Mechanism-Based Inhibitors of Prostaglandin ω-Hydroxylase: (*R*)- and (*S*)-12-Hydroxy-16-heptadecynoic Acid and 2,2-Dimethyl-12-hydroxy-16-heptadecynoic Acid

Alain Burger,[†] Joan E. Clark,[‡] Masazumi Nishimoto,[‡] A. Scott Muerhoff,[‡] Bettie Sue Siler Masters,[‡] and Paul R. Ortiz de Montellano*,[†]

Department of Pharmaceutical Chemistry, School of Pharmacy, and Liver Center, University of California, San Francisco, California 94143-0446, and Department of Biochemistry, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, Texas 78284-7760

Received December 28, 1992

12-Hydroxy-16-heptadecynoic acid has been shown to selectively inactivate cytochrome P450 4A4, a pulmonary cytochrome P450 enzyme that catalyzes the ω-hydroxylation of prostaglandins [Muerhoff, A. S.; Williams, D. E.; Reich, N. O.; CaJacob, C. A.; Ortiz de Montellano, P. R.; Masters, B. S. S. *J. Biol. Chem.* **1989**, *264*, 749–756]. Potent, specific inhibitors of this enzyme are required to explore its physiological role. In a continuing effort to develop such agents, the two enantiomers of 12-hydroxy-16-heptadecynoic acid have been stereospecifically synthesized, their absolute stereochemistry confirmed, and the dependence of enzyme inactivation on absolute stereochemistry determined using cytochrome P450 4A4 purified from the lungs of pregnant rabbits. The 12*S* enantiomer is roughly twice as active (K_I = 1.8 μM, $t_{1/2}$ = 0.7 min) as the 12*R* enantiomer (K_I = 3.6 μM, $t_{1/2}$ = 0.8 min), but the chirality of the hydroxyl group is not a major determinant of the specificity for the prostaglandin ω-hydroxylase. The flexibility of the acyclic skeleton of the inhibitor may account for the relatively low enantiomeric discrimination. 2,2-Dimethyl-12-hydroxy-16-heptadecynoic acid, an analogue that cannot undergo β-oxidation, has also been synthesized as a potential in vivo inhibitor of the enzyme and has been shown to inactivate the purified enzyme with K_I = 4.9 μM and $t_{1/2}$ = 1.0 min. These acetylenic agents, particularly the dimethyl analog, are promising in vivo inhibitors of cytochrome P450 4A4.

Prostaglandins are hydroxylated at the ω-position[1] in lung microsomes by a cytochrome P450 ω-hydroxylase known as $P450_{PG\omega}$[2] or P450 *p*-2[3] and classified by the current systematic nomenclature as CYP4A4.[4] This ω-hydroxylase is markedly elevated in pregnant rabbits[5,6] and in rabbits treated with progesterone.[7] The enzyme has been purified[2,3] and its amino acid sequence has been deduced from the cDNA sequence,[8] but its biological role and the reason for its induction remain unclear. One approach to clarifying the physiological role of this monooxygenase is to use agents that specifically inhibit or inactivate cytochrome P450 4A4 to examine its function. In a previous paper, we described the synthesis of several terminal acetylenic fatty acids and their activities as inhibitors of cytochrome P450 4A4.[9] Of the compounds tested, racemic 12-hydroxy-16-heptadecynoic acid [(±) 8] was found to possess the highest degree of selectivity for inactivation of the prostaglandin ω-hydroxylase in rabbit lung microsomes. One possible reason for the high specificity of this fatty acid analogue is that it bears the ω-5 hydroxyl function characteristic of the prostaglandins that are the substrates of the enzyme. However, the compound examined in the earlier study with microsomes prepared from lungs of pregnant rabbits was a racemic mixture of the *R* and *S* enantiomers, whereas the ω-5 hydroxyl group of the prostaglandins is exclusively in the *S* absolute configuration. The possibility therefore exists that the enzyme specifically or preferentially recognizes only one of the two enantiomers of the acetylenic fatty acid and is primarily or exclusively inactivated by it. The inhibitory profile of 12-hydroxy-16-heptadecynoic acid (8) is of further interest because recent studies have shown that it inhibits the pressure-induced vasoconstriction of renal vessels, probably by inhibiting the ω-hydroxylation of arachidonic acid by an as yet unspecified form of cytochrome P450.[10]

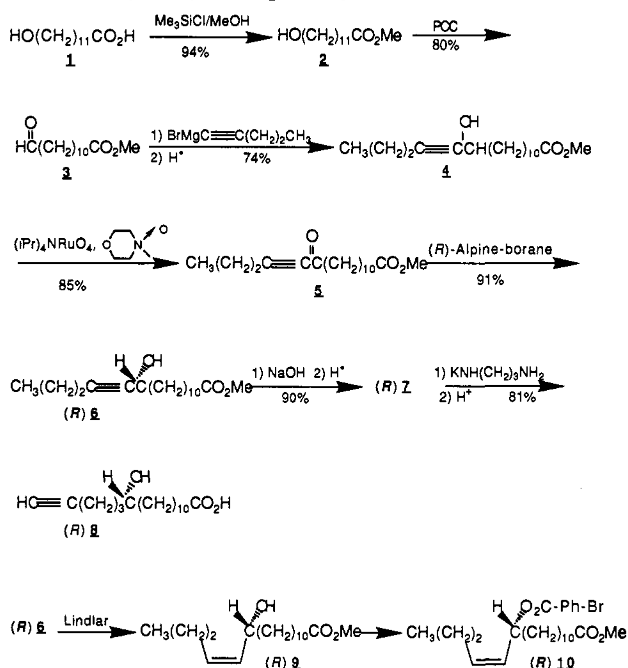
Rapid esterification and β-oxidation of fatty acids makes their use as in vivo probes of physiological mechanisms or as therapeutic agents difficult. For example, 10-undecynoic and 11-dodecynoic acids are effective in vitro inactivators of hepatic lauric acid ω-hydroxylase (CYP-4A1).[11] However, these agents exhibit very little inhibitory activity in the perfused liver and have no detectable activity in vivo.[12] The use of unsaturated fatty acid analogues as in vivo probes therefore requires the preparation of analogues that are resistant to metabolic degradation. Metabolic resistance was achieved in the case of the lauric acid ω-hydroxylase inactivators by placing two methyl groups α to the carboxyl group.[13] The two methyl groups block β-oxidation but apparently do not interfere with mechanism-based inactivation of lauric acid ω-hydroxylase.

We describe here synthesis of the individual *R* and *S* enantiomers of 12-hydroxy-16-heptadecynoic acid (8) as well as the 2,2-dimethylated derivative of the racemic compound. The results of inhibition studies using cytochrome P450 4A4 purified from lungs of pregnant rabbits indicate that the stereochemistry of the hydroxyl group is only a modest determinant of the isozyme specificity. Furthermore, they establish the potential utility of the 2,2-dimethyl compound as an in vivo inhibitor of the enzyme.

* Address correspondence to: Dr. Paul R. Ortiz de Montellano, School of Pharmacy, University of California, San Francisco, San Francisco, CA 94143-0446.
[†] University of California.
[‡] University of Texas Health Science Center.

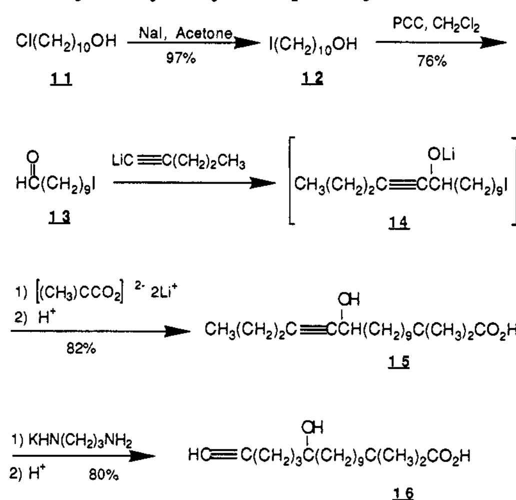
0022-2623/93/1836-1418$04.00/0 © 1993 American Chemical Society

Scheme I. Synthesis of the (12*R*)-12-Hydroxy-16-heptadecynoic Acid[a]



[a] The same scheme applies for synthesis of the 12*S* enantiomer.

Scheme II. Synthesis of 2,2-Dimethyl-12-hydroxy-16-heptadecynoic Acid



Synthesis

The *R* and *S* enantiomers of hydroxyl terminal acetylenic acid (**8**) were prepared by the seven-step synthesis shown in Scheme I. The key step in the scheme is reduction of the α,β-unsaturated prochiral ketone (**5**) to the chiral alcohol with the Midland reagent[14] which gives access to each of the two enantiomers of **6**. The overall yield of each of the enantiomers in this synthetic scheme is typically about 30%.

Conversion of 12-hydroxydodecanoic acid (**1**) to the α,β-unsaturated ketone **5** was accomplished by conventional methods. Methyl ester **2** was prepared in quantitative yield as described by Brook and Chan.[15] Oxidation of the esterified fatty acid alcohol to aldehyde **3** was achieved with pyridinium chlorochromate[16] in 80% yield. Addition of a slight excess of the Grignard reagent at −40 °C to a partially solubilized suspension of compound **3** in tetrahydrofuran gave propargylic alcohol **4** in 74% yield. The Grignard reagent is preferable to the lithium analogue because the lithiated derivative is much less selective and reacts with the ester group at temperatures as low as −78 °C. The propargylic alcohol was oxidized to the α,β-unsaturated ketone **5** in 85% yield using the method of Griffith et al.[17]

Several methods are now known for the reduction of α,β-ynones to chiral propargylic alcohols. Alpine-borane has proven useful for the asymmetric synthesis of these kind of compounds.[14] This reagent is desirable because it can be prepared from α-pinene, which is now available in 98% optical purity, in both *d* and *l* forms. However, in tetrahydrofuran solution, the reagent reacts relatively slowly with the conjugated ketone. Under these circumstances, the usual reduction by a cyclic mechanism is replaced by an alternative mechanism in which Alpine-borane dissociates to give pinene and the achiral 9-BBN. Reduction of the ketone by achiral 9-BBN produces the racemic alcohol and thus decreases the optical purity of the product. This side reaction can be overcome, as described recently by Brown and collaborators, by carrying out the reaction in more concentrated solution (neat).[18] Reduction with 2 equiv of neat, freshly-prepared (*R*)-Alpine-borane readily furnished the (*R*)-propargylic alcohol **6** in 91% yield. Hydrolysis of the methyl ester gave, in turn, the free acid (*R*)-**7** in 90% yield. Finally, the acetylenic function was shifted to the chain terminus by treatment with 10 equiv of potassium 3-aminopropylamide (KAPA).[19] Product (*R*)-**8** was thus obtained in 81% isolated yield.

The optical purity of (*R*)- and (*S*)-**6** (0.015 mmol in $CDCl_3$) was checked by ^1H NMR (500 MHz) with the chiral shift reagent, $Eu(hfc)_3$ (15% molar). It was determined to be 92% for the *R* isomer and 96% for its antipode. In each case the enantiomeric hydroxyl compound was added to verify the NMR peak assignment. In NMR spectra the signal of the carbinyl proton appeared as a broad singlet at 5.9 ppm for (*R*)-**6** and 6.1 ppm for (*S*)-**6**. The absolute configuration inferred from the mechanism proposed by Midland was confirmed by the Nakanishi rule.[20] The allylic *p*-bromobenzoate (*R*)-**10** was prepared as follows. The triple bond of (*R*)-**6** was semihydrogenated with Lindlar catalyst[21] to give (*R*)-**9**. The *Z* allylic alcohol was *p*-bromobenzoylated as described to give (*R*)-**10**.[20] The circular dichroism spectrum of (*R*)-**10**, with a negative Cotton effect ($c = 3.1 \times 10^{-5}$ M in MeOH, $\lambda_{max} = 243$ nm, De = −8 deg/M^{-1} cm^{-1}), and the large J_{vic} of 9 Hz between the olefinic and the carbinyl protons in the ^1H NMR (500 MHz, $CDCl_3$) are consistent with the reported analysis.[20] As described by Midland et al.,[22] no significant racemization of the optically-active propargylic alcohols (*R*)- and (*S*)-**7** occurred during isomerization with KAPA to, respectively, (*R*)- and (*S*)-**8**. Indeed, the optical purity of each of the final compounds was checked by ^1H NMR (500 MHz, $CDCl_3$) after esterification of the carboxylic acid with diazomethane and derivatization with Mosher's reagent.[23] In the NMR spectra of the diastereomers obtained from (*R*)- and (*S*)-**8**, the signals of the protons at C-17 appeared as a triplet ($J = 2.5$ Hz) at 1.97 and 1.95 ppm and the signal of the protons at C-15 appeared as a triplet of doublets ($J_1 = 7$ Hz, $J_2 = 2.5$ Hz) at 2.2 and 2.12 ppm, respectively.

The α-dimethyl analog of (±)-**8**, compound **16**, was prepared in four steps in 48% overall yield as shown in Scheme II. The starting material **11** was transformed into iodo aldehyde **13** in 74% yield by displacing the chloride with iodide and oxidizing the alcohol with pyridinium

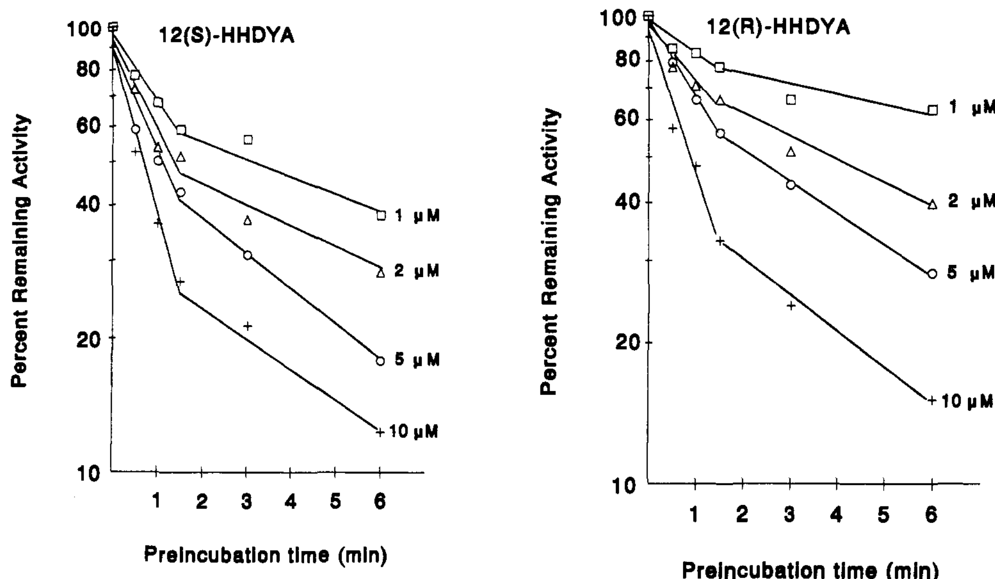


Figure 1. Time-dependence of the inactivation of prostaglandin E_1 ω-hydroxylase (CYP4A4) by (*S*)- and (*R*)-12-hydroxy-16-heptadecynoic acid. The concentrations of the inhibitors in the preincubation mixtures are indicated in the figures.

chlorochromate. Compound **15** was prepared from **13** in a one-pot synthesis. Addition of pentynyllithium to the aldehyde quantitatively yielded the lithiated propargylic alcohol **14**. This anion was directly condensed with dilithium isobutyrate[24,25] to produce the hydroxyl propargylic fatty acid **15**. This one-pot procedure furnished **15** in 82% yield. Finally, the internal triple bond of **15** was shifted to the terminal position with KAPA, as already described for (*R*)-**8**.

Biological Assays

Incubations of (*R*)- and (*S*)-**8** with purified rabbit lung CYP4A4 show that both enantiomers inhibit prostaglandin ω-hydroxylation (Figure 1). The *S* enantiomer inactivates the enzyme with $K_I = 1.8$ μM and $t_{1/2} = 0.7$ min and the *R* enantiomer with $K_I = 3.6$ μM and $t_{1/2} = 0.8$ min (Figure 2). The inactivation curves are biphasic, as is common for cytochrome P450.[9] The kinetic values were calculated from the rapid phase of the inactivation reaction. The K_I for the *R* enantiomer determined with the purified enzyme is approximately the same as that determined previously for the racemic mixture with the microsomal enzyme (K_I = 4.8 μM).[9] The present results show that the two enantiomers of **8** have similar activities as inactivating agents, although the *S* enantiomer is roughly twice as effective due to a higher affinity for the enzyme. The stereochemical tolerance implicit in this finding is surprising in view of the invariant chirality of the 15-hydroxyl group in the prostaglandins. The 15-hydroxyl group corresponds to the hydroxyl group in **8** in that it is five atoms away from the terminal methyl group that is hydroxylated by the enzyme. Indeed, the chirality at the ω-5 position of the more effective *S* inhibitor is opposite to that at the ω-5 position of the prostaglandins (note that the *S* configuration of the hydroxyl in the inhibitor corresponds to the *R* configuration in the prostaglandins). The poor discrimination between the two chiral hydroxyl groups may be due to the fact that the fatty acid skeleton of the inhibitor is relatively flexible compared to that of the prostaglandins. The absence of a strict stereochemical requirement for the hydroxyl group is consistent with the recent finding that cytochrome P450 4A4 oxidizes arachidonic acid nearly as effectively as it does the prostaglandins

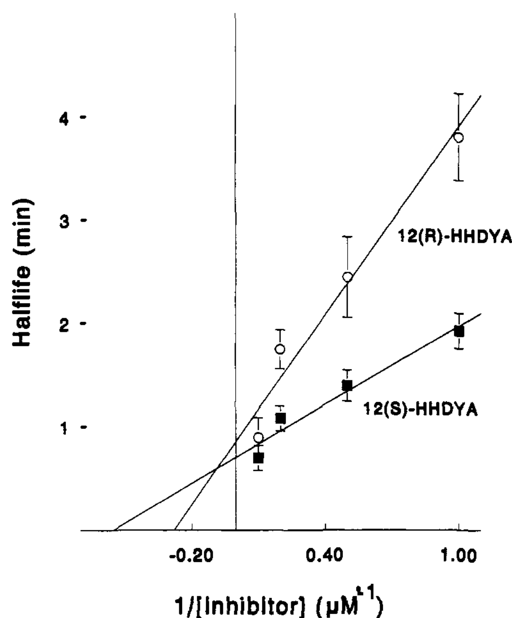


Figure 2. Determination of the K_I and $t_{1/2}$ values for the inactivation of prostaglandin ω-hydroxylase (CYP4A4) by the 12*S* and 12*R* enantiomers of 12-hydroxy-16-heptadecynoic acid. The *Y*-axis values for the points on the plot were obtained by weighed linear regression analysis of the time points between 0 and 1.5 min in Figure 1.

(unpublished results). The results demonstrate that the hydroxyl group conveys specificity for CYP4A4 but that this specificity depends on the presence, rather than the chirality, of the hydroxyl group.

In order to investigate the role of CYP4A4 in vivo it is not only desirable to have a specific inhibitor of that enzyme but also to have an inhibitor that is relatively resistant to metabolism. As noted earlier, we have shown that short-chain acetylenic fatty acids that inactivate lauric acid ω-hydroxylase in vitro are not detectably active in vivo, presumably because they are too rapidly metabolized or cleared from the circulation. This problem was circumvented in the work with CYP4A1 by introducing methyl groups α to the carboxyl group to block β-oxidation of the fatty acid chain. As shown in Figure 3, 2,2-dimethyl-12-hydroxy-16-heptadecynoic acid, the corresponding 2,2-

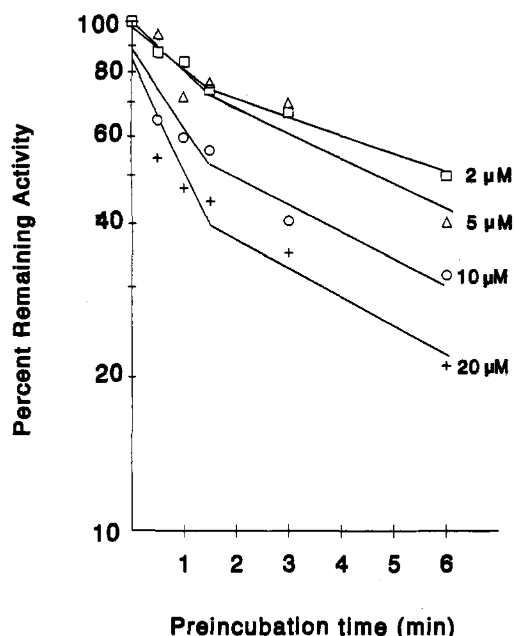


Figure 3. Time-dependence of the inactivation of prostaglandin E_1 ω-hydroxylase (CYP4A4) by 2,2-dimethyl-12-hydroxy-16-heptadecynoic acid. The concentrations of the inhibitor in the preincubation mixtures are indicated in the figure.

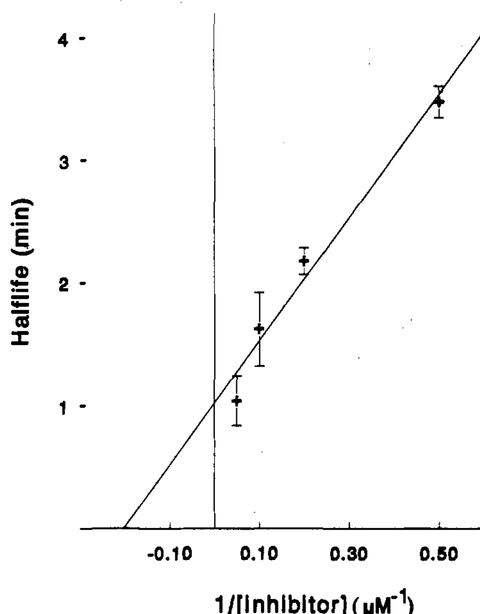


Figure 4. Determination of the K_I and $t_{1/2}$ values for the inactivation of prostaglandin ω-hydroxylase (CYP4A4) by 2,2-dimethyl-12-hydroxy-16-heptadecynoic acid. The Y-axis values for the points on the plot were obtained by weighed linear regression analysis of the time points between 0 and 1.5 min in Figure 3.

dimethylated analogue of 8 is an effective inactivating agent for CYP4A4 and therefore is attractive as an inactivating agent for this enzyme in intact cell systems or in vivo. The K_I and $t_{1/2}$ values, 4.9 μM and 1.0 min, respectively (Figure 4), show that the dimethyl groups do not impair the effectiveness of this agent as an inactivating agent. The similar experience with medium chain acetylenic fatty acid inactivators of CYP4A1 and the ω-5 hydroxylated long-chain acetylenic acid inhibitors of CYP4A4 suggests that the region α to the carboxyl group is not highly constrained in the active sites of the ω-hydroxylases.

Experimental Procedures

Melting points were measured on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Optical rotations [α] were measured at room temperature on a Perkin-Elmer 141 polarimeter. Circular dichroism spectra were recorded on a JASCO J-500A spectropolarimeter. Absorption spectra were recorded on a Hewlett-Packard 8450A UV/vis spectrophotometer. IR spectra were obtained on a Nicolet 5DX FT-IR instrument. ^{1}H NMR spectra were recorded on a General Electric 500-MHz instrument in deuterated chloroform using the chloroform signal at 7.26 ppm as an internal standard. Chemical shifts are reported in ppm downfield from tetramethylsilane. Electron-impact (70 eV) MS were obtained on a Kratos AEI-MS 25 spectrometer. Microanalyses were performed by the Microanalytical Laboratory of the Chemistry Department of the University of California, Berkeley.

Both *d*- and *l*-α-pinene (98% ee) and 9-BBN dimer were purchased from Aldrich. Solvents were freshly dried by distillation before use: acetone from P_2O_5, diaminopropane, dichloromethane, diisopropylamine, pentane, and tetrahydrofuran from CaH_2, and diethyl ether from lithium aluminum hydride (under argon). Air- or moisture-sensitive reactions were carried out in flame-dried glassware. Thin-layer chromatography was done on silica gel GF precoated plates (Analtech). The plates were developed by dipping in a solution of phosphomolybdic acid (25 g) and ceric sulfate tetrahydrate (10 g) in 94:6 H_2O–H_2SO_4 (1 L) and heating on a hot plate. Silica gel (230–400 mesh, 60 Å, Aldrich) was used for flash column chromatography.

Methyl 12-Hydroxydodecanoate (2). To a stirred solution under argon of compound 1 (8.6 g, 40 mmol) in dry methanol (fresh bottle, ACS grade, 200 mL) and dry hexane (fresh bottle, ACS grade, 50 mL) was added trimethylchlorosilane (12 mL, 94 mmol). After overnight stirring the reaction was neutralized by adding a freshly prepared solution of sodium methoxide in methanol (47 mL, 94 mmol). The mixture was evaporated to dryness and the crude residue was diluted in water (100 mL). The aqueous phase was extracted twice with ether (2 × 100 mL). The recollected organic layers were washed with brine (100 mL), dried ($MgSO_4$), filtered, and evaporated to dryness. The crude extract was dissolved in hot hexane (100 mL). After 3 h at room temperature and 24 h in the refrigerator, the crystalline product was filtered (4 °C) and was washed twice with small amounts of cold pentane (8 g, 87%). The mother liquor was concentrated in vacuo and stored overnight to yield an additional amount of compound 2 (total = 8.65 g, 94%): mp 34–35 °C (lit.[15] mp 31–32 and 33–34 °C).

Methyl 12-Oxododecanoate (3). Alcohol 2 (2.3 g, 10 mmol) in CH_2Cl_2 (5 mL) was added to a stirred orange suspension under argon of pyridinium chlorochromate (dried overnight over P_2O_5 in a dessicator under vacuum, 3.23 g, 15 mmol) and molecular sieves (activated by flame under vacuum, 40 beads, 4 Å) in dry CH_2Cl_2. After 4 h, dry ether (25 mL) was added to the brown mixture, and the mixture was then filtered through Celite. The brown residue was washed twice with dry ether (2 × 25 mL) and filtered. The volume of the combined organic layers was reduced under vacuum (30 °C). The slightly brown residue was flash chromatographed over silica gel (70 g) with pentane–ether (90–10) as eluent to yield 3 (1.82 g, 80%) as a colorless liquid: IR (neat) 2720 (w, OCH) and 1735 cm^{-1} (s, CO); ^{1}H NMR δ 1.27–1.61 (m, 16 H, CH_2), 2.31 (t, J = 7.5 Hz, 2 H, H-2), 2.42 (td, J_1 = 7.3 Hz, J_2 = 1.8 Hz, 2 H, H-11), 3.66 (s, 3 H, OMe), and 9.76 ppm (t, J = 1.8 Hz, 1 H, H-12); MS *m/z* (%) 200 (14), 197 (14), 185 (35), 153 (28), 143 (8), 74 (100). Anal. Calcd for $C_{13}H_{24}O_3$: C, 68.46; H, 10.60. Found: C, 68.24: H, 10.64.

Methyl 12-Hydroxy-13-heptadecynoate (4). EtMgBr in tetrahydrofuran (3.69 mL, 7.38 mmol) was added dropwise under argon to a stirred solution of pentyne (0.9 mL, 9 mmol) in dry tetrahydrofuran (20 mL). After 3 h of stirring, the solution of the pentyne Grignard was added dropwise under argon to a cooled mixture (−40 °C) of aldehyde 3 (1.35 g, 5.9 mmol) partially dissolved in dry tetrahydrofuran (45 mL). After 30 min, acetic acid in tetrahydrofuran (9 mL, 10%) was added to quench the reaction. The cold bath was then removed, brine (50 mL) was added, and the phases were separated. The ether layer was washed with brine (50 mL), dried ($MgSO_4$), filtered, and

evaporated to dryness. The crude extract was flash chromatographed over silica gel (200 g) with 87:13 pentane–ether as eluent to yield 4 (1.24 g, 74%) as a colorless liquid: IR (neat) 3465 (bm, OH), 2235 (w, CC), and 1743 cm^{-1} (s, CO); ^{1}H NMR, δ: 0.98 (t, J = 7.5 Hz, 3 H, H-17), 1.28–1.71 (m, 20 H, CH_2), 2.19 (bt, J = 7.5 Hz, 2 H, H-15), 2.30 (t, J = 7.5 Hz, 2 H, H-2), 3.67 (s, 3 H, OMe), and 4.34 ppm (m, 1 H, H-12); MS m/z (%) 279 (M^+ – OH, 4), 265 (2), 221 (15), 200 (47), 55 (100). Anal. Calcd for $C_{18}H_{32}O_3$: C, 72.94; H, 10.88. Found: C, 72.69; H, 10.69.

Methyl 12-Oxo-13-heptadecynoate (5). To a stirred solution under argon of dry N-methylmorpholine N-oxide (ground and activated at 75–80 °C under 2 mmHg vacuum for 3 h, 670 mg, 5.1 mmol) and propargyl alcohol 4 (1.01 g, 3.4 mmol) in dry CH_2Cl_2 (30 mL) were added molecular sieves (activated by flame under vacuum, 40 beads, 4 Å). After 10 min of stirring, dry tetrapropylammonium perruthenate (dried overnight over P_2O_5 in a dessicator under vacuum, 60 mg, 0.16 mmol) was added to the mixture. After 30 min the reaction mixture was diluted with CH_2Cl_2 (100 mL) and was filtered through a glass frit funnel. The organic layer was washed with a saturated aqueous solution of Na_2SO_3 (60 mL), brine (60 mL), and a saturated aqueous solution of $CuSO_4$ (100 mL) before it was dried ($MgSO_4$), filtered, and evaporated to dryness. The crude extract was flash chromatographed over silica gel (75 g) with hexane–ether (90–10) to pentane–ether (88–12) as eluent to yield 5 (0.85, 85%) as a colorless liquid: UV (CH_3CN): 219 nm (18 000); IR (neat) 2214 (m, CC), 1743 (s, CO_2Me), and 1680 cm^{-1} (s, CCCO); ^{1}H NMR δ 1.01 (t, J = 7.2 Hz, 3 H, H-17), 1.26–1.64 (m, 18 H, CH_2), 2.29 (t, J = 7.5 Hz, 2 H, H-2), 2.34 (t, J = 7.3 Hz, 2 H, H-15), 2.51 (t, J = 7.4 Hz, 2 H, H-11), and 3.65 ppm (s, 3 H, OMe); MS m/z (%) 263 (M^+ – MeO, 4), 219 (4), 95 (100). Anal. Calcd for $C_{18}H_{30}O_3$: C, 73.43; H, 10.27. Found: C, 73.43; H, 10.41.

Preparation of (*R*)- and (*S*)-12-Hydroxy Compounds. Alpine-borane was prepared as described by Brown and collaborators[18] with 9-BBN dimer and α-pinene (fresh bottle, degassed by bubbling with argon for 15 min, 1.2 equiv). (R)-Alpine-borane provided the (R)-propargyl alcohol 6 by reduction of 5 and (S)-Alpine-borane its enantiomer.[14] A typical procedure is described below for preparation of the (R)-propargyl alcohol.

Methyl 12(*R*)-Hydroxy-13-heptadecynoate (6). (R)-Alpine-borane (2 equiv) was added under a positive pressure of argon to the propargyl ketone (854 mg, 2.9 mmol) which had been deoxygenated by placing under vacuum and then under argon three times. The solution turned yellow during the addition. After overnight stirring, the reaction was cooled with an ice bath, and freshly distilled propanal (0.5 mL) was added. The excess propanal was pumped off over a 30-min period (0.4 mmHg) at 0 °C. The ice bath was replaced with an oil bath, and the yellow residue was heated (75 °C) under vacuum for 3 h to remove the liberated pinene. The residue was dissolved, under argon, in 10 mL of dry, argon-degassed ether. The solution was cooled (0 °C), and freshly distilled ethanolamine (degassed with argon, 0.4 mL, 6 mmol) was added dropwise. A white precipitate formed. After 15 min, the flask was opened to air. In a cold room, the white precipitate was separated from the cold ether solution by filtration. The precipitate was washed twice with a small amount of cold ether. The combined filtrate was washed with brine (15 mL), dried ($MgSO_4$), filtered, and evaporated to dryness. The crude extract was flash chromatographed over silica gel (100 g) with pentane–ether (90–10) to pentane–ether (87–13) as eluent to yield (R)-6 (785 mg, 91%) as a colorless liquid: $[\alpha]_D$ = + 6.7° (4.4 in Et_2O). Anal. Calcd for $C_{18}H_{32}O_3$: C, 72.94; H, 10.88. Found: C, 72.70; H, 10.64.

12(*R*)-Hydroxy-13-heptadecynoic Acid (7). To a stirred solution of ester (R)-6 (700 mg, 2.36 mmol) in tetrahydrofuran–MeOH (1–1, 10 mL) was added an aqueous solution of NaOH (4.7 mL, 4.7 mmol). After overnight stirring, the solution was evaporated to dryness. To the crude residue was added a saturated solution of NH_4Cl (4 mL) and CH_2Cl_2 (10 mL). The aqueous phase was acidified (pH = 4) with HCl (1 N). The phases were separated, and the aqueous phase was extracted twice with CH_2Cl_2 (2 × 10 mL). The combined organic layers were washed with brine (30 mL), dried ($MgSO_4$), filtered, and evaporated to dryness. The crude extract was purified by flash chromatography over silica gel (70 g) with 70:30 pentane–Et_2O to Et_2O as eluents to give (R)-7 as an amorphous solid (600 mg, 90%): mp 47.5–48 °C; $[\alpha]_D$ = + 5° (2.6 in tetrahydrofuran); IR (KBr) 3367–2600 (m, OH and CO_2H), 2235 (w, CC), and 1694 cm^{-1} (s, CO); ^{1}H NMR δ 0.97 (t, J = 7.4 Hz, 3 H, H-17), 1.27–1.71 (m, 20 H, CH_2), 2.19 (bt, J = 7.4 Hz, 2 H, H-15), 2.34 (t, J = 7.4 Hz, 2 H, H-2), and 4.35 ppm (m, 1 H, H-12); MS m/z (%) 221 (M^+ – 61, 1), 186 (2), 112 (34), 97 (100). Anal. Calcd for $C_{17}H_{30}O_3$: C, 72.30; H, 10.71. Found: C, 72.36; H, 10.75.

12(*R*)-Hydroxy-16-heptadecynoic Acid (8). Freshly distilled diaminopropane (7 mL) was added under argon to KH (35% in oil, 1.2 g, 10.5 mmol) which had been washed free of oil with dry pentane. After 2 h of stirring, (R)-7 (300 mg, 1.06 mmol) in diaminopropane (2 mL) was added dropwise to the brown solution of KAPA. The flask was washed with diaminopropane (0.5 mL), and the rinse solution was also added to the reaction solution. The conversion was complete after 24 h. The deep brown reaction mixture was poured into a cold saturated aqueous solution (4 °C) of NH_4Cl (100 mL). The reaction flask was washed twice with cold NH_4Cl (5 mL). The combined NH_4Cl fractions were washed with hexane (50 mL), acidified to pH = 1 with cold HCl (6 N, ~60 mL) and extracted with Et_2O (100 mL). The phases were separated, and the organic layer was washed with HCl (1 N, 100 mL) and brine (2 × 100 mL), dried ($MgSO_4$), filtered, and evaporated to dryness. The crude extract was flash chromatographed over silica gel (50 g) with pentane–ether (70–30) to pentane–ether–acetic acid (70–29–1) as eluent to yield (R)-8 as a crystalline compound (242 mg, 81%): mp 61 °C; $[\alpha]_D$ = –3.1° (3 in tetrahydrofuran); IR (KBr) 3342–2645 (m, OH and CO_2H), 3289 (s, C≡C), 2120 (w, CC), and 1702 cm^{-1} (s, CO); ^{1}H NMR δ 1.26–1.66 (m, 22 H, CH_2), 1.97 (t, J = 2.6 Hz, 1 H, H-17), 2.24 (td, J_1 = 6.6 Hz, J_2 = 2.6 Hz, 2 H, H-16), 2.36 (t, J = 7.4 Hz, 2 H, H-2), and 3.64 ppm (m, 1 H, H-12); MS m/z (%) 221 (9), 215 (13), 197 (73), 186 (14), 43 (100). Anal. Calcd for $C_{18}H_{32}O_3$: C, 72.30; H, 10.71. Found: C, 71.90; H, 10.50.

A sample of (R)-8 (0.01 mmol) was esterified with diazomethane in ether. The solvent was removed under vacuum, and the dry methyl ester was dissolved in dry $CDCl_3$ (0.5 mL) and dry pyridine (5 mL). The solution was filtered through cotton into a dry NMR tube (stored in a dessicator over KOH). To the solution was added Mosher's reagent (2 equiv) and a catalytic amount of N,N-dimethyl-4-aminopyridine. The conversion was complete after 12 h.

12(*S*)-Hydroxy-16-heptadecynoic Acid (8). In a similar manner as described above, the (S)-8 isomer was prepared starting with propargylic ketone 5: mp 62 °C; $[\alpha]_D$ = +3.4° (3.1 in tetrahydrofuran). Anal. Calcd for $C_{18}H_{32}O_3$: C, 72.30; H, 10.71. Found: C, 72.42; H, 10.82.

12-Iododecan-1-ol (12). Compound 11 (90%, 21.5 g, 0.1 mol) in acetone (25 mL) was added, under argon, to a solution of anhydrous NaI (30 g, 0.2 mol) in dry acetone (275 mL). The solution was heated to reflux in an oil bath. A white precipitate formed. The reaction was monitored by ^{1}H NMR (Varian 80 MHz, $CDCl_3$). Aliquots were taken and diluted 5-fold with ether, filtered from the precipitated salts through silica gel, and evaporated to dryness. The residues were dissolved in $CDCl_3$ and were filtered through cotton for NMR analysis. After 2 days, the reaction mixture was cooled to room temperature. Dry ether (300 mL) was added, and the mixture was filtered and evaporated to dryness (30 °C). The crude orange extract was partitioned between ether (250 mL) and water (50 mL). The phases were separated, and the ether layer was washed with Na_2SO_3 (1 M, 100 mL), water (50 mL), and brine (100 mL) before it was dried ($MgSO_4$), filtered, and evaporated to dryness (30 °C). The residue was flash chromatographed through a short column of silica gel (100 g) eluted first with pentane–ether (90–10), to remove remaining starting material, and then with pentane–ether (80–20) to give 12 as a pale yellow liquid that crystallized at 4 °C (27.6 g, 97%). The compound was shown by ^{1}H NMR to be sufficiently pure to continue the synthesis: mp 26–27 °C (–20 °C, pentane); IR (neat) 3336 cm^{-1} (m, OH); ^{1}H NMR δ 1.29–1.83 (m, 16 H, CH_2), 3.19 (t, J = 7 Hz, 2 H, H-10), and 3.64 ppm (t, J = 6.7 Hz, 2 H, H-1); MS m/z (%) 284 (M^+, 1), 157 (29), 128 (7), 127 (6), 97 (71), 55 (100).

12-Iododecanal (13). As described for compound 3, alcohol 12 (9.4 g, 33 mmol) was oxidized with pyridinium chlorochromate to give pure aldehyde 13 (7.1 g, 76%) after flash chromatography on silica gel (120 g) with 91:9 pentane–ether: IR (neat) 2718 (w,

OCH) and 1722 cm^{-1} (s, CO); ^{1}H NMR δ 1.30–1.81 (m, 14 H, CH_2), 2.43 (td, J_1 = 7.3 Hz, J_2 = 1.6 Hz, 2 H, H-2), 3.19 (t, J = 7 hz, 2 H, H-10), and 9.77 ppm (t, J = 1.6 Hz, 1 H, H-1); MS m/z (%) 282 (M^+, 1), 238 (3), 155 (57), 137 (76), 128 (12), 127 (12), 29 (100).

2,2-Dimethyl-12-hydroxy-13-heptadecynoic Acid (15). n-BuLi in hexane (13.7 mL, 22 mmol) was added dropwise under argon to a stirred and cooled solution (−78 °C) of pentyne (2.2 mL, 22 mmol) in dry tetrahydrofuran (10 mL). After 30 min, the reaction temperature was raised to −40 °C and a solution of compound **13** (5.64 g, 20 mmol) in dry tetrahydrofuran (5 mL) was added dropwise to the pentynyl lithium solution over a 15-min period. The flask containing the aldehyde was washed with tetrahydrofuran (2 mL), and the rinse was added to the reaction. TLC (hexane–ethyl acetate, 85–15) indicated the reaction was complete after 30 min. A solution of dilithium isobutyrate was prepared as follows. n-BuLi in hexane (41.2 mL, 66 mmol) was added under argon to a cooled solution (−78 °C) of dry diisopropylamine (9.2 mL, 66 mmol) in dry tetrahydrofuran (135 mL). After 30 min, the dry ice bath was replaced with an ice bath and isobutyric acid (fresh bottle, 3 mL, 33 mmol) was added dropwise to the LDA solution. After stirring for 30 min at 0 °C and 20 min at room temperature, the reaction was again cooled to 0 °C, and lithiated alcohol **14** was added dropwise, via a cannula, to the solution of dilithium isobutyrate. A white precipitate formed. The mixture was allowed to warm to room temperature and was stirred for an additional 3 h before it was cooled to 0 °C and quenched with cold water (100 mL). The phases were separated, and the organic layer was extracted twice with KOH (0.5 N, 2 × 75 mL). The combined aqueous phases were acidified to pH = 3–4 with cold HCl (6 N, ~32 mL) and were extracted with 150 mL of ether. The aqueous layer was washed with 25 mL of ether. The combined ether fractions were washed with brine (150 mL), dried ($MgSO_4$), filtered, and evaporated to dryness. The crude extract was flash chromatographed over silica gel (125 g) with pentane–Et_2O (1–1) as eluent to give **15** as a viscous oil (5.1 g, 82%): IR (neat) 3370–2560 (m, OH and CO_2H), 2234 (w, CC), and 1696 cm^{-1} (s, CO); ^{1}H NMR δ 0.98 (t, J = 7.4 Hz, 3 H, H-17), 1.18 (s, 6 H, H-2), 1.26–1.68 (m, 20 H, CH_2), 2.19 (td, J_1 = 7 Hz, J_2 = 1.6 Hz, 2 H, H-15), and 4.36 ppm (m, 1 H, H-12); MS m/z (%) 292 ($M^+ - H_2O$, 1), 264 (7), 236 (3), 221 (14), 214 (10), 43 (100).

2,2-Dimethyl-12-hydroxy-16-heptadecynoic Acid (16). As described for (R)-**8**, the internal triple bond of **15** (4.35 g, 40 mmol) was shifted to the terminal position with KAPA (9 equiv). **16** (3.5 g, 80%) was obtained as a viscous oil after flash chromatography over silica gel (150 g) with pentane–ether (70–30) to ether as eluent: IR (neat) 3428–2625 (m, OH and CO_2H), 3310 (CCH), 2120 (w, CC), and 1702 cm^{-1} (s, C=O); ^{1}H NMR 1.19 (s, 6 H, H-2), 1.27–1.70 (m, 22 H, CH_2), 1.96 (t, J = 2.5 Hz, 1 H, H-17), 2.23 (td, J_1 = 6.6 Hz, J_2 = 2.5 Hz, 2 H, H-16), and 3.64 ppm (m, 1 H, H-12); MS m/z (%) 310 (M^+, 1), 292 (1), 264 (4), 243 (4), 225 (22), 214 (13), 97 (100). Anal. Calcd for $C_{19}H_{34}O_3$: C, 73.49; H, 11.04. Found: C, 73.01; H, 11.04.

Bioassay Methods. Rabbit cytochrome P450 4A4 was purified from 25–28-day gestation pregnant rabbit lungs (Pelfreez Biologicals, Rogers, AZ) that had been perfused with saline, frozen in liquid nitrogen, and stored at −80 °C. The purification was carried out as described by Williams et al.[2] with the modifications of Muerhoff et al.[26] Reversed-phase HPLC analysis of PGE_1 [5,6-^{3}H(N)PGE_1 from New England Nuclear, Boston, MA] and its ω-hydroxylated product was carried out on a Beckman C-18 Ultrasphere ODS column (5 μm, 4.6 mm × 25 cm) connected with a 4.5-cm guard column as described by Okita et al.[27] HPLC and integration by Beckman software were performed with a Beckman System Gold HPLC interfaced with a Beckman 171 radioisotope detector. Preincubation mixtures contained, in 200 μL of 50 mM potassium phosphate (pH 7.4) buffer, 5 pmol of cytochrome P450 4A4, 50 pmol of pig liver cytochrome P450 reductase, 50 pmol of cytochrome b_5, 2.5 mM $MgCl_2$, 0.5 mM EDTA, 10 μg/mL dilauroyl L-α-phosphatidylcholine (DLPC), 1 mM NADPH, 20 mM isocitrate, and 0.05 unit of isocitrate dehydrogenase. The preincubation with inhibitor was initiated by adding one of the inhibitors (amounts given in figure legends) or an equal amount of solvent (methanol) as a control. At the various times indicated in the figure legends, a 35-μL aliquot was transferred from the inhibitor preincubation mixture to a reaction mixture (200 μL final volume after aliquot added) consisting of buffer, DLPC, NADPH, and the regenerating system, as above, plus 20 μM [^{3}H]PGE. After reaction times of 10 and 20 min, an 80-μL aliquot was transferred from the reaction mixture to a vial containing 50 μL of methanol, and the final solution was analyzed by HPLC. For the zero preincubation time point, the 35-μL aliquot was transferred to the reaction mixture prior to addition of inhibitor.

Acknowledgment. This research was supported by grants GM 25515 (P.R.O.M.) and GM 31296 (B.S.M.) from the National Institutes of Health. Mass spectra were obtained in the Biomedical, Bioorganic Mass Spectrometry Facility of the University of California at San Francisco (A. Burlingame, Director) supported by grants RR 01614 and P-30 DK 26743.

References

(1) Kupfer, D. Endogenous Substrates of Monoxygenases: Fatty Acids and Prostaglandins. *Pharmac. Ther.* **1980**, *11*, 469–496.
(2) Williams, D. E.; Hale, S. E.; Okita, R. T.; Masters, B. S. S. A Prostaglandin ω-Hydroxylase Cytochrome P-450 (P-450$_{PG\text{-}\omega}$) Purified from Lungs of Pregnant Rabbits. *J. Biol. Chem.* **1984**, *259*, 14600–14608.
(3) Yamamoto, S.; Kusunose, E.; Ogita, K.; Kaku, M.; Ichihara, K.; Kusunose, M. Isolation of Cytochrome P-450 Highly Active in Prostaglandin ω-Hydroxylation from Lung Microsomes of Rabbits Treated with Progesterone. *J. Biochem.* **1984**, *96*, 593–603.
(4) Nebert, D. W.; Nelson, D. R.; Coon, M. J.; Estabrook, R. W.; Feyereisen, R.; Fujii-Kuriyama, Y.; Gonzalez, F. J.; Guengerich, F. P.; Gunsalus, I. C.; Johnson, E. F.; Loper, J. C.; Sato, R.; Waterman, M. R.; Waxman, D. J. The P450 Superfamily: Update on New Sequences, Gene Mapping, and Recommended Nomenclature. *DNA Cell Biol.* **1991**, *10*, 1–14.
(5) Powell, W. S.; Solomon, S. Formation of 20-Hydroxyprostaglandins by Lungs of Pregnant Rabbits. *J. Biol. Chem.* **1978**, *253*, 4609–4616.
(6) Muerhoff, A. S.; Williams, D. E.; Leithauser, M. T.; Jackson, V. E.; Waterman, M. R.; Masters, B. S. S. Regulation of the Induction of a Cytochrome P-450 Prostaglandin ω-Hydroxylase by Pregnancy in Rabbit Lung. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7911–7914.
(7) Powell, W. S. ω-Oxidation of Prostaglandins by Lung and Liver Microsomes. *J. Biol. Chem.* **1978**, *253*, 6711–6716.
(8) Matsubara, S.; Yamamoto, S.; Sogawa, K.; Yokotani, N.; Fujii-Kuriyama, Y.; Haniu, M.; Shively, J. E.; Gotoh, O.; Kusunose, E.; Kusunose, M. cDNA Cloning and Inducible Expression during Pregnancy of the mRNA for Rabbit Pulmonary Prostaglandin ω-Hydroxylase (Cytochrome P-450$_{p\text{-}2}$). *J. Biol. Chem.* **1987**, *262*, 13366–13371.
(9) Muerhoff, A. S.; Williams, D. E.; Reich, N. O.; CaJacob, C. A.; Ortiz de Montellano, P. R.; Masters, B. S. S. Prostaglandin and Fatty Acid ω- and (ω-1)-Oxidation in Rabbit Lung. Acetylenic Fatty Acid Mechanism-Based Inactivators as Specific Inhibitors. *J. Biol. Chem.* **1989**, *264*, 749–756.
(10) Kauser, K.; Clark, J. E.; Masters, B. S.; Ortiz de Montellano, P. R.; Ma, Y.-H.; Harder, D. R.; Roman, R. J. Inhibitors of Cytochrome P-450 Attenuate the Myogenic Response of Dog Renal Arcuate Arteries. *Circulation Res.* **1992**, *68*, 1154–1163.
(11) Ortiz de Montellano, P. R.; Reich, N. O. Specific Inactivation of Hepatic Fatty Acid Hydroxylases by Acetylenic Fatty Acids. *J. Biol. Chem.* **1984**, *259*, 4136–4141.
(12) Reich, N. O.; Ortiz de Montellano, P. R. Dissociation of Increased Lauric Acid ω-Hydroxylase Activity from the Antilipidemic Action of Clofibrate. *Biochem. Pharmacol.* **1986**, *35*, 1227–1233.
(13) CaJacob, C. A.; Ortiz de Montellano, P. R. Mechanism-Based in Vivo Inactivation of Lauric Acid Hydroxylases. *Biochemistry* **1986**, *25*, 4705–4711.
(14) Midland, M. M.; Mc Dowell, D. C.; Hatch, R. L.; Tramontano, A. Reduction of α,β-Acetylenic Ketones with B-3-Pinanyl-9-borabicyclo[3.3.1]nonane. High Asymmetric Induction in Aliphatic Systems. *J. Am. Chem. Soc.* **1980**, *102*, 867–869.
(15) Brook, M. A.; Chan, T. H. A Simple Procedure for the Esterification of Carboxylic Acids. *Synthesis* **1983**, 201–203.
(16) Corey, E. J.; Suggs, J. W. Pyridinium Chlorochromate. An Efficient Reagent for Oxidation of Primary and Secondary Alcohols to Carbonyl Compounds. *Tetrahedron Lett.* **1975**, 2647–2650.
(17) Griffith, W. P.; Ley, S. V.; Whitecombe, G. P. Preparation and Use of Tetra-n-butylammonium Per-ruthenate (TBAP Reagent) and Tetra-n-propylammonium Per-ruthenate (TPAP Reagent) as New Catalytic Oxidants for Alcohols. *J. Chem. Soc., Chem. Commun.* **1987**, 1625–1627.

(18) Brown, H. C.; Pai, G. Selective Reductions. 37. Asymmetric Reduction of Prochiral Ketones with B-(3-Pinanyl)-9-borabicyclo-[3.3.1]nonane. *J. Org. Chem.* **1985**, *50*, 1384–1394.
(19) Brown, C. A.; Yamashita, A. The Acetylene Zipper. An Exceptionally Facile "Contrathermodynamic" Multipositional Isomerization of Alkynes with Potassium 3-Aminopropylamide. *J. Am. Chem. Soc.* **1975**, *97*, 891–892.
(20) Gonnella, N. C.; Nakanishi, K.; Martin, V. S.; Sharpless, K. B. General Method for Determining Absolute Configuration of Acyclic Allylic Alcohols. *J. Am. Chem. Soc.* **1982**, *104*, 3775–3776.
(21) Lindlar, H.; Dubuis, R. Palladium Catalyst for Partial Reduction of Acetylenes. *Org. Synth.* **1966**, *46*, 89–92.
(22) Midland, M. M.; Halterman, R. L.; Brown, C. A.; Yamaichi, A. The Isomerization of Optically-Active Propargyl Alcohols to Terminal Acetylenes. *Tetrahedron Lett.* 1981, *22*, 4171–4172.
(23) Dale, J. A.; Dull, D. L.; Mosher, H. S. α-Methoxy-α-trifluoromethylphenylacetic Acid, a Versatile Reagent for the Determination of Enantiomeric Composition of Alcohols and Amines. *J. Org. Chem.* **1969**, *34*, 2543–2549.
(24) Greger, P. L. Metalated Carboxylic Acids. I. Alkylation. *J. Am. Chem. Soc.* **1967**, *89*, 2500–2501.
(25) Greger, P. L. 2,2-Dimethyl-4-phenylbutyric Acid. *Org. Synth.* **1970**, *50*, 59–62.
(26) Muerhoff, A. S.; Williams, D. E.; Masters, B. S. S. Purification and Properties of Pregnancy-inducible Rabbit Lung Cytochrome P-450 Prostaglandin ω-Hydroxylase. *Methods Enzymol.* **1990**, *187*, 253–267.
(27) Okita, R. T.; Clark, J. E.; Okita, J. R.; and Masters, B. S. S. ω- and (ω-1)-Hydroxylation of Eicosanoids and Fatty Acids by High-Performance Liquid Chromatography. *Methods Enzymol.* 1991, *206*, 432–441.