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Synthesis of deuterium-labeled simvastatin

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This study describes the synthesis of deuterium-labeled simvastatin. The stable isotope-labeled compound was prepared starting from lovastatin in nine steps with 9% overall yield.

Keywords: deuterium-labeled; lovastatin; simvastatin; hypercholesterolemia

Introduction

Coronary artery disease is the leading cause of death worldwide. The primary risk factor for the disease is hypercholesterolemia.[1,2] The level of the microsomal enzyme 3-hydroxy-3-methylglutary-coenzyme A reductase (HMG-CoA reductase) controls the rate-limiting step in the cholesterol biosynthetic pathway.[3] This enzyme is a prime target for pharmacological intervention. Simvastatin, like all other statins, is an HMG-CoA reductase inhibitor used in the treatment of patients with hypercholesterolemia. It is a synthetic derivate of a fermentation product of *Aspergillus*.[4,5] Patients who receive treatment with simvastatin demonstrate reductions in serums levels of total cholesterol and low-density lipoprotein cholesterol.[6]

Identification and quantification of drugs and metabolites by liquid chromatography-mass spectrometry (LC-MS) relies largely on stable isotope-labeled analogs.[7,8] A renewed interest has been recently raised to develop a robust and validated LC-MS method to determine simvastatin in biological fluids. The preparation of the stable labeled version of the title compound with M + 6 was requested. Although ^{3}H-simvastatin and ^{14}C-simavastatin have been prepared for pharmacological studies,[9–11] the synthesis of its stable labeled internal standard has not been described in detail.[12] In this paper, the synthetic route to [$^{2}H_6$] simvastatin is described in detail.

Results and discussion

Although several methods are available for conversion of lovastatin to simvastatin,[13–18] the synthesis of deuterium-labeled simvastatin has not been described. Many approaches can be followed to prepare stable labeled versions of simvastatin. Initially, we chose the synthesis of [$^{2}H_3$]simvastatin (6). Based on protocols from the literature,[13–15] [$^{2}H_3$]simvastatin is synthesized in nine steps starting from lovastatin (1) (Scheme 1). Treatment of lovastatin (1) with butylamine under reflux followed by removal of the excess butylamine at reduced pressure and silylation of the resulting diol affords the protected amide (2) in one spot. Enolization of amide (2) with 2.3 equivalents of lithium pyrrolidide is followed by quenching with [$^{2}H_3$]methyl iodide to afford the [$^{2}H_3$]methylated derivative (3). Acid-catalyzed silyl transfer of [$^{2}H_3$]methylated derivative with methane sulfonic acid in aqueous methanol affords the dihydroxy amide (4). Hydrolysis of the amide (4) gives hydroxyl acid, which is converted to ammonium salt derivative (5) by ammonia. Lactonization of ammonium salt derivative affords [$^{2}H_3$]simvastatin (6). However, it is obvious that the [M + 3] internal standard is not sufficient to meet typical thresholds for cross-signal overlapping of a drug with a molecular mass >400. Thus, the end-user needed a target product with more than three deuteriums as internal standard. Therefore, we changed the synthesis strategy, and deuterium-labeled simvastatin could be obtained through esterification of deuterium-labeled 2,2-dimethylbutanoyl chloride (11) with monosilylated diol (13).[16] Although 2,2-dimethylbutanoyl chloride is a commercial material, the synthesis of [$^{2}H_6$]2,2-dimethylbutyryl chloride has not been described. Scheme 2 presents the general synthetic scheme for preparing compound (11). [$^{2}H_6$]Acetone (7) was alkylated with ethyl magnesium bromide, and then quenching with D_2O gave [$^{2}H_6$]2-methylbutane-2-ol (8). Solid Na_2CO_3 was employed as stabilizer while the solvent was evaporated and the product was distilled. Compound (8) was treated with 37% HCl solution to generate [$^{2}H_6$]2-chloro-2-methylbutane (9).[19] The reaction time and the quantity of concentrated HCl solution are crucial to avoid the scrambling of deuterium in (9). Compound (9) was treated with magnesium, and then gaseous CO_2 passing through a concentrated H_2SO_4 trap was bubbled to the reaction solution to afford [$^{2}H_6$]2,2-dimethylbutanoic acid (10).[20,21] The addition rate of (4) was very slow to avoid decomposition of *tert*-pentylmagnesium chloride. The flow rate of CO_2 was regulated so that the temperature of the solution did not rise above −5 °C when rapid stirring was in progress. Compound (10) was obtained in over 99% deuterium enrichment. Compound (10) was treated with oxalyl chloride to form [$^{2}H_6$]2,2-dimethylbutyryl chloride (11).

Scheme 3 illustrates the synthesis of [$^{2}H_6$]simvastatin (15). Treatment of lovastatin (1) with LiOH under reflux in water resulted in rapid opening of the lactone ring followed by slow

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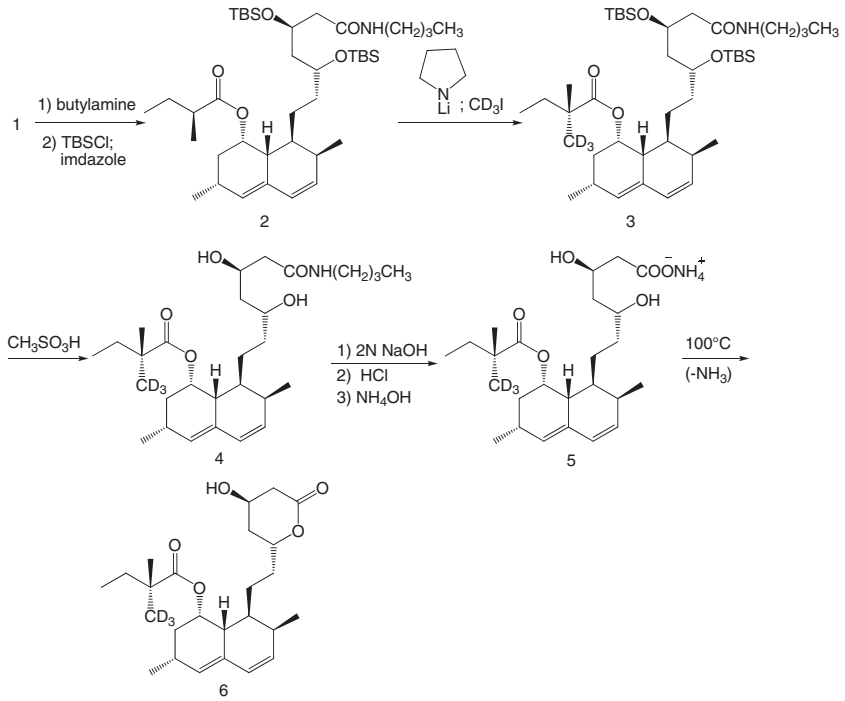
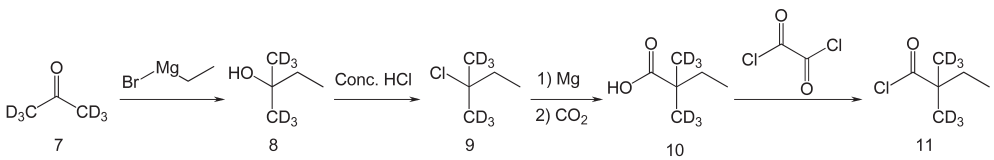


Scheme 1. Synthesis of [$^2H_3$]simvastatin.



Scheme 2. Synthesis of [$^2H_6$]2,2-dimethylbutyryl chloride.

saponification of the 2-methylbutyric ester over 68 h. Neutralization provided a dihydroxy acid, which when heated at reflux in toluene for 3 h, relactonized to afford lactone (12). Selective protection of the diol lactone (12) hydroxyl was accomplished by treatment with *t*-butyldimethylchlorosilane and imidazole in dry DMF to produce silyl ether derivative (13). Acylation of alcohol (13) was carried out with [$^2H_6$]2,2-dimethylbutyryl chloride (11) in anhydrous pyridine in the presence of 4-dimethylaminopyridine (DMAP) as catalyst to give [$^2H_6$]simvastatin derivative (14). Efforts were made to improve the yield for this step by optimizing reaction temperature and time. The best yield of 68% was obtained by heating the reaction at 90 °C for 8 h. Deprotection of (14) with tetrabutylammonium fluoride and acetic acid caused cleavage of the silyl ether protecting group to give [$^2H_6$]simvastatin (15) in 70.5% yield.

After purification by chromatography and recrystallization, the desired product (15) was obtained with 98% chemical purity. MS analysis of compound (15) revealed that the compound has over 98% deuterium enrichment. Compound (15) provided an excellent internal standard for LC-MS/MS studies.

Experimental

General

All reagents were obtained from Sigma-Aldrich and CDN Isotopes. Mass spectra were recorded using a Quattro micro API mass spectrometer. ^{1}H NMR spectra were recorded on a Bruker 300-MHz instrument. Chemical purities were determined with an Agilent 1200 HPLC with a XDB-C18 column (5 µm, 4.6 × 150 mm).

Synthesis of [$^2H_6$]2-methylbutan-2-ol (8)

To a stirred solution of [$^2H_6$]acetone (7) (25 g, 0.369 mol) in freshly distilled diethyl ether (125 mL) was added ethyl magnesium bromide in diethyl ether (3 N, 136.5 mL) over 2.5 h while the temperature was maintained at 8–12 °C by cooling with an ice–water bath as necessary. White solid was formed upon the addition of ethyl magnesium bromide. After addition, the mixture was stirred at 10 °C for 1 h. The reaction was quenched with D_2O (10.5 mL), and the resulting solution was stirred at 10 °C for 1 h. Then the solution was allowed to stand for 30 min, and the slightly milky diethyl ether solution on top was decanted. The resulting solid was stirred with diethyl ether (120 mL) for 30 min, and the suspension was allowed to stand for 30 min; the slightly milky diethyl ether solution on top was decanted. This process was repeated twice. The combined slightly milky diethyl ether solutions were washed with saturated Na_2CO_3 solution. The organic layer was separated, dried over Na_2SO_4, and filtered by gravity. The filtrate was concentrated to about 80 mL under reduced pressure after solid Na_2CO_3 (0.15 g) was added as stabilizer. Then the residue was transferred to a 100-mL distilling flask, into which fresh solid Na_2CO_3 (0.15 g) was added. The solution was distilled using a long water condenser. The

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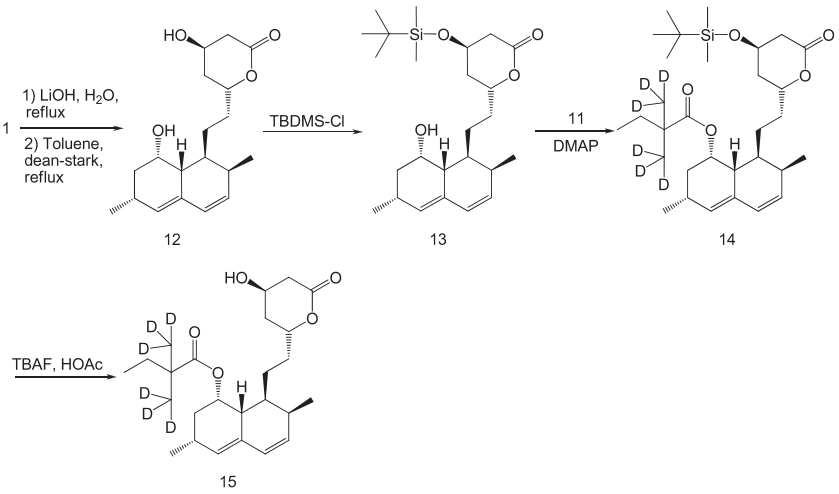

Scheme 3. Synthesis of [$^2H_6$]simvastatin.

fraction boiling at 100–102 °C was collected to give (8) as a colorless liquid (27 g, 78.6%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.47–1.53 (q, 2H), 1.23 (s, 1H), 0.90–0.94 (t, 3H).

Synthesis of [$^2H_6$]2-chloro-2-methylbutane (9)

A mixture of compound (8) (27 g, 0.286 mol) in concentrated HCl solution (12.2 M, 105.7 mL) was stirred at room temperature for 0.5 h. The mixture was cooled to 10 °C and diluted with H_2O (50 mL) and Et_2O (60 mL). The mixture was stirred for 5 min and then allowed to stand for 5 min. The organic layer was separated, and the aqueous layer was extracted with diethyl ether (30 mL). The combined organic layers were washed with 10% $NaHCO_3$ solution (35 mL), water (30 mL), and brine (20 mL). The organic layers were stirred with Na_2SO_4 (10 g) for 30 min and filtered by gravity. The resulting solution was distilled using a long water condenser. The fraction boiling at 85–86 °C was collected to give (9) as a colorless liquid (25 g, 77.4%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.58–1.72 (q, 2H), 0.95–0.99 (t, 3H).

Synthesis of [$^2H_6$]2,2-dimethylbutanoic acid (10)

To a suspension of Mg (2.16 g) turnings in freshly distilled diethyl ether was added (9) (0.5 g) and a crystal of iodine. The reaction mixture was stirred at 26 °C for 10 min. The solution became slightly milky. A solution of (9) (10 g, 89 mmol) in freshly distilled ether (20 mL) was added to the reaction solution over a period of 3 h. After addition, the reaction mixture was stirred at 26 °C for 1 h. The reaction was cooled by an ice–salt bath to −5 °C. The reaction mixture was stirred, and gaseous CO_2 was bubbled into the reaction after passing through two concentrated H_2SO_4 traps. This process was continued for 20 min. The flow rate of CO_2 was regulated so that the temperature of the mixture did not rise above −5 °C. The reaction was cooled with ice–water bath and acidified with 25% sulfuric acid (50 mL). The diethyl ether layer was separated and the aqueous layer was extracted with Et_2O (4 × 40 mL). The combined diethyl ether extracts were washed with 25% NaOH solution (4 × 20 mL). The combined alkaline aqueous layers were cooled with an ice–water bath and acidified with 25% sulfuric acid to pH 2. The aqueous layer was extracted with Et_2O (4 × 50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to give (10) as a colorless oil (3.5 g, 33.9%).

^{1}H NMR (DMSO-d6, 300 MHz): δ 1.56–1.62 (q, 2H), 0.87–0.91 (t, 3H).

Synthesis of [$^2H_6$]2,2-dimethylbutyryl chloride (11)

To a solution of (10) (2.00 g, 17.21 mmol) in CH_2Cl_2 (20 mL) was added oxalyl chloride (6.55 g, 52 mmol). Two drops of DMF was added, and carbon dioxide started to evolve immediately. The reaction solution was stirred at room temperature for 1 h. The reaction solution was evaporated to dryness under nitrogen to give crude product (11) as a colorless oil (2.1 g, 95%).

Synthesis of (4S,6R)-4-hydroxy-6-(2-((1S,2S,6R,8S,8aR)-8-hydroxy-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl)ethyl)tetrahydro-2H-pyran-2-one (12)

To a suspension of LiOH (8.88 g, 0.371 mol) in H_2O (900 mL) was added lovastatin (1) (15 g, 0.037 mol). The reaction mixture was stirred under nitrogen at reflux for 68 h. The reaction mixture was cooled to 0 °C and acidified with conc. HCl to pH 2 and then extracted with ether (3 × 300 mL). The combined organic layers were washed with H_2O (3 × 300 mL) and then with brine (300 mL), dried over Na_2SO_4, and concentrated under reduced pressure to give a yellow solid. The residue was recrystallized from ether to give an off-white solid (9.2 g, 73.7%). This solid was suspended in dry toluene (165.6 mL) and heated at reflux for 3 h in a Dean-Stark apparatus for azeotropic removal of water. The suspension became a clear off-white solution. After evaporation of the toluene, the residual oily solid was stirred at reflux in hexane (92 mL) for 0.5 h. After being cooled to 0 °C, the hexane solution was filtered and the desire product (12) was obtained as a beige solid (7.2 g, 82.6%).

Synthesis of (4S,6R)-4-(tert-butyldimethylsilyloxy)-6-(2-((1S,2S,6R,8S,8aR)-8-hydroxy-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl)ethyl)tetrahydro-2H-pyran-2-one (13)

To a solution of (12) (9.2 g, 28.71 mmol) in dry DMF (100 mL) was added imidazole (9.77 g, 0.144 mol) and TBDMSCl (10.82 g, 71.8 mmol). The reaction mixture was stirred at room temperature for 20 h. The reaction mixture was diluted with ether (700 mL) and washed successively with H_2O (350 mL), 2% HCl solution (350 mL), H_2O (350 mL), and saturated $NaHCO_3$ solution (350 mL). The organic layer was separated and concentrated under reduced pressure to give an off-white solid, which was recrystallized from hexane to afford (13) as a white solid (6.2 g, 49.7%).

MS-EI (*m/z*): 417 (42), 457 (MNa^+, 100), 458 (38), 508 (30).

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Synthesis of [$^2H_6$](1S,3R,7S,8S,8aR)-8-(2-((2R,4S)-4-(tert-butyldimethyl silyloxy)-6-oxotetrahydro-2H-pyran-2-yl)ethyl)-3,7-dimethyl-1,2,3,7,8, 8a- hexahydronaphthalen-1-yl 2,2-dimethylbutanoate (14)

A solution of (13) (1 g, 2.3 mmol) and DMAP (0.03 g, 1 mmol) in dry pyridine (10 mL) was stirred at 0 °C. To this stirred solution was added (11) (1.29 g, 9.2 mmol) in CH_2Cl_2 (10 mL) over 5 min. The solution was stirred at 0 °C for 10 min and then heated at 90 °C for 8 h. The reaction mixture was diluted with Et_2O (150 mL) and washed successively with 2% HCl solution (3 × 75 mL), saturated $NaHCO_3$ solution (75 mL), and brine (2 × 75 mL). The organic layer was separated and concentrated under reduced pressure to give a yellow oil. The crude product was purified by chromatography on a silica gel column and then eluted with ether/hexane (2:8) to afford (14) as a light yellow solid (0.83 g, 68.1%).

MS-EI (*m/z*): 539 (42), 540 (20), 561 (MNa^+, 100), 562 (38).

Synthesis of [$^2H_6$]simvastatin (15)

The reaction mixture of (14) (0.63 g, 1.17 mmol), acetic acid (0.25 g, 0.24 mL, 4.1 mmol), and tetrabutylammonium fluoride hydrate (0.82 g, 3 mmol) was stirred in dry THF (6 mL) at room temperature for 8 h. Excess TBAF was quenched with 2% HCl solution (30 mL), and the mixture was extracted with ether (2 × 30 mL). The combined organic extracts were washed with saturated $NaHCO_3$ (2 × 30 mL), dried over Na_2SO_4, and concentrated under reduced pressure to give a pale yellow oil. The crude product was purified by chromatography on a silica gel column and then eluted with CH_2Cl_2/Et_2O (9:1) to afford (15) as a white solid (0.35 g, 70.5%).

^{1}H NMR (DMSO-d6, 300 MHz): δ 5.97 (d, 1H, *J* = 9.0 Hz), 5.77 (dd, 1H, *J* = 6.0, 9.0 Hz), 5.50 (bt, 1H), 5. 20 (m, 1H), 4.46 (m, 1H), 4.10 (m, 1H), 2. 62 (m, 1H), 2.26–2.43 (m, 4H), 1.96 (dd, 1H, *J* = 12.0, 9.0 Hz), 1.54–1.85 (m, 5H), 1.42–1.52 (m, 2H), 1.20–1.36 (m, 3H), 1.02 (d, 3H, *J* = 6.0 Hz), 0.83 (d, 3H, *J* = 6.0 Hz), 0.77 (t, 3H).

MS-EI, (*m/z*): 447 (MNa^+, 38), 871.5 (100), 872.5 (55). HPLC (XDB-C18, ACN/H_2O = 76/24, 1.0 mL/min): t_R 5.35 min (98.6%). Isotopic enrichment determined by MS was over 98%.

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