Synthesis of Oleanolic Acid Dimers as Inhibitors of Glycogen Phosphorylase

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Recently, oleanolic acid was found to be an inhibitor of glycogen phosphorylase. For further structural modification, we have synthesized several dimers of oleanolic acid by using amide, ester, or triazole linkage with click chemistry. The click chemistry was shown to be the most efficient method for the dimer synthesis. Nearly quantitative yield of triazole-linked dimers was obtained. Biological evaluation of the synthesized dimers as inhibitors of glycogen phosphorylase has been described. Four of six dimers exhibited inhibitory activity against rabbit muscle glycogen phosphorylase a (RMGPa), with compounds **2** and **7** as the most potent inhibitors, which displayed an IC_{50} value (*ca.* 3 μM) lower than that of oleanolic acid ($IC_{50}=14$ μM).

Introduction. – Oleanolic acid (**1**; OA) is a naturally existing pentacyclic triterpene. It possesses some attractive pharmacological activities including protection of the liver against toxic injury, anti-inflammation, anti-HIV, antitumor, antioxidation, anti-hyperglycemia, and cardiovascular activities, and has been in active clinical use as an antihepatitis drug in China for over 20 years [1]. Recently, we have found that oleanolic acid and related pentacyclic triterpenes displayed also inhibitory activity against glycogen phosphorylases (GP) [2]. As a safe nonprescription drug for the treatment of hepatitis, OA may find its new clinical uses in treating diseases caused by disorders in glycogen metabolism. Since OA is widely distributed in the plant kingdom, and very cheap and easily available in large bulk, lead modification based on OA would be in a good position for further mechanistic studies and drug development. Various derivatives of OA have been synthesized as antitumor [3], antibacterial [4], or anti-osteoporosis agents [5], as percutaneous transport promoters [6], or as inhibitors of nitric oxide production [7].

Concerning glycogen phosphorylases, several structural classes of inhibitors have been reported [8], whose binding sites identified in GP include the catalytic site (which binds glucose-1-P, glycogen, glucose, and glucose analogues), the Ser14-phosphate recognition site, the purine inhibitory site (which binds purine derivatives as well as pentacyclic triterpenes [2]), the allosteric site (which binds the activator AMP and the inhibitor glucose-6-P), the glycogen storage site, a novel allosteric inhibitor site, and the newly discovered benzimidazole-binding site [9]. The catalytic site, a deep cavity

located at the center of the molecule, 15 Å from the protein surface, and close to the essential cofactor pyridoxal 5′-phosphate (PLP), has been probed with glucose and glucose analogue inhibitors [8]. Since there are several binding sites in GP, we have then designed the dimeric compounds in order to increase the binding specificity and strength by additional binding at allosteric sites. Appending a second pentacyclic triterpene may also increase the local concentration of the pentacyclic triterpene near the enzyme's binding sites (statistical rebinding mechanism) [10]. It is noteworthy that several symmetrical compounds have been screened as GP inhibitors, some of them bound at the dimer interface site of GP [11], others at the allosteric site of the enzyme [12]. It is to be noted that in medicinal chemistry, multivalent ligands have been used as inhibitors of several biological targets (*e.g.*, glycosidase inhibitors) [13].

In this article, we report the synthesis of OA dimers (*Fig.*) linked at C(3) or C(28) position *via* ester, amide, or triazole groups, as well as their inhibitory activities against glycogen phosphorylases. Our results showed that dimeric compounds **2** and **7** displayed IC_{50} values lower than that of the monomer **1**.

Results and Discussion. – *Synthesis.* For the comparison of the nature and the length of the chain linker between two oleanolic acids, we have synthesized compounds **2** to **4** linked at C(28) (*Scheme 1*). Compound **8** [11] (2 equiv.) was first treated with oxalyl chloride to give the corresponding acyl chloride, which was then reacted with diethylenetriamine (1 equiv.) in the presence of Et_3N, to afford the dimer **2** in 66% yield. Esterification of **1** (2 equiv.) with 1,6-dibromohexane (1 equiv.) gave directly the dimer **3** (57%). Compound **4** was prepared by an esterification reaction between the known 6-bromohexyl oleanolate **9** [14] and L-malic acid in the presence of K_2CO_3.

We have also synthesized the dimers **5** to **7** (*Scheme 2*) by the Cu^I-catalyzed *Huisgen* [2+3] dipolar cycloaddition reaction ('click chemistry') [15]. Reaction of propargyl oleanolate **10** [14] with 1,6-diazidohexane in the presence of catalytic amounts of sodium ascorbate and $CuSO_4 \cdot 5\ H_2O$ in CH_2Cl_2/H_2O afforded the dimer **5** in a very good yield (97%). Similarly, click reaction between benzyl 3-*O*-propargyl oleanolate **11** [14] with 1,6-diazidohexane led to the dimer **6**, which was debenzylated over Pd/C (10%) in THF/MeOH at room temperature to afford compound **7**. Compounds **2** to **7** have been fully characterized by ^{1}H- and ^{13}C-NMR spectroscopy and by mass spectrometry.

Bioactivity. The above synthesized derivatives were evaluated in the enzyme inhibition assay against rabbit muscle glycogen phosphorylase a (RMGPa) (*Table*) which shared considerable sequence similarity with human liver GPa. As described previously [16], the activity of rabbit muscle GPa was measured through detecting the release of phosphate from glucose-1-phosphate in the direction of glycogen synthesis.

The assay results show that compounds **2** (IC_{50} = 3.25 μM), **4** (IC_{50} = 12.3 μM), **5** (IC_{50} = 20.7 μM), and **7** (IC_{50} = 2.59 μM) were inhibitors of GPa. Among the oleanolic dimers linked at 28-position, the amide-linked dimer **2** displayed the most efficient inhibition, with a lower IC_{50} value than oleanolic acid **1** (IC_{50} = 14 μM). Compounds with a longer linker diminished the inhibitory potency (compounds **4** and **5** *vs.* **2**). Previous results showed that the esterified monomers **9** and **10** inhibited GPa with IC_{50} values of 32.7 and 405 μM, respectively [14]. However, the ester-linked dimer **3** was not active. For dimers linked at 3-position (compounds **6** and **7**), only the dimer **7** with a

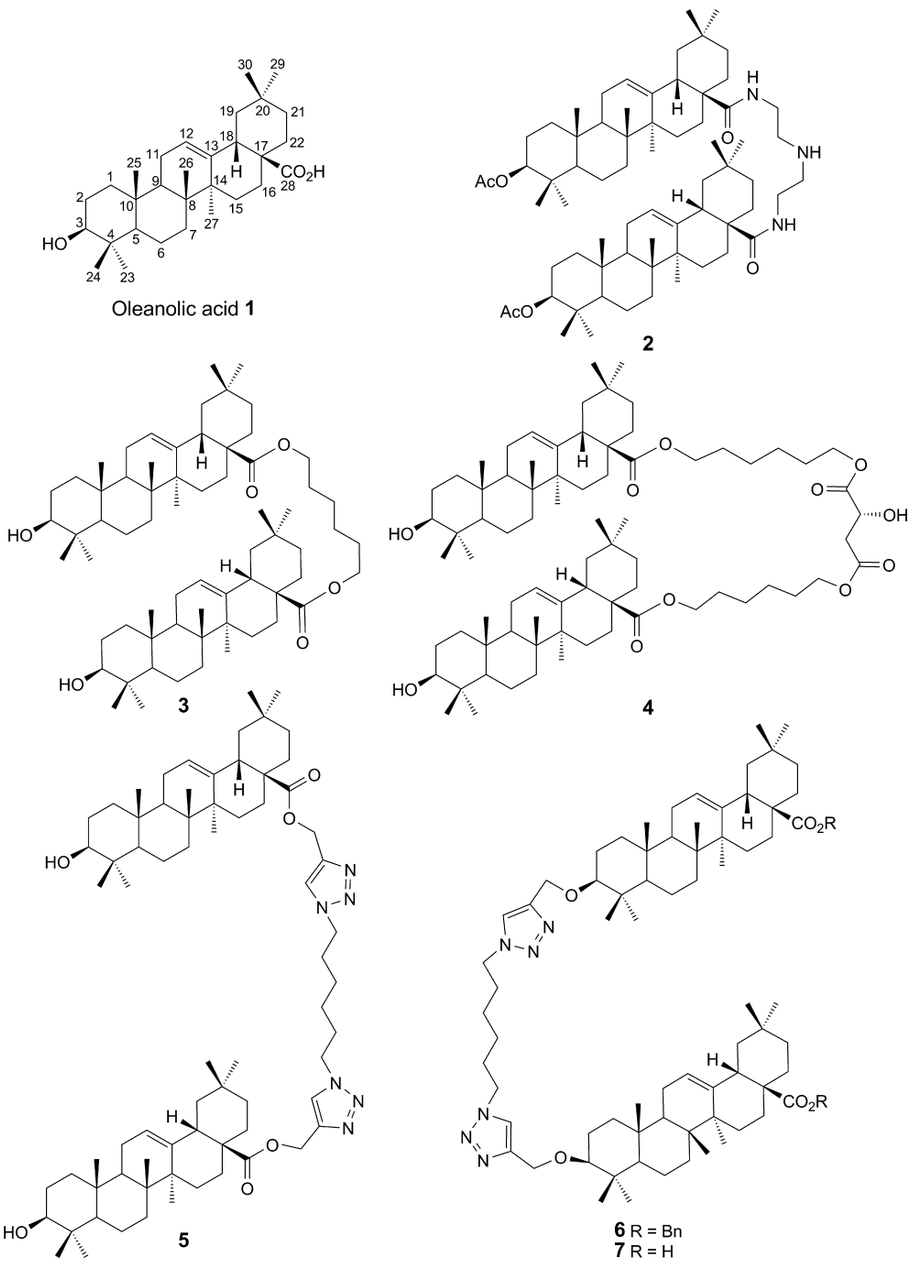
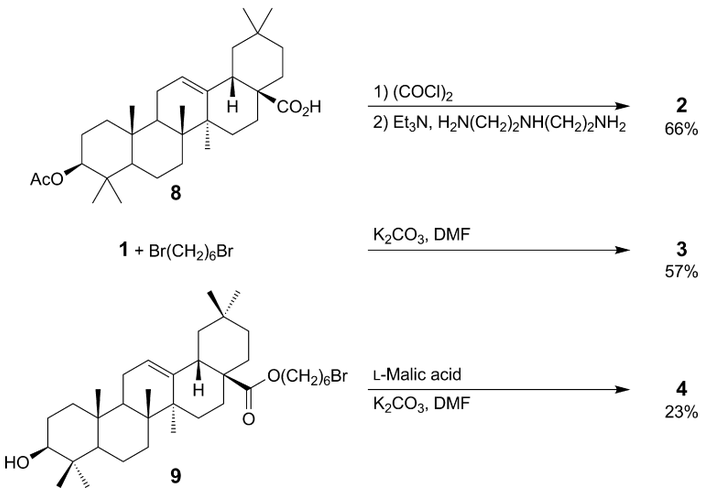
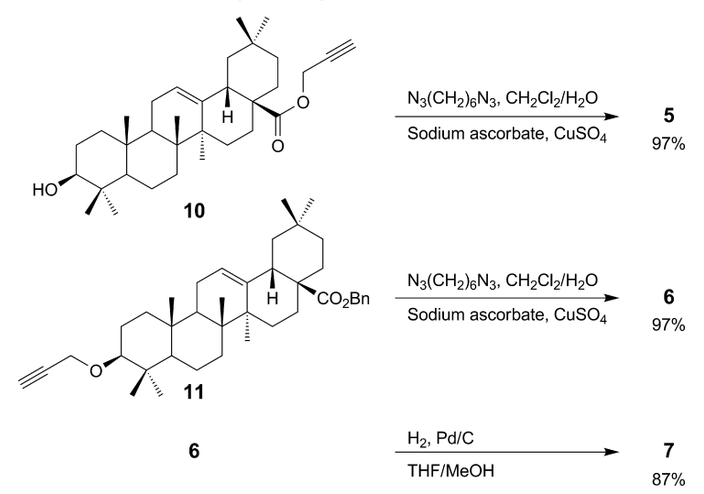


Figure. *Structure of oleanolic acid* (**1**) *and dimers* **2**–**7**

Scheme 1. *Synthesis of Oleanolic Acid Dimers* **2**–**4**



Scheme 2. *Synthesis of Oleanolic Acid Dimers* **5**–**7**



free carboxylic function inhibited GPa in spite of the inhibitory activity of the starting monomer **11** [14].

Conclusions. – Oleanolic acid dimers could be easily synthesized using amidification, esterification, and click chemistry. Nearly quantitative yield has been obtained for triazole-linked dimers. This result demonstrated once again the efficiency of this very popular reaction. The results of the GP inhibition assay indicate that efficient inhibitors

Table. IC_{50} *Values* [μM] *for RMGPa Inhibition Assay Results*

Compound	GPa IC_{50}[a])
1 [2]	14 ± 1.9
2	3.25 ± 0.27
3	na[b])
9 [14]	32.7 ± 2.9
4	12.3 ± 1.4
10 [14]	405 ± 15.6
5	20.7 ± 2.1
11 [14]	26.5 ± 1.5
6	na[b])
7	2.59 ± 1.5
Caffeine[c])	102.3 ± 6.7

[a]) Values are means of three experiments. [b]) na = No activity. [c]) Positive control.

can be obtained by introducing an appropriate linker at either C(3) or C(28) of oleanolic acid, with N-containing dimers **2** and **7** as the most potent inhibitors in this series of compounds. 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.

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Experimental Part

General. All commercially available solvents and reagents were used without further purification. Column chromatography (CC): *Merck* silica gel *60* (SiO_2; 230–400 mesh) or SiO_2 (200–300 mesh, *Qingdao Ocean Chemical Company*, P. R. China). M.p.: *RY-1* melting point apparatus. IR Spectra: *Shimadzu FTIR-8400S* spectrometer. ^{1}H- and ^{13}C-NMR spectra: *Bruker AV-300* spectrometer; chemical shifts are reported as values from an internal TMS standard. MS: *Agilent 1100 LC/DAD/MSD* or *Q-Tof Micro MS/MS* spectrometers.

Iminobis[ethane-2,1-diylimino(3β)-28-oxoolean-12-ene-28,3-diyl] Diacetate (**2**). To a soln. of **8** (0.5 g, 1 mmol) in dry CH_2Cl_2 (0.5 ml) was added oxalyl chloride (1.5 ml, 0.017 mol) at 0°. After stirring at 25° for 5 h, the mixture was evaporated, and co-evaporated with CH_2Cl_2 (3 × 1 ml). The residue was dissolved in dry CH_2Cl_2 (1 ml), and Et_3N (1.4 ml, 10 mmol) and $H_2N(CH_2)_2NH(CH_2)_2NH_2$ (0.1 ml, 1 mmol) were added at 0°. After stirring at r.t. for 24 h, the solvent was evaporated. The residue was taken up in 2M HCl (30 ml) and extracted with AcOEt (3 × 15 ml). The combined org. layers were washed with H_2O and brine, dried ($MgSO_4$), filtered, and concentrated to give a crude product. Purification by flash CC (CH_2Cl_2/MeOH 20:1) gave **2** as a white solid (0.39 g, 66%). M.p. 198–200°. R_f (CH_2Cl_2/MeOH, 7:1) 0.49. IR (KBr): 3415, 2946, 2876, 1735, 1659, 1518, 1465, 1368, 1246, 1027. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.76, 0.82, 0.86, 0.92, 1.16 (5*s*, 10 Me); 0.87 (*s*, 4 Me); 0.76–1.99 (*m*, 44 H); 2.05 (*s*, 2 MeCO); 2.52–2.57 (*m*, H–C(18), H–C(18′)); 2.78–2.80 (*m*, 2 CH_2N); 3.16–3.19 (*m*, $CONCH_2$); 3.43–3.49 (*m*, $CONCH_2$), 4.49 (*t*, *J* = 7.5, H–C(3), H–C(3′)); 5.39 (*s*, H–C(12), H–C(12′)). ^{13}C-NMR (75 MHz, $CDCl_3$): 15.9; 17.1; 17.4; 18.6; 21.7; 23.9; 24.2; 26.2; 27.8; 28.4; 31.1; 32.7; 33.0; 33.4; 34.5; 37.3;

38.1; 38.5; 38.6; 39.9; 42.3; 42.4; 46.8; 47.0; 47.9; 49.0; 55.7; 81.2; 123.6; 144.8; 171.4; 180.8. ESI-MS: 1064 ($[M+H]^+$), 1086 ($[M+Na]^+$).

Hexane-1,6-diyl (3β,3′β)-Bis(3-hydroxyolean-12-en-28-oate) (**3**). K_2CO_3 (1.4 g, 9.9 mmol) and 1,6-dibromohexane (0.51 ml, 3.3 mmol) were added to a soln. of oleanolic acid (3 g, 6.6 mmol) in DMF (20 ml). After stirring at r.t. for 12 h, the mixture was diluted with H_2O (80 ml) and extracted with AcOEt (3 × 40 ml). The combined org. layers were washed successively with 1N HCl, H_2O, sat. $NaHCO_3$, and brine, dried (Na_2SO_4), filtered, and concentrated to give a crude oil, which was purified by flash CC (AcOEt/petroleum ether (PE) 1:2) to obtain **3** as a white solid (1.87 g, 57%). M.p. 217–219°. R_f (AcOEt/PE 1:3) 0.45. IR (KBr): 3516, 2944, 2864, 1720, 1463, 1386, 1364, 1261, 1236, 1215, 1176, 1162, 1030, 1010, 995, 761, 667. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.73, 0.78, 0.92, 0.99, 1.13 (5*s*, 10 Me); 0.90 (*s*, 4 Me); 0.90–2.04 (*m*, 52 H); 2.84–2.90 (*dd*, *J* = 3.0, 13.3, H–C(18), H–C(18′)); 3.19–3.24 (*dd*, *J* = 4.2, 10.2, H–C(3), H–C(3′)); 4.01 (*t*, *J* = 6.3, 2 CH_2CO_2); 5.28 (*s*, H–C(12), H–C(12′)). ^{13}C-NMR (75 MHz, $CDCl_3$): 15.4; 15.6; 17.1; 18.4; 23.1; 23.5; 23.7; 25.8; 25.9; 27.3; 27.8; 28.2; 28.7; 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; 55.4; 64.1; 79.1; 122.4; 143.9; 177.7. ESI-MS: 1017 ($[M+Na]^+$).

*Bis(6-{[(3β)-3-hydroxy-28-oxoolean-12-en-28-yl]oxy}hexyl) (2*R*)-2-Hydroxybutanedioate* (**4**). K_2CO_3 (0.65 g, 4.7 mmol) was added to a soln. of **9** (0.42 g, 0.68 mmol) and L-malic acid (0.45 g, 3.4 mmol) in DMF (5 ml). After stirring at 50° for 48 h, the mixture was filtered, diluted with H_2O (40 ml), and extracted with CH_2Cl_2 (3 × 25 ml). The combined org. layers were washed with H_2O and brine, dried (Na_2SO_4), filtered, and concentrated to give a crude product, which was purified by flash CC (AcOEt/PE 1:6) to obtain **4** as a white solid (96 mg, 23%). M.p. 81–82°. R_f (AcOEt/PE 1:3) 0.53. IR (KBr): 3449, 2942, 2867, 1730. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.66, 0.71, 0.85, 0.92, 1.06 (5*s*, 10 Me); 0.83 (*s*, 4 Me); 0.66–1.82 (*m*, 60 H); 2.72–2.82 (*m*, CH_2O, H–C(18), H–C(18′)); 3.12–3.17 (*dd*, *J* = 4.5, 10.3, H–C(3), H–C(3′)); 3.94 (*t*, *J* = 6.3, 2 CH_2CO_2); 4.04 (*t*, *J* = 6.5, CH_2OCO); 4.14 (*t*, *J* = 6.6, CH_2OCO); 4.42 (br. *s*, CH); 5.21 (br. *s*, H–C(12), H–C(12′)). ^{13}C-NMR (75 MHz, $CDCl_3$): 15.3; 15.6; 17.0; 18.3; 23.0; 23.4; 23.6; 25.4; 25.5; 25.66; 25.7; 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; 63.9; 64.0; 65.0; 65.9; 67.3; 79.0; 122.3; 143.8; 170.5; 173.3; 177.7. ESI-MS: 1234 ($[M+Na]^+$).

*Hexane-1,6-diylbis(1*H*-1,2,3-triazole-1,4-diylmethanediyl) (3β,3′β)-Bis(3-hydroxyolean-12-en-28-oate)* (**5**). $CuSO_4 \cdot 5\,H_2O$ (0.042 g, 0.168 mmol) and sodium ascorbate (0.068 g, 0.34 mmol) were added to a soln. of **10** (0.21 g, 0.42 mmol) and 1,6-diazidohexane (0.035 g, 0.21 mmol) in CH_2Cl_2 (2 ml) and H_2O (2 ml). After stirring at r.t. for 12 h, the mixture was extracted three times with CH_2Cl_2. The combined org. layers were dried ($MgSO_4$), filtered, and concentrated to give **5** as a white solid (0.263 g, 97%). M.p. 134–136°. R_f (AcOEt/PE 1:1) 0.12. IR (KBr): 3437, 2945, 2846, 1723, 1717, 1463, 666. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.52, 0.74, 0.95, 1.08 (4*s*, 8 Me); 0.86 (*d*, *J* = 2.6, 6 Me); 0.52–1.92 (*m*, 52 H); 2.84 (*d*, *J* = 12.9, H–C(18), H–C(18′)); 3.15–3.20 (*m*, H–C(3), H–C(3′)); 4.28 (*t*, *J* = 6.8, 2 CH_2N); 5.14 (br. *s*, 2 CH_2CO_2); 5.24 (br. *s*, H–C(12), H–C(12′)); 7.55 (br. *s*, 2 CHN). ^{13}C-NMR (75 MHz, $CDCl_3$): 15.3; 15.6; 16.7; 18.3; 22.9; 23.3; 23.5; 25.7; 25.8; 27.1; 27.6; 28.0; 29.6; 29.9; 30.6; 32.3; 32.6; 33.0; 33.7; 36.9; 38.4; 38.7; 39.2; 41.2; 41.6; 45.8; 46.6; 47.5; 50.0; 55.1; 57.4; 78.8; 122.4; 143.5; 177.6. ESI-MS: 1157 ($[M+H]^+$), 1179 ($[M+Na]^+$).

*Dibenzyl (3β,3′β)-3,3′-[Hexane-1,6-diylbis(1*H*-1,2,3-triazole-1,4-diylmethanediyloxy)]bisolean-12-en-28-oate* (**6**). $CuSO_4 \cdot 5\,H_2O$ (0.075 g, 0.3 mmol) and sodium ascorbate (0.12 g, 0.6 mmol) were added to a soln. of **11** (0.288 g, 0.49 mmol) and 1,6-diazidohexane (0.042 g, 0.25 mmol) in CH_2Cl_2 (2 ml) and H_2O (2 ml). After stirring at r.t. for 12 h, the mixture was extracted three times with CH_2Cl_2. The combined org. layers were dried ($MgSO_4$), filtered, and concentrated to give a crude pink solid. Purification by CC (AcOEt/PE 2:1) afforded **6** as a white solid (0.29 g, 88%). M.p. 122–124°. R_f (AcOEt/PE 1:1) 0.27. IR (KBr): 2944, 2864, 1725, 1462, 1158, 1074, 736, 697. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.63, 0.81, 0.92, 0.95, 1.15 (5*s*, 10 Me); 0.93 (*s*, 4 Me); 0.63–2.08 (*m*, 52 H); 2.92–3.03 (*m*, H–C(18), H–C(18′), H–C(3), H–C(3′)); 4.36 (*t*, *J* = 6.8, 2 CH_2N); 4.61, 4.83 (*d*, *J* = 12.5, 2 CH_2O); 5.09, 5.11 (*d*, *J* = 12.5, 2 CH_2Ph); 5.32 (*s*, H–C(12), H–C(12′)); 7.36 (*m*, 10 arom. H); 7.53 (*s*, 2 CHN). ^{13}C-NMR (75 MHz, $CDCl_3$): 15.3; 16.4; 16.9; 18.2; 22.7; 23.0; 23.4; 23.6; 25.7; 25.8; 27.6; 28.1; 30.0; 30.7; 32.4; 32.7; 33.1; 33.8; 36.9; 38.3; 38.7; 39.3; 41.4; 41.7; 45.8; 46.7; 47.5; 49.9; 55.6; 63.3; 65.9; 86.5; 121.9; 122.5; 127.8; 127.9; 128.4; 136.4; 143.6; 146.5; 177.4. ESI-MS: 1338 ($[M+H]^+$), 1360 ($[M+Na]^+$).

*(3β,3′β)-3,3′-[Hexane-1,6-diylbis(1*H*-1,2,3-triazole-1,4-diylmethanediyloxy)]bisolean-12-en-28-oic Acid* (**7**). 10% Pd/C (0.009 g) was added to a soln. of **6** (0.045 g, 0.034 mmol) in THF (1.5 ml) and MeOH (1 ml). After stirring at r.t. under H_2 at atmospheric pressure for 12 h, the mixture was filtered through *Celite*. The filtrate was concentrated to give a crude product, which was purified by flash CC (AcOEt/PE 4:1) to obtain **7** as a white solid (0.034 g, 87%). M.p. 203–204°. R_f (AcOEt/PE 3:1) 0.09. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.69, 0.75, 0.90, 0.93, 0.95, 0.96, 1.10 (7*s*, 14 Me); 0.59–2.04 (*m*, 52 H); 2.82–2.85 (*m*, H–C(18), H–C(18′)); 2.95–2.99 (*m*, H–C(3), H–C(3′)); 4.26 (*t*, *J* = 7.0, CH_2N); 4.40 (*t*, *J* = 7.0, CH_2N); 4.42 (*d*, *J* = 11.8, CH_2O); 4.84 (*d*, *J* = 11.8, CH_2O); 5.27 (*s*, H–C(12), H–C(12′)); 7.47 (*s*, 2 CHN). ^{13}C-NMR (75 MHz, $CDCl_3$): 15.4; 17.1; 17.8; 18.2; 22.4; 22.8; 23.3; 23.6; 26.0; 26.2; 27.7; 28.5; 29.7; 30.2; 30.7; 32.3; 32.7; 33.1; 33.7; 37.2; 37.8; 38.8; 39.2; 40.8; 41.2; 45.7; 46.5; 47.3; 50.3; 55.4; 63.9; 77.2; 86.5; 121.3; 122.8; 128.0; 128.4; 143.6; 147.2; 185.1. ESI-MS: 1157 ($[M+H]^+$), 1179 ($[M+Na]^+$).

Enzyme Assay. The inhibitory activity of the test compounds against rabbit muscle glycogen phosphorylase a (RMGPa) was monitored using microplate reader (*BIO-RAD*) based on the published method [16]. In brief, GPa activity was measured in the direction of glycogen synthesis by the release of phosphate from glucose-1-phosphate. Each test compound was dissolved in DMSO and diluted at different concentrations for IC_{50} determination. The enzyme was added into 100 µl of buffer containing 50 mM *HEPES* (pH = 7.2), 100 mM KCl, 2.5 mM $MgCl_2$, 0.5 mM glucose-1-phosphate, 1 mg/ml glycogen, and the test compound in 96-well microplates (*Costar*). After the addition of 150 µl of 1M HCl containing 10 mg/ml ammonium molybdate and 0.38 mg/ml malachite green, reactions were run at 22° 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.

REFERENCES

[1] J. Liu, *J. Ethnopharmacol.* **1995**, *49*, 57; P. Dzubak, M. Hajduch, D. Vydra, A. Hustova, M. Kvasnica, D. Biedermann, L. Markova, M. Urban, J. Sarek, *Nat. Prod. Rep.* **2006**, *23*, 394.

[2] X. Wen, H. Sun, J. Liu, K. Cheng, P. Zhang, L. Zhang, J. Hao, L. Zhang, P. Ni, S. E. Zographos, D. D. Leonidas, K.-M. Alexacou, T. Gimisis, J. M. Hayes, N. G. Oikonomakos, *J. Med. Chem.* **2008**, *51*, 3540; J. Chen, J. Liu, L. Zhang, G. Wu, W. Hua, X. Wu, H. Sun, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2915; J. Hao, P. Zhang, X. Wen, H. Sun, *J. Org. Chem*. **2008**, *73*, 7405; K. Cheng, P. Zhang, J. Liu, J. Xie, H. Sun, *J. Nat. Prod*. **2008**, *71*, 1877.

[3] H.-J. Jeong, H.-B. Chai, S.-Y. Park, D. S. H. L. Kim, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1201.

[4] F. Hichri, H. B. Jannet, J. Cheriaa, S. Jegham, Z. Mighri, *C. R. Chim.* **2003**, *6*, 473.

[5] Y. Zhang, J.-X. Li, J. Zhao, S.-Z. Wang, Y. Pan, K. Tanaka, S. Kadota, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1629.

[6] L. Zaprutko, D. Partyka, B. Bednarczyk-Cwynar, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4723.

[7] T. Honda, B. V. Rounds, L. Bore, H. J. Finlay, F. G. Favaloro Jr., N. Suh, Y. Wang, M. B. Sporn, G. W. Gribble, *J. Med. Chem*. **2000**, *43*, 4233.

[8] L. Agius, *Best Pract. Res. Clin. Endocrin. Metab.* **2007**, *21*, 587; N. G. Oikonomakos, L. Somsák, *Curr. Opin. Invest. Drugs* **2008**, *9*, 379; L. Somsák, K. Czifrák, M. Tóth, É. Bokor, E. D. Chrysina, K.-M. Alexacou, J. M. Hayes, C. Tiraidis, E. Lazoura, D. D. Leonidas, S. E. Zographos, N. G. Oikonomakos, *Curr. Med. Chem.* **2008**, *15*, 2933; D. J. Baker, P. L. Greenhaff, J. A. Timmons, *Expert Opin. Ther. Pat.* **2006**, *16*, 459; N. G. Oikonomakos, *Curr. Protein Pept. Sci*. **2002**, *3*, 561.

[9] E. D. Chrysina, M. N. Kosmopoulou, C. Tiraidis, R. Kardakaris, N. Bischler, D. D. Leonidas, Z. Hadady, L. Somsák, T. Docsa, P. Gergely N. G. Oikonomakos, *Protein Sci.* **2005**, *14*, 873.

[10] R. J. Pieters, *Org. Biomol. Chem.* **2009**, *7*, 2013.

[11] K. Onda, R. Shiraki, Y. Yonetoku, K. Momose, N. Katayama, M. Orita, T. Yamaguchi, M. Ohta, S. Tsukamoto, *Bioorg. Med. Chem.* **2008**, *16*, 8627; V. L. Rath, M. Ammirati, D. E. Danley, J. L. Ekstrom, E. M. Gibbs, T. R. Hynes, A. M. Mathiowetz, R. K. McPherson, T. V. Olson, J. L. Treadway, D. J. Hoover, *J. Chem. Biol.* **2000**, *7*, 677.

[12] C. Tiraidis, K.-M. Alexacou, S. E. Zographos, D. D. Leonidas, T. Gimisis, N. G. Oikonomakos, *Protein Sci.* **2007**, *16*, 1773.

[13] J. E. Gestwicki, C. W. Cairo, L. E. Strong, K. A. Oetjen, L. L. Kiessling, *J. Am. Chem. Soc.* **2002**, *124*, 14922; D. Fournier, D. Poirier, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 693; A. L. LaFrate, K. E. Carlson, J. A. Katzenellenbogen, *Bioorg. Med. Chem.* **2009**, *17*, 3528; T. Wennekes, R. J. B. H. N. van den Berg, K. M. Bonger, W. E. Donker-Koopman, A. Ghisaidoobe, G. A. van der Marel, A. Strijland, J. M. F. G. Aerts, H. S. Overkleeft, *Tetrahedron: Asymmetry* **2009**, *20*, 836.
[14] K. Cheng, J. Liu, X. Liu, H. Li, H. Sun, J. Xie, *Carbohydr. Res.* **2009**, *344*, 841.
[15] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem., Int. Ed.* **2001**, *40*, 2004; C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002,** *67*, 3057; H. C. Kolb, K. B. Sharpless, *Drug Discovery Today* **2003**, *8*, 1128.
[16] W. H. Martin, D. J. Hoover, S. J. Armento, I. A. Stock, R. K. McPherson, D. E. Danley, R. W. Stevenson, E. J. Barrett, J. L. Treadway, *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 1776.

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