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(ω-2,ω-2,ω-3,ω-3)-Tetradeuterio-fatty acids for mechanistic studies of enzyme-catalyzed hydroxylation reactions

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Among the thousands of cytochrome P450 enzymes known, many selectively hydroxylate the hydrocarbon tail of fatty acids at the terminal (ω) position and the ω-1, ω-2, and ω-3 positions. A general method for synthesis of (ω-2,ω-2,ω-3,ω-3)-tetradeuterio-fatty acids that can be used in mechanistic studies of cytochromes P450 is illustrated by the synthesis of 9,9,10,10-tetradueteriododecanoic (lauric) acid and 13,13,14,14-tetradeuteriohexadecanoic (palmitic) acid. Deuterium is introduced early in the synthesis by reduction of the THP ether of 4-heptyn-1-ol with deuterium gas to give a common labeled intermediate, 4,4,5,5-tetradeuterioheptan-1-ol. This alcohol is converted to the corresponding tosylate that is used to alkylate O-protected (ω-1)-alkyn-1-ols to give, eventually, long-chain alcohols that are oxidized to the corresponding fatty acids. An important experimental detail is that relatively large amounts of Wilkinson's catalyst were used to limit isotopic scrambling.

Keywords: deuterium; alkyne; reduction; synthesis

Introduction

Fatty acid hydroxylation at unactivated C-H positions is a characteristic reaction of many cytochrome P450 (P450 or CYP) enzymes,[1] and the search for ω- and (ω-x)-hydroxylase enzymes drove early studies that led to the first isolations of P450s.[2] In mechanistic studies of P450-catalyzed hydroxylations, measurements of H/D kinetic isotope effects (KIEs) often have provided important information,[3,4] and one useful feature is a comparison of intermolecular and intramolecular KIEs. Intermolecular H/D KIEs are readily determined with non-deuterated and perdeuterated substrates, whereas intramolecular KIEs can be available from partially deuterated substrates. Several P450s selectively catalyze C-H hydroxylation reactions of fatty acids at the ω, ω-1, ω-2, and ω-3 positions, and fatty acids deuterated at carbons ω-2 and ω-3 are useful for intramolecular KIE studies. We describe a general method for the synthesis of such labeled fatty acids and the specific application of the approach for the preparation of 9,9,10,10-tetradeuteriododecanoic acid (lauric acid) and 13,13,14,14-tetradeuteriohexadecanoic acid (palmitic acid).

The synthetic design is shown in Scheme 1. The concept is to prepare a short labeled alcohol, 4,4,5,5-tetradeuterioheptan-1-ol, that can be converted to a tosylate that is used to alkylate an (ω-1)-alkyn-1-ol tetrahydropyranyl (THP) ether of any length. Similar approaches involving termination of alkyne alkylation followed by reduction of the alkyne with deuterium gas or alkylation of an alkyne with a deuterated iodoalkane followed by reduction with hydrogen gas have been reported,[5–9] but we are not aware of its use for the longer chain fatty acids we prepared in this work. The 1-alkyn-ω-ols are either commercially available or prepared from 4-pentyn-1-ol by alkylation of the protected alcohol and base-catalyzed isomerization of the alkynyl group from an internal position to the terminal ω-1 position.[8]

Results and discussion

Scheme 1 shows the sequence for preparation of labeled lauric acid. The THP ether **1** from 4-pentyn-1-ol was ethylated; the resulting THP ether **2** was reduced with deuterium using Wilkinson's catalyst to give THP **3**, which was hydrolyzed to give the tetradeuterated heptanol **4**, the key synthetic intermediate. Alcohol **4** was converted to tosylate derivative **5**, and the tosylate was used to alkylate the THP ether of 4-pentyn-1-ol (for laurate synthesis) or 8-nonyn-1-ol (for palmitate synthesis) to give alkynes **6**. Reductions of alkynes **6** gave the THP derivatives **7**, which were hydrolyzed to give the deuterium-labeled saturated alcohols **8**. Oxidations of **8** gave the desired fatty acids **9**, which were converted to the methyl esters **10** in this work for analysis. For the synthesis of labeled lauric acid (**9**, $n = 1$), the alcoholate that reacted with the deuterated heptanol derivative **5** was prepared from the THP ether of commercially available 4-pentyn-1-ol.

For the synthesis of labeled palmitic acid (**9**, $n = 5$), the alkynol used in the reaction with the deuterated heptanol **5** derivative was the THP derivative of 8-nonyn-1-ol, which was not readily

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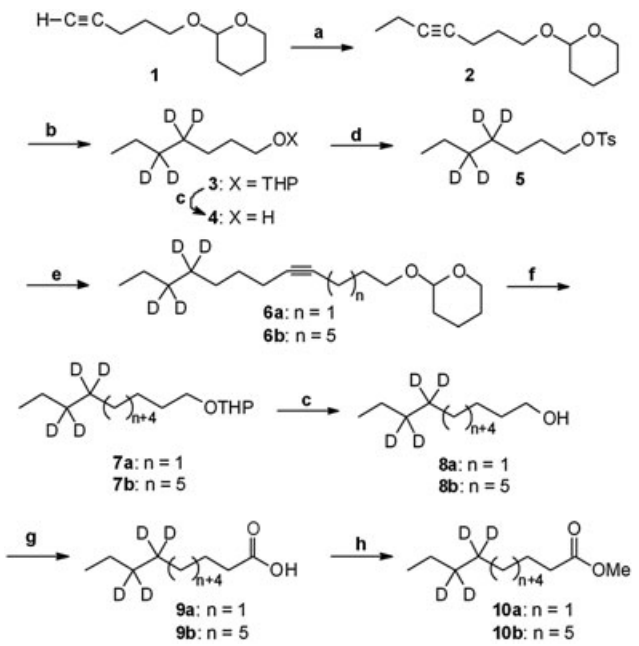


Scheme 1. Key: **a**: *n*-BuLi/THF, EtI; **b**: D_2, Wilkinson's catalyst; **c**: HCl/CH_3OH; **d**: *p*-TsCl, pyridine; **e**: 4-pentyn-1-ol THP ether or 8-nonyn-1-ol THP ether, *n*-BuLi/THF, NaI; **f**: H_2, Wilkinson's catalyst; **g**: $K_2Cr_2O_7$, H_2SO_4, acetone or PDC, DMF; **h**: CH_2N_2, Et_2O.

available. We prepared 8-nonyn-1-ol by the route in Scheme 2, which can be applied for a variety of (ω-1)-alkyn-1-ols that are either expensive or not available commercially. Thus, propargyl alcohol was protected to give THP **11**, which was deprotonated and alkylated with 1-iodohexane to give the THP ether **12**. Hydrolysis of **12** and isomerization of internal alkynol **13** gave the terminal alkynol, 8-nonyn-1-ol (**14**). It is noteworthy that the base-catalyzed isomerization of the β-alkynol we used followed the method reported previously for isomerization of γ-alkynols to (ω-1)-alkyn-1-ols.[10] The sequence in Scheme 2 employing propargyl alcohol can be used for synthesis of a wide range of (ω-1)-alkyn-1-ols. For example, alkylation of **11** with 1-iodooctane could be used to prepare, ultimately, labeled stearic acid.

One synthetic detail is important for highly regioselective incorporation of deuterium. We used a relatively large amount of Wilkinson's catalyst for the reduction of **2** (20 wt%) with deuterium gas. When smaller amounts of catalyst were used, 6–7 wt%, for example, the reduction was appreciably slowed overall, and deuterium was randomly scrambled into other positions in the chain as determined by NMR spectroscopy.

The tetradeuterated products can be analyzed by mass spectrometry to confirm that four deuterium atoms are incorporated in the product, but ^{13}C NMR spectroscopy is especially convenient for checking both the extent of deuterium incorporation and the absence of scrambling. Figure 1 shows the high field region in the ^{13}C NMR spectra of non-labeled dodecanoic acid and 9,9,10,10-tetradeuteriododecanoic acid. The signal at δ 32.0 and one of the signals at δ 29.6 in the non-labeled acid are shifted upfield by deuterium substitution as well as coupled to the deuterium in the labeled sample.

In summary, a general method for preparation of ω-3,ω-3, ω-2,ω-2-tetradeuterated fatty acids has been illustrated with the preparation of labeled dodecanoic acid (lauric acid) and hexadecanoic acid (palmitic acid). These labeled compounds are of use for measuring intramolecular KIEs in, for example, P450-catalyzed hydroxylation reactions.[11] A key step in the approach is the preparation of the tetradeuterated intermediate 4,4,5, 5-tetradeuterioheptan-1-ol tosylate that should be useful for preparation of any labeled saturated fatty acid of 10 or more carbon atoms.

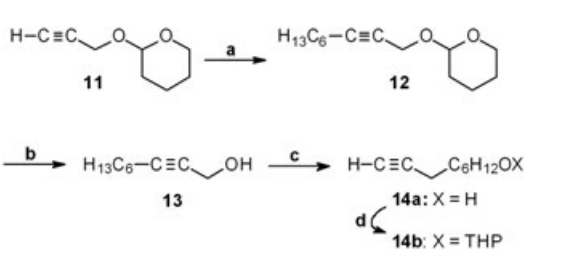
Experimental

Materials

Wilkinson's catalyst, *n*-butyllithium (BuLi), 4-pentyn-1-ol, iodoethane, and deuterium gas were purchased (Sigma-Aldrich, Wisconsin, U.S.A.) and used as obtained. *m*-Chloroperoxybenzoic acid was obtained from Sigma-Aldrich and recrystallized before use. Benzene was distilled from CaH_2 before use.

NMR spectra were obtained in $CDCl_3$ at 500 MHz (^1H) and referenced to internal tetramethylsilane or at 125 MHz (^{13}C) and referenced to the $CDCl_3$ at 77.0 ppm.

THP ethers **1** and **11** were prepared by reacting 4-pentyn-1-ol or 2-propyn-1-ol, respectively, with dihydropyran in methylene chloride at 0 °C. The ^1H and ^{13}C NMR spectra were consistent with data reported in the literature.[12]



Scheme 2. Key: **a**: *n*-BuLi/THF, *n*-$C_6H_{13}I$; **b**: *p*-TsOH, CH_3OH; **c**: Li, $H_2N(CH_2)_3NH_2$, KO-*t*-Bu; **d**: dihydropyran, *p*-TsOH.

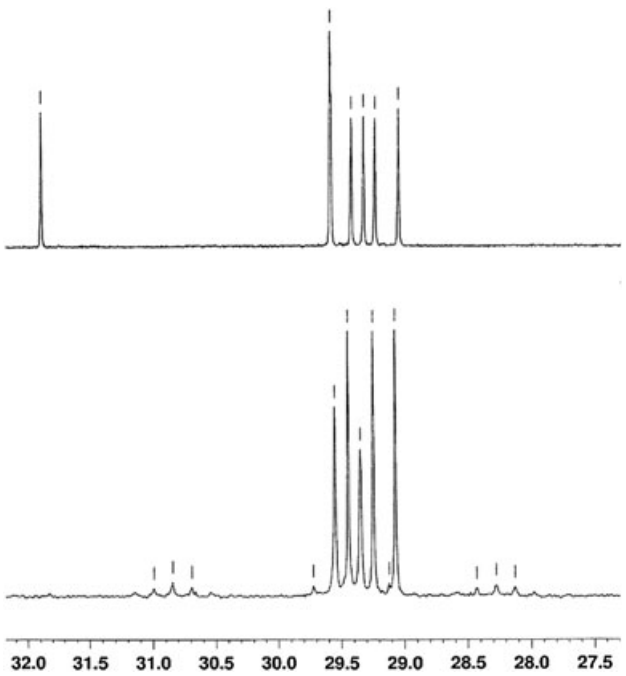


Figure 1. Upfield regions of the ^1H-decoupled ^{13}C NMR spectra of non-labeled dodecanoic acid (top) and 9,9,10,10-tetradeuteriododecanoic acid (bottom).

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*2-(Hept-4-ynyl-1-oxy)tetrahydropyran (**2**)*

THP ether **1** (5.0 g, 0.03 mol) was dissolved in THF (60 mL), and the solution was cooled in an ice bath under a nitrogen atmosphere. BuLi (23 mL, 1.6 M, 0.036 mol) was added slowly by syringe. The mixture was stirred at 0 °C for 15 min. Iodoethane (14 g, 0.09 mol) was added, and the mixture was heated at reflux for 15 h. The reaction mixture was poured into hexane (200 mL), and the resulting solution was filtered through silica. The solvent was removed by rotary evaporation, and the resulting oil was chromatographed on silica (hexane to 20:1 hexanes–ethyl acetate) to give **2** (5.2 g, 90%) as an oil. ^{1}H NMR: δ 1.05 (t, J = 7.5 Hz, 3H), 1.42–1.56 (m, 4H), 1.62–1.82 (m, 4H), 2.10 (m, 2H), 2.20 (m, 2H), 3.44 (m, 2H), 3.74 (m, 1H), 3.80 (m, 1H), 4.54 (m, 1H). ^{13}C NMR: δ 12.3 (CH_2), 14.2 (CH_3), 15.5 (CH_2), 19.4 (CH_2), 25.5 (CH_2), 29.2 (CH_2), 30.6 (CH_2), 62.0 (CH_2), 66.0 (CH_2), 78.6 (C), 81.8 (C), 98.6 (CH). Mass spectrum: *m/z* (intensity), 196 M^+ (0.6), 195 $(M-1)^+$, (4), 167 (15), 85 (100); HRMS (EI), calcd for $C_{12}H_{19}O_2$ $(M-1)^+$: 195.13851; found: 195.14000.

*2-(4,4,5,5,-Tetradeuterioheptyl-1-oxy)tetrahydropyran (**3**)*

Wilkinson's catalyst (0.4 g) was added to dry benzene (40 mL). Nitrogen was passed over the vigorously stirred solution for 10 min. Deuterium gas was passed slowly through the solution for approximately 15 min until the solution turned from dark red to orange. A gas burette was charged with deuterium and attached to the flask. THP ether **2** (2.0 g, 0.01 mol) was added. After 15 h, gas consumption ceased. The reaction mixture was poured into a 10:1 mixture of hexane and ethyl acetate, and the solution was filtered through silica. After solvent removal, the resulting oil was chromatographed on silica (20:1 hexanes–ethyl acetate) to give **3** (2.0 g, 96%) as an oil. ^{1}H NMR: δ 0.84 (t, J = 7.5 Hz, 3H), 1.20–1.35 (m, 4H), 1.45–1.60 (m, 6H), 1.66 (m, 1H), 1.80 (m, 1H), 3.35 (m, 1H), 3.46 (m, 1H), 3.46 (m, 1H), 3.70 (m, 1H), 3.83 (m, 1H), 4.54 (m, 1H). ^{13}C NMR: δ 14.0 (CH_3), 19.6 (CH_2), 22.3 (CH_2), 25.5 (CH_2), 25.9 (CH_2), 28.1 (pentet, J = 19 Hz, CD_2), 29.7 (CH_2), 30.7 (CH_2), 30.7 (pentet, J = 19 Hz, CD_2), 62.2 (CH_2), 67.6 (CH_2), 98.8 (CH). Mass spectrum, *m/z* (intensity), 204 M^+ (0.5), 203 $(M-1)^+$ (2.5), 85 (100); HRMS (EI), calcd for $C_{12}H_{19}D_4O_2$ $(M-1)$: 203.19491; found: 203.19552.

*4,4,5,5-Tetradeuterio-1-heptanol (**4**)*

THP ether **3** (1.8 g, 0.0088 mol) was dissolved in methanol (40 mL), and the mixture was cooled in ice. *p*-Toluenesulfonic acid (0.2 g) was added, and the mixture was stirred for 14 h at room temperature. Ether (100 mL) was added, and the solution was washed with aq $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$ and filtered. The solvent was removed by rotary evaporation to give **4** as a clear oil (0.75 g, 74% yield) that was used without further purification to prepare **5**. ^{1}H NMR: δ 0.87 (t, J = 7.5 Hz, 3H), 1.25–1.35 (m, 4H), 1.56 (m, 2H), 1.63 (s, br, 1H), 3.63 (t, J = 6.5 Hz, 2H). ^{13}C NMR: δ 14.0 (CH_3), 22.3 (CH_2), 25.5 (CH_2), 28.0 (pentet, J = 18 Hz, CD_2), 30.7 (pentet, J = 19 Hz, CD_2), 32.7 (CH_2), 63.0 (CH_2).

4,4,5,5-Tetradeuterio-1-heptyl-p-toluenesulfonate (**5**)

4,4,5,5-Tetradeuterio-1-heptanol (**4**) (0.75 g, 0.006 mol) was dissolved in pyridine (10 mL), and the mixture was cooled in an ice bath to 0°°C. *p*-Toluenesulfonyl chloride (1.5 g, 0.008 mol) was added slowly, and the mixture was held at 0 °C for 48 h. Methylene chloride (100 mL) was added, and the solution was washed with dilute HCl (0.5 M), aq $NaHCO_3$, and brine. The methylene chloride solution was dried over $MgSO_4$ and filtered. Removal of the solvent by rotary evaporation gave the crude tosylate that was chromatographed on silica (10:1 hexanes–ethyl acetate) to give **5** (1.2 g, 71% yield). ^{1}H NMR: δ 0.83 (t, J = 7.4 Hz, 3H), 1.23 (m, 4H), 1.60 (m, 6H), 1.66 (pentet, J = 6.5 Hz, 2H), 2.42 (s, 3H), 4.00 (t, J = 6.5 Hz, 2H), 7.38 (d, J = 8.2 Hz, 2H), 7.76 (d, J = 8.2 Hz, 2H) ^{13}C NMR: δ 13.9 (CH_3), 21.6 (CH_3), 22.2 (CH_2), 25.0 (CH_2), 27.5 (pentet, J = 19 Hz, CD_2), 28.8 (CH_2), 30.5 (pentet, (J = 19 Hz, CD_2), 70.7 (CH_2), 127.9 (CH), 129.8 (CH), 133.3 (C), 144.7 (C). Mass spectrum (TOF, ES^+), *m/z* (intensity), 297 $(M+Na)^+$ (100), 275 $(M-1)^+$ (20), 173 (64) 103 (29). HRMS (TOF, ES^+), calcd for $C_{14}H_{18}D_4SO_3Na$ (M + Na): 297.1438; found: 297.1434.

*2-(9,9,10,10-Tetradeuteriododec-4-ynyl-1-oxy)tetrahydropyran (**6a**)*

THP ether **1** (1.70 g, 0.01 mol) was dissolved in THF (20 mL) under N_2, and the mixture was cooled to 0 °C with an ice bath. BuLi (7.5 mL, 1.6 M in hexane, 0.012 mol) was added, and the mixture was stirred for 15 min. Tosylate **5** (1.1 g, 0.004 mol) and NaI (0.015 g) were added, and the mixture was heated at reflux for 40 h. The reaction mixture was poured into hexane (200 mL) and filtered through a pad of silica gel. The silica was washed with 10:1 hexanes–ethyl acetate. Solvent was removed from the filtrate by rotary evaporation, and the resulting oil was chromatographed on silica (20:1 hexanes–ethyl acetate) to give **6a** as a clear oil (0.96 g, 89%). ^{1}H NMR: δ 0.88 (t, J = 7.5 Hz, 3H), 1.28 (q, J = 7.4 Hz, 2H), 1.36 (m, 2H), 1.45 (m, 2H), 1.48–1.61 (m, 4H), 1.66–1.85 (m, 4H), 2.13 (tt, J = 7.1, 2.3 Hz, 2H), 2.26 (m, 2H), 3.44–3.52 (m, 2H), 3.82 (dt, J = 9.5, 6.4 Hz, 1H), 3.87 (ddd, J = 11.4, 8.2, 2.8 Hz, 1H), 4.60 (t, J = 3.4 Hz, 1H). ^{13}C NMR: δ 14.0 (CH_3), 15.6 (CH_2), 18.7 (CH_2), 19.5 (CH_2), 22.4 (CH_2), 25.5 (CH_2), 27.7 (pentet, J = 19 Hz, CD_2), 28.5 (CH_2), 29.0 (CH_2), 29.3 (CH_2), 30.6 (CH_2), 30.6 (pentet, J = 19 Hz, CD_2), 62.0 (CH_2), 66.0 (CH_2), 79.3 (C), 80.5 (C), 98.7 (CH). Mass spectrum *m/z* (intensity), 270 M^+, (0.3), 167 (0.26), 85 (100).

*2-(9,9,10,10-Tetradeuteriododecyl-1-oxy)tetrahydropyran (**7a**)*

Wilkinson's catalyst (0.2 g) was added to dry benzene (20 mL). Nitrogen was passed over the vigorously stirred solution for 10 min. Hydrogen gas was passed slowly through the solution for approximately 15 min until the solution turned from dark red to orange. A gas burette was charged with hydrogen and attached to the flask. THP ether **6a** (0.80 g, 0.0029 mol) was added. Small aliquots were periodically removed and analyzed by gas chromatography to monitor reaction progress. After 72 h, the reaction mixture was poured into 10:1 hexanes–ethyl acetate, and the solution was filtered through silica. After solvent removal, the resulting oil was chromatographed on silica (20:1 hexanes–ethyl acetate) to give **7a** (75 g, 92%) as an oil. ^{1}H NMR: δ 0.89 (t, J = 7.4 Hz, 3H), 1.20–1.38 (m, 14H), 1.46–1.62 (m, 6H), 1.66–1.74 (m, 1H), 1.76–1.86 (m, 1H), 3.35 (dt, J = 9.5, 6.7 Hz, 1H), 3.46 (m, 1H), 3.71 (dt, J = 9.6, 6.9 Hz, 1H), 3.85 (ddd, J = 11.4, 8.2, 2.7 Hz, 1H), 4.56 (t, J = 3.5 Hz, 1H). ^{13}C NMR: δ 14.0 (CH_3), 19.7 (CH_2), 22.4 (CH_2), 25.5 (CH_2), 26.2 (CH_2), 28.3 (pentet, J = 19 Hz, CD_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.62 (CH_2), 29.8 (CH_2), 30.8 (CH_2), 30.85 (pentet, J = 19 Hz, CD_2), 62.3 (CH_2), 67.7 (CH_2), 98.8 (CH). Mass spectrum: *m/z* (intensity), 274 M^+ (0.3), 273 (1.3), 101 (11), 85 (100); HRMS (EI), calcd for $C_{17}H_{29}D_4O_2$ $(M-1)^+$: 273.27316; found: 273.27309.

*9,9,10,10-Tetradeuteriododecan-1-ol (**8a**)*

THP ether **7a** (0.44 g, 0.0016 mol) was dissolved in methanol (15 mL), and the mixture was cooled in ice. HCl (three drops of conc HCl) was added, and the mixture was stirred for 16 h at room temperature. Methylene chloride (100 mL) was added, and the solution was washed with aq $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$ and filtered. The solvent was removed by rotary evaporation to give **8a** as a clear oil (0.28 g, 90% yield) that was used without further purification to prepare **9a**. ^{1}H NMR: δ 0.86 (t, J = 7.4 Hz, 3H), 1.20–1.38 (m, 14H), 1.55 (m, 3H), 3.61 (t, J = 6.6 Hz, 2H). ^{13}C NMR: δ 14.0 (CH_3), 22.4 (CH_2), 25.8 (CH_2), 28.2 (pentet, J = 19 Hz, CD_2), 29.4 (CH_2), 29.5 (CH_2), 29.61 (CH_2), 29.62 (CH_2), 30.9 (pentet, J = 19 Hz, CD_2), 32.8 CH_2), 62.0 (CH_2). Mass spectrum: *m/z* (intensity), 190 M^+ (0.01), 189 (0.08), 172 (4), 144 (18), 99 (34), 69 (75), 55 (100).

*9,9,10,10-Tetradeuteriododecanoic acid (**9a**)*

Alcohol **8a** (0.28 g, 0.0014 mol) was dissolved in acetone (15 mL), and the solution was cooled to 0 °C. Jones' reagent was added until the red color persisted. The mixture was stirred for 1 h. Water (100 mL) was added, and the solution was extracted with diethyl ether (3 × 100 mL). The organic layer was washed with brine and dried over $MgSO_4$. After filtration, the solvent was removed by rotary evaporation. Chromatography on silica (10:1 hexanes–ethyl acetate) gave a white solid (0.18 g, 59%, m.p. 38–40 °C). ^{1}H NMR: δ 0.87 (t, J = 7.6 Hz, 3H), 1.20–1.40 (m, 12H), 1.63 (pentet, J = 7.4 Hz, 2H), 2.34 (t, J = 7.6 Hz, 2H). ^{13}C NMR: δ 14.0 (CH_3),

22.4 (CH_2), 24.7 (CH_2), 28.3 (pentet, J = 19 Hz, CD_2), 29.1 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 30.8 (pentet J = 19 Hz, CD_2) 33.9 (CH_2), 180.2 (COOH). Mass spectrum: *m/z* (intensity), 204 M^+ (31), 175 (12), 161 (38), 129 (45), 87 (85), 73 (100); HRMS (EI), calcd for $C_{12}H_{20}D_4O_2$ (M^+): 204.20274; found: 204.20161.

*Methyl 9,9,10,10-tetradeuteriododecanoate (**10a**)*

Acid **9a** (0.020 g, 0.0001 mol) was dissolved in diethyl ether (50 mL). An ethereal solution of diazomethane (1 mL) was added, and the resulting yellow solution was allowed to stand at room temperature for 15 h. Solvent removal by rotary evaporation gave an oil (0.021 g, 100%). ^{1}H NMR: δ 0.86 (t, J = 7.4 Hz, 3H), 1.20–1.35 (m, 12H), 1.61 (m, 2H), 2.29 (t, J = 7.5 Hz, 2H), 3.66 (s, 3H). ^{13}C NMR: δ 14.0 (CH_3), 22.4 (CH_2), 25.0 (CH_2), 28.2 (pentet, J = 19 Hz, CD_2), 29.1 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 30.7 (pentet J = 19 Hz, CD_2), 34.1 (CH_2), 51.4 (CH_3), 174.3 (C). Mass spectrum: *m/z* (intensity), 218 M^+ (5.5), 187 (10), 175 (13), 143 (16), 87 (66), 74 (100). HRMS (EI), calcd for $C_{13}H_{22}D_4O_2$ (M^+): 218.21839; found: 218.21930.

*2-(13,13,14,14-Tetradeuteriohexadec-8-ynyl-1-oxy)tetrahydropyran (**6b**)*

THP ether **14b** (1.0 g, 0.0045 mol) was dissolved in THF (15 mL) under N_2, and the mixture was cooled to 0 °C in an ice bath. BuLi (3.3 mL, 1.6 M in hexane, 0.0052 mol) was added, and the mixture was stirred for 30 min. Tosylate **5** (0.95 g, 0.0034 mol) and NaI (0.15 g) were added, and the mixture was heated at reflux for 20 h. The reaction mixture was poured into hexane (200 mL), and the mixture was filtered through a pad of silica gel. The silica gel was washed with 10:1 hexanes–ethyl acetate. Solvent was removed from the filtrate by rotary evaporation, and the resulting oil was chromatographed on silica (5:1 hexanes–ethyl acetate) to give **6b** as a clear oil (1.05 g, 95%). ^{1}H NMR δ 0.84 (t, J = 7.4 Hz, 3H), 1.20–1.38 (m, 10H), 1.40–1.60 (m, 10H), 1.67 (m, 1H), 1.80 (m, 1H), 2.10 (t, J = 5.5 Hz, 2H), 3.33 (m, 1H), 3.46 (m, 1H), 3.69 (m, 1H), 3.83 (m, 1H), 4.54 (br s, 1H). ^{13}C NMR δ 13.9 (CH_3), 18.62 (CH_2), 18.64 (CH_2), 19.5 (CH_2), 22.2 (CH_2), 25.4 (CH_2), 26.1 (CH_2), 27.7 (pentet, J = 19 Hz, CD_2), 28.3 (CH_2), 28.6 (CH_2), 28.9 (CH_2), 29.02 (CH_2), 29.05 (CH_2), 29.6 (CH_2), 30.7 (CH_2), 30.7 (pentet, J = 19 Hz, CD_2), 61.9 (CH_2), 67.4 (CH_2), 79.9 (C), 80.0 (C), 98.6 (CH). Mass spectrum: *m/z* (intensity), 326 M^+ (0.006), 325 (.006), 101(15), 85(100). HRMS (EI), calcd for $C_{21}H_{34}D_4O_2$ (M^+): 326.31229; found: 326.31143.

*2-(13,13,14,14-Tetradeuteriohexadecyl-1-oxy)tetrahydropyran (**7b**)*

Wilkinson's catalyst (0.18 g) was added to dry benzene (20 mL). Nitrogen was passed over the vigorously stirred solution for 10 min. Hydrogen gas was passed slowly through the solution for approximately 15 min until the solution turned from dark red to orange. A gas buret was charged with hydrogen and attached to the flask. THP ether **6b** (0.75 g, 0.0023 mol) was added. Small aliquots were periodically removed and analyzed by GC to monitor reaction progress. After 15 h, the reaction mixture was poured into 10:1 hexane–ethyl acetate, and the solution was filtered through silica gel. After solvent removal, the resulting oil was chromatographed on silica gel (20:1 hexanes–ethyl acetate) to give **7b** (0.65 g, 86%) as an oil. ^{1}H NMR δ 0.85 (t, J = 7.4 Hz, 3H), 1.20–1.36 (m, 22H), 1.45–1.60 (m, 6H), 1.68 (m, 1H), 1.81 (m, 1H), 3.34 (dt, J = 9.5, 6.7 Hz, 1H), 3.46 (m, 1H), 3.71 (dt, J = 9.5, 6.9 Hz, 1H), 3.85 (m, 1H), 4.55 (t, J = 3.5 Hz, 1H). ^{13}C NMR: δ 14.0 (CH_3), 19.7 (CH_2), 22.4 (CH_2), 25.8 (CH_2), 26.3 (CH_2), 28.3 (pentet, J = 19 Hz, CD_2), 29.4 (CH_2), 29.5 (CH_2), 29.61 (CH_2), 29.62 (CH_2), 29.67 (CH_2), 29.68 (CH_2), 29.70 (CH_2), 29.8 (CH_2), 30.7 (CH_2), 30.8 (pentet, J = 19 Hz, CD_2), 62.2 (CH_2), 67.6 (CH_2), 98.8 (CH). Mass spectrum: *m/z* (intensity), 330 M^+ (0.1), 329 (0.4), 257 (0.5), 115 (3); 101(8), 85 (100). HRMS (EI), calcd for $C_{21}H_{38}D_4O_2$ (M^+), 330.34359; found: 330.34256.

*13,13,14,14-Tetradeuteriohexadecan-1-ol (**8b**)*

THP ether **7b** (0.53 g, 0.0016 mol) was dissolved in methanol (30 mL), and the mixture was cooled in an ice bath. *p*-Toluenesulfonic acid (0.1 g) was added, and the mixture was stirred for 16 h at room temperature. Methylene chloride (200 mL) was added, and the solution was washed with ($NaHCO_3$, aq) and brine. The organic layer was dried over $MgSO_4$ and filtered. The solvent was removed by rotary evaporation to give **8b** as a clear oil (0.37 g, 92% yield) that was used without further purification to prepare **9b**. ^{1}H NMR: δ 0.87 (t, J = 7.4 Hz, 3H), 1.20–1.38 (m, 22H), 1.56 (pentet, J = 7.4 Hz, 3H), 3.63 (t, J = 6.6 Hz, 2H). ^{13}C NMR: δ 14.1 (CH_3), 22.4 (CH_2), 25.7 (CH_2), 28.2 (pentet, J = 19 Hz, CD_2), 29.4 (CH_2), 29.61 (CH_2), 29.62 (CH_2), 29.67 (CH_2), 29.69 (CH_2), 29.70 (CH_2), 29.71 (CH_2), 30.9 (pentet, J = 19 Hz, CD_2), 32.8 CH_2), 63.1 (CH_2). Mass spectrum: *m/z* (intensity), 245 $(M-1)^+$ (3.6), 228 (27), 200 (20), 111 (100). HRMS (EI), calcd for $C_{16}H_{34}D_4O$ (M − 1): 245.27825; found: 245.27759.

*13,13,14,14-Tetradeuteriohexadecanoic acid (**9b**)*

Alcohol **8b** (0.24 g, 0.001 mol) was dissolved in anhydrous DMF (6 mL). Pyridinium dichromate (1.9 g, 0.005 mol) was added, and the mixture was stirred under N_2 for 3 days. The reaction mixture was poured into water (50 mL), and the mixture was extracted with hexane (3 × 40 mL). The combined hexane layer was washed with brine and dried over $MgSO_4$. After filtration, solvent was removed by rotary evaporation. The resulting solid was chromatographed on silica gel (10:1 hexanes–ethyl acetate) to give a white solid (0.14 g, 54%, m.p. 60–61 °C). ^{1}H NMR: δ 0.87 (t, J = 7.6 Hz, 3H), 1.20–1.40 (m, 20H), 1.61 (pentet, J = 7.2 Hz, 2H), 2.34 (t, J = 7.5 Hz, 2H). ^{13}C NMR: δ 14.0 (CH_3), 22.5 (CH_2), 24.7 (CH_2), 28.3 (pentet, J = 19 Hz, CD_2), 29.1 (CH_2), 29.3 (CH_2), 29.41 (CH_2), 29.44 (CH_2), 29.60 (CH_2), 29.65 (CH2), 29.68(CH_2), 29.70 (CH_2) 30.9 (pentet J = 19 Hz, CD_2), 34.1 (CH_2), 180.5 (COOH). HRMS (EI), calcd for $C_{16}H_{28}D_4O_2$ (M^+): 260.26534; found: 260.26617.

*Methyl 13,13,14,14-tetradeuteriohexadecanoate (**10b**)*

Acid **9b** (0.025 g, 0.0001 mol) was dissolved in diethyl ether (10 mL). An ethereal solution of diazomethane was added, and the resulting yellow solution was allowed to stand at room temperature for 2 h. Solvent removal by rotary evaporation gave an oil (0.023 g, 88%). ^{1}H NMR: δ 0.87 (t, J = 7.4 Hz, 3H), 1.27 (m, 20H), 1.61 (pentet, J = 7.1 Hz, 2H), 2.30 (t, J = 7.5 Hz, 2H), 3.66 (s, 3H). ^{13}C NMR: δ 14.1 (CH_3), 22.4 (CH_2), 25.0 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.65 (CH_2), 29.68 (CH_2), 29.7 (CH_2), 34.1 (CH_2), 51.4 (CH_3), 174.3 (C). Mass spectrum: *m/z* (intensity), 274 M^+ (100), 231 (31), 143 (56). HRMS (EI), calcd for $C_{17}H_{30}D_4O_2$ (M^+): 274.28099; found: 274.28197.

*2-(Non-2-ynyl-1-oxy)tetrahydropyran (**12**)*

THP ether **11** (7.0 g, 0.05 mol) was dissolved in THF (50 mL), and the mixture was cooled in an ice bath under a nitrogen atmosphere. BuLi (37 mL, 1.6 M, 0.06 mol) was added slowly by syringe. 1-Iodohexane (15.5 g, 0.07 mol) was added, and the mixture was heated at reflux for 15 h. The reaction mixture was poured into hexane (200 mL), and the resulting solution was filtered through silica gel. The solvent was removed by rotary evaporation, and the resulting oil was chromatographed on silica gel (hexane to 10:1 hexanes–ethyl acetate) to give **12** (8.1 g, 72%) as an oil. ^{1}H NMR: δ 0.84 (m, 3H), 1.42–1.56 (m, 4H), 1.24 (m, 4H), 1.34 (m, 2H), 1.48 (m, 4H), 1.58 (m, 2H), 1.68 (m, 1H), 1.80 (m, 1H), 2.17 (m, 2H), 3.48 (m, 1H), 3.80 (m, 1H), 4.17 (m, 1H), 4.25 (m, 1H), 4.78 (m, 1H). ^{13}C NMR: δ 14.0 (CH_3), 18.8 (CH_2), 19.1 (CH_2), 22.5 (CH_2), 25.4 (CH_2), 28.5 (CH_2), 28.6 (CH_2), 30.3 (CH_2), 31.3 (CH_2), 54.6 (CH_2), 61.9 (CH_2), 75.2 (C), 86.6 (C), 96.6 (CH).

*2-Nonyn-1-ol (**13**)*

THP ether **12** (4.5 g, 0.02 mol) was dissolved in methanol (50 mL), and the mixture was cooled in an ice bath. *p*-Toluenesulfonic acid (0.5 g) was added, and the mixture was stirred for 16 h at room temperature. Water (200 mL) was added, and the mixture was extracted with methylene chloride. The organic extract was washed with brine and dried over $MgSO_4$. Solvent was removed by rotary evaporation, and the resulting oil was chromatographed on silica gel (10:1 hexanes–ethyl acetate) to give **13** as a clear oil (2.2 g, 79%). ^{1}H NMR: δ 0.83 (t, J = 7.2 Hz, 3H), 1.16–1.27 (m, 4H), 1.28–1.35 (m, 2H), 1.43 (pentet, J = 7.3 Hz, 2H), 2.14 (t, J = 7.1 Hz, 2H), 2.81 (t, J = 5.3 Hz, 1H), 4.17 (m, 2H). ^{13}C NMR:

δ 14.0 (CH_3), 18.7 (CH_2), 22.5 (CH_2), 28.5 (CH_2), 28.6 (CH_2), 31.3 (CH_2), 51.0 (CH_2), 78.3 (C), 86.6 (C), 86.2 (C). HRMS (EI), calcd for $C_9H_{15}O$ $(M-1)^+$: 139.11230; found: 139.11295.

*8-Nonyn-1-ol (**14a**)*

Alcohol **14a** was prepared from **13** by the method of Hopf and Kruger.[10] Lithium wire (0.5 g) was washed in hexane and cut into small pieces that were placed in a 200-mL, 3-neck flask under N_2. 1,3-diaminopropane (30 mL) was added, and the mixture was stirred at room temperature for approximately 1 h to give a dark blue solution. The mixture was heated at 55–60 °C overnight. To the cooled mixture was added potassium *tert*-butoxide (4.4 g). After stirring for 20 min, 2-nonyn-1-ol (**13**, 1.4 g, 0.01 mol) was added, and mixture was stirred for 1.5 h. The mixture was extracted with hexane, and the hexane layer was washed with dilute HCl solution and dried over $MgSO_4$. Removal of hexane *in vacuo* gave 0.95 g (6.6 mmol, 66%) of **14a** as a light yellow oil that was used without further purification. The ^1H and ^{13}C NMR spectra were in agreement with the reported data.[10]

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Conflict of Interest

The authors did not report any conflict of interest.

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