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Research Article

Preparation of deuterium-labeled monounsaturated and saturated fatty acids for use as stable isotope metabolic tracers

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Summary
Synthetic routes adaptable to the preparation of a variety of d_7-labeled monounsaturated and saturated fatty acids are reported. Using these reaction sequences, d_7-oleic [18:1 (9c)], d_7-elaidic [18:1 (9t)], and d_7-stearic acid (18:0) were prepared for use in metabolism studies as stable isotope-labeled tracers. Copyright © 2006 John Wiley & Sons, Ltd.

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Key Words: fatty acid; metabolic syndrome; stable isotope; deuterium label

Introduction
The hallmarks of metabolic syndrome—blood dyslipidaemia, elevated blood pressure and insulin resistance/glucose intolerance, are an interrelated set of risk factors that contribute to the development of cardiovascular diseases and non-insulin dependent diabetes.[1a,b,c] It has been projected that obesity-induced metabolic syndrome and its associated pathologies will reach epidemic proportions in industrialized nations within the next several decades.[1a,b,c] The development of therapeutic agents for the treatment of the dyslipidaemia associated with metabolic syndrome will require delineation of the poorly understood molecular mechanisms that underlie its origin.[1d,e] Unfortunately, acute monitoring of the effects of obesity on lipid metabolism is complicated

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by interference from the endogenous lipid pool. This issue can be obviated by the use of radiolabeled tracers,[2a] however, stable isotopes are more attractive from handling and safety standpoints.[2b] We have recently reported a high-throughput LC-MS method for the quantitative analysis of deuterium labeled fatty acids in biological samples.[3] The requirement for a minimum incorporation of seven deuterium (d_7) atoms in these tracers arose out of the necessity to optimally discriminate the tracer from naturally occurring fatty acids present in biological samples. In order to support our studies in the area of metabolic syndrome-associated dyslipidaemia, we required access to a variety of d_7-labeled saturated and monounsaturated fatty acids for use as standards in an array of *in vivo* and *ex vivo* experiments. Herein, we report the synthetic routes that were developed to provide us with our requisite molecular tool kit.

Synthesis of d_7-labeled monounsaturated and saturated fatty acids

Our plan was to develop a flexible strategy whereby we could vary the chain length of a late stage alkyne intermediate that could subsequently be transformed into the corresponding series of saturated and monounsaturated (*cis*- and *trans*-) fatty acids. We also wished to position the deuterium labels as far as possible from the carboxylic acid functionality in order to monitor β-oxidation along the entire length of the hydrocarbon chain. In order to meet these requirements, we chose to assemble the fatty acid precursor from three components as illustrated in Figure 1. The adaptable sequence of chemical transformations that was developed to prepare both saturated and unsaturated d_7-labeled fatty acids is shown in Scheme 1. Scheme 2 details a similar reaction sequence that was designed to rapidly access saturated d_7-fatty acids. C_{18} fatty acids were prepared as illustrative examples in both instances.

Scheme 1 starts with a Wittig reaction of phosphonium salt **1** with d_7-butyraldehyde (**2**), prepared via PCC oxidation of commercially available d_7-butanol, to give olefin **3** (Z/E 11:1). Reduction of the double bond in **3** was accomplished by exposure to hydrogen (40 PSI) in the presence of Wilkinson's catalyst.[4a,†] Removal of the silyl ether protecting group in **4** with TBAF was followed by conversion of the alcohol **5** to the corresponding bromide **6** with HBr, and finally to iodide **7** under typical Finkelstein conditions. With the deuterium-labeled terminal C_{11}–C_{18} fragment of our fatty acid targets in hand, we then turned our attention to the preparation of the C_1–C_{10} fragment. Exposure of commercially available 2-decyn-1-ol to NaH in 1,3-diaminopro-

[†] Freshly prepared catalyst was used in this reaction in order to minimize the presence of rhodium oxides that are generated from Wilkinson's catalyst that has been exposed to atmospheric oxygen. These rhodium oxide species are capable of scrambling the deuterium labels in the final product as a consequence of competitive double bond isomerization during the hydrogenation reaction. For an in-depth discussion of this phenomenon. See references 4b and 4c.

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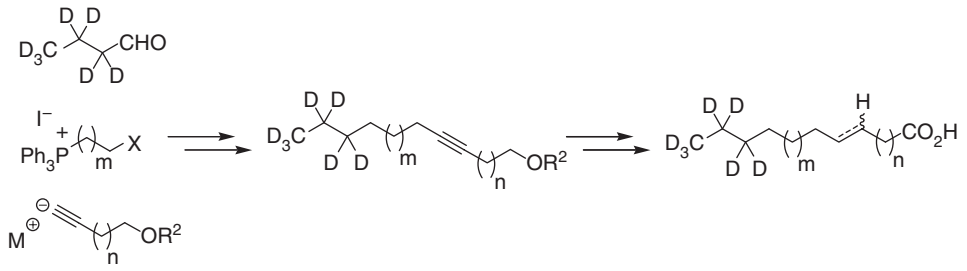
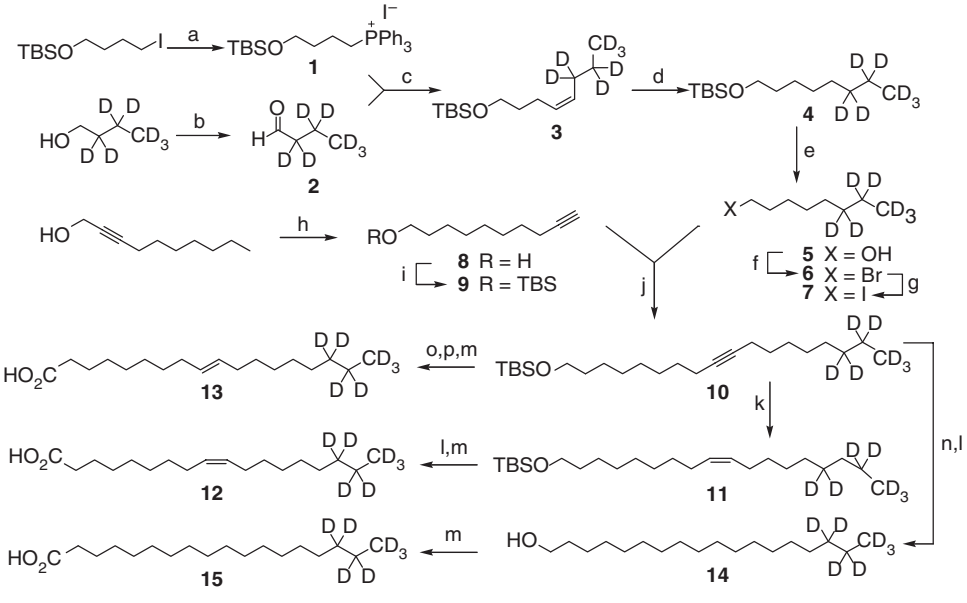


Figure 1. Strategy for the preparation of a variety of d_7-labeled saturated and monounsaturated fatty acids



Scheme 1. Conditions : (a) PPh_3, CH_3CN, reflux, > 99%; (b) PCC, CH_2Cl_2; (c) NaHMDS, THF, −78°C, 65%; (d) 40 PSI H_2, $(PPh_3)_3RhCl$, PhH, > 99%; (e) TBAF, THF, 94%; (f) 48% HBr, conc H_2SO_4, 100°C, 89%; (g) NaI, acetone, reflux, 89%; (h) 1,3-diaminopropane, NaH, 70°C, 88%; (i) TBSCI, DMAP, Et_3N, CH_2Cl_2, 75%; (j) *n*-BuLi, THF, hexanes, HMPA, –78°–0°C, 86% for X = I; (k) 1 atm H_2, Lindlar catalyst, quinoline, hexanes, > 99%; (l) TBAF, THF, 97%; (m) Jones oxidation (71% for 12, 65% for 13; 93% for 15); (n) 40 PSI H_2, 10% Pd/C, EtOAc, > 99%; (o) TBAF, THF, 78%; and (p) $LiAlH_4$, diglyme, 160°C, 90%

pane promoted an alkyne zipper reaction that resulted in isomerization of the internal alkyne to the terminal position to give **8**.[5] Protection of the alcohol in **8** as a TBS-ether in **9** was followed by removal of the acetylenic hydrogen with *n*-BuLi to generate the corresponding lithium acetylide, that was coupled to

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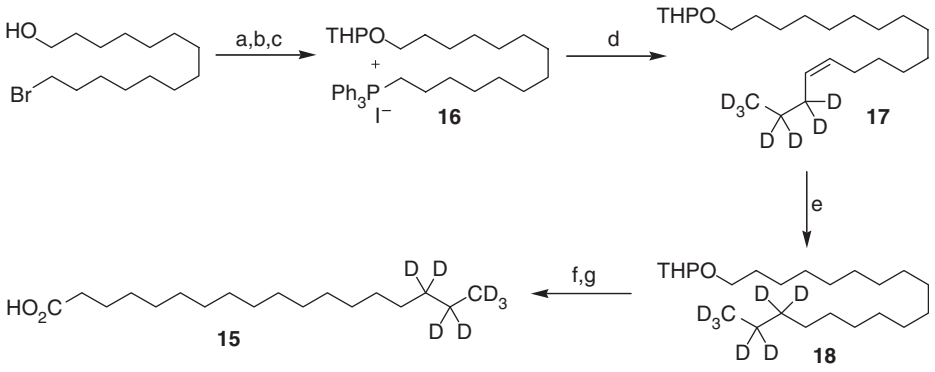

Scheme 2. Conditions: (a) dihydropyran, TsOH, CH_2Cl_2, 89%; (b) NaI, acetone, reflux, > 99%; (c) PPh_3, CH_3CN, reflux, > 99%; (d) NaHMDS, THF, 2, –78°–0°C, 78%; (e) 40 psi H_2, $(PPh_3)_3$ RhCl, PhH, 90%; (f) MeOH, TsOH, 50°C, 90%; and (g) Jones oxidation, 97%

iodide **7** to give **10** in 86% yield.[6] The coupling reaction could also be conducted with the corresponding bromide **6**, albeit in a more modest yield (59%). In the latter case, the product (**10**) was not easily separated from unreacted **9** using conventional silica gel chromatography. Key intermediate **10** could then be converted into the corresponding *cis*-, *trans*-, or saturated fatty acid depending on the reaction conditions chosen for reduction of the triple bond. Lindlar reduction of the alkyne in **10**, followed by removal of the TBS protecting group, and Jones oxidation afforded d_7-oleic acid (**12**). Liberation of the alcohol in **10** with TBAF, followed by conversion of the alkyne to the *trans*-alkene with $LiAlH_4$ in refluxing glyme,[7] and subsequent Jones oxidation, gave d_7-elaidic acid (**13**). Reduction of **10** with 40 PSI of hydrogen in the presence of Pd/C, followed by the same deprotection/oxidation sequence yielded d_7-stearic acid (**15**).

A more efficient approach leading to d_7-labeled saturated fatty acids, such as d_7-stearic acid (**15**), is detailed in Scheme 2. Essentially, the sequence of events in this instance is identical to the first phase of Scheme 1 leading to intermediate **4**. Use of a THP instead of a TBS protecting group allowed us to minimize the number of chromatographic purifications, and more readily access multi-gram quantities of d_7-stearic acid (**14**).

Conclusion

A synthetic route for the preparation of a diverse array of physiologically relevant monounsaturated and fully saturated d_7-labeled fatty acids from a common alkyne intermediate has been developed. A conceptually related chemical sequence was also devised to rapidly access d_7-labeled, saturated fatty acids. We plan to use these stable isotope-labeled compounds as tools to study

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lipid metabolism in animal models of obesity, diabetes and atherosclerosis. The results of these investigations will be reported in due course.

Experimental section

General

Unless specified otherwise, all reagents were obtained commercially and used without further purification. Jones reagent [2.7 M] was prepared by carefully adding 18 M H_2SO_4 (23 ml) to a solution of chromic acid (26.7 g, 267 mmol) in water (40 ml), and adjusting the final volume to 100 ml in a volumetric flask with water. *n*-Butyl-2,2,3,3,4,4,4-d_7-alcohol was purchased from CDN Isotopes (Pointe Claire, Quebec, Canada). Proton (^{1}H NMR) and carbon (^{13}C NMR) magnetic resonance spectra were recorded on a Bruker instrument at the indicated frequency. Spectra were recorded in $CDCl_3$, unless specified otherwise, using residual solvent as internal standard. Signal multiplicity was designated according to the following abbreviations: s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublet of doublets, t = triplet, q = quartet, dt = doublet of triplets, td = triplet of doublets, dq = doublet of quartets, m = multiplet, br = broad. High-resolution mass spectra were recorded on a Micromass QTof Premiere from Waters (Manchester, England) coupled to a Waters Acquity UPLC. Reactions were carried out with continuous stirring under a positive pressure of nitrogen except where noted. Flash chromatography was performed with silica gel 60, 230–400 mesh.

*(4-{tert-Butyl(dimethyl)silyl}oxy)butyl)(triphenyl)phosphonium iodide (**1**)*

A solution of *tert*-butyl(4-iodobutoxy)dimethylsilane (25.5 g, 81.2 mmol) and triphenylphosphine (21.3 g, 81.3 mmol) were heated together in dry acetonitrile (100 ml) at 85°C for 24 h. The mixture was evaporated, and the residue was triturated with anhydrous ether to afford the title compound as a colorless powder (44.2 g, 95%). ^{1}H NMR (CD_2Cl_2, 400 MHz) δ 7.93–7.73 (15 H, m), 3.71 (2 H, t, J = 5.2 Hz), 3.57–3.45 (2 H, m), 1.87–1.80 (4 H, m), 0.84 (9 H, s), 0.03 (6 H, s).

*2,2,3,3,4,4,4-d_7-Butyraldehyde, 20 wt% in CH_2Cl_2 (**2**)*

A solution of *n*-butyl-2,2,3,3,4,4,4-d_7-alcohol (21.80 g, 270 mmol) in CH_2Cl_2 (20 ml) was added dropwise at RT over 10 min to a slurry of PCC (73 g, 340 mmol, 1.25 eq.) and Celite (20 g) in CH_2Cl_2 (500 ml). The reaction mixture was then stirred at RT (mild spontaneous reflux controlled with a condenser) for 3 h prior to removal of the supernatant solution by decantation, and passage through a silica gel plug (6 × 9 cm) that had been prepacked using CH_2Cl_2. The plug was then washed with additional CH_2Cl_2 (125 ml), and the eluate was concentrated by fractional distillation under nitrogen through a

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12×0.75 inch Vigreux column using an oil bath (max temperature 60°C). The residue in the distillation flask (40.44 g, 3.7:1 CH_2Cl_2 : aldehyde by ^1H NMR, 38% yield) was used without further purification in subsequent reactions. ^1H NMR (acetone-d_6, 500 MHz) δ 9.74 (1 H, s), 5.63 (s, residual CH_2Cl_2).

*6,6,7,7,8,8,8-d$_7$-tert-Butyl(dimethyl)[(4Z)-oct-4-en-1-yloxy]silane (**3**)*

A solution of NaHMDS (1 M in THF, 22 ml) was added to a 0°C solution of **1** (12.67 g, 22.0 mmol) in THF (30 ml). The resulting orange solution was stirred at this temperature for 25 min prior to cooling to −78°C, and rapid addition of the 20 wt % solution of **2** (8.23 g, 21 mmol). After 20 min at −78°C, the reaction was quenched by the addition of 1 M pH 7 phosphate buffer (0.20 ml, 1:1 K_3PO_4/KH_2PO_4) followed by warming to RT. Solvents were removed by rotary evaporation, and the residue was stirred with 1:1 hexanes/H_2O. The solid triphenylphosphine oxide by product was removed by suction filtration, and the organic phase of the filtrate was separated, washed with water, and dried over Na_2SO_4. Concentration *in vacuo* and flash chromatography on silica gel eluting with 5/95 ether/hexanes afforded the title compound ($R_f = 0.50$; 1/99 ether/hexanes) as a colorless oil (3.35 g, 65%, *Z*:*E* 11:1). ^1H NMR (400 MHz) δ 5.40 (2 H, m), 3.63 (2 H, t, $J = 6.5$ Hz), 2.12–2.05 (2 H, m), 1.63–1.45 (2 H, m), 0.92 (9 H, s), 0.07 (6 H, s); discernable signals for the *E*-isomer: δ 5.42 (2 H, m).

Preparation of Wilkinson's catalyst ($(Ph_3P)_3RhCl$)[8]

$RhCl_3 \cdot 3H_2O$ (1.50 g, 5.70 mmol) and PPh_3 (9.00 g, 34.0 mmol, freshly recrystallized from ethanol) were heated together under a nitrogen atmosphere in nitrogen saturated (dispersion tube, 30 min) 95% ethanol (220 ml) for 2 h. The reaction mixture was then cooled to 40°C, and the solid was collected by suction filtration (glass frit) under a nitrogen atmosphere. The solid was washed in succession with nitrogen saturated absolute ethanol ($2\times$) and nitrogen saturated anhydrous ether ($3\times$), and was dried under a stream of nitrogen, then under hi-vacuum to yield a maroon powder (4.77 g, 90%).

*6,6,7,7,8,8,8-d$_7$-tert-Butyl(dimethyl)(octyloxy)silane (**4**)*

Wilkinson's catalyst (380 mg, 3 mol %) was suspended in nitrogen saturated benzene (70 ml) under a nitrogen atmosphere in a rubber septum capped Parr flask. Compound **3** (3.35 g, 13.5 mmol) was then added as a solution in nitrogen saturated benzene (10 ml), and the mixture was shaken under an atmosphere of hydrogen gas (40 PSI) for 16 h. Following confirmation of the completion of the reaction by ^1H NMR, the benzene was evaporated and the residue was purified by flash chromatography on silica gel eluting with 5/95 ether/hexanes to yield the title compound ($R_f = 0.78$; 2/98 ether/hexanes) as a

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colorless oil (3.38 g, >99%). ^{1}H NMR (400 MHz) δ 3.62 (2H, t, J = 6.6 Hz), 1.55–1.49 (2H, m), 1.34–1.26 (6H, m), 0.92 (9H, s), 0.07 (6H, s).

6,6,7,7,8,8,8-d_7-Octan-1-ol (**5**)

TBAF (20 ml, 1 M in THF) was added to a solution of **4** (3.38 g, 13.5 mmol) in THF (40 ml) with stirring at RT for 2 h. The THF was then evaporated, and the residue was taken up in ether, and washed with water and brine. The organic phase was dried over Na_2SO_4/$MgSO_4$, and filtered. Concentration of the filtrate, and flash chromatography of the residue on silica gel eluting with 1/3 ethyl acetate/hexanes gave the title compound (R_f = 0.42; 1/4 ethyl acetate/hexanes) as a colorless oil (1.73 g, 94%). ^{1}H NMR (400 MHz, acetone-d_6) δ 3.53 (2H, q, J = 6.2 Hz), 3.38 (1H, t, J = 5.3 Hz), 1.59–1.46 (2H, m), 1.41–1.27 (6H, m).

6,6,7,7,8,8,8-d_7-1-Bromooctane (**6**)

A mixture of **5** (1.73 g, 12.6 mmol), 48% HBr (16 g) and conc H_2SO_4 (4 g) were heated together with rapid stirring at 100°C for 1.5 h. The mixture was then cooled to RT, poured onto crushed ice and extracted with hexanes (2 ×). The organic phase was washed with water and saturated $NaHCO_3$ solution, and dried over Na_2SO_4/$MgSO_4$. Rotary evaporation of the solvents afforded a faint-yellow oil (2.23 g, 89%) that was used directly in the next step. R_f = 0.86 (1/3 ethyl acetate/hexanes); ^{1}H NMR (400 MHz) δ 3.21 (2H, t, J = 7.0 Hz), 1.83 (2H, quintet, J = 6.9 Hz), 1.46–1.36 (2H, m), 1.36–1.23 (4H, m).

6,6,7,7,8,8,8-d_7-1-Iodooctane (**7**)

Compound **6** (1.05 g, 5.25 mmol) was heated with NaI (1.23 g, 8.20 mmol) in refluxing acetone (15 ml) for 2 h. The mixture was then poured into water and extracted with hexanes (2 ×). The combined extracts were washed with water, dried over Na_2SO_4/$MgSO_4$, and concentrated. Flash chromatography on silica gel eluting with hexanes provided the title compound (R_f = 0.69) as a colorless oil (1.15 g, 89%). ^{1}H NMR (400 MHz) δ 3.21 (2 H, t, J = 7.0 Hz), 1.84 (2H, quintet, J = 7.0 Hz), 1.45–1.36 (2H, m), 1.36–1.23 (4H, m).

Dec-9-yn-1-ol (**8**)

In a 250 ml round bottom flask fitted with a reflux condenser, sodium hydride (6.44 g, 60 wt% in mineral oil, 160 mmol, washed free of the mineral oil with hexanes (3 × 20 ml)) was suspended in 1,3-diaminopropane (150 ml, distilled from barium oxide). The mixture was then heated carefully, to control foaming, at 55°C for 1 h. The resulting clear, brown solution was cooled to RT, and 2-decyn-1-ol (3.14 g, 20 mmol) was added dropwise followed by stirring at 55°C. After 18 h at 55°C, the reaction vessel contents were cooled to

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RT, poured into ice water, and extracted with ether (3 ×). The combined extracts were washed with 0.5 M HCl and brine, and then were dried over $Na_2SO_4/MgSO_4$. Concentration *in vacuo* afforded the title compound as a yellow oil (2.75 g, 88%) that was used directly in the next step. $R_f = 0.45$ (3/7 ethyl acetate/hexanes); ^{1}H NMR (500 MHz, acetone-d_6) δ 3.54 (2H, q, $J = 6.1$ Hz), 3.40 (1H, t, $J = 5.0$ Hz, OH), 2.32 (1H, t, $J = 2.7$ Hz), 2.19 (2H, td, $J = 7.0$, 2.5 Hz), 1.55–1.49 (4H, m), 1.34–1.28 (8H, m).

tert-Butyl(dimethyl)(dec-9-yn-1-yloxy)silane (**9**)

A solution of **8** (2.75 g, 17.9 mmol), *tert*-butyldimethylsilyl chloride (2.98 g, 19.9 mmol) and DMAP (0.20 g, 1.6 mmol) in CH_2Cl_2 (50 ml) was treated with triethylamine (3.0 ml, 22 mmol), and stirred at RT for 16 h. The mixture was then poured into water, and the layers were separated. The aqueous phase was extracted with additional CH_2Cl_2, and the combined organics were washed with water, dried over Na_2SO_4, and concentrated. Flash chromatography on silica gel eluting with 4/96 ether/hexanes provided the title compound ($R_f = 0.54$; 2/98 ether/hexanes) as a faint-yellow oil (3.54 g, 75%). ^{1}H NMR (500 MHz, acetone-d_6) δ 3.65 (2H, t, $J = 6.4$ Hz), 2.32 (1H, t, $J = 2.7$ Hz), 2.19 (2H, td, $J = 6.9$, 2.6 Hz), 1.55–1.49 (4H, m), 1.45–1.31 (8H, m), 0.92 (9H, d, $J = 2.7$ Hz), 0.07 (6H, s).

16,16,17,17,18,18,18-d$_7$-tert-Butyl(octadec-9-yn-1-yloxy)dimethylsilane (**10**)

A solution of **9** (574 mg, 2.14 mmol) in THF (3.0 ml) was treated dropwise at −78°C with *n*-BuLi (2.5 M in hexanes, 0.94 ml, 1.1 equiv). The resulting yellow solution was stirred at −78°C for 1 h prior to warming to 0°C. Compound **7** (580 mg, 2.35 mmol) was then added dropwise as a solution in HMPA (1.2 ml). After an additional 10 min of stirring at 0°C, 1 M pH 7 phosphate buffer (1:1 KH_2PO_4:K_3PO_4) was added to quench the reaction. The mixture was extracted with hexanes (2 ×), and the combined organics were washed with water, dried over $Na_2SO_4/MgSO_4$ and concentrated. Flash chromatography of the residue on silica gel eluting with 1/99 ether/hexanes yielded the title compound ($R_f = 0.36$) as a colorless oil (709 mg, 86%). ^{1}H NMR (400 MHz) δ 3.62 (2H, t, $J = 6.6$ Hz), 2.16 (4H, t, $J = 6.9$ Hz), 1.56–1.46 (6H, m), 1.38–1.28 (14H, m), 0.92 (9H, s), 0.07 (6H, s).

16,16,17,17,18,18,18-d$_7$-Oleic acid (**12**)

A mixture of **10** (150 mg, 390 μmol), Lindlar catalyst (5% Pd on $CaCO_3$ poisoned with lead, 40 mg) and quinoline (0.03 ml) in hexanes (8 ml) was stirred under an atmosphere of hydrogen (balloon) until the reaction was judged complete (4 h) by TLC analysis. The mixture was then filtered through Celite, and concentrated. Purification of the residue by flash chromatography

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on silica gel eluting with 1/99 ether/hexanes afforded 16,16,17,17,18,18,18-d_7-*tert*-butyl[(9*Z*)-octadec-9-en-1-yloxy]dimethylsilane (**11**) as a colorless oil (150 mg, 99%); R_f 0.64 (2/98 ether/hexanes); ^{1}H NMR (400 MHz) δ 5.37 (2H, m), 3.62 (2H, t, $J = 6.6$ Hz), 2.09–1.99 (4H, m), 1.58–1.49 (4H, m), 1.43–1.23 (16H, m), 0.92 (9H, s), 0.07 (6H, s). A solution of this compound (150 mg, 400 μmol) in THF (2 ml) was treated with TBAF (1 M in THF, 2.5 ml) with stirring at RT for 3 h. The mixture was then partitioned between ether and water, and the layers were separated. The aqueous phase was extracted with additional ether, and the combined extracts were washed with brine, before drying over Na_2SO_4/$MgSO_4$. Concentration of the filtrate, and flash chromatography on silica gel eluting with 1/4 ethyl acetate/hexanes gave 16,16,17,17,18,18,18-d_7-(9*Z*)-octadec-9-en-1-ol as a colorless oil (107 mg, 97%); R_f 0.47 (1/3 ethyl acetate/hexanes); ^{1}H NMR (400 MHz) δ 5.42–5.36 (2H, m), 3.73–3.61 (2H, m), 2.10–1.98 (4H, m), 1.61–1.55 (4H, m), 1.40–1.24 (16H, m). A solution of this alcohol (100 mg, 360 μmol) in acetone was cooled to 0°C, and treated with Jones reagent (2.7 M, 0.29 ml, 800 μmol) with stirring at this temperature for 20 min. Water was added, and the mixture was extracted with ether (2 ×). The combined extracts were washed with water (2 ×) and brine, and then were dried over Na_2SO_4/$MgSO_4$. The solvents were evaporated, and the residue was purified by flash chromatography on silica gel (1/4 ethyl acetate/hexanes containing 0.5% acetic acid) to yield the title compound as a colorless oil (75 mg, 71%); R_f 0.41; ^{1}H NMR (400 MHz) δ 5.41–5.33 (2H, m), 2.37 (2H, t, $J = 7.5$ Hz), 2.03 (4H, m), 1.68 (2H, m), 1.42–1.23 (16H, m); ^{13}C NMR (100 MHz) δ 180.5, 130.0, 129.7, 34.1, 30.7 (quintet, $J = 19$ Hz), 29.8, 29.7, 29.5, 29.3, 29.14, 29.07 (2C), 29.04, 27.22, 27.16, 24.7, 21.4 (quintet, $J = 19$ Hz), 12.9 (septet, $J = 19$ Hz); MS (-APCI) 288.2; HRMS calculated for $C_{18}H_{26}D_7O_2$ (M-H) 288.2925, found 288.2940.

*16,16,17,17,18,18,18-d_7-Elaidic acid (**13**)*

A solution of **10** (174 mg, 450 μmol) in THF (5 ml) was treated with TBAF (1 M in THF, 1.2 ml) with stirring at RT for 2 h. The mixture was then diluted with water, and extracted with ether (2 ×). The combined organics were washed with brine, dried over Na_2SO_4/$MgSO_4$, and concentrated. The residue was purified by flash chromatography on silica gel eluting with 1/3 ethyl acetate/hexanes ($R_f = 0.41$) to afford 16,16,17,17,18,18,18-d_7-octadec-9-yn-1-ol as a colorless oil (104 mg, 87% yield); ^{1}H NMR (400 MHz) δ 3.66 (2H, t, $J = 6.6$ Hz), 2.16 (4H, t, $J = 6.8$ Hz), 1.64–1.54 (2H, m), 1.54–1.45 (4H, m), 1.45–1.25 (14H, m). A solution of this alkynol (36 mg, 130 μmol) was dissolved in diglyme (3.5 ml), and treated with $LiAlH_4$ (62 mg, 1600 μmol, 12 eq.). The resulting slurry was then heated at 160 °C for 40 h. The excess hydride was quenched at RT by the addition of ethyl acetate followed by 2 M HCl. The

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mixture was partitoned between ethyl acetate and water, and the layers were separated. The organic phase was washed with brine, and dried over Na_2SO_4/$MgSO_4$. Concentration by rotary evaporation, and flash chromatography of the residue on silica gel eluting with 1/3 ethyl acetate/hexanes ($R_f = 0.47$) gave 16,16,17,17,18,18,18-d_7-(9*E*)-octadec-9-en-1-ol as a colorless, waxy solid (32 mg, 90%); ^{1}H NMR (500 MHz, acetone-d_6) δ 5.42 (2H, m), 3.56–3.52 (2H, m), 3.40 (1H, m, OH), 2.04–1.98 (4H, m), 1.54–1.47 (4H, m), 1.37–1.29 (16H, m). A solution of this alcohol (32 mg, 110 μmol) in acetone (2 ml) was treated with a 2.7 M solution of Jones reagent (0.10 ml, 270 μmol) with stirring at RT for 15 min. The reaction mixture was then diluted with water and extracted with ether (2 ×). The combined extracts were washed with water until no orange color persisted in the organic phase, followed by a brine wash, and drying over Na_2SO_4/$MgSO_4$. The organics were concentrated, and the residue was purified by flash chromatography on silica gel eluting with 1/4 ethyl acetate/hexanes containing 0.5% acetic acid ($R_f = 0.37$) to afford the title compound as a colorless, waxy solid (22 mg, 65%). ^{1}H NMR (400 MHz) δ 11.00 (1H, v br), 5.38 (2H, m), 2.37 (2H, t, J= 7.5 Hz), 2.03–1.95 (2H, m), 1.67–1.61 (2H, m), 1.43–1.24 (18H, m); ^{13}C NMR (100 MHz) 180.0, 130.5, 130.2, 34.0, 32.6, 32.5, 30.8 (quintet, J = 19 *Hz*), 29.7, 29.45, 29.36, 29.2, 29.1, 29.02, 28.96, 28.9, 24.7, 21.4 (quintet, J= 19 Hz), 21.9 (septet, J= 19 Hz); MS (-APCI) 288.1; HRMS calculated for $C_{18}H_{26}D_7O_2$ (M-H) 288.2925, found 288.2940.

*16,16,17,17,18,18,18-d$_7$-Octadeca-1-ol (***14***)*

A solution of **10** (2.26 g, 5.84 mmol) in ethyl acetate (50 ml) was added to 10% Pd/C (1.14 g) in a Parr flask under nitrogen. The reaction vessel contents were then shaken under a hydrogen atmosphere (40 PSI) for 6 h. Filtration through Celite and concentration of the filtrate afforded a colorless solid that was taken up in THF (20 ml), and treated with TBAF (1 M in THF, 4 ml) with stirring at RT for 2 h. The mixture was then partitioned between ether and water, and the layers were separated. The aqueous phase was extracted with additional ether, and the combined extracts were washed with brine before drying over Na_2SO_4/$MgSO_4$. Evaporation of the solvents, and flash chromatography on silica gel eluting with 1/4 ethyl acetate/hexanes (R_f= 0.38) afforded the title compound as a colorless powder (1.57 g, 97%). ^{1}H NMR (acetone-d_6, 500 MHz) δ 3.54 (2H, m), 3.38 (1H, t, J= 5.3 Hz), 1.56–1.47 (2H, m), 1.31 (26H, br s).

*16,16,17,17,18,18,18-d$_7$-Stearic acid (***15***)*

A solution of **14** (703 mg, 2.64 mmol) in acetone (35 ml) was treated with Jones reagent (2.7 M, 1.5 ml, 4.0 mmol) with stirring at RT for 30 min. Water (100 ml) was then added, and the mixture was stirred until all the chromium salts had dissolved. The remaining solid was collected by suction filtration, and

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washed well with water before being dissolved in ether and dried over Na_2SO_4/$MgSO_4$. Concentration *in vacuo* afforded the title compound as a colorless powder (718 mg, 93%). ^{1}H NMR (acetone-d_6, 500 MHz) δ 10.41 (1H, br), 2.30 (2H, t, $J = 7.42$ Hz), 1.68–1.56 (2H, m), 1.32 (24H, br s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.4, 34.1, 30.8 (pentet, $J = 19$ Hz), 29.70, 29.68, 29.65, 29.63, 29.60, 29.5, 29.4, 29.2, 29.13, 29.07, 24.7, 21.4 (pentet, $J = 19$ Hz), 12.9 (pentet, $J = 19$ Hz); MS (-APCI) 290.5; HRMS calculated for $C_{18}H_{28}D_7O_2$ (M-H) 290.3082, found 290.3075.

*Triphenyl[14-tetrahydro-2H-pyran-2-yloxy)tetradecyl]phosphonium iodide (***16***)*

Toluene-4-sulfonic acid monohydrate (0.50 g, 2.6 mmol) was added to a solution of 14-bromotetradecan-1-ol (9.90 g, 33.8 mmol) and freshly distilled dihydropyran (4.6 ml, 50 mmol) in CH_2Cl_2 (35 ml) with stirring at RT for 20 min. The reaction vessel contents were then mixed with saturated $NaHCO_3$ aqueous solution, and the layers were separated. The organic phase was washed with water, dried over Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel eluting with CH_2Cl_2 afforded 2-[(14-bromotetradecyl)oxy]tetrahydro-2*H*-pyran as a faint-yellow syrup (11.31 g, 89%); ^{1}H NMR (acetone-d_6, 500 MHz) δ 4.58–4.55 (1H, narrow m), 3.80 (1H, m), 3.69 (1H, dt, $J = 9.6$, 6.7 Hz), 3.51 (2H, t, $J = 6.81$ Hz), 3.45 (1H, m), 3.35 (1H, dt, $J = 9.6$, 6.4 Hz), 1.91–1.82 (2H, m), 1.80 (1H, m), 1.64 (1H, m), 1.59–1.43 (8H, m), 1.32 (18H, br s). A solution of this compound (11.31 g, 30 mmol) in acetone (80 ml) was treated with sodium iodide (6.0 g, 40 mmol) with heating at reflux for 1.5 h. The mixture was then cooled to RT, partitioned between water and ether, and the layers were separated. The aqueous phase was extracted with additional ether, and the combined organics were dried over Na_2SO_4/$MgSO_4$. Concentration *in vacuo* afforded the iodide as a faint-yellow syrup (12.72 g, >99%). A solution of this compound in acetonitrile (50 mL) was treated with triphenylphosphine (7.95 g, 30.0 mmol, 1 eq.) followed by heating at reflux for 16 h. The bulk of the acetonitrile was removed by rotary evaporation under reduced pressure, and the residue was placed under high vacuum (P < 1 mmHg) with heating at 60°C to remove the remaining solvent, and to afford the title compound as a thick, yellow syrup (20.58 g, >99%); ^{1}H NMR (acetonitrile-d_3, 500 MHz) δ 7.92–7.87 (3H, m), 7.76–7.69 (12H, m), 4.54 (1H, m), 3.81 (1H, m), 3.68 (1H, dt, $J = 9.6$, 6.8 Hz), 3.48 (1H, m), 3.36 (1H, dt, $J = 9.6$, 6.5 Hz), 3.24–3.14 (2H, m), 1.78 (1H, m), 1.70–1.59 (3H, m), 1.57–1.44 (8H, m), 1.40–1.22 (18H, m).

*16,16,17,17,18,18,18-d$_7$-2-[(14Z)-Octadec-14-en-1-yloxy]tetrahydro-2H-pyran (***17***)*

NaHMDS (1 M in THF, 31 ml) was added over a period of 5 min to a solution of **16** (20.58 g, 30 mmol) in THF (100 ml) at 0°C. The resulting cloudy, orange

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suspension was stirred at 0°C for an additional 15 min prior to cooling to −78°C. d_7-Butyraldehyde (**2**) (20 wt% in CH_2Cl_2, 3.00 g, 38 mmol) was then added rapidly via syringe with stirring at −78°C for 2 h, then at 0°C for 15 min. The reaction was quenched by the addition of 1 M pH 7 phosphate buffer (1:1 K_3PO_4/K_2HPO_4) and the THF was removed by rotary evaporation under reduced pressure. Water and hexanes were added with rapid stirring at RT, and the bulk of the solid triphenylphosphine oxide by-product was removed by suction filtration. The layers were separated, and the organic phase was washed with brine, and dried over $Na_2SO_4/MgSO_4$. Concentration of the organics, and flash chromatography on silica gel eluting with 1/99 ether/hexanes ($R_f = 0.88$, hexanes) gave the title compound as a colorless syrup (8.46 g, 78%); ^{1}H NMR (acetone-d_6, 500 MHz) δ 5.43–5.32 (2H, m), 4.56 (1H, narrow m), 3.80 (1H, m), 3.69 (1H, dt, $J = 9.6$, 6.7 Hz), 3.45 (1H, m), 3.35 (1H, dt, $J = 9.6$, 6.4 Hz), 2.04 (2H, overlapped m), 1.80 (1H, m), 1.65 (1H, m), 1.59–1.48 (6H, m), 1.32 (20H, br s).

*16,16,17,17,18,18,18-d_7-2-(Octadecyloxy)tetrahydro-2H-pyran (**18**)*

Wilkinson's catalyst (960 mg, 4 mol%) was suspended in nitrogen saturated benzene (80 ml) under a nitrogen atmosphere in a rubber septum capped Parr flask. Compound **17** (8.46 g, 23.5 mmol) was then added as a solution in nitrogen saturated benzene (20 ml), and the mixture was shaken under an atmosphere of hydrogen gas (50 PSI) for 4 days. Following confirmation of the completion of the reaction by ^{1}H NMR, the benzene was evaporated and the residue was purified by flash chromatography on silica gel eluting with 5/95 ethyl acetate/hexanes ($R_f = 0.43$) to yield the title compound as a colorless syrup (7.63 g, 90%). ^{1}H NMR (acetone-d_6, 500 MHz) δ 4.56 (1H, narrow m), 3.83–3.76 (1H, m), 3.69 (1H, dt, $J = 9.6$, 6.7 Hz), 3.45 (1H, m), 3.35 (1H, dt, $J = 9.6$, 6.4 Hz), 1.80 (1H, m), 1.65 (1H, m), 1.59–1.44 (4H, m), 1.43–1.24 (28H, s).

*16,16,17,17,18,18,18-d_7-Stearic acid (**15**) from scheme 2*

Compound **18** (7.63 g, 21.0 mmol) was heated at 50°C in methanol (150 ml) containing toluene-4-sulfonic acid monohydrate (190 mg, 1.00 mmol). After 30 min at this temperature, the mixture was cooled to RT, and treated with a saturated aqueous solution of $NaHCO_3$ (10 ml), followed by concentration by rotary evaporation under reduced pressure. The solid residue was suspended in water, and collected by suction filtration. Drying under a stream of dry nitrogen, and then under high vacuum overnight afforded **14** as a colorless powder (5.28 g, 90%); $R_f = 0.38$ (1/4 ethyl acetate/hexanes). A solution of **14** (5.28 g, 19.1 mmol) in acetone (250 ml) was added slowly over 15 min to a solution of Jones reagent (2.7 M, 16 ml, 43 mmol) in acetone (100 ml) at RT.

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After stirring at RT for an additional 45 min, water (500 ml) was added, and the mixture was stirred until all the chromium salts had dissolved. The remaining solid was collected by suction filtration, and was washed with water until no coloration was visible in the washings. The solid in the suction funnel was then dissolved in ether, and this solution was washed with water, brine, and was dried over $Na_2SO_4/MgSO_4$. Concentration *in vacuo* afforded the title compound as a colorless powder (5.40 g, 97%).

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