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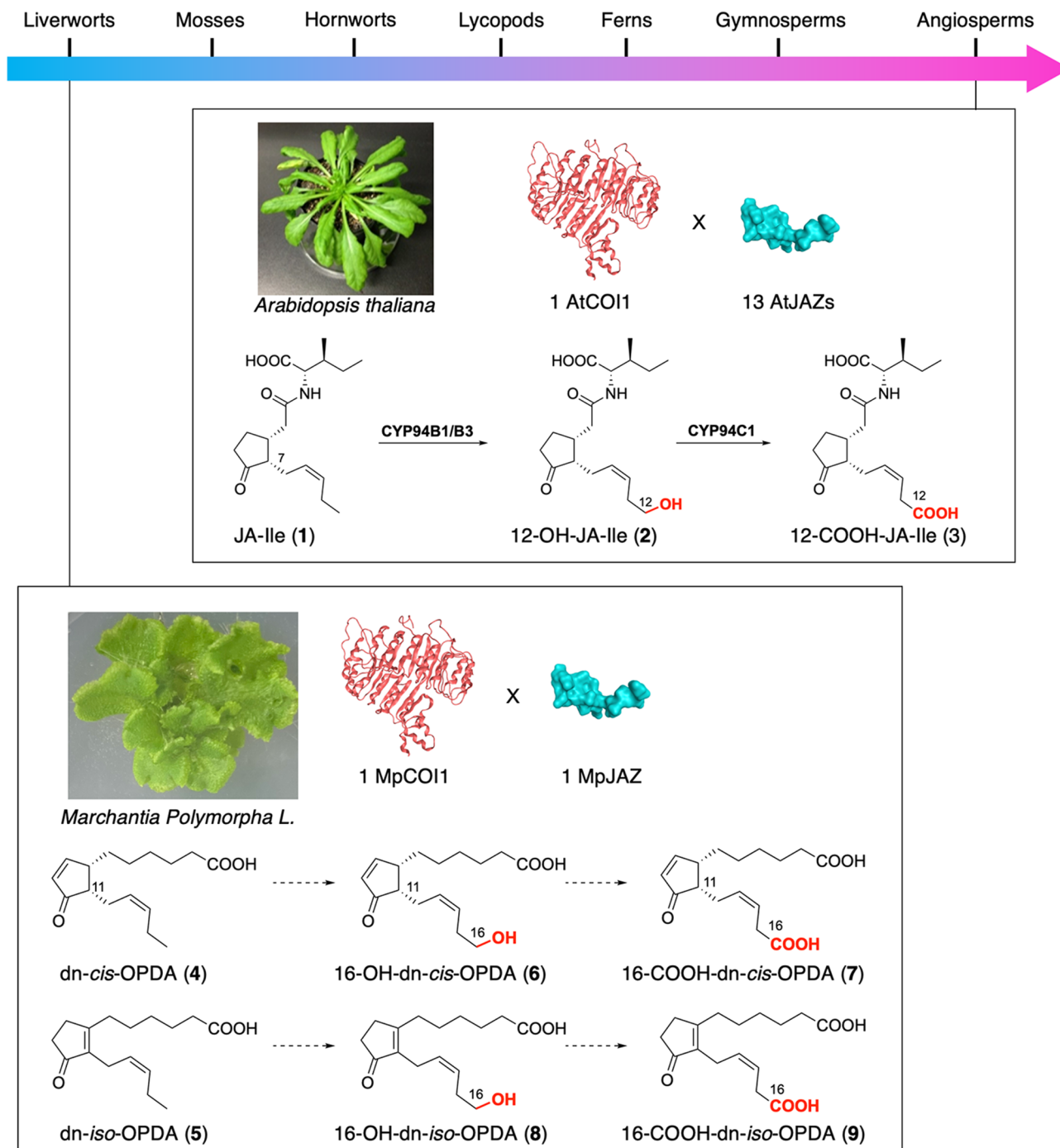
## Syntheses of dinor-*cis/iso*-12-oxo-phytodienoic acid (dn-*cis/iso*-OPDAs), ancestral jasmonate phytohormones of the bryophyte *Marchantia polymorpha* L., and their catabolites

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In recent years, the biology of the evolutionary origin of phytohormone signaling has made significant progress. Among them, the ligand-receptor co-evolution found in jasmonate signaling has attracted the attention of plant scientists. Dinor-*cis*-12-oxo-phytodienoic acid (dn-*cis*-OPDA, **4**) and dn-*iso*-OPDA (**5**) are ancestral plant hormones of the bryophyte *Marchantia polymorpha* L. We succeeded in the first practical synthetic supply of these hormones as well as their possible catabolites. These compounds are expected to be useful in the study of ancestral jasmonate signaling in bryophytes.

(+)-Jasmonoyl-L-isoleucine (JA-Ile, **1**) is a lipid-derived plant phytohormone, implicated in the regulation of plant growth, fertility, and defense against pathogens and insects<sup>1–3</sup>. JA-Ile-mediated signal transduction depends on the COI1-JAZ co-receptor system, composed of an F-box protein CORONATINE INSENSITIVE 1 (COI1) and JASMONATE ZIM-DOMAIN (JAZ) repressor protein<sup>4–6</sup>. After signal transduction, **1** is catabolized into 12-hydroxy-JA-Ile (12-OH-JA-Ile, **2**) by CYP94B1/B3, then 12-carboxy-JA-Ile (12-COOH-JA-Ile, **3**) by CYP94C1, and then deactivated within a few hours (Fig. 1)<sup>7–9</sup>. Biological studies of the evolution of phytohormone signaling is an important topic in plant science<sup>10–13</sup>, and recent achievements in the genomic sequencing of a myriad of plant species have enabled research into the evolutionary origins of phytohormones. *Marchantia polymorpha* L., a type of bryophyte, has attracted a great deal of attention due to its ancestral signaling module<sup>14</sup>, and its jasmonate signaling constitutes an intriguing example of ligand-receptor co-evolution, depending on the MpCOI1-MpJAZ co-receptor system (Fig. 1)<sup>15</sup>. However, JA-Ile, the usual ligand for the COI1-JAZ co-receptor system of vascular plants, cannot be perceived by MpCOI1-MpJAZ—dinor-*cis*-12-oxo-phytodienoic acid (dn-*cis*-OPDA, **4**) and dn-*iso*-OPDA (**5**) are the ligands of MpCOI1-MpJAZ co-receptor instead (Fig. 1). Genetic studies have revealed that the ligand-receptor pair of dn-*cis/iso*-OPDA and MpCOI1-MpJAZ participates in all the jasmonate responses of *M. polymorpha*, including defense responses<sup>16–18</sup>. However, biological studies require samples of **4** and **5**—in the past, **4** has been synthesized enzymatically<sup>19</sup> but is now out of stock; and the only known synthesis of **5** entails an electroorganic reaction<sup>20</sup> that cannot be accomplished using normal laboratory equipment (Scheme S1). Accordingly, we developed and report herein the first chemical synthesis of **4** and the first non-electroorganic synthesis of **5**. In addition, we synthesized their potent catabolites 16-hydroxy-dinor-*cis*-OPDA (16-OH-dn-*cis*-OPDA, **6**), 16-carboxy-dinor-*cis*-OPDA (16-COOH-dn-*cis*-OPDA, **7**), 16-hydroxy-dinor-*iso*-OPDA (16-OH-dn-*iso*-OPDA, **8**), 16-carboxy-dinor-*iso*-OPDA (16-COOH-dn-*iso*-OPDA, **9**) (Fig. 1). These potent catabolites will enable further studies on the catabolism of ancestral plant hormones.

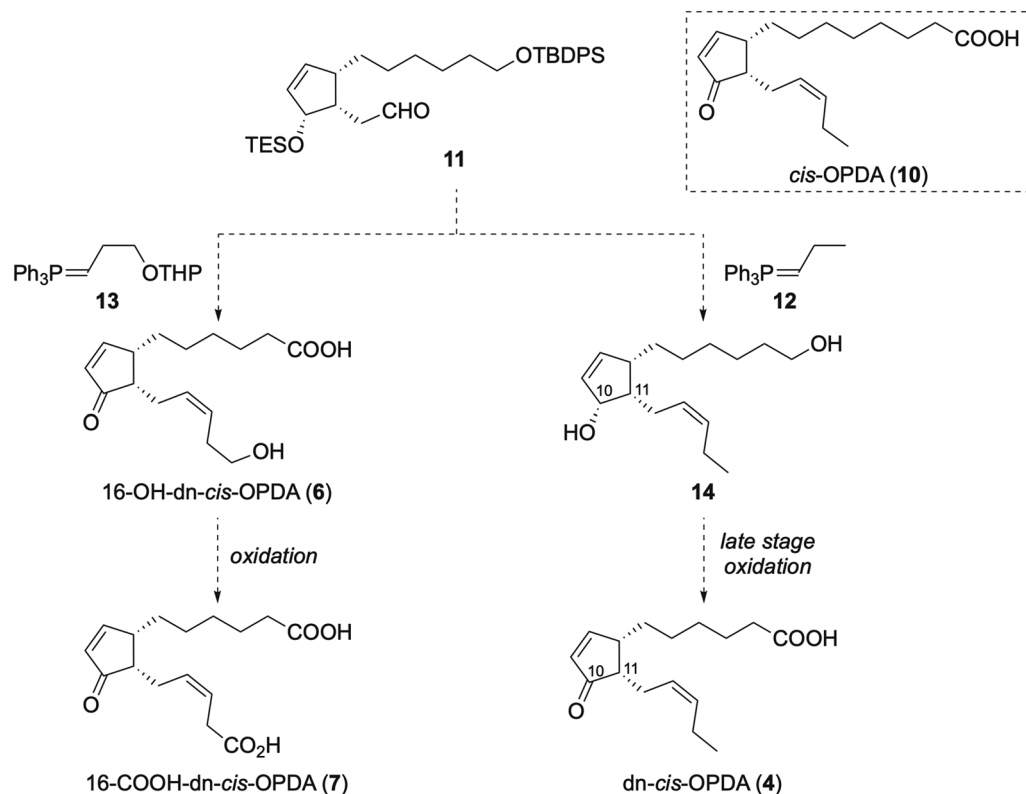
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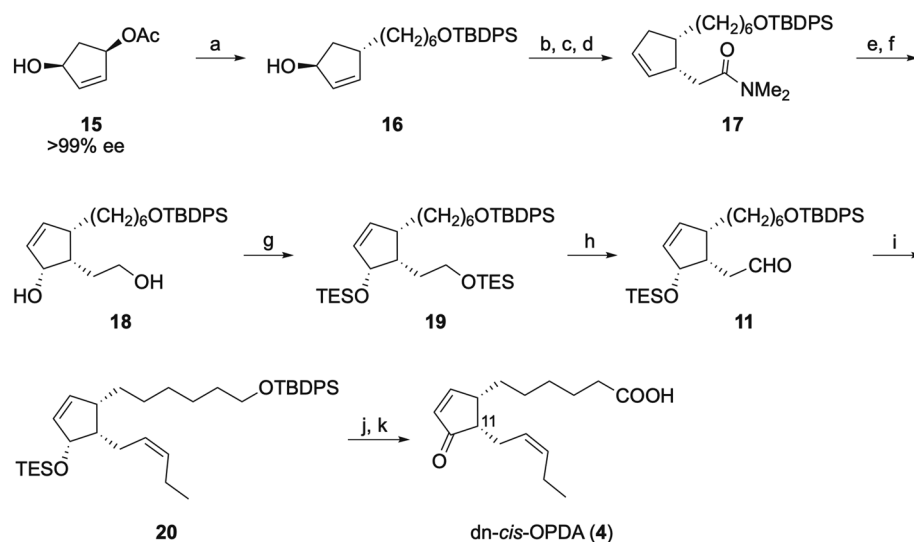
**Figure 1.** Ligand-receptor co-evolution of jasmonates, JA-Ile (1) in *Arabidopsis thaliana*, dn-*cis*-OPDA (4) and dn-*iso*-OPDA (5) in *Marchantia polymorpha* L., and their potent catabolites.

## Results and discussion

**Synthesis of dn-*cis*-OPDA (4) and its potent catabolites (6 and 7).** Our plan for the synthesis of dn-*cis*-OPDA (4) and its catabolites (6 and 7) is outlined in Scheme 1. A major initial concern in the syntheses of 4, 6 and 7 was the avoidance of epimerization at C11 which was anticipated to be facile in the presence of either acid or base based on the ready epimerization of the structurally similar 1 to give the more thermodynamically stable *trans*-1 in a ratio of *trans*:*cis* = 95:5<sup>21</sup>. We therefore planned to synthesize 4 according to a procedure similar to that used to synthesize OPDA (10), a congener of 4, developed by Kobayashi et al.<sup>22</sup>. They avoided epimerization by introducing the ketone at the late stage of synthesis<sup>23</sup>. Compounds 4 and 6 would be obtained from a common intermediate 11 by Wittig reaction using a different phosphonium salt (Scheme 1), and 7 would be obtained by oxidation of 6.

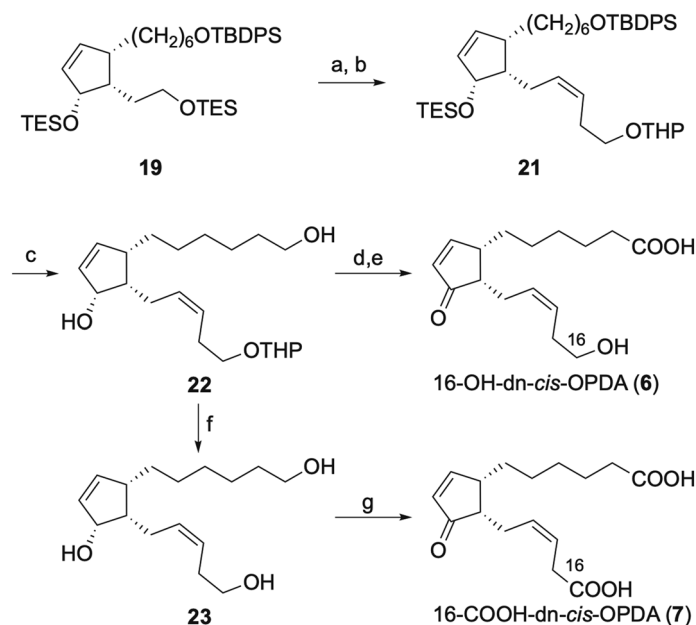


**Scheme 1.** Synthetic plan for dn-cis-OPDA (4) and its potent catabolites (6 and 7).



**Scheme 2.** Synthesis of dn-cis-OPDA (4). Reagents and conditions: (a) TBDPSO(CH<sub>2</sub>)<sub>6</sub>MgCl, CuCN, THF, -20 °C; (b) PPh<sub>3</sub>, AcOH, DIAD, toluene, -20 °C; (c) LiOH, THF, MeOH, H<sub>2</sub>O; (d) MeC(OMe)<sub>2</sub>NMe<sub>2</sub>, xylene, reflux, 50% (4 steps); (e) I<sub>2</sub>, buffer (pH 5.0), THF; (f) DBU, THF, reflux; LiAlH<sub>4</sub>, 78% (2 steps); (g) TESCl, imidazole, DMF, 92%; (h) (COCl)<sub>2</sub>, DMSO; NEt<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>; (i) [Ph<sub>3</sub>PPr]<sup>+</sup>Br<sup>-</sup>, NaHMDS, THF, 71% (2 steps); (j) TBAF, THF, reflux, 91%; (k) Jones reagent, acetone, -20 °C, 97%.

Our synthesis of dn-cis-OPDA (4) is summarized in Scheme 2. Allylic substitution of monoacetate 15, prepared by enzymatic reaction<sup>22</sup>, with TBDPSO(CH<sub>2</sub>)<sub>6</sub>MgCl, was performed in the presence of CuCN in THF, -20 °C. The Mitsunobu reaction of 16, hydrolysis of resulting acetate and Eschenmoser–Claisen rearrangement gave dimethylamide 17. Iodolactonization, elimination with DBU and subsequent reduction with LiAlH<sub>4</sub> afforded diol 18. In the iodolactonization step, use of water in place of buffer resulted in the removal of TBDPS group. TES



**Scheme 3.** Synthesis of 16-OH-dn-*cis*-OPDA (**6**) and 16-COOH-dn-*cis*-OPDA (**7**). Reagents and conditions: (a) (COCl)<sub>2</sub>, DMSO, NEt<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>; (b) [THPO(CH<sub>2</sub>)<sub>3</sub>PPh<sub>3</sub>]<sup>+</sup>Br<sup>-</sup>, NaHMDS, THF, 76% (2 steps); (c) TBAF, THF 72%; (d) Jones reagent, acetone, -20 °C, (e) MgBr<sub>2</sub>, Et<sub>2</sub>O, 72% (2 steps); (f) PPTS, MeOH, 35 °C, 54%; (g) Jones reagent, acetone, -20 °C, 73%.

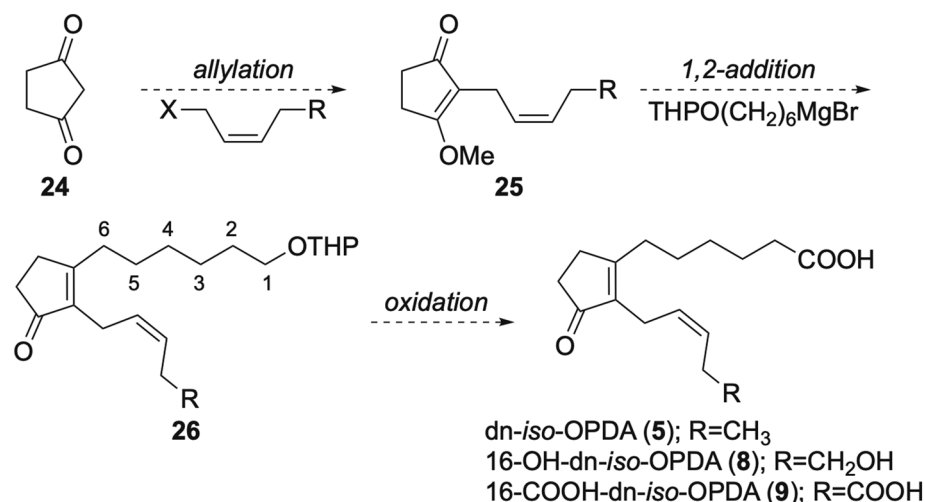
protection of diol **18** and regioselective Swern oxidation of the primary TESOCH<sub>2</sub> group<sup>24</sup> afforded the common intermediate **11**. In pursuit of **4**, **11** was treated with [Ph<sub>3</sub>PPr]<sup>+</sup>Br<sup>-</sup> and NaHMDS to give diene **20**. Deprotection of the silyl groups of **20** with TBAF and subsequent Jones oxidation at -20 °C afforded dn-*cis*-OPDA (**4**, 15 mg in 12 steps and 22% overall yield from **15**). In the work up of final Jones oxidation, removal of chromium compounds and sulfuric acid using silica gel caused epimerization of C11 (probably due to exothermic adsorption of sulfuric acid on silica gel)<sup>22</sup>, but this could be easily avoided by removal of the inorganic substances with water instead. The obtained **4** was quite stable at room temperature under neutral condition and no epimerization at C11 was observed even after 2 weeks<sup>25–27</sup>.

For the syntheses of 16-OH-dn-*cis*-OPDA (**6**) and 16-COOH-dn-*cis*-OPDA (**7**), [THPO(CH<sub>2</sub>)<sub>3</sub>PPh<sub>3</sub>]<sup>+</sup>Br<sup>-</sup> was used in the Wittig reaction with common intermediate **11**, leading to diene **21**. Deprotection of the silyl groups of **21** with TBAF afforded diol **22**, from which the target products **6** and **7** could be obtained by oxidation and deprotection, or vice versa (Scheme 3). Jones oxidation of **22** and subsequent THP deprotection using MgBr<sub>2</sub> afforded 16-OH-dn-*cis*-OPDA (**6**, 41 mg in 13 steps and 14% overall yield from **15**). In the THP deprotection step, some epimerization of C11 took place (*cis:trans* = ca. 94:6 by NMR). This epimerization and observed diastereomeric ratio were in good accordance with previous literature (*cis:trans* = ca. 92:8 in NMR)<sup>22</sup>. *cis*- and *trans*-**6** were easily separated by chiral HPLC using a CHIRALPAK IA column to obtain pure *cis*-**6** (22.3 mg). Conversely, THP deprotection of **22** using PPTS afforded triol **23**, Jones oxidation of which afforded 16-COOH-dn-*cis*-OPDA (**7**, 8.3 mg in 13 steps and 7.5% overall yield from **15**).

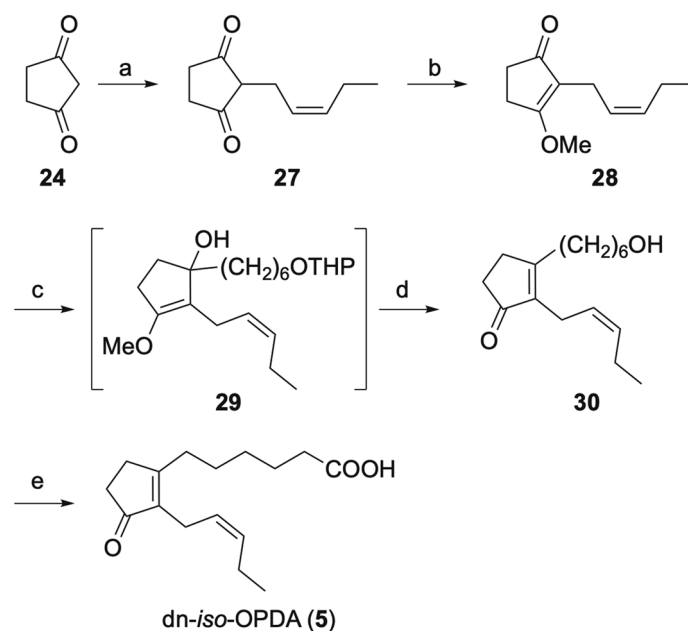
**Synthesis of dn-*iso*-OPDA (**5**) and its potent catabolites (**8** and **9**).** Our plan for the synthesis of dn-*iso*-OPDA (**5**) and its potent catabolites (**8** and **9**) is shown in Scheme 4. In the synthesis of [<sup>2</sup>H<sub>2</sub>]-tetrahydrodicanenone (*iso*-OPDA), Lauchli and Boland introduced the C1–8 side chain by the 1,4-addition using an organozinc reagent and CuCN<sup>28</sup>. However, organozinc reagent are difficult to prepare and CuCN is highly toxic. In contrast, Grignard reagents used for the 1,2-addition are easy to prepare and less toxic than CuCN. And potent catabolites 16-OH-dn-*iso*-OPDA (**8**) and 16-COOH-dn-*iso*-OPDA (**9**) could be obtained from the same starting material **24** by using a different allyl bromide.

Our synthesis of dn-*iso*-OPDA (**5**) is summarized in Scheme 5. Allylation of 1,3-cyclopentanone **24** followed by methylation of the resulting **27** gave cyclopentenone **28**. After the Grignard reaction of **28**, dilution of the reaction mixture with hydrochloric acid promoted hydrolysis of the enol ether and the deprotection of the THP group, to give alcohol **30**. Finally, Jones oxidation of **30** gave 61 mg of dn-*iso*-OPDA (**5**) in only 4 steps from **24**.

Next, we synthesized 16-OH-dn-*iso*-OPDA (**8**) and 16-COOH-dn-*iso*-OPDA (**9**) (Schemes 6, 7). Introduction of the C12–C16 side chain of **24** was first attempted by allylation, but the side chain could not be directly introduced using allylation in order to O-alkylation. Accordingly, we abandoned this approach and sought to construct the C12–C16 side chain by *Z*-selective cross metathesis<sup>29–31</sup>. Pd-mediated allylation<sup>32</sup> of **24** and subsequent methylation gave allylcyclopentenone **32**. Introduction of the C1–C6 side chain using THPO(CH<sub>2</sub>)<sub>6</sub>MgBr followed by hydrolysis and deprotection of THP group gave alcohol **33**. Finally, Jones oxidation of **33** followed by



**Scheme 4.** Synthetic plan for *dn-iso-OPDA* (5) and its potent catabolites (8 and 9).

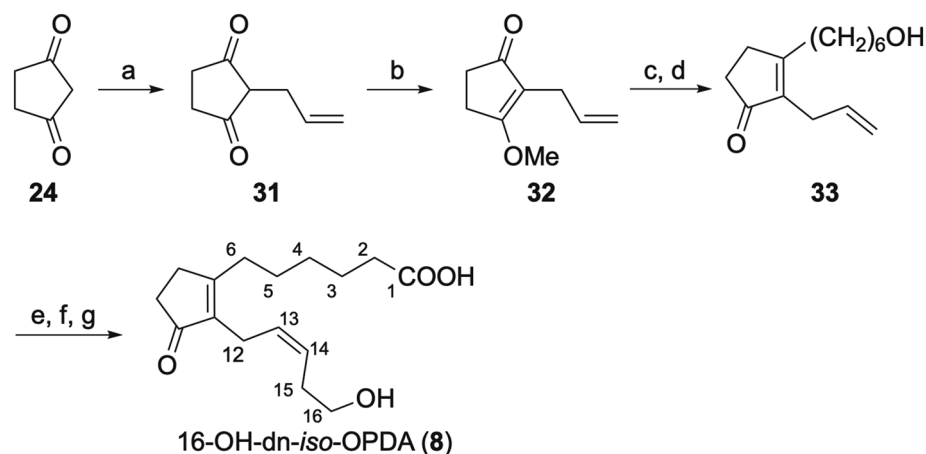


**Scheme 5.** Synthesis of *dn-cis-OPDA* (5). *Reagents and conditions:* (a) *cis*-1-bromopent-2-ene, K<sub>2</sub>CO<sub>3</sub>, H<sub>2</sub>O, 60 °C, 25%; (b) Me<sub>2</sub>SO<sub>4</sub>, K<sub>2</sub>CO<sub>3</sub>, acetone, reflux, quant.; (c) THPO(CH<sub>2</sub>)<sub>6</sub>MgBr, THF, reflux (d) 2 M HCl aq., 24%; (e) Jones reagent, acetone, 0 °C, 89%.

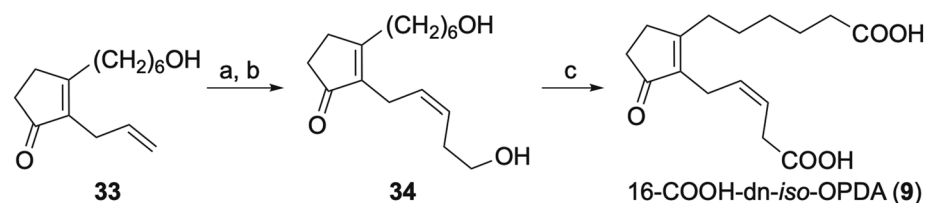
cross metathesis with CH<sub>2</sub>=CHCH<sub>2</sub>CH<sub>2</sub>OAc and deprotection of the acetyl group gave 16-OH-*dn-iso-OPDA* (8, 15.6 mg in 7 steps and 11% overall yield from 24) as a *Z/E* mixture (10:1 *Z/E*). *Z/E* isomers were easily separated by chiral HPLC using a CHIRALPAK IA column to obtain pure *Z*-8 (2.4 mg). 16-COOH-*dn-iso-OPDA* (9, 12:1 *Z/E*, 6.3 mg) was also obtained from allylcyclopentenone 33 by cross metathesis with CH<sub>2</sub>=CHCH<sub>2</sub>CH<sub>2</sub>OAc, hydrolysis of acetyl group and Jones oxidation (total 7 steps and 3.4% overall yield from 24). *Z/E* isomers were easily separated by HPLC to obtain pure *Z*-9 (2.6 mg).

## Conclusions

A synthetic supply of jasmonates is indispensable if their study is to be advanced<sup>20,30,33,34</sup>, and this work finally enables *dn-cis/iso-OPDAs* (4 and 5) and their potent catabolites (6–9) to be readily obtained. Our work is expected to accelerate biological studies of the signaling mechanisms of bryophyte hormones, which should lead to a better understanding of the evolutionary origins of phytohormone signaling. In particular, study of the catabolism of 4 and 5 should provide insights into the deactivation mechanism of ancestral plant hormones. Biological studies using synthetic 4–9 are now in progress.



**Scheme 6.** Synthesis of 16-OH dn-*iso*-OPDA (**8**). Reagents and conditions: (a) allyl acetate, [Pd(C<sub>3</sub>H<sub>5</sub>)Cl]<sub>2</sub>, dppe, BSA, NaOAc, THF, reflux, 81%; (b) Me<sub>2</sub>SO<sub>4</sub>, K<sub>2</sub>CO<sub>3</sub>, acetone, reflux, 98%; (c) THPO(CH<sub>2</sub>)<sub>6</sub>MgBr, THF, reflux; (d) 1 M HCl aq., reflux, 36% (2 steps); (e) Hoveyda–Grubbs catalyst M2001 Umicore, CH<sub>2</sub>=CHCH<sub>2</sub>CH<sub>2</sub>OAc; (f) Jones reagent, acetone, –20 °C; (g) LiOH, H<sub>2</sub>O, THF, 40% (3 steps).



**Scheme 7.** Synthesis of 16-COOH-dn-*iso*-OPDA (**9**). Reagents and conditions: (a) Hoveyda-Grubbs Catalyst M2001 Umicore, CH<sub>2</sub>=CHCH<sub>2</sub>CH<sub>2</sub>OAc. (b) NaOH, H<sub>2</sub>O, MeOH, 50 °C 32% (2 steps). (c) Jones reagent, acetone, –20 °C, 36%.

## Method

**Synthesis of dn-*cis*-OPDA (**4**).** To a solution of **20** (57.2 mg, 94.5 μmol) in THF (20 mL) was added 1 M TBAF in THF (1.6 mL, 1.60 mmol). After being stirred at reflux temperature for 1.5 h, the solvent was removed under reduced pressure. The residue was purified by medium-pressure chromatography (Isolera, eluent: 93:7 *n*-hexane/EtOAc to 40:60 *n*-hexane/EtOAc) to give a diol intermediate (22.3 mg, 94%) as a colorless oil. [α]<sub>D</sub><sup>22</sup> + 63.6 (c 1.09, CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ<sub>H</sub>: 6.22 (dd, *J* = 5.8, 2.6 Hz, 1H), 5.96 (brd, *J* = 5.8 Hz, 1H), 5.49–5.38 (m, 2H), 4.51 (dd, *J* = 5.8, 2.6 Hz, 1H), 3.63 (t, *J* = 6.6 Hz, 2H), 2.52–2.43 (m, 1H), 2.37–2.27 (m, 1H), 2.24–2.04 (m, 4H), 1.66–1.52 (m, 3H), 1.47–1.20 (m, 7H), 0.99 (t, *J* = 7.5 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ<sub>C</sub>: 141.64, 132.46, 132.01, 127.88, 76.55, 62.99, 46.13, 46.00, 33.52, 32.73, 29.70, 28.05, 25.69, 23.08, 20.78, 14.26; IR (neat) cm<sup>-1</sup>: 3356, 2931, 2861, 1458, 1053; HRMS (ESI, positive) *m/z* [M + Na]<sup>+</sup> Calcd. for C<sub>16</sub>H<sub>28</sub>NaO<sub>2</sub>; 275.1987, Found: 275.1976.

To a solution of diol intermediate (15.3 mg, 60.6 μmol) in acetone (5.2 mL) was added Jones reagent (4.0 M solution) at –20 °C until the orange color of the reagent persisted (30 drops). After 10 min of stirring at –20 °C, *i*-PrOH was added to quench the remaining reagent. Then, EtOAc/*n*-hexane (1/1, 20 mL) and H<sub>2</sub>O (20 mL) were added and the water layer was extracted with EtOAc. The combined organic layers were washed with saturated aqueous NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The residue was purified by medium-pressure chromatography (Isolera, eluent: 0.1:88:12 AcOH/*n*-hexane/EtOAc to 0.1:99.9 AcOH/EtOAc) to give **4** (116 mg, 97%) as a colorless oil. Diastereomeric purity of **4** was >99% by <sup>1</sup>H NMR spectroscopy (δ<sub>H</sub> = 7.72 (dd, *J* = 5.8, 2.7 Hz, 1H) for **4**; 7.59 (dd, *J* = 5.8, 2.6 Hz, 1H) for the *trans* isomer). [α]<sub>D</sub><sup>22</sup> + 135.5 (c 0.75, CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ<sub>H</sub>: 7.72 (dd, *J* = 5.8, 2.7 Hz, 1H), 6.18 (dd, *J* = 5.8, 1.6 Hz, 1H), 5.48–5.31 (m, 2H), 3.04–2.94 (m, 1H), 2.56–2.41 (m, 2H), 2.36 (brt, *J* = 7.1 Hz, 2H), 2.13 (dt, *J* = 15.8, 7.8 Hz, 1H), 2.05 (quintet, *J* = 7.5 Hz, 2H), 1.80–1.69 (m, 1H), 1.63 (brt, *J* = 7.1 Hz, 2H), 1.52–1.29 (m, 4H), 1.23–1.11 (m, 1H), 0.97 (t, *J* = 7.5 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ<sub>C</sub>: 210.86, 179.32, 166.96, 133.02, 132.50, 126.82, 49.79, 44.20, 33.84, 30.58, 29.18, 27.32, 24.49, 23.76, 20.78, 13.99; IR (neat) cm<sup>-1</sup>: 3487, 3178, 2935, 1709; HRMS (ESI, positive) *m/z* [M + Na]<sup>+</sup> Calcd. for C<sub>16</sub>H<sub>24</sub>NaO<sub>3</sub>; 287.1623, Found: 287.1618.

**Synthesis of 16-OH-dn-*cis*-OPDA (**6**).** To a solution of **22** (71.0 mg, 201 μmol) in acetone (20 mL) was added Jones reagent (4.0 M solution, 400 μL, 1.60 mmol) at –20 °C. After 20 min of stirring at –20 °C, *i*-PrOH was added to quench the remaining reagent. Then, EtOAc/*n*-hexane (1/1, 10 mL) and H<sub>2</sub>O (20 mL) were added



and the water layer was extracted with EtOAc. The combined organic layers were washed with saturated aqueous NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and filtered. The reaction mixture was concentrated under reduced pressure to give a carboxylic acid intermediate (86.3 mg, mixture) as a colorless oil. The crude product was used for the next reaction without further purification. To a solution of the carboxylic acid intermediate (86.3 mg, mixture) in Et<sub>2</sub>O (10 mL) was added MgBr<sub>2</sub> (90.5 mg, 510 μmol). The solution was stirred at room temperature for 20 min and diluted with Et<sub>2</sub>O and MeOH. Then, H<sub>2</sub>O was added and the water layer was extracted with AcOH/EtOAc (1/999). The combined organic layers were washed with saturated aqueous NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and filtered. The residue was purified by medium-pressure chromatography (Isolera, eluent: 0.1:84:16 AcOH/*n*-hexane/EtOAc to 0.1:99.9 AcOH/EtOAc) to give **6** (40.8 mg, 72% *cis/trans* = 92/8 mixture) as a colorless oil. *cis*- and *trans*-**6** were separated by chiral HPLC using a CHIRALPAK IA column (Daicel Co., Ltd., Japan, Φ20 × 250 mm, eluent: 0.1:20:80 AcOH/EtOH/*n*-hexane, flow rate: 8.0 mL/min, *cis*-**6**: Rt = 33.3 min, *trans*-**6**: Rt = 23.3 min) to afford pure *cis*-**6** (22.3 mg) as a colorless oil. [α]<sub>D</sub><sup>20</sup> + 34.8 (c 1.95, CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ<sub>H</sub>: 7.72 (dd, *J* = 5.8, 2.7 Hz, 1H), 6.18 (dd, *J* = 5.8, 1.6 Hz, 1H), 5.48–5.31 (m, 2H), 3.04–2.94 (m, 1H), 2.56–2.41 (m, 2H), 2.36 (brt, *J* = 7.1 Hz, 2H), 2.13 (dt, *J* = 15.8, 7.8 Hz, 1H), 2.05 (quintet, *J* = 7.5 Hz, 2H), 1.80–1.69 (m, 1H), 1.63 (brt, *J* = 7.1 Hz, 2H), 1.52–1.29 (m, 4H), 1.23–1.11 (m, 1H), 0.97 (t, *J* = 7.5 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ<sub>C</sub>: 210.86, 179.32, 166.96, 133.02, 132.50, 126.82, 49.79, 44.20, 33.84, 30.58, 29.18, 27.32, 24.49, 23.76, 20.78, 13.99; IR (neat) cm<sup>-1</sup>: 2935, 2873, 1107; HRMS (ESI, positive) *m/z* [M + Na]<sup>+</sup> Calcd. for C<sub>16</sub>H<sub>24</sub>NaO<sub>4</sub>: 303.1572, Found: 303.1565.

**Synthesis of 16-COOH-dn-*cis*-OPDA (7).** To a solution of **23** (10.3 mg, 38.4 μmol) in acetone (6.0 mL) was added Jones reagent (4.0 M solution) at –20 °C until the orange color of the reagent persisted (37 drops). After 20 min of stirring at –20 °C, *i*-PrOH was added to quench the remaining reagent. Then, EtOAc (10 mL) and H<sub>2</sub>O (60 mL) were added and the water layer was extracted with EtOAc. The combined organic layers were washed with saturated aqueous NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The residue was purified by medium-pressure chromatography (Isolera, eluent: 0.1:98:2 AcOH/CHCl<sub>3</sub>/MeOH to 0.1:80:20 AcOH/CHCl<sub>3</sub>/MeOH) to give **7** (8.3 mg, 73%) as a colorless oil. Diastereomeric purity of **7** was >98% by <sup>1</sup>H NMR spectroscopy (δ<sub>H</sub> = 7.74 (dd, *J* = 5.9, 2.7 Hz, 1H) for **7**; 7.62 (dd, *J* = 5.9, 2.4 Hz, 1H) for the *trans* isomer). [α]<sub>D</sub><sup>21</sup> + 117.6 (c 0.24, CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ<sub>H</sub>: 7.74 (dd, *J* = 5.9, 2.7 Hz, 1H), 6.18 (dd, *J* = 5.9, 1.6 Hz, 1H), 5.68–5.56 (m, 2H), 3.21 (dd, *J* = 17.2, 5.9 Hz, 1H), 3.11 (dd, *J* = 17.2, 3.9 Hz, 1H), 3.04–2.93 (m, 1H), 2.56–2.46 (m, 2H), 2.36 (brt, *J* = 7.1 Hz, 2H), 2.20 (ddd, *J* = 16.7, 10.7, 6.1 Hz, 1H), 1.78–1.54 (m, 3H), 1.52–1.22 (m, 4H), 1.22–1.07 (m, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ<sub>C</sub>: 210.45, 179.82, 177.53, 167.19, 132.47, 131.97, 121.59, 49.21, 44.26, 33.82, 32.94, 30.48, 29.03, 27.30, 24.24, 24.15; IR (neat) cm<sup>-1</sup>: 3448, 3205, 3031, 2935, 1708 (br); HRMS (ESI, positive) *m/z* [M + Na]<sup>+</sup> Calcd. for C<sub>16</sub>H<sub>22</sub>NaO<sub>5</sub>: 317.1365, Found: 317.1355.

**Synthesis of dn-*iso*-OPDA (5).** To a solution of THPO(CH<sub>2</sub>)<sub>6</sub>MgBr (0.85 M in THF, 3.3 mL, 2.80 mmol) was added a solution of **28** (198 mg, 1.10 mmol) in THF (6.5 mL) at reflux temperature under argon atmosphere. After being stirred at 60 °C for 3 h, the reaction mixture was allowed to cool to rt and 2 M HCl aq. (7 mL) was added. After 1.5 h of stirring, H<sub>2</sub>O was added and the water layer was extracted with EtOAc. The combined organic layers were washed with saturated aqueous NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. After evaporation, the residue was purified by medium-pressure chromatography (Isolera, eluent: 40:60 *n*-hexane/EtOAc to EtOAc) to give the oxidized compound. The compound was carried on to the next step.

To a solution of the mixture (79.6 mg) in acetone (6 mL) was added Jones reagent (4.0 M solution, 200 μL, 800 μmol) at 0 °C. After 3.5 h of stirring at 0 °C, *i*-PrOH was added to quench the remaining reagent. Then, H<sub>2</sub>O (20 mL) was added and the water layer was extracted with EtOAc. The combined organic layers were washed with saturated aqueous NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The residue was purified by medium-pressure chromatography (Isolera, eluent: 0.5:50:50 AcOH/*n*-hexane/EtOAc to 0.5:99.5 AcOH/EtOAc) to give **5** (61.4 mg, 89%) as a colorless oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ<sub>H</sub>: 5.37 (ddt, *J* = 18.0, 7.2, 1.2 Hz, 1H), 5.21 (ddt, *J* = 17.6, 7.2, 1.2 Hz, 1H), 2.93 (d, *J* = 7.2 Hz, 2H), 2.50 (t, *J* = 4.4 Hz, 2H), 2.44 (t, *J* = 7.6 Hz, 2H), 2.39–2.35 (m, 4H), 2.15 (quin, *J* = 7.6 Hz, 2H), 1.68 (quin, *J* = 6.4 Hz, 2H), 1.58 (quin, *J* = 8.0 Hz, 2H), 1.44–1.36 (m, 2H), 0.99 (t, *J* = 7.6 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ<sub>C</sub>: 209.56, 178.73, 174.01, 139.39, 132.34, 125.32, 34.19, 33.71, 31.10, 29.15, 29.12, 27.08, 24.44, 21.24, 20.61, 14.14; IR (neat) cm<sup>-1</sup>: 3502, 2935, 2866, 1697, 1631; HRMS (ESI, positive) *m/z* [M + Na]<sup>+</sup> Calcd. for C<sub>16</sub>H<sub>24</sub>O<sub>3</sub>: 287.1623, found: 287.1601.

**Synthesis of 16-OH-dn-*iso*-OPDA (8).** A 5 mL pear-shaped flask was charged with **33** (31.2 mg, 140 μmol), CH<sub>2</sub> = CHCH<sub>2</sub>CH<sub>2</sub>OAc (165 mg, 144 mmol) and Hoveyda–Grubbs Catalyst M2001 Umicore (4.6 mg, 7.27 μmol). After 24 h of stirring, the residue was roughly purified by medium-pressure chromatography (Isolera, eluent: 0.1:99:1 AcOH/CHCl<sub>3</sub>/MeOH to 0.1:90:10 AcOH/CHCl<sub>3</sub>/MeOH) to give a mixture (58.2 mg). The crude mixture was used for the next reaction without further purification. To a solution of the mixture (58.2 mg) in acetone (10 mL) was added Jones reagent (4.0 M solution, 95 μL, 380 μmol) at –20 °C. After 30 min of stirring at –20 °C, *i*-PrOH was added to quench the remaining reagent. Then, EtOAc and H<sub>2</sub>O were added and the water layer was extracted with EtOAc. The combined organic layers were washed with saturated aqueous NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The crude mixture (33.0 mg) was used for the next reaction without further purification. To a solution of the mixture (33.0 mg) in THF (4.5 mL) was added 1 M-LiOH solution (510 μL, 510 μmol) and the mixture was stirred for 1.5 h. The reaction mixture was quenched with 1 M HCl aq. and the aqueous layer was extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl, dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. After evaporation, the residue was purified medium-pressure chromatography (Isolera, eluent: 0.1:85:15 AcOH/*n*-hexane/EtOAc to 0.1:99.9 AcOH/EtOAc) to give 16-OH-dn-*iso*-OPDA (**8**) (15.6 mg, 40% in 3 steps) as a *Z/E* mixture (*Z/E* = 10/1). *Z/E* isomers were separated by

chiral HPLC using a CHIRALPAK IA column (Daicel Co., Ltd., Japan,  $\Phi 20 \times 250$  mm, eluent: 0.1:20:80 AcOH/EtOH/*n*-hexane, flow rate: 8.0 mL/min, *E*-8: Rt = 18.8 min, *Z*-8: Rt = 25.5 min) to afford pure *Z*-8 (2.4 mg) as a colorless oil.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{H}}$ : 5.43 (dt,  $J = 11.0, 7.2$  Hz, 1H), 5.37 (dt,  $J = 11.0, 6.6$  Hz, 1H), 3.71 (t,  $J = 6.2$  Hz, 2H), 2.98 (d,  $J = 6.6$  Hz, 2H), 2.52–2.50 (m, 2H), 2.49–2.44 (m, 4H), 2.38–2.36 (m, 4H), 1.69 (quintet,  $J = 7.6$  Hz, 2H), 1.57 (quintet,  $J = 7.6$  Hz, 2H), 1.40 (quintet,  $J = 7.6$  Hz, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{C}}$ : 210.23, 178.34, 174.92, 138.75, 128.63, 126.82, 61.97, 34.18, 33.69, 31.03, 30.71, 29.27, 28.94, 27.07, 24.35, 21.71; IR (neat)  $\text{cm}^{-1}$ : 3433, 2931, 2866, 1701, 1631; HRMS (ESI, positive)  $m/z$   $[\text{M} + \text{Na}]^+$  Calcd. for  $\text{C}_{16}\text{H}_{24}\text{NaO}_4$ : 303.1572, found: 303.1565.

**Synthesis of 16-COOH-dn-iso-OPDA (9).** To a solution of **35** (16.1 mg, 60.4  $\mu\text{mol}$ ) in acetone (1.5 mL) was added Jones reagent (4.0 M solution, 150  $\mu\text{L}$ , 600  $\mu\text{mol}$ ) at  $-20$  °C. After 1.5 h of stirring at  $-20$  °C, *i*-PrOH was added to quench the remaining reagent. Then, EtOAc and  $\text{H}_2\text{O}$  were added and the water layer was extracted with EtOAc. The combined organic layers were washed with saturated aqueous NaCl, dried over  $\text{Na}_2\text{SO}_4$  and concentrated under reduced pressure. The residue was purified by medium-pressure chromatography (Isolera, eluent: 0.1:98:2 AcOH/ $\text{CHCl}_3$ /MeOH to 0.1:80:20 AcOH/ $\text{CHCl}_3$ /MeOH) to give 16-COOH-dn-iso-OPDA (**9**) (6.3 mg, 36%) as a *Z/E* mixture (*Z/E* = 12/1). *Z/E* isomers were separated by HPLC using a Cholesterol column (Nacalai Tesque, Inc., Japan,  $\Phi 20 \times 250$  mm, eluent: 0.1:30:70 AcOH/MeCN/ $\text{H}_2\text{O}$ , flow rate: 8.0 mL/min, *E*-9: Rt = 25.0 min, *Z*-9: Rt = 25.9 min) to afford pure *Z*-9 (2.6 mg) as a colorless oil.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{H}}$ : 5.57 (dt,  $J = 10.6, 7.3, 1.5$  Hz, 1H), 5.48 (dt,  $J = 10.6, 7.4, 1.3$  Hz, 1H), 3.27 (d,  $J = 7.3$  Hz, 2H), 2.96 (d,  $J = 7.4$  Hz, 2H), 2.53–2.51 (m, 2H), 2.44 (t,  $J = 7.7$  Hz, 2H), 2.40–2.35 (m, 4H), 1.66 (quintet,  $J = 7.7$  Hz, 2H), 1.55 (quintet,  $J = 7.7$  Hz, 2H), 1.38 (quintet,  $J = 7.7$  Hz, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{C}}$ : 209.72, 179.46, 177.13, 175.07, 138.16, 129.97, 121.01, 34.13, 33.71, 32.95, 31.08, 29.27, 28.94, 27.05, 24.23, 21.58; IR (neat)  $\text{cm}^{-1}$ : 3518, 3190, 2935, 1705, 1628; HRMS (ESI, positive)  $m/z$   $[\text{M} + \text{Na}]^+$  Calcd. for  $\text{C}_{16}\text{H}_{22}\text{NaO}_5$ : 317.1365, found: 317.1340.

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## Author contributions

M.U. conceived, designed, and coordinated the research project. N.K. designed the synthetic route of all compounds. M.U., N.K. and T.K. wrote the main manuscript text and all figures. J.W. T.K. and N.K. synthesized dn-cis-12-OPDA and the derivatives, and H.S. and N.K. synthesized dn-iso-12-OPDA and the derivatives. All authors reviewed the manuscript.

## Competing interests

The authors declare no competing interests.

## Additional information

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