Synthesis of *gem*-Dideuterated Tetradecanoic Acids and Their Use in Investigating the Enzymatic Transformation of (*Z*)-11-Tetradecenoic Acid into (*E*,*E*)-10,12-Tetradecadienoic Acid

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Received June 4, 2001

We report the preparation of the deuterated tetradecanoic acids [2,2,3,3-$^2H_4$]-, [2,2,3,3,10,10-$^2H_6$]-, and [2,2,3,3,13,13-$^2H_6$]-tetradecanoic acids (**1**, **2**, and **3**, respectively) and their use to investigate the mechanism of the enzymatic transformation of (*Z*)-11-tetradecenoic acid into (*E*,*E*)-10,12-tetradecadienoic acid. Probes **2** and **3** were prepared from intermediate ketones **7** and **10**, which were transformed into the labeled bromides **17** and **18** by reduction with $NaBD_4$, tosylation of the resulting alcohol, replacement of the tosyloxy group by deuteride with $LiAlD_4$, hydrolysis, and reaction with *N*-bromosuccinimide. The resulting bromides were converted into the α-acetylenic esters **21** and **22**, respectively, and the additional deuterium labels were introduced by reduction of the conjugated triple bond with Mg in deuterated methanol. The same sequence of reactions starting with 11-bromoundecane afforded **27**. Saponification of the labeled esters **23**, **24,** and **27** gave the deuterated acids **2**, **3**, and **1**, respectively. The results of the biochemical experiments showed that C10−H removal, but not elimination of C13−H, was sensitive to deuterium substitution in the transformation of (*Z*)-11-tetradecenoic acid into (*E*,*E*)-10,12-tetradecadienoic acid, which is consistent with the hypothesis that this desaturase reaction involves a first slow, C10−H bond cleavage, with probable formation of an unstable allylic intermediate, followed by a second fast C13−H bond removal and concomitant rearrangement.

Introduction

Fatty acyl desaturases are non-heme iron-containing oxygen-dependent enzymes involved in the regio- and stereoselective introduction of double bonds in fatty acyl aliphatic chains.[1] Although most desaturases use saturated fatty acyl derivatives, desaturases that transform unsaturated substrates do also occur in nature. In the resulting polyunsaturated fatty acids, double bonds tend to be methylene-interrupted and in the (*Z*) configuration. However, fatty acids containing conjugated olefinic bonds with either (*Z*) or (*E*) double bonds have also been reported in algae,[2,3] plants, and insects. In plants, various conjugated linolenic acid isomers accumulate in seeds; examples include (*Z*,*E*,*E*)-(9,11,13)-octadecatrienoic acid,[4] (*Z*,*E*,*Z*)-(9,11,13)-octadecatrienoic acid, and (*Z*,*E*,*Z*)-(8,-10,12)-octadecatrienoic acid.[5,6] In insects, moth pheromone glands contain desaturases that catalyze the formation of conjugated dienoic acids, such as (*E*,*Z*)-10,-12-hexadecadienoic acid,[7,8] (*Z*,*Z*)-11,13-hexadecadienoic acid,[9] and (*E*,*E*)-8,10-dodecadienoic acid.[10]

The sex pheromone blend of the moth *Spodoptera littoralis* is an especially interesting example, since it contains two different conjugated diene systems: (*Z*,*E*)-9,11-tetradecadienoate[11,12] and (*E*,*E*)-10,12-tetradecadienoate.[13] Biosynthesis of these compounds takes place from myristic acid, which is desaturated to both (*E*)- and (*Z*)-11-tetradecenoic acid.[12] The (*E*)-isomer is then converted into the (*Z*,*E*)-9,11-tetradecadienoate,[12] whereas the (*Z*)-isomer is transformed into the (*E*,*E*)-10,12-tetradecadienoate.[13] In the (*E*)-monoene desaturation, both the location and stereochemistry of the substrate double bond are maintained in the diene product, whereas the (*Z*)-monoene desaturation involves the formation of a second double bond with concomitant rearrangement of the substrate monounsaturation.

In previous papers,[14,15] we have reported on the cryptoregiochemistry[14] and stereospecificity[15] of the above (*E*)-monoene desaturase. This reaction occurs by initial abstraction of the pro-(*R*) hydrogen atom at C9 followed by rapid elimination of the pro-(*R*) hydrogen atom at C10. In this paper, we report on the cryptoregiochemistry of the desaturation of (*Z*)-11-tetradecenoate into (*E*,*E*)-10,-12-tetradecadienoate. The putative existence of a single

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10.1021/jo010560w CCC: $20.00
Published on Web 10/30/2001

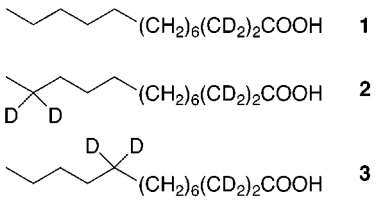
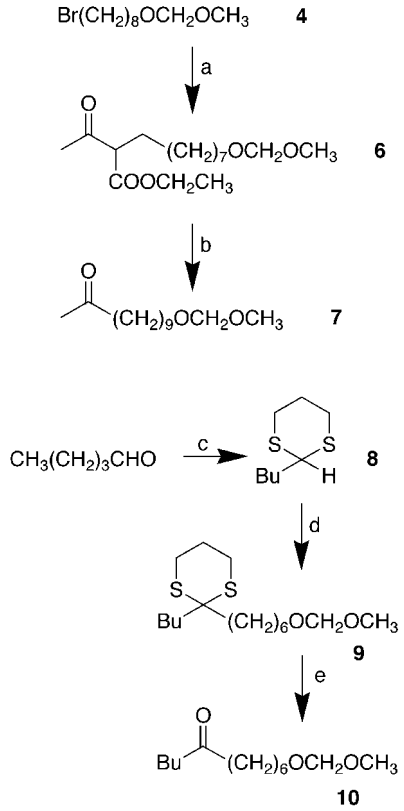


Figure 1. Deuterated myristic acids **1**−**3**.

Scheme 1. Synthesis of Ketones 7 and 10[a]



[a] Reagents and conditions: (a) Na/ethanol/ethyl acetoacetate, reflux, then **4**, reflux, 26 h (60%); (b) Na/ethanol, then ethanediol, then **6**/90 °C/30 min (60%); (c) BF_3·Et_2O/AcOH/propanedithiol/$CHCl_3$, reflux, 16 h (93%); (d) BuLi −30 °C/90 min, then $Br(CH_2)_6OCH_2OCH_3$ (**5**)/THF, −78 °C, 1 h (81%); (e) NBS, acetone/water, −20 °C (85%).

monoene desaturase that forms both the (*Z*,*E*)-9,11- and the (*E*,*E*)-10,12-diene systems from the corresponding (*E*)- and (*Z*)-11-monoene substrates is also discussed.

Results

Synthesis of Deuterium-Labeled Probes. The required deuterium-labeled compounds **1**−**3** (Figure 1) for the competition experiments were prepared as depicted in Schemes 2 and 3 from intermediate ketones **7** and **10**, obtained as shown in Scheme 1. Ketone **7** was obtained by coupling of bromoalkylderivative **4** with the anion of ethyl acetoacetate[16] generated with sodium ethoxide, afforded the *β*-ketoester **6**. Treatment of **6** with sodium ethoxide in ethylene glycol[17] at 90 °C furnished the expected ketone **7** in 60% yield. This procedure afforded better yields than other attempted dealkyloxycarbonylation conditions, such as DABCO/xylene[18] or lithium chloride/dimethylsulfoxide.[19,20]

Ketone **10** was prepared from properly functionalized dithiane **9** obtained by coupling reaction of 1-Bromo-7,9-dioxadecane (**5**) with the anion of dithiane **8**, generated by reaction with BuLi.[21] Final cleavage of **9** with NBS[22] afforded ketone **10** (Scheme 1).

Introduction of deuterium label was carried out in three steps (Scheme 2). Reduction of ketones **7** and **10** with $NaBD_4$,[23] followed by tosylation[24] of the resulting alcohols **11** and **12**, respectively, and replacement of the tosyloxy group by deuteride with $LiAlD_4$.[25] The alcohols **15** and **16** were recovered by hydrolysis[26] and then converted into the corresponding bromides[27] **17** and **18**, respectively. Reaction of bromoalkanes **17** and **18** with lithium acetylide[28] furnished the labeled terminal alkynes **19** and **20**. The same reaction starting from 1-bromoundecane gave 1-tridecyne (**25**) (Scheme 3). Reaction of methyl chloroformate with the lithium salts of terminal alkynes[29] **19**, **20**, and **25** afforded the α-acetylenic esters **21**, **22**, and **26**, respectively. The additional deuterium labels were introduced by reduction of the conjugated triple bond with Mg in deuterated methanol.[30] Labeled esters **23**, **24**, and **27** were thus obtained and were finally saponified to the deuterated acids **2**, **3**, and **1**, respectively.

The presence of deuterium in the probes synthesized was confirmed by GC−MS analysis of the corresponding methyl esters ($M^{\cdot+}$: **2** and **3**, *m*/*z* 248; **1**, *m*/*z* 246). Furthermore, the presence of deuterium at C2 and C3 in all probes was confirmed by the multiplicity and coupling constants of their corresponding ^{13}C NMR signals at 33.4 and 23.8 ppm, respectively (Table 1). Likewise, the occurrence of a CD_2 group at C13 of **2** was assessed by the signal at 21.8 ppm (quintet, $J = 19.0$ Hz) and the existence of a CD_2 moiety in **3** was confirmed by the signal at 24.2 ppm (quintet, $J = 20.0$ Hz) in the ^{13}C NMR spectra of these compounds.

Isotope Effect Experiments. The intermolecular primary kinetic isotope effects (KIE) are determined in competitive experiments between one substrate and the same substrate dideuterated at the appropriate sites of desaturation. In this case, probes were labeled myristic acids **1**, **2**, and **3**, which, after activation to the corre-

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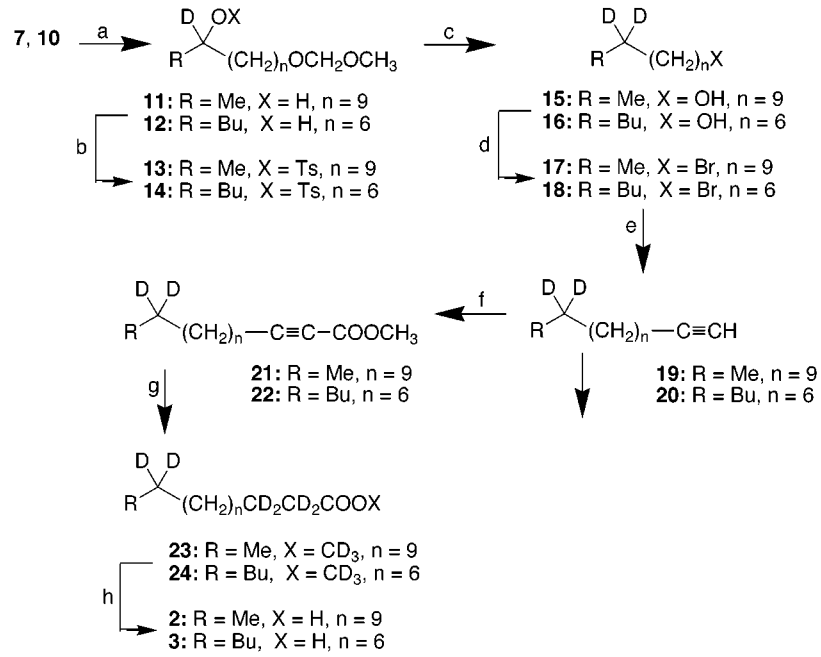
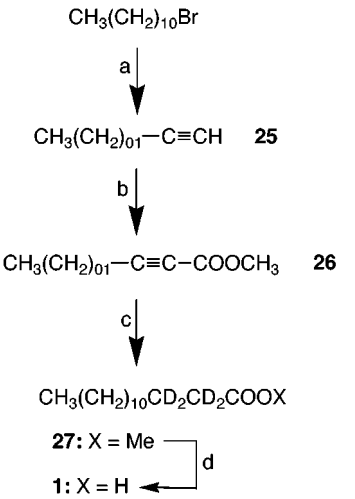
Scheme 2. Synthesis of Acids 2 and 3[a]



[a] Reagents and conditions: (a) $NaBD_4$/MeOH, 0 °C, 1 h **(11**, 95%; **12**, 92%); (b) $N(CH_3)_3$−HCl/TEA/*p*-TsCl/CH_2Cl_2, 0 °C, 90 min (**13**, 73%; **14**, 73%); (c) $LiAlD_4/Et_2O$, rt, 3 h **(15**, 78% **16**, 80%), then hydrolysis; (d) NBS/DMF, 50 °C, 15 min (**17**, 70%; **18**, 74%); (e) LiC≡CH/NH_3/DMSO, then **17** or **18**/DMSO, rt, 90 min (**19**, 60%; **20**, 58%); BuLi/THF, −78 °C, 30 min, then methyl chloroformate/THF, rt, 1 h (**21**, 77%; **22**, 78%); (g) Mg/CD_3OD, rt, 24 h **(23**, 70%; **24**, 72%); (h) KOH/MeOD, rt, 16 h **(2**, 70%; **3**, 72%).

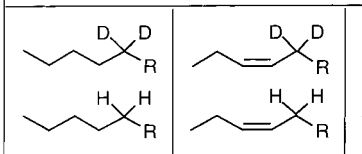
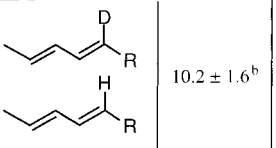
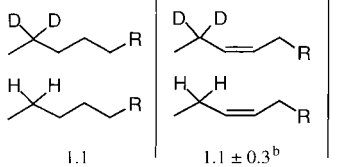
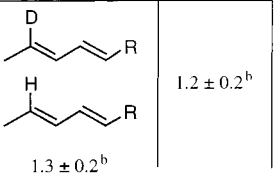
Scheme 3. Synthesis of Acid 1[a]



[a] Reagents and conditions: (a) LiC≡CH/NH_3/DMSO, rt, 90 min (82%); (b) BuLi/THF, −78 °C, 30 min, then methyl chloroformate/THF, rt, 1 h (87%); (c) Mg/CD_3OD, rt, 24 h (68%); (d) KOH/MeOD, rt, 12 h (74%).

sponding CoA esters, are transformed, at equal rates (Table 1), into the respective (*Z*)-11-tetradecenoic acid derivatives, which are the actual enzyme substrates. The base methanolyzed lipidic extracts, prepared after incubation of the glands with approximately 1/1 mixture of **1** and each of the hexadeuterated substrates **2** or **3**, were analyzed by GC−MS under the selected ion monitoring mode. Integration of peaks corresponding to the tetradeuterated and pentadeuterated methyl (*E*,*E*)-10,12-tetradecadienoates formed from each mixture afforded the data required to determine the KIEs and, subsequently, allowed to determine the site of initial oxidation in this desaturation reaction. As shown in Table 1, a large isotope effect was observed for the carbon−hydrogen bond cleavage at C10, but negligible isotope discrimination occurred in the removal of C13−H.

Table 1. Transformation of 1−3 into d_4 and d_6 (*Z*)-11-Tetradecenoic Acids and d_4 and d_5 (*E*,*E*)-10,12-Tetradecadienoic Acids in the Competitive Experiments

Probes ratio[a]	Δ11 → MD substrates ratio[a]	MD → MD products ratio[a]	KIE
 1.2	1.2 ± 0.2[b]	 12.2 ± 1.6[b]	10.2 ± 1.6[b]
 1.1	1.1 ± 0.3[b]	 1.3 ± 0.2[b]	1.2 ± 0.2[b]

[a] Ratios between d_4 and d_6 probes in the mixture used and ratios between d_4 and d_6 (*Z*)-11-tetradecenoic acids formed by the Δ^{11} desaturase (Δ11) and d_4 and d_5 dienes formed by the monoene desaturase (MD). [b] For the probes, the ratio corresponds to a single determination with a BF_3−MeOH-derivatized sample of the used mixture. For the metabolites, data are mean ± standard deviation of three different experiments. Product KIEs were calculated as described in the Experimental Section. R = $(CH_2)_6(CD_2)_2COOH$.

Discussion

The same methodology previously validated for other desaturases[14,31−33] was followed in this work to determine the site of initial oxidation in the transformation of (*Z*)-11-tetradecenoic acid into (*E*,*E*)-10,12-tetradecadienoic

Table 2. ^{13}C NMR Chemical Shifts for Probes 1−3

	carbon atom						
compd	C1	C2	C3	C4−C9, C11, C10	C12	C13	C14
1	180.5	33.4[a]	23.8[a]	29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 28.8	31.9	22.6	14.1
2	180.2	33.4[c]	23.8[b]	29.7, 29.6, 29.4, 29.1, 28.8	31.7	21.8[b]	13.8
3	180.6	33.4[a]	23.8[a]	29.5, 29.4, 29.2, 29.1, 28.8	31.8	22.6	14.1

[a] Quintet, J = 20.0 Hz. [b] Quintet, J = 19.0 Hz. [c] Quintet, J = 18.0 Hz.

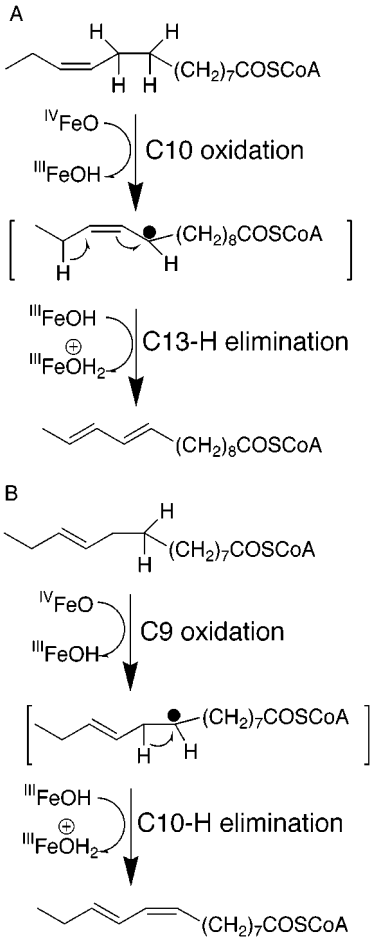


Figure 2. Mechanistic model for the formation of (*E*,*E*)-10,-12-tetradecadienoic acid from (*Z*)-11-tetradecenoic acid (A). The previously studied[14] enzymatic transformation of (*E*)-11-tetradecenoic acid into (*Z*,*E*)-9,11-tetradecadienoic acid is also shown (B) for comparison.

acid. The results of the competitive experiments showed that C10−H removal, but not elimination of C13−H, was sensitive to deuterium substitution. In agreement with the model proposed by Buist et al.,[31,34] these results are consistent with the hypothesis that this monoene desaturase reaction involves a first slow, isotope sensitive C10−H bond cleavage, with probable formation of an unstable allylic intermediate, followed by a second fast C13−H bond removal and concomitant rearrangement (Figure 2).

In contrast, the studies conducted on the (*Z*)-9 desaturation of (*E*)-11-tetradecenoic acid had shown that the rate-determining step was the rupture of C9−H,[14] followed by formation of a homoallylic intermediate and rapid loss of C10−H. The different cryptoregiochemistry found for both monoene desaturation reactions may arise from the proximity of either C9 or C10 to the oxidizing iron cluster at the active site of the enzyme. The different relationship of either C9 or C10 with respect to the iron cluster could result from the different conformations adopted by the substrates at the enzyme active site. Assuming the existence of two discrete monoene desaturases, the different substrate conformations would be imposed by the specific amino acid sequences of each enzyme. Nevertheless, it is also possible that the spatial location of C9 and C10 with respect to the iron cluster is determined by the geometry of the substrate double bond and that a single monoene desaturase is sufficient for the synthesis of both dienes. In this case, in the (*E*)-isomer, C9 would be closer to the oxidizing iron center, resulting in the formation of the homoallylic intermediate upon C9 oxidation. The (*Z*,*E*)-diene would finally result from elimination of C10−H. In contrast, (Z)-11-tetradecenoate would be accommodated in the desaturase active site in such a way that C10 would be near the iron cluster with formation of the allylic intermediate upon oxidation. Subsequent loss C13−H with rearrangement would afford the (*E*,*E*)-10,12-diene system. For the time being, there is no genetic evidence for the existence of a single monoene desaturase in *S. littoralis*. However, it has been demonstrated that both (*Z*)-11- and (*E*)-11-monoene fatty acids can be afforded by a single Δ^{11} desaturase and that the stereospecificity of this reaction depends on the substrate chain length.[33] Therefore, it is not unreasonable to propose that a single monoene desaturase might also be able to give both (*Z*,*E*)-9,11- and (*E*,*E*)-10,12-tetradecadienoates, depending on the geometry of the substrate double bond. Should this be the case, *S. littoralis* female moths would biosynthesize their complex pheromone blend by means of only two desaturases, a Δ^{11} desaturase, whose stereospecificity depends on the substrate chain length, and a monoene desaturase, whose cryptoregiochemistry, and hence the final reaction product, would depend on the geometry of the double bond of the substrate. In this way, the composition of the pheromone blend would be regulated not by the existence of several different desaturases, but by the supply of specific substrates to only two desaturase enzymes. Cloning and functional expression of the cDNA encoding the monoene desaturase(s) of *S. littoralis* will be conducted soon to test this hypothesis.

Experimental Section

General Methods. All ^{1}H NMR spectra were acquired at 300 MHz and ^{13}C NMR spectra at 75 M Hz in recently neutralized $CDCl_3$ solutions, and chemical shifts are given in

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ppm using as internal standards $Si(CH_3)_4$ for ^1H and $CDCl_3$ for ^{13}C. Gas chromatography coupled to mass spectrometry (GC−MS) analysis was performed by electron impact (EI) at 70 eV, using a nonpolar HP-1 capillary column (30 m × 0.20 mm i.d.). All IR spectra were run in film. Elemental analyses were obtained in the Microanalysis Service of IIQAB-CSIC, and they were conventional combustion analyses without discrimination between hydrogen and deuterium contents. $NaBD_4$ and $LiAlD_4$ (Deuterium content 99%) was obtained from Aldrich Chemical Co. The final deuterium contents of the labeled substrates were determined by GC−MS analysis of their respective methyl esters and were found to be as follows (%): **1**, 90.2 $^2H_4$, 8.5 $^2H_3$, and 1.3 $^2H_2$; **2**, 89.2 $^2H_6$, 7.9 $^2H_5$, and 2.9 $^2H_4$; **3**, 91.0 $^2H_6$, 6.2 $^2H_5$, and 2.8 $^2H_4$. Organic solutions were dried with anhydrous $MgSO_4$. The purification procedures were carried out by flash chromatography on silica gel (230−400 mesh) and products were mostly obtained as oils unless otherwise specified. Visualization of UV-inactive materials was accomplished with phosphomolybdic acid. Degree of purity of all new compounds was established by elemental analysis, except for the methyloxymethoxy derivatives **6**, **7**, **9**, and **10**−**14**, whose combustion analyses are not feasible, and purity was determined by GC and carbon NMR.

Dimethyl sulfoxide and Grace's medium were purchased from Sigma.

Synthesis of Probes. Preparation of Derivatives 4 and 5. To a stirred solution of the bromo alcohol in dimethoxymethane (1.5 mL/mmol of bromo alcohol) were added LiBr (0.2 equiv) and *p*-toluenesulfonic acid (0.1 equiv). The mixture was stirred at room temperature for 16 h. Brine was added, and the product was extracted with hexane. The organic layers were washed with brine and dried and concentrated at reduced pressure to give the expected products as oils, which were submitted to the following reaction without purification.

1-Bromo-9,11-dioxadodecane (4).[13] Yield: 97% (12.9 g, 51.2 mmol). ^1H NMR δ: 4.59 (s, 2H); 3.49 (t, J = 6.5 Hz, 2H); 3.38 (t, J = 7.0 Hz, 2H); 3.33 (s, 3H); 1.5−1.8 (m, 4H); 1.31 (br s, 8H). ^{13}C NMR δ: 96.3; 67.7; 55.0; 33.9; 32.7; 29.6; 29.2; 28.6; 28.0; 26.0. IR: 2931, 2856, 1465, 1440, 1386, 1215, 1147, 1110, 1047, 723 cm^{-1}.

1-Bromo-7,9-dioxadecane (5).[13] Yield: 96% (0.350 g, 1.9 mmol). ^1H NMR δ: 4.59 (s, 2H); 3.49 (t, J = 6.5 Hz, 2H); 3.38 (t, J = 7.0 Hz, 2H); 3.33 (s, 3H); 1.5−1.8 (m, 4H); 1.40 (m, 4H). ^{13}C NMR δ: 96.3; 67.5; 55.0; 33.7; 32.7; 29.5; 27.9; 25.3. IR: 2935, 2862, 1461, 1242, 1143, 1110, 1045 cm^{-1}.

Ethyl 2-(2,4-Dioxadodecyl)-3-oxobutanoate (6). To a solution of Na (46 mg, 2 mmol) in ethanol (1.3 mL) under argon was added ethyl acetoacetate (0.252 mL, 2 mmol). The reaction mixture was heated at reflux, 9,11-dioxa-1-bromooctane (0.504 g, 2 mmol) was added, and stirring was maintained at reflux for 26 h. The mixture was cooled, saturated NH_4Cl was added, and the product was extracted with hexane. The combined organic layers were washed with brine and dried. The crude residue obtained upon solvent removal was purified by column chromatography (hexane/diethyl ether 82:18) to obtain 0.360 g (1.2 mmol, 60%) of ketoester **6**. ^1H NMR δ: 4.57 (s, 2H); 4.15 (q, J = 7.0 Hz, 2H); 3.46 (t, J = 6.5 Hz, 2H); 3.34 (t, J = 6.0 Hz, 1H); 3.31 (s, 3H); 2.17 (s, 3H); 1.5−1.8 (m, 4H); 1.24 (br s, 12H); 1.22 (t, J = 7.0 Hz, 3H). ^{13}C NMR δ: 200.3; 169.8; 96.3; 67.7; 61.2; 55.0; 28.6; 29.6; 29.2; 29.1; 28.1; 27.3; 26.0; 14.0. IR: 2931, 2856, 1741, 1716, 1465, 1147, 1112, 1043 cm^{-1}.

12,14-Dioxa-2-pentadecanone (7). To a solution of Na (56 mg, 2.4 mmol) in ethanol (1.8 mL) was added freshly distilled ethanediol (4 mL) under argon. After 15 min at 85−90 °C, ketoester **6** (200 mg, 0.66 mmol) was added, and stirring was continued for 30 min. The mixture was cooled, aqueous saturated NH_4Cl was added, and the product was extracted with hexane. The combined organic layers were washed with brine and dried, and the solvent was removed to afford a crude that was purified by column chromatography (hexane/ethyl acetate 90:10) to give ketone **7** (90 mg, 0.39 mmol, 60%). ^1H NMR δ: 4.58 (s, 2H); 3.47 (t, J = 6.5 Hz, 2H); 3.32 (s, 3H); 2.37 (t, J = 7.0 Hz, 2H); 2.09 (s, 3H); 1.5−1.8 (m, 4H); 1.25 (br s, 10H). ^{13}C NMR δ: 209.3; 96.3; 67.8; 55.0; 43.7; 29.8; 29.6; 29.4; 29.3; 29.2; 29.1; 26.1; 23.7. IR: 2929, 2856, 1716, 1465, 1359, 1147, 1110, 1045, 918 cm^{-1}.

2-Butyl-1,3-dithiane (8). To a refluxing mixture of BF_3·Et_2O (2 mL), AcOH (4.1 mL), and $CHCl_3$ (8.2 mL) was added dropwise a solution of valeraldehyde (2.0 g, 23.5 mmol) and propanedithiol (2.8 mL, 25.9 mmol) in $CHCl_3$ (61 mL). Reflux was continued for 16 h. The solution was cooled to room temperature and washed sequentially with 10% KOH and brine. The organic layers were extracted with dichloromethane, and the combined organic layers were washed, dried, and concentrated at reduced pressure. The residue was distilled to obtain 3.7 g (21.0 mmol, 93%) of 2-butyl-1,3-dithiane. Bp = 122−123 °C/7.5 Torr.[35] ^1H NMR δ: 4.0 (t, J = 7.0 Hz, 1H); 2.8 (m, 4H); 2.1 (m, 2H); 1.2−1.8 (m, 6H); 0.8 (t, J = 7.0 Hz, 3H). ^{13}C NMR δ: 47.6; 35.1; 30.4; 26.0; 28.7; 22.2; 13.7. IR: 2954, 2931, 2898, 2858, 1465, 1421, 1274, 1242, 1182, 908 cm^{-1}.

2-Butyl-2-(7,9-dioxadecyl)-1,3-dithiane (9). A procedure previously reported[21] was followed. To a solution of dithiane **8** (0.370 g, 2.10 mmol) in dry THF (4 mL) and kept at −30 °C was added, under argon, 2.5 mL of a 1.34 M solution of *n*-BuLi in hexane. The mixture was stirred at −30 °C for 90 min and cooled to −78 °C, and then 7,9-dioxa-1-bromodecane (**5**) (0.375 g, 1.69 mmol) dissolved in 1 mL of THF was added. After being stirred for 1 h at −78 °C, the mixture was warmed to room temperature, THF was removed under vacuum, water was added, and the product was extracted with diethyl ether. The combined organic layers were washed with brine and dried and the crude resulting from evaporation of solvent was purified by column chromatography (hexane/diethyl ether 92:8) to afford 0.423 g (1.33 mmol, 81%) of dithioketal **9**. ^1H NMR δ: 4.58 (s, 2H); 3.47 (t, J = 6.5 Hz, 2H); 3.32 (s, 3H); 2.76 (m, 4H); 1.3−1.9 (m, 18H); 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR δ: 96.3; 67.6; 55.0; 53.2; 38.0; 37.8; 29.6; 29.5; 26.1; 26.0; 25.9; 25.5; 23.9; 22.8; 13.9.

12,14-Dioxa-5-pentadecanone (10). To a solution of *N*-bromosuccinimide (0.780 g, 4.38 mmol) in acetone/water 95:5 (13 mL) was added, at −20 °C, 180 mg of dithiane **9** (0.57 mmol) dissolved in the same solvent mixture (17 mL). Stirring was continued at −20 °C for 5 min and warmed to room temperature, and a 40% $NaHSO_3$ water solution was added until decoloration. Acetone was removed under vacuum, and the resulting residue was extracted with diethyl ether. The combined organic layers were washed with brine and dried, and the solvent was removed to furnish 111 mg (0.48 mmol, 85%) of ketone **10**. ^1H NMR δ: 4.57 (s, 2H); 3.47 (t, J = 6.5 Hz, 2H); 3.32 (s, 3H); 2.36 (t, J = 7.5 Hz, 2H); 2.35 (t, J = 7.5 Hz, 2H); 1.2−1.5 (m, 12H); 0.86 (t, J = 7.0 Hz, 3H). ^{13}C NMR δ: 211.5; 96.3; 67.6; 55.0; 42.6; 42.5; 29.5; 28.9; 26.0; 25.9; 23.7; 22.3; 13.8. IR: 2923, 2864, 1714, 1465, 1379, 1147, 1110, 1045, 919 cm^{-1}.

Reduction of Ketones 7 and 10. To a 1 M solution of the ketone in methanol was added, at 0 °C, $NaBD_4$ (2 molar equiv). The mixture was stirred at room temperature for 1 h and cooled to 0 °C, water was carefully added, and the product was extracted with dichloromethane. The organic layer was washed with brine and dried (Na_2SO_4), and the solvent was removed under vacuum to give the expected alcohols, which were submitted to the following reaction without purification.

[2-^{2}H]-12,14-Dioxa-2-pentadecanol (11). Yield: 95% (296 mg, 1.3 mmol). ^1H NMR δ: 4.58 (s, 2H); 3.47 (t, J = 6.5 Hz, 2H); 3.32 (s, 3H); 1.5−1.7 (m, 2H); 1.25 (br s, 17H); 1.13 (s, 3H). ^{13}C NMR δ: 96.3; 67.8; 67.6 (t, J = 21.5 Hz); 55.0; 39.2; 29.6; 29.3; 26.1; 25.6; 23.3. IR: 3440, 2927, 2856, 1465, 1373, 1149, 1112, 1045, 919 cm^{-1}.

[5-^{2}H]-12,14-Dioxa-5-pentadecanol (12). Yield: 92% (255 mg, 1.1 mmol). ^1H NMR δ: 4.57 (s, 2H); 3.47 (t, J = 6.5 Hz, 2H); 3.32 (s, 3H); 1.3−1.5 (m, 17H); 0.86 (t, J = 7.0 Hz, 3H). ^{13}C NMR δ: 96.3; 71.3 (t, J = 21.0 Hz); 67.7; 55.0; 37.2; 37;0; 29.6; 29.4; 27.7; 26.1; 25.5; 22.7; 14.0. IR: 3438, 2931, 2858, 1465, 1380, 1217, 1151, 1112, 1045, 919 cm^{-1}.

Preparation of Tosyl Esters 13 and 14. A 0.5 M solution of the alcohol in dichloromethane was treated with trimethyl-

(35) Seebach, D.; Wilka, E. *Synthesis* **1976**, 476−477.

amine hydrochloride (1 molar equiv) and anhydrous triethylamine (5 molar equiv). The mixture was cooled to 0 °C, and a 0.5 M solution of *p*-toluenesulfonyl chloride (1.5 molar equiv) in dichloromethane was added. The mixture was stirred at 0 °C for 90 min, and the product was extracted with ethyl acetate. The organic layers were washed with 1 N HCl and brine, dried, and concentrated to dryness to afford a crude that was purified by column chromatography (hexane/ethyl acetate 86:14) to obtain the expected products.

[2-^{2}H]-12,14-Dioxa-2-pentadecyl *p*-toluensulfonate (13). Yield: 73% (325 mg, 0.84 mmol). ^{1}H NMR δ: 7.76 (d, J = 8.5 Hz, 2H); 7.29 (d, J = 8.0 Hz, 2H); 4.58 (s, 2H); 3.48 (t, J = 6.5 Hz, 2H); 3.32 (s, 3H); 2.41 (s, 3H); 1.21 (s, 3H); 1.2−1.4 (m, 18H). ^{13}C NMR δ: 144.3; 134.5; 129.6; 127.6; 96.3; 80.2 (t, J = 22.0 Hz); 67.8; 55.0; 36.3; 29.7; 29.3; 29.2; 29.0; 26.1; 24.7; 21.5; 20.6. IR: 3031, 2929, 2856, 1598, 1463, 1359, 1178, 1110, 1045, 908 cm^{-1}.

[5-^{2}H]-12,14-Dioxa-5-pentadecyl *p*-toluensulfonate (14). Yield: 73% (0.238 g, 0.61 mmol). ^{1}H NMR δ: 7.76 (d, J = 8.5 Hz, 2H); 7.29 (d, J = 8.0 Hz, 2H); 4.58 (s, 2H); 3.45 (t, J = 6.5 Hz, 2H); 3.33 (s, 3H); 2.41 (s, 3H); 1.2−1.5 (m, 16H); 0.78 (t, J = 6.5 Hz). ^{13}C NMR δ: 144.3; 134.7; 129.6; 127.6; 96.3; 84,0 (t, J = 21.5 Hz); 67.7; 55.0; 33.8; 33.6; 29.5; 29.0; 26.7; 25.9; 24.5; 22.3; 21.5; 13.8. IR: 2935, 2864, 1598, 1465, 1359, 1178, 1110, 1045, 900 cm^{-1}.

Reaction of Tosylates 13 and 14 with $LiAlD_4$. The tosyl derivative was dissolved in dry diethyl ether (2 mL/mol) and treated with $LiAlD_4$ (3 molar equiv) dissolved in diethyl ether (1 mL/mol) for 3 h at room temperature. Water was added dropwise to the crude reaction mixture, and the product was extracted with hexane. The combined organic layers were washed with saturated $NaHCO_3$ and brine and dried. Evaporation of solvent afforded a crude that was dissolved in HCl 10% in methanol and stirred at room temperature for 16 h. The solvent was removed under vaccum, water was added, and the product was extracted with dichloromethane. The combined organic layers were treated as above and concentrated to give a crude that was purified by column chromatography (hexane/ethyl acetate 88:12) to afford the pure alcohols **15** and **16**.

[10,10-$^2H_2$]-1-Undecanol (15). Yield: 78% (108 mg, 0.62 mmol). ^{1}H NMR δ: 3.61 (t, J = 6.5 Hz, 2H); 1.54 (m, 3H); 1.23 (br s, 14H); 0.83 (s, 3H). ^{13}C NMR δ: 62.7; 32.6; 31.6; 29.6; 29.5; 29.4; 29.2; 21.8 (quintet, J = 19.0 Hz); 13.7. IR: 3344, 2925, 2854, 1461, 1377, 1348, 1163, 1056, 721 cm^{-1}. Anal. Calcd for $C_{11}H_{22}{}^2H_2O$: C, 75.86; H + D, 13.79. Found: C, 75.60; H, 13.71.

[7,7-$^2H_2$]-1-Undecanol (16). Yield: 80% (0.286 g, 1.64 mmol). ^{1}H NMR δ: 3.61 (t, J = 6.5 Hz, 2H); 1.54 (m, 2H); 1.23 (br s, 15H); 0.85 (t, J = 7.0 Hz, 3H). ^{13}C NMR δ: 62.8; 32.6; 31.8; 29.4; 29.3; 29.0; 28.7 (quintet, J = 19.0 Hz); 25.7; 22.6; 14.0. IR: 3360, 2956, 2923, 2854, 1460, 1178, 1110, 1056 cm^{-1}. Anal. Calcd for $C_{11}H_{22}{}^2H_2O$: C, 75.86; H + D, 13.79. Found: C, 75.72; H, 13.51.

Synthesis of Bromoalkanes 17 and 18. To a 0.5 M solution of the alcohol in dry dimethylformamide was added, under argon, *N*-bromosuccinimide (2 molar equiv). The mixture was heated at 50 °C for 15 min. Methanol (1 mL/mmol of alcohol) was added to remove the excess reagent, and stirring was maintained for 5 min. The product was extracted with diethyl ether, and the combined organic layers were washed with 1 N HCl and brine and dried. Evaporation of solvent afforded a crude that was purified by column chromatography (hexane).

[10,10-$^2H_2$]-1-Bromoundecane (17). Yield: 70% (127 mg, 0.66 mmol). ^{1}H NMR δ: 3.38 (t, J = 7.0 Hz, 2H); 1.83 (m, 2H); 1.23 (br s, 14H); 0.84 (s, 3H). ^{13}C NMR δ: 34.0; 32.8; 31.6; 29.6; 29.5; 29.4; 29.2; 28.7; 28.1; 21.8 (quintet, J = 19.0 Hz); 13.8. IR: 2958, 2923, 2854, 1461, 1163, 721 cm^{-1}. Anal. Calcd for $C_{14}H_{21}{}^2H_2Br$: C, 55.93; H + D, 9.74. Found: C, 55.60; H, 9.71.

[7,7-$^2H_2$]-1-Bromoundecane (18). Yield: 74% (286 mg, 1.48 mmol). ^{1}H NMR δ: 3.38 (t, J = 7.0 Hz, 2H); 1.83 (m, 2H); 1.23 (br s, 14H); 0.86 (t, J = 7.0 Hz, 3H). ^{13}C NMR δ: 34.0; 32.8; 31.8; 29.4; 29.3; 29.1; 28.7; 28.1; 22.6; 29.2 (quintet, J = 18.0 Hz); 14.1. IR: 2958, 2923, 2854, 1463, 1178, 1110, 1056 cm^{-1}. Anal. Calcd for $C_{14}H_{21}{}^2H_2Br$: C, 55.93; H + D, 9.74. Found: C, 55.82; H, 9.81.

Obtention of Terminal Alkynes 19 and 20. A suspension of lithium acetylide in liquid ammonia, prepared from lithium (2 molar equiv) and acetylene, was dissolved in dry dimethyl sulfoxide (0.5 mL/mmol Li). After evaporation of NH_3, the acetylide was treated with a 3 M solution of the bromoalkane in dry dimethyl sulfoxide. The mixture was stirred at room temperature for 90 min, cooled to 0 °C and saturated NH_4Cl was added. The product was extracted with hexane, and the combined organic layers were washed with brine and dried. Removal of solvent afforded a crude that was purified by column chromatography (hexane).

[12,12-$^2H_2$]-1-Tridecyne (19). Yield: 60% (56 mg, 0.31 mmol). ^{1}H NMR δ: 2.16 (dt, J_1 = 2.5 Hz, J_2 = 6.5 Hz, 2H); 1.91 (t, J = 2.5 Hz, 1H); 1.83 (m, 2H); 1.23 (br s, 14H); 0.84 (s, 3H). ^{13}C NMR δ: 84.6; 67.9; 31.7; 29.6; 29.5; 29.3; 29.1; 28.7; 28.5; 21.8 (quintet, J = 19.0 Hz); 18.3; 13.8. IR: 3315, 2925, 2854, 1465, 1163, 721 cm^{-1}. Anal. Calcd for $C_{13}H_{22}{}^2H_2$: C, 87.71; H + D, 13.18. Found: C, 87.60; H, 13.31.

[9,9-$^2H_2$]-1-Tridecyne (20). Yield: 58% (0.163 g, 0.89 mmol). ^{1}H NMR δ: 2.15 (dt, J_1 = 2.5 Hz, J_2 = 7.0 Hz, 2H); 1.90 (t, J = 2.5 Hz, 1H); 1.84 (m, 2H); 1.23 (br s, 14H); 0.85 (t, J = 7.0 Hz, 3H). ^{13}C NMR δ: 84.7; 67.9; 31.8; 29.5; 29.4; 29.2 (quintet, J = 18.0 Hz); 29.1; 28.4; 22.6; 18.3; 14.0. IR: 3315, 2954, 2923, 2854, 1465, 721 cm^{-1}. Anal. Calcd for $C_{13}H_{22}{}^2H_2$: C, 87.71; H + D, 13.18. Found: C, 87.58; H, 13.35.

1-Tridecyne (25).[36] Yield: 82% (500 mg, 2.73 mmol). ^{1}H NMR δ: 2.15 (dt, J_1 = 2.5 Hz, J_2 = 7.0 Hz, 2H); 1.91 (t, J = 2.5 Hz, 1H); 1.84 (m, 2H); 1.24 (br s, 16H); 0.85 (t, J = 7.0 Hz, 3H). ^{13}C NMR δ: 84.8; 67.9; 31.9; 29.6; 29.4; 29.3; 29.1; 28.7; 28.5; 22.6; 18.3; 14.1. IR: 3315, 2956, 2925, 2854, 1465, 721 cm^{-1}.

Synthesis of Esters 21, 22, and 26. A 0.5 M solution of the terminal alkyne in dry THF was treated, at −78 °C under argon, with a 1.6 M solution of BuLi in hexane (1.2 molar equiv) and stirred at −78 °C for 30 min. Methyl chloroformate (1 molar equiv) was added, the reaction mixture was warmed to room temperature and quenched with water, and the product was extracted with diethyl ether. The crude obtained upon solvent removal was purified by column chromatography (hexane/diethyl ether 97:3).

Methyl [13,13-$^2H_2$]-2-Tetradecynoate (21). Yield: 77% (35 mg, 0.14 mmol). ^{1}H NMR δ: 3.73 (s, 3H); 2.30 (t, J = 7.0 Hz, 2H); 1.54 (m, 2H); 1.23 (br s, 14H); 0.83 (s, 3H). ^{13}C NMR δ: 154.1; 89.8; 72.7; 52.4; 31.6; 29.5; 29.3; 29.2; 28.9; 28.7; 21.8 (quintet, J = 19.0 Hz); 18.5; 13.8. IR: 2952, 2927, 2854, 2237, 1722, 1434, 1253, 1076 cm^{-1}. Anal. Calcd for $C_{14}H_{24}{}^2H_2O_2$: C, 73.68; H + D, 11.40. Found: C, 73.42; H, 11.62.

Methyl [10,10-$^2H_2$]-2-Tetradecynoate (22). Yield: 78% (71 mg, 0.29 mmol). ^{1}H NMR δ: 3.73 (s, 3H); 2.30 (t, J = 7.0 Hz, 2H); 1.54 (m, 2H); 1.23 (br s, 14H); 0.85 (t, J = 7.0 Hz). ^{13}C NMR δ: 154.2; 90.0; 72.7; 52.5; 31.8; 29.3; 29.2 (quintet, J = 18.0 Hz); 29.1; 29.0; 28.8; 27.4; 22.6; 18.6; 14.1. IR: 2954, 2925, 2856, 2237, 1718, 1434, 1253, 1076, 752 cm^{-1}. Anal. Calcd for $C_{14}H_{24}{}^2H_2O_2$: C, 73.68; H + D, 11.40. Found: C, 73.29; H, 11.72.

Methyl 2-Tetradecynoate (26).[37] Yield: 87% (115 mg, 0.48 mmol). ^{1}H NMR δ: 3.73 (s, 3H); 2.30 (t, J = 7.0 Hz, 2H); 1.54 (m, 2H); 1.23 (br s, 14H); 0.85 (t, J = 7.0 Hz, 3H). ^{13}C NMR δ: 154.2; 90.0; 72.7; 52.5; 31.8; 29.5; 29.39; 29.30; 28.9; 28.8; 27.4; 22.6; 18.6; 14.1. IR: 2952, 2925, 2854, 2237; 1720; 1434, 1253, 1076, 752 cm^{-1}.

Reduction of Acetylenic Esters 21, 22, and 26 and Saponification. A mixture of the starting ester and Mg (5 molar equiv) in CD_3OD (10 mL/mmol of ester) was stirred at room temperature for 24 h. After this time, 37% HCl was added under stirring until complete solubilization of the suspension, and the product was extracted with diethyl ether. The combined organic layers were washed with brine and dried

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(37) Zehnter, R.; Gerlach, H. *Liebigs Ann. Org. Bioorg. Chem.* **1995**, *12*, 2209−2220.

and solvent was removed under vacuum. The resulting crude was stirred at room temperature for 16 h in 2.5 M KOH in deuterated methanol (0.25 mL/mmol of ester). Solvent was removed, and the product was extracted with dichloromethane. The organic layers were washed with brine and dried. Solvent removal afforded a crude that was purified by column chromatography (dichloromethane/methanol 98:2).

[2,2,3,3,13,13-$^2H_6$]-Tetradecanoic Acid (2). Yield: 70% (9 mg, 0.03 mmol). Mp: 50−52 °C. ^{1}H NMR δ: 9.01 (br s, 1H); 1.23 (br s, 18H); 0.84 (s, 3H). ^{13}C NMR δ: 180.2; 33.3 (quintet, J = 18 Hz); 31.7; 29.7; 29.6; 29.4; 29.1; 28.7; 23.8 (quintet, J = 20 Hz); 21.9 (quintet, J = 19 Hz); 13.8. IR: 2923, 2854, 1739, 1458, 1282, 1091 cm^{-1}. Anal. Calcd for $C_{14}H_{22}{}^2H_6O_2$: C, 71.76; H + D, 12.04. Found: C, 71.60; H, 11.71.

[2,2,3,3,10,10-$^2H_6$]-Tetradecanoic Acid (3). Yield: 72% (20 mg, 0.085 mmol). Mp: 50−52 °C. ^{1}H NMR δ: 11.02 (br s, 1H); 1.23 (br s, 18H); 0.85 (t, J = 7.0 Hz, 3H). ^{13}C NMR δ: 180.6; 33.4 (quintet, J = 20 Hz); 31.8; 29.5; 29.4; 29.2; 29.1; 28.8; 24.2 (quintet, J = 20 Hz); 23.8 (quintet, J = 20 Hz); 22.6; 14.1. IR: 2954, 2916, 2850, 1699; 1463, 1393, 1215, 908, 757 cm^{-1}. Anal. Calcd for $C_{14}H_{22}{}^2H_6O_2$: C, 71.76; H + D, 12.04. Found: C, 72.06; H + D, 12.25.

[2,2,3,3-$^2H_4$]-Tetradecanoic Acid (1). Yield: 74% (20 mg, 0.085). Mp: 51−53 °C. ^{1}H NMR δ: 10.99 (br s, 1H); 1.23 (br s, 18H); 0.85 (t, J = 7.0 Hz, 3H). ^{13}C NMR δ: 180.5; 33.4 (quintet, J = 20 Hz); 31.9; 29.7; 29.6; 29.5; 29.4; 29.3; 29.1; 28.8; 23.8 (quintet, J = 20 Hz); 22.6; 14.1. IR: 2953, 2918, 2850, 1696, 1466, 1305, 950 cm^{-1}. Anal. Calcd for $C_{14}H_{24}{}^2H_4O_2$: C, 71.37; H + D, 12.14. Found: C, 71.41; H + D, 12.05.

Kinetic Isotope Effect Experiments. Cryptoregiochemistry was determined in vitro following the previously reported procedure.[14] The experiments were carried out using round-bottom-96-well plates. To each well, a 5 μL drop of incubation medium was added. The incubation medium consisted of Grace's saline (135 μL) and a dimethyl sulfoxide solution (15 μL) of a 1:1 mixture of **2** and **1** or **3** and **1** (10 mg/mL each). *S. littoralis* pheromone glands were excised, carefully cleaned, and immersed into a drop of the incubation medium. Plates were sealed with adherent plastic covers and incubations proceeded for 3 h at 25 °C. After this time, pheromone glands were collected and soaked in chloroform/methanol (2:1) at 25 °C for 1 h. The lipidic extracts thus obtained were base methanolyzed as described elsewhere[12] to obtain the fatty acid methyl esters.

The extracts were analyzed by GC−MS, at 70 eV, on a Fisons gas chromatograph (8000 series) coupled to a Fisons MD-800 mass selective detector. The system was equipped with a nonpolar Hewlett-Packard HP-1 capillary column (30 m × 0.20 mm I. D.) using the following temperature program: from 120 °C to 180 °C at 5 °C/min and then to 260 °C at 2 °C/min after an initial delay of 2 min. Analyses were carried out by monitoring the ions 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, and 250. Dwell was set at 0.02 and mass span at 0.5.

Kinetic isotope effects (KIEs) were calculated from the ratios of product formed from unlabeled substrate and that produced from the deuterated analogues and were based on the abundance of the respective molecular ions of the various isotopomers of methyl (*E*,*E*)-10,12-tetradecadienoate (d_4, 242; d_5, 243). Isotope effects were corrected for the exact proportion of unlabeled and labeled substrates administered, which was determined by GC−MS analysis of a BF_3·MeOH derivatized sample of the applied mixture. Corrections were also made for incomplete deuterium incorporation in the substrates and for the natural abundance of carbon and oxygen isotopes in the ions monitored. These latter values were obtained from the GC−MS chromatograms of extracts of tissues incubated with the individual substrates.

Acknowledgment. We thank CAICYT (Grant No. AGF98-0844), Generalitat de Catalunya (Grant No. 99SGR-00187), and SEDQ S.A. for financial support and the Ministry of Education and Culture for a predoctoral fellowship to S.R. We thank Dr. Jesús Joglar for critically reading the manuscript and Germán Lázaro for rearing the insects used in this study.

JO010560W