# Supporting Information

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### SI Materials and Methods

**Microsome Preparation from Yeast.** The transformed colonies were inoculated into 10 mL of SGI medium (20 g/L glucose, 1 g/L bactocasamino acids, 6.7 g/L yeast nitrogen base without amino acid, and 40 mg/L DL-tryptophan) (1) and were grown at 30 °C in a shaking incubator (MMS-3010; EYELA) (160 rpm) until the cell density reached $4 \times 10^7$ cells/mL. Ten milliliters of SGI-cultured yeast medium was inoculated into 500 mL of YPGE medium [5 g/L glucose, 10 g/L yeast extract, 10 g/L bactopeptone, and 3% (vol/vol) ethanol] (1) and grown at 28 °C until the cell density reached $4 \times 10^7$ cells/mL. After 50 mL of sterile galactose solution (200 g/L) was added, YPGE-cultured yeast medium was incubated at 28 °C until the cell density reached $8 \times 10^7$ cells/mL. To prepare microsomal proteins, yeast cells were collected by centrifugation of $3{,}000 \times g$ for 5 min. Approximately 4 g of the cells was suspended in 40 mL of 0.1 M Tris-$SO_4$ buffer (pH 9.4) with 10 mM DTT and incubated at 30 °C for 15 min in a shaking incubator (MMS-3010) (70 rpm). Yeast cells were collected by centrifugation and suspended in 40 mL of 20 mM potassium-phosphate buffer (pH 7.4) with 1.2 M sorbitol twice. Spheroplast was prepared by incubation with 40 mg of Zymolyase-20T (Nacalai tesque) at 30 °C for 1 h in a shaking incubator (MMS-3010) (70 rpm), collected by centrifugation, and washed in 40 mL of 20 mM potassium-phosphate buffer (pH 7.4) with 1.2 M sorbitol twice. The spheroplast was suspended in 40 mL of 20 mM Tris·HCl buffer (pH 7.4) with 0.6 M sorbitol and 1 mM phenylmethylsulfonyl fluoride and broken by a Dounce Tissue Grinder (Wheaton). The broken cells were centrifuged at $3{,}000 \times g$ for 5 min, and the resulting supernatant was centrifuged at $10{,}000 \times g$ for 5 min and then centrifuged at $100{,}000 \times g$ for 1 h. The microsomal pellet fraction was suspended in 1 mL of 50 mM Tris·HCl buffer (pH 7.4) with 1 mM EDTA. Protein content was estimated using the BCA Protein Assay (TaKaRa Bio), and P450 content was estimated by the carbon monoxide difference spectrum (2).

**LC-MS/MS Analysis for Identification of Metabolites Converted by MAX1.** Each 30 μM of CL, 19-hydroxy-CL, and CLA was incubated with 38 pmol (in a 100-μL volume) of P450 in the presence of 500 μM NADPH at 28 °C for 1 h. The reaction mixture was extracted with 1 mL of ethyl acetate twice. The ethyl acetate phase was dehydrated using sodium sulfate and then evaporated to dryness under nitrogen gas. The residual solid was dissolved immediately in acetonitrile and subjected to LC-MS/MS analysis. LC-MS/MS analysis of proton adduct ions was performed with a triple quadrupole/linear ion trap instrument (LIT) (QTRAP5500; AB Sciex) with an electrospray source. MS/MS spectra were recorded in product ion scan mode using LIT. Ion source was maintained at 400 °C with curtain gas at 20, collisionally activated dissociation (CAD) gas at 7 psi (12 psi for LIT), ion source gas at 80 psi, and ion source gas2 at 70 psi. Ionspray voltage was set at 5,500 V in positive ion mode and −4,500 V in negative ion mode. Declustering, entrance, and collision cell exit potentials were maintained at 60, 10, and 15 V, respectively. Collision energies in SRM analysis were performed at 15 V for the transitions of *m/z* 301–283, 301–255, and 301–203 (19-hydroxy-CL), 20 V for *m/z* 301–185 (19-hydroxy-CL), 25 V for *m/z* 301–161 (19-hydroxy-CL), 30 V for *m/z* 301–133 (19-hydroxy-CL), −15 V for *m/z* 331–113 (CLA), and −40 V for 331–69 (CLA). Collision energies in product ion scan were performed at −15 V for CLA and 25 V for 19-hydroxy-CL. HPLC separation was performed on a UHPLC (Nexera X2; Shimadzu) equipped with an ODS column (Kinetex C18, ϕ 2.1 × 150 mm, 1.7 μm; Phenomenex) or a reversed phase column (Cosmosil 2.5 cholester, ϕ 2.0 × 50 mm; Nacalai tesque). The column oven temperature was maintained at 30 °C. The mobile phase consisted of acetonitrile (solvent A) and water (solvent B), both of which contained 0.1% (vol/vol) acetic acid. HPLC separation was conducted with a linear gradient of 35% A (0 min) to 95% A (20 min) for the Kinetex C18 column and 20% A (0 min) to 80% A (10 min) for the Cosmosil 2.5 cholester column at flow rate of 0.2 mL/min.

**Kinetic Assays.** Substrate *rac*-CL (0.04–5 μM), *rac*-19-hydroxy-CL (0.04–20 μM), 11(*R*)-CL (0.5 μM), and 11(*S*)-CL (0.5 μM) were incubated with 11–15 pmol (in a 100-μL volume) of P450 in the presence of 500 μM NADPH at 28 °C for 10 min. Reaction was stopped by addition of 1 mL of ethyl acetate, and 10 ng each of *rac*-[1-$^{13}CH_3$]CLA and *rac*-[1-$^{13}CH_3$]19-hydroxy-CL was spiked as internal standards. The reaction mixture was extracted with 1 mL of ethyl acetate twice. The ethyl acetate phase was dehydrated and then evaporated to dryness. The residual solid was dissolved in acetonitrile and subjected to LC-MS/MS analysis. The products CLA and 19-hydroxy-CL were quantified by the ratio between peak areas in the transitions of *m/z* 331.1 (CLA) and 332.1 (*rac*-[1-$^{13}CH_3$]CLA) to both 113.0 and in the transitions of *m/z* 301.2 (19-hydroxy-CL) and 302.2 (*rac*-[1-$^{13}CH_3$]19-hydroxy-CL) to 185.0 (19-hydroxy-CL) and 186.0 (*rac*-[1-$^{13}CH_3$]19-hydroxy-CL) on calibration curves, respectively. Kinetics parameter was determined using triplicate samples and calculated by the Michaelis–Menten equation using SigmaPlot software (Systat Software).

**Bioassays.** *Arabidopsis* seeds were sterilized in 1% sodium hypochlorite solution for 5 min and rinsed with sterile water. Sterilized seeds were sown on Murashige and Skoog (MS) medium (3) (pH 5.8) containing 2% sucrose and 0.2% gellan gum and stratified at 4 °C for 3 d. Seedlings were grown at 22 °C under a continuous light (fluorescence white light, 60–70 μmol/$m^2$/s) for 10 d. The grown seedlings were transplanted on soil [horticultural soil:vermiculite = 1:1 (vol/vol)], further grown under the same condition for additional 7 d, and treated with authentic samples. Authentic *rac*-CL, *rac*-19-hydroxy-CL, *rac*-CLA, *rac*-MeCLA, and GR24 in acetonitrile were used to prepare 10 μM solutions in water including 0.5% (vol/vol) acetonitrile. The 0.5% (vol/vol) acetonitrile solution without authentic sample was used as a mock treatment. Ten microliters of the solution was applied onto the basal region between the primary inflorescence and rosette leaves of *Arabidopsis* plants every second day for 2 wk. The number of lateral inflorescences from the basal region was measured ($n = 15$). The assays were performed using triplicate samples. Statistical analysis was performed using SigmaPlot software (Systat Software).

Germination assay on *O. minor* was conducted as reported previously (4) with the following modification. Authentic samples in acetonitrile were used to prepare respective concentrations in water. Conditioned *O. minor* seeds were treated on a filter paper with 650 μL of the test solution including 1% (vol/vol) acetonitrile.

**Identification of CLA in *Arabidopsis* and Rice Plants.** *Arabidopsis* seedlings were grown hydroponically as described previously (5). Sterilized seeds were stratified for 2 d at 4 °C and sown on the half-strength MS medium (pH 5.7) containing 1% sucrose and 1% agar. Seedlings were grown at 22 °C under a continuous light (60–70 μmol/$m^2$/s) for 14 d and then transferred to a glass pot containing 400 mL hydroponic solution (6) and grown under the same environmental condition for an additional 2 wk.

Rice seedlings were grown hydroponically as described previously (5). Sterilized rice seeds were incubated in sterile water at 28 °C in the dark for 2 d. The germinated seeds were transferred to hydroponic culture medium (7) without Pi solidified with 0.6% agar and cultured at 25 °C under a 16-h light (150 μmol/$m^2$/s)/8-h dark photoperiod for 5 d. The 1-wk-old seedlings were transferred to glass vials containing hydroponic culture media without Pi (13 mL), and further grown for an additional 1 wk.

To analyze CLA in root samples, the roots (1.5–2 g) were homogenized in 10 mL of acetone containing [1-$^{13}CH_3$]CLA as an internal standard. The filtrates were evaporated to dryness under nitrogen gas, dissolved in deionized water, and extracted with ethyl acetate twice. The ethyl acetate phase was evaporated to dryness under nitrogen gas. The extracts were then dissolved in 2-propanol and loaded onto Bond Elut DEA 1-mL cartridges (Agilent Technologies), washed with 2-propanol, and then eluted with acetic acid:2-propanol (1:99). For qualitative and quantitative analysis, the eluates were evaporated to dryness under nitrogen gas, dissolved in 50% (vol/vol) acetonitrile, and subjected to LC-MS/MS analysis.

To analyze SL-LIKE1 (MeCLA) in root samples, the roots were homogenized in 10 mL acetone and further purified as descrived in our previous report (5).

**Feeding of CL and CLA to *Arabidopsis* and Rice Plants.** *Arabidopsis* seedlings were grown on agar medium as described above. The *max4-7* single mutant and the *max1-4 max4-7* double mutant were grown for 2 wk on agar plates and for another 1 wk in the hydroponic culture system. The 3-wk-old plants were transferred to glass pots containing hydroponic culture media (400 mL) without Pi and further grown for 5 d. *Arabidopsis* plants grown hydroponically were transferred to glass vials containing Pi-free hydroponic culture media (7 mL) containing 1 μM [1-$^{13}CH_3$] 11*R*-CL, 1 μM [1-$^{13}CH_3$]*rac*-CLA and 0.02% acetone and were grown for an additional 2 d. Control plants were grown in the same volume of hydroponic culture media containing 0.02% acetone.

Rice seedlings were grown hydroponically for 1 wk as described above and then transferred to glass vials containing hydroponic culture media (13 mL) containing 1 μM [1-$^{13}CH_3$]*rac*-CLA and 0.02% acetone and incubated for 2 d. Hydroponic culture media were renewed every 2 d. Control plants were grown in the same volume of hydroponic culture media containing 0.02% acetone. All culture media contained 5 mM Mes and were adjusted to pH 5.7. Metabolites in root tissues of *Arabidopsis* and rice were extracted using the same method as described above. To analyze 4DO and orobanchol released from roots, the hydroponic culture media were collected and purified as described previously (5).

**LC-MS/MS Analysis of CLA, MeCLA, and SLs in *Arabidopsis* and Rice Plants.** LC-MS/MS analysis of CLA and MeCLA from root extract samples was carried out using a system containing of a quadrupole/time-of-flight tandem mass spectrometer (TripleTOF 5600; AB SCIEX) by high-resolution MRM mode (MRMHR) and a UHPLC (Nexera; Shimadzu) equipped with a reverse phase column (Acquity UPLC BEH-C18, ϕ 2.1 × 50 mm, 1.7 μm; Waters). For reverse phase chromatography of CLA, the elution of the samples was carried out using water (solvent A2) and acetonitrile (solvent B2), both of which contained 0.05% (vol/vol) acetic acid, with the following gradient (vol/vol): initially 30% B, 40% B at 1 min, 70% B at 6 min at a flow rate of 0.4 mL/min. The column temperature was maintained at 40 °C. MS/MS analysis conditions were as follows: declustering potential, −40; collision energy, −15 V; parent ion (*m*/*z*), 331.2 for unlabeled CLA and 332.2 for labeled CLA. Quantification was carried out using the channel for monitoring the transitions of *m*/*z* 331.2 (CLA) and 332.2 (*rac*-[1-$^{13}CH_3$]CLA) to both 113.02. For the analysis of MeCLA, the elution of the samples was carried out using water (solvent A2) and acetonitrile (solvent B2), both of which contained 0.05% (vol/vol) acetic acid, with the following gradient (vol/vol): initially 30% B, 75% B at 4 min keeping until 6 min at a flow rate of 0.4 mL/min. The column temperature was maintained at 40 °C. MS/MS analysis conditions were as follows: declustering potential, 55; collision energy, 20V; parent ion (*m*/*z*), 347.2 for unlabeled MeCLA and 348.2 for labeled MeCLA.

LC-MS/MS analysis of 4DO and orobanchol from rice root exudates was carried out according to the previously reported method (5).

**Functional Expression of AtD14 Protein.** The coding sequence for AtD14 was amplified by PCR using the primers (AtD14-F-blunt: 5′-ATGAGTCAACACAACATCTTAGAAG-3′; AtD14-R-EcoRI: 5′-TTTTGAATTCTCACCGAGGAAGAGC-3′). The PCR products were digested by EcoRI and cloned into modified pMALc5x (New England Biology) vector, which has His tag and HRV 3C protease site from pET49b (Novagen), to yield AtD14-pMALHis. *E.coli* Rosseta-gami 2 (Novagen) was used for the expression. Overnight growing culture (10 mL) was inoculated to fresh LB medium (1 L) containing 50 μg/mL ampicillin, 50 μg/mL streptomycin, 12.5 μg/mL tetracyclin, and 34 μg/mL chloramphenicol. After $OD_{600}$ reached 0.6, isopropyl β-D-1-thiogalactopyranoside (IPTG) was added at 0.1 mM concentration and further incubated at 15 °C for 20 h. The cells were collected by centrifugation and then suspended and sonicated in the lysis buffer [20 mM Tris·HCl (pH 8.0), 150 mM NaCl]. The supernatants from the resulting lysates were subjected to MBPTrap column chromatography (GE Healthcare). After washing by buffer [20 mM Tris (8.0), 150 mM NaCl], absorbed proteins were eluted using elution buffer [20 mM Tris (8.0), 150 mM NaCl, 10 mM maltose]. The elution fraction was further purified with TALON column chromatography (GE Healthcare). After washing by wash buffer [20 mM Tris (8.0), 300 mM NaCl, 2 mM imidazole], absorbed proteins were eluted using elution buffer [20 mM Tris (8.0), 300 mM NaCl, 50 mM imidazole]. The elution fraction was concentrated using Amicon Ultra-4 10 K (Millipore), and the concentration was adjusted to 5 μg/μL. Obtained recombinant proteins (MBP fusion) were divided into aliquots, immediately frozen in liquid nitrogen, and stored at −80 °C until use. atd14:S97A-pMALHis was prepared using the KOD-Plus-Mutagenesis Kit (TOYOBO) with AtD14-pMALHis as the template. Protein expression and purification was carried out as described above.

**Hydrolase Activity Tests of AtD14.** The hydrolase activity test of MBP-AtD14 was carried out at 30 °C for 3 h in 100 μL of a standard reaction buffer that containing 10 μg of recombinant protein and 10 μM of substrates (*rac*-CL, *rac*-CLA, *rac*-MeCLA, or *rac*-GR24) in 50 mM phosphate-Na buffer (pH 7.0) containing 2% acetone. The enzyme reaction was stopped by the addition of 100 μL of acetnitrile, and then the remaining substrate and HMB were analyzed by LC-MS/MS, respectively. The analysis of *rac*-CL, *rac*-CLA, *rac*-MeCLA, *rac*-GR24, and HMB was carried out using quadrupole/time-of-flight tandem mass spectrometer (TripleTOF 5600; AB SCIEX) and a UHPLC (Nexera; Shimadzu) equipped with a reverse phase column (Acquity UPLC BEH-phenyl, ϕ 2.1 × 50 mm, 1.7 μm; Waters). The elution of the samples was carried out using water (solvent A2) and acetonitrile (solvent B2), both of which contained 0.05% (vol/vol) acetic acid, with the following gradient (vol/vol): initially 1% B, 2% B at 2 min, and 98% B at 4.5 min at a flow rate of 0.4 mL/min. The column temperature was maintained at 50 °C. MS/MS analysis conditions were as follows: declustering potential, 40; collision energy, 17V; parent ion (*m*/*z*), 303.2 for CL, 333.2 for CLA, 347.2 for MeCLA, 299.2 for GR24, and 115.1 for HMB.

**Differential Scanning Fluorimetry Experiments.** DSF experiments were carried out according to reported method using Mx3000P (Agillent) (8). Sypro Orange (Ex/Em: 490/610 nm; Invitrogen) was used as the reporter dye. Reaction mixtures were prepared in 96-well plate, and each reaction was carried out in 20-μL scale in PBS buffer containing 10 μg protein, each concentration of CL derivatives or GR24 (acetone solution, final acetone concentration was 5%), and 0.014 μL Sypro Orange. In the control reaction, acetone was added instead of chemical solution. Samples were heated from 25 °C to 95 °C after incubation of 25 °C for 10 min in the absence of light. The denaturation curve was obtained using MxPro software.

**Chemical Synthesis of 19-Hydroxy-CL, CLA, MeCLA, and Their Isotopically Labeled Derivatives.** To synthesize 19-hydroxy-CL (Fig. S2), β-ionone was first oxidized with lead tetraacetate, and the resulting acetate was hydrolyzed with sodium carbonate in aqueous methanol to give hydroxyionone (9). Protection of the hydroxy group with *tert*-butyl(dimethyl)silyl (TBDMS) chloride in *N,N*-dimethylformamide with imidazole afforded the TBDMS-protected hydroxyionone. This silylated protected ketone was converted to TBDMSO-$C_{14}$-aldehyde via the Corey–Chaykovsky epoxidation with dimethylsulfonium methylide (10), followed by methylaluminium bis(4-bromo-2,6-di-*tert*-butylphenoxide)-promoted rearrangement of epoxide to aldehyde (11). *O*-Alkylation of the potassium enolate of aldehyde with bromobutenolide in the presence of 18-crown-6-ether furnished the silylated 19-hydroxy-CL and its (9*E*) geometric isomer in a ratio ~3:2 (12). After purification by preparative HPLC, 19-TBDMSO-CL was deprotected with either tetra-*n*-butylammonium fluoride or aqueous acetic acid to afford 19-hydroxy-CL. [1-$^{13}CH_3$]-19-hydroxy-CL was prepared from [1-$^{13}CH_3$]-β-ionone as above (Fig. S2) (5).

CLA was also synthesized from β-ionone as a starting material (Fig. S4*A*). β-Ionone was oxidized by sodium hypochlorite to the corresponding acid (13). The $C_{12}$-carboxylic acid was reduced by Red-Al, and the resulting alcohol was oxidized with manganese oxide to the $C_{12}$-aldehyde. The aldehyde was subjected to a Darzens reaction with ethyl chloroacetate and the glycidic ester so obtained was saponified and decarboxylated to give the $C_{13}$-aldehyde (14). This was subjected to a Pinnick oxidation to give the corresponding acid (15). The $C_{13}$-carboxylic acid was esterified with di-*tert*-butyl dicarbonate in the presence of dimethylaminopyridine in *tert*-butanol (16). Ester condensation of the *tert*-butyl ester with methyl formate followed by *O*-alkylation with bromobuteolide provided CLA *tert*-butyl ester. Mild deprotection of the *tert*-butyl group with triethylsilyl trifluoromethanesulfonate in the presence of 2,6-lutidine afforded CLA (17). [1-$^{13}CH_3$]-CLA was prepared from [1-$^{13}CH_3$]-β-ionone as above (Fig. S4*A*) (5).

MeCLA was obtained by oxidation of the $C_{13}$-aldehyde to the corresponding methyl ester with sodium cyanide and manganese oxide in methanol (18) followed by formylation and subsequent *O*-alkylation with bromobuteolide (Fig. S4*B*). [10-$^2H_1$]-MeCLA was prepared by formylation of the methyl ester with methyl deuterioformate followed by *O*-alkylation with bromobuteolide.

***General experimental procedure.*** Mass spectra were recorded on a JMS-700 instrument (JEOL), TripleTOF 5600 (AB SCIEX), or a GCMS-QP2010 Plus instrument (Shimadzu) in the direct injection mode. $^1$H- and $^{13}$C-NMR spectra were obtained with a JNM-AL400 NMR spectrometer (JEOL). Chemical shifts were referenced to tetramethylsilane as an internal standard. Column chromatography was performed with Kieselgel 60 (Merck), Chromatorex ODS (Fuji Silysia Chemical), Inertsil ODS-3 (ϕ 10 × 250 mm, 5 μm; GL Sciences), and Inertsil SIL-100A (ϕ 10 × 250 mm, 5 μm; GL Sciences).

***(3E)-2-Oxo-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-1-yl acetate (6a).*** To a solution of β-ionone **5a** (6.00 g, 31.2 mmol) in toluene (66 mL) was added lead tetraacetate (13.8 g, 31.2 mmol), and the mixture was stirred at 80 °C for 22 h under argon. Another lead tetraacetate (7.00 g, 15.8 mmol) was added, and stirring was continued for 8 h at the same temperature. Thereafter, lead tetraacetate (8.00 g, 18.0 mmol) was added, and the reaction was continued for an additional 15 h. The mixture was cooled to 0 °C and washed successively with water, saturated aqueous $NaHCO_3$ and water, and then dried over anhydrous $Na_2SO_4$. Purification by silica gel column chromatography eluted stepwise with *n*-hexane and ether [3% (vol/vol) increments] gave acetoxy-β-ionone **6a** (4.62 g, 18.5 mmol, 59%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.08 (6H, s, 6′-$CH_3$), 1.47–1.64 (4H, m, H-4′ and -5′), 1.79 (3H, s, 2′-$CH_3$), 2.09 (2H, t, $J$ = 6.3 Hz, H-3′), 2.20 (3H, s, -OAc), 4.87 (2H, s, H-1), 6.20 (1H, d, $J$ = 16.3 Hz, H-3), 7.46 (1H, d, $J$ = 16.3 Hz, H-4); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 18.8, 20.6, 21.8, 28.7, 33.8, 34.0, 39.8, 67.3, 125.3, 136.0, 138.4, 143.6, 170.4, 192.5; EI-MS *m/z*: 250 $[M]^+$, 235, 177; HREIMS *m/z*: 250.1550 $[M]^+$ (calcd. for $C_{15}H_{22}O_3$, *m/z* 250.1569).

***(3E)-1-Hydroxy-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one (7a).*** Acetoxy-β-ionone **6a** (4.44 g, 17.7 mmol) was dissolved in 1% (wt/vol) $Na_2CO_3$ in methanol–water (9:1, 220 mL) at −20 °C, and the mixture was stirred at 0 °C for 2 h. The reaction mixture was quenched by adding water (65 mL) and extracted with ether. The organic phase was washed with 2% (vol/vol) $H_2SO_4$, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. Purification by silica gel column chromatography eluted stepwise with *n*-hexane and ether [2% (vol/vol) increments] gave hydroxy-β-ionone **7a** (1.78 g, 8.6 mmol, 48%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.08 (6H, s, 6′-$CH_3$), 1.47–1.64 (4H, m, H-4′ and -5′), 1.80 (3H, s, 2′-$CH_3$), 2.10 (2H, t, $J$ = 6.3 Hz, H-3′), 4.45 (2H, s, H-1), 6.17 (1H, d, $J$ = 16.6 Hz, H-3), 7.58 (1H, d, $J$ = 16.6 Hz, H-4); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 18.8, 21.8, 28.8, 33.8, 34.1, 39.8, 66.7, 125.3, 135.9, 138.7, 143.8, 198.1; EI-MS *m/z*: 208 $[M]^+$, 193, 177, 149; HREIMS *m/z*: 208.1473 $[M]^+$ (calcd. for $C_{13}H_{20}O_2$, *m/z* 208.1463).

***(3E)-1-(tert-Butyldimethylsiloxy)-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one (8a).*** A mixture of hydroxy-β-ionone **7a** (500 mg, 2.4 mmol), *tert*-butyldimethylchlorosilane (728 mg, 4.80 mmol), and imidazole (654 mg, 9.60 mmol) in *N,N*-dimethylformamide (DMF, 6 mL) was stirred at room temperature for 2.5 h under argon. The reaction mixture was taken up in 2% (vol/vol) ether in *n*-hexane, washed with water, dried over anhydrous $Na_2SO_4$, and concentrated in vauo. Purification by silica gel column chromatography eluted stepwise with *n*-hexane and ether (2% (vol/vol) increments) gave *tert*-butyldimethylsilyloxy-β-ionone **8a** (610 mg, 1.89 mmol, 79%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 0.10 (6H, s, Si-$CH_3$ × 2), 0.93 (9H, s, Si-C$(CH_3)_3$), 1.09 (6H, s, 6′-$CH_3$), 1.46–1.63 (4H, m, H-4′ and -5′), 1.80 (3H, d, $J$ = 0.7 Hz, 2′-$CH_3$), 2.09 (2H, t, $J$ = 6.3 Hz, H-3′), 4.31 (2H, s, H-1), 6.53 (1H, d, $J$ = 16.3 Hz, H-3), 7.50 (1H, d, $J$ = 16.3 Hz, H-4); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: −5.4, 18.3, 18.8, 21.8, 25.8, 28.8, 33.9, 34.1, 40.0, 69.0, 124.5, 136.4, 138.0, 142.8, 199.3; EI-MS *m/z*: 322 $[M]^+$, 307, 265; HREIMS *m/z*: 322.2324 $[M]^+$ (calcd. for $C_{19}H_{34}O_2Si$, *m/z* 322.2328).

***2-[(tert-Butyldimethylsiloxy)methyl]-2-[(E)-2-(2,6,6-trimetylcyclohex-1-en-1-yl)ethenyl] oxirane (9a).*** To a solution of trimethylsulfonium iodide (988 mg, 4.85 mmol) in dimethyl sulfoxide (DMSO, 4.13 mL) was added tetrahydrofuran (THF, 4.13 mL) under argon to yield a finely divided suspension of sulfonium salt. This mixture was then cooled to −5 °C and treated with a solution of dimsyl sodium (4.4 M, 1.29 mL, 5.68 mmol). The resulting gray colored suspension was treated with a solutiuon of *tert*-butyldimethylsilyloxy-β-ionone **8a** (1.04 g, 3.23 mmol) in THF (1 mL). After stirring at −5 °C for 45 min, the mixture was warmed to room temperature, quenched by successively adding water (30 mL) and *n*-hexane (30 mL). The organic phase was washed with water, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo to give the epoxide **9a** (1.00 g, 2.98 mmol, 92%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 0.08 (6H, d, $J$ = 3.9 Hz, Si-$CH_3$ × 2), 0.89 (9H, s, Si-C$(CH_3)_3$), 0.97 (6H, s, 6″-$CH_3$), 1.42–1.62 (4H, m, H-4″ and -5″), 1.65 (3H, s, 2″-$CH_3$), 1.96 (2H, t, $J$ = 5.9 Hz, H-3″), 2.70 (1H, d, $J$ = 5.6 Hz, H-3α), 2.96 (1H, d, $J$ = 5.6 Hz, H-3β), 3.83 (2H, s, 2-$CH_2$), 5.43

(1H, d, $J$ = 16.3 Hz, H-1′), 6.24 (1H, d, $J$ = 16.3 Hz, H-2′); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: −5.4, 18.3, 19.2, 21.4, 25.9, 28.7, 32.7, 33.9, 39.4, 53.2, 58.8, 64.9, 129.0, 129.7, 130.3, 136.8; EI-MS $m/z$: 336 $[M]^+$, 279; HREIMS $m/z$: 336.2492 $[M]^+$ (calcd. for $C_{20}H_{36}O_2Si$, $m/z$ 336.2485).

***(3E)-2-[(tert-Butyldimethylsiloxy)methyl]-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-enal (10a).*** To a solution of 2,6-di-*tert*-butyl-4-bromophenol (3.40 g, 11.9 mmol) in dichloromethane (26 mL) was added at room temperature a 1.4 M hexane solution of trimethylaluminium ($Me_3Al$, 4.26 mL, 5.96 mmol), and the solution was stirred at room temperature for 1 h under argon. To a solution of the MABR (5.96 mmol) in dichloromethane was added a solution of epoxide **9a** (1.00 g, 2.98 mmol) in dichloromethane (2 mL) at −78 °C, and the resulting mixture was stirred at −78 °C for 30 min under argon. The reaction mixture was poured into water, and extracted with *n*-hexane. The organic phase was washed with saturated aqueous $NaHCO_3$, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was subjected to ODS column chromatography eluted stepwise with 70–100% (vol/vol) acetonitrile in water [5% (vol/vol) increments]; 85–90% acetonitrile eluates were combined, evaporated to water in vacuo, extracted with *n*-hexane, and evaporated to give the aldehyde **10a** (647 mg, 1.92 mmol, 64%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 0.06 (6H, s, Si-$CH_3$ × 2), 0.87 (9H, s, Si-C($CH_3$)$_3$), 0.97 (6H, s, 6′-$CH_3$), 1.41–1.60 (4H, m, H-4′ and -5′), 1.66 (3H, d, $J$ = 1.0 Hz, 2′-$CH_3$), 1.97 (2H, t, $J$ = 5.9 Hz, H-3′), 3.21–3.27 (1H, m, H-2), 3.88 (1H, dd, $J$ = 5.2, 10.1 Hz, H-1α), 3.99 (1H, dd, $J$ = 7.2, 10.1 Hz, H-1β), 5.33 (1H, dd, $J$ = 8.2, 16.2 Hz, H-3), 6.04 (1H, d, $J$ = 16.2 Hz, H-4), 9.69 (1H, d, $J$ = 2.4 Hz, 2-CHO); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: −5.5, 18.2, 19.2, 21.5, 25.8, 28.7, 32.6, 33.8, 39.3, 58.9, 62.7, 125.4, 129.3, 133.4, 137.1, 201.5; EI-MS $m/z$: 336 $[M]^+$, 321, 279; HREIMS $m/z$: 336.2483 $[M]^+$ (calcd. for $C_{20}H_{36}O_2Si$, $m/z$ 336.2485).

***5-{[(1E,3E)-2-(tert-Butyldimethylsiloxy)methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)buta-1,3-dien-1-yl]oxy}-3-methylfuran-2(5H)-one (TBDMSO-CL) (11a) and 5-{[(1Z,3E)-2-(tert-butyldimethylsiloxy)methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)buta-1,3-dien-1-yl]oxy}-3-methylfuran-2(5H)-one (TBDMSO-9E-CL) (12a).*** To a mixture of TBDMSO-$C_{14}$-aldehyde **10a** (250 mg, 0.753 mmol), phenotiazine (3.8 mg), (±)-4-bromo-2-methyl-2-buten-4-olide **4** (133 μL, 1.33 mmol), and 18-crown-6-ether (218 mg, 0.828 mmol) in THF (9.5 mL) potassium *tert*-butoxide (184 mg, 1.51 mmol) was added slowly under argon, and the reaction mixture was stirred at room temperature under argon. After stirring for 45 min, the mixture was poured into water (50 mL) and extracted with ether. The organic phase was washed with brine, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was subjected to silica gel column chromatography eluted stepwise with *n*-hexane and ether [3% (vol/vol) increments]. The 9–15% ether eluates containing TBDMSO-CL was purified by a semipreparative Inertsil ODS-3 HPLC (ϕ 10 × 250 mm, 5 μm; GL Sciences), using isocratic elution with 95% acetonitrile in water at a flow rate of 4.0 mL/min and monitored at 280 nm to give TBDMSO-CL **11a** [16.2 mg, 0.037 mmol, 5.0%, retention time (*Rt*) 14.0 min] and TBDMSO-9*E*-CL **12a** (11.4 mg, 0.026 mmol, 3.5%, *Rt* 14.9 min). TBDMSO-CL (**11a**) $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 0.08 (3H, s, Si-$CH_3$), 0.09 (3H, s, Si-$CH_3$), 0.90 (9H, s, Si-C($CH_3$)$_3$), 0.99 (6H, s, $CH_3$-16,17), 1.43–1.45 (2H, m, H-2), 1.56–1.62 (2H, m, H-3), 1.69 (3H, s, $CH_3$-18), 1.98 (3H, s, $CH_3$-15), 1.98–2.00 (2H, m, H-4), 4.26 (1H, d, $J$ = 12.4 Hz, H-19α), 4.32 (1H, d, $J$ = 12.4 Hz, H-19β), 6.01 (1H, s, H-11), 6.13 (1H, br d, $J$ = 16.6 Hz, H-7), 6.28 (1H, d, $J$ = 16.6 Hz, H-8), 6.39 (1H, s, H-10), 6.89 (1H, s, H-12); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: −5.3, 10.6, 18.3, 19.2, 21.7, 25.9, 28.85, 28.86, 32.9, 34.1, 39.4, 61.4, 100.3, 120.1, 124.7, 127.2, 128.7, 134.8, 138.0, 139.3, 142.2, 171.2; EI-MS $m/z$: 432 $[M]^+$, 335, 203, 97; HREIMS $m/z$: 432.2699 $[M]^+$ (calcd. for $C_{25}H_{40}O_4Si$, $m/z$ 432.2696). TBDMSO-9*E*-CL (**12a**) $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 0.058 (3H, s, Si-$CH_3$), 0.061 (3H, s, Si-$CH_3$), 0.88 (9H, s, Si-C($CH_3$)$_3$), 0.99 (3H, s, $CH_3$-16 or 17), 1.00 (3H, s, $CH_3$-16 or 17), 1.43–1.46 (2H, m, H-2), 1.57–1.61 (2H, m, H-3), 1.68 (3H, s, $CH_3$-18), 1.98 (2H, t, $J$ = 6.6 Hz, H-4), 2.00 (3H, t, $J$ = 1.3 Hz, $CH_3$-15), 4.39 (1H, d, $J$ = 11.3 Hz, H-19α), 4.47 (1H, d, $J$ = 11.3 Hz, H-19β), 5.74 (1H, d, $J$ = 16.1 Hz, H-8), 6.01 (1H, t, $J$ = 1.3 Hz, H-11), 6.30 (1H, br d, $J$ = 16.9 Hz, H-7), 6.38 (1H, s, H-10), 6.89 (1H, t, $J$ = 1.5 Hz, H-12); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: −5.3, −5.2, 10.7, 18.3, 19.3, 21.6, 25.9, 28.81, 28.86, 32.8, 34.2, 39.5, 55.8, 100.2, 122.6, 127.1, 128.3, 128.8, 134.9, 138.1, 141.4, 141.9, 171.0; EI-MS $m/z$: 432 $[M]^+$, 335, 203, 97; HREIMS $m/z$: 432.2671 $[M]^+$ (calcd. for $C_{25}H_{40}O_4Si$, $m/z$ 432.2696).

***5-{[(1E,3E)-2-(Hydroxymethyl)-4-(2,6,6-trimethylcyclohex-1-en-1-yl)buta-1,3-dien-1-yl]oxy}-3-methylfuran-2(5H)-one (19-hydroxy-CL) (1a).*** To a solution of TBDMSO-CL **11a** (16.2 mg, 0.037 mmol) and acetic acid (21.4 μL, 0.374 mmol) in THF (16 mL) was added at room temperature a 1.0 M THF solution of tetra-*n*-butylammonium fluoride (TBAF, 112.3 μL, 0.112 mmol) under argon. Another TBAF solution (112.3 μL, 0.112 mmol) was added at 1.5, 4.5, 6.5, 22.5, and 25 h after starting the reaction, and stirring was continued for an additional 5 h. The reaction mixture was poured into water and extracted with ether. The organic phase was washed with water, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was subjected to silica gel column chromatography eluted stepwise with *n*-hexane and ether [10% (vol/vol) increments]. The 70–90% ether eluates containing the deprotected product was purified by a semipreparative Inertsil SIL-100A (ϕ 10 × 250 mm, 5 μm; GL Sciences) using isocratic elution with 10% ethanol in *n*-hexane at a flow rate of 3 mL/min and monitored at 280 nm to give 19-hydroxy-CL **1a** (3.5 mg, 0.011 mmol, 30%, *Rt* 18.8 min). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.007 (3H, s, $CH_3$-16 or 17), 1.012 (3H, s, $CH_3$-16 or 17), 1.43–1.47 (2H, m, H-2), 1.57–1.62 (2H, m, H-3), 1.71 (3H, s, $CH_3$-18), 2.00 (3H, t, $J$ = 1.3 Hz, $CH_3$-15), 2.00 (2H, t, $J$ = 6.1 Hz, H-4), 4.29 (2H, d, $J$ = 3.2 Hz, H-19), 6.02 (1H, d, $J$ = 1.3 Hz, H-11), 6.29 (2H, s, H-7 and H-8), 6.45 (1H, s, H-10), 6.91 (1H, t, $J$ = 1.6 Hz, H-12); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 10.7, 19.3, 21.7, 28.9, 32.9, 34.2, 39.5, 61.3, 100.2, 120.3, 124.2, 127.9, 129.4, 135.0, 137.8, 140.3, 142.0, 171.1; EI-MS $m/z$ (rel. int): 318 $[M]^+$ (20), 221 (35), 203 (43), 175 (38), 97 (100); HREIMS $m/z$: 318.1815 $[M]^+$ (calcd. for $C_{19}H_{26}O_4$, $m/z$ 318.1831).

***[6′-$^{13}$CH$_3$]- (3E)-2-Oxo-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-1-yl acetate (6b).*** To a solution of [1-$^{13}$$CH_3$]-β-ionone **5b** (4.00 g, 20.8 mmol) in toluene (44 mL) was added lead tetraacetate (9.21 g, 20.8 mmol), and the mixture was stirred at 80 °C for 22 h under argon. The mixture was cooled to 0 °C, and washed successively with water, saturated aqueous $NaHCO_3$, and water, and then dried over anhydrous $Na_2SO_4$. Purification by silica gel column chromatography eluted stepwise with *n*-hexane and ether [3% (vol/vol) increments] gave $^{13}$C-acetoxy-β-ionone **6b** (1.69 g, 6.73 mmol, 32%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.08 (6H, d, appeared as two doublets, $^1J_{CH}$ = 125 Hz, $^3J_{CH}$ = 4.9 Hz, 6′-$^{13}$$CH_3$ and 6′-$CH_3$), 1.46–1.64 (4H, m, H-4′ and -5′), 1.79 (3H, s, 2′-$CH_3$), 2.09 (2H, t, $^3J_{HH}$ = 6.6 Hz, H-3′), 2.20 (3H, s, -OAc), 4.87 (2H, s, H-1), 6.20 (1H, d, $^3J_{HH}$ = 16.3 Hz, H-3), 7.46 (1H, d, $^3J_{HH}$ = 16.3 Hz, H-4); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 18.8 (d, $^3J_{CC}$ = 2.5 Hz), 20.6, 21.8, 28.7 (6′-$^{13}$$CH_3$), 33.8, 34.0 (d, $^1J_{CC}$ = 35.7 Hz), 39.8, 67.3, 125.3, 136.0, 138.4, 143.7, 170.4, 192.5; EI-MS $m/z$: 251 $[M]^+$, 236, 178; HREIMS $m/z$: 251.1585 $[M]^+$ (calcd. for $C_{14}$$^{13}$$CH_{22}O_3$, $m/z$ 251.1602).

***[6′-$^{13}$CH$_3$]-(3E)-1-Hydroxy-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one (7b).*** Acetoxy-β-ionone **6b** (1.69 g, 6.72 mmol) was dissolved in 1% (wt/vol) $Na_2CO_3$ in methanol–water (9:1, 84 mL) at −20 °C, and the mixture was stirred at 0 °C for 1.5 h. The reaction mixture was quenched by adding water (100 mL) and extracted with ether. The organic phase was washed successively with 2% (vol/vol) $H_2SO_4$ and water, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. Purification by silica gel column chromatography eluted with *n*-hexane and ether [2% (vol/vol)

increments] gave $^{13}$C-hydroxy-β-ionone **7b** (353 mg, 1.69 mmol, 25%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.09 (6H, d, appeared as two doublets, $^1J_{CH}$ = 126 Hz, $^3J_{CH}$ = 4.9 Hz, 6′-$^{13}CH_3$ and 6′-$CH_3$), 1.47–1.66 (4H, m, H-4′ and -5′), 1.80 (3H, s, 2′-$CH_3$), 2.10 (2H, t, $^3J_{HH}$ = 6.3 Hz, H-3′), 3.36 (1H, t, $^3J_{HH}$ = 4.6 Hz, 1-OH), 4.46 (2H, d, $^3J_{HH}$ = 4.6 Hz, H-1), 6.17 (1H, d, $^3J_{HH}$ = 16.3 Hz, H-3), 7.58 (1H, d, $^3J_{HH}$ = 16.3 Hz, H-4); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 18.7 (d, $^3J_{CC}$ = 2.5 Hz), 21.8, 28.8 (6′-$^{13}CH_3$), 33.8, 34.1 (d, $^1J_{CC}$ = 35.7 Hz), 39.8, 66.7, 125.3, 135.9, 138.8, 143.8, 198.1; EI-MS *m/z*: 209 $[M]^+$, 194, 178; HREIMS *m/z*: 209.1491 $[M]^+$ (calcd. for $C_{12}{}^{13}CH_{20}O_2$, *m/z* 209.1497).

***[6′-$^{13}CH_3$]-(3E)-1-(tert-Butyldimethylsiloxy)-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one (8b).*** A mixture of hydroxy-β-ionone **7b** (353 mg, 1.69 mmol), *tert*-butyldimethylchlorosilane (510 mg, 3.38 mmol), and imidazole (460 mg, 6.76 mmol) in *N,N*-dimethylformamide (DMF, 4 mL) was stirred at room temperature for 2.5 h under argon. The reaction mixture was taken up in 2% (vol/vol) ether in *n*-hexane, washed with water, dried over anhydrous $Na_2SO_4$, and concentrated in vauo. Purification by silica gel column chromatography eluted stepwise with *n*-hexane and ether [2% (vol/vol) increments] gave $^{13}$C-*tert*-butyldimethylsilyloxy-β-ionone **8b** (360 mg, 1.11 mmol, 66%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 0.10 (6H, s, Si-$CH_3$ × 2), 0.93 (9H, s, Si-C$(CH_3)_3$), 1.09 (6H, d, appeared as two doublets, $^1J_{CH}$ = 126 Hz, $^3J_{CH}$ = 4.9 Hz, 6′-$^{13}CH_3$ and 6′-$CH_3$), 1.46–1.63 (4H, m, H-4′ and -5′), 1.80 (3H, d, $^3J_{HH}$ = 0.7 Hz, 2′-$CH_3$), 2.09 (2H, t, *J* = 6.2 Hz, H-3′), 4.31 (2H, s, H-1), 6.53 (1H, d, $^3J_{HH}$ = 16.3 Hz, H-3), 7.50 (1H, d, $^3J_{HH}$ = 16.3 Hz, H-4); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: −5.4, 18.3, 18.8 (d, $^3J_{CC}$ = 1.7 Hz), 21.8, 25.8, 28.8 (6′-$^{13}CH_3$), 33.9, 34.1 (d, $^1J_{CC}$ = 33.2 Hz), 40.0, 69.0, 124.5, 136.3, 138.0, 142.8, 199.3; EI-MS *m/z*: 323 $[M]^+$, 308, 266; HREIMS *m/z*: 323.2380 $[M]^+$ (calcd. for $C_{18}{}^{13}CH_{34}O_2Si$, *m/z* 323.2362).

***[6′-$^{13}CH_3$]-2-[(tert-Butyldimethylsiloxy)methyl]-2-[(E)-2-(2,6,6-trimetylcyclohex-1-en-1-yl)ethenyl] oxirane (9b).*** To a solution of trimethylsulfonium iodide (341 mg, 1.67 mmol) in DMSO (1.42 mL) was added tetrahydrofuran (THF, 1.42 mL) under argon to yield a finely divided suspension of sulfonium salt. This mixture was then cooled to −5 °C and treated with a solution of dimsyl sodium (4.4 M, 445 μL, 1.96 mmol). The resulting gray colored suspension was treated with a solutiuon of $^{13}$C-*tert*-butyldimethylsilyloxy-β-ionone **8b** (360 mg, 1.11 mmol) in THF (344 μL). After stirring at −5 °C for 1 h, the mixture was warmed to room temperature, quenched by successively adding water (30 mL) and *n*-hexane (30 mL). The organic phase was washed with water, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo to give the epoxide **9b** (374 mg, 1.11 mmol, 100%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 0.07–0.10 (6H, m, Si-$CH_3$ × 2), 0.89 (9H, s, Si-C$(CH_3)_3$), 0.973 (3H, d, appeared as two doublets, $^1J_{CH}$ = 125 Hz, $^3J_{CH}$ = 4.9 Hz, 6″-$^{13}CH_3$), 0.976 (3H, d, appeared as two doublets, $^1J_{CH}$ = 125 Hz, $^3J_{CH}$ = 4.6 Hz, 6″-$^{13}CH_3$), 1.39–1.62 (4H, m, H-4″ and -5″), 1.66 (3H, s, 2″-$CH_3$), 1.97 (2H, t, $^2J_{HH}$ = 5.7 Hz, H-3″), 2.70 (1H, d, $^2J_{HH}$ = 5.6 Hz, H-3α), 2.97 (1H, d, $^2J_{HH}$ = 5.6 Hz, H-3β), 3.835 (1H, s, 2-$CH_2$), 3.837 (1H, s, 2-$CH_2$), 5.43 (1H, d, $^3J_{HH}$ = 16.3 Hz, H-1′), 6.24 (1H, dd, $^3J_{HH}$ = 16.3 Hz, $^5J_{HH}$ = 1.0 Hz, H-2′); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: −5.4, 18.3, 19.2 (d, $^3J_{CC}$ = 1.7 Hz), 21.4, 25.9, 28.7 (6′-$^{13}CH_3$), 32.7, 33.9 (d, $^1J_{CC}$ = 34.9 Hz), 39.4, 53.2, 58.8, 64.9, 129.0, 129.7, 130.3, 136.8; EI-MS *m/z*: 337 $[M]^+$, 280; HREIMS *m/z*: 337.2530 $[M]^+$ (calcd. for $C_{19}{}^{13}CH_{36}O_2Si$, *m/z* 337.2518).

***[6′-$^{13}CH_3$]-(3E)-2-[(tert-Butyldimethylsiloxy)methyl]-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-enal (10b).*** To a solution of 2,6-di-*tert*-butyl-4-bromophenol (1.27 g, 4.44 mmol) in dichloromethane (9.6 mL) was added at room temperature a 1.4 M hexane solution of trimethylaluminium ($Me_3Al$, 1.59 mL, 2.22 mmol), and the solution was stirred at room temperature for 1 h under argon. To a solution of the MABR (2.22 mmol) in dichloromethane was added a solution of expoxide **9b** (374 mg, 1.11 mmol) in dichloromethane (720 μL) at −78 °C, and the resulting mixture was stirred at −78 °C for 45 min under argon. The reaction mixture was poured into water and extracted with *n*-hexane. The organic phase was washed with saturated aqueous $NaHCO_3$, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was subjected to ODS column chromatography eluted stepwise with 70–100% (vol/vol) acetonitrile in water [5% (vol/vol) increments]; 85–90% acetonitrile eluates were combined, evaporated to water in vacuo, extracted with *n*-hexane, and evaporated to give the $^{13}$C-aldehyde **10b** (150 mg, 0.445 mmol, 41%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 0.06 (6H, s, Si-$CH_3$ × 2), 0.87 (9H, s, Si-C$(CH_3)_3$), 0.97 (6H, d, appeared as two doublets, $^1J_{CH}$ = 125 Hz, $^3J_{CH}$ = 4.9 Hz, 6′-$^{13}CH_3$ and 6′-$CH_3$), 1.36–1.63 (4H, m, H-4′ and -5′), 1.66 (3H, s, 2′-$CH_3$), 1.97 (2H, t, $^3J_{HH}$ = 5.9 Hz, H-3′), 3.21–3.27 (1H, m, H-2), 3.89 (1H, dd, $^3J_{HH}$ = 5.5 Hz, $^2J_{HH}$ = 9.9 Hz, H-1α), 3.99 (1H, dd, $^3J_{HH}$ = 7.1 Hz, $^2J_{HH}$ = 9.9 Hz, H-1β), 5.33 (1H, dd, $^3J_{HH}$ = 8.2 Hz, 16.0 Hz, H-3), 6.04 (1H, d, $^3J_{HH}$ = 16.0 Hz, H-4), 9.69 (1H, d, $^3J_{HH}$ = 2.0 Hz, 2-CHO); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: −5.4, 18.2, 19.2 (d, $^3J_{CC}$ = 1.7 Hz), 21.5, 25.8, 28.7 (6′-$^{13}CH_3$), 32.6, 33.8 (d, $^1J_{CC}$ = 36.5 Hz), 39.2, 58.9, 62.7, 125.4, 129.3, 133.4, 136.4, 201.5; EI-MS *m/z*: 337 $[M]^+$, 322, 280; HREIMS *m/z*: 337.2540 $[M]^+$ (calcd. for $C_{19}{}^{13}CH_{36}O_2Si$, *m/z* 337.2518).

***[6″-$^{13}CH_3$]-5-{[(1E,3E)-2-(tert-Butyldimethylsiloxy)methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)buta-1,3-dien-1-yl]oxy}-3-methylfuran-2(5H)-one ([1-$^{13}CH_3$]-TBDMSO-CL) (11b) and [6″-$^{13}CH_3$]-5-{[(1Z,3E)-2-(tert-butyldimethylsiloxy)methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)buta-1,3-dien-1-yl]oxy}-3-methylfuran-2(5H)-one ([1-$^{13}CH_3$]-TBDMSO-9E-CL) (12b).*** To a mixture of $^{13}$C-TBDMSO-$C_{14}$-aldehyde **10b** (150 mg, 0.445 mmol), phenotiazine (2.3 mg), (±)-4-bromo-2-methyl-2-buten-4-olide **4** (80 μL, 0.80 mmol), and 18-crown-6-ether (132 mg, 0.500 mmol) in THF (5.7 mL) was added slowly potassium *tert*-butoxide (101 mg, 0.90 mmol) under argon, and the reaction mixture was stirred at room temperature under argon. After stirring for 1 h, the mixture was poured into water (20 mL) and extracted with ether. The organic phase was washed with water, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was subjected to silica gel column chromatography eluted stepwise with *n*-hexane and ether [3% (vol/vol) increments]. The 12% and 15% ether eluates containing TBDMSO-CL was purified by a semipreparative Inertsil ODS-3 HPLC (ϕ 10 × 250 mm, 5 μm; GL Sciences), using isocratic elution with 95% acetonitrile in water at a flow rate of 4.0 mL/min and monitored at 280 nm to give [1-$^{13}CH_3$]-TBDMSO-CL **11b** (2.7 mg, 0.063 mmol, 1.4%, *Rt* 14.0 min) and [1-$^{13}CH_3$]-TBDMSO-9*E*-CL **12b** (1.6 mg, 0.037 mmol, 0.8%, *Rt* 14.9 min). [1-$^{13}CH_3$]-TBDMSO-CL (**11b**) $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 0.08 (3H, s, Si-$CH_3$), 0.09 (3H, s, Si-$CH_3$), 0.90 (9H, s, Si-C$(CH_3)_3$), 1.00 (6H, appeared as two doublets, $^1J_{CH}$ = 125 Hz, $^3J_{CH}$ = 4.9 Hz, $^{13}CH_3$-16,17), 1.42–1.46 (2H, m, H-2), 1.58–1.63 (2H, m, H-3), 1.69 (3H, s, $CH_3$-18), 1.98 (3H, t, $^{4,5}J_{HH}$ = 1.5 Hz, $CH_3$-15), 1.99 (2H, t, $^3J_{HH}$ = 6.3 Hz, $CH_3$-15), 4.25 (1H, dd, $^2J_{HH}$ = 12.4 Hz, $^4J_{HH}$ = 1.2 Hz, H-19α), 4.32 (1H, dd, $^2J_{HH}$ = 12.4 Hz, $^4J_{HH}$ = 1.2 Hz, H-19β), 6.01 (1H, t, $^{3,5}J_{HH}$ = 1.5 Hz, H-11), 6.14 (1H, br d, $^3J_{HH}$ = 16.6 Hz, H-7), 6.28 (1H, d, $^3J_{HH}$ = 16.6 Hz, H-8), 6.40 (1H, s, H-10), 6.90 (1H, p, $^{3,4}J_{HH}$ = 1.5 Hz, H-12); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: −5.3, 10.7, 18.3, 19.2, 21.7, 25.9, 28.86 ($^{13}CH_3$-16 or 17), 28.88 ($^{13}CH_3$-16 or 17), 32.9, 34.1 (d, $^1J_{CC}$ = 35.7 Hz), 39.4, 61.4, 100.3, 120.1, 124.7, 127.2, 128.9, 134.8, 138.1, 139.3, 142.2, 171.2; EI-MS *m/z*: 433 $[M]^+$, 336, 204, 97; HREIMS *m/z*: 433.2699 $[M]^+$ (calcd. for $C_{24}{}^{13}CH_{40}O_4Si$, *m/z* 433.2729). [1-$^{13}CH_3$]-TBDMSO-9*E*-CL (**12b**) $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 0.06 (3H, s, Si-$CH_3$), 0.07 (3H, s, Si-$CH_3$), 0.89 (9H, s, Si-C$(CH_3)_3$), 1.00 (3H, appeared as two doublets, $^1J_{CH}$ = 125 Hz, $^3J_{CH}$ = 4.9 Hz, $^{13}CH_3$-16 or 17),1.01 (3H, appeared as two doublets, $^1J_{CH}$ = 125 Hz, $^3J_{CH}$ = 4.6 Hz, $^{13}CH_3$-16 or 17), 1.43–1.47 (2H, m, H-2), 1.58–1.62 (2H, m, H-3), 1.68 (3H, s, $CH_3$-18), 1.99 (2H, t, $^3J_{HH}$ = 5.9 Hz, H-4), 2.00 (3H, t, $^{4,5}J_{HH}$ = 1.5 Hz, $CH_3$-15), 4.39 (1H, d, $^2J_{HH}$ = 11.4 Hz, H-19α), 4.47 (1H, d, $^2J_{HH}$ = 11.4 Hz, H-19β), 5.75 (1H, d, $^3J_{HH}$ = 16.5 Hz, H-8), 6.01 (1H, t, $^{3,5}J_{HH}$ = 1.5 Hz, H-11), 6.30 (1H, br d, $^3J_{HH}$ = 16.5 Hz, H-7), 6.38 (1H, s, H-10), 6.89 (1H, p, $^{3,4}J_{HH}$ = 1.5 Hz,

H-12); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: −5.24, −5.18, 10.7, 18.3, 19.3, 21.6, 26.0, 28.82 ($^{13}CH_3$-16 or 17), 28.88 ($^{13}CH_3$-16 or 17), 32.8, 34.2 (d, $^1J_{CC}$ = 35.7 Hz), 39.5, 55.9, 100.2, 122.6, 127.1, 128.4 (d, $^3J_{CC}$ = 1.7 Hz), 128.8, 135.0, 138.1, 141.5, 141.9, 171.0; EI-MS *m/z*: 433 $[M]^+$, 336, 204, 97; HREIMS *m/z*: 433.2759 $[M]^+$ (calcd. for $C_{24}{}^{13}CH_{40}O_4Si$, *m/z* 433.2729).

***[6″-$^{13}CH_3$]-5-{[(1E,3E)-2-(Hydroxymethyl)-4-(2,6,6-trimethylcyclohex-1-en-1-yl)buta-1,3-dien-1-yl]oxy}-3-methylfuran-2(5H)-one ([1-$^{13}CH_3$]-19-hydroxy-CL) (1b).*** To a solution of [1-$^{13}CH_3$]-TBDMSO-CL **11b** (2.7 mg, 6.3 μmol) in THF (0.9 mL) was added at room temperature acetic acid (2.7 mL) and water (0.9 mL), and the mixture was stirred for 6 h. The reaction mixture was poured into water and extracted with ether. The organic phase was washed with water, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. Purification by silica gel column chromatography eluted stepwise with *n*-hexane and ether [10% (vol/vol) increments] gave [1-$^{13}CH_3$]-19-hydroxy-CL **1b** (1.88 mg, 5.9 μmol, 94%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.01 (3H, appeared as two doublets, $^1J_{CH}$ = 125 Hz, $^3J_{CH}$ = 4.6 Hz, $^{13}CH_3$-16 or 17), 1.02 (3H, appeared as two doublets, $^1J_{CH}$ = 125 Hz, $^3J_{CH}$ = 4.6 Hz, $^{13}CH_3$-16 or 17) 1.43–1.47 (2H, m, H-2), 1.59–1.62 (2H, m, H-3), 1.71 (3H, s, $CH_3$-18), 2.00 (3H, t, $^{4,5}J_{HH}$ = 1.2 Hz, $CH_3$-15), 2.00 (2H, t, $^3J_{HH}$ = 6.1 Hz, H-4), 4.29 (2H, m, H-19), 6.02 (1H, d, $^{3,5}J_{HH}$ = 1.1 Hz, H-11), 6.29 (2H, s, H-7 and H-8), 6.45 (1H, s, H-10), 6.91 (1H, t, $^{3,4}J_{HH}$ = 1.3 Hz, H-12); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 10.7, 19.2 (d, $^3J_{CC}$ = 1.7 Hz), 21.8, 28.9 ($^{13}CH_3$-16,17), 32.9, 34.2 (d, $^1J_{CC}$ = 35.7 Hz), 39.4, 61.3, 100.2, 120.3, 124.2, 127.9, 129.4 (d, $^3J_{CC}$ = 1.7 Hz), 135.0, 137.8, 140.4, 142.0, 171.1; EI-MS *m/z* (rel. int): 319 $[M]^+$ (43), 222 (62), 204 (91), 176 (86), 97 (100); HREIMS *m/z*: 319.1853 $[M]^+$ (calcd. for $C_{18}{}^{13}CH_{26}O_4$, *m/z* 319.1865).

***(3E)-4-(2,6,6-Trimethylcyclohex-1-en-1-yl)but-3-enal (16a).*** (*E*)-3-(2,6,6-Trimethylcyclohex-1-enyl)acrylaldehyde ($C_{12}$-aldehyde) **15a** was prepared from β-ionone **5a** via oxidation (**13a**), reduction (**14a**), and oxidation (**15a**) according to the literature (12). To a mixture of $C_{12}$-aldehyde **15a** (2.38 g, 13.4 mmol), ethyl chloroacetate (2.0 mL, 18.8 mmol), pyridine (20 mL), and phenothiazine (7 mg) was slowly added sodium methoxide (1.1 g, 7.5 mmol) at −20 °C under argon. The mixture was stirred at −5 °C for 30 min, warmed to room temperature, and was then again cooled to −5 °C. After stirring for 2 h, 10% (wt/vol) sodium hydroxide in methanol (8 mL, 20 mmol) was slowly added at −5 °C. The mixture was stirred at 10 °C for 1 h and cooled to −20 °C. The reaction mixture was quenched by adding acetic acid (9.1 mL) at −20 °C, warmed to 10 °C, diluted with water, and extracted with ether. The organic phase was washed with water, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. Purification by silica gel column chromatography eluted with *n*-hexane gave the $C_{13}$-aldehyde **16a** (1.25 g, 6.51 mmol, 49%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 0.99 (6H, s, 6′-$CH_3$ × 2), 1.43–1.63 (4H, m, H-4′ and -5′), 1.68 (3H, s, 2′-$CH_3$), 1.98 (2H, t, *J* = 6.2 Hz, H-3′), 3.23 (2H, dt, *J* = 7.1, 1.7 Hz, H-2), 5.45 (1H, dt, *J* = 15.9, 7.1 Hz, H-3), 6.03 (1H, d, *J* = 15.9 Hz, H-4), 9.71 (1H, t, *J* = 2.1 Hz, H-1); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 19.2, 21.5, 28.7, 32.6, 33.8, 39.3, 47.9, 122.6, 129.2, 133.7, 137.0, 200.9; EI-MS *m/z*: 192 $[M]^+$, 177, 163; HREIMS *m/z*: 192.1509 $[M]^+$ (calcd. for $C_{13}H_{20}O$, *m/z* 192.1514).

***(3E)-4-(2,6,6-Trimethylcyclohex-1-en-1-yl)but-3-enoic acid (17a).*** To a rapidly stirred solution of $C_{13}$-aldehyde **16a** (1.50 g, 7.81 mmol) and 2-methyl-2-butene (8.3 mL, 78 mmol) in *tert*-butanol (40 mL) was added dropwise a solution of sodium chlorite (1.06 g, 10 mmol, 85%) in $NaH_2PO_4$ buffer (8 mL, pH 3.5) at room temperature under argon. After stirring for 2 h, the reaction mixture was made basic (pH 10) with 6 N NaOH solution and evaporated to water in vacuo. The aqueous residue was diluted with water (50 mL) and extracted with *n*-hexane. The aqueous phase was acidified with concentrated HCl and extracted with ether. The organic phase was washed successively with brine and water, dried over anhydrous $Na_2SO_4$, and evaporated to give $C_{13}$-carboxylic acid **17a** (1.27 g, 6.11 mmol, 78%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 0.98 (6H, s, 6′-$CH_3$ × 2), 1.42–1.62 (4H, m, H-4′ and -5′), 1.67 (3H, s, 2′-$CH_3$), 1.97 (2H, t, *J* = 6.1 Hz, H-3′), 3.18 (1H, d, *J* = 7.1 Hz, H-2α), 3.19 (1H, d, *J* = 7.1 Hz, H-2β), 5.47 (1H, dt, *J* = 15.9, 7.1Hz, H-3), 6.00 (d, *J* = 15.9 Hz, H-4); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 19.2, 21.3, 28.6, 32.6, 33.9, 38.3, 39.3, 124.2, 129.2, 132.4, 136.8, 178.3; EI-MS *m/z*: 208 $[M]^+$, 193; HREIMS *m/z*: 208.1452 $[M]^+$ (calcd. for $C_{13}H_{20}O_2$, *m/z* 208.1463).

***tert-Butyl (3E)-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-enoate (18a).*** To a solution of $C_{13}$-carboxylic acid **17a** (1.01 g, 4.86 mmol) and di-*tert*-butyl dicarbonate (1.47 mL, 6.74 mmol) in *tert*-butanol (10 mL) was added a solution of *N*,*N*-dimethyl-4-aminopyridine (DMAP, 59 mg, 0.48 mmol) in *tert*-butanol (1 mL) at room temperature under argon, and the reaction mixture was stirred for 2 h. After removal of solvent in vacuo, the residue was subjected to silica gel column chromatography eluted with *n*-hexane to give the $C_{13}$-carboxylic acid *tert*-butyl ester **18a** (870 mg, 3.30 mmol, 68%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 0.98 (6H, s, 6′-$CH_3$ × 2), 1.42–1.62 (4H, m, H-4′ and -5′), 1.45 (9H, s, $C(CH_3)_3$), 1.68 (3H, s, 2′-$CH_3$), 1.96 (2H, t, *J* = 6.2 Hz, H-3′), 3.027 (1H, d, *J* = 7.1 Hz, H-2α), 3.031 (1H, d, *J* = 7.1 Hz, H-2β), 5.44 (1H, dt, *J* = 15.9, 7.1 Hz, H-3), 5.94 (1H, *J* = 15.9 Hz, H-4); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 19.2, 21.4, 28.1, 28.6, 32.6, 33.9, 39.3, 40.3, 80.4, 126.0, 128.7, 131.2, 137.1, 171.4; EI-MS *m/z*: 264 $[M]^+$, 208, 193, 163, 57; HREIMS *m/z*: 264.2106 $[M]^+$ (calcd. for $C_{17}H_{28}O_2$, *m/z* 264.2089).

***(2E,3E)-tert-Butyl 2-((4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy)methylene)-4-(2,6,6-trimethylcyclohex-1-enyl)but-3-enoate (19a).*** To a suspension of sodium hydride (120 mg, 5.0 mmol) in DMF (3 mL) was added a solution of $C_{13}$-carboxylic acid *tert*-butyl ester **18a** (870 mg, 3.30 mmol) in DMF (4 mL) at room temperature under argon. Then methyl formate (1020 μL, 16.5 mmol) was added, and the mixture was stirred for 3 h. After cooling to 0 °C, (±)-4-bromo-2-methyl-2-buten-4-olide **4** (490 μL, 4.98 mmol) in DMF (1.5 mL) was added, and the reaction mixture was stirred at room temperature for 3 h under argon. The mixture was poured into ice-cooled 0.1 N HCl and extracted with ether. The organic phase was washed with water, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was subjected to silica gel chromatography eluted stepwise with *n*-hexane and ethyl acetate [10% (vol/vol) increments]. The 20% ethyl acetate eluate containing crude CLA *tert*-butyl ester **19a** was directly used in the next reaction. EI-MS *m/z*: 388 $[M]^+$, 291, 263, 235, 217, 97, 57.

***(2E,3E)-2-((4-Methyl-5-oxo-2,5-dihydrofuran-2-yloxy)methylene)-4-(2,6,6-trimethylcyclohex-1-enyl)but-3-enoic acid (CLA) (2a).*** To a solution of the 20% ethyl acetate eluate (497 mg) containing CLA *tert*-butyl ester **19a** and 2,6-lutidine (440 μL, 3.80 mmol) in dichloromethane (50 mL) was added triethylsilyl trifluoromethanesulfonate (TESOTf, 2.20 mL, 9.65 mmol) at 0 °C under argon, and the mixture was stirred at room temperature for 7 h. The reaction mixture was poured into ice-cooled 0.1 N HCl and extracted with dichloromethane. The organic phase was washed with water, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was subjected to silica gel column chromatography eluted stepwise with *n*-hexane and ethyl acetate [10% (vol/vol) increments]. The 40% ethyl acetate eluate containing CLA were combined and purified by a semipreparative Inertsil ODS-3 HPLC (ϕ 10 × 250 mm, 5 μm; GL Scieneces), using isocratic elution with 90% (vol/vol) acetonitrile in water containing 0.1% acetic acid at a flow rate of 2.4 mL/min, and monitoring at 325 nm to give CLA **2a** (20.6 mg, 0.062 μmol, 1.9% in two steps, *Rt* 9.5 min). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.00 (6H, s, $CH_3$-16 and 17), 1.43–1.47 (2H, m, H-2), 1.57–1.62 (2H, m, H-3), 1.71 (3H, s, $CH_3$-18), 1.99 (2H, br t, *J* = 6.1 Hz, H-4), 2.03 (3H, t, *J* = 1.3 Hz, $CH_3$-15), 6.06 (1H, d, *J* = 16.8 Hz, H-8), 6.18 (1H, t, *J* = 1.3 Hz, H-11), 6.80 (1H, d, *J* = 16.8 Hz, H-7), 6.97 (1H, t, *J* = 1.6 Hz, H-12), 7.64 (1H, s, H-10); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 10.7, 19.2, 21.6, 28.79, 28.81, 32.9, 34.1, 39.4, 100.5, 112.0, 121.2, 129.2, 133.1, 135.7, 138.3, 141.1, 153.0, 170.5, 172.5; EI-MS *m/z* (rel. int): 332 $[M]^+$ (22),

235 (39), 217 (100), 189 (51), 147 (61), 121 (52), 97 (46); HREIMS *m/z*: 332.1628 $[M]^+$ (calcd. for $C_{19}CH_{24}O_5$, *m/z* 332.1624).

***[6′-$^{13}CH_3$]-(2E)-3-(2,6,6-Trimethy-1-cyclohexen-1-yl)-2-propenoic acid (13b).*** [1-$^{13}CH_3$]-β-Ionone **5b** (1.00 g, 5.17 mmol) was added to a sodium hypochlorite solution (12% available chlorine, 12 mL) at room temperature under argon. The mixture was cooled to 0 °C, and then methanol (6.8 mL) was added. After stirring at 0 °C for 1 h, the mixture was warmed to room temperature and stirred for 23 h. After evaporation of methanol in vacuo, the aqueous residue was extracted with ether. The aqueous phase was acidified with concentrated HCl and extracted with ether. The organic phase was washed with brine, dried over anhydrous $Na_2SO_4$, and evaporated to give the $^{13}$C-enoic acid **13b** (730 mg, 3.74 mmol, 72%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.09 (6H, d, appeared as two doublets, $^1J_{CH}$ = 126 Hz, $^3J_{CH}$ = 4.9 Hz, 6′-$^{13}CH_3$ and 6′-$CH_3$), 1.46–1.64 (4H, m, H-4′ and -5′), 1.79 (3H, s, 2′-$CH_3$), 2.08 (2H, t, $^3J_{HH}$ = 6.1 Hz, H-3′), 5.85 (1H, d, $^3J_{HH}$ = 16.1 Hz, H-2), 7.56 (1H, d, $^3J_{HH}$ = 16.1 Hz, H-3); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 18.8 (d, $^3J_{CC}$ = 1.7 Hz), 21.7, 28.7 (6′-$^{13}CH_3$), 33.7, 34.0 (d, $^1J_{CC}$ = 35.7 Hz), 39.8, 120.5, 135.7, 137.3, 146.8, 172.4; EI-MS *m/z*: 195 $[M]^+$, 180; HREIMS *m/z*: 195.1350 $[M]^+$ (calcd. for $C_{11}{}^{13}CH_{18}O_2$, *m/z* 195.1340).

***[6′-$^{13}CH_3$]-(2E)-3-(2,6,6-Trimethy-1-cyclohexen-1-yl)-2-propen-1-ol (14b).*** To a solution of $^{13}$C-enoic acid **13b** (1.94 g, 9.93 mmol) in THF (21 mL) was slowly added Red-Al (3.4 M in toluene, 7.3 mL, 24.8 mmol) at 0 °C under argon, and the mixture was refluxed for 1 h. After cooled to room temperature, the reaction mixture was quenched by adding saturated aqueous $NH_4Cl$. The mixture was filtered and extracted with ether. The organic phase was successively washed with 5% (wt/vol) $NaHCO_3$ solution and water, dried over anhydrous $Na_2SO_4$, and evaporated to give the $^{13}$C-allyl alcohol **14b** (1.53 g, 8.44 mmol, 85%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.00 (6H, d, appeared as two doublets, $^1J_{CH}$ = 125 Hz, $^3J_{CH}$ = 4.9 Hz, 6′-$^{13}CH_3$ and 6′-$CH_3$), 1.43–1.63 (4H, m, H-4′ and -5′), 1.68 (3H, s, 2′-$CH_3$), 1.98 (2H, t, $^3J_{HH}$ = 6.5 Hz, H-3′), 4.21 (2H, d, $^3J_{HH}$ = 4.4 Hz, H-1), 5.62 (1H, dt, $^3J_{HH}$ = 15.9, 6.1 Hz, H-2), 6.11 (1H, dd, $^3J_{HH}$ = 15.9 Hz, $^5J_{HH}$ = 1.0 Hz, H-3); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 19.2 (d, $^3J_{CC}$ = 1.7 Hz), 21.4, 28.7 (6′-$^{13}CH_3$), 32.7, 34.0 (d, $^1J_{CC}$ = 35.7 Hz), 39.4, 64.3, 129.1 (d, $^3J_{CC}$ = 1.7 Hz), 129.8 (d, $^3J_{CC}$ = 1.7 Hz), 132.3, 136.6; EI-MS *m/z*: 181 $[M]^+$, 166; HREIMS *m/z*: 181.1532 $[M]^+$ (calcd. for $C_{11}{}^{13}CH_{20}O$, *m/z* 181.1548).

***[6′-$^{13}CH_3$]-(2E)-3-(2,6,6-Trimethy-1-cyclohexen-1-yl)-2-propenal (15b).*** A mixture of $^{13}$C-allyl alcohol **14b** (1.53 g, 8.44 mmol) and manganese (IV) oxide (14.7 g, 169 mmol) in dichloromethane (20 mL) was stirred at room temperature for 1 h under argon. The reaction mixture was filtered, and evaporated to give $^{13}$C-ene-aldehyde **15b** (1.07 g, 5.97 mmol, 71%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.11 (6H, d, appeared as two doublets, $^1J_{CH}$ = 125 Hz, $^3J_{CH}$ = 4.6 Hz, 6′-$^{13}CH_3$ and 6′-$CH_3$), 1.49–1.65 (4H, m, H-4′ and -5′), 1.82 (3H, s, 2′-$CH_3$), 2.13 (2H, t, $^3J_{HH}$ = 6.3 Hz, H-3′), 6.20 (1H, dd, $^3J_{HH}$ = 16.1, 7.8 Hz, H-2), 7.29 (1H, d, $^3J_{HH}$ = 16.3 Hz, H-3), 9.54 (1H, d, $^3J_{HH}$ = 7.8 Hz, H-1); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 18.7 (d, $^3J_{CC}$ = 2.5 Hz), 21.8, 28.7 (6′-$^{13}CH_3$), 34.00 (d, $^1J_{CC}$ = 34.0 Hz), 34.04, 39.9, 132.5, 136.6, 139.6, 152.8, 194.8; EI-MS *m/z*: 179 $[M]^+$, 164; HREIMS *m/z*: 179.1366 $[M]^+$ (calcd. for $C_{11}{}^{13}CH_{18}O$, *m/z* 179.1391).

***[6′-$^{13}CH_3$]-(3E)-4-(2,6,6-Trimethylcyclohex-1-en-1-yl)but-3-enal (16b).*** To a mixture of $^{13}$C-ene-aldehyde **15b** (320 mg, 1.79 mmol), ethyl chloroacetate (323 μL, 2.50 mmol), pyridine (2.7 mL), and phenothiazine (1 mg) was slowly added sodium methoxide (145 mg, 2.68 mmol) at −20 °C under argon. The mixture was stirred at −5 °C for 30 min, warmed to room temperature, and was then again cooled to −5 °C. After stirring for 1 h, 10% (wt/vol) sodium hydroxide in methanol (1.08 mL) was slowly added at −5 °C. The mixture was stirred at 10 °C for 1 h and cooled to −20 °C. The reaction mixture was quenched by adding acetic acid (1.22 mL) at −20 °C, warmed to 10 °C, diluted with water, and extracted with ether. The organic phase was washed successively with 0.1 N HCl and water, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. Purification by silica gel column chromatography eluted with *n*-hexane gave the $^{13}$C-$C_{13}$-aldehyde **16b** (115 mg, 0.595 mmol, 33%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 0.99 (6H, d, appeared as two doublets, $^1J_{CH}$ = 126 Hz, $^3J_{CH}$ = 4.9 Hz, 6′-$^{13}CH_3$ and 6′-$CH_3$), 1.43–1.63 (4H, m, H-4′ and -5′), 1.68 (3H, s, 2′-$CH_3$), 1.98 (2H, t, $^3J_{HH}$ = 6.1 Hz, H-3′), 3.24 (2H, dt, $^3J_{HH}$ = 7.1, 1.6 Hz, H-2), 5.45 (1H, dt, $^3J_{HH}$ = 15.9, 7.1 Hz, H-3), 6.03 (1H, d, $^3J_{HH}$ = 15.9 Hz, H-4), 9.72 (1H, t, $^3J_{HH}$ = 2.1 Hz, H-1); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 19.2 (d, $^3J_{CC}$ = 1.7 Hz), 21.5, 28.7 (6′-$^{13}CH_3$), 32.6, 33.8 (d, $^1J_{CC}$ = 35.7 Hz), 39.3, 48.0, 122.6, 129.2 (d, $^3J_{CC}$ = 1.7 Hz), 133.7 (d, $^3J_{CC}$ = 1.7 Hz), 137.0, 200.18; EI-MS *m/z*: 193 $[M]^+$, 178, 164; HREIMS *m/z*: 193.1538 $[M]^+$ (calcd. for $C_{12}{}^{13}CH_{20}O$, *m/z* 195.1548).

***[6′-$^{13}CH_3$]-(3E)-4-(2,6,6-Trimethylcyclohex-1-en-1-yl)but-3-enoic acid (17b).*** To a rapidly stirred solution of $^{13}$C-$C_{13}$-aldehyde **16b** (278 mg, 1.44 mmol) and 2-methyl-2-butene (1.53 mL, 14.4 mmol) in *tert*-butanol (7.4 mL) was added dropwise a solution of sodium chlorite (196 mg, 1.84 mmol, 85%) in $NaH_2PO_4$ buffer (1.48 mL, pH 3.5) at room temperature under argon. After stirring for 1.5 h, the reaction mixture was made basic (pH 10) with 6 N NaOH solution and evaporated to water in vacuo. The aqueous residue was diluted with water (10 mL) and extracted with *n*-hexane. The aqueous phase was acidified with 2 N HCl and extracted with ether. The organic phase was washed successively with brine and water, dried over anhydrous $Na_2SO_4$, and evaporated to give $^{13}$C-$C_{13}$-carboxylic acid **17b** (230 mg, 1.10 mmol, 76%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 0.98 (6H, d, appeared as two doublets, $^1J_{CH}$ = 125 Hz, $^3J_{CH}$ = 4.9 Hz, 6′-$^{13}CH_3$ and 6′-$CH_3$), 1.42–1.62 (4H, m, H-4′ and -5′), 1.68 (3H, s, 2′-$CH_3$), 1.97 (2H, t, $^3J_{HH}$ = 6.1 Hz, H-3′), 3.18 (1H, d, $^3J_{HH}$ = 7.1 Hz, H-2α), 3.19 (1H, d, $^3J_{HH}$ = 7.1 Hz, H-2β), 5.48(1H, dt, $^3J_{HH}$ = 15.9, 7.1Hz, H-3), 6.00 (d, $^3J_{HH}$ = 15.9 Hz, H-4); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 19.2 (d, $^3J_{CC}$ = 1.7 Hz), 21.6, 28.7 (6′-$^{13}CH_3$), 32.6, 33.9 (d, $^1J_{CC}$ = 35.7 Hz), 38.1, 39.3, 124.4, 129.2 (d, $^3J_{CC}$ = 2.5 Hz), 132.3, 136.8, 176.8; EI-MS *m/z*: 109 $[M]^+$, 194; HREIMS *m/z*: 209.1476 $[M]^+$ (calcd. for $C_{12}{}^{13}CH_{20}O_2$, *m/z* 209.1497).

***[6′-$^{13}CH_3$]-tert-Butyl (3E)-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-enoate (18b).*** To a solution of $^{13}$C-$C_{13}$-carboxylic acid **17b** (230 mg, 1.10 mmol) and di-*tert*-butyl dicarbonate (336 μL, 1.54 mmol) in *tert*-butanol (7.4 mL) was added a solution of *N*,*N*-dimethyl-4-aminopyridine (DMAP, 6.7 mg, 0.055 mmol) in *tert*-butanol (460 μL) at room temperature under argon. After stirring for 2 h, another solution of DMAP (6.7 mg, 0.055 mmol) in *tert*-butanol (460 μL) was added, and the mixture was stirred for an additional 30 min. After removal of solvent in vacuo, the residue was subjected to silica gel column chromatography eluted with *n*-hexane to give the $^{13}$C-$C_{13}$-carboxylic acid *tert*-butyl ester **18b** (195 mg, 0.736 mmol, 67%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 0.98 (6H, d, appeared as two doublets, $^1J_{CH}$ = 125 Hz, $^3J_{CH}$ = 5.1 Hz, 6′-$^{13}CH_3$ and 6′-$CH_3$), 1.42–1.62 (4H, m, H-4′ and -5′), 1.45 (9H, s, $C(CH_3)_3$), 1.68 (3H, s, 2′-$CH_3$), 1.97 (2H, t, $^3J_{HH}$ = 6.3 Hz, H-3′), 3.030 (1H, d, $^3J_{HH}$ = 7.1 Hz, H-2α), 3.033 (1H, d, $^3J_{HH}$ = 7.1 Hz, H-2β), 5.45 (1H, dt, $^3J_{HH}$ = 15.7, 7.1Hz, H-3), 5.94 (dd, $^3J_{HH}$ = 15.7 Hz, $^5J_{HH}$ = 1.0 Hz, H-4); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 19.2 (d, $^3J_{CC}$ = 1.7 Hz), 21.4, 28.1, 28.6 (6′-$^{13}CH_3$), 32.6, 33.9 (d, $^1J_{CC}$ = 35.7 Hz), 39.3, 40.3, 80.4, 126.0, 128.7, 131.2 (d, $^3J_{CC}$ = 1.7 Hz), 137.1, 171.4; EI-MS *m/z*: 265 $[M]^+$, 209, 164, 57; HREIMS *m/z*: 265.2137 $[M]^+$ (calcd. for $C_{16}{}^{13}CH_{28}O_2$, *m/z* 265.2123).

***[6′-$^{13}CH_3$]-(2E,3E)-tert-Butyl 2-((4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy) methylene)-4-(2,6,6-trimethylcyclohex-1-enyl)but-3-enoate (19b).*** To a suspension of sodium hydride (44 mg, 1.10 mmol) in DMF (900 μL) was added a solution of $^{13}$C-$C_{13}$-carboxylic acid *tert*-butyl ester **18b** (195 mg, 0.736 mmol) in DMF (900 μL) at room temperature under argon. Then methyl formate (228 μL, 3.68 mmol) was added, and the mixture was stirred for 3 h. After cooling to 0 °C, (±)-4-bromo-2-methyl-2-buten-4-olide **4** (162 μL, 1.62 mmol) in DMF (335 μL) was added, and the reaction mixture

was stirred at room temperature for 2.5 h under argon. The mixture was poured into ice-cooled 0.1 N HCl and extracted with ether. The organic phase was washed with water, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was subjected to silica gel chromatography eluted stepwise with *n*-hexane and ethyl acetate [10% (vol/vol) increments]. The 20% ethyl acetate eluate containing crude $^{13}$C-CLA *tert*-butyl ester **19b** was directly used in the next reaction. EI-MS *m/z*: 389 $[M]^+$, 292, 97, 57; HREIMS *m/z*: 389.2274 $[M]^+$ (calcd. for $C_{22}{}^{13}CH_{32}O_5$, *m/z* 389.2283).

***[6′-$^{13}CH_3$]-(2E,3E)-2-((4-Methyl-5-oxo-2,5-dihydrofuran-2-yloxy)methylene)-4-(2,6,6-trimethylcyclohex-1-enyl)but-3-enoic acid ([1-$^{13}CH_3$]-CLA) (2b).*** To a solution of the 20% ethyl acetate eluate (214 mg) containing $^{13}$C-CLA *tert*-butyl ester **19b** and 2,6-lutidine (191 μL, 1.65 mmol) in dichloromethane (20 mL) was added triethylsilyl trifluoromethanesulfonate (TESOTf, 934 μL, 4.13 mmol) at 0 °C under argon, and the mixture was stirred at room temperature for 3 h. The reaction mixture was poured into ice-cooled 0.1 N HCl and extracted with dichloromethane. The organic phase was washed with water, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was subjected to silica gel column chromatography eluted stepwise with *n*-hexane and ethyl acetate [5% (vol/vol) increments]. The 30–50% ethyl acetate eluates containing $^{13}$C-CLA were combined and purified by a semipreparative Inertsil ODS-3 HPLC (ϕ 10 × 250 mm, 5 μm; GL Scieneces), using isocratic elution with 90% (vol/vol) acetonitrile in water containing 0.1% acetic acid at a flow rate of 2.4 mL/min and monitored at 325 nm to give [1-$^{13}CH_3$]-CLA **2b** (12.7 mg, 0.038 mmol, 5.2% in two steps, *Rt* 9.6 min). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 0.997 (3H, appeared as two doublets, $^1J_{CH}$ = 125 Hz, $^3J_{CH}$ = 4.6 Hz, $^{13}CH_3$-16 or 17), 0.999 (3H, appeared as two doublets, $^1J_{CH}$ = 125 Hz, $^3J_{CH}$ = 4.4 Hz, $^{13}CH_3$-16 or 17), 1.43–1.47 (2H, m, H-2), 1.57–1.62 (2H, m, H-3), 1.71 (3H, s, $CH_3$-18), 2.00 (2H, br t, $^3J_{HH}$ = 6.3 Hz, H-4), 2.03 (3H, t, $^{4,5}J_{HH}$ = 1.5 Hz, $CH_3$-15), 6.06 (1H, d, $^3J_{HH}$ = 16.6 Hz, H-8), 6.18 (1H, t, $^{3,5}J_{HH}$ = 1.3 Hz, H-11), 6.80 (1H, d, $^3J_{HH}$ = 16.6 Hz, H-7), 6.97 (1H, t, $^{3,4}J_{HH}$ = 1.6 Hz, H-12), 7.64 (1H, s, H-10); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 10.7, 19.2 (d, $^3J_{CC}$ = 1.7 Hz), 21.6, 28.79 ($^{13}CH_3$-16 or 17), 28.81 ($^{13}CH_3$-16 or 17), 32.9, 34.1 (d, $^1J_{CC}$ = 35.7 Hz), 39.4, 100.5, 112.0, 121.2, 129.2 (d, $^3J_{CC}$ = 1.7 Hz), 133.0, 135.6, 138.3, 141.1, 153.0, 170.5, 172.2; EI-MS *m/z* (rel. int): 333 $[M]^+$ (23), 236 (40), 218 (99), 190 (74), 148 (48), 122 (45), 97 (100); HREIMS *m/z*: 333.1665 $[M]^+$ (calcd. for $C_{18}{}^{13}CH_{24}O_5$, *m/z* 333.1657).

***(E)-Methyl 4-(2,6,6-trimethylcyclohex-1-enyl)but-3-enoate (20a).*** A mixture of $C_{13}$-aldehyde **16a** (400 mg, 2.08 mmol), sodium cyanide (545 mg, 11.1 mmol), acetic acid (194 μL, 3.40 mmol), and manganese (IV) oxide (3.65 g, 42.0 mmol) in methanol (30 mL) was stirred at room temperature for 15.5 h under argon. The reaction mixture was filtered and evaporated. The residue was partitioned between ether and water. The organic layer was washed with water, dried over $MgSO_4$, and concentrated in vacuo. Purification by silica gel column chromatography eluted with *n*-hexane and 10% ether in *n*-hexane gave the $C_{13}$-carboxylic acid methyl ester **20a** (134 mg, 0.604 mmol, 29%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 0.98 (6H, s, 6′-$CH_3$), 1.42–1.63 (4H, m, H-4′ and -5′), 1.67 (3H, s, 2′-$CH_3$), 1.97 (2H, t, *J* = 6.2 Hz, H-3′), 3.138 (1H, d, *J* = 7.2 Hz, H-2α), 3.142 (1H, d, *J* = 7.2 Hz, H-2β), 3.69 (3H, s, -COOMe), 5.47(1H, dt, *J* = 15.7, 7.2 Hz, H-3), 5.97 (d, *J* = 15.7 Hz, H-4); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 19.2, 21.3, 28.6, 32.6, 33.9, 38.4, 39.3, 51.8, 125.1, 129.0, 131.8, 136.9, 172.6; EI-MS *m/z*: 222 $[M]^+$, 207, 175, 133; HREIMS *m/z*: 222.1639 $[M]^+$ (calcd. for $C_{14}H_{22}O_2$, *m/z* 222.1620).

***(2E,3E)-Methyl 2-((4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy)methylene)-4-(2,6,6-trimethylcyclohex-1-enyl)but-3-enoate (MeCLA) (3a).*** To a suspension of sodium hydride (8.6 mg, 0.36 mmol) in DMF (250 μL) was added a solution of $C_{13}$-carboxylic acid methyl ester **20a** (53 mg, 0.24 mmol) in DMF (250 μL) at room temperature under argon. Then methyl formate (74 μL, 1.2 mmol) was added, and the mixture was stirred for 13.5 h. After cooling to 0 °C, (±)-4-bromo-2-methyl-2-buten-4-olide **4** (36 μL, 0.36 mmol) in DMF (100 μL) was added, and the reaction mixture was stirred at room temperature for 3 h under argon. The mixture was poured into ice-cooled 0.1 N HCl and extracted with ether. The organic phase was washed with water, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was subjected to silica gel chromatography eluted stepwise with *n*-hexane and ethyl acetate [10% (vol/vol) increments]. The 20% ethyl acetate eluate containing crude MeCLA **3a** was purified by a semipreparative Inertsil SIL-100A HPLC (ϕ 10 × 250 mm, 5 μm; GL Sciences), using isocratic elution with 5% ethanol in *n*-hexane at a flow rate of 4.0 mL/min and monitored at 320 nm to give MeCLA **3a** (5.1 mg, 0.015 mmol, 6.3%, *Rt* 11.5 min). **MeCLA (3a)** $^1$H-NMR ($CDCl_3$, 400 MHz) δ: 1.000 (3H, s, $CH_3$-16 or 17), 1.003 (3H, s, $CH_3$-16 or 17), 1.43–1.47 (2H, m, H-2), 1.57–1.62 (2H, m, H-3), 1.71 (3H, d, *J* = 0.97 Hz, $CH_3$-18), 1.99 (2H, br t, *J* = 5.9 Hz, H-4), 2.02 (3H, t, *J* = 1.6 Hz, $CH_3$-15), 3.76 (3H, s, -COOMe), 6.08 (1H, d, *J* = 16.8 Hz, H-8), 6.14 (1H, t, *J* = 1.5 Hz, H-11), 6.79 (1H, br. d, *J* = 16.8 Hz, H-7), 6.95 (1H, t, *J* = 1.6 Hz, H-12), 7.50 (1H, s, H-10); $^{13}$C-NMR ($CDCl_3$, 100 MHz) δ: 10.7, 19.2, 21.6, 28.82, 28.84, 32.9, 34.1, 39.5, 51.7, 100.4, 113.0, 121.6, 129.1, 132.7, 135.5, 138.3, 141.3, 151.2, 167.1, 170.6; EI-MS *m/z* (rel. int): 346 $[M]^+$ (22), 249 (52), 217 (100), 189 (59), 161 (47), 147 (73), 121 (55), 97 (71); HRESIMS *m/z*: 347.1850 $[M+H]^+$ (calcd. for $C_{20}CH_{27}O_5$, *m/z* 347.1853).

***(2E,3E)-Methyl 2-(deuterio(4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy) methylene)-4-(2,6,6-trim ethylcyclohex-1-enyl)but-3-enoate ([10-$^2H_1$]-MeCLA) (3b).*** To a suspension of sodium hydride (8.6 mg, 0.36 mmol) in DMF (250 μL) was added a solution of $C_{13}$-carboxylic acid methyl ester **20a** (53 mg, 0.24 mmol) in DMF (250 μL) at room temperature under argon. Then methyl deuterioformate (75 μL, 1.2 mmol) was added, and the mixture was stirred for 13.5 h. After cooling to 0 °C, (±)-4-bromo-2-methyl-2-buten-4-olide **4** (36 μL, 0.36 mmol) in DMF (100 μL) was added, and the reaction mixture was stirred at room temperature for 3 h under argon. The mixture was poured into ice-cooled 0.1 N HCl and extracted with ether. The organic phase was washed with water, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was subjected to silica gel chromatography eluted stepwise with *n*-hexane and ethyl acetate [10% (vol/vol) increments]. The 20% ethyl acetate eluate containing crude [10-$^2H_1$]-MeCLA **3b** was purified by a semipreparative Inertsil SIL-100A HPLC (ϕ 10 × 250 mm, 5 μm; GL Sciences), using isocratic elution with 5% ethanol in *n*-hexane at a flow rate of 4.0 mL/min and monitored at 320 nm to give [10-$^2H_1$]-MeCLA **3b** (5.0 mg, 0.014 mmol, 5.8%, *Rt* 11.5 min). **[10-$^2H_1$]-MeCLA** EI-MS *m/z* (rel. int): 347 $[M]^+$ (22), 250 (46), 218 (100), 190 (55), 162 (37), 147 (50), 121 (54), 97 (56); HRESIMS *m/z*: 348.1913 $[M+H]^+$ (calcd. for $C_{20}CH_{27}D_1O_5$, *m/z* 348.1916).


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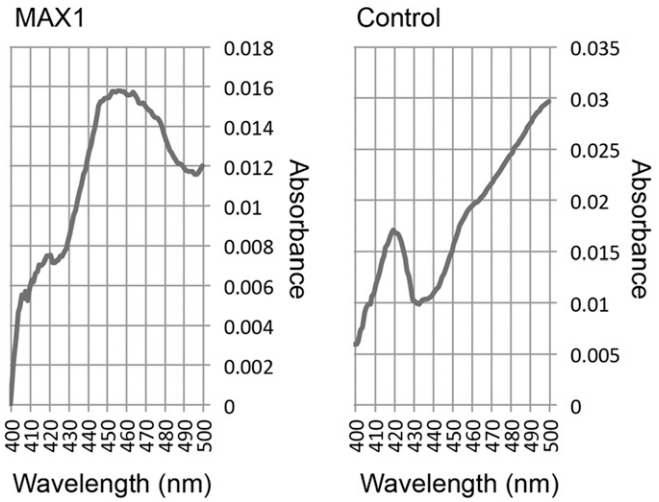


**Fig. S1.** Carbon monoxide difference spectra of recombinant MAX1. Microsomal proteins of yeast expressing MAX1 or having an empty vector (control) were used for spectrophotometric analysis.

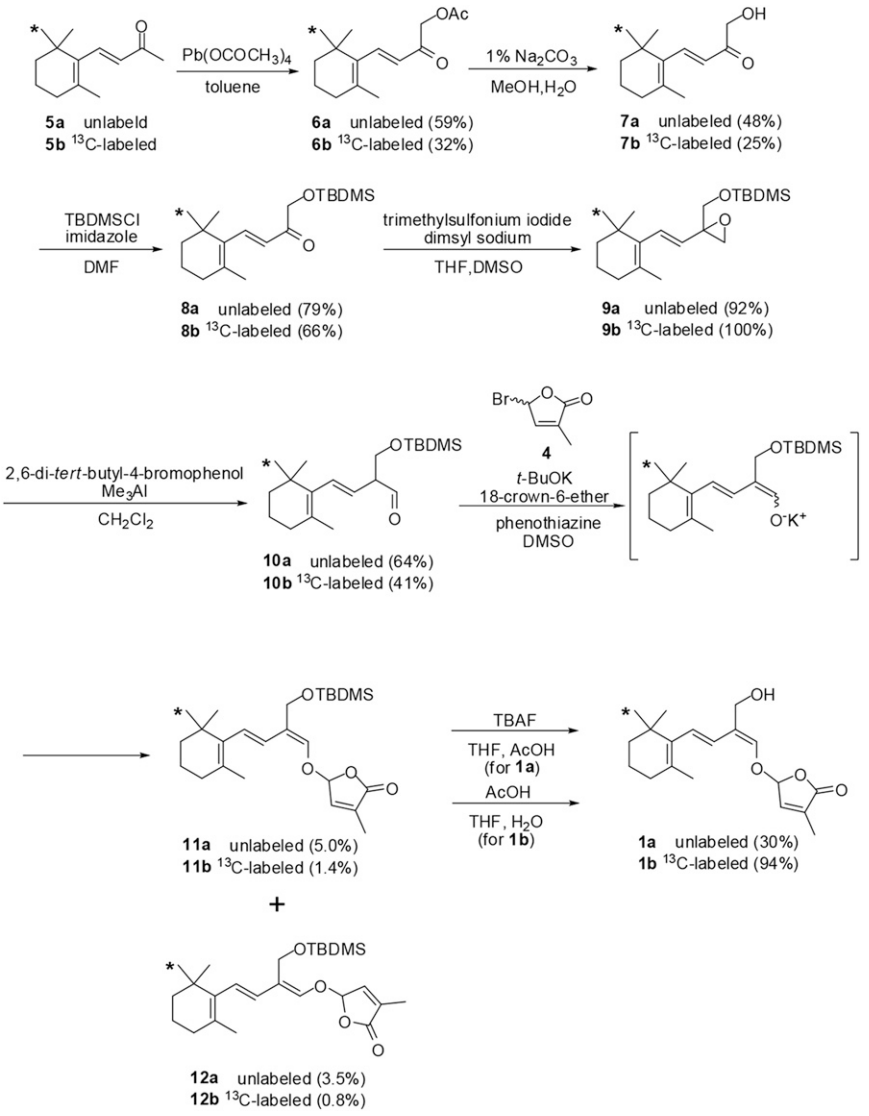


**Fig. S2.** Synthetic scheme of $^{13}$C-labeled and unlabeled 19-hydroxy-CL.

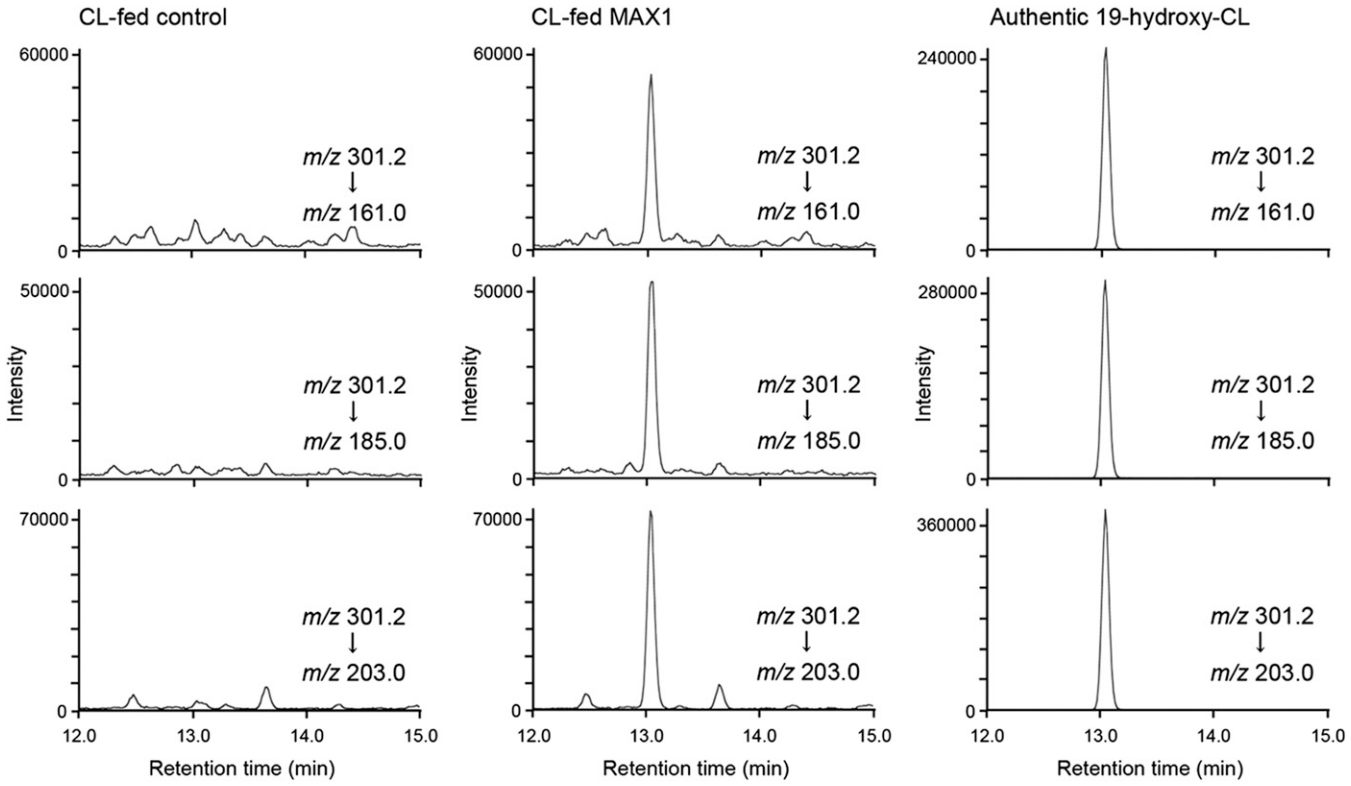


**Fig. S3.** 19-Hydroxy-CL produced from CL by recombinant MAX1. *rac*-CL was incubated with MAX1 and control microsomes. The extracts of the microsomes and authentic 19-hydroxy-CL were analyzed by ESI positive mode LC-MS/MS (QTRAP). SRM chromatograms of 19-hydroxy-CL are shown.

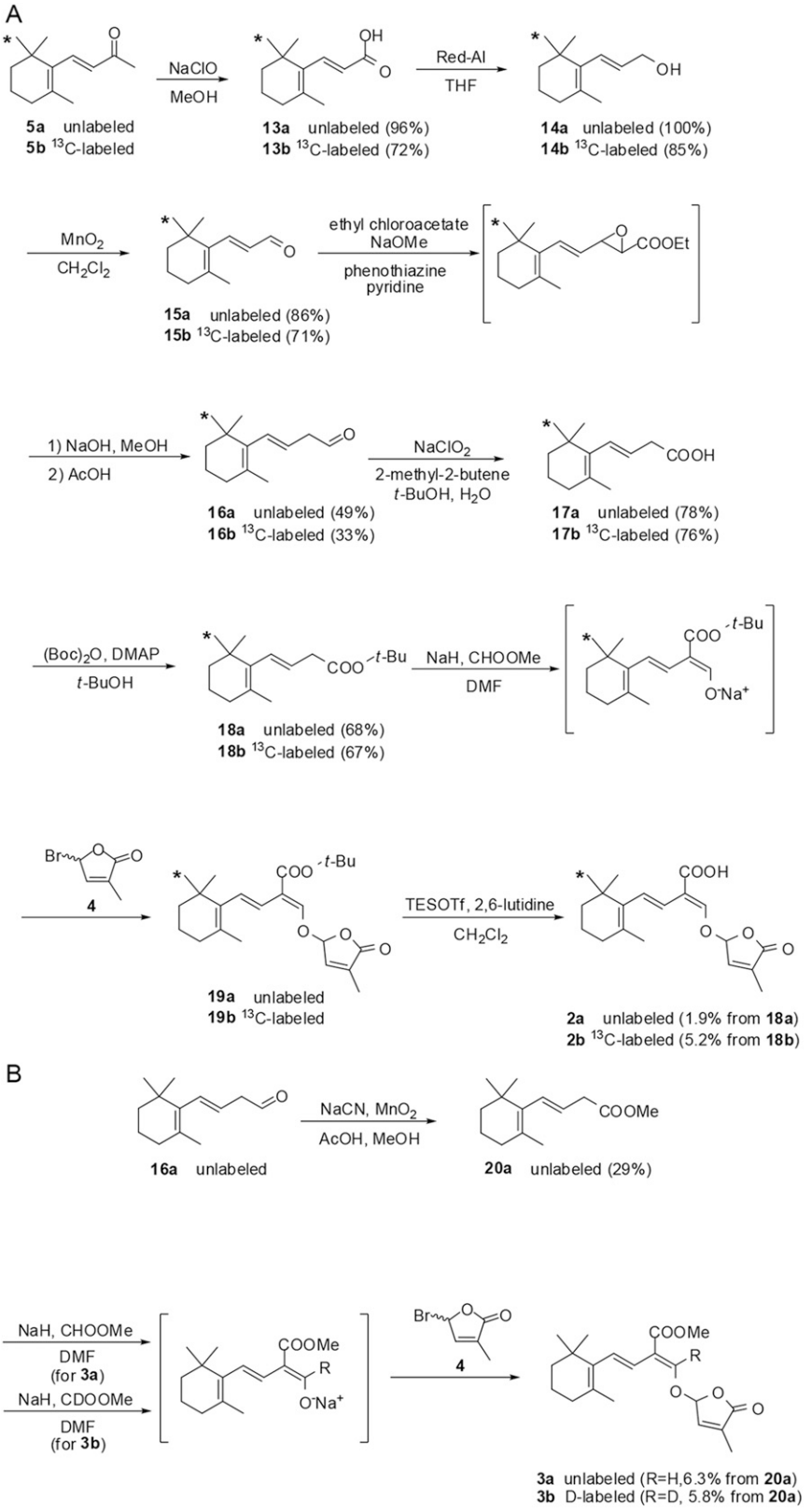


**Fig. S4.** Synthetic scheme of CLA and MeCLA. (*A*) $^{13}$C-labeled and unlabeled CLA. (*B*) $^{2}$H-labeled and unlabeled MeCLA.

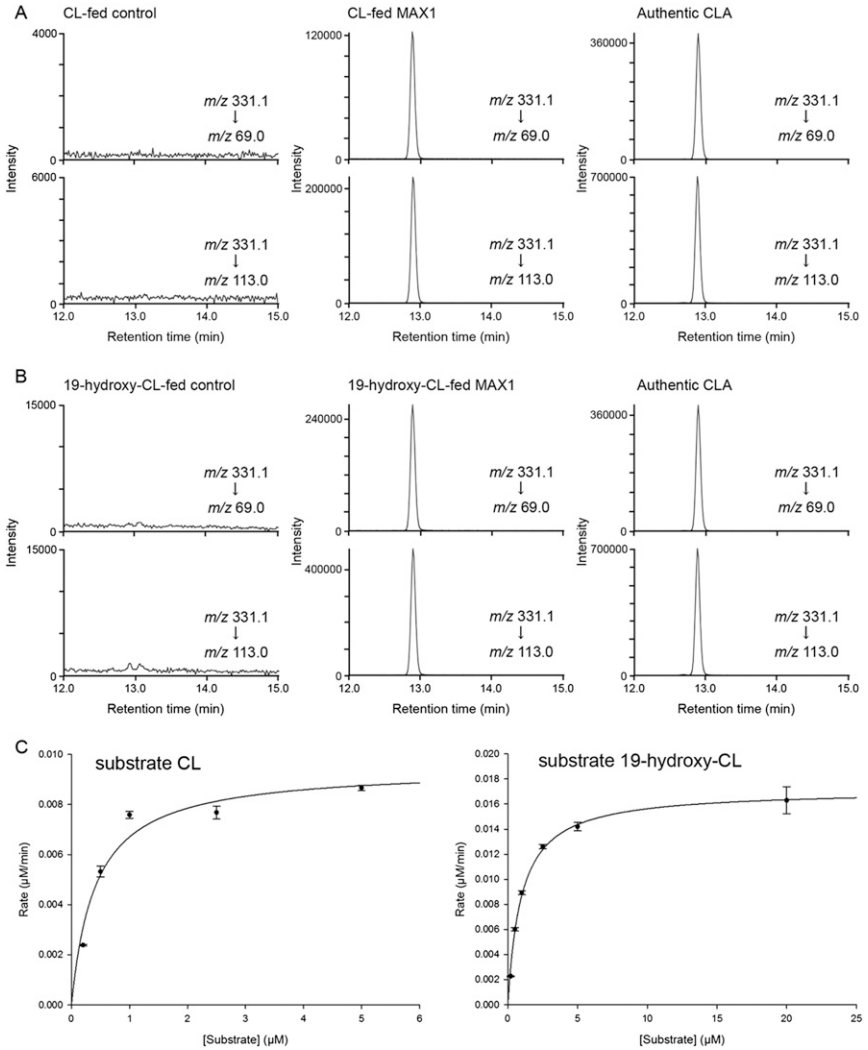


**Fig. S5.** CLA produced from CL and 19-hydroxy-CL by recombinant MAX1. *rac*-CL and *rac*-19-hydroxy-CL were incubated with MAX1 and control microsomes. The extracts of the microsomes and authentic CLA were analyzed by ESI negative mode LC-MS/MS (QTRAP). SRM chromatograms of CLA in (*A*) CL-fed and (*B*) 19-hydroxy-CL-fed samples are shown. (*C*) Reaction plots of CLA converted from CL and 19-hydroxy-CL are shown. The product CLA was quantified by LC-MS/MS (QTRAP) using [1-$^{13}CH_3$]*rac*-CLA as an internal standard. Michaelis–Menten equation was used to calculate kinetic parameter.

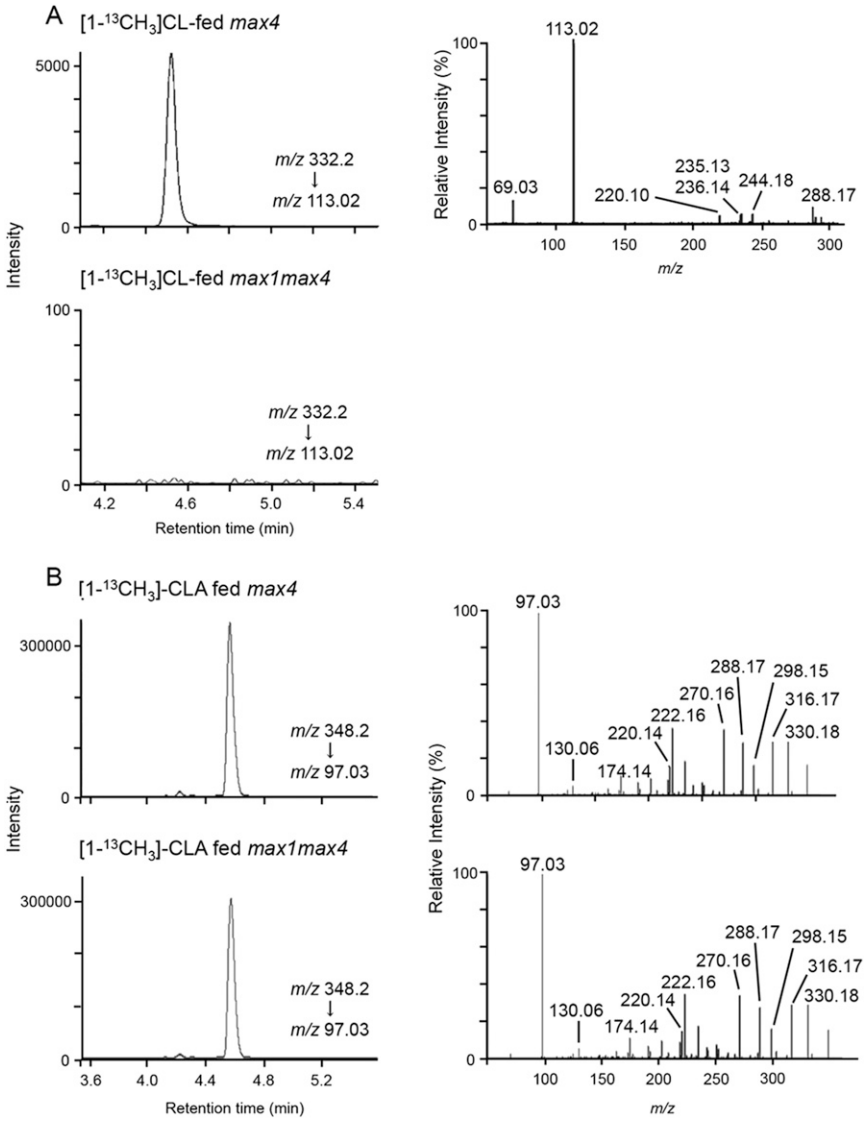


**Fig. S6.** Feeding of CL and CLA to *Arabidopsis* plants. [1-$^{13}CH_3$]11*R*-CL and [1-$^{13}CH_3$]*rac*-CLA were fed to the *max4* mutant and the *max1max4* double mutant, and their respective metabolites in roots were analyzed using LC-MS/MS (QTOF). (*A*) Ion traces from LC-MS/MS analysis (*Left*) of [$^{13}C_1$]-labeled CLA in [1-$^{13}CH_3$] 11*R*-CL-fed *max4* and *max1max4* are shown. The product ion spectrum (*Right*) of [$^{13}C_1$]-labeled CLA obtained from *max4* is shown. (*B*) Ion traces from LC-MS/MS analysis (*Left*) and product ion spectra (*Right*) of [$^{13}C_1$]-labeled MeCLA in [1-$^{13}CH_3$]CLA-fed *max4* and *max1max4* are shown.

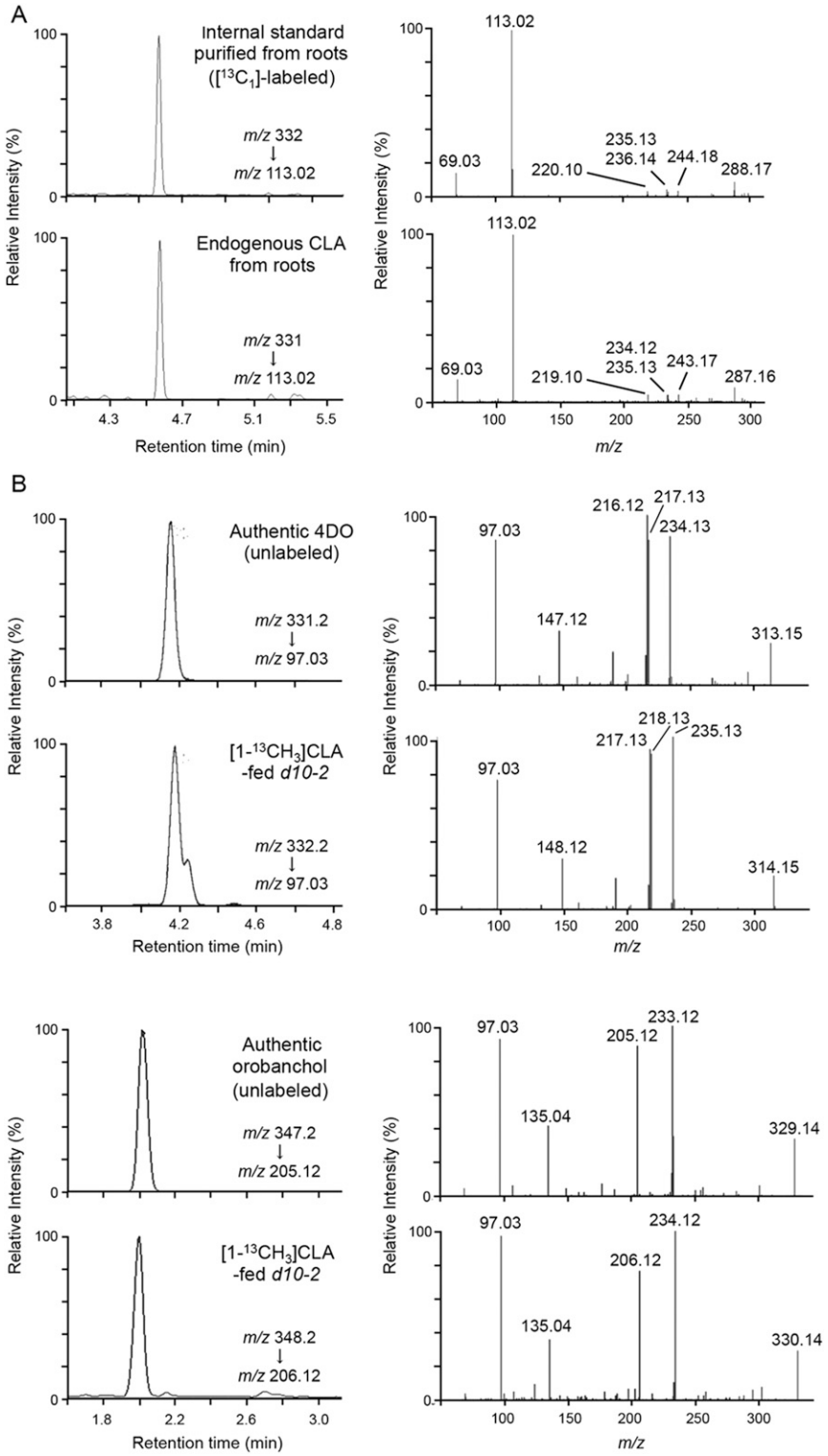


**Fig. S7.** Endogenous and exogenous analysis of CLA using rice plants. (*A*) Endogenous CLA in rice plants. Endogenous CLA was analyzed using rice Shiokari WT by LC-MS/MS (QTOF). Ion traces from LC-MS/MS analysis (*Left*) and product ion spectra (*Right*) of [1-$^{13}$C]CLA (internal standard) and endogenous CLA extracted from roots are shown. (*B*) Feeding of CLA to rice plants. CLA was fed to the rice *d10-2* mutant. [1-$^{13}CH_3$]CLA was added to culture media, and the metabolites were analyzed in root exudates using LC-MS/MS (QTOF). Ion traces from LC-MS/MS analysis (*Left*) and product ion spectra (*Right*) of [$^{13}C_1$]-labeled 4DO and [$^{13}C_1$]-labeled orobanchol extracted from root exudates and of those unlabeled authentic standards are shown.

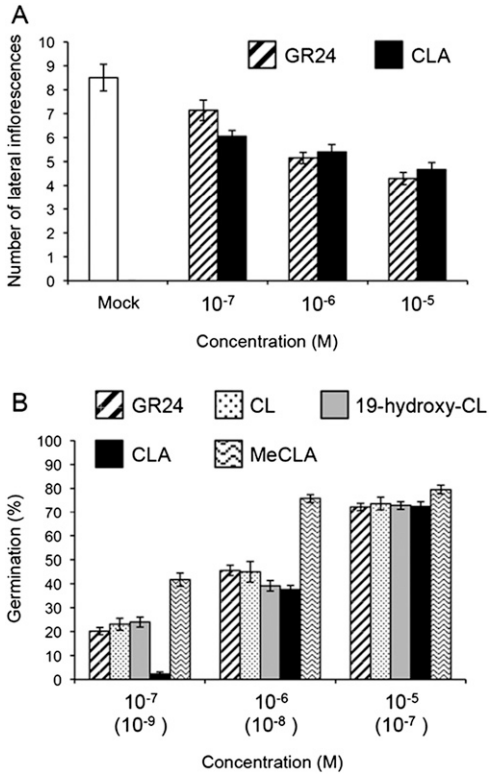


**Fig. S8.** Dose–responses of CL derivatives on bioassays. (*A*) Inhibitory effects of CLA and GR24 on increased lateral inflorescences of the *Arabidopsis max1* mutant. The solutions of each concentration were applied onto *max1* plants for 2 wk, and then the number of lateral inflorescences from rosette leaves was counted. The 0.5% (vol/vol) acetonitrile solution without authentic sample was used as a mock treatment. Values are mean ± SEM ($n = 15$). (*B*) Effects of CL, 19-hydroxy-CL, CLA, MeCLA, and GR24 on germination of *Orobanche minor* seeds. The solutions of each concentration were treated for 5 d, and then the germinated seeds were counted (~30 seeds per each solution were incubated). The concentration of GR24 is expressed in parentheses. Values are mean ± SEM ($n = 3$).

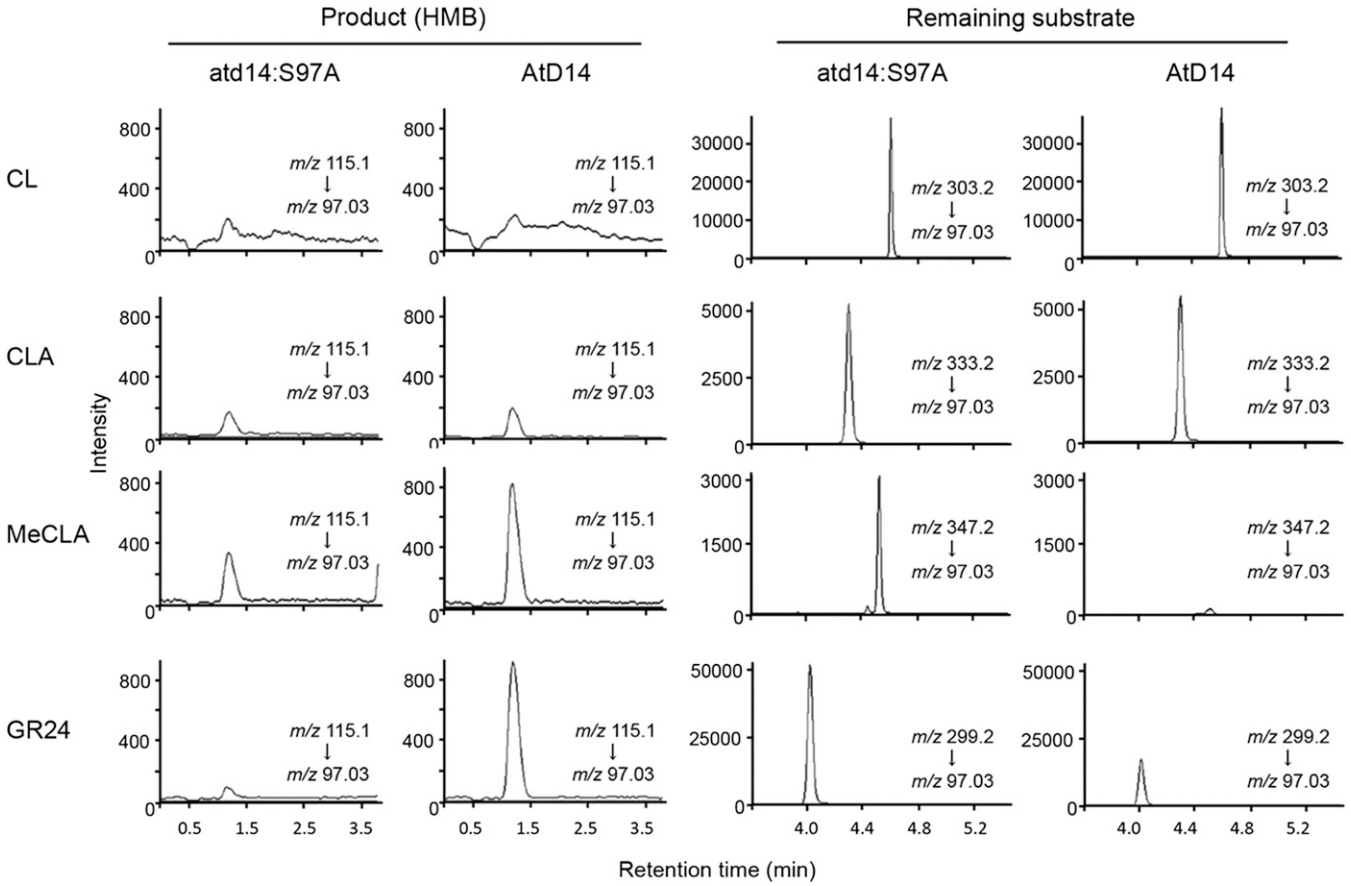


**Fig. S9.** Hydrolase activity of AtD14 on CL derivatives. Hydrolase activity of AtD14 was examined using a racemic mixture of CL, CLA, and MeCLA as a substrate. GR24 was used as a positive substrate. The active site mutant atd14:S97A was used as a negative control. A reaction product, hydroxymethylbutenolide (HMB), and each remaining substrate were analyzed by LC-MS/MS (QTOF). Ion traces from LC-MS/MS analysis are shown.