Practical Synthesis of Bredemolic Acid, a Natural Inhibitor of Glycogen Phosphorylase

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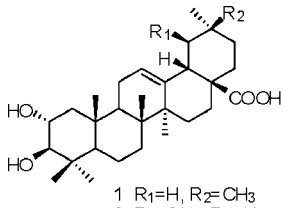
Received June 27, 2008

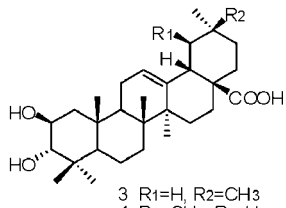
Bredemolic acid (**3**) is a naturally occurring 2β,3α-isomer of maslinic acid (**1**) that is an allosteric site inhibitor of glycogen phosphorylase (GP). A practical synthesis of **3** was accomplished (18% yield) in five steps starting from the readily available 2β,3β-diol **6a**. In a similar fashion, 2β,3α-dihydroxyurs-12-en-28-oic acid (**4**) was synthesized as a natural 2β,3α-isomer of corosolic acid (**2**). Compounds **3** and **4** exhibited significant inhibitory activity against rabbit muscle GPa with IC_{50} values of 6.25 and 1.1 μM, respectively.

Maslinic acid (**1**) and corosolic acid (**2**) have recently attracted much attention due to their reported antitumor, anti-HIV, anti-inflammation, antioxidation, antihyperglycemia, and cardiovascular activities.[1] Previously we reported that **1**, **2**, and related pentacyclic triterpenes represented a new class of allosteric site inhibitors of glycogen phosphorylases (GP), and their glucose-lowering activity could, at least in part, be due to modulation of glycogen metabolism.[2−6] Recently, Fukushima et al. proved that **2** exhibited a glucose-lowering effect on postchallenge plasma glucose levels in humans.[7] Both **1** and **2** have 2α,3β-dihydroxy functions, and the only structural difference lies in the positioning of E-ring dimethyl groups. In our previous studies,[2,3] it was shown that the configuration of 2,3-dihydroxy groups had an impact on GP inhibitory activity. In this regard, it seemed desirable to examine how isomeric compounds having 2β,3α-dihydroxy functions, rather than 2α,3β-dihydroxy groups as in **1** and **2**, would affect biological activity.

Bredemolic acid (**3**) (2β,3α-dihydroxyolean-12-en-28-oic acid) was obtained by acidic hydrolysis of a crude sapogenin from *Bredemeyera floribunda* (Polygalaceae).[8] To our knowledge, there has been no previous biological evaluation of this triterpene. 2β,3α-Dihydroxyurs-12-en-28-oic acid (**4**), an ursane type of counterpart of **3**, is claimed to have been isolated from *Lagerstroemia floribunda* (Lythraceae);[9] however, no spectroscopic data are available for **4**. Herein, we report a practical synthesis and biological evaluation of bredemolic acid (**3**) and 2β,3α-dihydroxyurs-12-en-28-oic acid (**4**), which are naturally occurring 2β,3α-isomers of **1** and **2**, respectively.



1 R_1=H, R_2=CH_3
2 R_1=CH_3, R_2=H



3 R_1=H, R_2=CH_3
4 R_1=CH_3, R_2=H

Results and Discussion

Tsehesche et al. carried out a partial synthesis of **3** by employing epoxidation of a triterpene diene followed by acid-catalyzed ring opening of the resulting epoxide as the key steps.[10,11] In our hands, however, preparations of the required triterpene dienes were very complex reactions, resulting in a variety of inseparable byproducts, and the overall yields were very poor. We therefore developed a new access to **3** and **4**, starting from the readily available 2β,3β-diols **6a** and **6b**,[2,3] respectively (Scheme 1).

As shown in Scheme 1, treatment of 2β,3β-diol **6a**, which was readily prepared from oleanolic acid (**5a**),[2] with *tert*-butyldimethylsilyl chloride (TBDMSCl) and imidazole in DMF gave the 2-O-silylated product **7a** as the major product (73%), together with the 3-O-silylated product **8a** as a minor product (19%). The observed stereoselectivity indicated that formation of **7a** was kinetically more favored than that of **8a**, possibly due to less steric hindrance at C2β-OH than at C3β-OH.[12] The relative configurations of **7a** and **8a** have been determined by NOE experiments (see the Supporting Information). Oxidation of **7a** with pyridinium chlorochromate (PCC) at slightly elevated temperature afforded ketone **9a** (66%). Considering that Meerwein−Pondorf reduction of the 3-oxo group of pentacyclic triterpenes has been proved to be an efficient method to prepare a 3α-hydroxy function,[2,13] this methodology was employed to provide the desired 3α-hydroxy function of the key intermediate **10a**. Reduction of **9a** in the presence of aluminum isopropoxide in isopropyl alcohol gave **10a** as the major product (46%), together with the 3β-hydroxy isomer **7a** as the minor product (40%). Deprotection of **10a** with tetrabutylammonium fluoride (TBAF) in THF at room temperature gave 2β,3α-diol **11a** in high yield (92%). Hydrogenolysis of **11a** over palladium−carbon in THF furnished bredemolic acid (**3**) in good yield (86%). In a similar fashion, 2β,3α-dihydroxyurs-12-en-28-oic acid (**4**) was synthesized, starting from 2β,3β-diol **6b**.

The synthesized natural triterpenes **3** and **4** were evaluated for their inhibitory activity against rabbit muscle GPa (RMGPa). The activity of RMGPa was measured by detecting the release of phosphate from glucose-1-phosphate in the direction of glycogen synthesis.[14] The bioassay results (Table 1) showed that both **3** (IC_{50} 6.25 μM) and **4** (IC_{50} 1.1 μM) exhibited significant inhibition against RMGPa. Interestingly, while **3** was more potent than its 2α,3β-isomer **1** (IC_{50} 28 μM),[6] **4** was more potent than its 2α,3β-counterpart **2** (IC_{50} 20 μM)[6] as well. That is to say, the 2β,3α-dihydroxy function in pentacyclic triterpenes appears to be more favorable than compounds having the 2α,3β-dihydroxy function in terms of GP inhibition.

Experimental Section

General Experimental Procedures. All commercially available solvents and reagents were used without further purification. Melting points of compounds were measured on a RY-1 melting point apparatus.

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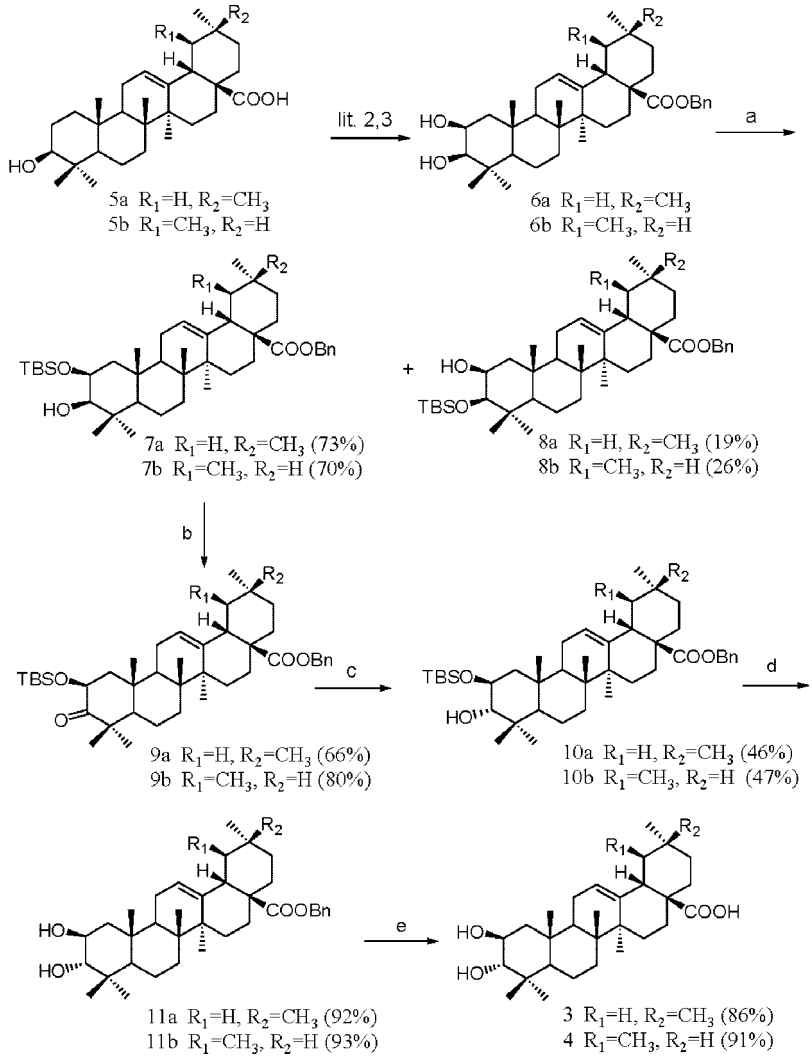
10.1021/np8003886 CCC: $40.75
Published on Web 10/11/2008

Scheme 1. Synthesis of Bredemolic Acid (**3**) and 2β,3α-Dihydroxyurs-12-en-28-oic acid (**4**)[a]



[a] (a) TBDMSCl, imidazole, DMF, 43 °C; (b) PCC, DCM, 40 °C; (c) Al(i-PrO)$_3$, i-PrOH, AlCl$_3$ (cat.), reflux; (d) TBAF, THF, rt; (e) H$_2$, 10% Pd−C, THF, rt.

Table 1. IC_{50} Values (μM) for the Inhibition of Rabbit Muscle GPa

compd	GPa IC_{50}[a]
3	6.25
4	1.1
caffeine	83.1

[a] Values are means of three experiments.

Column chromatography was carried out on silica gel (200−300 mesh, Qindao Ocean Chemical Company, China). IR spectra were recorded on a Shimadzu FTIR-8400S spectrometer. ^{1}H and ^{13}C NMR spectra were measured on Bruker AV-300 or AV-500 spectrometers. Chemical shifts are reported as δ values from an internal tetramethylsilane standard. Mass spectra were obtained on Agilent 1100 LC/DAD/MSD or Q-Tof Micro MS/MS spectrometers. Elemental analyses were measured on a Vario EL III instrument (Elementar, Germany).

Benzyl 2β-(*tert*-Butyldimethylsilyloxy)-3β-hydroxyolean-12-en-28-oate (7a). Imidazole (7.48 g, 0.11 mol) was added to a solution of **6a**[2] (7.5 g, 13 mmol) in DMF (180 mL) at room temperature. Then *tert*-butyldimethylsilyl chloride (TBDMSCl, 7.84 g, 52 mmol) was added. The reaction mixture was heated at 43 °C for 4 h. After cooling to room temperature, the mixture was diluted with water (200 mL) and extracted with EtOAc (3 × 150 mL), and the combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated to give a colorless oil. The oil was purified by flash chromatography (EtOAc/petroleum ether, 1:160) to afford **7a** as the major product (6.62 g, 73%), together with benzyl 3β-(*tert*-butyldimethylsilyloxy)-2β-hydroxyolean-12-en-28-oate (**8a**) as the minor product (1.7 g, 19%).

Compound 7a: white solid, mp 75−76 °C; $[\alpha]_D^{22}$ +57.9 (*c* 0.28, $CHCl_3$); IR (KBr) ν_{max} 2950, 1726, 696 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.63, 0.90, 0.92, 0.95, 1.00, 1.11, 1.16 (each 3H, s), 0.09 and 0.10 (each 3H, s, Si(CH$_3$)$_2$), 0.91 (9H, s, CH$_3$ of *tert*-butyl), 2.90 (1H, dd, J = 3.9, 13.6 Hz, H-18), 3.05 (1H, brs, H-3α), 4.06 (1H, m, H-2α), 5.06 (2H, s, CH$_2$Ar), 5.28 (1H, t, J = 3.5 Hz, H-12), 7.34 (5H, m, H-Ar); ^{13}C NMR ($CDCl_3$, 75 MHz) δ −5.4, −4.1, 16.2, 16.9, 17.0, 18.0, 18.1, 23.0, 23.4, 23.6, 25.9, 26.0, 27.5, 29.7, 30.7, 32.4, 32.7, 33.1, 33.9, 36.8, 38.3, 39.4, 41.4, 41.9, 44.7, 45.9, 46.7, 47.9, 55.3, 65.9, 72.0, 78.1, 122.4, 127.8, 127.9, 128.4, 136.4, 143.8, 177.4; ESIMS *m/z* 699.3 [M + Na]$^+$, 715.3 [M + K]$^+$; HRMS *m/z* 699.4796 (calcd for $C_{43}H_{68}NaO_4Si$, 699.4785); *anal*. calcd for $C_{43}H_{68}O_4Si\cdot 0.3H_2O$, C 75.67, H 10.13; found, C 75.42, H 10.18.

Compound 8a: white solid, mp 197−199 °C; $[\alpha]_D^{22}$ +51.8 (*c* 0.08, $CHCl_3$); IR (KBr) ν_{max} 2950, 1724, 696 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.63, 0.90, 0.97, 1.11, 1.21 (each 3H, s), 0.92 (6H, s), 0.08 and 0.10 (each 3H, s, Si(CH$_3$)$_2$), 0.94 (9H, s, CH$_3$ of *tert*-butyl), 2.90 (1H, dd, J = 3.7, 13.8 Hz, H-18), 3.25 (1H, d, J = 3.9 Hz, H-3α), 3.91 (1H, m, H-2α), 5.06 and 5.08 (each 1H, d, J = 12.5 Hz, CH$_2$Ar), 5.30 (1H, t, J = 3.5 Hz, H-12), 7.34 (5H, m, H-Ar); ESIMS *m/z* 699.5 [M + Na]$^+$, 715.5 [M + K]$^+$; *anal*. calcd for $C_{43}H_{68}O_4Si\cdot 0.3CH_3OH$, C 75.73, H 10.16; found, C 75.59, H 10.16.

Benzyl 2β-(*tert*-Butyldimethylsilyloxy)-3β-hydroxyurs-12-en-28-oate (7b). According to the procedure for preparation of **7a**, treatment of **6b**[2,3] (2.4 g, 4.3 mmol) with TBDMSCl afforded **7b** (2.0 g, 70%)

as the major product, together with benzyl 3β-(*tert*-butyldimethylsilyloxy)-2β-hydroxyurs-12-en-28-oate (**8b**) as the minor product (0.75 g, 26%).

Compound 7b: white solid, mp 92−93 °C; $[\alpha]_D^{22}$ +62.3 (*c* 0.13, $CHCl_3$); IR (KBr) ν_{max} 2950, 1723, 696 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.67, 0.94, 0.95, 1.00, 1.07, 1.18 (each 3H, s), 0.85 (3H, d, J = 6.5 Hz), 0.09 and 0.10 (each 3H, s, $Si(CH_3)_2$), 0.91 (9H, s, CH_3 of *tert*-butyl), 2.28 (1H, d, J = 11.4 Hz, H-18), 3.06 (1H, d, J = 3.7 Hz, H-3α), 4.07 (1H, m, H-2α), 5.02 and 5.06 (each 1H, d, J = 12.5 Hz, CH_2Ar), 5.25 (1H, t, J = 3.5 Hz, H-12), 7.34 (5H, m, H-Ar); ^{13}C NMR ($CDCl_3$, 75 MHz) δ −5.2, −4.0, 16.5, 16.9, 17.0, 17.1, 18.1, 18.2, 21.1, 23.5, 23.7, 24.4, 26.0, 28.0, 30.0, 30.8, 33.2, 36.7, 36.9, 38.4, 39.0, 39.2, 39.9, 42.4, 45.2, 48.0, 48.3, 53.1, 55.5, 66.0, 72.3, 78.3, 125.8, 127.9, 128.2, 128.4, 136,6, 138.4, 177.2; ESIMS *m/z* 675.5 $[M - H]^-$; HRMS *m/z* 699.4787 (calcd for $C_{43}H_{68}NaO_4Si$, 699.4785); *anal*. calcd for $C_{43}H_{68}O_4Si \cdot 0.1CH_3COOC_2H_5$, C 76.00, H 10.11; found, C 76.16, H 10.06.

Compound 8b: white solid, mp 134−135 °C; $[\alpha]_D^{22}$ +35.3 (*c* 0.09, $CHCl_3$); IR (KBr) ν_{max} 2940, 1723, 696 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.65, 0.91, 0.92, 0.97, 1.06, 1.23 (each 3H, s), 0.85 (3H, d, J = 6.4 Hz), 0.08 and 0.10 (each 3H, s, $Si(CH_3)_2$), 0.94 (9H, s, CH_3 of *tert*-butyl), 2.29 (1H, d, J = 11.5 Hz, H-18), 3.26 (1H, d, J = 3.9 Hz, H-3α), 3.93 (1H, m, H-2α), 5.00 and 5.09 (each 1H, d, J = 12.5 Hz, CH_2Ar), 5.25 (1H, t, J = 3.4 Hz, H-12), 7.35 (5H, m, H-Ar); ^{13}C NMR ($CDCl_3$, 75 MHz) δ −5.2, −4.1, 16.5, 17.0, 17.1, 17.8, 18.3, 21.1, 23.5, 23.6, 24.4, 26.0, 28.0, 30.0, 30.8, 33.2, 36.6, 36.7, 38.6, 39.0, 39.2, 39.8, 42.3, 43.3, 48.3, 53.1, 55.4, 66.0, 71.6, 80.3, 126.1, 128.0, 128.2, 128.4, 138.1, 177.2; ESIMS *m/z* 675.5 $[M - H]^-$; *anal*. calcd for $C_{43}H_{68}O_4Si \cdot 0.4\ CH_3COOC_2H_5$, C 75.20, H 10.07; found, C 75.52, H 9.89.

Benzyl 2β-(*tert*-Butyldimethylsilyloxy)-3-oxoolean-12-en-28-oate (9a). To a solution of **7a** (5.87 g, 8.7 mmol) in CH_2Cl_2 (26 mL) was added pyridinium chlorochromate (PCC, 3.59 g, 17.4 mmol) at 0 °C. Then the mixture was stirred at 40 °C for 12 h. The mixture was filtered through silica gel, and the insoluble material was washed several times with CH_2Cl_2. The filtrate was concentrated to give a crude product, which was purified by flash chromatography (EtOAc/petroleum ether, 1:40) to afford **9a** (3.85 g, 66%).

Compound 9a: white solid, mp 80−82 °C; $[\alpha]_D^{22}$ +81.8 (*c* 0.13, $CHCl_3$); IR (KBr) ν_{max} 2952, 1725, 697 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.61, 0.80, 0.91, 0.93, 1.07, 1.10, 1.19 (each 3H, s), 0.004 and 0.13 (each 3H, s, $Si(CH_3)_2$), 0.89 (9H, s, CH_3 of *tert*-butyl), 2.95 (1H, dd, J = 3.9, 13.7 Hz, H-18), 4.66 (1H, dd, J = 7.9, 11.2 Hz, H-2α), 5.06 and 5.08 (each 1H, d, J = 12.5 Hz, CH_2Ar), 5.33 (1H, *t*, J = 3.4 Hz, H-12), 7.33 (5H, m, H-Ar); ^{13}C NMR ($CDCl_3$, 75 MHz) δ −5.5, −4.7, 16.3, 18.0, 18.5, 19.6, 20.0, 23.1, 23.5, 23.6, 25.7, 25.8, 27.6, 29.9, 30.7, 31.8, 32.3, 33.1, 33.9, 36.9, 39.3, 41.7, 42.0, 45.9, 46.3, 46.9, 47.0, 51.4, 52.1, 66.0, 70.8, 122.5, 127.9, 128.0, 128.4, 136,4, 143.5, 177.3, 217.3; ESIMS *m/z* 697.3 $[M + Na]^+$, 713.3 $[M + K]^+$; *anal*. calcd for $C_{43}H_{66}O_4Si \cdot 1.3CH_3COOC_2H_5$, C 73.32, H 9.75; found, C 73.58, H 9.70.

Benzyl 2β-(*tert*-Butyldimethylsilyloxy)-3-oxours-12-en-28-oate (9b). According to the procedure for preparation of **9a**, oxidation of **7b** (2.0 g, 3 mmol) with PCC afforded **9b** (1.6 g, 80%).

Compound 9b: white solid, mp 93−95 °C; $[\alpha]_D^{22}$ +106.7 (*c* 0.12, $CHCl_3$); IR (KBr) ν_{max} 2932, 1725, 992 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.63, 0.81, 0.87, 0.96, 1.07, 1.10, 1.13 (each 3H, s), 0.005 and 0.13 (each 3H, s, $Si(CH_3)_2$), 0.90 (9H, s, CH_3 of *tert*-butyl), 2.31 (1H, d, J = 18.6 Hz, H-18), 4.67 (1H, dd, J = 13.1, 18.7 Hz, H-2α), 5.00 and 5.08 (each 1H, d, J = 20.7 Hz, CH_2Ar), 5.27 (1H, t, J = 6.0 Hz, H-12), 7.34 (5H, m, H-Ar); ^{13}C NMR ($CDCl_3$, 125 MHz) δ −5.3, −4.5, 16.6, 17.0, 18.1, 18.5, 19.7, 20.1, 21.1, 23.5, 23.6, 24.4, 25.9, 28.0, 30.0, 30.8, 32.2, 36.7, 37.0, 39.0, 39.3, 39.7, 42.4, 46.3, 47.1, 48.4, 51.7, 52.4, 53.3, 66.1, 70.9, 125.8, 128.0, 128.3, 128.4, 136.5, 138.2, 177.1, 217.1; ESIMS *m/z* 675.5 $[M + H]^+$, 697.4 $[M + Na]^+$; *anal*. calcd for $C_{43}H_{66}O_4Si \cdot 0.1H_2O$, C 76.30, H 9.86; found, C 75.94, H 9.89.

Benzyl 2β-(*tert*-Butyldimethylsilyloxy)-3α-hydroxyolean-12-en-28-oate (10a). Preparation of aluminum isopropoxide: finely cut aluminum (2.5 g, 0.09 mol) and anhydrous $AlCl_3$ (0.3 g, 2 mmol) was added to anhydrous isopropyl alcohol (60 mL), and the resulting mixture was refluxed until aluminum was deliquescent. Reflux was continued for 2 h, and then the mixture was cooled to room temperature.

A mixture of freshly prepared aluminum isopropoxide (17 mL, 26 mmol) and $AlCl_3$ (0.1 g, 0.7 mmol) was heated to 45 °C for half an hour and cooled to 30 °C, and then a solution of **9a** (2.8 g, 4.2 mmol) in anhydrous i-PrOH (36 mL) was added. After the reaction mixture was heated at reflux for 12 h, 1 M HCl (80 mL) was added at 0 °C, and then the mixture was extracted with EtOAc (3 × 80 mL). The combined organic layers were washed with water, saturated $NaHCO_3$, and brine, dried over Na_2SO_4, filtered, and concentrated to give a yellow solid, which was purified by flash chromatography (EtOAc/petroleum ether, 1:100) to afford **10a** as the major product (1.29 g, 46%), together with **7a** (1.12 g, 40%) as the minor product.

Compound 10a: white solid, mp 79−81 °C; $[\alpha]_D^{22}$ +82.6 (*c* 0.13, $CHCl_3$); IR (KBr) ν_{max} 2950, 1724, 770 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.61, 0.90, 0.91, 0.92, 1.03, 1.10, 1.14 (each 3H, s), 0.06 and 0.07 (each 3H, s, $Si(CH_3)_2$), 0.89 (9H, s, CH_3 of *tert*-butyl), 2.89 (1H, m, H-18), 3.54 (1H, d, J = 8.0 Hz, H-3β), 3.80 (1H, dd, J = 6.8, 14.8 Hz, H-2α), 5.06 and 5.07 (each 1H, d, J = 12.5 Hz, CH_2Ar), 5.31 (1H, t, J = 3.5 Hz, H-12), 7.33 (5H, m, H−Ar); ^{13}C NMR ($CDCl_3$, 75 MHz) δ −4.9, −4.2, 16.7, 18.0, 19.3, 20.0, 23.0, 23.2, 23.5, 23.7, 25.5, 25.9, 26.0, 27.6, 30.7, 32.4, 33.1, 34.0, 37.0, 37.5, 39.7, 41.6, 42.0, 46.0, 46.2, 46.9, 48.3, 50.4, 66.0, 71.4, 77.8, 122.7, 127.9, 128.0, 128.4, 136.5, 143.7, 177.4; ESIMS *m/z* 699.3 $[M + Na]^+$, 715.3 $[M + K]^+$; *anal*. calcd for $C_{43}H_{68}O_4Si \cdot 0.25\ CH_3COOC_2H_5$: C 75.53, H 10.09; found, C 75.97, H 9.88.

Benzyl 2β-(*tert*-butyldimethylsilyloxy)-3α-hydroxyurs-12-en-28-oate (10b). According to the procedure for preparation of **10a**, reduction of **9b** (1.5 g, 2 mmol) in the presence of aluminum isopropoxide afforded **10b** as the major product (0.7 g, 47%), together with **7b** as the minor product (0.62 g, 41%).

Compound 10b: white solid, mp 73−75 °C; $[\alpha]_D^{22}$ +77.2 (*c* 0.07, $CHCl_3$); IR (KBr) ν_{max} 2927, 1723, 758 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.64, 0.92, 0.95, 1.03, 1.08, 1.12 (each 3H, s), 0.86 (3H, d, J = 6.6 Hz), 0.08 (6H, s, $Si(CH_3)_2$), 0.90 (9H, s, CH_3 of *tert*-butyl), 2.25 (1H, d, J = 11.4 Hz, H-18), 3.52 (1H, d, J = 7.8 Hz, H-3β), 3.81 (1H, dd, J = 6.9, 14.1 Hz, H-2α), 5.00 and 5.08 (each 1H, d, J = 12.5 Hz, CH_2Ar), 5.26 (1H, t, J = 3.2 Hz, H-12), 7.34 (5H, m, H-Ar); ^{13}C NMR ($CDCl_3$, 75 MHz) δ −4.9, −4.3, 16.9, 17.0, 18.0, 19.1, 19.6, 21.1, 22.9, 23.3, 23.7, 24.3, 25.7, 25.9, 27.9, 30.7, 32.7, 36.7, 37.0, 37.4, 38.9, 39.2, 39.8, 42.3, 46.1, 48.2, 48.3, 50.3, 53.0, 66.0, 71.5, 77.9, 125.9, 128.0, 128.2, 128.4, 136.4, 138.2, 177.2; ESIMS *m/z* 675.5 $[M - H]^-$; *anal*. calcd for $C_{43}H_{68}O_4Si$, C 76.28, H 10.12; found, C 76.26, H 10.26.

Benzyl 2β,3α-Dihydroxyolean-12-en-28-oate (11a). To a solution of **10a** (0.29 g, 4.3 mmol) in THF (2 mL) was added dropwise 1 M TBAF (3 mL, 3 mmol). After stirring at room temperature for 8 h, the reaction mixture was diluted with water (20 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated to give a colorless oil, which was purified by flash chromatography (EtOAc/petroleum ether, 1:5) to afford **11a** (0.22 g, 92%).

Compound 11a: white solid, mp 106−108 °C; $[\alpha]_D^{22}$ +99.2 (*c* 0.08, $CHCl_3$); IR (KBr) ν_{max} 2947, 1723, 756 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.60, 0.92, 1.00, 1.06, 1.13 (each 3H, s), 0.90 (6H, s), 2.92 (1H, dd, J = 4.1, 13.7 Hz, H-18), 3.65 (1H, d, J = 10.4 Hz, H-3β), 3.75 (1H, m, H-2α), 5.06 and 5.07 (each 1H, d, J = 12.5 Hz, CH_2Ar), 5.31 (1H, t, J = 3.4 Hz, H-12), 7.33 (5H, m, H-Ar); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 16.6, 20.0, 20.8, 23.2, 23.3, 23.4, 23.6, 24.0, 25.9, 27.6, 29.7, 30.7, 32.3, 32.4, 33.1, 34.0, 37.4, 37.6, 39.7, 41.6, 42.0, 45.9, 46.9, 47.1, 48.3, 51.1, 66.0, 69.1, 78.3, 122.6, 127.9, 128.0, 128.4, 136.5, 143.7, 177.4; ESIMS *m/z* 585.5 $[M + Na]^+$, 561.5 $[M - H]^-$; *anal*. calcd for $C_{37}H_{54}O_4 \cdot 0.1H_2O$, C 78.71, H 9.68; found, C 78.43, H 9.53.

Benzyl 2β,3α-Dihydroxyurs-12-en-28-oate (11b). According to the procedure for preparation of **11a**, treatment of **10b** (0.5 g, 0.74 mmol) with TBAF afforded **11b** (0.39 g, 93%).

Compound 11b: white solid, mp 84−86 °C; $[\alpha]_D^{22}$ +80.2 (*c* 0.12, $CHCl_3$); IR (KBr) ν_{max} 3401, 1723, 757 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.63, 0.91, 0.94, 1.01 (each 3H, s), 0.86 (3H, d, J = 6.4 Hz), 1.08 (6H, s), 2.25 (1H, d, J = 11.2 Hz, H-18), 3.62 (1H, d, J = 10.4 Hz, H-3β), 3.73 (1H, m, H-2α), 5.00 and 5.08 (each 1H, d, J = 12.4 Hz, CH_2Ar), 5.26 (1H, t, J = 3.5 Hz, H-12), 7.33 (5H, m, H-Ar); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 16.8, 17.0, 19.9, 21.07, 21.14, 23.27, 23.33, 23.6, 24.0, 24.3, 27.9, 30.8, 32.6, 36.7, 37.3, 37.6, 38.9, 39.2, 39.8, 42.4, 47.4, 48.3, 51.1, 53.1, 66.0, 69.1, 78.3, 125.8, 128.0, 128.2, 128.4,

136.4, 138.2, 177.3; ESIMS *m/z* 561.3 [M − H]$^-$; *anal*. calcd for $C_{37}H_{54}O_4$, C 78.96, H 9.67; found, C 78.66, H 9.95.

2*β*,3α-Dihydroxyolean-12-en-28-oic acid (Bredemolic acid, 3). A mixture of **11a** (0.17 g, 0.3 mmol) and 10% Pd/C (0.13 g) in THF (3 mL) was stirred at room temperature under H_2 at atmospheric pressure for 24 h. The reaction mixture was filtered through Celite, and the insoluble substance was washed with THF (10 mL × 3). The filtrate was concentrated in vacuo to give a white solid, which was purified by flash chromatography (EtOAc/petroleum ether, 1:3) to afford **3** (0.12 g, 86%).

Compound 3: white solid, mp 292−294 °C (lit.[8] 288−292 °C); $[\alpha]_D^{22}$ +93.5 (*c* 0.17, pyridine) (lit.[8] $[\alpha]_D^{22}$ +100.5, *c* 1.0, pyridine); IR (KBr) ν_{max} 3397, 1741, 1007 cm^{-1}; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 0.93, 0.99, 1.06, 1.24, 1.28, 1.30, 1.31 (each 3H, s), 3.32 (1H, m, H-18), 3.99 (1H, d, J = 7.3 Hz, H-3β), 4.36 (1H, dd, J = 6.3, 12.9 Hz, H-2α), 5.51 (1H, s, H-12); ^{13}C NMR (pyridine-d_5, 75 MHz) δ 17.3, 19.8, 23.5, 23.8, 24.0, 26.2, 26.9, 28.3, 30.0, 31.0, 32.1, 33.1, 33.3, 34.4, 37.8, 37.9, 40.2, 42.2, 42.5, 46.0, 46.6, 46.8, 48.8, 51.0, 70.7, 78.4, 122.8, 144.9, 180.1; ESIMS *m/z* 495.4 [M + Na]$^+$, 471.5 [M − H]$^-$; HRMS *m/z* 471.3473 (calcd for $C_{30}H_{47}O_4$, 471.3474); *anal*. calcd for $C_{30}H_{48}O_4 \cdot 0.2H_2O$, C 75.65, H 10.24; found, C 75.21, H 10.64.

2*β*,3α-Dihydroxyolean-12-en-28-oic acid (4). According to the procedure for preparation of **3**, hydrogenolysis of **11b** (0.25 g, 4.4 mmol) afforded **4** (0.19 g, 91%).

Compound 4: white solid, mp 257−259 °C; $[\alpha]_D^{22}$ +60.9 (*c* 0.06, pyridine); IR (KBr) ν_{max} 3430, 1696, 664 cm^{-1}; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 0.96, 1.08, 1.19, 1.29 (each 3H, s), 1.01 (3H, d, J = 6.4 Hz), 1.30 (6H, s), 2.66 (1H, d, J = 11.2 Hz, H-18), 4.00 (1H, d, J = 7.5 Hz, H-3β), 4.36 (1H, dd, J = 6.6, 13.7 Hz, H-2α), 5.50 (1H, t, J = 3.3 Hz, H-12); ^{13}C NMR (pyridine-d_5, 75 MHz) δ 17.4, 17.5, 19.7, 19.9, 21.4, 23.5, 23.8, 23.9, 25.0, 27.0, 28.6, 31.2, 33.4, 37.5, 37.6, 37.9, 39.47, 39.54, 40.3, 42.8, 46.1, 48.2, 48.7, 50.9, 53.7, 70.7, 78.4, 125.9, 139.3, 179.9; ESIMS *m/z* 495.3 [M + Na]$^+$, 471.3 [M − H]$^-$; HRMS *m/z* 471.3484 (calcd for $C_{30}H_{47}O_4$, 471.3474); *anal*. calcd for $C_{30}H_{48}O_4 \cdot 0.5CH_3OH$, C 74.96, H 10.31; found, C 74.66, H 10.80.

Enzyme Assay. Inhibitory activity of the compounds against rabbit muscle glycogen phosphorylase a (GPa) was monitored using a microplate reader (BIO-RAD) based on the published method.[14] 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 μL 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 μL 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.

Acknowledgment. This program was financially supported by the National Natural Science Foundation (grants 30672523 and 90713037), research grants from the Ministry of Education (grants 706030 and 20050316008), and program for New Century Excellent Talents in University (NCET-05-0495).

Supporting Information Available: Copies of ^{1}H NMR and ^{13}C NMR spectra of **3**, **4**, and synthetic intermediates **7a**, **7b**, **8a**, **8b**, **9a**, **9b**, **10a**, **10b**, **11a**, and **11b**. Copies of NOE spectra of **7a** and **8a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP8003886