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The Synthesis of Carbon-14 Labeled Pravastatin

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Summary

An asymmetric route to [^{14}C]β-hydroxycompactin **1** bearing the (*S*)-2-methyl-[1-^{14}C]butanoate side chain has been developed. Methylation of [N-[1-^{14}C]butyryl]-4-(*S*)-phenylmethyl-2-oxazolidinone **4** afforded a 95:5 mixture of diastereomeric [N-(*S*,*R*)-2-methyl-[1-^{14}C]butyryl]-4-(*S*)-phenylmethyl-2-oxazolidinones **5**,**6** which were separated by preparative HPLC. Oxidative cleavage of **5** afforded optically pure (*S*)-2-methyl-[1-^{14}C]butanoic acid. Acylation of alcohol **9** with optically pure (*S*)-2-methyl-[1-^{14}C]butyryl chloride afforded ester **10**. Removal of the silyl ether produced diastereomerically pure compactin **11**. Hydroxylation was carried out by biotransformation with *Mucor hiemelus* to afford diastereomerically pure [[1-^{14}C]butanoate]β-hydroxycompactin, [^{14}C]Pravastatin **1**.

Key words: Pravastatin, Mevinolin, (*S*)-2-methyl-[1-^{14}C]butanoic acid, oxazolidinone

Results and Discussion

Compactin and Mevinolin have been shown to be competitive inhibitors of HMG-CoA reductase, an enzyme directly involved in the rate limiting step in the biosynthesis of cholesterol.[1] Initiated by these findings, years of research have culminated in the development of several mevinolin or compactin based hypocholesterolemic therapeutic agents. Herein we report the stereoselective synthesis of [[1-^{14}C]butanoate]β-hydroxycompactin.

Previous reports have shown the incorporation of a carbon-14 label into the ester side chain to be a viable, efficient route to isotopically labeled compactin analogs.[2] However, a tedious separation of diastereomers[2] was necessary when racemic 2-methyl-[1-^{14}C]butyric acid was used. With this in mind, the synthesis of optically pure (*S*)-2-methyl-[1-^{14}C]butyric acid utilizing Evan's oxazolidinone chemistry[3] was developed and is shown in Scheme 1. [1-^{14}C]Butyryl chloride **3** was prepared by treating sodium [1-^{14}C]butyrate **2** with oxalyl chloride and catalytic DMF in dichloromethane. The dichloromethane solution of **3** was added directly to 2.0 equivalents of the preformed 4-(*S*)-phenylmethyl-2-oxazolidinone anion in tetrahydrofuran. The resulting crude product was purified by column chromatography to afford pure [N-[1-^{14}C]butyryl]]-4-(*S*)-phenylmethyl-2-oxazolidinone **4** in 81% yield.

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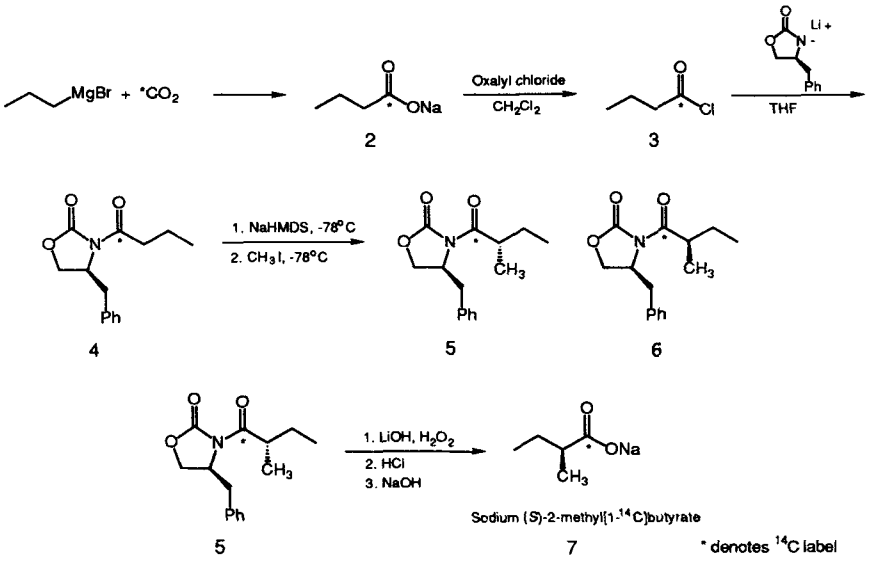
Deprotonation of **4** with sodium hexamethyldisilazide at -78° C followed by the addition of ten equivalents of methyl iodide afforded a 95:5 mixture of [N-(*S*,*R*)-2-methyl-[1-^{14}C]buytryl]-4-(*S*)-phenylmethyl-2-oxazolidinones **5** and **6**.[4] Separation by preparative HPLC provided diastereomerically pure **5** in 72% overall yield from **4**. Oxidative cleavage of **5** proceeded smoothly using lithium hydroxide and hydrogen peroxide[5] to afford sodium (*S*)-2-methyl[1-^{14}C]butyrate **7** in essentially quantitative yield.

Scheme 1

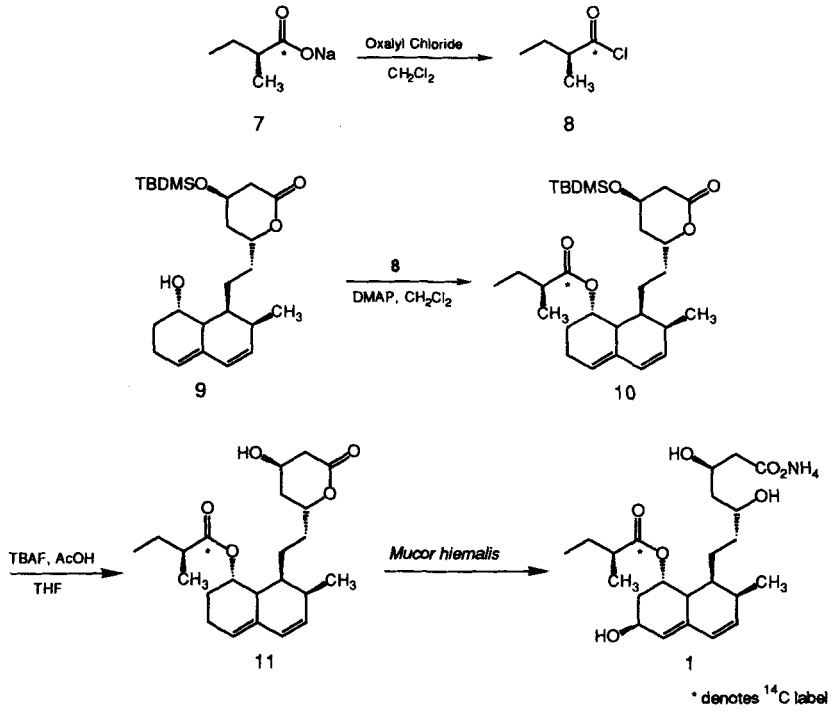


Two methods for coupling of cold **7** with the silyl-protected intermediate **9** were investigated. Esterification using dicyclohexylcarbodiimide and catalytic 4-dimethylaminopyridine (DMAP) in dichloromethane has been shown previously to proceed smoothly and in high yield using a large excess of 2-methylbutyric acid.[6] However, efforts to maximize the esterification yield based on one equivalent of acid resulted in only a 35% optimal conversion. Alternatively, a more efficient procedure proceeding from the acid chloride of **7** [2] was utilized (Scheme 2). **7** was treated with oxalyl chloride in dichloromethane and the resulting acid chloride **8** reacted with the alcohol **9** in the presence of excess DMAP to afford, after preparative chromatography, ^{14}C-labeled silyl-protected compactin **10** in a 80% yield. Desilylation of **10** was accomplished using tetrabutylammonium fluoride in tetrahyrofuran in the presence of acetic acid. Chromatographic purification of the crude isolate afforded [^{14}C]compactin **11** in 97% yield. Analysis of **11** by HPLC showed no racemization of the butyric acid side chain during oxidative cleavage or acylation.

The asymmetric hydroxylation of **11** to β-hydroxycompactin **1** was carried out by biotransformation with *Mucor hiemalis* MF-5021. The biotransformation was initiated by addition of **11** in dimethylsulfoxide to *Mucor hiemalis* MF5021 broth and the fermentation continued for 3 days. Following extraction of the broth, the crude extracts were purified by preparative HPLC to afford [^{14}C]β-hydroxycompactin **1** of 95% radiochemical purity in a 32% yield.

In summary, an asymmetric route was used to generate radioactive β-hydroxycompactin **1** bearing the (*S*)-2-methyl-[1-^{14}C]butanoate side chain in an overall yield of 14%. Furthermore, this methodology could be expanded to the synthesis of other compactin or mevinolin analogs.

Scheme 2



Experimental

Radioactivity measurments were carried out using Packard Tri-carb 1000 TR liquid scintillation spectrometer using Scintiverse I™ as scintillation medium. Analytical TLC was performed using silica gel 60 F-254 (E. Merck) with the radioactivity measurements carried out with a Berthold Model LB2760 Scanner. Analytical HPLC analyses were performed using a Dupont Zorbax RX C-8 (4.6 mm x 25 cm) or Whatman Partisil column (4.6 mm x 25 cm), Rainin UV-1 detector at 254 nm, Berthold LB-506-C radioactivity monitor, Spectra-Physics SP8810 LC pump and controller, and software run on an IBM PS/2 computer. Preparative HPLC were carried out using Altex pumps with a Beckman UV detector at 254 nm and either a Whatman M20 (22.1 mm x 25 cm) Partisil or Dupont (22.1 mm x 25 cm) Zorbax C-8 column. The identities of appropriate labeled intermediates as well as the final product were established by co-elution via HPLC or TLC with authentic material.

Sodium [1-^{14}C]butyrate

To diethyl ether (50 mL; dried over 4A sieves and purged with N_2 subsurface for 15 min), was added propylmagnesium chloride **1** (2M solution in ether, 2.75 mL, 5.5 mmol) by syringe. The flask was attached to a vacuum manifold and placed in a liquid nitrogen bath. The manifold was purged with N_2, evacuated (0.4 mmHg), and from a break seal flask, [^{14}C]CO_2 (Amersham CFQ 6283; 4.8 mmol, 250 mCi) was released. The valves were closed and the reaction warmed to -20° C and aged for 1 h. Following addition of H_2O (50 mL) and 1N NaOH solution (9.2 mL), the mixture was warmed to room temperature and the layers separated. The aqueous layer was washed with ether (3 x 10 mL), acidified with H_2SO_4 (11.55 mmol), and the product isolated by co-distillation

with water at 15 mmHg. The pH of the distillate was adjusted to 9.6 (0.1 N NaOH) using a pH meter. The water was removed *in vacuo* and the resulting residue dried at 0.1 mmHg and 80° C for 16 h to afford **2** (197 mCi, 79%) as a white solid.

[N-[1-^{14}C]butyryl]-4-(*S*)-phenylmethyl-2-oxazolidinone 4

To a slurry of dry sodium [1-^{14}C]butyrate **2** (663 mg, 165 mCi, 6.0 mmol) in CH_2Cl_2 (10.3 mL) in a screw capped culture tube was added oxalyl chloride (917 mg, 7.2 mmol) followed by a catalytic amount of DMF (6.6 mg, 0.09 mmol, 1.75 mL of a 0.05M solution of DMF in CH_2Cl_2). The mixture was aged at room temperature for 2 h with periodic venting. To a solution of 4-(*S*)-phenylmethyl-2-oxazolidinone[7] (2.13g, 12.0 mmol) in THF (48.2 mL) at -78° C was added a solution of n-BuLi in hexanes (4.8 mL of 2.5M, 12.0 mmol). After aging for 1 h at -78° C, the above solution of acid chloride **3** was added dropwise by cannulation to the anion solution over 5 min. The mixture was stirred at -78° C for 2.5 h, then quenched with saturated aqueous $NaHCO_3$ (12 mL). The mixture was diluted with H_2O (25 mL) and ether (25 mL), the layers separated, and the aqueous layer extracted with ether (2 x 15 mL). The combined organic layers were washed with aqueous $NaHCO_3$ (12 mL) and brine (12 mL), dried over $MgSO_4$, filtered, and the filtrate concentrated *in vacuo* to provide the crude product **4** as a yellow oil. Purification by silica gel chromatography (hexane:ethyl acetate; 8:1) afforded pure **4** (133 mCi, 537 mg, 81% yield).

[N-(*S*)-2-methyl-[1-^{14}C]buytryl]-4-(*S*)-phenylmethyl-2-oxazolidinone 5

To a solution of **4** (151 mg, 0.611 mmol, 32 mCi) in THF (5 mL) at -78° C was added sodium hexamethyldisilazide (670 uL, 0.670 mmol, 1.0 M). The mixture was aged 1 h at -78° C, then methyl iodide (0.867g, 6.11 mmol) dissolved in THF (2 mL) was added over 10 min. The mixture was aged 1 h at -78° C, warmed to room temperature, and quenched with saturated aqueous NH_4Cl (3.0 mL). The mixture was diluted with ether (10 mL), the layers separated, and the aqueous layer extracted with ether (5 mL). The combined organic layers were washed with 5% HCl (2 mL), saturated aqueous $NaHCO_3$ (2 mL), and brine (2 mL). The organic layer was then dried over $MgSO_4$, filtered, and concentrated. The diastereomers **5**,**6** were separated by preparative HPLC (Partisil, 22.4 mm x 25 cm, 20/80 CH_2Cl_2/hexane step gradient 10% CH_2Cl_2 every 45 min to 60/40 CH_2Cl_2/hexane) to afford diastereomerically pure [N-(*S*)-2-methyl-[1-^{14}C]buytryl]-4-(*S*)-phenylmethyl-2-oxazolidinone **5** (23.0 mCi, 116 mg, 72% yield). Analytical separation of diastereomers **5** and **6** was determined as follows; Whatman Partisil 10, 65% CH_2Cl_2/hexane, flow 1.5 mL/min, r_t (**5**) 16 min, r_t (**6**) 20 min.

Sodium (*S*)-2-methyl-[1-^{14}C]butyrate 7

To a solution of **5** at 0° C (23.0 mCi, 116 mg, 0.44 mmol) in 12 mL THF:H_2O 3:1 was added 30% aqueous H_2O_2 solution (200 uL, 1.77 mmol) and a solution of LiOH (37 mg, 0.88 mmol in H_2O (1.5 mL). The mixture was warmed to room temperature, aged for 1 hour, then quenched with 750 uL of 1.5M aqueous Na_2SO_3. $NaHCO_3$ (25 mg) was added and the THF was removed by nitrogen stream. To recover the chiral auxillary, the aqueous concentrate was extracted with CH_2Cl_2 (3 x 2 mL). The aqueous layer was acidified with conc. HCl at 0° C and the optically active acid extracted with CH_2Cl_2 (3 x 1 mL). The product was back extracted into H_2O (6.0 mL) using 1.0 N NaOH solution (440 uL). Concentration and drying of the residue at 60° C and 0.5 mmHg afforded sodium (*S*)-2-methyl[1-^{14}C]butyrate **7** (53.5 mg. 22.5 mCi, 98% yield).

6(*R*)-{2-[8-(*S*)-2-methylbutyryloxy-[1-^{14}C]]-2(*S*)-methyl-1,2,6,7,8,8a(*R*)-hexahydronaphthyl-1(*S*)]ethyl}-4(*R*)-*tert*-butyldimethylsilyloxy-3,4,5,6-tetrahydro-2H-pyran-2-one 10

To a slurry of **7** in a screw capped culture tube (74 mg, 0.596 mmol, 18 mCi) in CH_2Cl_2 (1.5 mL) was added oxalyl chloride (58 uL, 0.66 mmol). The mixture was aged at room temperature for 30 min, with periodic venting, followed by 1 h at 45° C. To a solution of **9** (251 mg, 0.596 mmol) and 4-dimethylaminopyridine (37 mg, 0.298 mmol) in pyridine (3.0 mL) was added the solution of acid chloride **8** and the mixture aged at room temperature for 4 h. An additional 2 equivalents of dimethylaminopyridine (74 mg) was added in three equal portions over a 3 h period. The reaction was quenched with 1 N HCl (10 mL) and poured into ether (40 mL). The layers were separated and the organic layer washed with 1.0N HCl (3 x 6.0 mL), saturated aqueous $NaHCO_3$ (2 x 5.0 mL), dried over $MgSO_4$, and concentrated. Purification by preparative HPLC (Zorbax C8, 22.4 mm x 25 cm, 40/60 CH_3CN/H_2O gradient to 80/20 CH_3CN/H_2O over 1.5h) afforded silyl-protected compactin **10** (14.7 mCi, 290 mg, 80% yield).

6(*R*)-{2-[8-(*S*)-2-methylbutyryloxy-[1-^{14}C]]-2(*S*)-methyl-1,2,6,7,8,8a(*R*)-hexahydronaphthyl-1(*S*)]ethyl}-4(*R*)-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-one 11

To a solution of **10** (14.7 mCi, 290 mg, 0.580 mmol) in THF (25 mL) was added acetic acid (139 mg, 2.32 mmol) followed by tetrabutylammonium fluoride (1.0 M, 1.75 mL, 1.75 mmol) and the mixture was aged at room temperature for 4 h. The reaction was concentrated *in vacuo* and the residue redissolved in ethyl acetate (10 mL). The solution was washed with aqueous $NaHCO_3$ (2 x 5 mL), brine (1 x 2 mL), dried over $MgSO_4$, filtered, and concentrated to a yellow oil. The oil was redissolved in 8:3 CH_2Cl_2:acetone (5 mL) and chromatographed through a silica column, eluting with 8:3 CH_2Cl_2:acetone. The fractions containing product were combined and concentrated to afford **11** (14.3 mCi, 221 mg, 97% yield) of 97% radiochemical purity as determined by HPLC analysis (Zorbax C8-RX, 60/40 CH_3CN/0.1% aqueous H_3PO_4, 1.0 mL/min, rt = 6.0 min). Analysis for diastereomeric purity was performed using different HPLC conditions (Zorbax C8-RX, 35/65 CH_3CN/H_2O for 45 min, step gradient to 40/60 CH_3CN/H_2O, 1.0 mL/min, rt = 69.25 min (*S*), rt = 70.28 min (*R*))

6(*R*)-{2-[8-(*S*)-2-methylbutyryloxy-[1-^{14}C]]-2(*S*)-methyl-6(*S*)hydroxy-1,2,6,7,8,8a(*R*)-hexahydronaphthyl-1(*S*)]ethyl}-3(*R*),5(*R*)-dihydroxyheptanoic acid, ammonium salt 1

The asymmetric hydroxylation of **11** to β-hydroxycompactin **1** was carried out by biotransformation with *Mucor hiemalis* MF-5021 (IFO-5834, Merck culture collection). Inoculum development and compactin bioconversion media were identical and contained per liter of distilled water: glucose, 10g; Hycase SF, 2g; beef extract, 1g; and corn steep, 3g. The medium is used as is (pH 5.3) after autoclaving for 20 min at 121° C. A frozen cell suspension (2 mL) was used to inoculate a 250 mL Erlenmeyer flask containing 50 mL of medium. The culture was incubated at 28°C with shaking (220 rpm) for 48 h. The hydroxylation was performed in 250 ml Erlenmeyer flasks containing 50 mL of the same medium. The flasks were inoculated with 5% inoculum and incubated for 24 h under the conditions described above. The biotransformation was initiated by addition of **11** (25 mCi/mmole, 120 mg, 7.2 mCi, dissolved in 9.5 mL dimethylsulfoxide, 6.3 mg/flask) to *Mucor hiemalis* MF5021 broth and the fermentation continued for 3 days. The whole broth was filtered through a pad of Celite, and the filtrate adjusted to pH 3.0 with 1.0M HCl and extracted with ethyl acetate (3 x 150 mL). The combined extracts were concentrated, and the [^{14}C]β-hydroxy compactin isolated by preparative HPLC

(Zorbax C8-RX 9 mm x 25 cm, 10/90 CH_3CN/0.05M aqueous NH_4OAc pH 7.6 buffer to 30/70 CH_3CN/0.05M aqueous NH_4OAc pH 7.6 buffer over 1 h, 10 mL/min) to afford [^{14}C]β-hydroxycompactin **1** (2.3 mCi, 32% yield) of 95% radiochemical purity as determined by HPLC analysis (Zorbax C8-RX, 30/70 CH_3CN/aqueous 0.1% H_3PO_4, 1.0 mL/min, rt = 7.2 min).

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