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Efficient synthesis of morolic acid and related triterpenes starting from betulin

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ABSTRACT

Morolic acid (**1**) is a naturally occurring pentacyclic triterpene whose derivatives exhibit promising anti-HIV and other biological activities. An efficient synthesis of **1** has been accomplished in 11 steps with a total yield of 24% starting from betulin. Some related natural triterpenes including moradiol (**4**), acridocarpusic acid D (**5**), acridocarpusic acid E (**6**), and moronic aldehyde (**7**) have also been synthesized. Biological assay results showed that **1**, **5**, and **6** exhibited moderate inhibitory activity against glycogen phosphorylase.

1. Introduction

Morolic acid (**1**)[1,2] and moronic acid (**2**)[3–6] (Fig. 1) are naturally occurring pentacyclic triterpenes, which possess various pharmacological properties such as cytotoxic, anti-HSV, anti-HIV, and anti-inflammatory activities.[4,7–10] Morolic acid derivative **3** exhibits more potent anti-HIV activity than the synthetic betulinic acid derivative PA-457,[11] which has successfully completed a Phase IIa clinical trial and is currently undergoing phase IIb clinical trial in HIV-infected patients.[12] The mechanism of action of this class of anti-HIV agents is different from many approved anti-HIV drugs, and very importantly, they are potent inhibitors of HIV isolates that are resistant to currently approved drugs.[13–16] On the other hand, we have recently reported that oleanolic acid, which is a double-bound position isomer of **1**, and related pentacyclic triterpenes represent a new class of allosteric site inhibitors of glycogen phosphorylases (GP), and their glucose-lowering activity might be, at least in part, due to modulation of glycogen metabolism.[17] Selected compounds from this triterpene family are currently under preclinical development in our laboratories as promising multi-target therapeutic agents against diabetes and ischemic diseases. Given the significant therapeutic potential of **1** and related triterpenes, it is highly desirable to establish an efficient access to **1** to fulfill further pharmacological research and drug development. Herein, we report an efficient synthesis of **1** in 11 steps with a total yield of 24% starting from betulin. Some related natural triterpenes including moradiol (**4**), acridocarpusic acid D (**5**), acridocarpusic acid E (**6**), and moronic aldehyde (**7**) have also been synthesized. Furthermore, the synthesized triterpenes were biologically evaluated as inhibitors of glycogen phosphorylase.

2. Results and discussion

2.1. Chemistry

Barton et al. carried out a partial synthesis of methyl morolate acetate based on methyl siaresinolate acetate.[18] However, the siaresinolic acid is not easily available, and the conversion of the

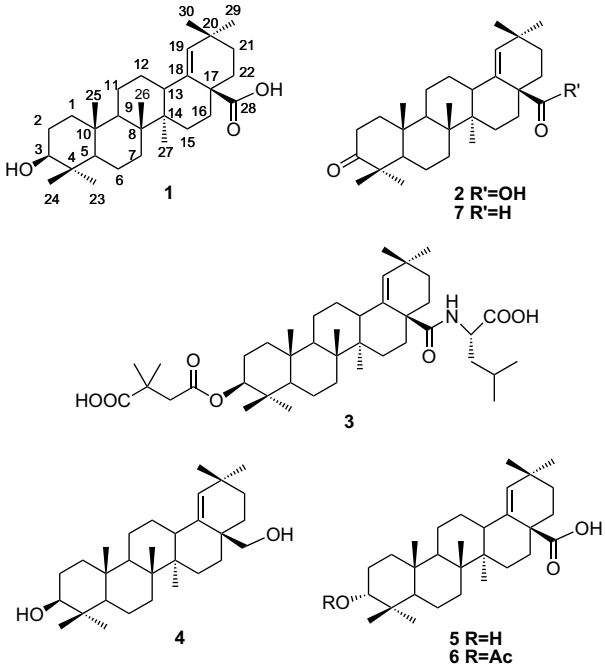

Figure 1. Morolic acid (**1**) and related triterpenes.

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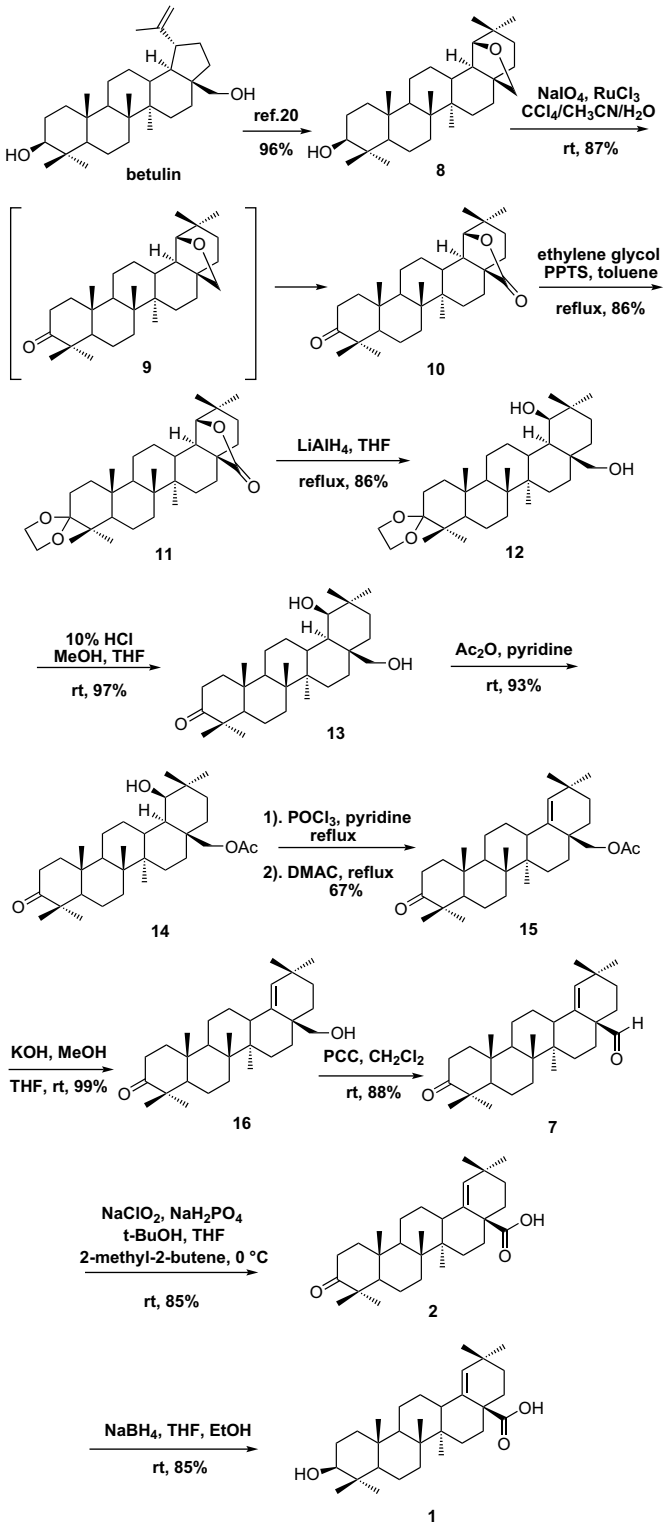
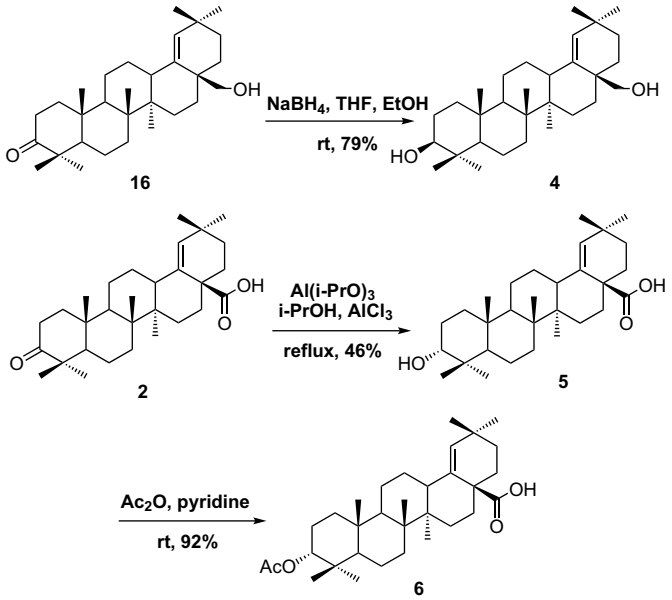
methyl ester to the corresponding carboxylic acid is difficult to implement by conventional hydrolysis methods (e.g., basic or acidic aqueous conditions). We therefore developed a new access to morolic acid, starting from easily available betulin (Scheme 1).

As shown in Scheme 1, allobetulonlactone (**10**) was employed as a key intermediate for preparation of **1**. According to the literatures,[19–21] the lactone ring system of **10** was built up from betulinic acid that has been found in certain plants only in small amounts. On the other hand, betulin is a cheap and easily available material that can be obtained by convenient extraction with ethanol from finely cut birch bark. We therefore used betulin as a starting material for preparation of **10**. One-pot oxidation of the 3-hydroxy group and tetrahydrofuran ring of allobetulin (**8**), which was readily prepared from betulin in 96% yield,[20] with sodium periodate/ruthenium trichloride[22,23] gave **10** in good yield (87%) via allobetulone (**9**) as a possible intermediate. In fact, when the reaction was quenched at early stage, **9** was isolated and its structure was determined by comparison with the literature data.[24] Treatment of **10** with ethylene glycol and PPTS in refluxing toluene[25] afforded cyclic ethylene ketal **11** (86%). Reductive ring-opening of lactone **11** was carried out with $LiAlH_4$ in refluxing THF[21] to afford the diol **12** in 86% yield. Deprotection of **12** with 10% HCl in THF/MeOH at room temperature gave ketone **13** in high yield (97%). Due to the high steric hindrance of C-19 alcohol, selective acetylation of the 28-hydroxyl group was accomplished in good yield (93%) with acetic anhydride in pyridine. According to Barton's work, dehydration of methyl dihydrosiaresinolate acetate using phosphorus oxychloride in refluxing pyridine could smoothly afford methyl morolate acetate.[18] However, it was found that direct dehydration of **14** under Barton's conditions[18] failed to provide **15** probably due to a more steric hindrance at 19β-hydroxyl group of **14** than at 19α-hydroxyl group of methyl dihydrosiaresinolate acetate. At this point, dehydration of **14** was attempted with several other conventional dehydration methods (e.g., $SOCl_2$/pyridine, H_2SO_4), however, the anticipated dehydration product **15** was not obtained by these methods. In fact, the steric hindrance of 19β-hydroxyl group in **14** was so strong that methylsulfonation or tosylation under very harsh conditions (e.g., high temperature, microwave irradiation) failed to provide the corresponding mesylate or tosylate. After extensive and very careful experimentation, we found that treatment of **14** with phosphorus oxychloride in hot pyridine for 1.5 h, followed by removing pyridine in vacuo, and then heating the residue in dimethylacetamide at high temperature (about 165 °C), furnished the desired dehydration product **15** in moderate yield (67%). Hydrolysis of **15** afforded alcohol **16** in 99% yield. Oxidation of **16** with pyridinium chlorochromate gave moronic aldehyde **7** (88%), which was further oxidized with sodium chlorite and sodium dihydrogenophosphate in a mixture of *t*-BuOH/THF/2-methyl-2-butene[26] to afford moronic acid **2** (85%). Stereoselective reduction of **2** with sodium borohydride produced morolic acid (**1**) (85%).



Scheme 1. Synthesis of morolic acid (**1**).



Scheme 2. Synthesis of moradiol (**4**), acridocarpusic acid D (**5**), and acridocarpusic acid E (**6**).

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

Compound	GPa IC_{50}[a]
1	70.3±8.5
2	100.5±9.7
4	173.3±10.6
5	34.5±2.4
6	32.7±3.3
7	558.5±40.5
Caffeine	83.1±7.6

[a] Values are means of three experiments.

Several related natural triterpenes have also been prepared as shown in Scheme 2. Stereoselective reduction of **16** with sodium borohydride produced moradiol (**4**) (79%). The Meerwein–Pondorf reduction[25] of **2** with aluminum isopropoxide in isopropyl alcohol afforded acridocarpusic acid D (**5**) in 46% yield together with 3β-hydroxy isomer **1** (30%). Acetylation of **5** with acetic anhydride in pyridine at room temperature afforded acridocarpusic acid E (**6**) (92%).

2.2. Biological activity

The synthesized natural triterpenes were biologically evaluated for their inhibitory activities against rabbit muscle GPa (RMGPa). The activity of RMGPa was measured through detecting the release of phosphate from glucose-1-phosphate in the direction of glycogen synthesis.[27] The bioassay results (Table 1) showed that only **1** (IC_{50}=70.3 μM), **5** (IC_{50}=34.5 μM), and **6** (IC_{50}=32.7 μM) exhibited moderate inhibition against RMGPa.

3. Conclusion

In summary, an efficient access to morolic acid (**1**) starting from betulin has been developed in 11 steps with an overall yield of 24%. Some related natural triterpenes such as moradiol (**4**), acridocarpusic acid D (**5**), acridocarpusic acid E (**6**), and moronic aldehyde (**7**) have also been synthesized based on this methodology. As betulin is an easily available material, this preparation opens a practical access to morolic acid and related triterpenes that are biologically active molecules with pharmaceutical potential as anti-HIV and antidiabetic agents. The synthesized natural triterpenes have been biologically evaluated as inhibitors of glycogen phosphorylase, and the result shows that **1**, **5**, and **6** are moderate GP inhibitors. Further structural modification and biological evaluation are ongoing, and the results will be reported in due course.

4. Experimental section

4.1. General

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

4.2. Allobetulonlactone (10)

To a solution of allobetulin **8**[20] (40 mg, 0.09 mmol) in CCl_4 (1.2 mL) and CH_3CN (1.2 mL) was added H_2O (1.8 mL), then $NaIO_4$ (150 mg, 0.72 mmol) and $RuCl_3$ (2 mg, 0.0072 mmol). The reaction mixture was allowed to stir at room temperature for 12 h. Additional $NaIO_4$ (150 mg, 0.72 mmol) and $RuCl_3$ (2 mg, 0.0072 mmol) were added. The reaction mixture was allowed to stir at room temperature for 12 h. At this point, the mixture was extracted with CH_2Cl_2, the CH_2Cl_2 layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate, 16:1) to give **10** as a white amorphous solid (36 mg, 87%). Mp>300 °C [lit.,[28] mp>300 °C]. $[\alpha]_D$ +55 (*c* 0.06, CH_2Cl_2) [lit.,[29] $[\alpha]_D$ +65 (*c* 0.7, CH_2Cl_2)]. IR (film, cm^{-1}): 2939, 2863, 2392, 2282, 1758, 1729, 1710, 1679, 1462, 1118, 1013, 966, 923, 738; ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, s), 0.94 (3H, s), 0.95 (3H, s), 0.96 (3H, s), 1.03 (6H, s), 1.08 (3H, s), 1.17–1.99 (22H, m), 2.43–2.52 (2H, m), 3.94 (1H, s); ^{13}C NMR ($CDCl_3$) δ 13.6, 15.3, 16.3, 19.5, 20.9, 21.4, 23.9, 25.5, 26.5, 26.7, 27.9, 28.7, 32.0, 32.3, 33.0, 33.6, 34.0, 36.1, 37.0, 39.9, 40.0, 40.5, 46.1, 46.7, 47.3, 50.6, 55.0, 85.9, 179.7, 217.8; ESI MS *m*/*z* 455.5 $[M+H]^+$. If the reaction was quenched at early stage, allobetulone (**9**) could be isolated as a white amorphous solid. Mp 228–230 °C [lit.,[24] mp 230–231 °C]. ^{1}H NMR ($CDCl_3$) δ 0.79 (3H, s), 0.91 (3H, s), 0.92 (3H, s), 0.93 (3H, s), 1.00 (3H, s), 1.02 (3H, s), 1.07 (3H, s), 1.08–1.67 (21H, m), 1.90–1.96 (1H, m), 2.42–2.50 (2H, m), 3.44 (1H, d, *J*=7.8 Hz), 3.52 (1H, s), 3.77 (1H, d, *J*=7.8 Hz).

4.3. 3,3-[1,2-Ethanediylbis(oxy)]-19-hydroxyolean-28-oic acid lactone (11)

To a solution of **10** (1.6 g, 3.52 mmol) in toluene (220 mL) was added ethylene glycol (5.5 mL), then PPTs (0.48 g, 1.91 mmol) was added. The reaction mixture was refluxed for 18 h. At this point, the mixture was extracted with EtOAc, the EtOAc layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate, 8:1) to give **11** as a white amorphous solid (1.50 g, 86%). Mp 292–291 °C. $[\alpha]_D$ +26.0 (*c* 0.265, CH_2Cl_2). IR (film, cm^{-1}): 2949, 2868, 1769, 1463, 1385, 1199, 1152, 1119, 1105, 1091, 1058, 968, 924, 738; ^{1}H NMR ($CDCl_3$) δ 0.82 (3H, s), 0.87 (6H, s), 0.91 (6H, s), 0.95 (3H, s), 1.02 (3H, s), 1.16–1.86 (24H, m), 3.89–3.99 (5H, m); ^{13}C NMR ($CDCl_3$) δ 13.7, 15.5, 16.3, 18.3, 19.9, 20.9, 22.8, 23.9, 25.5, 26.5, 26.9, 27.8, 28.7, 31.9, 32.3, 33.5, 33.6, 36.0, 37.1, 37.4, 40.0, 40.6, 42.2, 46.1, 46.7, 51.0, 53.5, 64.7, 64.9, 86.0, 113.2, 179.8; HRMS for $C_{32}H_{50}O_4$ calcd 498.3709, found 498.3706.

4.4. 3,3-[1,2-Ethanediylbis(oxy)]-oleanane-19(β),28-diol (12)

To a solution of **11** (300 mg, 0.602 mmol) in dry THF (15 mL) was added $LiAlH_4$ (110 mg, 3.01 mmol). The reaction mixture was refluxed for 40 min. At this point, the mixture was diluted with aqueous ether, and then extracted with CH_2Cl_2, the CH_2Cl_2 layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, dichloromethane/ethyl acetate, 15:1) to give **12** as a white amorphous solid (252 mg, 86%). Mp 288–290 °C. $[\alpha]_D$ +1.1 (*c* 0.07, CH_2Cl_2). IR (film, cm^{-1}): 3848, 3466, 3274, 3061, 2945, 2854, 2584, 2391, 2292, 1687, 1470, 1453, 1380, 1106, 1090, 1065, 1050, 1021, 941, 898, 724, 532; ^{1}H NMR (DMSO-d_6) δ 0.76 (3H, s), 0.84 (12H, s), 0.90 (3H, s), 0.99 (3H, s), 0.84–1.74 (23H, m), 1.81–1.88 (1H, m), 3.09 (1H, d, *J*=2.9 Hz), 3.37 (1H, dd, *J*=4.9, 11.6 Hz), 3.78–3.89 (5H, m), 4.00 (1H, t, *J*=5.1 Hz, exchangeable in D_2O), 4.17 (1H, d, *J*=5.0 Hz, exchangeable in D_2O); ^{13}C NMR (DMSO-d_6) δ 14.4, 15.5, 15.7, 18.0, 19.8, 20.4, 22.8, 23.8, 25.7, 26.0, 26.2, 28.6, 29.8, 31.1, 31.2, 31.7, 33.4, 35.0, 36.5, 36.8, 37.8, 40.6, 41.5, 41.6, 41.9, 49.6, 52.9, 58.6, 64.16, 64.24, 72.6, 112.1; HRMS for $C_{32}H_{54}O_4$ calcd 502.4022, found 502.4027.

4.5. 3-Oxooleanane-19,28-diol (13)

To a solution of **12** (20 mg, 0.0398 mmol) in THF (2 mL) were added MeOH (2.5 mL) and 10% HCl (1 mL). The reaction mixture

was stirred for 10 min. At this point, the mixture was neutralized with $NaHCO_3$ saturated solution, and then extracted with CH_2Cl_2, the CH_2Cl_2 layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, dichloromethane/ethyl acetate, 15:1) to give **13** as a white amorphous solid (17.6 mg, 97%). Mp 278–280 °C. $[\alpha]_D$ +40 (*c* 0.09, CH_2Cl_2). IR (film, cm^{-1}): 3139, 2945, 2871, 2686, 2272, 1706, 1509, 1461, 1385, 1087, 1034, 729, 509; ^1H NMR ($CDCl_3$) δ 0.93 (3H, s), 0.96 (6H, s), 0.97 (3H, s), 1.04 (3H, s), 1.08 (3H, s), 1.09 (3H, s), 1.18–2.04 (22H, m), 2.42–2.55 (2H, m), 3.33 (1H, s), 3.48 (1H, d, *J*=11.6 Hz), 4.15 (1H, d, *J*=11.5 Hz); ^{13}C NMR ($CDCl_3$) δ 14.6, 15.7, 15.9, 19.7, 21.1, 21.4, 24.5, 25.8, 26.6, 26.7, 28.0, 30.7, 32.7, 33.3, 33.4, 34.1, 34.4, 35.3, 36.9, 37.8, 39.6, 41.1, 42.0, 42.4, 47.4, 49.5, 55.1, 64.6, 74.7, 217.9; ESI MS *m*/*z* 481.3 $[M+Na]^+$; HRMS for $C_{30}H_{50}O_3$ calcd 458.3760, found 458.3772.

4.6. 28-Acetate-3-oxooleanane-19,28-diol (14)

To a solution of **13** (100 mg, 0.217 mmol) in pyridine (2.5 mL) was added Ac_2O (0.04 mL, 0.435 mmol). The reaction mixture was stirred at room temperature for 4 h. At this point, the mixture was neutralized with 3 N HCl, and then extracted with EtOAc, the EtOAc layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate, 15:1) to give **14** as a white amorphous solid (101 mg, 93%). Mp 204–206 °C. $[\alpha]_D$ +39.9 (*c* 0.18, CH_2Cl_2). IR (film, cm^{-1}): 3537, 2944, 2867, 1730, 1705, 1460, 1386, 1364, 1242, 1114, 1032, 970, 738, 704; ^1H NMR ($CDCl_3$) δ 0.91 (3H, s), 0.95 (6H, s), 0.96 (3H, s), 1.03 (3H, s), 1.08 (3H, s), 1.10 (3H, s), 0.79–1.96 (22H, m), 2.04 (3H, s), 2.35–2.55 (2H, m), 3.32 (1H, s), 4.30 (1H, d, *J*=11.9 Hz), 4.625 (1H, d, *J*=11.8 Hz); ^{13}C NMR ($CDCl_3$) δ 14.7, 15.8, 15.9, 19.7, 21.1, 21.4, 24.6, 25.7, 26.5, 26.6, 27.8, 29.5, 31.4, 31.6, 32.8, 33.3, 34.2, 35.3, 36.9, 37.1, 39.7, 41.1, 42.2, 42.6, 47.4, 49.6, 55.2, 62.6, 74.7, 171.3, 217.9; HRMS for $C_{32}H_{52}O_4$ calcd 500.3866, found 500.3864.

4.7. 28-Acetate-3-oxooleanane-18-en-28-ol (15)

To a solution of **14** (600 mg, 1.20 mmol) in pyridine (20 mL) was added $POCl_3$ (2.8 mL, 7.2 mmol). The reaction mixture was allowed to reflux for 1.5 h. At this point, the pyridine was removed, then dimethylacetamide (20 mL) was added, then the solution was refluxed for 0.5 h. At this point, the solution was added to 8 mL H_2O, and extracted with AcOEt, the AcOEt layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate, 30:1) to give **15** as a colorless oily product (390 mg, 67%). Mp 90–92 °C. $[\alpha]_D$ +33.1 (*c* 0.07, CH_2Cl_2). IR (film, cm^{-1}): 3393, 2945, 2865, 2676, 2287, 1738, 1706, 1453, 1383, 1365, 1232, 1107, 1033, 737; ^1H NMR ($CDCl_3$) δ 0.77 (3H, s), 0.95 (3H, s), 0.96 (6H, s), 1.03 (3H, s), 1.07 (3H, s), 1.09 (3H, s), 1.17–1.96 (20H, m), 2.06 (3H, s), 2.20–2.48 (3H, m), 3.99 (1H, d, *J*=11.1 Hz), 4.14 (1H, d, *J*=11.1 Hz), 5.06 (1H, s); ^{13}C NMR ($CDCl_3$) δ 14.6, 16.0, 16.5, 19.6, 20.9, 21.0, 21.6, 26.2, 26.8, 27.3, 29.4, 30.8, 31.30, 31.34, 32.2, 32.8, 33.8, 34.0, 36.9, 37.7, 39.0, 39.8, 40.7, 43.1, 47.2, 49.8, 50.5, 54.9, 65.8, 133.7, 138.0, 171.2; EIMS *m*/*z* 482 (12), 203 (100); HRMS for $C_{32}H_{50}O_3$ calcd 482.3760, found 482.3768.

4.8. 3-Oxooleanane-18-en-28-ol (16)

To a solution of **15** (1.05 g, 2.178 mmol) in THF (10 mL) was added KOH/MeOH (11.1 g/162 mL). The reaction mixture was allowed to stir at room temperature for 6 h. At this point, the reaction mixture was neutralized with 3 N HCl, and then extracted with AcOEt, the AcOEt layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate, 11:1) to give **16** as a white amorphous solid (0.94 g, 99%). Mp 191–194 °C. $[\alpha]_D$ +27.5 (*c* 0.235, CH_2Cl_2). IR (film, cm^{-1}): 3457, 2947, 2865, 1703, 1460, 1458, 1385, 1375, 1257, 1016, 849, 738, 595; ^1H NMR ($CDCl_3$) δ 0.79 (3H, s), 0.96 (6H, s), 0.99 (3H, s), 1.01 (3H, s), 1.06 (3H, s), 1.08 (3H, s), 1.10–1.96 (20H, m), 2.21 (1H, d, *J*=1.6 Hz), 2.44–2.51 (2H, m), 3.47 (1H, d, *J*=10.8 Hz), 3.64 (1H, d, *J*=10.7 Hz), 5.17 (1H, s); ^{13}C NMR ($CDCl_3$) δ 14.6, 16.0, 16.5, 19.7, 20.9, 21.6, 26.2, 26.9, 27.3, 29.8, 30.5, 31.2, 31.7, 32.2, 33.3, 33.9, 34.0, 36.9, 38.8, 39.6, 39.9, 40.8, 43.2, 47.3, 50.5, 54.9, 65.6, 134.6; ESI MS *m*/*z* 463.3 $[M+Na]^+$; HRMS for $C_{30}H_{48}O_2$ calcd 440.3654, found 440.3656.

4.9. Moronic aldehyde (7)

To a solution of **16** (600 mg, 1.36 mmol) in CH_2Cl_2 (60 mL) was added PCC (590 mg, 2.73 mmol). The reaction mixture was allowed to stir at room temperature for 1 h. At this point, the residue was removed by filtration and the solvent was evaporated under reduced pressure to give crude moronic aldehyde **7**. The residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate, 19:1) to give **7** as a white amorphous solid (525 mg, 88%). Mp 222–224 °C [lit.,[5] mp 145–148 °C]. $[\alpha]_D$ +71 (*c* 0.195, $CHCl_3$) [lit.,[5] $[\alpha]_D$ +46 (*c* 0.5, $CHCl_3$)]. IR (KBr, cm^{-1}): 2954, 2863, 2676, 2363, 1717, 1704, 1456, 1384, 1377, 1275, 1115, 976, 845, 585; ^1H NMR ($CDCl_3$) δ 0.79 (3H, s), 0.95 (3H, s), 0.99 (9H, s), 1.02 (3H, s), 1.07 (3H, s), 0.85–2.09 (21H, m), 2.43–2.49 (2H, m), 5.30 (1H, s), 9.38 (1H, s); ^{13}C NMR ($CDCl_3$) δ 14.7, 15.9, 16.5, 19.6, 21.0, 21.4, 26.5, 26.9, 28.8, 29.1, 29.4, 29.5, 30.6, 32.2, 33.1, 33.9, 34.1, 36.9, 39.9, 40.6, 41.3, 42.7, 47.3, 49.9, 51.5, 55.1, 134.7, 135.4, 204.9, 217.9; ESI MS *m*/*z* 439.2 $[M+H]^+$; HRMS for $C_{30}H_{46}O_2$ calcd 438.3498, found 438.3502.

4.10. Moronic acid (2)

To a solution of **7** (400 mg, 0.91 mmol) in *t*-BuOH (26 mL) and THF (5 mL) was added 2-mythyl-2-butene (8 mL). The mixture was allowed to stir in an ice bath. $NaClO_2$ and NaH_2PO_4 were dissolved into water, the solution was added to the mixture. The mixture was allowed to stir in an ice bath for 15 min, and then stirred at room temperature for 12 h. At this point, NH_4Cl was added, the mixture was extracted with EtOAc, the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate, 14:1) to give **2** as a white amorphous solid (350 mg, 85%). Mp 172–175 °C [lit.,[7] mp 210–212 °C]. $[\alpha]_D$ +61 (*c* 0.12, $CHCl_3$) [lit.,[4] $[\alpha]_D$ +59.3 (*c* 1.01, $CHCl_3$)]. IR (film, cm^{-1}): 3402, 2950, 2865, 2612, 1697, 1452, 1385, 1376, 1265, 1143, 1108, 1019, 951, 845, 738, 578; ^1H NMR ($CDCl_3$) δ 0.92 (3H, s), 1.07 (3H, s), 1.10 (3H, s), 1.13 (3H, s), 1.14 (3H, s), 1.15 (3H, s), 1.20 (3H, s), 0.96–2.39 (21H, m), 2.57–2.64 (2H, m), 5.30 (1H, s); ^{13}C NMR ($CDCl_3$) δ 14.8, 15.8, 16.5, 19.6, 20.9, 21.5, 26.0, 26.8, 29.1, 29.4, 30.3, 32.1, 33.3, 33.47, 33.53, 33.8, 34.0, 36.9, 39.8, 40.6, 41.5, 42.6, 47.3, 48.0, 50.5, 54.9, 133.4, 136.6, 181.2, 218.1; ESI MS *m*/*z* 453.3 $[M-H]^-$; HRMS for $C_{30}H_{46}O_3$ calcd 454.3447, found 454.3448.

4.11. Morolic acid (1)

To a solution of **2** (60 mg, 0.13 mmol) in THF (2 mL) and EtOH (0.5 mL) was added $NaBH_4$ (10 mg, 0.26 mmol). The reaction mixture was allowed to stir at room temperature for 3 h. At this point, the mixture was neutralized with 1 N HCl and extracted with EtOAc, the EtOAc layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate, 14:1) to give **1** as a white amorphous solid (50 mg, 85%). Mp 269–271 °C [lit.,[30] mp 273 °C]. $[\alpha]_D$ +31 (*c* 0.085, $CHCl_3$) [lit.,[4] $[\alpha]_D$ +39.8 (*c* 0.13, $CHCl_3$)]. IR (film, cm^{-1}): 3473, 2929, 2863, 2299, 1695,

1450, 1231, 1029, 848, 759, 604, 582; ^{1}H NMR ($CDCl_3$) δ 0.76 (3H, s), 0.78 (3H, s), 0.86 (3H, s), 0.97 (6H, s), 0.98 (3H, s), 1.00 (3H, s), 0.67–2.24 (23H, m), 3.20 (1H, dd, J=5.4, 10.9 Hz), 5.16 (1H, s); ^{13}C NMR ($CDCl_3$) δ 14.9, 15.4, 16.0, 16.6, 18.3, 20.9, 26.0, 27.4, 28.0, 29.1, 29.4, 30.3, 32.1, 33.4, 33.50, 33.54, 34.6, 37.3, 38.9, 39.0, 40.7, 41.4, 42.6, 48.0, 51.2, 55.6, 79.0, 133.2, 136.8, 181.0; ESI MS m/z 455.3 $[M-H]^-$; HRMS for $C_{30}H_{48}O_3$ calcd 456.3603, found 456.3607.

4.12. Moradiol (4)

To a solution of **16** (63 mg, 0.14 mmol) in THF (1 mL) and EtOH (0.25 mL) was added $NaBH_4$ (8 mg, 0.22 mmol). The reaction mixture was allowed to stir at room temperature for 4 h. At this point, the mixture was neutralized with 1 N HCl and extracted with EtOAc, the EtOAc layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate, 15:1) to give **4** as a white amorphous solid (50 mg, 79%). Mp 214–216 °C [lit.,[31] mp 218–220 °C]. $[\alpha]_D$ −7.2 (c 0.2, $CHCl_3$) [lit.,[31] $[\alpha]_D$ −8 ($CHCl_3$)]. IR (film, cm^{-1}): 3096, 2932, 2861, 2676, 2384, 2263, 1712, 1595, 1527, 1506, 1446, 1350, 1261, 1236, 1097, 1023, 848, 742, 585, 536; ^{1}H NMR ($CDCl_3$) δ 0.77 (3H, s), 0.78 (3H, s), 0.88 (3H, s), 0.96 (3H, s), 0.97 (6H, s), 1.06 (3H, s), 0.67–1.85 (22H, m), 2.21 (1H, d, J=10.8 Hz), 3.20 (1H, dd, J=5.4, 10.9 Hz), 3.46 (1H, d, J=10.7 Hz), 3.64 (1H, d, J=10.7 Hz), 5.16 (1H, s); ^{13}C NMR ($CDCl_3$) δ 14.7, 15.4, 16.1, 16.7, 18.3, 21.0, 26.2, 27.36, 27.43, 28.0, 29.9, 30.5, 31.3, 31.7, 32.2, 33.3, 34.6, 37.3, 38.7, 38.9, 39.0, 39.6, 40.9, 43.2, 51.2, 55.5, 65.6, 79.0, 134.5, 138.6; EIMS m/z 442 (14), 411 (100); HRMS for $C_{30}H_{50}O_2$ calcd 442.3811, found 442.3815.

4.13. Acridocarpusic acid D (5)

To a solution of **2** (150 mg, 0.33 mmol) in i-PrOH (5 mL) were added Al(i-PrO)$_3$ (202 mg, 0.99 mmol) and $AlCl_3$ (4.4 mg, 0.03 mmol). The reaction mixture was allowed to reflux for 4 h. At this point, the mixture was neutralized with 1 N HCl and extracted with EtOAc, the organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The crude product was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate, 18:1) to give **5** (67 mg, 46%) and 3β-hydroxy isomer **1** (44 mg, 30%). For **5**: a white amorphous solid. Mp 244–246 °C. $[\alpha]_D$ +4.3 (c 0.175, MeOH) [lit.,[8] $[\alpha]_D$ +17.5 (c 0.2, MeOH)]. IR (film, cm^{-1}): 3472, 2941, 2864, 2676, 2272, 1696, 1452, 1387, 1231, 1067, 844, 740, 606; ^{1}H NMR ($CDCl_3$) δ 0.79 (3H, s), 0.83 (3H, s), 0.87 (3H, s), 0.93 (3H, s), 0.98 (3H, s), 0.99 (3H, s), 1.00 (3H, s), 1.18–1.69 (18H, m), 1.91–2.03 (3H, m), 2.13–2.23 (2H, m), 3.40 (1H, br s), 5.17 (1H, s); ^{13}C NMR ($CDCl_3$) δ 14.8, 15.8, 16.3, 18.0, 20.6, 21.9, 25.2, 25.8, 28.0, 28.9, 29.1, 29.5, 30.2, 31.9, 33.2, 33.3, 33.4, 34.2, 37.2, 37.4, 40.7, 41.2, 42.5, 47.8, 49.0, 50.8, 76.1, 133.1, 136.7, 180.7; ESI MS m/z 455.2 $[M-H]^-$; HRMS for $C_{30}H_{48}O_3$ calcd 456.3603, found 456.3601.

4.14. Acridocarpusic acid E (6)

To a solution of **5** (32 mg, 0.070 mmol) in pyridine (=1 mL) was added Ac_2O (0.013 mL). The reaction mixture was stirred at room temperature for 18 h. At this point, the mixture was neutralized with 5 N HCl and extracted with EtOAc, the EtOAc layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate, 40:1) to give **6** as a white amorphous solid (32 mg, 92%). Mp 218–220 °C. $[\alpha]_D$ −14 (c 0.165, MeOH) [lit.,[8] $[\alpha]_D$ +2 (c 0.1, MeOH)]. IR (film, cm^{-1}): 3353, 2945, 2864, 2262, 1730, 1694, 1452, 1388, 1375, 1246, 1182, 1036, 986, 738; ^{1}H NMR ($CDCl_3$) δ 0.83 (6H, s), 0.87 (3H, s), 0.88 (3H, s), 0.97 (3H, s), 1.00 (6H, s), 1.21–2.04 (21H, m), 2.07 (3H, s), 2.15–2.24 (2H, m), 4.63 (1H, br s), 5.17 (1H, s); ^{13}C NMR (CDCl3) δ 15.1, 16.0, 16.4, 18.1, 20.8, 21.3, 21.7, 22.9, 26.0, 27.8, 29.1, 29.4, 30.3, 32.1, 33.4, 33.50, 33.55, 34.2, 34.4, 36.7, 37.3, 40.9, 41.4, 42.7, 48.0, 50.4, 51.0, 78.4, 133.2, 136.8, 170.8, 181.2; HRMS for $C_{32}H_{50}O_4$ calcd 498.3709, found 498.3712.

4.15. Enzyme assay

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

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.03.100.

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