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Enantiospecific Synthesis of 17- and 18-Hydroxyeicosatetraenoic Acids, Cytochrome P450 Arachidonate Metabolites

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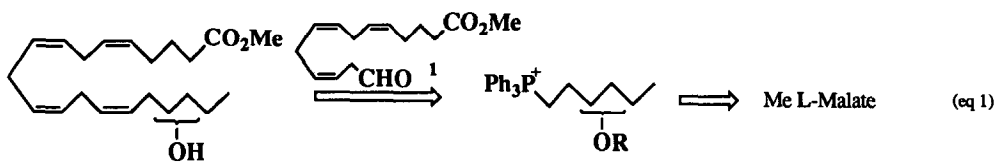
Abstract: The title bioactive eicosanoids were prepared from dimethyl L-malate by a convergent strategy exploiting the differential reactivity of ethereal dialkylcuprates towards tosylates versus bromides.

Arachidonic acid metabolism by microsomal cytochrome P450 proceeds via olefin epoxidation, allylic oxidation, or ω/ω-1 hydroxylation.[1] The latter monooxygenase activity is most prominent in lung,[2] kidney,[3] and bone marrow[4] where 20- and 19-hydroxyeicosatetraenoic acids (20- and 19-OH-AA, respectively) are the major cytochrome P450 arachidonate metabolites. The potent biological activities[5] of 20-/19-OH-AA and their correlation[6] with pathophysiologic disorders such as hypertension have stimulated wide interest in this pathway as a source of endogenous autacoids. Recently, additional monooxygenase metabolites have been isolated from a variety of mammalian tissues and their identities provisionally determined, in most cases, by mass spectroscopy.[7] To expedite current pharmacologic evaluations, we confirm herein the structural assignments of two of these, *viz.*, 18- and 17-OH-AA, and describe their enantiospecific total syntheses by a convergent strategy coupling the known aldehyde **1** with appropriately functionalized Wittig reagents derived from a single member of the chiral pool (eq 1).

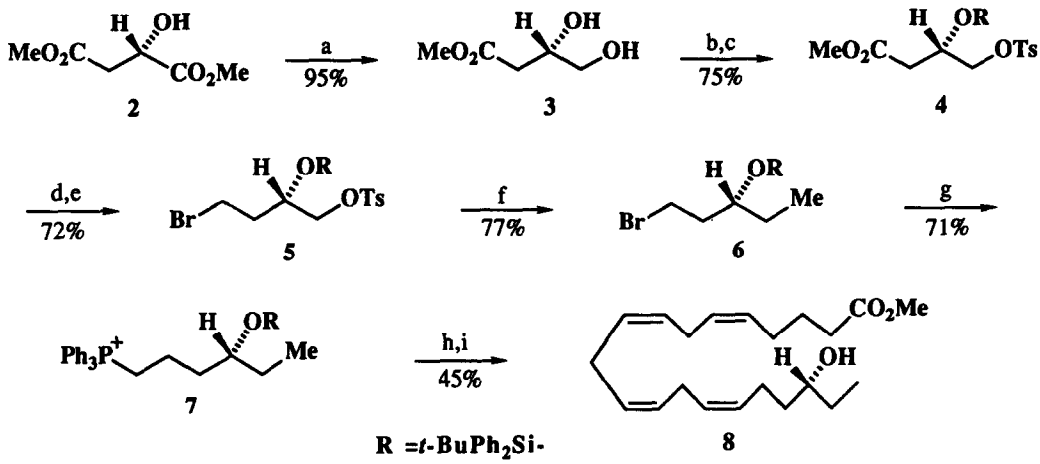


(eq 1)

Scheme 1 summarizes the preparation of methyl 18(**R**)-hydroxyeicosatetraenoate (**8**). Regiospecific reduction of dimethyl L-malate (**2**) according to literature procedure[8] afforded diol **3** from which **4**[9], mp 83-84°C, was obtained by selective primary tosylation followed by silylation of the remaining alcohol. Low temperature diisobutylaluminum hydride (DIBAL-H) reduction of the ester and alcohol-halide interconversion under standard conditions provided the key intermediate, bromo-tosylate **5**[10], mp 102-104°C. Preferential

displacement[11] of the tosylate in **5** using dimethyl cuprate in Et_2O gave rise to bromide **6** that in turn was homologated to phosphonium salt **7** by displacement with α-lithiomethylenetriphenylphosphorane[12]. Generation [$NaN(SiMe_3)_2$, THF, -40°C, 40 min] of the ylide from **7** and condensation with aldehyde **1**[13] gave, after fluoride mediated deprotection and HPLC purification[14], methyl 18(R)-OH-AA (**8**), $[\alpha]_D^{24}$= -4°(c 0.35, acetone). Mitsunobu inversion[15] ($PhCO_2H$, Ph_3P, DEAD) of **8** and methanolysis (NaOMe, MeOH, 24°C, 2h) of the resultant benzoate led to the 18(S)-isomer in good overall yield.

Scheme 1

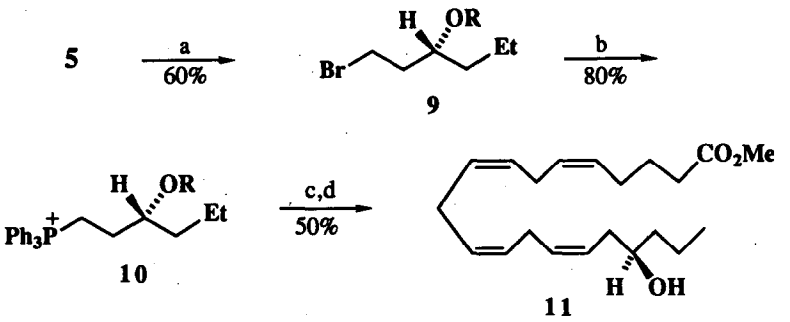


[a]BH_3•Me_2S,$NaBH_4$,THF,0→24°C,0.5h.[b]TsCl,C_5H_5N/CH_2Cl_2(1:12),0°C,12h.
[c]$tBuPh_2SiCl$,C_5H_5N/THF(1:3)$AgNO_3$ (1.5 equiv), 24°C,12h. [d]DIBAL-H,CH_2Cl_2,-78°C,2h.
[e]CBr_4/Ph_3P,CH_2Cl_2, 0°C,0.5h. [f]Me_2CuLi (3 equiv), Et_2O,0°C,4h. [g]Ph_3PCHLi,THF,
-78→-30°C,4h;HOAc.[h]$NaN(SiMe_3)_2$,THF,-40°C,40min; **1**,-78→0°C, 1h. [i]Bu_4NF,THF,24°C,10h.

Access to the C(17)-hydroxyl regioisomer was gained by selectively coupling tosylate **5** with diethyl cuprate in Et_2O to give bromide **9** (Scheme 2). Conversion to phosphonium salt **10** by standard procedure and union of the corresponding ylide with aldehyde **1** as described above furnished[14] methyl 17(R)-OH-AA (**11**),$[\alpha]_D^{24}$= +3.8° (c 1.5, acetone), following desilylation. The S-isomer was prepared as earlier using the Mitsunobu sequence.

Saponification of **8** and **11** and HPLC comparisons[14] of the resultant free acids with natural material derived from incubation[7a] of arachidonic acid with rat hepatic microsomal fractions as well as GC/NICI-MS analyses[16] of the corresponding pentafluorobenzyl ester trimethylsilyl ether derivatives corroborated the initial structural assignments. Details of biological testing and the elucidation of additional monooxygenase metabolites will be reported elsewhere.

Scheme 2



[a]Et_2CuLi (3equiv),Et_2O,0°C,4h. [b]Ph_3P,CH_3CN,65°C,30h. [c]$NaN(SiMe_3)_2$,THF,-40°C,40 min; 1,-78°→0°C,1h. [d]BuNF, THF, 24°C, 15h

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9. Satisfactory spectral data were obtained for all new compounds using chromatographically homogeneous samples.

10. Physical data for **5:** ^{1}H NMR ($CDCl_3$, 90 MHz) δ 0.95 (s, 9H), 1.88-2.20 (m, 2H), 2.44 (s, 3H) 3.30 (t, J~7Hz, 2H), 3.76-4.04 (m, 3H), 7.16-7.70 (m, 14H); TLC: SiO_2, EtOAc/hexane (1:4), R_f~0.32. **6:** ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.75 (t, J~7Hz, 3H), 0.85 (s, 9H), 1.20-1.50 (m, 2H), 1.80-2.00 (m, 2H), 3.37 (t, J~7H, 2H), 3.62-3.80 (m, 1H), 7.24-7.82 (m, 10H); TLC : SiO_2, hexane/CH_2Cl_2 (95:5), R_f~0.25. **8:** ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.95 (t, J~7.5 Hz, 3H), 1.38-1.79 (m, 8H), 2.03-2.24 (m, 3H), 2.33 (t, J~7.4Hz, 2H), 2.72-2.95 (m, 6H), 3.47-3.63 (m, 1H), 3.66 (s, 3H), 5.21-5.50 (m, 8H). **9:** ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.88 (t, J~7.4Hz, 3H), 1.07 (s, 9H), 1.15-1.47 (m, 4H), 1.91-2.09 (m, 2H), 3.41 (t, J~7.4Hz, 2H), 3.77-3.91 (m, 1H), 7.29-7.46 (m, 6H), 7.59-7.71 (m, 4H). **11:** ^{1}H NMR (C_6D_6, 250 MHz) δ 0.86 (t, J~7.4Hz, 3H), 1.13-1.67 (m, 6H), 1.90-2.26 (m, 6H), 2.61-2.94 (m, 6H), 3.36 (s, 3H), 3.38-3.50 (m, 1H), 5.19-5.63 (m, 8H).

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14. HPLC conditions: Waters μPorasil column (10 μm, 3.9 mm x150 mm) using a linear solvent gradient of hexane/2-propanol/HOAc (98.9:1.0:0.1 v/v/v) to hexane/2-propanol/HOAc (96.9:3.0:0.1 v/v/v) over 30 min at 3 ml/min with uv detection at 210 nm; **8** and **11** R_t~19.2 and 18.3 min, respectively.

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16. GC/NICI-MS conditions: Supelco SPB-20 fused silica capillary column (0.32 μm I.D., 0.25 μm coating thickness) programmed from 100 to 225°C at a rate of 10°C/min, then held at 225°C for 10 min. Helium and methane were carrier and reagent gas, respectively; PFB ester TMS ether derivatives of 17- and 18-OH-AA, R_t~20.6 and 21.2 min, respectively. The EI-MS spectra of the methyl ester trimethylsilyl ether derivatives of **8** and **11** were identical to published data (see ref. 7a).

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