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Synthesis of [^{14}C]Cerivastatin

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Summary

The title compound [^{14}C]Cerivastatin ([^{14}C]BAY w 6228) was synthesized in order to introduce the label into the metabolically stable 7-position of the side chain. Convergent synthesis was performed using a chiral aldehyde for alkene formation. Starting from [^{14}C]carbon dioxide and a suitable pyridine bromide the labelled phosphonate derivative was obtained in 5 steps.

The synthesis was completed in 4 additional steps and the radiochemical yield amounted to 10 %.

Key words

Sodium (E)-(3R,5S)-7-{4-(4-fluorophenyl)-2,6-diisopropyl-5-methoxymethyl-pyrid-3-yl}-3,5-dihydroxy-[7-^{14}C]hept-6-enoate, [^{14}C]Cerivastatin

Introduction

Cerivastatin (BAY w 6228) is a synthetic, pure enantiomer of the class of pyridines that specifically inhibits HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis [1, 2, 3]. For studies on pharmacokinetics and metabolism the carbon-14 labelled substance was necessary. This paper describes the synthesis of sodium (E)-(3R,5S)-7-{4-(4-fluorophenyl)-2,6-diisopropyl-5-methoxymethyl-pyrid-3-yl}-3,5-dihydroxy-[7-^{14}C]hept-6-enoate ([^{14}C]Cerivastatin, 1) and conditions suitable for purification and storage.

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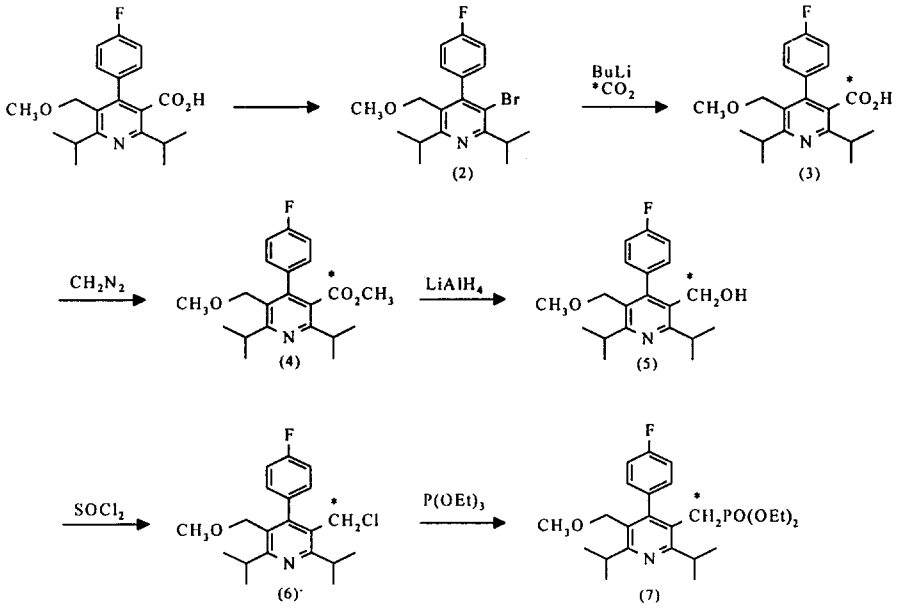
Results and discussion

The 7-position of the side chain was chosen for labelling with carbon-14. This position held several advantages. First, the label was placed on a metabolically stable carbon adjacent to the pyridine ring. Second, the labelled carbon could be introduced using [^{14}C]carbon dioxide (easily generated from barium [^{14}C]carbonate), which is inexpensive and readily available. Third, the precursor for introducing the label was obtained by degradation of a suitable intermediate. Therefore, no further synthetic steps with labelled material were necessary to obtain the highly substituted pyridine. Fourth, by using a convergent synthesis, the side chain containing both chiral centers was coupled in one step. Olefination was accomplished with an aldehyde derived from the chiral pool. The complete synthesis is summarized in schemes 1 - 2.

The non-labelled starting material was readily available by Hunsdiecker-type bromo-decarboxylation of the carboxylic acid [4, 5]. The carboxylic acid (3) was prepared easily by permanganate oxidation of the corresponding aldehyde [3] and was treated with thionyl chloride to yield the activated acid chloride. The carboxylic ester derived from N-hydroxy-pyridine-2-thione reacted with bromotrichloromethane in a radical chain reaction to give the arylbromide (2) in 50 - 60 % yield. An improvement with respect to yield and purity of (2) was obtained by use of a tungsten lamp to form radicals instead of high temperature (140 °C) and presence of AIBN as radical initiator. The preparation of [^{14}C]carboxylic acid (3) was achieved in 75 - 95 % yield by treatment of the arylbromide (2) with n-butyllithium at -73 °C, followed by carboxylation with [^{14}C]carbon dioxide generated by the action of conc. sulfuric acid on barium [^{14}C]carbonate.

Scheme 1: Synthesis of labelled phosphonate (7)

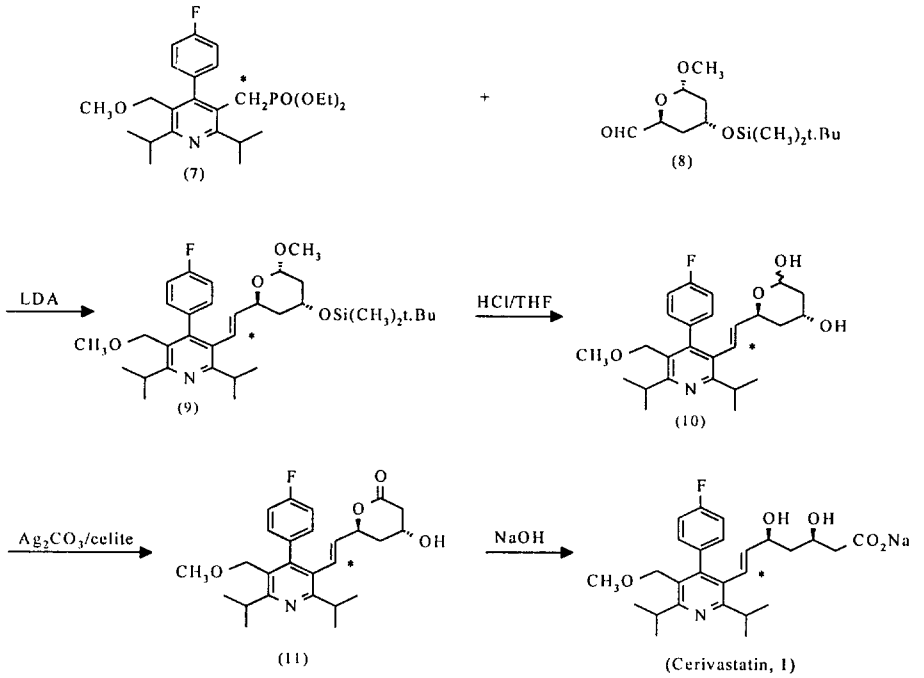


The ester (4) was formed quantitatively using diazomethane in ether, and reduction with lithium aluminium hydride furnished alcohol (5). The alcohol was converted to the arylmethylchloride (6) using thionyl chloride. The last 3 steps were performed without further purification of raw materials. The phosphonate (7) was prepared for use as reactant in a highly stereoselective Horner olefination with aldehyde (8) (scheme 2).

The arylmethylchloride (6) was heated at reflux in triethylphosphite to give after purification by column chromatography the desired phosphonate (7) in 87 % yield. The aldehyde (8) was derived from tri-O-acetyl-D-glucal by an 8 step synthesis according to literature procedures [6, 7, 8]. The enantiomerically pure aldehyde (8) was obtained in approx. 16 % yield.

The phosphonate (7) was transformed into the olefin (9) following formation of the carbanion with freshly prepared lithium diisopropylamide and addition of the aldehyde (8). After chromatography on silica gel the olefin (9) was obtained in 59 % yield. Cleavage of the protective groups was accomplished by heating the olefin (9) in tetrahydrofuran and aqueous hydrochloric acid. The resulting epimeric mixture of hemiacetals (10) was directly oxidized with silver carbonate on celite in refluxing toluene [6, 7, 8]. A loss of radioactive material was observed due to irreversible absorption on celite containing the silver salts. The lactone (11) was purified by recrystallization or chromatography on RP-18 material. In total an amount of 3141 MBq (84.9 mCi) [^{14}C]lactone (11) was available corresponding to a radiochemical yield of 12.7 % calculated on employed barium [^{14}C]carbonate as starting material. Saponification of lactone (11) to [^{14}C]Cerivastatin was achieved with aqueous sodium hydroxide in acetonitrile and purification was performed on Bond Elut® cartridges and by preparative HPLC on RP-18 material.

Scheme 2: Synthesis of [^{14}C]Cerivastatin (1) using chiral aldehyde (8).



Solutions of [^{14}C]Cerivastatin in mixtures of water/acetonitrile are stable for several months at 4 °C. If the labelled material is stored as solid or oily residue after evaporation, large amounts of the specific autoradiolysis product (sodium (E)-(3R)-7-{4-(4-fluorophenyl)-2,6-diisopropyl-5-methoxymethyl-pyrid-3-yl}-3-hydroxy-5-oxo-hept-6-enoate) were observed. This oxidation of the allylic alcohol to the corresponding ketone occurred within weeks.

Experimental

3-Bromo-4-(4-fluorophenyl)-2,6-diisopropyl-5-methoxymethyl pyridine (2)

A solution of 2.068 g (6.0 mmol) of non-labelled pyridine carboxylic acid (3) in 40 ml dichloromethane was treated with 2.0 ml (27.4 mmol) thionyl chloride and stirred at ambient temperature for 1 h. After addition of further 0.5 ml (5.6 mmol) thionyl chloride, stirring of the mixture was continued for 0.5 h. The solvent was evaporated in vacuo and the residue was rinsed twice with benzene and evaporation was repeated to remove traces of thionyl chloride and hydrochloric acid completely.

The acid chloride was obtained as a light yellow solid and dissolved in 20 ml bromotrichloromethane. This solution was added slowly (15 min) under nitrogen to a mixture of 1.06 g (7.1 mmol) sodium 2-mercaptopyridine-N-oxide and 80 mg (0.65 mmol) p-dimethylaminopyridine in 40 ml refluxing bromotrichloromethane. Irradiation of the reaction mixture with a simple 180 W tungsten lamp and simultaneous heating at reflux was performed for 2.5 h. The mixture was filtered and the filtercake rinsed with dichloromethane. The solvents were removed by vacuum distillation at 60 °C. The residue was purified by column chromatography on Lobar® C Si60 (Merck, Darmstadt, FRG) with n-hexane/dichloromethane 5:2 as eluent, a flow of 5 ml/min and UV detection at 285 nm. The appropriate fractions were combined and after evaporation a colorless oil remained. Yield: 1.26 g (3.3 mmol) = 55.0 %. Purity: 95 % (determined by GC).

Synthesis of sodium (E)-(3R,5S)-7-{4-(4-fluorophenyl)-2,6-diisopropyl-5-methoxymethyl-pyrid-3-yl}-3,5-dihydroxy-[7-^{14}C]hept-6-enoate ([^{14}C]Cerivastatin, 1) using chiral aldehyde (8)

4-(4-Fluorophenyl)-2,6-diisopropyl-5-methoxymethyl pyridine-[3-^{14}C]carboxylic acid (3)

A solution of 2.375 g (6.25 mmol) arylbromide (2) in 20 ml dry ether was cooled to -73 °C and treated with 4.64 ml n-butyllithium (1.6 M solution in hexane). The resultant mixture was stirred at -73 °C for 30 min and then frozen at -196 °C. The apparatus was connected to a high vacuum line and completely evacuated. [^{14}C]Carbon dioxide was generated from 1235 mg (6.26 mmol) barium[^{14}C]carbonate with a specific activity of 1990.6 MBq/mmol (53.8 mCi/mmol) by addition of 20 ml conc. sulfuric acid and condensed on the surface of the frozen reaction mixture containing the lithium organic compound. The mixture was stirred for 1 h at -73 °C and stirring was continued for 1 h, during which temperature increased from -73 °C to 0 °C. After additional stirring for 0.5 h at 0 °C 30 ml ether and 18 ml 2N sulfuric acid were added. The layers were separated and the aqueous layer was extracted 4 times with

15 ml ether, each. The organic layers were combined and extracted 4 times with 8 ml 1N sodium hydroxide, each. Acidification of this fraction using 22 ml 1N hydrochloric acid gave the carboxylic acid (3), which was extracted again into ether. The organic layer was dried over sodium sulfate and evaporated to dryness to give (3) as a slightly sticky, white solid. Yield: 1.92 g (5.54 mmol) = 88.5 %. Repetition of the experiment using 1272.0 mg barium [^{14}C]carbonate yielded additional 1.67 g (4.84 mmol) = 75 % of (3). Total activity: 20653.4 MBq (558.2 mCi).

Methyl 4-(4-fluorophenyl)-2,6-diisopropyl-5-methoxymethyl pyridine [3-^{14}C]carboxylate (4)

The solution of carboxylic acid (3) as raw material in 90 ml ether was treated with a solution of diazomethane in ether at 0 °C. The addition of diazomethane was continued until the yellow colour of the reaction mixture remained. The reaction was monitored by TLC (silica gel, eluent: ethyl acetate/petrolether 2:9) and stopped by evaporation in vacuo. The ester (4) was used directly in the next step.

4-(4-Fluorophenyl)-2,6-diisopropyl-3-hydroxy[^{14}C]methyl-5- methoxymethyl pyridine (5)

The ester (4) was dissolved in 40 ml dry ether and added slowly to a stirred suspension of 1.25 g (30.0 mmol) lithium aluminium hydride in 100 ml dry ether. The mixture was refluxed for 2 h under a nitrogen atmosphere and the reaction was monitored by TLC (silica gel, eluent: ethyl acetate/petrolether 2:9). The mixture was cooled in an ice bath, water was added dropwise and a clear solution was obtained after addition of 50 ml half conc. hydrochloric acid. The acidic aqueous layer was separated, extracted once more with ether and then 10 times with 10 ml dichloromethane, each. The extracts were combined, dried over sodium sulfate and evaporated to dryness in vacuo. Yield: 3.54 g (10.6 mmol) = 90 %. Purity: 95 % (determined by GC).

4-(4-Fluorophenyl)-3-chloro[^{14}C]methyl-2,6-diisopropyl-5-methoxymethyl pyridine (6)

3.54 g (10.6 mmol) of the alcohol (5) was dissolved in 35 ml chloroform and treated with a solution of 6.13 ml (84 mmol) thionyl chloride in 6 ml chloroform. The mixture was refluxed for 1.5 h and evaporated to dryness in vacuo. The residue was triturated twice with chloroform and evaporated to remove traces of thionyl chloride.
The title compound (6) was used directly in the next step.

Diethyl {4-(4-fluorophenyl)-2,6-diisopropyl-5-methoxymethyl pyrid-3-yl}-[^{14}C]methyl phosphonate (7)

The arylmethylchloride (6) was treated with 21 ml (130 mmol) triethylphosphite. The mixture was heated at reflux (bath temperature 180 - 200 °C) for 1.5 h and analyzed by TLC (silica gel, eluent: ether/n-hexane 5:1). The excess reagent was removed by distillation in vacuo at 70 °C. The residue was purified by column chromatography on 45 g silica gel Si60 using

ether/n-hexane as eluent, the appropriate fractions were combined and evaporated to dryness to give a colorless oil. Yield: 4.19 g (9.29 mmol) = 86.9 % (calculated from alcohol (5)). Purity: 94.7 % (determined by GC).

(E)-(2S,4R,6S)-6-{2-(4-(4-Fluorophenyl)-2,6-diisopropyl-3-methoxymethyl-pyrid-3-yl)-[2-^{14}C]ethenyl}-4-(tert. butyldimethyl-silyloxy)-2-methoxy-3,4,5,6-tetrahydro-2H-pyrane (9)

Lithium diisopropylamide was freshly prepared by treatment of 2.02 ml (14.3 mmol) diisopropylamine in 13 ml dry tetrahydrofuran with 8.25 ml (13.2 mmol) n-butyllithium (1.6 M in hexane) under argon atmosphere.
This reagent was added dropwise via syringe under argon purge to a solution of 2.335 g (5.18 mmol) of the [^{14}C]phosphonate (7) and 0.371 g (0.823 mmol) of non-labelled phosphonate in 30 ml dry tetrahydrofuran at -20 to -30 °C. After stirring the reaction mixture for 45 min at a temperature between -20 and -5 °C, 2.31 g (8.40 mmol) of the aldehyde (8), dissolved in 15 ml dry tetrahydrofuran was added at -10 °C. Stirring was continued for 1 h at -10 to 15 °C and for further 2 h at room temperature. After addition of 20 ml water, the aqueous layer was separated and extracted 4 times with 25 ml dichloromethane, each. The organic layers were combined, dried over sodium sulfate and evaporated to dryness in vacuo and 5.4 g of an oily residue were obtained.

The reaction was repeated under conditions described above, whereby 1.856 g (4.11 mmol) of labelled phosphonate (7) and 0.851 g (1.89 mmol) non-labelled phosphonate were used as starting material. After workup 5.9 g of raw material (9) was isolated. Both batches of raw material were combined and purified by repeated column chromatography on silica gel Si60 using different mixtures of n-hexane/ether (7:1/1:1/1:5) as eluent. Yield: 4.061 g (7.10 mmol) = 59 %.

(E)-(4R,6S)-6-{2(4-(4-Fluorophenyl)-2,6-diisopropyl-5-methoxy-methyl-pyrid-3-yl)-[2-^{14}C]ethenyl}-3,4,5,6-tetrahydro-4-hydroxy-2H-pyrane-2-one (11)

A solution of 1.526 g (2.67 mmol) of the protected alkene (9) in 40 ml tetrahydrofuran was treated with 27 ml 10 % hydrochoric acid and the stirred mixture was heated to 40 - 45 °C for 30 min. After removal of the solvents in vacuo the residue was triturated with saturated aqueous sodium bicarbonate and extraction with ether was performed. The combined organic fractions were dried over sodium sulfate, and evaporation yielded a yellow oil.

The crude hemiacetal (10) was dissolved in 110 ml toluene and after addition of 21 g (approx. 37 mmol) activated silver carbonate on celite the suspension was heated at reflux for 3 h. TLC (silica gel, eluent: ether) demonstrated that no starting material remained. The reaction mixture was cooled and suction filtered through a sodium sulfate pad. The solids were intensively rinsed with ether, the filtrate was concentrated and the residue was purified by column chromatography (silica gel Si60, eluent: ether) to afford 0.8 g. The experiment was repeated in the same manner using 1.65 g (2.90 mmol) of alkene (9) and 0.99 g of (11) as raw material were obtained. The residues yielded from both experiments were combined and

recrystallized several times from tert.-butylmethyl ether/n-heptane, whereby 200 mg of non-labelled lactone (11) were added. Yield: 960 mg (2.17 mmol). Total activity: 2475.3 MBq (66.9 mCi).

The mother liquor was further purified by chromatography on Lobar® B, RP-18 (Merck, Darmstadt, FRG) using acetonitrile/water 1:1 as eluent, a flow of 3.0 ml/min, and UV-detection of fractions was performed at 230 nm. Total activity: 666.4 MBq (18.0 mCi).

In summary 3141.7 MBq (84.9 mCi) of the lactone (11) were obtained. With respect to the labelled starting material barium[^{14}C]carbonate, the radiochemical yield of the synthesis amounted to 12.7 %.

Sodium (E)-(3R,5S)-7-{4-(4-fluorophenyl)-2,6-diisopropyl-5-methoxymethyl-pyrid-3-yl}-3,5-dihydroxy-[7-^{14}C]hept-6-enoate ([^{14}C]Cerivastatin, 1)

A solution of 69.6 mg lactone (11) (radiochemical purity: 74 %) in 2 ml water/acetonitrile 1:1 was treated with 200 µl (0.2 mmol) 1N aqueous sodium hydroxide and stirred overnight. The reaction mixture was diluted with water and adjusted to pH 4 with 1N hydrochloric acid. The resulting suspension was applied on a Mega Bond® cartridge preconditioned with water, methanol, acetonitrile and again water. Gradient elution with water/acetonitrile (20 % steps from 100 % aqueous to 100 % acetonitrile, 50 ml each) furnished a prepurified fraction (water/acetonitrile 40:60) which was further worked up by preparative HPLC. In general, further efforts to purify [^{14}C]Cerivastatin depended on the quality of the starting material (lactone, 11). Predominantly preparative HPLC was performed on RP 18 material using gradient elution with 0.2 % phosphoric acid or dist. water and acetonitrile.

[^{14}C]Cerivastatin, if synthesized according to the pathway and to purification procedures described above is characterized as follows: specific activity: 2.41 MBq/mg (65.1 µCi/mg), ee: ≥ 99 %, radiochemical purity: 99 %.

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