Supplementary material

for

Fluorophore-appended diosgenin inducing endoplasmic reticulum
stress and mitochondria-mediated apoptotic pathways

Meng Xin\textsuperscript{a1}, Dong Honghong\textsuperscript{a1}, Pan Yiwu\textsuperscript{a}, Man Shuli\textsuperscript{a*}, Ma Long\textsuperscript{a}, Liu Changxiao\textsuperscript{c},

Gao Wenyuan\textsuperscript{b*}

\textsuperscript{a}State Key Laboratory of Food Nutrition and Safety, Key Laboratory of Industrial Microbiology, Ministry of Education, Tianjin Key Laboratory of Industry Microbiology, National and Local United Engineering Lab of Metabolic Control Fermentation Technology, China International Science and Technology Cooperation Base of Food Nutrition/Safety and Medicinal Chemistry, College of Biotechnology, Tianjin University of Science & Technology, Tianjin, 300457, China

\textsuperscript{b}Tianjin Key Laboratory for Modern Drug Delivery and High Efficiency, School of Pharmaceutical Science and Technology, Tianjin University, Tianjin, 300072, China

\textsuperscript{c}The State Key Laboratories of Pharmacodynamics and Pharmacokinetics, Tianjin Institute of Pharmaceutical Research, Tianjin, China

*Corresponding authors: e-mail, biochemgao@163.com (Gao WY), Tel: 86-22-87401895, fax: 86-22-87401895; msl@tust.edu.cn (Man SL), Tel: +86 22 60601265, + Fax: 86 22 60602948.
Table of Contents

Intracellular localization of GND....................................................S3
Experimental procedures............................................................S4-S6
$^1$H NMR spectra for compound 3..................................................S8
$^{13}$C NMR spectra for compound 3...............................................S9
$^1$H spectra for compound 5........................................................S10
$^{13}$C NMR spectra for compound 5..............................................S11
**Intracellular localization of GND**

HepG2 cells were co-stained with different kinds of fluorescent dyes, containing the mitochondria, ER, lysosomal and Golgi apparatus after the cells incubated with GND for 6 h.

**Figure S1** Intracellular co-localization of GND with organelle-specific stains visualized by a fluorescence microscopy. HepG2 cells were co-stained with different kinds of fluorescent dyes, containing Mito tracker (100 nM), ER tracker (1 μM), Golgi tracker (5 μM) and lyso tracker (50 nM), respectively. Scale bars: 10 μm.
Experimental procedures

\[
\begin{align*}
\text{OAc} & \quad \text{OAc} & \quad \text{OAc} & \quad \text{CCl}_3 & \quad \text{NH}_2 \\
1 & + & 2 & \rightarrow & S1 & \quad \text{S1} & + & \text{Trillin}
\end{align*}
\]

**Diosgenyl β-D-glucopyranose (3)**

Trichloroacetimidate 1\(^1,2\) (68.7 mg, 139.3 µmol), diosgenin 2 (50.0 mg, 116.1 µmol) and 4Å molecular sieves (100.0 mg) were stirred in distilled CH\(_2\)Cl\(_2\) (3.0 mL) for 30 min at room temperature. The reaction mixture was cooled down to 0°C and TMSOTf (2.6 µL, 13.9 µmol) was added. The reaction mixture was allowed to warm to room temperature when the reaction was completed as indicated by TLC. Et\(_3\)N (0.1 mL) was added and the reaction mixture was filtered and concentrated. The crude was purified by column chromatography (PE: EA=8:1) to obtain S1 as white foam (60 mg, 68%). After that, the S1 (40.0 mg, 72.8 µmol) was dissolved in dry MeOH (2.0 mL) followed by the addition of NaOMe (1.6 mg, 28.9 µmol) to pH 9.0, then the mixture was stirred for 2 h at room temperature. The reaction mixture was neutralized with Dowex H\(^+\) resin, filtered, and concentrated. The crude residue was purified by column chromatography (CH\(_2\)Cl\(_2\): MeOH=10: 1) to give compound 3 as white foam (29.0 mg, 68%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 5.32–5.31 (d, 1H), 4.39–4.33 (m, 2H), 3.80–3.70 (m, 2H), 3.53–3.50 (m, 1H), 3.49–3.40 (m, 3H), 3.30–3.20 (m, 3H), 2.88–2.84 (d, 7H), 2.36–2.33 (m, 1H),
2.24–2.18 (m, 1H), 2.00–1.90 (m, 3H), 1.90–1.79 (m, 3H), 1.73–1.67 (m, 3H), 1.63–1.52 (m, 8H), 1.50–1.36 (m, 5H), 1.30–1.35 (m, 8H), 0.97–0.91 (m, 8H), 0.75–0.73 (m, 6H); $^{13}$C NMR (101Hz, CDCl$_3$) δ 139.27, 120.92, 108.49, 100.08, 79.92, 78.14, 76.45, 76.13, 75.30, 74.62, 72.48, 68.98, 65.88, 61.00, 60.85, 55.47, 49.06, 48.75, 48.53, 48.32, 48.10, 47.89, 47.68, 40.62, 39.28, 38.73, 37.71, 36.20, 35.85, 31.06, 30.75, 30.39, 30.33, 29.24, 28.70, 28.60, 27.71, 19.84, 18.34, 16.09, 15.26, 13.44.

**Diosgenyl 2-deoxy-2-phthalimido-β-D-glucopyranoside (5)**

Trichloroacetimidate 4$^3$ (60.0 mg, 104.2 µmol), diosgenin 2 (36.0 mg, 86.8 µmol) and 4Å molecular sieves (100 mg) were stirred in freshly distilled CH$_2$Cl$_2$ (3.0 mL) for 30 min at room temperature. The reaction mixture was cooled down to 0°C and TMSOTf (1.87 µl, 13.0 µmol) was added. Progress of the reaction was monitored by TLC. Upon consumption of the acceptor, the reaction mixture was quenched with triethylamine (0.1 mL), then was filtered and concentrated. The crude residue was purified by column chromatography (PE: EA = 8:1) to obtain S2 as a white foam (50.0 mg, 68%). Then, the intermediate S2 (50.0 mg) was dissolved in dry MeOH (1.5 mL), and NaOMe was added to reach to
pH 8.5. After the mixture was stirred for 30 min at room temperature, the reaction mixture was neutralized with Dowex H\(^+\) resin, filtered, and concentrated. The crude residue was purified by column chromatography (CH\(_2\)Cl\(_2\): MeOH= 30:1) to give the targeted 5 as a white solid (35.0 mg, 83\%). \(^1\)HNMR (400MHz, CDCl\(_3\)) \(\delta\) 7.75–7.73 (m, 2H), 7.64–7.63 (d, 2H), 5.25–5.23 (d,1H), 5.13 (s, 1H),4.46–4.30 (m, 2H), 4.30–4.16 (s, 1H), 4.10–3.96 (m, 2H), 3.86–3.77 (m, 2H), 3.70–3.61 (m, 1H), 3.42–3.21 (m, 5H), 2.00–1.90 (d, 2H), 1.90–1.72 (m, 5H), 1.71–1.59 (m, 5H), 1.58–1.46 (m, 6H), 1.41–1.29 (m, 9H), 1.11–0.92 (m, 3H), 0.90–0.85 (m, 4H), 0.84–0.72 (m, 6H), 0.71–0.69 (m, 3H), 0.68–0.63 (m, 3H); \(^{13}\)CNMR (101 Hz, CDCl\(_3\)) \(\delta\) 167.46, 139.20, 133.06, 130.64, 122.48, 120.74, 108.25, 95.94, 79.77, 78.07, 75.69, 74.45, 70.65, 70.38, 65.82, 61.07, 60.74, 55.88, 55.41, 48.94, 40.57, 39.21, 38.70, 37.54, 36.04, 35.67, 30.98, 30.50, 30.33, 29.27, 28.68, 28.35, 27.78, 21.67, 19.76, 18.25, 16.11, 15.22, 13.50, 13.10. ESIMS: m/z calcd for [C\(_{41}\)H\(_{55}\)NO\(_9\)+Na\(^+\)]: 728.3775, found: 728.3780.

References


$^1$H NMR spectra for compound 3 (Trillin)
$^{13}$C NMR spectra for compound 3 (Trillin)
$^1$H NMR spectra for compound 5 (GND)
$^{13}C$ NMR spectra for compound 5 (GND)