Drugs of the Future 1998, 23(8): 847-859

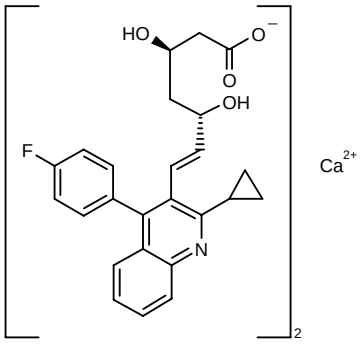
CCC: 0377-8282/98

NK-104

Hypolipidemic
HMG-CoA Reductase Inhibitor

Nisvastatin

(3*R*,5*S*)-7-[2-Cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl]-3,5-dihydroxy-6(*E*)-heptenoic acid calcium salt



$C_{50}H_{46}CaF_2N_2O_8$

CAS: 147526-32-7
CAS: 141750-63-2 (as lactone)

EN: 192009

Synthesis

NK -104 in its open and lactone forms has been synthesized by several different ways:

1) *Lactone form:* The reaction of 1(*R*),7,7-trimethylbicyclo[2.2.1]heptan-2-one (I) with 1-naphthylmagnesium bromide (II) gives the tertiary alcohol (III), which by reaction with $SOCl_2$ and then with $NaHCO_3$ yields 2-(1-naphthyl)-1(*R*),7,7-trimethylbicyclo[2.2.1]heptene (IV). Hydroboration of (IV) with BH_3 followed by oxidation with H_2O_2 affords 4(*S*),7,7-trimethyl-3*exo*-(1-naphthyl)bicyclo[2.2.1]heptan-2*exo*-ol (V), which is submitted to transesterification with methyl acetoacetate (VI) and dimethylaminopyridine (DMAP) to give the corresponding ester (VII). The condensation of (VII) with *N*-methoxy-*N*-methyl-3-[2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl]-2(*E*)-propenamide (VIII) by means of NaH yields the corresponding chiral 3,5-dioxoheptenoic acid ester (IX), which is selectively reduced first with diisobutylaluminum hydride acid (DIBAL) and then with diethylmethoxyborane and sodium borohydride affording the 3(*R*),5(*S*)-dihydroxyheptenoic ester (X). Finally, this compound is saponified with NaOH and treated with acetic acid/sodium acetate.

The intermediate amide (VIII) is obtained by condensation of 2-cyclopropyl-4-(4-fluorophenyl)quinoline-3-carbaldehyde (XI) with *N*-methoxy-*N*-methylacetamide (XII) by means of butyl lithium to the hydroxy propionamide (XIII), which is then dehydrated with methanesulfonyl chloride and triethylamine in the usual way (1, 2). Scheme 1.

2) *Lactone form:* The regioselective opening of (*R*)-2-(*tert*-butyldimethylsilylethynyl)oxirane (XIV) with KCN in ethanol gives 3(*S*)-hydroxy-5-(*tert*-butyldimethylsilyl)-4-pentynenitrile (XV), which is condensed with *tert*-butyl bromoacetate (XVI) by means of Zn in refluxing THF to afford the 5(*S*)-hydroxyketoester (XVII). The controlled reduction of (XVII) with $NaBH_4$/diethylmethoxyborane yields the 3(*R*),5(*S*)-dihydroxy ester (XVIII), which is deprotected with 2,2-dimethoxypropane and *p*-toluenesulfonic acid in THF/methanol to the protected heptynoic ester (XIX). The desilylation of (XIX) with tetrabutylammonium fluoride (TBAF) in THF affords the protected heptynoic ester (XX), which is condensed with 2-cyclopropyl-4-(4-fluorophenyl)-3-iodoquinoline (XXI, see Scheme 5) to give the protected NK-104 *tert*-butyl ester (XXII). Finally, this compound is treated with trifluoroacetic acid in dichloromethane (3, 4). Scheme 2.

3) The condensation of 3-(trimethylsilyl)propynal (XXIII) with the dialkaline salt of ethylacetoacetate (XXIV) in THF gives 5-hydroxy-3-oxo-7-(trimethylsilyl)-6-heptynoic acid ethyl ester (XXV), which is reduced with $NaBH_4$/diethylmethoxyborane to the racemic 3,5-dihydroxy-7-(trimethylsilyl)-6-heptynoic acid ethyl ester (XXVI). The protection and desilylation of (XXVI) with 2,2-dimethoxypropane and *p*-toluenesulfonic acid yields the protected heptynoic ester (XXVII), which is saponified to the corresponding acid (XXVIII) with NaOH. The optical resolution of the racemic acid (XXVIII) by treatment with 1(*R*)-(1-naphthyl)ethylamine (XXIX) and crystallization of the diastereomeric salts affords the protected (3*R*,5*S*)-isomer (XXX), which is esterified with ethyl iodide and DBU to the corresponding ester (XXXI). The condensation of (XXXI) with 2-cyclopropyl-4-(4-fluorophenyl)-3-iodoquinoline (XXI, see Scheme 5) by means of

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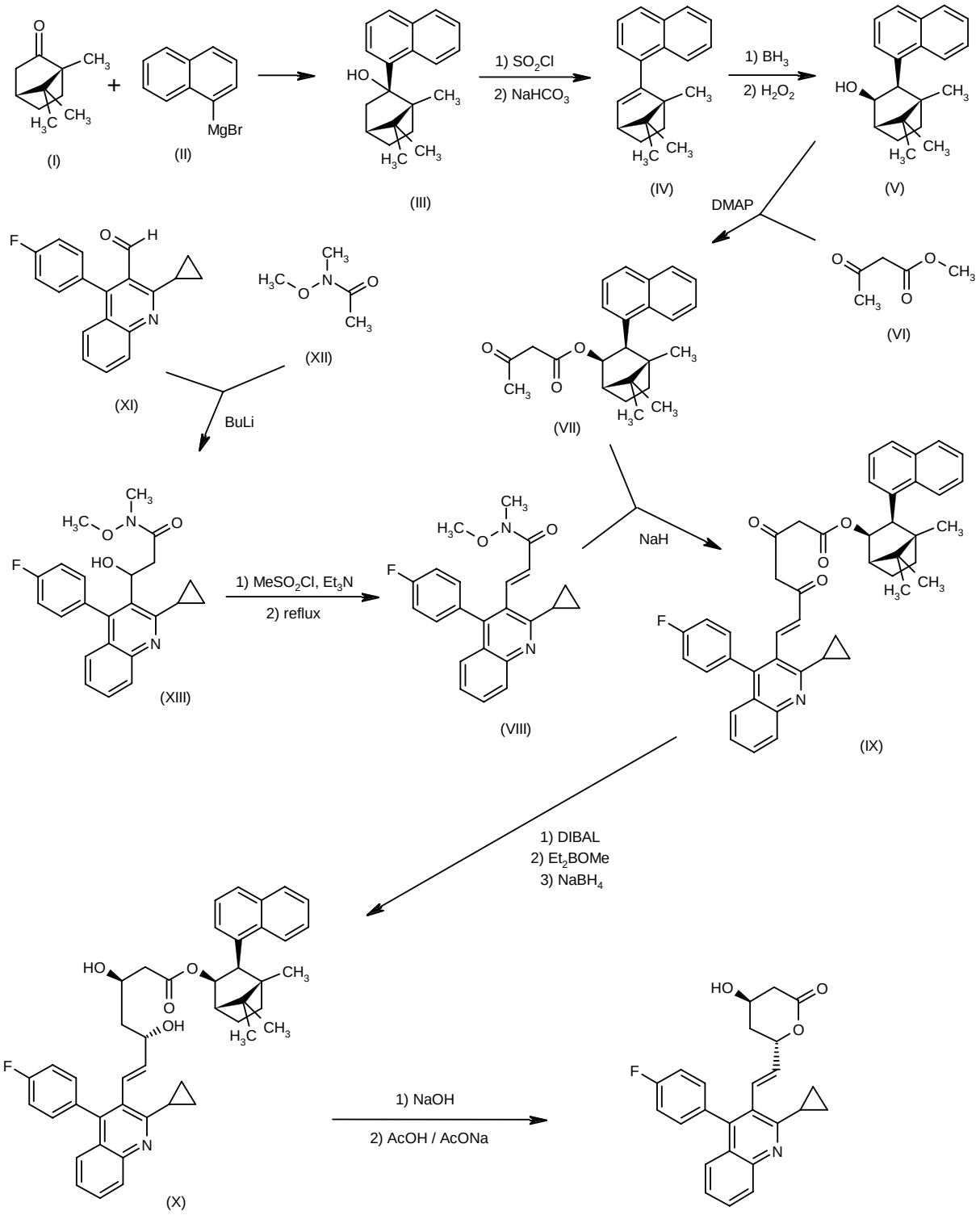
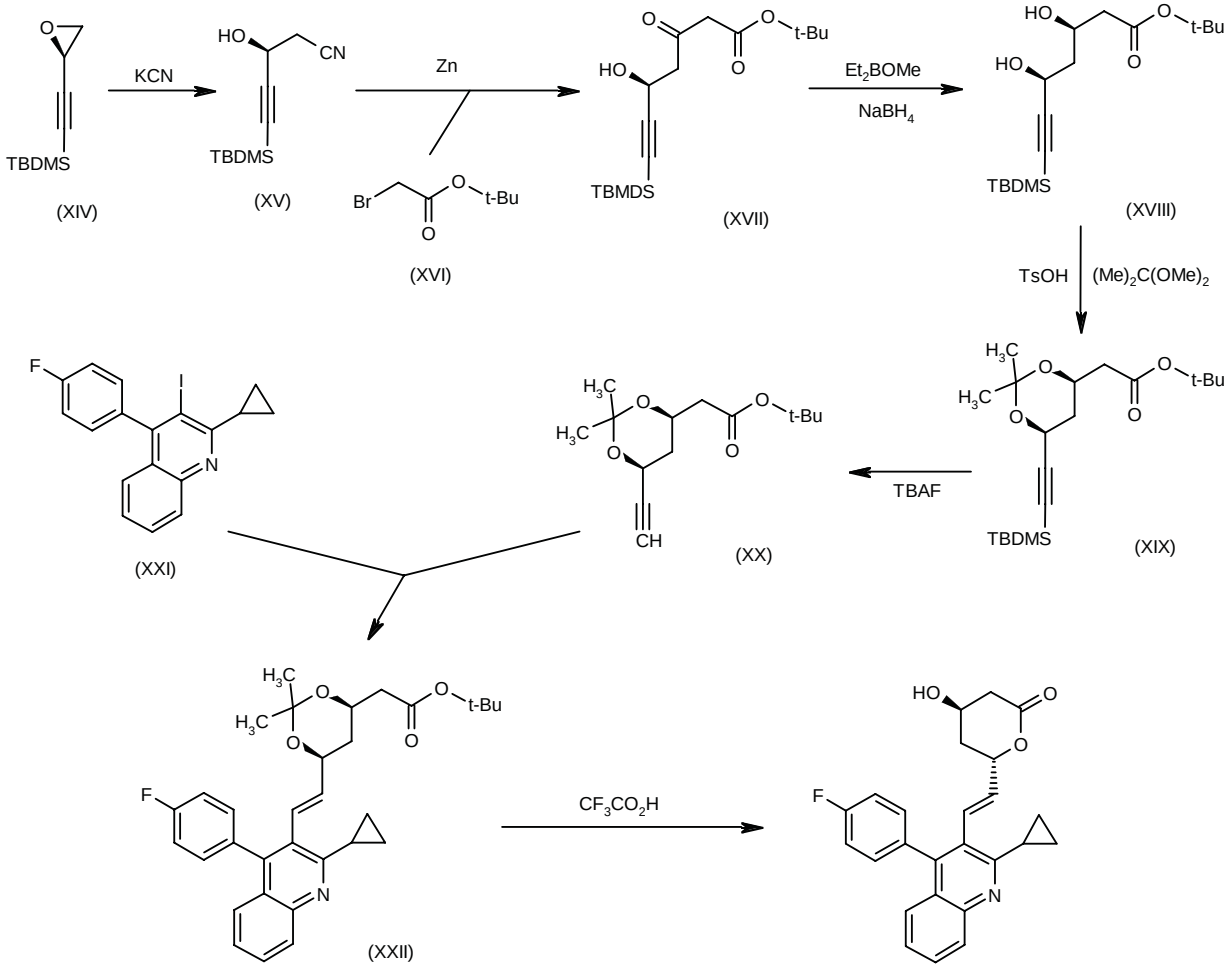
Scheme 1: Synthesis of the Lactone Form of NK-104



disiamylborane, NaOEt and $PdCl_2$ in acetonitrile gives the protected (3*R*,5*S*)-NK-104 ethyl ester (XXXII) (5). Scheme 3.

4) *Lactone form:* The condensation of 2(*S*)-(chloromethyl)oxirane (XXXIII) with trimethylsilylacetylene (XXXIV) by means of butyl lithium and BF_3 ethearate

Scheme 2: Synthesis of the Lactone Form of NK-104

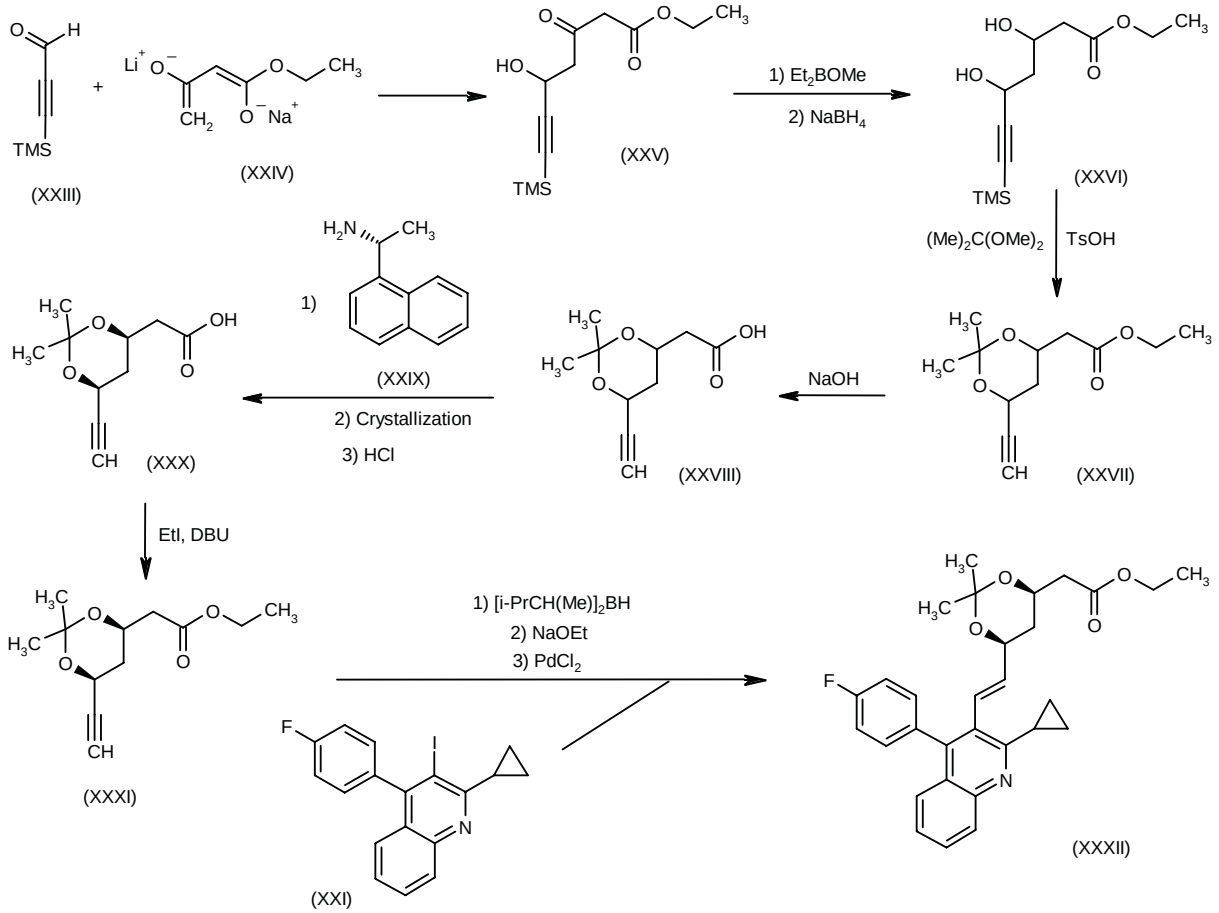


in THF gives 5-chloro-4(*S*)-hydroxy-1-(trimethylsilyl)-1-pentyne (XXXV), which is cyclized with KOH in THF to the chiral epoxide (XXXVI). The condensation of (XXXVI) with 2-cyclopropyl-4-(4-fluorophenyl)-3-(phenylsulfanylmethyl)quinoline (XXXVII, see Scheme 5) by means of butyl lithium in THF affords the silylated heptynol (XXXVIII), which is desilylated with K_2CO_3 in methanol to the terminal acetylene (XXXIX). The carboxylation of (XXXIX) with CO by means of $PdCl_2/CuCl_2$ in methanol yields the heptynoic acid ester (XL), which is selectively reduced with H_2 over the Lindlar catalyst in methanol to the *cis*-heptenoic ester (XLI). The cyclization of (XLI) with PPTS in refluxing toluene affords the (*S*)-unsaturated lactone (XLII), which is oxidized with *m*-chloroperbenzoic acid to the corresponding sulfinyl derivative (XLIII). Elimination of thiophenol from (XLIII) by means of $CaCO_3$ in refluxing toluene gives the unsaturated lactone (XLIV) with the (*E*)-vinylene bond. The α,β-epoxydation of the unsaturated lactone (XLIV) with H_2O_2 and NaOH in methanol/dichloromethane affords the monoepoxy lactone (XLV) regioselectively, which is finally submitted to a regioselective ring opening with diphenyl diselenide and $NaBH_4$ in THF (6). Scheme 4.

5) Alternative synthesis of the terminal acetylene (XXXIX): The condensation of 2(*R*)-(chloromethyl)oxirane (XLVII) with 2-cyclopropyl-4-(4-fluorophenyl)-3-(phenylsulfanylmethyl)quinoline (XXXVII, see Scheme 5) by means of butyl lithium in THF gives the 1-chloro-2(*R*)-butanol (XLVIII), which is treated with KOH in THF yielding the corresponding epoxide (XLIX). Finally, this compound is condensed with lithium acetylide (L) by means of ethylenediamine in DMSO to afford the terminal acetylene (XXXIX) (6). Scheme 4.

6) Synthesis of the quinoline (XXI): Anthranilic acid (LI) is tolylated with tosyl chloride and treated with PCl_5 in 1,2-dichloroethane to give the corresponding acyl chloride (LII), which is submitted to a Friedel Crafts condensation with fluorobenzene (LIII)/$AlCl_3$ yielding 2-amino-4'-fluorobenzophenone (LIV). The cyclization of (LIV) with ethyl 2-(cyclopropylcarbonyl)acetate (LV) [obtained by condensation of cyclopropyl methyl ketone (LVI) and

Scheme 3: Synthesis of Intermediate (XXXII)



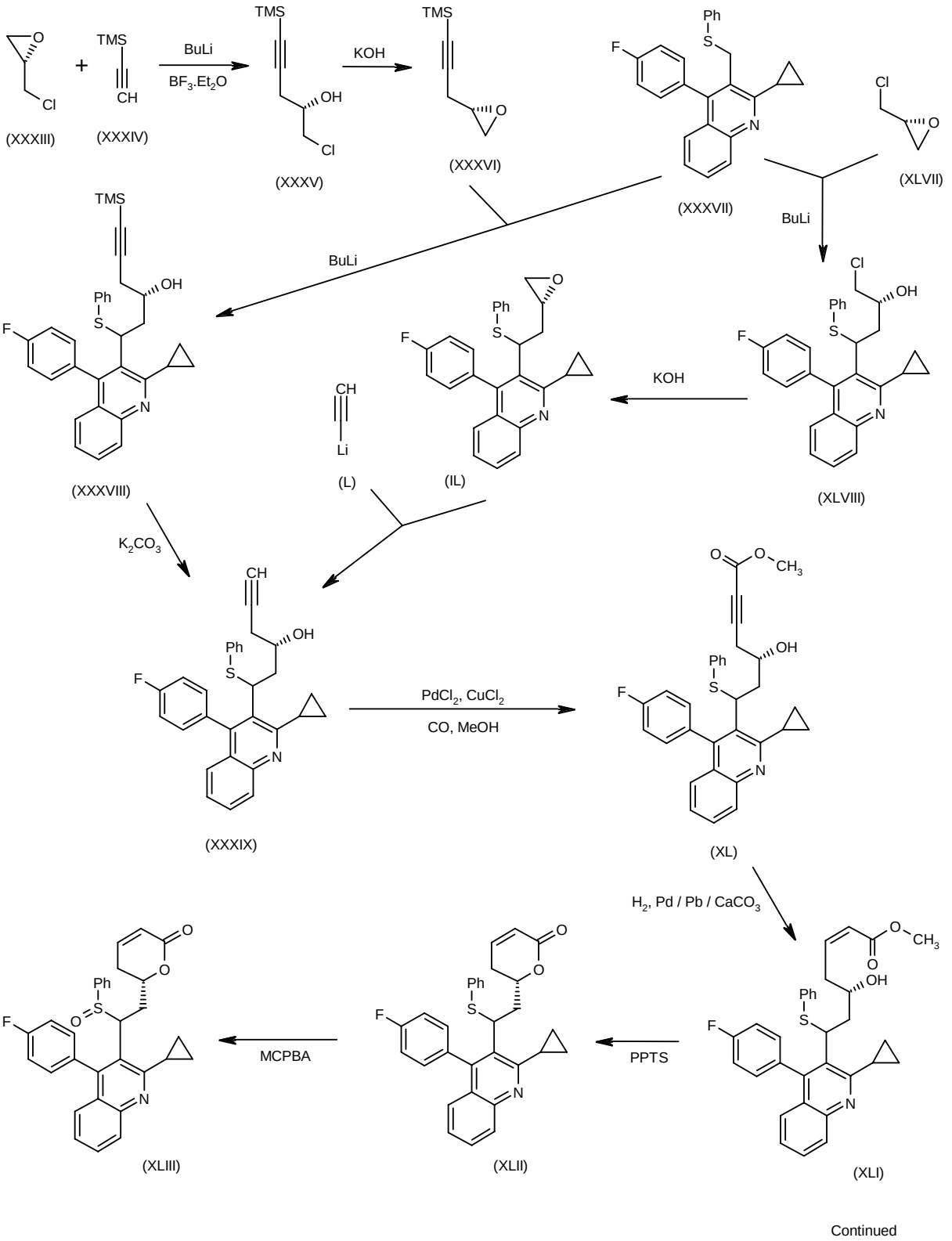
diethyl carbonate (LVII) with H_2SO_4] by means of *p*-toluenesulfonic acid yields 2-cyclopropyl-4-(4-fluorophenyl)-quinoline-3-carboxylic acid ethyl ester (LVIII), which is submitted to a decarboxylative iodination with I_2 and acetyl peroxide to afford 2-cyclopropyl-4-(4-fluorophenyl)-3-iodoquinoline (XXI) (5). Scheme 5.

7) Synthesis of the quinoline (XXXVII): The reduction of the quinolinecarboxylate (LVIII) with $LiAlH_4$ in THF gives the corresponding methanol derivative (LIX), which is then treated with diphenyl disulfide (LX) and tributylphosphine in pyridine to afford 2-cyclopropyl-4-(4-fluorophenyl)-3-(phenylsulfanylmethyl)quinoline (XXXVII) (6). Scheme 5.

8) *Open form:* The silylation of (*S*,*S*)-tartaric acid diisopropyl ester (LXI) with *tert*-butyldimethylsilyl chloride (TBDMS-Cl) and imidazole in DMF gives the bissilylated compound (LXII), which is condensed with the disodium salt of *tert*-butyl acetoacetate (LXIII) in THF, yielding (*S*,*S*)-7-(*tert*-butoxycarbonyl)-2,3-bis(*tert*-butyldimethylsilyloxy)-4,6-dioxoheptanoic acid isopropyl ester (LXIV). The selective reduction of (LXIV) with DIBAL in THF affords the monohydroxylated compound (LXV), which is further reduced with diethylmethoxyborane in THF to the dihydroxylated compound (LXVI). The protection of the OH groups of (LXVI) with 2,2-dimethoxypropane and *p*-toluenesulfonic acid gives the 1,3-dioxane derivative (LXVII), which is desilylated with TBAF in THF to the *gem*-diol (LXVIII). Oxidation of the diol (LXVIII) with sodium metaperiodate in water/ethyl ether affords the aldehyde (LXIX), which is then condensed with 2-cyclopropyl-4-(4-fluorophenyl)-quinolin-3-ylmethyl(diphenyl)phosphine oxide (LXX) by means of lithium 2,2,6,6-tetramethylpiperidine (TMPip-Li) (7) or butyl lithium (8) in THF, giving the protected *tert*-butyl ester (XXII). Finally, this compound is deprotected and hydrolyzed with trifluoroacetic acid in dichloromethane (7). Scheme 6.

9) The phosphine oxide (LXX) has been obtained as follows: 2-Cyclopropyl-4-(4-fluorophenyl)-3-(hydroxymethyl)quinoline (LIX, Scheme 5) was treated with PBr_3, yielding the corresponding bromomethyl derivative (LXXI), which was then treated with diphenyl(ethoxy)phosphorane in refluxing toluene (8). Scheme 6.

Scheme 4: Synthesis of the Lactone Form of NK-104



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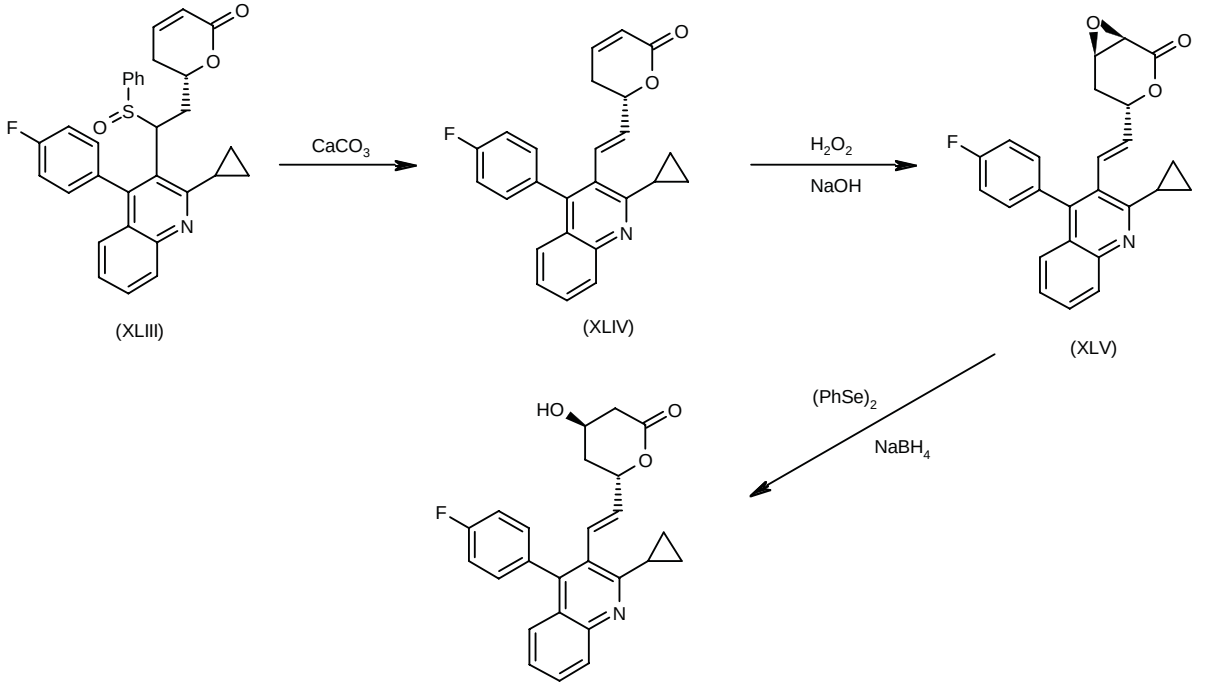
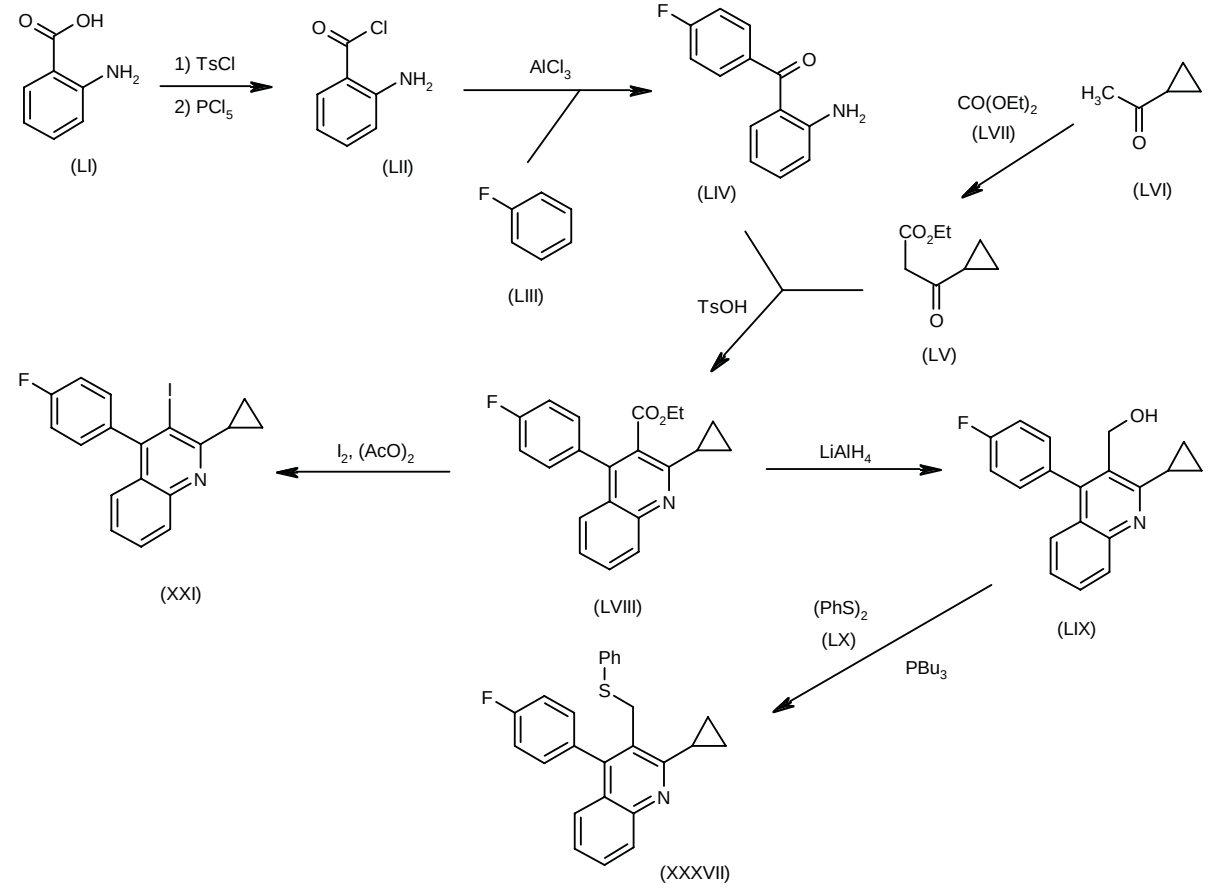
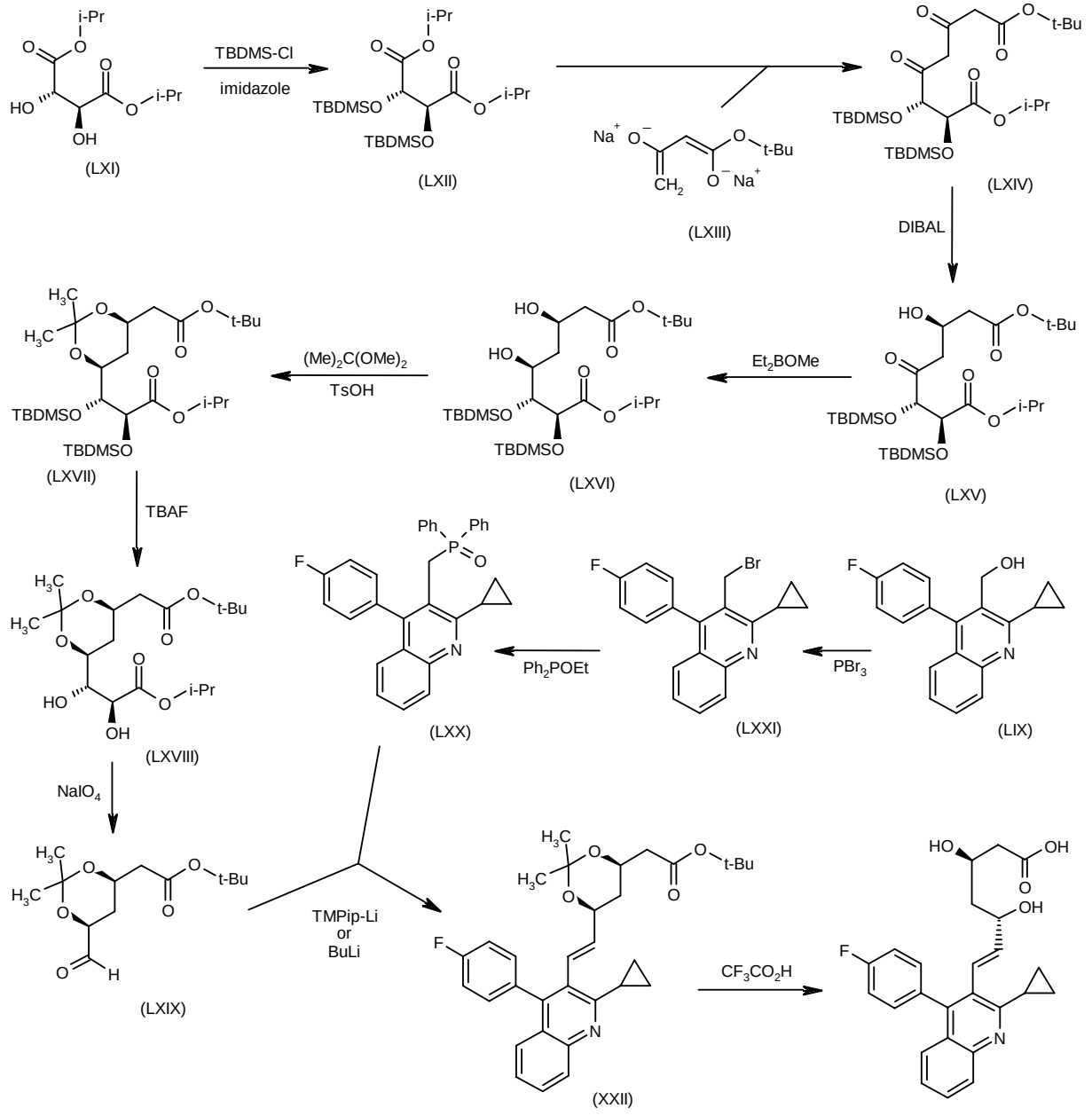
Scheme 4 (Cont.): Synthesis of the Lactone Form of NK-104



Scheme 5: Synthesis of Intermediates (XXI) and (XXXVII)



Scheme 6: Synthesis of NK-104



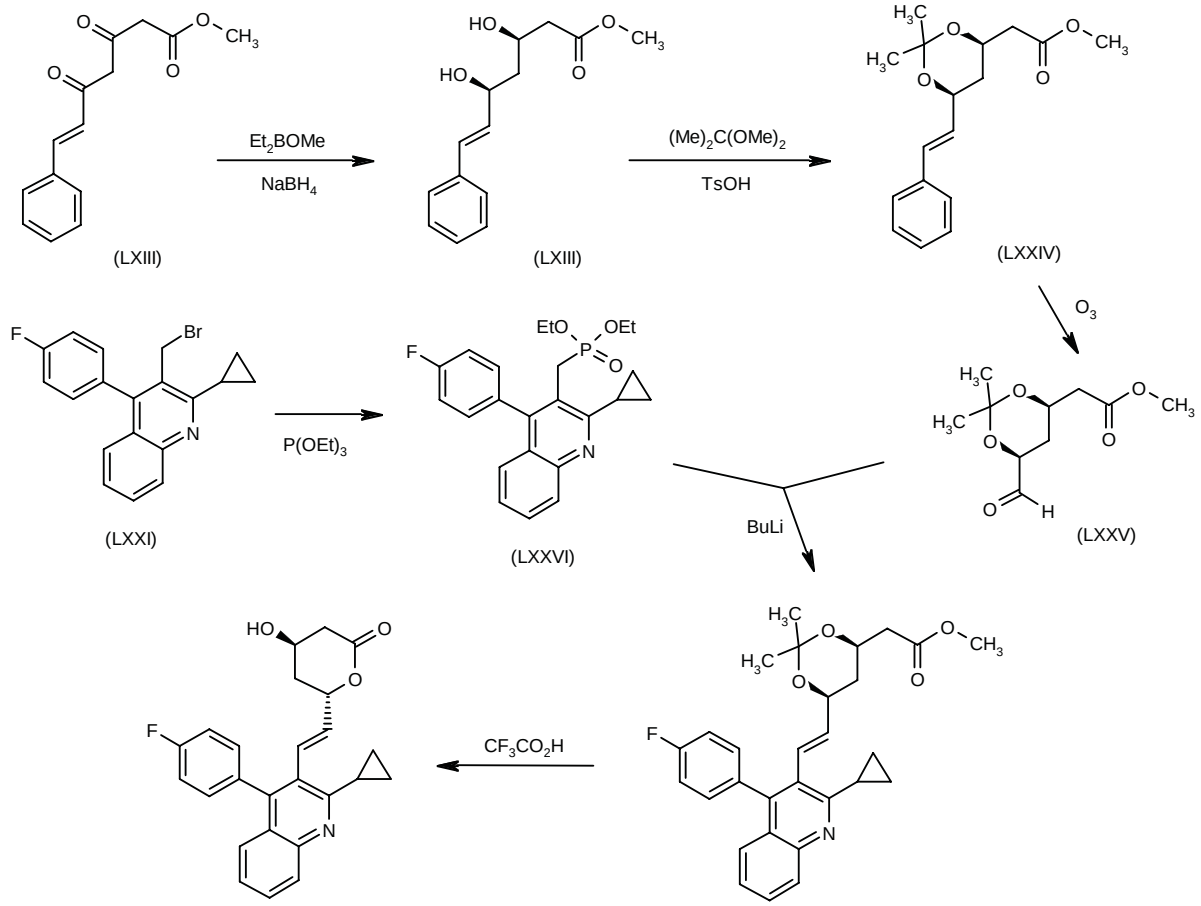
10) *Lactone form:* The reduction of 7-phenyl-3,5-dioxo-6(*E*)-heptenoic acid methyl ester (LXXII) with diethylmethoxyborane and $NaBH_4$ in THF/methanol gives the (3*R**,5*S**,6*E*)-dihydroxy ester (LXXIII), which by reaction with acetone dimethylacetal and *p*-toluenesulfonic acid yields the acetonide (LXXIV). The ozonolysis of (LXXIV) with O_3 and dimethylsulfide in methanol affords the aldehyde (LXXV), which is condensed with 2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-ylmethylphosphonic acid diethyl ester (LXXVI) by means of butyl lithium to give the acetonide of the methyl ester (LXXVII). Finally, this compound is treated with trifluoroacetic acid to yield the lactone (XLVI) (8). Scheme 7.

The phosphonate (LXXVI) has been obtained by reaction of the bromomethyl derivative (LXXI) with triethylphosphite (8). Scheme 7.

Introduction

Increased levels of total cholesterol (TC), low density lipoprotein (LDL)-cholesterol and very low density lipopro-

Scheme 7: Synthesis of the Lactone Form of NK-104



tein (VLDL)-cholesterol are identified as risk factors for the development of coronary heart disease, a major cause of death in Western societies. Reduction of TC and LDL-cholesterol concentrations in the plasma of hypercholesterolemic patients has been shown to reduce the incidence of cardiovascular mortality. Several drug strategies have been proposed to effectively control hyperlipidemia, including cholesterol absorption inhibitors (see monograph on F-1394 in the July issue of the journal) and cholesterol biosynthesis inhibitors.

The enzymatic biosynthesis of cholesterol is a complex process requiring more than 25 reaction steps. The four principal steps of the pathway include: conversion of acetic acid to mevalonic acid; conversion of mevalonic acid into squalene; conversion of squalene into lanosterol; and conversion of lanosterol into cholesterol. Inhibiting the synthesis of cholesterol has been established as one of the most effective approaches to lowering serum cholesterol. Several enzymes have been selected as targets for hypocholesterolemic drug design. Among these enzymes, HMG-CoA reductase [EC 1.1.1.34], the rate-limiting enzyme in cholesterol biosynthesis, catalyzes the reduction of HMG-CoA into mevalonic acid. Inhibition of this enzyme has proven to be an effective means for lowering serum TC and LDL levels in humans.

As shown in Table I, six HMG-CoA reductase inhibitors have been launched and at least three others are under clinical development. These include nisvastatin (NK-104; Nissan Chemical/ Kowa), S-4522/ZD-4522 (Shionogi; Zeneca) and bervastatin (Lipha). Table II presents the biological activity of HMG-CoA reductase inhibitors launched or in clinical development.

Pharmacological Actions

Nisvastatin (NK-104) is a novel, potent fully synthetic inhibitor of HMG-CoA reductase. Several *in vitro* and *in vivo* studies have demonstrated the ability of NK-104 to potently inhibit HMG-CoA reductase with a long duration of action. NK-104 was found to inhibit rat liver microsomal HMG-CoA reductase with a K_i of 1.7 nM. Moreover, the effect of NK-104 on de novo sterol synthesis determined by the the rate of [^{14}C]-acetate incoroporation was examined in male Wistar rats and compared to those of pravas-

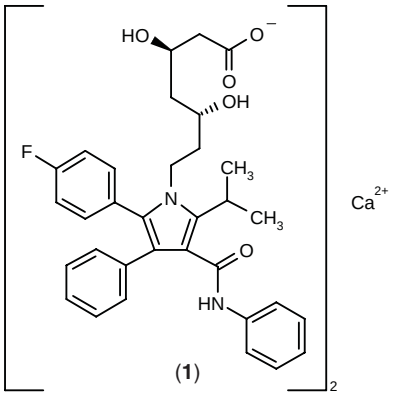
Table I: Chemical structures of HMG-CoA reductase inhibitors launched (year of introduction) and in clinical trials.

Launched

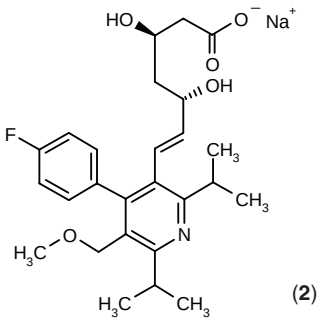
1. Atorvastatin calcium
   Parke-Davis; Pfizer (1997)
2. Cerivastatin sodium
   Bayer; SmithKline Beecham; Fournier (1997)
3. Fluvastatin sodium
   Novartis; Astra; Pierre Fabre; Tanabe Seiyaku (1994)
4. Lovastatin
   Merck & Co. (1987)
5. Pravastatin sodium
   Sankyo; Bristol-Myers Squibb; Rhône-Poulenc Rorer, Esteve (1989)
6. Simvastatin
   Merck & Co.; Mediolanum; Amrad (1988)

Clinical Trials

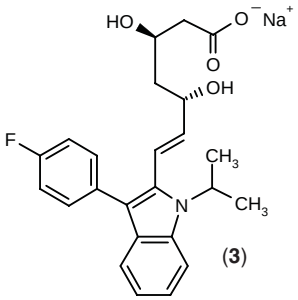
7. Bervastatin
   Lipha
8. NK-104
   Nissan Chemical; Kowa
9. S-4522/ZD-4522
   Shionogi; Zeneca



(**1**)



(**2**)



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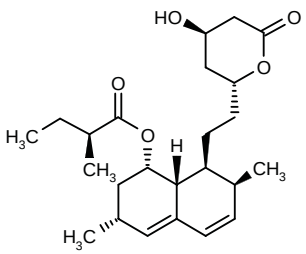

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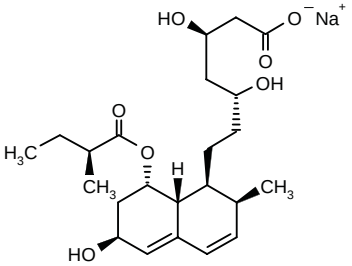
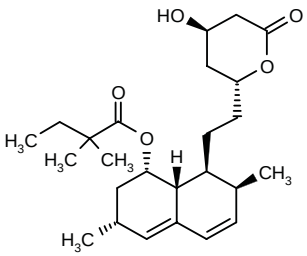


(**5**)



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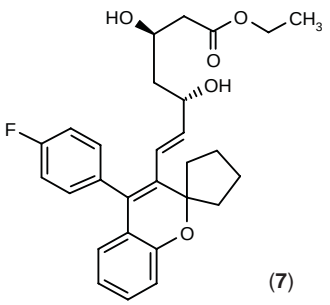

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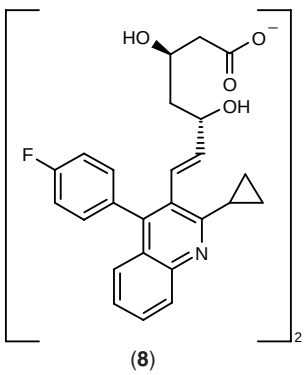

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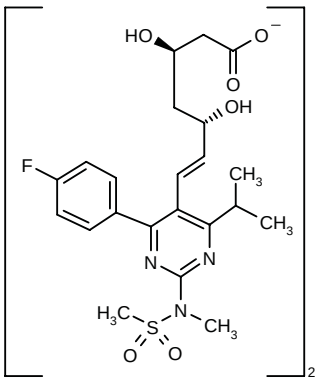

(**9**)

Source: Prous Science Ensemble database.

tatin and simvastatin. Animals received 1 mg/kg of the agents orally, followed by [^{14}C]-acetate injection (100 μCi/ml/kg i.p.). NK-104 significantly inhibited sterol synthesis in liver (ED_{50} = 0.13 g/kg) and ileum (ED_{50} = 0.20 mg/kg), and to a weaker extent in other tissues. While the inhibitory effects of pravastatin and simvastatin disap-

Table II: Biological activity of HMG-CoA reductase inhibitors launched and in clinical trials.

Compound	HMG-CoA reductase inhibition+ (IC_{50} nM)	Cholesterol synthesis inhibition (IC_{50} nM)	Material	Reference
Atorvastatin	7.0	-	-	28
Bervastatin	12	32	Rat hepatic microsomes	40
Cerivastatin	1.3*	1	Human Hep-G2 cells	35
Fluvastatin	31	-	-	29
	8	52	Rat hepatocytes	38
	2.5	79	Human Hep-G2 cells	30
	-	52	Rat hepatocytes	25
Lovastatin	27	32	Rat hepatocytes	25, 38
	13	29	Human Hep-G2 cells	33
	12	30	Human Hep-G2 cells	42
	9.2	50	Human Hep-G2 cells	30
	4	146	Rat hepatocytes	32
	2.3*	5	Human Hep-G2 cells	37
	2	-	-	34
	0.64*	-	-	36
NK-104	1.7*	0.13#	Rat liver	9
Pravastatin	40	-	-	26
	37.7	198	Rat hepatocytes	29
	26	100	Rat hepatocytes	32
	8.9	-	-	31, 37
	5.9*	2.4#	Rat liver	9
	2.3*	0.005	Rat hepatocytes	41
S-4522	11	1.12	Rat hepatocytes	29
Simvastatin	3	12	Human Hep-G2 cells	33
	0.94	-	-	27
	2.8*	0.36#	Rat liver	9
	0.2*	-	-	39

+In rat liver microsomes. *K_i nM. #Inhibition of sterol synthesis in rats *ex vivo* (ED_{50} mg/kg p.o.). Source: Prous Science MFLine database.

peared 6 h after administration, NK-104 suppression continued for over 6 h. The hypolipidemic actions of these agents were also compared in beagle dogs. After oral administration of the drugs for 2 weeks, NK-104 (doses of 0.1 mg/kg or higher) was found to decrease plasma triglycerides in addition to markedly reducing plasma cholesterol in a dose-dependent manner (13.1, 18.5 and 20.2% at doses of 0.1, 0.3, and 1 mg/kg, respectively). In contrast, although pravastatin (1 and 3 mg/kg) and simvastatin (3 mg/kg) lowered TC levels, plasma triglyceride levels remained unaltered (9).

Studies using WHHL rabbits have also demonstrated the cholesterol and triglyceride lowering ability of NK-104, in addition to its preventative effects on atherosclerosis. Rabbits were administered 0.5 mg/kg/day NK-104 in drinking water for 26 weeks. Significant reductions in the surface of the lesion areas were noted in treated individuals. Although LDL levels were unaltered by treatment, VLDL-C and IDL-C levels significantly decreased by 61.8 and 48.8%, respectively. Similarly, hepatic mRNA expression was enhanced. These data suggest that NK-104 may prevent the progression of atherosclerosis by reductions in plasma VLDL and IDL (10).

The ability of NK-104 to inhibit HMG-CoA was further demonstrated in studies using perfused liver of guinea pigs. Hepatic sterol synthesis was effectively suppressed by NK-104 treatment. Moreover, the effects of NK-104 were more potent and of a longer duration than those of simvastatin. Guinea pigs were orally administered 0.03, 0.3 or 3 mg/kg NK-104 or 30 mg/kg simvastatin for 2 weeks, after which livers were isolated and perfused. Results demonstrated the NK-104 decreased VLDL-triglycerides and VLDL-apolipoprotein (apo) B secretion in a dose-dependent manner. Significant reductions were observed with the NK-104 dose of 3 mg/kg, while simvastatin was ineffective (11).

One mechanism responsible for the NK-104 induced reduction in LDL has been shown to be increased expression of the hepatic LDL receptor. Studies performed *in vitro* using a hepatoma cell line (HepG2) revealed that NK-104 suppressed cholesterol synthesis with a lower IC_{50} as compared to simvastatin (9.0 x 10-9 M *vs.* 1.7 x 10-8 M, respectively). In addition, specific binding, internalization and degradation of 125I-LDL was dose-dependently increased approximately 3-fold. *In vivo* studies in which guinea pigs received NK-104 orally at a dose of 3 mg/kg for 3 weeks demonstrated a 45% decrease in LDL accompanied by a 1.6-fold increase in specific binding of ^{125}I-LDL in liver microsomal membrane. Simvastatin reduced LDL by 36% and increased LDL membrane binding in a similar manner. However, a 10-fold higher dose of simvastatin was necessary for the observed effects (12, 13).

Other studies have shown that NK-104 also is capable of contributing to plaque stability by suppressing foam cell formation of macrophages, an effect which triggers atherosclerotic plaque rupture. Studies revealed that NK-104 suppressed acetylated-, oxidized- and glycated-LDL-induced cholesterol ester deposition in J774 macrophages in a dose-dependent manner. Inhibition of denatured lipoprotein uptake by macrophages could be blocked by mevalonolactone and geranylgeraniol; farnesol, dolichol and ubquinone were ineffective in this respect. In addition, a 48-h pretreatment with 10 μM NK-104 inhibited cellular association and degradation of ^{125}I-acetylated-LDL by 62 and 97%, respectively; binding was unaffected by NK-104 treatment. Inhibitory effects were successfully blocked by mevalonolactone and geranylgeraniol although farnesol was ineffective. These results suggest that NK-104 may act through inhibition of geranylgeraniol moiety production to inhibit foam cell formation by macrophages (14).

NK-104 is capable of dose-dependently suppressing the smooth muscle cell growth responsible for neointimal thickening which accompanies atherosclerosis. NK-104 (1.0 mg/kg) maximally suppressed neointimal thickening by 69.5% in balloon-injured rabbit carotid artery, and the areas occupied by fibronectin and type I collagenase were significantly reduced from 81% and 38% in controls to 39% and 22%, respectively in NK-104 treated arteries (15, 16).

NK-104 has also been shown to modulate the secretion of apoB-100 in a human hepatoma cell line (HepG2). Preincubation of cells with 0.01-5 μM of NK-104 for 24 h, followed by an additional preincubation with oleate (0.8 mM), resulted in inhibition of [^{14}C]-acetate incorporation and a dose-dependent decrease in apoB-100 secretion. Further investigation has shown that modulation of apoB-100 secretion by NK-104 is post-translational; the cellular degradation of apoB-100 was reduced while synthesis remained unaltered (17). Moreover, NK-104 accelerated apolipoprotein A-1 secretion, thus further modulating cholesterol biosynthesis (18).

HMG-CoA reductase inhibitors were found to have differential time courses of action. In HepG2 cells, NK-104 was shown to increase mRNA for the LDL receptor and HMG-CoA reductase 4 h after incubation; simvastatin and compactin were ineffective. The NK-104-induced increase in mRNA levels remained unchanged up to 12 h, when levels were not significantly different from control untreated cells (19).

Pharmacokinetics

The pharmacokinetics of NK-104 were examined in rats, monkeys, dogs and humans. The plasma half-life in animals after oral treatment was approximately 4 h with an observed high bioavailability (80%) in rats, rabbits and dogs. The long half-life was attributed to the fact that the majority of the drug was excreted in bile and reabsorbed. The target organ for NK-104 was found to be the liver, and the drug was excreted in feces in rats, monkeys and dogs and in urine in rabbits. Examination of metabolites showed the presence of the lactone form and beta-oxidation products of NK-104. In humans, linear pharmacokinetics were observed since the maximum plasma concentration (C_{max}) and area under plasma concentration-*versus*-time curve (AUC) of the drug were proportional to the doses (0.5-8 mg/man). Although the half-life remained unchanged after 7 days of administration (11 h), the C_{max} of NK-104 increased 1.7-fold (20).

Clinical Studies

The effects of NK-104 as a hypocholesterolemic agent were evaluated in several clinical studies, confirming the efficacy and tolerability of NK-104 as an antihypercholesterolemic agent. Thirty-five hypercholesterolemic patients were orally administered 1, 2 or 4 mg/day, 1 mg twice daily or a placebo for 4 weeks. Significant differences were observed between treated and untreated patients. Treated patients exhibited marked decreases in TC (20, 28, 30 and 26% for respective doses) and LDL levels (32, 37, 43 and 41% for respective doses). No adverse side effects were observed with the exception of slight increases in GOT and GPT levels in some cases. The effective doses were determined to be 2 mg/day or 1 mg twice daily (21).

Similar results were also reported in a study involving 266 hypercholesterolemic patients administered 1, 2 or 4 mg NK-104 daily for 12 weeks. Decreases in TC of 23, 29 and 33% were observed with the 1-, 2- and 4-mg doses, respectively, and triglyceride levels were reduced by approximately 22-30%. These reductions were accompanied by a 6-8 mg/dl increase in high-density lipoprotein (HDL) levels at week 12. No adverse side effects were observed except 2.3, 3.8 and 5.4% increases in GOT, GPT and CK, respectively (22).

Clinical studies have also been performed to investigate the effects of NK-104 in patients with heterozygous familial hypercholesterolemia. Twenty-four patients were first administered 2 mg/day NK-104 for 8 weeks, followed by 4 mg/day for an additional 8 weeks. Decreases of 30% and 40% were observed in serum TC and LDL levels, respectively, after treatment with the initial dose of NK-104. The observed reductions were further increased following the subsequent 4 mg/day dose (36% and 47%, respectively). Moreover, serum triglyceride levels, apoB, C2, C3 and E levels significantly decreased after NK-104 treatment. No adverse side effects were reported (23).

NK-104 is in phase III trials for the once-daily oral treatment of hypercholesterolemia (24).

Manufacturer

Nissan Chemical Industries, Ltd. (JP), licensed to Kowa Co., Ltd. (JP).

References

1. Hiyama, T., Minami, T., Hanamoto, T., Reddy, G.B. (Sagami Chem. Res. Center). *Optically active esters of 7-substd. 3,5-difunctionalized 6-heptenoic acids.* EP 475627.

2. Kaiyama, T., Minami, T., Guntor, B., Sakota, R., Arai, K., Kobara, Y., Suzuki, M. (Sagami Chem. Res. Center). *Optically active β,δ-diketo acid esters and their reduced forms.* JP 94025092.

3. Takahashi, K., Minami, T., Ohara, Y., Hiyama, T. *A new synthesis of HMG-CoA reductase inhibitor NK-104 through hydrosilylation-cross coupling reaction.* Tetrahedron Lett 1993, 34: 8263-6.

4. Takahashi, K., Minami, T., Ohara, Y., Hiyama, T. *Synthesis of an artificial HMG-CoA reductase inhibitor NK-104 via a hydrosilylation-cross-coupling reaction.* Bull Chem Soc Jpn 1995, 68: 2649-56.

5. Miyachi, N., Yanagawa, Y., Iwasaki, H., Ohara, Y., Hiyama, T. *A novel synthetic method of HMG-CoA reductase inhibitor NK-104 via a hydroboration-cross-coupling sequence.* Tetrahedron Lett 1993, 34: 8267-70.

6. Takano, S., Kamikubo, T., Sugihara, T., Suzuki, M., Ogasawara, K. *Enantioconvergent synthesis of a promising HMG-CoA reductase inhibitor NK-104 from both enantiomers of epichlorohydrin.* Tetrahedron Asymmetry 1993, 4: 201-4.

7. Hiyama, T., Minami, T., Takahashi, K. *Synthesis of artificial HMG-CoA reductase inhibitors based on the olefination strategy.* Bull Chem Soc Jpn 1995, 68: 364-72.

8. Kaiyama, T., Minami, T., Yanagawa, E., Obara, Y. (Sagami Chem. Res. Center; Nissan Chem. Ind., Ltd.). *Condensed pyridines mevalonolactone intermediates and their preparation method.* JP 93310700.

9.Aoki, T., Nishimura, H., Nakagawa, S. et al. *Pharmacological profile of a novel synthetic inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase.* Arzneim-Forsch-Drug Res 1997, 47: 904-9.

10. Suzuki, H., Yamazaki, H., Aoki, T., Tamaki, T., Sato, F., Kitahara, M., Saito, Y. *Lipid-lowering and antiatherosclerotic effect of NK-104 (nisvastatin), a new synthetic HMG-CoA reductase inhibitor, in WHHL rabbits.* 13th Int Symp Drugs Affect Lipid Metab (May 30-June 3, Florence) 1998, 53.

11. Suzuki, H., Tamaki, T., Aoki, T., Sato, F., Kitahara, M., Saito, Y. *Inhibitory effect of NK-104 (nisvastatin), a new synthetic HMG-CoA reductase inhibitor, on VLDL secretion in guinea pigs. Liver perfusion studies after 2-week dosage.* 13th Int Symp Drugs Affect Lipid Metab (May 30-June 3, Florence) 1998, 54.

12. Aoki, T., Nakagawa, S., Suzuki, H., Tamaki, T., Wada, Y., Yokoo, N., Kitahara, M., Saito, Y. *Pharmacological studies on NK-104, a novel potent HMG-CoA reductase inhibitor (2). Plasma lipid-lowering effects in guinea pigs.* Jpn J Pharmacol 1995, 67(Suppl. 1): Abst P1-54.

13. Aoki, T., Nakagawa, S., Suzuki, H., Tamaki, T., Sato, F., Kitahara, M., Saito, Y. *LDL-receptor enhancing effect of NK-104 (nisvastatin) in HepG2 cells (in vitro) and in guinea pigs (in vivo).* 13th Int Symp Drugs Affect Lipid Metab (May 30-June 3, Florence) 1998, 47.

14. Kitahara, M., Kanaki, T., Tamaki, T., Tanaka, S., Saito, Y. *NK-104 (nisvastatin) suppresses foam cell formation induced by denatured low density lipoprotein in J774 macrophages.* 13th Int Symp Drugs Affect Lipid Metab (May 30-June 3, Florence) 1998, 49.

15. Kitahara, M., Kanaki, T., Miyakoshi, C., Kamon, J., Tamaki, T., Tanaka, S., Saito, Y. *NK-104 (nisvastatin) suppresses arterial neointimal thickening by endothelial injury in rabbits via inhibiting smooth muscle cell growth and fibronectin production.* 13th Int Symp Drugs Affect Lipid Metab (May 30-June 3, Florence) 1998, 95.

16. Kitahara, M., Kanaki, T., Toyoda, K., Miyakoshi, C., Tanaka, S., Tamaki, T., Saito, Y. *NK-104, a newly developed HMG-CoA reductase inhibitor, suppresses neointimal thickening by inhibiting smooth muscle cell growth and fibronectin production in balloon-injured rabbit carotid artery.* Jpn J Pharmacol 1998, 77: 117-28.

17. Ooyen, C., Bersino, A.M., Catapano, A.L. *NK-104, a potent HMG-CoA reductase inhibitor, increases intracellular degradation of apo B-100 and decreases its secretion in HepG2 cells.* Atherosclerosis 1997, 134(1-2): Abst 2.P.71.

18. Yanagida, A., Hara, E., Yotsumoto, H., Yamamoto, M. *Influence of NK-104, a novel HMG-CoA reductase inhibitor (HCRI), on inhibition of apoprotein B100-secretion and acceleration of apoprotein A-1-secretion from HepG2 cells.* J Jpn Atheroscler Soc 1998, 25(Suppl.): Abst 80.

19. Umetani, M., Morikawa, S., Kodama, T., Saito, Y. *NK-104 (nisvastatin): Comparative study on the time course of mRNA induction for LDL receptor and HMG-CoA reductase in HepG2 cells by HMG-CoA reductase inhibitors.* 13th Int Symp Drugs Affect Lipid Metab (May 30-June 3, Florence) 1998, 128.

20. Kimata, H., Kojima, J., Fujino, H., Koide, T., Yamada, Y., Yoshimura, M., Nakabeppu, H., Nakaya, N., Saito, Y. *Pharmacokinetics of NK-104 (nisvastatin) in animals and human.* 13th Int Symp Drugs Affect Lipid Metab (May 30-June 3, Florence) 1998, 63.

21. Nakaya, N., Kojima, J., Kimata, H., Kuwahata, R., Narusima, H. *NK-104: Efficacy and tolerance of a new synthetic HMG-CoA reductase inhibitor in hypercholesterolemic volunteers. 1*2th Int Symp Drugs Affect Lipid Metab (Nov 7-10, Houston) 1995, 137.

22. Yamada, N., Goto, Y., Saito, Y. *Clinical effect of NK-104 (nisvastatin) on hyperlipidemia – Double-blind comparative dose-finding study in three parallel groups.* 13th Int Symp Drugs Affect Lipid Metab (May 30-June 3, Florence) 1998, 129.

23. Kajinami, K., Koizumi, J., Miyamoto, S. et al. *Efficacy of NK-104 (nisvastatin), a new totally synthetic HMG-CoA reductase inhibitor, in heterozygous familial hypercholesterolemia.* 13th Int Symp Drugs Affect Lipid Metab (May 30-June 3, Florence) 1998, 63.

24. *NK-104 development status.* Kowa Co., Ltd. Company Communication 1998, July 7.

25. Sit, S.Y., Parker, R.A., Motoc, I., Han, W., Balasubramanian, N., Catt, J.D., Brown, P.J., Harte, W.E., Thompson, M.D., Wright, J.J. *Synthesis, biological profile, and quantitative structure-activity relationship of a series of novel 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors.* J Med Chem 1990, 33: 2982-99.

26. Roth, B.D., Bocan, T.M.A., Blankley, C.J. et al. *Relation between tissue selectivity and lipophilicity for inhibitors of HMG-CoA reductase.* J Med Chem 1991, 34: 463-6.

27. Pietro, D.A., Mantell, G. *Simvastatin: A new HMG-CoA reductase inhibitor.* Cardiovasc Drug Rev 1990, 8: 220-8.

28. Roth, B.D., Blankley, C.J., Chucholowski, A.W. et al. *Inhibitors of biosynthesis. 3. Tetrahydro-4-hydroxy-6-[2-(1H-pyrrol-1-yl)ethyl]-2H-pyran-2-one inhibitors of HMG-CoA reductase. 2. Effects of introducing substituents at positions three and four of the pyrrole nucleus.* J Med Chem 1991, 34: 357-66.

29. Watanabe, M., Koike, H., Ishiba, T., Okada, T., Seo, S., Hirai, K. *Synthesis and biological activity of methanesulfonamide pyrimidine- and N-methylsulfonyl pyrrole-substituted 3,5-dihydroxy-6-heptenoates, a novel series of HMG-CoA reductase inhibitors.* Bioorg Med Chem 1997, 5: 437-44.

30. Connolly, P.J., Westin, C.D., Loughney, D.A., Minor, L.K. *HMG-CoA reductase inhibitors: Design, synthesis, and biological activity of tetrahydroindazole-substituted 3,5-dihydroxy-6-heptenoic acid sodium salts.* J Med Chem 1993, 36: 3674-85.

31. Regan, J.R., Bruno, J.G., Gustafson, K., Amin, D., Neuenschwander, K., Perrone, M. *Inhibitors of HMG-CoA reductase. Biological effects of a 6-[2-[2-(4-fluoro-3-methylphenyl)-4-substituted cyclohexe-1-en-1-yl]ethenyl]-4-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-one.* Eur J Med Chem 1992, 27: 735-42.

32. Karanewsky, D.S., Badia, M.C., Ciosek, C.P. Jr., Robl, J.A., Sofia, M.J., Simpkins, L.M., DeLange, B., Harrity, T.W., Biller, S.A., Gordon, E.M. *Phosphorus-containing inhibitors of HMG-CoA reductase. 1. 4-[(2-Arylethyl)hydroxyphosphinyl]-3-hydroxybutanoic acids: A new class of cell-selective inhibitors of cholesterol biosynthesis.* J Med Chem 1990, 33: 2952-6.

33. Bone, E.A., Davidson, A.H., Lewis, C.N., Todd, R.S. *Synthesis and biological evaluation of dihydroeptastatin, a novel inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase.* J Med Chem 1992, 35: 3388-93.

34. Amin, D., Rutledge, R.Z., Needle, S.N. et al. *RPR 107393, a potent squalene synthase inhibitor and orally effective cholesterol-lowering agent: Comparison with inhibitors of HMG-CoA reductase.* J Pharmacol Exp Ther 1997, 281: 746-52.

35. Bischoff, H., Angerbauer, R., Bender, J., Bischoff, E., Faggiotto, A., Petzinna, D., Pfitzner, J., Porter, M.C., Schmidt, D., Thomas, G. *Cerivastatin: Pharmacology of a novel synthetic and highly active HMG-CoA reductase inhibitor.* Atherosclerosis 1997, 135: 119-30.

36. Alberts, A.W., Chen, J., Kuron, G. et al. *Mevinolin: A highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent.* Proc Natl Acad Sci USA 1980, 77: 3957-61.

37. Amin, D., Gustafson, S.K., Weinacht, J.M., Cornell, S.A., Neuenschwander, K., Kosmider, B., Scotese, A.C., Regan, J.R., Perrone, M.H. *RG 12561 (dalvastatin): A novel synthetic inhibitor of HMG-CoA reductase and cholesterol-lowering agent.* Pharmacology 1993, 46: 13-22.

38. Parker, R.A., Clark, R.W., Sit, S.-Y., Lanier, T.L., Grosso, R.A., Wright, J.J.K. *Selective inhibition of cholesterol synthesis in liver versus extrahepatic tissues by HMG-CoA reductase inhibitors.* J Lipid Res 1990, 31: 1271-82.

39. McTavish, D., Sorkin, E.M. *Pravastatin. A review of its pharmacological properties and therapeutic potential in hypercholesterolaemia.* Drugs 1991, 42: 65-89.

40. Festal, D. *Bervastatin.* Drugs Fut 1995, 20: 1217-9.

41. Tsujita, Y., Kuroda, M., Shimada, Y. et al. *CS-514, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase: Tissue-selective inhibition of sterol synthesis and hypolipidemic effect on various animal species.* Biochim Biophys Acta 1986, 877: 50-60.

42. Kramer, W., Wess, G., Enhsen, A. et al. *Bile acid derived HMG-CoA reductase inhibitors.* Biochim Biophys Acta 1994, 1227: 137-54.