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Synthesis and Use of Stereospecifically Deuterated Analogues of Palmitic Acid To Investigate the Stereochemical Course of the Δ^{11} Desaturase of the Processionary Moth

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Thaumetopoea pityocampa pheromone glands contain desaturases that, after several sequential reactions from palmitic acid, catalyze the formation of a unique enyne fatty acid, which is the immediate sex pheromone precursor. In this article, we describe the synthesis of different stereospecifically deuterium-labeled and isotopically tagged palmitic acid probes needed to decipher the stereochemical course of the *T. pityocampa* Δ^{11} desaturase. The synthesis of probes has been carried out by a chemoenzymatic route, in which the key step is the kinetic lipase-catalyzed resolution of racemic mixtures of secondary propargyl alcohols. The presence of the acetylenic bond simplifies the absolute configuration determination of the resolved alcohols. Moreover, it allows the introduction of the isotopic tag by deuteration. By use of the probes thus prepared, experimental evidence is presented that the Δ^{11} desaturase of *T. pityocampa* transforms palmitic acid into (*Z*)-11-hexadecenoic acid by removal of the *pro*-(*R*)-hydrogen atoms from both C11 and C12.

Introduction

Biosynthesis of *Thaumetopoea pityocampa* sex pheromone, (*Z*)-13-hexadecen-11-inyl acetate (**IV**), occurs from palmitic acid by the combined action of different desaturases.[1] Thus, a Δ^{11} desaturase transforms palmitic acid into (*Z*)-11-hexadecenoic acid and then this monoene is converted into 11-hexadecynoic acid by a Δ^{11} acetylenase. On the other hand, (*Z*)-11-hexadecenoic acid gives rise to (*Z*,*Z*)-11,13-hexadecadienoic acid and 11-hexadecynoic acid to (*Z*)-13-hexadecen-11-ynoic acid, the immediate pheromone precursor, by the action of a Δ^{13} desaturase (Figure 1).

In-depth studies of the desaturase enzymes involved in this biosynthetic pathway include their cloning and functional expression, as well as the investigation of their mechanistic features.

Besides insect cells, in which different Δ^{11} desaturases have been cloned and sequenced,[2] a Δ^{11} desaturase has been identified recently in the microalga *Thalassiosira pseudonana*.[3] Interestingly, the latter enzyme has a cytochrome *b*5 domain in its *N*-terminal region, whereas this domain is not present in the insect Δ^{11} desaturases. This represents a major primary structure difference between the enzymes. Alignment of the desaturase domain of *T. pseudonana* with the full sequence of insect Δ^{11} desaturases showed an identity of 20%.

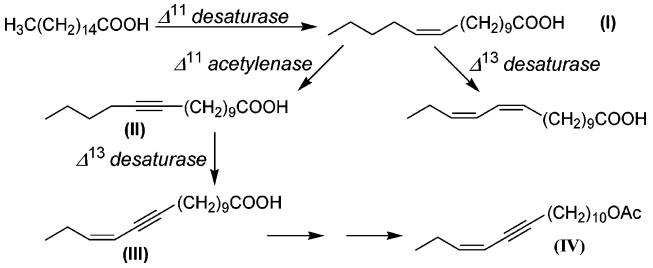

FIGURE 1. Biosynthetic pathway of the sex pheromone of the processionary moth *T. pityocampa*.

Several works have been published about the stereochemical course of Δ^{11} desaturases.[4−6] In addition, the stereochemical course of other desaturases have recently been reported.[7−9] In all the reported examples, the reactions occur by removal of both *pro*-(*R*)-hydrogen atoms from the positions to be oxidized. This work was undertaken to determine if the processionary moth Δ^{11} desaturase complies with this general rule.

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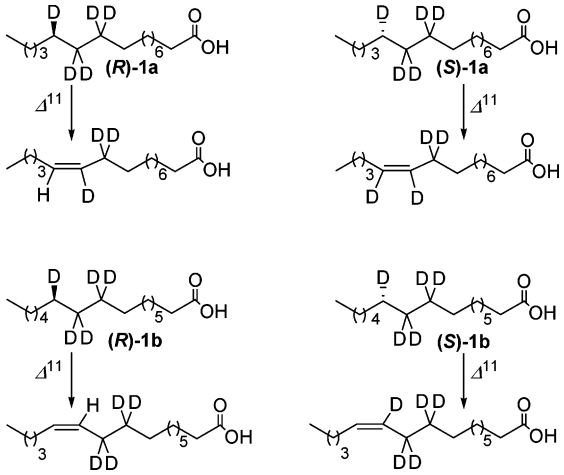

FIGURE 2. Enantiomerically pure deuterated probes and their Δ^{11} desaturation products in *T. pityocampa*.

To carry out this investigation, the synthesis of chiral deuterium-labeled fatty acids as probes was required. In a previous article,[10] we reported on a novel chemoenzymatic strategy for the synthesis of enantiomerically pure secondary alcohols with sterically similar substituents. The key step is the kinetic lipase-catalyzed resolution of racemic mixtures of substituted propargylic alcohols. Two strategies were tested. In the first one, racemic 1-alkynyl-3-ols were efficiently resolved enzymatically with *Candida antarctica* lipase B (CAL-B) and then elongated with the suitable alkyl bromoderivative. Alternatively, disubstituted propargyl alcohols were resolved biocatalytically with *Thermomyces lanuginosus* lipase (HLL). In this article, these two strategies have been applied to prepare isotopically tagged and stereotopically deuterated palmitic acid probes (Figure 2). Using these substrates, in the present paper we show that the Δ^{11} desaturase of *T. pityocampa* exhibits the same stereospecificity reported for other Δ^{11} desaturase enzymes.

Results and Discussion

Synthesis of Probes. To study the stereospecificities of fatty acid desaturases, the synthesis of chiral deuterium-labeled fatty acids as probes is required. For preparation of pure enantiomers of the monodeuterated acids, chiral alcohols are suitable intermediates that afford the deuterated stereogenic centers by transformation of the hydroxyl functionality into a good leaving group and its subsequent replacement by the deuterium label. A good approach to obtain the chiral alcohols is the kinetic enzymatic resolution of the corresponding racemic precursors with lipases. However, biocatalytic racemic resolutions become more difficult as the hydroxyl group is displaced toward the middle position of the aliphatic chain and the chain length is enlarged, making the biocatalytic approaches useless for preparation of some enantiomerically pure deuterated compounds. To solve this problem, we have developed a general methodology by combining the reduced steric requirements of the acetylenic moiety for enzymatic chiral discrimination and the chemical versatility of this group for its easy conversion to aliphatic chains. The approach is based on the previous experimental evidence that unsaturated substituents in the hydroxymethine functionality enhanced the enantioselectivity of some lipases.[11−13] This synthetic strategy, which starts with commercially available racemic mixtures of substituted secondary propargyl alcohols, has been applied to the obtention of the enantiomerically pure and tagged deuterated acids (*R*)-**1a,b** and (*S*)-**1a,b** needed in this study (Schemes 1 and 2). According to Scheme 1, a double enzymatic resolution (45% and 90% conversions, respectively) of the propargyl alcohol **2** with *Candida antarctica* lipase in anhydrous diisopropyl ether in the presence of vinyl acetate afforded the enantiomerically pure (>98% ee) acetate (*S*)-**3** and partially resolved alcohol (*R*)-**2** (80% ee). The alkynyllithium salt of this alcohol was then formed with *n*-butyllithium and hexamethylphosphoramide (HMPA), and its coupling with alkyl bromoderivative **5** afforded the elongated intermediate alcohol (*R*)-**6**. A new enzymatic resolution of (*R*)-**6** with *Thermomyces lanuginosus* lipase immobilized on EP-100 with the same reagents and conditions as above afforded the enantiomerically pure acetate (*R*)-**7**. Both acetates, (*S*)-**3** and (*R*)-**7**, were saponified with a methanolic potassium carbonate solution to their corresponding enantiomerically pure alcohols, which were further protected to the methoxymethyl derivatives under standard conditions[14] to give rise to compounds (*S*)-**4** and (*R*)-**8**. Finally, coupling of the alkynyllithium salt of (*S*)-**4** with the alkyl bromoderivative **5** afforded (*S*)-**8** in high yields.

The presence of the alkyne functionality has simplified the absolute configuration determination of the resolved alcohols **2** and **6** by ^{1}H NMR after derivatization of the hydroxyl functionality with (*R*)-(−)-α-methoxy-α-phenylacetic acid [(*R*)-MPA].

Furthermore, the acetylenic bond is susceptible to being deuterated for isotopic tagging of the final probe. The tagging strategy is necessary to avoid putative interferences by the endogenous natural materials in the gas chromatography/mass spectrometry (GC/MS) analyses, which prevent the obtention of reliable data. Each of the enantiomers of **8** was isotopically tagged and transformed into the final probes as exemplified in Scheme 2 for (*R*)-**1a,b**. Thus, introduction of deuterium into **8** to furnish compounds **9** was achieved by nonscrambling deuteration of the triple bond by use of the Wilkinson catalyst. It is worth mentioning that the deuteration reaction had to be carried out with the methoxymethyl-protected alcohol intermediates **8** since the unprotected alcohols **6** rendered important amounts of ketone[10,15] and in order to reduce the deuterium scattering.[16] Under the optimum synthetic conditions, mean ratios of d_6, d_5, d_4, d_3, and d_2 fatty acids were,

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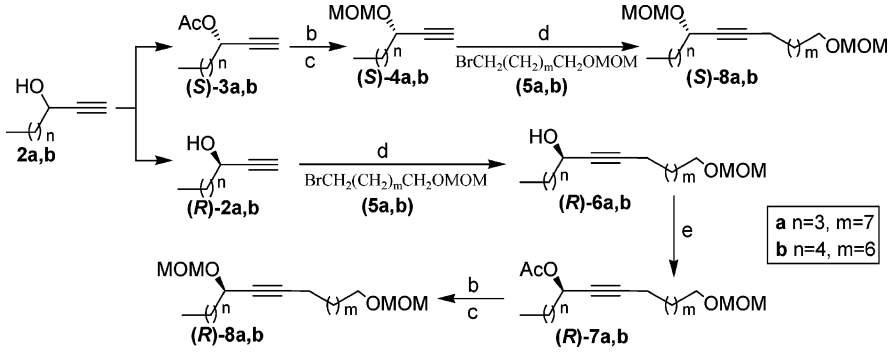
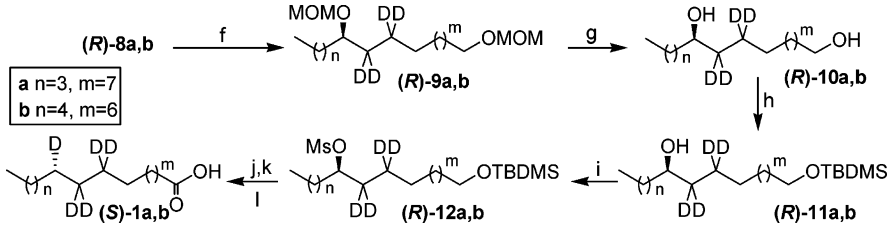
SCHEME 1. Synthesis of (*R*)-8a,b and (*S*)-8a,b[a]



[a] Reagents and conditions: (a) CAL-B/diisopropyl ether/vinyl acetate; (b) K_2CO_3/MeOH; (c) BrLi/TsOH/ dimethoxymethane; (d) BuLi/ THF/HMPA; (e) HLL/diisopropyl ether/vinyl acetate.

SCHEME 2. Transformation of (*R*)-8a,b in Probes (*R*)-1a,b[a]



[a] Reagents and conditions: (f) Wilkinson catalyst/D_2/benzene; (g) HCl/MeOH; (h) TBMSCl/DBU/CH_2Cl_2; (i) NEt_3/MsCl/CH_2Cl_2; (j) $LiAlD_4$/Et_2O; (k) HCl/MeOH; (l) PDC/DMF.

respectively, 11.8, 78.4, 6.6, 2.4, and 0.8 (by GC/MS of the corresponding fatty acid methyl esters).

Deprotection of the hydroxyl groups in acidic media gave rise to the corresponding diols **10**, which could be regioselectively protected in the primary alcohol group with *tert*-butyldimethylsilyl chloride (TBDMSCl) in the presence of DBU, thus affording compounds **11**.[17] The stereotopic deuterium label was introduced in two steps with absolute configuration inversion of the stereogenic center by mesylation of the secondary hydroxyl group, followed by reaction of **12** with $LiAlD_4$.[18,19] Finally, treatment with HCl/MeOH and further PDC oxidation with *N*,*N*-dimethylformamide (DMF)[20] as solvent afforded each one of the enantiotopically deuterated palmitic acids **1a,b**, which also bear a tetradeuterium tag for unambiguous GC/MS investigation of their metabolic fate.

The number and the presence of the deuterium labels was as expected from the analyses of ^{13}C NMR (presence of two quintuplets for CD_2 in compounds **9**−**12** and an additional triplet for CHD in compounds **13**, **14**, and **1**) and mass spectra. In addition, NMR comparison of the deuterated compounds **1a,b** and **14a,b** with the corresponding nondeuterated counterparts (palmitic acid and cetyl alcohol, respectively) showed some differences owing to the presence of the deuterium atoms. Thus, the most important dissimilarities include the minor integration of the signal at 1.25 ppm in the ^1H NMR spectra, revealing the replacement of five hydrogen atoms ($2CH_2$ and CH) by deuterium, as well as some chemical shift changes in the 30−28.6 ppm range in the ^{13}C NMR spectra.

The absolute configuration and enantiomeric excesses (ee) of the different unsaturated and saturated alcohol intermediates were determined by NMR, as in previous articles,[10,18] after derivatization with either (*R*)-MPA[21] or synthetic (*R*)-(−)-α-methoxy-α-(9-anthryl)acetic acid, [(*R*)-AMA, 97% ee], respectively.[22] In the case of synthetic series **b**, the absolute configuration was verified by (*R*)-MPA derivatization of commercially available enantiomers (*R*)-**2b** and (*S*)-**2b**.

Stereospecificity Studies. For the unequivocal determination of the stereospecificity of the Δ^{11} desaturase, the fate of the individual hydrogen/deuterium atoms bound to C11 and C12 of both enantiomers of **1a** and **1b** was investigated. The pheromone glands of *T. pityocampa* females were incubated with each probe, and tissues were extracted and methanolyzed. The fatty acid methyl ester extracts thus obtained were analyzed by chemical ionization GC/MS with methane as ionization gas. This mode affords the $M^{\bullet +} + 1$ ion as the most abundant fragment in the mass spectra (Figure 3). From incubations with (*R*)-**1a**, the resulting labeled methyl dienoate displayed a $M^{\bullet +} + 1$ isotopic cluster centered at *m*/*z* 272. This implicates the loss of two deuterium atoms from the saturated, deuterium-labeled precursor (*R*)-**1a**. Conversely, after incubations with (*S*)-**1a**, the $M^{\bullet +} + 1$ ion of the corresponding methyl dienoate was centered on

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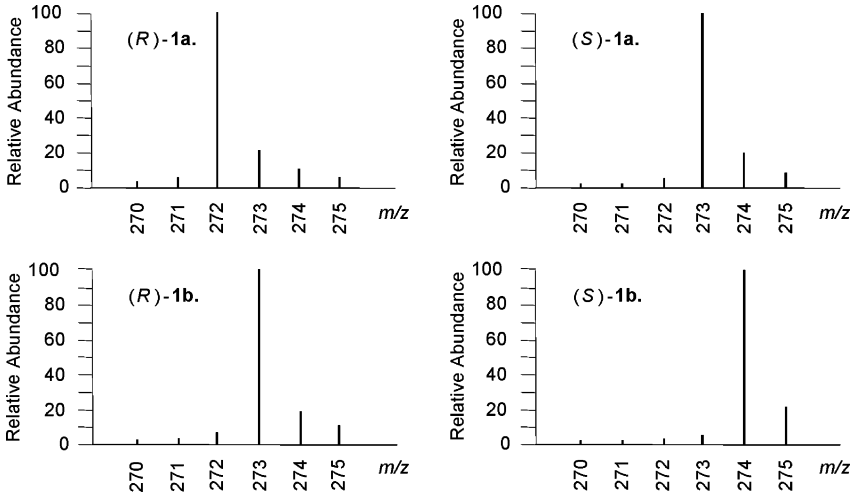

FIGURE 3. Isotopomers of the $M^{\bullet+} + 1$ ion clusters corresponding to the methyl (*Z*)-11-hexadecenoates present in the fatty acid methyl ester extracts from pheromone glands of *T. pityocampa* cultured in the presence of the indicated enantiomerically pure deuterated probes **1a,b**.

m/*z* 273, indicating the loss of the C11 deuterium atom along with the C12 hydrogen from the saturated precursor. In the experiments with (*R*)-**1b**, the $M^{\bullet+} + 1$ ion cluster of the resulting dienoate was centered at *m*/*z* 273, whereas (*S*)-**1b** gave rise to a dienoate in which the most abundant $M^{\bullet+} + 1$ isotopomer was that at *m*/*z* 274. This implies the loss of either deuterium or hydrogen atoms from (*R*)-**1b** and (*S*)-**1b**, respectively. These overall results indicate that the Δ^{11} desaturation of palmitic acid in the moth *T. pityocampa* occurrs with removal of both *pro*-(*R*)-hydrogen atoms from C11 and C12 of the substrate.

The overall results obtained in the biochemical studies with the enantiomerically pure deuterated probes **1a** and **1b** are a clear indication of a *syn*-elimination process occurring with removal of both *pro*-(*R*)-hydrogen atoms from C11 and C12 of the precursor acid. The observed stereochemistry corresponds to the previously established stereochemistry of the desaturation step in *Mamestra brassicae*,[4] *Spodoptera littoralis*,[23] *Bombyx mori*,[6] and *Manduca sexta*.[6] Thus, the present work provides a new example of the stereospecificity course of Δ^{11} desaturases from moth females.

Such a steric course probably arises from the conformation adopted by the substrate at the enzyme active site in that the hydrophobic chain of the fatty acid is twisted around the carbon atoms to be oxidized to form a U-shape. This particular orientation of the substrate exposes the two *pro*-(*R*)-hydrogen atoms to the enzyme diiron center, thus allowing the *syn*-elimination of both hydrogen atoms and the formation of the *Z* isomer of the resulting olefinic product.

It is worth mentioning that the relative amounts of deuterated olefinic products formed from either (*R*)-**1a**, (*S*)-**1a**, and (*R*)-**1b** were similar and approximately 6 times lower than those produced from the (*S*)-**1b** precursor. This result suggests that the abstraction of C11-H is sensitive to isotopic substitution and it occurs with a primary isotope effect of ca. 6. This result agrees with the mechanism found for the Δ^{11} desaturase present in *S. littoralis*, in which the rate-limiting step is oxidation at C11.[24] A more detailed study of the cryptoregiochemistry (site of initial oxidation)[25] of this Δ^{11} desaturase is currently under way.

Conclusions

In summary, we have reported a procedure to obtain enantiomerically pure deuterated palmitic acids with a tetradeuterium tag and their use to decipher the stereospecificity of the processionary moth Δ^{11} desaturase. The synthesis of probes has been carried out by application of a chemoenzymatic strategy previously reported, in which the key step is the kinetic lipase-catalyzed resolution of racemic mixtures of secondary propargylic alcohols. The presence of the acetylenic bond simplifies the absolute configuration determination of the resolved alcohols and, on the other hand, allows the introduction of the isotopic tag by deuteration. Using the substrates thus prepared, we show that the Δ^{11} desaturation of palmitic acid in *T. pityocampa* occurs with removal of both *pro*-(*R*)-hydrogen atoms from C11 and C12 of the substrate and, therefore, it has the same stereospecificity reported for other (*Z*)-11-desaturase enzymes.

Experimental Section

Enzyme Deposition and Absorption. *C. antarctica* was supplied as an immobilized preparation on a macroporous acrylic resin. Adsorption of *T. lanuginosus* lipase onto polypropylene support (EP100) was carried out following the methodology described by Gitlesen et al.[26] The lipase (10 g) was dissolved in aqueous phosphate buffer (200 mL, 0.1 M, pH 7.0) and mixed with the solid support EP100 (10 g), containing ethanol/water (30 mL), 24:1 v/v. The mixture was reciprocally shaken (120 rpm) for 24 h at 25 °C. After this period, the enzyme−support preparation was filtered off and dried under vacuum until a constant weight was obtained.

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The activity of both immobilized lipase preparations was measured by use of the acylation of 1-dodecanol in organic media.[10] The specific activities (units per milligram of immobilized preparation) of the immobilized preparations were 5.05 for CAL-B, and 0.30 for HLL [where 1 unit corresponds to the amount of enzyme that converts 1 μmol of dodecyl acetate from 1-dodecanol and vinyl acetate (0.2 mmol) in anhydrous diisopropyl ether at 25 °C].

Deuteration of Methoxymethane-Protected Alkynols 8: General Procedure. Preparation of Wilkinson's catalyst:[27] A mixture of 2.1 g (8 mmol) of triphenylphosphine and 0.25 g (1.2 mmol) of $RhCl_3$ in 60 mL of ethanol was refluxed under an argon atmosphere. Heating was continued for 5 days until red crystals precipitated; the mixture was then allowed to cool to room temperature. The precipitate was filtered out, washed with methanol and ether, and dried at reduced pressure to afford 1.08 g (1.16 mmol, 98% yield) of Wilkinson's catalyst that was stored at 4 °C at dark under argon atmosphere. The product prepared under these conditions has been active after 1 year of storage.

To a mixture of 1.71 g of **8** (5 mmol) and 275 mg (0.3 mmol) of $RhCl(PPh_3)_3$ was added 20 mL of degassed benzene under an argon atmosphere to get a reddish solution. The system was purged by passing a stream of D_2 through, then the D_2 atmosphere was kept from the balloon and the solution was stirred for 36 h. The mixture was filtered through a bed of Celite and the solvent was evaporated. Residue was purified by flash chromatography on silica gel (0−3% MTBE/hexane) to give product **9** (92−96% yield).

[6,6,7,7-$^2H_4$]-5-Methoxymethyloxy-17,19-dioxaeicosane (9a). Compounds (*S*)-**9a** (1.61 g, 92%) and (*R*)-**9a** (1.63 g, 93%) were obtained from compounds (*S*)-**8a** and (*R*)-**8a**, respectively: IR 2925, 2855, 1465, 1150, 1110, 1045, 920 cm^{-1}; ^{1}H NMR δ 4.65 (s, 2H), 4.62 (s, 2H), 4.65−4.55 (1H), 3.52 (t, J = 6.5 Hz, 4H), 3.38 (s, 3H), 3.36 (s, 3H), 1.65−1.53 (2H), 1.53−1.20 (18H), 0.90 (t, J = 7 Hz, 3H); ^{13}C NMR δ 96.3 (CH_2), 95.2 (CH_2), 77.2 (CH), 67.8 (CH_2), 55.3 (CH_3), 55.0 (CH_3), 33.9 (CH_2), 33.5 (quint, J = 18 Hz), 29.7 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 27.4 (CH_2), 26.1 (CH_2), 24.2 (quint, J = 18 Hz), 22.8 (CH_2), 14.1 (CH_3); MS m/z 349 ($M^{\bullet+}$ − 1, 1), 317 (2), 289 (25), 275 (10), 257 (100), 239 (15), 217 (10), 187 (10), 169 (5), 141 (10), 113 (12), 95 (20), 85 (20); (*S*)-(−)-**9a** [α] = −2.0 (c = 2, $CHCl_3$); (*R*)-(+)-**9a** [α] = +1.9 (c = 2, $CHCl_3$).

Primary Alcohol Protection: General Procedure. ^{t}Butyldimethylsiloxy (TBDMS) protecting group was regioselectively introduced at the primary alcohol by the procedure reported by Aizpurua and Palomo with minor modifications.[17] Thus, a mixture of the diol **10** (525 mg, 2 mmol), *tert*-butyldimethylsilyl chloride (TBDMSCl) (360 mg, 2.4 mmol), and DBU (456 mg, 3 mmol) in dry CH_2Cl_2 (20 mL) was stirred under an argon atmosphere for 1 h. Then the resulting mixture was poured into 10 mL of an aquous solution saturated with CO_2 gas and extracted with CH_2Cl_2 (2 × 3 mL), and the combined organic fractions were evaporated to obtain a residue that was purified by column chromatography (hexanes/MTBE 10:1) on silica gel to afford the silylated product **11** (84−91% yield).

[6,6,7,7-$^2H_4$]-16-*O*-(*tert*-Butyldimethylsilyl)hexadecan-5-ol (11a). Compounds (*S*)-**11a** (639 mg, 85%) and (*R*)-**11a** (677 mg, 90%) were obtained from 525 mg of (*S*)-**10a** and 525 mg of (*R*)-**10a**: IR 3355, 2955, 2925, 2855, 1465, 1255, 1100, 835 cm^{-1}; ^{1}H NMR δ 3.60 (t, J = 6.5 Hz, 4H), 1.66−1.20 (20H), 0.91 (t, J = 7 Hz, 3H), 0.90 (s, 9H), 0.05 (s, 6H); ^{13}C NMR δ 71.8 (CH), 63.3 (CH), 37.1 (CH_2), 36.4 (quint, J = 19 Hz, CD_2), 32.8 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 27.8 (CH_2), 25.9 (CH_2), 25.8 (CH_2), 24.6 (quint, J = 19 Hz), 22.7 (CH_2), 18.3 (C), 14.1 (CH_3), −5.3 (CH_3); MS m/z 377 ($M^{\bullet+}$ + 1, 20), 359 (100), 342 (45), 319 (35), 301 (50), 273 (10), 245 (15), 227 (35), 173 (10), 156 (15), 140 (20), 133 (75), 127 (25), 115 (25), 99 (30); (*S*)-(+)-**11a** [α] = +0.6 (c = 3, $CHCl_3$); (*R*)-(−)-**11a** [α] = −0.6 (c = 3, $CHCl_3$).

Reduction of Mesylates 12: General Procedure. The mesyl derivative was dissolved in Et_2O (4 mL) and treated with $LiAlD_4$ (8 molar equiv) for 16 h at 20 °C (TLC monitoring). H_2O was added dropwise to the crude reaction mixture and the resulting white precipitate was filtered through Celite and concentrated to give a residue that, after purification by flash chromatography on silica gel with a gradient of 0−10% MTBE in hexane, gave the corresponding pure deuterated products **13** with 61−68% yields. In this reaction some desilylated alcohol was obtained (20−28%) that could be combined with what came from the next reaction.

[10,10,11,11,12-$^2H_5$]-1-*O*-(^{t}Butydimethylsilyl)hexadecane (13a). Compounds (*S*)-**13a** (161 mg, 68%) and (*R*)-**13a** (138 mg, 62%) were obtained from 290 mg of (*S*)-**12a** and 272 mg of (*R*)-**12a**: IR 2955, 2925, 2855, 1465, 1380, 1360, 1255, 1100, 835, 775 cm^{-1}; ^{1}H NMR δ 3.60 (t, J = 6.5 Hz, 2H), 1.57−1.44 (2H), 1.40−1.18 (21H), 0.89 (s, 9H), 0.88 (t, J = 7 Hz, 3H), 0.05 (s, 6H); ^{13}C NMR δ 63.4 (CH), 32.9 (CH_2), 31.9 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.2 (CH_2), 29.2 (t, J = 19 Hz, CHD), 28.7 (quint, J = 19 Hz), 28.6 (quint, J = 19 Hz), 26.0 (CH_2), 25.8 (CH_2), 22.7 (CH_2), 18.4 (C), 14.1 (CH_3), −5.3 (CH_3); MS m/z 390 ($M^{\bullet+}$ + 18, 10), 360 ($M^{\bullet+}$-2, 90), 346 (80), 304 (100), 228 (30), 133 (45), 127 (35), 115 (10), 99 (10).

Preparation of Carboxylic Acids 1: General Procedure. According to the procedure reported by Corey and Schmidt,[20] alcohols obtained previously were stirred in a solution of PDC (3 equiv) in DMF (0.2 M) for 3 days. At this time, the reaction mixture was treated with $Na_2S_2O_3$ solution until a green solution was obtained; then 2 mL of HCl (1 M) was added and the mixture was extracted with CH_2Cl_2, dried, and concentrated to a residue that was purified by flash chromatography on silica gel with hexane/MTBE 85:15 to give the corresponding acids in 65−70% yields.

[10,10,11,11,12-$^2H_5$]Hexadecanoic Acid (1a). Compounds (*S*)-[10,10,11,11,12-$^2H_5$]hexadecanoic acid [(*S*)-**1a**] (56 mg, 68%) and (*R*)-[10,10,11,11,12-$^2H_5$]hexadecanoic acid [(*R*)-**1a**] (59 mg, 70%) were obtained from 78 mg of (*S*)-**14a** and 80 mg of (*R*)-**14a**: mp 59−61 °C; IR 2920, 2855, 1705, 1460, 1415, 1295, 1230, 910, 735 cm^{-1}; ^{1}H NMR δ 2.35 (t, J = 7.5 Hz, 2H), 1.70−1.55 (2H), 1.40−1.14 (21H), 0.88 (t, J = 7 Hz, 3H); ^{13}C NMR δ 180.4 (CO), 34.1 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 29.0 (t, J = 19 Hz, CHD), 28.6 (quint, J = 19 Hz), 28.5 (quint, J = 19 Hz), 24.7 (CH_2), 22.7 (CH_2), 14.1 (CH_3). Anal. Calcd for $C_{16}H_{27}{}^2H_5O_2$: C, 73.50; H, 12.34. Found: C, 73.53; H, 12.12.

In Vitro Gland Culture Procedure: Determination of Stereospecificity. These experiments were carried out in round-bottom 96-well plates. In these experiments, 1-day-old virgin *T. pityocampa* females were briefly anesthetized on ice and pheromone glands were everted and excised, carefully cleaned, and immersed (1 gland/well). To each well, a 10 μL drop of incubation medium was added. The incubation medium consisted of the commercial Grace's insect medium (135 μL) and a dimethyl sulfoxide (DMSO) solution (15 μL) of stereoespecifically deuterated probe **1** (10 mg/mL each) for treated tissues or a DMSO solution for controls. Plates were sealed with adherent plastic covers, and incubations proceeded for 3 h at 25 °C. After this time, to obtain the methyl ester derivatives of the gland lipids for analysis, pheromone glands were soaked in chloroform/methanol (2:1) at 25 °C for 1 h and base-methanolized in 0.5 M KOH for 30 min, and then the organic solution was neutralized with 1 N HCl and extracted with hexane containing methyl pentadecanoate (10 ng/gland) as internal standard for quantification. Five glands were used for each sample.

Instrumental Analysis of the Biological Extracts. GC/MS analysis of extracts was performed by chemical ionization (CI) with methane as ionization gas. The system was equipped with a nonpolar capillary column (30 m × 0.25 mm i.d., 0.25

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μm stationary phase thickness) and used the following program: from 120 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 on methanolyzed lipidic extracts from pheromone glands with the equipment and conditions described above. Stereospecificity was determined from the abundance of ions in the range *m*/*z* 270−275, which afforded a cluster of ions in which the most abundant corresponded to the molecular ion of the resulting isotopomer of Δ^{11}-16-acid, analyzed as methyl ester, formed from each probe.

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Supporting Information Available: General experimental section and copies of ^{1}H and ^{13}C NMR and DEPT spectra for compounds **1b**, **2**−**4**, and **6**−**14** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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