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Characterization and preparation of Process Related Substances of Rosuvastatin-Calcium an anti lipidemic drug

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ABSTRACT

Rosuvastatin belongs to a group of medicines known as statins. It regulates the amount of cholesterol and other lipids made by your body, and helps to reduce the risk of heart and blood vessel disease. The dose range 5 to 40 mg orally once daily for a longer period . Presence of higher level of related substances or impurties may have harmful effect on body, hence needed to be identified ,synthesised & charateised for safer use of the medicine. During process optimization of Rosuvastatin calcium(Anti lipidemic drug), impurities ranging from 0.03 to 0.3 % were observed. These related substances or impurities were isolated, characterized and proposed structure was confirmed by chemical synthesis. The structure of these impurities were assigned as Bis 7-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methanesulfonylaminopyrimidin)-5-yl]-3R-hydroxy -5-oxo-(E)-6-heptenoate calcium (5-keto acid), 6-[(E)-2-[4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methanesulfonylaminopyrimidin)-5-yl] vinyl-4-hydroxytetrahydro-2H-pyran-2-one (Lactone), Bis (+) 7-[4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methane sulfonylaminopyrimidin)-5-yl] (3R, 5R) - dihydroxy-(E)-6-heptenoate calcium (3R, 5R-isomer), Bis (+) 7-[4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methane sulfonylaminopyrimidin)-5-yl] (3S, 5R) - dihydroxy-(E)-6-heptenoate calcium (3S, 5R-isomer) respectively. Investigation for the cause of these impurities helped in improvement of yield in bulk preparation of drug. The formation, synthesis and characterization of the Rosuvastatin calcium impurities are discussed.

Keywords: Rosuvastatin calcium• impurity profile • related substances • Enantiomers• Diastereoisomers

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INTRODUCTION

Rosuvastatin is used for Lowering high cholesterol and triglycerides in certain patients. It also increases high-density lipoprotein (HDL) ("good") cholesterol levels. It is used to slow atherosclerosis (narrowing of the arteries) in patients with high blood cholesterol levels. It is used in certain patients to reduce the risk of heart attack or stroke. It is also used in certain patients to reduce the need for medical procedures to open blocked heart vessels. It is used along with an appropriate diet. It may also be used for other conditions as determined by your doctor. Rosuvastatin Calcium (CRESTOR) is a synthetic, enantiomerically pure lipid-lowering agent. It is a selective, potent and competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in cholesterol biosynthesis.Studies have shown that rosuvastatin lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and cholesterol synthesis in the liver by increasing the number of hepatic Low Density Lipoprotein (LDL) receptors on the cell-surface for enhanced uptake and catabolism of LDL. Additionally, rosuvastatin inhibits the hepatic synthesis of Very Low Density Lipoprotein (VLDL), thereby reducing the total number of VLDL and LDL particles.

The HPLC analysis of Rosuvastatin Calcium displayed four impurity peaks in the range of 0.03 to 0.3% Levels along with the Rosuvastatin peak. As per the guidelines recommended by ICH, the acceptable level for a known or unknown related compound (impurity) is less than 0.15 and 0.10 % respectively in a drug substance. In order to meet the stringent regulatory requirements, the impurities present in the drug substance must be identified and characterized. Present work deals with the identification, synthesis and characterization of impurities/related substances of Rosuvastain Calcium [1-7].

MATERIALS AND METHODS

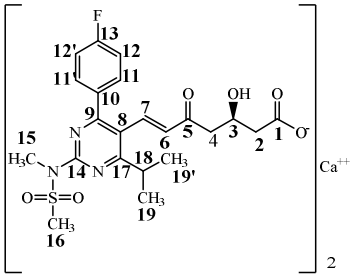
2.1Analytical

HPLC was carried out using Waters HPLC having 2487 UV detector with empower chromatography software. Column symmetry used is C18, 158× 4.6 mm, 5 µm with UV- Detector at 250 nm. Flow rate is maintained at 1.0 ml/min with injection volume of 10 µL using diluent Acetonitrile. The ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO on a Bruker Advance 300 spectrometer. The chemical shifts are reported in δ ppm relative to TMS (δ 0.00) and DMSO and D_2O as internal standards respectively. Electron Spray Ionization-Mass spectra (ESI-MS) of isolated compounds were measured using Agilent 1100 LC/MSD Trap SL instrument.

2.2Chemicals

All the chemicals and reagents used were of commercial grade.

Table 1: NMR data



Position	^{1}H	δ (ppm)	J (Hz)	^{13}C, J(Hz)	DEPT
1	-	-	-	177.53	-
2	2H	2.01-2.19	m	43.75	CH_2
3	1H	4.15	br, m	65.24	CH
4	2H	2.59 – 2.73	m	47.56	CH_2
5	-	-	-	198.34	-
6	1H	7.62 -7.72	m	134.53	CH
7	1H	6.14	d (16.5)[1]	136.98	CH
8	-	-	-	119.70	-
9	-	-	-	163.55	-
10	-	-	-	133.82	-
11, 11’	2H	7.62- 7.72	m	132.08, d(8.7)[3]	CH
12, 12’	2H	7.31	t(8.9)[1,2]	115.28, d(21.6)[3]	CH
13	-	-	-	162.86, d(246.6)[3]	-
14	-	-	-	157.45	-
15	3H	3.56	s	41.58	CH_3
16	3H	3.47	s	33.12	CH_3
17	-	-	-	174.37	-
18	1H	3.35-3.39	m	31.72	CH
19, 19’	6H	1.23	d(6.0)[1]	21.43	CH_3

NMR (DMSO – d_6, 300 MHz)

s- singlet, d- doublet, t- triplet, m-multiple, br- broad

[1] 1H- 1H Coupling constant, [2] 1H – 19F Coupling constant, [13]C – 19F Coupling constant

2.3Synthesis

2.3.1 Preparation of Bis 7-(4-fluorophenyl)-6-isopropyl-2-(N-methyl methanesulfonylaminopyrimidin)-5-yl]-3R-hydroxy -5-oxo-(E)-6-heptenoate calcium (Ca salt of 5-Keto acid)

To a solution of Methyl 7-[4-(4-flourophenyl)-6-isopropyl-2-(N-methyl-N-methylsulfonylamino)-pyrimidin-5-yl] – (3R)-3hydroxy-5-oxo-(E)-6-heptenate (10.0 g , 0.02 mol) in ethyl alcohol (100 ml) and tetrahydrofuran (40 ml), was added solution sodium hydroxide (0.8 g) in water (40 ml).The reaction mixture was stirred at room temperature for 2 h. The reaction mass was concentrated under reduced pressure to get residue. Water (100 ml) dichloromethane (100 ml) is added to the resulting residue and was cooled to 10^{o}C. pH was adjusted to 3.0 with ~ 35 % aqueous

hydrochloric acid solution and the layers were stirred and separated. The organic layer was washed with brine solution, dried and concentrated to get residue. To the residue added ethyl acetate and water and was cooled to 10^{o}C. pH was adjusted 9.0- 11.0 with ~ 4 % aqueous sodium hydroxide solution, stirred and the layers were separated. The aqueous layer was washed with ethyl acetate. To the aqueous layer was added a solution of Calcium acetate (1.97 g, 0.0124 mol) in water (50 ml).The suspension was stirred for 3 h at room temperature. The suspension was filtered and washed with water, dried to give of Bis 7-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methanesulfonylaminopyrimidin)-5-yl]-3R-hydroxy -5-oxo-(E)-6-heptenoate calcium (4.0 g, 40 %). MASS m/z 480 (M+H), 502 (M+Na) IR (cm $^{-1}$) 3428(OH stretching), 2968, 2929 (Aliphatic C-H stretching), 1509 (C=N stretching), 1382, 1338 (O=S=O stretching), 1231 (C-F stretching), 964 (Out of plane C-H bending), 846, 776 (Aromatic C-H bending), 566, 521 (C-S stretching); UV spectra (λ nm) 295, 256, 202

2.3.2 Preparation of Bis (+) 7-[4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methane sulfonylaminopyrimidin)-5-yl] (3S, 5R) - dihydroxy-(E)-6-heptenoate calcium (3S, 5R-isomer)

Preparation of this impurity is divided into 2 parts

(a) First Methyl (3S)-3-(tert-butyldimethylsilyloxy)-5-oxo-6-triphenylphosphoranylidene hexanoate is prepared

(b) Then Methyl (3S)-3-(tert-butyldimethylsilyloxy)-5-oxo-6-triphenylphosphoranylidene hexanoate is used for preparation of 3S, 5R isomer

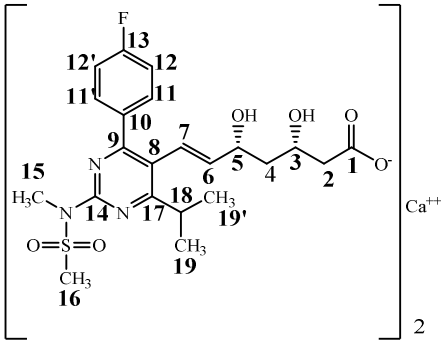
2.3.3 Preparation of Methyl (3S)-3-(tert-butyldimethylsilyloxy)-5-oxo-6 triphenyl phosphoranylidene hexanoate

A solution of Benzyl (S) Mandelate (50.0 g, 0.2 mol) in THF (1000 ml) was cooled to -78 ^{o}C, and a solution of 1.6 M BuLi in hexane (137 ml, 0.218 mol) was added dropwise, and the mixture was stirred for 30 min. To the reaction mixture was added a solution of 3-[(tert-butyldimethylsilyl) oxy] pentanedioic anhydride (50.4 g 0.2 mol) in THF 200 ml, and the resulting mixture was stirred for 120 min. The reaction mixture was acidified with 1:1 aqueous hydrochloric acid solution and the product was extracted with ethyl acetate. The organic layer was washed with water and sodium chloride solution. The organic layer was concentrated to give 3-[(tert-butyldimethylsilyl)oxy] pentanedioic acid, 1- [Benzyl(S) Mandelate] ester (101 g, 100 %). To a solution of 3-[(tert-butyldimethylsilyl) oxy] pentanedioic acid, 1- [Benzyl(S) Mandelate] ester (100 g, 0.205 mol) in ethyl acetate (1000 ml) was added 5 % Palladium on charcoal, and the mixture was stirred at room temperature in a hydrogen atmosphere for 120 min.The reaction mixture was filtered to remove the catalyst, and the filtrate was extracted with 5 % sodium bicarbonate solution (500 ml). The organic layer was extracted again with 300 ml 5 % sodium bicarbonate solution and the resulting aqueous layer was washed with ethyl acetate (500 ml). After the aqueous layer was partitioned with dichloromethane (500 ml) and acidified with ~ 36 % aqueous hydrochloric acid solution, the aqueous layer was extracted with dichloromethane and the organic layer was washed with water. The combine organic extracts were dried and concentrated to give (3S) 3-[(tert-butyldimethylsilyl) oxy] pentanedioic acid, 1- [(S) Mandelic acid] ester (55.0 g, 68 %). A solution of sodium methoxide (68.6 g , 1.27 mol) in methanol (240 ml) was added dropwise a solution of (3S) 3-[(tert-butyldimethylsilyl) oxy] pentanedioic acid, 1- [(S) Mandelic acid] ester (50.0 g, 0.126 mol) in methanol (100 ml) below 7 ^{o}C. Stirred for 60 min at same temperature and the mixture was poured into the115 ml ~ 36 % % aqueous hydrochloric acid solution, 230 ml water and 385 ml dichloromethane mixture. The organic layer was washed with water three times, dried and concentrated to give Hydrogen (3S) -1-Methyl-3-[(tert-butyldimethylsilyl)-oxy]pentanedioate (35.0 g, 100 %). A solution of Hydrogen (3S) -1-Methyl-3-[(tert-butyldimethylsilyl)-oxy] pentanedioate (16.5 g , 0.059 mol) in hexane (300 ml) and triethylamine (10.87 g, 0.107 mol) was cooled to -40^{o}C, ethylchlorocarbonate (9.71 g , 0.089 mol) was added drop wise in 2 h at same temperature. The reaction mixture was stirred for 120 min at same temperature to give Mixed anhydride of Hydrogen (3S) -1-Methyl-3-[(tert-butyldimethylsilyl)-oxy] pentanedioate. In another flask a suspension of methyltriphenylphosphonium bromide (59.7 g, 0.167 mol) in Tetrahydrofuran (350 ml) was cooled to 0^{o}C , and 1.6 M BuLi (97 ml , 0.155 mol) in hexane was added drop wise over 30 min. The reaction mass was stirred for 2 h at 20-25 ^{o}C.The mixture was cooled to – 80 ^{o}C. Mixed anhydride solution was added in 15 min. The resulting reaction mixture was stirred for 2 h at -80 ^{o}C.Then the reaction mass was poured into water. The organic layer was concentrated till get thick residue. The residue was taken into diisopropyl ether and washed with water, sodium bicarbonate solution and brine solution. Organic layer was dried and concentrated to give Methyl (3S)-3-(tert-butyldimethylsilyloxy)-5-oxo-6-triphenylphosphoranylidene hexanoate (30.0 g, 93 %).

2.3.4 Preparation of Bis (+) 7-[4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methane sulfonylaminopyrimidin)-5-yl] (3S, 5R) - dihydroxy-(E)-6-heptenoate calcium (3S, 5R-isomer)

A solution of 4-(4-flourophenyl)-6-isopropyl-2-(N-methyl-N-methylsulfonyl amino)-5-pyrimidine carbaldehyde (25.0 g, 0.071 mol), Methyl (3S)-3-(tert-butyldimethylsilyloxy)-5-oxo-6-triphenylphosphoranylidene hexanoate (53.25 g, 0.099 mol) is refluxed in acetonitrile (100 ml) under heating for 20 h and evaporated under reduced pressure to distill off acetonitrile. The resulting residue was taken into cyclohexane and stirred for 2 h at 15^{o}C. Filter the reaction mass to remove Triphenylphosphine oxide. Concentrate the filtrate containing methyl (3S)-3-(tert-butyldimethylsilyloxy)-5-oxo-6-triphenylphosphoranylidene hexanoate under reduced pressure to get thick residue.

Table 2: NMR data



Position	1H	δ (ppm)	J (Hz)	^{13}C, J(Hz)	DEPT
1	-	-	-	178.07	-
2Ha	1H	1.89-1.97	m	43.76	CH_2
2Hb	1H	2.07-2.11	m		
3	1H	3.71	-	65.73	CH
4Ha	1H	1.32	m	43.99	CH_2
4Hb	1H	1.48-1.50	m		
5	1H	4.19-4.21	-	68.61	-
6	1H	5.49-5.56	dd(16.1, 5.3)[1]	141.55	CH
7	1H	6.50	d (15.9)[1]	120.88	CH
8	-	-	-	121.87	-
9	-	-	-	162.86	-
10	-	-	-	134.45	-
11, 11’	2H	7.71	t(6.8)[1,2]	132.11, d(8.6)[3]	CH
12, 12’	2H	7.28	t(8.6)[1,2]	114.98, d(21.2)[3]	CH
13	-	-	-	162.53, d(245.6)[3]	-
14	-	-	-	156.78	-
15	3H	3.55	s	41.55	CH_3
16	3H	3.44	-	33.20	CH_3
17	-	-	-	174.27	-
18	1H	3.44	-	31.28	CH
19, 19’	6H	1.23	d(6.0)[1]	21.46	CH_3
OH	1H	5.04	br, s	-	-
OH	1H	6.47-6.53	br, s	-	-

NMR (DMSO – d_6, 300 MHz)
s- singlet, d- doublet, dd – doublet of doublet, t- triplet, m-multiplet, br- broad
^{1}H- ^{1}H Coupling constant
^{1}H – ^{19}F Coupling constant
^{13}C – ^{19}F Coupling constant

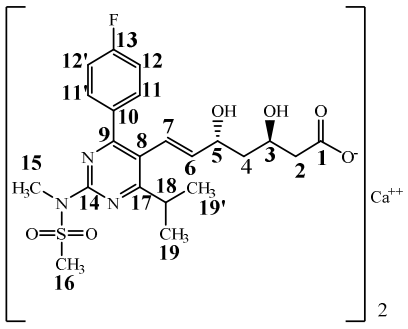
The residue was taken into acetonitrile (87 ml) was cooled to 0^{o}C, a solution of 82.5 ml ~ 40 % aqueous HF in acetonitrile (87 ml) was added dropwise at 0^{o}C, and the mixture was warmed to room temperature and stirred for 1.5 h.To the reaction mixture was added dichloromethane (500 ml) and washed the reaction mass thrice with water, then with saturated sodium bicarbonate solution and brine solution. The organic layer was dried and concentrated to give Methyl 7-[4-(4-flourophenyl)-6-isopropyl-2-(N-methyl-N-methylsulfonylamino)-pyrimidin-5-yl] – (3S)-3hydroxy-5-oxo-(E)-6-heptenate (30.0 g, 85 %). To 500 ml anhydrous tetrahydrofuran added 2.5 g sodium borohydride at -78 ^{o}C, to this mixture added a solution of 57 ml of 1 M diethylmethoxyborane-THF is added at -78 ^{o}C, and the mixture is stirred at same temperature for 30 minutes. To this mixture slowly added 25 g of the compound (**2**) dissolved in 500 ml of anhydrous THF and 175 ml of methanol and the mixture is stirred for 3 h. After conforming the reaction by TLC 31ml acetic acid is added and the mixture is adjusted to pH 8 with saturated sodium bicarbonate and extracted with ethyl acetate (250 ml). The organic layer is washed with water, dried and the ethyl acetate is evaporated under reduced pressure. To the resulting residue methanol is added and the mixture is evaporated under reduced pressure for three times to give Methyl-7-[4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methylsulfonylamino) pyrimidin-5-yl]-(3S, 5R)-dihydroxy-(E)-6-heptenate (25.0 g, 99 %). To a solution of Methyl-7-[4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methylsulfonylamino) pyrimidin-5-yl]-(3S, 5R)-dihydroxy-(E)-6-heptenate (25.0 g, 0.05 mol) in ethanol (150 ml) is added solution of sodium hydroxide (2.0 g, 0.05 mol) under ice cooling. The reaction mixture is warmed to room temperature and stirred for 2 hrs. The solvent is distilled off under reduced pressure.Water (125 ml) and ethyl acetate (250 ml) is added to the resulting residue and was cooled to 10 ^{o}C. pH was adjusted 3.0 with ~ 35 % aqueoushydrochloric acid solution stirred and the layers were separated. The organic layer is washed with brine solution, dried and concentrated to get residue. To the residue added methyl tert-butyl ether and water and was cooled to 10 ^{o}C. Adjusted pH to 9.0-11.0 with ~ 4 % Aq.sodium hydroxide solution

stirred and separated the layers. Washed the aqueous layer with Methyl tert-butyl ether. Degassed the aqueous layer under vacuum to remove the traces of MTBE.To the aqueous layer added solution of calcium acetate (8.0 g, 0.05 mol) in water (125 ml).Stirred the suspension for 3 hrs at room temperature. Filter and washed with water, dried to give Bis (+) 7-[4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methane sulfonylaminopyrimidin)-5-yl] (3S, 5R) - dihydroxy-(E)-6-heptenoate calcium (3S, 5R-isomer) (15.0 g, 59 %). MASS m/z = 482 (M+ H), IR 3430 (OH stretching), 2967, 2929 (CH stretching), 1510 (C=N stretching), 1382 (aliphatic CH stretching), 1337 (C-N stretching), 1229, 1156 (C-F stretching), 966 (=CH bending), 844, 776 (Aromatic C-H bending), 577, 519 (C-S stretching); UV spectrum (λ nm) 244, 202

2.3.5 Preparation of Bis (+) 7-[4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methane sulfonylaminopyrimidin)-5-yl] (3R, 5R) - dihydroxy-(E)-6-heptenoate calcium (3R, 5R-isomer)

To 500 ml anhydrous tetrahydrofuran, was added 2.5 g sodium borohydride at -78 ^{o}C, and the mixture was stirred at same temperature for 30 minutes. To this mixture, was slowly added 25 g of the compound (**2**) dissolved in 500 ml of anhydrous THF and 175 ml of methanol and the mixture was stirred for 3 h. After conforming the reaction by TLC 31 ml acetic acid is added, and the mixture is adjusted to pH 8 with saturated sodium bicarbonate and extracted with ethyl acetate (250 ml). The organic layer is washed with water, dried and the ethyl acetate is evaporated under reduced pressure. To the resulting residue methanol is added and the mixture is evaporated under reduced pressure for three times to give Methyl-7-[4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methylsulfonylamino) pyrimidin-5-yl]-(3R, 5R)-dihydroxy-(E)-6-heptenate (25.0 g, 99 %). To a solution of Methyl-7-[4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methylsulfonylamino) pyrimidin-5-yl]-(3R, 5R)-dihydroxy-(E)-6-heptenate (25.0 g, 0.05 mol) in ethanol (150 ml) is added solution of sodium hydroxide (2.0 g , 0.05 mol) under ice cooling. The reaction mixture is warmed to room temperature and stirred for 2 h. The solvent is distilled off under reduced pressure. Water (125 ml) ethyl acetate (250 ml) is added to the resulting residue and the mixture was cooled to 10^{o}C. pH was adjusted to 3.0 with ~ 35 % aqueous hydrochloric acid solution stirred and the layers are separated. The organic layer was washed with brine solution, dried and concentrated to get residue. To the residue was added methyl tert-butyl ether and water and was cooled to 10^{o}C. pH was adjusted 9.0-11.0 with ~ 4 % aqueous sodium hydroxide solution, stirred and the layers were separated. Aqueous layer was washed with Methyl tert-butyl ether. Aqueous layer was degassed under vacuum to remove the traces of MTBE. To the aqueous layer was added a solution of Calcium acetate (8.0 g, 0.05 mol) in water (125 ml). The suspension was stirred for 3 h at room temperature. Filter the reaction mass and washed with water, dried to give Bis (+) 7-[4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methane sulfonylaminopyrimidin)-5-yl] (3R, 5R) - dihydroxy-(E)-6-heptenoate calcium (3R, 5R-isomer) (15.0 g, 59 %), MASS m/z = 482 (M+H), IR (cm^{-1}) 3393 (OH stretching), 2968, 2934 (Aliphatic CH stretching), 1510 (Aromatic C=C, C=N stretching), 1382, (Aliphatic CH bending), 1336 (O=S=O Asymmetrical stretching), 1230 (C-F stretching), 1155 (O=S=O symmetrical stretching), 965 (Out of plane C-H bending), 845, 776 (Aromatic C-H bending), 576, 567, 520 (C-S stretching), UV λ(nm) 243, 204

Table 3: NMR data



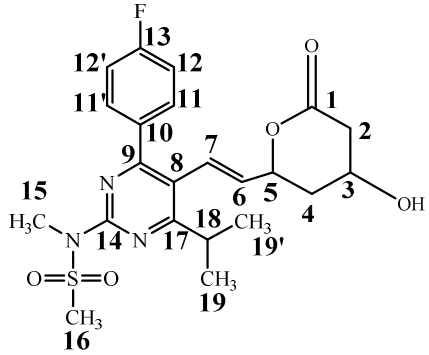
Position	1H	δ (ppm)	J (Hz)	^{13}C, J(Hz)	DEPT
1	-	-	-	178.37	-
2Ha	1H	1.89-1.97	m	43.90	CH_2
2Hb	1H	2.04-2.10	m		
3	1H	3.87	br, m	64.57	CH
4	2H	1.29-1.31	m	44.31	CH_2
5	1H	4.22	br	67.42	-
6	1H	5.50-5.57	dd(15.9, 5.1)[1]	142.42	CH
7	1H	6.50	d (16.2)[1]	120.30	CH
8	-	-	-	121.90	-
9	-	-	-	162.76	-
10	-	-	-	134.44, d(3.0)[3]	-
11, 11’	2H	7.69-7.74	dd(8.4, 5.7)[1,2]	132.10, d(8.6)[3]	CH
12, 12’	2H	7.28	t(8.7)[1,2]	114.91, d(21.5)[3]	CH
13	-	-	-	162.50, d(245.9)[3]	-
14	-	-	-	156.75	-
15	3H	3.54	s	41.52	CH_3
16	3H	3.45	s	33.15	CH_3
17	-	-	-	174.23	-
18	1H	3.37-3.45	m	31.26	CH
19, 19’	6H	1.21	d(6.6)[1]	21.42	CH_3
OH	2H	4.94 & 6.22-6.26	br, s	-	-

NMR (DMSO – d_6, 300 MHz)
s- Singlet, d- doublet, dd – doublet of doublet, t- triplet, m-multiplet, br- broad
^{1}H- ^{1}H Coupling constant
^{1}H – ^{19}F Coupling constant
^{13}C – ^{19}F Coupling constant

2.3.6 Preparation of 6-[(E)-2-[4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methanesulfonylaminopyrimidin)-5-yl] vinyl-4-hydroxytetrahydro-2H-pyran-2-one (Lactone)

To 500 ml ethyl acetate, was added 20.0 g rosuvastatin calcium, and to this 250 ml water was added. The mixture was adjusted to pH 3.0 with aqueous hydrochloric acid solution. Stirred and the layers are separated. Washed the organic layer with water and brine solution, dried and concentrated under reduced pressure to give thick residue. To the residue was added 200 ml toluene, and heated the content to the reflux for 5 h using Dean stark apparatus. Cooled the solution to 0^{o}C, stirred, filtered and washed with toluene. Dried the compound to get 10.2 g 6-[(E)-2-[4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methanesulfonylaminopyrimidin)-5-yl] vinyl-4-hydroxytetrahydro-2H-pyran-2-one (Lactone). MASS m/z 464 (M+H), 486 (M+ Na); IR 3468 (OH stretching), 2968, 2932 (Aliphatic CH stretching), 1742, 1714 (C=O stretching), 1604, 1548, 1509 (Aromatic C=C, C=N stretching), 1438, 1382 (Aliphatic CH bending), 1338 (O=S=O Asymmetrical stretching), 1235 (C-F stretching), 1156 (O=S=O symmetrical stretching), 963 (Out of plane C-H bending), 845, 778 (Aromatic C-H bending), 576, 521 (C-S stretching), UV λ(nm) 244, 204

Table 4: NMR data

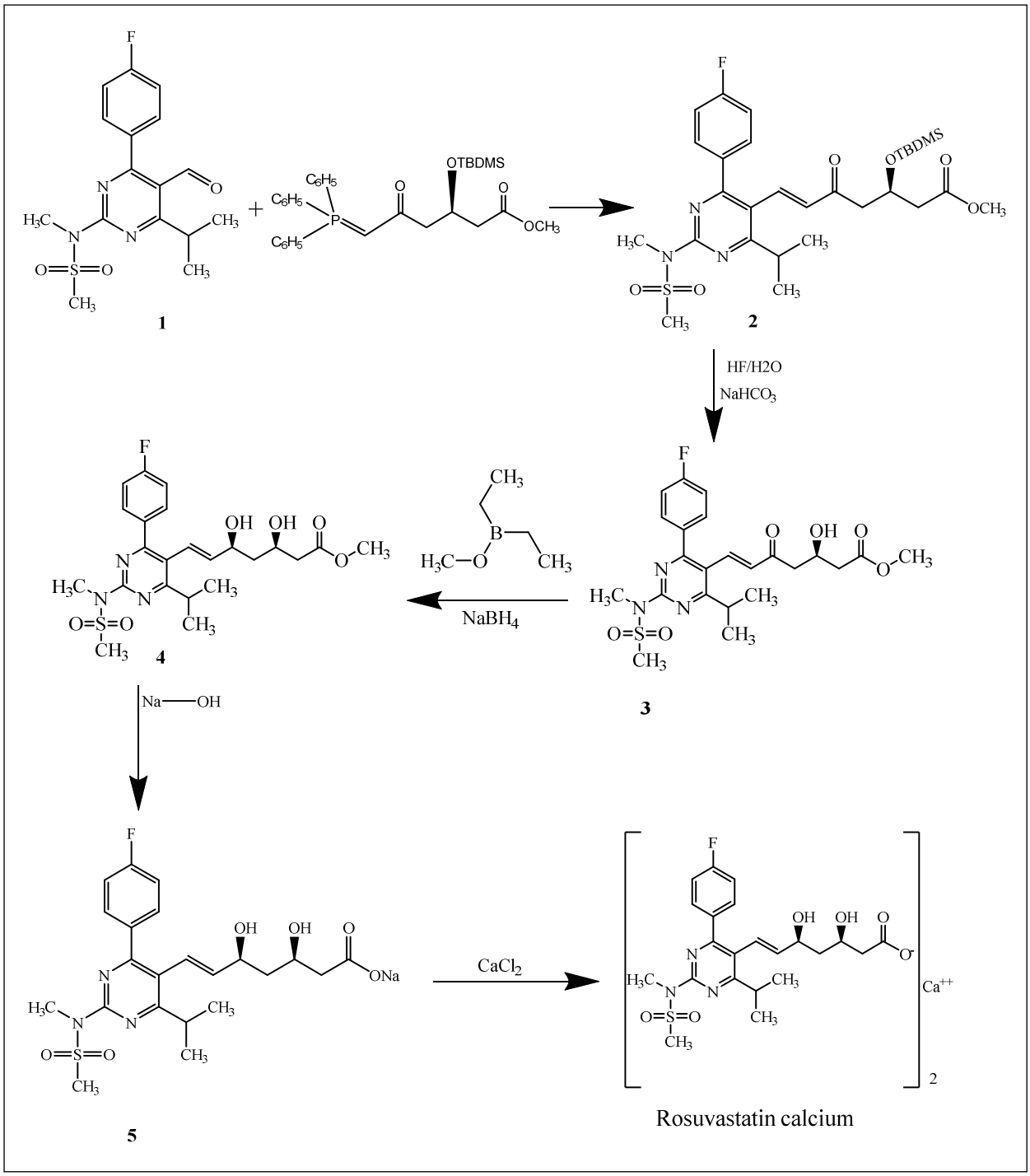


Position	1H	δ (ppm)	J (Hz)	13C, J(Hz)	DEPT
1	-	-	-	169.65	-
2Ha	1H	2.36-2.43	dd (17.1, 2.4)	34.86	CH_2
2Hb	1H	2.62-2.69	dd (17.4, 4.5)		
3	1H	4.08	br, m	61.04	CH
4	2H	1.60-1.78	m	38.51	CH_2
5	1H	5.12-5.19	m	75.38	-
6	1H	5.53-5.60	dd(16.2, 6.6)[1]	133.65	CH
7	1H	6.75	d (16.2)[1]	124.83	CH
8	-	-	-	121.01	-
9	-	-	-	163.09	-
10	-	-	-	134.24	-
11, 11’	2H	7.67-7.72	dd(8.7, 5.4)[1,2]	132.04, d(8.6)3	CH
12, 12’	2H	7.28-7.34	m	114.97, d(21.5)3	CH
13	-	-	-	162.54, d(245.4)3	-
14	-	-	-	157.06	-
15	3H	3.55	s	33.12	CH_3
16	3H	3.46	s	41.53	CH_3
17	-	-	-	174.19	-
18	1H	3.33-3.40	m	31.56	CH
19, 19’	6H	1.23	d(6.6)[1]	21.18, 21.25	CH_3
OH	1H	5.25	d(2.4)	-	-

NMR (DMSO – d_6, 300 MHz)
s- singlet, d- doublet, dd – doublet of doublet, t- triplet, m-multiplet, br- broad
1 ^1H- ^1H Coupling constant
2 ^1H – ^{19}F Coupling constant
3 ^{13}C – ^{19}F Coupling constant

RESULTS AND DISCUSSION

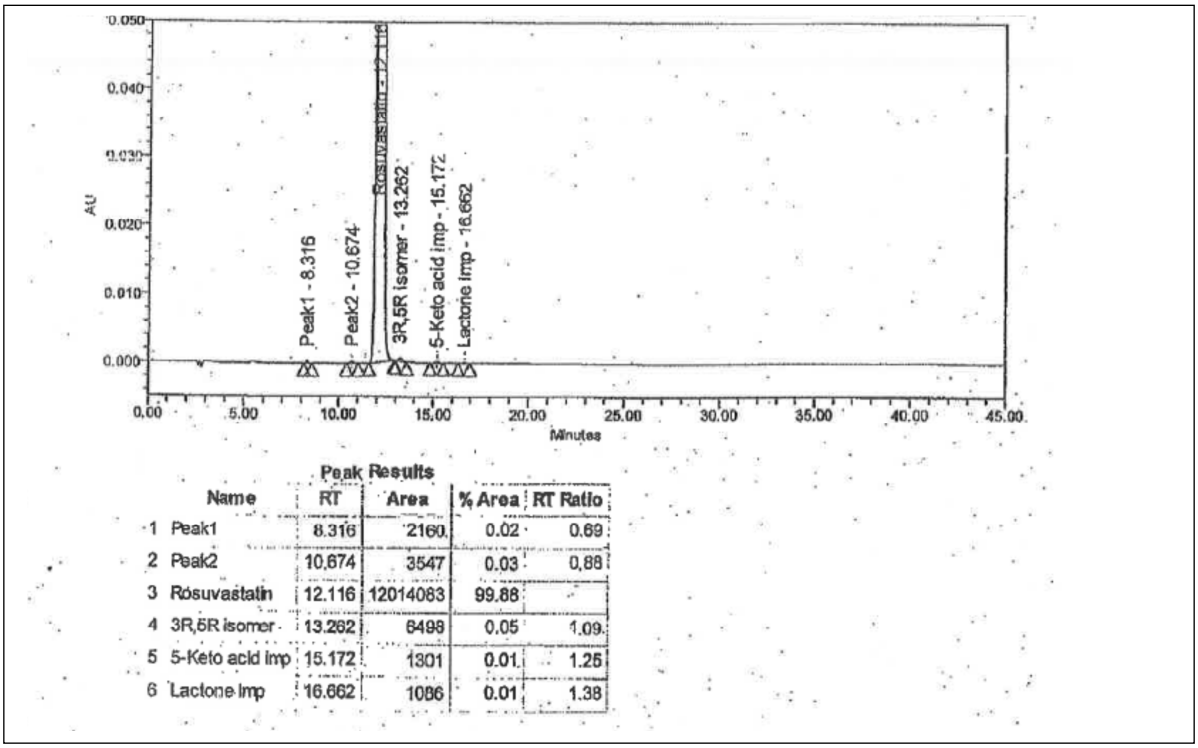
In the literature, many processes are described for the synthesis of Rosuvastatin calcium (Kentaro et al,. 1992, Lim et al,. 2003, De et al,. 2004, Niddam-Hildesheim et al,. 2005, Balanov et al,. 2006, Diorazio et al,. 2000, Okada et al,. 2005). An important route for the synthesis of Rosuvastatin is shown in Scheme 1 in which 4-(4-flourophenyl)-6-isopropyl-2-(N-methyl-N-methylsulfonyl amino)-5-pyrimidine carbaldehyde is reacted with Methyl (3R)-3-(tert-butyldimethylsilyloxy)-5-oxo-6-triphenylphosphoranylidene hexanoate, then cleavage of OTBDMS group with hydrofluoric acid , then selective reduction of keto group by using diethyl methoxyborane and sodium borohydride , then reaction with sodium hydroxide to cleave methyl ester then with Calcium source (Calcium chloride Or Calcium acetate) to get Rosuvastatin calcium.



Scheme 1.Synthetic scheme followed for preparation of Rosuvastatin Calcium

A typical analytical LC chromatogram of a laboratory sample of rosuvastatin Calcium displayed impurities/related compounds over a range of 0.03-0.30%. These impurities were identified, synthesized and characterized by spectral analysis (Figure 1). An HPLC chromatogram of rosuvastatin with impurities is given below (Figure 2). 5- keto acid impurity is observed at relative retention time (RRT)-1.09, Lactone impurity at RRT- 1.38 and 3R, 5R isomer at RRT- 1.09. A separate chiral method was used to determine the chiral isomer i.e. 3S, 5R isomer of rosuvastatin (Figure 3). 3S, 5R isomer was observed at RRT – 1.19.

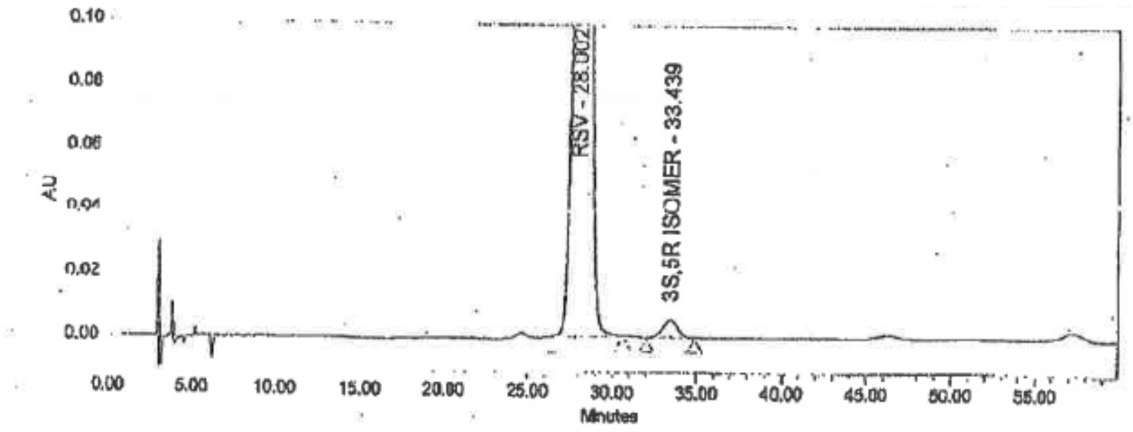
Fig. 1Impurities of Rosuvastatin calcium



Peak Results

	Name	RT	Area	% Area	RT Ratio
1	Peak1	8.316	2160	0.02	0.69
2	Peak2	10.674	3547	0.03	0.88
3	Rosuvastatin	12.116	12014083	99.88	
4	3R,5R isomer	13.262	6498	0.05	1.09
5	5-Keto acid Imp	15.172	1301	0.01	1.25
6	Lactone Imp	16.662	1086	0.01	1.38

Fig. 2Chromatogram of Rosuvastatin with related substances

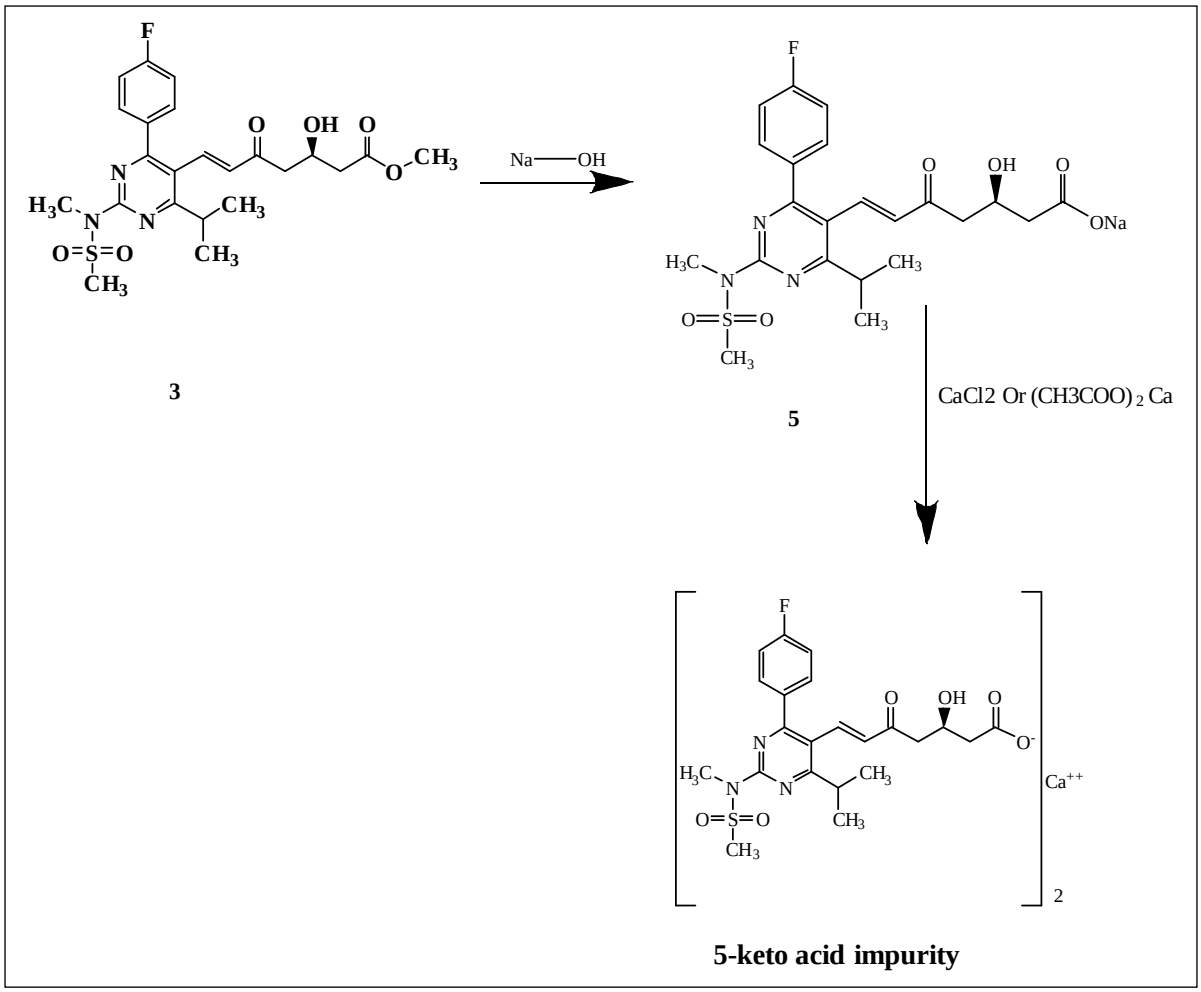


Peak Results

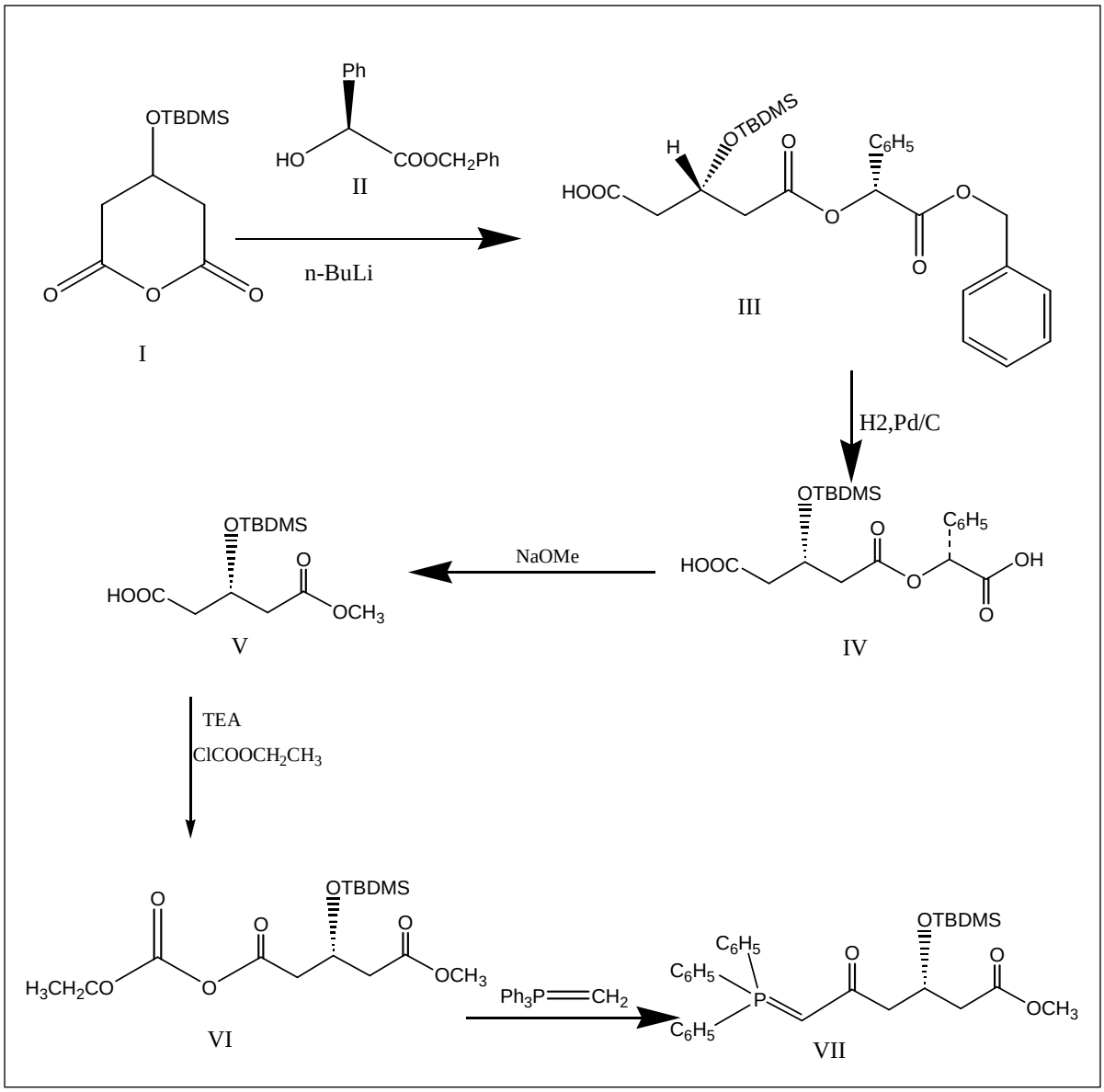
	Name	RT	Area	% Area	RT Ratio	USP Resolution	USP Tailing	USP Plate Count
1	RSV	28.00	23035017	98.53			1.18	6664
2	3S,5R ISOMER	33.44	342488	1.47	1.19	3.57	1.03	6922
Sum				100				

Fig. 3Chiral chromatogram of Rosuvastatin with 3S, 5R isomer

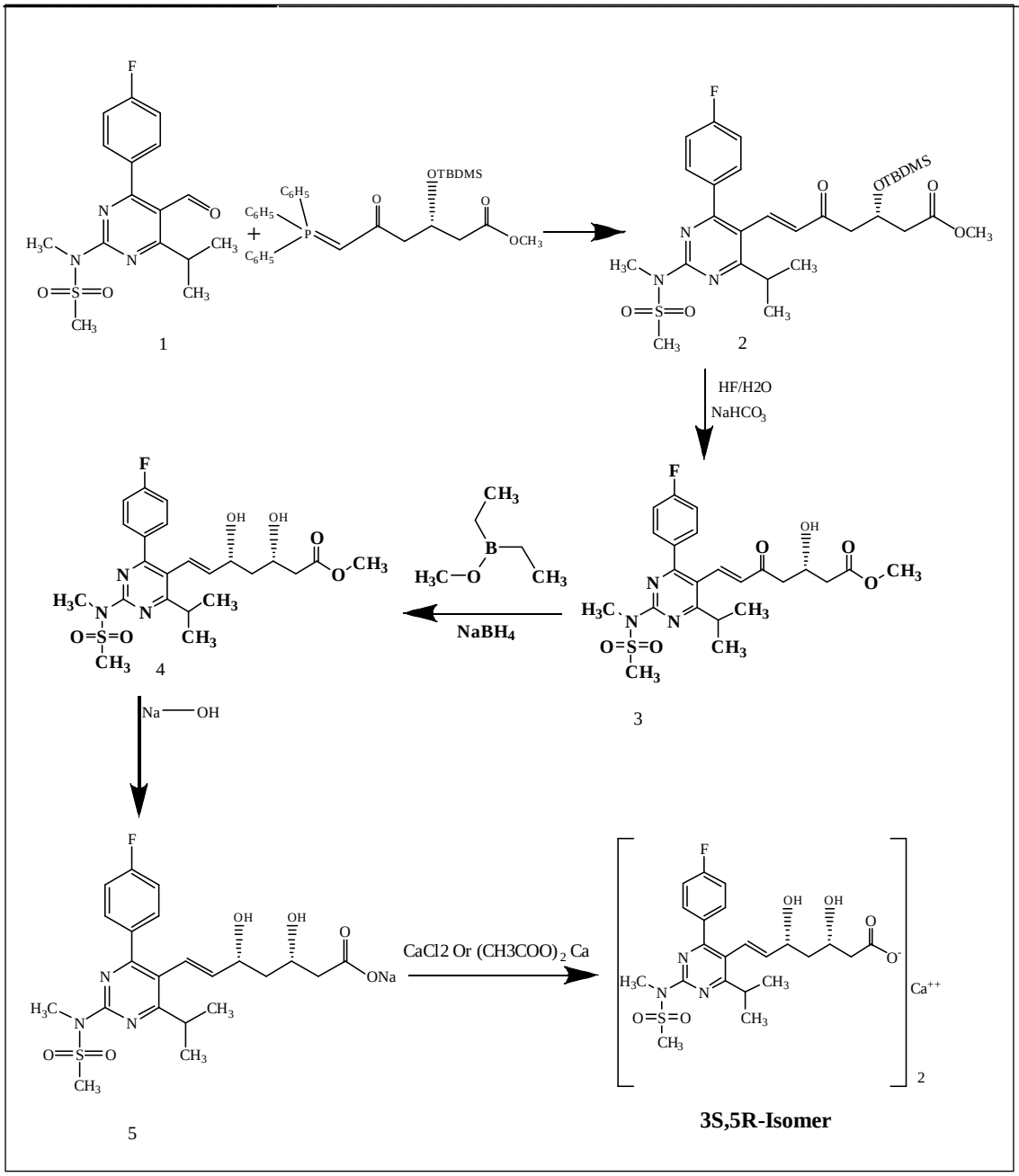
5-Keto acid impurity will form as an impurity during the synthesis of rosuvastatin Calcium if unreacted **3 is** present in the preparation of compound **4**. And also it will increase during the storage of the drug due to air oxidation. (3S, 5R) - isomer impurity was prepared by reaction of intermediate **3** by the following process(Scheme 2). This impurity is prepared by reaction of Methyl 7-[4-(4-flourophenyl)-6-isopropyl-2-(N-methyl-N-methylsulfonylamino)-pyrimidin-5-yl] – (3R)-3hydroxy-5-oxo-(E)-6-heptenate with sodium hydroxide followed by reaction with calcium acetate. This impurity will form if the methyl (3R)-3-(tert-butyldimethylsilyloxy)-5-oxo-6-triphenylphosphoranylidene hexanoate side chain has 3R-Isomer. This impurity formation was prepared by using Methyl (3S)-3-(tert-butyldimethylsilyloxy)-5-oxo-6-triphenylphosphoranylidene hexanoate side chain (Scheme 3 and 4). (3R, 5R) isomer impurity will form during selective keto reduction. This impurity was prepared by using intermediate **3** without using selective reducing agent (Scheme 5). Lactone impurity will be formed in acidic conditions. This impurity was prepared using Rosuvastatin calcium by refluxing in acidic pH (Scheme 6).



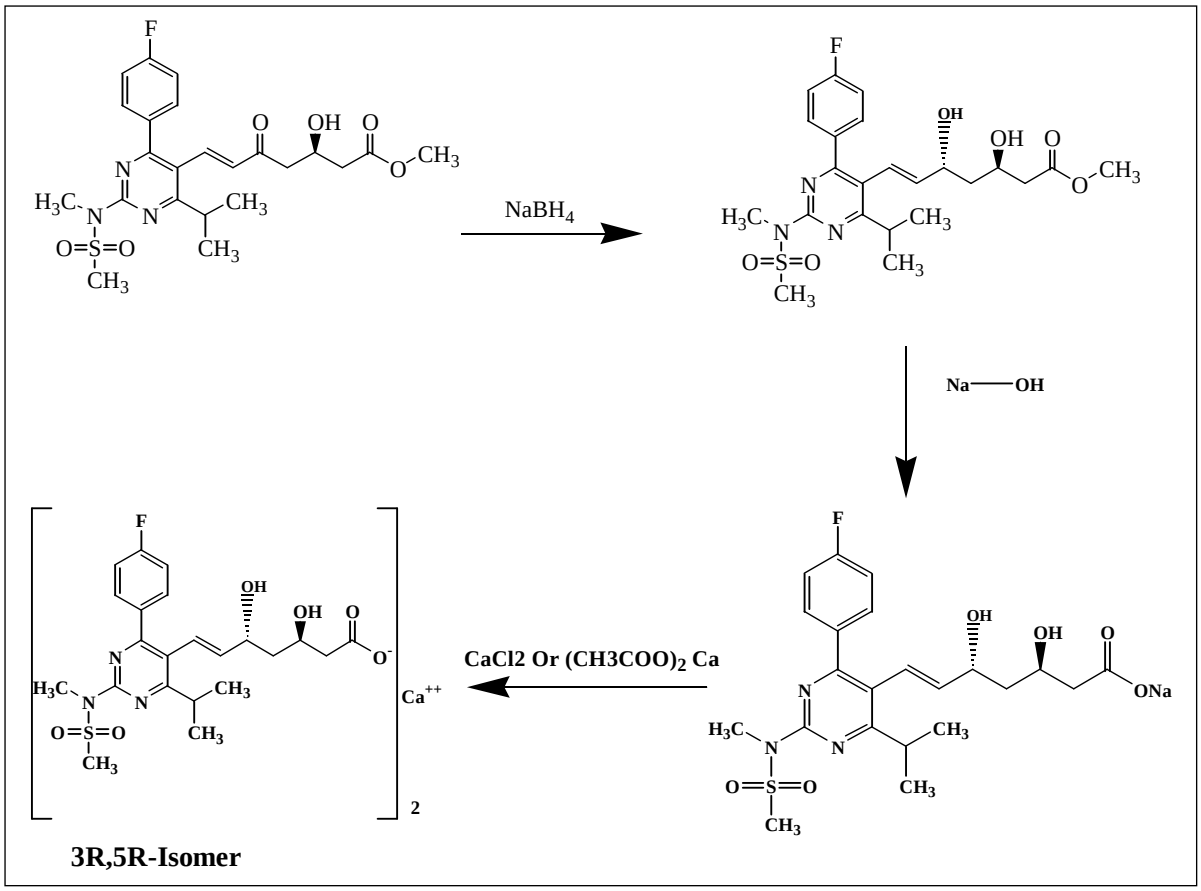
Scheme 2. Descriptionof Synthetic route for preparation of 5- keto acid impurity



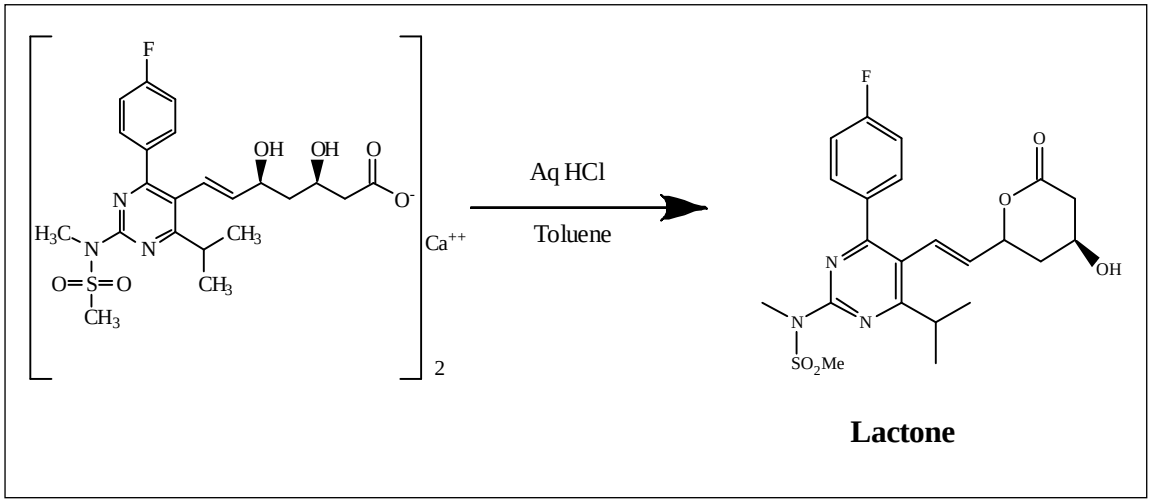
Scheme 3. Preparation of Methyl (3S)-3-(tert-butyldimethylsilyloxy)-5-oxo-6-triphenylphosphoranylidene hexanoate



Scheme 4Synthetic scheme for preparation of Bis (+) 7-[4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methane sulfonylaminopyrimidin)-5-yl] (3S, 5R) - dihydroxy-(E)-6-heptenoate calcium (3S, 5R-isomer)



Scheme 5 Synthetic scheme for preparation of 3R, 5R isomer



Scheme 6Synthetic route for preparation of Lactone impurity

CONCLUSION

During preparation of Rosuvastatin calcium, impurities in the range of 0.03 to 0.3 % were observed. These impurities were identified by their mass numbers in LC-MS and were then prepared and isolated. Identification of these impurities could help in improvement of yield and quality of Rosuvastatin prepared.

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