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A chemoenzymatic route to quasisymmetrical chiral sulfoxides and their phospholipid derivatives

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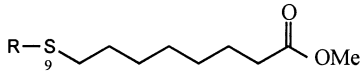
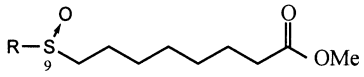
Abstract—The chain-length dependence of yeast Δ^9 desaturase-mediated sulfoxidation was examined. Methyl (*R*)-9-thiahexadecanoate *S*-oxide (95% ee) and the corresponding phosphatidylcholine diester was synthesized.

It has been shown previously that fatty acid desaturases[1] can function as highly enantioselective sulfoxidases if the thia substrate analogues bear a sulfur atom corresponding to the site at which the parent dehydrogenation reaction is initiated.[2,3] The stereochemistry of the oxo transfer matches the known preference for pro *R* hydrogen removal[4] in the desaturation process (Scheme 1). Little overoxidation to the sulfone has been observed in these experiments. Given the continued interest in functionalized lipids and the effect of mid-chain stereochemistry on self assembly,[5] we wished to explore the feasibility of synthesizing chiral, non-racemic, sulfoxy fatty acids on a preparative scale. Herein, we report the results of an investigation in which we determine how the efficiency of yeast Δ^9 desaturase-mediated sulfoxidation varies as a function of substrate chain length. A model synthesis of a phospholipid bearing enantiomerically enriched, sulfoxy-containing side chains is also reported.

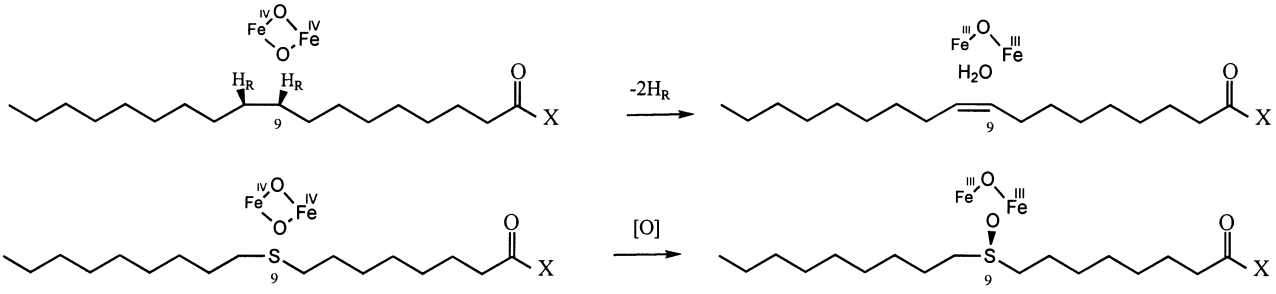
A homologous series of 9-thiafatty acids (**1a**–**k**) ranging in chain length from C-10 to C-20 was synthesized by alkylation of 8-thiooctanoic acid with the appropriate alkyl bromide using previously published procedures.[6] Sulfoxide reference standards (*R*,*S*)-**2a**–**k** were prepared by oxidation of the corresponding sulfide methyl ester using one equivalent of *meta*-chloroperbenzoic acid (MCPBA).[7] The analytical data (^{1}H and ^{13}C NMR, MS) of the substrates and sulfoxy derivatives were in accord with previous structural assignments.[2,7]



1a-k, **a**: R = methyl, **b**: R = ethyl.... **k**: R=undecyl



2a-k, **a**: R = methyl, **b**: R = ethyl.... **k**: R=undecyl



Scheme 1. Relationship between Δ^9 desaturation of long chain fatty acyl derivatives and the corresponding sulfoxidation of thia analogues (X = Coenzyme A or phospholipid ester).

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Each substrate methyl ester (~25 mg, ethanol) was incubated separately with actively growing cultures (200 mL) of wild type *S. cerevisiae* #5288C for 24 h. After centrifugation of the yeast cells (6000 rpm, 20 min), the supernatant was acidified to pH 3 and extracted with $CHCl_3$ (4×100 mL). The procedures used have been outlined in an earlier account.[2] The combined extracts were dried over Na_2SO_4, evaporated to constant weight and the amount of sulfoxide produced in each case quantitated by ^1H NMR analysis. The latter was accomplished by integration of the α-sulfinyl resonances at δ 2.55–2.70 ppm relative to an internal standard – methyl 2-methoxy-2-phenylethanoate (singlet, δ 4.76 ppm). The conversion of sulfide to sulfoxide for each substrate is compared in Table 1.

Inspection of the data in Table 1 reveals that useful levels of desaturase-catalyzed 9-sulfoxidation were observed for substrates with chain lengths ranging from C-14 to C-19. These results correlate well with an earlier in vitro study in which the chain length dependence of maximal enzyme velocity (Vmax) for a closely related, hepatic Δ^9 desaturase was examined (Table 1).[8] We have also observed similar trends for yeast-mediated Δ^9-desaturation of a homologous series of 5-thia fatty acids (unpublished results). The very high yield observed for sulfoxidation of the C-15 substrate is somewhat surprising; a more detailed analysis of structure/activity relationships must await the results of ongoing efforts to determine the structure of the yeast Δ^9 desaturase.

Having defined the boundaries of sulfoxidase activity, we chose to scale up the production of 9-thiahexadecanoate *S*-oxide **2g** in order to facilitate potential comparison with naturally occurring, non-thia, C-16 (palmitoyl)-containing phospholipid systems. Thus methyl 9-thiahexadecanoate (**1g**, 500 mg) was incubated with *S. cerevisiae* in batch culture under conditions similar to that used in the trial experiment. Extracts of the medium were treated with diazomethane/ether (CAUTION: *diazomethane is toxic and explosive*) and the crude methyl 9-thia hexadecanoate *S*-oxide so obtained was purified by flash chromatography (SiO_2, 100% EtOAc) to yield ~200 mg of the desired product (40% yield) as a white solid. The analytical data for this material (^1H and ^{13}C NMR, IR, MS and HRMS (EI): *m/z* calcd for $C_{16}H_{31}O_2S$ (M^+–OH, base peak) 287.2045; found: 287.2044) were identical to those of the reference standard prepared by MCPBA oxidation of the parent sulfide.

The stereochemical analysis of biosynthetic **2g** was achieved via application of the methodology developed[2] to analyze the corresponding C-18 sulfoxy analogue **2i**. This approach involves the use of (*S*)-(+)-α-methoxyphenylacetic acid (MPAA) as a chiral NMR shift reagent.[9] Application of a Pirkle-type[10] complexation model, which has been validated by synthesis of chiral reference standards,[2,9] allows prediction of the absolute configuration at the sulfinyl centre via the observation of differential upfield shielding effects (Fig. 1). Due to the complexity of the ^1H NMR spectrum in the sulfoxide region of **2g**, we elected to use the two α-sulfoxy ^{13}C signals as our reporter resonances.[†] As depicted in Fig. 2, our analysis clearly shows that the yeast-derived product **2g** was highly enriched (~95% ee) in the *R*-enantiomer—a result which is consistent with a diverted Δ^9 desaturase-catalyzed process (Scheme 1).

The ready availability of long chain chiral sulfoxides paves the way for an examination of how asymmetry

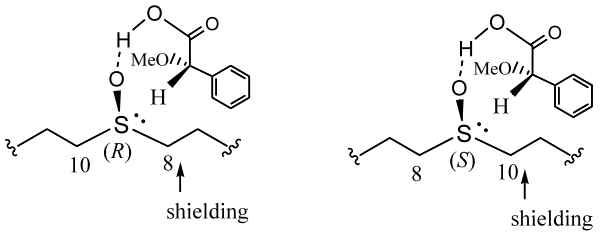


Figure 1. Binding model for the interaction of (*S*)-MPAA with the two enantiomers of **2g**.

Table 1. Effect of substrate chain length on the efficiency of baker's yeast-mediated sulfoxidation of 9-thia fatty acid methyl esters

Substrate	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**1h**	**1i**	**1j**	**1k**
Chain length	10	11	12	13	14	15	16	17	18	19	20
Conversion (%)[a]	<5	<5	<5	<5	19	90	40	35	11[b]	30	<5
Vmax[c]	<1	ND[d]	7	50	69	ND	86	103	100	103	<1

[a] % Conversion was evaluated by quantation of the sulfoxy product found in the supernatant. Previous experiments[2,7] have shown that very little sulfoxide is found in the cells. The sulfoxide is produced as the free acid via a yeast-mediated hydrolysis reaction.

[b] This value is in good agreement with previously measured[2] conversions (8, 9%) of methyl 9-thiaoctadecanoate **2i**.

[c] Relative maximal velocity of Δ^9 desaturation of *n*-alkanoate derivatives.[8]

[d] ND = not determined.

[†] The ^{13}C chemical shifts for C-8 (δ 52.40 ppm) and C-10 (δ 52.53 ppm) of **2g** were assigned based on the similarity of these values with those previously attributed[2] to the corresponding carbons of the C-18 analogue **2i**: C-8 (δ 52.42 ppm) and C-10 (δ 52.56 ppm). The latter resonances were unambiguously assigned via regiospecific deuterium labeling.[2]

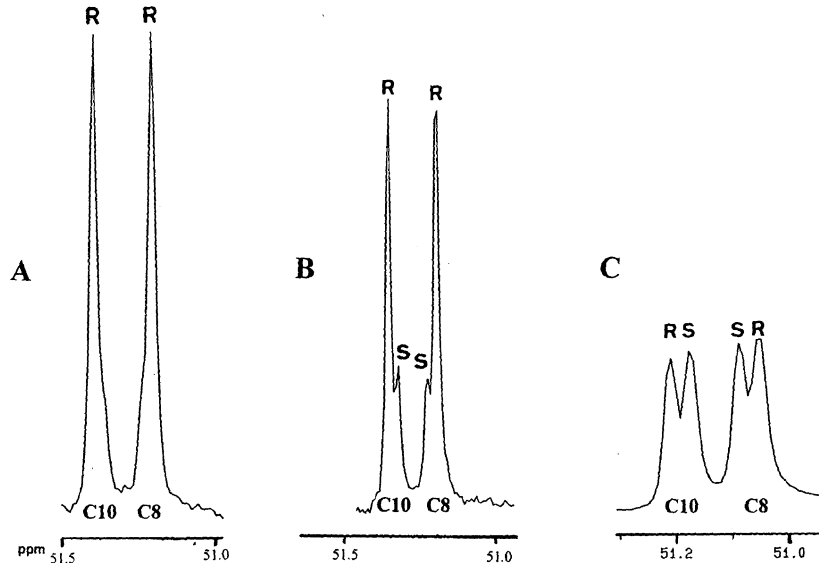
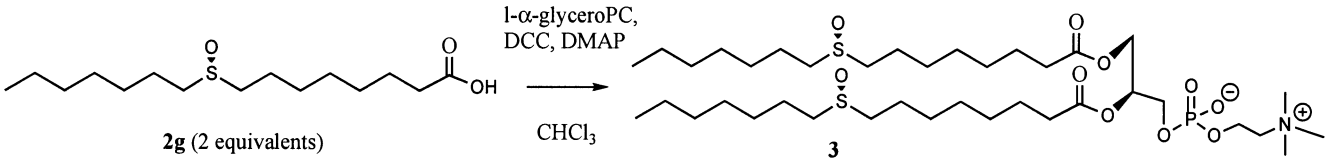


Figure 2. Effect of addition of 3 equiv. of (*S*)-MPAA on ^{13}C NMR (100.6 MHz) resonances due to the α-sulfinyl carbons of (**A**) biosynthetic methyl 9-thiahexadecanoate S-oxide (95% ee); (**B**) a 2:1 mixture of biosynthetic methyl 9-thiahexadecanoate S-oxide (95% ee) with corresponding racemate (resultant 63% ee, calculated = 63% ee, observed); (**C**) racemic methyl 9-thiahexadecanoate S-oxide.



Scheme 2. Synthesis of the phosphatidyl choline ester of (*R*)-9-thiahexadecanoate *S*-oxide.

affects self assembly. Since such studies are frequently carried out using esters of phosphatidyl choline, biosynthetic **2g** was hydrolyzed (2N KOH/EtOH) and the resultant acid coupled with l-α-glycerophosphorylcholine under standard anhydrous conditions ($CHCl_3$, DCC, DMAP catalysis).[11] The desired phospholipid **3** was obtained as a white solid[12] in 46% yield after purification by gradient flash chromatography (10% MeOH/$CHCl_3$ (1:10), H_2O/MeOH/$CHCl_3$ (4:35:65) (Scheme 2). That the stereochemical purity of the sulfinyl centers had not decreased measurably during the hydrolysis/coupling sequence was demonstrated by ^{13}C NMR analysis ($CDCl_3$, 6 equiv. of (*S*)-MPAA) as discussed above. Preliminary Langmuir film studies of **3**, and the corresponding material synthesized from racemic **2g** suggest that a 'homochiral' lipid exhibits improved packing characteristics relative to a mixture of diastereomers. A more detailed analysis using DSC (differential scanning calorimetry) measurements is in progress.

In summary, we have demonstrated the feasibility of generating a series of novel, chiral sulfoxide-containing phospholipids by a relatively straightforward combination of enzymatic and chemical synthesis. If so desired, the position of the sulfoxy function along the hydrocarbon chain can be altered by taking advantage of the wide range of naturally occurring desaturase regioselectivities[1] and the ease with such enzymes can be expressed in microbial hosts.

Acknowledgements

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12. **1,2-(9′*R*,9″*R*)-Di-9-thiapalmitoyl-*S*-oxide-*sn*-glycero-3-phosphatidylcholine**: R_f 0.22 (SiO_2, H_2O/MeOH/$CHCl_3$ (4:35:65)); ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.90 (t, *J* 6.8 Hz, 6H, 2×RCH_3) 1.30–1.44 (m, 28H, methylene envelope) 1.60 (m, 4H, 2×O(O)CCH_2CH_2) 1.76 (m, 8H, 2×CH_2CH_2SOCH_2CH_2) 2.31 (t, *J* 7.5 Hz, 4H, (O(O)CCH_2) 2.67 (m, 8H, 2×CH_2SOCH_2), 3.37 (s, 9H, N(CH_3)$_3$), 3.81 (m, 2H, $(CH_3)_3$NCH_2), 3.97 (m, 2H, OPO_2OCH_2CH), 4.14 (dd, *J* 12.0 Hz, *J* 7.2 Hz, 1H, HC-C$H_a$$H_b$O(O)C), 4.34 (m, 2H, $(CH_3)_3$NCH_2CH_2O-), δ 4.41 (dd, ^2J 12.0 Hz, ^3J 2.5 Hz, 1H, HCC$H_a$$H_b$)O(O)C), 5.21 (m, 1H, OPO_2OCH_2CH); ^{13}C NMR (100.6 MHz) δ 14.04 (R-*C*H_3), 22.56, 22.62, 22.64, 24.69, 24.73, 28.65, 28.74, 28.79, 28.85, 28.88, 31.55, 33.97 (C(O)O*C*H_2), 34.17 (C(O)O*C*H_2), 52.33 (2×*C*H_2SO$(CH_2)_6CH_3$), 52.54 (2×SO*C*$H_2$$(CH_2)_5CH_3$), 54.56 ($(H_3C)_3$NR), 59.25 (d, $^2J_{CP}$ 4.9 Hz, $(CH_3)_3$N(CH_2)*C*H_2O-PO_2O), 62.95 (OHC*C*H_2OC(O), 63.40 (d, $^2J_{CP}$ 5.2 Hz, (OPO_2-O*C*H_2R)), 66.49 (d, $^3J_{CP}$ 6.0 Hz, $(CH_3)_3$N*C*H_2-CH_2OPO_2), 70.61 (d, $^3J_{CP}$ 7.6 Hz, (OPO_2O-CH_2*C*HO), 173.07 (R*C*(O)OR), 173.42 (R*C*(O)OR); MS (electrospray) *m*/*z* 825 (MNa^+), 802 (MH^+).