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Synthesis of glucoconjugates of oleanolic acid as inhibitors of glycogen phosphorylase

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ABSTRACT

Synthesis and biological evaluation of glucoconjugates of oleanolic acid, linked by either a triazole moiety or an ester function, as novel inhibitors of glycogen phosphorylase have been described. Several triterpene–glycoside conjugates exhibited moderate-to-good inhibitory activity against rabbit muscle GPa. Compound **12** showed the best inhibition with an IC_{50} value of 1.14 μM. Structure–activity relationship (SAR) analysis of these inhibitors is also discussed. Possible binding modes of compound **12** were explored by molecular docking simulations.

1. 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. Inhibition of glycogen phosphorylases (GP) has been regarded as a therapeutic strategy for blood glucose control in diabetes, and various studies have shown the efficacy of GP inhibitors in lowering blood glucose in animal models of diabetes and in clinical trials.[1] Several structural classes of GP inhibitors have been reported, and at least six potential regulatory binding sites have been identified in GP.[2] A series of glucose analogues have developed as GP inhibitors that bind at the catalytic site of the enzyme.[1c,e,3,4] On the other hand, we have recently reported that oleanolic acid (**1**, OA) and related pentacyclic triterpenes represent a new class of GP inhibitors,[5,6] and the results of X-ray crystallographic studies disclose the molecular basis of the pentacyclic triterpenes binding to GP at the allosteric site.[5]

Oleanolic acid (**1**, OA), which has been in active clinical use as an anti-hepatitis drug in China for over 20 years, possesses some attractive biological activities including protection of the liver against toxic injury, anti-inflammation, anti-HIV, anti-tumor, anti-oxidation, anti-hyperglycemia, and cardiovascular activities.[7] Given the biological importance and clinical significance of OA, lead modification based on OA is highly desirable for the study of structure–activity relationships and drug development. Considering that OA might bind to GP at the allosteric site,[5] and glucose analogue inhibitors bind to GP at the catalytic site, it would be interesting to design glucoconjugates of OA in order to find more potent GP inhibitors. Recent investigation on the click chemistry,[8] the Cu(I)-catalyzed Huisgen [2+3] dipolar cycloaddition reaction between an organic azide and an alkyne, has attracted much attention in drug design. This transformation is especially useful for drug discovery since it is a reliable conjugating reaction. However, to the best of our knowledge, the application of this approach to pentacyclic triterpenes has not been explored. Herein, we report the synthesis of glucoconjugates of oleanolic acid by conjugating together OA and a glycoside through a triazole or ester linkage. Biological evaluation of the synthesized glucoconjugates as new GP inhibitors is also presented. Moreover, the possible binding mode of the most active compound **12** was investigated by molecular docking simulation.

2. Results and discussion

The syntheses are summarized in Schemes 1–4. To realize click chemistry between oleanolic acid and glycoside derivatives, azide

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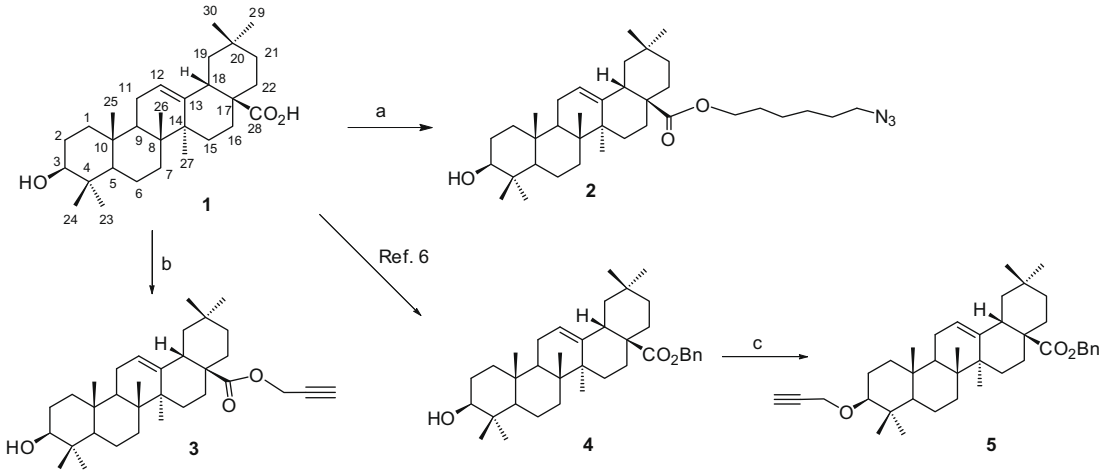
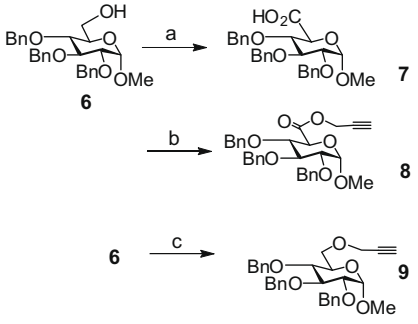


Scheme 1. Reagents and conditions: (a) K_2CO_3, $Br(CH_2)_6N_3$, DMF, rt, 92%. (b) K_2CO_3, propargyl bromide, DMF, rt, 97%; and (c) NaH, propargyl bromide, THF, 0 °C to reflux, 88%.



Scheme 2. Reagents and conditions: (a) TEMPO, KBr, NaOCl, $NaHCO_3$, acetone, rt, 92%; (b) K_2CO_3, propargyl bromide, DMF, rt, 97%; and (c) NaH, propargyl bromide, THF, 0 °C to rt, 86%.

or alkyne functions were first introduced into the 3- and 28-position of oleanolic acid **1** (Scheme 1). Esterification of **1** with 1-azido-6-bromohexane or propargyl bromide gave, respectively, azide **2** (92%) and alkyne **3** (97%). Protection of oleanolic acid with benzyl chloride, followed by an etherification with propargyl bromide, afforded the alkyne **5** (88%).

Two alkyne-functionalized glycoside derivatives **8** and **9** have been prepared as shown in Scheme 2. Oxidation of the primary hydroxyl group of methyl glucoside **6** by TEMPO–KBr–NaOCl–$NaHCO_3$ in acetone afforded carboxylic acid **7** in 92% yield.[9] Esterification of **7** with propargyl bromide afforded alkyne **8** (97%). Etherification of **6** with propargyl bromide afforded alkyne **9**[10] (86%).

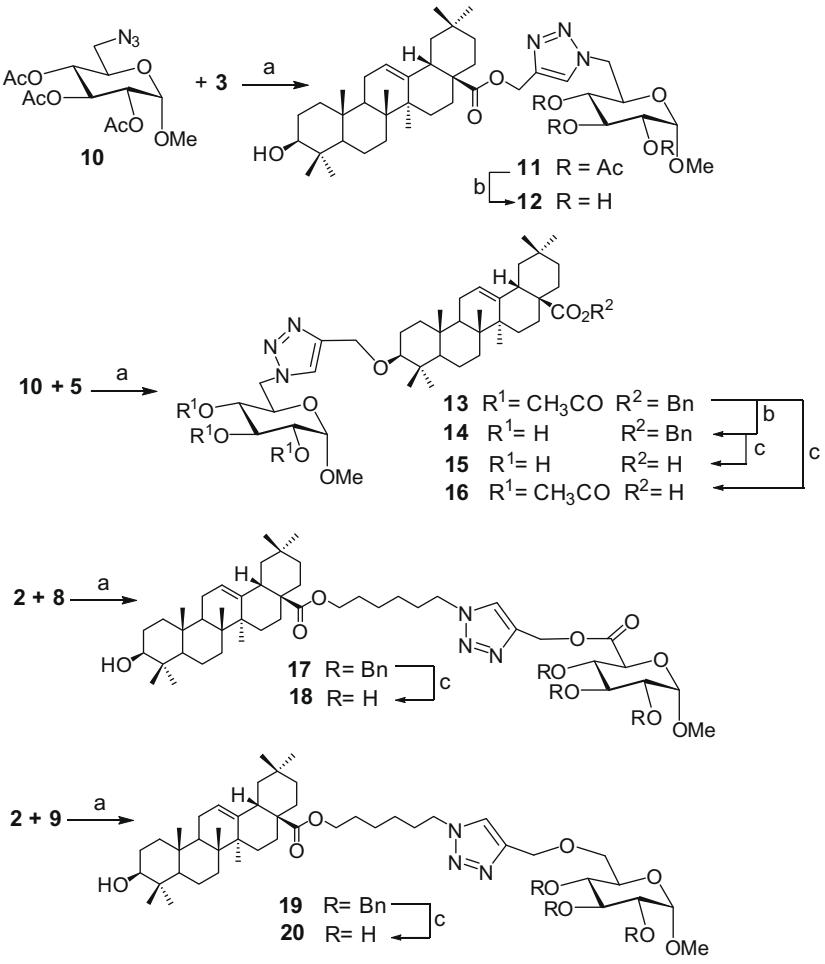
Click chemistry was handled as shown in Scheme 3. The corresponding azide and alkyne were dissolved in CH_2Cl_2–H_2O, followed by the addition of catalytic sodium ascorbate and $CuSO_4{\cdot}5H_2O$. Reaction of the known azide **10**[11] with alkyne **3** afforded triazole **11** in 93% yield. Similarly, the oleanolate–glycoside conjugates **13**, **17,** and **19** have been prepared in more than 83% yield. Cleavage of acetyl groups in **11** and **13** with MeONa–MeOH gave triazoles **12** (43%) and **14** (78%), respectively. The poor yield of **12** can be explained by a partial removal of the acetyl groups. Hydrogenolysis of **13**, **14**, **17,** and **19** over Pd/C (10%) in THF–MeOH at room temperature gave the corresponding triterpene derivatives **15** (81%), **16** (85%), **18** (76%), and **20** (38%), respectively.

Ester-linked oleanolate–glycoside conjugates have also been prepared (Scheme 4). Esterification of **1** with 1,6-dibromohexane afforded bromide **21** (52%). Further esterification of **21** with sugar carboxylic acid **7** or **24**[12] afforded protected glucoconjugates **22** (88%) and **25** (94%), respectively. Treatment of **4** with 2-chloroethyl chloroformate led to the carbonate **27** (92%) that was then treated with **7** to afford glucoconjugate **28** (91%). Hydrogenolysis of **22**, **25,** and **28** with Pd/C (10%) in THF–MeOH at room temperature gave the target compounds **23** (85%), **26** (86%), and **29** (81%), respectively.

The above-synthesized derivatives were evaluated in an enzyme inhibition assay against rabbit muscle glycogen phosphorylase a (RMGPa) (Table 1), an enzyme that shares considerable sequence similarity with human liver GPa. As described previously,[13] the activity of rabbit muscle GPa was measured through detecting the release of phosphate from glucose-1-phosphate in the direction of glycogen synthesis.

It is interesting to notice that glucosides modified at 6-position (compounds **8** and **9**) also exhibit GP inhibition activity, since only anomerically modified glucose derivatives have been reported as GP inhibitors.[14] Compared to oleanolic acid **1**, modification at either 3- or 28-position with an alkyl group diminished the inhibitory activity (**1** vs **3**, **5,** and **21**). Conjugation of oleanolic acid with glucosides exhibited moderate to good inhibitory activity against rabbit muscle GPa. SAR analysis shows that the distance between the triterpene moiety and sugar moiety is important: a shorter linker led to a better GPa inhibitory activity (e.g., **12** vs **18**, **20**, **23,** and **26**). The different potency between compounds **17** vs **18**, **19** vs **20**, **22** vs **23**, and **25** vs **26** indicated that with a long carbon-chain linker, benzyl-protected sugar derivatives displayed stronger affinity toward GPa. With a shorter linker, hydrophilic conjugates showed better inhibitory activity (**11** vs **12**, **14** vs **15**, and **28** vs **29**). Presence of an ester function at the C-6 position of the sugar is also beneficial (**17** vs **19**, **8** vs **9**). The 3-hydroxyl and 28-carboxyl groups of triterpene moiety seem to contribute to the GPa enzyme inhibitory activity (**12** vs **14** and **15**), although it is less predictable. Nevertheless, modification at the 28-carboxyl group led to more potent inhibitors (**12** and **17**) than those modified at the 3-position (**15** and **29**). The presence of an azido group was not favorable to inhibition (e.g., **2** and **10**). The acetyl group in the sugar moiety could decrease the potency (**15** vs **16**). Within this series of compounds, triterpene–glucoside conjugate **12** is the most potent GP inhibitor with an IC_{50} value of 1.14 μM. This value is much lower than that of the parent oleanolic acid (IC_{50} = 14 μM).[5,6]

To explore the corresponding binding site and mode of the glucoconjugates of oleanolic acid for GP inhibition, the most potent compound **12** was selected for subsequent molecular docking simulation. Both the dimer interface site and the catalytic site are buried deeply in their respective binding cavities and are extremely spatially constrained, indicating a low compatibility between the



Scheme 3. Reagents and conditions: (a) $CuSO_4$, sodium ascorbate, CH_2Cl_2–H_2O, rt, 83–93%; (b) MeONa, MeOH, rt, for **12**: 43%; for **14**: 78%; and (c) H_2, Pd/C (10%), THF–MeOH, rt, 38–85%.

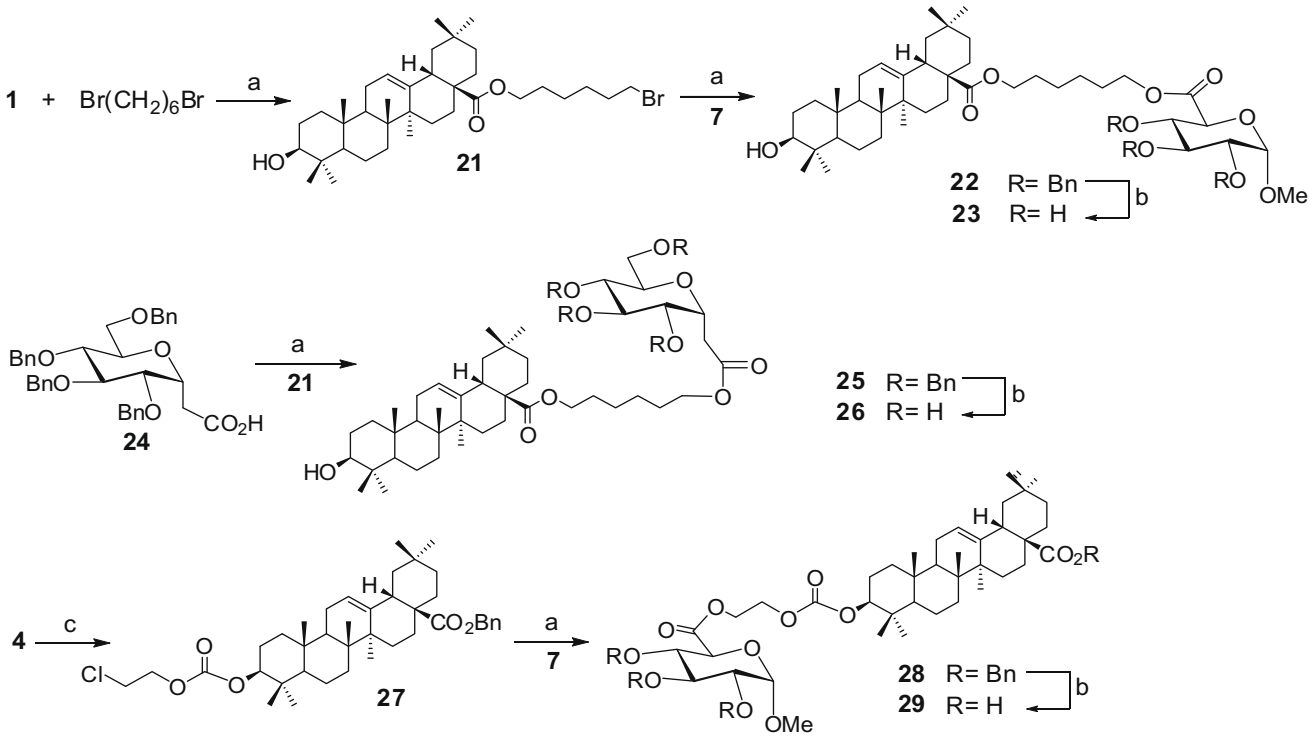
highly extended and flexible glucoconjugates of oleanolic acid and these two binding sites. This assumption was proved by molecular docking simulations during which no rational binding position of compound **12** was observed for either site. Compound **12** was predicted to bind at the T-state allosteric site exclusively as shown in Figure 1a due to the steric and volume incompatibility with the catalytic site, and no reasonable binding attitudes against this site were observed from the docking results (data not shown). The top-ranked binding position of **12** at the T-state allosteric site of GP was determined as a preferred docking mode with the calculated Glide GScore of −10.086 (as shown in Fig. 1), which is superior to the docking result against the inhibitor site with GScore value of −7.31. The predicted binding position of the oleanolic acid moiety of compound **12** occupied the same location as the T-state allosteric site of asiatic acid[5] in the crystal structure (see Fig. 1c). However, the newly attached sugar moiety shielded the carboxyl group of oleanolic acid from forming the salt bridge with Arg310 (A), and consequently reversed the orientation of the oleanolic acid as well as propelled the sugar moiety to extend more deeply into the alloteric dimer interface. As a result, a new hydrogen-bonding network formed between the sugar hydroxyls and the carboxyl of Asp227 (A) plus the guanidino of Arg193 (A), respectively, enhancing their contribution to the stability of the complex (Fig. 1b). This preferred binding pose was stabilized by extra hydrogen-bonding networks involving the 3-hydroxyl of the triterpene moiety and the guandino groups of Arg242 (A) and Arg309 (A) plus the carboxyl of Asp306 (A). These hydrogen-bonding networks were critical for maintaining the good activity and highlighted the contribution of unshielded hydroxyls in the sugar moiety (**12** vs **11**). Other interactions contributing to the high affinity of **12** included hydrophobic contacts between pentacyclic triterpenes and Tyr75 (A), Val45 (B), and Phe196 (A).

In summary, a series of glucoconjugates of oleanolic acid have been synthesized using click chemistry and esterification methods. The results of GP inhibition assay and SAR analysis indicate a clear preference for hydrophobic groups with a long chain linker and hydrophilic groups with a short chain linker. Further research and drug development on pentacyclic triterpenes as promising modulators of glycogen metabolism are ongoing in our laboratories, and the results will be reported in due course.

3. Experimental

3.1. Chemistry

All commercially available solvents and reagents were used without further purification. Melting points were measured on a RY-1 melting point apparatus. Column chromatography was carried out on E. Merck Silica Gel 60 (230–400 mesh) or on silica gel (200–300 mesh, Qindao Ocean Chemical Company, China). IR spectra were recorded on a Shimadzu FTIR-8400S spectrometer. ^1H and ^{13}C NMR spectra were measured on Bruker AV-300 or AGH-250 spectrometers. Chemical shifts are reported as values from an internal tetramethylsilane standard. Mass spectral data



Scheme 4. Reagents and conditions: (a) K_2CO_3, DMF, rt or 40 °C, 52–94%; (b) H_2, Pd/C (10%), THF–MeOH, rt, 81–86%; and (c) $ClCH_2CH_2OCOCl$, K_2CO_3, DMAP, CH_2Cl_2, 35 °C, 92%.

Table 1
IC_{50} values (μM) for RMGPa inhibition assay results

Compd	GPa IC_{50}[a]	Compd	GPa IC_{50}[a]
1[b]	14	19	136.5
2	na[c]	20	na
3	405	22	50.4
4[b]	461	23	na
21	32.7	25	41.6
6	na	26	na
7	na	5	26.5
8	37.2	13	466.9
9	289.3	14	na
10	na	15	66.2
24	na	16	na
11[6]	na	27	574.7
12	1.14	28	na
17	11.6	29	62.6
18	na	Caffeine	83.1

[a] Values are means of three experiments.
[b] See Ref.[6].
[c] na = no activity.

were obtained on an Agilent 1100 LC/DAD/MSD spectrometer or Q-Tof Micro MS/MS spectrometer. Elementary analysis was measured on a Vario EL III instrument (Elementar, Germany). Optical rotations were measured using a Perkin–Elmer 141 polarimeter or Jasco P-2000 polarimeter.

3.1.1. General procedure for the esterification of oleanolic acid or uronic acid

To a solution of the carboxylic acid (3.4 mmol) in DMF (8 mL) were added alkyl bromide (3.8 mmol) and K_2CO_3 (6.8 mmol). The reaction mixture was stirred at rt for 6–18 h, then concentrated. The residue was diluted with EtOAc (50 mL), and the extract was washed successively with 1 N HCl, water, satd $NaHCO_3$, water and brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography.

3.1.2. General procedure for the O-alkylation of oleanolic acid or glucoside

A solution of alcohol (0.22 mmol) in anhyd THF (2 mL) was added dropwise to a suspension of NaH (60%, 0.56 mmol) in anhyd THF (2 mL) at 0 °C. After 30 min of further stirring at 0 °C, alkyl bromide (0.48 mmol) was added dropwise, and the mixture was stirred at rt for 12 h. MeOH (0.1 mL) was added at 0 °C. After stirring for 15 min, the mixture was concentrated under reduced pressure. The residue was diluted in EtOAc, washed successively with water and brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography.

3.1.3. General procedure for the click reaction

To a solution of alkyne (0.51 mmol) and azide (0.51 mmol) in CH_2Cl_2 (2 mL) and H_2O (2 mL) were added $CuSO_4{\cdot}5H_2O$ (0.2 mmol) and sodium ascorbate (0.4 mmol). The resulting solution was stirred at rt for 8 h. The reaction mixture was extracted with CH_2Cl_2 (3×). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography.

3.1.4. General procedure for the deacetylation

To a solution or suspension of the acetate (0.2 mmol) in dry MeOH (10 mL) was added a solution of MeONa (0.2 M in MeOH, 40 mL) with stirring at rt for 24 h. The mixture was concentrated in vacuo, and the residue was taken up in EtOAc (50 mL), washed successively with 1 N HCl (3 × 25 mL), water (3 × 15 mL) and brine (3 × 15 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography.

3.1.5. General procedure for the debenzylation

To a solution of benzyl ether or ester (0.117 mol) in THF (1.5 mL) and MeOH (1.5 mL) was added 10% Pd/C (0.09 g), with stirring under hydrogen atmosphere at rt for 12 h. The catalyst was filtered off, and the filtrate was concentrated to give a crude product that was purified by flash column chromatography.

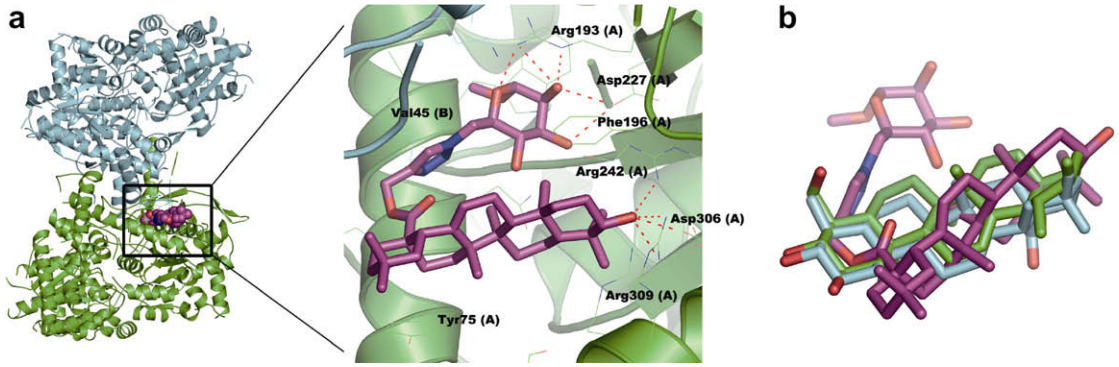


Figure 1. Stereoview of the binding mode of compound **12** at the dimer allosteric site of RMGP (a) and enlarged view of binding site (b) with residues around the docked position within 4.0 Å (labeled by capital letter A or B to indicate which chain they belong to). The comparison of docking modes of **12** and oleanolic acid, and asiatic acid crystallized conformation of 2QN1 is shown in c. The RMGP structure is presented in the cartoon with green and cyan indicating each monomer, respectively. The residues of the binding site are shown in line format, and compound **12**, oleanolic acid and asiatic acid are shown as stick structures. The coloring rules for **12** are as follows: carbon atoms, magenta; oxygen atoms, red; nitrogen atoms, blue; respectively. The carbon atoms of oleanolic acid and asiatic acid are colored in cyan and green, respectively. All hydrogen atoms are omitted for clarity. The hydrogen bonds in b are represented by red-dashed lines.

3.1.6. 6-Azidohexyl 3β-hydroxyolean-12-en-28-oate (2)

This compound was prepared from **1** (1.55 g, 3.4 mmol) and 1-azido-6-bromohexane (0.78 g, 3.8 mmol) according to the general procedure 3.1.1. The residue was purified by column chromatography (1:6 EtOAc–petroleum ether). Yield: 1.82 g, 92%; white solid, mp 73–75 °C; R_f 0.5 (2:7 EtOAc–petroleum ether). $[\alpha]_D$ +53.9 (*c* 0.55, CH_2Cl_2). IR (KBr, cm^{-1}): 3514, 3402, 2942, 2863, 2095, 1721, 770, 759. ^1H NMR (300 MHz, $CDCl_3$): δ 0.73, 0.78, 0.90, 0.91, 0.92, 0.99, 1.13 (7s, each 3H, 7 × CH_3), 0.73–1.97 (m, 30H), 2.88 (dd, 1H, $J_{18,19a}$ 3.9, $J_{18,19b}$ 13.5 Hz, H-18), 3.20 (t, 1H, *J* 5.7 Hz, H-3), 3.27 (t, 2H, *J* 6.8 Hz, CH_2N_3), 4.01 (t, 2H, *J* 6.4 Hz, CO_2CH_2), 5.27 (br s, 1H, H-12). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.3, 15.6, 17.1, 18.3, 23.1, 23.4, 23.6, 25.7, 25.9, 26.4, 27.2, 27.7, 28.1, 28.5, 28.8, 30.7, 32.5, 32.8, 33.1, 33.9, 37.1, 38.5, 38.8, 39.4, 41.4, 41.7, 45.9, 46.7, 47.6, 51.4, 55.3, 64.0, 79.0, 122.4, 143.9, 177.7. ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{36}H_{59}N_3NaO_3$: 604.4; found: 604.5.

3.1.7. Propargyl 3β-hydroxyolean-12-en-28-oate (3)

This compound was prepared from **1** (1 g, 2.2 mmol) and propargyl bromide (0.27 mL, 2.4 mmol) according to the general procedure 3.1.1. The residue was purified by column chromatography (1:6 EtOAc–petroleum ether). Yield: 1.05 g, 97%; white solid, mp 121–122 °C; R_f 0.33 (1:4 EtOAc–petroleum ether). $[\alpha]_D$ +67.9 (*c* 0.50, CH_2Cl_2). IR (KBr, cm^{-1}): 3308, 2945, 2866, 1731, 1157, 1032, 739. ^1H NMR (300 MHz, $CDCl_3$): δ 0.74, 0.77, 0.92, 0.98, 1.13 (5s, each 3H, 5 × CH_3), 0.90 (s, 6H, 2 × CH_3), 0.71–2.04 (m, 22H), 2.41 (t, 1H, *J* 2.6 Hz, CH), 2.87 (dd, 1H, $J_{18,19a}$ 4.1, $J_{18,19b}$ 9.5 Hz, H-18), 3.21 (dd, 1H, $J_{2a,3}$ 5.1, $J_{2b,3}$ 10.7 Hz, H-3), 4.56 (dd, 1H, *J* 2.6, 15.4 Hz, CO_2CH_2), 4.68 (dd, 1H, *J* 2.6, 15.4 Hz, CO_2CH_2), 5.30 (t, 1H, *J* 3.5 Hz, H-12). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.3, 15.6, 17.1, 18.3, 23.0, 23.4, 23.6, 25.8, 27.2, 27.7, 28.1, 30.7, 32.2, 32.8, 33.1, 33.8, 37.0, 38.5, 38.8, 39.4, 41.3, 41.7, 45.9, 46.8, 47.6, 51.6, 55.2, 74.4, 78.1, 79.0, 122.63, 143.4, 176.8. ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{33}H_{50}NaO_3$: 517.4; found: 517.3.

3.1.8. Benzyl 3-*O*-propargyl-3β-hydroxyolean-12-en-28-oate (5)

This compound was prepared from **4** (1.0 g, 1.8 mmol) and propargyl bromide (0.8 mL, 7.2 mmol) according to the general procedure 3.1.2. The residue was purified by column chromatography (1:40 EtOAc–petroleum ether). Yield: 0.94 g, 88%; white solid, mp 153–155 °C; R_f 0.51 (1:6 EtOAc–petroleum ether). $[\alpha]_D$ +71.0 (*c* 0.48, CH_2Cl_2). IR (KBr, cm^{-1}): 3308, 2945, 2865, 1725, 1076, 758, 696, 666. ^1H NMR (300 MHz, $CDCl_3$): δ 0.61, 0.78, 0.89, 0.90, 0.92, 0.99, 1.13 (7s, each 3H, 7 × CH_3), 0.61–1.85 (m, 22H), 2.36 (t, 1H, *J* 2.4 Hz, CH), 2.91 (dd, 1H, $J_{18,19a}$ 4.1, $J_{18,19b}$ 13.9 Hz, H-18), 3.01 (dd, 1H, $J_{2a,3}$ 4.3, $J_{2b,3}$ 11.7 Hz, H-3), 4.14 (dd, 1H, *J* 2.4, 16.0 Hz, CH–O), 4.23 (dd, 1H, *J* 2.4, 15.9 Hz, CH-O), 5.06 (d, 1H, *J* 12.5 Hz, CHPh), 5.08 (d, 1H, *J* 12.5 Hz, CHPh), 5.29 (t, 1H, *J* 3.5 Hz, H-12), 7.31–7.35 (m, 5H, Ph-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.3, 16.4, 17.0, 18.4, 22.6, 23.2, 23.5, 23.7, 25.9, 27.8, 28.2, 30.7, 32.5, 32.9, 33.1, 34.0, 37.1, 38.5, 38.6, 39.5, 41.6, 41.9, 46.1, 46.9, 47.8, 56.0, 56.5, 66.0, 73.3, 73.4, 81.1, 86.1, 122.7, 127.9, 128.0, 128.4, 136.6, 143.8, 177.4. ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{40}H_{56}NaO_3$: 607.4; found: 607.3.

3.1.9. Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosiduronic acid (7)

KBr (0.028 g, 0.24 mmol), $NaHCO_3$ (5%, 10 mL), 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO, 0.374 g, 2.4 mmol) and NaOCl (5%, 1.8 mL) were added at 0 °C to a solution of **6** (1.0 g, 2.2 mmol) in acetone (15 mL). After the mixture had been stirred for 1 h at 0 °C, additional NaOCl (5%, 4.9 mL) was added, and the mixture was stirred further at rt for 20 h. The solution was concentrated and diluted in EtOAc (30 mL), washed with 10% HCl and brine, dried ($MgSO_4$), filtered, and concentrated to give a crude yellow oil. Purification by flash column chromatography (1:1 EtOAc–petroleum ether) gave **7** (1.0 g, 92%) as a yellow oil. R_f 0.7 (7:1 CH_2Cl_2–MeOH). IR (KBr, cm^{-1}): 3063, 3031, 2932, 2874, 1748, 1731, 1089, 1071, 1050, 1029, 770, 737, 698. ^1H NMR (300 MHz, $CDCl_3$): δ 3.48 (s, 3H, OCH_3), 3.62 (dd, 1H, $J_{1,2}$ 3.5, $J_{2,3}$ 9.7 Hz, H-2), 3.74 (dd, 1H, *J* 8.8, 9.9 Hz, H-4), 4.06 (t, 1H, *J* 9.4 Hz, H-3), 4.28 (d, 1H, *J* 9.9 Hz, H-5), 4.66–5.03 (m, 7H, H-1, 3 × $PhCH_2$), 7.27–7.40 (m, 15H, Ph-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.8, 69.4, 73.6, 75.3, 75.9, 79.1, 79.3, 81.4, 98.6, 127.7, 127.93, 127.96, 128.1, 128.11, 128.18, 128.4, 128.42, 128.5, 137.4, 137.8, 138.4, 173.0. ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{28}H_{30}NaO_7$: 501.2; found: 501.1.

3.1.10. Propargyl (methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosid)uronate (8)

This compound was prepared from **7** (0.28 g, 0.59 mmol) and propargyl bromide (0.072 mL, 0.64 mmol) according to the general procedure 3.1.1. The residue was purified by column chromatography (1:7 EtOAc –petroleum ether). Yield: 0.29 g, 97%, colorless oil; R_f 0.48 (1:4 EtOAc–petroleum ether). $[\alpha]_D$ +34.1 (*c* 0.71, CH_2Cl_2). IR (KBr, cm^{-1}): 3281, 2917, 1752, 1609, 1509, 1452, 1359, 1180, 1091, 1071, 1044, 697. ^1H NMR (250 MHz, $CDCl_3$): δ 2.37 (t, 1H, *J* 2.3 Hz, CH), 3.33 (s, 3H, OCH_3), 3.50 (dd, 1H, $J_{1,2}$ 3.6, $J_{2,3}$ 9.6 Hz, H-2), 3.67 (t, 1H, *J* 9.5 Hz, H-4), 3.92 (t, 1H, *J* 9.4 Hz, H-3), 4.18 (d, 1H, *J*

10.0 Hz, H-5), 4.53 (d, 1H, $J_{1,2}$ 3.3 Hz, H-1), 4.54–4.91 (m, 8H, CO_2CH_2 and 3 × $PhCH_2$), 7.16–7.28 (m, 15H, Ph-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 52.8, 55.7, 70.1, 73.5, 75.1, 75.5, 75.8, 76.8, 79.2, 79.4, 81.3, 98.7, 127.6, 127.7, 127.8, 127.92, 127.97, 128.1, 128.25, 128.3, 128.4, 137.8, 137.9, 138.5, 168.8. ESIMS *m/z*: $[M+NH_4]^+$ calcd for $C_{31}H_{36}NO_7$: 534.2; found: 534.3; $[M+Na]^+$ calcd for $C_{31}H_{32}NaO_7$: 539.2; found: 539.2.

3.1.11. Methyl 2,3,4-tri-*O*-benzyl-6-*O*-propargyl-α-D-glucopyranoside (9)

This compound was prepared from **6** (0.1 g, 0.22 mmol) and propargyl bromide (0.052 mL, 0.48 mmol) according to the general procedure 3.1.2. The residue was purified by column chromatography (1:6 EtOAc–petroleum ether). Yield: 93 mg, 86%, colorless oil; R_f 0.4 (1:2 EtOAc–petroleum ether,). IR (KBr, cm^{-1}): 3292, 2917, 2872, 1728, 1510, 1453, 1359, 1159, 1134, 1094, 1070, 1049, 1030, 740, 698. ^1H NMR (300 MHz MHz, $CDCl_3$): δ 2.43 (t, 1H, *J* 2.4 Hz, CH), 3.44 (s, 3H, OCH_3), 3.62 (dd, 1H, $J_{1,2}$ 3.7, $J_{2,3}$ 9.6 Hz, H-2), 3.65 (t, 1H, *J* 10.0 Hz, H-4), 3.70 (dd, 1H, $J_{5,6}$ 2.0, J_{gem} 10.0 Hz, H-6), 3.80–3.85 (m, 1H, H-5), 3.91 (dd, 1H, $J_{5,6'}$ 3.5, J_{gem} 10.1 Hz, H-6′), 4.05 (t, 1H, *J* 9.2 Hz, H-3), 4.18 (dd, 1H, *J* 2.4, 16.0 Hz, OCHC≡C), 4.27 (dd, 1H, *J* 2.6, 15.8 Hz, OCHC≡C), 4.67 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 4.70–5.07 (m, 6H, 3 × $PhCH_2$), 7.30–7.47 (m, 15H, Ph-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.1, 58.5, 68.0, 69.8, 73.3, 74.8, 74.9, 75.6, 79.4, 79.7, 82.0, 98.2, 127.5, 127.6, 127.82, 127.83, 128.1, 128.3, 128.4, 138.1, 138.4, 138.8. ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{31}H_{34}NaO_6$: 525.2; found: 525.2.

3.1.12. [1-(Methyl 2,3,4-tri-*O*-acetyl-6-deoxy-α-D-glucopyranosid-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl 3β-hydroxyolean-12-en-28-oate (11)

This compound was prepared from **3** (0.25 g, 0.51 mmol) and **10** (0.18 g, 0.51 mmol) according to the general procedure 3.1.3. The residue was purified by column chromatography (3:2 EtOAc–petroleum ether). Yield: 0.39 g, 93%, white solid, mp 138–139 °C. R_f 0.13 (1:2 EtOAc–petroleum ether). $[\alpha]_D$ +95.2 (*c* 0.11, $CHCl_3$). IR (KBr, cm^{-1}): 2946, 2866, 1750, 1246, 1221, 1046, 769. ^1H NMR (250 MHz, $CDCl_3$): δ 0.54, 0.71, 0.83, 0.91, 1.04 (5s, each 3H, 5 × CH_3), 0.81 (s, 6H, 2 × CH_3), 0.54–1.82 (m, 22H), 1.94, 2.00, 2.03 (3s, each 3H, 3 × CH_3CO), 2.75 (m, 1H, H-18), 3.03 (s, 3H, OCH_3), 3.11–3.17 (m, 1H, H-3), 4.03–4.14 (m, 1H, H-5^{Glc}), 4.20–4.29 (m, 1H, H-6^{Glc}), 4.51 (dd, 1H, $J_{5,6'}$ 1.9, J_{gem} 14.1 Hz, H-6′^{Glc}), 4.74–4.80 (m, 2H, H-2^{Glc},4^{Glc}), 4.83 (d, $J_{1,2}$ 3.5 Hz, H-1^{Glc}), 5.10 (s, 2H, CO_2CH_2), 5.21 (br s, 1H, H-12), 5.41 (t, 1H, *J* 9.6 Hz, H-3^{Glc}), 7.64 (s, 1H, CH=N). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.3, 15.5, 16.8, 18.3, 20.5, 20.6, 22.9, 23.3, 23.5, 25.8, 27.2, 27.6, 28.1, 30.6, 32.3, 32.7, 33.0, 33.8, 37.0, 38.4, 38.7, 39.3, 41.3, 41.7, 45.8, 46.6, 47.5, 50.7, 55.2, 55.4, 57.4, 67.8, 69.6, 70.0, 70.7, 78.9, 96.6, 122.5, 125.4, 143.2, 143.5, 169.78, 169.81, 170.0, 177.5. ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{46}H_{69}N_3NaO_{11}$:862.5; found: 862.3. $[M+H]^+$ calcd for $C_{46}H_{70}N_3O_{11}$: 840.5; found: 840.4. Anal. Calcd for $C_{46}H_{69}N_3O_{11}$: C, 65.77; H, 8.28; N, 5.00. Found: C, 65.21; H, 8.30; N, 4.89.

3.1.13. 3-*O*-[1-(Methyl 6-deoxy-α-D-glucopyranosid-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl 3β-hydroxyolean-12-en-28-oate (12)

This compound was prepared from **11** (0.18 g, 0.2 mmol) according to the general procedure 3.1.4. The residue was purified by column chromatography (8:1 EtOAc–petroleum ether). Yield: 66 mg, 43%, white solid, mp 142–143 °C. R_f 0.15 (EtOAc). $[\alpha]_D$ +56.8 (*c* 0.17, MeOH). IR (KBr, cm^{-1}): 3416, 2944, 2873, 1727, 1157, 1049, 1009. ^1H NMR (300 MHz, $CDCl_3$ + D_2O): δ 0.63, 0.78, 0.98, 1.12, 1.24 (5s, each 3H, 5 × CH_3), 0.89 (s, 6H, 2 × CH_3), 0.63–1.87 (m, 22H), 2.83 (d, 1H, *J* 11.0 Hz, H-18), 3.10–3.16 (m, 2H), 3.19 (3H, s, OCH_3), 3.41 (1H, dd, $J_{1,2}$ 2.9, $J_{2,3}$ 9.4 Hz, H-2^{Glc}), 3.73 (t, 1H, *J* 9.0 Hz, H-4^{Glc}), 3.88 (t, 1H, *J* 7.0 Hz, H-3^{Glc}), 4.55 (m, 1H), 4.68–4.77 (m, 2H), 5.14 (d, 1H, J_{gem} 12.7 Hz, CO_2CH), 5.18 (d, 1H, J_{gem} 12.7 Hz, CO_2CH), 5.27 (s, 1H, H-12), 7.74 (s, 1H, CH=N). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.4, 15.6, 16.9, 18.4, 23.1, 23.4, 23.7, 25.8, 27.2, 27.7, 28.2, 29.7, 30.7, 32.4, 32.8, 33.0, 33.9, 37.1, 38.6, 38.8, 39.4, 41.4, 41.8, 45.9, 46.8, 47.7, 55.3, 55.7, 57.6, 77.2, 79.0, 122.6, 143.6. ESIMS *m/z*: $[M+K]^+$ calcd for $C_{40}H_{63}KN_3O_8$: 752.4; found: 752.4. $[M+H]^+$ calcd for $C_{40}H_{64}N_3O_8$: 714.5; found: 714.5.

3.1.14. Benzyl 3-*O*-{[(methyl 2,3,4-tri-*O*-acetyl-6-deoxy-α-D-glucopyranosid-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl} 3β-hydroxyolean-12-en-28-oate (13)

This compound was prepared from **5** (0.4 g, 0.68 mmol) and **10** (0.239 g, 0.69 mmol) according to the general procedure 3.1.3. The residue was purified by column chromatography (1:1 EtOAc–petroleum ether). Yield: 0.53 g, 83%, white solid, mp 107–108 °C. R_f 0.18 (1:2 EtOAc–petroleum ether). $[\alpha]_D$ +102.5 (*c* 0.11, $CHCl_3$). IR (KBr, cm^{-1}): 2947, 2866, 1748, 1368, 1247, 1221, 1046, 760. ^1H NMR (250 MHz, $CDCl_3$): δ 0.42, 0.57, 0.72, 0.74, 0.93 (5s, each 3H, 5 × CH_3), 0.70 (s, 6H, 2 × CH_3), 0.42–1.66 (m, 22H), 1.83, 1.89, 1.92 (3s, each 3H, 3 × $COCH_3$), 2.76–2.81 (m, 2H, H-3, H-18), 2.94 (s, 3H, OCH_3), 3.96–4.04 (m, 1H, H-5^{Glc}), 4.17 (dd, 1H, $J_{5,6}$ 8.6, J_{gem} 14.1 Hz, H-6^{Glc}), 4.35–4.44 (2H, m, H-6′^{Glc}, OCH), 4.57–4.73 (m, 4H, H-1^{Glc}, H-2^{Glc}, H-4^{Glc}, OCH), 4.88 (d, 1H, J_{gem} 12.5 Hz, PhCH), 4.90 (d, 1H, J_{gem} 12.5 Hz, PhCH), 5.12 (t, 1H, *J* 4.0 Hz, H-12), 5.30 (t, 1H, *J* 9.6 Hz, H-3^{Glc}), 7.13–7.17 (m, 5H, Ph-H), 7.46 (s, 1H, CH=N). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.3, 16.4, 16.8, 18.2, 20.6, 20.61, 20.63, 22.64, 23.0, 23.4, 23.6, 25.8, 27.6, 28.1, 30.7, 32.3, 32.7, 33.1, 33.8, 36.9, 38.2, 38.6, 39.3, 41.4, 41.6, 45.8, 46.7, 47.6, 50.7, 55.4, 55.6, 63.0, 65.9, 67.9, 69.7, 70.1, 70.7, 86.6, 96.6, 122.5, 123.7, 127.8, 127.9, 128.3, 136.4, 143.6, 146.6, 169.8, 169.82, 170.1, 177.4. ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{53}H_{75}N_3NaO_{11}$: 952.5; found: 952.5. $[M+H]^+$ calcd for $C_{53}H_{76}N_3O_{11}$: 930.5; found: 930.5. Anal. Calcd for $C_{53}H_{75}N_3O_{11}$: C, 68.44; H, 8.13; N, 4.52. Found: C, 68.16; H, 8.15; N, 4.40.

3.1.15. Benzyl 3-*O*-{[1-(methyl 6-deoxy-α-D-glucopyranosid-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl} 3β-hydroxyolean-12-en-28-oate (14)

This compound was prepared from **13** (0.2 g, 0.22 mmol) according to the general procedure 3.1.4. The residue was purified by column chromatography (EtOAc). Yield: 0.134 g, 78%, white solid, mp 116–118 °C, R_f 0.43 (7:1 CH_2Cl_2–MeOH). $[\alpha]_D$ +85.5 (*c* 0.11, $CHCl_3$). IR (KBr, cm^{-1}): 3419, 2943, 2865, 1725, 1156, 1097, 1052, 1011, 751, 696. ^1H NMR (300 MHz, $CDCl_3$ + D_2O): δ 0.60, 0.74, 0.87, 0.91, 1.11 (5s, each 3H, 5 × CH_3), 0.89 (s, 6H, 2 × CH_3), 0.60–1.97 (m, 22H), 2.88–2.96 (m, 2H, H-18, H-3), 3.14 (s, 3H, OCH_3), 3.18 (m, 1H, H-2^{Glc}), 3.43 (dd, 1H, *J* 3.0, 9.6 Hz, H-5^{Glc}), 3.72 (t, 1H, *J* 9.2 Hz, H-4^{Glc}), 3.86 (t, 1H, *J* 7.3 Hz, H-3^{Glc}), 4.46–4.57 (m, 2H, H-6^{Glc}), 4.66 (d, 1H, $J_{1,2}$ 3.2 Hz, H-1^{Glc}), 4.76 (d, 2H, *J* 12.9 Hz, OCH_2), 5.05 (d, 1H, J_{gem} 12.4 Hz, PhCH), 5.07 (d, 1H, J_{gem} 12.4 Hz, PhCH), 5.29 (br s, 1H, H-12), 7.28–7.33 (m, 5H, Ph-H), 7.68 (s, 1H, CH=N). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.3, 16.5, 17.0, 18.3, 22.8, 23.1, 23.5, 23.7, 25.9, 27.7, 28.2, 30.7, 32.4, 32.8, 33.1, 33.9, 37.0, 38.4, 38.8, 39.4, 41.5, 41.8, 46.0, 46.8, 47.7, 51.0, 55.5, 55.8, 63.2, 65.9, 70.2, 71.0, 72.1, 74.1, 87.0, 99.7, 122.6, 124.2, 127.9, 128.0, 128.4, 136.5, 143.7, 146.3, 177.4. ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{47}H_{69}NaN_3O_8$: 826.5; found: 826.5. $[M+H]^+$ calcd for $C_{47}H_{70}N_3O_8$: 804.5; found: 804.5. HRESIMS: $[M+H]^+$ calcd for $C_{47}H_{70}N_3O_8$: 804.5157; Found: 804.5183.

3.1.16. 3-*O*-{[1-(Methyl 6-deoxy-α-D-glucopyranosid-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl} 3β-hydroxyolean-12-en-28-oic acid (15)

This compound was prepared from **14** (0.094 g, 0.117 mol) according to the general procedure 3.1.5. The residue was purified by column chromatography (EtOAc). Yield: 67 mg, 81%, white solid, mp 249–251 °C, R_f 0.17 (15:1 CH_2Cl_2–MeOH). $[\alpha]_D$ +147 (*c*

0.06, MeOH). IR (KBr, cm^{-1}): 3416, 2942, 2874, 1710, 1464, 1386, 1147, 1099, 1051, 1035, 1011. ^1H NMR (300 MHz, pyridine-d_5): δ 0.83, 0.86, 0.96, 1.00, 1.01, 1.02, 1.27 (7s, each 3H, 7 × CH_3), 0.83–2.14 (m, 22H), 3.05 (dd, 1H, *J* 3.5, 11.0 Hz, H-18), 3.24 (s, 3H, OCH_3), 3.31 (dd, 1H, *J* 3.3, 13.3 Hz, H-3), 3.82 (t, 1H, *J* 9.3 Hz, H-4^{Glc}), 4.03 (dd, 1H, $J_{1,2}$ 3.6, $J_{2,3}$ 9.5 Hz, H-2^{Glc}), 4.44–4.50 (m, 2H, H-6^{Glc}), 4.79 (d, 1H, J_{gem} 12.0 Hz, OCH), 4.85 (q, 1H, *J* 8.1 Hz, H-5^{Glc}), 4.99 (d, 1H, J_{gem} 12.2 Hz, OCH), 5.06 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1^{Glc}), 5.30 (m, 1H, H-3^{Glc}), 5.49 (br s, 1H, H-12), 8.23 (s, 1H, CH=N). ^{13}C NMR (75 MHz, pyridine-d_5): δ 15.5, 16.9, 17.5, 18.7, 23.0, 23.8, 26.2, 28.4, 31.0, 33.3, 34.4, 37.3, 38.6, 39.0, 39.9, 42.1, 42.3, 46.6, 46.8, 48.1, 52.1, 55.2, 56.1, 63.3, 71.9, 73.0, 73.7, 75.2, 86.2, 101.6, 122.6, 124.7, 144.9, 146.3, 180.1. Negative-mode ESIMS *m/z*: $[M-H]^+$ calcd for $C_{40}H_{62}N_3O_8$: 712.5; found: 712.6. Positive-mode ESIMS *m/z*: $[M+H]^+$ calcd for $C_{40}H_{64}N_3O_8$: 714.5; found: 714.4 $[M+H]^+$. Anal. Calcd for $C_{40}H_{63}N_3O_8{\cdot}0.5H_2O$: C, 66.45; H, 8.92; N, 5.81. Found: C, 66.41; H, 9.02; N, 5.79.

3.1.17. 3-*O*-{[1-(Methyl 2,3,4-tri-*O*-acetyl-6-deoxy-α-D-glucopyranosid-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl} 3β-hydroxyolean-12-en-28-oic acid (16)

This compound was prepared from **13** (0.1 g, 0.108 mmol) according to the general procedure 3.1.5. The residue was purified by column chromatography (2:1 EtOAc–petroleum ether). Yield: 0.077 g, 85%, white solid, mp 262–263 °C, R_f 0.61 (3:1 EtOAc–petroleum ether). $[\alpha]_D$ +237.8 (*c* 0.06, MeOH). IR (KBr, cm^{-1}): 3438, 2945, 2873, 1755, 1463, 1368, 1223, 1075, 1046, 602. ^1H NMR (300 MHz, $CDCl_3$): δ 0.74, 0.75, 0.88, 0.90, 0.91, 0.92, 1.11 (7s, each 3H, 7 × CH_3), 0.74–1.87 (m, 22H), 2.00, 2.06, 2.10 (3s, each 3H, 3 × $COCH_3$), 2.81 (dd, 1H, *J* 3.5, 13.0 Hz, H-18), 2.98 (dd, 1H, *J* 3.8, 11.3 Hz, H-3), 3.12 (s, 3H, OCH_3), 4.18 (t, 1H, *J* 8.4 Hz, H-4^{Glc}), 4.34 (dd, 1H, $J_{5,6}$ 8.6, J_{gem} 14.0 Hz, H-6^{Glc}), 4.54–4.61 (m, 2H, H-$6'^{Glc}$, H-1^{Glc}), 4.75–4.91 (m, 4H, OCH_2, H-2^{Glc}, H-5^{Glc}), 5.27 (br s, 1H, H-12), 5.47 (t, 1H, *J* 9.7 Hz, H-3^{Glc}), 7.63 (s, 1H, CH=N). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.3, 16.4, 17.2, 18.3, 20.6, 22.8, 23.0, 23.5, 23.6, 25.9, 27.7, 28.2, 30.7, 32.5, 32.7, 33.0, 33.9, 37.1, 38.4, 38.8, 39.4, 41.1, 41.7, 45.9, 46.7, 47.7, 50.8, 55.5, 55.8, 63.2, 68.0, 69.8, 70.2, 70.9, 86.7, 96.7, 122.7, 123.7, 143.6, 146.7, 169.8, 170.1, 182.5. ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{46}H_{69}NaN_3O_{11}$: 862.5; found: 862.5. $[M+H]^+$ calcd for $C_{46}H_{70}N_3O_{11}$: 840.5; found: 840.5. Anal. Calcd for $C_{46}H_{69}N_3O_{11}{\cdot}0.5H_2O$: C, 65.07; H, 8.31; N, 4.95. Found: C, 64.93; H, 8.17; N, 5.09.

3.1.18. 6-[4-(Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosiduronylmethyl)-1*H*-1,2,3-triazol-1-yl]hexyl 3β-hydroxyolean-12-en-28-oate (17)

This compound was prepared from **2** (0.162 g, 0.28 mmol) and **8** (0.144 g, 0.28 mmol) according to the general procedure 3.1.3. The residue was purified by column chromatography (3:2 EtOAc–petroleum ether). Yield: 0.278 g, 88%, white solid, mp 73–74 °C, R_f 0.15 (1:2 EtOAc–petroleum ether). $[\alpha]_D$ +23.1 (*c* 0.07, $CHCl_3$). IR (KBr, cm^{-1}): 3027, 2942, 2864, 1747, 1721, 1180, 1092, 1071, 1047, 754, 698, 666. ^1H NMR (250 MHz, $CDCl_3$): δ 0.65, 0.70, 0.85, 0.91, 1.06 (5s, each 3H, 5 × CH_3), 0.82 (s, 6H, 2 × CH_3), 0.65–1.97 (m, 30H), 2.89 (m, 1H, H-18), 3.26 (m, 1H, H-3), 3.31 (s, 3H, OCH_3), 3.49 (dd, 1H, $J_{1,2}$ 3.5, $J_{2,3}$ 9.5 Hz, H-2^{Glc}), 3.68 (t, 1H, *J* 9.5 Hz, H-4^{Glc}), 3.90 (t, 1H, *J* 9.1 Hz, H-3^{Glc}), 3.91 (t, 2H, *J* 6.4 Hz, CH_2N), 4.03–4.10 (m, 1H, H-5^{Glc}), 4.11 (t, 2H, *J* 9.9 Hz, CO_2CH_2), 4.52 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1^{Glc}), 4.34–4.89 (m, 6H, 3 × $PhCH_2$), 5.18–5.22 (m, 3H, $NCCH_2O$, H-12), 7.19–7.28 (m, 15H, Ph-H), 7.32 (s, 1H, CH=N). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.3, 15.5, 17.0, 18.3, 23.0, 23.4, 23.6, 25.4, 25.8, 26.1, 27.2, 27.6, 28.1, 28.4, 30.0, 30.7, 32.5, 32.7, 33.0, 33.8, 37.0, 38.4, 38.7, 39.3, 41.3, 41.7, 45.8, 46.6, 47.6, 50.1, 55.2, 55.7, 58.7, 63.8, 70.2, 73.6, 74.8, 75.8, 78.9, 79.3, 79.6, 81.3, 98.8, 122.3, 123.7, 127.5, 127.6, 127.7, 127.9, 128.0, 128.1, 128.2, 128.3, 128.5, 137.9, 138.0, 138.5, 142.1, 143.8, 169.7, 177.6. ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{67}H_{91}NaN_3O_{10}$: 1120.7; found: 1120.6. $[M+H]^+$ calcd for $C_{67}H_{92}N_3O_{10}$: 1098.7; found: 1098.6. Anal. Calcd for $C_{67}H_{91}N_3O_{10}{\cdot}0.5H_2O$: C, 72.66; H, 8.37; N, 3.79. Found: C, 72.66; H, 8.42; N, 3.68.

3.1.19. 6-[4-(Methyl α-D-glucopyranosiduronylmethyl)-1*H*-1,2,3-triazol-1-yl]hexyl 3β-hydroxyolean-12-en-28-oate (18)

This compound was prepared from **17** (0.18 g, 0.164 mmol) according to the general procedure 3.1.5. The residue was purified by column chromatography (10:1 CH_2Cl_2–MeOH). Yield: 0.103 g, 76%, white solid, mp 98–100 °C. R_f 0.16 (20:1 CH_2Cl_2–MeOH), $[\alpha]_D$ −9.3 (*c* 0.08, MeOH). IR (KBr, cm^{-1}): 3419, 2941, 2863, 1747, 1726, 1177, 1048. ^1H NMR (300 MHz, $CDCl_3$ + D_2O): δ 0.71, 0.77, 0.92, 0.98, 1.13 (5s, each 3H, 5 × CH_3), 0.89 (s, 6H, 2 × CH_3), 0.71–1.92 (m, 30H), 2.83–2.86 (m, 1H, H-18), 3.20 (dd, 1H, *J* 5.3, 10.4 Hz, H-3), 3.46 (s, 3H, OCH_3), 3.62 (dd, 1H, $J_{1,2}$ 3.8, $J_{2,3}$ 9.3 Hz, H-2^{Glc}), 3.68 (t, 1H, *J* 9.1 Hz), 3.79 (t, 1H, *J* 8.9 Hz), 3.99 (t, 2H, *J* 6.4 Hz, CO_2CH_2), 4.20 (d, 1H, *J* 9.5 Hz), 4.34 (t, 2H, *J* 7.2 Hz), 4.76 (br s, 1H), 4.87 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1^{Glc}), 5.27 (br s, 1H, H-12), 5.54 (d, 1H, *J* 13.2 Hz), 7.62 (1H, s, CH=N). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.3, 15.6, 17.1, 18.4, 23.1, 23.5, 23.6, 25.5, 25.9, 26.1, 27.2, 27.7, 28.1, 28.4, 30.1, 30.7, 32.6, 32.8, 33.0, 33.9, 37.1, 38.5, 38.8, 39.4, 41.4, 41.8, 46.0, 46.8, 47.7, 50.5, 55.3, 56.0, 58.4, 63.9, 71.1, 71.7, 71.8, 73.6, 79.0, 99.8, 122.4, 123.0, 143.8, 169.8, 177.7. ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{46}H_{73}NaN_3O_{10}$: 850.5; found: 850.5. $[M+H]^+$ calcd for $C_{46}H_{74}N_3O_{10}$: 828.5; found: 828.5. Anal. Calcd for $C_{46}H_{73}N_3O_{10}$: C, 66.72; H, 8.89; N, 5.07. Found: C, 66.54; H, 9.08; N, 4.91.

3.1.20. 6-[4-(Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosid-6-yloxy)-methyl-1*H*-1,2,3-triazol-1-yl]hexyl 3β-hydroxyolean-12-en-28-oate (19)

This compound was prepared from **2** (0.108 g, 0.19 mmol) and **9** (0.093 g, 0.19 mmol) according to the general procedure 3.1.3. The residue was purified by column chromatography (2:1 EtOAc–petroleum ether). Yield: 0.175 g, 88%, white solid, mp 72–74 °C, R_f 0.23 (1:1 EtOAc–petroleum ether), $[\alpha]_D$ +59.2 (*c* 0.05, $CHCl_3$). IR (KBr, cm^{-1}): 3498, 2941, 2864, 1721, 1454, 1047, 767, 698. ^1H NMR (250 MHz, $CDCl_3$): δ 0.65, 0.70, 0.85, 0.91, 1.07 (5s, each 3H, 5 × CH_3), 0.82 (s, 6H, 2 × CH_3), 0.65–1.96 (m, 30H), 2.76–2.80 (m, 1H, H-18), 3.09–3.16 (m, 1H, H-3), 3.29 (s, 3H, OCH_3), 3.46 (dd, 1H, $J_{1,2}$ 3.5, $J_{2,3}$ 9.8 Hz, H-2^{Glc}), 3.53–3.74 (m, 4H, H-4^{Glc}, H-5^{Glc}, H-6^{Glc}), 3.87–3.94 (m, 3H, H-3^{Glc}, CH_2N), 4.16 (t, 2H, *J* 7.3 Hz, CO_2CH_2), 4.54 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1^{Glc}), 4.41–4.92 (m, 8H, 3 × $PhCH_2$, $NCCH_2O$), 5.20 (br s, 1H, H-12), 7.12–7.25 (m, 15H, Ph-H), 7.37 (s, 1H, CH=N). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1, 15.3, 15.5, 17.0, 18.3, 21.0, 23.0, 23.4, 23.6, 23.8, 25.4, 25.5, 25.8, 26.1, 27.1, 27.6, 28.1, 28.3, 30.1, 30.6, 32.4, 32.7, 33.0, 33.8, 37.0, 38.4, 38.7, 39.3, 41.3, 41.7, 45.8, 46.6, 47.6, 50.1, 53.4, 55.1, 55.2, 60.3, 63.8, 65.0, 67.9, 68.8, 70.0, 73.3, 74.9, 75.7, 78.9, 79.8, 82.0, 98.2, 122.1, 122.3, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.31, 128.33, 128.37, 138.1, 138.2, 138.7, 143.8, 144.9, 177.6. ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{67}H_{93}NaN_3O_9$: 1106.7; found: 1106.6. $[M+H]^+$ calcd for $C_{67}H_{94}N_3O_9$: 1084.7; found: 1084.7. Anal. Calcd for $C_{67}H_{93}N_3O_9{\cdot}0.5H_2O$: C, 73.59; H, 8.66; N, 3.84. Found: C, 73.27; H, 8.64; N, 3.66.

3.1.21. 6-[4-(Methyl α-D-glucopyranosid-6-yloxy)methyl-1*H*-1,2,3-triazol-1-yl]hexyl 3β-hydroxyolean-12-en-28-oate (20)

This compound was prepared from **19** (0.045 g, 0.042 mmol) according to the general procedure 3.1.5. The residue was purified by column chromatography (10:1 CH_2Cl_2–MeOH). Yield: 13 mg, 38%, colorless oil, R_f 0.1 (21:1 CH_2Cl_2–MeOH). ^1H NMR (300 MHz, $CDCl_3$): δ 0.71, 0.77, 0.88, 0.89, 0.91, 0.98, 1.12 (7s, each 3H, 7 × CH_3), 0.71–2.04 (m, 30H), 2.85 (dd, 1H, *J* 3.8, 13.3 Hz, H-18), 3.21 (dd, 1H, *J* 5.0, 10.4 Hz, H-3), 3.41 (s, 3H, OCH_3), 3.54–3.91

(m, 6H, H-2^{Glc}, H-3^{Glc}, H-4^{Glc}, H-5^{Glc}, H-6^{Glc}), 3.99 (t, 2H, *J* 6.5 Hz, NCH_2), 4.33 (t, 2H, *J* 7.2 Hz, CO_2CH_2), 4.67 (d, 1H, *J* 12.8 Hz, NCCHO), 4.75 (d, 1H, *J* 12.5 Hz, NCCHO), 4.77 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1^{Glc}), 5.26 (t, 1H, *J* 3.3 Hz, H-12), 7.51 (s, 1H, CH=N). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.4, 15.6, 17.1 18.4, 23.1, 23.5, 23.7, 25.6, 25.9, 26.2, 27.3, 27.7, 28.2, 28.5, 29.7, 30.2, 30.7, 32.6, 32.9, 33.1, 34.0, 37.1, 38.6, 38.8, 39.5, 41.5, 41.8, 46.0, 46.8, 47.7, 50.4, 55.3, 55.4, 63.9, 64.8, 69.8, 70.4, 70.6, 72.4, 74.7, 77.2, 79.1, 99.6, 122.0, 122.4, 143.9, 177.8. ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{46}H_{75}NaN_3O_9$: 836.5; found: 836.5. $[M+H]^+$ calcd for $C_{46}H_{76}N_3O_9$: 814.6; found: 814.5. Anal. Calcd for $C_{46}H_{75}N_3O_9$: C, 67.87; H, 9.29; N, 5.16. Found: C, 67.33; H, 9.14; N, 5.06.

3.1.22. 6-Bromohexyl 3β-hydroxyolean-12-en-28-oate (21)

This compound was prepared from **1** (1.87 g, 4.1 mmol) and 1,6-dibromohexane (0.63 mL, 4.1 mmol) according to the general procedure 3.1.1. The residue was purified by column chromatography (1:6 EtOAc–petroleum ether). Yield: 1.3 g, 52%, white solid, mp 71–73 °C, R_f 0.7 (1:3 EtOAc–petroleum ether). IR (KBr, cm^{-1}): 3515, 2931, 2864, 1722, 1463, 1386, 1364, 1261, 1176, 1161, 1031, 1001, 763, 667. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.73, 0.78, 0.92, 0.98, 1.13 (5s, each 3H, 5 × CH_3), 0.90 (s, 6H, 2 × CH_3), 0.73–2.04 (m, 30H), 2.85 (dd, 1H, *J* 3.2, 13.5 Hz, H-18), 3.20 (dd, 1H, *J* 4.0, 10.1 Hz, H-3), 3.41 (t, 2H, *J* 6.7 Hz, $BrCH_2$), 4.02 (t, 2H, *J* 6.3 Hz, CO_2CH_2), 5.28 (s, 1H, H-12). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.3, 15.6, 17.1, 18.4, 23.1, 23.5, 23.6, 25.3, 25.9, 27.3, 27.75, 27.82, 28.2, 28.5, 30.7, 32.6, 32.7, 32.9, 33.1, 33.4, 34.0, 37.1, 38.6, 38.8, 39.5, 41.5, 41.8, 46.0, 46.8, 47.7, 55.4, 64.0, 77.2, 79.1, 122.4, 143.9, 177.7. ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{36}H_{59}BrNaO_3$: 641.4; found: 641.4.

3.1.23. 6-(Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosiduronyl) hexyl 3β-hydroxyolean-12-en-28-oate (22)

This compound was prepared from **21** (0.26 g, 0.4 mmol) and **7** (0.2 g, 0.4 mmol) according to the general procedure 3.1.1. The residue was purified by column chromatography (1:6 EtOAc–petroleum ether). Yield: 0.38 g, 88%, white solid, mp 59–61 °C, R_f 0.27 (1:6 EtOAc–petroleum ether). IR (KBr, cm^{-1}): 3531, 2940, 2864, 1747, 1720, 1455, 1369, 1259, 1194, 1071, 1046, 734, 697, 463. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.72, 0.77, 0.92, 0.97, 1.13 (5s, each 3H, 5 × CH_3), 0.89 (s, 6H, 2 × CH_3), 0.72–1.97 (m, 30H), 2.85 (dd, 1H, *J* 4.4, 13.5 Hz, H-18), 3.20 (dd, 1H, *J* 4.8, 10.7 Hz, H-3), 3.41 (s, 3H, OCH_3), 3.58 (dd, 1H, $J_{1,2}$ 3.5, $J_{2,3}$ 10.0 Hz, H-2^{Glc}), 3.73 (t, 1H, *J* 9.5 Hz, H-4^{Glc}), 3.95–4.14 (m, 5H, 2 × CO_2CH_2, H-3^{Glc}), 4.19 (d, 1H, $J_{4,5}$ 10.1 Hz, H-5^{Glc}), 4.55–4.97 (m, 7H, 3 × $PhCH_2$, H-1^{Glc}), 5.27 (t, 1H, *J* 3.5 Hz, H-12), 7.24–7.34 (m, 15H, Ph-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.3, 15.7, 17.1, 18.4, 23.1, 23.4, 23.6, 25.5, 25.7, 25.9, 27.2, 27.7, 28.1, 28.4, 28.5, 30.7, 32.5, 32.8, 33.1, 33.9, 37.1, 38.5, 38.8, 39.4, 41.4, 41.7, 45.9, 46.7, 47.7, 55.3, 55.6, 64.0, 65.6, 70.3, 73.6, 75.0, 75.8, 79.0, 79.5, 79.7, 81.4, 98.8, 122.4, 127.7, 127.9, 128.0, 128.1, 128.3, 128.4, 128.5, 138.0, 138.1, 138.6, 143.8, 169.8, 177.7. ESIMS *m/z*: $[M+K]^+$ calcd for $C_{64}H_{88}KO_{10}$: 1055.6; found: 1055.8.

3.1.24. 6-(Methyl α-D-glucopyranosiduronyl)hexyl 3β-hydroxyolean-12-en-28-oate (23)

This compound was prepared from **22** (0.24 g, 0.24 mmol) according to the general procedure 3.1.5. The residue was purified by column chromatography (20:1 CH_2Cl_2–MeOH). Yield: 0.15 g, 85%, white solid, mp 84–86 °C, R_f 0.23 (20:1 CH_2Cl_2–MeOH), $[\alpha]_D$ +73.1 (*c* 0.06, MeOH). IR (KBr, cm^{-1}): 3431, 2940, 1732, 1048. ^{1}H NMR^{1}H NMR (300 MHz, $CDCl_3$): δ 0.72, 0.78, 0.92, 0.98, 1.13 (5s, each 3H, 5 × CH_3), 0.90 (s, 6H, 2 × CH_3), 0.72–1.99 (m, 30H), 2.85 (dd, 1H, *J* 3.6, 13.7 Hz, H-18), 3.21 (dd, 1H, *J* 4.8, 10.2 Hz, H-3), 3.50 (s, 3H, OCH_3), 3.68–3.82 (m, 3H, H-2^{Glc}, H-3^{Glc}, H-4^{Glc}), 4.01 (t, 2H, *J* 6.1 Hz, CO_2CH_2), 4.12 (d, 1H, $J_{4,5}$ 9.4 Hz, H-5^{Glc}), 4.22 (2H, t, *J* 6.8 Hz, CH_2OCO), 4.85 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1^{Glc}), 5.27 (br s, 1H, H-12). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.3, 15.6, 17.0, 18.3, 23.0, 23.4, 23.6, 25.3, 25.6, 25.9, 27.2, 27.7, 28.1, 28.4, 28.5, 29.7, 30.7, 32.5, 32.8, 33.1, 33.9, 37.0, 38.5, 38.7, 39.4, 41.3, 41.7, 45.9, 46.7, 47.6, 55.2, 55.9, 64.0, 65.8, 70.4, 71.7, 73.8, 79.0, 99.6, 122.4, 130.6, 143.8, 170.2, 177.8. ESIMS *m/z*: $[M+Cl]^+$ calcd for $C_{43}H_{70}ClO_{10}$: 781.5; found: 781.5. Anal. Calcd for $C_{43}H_{70}O_{10}\cdot H_2O$: C, 67.51; H, 9.49. Found: C, 67.77; H, 9.81.

3.1.25. 6-[2-(Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl) ethanoyl]hexyl 3β-hydroxyolean-12-en-28-oate (25)

This compound was prepared from **21** (0.12 g, 0.19 mmol) and **24** (0.11 g, 0.19 mmol) according to the general procedure 3.1.1. The residue was purified by column chromatography (1:6 EtOAc–petroleum ether). Yield: 0.20 g, 94%, white solid, mp 53–55 °C, R_f 0.21 (1:6 EtOA –petroleum ether). IR (KBr, cm^{-1}): 3509, 2942, 2865, 1727, 1457, 1090, 737. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.73, 0.77, 0.92, 0.98, 1.13 (5s, each 3H, 5 × CH_3), 0.90 (s, 6H, 2 × CH_3), 0.73–1.97 (m, 30H), 2.69–2.78 (m, 2H, CH_2CO_2), 2.87 (dd, 1H, *J* 3.8, 13.9 Hz, H-18), 3.17 (dd, 1H, *J* 5.0, 10.8 Hz, H-3), 3.62–3.81 (m, 6H, H-2^{Glc}, H-3^{Glc}, H-4^{Glc}, H-5^{Glc}, H-6^{Glc}), 4.00–4.03 (m, 4H, 2 × CO_2CH_2), 4.44–4.93 (m, 9H, H-1^{Glc}, 4x$PhCH_2$), 5.28 (br s, 1H, H-12), 7.11–7.32 (m, 20H, Ph-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.25, 15.32, 15.6, 17.1, 18.4, 23.1, 23.5, 23.6, 25.6, 25.8, 25.9, 27.3, 27.7, 28.1, 28.5, 30.7, 32.5, 32.8, 33.1, 33.9, 37.1, 38.5, 38.8, 39.4, 41.4, 41.8, 46.0, 46.7, 47.7, 55.3, 64.1, 64.6, 68.9, 71.5, 72.3, 73.2, 73.5, 75.0, 75.4, 79.0, 79.3, 82.2, 122.4, 127.6, 127.7, 127.8, 127.83, 127.9, 128.3, 128.4, 138.0, 138.1, 138.2, 138.6, 143.9,171.2, 177.7. ESIMS *m/z*: $[M+K]^+$ calcd for $C_{72}H_{96}KO_{10}$:1159.7; found: 1159.9.

3.1.26. 6-[2-(Methyl α-D-glucopyranosyl) ethanoyl]hexyl 3β-hydroxyolean-12-en-28-oate (26)

This compound was prepared from **25** (0.14 g, 0.12 mmol) according to the general procedure 3.1.5. The residue was purified by column chromatography (15:1 CH_2Cl_2–MeOH). Yield: 0.082 g, 86%, white solid, mp 89–91 °C, R_f 0.09 (20:1 CH_2Cl_2–MeOH). $[\alpha]_D$ +72.8 (*c* 0.10, MeOH). IR (KBr, cm^{-1}): 3402, 2940, 2863, 1722, 1635, 1386, 1176, 1067, 1033. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.72, 0.77, 0.92, 0.97, 1.13 (5s, each 3H, 5 × CH_3), 0.90 (s, 6H, 2 × CH_3), 0.60–2.00 (m, 30H), 2.67–2.72 (m, 2H, CH_2CO_2), 2.86 (d, 1H, *J* 10.3 Hz, H-18), 3.23 (t, 1H, *J* 7.1 Hz, H-3), 3.50–3.81 (m, 6H, H-2^{Glc}, H-3^{Glc}, H-4^{Glc}, H-5^{Glc}, H-6^{Glc}), 3.94–4.07 (m, 4H, 2xCO_2CH_2), 4.51 (br s, 1H, H-1^{Glc}), 5.27 (br s, 1H, H-12). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.3, 15.7, 17.1, 18.4, 23.1, 23.5, 23.7, 25.4, 25.8, 25.9, 27.1, 27.7, 28.2, 28.5, 28.6, 29.7, 30.7, 31.9, 32.6, 32.8, 33.1, 33.9, 37.1, 38.5, 38.8, 39.4, 41.4, 41.7, 45.9, 46.8, 47.6, 55.2, 61.6, 64.1, 65.1, 72.9, 73.2, 77.2, 79.0, 122.4, 143.9, 172.2, 177.9. ESIMS *m/z*: $[M+HCOO]^+$ calcd for $C_{45}H_{73}O_{12}$: 805.5; found: 805.5. Anal. Calcd for $C_{44}H_{72}O_{10}\cdot H_2O$: C, 67.84; H, 9.57. Found: C, 67.19; H, 9.76.

3.1.27. Benzyl 3-*O*-(2-chloroethoxycarbonyl)-3β-hydroxyolean-12-en-28-oate (27)

To a solution of benzyl oleanolate **4** (2.6 g, 4.8 mmol) in anhyd CH_2Cl_2 (100 mL) were added K_2CO_3 (13 g, 0.095 mol), DMAP (0.06 g, 0.48 mmol), and then 2-chloroethyl chloroformate (9.8 mL, 0.095 mol) in three portions during 9 h. After stirring at 35 °C for 12 h, the reaction mixture was filtered, and the filtrate was diluted with water (200 mL), and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were washed with water and brine, dried (Na_2SO_4), filtered, and concentrated. Purification by flash column chromatography (1:60 EtOAc–petroleum ether) afforded **27** (2.87 g, 92%) as a white solid. mp 61–63 °C, R_f 0.61 (1:8 EtOAc–petroleum ether). IR (KBr, cm^{-1}): 2947, 2878, 1743, 1460, 1391, 1157, 1253, 1011, 966, 739, 457. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.60, 0.87, 0.92, 1.01, 1.12 (5s, each 3H, 5 × CH_3), 0.90 (s, 6H, 2 × CH_3), 0.60–1.99 (m, 22H), 2.91 (dd, 1H, *J* 3.9, 13.7 Hz, H-18), 3.70 (t, 2H, *J* 5.8 Hz, CH_2Cl), 4.32–4.43 (m,

3H, H-3, CO_2CH_2), 5.06 (d, 1H, J_{gem} 12.6 Hz, PhCH), 5.08 (d, 1H, J_{gem} 12.6 Hz, PhCH), 5.28 (t, 1H, J 3.3 Hz, H-12). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.3, 16.6, 16.9, 18.2, 23.1, 23.4, 23.5, 23.6, 25.8, 27.6, 28.0, 30.7, 32.4, 32.7, 33.1, 33.9, 36.9, 38.0, 38.1, 39.3, 41.0, 41.2, 41.4, 41.7, 45.9, 46.8, 47.6, 55.4, 65.9, 66.8, 67.5, 77.2, 86.0, 122.4, 127.9, 128.0, 128.4, 136.5, 143.7, 154.4, 154.9, 177.4. ESIMS m/z: $[M+K]^+$ calcd for $C_{40}H_{57}ClKO_5$: 691.4; found: 691.5. $[M+Na]^+$ calcd for $C_{40}H_{57}ClNaO_5$: 675.4; found: 675.6.

3.1.28. Benzyl 3-*O*-[2-*O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosiduronyl ethoxycarbonyl]-3β-hydroxyolean-12-en-28-oate (28)

This compound was prepared from **27** (0.13 g, 0.2 mmol) and **7** (0.19 g, 0.4 mmol) according to the general procedure 3.1.1. The residue was purified by column chromatography (1:7 EtOAc–petroleum ether). Yield: 0.20 g, 91%, white solid, mp 62–63 °C, R_f 0.33 (1:5 EtOAc–petroleum ether). IR (KBr, cm^{-1}): 2947, 1745, 1454, 1365, 1256, 754, 698. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.60, 0.80, 0.88, 0.92, 1.12 (5s, each 3H, 5 × CH_3), 0.90 (s, 6H, 2 × CH_3), 0.60–1.97 (m, 22H), 2.89–2.92 (m, 1H, H-18), 3.40 (s, 3H, OCH_3), 3.57 (dd, 1H, $J_{1,2}$ 3.5, $J_{2,3}$ 9.6 Hz, H-2^{Glc}), 3.74 (t, 1H, J 9.5 Hz, H-4^{Glc}), 3.99 (t, 1H, J 9.3 Hz, H-3^{Glc}), 4.19–4.38 (m, 6H, H-3, H-5^{Glc}, $CO_2CH_2CH_2$), 4.59–4.97 (m, 7H, H-1^{Glc}, 3 × $PhCH_2$), 5.06 (d, 1H, J_{gem} 12.6 Hz, PhCH), 5.08 (d, 1H, J_{gem} 12.5 Hz, PhCH), 5.29 (br s, 1H, H-12), 7.22–7.34 (m, 20H, Ph-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.3, 16.6, 16.9, 18.2, 23.1, 23.4, 23.6, 25.8, 27.6, 28.0, 30.7, 32.4, 32.7, 33.1, 33.9, 36.9, 37.9, 38.1, 39.3, 41.4, 41.7, 45.9, 46.8, 47.6, 55.3, 55.7, 63.1, 64.8, 65.9, 70.2, 73.6, 75.1, 75.8, 77.2, 79.4, 79.6, 81.4, 85.9, 98.8, 122.4, 127.6, 127.7, 127.8, 127.9, 127.99, 128.0, 128.1, 128.3, 128.37, 128.40, 128.5, 138.0, 138.6, 143.7, 155.0, 169.5, 177.4. ESIMS m/z: $[M+K]^+$ calcd for $C_{68}H_{86}KO_{12}$: 1133.6; found: 1133.3.

3.1.29. 3-*O*-[2-*O*-(Methyl α-D-glucopyranosiduronyl) ethoxycarbonyl]-3β-hydroxy olean-12-en-28-oic acid (29)

This compound was prepared from **28** (0.12 g, 0.11 mmol) according to the general procedure 3.1.5. The residue was purified by column chromatography (23:1 CH_2Cl_2–MeOH). Yield: 0.065 g, 81%, white solid, mp 204–206 °C. R_f 0.14 (20:1 CH_2Cl_2–MeOH). $[\alpha]_D$ +91.1 (c 0.07, MeOH). IR (KBr, cm^{-1}): 3412, 2942, 1744, 1692, 1273, 1051, 1033, 1014. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.76, 0.87, 0.93, 0.97, 1.15 (5s, each 3H, 5 × CH_3), 0.96 (s, 6H, 2 × CH_3), 0.76–2.04 (m, 22H), 2.84–2.88 (m, 1H, H-18), 3.49 (s, 3H, OCH_3), 3.67 (m, 1H, H-2^{Glc}), 3.75–3.83 (m, 2H, H-3^{Glc}, H-4^{Glc}), 4.19 (d, 1H, J 9.3 Hz, H-5^{Glc}), 4.34–4.47 (m, 5H, H-3, $CO_2CH_2CH_2$), 4.86 (br s, 1H, H-1^{Glc}), 5.30 (br s, 1H, H-12). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.4 16.6, 17.5, 18.2, 22.8, 23.4, 23.5, 23.6, 26.0, 27.7, 28.0, 29.7, 30.7, 32.5, 32.6, 33.1, 33.8, 37.0, 38.0, 39.3, 40.9, 41.5, 45.8, 46.5, 47.5, 55.3, 55.9, 63.5, 65.0, 70.8, 71.6, 73.5, 85.9, 99.8, 122.6, 143.7, 155.1, 169.8, 184.0. Negative-mode ESIMS m/z: $[M-H]^+$ calcd for $C_{40}H_{61}O_{12}$: 733.4; found: 733.4. Anal. Calcd for $C_{40}H_{62}O_{12}\cdot H_2O$: C, 63.81; H, 8.57. Found: C, 63.69; H, 8.70.

3.2. Enzyme assay

The inhibitory activity of the test compounds against rabbit muscle glycogen phosphorylase a (RMGPa) was monitored using a microplate reader (BIO-RAD) based on the published method.[13] In brief, the 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.

3.3. Molecular docking simulation

To further identify the putative binding site and corresponding binding poses of the compounds, molecular docking simulation was performed against the GP protein. Glide 4.0[15] was used. The high-resolution crystal structure of GP complexed with asiatic acid at the allosteric site was received from the Protein Data Bank[16] (PDB code: 2QN1[5]), and the corresponding dimer was manually generated as the receptor according to the symmetry information provided in the PDB file. All the co-crystallized ligands and waters were removed. Preparation of the target protein with the Protein Preparation Wizard script in Maestro[17] involved the addition of hydrogen atoms to the macromolecule and optimization of the states for hydroxyl, Asn, Gln, and His residues. Three-dimensional affinity grids were positioned around the allosteric site, inhibitor site, catalytic site, and dimer interface site, and calculated with Receptor Grid Generation module of Glide, respectively. The starting 3D conformations of compound **12** and olealonic acid were prepared with LigPrep.[18] The extra precision (XP) mode of Glide was applied in molecular docking, and positions with a Coulomb–van der Waals score greater than 0 kcal/mol were rejected. All the docking positions were evaluated with Glide GScore. The best-ranked position from each of the binding sites was determined by selecting the one with the lowest calculated GScore values.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2009.02.012.

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