Site-specific ^{2}H-labeled oleic acid and derived esters for use as tracers of ethyl oleate metabolism in honey bees

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The inventory of labeled fatty acids and protocols for their syntheses are constantly increasing, but site-specific labeled precursors in biosynthetic studies are still needed. Ethyl oleate (EO) is an important primer pheromone in honeybees, which is responsible for the regulation of behavioral maturation. During our biosynthetic studies on EO, a site-specific labeled oleic acid precursor was required. In this report, a synthetic route adaptable to the preparation of [9,10,11,11-D$_4$] oleic acid and its derived esters for use as tracers of EO metabolism in honey bees is presented.

Keywords: (Z)-9-octadecenoic acid; ethyl ester; *N*-acetyl cysteamide thioester; triacylglycerol

Introduction

The cohesion and distribution of tasks in a honey bee (*Apis mellifera* L.) hive is controlled mainly by pheromone blends.[1] For example, the transition of worker bees from nursing to foraging tasks is influenced by the presence of old workers: a large number of foragers delay the transition of the younger workers, whereas a scarce population of foragers causes the opposite effect. An inhibitory primer pheromone that controls this process was identified as ethyl oleate (EO).[2] In that report, it has been demonstrated that EO is biosynthesized *de novo* by foragers from glucose. In order to identify and characterize the possible enzymes involved the metabolism of EO, stable isotope labeled oleic acid was required, as a substrate and also as a synthetic precursor to the *N*-acetylcysteamide thioester, monoglycerides, and triglycerides and of EO standards.

There are three reasons for the choice of site-specific deuteration of oleic acid. First, a GC-MS method for the quantitative analysis of deuterium-labeled oleate in biological samples was developed in our studies (unpublished results). In order to optimally discriminate the tracer from naturally occurring fatty acids present in bee samples, a minimum incorporation of four deuterium atoms was required because of good isotope fractionation from non-labeled endogenous material. Second, on the basis of our genetic and bioconversion studies, a desaturase gene is highly expressed in the esophagus of worker bees and is upregulated upon exposure of honey bees to high doses of EO (unpublished results), suggesting a possible metabolic pathway shown in Scheme 1. In our hypothesis, the EO in honey bees could be further desaturated to the ethyl ester of an unsaturated fatty acid with one (Z) double bond at the position 9 and the second and third double bonds at positions 6, 12, or 15. With the [9,10,11,11-D$_4$] labeling pattern, a labeled tri-unsaturated fatty acid ethyl ester would give diagnostic fragment ions,[3,4] which could tell us if the three methylene-interrupted double bonds

exist in the molecule or not. Third, we also wished to potentially monitor beta-oxidation and omega oxidation of the oleate and derived fatty acids in the first a few oxidation cycles and, to detect products, we needed to place the deuterium labels after position 8 in oleate and before the end of the chain.

By the combination of the above three requirements, a [9,10,11,11-D$_4$] oleic acid precursor was highly desirable. In this report, we present a synthetic route that was developed to provide us with the specifically labeled molecular probe and its derived esters.

Experimental

General

Commercial-grade solvents were distilled under nitrogen prior to use, and reagents were used without further purification with the following exceptions: triethylamine was distilled and stored over NaOH. CH$_2$Cl$_2$ was distilled over CaCl$_2$ and stored over molecular sieves 3 Å. Dried THF (MBraun Inc. Stratham, New Hampshire, USA) was obtained from a MBRAUN LTS 350 solvent purification system. The ^{1}H and ^{13}C NMR spectra were recorded in CDCl$_3$ on Bruker 400 and 500 MHz spectrometers. The reference for NMR chemical shifts was the residue peaks of solvents. Data are given as chemical shift (δ), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), and coupling constants (s) (Hz). Mass spectra were acquired on a Varian Saturn 2000 ion trap mass spectrometer, interfaced with a Varian GC, in EI mode [2 μscans (0.55 s/scan), emission current (30 μA),

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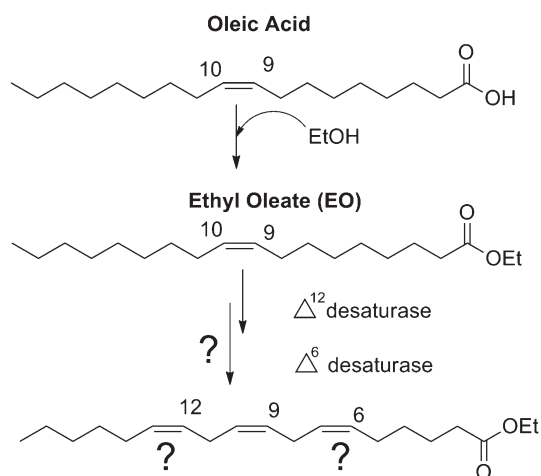
Scheme 1. Formation of EO from oleic acid in honey bee gut fluid and desaturation of excess EO in honey bee tissue to an ethyl ester of an unsaturated fatty acid with a Δ9 double bond and two more double bonds at unknown positions.

scanning SIS (49–375 m/z)]. The GC-MS instrument was also equipped with a SPB-5 column and was programmed 100 °C (5 min), 10 °C /min, 200 °C (4.0 min), 15 °C/min, and 250 °C (14.0 min). Flash column chromatography was performed with Kieselgel 60 (230–400 mesh ASTM).

Synthesis of 1-[1,1-D$_2$] octanol (2)

Compound **2** was synthesized from octanoic acid (**1**) (4 g, 27.7 mmol) according to the procedure reported by Rolla and co-workers.[5] The title compound was obtained as a colorless oil (3.6 g, 97%). Total deuterium incorporation rate: 98% (calculated by the residual signal of -CH$_2$OH at 3.61 ppm). ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.54 (2H, t, $J = 6.8$ Hz), 1.35–1.27 (10H, m), (0.87, t, $J = 6.8$ Hz); ^{13}C NMR (100 MHz, CDCl$_3$) δ 62.24 (quintet), 32.55, 31.78, 29.37, 29.24, 25, 67, 22.61, 14.04.

Synthesis of [1,1-D$_2$] octane-1-yl-methanesulfonyl ester (3)

Compound **2** was synthesized from **3** (3 g, 22.7 mmol) according to the procedure that Mosher and Williams[6] was utilized for the unlabeled **3**. The title compound was obtained as light yellow oil (4.5 g, 96%). Total deuterium incorporation rate: 98% (calculated by the residual signal of -CH$_2$OMs at 4.23 ppm). The ^{1}H NMR of **3** was compared to literature.[6] ^{1}H NMR (400 MHz, CDCl$_3$) δ 3.00 (3H, s), 1.73 (2H, t, $J = 6.8$ Hz), 1.41–1.27 (10H, m), 0.88 (3H, t, $J = 6.8$ Hz); ^{13}C NMR (100 MHz, CDCl$_3$) δ 69.41 (quint), 37.38, 31.69, 29.06, 28.97, 28.90, 25.34, 22.59, 14.04.

Synthesis of 1-iodo-[1,1-D$_2$] octane (4)

A similar procedure to that of Organ and co-workers was utilized.[7] A solution of **3** (4.5 g, 21.6 mmol) in dry acetone was added sodium iodide (7.9, 52.8 mmol). The reaction mixture was heated at gentle reflux for 4 h. The mixture was allowed to cool to room temperature. The acetone was removed by rotary evaporation in a 20–25 °C water bath. The residue was partitioned between 50 mL of hexanes and 50 mL of 10% sodium thiosulfate solution by swirling the flask until all the precipitate dissolved. The aqueous phase was discarded, and the organic layer was washed with 50 mL of brine, dried over Na$_2$SO$_4$ and concentrated *in vacuo*. The residue was purified

by flash chromatography on silica gel eluting with 5% EtOAc in hexanes to afford the title compound as light red oil (4.3 g, 83%). Deuterium incorporation rate: 98% (calculated by the residual signal of -CH$_2$I at 3.20 ppm) ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.81 (2H, t, $J = 6.8$ Hz), 1.40–1.28 (10H, m), (0.88, t, $J = 6.8$ Hz); ^{13}C NMR (100 MHz, CDCl$_3$) δ 33.33, 31.74, 30.44, 29.07, 28.51, 22.61, 14.06, 7.10 (quintet).

Synthesis of dec-9-yn-1-ol (6)

Compound **6** was synthesized from 2-decyn-1-ol (**5**) (1 g, 6.5 mmol) with the previously described procedure.[9] The title compound was obtained as a yellow oil (0.9 g, 90%), which was used directly for the next step without further purification. ^{1}H NMR (500 MHz, CDCl$_3$) 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).

Synthesis *tert*-butyl (dec-9-ynyloxy)dimethylsilane (7)

Compound **7** was synthesized from **6** (1 g, 6.5 mmol) with the previously described procedure.[9] The title compound was obtained as a light yellow oil (1.2 g, 75%). ^{1}H NMR (500 MHz, CDCl$_3$) 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).

Synthesis of [11,11-D$_2$] octadec-9-yn-1-yloxy-*tert*-butyl dimethylsilane (8)

The general procedure of Cran and co-workers was utilized.[9] The title compound was obtained from **4** (1.2 g, 5.1 mmol) and **7** (1.2 g, 4.3 mmol) as a light yellow oil (1.0 g, 61%). Deuterium incorporation analyzed by GC-MS: D$_2$ 97.8%; D$_1$ 1.5%; D$_0$ 0.6%. ^{1}H NMR (400 MHz, CDCl$_3$) δ 3.62 (2H, t, $J = 6.8$ Hz); 1.98 (2H, t, $J = 6.8$ Hz); 1.68–1.30 (24H, m); 0.92 (9H, s), 0.90 (3H, t, $J = 6.8$ Hz), 0.07 (6H, s); ^{13}C NMR (100 MHz, CDCl$_3$) δ 68.04, 63.33, 63.31, 32.88, 32.86, 31.86, 29.36, 29.29, 29.23, 29.17, 29.15, 28.83, 28.70, 28.48, 25.99, 25.76, 22.67, 18.76, 18.40 (quintet), 14.11, −5.25.

Synthesis of [9,10,11,11-D$_4$] (9Z)-octadec-9-en-1-yloxyl-*tert*-butyl dimethylsilane (9)

The general procedure of Cran and co-workers was utilized.[9] The title compound was obtained from **8** (150 mg, 0.39 mmol) as a light yellow oil (140 mg, 92%). Total deuterium incorporation rate: 97% (calculated by the residual signal of vinylic at 5.42 ppm); Deuterium incorporation analyzed by GC-MS: D$_4$ 98.4%; D$_3$ 0.2%; D$_2$ 0.9%; D$_1$ 0.4%; D$_0$ 0.1%. ^{1}H NMR (400 MHz, CDCl$_3$) δ 3.60 (2H, t, $J = 6.8$ Hz); 2.04 (2H, t, $J = 6.8$ Hz); 1.68–1.30 (24H, m); 0.92 (9H, s), 0.90 (3H, t, $J = 6.8$ Hz), 0.07 (6H, s); ^{13}C NMR (100 MHz, CDCl$_3$) δ 63.34, 32.90, 31.91, 29.76, 29.66, 29.59, 29.58, 29.54, 29.46, 29.43, 29.33, 29.28, 29.27, 25.99, 25.81, 22.69, 18.39, 14.11, −5.21.

Synthesis of [9,10,11,11-D$_4$] (9Z)-octadec-9-en-1-ol (10)

The general procedure of Cran and co-workers was utilized.[9] The title compound was obtained from **9** (150 mg, 0.39 mmol) as a light yellow oil (75 mg, 70%). Deuterium incorporation rate: 97% (calculated by the residual signal of vinylic H at 5.42 ppm). ^{1}H NMR (400 MHz, CDCl$_3$) δ 3.66 (2H, t, $J = 6.8$ Hz);

2.03 (2H, t, $J = 6.8$ Hz); 1.62–1.55 (2H, m), 1.39–1.29 (22H, m); 0.90 (3H, t, $J = 6.8$ Hz); ^{13}C NMR (100 MHz, CDCl$_3$) δ 129.39 (m), 63.33, 32.82, 31.91, 29.75, 29.58, 29.54, 29.51, 29.41, 29.33, 29.28, 29.24, 27.07, 25.74, 22.69, 14.11.

Synthesis of [9,10,11,11-D$_4$] oleic acid (11)

The procedure described by Cran and co-workers was utilized.[9] The title compound was obtained from **10** (75 mg, 0.26 mmol) as a light yellow oil (60 mg, 82%). Deuterium incorporation rates: 97% (calculated by the residual signal of vinylic protons at 5.42 ppm); 98% (calculated by the residual signal of -CD=H; CDCH$_2$ protons at 2.12 ppm); total D$_4$ incorporation at 97.5%. Deuterium incorporation analyzed by GC-MS: D$_4$ 82.7%; D$_3$ 15.4%; D$_2$ 0.9%; D$_1$ 0.2%; D$_0$ 0.9%. ^{1}H NMR (400 MHz, CDCl$_3$) δ 11.19 (1H, bs), 2.35 (2H, t, $J = 6.8$ Hz); 2.01 (2H, t, $J = 6.8$ Hz); 1.67–1.60 (2H, m), 1.31–1.27 (20H, m); 0.88 (3H, t, $J = 6.8$ Hz); ^{13}C NMR (100 MHz, CDCl$_3$) δ 180.32, 129.45 (m), 34.09, 31.92, 29.68, 29.58, 29.54, 29.33, 29.28, 29.15, 29.07, 29.04; 27.03, 26.41 (weak signal), 24.67, 22.69, 14.11. GC-MS (derivatized with BSTFA): m/z 359.4 (M + 1$^+$, 57.5%), 343.5 (70%), 129.1 (95%), 117.1 (100%), 75.1 (82.5%).

Synthesis of 13

A solution of d_4-oleic acid (50 mg, 0.18 mmol) in 5 mL of CH$_2$Cl$_2$ was added N-acetyl cysteamine (31 mg, 0.26 mmol), followed by DMAP (2 mg, 0.018 mmol) and EDC (69 mg, 0.36 mmol). The mixture was allowed to stir under N$_2$ at room temperature overnight. The reaction solution was diluted with 20 mL of CH$_2$Cl$_2$, which was washed with saturated NH$_4$Cl. The organic phase was separated. The remaining aqueous phase was extracted with two portions of CH$_2$Cl$_2$. The combined organic phase was washed with brine, dried over Na$_2$SO$_4$, and then concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (hexanes/EtOAc 4:1) to afford the tile compound as a light yellow solid (57 mg, 82%). Deuterium incorporation rates: 95% (calculated by the residual signal of vinylic protons at 5.36 ppm). ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.91 (1H, bs, -NH), 3.42 (2H, q, $J = 5.0$ Hz), 3.01 (2H, t, $J = 5.0$ Hz), 2.56 (2H, t, $J = 5.0$ Hz), 1.99–1.93 (2H, m), 1.95 (3H, s), 1.64 (2H, m), 1.30–1.25 (20H, m), 0.87 (3H, t, $J = 5.0$ Hz); ^{13}C NMR (125 MHz, CDCl$_3$) 200.25, 170.22, 129.87 (m, the residue of vinylic protons), 44.09, 39.74, 32.34, 31.87, 29.46, 29.42, 29.28, 29.10, 29.05, 28.86, 28.40, 25.62, 23.18, 22.65, 14.09, (note: the signal of -CD$_2$ was not observed).

Synthesis of 15

A solution of d_4-oleic acid (**11**) (38 mg, 0.132 mmol) in 5 mL of CH$_2$Cl$_2$ was added glycerol (3 mg, 0.033 mmol), followed by DMAP (4 mg, 0.033 mmol) and EDC (25 mg, 0.132 mmol). The mixture was allowed to stir under N$_2$ at room temperature overnight. The reaction solution was diluted with 20 mL of CH$_2$Cl$_2$, which was washed with saturated NH$_4$Cl. The organic phase was separated. The remaining aqueous phase was extracted with two portions of CH$_2$Cl$_2$. The combined organic phase was washed with brine, dried over Na$_2$SO$_4$, and then concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (hexanes/EtOAc 9:1) to afford the title compound as a colorless oil (17 mg, 60%). Deuterium incorporation percentages: 95% (calculated by the residual signal of vinylic protons at

5.38 ppm); ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.26 (1H, quintet, $J = 5.0$, 10.0 Hz), 4.29 (2H, dd, $J = 5.0$, 15.0 Hz); 4.14 (2H, dd, $J = 5.0$, 15.0 Hz); 2.31 (6H, m, $J = 5.0$ Hz); 2.00 (6H, t, $J = 5.0$ Hz); 1.61–1.57 (6H, m), 1.30–1.26 (60H, m); 0.88 (9H, t, $J = 5.0$ Hz); ^{13}C NMR (125 MHz, CDCl$_3$) δ 173.29 (overlapped), 172.87, 129.40 (m), 68.80, 62.11 (overlapped), 34.21, 34.04, 31.92, , 29.58, 29.55, 29.34, 29.29, 29.22, 29.20, 29.14, 29.10, 29.07; 27.05, 26.34 (m, weak signal), 24.90, 24.86, , 22.70, 14.13.

Synthesis of isoproylideneglycerol (16)

Compound **16** was synthesized from acetone (47.4 g, 0.82 mol), glycerol (20 g, 0.22 mol), p-TsOH (0.6 g, 3.1 mmol), and 100 mL of petroleum ether (30–60 °C) with the previously described procedure.[11] The tile compound was obtained as a colorless oil (23 g, 80%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.23 (1H, m), 4.03 (1H, dd, $J = 8.0$ Hz), 3.78 (1H, dd, $J = 8.0$ Hz), 3.72 (1H, m), 3.58 (1H, m), 2.04 (OH, t, $J = 8.0$ Hz), 1.42 (3H, s), 1.36 (3H, s); ^{13}C NMR (100 MHz, CDCl$_3$) δ 109.40, 76.16, 65.70, 63.00, 26.69, 25.25.

Synthesis of 17

A solution of d_4-oleic acid (**11**) (20 mg, 0.07 mmol) in 5 mL of CH$_2$Cl$_2$ was added **16** (14 mg, 0.10 mmol), followed by DMAP (2 mg, 0.014 mmol) and EDC (27 mg, 0.14 mmol). The mixture was allowed to stir under N$_2$ at room temperature overnight. The reaction solution was diluted with 20 mL of CH$_2$Cl$_2$, which was washed with saturated NH$_4$Cl. The organic phase was separated. The remaining aqueous phase was extracted with two portions of CH$_2$Cl$_2$. The combined organic phase was washed with brine, dried over Na$_2$SO$_4$, and then concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (hexanes/EtOAc 9:1) to afford the tile compound as a colorless oil (24 mg, 85%). Deuterium incorporation percentages: 95% (calculated by the residual signal of vinylic protons at 5.40 ppm); ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.31 (1H, quintet, $J = 4.0$, 12.0 Hz), 4.16 (1H, dd, $J = 4.0$, 12.0 Hz), 4.08 (2H, m, $J = 4.0$, 12.0 Hz), 3.73 (1H, dd, $J = 4.0$, 12.0 Hz), 2.34 (2H, t, $J = 8.0$ Hz), 2.00 (2H, t, $J = 8.0$ Hz), 1.64 (2H, t, $J = 8.0$ Hz), 1.43 (3H, s), 1.37 (3H, s); 1.26–1.30 (20H, m), 0.88 (3H, t, $J = 8.0$ Hz); ^{13}C NMR (100 MHz, CDCl$_3$) δ 173.60, 109.81, 73.66, 66.36, 64.52, 34.10, 31.89, 29.67, 29.56, 29.52, 29.31, 29.26, 29.14, 29.09, 27.02, 26.69, 25.39, 24.88, 22.67, 14.10, (note: signals of carbon labeled by deuterium were not observed).

Synthesis of 18

Compound **18** was prepared from **17** by following a reported procedure.[12] Deuterium incorporation rates: 96% (calculated by the residual signal of vinylic protons at 5.36 ppm). ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.61 (1H, m, $J = 4.0$, 12.0 Hz), 4.28 (1H, t, $J = 8.0$ Hz), 4.18 (2H, m, $J = 4.0$, 12.0 Hz), 3.96 (1H, m, $J = 4.0$, 12.0 Hz), 2.37 (2H, t, $J = 8.0$ Hz), 2.02 (2H, t, $J = 8.0$ Hz), 1.65 (2H, t, $J = 8.0$ Hz); 1.20–1.31 (20H, m), 0.90 (3H, t, $J = 8.0$ Hz); ^{13}C NMR (100 MHz, CDCl$_3$) δ 173.59, 73.47, 66.77, 65.42, 61.23, 34.11, 31.91, 29.69, 29.57, 29.54, 29.32, 29.28, 29.16, 29.10, 27.04, 24.88, 22.69, 14.10 (note: signals of carbon labeled by deuterium were not observed).

Synthesis of 19

A solution of oleic acid (63 mg, 0.22 mmol) in 5 mL of CH_2Cl_2 was added **18** (20 mg, 0.055 mmol), followed by DMAP (3 mg, 0.022 mmol) and EDC (42 mg, 0.22 mmol). The mixture was allowed to stir under N_2 at room temperature overnight. The reaction solution was diluted with 20 mL of CH_2Cl_2, which was washed with saturated NH_4Cl. The organic phase was separated. The remaining aqueous phase was extracted with two portions of CH_2Cl_2. The combined organic phase was washed with brine, dried over Na_2SO_4, and then concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (hexanes/EtOAc 9:1) to afford the title compound as a colorless oil (30 mg, 63%). ^1H NMR (400 MHz, $CDCl_3$) δ 5.37 (4H, m,), 5.26 (1H, m) 4.61 (1H, m, $J = 4.0$, 12.0 Hz), 4.32 (1H, dd, $J = 8.0$ Hz), 4.17 (2H, dd, $J = 4.0$, 12.0 Hz), 2.33 (6H, m, $J = 4.0$ Hz), 2.04 (10H, m), 1.61–1.58 (6H, m), 1.32–1.28 (60H, m), 0.90 (9H, t, $J = 8.0$ Hz); NMR (100 MHz, $CDCl_3$) δ 173.27, 172.85, 130.04, 130.02, 129.72, 129.69, 68.88, 68.21, 34.21, 34.04, 31.91, 30.93, 29.78, 29.71, 29.54, 29.19, 29.13, 29.10, 27.28, 27.13, 24.85, 22.69, 14.12 (note: signals of carbon labeled by deuterium were not observed).

Results and discussion

As discussed in the Introduction section, our plan was to develop a d_4-labeled oleic acid precursor with two of deuterium located at position 11 specifically for use as a tracer in a GC-MS based bioanalytical assay. To meet the requirement for our research plan, we chose to assemble the labeled acid from two components, in which the first two site-specific deuterium atoms at position 11 were introduced by lithium aluminum deuteride ($LiAlD_4$) reduction, followed by the catalytic semi-deuteration using D_2 and Lindlar catalyst to introduce the second two deuterium atoms (Figure 1).

The synthesis (Scheme 2) starts with a reduction of octanoic acid (**1**) with $LiAlD_4$ to give d_2-octanol (**2**).[5] After mesylation of d_2-octanol, d_2-octane iodide (**4**) was prepared by the treatment of mesylate **3** with NaI in acetone.[6,7] With the first deuterium-labeled fragment **4** in hand, we turned our attention to the second fragment. Treatment of commercially available dec-2-yn-ol (**5**) with NaH in 1,3-diaminopropane by an alkyne zipper reaction that gave rise to isomerization of the internal alkyne to the terminal position afforded dec-9-yn-1-ol (**6**).[8,9] The alcohol functionality on **6** was then protected as the TBDMS silyl ether **7**.[9] Treatment of the protected alcohol **7** with *n*-BuLi generated the corresponding lithium acetylide, which was coupled to iodide **4** to afford **8** in 80% yield.[9,10] The obtained key intermediate **8** was then partially reduced with D_2 to **9** over Lindlar catalyst.[9] After removing the silyl group, the resulting alcohol **10** was treated with Jones reagent to give the desired d_4-oleic acid (**11**).[9]
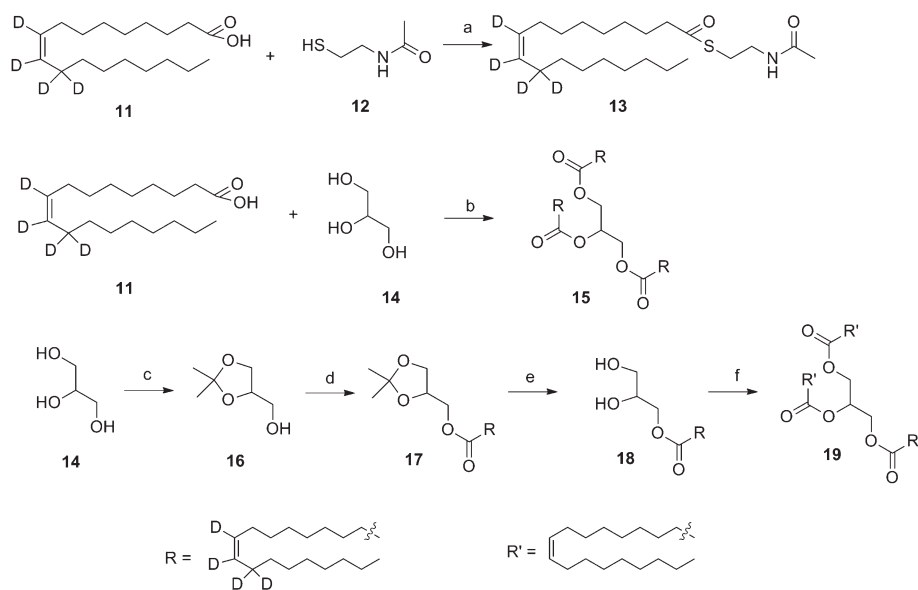
With the site-specific d_4-oleic acid in hand, three derived esters were also prepared. Thioester **13** and ester **15** were synthesized directly by coupling **11** with the *N*-acetyl cysteamine (**12**) and glycerol (**14**), respectively (Scheme 3).
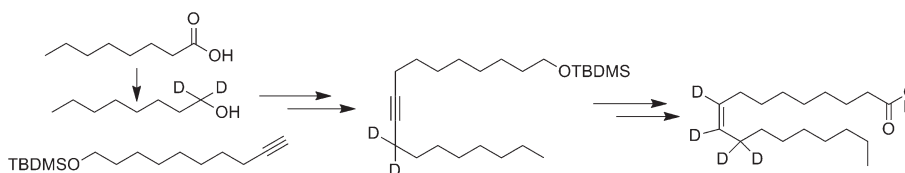
Figure 1. Strategy for the preparation the site-specific deuterium labeled oleic acid.
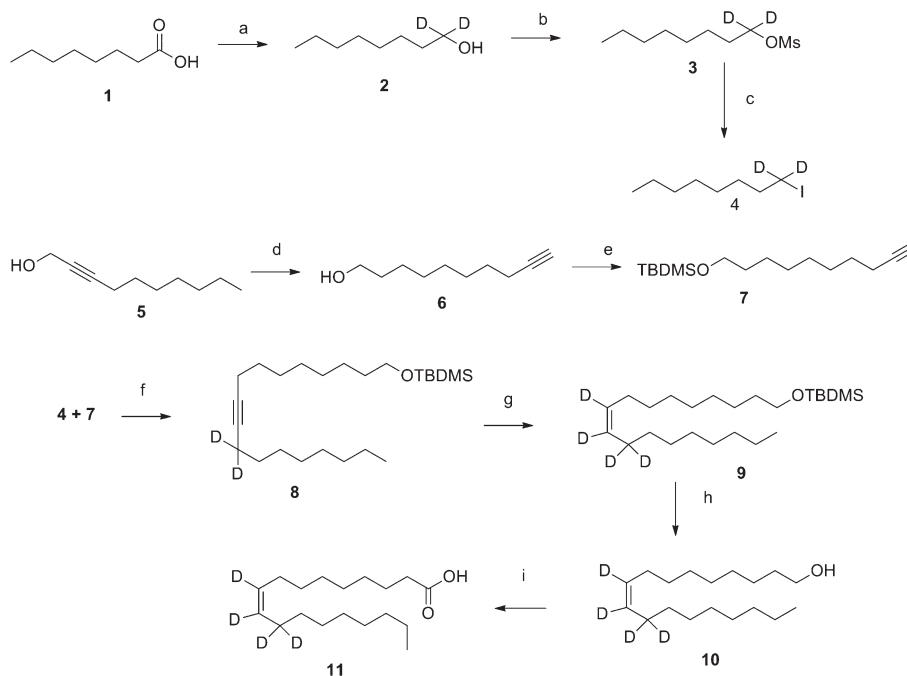
Scheme 2. (a) $LiAlD_4$, THF, reflux; (b) TEA, MsCl, CH_2Cl_2, rt; (c) NaI, acetone, reflux; (d) 1,3-diaminopropane, NaH, 60 °C; (e) TBDMSCl, TEA, DMAP, CH_2Cl_2, rt; (f) *n*-BuLi, THF-HMPA, -78 °C-0 °C; (g) Lindlar catalyst, D_2, hexanes, rt; (h) TBAF, THF, rt; (i) Jones reagent, acetone, 0 °C.

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Scheme 3. (a) EDC, DMAP, CH_2Cl_2, rt; (b); EDC, DMAP, CH_2Cl_2, rt; (c) acetone, p-TsOH, petroleum ether (30–60 °C), reflux; (d) d_4-oleic acid, EDC, DMAP, CH_2Cl_2, rt; (e) triethylborate, trifluoroethanol, and TFA in 8:1:1 ratio, rt; (f) oleic acid, EDC, DMAP, CH_2Cl_2, rt.

To synthesize compound **19**, glycerol (**14**) was first treated with acetone in the presence of catalytic p-TsOH to afford 2,3-isopropylideneglycerol (**16**).[11] Compound **16** was coupled with d_4-oleic acid (**11**) in the presence of EDC and DMAP to give **17**. The product **17** was treated with TFA in trifluoroethanol/triethyl borate (1:8 v/v) solution at room temperature to afford **18**,[12] which was subsequently coupled with oleic acid to yield the final product **19**.

In conclusion, a synthetic route for the efficient preparation of a site-specific deuterium labeled oleic acid from readily available starting materials and reagents has been developed. Several derived esters were also prepared. The labeled precursors have been used as powerful probes in the study of biosynthesis of EO in honeybees. The results of these investigations will be reported in due course.

Conflict of Interest

The authors did not report any conflict of interest.

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