Stereochemistry of Δ^4 dehydrogenation catalyzed by an ivy (*Hedera helix*) Δ^9 desaturase homolog

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The stereochemistry of palmitoyl-ACP Δ^4 desaturase-mediated dehydrogenation has been examined by tracking the fate of deuterium atoms located on stereospecifically monodeuterated substrates-(4*S*)- and (4*R*)-[4-$^2H_1$]-palmitoyl-ACP and (5*S*)- and (5*R*)-[5-$^2H_1$]-palmitoyl-ACP. It was found that the introduction of the (*Z*)-double bond between C-4 and C-5 of a palmitoyl substrate occurs with *pro-R* enantioselectivity—a result which matches that obtained for a closely related homolog-castor stearoyl-ACP Δ^9 desaturase. These data show that despite the difference in regioselectivity between the two enzymes, the stereochemistry of hydrogen removal is conserved.

Introduction

Fatty acid desaturases catalyze the highly selective, O_2-dependent, 1,2-dehydrogenation of lipidic substrates.[1] The most common example of this important biological reaction features the insertion of a C9–C10 double bond into long-chain fatty acids as shown in Scheme 1. Interestingly, a number of regiochemical variations of Δ desaturation have been identified in a wide variety of aerobic life forms (Scheme 1).[2] The occurrence of fatty acids with various double bond position or chain lengths is associated with cold acclimation, protection from herbivory and biological signaling.[3]

Elucidation of the structural determinants that control desaturase regioselectivity is an intriguing research problem of intrinsic interest in the area of protein engineering.[4] However, to date, only soluble stearoyl-ACP Δ^9 desaturases (ACP = acyl carrier protein) have been purified in sufficient amounts to permit analysis by X-ray crystallography.[5] Recently, a closely related homolog, palmitoyl-ACP Δ^4 desaturase (74% sequence identity), found in ivy (*Hedera helix* L.) with promising biophysical characteristics has been overexpressed.[6] The availability of two stable, structurally related desaturases with differing regioselectivities offers a unique opportunity to compare active site topographies. A critical element of such an investigation involves determining the enantioselectivity of desaturation for each enzyme in order to validate crystallographic models of substrate–enzyme complexes. In the case of the castor Δ^9 desaturase-mediated dehydrogenation, we have shown that it is the *pro-R* hydrogens at C-9 and C-10 of substrate that are removed.[7] In this paper, we extend this approach to the stereochemical analysis of the related Δ^4 desaturation.

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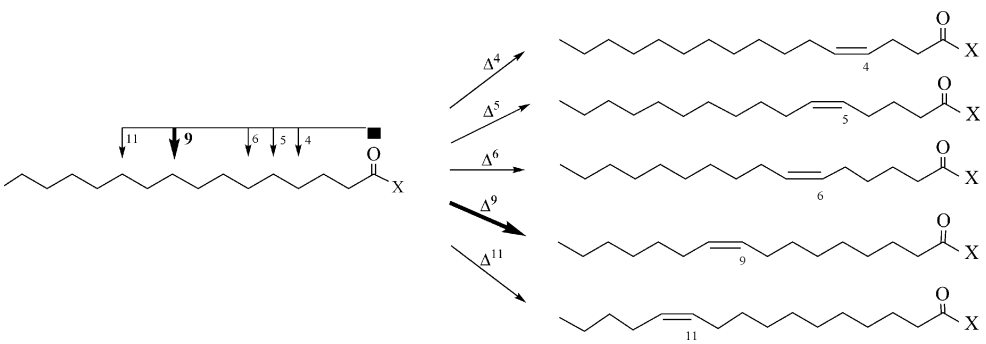
Results and discussion

The enantioselectivity of Δ^4 desaturase-mediated oxidation was determined by mass spectrometric examination of products derived from C-4,5-dehydrogenation of stereospecifically monodeuterated palmitoyl-ACP derivatives. The methodology for enzymatic preparation of palmitoyl-ACP derivatives from the corresponding carboxylic acid has been developed previously.[8] The synthesis of the required labeled palmitates (4*R*)-[4-$^2H_1$]-**1**, (4*S*)-[4-$^2H_1$]-**1**, (5*R*)-[5-$^2H_1$]-**1** and (5*S*)-[5-$^2H_1$]-**1** is shown in Scheme 2A,B. It should be noted that *de novo* synthesis of these compounds was required because naturally occurring palmitates bearing appropriately situated chiral functionality such as the mid-chain hydroxyl group are not available from natural sources. A number of routes to stereospecifically labelled fatty acids have been reported;[9] we decided to take advantage of the convenient Jacobson epoxide resolution methodology[10] to prepare the four required compounds (Scheme 2A,B). Thus chiral



Scheme 1 Some naturally occurring variations on the prototypical Δ^9 desaturation of hexadecanoate.

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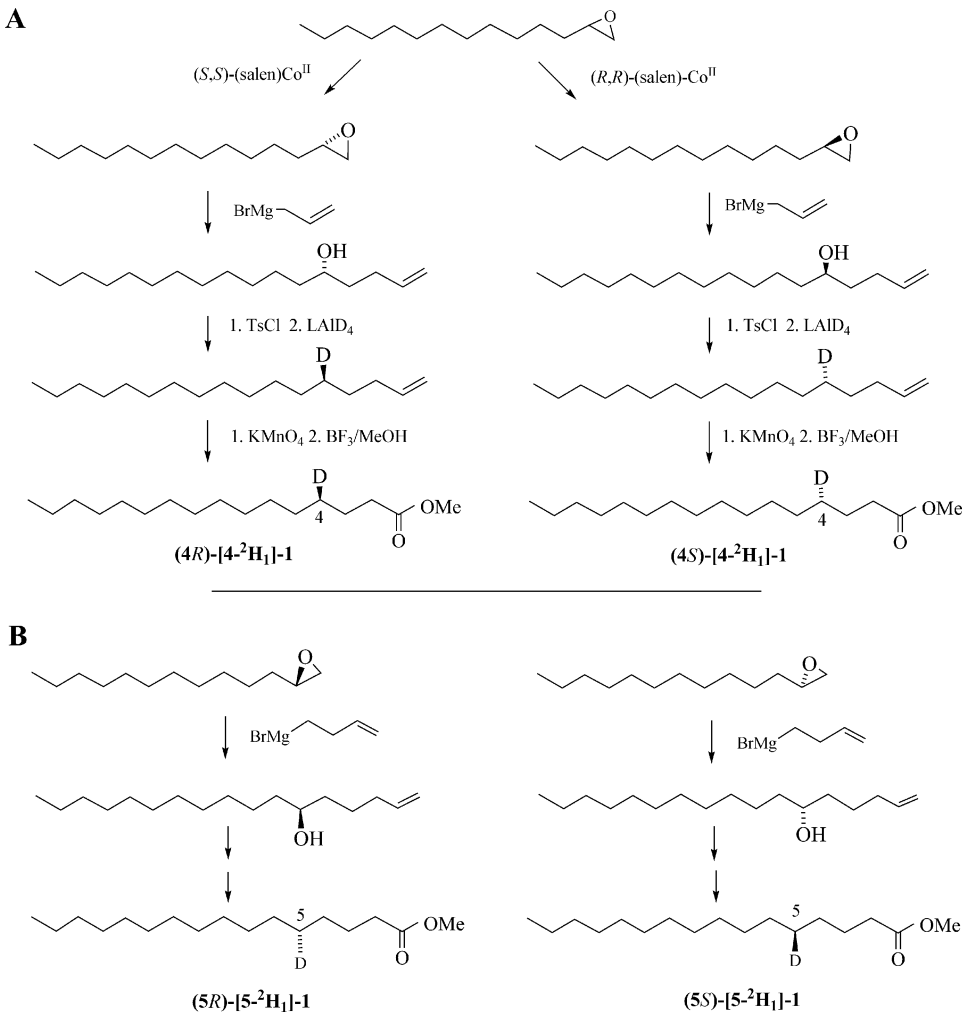
epoxides of appropriate chain length were opened with either allyl or 4-but-1-enyl Grignard to give two pairs of regioisomeric, hydroxyheptadec-1-enyl enantiomers. The enantiomeric purity of these intermediates was estimated to be >98% in each case as determined by examination of the ^{1}H NMR spectrum of the corresponding (*S*)-(+)-*O*-acetylmandelate derivatives[11] ($\Delta\delta^{RS}$ = 0.21 ppm for the ^{1}H NMR resonances assigned to the C-2 vinyl hydrogen). Stereospecific introduction of the monodeuterium label was achieved by a standard tosylation/$LiAlD_4$ displacement sequence. Mass spectrometric evaluation of the resultant monodeuterated terminal alkenes indicated that the isotopic purity of these compounds was very high (>98% d_1 species, Table 1). The desired monodeuterated palmitates were obtained by oxidative cleavage[12] of the corresponding heptadec-1-enes and purified by flash chromatography as the methyl esters. The analytical data for these compounds was in accord with the assigned structures; the location of the deuterium label in each case was confirmed by comparison of the ^{13}C NMR spectral data with that of unlabeled methyl palmitate (See Experimental section).

The ACP derivative of each enantiomer was prepared as previously described and incubated with ivy palmitoyl-ACP Δ^4 desaturase under conditions that maximized olefin production.



Scheme 2 Synthesis of stereospecifically monodeuterated palmitates.

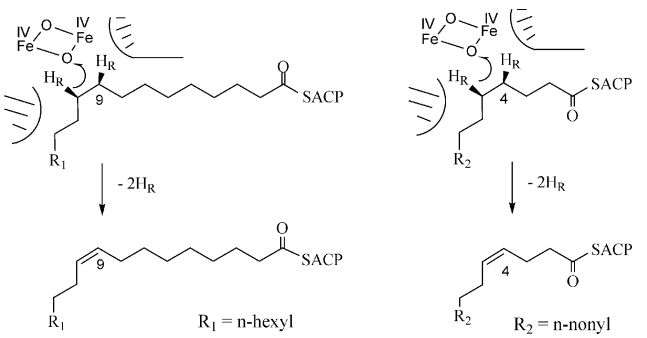
Table 1 Isotopic content[a] of stereospecifically monodeuterated palmitates and Δ^4 desaturated-products

	Substrates		Products		
	%d_0	%d_1	%d_0	%d_1	%Retention of label[b]
(4*R*)-[4-^{2}H$_1$]-**1**	0.9 ± 0.2	99.1 ± 0.2	99.4 ± 0.4	0.6 ± 0.4	0.6 ± 0.4
(4*S*)-[4-^{2}H$_1$]-**1**	1.1 ± 0.2	98.9 ± 0.2	2.4 ± 0.6	97.6 ± 0.5	98.7 ± 0.5
(5*R*)-[5-^{2}H$_1$]-**1**	1.0 ± 0.2	99.0 ± 0.2	98.9 ± 0.5	1.1 ± 0.5	1.1 ± 0.5
(5*S*)-[5-^{2}H$_1$]-**1**	1.3 ± 0.2	98.7 ± 0.2	3.5 ± 0.2	96.5 ± 0.2	97.8 ± 0.3

[a] Each incubation was repeated two times and the deuterium content is given as an average value ± standard deviation of three independent GC-MS analyses. [b] % Retention of label = [%d_1 (product)/(%d_1 (substrate) × 100].

In each case, the enzymatic ACP product was isolated as the corresponding methyl ester after treatment of the quenched reaction with sodium methoxide solution. GC-MS analysis of the organic extracts allowed determination of the deuterium content of the products. The mass spectrometric data is shown in Table 1 and clearly demonstrate that Δ^4 desaturation involves removal of the *pro-R* hydrogens at C-4 and C-5. That is, essentially complete loss of deuterium was observed upon desaturation of (*R*)-deuterated substrates while the olefinic product derived from (*S*)-labelled palmitates retained deuterium label to a very high degree.

The enantioselectivity displayed by the ivy Δ^4 enzyme is identical to that elucidated for the homologous castor Δ^9 desaturase.[7] Given the high degree of sequence homology, it is tempting to account for the observed conservation of stereochemical preference in terms of a common active site architecture in the region of the putative non-heme diiron dioxo oxidant[1] (Scheme 3). *In silico* analysis of the X-ray structure for castor Δ^9 desaturase[5] has shown that the diiron centre is proximal to the *pro-R* hydrogens of a docked stearoyl substrate residing in a bent hydrophobic binding pocket. Mechanistic studies using oxygen, sulfur-[13] and fluorine-substituted substrate analogues[7] have suggested that the C-10 *pro-R* hydrogen of stearoyl-ACP may be removed first in the castor Δ^9 desaturase-catalyzed reaction.[14] If the location of oxidant relative to bound substrate is strictly conserved as implied in Scheme 3, then one would predict that the site of initial oxidation would be C-5 for the Δ^4 desaturase-catalyzed reaction. Experiments designed to test this prediction are planned. It is hoped that the results of these efforts, together with X-ray crystallographic data of enzyme–substrate complexes can be used to gain more insight into this fascinating set of ultra selective reactions.



Scheme 3 Enantioselectivity of desaturation catalyzed by two structurally related, soluble desaturase homologs.

Conclusions

1. Stereospecifically monodeuterated palmitates can be prepared in high isotopic and enantiomeric purity from readily available chiral epoxides. This synthetic route constitutes a general approach to compounds of this type.

2. The stereochemistry of dehydrogenation mediated by two structurally related, soluble plant desaturases with differing regioselectivity has been compared for the first time. The Δ^4 palmitoyl desaturase isolated from English ivy (*Hedera helix* L.) removes the vicinal *pro-R* hydrogens from substrate to generate a (4*Z*)-palmitoyl product. Despite the difference in regioselectivity (Δ^4 *versus* Δ^9) between the ivy and castor desaturases, the observed enantioselectivity is strictly conserved.

Experimental

General methods

^{1}H and ^{13}C NMR spectra were obtained at 300 and 75.5 MHz respectively on a Brüker Avance 300 spectrometer with the use of dilute $CDCl_3$ solutions. Chemical shifts are expressed in ppm (δ) and are referenced to tetramethylsilane. *J*-values are reported in Hertz (Hz).

Mass spectra of synthetic intermediates were obtained by GC/MS using a Kratos 1H mass spectrometer coupled to a HP 5980 Series 2 gas chromatograph equipped with a J. & W. DB-5 capillary column (30 m × 0.21 mm), temperature programmed from 120 °C to 320 °C at 10 °C min^{-1}. GC-MS analysis of enzymatic products was carried out using a HP5973 mass spectrometer coupled to a HP6890 GC equipped with a SP2340 capillary column (60 m × 0.25 mm), temperature programmed from 100 °C to 160 °C at 25 °C min^{-1} and 160 °C to 240 °C at 10 °C min^{-1}. The isotopic content of analytes was estimated by scanning several times per GC peak; the integrated intensities of the individual ions in the pertinent ion cluster were analyzed with the use of HP-ChemStation software and have been corrected for natural isotopic abundances. Care was taken to include the entire GC peak in the integration procedure in order to prevent errors due to fractionation of isotopic species during chromatography.

Flash chromatography with silica gel (230–400 mesh) was used to purify all intermediates and substrates. Visualization of UV-inactive materials on silica gel TLC was accomplished by a combination of water spray or I_2 vapor as appropriate.

All reagents and starting materials for organic synthesis were purchased from Sigma-Aldrich and used without purification. Tetrahydrofuran (THF) and diethyl ether (Et_2O) were freshly distilled from Na-benzophenone ketyl. All air- and moisture-sensitive reactions were performed under N_2. Organic extracts were typically dried by gravity filtration through anhyd. Na_2SO_4 and solvents were evaporated *in vacuo* on a Büchi RE 111 Rotavapor.

All buffers and salts, NADH, BSA and other biochemicals were purchased from Sigma-Aldrich. Protein concentrations were measured by the method of Bradford[15] with the use of bovine serum albumin as standard protein. The purification of ivy palmitoyl-ACP Δ^4 desaturase and required cofactors and the synthesis of substrate ACP derivatives has been previously described.[6,8]

Synthesis of substrates

Preparation of chiral epoxides

1,2-Epoxytetradecane. To 1-tetradecene (3.92 g, 20 mmol) in dichloromethane (15 ml) in a RBF at 0 °C was added *m*-chloroperbenzoic acid (7.92 g, 55%, 45.4 mmol) dissolved in dichloromethane (300 mL). The solution was left to react for 18 h at 4 °C and then washed with sat. $NaHCO_3$ (3 × 75 mL), 10% NaOH (1 × 75 mL) and sat. NaCl (3 × 75 mL), dried and evaporated to give the title compound (4.06 g, 96%) as a colourless oil at room temperature. The analytical data for this compound

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is similar to that reported previously.[16] R_f 0.34 (5% EtOAc in hexanes); ^{1}H NMR δ 2.90 (m, 1H), 2.74 (dd, J 5.0 Hz, 4.1 Hz, 1H), 2.46 (dd, J 5.1 Hz, 3.0 Hz, 1H), 1.26–1.54 (m, 22H), 0.88 (t, J 6.8 Hz, 3H). ^{13}C NMR δ 52.41, 47.13, 32.51, 31.93, 29.68, 29.64, 29.64, 29.56, 29.56, 29.46, 29.36, 26.02, 22.69, 14.11; EI MS (rel. intensity) m/z 169 (1), 137 (2), 123 (4), 109 (12), 95 (31), 82 (56), 71 (100), 55 (88), 43 (89).

1,2-Epoxytridecane. From 1-tridecene and MCPBA. A colourless oil at room temperature.[17] Analytical data similar to that of 1,2-epoxytetradecane except for ^{13}C NMR δ 52.43, 47.11, 32.47, 31.89, 29.61, 29.60, 29.53, 29.53, 29.42, 29.32, 25.94, 22.66, 14.07; EI MS (rel intensity) m/z 155 (1), 137 (2), 123 (8), 109 (13), 95 (33), 82 (52), 71 (100), 55 (80), 43 (75).

(*S*)-(−)-1,2-Epoxytetradecane. Glacial acetic acid was added (62.5 μL, 1.10 mmol) to a solution of [(*S*,*S*)-*N*,*N*′-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminato(2−)] cobalt(II) (63 mg, 0.10 mmol) in dichloromethane (2 mL) and the solution was stirred 30 minutes and then concentrated *in vacuo* to give a crude brown solid. This residue was cooled to 0 °C and a solution of 1,2-epoxytetradecane (4.40 g, 20.7 mmol) in isopropanol (1 mL) was added. H_2O (375 μL, 21 mmol) was added dropwise with stirring. The reaction mixture was allowed to warm to room temperature; the reaction was allowed to proceed for 48 h. Hexanes (40 mL) were added to the product mixture and the turbid solution filtered to remove a solid precipitate. The filtrate was concentrated and purified by flash chromatography (2.5% EtOAc in hexanes) to yield the title compound (1.09 g, 5.15 mmol, 25%), a colourless oil at room temperature. R_f 0.34 (5% EtOAc in hexanes). All spectral data were similar to that of (*R*,*S*)-1,2-epoxytetradecane. $[\alpha]_D^{21} = -5.9°$ (c 1.36, $CHCl_3$).

(*R*)-(+)-1,2-Epoxytetradecane. From [(*R*,*R*)-*N*,*N*′-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminato(2−)] cobalt(II) and 1,2-epoxytetradecane. Colourless oil at room temperature. R_f 0.34 (5% EtOAc in hexanes); All spectral data were similar to that of (*R*,*S*)-1,2-epoxytetradecane. $[\alpha]_D^{21} = +5.8$ (c 1.48, $CHCl_3$) lit.[18] + 4.31° (c 1.42, $CHCl_3$).

(*S*)-(−)-1,2-Epoxytridecane. From [(*S*,*S*)-*N*,*N*′-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminato(2−)] cobalt(II) and 1,2-epoxytridecane. Colourless oil at room temperature. R_f 0.34 (EtOAc/hexanes 1 : 20). All spectral data were similar to that of (*R*,*S*)-1,2-epoxytridecane. $[\alpha]_D^{21} = -6.7$ (c 1.16, $CHCl_3$).

(*R*)-(+)-1,2-Epoxytridecane. From [(*R*,*R*)-*N*,*N*′-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminato(2−)] cobalt(II) and 1,2-epoxytridecane. Colourless oil at rt. R_f 0.34 (5% EtOAc in hexanes). All spectral data were similar to that of (*R*,*S*)-1,2-epoxytridecane. $[\alpha]_D^{21} = +6.5$ (c 1.65, $CHCl_3$).

Preparation of chiral alcohols

(*S*)-5-Hydroxy-1-heptadecene. To allyl magnesium bromide (1 M in THF, 6.6 mL, 6.6 mmol) at 0 °C was added, slowly with stirring, (*S*)-(−)-1,2-epoxytetradecane (906 mg, 4.27 mmol) dissolved in THF (1.5 mL), followed by dilithium tetrachlorocuprate solution (0.3 mL, 24 mg LiCl and 34 mg $CuCl_2$ in 2.5 mL THF). The mixture was allowed to warm to rt and stirred for 2 h. The reaction was quenched by the addition of crushed ice (5 g). The mixture was transferred to a separatory funnel, and the reaction vessel rinsed with Et_2O (10 mL) and 3 M HCl (3 mL). The aqueous layer was extracted with Et_2O (3 × 6 mL) and the combined organic layers were washed with 10% NH_4OH (3 × 6 mL), 10% $Na_2S_2O_3$ (1 × 4 mL), H_2O (2 × 4 mL) and sat. NaCl (1 × 5 mL). The solution was dried and concentrated *in vacuo* to yield the title compound (1.05 g, 4.14 mmol, 97%) as a low melting solid. Analytical data similar to that reported for corresponding racemic compound.[19] R_f 0.08 (5% EtOAc in hexanes); ^{1}H NMR δ 5.85 (ddt, J 17.1, 10.2, 6.8 Hz 1H), 5.05 (dm, J 17.1 Hz, 1H), 4.99 (dm, J 10.2 Hz, 1H,), 3.62 (m, 1H), 2.06–2.27 (m, 2H), 1.18–1.64 (m, 24H), 0.88 (t, J 6.6 Hz, 3H). ^{13}C NMR δ 138.68, 14.71, 71.53, 37.51, 36.48, 31.93, 31.10, 29.69, 29.69, 29.67, 29.66, 29.63, 29.63, 29.37, 25.64, 22.70, 14.22; EI MS (rel. intensity) TMS derivative m/z 311 (6, $[M - 15]^+$), 271 (54, $[TMSOCH–(CH_2)_9CH_3]^+$), 157 (100, $[TMSOCH–CH_2–CH_2–CH=CH_2]^+$); >98% ee (^{1}H NMR of (*S*)-(+)-*O*-acetylmandelate derivative[11]).

(*R*)-5-Hydroxy-1-heptadecene. From (*R*)-(+)-1,2-epoxytetradecane and allyl magnesium bromide. Low melting solid at rt. R_f 0.08 (5% EtOAc in hexanes). Analytical data similar to that reported for corresponding racemic compound.[19] >98% ee (^{1}H NMR of (*S*)-(+)-*O*-acetylmandelate derivative[11]).

(*S*)-6-Hydroxy-1-heptadecene. To a 3-necked RBF equipped with magnetic stir bar, N_2 inlet and two rubber septa, was added dry magnesium turnings (247 mg, 10.3 mmol). The vessel was flushed with N_2 for 5 min and 4-bromo-1-butene (1.15 g, 8.5 mmol) dissolved in THF (7 mL) was added. The mixture was refluxed until all metal was consumed (1.5 h) after which the solution was cooled to 0 °C. (*S*)-(−)-1,2-epoxytridecane (1.12 g, 5.63 mmol) dissolved in THF (1 mL) was slowly added to the reaction vessel *via* syringe, followed by dilithium tetrachlorocuprate solution (0.3 mL, 24.2 mg LiCl and 34 mg $CuCl_2$ in 2.5 mL THF). The mixture was allowed to warm to room temperature and stirred for 2 h. The reaction was quenched with crushed ice (5 g) and the resultant mixture transferred to a separatory funnel, rinsing the reaction vessel with Et_2O (7.5 mL) and 3 M HCl (3 mL). The aqueous layer was extracted with Et_2O (3 × 6 mL). The combined organic layers were washed with 10% NH_4OH (3 × 6 mL), 10% $Na_2S_2O_3$ (1 × 4 mL), H_2O (2 × 4 mL) and sat. NaCl (1 × 5 mL). The solution was dried and concentrated *in vacuo* to yield title compound (1.21 g, 85% yield) as a low melting solid. Analytical data similar to that reported in the literature[20] for this compound. R_f 0.08 (5% EtOAc in hexanes); ^{1}H NMR δ 5.81 (ddt, J 18, 10.5, 7.5 Hz 1H), 4.96–5.06 (dm, J 18 Hz, 1H), 4.92–4.99 (dm, J 9 Hz, 1H), 3.59 (m, 1H), 2.03–2.10 (m, 2H), 1.18–1.64 (m, 24H), 0.88 (t, J 6.6 Hz, 3H). ^{13}C NMR δ 138.77, 114.55, 71.84, 37.54, 36.88, 33.75, 31.92, 29.71, 29.67, 29.64, 29.64, 29.62, 29.35, 25.65, 24.93, 22.69, 14.11; EI MS (rel. intensity) TMS derivative m/z 311 (6, $[M - 15]^+$), 257 (93, $[TMSOCH–(CH_2)_8CH_3]^+$), 171 (29, $[TMSOCH–(CH_2)_3–CH=CH_2]^+$). >98% ee (^{1}H NMR of (*S*)-(+)-*O*-acetylmandelate derivative[11]).

(*R*)-6-Hydroxy-1-heptadecene. From (*R*)-(+)-1,2-epoxytridecane and 4-bromo-1-butene. A low melting solid. R_f 0.08 (5% EtOAc in hexanes). Analytical data similar to that reported for corresponding enantiomer.[20] >98% ee (^{1}H NMR of (*S*)-(+)-*O*-acetylmandelate derivative[11]).

Preparation of stereospecifically deuterated palmitates

Methyl (4*R*)-[4-$^2H_1$]-palmitate ((4*R*)-[4-$^2H_1$]-1). A solution of (*S*)-5-hydroxy-1-heptadecene (906 mg, 3.57 mmol) in dry pyridine (7.5 mL) was treated with TsCl (1.41 g, 7.14 mmol) at 0 °C. The solution was stirred for 1 h at 0 °C and left at 4 °C (3 days). The precipitated pyridinium hydrochloride salt was dissolved by adding H_2O (25 mL) to the reaction mixture and the aqueous layer extracted with Et_2O (3 × 30 mL). The combined organics were washed with sat. $CuSO_4$ (4 × 15 mL), H_2O (2 × 15 mL), 5% $NaHCO_3$ (2 × 15 mL), sat NaCl (1 × 15 mL), dried and concentrated *in vacuo* to give the tosylate (1.29 g) as a viscous oil. ^{1}H NMR δ 7.79 (d, *J* 9.0 Hz, 2H), 7.33 (d, *J* 9.0 Hz, 2H), 5.70 (m, 1H), 4.98 (dm, *J* 5.1 Hz, 1H), 4.92 (m, 1H), 4.57 (p, *J* 6.0 Hz, 1H), 2.44 (s, 3H), 1.94–2.06 (m, 2H), 1.61–1.73 (m, 2H), 1.15–1.33 (m, 22H), 0.88 (t, *J* 6.6 Hz, 3H). The tosylate intermediate (1.14 g, *ca.* 2.8 mmol) was dissolved in dry ether (5 mL) and lithium aluminium deuteride (372 mg, 8.8 mmol) was added; the reaction mixture was stirred for 5 h at rt under N_2. The reaction was quenched with H_2O (12 mL) and 6 M HCl (21 mL) and then extracted with Et_2O (3 × 30 mL). The combined ethereal layers were washed with H_2O (1 × 15 mL), dried and concentrated *in vacuo* to give the crude monodeuterated 1-heptadecene (686 mg) as a low melting solid: ^{1}H NMR δ 5.81 (ddt, *J* 18, 10.5, 7.5 Hz, 1H), 4.99 (dm, *J* 18 Hz, 1H), 4.92 (dm, *J* 9 Hz, 1H), 2.04 (dt, *J* 9.0, 6.0 Hz, 2H), 1.26 (br s, 25 H), 0.88 (t, *J* 6.0, 3H); MS (EI, 70 eV) *m/z* 239 (M^+), 211 ($M^+ - 28$). A portion of the monodeuteroheptadec-1-enyl intermediate so obtained (289 mg, *ca.* 1.26 mmol) was oxidized with aqueous $KMnO_4$ (800 mg, 5 mmol in 19 mL) containing hexadecyltributyl phosphonium bromide (11 mg) for 5 h at rt with vigorous stirring. The product mixture was treated with sodium sulfite (1.5 g) in 15 mL 4 M HCl and then extracted with hexanes (3 × 30 mL) followed by washing with H_2O, (1 × 20 mL) and sat. NaCl (1 × 20 mL) to give the crude carboxylic acid (319 mg) after drying and evaporation of the organic layer. A portion of this material (232 mg) was subsequently methylated by BF_3/MeOH and purified by flash chromatography (2.5% EtOAc/hexanes) to give the title compound as a white solid (125 mg, 0.46 mmole, 44% overall yield from (*S*)-5-hydroxy-1-heptadecene). R_f 0.18 (2.5% EtOAc in hexanes). ^{1}H NMR δ 3.66 (s, 3H), 2.30 (t, *J* 7.4, 2H), 1.61 (m, 2H,), 1.26 (br s, 23H), 0.88 (t, *J* 6.7, 3H); ^{13}C NMR δ 174.37, 51.44, 34.11, 31.94, 29.71, 29.71, 29.71, 29.67, 29.67, 29.62, 29.45, 29.38, 28.77 (C4, t, J_{CD} 19 Hz, upfield α-deuterium isotope shift[21] (0.41 ppm)), 29.18 (C5, upfield β-deuterium isotope shift (0.1 ppm)) 24.88 (C-3, upfield β-deuterium isotope shift (0.1 ppm)), 22.71, 14.14; MS (EI, 70 eV) *m/z* 271 (M^+), 240 ($M^+ - CH_3O$).

Methyl (4*S*)-[4-$^2H_1$]-palmitate ((4*S*)-[4-$^2H_1$]-1). From (*R*)-5-hydroxy-1-heptadecene. Obtained as a white solid. The spectral data of the title compound was identical to that of corresponding (*R*)-enantiomer.

Methyl (5*R*)-[5-$^2H_1$]-palmitate ((5*R*)-[5-$^2H_1$]-1). From (*S*)-6-hydroxy-1-heptadecene. Obtained as a white solid. The spectral data of the title compound were similar to those reported for the isotopomer above except for: ^{13}C NMR δ 174.36, 51.44 34.13, 31.94, 29.70, 29.70, 29.70, 29.69, 29.67, 29.67, 29.38 ((C6, upfield β-deuterium isotope shift (0.1 ppm)) 29.38 (C13), 28.86 (C5, t, J_{CD} 19 Hz, upfield α-deuterium isotope shift (0.4 ppm)), 29.07 (C4, upfield β-deuterium isotope shift (0.1 ppm)) 24.95, 22.71, 14.13.

Methyl (5*S*)-[5-$^2H_1$]-palmitate ((5*S*)-[5-$^2H_1$]-1). From (*R*)-6-hydroxy-1-heptadecene. Obtained as a white solid. The spectral data of the title compound was identical to that of the corresponding (*R*)-enantiomer.

Desaturase assay. Reactions of acyl-ACP derivatives with Δ^4 desaturase were carried out at room temperature. Each reaction mixture consisted of ivy Δ^4 desaturase dimer (2.4 nmol), dithiothreitol (400 nmol), bovine liver catalase (16 nmol), *Anabaena* vegetative ferredoxin (4.7 nmol), maize root NADPH:ferredoxin reductase (0.3 nmol), acyl-ACP (10.6 nmol) in a total volume of 660 μL of buffer. The reaction was initiated by the addition of NADPH (0.7 μmol) in buffer (32 μL) and allowed to continue for 30 min. The reaction was terminated with the addition of toluene (1 mL) and the thioester linkage was transesterifed to give the corresponding methyl ester with freshly prepared 0.5 M NaOMe at 55 °C for 30 min. The residue was acidified with acetic acid (100 μL) and extracted with hexane (2 × 2 mL). The phases were separated by centrifugation and the combined organics were evaporated under a steady stream of N_2 and the residue was diluted with 100 μL of hexane for analysis by GC-MS.

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