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In Pursuit of Higher Purity: Use of ^{13}C NMR and ^{13}C-Enriched Substrates To Trace Impurity Generation and Removal in the Synthesis of Atorvastatin

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Abstract:

The use of ^{13}C-labeled starting materials and intermediates in the synthesis of the lipid regulator atorvastatin is described. ^{13}C NMR of the ^{13}C-enriched intermediates allowed monitoring of problematic nonchromophoric low-level impurities which were unobservable using standard analytical techniques.

Introduction

Atorvastatin {[*R*-(*R**,*R**)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrole-1-heptanoic acid calcium salt (2:1)} (**6**), has recently been approved by the FDA for the treatment of serum dyslipidemia. Atorvastatin competitively inhibits hydroxymethylglutaryl-CoA (HMG-CoA) reductase, the enzyme that catalyzes the rate-limiting step in cholesterol biosynthesis. Inhibition of this enzyme has proven to be an effective means for lowering total and low-density lipoprotein cholesterol in humans.[1] The ability of cholesterol-lowering drugs to reduce the incidence of coronary heart disease or delay its progression is well established.[2]

The synthesis of amorphous atorvastatin used in early toxicology and clinical lots (phases I and II: batch size up to 32 kg) has been described.[3] Approximately 40% of these batches had to be reworked to pass release specifications. Three issues were problematic for further production and scale-up: product isolation, residual solvent levels, and purity of the bulk pharmaceutical chemical (BPC). Typically, the calcium salt was extracted from an aqueous solvent matrix with a hot ethyl acetate and heptane solution. Further addition of heptane to the product mixture caused *precipitation* of the BPC as a white, gelatinous mass. Isolation of 10−12 kg of BPC (dry weight) in multiple loads from a 240 L capacity centrifuge often took >16 h. Vacuum tray drying usually took 3−4 weeks under high vacuum at elevated temperatures to reduce residual heptanes to <0.5%. Rotary, fluid-bed, or Nutsche drying were not practical either because of high initial organic solvent levels or because these methods resulted in BPC with undesirable physical characteristics.

The synthesis of a new, highly purified analytical reference standard of atorvastatin coincided with bulk drug requests totaling more than 70 kg of BPC. Given problems already noted, we decided to produce this material in three smaller lots. However, the BPC from all three batches substantially failed the minimum HPLC w/w specification of 97%.[4] Moreover, the usual rework method[5] failed to significantly increase the w/w purity of laboratory samples of these batches when measured against a recently produced analytical reference standard. Thus, it became imperative to develop a new BPC rework procedure and to identify or at least eliminate these impurities through synthetic route modifications.

Analytically, the IR spectra of these failed batches matched the reference standard, as did the HPLC impurity profiles (UV detection),[6] optical rotation, and the calcium and sodium levels. These samples also gave normal residue on ignition tests, elemental analyses, acidic and basic titration, and TLC profile. The 400 MHz ^1H NMR spectra hinted at traces of aliphatic impurities, but no clear signal separation from existing resonances was observed.

This became known as the "missing mass" problem due to the discrepancy in the w/w analyses and the near total analytical "invisibility" of the impurity or impurities. The best tool for detecting the impurities, aside from HPLC w/w analyses, was the use of photodiode array detection during the HPLC impurity profiles. These chromatograms showed several *minor* absorptions at 203 nm when compared to an analytical reference standard.

We reasoned that we were probably dealing with several minor impurities derived from the aliphatic portion (non-^{13}C-labeled equivalents of **1c** and **2**; Scheme 1) of the molecule. These putative impurities lack aromatic and/or conjugated chromophores and thus would be transparent at the wavelength (245 nm) at which atorvastatin is normally analyzed. In addition, the NMR signals from these impurities would be expected to be masked by existing resonances from the aliphatic portion of atorvastatin.

Results and Discussion

Rework of the low w/w BPC was successfully accomplished by converting back to atorvastatin lactone (**5**), which was crystallized from toluene. HPLC analysis of residue from the toluene filtrates utilizing photodiode array

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(4) Parke-Davis guidelines specify a minimum HPLC w/w purity of 98% for clinical lots. The level was initially set at 97% for atorvastatin due to difficulties in removing the corresponding lactone.

(5) The calcium salt was converted to the sodium salt via the carboxylic acid. The sodium salt was refluxed at high pH prior to reconversion to the calcium salt.

(6) HPLC refractive index detection could not be used due to the relative insolubility of atorvastatin in the mobile phase.

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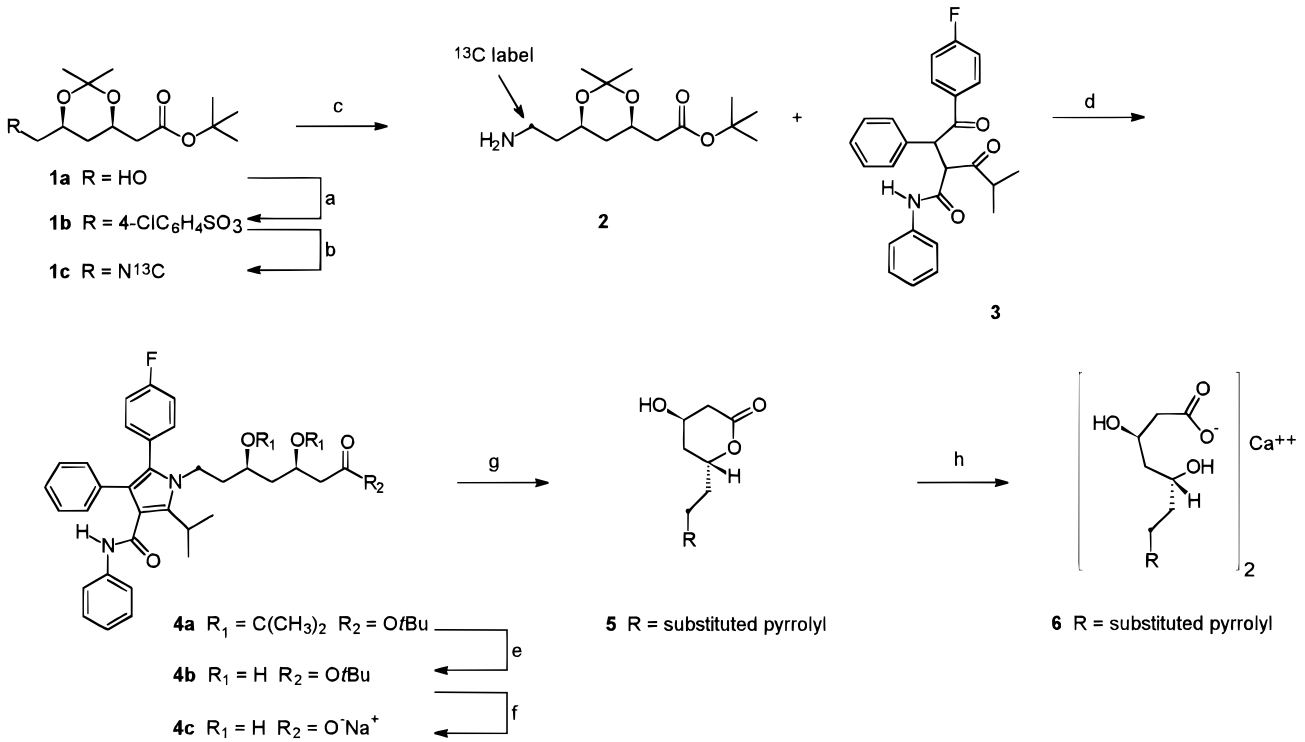
Scheme 1[a]



[a] Reagents: (a) 4-chlorobenzenesulfonyl chloride, TEA, CH_2Cl_2; (b) $K^{13}CN$, $MeSO_3H$ (cat.), DMSO; (c) sponge Ni; 50 psi of H_2, isopropyl alcohol; (d) $C(CH_3)_3CO_2H$, THF/hexanes; (e) HCl, MeOH; (f) NaOH/H_2O; (g) HCl, toluene, heat; isolate; (h) NaOH, H_2O/MeOH/MTBE, then $Ca(OAc)_2$.

detection showed some enrichment of the 203 nm absorptions. That the lactone filtrates contained the putative impurities responsible for low w/w assay of BPC was demonstrated by conversion of lactone **5** back into high w/w atorvastatin. Thus, we had in hand an effective rework method. Since lactone crystallization eventually afforded pure BPC, the existing atorvastatin synthesis was then modified to incorporate lactone isolation (Scheme 1) and was designated the "lactone route".

^{13}C NMR spectroscopy in conjunction with ^{13}C-labeling experiments has been extensively used to trace biosynthetic pathways.[7] In a similar manner, we decided to incorporate ^{13}C-labeled aliphatic material into the atorvastatin synthesis and monitor functional group transformations with ^{13}C NMR. If the ^{13}C label is introduced as the nitrile carbon in nitrile ester **1c**, the chemical shift difference between a carbon adjacent to a pyrrole ring (e.g., **4a** or **5**) and a carbon adjacent to an amide or similar functionality in an impurity should be distinguishable.[6] With 100% ^{13}C label incorporation, any singly ^{13}C labeled impurity occurring around the 1% level in isolated intermediates or product should produce a ^{13}C NMR spectrum containing extra aliphatic peaks compared to non-^{13}C-labeled material. The extra peaks should have an average peak height approximately the same as that of nonlabeled natural abundance (1.1% ^{13}C) aliphatic carbons, and thus be readily discernible.

Synthesis of ^{13}C-labeled nitrile **1c** was achieved in two steps. The commercially available alcohol (**1a**)[8] was converted to the unstable 4-chlorobenzenesulfonate **1b**. Displacement of the 4-chlorobenzensulfonate with 99% ^{13}C enriched KCN in DMSO gave nitrile **1c** in high yield. This displacement was aided by a catalytic amount of methanesulfonic acid, which in identical unlabeled experiments prevented extensive product decomposition.

With ^{13}C-enriched nitrile **1c** in hand, we conducted parallel atorvastatin syntheses in order to compare intermediates and BPC obtained from the original synthetic method[3] (no lactone isolation) with those obtained from the lactone route (Scheme 1). Subsequent reduction of the ^{13}C-labeled nitrile **1c** with hydrogen and sponge nickel under slightly varying conditions quantitatively afforded amino ester **2** with equivalent $^{13}C(^1H)$ NMR spectra (Figure 1b: only methylene resonances are shown).

The ^{13}C-enriched C7 allows unambiguous assignment for the C6, C4, and C2 resonances. The one-bond ($^1J_{C7C6}$) coupling of 35.4 Hz and the three-bond ($^3J_{C7C4}$) coupling of 3.4 Hz are consistent with typical literature carbon−carbon coupling constants.[9] Several resonances of undetermined origin are readily apparent. However, it is not likely that these unknown resonances represent the source of the "missing mass". Control experiments using the original atorvastatin syntheses using distilled amine **2** versus undistilled amine both produced low w/w atorvastatin, strongly suggesting that the responsible impurities are formed at a later point in the synthesis.

Reaction of amino ester **2** with diketone **3** under Paal−Knorr conditions[10] gave pyrrolic ester **4a**. After workup to remove any traces of unreacted amino ester **2**, the $^{13}C(^1H)$ NMR spectra (Figure 2b) of **4a** produced either by the original atorvastatin synthesis or by the lactone route were

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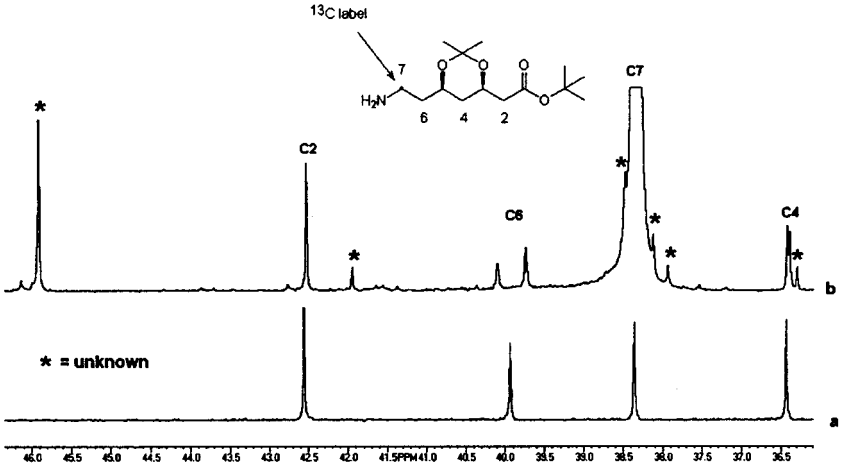


Figure 1. **Portions of the 100 MHz ^{13}C NMR spectra of (a) unlabeled and (b) ^{13}C-labeled amino ester 2 in $CDCl_3$. Spectrum b is vertically truncated to allow for ready comparison of unenriched ^{13}C resonances. Unidentified resonances due to synthetic impurities are marked with a asterisks.**

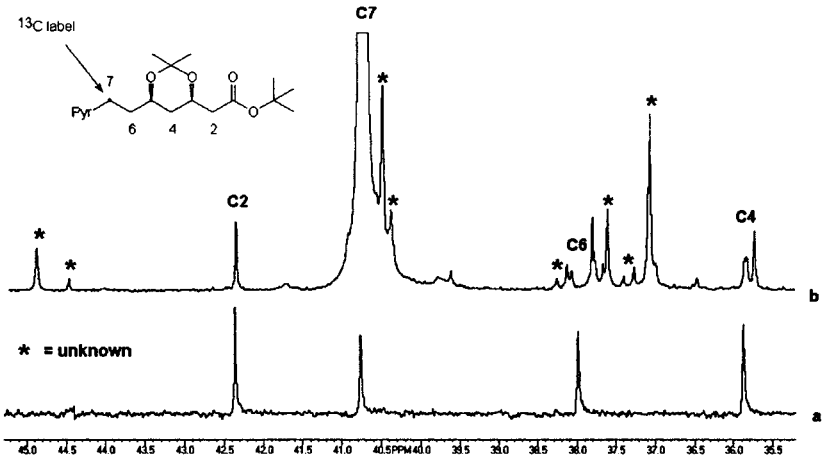
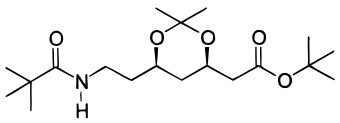


Figure 2. **Portions of the 100 MHz ^{13}C NMR spectra of (a) unlabeled and (b) ^{13}C-labeled pyrrolic ester 4a in $CDCl_3$. Spectrum b is vertically truncated to allow for ready comparison of unenriched ^{13}C resonances. Unidentified resonances due to synthetic impurities are marked with a asterisks.**

again essentially identical. The C7 resonance, now adjacent to a pyrrole ring, has shifted downfield as earlier predicted. Comparison with the $^{13}C(^1H)$ NMR of unenriched **4a** (Figure 2a) reveals several unknown resonances, at least three of which have approximately the same signal intensity of the natural abundance ^{13}C carbons. The large unknown resonance at about 37 ppm is likely due to pivalamide **7**, on the basis of ^{13}C NMR comparison with an authentic sample. This



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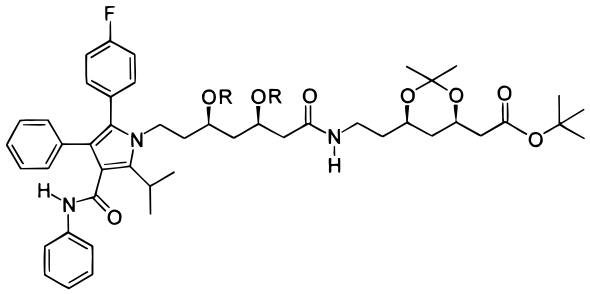
byproduct is produced through the condensation of amine **2** and the pivalic acid used to catalyze the Paal−Knorr reaction. Indeed, if diketone **3** is left out of the Paal−Knorr condensation, about 1−3% of pivalamide **7** (based on amine **2** present) is formed as the only product.

The large unknown resonance adjacent to the ^{13}C-labeled C7 resonance in Figure 2b is likely due to ^{13}C-labeled pyrrole **8a**, on the basis of ^{13}C NMR comparison with an authentic sample of **8b**.[11]

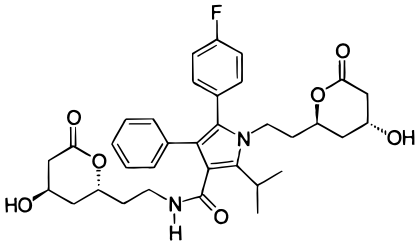


8a R = $C(CH_3)_2$
8b R = H

It is interesting to note that nitrogen acylation in amine **2** with pivalic acid versus acylation with pyrrole **4a** produces distinctly different C7 resonances. Structure **8a** is further supported by the fact that the sodium/calcium salt of fully deprotected **8a** or **8b** is a known low-level impurity removed later in the synthesis. Another impurity identified in isolated lactone **5** is dilactone **9**.[12] Its suitably functionalized di-*tert*-

(11) **8b** was generated via the condensation of amine **2** with lactone **5.**
(12) Dilactone **9** is typically seen at about 0.5−1 area % in isolated lactone **5**.

butyl precursor is also likely responsible for some of the unknown resonances in Figure 2b. The formation of the carbon backbone for either pyrrole **8a** or **9** can be envisaged as arising from the condensation of excess amine **2** with pyrrole **4a**.



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^{13}C-labeled pyrrole **4a** was converted via the intermediate diol (**4b**) and sodium salt (**4c**) into lactone **5**, which was isolated as a crystalline solid from toluene. We had previously determined that an additional lactone crystallization from toluene was necessary in order to consistently produce high-purity atorvastatin. We could not eliminate this recrystallization despite systematic attempts to vary the Paal−Knorr reaction conditions. The necessity for lactone recrystallization is further corroborated by examination of the ^{13}C(^{1}H) NMR of the ^{13}C-enriched crude and recrystallized lactone filtrates (Figure 3c,e) compared to either singly crystallized ^{13}C-enriched lactone (Figure 3b), doubly crystallized ^{13}C-labeled lactone (Figure 3d), or unlabeled lactone (Figure 3a). Significant extraneous ^{13}C resonances are still present in the recrystallized lactone filtrate (Figure 3e). Concentrated crude lactone filtrate (Figure 3c) shows mostly lactone **5** by HPLC with UV detection, yet analyzes at less than 60% on a w/w basis, again indicating that it contains significant levels of nonchromophoric material. Clearly, isolation and recrystallization of atorvastatin lactone (**5**) has removed significant quantities of ^{13}C-labeled impurities.

Conversion of ^{13}C-labeled pyrrole **4a** into atorvastatin, without intermediate lactone isolation, was readily accomplished using the original synthesis. Since this original synthetic method was producing the low-assay atorvastatin, we had hoped to see extraneous ^{13}C-enriched resonances due to the impurities. Unfortunately, this was not the case and ^{13}C-enriched resonances due to the impurities were not resolved from existing atorvastatin. In fact, high-assay atorvastatin produced from ^{13}C-labeled lactone **5** gave a comparable ^{13}C NMR spectrum. Apparently, minute changes in local diamagnetic anisotropy caused by the conformational differences of lactone versus atorvastatin were all that allowed resolution of the ^{13}C-enriched impurities in lactone **5**.

Conclusions

^{13}C-enriched substrates and ^{13}C NMR monitoring of the modified atorvastatin synthesis allowed us to track generation and removal of some process impurities essentially analytically invisible by conventional methods. Although isotopic labeling is commonly used to trace biosynthetic pathways, this set of experiments shows that it can be a useful tool for the process chemist as well. In our case, this method provided further *qualitative* support to the *quantitative* improvements in atorvastatin purity already observed by switching to the lactone route.

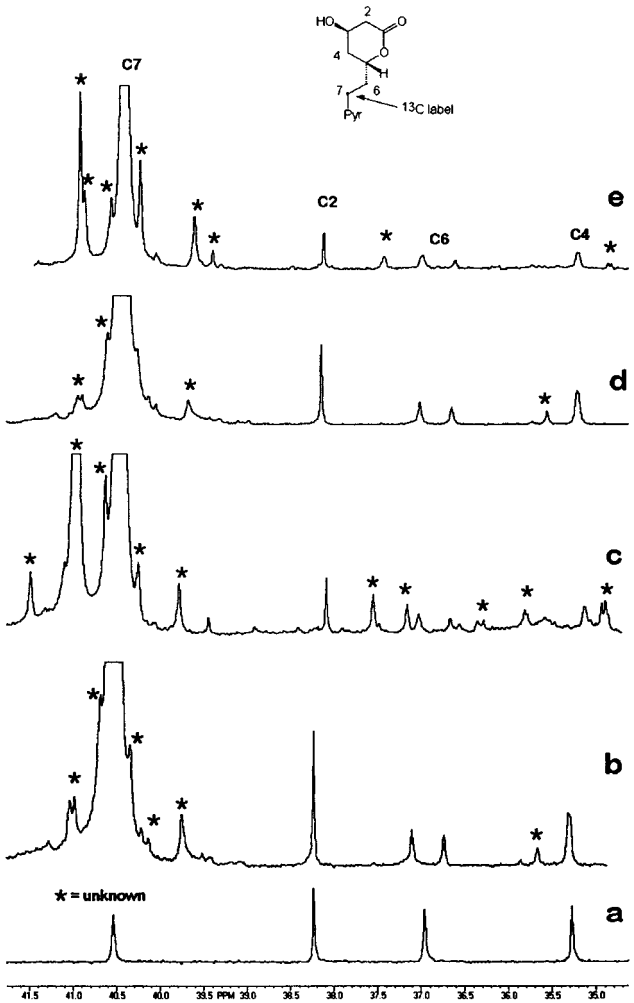

Figure 3. **Portions of the 100 MHz ^{13}C NMR spectra of (a) unlabeled and (b) ^{13}C-labeled crude (unrecrystallized) lactone 5 in $CDCl_3$. Spectrum c is the ^{13}C NMR spectrum of the filtrate residue from the first lactone crystallization. Spectrum d is the ^{13}C NMR spectrum of the recrystallized lactone. Spectrum e is the ^{13}C NMR spectrum of the filtrate residue from recrystallized lactone. Spectra b−e are vertically truncated to allow for ready comparison of unenriched ^{13}C resonances. Unidentified resonances due to synthetic impurities are marked with a asterisks.**

Experimental Section

General Procedures. Common laboratory solvents were used without further purification. All ^{1}H NMR spectra were obtained at 200 MHz (Varian Gemini 200) with tetramethylsilane as internal reference. ^{13}C NMR spectra were obtained at 100 MHz (Varian Unity 400) with the solvent ($CDCl_3$) as reference. Mass spectra were obtained on a Finnegan 4500 mass spectrometer using DCI with isobutane as CI gas. All melting points are uncorrected. The abbreviation w/w % indicates analysis compared to a reference standard.

Preparation of ^{13}C-Labeled (4*R*-*cis*)-1,1-Dimethylethyl 6-(Cyanomethyl)-2,2-dimethyl-1,3-dioxane-4-acetate (1c). (4*S*-*cis*)-1,1-Dimethylethyl 6-(hydroxymethyl)-2,2-dimethyl-1,3-dioxane-4-acetate (**1a**, 50 g, 192 mmol) was added to a cooled (about 0 °C) solution of 43 g (204 mmol) of

4-chlorobenzenesulfonyl chloride (97% purity, Aldrich) and 35 g (350 mmol) of triethylamine in methylene chloride (250 mL). The solution was allowed to warm to room temperature and stirred for 6 h. Water (250 mL) was added, the layers were separated, and the lower organic layer was concentrated *in vacuo*. The yellow oil was dissolved in DMSO (250 mL) and transferred to a flask containing 14.5 g (220 mmol) of $K^{13}CN$. Anhydrous methanesulfonic acid (1.1 g, 11 mmol) was added, and the stirred mixture was heated to 40−50 °C for about 60 h. After cooling to room temperature, ethyl acetate and water (500 mL each) were added, the layers were separated, and the organic layer was concentrated *in vacuo*. The residue was dissolved in hexane (500 mL) and washed with water (3 × 200 mL) and the solution cooled to about 5 °C for about 24 h. The crystallized product was isolated by filtration, washed with cooled hexane, and air-dried, resulting in nitrile **1c** (41 g, 79%) as an off-white solid. ^{1}H NMR (200 MHz in $CDCl_3$) δ 1.36 (m, 1H), 1.42 (s, 3H), 1.49 (s, 9H), 1.50 (s, 3H), 1.79 (dt, J = 2.5, 12.1 Hz, 1H), 2.40 (dd, J = 6.2,15.4 Hz, 1H), 2.5−2.7 (m, 1H), 2.55 (d, J = 6.1 Hz, 2H), 4.18 (m, 1H), 4.32 (m, 1H); MS DCI (isobutane) m/z 271 $[MH]^+$.

Preparation of ^{13}C-Labeled (2*R*-*trans*)-5-(4-Fluorophenyl)-2-(1-methylethyl)-*N*,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2*H*-pyran-2-yl)ethyl]-1*H*-pyrrole-3-carboxamide (5). A mixture of **1c** (20.0 g, 74 mmol), Activated Metals A7000 sponge nickel (5.3 g water-wet), isopropyl alcohol (150 mL), and 28% ammonia solution (55 mL) was placed in a 500 mL Parr pressure vessel, made inert, and reduced overnight with 50 psi of hydrogen. The mixture was filtered and concentrated *in vacuo*. The resulting oil was dissolved in warm toluene (100 mL), water washed, and again concentrated *in vacuo* to give amino ester **2.** Crude **2**, diketone **3** (34.0 g, 81 mmol), pivalic acid (5.0 g, 49 mmol), THF (55 mL), and hexanes (55 mL) were placed in a 500 mL pressure vessel, made inert with an argon blanket, sealed, and heated at 75 °C for 96 h. After cooling, the solution was diluted with *tert*-butyl methyl ether (MTBE, 160 mL), sequentially washed with dilute aqueous sodium hydroxide and dilute aqueous HCl, and concentrated *in vacuo* to give acetonide ester **4a.** Crude **4a** was dissolved in warm methanol (110 mL) and aqueous hydrochloric acid (2.0 g of 37% HCl in 30 mL of H_2O) added, and the mixture stirred overnight at 30 °C to produce diol ester **4b** as a thick white slurry. MTBE (40 mL) and aqueous sodium hydroxide (60 mL of H_2O and 10.0 g of 50% aqueous sodium hydroxide) were added, and the mixture was stirred overnight at 30 °C to produce sodium salt **4c**. Water (240 mL) was added, the mixture washed with MTBE (2 × 175 mL), and residual MTBE removed from the aqueous layer by distillation. After heating overnight at 80 °C, the solution was cooled, acidified, and extracted into toluene (350 mL). The toluene was heated at reflux for 4 h and water azeotropically removed. After cooling, filtration, and recrystallization from toluene, lactone **5** was isolated as an off-white solid, 20.03 g (50% from nitrile **1c**): mp 157−159 °C; ^{1}H NMR δ 1.5−1.6 (m, 7H), 1.8−1.7 (m, 2H), 1.9−1.8 (m, 1H), 2.6−2.5 (m, 2H), 3.4−2.9 (br s, 1H), 3.52 (septet, J = 7 Hz, 1H), 4.0 (doublet of multiplets, $J(^{13}C-^1H)$ = 130 Hz, 1H), 4.18 (doublet of multiplets, $J(^{13}C-^1H)$ = 130 Hz, 1H), 4.23 (m, 1H), 4.55 (m, 1H), 7.3−6.9 (m, 14H). Anal. Calcd for $^{13}C_1{}^{12}C_{32}H_{33}FN_2O_4$: C, 73.36; H, 6.14; N, 5.17. Found: C, 72.93; H, 6.20; N, 5.21.

Preparation of ^{13}C-Labeled Atorvastatin {[*R*-(*R,*R**)]-2-(4-fluorophenyl)-*β*,*β*-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrole-1-heptanoic Acid Calcium Salt (2:1)} (6).** To lactone **5** (18.0 g, 33 mmol) dissolved in MTBE (100 mL) and methanol (45 mL) was added sodium hydroxide (1.37 g, 34 mmol) dissolved in water (230 mL). The solution was heated to 50 °C, stirred for 1 h, and phased, and the aqueous layer was washed with MTBE (70 mL). To the aqueous layer were added MTBE (7 mL) and calcium acetate hydrate (2.9 g, 18 mmol) dissolved in water (100 mL) and atorvastatin trihydrate seeds (0.3 g). The resulting suspension was stirred at 50 °C for about 15 min, cooled, filtered, rinsed with aqueous methanol followed by water, and dried *in vacuo* to give atorvastatin (18.9 g, 94%) as a white crystalline trihydrate (HPLC w/w = 98.2%); ^{1}H and ^{13}C NMR were in accord with the structure.[3]

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