Stereochemistry of 10-sulfoxidation catalyzed by a soluble Δ^9 desaturase†

Amy E. Tremblay,[a] Nigel Tan,[a] Ed Whittle,[b] Derek J. Hodgson,[c] Brian Dawson,[c] Peter H. Buist*[a] and John Shanklin*[b]

Received 19th October 2009, Accepted 5th December 2009
First published as an Advance Article on the web 7th January 2010
DOI: 10.1039/b921753c

The stereochemistry of castor stearoyl-ACP Δ^9 desaturase-mediated 10-sulfoxidation has been determined. This was accomplished by ^{19}F NMR analysis of a fluorine-tagged product, 18-fluoro-10-thiastearoyl ACP S-oxide, in combination with a chiral solvating agent, (*R*)-AMA. Sulfoxidation proceeds with the same stereoselectivity as hydrogen removal from the parent stearoyl substrate. These data validate the use of thia probes to determine the stereochemistry and cryptoregiochemistry of desaturase-mediated oxidations.

Introduction

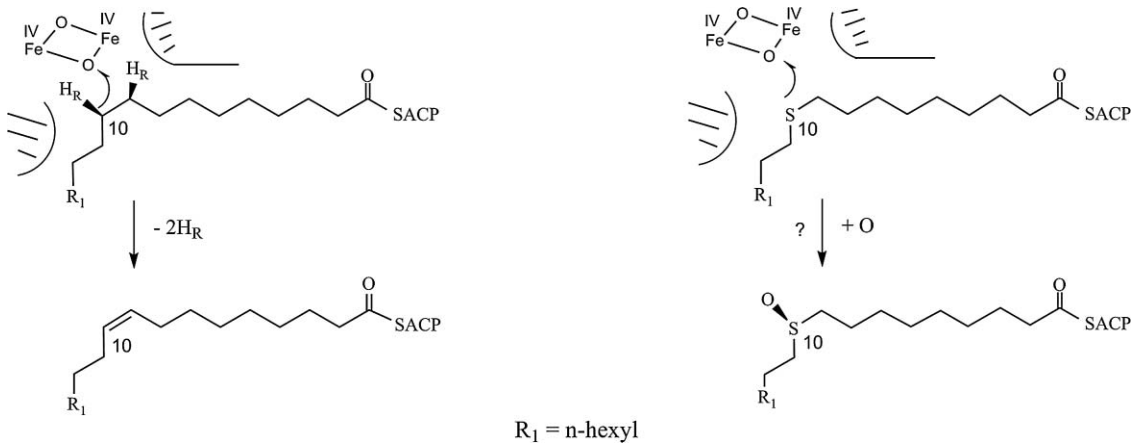
The desaturase family of enzymes catalyze the highly selective, O_2-dependent, dehydrogenation of lipidic substrates and play critically important roles in a diverse array of biological functions.[1] These include cold acclimation, biodefence and biosignaling. Desaturases, like the closely related hydroxylases, are capable of attacking a variety of unactivated C–H bonds. A prototypical example of this intriguing biological chemistry is the pro *R*-selective 9,10-desaturation of long-chain fatty acid derivatives by soluble plant stearoyl ACP Δ^9 desaturases (Scheme 1A).[2] An identical reaction, which is also pro *R*-selective, is catalyzed by ubiquitous, membranous Δ^9 desaturases as exemplified by the yeast stearoyl CoA desaturase.[3] The soluble and membrane-bound subclasses of desaturases are not structurally related with respect to primary sequence, but are thought to share a common, non-heme diiron catalytic core.[4]

A critically important mechanistic issue that relates to all desaturases involves determining the order of hydrogen removal—the site of initial oxidation (cryptoregiochemistry).[5] In the case of membrane-bound Δ^9 desaturases, it was readily determined, through the use of KIE methodology, that C9–H cleavage preceded C10–H bond rupture.[5] This result was corroborated by showing that the putative diiron dioxo oxidant oxygenated a 9-thia substrate analogue more efficiently than the corresponding 10-thiaoctadecanoyl CoA ester.[6] The notion that the enzymatic oxidant is located near C-9 was further strengthened by demonstrating that the stereochemistry of yeast (membranous) Δ^9 desaturase-mediated sulfoxidation matched that of H-removal for the parent reaction.[7] In contrast, it has been found that soluble castor stearoyl ACP Δ^9 desaturase sulfoxidizes S-10 substrate analogues efficiently (Scheme 1B) whereas S-9 analogues primarily undergo chain cleavage with this enzyme, presumably by C–H activation at C-10.[8] Taken together, these and other results[2,9] point to abstraction of the C-10 H_R substrate as the initial event for soluble Δ^9 desaturase oxidation of the parent stearoyl substrate. A critical test of this interpretation would be to determine the stereochemistry of soluble Δ^9 desaturase-mediated 10-sulfoxidation. This is a nontrivial task in the case of soluble desaturases since typically only nanomole amounts of enzymatic product are available for analysis. In this paper, we report on how our recently developed ^{19}F NMR methodology,[10,11] in combination with the appropriate chiral NMR solvating agent, was used to solve this problem.

Results and discussion

The stereochemical analysis of desaturase-mediated sulfoxidation requires a determination of the absolute configuration of the sulfoxy product. Previously, enantiomers of fatty acid sulfoxides could be distinguished using ^{1}H or ^{13}C NMR analysis in combination with a chiral solvating reagent, (*S*)-(+)-MPAA. Differential shielding effects on ^{1}H or ^{13}C reporter atoms could be evaluated using a Pirkle-type binding model[12] that had been validated with synthetic chiral reference standards (Fig. 1A).[7,13] This approach was feasible because milligram amounts of analyte could be produced using a convenient *in vivo* desaturating system.[6] However, to analyze the nanomole amounts of sulfoxide produced by *in vitro* stearoyl ACP Δ^9 desaturase oxidations, use of ^{1}H-decoupled ^{19}F NMR and a fluorine-tagged substrate appeared to be the method of choice. This methodology features several advantages, namely, the lack of naturally occurring interferences, inherently high sensitivity and wide chemical shift range. Indeed, we have been able to monitor the sulfoxidation of 18-fluoro-thiaoctadecanoyl ACP derivatives at the nanomole level of detection.[8b,e] To apply our stereochemical methodology in this case, we require that the shielding effects of a Pirkle-type solvating agent (Fig. 1A) extend out to the CH_2F reporting group located several carbons away from the sulfoxy center. A systematic study was carried out that involved testing four chiral H-bonding aromatic compounds (Fig. 1B) with a set of analytes of varying chain length, $(CH_3(CH_2)_nCH_2S(O)(CH_2)_7CO_2Me$. We chose to use 9-sulfoxy fatty acids in these experiments since the corresponding 9-thia precursors were readily synthesized from commercially available 8-bromooctanoic acid. As is clear from Fig. 1C, the

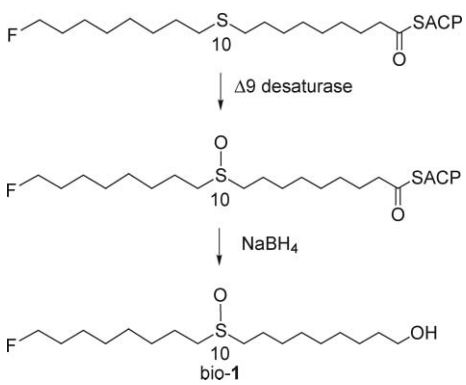
[a]*Department of Chemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario, Canada K1S 5B6*
[b]*Brookhaven National Laboratory, Department of Biology, Upton, NY 11973, USA*
[c]*Centre for Biologics Research, Biologics and Genetic Therapies Directorate, Health Canada, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2*
† Electronic supplementary information (ESI) available: Experimental details and characterization data. See DOI: 10.1039/b921753c



Scheme 1 (**A**) Δ^9 Desaturation of stearoyl ACP. (**B**) Δ^9 Desaturase-mediated sulfoxidation of 10-thiastearoyl ACP.

ability to use the terminal methyl group to discriminate between sulfoxide enantiomers ($\delta\Delta$) falls off uniformly as a function of increasing distance between the reporting atom and sulfoxy center. Importantly, it appeared that use of (*S*) or (*R*)- AMA ((9-anthryl) methoxy acetic acid) would offer the best chance of success in the stereochemical analysis of an enzymatically produced 18-fluoro-10-sulfoxide ($n = 6$, where $n + 1$ = number of methylene groups between reporter group and sulfoxy centre).

Sufficient quantities of sulfoxy analyte were obtained by scaling up (15×) a typical stearoyl ACP Δ^9 desaturase assay in order to convert approximately 500 nmol of 18-fluoro-10-thiaoctadecanoyl ACP to the corresponding sulfoxide (Scheme 2). The product was isolated from the enzymatic mixture as the primary alcohol (bio-**1**, 18-fluoro-10-thiaoctadecan-1-ol S-oxide) by methylene chloride extraction after reductive ($NaBH_4$) workup. Evaporation of the combined methylene chloride extracts yielded a residue that was taken up in $CDCl_3$ (1 mL) and analyzed by ^{1}H-decoupled ^{19}F NMR, and shown to exhibit a signal with adequate signal to noise, at the expected chemical shift (–218.375 ppm, reference standard[14]) (Fig. 2A). The appearance of this ^{19}F resonance did not change (Fig. 2B) upon the addition of (*R*)-AMA (50 mg ml^{-1})—an amount, which in a parallel trial experiment, resulted in induced nonequivalence ($\delta\Delta = 0.005$ ppm) of the ^{19}F resonances for a racemic, synthetic sample of **1**[14] (Fig. 3A). These results indicated that the enzymatic product, bio-**1**, was of high enantiomeric purity.



Scheme 2 Desaturase-mediated sulfoxidation of 18-fluoro-10-thiaoctadecanoyl ACP.

The absolute configuration of bio-**1** was inferred by spiking the biological sample with an approximately equimolar amount of racemic **1** (Fig. 2C). The resonance of highest intensity, belonging to bio-**1**, can be assigned to the *S* enantiomer[15] by application of the Pirkle-type binding model combined with our recent discovery[11] that ^{19}F NMR resonances, unlike ^{1}H NMR resonances, are shifted *downfield* under the influence of the proximal aromatic ring of aromatic acids such as MPAA and AMA (Fig. 4).

Further support for our stereochemical assignment was obtained by synthesizing samples of (*R*)- and (*S*)-**1** as authentic reference samples. These were synthesized from 1-bromo-8-fluorooctane (Scheme 3) using the method of Alcudia *et al.*[16] The key intermediate in this synthetic sequence was diastereomerically enriched DAG-(*S*)- or (*R*)-ω-fluorobutanesulfinates (de = 66 and 82%, respectively) (DAG = diacetone-D-glucose). The absolute configuration of the sulfinates was confirmed by a comparison of the chiroptical and NMR data (characteristic ^{1}H resonances (H2) and ^{13}C resonances (C1–C4) for these compounds with that of literature values reported for the two diastereomeric DAG n-propylsulfinates[16] and DAG n-butylsulfinates.[17] Treatment of DAG-(*S*)- or (*R*)-ω-fluorooctanesulfinates with 3 equivalents of 8-OTBS-1-nonylmagnesium bromide yielded, after deprotection, enantiomerically enriched samples of (*R*)- and (*S*)-**1** respectively. This substitution reaction is known to proceed with inversion of configuration at the sulfinyl centre of the DAG-alkylsulfinates.[16]

With reference standards of known absolute configuration in hand, we were able to simulate the ^{19}F NMR spectrum of the spiked biological sample (Fig. 2C) using samples of known enantiomeric composition. The ^{1}H-decoupled ^{19}F NMR spectrum of (*R*)-3 and (*S*)-**1** (2 : 1 ratio) or (*R*)-3 and (*S*)-**1** (1 : 2 ratio), to which (*R*)-AMA (4 equivalents) was added, are shown in Fig. 3B and C, respectively. A comparison of these spectra with the spectrum shown in Fig. 2C clearly demonstrates that enzymatically produced **1** bears the *S* configuration.

Conclusions

1. The enantioselectivity of soluble castor Δ^9 stearoyl ACP-mediated 10-sulfoxidation matches that of hydrogen removal for the parent reaction (compare Scheme 1A and 1B). This result validates the use of thia probes to report on the stereochemistry

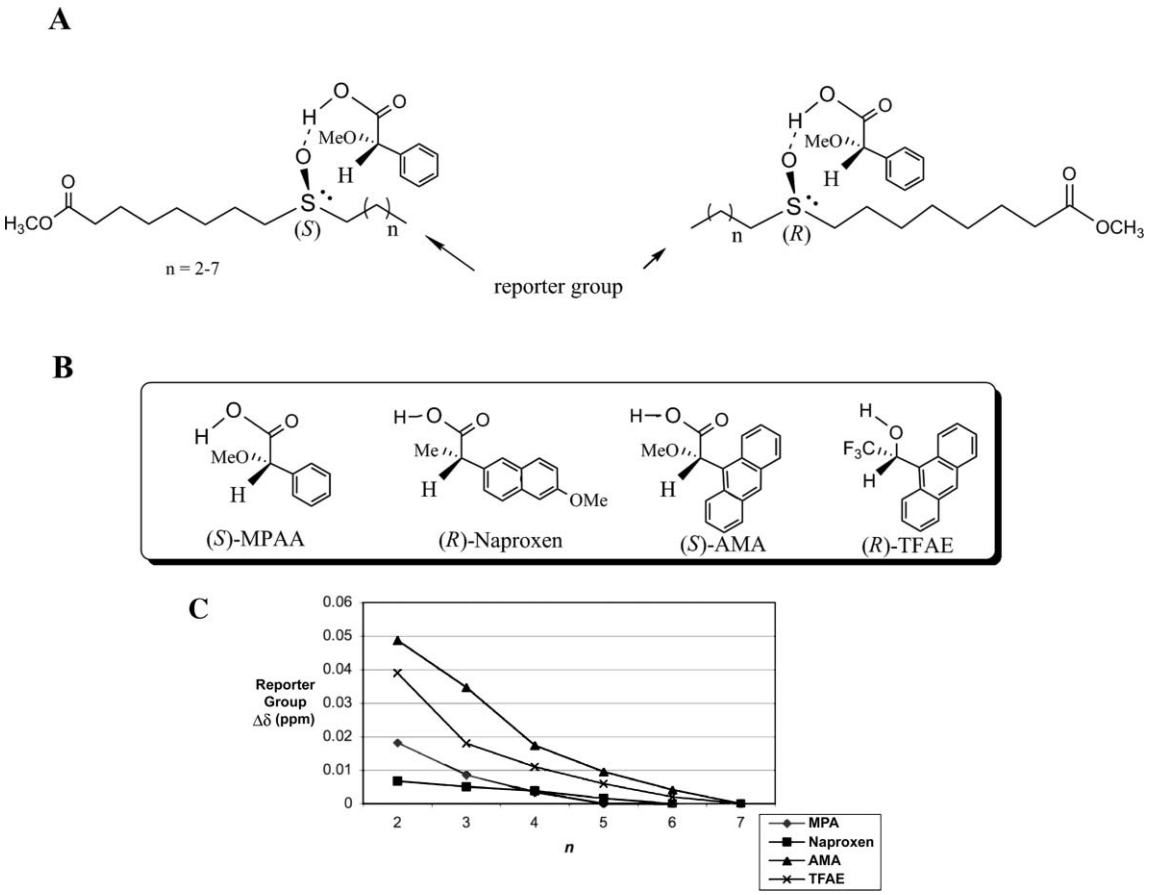


Fig. 1 (**A**) Pirkle-type complexation model featuring differential shielding effects of (*S*)-(+)-MPAA on ^{1}H NMR signals of a methyl reporter group; (**B**) various chiral solvating agents that are useful in probing stereochemistry of fatty acid sulfoxides; (**C**) magnitude of induced non-equivalence as a function of the number of methylene units (*n*) between the reporting group and C10 of 9-sulfoxide.

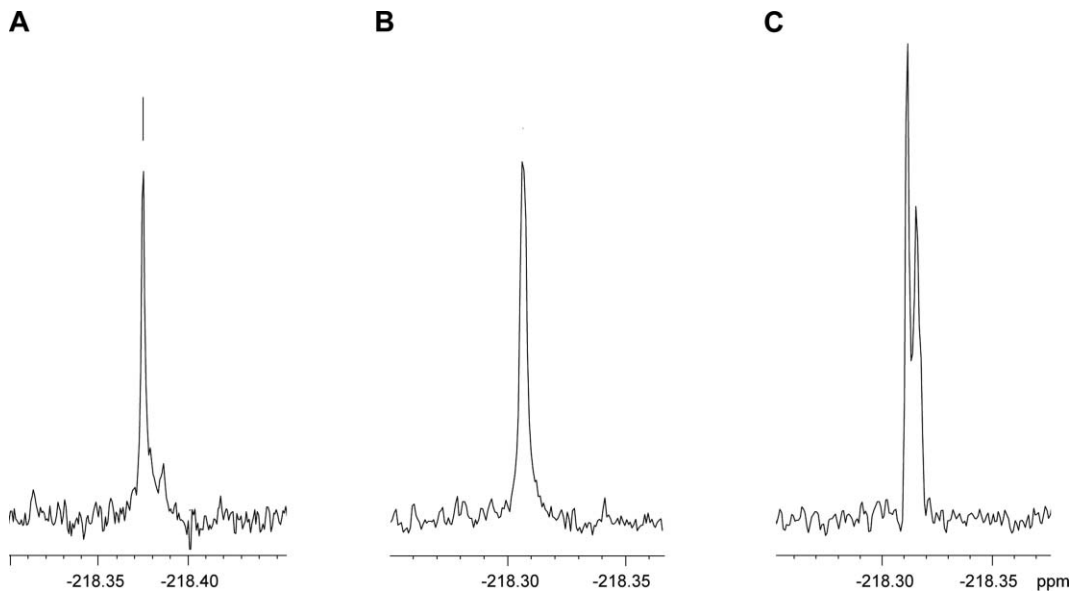


Fig. 2 ^{1}H-decoupled ^{19}F NMR signal of (**A**) enzymatically generated sample of 18-fluoro-10-thiaoctadecan-1-ol (*bio*-**1**); (**B**) (*bio*-**1**) + (*R*)-AMA (50 mg mL^{-1}); (**C**) (*bio*-**1**) spiked with *racemic* **1** + (*R*)-AMA (50 mg mL^{-1}).

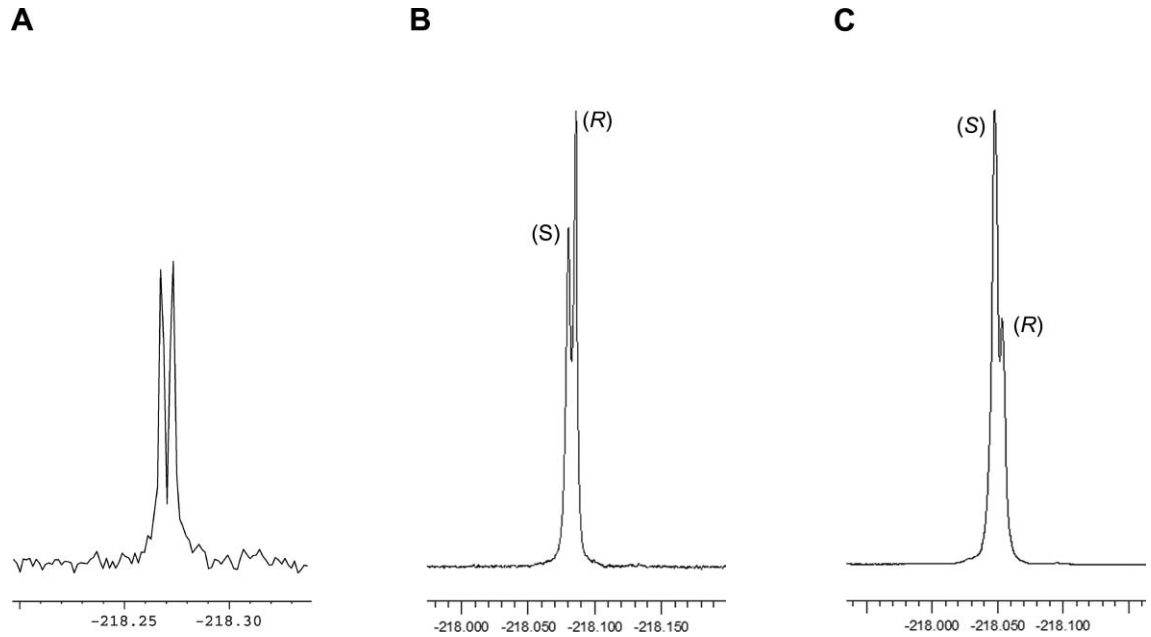


Fig. 3 ^{1}H-decoupled ^{19}F NMR signal of (**A**) racemic 18-fluoro-10-thiaoctadecan-1-ol (*racemic*-**1**); (**B**) (*R*)-**1** : (*S*)-**1**, 2 : 1 mixture + (*R*)-AMA; (**B**) (*R*)-**1** : (*S*)-**1**, 1 : 2 mixture + (*R*)-AMA.

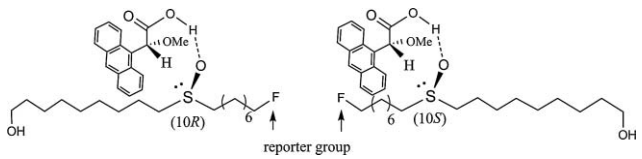


Fig. 4 Pirkle-type binding model showing collision complex between (*R*)-AMA and enantiomers of 18-fluoro-10-thia-octadecan-1-ol.

of desaturase-mediated reactions and gives valuable information on the topology of the active site.

2. ^{1}H-decoupled ^{19}F NMR in combination with the use of fluorine-tagged substrates and the appropriate chiral solvating agent constitutes powerful methodology for stereochemical analysis of *in vitro* desaturase-mediated sulfoxidation at the nanomole level of detection (~1000 fold increase in sensitivity). This approach may have application to other enzymatic transformations in cases where fluorine tagging is feasible.

3. The scope and limitations of Pirkle-type NMR solvating agents in probing sulfoxide stereochemistry have been delineated. Thus, it appears that reagents bearing phenyl, 2-naphthyl or 9-anthryl substituents are able to induce nonequivalence of reporter groups that are situated up to 7 carbons from the sulfinyl centre. Use of solvating agents with larger aromatic ring systems would potentially extend the range at which the fluorine tag can function.

Experimental

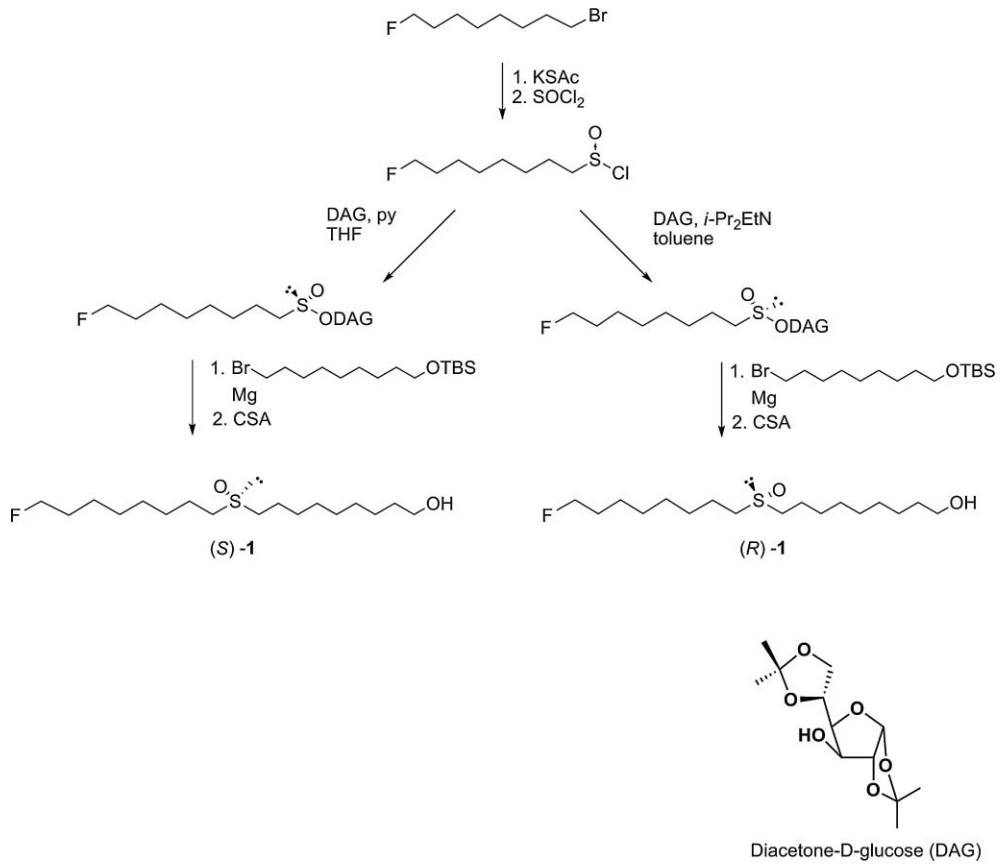
General methods

Routine ^{1}H and ^{13}C NMR spectra were obtained at 300.2 and 75.5 MHz respectively on a Brüker Avance 300 spectrometer. Some ^{13}C NMR spectra were recorded at 53.6 MHz on a Gemini 200 spectrometer as indicated. Preliminary ^{1}H NMR experiments involving chiral NMR solvating agents were carried out using a Brüker AMX 400 spectrometer while the corresponding work using ^{1}H-decoupled ^{19}F NMR was accomplished at 282.4 MHz on the Brüker Avance 300 spectrometer. ^{1}H-decoupled ^{19}F NMR spectra of biological samples were recorded on a Brüker AM 400 instrument at 376.5 MHz; ^{1}H and ^{13}C chemical shifts are expressed in ppm (δ) and are referenced to tetramethylsilane. ^{19}F NMR chemical shifts (ppm) are referenced to external $CFCl_3$. *J*-values are reported in Hertz (Hz). The spectra of all samples were recorded as dilute $CDCl_3$ solutions.

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 to 320 °C at 10 °C min^{-1}. GC-MS analysis of the enzymatic reaction (disappearance of starting material) 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 to 160 °C at 25 °C min^{-1} and from 160 to 240 °C at 10 °C min^{-1}.

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. 8-Thiooctanoic acid was prepared from 8-bromooctanoic acid as previously described.[18] 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 anh. Na_2SO_4, and solvents were evaporated *in vacuo* on a Büchi RE 111 Rotavapor.



Scheme 3 Synthesis of (*R*)- and (*S*)-18-fluoro-10-thiaoctadecan-1-ol.

Chiral solvating reagents, MPAA, Naproxen and TFAE, are available from Sigma-Aldrich. (*R*)- and (*S*)-AMA were kindly provided by Dr Jose Abad (IIQAB. CSIC. Barcelona).

All buffers and salts, NADH, BSA and other biochemicals were purchased from Sigma-Aldrich. Protein concentrations were measured by the method of Bradford[19] with the use of bovine serum albumin as standard protein. The purification of castor stearoyl-ACP Δ^9 desaturase and required cofactors and the synthesis of substrate ACP derivatives has been previously described.[20] The ACP used was Spinach ACP isoform I.[20c]

Synthesis of diastereomerically enriched fluorine-tagged alkyl sulfinates

1-Bromo-8-fluorooctane. To (diethylamino)sulfur trifluoride (DAST) (2.57 ml, 3.16 g, 19.6 mmol) at room temperature under N_2, was added 8-bromo-1-octanol (1.68 ml, 2.05 g, 9.8 mmol) dropwise. The solution was heated to 50 °C for 4 h with stirring, quenched with ice water (150 ml) and extracted with CH_2Cl_2 (3 × 50 ml). The combined extracts were washed with sat. $NaHCO_3$ (3 × 30 ml) and H_2O (50 ml), then dried (Na_2SO_4) and filtered through Florisil. The solvent was removed by roto-evaporation to obtain the title compound (1.97 g, 9.3 mmol, 95%) as an amber liquid. TLC (EtOAc–hexane 15 : 85): R_f 0.63. B.p. 120 °C (22.5 mm Hg) (lit 118–120 °C (22.5 mm Hg)[21]). IR (film): 2933, 2857, 1464, 1247, 1047, 1002, 724, 645 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.43 (*dt*, $^2J_{HF}$ = 47.3, $^3J_{HH}$ = 6.2, 2H), 3.41 (*t*, *J* = 6.8, 2H), 1.86 (*p*, *J* = 7.1, 2H), 1.69 (*dm*, $^3J_{HF}$ = 24.9, 2H), 1.34-1.50 (*m*, 8H). ^{13}C-NMR (75 MHz, $CDCl_3$): 84.12 (*d*, *J* = 163.1), 33.90, 32.75, 30.35 (*d*, *J* = 19.2), 29.03, 28.63, 28.05, 25.08 (*d*, *J* = 5.4). ^{19}F NMR (282.4 MHz, $CDCl_3$): –218.30.

Thioacetic acid *S*-(8-fluoro-octyl) ester. To a solution of potassium thioacetate (1.08 g, 9.5 mmol) in anh. DMF (25 ml) was added 1-bromo-8-fluorooctane (1.97 g, 9.3 mmol) dissolved in DMF (15 ml) under N_2 at room temperature. After stirring at room temperature for 15 h, the reaction mixture was diluted with CH_2Cl_2 (100 ml), washed with dH_2O (5 × 60 ml), sat. NaCl (75 ml), dried (Na_2SO_4) and roto-evaporated. The resulting oil was then partitioned between hexanes (2 × 20 ml) and dH_2O (20 ml), and the combined organic layers were dried (Na_2SO_4) and evaporated to give the title compound (1.87 g, 9.1 mmol, 97%) as an amber oil. TLC (EtOAc–hexane 15 : 85): R_f 0.54. IR (film): 2932, 2857, 1693 (C=O), 1464, 1430, 1354, 1135, 1047, 1003, 956, 725, 627 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.41 (*dt*, $^2J_{HF}$ = 47.3, $^3J_{HH}$ = 6.2, 2H), 2.85 (*t*, *J* = 7.3, 2H), 2.31 (*s*, 3H), 1.5-1.8 (*m*, 4H), 1.23–1.45 (*m*, 8H). ^{13}C-NMR (75 MHz, $CDCl_3$): 195.94, 84.09 (*d*, *J* = 163.2), 30.58, 30.33 (*d*, *J* = 19.3), 29.43, 29.06, 29.02, 28.93, 28.63, 25.05 (*d*, *J* = 5.4). ^{19}F NMR (282.4 MHz, $CDCl_3$): –218.27.

8-Fluorooctanesulfinyl chloride. Thioacetic acid *S*-(8-fluoro-octyl) ester (1.84 g, 8.9 mmol) and acetic anhydride (0.84 ml, 0.91 g, 8.9 mmol) were combined in a 3-neck RBF equipped with a magnetic stirrer bar and N_2 inlet. The reaction vessel was cooled to –10 °C and sulfuryl chloride (1.44 ml, 2.40 g, 17.8 mmol) was added dropwise over a half hour. Stirring was continued for another half hour at –10 °C. The solution was roto-evaporated to

give the title compound (1.88 g, crude) as an amber oil: 4.44 (*dt*, $^2J_{HF} = 47.3$, $^3J_{HH} = 6.1$, 2H), 3.39 (*m*, 2H), 1.93 (*m*, 2H), 1.6-1.8 (*m*, 2H), 1.3-1.6 (*m*, 8 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 84.08 (*d*, $J = 163.3$), 64.43, 30.32 (*d*, $J = 19.3$), 29.05, 28.86, 28.25, 25.08 (*d*, $J = 5.2$), 22.22. ^{19}F NMR (282.4 MHz, $CDCl_3$): –218.40.

1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranosyl (+)-(*R*)-8-fluorooctanesulfinate. To diacetone-D-glucose (0.77 g, 2.9 mmol) in dry THF (12 ml) under N_2 gas at -78 °C was added pyridine (0.29 ml, 3.5 mmol) followed by 8-fluorooctanesulfinyl chloride (3.52 mmol). The mixture was stirred for 3 h (–78 °C). The reaction was quenched dropwise with water (5 ml) then dissolved in DCM (50 ml). The resulting mixture was washed with 5% HCl (50 ml), 2% $NaHCO_3$ (50 ml), and sat. NaCl (50 ml), then dried over Na_2SO_4 and concentrated by roto-evaporation. The title compound was obtained by flash chromatography (40% Et_2O–hexanes), to obtain a thick, pale oil (0.79 g, 1.8 mmol, 62%, dr *R*:*S*: 91:9). $[\alpha]_D^{21} = +1.39$ deg cm^3 g^{-1} dm^{-1}(*c* 1.10, acetone). TLC (EtOAc–hexane 1:1): R_f 0.70. IR (film): 2987, 2935, 2859, 1457, 1374, 1256, 1217, 1165, 1136, 1076, 1023, 955, 887, 837, 754, 730, 687 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$): 5.91 (*d*, $J = 3.5$, 1H), 4.78 (*d*, $J = 3.5$, 1H), 4.71 (*d*, $J = 1.2$, 1H), 4.43 (*dt*, $^2J_{HF} = 47.3$, $^3J_{HH} = 6.1$, 2H), 3.96-4.17 (*m*, 4H), 2.81 (*2dt*, $J = 7.8, 13.4$, 2H), 1.70 (*m*, 2H), 1.28-1.47 (*m*, 10H), 1.50 (*s*, 3H), 1.41 (*s*, 3H), 1.32 (*s*, 3H), 1.30 (*s*, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): 112.40, 109.42, 105.35, 84.07 (*d*, $J = 163.1$), 83.84, 83.05, 80.93, 72.14, 67.70, 57.86, 30.33 (*d*, $J = 19.3$), 29.09, 28.93, 28.66, 26.86, 26.74, 26.19, 25.29, 25.10 (*d*, $J = 5.3$), 21.03. ^{19}F NMR (282.4 MHz, $CDCl_3$): –218.36 EI-MS: *m*/*z* 423 (27 [M – 15]$^+$), 365 (3), 249 (7), 185 (19), 170 (14, [$FC_8H_{16}SO$]$^+$), 127 (28, [$C_6H_7O_3$]$^+$), 101 (100, [$C_5H_9O_2$]$^+$), 85 (13), 59 (20), 43 (39, [C_2H_3O]$^+$); HR-EI-MS *m*/*z* 423.1851 ([M–CH_3]$^+$, $C_{19}H_{32}O_7FS$ requires 423.1853).

1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranosyl (–)-(*S*)-8-fluorooctanesulfinate. Obtained as for 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranosyl-(+)-(*R*)-8-fluorooctanesulfinate, except for the solvent (toluene) and the base (*i*-Pr_2NEt). (30% yield, dr *R*:*S*: 17:83). $[\alpha]_D^{21} = -36.2$ deg cm^3 g^{-1} dm^{-1} (*c* 1.20, acetone). TLC (EtOAc–hexane 1:1): R_f 0.67. IR (film): 2988, 2934, 2859, 1714, 1457, 1373, 1256, 1220, 1165, 1135, 1075, 1022, 830. ^{1}H-NMR (300 MHz, $CDCl_3$): 5.91 (*d*, $J = 3.6$, 1H), 4.74 (*d*, $J = 2.3$, 1H), 4.60 (*d*, $J = 3.7$, 1H), 4.43 (*dt*, $^2J_{HF} = 47.3$, $^3J_{HH} = 6.1$, 2H), 3.98-4.32 (*m*, 4H), 2.78 (*m*, 2H), 1.60-1.79 (*m*, 2H), 1.30-1.48 (*m*, 10H), 1.51 (*s*, 3H), 1.43 (*s*, 3H), 1.34 (*s*, 3H), 1.31 (*s*, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): 112.46, 109.24, 104.97, 84.08 (*d*, $J = 163.1$), 83.61, 80.38, 79.21, 72.40, 66.71, 57.37, 30.32 (*d*, $J = 19.5$), 29.06, 28.92, 28.64, 26.74, 26.71, 26.27, 25.19, 25.10 (*d*, $J = 5.3$), 21.26. ^{19}F NMR (282.4 MHz, $CDCl_3$): –218.37 EI-MS: *m*/*z* 423 (31 [M – 15]$^+$), 367 (4). 365 (3), 249 (3), 185 (17), 127 (30, [$C_6H_7O_3$]$^+$), 101 (100, [$C_5H_9O_2$]$^+$), 85 (12), 59 (19), 43 (44, [C_2H_3O]$^+$); HR-EI-MS *m*/*z* 423.1858 ([M–CH_3]$^+$, $C_{19}H_{32}O_7FS$ requires 423.1853).

(9-Bromo-nonyloxy)-*tert*-butyldimethylsilane. 9-Bromo-1-nonanol (2.33 g, 10.4 mmol), *tert*-butyldimethylsilyl chloride (1.85 g, 12.3 mmol) and imidazole (1.85 g, 27.1 mmol) were combined and DMF (2.6 ml) was added. The mixture was stirred at room temperature under N_2 overnight. The resulting cloudy yellow mixture was diluted with ether (10 ml) and washed with sat. NaCl (10 ml). The organic phase was evaporated *in vacuo* and the resulting oil was partitioned between hexanes (3 × 10 ml) and dH_2O (10 ml). The combined organic phases were washed with sat. NaCl (10 ml), dried (Na_2SO_4) and concentrated *in vacuo* to yield the title compound (2.97 g, 8.8 mmol, 85%) as a pale oil. TLC (EtOAc–hexane 15:85): R_f 0.67; ^{1}H-NMR (300 MHz, $CDCl_3$): 3.60 (*t*, $J = 6.6$, 2 H), 3.42 (*t*, $J = 6.8$, 2 H), 1.86 (*m*, 2 H), 1.47-1.59 (*m*, 2 H), 1.32 (*m*, 10H), 0.89 (*s*, 9 H), 0.05 (*s*, 6 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 63.29, 34.03, 32.86, 29.42, 29.33, 29.72, 28.17, 31.60, 25.77, 18.90, –5.24.

***tert*-Butyl-[10-(8-fluoro-octane-1-(*S*)-sulfinyl)-decyloxy]-dimethylsilane.** Freshly ground magnesium turnings (0.14 g, 5.9 mmol) were covered with dry THF (1.5 ml) under N_2 gas. To this mixture, was added (9-bromo-nonyloxy)-*tert*-butyldimethylsilane (1.49 g, 4.4 mmol) in dry THF (7 ml). The resulting mixture was refluxed for 7 h and then cooled to room temperature.

In another vessel, the (*R*)-DAG sulfinate ester prepared above (0.33 g, 0.77 mmol) was dissolved in toluene (16 ml) and cooled to 0 °C. To this solution was added a portion of the Grignard reagent (4.5 ml, 2.6 mmol) and the reaction mixture was stirred for 30 min at 0 °C, allowed to warm to room temperature and stirred for 24 h. The solution was quenched with sat. NH_4Cl (10 ml) and then extracted with DCM (2 × 10 ml). The combined organic phases were dried over Na_2SO_4 and concentrated by roto-evaporation. The title compound was obtained by flash chromatography (60% EtOAc–hexanes) to obtain a white solid (0.27 g, 0.61 mmol, 80%). TLC (EtOAc–hexane 75:25): R_f 0.30. IR (KBr): 3434, 2927, 2854, 1636, 1471, 1388, 1361, 1256, 1103, 1017, 934, 837, 776, 726, 663 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.42 (*dt*, $^2J_{HF} = 47.3$, $^3J_{HH} = 6.1$, 2H), 3.58 (*t*, $J = 6.6$, 2H), 2.64 (*m*, 4H), 1.68-1.84 (*m*, 4H), 1.58-1.84 (*m*, 2H), 1.22-1.54 (*m*, 20H), 0.88 (*s*, 9H), 0.03 (*s*, 6H). ^{13}C-NMR (75 MHz, $CDCl_3$): 84.09 (*d*, $J = 163.2$), 63.26, 52.47, 52.38, 52.38, 32.82, 30.32 (*d*, $J = 19.2$), 29.33, 29.30, 29.15, 29.08, 28.94, 28.87, 28.76, 25.97, 25.97, 25.97, 25.74, 25.07 (*d*, $J = 5.3$), 22.60, 22.58, 18.36, -5.27, –5.27. ^{19}F NMR (282.4 MHz, $CDCl_3$): –218.36. EI-MS: *m*/*z* 419 (2 [M – OH]$^+$), 379 (100, [M–*t*-Bu]$^+$), 359 (3), 199 (3), 101 (7), 89 (3), 75 (24), 69 (16), 55 (16), 41 (10).

***tert*-Butyl-[10-(8-fluoro-octane-1-(*R*)-sulfinyl)-decyloxy]-dimethylsilane.** Obtained as for *tert*-butyl-[10-(8-fluoro-octane-1-(*S*)-sulfinyl)-decyloxy]-dimethylsilane, using (*S*)-DAG sulfinate ester (69% yield). Structural data identical for that of *tert*-butyl-[10-(8-fluoro-octane-1-(*S*)-sulfinyl)-decyloxy]-dimethylsilane.

10-(8-Fluoro-octane-1-(*S*)-sulfinyl)-decan-1-ol. To a solution of (*S*)-OTBS-sulfoxide (18 mg, 0.041 mmol) in MeOH (1.4 ml) at 0 °C was added (1*S*)-(+)-10-camphorsulfonic acid (2 mg, 9 μmol) with stirring. The resulting solution was stirred for 45 min. The reaction was quenched with sat. $NaHCO_3$ (3.5 ml) and extracted with DCM (3 × 2 ml). The extracts were dried (Na_2SO_4) and dried under a gentle flow of N_2 to obtain the title compound as a white solid (11 mg, 0.033 mmol, 81%). TLC (EtOAc–hexane 75:25): R_f 0.03. IR (KBr): 3437, 2923, 2850, 1636, 1468, 1417, 1063, 1018, 932, 879, 727 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.43 (*dt*, $^2J_{HF} = 47.3$, $^3J_{HH} = 6.1$, 2H), 3.63 (*t*, $J = 6.5$, 2H), 2.65 (*m*, 4H), 1.28-1.84 (*m*, 26H). ^{13}C-NMR (75 MHz, $CDCl_3$): 84.14 (*d*, $J = 163.0$), 62.95, 52.44, 52.39, 52.39, 32.73, 30.33 (*d*, $J = 19.3$), 29.25, 29.25, 29.09, 29.09, 28.95, 28.82, 28.76, 25.67, 25.08 (*d*, $J = 5.2$), 22.59, 22.59. ^{19}F NMR (282.4 MHz, $CDCl_3$): –218.34. EI-MS (pk intensity, fragment): *m*/*z* 305 (72 [M – OH]$^+$), 157 (27), 143

(20, $[C_9H_{18}OH]^+$), 83 (33), 69 (100), 55 (78), 41 (36). HR-EI-MS *m/z* 305.2319 ($[M - OH]^+$, $C_{17}H_{34}OFS$ requires 305.2314).

10-(8-Fluoro-octane-1-(*R*)-sulfinyl)-decan-1-ol. Obtained as for (*S*) sulfoxide, using (*R*)-OTBS sulfoxide (83% yield). The analytical data for this compound is identical to the other enantiomer.

Δ^9 Desaturase-mediated sulfoxidation of 18-fluoro-10-thia-octadecanoyl-ACP

Incubation of 18-fluoro-10-thia-octadecanoyl-ACP with Δ^9 desaturase was carried out at room temperature and reaction progress was monitored for disappearance of starting material by GC-MS analysis of silylated aliquots (0.1 ml TMCS + BSTFA). The reaction mixture consisted of castor Δ^9 desaturase dimer (175 mg ml^{-1}, 20 µl), *Anabaena* vegetative ferredoxin (2 mg ml^{-1}, 300 µl), maize root NADPH:ferredoxin reductase (0.25 mg ml^{-1}, 150 µl), 18-fluoro-10-thia-octadecanoyl-ACP (3.5 mg ml^{-1}, 1.6 ml) in a total volume of 7.5 ml of buffer. The reaction was initiated by the addition of NADPH (21 mg l^{-1}, 1.5 ml) and allowed to continue for 30 min. At this time, additional aliquots of ferredoxin (150 µl), reductase (75 µl), NADPH (750 µl), desaturase (6 µl) were added and the reaction allowed to continue for a further 30 min. The reaction was terminated with the addition of THF (1.5 ml) and the thioester linkage was reduced to the corresponding primary alcohol with the addition of NaBH4 (9 mg). The mixture was diluted with water (3 ml) and extracted with dichloromethane (4 × 6 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 1 ml of $CDCl_3$ for analysis by ^{19}F NMR.

Acknowledgements

This work was supported by an NSERC PGS1 (to A. T), NSERC USRA (to N. T.); N.S.E.R.C. Grant OGP-2528 (to P. H. B.); by the Office of Basic Energy Sciences of the United States Department of Energy (to J. S.). We are grateful Dr Jose Abad (IIQAB, CSIC, Barcelona, SP) for initially providing us with samples of (*S*)- and (*R*)-AMA and procedures for their preparation.

Notes and references

1 J. Shanklin and E. B. Cahoon, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1998, **49**, 611; J. Shanklin, J. E. Guy, G. Mishra and Y. Lindqvist, *J. Biol. Chem.*, 2009, **284**, 18559; P. H. Buist, *Nat. Prod. Rep.*, 2004, **21**, 249; P. H. Buist, Chemistry of Fatty Acid Desaturases, *Wiley Encyclopedia of Chemical Biology*, 2008, Vol. 2, p. 1, John Wiley & Sons, Hoboken, NJ.
2 B. Behrouzian, C. K. Savile, B. Dawson, P. H. Buist and J. Shanklin, *J. Am. Chem. Soc.*, 2002, **124**, 3277.
3 G. J. Schroepfer and K. Bloch, *J. Biol. Chem.*, 1965, **240**, 54.
4 B. G. Fox, J. Shanklin, J. Y. Ay, T. M. Loehr and J. Sanders-Loehr, *Biochemistry*, 1994, **33**, 12776; J. Shanklin, C. Achim, H. Schmidt, B. G. Fox and E. Munck, *Proc. Natl. Acad. Sci. U. S. A.*, 1997, **94**, 2981.
5 P. H. Buist and B. Behrouzian, *J. Am. Chem. Soc.*, 1996, **118**, 6295.
6 P. H. Buist, H. G. Dallmann, R. R. Rymerson, P. M. Seigel and P. Skala, *Tetrahedron Lett.*, 1988, **29**, 435.
7 P. H. Buist and D. M. Marecak, *J. Am. Chem. Soc.*, 1992, **114**, 5073.
8 (*a*) B. Behrouzian, P. H. Buist and J. Shanklin, *Chem. Commun.*, 2001, 401; (*b*) B. Behrouzian, D. Hodgson, C. K. Savile, B. Dawson, P. H. Buist and J. Shanklin, *Magn. Reson. Chem.*, 2002, **40**, 524; (*c*) C. E. Rogge and B. G. Fox, *Biochemistry*, 2002, **41**, 10141; (*d*) R. D. White and B. G. Fox, *Biochemistry*, 2003, **42**, 7828; (*e*) A. E. Tremblay, P. H. Buist, D. Hodgson, B. Dawson, E. Whittle and J. Shanklin, *Magn. Reson. Chem.*, 2006, **44**, 629.
9 B. G. Fox, K. S. Lyle and C. E. Rogge, *Acc. Chem. Res.*, 2004, **37**, 421.
10 K. Y. Y. Lao, D. J. Hodgson, B. Dawson and P. H. Buist, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 2799.
11 A. E. Tremblay, K. Y. Y. Lao, D. J. Hodgson, B. Dawson and P. H. Buist, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 5146.
12 W. H. Pirkle, S. D. Beare and R. L. Muntz, *Tetrahedron Lett.*, 1974, **15**, 2295.
13 P. H. Buist, D. Marecak, H. L. Holland and F. M. Brown, *Tetrahedron: Asymmetry*, 1995, **6**, 7.
14 A sample of racemic **1** was prepared by $LiAlH_4$ reduction of the corresponding methyl ester that was available from a previous study: D. J. Hodgson, K. Y. Y. Lao, B. Dawson and P. H. Buist, *Helv. Chim. Acta*, 2003, **86**, 3688.
15 It should be noted that the presence of the fluorine tag in **1** results in a change in priority of the pendant alkyl group (C8–C18) relative to the nonfluorinated compound. This results in assignment of the (*S*)-configuration to the sulfoxy centre of bio-**1** whereas the corresponding non-fluorinated parent compound is *R* configured.
16 I. Fernández, N. Khiar, J. M. Llera and F. Alcudia, *J. Org. Chem.*, 1992, **57**, 6789.
17 F. Daligault, A. Arboré, C. M. Nugier-Chauvin and H. Patin, *Tetrahedron: Asymmetry*, 2004, **15**, 917.
18 P. H. Buist, H. G. Dallmann, R. R. Rymerson and P. M. Seigel, *Tetrahedron Lett.*, 1987, **28**, 857.
19 M. M. Bradford, *Anal. Biochem.*, 1976, **72**, 248.
20 (*a*) J. M. Studts and B. G. Fox, *Protein Expression Purif.*, 1999, **16**, 109; (*b*) J. Shanklin, *Protein Expression Purif.*, 2000, **19**, 319; (*c*) J. A. Broadwater and B. G. Fox, *Protein Expression Purif.*, 1999, **15**, 314.
21 F. L. M Pattison, *J. Org. Chem.*, 1956, **21**, 748–53.

