Naturally Occurring Pentacyclic Triterpenes as Inhibitors of Glycogen Phosphorylase: Synthesis, Structure−Activity Relationships, and X-ray Crystallographic Studies†

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Twenty-five naturally occurring pentacyclic triterpenes, 15 of which were synthesized in this study, were biologically evaluated as inhibitors of rabbit muscle glycogen phosphorylase a (GPa). From SAR studies, the presence of a sugar moiety in triterpene saponins resulted in a markedly decreased activity (**7**, **18**−**20**) or no activity (**21**, **22**). These saponins, however, might find their value as potential natural prodrugs which are much more water-soluble than their corresponding aglycones. To elucidate the mechanism of GP inhibition, we have determined the crystal structures of the GPb−asiatic acid and GPb−maslinic acid complexes. The X-ray analysis indicates that the inhibitors bind at the allosteric activator site, where the physiological activator AMP binds. Pentacyclic triterpenes represent a promising class of multiple-target antidiabetic agents that exert hypoglycemic effects, at least in part, through GP inhibition.

Introduction

Type 2 diabetes is associated with disorders in glucose metabolism by the liver and periphery, and an ideal antidiabetic agent should be capable of lowering blood glucose in both fed and fasted states. Control of the hepatic and peripheral glycogen metabolism is one of the key events through which insulin maintains blood glucose homeostasis.[1] Aiston et al. demonstrated that inactivation of glycogen phosphorylase (GP)[a] but not inhibition of glycogen kinase synthase-3 (GSK-3) could mimic insulin stimulation of hepatic glycogen synthesis and that a signaling pathway involving dephosphorylation of GPa leading to both activation and translocation of glycogen synthase (GS) was a critical component of the mechanism by which insulin stimulated hepatic glycogen synthesis.[2] In this regard, inactivation of GP would not only reduce glycogenolysis but would also stimulate glycogen synthesis. GP inhibition has been regarded as a therapeutic strategy for blood glucose control in diabetes,[3] and various studies have shown the efficacy of GP inhibitors at lowering blood glucose in animal models of diabetes[4,5] and in clinical trials.[6] To date, several structural classes of GP inhibitors have been described, and at least six potential regulatory binding sites have been identified in GP.[7]

Pentacyclic triterpenes are widely distributed throughout the plant kingdom, and a variety of biological properties have been ascribed to this class of compounds.[8] Pentacyclic triterpenes can be classified into three major types based on their structural skeleton (Figure 1): (a) oleanane type of triterpenes (e.g., **1**−**9**, **18**); (b) ursane type of triterpenes (e.g., **10**−**17**, **19**−**22**); (c) lupane type of triterpenes (e.g., **23**−**25**). The most well-known member of this family of compounds is probably oleanolic acid (**1**),[9] which has been clinically used as a hepatoprotective/antihepatitis drug in China for more than 20 years. Glycyrrhizic acid (**7**) has also been marketed as an antihepatitis drug in China and Japan. Moreover, several other pentacyclic triterpenes have entered clinical trials as anti-HIV or antitumor agents.[10−12] In a recent human test, the Japanese researchers proved for the first time that corosolic acid (**12**) exhibited a glucose-lowering effect on postchallenge plasma glucose levels in humans.[13] In previous communications,[14−17] we first reported that pentacyclic triterpenes (e.g., **1**, **3**, and **12**) represented a new class of inhibitors of glycogen phosphorylase. Herein, we disclose the detailed procedures for the semisynthesis of some natural pentacyclic triterpenes, including maslinic acid (**3**), 3-epimaslinic acid (**4**), augustic acid (**5**), corosolic acid (**12**), pygenic acid A (**13**), and 2-epicorosolic acid (**14**) (Figure 1). Moreover, SAR analysis of a series of naturally occurring pentacyclic triterpenes as GP inhibitors is discussed. The results of our X-ray crystallographic studies are also presented to disclose the molecular basis of the pentacyclic triterpenes binding to GP.

Results and Discussion

Chemistry. 11-Deoxyglycyrrhetinic acid (**9**) was prepared by deoxygenation of glycyrrhetinic acid (**8**) following the literature procedure.[18] Betulin (**23**),[19] betulinic acid (**24**),[20] and 23-hydroxybetulinic acid (**25**)[21] were obtained following the literature procedures. Oleanonic acid (**6**) and ursonic acid (**17**)

† The coordinates of the new structures have been deposited with the RCSB Protein Data Bank (http://www.rcsb.org/pdb) with the codes 2QN1 (GPb−asiatic acid complex) and 2QN2 (GPb−maslinic acid complex).

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[a] Abbreviations: GP, glycogen phosphorylase; 1,4-α-D-glucan, orthophosphate α-glucosyltransferase (EC 2.4.1.1); GPb, rabbit muscle glycogen phosphorylase b; GPa, rabbit muscle glycogen phosphorylase a; hlGPa, human liver glycogen phosphorylase a; PLP, pyridoxal 5′-phosphate; glucose, α-D-glucose; Glc-1-P, α-D-glucose 1-phosphate; Glc-6-P, D-glucose 6-phosphate; W1807, (−)-(*S*)-3-isopropyl 4-(2-chlorophenyl)-1,4-dihydro-1-ethyl-2-methylpyridine-3,5,6-tricarboxylate; FR258900, (2*R*,3*S*)-2,3-bis((*E*)-3-(4-hydroxyphenyl)acryloyloxy)pentanedioic acid; rms deviation, root-mean-squared deviation.

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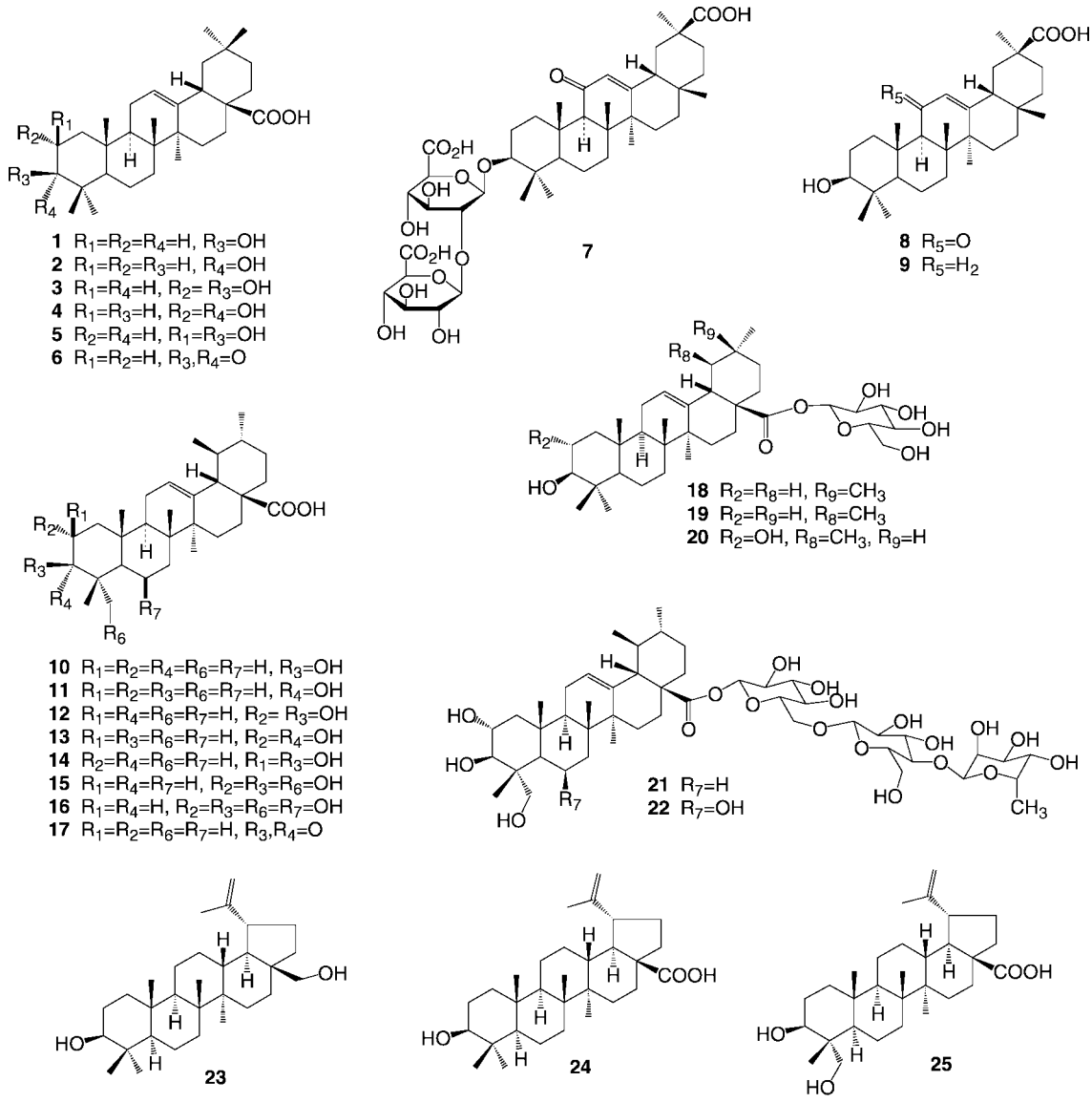


Figure 1. Chemical structures of some naturally occurring pentacyclic triterpenes **1**−**25**.

were easily prepared by oxidation of oleanolic acid (**1**) and ursolic acid (**10**) with PCC, respectively.

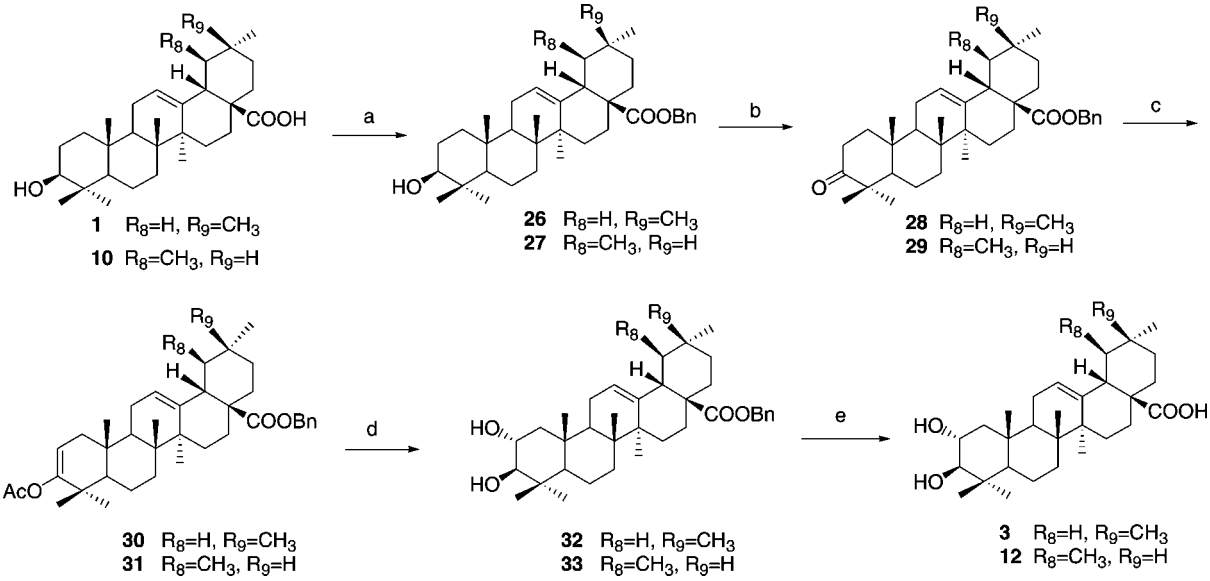
In an initial effort[14] for synthesis of **3** and **12**, a modified procedure based on Caglioti's methodology[22] was employed using hydroboration−oxidation of enol acetates **30** and **31** as the key step (Scheme 1). The disadvantages of the above hydroboration−oxidation methodology were that the overall yields were poor and that the cost (mainly due to the cost of borane reagents) was relatively high in terms of large-scale production, and thus, we explored another approach to **3** and **12** based on a ketone-hydroxylation strategy (Scheme 2).[16,17,23]

As depicted in Scheme 2, stereoselective hydroxylation of **28** with mCPBA catalyzed by H_2SO_4 in MeOH−CH_2Cl_2 at 0 °C gave 2α-hydroxyl ketone **34** (80%). Reduction of **34** with $NaBH_4$ in THF at 0 °C gave 2α,3β-diol **32** (77%) as the major product, together with 2α,3α-diol **36** (18%) as a minor product. On the other hand, Meerwein−Pondorf reduction[24] of **34** gave 2α,3α-diol **36** as the major product (50%) together with **32** as the minor product. Hydrogenolysis of **32** or **36** over palladium/carbon in THF furnished maslinic acid (**3**) or 3-epimaslinic acid (**4**) in quantitative yields, respectively. In the same fashion, corosolic acid (**12**) and pygenic acid A (**13**) were synthesized using α-hydroxyl ketone **35** as the key intermediate.

Stereoselective syntheses of augustic acid (**5**) and 2-epicorosolic acid (**14**) are summarized in Scheme 3. Treatment of ketone **28** with a large excess of *t*-BuOK under air at room temperature gave diketone **38**, which existed in the form of α, β-unsaturated ketone. Alternatively, treatment of α-hydroxyl ketone **34** with KOH in MeOH−DMF at room temperature also afforded **38** in good yield. Reduction of **38** with $NaBH_4$ gave 2β,3β-diol **40** in 61% yield. Interestingly, when **34** was treated with $NaHCO_3$ instead of KOH, it was found that a rearrangement product was formed to give 3β-hydroxyketone **42**. Reduction of **42** with $NaBH_4$ gave 2β,3β-diol **40**, which was identical to the product obtained by $NaBH_4$ reduction of **38**. Hydrogenolysis of **40** over palladium/carbon in THF furnished augustic acid (**5**). In the same fashion, 2-epicorosolic acid (**14**) was synthesized via $NaBH_4$ reduction of **39** (prepared by treatment of ketone **29** with a large excess of *t*-BuOK as described for preparation of **38**), followed by hydrogenolysis of the resulting 2β,3β-diol **41**.

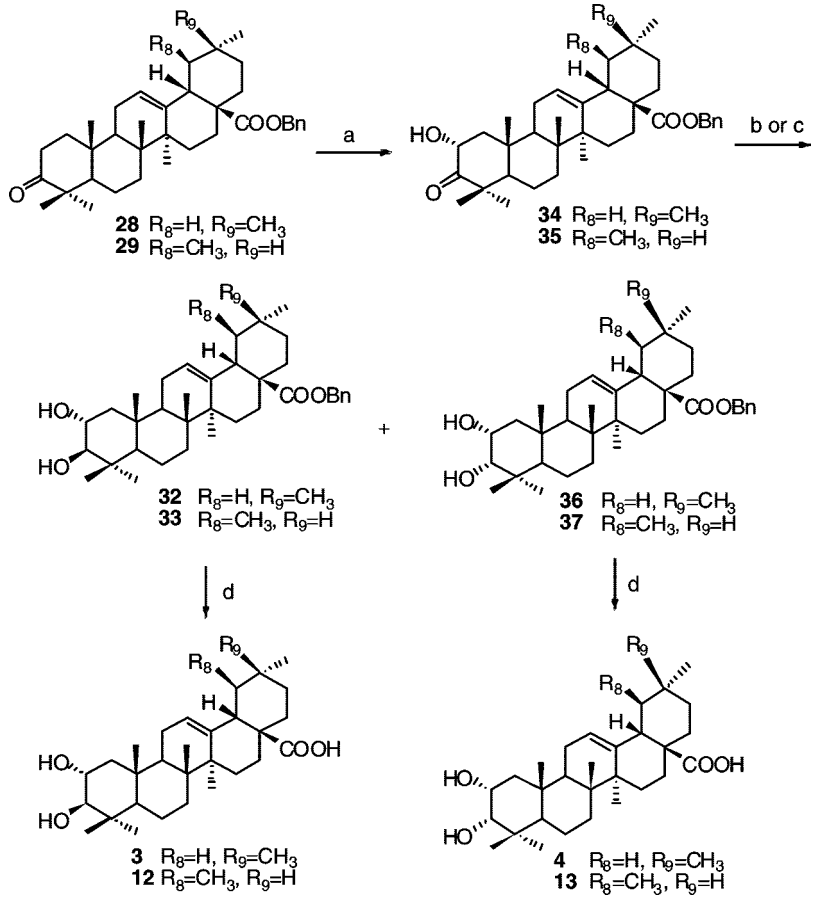
The synthetic route to 3-epioleanolic acid (**2**) and 3-epiursolic acid (**11**) is outlined in Scheme 4. Meerwein−Pondorf reduction of benzyloleanonic acid (**28**) afforded benzyl-3-epioleanolic acid (**43**) as the major product (57%). Hydrogenolysis of **43** over palladium/carbon in THF furnished 3-epioleanolic acid (**2**)

Scheme 1. Synthesis of Maslinic Acid (**3**) and Corosolic Acid (**12**) Based on a Hydroboration−Oxidation Strategy[a]



[a] Reagents and conditions: (a) K_2CO_3, BnCl, DMF, 55 °C; (b) PCC, CH_2Cl_2; (c) AcOCH=CH_2, concentrated H_2SO_4 (cat.), 100 °C; (d) (a) BH_3−THF; (b) H_2O_2, NaOH; (e) H_2, Pd/C, THF, room temp.

Scheme 2. Synthesis of Maslinic Acid (**3**), 3-Epimaslinic Acid (**4**), Corosolic Acid (**12**), and Pygenic Acid A (**13**) Based on a Ketone-Hydroxylation Strategy[a]

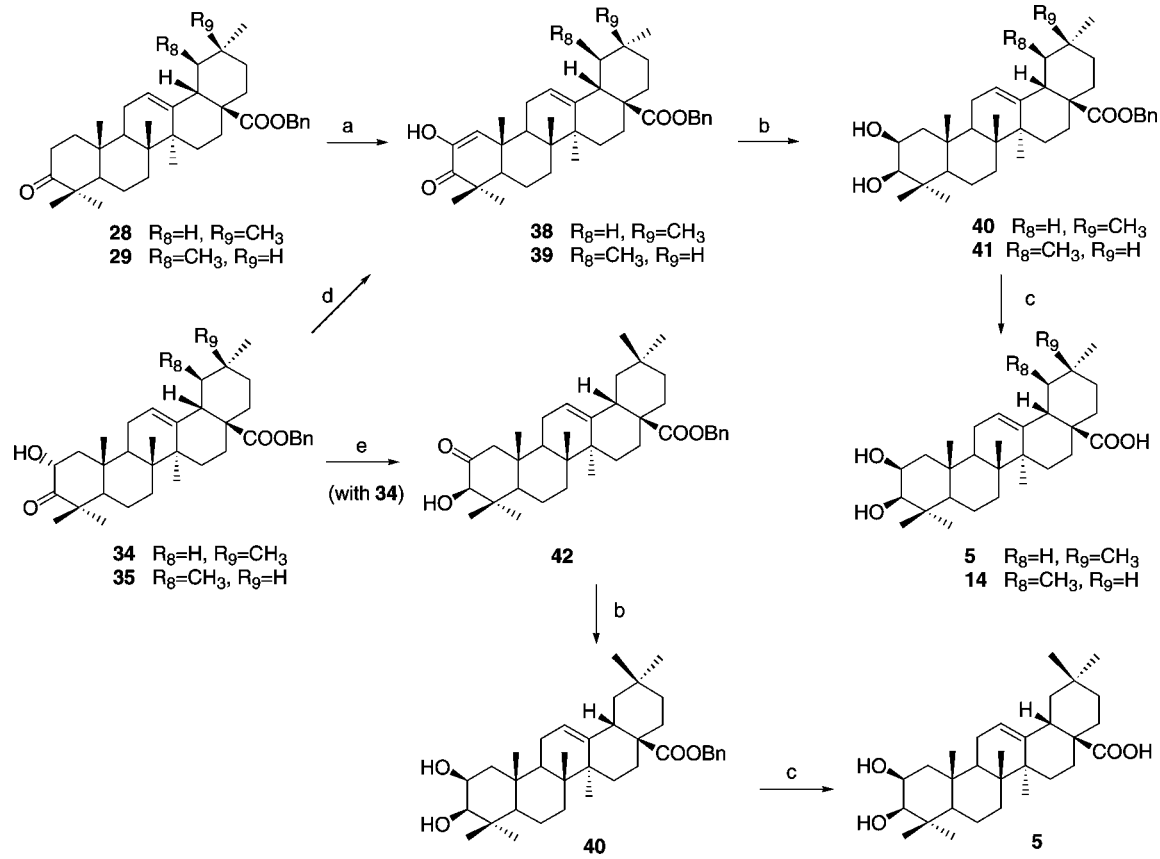


[a] Reagents and conditions: (a) mCPBA, concentrated H_2SO_4 (cat.), MeOH−CH_2Cl_2, 0 °C; (b) $NaBH_4$, THF, 0 °C; (c) Al(O-*i*-Pr)$_3$, *i*-PrOH, reflux; (d) H_2, Pd/C, THF, room temp.

(90%). In the same manner, 3-epiursolic acid (**11**) was prepared via Meerwein−Pondorf reduction of benzylursonic acid (**29**), followed by hydrogenolysis of the resulting benzyl ester **44**.

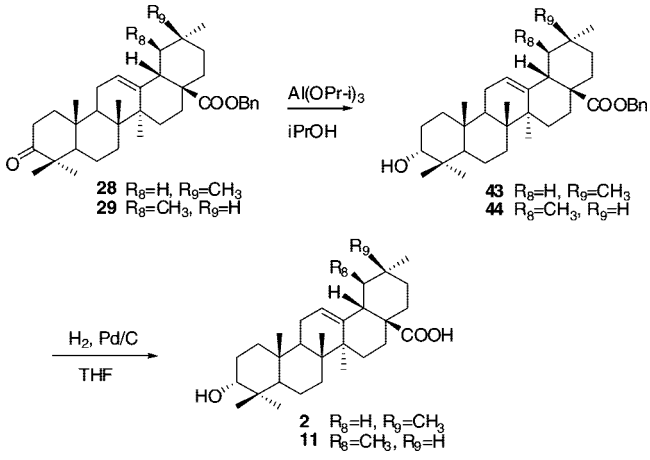
For the preparation of triterpene acid β-D-glucopyranosyl ester compounds, direct esterification of **1** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucosyl bromide (**45**)[25] afforded oleanolic acid 2,3,4,6-

Scheme 3. Synthesis of Augustic Acid (**5**) and 2-Epicorosolic Acid (**14**)[a]



[a] Reagents and conditions: (a) *t*-BuOK, *t*-BuOH, air, room temp; (b) $NaBH_4$, THF/MeOH, 0 °C; (c) H_2, Pd/C, THF, room temp; (d) KOH, MeOH-DMF, room temp; (e) $NaHCO_3$, THF, room temp.

Scheme 4. Synthesis of 3-Epioleanolic Acid (**2**) and 3-Epiursolic Acid (**11**)



tetra-*O*-acetyl-β-D-glucopyranosyl ester (**46**), which was hydrolyzed with NaOH in MeOH/THF to give oleanolic acid β-D-glucopyranosyl ester **18** (85% for two steps) (Scheme 5). In the same fashion, ursolic acid β-D-glucopyranosyl ester **19** and corosolic acid β-D-glucopyranosyl ester **20** were prepared.

Enzyme Assay and SAR Analysis. . Twenty-five naturally occurring pentacyclic triterpenes were biologically evaluated for their inhibitory activity against rabbit muscle GPa (RMGPa). The activity of RMGPa was measured through detection of the release of phosphate from glucose 1-phosphate in the direction of glycogen synthesis.[4] The bioassay results are summarized in Table 1. Most of the tested triterpenes exhibited moderate inhibitory activity against GPa. It seems that the diversity of structural skeleton of pentacyclic triterpenes does not have a significant impact on GP inhibition, since all the three types of tested triterpenes, including oleanane triterpenes (e.g., **1**−**9**, **18**), ursane triterpenes (e.g., **10**−**15**, **17**, **19**, **20**), and lupane triterpenes (e.g., **23**−**25**), exhibited inhibitory activity against GPa.

The amount and positioning of hydroxyl groups of triterpenes seemed to have some impact on potency. Both 2α-hydroxyoleanolic acid (**3**, $IC_{50} = 28\ \mu M$) and 2β-hydroxyoleanolic aicd (**5**, $IC_{50} = 34\ \mu M$) were less active than the parent compound **1** ($IC_{50} = 14\ \mu M$), suggesting that the introduction of hydroxyl group at C-2 resulted in a loss of potency. The same tendency was also observed with ursolic acid (**10**, $IC_{50} = 9\ \mu M$), since both 2α-hydroxyursolic acid (**12**, $IC_{50} = 20\ \mu M$) and 2β-hydroxyursolic aicd (**14**, $IC_{50} = 116\ \mu M$) were less potent than **10**. On the other hand, in two cases examined (**12** vs **15**; **24** vs **25**), incorporation of a hydroxyl group at C-23 resulted in slight increases in potency. Surprisingly, as in the case of madecassic acid (**16**), incorporation of hydroxyl group at C-6 resulted in a complete loss of potency.

The effects of configuration of hydroxyl groups on GP inhibition were further examined. In contrast to **3** or **12** having 2α,3β configuration, the potency decreased when both 2-hydroxyl and 3-hydroxyl groups were in the same side of A-ring (e.g., **3** vs **4** and **5**; **12** vs **13** and **14**). Conversion of 3β hydroxyl group of oleanolic acid (**1**) or ursolic acid (**10**) to 3α hydroxyl group resulted in a slight loss of potency (**1** vs **2**; **10** vs **11**). Conversion of 3β hydroxyl group of **1** or **10** to 3-carbonyl group did not result in a significant loss of potency (**1** vs **6**; **10** vs **17**).

Scheme 5. Synthesis of Triterpene Acid β-D-Glucopyranosyl Esters **18**−**20**

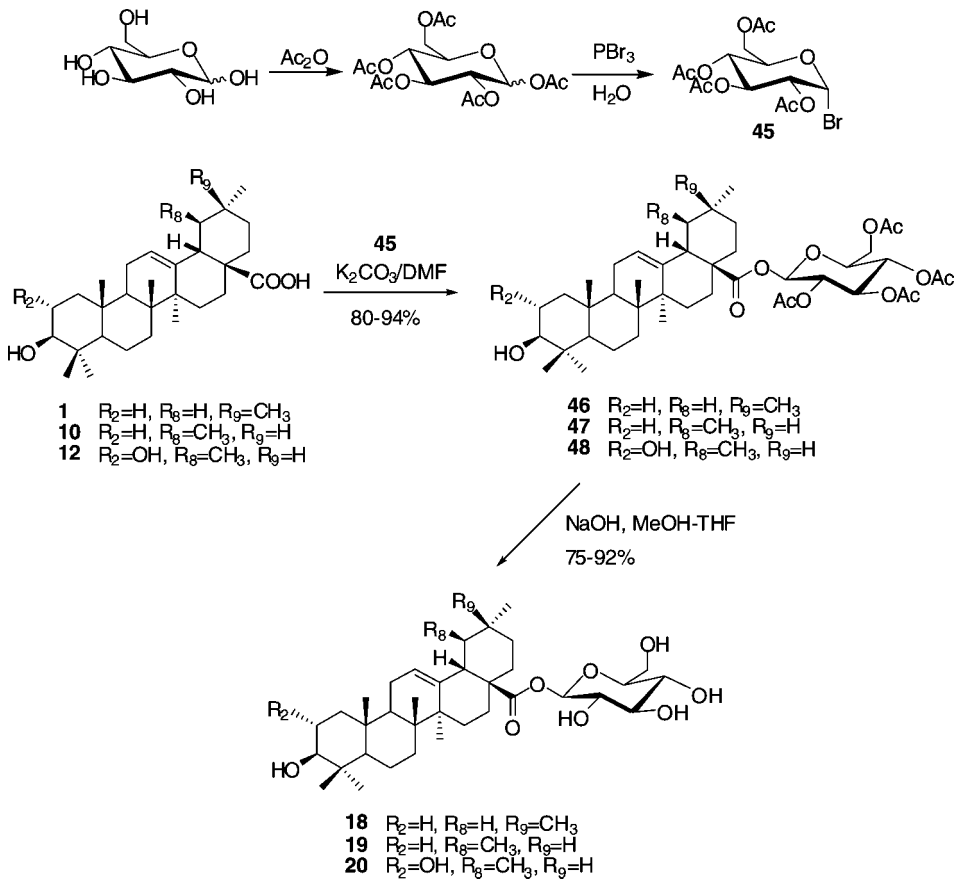


Table 1. IC_{50} Values (μM) for the Inhibition of Rabbit Muscle GPa

compd	GPa IC_{50}[a]	compd	GPa IC_{50}[a]
1	14	**14**	116
2	21	**15**	17
3	28	**16**	na[b]
4	144	**17**	57
5	34	**18**	293
6	18	**19**	97
7	822	**20**	106
8	66	**21**	na[b]
9	82	**22**	na[b]
10	9	**23**	17
11	19	**24**	43
12	20	**25**	16
13	213	caffeine	114

[a] Values are the mean of three experiments. [b] na = no activity.

The presence of a sugar moiety in triterpene saponins resulted in a markedly decreased activity (**7**, **18**−**20**) or no activity (**21**, **22**). Glycyrrhizic acid (**7**, IC_{50} = 822 μM) was 12-fold less potent than its aglycone **8** (IC_{50} = 66 μM). 3β-Hydroxyolean-12-en-28-oic acid β-D-glucopyranosyl ester (**18**, IC_{50} = 293 μM) was 20-fold less potent than its aglycone **1** (IC_{50} = 14 μM). The potency difference between **15** (IC_{50} = 17 μM) and **21** (a saponin derived from **15**) was quite intriguing because of the fact that while **15** was relatively quite active, **21** was inactive at 2000 μM. Despite of the low potency or no activity of the triterpene saponins, these saponins might find their value as potential natural prodrugs which are much more water-soluble than the corresponding aglycones. For example, asiaticoside (**21**) was reported to undergo degradation in human bodies to release the biologically active asiatic acid (**15**).[26]

Although some allosteric inhibitors such as phthalic acids and dihydropyridine diacid were reported to have a higher potency for the liver isoform than the muscle isoform,[27] we did not observe a significant selectivity between liver GP inhibition and muscle GP inhibition by the triterpenes.[14] In our view, however, GP subtype selectivity is not an essential issue here because of the fact that pentacyclic triterpenes are mild GP inhibitors, and thus, mechanism-related toxicity is avoidable.[28] On the other hand, it has been suggested that since the liver is the major site of exposure to drugs administered orally, it should be possible to achieve an appropriate drug dosage that targets predominantly hepatic GP.[29] In fact, not only hepatic glycogen metabolism (mainly modulated by liver GP) contributes in glucose metabolism but also peripheral glycogen metabolism (mainly modulated by muscle GP and brain GP) plays an important role in maintaining blood glucose homeostasis, especially under fed-state conditions.

X-ray Crystallography. In order to elucidate the structural basis of GP inhibition by pentacyclic triterpenes, we have determined the crystal structures of GPb in complex with both asiatic acid (**15**) and maslinic acid (**3**) (Figure 2). A summary of crystallographic data collection and refinement statistics for the GPb−asiatic acid and GPb−maslinic acid complex structures is given in Supporting Information (SI, Tables S2 and S3). The $2F_o - F_c$ Fourier electron density maps indicated that both **15** and **3** were bound at the allosteric site (Figure 3). Portions of the $2F_o - F_c$ electron density maps for asiatic acid and maslinic acid molecules are shown in Figure 4.

Binding of Asiatic Acid (15). Asiatic acid and maslinic acid both contain five fused six-membered rings (A−E) in a steroid-like conformation, with ring E being cis fused to the 17−18 bond of ring D and containing an axial carboxylate substituent in position 17 (Figure 2). Asiatic acid (**15**) binds at the interface of the dimer forming the allosteric (AMP) site some 30 Å from

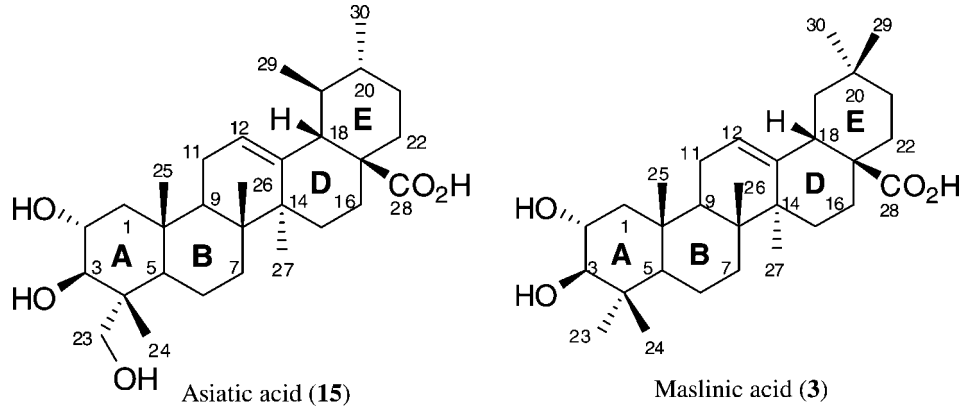


Figure 2. Chemical structures of compounds **15** and **3** showing the numbering system used.

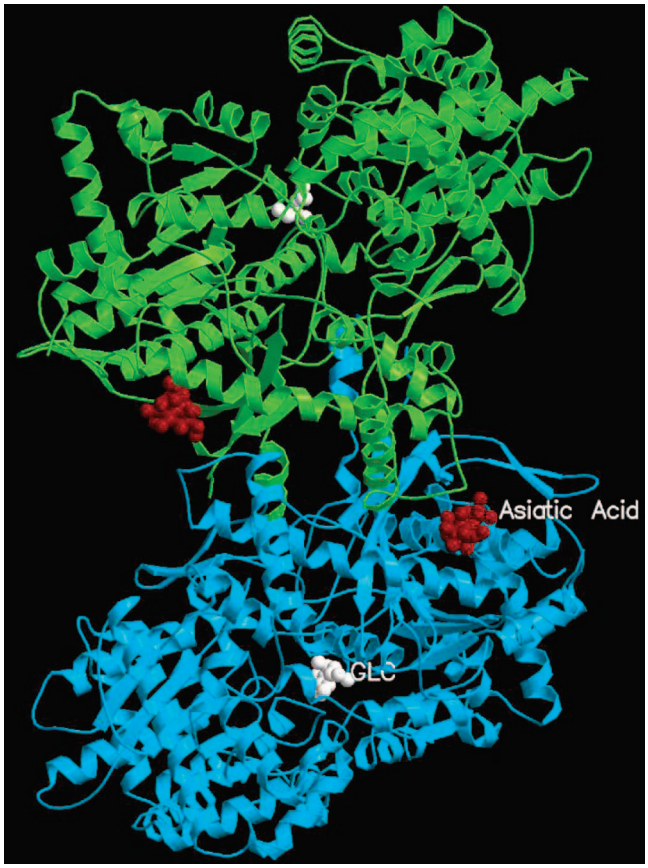


Figure 3. Schematic diagram of the T-state GPb dimeric molecule, for residues 10−837, viewed down the molecular dyad, showing the positions for the catalytic and the allosteric binding sites. The catalytic site (marked by glucose, shown in white), which includes the essential cofactor PLP (not shown), is buried at the center of the subunit accessible to the bulk solvent through a 15 Å long channel. The allosteric site, which binds the activator AMP and the allosteric inhibitor asiatic acid (shown in red), is situated at the subunit−subunit interface some 30 Å from the catalytic site.

the catalytic site.[30,31] On binding at the allosteric site, **15** makes a total of six hydrogen bonds to the protein, involving all potential hydrogen bonding groups except the hydroxyl group O2, and 48 van der Waals interactions (8 polar/polar, 26 polar/nonpolar, and 14 nonpolar/nonpolar interactions) (SI, Tables S4 and S6). There are 13 contacts to residues Asp42′, Asn44′, and Val45′ from the symmetry related subunit. The hydrogen bonding interactions formed between the ligand and the protein are illustrated in Figure 5a. Specifically, hydroxyl O3 and hydroxyl O23 make hydrogen bonding interactions with Gln72 NE2, Asp42′ OD1, and Asp42′ OD2. Carboxylate oxygens O28 and O29 exploit the allosteric effector phosphate-recognition subsite by forming hydrogen bonding interactions with Arg310 NE and NH1. The phosphate-recognition subsite recognizes the phosphate of a variety of phosphorylated compounds, such as AMP, ATP, Glc-6-P, and the carboxylate of the nonphysiological inhibitors W1807 and FR258900.[32–34] The inhibitor becomes buried on forming the complex with GPb. On the protein surface, a total of 367 Å^2 (309 Å^2 in one subunit and 58 Å^2 in the symmetry related subunit) solvent accessible surface area becomes inaccessible on binding of the ligand. The total buried surface area (protein plus ligand) for the GPb−ligand complex is 854 Å^2.

Comparison with the Native T-State Structure. Superposition of the activation locus, residues 24−78, 94−111, and 118−125 from both subunits[35] of the structure of the native T-state GPb with the activation locus of structure of the GPb−**15** complex gave an rms deviation of 0.34 Å for Cα atoms, indicating that the two structures have very similar overall conformations within the limits of the 2.4 Å resolution data. The major conformational changes on binding of **15** to rmGPb occur in the vicinity of the allosteric site. Shifts for Cα atoms are observed for residues 41′−48′ (between 0.4 and 0.7 Å) and residues 71−78 (between 0.4 and 0.7 Å) that affect the subunit−subunit interface in the region between the cap′ and the α2 helix. The major shifts for the side chain atoms are observed for residue 72 by about 0.9−1.9 Å and for residue 196 by about 0.6−1.7 Å to optimize their hydrogen bonding and van der Waals interactions with ligand. Similar shifts were observed previously on binding of Glc-6-P,[36] W1807,[32,33,37] phenoxy-phthalates,[38] acylureas,[39,40] and FR258900[34] to the allosteric site. A comparison of the two structures in the vicinity of the allosteric site is shown in Figure 6a.

Comparison with R-State GPa. Comparison of the GPb−**15** complex with the R-state GPa[30] suggests that the inhibitor is likely to have lower affinity for the R-state conformation (Figure 6b). Superposition of the activation loci of the structure of the R-state GPa (subunit A) with the activation locus of structure of the GPb−**15** complex gave an rms deviation of 1.18 Å for Cα atoms. If **15** were to be superimposed into the R-state GPa, that would result in clashes with Asp42′ atoms OD1, OD2, CG, and CB, Asn44′ atom CG, and Asn72 atoms CA, CB, and NE2. Hence, it would be anticipated that the affinity of **15** for the R state would be less than that of the T state, but there is no direct experimental evidence.

Comparison with AMP. A structural comparison of the R-state hlGPa−AMP complex[41] with T-state GPb−**15** com-

plex is shown in Figure 7. The adenine and ribose partially overlap with A and B rings of the steroid-like structure, and the phosphate group partially overlaps with the D ring of the cis-fused decalin moiety, while the carboxylate and the phosphate groups are positioned very close to each other in the phosphate-recognition subsite (P/C28 and O1P/O29 separations are respectively 2.2 and 1.8 Å).

Comparison with Other Types of Allosteric Site Inhibitors. Compounds W1807, Novo4j,[38] and AVE#21[39] (SI, Figure S1) are known AMP-site allosteric inhibitors for GP. Comparisons of GPb−**15** with GPb−W1807 (SI, Figure S2), GPb−Novo4j (SI, Figure S3), and hlGPa−AVE#21 (SI, Figure S4) were performed. The detailed comparisons are presented in the Supporting Information. The results show that the allosteric site displays broad specificity. For example, AVE#21 does not exploit the allosteric phosphate-recognition site (composed of Arg309, Arg310, and the more distant Arg242), which recognizes phosphorylated compounds (e.g., AMP, ATP and Glc-6-P) and the nonphysiological inhibitors such as W1807 and Novo4j. Upon binding of the inhibitors W1807, Novo4j and AVE#21, there are conformational changes in the vicinity of the allosteric site that affect the plasticity of the site and appear important in stabilizing an inactive T-state conformation. These results show the ability of GP to distinguish among various specific potent inhibitors, and this appears to originate in protein conformational flexibility in the region of the allosteric site.[42] Understanding such protein flexibility will be important in the design of more specific inhibitors. The use of rabbit GP is a good surrogate for human GP given the generally high conservation of amino acid sequence for glycolytic enzymes between different species. With the exception of Cys318 (which is serine in the human liver isoenzyme), all AMP (or Glc-6-P) binding residues are identical in the rabbit muscle and human liver GPs.[43]

Binding of Maslinic Acid (3). The position and conformation of **3**, bound at the allosteric site, are also similar to those observed for **15** (Figures 5 and 8). The electron density observed for maslinic acid exhibits many common features with the corresponding density of asiatic acid. Interestingly, a similar lack of density for C21, C22, and the methyl C24 is also observed. As expected, the main difference between the two structures is the lack of density for O23 that is absent in maslinic acid, although no density is observed for the existing C23 either. The pattern of polar and nonpolar interactions between the inhibitor and the enzyme is maintained when comparing the complex structures of derivatives **15** and **3** except that there is no a hydrogen bonding interaction between O23 and Asp42′ OD1 in the **3** complex (SI, Tables S5 and S7).

Conclusions

In this study, 25 naturally occurring pentacyclic tritepenes, 15 of which were efficiently synthesized starting from commercial products, were biologically evaluated as inhibitors of glycogen phosphorylase. The bioassay results show that most of the tested triterpenes exhibit moderate inhibitory activity against GPa. X-ray analyses of the GPb−asiatic acid and GPb−maslinic acid complexes show that compounds **15** and **3**, on binding to GPb, promote conformational changes that stabilize the inactive T-state quaternary conformation of the enzyme, form direct hydrogen bonds with Gln72, Arg310, Asp42′, and make nonpolar interactions with the side chains of Tyr75, Phe196 and Val45′ (from the symmetry related subunit). These interactions appear important in stabilizing the inactive T state and may explain why **15** and **3** are micromolar inhibitors of the enzyme.

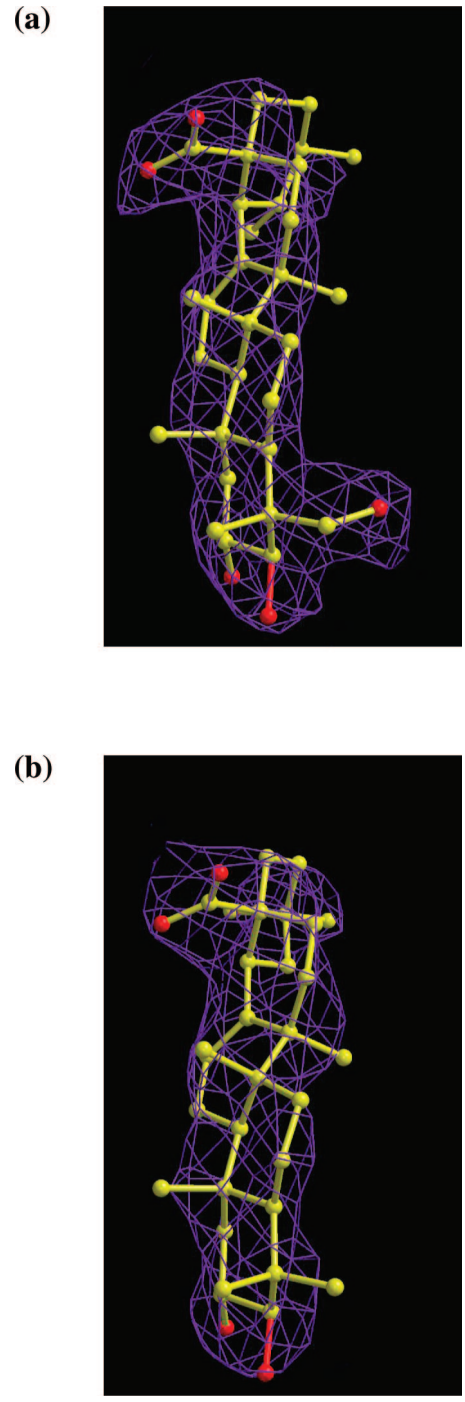


Figure 4. Diagrams of the $2F_o - F_c$ electron density map, contoured at 1σ, for the bound compounds asiatic acid (a) and maslinic acid (b) at the allosteric site of GPb. Electron density maps were calculated using the standard protocol as implements in REFMAC[65] before incorporating ligand molecules.

Taken together, pentacyclic triterpenes may exert hypoglycemic effects,[9,13–15,44–46] at least in part, through GP inhibition. Other mechanisms[47–49] may also account for the hypoglycemic activity of pentacyclic triterpenes. Further studies are needed to address the detailed molecular mechanisms and to biologically evaluate natural and synthetic pentacyclic triterpenes as promising antidiabetic agents with preventive effects against diabetic complications such as ischemic heart and brain diseases.

Experimental Section

Chemistry. Materials and General Methods. All commercially available solvents and reagents were used without further purifica-

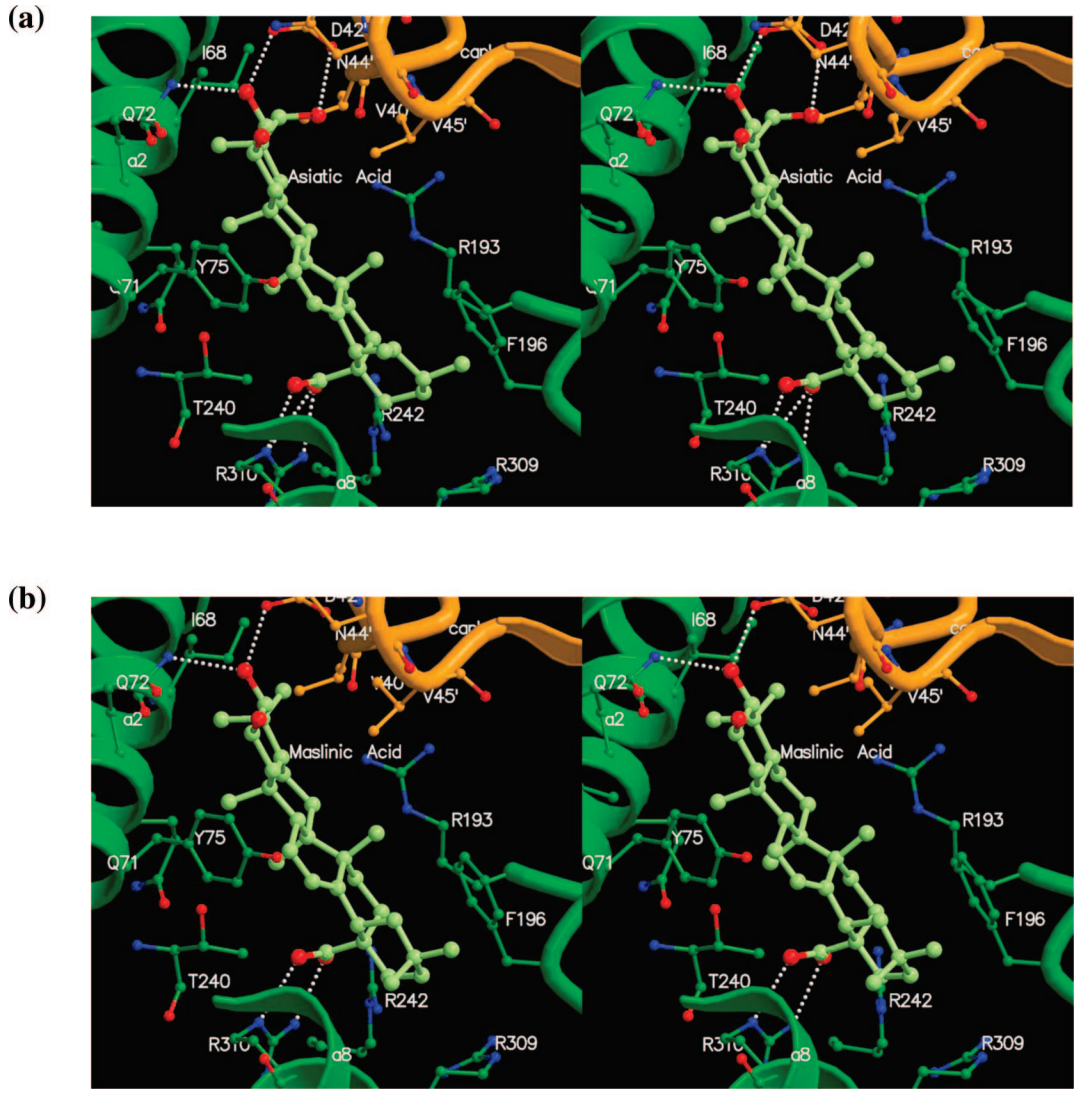


Figure 5. (a) Interactions between asiatic acid and GPb at the allosteric site. (b) Interactions between maslinic acid and GPb at the allosteric site. Residues from subunit 1 are shown in green, and residues from subunit 2 are shown in orange.

tion. The purity of commercial triterpene products (**1**, **7**, **8**, **10**, **15**, **16**, **21**, **22**) used in this study was at least 95%. Melting points of compounds were measured on a RY-1 melting point apparatus. Column chromatography was carried out on silica gel (200−300 mesh, Qindao Ocean Chemical Company, China). IR spectra were recorded on Shimadzu FTIR-8400S spectrometer. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AV-300 or AV-500 spectrometer. Chemical shifts are reported as δ values from an internal tetramethylsilane standard. Mass spectral data were obtained on Agilent 1100 LC/DAD/MSD or Q-Tof Micro MS/MS spectrometer. Elemental analysis was carried out on Vario EL III instrument (Elementar, Germany).

3-Oxoolean-12-en-28-oic Acid (Oleanonic Acid, 6). To a mixture of oleanolic acid (0.46 g, 1.01 mmol) and pyridine (10 mL) was added PCC (0.52 g, 2.42 mmol) at room temperature. After the mixture was stirred at room temperature for 48 h, EtOAc was added to the reaction mixture. The resulting mixture was washed with 1 N HCl and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by column chromatography [petroleum ether−EtOAc (6:1)] to give **6** as a white solid (0.35 g, 76%). Mp: 199−201 °C. IR (KBr, cm^{-1}): 3449, 2945, 2866, 1701, 1460, 1385, 1363, 1267, 1161, 1009, 642. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.81, 0.91, 0.93, 1.03, 1.05, 1.09, 1.15 (each 3 H, *s*), 2.39 and 2.53 (each 1 H, m), 2.82 (1 H, dd, J = 4.1, 13.8 Hz), 5.30 (1 H, brs). ESI-MS *m*/*z*: 453.3 [M − H].

3-Oxours-12-en-28-oic Acid (Ursonic Aicd, 17). Following the procedure for preparation of **6**, ursolic acid was oxidized by PCC to give **17** as a white solid (66%). Mp: 265−267 °C. IR (KBr, cm^{-1}): 2975, 2940, 2871, 1691, 1459, 1385, 1316, 1276, 1258, 1235, 1111, 747. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.85, 1.04, 1.06, 1.095, 1.104 (each 3 H, s), 0.88 (3 H, d, J = 6.4 Hz), 0.95 (3 H, d, J = 6.2 Hz), 2.23 (1 H, d, J = 11.2 Hz), 2.41 and 2.54 (each 1 H, m), 5.28 (1 H, t, J = 3.5 Hz). ESI-MS *m*/*z*: 477.3 [M + Na]$^+$.

Benzyl-3β-hydroxyolean-12-en-28-oic Acid (26). A mixture of oleanolic acid (100 g, 0.22 mol) and K_2CO_3 (61 g, 0.44 mol) in DMF (800 mL) was heated to 55 °C, and then benzyl chloride (33 mL, 0.29 mol) was added dropwise over a period of 20 min. After being stirred at 55 °C for 4 h, the reaction mixture was cooled to room temperature, filtered, and washed with DMF (50 mL × 3). The filtrate was poured into ice−water to give a white precipitate. The precipitate was filtered, washed with water, and dried to give **7** (115 g, 96%), which was almost a pure product, and was used for the next reaction without further purification. Pure product was obtained by recrystallization of the crude product from EtOH. Mp: 190−192 °C. IR (KBr, cm^{-1}): 3415, 2941, 1695, 1253, 1170. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.62, 0.78, 0.88, 0.90, 0.92, 0.98, 1.13 (each 3 H, s), 2.91 (1 H, dd, J = 4.4, 13.9 Hz), 3.20 (1 H, dd, J = 4.5, 11.2 Hz), 5.04 and 5.09 (each 1 H, d, J = 12.5 Hz), 5.28 (1 H, t, J = 3.6 Hz), 7.28−7.36 (5 H, m). ESI-MS *m*/*z*: 569 [M + Na]$^+$.

Benzyl-3β-hydroxyurs-12-en-28-oic Acid (27). Following the procedure for preparation of **26**, benzylation of ursolic acid gave **27** as a white solid. Mp: 180−182 °C. IR (KBr, cm^{-1}): 2924, 2870, 1721, 1455, 1377, 1267, 1224, 1138, 1110, 1029, 996, 749, 696. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.67, 0.81, 0.95, 1.01, 1.10 (each

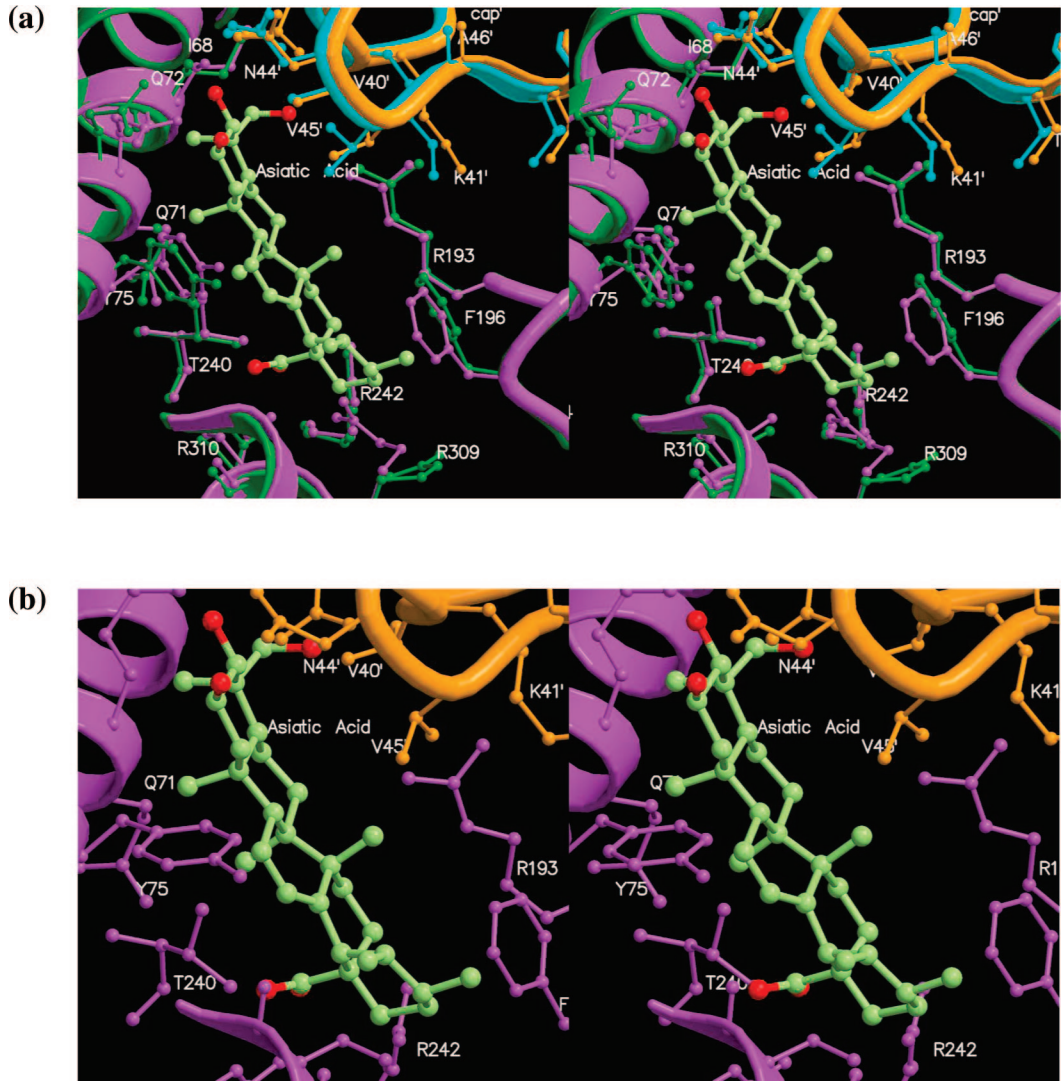


Figure 6. (a) Comparison between GPb−asiatic acid complex (shown in green-subunit 1 and orange-subunit 2) and T-state GPb (shown in mauve-subunit 1 and cyan-subunit 2). (b) GPa complex in the R state (shown in mauve-subunit 1 and orange-subunit 2) viewed in an orientation similar to that of the GPb−asiatic acid complex. The position of asiatic acid is superimposed.

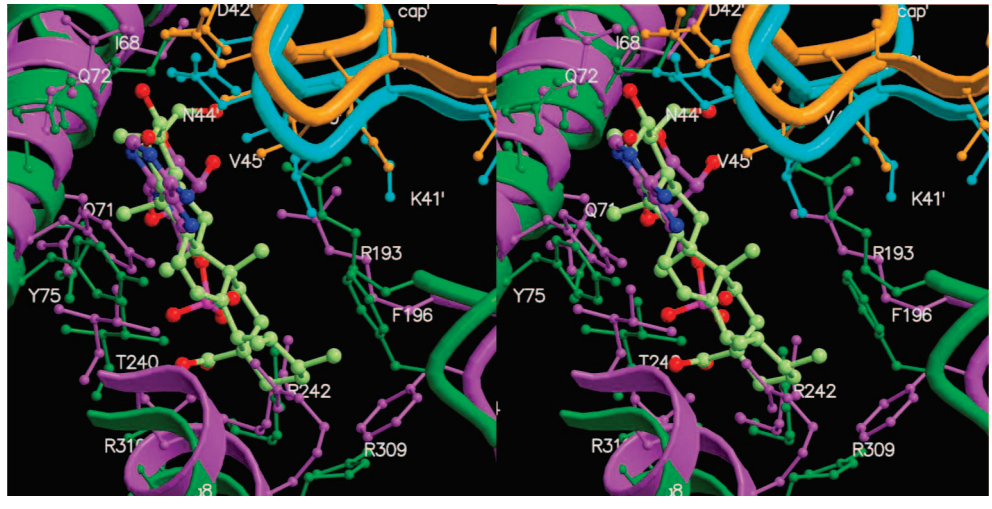


Figure 7. Comparison between GPb−asiatic acid complex (shown in green-subunit 1 and orange-subunit 2) and R-state hlGPa−AMP (shown in mauve-subunit 1 and cyan-subunit 2).

3 H, s), 0.89 (3 H, d, $J = 6.4$ Hz), 0.97 (3 H, d, $J = 5.4$ Hz), 2.31 (1 H, d, $J = 11.3$ Hz), 3.23 (1 H, dd, $J = 4.6$, 10.4 Hz), 5.03 and 5.11 (each 1 H, d, $J = 12.5$ Hz), 5.26 (1 H, *t*, $J = 3.5$ Hz), 7.35 (5 H, m). ESI-MS m/*z*: 547.1 $[M + H]^+$.

Benzyl-3-oxoolean-12-en-28-oic Acid (28). To a solution of **26** (111 g, 0.2 mol) in CH_2Cl_2 (588 mL) was added PCC (60.4 g, 0.28 mol) at 0 °C. After being stirred at 0 °C for 4 h, the reaction mixture was warmed to room temperature and stirred overnight.

Figure 8. Comparison of the position of the asiatic acid (red) to that of the maslinic acid (orange) at the allosteric site after superimposing the GPb−maslinic acid complex structure onto the GPb−asiatic acid complex structure.

The mixture was filtered through a pad of Celite and washed with CH_2Cl_2 (50 mL × 8). The filtrate was concentrated in vacuo to give a brown solid. Crystallization from ethanol gave **28** as a white solid (96.8 g, 89%). Mp: 160−162 °C. IR (KBr, cm^{-1}): 3417, 2935, 1724, 1460. ^1H NMR ($CDCl_3$, 500 MHz): δ 0.62, 0.90, 0.92, 1.01, 1.04, 1.08, 1.13 (each 3 H, s), 2.36−2.51 (2 H, m), 2.92 (1 H, dd, J = 4.3, 13.8 Hz), 5.07 and 5.08 (each 1 H, d, J = 12.5 Hz), 5.30 (1 H, t, J = 3.6 Hz), 7.25−7.36 (5 H, *m*). ESI-MS *m/z*: 567 $[M + Na]^+$. HRMS calcd for $C_{37}H_{51}O_3$ [M − H]: 543.3838. Found: 543.3865.

Benzyl-3-oxours-12-en-28-oic Acid (29). Following the procedure for preparation of **28**, ursolic acid benzyl ester **27** was oxidized with PCC to give **29** as a white solid. Mp: 156−157 °C. IR (KBr, cm^{-1}): 2924, 2869, 1722, 1704, 1454, 1383, 1268, 1225, 1140, 1110, 1030, 749, 697. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.71, 1.05, 1.07 (each 3 H, s), 0.89 (3 H, d, J = 6.4 Hz), 0.97 (3 H, d, J = 5.6 Hz), 1.11 (6 H, s), 2.29 (1 H, d, J = 11.3 Hz), 2.41 and 2.54 (each 1 H, m), 5.04 and 5.12 (each 1 H, d, J = 12.5 Hz), 5.28 (1 H, t, J = 3.5 Hz), 7.37 (5 H, m). ESI-MS *m/z*: 567 $[M + Na]^+$. HRMS Calcd for $C_{37}H_{51}O_3$ [M − H]: 543.3838. Found: 543.3851.

Benzyl-2α-hydroxy-3-oxoolean-12-en-28-oic Acid (34). To a mixture of **28** (10.88 g, 0.02 mol), CH_2Cl_2 (100 mL), methanol (200 mL), and concentrated sulfuric acid (0.1 mL) was added m-CPBA (85%, 6.2 g, 0.03 mol) at 0 °C. The reaction mixture was stirred in darkness at room temperature for 21 h. To the reaction mixture was added an aqueous solution containing $NaHSO_3$ (1.2 g, 0.012 mol), and the resulting mixture was stirred for 30 min. After most organic solvents were removed by evaporation in vacuo at 35 °C, the residue was acidified with 1 N HCl (100 mL) and extracted with EtOAc. The combined extract was washed with saturated $NaHCO_3$ (125 mL × 3) and brine (125 mL × 3), dried over Na_2SO_4, filtered, and concentrated in vacuo to afford a yellow oil, which was purified by column chromatography [petroleum ether−EtOAc (30:1)] to give **34** as a white solid (8.9 g, 80%). Mp: 180−182 °C. ^1H NMR ($CDCl_3$, 300 MHz):δ 0.59, 0.83, 0.89, 1.09, 1.10, 1.15, 1.23 (each, 3 H, s), 2.38 (1 H, dd, J = 6.6, 12.5 Hz), 2.90 (1 H, dd, J = 4.1, 13.8 Hz), 4.54 (1 H, dd, J = 6.6, 12.6 Hz), 5.07 and 5.08 (each 1 H, *d*, J = 12.5 Hz), 5.29 (1 H, m), 7.34 (5 H, m). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 216.6, 177.3, 143.8, 136.4, 128.4, 128.0, 127.9, 122.0, 69.1, 65.9, 57.7, 49.5, 47.3, 45.8, 41.3, 33.8, 33.0, 32.4, 32.3, 30.6, 27.6, 25.8, 24.7, 23.6, 23.5, 23.0, 21.6, 19.1, 17.0, 15.9. EI-MS: 561 $[M + H]^+$. HRMS calcd for $C_{37}H_{51}O_4$ [M − H]: 559.3787. Found: 559.3815. Anal. Calcd for $C_{37}H_{52}O_4$: C, 79.24, H, 9.35. Found: C, 78.98, H, 9.31.

Benzyl-2α-hydroxy-3-oxours-12-en-28-oic Acid (35). Following the procedure for preparation of **34**, hydroxylation of **29** with m-CPBA gave **35** as a white solid (70%). Mp: 95−97 °C. IR (KBr, cm^{-1}): 3479, 2945, 2926, 2872, 1718, 1456, 1387, 1263, 1225, 1144, 1103, 993, 744, 596. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.68, 1.04, 1.11, 1.15, 1.24 (each 3 H, s), 0.85 (3 H, d, J = 6.4 Hz), 0.94 (3 H, d, J = 5.9 Hz), 2.30 (1 H, d, J = 11.1 Hz), 2.43 (1 H, dd, J = 6.6, 12.5 Hz), 4.53 (1 H, dd, J = 6.6, 12.6 Hz), 5.01 and 5.09 (each 1 H, d, J = 12.5 Hz), 5.24 (1 H, t, J = 3.6 Hz), 7.34 (5 H, m). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 16.1, 17.0, 17.2, 19.2, 21.1, 21.6, 23.45, 23.53, 24.2, 24.8, 27.9, 30.6, 32.7, 36.6, 37.6, 38.8, 39.1, 39.7, 42.1, 47.3, 47.7, 48.1, 49.7, 52.8, 57.8, 66.0, 69.2, 125.2, 128.0, 128.2, 128.4, 136.4, 138.4, 177.2, 216.6. ESI-MS *m/z*: 561.4 $[M + H]^+$, 583.4 $[M + Na]^+$, 599.4 $[M + K]^+$. HRMS Calcd for $C_{37}H_{51}O_4$ [M − H]: 559.3787. Found: 559.3811. Anal. Calcd for $C_{37}H_{52}O_4$: C, 79.24, H, 9.35. Found: C, 79.10, H, 9.30.

Benzyl-2α,3β-dihydroxyolean-12-en-28-oic Acid (32). To a solution of **34** (8.9 g, 0.016 mol) in THF (100 mL) and ethanol (20 mL) was added $NaBH_4$ (0.7 g, 0.018 mol) at 0 °C. After the mixture was stirred at 0 °C for 5 h, 1 N HCl (200 mL) was added dropwise, and the mixture was extracted with EtOAc (150 mL × 1, 80 mL × 3). The organic layer was washed with saturated $NaHCO_3$ (80 mL × 3) and brine (80 mL × 3), dried over Na_2SO_4, filtered, and concentrated to afford a white powder, which was purified by flash chromatography [petroleum ether−EtOAc (6:1)] to give **32** as a white solid (6.94 g, 77%), together with **36** as a minor product (1.62 g, 18%). Data for **32**: mp 155−157 °C. IR (KBr, cm^{-1}): 3371, 2945, 1725, 1461, 1385, 1261, 1159, 1122, 1049, 1031, 994, 745, 697. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.60, 0.82, 0.90, 0.92, 0.95, 1.02, 1.12 (each 3 H, s), 2.92 (1 H, dd, J = 4.1, 13.7 Hz), 3.01 (1 H, d, J = 9.5 Hz), 3.68 (1 H, m), 5.06 and 5.08 (each 1 H, d, J = 12.6 Hz), 5.29 (1 H, t, J = 3.5 Hz), 7.34 (5 H, m). ESI-MS *m/z*: 585.2 $[M + Na]^+$. HRMS Calcd for $C_{37}H_{53}O_4$[M − H]: 561.3944. Found: 561.3929.

Benzyl-2α,3β-dihydroxyurs-12-en-28-oic Acid (33). Reduction of **35** with $NaBH_4$ was carried out following the procedure for reduction of **34** with $NaBH_4$ to give **33** (68%) as a major product, together with **37** (14%) as a minor product. Data for **33**: mp 163−165 °C. IR (KBr, cm^{-1}): 3401, 2925, 2870, 1723, 1455, 1377, 1267, 1225, 1140, 1109, 1048, 1031, 995, 961, 746, 696. ^1H NMR ($CDCl_3$, 500 MHz): δ 0.63, 0.82, 0.96, 1.03, 1.07 (each 3 H, s), 0.85 (3 H, d, J = 6.4 Hz), 0.94 (3 H, d, J = 6.3 Hz), 2.28 (1 H, d, J = 11.3 Hz), 3.00 (1 H, d, J = 9.4 Hz), 3.69 (1 H, m), 5.00 and 5.08 (each 1 H, d, J = 12.5 Hz), 5.24 (1 H, t, J = 3.5 Hz), 7.28−7.37 (5 H, m). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 16.7, 16.8, 16.97, 17.00, 18.3, 21.1, 23.3, 23.6, 24.2, 27.9, 28.6, 30.6, 32.9, 36.6, 38.2, 38.8, 39.07, 39.13, 39.6, 42.1, 46.6, 47.5, 48.1, 52.8, 55.3, 66.0, 69.0, 83.9, 125.5, 127.9, 128.1, 128.4, 136.3, 138.2, 177.3. ESI-MS *m/z*: 585 $[M + Na]^+$. HRMS Calcd for $C_{37}H_{53}O_4$[M − H]: 561.3944. Found: 561.3969.

2α,3β-Dihydroxyolean-12-en-28-oic Acid (Maslinic Acid) (3). A mixture of **32** (2.8 g, 5 mmol) and 10% Pd/C (0.4 g) in THF (27 mL) was stirred at room temperature under H_2 at atmospheric pressure for 18 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 **3** as a white solid (2.2 g, 95%). Mp: 269−271 °C (lit. mp 266−269 °C[50]). IR (KBr, cm^{-1}): 3414, 2943, 2878, 1695, 1460, 1389, 1366, 1269, 1184, 1051, 1031, 822, 658. ^1H NMR (pyridine-d_5, 300 MHz): δ 0.93, 0.98, 0.99, 1.01, 1.06 (each 3 H, s), 1.26 (6 H, s), 3.30 (1 H, dd, J = 3.7, 13.5 Hz), 3.39 (1 H, d, J = 9.3 Hz), 4.08 (1 H, m), 5.46 (1 H, brs). ^{13}C NMR (pyridine-d_5, 75 MHz): δ 16.9, 17.5, 17.7, 18.9, 23.7, 23.8, 23.9, 26.2, 28.3, 29.3, 31.0, 33.2, 33.3, 34.3, 38.6, 39.8, 42.0, 42.2, 46.5, 46.7, 47.8, 48.2, 55.9, 68.6, 83.8, 122.5, 144.9, 180.2. ESI-MS *m/z*: 471 [M − H]. HRMS Calcd for $C_{30}H_{47}O_4$ [M − H]: 471.3474. Found: 471.3465.

2α,3β-Dihydroxyurs-12-en-28-oic Acid (Corosolic Acid) (12). Corosolic acid was prepared in quantitative yield via hydrogenolysis of **33** following the procedure for preparation of **3**. Mp 253−255 °C (lit. mp 255−258 °C[51]). IR (KBr, cm^{-1}): 3421, 2966, 2945, 2926, 2872, 1695, 1456, 1389, 1230, 1049, 1032, 997, 662. ^{1}H NMR (pyridine-d_5, 300 MHz): δ 0.96, 1.02, 1.05, 1.19, 1.25 (each 3 H, s), 0.94 (3 H, d, J = 6.0 Hz), 0.97 (3 H, d, J = 4.7 Hz), 2.61 (1 H, d, J = 11.3 Hz), 3.38 (1 H, d, J = 9.4 Hz), 4.06 (1 H, m), 5.44 (1 H, t, J = 3.1 Hz). ^{13}C NMR (pyridine-d_5, 75 MHz): δ 17.0, 17.50, 17.52, 17.7, 18.9, 21.4, 23.8, 23.9, 24.9, 28.7, 29.4, 31.1, 33,5, 37.5, 38.5, 39.4, 39.5, 39.8, 40.1, 42.5, 48.0, 48.06, 48.13, 53.6, 56.0, 68.6, 83.8, 125.6, 139.3, 179.8. ESI-MS *m/z*: 495.4 $[M + Na]^+$. HRMS Calcd for $C_{30}H_{47}O_4$[M − H]: 471.3474. Found: 471.3453.

Benzyl-2α,3α-dihydroxyolean-12-en-28-oic Acid (36). A mixture of **34** (1.2 g, 2.14 mmol), freshly prepared Al(O-*i*-Pr)$_3$ (42.8 mmol), catalytic amount of $AlCl_3$, and dry *i*-PrOH (20 mL) was refluxed for 4 h. After the mixture was cooled to room temperature, 1 N HCl (50 mL) was added to the reaction mixture, and the mixture was extracted with EtOAc (50 mL × 3). The combined extract was washed with saturated $NaHCO_3$ (50 mL × 3) and brine (50 mL × 3), dried over Na_2SO_4, filtered, and concentrated to afford a yellow solid, which was purified by flash chromatography [petroleum ether−EtOAc (6:1)] to give **36** as a white solid (0.6 g, 50%), together with **32** as a minor product. Data for **36**: mp 110−112 °C. IR (KBr, cm^{-1}): 3447, 2947, 2870, 1726, 1632, 1460, 1387, 1258, 1161, 1036, 941, 824, 743, 694, 596, 465. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.60, 0.85, 0.90, 0.919, 0.925, 1.01, 1.16 (each 3 H, s), 2.92 (1 H, dd, J = 4.6, 14.1 Hz), 3.42 (1 H, d, J = 2.6 Hz), 4.00 (1 H, m), 5.06 and 5.07 (each 1 H, d, J = 12.6 Hz), 5.29 (1 H, t, J = 3.5 Hz), 7.35 (5 H, m). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 16.3, 16.9, 18.0, 21.8, 23.0 23.4, 23.6, 26.0, 27.6, 28.5, 30.7, 32.4, 32.5, 33.1, 33.9, 38.2, 38.4, 39.5, 41.4, 41.7, 41.8, 45.9, 46.7, 47.3, 48.1, 65.9, 66.5, 78.9, 122.3, 127.87, 127.94, 128.4, 136.5, 143.8, 177.4. ESI-MS *m/z*: 585.3 $[M + Na]^+$. HRMS Calcd for $C_{37}H_{53}O_4$ [M − H]: 561.3944. Found: 561.3962.

Benzyl-2α,3α-dihydroxyurs-12-en-28-oic Acid (37). Meerwein−Pondorf reduction of **35** was carried out following the procedure for Meerwein−Pondorf reduction of **34** to give **37** as a major product (51%), together with **33** (6%) as a minor product. Data for **37**: mp 106−108 °C. IR (KBr, cm^{-1}): 3450, 3431, 2947, 2926, 2870, 1724, 1454, 1383, 1271, 1225, 1140, 1036, 993, 746, 696, 665, 598. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.63, 0.85, 0.94, 1.01, 1.08 (each 3 H, s), 0.84 (3 H, d, J = 5.4 Hz), 0.93 (3 H, d, J = 4.7 Hz), 2.29 (1 H, d, J = 11.3 Hz), 3.43 (1 H, d, J = 2.7 Hz), 4.01 (1 H, m), 5.00 and 5.08 (each 1 H, d, J = 12.5 Hz), 5.24 (1 H, t, J = 3.5 Hz), 7.34 (5 H, m). ^{13}C NMR ($CDCl_3$, 75 MHz): δ16.5, 17.0, 17.1, 18.1, 21.2, 21.9, 23.3 23.7, 24.3, 28.0, 28.5, 30.7, 32.9, 36.7, 38.3, 38.4, 38.9, 39.1, 39.8, 42.1, 42.2, 47.4, 48.2, 53.0, 66.0, 66.5, 79.0, 125.6, 127.9, 128.2, 128.4, 136,5, 138.3, 177.3. ESI-MS *m/z*: 585.3 $[M + Na]^+$, 601.2 $[M + K]^+$. HRMS Calcd for $C_{37}H_{53}O_4$ [M − H]: 561.3944. Found: 561.3964.

2α,3α-Dihydroxyolean-12-en-28-oic Acid (4). Hydrogenolysis of **36** was carried out in quantitative yield following the procedure for hydrogenolysis of **32** to give **4** as a white solid. Mp: 295−297 °C. IR (KBr, cm^{-1}): 3445, 2947, 2874, 1693, 1458, 1385, 1267, 1182, 1036, 995, 941, 881, 822, 667, 646, 567. ^{1}H NMR (pyridine-d_5, 300 MHz): δ 0.89, 0.91, 0.96, 0.98, 1.01, 1.17, 1.26 (each 3 H, s), 3.26 (1 H, dd, J = 4.4, 13.9 Hz), 3.75 (1 H, d, J = 2.6 Hz), 4.29 (1 H, m), 5.46 (1 H, t, J = 3.3 Hz). ^{13}C NMR (pyridine-d_5, 75 MHz): δ 16.6, 17.5, 18.5, 22.3, 23.7, 23.8, 23.9, 26.1, 28.3, 29.5, 30.9, 33.2, 34.3, 38.7, 38.8, 40.0, 42.0, 42.3, 42.8, 46.5, 46.7, 48.0, 48.8, 66.1, 79.4, 122.5, 144.9, 180.2. ESI-MS *m/z*: 473.4 $[M + H]^+$, 495.4 $[M + Na]^+$, 511.3 $[M + Na]^+$. HRMS Calcd for $C_{30}H_{47}O_4$ [M − H]: 471.3474. Found: 471.3495.

2α,3α-Dihydroxyurs-12-en-28-oic Acid (Pygenic Acid A) (13). Hydrogenolysis of **37** was carried out following the procedure for hydrogenolysis of **32** to give **13**[52] as a white solid. Mp: 230−232 °C. IR (KBr, cm^{-1}) 3454, 2928, 1688, 1630, 1383. ^{1}H NMR (pyridine-d_5, 300 MHz): δ 0.88, 0.94, 1.03, 1.11, 1.25 (each 3 H, s), 0.92 (3 H, d, J = 6.2 Hz), 0.96 (3 H, d, J = 6.3 Hz), 2.61 (1 H, d, J = 11.1 Hz), 3.74 (1 H, d, J = 2.6 Hz), 4.28 (1 H, m), 5.44 (1 H, t, J = 3.3 Hz). ^{13}C NMR (pyridine-d_5, 75 MHz): δ 16.8, 17.4, 17.5, 18.5, 21.4, 22.3, 23.7, 23.9, 24.9, 28.7, 29.5, 31.1, 33.5, 37.4, 38.6, 38.8, 39.4, 39.5, 40.2, 42.6, 43.0, 47.9, 48.1, 48.7, 53.6, 66.1, 79.4, 125.6, 139.3, 179.9. ESI-MS *m/z*: 495.3 $[M + Na]^+$, 511.3 $[M + K]^+$. HRMS Calcd for $C_{30}H_{47}O_4$ [M − H]: 471.3474. Found: 471.3485.

Benzyl-2-hydroxy-3-oxoolean-1,12-dien-28-oic Acid (38). Method A (Preparation from 34). A mixture of **34** (0.6 g, 1.07 mmol), DMF (8 mL), MeOH (8 mL), and KOH (1.5 g, 27 mmol) was stirred at room temperature for 4 h. The reaction was quenched by adding 2 N HCl (20 mL), and the sample was extracted with EtOAc (50 mL × 3). The combined extract was washed with saturated $NaHCO_3$ (50 mL × 3) and brine (50 mL × 3), dried over Na_2SO_4, filtered, and concentrated to afford a yellow oil, which was purified by flash chromatography [petroleum ether−EtOAc (30/1, v/v)] to give **38** as a white solid (0.55 g, 92%).

Method B (Preparation from 28). To a solution of **28** (2.0 g, 3.68 mmol) in *t*-BuOH (80 mL) and THF (10 mL) was added *t*-BuOK (2.5 g, 22.28 mmol) at room temperature under air. After the mixture was stirred overnight, most of the solvents were removed by evaporation in vacuo, and the residue was diluted with water, acidified with 1 N HCl, and extracted with EtOAc. The combined extract was washed with saturated brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to afford a pale-yellow oil, which was purified by column chromatography [petroleum ether−EtOAc (30:1)] to give **38** as a white solid (1.72 g, 84%). Mp: 88−90 °C. IR (KBr, cm^{-1}): 3441, 2945, 2870, 1726, 1666, 1460, 1389, 1236. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.64, 0.90, 0.93, 1.115, 1.122, 1.19, 1.21 (each 3 H, s), 2.95 (1 H, dd, J = 4.4, 13.6 Hz), 5.07 and 5.09 (each 1 H, d, J = 12.5 Hz), 5.34 (1 H, t, J = 3.5 Hz), 5.92 (1 H, s), 6.33 (1 H, s), 7.34 (5 H, m). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.3, 18.7, 19.6, 21.8, 23.0, 23.4, 23.6, 25.8, 27.2, 27.6, 32.3, 32.5, 33.1, 33.9, 38.4, 40.0, 41.5, 42.1, 43.1, 43.9, 45.7, 46.8, 53.9, 66.0, 121.9, 127.95, 128.04, 128.2, 128.4, 136.4, 143.7, 144.1, 177.4, 201.1. ESI-MS *m/z*: 559.4 $[M + H]^+$, 581.3 $[M + Na]^+$, 597.3 $[M + K]^+$. HRMS Calcd for $C_{37}H_{49}O_4$ [M − H]: 557.3631. Found: 557.3657.

Benzyl-2-hydroxy-3-oxours-1,12-dien-28-oic Acid (39). In the same fashion as described for preparation of **38**, treatment of **35** with KOH gave **39** (62%) as a white solid. Alternatively, direct oxidation of **29** in the presence of *t*-BuOK also afforded **39** (85%). Mp: 90−92 °C. IR (KBr, cm^{-1}): 3435, 2970, 1724, 1664, 1454, 1404, 1232, 1144. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.69, 1.06, 1.12, 1.20, 1.21 (each 3 H, s), 0.86 (3 H, d, J = 6.4 Hz), 0.95 (3 H, d, J = 6.1 Hz), 2.31 (1 H, d, J = 11.1 Hz), 5.01 and 5.09 (each 1 H, d, J = 12.4 Hz), 5.29 (1 H, t, J = 3.5 Hz), 5.93 (1 H, s), 6.35 (1 H, s), 7.34 (5 H, m). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.0, 17.6, 18.7, 19.7, 21.1, 21.8, 23.3, 23.5, 24.3, 27.2, 28.0, 30.7, 32.9, 36.6, 38.3, 38.9, 39.1, 40.3, 42.5, 43.1, 43.9, 48.2, 53.1, 54.0, 66.0, 125.1, 128.0, 128.2, 128.4, 136.4, 138.7, 143.8, 177.1, 201.1. ESI-MS *m/z*: 559.4 $[M + H]^+$, 581.4 $[M + Na]^+$, 597.4 $[M + K]^+$. HRMS Calcd for $C_{37}H_{49}O_4$[M − H]: 557.3631. Found: 557.3651.

Benzyl-2β,3β-dihydroxyolean-12-en-28-oic Acid (40). To a solution of **38** (4 g, 7.2 mmol) in THF (43 mL) and ethanol (8.9 mL) was added $NaBH_4$ (0.62 g, 15.7mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 5 h, and then 1 N HCl (100 mL) was added, extracted with EtOAc (100 mL × 1, 50 mL × 3). The combined extract was washed with saturated $NaHCO_3$ (50 mL × 3) and brine (50 mL × 3), dried over Na_2SO_4, filtered, and concentrated to afford a white powder, which was purified by flash chromatography [petroleum ether−EtOAc (8:1)] to give **40** as a white solid (2.47 g, 61%). Mp: 98−100 °C. IR (KBr, cm^{-1}): 3448, 2947, 2876, 1726, 1632, 1458, 1383, 1159. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.63, 0.90, 0.92, 1.00, 1.01, 1.12, 1.21 (each 3 H, s), 2.93 (1 H, dd, J = 4.1, 13.8 Hz), 3.21 (1 H, d, J = 3.9 Hz), 4.09 (1 H, m), 5.07 and 5.08 (each 1 H, d, J = 12.5 Hz), 5.30 (1 H, t, J = 3.5 Hz), 7.34 (5 H, m). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 16.3, 16.9, 17.3, 18.1, 23.1, 23.5, 23.6, 25.9, 27.5, 29.7, 30.7, 32.4, 32.7, 33.1, 33.9, 36.7, 38.1, 39.4, 41.4, 41.8, 44.1, 45.9, 46.8, 48.1, 55.2, 65.9, 71.1, 78.5, 122.6, 127.9, 128.0, 128.4, 136.5, 143.7, 177.4. ESI-

MS *m/z*: 585.3 $[M + Na]^+$, 601.3 $[M + K]^+$. HRMS Calcd for $C_{37}H_{53}O_4$ [M − H]: 561.3944. Found: 561.3971.

Benzyl-2*β*,3*β*-dihydroxyurs-12-en-28-oic Acid (41). In the same fashion as described for the preparation of **40**, reduction of **39** with $NaBH_4$ gave **41** as a white solid (69%). Mp: 85−87 °C. IR (KBr, cm^{-1}): 3452, 2924, 2872, 1724, 1456, 1381, 1225, 1142. ^1H NMR ($CDCl_3$, 300 MHz): *δ* 0.66, 1.00, 1.01, 1.06, 1.22 (each 3 H, s), 0.86 (3 H, d, *J* = 6.4 Hz), 0.94 (3 H, d, *J* = 5.9 Hz), 2.29 (1 H, d, *J* = 11.3 Hz), 3.43 (1 H, d, *J* = 4.0 Hz), 4.01 (1 H, m), 5.00 and 5.09 (each 1 H, d, *J* = 12.5 Hz), 5.24 (1 H, t, *J* = 3.6 Hz), 7.34 (5 H, m). ^{13}C NMR ($CDCl_3$, 75 MHz): *δ* 16.4, 16.95, 17.00, 17.3, 18.1, 21.1, 23.4 23.6, 24.2, 27.8, 29.7, 30.7, 33.0, 36.6, 36.7, 38.1, 38.8, 39.1, 39.7, 42.2, 44.3, 48.0, 48.1, 52.9, 55.2, 66.0, 71.1, 78.5, 125.8, 127.9, 128.2, 128.4, 136,4, 138.1, 177.2. ESI-MS *m/z*: 585.2 $[M + Na]^+$, 601.2 $[M + K]^+$. HRMS Calcd for $C_{37}H_{53}O_4$ [M − H]: 561.3944. Found: 561.3959.

2*β*,3*β*-Dihydroxyolean-12-en-28-oic Acid (Augustic Acid) (5). Hydrogenolysis of **40** was carried out following the procedure for hydrogenolysis of **32** to give **5**[53,54] as a white solid. Mp: 308−310 °C. IR (KBr, cm^{-1}): 3485, 3433, 2949, 1703, 1464, 1385, 1263, 1194, 1063. ^1H NMR (pyridine-d_5, 300 MHz): *δ* 0.98, 1.04, 1.11, 1.29, 1.34, 1.38, 1.54 (each 3 H, s), 3.35 (1 H, dd, *J* = 3.4, 13.4 Hz), 3.46 (1 H, d, *J* = 3.6 Hz), 4.42 (1 H, m), 5.54 (1 H, brs). ^{13}C NMR (pyridine-d_5, 75 MHz): *δ* 16.6, 17.5, 18.1, 18.7, 23.8, 24.0, 26.3, 28.3, 30.3, 31.0, 33.3, 33.4, 34.3, 37.4, 38.8, 40.0, 42.1, 42.4, 45.0, 46.6, 46.7, 48.6, 56.0, 71.5, 78.4, 122.8, 144.9, 180.2. ESI-MS *m/z*: 495.3 $[M + Na]^+$. HRMS Calcd for $C_{30}H_{47}O_4$ [M − H]: 471.3474. Found: 471.3470.

2*β*,3*β*-Dihydroxyurs-12-en-28-oic Acid (2-Epicorosolic Acid) (14). Hydrogenolysis of **41** was carried out following the procedure for hydrogenolysis of **32** to give **14**[55] as a white solid. Mp: 284 °C (dec). IR (KBr, cm^{-1}): 3439, 2926, 1691, 1634, 1454, 1381, 1051, 999. ^1H NMR (pyridine-d_5, 300 MHz): *δ* 0.97 (3 H, d, *J* = 5.7 Hz), 1.03 (3 H, d, *J* = 6.4 Hz), 1.11, 1.25, 1.27, 1.36, 1.51 (each 3 H, s), 2.66 (1 H, d, *J* = 11.3 Hz), 3.46 (1 H, d, *J* = 3.9 Hz), 4.41 (1 H, m), 5.50 (1 H, brs). ^{13}C NMR (pyridine-d_5, 75 MHz): *δ* 16.8, 17.5, 18.2, 18.7, 21.4, 23.9, 24.0, 25.0, 28.7, 30.3, 31.2, 33.7, 37.3, 37.5, 38.8, 39.5, 39.6, 40.2, 42.8, 45.2, 48.1, 48.5, 53.7, 56.0, 71.5, 78.4, 125.9, 139.3, 179.9. ESI-MS *m/z*: 495.2 $[M + Na]^+$, 511.2 $[M + K]^+$. HRMS Calcd for $C_{30}H_{47}O_4$ [M − H]: 471.3474. Found: 471.3481.

Benzyl-3*β*-hydroxy-2-oxoolean-12-en-28-oic Acid (42). A mixture of **34** (0.54 g, 0.96 mmol), $NaHCO_3$ (2 g), CH_2Cl_2 (20 mL), MeOH (20 mL), and water (10 mL) was heated at reflux for 48 h. The reaction mixture was then evaporated under reduced pressure, and to the residue was added water, extracted with EtOAc (50 mL × 3). The combined extract was washed with 1 N HCl and brine, dried over Na_2SO_4, filtered, and concentrated. Purification by flash chromatography [petroleum ether−EtOAc (10:1)] and recrystallization from EtOH gave **42** as a white crystal (80 mg, 15%). Mp: 184−186 °C. IR (KBr, cm^{-1}): 3483, 2959, 2912, 2876, 1720, 1705, 1454, 1389, 1304, 1254, 1213, 1157. ^1H NMR ($CDCl_3$, 300 MHz): *δ* 0.58, 0.69, 0.84, 0.90, 0.92, 1.17, 1.18 (each 3 H, s), 2.07 (1 H, d, *J* = 12.1 Hz), 2.43 (1 H, d, *J* = 12.2 Hz), 2.94 (1 H, m), 3.88 (1 H, s), 5.06 and 5.07 (each 1 H, d, *J* = 12.5 Hz), 5.29 (1 H, brs), 7.34 (5 H, m). ^{13}C NMR ($CDCl_3$, 75 MHz): *δ* 16.1, 16.45, 16.53, 18.5, 23.0, 23.3, 23.6, 25.8, 27.6, 29.4, 30.7, 32.3, 32.4, 33.0, 33.8, 39.7, 41.4, 41.8, 43.6, 45.6, 45.9, 46.7, 47.6, 53.1, 54.5, 65.9, 82.8, 121.7, 127.9, 128.0, 128.4, 136.4, 143.9, 177.2, 210.9. ESI-MS *m/z*: 561.3 $[M + H]^+$, 583.3 $[M + Na]^+$, 599.3 $[M + K]^+$. HRMS Calcd for $C_{37}H_{51}O_4$ [M − H]: 559.3787. Found: 557.3808. Anal. Calcd for $C_{37}H_{52}O_4$: C, 79.24, H, 9.35. Found: C, 79.14, H, 9.20.

Preparation of 40 via Reduction of 42. Reduction of **42** with $NaBH_4$ was carried out following the procedure described for reduction of **38** to give 2*β*,3*β*-diol **40**, which was identical with the product obtained by $NaBH_4$ reduction of **38**.

Benzyl-3α-hydroxyolean-12-en-28-oic Acid (43). Meerwein−Pondorf reduction of **28** was carried out following the procedure described for the preparation of **36** to afford **43** as a white solid (57%). Mp: 196−198 °C. IR (KBr, cm^{-1}): 2946, 2863, 1723, 1456, 1386, 1365, 1262, 1159. ^1H NMR ($CDCl_3$, 300 MHz): *δ* 0.61, 0.83, 0.89, 0.90, 0.92, 0.95, 1.14 (each 3 H, s), 2.89 (1 H, dd, *J* = 4.6, 13.6 Hz), 3.40 (1 H, t, *J* = 2.7 Hz), 5.06 and 5.08 (each 1 H, d, *J* = 12.6 Hz), 5.29 (1 H, t, *J* = 3.5 Hz), 7.34 (5 H, m). ESI-MS *m/z*: 569.3 $[M + Na]^+$.

Benzyl-3α-hydroxyurs-12-en-28-oic Acid (44). Meerwein−Pondorf reduction of **29** was carried out following the procedure described for the preparation of **36** to afford **44** as a white solid (52%). Mp: 103−105°. IR (KBr, cm^{-1}): 3359, 2923, 2870, 1722, 1454, 1386, 1274, 1226, 1139, 1108. ^1H NMR ($CDCl_3$, 300 MHz): *δ* 0.65, 0.84, 0.91, 0.95, 1.09 (each 3 H, s), 0.86 (3 H, d, *J* = 6.8 Hz), 0.94 (3 H, d, *J* = 6.3 Hz), 2.28 (1 H, d, *J* = 11.4 Hz), 3.40 (1 H, t, *J* = 2.8 Hz), 5.00 and 5.08 (each 1 H, d, *J* = 12.5 Hz), 5.24 (1 H, t, *J* = 3.6 Hz), 7.34 (5 H, m). ESI-MS *m/z*: 569.4 $[M + Na]^+$.

3α-Hydroxyolean-12-en-28-oic Acid (3-Epioleanolic Acid) (2). Hydrogenolysis of **43** was carried out following the procedure for hydrogenolysis of **32** to give **2**[56,57] as a white solid (90%). Mp: 293 °C (dec). IR (KBr, cm^{-1}): 3450, 2947, 2868, 1691, 1462, 1385, 1279, 1209, 1068. ^1H NMR (pyridine-d_5, 300 MHz): *δ* 0.88, 0.91, 0.92, 0.99, 1.05, 1.18, 1.22 (each 3 H, s), 3.27 (1 H, dd, *J* = 4.0, 13.8 Hz), 3.60 (1 H, brs), 5.49 (1 H, t, *J* = 3.3 Hz). ESI-MS *m/z*: 479.4 $[M + Na]^+$.

3α-Hydroxyurs-12-en-28-oic Acid (3-Epiursolic Acid) (11). Hydrogenolysis of **44** was carried out following the procedure for hydrogenolysis of **32** to give **11**[58] as a white solid (94%). Mp: 279 °C (dec). IR (KBr, cm^{-1}): 3448, 2945, 2926, 2870, 1697, 1454, 1383, 1311, 1232, 1067. ^1H NMR (pyridine-d_5, 300 MHz): *δ* 0.88, 0.92, 1.07, 1.11, 1.22 (each 3 H, s), 0.93 (3 H, d, *J* = 3.7 Hz), 0.95 (3 H, d, *J* = 6.5 Hz), 2.60 (1 H, d, *J* = 11.5 Hz), 3.60 (1 H, brs), 5.49 (1 H, t, *J* = 3.4 Hz). ESI-MS *m/z*: 479.4 $[M + Na]^+$.

2,3,4,6-Tetra-*O*-acetyl-*β*-D-pyranoglucosyl 3*β*-Hydroxyolean-12-en-28-oate (46). A mixture of oleanolic acid (**1**) (2 g), 2,3,4,6-tetra-*O*-acetyl-α-D-glucosyl bromide[25] (**45**, 3.51 g), K_2CO_3 (2.42 g), and DMF (40 mL) was stirred overnight at room temperature. The solids were filtered off, and to the filtrate was added 200 mL of water. The resulted precipitate was collected by filtration, washed with water, and dried. The crude product was purified by column chromatography to give **46** as a white solid (3.15 g, 92%). Mp: 163−165 °C. IR (KBr, cm^{-1}): 2647, 1759, 1462, 1366, 1226, 1076, 1037. ^1H NMR (300 MHz, $CDCl_3$): *δ* 0.74, 0.78, 0.98, 1.13 (each, 3 H, s), 0.91 (9 H, s), 2.01 (3 H, s), 2.02 (6 H, s), 2.07 (3 H, s), 2.80 (1 H, dd, *J* = 4.5, 13.9 Hz), 3.20 (1 H, dd, *J* = 4.3, 11.0 Hz), 3.80 (1 H, m), 4.07 (1 H, dd, *J* = 2.1, 12.4 Hz), 4.26 (1 H, dd, *J* = 4.4, 12.5 Hz), 5.13 (1 H, t, *J* = 9.2 Hz), 5.18 (1 H, t, *J* = 7.8 Hz), 5.25 (1 H, t, *J* = 9.1 Hz), 5.28 (1 H, brs), 5.57 (1 H, d, *J* = 7.8 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): *δ* 15.3,15.6, 16.9, 18.3, 20.6, 20.7, 22.8, 23.38, 23.42, 25.7, 27.2, 27.7, 28.1, 29.7, 30.6, 31.7, 32.9, 33.0, 33.7, 37.0, 38.5, 38.7, 39.3, 41.0, 41.7, 45.7, 46.8, 47.6, 55.2, 61.5, 68.0, 69.9, 72.4, 72.8, 79.0, 91.6, 122.9, 142.8, 168.9, 169.4, 170.1, 170.6, 175.6. ESI-MS *m/z*: 809.4 $[M + Na]^+$.

2,3,4,6-Tetra-*O*-acetyl-*β*-D-pyranoglucosyl 3*β*-Hydroxyurs-12-en-28-oate (47). Reaction of ursolic acid (**10**) with **45** was carried out following the procedure described for the preparation of **46** to afford **47** as a white solid (90%). Mp: 174−175 °C. IR (KBr, cm^{-1}): 2926, 1758, 1454, 1367, 1224, 1069, 1037. ^1H NMR ($CDCl_3$, 300 MHz): *δ* 0.77, 0.78, 0.92, 0.95, 0.98, 1.07 (each 3 H, s), 0.85 (3 H, d, *J* = 6.4 Hz), 2.01, 2.02, 2.03, 2.06 (each 3 H, s), 2.21 (1 H, d, *J* = 11.8 Hz), 3.20 (1 H, dd, *J* = 4.7, 10.4 Hz), 3.78 (1 H, m), 4.05 (1 H, dd, *J* = 2.3, 12.3 Hz), 4.25 (1 H, dd, *J* = 4.4, 12.3 Hz), 5.11 (1 H, t, *J* = 9.5 Hz), 5.16 (1 H, t, *J* = 8.5 Hz), 5.24 (1 H, t, *J* = 9.2 Hz), 5.28 (1 H, t, *J* = 3.9 Hz), 5.54 (1 H, d, *J* = 7.9 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): *δ* 15.5, 15.6, 16.9, 17.1, 18.3, 20.58, 20.64, 20.7, 21.1, 23.3, 23.4, 24.1, 27.3, 28.2, 30.6, 33.3, 36.0, 37.0, 38.7, 38.8, 38.9, 39.1, 39.6, 42.1, 47.6, 48.2, 52.7, 55.3, 61.7, 68.2, 70.0, 72.5, 73.0, 79.0, 91.6, 126.2, 137.3, 169.0, 169.4, 170.1, 170.6, 175.4. ESI-MS *m/z*: 809.1 $[M + Na]^+$.

2,3,4,6-Tetra-*O*-acetyl-*β*-D-pyranoglucosyl 2α,3*β*-Dihydroxyurs-12-en-28-oate (48). Reaction of corosolic acid (**12**) with **45** was carried out following the procedure described for the preparation of **46** to afford **48** as a white solid (94%). Mp: 165−167 °C. IR (KBr, cm^{-1}): 2925, 1758, 1454, 1367, 1224, 1068, 1037, 749.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.76, 0.82, 0.95, 0.99, 1.03, 1.07 (each, 3 H, s), 0.84 (3 H, d, J = 6.4 Hz), 2.01 (3 H, s), 2.02 (6 H, s), 2.06 (3 H, s), 2.17 (1 H, d, J = 11.4 Hz), 3.01 (1 H, d, J = 9.5 Hz), 3.69 (1 H, m), 3.77 (1 H, m), 4.05 (1 H, dd, J = 2.2, 12.4 Hz), 4.25 (1 H, dd, J = 4.4, 12.4 Hz), 5.12 (1 H, t, J = 9.5 Hz), 5.16 (1 H, t, J = 8.5 Hz), 5.25 (1 H, t, J = 9.1 Hz), 5.28 (1 H, t, J = 3.4 Hz), 5.54 (1 H, d, J = 7.9 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 16.8, 16.9, 17.1, 18.3, 20.57, 20.60, 20.67, 20.69, 21.1, 23.3, 24.1, 28.1, 28.6, 30.5, 33.2, 36.0, 38.2, 38.8, 39.06, 39.13, 39.7, 42.1, 46.7, 47.5, 48.2, 52.6, 55.3, 61.6, 68.1, 69.0, 70.0, 72.5, 72.9, 84.0, 91.6, 125.9, 137.3, 168.9, 169.4, 170.1, 170.6, 175.3. ESI-MS m/z: 825.4 $[M + Na]^+$.

β-D-Pyranoglucosyl 3β-Hydroxyolean-12-en-28-oate (18). To a solution of **46** (0.14 g, 0.19 mmol) in THF (3 mL) and MeOH (2 mL) was added dropwise 4 N NaOH aqueous solution (0.5 mL), and then the reaction mixture was stirred at room temperature for 45min. The mixture was diluted with water (30 mL) and extracted with EtOAc−THF (2:1) three times (each time 30 mL). The combined extract was washed with 1 N HCl (20 mL) and brine (20 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography [CH_2Cl_2−MeOH (20:1)] to give **18**[59] as a white solid (0.11 g, 92%). Mp: 224−226 °C. IR (KBr, cm^{-1}): 3435, 2944, 2873, 1735, 1460, 1385, 1072, 1029. ^{1}H NMR (pyridine-d_5, 300 MHz): δ 0.88, 0.90, 0.92, 1.02, 1.03, 1.22, 1.24 (each, 3 H, s), 3.22 (1 H, dd, J = 4.5, 13.9 Hz), 3.43 (1 H, dd, J = 5.6, 10.0 Hz), 4.02 (1 H, m), 4.19 (1 H, t, J = 8.3 Hz), 4.27 (1 H, t, J = 8.6 Hz), 4.34 (1 H, t, J = 8.9 Hz), 4.40 (1 H, dd, J = 4.2, 11.8 Hz), 4.44 (1 H, dd, J = 2.5, 11.9 Hz), 5.45 (1 H, brs), 6.31 (1 H, d, J = 7.9 Hz). ^{13}C NMR (pyridine-d_5, 75 MHz): δ 15.7, 16.5, 17.6, 18.9, 23.5, 23.7, 23.9, 26.1, 28.1, 28.3, 28.8, 30.8, 32.6, 33.1, 33.3, 34.1, 37.4, 39.0, 39.4, 40.0, 41.8, 42.2, 46.3, 47.1, 55.9, 62.3, 71.2, 74.2, 78.2, 79.0, 79.3, 95.8, 144.2, 176.4. ESI-MS m/z: 641.4 $[M + Na]^+$.

β-D-Pyranoglucosyl 3β-Hydroxyurs-12-en-28-oate (19). Hydrolysis of **47** was carried out following the procedure described for the preparation of **18** to afford **19**[60] as a white solid (75%). Mp: 186−188 °C. IR (KBr, cm^{-1}): 3437, 2925, 1736, 1458, 1388, 1072, 1028, 996. ^{1}H NMR (pyridine-d_5, 300 MHz): δ 0.91, 1.03, 1.18, 1.19, 1.24 (each, 3 H), 0.95 (6 H, s), 0.98 (3 H, d, J = 7.6 Hz), 2.52 (1 H, d, J = 11.4 Hz), 3.45 (1 H, m), 4.04 (1 H, m), 4.11 (1 H, t, J = 8.1 Hz), 4.28 (1 H, t, J = 8.6 Hz), 4.26 (1 H, t, J = 9.0 Hz), 4.40 (1 H, m), 4.35 (1 H, m), 5.47 (1 H, brs), 6.26 (1 H, d, J = 7.8 Hz). ^{13}C NMR (pyridine-d_5, 75 MHz): δ 15.8, 16.6, 17.4, 17.7, 18.8, 21.3, 23.7, 23.8, 24.7, 28.2, 28.7, 28.8, 30.9, 33.6, 36.8, 37.3, 39.17, 39.22, 39.4, 40.2, 42.5, 48.2, 48.4, 53.4, 55.9, 62.4, 71.3, 74.1, 78.2, 78.9, 79.2, 95.8, 126.2, 138.5, 176.2. ESI-MS m/z: 641.1 $[M + Na]^+$.

β-D-Pyranoglucosyl 2α,3β-Dihydroxyurs-12-en-28-oate (20). Hydrolysis of **48** was carried out following the procedure described for the preparation of **18** to afford **20**[61] as a white solid. Mp: 275 °C (dec). IR (KBr, cm^{-1}): 3414, 2925, 2873, 1730, 1455, 1380, 1072, 1030, 997. ^{1}H NMR (pyridine-d_5, 300 MHz): δ 0.82, 0.96, 1.00, 1.18 (each, 3 H, s), 0.85 (3 H, d, J = 6.4 Hz), 1.10 (6 H, s), 2.43 (1 H, d, J = 11.3 Hz), 3.29 (1 H, d, J = 9.4 Hz), 3.95 (2 H, m), 4.11 (1 H, t, J = 8.2 Hz), 4.19 (1 H, t, J = 8.5 Hz), 4.26 (1 H, t, J = 9.2 Hz), 4.31 (1 H, dd, J = 4.3, 11.7 Hz), 4.35 (1 H, dd, J = 2.8, 11.8 Hz), 5.36 (1 H, brs), 6.17 (1 H, d, J = 7.9 Hz). ^{13}C NMR (pyridine-d_5, 75 MHz): δ 17.1, 17.4, 17.7, 17.8, 18.9, 21.3, 23.8, 24.7, 28.7, 29.4, 30.9, 33.6, 36.8, 38.5, 39.2, 39.4, 39.8, 40.3, 42.6, 48.1, 48.2, 48.4, 53.3, 56.0, 62.4, 68.6, 71.3, 74.1, 78.9, 79.2, 83.9, 95.8, 126.1, 138.5, 176.2; ESI-MS m/z: 657.1 $[M + Na]^+$.

Enzyme Kinetics. The inhibitory activity of the test compounds against rabbit muscle glycogen phosphorylase a (GPa) was monitored using microplate reader (BIO-RAD) based on a published method.[4] 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 to 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.

X-ray Crystallography. Rabbit muscle GPb was isolated, purified, recrystallized, and assayed as described.[62] Binding studies performed by diffusion of pentacyclic triterpenes into freshly prepared GPb crystals, grown in the tetragonal lattice, space group $P4_32_12$, as described previously,[63] resulted in crystal dissolution. Crystal dissolution was in part overcome by using old preformed GPb crystals, since in most experiments soaking resulted in calculated $2F_o - F_c$ and $F_o - F_c$ electron density maps with very poor or no electron density for the bound ligand. Good results were obtained with asiatic (**15**) and maslinic (**3**) acids. Binding of **15** at the allosteric site showed a concentration and soaking time dependence. Thus, at 10 mM and a soaking time of 2.5 h, there was no binding of either **15** or **3** at the allosteric site. Also, with 2.5 and 5 mM **15** and a soaking time of 2.5−85 h there was no ligand binding. As the concentration of **15** was increased, binding was observed at 10 mM or higher. Similarly with 10 mM **3** and a soaking time of 2.5 h, there was no binding. Finally, soaking with corosolic acid (**12**), glycyrrhetinic acid (**8**), ursolic acid (**10**), and oleanolic acid (**1**) at the highest concentration achieved (5 mM) and at a soaking time of 7 h resulted in no binding of these ligands. The best crystallographic results were obtained with 10 mM **15** (soaked for 7 h) in a buffered solution (10 mM Bes, 10% DMSO, pH adjusted to ∼8 with NaOH) prior to data collection. Similarly, crystallographic data for the GPb−maslinic acid complex were collected from a single crystal soaked with 10 mM maslinic acid (for 7 h) in 10 mM Bes, 10% DMSO, pH adjusted to ∼8 with NaOH. The high B-factors for both inhibitors probably indicate a rather low occupancy of the allosteric site. Diffraction data were collected either at Daresbury Laboratory SRS (beamline PX10.1) or at EMBL-Hamburg outstation (beamline X13). Crystal orientation, integration of reflections, interframe scaling, partial reflection summation, data reduction, and postrefinement were all performed using DENZO and SCALEPACK.[64]

Crystallographic refinement of the complexes was performed by maximum-likelihood methods using REFMAC.[65] The starting model employed for the refinement of the complexes was the structure of the native T state GPb complex determined at 1.9 Å resolution (Oikonomakos et al., unpublished results). The $2F_o - F_c$ and $F_o - F_c$ electron density maps calculated were visualized using the program for molecular graphics "O".[66] Ligand models of asiatic acid (**15**) and maslinic acid (**3**), optimized by quantum mechanics using DFT (B3LYP/6-31G*) with Jaguar 7.0, were fitted to the electron density maps after adjustment of their torsion angles. Alternate cycles of manual rebuilding with "O" and refinement with REFMAC improved the quality of the models.

The stereochemistry of the protein residues was validated by PROCHECK.[67,68] Hydrogen bonds and van der Waals interactions were calculated with the program CONTACT as implemented in CCP4,[68] applying a distance cutoff of 3.3 and 4.0 Å, respectively. Protein structures were superimposed using LSQKAB.[68] Solvent-accessible areas were calculated with the program NACCESS.[69] The figures were prepared with the program MolScript[70] and rendered with Raster3D[71] and with the program MG.[72,73] The coordinates of the new structures have been deposited with the RCSB Protein Data Bank (http://www.rcsb.org/pdb) with codes 2QN1 (GPb−asiatic acid complex) and 2GN2 (GPb−maslinic complex).

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Supporting Information Available: Crystallographic data collection details for compounds **15** and **3**, hydrogen bonding and van der Waals interactions between the inhibitors and GPb residues at the catalytic site, detailed structural comparisons of GPb−asiatic acid with GPb−W1807, GPb−Novo4j and hlGPa−AVE#21, and analytical data for the synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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