Synthesis and Biological Evaluation of Asiatic Acid Derivatives as Inhibitors of Glycogen Phosphorylases

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Twenty-four asiatic acid derivatives have been synthesized and biologically evaluated as inhibitors of glycogen phosphorylase (GP). Within this series of compounds, asiatic acid benzyl ester (**23**; IC_{50} = 3.8 μM) exhibited more potent activity than its parent compound **1** (IC_{50} = 17 μM). SAR Analysis showed that asiatic acid (**1**) possessing a 2α-OH function exhibited more potent GP inhibitory activity than eriantic acid B (**27**) which possesses a 2β-OH function. Further lead optimization based on **1** is needed to find more effective asiatic acid derivatives as antidiabetic agents with protective effects against ischemic diabetic complications.

Introduction. – Asiatic acid (**1**) is a member of the ursane family of pentacyclic triterpenoids isolated from *Centella asiatica*, which is extensively used as a medicinal herb in many countries [1]. Compound **1** was reported to possess a wide spectrum of biological activities including antioxidation, anti-inflammation, antitumor, antidepression, anti-*Alzheimer*'s disease, cardiovascular protection, and hepatoprotective effect [2–8]. Plant extracts containing **1** and related saponins have been in clinical uses for decades mainly for treating injured skin (wound healing) and chronic ulcer deformation of skin [9].

In previous communications [10–13], we first reported that pentacyclic triterpenes such as maslinic acid (MA) and corosolic acid (CA) (*Fig.*) represented a new class of inhibitors of glycogen phosphorylases (GP), and their glucose-lowering activity might

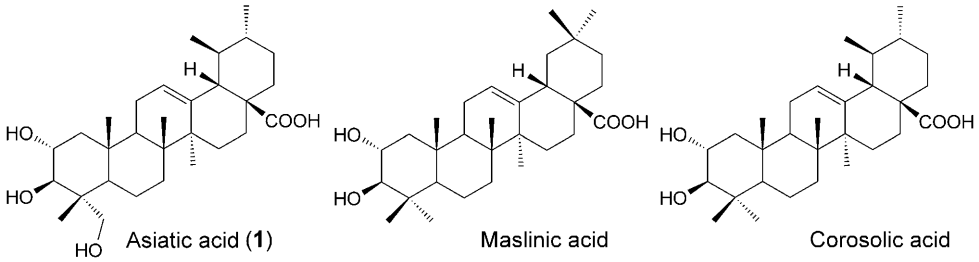


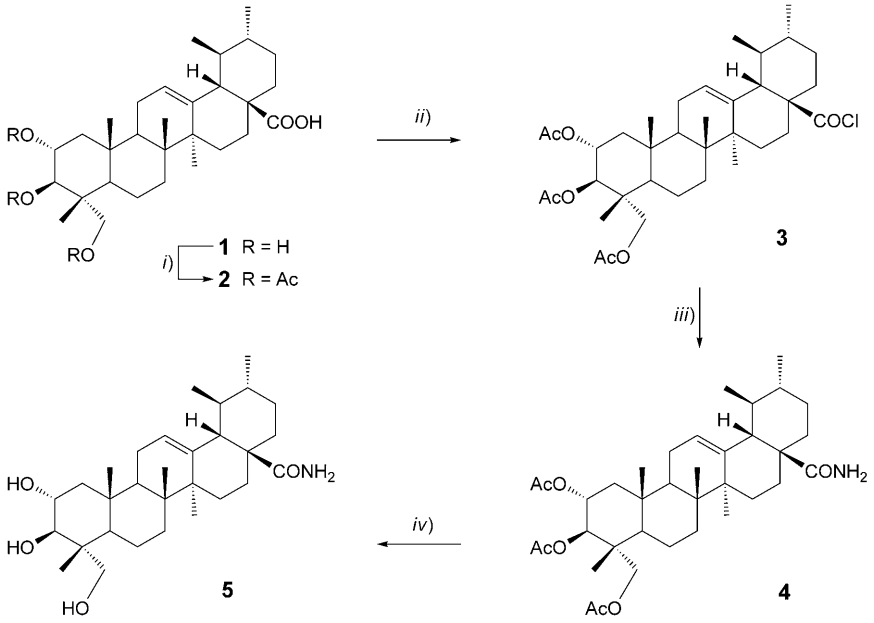
Figure. *Structures of asiatic acid* (**1**)*, maslinic acid, and corosolic acid*

be, at least in part, due to modulation of hepatic and peripheral glycogen metabolism. GP Inhibition has been regarded as a therapeutic approach to treating diabetes [14][15], and various studies have shown the efficacy of GP inhibitors at lowering blood glucose in animal models of diabetes [16] and in clinical trials [17].

Based on our previous studies, we tested the GP inhibition activity of **1** which is a close analog of MA and CA, and, not surprisingly, the result showed that **1** was a moderate GP inhibitor ($IC_{50}=17\ \mu M$) [18]. To elucidate the mechanism of GP inhibition by pentacyclic triterpenes, we have determined the X-ray crystal structure of **1** binding to GPb, which demonstrates that **1** binds at the allosteric activator site, where the physiological activator AMP binds [18]. In this study, lead optimization based on **1** was carried out in order to find more potent triterpene GP inhibitors with good pharmacokinetic properties. In this regard, amino acid derivatives of **1** were synthesized in order to improve water solubility of **1**. On the other hand, a series of C(28) ester derivatives of **1** were synthesized in order to further explore structure–activity relationships (SAR) at C(28) of **1**. Moreover, eriantic acid B (**27**) [19], the naturally occurring *C(2)*-epimer of **1**, was synthesized to check the effect of 2-OH configuration on GP inhibition.

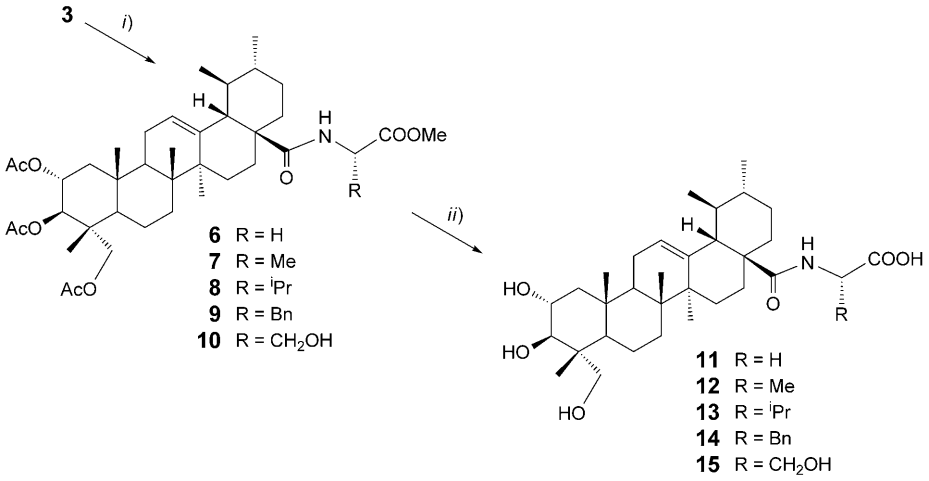
Results and Discussion. – 1. *Synthesis.* As shown in *Schemes 1* and *2*, asiatic amide **5** and a series of amino acid derivatives of **1** were synthesized. Full acetylation of **1** afforded 2α,3β,23-*O*-triacetylasiatic acid (**2**) in good yield. Treatment of **2** with $SOCl_2$ afforded the corresponding acyl chloride **3**, which was used for the next reactions

Scheme 1



i) Ac_2O/Pyr; 78%. *ii*) $SOCl_2$. *iii*) $NH_3\cdot H_2O$, THF; 83% over 2 steps. *iv*) 4N NaOH, MeOH/THF; 86%.

Scheme 2



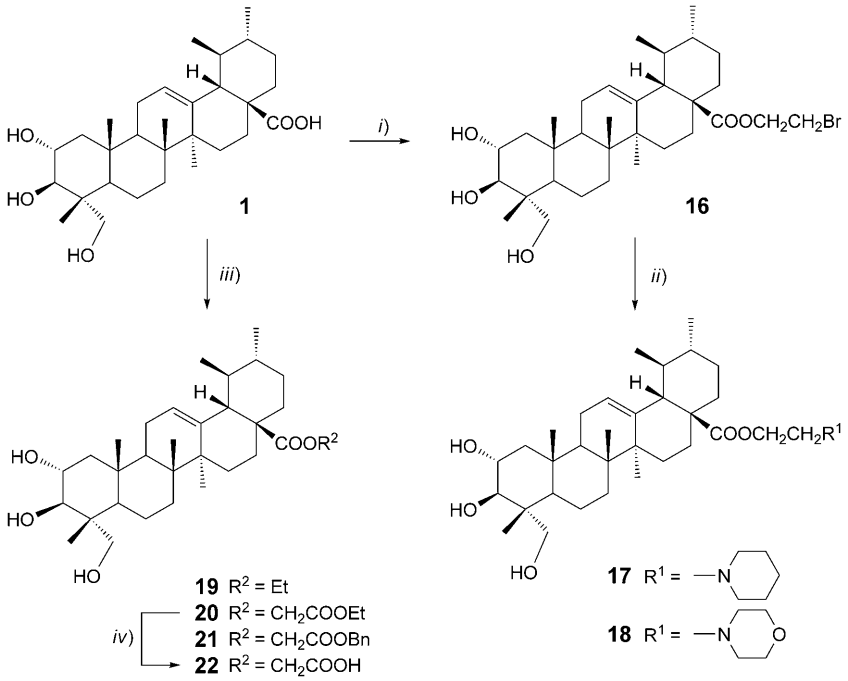
i) $RCH(NH_2)COOMe \cdot HCl$, 4-(dimethylamino)pyridine (DMAP)/CH_2Cl_2; 52–87%. *ii*) 4N NaOH, MeOH/THF; 68–89%.

without further purification. Reaction of **3** with concentrated NH_3 solution at 0° furnished amide **4**, which was hydrolyzed with aqueous NaOH to give asiatic amide **5** (*Scheme 1*). The amino acid derivatives **6**–**15** were readily prepared as described above, using corresponding amino acid methyl esters as amine reactants (*Scheme 2*).

A series of C(28) ester derivatives of **1** were synthesized as depicted in *Scheme 3*. Alkylation of **1** with 1,2-dibromoethane in the presence of K_2CO_3 in DMF gave 2-bromoethyl ester **16** in 82% yield. Reaction of **16** with piperidine or morpholine gave 2-(amino)ethyl esters **17** or **18**, respectively. Similar to the preparation of **16**, esters **19**, **20**, and **21** were prepared in high yields. Ester **20** was further converted to the free acid **22** (59%) by saponification with 4N NaOH aqueous solution without affecting the C(28) ester group.

It was noticed that the configurations of the OH groups at C(2) and C(3) had an impact on GP inhibitory potency of pentacyclic triterpenes [13]. In the light of this observation, it should be interesting to see how a 2β-OH function would affect the potency in contrast to **1** with a 2α-OH function. In this regard, eriantic acid B (**27**), possessing a 2β-OH function, was synthesized (*Scheme 4*). Reaction of **1** with BnCl (Bn = $PhCH_2$) in the presence of K_2CO_3 in DMF at 60° gave benzyl ester **23** in high yield. Treatment of **23** with 2,2-dimethoxypropane in the presence of TsOH afforded **24**. Oxidation of **24** with pyridinium chlorochromate (PCC) in CH_2Cl_2 at room temperature afforded ketone **25**, which was used for the next reaction without further purification. Reduction of **25** with $NaBH_4$ in THF at 0°, followed by acidic deprotection, gave eriantic acid B benzyl ester **26** in 75% yield. Hydrogenolysis of **26** over Pd/C in THF furnished **27** in 92% yield. The spectroscopic data of **27** were identical with those reported in [19].

Scheme 3

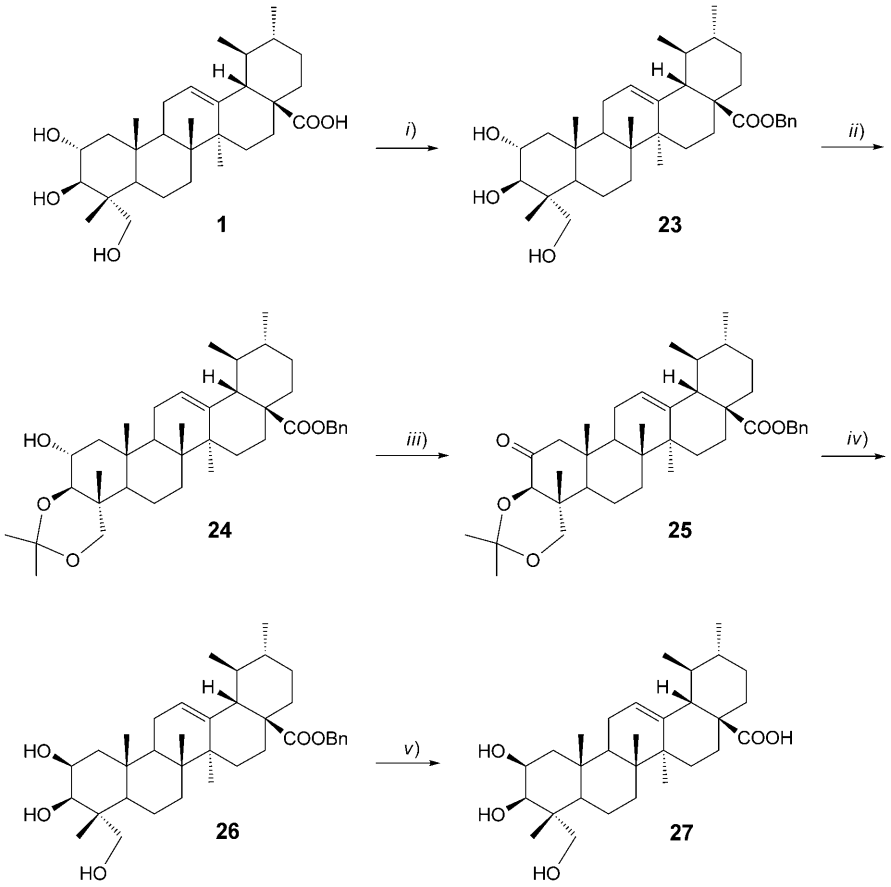


i) $BrCH_2CH_2Br$, K_2CO_3, DMF, 40°; 82%. *ii*) Piperidine or morpholine, K_2CO_3, acetone, reflux; 72–77%. *iii*) R^2Br, K_2CO_3, DMF, r.t.; 82–92%. *iv*) 4N NaOH, MeOH/THF; 59%.

2. *Biological Activity.* The synthesized asiatic acid derivatives were evaluated in the enzyme-inhibition assay against rabbit muscle glycogen phosphorylase a (RMGPa). The activity of RMGPa was measured through detecting the release of phosphate from glucose-1-phosphate in the direction of glycogen synthesis [16]. Caffeine was routinely used as a positive control in GP assay in our studies and others [10–13][16][18]. Although caffeine is not a very potent GP inhibitor, it is a fairly convincing positive control. More importantly, caffeine is a known allosteric GP inhibitor, and thus it shares the same binding site with the triterpene compounds, since our X-ray crystal-structure study shows that **1** binds at the allosteric site of GP [18].

The bioassay results are summarized in the *Table.* Some of the tested triterpene compounds exhibited good-to-moderate inhibitory activity against GPa. Within this series of compounds, **23** (IC_{50} = 3.8 μM) was the most potent (more potent than its parent compound **1** (IC_{50} = 17 μM)). Incorporation of amino acid moiety at C(28) of **1** resulted in loss of potency (*e.g.*, **11**–**15**). Even though benzyl ester **23** exhibited strong GP inhibition activity, data analysis indicated no clear SAR for structural modifications at C(28). Interestingly, 2β-OH function of **27** (2-epiasiatic acid; IC_{50} = 92.8 μM) resulted in a significant decrease in potency compared with 2α-OH function of **1** (IC_{50} = 17 μM). Introduction of hydrophilic groups into the side chain at C(28) was carried out in order

Scheme 4



i) K_2CO_3, BnCl, DMF (95%). *ii*) 2,2-Dimethoxypropane/TsOH (86%). *iii*) Pyridinium chlorochromate (PCC), CH_2Cl_2. *iv*) 1. $NaBH_4$, THF; 2. 20% HCl (44%, over 2 steps). *v*) H_2, 10% Pd/C, THF, r.t. (92%).

to improve H_2O solubility of **1**; however, the resulting compounds exhibited a markedly decreased activity (*i.e.*, **17**) or no activity (*i.e.*, **11**–**15**, **18**).

Conclusions. – In this study, 24 asiatic acid derivatives have been synthesized and biologically evaluated as inhibitors of glycogen phosphorylase. Within this series of compounds, asiatic acid benzyl ester (**23**; $IC_{50}=3.8$ μM) exhibited more potent activity than its parent compound **1**. SAR Analysis showed that asiatic acid (**1**) possessing a 2α-OH function exhibited more potent GP inhibitory activity than eriantic acid B (**27**) which possesses a 2β-OH function. Extensive lead optimization based on **1**, aiming at improvement of potency and pharmacokinetic properties, is ongoing in our laboratory, and more results will be reported in due time.

Table. *Rabbit Muscle GPa Inhibition-Assay Results for Asiatic Acid Derivatives*

Compound	IC_{50} [a]) [μM]	Compound	IC_{50} [a]) [μM]	Compound	IC_{50} [a]) [μM]
1	17 ± 1.2	**11**	n.i.[b])	**21**	n.i.
2	33 ± 2.9	**12**	n.i.	**22**	116 ± 12.2
3	n.d.[c])	**13**	n.i.	**23**	3.8 ± 0.9
4	36 ± 3.6	**14**	n.i.	**24**	n.i.
5	n.i.	**15**	n.i.	**25**	n.d.
6	101 ± 12.2	**16**	n.d.	**26**	55 ± 3.2
7	112 ± 17.3	**17**	132 ± 15.3	**27**	93 ± 7.1
8	n.i.	**18**	173 ± 27.4	Caffeine[d])	114 ± 14.5
9	104 ± 11.6	**19**	n.i.		
10	n.i.	**20**	n.i.		

[a]) Each value represents the mean ± S.D. of three determinations. [b]) n.i.: No inhibition. [c]) n.d.: Not determined. [d]) Positive control.

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Experimental Part

General. Chemicals: *Shanghai Chemical Reagent Company.* Column chromatography (CC): silica gel *60* (200–300 mesh, *Qingdao Ocean Chemical Company*, China). TLC: *60 F_{254}* silica-gel plates (250 μm, *Qingdao Ocean Chemical Company*, China). M.p.: *RY-1* melting-point tester; in cap. tube; uncorrected. IR Spectra: *Shimadzu FTIR-8400S* spectrometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *ACF* 300Q Bruker*, $CDCl_3$ unless otherwise indicated; δ in ppm, J in Hz. LR-MS: *Hewlett-Packard 1100 LC/MSD* spectrometer.

2α,3β,23-Triacetoxyurs-12-en-28-oic acid (**2**). To a soln. of *asiatic acid* (**1**; 0.20 g, 0.40 mmol) in 10 ml of anh. pyridine, Ac_2O (76 mg, 0.74 mmol) was slowly added while cooling and stirring. The mixture was then stirred overnight at r.t. After the addition of AcOEt (20 ml), the mixture was worked up with 1N HCl, sat. $NaHCO_3$, and brine in sequence, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by CC (petroleum ether (PE)/AcOEt 10 : 1) to give **2** (170 mg, 78%). White solid. M.p. 93–95°. IR (KBr): 3462, 2950, 1745, 1234. ^{1}H-NMR (300 MHz): 0.78 (*s*, 3 H); 0.85 (*d*, J = 6.4, 3 H; 0.88 (*s*, 3 H); 0.94 (*d*, J = 5.9, 3 H); 1.07, 1.11 (2*s*, each 3 H); 1.98, 2.02, 2.08 (3*s*, each 3 H); 2.19 (*d*, J = 11.3, 1 H); 3.58 (*d*, J = 11.8, 1 H); 3.85 (*d*, J = 11.8, 1 H); 5.07 (*d*, J = 10.3, 1 H); 5.12–5.17 (*m*, 1 H); 5.25 (br. *s*, 1 H). ^{13}C-NMR (75 MHz): 182.9; 170.9; 170.5; 170.4; 138.0; 125.3; 77.4; 77.0; 76.6; 74.8; 69.9; 65.3; 52.5; 47.9; 47.6; 47.5; 43.7; 42.0; 41.9; 39.5; 39.0; 38.8; 37.8; 36.6; 32.4; 30,6; 27.9; 24.0; 23.4; 23.3; 21.2; 21.1; 20.9; 20.8; 17.9; 17.0; 17.0; 16.9; 13.9. ESI-MS: 637 ($[M+Na]^+$).

2α,3β,23-Triacetoxyurs-12-en-28-amide (**4**). A mixture of **2** (60 mg, 0.098 mmol) and $SOCl_2$ (2 ml) was refluxed for 2 h, and excess reagent was removed under vacuum to give acyl chloride **3**. Without purification, a soln. of **3** in THF (10 ml) was added dropwise to a stirred soln. of conc. NH_3 (5 ml) at 0°, and the mixture was stirred overnight. The mixture was concentrated under vacuum to remove the solvent, and AcOEt was added. The org. layer was washed with 1N HCl, sat. $NaHCO_3$, and brine in sequence, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by CC (PE/AcOEt 3 : 1) to afford **4** (49 mg, 83% for two steps). Pale yellow solid. M.p. 142–146°. IR (KBr): 2924, 1738, 1667, 1236. ^{1}H-NMR (300 MHz): 0.86, 0.88, 0.89, 0.96 (4*s*, each 3 H); 1.11 (*s*, 6 H); 1.98, 2.02, 2.08 (3*s*, each 3 H); 3.58 (*d*, J = 11.8, 1 H); 3.85 (*d*, J = 11.8, 1 H); 5.08 (*d*, J = 10.3, 1 H); 5.12–5.20 (*m*, 1 H); 5.32 (*t*, J = 3.5, 1 H); 5.84 (br. *s*, 1 H). ^{13}C-NMR (75 MHz): 180.8; 170.7; 170.4; 170.2; 140.0; 125.2; 74.9; 69.9; 65.4; 54.3; 47.8; 47.7; 43.9; 42.6; 42.0; 39.8; 39.6; 39.1; 37.9; 37.2; 32.4; 30.9; 29.7; 27.9; 24.9; 23.5; 23.2; 21.2; 21.0; 20.8; 20.7; 18.0; 17.2; 17.1; 13.9. ESI-MS: 636.5 ($[M+Na]^+$).

2α,3β,23-Trihydroxyurs-12-en-28-amide (**5**). To a soln. of **4** (100 mg, 0.16 mmol) in MeOH (2 ml) and THF (3 ml), 4N NaOH (0.6 ml) was added dropwise, and the resulting mixture was stirred at r.t. for 2 h. The mixture was acidified with aq. HCl and extracted with AcOEt. The org. layer was washed with sat. $NaHCO_3$ and brine in sequence, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by CC (CH_2Cl_2/MeOH 40:1) to give **5** (68 mg, 86%). White solid. M.p. 248–252°. IR (KBr): 3402, 2923, 1654, 1048. ^{1}H-NMR (300 MHz, CD_3OD): 0.69, 0.87 (2*s*, each 3 H); 0.90 (*d*, *J* = 6.4, 3 H); 0.97, 1.04, 1.14 (3*s*, each 3 H); 2.08 (*d*, *J* = 11.3, 1 H); 3.25 (*d*, *J* = 11.1, 1 H); 3.34 (*d*, *J* = 9.6, 1 H); 3.49 (*d*, *J* = 11.2, 1 H); 3.65–3.73 (*m*, 1 H); 5.33 (*t*, *J* = 3.3, 1 H). ^{13}C-NMR (75 MHz, CD_3OD): 183.6; 140.2; 127.0; 78.5; 69.7; 66.8; 54.6; 49.8; 48.1; 48.0; 44.1; 43.5; 40.9; 40.8; 40.3; 39.0; 38.7; 33.6; 31.9; 29.0; 25.3; 24.5; 24.1; 21.5; 19.1; 17.9; 17.7; 13.8. ESI-MS: 488.4 ($[M+H]^+$).

N-*(2α,3β,23-Acetoxyurs-12-en-28-oyl)glycine Methyl Ester* (**6**). A mixture of **2** (0.25 g, 0.40 mmol) and $SOCl_2$ (5 ml) was refluxed for 2 h, and the excess reagent was removed under vacuum to give **3** which was re-dissolved in 10 ml of CH_2Cl_2. The soln. thus prepared was added dropwise to a mixture of glycine methyl ester hydrochloride (0.15 g, 1.20 mmol) and DMAP (0.03 g, 0.22 mmol) in CH_2Cl_2 (10 ml) at 0°. The mixture was then stirred overnight at r.t., and, after washing with 1N HCl, sat. $NaHCO_3$, and brine in sequence, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by CC (PE/AcOEt 4:1) to give **6** (244 mg, 87%). White solid. M.p. 175–178°. IR (KBr): 2924, 2870, 1744, 1369, 1235. ^{1}H-NMR (300 MHz): 0.73, 0.87, 0.89, 0.96 (4*s*, each 3 H); 1.10 (*s*, 6 H); 1.98, 2.02, 2.08 (3*s*, each 3 H); 3.58 (*d*, *J* = 11.8, 1 H); 3.76 (*s*, 3 H); 3.81–3.88 (*m*, 2 H); 4.05–4.13 (*m*, 1 H); 5.06–5.21 (*m*, 2 H); 5.41 (*t*, *J* = 3.4, 1 H); 6.45 (*t*, *J* = 4.2, 1 H). ^{13}C-NMR (75 MHz): 177.9; 170.7; 170.6; 170.3; 170.2; 139.3; 125.8; 75.0; 69.9; 65.4; 53.8; 52.2; 47.9; 47.8; 47.7; 44.0; 42.5; 42.1; 41.6; 39.8; 39.1; 37.9; 37.1; 32.4; 30.9; 27.9; 24.9; 23.6; 23.3; 21.2; 21.0; 20.7; 18.0; 17.1; 17.0; 16.6; 13.9. ESI-MS: 686.4 ($[M+H]^+$).

N-*(2α,3β,23-Acetoxyurs-12-en-28-oyl)*-L-*alanine Methyl Ester* (**7**). As described for the preparation of **6**, treatment of **2** (0.25 g, 0.40 mmol) with L-alanine methyl ester hydrochloride (0.17 g, 1.21 mmol) afforded 0.15 g (54%) of **7**. Pale yellow solid. M.p. 220–222°. IR (KBr): 2924, 1743, 1370, 1235. ^{1}H-NMR (300 MHz): 0.70 (*s*, 3 H); 0.88 (*s*, 6 H); 0.96 (*s*, 3 H); 1.09 (*s*, 9 H); 1.98, 2.02, 2.08 (3*s*, each 3 H); 3.57 (*d*, *J* = 11.8, 1 H); 3.75 (*s*, 3 H); 3.84 (*d*, *J* = 11.8, 1 H); 4.45–4.49 (*m*, 1 H); 5.07 (*d*, *J* = 10.3, 1 H); 5.12–5.17 (*m*, 1 H); 5.40 (br. *s*, 1 H); 6.57 (*d*, *J* = 5.9, 1 H). ^{13}C-NMR (75 MHz): 177.2; 173.7; 170.7; 170.3; 170.2; 138.7; 126.0; 75.0; 70.0; 65.4; 53.8; 52.3; 48.2; 47.8; 47.7; 44.0; 42.4; 42.1; 39.8; 39.7; 39.1; 37.9; 37.4; 32.5; 30.9; 29.7; 27.9; 24.7; 23.5; 23.3; 21.1; 21.0; 20.7; 20.7; 18.7; 18.0; 17.1; 16.7; 13.9. ESI-MS: 700.5 ($[M+H]^+$).

N-*(2α,3β,23-Acetoxyurs-12-en-28-oyl)*-L-*valine Methyl Ester* (**8**). As described for the preparation of **6**, treatment of **2** (0.25 g, 0.40 mmol) with L-valine methyl ester hydrochloride (0.21 g, 1.25 mmol) afforded 0.15 g (52%) of **8**. White solid. M.p. 205–208°. IR (KBr): 2953, 1744, 1370, 1232. ^{1}H-NMR (300 MHz): 0.68 (*s*, 3 H); 0.88–0.97 (*m*, 15 H); 1.09 (*s*, 6 H); 1.98, 2.02, 2.08 (3*s*, each 3 H); 3.57 (*d*, *J* = 11.8, 1 H); 3.70 (*s*, 3 H); 3.84 (*d*, *J* = 11.8, 1 H); 4.42–4.46 (*m*, 1 H); 5.07 (*d*, *J* = 10.3, 1 H); 5.11–5.19 (*m*, 1 H); 5.38 (br. *s*, 1 H); 6.36 (*d*, *J* = 7.4, 1 H). ^{13}C-NMR (75 MHz): 177.4; 172.5; 170.6; 170.3; 170.2; 138.6; 126.0; 75.0; 70.0; 65.5; 57.2; 54.1; 51.8; 48.1; 47.8; 44.0; 42.5; 42.1; 39.8; 39.8; 39.2; 37.9; 37.8; 32.6; 32.0; 30.9; 27.9; 24.7; 23.5; 23.3; 21.1; 21.0; 20.7; 18.7; 18.3; 18.0; 17.1; 17.0; 16.8; 13.9. ESI-MS: 728.5 ($[M+H]^+$).

N-*(2α,3β,23-Acetoxyurs-12-en-28-oyl)*-L-*phenylalanine Methyl Ester* (**9**). As described for the preparation of **6**, treatment of **2** (0.25 g, 0.40 mmol) with L-phenylalanine methyl ester hydrochloride (0.26 g, 1.21 mmol) afforded 0.18 g (56%) of **9**. White solid. M.p. 120–124°. IR (KBr): 3418, 2952, 1747, 1237. ^{1}H-NMR (300 MHz): 0.60, 0.83, 0.88, 0.94, 1.05, 1.06 (6*s*, each 3 H); 1.97, 2.02, 2.08 (3*s*, each 3 H); 3.00–3.17 (*m*, 2 H); 3.56 (*d*, *J* = 11.7, 1 H); 3.68 (*s*, 3 H); 3.84 (*d*, *J* = 12.0, 1 H); 4.71 (*dd*, *J* = 6.0, 12.0, 1 H); 5.06 (*d*, *J* = 10.2, 1 H); 5.10–5.19 (*m*, 1 H); 5.25 (*t*, *J* = 3.0, 1 H); 6.34 (*d*, *J* = 6.0, 1 H); 7.08–7.31 (*m*, 5 H). ^{13}C-NMR (75 MHz): 177.3; 172.2; 170.7; 170.3; 170.2; 138.5; 136.3; 129.4; 128.4; 127.0; 126.0; 75.0; 70.0; 65.4; 53.7; 53.6; 52.0; 47.8; 47.7; 47.7; 43.9; 42.4; 42.0; 39.7; 39.7; 39.1; 38.3; 37.9; 37.3; 32.4; 30.9; 29.7; 27.8; 24.8; 23.4; 23.3; 21.1; 21.0; 20.7; 17.9; 17.1; 16.6; 13.9. ESI-MS: 776.4 ($[M+H]^+$).

N-*(2α,3β,23-Acetoxyurs-12-en-28-oyl)*-L-*serine Methyl Ester* (**10**). As described for the preparation of **6**, treatment of **2** (0.25 g, 0.40 mmol) with L-serine methyl ester hydrochloride (0.14 g, 1.20 mmol) afforded 0.19 g (68%) of **10**. White solid. M.p. 217–219°. IR (KBr): 2924, 1744, 1369, 1235. ^{1}H-NMR (300 MHz): 0.70, 0.88, 0.88, 0.97, 1.09, 1.10 (6*s*, each 3 H); 1.98, 2.02, 2.08 (3*s*, each 3 H); 2.26 (*d*, *J* = 17.1, 1 H); 3.57 (*d*, *J* = 11.8, 1 H); 3.79 (*s*, 3 H); 3.82–3.86 (*m*, 2 H); 3.94–3.99 (*m*, 1 H); 4.52–4.53 (*m*, 1 H); 5.07 (*d*, *J* = 10.3, 1 H); 5.12–5.16 (*m*, 1 H); 5.43 (*t*, *J* = 3.0, 1 H); 6.85 (*d*, *J* = 5.1, 1 H). ^{13}C-NMR

(75 MHz): 179.1; 170.9; 170.7; 170.4; 170.3; 138.5; 126.2; 74.9; 69.9; 65.4; 64.4; 55.7; 53.8; 52.7; 48.0; 47.7; 47.7; 43.9; 42.4; 42.0; 39.8; 39.7; 39.0; 37.9; 37.6; 32.4; 30.9; 29.7; 27.9; 24.7; 23.5; 23.3; 21.1; 21.0; 20.8; 20.7; 17.9; 17.1; 16.6; 13.9. ESI-MS: 716.5 ($[M+H]^+$).

N-*(2α,3β,23-Hydroxyurs-12-en-28-oyl)glycine* (**11**). As described for the preparation of **5**, **11** was obtained in 89% yield. White solid. M.p. 269° (dec.). IR (KBr): 3403, 2924, 1633, 1048. ^{1}H-NMR (300 MHz, (D_6)DMSO + D_2O): 0.55, 0.65 (2*s*, each 3 H); 0.84 (*d*, *J* = 6.2, 3 H); 0.92 (*s*, 6 H); 1.04 (*s*, 3 H); 2.09 (*d*, *J* = 10.7, 1 H); 3.06 (*d*, *J* = 10.6, 1 H); 3.18 (*d*, *J* = 9.5, 1 H); 3.30 (*d*, *J* = 10.9, 1 H); 3.49–3.53 (*m*, 1 H); 3.59 (*d*, *J* = 17.3, 1 H); 3.74 (*d*, *J* = 17.5, 1 H); 5.24 (br. *s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 176.4; 171.2; 138.3; 124.9; 75.9; 67.4; 64.3; 52.2; 47.1; 47.0; 46.6; 46.3; 42.5; 41.7; 41.1; 40.5; 38.8; 38.5; 37.3; 36.7; 32.2; 30.4; 27.3; 23.8; 23.3; 23.0; 21.1; 17.5; 17.1; 16.9; 16.6; 13.6. ESI-MS: 544.3 ($[M-H]^-$).

N-*(2α,3β,23-Hydroxyurs-12-en-28-oyl)*-L-*alanine* (**12**). As described for the preparation of **5**, **12** was obtained in 79% yield. White solid. M.p. 226–230°. IR (KBr): 3402, 2926, 1636, 1048. ^{1}H-NMR (300 MHz, (D_6)DMSO + D_2O): 0.51, 0.62 (2*s*, each 3 H); 0.80 (*d*, *J* = 5.3, 3 H); 0.88 (*s*, 6 H); 1.00 (*s* 3 H); 1.19 (*d*, *J* = 5.7, 3 H); 2.04 (*d*, *J* = 10.5, 1 H); 3.02 (*d*, *J* = 10.5, 1 H); 3.13 (*d*, *J* = 9.1, 1 H); 3.26 (*d*, *J* = 10.7, 1 H); 3.48 (*t*, *J* = 10.5, 1 H); 4.06 (*d*, *J* = 6.3, 1 H); 5.20 (br. *s*, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 175.6; 174.0; 137.9; 125.1; 75.7; 67.3; 64.2; 52.3; 47.6; 47.0; 46.8; 46.4; 46.1; 42.3; 41.6; 40.3; 40.1; 38.9; 38.6; 38.3; 37.1; 36.6; 32.1; 30.2; 27.2; 23.5; 23.1; 22.9; 20.9; 17.6; 17.3; 16.9; 16.7; 16.5; 13.5. ESI-MS: 558.3 ($[M-H]^-$).

N-*(2α,3β,23-Hydroxyurs-12-en-28-oyl)*-L-*valine* (**13**). As described for the preparation of **5**, **13** was obtained in 85% yield. White solid. M.p. 214–218°. IR (KBr): 3407, 2928, 1637, 1048. ^{1}H-NMR (300 MHz, (D_6)DMSO + D_2O): 0.54, 0.63 (2*s*, each 3 H); 0.83–0.91 (*m*, 15 H), 1.04 (*s*, 3 H); 2.09 (*d*, *J* = 10.5, 1 H); 3.03 (*d*, *J* = 10.5, 1 H); 3.16 (*d*, *J* = 9.3, 1 H); 3.29 (*d*, *J* = 10.5, 2 H); 4.00 (*t*, *J* = 6.6, 1 H); 5.20 (br. *s*, 1 H); 6.90 (*d*, *J* = 7.5, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 176.3; 172.7; 137.8; 125.4; 75.7; 67.4; 64.1; 57.3; 52.6; 47.1; 47.0; 46.9; 46.2; 42.4; 41.8; 40.3; 38.8; 38.7; 38.4; 37.3; 37.0; 32.3; 30.5; 30.4; 27.3; 23.7; 23.2; 23.0; 20.9; 18.9; 18.5; 17.4; 17.0; 16.9; 16.7; 13.6. ESI-MS: 588.3 ($[M+H]^+$).

N-*(2α,3β,23-Hydroxyurs-12-en-28-oyl)*-L-*phenylalanine* (**14**). As described for the preparation of **5**, **14** was obtained in 68% yield. White solid. M.p. 210–214°. IR (KBr): 3405, 2926, 1632, 1049. ^{1}H-NMR (300 MHz, (D_6)DMSO + D_2O): 0.52, 0.76, 0.78, 0.83, 0.87, 0.94 (6*s*, each 3 H); 2.88–2.91 (*m*, 1 H); 2.92–2.96 (*m*, 2 H); 3.13 (*d*, *J* = 9.3, 1 H); 3.27 (*d*, *J* = 10.2, 1 H); 4.16 (br. *s*, 1 H); 5.01 (*t*, *J* = 3.6, 1 H); 7.20 (*s*, 5 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 176.3; 173.0; 138.0; 129.4; 128.0; 126.2; 125.1; 75.8; 67.4; 64.3; 54.0; 52.5; 47.1; 46.9; 46.7; 46.3; 42.5; 41.7; 40.3; 40.1; 39.1; 38.9; 38.8; 38.7; 38.6; 37.3; 36.5; 32.0; 30.4; 27.3; 23.8; 23.3; 23.0; 21.1; 17.4; 17.1; 16.9; 16.2; 13.6. ESI-MS: 636.3 ($[M+H]^+$).

N-*(2α,3β,23-Hydroxyurs-12-en-28-oyl)*-L-*serine* (**15**). As described for the preparation of **5**, **15** was obtained in 82% yield. White solid. M.p. 224–227°. IR (KBr): 3403, 2925, 1631, 1047. ^{1}H-NMR (300 MHz, (D_6)DMSO + D_2O): 0.50, 0.60 (2*s*, each 3 H); 0.79 (*d*, *J* = 6.2, 3 H); 0.86, 0.87, 1.00 (3*s*, each 3 H); 2.99 (*d*, *J* = 10.6, 1 H); 3.11 (*d*, *J* = 9.3, 1 H); 3.24 (*d*, *J* = 11.0, 1 H); 3.45–3.54 (*m*, 2 H); 4.10–4.11 (*m*, 1 H); 5.21 (br. *s*, 1 H); 6.96 (*d*, *J* = 6.1, 1 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 176.2; 172.1; 138.1; 125.4; 75.8; 67.6; 64.1; 61.7; 61.6; 54.7; 52.6; 47.2; 46.9; 46.2; 42.6; 41.9; 38.5; 37.4; 36.8; 32.3; 30.5; 27.5; 23.9; 23.4; 23.4; 23.1; 21.2; 17.5; 17.2; 17.0; 16.9; 16.6; 13.8. ESI-MS: 574.2 ($[M-H]^-$).

2-Bromoethyl 2α,3β,23-Trihydroxyurs-12-en-28-oate (**16**). According to the literature methods [11][12], alkylation of **1** with 1,2-dibromoethane in the presence of K_2CO_3 in DMF gave **16** (82%). White solid. M.p. 183–185°. IR (KBr): 3442, 2923, 1724, 1639. ^{1}H-NMR (300 MHz): 0.77 (*s*, 3 H); 0.86 (*d*, *J* = 6.4, 3 H); 0.90 (*s*, 3 H); 0.96 (*d*, *J* = 10.4, 3 H); 1.04, 1.09 (2*s*, each 3 H); 2.24 (*d*, *J* = 11.2, 1 H); 3.40–3.50 (*m*, 4 H); 3.69 (*d*, *J* = 10.4, 1 H); 3.73–3.79 (*m*, 1 H); 4.31 (*t*, *J* = 6.0, 2 H); 5.28 (*t*, *J* = 3.3, 1 H). ESI-MS: 595 ($[M+H]^+$).

2-(Piperidin-1-yl)ethyl 2α,3β,23-Trihydroxyurs-12-en-28-oate (**17**). According to the literature methods [11][12], reaction of **16** with piperidine gave **17** (77%). M.p. 178–180°. IR (KBr): 3417, 2929, 1726, 1047. ^{1}H-NMR (300 MHz): 0.75 (*s*, 3 H); 0.84 (*d*, *J* = 6.4, 3 H); 0.90 (*s*, 3 H); 0.95 (*d*, *J* = 4.7, 3 H); 1.04, 1.07 (2*s*, each 3 H); 2.22 (*d*, *J* = 11.2, 1 H); 2.47 (br. *s*, 4 H); 2.60 (*t*, *J* = 5.9, 2 H); 3.42 (*t*, *J* = 7.9, 2 H); 3.68 (*d*, *J* = 10.5, 1 H); 3.71–3.79 (*m*, 1 H); 4.14 (*t*, *J* = 6.1, 2 H); 5.23 (*t*, *J* = 3.4, 1 H). ESI-MS: 600 ($[M+H]^+$).

2-(Morpholin-4-yl)ethyl 2α,3β,23-Trihydroxyurs-12-en-28-oate (**18**). According to the literature methods [11][12], reaction of **16** with morpholine gave **18** (72%). M.p. 173–175°. IR (KBr): 3427, 2923,

1724, 1116. ^{1}H-NMR (300 MHz): 0.76 (*s*, 3 H); 0.85 (*d*, *J* = 6.4, 3 H); 0.90 (*s*, 3 H); 0.94 (*d*, *J* = 4.6, 3 H); 1.04, 1.08 (2*s*, each 3 H); 2.53 (br. *s*, 4 H); 2.63 (br. *s*, 2 H); 2.22 (*d*, *J* = 11.1, 1 H); 3.43 (*m*, 2 H); 3.60–3.80 (*m*, 6 H); 4.16 (br. *s*, 2 H); 5.24 (*t*, *J* = 3.3, 1 H). ESI-MS: 602 ($[M + H]^+$).

Asiatic Acid Ethyl Ester (**19**). According to the literature methods [11][12], reaction of **1** with EtBr gave **19** (82%). M.p. 182–184°. IR (KBr): 3444, 2925, 1724, 1035. ^{1}H-NMR (300 MHz): 0.76 (*s*, 3 H); 0.85 (*d*, *J* = 6.4, 3 H); 0.90 (*s*, 3 H); 0.93 (*d*, *J* = 4.6, 3 H); 1.04, 1.08 (2*s*, each 3 H); 1.21 (*t*, *J* = 7.1, 3 H); 2.23 (*d*, *J* = 11.2, 1 H); 3.40–3.46 (*m*, 2 H); 3.68 (*d*, *J* = 10.4, 1 H); 3.73–3.80 (*m*, 1 H); 4.05 (*q*, *J* = 7.1, 2 H); 5.25 (*t*, *J* = 3.4, 1 H). ESI-MS: 539 ($[M + Na]^+$).

(Ethoxycarbonyl)methyl 2α,3β,23-Trihydroxyurs-12-en-28-oate (**20**). According to the literature methods [11][12], reaction of **1** with $BrCH_2COOEt$ gave **20** (92%) as a white solid. M.p. 208–210°. IR (KBr): 3444, 2925, 1762, 1737, 1047. ^{1}H-NMR (300 MHz): 0.74 (*s*, 3 H); 0.86 (*d*, *J* = 6.4, 3 H); 0.91 (*s*, 3 H); 0.95 (*d*, *J* = 5.9, 3 H); 1.04, 1.09 (2*s*, each 3 H); 1.26 (*t*, *J* = 7.1, 3 H); 2.26 (*d*, *J* = 11.5, 1 H); 3.42 (*m*, 2 H); 3.69 (*d*, *J* = 10.4, 1 H); 3.75 (*m*, 1 H); 4.19 (*q*, *J* = 7.1, 2 H); 4.46 (*d*, *J* = 15.7, 1 H); 4.56 (*d*, *J* = 15.7, 1 H); 5.26 (*t*, *J* = 3.3, 1 H). ESI-MS: 597 ($[M + Na]^+$).

(Benzyloxycarbonyl)methyl 2α,3β,23-Trihydroxyurs-12-en-28-oate (**21**). According to the literature method [11][12], reaction of **1** with $BrCH_2COOBn$ gave **21** (89%). White solid. M.p. 215–217°. IR (KBr): 3407, 2923, 1762, 1733, 1049. ^{1}H-NMR (300 MHz): 0.72 (*s*, 3 H); 0.85 (*d*, *J* = 6.4, 3 H); 0.90 (*s*, 3 H); 0.94 (*d*, *J* = 5.0, 3 H); 1.03, 1.08 (2*s*, each 3 H); 2.25 (*d*, *J* = 11.1, 1 H); 3.42 (*m*, 2 H); 3.76 (*m*, 1 H); 3.69 (*d*, *J* = 10.5, 1 H); 4.52 (*d*, *J* = 15.8, 1 H); 4.63 (*d*, *J* = 15.8, 1 H); 5.17 (*s*, 2 H); 5.25 (*t*, *J* = 3.5, 1 H); 7.30–7.39 (*m*, 5 H). ESI-MS: 659 ($[M + Na]^+$).

Carboxymethyl 2α,3β,23-Trihydroxyurs-12-en-28-oate (**22**). According to the literature method [11][12], reaction of **20** with aq. NaOH gave **22** (59%). White solid. M.p. 252–254°. IR (KBr): 3414, 2932, 1611, 1047. ^{1}H-NMR (300 MHz, CD_3OD): 0.70, 0.78 (2*s*, each 3 H); 0.89 (*d*, *J* = 6.4, 3 H); 0.95, 1.04, 1.13 (3*s*, each 3 H); 2.31 (*d*, *J* = 10.9, 1 H); 3.26 (*d*, *J* = 11.0, 1 H); 3.35 (*d*, *J* = 9.6, 1 H); 3.50 (*d*, *J* = 11.0, 1 H); 3.65–3.74 (*m*, 1 H); 4.33 (*dd*, *J* = 15.0, 34.0, 2 H); 5.25 (*t*, *J* = 3.6, 1 H). ^{13}C-NMR (75 MHz, CD_3OD): 178.3; 171.1; 139.4; 126.9; 78.3; 69.6; 66.6; 61.3; 54.2; 49.6; 48.4; 48.3; 48.0; 44.0; 43.3; 40.8; 40.3; 40.2; 38.9; 37.6; 33.6; 31.7; 29.0; 25.3; 24.4; 24.2; 21.6; 19.1; 17.8; 17.7; 17.6; 13.9. ESI-MS: 545.3 ($[M - H]^-$).

Asiatic Acid Benzyl Ester (**23**). To a mixture of asiatic acid (**1**; 1.00 g, 2.05 mmol) and K_2CO_3 (0.57 g, 5.4 mmol) in DMF (8 ml) was added BnCl (0.25 ml, 2.18 mmol). The mixture was stirred at 60° for 2 h and then cooled, and ice H_2O (20 ml) was added. The resulting solid was filtered, washed with H_2O, and dried to yield **23** (1.13 g, 95%). White solid. M.p. 189–190°. IR (KBr): 3418, 2925, 1723, 1049. ^{1}H-NMR (300 MHz): 0.63, 0.84, 0.85, 0.94, 1.00, 1.07 (6*s*, each 3 H); 2.26 (*d*, *J* = 11.2, 1 H); 2.92 (br. *s*, 1 H); 3.38–3.42 (*m*, 3 H); 3.62 (*d*, *J* = 10.6, 1 H); 3.71–3.80 (*m*, 1 H); 4.97 (*d*, *J* = 12.5, 1 H); 5.09 (*d*, *J* = 12.5, 3 H); 5.23 (br. *s*, 1 H); 7.33 (*s*, 5 H). ^{13}C-NMR (75 MHz): 177.2; 138.2; 136.4; 128.4; 128.1; 127.9; 125.4; 80.4; 77.4; 70.4; 68.8; 66.0; 52.9; 49.1; 48.1; 47.5; 46.3; 42.5; 42.1; 39.6; 39.1; 38.8; 38.1; 36.6; 32.7; 30.7; 27.9; 24.2; 23.6; 23.3; 21.1; 18.3; 17.1; 17.0; 17.0; 12.8. ESI-MS: 577.5 ($[M - H]^-$).

Benzyl 2α-Hydroxy-3β,23-(isopropylidenedioxy)urs-12-en-28-oate (**24**). To a mixture of **23** (1.00 g, 1.73 mmol) and TsOH (35 mg, 0.20 mmol) in anh. DMF (20 ml), 2,2-dimethoxypropane (0.7 ml, 5.71 mmol) was added by injection. The mixture was stirred at r.t. for 7 h. After neutralization with 5% aq. NaOH to pH 7–8, the mixture was extracted with AcOEt and washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by CC (PE/AcOEt 8:1) to give **24** (0.92 g, 86%). White solid. M.p. 88–91°. IR (KBr): 3487, 2924, 1722, 1454. ^{1}H-NMR (300 MHz): 0.61, 0.86, 0.93, 1.00, 1.06, 1.08 (6*s*, each 3 H); 1.44 (*s*, 6 H); 2.27 (*d*, *J* = 11.3, 1 H); 3.28 (*d*, *J* = 8.7, 1 H); 3.43 (*d*, *J* = 10.6, 1 H); 3.49 (*d*, *J* = 10.6, 1 H); 3.73–3.81 (*m*, 1 H); 4.97 (*d*, *J* = 12.5, 1 H); 5.08 (*d*, *J* = 12.5, 1 H); 5.23 (*t*, *J* = 3.1, 1 H); 7.33 (*s*, 5 H). ^{13}C-NMR (75 MHz): 177.2; 138.0; 136.3; 128.4; 128.1; 127.9; 125.4; 99.5; 82.1; 76.6; 72.7; 65.9; 65.2; 52.8; 51.4; 48.0; 47.6; 46.5; 42.0; 39.6; 39.1; 38.8; 37.9; 36.9; 36.6; 32.4; 30.6; 29.7; 27.9; 24.2; 23.6; 23.1; 21.1; 19.4; 17.9; 17.5; 17.0; 16.9; 13.5. ESI-MS: 641.4 ($[M + Na]^+$).

Benzyl 2β,3β,23-Trihydroxyurs-12-en-28-oate (**26**). To a soln. of **24** (0.55 g, 0.89 mmol) in CH_2Cl_2 (7 ml) was added PCC (0.21 g) at 0°, and the mixture was stirred at r.t. for 12 h. The mixture was then filtered through *Celite*, and the filtrate was evaporated under vacuum to give ketone **25**, which was used for next reaction without further purification. Ketone **25** was dissolved in THF (15 ml), and $NaBH_4$ (41 mg, 1.07 mmol) was added at 0°. After stirring at r.t. for 7 h, 20% aq. HCl was added to the mixture, which was then extracted with AcOEt. After washing with sat. $NaHCO_3$ and brine, the org. layer was

dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by CC (PE/AcOEt 3:1) to give **26** (0.23 g, 44% for two steps). White solid. M.p. 117–119°. IR (KBr): 3395, 2923, 1725, 1107. ^{1}H-NMR (300 MHz): 0.64 (*s*, 3 H); 0.83–0.88 (*m*, 6 H); 0.94 (*s*, 3 H); 1.06 (*s*, 3 H); 1.11 (*s*, 3 H); 3.41 (*d*, *J* = 10.0, 1 H); 3.61 (*d*, *J* = 3.9, 1 H); 3.71 (*d*, *J* = 10.2, 1 H); 4.10–4.11 (*m*, 1 H); 4.97 (*d*, *J* = 12.4, 1 H); 5.10 (*d*, *J* = 12.5, 1 H); 5.24 (*t*, *J* = 3.0, 1 H); 7.34 (*s*, 5 H). ^{13}C-NMR (75 MHz): 177.3; 138.1; 136.4; 128.4; 128.2; 128.0; 125.8; 76.2; 73.3; 70.8; 66.0; 52.9; 49.80; 48.0; 43.7; 42.2; 41.1; 39.8; 39.1; 38.8; 36.6; 36.5; 32.8; 31.9; 30.7; 29.7; 29.4; 27.8; 24.2; 23.6; 23.3; 22.7; 21.2; 18.2; 17.0; 16.9; 14.1; 13.1. ESI-MS: 601.3 ($[M + Na]^+$).

Eriantic Acid B (**27**). A mixture of **26** (64 mg, 0.11 mmol) and 10% Pd/C (8 mg) in THF (2 ml) was stirred at r.t. under H_2 for 12 h. The mixture was filtered through *Celite*, and the insoluble substance was washed with THF. The filtrate was concentrated under reduced pressure to give **27** (51 mg, 92%). White solid. M.p. 259–263°. IR (KBr): 3462, 2920, 1678, 1048. ^{1}H-NMR (300 MHz, (D_5)Pyridine): 0.73 (*s*, 3 H); 0.78 (*d*, *J* = 6.3, 3 H); 0.91 (*s*, 3 H); 0.98 (*s*, 3 H); 1.14 (*s*, 3 H); 1.37 (*s*, 3 H); 2.61 (*d*, *J* = 10.9, 1 H); 3.70 (*d*, *J* = 10.4, 1 H); 4.15 (*d*, *J* = 10.4, 1 H); 4.25 (*d*, *J* = 4.1, 1 H); 4.31–4.32 (*m*, 1 H); 5.48 (*t*, *J* = 3.6, 1 H). ^{13}C-NMR (75 MHz, (D_5)Pyridine): 179.9; 139.3; 125.9; 73.2; 71.6; 68.0; 53.7; 48.5; 48.2; 48.1; 45.1; 42.8; 42.4; 40.2; 39.5; 39.5; 37.5; 37.2; 33.4; 31.2; 30.0; 28.7; 25.0; 24.0; 24.0; 21.4; 18.4; 17.6; 17.5; 14.6. ESI-MS: 487.4 ($[M - H]^-$).

Enzymatic Activity Assays. The inhibitory activity of the test compounds against rabbit muscle glycogen phosphorylase was monitored using microplate reader (*BIORAD*) based on the method described in [16]. 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 6 different concentrations (100, 50, 25, 12.5, 6.25, 3.125 μM) for IC_{50} determination. The enzyme (GPa) 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 1M HCl containing 10 mg/ml ammonium molybdate and 0.38 mg/ml malachite green, reactions were run at 22° for 25 min. 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. The results are listed in the *Table*. Values were expressed as mean ± standard error of the mean.

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