Stereospecificity of an Enzymatic Monoene 1,4-Dehydrogenation Reaction: Conversion of (*Z*)-11-Tetradecenoic Acid into (*E*,*E*)-10,12-Tetradecadienoic Acid

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In this article, we report the first stereochemical study of an enzymatic 1,4-dehydrogenation reaction, namely, the transformation of (*Z*)-11-tetradecenoic acid into (*E*,*E*)-10,12-tetradecadienoic acid, involved in the sex pheromone biosynthesis of the moth *Spodoptera littoralis*. The investigation was carried out using the labeled substrates (*R*)-[10-^{2}H]- and (*S*)-[10-^{2}H]-tridecanoic acids ((*R*)-**2** and (*S*)-**2**, respectively) and (*R*)-[2,2,3,3,13-$^2H_5$]- and (*S*)-[2,2,3,3,13-$^2H_5$]-tetradecanoic acids ((*R*)-**1** and (*S*)-**1**, respectively). Probes (*R*)-**2** and (*S*)-**2** were prepared as described in a previous article.[1] The synthesis of the pentadeuterated chiral substrates (*R*)-**1** and (*S*)-**1** was accomplished by kinetic resolution of the racemic 12-tridecyn-2-ol (**6**) with immobilized porcine pancreatic lipase. The enantiomerically pure alcohols (*R*)-**6** and (*S*)-**6** were transformed into the final acids (*S*)-**1** and (*R*)-**1**, respectively, by a sequence of well-established reactions. The analyses of methanolyzed lipidic extracts from glands incubated separatedly with each individual probe showed that in the transformation of (*Z*)-11-tetradecenoic acid into (*E*,*E*)-10,12-tetradecadienoic acid, both *pro*-(*R*) hydrogen atoms at C-10 and C-13 are removed from the substrate. This is the first example reported of a desaturase with *pro*-(*R*)/*pro*-(*R*) stereospecificities that gives rise to (*E*)-double bonds. A mechanistic explanation for the stereochemical outcome of this reaction is advanced.

Introduction

Nature provides us with hundreds of different fatty acids, many of which are useful for industrial and pharmaceutical purposes. In general, the structural diversity of these compounds lies in the different number and location of double and triple bonds and other functional groups such as hydroxyl and epoxy functionalities.[2] Biosynthesis of all these various fatty acids involves the activity of a family of structurally related enzymes, including desaturases, hydroxylases, epoxygenases, acetylenases, and conjugases, which contain the fatty acid desaturase domain.[2]

In general, polyunsaturated fatty acids have methylene-interrupted (*Z*) unsaturations. In addition, fatty acids with conjugated double bonds and different olefin geometries also occur in nature.[3−16] This type of fatty acid originates from unsaturated precursors either by direct formation of an additional olefinic bond[7,9,13] or by 1,4-dehydrogenation, which occurs, formally, with rearrangement of the substrate double bond(s).[5,6,10,11,16]

The stereospecificity of desaturases that afford polyunsaturated fatty acids has been investigated in only a few cases.[17,18] In all instances, the two *pro*-(*R*) hydrogen atoms are removed to give the double bond. Besides its academic interest, the mechanistics of fatty acid desaturation has also attracted attention for the possible biotechnological preparation of unsaturated fatty acids of high added value. In this article, we report the first stereospecificity study of a 1,4-dehydrogenation type of transformation, namely, the conversion of (*Z*)-11-tetradecenoic acid into (*E*,*E*)-10,12-tetradecadienoic acid, occurring in the biosynthesis of *Spodoptera littoralis* (Lepidoptera: Noctuidae) sex pheromone. We also describe the synthesis of enantiomerically pure (*R*)-(2,2,3,3,-13-$^2H_5$) ((*R*)-**1**) and (*S*)-(2,2,3,3,13-$^2H_5$) ((*S*)-**1**) tetra-

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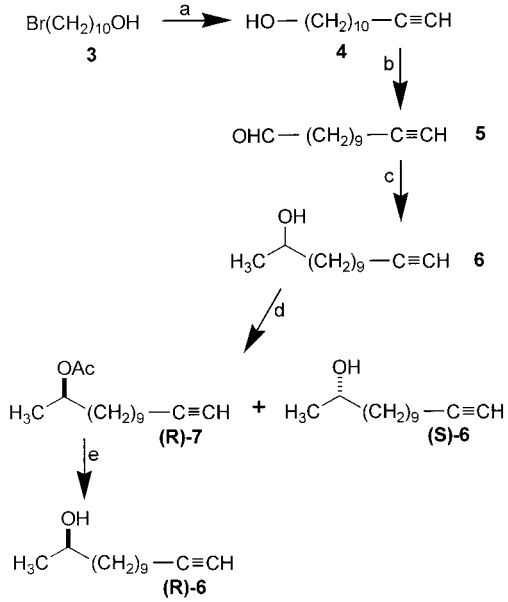
Scheme 1. Synthesis of (*R*)-6 and (*S*)-6[a]



[a] Reagents and conditions: (a) Li/NH_3/acetylene/DMSO (97%); (b) $(COCl)_2$/DMSO/Et_3N/CH_2Cl_2 (71%); (c) THF/MeMgBr/from −50 to 0° C (75%); (d) vinyl acetate/PPL-EP100/diisopropyl ether/25° C/26 h; (e) K_2CO_3/MeOH/rt/3 h (95%).

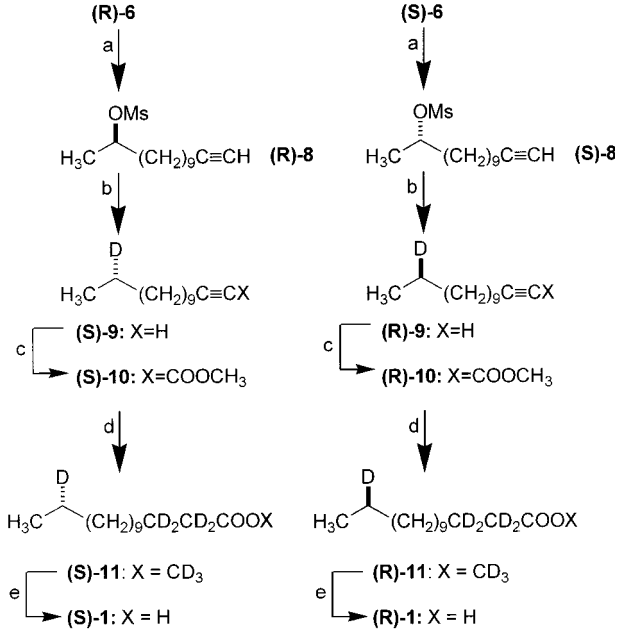
decanoic acids, which were used as probes in this investigation along with the previously reported (*R*)-[10-^{2}H]- and (*S*)-[10-^{2}H]-tridecanoic acids ((*R*)-**2** and (*S*)-**2**, respectively).[1]

Results

Synthesis of Probes. Enantiomerically pure deuterated acids (*R*)-**1** and (*S*)-**1** were prepared as outlined in Schemes 1 and 2. Reaction of 11-dodecyn-1-al (**5**), obtained by Swern oxidation[19] of 11-dodecyn-1-ol (**4**), with methylmagnesium bromide afforded the intermediate alcohol **6** (Scheme 1). Then, this racemic alcohol **6** was resolved by an enzymatic transesterification reaction in anhydrous diisopropyl ether.[20,21] Commercially available lipases, either free or deposited onto solid supports, were tested as catalysts using vinyl acetate as an acyl donor. The results obtained are summarized in Table 1. Porcine pancreatic lipase (PPL) deposited onto EP100 gave the best conversion, and enantiomeric excesses and this biocatalyst were used for the reaction scale-up.

Each of the separated enantiomers was transformed into the final probes as depicted in Scheme 2. Introduction of label at the stereogenic center was achieved by mesylation[22] followed by reaction with $LiAlD_4$.[23] The terminal acetylenic bond was then converted into the α,β-acetylenic ester **9** by reaction of the corresponding lithium acetylide with methyl chloroformate.[24] Introduction of labels at the α and β positions was carried out by reaction of **9** with Mg in deuterated methanol.[25] Final saponification of the labeled esters **10** afforded the expected acids (*R*)-**1** and (*S*)-**1**.

Scheme 2. Synthesis of (*R*)-1 and (*S*)-1[a]



[a] Reagents and conditions: (a) $(CH_3)_3N$·HCl/Et_3N/MsCl/CH_2Cl_2/0 °C/1 h ((*S*)-**7**, 82%; (*R*)-**7**, 81%); (b) $LiAlD_4$/Et_2O/from 0 °C to rt/3 h ((*R*)-**8**, 78%; (*S*)-**8**, 70%); (c) BuLi/THF/−60 °C/10 min, then $ClCO_2Me$/THF/rt/1 h ((*S*)-**9**, 83%; (*R*)-**9**, 70%); (d) Mg/CD_3OD/rt/16 h ((*S*)-**10**, 67%; (*R*)-**10**, 56%); (e) KOH/CD_3OD/D_2O/rt/16 h ((*S*)-**1**, 84%; (*R*)-**1**, 87%).

Table 1. Lipase-Catalyzed Resolution of the Racemic Alcohol 6

biocatalyst[a] (amount, mg)	% conversion[b] (time, h)	% ee alcohol[c] (*S*)	% ee ester (*R*)
PPL (63)	24 (47)	42	95
PPL-Celite (100)	34 (47)	33	>95
PPL-EP100 (50)	50 (23)	90	95
RML (100)	55 (23)	86	85
RML (100)	43 (8)	60	90
CAL (100)	100 (23)	0	0
CRL-Celite (100)	37 (191)	23	16
CRL-EP100 (100)	9 (95)		
PSL-EP100 (100)	90 (4)	0	0
ANL-EP100 (100)	13 (23)		

[a] Lipases abbreviations: ANL, *A. niger* lipase; CAL, *C. antarctica* lipase; CRL, *C. rugosa* lipase; PPL, porcine pancreatic lipase; PSL, *Pseudomonas sp.* lipase; RML, *R. miehei* lipase. Immobilization procedures and enzyme loading onto the support are described in the Experimental Section. [b] Substrate conversion was measured by GC and calculated from the peak areas of alcohol **6** and acetylated alcohol **7** by addition of an external standard. [c] Enantiomeric excesses of alcohol **6** and ester were measured by NMR spectroscopy of the diastereomeric Mosher esters.

Stereospecificity Study. Although labeled (*Z*)-11-tetradecenoyl CoA would be the actual substrate of the desaturase studied here, this compound is intracelullarly biosynthesized from the administered saturated precursors (*R*)-**1** and (*S*)-**1** (Scheme 3).[26] Tridecanoic acid derived probes, such as **2**, are also suitable tracers, since

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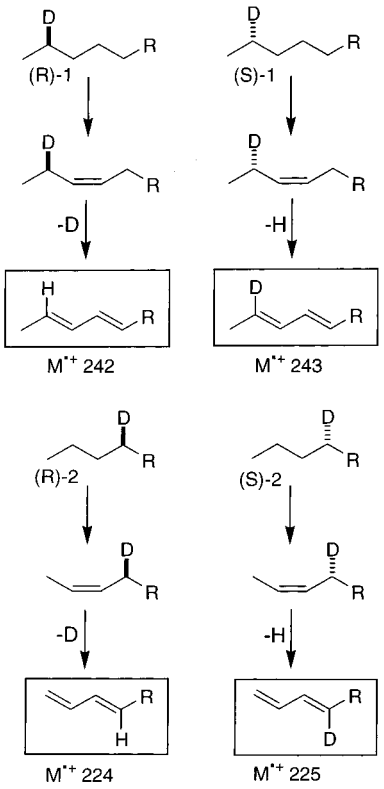
Scheme 3. Transformation of Probes 1 and 2 into (*E*,*E*)-10,12-Dienoates



1: R = $(CH_2)_6CD_2CD_2COOH$
2: R = $(CH_2)_8COOH$

Table 2. Absolute Stereochemistry of Hydrogens Removed at C-10 and C-13 in the Transformation of (*Z*)-11-Tetradecenoic Acid into (*E*,*E*)-10,12-Tetradecadienoic Acid

	isotopomer (%)[a]			
substrate	d_0	d_1	d_4	d_5
(*R*)-**2**	93.0 ± 2.8	7.0 ± 0.1		
(*S*)-**2**	4.9 ± 0.3	95.1 ± 5.2		
(*R*)-**1**			92.6 ± 3.7	7.4 ± 0.8
(*S*)-**1**			9.2 ± 0.9	90.8 ± 2.9

[a] Percentages of isotopomers correspond to the average ± standard deviation of three independent experiments performed with groups of three glands, except for the experiment with (*R*)-**2**. In this case, data correspond to two determinations with pools of 16 glands. Percentages were corrected for the abundances of $M^{\bullet+} + 1$ and $M^{\bullet+} - 1$ ions in the substrates. Products were analyzed as methyl esters as described in the text.

they are also precursors of 10,12-diene products (Scheme 3).[18,27] The results obtained in the deuterium-labeling experiments with both **1** and **2** are summarized in Table 2. The relative amounts of each isotopomer of the (*E*,*E*)-10,12-dienoate formed from each individual probe were measured by gas chromatography−mass spectrometry analysis as described in previous articles.[18,27] Dienes formed from either (*S*)-**1** or (*S*)-**2** presented clusters in which the most abundant ions were those at *m*/*z* 243 (tetradecadienoate-d_5) and 225 (tridecadienoate-d_1), respectively, corresponding to the diene products with all the deuterium atoms present in the substrates (see Scheme 3). Conversely, dienes formed from either (*R*)-**1** or (*R*)-**2** exhibited clusters in which the ions at *m*/*z* 242 (tetradecadienoate-d_4) and 224 (tridecadienoate-d_0), respectively, were the major ones. These results indicate that both *pro*-(*R*) hydrogen atoms are removed at C-10 and C-13 from the natural substrate, (Z)-11-tetradecenoic acid, to give (*E*,*E*)-10,12-tetradecadienoic acid (Scheme 3).

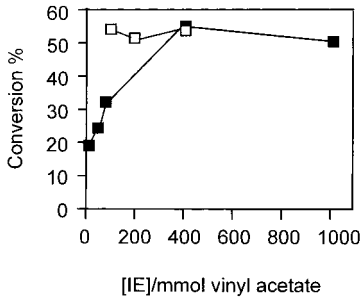


Figure 1. Lipase-catalyzed resolution of the racemic alcohol **6**. Influence of the immobilized enzyme concentration (mg/mL) to mmol of vinyl acetate ratio on the racemic alcohol **6** conversion using RML (filled symbols) and PPL on EP100 (empty symbols). Reactions were performed as described in the Experimental Section.

Discussion

As mentioned above, the synthesis of both enantiomerically pure probes (*R*)-**1** and (*S*)-**1** was carried out through lipase-catalyzed resolution of the intermediate racemic alcohol **6.** This enzymatic resolution had some interesting features to consider (Table 1). Among the lipases tested, only PPL and *Rizhomucor miehei* (RML) showed selectivity toward the substrate **6**. We also found that the activity of PPL increased when used deposited or adsorbed onto a support. Adsorption onto EP100 provided the best reaction rates and enantiomeric excesses. Working with RML, we found that the reaction performance depended on the ratio between the concentration of immobilized enzyme and vinyl acetate in the medium (Figure 1). Thus, as shown in Figure 1, the substrate conversion and RML enzymatic activity decreased upon decreasing this ratio. Interestingly, this behavior was not observed when using PPL on EP100 as the catalyst. The results found with RML suggested that vinyl acetate might be spontaneously hydrolyzed on contact with the ionic exchange resin used as a support, leading to the formation of acetaldehyde, which would finally cause the enzyme inactivation.

The stereospecificity found for the desaturase studied here is identical to that reported for the (*Z*)-9 stearoyl CoA desaturase from several systems,[17,28] as well as that of (*Z*)-12 oleoyl CoA[17] and (*Z*)-11 acyl CoA desaturases.[29,30] Furthermore, the same stereospecificity has been recently reported for the (*Z*)-9 desaturation of (*E*)-11-tetradecenoic acid,[18] which also occurs in the biosynthesis of *S. littoralis* sex pheromone.

It is worth mentioning that in all cases investigated so far, those desaturases with *pro*-(*R*)/*pro*-(*R*) stereospecificities afford (*Z*)-double bonds, whereas the isomeric (*E*)-olefins arise from enzymes with *pro*-(*R*)/*pro*-(*S*) stereospec-

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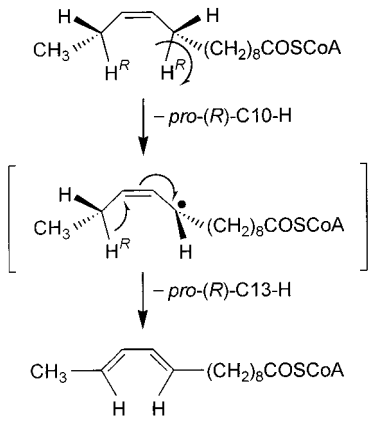

Figure 2. Transformation of (*Z*)-11-tetradecenoic acid into (*E*,*E*)-10,12-tetradecadienoic acid. According to the cryptoregiochemistry of the reaction[34] and the stereospecificity found here, the conversion of (*Z*)-11-tetradecenoic acid into (*E*,*E*)-10,12-tetradecadienoic acid involves a first rate-determining cleavage of the C-10-*pro*-(*R*)-H bond with formation of an unstable allylic intermediate (within brackets in the figure), followed by fast elimination of C-13-*pro*-(*R*)-H and concomitant rearrangement. The stereochemical outcome of the conversion is determined by the syn relationship adopted by the *pro*-(*R*) hydrogen atoms removed from the substrate at the enzyme active site.

ificities.[30,31] Interestingly, the enzyme studied here gives rise to a (*E*,*E*)-conjugated dienoic system upon removal of two *pro*-(*R*) hydrogen atoms. A possible explanation for this fact arises from considering that enzymatic desaturations are syn dehydrogenation processes.[32] Therefore, both *pro*-(*R*) C-10-H and *pro*-(*R*) C-13-H probably adopt a syn relationship at the enzyme active site (Figure 2). Considering the previously investigated cryptoregiochemistry[33] of this reaction,[34] the desaturation of (*Z*)-11-tetradecenoic acid takes place in two steps. First, a rate-determining oxidation of C-10 (loss of *pro*-(*R*) C-10-H as found here) with formation of an allylic intermediate occurs. Second, the intermediate rapidly collapses into the final diene by elimination of C-13-H (*pro*-(*R*) as shown here). The occurrence of bond rotations in the reaction course would give rise to dienes of different double bond geometries, (*Z*) or (*E*). However, since the intermediates formed in desaturation reactions are highly unstable and extremely short lived,[35] bond rotations are very unlikely and, therefore, the resulting diene is compelled to have an (*E*,*E*)-geometry. A similar mechanism was proposed by Fritsche et al.[36] in the formation of calendic acid from linoleic acid in seed oils. In this case, a 1,4-dehydrogenation with respect to the substrate C9 double bond afforded a conjugated 8,10,12-trienoic system.

In conclusion, this is the first reported study of the stereospecificity of an enzymatic 1,4-dehydrogenation reaction. Work is in progress to investigate the stereospecificities of other 1,4-dehydrogenation enzymatic processes that occur with formation of conjugated dienes of different geometries to gain a general insight into these unique biotransformations.

Experimental Section

General Methods. General methods can be found in previous articles.[1] All ^{1}H NMR spectra were acquired at 300 MHz, ^{13}C NMR spectra at 75 MHz, and ^{19}F NMR at 282 MHz in $CDCl_3$ solutions. Chemical shifts are given in parts per million using the internal standard $Si(CH_3)_4$ for ^{1}H, $CDCl_3$ for ^{13}C, and $CFCl_3$ for ^{19}F NMR. 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. GC-MS was performed 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.). In the biochemical experiments, the following temperature program was used: 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, and analyses were carried out under the selected ion monitoring (SIM) mode. Selected ions were 223, 224, 225, 226, 227, 240, 241, 242, 243, and 244. The dwell was set at 0.02 and the mass span at 0.5. Column chromatography was carried out by flash chromatography on silica gel (230−400 mesh). $LiAlD_4$ (deuterium content 99%) was obtained from Aldrich Chemical Co., and dimethyl sulfoxide was from Sigma. The probes (10*R*)-(10-^{2}H) tridecanoic acid ((*R*)-**2**) and (10*S*)-(10-^{2}H) tridecanoic acid ((*S*)-**2**) were synthesized in the laboratory as reported.[1]

Enzymes. *Aspergillus niger* lipase A (ANL) (136 000 U/g Amano's method) and *Pseudomonas* sp. lipase PS (PSL) (32 100 U/g Amano's method) were a generous gift of Amano Pharmaceuticals Co., Ltd. (Nagoya, Japan). *Candida rugosa* lipase (CRL) (240 U/mg, 1 unit (U) corresponds to 1 μmol of fatty acid liberated in the assay conditions described by Sanchez et al.[37]) was donated by the Department of Chemical Engineering (UAB) (Bellaterra, Spain). *Candida antarctica* lipase, Novozym 435 (CAL) (7000 PLU/g, PLU = propyl laurate unit), and *R. miehei* lipase, Lipozyme IM (RML) (5−6 BAUN/g, BAUN = batch acidolysis units Novo), were a generous gift of Novozymes A/S (Bagsvaerd, Denmark). PPL Type II, crude (60 U/mg, 1 unit hydrolyzes 1 μequiv of triacetin in 1 h at pH 7.7 at 37 °C), was purchased from Sigma (St. Louis, MO).

11-Dodecyn-1-ol (4).[38] Compound **4** was obtained from 10-bromo-1-decanol (**3**) following a previously reported procedure:[39] yield 97% (7.5 g, 41.1 mmol); ^{1}H NMR δ 3.61 (t, J = 6.5 Hz, 2H), 2.15 (dt, J = 2.5, 7.0 Hz, 2H), 1.91 (t, J = 2.5 Hz, 1H), 1.84 (m, 2H), 1.27 (b, 15H); ^{13}C NMR δ 84.7, 68.0, 63.0, 32.7, 29.5, 29.3, 29.0, 28.7, 28.4, 25.7, 18.3; IR 3311, 2927, 2854, 2114, 1461, 1058 cm^{-1}.

11-Dodecyn-1-al (5).[40] Aldehyde **5** was obtained by Swern oxidation of **4** (4.46 g, 24.4 mmol) using 3.2 mL of $(COCl)_2$ (36.7 mmol), 2.6 mL (36.7 mmol) of DMSO, and 10 mL (73.4 mmol) of dry Et_3N in a final volume of 50 mL of anhydrous CH_2Cl_2. Extraction of the reaction product with pentane afforded a crude that was purified by column chromatography (hexane/Et_2O 94:6) to give compound **5** (3.1 g, 17.2 mmol) in 71% yield: ^{1}H NMR δ 9,73 (t, J = 2.0 Hz, 1H), 2.39 (dt, J = 2.0, 7.5 Hz, 2H), 2.15 (dt, J = 2.5, 7.0 Hz, 2H), 1,91 (t, J = 2.5 Hz, 1H), 1.59 (m, 2H), 1.47 (m, 2H), 1.27 (b, 10H); ^{13}C NMR δ 202.9, 84.7, 68.0, 43.8, 29.24, 29.22, 29.0, 28.9, 28.6, 28.4, 22.0, 18.3; IR 3309, 2930, 2856, 2719, 1725, 1457 cm^{-1}.

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12-Tridecyn-2-ol (6).[41] To a solution of aldehyde **5** (2.54 g, 14.0 mmol) in dry THF (6 mL) kept at −50 °C was added, under argon, 5.6 mL (17 mmol) of a 3 M solution of MeMgBr in Et_2O. The mixture was stirred at −50 °C for 10 min and warmed to 0 °C; to the mixture was added 1 N HCl until complete solubilization of the crude, and the product was extracted with ethyl acetate. The combined organic layers were washed with brine and dried, and the crude product resulting from evaporation of the solvent was purified by column chromatography (hexane/Et_2O 80:20) to afford 2.06 g (10.5 mmol, 75%) of alcohol **6**: ^{1}H NMR δ 3.75 (c, 1H), 2.15 (dt, J = 2.5, 7.0 Hz, 2H), 1.91 (t, J = 2.5 Hz, 1H), 1.49 (m, 2H), 1.26 (b, 14H), 1,15 (d, J = 6.5 Hz, 3H); ^{13}C NMR δ 84.7, 68.1, 68.0, 39.3, 29.5, 29.3, 29.0, 28.7, 28.4, 25.7, 18.3, 23.4; IR 3388, 3315, 2929, 2902, 2860, 1466, 1378, 1133 cm^{-1}.

Mosher Esters Preparation. Oxalyl chloride (16 μL, 0.18 mmol) was added to a mixture of (*S*)-(−)-methoxytrifluoromethylacetic acid (8 mg, 0.03 mmol) and DMF (8 μL, 0.1210 mmol) in dry hexane (1 mL) under argon at room temperature. After 2 h, the formed precipitate was filtered off and solvent was evaporated to dryness at reduced pressure. This residue was dissolved in 0.350 mL of dry CH_2Cl_2 and added to a mixture of the nonracemic alcohol **6** (5 mg, 0.025 mmol), dry Et_3N (10 μL, 0.14 mmol), and DMAP (catalytic amount). After 2 h of stirring at room temperature, the solvent was removed under vacuum and $CDCl_3$ was added: (*S*)-6-MTPA, ^{19}F NMR δ −71.945; (*R*)-6-MTPA, ^{19}F NMR δ −71.989.

Enzyme Deposition and Adsorption. The general procedure for the deposition of the enzymes onto solid support materials was the following. An enzyme solution in 50 mM aqueous phosphate buffer pH 7.0 (1 mL) was mixed thoroughly with the support (1 g). The mixture was then evaporated under vacuum overnight. For *A. niger* (ANL), *Pseudomonas sp.* (PSL), *C. rugosa* lipase (CRL), and PPL, the enzyme loading was 100 mg of enzyme/g of Celite. Adsorption onto polypropylene was carried out following the methodology described by Gitlesen et al.[42] *C. antarctica* and *R. miehei* lipases were supplied as immobilized preparations on a macroporous acrylic resin and a macroporous anion exhange resin, respectively.

Kinetic Resolution of 6. Reactions at the analytical level were performed in a 10 mL closed flask. Unless otherwise stated, vinyl acetate (18 μL, 0.19 mmol) and the racemic alcohol **6** (25 mg, 0.12 mmol) were dissolved in diisopropyl ether (1.25 mL). To this solution was added free or immobilized lipase (Table 1). The mixture was placed on a reciprocal shaker (125 rpm) thermostated at 25 °C.

Reactions at the preparative level were performed as follows. Vinyl acetate (0.7 mL, 7.5 mmol) and the racemic alcohol **6** (0.99 g, 5.0 mmol) were dissolved in diisopropyl ether (50 mL). To this solution was added PPL adsorbed on EP100 (2 g). The mixture was placed on a reciprocal shaker (125 rpm) at 25 °C for 26 h. The suspension was filtered, and the solid was washed with Et_2O; the solvent was evaporated under vacuum, and the residue was purified by column chromatography on silica. Elution with hexane/Et_2O (94:6) afforded (*R*)-(−)-1-methyl-11-dodecinyl acetate ((*R*)-**7**) (486 mg, 2.0 mmol), and elution with hexane/Et_2O (80:20) gave (*S*)-(+)-12-tridecyn-2-ol ((*S*)-**6**) (385 mg, 1.9 mmol). (*S*)-**6**: ^{1}H NMR δ 3.76 (c, 1H), 2.15 (dt, J = 2.5, 7.0 Hz, 2H), 1.91 (t, J = 2.5 Hz, 1H), 1.26 (b, 16H), 1.15 (d, J = 6,5 Hz, 3H); ^{19}F NMR δ −71.945 (>95% (*S*)-enantiomer); $[\alpha]^{20}_D$ +4 (*c* 5, $CHCl_3$); ee > 95%. (*R*)-**7**: ^{1}H NMR δ 4,85 (tq, J = 6.0, 6.5 Hz, 1H), 2.15 (dt, J = 2.5, 7.0 Hz, 2H), 1.99 (s, 3H), 1.91 (t, J = 2.5 Hz, 1H), 1.24 (b, 16H), 1.16 (d, J = 6.5 Hz, 3H); ^{13}C NMR δ 170.7, 84.7, 71.0, 68.0, 35.8, 29.4, 29.38, 29.36, 29.0, 28.6, 28.4, 25.3, 18.3, 21.3, 19.9; IR 3301, 2978, 2931, 2857, 1737, 1369, 1244 cm^{-1}; $[\alpha]^{20}_D$ −1 (*c* 5, $CHCl_3$); ee 84%. Anal. Calcd for $C_{15}H_{26}O_2$: C, 75.58; H, 10.99. Found: C, 75.66; H, 11.12.

(*R*)-(−)-12-Tridecyn-2-ol (R-6). A mixture of acetate (*R*)-**7** (486 mg, 2.0 mmol), K_2CO_3 (1.1 g, 8.1 mmol), and methanol (9 mL) was stirred at room temperature for 3 h. The mixture was cooled at 0 °C, and 1 M HCl was added until complete solubilization of the suspension was achieved. Methanol was evaporated, and the product was extracted with ethyl acetate. The reaction crude was purified by column chromatography using hexane/Et_2O (80:20) to obtain the expected alcohol (*R*)-**6** (377 mg, 1.9 mmol, 95%): ^{1}H NMR δ 3.77 (c, 1H), 2.15 (dt, J = 2.5, 7.0 Hz, 2H), 1.91 (t, J = 2.5 Hz, 1H), 1.49 (m, 2H), 1.26 (b, 14H), 1.16 (d, J = 6.5 Hz, 3H); ^{19}F NMR δ −71.945 (8%, (*S*)-enantiomer), −71.989 (92% (*R*)-enantiomer); $[\alpha]^{20}_D$ −5 (*c* 5, $CHCl_3$); ee 84%.

12-Tridecyn-2-yl Methanesulfonate (8). Compound **8** was obtained from **6** following a previously reported procedure.[34] *S*-(+)-**8**: yield 82% (437 mg, 1.6 mmol) after column chromatography (hexane/Et_2O (80:20)); ^{1}H NMR δ 4.76 (tq, J = 6.0, 6.5 Hz, 1H), 2.96 (s, 3H), 2.15 (dt, J = 2.5, 7.0 Hz, 2H), 1.91 (t, J = 2.5 Hz, 1H), 1.38 (d, J = 6.0 Hz, 3H), 1.25 (b, 16H); ^{13}C NMR δ 84.7, 80.4, 68.0, 38.6, 36.6, 29.3, 29.1, 28.9, 28.6, 28.4, 25.1, 18.3, 21.3; IR 3291, 2931, 2856, 1464, 1352, 1176, 971, 915 cm^{-1}; $[\alpha]^{20}_D$ +6 (*c* 5, $CHCl_3$). *R*-(−)-**8**: yield 81% (418 mg, 1.5 mmol); $[\alpha]^{20}_D$ −6 (*c* 5, $CHCl_3$).

[12-^{2}H]-1-Tridecyne (9). Compound **9** was obtained from **7** following a previously reported procedure.[34] (*R*)-**9**: yield 78% (211 mg, 1.2 mmol) after column chromatography (hexane); ^{1}H NMR δ 2.16 (dt, J = 2.5, 7.0 Hz, 2H), 1.91 (t, J = 2.5 Hz, 1H), 1.23 (b, 17H), 0.84 (d, J = 5.5 Hz, 3H); ^{13}C NMR δ 84.7, 67.9, 31.8, 29.6, 29.5, 29.3, 29.1, 28.7, 28.4, 25.1, 18.3, 22.28 (t, J = 19.0 Hz), 13.9; IR 3315, 2926, 2854, 1458, 1238 cm^{-1}. Anal. Calcd for $C_{13}H_{23}D$: C, 86.11; H + D, 13.34. Found: C, 85.78; H + D, 13.21. (*S*)-**9**: yield 70% (193 mg, 1.1 mmol). Anal. Calcd for $C_{13}H_{23}D$: C, 86.11; H + D, 13.34. Found: C, 85.69; H + D, 13.54.

[13-^{2}H]-Methyl 2-Tetradecynoate (10). Compound **10** was obtained from **9** following a previously reported procedure[34] and purified by column chromatography (hexane/Et_2O (97:3)). (*S*)-**10**: yield 83% (206 mg, 0.86 mmol); ^{1}H NMR δ 3.73 (s, 3H), 2.29 (t, J = 7.0 Hz, 2H), 1.54 (m, 2H), 1.22 (b, 15H), 0.84 (d, J = 6.0 Hz, 3H); ^{13}C NMR δ 154.28, 89.9, 72.7, 52.5, 31.7, 29.5, 29.3, 29.2, 28.9, 28.7, 27.4, 18.6, 22, 25 (t, J = 19.0 Hz), 13.9 (CH_3); IR 2924, 2855, 2238, 1722, 1254, 1077 cm^{-1}. Anal. Calcd for $C_{15}H_{25}DO_2$: C, 75.26; H + D, 10.94. Found: C, 74.98; H + D, 11.22. (*R*)-**10**: yield 70% (188 mg, 0.78 mmol). Anal. Calcd for $C_{15}H_{25}DO_2$: C, 75.26; H + D, 10.94. Found: C, 74.80; H + D, 11.06.

[1,1,1-$^2H_3$]-Methyl [2,2,3,3,13-$^2H_5$]-Tetradecanoate (11). Compound **11** was obtained from **10** following a previously reported procedure.[34] (*S*)-**11**: yield 67% (139 mg, 0.55 mmol) after column chromatography (hexane/Et_2O (97:3)); ^{1}H NMR δ 1.23 (b, 19H), 0.84 (d, J = 5.5 Hz, 3H); IR 2924, 2854, 1739, 1456, 1283, 1089 cm^{-1}. (*R*)-**11**: yield 56% (109 mg, 0.44 mmol).

[2,2,3,3,13-$^2H_5$]-Tetradecanoic Acid (1). Compound **1** was obtained by saponification of **10** following a previously reported procedure.[34] (*S*)-**1**: yield 84% (79 mg, 0.34 mmol) after column chromatography (CH_2Cl_2/MeOH (98:2)); mp 51−53 °C; ^{1}H NMR δ 1.23 (b, 19H), 0.85 (d, J = 5.5 Hz, 3H); ^{13}C NMR δ 180.5, 31.8, 29.66, 29.63, 29.5, 29.4, 29.3, 29.1, 28.7, 33.3 (quintet, J = 20.0 Hz), 23.83 (quintet, J = 21.0 Hz), 22.28 (t, J = 19.0 Hz), 13.9. IR 2952, 2917, 2850, 1696, 1310, 952 cm^{-1}. Anal. Calcd for $C_{14}H_{23}D_5O_2$: C, 72.06; H + D, 12.09. Found: C, 72.40; H, 12.48. (*R*)-**1**: yield 87% (88 mg, 0.38 mmol); mp 51−53 °C. Anal. Calcd for $C_{14}H_{23}D_5O_2$: C, 72.06; H + D, 12.09. Found: C, 72.25; H, 12.38.

The following are the final deuterium contents (%) of the labeled substrates, as determined by GC-MS analysis of their respective methyl esters: (*R*)-**1**, 90.4 $^2H_5$, 8.3 $^2H_4$, 1.3 $^2H_3$; (*S*)-**1**, 89.9 $^2H_5$, 7.4 $^2H_4$, 2.8 $^2H_3$.

Biochemical Experiments. The procedure of Abad et al.[18] was followed. Pheromone glands were treated with the probes dissolved in dimethyl sulfoxide (0.1 μL, 10 μg/μL). A total dose of 4 μg was given in four subsequent 60 min incubations with 1 μg each. After each treatment, incubations were performed in chambers at 25 °C and 65% humidity. The glands were dissected 60 min after the last application, and tissues were

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soaked in chloroform/methanol (2:1) at 25 °C for 1 h. The lipidic extract thus obtained was base methanolyzed, and the extracts were analyzed by GC-MS using the equipment mentioned in General Methods. Stereospecificity was determined from the abundance of ions in the *m*/z range from 223 to 227 (treatments with (*R*)-**2** or (*S*)-**2**) or 240 to 244 (treatments with (*R*)-**1** or (*S*)-**1**), which afforded a cluster of ions in which the most abundant product corresponded to the molecular ion of the resulting isotopomer of the 10,12-conjugated dienoate (nondeuterated (*E*,*E*)-10,12-tridecadienoate, 224; monodeuterated (*E*,*E*)-10,12-tridecadienoate, 225; pentadeuterated (*E*,*E*)-10,-12-tetradecadienoate, 243; tetradeuterated (*E*,*E*)-10,12-tetradecadienoate, 242).

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Note Added after ASAP Posting. The version of this paper posted on March 3, 2002, had incorrect structures in Scheme 3. The version with the corrected Scheme 3 was posted on March 11, 2002.

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