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Synthesis of [16,16,16-$^2H_3$] 11-Hexadecynoic Acid and [15,15,16,16,16-$^2H_5$] (Z,Z)-11,13-Hexadecadienoic Acid and their use as Tracers in a Key Step of the Sex Pheromone Biosynthesis of the Processionary Moth.

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Abstract: The synthesis of deuterium labeled 11-hexadecynoic acid and (Z,Z)-11,13-hexadecadienoic acid and their use to investigate the biosynthetic pathway of the processionary moth sex pheromone is reported. In [16,16,16-$^2H_3$] 11-hexadecynoic acid, deuterium atoms were introduced by reaction of iodoalkyne **6b** with $(CD_3)_2CuLi$. Alkylation of terminal diyne **14b** with CD_3CD_2I followed by stereoselective reduction of the corresponding diyne to the corresponding (Z,Z) diene afforded [15,15,16,16,16-$^2H_5$] (Z,Z)-11,13-hexadecadienoic acid. GLC-MS analyses of extracts from pheromone glands incubated with these tracers revealed that the acetylenic acid, but not the dienoic acid, is a precursor of the pheromone enyne system.

Some years ago, (Z)-13-hexadecen-11-ynyl acetate (**1**) was identified as the major component of the female sex pheromone of the processionary moth, *Thaumetopoea pityocampa*, in this laboratory[1]. Ongoing research on the biosynthesis of this compound and other putative minor components, so far undetected in pheromonal gland secretion, led to the establishment of pathways depicted in Fig. 1[2,3]. By application of selectively labeled precursors in *in vivo* experiments[3], it was inferred that (Z)-11-hexadecenoic acid was formed

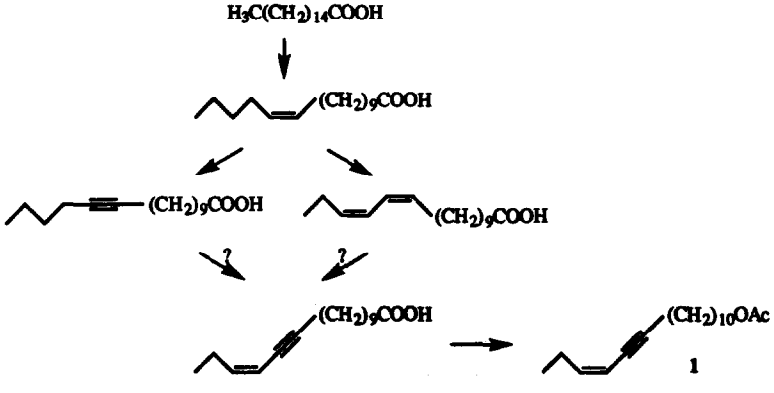


Figure 1

from palmitic acid by the action of a Z11 desaturase. This intermediate can be further desaturated at C-11 to give 11-hexadecynoic acid or transformed into (Z,Z)-11,13-hexadecadienoic acid by an unique Z13 desaturase. Although preliminary results suggested that the acetylenic acid might be a biosynthetic precursor of the major pheromone component, definitive proofs for this hypothesis and the alternative fate of the dienoic acid were lacking. In the present paper we report on the synthesis of [16,16,16-$^2H_3$] 11-hexadecynoic acid (**2**) and [15,15,16,16,16-$^2H_5$] (Z,Z)-11,13-hexadecadienoic acid (**4**) (Fig. 2) and their use as tracers in both key steps of this biosynthetic pathway.

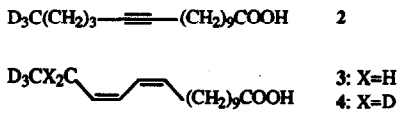
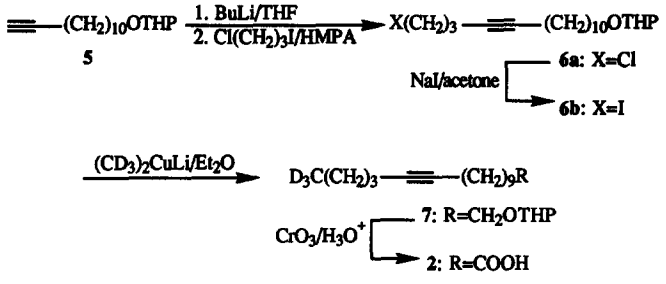


Figure 2

RESULTS AND DISCUSSION

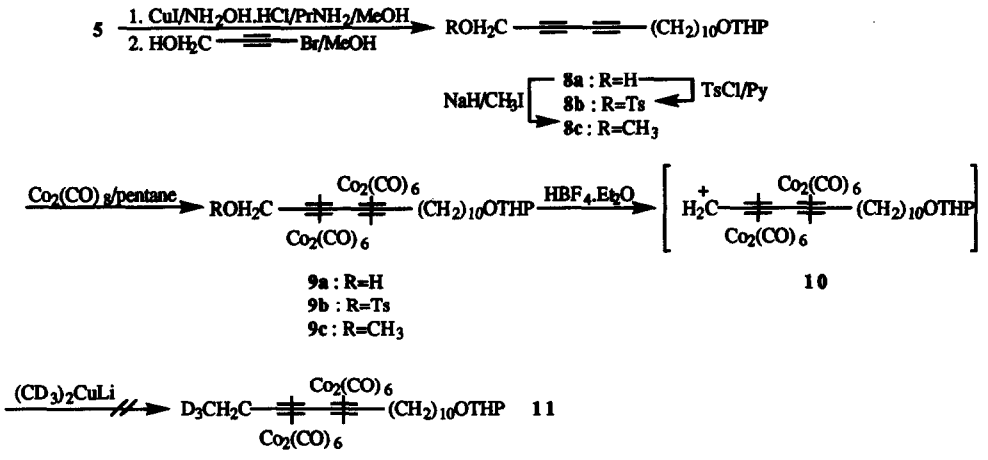
The preparation of labeled acid **2** was carried out as summarized in Scheme 1. Coupling of 1-chloro-3-iodopropane with the lithium derivative of **5** in the presence of HMPA afforded a high yield of compound **6a**, which was further transformed into the iodoalkyne **6b** by treatment with NaI in acetone. Introduction of the trideuteromethyl group was carried out by reaction of **6b** with $(CD_3)_2CuLi$, easily prepared from commercially available CD_3Li and CuI in THF. Finally, Jones oxidation of **7** afforded the expected acid **2** in high yield. This synthetic scheme requires a shorter number of steps and proceeds in higher overall yields than other previously reported syntheses of ω-trideuterated unsaturated fatty acids[4-6]. Furthermore, it can also be applied to the preparation of any ω-trideuterated alkynoic or alkenoic fatty acid.



Scheme 1

Synthesis of labeled diene **3** was attempted as indicated in Scheme 2. The Cadiot-Chodkiewick reaction of 3-bromo-2-propyn-1-ol with THP-derivative **5** afforded alkynol **8a**, which was then used to prepare the corresponding tosylate **8b** and methyl ether **8c** derivatives by standard procedures. Direct reaction of tosylate **8b** with $(D_3C)_2CuLi$ was expected to lead to the cumulene system arising from conjugated nucleophilic substitution[7]. Alternatively, we anticipated that protection of the diacetylenic system with $Co_2(CO)_8$ might favour the direct substitution of the tosylate group[8]. However, reaction of **8b** with $Co_2(CO)_8$ always led to alcohol **9a**,

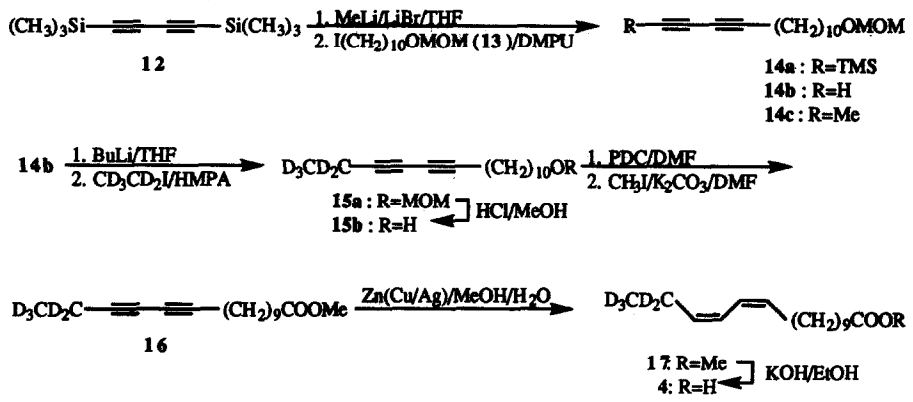
probably arising from hydrolytic quenching of the (propargyl)dicobalt hexacarbonyl cation **10**, formed by hydrolysis of the tosylate function. Consequently, we sought to achieve the introduction of the trideuteromethyl group by direct treatment of this cation with $(D_3C)_2CuLi$[9]. Unfortunately, reaction of **10**, prepared by treatment of either alkynol **9a** or methyl ether **9c** with $HBF_4.Et_2O$[10], with $(D_3C)_2CuLi$ never led to the expected compound **11**.



Scheme 2

In view of the above results, we envisaged that labeled dienoic acid **4** would also be an useful alternative tracer. Synthesis of this compound was carried out according to Scheme 3. The key intermediate diyne **15b** was prepared following the procedure described by Xu *et al*[11] for the synthesis of a series of long chain conjugated diacetylenic alcohols. Thus, monodesilylation of 1,4-bis(trimethylsilyl)-1,3-butadiyne (**12**) with CH_3Li-LiBr in THF afforded lithium (trimethylsilyl)butadiyne, which was then coupled with the MOM-protected 10-iodo-1-decanol (**13**) using DMPU as polar aprotic solvent. Surprisingly, simultaneous deprotection of the terminal diyne **14a** occurred during this coupling reaction. It is worth mentioning that, in our hands, the alternative use of HMPA as co-solvent in this reaction did not lead to the expected TMS-protected terminal diyne **14a**, as reported by Xu *et al*[11], but to the methylated product **14c** in considerable yield. Although the reason for this difference is currently being investigated, it appears that the molar ratios between **12**, MeLi-LiBr and **13** are crucial to obtain either the expected or the methylated product. Treatment of the lithium salt of **14b** with perdeuterated ethyl iodide in the presence of HMPA afforded the expected unsymmetrical conjugated diyne **15a** in 75% yield. The corresponding acetylenic alcohol **15b** was easily prepared by treatment of **15a** with concentrated HCl in methanol at room temperature. Oxidation of **15b** with pyridinium dichromate in dimethylformamide, followed by treatment with K_2CO_3 and methyl iodide in dimethylformamide afforded the corresponding methyl ester **16**. Reduction of **16** into the conjugated diene **17** was carried out by treatment with Zn(Cu/Ag)[12,13]. It is worth noting that the outcome of the transformation was influenced by the source of the metal used and that, in any case, previous activation of Zn with diluted HCl was necessary to achieve full reduction of the diyne to the diene

system. The use of unactivated Zn led to a mixture of the two possible enynes, which were not further reduced to the diene system. Finally, hydrolysis of ester **17** under usual conditions afforded the expected acid **4**.



Scheme 3

Characterization of compounds **2** and **4** was accomplished by a combination of ^{1}H and ^{13}C NMR and GC-MS methods. Interpretation of the NMR spectra was carried out by comparison with those of the corresponding undeuterated compounds[14].

Incubation of pheromone glands with the above labeled tracers, under previously reported conditions[3], revealed that 11-hexadecynoic acid, but not (Z,Z)-11,13-hexadecadienoic acid, was a precursor of the enyne system. In these studies, pheromone glands were first incubated with the tracers and, after a 3 h period, the tissues were dissected. Lipids were then extracted, methanolyzed and finally epoxydized and the extracts thus obtained were analyzed by capillary GC-MS. As indicated in our previous work[3], epoxidation of the fatty acid methyl ester extracts obtained by methanolysis of the gland lipids was necessary for reliable GC-MS analyses. In these analyses, in agreement with previous results[3], labeled epoxyacetylene **18a** (Fig. 3 and 4) was detected in extracts after incubations with deuterated acetylene **2**. However, labeled **18b** (Fig. 3 and 4) was not formed after treatment with the labeled diene **4** under identical experimental conditions. These experiments clearly indicate that the biosynthesis of enyne **1** occurs via 11-hexadecynoic acid, but not via (Z,Z)-11,13-hexadecadienoic acid. Thus, although the delta-13 desaturase present in the pheromone gland desaturates both 11-hexadecynoic and Z-11-hexadecenoic acids, the Z-11-ene desaturase appears to be specific for Z-11-hexadecenoic acid, whereas (Z,Z)-11,13-hexadecadienoic acid remains unaffected.

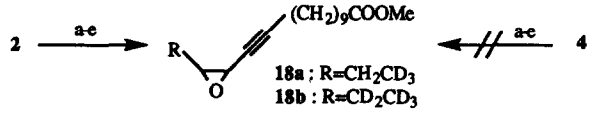


Figure 3. Bioassays with tracers **2** and **4**. a, Incubation with pheromone glands; b, Dissection of tissues; c, Extraction of lipids; d, KOH/MeOH; e, MCPBA/CH_2Cl_2.

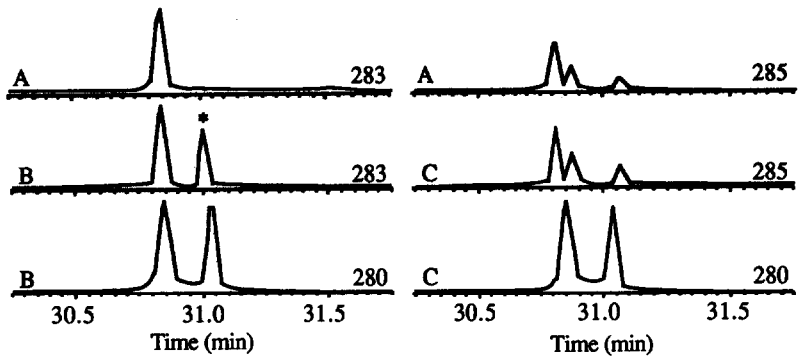


Figure 4. Capillary GC-MS traces of methanolyzed and further epoxydized lipidic extracts of *T. pityocampa* pheromone glands treated with: A, DMSO (controls); B, 2 and C, 4. The ions monitored were 280, 283 and 285, corresponding to natural, d_3 labeled and d_5 labeled methyl 12,13-epoxy-11-hexadecynoate, respectively. A synthetic sample of methyl 12,13-epoxy-11-hexadecynoate eluted at 31.03 min. Asterisk indicates d_3 labeled methyl 12,13-epoxy-11-hexadecynoate.

EXPERIMENTAL SECTION

Synthesis

Elemental analyses were performed on a Carlo Erba 1106 apparatus. FT-IR spectra were recorded in film with a Michelson Bomem MB-120 spectrometer. ^1H and ^{13}C NMR spectra were obtained in $CDCl_3$ with Varian instruments (XL200 or Unity 300 Models) at 200 or 300 MHz, respectively, for ^1H and 50 or 75 MHz for ^{13}C, respectively. Chemical shifts are given in ppm downfield from TMS. Low resolution mass spectra were determined on a Fisons MD800 mass spectrometer coupled to a gas chromatograph equipped with a 30-m HP-1 bonded-phase capillary column. HRMS were measured on an Autospec-Q mass spectrometer.

2-(15-chloro-11-pentadecynyloxy)tetrahydropyran (6a). To a solution of **5** (1 g, 3.76 mmol) in 8 mL of THF maintained at -10°C was added, under argon, 3.2 mL (3.8 mmol) of a 1.2 M solution of BuLi in hexane. The resulting solution was stirred at -10°C for 90 min and then treated with 0.5 mL (4.6 mmol) of 1-chloro-3-iodopropane dissolved in 4 mL of HMPA. After stirring for 2 h at rt, the mixture was poured onto ice and extracted with hexane. The combined organic layers were washed with brine and dried to afford, after evaporation of the solvent, 1.04 g (3.04 mmol, 81%) of **6a**, which was utilized in the next reaction without purification. Anal: calcd for $C_{20}H_{35}ClO_2$: C, 70.05; H, 10.29; Cl, 10.33; found: C, 69.95; H, 10.32; Cl, 10.33. IR 2925, 2852, 1440, 1351, 1120, 1078, 1033 cm^{-1}. ^1H NMR (300 MHz) ∂ 4.56 (c, 1H); 3.64 (t, J = 6.4 Hz, 2H); 3.31-3.90 (c, 4H); 2.32 (tt, J = 6.7 and 2.1 Hz, 2H); 2.12 (tt, J = 6.9 and 2.1 Hz, 2H); 1.91 (m, J = 6.6 Hz, 2H); 1.22-1.60 (c, 22H). ^{13}C NMR (75 MHz) ∂ 98.82 (C-2); 81.44 (C-11'); 77.96 (C-12'); 67.67 (C-1'); 62.35 (C-6); 43.82 (C-15'); 31.75 (C-14'); 30.76 (C-2'); 29.73, 29.52, 29.45, 29.10, 29.00, 28.83 (C-4 and C-4' to C-9'); 26.21 (C-3'); 25.47 (C-5); 19.69 (C-3); 18.67 (C-10'); 16.19 (C-13').

2-(15-iodo-11-pentadecynyloxy)tetrahydropyran (6b). A solution of **6a** (1.04 g, 3.04 mmol) in 15 mL of acetone was treated with 4 g (26 mmol) of NaI under reflux for 20 h. After this time, acetone was evaporated, the residue was dissolved in water and extracted with hexane. The combined organic layers were washed with brine and dried and the solvent evaporated, affording 1.23 g (2.84 mmol, 93%) of iodide **6b**, which was submitted to the next reaction without purification. Anal: calcd for $C_{20}H_{35}IO_2$: C, 55.30; H, 8.12; I, 29.21; found: C, 55.27; H, 8.16; I, 29.21. IR 2927, 2852, 1440, 1350, 1120, 1078, 1033 cm^{-1}.^1H NMR (300 MHz) ∂ 4.55 (c, 1H); 3.31-3.90 (c, 4H); 3.27 (t, J = 6.6 Hz, 2H); 2.26 (tt, J = 6.6 and 2.4 Hz, 2H); 2.10 (tt, J = 6.6 and 2.4 Hz, 2H); 1.92 (m, J = 6.6 Hz, 2H); 1.22-1.80 (c, 22H). ^{13}C NMR (75 MHz) ∂ 98.72 (C-2); 81.52 (C-11'); 77.61 (C-12'); 67.57 (C-1'); 62.21 (C-6); 32.46 (C-14'); 30.70 (C-2'); 29.68, 29.46, 29.40, 29.05, 28.93, 28.77 (C-4 and C-4' to C-9'); 26.17 (C-3'); 25.44 (C-5); 19.73 (C-3); 19.62 (C-13'); 18.64 (C-10'); 5.57 (C-15').

[16,16,16-$^2H_3$] 11-hexadecynoic acid (2). A solution of iodide **6b** (0.4 g, 0.92 mmol) in 1.5 mL of THF was treated, under Argon at -10°C, with a 0.2 M THF solution of $(CD_3)_2CuLi$, previously prepared from CuI (0.27g, 1.4 mmol) and a 0.5 M solution of CD_3Li in hexane (3 mL, 1.5 mmol). The reaction mixture was stirred at 0°C for 30 min and then at rt for 14 h. After this time, a saturated solution of NH_4Cl was added and the aqueous layer was extracted with hexane. Removal of solvent furnished 0.29 g of an oil, which was dissolved in 5 mL of acetone and treated, at -5°C, with a solution of CrO_3 (1.05 g, 10.5 mmol) and H_2SO_4 (0.81 mL) in water (2.7 mL). After stirring for 20 h, acetone was evaporated and the residue extracted with CH_2Cl_2. The organic layers were washed with brine and dried. Evaporation of the solvent furnished 0.19 g (0.75 mmol, 82%) of acid **2**. Anal: calcd for $C_{17}H_{27}D_3O_2$ (Methyl ester): C, 75.79; H/D, 11.35; found: C, 75.78; H/D, 11.40. IR 2927, 2856, 2213, 2125, 2075, 1710, 1461, 1433, 1411 cm^{-1}. ^1H NMR (300 MHz) ∂ 10.81 (b, 1H); 2.33 (t, J = 7.5 Hz, 2H); 2.13 (c, 4H); 1.62 (c, 2H); 1.23-1.49 (c, 16H). ^{13}C NMR (75 MHz) ∂ 180.36 (C-1); 80.17, 80.11 (C-11 and C-12); 34.07 (C-2); 31.16 (C-14); 29.28, 29.15, 29.10, 29.04, 29.01, 28.77 (C-4 to C-9); 24.63 (C-3); 21.63 (C-15); 18.70, 18.42 (C-10 and C-13); 12.71 (hept, J = 19 Hz, C-16).

15-(2-tetrahydropyranyloxy)-2,4-pentadecadiyn-1-ol (8a). A solution of 0.37 g (1.91 mmol) of CuI and 0.75 g (10.8 mmol) of $NH_2OH.HCl$ in 9 mL (6.5 g, 0.12 mol) of $PrNH_2$, cooled at 0°C, was treated with 4 g (15.0 mmol) of **5** dissolved in 64 mL of MeOH. The mixture was stirred at 0°C for 2 h and then was added a solution of 3-bromo-2-propyn-1-ol (2.25 g, 16.5 mmol) in 8 mL of MeOH. The reaction mixture was stirred at 50 °C for 16 h, cooled to room temperature and treated with a solution of NaCN (2.4 g, 49 mmol) in H_2O (200 mL) and extracted with CH_2Cl_2. Evaporation of the solvent gave an oil, which was purified by column chromatography on Al_2O_3 eluting with CH_2Cl_2:MeOH (95/5) to afford 2.5 g (7.8 mmol, 50%) of pure **8a**. Anal: calcd for $C_{20}H_{31}O_3$: C, 74.96; H, 10.06; found: C, 74.67; H, 10.10. IR 3407, 2927, 2854, 2254, 1351, 1031 cm^{-1}. ^1H NMR (300 MHz) ∂ 4.58 (c, 1H); 4.31 (s, 2H); 3.32-3.94 (c, 4H); 2.28 (t, J = 6.8 Hz, 2H); 1.22-1.84 (c, 22H). ^{13}C NMR (50 MHz) ∂ 98.71 (C-2'); 81.37, 73.90, 70.40, 64.40 (C-2 to C-5); 67.65 (C-15); 62.21 (C-6'); 51.10 (C-1); 30.63 (C-14); 29.58, 29.34, 29.30, 29.23, 28.84, 28.61, 27.99 (C-7 to C-12 and C-4'); 26.10 (C-13); 25.35 (C-5'); 19.50 (C-3'); 19.11 (C-6).

15-(2-tetrahydropyranyloxy)-2,4-pentadecadiynyl p-toluenesulphonate (8b). Treatment of alcohol **8a** (1.5 g, 4.7 mmol) with TsCl (1.0 g, 5.3 mmol) and 85% KOH (1.8 g, 27 mmol) in 20 mL of Et_2O at -60°C for 30 min afforded, after extraction with hexane, 1.9 g (4 mmol, 85%) of **8b**. Anal: calcd for $C_{27}H_{37}SO_6$: C, 68.32; H, 8.07; S, 6.75; found: C, 68.03; H, 8.08; S, 6.51. IR 2927, 2854, 2256, 1371, 1176 cm^{-1}. ^1H NMR (200 MHz) ∂ 7.81 (d, J = 8.2 Hz, 2H); 7.35 (d, J = 8.2 Hz, 2H); 4.75 (s, 2H); 4.57 (c, 1H); 3.31-3.94 (c, 4H); 2.45 (s, 3H); 2.25 (t, J = 6.8 Hz, 2H); 1.20-1.85 (c, 22H). ^{13}C NMR (50 MHz) ∂ 145.11, 132.75, 129.79, 128.08 (Ar-C); 98.80 (C-2'); 83.50, 73.62, 66.36, 63.81 (C-2 to C-5); 67.60 (C-15); 62.31 (C-6'); 58.17 (C-1); 30.73 (C-14); 29.68, 29.43, 29.38, 29.32, 28.95, 28.71, 27.91 (C-7 to C-12 and C-4'); 26.17 (C-13); 25.44 (C-5'); 21.63 (CH_3-) 19.66 (C-3'); 19.11 (C-6).

15-(2-tetrahydropyranyloxy)-2,4-pentadecadiynyl methyl ether (8c). To a dispersion of NaH (98 mg, 2.43 mmol) in THF (2 mL) was added, at 45°C, 0.160 mL (2.62 mmol) of CH_3I and then 0.46 g (1.44 mmol) of **8a** dissolved in 1.5 mL of THF. After 30 min of stirring at 45°C, the mixture was extracted with Et_2O to furnish 0.47 g (0.41 mmol, 97%) of ether **8c**. Anal: calcd for $C_{21}H_{34}O_3$: C, 75.40; H, 10.24; found: C, 75.52; H, 10.30. IR 2927, 2854, 2254, 1353, 1186, 1120, 1101, 1033 cm^{-1}. ^1H NMR (200 MHz) ∂ 4.54 (c, 1H); 4.11 (s, 2H); 3.25-3.90 (c, 4H); 3.35 (s, 3H); 3.24 (t, J = 6.8 Hz, 2H); 1.20-1.86 (c, 22H). ^{13}C NMR (50 MHz) ∂ 98.73 (C-2'); 81.12, 71.43, 64.38 (C-2 to C-5); 67.65 (C-15); 62.21 (C-6'); 60.10 (C-1); 57.55 (CH_3); 30.70 (C-14); 29.65, 29.42, 29.35, 29.30, 28.96, 28.72, 28.04 (C-7 to C-12 and C-4'); 26.13 (C-13); 25.42 (C-5'); 19.60 (C-3'); 19.14 (C-6).

Dicobalthexacarbonyl complex 9a. Protection reactions of **8a-c** with $Co_2(CO)_8$ and preparation of (propargyl)dicobalt hexacarbonyl cation **10** from **9a** and **9c** were carried out as reported elsewhere[15,16]. Anal: calcd for $C_{32}H_{32}Co_4O_{15}$: C, 43.07; H, 3.61; found: C, 42.97; H, 3.68. IR 3438 (bb), 2929, 2854, 2098, 2079, 2052, 2019, 1458, 1120, 1026 cm^{-1}. ^1H NMR (300 MHz) ∂ 4.90 (d, J = 5.7 Hz, 2H); 4.58 (c, 1H); 3.34-3.92 (c, 4H); 2.83 (t, J = 8 Hz, 2H); 1.2-2.0 (c, 22H). ^{13}C NMR (50 MHz) ∂ 199.22 (bb, $2(CO)_6$); 107.00, 102.32, 90.73, 90.23 (C-2 to C-5); 98.87 (C-2'); 67.69 (C-15); 63.63 (C-1); 62.31 (C-6'); 33.58, 31.84 (C-1 and C-6); 30.84 (C-14); 29.78, 29.54, 29.50, 29.43, 29.34 (C-9 to C-12 and C-4'); 26.24 (C-13); 25.57 (C-5'); 19.71 (C-3').

1-Iodo-10-(methoxymethoxy)decane (13). To a solution of 2.6 g (11.0 mmol) of 10-bromodecan-1-ol in 20 mL of dimethoxymethane was added 0.27 g (3.1 mmol) of LiBr and a catalytic amount of TsOH. The mixture was stirred at room temperature for 20 h and then treated with a saturated solution of NaCl and extracted with

hexane. Evaporation of the solvent afforded 2.96 g of a residue, which was treated with NaI/acetone, as described for the preparation of **6b**, to afford 2.2 g (6.7 mmol, 92%) of **13**. Anal: calcd for $C_{10}H_{21}IO$: C, 42.27; H, 7.45; I, 44.66; found: C, 42.20; H, 7.50; I, 44.65. IR 2927, 2854, 1464, 1149, 1110, 1047, 920 cm^{-1}. ^1H NMR (200 MHz) ∂ 4.56 (s, 2H); 3.46 (t, J = 6.5Hz, 2H); 3.30 (s, 3H); 3.13 (t, J = 7.1 Hz); 1.77 (m, 2H); 1.53 (m, 2H); 1.20-1.37 (c, 12H). ^{13}C NMR (50 MHz) ∂ 96.20 (OCO); 67.64 (C-10); 54.88 (CH_3); 33.40 (C-2); 30.34 (C-3); 29.58, 29.32, 29.23, 28.28 (C-4 to C-7); 26.04 (C-8); 7.01 (C-1).

14-(Methoxymethoxy)-1,3-tetradecadiyne (14b). A solution of **12** (0.2 g, 1.03 mmol) in 4 mL of THF was cooled to -78°C and 0.7 mL (1.05 mmol) of a 1.5 M solution of MeLi-LiBr in Et_2O was added under Ar. The mixture was stirred at room temperature for 4 h, cooled to -78°C and treated with 0.357 g (1.09 mmol) of **13** dissolved in 2 mL of DMPU. The resulting mixture was stirred at room temperature for 18 h, treated with a saturated solution of NH_4Cl and extracted with hexane. Solvent removal afforded 0.2 g (0.8 mmol, 78%) of diacetylene **14b**. Anal: calcd for $C_{14}H_{22}O$ (Alcohol): C, 81.50; H, 10.75; found: C, 81.43; H, 10.76. IR 3313, 3238, 2927, 2854, 2225, 1465, 1145, 1110, 1043, 918 cm^{-1}. ^1H NMR (300 MHz) ∂ 4.62 (s, 2H); 3.51 (t, J=6.6 Hz, 2H); 3.36 (s, 3H); 2.25 (dt, J=7.0 and 1.2 Hz, 2H); 1.96 (t, J=1.2 Hz, 1H); 1.27-1.60 (c, 16H). ^{13}C NMR (75 MHz) ∂ 96.32 (OCO), 78.53 (C-4), 68.47 (C-2), 67.84 (C-14); 64.61 (C-3), 64.43 (C-1), 55.03 (CH3), 29.72 (C-13), 29.47, 29.36, 29.34, 28.98, 28.75, 27.94 (C-6 to C-11), 26.16 (C-12), 19.00 (C-5).

[1,1,1,2,2-$^2H_5$] 11,13-hexadecadiyn-1-ol (15b). To a solution of 0.2 g (0.8 mmol) of diyne **14b** in 3 mL of THF, cooled at -20°C, was added, under Ar, 0.68 mL (0.85 mmol) of a 1.25 M solution of BuLi in hexane. Stirring was maintained at this temperature for 1.5 h and then was added 0.07 mL (0.88 mmol) of D_3CD_2CI dissolved in 2 mL of HMPA. The mixture was stirred at room temperature for 3 h, treated with a saturated solution of NH_4Cl and extracted with hexane to afford 0.17 g of crude, which was treated with 1.5 mL of 37% HCl in 15 mL of MeOH for 16 h. After this time, solvent was removed and the residue was extracted with CH_2Cl_2, furnishing 0.143 g (0.6 mmol, 75%) of **15b**. Anal: calcd for $C_{16}H_{21}D_5O$: C, 80.29; H/D, 11.18; found: C, 80.10; H/D, 11.30. IR 3350, 2927, 2854, 2238, 1465, 1055 cm^{-1}. ^1H NMR (300 MHz) ∂ 3.58 (t, J=6.6 Hz, 2H); 2.20 (t, J=6.9 Hz, 2H); 1.49 (c, 4H); 1.20-1.40 (c, 12H). ^{13}C NMR (75 MHz) ∂ 78.46 (C-14), 77.45 (C-11), 65.07 (C-12), 64.58 (C-13), 62.80 (C-1), 32.63 (C-2), 29.43, 29.30, 28.96, 28.70, 28.21 (C-4 to C-9), 25.63 (C-3), 19.05 (C-10), 18.90 (quint, J=22 Hz, C-15), 12.29 (hept, J=20 Hz, C-16).

Methyl [15,15,16,16,16-$^2H_5$] 11,13-hexadecadiynoate (16). Compound **15b** (45 mg, 0.19 mmol) was treated with a solution of 0.57 g (1.52 mmol) of pyridinium dichromate in dimethylformamide (2 mL). After stirring for 16 h at rt, the mixture was acidified with HCl 3N and extracted with CH_2Cl_2. The organic layer was washed with water and then with brine and dried (Na_2SO_4). Solvent removal afforded a crude that was dissolved in 1 mL of dimethylformamide and treated with K_2CO_3 (0.1 g) for 10 min and then with 50 µL de CH_3I. The reaction mixture was stirred at rt for 16 h. After this time, extraction with hexane furnished crude ester **16**, which was purified by flash chromatography. Elution with hexane/AcOEt (20:1) gave 24 mg (0.09 mmol, 48%) of ester **16**. HRMS: calcd for $C_{17}H_{21}D_5O_2$: 267.224664; found: 267.224822. IR 2927, 2858, 2238, 1739, 1259, 1016, 800 cm^{-1}. ^1H NMR (300 MHz) ∂ 3.66 (s, 3H); 2.30 (t, J = 7.5 Hz, 2H); 2.24 (t, J = 6.9 Hz, 2H); 1.27-1.62 (c, 14H). ^{13}C NMR (75 MHz) ∂ 174.22 (C-1); 78.50 (C-14); 77.46 (C-11); 65.13 (C-12); 64.60 (C-13); 51.36 (CH_3); 34.01 (C-2); 29.18, 29.11, 29.03, 28.93, 28.70, 28.23 (C-4 to C-9); 24.86 (C-3); 19.09 (C-10).

Methyl [15,15,16,16,16-$^2H_5$] (Z,Z) 11,13-hexadecadienoate (17). Activated Zn (2 g) was suspended in water (3 mL) and was added, under Ar, 50 mg of $Cu(OAc)_2$ H_2O. After 15 min of stirring was added $AgNO_3$ (50 mg) and stirring was maintained for 30 more min. After this time, the metal was filtered under vacuum and Ar and washed, subsequently, with water (2 x 9 mL), MeOH (2 x 9 mL), acetone (2 x 9 mL) and Et_2O (2 x 9 mL). The Zn thus prepared was immediately transferred to a flask and suspended in 10 mL of MeOH/H_2O (1:1) and a solution of **16** (115 mg, 0.43 mmol) in MeOH (1 mL) was added. The mixture was stirred at rt under Ar for 72 h, and then filtered through celite using MeOH. The solvent was removed under vacuum, the residue was treated with water and extracted with hexane and washed with brine. Removal of the solvent gave 63 mg (0.23 mmol, 54%) of **17**. HRMS: calcd for $C_{17}H_{25}D_5O_2$: 271.255964; found: 271.256032. IR 3033, 2999, 2925, 2854, 2223, 2131, 2069, 1741, 1596, 1461, 1434, 1195, 1170, 1114, 1056, 702 cm^{-1}. ^1H NMR (300 MHz) ∂ 6.15-6.28 (c, 2H); 5.31-5.49 (c, 2H); 3.65 (s, 3H); 2.28 (t, J = 7.3 Hz, 2H); 2.14 (c, 2H); 1.60 (c, 2H), 1.20-1.40 (c, 12H). ^{13}C NMR (75 MHz) ∂ 174.23 (C-1), 133.43 (C-14), 132.00 (C-11); 123.40 (C-12); 122.99 (C-13); 51.36 (OCH_3); 34.04 (C-2); 29.57, 29.37, 29.33, 29.19, 29.14, 29.08 (C-4 to C-9); 27.40 (C-10); 24.89 (C-3); 19.80 (quint, J=19 Hz, C-15); 13.06 (hept, J=19 Hz, C-16).

[15,15,16,16,16-$^2H_5$] (Z,Z) 11,13-hexadecadienoic acid (4). Treatment of **17** (55 mg, 0.2 mmol) with 0.5 mL of 2.5M KOH in 80% EtOH for 18 h furnished, after the usual work up, 46 mg (0.18 mmol, 90%) of

labeled acid **2**. HRMS: calcd for $C_{16}H_{23}D_5O_2$: 257.240314; found: 257.241041. IR 3078, 3035, 3001, 2925, 2854, 2223, 2069, 1708, 1456, 1411, 1286, 943, 702 cm^{-1}. ^1H NMR (300 MHz) ∂ 6.17-6.29 (c, 2H); 5.40-5.48 (c, 2H); 2.34 (t, J = 7.5 Hz, 2H); 2.16 (c, 2H); 1.62 (c, 2H), 1.25-1.39 (c, 12H) ^{13}C NMR (75 MHz) ∂ 180.34 (C-1), 133.50 (C-14), 132.06 (C-11); 123.43 (C-12); 123.02 (C-13); 34.07 (C-2); 29.60, 29.40, 29.34, 29.21, 29.19, 29.01 (C-4 to C-9); 27.43 (C-10); 24.64 (C-3).

Bioassays

Pheromone glands were topically treated with either tracer **2** or **4** following previously reported procedures[3]. Lipids extraction and methanolysis were performed as described elsewhere[3]. Preparation of epoxyfatty acid methyl esters was carried out by treatment of the methanolyzed extracts (5 female equivalents) with 40 µL of a 0.1 M solution of MCPBA in CH_2Cl_2 at room temperature for 60 min. Solvent was then removed under nitrogen, hexane was added and the solution was wased subsequently with saturated solutions of $NaHCO_3$ and NaCl. The resulting hexane solution was concentrated and 1 female equivalent analyzed by capillary GC-MS as reported previously[3].

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