Synthesis of Carbon-14 Labeled Fluvastatin (Lescol®)

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Summary: [R*, S*]-(±)-7-[3-(4-Fluorophenyl)-1-(1-methylethyl)-1*H*-indol-2-yl-3-^{14}C]-3,5-dihydroxy -6-heptenoic acid, sodium salt (fluvastatin, **4**) was prepared from [^{14}C] bromoacetyl chloride in a six step synthesis with an overall radiochemical yield of 13.2%. This synthetic route was chosen because it puts the label in the metabolically stable 3-position of the indole ring.

Introduction

Coronary heart disease (CHD) continues to be a major health issue in developed countries. A significant link has been established between high blood sereum levels of total cholesterol (Low Density Lipoprotein Cholesterol, LDL-C, and Very Low Density Lipoprotein Cholesterol, VLDL-C) and CHD[2]. For patients that are unresponsive to dietary interventions, the treatment of choice has become therapeutic agents that are inhibitors of the enzyme β-Hydroxy-β-Methyl-Glutaryl-CoA reductase (HMG-CoA reductase, EC 1.1.1.34), which controls an early step in cholesterol biosynthesis in humans[3]. One of these pharmaceuticals is fluvastatin[4] (XU 62-320 na, Lescol®), **1**, the first totally synthetic "statin" to reach market. In this report, we discuss the synthesis of a carbon-14 labeled isotopomer that was used in ADME (Adsorption, Distribution, Metabolism, and Excretion) studies.

Results and Discussion

A preliminary synthesis of carbon-14 labeled fluvastatin was undertaken wherein the label was situated in the 3-position of the 3,5-dihydroxyhept-6-enoate side chain, giving compound **2**.

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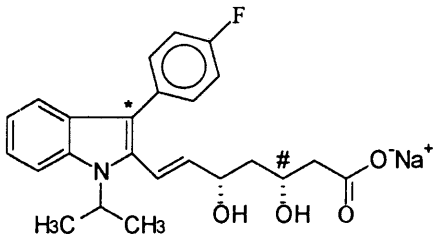
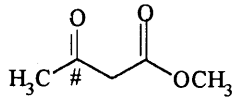


1, *,# =^{12}C; 2_ * =^{12}C, # =^{14}C; 4 * =^{14}C,# =^{12}C

While this synthesis[5] was readily accomplished using 3-oxobutanoic acid-3-^{14}C methyl ester, **3**[6],



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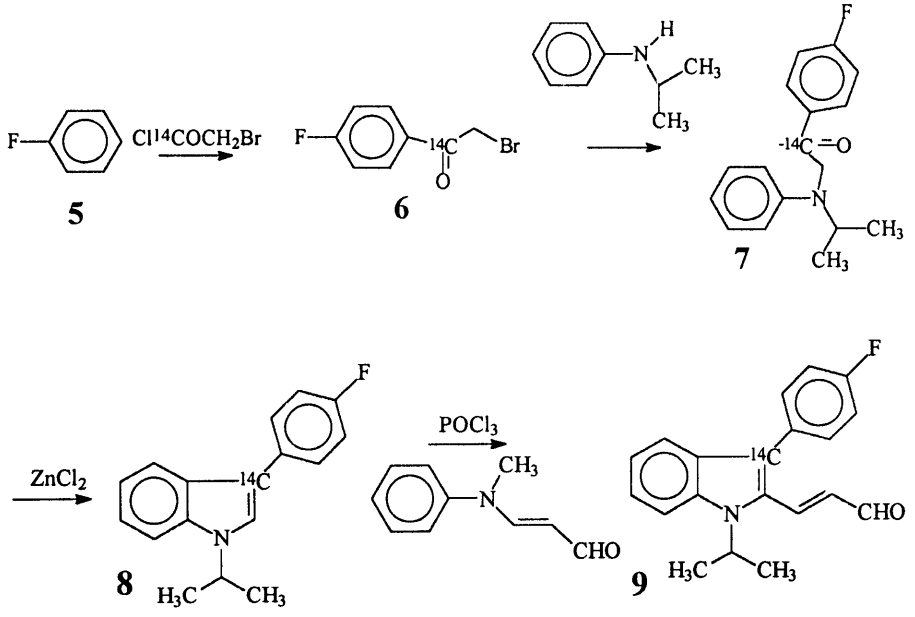
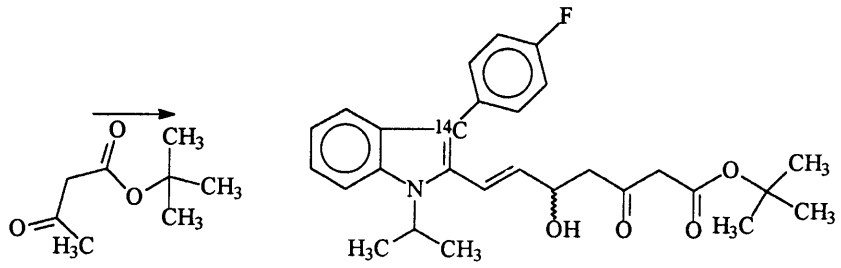
= indicates location of label

the labeled site in the drug substance proved to be biologically unstable. To circumvent this problem, it was decided to bury the label deep within the indole moiety to protect it from metabolic attack. Thus the synthesis of compound **4** was undertaken. The path followed in the construction of this material is shown below.





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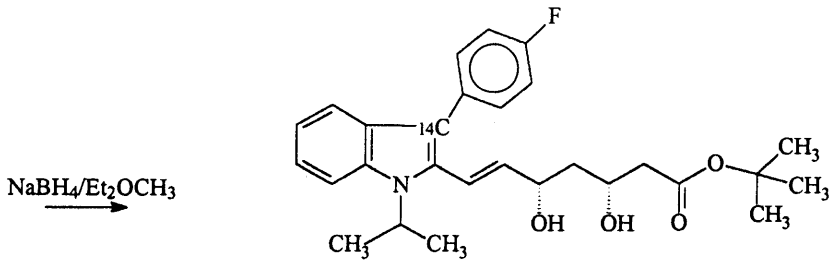
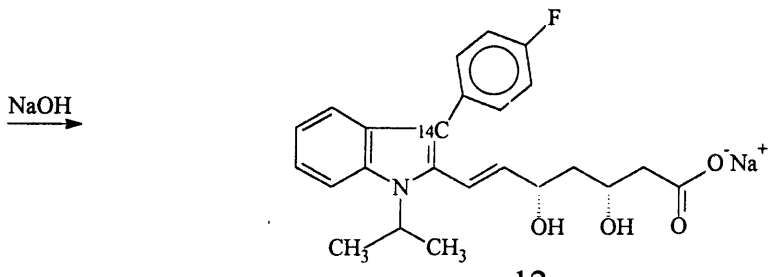


11* For sake of clarity, only one of the two syn-enantiomers is shown



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The methodology chosen was adapted from a "cold" synthesis of fluvastatin. Thus bromoacetyl-1-[^{14}C]-chloride[7] was reacted with fluorobenzene, **5**, under Friedel-Craft[8] conditions to give exclusively the *para*-substituted fluoro ethanone **6** in 85% radiochemical yield. It was imperative that the starting bromo-acid chloride was completely free of elemental bromine, as this led to indiscriminate bromination of the aromatic ring under the reaction conditions. These brominated compounds proved difficult to separate at this point or other advanced stages. The purified ethanone **6** was reacted with two equivalents of isopropylaniline, yielding the phenyl-amino intermediate **7**. Interestingly, this material could not be converted to the indole **8** in greater than 50% yield in a one-pot operation, regardless

of the amount of zinc chloride used. This is in contrast to the cold synthesis, where the one-pot procedure affords the corresponding indole in greater than 90% yield. Fortunately, it was discovered that if the excess isopropylaniline was removed prior to introduction of zinc chloride, the conversion of **6** to indole **8** proceeded in 58% overall radiochemical yield. The subsequent vinylogous Villsmeyer homologation[9] proceeded without incident to give **9** in 80% radiochemical yield. It is advisable to use *N*-phenyl-*N*-methylaminoacrolein of high purity to obtain maximal yields. The aldol condensation of aldehyde **9** with the dianion of *t*-butyl acetoacetate proved to be very sensitive to the stoichiometry of NaH, *n*-BuLi and substrate and careful titration of the reactants was required to optimize the reaction at 71% radiochemical yield. *Syn*-reduction of the keto-alcohol **10** was performed according to the method of Prasad *et al*[10] to give the diol **11** in 86% radiochemical yield. Final saponification afforded the labeled drug substance in 91% radiochemical yield. The overall radiochemical yeild from [^{14}C] bromoacetyl chloride was 13.2%

Experimental

Bromoacetyl-1-^{14}C-chloride was purchased from American Radiclabeled Chemicals, Inc. Chemical ionization mass spectroscopy was performed on a Finnigan 4600 mass spectrometer utilizing isobutane as the reagent gas. Radio-TLC chromatograms were conducted on 5 x 20 cm E. Merck silica gel F-254 plates (250 micron thickness). Radiochemical purities were determined by scanning the chromatograms for radioactivity with a Vanguard gas proportional scanner with a 1 mm x 10 mm collimator, as well as radio-HPLC. Identities of intermediates were determined by comparative TLC and HPLC versus non-labeled standards that were identified by mass spectroscopy as well as 300 or 500 MHz ^{1}H-NMR and 75 or 125 MHz ^{13}C-NMR spectroscopy. Specific activities were determined by the "weight-in-volume" assay.

2-Bromo-(4-fluorophenyl)-ethanone-1-[^{14}C], 6

A 100-mL 3-neck round bottomed flask equipped with a magnetic stirring bar, thermometer, dropping funnel and reflux condensor was charged with 1.33 g of fluorobenzene, **5**, and 466.7 mg of anhydrous aluminum chloride. The reaction mixture was heated to 75 ^{0}C and to it was added a solution of bromoacetyl-1-[^{14}C]-chloride (112.8 mCi, *ca.* 55 mCi/mmol) in 1 g of fluorobenzene. The resultant mixture was refluxed at 80 ^{0}C for one hour. At this point, the reaction mixture was cooled to 0^{0}C and quenched by the addition of 5 mL of 6N HCl

solution. The organic layer was separated and washed with three 10 mL portions of brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure to give 96 mCi of **6** that was used in the next step without further manipulation. TLC: 3% ethyl acetate in hexane.

3-(4-Fluorophenyl)-1-(1-methylethyl)-1*H*-indole-3-[^{14}C], 8

Under a blanket of nitrogen, 403.5 mg (3.42 mmol) of *N*-isopropylaniline, 96 mCi of 2-bromo-(4-fluorophenyl)-ethanone-1-[^{14}C], **6**, and 15 mL of absolute ethanol were combined and heated at 78 ^{0}C for one hour. Solvent was distilled off and the resultant residue was eluted through silica gel (20% CH_2Cl_2 in CCl_4) to remove excess *N*-isopropylaniline. The isolated intermediate, 1-(4-fluorophenyl)-2-[(1-methylethyl)phenylamino]-ethanone-1-[^{14}C], **7**, was immediately dissolved in 15 mL absolute ethanol. To this was added 466.1 mg (3.42 mmol) of $ZnCl_2$ and the solution was refluxed for five hours. The mixture was allowed to cool to room temperature and concentrated under reduced pressure. The residue was chromatographed on silica gel (20% CH_2Cl_2 in CCl_4) to give 56 mCi of the title compound. The specific activity of this material was adjusted to approximately 28 mCi/mmol by co-crystallization with 253 mg of "cold" **8**.

3-[3-(4-Fluorophenyl)-1-(1-methylethyl)-1*H*-indol-2-yl-3-^{14}C]-2-propenal, 9

Under a nitrogen atmosphere, a solution of *N*-methyl-*N*-phenyl-3-aminoacrolein (942 mg, 5 mmol) in 1 mL of acetonitrile was added over a 45 minute period to a solution of phosphorus oxychloride (908 mg, 5.92 mmol) in 2 mL of acetonitrile at -5 ^{0}C. After the addition was completed, a solution of 3-(4-fluorophenyl)-1-(1-methylethyl)-1*H*-indole-3-[^{14}C], **8**, (457 mg, 56 mCi, 2 mmol) in 5 mL of acetonitrile was injected rapidly into the mixture and the reaction contents were heated at reflux for three hours. The mixture was allowed to cool to room temperature and quenched by the addition of 5 mL of water. The solution was warmed to 55 ^{0}C for two hours and then cooled to 15 ^{0}C and diluted with 20 mL of toluene. The organic layer was separated and washed with two 50 mL portions of water, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The oily residue was purified by silica gel chromatography (1:1 CH_2Cl_2 : CCl_4). The fractions of interest were pooled and evaporated, and the residue was crystallized from isopropanol to provide 45 mCi (80% radiochemical yield, 503 mg) of 3-[3-(4-fluorophenyl)-1-(1-methylethyl)-1*H*-indol-2-yl-3-^{14}C]-2-propenal, **9**, as pale-yellow crystals that behaved in identical fashion as the unlabeled standard in TLC and HPLC chromatograms.

3-[3-(4-Fluorophenyl)-1-(1-methylethyl)-1*H*-indol-2-yl-3-^{14}C]-5-hydroxy-3-oxo-6-heptenoic acid, *t*-butyl ester, 10

Under anhydrous conditions, 128.0 mg (3.2 mmol) of sodium hydride (60 % dispersion in mineral oil) was added to 1 mL of dry THF at -15 ^{0}C. To this was added a solution of *t*-butyl acetoacetate (474.6 mg, 3 mmol) in 0.5 mL of THF at such a rate that the temperature was maintained at -15 ^{0}C. After stirring for 1 hour at this temperature, 1.8 mL (3 mmol) of a 1.6 M solution of n-BuLi was added, again at such a rate that the temperature was maintained at -15 ^{0}C. After the addition was completed, a solution of 3-[3-(4-fluorophenyl)-1-(1-methylethyl)-1*H*-indol-2-yl-3-^{14}C]-2-propenal, **9**, (503 mg, 45 mCi) in 2 mL of THF was added. The resultant mixture was stirred at -10 ^{0}C for 30 minutes. The reaction was quenched by the addition of 0.5 mL of conc. HCl in 3 mL of water. The organic phase was separated and the aqueous phase was extracted with two 25 mL portions of a 1:1 mixture of toluene/ ethyl acetate. The combined organic phases were washed with brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was chromatographed on silica gel (3-10% ethyl acetate/hexane) to give 479 mg, 32 mCi (71% radiochemical yield) of the title compound as a pale-yellow powder that behaved in identical fashion as the unlabeled standard in TLC and HPLC chromatograms.

[R*, S*]-(±)-7-[3-(4-Fluorophenyl)-1-(1-methylethyl)-1*H*-indol-2-yl-3-^{14}C]-3,5-dihydroxy-6-heptenoic acid, *t*-butyl ester, 11

To a stirred solution of $NaBH_4$ (116.4 mg, 3.08 mmol) in 1.2 mL of THF and 0.33 mL of methanol at -78 ^{0}C was added 0.25 mL of a 50% (5 M) solution of diethylmethoxyborane in THF. The resultant solution was allowed to stir at this temperature for 10 minutes and to it was then added a solution of 3-[3-(4-fluorophenyl)-1-(1-methylethyl)-1*H*-indol-2-yl-3-^{14}C]-5-hydroxy-3-oxo-6-heptenoic acid, *t*-butyl ester, **10**, (479 mg, 32 mCi) in a mixture of 0.05 mL of methanol in 0.21 mL of THF. The reaction mixture was stirred at this temperature for three hours and quenched by the addition of a mixture consisting of 5 mL of saturated $NaHCO_3$ solution, 15 mL of ethyl acetate and 5 mL of water. The organic phase was removed and washed with 20 mL of brine and dried over anhydrous sodium sulfate. After concentration, the residue was taken up in 25 mL of ethyl acetate and stirred with 1 ml of 30 % hydrogen peroxide solution for 90 minutes at room temperature. The reaction vessel contents were transferred to a separatory funnel and washed with 5 mL of saturated $NaHCO_3$ solution, followed by 20 mL of water. The separated organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure to give a powdery yellow substance that was purified by silica gel chromatography (15-30 % ethyl acetate : hexane). The residue was crystallized from 3% isopropanol-hexane to give 27.5 mCi (86 % radiochemical yield) of the diol **11** as white crystals that behaved in identical fashion as the unlabeled standard in TLC and HPLC chromatograms.

[R*, S*]-(±)-7-[3-(4-Fluorophenyl)-1-(1-methylethyl)-1*H*-indol-2-yl-3-^{14}C]-3,5-dihydroxy -6-heptenoic acid, sodium salt, 4

[R*,S*]-(±)-7-[3-(4-Fluorophenyl)-1-(1-methylethyl)-1*H*-indol-2-yl-3-^{14}C]-3,5-dihydroxy-6-heptenoic acid, *t*-butyl ester, **11**, (250 mg, 16.25 mCi) was dissolved in 20 mL of methanol and to it was added 0.526 mL of 0.95 N NaOH solution. The mixture was stirred at room temperature for two hours. At this point the mixture was concentrated under reduced pressure and the residue was taken up in 15 mL of water. The aqueous solution was partitioned with 15 mL of methyl *t*-butyl ether and stirred vigorously for 10 minutes. The layers were separated and the aqueous phase was subjected to this procedure three more times, followed by lyophilization for 72 hours under high vacnum to afford 208 mg, 14.93 mCi (91% radiochemical yield) of a very pale-yellow powder of the title compound that showed identical behavior as unlabeled standard in TLC and HPLC systems.

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