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Short communication

Structural elucidation of an unknown Simvastatin by-product in industrial synthesis starting from Lovastatin

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Abstract

Unknown by-product in Simvastatin synthesis from Lovastatin was found. The elucidation of this molecular structure by means of ^{1}H and ^{13}C NMR spectroscopy, HPLC/MS, MS/MS and FT-IR was shown. The mentioned by-product, originated during Merck Sharp and Dhome synthesis scheme was isolated in the second-last step replacing butylamine with benzylamine. The spectroscopic results agreed with a molecular formula $C_{32}H_{43}NO_3$. The proposed structure of this compound, characterised by the presence of a conjugated dienic system in the heptanoic acid amide residue, was α,β,γ,δ unsaturated Simvastatin *N*-benzylamide.

Keywords: Simvastatin; By-product; Structural elucidation; Dienic-derived compound; HPLC/MS/MS; NMR

1. Introduction

Simvastatin, [1*S*-[1α,3α,7β,8β(2*S**,4*S**),8αβ]]-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetra-hydro-4-hydroxy-6-*oxo*-2*H*-pyran-2-yl)ethyl]-1-naphtalenyl-2,2-dimethylbutanoate, is a semi-synthetic [1] derivative of a natural fungal metabolite [2]: Lovastatin, a cholesterol-lowering agent [3] used in hypercholesterolemia treatment. After oral administration Simvastatin is quickly hydrolyzed to the corresponding β-hydroxy acid, the effective active ingredient, which is a regulator of cholesterol synthesis by 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibition [4].

Several methods [5–9] for the synthesis of Simvastatin **2** from Lovastatin **1** are available. The industrial process object of our study was a Merck Sharp and Dhome [5] modified method consisting in eight steps (Scheme 1) with an overall yield from **1** to **2** of 49%. Moving from Lovastatin **1** the reaction with benzylamine led to the amide **3**, which was then protected by silylation of the hydroxyl groups giving compound **4**. This intermediate was methylated, deprotected, the amide hydrolyzed and than lactonized affording Simvastatin **2**. Following this synthetic scheme, an unknown by-product was isolated from ammonium salt **6** mother liquor, which did not correlate with any Simvastatin impurities in literatures [10,11] described.

The main purpose of this work was the structural identification of this by-product (Fig. 1) through different spectroscopic techniques.

2. Experimental

2.1. Apparatus

The ^{1}H and ^{13}C NMR and bi-dimensional analyses, Cosy, HSQC and Noesy phase sensitive were taken on a Bruker Avance 500 (Billerica, MA, USA) operating at 500 MHz. Chemical shifts were expressed as ppm (δ). Samples of about 5 mg were dissolved in 0.7 ml of $CDCl_3$.

HPLC/DAD analyses were performed using a Waters HPLC apparatus mod. Delta PREP600E equipped with a manual injector (loop 20 μl), an online degasser and a column block heater. The detector was a Diode Array (Waters mod. 2996, MA, USA) operating at 240 nm. The column employed was a Symmetry Shield RP 18 (Waters, MA, USA), 250 mm × 4.6 mm i.d. (5 μm particle size); mobile phase was a mixture of acetonitrile/water 85:15 (v/v) eluted at 0.8 ml/min with a temperature maintained

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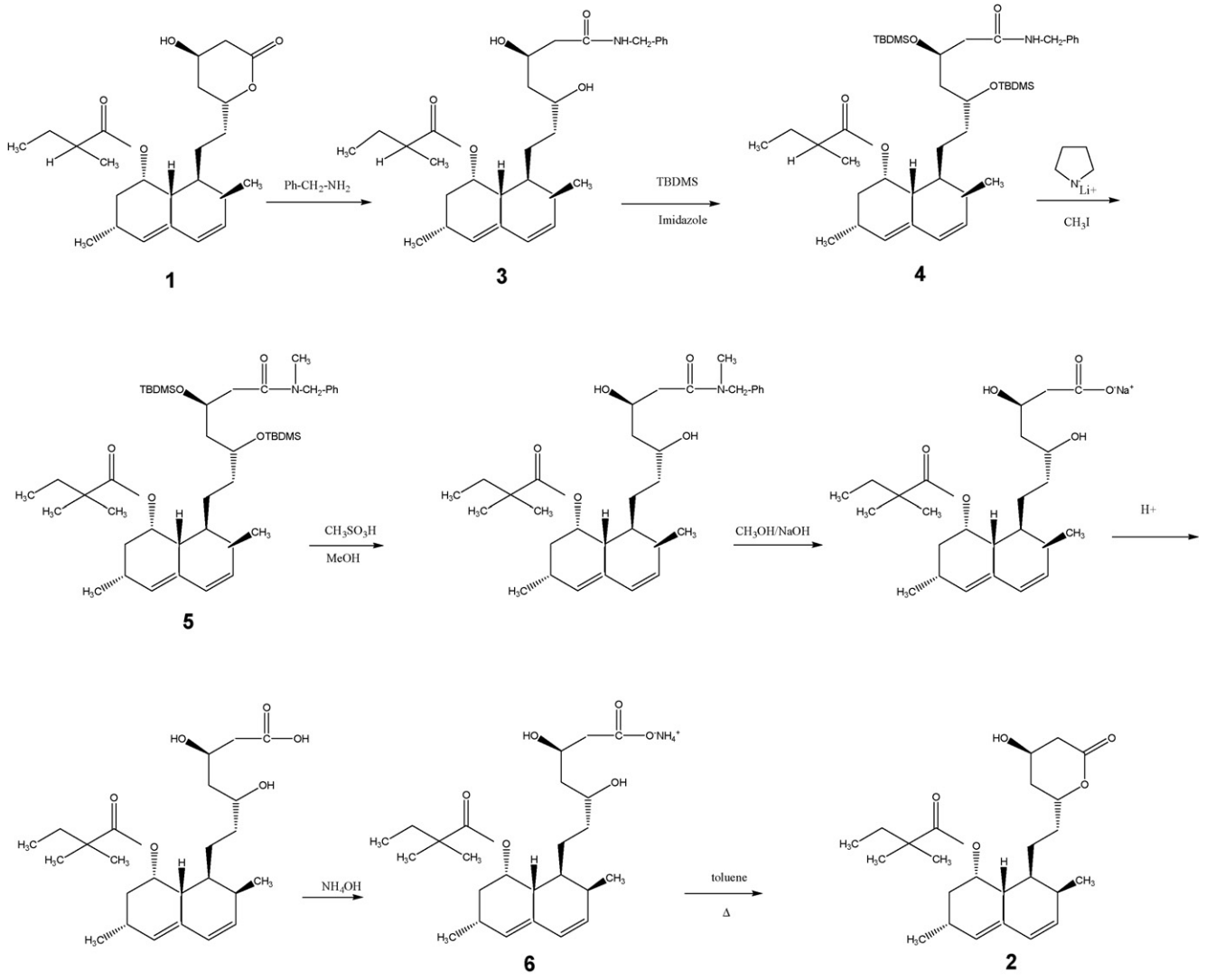


Scheme 1. Synthesis of Simvastatin **2** from Lovastatin **1**.

at 30 °C. The UV–vis peaks spectra were recorded ranging from 200 to 400 nm.

LC/DAD/MS analyses were performed by using a Thermo Finnigan (MA, USA) LCQ Advantage system equipped with a quaternary pump, Diode Array Detector (working wavelength 240 nm) and MS spectrometer with an Electrospray ionisation source and an "Ion Trap" mass analyser. The LC column employed was a Symmetry Shield RP 18 (Waters, MA, USA), 250 mm × 4.6 mm i.d. (5 μm particle size). The chromatographic conditions were the same used in LC/DAD experiments.

The LC–MS chromatograms and MS spectra were obtained under following conditions: ionisation, ESI positive; capillary temperature, 250 °C; source voltage, 5.50 kV; source current, 4.00 μA; multipoles 1 and 2 offset, −5.50 and −7.50 V respectively; intermultipole lens voltage, −16.00 V; trap dc offset voltage, −10.00 V.

FT-IR spectra were collected by using a Perkin Elmer (MA, USA) FT-IR Spectrometer "Spectrum One" in a spectral region between 4000 and 450 cm^{-1}. Samples were mixed in a mortar with KBr (1:100) and pressed in a hydraulic press (14 tonnes) to small tablets, which were then analysed by transmittance technique with 32 scansions and 4 cm^{-1} resolution.

2.2. Chemicals

All reagents and spectroscopic solvents grade employed for HPLC e LC/MS analyses and Chloroform-d_3 for NMR were purchased from Aldrich Chemical.

2.3. Synthesis of *N*-benzyl-7-[1,2,6,7,8,8a]-(R)-hexahydro-2(S), 6(R)-dimethyl-8(S)-[[2,2-dimethyl butanoyl] oxy]-[1(S)-naphtyl]-3(R)-5(R)-bis[(tert-butyldimethylsilyl)oxy]-heptanoic acid amide (*5*)

Operating under inert atmosphere of N_2 gas, a solution of *n*-butyllitium in hexane (2.5 M, 100 ml, 0.25 mol) was cooled to −20 °C and, keeping the temperature between −15 and −20 °C, a solution of sieve-dried pyrrolidine (22.7 ml, 0.27 mol) in abso-

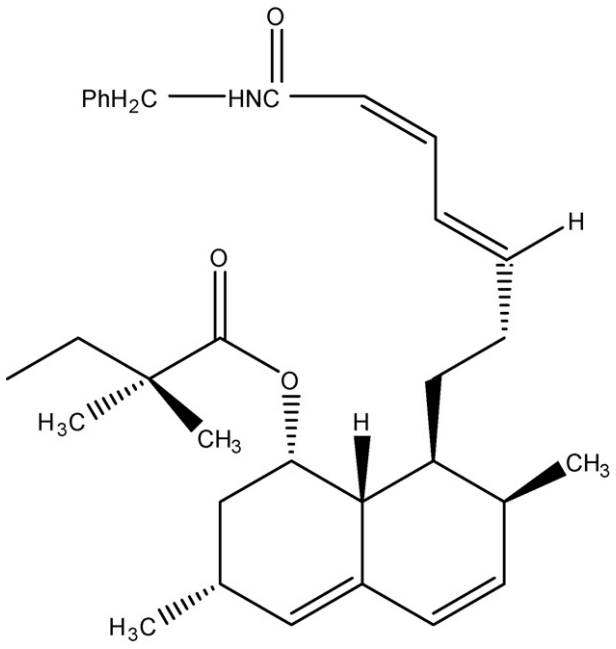


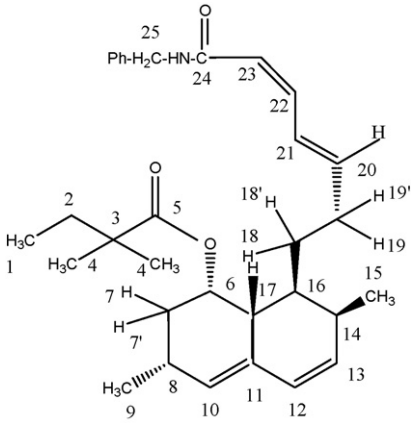
Fig. 1. Molecular structure proposed for the unknown by-product.

lute THF (190 ml) was added in a maximum time of 20 min. The reaction mixture was stirred at this temperature for 2 h and then cooled to −70 °C. A precooled (−30 °C) solution of crude amide **4** (54.7 g, 0.074 mol) in anhydrous THF (270 ml) was added at such a rate to maintain the temperature below −30 °C and the mixture was stirred for 2 h. Methyl iodide (10.9 ml, 0.175 mol) in one portion was added: after an initial −5 °C exotherm, the solution was recooled at −30 °C and stirred for 30 min, warmed up to −10 °C and stirred for 20 min and finally stored at 0 °C for one night. Keeping at 5 °C, water (165 ml) was added and the mixture stirred for 10 min. The phases were separated, and the organic layer was washed with 10 °C 1 N HCl (172 ml) solution; the resulting organic phase was evaporated under reduced pressure (under 40 °C) and the residue directly used without further purification.

*2.4. Synthesis of N-benzyl-7-[1,2,6,7,8,8a]-(R)-hexahydro-2(S),6(R)-dimethyl-8(S)-[[2,2-dimethylbutanoyl]oxy]-[1(S)-naphtyl]-3(R)-5(R)-dihydroxy-heptanoic acid ammonium salt (**6**)*

Methanol (450 ml), water (30 ml) and methanesulfonic acid were added to the crude heptanoic amide (**5**) and the mixture was stirred at 30 °C for 5 h. NaOH 2 N (200 ml) was added and the solution was heated while the distillate was collected at atmospheric pressure. The distillation, continued until the pot temperature was 78–80 °C, was then interrupted and the solution refluxed for 2 h. After cooling at room temperature, methanol was distilled under reduced pressure; the mixture was diluted with distilled water (150 ml) and cooled to 10 °C, adjusting the pH to 6–7 with HCl 3 N. Ethyl acetate was then added (500 ml) and the pH further adjusted to 5.0 with HCl 3 N. After stirring, the organic layer was separated and the aqueous layer extracted again with ethyl acetate (100 ml). To the combined organic phases methanol (53 ml) and aqueous ammonium hydroxide (13 ml, 33%) in methanol (37 ml) were added; the mixture was stirred at 20–25 °C for 1 h and then between −10 and −15 °C for 2.5 h. The ammonium salt crystals thus precipitated were filtered off, washed with ethyl acetate and dried overnight at 35 °C affording the ammonium salt (**6**) with 49% yield.

Table 1
^{1}H and ^{13}C chemical shifts of the unknown by-product



Position	^{1}H (ppm, multiplicity)	^{13}C (ppm)
1	0.86 (t, 3H), J = 7.5 Hz	8.6
2	1.51–1.64 (AB of system ABX_3) overlap with 18′	32.4
3	–	42.25
4	1.14 (s, 6H)	23.9 and 24.0
5	–	177.1
6	5.31 (m,1H)	67.4
7	1.96 (A of system ABXY), J = 15, J = 8.0, J = 3.0 Hz	31.9
7′	2.02 (B of system ABXY), J = 15, J = 3.0, J = ∼0 Hz overlap with 19	31.9
8	2.40–2.50 (m, 2H) overlap with H_{14}	26.6
9	1.09 (d, 3H), J = 7.4 Hz	22.3
10	5.51 (dd, 1H), J = 5.5, J = 3.0 Hz	128.8
11	–	131.1
12	6.01 (d, 1H), J = 9.6 Hz	125.6–127.9
13	5.82 (dd, 1H), J = 3.4 and 9,6 Hz	132.5
14	2.40–2.45 (m, 2H) overlap with H_8	29.6
15	0.90 (d,3H), J = 7.0 Hz	13.2
16	1.75 (m,1H), J = 12.0 Hz	35.6
17	2.26 (dm, 1H), J = 12.0	36.7
18	1.20–1.30 (m, 1H)	27.1
18′	1.50–1.60 (m, 3H) overlap with 2	27.1
19	1.95–2.04 (m, 3H) overlap with 7′	29.3
19′	2.30–2.34 (m, 1H)	29.4
20	5.92 (ddd, 1H), J = 7.1, J = 7.6, J = 15.1 Hz	142.6
21	7.51 (dd, 1H), J = 11.4 and 15.1 Hz	126.5
22	6.38 (t, 1H), J = 11.4 Hz	141.2
23	5.49 (d, 1H), J = 11.4 Hz	117.6
24	–	165.5
25	4.52 (d, 2H), J = 5.7 Hz	42.7
NH	5.78 (t, 1H), J = 5.7 Hz	–
CH arom.	7.30–7.38 (m, 5H)	125.6–127.9; 137.7

^{1}H and ^{13}C assignments: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; m, multiplet; J, coupling constant.

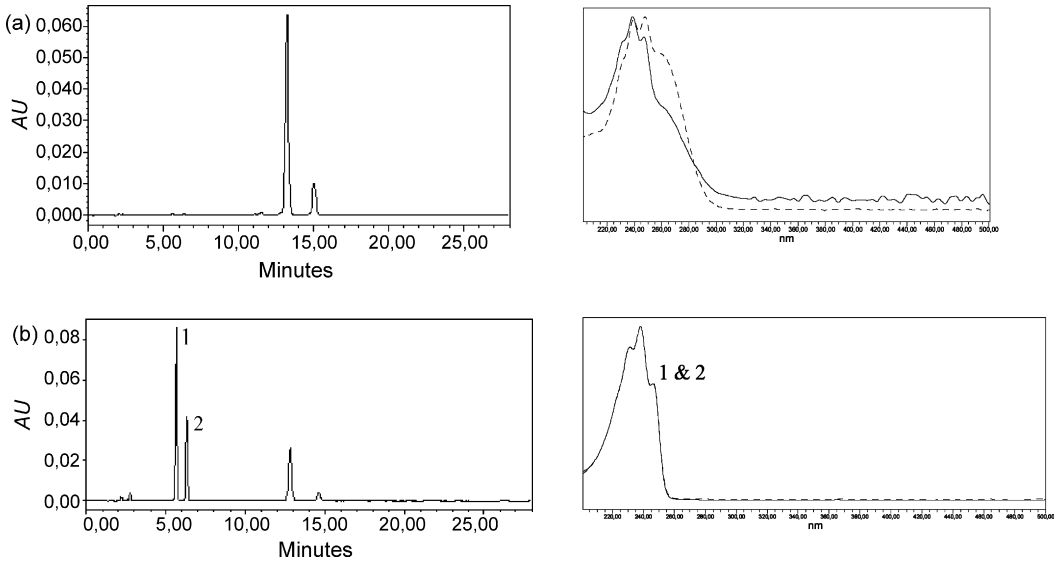


Fig. 2. HPLC chromatograms and UV–vis absorption spectra of by-products (a) and by-products spiked with Lovastatin (**1**) and Simvastatin (**2**) (b).

2.5. Isolation of N-benzyl-7-[1,2,6,7,8,8a]-(R)-hexahydro-2(S),6(R)-dimethyl-8(S)-[[2,2-dimethyl butanoyl]oxy]-[1(S)-naphtyl]-2,4-heptanoic acid amide (Fig. 2)

The mother liquor (ethyl acetate) obtained from the precipitation of the ammonium salt (**6**) where diluted with methanol (80 ml), stirred at room temperature for 1 h, decanted and filtered. The filtrate was stirred at 60 °C and diluted with distilled water (20 ml); when room temperature was reached, a precipitate separated. The crystals were filtered, washed with water/methanol 80/20 v/v and dried under reduced pressure giving of α,β,γ,δ unsaturated Simvastatin benzylamide (yield 50%).

$[M+H]^+$: 490 *m/z*; IR (cm^{-1}): 3420, 3014, 2961, 2931, 1716, 1665–1636, 1600 and 1548–1496.

^1H and ^{13}C are reported in Table 1.

3. Results and discussion

Merck S. and D synthesis method is one of the available industrial preparation procedures of Simvastatin. The first step of this scheme requested the employment of butyl amine as nucleophilic agent to afford delactonisation of Lovastatin; then, after hydroxyl group's protection, a solution of lithium pyrrolidine was added as base for methylation with methyl iodide. The method we followed, provided the use of benzylamine, the same hydroxyl groups protection and the subsequent addiction to a solution of lithium pyrrolidine. From this step, the reaction proceeded following Scheme 1 with an overall yield from **1** to **2** of 49% despite the Merck S. and D 90.9% yield.

In the Simvastatin ammonium salt preparation step, the reaction product precipitated in the medium while the unknown product object of this study was isolated from mother liquor

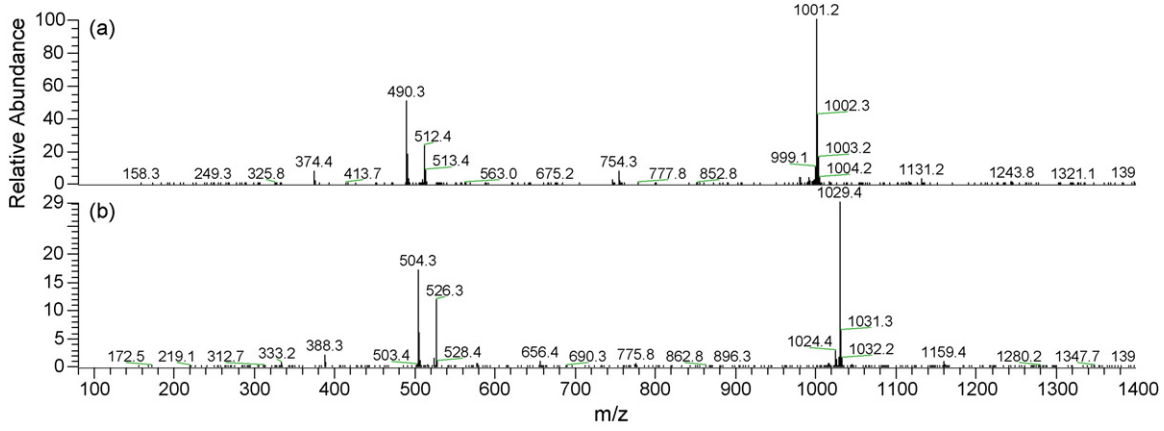


Fig. 3. Full mass spectra obtained from LC/MS analysis of α,β,γ,δ unsaturated Simvastatin *N*-benzylamide (a) and α,β,γ,δ unsaturated Simvastatin *N*-benzylmethylamide (b).

evaporation. The product has been characterized by analytical and spectroscopic techniques.

3.1. HPLC/DAD analysis

The chromatograms obtained from the isolated by-product showed the presence of a peak at retention time of 12.8 min with absorption λ_{max} at 239, 248 nm coupled with a peak at retention time of 14.6 min with λ_{max} at 239, 248 nm (Fig. 2a). The spiked chromatogram of this compounds confirmed the detection of new peaks imputable neither Lovastatin ($t_R = 5.6$ min, λ_{max} at 231, 238, 247 nm) nor Simvastatin ($t_R = 6.3$ min, λ_{max} at 231, 238, 247 nm) (Fig. 2b).

3.2. FT-IR

The infrared spectrum showed some absorption bands suggesting that the compound was not referable neither to Lovastatin nor Simvastatin. In fact from comparative IR spectra of the new dienic compound, Lovastatin and Simvastatin in the region between 3600 and 2700 cm^{-1} the free and associated O–H stretchings at 3542 cm^{-1} (Lovastatin) or 3551 cm^{-1} (Simvastatin) disappeared. A new sharp absorption band at 3420 cm^{-1} referred to an N–H stretching was revealed. Furthermore the spectrum showed a new system of bands over 3000 cm^{-1} corresponding to C–H sp^2 stretchings and a weak combination of overtone bands between 2000 and 1800 cm^{-1} corresponding to an aromatic system. Between 1750 and 1490 cm^{-1} the absorption bands system showed many changes; particularly diagnostic were the modifications in the C=O and C=C vibration stretchings in addition to 1665, 1650 and 1636 cm^{-1} bands due to C=O stretching and N–H bending of an amidic group. Besides, the C–O stretching bands lower intensity at 1221 cm^{-1} confirmed the absence of lactonic system contribution.

3.3. NMR

The ^{1}H NMR spectrum showed the presence of four different signals in the olefinic shift region exhibiting their resonance at δ 7.51(dd, 1H, $J = 11.4$; 15.1 Hz), δ 6.38 (t, 1H, $J = 11.4$ Hz), δ 5.92 (ddd, 1H, $J = 7.1$; 7.6; 15.1 Hz) and δ 5.49 (d, 1H, $J = 11.4$ Hz). The correct attribution of these signals to H in 21, 22, 20 and 23 positions respectively was possible using Cosy bi-dimensional technique. Besides, the spectrum revealed a triplet at 5.78 ppm exchanging by the addiction of D_2O, consistent with one amidic proton.

The use of heteronuclear correlation (HSQC) experiment allowed assigning the ^{13}C spectrum peaks at 142.6, 141.2, 126.5 and 117.6 ppm to CH in 20, 22, 21 and 23 positions respectively.

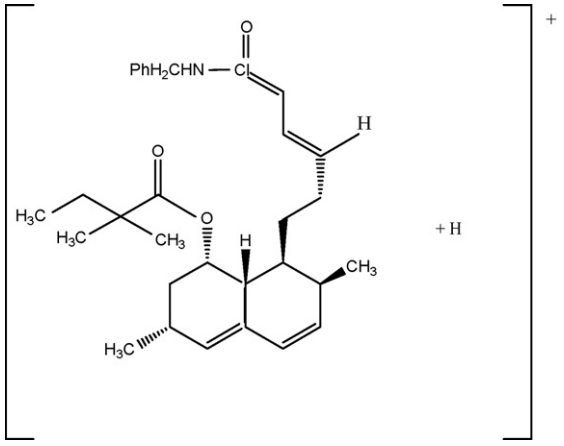
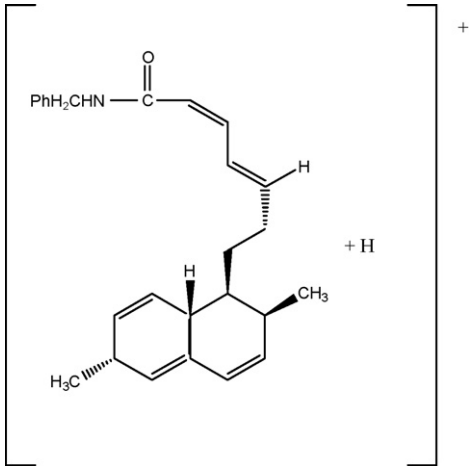
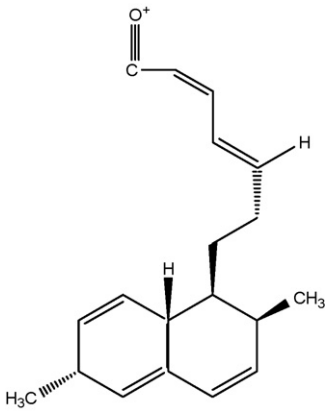
Bi-dimensional Noesy phase sensitive analysis allowed us to confirm that the stereochemistries of Lovastatin, used as starting material, and that of the by-product were the same.

3.4. LC/MS and MS/MS

The LC/MS analysis gave the possibility to directly collect the molecular weight of the analytes eluted (Fig. 3). The by-product full mass spectra (a) revealed the presence of a molecular ion peak at m/z 490 $[M+H]^+$ indicating a molecular weight for this compound at 489 m/z. The full mass spectra of the small coupled peak (b) (RT 14.6 min) showed a molecular ion peak at m/z 504 $[M+H]^+$; both peaks were associated with three diagnostic peaks corresponding to the $[M+Na]^+$ and the $[2M+Na]^+$ adducts and the first fragment due to the ester residue α-cleavage.

The MS/MS fragmentation pattern of the m/z 490 peaks is reported in Table 2.

Table 2
MS/MS fragmentation pattern of compound **6**

m/z	Fragment
490	
374	
267	

4. Conclusion

The new molecule generated during the Merck S. and D. Modified method we used, was identified as α,β,γ,δ unsaturated Simvastatin *N*-benzylamide and characterized by using various spectroscopic techniques; to our knowledge this is a new compound never in literature described.

LC/MS chromatograms has also shown the presence of small amount (15%) of another product corresponding to α,β,γ,δ unsaturated Simvastatin *N*-benzylmethylamide.

As reported in literatures [12–14] β carbonyl-silyloxy derivatives could give elimination reactions. Therefore, α,β,γ,δ unsaturated Simvastatin benzylamide probably generated during silyloxy compound **4** addiction to the lithium pyrrolidine solution; the strong basic conditions allowed also C21 and C23 deprotonation followed by the $TBDMSO^{-}Li^{+}$ loss, generating the new dienic compound object of our characterization.

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