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A micromethod for the stereochemical analysis of fatty acid desaturase-mediated sulfoxidation reactions

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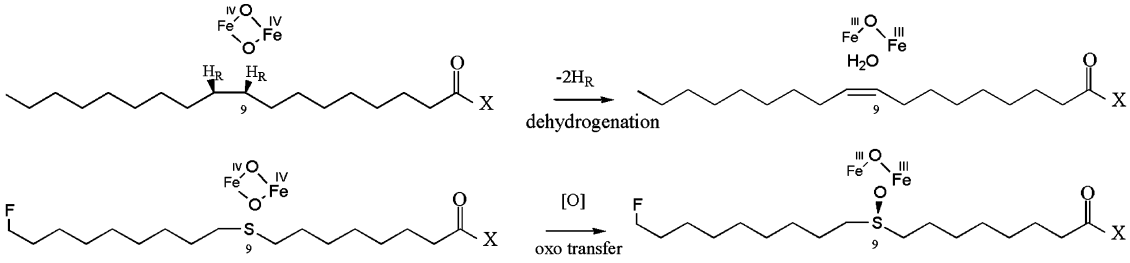
Abstract—The stereochemistry of fatty acid desaturase-mediated sulfoxidation can be evaluated at micromolar concentrations of analyte using ^{19}F NMR in combination with a chiral shift reagent: (*S*)-(+)-MPAA.

Dehydrogenation of long-chain fatty acyl derivatives is a topic of current interest to medicinal chemists following the discovery that upregulation of stearoyl CoA desaturase (SCD) activity may play a role in the metabolic syndrome and associated disorders (Scheme 1).[1] Research in this area is hampered by the lack of structural information on desaturases of this type and indirect methods for mapping active site topology have been developed.[2] The use of ω-fluoro-thiafatty acids was recently validated[3] as a method of probing the cryptoregiochemistry (site of initial oxidation)[4] of a yeast SCD (Scheme 1) as well as a plant Δ^9 desaturase.[5] In this approach, the sulfur atom is used as a methylene isostere to probe for oxo transfer from the putative diiron cluster and a remote fluorine substituent reports the oxidation event using ^{19}F NMR. Herein, we provide a 'proof of concept' demonstration that this methodology can be adapted to determine the enantioselectivity of desaturase-mediated oxo transfer.

Methyl 15-fluoro-11-thiapentadecanoate **1** was selected as a test substrate on the basis of the known characteristics of the commercially available chiral NMR shift reagent (*S*)-(+)-α-methoxyphenylacetic acid (MPAA)[6] and the substrate profile of a convenient whole cell yeast Δ^9 desaturating system.[7] Compound **1** was synthesized by *S*-alkylation of 10-mercaptodecanoic acid[8] with 1-bromo-4-fluorobutane followed by methylation (BF_3/MeOH) using previously published procedures.[3] The crude product was purified by flash chromatography (SiO_2, 5% EtOAC/hexane) to give **1** in an overall yield of 8% based on 10-bromodecanoic acid. The corresponding racemic sulfoxide **2** and sulfone **3** were prepared by oxidation of **1** with one and two equivalents



Scheme 1. The oxidation reactions catalyzed by Stearoyl CoA Δ^9 desaturase. (A) desaturation; (B) sulfoxidation (CoA = Coenzyme A).

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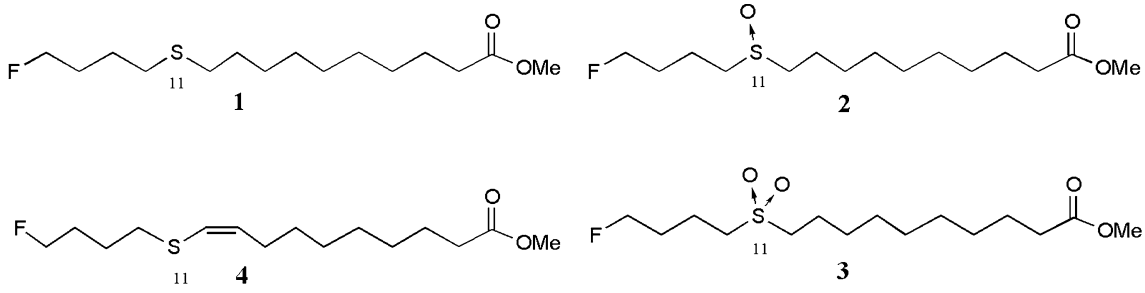

of *meta*-chloroperbenzoic acid (MCPBA), respectively. The analytical and spectral data of **1**, **2**, and **3** were consistent with the assigned structures.[9]

The ^{1}H-decoupled ^{19}F NMR spectrum of each individual compound was recorded in $CDCl_3$ and values for the chemical shifts were found to be as follows: **1** (δ −218.82 ppm), **2** (δ −219.24 ppm), and **3** (δ −219.73 ppm). For the purposes of comparison, the ^{19}F resonances of **1**, **2**, and **3** (ca. 1:1:1 mixture) are displayed in Figure 1A. The effect of sulfur oxidation on the reporter ^{19}F NMR signal of ω-fluorothia-analogues was consistent with previous observations[3] both with respect to direction (upfield) and absolute magnitude of the substituent effect ($\Delta\delta_{SO-S} = 0.42$ ppm $< \Delta\delta_{SO_2-S} = 0.91$ ppm). It should be noted that $\Delta\delta_{SO-S}$ and $\Delta\delta_{SO_2-S}$ in the case of **2** and **3** is substantially greater than the corresponding values for systems where the reporter fluorine is further away from the sulfur centre (e.g., 8–11 sigma bonds: $\Delta\delta_{SO-S} = 0.05$–$0.16$ ppm).[3]

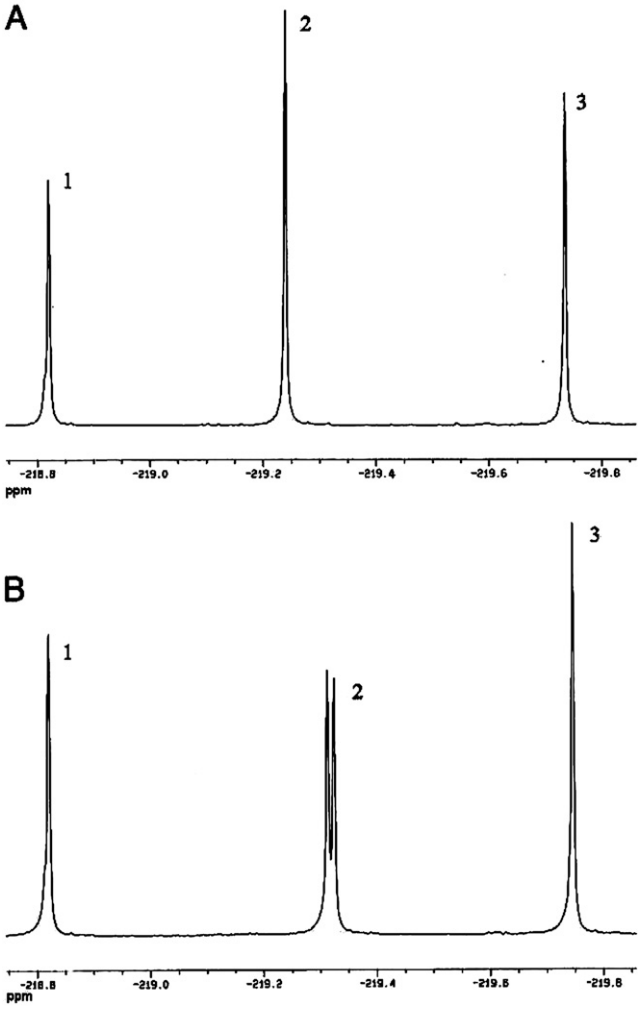

Figure 1. ^{1}H-decoupled ^{19}F NMR spectrum of methyl 15-fluoro-11-thiapentadecanoate **1** and its oxidized derivatives **2** and **3** before addition of (*S*)-MPAA (A): **1** (δ −218.82 ppm); **2** (δ −219.24 ppm); **3** (δ −219.73 ppm) and after addition of (*S*)-MPAA (B): **1** (δ −218.82 ppm); **2** (δ −219.31, −219.32 ppm); **3** (δ −219.75 ppm).

The ^{1}H-decoupled ^{19}F NMR spectrum of the mixture of **1**, **2**, and **3** (ca. 2.5 μmol each) to which (*S*)-(+)-MPAA (70 μmol) was added is shown in Figure 1B. As expected, only the resonance due to racemic sulfoxide is shifted upfield significantly (0.08 ppm) and split ($\Delta\delta = 0.01$ ppm) due to Pirkle-type interaction of racemic **2** with this shift reagent (Fig. 2).[6,10] Similar doubling of the corresponding CH_2F resonances in the ^{1}H NMR spectrum of a sulfoxide **2**/(*S*)-(+)-MPAA mixture was also observed (data not shown). The ^{13}C resonances (d, $J_{CF} = 166$ Hz) of the terminal CH_2F group of racemic **2** were broadened but not differentiated under these conditions.

To evaluate the applicability of this methodology to the stereochemical analysis of enantiomerically enriched sulfoxides isolated from a biological matrix, we incubated **1** (25 mg) with actively growing cultures (200 mL) of *Saccharomyces cerevisiae* S522C under a standard set of conditions (YPED medium,[11] 24 h, 30 °C, 150 rpm).[3] Previous experiments have shown that this whole cell system efficiently incorporates fatty acyl substrates from the medium into the intracellular CoA thioester substrate pool[12] where they are processed by the endogenous Δ^9 desaturase. Polar sulfoxy products produced in this manner are then hydrolyzed and excreted into the medium as the free acid. Thus, centrifu-

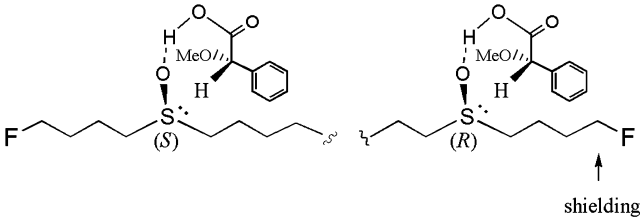

Figure 2. Binding model for the interaction of (*S*)-MPAA with the two enantiomers of methyl 15-fluoro-11-thiapentadecanoate *S*-oxide **2**.

gation of the yeast culture at the end of the incubation period (10,000 rpm, 15 min) yielded a clear supernatant, which was acidified to pH 2 and extracted with methylene chloride. The organic layer was dried (anhyd sodium sulfate), evaporated in vacuo and the fatty acids converted to the corresponding methyl esters by brief (10 min, 0 °C) exposure to freshly prepared ethereal diazomethane. (*Caution*: diazomethane is toxic and explosive.)

^{19}F NMR analysis of the CH_2Cl_2 extract revealed the presence of strong signals corresponding to residual **1** and the corresponding sulfoxide **2**. No overoxidation of **2** to ω-fluoro-sulfone **3** could be detected in this mixture (<1% of total fluorinated analytes). To expedite the stereochemical analysis of sulfoxide **2**, the extract was chromatographed on a short flash chromatography column (SiO_2, EtOAc) to remove the majority of unidentified fluorinated byproducts. The latter were presumably derived from chain cleavage of a 9,10-dehydro-11-sulfide **4** produced in turn by Δ^9 desaturation of **1**. The ^{1}H-decoupled ^{19}F NMR spectrum of purified sulfoxide **2** dissolved in dry $CDCl_3$ is presented in Figure 3A; the amount of **2** (40 nmol, 12 μg) was quantitated through the use of a calibrated, external reference standard (fluperolone acetate).

Stereochemical analysis of biosynthetic sulfoxide **2** was carried out by measuring the effect of (*S*)-(+)-MPAA (4 mg) addition on the ^{19}F reporter resonance (Fig. 3B). The predominant enantiomer (32% ee) was found to be (*S*) according to a Pirkle-type binding model (Fig. 3) that has been validated using a large number of sulfoxides of known configuration.[6b] The observed enantioselectivity of yeast desaturase-mediated sulfoxidation is consistent with that observed using a number of related substrates[13] and corresponds to the known stereochemistry of hydrogen removal (H_R) for the parent dehydrogenation reaction (Scheme 1). The low enantiomeric enrichment of the biosynthetic sulfoxide obtained in this experiment was not unexpected and may correlate with our observation that the efficiency of Δ^9 desaturase-mediated oxo transfer is markedly reduced for thiasubstrates bearing sulfur in positions other than 'C-9' and 'C-10'.[3]

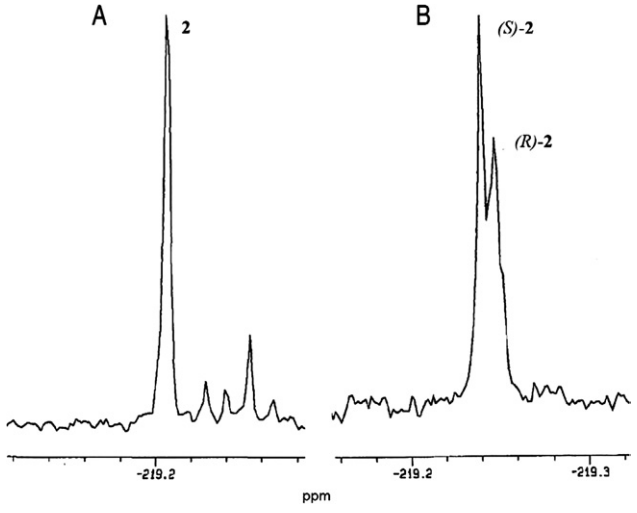

Figure 3. ^{1}H-decoupled ^{19}F NMR spectrum of biosynthetic methyl 15-fluoro-11-thiapentadecanoate *S*-oxide **2** before (A) and after (B) addition of (*S*)-MPAA. Calculation of % ee was corrected for the presence of minor byproducts.

The inherently high sensitivity of ^{1}H-decoupled ^{19}F NMR in combination with suitable remote fluorine labeling has allowed the stereochemical analysis of low-level biosulfoxidation for the first time. This approach would seem to be particularly useful for in vitro applications where analyte concentrations are in the micromolar range or for in vivo mechanistic studies where the efficiency of sulfoxidation is very low.[14] In addition, application of this work to substrates with longer chain length such as stearoyl CoA (Scheme 1) or stearoyl ACP[5] would be an obvious next step; the availability of chiral NMR shift reagents analogous to MPAA but bearing naphthyl or anthryl groups[15] would appear to be promising in this regard. Finally, we would like to point out that the unique characteristics of a ^{19}F NMR-based approach and the relative ease with which ω-fluorinated analogues can be synthesized should permit one to track many other biological mid-chain fatty acid modifications[16] at the trace analytical level.

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8. Prepared from commercially available 10-bromodecanoic acid.[3]

9. The analytical data for **1**, **2**, and **3** are as follows: *Methyl 15-fluoro-11-thiapentadecanoate* (**1**): colorless oil at rt. Mp 12–13 °C; R_f = 0.38 (SiO_2, hexane/EtOAc 90:10); ^{1}H NMR ($CDCl_3$, 400.1 MHz): δ 4.47 (dt, $^2J_{HF}$ = 47.3, $^3J_{HH}$ = 7.5, 2H, CH_2F), 3.67 (s, 3H), 2.56 (t, $^3J_{HH}$ = 7.0, 2H), 2.50 (t, $^3J_{HH}$ = 7.4, 2H), 2.30 (t, $^3J_{HH}$ = 7.5, 2H), 1.53–1.86 (m, 8H), 1.25–1.40 (m, 10H); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 174.33, 83.68 (d, $^1J_{CF}$ = 164.8), 51.46, 34.09, 32.06, 31.66, 29.64, 29.55 (d, $^2J_{CF}$ = 19), 29.31, 29.19, 29.17, 29.11, 28.88, 25.39 (d, $^3J_{CF}$ = 4.7), 24.93; ^{19}F NMR (376.5 MHz, $CDCl_3$): δ −218.82; IR (film): ν_{max} 2928, 2855, 1740 (C═O), 1436, 1436, 1253, 1197, 1172 cm^{-1}; EI-MS: *m/z* 292 (22, M^+), 261 (17, $[M-31]^+$), 185 (56, $[(CH_2)_9CO_2CH_3]^+$), 121 (25), 87 (61, $[CH_2{=}CH(CH_2)_2S]^+$), 74 (64), 55 (100); HR-EI-MS *m/z* 292.1859 ($[M]^+$, $C_{15}H_{29}O_2FS$ requires 292.1872). (*R,S*)-*Methyl 15-fluoro-11-thiapentadecanoate S-oxide* (**2**): amorphous white crystals (recrystallized from hexane/EtOAc). Mp 69–70 °C; R_f 0.13 (SiO_2, EtOAc); IR (KBr): ν_{max} 2920, 2848, 1731 (C═O), 1471, 1438, 1198, 1176, 1081 (SO) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400.1 MHz): δ 4.50 (dt, $^2J_{HF}$ = 47.8, $^3J_{HH}$ = 7.5, 2H), 3.67 (s, 3H), 2.59– 2.75 (m, 4H), 2.30 (t, $^3J_{HH}$ = 7.6, 2H), 1.58–1.98 (m, 8H), 1.38–1.52 (m, 2H), 1.25–1.38 (m, 8H); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 174.33, 83.42 (d, $^1J_{CF}$ = 165.6), 52.51, 51.80, 51.49, 34.07, 29.51 (d, $^2J_{CF}$ = 20.0), 29.12 (3C's), 29.06, 28.82, 24.90, 22.59, 19.08 (*d*, $^3J_{CF}$ = 4.7); ^{19}F NMR ($CDCl_3$, 376.5 MHz): δ −219.24; EI-MS: *m/z* 291 (17, $[M-OH]^+$), 277 (5), 235 (4), 202 (3), 162 (4), 111 (5), 83 (9), 69 (63) 55 (100); HR-EI-MS *m/z* 291.1787 ($[M-OH]^+$, $C_{15}H_{28}O_2FS$ requires 291.1794).
Methyl 15-fluoro-11-thiapentadecanoate S,S-dioxide (**3**): amorphous white solid. Mp 59–60 °C; R_f 0.23 (SiO_2, hexane/EtOAc 75:25); IR (film): ν_{max} 2915, 2848, 1735 (C═O), 1466, 1416, 1328, 1267, 1246, 1124 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400.1 MHz): δ 4.51 (dt, $^2J_{HF}$ = 47.2, $^3J_{HH}$ = 7.5, 2H), 3.67 (s, 3H), 3.02 (m, 2H), 2.96 (m, 2H), 2.31 (t, *J* = 7.5, 2H), 1.8–2.06 (m, 6H), 1.61 (m, 2H), 1.44 (m, 2H), 1.25–1.38 (m, 8H); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 174.30, 83.23 (d, $^1J_{CF}$ = 165.8), 52.89, 52.10, 51.49, 34.06, 29.15 (d, $^2J_{CF}$ = 19), 29.09, 29.03 (2C's), 28.98, 28.46, 24.88, 21.91, 18.55 (d, $^3J_{CF}$ = 4.2); ^{19}F NMR ($CDCl_3$, 376.5 MHz): δ −219.73. EI-MS *m/z* 293 (9, $[M-OCH_3]^+$), 273 (<1), 251 (4), 185 (9, $[(CH_2)_9CO_2CH_3]^+$), 153 (18), 141 (20), 98 (81), 69 (74), 55 (100); HR-EI-MS: *m/z* 293.1587 ($[M-OCH_3]^+$, $C_{14}H_{26}O_3FS$ requires 293.1587).
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