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Conformational Studies on the Δ^8(*E*,*Z*)-Sphingolipid Desaturase from *Helianthus annuus* with Chiral Fluoropalmitic Acids As Mechanistic Probes

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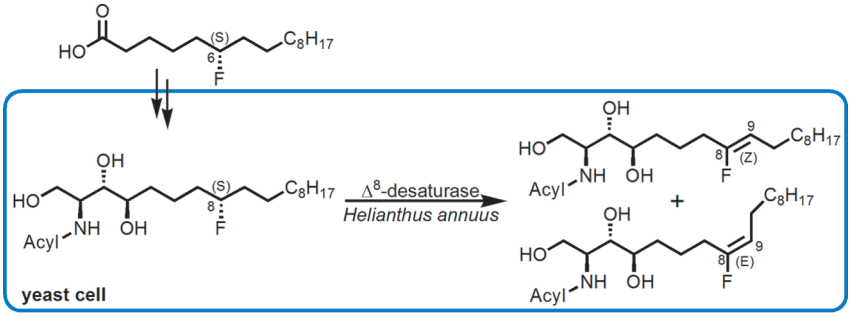

The Δ^8-sphingolipid desaturase from sunflower (*Helianthus annuus*) converts phytosphinganine into a mixture of Δ^8-(*E*)- and -(*Z*)-phytosphingenines by removal of two *syn*-hydrogen atoms from *anti*-, and *gauche*-conformations of the substrate. With chiral (*R*)-6-, (*S*)-6-, (*R*)-7-, and (*S*)-7-fluoropalmitic acids the importance of conformations for the formation of (*E*)- and (*Z*)-isomers was investigated by using growing yeast cells expressing the desaturase from *H. annuus*. The fluoropalmitic acids were readily incorporated into a series of fluorinated phytosphinganines. The desaturation products of the major C_{18}-fluorophytosphinganine demonstrate that different conformations of the relevant aliphatic segment of the sphingolipids can be exposed to the active center of the enzyme resulting in (*E*)- or (*Z*)-fluoroalkenes. The presence of a fluorine atom at the position of the initial hydrogen removal C8−H_R led to a complete suppression of the desaturation reaction, while replacement of C8−H_S with fluorine generated a mixture of mainly (*Z*)- and trace amounts of (*E*)-fluoroolefine. Fluorine at C9 of the phytosphinganine precursors did not interfere with the initial C−H activation step and produced (*E*)- and (*Z*)-fluoroalkenes in the same ratio as observed for the nonfluorinated precursors. Hydroxylated byproducts of the desaturation process were not observed. These results strongly support the importance of conformations of the transition states during desaturation as the relevant criterion for the relative ratio of (*E*)- and (*Z*)-alkenes.

Introduction

Sphingolipids are characteristic lipid components of all eukaryotic cells in which they have structural roles as membrane components and signaling functions in many regulatory circuits.[1−3] The sphingolipids are generally composed of a long-chain base (LCB), an amide-linked fatty acyl chain of varying chain length, and a polar head.[4] The predominant LCBs are C_{18} amino alcohols such as sphinganine (**3**) and 4-hydroxysphinganine

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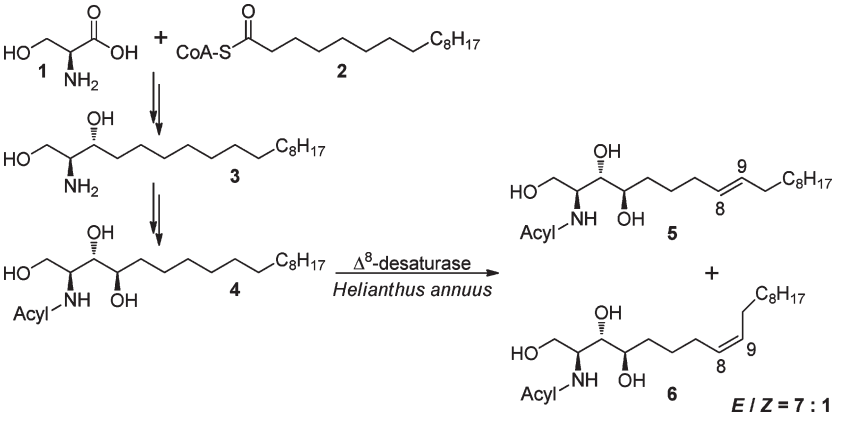
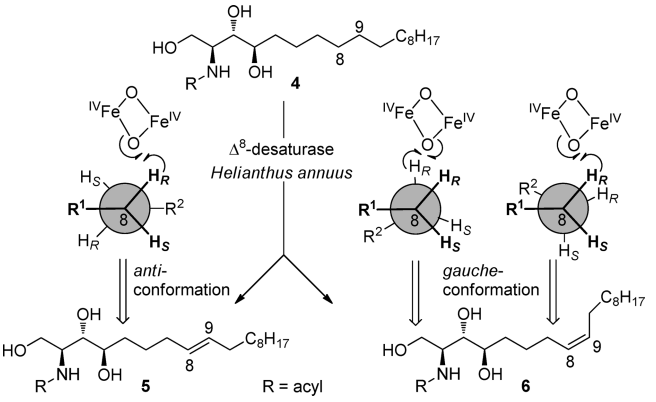
SCHEME 1. Biosynthesis of Unsaturated (8*E*,*Z*)-Phytoceramides



found in many organisms. Many of them, and in particular plants and fungi, can introduce additional double bonds into their LCB resulting in additional variation, with the unsaturated 4-hydroxy-8-sphingenine prevailing in plants.[5] The first and committed step in the de novo sphingolipid synthesis is catalyzed by a serine palmitoyltransferase (EC 2.3.1.50), which condenses serine (**1**) and palmitoyl-CoA (**2**) to 3-ketosphinganine. The 3-ketosphinganine is then reduced to the LCB sphinganine (**3**). Following acyl-transfer (ceramide synthase) and hydroxylation at C4, a phytoceramide (*N*-acyl 4-hydroxysphinganine) **4** is obtained.

In plants, the LCBs can be additionally desaturated between C8 and C9 yielding a mixture of (*E*)- and (*Z*)-isomers of Δ^8-unsaturated LCBs **5** and **6**, namely (8*E*/*Z*)-4-hydroxy-8-sphingenines (phytosphingenines).[6] Heterologous expression of plant-derived cDNAs encoding the Δ^8-desaturases in yeast cells (*S. cerevisiae*) resulted in significant proportions of both (*E*)- and (*Z*)-isomers of 4-hydroxy-8-sphingenines (Scheme 1). Since these compounds were not produced by the wild-type yeasts, their formation in the transgenic cells could be clearly attributed to the activity of the expressed nonstereospecific sphingolipid Δ^8-desaturase. Moreover, depending on the origin of the Δ^8-desaturase, different but characteristic (*E*/*Z*)-ratios ranging from pure (*E*)-isomers (from fungi[7]) via 7:1 and 1:1 to predominating (*Z*)-isomers (from plants[8−14]) were observed with the same expression system.[5] The deduced amino acid sequences of the proteins show high similarities to membrane-bound acyl lipid desaturases bearing a highly conserved multihistidine di-iron coordination site.

SCHEME 2. Proposed Mechanism for a Nonstereospecific Sphingolipid Δ^8-Desaturase from Plants



The desaturases are assumed to convert the saturated substrate into an olefin by a two-step radical mechanism: Initial C−H activation by a bis(μ-oxo)-diiron complex (Scheme 2) removes the first hydrogen atom from the carbonyl-proximal carbon. This step is rate limiting and displays a high intrinsic primary kinetic isotope effect (KIE, $k_{H/D} \approx 3-8$), whereas the second hydrogen atom is lost without a significant KIE. Desaturation proceeds suprafacially and removes two *syn*-oriented vicinal hydrogen atoms from the substrate.[15−17] The simultaneous formation of (*E*,*Z*)-olefins by a single enzyme was rationalized by removal of two vicinal *syn*-oriented hydrogen atoms from the substrate in different conformations. In sunflower Δ^8-sphingolipid desaturases a low but distinct kinetic isotopic effect suggested a preferential attack of the reactive di-iron center onto the C8−H_R ($k_{H/D} = 1.91$) of the substrate to yield the (*E*)-isomer after loss of the neighboring C9−H_S ($k_{H/D} = 1.16$) via an energetically favored *anti*-orientation. (*Z*)-Isomers are produced via two different *gauche*-orientations after initial attack onto either the C9−H_R ($k_{H/D} = 3.97$) or the C8−H_R ($k_{H/D} = 2.07$) followed by loss of the vicinal hydrogen atom[18] (Scheme 2).

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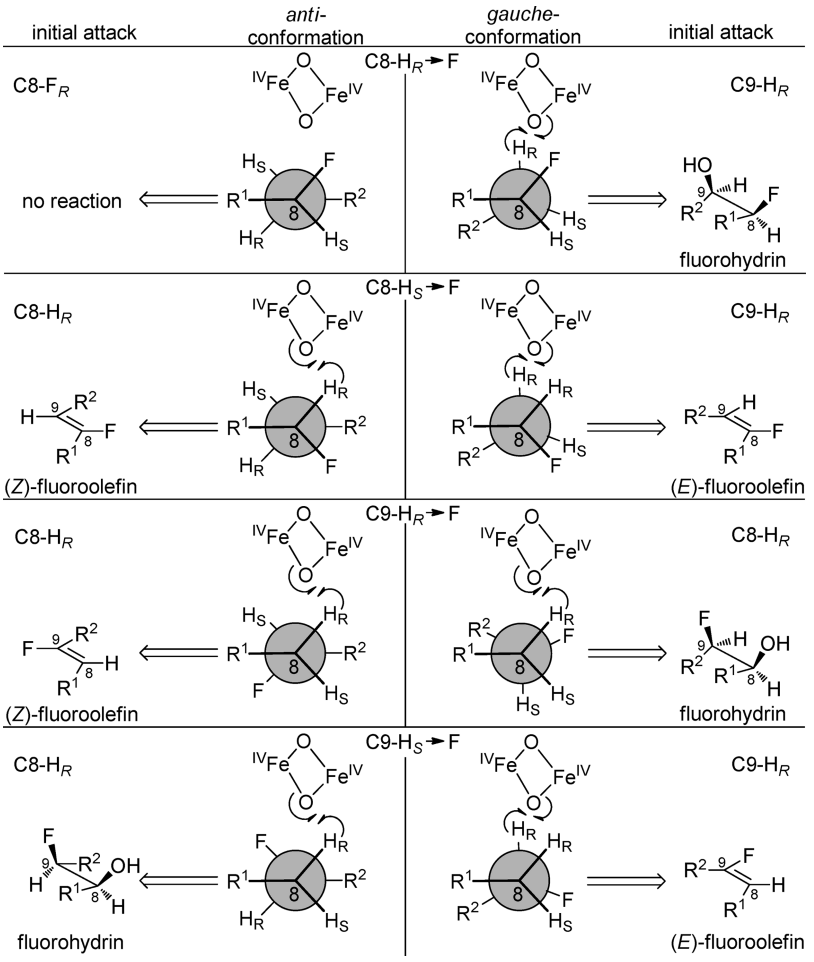
SCHEME 3. Mechanistic Alternatives for the Desaturation of Chiral 8- or 9-Fluorophytosphinganines by the Δ^8-Desaturase from *Helianthus annuus*[a]



[a]Only one *gauche*-conformation is shown.

According to this model, the final ratio of the (*E*/*Z*)-olefins will be determined by the ratio of the differently populated conformations of the substrate with either *anti*- or *gauche*-orientation around the C8−C9 axis. Since the enzyme accepts both substrate conformations, the controlled manipulation of steric or electronic effects within the substrate can be expected to alter the products from various conformations in a predictable fashion. In particular, fluorinated substrates are promising tools for this type of modification since the exchange of a hydrogen atom by fluorine does not greatly affect the size or shape of the substrate but strongly modifies the electronic properties of the adjacent bonds. Certain conformations of the chiral fluorosubstrates will expose the inert fluorine atom to the active site of the desaturase, which will not yield a product. Only after a conformational change can desaturation products be expected and, hence, the fluorinated substrates can be used as valuable mechanistic probes. With respect to the established major reaction pathway to (*E*)-olefins implying an initial attack onto the C8−H$_R$ of the *anti*-oriented substrate followed by *syn*-elimination of the C9−H$_S$ (Scheme 2), different stereochemical outcomes can be envisaged for the transformation of chiral 8- or 9-fluorophytosphinganines by the sunflower desaturase (Scheme 3).

1. (*R*)-8-Fluorophytosphinganine: The substrate conformation en route to the major (*E*)-isomer exposes the fluorine atom to the active center of the desaturase. The initial attack onto the C8−F$_R$ is, thus, prohibited and no olefin should be formed. Instead, the formation of a fluorohydrin[19,20] should be possible from a *gauche*-conformation with initial attack at C9.

2. (*S*)-8-Fluorophytosphinganine: The substrate can be directly converted into a (*Z*)-fluoroalkene via the preferred *anti*-orientation of the substituents R^1 and R^2. The formation of an (*E*)-fluoroalkene would require a *gauche*-conformation. Note that due to priorities in nomenclature assignment the (*Z*)-fluoroalkene has the same architecture of the carbon skeleton as the nonfluorinated (*E*)-alkene.

3. (*R*)-9-Fluorophytosphinganine: Expected products are the (*Z*)-fluoroalkene from the *anti*-conformer and a fluorohydrin from the two *gauche*-conformers.

4. (*S*)-9-Fluorophytosphinganine: The *anti*-conformer is expected to generate a fluorohydrin. The two *gauche*-conformations should produce an (*E*)-fluoroalkene as illustrated in Scheme 3.

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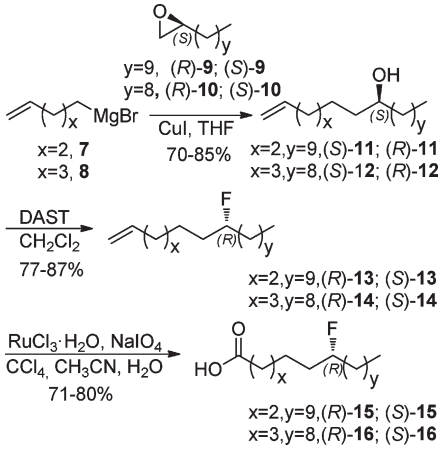
SCHEME 4. Synthesis of Chiral Fluoropalmitic Acids



In summary, depending on the configuration of the 8- or 9-fluorosphinganine either (*E*)- or (*Z*)-fluoroolefins should be generated in a predictable fashion proving the concept that different substrate conformations lead to the nonstereospecific generation of (*E*,*Z*)-olefins by a single enzyme. Here we report on the stereochemical analysis of the Δ^8-sphingolipid desaturase from sunflower (*Helianthus annuus*) using chiral 8- and 9-fluorosphinganines as substrates. The enantiospecific synthesis of chirally fluorinated palmitic acids as precursors for the fluorinated phytoceramides and their conversion to fluorinated sphingolipids as well as the mechanistic consequences of the F/H-exchange on the desaturation are described.

Results

Synthesis of Chiral Fluoropalmitic Acids. Fluorinated palmitic acids can be used as distinguished mechanistic probes for the desaturases since the transgenic yeast generates the required 8- and 9-fluorophytosphinganines from these substrates (Scheme 1). The synthesis of the chiral fluoropalmitic acids was achieved along the protocol of Habel et al.[21] as outlined in Scheme 4. The readily available chiral terminal epoxides **9** (**10**) (>99% ee)[22,23] were alkylated at C1 with terminally unsaturated Grignard reagents in the presence of Cu(I). The resulting secondary alcohols **11** (**12**) were obtained in high yield and high ee (>99%)[21] as confirmed by Mosher ester derivatization (see the Supporting Information).

After recrystallization from pentane at −20 °C, the alcohols were converted with diethylaminosulfur trifluoride (DAST) at −78 °C into the corresponding fluoroalkenes **13** (**14**) with inversion of configuration.[24,25] The olefins were converted to the chiral fluoropalmitic acids (*R*)-**15**, (*S*)-**15**, (*R*)-**16**, and (*S*)-**16** by oxidative degradation with $RuCl_3$ and $NaIO_4$.[26]

Administration of Fluoropalmitic Acids to Transgenic Yeast Cells. Cultures of the yeast strain *S. cerevisiae* INVSc1 (Invitrogen) containing the Δ^8-sphingolipid desaturase from *Helianthus annuus* (pHaDES8) were grown at 30 °C for 48 h in complete minimal-dropout-uracil medium.[14] For desaturase expression the cultures were induced with galactose and incubated in the presence of cerulenin[27] and a chiral fluoropalmitic acid for 5 d at 28 °C. Cerulenin was added to avoid channeling of the fluoropalmitic acid into the general fatty acid biosynthesis of the yeast. After optimization of the growth conditions with respect to temperature, nutrient composition, inhibitor and substrate concentrations, as well as time (5 d at 28 °C), fluorinated 4-hydroxysphinganines and 4-hydroxysphing-8-enines (>95% labeled) could be isolated from the cultures. The optimization was essential, since the fluoropalmitic acids, unlike palmitic acid, reduced the growth of the yeast cultures which ceased completely at substrate concentrations >90 μg mL^{-1}.

Analysis of Fluorinated Sphingolipids. Established workup procedures of complex sphingolipids from plant tissue make use of a strong base such as $Ba(OH)_2$ to liberate the LCBs from the ceramides.[28,29] Since these procedures gave only low yields for the fluorinated LCBs, an alternative approach without cleavage of the amide bond of the phytoceramide moiety was developed. The presence of a vicinal diol in the phytoceramide moieties allows a selective and highly efficient cleavage of the phytoceramide **17** in simple as well as in more complex sphingolipids between the hydroxy groups at C3 and C4 by using $Pb(OAc)_4$.[30] This procedure gave high yields of fluoroaldehydes which after prepurification by passage over Florisil were converted into the corresponding *N*,*N*-dimethylhydrazones (DMZ). Unlike the free fluoroaldehydes the *N*,*N*-dimethylhydrazones show characteristic ion fragments that allow a rapid identification and a detailed analysis of the metabolites by GLC-MS (Figure 1). All hydrazones of the saturated fluoroaldehydes display an intense molecular ion (Figure 1A) and a major fragment at *m*/*z* 85 resulting from allylic cleavage next to the N=C double bond and a fragment at *m*/*z* 86 corresponding to the McLafferty rearrangement of the hydrazone moiety.[31,32] The other minor fragment ions are generated mainly by repeated alkyl cleavage. The typical EI-induced elimination of HF was not observed with the *N*,*N*-dimethylhydrazones representing an additional major advantage of this procedure. The molecular ions of the derivatized fluoroolefins (Figure 1B) are less abundant, but still allow identification of the compounds.

The stereochemistry of the unsaturated 5- and 6-fluoroaldehydes was determined by GLC with synthetic references. The stereo- and regioisomers display different retention times and allow the unequivocal determination of the configuration of the enzymatically introduced double bonds (see the Supporting Information).

Administration of Chiral Fluoropalmitic Acids and Analysis of Fluorinated Metabolites. Despite their effects on culture growth the fluorinated palmitic acids were taken up by the yeasts, and under optimized conditions acceptable yields of fluorinated sphingolipids were obtained. The chiral fluoropalmitic acids **15** and **16** were clearly incorporated into the LCB backbone as indicated by analysis of the corresponding *N*,*N*-dimethylhydrazones obtained after treatment of the

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crude lipid extracts with $Pb(OAc)_4$ and *N*,*N*-dimethylhydrazine (see Experimental Section). The main product was the expected hydrazone **18** of 5-fluoropentadecanal resulting from diol-cleavage of 8-fluorophytosphinganine **17** (Figure 1). In addition we found minor amounts of the *N*,*N*-dimethylhydrazones of saturated fluorinated C_{16}- to C_{18}-aldehydes derived from C_{19}- to C_{21}-fluorophytosphinganines, probably resulting from a combination of chain elongation by acetate units and α-oxidation of the fatty acid precursor. Moreover, fluorinated *N*,*N*-dimethylhydrazones from long-chain C_{25}- and C_{26}-aldehydes (derived from C_{28}- and C_{29}-fluorophytosphinganines) were detected. Nonfluorinated hydrazones derived from C_{18}- and C_{20}-phytosphinganines were only found in trace amounts due to the inhibition of fatty acid elongation by cerulenin. All *N*,*N*-dimethylhydrazones were identified by references and their molecular ions with high-resolution mass spectroscopy (HRMS). The stereochemistry of the unsaturated fluoroaldehydes, resulting from desaturation of the fluorinated phytosphinganines by the *H. annuus* enzyme, was determined by GLC comparison with synthetic references (see the Supporting Information). Thus, administration of (*R*)-6-fluoropalmitic acid ((*R*)-**15**) generated (*R*)-8-fluorophytosphinganine and administration of (*S*)-6-fluoropalmitic acid ((*S*)-**15**) resulted in (*S*)-8-fluorophytosphinganine (**27**), which was predominantly transformed to (*Z*)-8-fluoro-8-phytosphingenine (**30**) along with only trace amounts of the (*E*)-8-fluoro-8-phytosphingenine (**31**). (*R*)-8-Fluorophytosphinganine (**26**) did not yield any desaturation product.

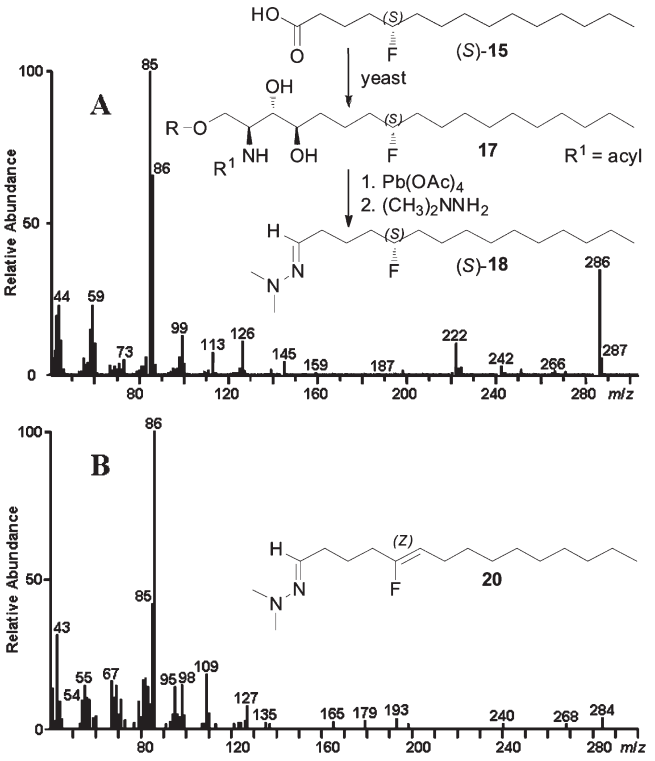


FIGURE 1. Mass spectra of (un)saturated *N*,*N*-dimethylhydrazones **18** and **20**. The fluorinated metabolites were analyzed by GLC-MS after oxidative cleavage of the phytosphinganine to fluoroaldehydes and derivatization with *N*,*N*-dimethylhydrazine.

According to Figure 2 and Table 1 the administration of (*S*)-7-fluoropalmitic acid ((*S*)-**16**) resulted predominantly in the formation of (*E*)-9-fluoro-8-phytosphingenine (**33**) as the major unsaturated product deduced from the signal of the *N*,*N*-dimethylhydrazone-derivative **23** of (*E*)-6-fluoro-5-pentadecenal (Figure 2). A summary of the fluorinated cleavage products and their fluorophytosphinganine precursors resulting from the incorporation of the chiral fluoropalmitic acids is given in Table 1.

According to Scheme 3 the formation of *erythro*- or *threo*-fluorohydrins is in principle a feasible reaction pathway. To detect these compounds, the cleavage products were additionally derivatized with MSTFA and analyzed by GLC-MS. However, no evidence for the generation of fluorohydrins was found. Also the direct analysis of the crude reaction mixture of the fluorophytosphinganines by LC-MS analysis[13] gave no hint for the formation of fluorohydrins.

Discussion

Expression of the gene, coding for the phytosphinganine Δ^8-desaturase from sunflower in the yeast *S. cerevisiae*,

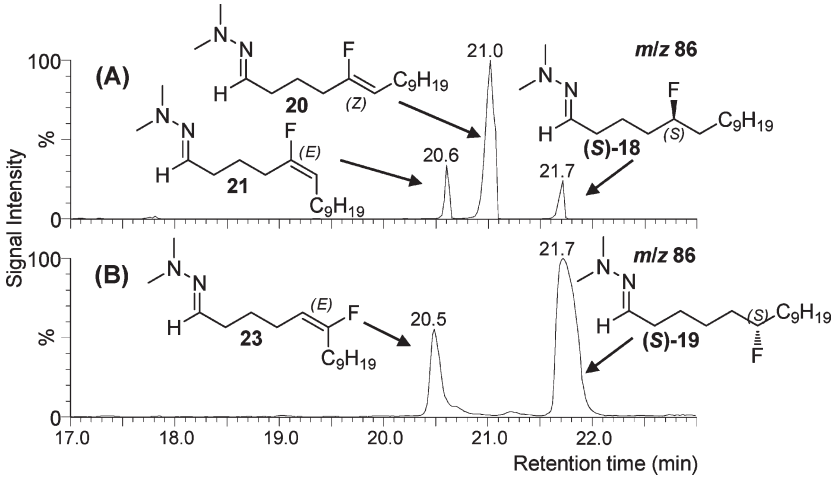
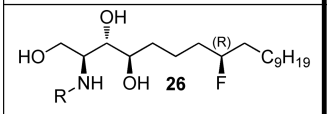
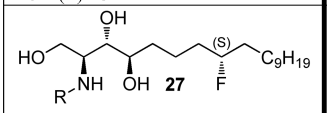
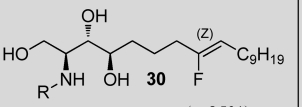
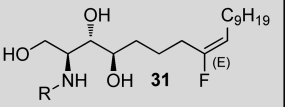
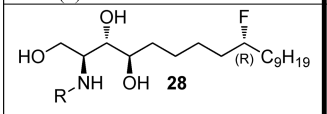
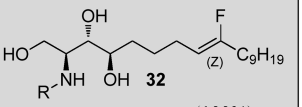
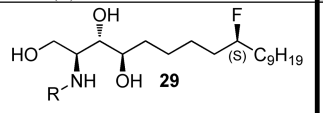
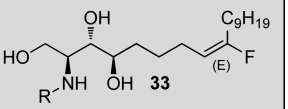


FIGURE 2. Chromatographic separation of *N*,*N*-dimethylhydrazones of fluoroaldehydes. Separation of a mixture of synthetic references *N*,*N*-dimethylhydrazones: (A) (*S*)-5-fluoropentadecanal ((*S*)-**18**), (*Z*)-5-fluoropentadec-5-enal (**20**), and (*E*)-5-fluoropentadec-5-enal (**21**). The 5/6 fluoro regioisomers of the *N*,*N*-dimethylhydrazones of *E*- and *Z*-fluoropentadecanals respectively show identical retention times. (B) Representative GLC-MS chromatogram of fluorinated metabolites obtained after administration and metabolism of (*S*)-7-fluoropalmitic acid ((*S*)-**16**). (Ion traces of *m*/*z* 86 are shown, complete analysis for all incubations is provided in the Supporting Information, Figure S1).

TABLE 1. Unsaturated Fluoroaldehydes from Fluorosphingenine Metabolites

substrate	fluorophyto-sphinganine	(*E*)-5-fluoro-5-pentadecenal (%)	(*Z*)-5-fluoro-5-pentadecenal (%)	(*E*)-6-fluoro-5-pentadecenal (%)	(*Z*)-6-fluoro-5-pentadecenal (%)
(*R*)-**15**	**26**				
(*S*)-**15**	**27**	5	95		
(*R*)-**16**	**28**				100
(*S*)-**16**	**29**			100	

TABLE 2. Product Spectrum of the Nonstereospecific Δ^8(*E*,*Z*)-Desaturase from *H. annuus*[a]

Phytosphinganins	*anti* - conformation	*gauche* - conformation
 from (*R*)-**15**	(no reaction)	no formation of fluorohydrins
 from (*S*)-**15**	 (> 95%)	 (< 5%)
 from (*R*)-**16**	 (100%)	no formation of fluorohydrins
 from (*S*)-**16**	no formation of fluorohydrins	 (100%)

[a]Product spectrum observed after application of chirally fluorinated fatty acids. Gray background highlights the observed products (R = acyl).

resulted in the parallel formation of (*E*)- and (*Z*)-8-phytosphingenine in a ratio of 88:12. Different population of *anti*- and *gauche*-conformations at the active site of the enzyme in combination with a *syn*-elimination of the relevant hydrogen atoms were assumed to control this stereochemical outcome.[18] To evaluate the importance of conformations in the transiion state of the desaturation, we administered chiral fluoropalmitic acids to the transgenic yeast. As expected, the palmitic acids were converted to chiral fluorophytosphinganines after suppression of the yeast fatty acid elongation by cerulenin. These chiral fluorophytosphinganines served as precursors for the Δ^8-desaturase and yielded (*E*,*Z*)-fluorophytosphingenines with predictable configurations as outlined in Scheme 3. The stereochemistry of the desaturated products was analyzed after cleavage of the vicinal diol of the phytoceramide moieties by $Pb(OAc)_4$ followed by derivatization of the resulting aliphatic fragments with *N*,*N*-dimethylhydrazine to the corresponding *N*,*N*-dimethylhydrazones. This procedure allowed both sensitive detection of the relevant fragments and determination of their configuration by GLC-MS with authentic references.

According to the product distribution compiled in Table 2 the stereochemistry of all fluoroalkene products was in agreement with the considerations and predictions based in Scheme 3. Although fluorohydrins have been reported previously as trace products of a desaturase reaction,[33] the current study gave no evidence for their formation.

If a fluorine atom replaces the C8−H_R in **26** no product is observed since none of the known desaturases is able to remove a fluorine radical. Also the initial attack onto the C9−H_R of **26** in combination with a *gauche*-conformation does not lead to an alkene since fluorine is a bad leaving group. The alternative formation of a fluorohydrin was also not observed. Without the presence of the fluorine atom, the initial attack onto the C9−H_R in combination with a *gauche*-conformation of the substrate would generate a (*Z*)-8-phytosphingenine.[18] The desaturation of the diastereomeric (*S*)-8-fluorophytosphinganine (**27**) delivers both (*Z*)- and (*E*)-8-fluoro-8-phytosphingenines (**30** and **31**) with the (*Z*)-isomer prevailing (*E*:*Z* = 5:95). Accordingly, the fluorinated and the nonfluorinated substrates are converted via the same geometry of the transition state into the unsaturated products. Only the population of the respective *anti*- and *gauche*-conformations is slightly different. While the desaturation of

(33) Carvalho, F.; Gauthier, L. T.; Hodgson, D. J.; Dawson, B.; Buist, P. H. *Org. Biomol. Chem.* **2005**, *3*, 3979–3983.

the natural phytosphinganine by the sunflower desaturase generates a 88:12 ratio of the (*E*)- and (*Z*)-olefins,[14] the (*S*)-8-fluorophytosphinganine (**27**) is converted to the (*Z*)- and (*E*)-8-fluoro-8-phytosphingenines (**30** and **31**) in a ratio of 95:5. This altered ratio indicates that the fluorinated substrate strongly favors the *anti*-configuration at the active center of the desaturase (Scheme 3). An exclusive production of the (*Z*)-9-fluoro-8-phytosphingenine (**32**) was observed after administration of the (*R*)-9-fluorophytosphinganine (**28**). This result is easily explained by an initial attack onto the C8−H_R and *syn*-elimination of two vicinal hydrogen atoms via an *anti*-conformation of the substrate. An initial attack on the C9−F is excluded and generates no product. As expected, administration of the (*S*)-7-fluoropalmitic acid ((*S*)-**16**) results in the formation of (*S*)-9-fluorophytosphinganine (**29**), which produces the (*E*)-9-fluoro-8-phytosphingenine (**33**) as the sole reaction product. In this case only the initial attack on the C9−H_R in combination with the *gauche*-conformation of the substrate is productive.

Conclusion

Taken together, our results clearly demonstrate that the nonstereospecific desaturase from sunflower simultaneously generates (*E*)- and (*Z*)-alkenes via different conformations of the substrate at the active center of the enzyme. Replacement of one of the involved hydrogen atoms by fluorine either prevents the removal of a hydrogen atom, and/or directs the conformation of the substrate to *anti* or *gauche*, which generated one isomer predominantly (entries **27** and **29** of Table 2). These findings clearly demonstrate that not the intrinsic mechanism of the desaturation at the di-iron center, but instead the conformation of the substrate determines the stereochemistry of the resulting alkenes. Most likely the shape of the active site also determines the stereochemistry of specific (*E*)- or (*Z*)-desaturases by allowing only one defined conformation in the substrate protein interactions.

Experimental Section

Synthesis of Substrates. (*R*)-Heptadec-1-en-7-ol ((*R*)-11): The synthesis was performed according to a published procedure[21] from Mg turnings (0.17 g, 7 mmol), 5-bromo-1-pentene (**7**) (0.9 g, 6 mmol), CuI (0.1 g, 0.53 mmol), and (*R*)-1,2-epoxydodecane ((*R*)-**9**) (0.65 g, 3.5 mmol). Yield: 0.76 g (85%). ee: > 97% according to ^{1}H NMR of the corresponding Mosher ester (see the Supporting Information). IR (cm^{-1}): 3329, 3239, 3081, 2958, 2921, 2873, 2848, 1643, 1467, 1379, 1345, 1132, 1102, 1066, 1044, 1021, 990, 931, 913, 865, 839, 721, 640. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, J = 6.8 Hz, 3H), 1.52−1.16 (m, 24H), 2.00−2.11 (m, 2H), 3.52−3.63 (m, 1H), 4.89−5.05 (m, 2H), 5.81 (ddt, J = 16.9, 10.2, 6.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 22.7, 25.1, 25.7, 29.0, 29.3, 29.6, 29.63, 29.64, 29.7, 31.9, 33.7, 37.3, 37.5, 71.9, 114.3, 138.9. EIMS (70 eV) m/z: 253 (0.5, $M^+ - H$), 236 (2), 208 (11), 180 (2), 171 (12), 152 (3), 138 (3), 124 (6), 113 (10), 111 (14), 97 (41), 95 (100), 83 (32), 69 (40), 57 (25), 55 (37). HRMS (EI) m/z: calcd for $C_{17}H_{34}O$ 254.260966, found 254.262222.

(*S*)-Heptadec-1-en-7-ol ((*S*)-11): Prepared from Mg turnings (0.33 g, 13.4 mmol), 5-bromo-1-pentene (**7**) (1.8 g, 12.2 mmol), CuI (0.19 g, 1 mmol), and (*S*)-1,2-epoxydodecane ((*S*)-**9**) (1.5 g, 8.1 mmol). Yield: 1.73 g (84%). ee: > 97%, according to ^{1}H NMR of the corresponding Mosher ester (see the Supporting Information). Spectroscopic data are in agreement with (*R*)-**11**.

(*R*)-Heptadec-1-en-8-ol ((*R*)-12): Prepared from Mg turnings (0.12 g, 4.9 mmol), 6-bromo-1-hexene (**8**) (0.72 g, 4.4 mmol), CuI (72 mg, 0.38 mmol), and (*R*)-1,2-epoxyundecane ((*R*)-**10**) (0.5 g, 2.9 mmol). Yield: 0.52 g (70%). ee: > 97%, according to ^{1}H NMR of the corresponding Mosher ester (see Supporting Information). IR (cm^{-1}): 3319, 3243, 3080, 2955, 2924, 2873, 2851, 1643, 1466, 1378, 1352, 1133, 1101, 1068, 1046, 1020, 992, 912, 868, 821, 722, 642. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, J = 6.8 Hz, 3H), 1.16−1.50 (m, 24H), 1.96−2.11 (m, 2H), 3.51−3.63 (m, 1H), 4.84−5.05 (m, 2H), 5.81 (ddt, J = 16.9, 10.2, 6.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 22.7, 25.5, 25.7, 28.9, 29.2, 29.3, 29.57, 29.63, 29.7, 31.9, 33.7, 37.4, 37.5, 72.0, 114.2, 139.1. EIMS (70 eV) m/z: 253 (0.3, $M^+ - H$), 236 (2), 208 (6), 194 (3), 183 (3), 166 (2), 157 (16), 155 (8), 138 (5), 127 (13), 109 (100), 97 (46), 95 (23), 83 (89), 81 (36), 69 (50), 67 (72), 57 (47), 55 (77). HRMS (EI) m/z: calcd for $C_{17}H_{34}O$ 254.260966, found 254.260286.

(*S*)-Heptadec-1-en-8-ol ((*S*)-12): Prepared from Mg turnings (0.18 g, 7.4 mmol), 6-bromo-1-hexene (**8**) (1 g, 6.4 mmol), CuI (0.11 g, 0.56 mmol), and (*S*)-1,2-epoxyundecane ((*S*)-**10**) (0.73 g, 4.3 mmol). Yield: 0.86 g (79%). ee: > 97%, according to ^{1}H NMR of the corresponding Mosher ester (see the Supporting Information). Spectroscopic data are in agreement with (*R*)-**12**.

Fluoro-1-alkenes, Representative Procedure. (*S*)-7-Fluoroheptadec-1-ene ((*S*)-13): A cold solution (−78 °C) of (diethylamino)sulfur trifluoride (DAST, 0.56 g, 3.5 mmol) in CH_2Cl_2 (20 mL) was treated dropwise with a solution of (*R*)-**11** (0.6 g, 2.3 mmol) in the same solvent (2 mL). Stirring was continued overnight at room temperature. After hydrolysis with water (15 mL) the product was extracted with CH_2Cl_2, washed with sat. $NaHCO_3$ solution, and dried over anhydrous Na_2SO_4. Purification was achieved by column chromatography (SiO_2) with hexane for elution. Yield: 0.53 g (87%). IR (cm^{-1}): 3078, 2927, 2856, 1641, 1464, 1378, 1353, 1130, 993, 910, 723, 639. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, J = 7.0 Hz, 3H), 1.68−1.22 (m, 24H), 2.09−2.01 (m, 2H), 4.45 (dtt, J = 49.12, 7.89, 3.99 Hz, 1H), 4.94 (ddt, J = 10.18, 2.31, 1.23 Hz, 1H), 5.00 (ddt, J = 17.12, 2.10, 1.58 Hz, 1H), 5.81 (ddt, J = 16.94, 10.19, 6.68 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 139.0, 114.3, 95.2, 93.9, 35.3, 35.2, 35.1, 35.04, 33.7, 31.9, 29.54, 29.53, 29.52, 29.3, 29.0, 28.8, 25.15, 25.12, 25.01, 24.97, 22.7, 14.1. EIMS (70 eV) m/z: 256 (0.1, M^+), 236 (3), 208 (23), 194 (3), 180 (4), 165 (5), 152 (9), 151 (11), 137 (12), 124 (19), 123 (22), 110 (26), 109 (38), 95 (96), 82 (84), 67 (100), 54 (70). HRMS (EI) m/z: calcd for $C_{17}H_{33}F$ 256.256630, found 256.255409.

(*R*)-7-Fluoroheptadec-1-ene ((*R*)-13): Prepared from (*S*)-**11** (1.4 g, 5.5 mmol) and DAST (1.32 g, 8.2 mmol). Yield: 1.17 g (83%). Spectroscopic data are in agreement with (*S*)-**13**.

(*S*)-8-Fluoroheptadec-1-ene ((*S*)-14): Prepared from (*R*)-**12** (0.30 g, 1.2 mmol) and DAST (0.29 g, 1.8 mmol). Yield: 0.25 g (83%). IR (cm^{-1}): 3078, 2927, 2856, 1642, 1464, 1379, 1354, 1131, 994, 911, 723. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, J = 7.0 Hz, 3H), 1.69−1.21 (m, 24H), 2.09−2.02 (m, 2H), 4.45 (ddt, J = 23.23, 7.93, 4.02 Hz, 1H), 5.03−4.91 (m, 2H), 5.81 (ddt, J = 16.90, 10.16, 6.67 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 139.0, 114.3, 95.2, 93.9, 35.3, 35.2, 35.1, 35.0, 33.7, 31.9, 29.54, 29.53, 29.52, 29.3, 29.0, 28.8, 25.15, 25.11, 25.01, 24.97, 22.7, 14.1. EIMS (70 eV) m/z: 256 (0.1, M^+), 236 (7), 208 (22), 195 (3), 180 (5), 166 (6), 152 (14), 137 (17), 123 (26), 111 (41), 110 (42), 96 (95), 82 (100), 80 (69), 69 (45), 67 (22), 56 (61), 55 (55). HRMS (EI) m/z: calcd for $C_{17}H_{33}F$ 256.256629, found 256.256510.

(*R*)-8-Fluoroheptadec-1-ene ((*R*)-14): Prepared from (*S*)-**12** (0.8 g, 3.1 mmol) and DAST (0.75 g, 4.7 mmol). Yield: 0.62 g (77%). Spectroscopic data are in agreement with (*S*)-**14**.

Preparation of Fluoropalmitic Acids, Representative Procedure. (*S*)-6-Fluorohexadecanoic Acid ((*S*)-15): Ruthenium trichloride hydrate (9 mg, 2.2 mol %) was added to a solution of sodium metaperiodate (1.73 g, 8.1 mmol) and (*S*)-**13** (0.5 g, 2 mmol) in a solvent mixture of CCl_4 (4 mL), CH_3CN (4 mL), and H_2O (6 mL). The mixture was stirred vigorously for 2 h at

room temperature. Then, CH_2Cl_2 (10 mL) was added and the phases were separated. The aqueous phase was extracted with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by flash chromatography on silica gel with Et_2O/hexane/formic acid 1:1:0.01 for elution. Yield: 0.43 g (80%). IR (cm^{-1}): 3060, 2953, 2944, 2915, 2870, 2847, 1695, 1469, 1443, 1407, 1316, 1274, 1258, 1234, 1209, 1132, 1098, 1065, 1021, 990, 971, 930, 856, 828, 786, 733, 719. ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, $J = 6.8$ Hz, 3H), 1.17−1.75 (m, 24H), 2.37 (t, $J = 7.43$ Hz, 2H), 4.35−4.57 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 22.7, 24.5, 24.6, 24.7, 25.1, 25.1, 29.3, 29.49, 29.53, 29.57, 29.59, 31.9, 33.8, 34.7, 34.9, 35.1, 35.3, 93.4, 95.0, 179.2. EIMS (70 eV) *m*/*z*: 254 (8, $M^+ - HF$), 236 (58), 192 (21), 165 (7), 151 (10), 138 (12), 123 (15), 111 (28), 97 (58), 83 (72), 69 (71), 57 (63), 55 (100). HRMS (EI) *m*/*z*: calcd for $C_{16}H_{30}O_2$ ($M^+ - HF$) 254.224580, found 254.224823.

(*R*)-6-Fluorohexadecanoic Acid ((*R*)-15): Prepared as above from (*R*)-**13** (0.5 g, 2 mmol), $NaIO_4$ (1.73 g, 8.1 mmol), and $RuCl_3 \cdot H_2O$ (9 mg, 2.2 mol %). Yield: 0.4 g (75%). Spectroscopic data are in agreement with (*S*)-**15**.

(*S*)-7-Fluorohexadecanoic Acid ((*S*)-16): Prepared as above from (*S*)-**14** (0.2 g, 0.8 mmol), $NaIO_4$ (0.68 g, 3.2 mmol), and $RuCl_3 \cdot H_2O$ (5 mg, 2.2 mol %). Yield: 0.15 g (71%). IR (cm^{-1}): 3060, 2953, 2915, 2848, 1695, 1470, 1444, 1407, 1315, 1296, 1274, 1240, 1210, 1129, 1100, 1066, 1044, 1019, 967, 893, 858, 807, 719. ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, $J = 6.85$ Hz, 3H), 1.20−1.71 (m, 24H), 2.33 (t, $J = 7.47$ Hz, 2H), 4.45 (dtt, $J = 48.70$, 7.70, 3.68 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 22.7, 24.6, 24.77, 24.82, 25.1, 25.2, 28.9, 29.3, 29.50, 29.53, 31.9, 33.6, 34.8, 35.03, 35.07, 35.3, 93.6, 95.2, 178.2. EIMS (70 eV) *m*/*z*: 254 (6, $M^+ - HF$), 236 (52), 192 (15), 165 (7), 152 (12), 138 (12), 125 (17), 111 (24), 97 (49), 83 (62), 69 (70), 57 (45), 55 (100). HRMS (EI) *m*/*z*: calcd for $C_{16}H_{30}O_2$ ($M^+ - HF$) 254.224580, found 254.224739.

(*R*)-7-Fluorohexadecanoic Acid ((*R*)-16): Prepared as above from (*R*)-**14** (0.4 g, 1.6 mmol), $NaIO_4$ (1.37 g, 6.4 mmol), and $RuCl_3 \cdot H_2O$ (10.5 mg, 2.2 mol %). Yield: 0.31 g (73%). Spectroscopic data are in agreement with (*S*)-**16**.

Administration of Fluoropalmitic Acids to Growing Cultures of *S. cerevisiae*. The galactose-inducible strain of *S. cerevisiae* INVSc1 (Invitrogen) containing the Δ^8-sphingolipid desaturase from *Helianthus annuus* (pHaDES8) has been described earlier.[14] A starter culture (5 mL) of transformed *S. cerevisiae* cells was grown at 30 °C for 48 h in complete minimal-dropout-uracil medium containing 2% (w/v) raffinose (ForMedium). This culture was used to inoculate 100 mL of medium, containing 1% Tergitol in a 500 mL Erlenmeyer flask. The cultures were directly induced with 10 mL of galactose (20% solution, w/v) to a final concentration of 2%. Cerulenin (925 μL of 3 mM EtOH solution) and the fluoropalmitic acid (280 μL of 100 mM EtOH solution) were added to the culture flask and the inoculated cultures were incubated for 5 d at 28 °C in a rotary incubator/shaker set at 220 rpm. The yeast cells were harvested by centrifugation, resuspended in 4 mL of $CHCl_3$/MeOH (1:1; v:v), and mechanically disrupted with glass beads by vortexing for 3 min, followed by stirring for 2 h at 4 °C. Yeast cells were separated from solvent and the extraction was repeated with $CHCl_3$/MeOH (2:1; v:v). After separation of the solvent, yeast cells were resuspended in 5 mL of $CHCl_3$/MeOH (2:1; v:v) and stirred overnight at 4 °C. The combined organic extracts were filtered and evaporated.

Analysis of Phytosphingolipids. The residue was dissolved in 2 mL of CH_2Cl_2 and cooled to 4 °C. $Pb(OAc)_4$ (5−10 mg) was added followed by an incubation for 2 h at room temperature. The solution was then passed through a short plug of silica gel (Pasteur pipet) for removal of residual $Pb(OAc)_4$ and the plug was washed with Et_2O (5 mL). The solvent was evaporated under a stream of argon.

Derivatization of Released Aldehydes. The residue was dissolved in hexane (1 mL) and *N*,*N*-dimethylhydrazine (5 μL) was added followed by incubation for 1 h. The solvent was evaporated under a stream of argon, the residue was redissolved in CH_2Cl_2, and an aliquot was applied to GC/MS.

Instrumental Analysis of the Biological Extracts. GC/MS analysis of *N*,*N*-dimethylhydrazones was performed on a GC-TOF mass spectrometer, a 30 m DB-1 column (ZB-1MS, 30 m × 0.25 mm, film thickness 0.25 μm; Phenomenex, Torance, CA, USA) installed. Helium was used as carrier gas (1 mL min^{-1}); injector temperature: 220 °C; split less mode: GC oven temperature program: 50 °C (maintained for 2 min) at 10 deg min^{-1} to 180 °C followed by 5 deg min^{-1} to 250 °C followed by 30 deg min^{-1} to 280 °C (maintained for 2 min). Mass spectra were recorded in positive EI mode (70 eV).

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Supporting Information Available: Experimental details and ^1H NMR, ^{13}C NMR, infrared, and mass spectra of substrates and reference compounds. This material is available free of charge via the Internet at http://pubs.acs.org.