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Research Article

Synthesis of d_3-cerivastatin for use as internal standard in a LC/MS/MS method developed for quantitation of the drug in human serum

Bang-Chi Chen[1,*], Mark S. Bednarz[1], Huiping Zhang[1], Peng Guo[1], Mohammed Jemal[2], Jeffrey A. Robl[1], Scott A. Biller[1], Joseph E. Sundeen[1], Balu Balasubramanian[1] and Joel C. Barrish[1]

[1] *Discovery Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543-4000, USA*

[2] *Metabolism and Pharmacokinetics, Bristol-Myers Squibb Pharmaceutical Research Institute, New Brunswick, NJ 08903-0191, USA*

Summary

d_3-Cerivastatin was synthesized as an internal standard for use in a LC/MS/MS method developed for the simultaneous quantitative determination of the drug in human serum. d_3-Cerivastatin was efficiently prepared on large scale from d_3-iodomethane using improved procedures. Copyright © 2006 John Wiley & Sons, Ltd.

Key Words: cerivastatin; deuterium; Horner olefination

Introduction

Cerivastatin, sodium (E)-(+)-(3R,5S)-7-[4-(4-fluorophenyl)-2,6-diisopropyl-5-methoxymethyl)-pyrid-3-yl]-3,5-dihydroxyhept-6-enoate, is a new synthetic inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase effective for the treatment of high serum cholesterol.[1–3] A LC method, based on post-column induced fluorometric detection, has been reported for the quantitation of cerivastatin in human plasma.[4] Methods based on radio-immunoassay, HMG-CoA reductase inhibition assay and capillary gas chromatography for the quantitation of cerivastatin in human plasma have also been reported.[5] For a project aimed at the development of a bioanalytical method based on high-performance liquid chromatography (LC) with electrospray tandem mass spectrometry (MS/MS) for the simultaneous

*Correspondence to: Bang-Chi Chen, Bristol-Myers Squibb, Princeton, NJ 08543, USA.
E-mail: bangchi.chen@bms.com

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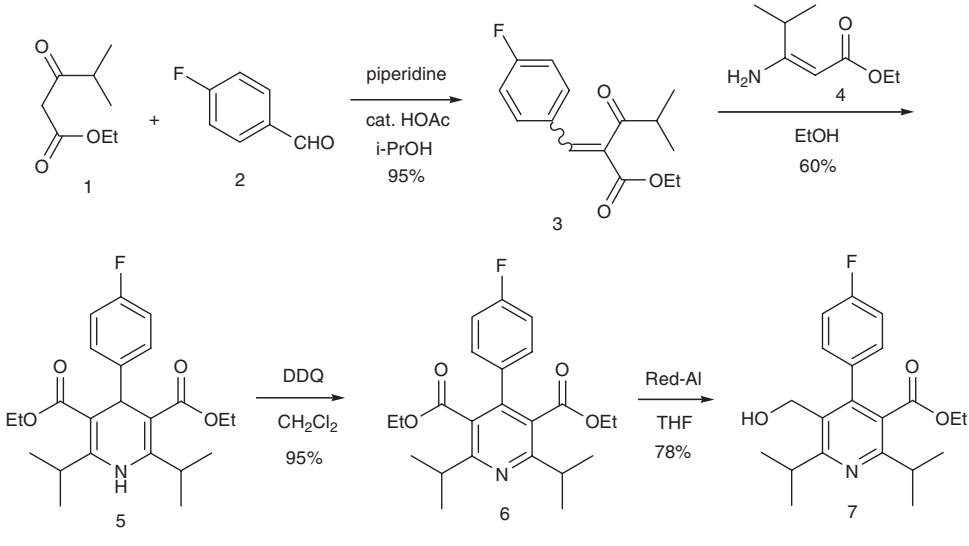
quantitative determination of cerivastatin in human serum,[6] deuterium labeled cerivastatin was required. While stable isotope labeled cerivastatin has the same solubility, extraction and chromatographic behavior as its non-labeled counterpart, its difference in molecular weight makes it distinguishable in LC/MS/MS from the non-labeled counterpart. Therefore, stable isotope labeled cerivastatin is an ideal internal standard in a LC/MS/MS assay used for the quantitation of the drug in biological matrices.

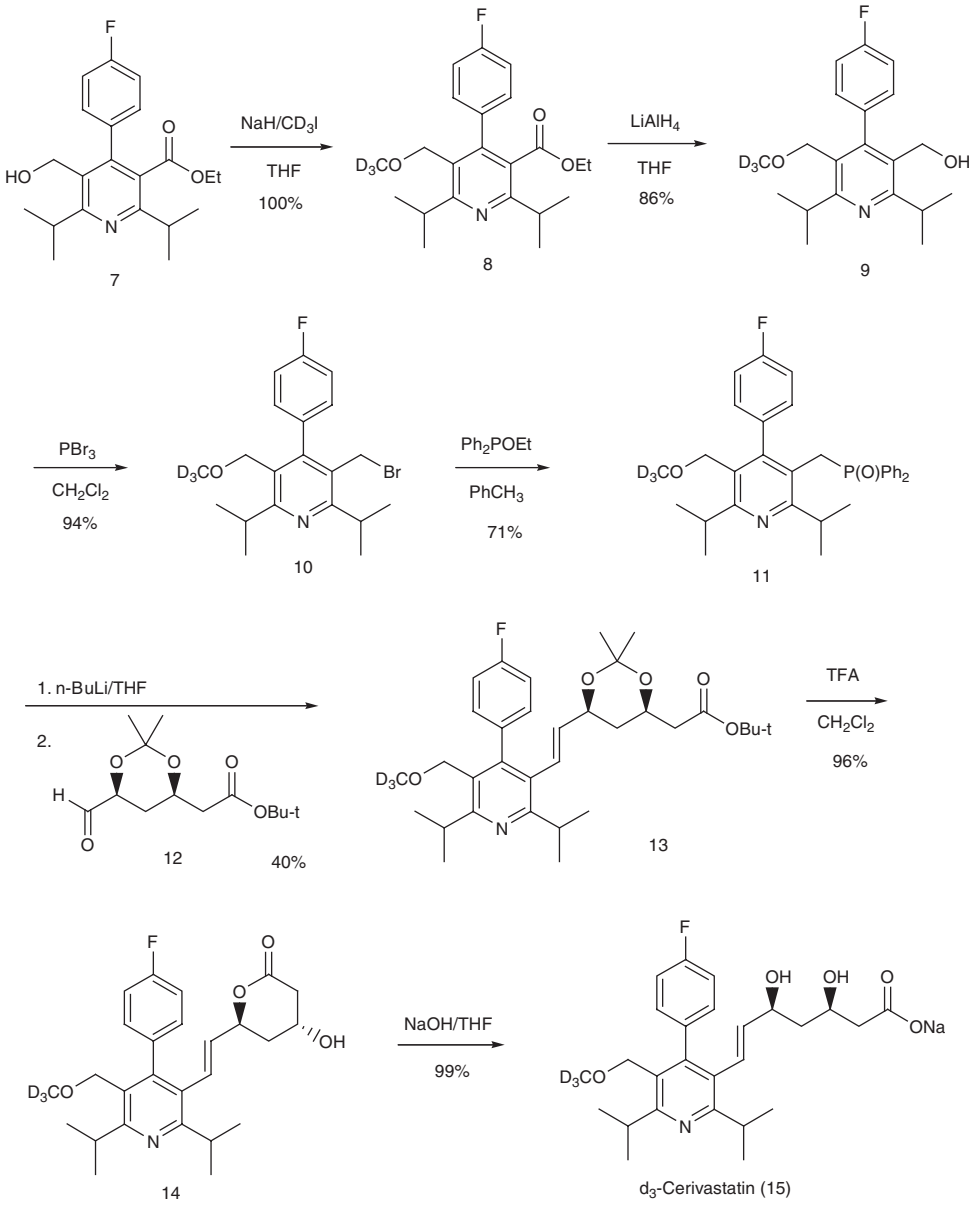
Results and discussion

The methoxy site of cerivastatin appears to be appropriate for labeling with deuterium because of the ready availability of d_3-iodomethane. While the previously reported procedures for the synthesis of cerivastatin[7,8] could be adapted, significant modifications were needed and new procedures were developed for the preparation of the deuterated intermediates and d_3-cerivastatin (**15**) (Schemes 1 and 2). This new and improved synthesis is featured by a Horner type olefination using diphenyl phosphine oxide (**11**) and the readily available chiral non-racemic aldehyde (**12**),[9] to avoid the chemical and chromatographic resolutions or the lengthy preparation of chiral non-racemic side chain needed in the previously reported methods.[7,8]

Thus, treatment of ethyl isobutyryl acetate (**1**) with 4-fluorobenzaldehyde (**2**) in isopropanol in the presence of catalytic amounts of acetic acid and piperidine gave ethyl 2-(4-fluorobenzylidene)-4-methyl-3-oxopentanoate (**3**) in 95% yield as a mixture of *cis*/*trans*-isomers. Condensation of **3** with ethyl



Scheme 1.



Scheme 2.

3-amino-4-methyl-2-pentenoate (**4**)[10] in refluxing ethanol provided dihydropyridine diester (**5**) in 60% yield. Oxidation of **5** with DDQ gave pyridine diester (**6**) in 95% yield. The diester **6** was then selectively reduced with Red-Al to give hydroxy ester (**7**) in 78% yield.

The d_3-methyl group was then introduced by treatment of hydroxy ester (**7**) with d_3-iodomethane using NaH as the base to give d_3-methoxy ester (**8**) in quantitative yield. Further reduction of d_3-methoxy ester (**8**) with lithium

aluminum hydride gave d_3-methoxy alcohol (**9**) in 86% yield. Treatment of **9** with phosphorous tribromide in methylene chloride afforded d_3-methoxy bromide (**10**) in 91% yield. Reaction of the bromide (**10**) with ethyl diphenylphosphinite in refluxing toluene gave d_3-methoxy phosphine oxide (**11**) in 71% yield. Treatment of **11** with *n*-BuLi followed by addition of the chiral non-racemic aldehyde (**12**) gave d_3-methoxy enoate (**13**) in 40% isolated yield and $>98\%$ E stereochemical purity, which was consistently with the extraordinarily high E selectivity reported previously for this type of Horner olefination between a related diphenyl phosphine oxide and **12**.[9] The enoate (**13**) was then directly treated with TFA to give d_3-cerivastatin lactone (**14**) in 96% yield. d_3-Cerivastatin sodium salt (**15**) was finally obtained in 99% yield by reaction of **14** with 1 equiv of sodium hydroxide. The structure of **15** was confirmed by comparison of the NMR spectral data with those reported for the non-labeled Cerivastatin and mass spectrometric studies.[3,6]

Experimental

Enamine (**4**)[10] and the aldehyde (**12**)[9] were prepared according to literature procedures. d_3-Iodomethane (99+ atom% D) was purchased from Aldrich Company. ^{1}H-NMR spectra were recorded in $CDCl_3$ or CD_3OD on a Bruker ARX-400, Joel Eclipse 270 or 500 instrument using tetramethylsilane as internal standard. The reactions were monitored by HPLC using a Shimadzu LC-10AS system. LC/MS analyses were conducted using a Hewlett-Packard 1090L HPLC system and a Finigan TSQ-7000 triple quadruple mass spectrometer.[11]

Ethyl 2-(4-fluorobenzylidene)-4-methyl-3-oxopentanoate (**3**)

In a 3 l round bottomed flask equipped with a magnetic stirrer was placed ethyl isobutyrylacetate (**1**, 211.0 g, 1.33 mol), 4-fluorobenzaldehyde (161.3 g, 1.30 mol), isopropanol (760 ml), pyperidine (7.2 ml) and glacious acetic acid (4.2 ml). The reaction mixture was stirred at room temperature under nitrogen for 22 h. The solvent was removed under reduced pressure. The residue was dissolved with methylene chloride (500 ml) and washed with sat. $NaHCO_3$ (3×300 ml) and dried over anhydrous $MgSO_4$. Removal of $MgSO_4$ and solvent afforded ethyl 2-(4-fluorobenzylidene)-4-methyl-3-oxopentanoate **3** (319.0 g) in 95% yield as a mixture of *cis*/*trans*-isomers which was used in the subsequent step without further purification. ^{1}H NMR spectral data of **3** were consistent with those reported previously.[12]

Diethyl 4-(4-fluorophenyl)-2,6-diisopropyl-1,4-dihydropyridine-3,5-dicarboxylate (**5**)

In a 2 l round bottomed flask equipped with a magnetic stirrer and a condenser was placed ethyl 2-(4-fluorobenzylidene)-4-methyl-3-oxopentanoate

(**3**, 237.8 g, 0.90 mol), enamine (**4**, 141.5 g, 0.90 mol) and absolute ethanol (750 ml). The reaction mixture was heated to reflux and stirred under nitrogen for 16.5 h. The solvent was distilled until the pot temperature reached to 170°C. The reaction mixture was then stirred at 170–173°C for 4 h and cooled to 60°C. Hexane (950 ml) was added. The resulting slurry was stirred at room temperature for 0.5 h, filtered, washed with hexane (3 × 200 ml) and suction dried at room temperature for 17 h to give **5** (217.8 g) in 60.3% yield. ^{1}H NMR ($CDCl_3$) δ 7.15–7.26 (m, 2H), 6.84–6.88 (m, 2H), 6.12 (s, 1H), 4.98 (s, 1H), 4.12–4.22 (m, 2H), 4.04–4.11 (m, 4H), 1.15–1.25 (m, 18H).

Diethyl 4-(4-fluorophenyl)-2,6-diisopropylpyridine-3,5-dicarboxylate (**6**)

In a 3 l round bottomed flask equipped with a mechanical stirrer and a condenser was placed dihydropyridine (**5**, 217.7 g, 0.54 mol), methylene chloride (1.7 l) and DDQ (123.1 g, 0.54 mol). The reaction was stirred at room temperature for 1 h. The reaction mixture was filtered through of a pad of silica gel (310 g) and the pad washed with methylene chloride (3 × 350 ml). The filtrate was concentrated under reduced pressure. The resulting solid was dried under vacuum to give pyridine **6** (206.5 g) in 95% yield. ^{1}H NMR ($CDCl_3$) δ 7.15–7.19 (m, 2H), 6.93–6.99 (m, 2H), 3.92 (q, $J = 7.3$ Hz, 4H), 2.97–3.05 (m, 2H), 1.21 (d, $J = 6.8$ Hz, 12H), 0.88 (t, $J = 7.3$ Hz, 6H).

Ethyl 4-(4-fluorophenyl)-5-(hydroxymethyl)-2,6-diisopropylnicotinate (**7**)

In a 5 l three-necked round-bottomed flask equipped with a mechanical stirrer, pressure-equalized addition funnel and rubber septum was placed pyridine ester (**6**, 94.29 g, 0.23 mol) and tetrahydrofuran (1 l). The reaction mixture was stirred to give a solution under nitrogen and cooled to −11°C. Red-Al (215 ml, 65 wt% in toluene, 0.69 mol) was added over 18 min while keeping the reaction temperature below −8°C. After 22.5 h, an additional Red-Al (40 ml, 65 wt% in toluene, 0.13 mol) was added and the reaction was stirred at rt for another 22 h. The reaction mixture was cooled to −10°C and water (1.1 l) was carefully added to destroy any excess Red-Al. The reaction mixture was transferred into a separation funnel and the phases separated. The aqueous phase was extracted with ethyl acetate (2 × 1 l). The organic layers were combined and dried over anhydrous $MgSO_4$. Removal of drying agent and solvent afforded a crude product which was purified by flash chromatography using 4:1 heptane:ethyl acetate as the eluent to give hydroxy ester **7** (65.69 g) in 78% yield. ^{1}H NMR ($CDCl_3$) δ 7.00–7.30 (m, 4H), 4.41 (s, 2H), 3.94 (q, $J = 7.0$ Hz, 2H), 3.37–3.52 (m, 1H), 2.95–3.10 (m, 1H), 1.29 (d, $J = 6.8$ Hz, 6H), 1.24 (d, $J = 6.8$ Hz, 6H), 0.94 (t, $J = 7.2$ Hz, 3H).

Ethyl 4-(4-fluorophenyl)-2,6-diisopropyl-5-(d_3-methoxymethyl)nicotinate (**8**)

In a 2 l three-necked round bottomed flask equipped with a magnetic stirrer and rubber septum was placed hydroxy ester (**7**, 34.84 g, 97 mmol) and tetrahydrofuran (1 l). The reaction mixture was stirred to give a solution under nitrogen and cooled to −53°C. d_3-Iodomethane (25.06 g, 173 mmol) was added followed by sodium hydride (5.84 g, 60 wt%). The reaction mixture was then allowed to warm to rt. After stirring over weekend, the reaction mixture was carefully quenched by water (500 ml) and concentrated under reduced pressure to a volume of ~700 ml. This mixture was extracted with methyl *t*-butyl ether (2 × 400 ml). The combined extract was dried over anhydrous $MgSO_4$. Removal of drying agent and solvent afforded d_3-methoxy ester **8** (38.19 g) in quantitative yield. ^{1}H NMR ($CDCl_3$) δ 7.00–7.30 (m, 4H), 4.07 (s, 2H), 3.96 (q, J = 7.2 Hz, 2H), 3.30–3.45 (m, 1H), 2.96–3.08 (m, 1H), 1.30 (d, J = 6.8 Hz, 6H), 1.26 (d, J = 6.8 Hz, 6H), 0.93 (t, J = 7.2 Hz, 3H).

(4-(4-Fluorophenyl)-2,6-diisopropyl-5-(d_3-methoxymethyl)pyridin-3-yl) methanol (**9**)

In a 2 l three-necked round bottomed flask equipped with a magnetic stirrer, reflux condenser and rubber septum was placed lithium aluminum hydride (6.02 g, 95%, 151 mmol) and dry tetrahydrofuran (240 ml). The suspension was stirred under argon and was heated to 60°C. A solution of d_3-methoxy ester (**8**, 38.02 g, 101 mmol) in dry tetrahydrofuran (350 ml) was added. The reaction was heated to reflux and stirred for 50 min. The pinkish gray suspension was allowed to cool to 35°C and was quenched carefully by aqueous KOH (40 ml, 17 w/w%). Water (800 ml) was then added and the mixture was stirred for 10 min. The mixture was transferred into a separation funnel and extracted with methyl *t*-butyl ether (2 × 400 nml). The combined extract was washed with saturated aqueous brine (500 ml). Removal of solvent afforded a crude product which was purified by flash chromatography using 9:1 heptane:ethyl acetate as the eluent to give d_3-methoxy alcohol **9** (27.97 g) in 86% yield. ^{1}H NMR ($CDCl_3$) δ 7.05–7.26 (m, 4H), 4.33 (s, 2H), 4.00 (s, 2H), 3.29–3.46 (m, 2H), 1.41 (s, 1H), 1.31 (d, J = 6.7 Hz, 6H), 1.28 (d, J = 6.7 Hz, 6H).

3-(Bromomethyl)-4-(4-fluorophenyl)-2,6-diisopropyl-5-(d_3-methoxymethyl) pyridine (**10**)

In a 2 l round bottomed flask equipped with a magnetic stirrer, a pressure-equalized addition funnel and rubber septum was placed d_3-methoxy alcohol (**9**, 27.80 g, 83 mmol) and methylene chloride (750 ml). The mixture was stirred to give a solution at room temperature. Phosphorous tribromide (16 ml, 170 mmol) was added over 3 min while keeping the reaction temperature below

28°C. After stirring for 1 h, the reaction was carefully quenched by saturated aqueous sodium bicarbonate (500 ml). Additional methylene chloride (500 ml) was added. The organic phase was separated and dried over anhydrous $MgSO_4$. Removal of the drying agent and solvent afforded d_3-methoxy bromide **10** (31.17 g) in 94% yield. ^{1}H NMR ($CDCl_3$) δ 7.24–7.29 (m, 2H), 7.11–7.18 (m, 2H), 4.20 (s, 2H), 3.97 (s, 2H), 3.34–3.39 (m, 2H), 1.32 (d, $J = 6.7$ Hz, 6H), 1.29 (d, $J = 6.7$ Hz, 6H).

3-((Diphenylphosphoryl)methyl)-4-(4-fluorophenyl)-2,6-diisopropyl-5-(d_3-methoxymethyl)pyridine (**11**)

In a 1 l three necked round bottomed flask equipped with a magnetic stirrer, a reflux condenser and rubber septum was placed d_3-methoxy bromide (**10**, 19.43 g, 49 mmol), toluene (200 ml) and ethyl diphenylphosphinite (10.5 ml, 49 mmol). The solution was heated to reflux (~116°C) and stirred under nitrogen for 4 h and allowed to cool to room temperature overnight. The solvent was removed to give a pasty off white solid which was dissolved in refluxing heptane (500 ml). This turbid solution was polish filtered while hot. The filtrate was stirred and allowed to cool to rt. The resulting heavy slurry was diluted with heptane (100 ml). After cooling to ~15°C, the slurry was filtered, washed with heptane and air dried to afford d_3-methoxy phosphine oxide **11** (17.90 g) in 71% yield. ^{1}H NMR ($CDCl_3$) δ 7.43–7.47 (m, 2H), 7.29–7.33 (m, 8H), 6.80–6.84 (m, 2H), 6.73–6.76 (m, 2H), 3.87 (s, 2H), 3.62 (d, $J = 13.5$ Hz, 2H), 3.19–3.31 (m, 2H), 1.28 (d, $J = 6.7$ Hz, 6H), 1.12 (d, $J = 6.1$ Hz, 2H).

tert-Butyl 2-((4R,6S)-6-((E)-2-(4-(4-fluorophenyl)-2,6-diisopropyl-5-(d_3-methoxymethyl)pyridin-3-yl)vinyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetate (**13**)

In a 500 ml oven dried round-bottomed flask equipped with a magnetic stirrer and rubber septum was placed d_3-methoxy phosphine oxide (**11**, 17.90 g, 34.5 mmol) and dry tetrahydrofuran (180 ml). The reaction mixture was stirred to give a solution under nitrogen and cooled to −9°C. *n*-BuLi (14 ml, 2.5 M in hexane, 35 mmol) was added over 3 min while keeping the reaction temperature below 3°C. The resulting red–orange solution was stirred at 3–5°C for 50 min.

In another oven dried 1 l round-bottomed flask equipped with a magnetic stirrer and rubber septum was placed activated 4 A molecular sieves (28.5 g), aldehyde (**12**, 11.27 g, 61 mmol) and dry tetrahydrofuran (100 ml). After stirring at ~0°C for 20 min, the aldehyde/THF solution was added via a cannulation into the above prepared phosphine oxide anion solution over 4 min while keeping the pot temperature below 5°C. After addition, the reaction mixture was stirred at 0°C for 10 min and rt for 1 h. The reaction was then quenched by saturated aqueous brine (200 ml) and diluted with methyl

t-butyl ether (200 ml). The mixture was filtered. The filtrate was transferred into a separation funnel and the phases separated. The aqueous phase was extracted with methyl *t*-butyl ether (200 ml). The combined organic was dried over anhydrous $MgSO_4$. Removal of drying agent and solvent afforded a crude product which was slurried in a mixture of 4:1 heptane:ethyl acetate (95 ml) at $\sim 42^{\circ}C$. The resulting solids were removed by filtration. The filtrate was concentrated and purified by flash chromatography using 4:1 heptane:ethyl acetate as the eluent to give d_3-methoxy enoate **13** (14.96 g) in 40% yield. ^1H NMR ($CDCl_3$) δ 6.98–7.12 (m, 4H), 6.17 (d, $J = 16.1$ Hz, 1H), 5.19 (dd, $J = 6.1$, 16.1 Hz, 1H), 4.14–4.23 (m, 2H), 4.00–4.06 (m, 2H), 3.26–3.35 (m, 2H), 2.19–2.39 (m, 2H), 0.8–1.45 (m, 29H).

(4R,6S,E)-6-(2-(4-(4-fluorophenyl)-2,6-diisopropyl-5-(d_3-methoxymethyl)-pyridin-3-yl)vinyl)-4-hydroxy-tetrahydropyran-2-one **(14)**

In a 500 ml round-bottomed flask equipped with a magnetic stirrer was placed d_3-methoxy enoate (**13**, 6.88 g, 14.2 mmol) and methylene chloride (300 ml). The reaction mixture was stirred under nitrogen to give a pale yellow solution and trifluoroacetic acid (28 ml) was then added. After stirring at room temperature for 16.25 h, the reaction mixture was concentrated under reduced pressure. The residue was redissolved in methylene chloride (200 ml), washed with pH 7.5 sodium phosphate buffer (3×200 ml) and dried over anhydrous $MgSO_4$. Removal of drying agent and solvent afforded a crude product which was purified by flash chromatography using 4:1 to 2:1 heptane:ethyl acetate as the eluent to afford d_3-methoxy lactone **14** (4.43 g) in 81% yield. ^1H NMR ($CDCl_3$) δ 7.05–7.14 (m, 4H), 6.39 (d, $J = 16.2$ Hz, 1H), 5.27 (dd, $J = 6.4$, 16.2 Hz, 1H), 5.02–5.08 (m, 1H), 4.12–4.18 (m, 1H), 4.03–4.09 (m, 2H, 3.20–3.40 (m, 2H), 2.50–2.68 (m, 2H), 1.96 (s, 1H), 1.63–1.71 (m, 1H), 1.45–1.54 (m, 1H), 1.32 (d, $J = 6.8$ Hz, 6H), 1.23 (d, $J = 6.4$ Hz, 6H).

Sodium (3R,5S,E)-7-(4-(4-fluorophenyl)-2,6-diisopropyl-5-(d_3-methoxymethyl)pyridin-3-yl)-3,5-dihydroxyhept-6-enoate (d_3-cerivastatin, **(15)**

In a 200 ml round-bottomed flask equipped with a magnetic stirrer and rubber septum was placed d_3-methoxy lactone (**14**, 1.00 g, 2.25 mmol) and freshly distilled inhibitor-free tetrahydrofuran (8 ml). The reaction mixture was stirred to give a pale yellow solution. Then a diluted solution of sodium hydroxide (2.28 ml, 0.996 N) in distilled water (6 ml) was added. After stirring at room temperature for 1.25 h, the reaction was concentrated under reduced pressure to remove the bulk of the tetrahydrofuran. The residue was lyophilized to afford d_3-cerivastatin **15** (1.08 g) in 99% yield. ^1H NMR (CD_3OD) δ 7.15–7.26 (m, 4H), 6.36 (d, $J = 16.2$ Hz, 1H), 5.40 (dd, $J = 6.5$, 16.2 Hz, 1H), 4.93 (m, 3H), 4.20–4.17 (m, 1H), 4.12–4.18 (m, 2H), 3.81–3.89 (m, 1H), 3.35–3.53

(m, 2H), 2.10–2.38 (m, 2H), 1.54–1.63 (m, 1H), 1.35 (d, $J = 6.4$ Hz, 6H), 1.30 (d, $J = 6.6$ Hz, 6H).

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