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Development of a Practical and Efficient Synthesis of SIPI-4884, a HMG CoA Reductase Inhibitor for the Treatment of Hypercholesterolemia

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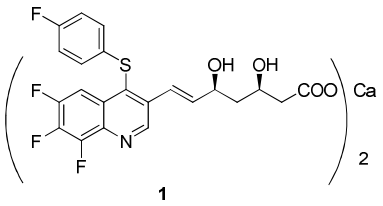
ABSTRACT: An improved process of the novel HMG CoA reductase inhibitor SIPI-4884 has been developed for early preclinical pharmacology and safety studies and it was made up with an efficient nine-step and scalable process. Significant improvements in the nucleophilic substitution, reduction, Witting-Horner reaction and preparation of calcium salt were demonstrated. The overall yield was improved to 17.2%.

INTRODUCTION

Hypercholesterolemia is a well-known risk factor for atherosclerotic diseases, especially for coronary heart disease.[1] Currently, the most common method of treating hypercholesteromia is the use of 3-hydroxy-3-methyl glutaryl-coenzyme A (HMG-CoA) reductase inhibitors, also known as statins, including lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin,

rosuvastatin and pitavastatin. Their mechanism of action is based on the reversible and competitive inhibition of HMG-CoA reductase which is the rate-limiting enzyme of cholesterol biosynthesis in the liver and nonhepatic tissues, ultimately resulting in up-regulation of the low density lipoprotein (LDL) receptor in liver, clearing of LDL particles from the blood stream and decreased levels of circulating cholesterol.[2]

Figure 1: Structure of SIPI-4884



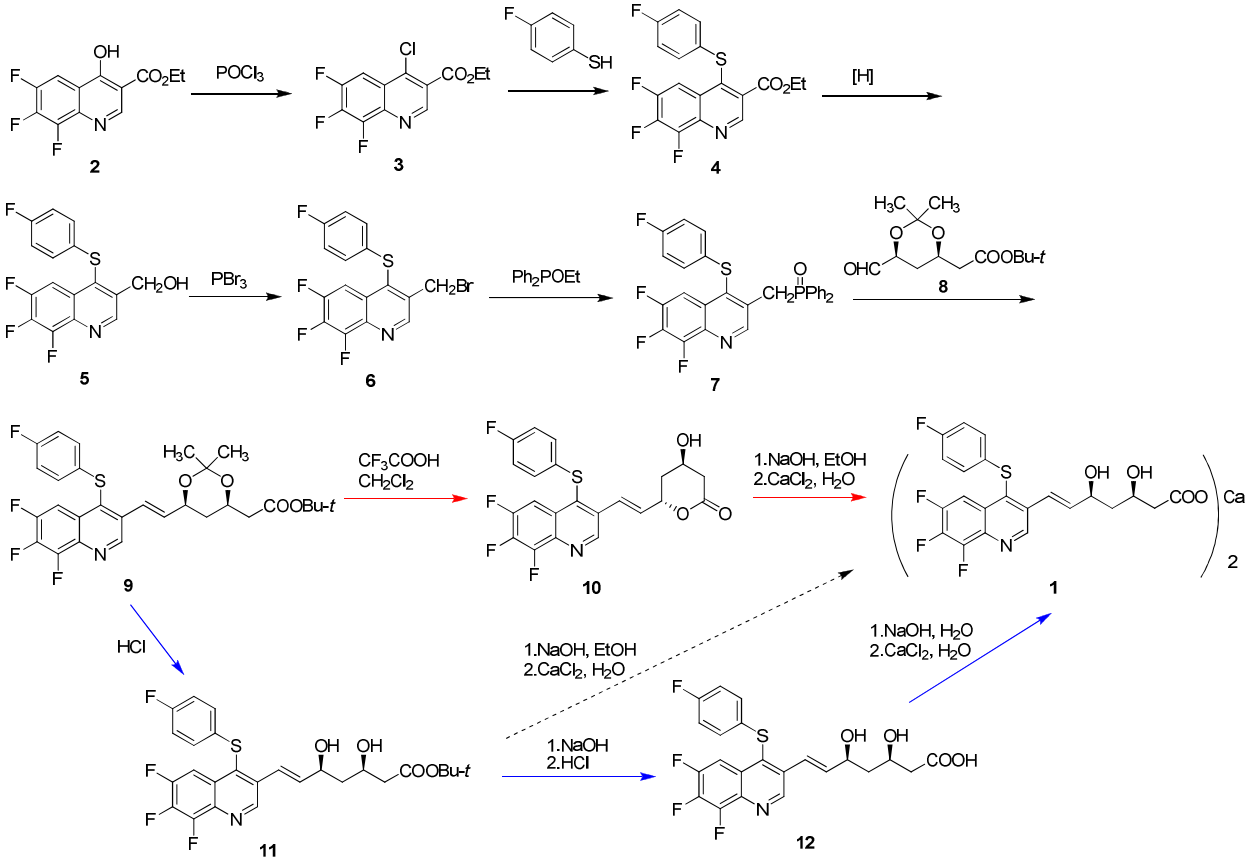
1

Our efforts in this area resulted in the identification of Calcium (3*R*,5*S*,6*E*)-7-[6,7,8-trifluoro-4-(4-fluoro-phenylthio)-quinoline-3-yl]-3,5-dihydroxy-hept-6-enoate (SIPI-4884, **1**) as a novel HMG CoA reductase inhibitor for the treatment of hypercholesterolemia.[3] Herein we describe the original synthesis used to prepare SIPI-4884 and the optimization of the reaction conditions for this synthesis to support scale-up from laboratory to pilot plant campaign and future preclinical pharmacology and safety studies.

RESULTS AND DISCUSSION

The synthesis utilized for the optimization and scale-up campaign is shown in Scheme 1, wherein three key building blocks, ethyl 6,7,8-trifluoro-4-hydroxy-quinoline-3-carboxylate (**2**), 4-fluoro-thiophenol, and *tert*-butyl (3*R*,5*S*)-6-oxo-3,5-dihydroxy-3,5-*O*-isopropylidene-hexanoate (**8**), are combined in an nine-step sequence. The optimization focuses on three modest yield steps, including the second step (nucleophilic substitution), third step (reduction) and sixth step (Witting-Horner reaction). In addition, the other steps also require development.

Scheme 1. Synthesis routes of 1



→ Synthesis route in the first laboratory campaign

→ The optimized synthesis route

2.1 Preparation of ethyl 4-chloro-6,7,8-trifluoroquinoline-3-carboxylate (3)

This step in the preliminary procedure used excess phosphorylchloride about 10 equivalents (equiv) as both chlorination reagent and solvent. The process was improved with toluene as solvent and the amount of $POCl_3$ was decreased to 1.5 equiv. The isolation yield was 80% after recrystallization.

2.2 Preparation of ethyl 4-(4-fluoro-phenylthio)-6,7,8-trifluoroquinoline-3-carboxylate (4)

It was known that in general, the nucleophilic reaction was carried out in a polar aprotic solvent in the presence of base[4]. The substitution of compound **3** with 4-fluoro-thiophenol was conducted in *N,N*-dimethylformamide (DMF) with Et_3N in the preliminary test and it was found

that the main product was 4,7-disubstituted quinoline **13** (Table 1, entry 1). If the reaction temperature was decreased to -15℃, the desired product **4** was obtained in very low yield after column chromatography (Table 1, entry 2). Then with the low-polar solvent tetrahydrofuran (THF) as solvent, a variety of bases including inorganic and organic, were screened, the product was contaminated with byproduct **13**. After column chromatography, product **4** was obtained (Table 1, entries 3-8).

When the reaction was carried out in DMF or dimethyl sulfoxide (DMSO) without base, no desired product was found and the main spot was identified as the starting material **3** by thin layer chromatography (TLC) (Table 1, entries 9-10). Surprisingly, the reaction went quite well in a non-polar solvent dichloromethane in the absence of base, no resulting in formation of compound **13**, although a little of starting material **3** remained after nineteen hours reflux. We reasoned that, because of the presence of HCl generated, it was difficult to drive the substitution to completion without using any bases. Thus the distillation of about half of the solvent, which took HCl away, was conducted after nineteen hours, and the reaction was continued for another hour under reflux to complete the reaction with high yield 86% after recrystallization (Table 2, entry 11).

The scale-up with 5.4 kg of compound **3** gave the better result (yield 91%, Table 1, entry 12). During the distillation, the reaction went to the end without additional reflux.

Table 1. Screening of bases and solvents for the monosubstitution

entry	solvent	base	Temp (℃)	Time (h)	yield (%)[a]
1	DMF	Et_3N	20	1	70 [b]
2	DMF	Et_3N	-15	1	30
3	THF	Et_3N	-15	4	38

4	THF	4-Methylmorpholine	-15	4	45
5	THF	pyridine	-15	1	55
6	THF	K_2CO_3	-15	1	40
7	THF	Na_2CO_3	-15	1	51
8	THF	$NaHCO_3$	-15	1	63
9	DMF	—	-15	2	[c]
10	DMSO	—	-15	2	[c]
11	CH_2Cl_2	—	reflux	19+1	86
12[d]	CH_2Cl_2	—	reflux	19	91

[a] Isolated yield

[b] yield of ethyl 4,7-di(4-fluoro-phenylthio)-6,8-difluoroquinoline-3-carboxylate (13)

[c] No desired product was found, the main spot was compound **3** by TLC.

[d] scale-up with 5.4 kg

2.3 Preparation of 4-(4-fluoro-phenylthio)-6,7,8-trifluoro-quinoline-3-methanol (5)

Several reducing agents were screened for the reduction of compound **4** and the results were shown in Table 2. No product was found with $LiAlH_4$ or $NaBH_4$ (Table 2, entries 1-2). When diisobutyl aluminium hydride (DIBALH, 4 equiv) was served, the yield of **5** was 52% after purification by chromatography (Table 2, entry 3), meanwhile one over-reductive byproduct **14** was found (Table 4). It prompted us to reduce the amount of DIBALH. Finally, slight excess (2.2 equiv) was proved to be suitable (Table 2, entry 5) to avoid the impurity **14** with the yield 83% after recrystallization by toluene and heptane.

Table 2. Screening of reducing agent for the reduction

entry	Reducing agent	Equiv of reductant	yield (%)
1	$LiAlH_4$	0.5	[a]

2	$NaBH_4$	1~4	[d]
3	DIBALH	4.0	52[b]
4	DIBALH	2.5	74[b]
5	DIBALH	2.2	83[c]
6	DIBALH	2.1	76[c]
7	$ZnCl_2$/ KBH_4(1:2)	1.25	46[c]

[a] the reaction was complicated, so the product was not isolated

[b] Isolated yield after column chromatography

[c] Isolated yield after recrystallization

[d] The starting material remain

On the other hand, modest yield (46%) was received by using $ZnCl_2/KBH_4$ (1.25 equiv, Table 2, entry 7). Although the yield was lower than in DIBALH condition, $ZnCl_2/KBH_4$ provided an alternative method to minimize the total cost (since the reagents were much cheaper than DIBALH) and to avoid the potential risk related with DIBALH.

2.4 Preparation of 4-(4-fluoro-phenylthio)-6,7,8-trifluoro-3-(diphenyl-oxo-phosphonyl-methyl)-quinoline (7)

Bromination of compound **5** with PBr_3 in dichloromethane gave 3-bromomethyl-4-(4-fluoro-phenylthio)-6,7,8-trifluoro-quinoline (**6**) in 90% yield, which was used to the next step without any purification.

The phosphorylation of compound **6** was performed in reflux toluene with diphenyl ethoxyphosphine (1.5 equiv). The yield of **7** was 95%.

2.5 Preparation of *tert*-Butyl (3*R*,5*S*,6*E*)-7-[4-(4-fluoro-phenylthio)-6,7,8-trifluoro-quinoline -3-yl]-3,5-dihydroxy-3,5-*O*-isopropylidene-6-heptenoate (9)

In order to construct the *E*-olefine by the Wittig-Horner reaction of compound **7** with *tert*-butyl (3*R*,5*S*)-6-oxo-3,5-dihydroxy-3,5-*O*-isopropylidene-hexanoate (**8**), a series of conditions were tested and the data were shown in Table 3, from which lithium tetramethylpiperidine in THF was

identified as the preferred base (Table 3, entry 1) although K_2CO_3 in DMF also produced an acceptable yield (entry 6).

Table 3. Screening of bases (1.5 equiv) and solvents for the Wittig-Horner reaction

entry	solvent	base	*E*:*Z*	yield [a] (%)
1	THF	*n*-BuLi +tetramethylpiperidine	95:5	66
2	THF	*n*-BuLi	77:23	[b]
3	THF	*n*-BuLi+4-butylaniline	89:11	55
4	THF	*n*-BuLi+HMDS	79:21	b
5	THF	NaH	89:11	40
6	DMF	K_2CO_3	95:5	59
7	2-Me THF	*n*-BuLi +tetramethylpiperidine	90:10	60
8	$CH_3OCH(CH_3)_3$	*n*-BuLi +tetramethylpiperidine	—	—[b]
9	CH_2Cl_2	*n*-BuLi +tetramethylpiperidine	—	—[b]
10	THF	*n*-BuLi +tetramethylpiperidine[c]	95:5	82[f]

[a] Isolated yield of *E*-form after silica gel chromatography (petroleum ether : EtOAc, 6 : 1)

[b] Unreacted

[c] 1.1 equiv of the base was used.

[f] Yield of *E*-form after crystallization.

After identifying the base and solvent, experiments on the stoichiometry of lithium tetramethylpiperidine showed that 1.1 equiv of the base was optimal with a yield of 82% (Table 3, entry 10).

2.6 Preparation of calcium salt 1

In the first laboratory campaign, compound **9** was converted to lactone **10** by F_3CCOOH (TFA), the lactone **10** in THF was hydrolyzed to the corresponding carboxylate sodium salt at 0 ℃ by saponification with NaOH aqueous solution, then HCl solution was added to adjust pH

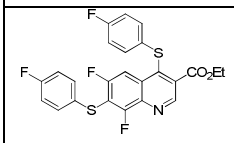
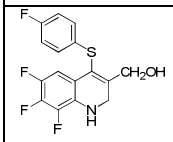
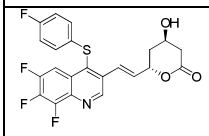
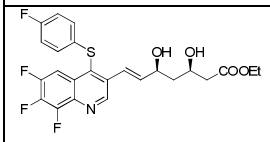
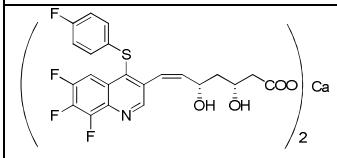
7-8, the solution was concentrated under reduced pressure to evaporate THF at 40℃, and $CaCl_2$ aq solution was added to generate calcium salt **1**. In the process, especially during the evaporation of THF, it was found that the product was contaminated with the *Z*-isomer. Sometimes this impurity contributed 3-5 percentages determined by HPLC. This impelled us to explore another method. In the optimized process, the deprotection of compound **9** with hydrochloric acid instead of highly corrosive TFA generated a new intermediate *tert*-butyl (3*R*,5*S*,6*E*)-7-[6,7,8-trifluoro-4-(4-fluoro-phenylthio)-quinoline-3-yl]-3,5-dihydroxy-hept-6-enoate (**11**, Scheme 1). Two choices were attempted to obtain the final product: acidic deprotection from **9** to generated **11** first, and then basic hydrolysis from **11** and generation of calcium salt were conducted in one pot. Alternatively, deprotection and hydrolysis were carried out in one pot, then acidified to form the carboxylic acid **12**, which was transferred to the calcium salt **1** (Scheme 1). The experiments showed that the purity of either **11** or **12** must be more than 98% in order to assure the calcium salt **1** as an active pharmaceutical ingredient (API) with purity more than 99%. The efforts of controlling the quality of **11** were unsatisfactory. The purity of **11** reached above 98% after recrystallization by ethanol, but the yield was 43%. The main impurities were identified as the lactone **10** and carboxylic acid **12**.

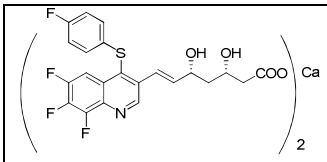
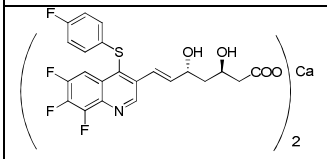
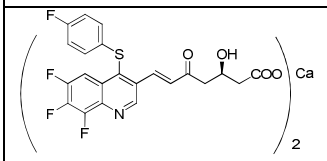
So the second choice was adopted. Acid **12** was prepared from the acidic deprotection followed by basic hydrolysis from **9** and recrystallized by ethanol with high purity (99%). The calcium salt **1** was generated via the sodium salt from **12**. An extensive screen of solvents for recrystallization of the final product was made, it was found that calcium salt **1** was recrystallized in 10% aqueous acetone with suitable solid form properties for downstream processing and the purity of more than 99% by HPLC.

2.7 Impurity Control[5]

Detection, identification, and control of impurities in the API are of primary importance during the development of any drug candidate. Table 4 summarizes several impurities that have been identified during the development process. 5-oxo product (**19**) was a main impurity detected in the API stored more than 2 years, inferring the formation owing to oxidation. The diastereoisomers were not found in API. All the impurities can be controlled effectively in the process.

Table 4. Impurity summary and control strategy

Structure	Common Name	Control Strategy	Comments
	4,7-disubstituted product **13**	Specified in **3** at NMT 0.1%, controlled in crystallization of monosubstituted product	Well controlled by the reaction
	over-reductive product **14**	Controlled in crystallization by specifying NMT 0.5% of **5**.	Reduce the amount of reducing agent
	lactone **10**	Hydrolysis in the process	This impurity was found in deprotection step.
	ethyl ester **16**	Hydrolysis in the process	This impurity was found in crystallization of **12** by ethanol and may be converted to the final product in the following step..
	*Z*-isomer **17**	Specified in API at NMT 0.1%, controlled in crystallization of **1**	Most *Z*-olefine was controlled by crystallization after Wittig-Horner reaction

	Enantiomer **18**	Specified in API at NMT 0.1%, controlled in crystallization of **1**	
	Diastereoisomer **19**	Specified in API at NMT 0.1%.	Diastereoisomers was not detected in API.
	5-oxo product **15**	Specified in API at NMT 0.2%, controlled in crystallization of **1.**	Generated by oxidation in the air. The API must be storaged in the absence of air.

CONCLUSION

In summary, an efficient nine-step and scalable process was developed for the preparation of SIPI-4884 (**1**). Significant improvements in the nucleophilic substitution, reduction, Witting-Horner reaction and preparation of calcium salt were demonstrated. The reaction of **3** with 4-fluro-thiophenol was carried out in CH_2Cl_2 without base, the formation of disubstituted by-product **13** was avoided and the yield of compound 4 was increased to 91% after recrystallization. The reduction with DIBALH gave a yield of 83%, and the method by $ZnCl_2/KBH_4$ might be cheaper and safer. The final product **1** was synthesized from carboxylic acid **12**, recrystallized by acetone-ethanol (8:1), and the yield and purity (HPLC>99%) were much better than the result from other ways. The overall yield was improved to 17.2% (from compound **2**). A total 1.2 kg of API was produced for preclinical studies, utilizing the route shown in Scheme 1.

EXPERIMENTAL SECTION

Melting points were determined on a capillary melting point apparatus and were uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian INOVA-400 spectrometer with chemical shifts (δ) and coupling constants (J) given in ppm and Hz

respectively relative to TMS as an internal standard. Mass spectra were recorded by an Q-TOF Mass Spectrometer using electrospray ionization (ESI). Optical rotations were measured with a Rudolph Autopol IV. Solvents and reagents were used without any pretreatment. Achiral HPLC analyses were carried out on an Agilent 1100 series using Welch column (4.6 × 250 mm, 5μm) with THF-methanol-buffer (0.02 M ammonium acetate, adjusted to pH 4.5 with acetic acid) 13-48-39 as the mobile phase, 0.8 mL/min at 30 ^{o}C with detection at 255 nm. HPLC retention times: **2** = 6.85 min, **17** = 19.87 min, **15** = 20.37 min, **1** = 21.42 min, **19** = 23.31 min, **3** = 27.09 min, **5** = 29.06 min, **7** = 34.43 min, **16** = 43.19 min, **4** = 53.13 min. Achiral HPLC analyses of compound **9** and **11** were carried out on an Agilent 1100 series using Welch column (4.6 × 250 mm, 5μm) with THF - buffer (0.02 M ammonium acetate, adjusted to pH 4.5 with acetic acid) 52-48 as the mobile phase, 1 mL/min at 30 ^{o}C with detection at 255 nm. HPLC retention times: **1** = 3.49 min, **9** = 7.84 min, **11** = 16.66 min. Chiral HPLC analyses were carried out on an Agilent 1100 series using CHIRALCEL AS-H column (4.6 × 250 mm, 5μm) with hexane-ethanol-trifluoroacetic acid (80-20-0.1) as the mobile phase, 0.8 mL/min at 30 ^{o}C with detection at 255 nm. HPLC retention times: **18** = 6.81 min, **1** = 9.73 min. HPLC purity is reported by area %.

Ethyl 4-chloro-6,7,8-trifluoro-quinoline-3-carboxylate (3). A suspension of ethyl 4-hydroxyl-6,7,8-trifluoroquinoline-3-carboxylate **2** (10.0 kg, 36.87 mol) in toluene (50 L) was stirred while phosphorylchloride (8.49 kg, 55.35mol) was added at room temperature. After addition, it was heated under refluxing for 2 h and became a clear solution. After concentrated under reduced pressure, the residue was treated with ice water (20 L) and Na_2CO_3 (4.17 kg) to adjust pH 8, then the solid was filtrated, and washed with water (3 × 8 L) and heptane (4 L) to afford a off-white solid after drying at 80 ^{o}C for 5 h. The obtained solid was charged into ethyl

acetate (20 L) and heated to reflux for 20 min. The clear solution was cooled to 0-10 ^{o}C, stirred for 5 h and the precipitated solid was filtrated and washed with ethyl acetate (3 L), then dried at 60 ℃ for 10 h to furnish title compound **3** as a white acicular crystal (8.67 kg, 81.2% yield), mp: 116-119 ^{o}C, ^{1}H-NMR (400MHz, $CDCl_3$) δ (ppm): 9.21(s, 1H), 8.01-7.96(m, 1H), 4.51(q, J=7.2, 2H), 1.46(t, J=7.2, 3H), MS (ESI^+) : m/z, 290.10 ($[M+H]^+$).

Ethyl 4-(4-fluoro-phenylthio)-6,7,8-trifluoro-quinoline-3-carboxylate (4). Ethyl 4-chloro-6,7,8-trifluoro-quinoline-3-carboxylate **3** (5.4 kg, 18.7 mol) and 4-fluorophenthiol (2.5 kg, 19.2 mol) was dissolved in CH_2Cl_2 (50.0 L) at room temperature and the mixture was heated to reflux for 19 h. Then the solution was concentrated to dryness, toluene (13 L) and heptane (8.7 L) were added to the residue, and the mixture was heated to reflux to give a solution. It was cooled to 10 ^{o}C over 10 h. The solid was isolated by filtration, washed with a mixture of toluene (3 L) and heptane (3 L), then washed twice with water (5 L) , washed twice with heptane, and then dried for 10 h at 60 ^{o}C to afford the product **4** as a yellow solid (6.5 kg, 91.0%), mp 131-133℃. ^{1}H-NMR (400MHz, $CDCl_3$) δ (ppm): 9.04 (s, 1H), 8.05-8.00 (m, 1H), 7.24-7.20(m, 2H), 6.99-6.94(m, 2H), 4.30(q, 2H, J=7.2), 1.32(t, 3H, J=7.2), ^{13}C NMR (100MHz, $CDCl_3$) δ (ppm): 165.2, 163.5, 161.0, 152.2, 149.6, 149.3, 147.3, 144.8, 142.7, 140.1, 137.2, 132.1, 130.7, 129.3, 124.6, 116.7, 107.0, 62.3, 13.9 ($CDCl_3$), 77.3, 77.0, 76.7. MS (ESI^+) : m/z, 382.05 ($[M+H]^+$).

4-(4-Fluoro-phenylthio)-6,7,8-trifluoro-quinoline-3-methanol (5). (DIBALH method) A suspension of **4** (1500 g, 3.94 mol) in a mixture of toluene (6000 mL) was stirred at -5 ^{o}C while the solution of DIBALH in toluene (1 M, 8.67 L, 8.67 mol) was added during 4 h under an atmosphere of nitrogen. The solution was stirred at 0 ^{o}C for 3 h, then it was slowly poured into a mixture of ice water (10L) and EtOAc (7.5 L), 6 M HCl (3.1 L) was added to adjust pH 4-5. The

organic phase was separated, and the aqueous phase was extracted with ethyl acetate twice (2×2.5 L). The combined organic phase was washed with water (3×2.5 L), dried over anhydrous Na_2SO_4, filtered and washed with EtOAc (2 L), concentrated under reduced pressure to dryness. The residue was crystallized with toluene (8100 mL) and heptane (5400 mL), and stirred at room temperature for 10 h, and the solids were isolated by filtration, washed with 2 L of toluene and heptane (3:5), and dried at 60 ^{o}C for 10 h to give the title compound **5** as a yellow solid (1335 g, 83.2% yield). mp：149-151 ^{o}C。^{1}H NMR(400 MHz, $CDCl_3$) δ (ppm): 9.14 (s, 1H), 8.00-7.95 (m, 1H), 7.07-7.03 (m, 2H), 6.95-6.91 (m, 2H), 5.04 (s, 2H), 2.26 (br, 1H), ^{13}C NMR (100MHz, $CDCl_3$) δ (ppm): 190.1, 163.2, 160.7, 152.2, 150.7, 149.7, 147.2, 144.7, 141.9, 139.4, 139.3, 138.8, 136.1, 132.5, 131.3, 130.4, 129.4, 128.8, 125.6, 117.0, 116.7, 116.4, 109.5, 106.4, 62.6, 61.6, 44.6 ($CDCl_3$), 77.3, 77.0, 76.7. MS (ESI^+) : m/z, 340.20 ($[M+H]^+$).

($ZnCl_2$/ KBH_4 method) KBH_4 (352.1 g, 6.52 mol), anhydrous $ZnCl_2$ (445.0 g, 3.26 mol) and THF (7 L) were charged into reactor at room temperature and stirred for 2 h. Compound **4** (1000 g, 2.63 mol) in toluene (10.5 L) were added to the above suspension. And then it was heated to reflux for 3.5 h and cooled to room temperature. Some solid was removed by filtration. Ice water (5 L) and EtOAc (5 L) was added to the filtrate, 6 M HCl (1750 mL) was added to adjusted pH 1. The organic phase was separated, the aqueous layer was extracted with EtOAc (2 ×2 L). The combined organic phase was washed with saturated aqueous sodium hydrogen carbonate (5 L) and water (2× 5 L), dried over anhydrous Na_2SO_4, filtered, washed with EtOAc (1 L) and concentrated to dryness. After crystallization in toluene (3 L) and heptane (5 L), a yellow solid **5** (414.6 g, 46.6% yield) was given. mp: 148-150 ℃.

4-(4-fluoro-phenylthio)-6,7,8-trifluoro-3-(diphenyl-oxo-phosphonyl-methyl)-quinoline (7). A solution of PBr_3 (173 mL, 1.8 mol) in CH_2Cl_2 (2 L) was added to the mixture of **5** (520 g, 1.53

mol) in CH_2Cl_2 (3 L) at 0 ^oC. The resulting mixture was stirred at room temperature for 2 h. The solution was concentrated under vacuum to an oil, and then saturated Na_2CO_3 aqueous solution was slowly added to adjust pH 7-8.[6] The solids were filtrated, washed with water (3 ×1 L), and dried at 80 ^oC for 15 h to afford a yellow solid **6** (556.2 g, 90.2% yield) which was used to the next reaction without any purification. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.04 (s, 1H), 8.00-7.90 (m, 1H), 7.13-7.10 (m, 2H), 6.98-6.93 (m, 2H), 4.91 (s, 2H), ^{13}C NMR (100MHz, $CDCl_3$) δ (ppm): 163.3, 160.8, 152.2, 150.0, 147.5, 144.9, 142.4, 140.2, 136.8, 130.7, 129.3, 125.6, 117.1, 107.1, 32.5, 28.2 ($CDCl_3$), 77.3, 77.0, 76.7. MS (ESI^+) : m/z, 402.55 ($[M+H]^+$).

6 (530 g, 1.32 mol) and diphenyl ethoxyphosphine (455.4 g, 1.98mol) were dissolved in toluene (2650 mL). The solution was refluxed for 2 h during which time the precipitated solid developed. The slurry was cooled to room temperature, heptane (1.3 L) was added and stirred for 5 h. The solid was isolated by filtration and washed with toluene (2×500 mL). After drying at 80 ^oC for 10 h, the desired product **7** as a white solid (655.7 g, 95.1% yield) was afforded, mp: 244-245 ^oC, ^1H NMR(400 MHz, $CDCl_3$) δ(ppm): 8.83 (s, 1H), 7.76-7.73 (m, 5H), 7.54-7.50 (m, 2H), 7.46-7.41 (m, 4H), 6.91-6.83 (m, 4H), 4.28 (d, 2H, *J*=13.6 Hz), ^{13}C NMR (100MHz, $CDCl_3$) δ (ppm): 162.8, 160.4, 152.1, 151.9, 149.5, 147.5, 144.8, 141.6, 140.7, 139.1, 163.0, 132.3, 132.0, 131.2, 131.1, 130.2, 129.7, 128.8, 125.5, 125.4, 116.8, 116.6, 107.1, 106.9, 35.5, 34.9 ($CDCl_3$), 77.3, 77.0, 76.7. MS (ESI^+) : m/z, 340.20 ($[M+H]^+$).

***tert*-Butyl (3*R*,5*S*,6*E*)-7-[4-(4-fluoro-phenylthio)-6,7,8-trifluoro-quinoline -3-yl]-3,5-dihydroxy-3,5-*O*-isopropylidene-6-heptenoate (9).** 287.1 ml (0.84 mol) of 2.93 M hexane solution of *n*-BuLi was added to a solution of 2,2,6,6-tetramethylpiperidine (129.4 g, 0.92 mol) in THF (4 L) at 0 ^oC and the solution stirred for 1 h under an atmosphere of nitrogen.

Then compound **7** (400 g, 0.76 mol) was added and stirred for 1 h at 0 °C, the solution of tert-butyl (3*R*,5*S*)-6-oxo-3,5-dihydroxy-3,5-*O*-isopropylidene-hexanoate (**8)** (258 g, 0.92 mol) in THF (400 ml) was added to the reaction solution and stirred for 3 h at room temperature. The solution was quenched with ice water (4 L), EtOAc (4 L) was added and the organic layer was separated. The aqueous phase was extracted with EtOAc (2×1.2 L), the combined organic layer was washed with water (3×1.2 L) and dried over sodium sulfate. The organic solvent was removed via rotary evaporation, and then ethanol (1200 mL) and heptane (2400 mL) was added and heated to reflux for 1 h, after which the solution was filtered hot. The filtrate was allowed to cool to room temperature. The resulting slurry was stirred for 10 h and the solids were isolated by filtration, washed with mixture of ethanol (150 mL) and heptane (300 mL), and dried at 60 °C for 15 h to give the product **9** as a off-white solid (354.4 g, 82.3% yield), mp：133-135℃, $[\alpha]_D^{20}$ =8.3 (c 1, CH_2Cl_2)。HPLC purity (area %)：99.42%。^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.08 (s, 1H), 8.08-8.03 (m, 1H), 7.25 (d, 1H, *J*=16.0), 7.08-7.05 (m, 2H), 6.94-6.90 (m, 2H), 6.32 (dd, 1H, *J*=16.0, 5.4)，4.57-4.53 (m, 1H), 4.32-4.28 (m, 1H), 2.49-2.28(m, 2H), 1.67-1.21 (m, 17H), ^{13}C NMR (100MHz, $CDCl_3$) δ (ppm): 150.0, 163.1, 160.6, 152.3, 149.8, 149.3, 147.5, 144.9, 141.8, 139.2, 138.1, 131.1, 130.9, 129.9, 125.9, 125.7, 116.7, 116.5, 116.4, 106.7, 106.4, 99.1, 80.7, 69.4, 65.9, 42.5, 36.4, 30.0, 28.1, 19.6 ($CDCl_3$), 77.3, 77.0, 76.7. MS (ESI^+) : m/z, 564.20 ($[M+H]^+$).

(3*R*,5*S*,6*E*)-7-[4-(4-fluoro-phenylthio)-6,7,8-trifluoro-quinoline -3-yl]-3,5-dihydroxy-6-heptenoic acid (12). To a solution of compound **9** (350g, 0.62 mol) in THF (3.5L) was added dropwise 6 M HCl solution (700 mL) at 20-30 °C, and stirred for 3 h. NaOH (252 g, 6.3 mol) in water (800 mL) was added dropwise to the solution at 0 °C and stirred for 1 h. 6 M HCl solution (300 ml) was added to the reaction mixture at 0-5 °C, EtOAc (3.5 L)

was added and the organic layer was separated, the aqueous phase was extracted with EtOAc (2×1 L). The organic layer was combined, washed with saturated NaCl aqueous solution (1 L), water (2×1L) and dried over sodium sulfate, the solution was concentrated to dryness. Ethanol (3.5 L) was added to the residue and heated to reflux for 1 h, after which the clear solution was cooled at room temperature. The resulting slurry was stirred at room temperature for 10 h, and the solid was isolated by filtration, washed with ethanol (300 mL), and dried in a vacuum oven at 60 ^{o}C for 15 h to afford the product **12** as a light yellow solid (244.1 g, 84.1% yield), mp : 172-174 ^{o}C, $[\alpha]_{D}^{20}$=30.4 (c 1, THF), HPLC purity (area %): 99.27%. ^{1}H NMR(400 MHz, DMSO-d_6) δ (ppm) : 9.30 (s, 1H), 8.12-8.07 (m, 1H), 7.22-7.11 (m, 2H), 7.09-7.08 (m, 2H), 7.28 (d, 1H, *J*=15.6), 6.76 (dd, 1H, *J*=15.8, 5.2), 5.09-5.08 (m, 1H), 4.71 (m, 1H), 4.38 (m, 1H), 4.01-4.00 (m, 1H), 2.42-2.25 (m, 2H), 1.70-1.57 (m, 2H), ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 193.0, 162.6, 160.1, 151.4, 150.1, 148.9, 147.0, 144.4, 141.6, 140.9, 138.4, 136.2, 136.0, 134.9, 131.0, 130.1, 125.8, 123.3, 117.0, 116.8, 106.7, 68.9, 65.4, 56.3, 44.4, 42.7, 18.6 ($CDCl_3$), 140.3-39.1. MS (ESI^+): m/z, 468.10 ($[M+H]^+$)

Calcium (3*R*,5*S*,6*E*)-7-[6,7,8-trifluoro-4-(4-fluoro-phenylthio)-quinoline-3-yl]-3,5-dihydroxy-hept-6-enoate (1). NaOH aqueous solution (21.6 g, 0.54 mol, 1.2 L) was added to the slurry of carboxylic acid **12** (240 g, 0.51 mol) in water[7] (2.4 L) at 0-10 ^{o}C for 3 h to give the clear solution. 1 M HCl solution (ca. 30 mL) was added dropwise below 10 ^{o}C and calcium chloride aqueous solution (31.2 g, 0.28 mol, 200 mL) was slowly added to the sodium salt solution. After stirring for 1 h, the precipitate was filtrated and then slurried with water (2 L) for 30 min, filtrated again and washed with water (1 L). The resulting solid was dried in a vacuum oven at 60

°C for 15 h to afford the crude product **1**. Crude **1** (245 g) was suspended in a mixture of acetone (1960 mL) and water (245 mL). When the mixture was heated to reflux to give solution, activated charcoal (12 g) was added. After stirring 20 min, the hot solution was filtrated, washing with acetone (200 ml). The filtered solution was cooled to 20 °C. The resulting slurry was stirred for 24 h, then the solid was isolated by filtration, washed twice with a mixture of acetone (160 mL) and water (20 mL).The solid was dried in a vacuum oven at 60 °C for 15 h to afford the calcium salt **1** as a white solid (210.7 g, 84.3% yield), mp : 170 °C decomposed, $[\alpha]_D^{20}$=26.4 (c 1, DMF), HPLC purity (area %): 99.06%.IR (thin film): cm^{-1} 3344.1, 2919.5, 1508.1, 1489.0, 1562.9, 1094.5, 829.4, 1231.9, 1395.5, 969.9. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.25 (s, 1H), 8.06-8.02 (m, 1H), 7.27-7.07 (m, 5H), 6.77 (dd, 1H, J=16.4, 5.2), 4.42-4.44 (m, 1H), 3.96-3.94 (m, 1H), 2.24-2.06 (m, 2H), 1.66-1.52 (m, 2H), MS (ESI^+) : m/z, 973.10 ($[M+H]^+$), Anal. Calcd For $C_{44}H_{32}F_8N_2O_8S_2Ca$: C, 53.3; H, 3.43; N, 2.82. Found: C, 53.01; H, 3.31; N 2.66

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Grundy, S.M. *Intern. Med*. **1997**, *241*, 295-306.

(2) Endo, A. *J. Lipid. Res.* **1992**, *33*, 1569-1582.

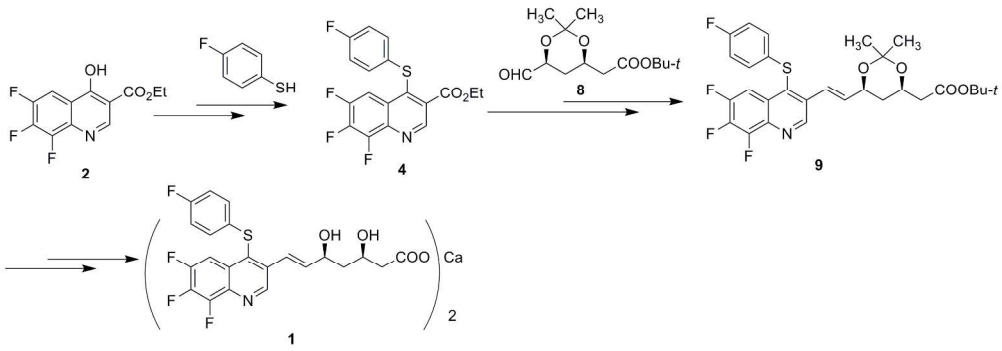
(3) (a) Cai, Z.; Zhou, W.; Sun, L. *Bioorg. Med. Chem.* **2007**, 15, 7809-7829. (b) Hao, Q.; Cai, Z.; Pan, J.; Li, Y.; Xia, Y.; Min, Y.; Sheng,Y.; Zhou, W. *Chem. Biol. Drug. Des.* **2011**, *78*, 730-733. (c) Zhao, S.; Zhou, W.; Liu, J. *Bioorg. Med. Chem.* **2007**, 17, 7915-7923.

(4) Zhao, S.; Zhou, W. *Syn. Commun.* **2011**, *41*, 20-32.

(5) The synthesis of impurities see: (a) Hao, Q.; Cai, Z.; Pan, J.; Li, Y.; Zhou, W. *Chin. J. Pharm*. **2010**, *41*, 884-890. The determination of the related substances see: (b) Li, Y.; Zhang, S.; Hao, Q.; Cai, Z.; Zhou, W. *Chin. J. Pharm*. **2012**, *43*, 281-285.

(6) The HBr salt was generated after concentration, Na_2CO_3 solution was added to affor the free base.

(7) The water used in this step is deionized water.



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