# Use of Biological Catalysts for the Preparation of Chiral Molecules. 8. Preparation of Propargylic Alcohols. Application in the Total Synthesis of Leukotriene $B_4$

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Leukotriene $B_4$ ($LTB_4$) (1) was synthesized from two chiral propargylic alcohols 2 and 3 obtained by enantioselective enzymatic hydrolysis and enantiogenic microbial reduction, respectively. Condensation of these two synthons using a rapid and reproducible method not involving a Wittig reaction led to a compound with identical biological activity to that of natural $LTB_4$.

## Introduction

Leukotriene $B_4$ or $LTB_4$ (1) is formed in vivo via the action of 5-lipoxygenase on arachidonic acid.[1] It is considered to be an important mediator of inflammation and is one of the strongest chemotactic substances known.[2] The biochemical importance of this compound and the difficulty in obtaining the purified substance in sufficient quantities from biological fluids has prompted several groups to synthesize natural $LTB_4$.[3] However, since only the conformer (*E,E,Z*) with absolute configuration (5*S*,12*R*) is biologically active,[4] the chirality of the asymmetric carbons and the geometry of the triene system must be accurately reproduced.

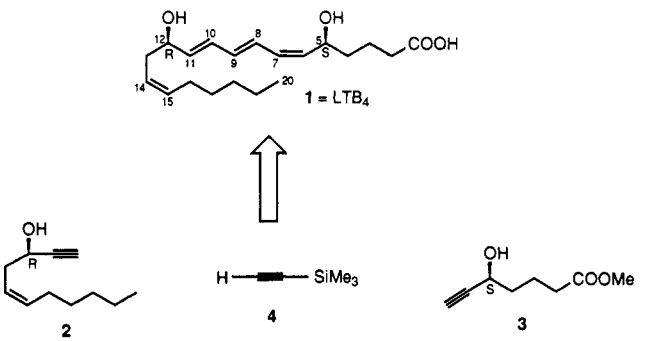
Most synthetic approaches to this compound have employed sugar precursors as the source for chirality,[3a-e,h,5a] and the trienic system was built through a rather nonstereoselective Wittig reaction which often produced a *Z*/*E* mixture[3a-h,j,k].

We propose here a retrosynthetic route (Scheme I) based on the methods described by Pougny[5a] and Linstrumelle[5b] in which the construction of the conjugated triene system (*E,E,Z*) is produced by a stereoselective coupling which does not involve a Wittig reaction. The enantioselectivity of two crucial steps, syntheses of synthons 2 and 3 in Scheme I, is accomplished by microbiological and enzymatic reactions.

## Results and Discussion

Microorganisms and enzymes are of particular interest in the regio- and stereoselective transformation of polyfunctional molecules under mild conditions. Following our general studies on the microbiological reduction of carbonyl groups, we were interested in the stereoselective reduction of propargylic ketones. It is reportedly difficult to accomplish this transformation using microorganisms,[7] however, several methods using chemical catalysts for the enantiogenic reduction of propargylic ketones have been described.[3f,6] Yet, after a short screening program, we found that a wide variety of microorganisms in their metabolic resting phase could carry out this transformation. The enantiomeric alcohols of a series of aliphatic propargylic alcohols were obtained using *Saccharomyces cerevisiae* (*S*), *Geotrichum candidum* (*R* and *S*), *Aspergillus niger* (*R*) or *Mortierella isabellina* (*S*). The fungus

**Scheme I**




*G. candidum* was found to give the best optical and chemical yields. Furthermore, this strain contains many alcohol dehydrogenases, and we observed different stereospecificities under different reaction conditions.[8] We were able, therefore, to obtain synthon 3 (5*S*) under anaerobic conditions and the (5*R*) enantiomer under aerobic conditions via the actions of different dehydrogenases. The preparation of synthon 2 was not pursued using this method since we observed a decrease in stereospecificity with increasing chain length. A further

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§ In memory of Professor Pougny (1947–1988).

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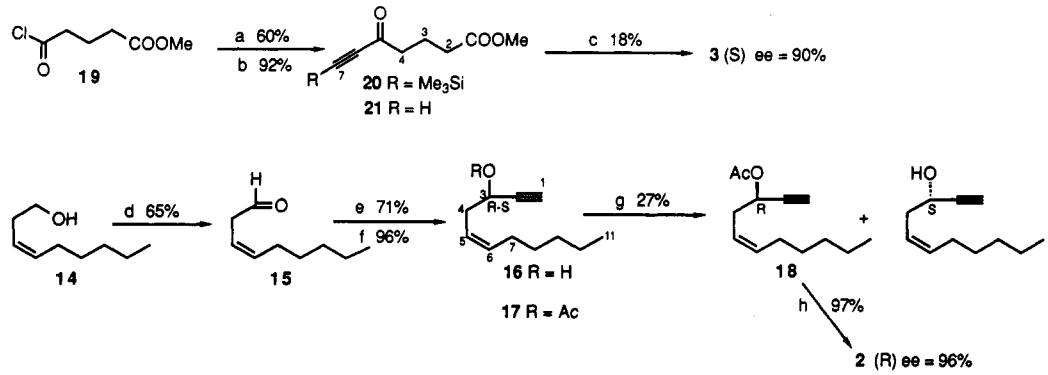
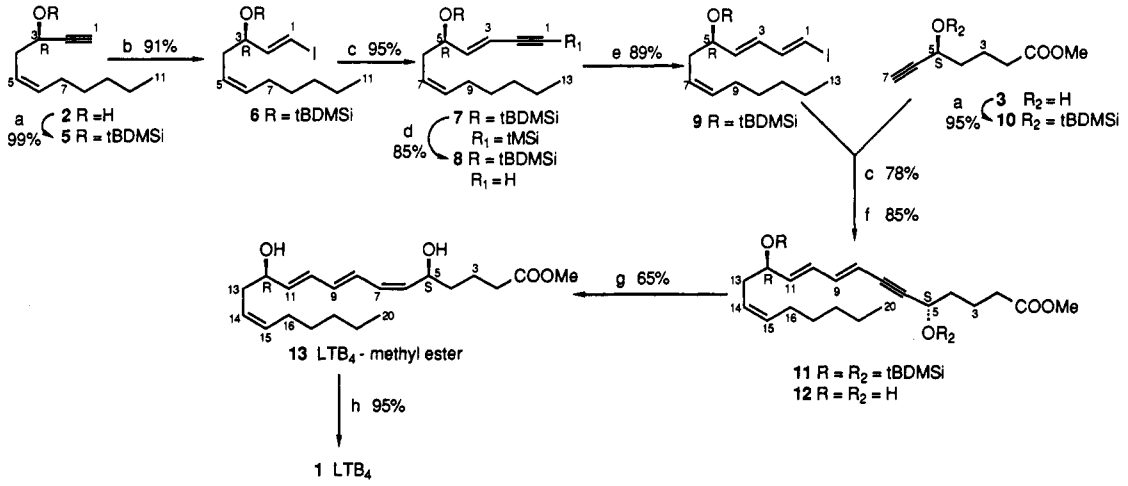
**Scheme II**[a]




[a] Key: (a) $Me_3SiC{\equiv}CSiMe_3$, $AlCl_3$, $CH_2Cl_2$, 0 °C; (b) borax (0.01 M), HCl, MeOH, 0 °C; (c) *G. candidum*, anaerobic conditions, 27 °C, 17 h; (d) PCC, $CH_2Cl_2$, 1 h; (e) $HC{\equiv}CLi$, THF, −78 °C to rt; (f) $Ac_2O$, DMAP, $Et_3N$; (g) PPL, phosphate buffer (0.1 M, pH 7), 25 °C, 24 h; (h) $K_2CO_3$, $MeOH/H_2O$, 25 °C, 2 h.

**Scheme III**[a]




[a] Key: (a) *t*-BDMSiCl, imidazole, DMF, 35 °C; (b) $Cp_2Zr(Cl)H$, $C_6H_6$, 40 °C; $I_2$; (c) $HC{\equiv}CSiMe_3$, $[(Ph_3P)_4Pd]$, $C_6H_6$, *n*-$BuNH_2$, CuI; (d) $AgNO_3$, $EtOH/H_2O$, 30 °C; KCN, $H_2O$; (e) $Cp_2Zr(Cl)H$, $C_6H_6$/THF, 35 °C; $I_2$; (f) $Bu_4NF$, THF; $CH_2N_2$; (g) Zn, $MeOH/H_2O$, 30 °C; (h) $K_2CO_3$, $MeOH/H_2O$.

obstacle was the instability of the corresponding propargylic ketone. Synthon **2** was therefore prepared by enzymatic resolution of the racemic alcohol using a lipase. Numerous groups have shown that enzymatic or microbial hydrolysis of racemic acetylenic alcohol acetates can liberate alcohols in either the *R* or the *S* configuration.[9] We prepared alcohol **2** directly using a lipase from *Candida cylindracea*, but with a poor enantiomeric excess. Improved stereoselectivity was obtained using porcine pancreatic lipase (PPL), but it liberated the alcohol of (3*S*) configuration. However, the synthon **2** (3*R*) was obtained with a high enantiomeric excess from remaining (*R*)-acetate **18** after deprotection. The synthesis of the acetylenic alcohols **2** and **3** is shown in Scheme II.

Commercially available alcohol **14** was oxidized to the corresponding aldehyde **15** with pyridinium chlorochromate (65%). Treatment of aldehyde **15** with monolithium acetylene[10] (71%) and acetylation (96%) of the resultant propargylic alcohol **16** gave **17**. The acetylated derivative **17** was hydrolyzed enantioselectively (70% hydrolysis) by porcine pancreatic lipase (PPL). The remaining (*R*)-acetate **18** ($[\alpha]^{20}_J$ = +36.3°) afforded the optically active propargylic alcohol **2** ($[\alpha]^{20}_J$ = +34°). The enantiomeric excess of **2** was determined by GC analysis of the corresponding Mosher ester[11] (ee = 96%).

Synthon **3** was obtained (18%) by microbial reduction of the acetylenic ketone **21** by *G. candidum* under anaerobic conditions (ee = 90%; $[\alpha]^{20}_J$ = −18.5°). Compound **21** was prepared by condensation of the commercially available acid chloride **19** with bis(trimethylsilyl)acetylene in the presence of aluminum chloride,[3f,12] followed by desilylation of the intermediate ketone **20**.

We were able, therefore, to prepare synthons **2** and **3** in four and three steps, respectively, from commercially available starting compounds. The previous syntheses of synthons **2** and **3** as described by Pougny[5a] from D-xylose were carried out in 10 and 13 steps, respectively. The previously reported optical rotations are $[\alpha]_D$ = +26° for

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(11) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543–2549. **Preparation of derivatives** (using modified procedure). 5 mg of (+)-MTPA-Cl* ($2 \times 10^{-2}$ mmol) in 0.5 mL of anhydrous $CCl_4$ are mixed under strictly anhydrous conditions with $10^{-2}$ mmol of the alcohol to be derivatized. After addition of 0.5 mL of a solution of DMAP (0.04 M) in $CCl_4$, the mixture is agitated for 10 min. A few drops of methanol are added to eliminate excess acid chloride. After being dried under a stream of argon, the residue is taken up in 0.2 mL of ether and filtered through a Sep-Pak cartridge (silica). After elution with ether ($2 \times 1$ mL), the ether extract is injected directly into the GC apparatus. *(+)-MTPA-Cl = (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid chloride.

(12) Walton, D. R. M.; Waugh, F. *J. Organomet. Chem.* **1972**, *37*, 45–56.

2 and $[\alpha]_D = -9°$ for 3. The new scheme thus also improved the enantiomeric excesses.

The other feature of this scheme was the production of a pure triene system. This was accomplished by using $Pd^0/Cu[Pd(PPh_3)_4/CuI]$ in the condensation of the terminal acetylenic derivative with the vinyl halide.[5b-13] The formation of the $C_9$–$C_{10}$ and $C_7$–$C_8$ bonds and the details of the synthesis are illustrated in Scheme III. The geometry of the triene system (*E,E,Z*) was accurately reproduced, thus obviating purification of the final product 1 or intermediates by HPLC.

Vinyl iodide 6 was produced in 91% yield from 5 by hydrozirconation[14] followed by iodination of the intermediate organometallic. The $C_9$–$C_{10}$ bond was formed by condensing 6 with (trimethylsilyl)acetylene (4) in the presence of $Pd^0/Cu$ (yield > 95%). The enzyme 8 was obtained in 85% yield by specific desilylation of the triple bond of compound 7 according to the method described by Schmidt and Arens.[15] Hydrozirconation followed by iodination of 8 afforded the vinyl iodide 9 (86%), which was condensed with the terminal acetylene group of compound 10, thereby forming the $C_7$–$C_8$ bond in a yield of 78%. The dienyne 12 was obtained by desilylation using tetrabutylammonium fluoride followed by a partial reduction to give the methyl ester of $LTB_4$ 13 (65%) using activated zinc in a method adapted to $LTB_4$ synthesis in our laboratory[16] from that described by Boland.[17] $LTB_4$ 1 was obtained by hydrolysis of the methyl ester 13 using $K_2CO_3$ (95%).

## Conclusion

The optical purity of $LTB_4$ (ee = 96%) prepared in this work shows that biological catalysts are well suited for this type of organic synthesis. Synthons 2 and 3 were obtained in improved enantiomeric excess in four and three stages, respectively. The construction of the triene system was novel as it avoided use of the Wittig reaction, which is often not readily implemented and nearly always leads to a mixture of the *E/Z* isomers. Most of the published procedures for preparing $LTB_4$ give several geometrical isomers that required separation. Using the hydrozirconation/iodination technique associated with the Linstrumelle coupling reaction,[5b,13] a high geometrical purity of the expected product was obtained. Thus, this synthesis resulted in a single product and the overall yield was not diminished by the coproduction of undesirable isomers that require elimination. Preliminary experiments with racemic synthons have shown that $LTB_4$ can be obtained in gram quantities using this method. Optimization of the bioconversion conditions should enable large-scale production of the desired isomer of $LTB_4$ (5*S*,12*R*).

The biological activity of the $LTB_4$ synthesized was tested.[18] The metabolites produced by its action on human leucocytes were qualitatively and quantitatively identical to those produced by the natural product.[18]

## Experimental Section

The $^1H$ NMR spectra were recorded in $CDCl_3$ without an internal reference. Optical rotations were obtained at 20 °C using the J band of mercury (578 nm). Concentrations are expressed in g/mL in the indicated solvent. The solvents were freshly distilled over drying agents under argon. All reactions were checked by TLC using a fluorescence indicator (Merck 60 $F_{254}$). Different reactions were used to detect the aldehydes[19] and the propargylic alcohols.[20] Enzymatic hydrolyses were followed by gas chromatography (GC; OV1 capillary column; 0.2-mm diam, 25-m long). The enantiomeric excess (ee) was determined by the GC separation of Mosher derivatives with the same column.[11] Compounds were purified by flash chromatography,[21] on silica gel columns (Kieselgel 60 (230–400 mesh ASTM 0.040–0.063 mm). Yields are given after checking purity of chromatographic separations by $^1H$ NMR at 300 MHz and GC.

**(3*Z*)-Non-3-enal (15).** $CH_2Cl_2$ (10 mL) was added under argon to 1.14 g (5.3 mmol) of finely ground PCC. A mixture of 500 mg (3.5 mmol) of alcohol 14 suspended in 2 mL of $CH_2Cl_2$ was quickly added with vigorous stirring. The orange suspension quickly turned dark brown. The reaction was stirred for 1 h at room temperature and then diluted with 50 mL of anhydrous ether. The organic phase was removed, and the residual tars were ground up twice in 10 mL of ether. The organic phases were filtered through Florisil, and the solvent evaporated under reduced pressure. Crude distillation of the reaction mixture (70 °C/10 mmHg) afforded 321 mg (65.4%) of colorless product 15. The last fractions contained 24.5 mg (5%) of nonen-2-al (85 °C/10 mmHg). The highly unstable compound 15 was used immediately for the following reaction. Only its IR spectrum was recorded: IR (film) $\nu$ 3020 (w), 2720 (m), 1730 (s) $cm^{-1}$. A single peak was observed by GC.

**(±)-(3*Z*)-5-Undecen-1-yn-3-ol (16).** Anhydrous THF (5 mL) was placed in a thoroughly dried, round-bottomed flask under argon and cooled to −78 °C. With continuous stirring, 100 mL (4.46 mmol) of acetylene (readily dissolved in cold THF) was added. A 1.4-mL (2.24 mmol) portion of *n*-BuLi (1.6 M/hexane) was then added dropwise. After stirring for 20 min at −78 °C, the aldehyde 15 (314 mg, 2.24 mmol) in 1 mL of anhydrous THF was added slowly. After 20 min of additional stirring at −78 °C, the temperature of the mixture was allowed to rise to room temperature. The mixture was hydrolyzed with 7 mL of 5% aqueous $NH_4Cl$ and then extracted with ether (3 × 5 mL). The combined organic phases were washed with 5 mL of $H_2O$ and 5 mL of saturated NaCl, dried, and concentrated. A yellowish oil was obtained. After chromatography (15 g silica; hexane/ethyl acetate (8:2) v/v; $R_f$ = 0.33) 264 mg (71%) of compound 16 was isolated as a colorless oil; high-resolution MS (CI-$NH_3$) $[M + 18]^+$ found *m/z* 184.1701, calcd for $C_{11}H_{22}NO$ 184.1702; IR (film) $\nu$ 3500–3300 (s), 3300 (s), 3010 (m), 2100 (w) $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 5.64 (1 H, dtt, $J$ = 10.9, 6, 1.5 Hz, $H_6$), 5.45 (1 H, dtt, $J$ = 10.9, 6.5, 1.5 Hz, $H_5$), 4.38 (1 H, td, $J$ = 6.5, 2 Hz, $H_3$), 2.5 (2 H, ddd, $J$ = 6.5, 1.5 Hz, $H_4$), 2.45 (1 H, *d*, J = 2 Hz, $H_1$), 2.06 (2 H, tdd, $J$ = 6, 1.5 Hz, $H_7$), 1.8 (1 H, s, OH), 1,3 (6 H, m, $H_{8-9-10}$), 0.87 (3-H, t, $J$ = 6.8 Hz, $H_{11}$).

**(±)-(5*Z*)-3-Acetoxy-5-undecen-1-yne (17).**[22] Triethylamine (2 mL, 14.4 mmol), 1.66 g of alcohol 16 (10 mmol), and, in order, 1.9 mL (20 mmol) of acetic anhydride were added under argon to 12 mg (0.1 mmol) of DMAP. After being stirred overnight at room temperature, the reaction mixture was diluted with 20 mL of ether. After hydrolysis with 1 N aqueous HCl, the ether phase was recovered and the aqueous phase extracted with ether (2 × 20 mL). The ether phases were combined, washed with saturated

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(14) Schwartz's reagent ($Cp_2Zr(Cl)H$) was used for the hydrozirconation reaction. For reviews, see: (a) Schwartz, J.; Labinger, J. A. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 333–340. (b) Negishi, E.; Takahashi, T. *Synthesis* **1988**, *1*, 1–19.

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(16) Avignon-Tropis, M.; Pougny, J. R. *Tetrahedron Lett.* **1989**, *30*, 4951–4952.

(17) Boland, W.; Schroer, N.; Sieler, C. *Helv. Chim. Acta.* **1987**, *70*, 1025–1040.

(18) $LTB_4$ was metabolized by human polymorphonuclear leucocytes into ($\omega$OH, $\omega$COOH $LTB_4$, etc.). The metabolites (analyzed by reversed-phase HPLC) were qualitatively and quantitatively identical to those produced in a control sample of natural $LTB_4$ and $LTB_4$ supplied by Rokach. We would like to thank Dr. Delaforge and his co-workers (Université René Descartes, Paris) for carrying out the biological tests.

(19) **Solution I**: 2 mL of saturated aqueous $AgNO_3$ is made up to 100 mL with acetone. **Solution II**: 2.8 g of KOH in 2 mL of water is made up to 100 mL with ethanol. **Visualization**: the TLC plates are immersed in solution I, dried, and then immersed in solution II. Spots appear immediately.

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(22) Höfle, G.; Steglich, W.; Vorbrüggen, H. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 569–583.

$NaHCO_3$ and saturated NaCl, dried, and concentrated. A total 1.99 g (9.6 mmol) of the pure acetate **17** (96%) was isolated after chromatography of the crude reaction mixture (100 g silica; hexane/ethyl acetate (9:1) v/v; $R_f$ = 0.38): high-resolution MS (CI-$NH_3$) $[M + 18]^+$ found $m/z$ 226.1811, calcd for $C_{13}H_{24}NO_2$ 226.1807; IR (film) $\nu$ 3309 (s), 3017 (m), 2122 (w), 1745 (s) $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 5.59 (1 H, dtt, $J$ = 10.9, 6, 1.5 Hz, $H_6$), 5.42 (1 H, dtt, $J$ = 10.9, 6.5, 1.5 Hz, $H_5$), 5.36 (1 H, td, $J$ = 6.5, 2 Hz, $H_3$), 2.55 (2 H, ddd, $J$ = 6.5, 1.5 Hz, $H_4$), 2.47 (1 H, d, $J$ = 2 Hz, $H_1$), 2.1 (3 H, s, Ac), 2.06 (2 H, tdd, $J$ = 6, 1.5 Hz, $H_7$), 1.3 (6 H, m, $H_{8-9-10}$), 0.9 (3 H, t, $J$ = 6.5 Hz, $H_{11}$).

**(+)-(5*Z*)-(3*R*)-3-Acetoxy-5-undecen-1-yne (18) (Enzymatic Resolution).** A 1-g portion of porcine pancreatic lipase (Sigma; triacylglycerol lipase EC 3.1.1.3; 36 units/mg prot.) was added to a solution of 1 g (4.8 mmol) of the acetate **17** in 200 mL of phosphate buffer (0.1 M, $K_2HPO_4$, pH 7). The mixture was stirred vigorously at 25 °C to achieve 70% conversion (around 24 h) as determined using GC. The mixture was filtered through Celite under reduced pressure and extracted three times with ether. The Celite was ground up in ether and then filtered. The combined ether fractions were dried and concentrated. A total of 270 mg (1.3 mmol) of acetate **18** (27%) was isolated as a colorless oil $[\alpha]^{20}_J$ = +36.3° (*c* 0.01; $CCl_4$) by chromatography using the same conditions as described for compound **17**. The other physicochemical properties of **18** MS, IR, $^1H$ NMR are identical to those of the racemic mixture **17**.

**(+)-(5*Z*)-(3*R*)-5-Undecen-1-yn-3-ol (Synthon 2).** $K_2CO_3$ (10 mg, 0.07 mmol) was added to a solution of 250 mg (1.2 mmol) of acetate **18** in 10 mL of aqueous methanol (10% water). Complete deprotection was achieved after stirring for 2 h at 25 °C (checked by GC). The methanol was evaporated under reduced pressure the reaction mixture diluted with 50 mL of water and extracted three times with ether. After being washed, the crude reaction mixture was purified by chromatography under the same conditions as those described for compound **16**. A total of 195 mg (1.17 mmol) of pure synthon **2** (97%) was isolated as a colorless oil $[\alpha]^{20}_J$ = +34° (*c* 0.008; $CCl_4$) (ee = 96%)[11] lit.[5a] $[\alpha]_D$ = +26°). The other physicochemical properties of synthon **2** (MS; IR; $^1H$ NMR) are identical to those of the racemic mixture **16**.

**(+)-(5*Z*)-(3*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-5-undecen-1-yne (5).**[23a] A 204-mg (3-mmol) portion of imidazole, followed by 217 mg (1.44 mmol) of *tert*-butyldimethylchlorosilane, was added rapidly to a cooled solution (0 °C) of 200 mg (1.2 mmol) of synthon **2** in 1 mL of anhydrous DMF. The reaction was stirred for 3 h at 35 °C under argon and diluted in 10 mL of hexane, and the excess of reagent was hydrolyzed with 10 mL of water. The organic phase was collected and the aqueous phase extracted twice with hexane. The combined organic phases were washed, dried, and concentrated. Pure compound **5** (332 mg, 1.19 mmol) in the form of a colorless oil (99%) was isolated after chromatography (8 g of silica; hexane; $R_f$ = 0.3) of $H_{8-9-10}$), crude reaction mixture: $[\alpha]^{20}_J$ = +28.5° (*c* 0.02; $CCl_4$); MS (CI-$NH_3$) $[M + 18]^+$ at $m/z$ 298 (100); IR (film) $\nu$ 3313 (s), 3010 (m), 2100 (w) $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 5.64 (1 H, dtt, $J$ = 10.9, 6, 1.5 Hz, $H_6$), 5.45 (1 H, dtt, $J$ = 10.9, 6.5, 1.5 Hz, $H_5$), 4.38 (1 H, td, $J$ = 7.5, 2 Hz, $H_3$), 2.45 (2 H, td, $J$ = 7.5, 1.5 Hz, $H_4$), 2.4 (1 H, d, $J$ = 2 Hz, $H_1$), 2.04 (2 H, qd, $J$ = 6, 1.5 Hz, $H_7$), 1.4–1.26 (6 H, m, $H_{8-9-10}$), 0.89 (12 H, m, $H_{11}$ and *t*-Bu), 0.05–0.03 (6 H, s + s, $Me_2Si$).

**Methyl 5-Oxo-7-(trimethylsilyl)-6-heptynoate (20).** A suspension of 800 mg (6.6 mmol) of $AlCl_3$ in 2 mL of $CH_2Cl_2$ was added under argon to a $CH_2Cl_2$ solution (18 mL) of 1 g (6 mmol) of methyl 4-(chloroformyl)butyrate (**19**) and 0.77 mL (6 mmol) of bis(trimethylsilyl)acetylene. The mixture was stirred for 3 h from 0 °C to room temperature and then hydrolyzed with 10% aqueous hydrochloric acid. The aqueous phase was extracted three times with ether. After washing and concentration, the residue was purified by chromatography (50 g silica; hexane/ethyl acetate (75:25) v/v; $R_f$ = 0.34) producing 815 mg (60%) of pure compound **20** as a colorless oil. MS (CI-$NH_3$) $[M + 18]^+$ $m/z$ 244; IR (film) $\nu$ 2150 (m), 1730 (s), 1670 (s), 845–760 (s) $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 3.7 (3 H, s, $CH_3OCO$), 2.67 (2 H, t, $J$ = 7.2 Hz, $H_4$), 2.39 (2 H, t, $J$ = 7.2 Hz, $H_2$), 2 (2 H, tt, $J$ = 7.2 Hz, $H_3$), 0.27 (9 H, s, TMS). Anal. Calcd for $C_{11}H_{18}O_3Si$: C, 58.37; H, 8.01. Found: C, 58.52; H, 8.16.

**Methyl 5-Oxo-6-heptynoate (21).** A 5.5-mL portion of a 0.01 M aqueous solution of borax was added to 800 mg (3.54 mmol) of ketone **20** in 45 mL of methanol. After 10 min of stirring at room temperature, the reaction mixture was cooled to 0 °C and acidified with 10% hydrochloric acid, which decolorizes the mixture. The methanol was evaporated, and the mixture was extracted three times with ether. The combined organic phases were dried and concentrated. A total of 503 mg (3.26 mmol) of the pure acetylenic compound **21** (92%) were isolated as a colorless oil after chromatographic purification (30 g silica; hexane/ethyl acetate (85:15) v/v; $R_f$ = 0.35). MS (CI-$NH_3$) $[M + 18]^+$ $m/z$ 172; IR (film) $\nu$ 3250 (s), 2100 (s), 1730 (s), 1680 (s) $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 3.68 (3 H, s, $CH_3OCO$), 3.34 (1 H, s, $H_7$), 2.69 (2 H, t, $J$ = 7.2 Hz, $H_4$), 2.37 (2 H, t, $J$ = 7.2 Hz, $H_2$), 1.98 (2 H, tt, $J$ = 7.2 Hz, $H_3$). Anal. Calcd for $C_8H_{10}O_3$: C, 62.32; H, 6.54. Found: C, 62.44; H, 6.42.

**(−)-(5*S*)-Methyl 5-Hydroxy-6-heptynoate (Synthon 3): Microbial Reduction.** *G. candidum* CBS 233-76 was cultured in a 2-L fermenter in the following medium: 50 g of glucose, 10 g of yeast extract, 10 g of peptone, 1 L of $H_2O$, pH 7.1. After 48 h of growth at 27 °C, the cells were separated from the culture medium by filtration (pH 4.7) and washed repeatedly with saline. A 50-g portion of wet cells was placed in a screw-topped flask containing 500 mL of distilled water previously degassed by autoclaving. Ketone (500 mg, 3.24 mmol) was added under a stream of nitrogen. The hermetically sealed flask was stirred for 17 h at 27 °C. After incubation, the cells were filtered off, and the filtrate was continuously extracted with ether for 24 h. A total of 108 mg (0.59 mmol) of the pure *S* alcohol **3** (18%) was obtained as a colorless oil after chromatography (4 g silica; $CH_2Cl_2$/ethyl acetate (95:5); $R_f$ = 0.36): $[\alpha]^{20}_J$ = −18.5° (*c* 0.01; $CCl_4$) (enantiomeric excess[11] = 90%) (lit.[5a] $[\alpha]_D$ = −9°); MS (CI-$NH_3$) $[M + 18]^+$ $m/z$ 174; high-resolution MS (EI) of TMS derivative $(M - 15)^+$ found $m/z$ 213.0958, calcd for $C_{10}H_{17}O_3Si$ 213.0947; IR (film) $\nu$ 3500–3300 (s), 3400 (s), 2100 (w), 1730 (s) $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 4.4 (1 H, m, $H_5$), 3.7 (3 H, s, $CH_3OCO$), 2.5 (1 H, d, $J$ = 2 Hz, $H_7$), 2.38 (2 H, t, $J$ = 7.5 Hz, $H_2$), 1.9–1.7 (5 H, m, $H_3$–$H_4$–OH).

**(−)-(5*S*)-Methyl 5-[(*tert*-Butyldimethylsilyl)oxy]-6-heptynoate (10).**[23a] The procedure described for the preparation of compound **5** was employed on 100 mg (0.54 mmol) of alcohol **3**. A 139.4-mg (0.52 mmol) portion of the pure silylated derivative (95%) was isolated after chromatography (6 g silica; hexane/ethyl acetate (95:5); $R_f$ = 0.37) of the crude reaction mixture: $[\alpha]^{20}_J$ = −38.5° (*c* 0.006; $CCl_4$); MS (CI-$NH_3$) $[M + 18]^+$ $m/z$ 288; IR (film) $\nu$ 3300 (s), 2110 (w), 1730 (s), 840 and 776 (s) $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 4.35 (1 H, td, $J$ = 6, 2 Hz, $H_5$), 3.66 (3 H, s, $CH_3OCO$), 2.37 (1 H, d, $J$ = 2 Hz, $H_7$), 2.35 (2 H, t, $J$ = 7.5 Hz, $H_2$), 1.80–1.65 (4 H, m, $H_3$–$H_4$), 0.89 (9 H, s, *t*-Bu), 0.13–0.10 (6 H, s + s, $Me_2Si$).

**(+)-(1*E*,5*Z*)-(3*R*)-1-Iodo-3-[(*tert*-butyldimethylsilyl)oxy]-1,5-undecadiene (6).** Zirconium hydride ($Cp_2Zr(Cl)H$) (309.5 mg, 1.2 mmol) and 2 mL of freshly distilled benzene were introduced into a round-bottomed flask in the dark and under argon. A total of 300 mg (1 mmol) of the acetylenic derivative **5** in 1 mL of benzene was then added. After being warmed to 40 °C with vigorous stirring, the white suspension cleared within a few minutes to give a pale yellow solution, indicating the end of the reaction (checked by TLC). A few crystals of iodine were then added until a brown color persisted. The reaction mixture was poured into 10 mL of hexane and stirred for 5 min until the zirconium salt precipitated. The suspension was filtered over Florisil and then concentrated. A total of 372 mg (0.91 mmol) of vinyl iodide **6** (91%) was obtained as a colorless oil after chromatographic purification (15 g silica; hexane; $R_f$ = 0.5) of the crude oil: $[\alpha]^{20}_J$ = +4.8° (*c* 0.009; $CCl_4$); IR (film) $\nu$ 3013 (m), 1607 (m), 845 and 770 (s) $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 6.50 (1 H, dd, $J$ = 14.4, 6 Hz, $H_2$), 6.17 (1 H, dd, $J$ = 14.4, 1.2 Hz, $H_1$), 5.45 (1 H, dtt, $J$ = 10.5, 7, 1 Hz, $H_6$), 5.34 (1 H, dtt, $J$ = 10.5, 6, 1.5 Hz, $H_5$), 4.06 (1 H, tdd, $J$ = 6, 1.2 Hz, $H_3$), 2.30 (2 H, dd, $J$ = 6 Hz, $H_4$), 2.05 (2 H, td, $J$ = 7 Hz, $H_7$), 1.4–1.28 (6 H, m, $H_{8-9-10}$), 0.89 (12 H, m, $H_{11}$–*t*-Bu), 0.05–0.03 (6 H, s + s, $Me_2Si$).

**(+)-(3*E*,7*Z*)-(5*R*)-5-[(*tert*-Butyldimethylsilyl)oxy]-1-(trimethylsilyl)-3,7-tridecadien-1-yne (7).** Tetrakis(triphenylphosphine)palladium ($[(Ph_3P)]_4Pd$) (25 mg, 0.02 mmol)

(23) (a) Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190–6192. (b) Corey, E. J.; Snider, B. B. *J. Am. Chem. Soc.* **1972**, *94*, 2549–2550 and ref 4f.

was introduced under argon into a solution of 300 mg (0.73 mmol) of the vinyl iodide **6** in 3 mL of freshly distilled benzene. After the crystals were completely dissolved, 0.8 mL (8 mmol) of *n*-butylamine followed by 10 mg (0.05 mmol) of copper iodide were added. A total of 0.5 mL (3.54 mmol) of (trimethylsilyl)acetylene was then added, and the ensuing exothermic reaction was completed in 30 min with stirring (checked by TLC). The reaction mixture was hydrolyzed with 10 mL of 10% aqueous ammonium chloride and extracted with ether (3 × 10 mL). The combined ether layers were washed with saturated NaCl, dried, and concentrated. A total of 263 mg (0.69) of compound **7** was isolated as a colorless oil (95%) after chromatography (16 g silica; hexane; $R_f$ = 0.45) of the crude reaction mixture: $[\alpha]^{20}_J$ = +11.4° (*c* 0.01; $CCl_4$); MS (CI-$NH_3$) $[M + 1]^+$ *m/z* = 379; high-resolution MS (EI) found for $[M - C_4H_9]^+$ *m/z* 321.2077, calcd for $C_{18}H_{33}OSi_2$ 321.2070; IR (film) $\nu$ 3010 (w), 2161 (m), 840 and 770 (s) $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 6.24 (1 H, dd, *J* = 15.9, 5 Hz, $H_4$), 5.65 (1 H, dd, *J* = 15.9, 2.5 Hz, $H_3$), 5.45 (1 H, dtt, *J* = 10.5, 7, 1 Hz, $H_8$), 5.35 (1 H, dtt, *J* = 10.5, 6, 1.5 Hz, $H_7$), 4.17 (1 H, td, *J* = 6, 5 Hz, $H_5$), 2.25 (2 H, dd, *J* = 6 Hz, $H_6$), 1.99 (2 H, td, *J* = 6.7, $H_9$), 1.35–1.25 (6 H, m, $H_{10-11-12}$), 0.89 (12 H, m, $H_{13}$ + *t*-Bu), 0.18 (9 H, s, TMS), 0.04–0.025 (6 H, s + s, $Me_2Si$).

**(+)-(3*E*,7*Z*)-(5*R*)-5-[(*tert*-Butyldimethylsilyl)oxy]-3,7-tridecadien-1-yne (8).** A solution of 299 mg (1.76 mmol) of $AgNO_3$ in 1.6 mL of aqueous ethanol (25% water) was added dropwise to 250 mg (0.66 mmol) of the silylated acetylenic derivative **7** in 3 mL of ethanol. A yellow precipitate of the silver acetylide appeared. After the solution was stirred for 30 min, 550 mg (8.45 mmol) of KCN in 1 mL of water was added after which the solution became homogeneous. The mixture was extracted with pentane (3 × 10 mL), and the combined organic phases were washed with saturated NaCl, dried, and concentrated. A total of 172 mg (0.56 mmol) of the acetylenic derivative **8**[24] (85%) was obtained as a colorless oil after chromatography (7 g silica; hexane; $R_f$ = 0.36) of the crude oil: $[\alpha]^{20}_J$ = +16.9° (0.013; $CCl_4$); high-resolution MS (CI-$NH_3$) $[M + 1]^+$ found *m/z* 307.2463; calculated for $C_{19}H_{35}OSi$ = 307.2457; IR (film) $\nu$ 3314 (s), 3012 (w), 2103 (w), 836 and 776 (s) $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 6.24 (1 H, dd, *J* = 15.9, 5 Hz, $H_4$), 5.65 (1 H, ddd, *J* = 15.9, 2.5 Hz, $H_3$), 5.45 (1 H, dtt, *J* = 10.5, 7, 1 Hz, $H_8$), 5.35 (1 H, dtt, *J* = 10.5, 6, 1.5 Hz, $H_7$), 4.17 (1 H, td, *J* = 6, 5 Hz, $H_5$), 2.85 (1 H, d, *J* = 2.5 Hz, $H_1$), 2.25 (2 H, dd, *J* = 6 Hz, $H_6$), 1.99 (2 H, td, *J* = 6.7 Hz, $H_9$), 1.35–1.25 (6 H, m, $H_{10-11-12}$), 0.89 (12 H, m, $H_{13}$ + *t*-Bu), 0.04–0.025 (6 H, s + s, $Me_2Si$).

**(+)-(1*E*,3*E*,7*Z*)-(5*R*)-1-Iodo-5-[(*tert*-butyldimethylsilyl)oxy]-1,3,7-tridecatriene (9).** A 76-mg (0.29 mmol) portion of $Cp_2Zr(Cl)H$ was quickly added in the dark to 150 mg (0.49 mmol) of the acetylenic derivative **8** in 0.75 mL of benzee and 0.75 mL of THF (both solvents anhydrous and freshly distilled) with vigorous stirring under argon. After 15 min at 35 °C, the white suspension became a clear yellow solution. An additional 76 mg (0.29 mmol) of $Cp_2Zr(Cl)H$ was added. After complete disappearance of the suspension (around 15 min), crystals of iodine were added until the color of the solution remained brown. The mixture was diluted with 5 mL of hexane, filtered over Florisil under reduced pressure, rinsed with hexane (3 × 5 mL), and concentrated. After chromatographic purification (10 g silica; hexane; $R_f$ = 0.47), the vinyl iodide **9** (189.5 mg, 0.436 mmol) was obtained as a colorless oil (89%) which turns brown in the light: IR (film) $\nu$ 3080 (w), 3040 (w), 1600 (w), 845 and 780 (s) $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 7 (1 H, dd, *J* = 14.3, 10.7 Hz, $H_2$), 6.26 (1 H, d, *J* = 14.3 Hz, $H_1$), 6.09 (1 H, dd, *J* = 15.2, 10.6 Hz, $H_3$), 5.70 (1 H, dd, *J* = 15.2, 5.8 Hz, $H_4$), 5.45 (1 H, dtt, *J* = 10.5, 7, 1 H, $H_8$), 5.34 (1 H, dtt, *J* = 10.5, 6, 1.5 Hz, $H_7$), 4.12 (1 H, td, *J* = 5.8 Hz, $H_5$), 2.25 (2 H, dd, *J* = 6 Hz, $H_6$), 1.98 (2 H, td, *J* = 6.7 Hz, $H_9$), 1.35–1.28 (6 H, m, $H_{10-11-12}$), 0.89 (12 H, m, $H_{13}$-*t*-Bu), 0.04–0.025 (6 H, s + s, $Me_2Si$).

**(−)-(8*E*,10*E*,14*Z*)-(5*S*,12*R*)-Methyl 5,12-Bis[(*tert*-butyldimethylsilyl)oxy]eicosa-8,10,14-trien-6-ynoate (11).** A total of 13.3 mg (0.0115 mmol) of $Pd[P(Ph_3)]_4$ was introduced under argon into a flask containing 100 mg (0.23 mmol) of the vinyl iodide **9** in 2 mL of benzene. After the catalyst was completely dissolved, 0.045 mL (0.46 mmol) of *n*-butylamine followed by 4.38 mg (0.023 mmol) of copper iodide were added rapidly. 124.5 mg (0.46 mmol) of the acetylenic derivative **10** in 1 mL of benzene were then added. The reaction was complete (checked by TLC) after 3 h of stirring at room temperature. The reaction mixture was hydrolyzed with 10 mL of aqueous ammonium chloride (10%) and extracted with ether (3 × 10 mL). The combined ether layers were washed with saturated NaCl, dried, and concentrated. A total of 103.8 mg (0.18 mmol) of pure compound **7** was isolated (78%) as a colorless oil after chromatography (6 g silica; hexane/ethyl acetate (95:5) v/v; $R_f$ = 0.45): $[\alpha]^{20}_J$ = −29.6° (*c* 0.011; $CCl_4$); IR (film) $\nu$ 3010 (w), 2123 (w), 1742 (s), 837 and 777 (s) $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 6.55 (1 H, dd, *J* = 15.5, 11 Hz, $H_9$), 6.18 (1 H, dd, *J* = 15.2, 11 Hz, $H_{10}$), 5.77 (1 H, dd, *J* = 15.2, 6 Hz, $H_{11}$), 5.55 (1 H, d, *J* = 15.5 Hz, $H_8$), 5.43 (1 H, dt, *J* = 10.5, 7 Hz, $H_{15}$), 5.34 (1 H, dt, *J* = 10.5, 6 Hz, $H_{14}$), 4.51 (1 H, m, $H_5$), 4.17 (1 H, td, *J* = 6 Hz, $H_{12}$), 3.67 (3 H, s, $CH_3OCO$), 2.37 (2 H, t, *J* = 6.5 Hz, $H_2$), 2.25 (2 H, m, $H_{13}$), 1.98 (2 H, td, *J* = 7 Hz, $H_{16}$), 1.86–1.7 (4 H, m, $H_{3-4}$), 1.35–1.27 (6 H, m, $H_{17-18-19}$), 0.89 (21 H, s, $H_{20}$ 2 × *t*-Bu), 0.13–0.10 (6 H, s + s, $Me_2Si$), 0.04–0.02 (6 H, s + s, $Me_2Si$).

**(−)-(8*E*,10*E*,14*Z*)-(5*S*,12*R*)-Methyl 5,12-Dihydroxy-8,10,14-eicosatrien-6-ynoate (12).**[23b] A 1.73-mL (1.73 mmol) portion of tetrabutylammonium fluoride (1 M/THF) was injected slowly under argon into a solution of 100 mg (0.173 mmol) of the silylated derivative **11** in 5 mL of THF cooled in an ice bath. After being stirred 2 h at room temperature, the mixture was acidified with 5 mL of 5% hydrochloric acid and extracted with ether (3 × 5 mL). The organic phases were washed with 10 mL of saturated NaCl and dried. An excess of diazomethane was added to regenerate the hydrolyzed methyl ester. After concentration, the crude reaction mixture was purified by chromatography (2.5 g silica; $CH_2Cl_2$/ethyl acetate (70:30) v/v; $R_f$ = 0.35) to afford 51 mg (0.146 mmol) of the pure hydroxylated compound **12** (85%). $[\alpha]^{20}_J$ = −4.5° (0.008; $CCl_4$); MS (CI-$NH_3$) $[M + 18]^+$ found *m/z* 366; IR (film) $\nu$ 3381 (s), 3010 (w), 2120 (w), 1740 (s), 1640 (w) $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 6.55 (1 H, dd, *J* = 15.5, 11 Hz, $H_9$), 6.18 (1 H, dd, *J* = 15.2, 11 Hz, $H_{10}$), 5.77 (1 H, dd, *J* = 15.2, 6 Hz, $H_{11}$), 5.55 (1 H, d, *J* = 15.5 Hz, $H_8$), 5.43 (1 H, dt, *J* = 10.5, 7 Hz, $H_{15}$), 5.34 (1 H, dt, *J* = 10.5, 6 Hz, $H_{14}$), 4.51 (1 H, m, $H_5$), 4.17 (1 H, td, *J* = 6 Hz, $H_{12}$), 3.67 (3 H, s, $CH_3OCO$), 2.37 (2 H, t, *J* = 6.5 Hz, $H_2$), 2.25 (2 H, m, $H_{13}$), 1.98 (2 H, td, *J* = 7 Hz, $H_{16}$), 1.86–1.6 (6 H, m, $H_{3-4}$ + 2(OH)), 1.35–1.37 (6 H, m, $H_{17-18-19}$), 0.89 (3 H, t, *J* = 6 Hz, $H_{20}$).

**(+)-(6*Z*,8*E*,10*E*,14*Z*)-(5*S*,12*R*)-Methyl 5,12-Dihydroxy-6,8,10,14-eicosatetraenoate (13).** Dienyne **12** (50 mg, 0.143 mmol) in 0.2 mL of methanol was added to 300 mg of activated zinc[16] suspended in 1 mL of methanol/water (1:1). On completion of the reaction (checked by TLC) after stirring at 30 °C for 5 h, the reaction mixture was filtered through Celite 545, rinsed with methanol (2 × 1 mL), concentrated, and coevaporated with toluene. A total of 28 mg (0.08 mmol) of the pure methyl ester of $LTB_4$ **13**[25] (65%) was obtained after chromatography (1 g of silica; $CH_2Cl_2$/ethyl acetate (65:35) v/v; $R_f$ = 0.35): $[\alpha]^{20}_J$ = +4.5° (*c* 0.001; $CCl_4$); MS of the di-TMS derivative (CI-$NH_3$) $[M + 18]^+$ found *m/z* 512; IR (film) $\nu$ 3400 (s), 3008 (w), 1737 (s), 1638, 1613–1589 (w) $cm^{-1}$; $^1H$ NMR (300 MHz) $\delta$ 6.45 (1 H, dd, *J* = 14.5, 11 Hz, $H_9$), 6.29 (1 H, dd, *J* = 15, 10.5 Hz, $H_{10}$), 6.22 (1 H, dd, *J* = 14.5, 10.5 Hz, $H_8$), 6.07 (1 H, dd, *J* = 11 Hz, $H_7$), 5.77 (1 H, dd, *J* = 15, 6.3 Hz, $H_{11}$), 5.54 (1 H, m, $H_{15}$), 5.4 (1 H, dd, *J* = 11, 9.5 Hz, $H_6$), 5.37 (1 H, m, $H_{14}$), 4.57 (1 H, td, *J* = 9.5 Hz, $H_5$), 4.20 (1 H, td, *J* = 6.3 Hz, $H_{12}$), 3.65 (3 H, s, $CH_3OCO$), 2.33 (4 H, m, $H_{2-13}$), 2.02 (2 H, td, *J* = 7 Hz, $H_{16}$), 1.73–1.5 (6 H, m, $H_{4-3}$ and 2(OH)), 1.37–1.21 (6 H, m, $H_{17-18-19}$), 0.87 (3 H, t, *J* = 6.6 Hz, $H_{20}$).

**(+)-(6*Z*,8*E*,10*E*,14*Z*)-(5*S*,12*R*)-5,12-Dihydroxy-6,8,10,14-eicosatetraenoic Acid ($LTB_4$, 1).** A solution of 1 mg ($2.85 \cdot 10^{-3}$ mmol) of $LTB_4$ methyl ester **13** in 0.4 mL of methanol and 0.1 mL of water containing 3.94 mg ($2.85 \times 10^{-2}$ mmol) of $K_2CO_3$ was stirred overnight under argon at room temperature. After the methanol was evaporated using a stream of argon, the residue was dissolved in 0.2 mL of water and introduced on a reversed-phase Sep-Pak cartridge (Millipore $C_{18}$) previously washed with 5 mL of methanol and 5 mL of water. The Sep-Pak cartridge was rinsed with 4 mL of water (until neutral pH) before $LTB_4$

(24) The enantiomeric excess was conserved in the Mosher ester of the desilylated compound **8** (GC; ee = 96%).

(25) Traces of the diastereoisomer (5*R*,12*R*) ($R_f$ = 0.4) were eliminated on chromatography,[4k] thus conserving the enantiomeric excess of the synthon **2** (ee = 96%).

was eluted with 4 mL of methanol. After evaporation of the methanol, 0.9 mg ($2.68 \times 10^{-3}$ mmol) of $LTB_4$ was obtained (95%): $[\alpha]^{20}_J$ = +12.8° (c 0.0009; $CCl_4$) (Lit.[3g] $[\alpha]^{25}_D$ = +12.6°. The physicochemical characteristics (MS, IR, $^1H$ NMR, UV) were identical to literature values. The biological activity was comparable (see ref 18) to that of $LTB_4$ synthesized by Rokach.

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# Analogues of Tri-*o*-thymotide (TOT) and Tri-*o*-carvacrotide (TOC) by Direct Bromination of TOT and TOC[1]

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Mono-, di-, tri-, tetra-, and pentabrominated tri-*o*-thymotide (TOT) analogues (3–7) and mono-, di-, tri-, and tetrabrominated tri-*o*-carvacrotide (TOC) analogues (9–12) were synthesized by direct bromination of TOT and TOC. Total yields of about 60% were realized from TOT or TOC. The degree of bromination of the methyl group depended upon the reaction temperature and ratio of TOT (TOC):NBS. Tribromo-TOT (5) was generated in the greatest yield using a 1:18 ratio of TOT:NBS. No bromination occured in the isopropyl group. Structure assignments were made for all new analogues. The conformational mobility of some of these analogues as well as the effect of solvents on the proton chemical shifts were studied by NMR techniques.

## Introduction

The recent commercial availability of *o*-thymotic acid[2] allows TOT to be prepared more readily and makes feasible its direct conversion into other analogues by functional group transformation. The placement of one or more halogen atoms onto the TOT framework (specifically into the isopropyl or methyl groups appended to the aromatic rings) would not only provide new halogenated TOT analogues, but would afford an opportunity to further prepare a large number of substituted analogues by nucleophilic displacement reactions. A study of the bromination of TOT (1) and TOC (2) is described in this paper.

## Results and Discussion

**Bromination Studies.** In refluxing $CCl_4$ (77 °C), using 1–45 equiv of NBS, TOT affords a complex mixture of polybrominated and dehydrobrominated products. The components of this mixture were extremely difficult to separate. Integration of various NMR spectra of these reaction mixtures showed that the absorptions of the primary and tertiary isopropyl group hydrogen atoms in the products decreased relative to those in TOT. In addition the intensity of the Me group absorption decreased with the appearance of an AB quartet at δ 4.5–4.8. This indicated that bromination of TOT at elevated temperature took place both in the isopropyl and methyl groups. Dehydrohalogenation of some of these brominated compounds also occurred as demonstrated by the appearance of absorptions in the olefin region of the NMR spectra (δ 5–5.2). Lowering the temperature to 0 °C, under otherwise identical conditions, caused solubility problems with NBS and suggested further study.

The bromination of TOT could be controlled and made to take place in the methyl group(s) only by using acetone/$H_2O$ as the solvent and by carrying out the reaction at 50 °C to minimize dehydrohalogenation. Using a variety of conditions (as indicated in Table I), mono- (3), di- (4), tri- (5), tetra- (6), and even pentabrominated TOT (7) analogues were isolated. The total yield of brominated products, and the ratios between each of the brominated products, depended mainly on the temperature and the number of equivalents of NBS used.

The best conditions for the bromination of the Me groups in TOT is shown in runs 5–7 (Table I). These conditions afford the largest yield of tribrominated TOT (5) and the smallest amount of unreacted TOT. Under similar conditions TOC (2) also underwent bromination, affording 9–12 (Table I, runs 10 and 11).

Generally, the benzylic methine hydrogen atom (tertiary) of an isopropyl group is more easily brominated than a primary hydrogen atom of a benzylic methyl group.[4] However, in TOT, using the above conditions, the methyl group is brominated exclusively. Even when the three methyl groups in TOT are already brominated (as in 5), continued bromination occurs at the bromomethyl group ($CH_2Br$) and not at the formally more active isopropyl group, giving the tetra- and pentabrominated TOT analogues 6 and 7. We believe that steric inhibition of reso-

(1) This is the second paper in a series dealing with the effects of substituent modification on the host properties of TOT. See: Harris, T. D.; Oruganti, S. R.; Davis, L. D.; Keehn, P. M.; Green, B. S. *Tetrahedron* 1987, *43*, 1519–1540.

(2) (a) Aldrich Chemical Co. (b) Gnaim, J. M.; Green, B. S.; Arad-Yellin, R.; Keehn, P. M. *J. Org. Chem.* 1991, *56*, 4525.

(3) Each of the reaction products of the bromination of TOT is believed to be a mixture of the two propeller shaped chiral enantiomers. This was substantiated by using Pirkle's reagent. The AB q of the hydrogen atoms in the bromomethyl group in tribromo-TOT (5) was split in two when the ratio of Pirkle's reagent to solute was 1.14:1 (300 MHz, room temperature). Resolution of 5 has not yet been attempted.

(4) (a) Pizey, J. S. *Synthetic Reagents*; John Wiley & Sons Inc.: New York, 1974; Vol. 2, pp 5–6. (b) Barnes, R. A.; Buckwalter, G. R. *J. Am. Chem. Soc.* 1951, *73*, 3858. (c) Offermann, W.; Vogtle, F. *Angew. Chem., Int. Ed. Engl.* 1980, *19*, 464. (d) Cram, D. J.; Helgeson, R. C. *J. Am. Chem. Soc.* 1966, *88*, 3515.

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