Supplemental Information for *BPB Reports*

Oleanolic acid-3-(1′2′orthoacetate-glucoside)-28-glucoside alleviates methylmercury toxicity *in vitro* and *in vivo*

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I.Chemistry

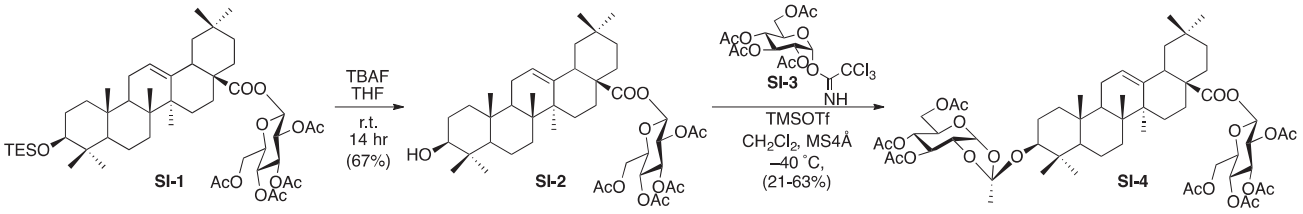
Our synthetic study was started from the deprotection of C3-TES group in C28-acetylated glucoside **SI-1** reported by previous report.[S1)] The orthoester formation of C3-OH was performed with acetylated imidate donor which usually form a orthoester at a glycosylation condition, to give the desired sugar orthoester **SI-3** in modulated yield.

Having the protected orthoester **SI-3** in hand, we focused on the deprotection process of the resulting acetyleted sugar orthoester (SI-table-1). The deprotection reaction was performed by methanolysis with NaH in MeOH. Although, in the reaction medium, the desired free sugar orthoester was not found in the reaction medium by ESI mass analysis., compound was not obtained after the treatment of protonic resin (Dowex) for a neutralization due to the cleavage of orthoester (entry 1).

Changing the neutralization process of the reaction medium, a synthetic polystyrenic adsorbent, Diaion® HP20, was suitable for this purpose, in which an amphiphilic molecule such as a saponin was adsorbed on a HP20 column and eluted using an alcoholic solvent. Therefore, the neutralization procedure was replaced to column chromatography using HP 20 for removal of a basic component such as NaOH.

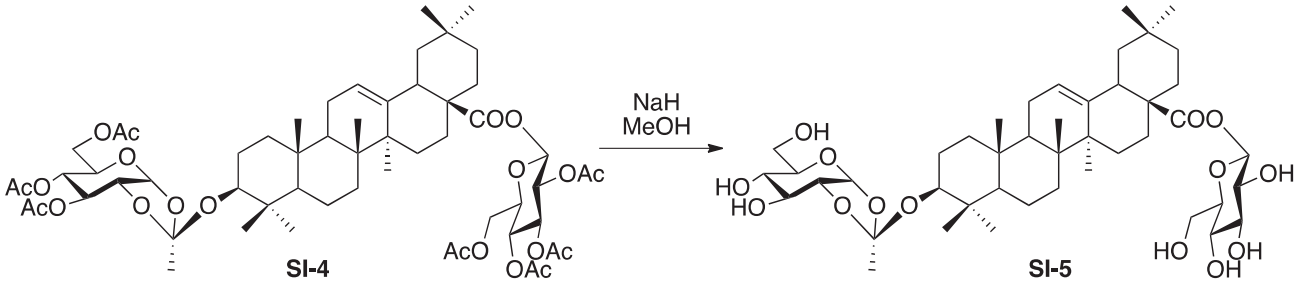
The final deprotection reaction medium was diluted with H_2O, resulted in subjecting to HP 20 column chromatography to afford H_2O, 50% MeOH fractions. The desired compound **SI-5** was detected in 50% MeOH fraction by ESI mass analysis. However, the same type of cleavage of orthoester moiety was observed during a purification of the 50% MeOH fraction using a silica gel (entry 2-4). Thus, the purification was performed by preparative HPLC with ODS column to isolated compound **SI-5** (entry 5).

The molecular formula $C_{44}H_{70}NaO_{14}$ was determined by the HR-ESI-MS data, which showed a positive ion at *m/z* 845.4670 $[M+Na]^+$ (Calcd. for C44H70NaO14: 845.4663). The ^{1}H-NMR spectra displayed typical resonances for an orthoester moiety at δ H 5.65 (d, *J* = 4.9 Hz, 1'-H), 4.13 (t, *J* = 4.9 Hz, 2'-H) and 1.62 (s, CH_3 in orthoester). Thus, the structure of compound **SI-5** including orthoester moiety.



SI-scheme 1 Synthesis of orthoester SI-4.

SI-Table 1



Entry	Reaction time (min)	Treatment for neutralization	Purification	Yield (%)
1	5	Dowex® 50W-X8	Silica gel	ND
2	40	DIAION™ HP20	Silica gel	21[a)]
3	15	DIAION™ HP20	Silica gel	17[a)]
4	15	DIAION™ HP20	Silica gel	13[a)]
5	15	DIAION™ HP20	ODS[b)]	86

a)Part of decompositon of orthoester was observed during purification process. b)Preparative HPLC was used. HPLC condition; column: YMC-Pack Pro C18, Eluent: H_2O : MeOH = 1 :9, flow rate = 8.0 mL/min, retention time = 6.8 min.

II. General for chemical procedure

All reactions were carried out under argon atmosphere in dried glassware, unless otherwise noted. All reagents were purchased from Tokyo Kasei Kogyo, Kanto Chemical, Fluka or Aldrich companies and used without further purification, unless otherwise noted. Dry THF, toluene, and CH_2Cl_2 were purchased from Kanto Chemical Co. Diethyl ether was freshly distilled from sodium and benzophenone. Precoated silica gel plates with a fluorescent indicator (Merck 60 F254) were used for analytical and preparative thin layer chromatography. Flash column chromatography was carried out with Kanto Chemical silica gel (Kanto Chemical, silica gel 60N, spherical neutral, 0.040-0.050 mm, Cat.-No. 37 563-84). Powdered and pre-dried molecular sieves 4Å, and 5Å were

used in glycosylation. ^{1}H NMR spectra were recorded at 300, 400 and 600 MHz and ^{13}C NMR spectra were recorded at 75 or 100, 150 MHz on Varian VXR-300 (300 MHz), Varian XL-400 (400 MHz), Varian UNITY-400 (400 MHz), or Varian INOVA (600 MHz) spectrometers. The chemical shifts are expressed in ppm downfield from the internal solvent peaks for $CHCl_3$ (7.26 ppm, ^{1}H NMR), CH_3OH (3.31, 4.84 ppm, ^{1}H NMR), C_6H_6 (7.27 ppm, ^{1}H NMR), $CDCl_3$ (77.0 ppm, ^{13}C NMR), CD_3OD (49.0 ppm, ^{13}C NMR), or C_6D_6 (128.0 ppm, ^{13}C NMR) and J values are given in Hertz. The coupling patterns are denoted s (singlet), d (doublet), dd (double doublet), ddd (double double doublet), t (triplet), dt (double triplet), q (quartet), m (multiplet), or br (broad). High-performance liquid chromatography (HPLC) was carried out using a Senshu UV-vis Detector (SSC-5410) and Senshu HPLC-pump (SSC-3461, UV; 254 nm) with Senshu Pak PEGASIL Silica 60-5 (normal phase: 4.6 ø x 250 mm) and Senshu Pak PEGASIL Silica SP100 (normal phase: 4.6 ø x 250 mm). All infrared spectra were measured on a JASCO FT/IR-460 spectrometer. High- and low-resolution mass spectra were measured on a JEOL JMS-T100 LP and JEOL JMS-AX505 HA spectrometer. Optical rotations were measured by using JASCO DIP-370 polarimeter. A 0.1 M solution of $TMSClO_4$ in Et_2O was prepared as below; to a solution of $AgClO_4$ (38.4 mg, 185 μmol) in Et_2O (1.85 mL) at 0 ˚C was added TMSCl (20.5 mg, 189 μmol) and this mixture were stirred. After the mixture was left standing for 10 min without stirring, the supernatant was used for glycosylation as a catalyst.

II. Synthetic procedure

Olean-12-en-28-oic acid, 3-hydroxy-, 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl ester (SI-2).

To a solution of **SI-1** (300 mg, 0.331 mmol) in THF (0.1 mL) at ambient temperature was added TBAF 1M THF solution (1.7 mL, 1.66 mmol). The reaction mixture was stirred at ambient temperature for 14 hr before it was added sat. aq. NH_4Cl (10 mL). The mixture was extracted with CH_2Cl_2 (2 x 30 mL). The resultant organic layer was washed with sat. aq. $NaHCO_3$ (30 mL) and brine (30 mL), dried with sodium sulfate, filtered, and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, hexane : AcOEt = 2 : 1) afforded **SI-2** (176 mg, 0.224 mmol, 67%) as a white solid. R*f* = 0.40 (hexane : AcOEt = 1 : 1); $[\alpha]_D^{22}$ +35.4 (*c* 1.00, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ : 5.58 (d, *J* = 8.0 Hz, 1H, 1'-H), 5.31 (t, *J* = 3.5 Hz, 1H, 12-H), 5.25 (t, *J* = 9.3 Hz, 1H, 3'-H), 5.18 (dd, *J* = 9.3 Hz, 8.0 Hz, 1H, 2'-H), 5.13 (dd, *J* = 10.0 Hz, 9.3 Hz, 1H, 4'-H), 4.27 (dd, *J* = 12.5 Hz, 4.4 Hz, 1H, 6'-H), 4.05 (dd, *J* = 12.5 Hz, 2.4 Hz, 1H, 6'-H), 3.79 (ddd, *J* = 10.0 Hz, 4.4 Hz, 2.4 Hz, 1H, 5'-H), 3.19 (m, 1H, 3-H), 2.78 (m, 1H, 18-H), 2.06 (s, 3H, -OCOC<u>H</u>$_3$), 2.02 (s, 3H, -OCOC<u>H</u>$_3$), 2.01 (s, 6H, -OCOC<u>H</u>$_3$ x2), 1.95 (m, 1H, 11-H), 1.84 (m, 2H, 16-H), 1.60 (m, 1H, 19-H), 1.58 (m, 2H, 22-H), 1.56 (m, 1H, 15-H), 1.54 (m, 1H, 1-H), 1.53 (m, 1H, 11-H), 1.48 (m, 2H, 6-H, 9-H), 1.44 (m, 2H, 2-H), 1.40 (m, 1H, 7-H), 1.31 (m, 1H, 6-H), 1.29 (m, 1H, 21-H), 1.18 (m, 1H, 21-H), 1.15 (m, 1H, 7-H), 1.14 (m, 1H, 19-H), 1.10 (s, 3H, 27-H), 1.01 (m, 1H, 15-H), 0.89 (m, 1H, 1-H), 0.88 (s, 6H, 23-H, 30-H), 0.87 (s, 6H, 25-H, 29-H), 0.72 (s, 3H, 24-H), 0.69 (s, 3H, 26-H), 0.65 (m, 1H, 5-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ : 175.6 (C-28), 170.5

(-O<u>C</u>OCH$_3$), 170.0 (-O<u>C</u>OCH$_3$), 169.4 (-O<u>C</u>OCH$_3$), 168.9 (-O<u>C</u>OCH$_3$), 142.8 (C-13), 122.8 (C-12), 91.5 (C-1'), 78.8 (C-3), 72.8 (C-3'), 72.4 (C-5'), 69.9 (C-2'), 68.0 (C-4'), 61.5 (C-6'), 55.1 (C-5), 47.5 (C-9), 46.8 (C-17), 45.7 (C-19), 41.6 (C-14), 39.3 (C-18), 38.7 (C-8), 38.4 (C-4), 36.8 (C-1), 33.7 (C-10), 32.9 (C-21), 32.8 (C-29), 32.8 (C-7), 31.7 (C-22), 30.5 (C-20), 28.0 (C-23), 27.7 (C-15), 27.1 (C-2), 25.6 (C-27), 23.4 (C-30), 23.3 (C-16), 22.8 (C-11), 20.6 (-OCO<u>C</u>H$_3$), 20.5 (-OCO<u>C</u>H$_3$), 20.5 (-OCO<u>C</u>H$_3$), 20.5 (-OCO<u>C</u>H$_3$), 18.2 (C-6), 16.9 (C-26), 15.5 (C-24), 15.3 (C-25); IR (KBr) cm^{-1} ν : 3445 (-O-H), 2933 (=C-H), 1760 (-C=O), 1036 (-C-O-); HR-MS (ESI$^+$) *m/z* 809.3822[M+Na]$^+$, Calc'd for $C_{44}H_{66}O_{12}Na$: 809.4452.

Olean-12-en-28-oic acid, 3-[1,2-(3,4,6,-tri-*O*-acetyl-β-D-glucopyranosyl) orthoacetate]-, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl ester (SI-4)

To a solution of **SI-2** (26.5 mg, 0.0337 mmol), **SI-3** (57.7 mg, 0.101 mmol) and MS4Å (50 mg) in CH_2Cl_2 (1.0 mL) at –20 ˚C was added a solution of TMSOTf (0.61 μL, 3.4 μmol) in CH_2Cl_2 (1.5 mL) dropwise. The reaction mixture was stirred for 25 min at –40 ˚C and added TMSOTf (0.30 μL, 1.7 μmol) in CH_2Cl_2 (0.75 mL) dropwise. Furthermore, the reaction mixture was stirred for 30 min at –40 ˚C before it was quenched by addition of triethylamine (2.0 μL). This mixture was filtered through Celite, rinsed with AcOEt (10 mL). The resulting solution was concentrated under reduced pressure. Purification by flash column chromatography (silica gel, hexane : AcOEt = 3 : 1) afforded **SI-4** (23.8 mg, 0.0212 mmol, 63%) as colorless oil. R*f* = 0.72 (hexane : AcOEt = 2 : 1); $[\alpha]_D^{24}$ +29.8 (*c* 1.00, CH_3OH); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.67 (d, *J* = 5.2 Hz, 1H, 1'-H), 5.57 (d, *J* = 8.1 Hz, 1H, 1"-H), 5.31 (t, *J* = 3.3 Hz, 1H, 12-H), 5.25 (m, 1H, 4"-H), 5.22 (m, 1H, 4'-H), 5.18 (m, 1H, 2"-H), 5.15 (m, 1H, 2'-H), 5.13 (m, 1H, 3"-H), 5.10 (m, 1H, 3'-H), 4.88 (ddd, *J* = 9.5 Hz, 3.3 Hz, 0.62 Hz, 1H, 6'-H), 4.33 (ddd, *J* = 5.2 Hz, 3.3 Hz, 0.75 Hz, 1H, 6'-H), 4.26 (dd, *J* = 12.5 Hz, 4.3 Hz, 1H, 6"-H), 4.03 (dd, *J* = 12.5 Hz, 2.3 Hz, 1H, 6"-H), 3.95 (m, 1H, 5'-H), 3.79 (ddd, *J* = 10.0 Hz, 4.3 Hz, 2.3 Hz, 1H, 5"-H), 3.13 (dd, *J* = 11.2 Hz, 4.5 Hz, 1H, 3-H), 2.80 (dd, *J* = 13.9 Hz, 3.8 Hz, 1H, 18-H), 2.10 (s, 3H, -OCOC<u>H</u>$_3$), 2.08 (s, 3H, -OCOC<u>H</u>$_3$) 2.07 (s, 3H, -OCOC<u>H</u>$_3$), 2.05 (s, 3H, -OCOC<u>H</u>$_3$), 2.03 (s, 3H, -OCOC<u>H</u>$_3$), 2.01 (s, 3H, -OCOC<u>H</u>$_3$), 2.00 (s, 6H, -OCOC<u>H</u>$_3$ x2), 1.95 (m, 1H, 11-H), 1.84 (m, 2H, 16-H), 1.60 (m, 1H, 19-H), 1.58 (m, 2H, 22-H), 1.56 (m, 1H, 15-H), 1.54 (m, 1H, 1-H), 1.53 (m, 1H, 11-H), 1.48 (m, 2H, 6-H, 9-H), 1.44 (m, 2H, 2-H), 1.40 (m, 1H, 7-H), 1.31 (m, 1H, 6-H), 1.29 (m, 1H, 21-H), 1.18 (m, 1H, 21-H), 1.15 (m, 1H, 7-H), 1.14 (m, 1H, 19-H), 1.10 (s, 3H, 27-H), 1.01 (m, 1H, 15-H), 0.89 (m, 1H, 1-H), 0.88 (s, 6H, 23-H, 30-H), 0.87 (s, 6H, 25-H, 29-H), 0.72 (s, 3H, 24-H), 0.69 (s, 3H, 26-H), 0.65 (m, 1H, 5-H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ : 175.6 (C-28), 171.1 (-O<u>C</u>OCH$_3$), 170.7 (-O<u>C</u>OCH$_3$), 170.6 (-O<u>C</u>OCH$_3$), 170.1 (-O<u>C</u>OCH$_3$), 169.6 (-O<u>C</u>OCH$_3$), 169.4 (-O<u>C</u>OCH$_3$), 169.2 (-O<u>C</u>OCH$_3$), 168.9 (-O<u>C</u>OCH$_3$), 142.8 (C-13), 122.1 (C-12), 97.0 (C-1'), 91.5 (C-1"), 81.2 (C-3), 73.3 (C-4'), 72.8 (C-4"), 72.4 (C-5"), 70.4 (C-5'), 70.0 (C-2'), 69.8 (C-2"), 68.0 (C-3"), 67.9 (C-3'), 61.5 (C-6"), 60.3 (C-6'), 47.5 (C-5), 46.7 (C-9), 45.7 (C-17), 41.7 (C-19), 41.0 (C-14), 39.2 (C-18), 38.4 (C-8), 38.4 (C-4), 36.8 (C-1), 33.7 (C-10), 32.9 (C-21), 32.9 (C-29), 32.8 (C-7), 31.7 (C-22), 30.7 (C-20), 28.4 (C-23), 27.6 (C-15), 27.6 (C-2), 25.6 (C-27),

23.4 (C-30), 22.8 (C-11), 21.0 (-OCO<u>C</u>H$_3$), 20.8 (-OCO<u>C</u>H$_3$), 20.8 (-OCO<u>C</u>H$_3$), 20.8 (-OCO<u>C</u>H$_3$), 20.7 (-OCO<u>C</u>H$_3$), 20.6 (-OCO<u>C</u>H$_3$), 20.6 (-OCO<u>C</u>H$_3$), 20.5 (-OCO<u>C</u>H$_3$), 18.4 (C-6), 16.9 (C-26), 16.5 (C-24), 15.3 (C-25); IR (KBr) cm^{-1} *v*: 2950 (=C-H), 1752 (-C=O), 1039 (-C-O-); HR-MS (ESI$^+$) *m/z* 1139.5395[M+Na]$^+$, Calc'd for $C_{58}H_{84}O_{21}SiNa$: 1139.5505.

Olean-12-en-28-oic acid, 3-(1,2-*β*-D-glucopyranose orthoacetate)-, *β*-D-glucopyranosyl ester (SI-5)

To a solution of **SI-4** (47.9 mg, 0.0429 mmol) in MeOH (1.5 mL) at ambient temperature was added NaH (85.8 μg, 2.15 μmol, 60% disp.). The reaction mixture was stirred at ambient temperature for 15 min before it was quenched by diluting H_2O (5 mL). The resultant mixture was fractionated by column chromatography using Diaion HP20® (15 cc) in MeOH-H_2O mixture [H_2O (100 mL), 50% MeOH (150 mL)]. The 50% MeOH fraction was concentrated. The crude product was purified by HPLC (YMC-Pack Pro C18, H_2O : MeOH = 1 :9, flow rate = 8.0 mL/min, retention time = 6.8 min) to afford **SI-5** (28.8 mg, 0.0369 mmol, 86%) as a colorless solid. R*f* = 0.25 ($CHCl_3$: MeOH = 5 : 1); $[\alpha]_D^{22}$ +33.6 (*c* 0.28, CH_3OH); ^{1}H-NMR (400 MHz, CD_3OD) δ : 5.65 (d, *J* = 4.9 Hz, 1H, 1'-H), 5.31 (d, *J* = 8.1 Hz, 1H, 1"-H), 5.28 (t, *J* = 3.4 Hz, 1H, 12-H), 4.13 (t, *J* = 4.9 Hz 1H, 2'-H), 3.8-3.7 (m, 2H, 6"-H), 3.78 (m, 1H, 5"-H), 3.75 (dd, *J* = 4.9 Hz, 1.6 Hz, 1H, 3'-H), 3.65 (m, 2H, 4'-H, 3"-H), 3.57 (m, 1H, 5'-H), 3.54 (m, 1H, 6'-H), 3.43 (m, 1H, 6'-H), 3.41 (m, 1H, 4"-H), 3.27 (m, 1H, 2"-H), 3.16 (dd, *J* = 3.8 Hz, 11.2 Hz, 1H, 3-H), 2.82 (dd, *J* = 3.3 Hz, 13.6 Hz, 1H, 18-H), 2.01 (m, 1H, 11-H), 1.87 (m, 2H, 16-H), 1.80 (m, 1H, 15-H), 1.73 (m, 1H, 22-H), 1.72 (m, 1H, 11-H), 1.71 (m, 1H, 19-H), 1.65 (m, 1H, 9-H), 1.63 (m, 1H, 22-H), 1.62 (s, 3H, 27-H), 1.62 (m, 2H, 1-H, 2-H), 1.57 (m, 1H, 2-H), 1.54 (m, 1H, 6-H), 1.52 (m, 1H, 7-H), 1.48 (m, 1H, 6-H), 1.34 (m, 1H, 21-H), 1.32 (m, 1H, 7-H), 1.24 (m, 1H, 21-H), 1.16 (s, 3H, 23-H), 1.12 (m, 1H, 19-H), 1.07 (m, 1H, 15-H), 0.98 (m, 1H, 1-H), 0.91 (s, 6H, 25-H, 30-H), 0.87 (s, 3H, 29-H), 0.76 (s, 3H, 24-H), 0.75 (m, 1H, 5-H); ^{13}C-NMR (400 MHz, CD_3OD) δ : 176.6 (C-28), 143.3 (C-13), 122.3 (C-12), 97.5 (C-1"), 94.1 (C-1'), 80.1 (C-3), 78.1 (C-3"), 78.1 (C-3'), 77.2 (C-4"), 76.9 (C-4'), 73.8 (C-2"), 73.7 (C-2'), 69.7 (C-5"), 68.6 (C-5'), 61.6 (C-6"), 60.9 (C-6'), 55.8 (C-5), 47.2 (C-9), 46.6 (C-17), 45.8 (C-19), 41.5 (C-14), 41.3 (C-18), 39.3 (C-8), 38.5 (C-4), 38.1 (C-1), 36.6 (C-10), 33.5 (C-21), 32.5 (C-7), 32.2 (C-29), 31.7 (C-22), 30.1 (C-20), 27.7 (C-15), 27.4 (C-23), 25.2 (C-2), 25.0 (C-27), 23.4 (C-16), 23.2 (C-30), 22.6 (C-11), 18.2 (C-6), 16.3 (C-26), 15.7 (C-24), 14.7 (C-25)
IR (KBr) cm^{-1} *v* : 3423 (-O-H), 2946 (=C-H), 1742 (-C=O), 1073 (-C-O-); HR-MS (ESI$^+$) *m/z* 803.4685[M+Na]$^+$, Calc'd for $C_{42}H_{68}O_{13}SiNa$: 803.4660.

IV. Reference

S1) Konishi N, Shirahata T, Yokoyama M, Katsumi T, Ito Y, Hirata N, Nishino T, Makino K, Sato N, Nagai T, Kiyohara H, Yamada H, Kaji E, Kobayashi Y. Synthesis of Bisdesmosidic Oleanolic Acid Saponins via a Glycosylation-Deprotection Sequence under Continuous Microfluidic/Batch Conditions. *J. Org. Chem.* 82, 6703-6719, (2017).