of the cytotoxic betulinic acid 3-*O*-α-L-rhamnopyranoside featuring a monomethylated rhamnoside residue were synthesized. An improved catalytic procedure was involved to functionalize the *O*-3 position of the monosaccharide in a site-selective fashion. The cytotoxicity of the novel compounds was evaluated in vitro to highlight the moderate impact of carbohydrate monomethylation on the biological activity of betulinic acid 3-*O*-α-L-rhamnopyranoside.



INTRODUCTION

Partially methylated carbohydrates occur in various families of bioactive natural glycosides. For instance, several antibiotic macrolides, such as erythromycin, display various partially methylated carbohydrates. In the same fashion, calicheamicin γ1—the payload of clinically relevant anticancer antibody−drug conjugates—displays a 3-*O*-methyl-α-L-rhamnopyranoside. The presence and position of methyl groups on carbohydrates influence the pharmacological properties in several cases.[1] For instance, partially methylated cardiac glycosides isolated from *Streblus asper* showed various degrees of cytotoxicity depending on the nature and methylation pattern of carbohydrates.[2] Strikingly, the monomethylated quercitrin derivative **1a**, isolated from *Excoecaria agallocha*, inhibited the Hedgehog signaling pathway, which translated into a potent cytotoxicity against pancreatic (PANC1) and prostatic (DU-145) cancer cells, whereas quercitrin **1b** was inactive (Figure 1).[3] Furthermore, the absence of the 4-*O*-methyl group on the novobiose of the antibacterial aminocoumarin novobiocin resulted in a reduced activity against methicillin-resistant *Staphylococcus aureus*.[4] The partial methylation of saponins, more specifically chlorogenin and methyl ursolate chacotriosides, was also shown to change their antiviral activity against H5N1 influenza viruses.[5] For these reasons, the partial methylation of carbohydrates in bioactive glycosides could be an attractive pharmacomodulation strategy.

In the course of a research program on the antitumor properties of synthetic saponins,[6] we performed a screening of pyranoses that demonstrated the pivotal role of the carbohydrate structure on the biological activity of the resulting glycosides. These studies highlighted the peculiar properties of L-rhamnose as this carbohydrate improved the cytotoxicity of various triterpenes. For example, the betulinol 3,28-di-*O*-α-L-rhamnopyranoside[7] exhibited relevant antitumor activity in vivo.[8] In the same fashion, we focussed on betulinic acid, a natural lupane-type triterpenoid that exhibited appealing cytotoxic, anti-inflammatory, antimalarial, and anti-HIV activities.[9] The betulinic acid 3-*O*-α-L-rhamnopyranoside **2**[10] (Figure 1) was both more active and more selective toward

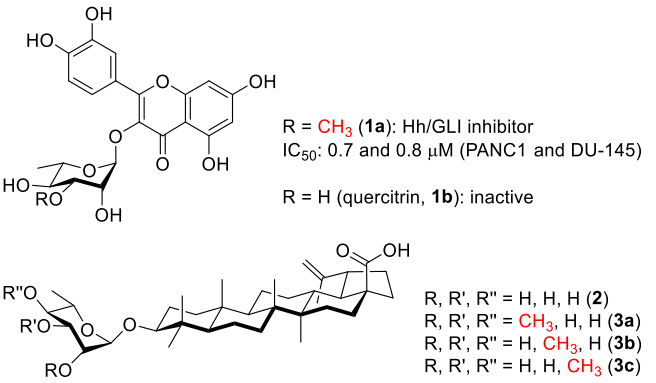


Figure 1. Structure of the Hedgehog/GLI1 (Hh/GLI1) signaling pathway inhibitor **1a** and its inactive counterpart **1b**. Structure of the cytotoxic betulinic acid 3-*O*-α-L-rhamnopyranoside **2** and its monomethylated analogues **3a−c** studied here.

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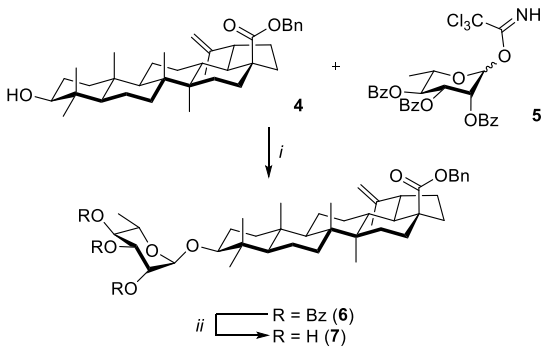
cancer cells than the corresponding triterpene and devoid of the hemolytic activity that is commonly associated with saponins.[11]

Within this framework, we wondered if the activity of the betulinic acid 3-*O*-*α*-L-rhamnopyranoside **2** could be tuned by monomethylation of its monosaccharide. Along these lines, we devise the synthesis and cytotoxic evaluation of the monomethylated glycosides **3a**−**c**. The impact of this subtle structural modification on the cytotoxicity of 3-*O*-*α*-L-rhamnopyranoside is also discussed. In addition, the preparation of the 3-*O*-methyl-*α*-L-rhamnopyranoside **3b** was performed following an unprecedented catalytic site-selective methylation. The reaction was derived from a method that used to be restricted to more reactive electrophiles and simpler substrates.

RESULTS AND DISCUSSION

The starting point for the synthesis of saponins **3a**−**c** was benzyl betulinate **4**.[12] The carbohydrate moiety was introduced following the Schmidt glycosylation procedure using 2,3,4-tri-*O*-benzoyl-*α*-L-rhamnopyranoside trichloroacetimidate **5**[10] in the presence of a catalytic amount of trimethylsilyl trifluoromethanesulfonate (TMSOTf) (Scheme 1). The

Scheme 1. Synthesis of the Triol 7[a]

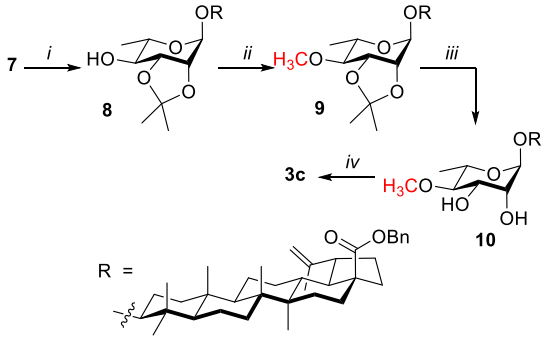


[a]Reagents and conditions: (i) TMSOTf, 4 Å molecular sieve, 0 °C to RT, 40 min (78%); (ii) CH_3ONa, CH_2Cl_2/MeOH, 0 °C to RT, 4 h (90%).

stereochemistry of the glycosidic bond in the protected intermediate **6** was secured by the participation of the benzoate protective group on the *O*-2 position of the carbohydrate in the mechanism of the reaction.[13] Benzoates were then removed by basic transesterification using sodium methanolate in a mixture of dichloromethane and methanol to afford the glycoside **7** displaying three free hydroxyls on its rhamnoside moiety.

The synthesis of the 4-*O*-methyl-*α*-L-rhamnopyranoside **3c** was performed following a classical sequence (Scheme 2) using an isopropylidene protective group to block the *O*-2 and *O*-3 positions of the carbohydrate. The triol **7** was treated in the presence of 2,2-dimethoxypropane and 4-toluenesulfonic acid (*p*-TSA) in acetone to provide the isopropylidene **8**. The hydroxyl function was then methylated using iodomethane and sodium hydride in dimethylformamide (DMF) to afford the 2,3-isopropylidene-4-*O*-methyl-*α*-L-rhamnopyranoside **9**. Deprotection of the isopropylidene was then achieved under acidic transacetalyzation conditions prior to the catalytic hydrogenolysis of the benzyl ester to afford the expected

Scheme 2. Synthesis of the 4-*O*-Methyl-*α*-L-rhamnopyranoside 3c[a]

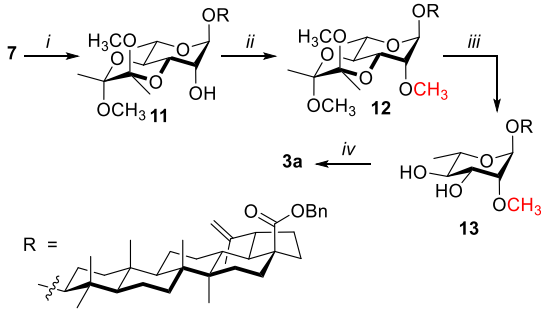


[a]Reagents and conditions: (i) 2,2-dimethoxypropane, *p*-TSA, acetone, RT, 4 h (91%); (ii) CH_3I, NaH, DMF, RT, 18 h (68%); (iii) *p*-TSA, CH_2Cl_2/MeOH, RT, 4.5 h (76%); (iv) H_2, Pd/C, AcOEt, RT, 18 h (50%, 80% based on the reisolated starting material).

methylated rhamnoside **3c** in a satisfying 38% yield over four steps.

The 2-*O*-methylrhamnoside **3a** was also obtained following a conventional approach (Scheme 3). Briefly, the *O*-3 and *O*-4

Scheme 3. Synthesis of the 2-*O*-Methyl-*α*-L-rhamnopyranoside 3a[a]



[a]Reagents and conditions: (i) 2,3-butanedione, CSA, CH_2Cl_2, 21 h, RT (51%); (ii) CH_3I, NaH, DMF, 18 h, RT (52%); (iii) *p*-TSA, CH_2Cl_2/MeOH, reflux, 2 h (47%); (iv) H_2, Pd/C, MeOH, reflux, 2 h (54%).

positions of the carbohydrate moiety of triol **7** were converted into the bis-acetal **11** using 2,3-butanedione in the presence of camphorsulfonic acid (CSA). The *C*-2 position was then methylated following the same procedure as described above. The bis-acetal **12** was then removed by transacetalyzation under acidic conditions prior to the catalytic hydrogenolysis of the benzyl protective group. Following this four-step sequence, the glycoside **3a** featuring a methyl group at the *O*-2 position of its monosaccharide moiety was obtained in a modest 7% overall yield, yet providing sufficient amounts of the final product to perform its biological evaluation.

The methylation of the *O*-3 position of rhamnosides has been commonly achieved following a two-step sequence, through the formation of a stannylene acetal between the *O*-2 and *O*-3 positions of the carbohydrate using a stoichiometric amount of dibutyltin oxide.[14] However, our preliminary attempts to generate a stannylene acetal from the triol **7** were unsuccessful. It is worthy of note that the group of Lowary also reported unfruitful selective methylations of oligomannosides through stannylene acetal intermediates.[15]

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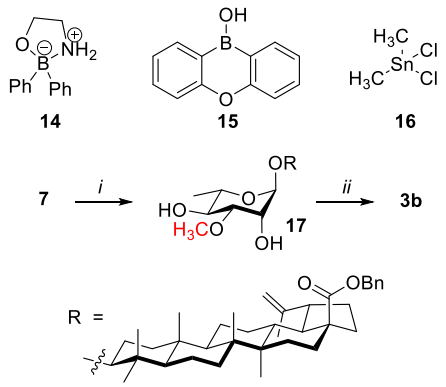
We therefore considered the option of circumventing the use of stoichiometric amounts of the harmful tin reagent.[16] To this end, we implemented a catalytic site-selective methylation step. Various catalytic systems were indeed described during the last few years to enable the site-selective acylation, alkylation, sulfonylation, silylation,[17] or carbamoylation[18] of *cis* 1,2-diols in carbohydrates (Scheme 4). Recent reports on site-selective

Scheme 4. Structure of the Catalysts Envisioned for the Site-Selective Modification of the Triol 7 and Synthesis of the 3-*O*-Methyl-α-L-rhamnopyranoside 3b[a]



[a]Reagents and conditions: (i) CH_3I, tetrabutylammonium iodide, K_2CO_3, **16** (0.2 equiv), DMF, 90 °C, 8 h (37%); (ii) H_2, Pd/C, MeOH, reflux, 2 h (63%).

alkylation include the use of iron,[19−21] tin,[22,23] or boron[24−27]-based catalysts. However, to the best of our knowledge, no convenient catalytic site-selective methylation has been described to date.[1] Indeed, the only reported studies involved diazomethane as the methylation reagent and afforded mixtures of monomethylated products.[28,29] As pointed out by Chan and Taylor, the catalytic site-selective alkylation of pyranosides depends on the reactivity of the electrophile.[24] Activated benzyl or allyl halides such as benzylbromide were shown to be essential for efficient alkylation reactions catalyzed by aminoethyl diphenylborinate **14**, and methyl iodide did not react under the tested conditions.

We confirmed this limitation as no reaction was observed when the triol **7** was treated with iodomethane in the presence of K_2CO_3, even with the oxaboraanthracene[26] catalyst **15** in DMF at 80 °C. These ineffective attempts emphasized the challenging character of the intended transformation. On the other hand, when the methylation was carried out with 20 mol % of dimethyltin dichloride **16**, 37% of the expected 3-*O*-methylrhamnoside **17** were successfully isolated. The transformation was selective, and no trace of other methylation products was observed on the ^1H NMR spectrum of the crude mixture. The site selectivity of the reaction was attributed to the transient formation of a stannylene acetal between the *O*-2 and *O*-3 positions of the rhamnoside, followed by the methylation of the more nucleophilic equatorial position. The modest yield was considered as satisfactory as the reaction circumvented a two-step procedure involving stoichiometric amounts of a harmful tin reagent and was performed on a complex, polyfunctional glycoside featuring a triol and a sensitive triterpenic framework. Furthermore, our result could be referred to as the first catalytic site-selective methylation of an unprotected glycoside using iodomethane. This unprecedented extension of an alkylation protocol to methylation could therefore pave the way to an alternative strategy to the conventional stepwise, stoichiometric sequence. The final debenzylation provided the 3-*O*-methylrhamnoside **3b**. The position of the methyl group was confirmed by careful analysis of NMR spectra of the 3-*O*-methylrhamnoside **3b**. Especially, a correlation was observed on the HMBC spectrum between the *C*-3 of rhamnose at 81.6 ppm and the three protons of the methoxy group (singlet at 3.46 ppm integrating for 3 H) (see Figure S27 in the Supporting Information).

The cytotoxicity of monomethylated derivatives **3a−c** was evaluated on two malignant (A549 and DLD-1) and one normal (WS1) human cell lines and compared to the parent rhamnoside **2**. The concentrations inhibiting 50% of cell growth (IC_{50}) were calculated and are reported in Table 1.

Table 1. In Vitro Cytotoxicity Evaluation of Saponins 3a−c

	IC_{50} (μM)[a]		
compound	A549	DLD-1	WS1
2	3.3 ± 0.3	2.5 ± 0.2	9 ± 1
3a	4.2 ± 0.4	5.4 ± 0.6	22 ± 2
3b	10 ± 1	10 ± 2	41 ± 5
3c	2.5 ± 0.2	4.3 ± 0.5	21 ± 1
etoposide[b]	4.1 ± 0.5	1.3 ± 0.1	5 ± 1

[a]IC_{50} values ± SD (n = 3) are representative of two different experiments. [b]Used as a positive control. A549: human lung adenocarcinoma cells; DLD-1: human colon cancer cells; WS-1: human skin fibroblast.

Unlike the nature of the carbohydrate, which had a critical impact on the cytotoxicity of the corresponding betulinic acid glycosides,[10] monomethylation had a moderate influence on the bioactivity of the saponin **2**. *O*-2 methylation (compound **3a**) slightly decreased cytotoxicity against all tested lines by a factor of approximately two. This effect was more pronounced with *O*-3 methylation (compound **3b**) that was three to four times less active than the parent derivative **2** while preserving a comparable selectivity between cell lines. On the other hand, the 4-*O*-methylrhamnoside **3c** displayed a similar cytotoxicity against cancer cell lines as the rhamnoside **2**. Furthermore, the selectivity of the 4-*O*-methylrhamnoside **3c** toward malignant cells was slightly improved as it showed a 21 ± 1 μM IC_{50} against WS-1 normal cells compared to 9 ± 1 μM for the rhamnoside **2**. Therefore, *O*-4 methylation of **2** moderately improved its selective cytotoxicity toward cancer cells.

CONCLUSIONS

Altogether, we devised the synthesis of three novel partially methylated analogs of the cytotoxic betulinic acid 3-*O*-α-L-rhamnopyranoside **2**. The preparation of the 3-*O*-methylrhamnoside **3b** was performed following an unprecedented catalytic site-selective methylation reaction. Monomethylation of its carbohydrate moiety moderately altered the cytotoxicity of the saponin **2**. Nevertheless, the monomethylation pattern influenced the cytotoxicity. Especially, the 4-*O*-methylrhamnoside **3c** was slightly more selective toward malignant cell lines than the parent rhamnoside **2**. 2- and 3-*O*-methylation both decreased cytotoxicity. We also think that a convenient catalytic protocol for the site-selective methylation of unprotected glycosides could stem from the preliminary result devised for the synthesis of compound **3b**.

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METHODS

General Information. All starting materials and reagents were purchased from commercial sources and used as received without further purification. Unless otherwise noted, reactions were conducted using anhydrous commercial solvents under an argon atmosphere. Anhydrous solvents, supplied over molecular sieves, were used as received. Saponin **2**[10] were prepared following published procedures. Reactions were monitored by thin-layer chromatography (TLC) with silica gel (60 Å F254 0.25 mm precoated aluminum foil plates) and visualized under UV light (254 nm) or with an acid solution of cerium ammonium molybdate. All flash chromatographic purifications were performed using 60 Å silica (SiO_2) (40−63 μm). Optical rotations were determined at the sodium D line (590 nm) on a Rudolph Research Analytical Autopol IV automatic polarimeter. NMR spectra were recorded with a Bruker Avance 400 spectrometer at 400 MHz for ^{1}H nucleus and 101 MHz for ^{13}C nucleus, using deuterated chloroform ($CDCl_3$), deuterated methanol (CD_3OD), or deuterated dimethyl sulfoxide (DMSO-d_6) as the solvent. Chemical shifts were reported in ppm relative to the solvent residual peak ($CDCl_3$: δ = 7.26 ppm for ^{1}H and 77.16 ppm for ^{13}C; CD_3OD: δ = 3.31 ppm for ^{1}H and 49.00 ppm for ^{13}C; DMSO-d_6: δ = 2.50 ppm for ^{1}H and 39.52 ppm for ^{13}C)[30] and coupling constants J in Hertz (Hz). Multiplicities were reported using the following abbreviations: s, singlet; d, doublet, t, triplet; q, quartet; m, multiplet.

Cytotoxicity Assays. The A549 (human lung cancer, ATCC # CCL-185), DLD-1 (human colon cancer, ATCC # CCL-221), and WS-1 (human skin fibroblasts, ATCC # CRL-1502) cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cell lines were grown in minimum essential medium containing Earle's salt (Mediatech Cellgro, Herndon, VA, USA), supplemented with 10% fetal calf serum (Hyclone, Logan, UT, USA), 1 × solution of vitamins, 1 × sodium pyruvate, 1 × non-essential amino acids, and 100 I.U. of penicillin and 100 μg/mL of streptomycin (Mediatech Cellgro). Cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were plated at a density of 5 × 10^3 cells per well in 96-well microplates (BD Falcon) in culture medium (100 μL) and were allowed to adhere for 16 h before treatment. Then, cells were incubated for 48 h in the presence or absence of 100 μL of increasing concentrations of compounds dissolved in culture medium and DMSO. The final concentration of DMSO in the culture medium was maintained at 0.5% (v/v) to avoid toxicity. Cytotoxicity was assessed using Hoechst 33342 (bisbenzimide) fluorometric assay.[31] It was expressed as the concentration of drug-inhibiting cell growth by 50% (IC_{50}).

*3-O-(2,3,4-Tri-O-benzoyl-α-L-rhamnopyranosyl)-28-O-benzyl Betulinate (**6**).* To a cooled solution (0 °C, ice/water bath) of benzyl betulinate (**4**)[12] (401.0 mg, 0.73 mmol) and 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranoside trichloroacetimidate (**5**)[10] (548.1 mg, 0.88 mmol) in anhydrous dichloromethane (15 mL) with 4 Å molecular sieves was added trimethylsilyl trifluoromethanesulfonate (3.2 μL, 0.073 mmol). The mixture was stirred at room temperature for 40 min, diluted in dichloromethane (15 mL) then quenched with 30 mL of a saturated aqueous $NaHCO_3$ solution. Molecular sieves were filtered off, and the aqueous layer was extracted with 3 × 30 mL of dichloromethane. The organic layers were combined and dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by silica gel flash chromatography with a gradient from 0 to 20% of ethyl acetate in hexanes as the eluent to afford the title compound **6** as a white solid (568.3 mg, 0.57 mmol, 78%). R_f = 0.40 (Hex/EtOAc, 9:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, J = 7.3 Hz, 2H), 7.99 (d, J = 7.3 Hz, 2H), 7.84 (d, J = 7.3 Hz, 2H), 7.64−7.57 (m, 1H), 7.55−7.46 (m, 3H), 7.46−7.30 (m, 8H), 7.29−7.23 (m, 2H), 5.82 (dd, J = 3.3, 10.2 Hz, 1H), 5.68 (t, J = 10.0 Hz, 1H), 5.65−5.61 (m, 1H), 5.17 (d, J = 12.3 Hz, 1H), 5.09 (d, J = 12.3 Hz, 1H), 5.07 (d, J = 1.3 Hz, 1H), 4.73 (d, J = 1.9 Hz, 1H), 4.59 (m, 1H), 4.36−4.26 (m, 1H), 3.24−3.15 (m, 1H), 3.03 (td, J = 4.6, 10.6 Hz, 1H), 2.30 (d, J = 12.3 Hz, 1H), 2.19 (td, J = 3.3, 12.5 Hz, 1H), 1.95−1.75 (m, 4H), 1.73−1.69 (m, 1H), 1.63−1.48 (m, 3H), 1.46−1.21 (m, 10H), 1.32 (d, J = 6.3, 3H), 1.21−1.07 (m, 2H), 1.03−0.98 (m, 1H), 1.68 (s, 3H), 1.04 (s, 3H), 0.95 (s, 3H), 0.92 (s, 3H), 0.86 (s, 3H), 0.77 (s, 3H), 0.74−0.66 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.95, 165.99, 165.83, 165.72, 150.74, 136.63, 133.54, 133.40, 133.19, 130.03, 129.88, 129.81, 129.69, 129.50, 129.43, 128.70, 128.63, 128.54, 128.39, 128.20, 109.72, 99.87, 90.20, 72.12, 71.37, 70.34, 66.90, 65.87, 56.69, 55.68, 50.65, 49.59, 47.09, 42.54, 40.82, 39.30, 38.82, 38.33, 37.09, 34.42, 32.25, 30.72, 29.72, 28.40, 25.80, 25.67, 21.07, 19.50, 18.43, 17.72, 16.54, 16.34, 15.99, 14.82. NMR data were consistent with the literature description.[32]

*3-O-α-L-Rhamnopyranosyl-28-O-benzyl Betulinate (**7**).* To a cooled solution (0 °C, ice/water bath) of intermediate **6** (568.3 mg, 0.57 mmol) in anhydrous CH_2Cl_2 (14 mL) and anhydrous methanol (14 mL), sodium was added (16.3 mg, 0.71 mmol), and the mixture was stirred at room temperature for 4 h. The mixture was then neutralized with weakly acid Amberlite resin, filtered, and concentrated under reduced pressure. The crude residue was purified by normal phase silica gel flash chromatography with a gradient from 0 to 10% of methanol in dichloromethane as the eluent to afford the title compound **7** as a white solid (609 mg, 0.51 mmol, 90%). R_f = 0.20 (CH_2Cl_2/MeOH 95:5); ^{1}H NMR (400 MHz, $CDCl_3$:CD_3OD, 1:2) δ 7.44−7.25 (m, 5H), 5.14 (d, J = 12.3 Hz, 1H), 5.09 (d, J = 12.3 Hz, 1H), 4.72 (d, 1H), 4.69 (br s, 1H), 4.56 (br s, 1H), 3.84 (br s, 1H), 3.78−3.62 (m, 2H), 3.41−3.31 (m, 1H), 3.10−2.92 (m, 2H), 2.28 (d, J = 10.5 Hz, 1H), 2.16 (t, J = 12.4 Hz, 1H), 1.94−1.79 (m, 2H), 1.79−1.53 (m, 5H), 1.65 (s, 3H), 1.48−1.19 (m, 11H), 1.22 (d, J = 6.3 Hz, 3H), 1.19−1.03 (m, 2H), 1.02−0.95 (m, 1H), 0.93 (s, 3H), 0.88 (s, 3H), 0.79 (s, 3H), 0.72 (s, 3H), 0.71 (s, 3H), 0.69−0.63 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$:CD_3OD, 1:2) δ 176.89, 150.97, 136.99, 129.05, 128.94, 128.71, 110.11, 103.48, 89.82, 73.52, 72.01, 71.70, 69.01, 66.43, 57.24, 56.15, 51.18, 50.04, 47.71, 42.96, 41.26, 39.61, 38.92, 37.48, 31.11, 30.13, 28.41, 26.13, 19.58, 17.61, 16.56, 16.53, 16.25, 15.05. NMR data were consistent with the literature description.[32]

*3-O-(2,3-O-Isopropylidene-4-O-methyl-α-L-rhamnopyranosyl)-28-O-benzyl Betulinate (**9**).* To a solution of triol **7** (31.6 mg, 0.046 mmol) in acetone (HPLC grade, 1 mL) under an argon atmosphere, 4-toluenesulfonic acid (1.3 mg, 0.007 mmol) and 2,2-dimethoxypropane (56.4 μL, 0.46 mmol) were added, and the reaction mixture was stirred for 4 h. The acid was then neutralized with triethylamine (1.5 μL) and the solvent was evaporated under reduced pressure. The crude residue was purified by flash chromatography over silica gel using a gradient from 0 to 20% of ethyl acetate in hexanes affording the title compound **8** containing an unidentified

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impurity as a white solid (30.6 mg). The mixture was engaged in the next step without further purification R_f = 0.45 (Hex/EtOAc, 75:25). To a cooled solution (0 °C, ice/water bath) of crude alcohol **8** (225.4 mg, 0.31 mmol) in anhydrous DMF (1.6 mL) under an argon atmosphere, sodium hydride 60% (25.8 mg, 0.64 mmol) was added and stirred for 10 min at 0 °C before introducing iodomethane (38.3 μL, 0.62 mmol). After 18 h of stirring at room temperature, TLC monitoring or the reaction showed partial conversion of alcohol **8**. The reaction mixture was cooled to 0 °C for the addition of sodium hydride (22.3 mg, 0.56 mmol) and iodomethane (38.3 μL, 0.62 mmol). After 3 h of stirring at room temperature, the reaction was quenched using MeOH (63 μL). The mixture was diluted with toluene (15 mL) and ethyl acetate (15 mL) and then washed with 2 × 30 mL of a saturated aqueous solution of NH_4Cl, 2 × 30 mL of a saturated aqueous solution of $NaHCO_3$, and 30 mL of brine. The organic layer was dried with anhydrous Na_2SO_4 and filtered, and volatiles were evaporated under reduced pressure. The crude residue was purified by flash chromatography over silica gel using a gradient from 0 to 10% of ethyl acetate in hexane to provide the title compound **9** as a white solid (154.9 mg, 0.21 mmol, 62%, 2 steps). R_f = 0.85 (Hex/EtOAc, 75:25); $[\alpha]^{20}_D$ − 14.8 (*c* 0.21, $CHCl_3$); HRMS (ESI) m/z calcd for $C_{47}H_{71}O_7$ $[M + H]^+$ 747.5194, found 747.5204; ^1H NMR (400 MHz, $CDCl_3$) δ 7.38−7.29 (m, 5H, Ar), 5.14 (d, *J* = 12.3, 1H), 5.08 (d, *J* = 12.3, 1H), 4.97 (s, 1H), 4.72 (d, *J* = 2.0, 1H), 4.59 (s, 1H), 4.15−4.09 (m, 2H), 3.77−3.69 (m, 1H), 3.53 (s, 3H, O-CH_3), 3.08 (dd, *J* = 4.7, 11.3 Hz, 1H), 3.01 (td, *J* = 4.5, 11.1 Hz, 1H), 2.96 (dd, *J* = 6.4, 9.9 Hz, 1H), 2.31−2.23 (m, 1H), 2.17 (td, *J* = 3.1, 12.3 Hz, 1H), 1.94−1.80 (m, 2H), 1.73−1.63 (m, 3H), 1.67 (s, 3H), 1.63−1.57 (m, 2H), 1.54 (s, 3H), 1.48−1.42 (m, 1H), 1.42−1.38 (m, 2H), 1.37 (s, 3H), 1.35−1.28 (m, 4H), 1.27−1.14 (m, 4H), 1.23 (d, *J* = 6.3 Hz, 3H), 1.12−0.96 (m, 2H), 0.93 (s, 3H), 0.90 (s, 3H), 0.88−0.83 (m, 1H), 0.79 (s, 3H), 0.74 (s, 3H), 0.73 (s, 3H), 0.66 (br d, *J* = 9.1 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.93, 150.70, 136.62, 128.62, 128.37, 128.18, 109.72, 109.11, 99.65, 88.92, 83.89, 78.56, 76.42, 65.85, 64.66, 59.60, 56.69, 55.61, 50.65, 49.58, 47.08, 42.51, 40.79, 39.29, 38.33, 37.08, 37.01, 32.26, 28.40, 28.27, 26.61, 25.67, 19.51, 17.65, 16.39, 16.31, 15.96, 14.81.

*3-O-(4-O-Methyl-α-L-rhamnopyranosyl)-28-O-benzyl Betulinate (**10**).* To a solution of isopropylidene **9** (128.0 mg, 0.17 mmol) in anhydrous CH_2Cl_2 (2 mL) and MeOH (4 mL), 4-toluenesulfonic acid (33.1 mg, 0.17 mmol) was added, and the mixture was stirred for 4.5 h at room temperature. The acid was neutralized with triethylamine (26.7 μL), and the solvent was evaporated under reduced pressure. The crude residue was purified by flash chromatography over silica gel using a gradient from 0 to 2% of MeOH in $CHCl_3$ to provide the title compound **10** as a white solid (91.7 mg, 0.13 mmol, 76%). R_f = 0.40 ($CHCl_3$/CH_3OH, 95:5); $[\alpha]^{20}_D$ − 20.3 (*c* 0.35, $CHCl_3$); HRMS (ESI) m/z calcd for $C_{44}H_{67}O_7$ $[M + H]^+$ 707.4881, found 707.4877; ^1H NMR (400 MHz, $CDCl_3$) δ 7.37−7.27 (m, 5H, Ar), 5.13 (d, *J* = 12.3 Hz, 1H), 5.08 (d, *J* = 12.3 Hz, 1H), 4.77 (s, 1H), 4.71 (d, *J* = 1.7 Hz, 1H), 4.58 (s, 1H), 3.93 (s, 1H), 3.87−3.80 (m, 1H), 3.80−3.71 (m, 1H), 3.55 (s, 3H, O-CH_3), 3.10−2.96 (m, 3H), 2.79 (s, 2H), 2.31−2.22 (m, 1H), 2.16 (td, *J* = 3.4, 12.6 Hz, 1H), 1.93−1.79 (m, 2H), 1.76−1.68 (m, 1H), 1.67 (s, 3H), 1.64−1.52 (m, 3H), 1.47−1.13 (m, 12H), 1.26 (d, *J* = 6.3 Hz, 3H), 1.12−0.95 (m,2H), 0.92 (s, 3H), 0.86 (s, 3H), 0.85−0.80 (m, 1H), 0.77 (s, 3H), 0.73 (s, 3H), 0.70 (s, 3H), 0.63 (br d, *J* = 9.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.91, 150.63, 136.56, 128.57, 128.33, 128.14, 109.70, 102.01, 89.48, 83.72, 71.56, 71.50, 67.27, 65.81, 60.89, 56.63, 55.56, 50.59, 49.53, 47.02, 42.45, 40.73, 39.10, 38.72, 38.26, 37.02, 36.96, 34.35, 32.19, 30.67, 29.64, 28.25, 25.60, 25.52, 20.96, 19.46, 18.34, 17.91, 16.30, 16.22, 15.90, 14.75.

*Betulinic Acid 3-O-(4-O-Methyl-α-L-rhamnopyranoside) (**3c**).* To a cooled solution (0 °C, ice/water bath) of benzyl ester **10** (91.7 mg, 0.13 mmol) in anhydrous ethyl acetate (2.6 mL) was added palladium on activated charcoal (10.8 mg). The mixture was alternatingly bubbled with H_2 at 0 °C during 30 min every 2 h for a total of 8 h and was stirred under a H_2 atmosphere without bubbling overnight. Pd/C was then filtered on a C18 solid-phase extraction cartridge (1 g), and the solvent was evaporated under reduced pressure. The crude residue was purified by flash chromatography over silica gel using a gradient from 0 to 2% of MeOH in $CHCl_3$ to obtain the title compound **3c** as a white solid (39.8 mg, 0.065 mmol, 50%). 35.0 mg of starting material **10** were also recovered, providing an 80% yield based on the reisolated starting material. R_f = 0.35 ($CHCl_3$/CH_3OH, 95: 5); $[\alpha]^{20}_D$ − 33.9 (*c* 0.18, $CHCl_3$); HRMS (ESI) m/z calcd for $C_{37}H_{59}O_7$ $[M-H]^-$ 615.4266, found 615.4288; ^1H NMR (400 MHz, $CDCl_3$) δ 4.79 (s, 1H), 4.73 (s, 1H), 4.60 (s, 1H), 3.95 (s, 1H), 3.84 (dd, *J* = 2.8, 9.1 Hz, 1H), 3.81−3.72 (m, 1H), 3.56 (s, 3H, O-CH_3), 3.10−2.94 (m, 3H), 2.26 (d, *J* = 12.2 Hz, 1H), 2.17 (td, *J* = 3.1, 12.2 Hz, 1H), 2.02−1.89 (m, 2H), 1.79−1.71 (m, 1H), 1.68 (s, 3H), 1.65−1.55 (m, 3H), 1.53−1.37 (m, 5H), 1.21−1.13 (m, 3H), 1.12−0.99 (m, 2H), 0.96 (s, 3H), 0.91 (s, 3H), 0.87 (s, 3H), 0.86 (m, 1H), 0.80 (s, 3H), 0.71 (s, 3H), 0.67 (br d, *J* = 9.7 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 181.67, 150.61, 109.80, 102.01, 89.60, 83.85, 71.62, 71.56, 67.32, 60.99, 56.52, 55.61, 50.58, 49.41, 47.06, 42.54, 40.81, 39.16, 38.76, 38.52, 37.05, 29.84, 28.30, 25.60, 20.99, 19.52, 18.37, 17.93, 16.33, 16.26, 16.17, 14.81.

*3-O-(3,4-O-(2,3-Dimethoxybutane-2,3-diyl)-α-L-rhamnopyranosyl)-28-O-benzyl Betulinate (**11**).* To a solution of triol **7** (150.0 mg, 0.216 mmol) in anhydrous dichloromethane (2 mL) and methanol (2 mL), 2,3-butadione (22.8 μL, 0.26 mmol), trimethyl orthoformate (96.3 μL, 0.88 mmol), and camphor sulfonic acid (3.2 mg, 0.014 mmol) were added at room temperature, and the mixture was refluxed for 21 h. After cooling the mixture to room temperature, the reaction was quenched using triethylamine (3.1 μL, 0.022 mmol). Volatiles were then evaporated under reduced pressure, and the crude residue was purified by flash chromatography over silica gel (12 g SiO_2) using a gradient from 0 to 2% of methanol in dichloromethane to give the title compound **11** as a white solid (87.8 mg, 0.11 mmol, 50%). R_f = 0.80 (CH_2Cl_2/CH_3OH, 95:5); $[\alpha]^{20}_D$ − 78.5 (*c* 0.14, $CHCl_3$); HRMS (ESI) m/z calcd for $C_{49}H_{74}O_9Na$ $[M + Na]^+$ 829.5225, found 829.5227; ^1H NMR (400 MHz, $CDCl_3$) δ 7.39−7.27 (m, 5H), 5.13 (d, *J* = 12.3 Hz, 1H), 5.07 (d, *J* = 12.3 Hz, 1H), 4.79 (s, 1H), 4.71 (s, 1H), 4.58 (s, 1H), 3.97−3.89 (m, 3H), 3.68 (t, *J* = 9.9 Hz, 1H), 3.25 (s, 3H), 3.22 (s, 3H), 3.07−2.96 (m, 2H), 2.26 (d, *J* = 12.3 Hz, 1H), 2.16 (m, 1H), 1.95−1.80 (m, 2H), 1.78−1.67 (m, 1H), 1.66 (s, 3H), 1.65−1.52 (m, 3H), 1.47−1.32 (m, 7H), 1.32−1.28 (m, 1H), 1.30 (s, 3H), 1.28 (s, 3H), 1.27−1.14 (m, 8H), 1.10−0.95 (m, 2H), 0.92 (s, 3H), 0.89−0.86 (m, 1H), 0.85 (s, 3H), 0.77 (s, 3H), 0.73 (s, 3H), 0.68 (s, 3H), 0.64 (br d, *J* = 9.1 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.85, 150.61, 136.56, 128.55, 128.33, 128.12, 109.68, 102.67, 100.22, 99.82, 89.50, 70.26, 68.62, 68.47, 66.53, 65.79, 56.62,

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55.54, 50.58, 49.53, 47.95, 47.72, 47.02, 42.45, 40.73, 39.11, 38.27, 37.02, 36.96, 32.20, 30.68, 28.26, 25.62, 20.96, 19.47, 18.37, 17.93, 17.79, 16.48, 16.35, 16.25, 15.90, 14.75.

*3-O-(2-O-Methyl-3,4-O-(2,3-dimethoxybutane-2,3-diyl)-α-L-rhamnopyranosyl)-28-O-benzyl Betulinate (**12**).* To a solution of alcohol **11** (50.3 mg, 0.062 mmol) in anhydrous DMF (0.5 mL) cooled at 0 °C was added NaH (60% dispersion in mineral oil, 9.1 mg, 0.23 mmol). The mixture was stirred at 0 °C for 10 min and allowed to warm to room temperature before adding iodomethane (13.4 μL, 0.22 mmol). The reaction was then stirred at room temperature for 18 h. The mixture was diluted with ethyl acetate (4 mL) and toluene (4 mL) and washed with 2 × 8 mL of a saturated ammonium chloride solution (NH_4Cl), 2 × 8 mL of a saturated sodium bicarbonate solution ($NaHCO_3$), and 8 mL of brine. The organic layer was dried with anhydrous sodium sulfate and filtered, and volatiles were evaporated under reduced pressure. The crude residue was purified by flash chromatography over silica gel (4 g SiO_2) using a gradient from 0 to 7% of ethyl acetate in hexanes to obtain the title compound **12** as a white solid (26 mg, 0.032 mmol, 52%). R_f = 0.68 (Hex/EtOAc, 75:25); $[\alpha]^{20}_D$ − 87.2 (*c* 0.26, $CHCl_3$); HRMS (ESI) m/z calcd for $C_{50}H_{76}O_9Na$ $[M + Na]^+$ 843.5382, found 843.5388; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40−7.28 (m, 5H), 5.14 (d, *J* = 12.3, 1H), 5.09 (d, *J* = 12.3, 1H), 4.82 (s, 1H), 4.72 (s, 1H), 4.59 (s, 1H), 3.98 (dd, *J* = 2.9, 10.3 Hz, 1H), 3.93−3.84 (m, 1H), 3.73−3.65 (t, *J* = 10.1 Hz, 1H), 3.48 (s, 3H), 3.45 (dd, *J* = 1.4, 2.5 Hz, 1H), 3.26 (s, 3H), 3.24 (s, 3H), 3.07−2.97 (m, 2H), 2.27 (d, *J* = 12.3 Hz, 1H), 2.16 (td, *J* = 3.2, 12.1 Hz, 1H), 1.94−1.79 (m, 2H), 1.78−1.69 (m, 1H), 1.67 (s, 3H), 1.65−1.53 (m, 3H), 1.49−1.33 (m, 6H), 1.33−1.28 (m, 2H), 1.31 (s, 3H), 1.29 (s, 3H), 1.26−1.19 (m, 7H), 1.11−0.96 (m, 2H), 0.93 (s, 3H), 0.87 (s, 3H), 0.86−0.83 (m, 1H), 0.78 (s, 3H), 0.74 (s, 3H), 0.70 (s, 3H), 0.65 (br d, *J* = 8.7 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.93, 150.70, 136.62, 128.61, 128.38, 128.18, 109.72, 100.83, 99.91, 99.54, 89.66, 79.18, 68.97, 68.69, 67.04, 65.85, 59.04, 56.69, 55.62, 49.58, 47.87, 47.76, 47.07, 42.51, 40.79, 39.16, 38.33, 37.09, 37.03, 32.26, 29.70, 28.31, 25.67, 21.01, 19.52, 18.45, 18.03, 17.98, 16.66, 16.42, 16.29, 15.96, 14.81.

*3-O-(2-O-Methyl-α-L-rhamnopyranosyl)-28-O-benzyl Betulinate (**13**).* To a solution of diacetal **12** (26 mg, 0.032 mmol) in dichloromethane (1 mL) and methanol (2 mL) was added 4-toluenesulfonic acid (12.7 mg, 0.067 mmol), and the reaction mixture was stirred under reflux for 9 h. Then, 4-toluenesulfonic acid (4.2 mg, 0.022 mmol) was added, and the reaction was refluxed for 2 h. The acid was neutralized with triethylamine (13.4 μL, 0.096 mmol), and volatiles were evaporated under reduced pressure. The crude residue was purified by flash chromatography over silica gel (4 g SiO_2) using a gradient from 0 to 2% of methanol in dichloromethane to obtain the title compound **13** as a white solid (10.4 mg, 0.015 mmol, 47%). R_f = 0.45 (CH_2Cl_2/CH_3OH, 95:5); $[\alpha]^{20}_D$ − 12.6 (*c* 0.068, $CHCl_3$); HRMS (ESI) m/z calcd for $C_{44}H_{67}O_7$ $[M + H]^+$ 707.4881, found 707.4872; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45−7.29 (m, 5H, Ar), 5.14 (d, *J* = 12.3, 1H), 5.08 (d, *J* = 12.3, 1H), 4.88 (s, 1H), 4.72 (s, 1H), 4.59 (s, 1H), 3.80−3.65 (m, 2H), 3.47 (s, 1H), 3.45 (s, 3H, O-CH_3), 3.39−3.30 (m, 2H), 3.09−2.96 (m, 2H), 2.38−2.24 (m, 2H), 2.22−2.10 (m, 1H), 1.94−1.79 (m, 2H), 1.78−1.71 (m, 1H), 1.67 (s, 3H), 1.64−1.53 (m, 3H), 1.47−1.30 (m, 6H), 1.30−1.17 (m, 9H), 1.12−0.96 (m, 2H), 0.93 (s, 3H), 0.88 (s, 3H), 0.86−0.83 (m, 1H), 0.79 (s, 3H), 0.74 (s, 3H), 0.73 (s, 3H), 0.65 (br d, *J* = 9.5 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.95, 150.72, 136.62, 128.63, 128.39, 128.20, 109.73, 98.81, 89.64, 80.68, 74.40, 71.80, 67.74, 65.87, 58.76, 56.69, 55.66, 50.68, 49.59, 47.07, 42.51, 40.80, 39.22, 38.82, 38.33, 37.09, 37.05, 34.42, 32.26, 30.73, 29.85, 29.70, 28.27, 25.67, 21.02, 19.52, 18.42, 17.51, 16.36, 16.29, 15.96, 14.80.

*Betulinic Acid 3-O-(2-O-Methyl-α-L-rhamnopyranoside) (**3a**).* To a solution of benzyl ester, **13** (19.5 mg, 0.028 mmol) in anhydrous methanol (2 mL) was added palladium on activated charcoal (4.2 mg). The mixture was stirred for 3 h under a H_2 atmosphere at gentle reflux. After cooling to room temperature, the Pd/C was filtered on a C18 solid phase extraction cartridge (1 g) and washed with methanol. The solvent was evaporated under reduced pressure. The crude residue was purified by flash chromatography over silica gel (4 g, SiO_2) with a gradient from 0 to 25% of ethyl acetate in chloroform to obtain the title compound **3a** as a white solid (9.1 mg, 0.015 mmol, 54%). R_f = 0.35 (CH_2Cl_2/MeOH, 95:5); $[\alpha]^{20}_D$ − 10.5 (*c* 0.038, $CHCl_3$); HRMS (ESI) m/z calcd for $C_{37}H_{59}O_7$ $[M-H]^-$ 615.4266, found 615.4284; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.89 (s, 1H), 4.74 (s, 1H), 4.61 (s, 1H), 3.82−3.68 (m, 2H), 3.51−3.43 (m, 1H), 3.46 (s, 3H, O-CH_3), 3.36 (d, *J* = 9.1 Hz, 1H), 3.07 (dd, *J* = 4.7, 11.3 Hz, 1H), 3.00 (td, *J* = 3.9, 10.0 Hz, 1H), 2.27 (d, *J* = 12.4 Hz, 1H), 2.18 (td, *J* = 3.4, 12.5 Hz, 1H), 2.03−1.91 (m, 2H), 1.78−1.72 (m, 1H), 1.69 (s, 3H), 1.67−1.57 (m, 3H), 1.54−1.45 (m, 3H), 1.45−1.38 (m, 3H), 1.38−1.33 (m, 3H), 1.29−1.23 (m, 5H), 1.22−1.14 (m, 2H), 1.09−1.00 (m, 1H), 0.96 (s, 3H), 0.92 (s, 3H), 0.89 (s, 3H), 0.88−0.86 (m, 1H), 0.82 (s, 3H), 0.74 (s, 3H), 0.68 (br d, *J* = 9.6 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 181.10, 150.54, 109.84, 98.83, 89.64, 80.70, 74.38, 71.84, 67.79, 58.77, 56.49, 55.68, 50.65, 49.44, 47.05, 42.57, 40.85, 39.25, 38.84, 38.54, 37.20, 37.10, 34.46, 30.73, 29.85, 28.30, 25.70, 25.65, 21.02, 19.54, 18.43, 17.52, 16.37, 16.29, 16.19, 14.83.

*3-O-(3-O-Methyl-α-L-rhamnopyranosyl)-28-O-benzyl Betulinate (**17**).* To a solution of triol **7** (73.1 mg, 0.105 mmol) in dry DMF (1 mL) were added dimethyltin dichloride (5.1 mg, 0.023 mmol), tetrabutylammonium iodide (4.1 mg, 0.011 mmol), potassium carbonate (21.8 mg, 0.16 mmol), and iodomethane (19.4 μL, 0.315 mmol). The mixture was stirred at 90 °C for 3 h. The reaction was then cooled to room temperature, and iodomethane was added (19.4 μL, 0.315 mmol) as the TLC monitoring of the reaction showed partial conversion of triol **7**. The mixture was stirred at 90 °C for 4 h. The mixture was cooled to room temperature, diluted with toluene (7.5 mL) and ethyl acetate (7.5 mL), and washed with a saturated ammonium chloride solution (2 × 13 mL), a saturated sodium carbonate solution (2 × 13 mL), and brine. The organic layer was dried with anhydrous sodium sulfate and filtered, and volatiles were evaporated under reduced pressure. The crude residue was purified by flash chromatography over silica gel (4 g SiO_2) using a gradient from 0 to 2% of MeOH in CH_2Cl_2 to provide the title compound **17** as a white solid (27.5 mg, 0.039 mmol, 37%). R_f = 0.40 (CH_2Cl_2/CH_3OH, 95:5); $[\alpha]^{20}_D$ − 14.8 (*c* 0.19, $CHCl_3$); HRMS (ESI) m/z calcd for $C_{44}H_{67}O_7$ $[M + H]^+$ 707.4881, found 707.4882; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40−5.28 (m, 5H, Ar), 5.14 (d, *J* = 12.3 Hz, 1H), 5.08 (d, *J* = 12.3 Hz, 1H), 4.86 (s, 1H), 4.72 (d, *J* = 1.3 Hz, 1H), 4.59 (s, 1H), 4.06 (s, 1H), 3.87−3.77 (m, 1H), 3.53−3.43 (m, 1H), 3.46 (s, 3H, O-CH_3), 3.38 (dd, *J* = 3.0, 9.2 Hz, 1H), 3.10−2.96 (m, 2H), 2.37−2.23 (m, 3H), 2.17 (td, *J* = 3.2, 12.6 Hz, 1H), 1.95−1.80 (m, 2H), 1.80−1.71 (m, 1H),

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1.67 (s, 3H), 1.66−1.53 (m, 3H), 1.48−1.15 (m, 12H), 1.27 (d, J = 6.3 Hz, 3H), 1.12−0.96 (m, 2H), 0.93 (s, 3H), 0.89 (s, 3H), 0.87−0.82 (m, 1H), 0.80 (s, 3H), 0.74 (s, 6H), 0.66 (br d, J = 8.6 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.94, 150.68, 136.61, 128.60, 128.37, 128.17, 109.73, 102.08, 89.45, 81.59, 71.94, 67.72, 67.19, 65.85, 57.01, 56.68, 55.58, 50.63, 49.57, 47.07, 42.50, 40.78, 39.19, 38.32, 37.02, 32.24, 30.71, 29.69, 28.35, 25.64, 21.01, 19.50, 18.41, 17.61, 16.41, 16.29, 15.96, 14.79.

*Betulinic Acid 3-O-(3-O-Methyl-α-L-rhamnopyranoside) (**3b**).* To a solution of benzyl ester, **17** (27.0 mg, 0.038 mmol) in anhydrous methanol (2 mL) was added palladium on activated charcoal (6.2 mg). The mixture was stirred under a H_2 atmosphere at gentle reflux for 2 h. The mixture was then cooled to room temperature. Pd/C was filtered on a C18 solid-phase extraction cartridge (1 g) and washed with methanol. The solvent was evaporated under reduced pressure. The crude residue was purified by flash chromatography over silica gel (4 g, SiO_2) with a gradient from 0 to 50% of ethyl acetate in hexanes to obtain the title compound **3b** as a white solid (15.0 mg, 0.024 mmol, 63%). R_f = 0.35 (CH_2Cl_2/CH_3OH, 95:5); $[\alpha]^{20}_D$ − 12.8 (c 0.036, $CHCl_3$); HRMS (ESI) m/z calcd for $C_{37}H_{59}O_7$ $[M-H]^-$ 615.4266, found 615.4279; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.08 (br s, 1H), 4.85 (d, J = 5.5 Hz, 1H), 4.74 (s, 1H), 4.68 (s, 1H), 4.60 (s, 1H), 4.56 (s, 1H), 3.83 (br s, 1H), 3.57−3.46 (m, 1H), 3.29 (s, 3H, O-CH_3), 3.27−3.21 (m, 1H), 3.08 (dd, J = 2.8, 9.3 Hz, 1H), 3.03−2.90 (m, 2H), 2.21 (t, J = 10.9 Hz, 1H), 2.14−2.05 (m, 1H), 1.85−1.75 (m, 2H), 1.64 (s, 3H), 1.61−1.49 (m, 3H), 1.47−1.20 (m, 12H), 1.20−1.13 (m, 2H), 1.09 (d, J = 6.2 Hz, 3H), 1.03−0.96 (m, 1H), 0.93 (s, 3H), 0.87 (s, 3H), 0.86 (s, 3H), 0.83−0.80 (m, 1H), 0.78 (s, 3H), 0.70 (s, 3H), 0.75−0.66 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.24, 150.31, 109.67, 102.85, 87.49, 80.85, 70.76, 68.56, 66.50, 56.28, 55.42, 54.67, 49.83, 48.54, 46.62, 42.02, 40.25, 38.66, 38.06, 37.58, 36.48, 36.34, 33.82, 31.70, 30.10, 29.20, 27.81, 25.09, 20.46, 18.96, 17.87, 17.78, 16.24, 15.94, 15.71, 14.37.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c04301.

NMR spectra of saponins **3a−c** and intermediates **9−13** and **17** (PDF)

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Notes

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