

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

Minoru Ueda,*^a Gangqiang Yang,^a Yuuki Nukadzuka,^a Miyuki Shigenaga,^a Satoru Tamura,^a Yasuhiro Ishimaru,^a and Yoshiyuki Manabe^a

^a Laboratory of Organic Chemistry, Department of Chemistry, Tohoku University, Aoba-ku, Sendai 980-8578, Japan

Table of Contents:

Figures S1-S5	SI 2
Materials and Methods	SI 7
Experimental Procedures	SI 8
References	SI 23
¹ H and ¹³ C NMR Spectra	SI 24

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

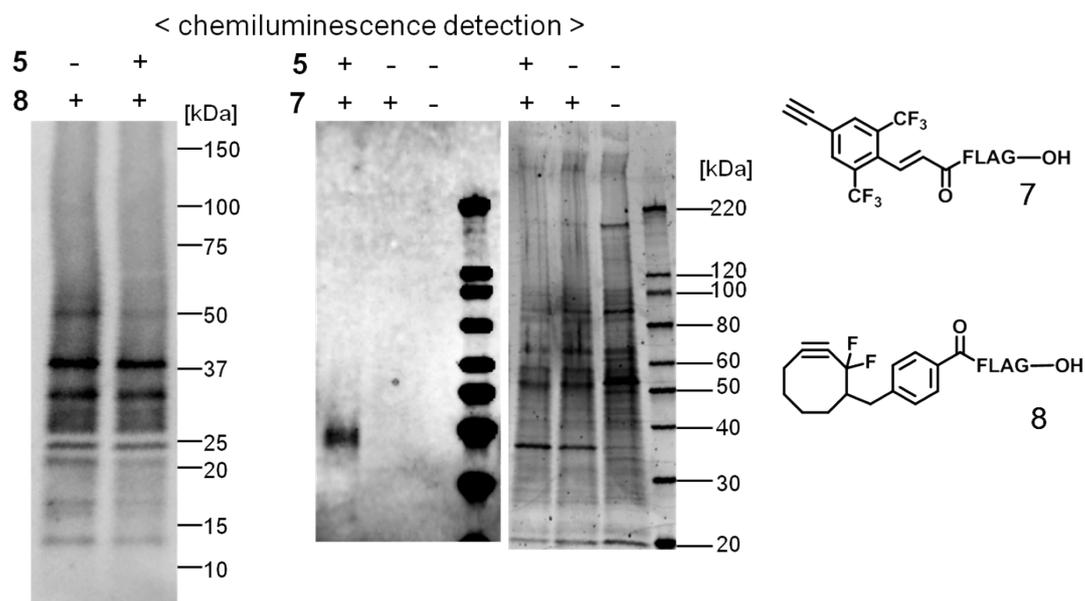


Figure S1. Introduction of a FLAG tag on CMP **5**-labeled proteins using click chemistry MTJG in living cells was labeled by photoaffinitylabeling with CMP **5** (5×10^{-4} M) and subsequent introduction of a FLAG tag by copper-free click chemistry (CFCC) using **8** (1×10^{-4} M, left) or by Cu-catalyzed azide alkyne cycloaddition (CuAAC) using **7** (5×10^{-4} M, right). Microsomal fraction of cells was used for SDS-PAGE (full range) on chemiluminescence detection of MTJG. The same PVDF membrane in chemiluminescence detection was used to check protein loading amount by Colloidal Gold Total Protein Stain.

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

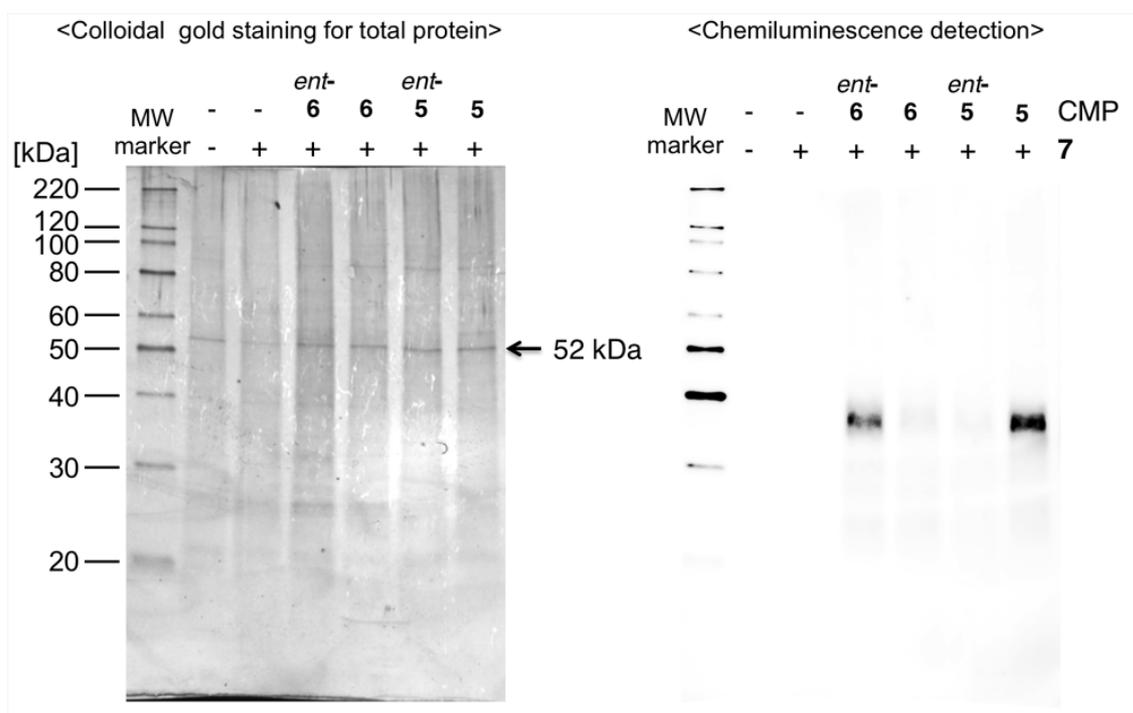


Figure S2. Photoaffinitylabeling of MTJG using living *Samanea* motor cell with CMP

Photoaffinitylabeling of MTJG using living *Samanea* motor cell with CMP (**5**, *ent*-**5**, **6** and *ent*-**6**: 4×10^{-4} M, respectively). Introduction of a FLAG tag was performed by CuAAC using **7** (5×10^{-4} M). Microsomal fraction of cells was used for SDS-PAGE (full range) on chemiluminescence detection of MTJG. The same PVDF membrane in chemiluminescence detection was used to check protein loading amount by Colloidal Gold Total Protein Stain.

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

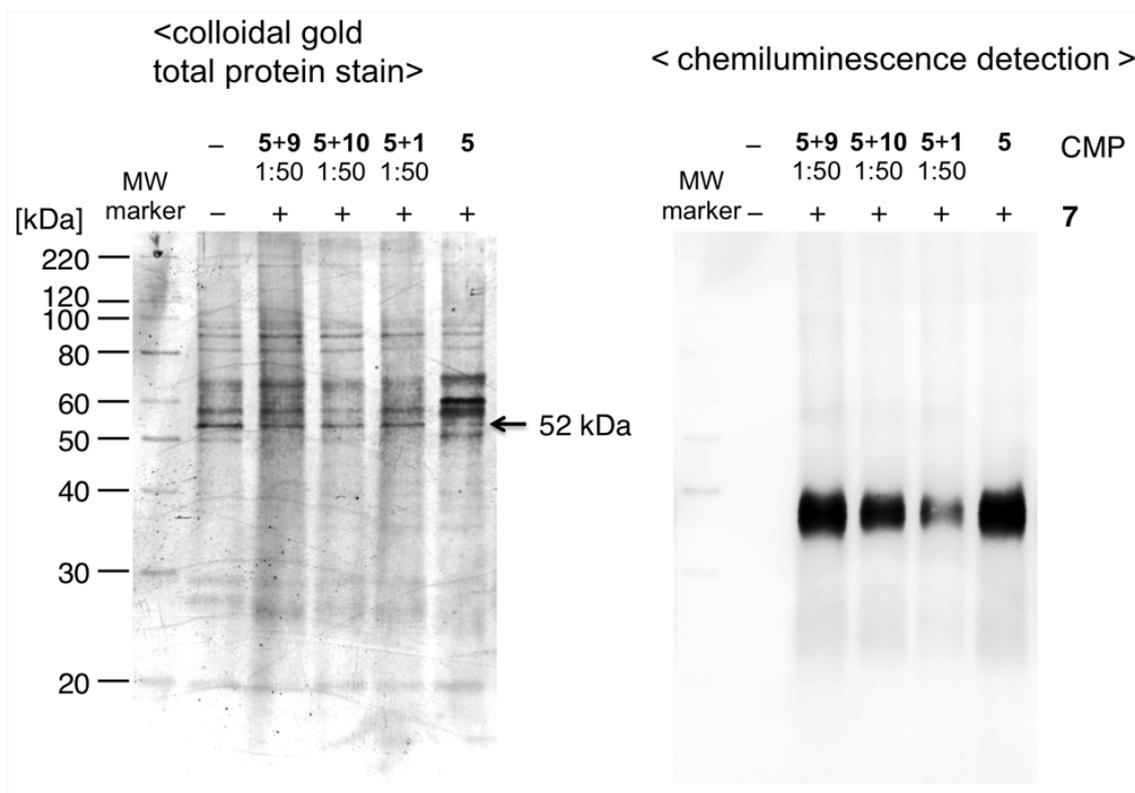


Figure S3. Competitive inhibition of binding between **5** and MTJG by using **1** or **9** or **10**

Competitive inhibition of binding between **5** (4×10^{-4} M) and MTJG was performed by using **1** or **9** or **10** (2×10^{-2} M, respectively). Introduction of a FLAG tag was performed by CuAAC using **7** (5×10^{-4} M). Microsomal fraction of cells was used for SDS-PAGE (full range) on chemiluminescence detection of MTJG. The same PVDF membrane in chemiluminescence detection was used to check protein loading amount by Colloidal Gold Total Protein Stain.

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

Figure S4. Important ROE correlations and ROESY spectrum of CMP *ent*-6.

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

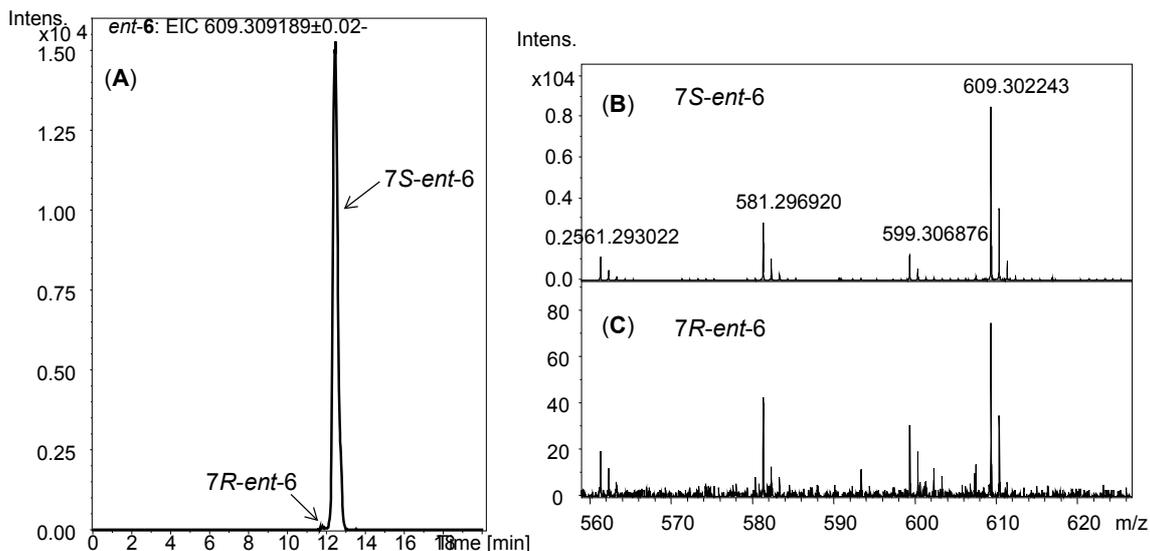


Figure S5. LC/ESI TOF MS analysis for equilibrium mixture of *7S-ent-6* and *7R-ent-6*. (A) Extracted ion chromatogram of [M-H]⁻ ion from *ent-6* (mass window ±0.02 Da) indicating *7S-ent-6* (12.5 min) and *7R-ent-6* (11.8 min) in ratio of 99.5:0.5. (B) and (C) Compound spectra of *ent-6* averaged over retention time windows of (B) 12.5-12.7 min and (C) 11.8 min. [M-H]⁻ ions are labeled with corresponding *m/z* values.

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

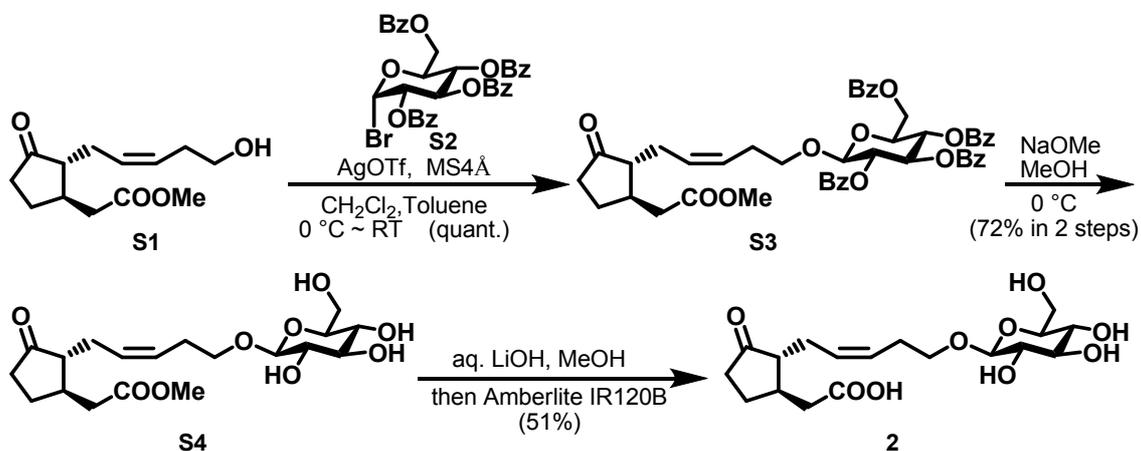
Materials and Methods.

Unless otherwise stated, reactions were performed in flame-dried glassware under an argon or nitrogen atmosphere using dry solvents. Solvents were dried over activated molecular sieves under argon. All the starting materials were purchased from commercial sources and used as received, unless otherwise stated. Liquids and solutions were transferred via syringe or positive-pressure cannula. Brine solutions refer to saturated aqueous sodium chloride solutions. Reaction temperatures were controlled by an EYELA temperature modulator (Tokyo Rikakikai Ltd.). Thin-layer chromatography (TLC) was performed using Merck silica gel 60 F₂₅₄ precoated plates (0.25 mm) or Merck RP-18F_{254S} (0.25 mm) and visualized by UV fluorescence quenching, anisaldehyde, or H₃(PMo₁₂O₄₀) staining. Kanto Reagents Silica Gel 60N (particle size 63–210 μm) was used for column chromatography. HPLC purifications were carried out using PU-2089 with UV-2075 detector (Jasco Ltd.) equipped with Cosmosil 5C18AR (φ 20×250 mm, Nakalai. Tesque Ltd.) at a flow rate of 5.0 mL/min. ¹H and ¹³C NMR spectra were recorded on ECA400 and Alpha 500 spectrometers (JEOL Ltd.) and chemical shifts are given in parts per million (ppm) relative to Me₄Si (¹H: 0.0 ppm) in CDCl₃ (¹³C: 77.0 ppm) or CD₂HOD in CD₃OD (¹H: 3.30 ppm, ¹³C: 49.0 ppm). Data for ¹H NMR spectra are reported as follows: chemical shift (ppm) (multiplicity, coupling constant (Hz), integration). Multiplicity and qualifier abbreviations are as follows: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br = broad. IR spectra were recorded on FT/IR-4100 spectrometer (Jasco Co., Ltd.) using dry KBr as a standard reference and are reported in frequency of absorption (cm⁻¹). HR MS were recorded on an ESI-mode by using MicrOTOF-II spectrometers (Bruker Daltonics Ltd.). LC-MS analyses were carried out using MicrOTOF-II spectrometers (Bruker Daltonics Ltd.) with Agilent 1290 Infinity HPLC system (Agilent Technologies) using an Agilent Eclipse Plus C18 column (1.8 μm, φ 2.1×50 mm, Agilent Technologies). Optical rotation was recorded on a DIP-1000 spectrometer (Jasco Co., Ltd.) using 100-mm cell.

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

Experimental Procedures

Scheme S1. Synthesis of 12-O- β -L-glucopyranosyljasmonic acid (**2**)



Compound **S3**

To a suspension of **S1** (33.6 mg, 0.140 mmol), **S2** (190.0 mg, 0.288 mmol), and MS4Å (0.300 g) in dehydrated CH₂Cl₂ (1.4 mL) was slowly added AgOTf (88.2 mg, 0.343 mmol) in dehydrated toluene (1.4 mL) at 0 °C under an Ar atmosphere. After being stirred for 1.5 h at RT in dark, the reaction mixture was diluted with CHCl₃ and filtered through a pad of Celite. The filtrate was washed with sat. NaHCO₃ aq., dried over Na₂SO₄, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (*n*-hexane/EtOAc = 3/1 -2/1 -4/3 -1/1) gave **S3** (124.1 mg, quant.) as a colorless viscous oil.

¹H NMR (400 MHz, CDCl₃) δ 8.02 (dd, J = 8.4, 1.3 Hz, 2H), 7.95 (dd, J = 8.4, 1.3 Hz, 2H), 7.90 (dd, J = 8.4, 1.3 Hz, 2H), 7.83 (dd, J = 8.4, 1.3 Hz, 2H), 7.56-7.26 (m, 12H), 5.90 (dd, J = 9.6, 9.6 Hz, 1H), 5.68 (dd, J = 9.6, 9.6 Hz, 1H), 5.52 (dd, J = 9.6, 7.8 Hz, 1H), 5.33 (dt, J = 11.0, 7.3 Hz, 1H), 5.20 (dt, J = 11.0, 7.3 Hz, 1H), 4.87 (d, J = 7.8 Hz, 1H), 4.65 (dd, J = 12.1, 3.2 Hz, 1H), 4.50 (dd, J = 12.1, 5.3 Hz, 1H), 4.18 (ddd, J = 9.6, 5.3, 3.2 Hz, 1H), 3.93 (dt, J = 9.6, 6.9 Hz, 1H), 3.68 (s, 3H), 3.56 (dt, J = 9.6, 6.9 Hz, 1H), 2.60 (dd, J = 14.0, 2.8 Hz, 1H), 2.35-2.15 (m, 8H), 2.03 (ddd, J = 19.0, 11.0, 8.7 Hz, 1H), 1.81 (dt, J = 9.2, 5.5 Hz, 1H), 1.50-1.40 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 218.7, 172.4, 166.1, 165.8, 165.2, 165.0, 133.4, 133.2, 133.1, 133.1, 129.8 (2 C), 129.7 (6 C), 129.6, 129.4, 128.8, 128.8, 128.4 (2 C), 128.3 (2 C), 128.3 (2 C), 128.2 (2 C), 127.8, 127.4, 101.2, 72.9, 72.2, 71.8, 69.8, 69.6, 63.2, 53.8, 51.6, 38.6, 37.8, 37.6, 27.7, 27.1, 25.4; IR (film) 2953, 1733, 1602, 1452, 1370, 1315, 1268, 1177, 1107, 1095,

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

1069, 1027, 757, 710; $[\alpha]_D^{22} +38.98$ (c 1.44, CHCl_3); HRMS (ESI, positive) m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{47}\text{H}_{46}\text{O}_{13}\text{Na}$ 841.2831, found 841.2834.

Compound **S4**

To a solution of **S3** (58.8 mg) in MeOH (2.9 mL) was added NaOMe (39.8 mg, 0.737 mmol) at 0 °C under an Ar atmosphere. After being stirred for 1.5 h at RT, the reaction mixture was neutralized with Amberlite IR120B, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O} = 30/3/1$, lower layer - 15/3/1, lower layer) to afford **S4** (19.1 mg, 0.0475 mmol, 72% in 2 steps) as a colorless viscous oil.

^1H NMR (400 MHz, CD_3OD) δ 5.52 (dt, $J = 11.0, 7.3$ Hz, 1H), 5.39 (dt, $J = 11.0, 7.8$ Hz, 1H), 4.26 (d, $J = 7.8$ Hz, 1H), 3.88 (dt, $J = 9.6, 7.3$ Hz, 1H), 3.87-3.84 (m, 1H), 3.68 (s, 3H), 3.68-3.64 (m, 1H), 3.55 (dt, $J = 9.6, 6.9$ Hz, 1H), 3.34 (dd, $J = 8.7, 8.7$ Hz, 1H), 3.27-3.26 (m, 2H), 3.16 (dd, $J = 8.7, 7.8$ Hz, 1H), 2.72 (dd, $J = 14.9, 3.7$ Hz, 1H) 2.45-2.29 (m, 7H), 2.23-2.16 (m, 1H), 2.09 (ddd, $J = 19.0, 10.5, 8.7$ Hz, 1H), 1.99 (dtd, $J = 10.1, 5.3, 0.9$ Hz, 1H), 1.57-1.46 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 221.6, 174.5, 129.0, 128.9, 104.4, 78.1, 77.9, 75.1, 71.6, 70.2, 62.8, 55.0, 52.1, 39.5, 39.2, 38.6, 29.0, 28.1, 26.4; IR (film) 3402, 2952, 2890, 1734, 1438, 1232, 1164, 1079, 1037, 754; $[\alpha]_D^{22} -26.33$ (c 0.61, MeOH); HRMS (ESI, positive) m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{19}\text{H}_{30}\text{O}_9\text{Na}$ 425.1782, found 425.1782.

Compound **2**

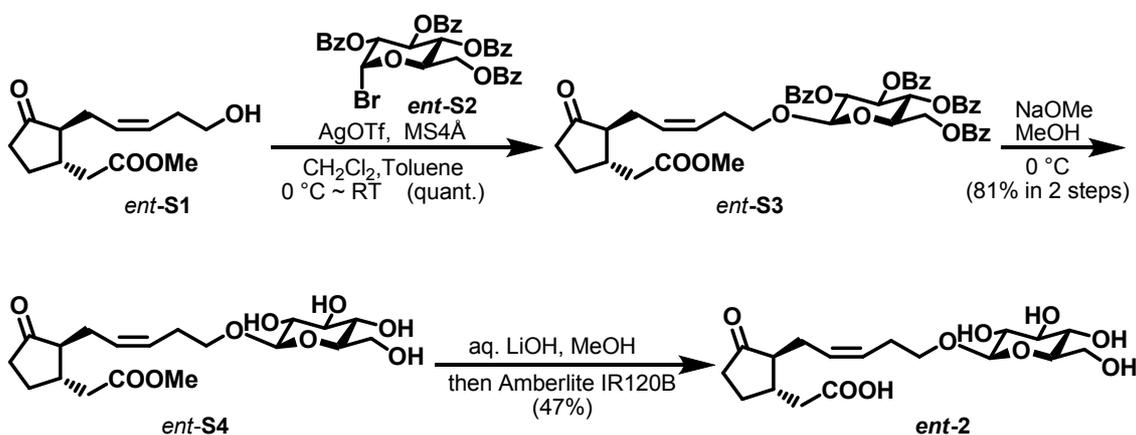
To a solution of **S4** (19.1 mg, 0.0475 mmol) in MeOH (0.48 mL) was added 1 N LiOH aq. (0.96 mL) at 0 °C. After being stirred for 10 min at RT, the mixture was added Amberlite IR120B, stirred for 20 min at RT, filtered and concentrated *in vacuo*. The residue was purified by HPLC, using a Cosmosil 5C18AR column ($\phi 20 \times 250$ mm, Nacalai Tesque Co.) with 13% MeCN aq. containing 0.05% TFA to afford **2** (9.3 mg, 0.0240 mmol, 51%) as a colorless viscous oil.

^1H NMR (400 MHz, CD_3OD) δ : 5.51 (dt, $J = 11.0, 7.3$ Hz, 1H), 5.41 (dt, $J = 11.0, 7.3$ Hz, 1H), 4.26 (d, $J = 7.8$ Hz, 1H), 3.88 (dt, $J = 9.6, 6.9$ Hz, 1H), 3.87-3.84 (m, 1H), 3.68-3.63 (m, 1H),

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

3.55 (dt, $J = 9.6, 6.9$ Hz, 1H), 3.34 (dd, $J = 9.6, 7.8$ Hz, 1H), 3.28- 3.26 (m, 2H), 3.16 (dd, $J = 8.7, 7.8$ Hz, 1H), 2.68 (dd, $J = 19.2, 8.7$ Hz, 1H), 2.46-2.20 (m, 8H), 2.09 (ddd, $J = 19.2, 10.5, 8.7$ Hz, 1H), 1.99 (dt, $J = 9.2, 5.5$ Hz, 1H), 1.58-1.48 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 221.8, 176.0, 129.0, 128.9, 104.4, 78.1, 77.9, 75.1, 71.7, 70.2, 62.8, 55.1, 39.8, 39.2, 38.6, 29.0, 28.2, 26.4; IR (film) 3388, 2924, 1730, 1406, 1260, 1160, 1072, 1033, 748; $[\alpha]_{\text{D}}^{20}$ -31.89 (c 0.46, MeOH); HRMS (ESI, negative) m/z $[\text{M}-\text{H}]^-$ calcd for $\text{C}_{18}\text{H}_{27}\text{O}_9$ 387.1661, found 387.1664.

Scheme S2. Synthesis of 12-O- β -D-glucopyranosyl-*ent*-jasmonic acid



Compound *ent*-S3

To a suspension of *ent*-S1 (30.3 mg, 0.125 mmol), *ent*-S2 (175.4 mg, 0.266 mmol), and MS4A (0.270 g) in dehydrated CH_2Cl_2 (1.2 mL) was slowly added AgOTf (85.6 mg, 0.333 mmol) in dehydrated toluene (1.2 mL) at 0°C under an Ar atmosphere. After being stirred for 75 min at RT in dark, the reaction mixture was diluted with CHCl_3 and filtered through a pad of Celite. The filtrate was washed with sat. NaHCO_3 aq., dried over Na_2SO_4 , filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (n -hexane/EtOAc = 3/1 -2/1 -4/3 -1/1) gave *ent*-S3 (131.6 mg, quant.) as a colorless viscous oil.

^1H NMR (400 MHz, CDCl_3) δ 8.02 (dd, $J = 8.5, 1.4$ Hz, 2H), 7.95 (dd, $J = 8.5, 1.4$ Hz, 2H), 7.90 (dd, $J = 8.2, 1.4$ Hz, 2H), 7.83 (dd, $J = 8.2, 1.4$ Hz, 2H), 7.56-7.25 (m, 12H), 5.90 (dd, $J = 9.6, 9.6$ Hz, 1H), 5.68 (dd, $J = 9.6, 9.6$ Hz, 1H), 5.52 (dd, $J = 9.6, 7.8$ Hz, 1H), 5.33 (dt, $J = 11.0,$

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

7.3 Hz, 1H), 5.20 (dt, $J = 11.0, 7.3$ Hz, 1H), 4.88 (d, $J = 7.8$ Hz, 1H), 4.65 (dd, $J = 12.0, 3.2$ Hz, 1H), 4.50 (dd, $J = 12.0, 5.5$ Hz, 1H), 4.18 (ddd, $J = 9.6, 5.5, 3.2$ Hz, 1H), 3.93 (dt, $J = 9.6, 6.4$ Hz, 1H), 3.68 (s, 3H), 3.56 (dt, $J = 9.6, 6.9$ Hz, 1H), 2.59 (dd, $J = 14.2, 2.8$ Hz, 1H), 2.35-2.15 (m, 8H), 2.03 (ddd, $J = 18.9, 11.0, 8.7$ Hz, 1H), 1.81 (dt, $J = 9.6, 5.5$ Hz, 1H), 1.50-1.40 (m, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 218.6, 172.4, 166.1, 165.8, 165.2, 165.0, 133.3, 133.1, 133.1, 129.8 (2 C), 129.7 (6 C), 129.6, 129.4, 128.8, 128.8, 128.3 (2 C), 128.3 (2 C), 128.3 (2 C), 128.2 (2 C), 127.4, 127.4, 101.2, 72.9, 72.2, 71.9, 69.8, 69.6, 63.2, 53.8, 51.6, 38.6, 37.8, 37.6, 27.7, 27.1, 25.4; IR (film) 2954, 1733, 1602, 1451, 1370, 1315, 1267, 1177, 1108, 1095, 1069, 1027, 755, 710; $[\alpha]_{\text{D}}^{22}$ -38.73 (c 1.33, CHCl_3); HRMS (ESI, positive) m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{47}\text{H}_{46}\text{O}_{13}\text{Na}$ 841.2831, found 841.2831.

Compound ent-S4

To a solution of *ent*-S3 (62.9 mg) in MeOH (3.1 mL) was added NaOMe (44.6 mg, 0.826 mmol) at 0 °C under an Ar atmosphere. After being stirred for 1.5 h at RT, the reaction mixture was neutralized with Amberlite IR120B, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O} = 30/3/1$, lower layer - 15/3/1, lower layer) to afford *ent*-S4 (19.5 mg, 0.0485 mmol, 81% in 2 steps) as a colorless viscous oil.

^1H NMR (400 MHz, CD_3OD) δ 5.51 (dt, $J = 11.0, 7.3$ Hz, 1H), 5.39 (td, $J = 11.0, 7.8$ Hz, 1H), 4.26 (d, $J = 7.8$ Hz, 1H), 3.88 (dt, $J = 9.6, 7.3$ Hz, 1H), 3.87-3.84 (m, 1H), 3.68 (s, 3H), 3.68-3.64 (m, 1H), 3.55 (dt, $J = 9.6, 6.9$ Hz, 1H), 3.36-3.32 (m, 1H), 3.28-3.26 (m, 2H), 3.16 (dd, $J = 8.7, 7.8$ Hz, 1H), 2.72 (dd, $J = 14.9, 3.7$ Hz, 1H), 2.45-2.29 (m, 7H), 2.23-2.13 (m, 1H), 2.09 (ddd, $J = 19.1, 10.5, 8.7$ Hz, 1H), 1.99 (dtd, $J = 10.0, 5.3, 0.9$ Hz, 1H), 1.57-1.46 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 221.6, 174.5, 129.0, 128.9, 104.4, 78.1, 77.9, 75.1, 71.6, 70.2, 62.8, 55.0, 52.1, 39.5, 39.2, 38.6, 29.0, 28.1, 26.4; IR (film); 3406, 2952, 2891, 1734, 1438, 1232, 1164, 1078, 1037, 755; $[\alpha]_{\text{D}}^{22}$ +26.51 (c 0.77, MeOH); HRMS (ESI, positive) m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{19}\text{H}_{30}\text{O}_9\text{Na}$ 425.1782, found 425.1782.

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

Compound *ent-2*

To a solution of *ent-S4* (19.5 mg, 0.0485 mmol) in MeOH (0.48 mL) was added 1 N LiOH aq. (0.97 mL) at 0 °C. After being stirred for 30 min at RT, the mixture was added Amberlite IR120B, stirred for 20 min at RT, filtered and concentrated *in vacuo*. The residue was purified by HPLC, using a Cosmosil 5C18AR column ($\phi 20 \times 250$ mm, Nacalai Tesque Co.) with 13% MeCN aq. containing 0.05% TFA to afford *ent-2* (8.9 mg, 0.0229 mmol, 47%) as a colorless viscous oil.

^1H NMR (400 MHz, CD_3OD) δ : 5.51 (dt, $J = 11.0, 7.3$ Hz, 1H), 5.41 (dt, $J = 11.0, 7.8$ Hz, 1H), 4.26 (d, $J = 7.8$ Hz, 1H), 3.88 (td, $J = 9.2, 6.9$ Hz, 1H), 3.87-3.84 (m, 1H), 3.68-3.64 (m, 1H), 3.55 (dt, $J = 9.2, 7.3$ Hz, 1H), 3.34 (dd, $J = 9.2, 7.8$ Hz, 1H), 3.28- 3.26 (m, 2H), 3.16 (dd, $J = 9.2, 7.8$ Hz, 1H), 2.68 (dd, $J = 19.0, 8.7$ Hz, 1H), 2.46-2.20 (m, 8H), 2.09 (ddd, $J = 19.1, 10.5, 8.7$ Hz, 1H), 1.99 (dt, $J = 9.2, 5.5$ Hz, 1H), 1.58-1.48 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 221.8, 176.1, 129.0, 129.0, 104.4, 78.1, 77.9, 75.1, 71.7, 70.2, 62.8, 55.1, 39.9, 39.2, 38.7, 29.0, 28.2, 26.4; IR (film) 3388, 2927, 1726, 1407, 1261, 1164, 1078, 1033, 752; $[\alpha]_{\text{D}}^{21} +31.63$ (c 0.47, MeOH); HRMS (ESI, negative) m/z $[\text{M}-\text{H}]^-$ calcd for $\text{C}_{18}\text{H}_{27}\text{O}_9$ 387.1661, found 387.1658

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

mixture was filtered through a pad of Celite, and concentrated *in vacuo* to afford **S6** (148.8 mg, 0.670 mmol, 98 %) as a colorless viscous oil.

¹H NMR (400 MHz, CD₃OD) δ 4.16 (ddd, *J* = 7.3, 6.0, 6.0 Hz, 1H), 4.06 (dd, *J* = 8.6, 6.0 Hz, 1H), 3.95 (dd, *J* = 8.6, 6.0 Hz, 1H), 3.76 (ddd, *J* = 6.0, 4.6, 4.6 Hz, 1H), 3.73 (dd, *J* = 4.6, 2.1 Hz, 1H), 3.67 (dd, *J* = 11.3, 4.6 Hz, 1H), 3.64 (dd, *J* = 7.3, 2.1 Hz, 1H), 3.59 (dd, *J* = 11.3, 6.0 Hz, 1H), 1.37 (s, 3H), 1.32 (s, 3H); ¹³C NMR (100 MHz, CD₃OD) δ 110.1, 77.1, 74.8, 73.9, 71.4, 67.9, 64.0, 27.1, 25.7; IR (film) 3388, 2986, 2935, 2935, 2898, 2889, 1373, 1253, 1217, 1066, 846; [α]_D²³ +4.08 (*c* 1.08, MeOH); HRMS (ESI, negative) *m/z* [M-H]⁻ calcd for C₉H₁₇O₆ 221.1030, found 221.1030.

Compound **S7**

To a solution of **S6** (111.1 mg, 0.500 mmol) in dehydrated DMF (4.7 mL) was added imidazole (103.1 g, 1.51 mmol) and TBDPSCl (310.0 mg, 1.13 mmol) at RT under an Ar atmosphere. After being stirred for 1.7 h at RT, the reaction mixture was added brine, and the mixture was extracted with CHCl₃, washed with MilliQ and brine, dried over Na₂SO₄, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (CHCl₃/MeOH/H₂O = 100/3/1, lower layer) to afford silyl ether (246.2 mg) as a colorless viscous oil.

To a solution of silyl ether (246.2 mg) in dehydrated CH₂Cl₂ (5.4 mL) was added *N,N*-diisopropylethylamine (1.66 g, 12.8 mmol) and MOMCl (0.775 g, 9.63 mmol) at 0 °C under an Ar atmosphere. After being stirred for 5 h under reflux, the mixture was quenched with brine, extracted with EtOAc, dried over Na₂SO₄, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 4/1 -3/1 -2/1) to afford MOM ether (276.2 mg) as a pale yellow oil.

To a solution of MOM ether (276.2 mg) in dehydrated THF (4.7 mL) was added TBAF (1.0 M in THF, 1.4 mL, 1.40 mmol) at RT under an Ar atmosphere. After being stirred for 2 h at RT, the mixture was added brine, extracted with CHCl₃, dried over Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

hexane/EtOAc = 4/3 -1/1 -1/2 -1/3 - 0/1) to afford **S7** (160.8 mg, 0.454 mmol, 97%) as a pale yellow oil.

¹H NMR (400 MHz, CDCl₃) δ 4.83 (d, *J* = 6.4 Hz, 1H), 4.78-4.72 (m, 5H), 4.23 (dt, *J* = 6.9, 6.0 Hz, 1H), 4.09 (dd, *J* = 8.0, 6.0 Hz, 1H), 3.98 (dd, *J* = 8.0, 6.9 Hz, 1H), 3.93 (dd, *J* = 5.5, 4.5 Hz, 1H), 3.87-3.73 (m, 4H), 3.44 (s, 3H), 3.43 (s, 3H), 3.39 (s, 3H), 1.40 (s, 3H), 1.35(s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 108.5, 98.3, 97.8, 97.4, 80.9, 78.1, 77.7, 75.4, 66.2, 62.2, 56.1, 55.9, 55.6, 26.1, 24.9; IR (film) 3482, 2986, 2938, 2896, 2825, 1456, 1371, 1214, 1153, 1028, 919, 859; [α]_D¹⁹ +31.92 (*c* 2.63, CHCl₃); HRMS (ESI, positive) *m/z* [M+Na]⁺ calcd for C₁₅H₃₀O₉Na 377.1782, found 377.1782.

Compound **S8**

To a solution of **S7** (315.8 mg, 0891 mmol) in dehydrated DME (5.9 mL) was added 15-crown-5 (1.2 g, 5.35 mmol) and NaH (123 mg, 5.12 mmol) at 0 °C under an Ar atmosphere. After being stirred for 30 min at RT, the mixture was added allyl bromide (863 mg, 7.13 mmol) at 0 °C under an Ar atmosphere. After being stirred for 25 min at RT, the mixture was added sat. NH₄Cl aq., extracted with EtOAc, dried over Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 4/1 -3/1 -2/1 -1/1 -2/3 -2/3 -3/4 -0/1) to afford allyl ether (351.5 mg) as a colorless oil.

To a solution of allyl ether (359.4 mg, 0.911 mmol) in dehydrated THF (9.1 mL) was added 9-BBN (0.5 M in THF, 5.46 mL, 2.73 mmol) at 0 °C under an Ar atmosphere. After being stirred for 45 min at 0 °C, the mixture was added 3 N NaOH aq.(12 mL) and H₂O₂ (35%, 12 mL) at 0 °C. Further stirred for 2 h at RT, the mixture was extracted with CHCl₃, washed with sat. Na₂S₂O₃, dried over Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 5/1 -4/1 -3/1 -2/1) to afford **S8** (336.8 mg, 0.817 mmol, 90%) as a colorless oil.

¹H NMR (400 MHz, CDCl₃) δ 4.82 (d, *J* = 6.9 Hz, 1H), 4.77 (s, 2H), 4.76 (d, *J* = 6.9 Hz, 1H), 4.75 (d, *J* = 6.9 Hz, 1H), 4.73 (d, *J* = 6.9 Hz, 1H), 4.26 (ddd, *J* = 7.0, 6.4, 5.5 Hz, 1H), 4.09 (dd, *J* = 8.2, 6.4 Hz, 1H), 3.99 (dd, *J* = 8.2, 7.0 Hz, 1H), 3.96 (ddd, *J* = 5.5, 5.5, 4.1 Hz, 1H), 3.92 (dd, *J* = 5.5, 4.5 Hz, 1H), 3.88 (dd, *J* = 5.5, 4.5 Hz, 1H), 3.76-3.73 (m, 2H), 3.73-3.66 (m, 2H),

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

3.63 (t, $J = 6.0$ Hz, 2H), 3.43 (s, 3H), 3.40 (s, 3H), 3.39 (s, 3H) 2.65 (br s, 1H), 1.82 (quin. like, $J = 6.0$ Hz, 2H), 1.41 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 108.7, 98.6, 98.1, 97.1, 77.8, 77.8, 77.0, 75.8, 70.8, 69.7, 66.4, 61.1, 56.4, 56.2, 55.8, 32.0, 26.4, 25.1; IR (film) 3495, 2985, 2935, 2893, 2825, 1457, 1371, 1260, 1214, 1153, 1103, 1029, 919, 860; $[\alpha]_{\text{D}}^{22} +29.19$ (c 1.08, CHCl_3); HRMS (ESI,positive) m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{18}\text{H}_{36}\text{O}_{10}\text{Na}$ 435.2201, found 435.2201.

Compound S9

To a solution of **S8** (52.4 mg, 0.127 mmol) in dehydrated pyridine (1.3 mL) was added MsCl (46.4 mg, 0.405 mmol) at 0 °C under an Ar atmosphere. After being stirred for 1 h at RT, the mixture was added brine, extracted with EtOAc, washed with 1N HCl aq., sat. NaHCO_3 aq. and brine, dried over Na_2SO_4 , filtered and concentrated *in vacuo* to afford crude mesylate (56.6 mg).

To a solution of crude mesylate (45.4 mg) in dehydrated DMF (1.2 mL) was added tetrabutylammonium bromide (100.2 mg, 0.311 mmol) at RT under an Ar atmosphere. After being stirred for 4 h at 50 °C to 60 °C, the mixture was added brine, extracted with EtOAc, washed with MilliQ and brine, dried over Na_2SO_4 , filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 3/1 -2/1 -1/1 -1/2) to afford **S9** (39.3 mg, 0.0827 mmol, 80%) as a colorless oil.

^1H NMR (400 MHz, CDCl_3) δ 4.82 (d, $J = 6.4$ Hz, 1H), 4.78 (d, $J = 6.4$ Hz, 1H), 4.77 (s, 2H), 4.76 (d, $J = 6.9$ Hz, 1H), 4.73 (d, $J = 6.9$ Hz, 1H), 4.28 (ddd, $J = 6.4, 6.4, 5.9$ Hz, 1H), 4.09 (dd, $J = 8.2, 6.4$ Hz, 1H), 3.99 (dd, $J = 8.2, 6.4$ Hz, 1H), 3.96 (ddd, $J = 5.5, 5.0, 3.7$ Hz, 1H), 3.91 (dd, $J = 5.0, 4.1$ Hz, 1H), 3.89 (dd, $J = 5.9, 4.1$ Hz, 1H), 3.74 (dd, $J = 10.5, 3.7$ Hz, 1H), 3.66 (dd, $J = 10.5, 5.5$ Hz, 1H), 3.62-3.54 (m, 2H), 3.51 (t, $J = 6.4$ Hz, 2H), 3.43 (s, 3H), 3.40 (s, 3H), 3.39 (s, 3H), 2.13-2.07 (m, 2H), 1.41 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 108.6, 98.5, 98.1, 97.1, 78.0, 77.7, 77.2, 75.7, 71.0, 67.6, 66.5, 56.4, 56.2, 55.8, 41.9, 32.6, 26.5, 25.2; IR (film) 2983, 2930, 2893, 2858, 2824, 1726, 1451, 1370, 1261, 1214, 1154, 1105, 1029, 919, 860, 801; $[\alpha]_{\text{D}}^{19} +1.61$ (c 0.27, CHCl_3); HRMS (ESI,positive) m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{18}\text{H}_{35}\text{O}_9\text{BrNa}$ 497.1357, found 497.1357.

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

Compound S11

To a solution of **S9** (236.2 mg, 0.497 mmol) in dehydrated MeCN (5.0 mL) was added PPh₃ (260.7 mg, 0.994 mmol) and *N,N*-diisopropylethylamine (385.2 mg, 2.98 mmol) at RT under an Ar atmosphere. After being stirred for 3 d under reflux, the mixture was added PPh₃ (136.9 mg, 0.522 mmol). After being stirred for 1 d under reflux, the mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 2/1 -1/1 - CHCl₃/MeOH = 20/1 -10/1- 5/1) to afford Wittig salt (355.3 mg, 0.481 mmol, 97%) as a pale yellow oil.

To a solution of Wittig salt (72.0 mg, 0.0975 mmol) and 18-crown-6 (46.9 mg, 0.177 mmol) in dehydrated THF (1.2 mL) was added KHMDS (0.5 M in THF, 0.234 mL, 0.117 mmol) at 0 °C under an Ar atmosphere. After being stirred for 30 min at RT, the reaction mixture was added **S10** (0.08 M in THF, 92.4 mg, 0.293 mmol) dropwisely at -78 °C under an Ar atmosphere. Further stirred for 40 min, the reaction mixture was quenched with sat. NH₄Cl aq. at 0 °C, extracted with EtOAc, dried over Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 10/1 -8/1 -5/1 -4/1 -3/1 -2/3) to afford **S11** (40.7 mg, 0.0587 mmol, 60%) as a colorless viscous oil.

¹H NMR (400 MHz, CDCl₃) δ 5.50 (dt, *J* = 11.0, 7.8 Hz, 1H), 5.38 (dt, *J* = 11.0, 6.9 Hz, 1H), 4.81 (d, *J* = 6.9 Hz, 1H), 4.79-4.75 (m, 4H), 4.73 (d, *J* = 6.9 Hz, 1H), 4.28 (ddd, *J* = 6.4, 6.4, 5.9 Hz, 1H), 4.18-4.15 (m, 1H), 4.19 (dd, *J* = 8.0, 6.4 Hz, 1H), 4.00 (dd, *J* = 8.0, 6.4 Hz, 1H), 3.98-3.94 (m, 1H), 3.93 (dd, *J* = 5.9, 4.6 Hz, 1H), 3.89 (dd, *J* = 5.5, 4.6 Hz, 1H), 3.72 (dd, *J* = 10.5, 3.7 Hz, 1H), 3.66 (s, 3H), 3.64 (dd, *J* = 10.5, 5.9 Hz, 1H), 3.48-3.43 (m, 2H), 3.43 (s, 3H), 3.40 (s, 3H), 3.39 (s, 3H), 2.52 (dd, *J* = 13.1, 2.8 Hz, 1H), 2.33 (q, *J* = 6.9 Hz, 2H), 2.18-2.01 (m, 5H), 1.76-1.68 (m, 1H), 1.63-1.56 (m, 1H), 1.41 (s, 3H), 1.35 (s, 3H), 1.34-1.21 (m, 2H), 0.87 (s, 9H), 0.04 (s, 3H), 0.02 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 173.7, 130.7, 125.9, 108.6, 98.5, 98.1, 97.1, 77.9, 77.7, 76.8, 75.8, 74.5, 71.0, 70.9, 66.3, 56.4, 56.2, 55.8, 51.6, 51.4, 39.6, 38.6, 33.8, 28.9, 28.0, 26.4, 25.8 (4 C), 25.2, 18.0, -4.3, -5.0; IR (film) 2953, 2932, 2864, 2857, 2824, 1738, 1472, 1463, 1439, 1370, 1254, 1214, 1155, 11.07, 1031, 919, 837, 774; [α]_D²¹ +55.71 (*c* 0.86, CHCl₃); HRMS (ESI, positive) *m/z* [M+Na]⁺ calcd for C₃₄H₆₄O₁₂SiNa 715.4059, found 715.4060.

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

Compound S12

To a solution of **S11** (58.9 mg, 0.0850 mmol) in dehydrated THF (1.1 mL) was added TBAF (1.0 M in THF, 0.425 mL, 0.425 mmol) at RT under an Ar atmosphere. After being stirred for 2 h at 40 °C, the mixture was added TBAF (1.0 M in THF, 0.425 mL, 0.425 mmol). Further stirred for 5.5 h at 40 °C, the mixture was quenched with sat. NH₄Cl aq., extracted with CHCl₃, dried over Na₂SO₄, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 2/1 -1/1 -1/2 -1/3) to afford alcohol (47.3 mg) as a pale yellow viscous oil.

To a solution of alcohol (47.3 mg) in *tert*-BuOH (1.6 mL) was added IBX (46.1 mg, 0.165 mmol) at RT under an Ar atmosphere. After being stirred for 4 h at 80 °C, the mixture was quenched with sat. NaHCO₃ aq. and sat. Na₂S₂O₃ aq. at RT, extracted with CHCl₃, dried over Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (CH₂Cl₂/acetone = 30/1 -20/1 -10/1 -6/1 -5/1) to afford **S12** (41.0 mg, 0.0711 mmol, 87%) as a colorless viscous oil.

¹H NMR (400 MHz, CDCl₃) δ 5.48 (dt, *J* = 10.5, 6.8 Hz, 1H), 5.39 (dt, *J* = 10.5, 7.3 Hz, 1H), 4.81 (d, *J* = 6.4 Hz, 1H), 4.79-4.76 (m, 3H), 4.76 (d, *J* = 6.4 Hz, 1H), 4.72 (d, *J* = 6.4 Hz, 1H), 4.28 (ddd, *J* = 7.3, 6.4, 5.9 Hz, 1H), 4.09 (dd, *J* = 8.2, 6.4 Hz, 1H), 4.00 (dd, *J* = 8.2, 7.3 Hz, 1H), 3.98-3.91 (m, 2H), 3.88 (dd, *J* = 5.5, 4.1, Hz, 1H), 3.73-3.70 (m, 1H), 3.70 (s, 3H), 3.64, (dd, *J* = 10.3, 5.9 Hz, 1H), 3.50-3.42 (m, 2H), 3.42 (s, 3H), 3.40 (s, 3H), 3.39 (s, 3H), 2.69 (dd, *J* = 19.0, 8.7 Hz, 1H), 2.40-2.01 (m, 8H), 2.11 (ddd, *J* = 19.0, 10.5, 8.7 Hz, 1H), 1.93-1.88 (m, 1H), 1.56-1.45 (m, 1H), 1.40 (s, 3H), 1.35 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 218.6, 172.4, 128.1, 127.5, 108.5, 98.5, 98.1, 97.1, 77.8, 77.6, 76.8, 75.8, 70.8, 70.7, 66.3, 56.3 56.1, 55.8, 53.8, 51.6, 38.7, 37.9, 37.6, 27.9, 27.1, 26.4, 25.6, 25.1; IR (film) 2984, 2951, 2932, 2894, 2824, 1739, 1440, 1371, 1260, 1213, 1154, 1105, 1027, 919, 858; [α]_D²¹ -10.69 (*c* 1.86, CHCl₃); HRMS (ESI, positive) *m/z* [M+Na]⁺ calcd for C₂₈H₄₈O₁₂Na 599.3038, found 599.3038.

Compound 4

To a solution of **S12** (37.3 mg, 0.0647 mmol) in dehydrated CH₂Cl₂ (1.3 mL) was added *B*-bromocatecholborane (0.2 M in CH₂Cl₂, 1.78 mL, 0.356 mmol) at -20 °C under an Ar

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

atmosphere. After being stirred for 3 h at -20 °C, the mixture was added *B*-bromocatecholborane (0.2 M in CH₂Cl₂, 0.6 mL, 0.120 mmol) at -20 °C. After being stirred for 30 min at -20 °C, the mixture was added MilliQ at 0 °C, further stirred for 20 min at RT and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 2/1 -4/3 -1:1- 1/2- CHCl₃ only -CHCl₃/MeOH/H₂O = 100/3/1, lower layer -50/3/1, lower layer -30/3/1, lower layer -15/3/1, lower layer -7/3/1, lower layer) to afford pentaol (13.0 mg, 0.0321 mmol) as a pale yellow oil.

To a solution of pentaol (13.0 mg, 0.0321 mmol) in MeOH(0.32 mL) was added 1 N LiOH aq.(0.64 mL) at 0 °C. After being stirred for 25 min at RT, the mixture was added Amberlite IR120B, stirred for 20 min at RT, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (CHCl₃/MeOH/H₂O = 50/3/1, lower layer -30/3/1, lower layer -15/3/1, lower layer -7/3/1, lower layer -6/4/1) and HPLC, using a Develosil ODS-HG-5 column (□20 × 250 mm, Nomura Chemical Co., Ltd.) with 30% MeOH aq. containing 0.05% TFA to afford **4** (7.6 mg, 0.0195 mmol, 30%) as a colorless oil.

¹H NMR (400 MHz, CD₃OD) δ 5.49 (dt, *J* = 11.0, 6.9 Hz, 1H), 5.40 (dt, *J* = 11.0, 7.3 Hz, 1H), 3.90-3.85 (m, 2H), 3.77 (dd, *J* = 11.0, 3.2 Hz, 1H), 3.72-3.68 (m, 1H), 3.63-3.57 (m, 3H), 3.53-3.47 (m, 3H), 2.68 (dd, *J* = 19.0, 8.4 Hz, 1H), 2.40-2.22 (m, 8H), 2.09 (ddd, *J* = 18.9, 11.0, 8.2 Hz, 1H), 2.00-1.96 (m, 1H), 1.58-1.48 (m, 1H); ¹³C NMR (100 MHz, CD₃OD) δ 221.8, 176.1, 129.2, 128.9, 73.7, 73.5, 73.2, 73.0, 72.0, 71.0, 64.8, 55.1, 39.7, 39.2, 38.7, 28.9, 28.2, 26.4; IR (film) 3388, 2955, 2926, 2871, 2856, 1732, 1457, 1407, 1260, 1096, 1035, 802, 748; [α]_D²³ -33.25(*c* 0.38, MeOH); HRMS (ESI, negative) *m/z* [M-H]⁻ calcd for C₁₈H₂₉O₉389.1817, found 389.1815

Cell-shrinking assay using living extensor motor cell protoplasts of *S. saman*

The protoplasts were prepared from *S. saman* using the extensor (adaxial) part of the tertiary pulvini on the 2nd or 3rd branch from the short apex according to the previously reported method¹⁻⁵ with modifications. About 100 pieces of the extensor from tertiary pulvini were placed in 1 mL of predigestion solution (Gamborg's B-5, 0.3 M sorbitol, 50 mM MES-KOH (pH 5.5), 0.2% BSA, 8 mM CaCl₂). The osmotic pressure of the predigestion solution was then raised to the desired level (0.6 M sorbitol) in two steps

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

over 20 min with osmotic adjustment solution (Gamborg's B-5, 4.0 M sorbitol, 50 mM MES-KOH (pH 5.5), 0.2% BSA, 8 mM CaCl₂). Then the tissues were moved into the 35 mm tissue culture dish with 1.6 mL filtered enzyme solution (Gamborg's B-5, 50 mM MES-KOH (pH 5.5), 0.4 M sorbitol, 0.2% BSA, 8 mM CaCl₂, 3% (w/v) each of Driselase (Aska Pharmaceutical Co. Ltd.), Macerozyme R-10 and cellulase Onozuka RS, 0.3% pectolyase Y-23 (Yakult Pharmaceutical Industry Co., Ltd.)). The enzyme solution was penetrated into the tissues by shaking gently for 1 h at 30 °C. The tissues subsequently digested for further 1 h without agitation at 30 °C. Then the enzyme solution was discarded with three 1 mL rinses of recovering solution (Gamborg's B-5, 0.35 M sorbitol, 20 mM MES-Tris (pH 5.5), 100 mM KCl, and 1 mM CaCl₂). The protoplasts were released in 1.6 mL recovering solution for 0.5 h at 30 °C. Debris was removed by filtration of the protoplast suspension through a 50 µm nylon mesh. These two steps were repeated twice. The collected protoplasts were store on ice and concentrated on a sucrose cushion (0.57 M sucrose, 20 mM MES-Tris (pH 5.5), 10 mM KCl, 1 mM CaCl₂) by centrifugation at 60 × g, 4 °C for 5 min and subsequently further purified on sucrose gradient: protoplasts were suspended with 0.8 mL ~ 80% sucrose cushion in 2 mL eppendorf tube, then 0.5 mL mix solution (sucrose cushion : wash solution = 4 : 3, wash solution: 0.57 M sorbitol, 20 mM MES-Tris (pH 5.5), 10 mM KCl, 1 mM CaCl₂) was layered on top of the protoplast suspension and 0.5 mL wash solution was layered in upper part. The gradient was centrifuged at 130 × g, 4 °C for 10 min. The purified protoplasts were collected at the interphase between wash solution and mix solution and suspended in the wash solution on ice in dark.

The prepared protoplasts in 350 µL wash solution were sealed in a chamber cover (24.5 mm × 53.9 mm, 8 well, Matsunami Glass IND., LTD), placed under an inverted microscope (IX-71, Olympus, Tokyo, Japan), and monitored at 24 °C ± 1 °C under continuous irradiation with light (50 µmol m⁻² s⁻¹ PAR) passed through a green filter (43IF550-W45, Olympus, Tokyo, Japan), as previously reported^{4, 5}. Following incubation on the microscope for 10 min, 50 µL of a solution of the different compounds (each at 4 × 10⁻⁸ mol in wash solution) was added to the protoplast suspension. The status of the protoplasts was recorded with time-lapse photography (10 min intervals) for 40 min using a digital camera (DP 72, Olympus, Tokyo, Japan) and DP2-BSW analysis software (DP 72, Olympus, Tokyo, Japan). Finally, 200 µL of

0.02% fluorescein diacetate (FDA) in wash solution was added to enable the selection of living protoplasts⁵. The semidiameter for each magnified image of living protoplasts was measured by DP2-BSW analysis software (DP 72, Olympus, Tokyo, Japan).

Introduction of a FLAG tag on CMP 5-labeled proteins using click chemistry

The *Samanea* motor cell protoplast was prepared according to previous method³. To a suspension of freshly protoplasts (about 1×10^4 protoplasts in 19 μL wash solution) was added **5** (1×10^{-8} mol in 1 μL DMSO) and the mixture was incubated for 5 min on ice. After cross-linking by irradiation with UV light (365 nm, irradiated from ca. 1 cm above the surface, handy UV lamp LUV-16 [AS ONE, Co., Ltd]) for 20 min on ice and adding 100 μL wash solution, the cross-linked protoplasts were sedimented by centrifugation ($110 \times g$, 5 min, 4 $^{\circ}\text{C}$) and the supernatant was decanted. Then the cross-linked protoplasts were resuspended 1) in 99 μL wash solution in CFCC and added FLAG unit **8** (1×10^{-8} mol in 1 μL DMSO), or 2) in 18 μL wash solution in CuAAC and added FLAG unit **7** (1×10^{-8} mol in 1 μL DMSO), ascorbic acid (2×10^{-8} mol in 0.5 μL water) and ligation buffer (CuSO_4 2×10^{-8} mol and THPTA ligand 2×10^{-8} mol in 0.5 μL DMSO). After incubating for 1) 1 h in 30 $^{\circ}\text{C}$ or 2) 30 min on ice, the protoplasts were homogenized in 500 μL extraction buffer (0.25 M saccharose, 3 mM EDTA-2K, 2.5 mM DTT, 25 mM Tris-MES (pH 7.2) and one tablet of completeTM (Roche Co., Ltd.)/50 mL) using a plastic pestle. Centrifuging the lysate twice (1st: $3,000 \times g$, 15 min, 4 $^{\circ}\text{C}$, Kokusan H-9R with AN rotor [Kokusan Co. Ltd.]; 2nd: $150,000 \times g$, 1 h, 4 $^{\circ}\text{C}$, Beckman Coulter Optima TLX [Beckman Coulter Inc.]) gave a microsomal fraction pellet. The microsomal fractions were suspended in 15 μL of sample buffer (0.07 M Tris-HCl (pH6.8), 2% SDS, 6% glycerol, 1.86% DTT, bromophenol blue) and the solution was heated at 95 $^{\circ}\text{C}$ for 5 min. The reaction mixture was analyzed by SDS-PAGE (Ready Any KDTM resolving polyacrylamide gels, Bio-Rad Laboratories, Inc.) with a molecular weight marker (MagicMarkTM XP Western Protein Standard, Invitrogen). After western blotting using Hybond-P PVDF membrane (GE Healthcare UK, Ltd.), this membrane was treated with anti-FLAG antibody from rabbit (Delta Biolabs) and anti rabbit IgG antibody-HRP from goat (Santa Cruz Biotechnology Inc). The protein bands were detected by chemiluminescence using an ECL Advance western

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

blotting detection kit (GE Healthcare UK, Ltd.) with an LAS-4000 Bioimager (Fuji Film Co., Ltd.). The same PVDF membrane was further stained by Colloidal Gold Total Protein Stain (Bio-Rad Laboratories, Inc.).

Photoaffinitylabeling of MTJG using living *Samanea* motor cell with CMP

To a suspension of freshly protoplasts (about 1.5×10^4 protoplasts in 24 μL wash solution) was added **5** (1×10^{-8} mol in 1 μL DMSO), *ent-5* (1×10^{-8} mol in 1 μL DMSO), **6** (1×10^{-8} mol in 1 μL DMSO) or *ent-6* (1×10^{-8} mol in 1 μL DMSO) and the mixture was incubated for 5 min on ice. Following process was the same as described above.

Competition inhibition of binding between **5 and MTJG by using **1** or **9** or **10****

To a suspension of freshly protoplasts (about 1.5×10^4 protoplasts in 24 μL wash solution) was added **5** (1×10^{-8} mol in 1 μL DMSO), **5+1** (**5**, 1×10^{-8} mol; **1**, 5×10^{-7} mol in 1 μL DMSO), **5+9** (**4**, 1×10^{-8} mol; **9**, 5×10^{-7} mol in 1 μL DMSO) or **5+10** (**5**, 1×10^{-8} mol; **10**, 5×10^{-7} mol in 1 μL DMSO) and the mixture was incubated for 5 min on ice. Following process was the same as described above.

LC-MS analysis of *ent-6*

Ent-6 (1×10^{-4} M) was solved in 50%(v/v) MeCN in water for LC/ ESI-TOF-MS analysis using MicrOTOF-II spectrometers (Bruker Daltonics Ltd.) with Agilent 1290 Infinity HPLC system (Agilent Technologies). The equilibrium mixture of two isomers of *ent-6* was separated on an Agilent Eclipse Plus C18 column (1.8 m, ϕ 2.1 \times 50 mm, Agilent Technologies) using a gradient of 0.1% TFA (solvent A) and MeCN containing 0.1% TFA (solvent B). The gradient consisted of a linear increase from 26% B/74% A to 28% B/72% A in 10 min, a ramp to 90% B/10% A in 2 min and maintenance of 90% B/10% A in 2 min. Finally, the column was re-equilibrated for 2 min. 1 μL of *ent-6* was injected with the flow rate at 0.2 mL min⁻¹. The mass spectrometer was operated in

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

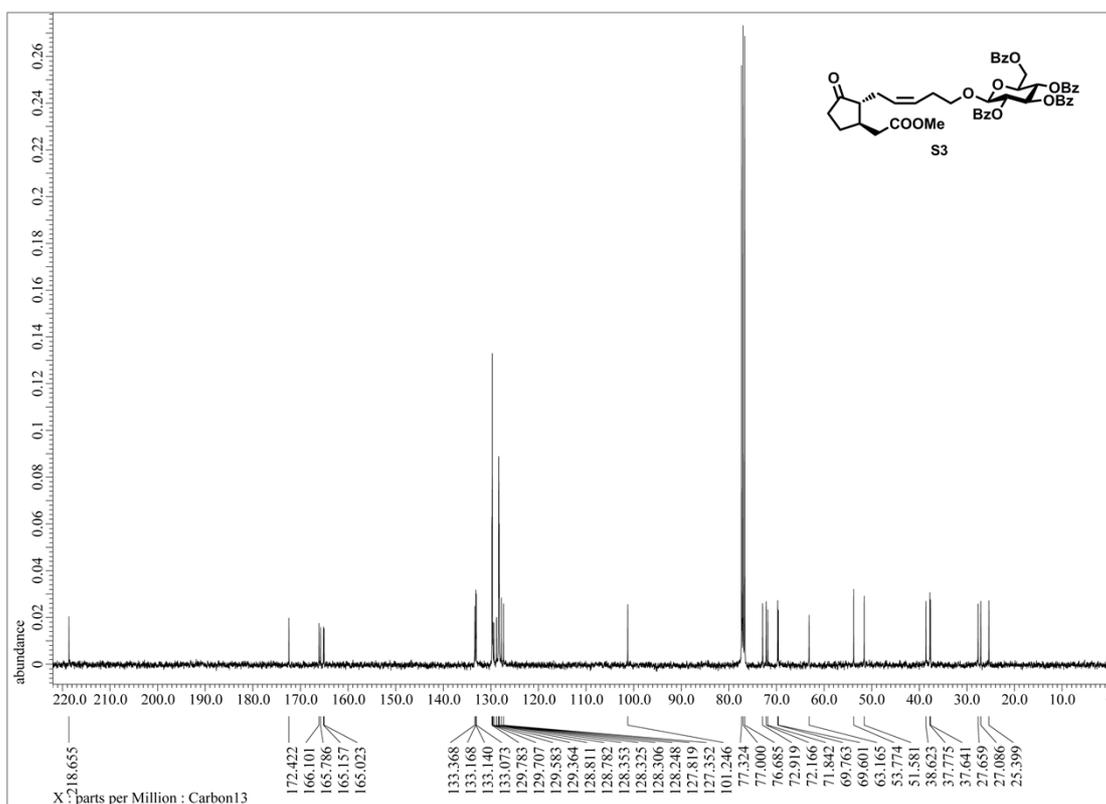
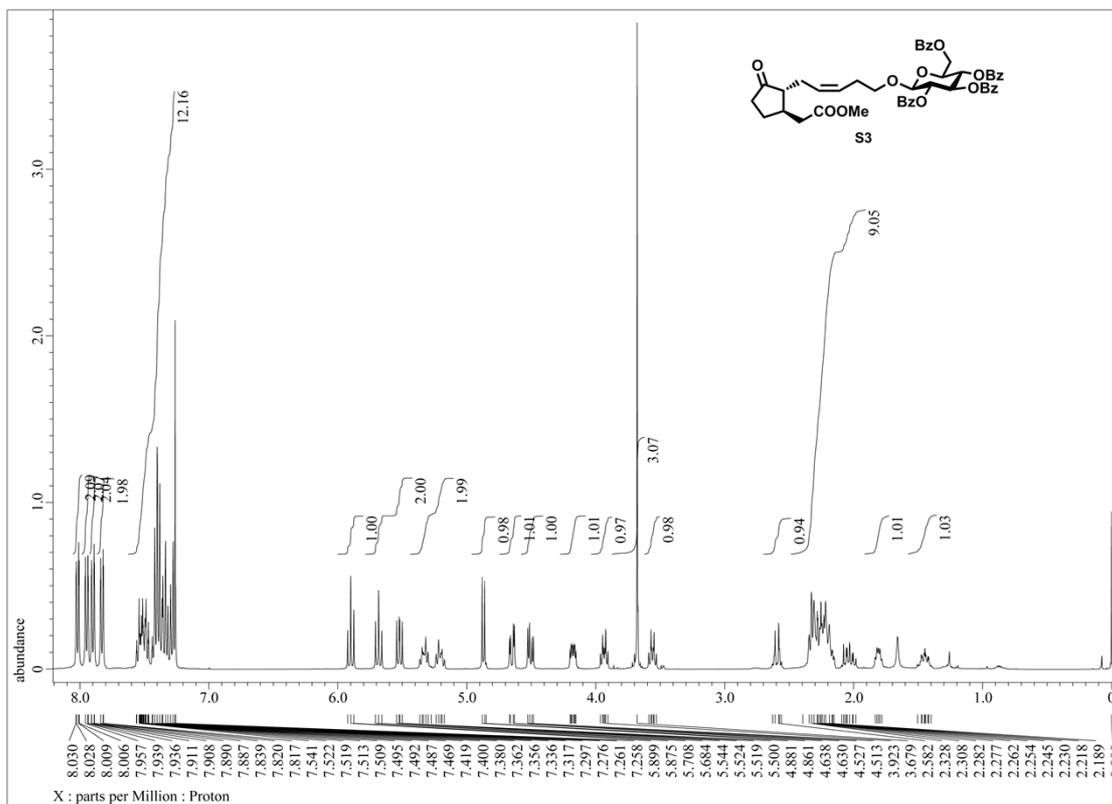
negative ion mode, with a capillary voltage of +3500 V, dry gas flow rates at 8.0L/min and dry temperature at 180 °C.

References

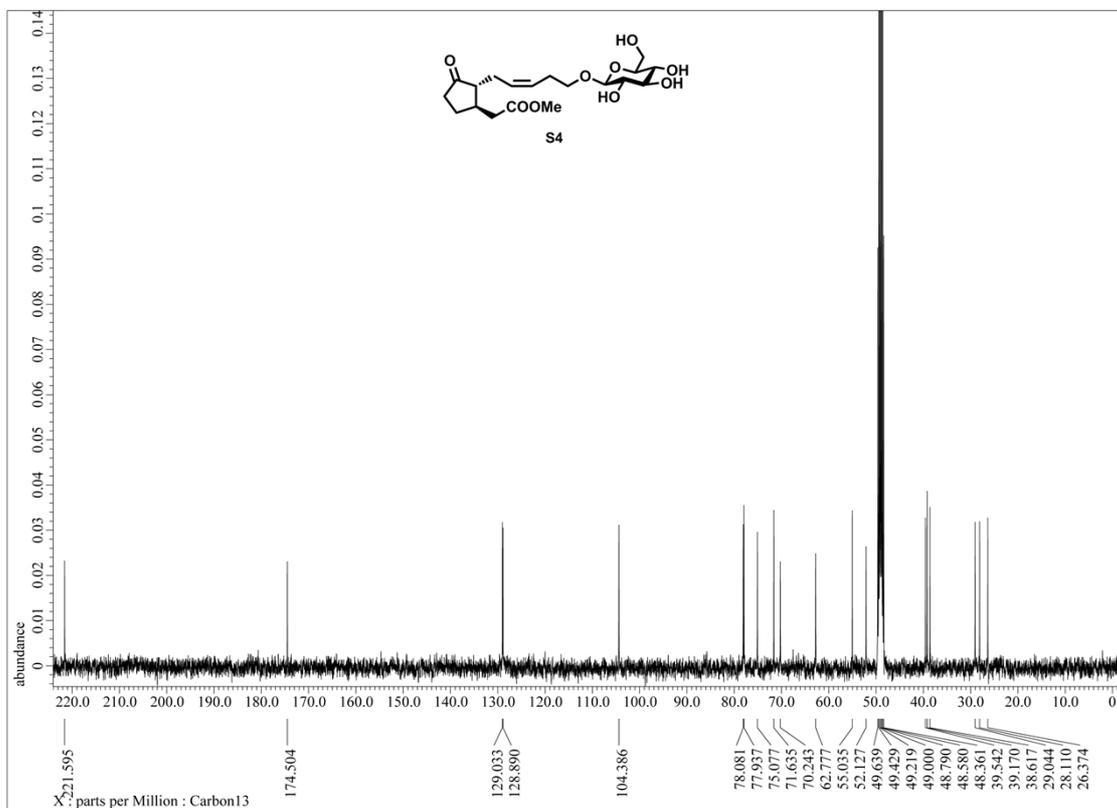
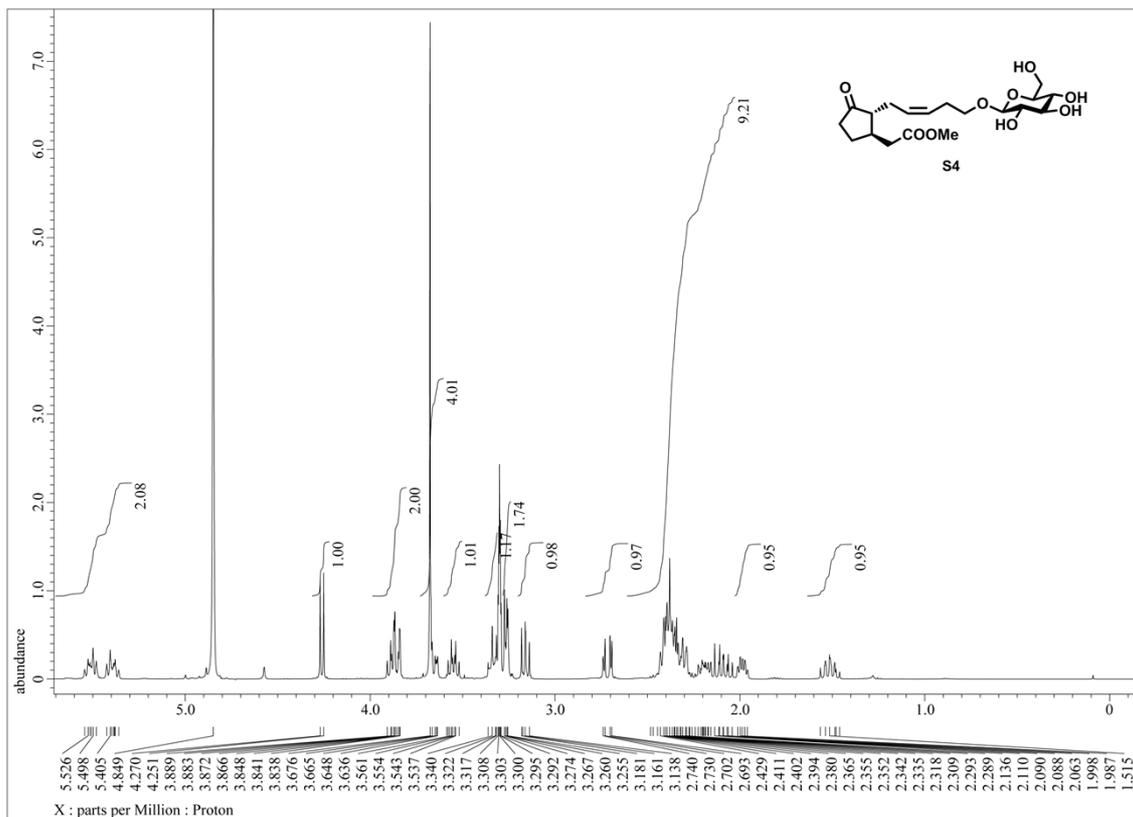
1. H. L. Gorton and R. L. Satter, *Plant Physiol.*, 1984, **76**, 680-684.
2. N. Moran, D. Fox and R. L. Satter, *Plant Physiol.*, 1990, **94**, 424-431.
3. Y. Nakamura, R. Miyatake and M. Ueda, *Angew. Chem. Int. Ed. Engl.*, 2008, **47**, 7289-7292.
4. Y. Nakamura, A. Mithofer, E. Kombrink, W. Boland, S. Hamamoto, N. Uozumi, K. Tohma and M. Ueda, *Plant Physiol.*, 2011, **155**, 1226-1236.
5. M. Ueda, G. Yang, Y. Ishimaru, T. Itabashi, S. Tamura, H. Kiyota, S. Kuwahara, S. Inomata, M. Shoji and T. Sugai, *Bioorg. Med. Chem.*, 2012, **20**, 5832-5843.

Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

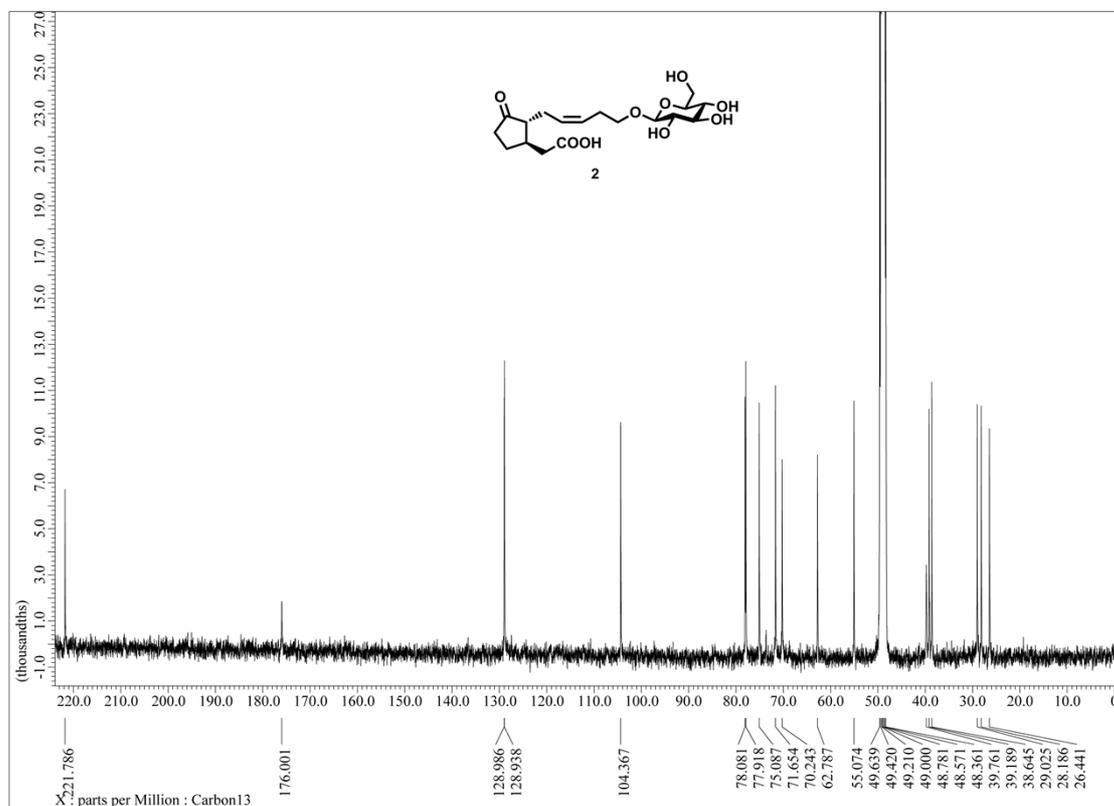
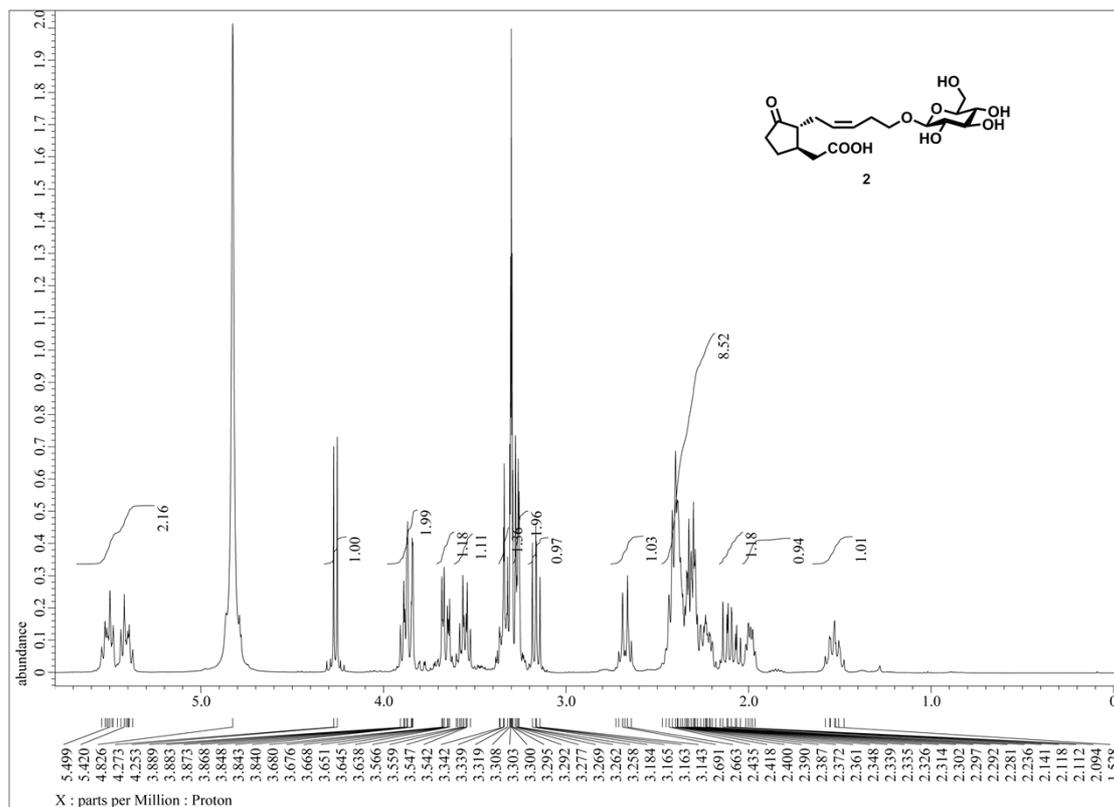
¹H and ¹³C NMR Spectra



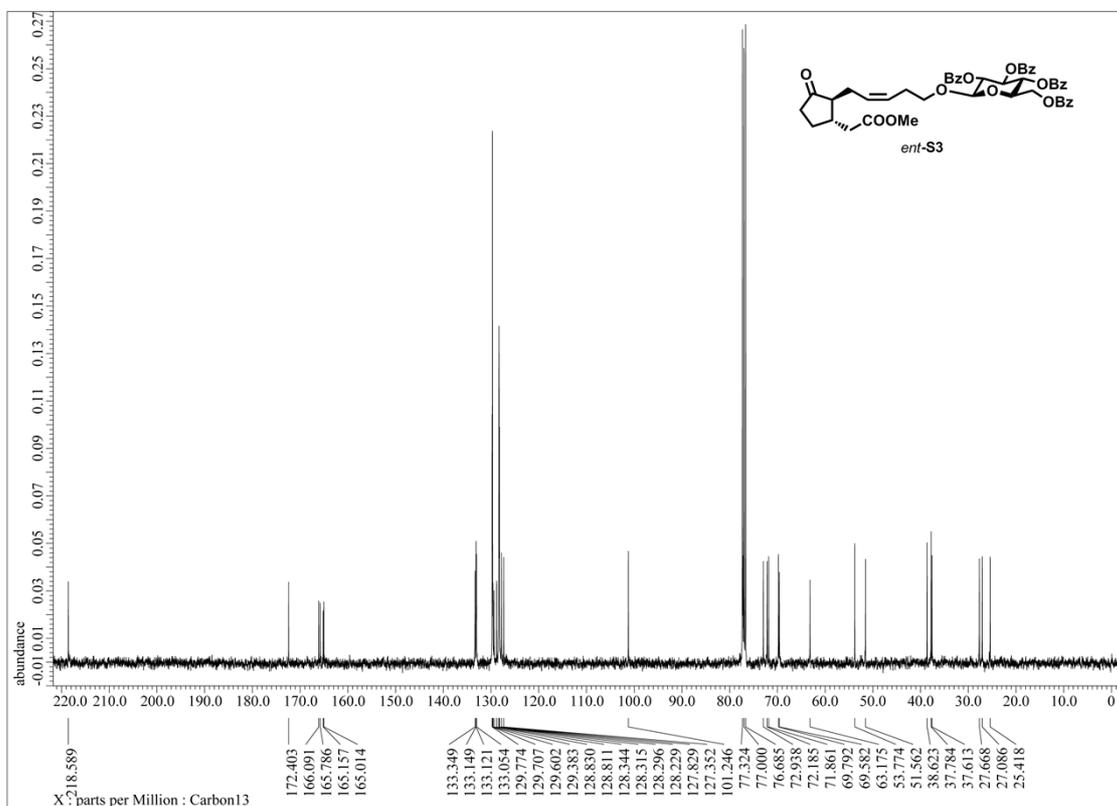
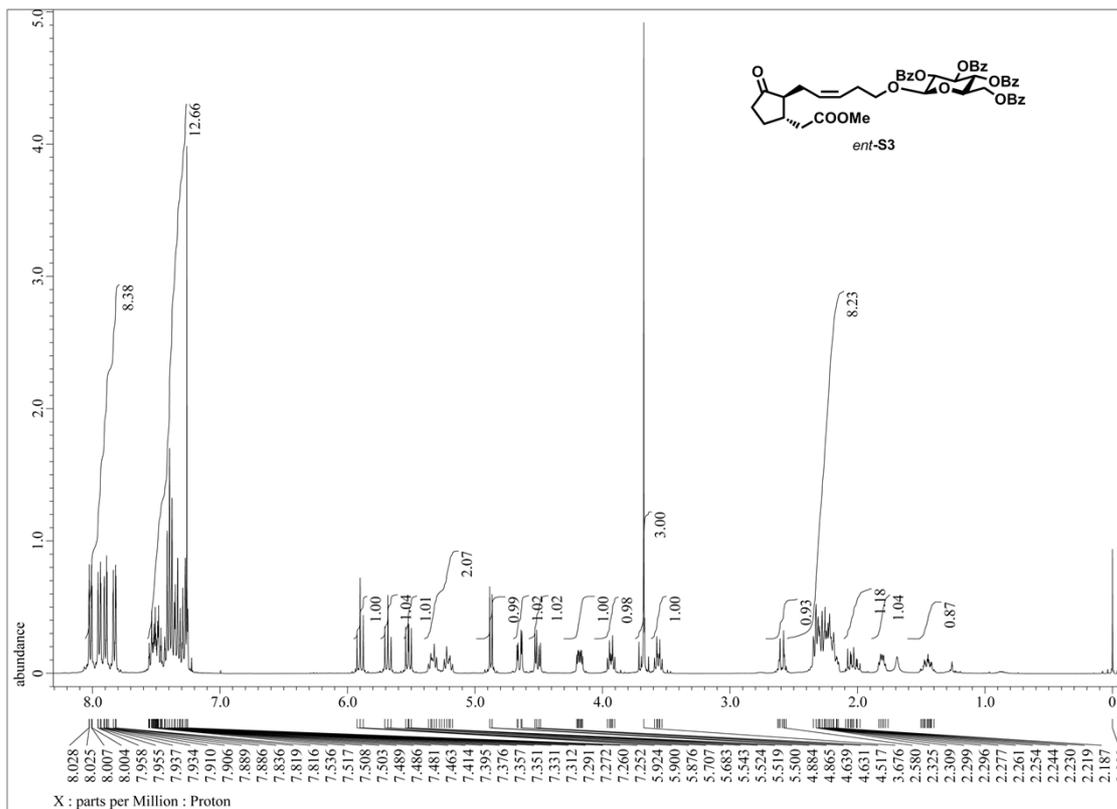
Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside



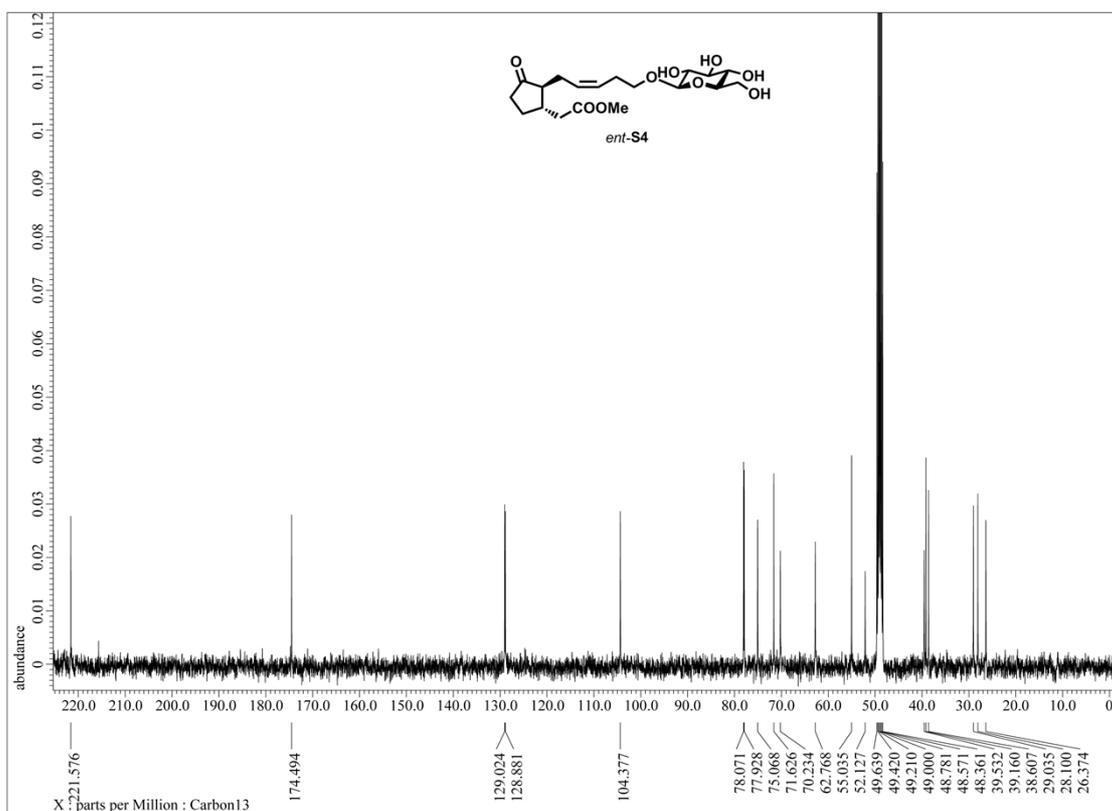
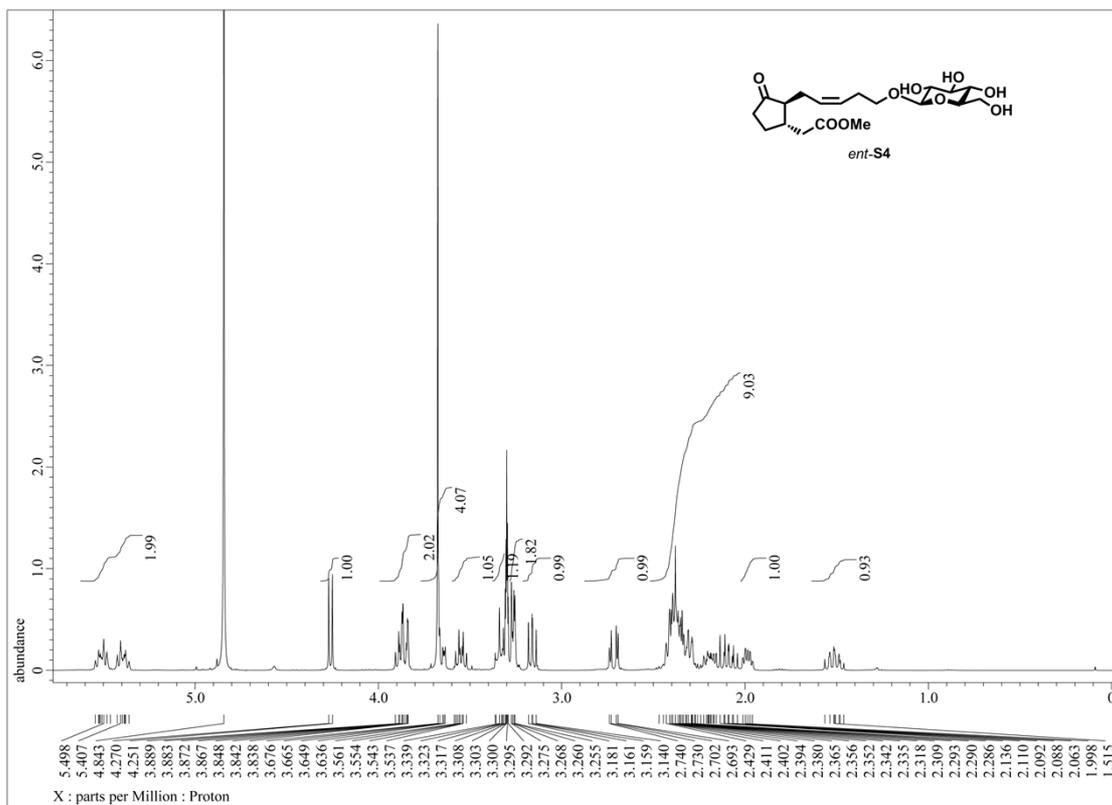
Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside



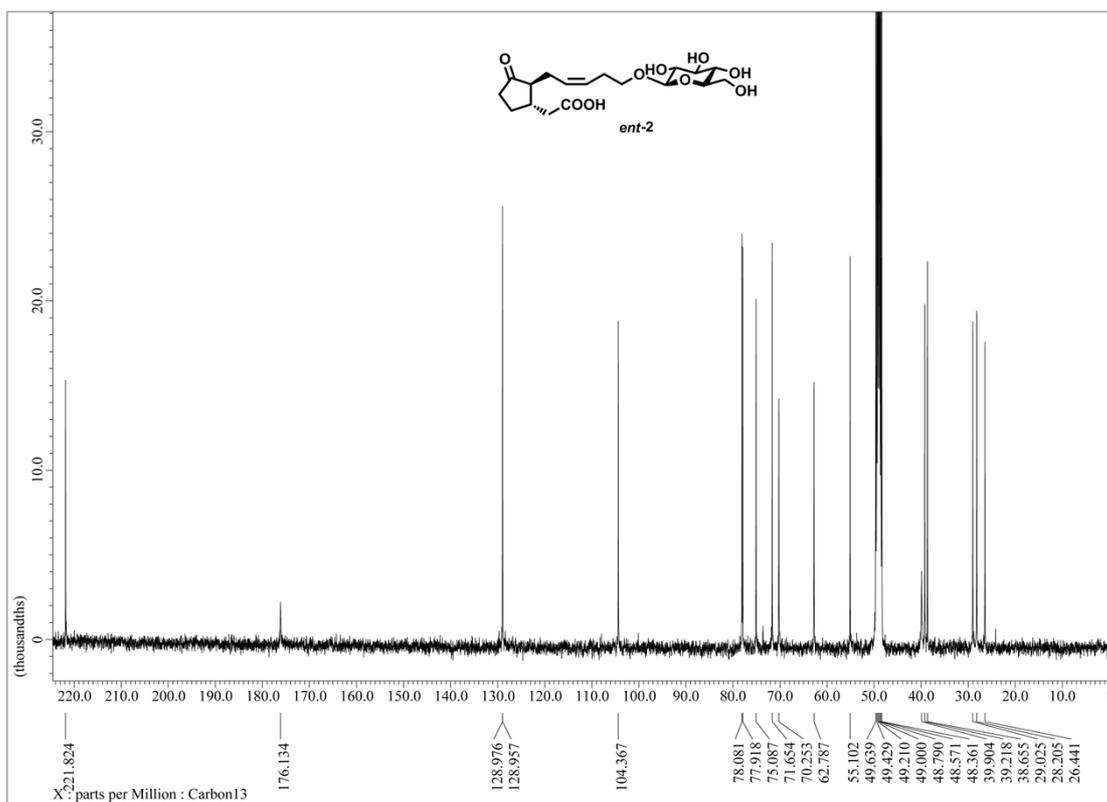
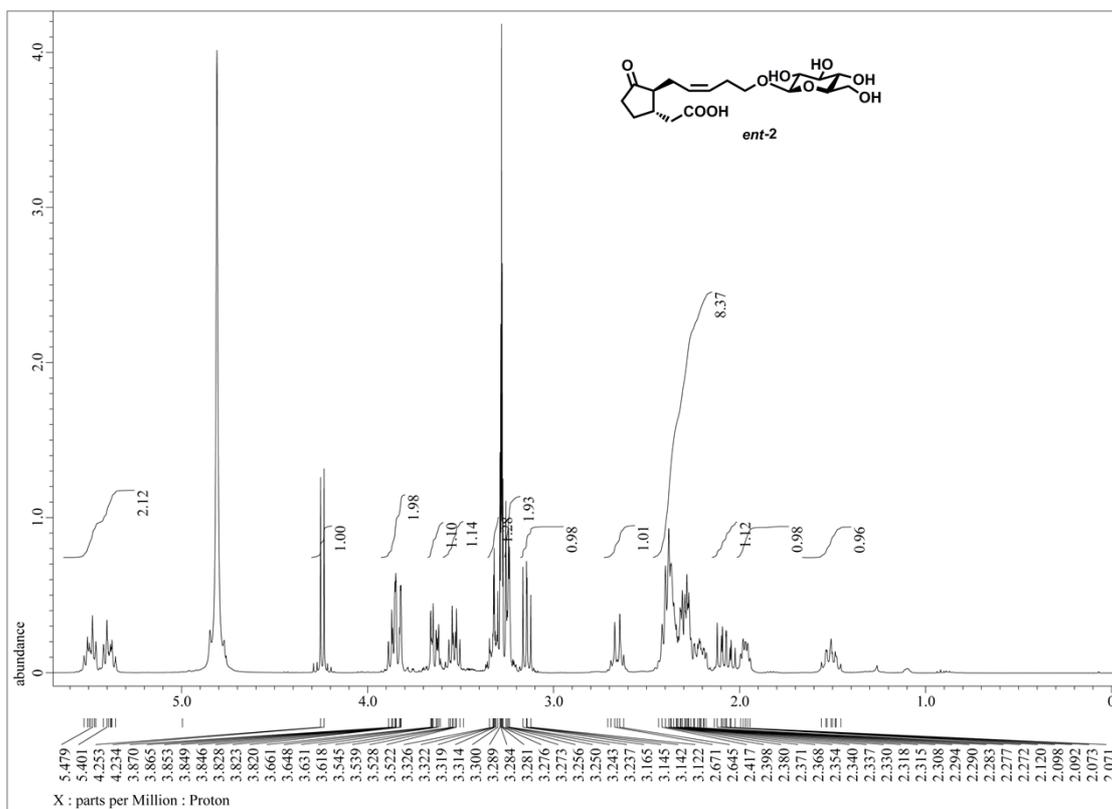
Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside



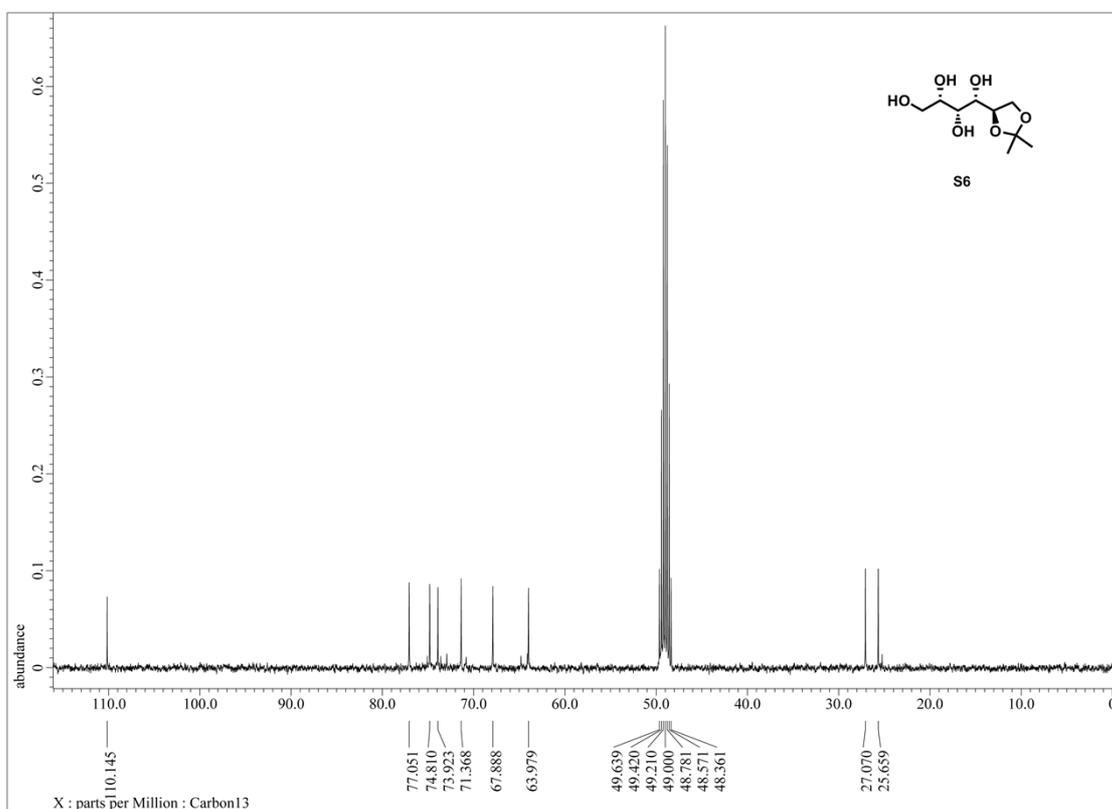
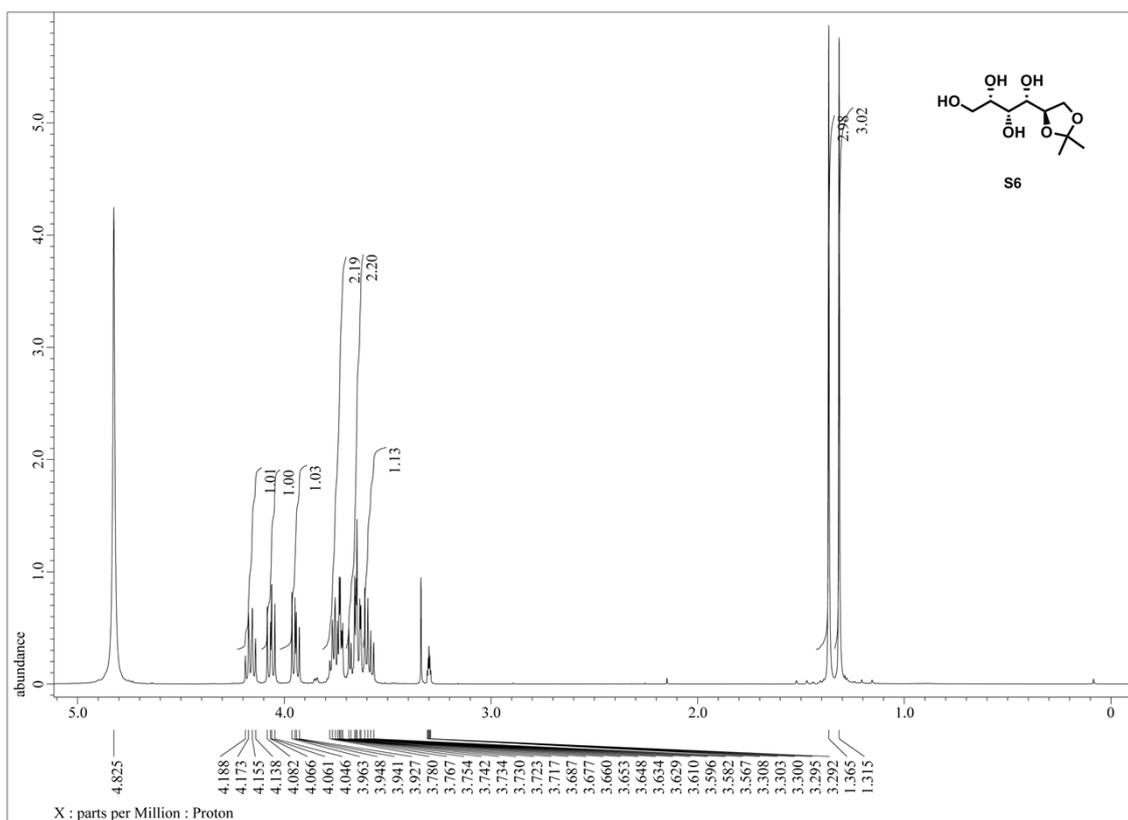
Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside



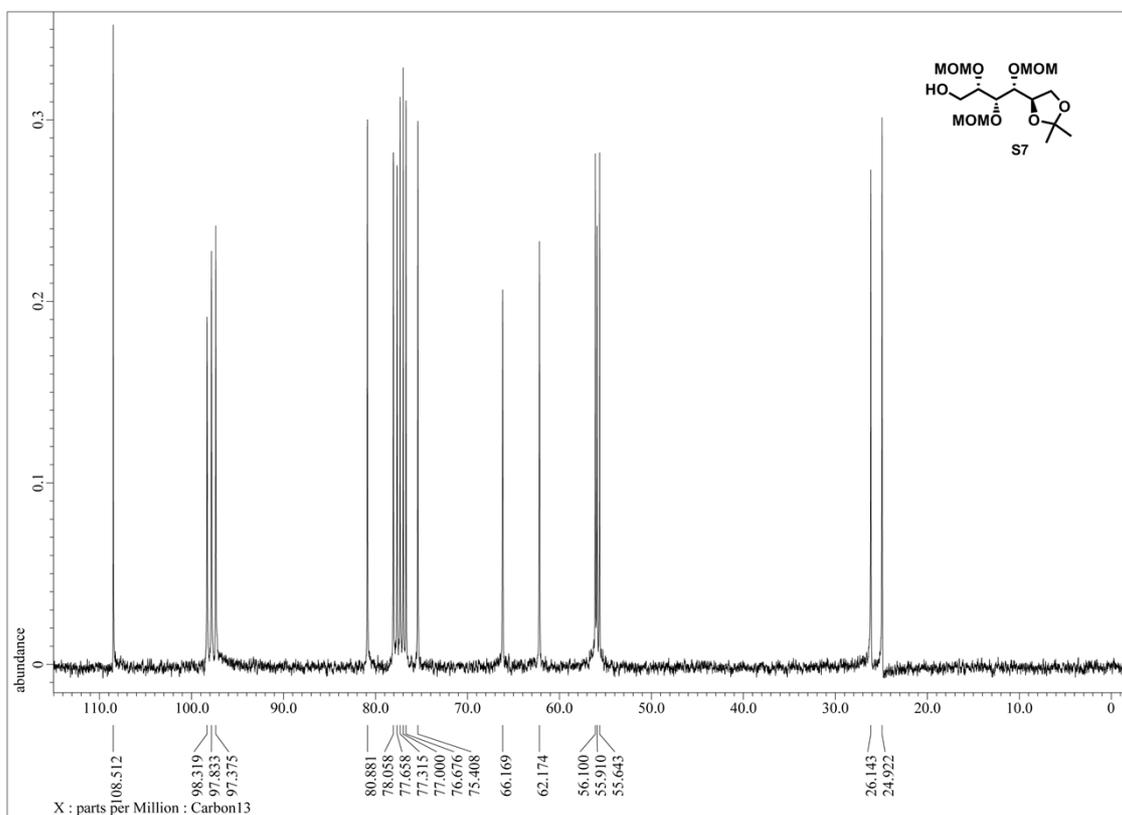
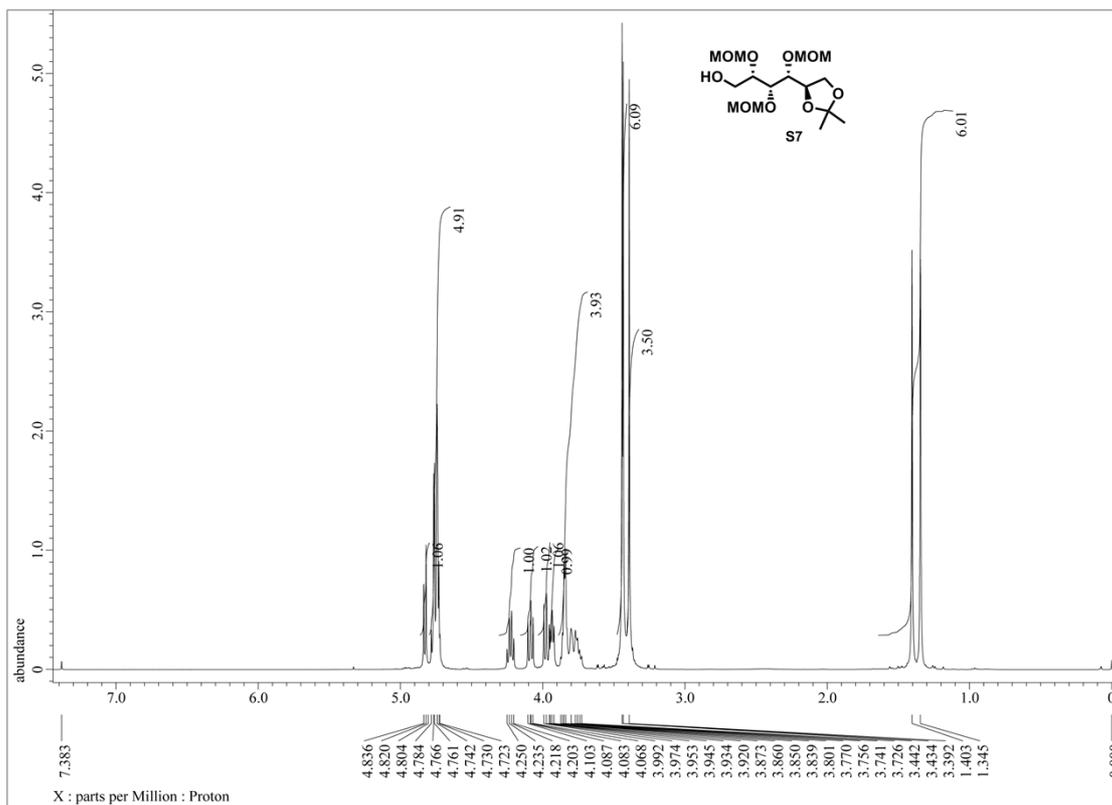
Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside



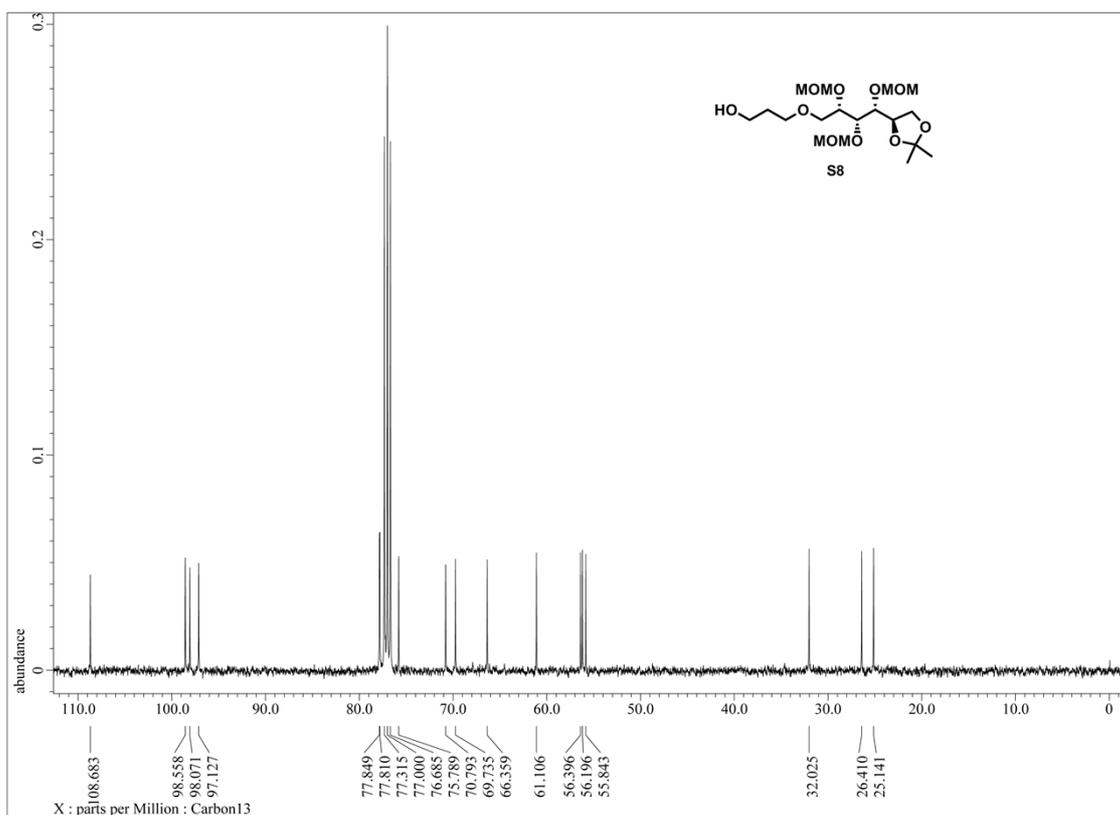
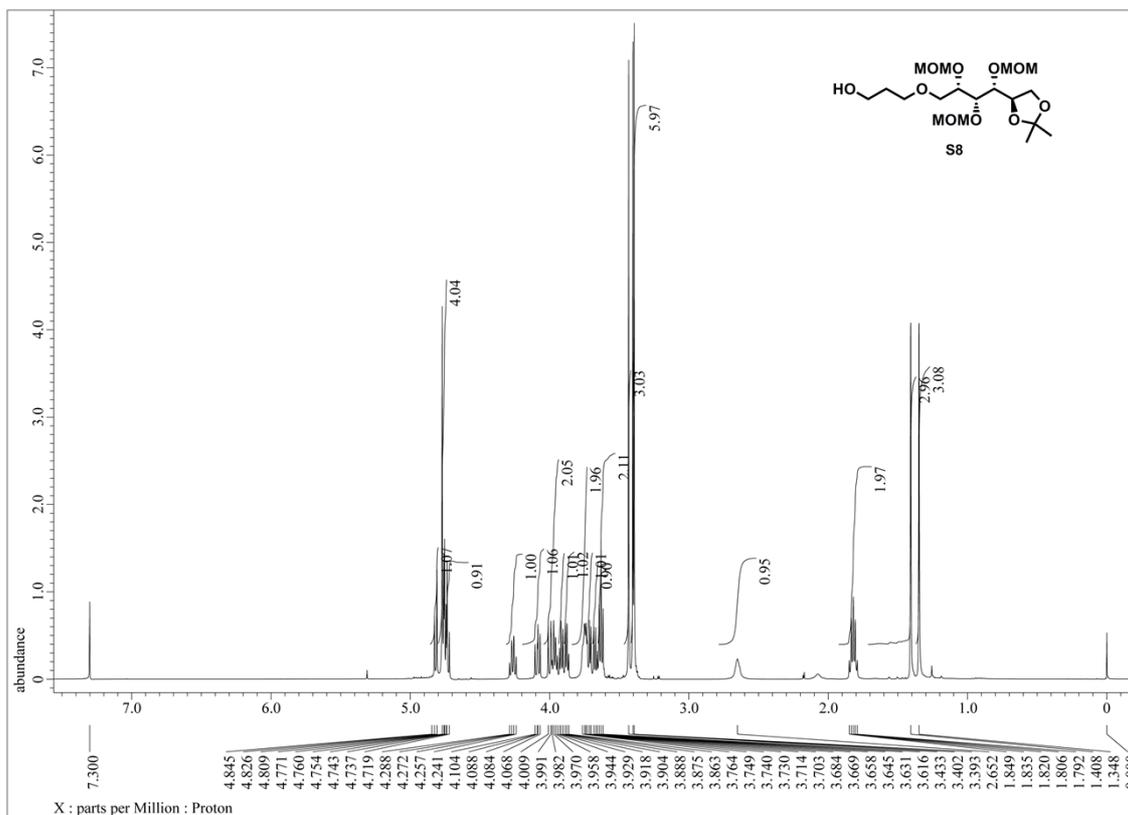
Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside



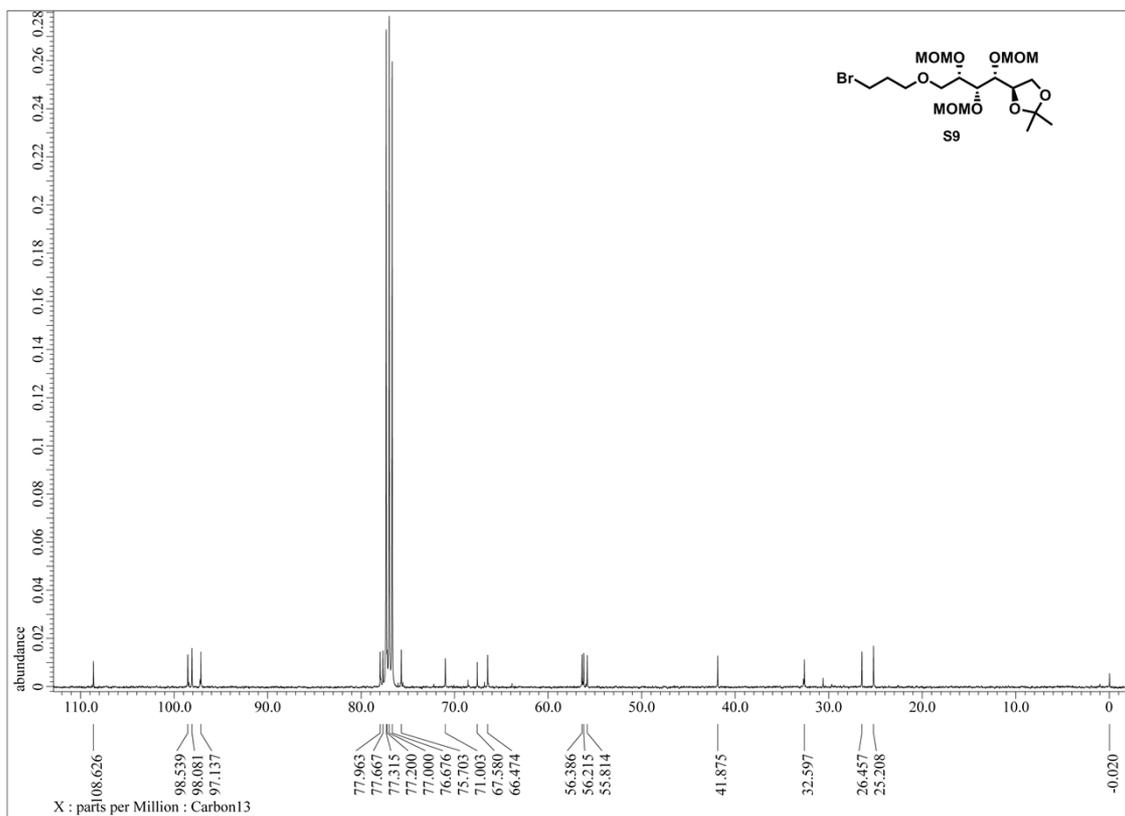
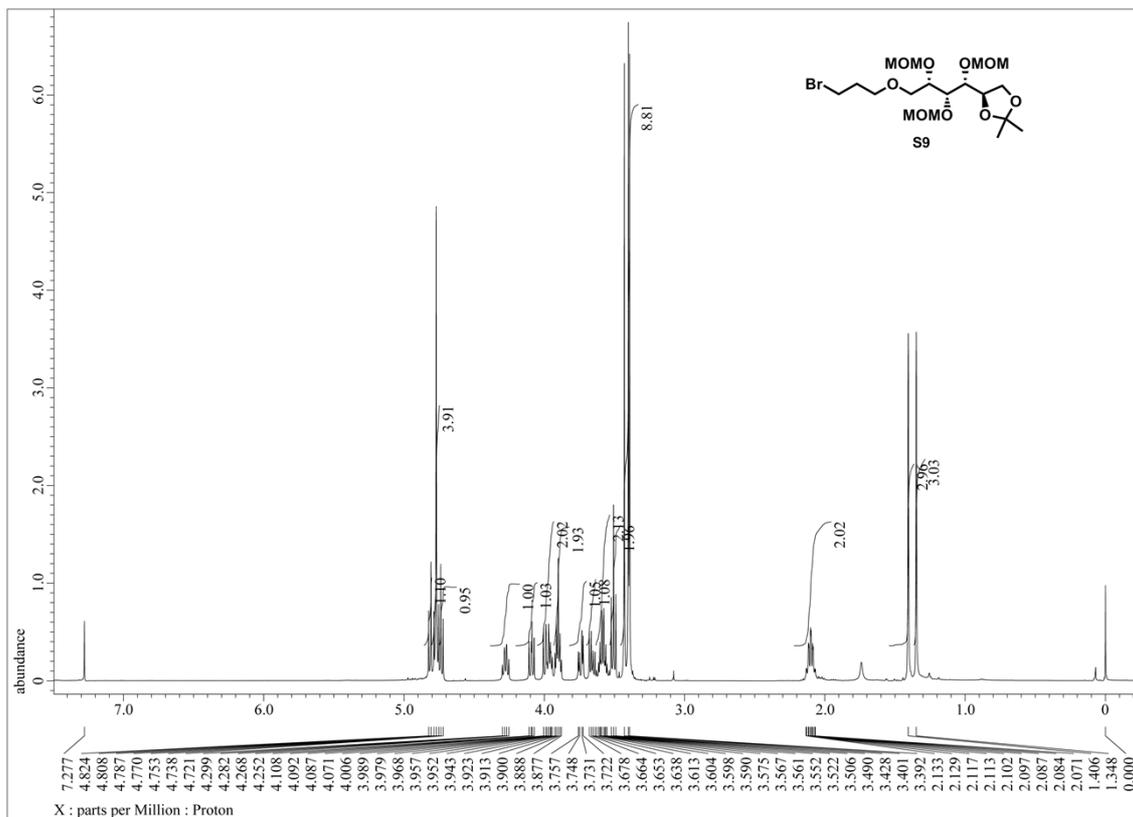
Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside



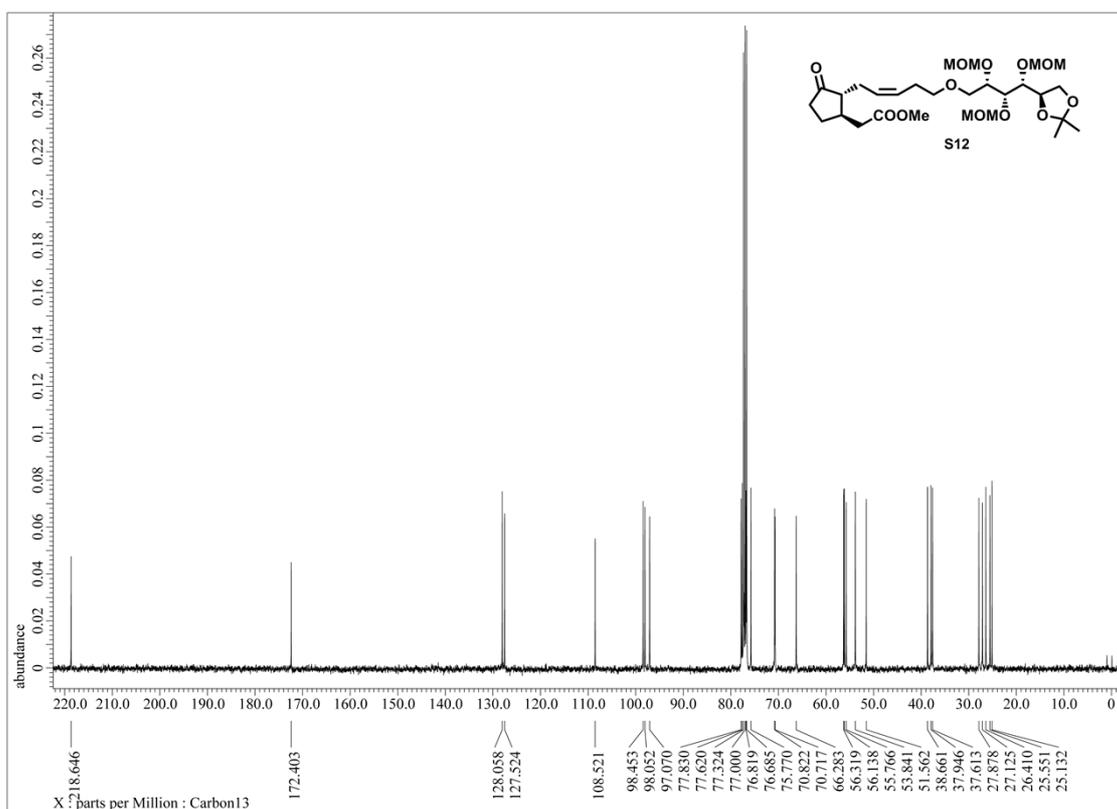
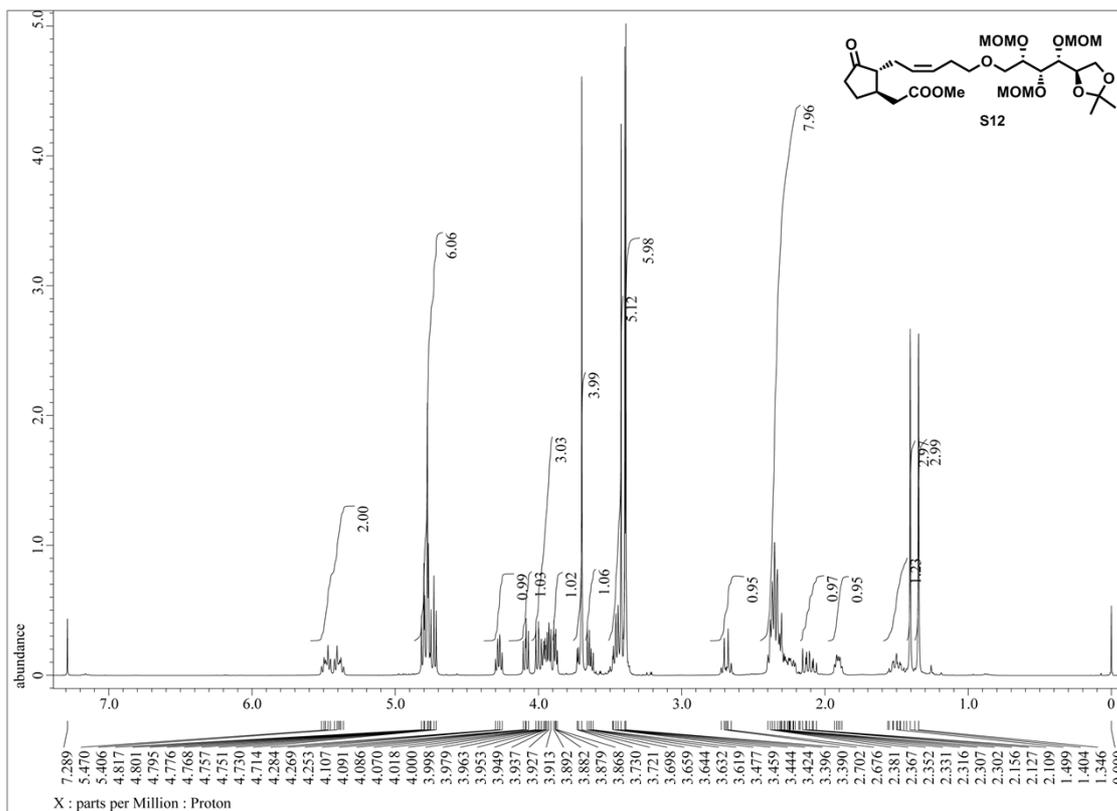
Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside



Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside



Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside



Supporting Information for Ueda, Yang, Nukadzuka, Shigenaga, Tamura, Ishimaru, and Manabe; Importance of D-glycopyranoside structure to the bioactivity and target affinity of jasmonic acid glucoside

