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Synthesis of Tritium Labelled (3R*,5S*)-3,5-Dihydroxy-9,9-diphenyl-6,8-nonadienoate

Lewin T. Wint and Peter A. McCarthy*

Central Research, Pfizer Inc., Groton, Connecticut 06340

SUMMARY

Methyl (3*R**,5*S**)-(E)-3,5-dihydroxy-9,9-diphenyl-6,8-nonadienoate (1) is a competitive inhibitor of HMG-CoA reductase, the rate-limiting step in cholesterol biosynthesis. We synthesized 1 with a tritium label at C_3 in order to investigate tissue selectivity. The synthesis began with β-phenyl- cinnamaldehyde which was homologated to (E)-5,5-diphenyl-2,4-pentadienal in three steps. Addition of the dianion of methyl acetoacetate gave methyl (E)-5-hydroxy-9,9-diphenyl-3-oxo-6,8-nonadienoate. Diastereoselective introduction of the tritium label was achieved using tritium labelled sodium borohydride, triethylborane and a catalytic amount of pivalic acid. The radiochemical yield for this step was 7.5 %, the specific activity was 13.3 mCi/mmole, and the product was >95% radiochemically pure. This method may be applicable to the preparation of other radiolabelled HMG-CoA reductase inhibitors which possess a 3,5-dihydroxy acid moiety.

Keywords: HMG CoA reductase inhibition, tissue selectivity, diastereoselective reduction.

INTRODUCTION

Reduction of serum cholesterol is a proven therapeutic means of halting and reversing atherosclerosis[1] and reducing the incidence of myocardial infarctions.[2] Since de novo synthesis of cholesterol accounts for the majority of cholesterol in man,[3] inhibition of cholesterol biosynthesis has long been an attractive goal for intervention. Recently, inhibition of HMG-CoA reductase has

* - Author to whom correspondences should be addressed.

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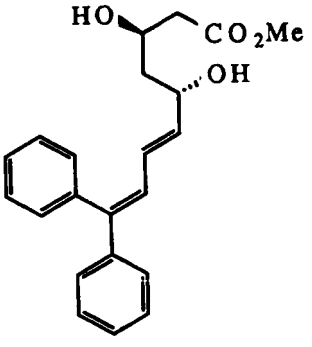
emerged as the preeminent approach to inhibiting cholesterol biosynthesis.[4] Concerns have been raised, however, that these agents may have adverse effects if they inhibit cholesterol biosynthesis in tissues other than the liver and gut, particularly the adrenals, lens, or ovaries.[5] Cognizant of this potential problem, we sought to evaluate the tissue selectivity of a prototypical HMG-CoA reductase inhibitor by synthesizing it containing a radioactive nucleus.



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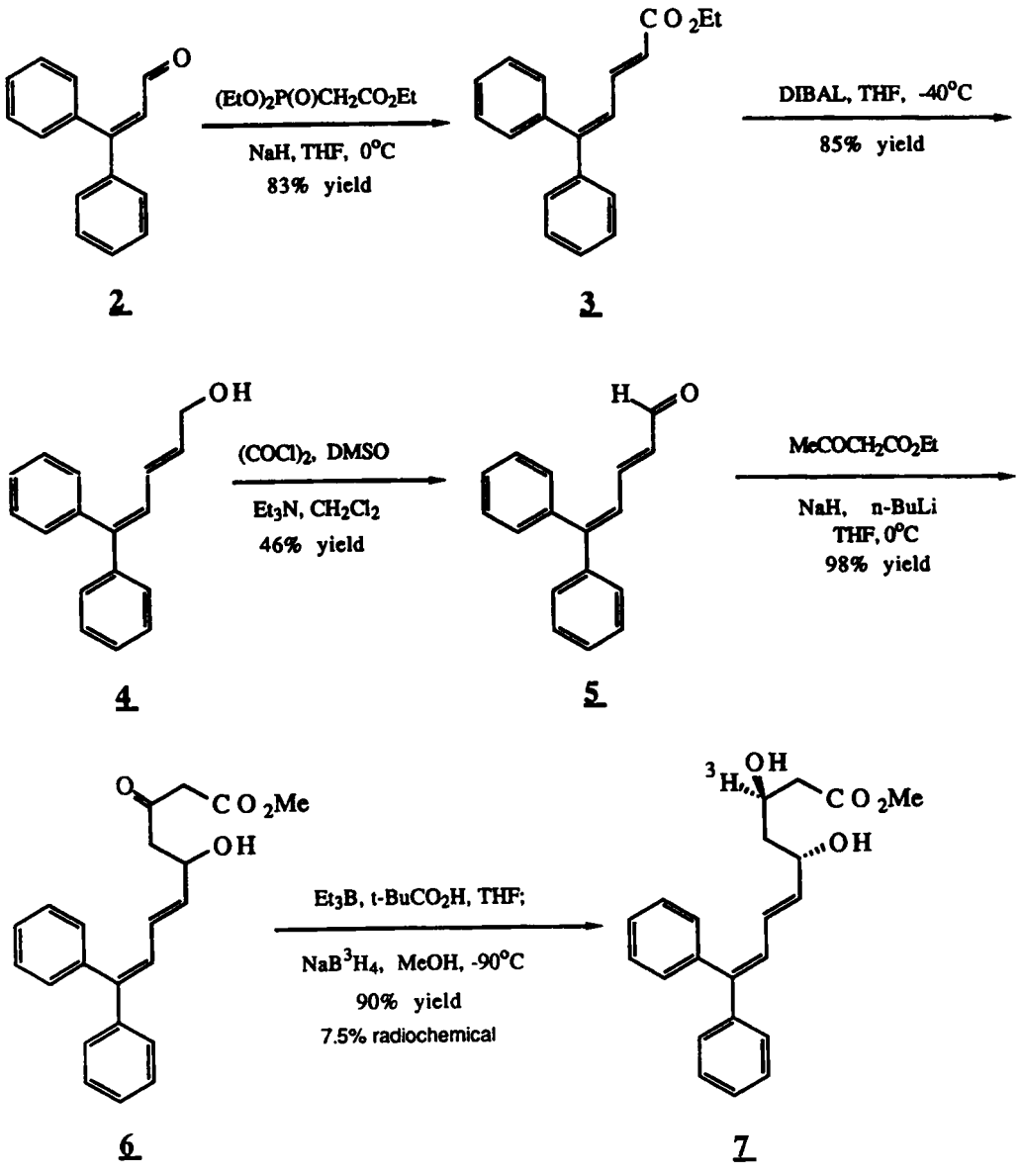
DISCUSSION

The compound we chose to label was methyl (3*R**,5*S**)-(E)-3,5-dihydroxy-9,9-diphenyl-6,8-nonadienoate (1). As an initial approach, we decided to introduce a tritium at C_3 giving 7 (see Scheme). As described below, this was very readily accomplished for 7 and should be equally facile for analogs of 1 or any HMG-CoA reductase inhibitor containing the ubiquitous (3*R**,5*S**)-3,5-dihydroxyalkanoate moiety. Such synthetic accessibility greatly facilitates study of the relationship between structure and tissue distribution. Although the metabolic lability of this tracer may undermine the validity of such studies, within a given structural series and at early time points, comparison of the observed tissue distribution should be an accurate reflection of relative tissue distribution since the metabolic fates of the compounds should be similar. Thus, this procedure should allow for identification of analogs with optimum tissue distribution characteristics. Studies of this type using a more metabolically stable tracer would be difficult, if not impossible, given the broad range of analogs one would want to examine. Nonetheless, once the better analogs were identified, it would be appropriate to invest the effort needed to synthesize these analogs with a more metabolically stable tracer in

Scheme



order to validate earlier studies and allow for comparison to structurally diverse inhibitors.

The procedure used to synthesize [3-^{3}H] methyl (3*R**,5*S**)-(E)-3,5-dihydroxy-9,9-diphenyl-6,8-nonadienoate (7) is shown in the Scheme. Wadsworth-Emmons reaction[6] of triethyl phosphonoacetate with β-phenylcinnamaldehyde (2) provided

the homologated ester (3). Reduction to the corresponding alcohol (4) followed by Swern oxidation[7] gave 5,5-diphenyl-2,4-pentadienal (5). Addition of the dianion of methyl acetoacetate to aldehyde 5 under carefully controlled conditions gave ketoester 6, the precursor for the diastereoselective reduction.

The diastereomeric reduction to introduce the tritium at C_3 essentially follows a published modification[8] of the original Narasaka procedure.[9] In hopes of avoiding possible tritium gas release during acidic workup, we investigated workup under basic conditions for this diastereoselective reduction using unlabelled sodium borohydride. Unfortunately, such a procedure led to complete decomposition of the desired product. Since we were confined to acidic workup conditions, we took steps to maximize the incorporation of tritium into our compound, while at the same time minimizing tritium gas release. We allowed labelled sodium borohydride to react completely with ketoester 6 before finishing the reaction with unlabelled sodium borohydride. As a final precaution, excess borohydride was decomposed with acetone followed by hydrogen peroxide prior to acidic workup. The reduction of ketoester 6 proceeded so cleanly that the desired product 7 did not need to be purified. As a crude product, 7 was homogeneous by TLC, and >95% radiochemically pure. The radiochemical yield was 7.5% and the specific activity was 13.3 mCi/mmole.

In summary, we achieved a diastereoselective introduction of tritium into HMG-CoA reductase inhibitor, 1. Furthermore, this labelled inhibitor (7) has been successfully utilized to probe tissue selectivity, and these studies will be reported elsewhere.[10] The synthetic methodology described in this communication is suitable for the preparation of other tritiated members of the class of (3*R**,5*S**)-3,5-dihydroalkanoate HMG CoA reductase inhibitors (e.g. compactin, mevinolin),[11] thereby allowing tissue distribution and specificity to be determined.

EXPERIMENTAL

The synthesis of [3-^{3}H] methyl (3*R**,5*S**)-(E)-3,5-dihydroxy-9,9-diphenyl-6,8-nonadienoate (7) was achieved using tritium labelled sodium borohydride purchased from New England Nuclear. The synthesis was fully explored using

unlabelled material prior to the radiolabelled synthesis. Tetrahydrofuran was distilled from sodium-benzophenone ketal immediately prior to use, and methylene chloride was distilled from calcium hydride and stored over 4A molecular sieves. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Radiochemical purities were determined using a Berthold L2842 TLC Linear Analyzer. ^{1}H and ^{13}C NMR spectra were recorded on either a Varian XL-300 FT-NMR Spectrometer or a Bruker WM-250 FT-NMR Spectrometer. Infrared spectra were recorded on a Perkin-Elmer 283B Infrared Spectrometer. Exact masses were determined using an AEI-MS30 Mass Spectrometer. Elemental analyses were determined by Pfizer's Central Research Analytical Department.

Ethyl (E)-5,5-Diphenyl-2,4-pentadienoate (3): A 60% dispersion of sodium hydride (3.0 g of dispersion, 1.8 g of NaH, 75 mmole) was washed with anhydrous THF (3 X 60 ml) and suspended in anhydrous THF (200 ml). The mixture was cooled to 0°C and triethyl phosphonoacetate (15.99 g, 71.3 mmole) was added dropwise via syringe. While stirring at 0°C for 1 h, the suspension changed to a clear solution. 3-Phenylcinnamaldehyde (9.9 g, 47.5 mmole) was added dropwise and the resulting mixture was then stirred for 1 h at 0°C. The reaction was quenched by pouring the mixture into water (150 ml). The phases were separated and the aqueous phase was extracted with ether (3 X 50 ml). The combined organic phases were washed with water (2 X 50 ml), dried with anhydrous magnesium sulfate, filtered and concentrated *in vacuo* to give 13.12 g (99% crude yield) of ethyl (E)-5,5-diphenyl-2,4-pentadienoate (3). A sample from this lot (0.350 g) was purified by PTLC (2:3 ether:hexane) to give 0.290 g (83% recovery) of pure product. High resolution mass spectra: m/e found 278.1276, calc. for $C_{19}H_{18}O_2$ 278.1307. ^{1}H NMR ($CDCl_3$), δ: 7.44-7.20 (m, 11 H); 6.78 (d, 12 Hz, 1 H); 6.04 (d, 15 Hz, 1 H); 4.08 (q, 7 Hz, 2 H); 1.14 (t, 7 Hz, 3 H). ^{13}C NMR ($CDCl_3$), δ: 167.1, 150.8, 142.2, 141.4, 138.6, 130.4, 128.7, 128.1, 125.4, 122.4, 60.2, 14.3. IR ($CHCl_3$) cm $^{-1}$: 3046, 2976, 1697, 1616, 1270.

(E)-5,5-Diphenyl-2,4-pentadien-1-ol (4): To a -40°C solution of ethyl (E)-5,5-diphenyl-2,4-pentadienoate (3, 12.8 g, 46 mmole) in anhydrous THF (250 ml) was added 1M diisobutylaluminum hydride in hexanes (122.3 ml, 122.3 mmole, 2.6 equiv) dropwise via syringe. The reaction mixture was stirred at -40°C for

2 h, then cautiously quenched by dropwise addition of ethanol (22 ml). The cooling bath was removed and saturated aqueous sodium chloride (45 ml) was added. The resulting mixture was diluted with ether (450 ml) and stirred at RT for 1 h. A gelatinous solid formed during this period. The mixture was dried with anhydrous magnesium sulfate, filtered through celite, and concentrated in vacuo to give 13.1 g of crude product. Purification by flash chromatography (1:4 ethyl acetate:hexane) gave 9.2 g (85% yield) of (E)-5,5-diphenyl-2,4-pentadien-1-ol (4) which was sufficiently pure to be used in the next step. A sample of this lot (0.300 g) was further purified by PTLC (1:2 ether:hexane) to give 0.276 g (92% recovery) of material which gave the following spectral data. High resolution mass spectra: m/e found 236.1216, calc. for $C_{17}H_{16}O$ 236.1201. ^1H NMR ($CDCl_3$), δ: 7.40-7.20 (m, 10 H); 6.85 (d, 12 Hz, 1 H); 6.35 (ddt, 15, 12 & 1 Hz, 1 H); 6.00 (dt, 15 & 7 Hz, 1 H); 4.17 (ddd, 7, 7 & 1 Hz, 2 H); 1.68 (t, 7 Hz, 1 H). ^{13}C NMR ($CDCl_3$), δ: 143.2, 139.5, 133.7, 130.4, 129.6, 128.2, 128.1, 127.5, 127.4, 127.0, 63.6. IR ($CHCl_3$) cm $^{-1}$: 3667, 3592, 3033, 2990, 2920, 1600.

(E)-5,5-Diphenyl-2,4-pentadienal (5): To a -78°C solution of oxalyl chloride (5.94 g, 46.8 mmole) in anhydrous methylene chloride (100 ml) was added dimethyl sulfoxide (7.03 g, 90 mmole) dropwise. After stirring at -78°C for 30 min, this mixture was addod via cannula to a -78°C solution of (E)-5,5-diphenyl-2,4-pentadien-1-ol (4, 8.70 g, 36.8 mmole) in anhydrous methylene chloride (100 ml). After stirring at -78°C for 3 h, triethylamine (29 ml, 205 mmole) was added and the resulting mixture was stirred for an additional 30 min. The mixture was allowed to warm to RT and diluted with 1:4 methylene chloride:hexane (600 ml). The resulting mixture was washed with 10% aqueous sodium bisulfate (4 X 200 ml) and water (2 X 100 ml), dried with sodium sulfate, filtered and concentrated in vacuo to give 8.85 g of crude product contaminated with an unidentified less polar material. Purification by flash chromatography (1:4 ethyl acetate:hexane) gave 3.98 g (46% yield) of (E)-5,5-diphenyl-2,4-pentadienal (5) as a yellow solid, mp 71-72°C. High resolution mass spectra: m/e found 234.1041, calc. for $C_{17}H_{14}O$ 234.1045. ^1H NMR ($CDCl_3$), δ: 9.46 (d, 9 Hz, 1 H); 7.42-7.20 (m, 10 H); 7.20-7.17 (dd, 15 & 12 Hz, 1 H); 6.94 (d, 12 Hz, 1 H); 6.30 (dd, 15 & 9 Hz, 1 H). ^{13}C NMR ($CDCl_3$), δ: 193.9, 153.2, 149.8, 140.9, 138.4, 132.5, 130.5, 129.8, 129.3, 128.8, 128.7, 128.4, 128.2, 127.6, 127.5, 127.4, 127.3. IR ($CHCl_3$) cm $^{-1}$: 3000, 1720, 1598, 1205.

Methyl (E)-5-Hydroxy-9,9-diphenyl-3-oxo-6,8-nonadienoate (6): A 60% dispersion of sodium hydride (0.095 g of dispersion, 0.057 g of NaH, 2.4 mmole) was triturated with anhydrous THF (3 X 20 ml). The pure sodium hydride was then suspended in anhydrous THF (10 ml) and cooled to 0°C. Methyl acetoacetate (0.227 g, 1.95 mmole) was added dropwise. After stirring the resulting colorless solution for 10 min at 0°C, n-butyl lithium in hexanes (0.96 ml, 2.5 M, 2.4 mmole) was added dropwise via syringe. After stirring the reaction mixture for another 10 min at 0°C, a solution of 5,5-diphenyl-2,4-pentadienal (5, 0.410 g, 1.75 mmole) in anhydrous THF (10 ml) was added slowly via syringe. After completion of the addition, the reaction was stirred for 10 min at 0°C. The reaction mixture was then quenched by pouring it into saturated aqueous ammonium chloride. After stirring vigorously for 5 min, the phases were separated and the aqueous phase was extracted with ether (3 X 25 ml). The combined organic phases were washed with saturated aqueous sodium bicarbonate (40 ml) and water (2 X 40 ml), dried with anhydrous magnesium sulfate and concentrated in vacuo to give 0.610 g (98% yield) of methyl (E)-5-hydroxy-9,9-diphenyl-3-oxo-6,8-nonadienoate (6) as a viscous liquid. Since this material was chromatographically homogenous and is known to be unstable on silica, it was carried onto the next step without purification. Another lot was purified by PTLC (1:2 ethyl acetate:hexane, 10% recovery) and gave the following spectral data: High resolution mass spectra: m/e 350.1554, calc. for $C_{22}H_{22}O_4$ 350.1518. ^1H NMR ($CDCl_3$), δ: 7.40-7.15 (m, 10H); 6.65 (d, 12 Hz, 1 H); 6.50 (dd, 16 & 12 Hz, 1 H); 5.90 (dd, 16 & 6.6 Hz, 1 H); 4.65 (ddd, 6.6, 6.0 & 1 Hz, 1 H); 3.70 (s, 3 H); 3.45 (s, 2 H); 2.77 (dd, 15 & 6.0 Hz, 1 H); 2.73 (dd, 15 & 1 Hz, 1 H). ^{13}C NMR ($CDCl_3$), δ: 202.4, 167.3, 143.1, 139.5, 135.0, 130.4, 129.6, 129.1, 128.3, 127.6, 126.8, 68.5, 52.5, 49.8, 49.6. IR ($CHCl_3$) cm^{-1}: 3580, 3019, 2949, 1748, 1714, 1629, 1491, 1363.

Methyl (3*R**,5*S**)-(E)-[3-^3H]3,5-Dihydroxy-9,9-diphenyl-6,8-nonadienoate (7): To a RT solution of (E)-5-hydroxy-9,9-diphenyl-3-oxo-6,8-nonadienoic acid (6, 0.055 g, 15.7 mmoles) in anhydrous THF (2 ml) was added triethylborane (0.220 ml of 1.0 M solution in THF, 22.0 mmoles) and pivalic acid (0.009 g, 0.09 mmole) under nitrogen. The pale yellow solution was stirred at RT under nitrogen for 2 h. The resulting complex was cooled to -90°C and tritium-labelled sodium borohydride (100 mCi, 15 Ci/mmole, 0.25 mg, 0.0067 mmole) and methanol (1 drop)

were added. After stirring an additional 30 min, sodium borohydride (0.026 g, 0.70 mmoles, approx. 100-fold dilution) and methanol (1 ml) were added. After stirring for 30 min at -90°C, acetone (1 ml, 13.6 mmole) was added and the reaction stirred for 30 min. At this point, buffered, aqueous hydrogen peroxide (0.5 ml of 30% H_2O_2 mixed with 0.5 ml of saturated aqueous sodium bicarbonate) was added and the cooling bath was removed. After stirring for 45 min at RT, isopropanol (1 ml) and ethyl acetate (5 ml) were added. The resulting mixture was washed with saturated aqueous ammonium chloride (2 X 5 ml) and brine (2 X 5 ml), dried over anhydrous magnesium sulfate, filtered through fiuted filter paper and concentrated. The resulting oil was redissolved in chloroform (5 ml, previously passed through alumina) and reconcentrated to give 0.050 g (90% chemical yield, 7.5% radiochemical yield, 13.3 mCi/mmole specific activity) of [3-^{3}H] methyl (3*R**,5*S**)-(E)-[3-^{3}H]3,5-dihydroxy-9,9-diphenyl-6,8-nonadienoate (7), which did not require any further purification. This material co-migrated with an authentic sample of methyl (3*R**,5*S**)-(E)-[3-^{3}H]3,5-dihydroxy-9,9-diphenyl-6,8-nonadienoate (1), m.p. 87-89°C. High resolution mass spectra: m/e 352.1662, calc. for $C_{22}H_{24}O_4$ 352.1674. ^{1}H NMR($CDCl_3$), δ: 7.4-7.1 (m, 10 H); 6.64 (d, 12 Hz, 1 H); 6.30 (dd, 15 & 12 Hz, 1 H); 5.85 (dd, 15 & 6.6 Hz, 1 H); 4.35 (m, 1 H); 4.22 (m, 1 H); 3.82 (s, 1 H); 3.65 (s, 3 H); 3.32 (s, 1 H); 2.52 (dd, 5 & 15 Hz, 1 H); 2.43 (dd, 2 & 15 Hz, 1H); 1.65 (m, 2 H). ^{13}C NMR ($CDCl_3$), δ: 172.8, 143.4, 142.1, 137.7, 130.3, 128.5, 128.2, 128.1, 127.9, 127.8, 127.6, 127.5, 127.0, 72.6, 68.3, 51.8, 42.5, 41.5. IR ($CHCl_3$) cm^{-1}: 3330, 3040, 1745, 1595, 1495. By use of an unselective sodium borohydride reduction, a 1.4:1.0 mixture of the 3*R**,5*S**:3*R**,5*R** products was obtained. After repeated flash chromatography, small samples enriched in (3*R**,5*R**) isomer of 1 could be obtained. This diastereomer gave rise to several new ^{1}H NMR peaks, but the following were diagnostic for the presence of this compound: 6.62 (d, 12 Hz, 1 H); 6.23 (dd, 15 & 12 Hz, 1 H); 5.65 (dd, 15 & Hz, 1 H).

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