ω-Fluoro Thiafatty Acids: New Mechanistic Probes of Desaturase-Mediated Reactions

by **Derek J. Hodgson**[a][b], **Kim Y. Y. Lao**[a], **Brian Dawson**[b], and **Peter H. Buist***[a]

[a]) Department of Chemistry, Carleton University, Ottawa, ON Canada K1S 5B6
(phone: ++1613-520-2600 (3643); fax: ++1613-520-3749; e-mail: pbuist@ccs.carleton.ca)
[b]) Centre for Biologics Research, Biologics and Genetic Therapies Directorate, Health Canada, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

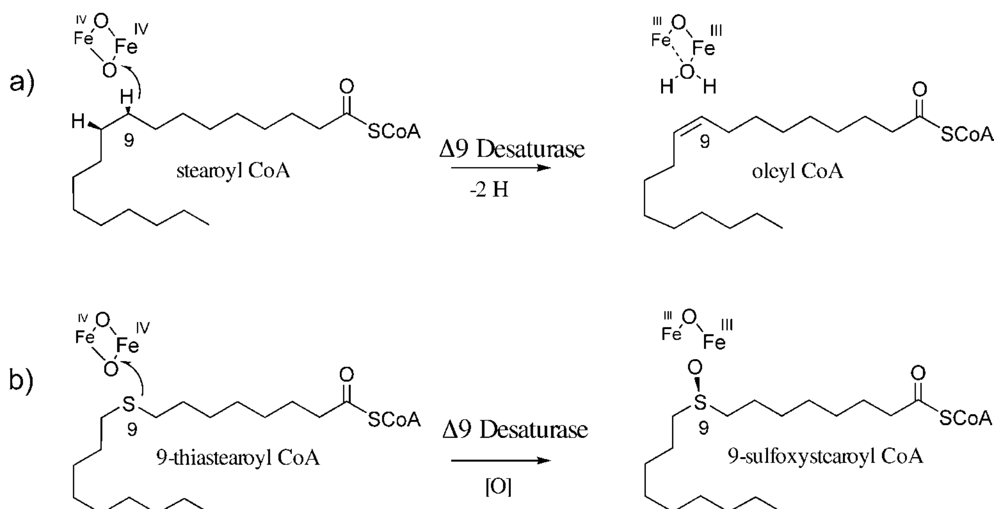
Dedicated to *Duilio Arigoni* on the occasion of his 75th birthday

A series of 18-fluoro thiastearates were prepared and incubated with a yeast Δ9-desaturating system. The relative efficiency of desaturase-mediated sulfoxidation was monitored *via* ^{19}F-NMR analysis of the sulfoxide products, and a strong preference for oxo transfer to the S-atom occupying the 9-position was confirmed. The oxidation profile obtained in this manner matched that of analogous experiments with non-fluorinated substrates. These results form the basis of a versatile ^{19}F-NMR-based method for mapping the position of the putative diiron oxidant relative to substrate, and has potential application to the study of membrane-bound desaturases *in vitro*.

1. Introduction. – Fatty acid desaturases are an important superfamily of enzymes found in a wide variety of aerobic organisms [1]. The first desaturation reaction to be studied in detail was the conversion of stearoyl CoA to oleyl CoA as it occurs in baker's yeast (*Scheme 1,a*) [2]. Interest in this particular enzyme system has risen recently with the discovery that enhanced stearoyl CoA Δ9 desaturase (SCD) activity in mammals occurs in a number of disorders, including obesity, diabetes, and other metabolic diseases [3]. Thus, the development of mechanism-based SCD inhibitors would have potential therapeutic significance. A major stumbling block to progress in this area is that stearoyl CoA Δ9 desaturase is membrane-bound and difficult to isolate in purified form. However, some important mechanistic information has been obtained on the structurally related yeast enzyme [4], which can be conveniently studied *in vivo* by an appropriate experimental design. A KIE study showed that the H-atom at C(9) of substrate is removed first in an isotopically sensitive step, followed by rapid cleavage of the C−H bond at C(10) [5]. This result correlates well with the observation that desaturase-mediated oxo transfer occurs most efficiently when a S-atom occupies the 9-position of a thiastearoyl substrate (*Scheme 1,b*) [6]; the enantioselectivity of sulfoxidation matches the stereochemistry (*syn*, *pro-R*) of the corresponding parent reaction [7][8]. Taken together, these results strongly suggest that the putative diiron oxidant involved in yeast Δ9 desaturase mediated oxidation is asymmetrically located between C(9) and C(10) of substrate.

The success of the thia-analogue approach in providing critically important information on desaturase-active-site topology prompted us to consider modifications to the probe that would obviate the need to isolate milligram amounts of product for

Scheme 1. *The Oxidation Reactions Catalyzed by Stearoyl CoA Desaturase. a*) Desaturation; *b*) sulfoxidation (CoA = Coenzyme A).

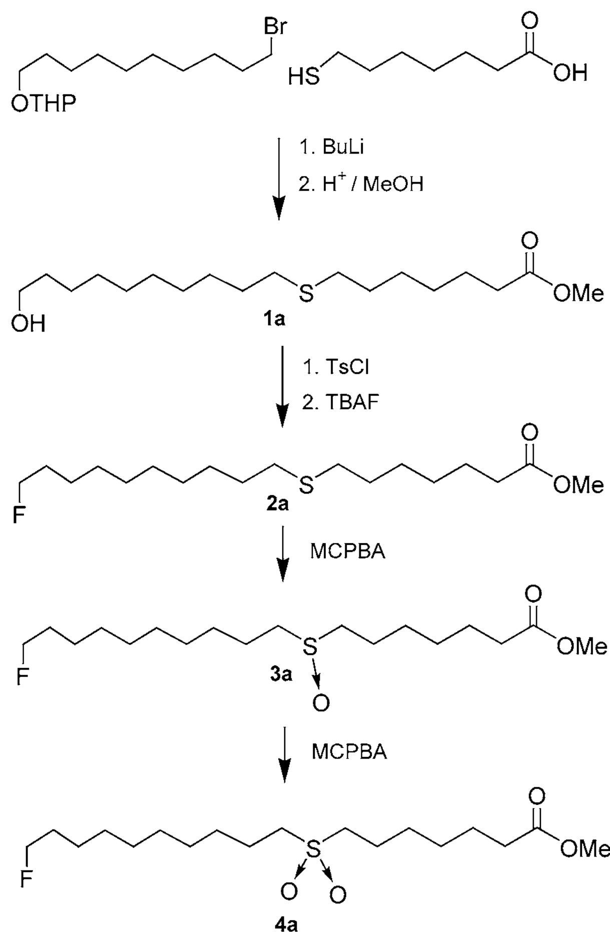


analysis [6][8]. Development of a more-convenient analytical method would greatly enhance the versatility of the 'thia-test' by permitting the study of membrane-bound desaturases such as SCD *in vitro*. As a first step in this direction, we report here the validation of a ^{19}F-NMR-based method for monitoring Δ9-desaturase-mediated sulfoxidation of F-tagged thiasubstrates. A preliminary publication of portions of this work has appeared [9].

2. Results. – The required methyl ω-fluoro-thiaoctadecanoates **2a**–**2d** were synthesized by fluorination of the corresponding methyl 18-hydroxy-thiaoctadecanoates **1a**–**1d** *via* a conventional tosylation/Bu_4NF(TBAF) sequence (*Scheme 2*). Use of DAST ((diethylamino)sulfur trifluoride) in this step was compromised by the fact that a significant reaction at the S-atom occurred to give the sulfoxy by-product. The intermediate alcohols **1a**–**1d** were readily available *via* alkylation of the appropriate ω-thioacid [10] with the 3,4,5,6-tetrahydro-2*H*-pyran-2-yl (THP)-protected ω-hydroxy alkyl bromides of the correct chain length. Oxidation of **2a**–**2d** with 1 or 2 equiv. of *m*-chloroperbenzoic acid (MCPBA) furnished the corresponding sulfoxides **3a**–**3d** and sulfones **4a**–**4d** [6], respectively. The analytical and spectral data of all thiastearates and their oxidized derivatives were consistent with the assigned structures (see *Exper. Part*). The ^{1}H-decoupled ^{19}F-NMR spectra of these compounds were recorded in $CDCl_3$, and the trends in chemical shift for sulfide, sulfoxide, and sulfone as a function of the position of the S-atom are presented in *Fig. 1*. ^{19}F-NMR Resonances of each series of ω-fluorothiastearate and the corresponding oxidized derivatives were resolved to baseline in all cases. The absolute value of the ^{19}F chemical shifts for each analyte is given in the *Exper. Part*.

To evaluate the suitability of the ω-fluorothiastearates as substrates for Δ9 desaturation by SCD-type enzymes, we turned to our well-characterized yeast model

Scheme 2. *Synthesis of ω-Fluoro Thiaoctadecanoate Isomers and Their Oxidized Derivatives*



system. Each ω-fluorothiastearate **2a – d** (25 mg) was incubated separately with actively growing cultures (200 ml) of *Saccharomyces cerevisiae S522C* to maximize production of the corresponding sulfoxide. Our whole-cell system allows us to use methyl ester substrates that are presumably converted intracellularly to the corresponding CoA thioesters prior to desaturase-mediated sulfoxidation. The product sulfoxide is then hydrolyzed and excreted into the medium. Control experiments with a yeast mutant have demonstrated previously that sulfoxidation of thiastearate is dependent on active expression of the Δ9 desaturase [11].

^{19}F-NMR Analysis of the CH_2Cl_2 extracts of the supernatant derived from each ω-fluorothiastearate incubation revealed the presence of sulfide and the corresponding sulfoxide as the major analytes in each case. The sulfoxy products were identified on the

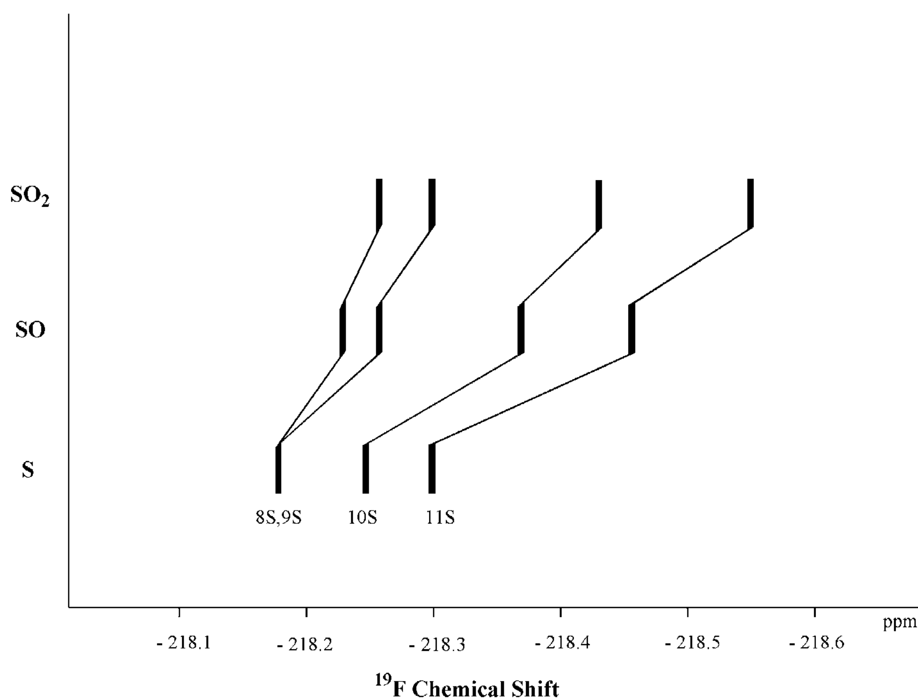


Fig. 1. *The effect of sulfur oxidation on the* [19]*F chemical shifts of methyl ω-fluoro thiastearates as a function of S-position*

basis of ^{19}F-NMR chemical-shift data (agreement within 0.01 ppm) as well as TLC analysis of each extract by using authentic standards. Only small (< 5% of total) amounts of *ω*-fluoro sulfone were observed; in the case of **2d** oxidation, three additional minor, unidentified products (12% of total), presumably derived from chain cleavage of a 9,10-dehydro-11-thia derivative, were observed in the ^{19}F-NMR spectrum of the extract.

The relative amounts of sulfoxidized product were quantitated by the use of an external standard (flunisolide), and the % sulfoxidation of **2a**, **2b**, **2c**, and **2d** was calculated to be 1.2, 7, 4.5, 2.0%, respectively. These data are presented in graphical form (normalized to % sulfoxidation of **2b**) in *Fig. 2* along with data obtained previously for the parent, non-fluorinated thiastearoyl substrates.

3. Discussion. – The success of a ^{19}F-NMR-based approach to studying desaturase-mediated sulfoxidation depends on three considerations: *1*) synthetic accessibility of the fluorinated probes; *2*) sensitivity of the remote ^{19}F-NMR reporter resonance to changes to sulfide oxidation state; *3*) nonperturbing nature of the fluorine tag. We were pleased to see that all of these criteria were met in these experiments:

1) The synthetic scheme we employed to generate **2a** – **2d** is straightforward and can be easily adapted to generate a number of *ω*-fluoro thia-analogues that vary in S-

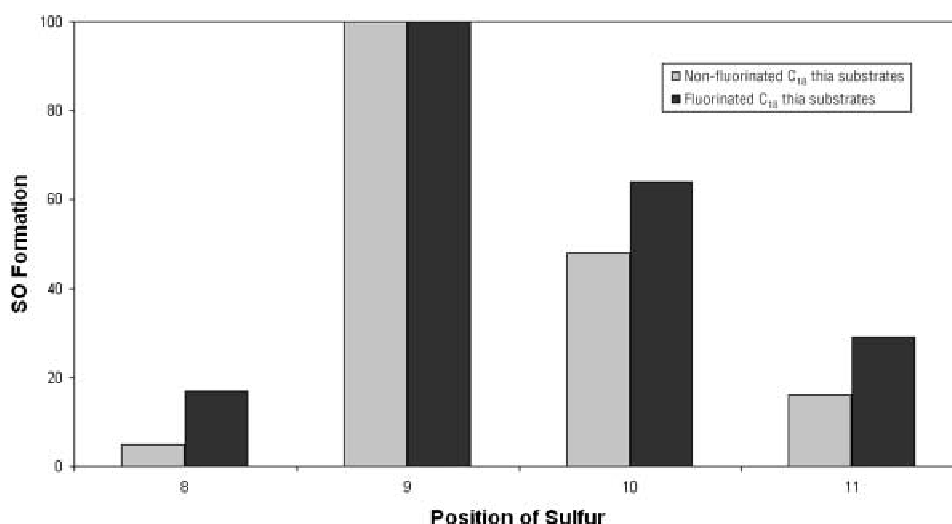


Fig. 2. *Comparison of yeast-mediated % sulfoxidation of non-fluorinated and fluorinated 8-, 9- 10-, and 11-thiastearates* **2a – d**

position and chain length. This will allow further study of desaturases with different positional specificities [12][13].

2) The $\Delta\delta$ between **1a – 1d** and the corresponding sulfoxy products is sufficiently different to permit facile monitoring of desaturase-mediated sulfoxidation. As might be expected, the substituent effects on the ^{19}F chemical shifts decreased in magnitude as the distance (seven to eleven bonds) between the S-atom and the F-nucleus increases (*Fig. 1*). The direction of the $\Delta\delta$ effects for increasingly electron-withdrawing substituents (shielding) match previous observations for rigid cycloalkyl systems [14].

3) Perhaps the most important finding of this work was the good agreement between the regioselectivity profile of sulfoxidation for F-tagged thiastearates and the corresponding non-fluorinated substrates (*Fig. 2*). The latter data set was obtained by mass spectrometric analysis of sulfoxide mixtures isolated by silica-gel chromatography from direct competition experiments between non-fluorinated thiastearates. It is clear that the presence of a substrate ω-F-tag did not significantly affect the pronounced preference of the desaturase system to oxygenate 9-thia substrate analogues as we have previously documented (see *Introduction*). The ratio of 9- to 10-sulfoxidized products (1.6 : 1) obtained in this work compares favorably with the values obtained from both direct competition (2.1 : 1 [6]) and noncompetitive incubation of thiastearates (1.7 [5]). Good correlation between sulfoxidation ratios derived from experiments with non-fluorinated and ω-fluorinated thiastearates have also been obtained for a soluble, plant Δ9-desaturase system [9][15].

The relatively low levels of 8- and 11-sulfoxidation observed in these experiments (*Fig. 2*) may be due to autoxidation and/or oxidation by unselective bio-oxidation by non-desaturase-related enzymes, since the 8- and 11-positions are not expected to be accessible to the Δ9-desaturase oxidant. A more-detailed assessment of the fate of 8-

and 11-thiastearoyl substrates must await development of an *in vitro* assay; the predicted C(9)=C(10) sulfide products are not expected to be stable under our *in vivo* conditions.

4. Conclusions. – The results presented in this paper open a new window of opportunity in the mechanistic characterization of desaturases. In principle, it should be possible to generate a ^{19}F-NMR-based sulfoxidation profile for any given desaturase in a single competition experiment provided the sulfoxide products derived from the ω-fluorinated thiafatty acid mixtures have unique chemical shifts. A number of possible applications of this approach are envisaged. For example, it should be possible to take advantage of the excellent inherent sensitivity of ^{1}H-decoupled ^{19}F-NMR (μM concentration of fluorinated analyte) coupled with the lack of interference to obtain the *in vitro* oxidation signature of medically important desaturases such as SCD. Furthermore, it would be interesting to characterize the regioselectivity of sulfoxidation for desaturases with novel cryptoregiochemistry as determined by KIE experiments [16]. Finally, our ^{19}F-NMR-based method may offer a unique way of tracking site-directed-mutagenesis experiments by obtaining a snapshot of the position of the diiron oxidant relative to substrate for a series of modified desaturases.

Experimental Part

General. Abbreviations: MCPBA: *meta*-chloroperbenzoic acid, FC: flash chromatography. THF was freshly distilled from Na before use. MeCN was dried over 4-Å molecular sieves. Pyridine was dried over NaOH. Unless otherwise noted, all reagents and starting materials for organic synthesis were purchased from *Sigma-Aldrich* and used without purification. TsCl was recrystallized from hexane/$CHCl_3$ before use. 8-Sulfanyloctanoic and 10-sulfanyldecanoic acids were prepared from the corresponding, commercially available bromo acids as described in [10]. In a similar manner, 7-sulfanylheptanoic and 9-sulfanylnonanoic acid were obtained from 7-bromoheptanoic acid and 9-bromononanoic acid, resp.; the latter two compounds were available from the corresponding ω-bromoalkan-1-ol *via Jones* oxidation [17]. Protection of the required ω-bromoalkan-1-ols as the THP ethers was carried out according to a standard procedure [18]. All air- and moisture-sensitive reactions were performed under N_2. Org. extracts were typically shaken with sat. NaCl soln., dried (Na_2SO_4) and solvents were evaporated *in vacuo* on a *Büchi RE 111 Rotavapor*. FC: *Merck* silica gel (230–400 mesh, at *ca.* 0.5 atm). TLC: *Merck* silica gel *60 F254* plates; visualization by a combination of water spray and I_2 vapor. M.p.: *Fisher-Johns* melting-point apparatus; uncorrected. IR Spectra: *ABB Bomem MB* spectrometer; in cm^{-1}. NMR Spectra: *Bruker AM-400* (^{1}H-decoupled ^{19}F: 376.5 MHz); *Bruker AMX-400* (^{1}H: 400 MHz, ^{13}C: 100 MHz); *Bruker Avance 700* (^{1}H: 700 MHz, ^{13}C: 176 MHz); ^{1}H and ^{13}C chemical shifts (δ) in ppm downfield from internal TMS ($\delta = 0.00$ ppm); ^{19}F chemical shifts (δ) in ppm downfield from external $CFCl_3$ ($\delta = 0.00$ ppm), J values in Hz. EI-MS: *Kratos Concept 1 H* mass spectrometer; direct introduction or *via* GC (*HP 5980 Series 2* gas chromatograph (*J. & W.* 30 m × 0.21 mm, *DB-5* capillary column)). *Saccharomyces cerevisiae S288C* cultures were obtained from the Yeast Genetic Stock Center, University of California at Berkeley and cultured as described in [6].

Methyl 18-Hydroxy-8-thiaoctadecanoate (**1a**). To a soln of 7-sulfanylheptanoic acid (1.63 g, 10.0 mmol) in THF/HMPA 3:1 (66 ml) was added a 1.6M BuLi (14.0 ml) reagent *via* syringe, under N_2 at 0°. After stirring the mixture at 0° for 30 min, a soln of 10-bromodecan-1-ol THP ether (3.23 g, 10.1 mmol) was added *via* syringe. The mixture was stirred at r.t. for 22 h, and the reaction was quenched by the addition of H_2O (60 ml), and the mixture was acidified to a pH of 2 with 3M HCl (15 ml) and extracted with hexanes (4 × 60 ml). The combined org. extracts were washed with sat. aq. NaCl soln., dried (Na_2SO_4) and evaporated to give crude ω-hydroxy-8-thiastearic acid THP ether (3.19 g). This compound was deprotected and methylated with TsOH (115 mg) / MeOH (30 ml; reflux) and, after evaporation of the reaction solvent, the crude product (1.99 g) was purified by FC (20% AcOEt/hexanes) to give **1a** (558 mg, 17% based on 7-sulfanylheptanoic acid). White solid. TLC (hexane/AcOEt 40:60): R_f 0.25. M.p. 43–45° (amorphous crystals, recrystallized from hexane). IR (KBr):

3340, 2931, 2851, 1738, 1471, 1462, 1437, 1254, 1176, 1171, 1073. ^{1}H-NMR (400 MHz, $CDCl_3$): 3.66 (*s*, 3 H); 3.63 (*t*, *J* = 6.6, 2 H); 2.489, 2.487 (partially resolved overlapping *t*, *J* = 7.4, 4 H); 2.30 (*t*, *J* = 7.5, 2 H); 1.53 – 1.67 (*m*, 9 H); 1.25 – 1.45 (*m*, 16 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 174.23; 63.10; 51.47; 34.03; 32.82; 32.23; 32.11; 29.73; 29.53; 29.53; 29.45; 29.41; 29.24; 28.94; 28.78; 28.55; 25.74; 24.84. EI-MS: 332 (8, M^+), 301 (11, $[M-31]^+$), 283 (2), 259 (4), 231 (3), 203 (6), 189 (16, $[HO(CH_2)_{10}S]^+$), 171 (51, $[CH_2{=}CH(CH_2)_8S]^+$), 159 (10), 143(89, $[(CH_2)_6CO_2Me]^+$), 129 (8), 111 (26),101 (25), 87 (54), 69 (60), 55 (100), 41 (66). HR-EI-MS: 332.2392 (M^+, $C_{18}H_{36}O_3S$; calc. 332.2385).

Methyl 18-Hydroxy-9-thiaoctadecanoate (**1b**). From 8-sulfanyloctanoic acid and 9-bromononan-1-ol THP ether: white solid. TLC (hexane/AcOEt 40 : 60): R_f 0.25. M.p. 42 – 43° (plates, recrystallized from hexane). IR (KBr): similar to that of **1a**. ^{1}H-NMR (400 MHz, $CDCl_3$): 3.66 (*s*, 3 H); 3.63 (*t*, *J* = 6.6, 2 H); 2.49 (unresolved overlapping *t*, $J \approx 7$, 4 H); 2.30 (*t*, *J* = 7.4, 2 H); 1.53 – 1.66 (*m*, 9 H); 1.5 – 1.45 (*m*, 16 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 174.31; 63.08; 51.49; 34.07; 32.80; 32.19; 32.14; 29.70; 29.64; 29.47; 29.36; 29.18; 29.03; 28.91; 28.89; 28.74; 25.72; 24.89. EI-MS: 332 (5, M^+), 301 (10, $[M-31]^+$), 175 (8, $[HO(CH_2)_9S]^+$), 157 (100, $[CH_2{=}CH(CH_2)_7S]^+$, $[(CH_2)_7CO_2Me]^+$), 87 (47), 69 (37) , 55 (100), 41 (53). HR-EI-MS: 332.2389 (M^+, $C_{18}H_{36}O_3S$; calc. 332.2385).

Methyl 18-Hydroxy-10-thiaoctadecanoate (**1c**). From 9-sulfanylnonanoic acid and 8-bromooctan-1-ol THP ether: a white solid. TLC (hexane/AcOEt 40 : 60): R_f 0.25. M.p. 41.5 – 42.5° (amorphous crystals, recrystallized from hexane). IR (KBr): similar to that of **1a**. ^{1}H-NMR (400 MHz, $CDCl_3$): 3.66 (*s*, 3 H); 3.63 (*t*, *J* = 6.6, 2 H); 2.49 (unresolved overlapping *t*, $J \approx 7$, 4 H); 2.30 (*t*, *J* = 7.5, 2 H); 1.53 – 1.66 (*m*, 9 H); 1.25 – 1.45 (*m*, 16 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 174.36; 63.05; 51.48; 34.09; 32.77; 32.17; 32.17; 29.69; 29.69; 29.30; 29.21; 29.14; 29.08; 29.05; 28.86; 28.86; 25.68; 24.92. EI-MS: 332 (4, M^+), 301 (11, $[M-31]^+$), 171(42, $[(CH_2)_8CO_2Me]^+$), 161 (6, $[HO(CH_2)_8S]^+$), 143 (64, $[CH_2{=}CH(CH_2)_6S]^+$), 87 (54), 69 (59), 55 (100), 41 (57). HR-EI-MS: 332.2383 (M^+, $C_{18}H_{36}O_3S$; calc. 332.2385).

Methyl 18-hydroxy-11-thiaoctadecanoate (**1d**). From 10-sulfanyldecanoic acid and 7-bromoheptan-1-ol THP ether: a white solid. TLC (hexane/AcOEt 40 : 60): R_f 0.25. M.p. 40 – 41° (amorphous crystals, recrystallized from hexane). IR (KBr): similar to that of **1a**. ^{1}H-NMR (400 MHz, $CDCl_3$): 3.66 (*s*, 3 H); 3.63 (*t*, *J* = 6.6, 2 H); 2.495, 2.501 (partially resolved, overlapping *t*, *J* = 7.4, 4 H); 2.30 (*t*, *J* = 7.5, 2 H); 1.53 – 1.66 (*m*, 9 H); 1.25 – 1.45 (*m*, 16 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 174.38; 63.02; 51.48; 34.11; 32.73; 32.19; 32.15; 29.70; 29.64; 29.32; 29.20; 29.20; 29.13; 29.05; 28.91; 28.89; 25.64; 24.95. EI-MS: 332 (4, M^+), 301 (13, $[M-31]^+$), 185 (28, $[(CH_2)_9CO_2Me]^+$), 147 (5, $[HO(CH_2)_7S]^+$), 129 (65, $[CH_2{=}CH(CH_2)_6S]^+$), 87 (52), 69 (47) , 55 (100), 41 (51). HR-EI-MS: 332.2386 (M^+, $C_{18}H_{36}O_3S$; calc. 332.2385).

Methyl 18-Fluoro-8-thiaoctadecanoate (**2a**). To a soln of **1a** (0.307 g, 0.92 mmol) in dry pyridine (2 ml) was added TsCl (0.352 g) under N_2 at r.t. After stirring at r.t. for 3 h, the reaction was quenched with the addition of H_2O (20 ml), and the mixture was extracted with Et_2O (4 × 30 ml). The combined org. layers were washed with 2M HCl (2 × 20 ml), dried (Na_2SO_4) and evaporated to give the crude tosylate (0.266 g, 59% est.): TLC (hexane/AcOEt 30 : 70): R_f 0.35. ^{1}H-NMR (200 MHz , $CDCl_3$): 7.79 (*d*, *J* = 8.3, 2 H); 7.35 (*d*, *J* = 8.3, 2 H); 4.01 (*t*, *J* = 6.3, 2 H); 3.67 (*s*, 3 H); 2.49 (overlapping *t*, *J* = 6.7, 4 H); 2.45 (*s*, 3 H), 2.31 (*t*, *J* = 7.3, 2 H); 1.45 – 1.70 (*m*, 8 H); 1.10 – 1.40 (*m*, 14 H). This compound was stirred at r.t. with 6 equiv. of TBAF in dry THF (50 ml) over molecular sieves (0.5 g), for 3 h. THF was removed *in vacuo*, and the residue was partitioned between H_2O (10 ml) and hexane (4 × 20 ml). The hexane layer was washed with sat. aq. NaCl soln. (1 × 50 ml), dried (Na_2SO_4) and evaporated. The crude product was purified by FC with 5% AcOEt/hexanes to give **2a** (111 mg, 36% based on **1a**). Colorless oil. TLC (hexane/AcOEt 90 : 10): R_f 0.34. M.p. 24 – 24.5°. IR (film): 2928, 2855, 1741, 1463, 1436, 1248, 1198, 1171. ^{1}H-NMR (400 MHz , $CDCl_3$): 4.44 (*dt*, *J* = 47.4, 2 H); 3.67 (*s*, 3 H); 2.497, 2.494 (partially resolved overlapping *t*, *J* = 7.4, 4 H); 2.31 (*t*, *J* = 7.5, 2 H); 1.53 – 1.80 (*m*, 8 H); 1.25 – 1.45 (*m*, 16 H). ^{13}C-NMR (100 MHz , $CDCl_3$): 174.23; 84.25 (*d*, *J* = 163.9); 51.49; 34.01; 32.04; 32.20; 30.41 (*d*, *J* = 19.3); 29.71; 29.50; 29.45; 29.42; 29.22; 29.22; 28.94; 28.76; 28.55; 25.15 (*d*, *J* = 5.5); 24.83. ^{19}F NMR (376.5 MHz, $CDCl_3$): −218.18. EI-MS: 334 (24, M^+), 303 (10, $[M-31]^+$), 191(49, $[F(CH_2)_{10}S]^+$), 171 (22, $[CH_2{=}CH(CH_2)_8S]^+$), 143 (100, $[(CH_2)_6CO_2Me]^+$), 111 (38), 87 (45), 69(62), 55 (95), 41 (71). HR-EI-MS: 334.2323 (M^+, $C_{18}H_{35}O_2FS$; calc. 334.2342).

Methyl 18-Fluoro-9-thiaoctadecanoate (**2b**). From **1b**: white plates. TLC (hexane/AcOEt 90 : 10): R_f 0.34. M.p. 22.5 – 23.5°. IR (film): similar to that of **2a**. ^{1}H-NMR (400 MHz , $CDCl_3$): 4.43 (*dt*, *J* = 47.4, 2 H); 3.67 (*s*, 3 H); 2.50 (unresolved overlapping *t*, *J* = 7.4, 4 H); 2.30 (*t*, *J* = 7.5, 2 H); 1.53 – 1.83 (*m*, 8 H); 1.25 – 1.45 (*m*, 16 H). ^{13}C-NMR (100 MHz , $CDCl_3$): 174.25; 84.21 (*d*, *J* = 164.0); 51.45; 34.04; 32.17; 32.12; 30.39 (*d*, *J* = 19.3); 29.68; 29.62; 29.37; 29.16; 29.13; 29.01; 28.89; 28.87; 28.72; 25.13 (*d*, *J* = 5.5); 24.87. ^{19}F-NMR (376.5 MHz, $CDCl_3$): −218.18. EI-MS: 334 (37, M^+), 303 (20, $[M-31]^+$), 189 (28, $[S(CH_2)_7CO_2Me]^+$), 177 (63,

[$F(CH_2)_9S]^+$), 157 (100, [$CH_2{=}CH(CH_2)_7S]^+$, [$(CH_2)_7CO_2Me]^+$), 125 (56), 87 (62), 55 (92), 41 (63). HR-EI-MS: 334.2352 (M^+, $C_{18}H_{35}O_2FS$; calc. 334.2342).

Methyl 18-Fluoro-10-thiaoctadecanoate (**2c**). From **1c**: colorless oil at r.t. TLC (hexane/AcOEt 90:10): R_f 0.34. M.p. 19.5–20°. IR (film): similar to that of **2a**. ^{1}H-NMR (400 MHz, CDCl$_3$): 4.44 (*dt*, $J = 47.4$, 2 H); 3.67 (*s*, 3 H); 2.500, 2.498 (partially resolved, overlapping *t*, $J = 7.4$, 4 H); 2.30 (*t*, $J = 7.5$, 2 H); 1.53–1.80 (*m*, 8 H); 1.25–1.45 (*m*, 16 H). ^{13}C-NMR (100 MHz, CDCl$_3$): 174.24; 84.06 (*d*, $J = 164.1$); 51.39; 34.04; 32.14; 32.14; 30.33 (*d*, $J = 19.3$); 29.65; 29.64; 29.10; 29.09; 29.04; 29.01; 28.87; 28.82; 28.78; 25.07 (*d*, $J = 5.4$); 24.88. ^{19}F-NMR (376.5 MHz, CDCl$_3$): −218.24. EI-MS: 334 (47, M^+), 303 (32, [$M-31]^+$), 203 (22, [$S(CH_2)_8CO_2Me]^+$), 171 (100, [$(CH_2)_8CO_2Me]^+$), 163 (60, [$F(CH_2)_8S]^+$), 143 (65, [$CH_2{=}CH(CH_2)_7S]^+$), 109 (32) 87 (69), 69 (73), 55 (72), 41 (51). HR-EI-MS: 334.2330 (M^+, $C_{18}H_{35}O_2FS$; calc. 334.2342).

Methyl 18-Fluoro-11-thiaoctadecanoate (**2d**). From **1d**: colorless oil at r.t. TLC (hexane/AcOEt 90:10): R_f 0.34. M.p. 24–25°. IR (film): similar to that of **2a**. ^{1}H-NMR (400 MHz, CDCl$_3$): 4.44 (*dt*, $J = 47.4$, 2 H); 3.67 (*s*, 3 H); 2.503, 2.496 (unresolved, overlapping *t*, $J = 7.4$, 4 H); 2.30 (*t*, $J = 7.5$, 2 H); 1.53–1.80 (*m*, 8 H); 1.25–1.45 (*m*, 16 H). ^{13}C-NMR (100 MHz, CDCl$_3$): 174.34; 84.16 (*d*, $J = 164.1$); 51.46; 34.22; 32.22; 32.15; 30.35 (*d*, $J =$ 19.6); 29.72; 29.60; 29.33; 29.19; 29.19; 29.13; 28.92; 28.87; 28.79; 25.08 (*d*, $J = 5.4$); 24.95. ^{19}F-NMR (376.5 MHz, CDCl$_3$): −218.30. EI-MS: 334 (40, M^+), 303 (37, [$M-31]^+$), 185 (69, [$(CH_2)_9CO_2Me]^+$), 149 (45, [$F(CH_2)_7S]^+$), 129 (18, [$CH_2{=}CH(CH_2)_7S]^+$), 95 (37) 87 (69), 55 (100), 41 (69). HR-EI-MS: 334.2329 (M^+, $C_{18}H_{35}O_2FS$; calc. 334.2342).

*(*RS*)-Methyl 18-Fluoro-8-thiaoctadecanoate* S*-Oxide* (**3a**). To a soln. of **2a** (16.6 mg, 0.044 mmol) in CH_2Cl_2 (1 ml) was added MCPBA (0.5 equiv., 8.0 mg) at 0°. After standing at 0° for 30 min, the mixture was filtered to remove a precipitated white solid (MCPBA), and the filtrate was washed with 3M NaOH (2 × 2.5 ml), dried (Na_2SO_4) and evaporated to give the crude sulfoxide, which was purified by recrystallization from AcOEt/hexanes to give **3a** (6.4 mg, 42%). White solid. TLC (AcOEt): R_f 0.40. M.p. 66–66.5° (amorphous crystals, recrystallized from hexane/AcOEt). IR (KBr): 2921, 2850, 1737, 1464, 1437, 1254, 1207, 1174, 1011. ^{1}H-NMR (400 MHz, CDCl$_3$): 4.44 (*dt*, $J = 47.4$, 2 H); 3.67 (*s*, 3 H); 2.57–2.73 (*m*, 4 H); 2.32 (*t*, $J = 7.5$, 2 H); 1.78, 1.76 (overlapping *m*, 4 H); 1.60–1.68 (*m*, 4 H), 1.24–1.56 (*m*, 16 H). ^{13}C-NMR (100 MHz, CDCl$_3$): 174.07; 84.23 (*d*, $J = 164.0$); 52.51; 52.32; 51.53; 33.90; 30.40 (*d*, $J = 19.3$); 29.38; 29.24; 29.18; 29.18; 28.88; 28.71; 28.54; 25.14 (*d*, $J = 5.4$); 24.64; 22.62; 22.48. ^{19}F-NMR (376.5 MHz, CDCl$_3$): −218.23. EI-MS: 333 (100, [$M-OH]^+$), 303(3), 191(18), 160 (57), 143 (32, [$(CH_2)_6CO_2Me]^+$), 111 (32), 55 (95). HR-EI-MS: 333.2263 ([$M-OH]^+$, $C_{18}H_{34}FO_2S^+$; calc. 333.2264).

*(*RS*)-Methyl 18-Fluoro-9-thiaoctadecanoate* S*-Oxide* (**3b**). From **2b**: a white solid. TLC (AcOEt): R_f 0.40. M.p. 66–66.5° (amorphous crystals, recrystallized from hexane/AcOEt). IR (KBr): similar to that of **3a**. ^{1}H-NMR (400 MHz, CDCl$_3$): 4.44 (*dt*, $J = 47.4$, 2 H); 3.67 (*s*, 3 H); 2.57–2.73 (*m*, 4 H); 2.31 (*t*, $J = 7.5$, 2 H); 1.78 (*m*, 4 H); 1.6–1.68 (*m*, 4 H), 1.24–1.56 (*m*, 16 H). ^{13}C-NMR (100 MHz, CDCl$_3$): 174.20; 84.20 (*d*, $J = 164.0$); 52.48; 52.41; 51.42; 33.99; 30.38 (*d*, $J = 19.2$); 29.23; 29.11; 28.86; 28.86; 28.85; 28.85; 28.69; 25.13 (*d*, $J = 5.4$); 24.80; 22.62; 22.57. ^{19}F-NMR (376.5 MHz, CDCl$_3$): −218.25. EI-MS: 333 (26, [$M-OH]^+$), 303 (8), 177 (49), 174 (16), 157 (73, [$(CH_2)_7CO_2Me]^+$), 125 (41), 55 (100). HR-EI-MS: 333.2256 ([$M-OH]^+$, $C_{18}H_{34}O_2FS$; calc. 333.2264).

*(*RS*)-Methyl 18-Fluoro-10-thiaoctadecanoate* S*-oxide* (**3c**). From **2c**: a white solid. TLC (AcOEt): R_f 0.41. M.p. 65.5–66.5° (amorphous crystals, recrystallized from hexane/AcOEt). IR (KBr): similar to that of **3a**. ^{1}H-NMR (400 MHz, CDCl$_3$): 4.44 (*dt*, $J = 47.4$, 2 H); 3.67 (*s*, 3 H); 2.57–2.73 (*m*, 4 H); 2.30 (*t*, $J = 7.5$, 2 H); 1.77, 1.76 (two overlapping *m*, 4 H); 1.6–1.68 (*m*, 4 H), 1.24–1.56 (*m*, 16 H). ^{13}C-NMR (100 MHz, CDCl$_3$): 174.27; 84.14 (*d*, $J = 164.3$); 52.64; 52.44; 51.48; 34.05; 30.35 (*d*, $J = 19.2$); 29.11; 29.01; 29.01; 28.99; 28.96; 28.82; 28.79; 28.69; 25.10 (*d*, $J = 5.4$); 24.87; 22.61; 22.61. ^{19}F-NMR (376.5 MHz, CDCl$_3$): −218.37. EI-MS: 333 (26, [$M-OH]^+$), 303 (9), 188(12), 171 (49, [$(CH_2)_8CO_2Me]^+$), 163 (26), 139 (30), 55 (100). HR-EI-MS: 333.2247 ([$M-OH]^+$, $C_{18}H_{34}O_2FS$; calc. 333.2264).

*(*RS*)-Methyl 18-Fluoro-11-thiaoctadecanoate* S*-Oxide* (**3d**). From **2d**: a white solid. TLC (AcOEt): R_f 0.41. M.p. 59–60° (amorphous crystals, recrystallized from hexane/AcOEt). IR (KBr): similar to that of **3a**. ^{1}H-NMR (400 MHz, CDCl$_3$): 4.44 (*dt*, $J = 47.3$, 2 H); 3.67 (*s*, 3 H); 2.57–2.73 (*m*, 4 H); 2.30 (*t*, $J = 7.5$, 2 H); 1.78,1.76 (two overlapping *m*, 4 H); 1.58–1.68 (*m*, 4 H); 1.58–1.68 (*m*, 4 H); 1.38–1.52 (*m*, 8 H); 1.24–1.38 (*m*, 8 H). ^{13}C-NMR (176 MHz, CDCl$_3$): 174.34; 84.07 (*d*, $J = 164.4$); 52.50; 52.38; 51.49; 34.09; 30.29 (*d*, $J = 19.2$); 29.15; 29.14; 29.08; 28.86; 28.86; 28.78; 28.69; 25.01 (*d*, $J = 5.7$); 24.92; 22.62; 22.57. ^{19}F-NMR (376.5 MHz, CDCl$_3$): −218.46. EI-MS: 333 (44, [$M-OH]^+$), 303 (3), 202 (25), 185 (6, [$(CH_2)_9CO_2Me]^+$), 153 (19), 149 (7), 55 (100). HR-EI-MS: 333.2271 ([$M-OH]^+$, $C_{18}H_{34}O_2FS$; calc. 333.2264).

Methyl 18-Fluoro-8-thiaoctadecanoate S,S*-Dioxide* (**4a**). To a soln. of **3a** (14.8 mg, 0.044 mmol) in CH_2Cl_2 (1 ml) was added MCPBA (2 equiv., 31.4 mg) at 0°. After standing at 0° for 30 min, the mixture was filtered to

remove a precipitated white solid (MCPBA), and the filtrate was washed with 3M NaOH (2×2.5 ml), dried (Na_2SO_4) and evaporated to give the crude sulfone, which was purified by FC with 25% AcOEt/hexanes to give **4a** (14.8 mg, 92%). White solid. TLC (Hexane/AcOEt 75:35): R_f 0.19. M.p. 67–68° (amorphous crystals, recrystallized from hexane/AcOEt). IR (KBr): 2938, 2850, 1738, 1474, 1438, 1262, 1213, 1174, 1137. ^{1}H-NMR (400 MHz, $CDCl_3$): 4.44 (*dt*, *J* = 47.4, 2 H); 3.67 (*s*, 3 H); 2.940 (*m*, 4 H); 2.32 (*t*, *J* = 7.5, 2 H); 1.85, 1.83 (overlapping *m*, 4 H); 1.6–1.78 (*m*, 4 H); 1.28–1.52 (*m*, 16 H). ^{13}C-NMR (100 MHz , $CDCl_3$): 173.98; 84.21 (*d*, *J* = 164.1); 52.82; 52.61; 51.55; 33.82; 30.39 (*d*, *J* = 19.3); 29.35; 29.15; 29.15; 29.04; 28.56; 28.51; 28.20; 25.14 (*d*, *J* = 5.4); 24.52; 21.94; 21.78. ^{19}F-NMR (376.5 MHz, $CDCl_3$): –218.26. EI-MS: 335 (7, [*M* – MeO]$^+$), 315 (3), 293 (9), 143 (85, [$(CH_2)_6CO_2Me$]$^+$), 111 (73), 83 (100), 55 (91). HR-EI-MS: 335.2039 ([*M* – MeO]$^+$, $C_{17}H_{32}O_3FS$; calc. 335.2056).

Methyl 18-Fluoro-9-thiaoctadecanoate S,S-*Dioxide* (**4b**). From **3b**: a white solid. TLC (hexane/AcOEt 75:25): R_f 0.22. M.p. 69–70° (plates, recrystallized from hexane/AcOEt). IR (KBr): similar to that of **4a**. ^{1}H-NMR (400 MHz, $CDCl_3$): 4.44 (*dt*, *J* = 47.4, 2 H); 3.67 (*s*, 3 H); 2.94 (*m*, 4 H); 2.31 (*t*, *J* = 7.5, 2 H); 1.83(*m*, 4 H); 1.6–1.78 (*m*, 4 H); 1.28–1.52 (*m*, 16 H). ^{13}C-NMR (100 MHz , $CDCl_3$): 174.14; 84.17 (*d*, *J* = 164.1); 52.77; 52.70; 51.51; 33.95; 30.37 (*d*, *J* = 19.6); 29.13; 29.01; 28.73; 28.73; 28.73; 28.49; 28.32; 25.13 (*d*, *J* = 5.4); 24.87; 21.93; 21.86. ^{19}F-NMR (376.5 MHz, $CDCl_3$): –218.33. EI-MS: 335 (9, [*M* – MeO]$^+$), 315 (13), 293 (19), 157 (59, [$(CH_2)_7CO_2Me$]$^+$), 125 (89), 55 (100). HR-EI-MS: 335.2037 ([*M* – MeO]$^+$, $C_{17}H_{32}O_3FS$; calc. 335.2056).

Methyl 18-Fluoro-10-thiaoctadecanoate S,S-*Dioxide* (**4c**). From **3c**: a white solid. TLC (hexane/AcOEt 75:25): R_f 0.24. M.p. 66–67° (plates, recrystallized from hexane/AcOEt). IR (KBr): similar to that of **4a**. ^{1}H-NMR (400 MHz, $CDCl_3$): 4.44 (*dt*, *J* = 47.4, 2 H); 3.67 (*s*, 3 H); 2.94 (*m*, 4 H); 2.30 (*t*, *J* = 7.5, 2 H); 1.84, 1.83 (overlapping *m*, 4 H); 1.6–1.78 (*m*, 4 H); 1.28–1.52 (*m*, 16 H). ^{13}C-NMR (100 MHz , $CDCl_3$): 174.23; 84.10 (*d*, *J* = 164.1); 52.76; 52.72; 51.48; 34.02; 30.32 (*d*, *J* = 19.4); 28.96; 28.96; 28.88; 28.87; 28.87; 28.45; 28.42; 25.09 (*d*, *J* = 5.2); 24.83; 21.92; 21.90. ^{19}F-NMR (376.5 MHz, $CDCl_3$): –218.43. EI-MS: 335 (11, [*M* – MeO]$^+$), 334 (14, [*M* – HOMe]$^+$), 315 (9), 293 (8), 171 (37, [$(CH_2)_8CO_2Me$]$^+$), 139 (89), 55 (100). HR-EI-MS: 334.1964 ([*M* – HOMe]$^+$, $C_{17}H_{31}O_3FS$; calc. 335.1978).

Methyl 18-Fluoro-11-thiaoctadecanoate S,S-*Dioxide* (**4d**). From **3d**: a white solid. TLC (hexane/AcOEt 75:25): R_f 0.22. M.p. 66.5–67° (plates, recrystallized from hexane/AcOEt). IR (KBr): similar to that of **4a.** ^{1}H-NMR (400 MHz, $CDCl_3$): 4.44 (*dt*, *J* = 47.3, 2 H); 3.67 (*s*, 3 H); 2.944, 2.938 (overlapping *m*, 4 H); 2.30 (*t*, *J* = 7.5, 2 H); 1.6–1.78 (*m*, 4 H); 1.28–1.52 (*m*, 16 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 174.30; 83.99 (*d*, *J* = 164.2); 52.81; 52.66; 51.47; 34.07; 30.25 (*d*, *J* = 19.4); 29.09; 29.04; 29.04; 28.99; 28.72; 28.50; 28.43; 24.95 (*d*, *J* = 5.4); 24.89; 21.94; 21.85. ^{19}F-NMR (376.5 MHz, $CDCl_3$): –218.56. EI-MS: 335 (11, [*M* – MeO]$^+$), 315 (1), 293 (13), 185 (27, [$(CH_2)_9CO_2Me$]$^+$), 153 (48), 55 (100). HR-EI-MS: 335.2058 ([*M* – MeO]$^+$; $C_{17}H_{31}O_3FS$; calc. 335.2056).

Incubation Experiments with S. cerevisiae. A typical feeding experiment was carried out as follows: the strain of baker's yeast used in this experiment was wild type *Saccharomyces cerevisiae* # S288C. Cultures were grown in YEPD medium (1.0% yeast extract, 2.0% bactopeptone, 2.0% D-glucose), at 30° in a rotary incubator-shaker set at 150 rpm. The starter culture (1 ml) was used to innoculate 200 ml of sterile medium contained in a 1-l *Erlenmeyer* flask. A 25-mg portion of thiafatty acid methyl ester was added as a soln. (5% (*w*/*v*)) in 95% EtOH to each culture flask, and the innoculated cultures were then incubated for 24 h. The addition of exogenous fatty acid did not affect the growth of the organism with respect to control cultures.

Culture media was collected by centrifugation ($6000 \times g$, 10 min), and the supernatant was adjusted to pH 3.5 with AcOH (*ca.* 5 ml) and extracted with five 80-ml portions of CH_2Cl_2. The combined org. layers were dried and the solvent was evaporated *in vacuo* to give a crude extract, which was methylated with an ethereal CH_2N_2 soln. The residue was dissolved in AcOEt and passed through a small column of *Fluorosil* to give a final extract (*ca.* 25 mg). TLC Analysis of the extract was carried out on silica-gel plates with 80% AcOEt/hexane. The presence of sulfoxide product was confirmed by comparison with R_f values of authentic synthetic standards.

This work was supported by *NSERC* Grant OGP-2528 to *P. H. B.* The technical assistance of *Frances Carvalho* (Carleton University) in performing the yeast incubations is gratefully acknowledged. We thank *Clem Kazakoff* (University of Ottawa Mass Spectrometry Centre) and *Keith Bourque* (Carleton University) for recording the mass and NMR spectra, respectively.

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Received August 19, 2003