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Structure–activity relationship study of flowering-inducer FN against *Lemna paucicostata*

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ABSTRACT

FN1 (**1**) and FN2 (**2**), cycloadducts of α-ketol octadecadienoic acid (**3**) with norepinephrine (NE), induce flowering in *Lemna paucicostata*. In order to broaden our understanding of structural requirements of FN for flower induction, nine analogs of **3** (**4**–**12**) were synthesized and reacted with NE under basic conditions. These analogs, except for **8**, **10**, and **12**, exhibited significant activity regarding to floral induction in *L. paucicostata*. Similar experiments were carried out by using **3** and epinephrine, and it was demonstrated that these products also possessed biological activity.

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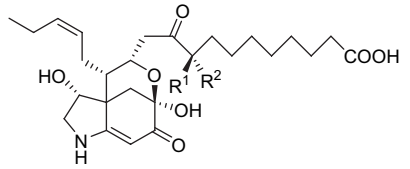
1. Introduction

Flowering time in plants is controlled by coincidence of internal and environmental signals. These different pathways converge to regulate a set of genes related to floral initiation. Many studies have suggested that *FLOWERING LOCUS T* (*FT*) is a major floral activator and a candidate for encoding florigen.[1] Very recently, FT protein was determined as a mobile flowering signal in *Arabidopsis thaliana*.[2] The protein encoded by *Hd3a*, a rice ortholog of *FT*, was also shown to be a florigen.[3] Therefore, FT/Hd3a protein should be a general signal that regulates the transition from vegetative to floral phases in higher plants. However, taking into consideration the agrochemical usage, these proteins seem to be unfavorable due to the difficulties in their application. Thus, the development of chemicals having such an activity is very important to control flowering in plants.

In course of screening for endogenous flowering inducers, (12*Z*,15*Z*)-9-hydroxy-10-oxooctadeca-12,15-dienoic acid (**3**) (Fig. 1), an oxylipin, was isolated from *Lemna paucicostata*.[4] This fatty acid, however, needs norepinephrine (NE) as a co-activator to show its activity. Further investigations have revealed that FN1 (**1**) and FN2 (**2**) are truly active compounds, which are expected to be formed by cycloaddition between 12-olefin of **3** and α,β-unsaturated carbonyl of noradrenochrome, an oxygenated form of NE.[5,8] Previous work to gain a better understanding of the structure–activity relationship (SAR) study focused on altering the fatty acid part of FN.[4] This attempt provided a suggestion that the conjugated diene, α-ketol, and carboxy groups in fatty acids are important to induce the flowering in *L. paucicostata*. However, since the structural element in all test compounds is rather different, it seems to be difficult to interpret the results obtained in that study. Few analogs of **3** with



1: R^1= OH, R^2= H
2: R^1= H, R^2= OH

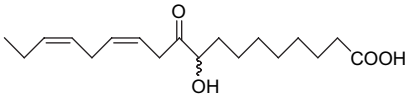


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Figure 1. Structures of **1**–**3**.

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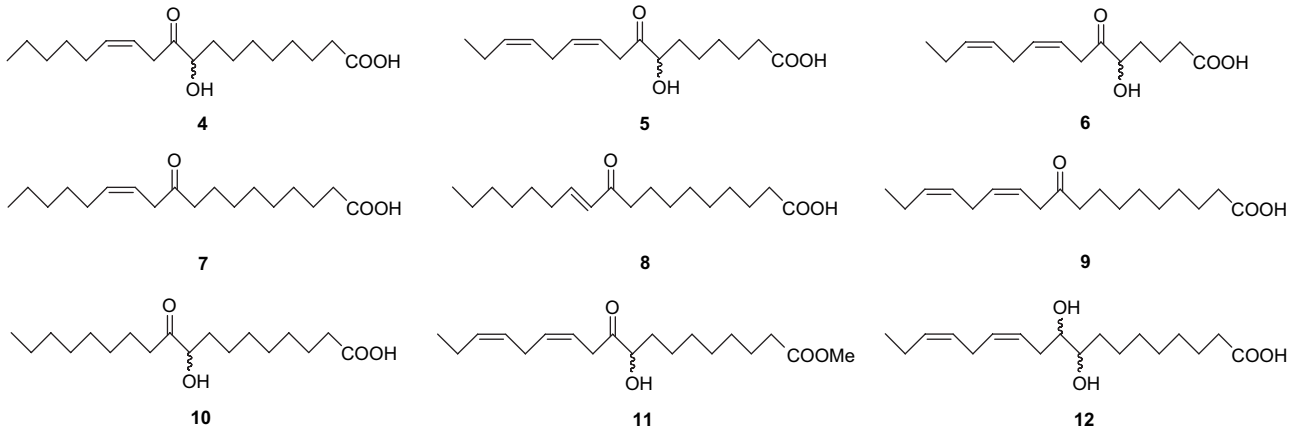


Figure 2. Structures of analogs **4**–**12**.

alteration at the respective structural moiety have been synthesized and tested for biological activity. Therefore, clearly much work needs to be done before we have sufficient knowledge of the structural requirements of FN for its activity. This effort is also important to identify FN's mode of action in floral development.

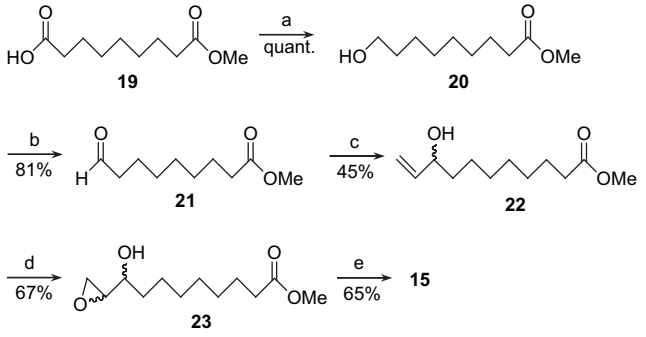
We report here the SAR study of the fatty acid moiety of FN for flowering in *L. paucicostata* by using series of **3** analogs shown in Figure 2 (**4**–**12**). Firstly, we synthesized nine fatty acid analogs, where single or combinative alterations have been made to the structural components of **3**. Because we were also interested in whether FN analogs derived from **3** and epinephrine (Epi) induce flowering, the analogs were prepared and tested for their ability to induce flowering. We used a new method to prepare FN derivatives from corresponding fatty acid analogs, which improve the yield of cycloaddition between fatty acid and NE.

2. Results and discussion

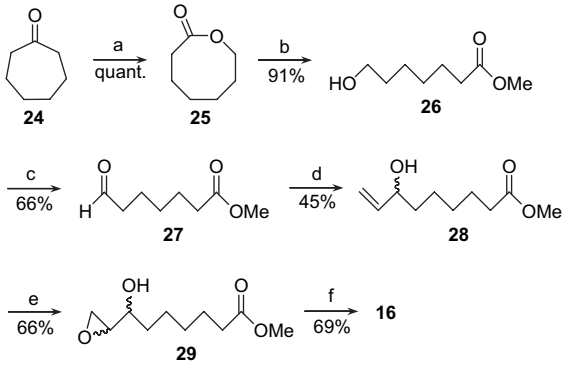
2.1. Synthesis of analogs 4–12

In order to know the structural requirements of FN for flower induction, we designed nine structural analogs of **3** as shown in Figure 2. Our general synthetic strategy for the synthesis of **4**–**9** is based on the earlier work, where the key reaction is coupling of epoxides **15**–**18** with 1-heptyne (**13**) or 1,4-heptadiyne (**14**) (Scheme 1).[6] Preparation of analogs **10** and **11** were accomplished as described in the previous report.[5] Diol **12** was obtained by reducing **3** with $NaBH_4$ quantitatively.

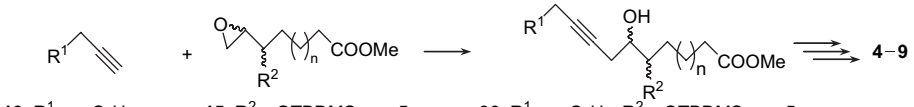
Synthesis of epoxy building blocks **15**–**18** are summarized in Schemes 2–4. Treatment of mono methyl azelate (**19**) with BH_3·THF complex in THF afforded **20** quantitatively, and subsequent oxidation of **20** with PDC in CH_2Cl_2 yielded **21**. Grignard reaction of **21** with vinylmagnesium bromide in THF at −78 °C gave an allyl alcohol **22** in a yield of 45%. Treatment of **22** with *m*-CPBA in CH_2Cl_2 containing saturated aq $NaHCO_3$ afforded a diastereomeric mixture of epoxide **23**. The hydroxy group of **23** was then protected as TBDMS ether to give the desired epoxide **15**. The overall yield of **15** was 16% based on **19** (5 steps), this being slightly better than the previous method (12%).[6] Compound **26** was obtained from cycloheptanone (**24**), which was first transformed into lactone **25** via a Baeyer–Villiger reaction (Scheme 3). The lactone ring of **25** was



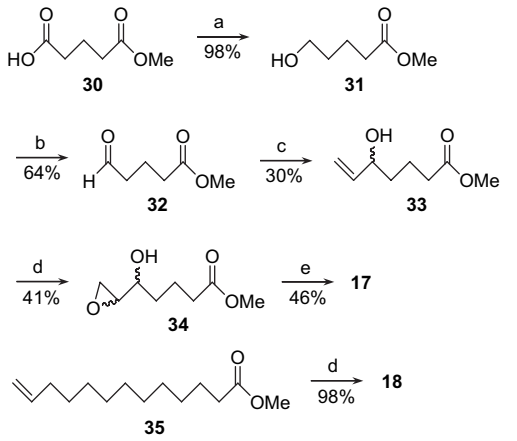
Scheme 2. Synthesis of epoxide **15**. Reagents and conditions: (a) BH_3·THF, THF, −18 °C to rt; (b) PDC, CH_2Cl_2, rt; (c) vinylmagnesium bromide, THF, −78 °C to rt; (d) *m*-CPBA, saturated aq $NaHCO_3$, CH_2Cl_2, rt; (e) TBDMS–Cl, imidazole, DMF, 0 °C to rt.



Scheme 3. Synthesis of epoxide **16**. Reagents and conditions: (a) *m*-CPBA, CH_2Cl_2, rt; (b) concd H_2SO_4, MeOH, rt; (c) PDC, CH_2Cl_2, rt; (d) vinylmagnesium bromide, THF, −78 °C to rt; (e) *m*-CPBA, saturated aq $NaHCO_3$, CH_2Cl_2, rt; (f) TBDMS–Cl, imidazole, DMF, 0 °C to rt.



Scheme 1.



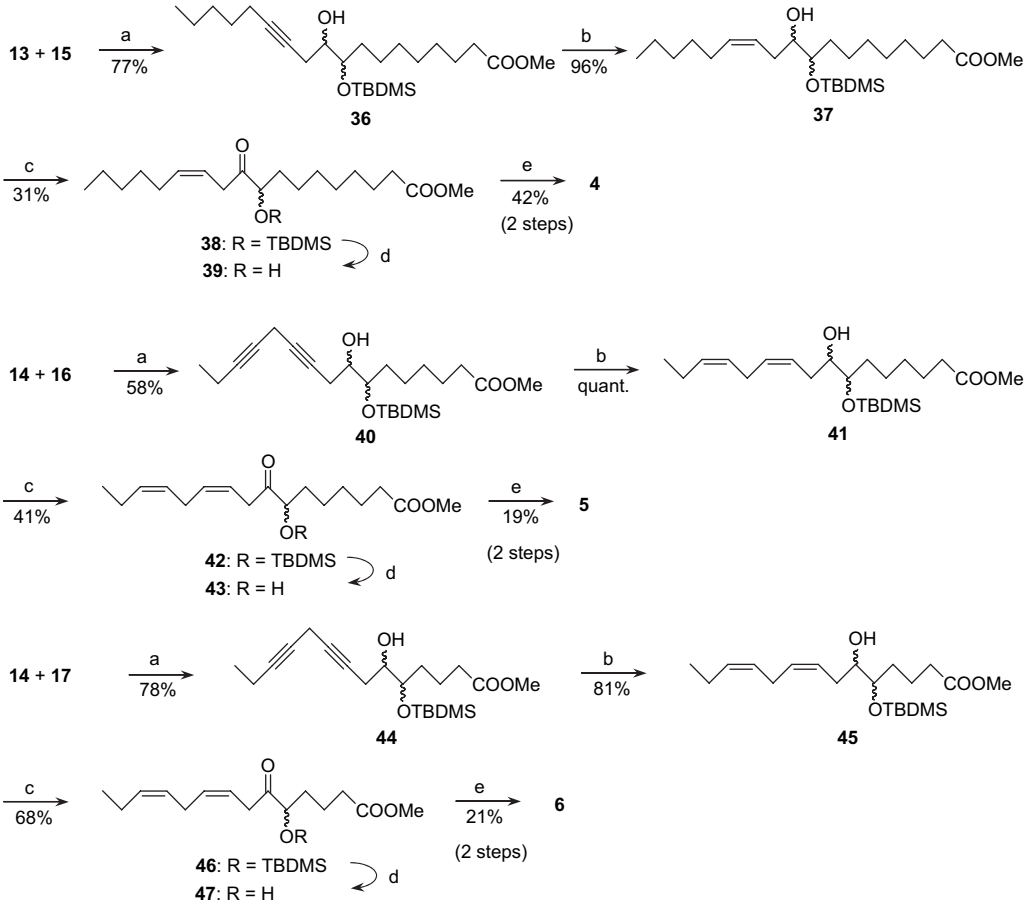
Scheme 4. Synthesis of epoxides **17** and **18**. Reagents and conditions: (a) BH_3·THF, THF, −18 °C to rt; (b) PDC, CH_2Cl_2, rt; (c) vinylmagnesium bromide, THF, −78 °C to rt; (d) *m*-CPBA, saturated aq $NaHCO_3$, CH_2Cl_2, rt; (e) TBDMS–Cl, imidazole, DMF, 0 °C to rt.

then opened to corresponding hydroxyester **26** with concd H_2SO_4/MeOH (91% yield). According to the synthesis of **15** from **20**, desired epoxide **16** was synthesized from **26**. Compound **16** was obtained in an overall yield of 12% from **24**. Similarly, epoxide **17** was synthesized from mono methyl glutarate (**30**) as shown in Scheme 4. The overall yield of **17** was 3.5% based on **30** (6 steps). Epoxide **18** was easily prepared from methyl undec-10-enoate (**35**) by epoxidation with *m*-CPBA in a yield of 98%.

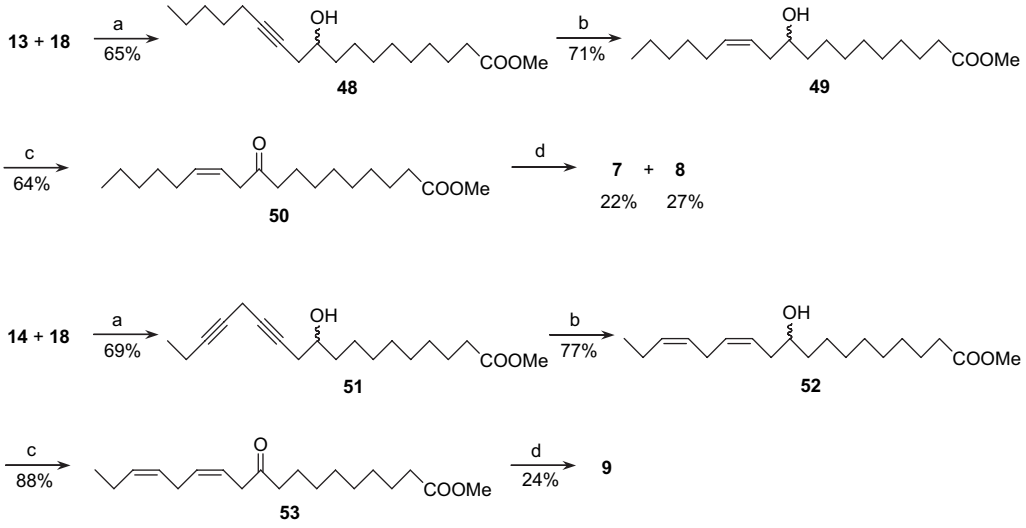
These epoxide building blocks were transformed into corresponding fatty acid analogs of **3** as shown in Schemes 5 and 6. Treatment of **15** with 1-heptyne (**13**) and *n*-BuLi in THF in the presence of BF_3·Et_2O at −78 °C afforded a coupled product **36** in a yield of 77%. Catalytic hydrogenation of compound **36** over Lindlar's catalyst easily gave **37** and subsequent oxidation furnished a ketone **38**. Deprotection of TBDMS group of **38** with 46% aq HF–MeCN yielded **39**. Ester hydrolysis of **39** with lipase PS provided the desired product **4** in a 9.6% overall yield (5 steps). During demethylation and purification processes, the double bond in **4** migrated from 12- to more stable 10-position to afford an α,β-unsaturated ketone, which reduced the yield of the desired compound. Following the above strategy, the diene analogs **5** and **6** were also synthesized from corresponding epoxides as shown in Scheme 5 (five-step yield, **5**: 4.5%; **6**: 9.0%). Similarly, analogs **7** and **9** were prepared as shown in Scheme 6. The overall yields of **7** and **9** were 7.2% and 11%, respectively. The migration of olefinic bound in **7** from C-12 to C-11 yielded analog **8** during demethylation with lipase PS.

2.2. Cycloaddition of fatty acid analogs with NE/Epi

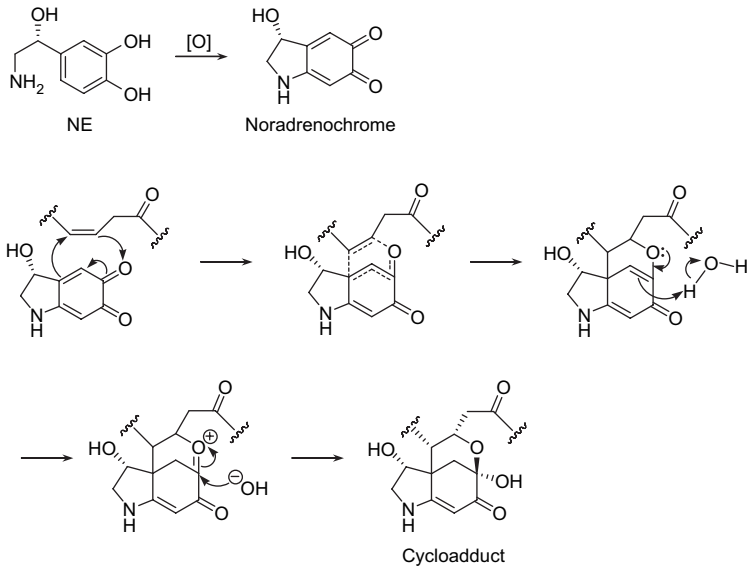
In a previous study,[5] the tricyclic structure of FNs was suggested to be formed by an intramolecular cycloaddition of the fatty acid-derived olefin across a preformed α,β-unsaturated carbonyl moiety derived from an oxidized NE, noradrenochrome. A plausible mechanism of this cycloaddition is proposed as shown in Scheme 7. Fatty acid and noradrenochrome concertedly form six-membered ring intermediate, into which an H_2O molecule is incorporated to yield an FN-like compound. From this, a significant implication concerned that the reaction should be promoted under O_2 atmosphere. Along this line, we carried out the cycloaddition reaction under several atmospheric conditions and calculated the yields



Scheme 5. Synthesis of **4**–**6**. Reagents and conditions: (a) BF_3·Et_2O, *n*-BuLi, THF, −78 °C; (b) H_2, Lindlar's cat., toluene, rt; (c) (1) DMSO, $(COCl)_2$, CH_2Cl_2, −60 °C, (2) Et_3N, −60 to −45 °C; (d) 46% aq HF, MeCN, rt; (e) lipase PS, 0.1 M phosphate buffer (pH 7)–acetone (1:1), rt.



Scheme 6. Synthesis of **7**–**9**. Reagents and conditions: (a) $BF_3{\cdot}Et_2O$, *n*-BuLi, THF, −70 °C; (b) H_2, Lindlar's cat., toluene, rt; (c) (1) DMSO, $(COCl)_2$, CH_2Cl_2, −60 °C, (2) Et_3N, −60 to −45 °C; (d) lipase PS, 0.1 M phosphate buffer (pH 7)–acetone (1:1), rt; (e) $BF_3{\cdot}Et_2O$, *n*-BuLi, THF, −70 °C; (f) H_2, Lindlar's cat., toluene, rt; (g) (1) DMSO, $(COCl)_2$, CH_2Cl_2, −60 °C, (2) Et_3N, −60 to −45 °C; (h) lipase PS, 0.1 M phosphate buffer (pH 7)–acetone (1:1), rt.



Scheme 7. Proposed reaction scheme for cycloaddition of **3** and NE.

based on the standard curve. Although previous method gave FNs in a yield of 2.3%, the reaction under O_2 atmosphere more effectively afforded the desired products (13% yield). The conditions under N_2 atmosphere showed no significant effect on yield of FNs (2.6% yield). These results indicated that oxidation of NE is inevitable for cycloaddition between **3** and NE. The cycloadducts were easily separated from byproducts in the reaction mixture by extraction with EtOAc (Fig. 3). It was, therefore, presumed that the structural requirements of FNs for the biological activity can be examined by using the EtOAc extracts of reaction products without further purifications.

In accordance with the above method, we prepared reaction products from analogs **4**–**12** with NE and analyzed them by using an LC–PDA/MS. The results are summarized in Table 1. Fatty acid analogs having β,γ-unsaturated carbonyl group gave a peak that showed characteristic UV adsorptions for FNs in LC–PDA analysis, whereas others did not. For example, the product of **4** with NE showed λ_{max} at 236, 295, and 336 nm. Furthermore, ESI-MS analysis enabled us to detect the desired ions, $[M+H]^+$, $[M+Na]^+$, and $[M+H-H_2O]^+$, of the respective peaks. Fatty acid **3** was revealed to be reacted with Epi to give possible cycloadducts as summarized in Table 1. All products obtained were shown to have the molecular formulae, which were consistent with that of the desired

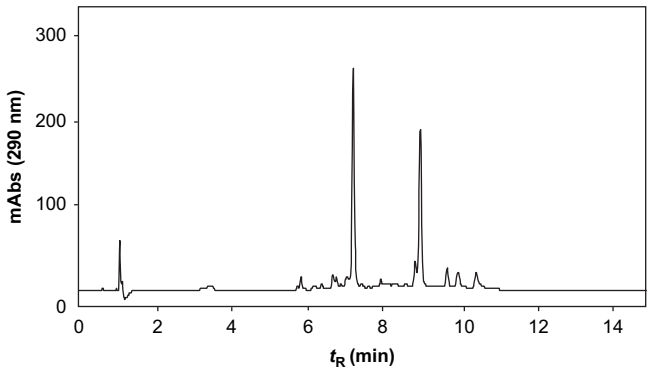


Figure 3. HPLC chromatogram of the EtOAc extract of the reaction mixture of **3** and NE. FNs were detected in a peak at t_R=7.26 min.

Table 1
LC-PDA/MS and HRMS analyses of the cycloadducts in the reaction mixtures of fatty acids **3**–**12** and NE/Epi

Substrates	Cycloadduct	λ_{max} (nm)	MS (*m*/*z*)	HRMS (*m*/*z*)	Molecular formula (calcd mass)	Yield[a] (%)
3/NE	**1**/**2**	294, 336, 347	516 $[M+Na]^+$, 494 $[M+H]^+$, 476 $[M+H-H_2O]^+$	516.2573 $[M+Na]^+$	$C_{26}H_{39}NNaO_8$ (516.2573)	13
4/NE	**54**	236, 295, 336	518 $[M+Na]^+$, 496 $[M+H]^+$, 478 $[M+H-H_2O]^+$	518.2728 $[M+Na]^+$	$C_{26}H_{41}NNaO_8$ (518.2730)	18
5/NE	**55**	240, 295, 343	488 $[M+Na]^+$, 466 $[M+H]^+$, 448 $[M+H-H_2O]^+$	488.2267 $[M+Na]^+$	$C_{24}H_{35}NNaO_8$ (488.2260)	18
6/NE	**56**	237, 296, 336	460 $[M+Na]^+$, 438 $[M+H]^+$, 420 $[M+H-H_2O]^+$	460.1952 $[M+Na]^+$	$C_{22}H_{31}NNaO_8$ (460.1947)	9.0
7/NE	**57**	293, 336, 347	502 $[M+Na]^+$, 480 $[M+H]^+$, 462 $[M+H-H_2O]^+$	502.2783 $[M+Na]^+$	$C_{26}H_{41}NNaO_7$ (502.2781)	8.8
8/NE	—[b]	—	—	—	—	0
9/NE	**58**	293, 336, 344	500 $[M+Na]^+$, 478 $[M+H]^+$, 460 $[M+H-H_2O]^+$	500.2624 $[M+Na]^+$	$C_{26}H_{39}NNaO_7$ (500.2624)	6.0
10/NE	—	—	—	—	—	0
11/NE	**59**	249, 293, 332	530 $[M+Na]^+$, 508 $[M+H]^+$, 490 $[M+H-H_2O]^+$	530.2727 $[M+Na]^+$	$C_{27}H_{41}NNaO_8$ (530.2730)	15
12/NE	—	—	—	—	—	0
3/Epi	**60**	193, 232, 301	530 $[M+Na]^+$, 508 $[M+H]^+$, 490 $[M+H-H_2O]^+$	530.2732 $[M+Na]^+$	$C_{27}H_{41}NNaO_8$ (530.2730)	20

[a] Yield was calculated by standard curve.
[b] Not detected/determined.

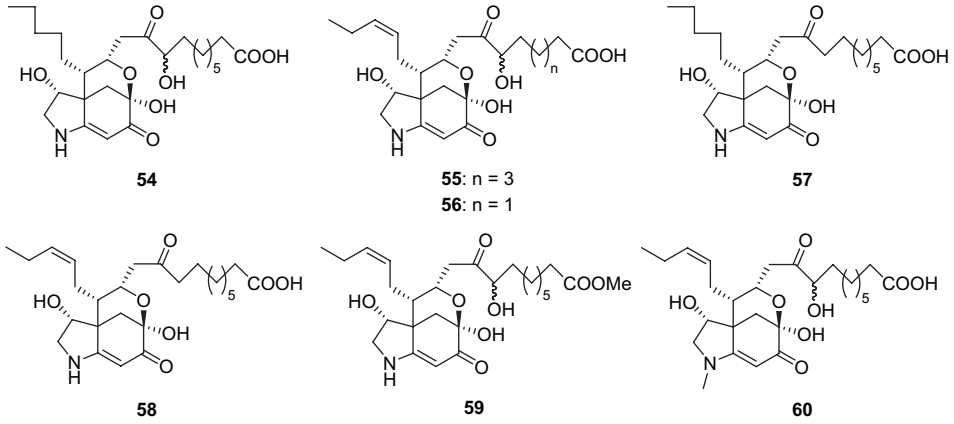


Figure 4. Structures of cycloadducts **54**–**60**.

cycloadducts, by HRMS analysis. These data strongly suggested that the desired derivatives of FN (**54**–**60**, Fig. 4) were obtained from fatty acid analogs and NE/Epi in yields ranging from 6 to 20%. Further experiment to completely identify their structures is underway.

2.3. Biological activity of cycloadducts

The above analogs were evaluated for their ability to induce flowering in *L. paucicostata*. With the exception of compounds **8**, **10**, and **12**, all these analogs proved to have a flowering activity after reacting with NE (Fig. 5). Compound **4**, in which 15-olefinic bond is saturated, displayed high activity of same magnitude as **3**. This suggested that olefinic bond at 15-position in compound **3** is not important for activity. Similar event was observed in the activities between 9-deoxy analogs **7** and **9**, where no significant difference was detected within a concentration range tested in the experiments. The effect of 9-hydroxy group on flowering activity was also investigated with these analogs. Compounds **7** and **9** displayed a significant activity but less in magnitude as the parent compound **3**. This implied that 9-hydroxy group may not be involved in primary recognition of the target whereas the presence is favorable to show high activity. Compounds **5** and **6**, in which their alkyl chains are shortened, displayed flowering activity at a concentration of more than 1 μM. In addition to this, the biological result obtained with methyl ester **11** indicated that recognition of the aliphatic chain and terminal carboxy group in FNs is relatively obscure. On the other hand, changing of β,γ-unsaturated carbonyl moiety led to complete loss of activity in *L. paucicostata*. The significant activity of compounds **8**, **10**, and **12** could not be observed even at a concentration of 10 μM. This result showed that cycloaddition of fatty acid with NE is inevitable process to induce flowering in *L. paucicostata*. The activity of cycloadduct of **3** with Epi was comparable to that of **3** with NE at high concentrations, whereas a significant reduction in flowering was observed at lower concentrations. This indicated that secondary amine in FNs is not essential for biological activity, but the presence of methyl group at this position may hamper their recognition by target protein.

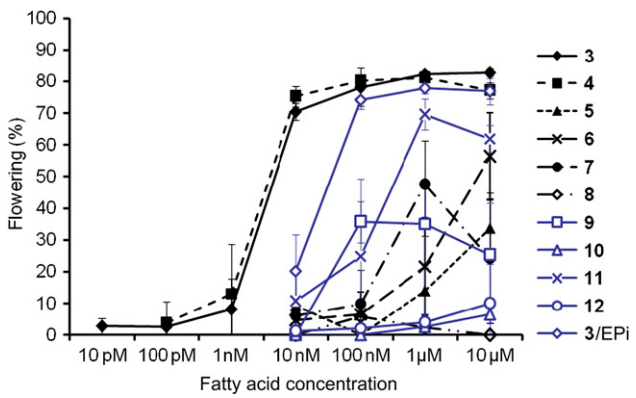


Figure 5. Flower-inducing activity of fatty acids **3**–**12** after reacting with NE/Epi. The error bars indicate the standard deviations of three replicates.

In previous SAR studies,[4,5] the strong flowering activity was observed only when fatty acid **3** was reacted with several catecholamines. The present study, however, provided the compelling results for analogs **4** and **11** in the induction of flowering. This is explainable based on the following reasons: (1) olefinic bond in analogs **4** and **11** migrated from β,γ- to α,β-position of 10-carbonyl before reaction with NE, and the resulting compounds never formed the desired adducts; (2) cycloaddition between these fatty

acids and NE was not conducted since the previous method is difficult to give the adducts in sufficient amount. The loss of their activity in previous report could be because of either of these things. Biological evaluation of purified cycloadducts is in progress, the result of which will be reported elsewhere in near future.

3. Conclusions

In the present report, we synthesized nine analogs of **3**, and evaluated their ability to induce flowering in *L. paucicostata* after the reaction with catecholamine. We observed that all the analogs possessing β,γ-unsaturated carbonyl group were cycloadducted with catecholamine and showed the activity of flowering induction. From the above data, tricyclic structure derived from conjugation of fatty acid with catecholamine was suggested to be inevitable to show an FN-like activity. To date, it is unclear how the derivatives of FN initiate the flowering signals in *L. paucicostata*. This work will serve as an entry point for the future study of chemical control of flowering in plants. Efforts are underway to further investigate the SAR of FN in flowering.

4. Experimental

4.1. General

^{1}H and ^{13}C NMR spectra were recorded on a JNM EX-270 spectrometer (JEOL, Tokyo, Japan) using TMS in $CDCl_3$ as an internal standard. LC–PDA/MS analysis was conducted with an LC-10VP system equipped with an LCMS 2010A mass spectrometer (Shimadzu, Kyoto, Japan). Mass spectra were recorded with JMS-DX303HF (JEOL) and LCMS 2010A mass spectrometers. High-resolution mass spectra were obtained with a JMS-T100LC AccuTOF mass spectrometer (JEOL). HPLC separation was performed with a JASCO (Tokyo, Japan) LC system. Solvents for HPLC were purchased from Kanto Chemical (Tokyo, Japan). A three-solvent system was used to generate the mobile phase for HPLC: solvent A, 0.05% aq formic acid; solvent B, 0.05% aq TFA; solvent C, MeCN. Column chromatography was performed on silica gel 60N (Kanto Chemical) or Wakogel C-200 (Wako Pure Chemical, Osaka, Japan).

4.2. Synthesis of (*Z*)-9-hydroxy-10-oxooctadec-12-enoic acid (4)

4.2.1. Methyl 9-hydroxynonanoate (*20*)

To a solution of mono methyl azelate (**19**; 10 g, 49.4 mmol) in dry THF (25 mL), BH_3·THF complex (0.9 M in THF; 54.9 mL, 49.4 mmol) was added dropwise at −18 °C over 20 min, and the mixture was stirred for 4 h at rt. After the reaction was quenched with water and K_2CO_3 (11.5 g, 83.9 mmol) at 0 °C, the mixture was extracted with Et_2O (3×100 mL). Combined organic layer was washed with brine and dried over Na_2SO_4. Evaporation of the solvent under vacuum gave **20** as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.24–1.88 (8H), 1.50–1.64 (4H), 2.30 (2H, t, J=7.6 Hz), 3.63 (2H, t, J=6.6 Hz), 3.67 (3H, s). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 24.8, 25.5, 29.0, 29.1 (C×2), 32.6, 34.0, 51.4, 62.9, 174.3. MS (ESI$^+$) m/z 189 $[M+H]^+$.

4.2.2. Methyl 9-oxononanoate (*21*)

A solution of **20** (49.4 mmol) in CH_2Cl_2 (15 mL) was added dropwise to a stirring suspension of PDC (27.8 g, 74.1 mmol) and Celite (10 g) in CH_2Cl_2 (80 mL). The mixture was stirred for 4 h at rt and the solvent removed under vacuum. The residue was purified by column chromatography (hexane–EtOAc, 8:2) to give **21** as a colorless oil (7.47 g, 40.1 mmol, 81%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.29–1.39 (6H), 1.58–1.68 (4H), 2.28 (2H, t, J=7.5 Hz), 2.42 (2H, t, J=7.3 Hz), 3.67 (3H, s), 9.78 (1H, br). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 21.9, 24.8, 28.9 (C×2), 34.0, 43.8, 51.4, 60.3, 174.2, 202.7. MS (ESI$^+$) m/z 187 $[M+H]^+$.

4.2.3. Methyl 9-hydroxyundec-10-enoate (*22*)

A solution of vinylmagnesium bromide (1 M in THF; 105 mL, 105 mmol) was added dropwise to a solution of **21** (17.8 g, 95.5 mmol) in dry THF (200 mL) at −78 °C under Ar. After stirring for 5 h at −25 °C, the reaction was quenched with saturated aq NH_4Cl (200 mL), and extracted with Et_2O (3×200 mL). The organic layer was washed with brine and dried over Na_2SO_4, and the solvent was removed under vacuum. The concentrate was purified by column chromatography (hexane–EtOAc, 8:2) to give **22** as a colorless oil (9.18 g, 42.8 mmol, 45%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.23–1.45 (8H), 1.49–1.65 (4H), 2.30 (2H, t, J=7.3 Hz), 3.66 (3H, s), 5.10 (1H, d, J=9.2 Hz), 5.21 (1H, d, J=17.0 Hz), 5.87 (1H, ddd, J=6.5, 9.2, 17.0 Hz). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 24.9, 25.2, 29.0, 29.1, 29.3, 34.0, 37.0, 51.4, 73.2, 114.5, 141.3, 174.3. MS (ESI$^+$) m/z 215 $[M+H]^+$.

4.2.4. Methyl 9-hydroxy-9-(oxiran-2-yl)nonanoate (*23*)

A solution of **22** (9.18 g, 42.8 mmol), *m*-CPBA (14.7 g, 85.6 mmol), and saturated aq $NaHCO_3$ (30 mL) in CH_2Cl_2 (120 mL) was stirred for 6 h at rt. The reaction mixture was washed with saturated aq $NaHCO_3$ and brine, and the organic layer was dried over Na_2SO_4. After concentration of organic layer under vacuum, the residue was purified by column chromatography (hexane–EtOAc, 7:3) to give **23** as a colorless oil (6.63 g, 28.7 mmol, 67%, diastereomeric mixture). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.23–1.64 (12H), 2.30 (2H, t, J=7.6 Hz), 2.72 (0.5H, m), 2.80 (0.5H, m), 3.00 (0.5H, m), 3.42 (0.5H, m), 3.61–3.72 (0.5H, m), 3.66 (3H, s), 3.83 (0.5H, m), 4.35–4.51 (0.5H, m). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 24.80, 24.83, 25.15, 28.93, 28.96, 29.30, 29.36, 33.37, 34.00, 34.28, 43.40, 45.13, 51.41, 54.51, 55.37, 68.41, 71.62, 174.30. MS (ESI$^+$) m/z 231 $[M+H]^+$.

4.2.5. Methyl 9-[(tert-butyldimethylsilyl)oxy]-9-(oxiran-2-yl)-nonanoate (*15*)

To a solution of **23** (3.84 g, 16.5 mmol) in dry DMF (100 mL), TBDMS–Cl (3.23 g, 21.4 mmol) and imidazole (1.45 g, 21.4 mmol) was added at 0 °C. After stirring the reaction mixture overnight at rt, it was diluted with $CHCl_3$ (200 mL), washed with 1 M HCl and brine, and dried over Na_2SO_4. The organic layer was concentrated under vacuum and purified by column chromatography (hexane–EtOAc, 95:5) to give **15** as a colorless oil (3.74 g, 10.8 mmol, 65.4%, diastereomeric mixture). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.03–0.10 (6H), 0.86–0.90 (9H), 1.20–1.70 (14H), 2.30 (2H, t, J=7.6 Hz), 2.54 (0.6H, m), 2.64 (0.4H, m), 2.69 (0.4H, m), 2.77 (0.6H, t, J=5.1 Hz), 2.83–2.93 (1H, m), 3.24 (0.6H, m), 3.54 (0.4H, m). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −5.00, −4.87, −4.38, 18.17, 24.78, 24.89, 24.91, 25.21, 25.63, 25.80, 25.85, 29.05, 29.13, 29.43, 29.52, 34.06, 34.67, 35.23, 44.85, 51.40, 54.66, 55.96, 71.33, 74.54, 174.25. MS (ESI$^+$) m/z 345 $[M+H]^+$.

4.2.6. Methyl 9-[(tert-butyldimethylsilyl)oxy]-10-hydroxyoctadec-12-ynoate (*36*)

A solution of *n*-BuLi (1.57 M in hexane; 19.5 mL, 30.6 mmol) was added dropwise to a solution of **13** in dry THF (100 mL) at −78 °C under Ar. After stirring for 1 h at −78 °C, a solution of **15** (5.30 g, 15.3 mmol) in dry THF (50 mL) and BF_3·Et_2O complex (1.88 mL, 15.3 mmol) were added dropwise to the reaction mixture. The mixture was stirred for 1.5 h at −78 °C under Ar and then poured into saturated aq NH_4Cl (100 mL). The mixture was extracted with Et_2O (3×100 mL), washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by column chromatography (hexane–EtOAc, 95:5) to give **36** as an orange oil (5.23 g, 11.8 mmol, 77%, diastereomeric mixture). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.07–0.10 (6H), 0.87–0.92 (12H), 1.22–1.63

(18H), 2.12–2.40 (7H), 3.52–3.84 (2H), 3.66 (3H, s). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −4.70, −4.51, −4.46, −4.25, 13.96, 14.17, 18.07, 18.68, 18.70, 22.20, 22.71, 24.51, 24.90, 25.19, 25.86, 28.67, 28.69, 29.07, 29.09, 29.19, 29.54, 29.66, 31.06, 31.49, 33.77, 34.06, 34.07, 51.41, 71.33, 72.60, 72.67, 73.93, 75.97, 76.38, 76.52, 82.44, 82.85, 174.24, 174.26. HRMS (ESI$^+$) m/z 463.3250 [M+Na]$^+$ (calcd for $C_{25}H_{48}NaO_4Si$, 463.3220).

4.2.7. (Z)-Methyl 9-[(tert-butyldimethylsilyl)oxy]-10-hydroxy-octadec-12-enoate (**37**)

A solution of **36** (5.23 g, 11.8 mmol) in toluene (80 mL) was added to a suspension of Lindlar's catalyst (5% Pd–$CaCO_3$–Pb^{2+}, 523 mg) in toluene (10 mL). After stirring for 5 h at rt under H_2, the mixture was filtered through Celite, and filtrate was evaporated to dryness to give **37** as a colorless oil (5.01 g, 11.3 mmol, 96%, diastereomeric mixture). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.07–0.09 (6H), 0.86–0.91 (12H), 1.14–1.77 (18H), 2.02–2.41 (6H), 3.39–3.66 (2H), 3.67 (3H, s), 5.36–5.55 (2H). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −4.61, −4.42, −4.09, 14.03, 18.10, 22.55, 24.91, 25.06, 25.49, 25.88, 27.39, 27.48, 29.07, 29.19, 29.28, 29.31, 29.66, 29.87, 31.14, 31.53, 31.93, 33.73, 34.07, 51.42, 72.67, 74.28, 74.50, 74.87, 125.26, 125.50, 132.36, 132.82, 174.26. HRMS (ESI$^+$) m/z 465.3371 [M+Na]$^+$ (calcd for $C_{25}H_{50}NaO_4Si$, 465.3376).

4.2.8. (Z)-Methyl 9-[(tert-butyldimethylsilyl)oxy]-10-oxooctadec-12-enoate (**38**)

A solution of DMSO (2.80 mL, 39.5 mmol) in dry CH_2Cl_2 (8 mL) was added dropwise to a solution of $(COCl)_2$ (2.90 mL, 33.9 mL) in dry CH_2Cl_2 (30 mL) at −60 °C under Ar. After stirring for 10 min at −60 °C, a solution of **37** (5.01 g, 11.3 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise to the above solution. The mixture was stirred for 15 min at −60 °C and allowed to warm to −45 °C. Et_3N (9.43 mL, 67.8 mmol) was added to the mixture, which was stirred for 10 min at rt. After quenching the reaction with saturated aq NH_4Cl (100 mL), the mixture was extracted with CH_2Cl_2 (3×100 mL), and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by column chromatography (hexane–EtOAc, 95:5) to give **38** as an orange oil (1.51 g, 3.46 mmol, 31%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.05 (3H, s), 0.06 (3H, s), 0.88 (3H, t, J=6.9 Hz), 0.92 (9H, s), 1.10–1.37 (14H), 1.46–1.70 (4H), 1.99 (2H, q-like), 2.29 (2H, t, J=7.3 Hz), 3.32 (2H, m), 3.66 (3H, s), 4.04 (1H, m), 5.49–5.64 (2H). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −4.9, 14.0, 18.1, 22.5, 24.7, 24.9, 25.3 (C×3), 27.6, 29.0 (C×2), 29.3, 27.6, 31.5, 34.1, 35.0, 36.0, 51.4, 78.7, 120.7, 133.4, 174.2, 211.9. HRMS (ESI$^+$) m/z 463.3224 [M+Na]$^+$ (calcd for $C_{25}H_{48}NaO_4Si$, 463.3220).

4.2.9. (Z)-Methyl 9-hydroxy-10-oxooctadec-12-enoic acid (**39**)

A solution of **38** (1.51 g, 3.46 mmol) in 46% aq HF–MeCN (100 mL, 1:19) was stirred at rt for 1 h. The reaction was quenched with saturated aq $NaHCO_3$ (100 mL), and the product was extracted with Et_2O (3×100 mL). After evaporation, the residual oil is **39** (1.12 g, 3.43 mmol, orange oil), which was used in next reaction without any purification step. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.89 (3H, t, J=6.6 Hz), 1.20–1.70 (17H), 1.81 (1H, m), 2.02 (2H, q-like), 2.30 (2H, t, J=7.6 Hz), 3.24 (2H, m), 3.67 (3H, s), 4.23 (1H, m), 5.52 (1H, m), 5.70 (1H, m). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.5, 23.0, 25.2, 25.3, 28.0, 29.4, 29.5 (C×2), 29.7, 31.9, 34.1, 34.5, 37.2, 51.9, 76.5, 120.1, 134.9, 174.7, 210.9. HRMS (ESI$^+$) m/z 349.2357 [M+Na]$^+$ (calcd for $C_{19}H_{34}NaO_4$, 349.2355).

4.2.10. (Z)-9-Hydroxy-10-oxooctadec-12-enoic acid (**4**)

A solution of **39** (972 mg, 2.98 mmol) and lipase PS Amano SD (972 mg, Wako Pure Chemical) in 0.1 M phosphate buffer (pH 7.0)–acetone (60 mL, 1:1) was stirred at rt for 30 min. The mixture was diluted with water (60 mL) and extracted with EtOAc (4×60 mL). The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by column chromatography (hexane–EtOAc, 6:4) and preparative HPLC [column, CAPCELL PAK UG120 20×250 mm (Shiseido, Tokyo, Japan); solvent, 60% C/(B+C); flow rate, 10 mL/min] to give **4** as a white solid (395 mg, 1.26 mmol, 42%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.89 (3H, t, J=6.6 Hz), 1.19–1.93 (18H), 2.02 (2H, q-like), 2.35 (2H, t, J=7.6 Hz), 3.24 (2H, m), 4.25 (1H, m), 5.51 (1H, m), 5.64 (1H, m). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.0, 22.5, 24.6, 24.7, 27.6, 28.9 (C×2), 29.0, 29.2, 31.4, 33.6, 33.8, 36.8, 76.0, 119.5, 134.5, 179.0, 210.4. HRMS (ESI$^+$) m/z 335.2198 [M+Na]$^+$ (calcd for $C_{18}H_{32}NaO_4$, 335.2198).

4.3. Synthesis of (10*Z*,13*Z*)-7-hydroxy-8-oxohexadaca-10,13-dienoic acid (5)

4.3.1. 7-Heptanolide (**25**)

To a solution of *m*-CPBA (9.2 g, 53.5 mmol) in CH_2Cl_2 (100 mL), compound **24** was added at 0 °C. After stirring for 5 days at rt, the reaction mixture was filtered, washed with saturated aq $NaHCO_3$ and water, and dried over Na_2SO_4. The organic layer was evaporated under vacuum to give **25** as a colorless oil quantitatively. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.53–1.92 (8H, m), 2.56 (2H, t, J=6.2 Hz), 4.36 (2H, t, J=5.7 Hz). MS (EI$^+$) m/z 345 M$^+$.

4.3.2. Methyl 7-hydroxyheptanoate (**26**)

Lactone **25** (106.9 mmol) was opened with MeOH (150 mL) in the presence of concd H_2SO_4 (1 mL) at rt in 8 h. After removing the solvent, the residue was dissolved in Et_2O (150 mL), washed with water twice, and dried over Na_2SO_4. Et_2O layer was concentrated and dried to give **26** as a colorless oil (13 g, 81.1 mmol, 91%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.35–1.38 (4H), 1.56–1.67 (4H), 2.32 (2H, t, J=7.6 Hz), 3.65 (2H, t, J=6.3 Hz), 3.67 (3H, s). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 24.8, 25.3, 28.8, 32.3, 33.9, 51.5, 62.7, 174.3. MS (FAB$^+$) m/z 161 [M+H]$^+$.

4.3.3. Methyl 7-oxoheptanoate (**27**)

Reaction procedure as in Section 4.2.2 was followed. Chromatography: hexane–EtOAc (8:2). Colorless oil (66%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.38 (2H, m), 1.60–1.68 (4H, m), 2.32 (2H, t, J=7.3 Hz), 2.45 (2H, t, J=7.6 Hz), 3.67 (3H, s), 9.78 (1H, br). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 21.7, 24.6, 28.6, 33.8, 43.6, 51.5, 173.9, 202.4. MS (FAB$^+$) m/z 159 [M+H]$^+$.

4.3.4. Methyl 7-hydroxynon-8-enoate (**28**)

Reaction procedure as in Section 4.2.3 was followed. Chromatography: hexane–EtOAc (7:3). Colorless oil (45%).^{1}H NMR (270 MHz, $CDCl_3$) δ 1.29–1.79 (8H), 2.31 (2H, t, J=7.6 Hz), 3.67 (3H, s), 4.09 (1H, dt, J=7.3, 14.0 Hz), 5.10 (1H, d, J=10.5 Hz), 5.21 (1H, d, J=17.0 Hz), 5.87 (1H, ddd, J=7.3, 10.5, 17.0 Hz). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 24.8, 24.9, 29.0, 33.9, 36.7, 51.4, 73.0, 114.5, 141.2, 174.2. MS (ESI$^+$) m/z 187 [M+H]$^+$.

4.3.5. Methyl 7-hydroxy-7-(oxiran-2-yl)heptanoate (**29**)

Reaction procedure as in Section 4.2.4 was followed. Chromatography: hexane–EtOAc (6:4). Colorless oil (diastereomeric mixture, 66%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.30–1.70 (8H), 2.32 (2H, t, J=7.6 Hz), 2.72 (0.5H, m), 2.81 (0.5H, m), 3.00 (0.5H, m), 3.44 (0.5H, m), 3.67 (3H, s), 3.82 (0.5H, m). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 24.72, 24.75, 24.92, 29.00, 29.07, 33.92, 33.93, 34.15, 43.37, 45.12, 51.47, 54.45, 55.30, 60.38, 68.30, 71.47, 174.17. MS (ESI$^+$) m/z 203 [M+H]$^+$.

4.3.6. Methyl 7-[(tert-butyldimethylsilyl)oxy]-7-(oxiran-2-yl)-heptanoate (**16**)

Reaction procedure as in Section 4.2.5 was followed. Chromatography: hexane–EtOAc (85:15). Orange oil (diastereomeric

mixture, 69%). ^{1}H NMR (270 MHz, $CDCl_3$) δ −0.01–0.07 (6H), 0.82–0.86 (9H), 1.22–1.62 (8H), 2.27 (2H, t, J=7.6 Hz), 2.49 (0.5H, m), 2.59–2.62 (0.5H, m), 2.66 (0.5H, m), 2.75–2.89 (0.5H, m), 3.20 (0.5H, m), 3.49–3.55 (0.5H, m). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −5.04, −4.90, −4.40, 18.13, 20.98, 24.50, 24.80, 24.94, 25.62, 25.77, 25.82, 29.12, 29.22, 31.55, 33.95, 34.47, 35.04, 44.80, 44.87, 51.41, 54.58, 55.90, 60.34, 71.25, 74.45, 171.08, 174.11. MS (ESI$^+$) m/z 317 $[M+H]^+$.

4.3.7. Methyl 7-[(tert-butyldimethylsilyl)oxy]-8-hydroxyhexadeca-10,13-diynoate (**40**)

1,4-Heptadiyne (**14**) was freshly prepared from ethylmagnesium bromide and propargyl bromide in the presence of copper(I) chloride as reported previously.[7] Synthesis of **40** was conducted by using **14** instead of **13**. Reaction procedure as in Section 4.2.6 was followed. Chromatography: hexane–EtOAc (85:15). Orange oil (diastereomeric mixture, 58%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.07–0.10 (6H), 0.88 (9H), 1.11 (3H, t, J=7.5 Hz), 1.28–1.65 (8H), 2.12–2.20 (2H), 2.27–2.40 (4H), 3.11 (2H, m), 3.60–3.78 (2H), 3.66 (3H, s). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −4.71, −4.53, −4.47, −4.25, 9.70, 9.74, 12.33, 13.85, 13.87, 14.17, 18.05, 22.68, 24.45, 24.57, 24.85, 24.88, 25.85, 29.24, 29.35, 31.40, 33.57, 33.97, 34.01, 51.47, 71.10, 72.40, 72.67, 73.36, 73.44, 73.84, 76.74, 76.79, 77.21, 81.91. 82.00, 174.16, 174.21. HRMS (ESI$^+$) m/z 431.2636 $[M+Na]^+$ (calcd for $C_{23}H_{40}NaO_4Si$, 431.2594).

4.3.8. (10Z,13Z)-Methyl 7-[(tert-butyldimethylsilyl)oxy]-8-hydroxyhexadeca-10,13-dienoate (**41**)

Reaction procedure as in Section 4.2.7 was followed. Orange oil (diastereomeric mixture, quantitatively). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.07–0.09 (6H), 0.91 (9H), 0.97 (3H, t, J=7.3 Hz), 1.21–1.75 (6H), 2.04–2.35 (6H), 2.73–2.95 (2H), 3.45–3.74 (1H), 3.67 (3H, s), 5.26–5.54 (4H). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −4.60, −4.42, −4.10, 13.96, 14.21, 18.08, 20.43, 20.57, 24.90, 25.19, 25.68, 25.87, 51.42, 72.65, 74.17, 74.48, 74.83, 125.28, 125.64, 125.84, 126.85, 129.02, 130.48, 130.88, 132.15, 174.11, 174.15. HRMS (ESI$^+$) m/z 435.2910 $[M+Na]^+$ (calcd for $C_{23}H_{44}NaO_4Si$, 435.2907).

4.3.9. (10Z,13Z)-Methyl 7-[(tert-butyldimethylsilyl)oxy]-8-oxohexadeca-10,13-dienoate (**42**)

Reaction procedure as in Section 4.2.8 was followed. Chromatography: hexane–EtOAc (95:5). Orange oil (41%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.05 (3H, s), 0.06 (3H, s), 0.93 (9H, s), 0.97 (3H, t, J=7.5 Hz), 1.20–1.40 (4H), 1.50–1.70 (4H), 2.06 (2H, m), 2.29 (2H, t, J=7.5 Hz), 2.75 (2H, t, J=5.6 Hz), 3.35 (2H, m), 3.66 (3H, s), 4.05 (1H, t, J=5.9 Hz), 5.24–5.45 (2H), 5.52–5.63 (2H). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −4.9, 14.2, 18.1, 20.6, 24.5, 24.7, 25.7 (C×3), 25.8, 29.0, 33.9, 34.8, 36.0, 51.4, 78.6, 121.0, 126.4, 131.5, 132.4, 174.1, 211.6. HRMS (ESI$^+$) m/z 433.2750 $[M+Na]^+$ (calcd for $C_{23}H_{42}NaO_4Si$, 433.2750).

4.3.10. (10Z,13Z)-7-Hydroxy-8-oxohexadeca-10,13-dienoate (**5**)

Reaction procedure as in Sections 4.2.9, 4.2.10 was followed. Chromatography: hexane–EtOAc (6:4), HPLC [CAPCELL PAK UG120 20×250 mm, 60% C/(B+C), 10 mL/min]. White solid (66%, 2 steps). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.98 (3H, t, J=7.6 Hz), 1.20–1.75 (7H), 1.84 (1H, m), 2.06 (2H, m), 2.37 (2H, t, J=7.3 Hz), 2.78 (2H, t, J=7.0 Hz), 3.28 (2H, t-like), 4.27 (1H, m), 5.29 (1H, m), 5.42 (1H, m), 5.54 (1H, m), 5.63 (1H, m). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.2, 20.6, 24.4, 24.5, 25.8, 28.7, 33.3, 33.7, 36.7, 76.0, 119.9, 125.9, 132.7, 132.8, 179.1, 210.1. HRMS (ESI$^+$) m/z 305.1730 $[M+Na]^+$ (calcd for $C_{16}H_{26}NaO_4$, 305.1729).

4.4. Synthesis of (8*Z*,11*Z*)-5-hydroxy-6-oxotetradeca-8,11-dienoate (6)

4.4.1. Methyl 5-hydroxypentanoate (**31**)

Reaction procedure as in Section 4.2.1 was followed. Colorless oil (98%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.54–1.78 (5H), 2.36 (2H, t, J=6.9 Hz), 3.65 (2H, t, J=5.9 Hz), 3.68 (3H, s). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 21.0, 32.0, 33.6, 51.5, 62.2, 174.2. MS (FAB$^+$) m/z 133 $[M+H]^+$.

4.4.2. Methyl 5-oxopentanoate (**32**)

Reaction procedure as in Section 4.2.2 was followed. Chromatography: hexane–EtOAc (6:4). Colorless oil (64%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.96 (2H, t, J=7.2 Hz), 2.38 (2H, t, J=7.2 Hz), 2.54 (2H, t, J=7.2 Hz), 3.68 (3H, s), 9.78 (1H, br). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 17.3, 32.9, 42.9, 51.6, 173.3, 201.4. MS (FAB$^+$) m/z 131 $[M+H]^+$.

4.4.3. Methyl 5-hydroxyhept-6-enoate (**33**)

Reaction procedure as in Section 4.2.3 was followed. Chromatography: hexane–EtOAc (6:4). Orange oil (30%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.52–1.77 (4H), 2.36 (2H, t, J=7.2 Hz), 3.67 (3H, s), 4.12 (1H, m), 5.14 (1H, d, J=11.5 Hz), 5.24 (1H, d, J=15.8 Hz), 5.86 (1H, m). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 20.7, 33.8, 36.2, 51.5, 72.7, 114.9, 140.8, 174.0. MS (FAB$^+$) m/z 159 $[M+H]^+$.

4.4.4. Methyl 5-hydroxy-5-(oxiran-2-yl)pentanoate (**34**)

Reaction procedure as in Section 4.2.4 was followed. Chromatography: hexane–EtOAc (5:5). Colorless oil (diastereomeric mixture, 41%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.57–1.88 (4H), 2.38 (2H, m), 2.71–2.76 (1H), 2.80–2.84 (1H), 2.97–3.03 (1H), 3.46 (0.5H, m), 3.84 (0.5H, m). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 20.68, 20.73, 32.66, 33.64, 33.71, 33.76, 43.45, 45.06, 51.56, 54.32, 55.19, 68.12, 71.16, 173.94. MS (FAB$^+$) m/z 175 $[M+H]^+$.

4.4.5. Methyl 5-[(tert-butyldimethylsilyl)oxy]-5-(oxiran-2-yl)-pentanoate (**17**)

Reaction procedure as in Section 4.2.5 was followed. Chromatography: hexane–EtOAc (95:5). Colorless oil (diastereomeric mixture, 46%). ^{1}H NMR (270 MHz, $CDCl_3$) δ −0.04–0.11 (6H), 0.87–0.90 (9H), 1.50–1.86 (4H), 2.30–2.36 (2H), 2.64 (0.5H, m), 2.70 (0.5H, m), 2.77 (0.5H, t-like), 2.84–2.94 (1H), 3.27 (0.5H, m), 3.57 (0.5H, m), 3.66 (3H, s). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −5.06, −4.91, −4.38, 18.13, 20.37, 20.78, 25.63, 25.78, 25.83, 33.92, 34.06, 34.56, 44.78, 44.88, 51.49, 54.40, 55.75, 70.95, 74.24, 173.81. HRMS (ESI$^+$) m/z 311.1653 $[M+Na]^+$ (calcd for $C_{14}H_{28}NaO_4Si$, 311.1654).

4.4.6. Methyl 5-[(tert-butyldimethylsilyl)oxy]-6-hydroxytetradeca-8,11-diynoate (**44**)

Reaction procedure as in Section 4.2.6 was followed. Chromatography: hexane–EtOAc (9:1). Orange oil (diastereomeric mixture, 78%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.08–0.11 (6H), 0.89 (9H), 1.11 (3H, t, J=7.2 Hz), 1.40–1.80 (4H), 2.12–2.41 (6H), 3.11–3.13 (2H), 3.55–3.81 (2H), 3.66 (3H, s). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −4.71, −4.56, −4.48, −4.30, 9.71, 9.75, 12.38, 13.86, 14.19, 18.05, 21.47, 22.85, 24.38, 25.86, 31.127, 33.17, 33.99, 34.12, 51.51, 71.16, 72.25, 72.41, 73.35, 73.55, 76.00, 76.41, 81.95, 82.03, 173.76. HRMS (ESI$^+$) m/z 403.2279 $[M+Na]^+$ (calcd for $C_{21}H_{36}NaO_4Si$, 403.2281).

4.4.7. (8Z,11Z)-Methyl 5-[(tert-butyldimethylsilyl)oxy]-6-hydroxytetradeca-8,11-dienoate (**45**)

Reaction procedure as in Section 4.2.7 was followed. Chromatography: hexane–EtOAc (9:1). Orange oil (diastereomeric mixture, 81%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.08–0.09 (6H), 0.90 (9H), 0.97 (3H, t, J=7.2 Hz), 1.23–1.70 (5H), 1.90–2.34 (5H), 2.72–2.84 (2H, m), 3.39–3.70 (2H), 3.66 (3H, s), 5.25–5.56 (4H). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −4.61, −4.47, −4.17, 14.04, 14.18, 14.22, 18.08, 20.57, 20.62, 25.52, 25.68, 25.86, 29.27, 29.30, 31.52, 31.75, 33.94, 34.12, 51.49, 72.67, 74.12, 74.47, 74.91, 125.49, 125.70, 126.82, 126.89, 130.60, 131.00, 132.17, 132.56, 173.79, 173.92. HRMS (ESI$^+$) m/z 407.2594 $[M+Na]^+$ (calcd for $C_{21}H_{40}NaO_4Si$, 407.2594).

*4.4.8. (8Z,11Z)-Methyl 5-[(tert-butyldimethylsilyl)oxy]-6-oxotetradeca-8,11-dienoate (**46**)*

Reaction procedure as in Section 4.2.8 was followed. Chromatography: hexane–EtOAc (95:5). Orange oil (68%).^{1}H NMR (270 MHz, $CDCl_3$) δ 0.06 (3H, s), 0.07 (3H, s), 0.89–0.96 (12H), 1.57–1.69 (4H), 1.95–2.08 (4H), 2.31 (2H, t-like), 3.35 (2H, m), 3.66 (3H, s), 4.07 (1H, m), 5.24–5.65 (4H). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ −5.0, 14.2, 18.1, 20.5, 25.6, 25.7 (C×3), 25.9, 29.4, 31.5, 33.8, 34.2, 51.5, 78.3, 120.8, 126.3, 131.2, 132.4, 173.5, 211.1. HRMS (ESI$^+$) *m*/*z* 405.2433 [M+Na]$^+$ (calcd for $C_{21}H_{38}NaO_4Si$, 405.2437).

*4.4.9. (8Z,11Z)-5-Hydroxy-6-oxotetradeca-8,11-denoate (**6**)*

Reaction procedure as in Sections 4.2.9, 4.2.10 was followed. Chromatography: HPLC [CAPCELL PAK UG120 20×250 mm, 39% C/(B+C), 10 mL/min]. White solid (21%, 2 steps). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.97 (3H, t, *J*=7.6 Hz), 1.89 (2H, m), 2.06 (2H, quint, *J*=7.6 Hz), 2.45 (2H, m), 2.78 (2H, t, *J*=6.3 Hz), 3.44 (2H, dd, *J*=1.3, 5.6 Hz), 5.27 (1H, m), 5.40 (1H, m), 5.50–5.69 (2H). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.1, 20.6, 24.4, 25.8, 29.6, 32.4, 36.7, 75.7, 119.4, 125.8, 132.7, 133.0, 170.4, 205.2. HRMS (ESI$^+$) *m*/*z* 253.1434 [M+Na]$^+$ (calcd for $C_{14}H_{21}O_4$, 253.1440).

4.5. Synthesis of (*Z*)-10-oxooctadec-12-enoic acid (7) and (*E*)-10-oxooctadec-11-enoic acid (8)

*4.5.1. Methyl 9-(oxiran-2-yl)nonanoate (**18**)*

A solution of **35** (10 g, 50.4 mmol), *m*-CPBA (17.3 g, 100.8 mmol), and saturated aq $NaHCO_3$ (40 mL) in CH_2Cl_2 (120 mL) was stirred for 6 h at rt. The CH_2Cl_2 layer was washed with saturated aq $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated under vacuum. The concentrate was purified by column chromatography (hexane–EtOAc, 9:1) to give **18** as a colorless oil (1.06 g, 43.9 mmol, 98%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.23–1.64 (14H), 2.31 (2H, t, *J*=7.5 Hz), 2.47 (1H, m), 2.76 (1H, dd, *J*=4.2, 4.6 Hz), 2.92 (1H, m), 3.67 (3H, s). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 24.9, 25.9, 29.0, 29.1, 29.2, 29.3, 32.4, 34.0, 47.1, 52.4, 51.4, 174.3. MS (ESI$^+$) *m*/*z* 214 [M+H]$^+$.

*4.5.2. Methyl 10-hydroxyoctadec-12-ynoate (**48**)*

Reaction procedure as in Section 4.2.6 was followed. Chromatography: hexane–EtOAc (9:1). Colorless oil (65%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.90 (3H, t, *J*=7.1 Hz), 1.23–1.64 (16H), 2.13–2.45 (6H), 3.67 (3H, s), 3.69 (1H, m). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 13.9, 18.7, 22.2, 24.9, 25.6, 27.7, 28.7, 29.0, 29.1, 29.3, 29.5, 31.0, 34.1, 36.1, 51.4, 70.2, 76.0, 83.2, 174.3. HRMS (ESI$^+$) *m*/*z* 333.2394 [M+Na]$^+$ (calcd for $C_{19}H_{34}NaO_3$, 333.2406).

*4.5.3. (Z)-Methyl 10-hydroxyoctadec-12-enoate (**49**)*

Reaction procedure as in Section 4.2.7 was followed. Chromatography: hexane–EtOAc (9:1). Colorless oil (71%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.88 (3H, t, *J*=6.6 Hz), 1.23–1.62 (12H), 2.05 (2H, m), 2.21 (2H, t, *J*=6.6 Hz), 2.30 (2H, t, *J*=7.6 Hz), 3.61 (1H, m), 3.67 (3H, s), 5.40 (1H, m), 5.57 (1H, m). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.0, 22.5, 24.9, 25.7, 27.4, 29.1, 29.2, 29.3, 29.4, 29.6, 31.5, 34.1, 35.4, 36.8, 51.4, 71.5, 125.1, 133.6, 174.3. HRMS (ESI$^+$) *m*/*z* 335.2545 [M+Na]$^+$ (calcd for $C_{19}H_{36}NaO_3$, 335.2562).

*4.5.4. (Z)-Methyl 10-oxooctadec-12-enoate (**50**)*

Reaction procedure as in Section 4.2.8 was followed. Chromatography: hexane–EtOAc (95:5). Orange oil (64%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.88 (3H, t, *J*=7.0 Hz), 1.23–1.64 (12H), 2.05 (2H, m), 2.30 (2H, t, *J*=7.6 Hz), 2.42 (2H, t, *J*=7.3 Hz), 3.15 (2H, d, *J*=6.2 Hz), 3.67 (3H, s), 5.51–5.62 (2H, m). MS (ESI$^+$) *m*/*z* 311 [M+H]$^+$.

*4.5.5. (Z)-10-Oxooctadec-12-enoic acid (**7**)*

Reaction procedure as in Section 4.2.10 was followed. Chromatography: Chromatography: HPLC [CAPCELL PAK UG120 20×250 mm, 39% C/(B+C), 10 mL/min]. Orange oil (22%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.89 (3H, t, *J*=6.9 Hz), 1.30–1.42 (14H), 1.53–1.65 (4H), 2.02 (2H, q-like), 2.34 (2H, t, *J*=7.6 Hz), 2.43 (2H, t, *J*=7.6 Hz), 3.15 (2H, d, *J*=5.9 Hz), 5.48–5.64 (2H, m), 6.47 (1H, br). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.0, 22.5, 23.7, 24.6, 27.5, 29.0 (C×3), 29.1 (C×2), 31.5, 33.9, 41.7, 42.2, 120.9, 133.7, 179.4, 209.4. HRMS (ESI$^+$) *m*/*z* 319.2246 [M+Na]$^+$ (calcd for $C_{18}H_{32}NaO_3$, 319.2249).

*4.5.6. (E)-10-Oxooctadec-11-enoic acid (**8**)*

Orange oil (27%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.89 (3H, t, *J*=6.6 Hz), 1.10–1.70 (20H), 2.22 (2H, q-like), 2.36 (2H, t, *J*=7.3 Hz), 2.54 (2H, t, *J*=7.3 Hz), 6.11 (1H, d, *J*=15.8 Hz), 6.86 (1H, dt, *J*=6.9, 15.8 Hz), 7.31 (1H, br). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.0, 22.5, 24.4, 24.6, 28.0, 28.8, 28.9, 29.0, 29.1, 29.2, 31.5, 32.5, 33.9, 39.9, 130.1, 148.5, 179.7, 202.2. HRMS (ESI$^+$) *m*/*z* 319.2251 [M+Na]$^+$ (calcd for $C_{18}H_{32}NaO_3$, 319.2249).

4.6. Synthesis of (12*Z*,15*Z*)-10-oxooctadeca-12,15-dienoic acid (9)

*4.6.1. Methyl 10-hydroxyoctadeca-12,15-diynoate (**51**)*

Reaction procedure as in Section 4.3.7 was followed. Chromatography: hexane–EtOAc (9:1, 8:2). Orange oil (51%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.12 (3H, t, *J*=7.6 Hz), 1.20–1.64 (14H), 2.17 (2H, m), 2.30 (2H, t, *J*=7.6 Hz), 2.38 (2H, m), 3.19 (2H, m), 3.67 (3H, s), 3.70 (1H, m). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 9.7, 12.3, 13.8, 24.9, 25.5, 27.7, 29.1 (C×2), 29.3, 29.4, 34.1, 36.2, 51.4, 70.1, 73.4, 76.6, 77.5, 82.0, 174.3. HRMS (ESI$^+$) *m*/*z* 329.2088 [M+Na]$^+$ (calcd for $C_{19}H_{30}NaO_3$, 329.2093).

*4.6.2. (12Z,15Z)-Methyl 10-hydroxyoctadeca-12,15-dienoate (**52**)*

Reaction procedure as in Section 4.2.7 was followed. Chromatography: hexane–EtOAc (8:2). Orange oil (77%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.97 (3H, t, *J*=7.6 Hz), 1.20–1.50 (12H), 1.62 (2H, t, *J*=7.0 Hz), 2.02–2.13 (4H), 2.19–2.35 (4H), 2.81 (2H, t, *J*=6.6 Hz), 3.63 (1H, m), 3.67 (3H, s), 5.25–5.60 (4H, m). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.2, 20.5, 24.9, 25.7 (C×2), 29.0, 29.1, 29.3, 29.5, 34.1, 35.3, 36.8, 51.4, 71.4, 125.5, 126.8, 131.4, 132.1, 174.3. HRMS (ESI$^+$) *m*/*z* 333.2405 [M+Na]$^+$ (calcd for $C_{19}H_{34}NaO_3$, 333.2406).

*4.6.3. (12Z,15Z)-Methyl 10-oxooctadeca-12,15-dienoate (**53**)*

Reaction procedure as in Section 4.2.8 was followed. Chromatography: hexane–EtOAc (9:1). Orange oil (88%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.98 (3H, t, *J*=7.6 Hz), 1.08–1.23 (8H), 1.56–1.65 (4H), 2.04 (2H, quint, *J*=7.6 Hz), 2.30 (2H, t, *J*=7.6 Hz), 2.42–2.52 (4H), 2.78 (2H, t, *J*=5.7 Hz), 3.18 (2H, m), 3.67 (3H, s), 5.31–5.59 (4H). HRMS (ESI$^+$) *m*/*z* 331.2248 [M+Na]$^+$ (calcd for $C_{19}H_{32}NaO_3$, 331.2249).

*4.6.4. (12Z,15Z)-10-oxooctadeca-12,15-dienoic acid (**9**)*

Reaction procedure as in Section 4.2.10 was followed. Chromatography: HPLC [CAPCELL PAK UG120 20×250 mm, 55% C/(B+C), 10 mL/min]. Colorless oil (71%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.98 (3H, t, *J*=7.6 Hz), 1.09–1.13 (8H), 1.57–1.63 (8H), 2.07 (2H, m), 2.35 (2H, t, *J*=7.6 Hz), 2.44 (2H, t, *J*=7.3 Hz), 2.78 (2H, t-like), 3.20 (2H, d, *J*=5.1 Hz), 5.28 (1H, m), 5.40 (1H, m), 5.51–5.63 (2H). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.1, 20.6, 23.7, 24.3, 25.7, 28.9 (C×2), 29.0, 29.1, 33.9, 41.6, 42.3, 121.2, 126.2, 131.8, 132.4, 179.7, 209.3. HRMS (ESI$^+$) *m*/*z* 317.2090 [M+Na]$^+$ (calcd for $C_{18}H_{30}NaO_3$, 317.2093).

4.7. Synthesis of 9-hydroxy-10-oxooctadecanoic acid (10)

To a solution of **3** (184 mg, 0.592 mmol) in Et_2O (5 mL), Pd/C (91 mg) was added at 0 °C. After stirring for 2 h at rt under H_2, the suspension was filtered with Celite, and the filtrate was concentrated under vacuum and purified by column chromatography

(hexane–EtOAc, 8:2) to give **10** as a white powder (100 mg, 0.318 mmol, 54%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.88 (3H, t, J=6.9 Hz), 1.24–1.63 (23H), 1.80 (1H, m), 2.34 (2H, t, J=7.6 Hz), 2.45 (2H, m), 4.16 (1H, m). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.1, 22.6, 23.6, 24.6, 24.8, 28.9, 29.0, 29.1, 29.2, 29.3, 31.8, 33.7, 33.9, 37.9, 76.3, 179.1, 212.5. HRMS (ESI$^+$) m/z 337.2351 $[M+Na]^+$ (calcd for $C_{18}H_{34}NaO_4$, 337.2355).

4.8. Synthesis of methyl (12*Z*,15*Z*)-9-hydroxy-10-oxooctadeca-12,15-dienoate (11)

To a solution of **3** (415 mg, 133 mmol) in MeOH (5 mL), a solution of (trimethylsilyl)diazomethane (2 M in hexane; 3 mL) was added dropwise and stirred for 5 min. After removing the solvent and reagent under vacuum, the resulting oil is purified by HPLC [CAPCELL PAK UG120 20×250 mm, 70% C/(B+C), 10 mL/min] to give **11** as an orange oil (345 mg, 1.06 mmol, 80%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.98 (3H, t, J=7.6 Hz), 1.26–1.40 (8H), 1.50 (1H, m), 1.61 (2H, m), 1.84 (1H, m), 2.07 (2H, m), 2.30 (2H, t, J=7.6 Hz), 2.78 (2H, dt, J=0.7, 6.3 Hz), 3.27 (2H, m), 3.67 (3H, s), 4.23 (1H, m), 5.28 (1H, m), 5.43 (1H, m), 5.55 (1H, m), 5.64 (1H, m). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.2, 20.6, 24.7, 24.8, 25.8, 29.0 (C×2), 29.2, 33.6, 34.0, 36.7, 51.4, 76.0, 120.0, 125.9, 132.5, 132.7, 174.2, 210.1. MS (ESI$^+$) m/z 325 $[M+H]^+$.

4.9. Synthesis of (12*Z*,15*Z*)-9,10-dihydroxyoctadeca-12,15-dienoate (12)

A solution of **3** (3 mg, 9.7 μmol) and $NaBH_4$ (1 mg, 24.6 μmol) in EtOH (300 μL) was stirred for 30 min at rt. The reaction was quenched with water (1.5 mL) and extracted with EtOAc (3×2 mL). The EtOAc layer was washed with 1 M HCl and brine, and dried over Na_2SO_4. After removal of solvent, **12** was obtained quantitatively as white solid. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.96 (3H, t, J=7.6 Hz), 1.33 (8H, m), 1.45 (2H, m), 1.59 (2H, m), 2.10 (2H, m), 2.14 (2H, t, J=7.6 Hz), 2.22 (1H, m), 2.34 (1H, m), 2.81 (2H, t, J=6.7 Hz), 3.43 (1H, m), 3.60 (1H, m), 5.30 (1H, m), 5.37 (1H, m), 5.43 (1H, m), 5.49 (1H, m). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.6, 21.5, 26.6, 27.0, 27.8, 30.6, 30.7, 30.8, 32.1, 34.2, 39.3, 74.6, 75.2, 127.3, 128.3, 130.9, 132.7, 177.8. MS (ESI$^+$) m/z 312 $[M+H]^+$.

4.10. Cycloaddition of fatty acid with NE/Epi

To a solution of fatty acids **3**–**12** (5 mg) in water (5 mL), NE/Epi (10 mM in water; 1.5 mL) and Tris–HCl buffer (1 M, pH 8.0, 7.5 mL) were added. The reaction was carried out at 25 °C for 15 h under O_2 atmosphere. After acidification of reaction mixture with 1% aq HCOOH, the products were extracted with EtOAc (3×10 mL). EtOAc layer was washed with brine and dried over Na_2SO_4. LC–PDA/MS analysis of the products was performed with following conditions: column, CAPCELL PAK UG120 2×75 mm; flow rate, 200 μL/min; solvent, 10–90% A/(A+C) for 15 min and thereafter 90% A/(A+C) within 5 min; temperature, 40 °C; MS, positive ion mode.

4.11. Flower induction assay

The flower inducing activity was measured according to the method described previously with some modifications.[4] All samples were dried and stored at −30 °C under N_2, and dissolved in EtOH immediately before use. All experiments were conducted with negative and positive controls. Positive control experiments were performed in the presence of 1 μM 6-benzylaminopurine. The final concentration of EtOH in bioassays was ≤0.03%. A three-frond colony of *L. paucicostata* 151 (P151, a gift from Professor O. Tanaka) was planted on E medium containing a test sample, and incubated on for 10 days at 25 °C under continuous light. The percentage of fronds with flowers was determined. All experiments were performed with three replicates and reproducibility was checked on different days.

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8. Yamaguchi et al.[5] reported that strong flowering induction was observed only when **1**, but not its C-9 epimer **2**, was assayed. However, we could not observe such a difference between these isomers with respect to biological activity. Compounds **1** and **2** purified by HPLC showed identical activity within a concentration tested. Therefore, we determined that **2** is also active to induce flowering in *L. paucicostata*.