

SUPPORTING INFORMATION

Investigating Inner Sphere Reorganization via Secondary Kinetic Isotope Effects in the C–H Cleavage Reaction Catalyzed by Soybean Lipoxygenase: Tunneling in the Substrate Backbone as well as the Transferred Hydrogen

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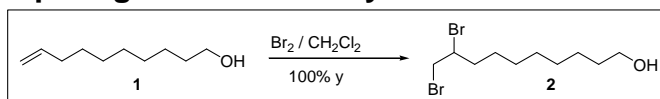
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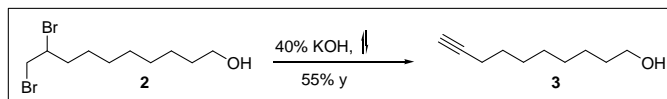
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Procedure for Preparing Combinatorially Labeled Linoleic Acid (9a)



9,10-Dibromodecanol (2)

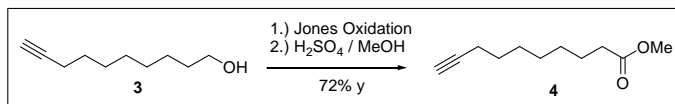
9-Decenol (43.8 g, 50.0 mL, 280 mmol) was dissolved in 200 mL of dichloromethane and cooled to -45°C in a 500 mL round bottom flask fitted with a 125 mL addition funnel. Liquid bromine (44.8 g, 14.4 mL, 280 mmol) was dissolved in 100 mL of dichloromethane and added to the addition funnel. The bromine solution was added dropwise to prevent warming of the reaction. After complete addition of the bromine, the reaction was allowed to warm to room temperature. Solvent was then removed to yield **2** (88.3 g, 279 mmol) as a yellow oil. ^1H NMR (300 MHz, CDCl_3) δ : 1.31 (m, 10H), 1.55 (m, 3H), 1.76 (m, 1H), 2.11 (m, 1H), 3.61 (t, $J = 10.1$ Hz, 1H), 3.62 (t, $J = 6.6$ Hz, 2H), 3.83 (dd, $J = 4.4$ Hz, $J = 10.2$ Hz, 1H), 4.15 (m, 1H).



9-Decynol (3)

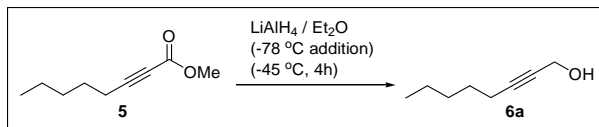
Potassium hydroxide (127 g, 2.26 mol) was dissolved in 75 mL water in a 2-neck 250 mL round bottom flask fitted with a reflux condenser. 9,10-Dibromodecanol (**2**) (280 mmol, 88.5 g) was then added to the reaction flask via the side neck. The reaction was heated to reflux and refluxed vigorously for 5 h under nitrogen and was then allowed to return to room temperature, then added to 250 mL water in a beaker cooled to 0°C in an ice bath. The contents of the beaker were extracted with 5 washes of 50 mL diethyl

ether. The diethyl ether washes were collected and extracted with 50 mL water, 50 mL brine, then dried with sodium sulfate. The diethyl ether was removed to yield a yellow oil. This material was distilled under 5 mm Hg. Fractions containing the pure liquid boiled at 85 °C. The pure fractions were collected to yield **3** (167 mmol, 25.7 g) as a clear liquid. ¹H NMR (300 MHz, CDCl₃) δ: 1.31 (m, 9H), 1.51 (m, 4H), 1.91 (t, J = 2.6 Hz, 1H), 2.16 (dt, J = 7.1 Hz, J = 2.6 Hz, 2H), 3.62 (t, J = 6.6 Hz, 2H).



Methyl 9-octynoate (**4**)

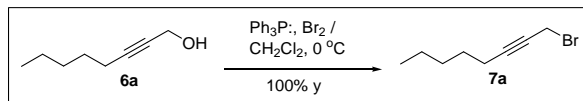
In a 100 mL beaker 14 mL conc. H₂SO₄ was added to 45 mL water and cooled to 0 °C. This was added to a 500 mL 3-neck round bottom flask charged with (16.2 g, 162 mmol) chromium trioxide and fitted with reflux condenser and a 125 mL addition funnel to yield a deep orange solution. The 500 mL flask was placed in an ice/water bath and the addition funnel was charged with 100 mL acetone and (10.0 g, 64.8 mmol) 9-octyn-1-ol (**3**). The 9-octyn-1-ol solution was added dropwise over about 1 h, with continued cooling of the reaction. The reaction was then allowed to come to room temperature over about 30 min. and was then taken to reflux for 1 h. The reaction was added to about 500 mL ice water and extracted with four 50 mL quantities of diethyl ether. Upon addition of 50 g NaCl to the aqueous layer, an organic layer separated. This layer was combined with the previous ether layers. The combined organic layers were dried with anhydrous sodium sulfate, filtered, and the solvent was removed to yield a colorless liquid which was a combination of about 10% 9'-decynyl 9-decynoate and 90% 9-decynoic acid. This material was then dissolved in about 50 mL methanol and placed in a 100 mL round bottom flask to which about 5 drops concentrated sulfuric acid was added. The reaction was followed by TLC. When no more free acid appeared by TLC, a small spatula full of potassium carbonate was added to the solution to neutralize the sulfuric acid. The reaction was then filtered and solvent was removed. The resulting clear liquid, now a mixture of methyl 9-decynoate (**11**) and 9-decyn-1-ol (**10**) was purified by flash chromatography on a 14" X 2" column loaded with silica 60. Eluents of increasing polarity were used to resolve the alcohol from the methyl ester: 1000 mL of 30% diethyl ether/70% hexanes, 500 mL of 50% diethyl ether/50 % hexanes, and 500 mL of 70% diethyl ether/30% hexanes. The alcohol and methyl ester were completely resolved with the methyl ester eluting first. The solvent was removed from the fractions containing (**4**) to yield (8.52 g, 46.8 mmol) of (**4**) as a clear liquid. ¹H NMR (300 MHz, CDCl₃) δ: 1.20-1.65 (m, 10H), 1.915 (t, J = 2.7 Hz, 1H), 2.16 (dt, J = 2.6 Hz, J = 7.1 Hz, 2H), 2.28 (t, J = 7.6 Hz, 2H), 3.64 (s, 3H).



2-Octynol (**6a**)

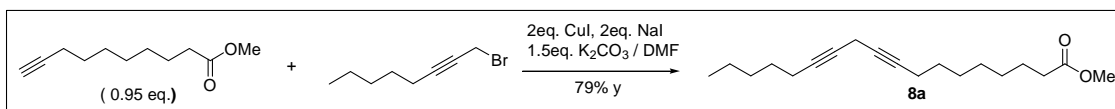
A dry 500 mL 2-neck round bottom flask fitted with a 125 mL addition funnel was charged with 100 mL diethyl ether and purged with nitrogen. To this, LiAlH₄ (2.28 g, 60.1 mmol) was added via the side neck. The addition funnel was charged with (12.34 g, 13.41 mL, 80.0 mmol) methyl 2-octynoate and 50 mL diethyl ether. The flask was cooled to -78 °C in a dry ice/acetone bath. The ester was added dropwise with stirring at the rate of about 1 drop/second to avoid raising the temperature of the reaction. After complete addition of the ester, the reaction was placed in a -45 °C dry ice/acetonitrile slurry and allowed to stir for 1 h. The reaction was quenched at -45 °C while stirring by adding 2.28 mL water, then 2.28 mL 15% NaOH, and then 6.84 mL water, dropwise. The reaction was kept under an atmosphere of nitrogen for the duration of the reaction and the quenching. The resulting white slurry was filtered and washed with about 50 mL diethyl ether. The solvent was removed from the filtrate to yield a mixture of 2-octynol and 2-(Z)-octenol (2-10%). This mixture was placed in about 200 mL chloroform in a 500 mL round bottom flask, to which 10 equivalents (based on moles of 2-(Z)-octenol) of 3-chloroperoxybenzoic acid (mCPBA) were added. The reaction was stirred at room temperature for 12 h. After reaction, the chloroform solution was taken up into two volumes of ether and extracted with three washes of 100 mL 15% NaOH and 100 mL brine. The epoxide resulting from oxidation of the 2-(Z)-octenol was then separated from the desired reaction product by flash chromatography (10-12" X 2" column of silica 60) using three eluents of increasing polarity: 1000

mL 30% Et₂O/70% hexanes; 500 mL 40% Et₂O/60% hexanes; 500 mL 50% Et₂O/50% hexanes. The impurity and the 2-octynol were completely resolved and the solvent was removed to yield **6a** (9.937 g, 78.7 mmol) as a colorless liquid. ¹H NMR (300 MHz, CDCl₃) δ: 0.89 (t, J = 7.2 Hz, 3H), 1.19 (t, J = 7.0 Hz, 1H), 1.30 (m, 4H), 1.49 (m, 2H), 2.19 (tt, J = 7.2 Hz, J = 2.2 Hz, 2H), 4.23 (t, J = 2.2 Hz, 2H).



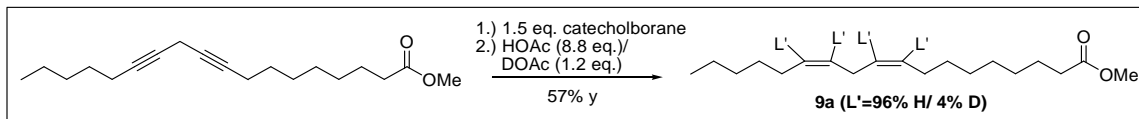
1-Bromo-2-octyne (**7a**)

A 500 mL round bottom flask was charged with 220 mL dichloromethane and (22.50 g, 85.8 mmol) of triphenylphosphine and cooled to 0 °C in an ice/water bath. To this, a slight excess of bromine dissolved in dichloromethane was added dropwise via addition funnel until a light red color persisted. An additional pellet of triphenylphosphine was added to return the solution to a yellow-white cloudy appearance. The (10.0 g, 79.2 mmol) 2-octynol (**6a**) was then added dropwise via addition funnel while maintaining temperature between 0 and 5 °C. The reaction was stirred at 0 °C for 30 min and was placed in a 1 L Erlenmeyer to which 440 mL pentane was added. Upon waiting for about 30 min, the triphenylphosphine oxide by-product precipitated out in the pentane. The mixture was then passed through a 2" X 3" silica plug and washed with 50 mL pentane. Solvent was removed to yield **7a** (14.90 g, 78.8 mmol) as a colorless liquid. ¹H NMR (300 MHz, CDCl₃) δ: 0.88 (t, J = 7.0 Hz, 3H), 1.32 (m, 4H), 1.49 (m, 2H), 1.57 (s, 1H), 2.21 (tt, J = 7.0 Hz, 2.4 Hz 2H), 3.91 (t, 2.4 Hz, 2H).



Methyl 9,12-octadecadiynoate (**8a**)

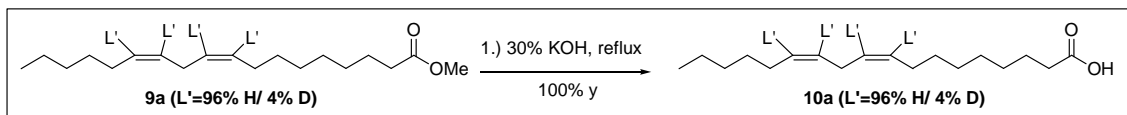
In a dried and nitrogen-purged 100 mL round bottom flask, (6.04 g, 31.7 mmol) CuI, (4.76 g, 31.7 mmol) NaI, and (3.29 g, 23.8 mmol) potassium carbonate were added to 30 mL of dimethylformamide that had been distilled over calcium hydride. To this, was added (2.75 g, 15.1 mmol) of (**4**) and then (3.00 g, 15.9 mmol) (**7a**). The reaction was allowed to stir for 12h under nitrogen and was poured into a solution made by mixing 100 mL saturated ammonium chloride and 100 mL water and extracted with five 50 mL portions of diethyl ether. The collected ether layers were dried with anhydrous sodium sulfate and filtered. The solvent was removed to give a combination of (**7a**) and methyl 9,12-octadecadiynoate (**8a**) which was further purified using flash chromatography in a 12" X 2" column of silica 60. Eluents of increasing polarity were used to achieve resolution: 1000 mL hexanes, 500 mL 90% hexanes/10% Et₂O, and 70% hexanes/30% Et₂O. Removal of solvent yielded **8a** (3.47 g, 11.9 mmol) as a clear liquid. ¹H NMR (300 MHz, CDCl₃) δ: 0.87 (t, J = 7.1 Hz, 3H), 1.29 (m, 10H), 1.47 (m, 4H), 1.60 (m, 2H), 2.13 (tt, J = 7.0 Hz, J = 2.3 Hz, 4H), 2.28 (t, J = 7.6 Hz, 2H), 3.10 (quin, J = 2.4 Hz, 2H), 3.65 (s, 3H).



Combinatorially labeled methyl 9,12-octadecadienoate (**9a**)

In a dried and nitrogen-purged 100 mL 2-neck round bottom flask, fitted with a reflux condenser and a septum, (5.74 g, 5.10 mL, 47.9 mmol) of catechol borane was added via syringe to (3.97 g, 13.7 mmol) of **8a**. The reaction was run under nitrogen atmosphere for 5 h at about 60 °C. Upon cooling the reaction to room temperature, a mixture of (26.29 g, 25.05 mL, 437.8 mmol) acetic acid and (6.68 g, 6.31 mL, 109.4 mmol) d-acetic acid was added to the reaction and then taken to mild reflux for about 5 h and was allowed to stir at room temperature for 12 h. The reaction was added to 250 mL ice/water and extracted with five 20 mL quantities of Et₂O. The ether layers were combined, dried with anhydrous sodium sulfate, and filtered. Solvent was removed to yield a viscous brown liquid. This was purified by flash chromatography using a 12" X 2" column of silica 60 and eluents of increasing polarity: 1000 mL hexanes, 500 mL 80%

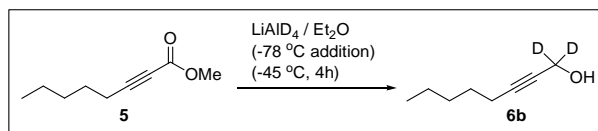
hexanes/20 % Et₂O, and 500 mL 60% hexanes/40% Et₂O. The product of interest eluted ahead of the main contaminants. Solvent was removed to yield **9a** (2.30 g, 7.8 mmol) as a clear liquid. ¹H NMR (300 MHz, CDCl₃) δ: 0.87 (t, J = 6.9 Hz, 3H), 1.29 (m, 14H), 1.60 (m, 2H), 2.01 (m, 4H), 2.28 (t, J = 7.7 Hz, 2H), 2.75 (t, J = 5.8 Hz, 2H), 3.65 (s, 3H), 5.35 (m, 4H).



Combinatorially labeled 9,12-octadecadienoic (linoleic) acid (**10a**)

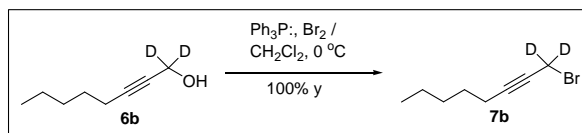
A 100 mL round bottom flask fitted with a reflux condenser was charged with 15 g of potassium hydroxide and 35 mL of water. To this, (1.62 g, 5.5 mmol) **9a** was added. The reaction was refluxed and followed by TLC. The end of the reaction was accompanied by a large degree of foaming. Complete reaction was usually achieved in about 1 h. The reaction was allowed to cool to room temperature and was added to a mixture of 240 mL ice/water and 85% phosphoric acid. This was then extracted with five 20 mL quantities of Et₂O. The solvent was removed and any remaining water was eliminated by adding benzene to the resulting liquid and removing by rotary evaporation to yield **10a** (1.54 g, 5.5 mmol) as a clear liquid. ¹H NMR (300 MHz, CDCl₃) δ: 0.87 (t, J = 7.1 Hz, 3H), 1.29 (m, 14H), 1.62 (m, 2H), 2.03 (q, J = 6.6 Hz, 4H), 2.33 (t, J = 7.4 Hz, 2H), 5.34 (m, 4H), 10.42 (s, 1H).

Procedure for Preparing 11,11-*d*₂-Linoleic Acid (**9b**)



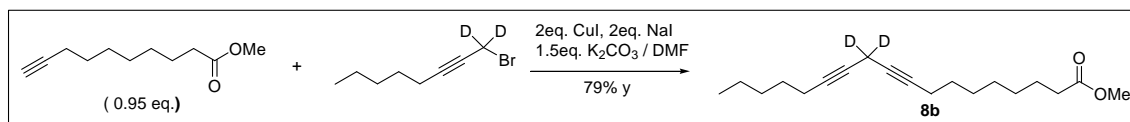
1,1-*d*₂-2-Octynol (**6b**)

A dry 500 mL 2-neck round bottom flask fitted with a 125 mL addition funnel was charged with 100 mL diethyl ether and purged with nitrogen. To this, LiAlD₄ (2.50 g, 59.5 mmol) was added via the side neck. The addition funnel was charged with (12.34 g, 13.41 mL, 80.0 mmol) methyl 2-octynoate (**5**) and 50 mL diethyl ether. The flask was cooled to -78 °C in a dry ice/acetone bath. The ester was added dropwise with stirring at the rate of about 1 drop/second to avoid raising the temperature of the reaction. After complete addition of the ester, the reaction was placed in a -45 °C dry ice/acetonitrile slurry and allowed to stir for 1 h. The reaction was quenched at -45 °C while stirring by adding 2.28 mL water, then 2.28 mL 15% NaOH, then 6.84 mL water, dropwise and was kept under an atmosphere of nitrogen for the duration of the reaction and the quenching. The resulting white slurry was filtered and washed with about 50 mL diethyl ether. The solvent was removed from the filtrate to yield a mixture of 1,1-*d*₂-2-octynol and 1,1,3-*d*₃-2-(*Z*)-octenol (< 1%). This mixture was then placed in about 20 mL chloroform in a 50 mL round bottom flask, to which 10 equivalents (based on moles of 1,1,3-*d*₃-2-(*Z*)-octenol) of mCPBA were added. The reaction was stirred at room temperature for 12 h. After reaction, the chloroform solution was taken up into two volumes of ether and extracted with three washes of 100 mL 15% NaOH and 100 mL brine. The epoxide resulting from oxidation of the 1,1,3-*d*₃-2-(*Z*)-octenol was then separated from the desired reaction product by flash chromatography (10-12" X 2" column of silica 60) using three eluents of increasing polarity: 1000 mL 30% Et₂O/70% hexanes; 500 mL 40% Et₂O/60% hexanes; 500 mL 50% Et₂O/50% hexanes. The impurity and the 1,1-*d*₂-2-octynol were completely resolved and the solvent was removed to yield **6** (10.24 g, 79.9 mmol) as a colorless liquid. ¹H NMR (300 MHz, CDCl₃) δ: 0.87 (t, J = 7.0 Hz, 3H), 1.31 (m, 4H), 1.47 (q, J = 7.0 Hz, 2H), 1.57 (s, 1H), 2.18 (t, J = 7.0, 2H).



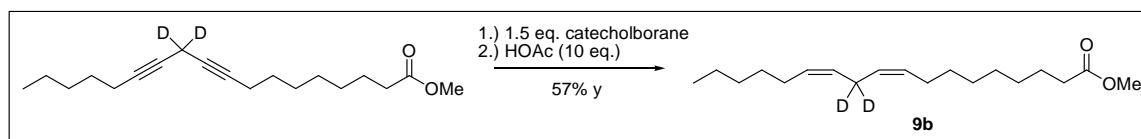
1,1-d₂-1-Bromo-2-octyne (**7b**)

A 500 mL round bottom flask was charged with 220 mL dichloromethane and (22.50 g, 85.8 mmol) of triphenylphosphine and cooled to 0 °C in an ice/water bath. To this, a slight excess of bromine dissolved in dichloromethane was added dropwise via addition funnel until a light red color persisted. An additional pellet of triphenylphosphine was added to return the solution to a yellow-white cloudy appearance. The (10.1 g, 79 mmol) 2-octynol (**6b**) was added dropwise via addition funnel while maintaining temperature between 0 and 5 °C. The reaction was stirred at 0 °C for 30 min and was then placed in a 1 L Erlenmeyer to which 440 mL pentane was added. Upon waiting for about 30 min., the triphenylphosphine oxide by-product precipitated out in the pentane. The mixture was then passed through a 2" X 3" silica plug and washed with 50 mL pentane. Solvent was removed to yield **7b** (15.04 g, 78.7 mmol) as a colorless liquid. ¹H NMR (300 MHz, CDCl₃) δ: 0.86 (t, J = 7.0 Hz, 3H), 1.32 (m, 4H), 1.50 (m, 2H), 2.21 (t, J = 7.0 Hz, 2H).



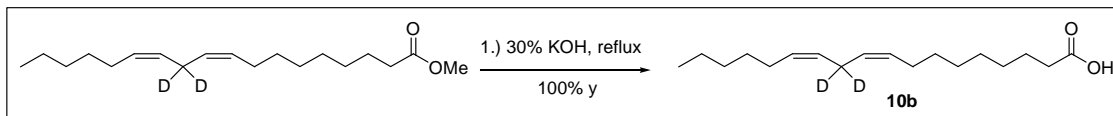
Methyl 11,11-d₂-9,12-octadecadiynoate (**8b**)

In a dried and nitrogen-purged 100 mL round bottom flask, (6.04 g, 31.7 mmol) CuI, (4.76 g, 31.7 mmol) NaI, and (3.29 g, 23.8 mmol) potassium carbonate were added to 30 mL of dimethylformamide that had been distilled over calcium hydride. To this was added (2.75 g, 15.1 mmol) of (**4**) and then (3.03 g, 15.9 mmol) (**7b**). The reaction was allowed to stir for 12h under nitrogen and was poured into a solution made by mixing 100 mL saturated ammonium chloride and 100 mL water and extracted with five 50 mL portions of diethyl ether. The collected ether layers were dried with anhydrous sodium sulfate and filtered. The solvent was removed to yield methyl 11,11-d₂-9,12-octadecadiynoate (**8b**) which was further purified using flash chromatography in a 12" X 2" column of silica 60. Eluents of increasing polarity were used to achieve resolution: 1000 mL hexanes, 500 mL 90% hexanes/10% Et₂O, and 70% hexanes/30% Et₂O. Removal of solvent yielded **8b** (3.53 g, 12.1 mmol) as a clear liquid. ¹H NMR (300 MHz, CDCl₃) δ: 0.87 (t, J = 7.0 Hz, 3H), 1.29 (m, 10H), 1.46 (m, 4H), 1.60 (m, 2H), 2.13 (t, J = 7.1 Hz, 4H), 2.28 (t, J = 7.5 Hz, 2H), 3.64 (s, 3H).



Methyl 11,11-d₂-9,12-octadecadienoate (**9b**)

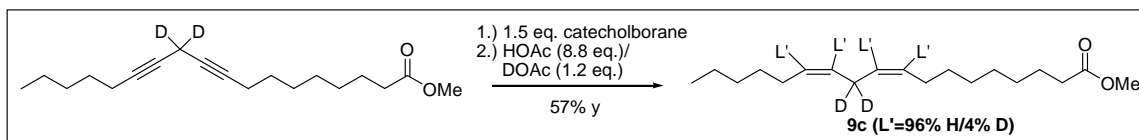
In a dried and nitrogen-purged 100 mL 2-neck round bottom flask fitted with a reflux condenser and a septum, (5.74 g, 5.10 mL, 47.9 mmol) of catechol borane was added via syringe to (4.00 g, 13.7 mmol) of **8b**. The reaction was run under nitrogen atmosphere for 5 h at about 60 °C. Upon cooling the reaction to room temperature, a mixture of (28.76 g, 27.42 mL, 479.0 mmol) acetic acid was added to the reaction and then taken to mild reflux for about 5 h and was allowed to stir at room temperature for 12 h. The reaction was then added to 250 mL ice/water and extracted with five 20 mL quantities of Et₂O. The ether layers were combined, dried with anhydrous sodium sulfate, and filtered. Solvent was removed to yield a viscous brown liquid. This was purified by flash chromatography using a 12" X 2" column of silica 60 and mobile phases of increasing polarity: 1000 mL hexanes, 500 mL 80% hexanes/20% Et₂O, and 500 mL 60% hexanes/40% Et₂O. The product of interest eluted ahead of the main contaminants. Solvent was removed to yield **9b** (2.25 g, 7.6 mmol) as a clear liquid. ¹H NMR (300 MHz, CDCl₃) δ: 0.87 (t, J = 7.0 Hz, 3H), 1.29 (m, 14H), 1.60 (m, 2H), 2.01 (m, 4H), 2.28 (t, J = 7.6 Hz, 2H), 3.65 (s, 3H), 5.34 (m, 4H).



11,11-*d*₂- 9,12-Octadecadienoic acid (**10b**)

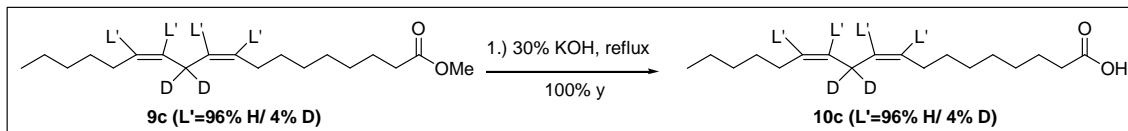
A 100 mL round bottom flask fitted with a reflux condenser was charged with 15 g of potassium hydroxide and 35 mL of water. To this, (1.80 g, 6.1 mmol) **9b** was added. The reaction was refluxed and followed by TLC. The end of the reaction was usually accompanied by a large degree of foaming. Complete reaction was usually achieved in about 1 h. The reaction was allowed to cool to room temperature and was added to a mixture of 240 mL ice/water and 85% phosphoric acid. This was then extracted with five 20 mL quantities of Et₂O. The solvent was removed and any remaining water was eliminated by adding benzene to the resulting liquid and removing by rotary evaporation to yield **10b** (1.70 g, 6.0 mmol) as a clear liquid. ¹H NMR (300 MHz, CDCl₃) δ: 0.87 (t, J = 7.1 Hz, 3H), 1.29 (m, 14H), 1.61 (m, 2H), 2.03 (q, J = 6.7 Hz, 4H), 2.33 (t, J = 7.6 Hz, 2H), 2.75 (t, J = 5.8 Hz, 2H), 5.34 (m, 4H), 10.81 (s, 1H).

Procedure for Preparing Combinatorially Labeled 11,11-*d*₂-Linoleic Acid (**9c**)



Combinatorially labeled 11,11-*d*₂-methyl 9,12-octadecadienoate (**9c**)

In a dried and nitrogen-purged 100 mL 2-neck round bottom flask fitted with a reflux condenser and a septum, (5.74 g, 5.10 mL, 47.9 mmol) of catechol borane was added via syringe to (4.00 g, 13.7 mmol) of **8b**. The reaction was run under nitrogen atmosphere for 5 h at about 60 °C. Upon cooling the reaction to room temperature, a mixture of (26.29 g, 25.05 mL, 437.8 mmol) acetic acid and (6.68 g, 6.31 mL, 109.4 mmol) d-acetic acid was added to the reaction and then taken to mild reflux for about 5 h and was allowed to stir at room temperature for 12 h. The reaction was added to 250 mL ice/water and extracted with five 20 mL quantities of Et₂O. The ether layers were combined, dried with anhydrous sodium sulfate, and filtered. Solvent was removed to yield a viscous brown liquid. This was purified by flash chromatography using a 12" X 2" column of silica 60 and eluents of increasing polarity: 1000 mL hexanes, 500 mL 80% hexanes/20% Et₂O, and 500 mL 60% hexanes/40 % Et₂O. The product of interest eluted ahead of the main contaminants. Solvent was removed to yield **9c** (2.25 g, 7.6 mmol) as a clear liquid. ¹H NMR (300 MHz, CDCl₃) δ: 0.87 (t, J = 7.0 Hz, 3H), 1.29 (m, 14H), 1.60 (m, 2H), 2.01 (m, 4H), 2.28 (t, J = 7.6 Hz, 2H), 3.65 (s, 3H), 5.34 (m, 4H).

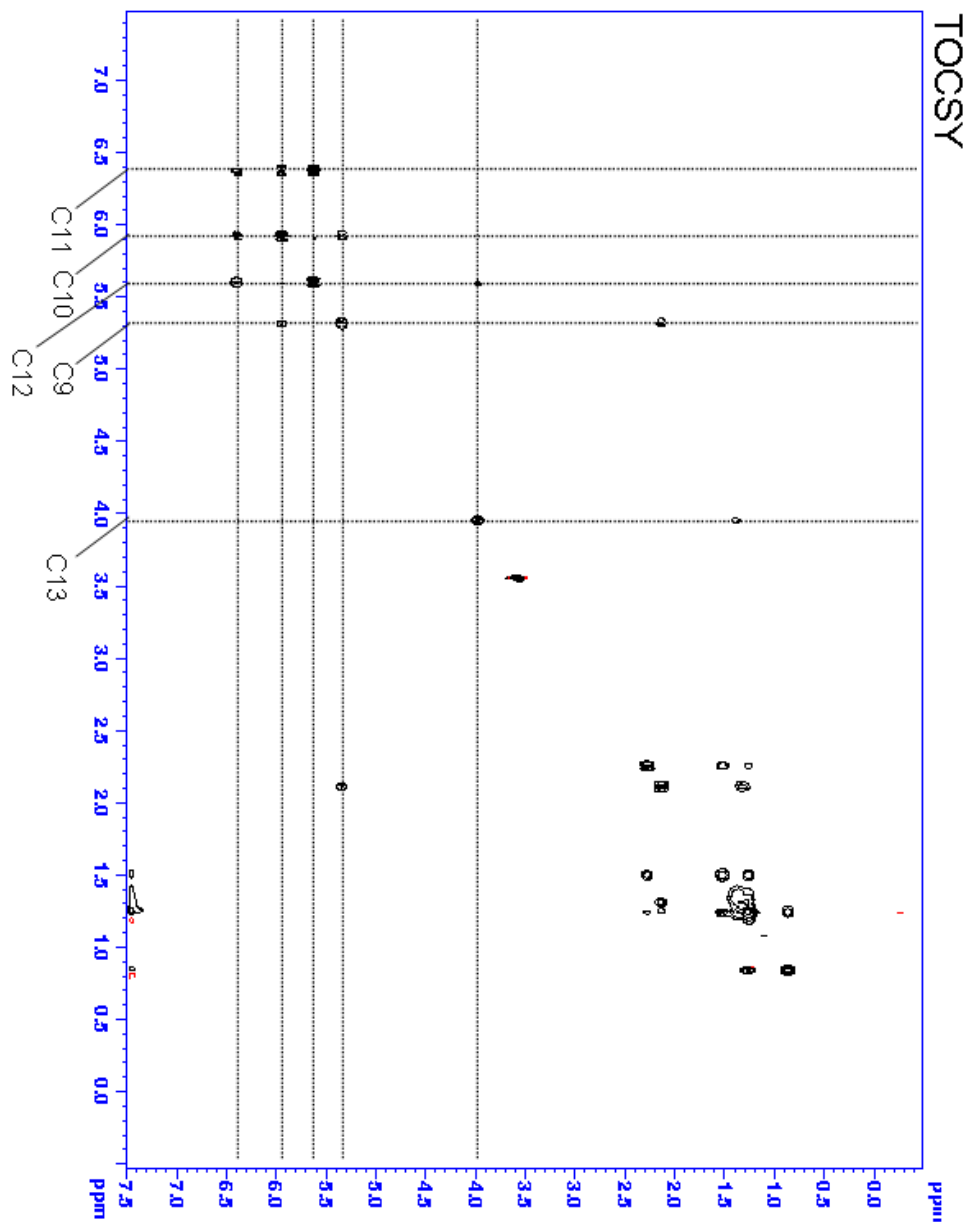


11,11-*d*₂- 9,12-Octadecadienoic acid (**10c**)

A 100 mL round bottom flask fitted with a reflux condenser was charged with 15 g of potassium hydroxide and 35 mL of water. To this, (1.80 g, 6.1 mmol) **9c** was added. The reaction was refluxed and followed by TLC. The end of the reaction was usually accompanied by a large degree of foaming. Complete reaction was usually achieved in about 1 h. The reaction was allowed to cool to room temperature and was added to a mixture of 240 mL ice/water and 85% phosphoric acid. This was extracted with five 20 mL quantities of Et₂O. The solvent was removed and any remaining water was eliminated by adding benzene to the resulting liquid and removing by rotary evaporation to yield **10c** (1.70 g, 6.0 mmol) as a clear liquid. ¹H NMR (300 MHz, CDCl₃) δ: 0.87 (t, J = 7.1 Hz, 3H), 1.29 (m, 14H), 1.61 (m, 2H), 2.03 (q, J = 6.7 Hz, 4H), 2.33 (t, J = 7.6 Hz, 2H), 2.75 (t, J = 5.8 Hz, 2H), 5.34 (m, 4H), 10.81 (s, 1H).

Figure S1. Spectra used for the assignment of ^2H and ^{13}C NMR peaks for MHOD

A. TOCSY spectrum used to establish $^1\text{H}/^1\text{H}$ resonance correlations and make ^2H NMR assignments. Dotted lines are used to illustrate correlations.



B. HMQC spectrum used to establish $^1\text{H}/^{13}\text{C}$ resonance correlations and make ^{13}C spectral assignments. Dotted lines are used to show correlations.

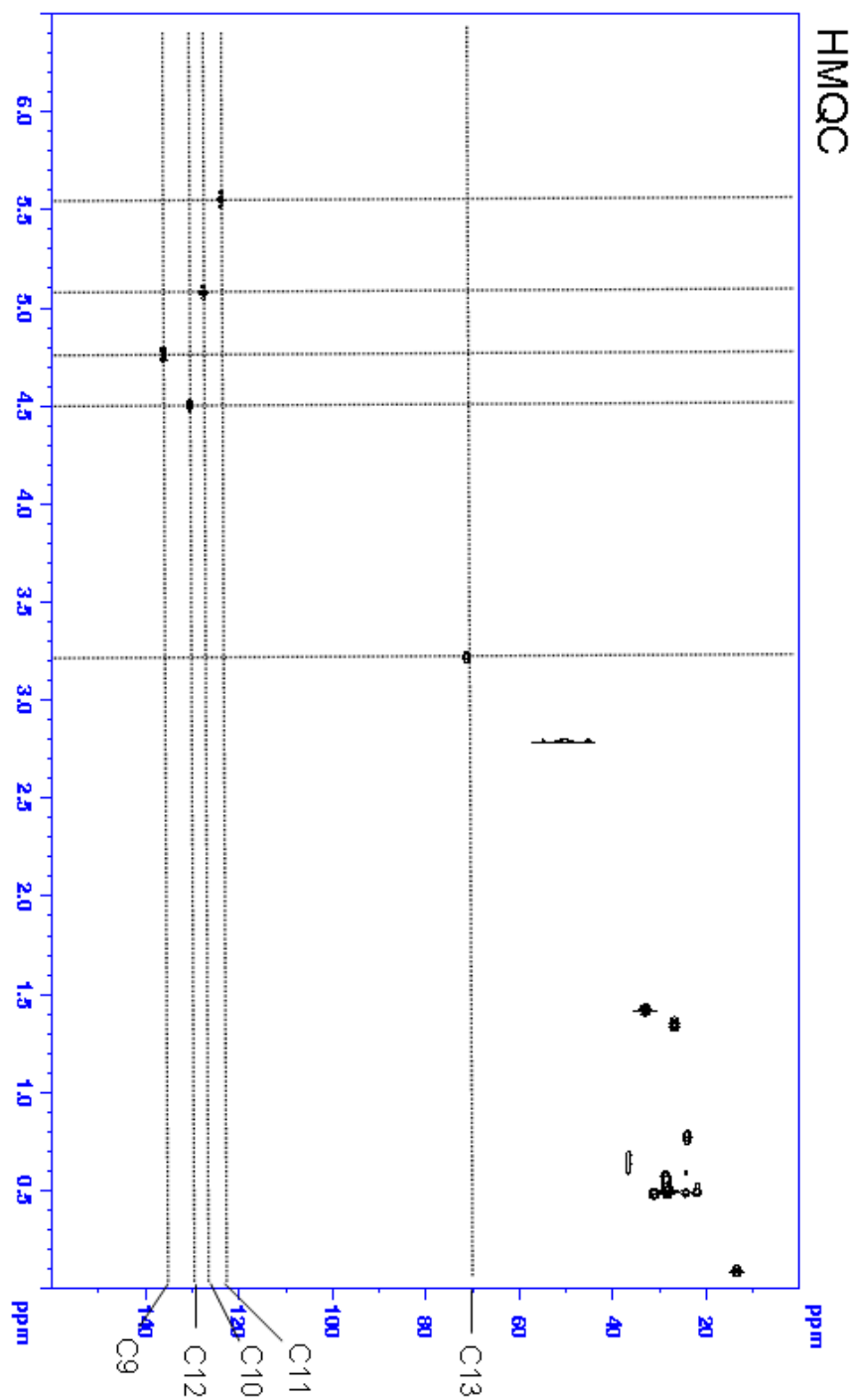
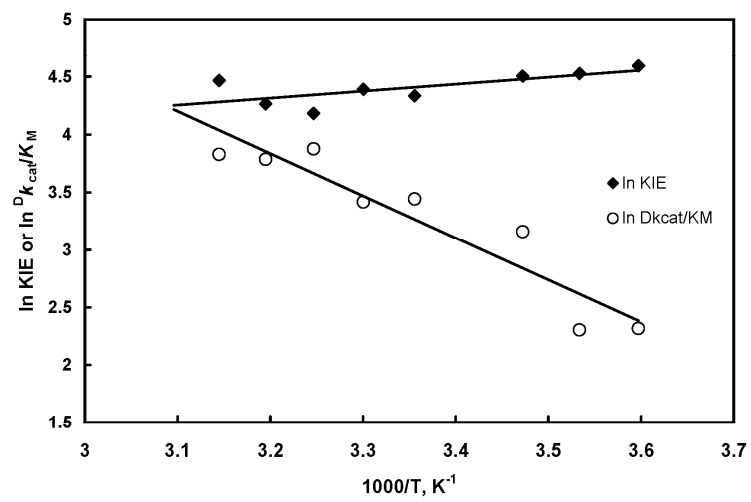


Figure S2. Plot of $^Dk_{\text{cat}}$ versus $^Dk_{\text{cat}}/K_m$ for SLO-1.



Procedure for Running Partial Conversion ($F \approx 0.35$) Reactions

These reactions were performed between 21 and 23 °C. A 12 L 3-neck round-bottomed flask was charged with 10 L of 0.1 M borate (pH 9.0). To this was added 627.5 μ L of the combinatorially labeled 11,11-*d*₂-linoleic acid or linoleic acid dissolved in 100 mL methanol. This mixture was allowed to stir slowly for about 1 hour before spectrophotometrically determining the initial concentration of linoleic acid. Four aliquots of 500 μ L were taken before the reaction was initiated by the addition of SLO-1. These aliquots were diluted with 500 μ L 0.1 M borate (pH 9.0). A 495 μ L aliquot of this diluted solution was added to a cuvette, the absorbance was zeroed, and 5 μ L of approximately 1 mg/mL SLO-1 in 0.1 M borate (pH 9.0) was added to the cuvette. The absorbance was followed at 234 nm. The final absorbance (corrected for the absorbance of SLO-1) was used to compute the initial concentration of substrate in the reaction using an extinction coefficient for the product of 23,600 AU M⁻¹ cm⁻¹. Three reactions were then taken to approximately 35% conversion. Conversion was monitored by taking a 500 μ L aliquot of the reaction mixture, diluting it 1:1 with 500 μ L of 0.1 M borate (pH 9.0) and assaying for remaining linoleic acid by observing absorbance change at 234 nm after adding 5 μ L of 1 mg/mL SLO-1 to 495 μ L of the diluted reaction mixture. These partial conversion reactions usually required approximately 1 mg and 10 mg SLO-1 to achieve the appropriate fractional conversion of linoleic acid and 11,11-*d*₂-linoleic acid, respectively. These substrates were utilized for the determination of ¹³C KIEs. Substrates that were combinatorially labeled at the 9, 10, 12, and 13 positions with approximately 4% deuterium were used in ²H KIE determinations. Combinatorial labeling did not noticeably affect reaction time or the amount of SLO-1 required to achieve the desired conversion.

The above reactions were quenched when the reaction reached the desired 35% completion mark. The quenching process involved the addition of about 600 mL diethyl ether followed by the addition of 3.9 g NaBH₄, 136.9 g KH₂PO₄, and 584.4 g NaCl. The NaBH₄ was used to reduce 13-hydroperoxy-9,11-(*Z,E*)-octadecadienoic acid to 13-hydroxy-9,11-(*Z,E*)-octadecadienoic acid (HOD). The KH₂PO₄ was used to acidify the solution to aid in extraction of linoleic acid and the reduced product. The NaCl was also found to aid in extraction. It should be noted here that KH₂PO₄ should be added slowly because NaBH₄ evolves hydrogen rapidly in the presence of acid, and foaming can occur due to the soapy nature of the substrate and product. The quenched reactions were separated into 3 approximately equivalent volumes and extracted in a 4 L separatory funnel with 6 \times 200 mL volumes of diethyl ether. After extraction, the combined ether layers were washed with approximately 200 mL brine. The ether was then removed using rotary evaporation. The residual oil containing linoleic acid and HOD was methylated using diazomethane prepared via the addition of N-methyl-N-Nitrosourea to a biphasic mixture of 4.5 mL 15% KOH and 10 mL diethyl ether. The methyl esters of the linoleic acid and HOD were separated via flash chromatography using 200 mesh neutral silica as the stationary phase in a 2.5'' \times 14'' column. The mobile phase consisted of (in order) 1000 mL of 20% diethyl ether/80% hexanes, then 500 mL 30% diethyl ether/70% hexanes, then 500 mL 50% diethyl ether/50% hexanes. About 160 fractions of about 10 mL were collected. The methyl linoleate typically eluted from fraction 15 to fraction 30, and the methyl ester of HOD (MHOD) eluted between fractions 100 and 140.

Procedure for Running Full Conversion ($F=1.00$) Reactions

These reactions were carried out as described above. Full conversion of linoleic acid required 3 mg of SLO-1, while full conversion of 11,11-*d*₂-linoleic acid required 30 mg of SLO-1. Reactions were analyzed for progress as described above. When no further absorbance change was detected from 1:1 diluted aliquots treated with 1 mg/mL SLO-1, the reaction was considered to have proceeded to completion. Methyl linoleate was not detectable after methylation and purification of the converted substrate. MHOD was the only material detectable.

¹³C Kinetic Isotope Effect Results and Calculations

¹³C NMR Measurements. Quantitative ¹³C NMR spectra were obtained using a Bruker DRX-500 spectrometer equipped with a broad band probe at 125.77 MHz in a 5mm NMR tube containing approximately 150 mg of MHOD in *d*₆-DMSO filled to 5 cm. The temperature was held to 23.2 °C for all samples. The overall relaxation time was determined by inversion recovery. Delays between acquisitions were set to greater than 5×T₁ for the center of interest with the longest relaxation time. Over approximately 13 hours, 24 acquisitions of 64 scans each were acquired while locked on the deuterium signal in *d*₆-DMSO. Scans of 131072 points acquired over 2.12 s were taken with a total delay of 32.12 s between excitation pulses. Inverse gated decoupling of proton signals was used to decouple while not inducing spurious NOE contributions to the ¹³C resonances. A $\pi/6$ pulse was used for excitation. The 24 FIDs acquired during each experiment were added together. Processing was performed by zero filling the FID with 128K points. Fourier transforms were performed with a line broadening of 0.75 Hz. Integrations for C2 were included as a check upon overall validity. Although there is significant dispersion in integrations and propagated KIEs between samples, the chosen internal standard, C7, and other control positions should yield little or no significant KIE. The average value for the KIE for positions other than those of interest (C9-C13) is 0.994 with a standard deviation of 0.017, suggesting that, when averaged, the KIEs reported here converge to a number close to the true mean.

Computing ¹³C KIEs. Kinetic isotope effects were computed from the fractional conversion attained in partial conversion reactions (*F*), quantitative ¹³C integrations in the product isolated from partial conversion reactions (*R_p*) and quantitative ¹³C integrations in the product isolated from full conversion reactions (*R₀*). Integrations at the C2 position were used as the internal standard and scaled to 1000 in both partial and full conversion reactions. *F*, *R_p*, and *R₀* were used as input into eq. 1. KIEs were computed from the three partial conversion reactions and two standard reactions to yield six estimates of the KIE. The error associated with these estimates was computed using eq. 2, where N=6.

$$KIE_p = \frac{\ln(1-F)}{\ln[1-F(R_p/R_0)]} \quad (S1)$$

$$\Delta KIE_p = \sqrt{\frac{1}{N-1} \sum_{i=1}^6 (KIE_{p,i} - \overline{KIE_p})^2} \quad (S2)$$

Integrations of ^{13}C Spectra of 11-*d*-MHOD

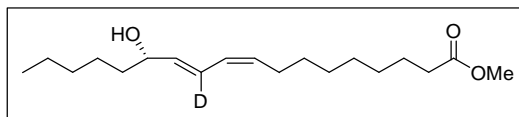


Table S1. Average ^{13}C integrations for reisolated product from the conversion of 11,11- d_2 -linoleic acid.

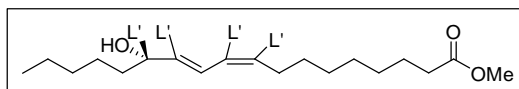
Reaction →	F=0.364	F=0.340	F=0.365	Standard 1 (F=1.00)	Standard 2 (F=1.00)
Position ↓					
C7 (Int. Ref.)	1000	1000	1000	1000	1000
C9	1043.13	1068.07	1078.09	1097.67	1096.80
C10	1009.54	998.43	994.37	1015.44	1021.11
C11	1082.102	1073.40	1085.78	1090.50	1151.60
C12	1006.86	1016.32	1012.13	1038.29	1046.73
C13	1014.76	1025.14	1025.76	1039.95	1035.80
C2	1040.36	1017.07	1019.07	1027.72	1035.68
peak 10	1016.32	1023.89	1027.57	1021.18	1026.83
peak 11	1014.36	1047.60	1029.24	984.42	985.26
peak 15	1015.51	999.23	1004.57	999.75	996.42
peak 16	1027.32	998.94	1013.01	1002.46	1017.22
peak 17	1026.00	1001.36	1011.70	1017.24	1022.72
peak 18	1019.16	1017.18	1012.76	1007.27	1010.05

Table S2. Relative fractionation (R_p/R_0) for reisolated product.

Reaction →	F=0.364	F=0.340	F=0.365	F=0.364	F=0.340	F=0.365
	Relative to Standard 1			Relative to Standard 2		
Position ↓						
C7 (Int. Ref.)	1.000	1.000	1.000	1.000	1.000	1.000
C9	0.950325	0.973050	0.982172	0.951072	0.973814	0.982943
C10	0.994273	0.983331	0.979326	0.988675	0.977795	0.973812
C11	0.992778	0.984796	0.996148	0.939655	0.932100	0.942844
C12	0.969740	0.978849	0.974820	0.961912	0.970947	0.966950
C13	0.975779	0.985760	0.986360	0.979693	0.989714	0.990316
C2	1.01231	0.989646	0.991591	1.00456	0.982071	0.984001
peak 10	0.995288	1.00270	1.00630	0.989782	0.997156	1.00074
peak 11	1.03045	1.06422	1.04557	1.02953	1.06327	1.04463
peak 15	1.01577	0.999486	1.00483	1.01918	1.00284	1.00820
peak 16	1.02480	0.996492	1.01052	1.00993	0.982029	0.995856
peak 17	1.00862	0.984396	0.994554	1.00324	0.979149	0.989253
peak 18	1.01180	1.00984	1.00545	1.00902	1.00706	1.00269

Computing ^2H KIEs. ^2H KIEs were computed in the same way as the ^{13}C KIEs using eqs. S1 and S2.

Integrations of ^2H Spectra of Combinatorially Labeled MHOD



Reaction →	F=0.328	F=0.348	F=0.337	Standard 1 (F=1.00)	Standard 2 (F=1.00)
Position ↓					
aromatic	318.97	355.19	344.26	153.36	148.24
C(=O)OMe	300	300	300	300	300

Reaction →	F=0.328	F=0.348	F=0.337	Standard 1 (F=1.00)	Standard 2 (F=1.00)
Position ↓					
Ph-CD ₃	1000	1000	1000	1000	1000
C9	14.3880	13.4699	13.2569	33.0570	35.1617
C10	19.0084	17.5164	17.6412	42.4156	45.0334
C12	19.9214	18.5278	18.6124	47.5182	50.2801
C13	12.1246	11.4175	11.2399	26.7538	27.7068

Reaction →	F=0.328	F=0.348	F=0.337	F=0.328	F=0.348	F=0.337
	Relative to Standard 1			Relative to Standard 2		
Position ↓						
Ph-CD ₃	1.000	1.000	1.000	1.000	1.000	1.000
C9	0.9053	0.9437	0.9002	0.8804	0.9179	0.8755
C10	0.9321	0.9565	0.9336	0.9082	0.9320	0.9097
C12	0.8720	0.9030	0.8792	0.8525	0.8829	0.8596
C13	0.9426	0.9884	0.9431	0.9416	0.9873	0.9421

Integrations of ^2H Spectra of Combinatorially Labeled 11,11-*d*-MHOD

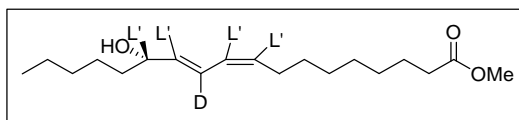


Table S6. Average ^1H integrations used to determine the ratio of external standard ($\alpha,\alpha,\alpha\text{-d}_3\text{-toluene}$) to combinatorially labeled 11-*d*-MHOD.

Reaction →	F=0.359	F=0.307	F=0.328	Standard 1 (F=1.00)	Standard 2 (F=1.00)
Position ↓					
aromatic	250.76	363.53	289.53	171.28	161.09
C(=O)OMe	300	300	300	300	300

Table S7. Average ^2H integrations used to determine R_p and R_0 .

Reaction →	F=0.359	F=0.307	F=0.328	Standard 1 (F=1.00)	Standard 2 (F=1.00)
Position ↓					
Ph-CD ₃	1000	1000	1000	1000	1000
C9	20.3488	12.7941	16.4822	33.9479	36.4389
C10	43.8343	27.7506	37.4242	77.5908	84.9736
C12	31.9017	20.7739	28.1901	54.0345	57.8513
C13	13.5732	9.6106	11.0141	20.2374	22.24574613

Table S8. Relative fractionation (R_p/R_0) for reisolated product.

Reaction →	F=0.359	F=0.307	F=0.328	F=0.359	F=0.307	F=0.328
	Relative to Standard 1			Relative to Standard 2		
Position ↓						
Ph-CD ₃	1.000	1.000	1.000	1.000	1.000	1.000
C9	0.8776	0.7999	0.8207	0.8693	0.7924	0.8130
C10	0.8271	0.7591	0.8153	0.8030	0.7370	0.7916
C12	0.8644	0.8160	0.8819	0.8584	0.8104	0.8758
C13	0.9820	1.008	0.9200	0.9498	0.9750	0.8899

EIEs from Computed Structures

The optimized structure corresponding to the reactant model, 2,5-heptadiene, and the constrained intermediate model, 2,5-heptadienyl radical, were used to compute frequencies using the same density functional (B3LYP) and basis set [6-31+G(d,p)] used in the optimization. The 'freq' keyword in Gaussian03¹ was utilized, thus yielding frequencies under the harmonic approximation. The resulting frequencies were used to compute the ratio of equilibrium constants using the ratios of partition functions in conjunction with the Teller-Redlich product rule (eqs S3 and S4). The following terms are the products corresponding to the $3N^{\text{react}}-6$ real frequencies in the reactant and the $3N^{\text{int}}-6$ real frequencies in the intermediate. N^{react} is the number of atoms in the reactant; N^{int} is the number of atoms in the intermediate.

$$\frac{K_H}{K_D} = \prod_i^{3N^{\text{int}}-6} \left(\frac{e^{\frac{u_{i,D}^{\text{int}}}{2}}}{e^{\frac{u_{i,H}^{\text{int}}}{2}}} \right) \left(\frac{1 - e^{-u_{i,D}^{\text{int}}}}{1 - e^{-u_{i,H}^{\text{int}}}} \right) \prod_j^{3N^{\text{react}}-6} \left(\frac{e^{\frac{u_{j,H}^{\text{react}}}{2}}}{e^{\frac{u_{j,D}^{\text{react}}}{2}}} \right) \left(\frac{1 - e^{-u_{j,H}^{\text{react}}}}{1 - e^{-u_{j,D}^{\text{react}}}} \right) \quad (\text{S3})$$

$$u_{j,H}^{\text{react}} = \frac{h\nu_{j,H}^{\text{react}}}{kT} \quad (\text{S4})$$

Computed Structures

All quantum mechanical calculations were performed with Gaussian03.¹ The 2,5-heptadiene reactant model was fully optimized using the Becke3LYP hybrid functional together with the 6-31+G(d,p) basis set. The 2,5-heptadienyl radical model for the intermediate was optimized with the dihedral angles fixed to those values observed in models of linoleic acid bound to the active site of SLO-1. Vibrational analyses were carried out on both structures, and no imaginary frequencies were observed.

Calculation of ^2H KIEs Arising from Inner Sphere Reorganization

	E_s 19					E_s 19					
$v(H)$	E_c	ΔG	$y(H)$	$F(H)$		$v(D)$	E_c	ΔG	$y(D)$	$F(D)$	KIE
3.741501	0.05	-6	-0.31577	-3.78377		2.645641	0.05	-6	-0.31572	-3.79584	1.012147
$w(H)$	0.1	-6	-0.31576	-3.81526		$w(D)$	0.1	-6	-0.31565	-3.8394	1.024441
5.84E+14	0.15	-6	-0.31574	-3.84674		4.13E+14	0.15	-6	-0.31559	-3.88297	1.036886
	0.2	-6	-0.31573	-3.87823			0.2	-6	-0.31552	-3.92653	1.049483
	0.25	-6	-0.31571	-3.90972			0.25	-6	-0.31545	-3.97009	1.062234
	0.3	-6	-0.3157	-3.94121			0.3	-6	-0.31538	-4.01366	1.07514
	0.35	-6	-0.31568	-3.97269			0.35	-6	-0.31531	-4.05722	1.088204
	0.4	-6	-0.31566	-4.00418			0.4	-6	-0.31525	-4.10079	1.101427
	0.45	-6	-0.31565	-4.03567			0.45	-6	-0.31518	-4.14436	1.114812
	0.5	-6	-0.31563	-4.06716			0.5	-6	-0.31511	-4.18792	1.12836
	0.55	-6	-0.31562	-4.09864			0.55	-6	-0.31505	-4.23149	1.142074
	0.6	-6	-0.3156	-4.13013			0.6	-6	-0.31498	-4.27506	1.155955
	0.65	-6	-0.31559	-4.16162			0.65	-6	-0.31491	-4.31863	1.170005
	0.7	-6	-0.31557	-4.19311			0.7	-6	-0.31484	-4.3622	1.184228
	0.75	-6	-0.31556	-4.2246			0.75	-6	-0.31478	-4.40577	1.198624
	0.8	-6	-0.31554	-4.25608			0.8	-6	-0.31471	-4.44934	1.213196
	0.85	-6	-0.31553	-4.28757			0.85	-6	-0.31464	-4.49292	1.227946
	0.9	-6	-0.31551	-4.31906			0.9	-6	-0.31457	-4.53649	1.242676
	0.95	-6	-0.31549	-4.35055			0.95	-6	-0.31451	-4.58006	1.257988
	1	-6	-0.31548	-4.38204			1	-6	-0.31444	-4.62364	1.273286
	1.05	-6	-0.31546	-4.41352			1.05	-6	-0.31437	-4.66721	1.28877
	1.1	-6	-0.31545	-4.44501			1.1	-6	-0.31431	-4.71079	1.304443
	1.15	-6	-0.31543	-4.4765			1.15	-6	-0.31424	-4.75437	1.320308
	1.2	-6	-0.31542	-4.50799			1.2	-6	-0.31417	-4.79794	1.336367
	1.25	-6	-0.3154	-4.53948			1.25	-6	-0.31411	-4.84152	1.352621
	1.3	-6	-0.31539	-4.57097			1.3	-6	-0.31404	-4.8851	1.369075
	1.35	-6	-0.31537	-4.60245			1.35	-6	-0.31397	-4.92868	1.38573
	1.4	-6	-0.31535	-4.63394			1.4	-6	-0.31391	-4.97226	1.402588
	1.45	-6	-0.31534	-4.66543			1.45	-6	-0.31384	-5.01584	1.419652
	1.5	-6	-0.31532	-4.69692			1.5	-6	-0.31377	-5.05943	1.436925
	1.55	-6	-0.31531	-4.72841			1.55	-6	-0.31371	-5.10301	1.454409
	1.6	-6	-0.31529	-4.7599			1.6	-6	-0.31364	-5.14659	1.472107
	1.65	-6	-0.31528	-4.79139			1.65	-6	-0.31357	-5.19018	1.490022
	1.7	-6	-0.31526	-4.82287			1.7	-6	-0.31351	-5.23376	1.508155
	1.75	-6	-0.31525	-4.85436			1.75	-6	-0.31344	-5.27735	1.52651
	1.8	-6	-0.31523	-4.88585			1.8	-6	-0.31338	-5.32093	1.545089
	1.85	-6	-0.31522	-4.91734			1.85	-6	-0.31331	-5.36452	1.563896
	1.9	-6	-0.3152	-4.94883			1.9	-6	-0.31324	-5.40811	1.582932
	1.95	-6	-0.31518	-4.98032			1.95	-6	-0.31318	-5.4517	1.602202
	2	-6	-0.31517	-5.01181			2	-6	-0.31311	-5.49529	1.621707

Example calculation:

Solving the saddle point condition below with inputs of $\Delta G = -6.0$ kcal/mol, $\lambda_{in} = 0.2$ kcal/mol, $\lambda_{out} = 19$ kcal/mol, $T = 298.15$ K, and $\omega_H = 5.84 \times 10^{14} \text{ s}^{-1}$ yields $y_H = -0.31573$. Solving the same condition with $\omega_D = 4.13 \times 10^{14} \text{ s}^{-1}$ yields $y_D = -0.31552$.

$$\Delta G = \lambda_{out} y_H + \lambda_{in} \left(\frac{\sinh(\hbar \omega_H y_H / 2k_B T)}{\sinh(\hbar \omega_H / 2k_B T)} \right)$$

The values of the parameters listed above can then be used along with the computed values of y_H and y_D to arrive at values for $F_H = -3.87823$ and $F_D = -3.92653$ using the equation below.

$$F_H = \frac{\lambda_{out}}{4k_B T} (y_H^2 - 1) - \frac{\Delta G}{2k_B T} (y_H + 1) + \frac{\lambda_{in}}{\hbar \omega_H} \left[\frac{\cosh(\hbar \omega_H y_H / 2k_B T)}{\sinh(\hbar \omega_H / 2k_B T)} \right] - \coth(\hbar \omega_H / 2k_B T)$$

These values can then be used to yield $k_H/k_D = 1.04948$ using the equation below:

$$k_H/k_D = \exp \left[F(\Delta G, \lambda_{in}, \lambda_{out}, T, \omega_D, y_D) - F(\Delta G, \lambda_{in}, \lambda_{out}, T, \omega_H, y_H) \right]$$

2,5-Heptadienyl, UB3LYP/6-31+G(d,p)

E(UB+HF-LYP) = -273.316574006

Zero-point correction= 0.155531
(Hartree/Particle)

Thermal correction to Energy= 0.163784
 Thermal correction to Enthalpy= 0.164728
 Thermal correction to Gibbs Free Energy= 0.122095
 Sum of electronic and zero-point Energies= -273.161043
 Sum of electronic and thermal Energies= -273.152790
 Sum of electronic and thermal Enthalpies= -273.151846
 Sum of electronic and thermal Free Energies= -273.194479

	E (Thermal)		CV	S
	KCal/Mol		Cal/Mol-Kelvin	Cal/Mol-Kelvin
Total	102.776		29.353	89.728
C	1.138623	0.950381	-0.621339	
C	1.898249	-0.186875	-0.401276	
C	1.677483	-1.248163	0.633659	
C	0.020905	1.436990	0.080826	
C	-0.955266	0.693482	0.880131	
C	-1.822654	-0.244036	0.443008	
C	-1.889660	-0.842060	-0.932058	
H	1.560766	1.648835	-1.345733	
H	2.757432	-0.327237	-1.055052	
H	2.637217	-1.615991	1.014838	
H	1.150118	-2.118731	0.215901	
H	1.081593	-0.887182	1.474969	
H	-0.156077	2.511619	0.018387	
H	-1.127560	1.077717	1.889337	
H	-2.553918	-0.617611	1.159867	
H	-1.190665	-0.363881	-1.621769	
H	-2.903554	-0.749747	-1.342339	
H	-1.661426	-1.916100	-0.906111	

2,5-Heptadiene, B3LYP/6-31+G(d,p)

E(RB+HF-LYP) = -273.959587482

Zero-point correction= 0.169737
(Hartree/Particle)

Thermal correction to Energy= 0.178735
 Thermal correction to Enthalpy= 0.179679
 Thermal correction to Gibbs Free Energy= 0.134859
 Sum of electronic and zero-point Energies= -273.789851
 Sum of electronic and thermal Energies= -273.780853
 Sum of electronic and thermal Enthalpies= -273.779909
 Sum of electronic and thermal Free Energies= -273.824729

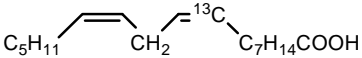
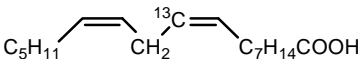
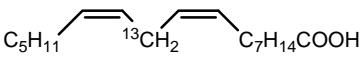
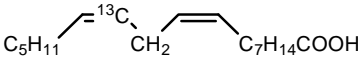
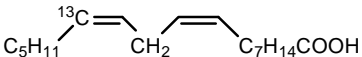
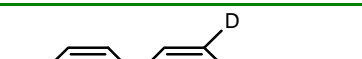
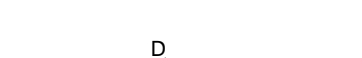
	E (Thermal)		CV	S
	KCal/Mol		Cal/Mol-Kelvin	Cal/Mol-Kelvin
Total	112.158		31.174	94.332
C	-1.253299	-1.009660	-0.054331	
C	-2.280478	-0.201970	-0.354049	

C	-2.454518	1.248938	0.002276
C	0.000002	-0.670570	0.720594
C	1.253174	-1.009646	-0.054572
C	2.280415	-0.201994	-0.354197
C	2.454656	1.248809	0.002460
H	-1.307156	-2.040840	-0.405411
H	-3.101083	-0.635395	-0.926449
H	-3.352984	1.393776	0.616106
H	-2.591825	1.855729	-0.901856
H	-1.604992	1.658477	0.554272
H	0.000091	-1.263804	1.649742
H	0.000035	0.377255	1.027780
H	1.306894	-2.040753	-0.405877
H	3.100915	-0.635391	-0.926771
H	1.604998	1.658438	0.554185
H	2.592487	1.855735	-0.901500
H	3.352901	1.393331	0.616690

Transition State Theory Computations. A structure² of linoleic acid docked³ to the active site of the 1F8N.pdb⁴ crystal structure of SLO-1 was abbreviated to 2,5-heptadiene. However, the four internal carbon skeleton dihedrals present in the bound ground state are preserved in the transition state. The transition state for hydrogen atom abstraction from 2,5-heptadiene to cyclopentenyl radical was computed using the Gaussian 98W suite.⁵ For the computation of ground state 2,5-heptadiene, the most stable structure was sought, and no constraints were placed on the reactant structure. The justification for this step rests in the nature of the isotope effect studies carried out herein. These competitive KIEs explore the isotopic fractionation experienced in going from the free substrate to the transition state structure of the rate-determining step. Density functional theory was employed for these calculations using the B3LYP functional; the 6-31+G(d,p) basis set was utilized. Frequency calculations were then performed on the optimized structures. The transition state was found to have exactly one imaginary frequency corresponding to $1778.2i\text{ cm}^{-1}$ for all light isotopes. A perl program was written to compute kinetic isotope effects from the force constant matrices generated in the frequency calculations. First, the perl program extracts and parses the force constant

matrix for the reactant and transition state structures. Then, according to the isotopic substitution desired, the force constant matrix is weighted according to mass. The mass-weighted force constant matrix is then diagonalized using the Math::MatrixReal module available on CPAN.⁶ The resulting frequency eigenvalues (after discarding rotational and translational eigenvalues) were then placed into the Bigeleisen equation (*vide infra*). The Bell correction was then employed to correct for tunneling. The Bell correction employed here was of the correct form provided by Northrop,⁷ where 20 terms were computed in the interest of avoiding the non-monotonic behavior of the Bell correction with respect to reduced imaginary frequency ($h\nu^\ddagger/kT$) for high imaginary frequencies when only the first or first few terms are used.

Table S9. Computed KIEs and Bell tunnel correction for model reaction between 2,5-heptadiene and cyclopentadienyl radical.

<i>Heavy Isotopolog Represented by Model^{a, b}</i>	<i>Imaginary Frequency $\nu(\text{cm}^{-1})$</i>	<i>KIE (no tunneling)</i>	<i>$Q_{\text{light}}/Q_{\text{heavy}}$</i>	<i>KIE (Bell Correction)^c</i>
	1778.60 <i>i</i>	1.002	1.000	1.002 (1.002)
	1778.34 <i>i</i>	1.003	1.003	1.006 (1.000)
	1774.49 <i>i</i>	1.017	1.041	1.059 (1.083)
	1778.35 <i>i</i>	1.005	1.003	1.008 (1.008)
	1778.62 <i>i</i>	1.003	1.000	1.003 (1.003)
	1778.63 <i>i</i>	1.02	1.00	1.02 (1.02)
	1777.35 <i>i</i>	1.02	1.01	1.03 (1.04)

	1778.41 <i>i</i>	0.98	1.00	0.99 (0.99)
	1778.56 <i>i</i>	1.00	1.00	1.01 (1.01)
	1349.61 <i>i</i>	5.51	136.00	751.00

^a Carbon positions correspond to linoleic acid numbering. Deuterium and protium numbering refer to the carbon bound to these atoms.

^b Light isotopolog is all ¹H- and ¹²C-labeled linoleic acid.

^c The numbers in parentheses are for conversion of compound dideuterated at carbon 11. The reaction coordinate imaginary frequency in this calculation was 1349.56*i*.

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- 2) This was provided by Prof. Sharon Hammes-Schiffer and Elizabeth Hatcher at Pennsylvania State University.
- 3) Using Autodock 3.0: Morris, G. M.; Goodsell, D. S.; Halliday, R.S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comp. Chem.* **1998**, *19*, 1639-1662.4) Tomchick, D. R.; Phan, P.; Cymborowski, C.; Minor, W.; Holman, T. *Biochemistry* **2001**, *40*, 7509-7517.5) Gaussian 98, Revision A.11.4, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, N. Rega, P. Salvador, J. J. Dannenberg, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson,

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