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(72) Inventeurs/Inventors:

NIDDAM-HILDESHEIM, VALERIE, IL;
BALANOV, ANNA, IL;
VEINBERG, IRENA, IL

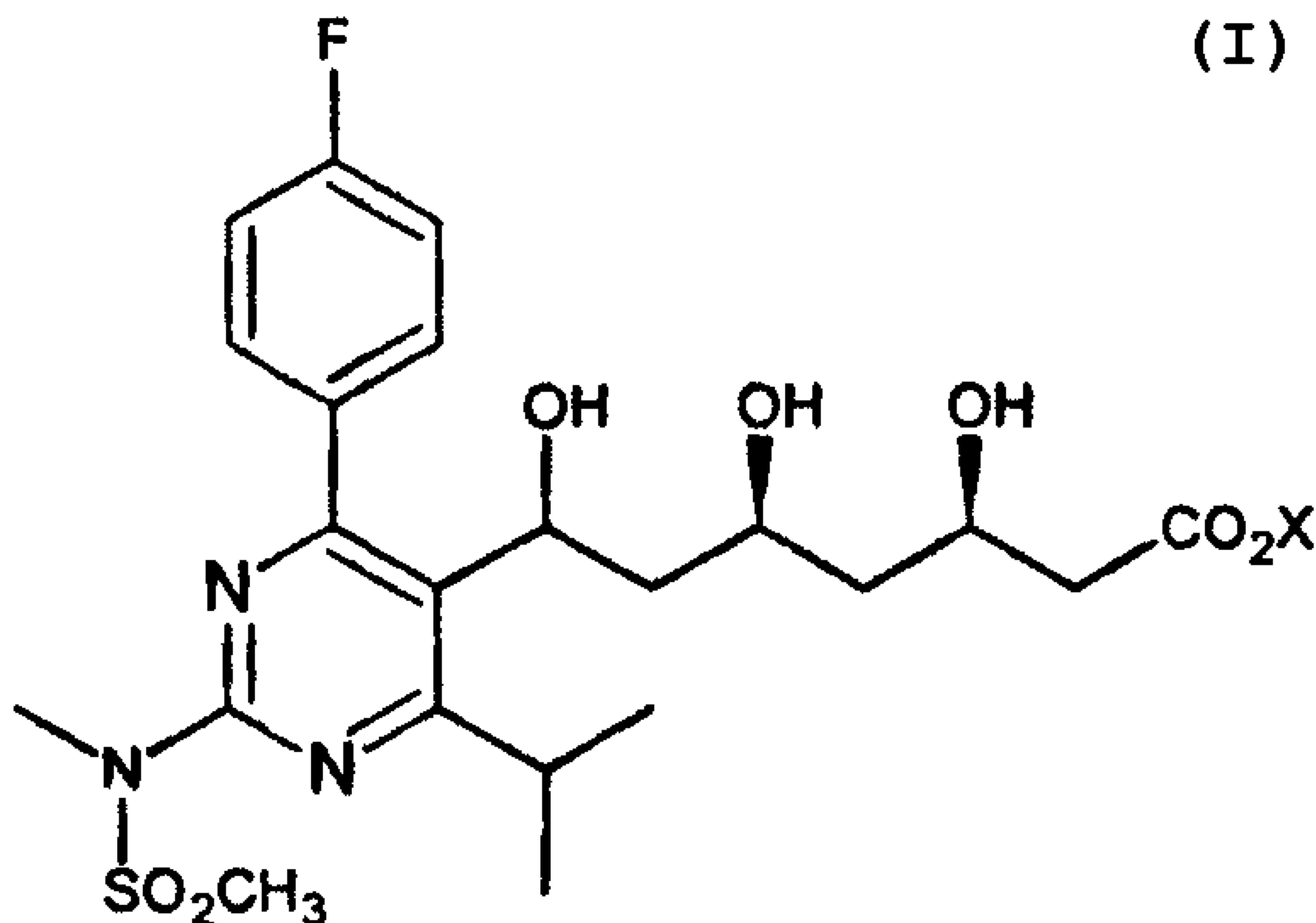
(73) Propriétaire/Owner:

TEVA PHARMACEUTICAL INDUSTRIES LTD., IL

(74) Agent: HEENAN BLAIKIE LLP

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(74) Agents: **MORADIAN, Payam** et al.; Kenyon & Kenyon
LLP, One Broadway, New York, NY 10004-1050 (US).

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(71) Applicant (*for all designated States except BB, US*):
TEVA PHARMACEUTICAL INDUSTRIES LTD.
[IL/IL]; 5 Basel Street, P.O. Box 3190, 49131 Petah Tiqva (IL).

(71) Applicant (*for BB only*): **TEVA PHARMACEUTICALS USA, INC.** [US/US]; 1090 Horsham Road, P.O. Box 1090, North Wales, PA 19454-1090 (US).

(72) Inventors; and

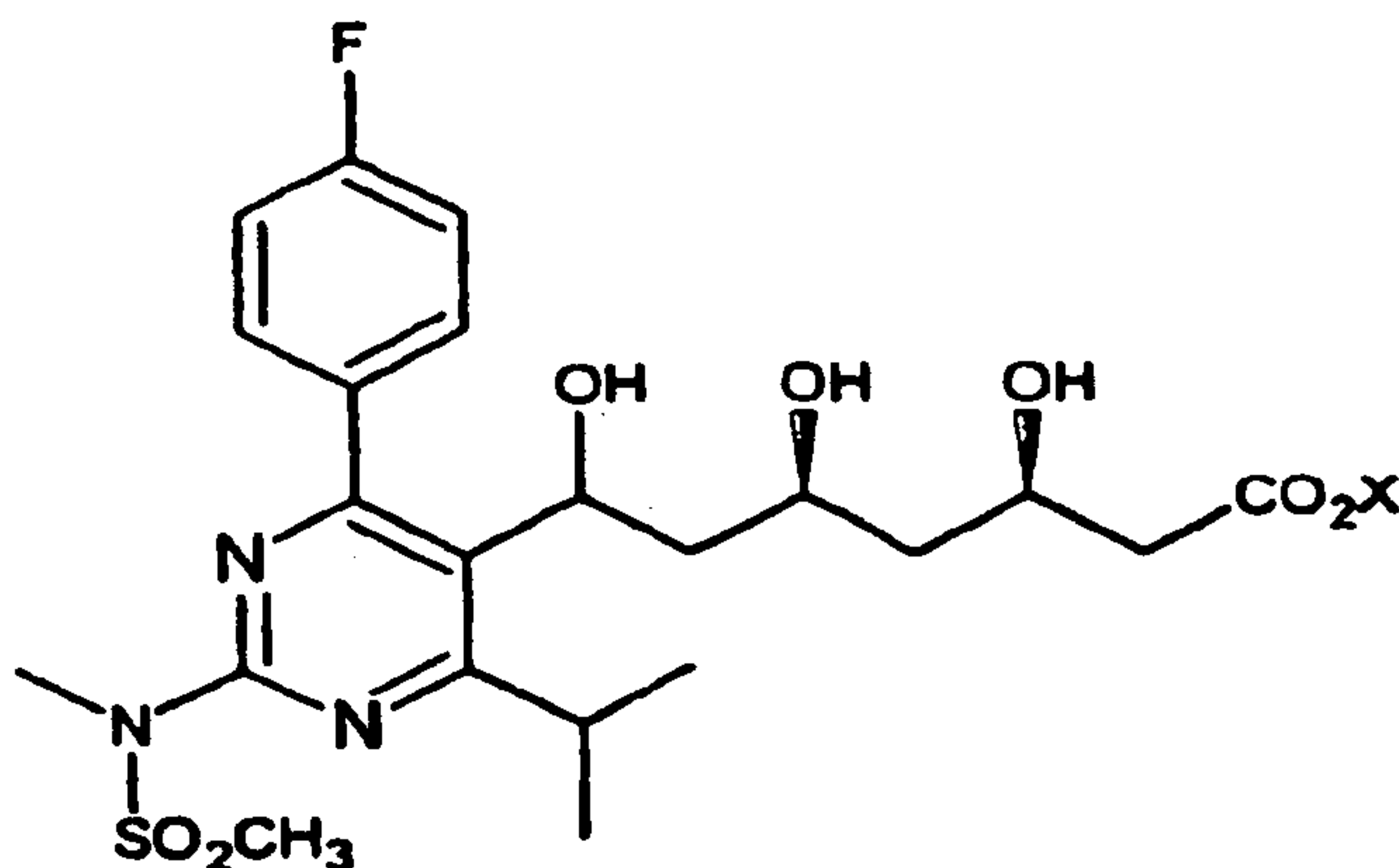
(75) Inventors/Applicants (*for US only*): **NIDDAM-HILDESHEIM, Valerie** [IL/IL]; P.O. Box 3948, 60920 Kadima (IL). **BALANOV, Anna** [IL/IL]; Keselman 3/7, 76573 Rehovot (IL). **VEINBERG, Irena** [IL/IL]; 42 Hanassi Harishon St., Apt.28, 76302 Rehovot (IL).

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TRIOL FORM OF ROSUVASTATIN**CROSS-REFERENCE TO RELATED APPLICATIONS**

This application claims the benefit of U.S. Provisional Application Nos. 60/906,914 filed on March 13, 2007 and 60/918,466 filed on March 15, 2007.

FIELD OF THE INVENTION

The present invention relates to rosuvastatin triol and its use as a reference standard for analysis of rosuvastatin.

BACKGROUND OF THE INVENTION

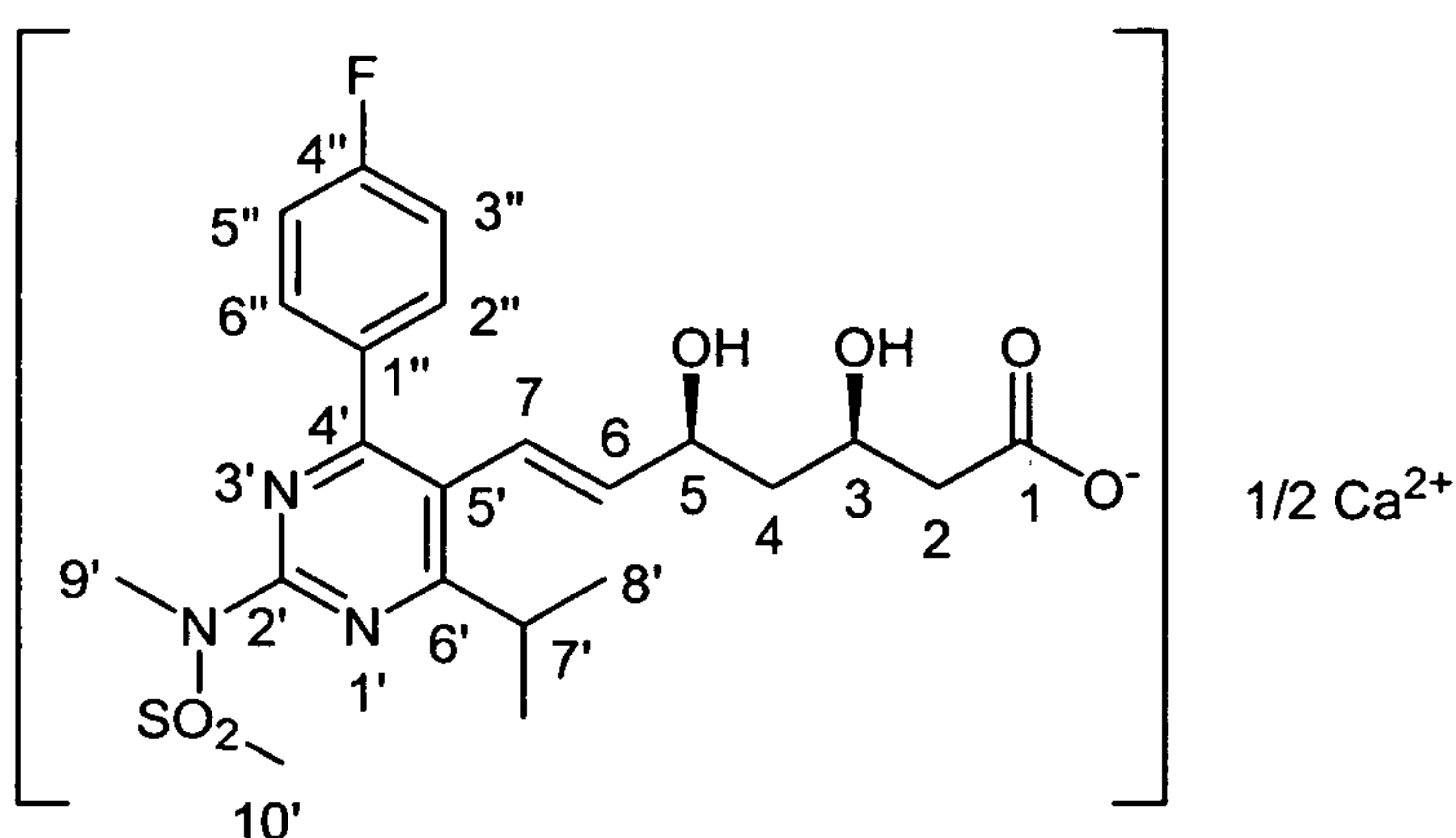
Statins are currently the most therapeutically effective drugs available for reducing low-density lipoprotein (LDL) particle concentration in the blood stream of patients at risk for cardiovascular disease. Thus, statins are used in the treatment of hypercholesterolemia, hyperlipoproteinemia, and atherosclerosis. A high level of LDL in the bloodstream has been linked to the formation of coronary lesions that obstruct the flow of blood and can rupture and promote thrombosis. Goodman and Gilman, The Pharmacological Basis of Therapeutics, page 879 (9th Ed. 1996).

Statins inhibit cholesterol biosynthesis in humans by competitively inhibiting the 3-hydroxy-3-methyl-glutaryl-coenzyme A ("HMG-CoA") reductase enzyme. HMG-CoA reductase catalyzes the conversion of HMG to mevalonate, which is the rate-determining step in the biosynthesis of cholesterol. Decreased production of cholesterol causes an increase in the number of LDL receptors and corresponding reduction in the concentration of LDL particles in the bloodstream. Reduction in the LDL level in the bloodstream reduces the risk of coronary artery disease. J.A.M.A., 1984, 251, 351-74.

Currently available statins include lovastatin, simvastatin, pravastatin, fluvastatin, cerivastatin and atorvastatin. Lovastatin (disclosed in U.S. Pat. No. 4,231,938) and simvastatin (disclosed in U.S. Pat. No. 4,444,784) are administered in the lactone form. After absorption, the lactone ring is opened in the liver by chemical or enzymatic hydrolysis, and the active hydroxy acid is generated.

Pravastatin (disclosed in U.S. Pat. No. 4,346,227) is administered as the sodium salt. Fluvastatin (disclosed in U.S. Pat. No. 4,739,073) and cerivastatin (disclosed in U.S. Pat. Nos. 5,006,530 and 5,177,080), also administered as the sodium salt, are entirely synthetic compounds that are in part structurally distinct from the fungal derivatives of this class that contain a hexahydronaphthalene ring. Atorvastatin and two new "superstatins," rosuvastatin and pitavastatin, are administered as calcium salts.

Rosuvastatin calcium (monocalcium bis (+) 7-[4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methylsulfonylamino)pyrimidin-5-yl]-(3R,5S)-dihydroxy-(E)-6-heptenoate) is an HMG-CoA reductase inhibitor, developed by Shionogi for the once daily oral treatment of hyperlipidaemia (Ann Rep, Shionogi, 1996; Direct communications, Shionogi, 8 Feb 1999 & 25 Feb 2000). Rosuvastatin calcium has the following chemical formula:



Rosuvastatin calcium is marketed under the name CRESTOR[®] for treatment of a mammal such as a human. According to the maker of CRESTOR[®], it is administered in a daily dose of from about 5 mg to about 40 mg. For patients requiring less aggressive LDL-C reductions or who have pre-disposing factors for myopathy, the 5 mg dose is recommended, while 10 mg dose is recommended for the average patient, 20 mg dose for patients with marked hyper-cholesterolemia and aggressive lipid targets (>190 mg/dL), and the 40 mg dose for patients who have not been responsive to lower doses.

U.S. Pat. No. 5,260,440 discloses and claims rosuvastatin, its calcium salt (2:1), and its lactone form. The process of the '440 patent prepares rosuvastatin by reacting 4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methylsulfonylamino)-5-

pyrimidinecarbaldehyde with methyl (3R)-3-(tert-butyldimethylsilyloxy)-5-oxo-6-triphenylphosphoranylidene hexanate in acetonitrile under reflux. The silyl group is then cleaved with hydrogen fluoride, followed by reduction with sodium borohydride (NaBH_4) and diethylmethoxyborane in tetrahydrofuran (THF) to obtain a methyl ester of rosuvastatin.

The ester is then hydrolyzed with sodium hydroxide (NaOH) in ethanol at room temperature, followed by removal of ethanol and addition of ether, to obtain the sodium salt of rosuvastatin. The sodium salt is then converted to the calcium salt. The sodium salt is dissolved in water and maintained under a nitrogen atmosphere. Calcium chloride is then added to the solution, resulting in precipitation of rosuvastatin calcium (2:1).

The product mixture of a reaction rarely is a single compound pure enough to comply with pharmaceutical standards. Side products and byproducts of the reaction and adjunct reagents used in the reaction will, in most cases, be present. At certain stages during processing of the rosuvastatin contained in the product mixture into an active pharmaceutical ingredient ("API"), the rosuvastatin must be analyzed for purity, typically by HPLC or GC analysis, to determine if it is suitable for continued processing or ultimately for use in a pharmaceutical product. The rosuvastatin does not need to be absolutely pure. Absolute purity is a theoretical ideal that is unattainable. Rather, there are purity standards intended to ensure that an API is not made less safe for clinical use because of the presence of impurities. In the United States, the Food and Drug Administration guidelines recommend that applicants limit some impurities to below 0.1%.

Generally, side products, byproducts and adjunct reagents (collectively "impurities") are identified spectroscopically and by other physical methods and then the impurities are associated with a peak position in a chromatogram (or a spot on a TLC plate). (Strobel p. 953) (Strobel, H.A.; Heineman, W.R., *Chemical Instrumentation: A Systematic Approach*, 3rd ed. (Wiley & Sons: New York 1989)). Thereafter, the impurity can be identified by its position in the chromatogram, which is conventionally measured in minutes between injection of the sample on the column and elution of the particular component through the detector, known as the "retention time." This time period varies daily based upon the condition of the instrumentation and many other factors. To mitigate the effect that such variations have upon accurate

identification of an impurity, practitioners use “relative retention time” (“RRT”) to identify impurities. (Strobel p. 922). The RRT of an impurity is its retention time divided by the retention time of some reference marker. In theory, rosuvastatin itself could be used as the reference marker, but as a practical matter it is present in such overwhelming proportion in the mixture that it tends to saturate the column, leading to irreproducible retention times, i.e., the maximum of the peak corresponding to rosuvastatin tends to wander (Strobel Fig. 24.8(b) p. 879, contains an illustration of the sort of asymmetric peak that is observed when a column is overloaded). Thus, it is sometimes desirable to select an alternative compound that is added to, or is present in, the mixture in an amount significant enough to be detectable and sufficiently low as not to saturate the column and to use that compound as the reference marker.

A compound in a relatively pure state can be used as a “reference standard” (a “reference marker” is similar to a reference standard but it is used for qualitative analysis) to quantify the amount of the compound in an unknown mixture. When the compound is used as an “external standard,” a solution of a known concentration of the compound is analyzed by the same technique as the unknown mixture. (Strobel p. 924, Snyder p. 549) (Snyder, L.R.; Kirkland, J.J. *Introduction to Modern Liquid Chromatography*, 2nd ed. (John Wiley & Sons: New York 1979)). The amount of the compound in the mixture can be determined by comparing the magnitude of the detector response. *See also* USP 6,333,198.

The reference standard compound also can be used to quantify the amount of another compound in the mixture if the “response factor,” which compensates for differences in the sensitivity of the detector to the two compounds, has been predetermined. (Strobel p. 894). For this purpose, the reference standard compound may be added directly to the mixture, in which case it is called an “internal standard.” (Strobel p. 925, Snyder p. 552).

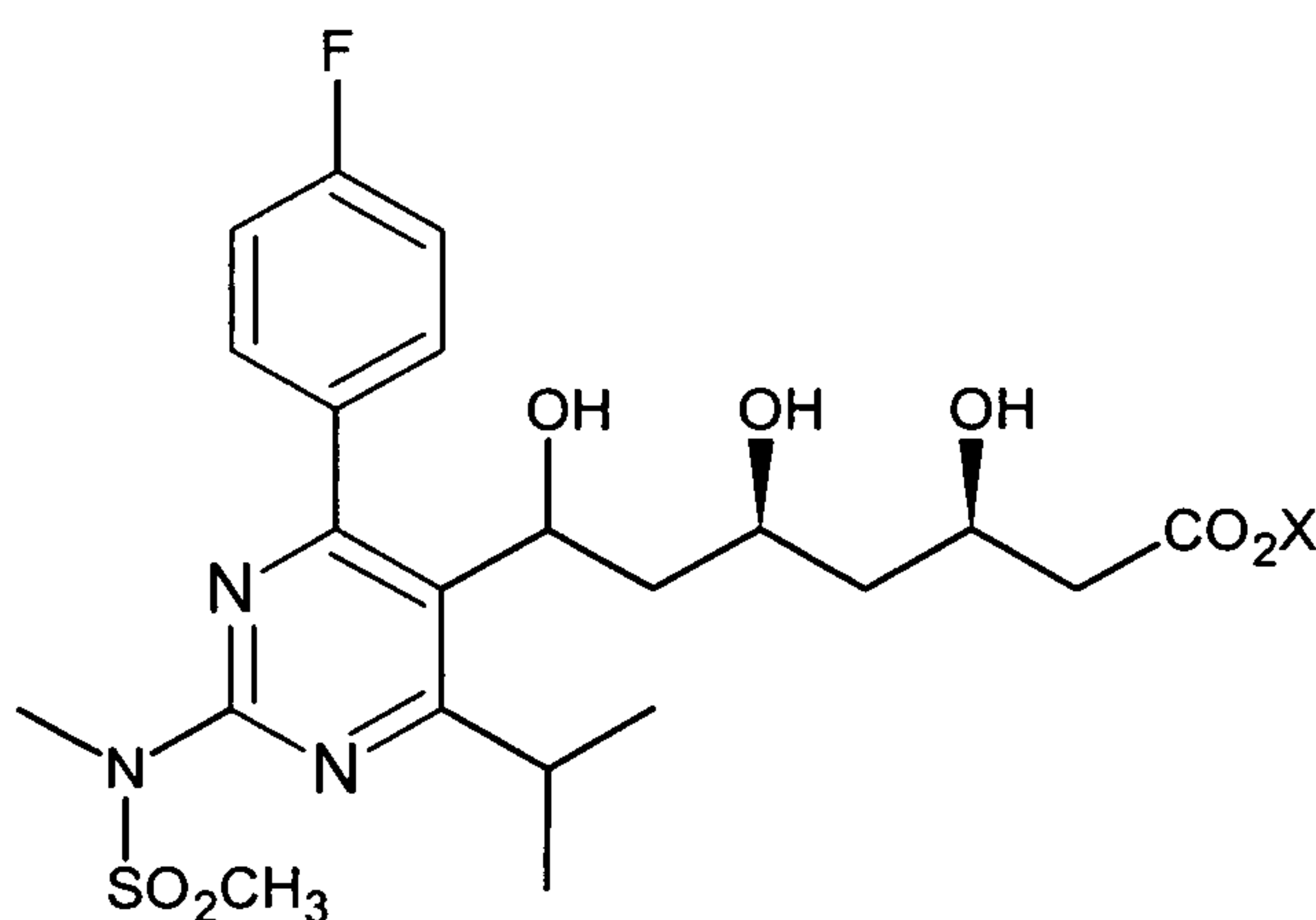
The reference standard compound can even be used as an internal standard when the unknown mixture contains some of the reference standard compound by using a technique called “standard addition,” wherein at least two samples are prepared by adding known and differing amounts of the internal standard. (Strobel pp. 391-393, Snyder pp. 571, 572). The proportion of detector response due to the reference standard compound that is originally in the mixture can be determined by extrapolation of a plot of detector response versus the amount of the reference

standard compound that was added to each of the samples to zero. (e.g. Strobel, Fig. 11.4 p. 392).

The present invention provides compounds that can be used as a reference standard and reference marker for quantification and identification of rosuvastatin and impurities present in batches of rosuvastatin.

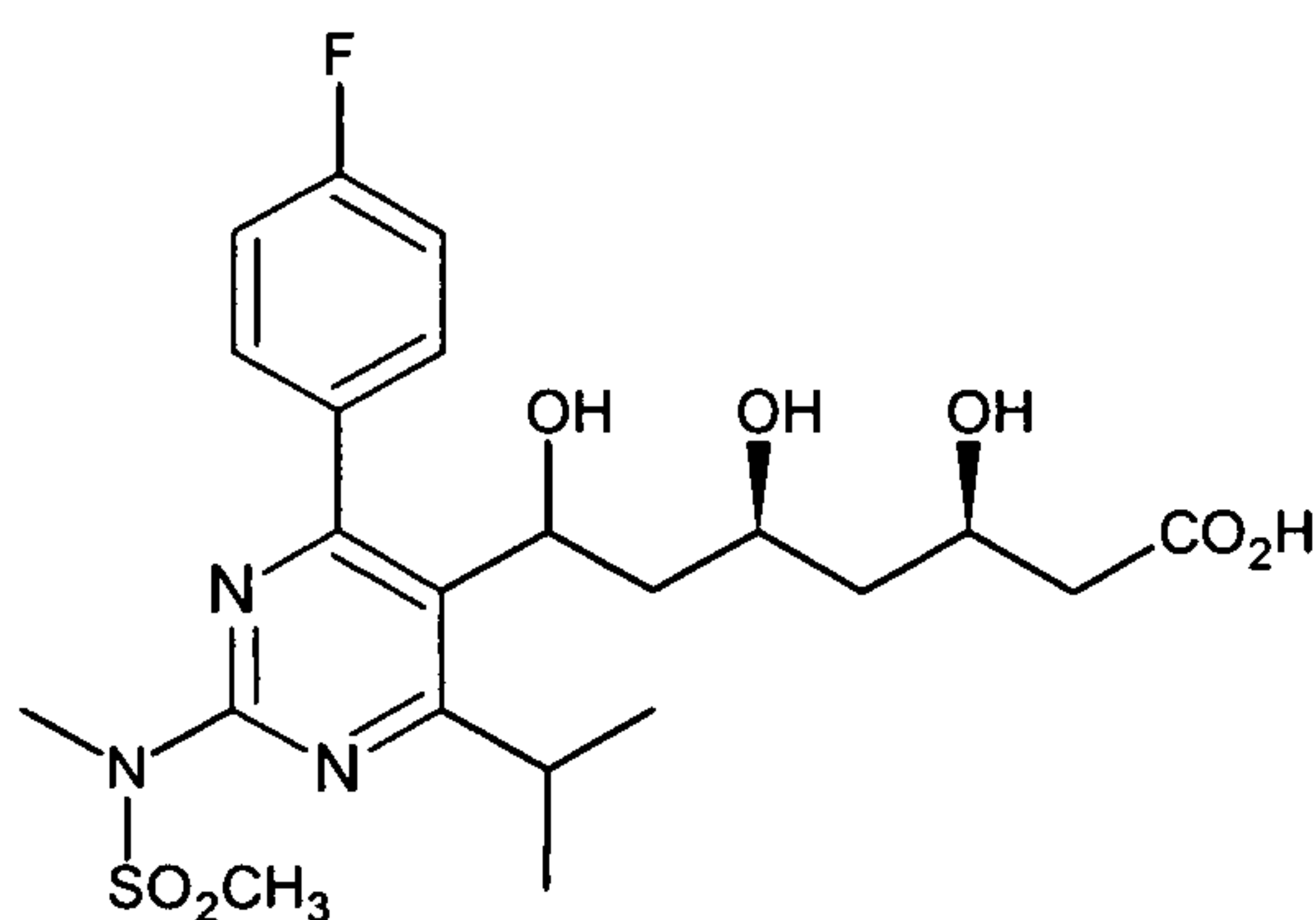
SUMMARY OF THE INVENTION

In one embodiment, the present invention provides a rosuvastatin triol having the following structure:

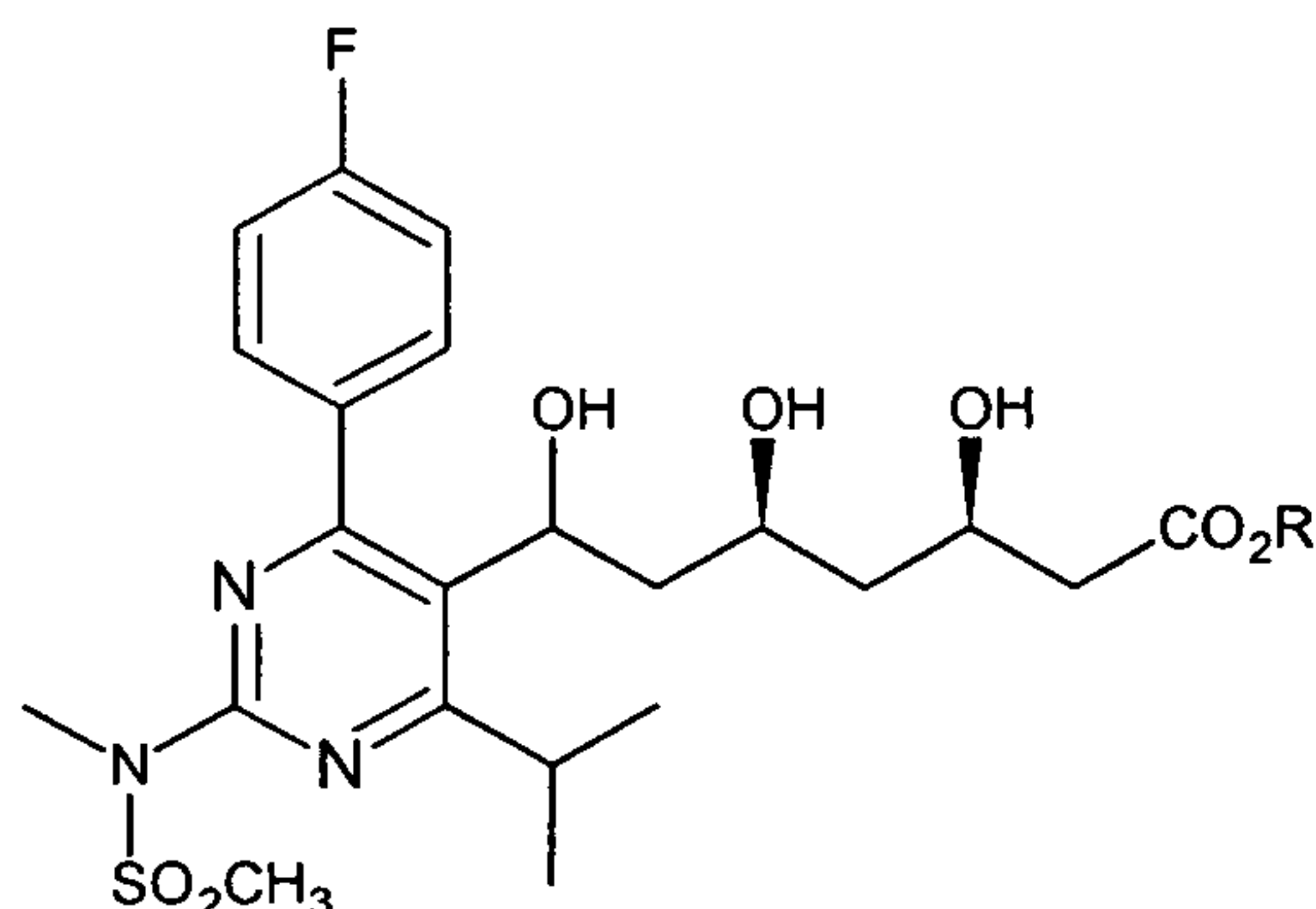


Wherein X is a hydrogen, a C₁-C₄ alkyl group, or an alkali or alkaline earth metal cation, with the proviso that when X is an alkaline earth metal, two molecules of rosuvastatin are present to one of the metal cation.

In another embodiment, the present invention provides a rosuvastatin triol in acid form has the following structure:

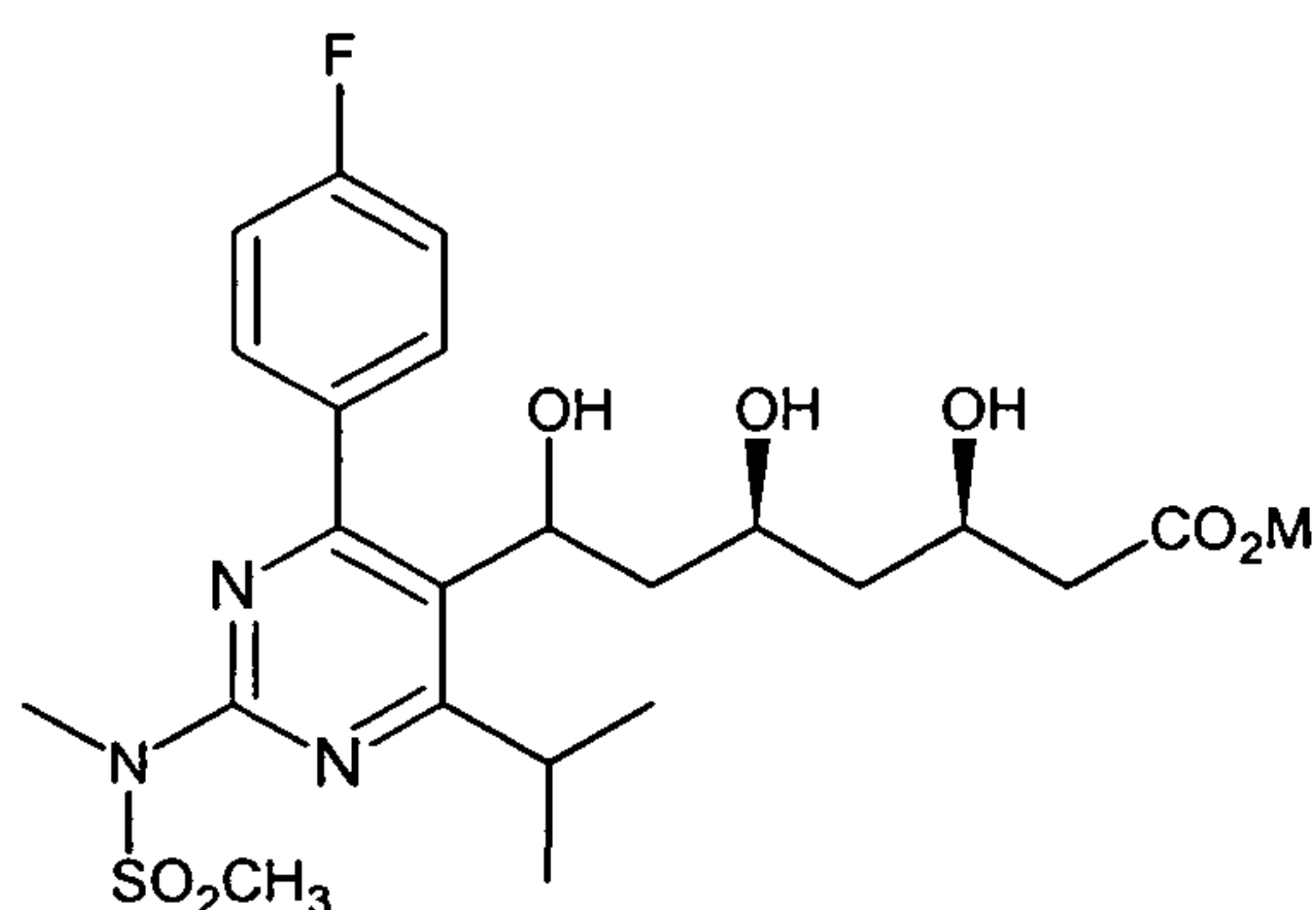


In yet another embodiment, the present invention provides a rosuvastatin triol in ester form having the following structure:



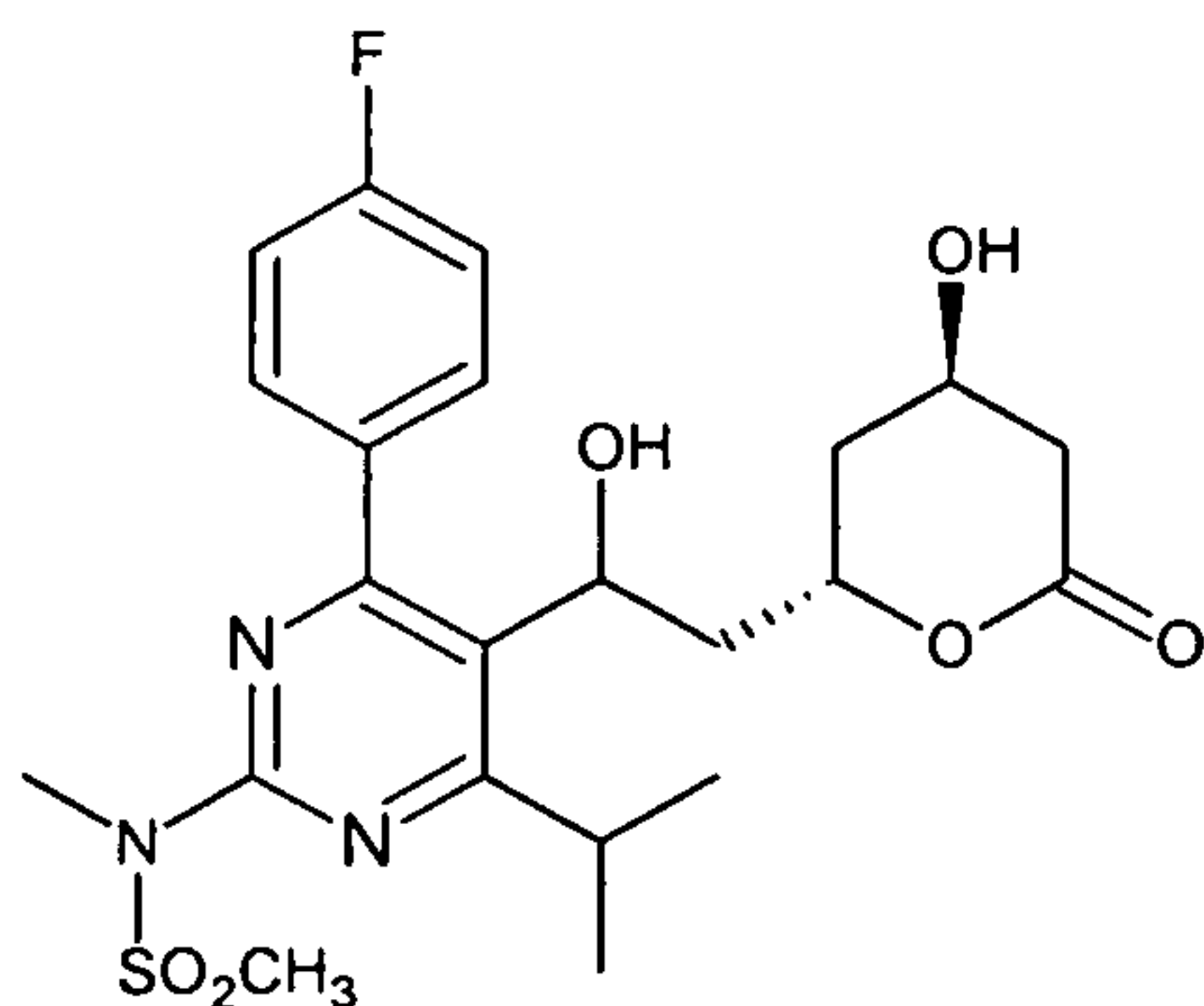
Wherein R is a C₁-C₄ alkyl ester.

In one embodiment, the present invention provides a rosuvastatin triol in salt form having the following structure:



Wherein M is an alkali or alkaline earth metal cation, with the proviso that when X is an alkaline earth metal, two molecules of rosuvastatin are present to one of the metal cation.

In one embodiment, the present invention provides a rosuvastatin triol in lactone form having the following structure:



In another embodiment the present invention provides each of the above forms of the triol in isolated or purified form, substantially free of the corresponding rosuvastatin diol form.

In yet another embodiment, the present invention provides a process for preparing a rosuvastatin triol C₁-C₄ ester comprising combining rosuvastatin C₁-C₄ ester with a solution of borane dimethylsulfide complex in a suitable organic solvent to obtain a reaction mixture, combining the resulting reaction mixture with a solution of NaOH in water, adding hydrogen peroxide (H₂O₂) and recovering the triol ester.

In another embodiment the present invention provides a process for preparing rosuvastatin triol C₁-C₄ ester comprising oxidizing rosuvastatin diol C₁ to C₄ ester to obtain the rosuvastatin triol ester with a hydroxyl group at position 7.

In another embodiment the present invention provides a process comprising combining rosuvastatin C₁-C₄ ester with a solution of a borane in an organic solvent to obtain a reaction mixture, combining the resulting reaction mixture with a solution of an inorganic base in water, and adding peroxide and recovering the triol ester.

In one embodiment the present invention provides a process for reducing amount of impurities present in rosuvastatin calcium by measuring amount of rosuvastatin calcium triol in batches of rosuvastatin calcium, selecting batches of the rosuvastatin calcium with desirable level of the triol and preparing pharmaceutical compositions with the selected rosuvastatin calcium batch.

In another embodiment, the present invention provides a process for reducing amount of rosuvastatin triol calcium present in a mixture comprising rosuvastatin diol calcium and rosuvastatin triol calcium comprising measuring amount of rosuvastatin triol C₁-C₄ ester in batches of rosuvastatin diol C₁-C₄ ester, selecting batches of the rosuvastatin diol C₁-C₄ ester with of the triol C₁-C₄ ester and preparing pharmaceutical compositions of rosuvastatin diol calcium with the selected rosuvastatin diol C₁-C₄ ester batch.

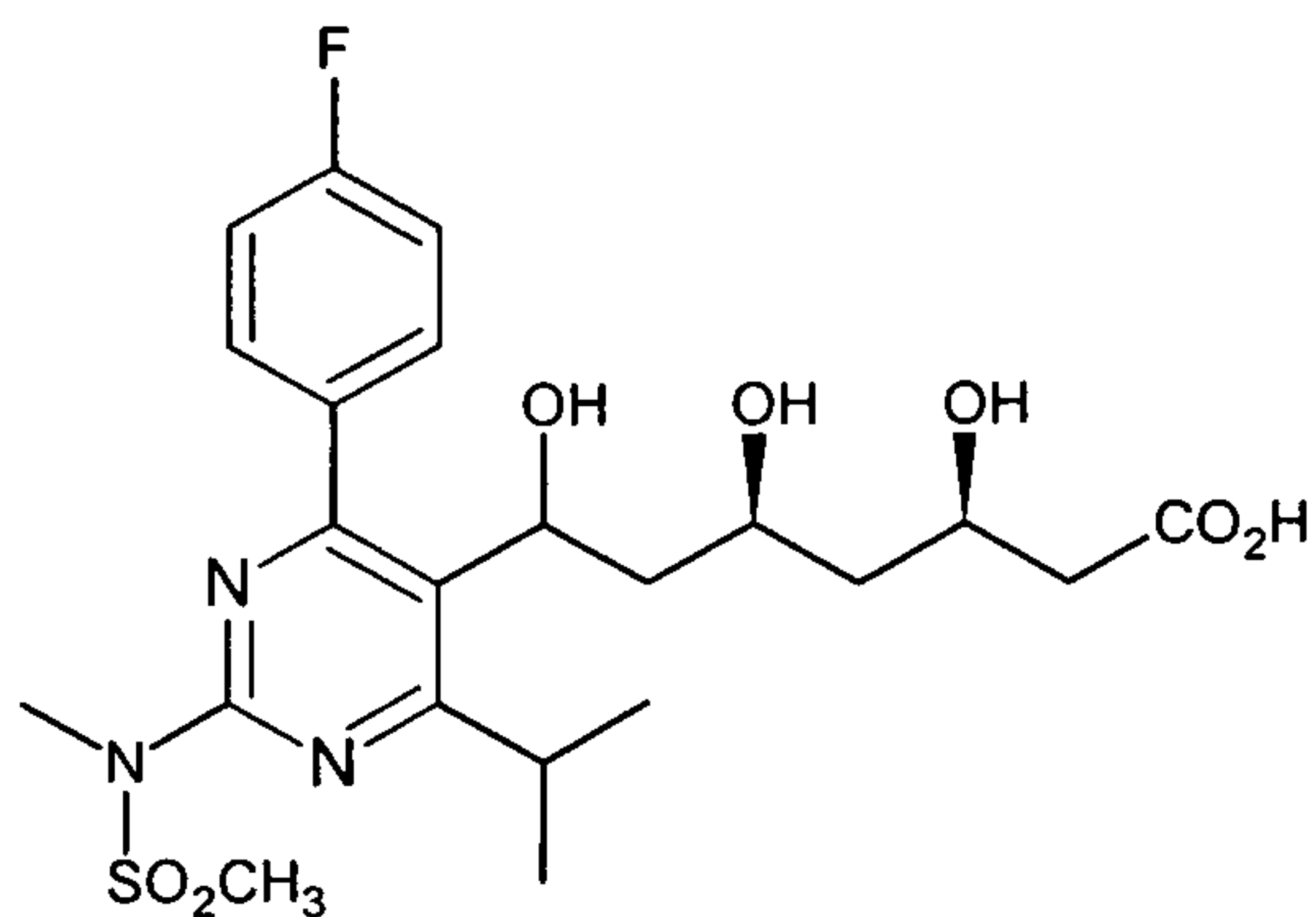
In another embodiment, the present invention provides a method of determining the amount of an impurity in a sample of rosuvastatin ester (preferable t-butyl) comprising measuring by GC or HPLC the area under a peak corresponding to rosuvastatin triol ester in a reference standard comprising a known amount of rosuvastatin triol ester (preferably t-butyl); measuring by GC or HPLC the area under a peak corresponding to rosuvastatin triol ester in a sample comprising rosuvastatin triol and rosuvastatin diol esters (preferably t-butyl); and determining the amount of the rosuvastatin triol ester in the sample by comparing the area of reference standard with that of the test sample.

In yet another embodiment, the present invention provides a method of determining the amount of an impurity in a sample of rosuvastatin calcium comprising measuring by GC or HPLC the area under a peak corresponding to rosuvastatin triol calcium in a reference standard comprising a known amount of rosuvastatin triol calcium; measuring by GC or HPLC the area under a peak corresponding to rosuvastatin triol calcium in a sample comprising rosuvastatin triol and rosuvastatin diol calcium salts; and determining the amount of the triol calcium in the sample by comparing the area of reference standard with that of the test sample.

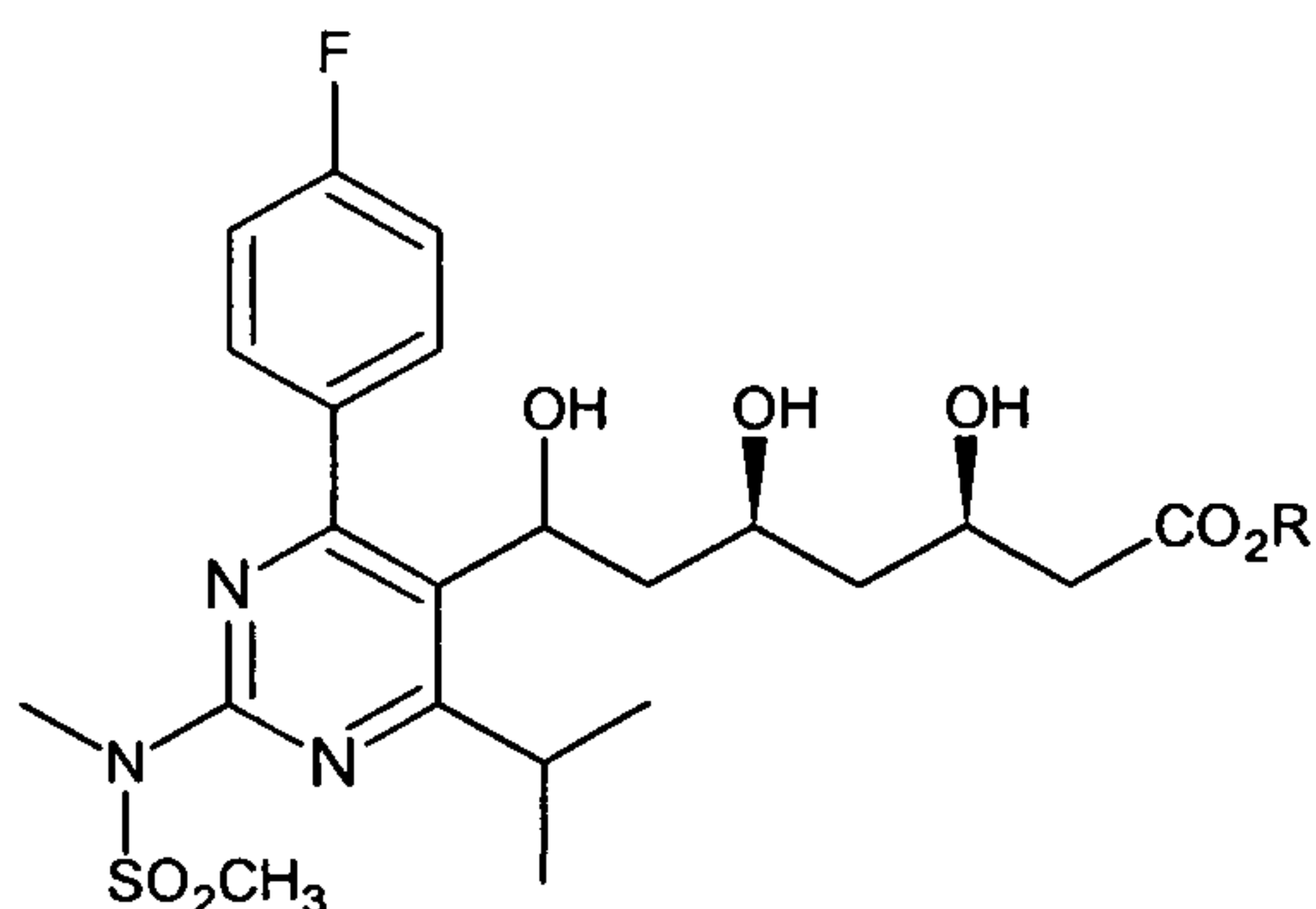
In one embodiment, the present invention provides a method of identifying the relative retention time (RRT) of an impurity in a sample of rosuvastatin diol ester (preferably t-butyl) comprising measuring by GC or HPLC the relative retention time (RRT) corresponding to rosuvastatin triol ester in a reference marker sample; carrying out GC or HPLC with a test sample comprising of the rosuvastatin diol ester and rosuvastatin triol ester to obtain an GC or HPLC chromatogram with retention times; and determining the relative retention time (RRT) of the triol ester in the sample by comparing the relative retention time (RRT) of the reference marker to the relative retention time (RRT) of the test sample.

In another embodiment, the present invention provides a method of identifying the relative retention time (RRT) of an impurity in a sample of rosuvastatin diol calcium comprising measuring by GC or HPLC the relative retention time (RRT) corresponding to rosuvastatin triol calcium in a reference marker sample; carrying out GC or HPLC with a test sample comprising of the rosuvastatin diol and rosuvastatin triol calcium salts to obtain an GC or HPLC chromatogram with retention times; and determining the relative retention time (RRT) of the triol calcium in the sample by comparing the relative retention time (RRT) of the reference marker to the relative retention time (RRT) of the test sample.

In one embodiment the present invention provides a process for preparing rosuvastatin triol acid with the following structure:

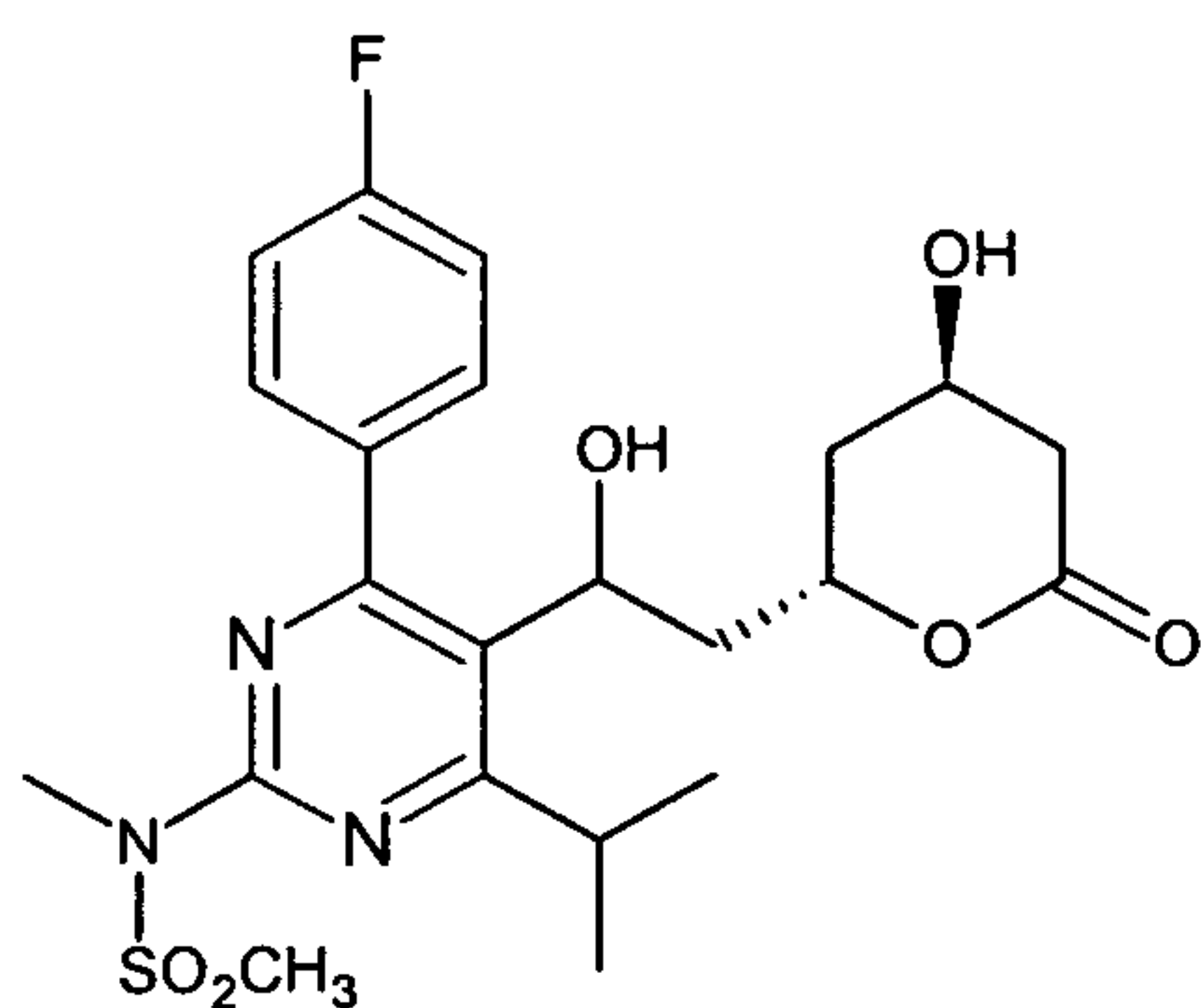


comprising hydrolyzing an ester of the following structure

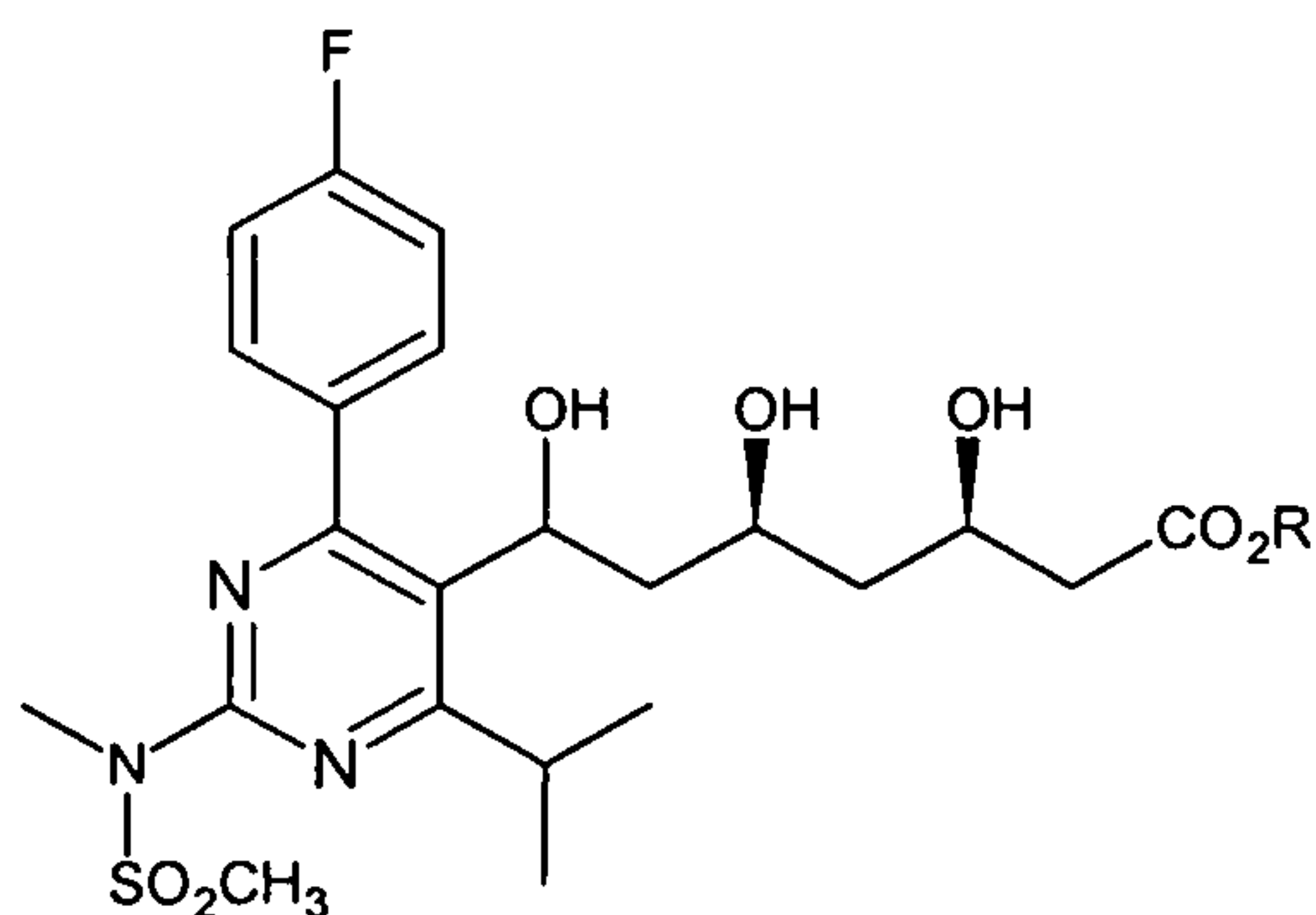


and converting the hydrolyzed ester with an acid, wherein R is a C₁-C₄ group.

In one embodiment the present invention provides a process for preparing rosuvastatin triol lactone with the following structure:

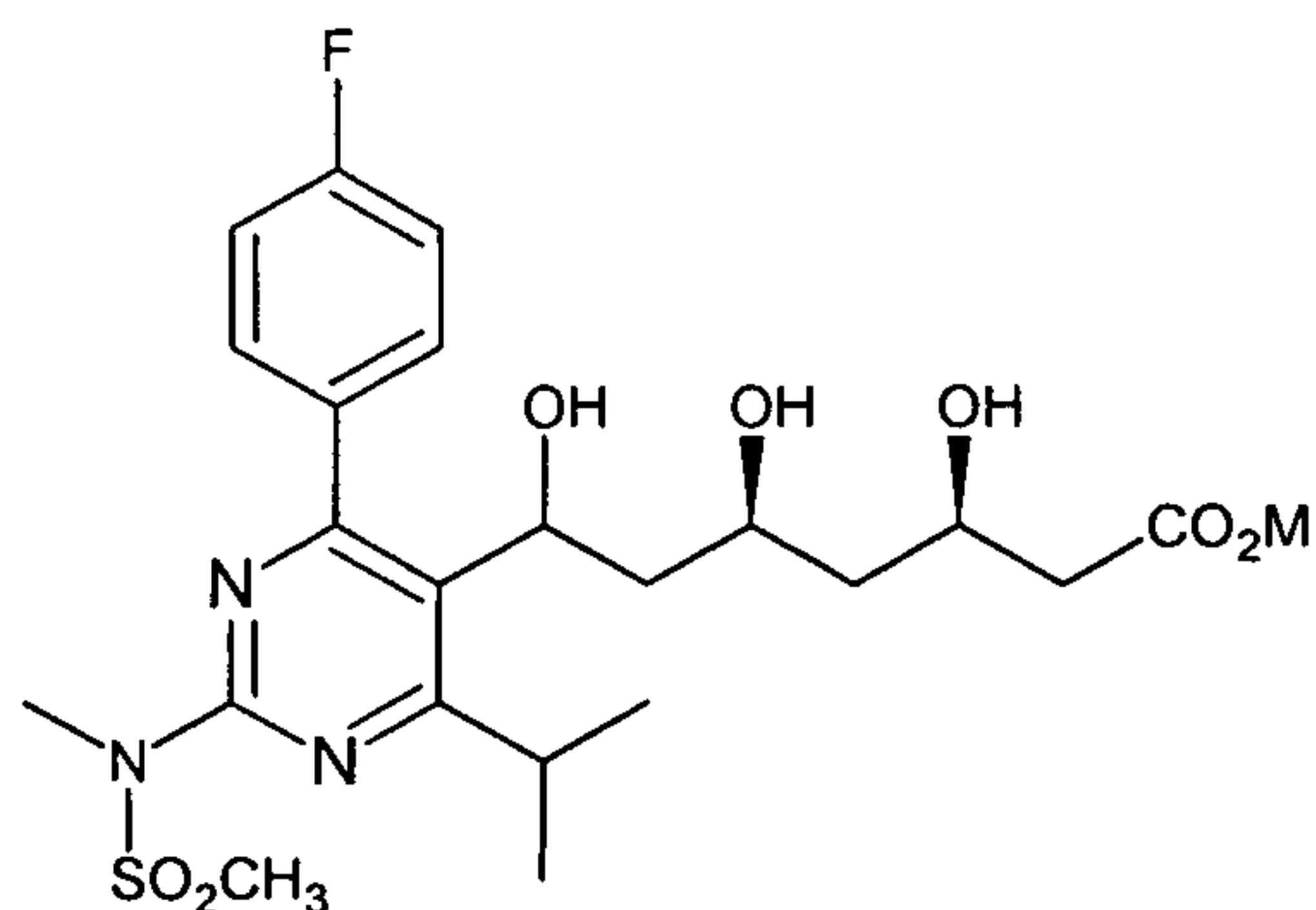


comprising hydrolyzing an ester of the following structure

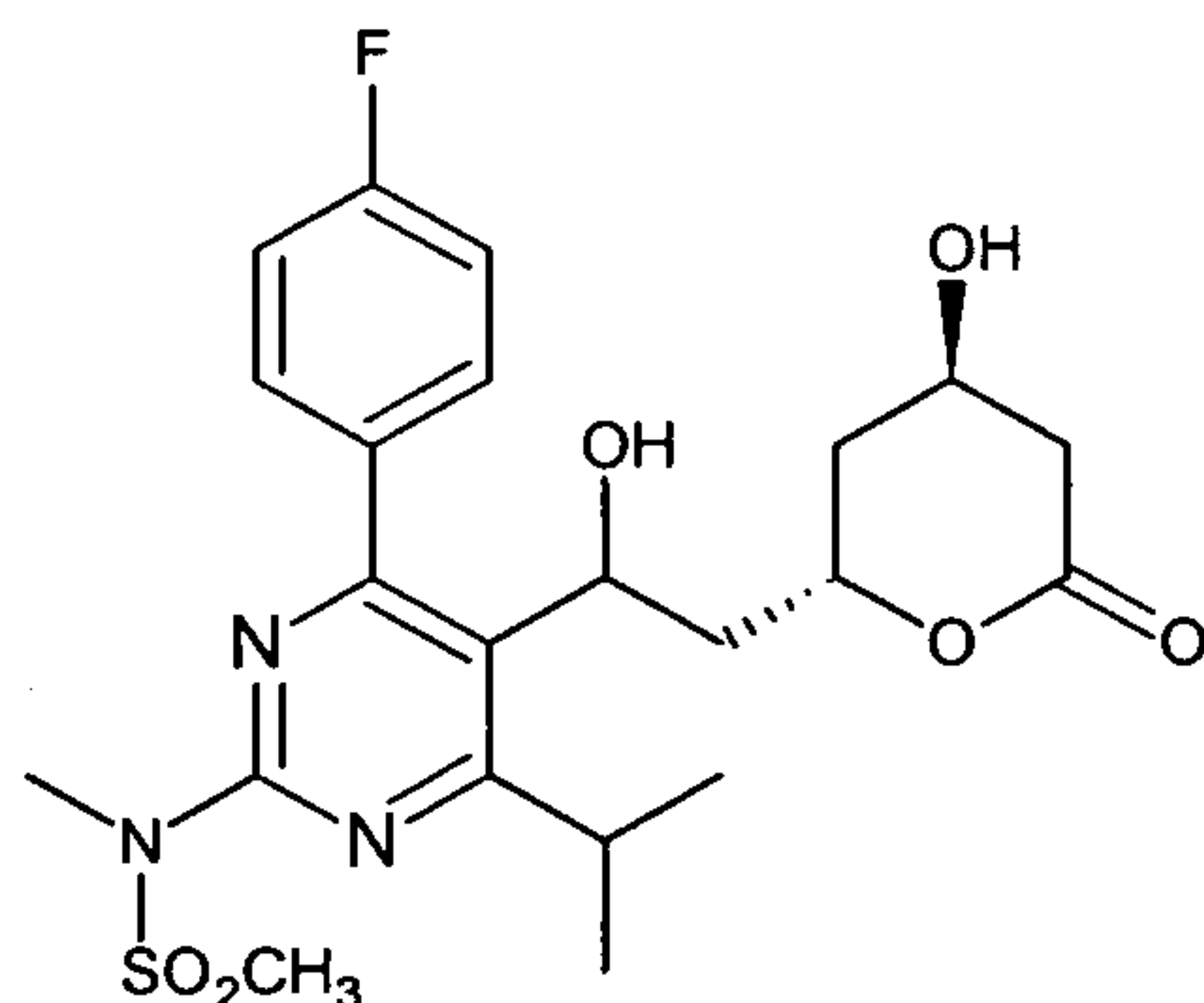


and converting the hydrolyzed ester to a lactone, wherein R is a C₁-C₄ ester.

In one embodiment the present invention provides a process for preparing rosuvastatin triol acid with the following structure:

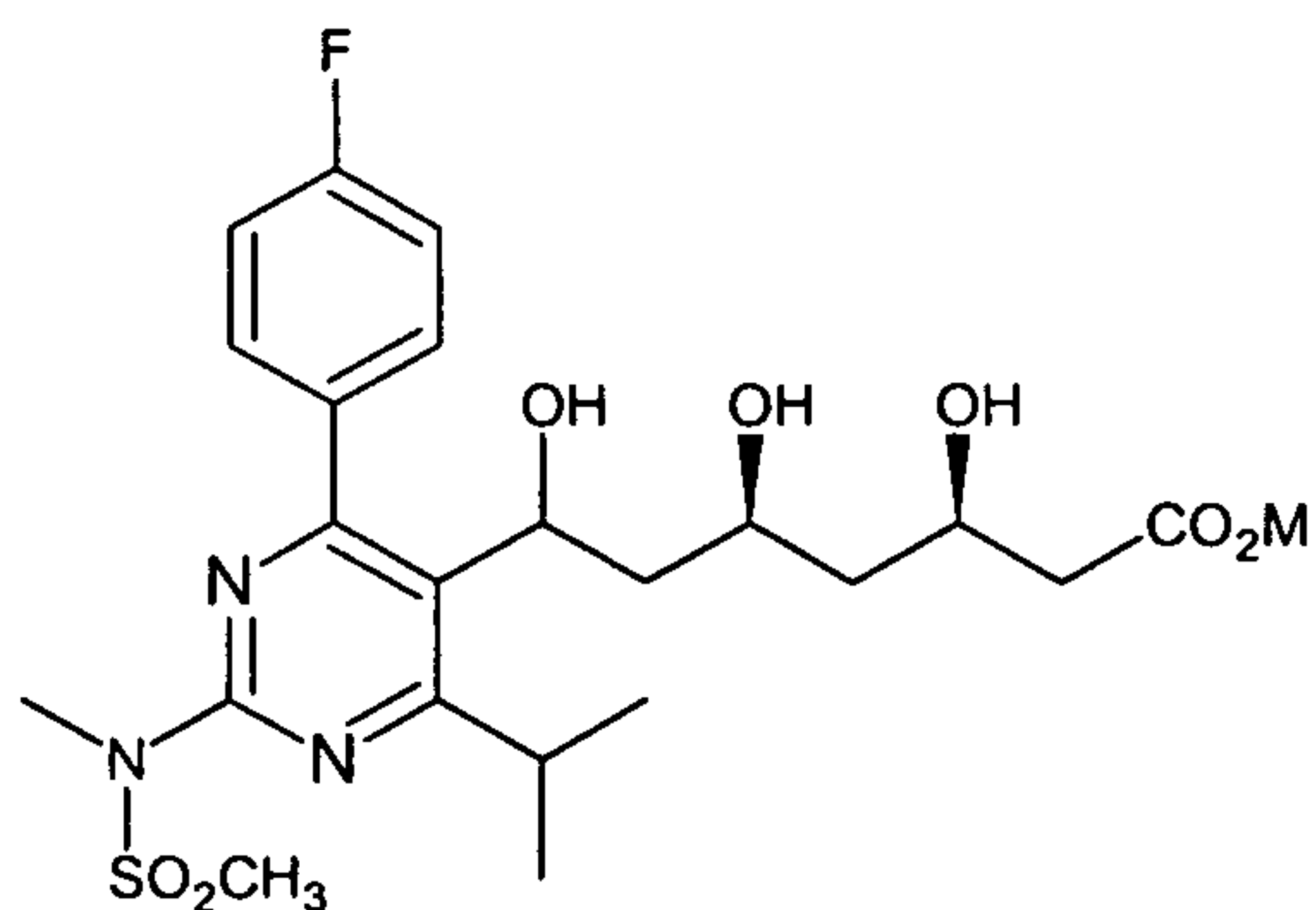


comprising hydrolyzing a lactone having the following structure

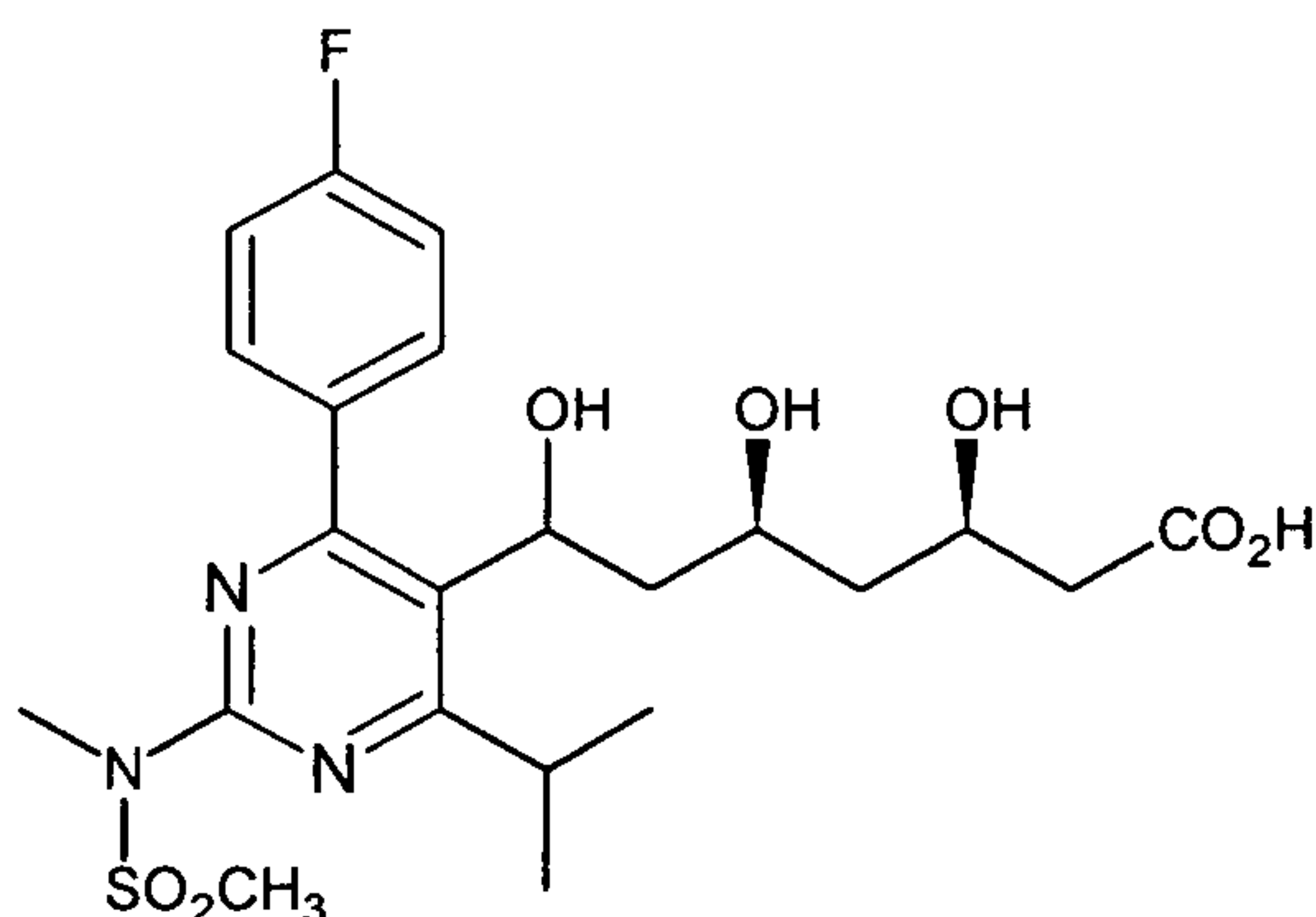


and converting the hydrolyzed lactone to an the salt, wherein M is an alkali metal or an alkaline earth metal with the proviso that if the metal cation is an alkaline earth metal, two molecules of rosuvastatin are present for each cation. .

In one embodiment the present invention provides a process for preparing rosuvastatin triol salt with the following structure:



Comprising contacting an acid with the following structure



with a base, with the proviso that if the metal cation is an alkaline earth metal, two molecules of rosuvastatin are present for each cation.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is an NMR of TBRE (t-butyl Rosuvastatin Ester) triol.

Figure 2 is an HPLC chromatogram illustrating use of rosuvastatin triol calcium as a reference standard (including a reference marker).

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term “diol” refers to the two hydroxyl groups present on rosuvastatin. Diol rosuvastatin is used herein synonymously as rosuvastatin.

As used herein the term “substantially free” refers to having less than about 30% of the corresponding compound (*e.g.*, diol or diastereoisomer), more preferably less than about 20%, even more preferably less than about 10%, and most preferably less than about 5%, based on area percentage HPLC.

As used herein, the term “triol lactone” refers to the lactone of rosuvastatin triol.

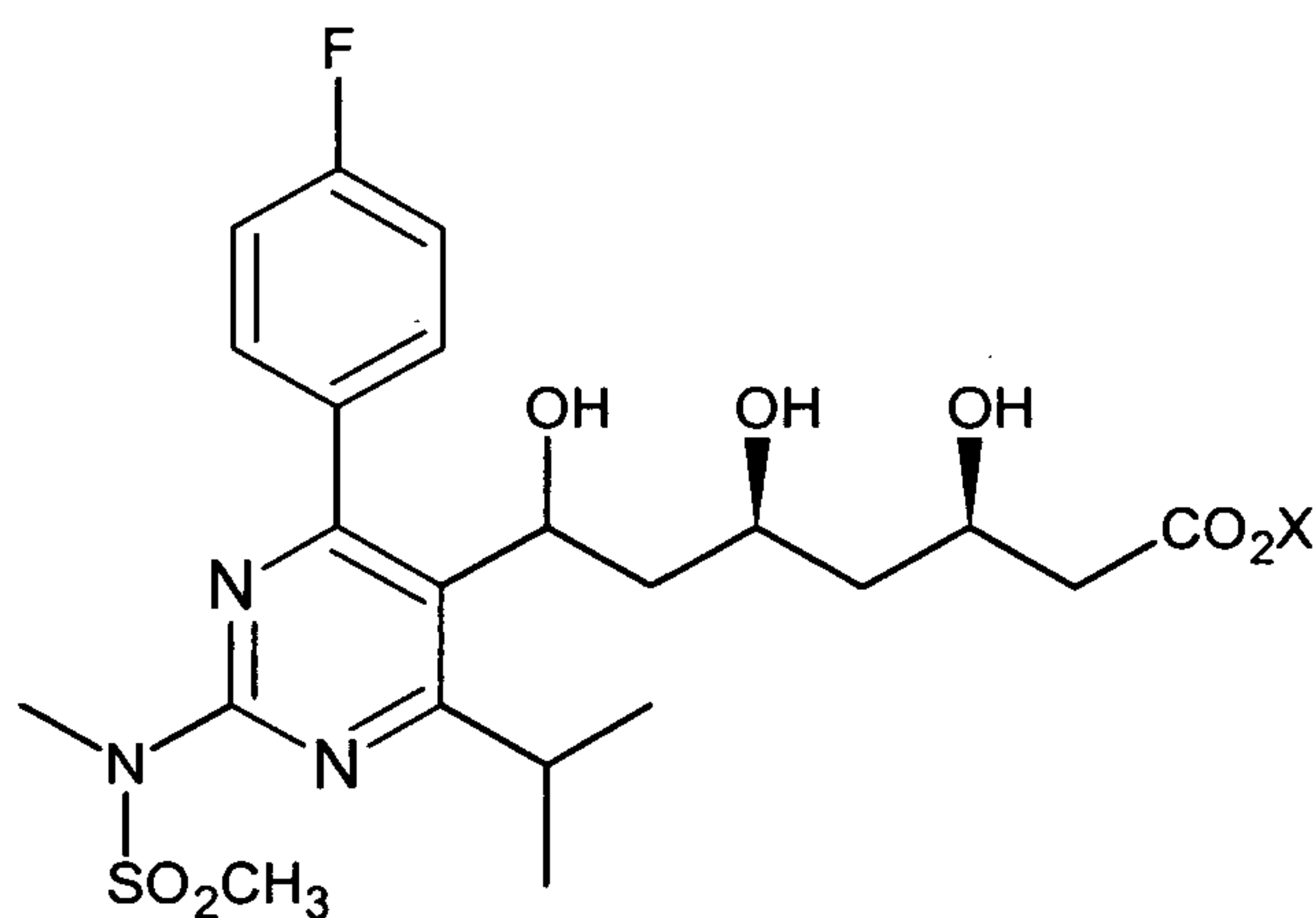
As used herein, the term “reference standard” refers to a compound that may be used both for quantitative and qualitative analysis of an active pharmaceutical ingredient. For example, the retention time of the compound in HPLC allows for setting a relative retention time, thus making qualitative analysis possible. The concentration of the compound in solution before injection into an HPLC column allows for comparison of the areas under the peaks in an HPLC chromatogram, thus making quantitative analysis possible.

A “reference marker” is used in qualitative analysis to identify components of a mixture based upon their position, *e.g.* in a chromatogram or on a Thin Layer Chromatography (TLC) plate (Strobel pp. 921, 922, 953). For this purpose, the

compound does not necessarily have to be added to the mixture if it is present in the mixture. A “reference marker” is used only for qualitative analysis, while a reference standard may be used for quantitative or qualitative analysis, or both. Hence, a reference marker is a subset of a reference standard, and is included within the definition of a reference standard.

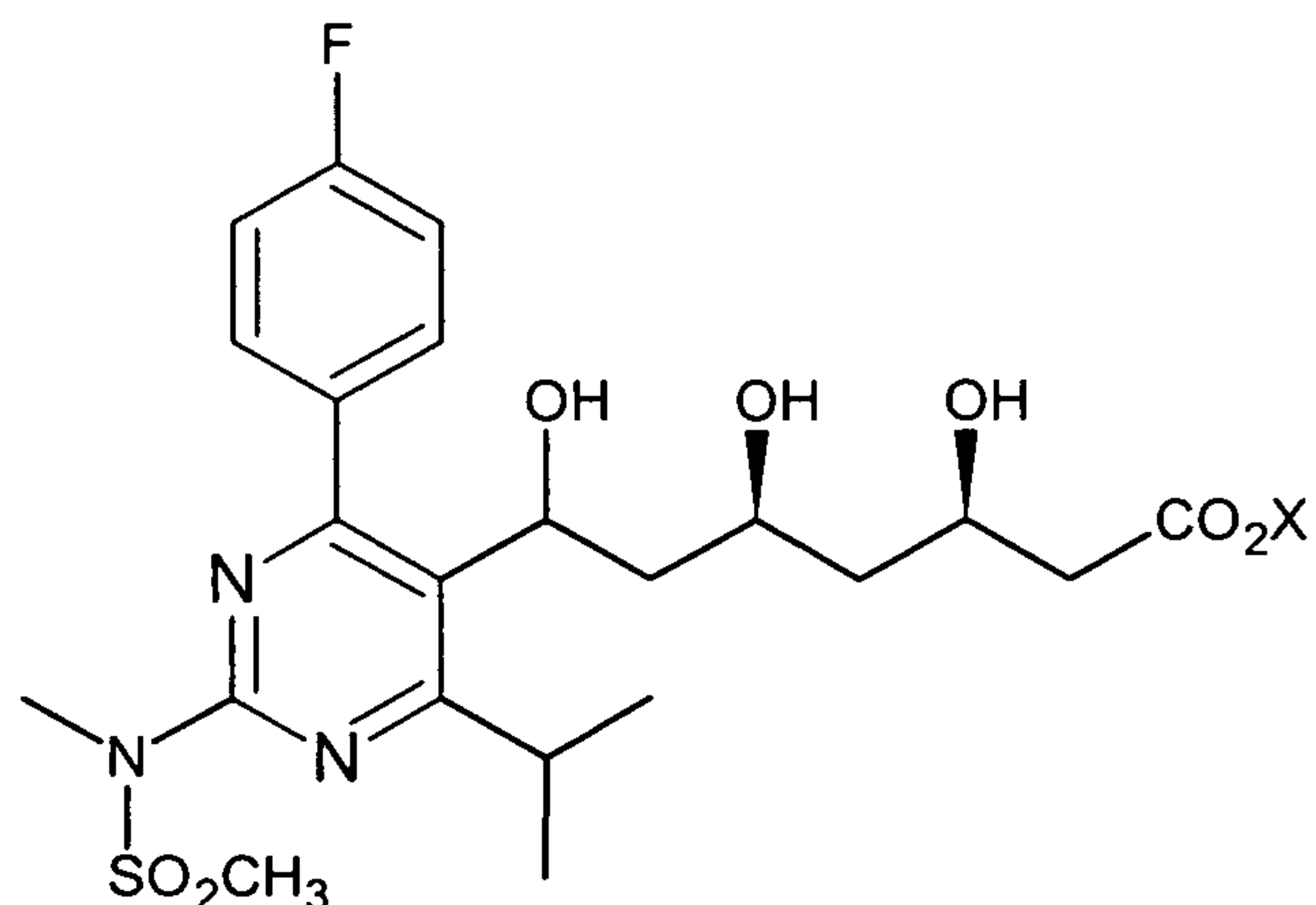
Although some of the knowledge of those in the art regarding reference standards has been described in general terms up to this point, those skilled in the art also understand that the detector response can be, for example, the peak heights or integrated peak areas of a chromatogram obtained, e.g. by UV or refractive index detection, from the eluent of an HPLC system or, e.g. flame ionization detection or thermal conductivity detection, from the eluent of a gas chromatograph, or other detector response, e.g. the UV absorbance, of spots on a fluorescent TLC plate. The position of the reference standard may be used to calculate the relative retention time for rosuvastatin and other impurities.

The present invention provides a rosuvastatin triol having the following structure:



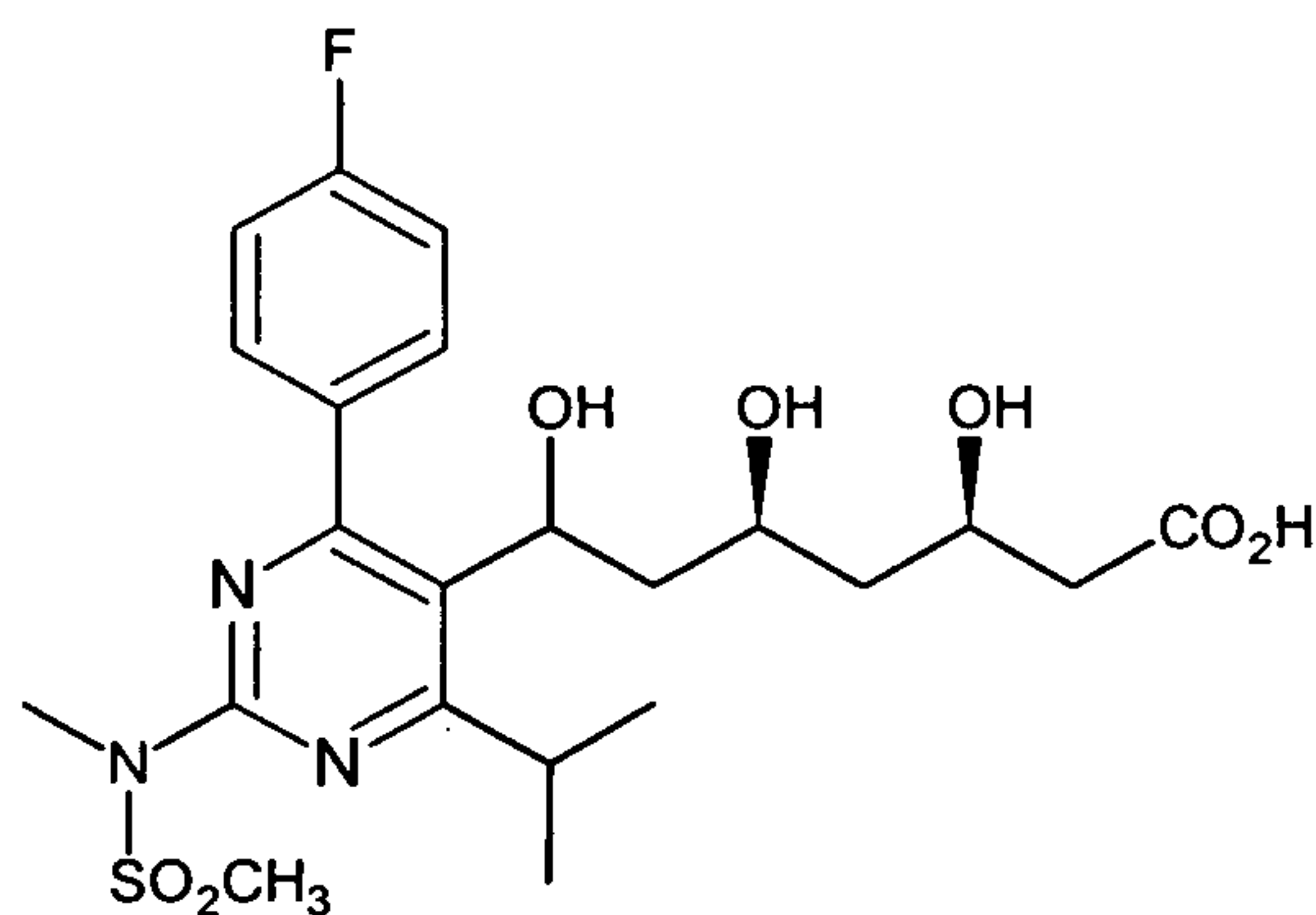
wherein X is a hydrogen, an alkali or alkaline earth metal or a C₁-C₄ alkyl group. Preferably X is hydrogen (i.e. rosuvastatin triol acid), calcium (Ca²⁺) (i.e. rosuvastatin triol calcium) or tert-butyl (i.e. rosuvastatin triol tert-butyl ester (“TBRE”)).

The present invention provides a rosuvastatin triol having the following structure:

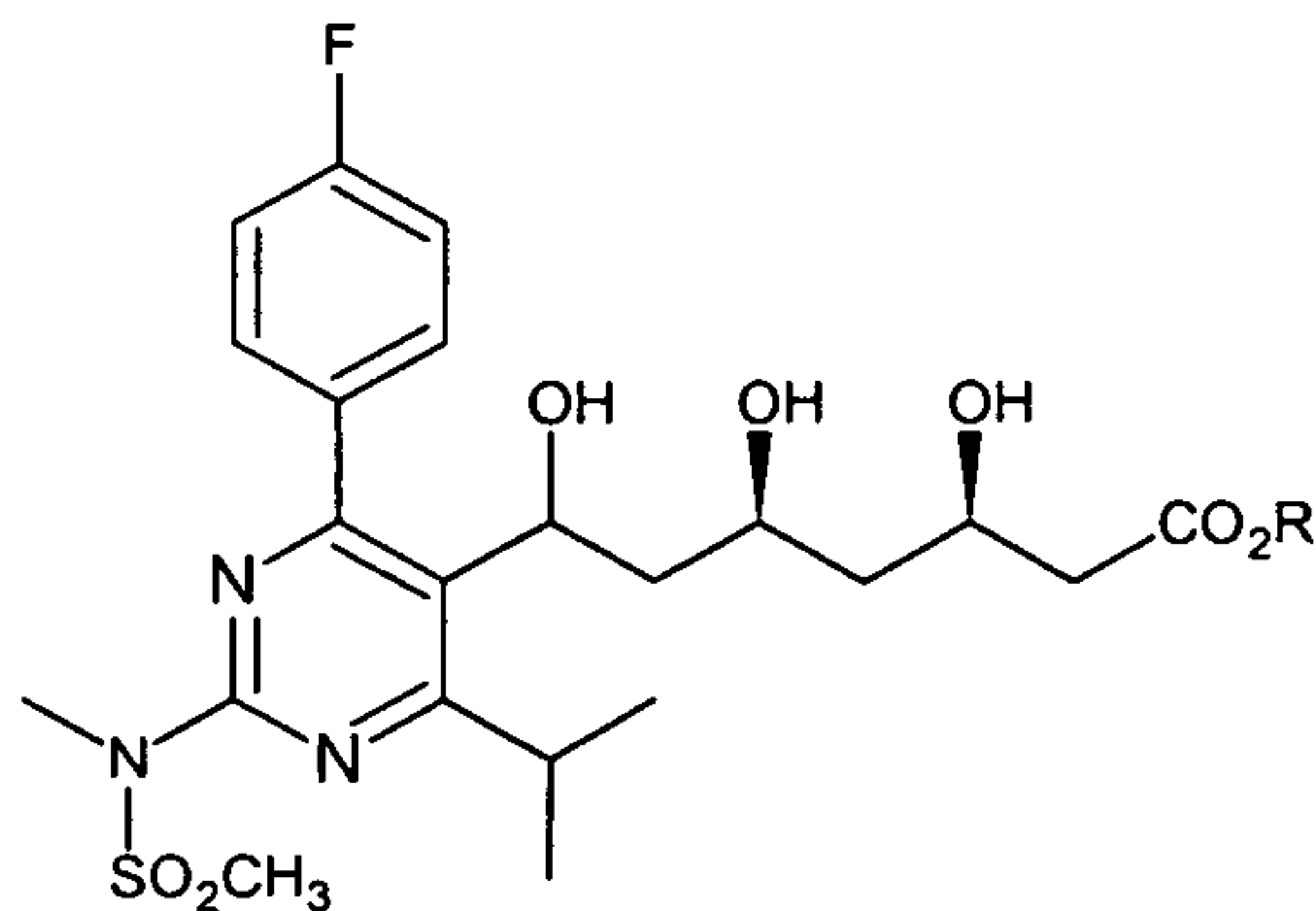


wherein X is a hydrogen, an alkali or alkaline earth metal or a C₁-C₄ alkyl group. Preferably X is hydrogen (i.e. rosuvastatin triol acid), calcium (Ca²⁺) (i.e. rosuvastatin triol calcium) or tert-butyl (i.e. rosuvastatin triol tert-butyl ester ("TBRE")) in its isolated form.

The present invention provides a rosuvastatin triol in an acid form having the following structure:

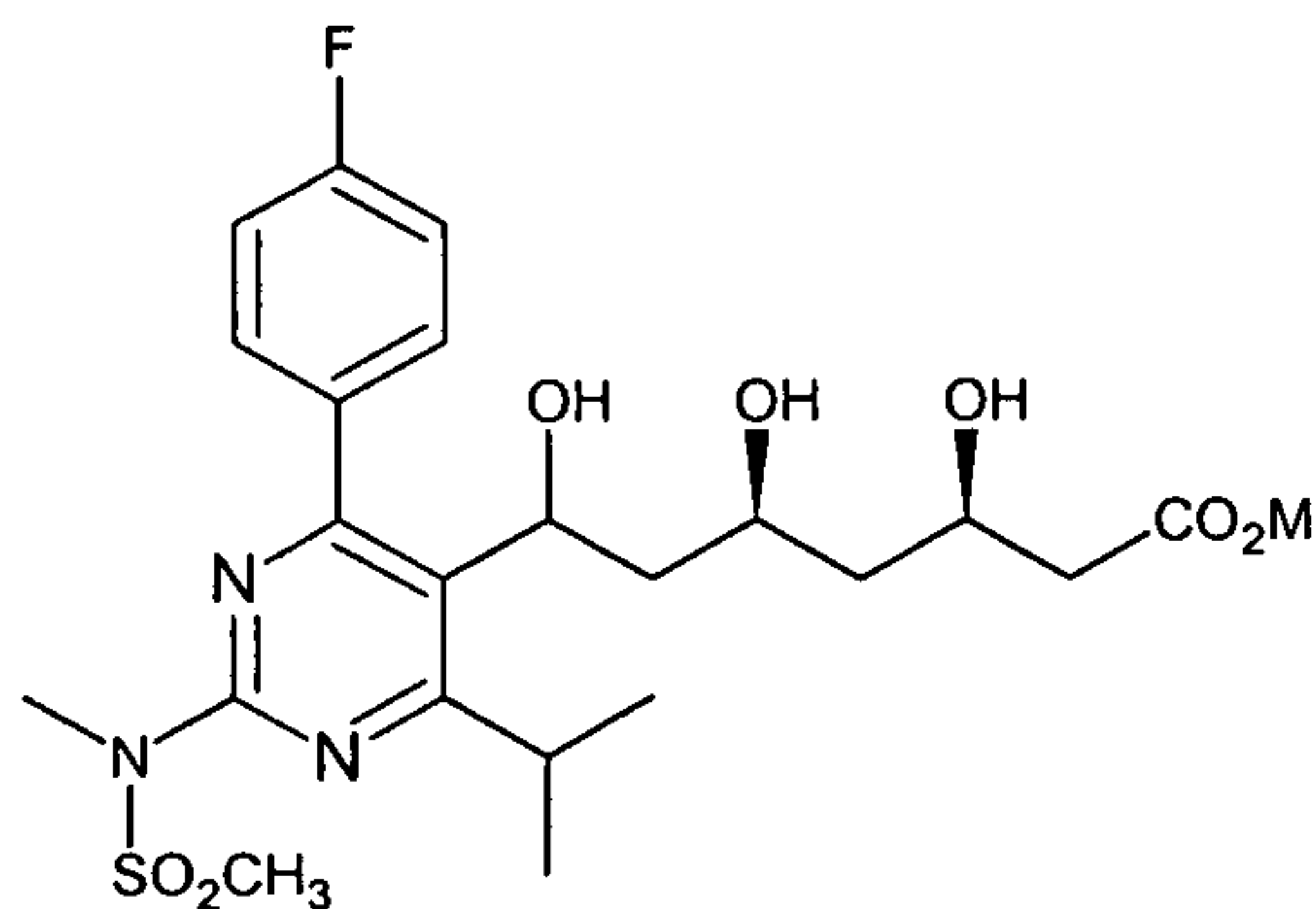


The present invention provides rosuvastatin triol in ester form having the following structure

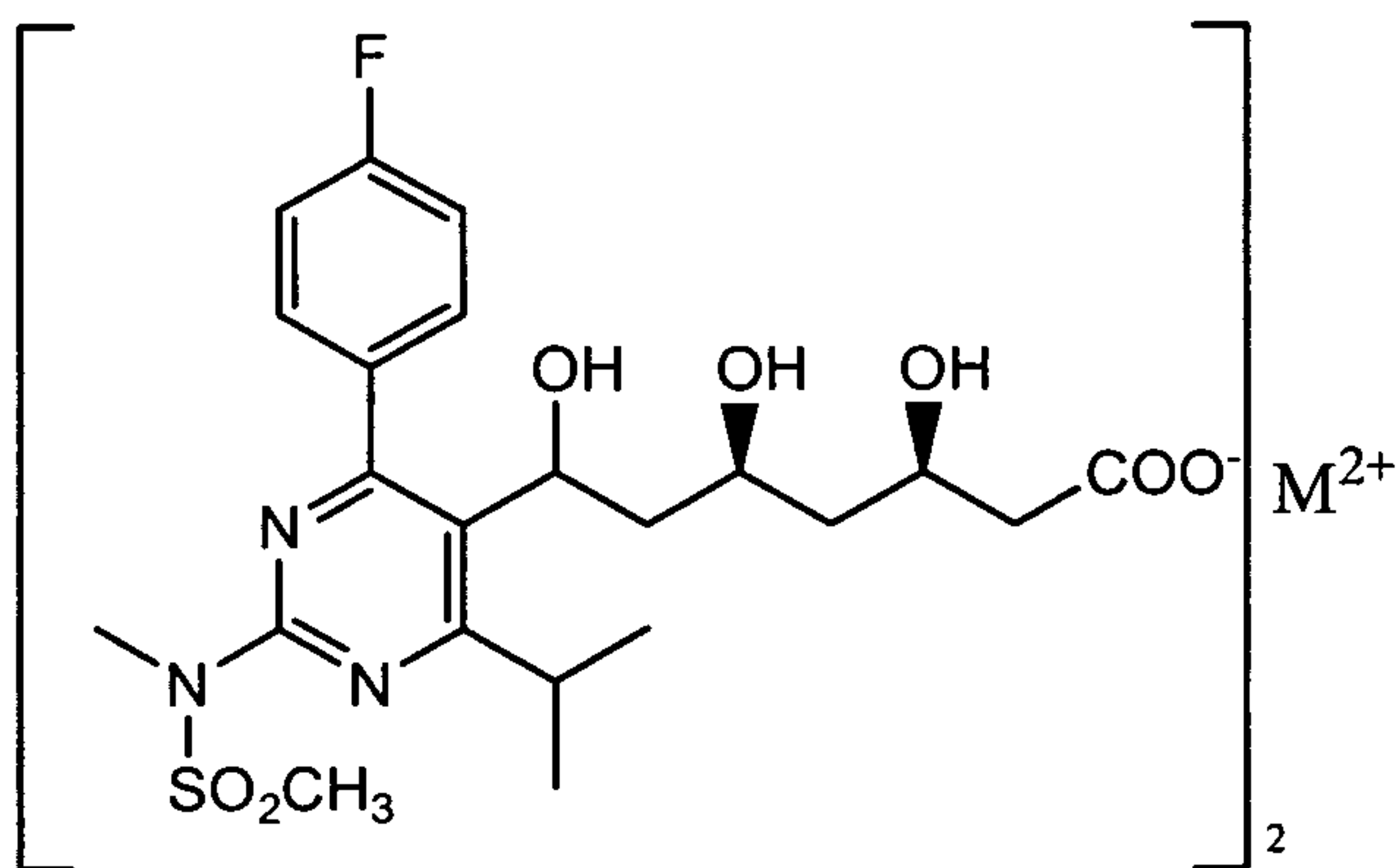


wherein R is a C₁-C₄ alkyl group. Preferably, R is a t-butyl or methyl group. More preferably, the R is t-butyl.

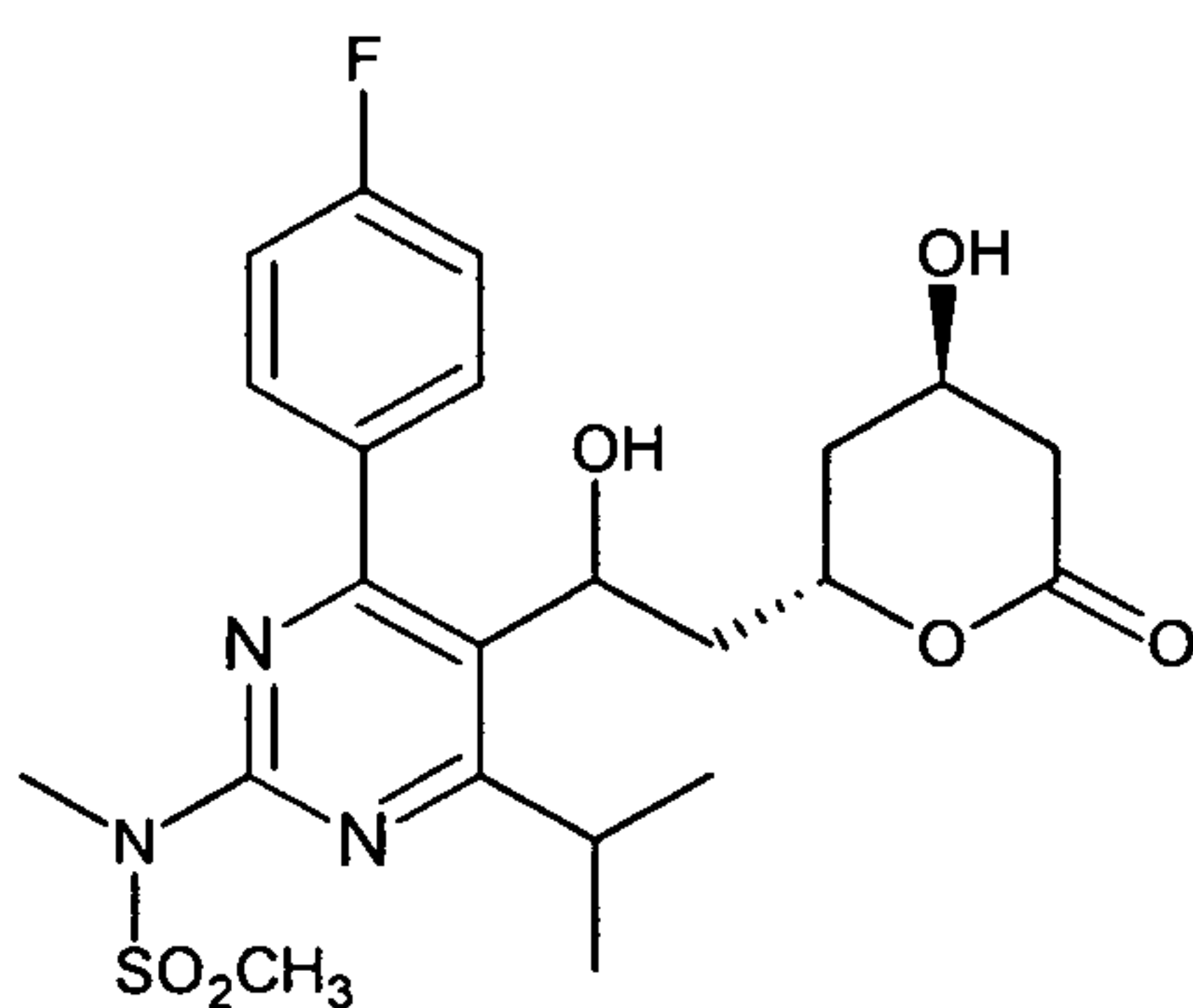
The present invention provides rosuvastatin triol in a salt form having the following structure:



wherein M is an alkali metal or alkaline earth metal cation. Preferably, M is calcium. One of ordinary skill of art would appreciate that when M is an alkaline earth metal cation, such as calcium, the salt would be a hemi calcium salt (2:1 ratio):



The present invention further provides rosuvastatin triol in lactone form has the following structure:



The present invention also provides each of the above forms of the rosuvastatin triol substantially free of the corresponding rosuvastatin diol form. Thus, the present invention provides:

- a) Rosuvastatin triol C₁-C₄ ester substantially free of rosuvastatin diol C₁-C₄ ester. Also provided is rosuvastatin triol t-butyl ester substantially free of rosuvastatin diol t-butyl ester;
- b) Rosuvastatin triol acid substantially free of rosuvastatin diol acid;
- c) Rosuvastatin triol salt (preferably calcium salt) substantially free of rosuvastatin diol salt (preferably calcium salt); and
- d) Rosuvastatin triol lactone substantially free of rosuvastatin diol lactone.

The present invention also provides each of the above forms of the rosuvastatin triol in racemic, (7S) and (7R) configuration. The (7S) and (7R) configurations are diastereoisomers.

Specifically, the present invention provides:

- a) Rosuvastatin triol C₁-C₄ ester, preferably t-butyl ester, in racemic, (7S) and (7R) forms. In one embodiment, the (7S) form is substantially free of the (7R) form. In one embodiment the (7R) form is substantially free of the (7S) form.
- b) Rosuvastatin triol acid in racemic, (7S) and (7R) forms. In one embodiment, the (7S) form is substantially free of the (7R) form. In one embodiment the (7R) form is substantially free of the (7S) form.
- c) Rosuvastatin triol salt (such as calcium), in racemic, (7S) and (7R) forms. In one embodiment, the (7S) form is substantially free of the (7R) form. In one embodiment the (7R) form is substantially free of the (7S) form.
- d) Rosuvastatin triol lactone in racemic, (7S) and (7R) forms. In one embodiment, the (7S) form is substantially free of the (7R) form. In one embodiment, the (7R) form is substantially free of the (7S) form.

The present invention also provides a method for preparing rosuvastatin triol ester. The triol ester can be prepared by oxidizing rosuvastatin C₁-C₄ ester, particularly t-butyl ester. The oxidation of the ester can be carried out by combining rosuvastatin C₁-C₄ ester, particularly t-butyl ester, with borane (*e.g.* BH₃, B₂H₆). Complexes of borane, as well as various monoalkyl (C₁-C₈)- and dialkyl (C₁-C₈)-boranes may be used. Preferably, a solution of borane dimethylsulfide complex in a suitable organic solvent is combined with the ester. The reaction mixture can be stirred. A solution of an inorganic base, preferably NaOH, in water is then combined with the reaction mixture followed by addition of H₂O₂ (preferably about 30% in

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water). The H_2O_2 is preferably added dropwise. The temperature during H_2O_2 addition is preferably kept below about 50°C .

In addition to H_2O_2 , other oxidation reagents can be used. For example, any other peroxides can be used including t-Butyl Hydroperoxide (TBHP) and Magnesium monoperoxyphthalate hexahydrate (MMPP).

The inorganic base is preferably an alkali metal base, more preferably a hydroxide base, such as NaOH , KOH and LiOH . Another base that can be used is NH_4OH . The organic solvent may be a $\text{C}_3\text{-C}_8$ ether such as tetrahydrofuran.

The organic phase can be separated and washed with water and/or brine to remove water miscible by-products such as borane by products (e.g.: H_3BO_3). It can also be washed with sodium sulphite to remove excess hydrogen peroxide. The organic phase can then be concentrated to obtain a residue. Concentration can be done by reducing the pressure to less than 1 atmosphere such as less than about 100mmHg.

After the reaction, if desired, a precipitating agent, such as ammonium chloride or another salt can be added to precipitate impurities out of the reaction mixture. Ammonium chloride is used to remove H_3BO_3 , the reaction-by-product. Instead of using ammonium chloride, an acid such as acetic acid or HCl can be used to neutralize the basic mixture. The H_3BO_3 can be removed by washing with water.

The rosuvastatin triol ester can then be purified and isolated from the corresponding rosuvastatin diol ester by chromatography.

The present invention provides rosuvastatin triol ester in its isolated form.

The triol ester can then be converted to the corresponding acid, salt or lactone.

The triol ester can be converted to the triol salt by hydrolysis of the ester and addition of a suitable source of ions. To obtain the calcium salt either a combination of sodium hydroxide and calcium chloride can be used, or calcium hydroxide can be used.

The rosuvastatin ester can be converted to the salt by suspending the ester in a mixture of an organic solvent and water mixture and combined with a base such as sodium hydroxide to obtain a solution. The organic solvent may be $\text{C}_1\text{-C}_4$ alcohol, preferably ethanol. The organic solvent is then evaporated under reduced pressure followed by addition of calcium chloride, which results in precipitation of the calcium salt of the triol. The precipitate can be recovered by conventional techniques such as filtration.

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The present invention provides rosuvastatin triol salt in its isolated form.

To obtain the rosuvastatin triol acid, the salt is combined with an acid, such as hydrochloric or sulfuric acid. In one embodiment Rosuvastatin triol calcium is suspended in an organic solvent such as dichloromethane, to which aqueous HCl is added. The rosuvastatin triol acid is then isolated from the reaction mixture, such as by separation of the organic phase followed by removal of organic solvent, such as by evaporation under reduced pressure.

The acid can also be obtained after hydrolysis of the ester, by acidification of the reaction mixture instead of addition of calcium chloride. Inorganic acids such as HCl and H₂SO₄ can be used.

The present invention provides rosuvastatin triol acid in its isolated form. The triol acid can be converted to the triol salt by contacting the acid with a base.

The rosuvastatin lactone can then be obtained from the acid under conditions that favor lactonization. In one embodiment, rosuvastatin triol calcium is dissolved in an organic solvent such as acetonitrile, to which aqueous HCl is added. The reaction mixture can then be stirred. The organic solvent and water can then be removed, such as by evaporation under reduced pressure to obtain the lactone.

As stated above, these compounds, namely rosuvastatin triol acid, salt, lactone and ester can be used as reference marker/standards. Figure 2 illustrates that the compounds of the present invention can be used as reference standards to both quantify and identify amount of impurities present in a composition of rosuvastatin. Rosuvastatin triol calcium is close to rosuvastatin diol calcium on the column, yet does not overlap with the peak for rosuvastatin. This lack of overlap is ideal since it can make quantification easier.

The present invention provides rosuvastatin triol lactone in its isolated form.

The present invention provides a process for reducing amount of rosuvastatin triol calcium present in a mixture comprising rosuvastatin calcium and rosuvastatin triol calcium comprising measuring amount of rosuvastatin calcium triol in batches of rosuvastatin diol calcium, selecting batches of the rosuvastatin diol with desirable level of the triol and preparing pharmaceutical compositions with the selected rosuvastatin diol batch. Salts in general other than calcium can also be used in this process.

The present invention provides a process for reducing amount of rosuvastatin triol calcium present in a mixture comprising rosuvastatin diol calcium and rosuvastatin triol calcium comprising measuring amount of rosuvastatin triol C₁-C₄

ester in batches of rosuvastatin diol C₁-C₄ ester, selecting batches of the rosuvastatin diol C₁-C₄ ester with of the triol C₁-C₄ ester and preparing pharmaceutical compositions of rosuvastatin diol calcium with the selected rosuvastatin diol C₁-C₄ ester batch. The ester is preferably t-butyl.

The present invention provides a process for reducing amount of rosuvastatin triol calcium present in a mixture comprising rosuvastatin diol calcium and rosuvastatin triol calcium comprising measuring amount of rosuvastatin triol lactone in batches of rosuvastatin diol lactone, selecting batches of the rosuvastatin diol lactone with desirable level of the triol lactone and preparing pharmaceutical compositions of rosuvastatin diol calcium with the selected rosuvastatin diol lactone batch.

The present invention provides a process for reducing amount of rosuvastatin triol calcium present in a mixture comprising rosuvastatin diol calcium and rosuvastatin triol calcium comprising measuring amount of rosuvastatin triol acid in batches of rosuvastatin diol acid, selecting batches of the rosuvastatin diol acid with desirable level of the triol acid and preparing pharmaceutical compositions of rosuvastatin diol calcium with the selected rosuvastatin diol acid batch.

The present invention provides a method of determining the amount of an impurity in a sample of rosuvastatin diol ester (preferable t-butyl ester) comprising measuring by GC or HPLC the area under a peak corresponding to rosuvastatin triol ester in a reference standard comprising a known amount of rosuvastatin triol ester (preferably t-butyl); measuring by GC or HPLC the area under a peak corresponding to rosuvastatin triol ester in a test sample comprising rosuvastatin triol and rosuvastatin diol esters (preferably t-butyl); and determining the amount of the triol ester in the sample by comparing the area of reference standard with that of the test sample.

The present invention provides a method of determining the amount of an impurity in a sample of rosuvastatin calcium comprising measuring by GC or HPLC the area under a peak corresponding to rosuvastatin triol calcium in a reference standard comprising a known amount of rosuvastatin triol calcium; measuring by GC or HPLC the area under a peak corresponding to rosuvastatin triol calcium in a test sample comprising rosuvastatin triol and rosuvastatin diol calcium salts; and determining the amount of the triol calcium in the sample by comparing the area of

reference standard with that of the test sample. Salts in general other than calcium can also be used in this process.

The present invention provides a method of determining the amount of an impurity in a sample of rosuvastatin acid comprising measuring by GC or HPLC the area under a peak corresponding to rosuvastatin triol acid in a reference standard comprising a known amount of rosuvastatin triol acid; measuring by GC or HPLC the area under a peak corresponding to rosuvastatin triol acid in a test sample comprising rosuvastatin acid and rosuvastatin diol acid; and determining the amount of the triol acid in the sample by comparing the area of reference standard with that of the test sample.

The present invention provides a method of determining the amount of an impurity in a sample of rosuvastatin lactone comprising measuring by GC or HPLC the area under a peak corresponding to rosuvastatin triol lactone in a reference standard comprising a known amount of rosuvastatin triol lactone; measuring by GC or HPLC the area under a peak corresponding to rosuvastatin triol lactone in test a sample comprising rosuvastatin triol lactone and rosuvastatin diol lactone; and determining the amount of the triol lactone in the sample by comparing the area of reference standard with that of the test sample.

The present invention provides a method of identifying the relative retention time (RRT) of an impurity in a sample of rosuvastatin diol ester (preferably t-butyl) comprising measuring by GC or HPLC the relative retention time (RRT) corresponding to rosuvastatin triol ester in a reference marker sample; carrying out GC or HPLC with a test sample comprising of the rosuvastatin ester and rosuvastatin triol ester to obtain an HPLC or GC chromatogram with retention times; and determining the relative retention time (RRT) of the triol ester in the sample by comparing the relative retention time (RRT) of the reference marker to the relative retention time (RRT) of the test sample. Preferably the rosuvastatin diol and rosuvastatin triol ester are tert-butyl esters.

Accordingly in another embodiment, the present invention provides a method of determining the relative retention time (RRT) of an impurity in a sample of rosuvastatin diol calcium comprising measuring by GC or HPLC the relative retention time (RRT) corresponding to rosuvastatin triol calcium in a reference marker sample; carrying out GC or HPLC with a test sample comprising of the rosuvastatin diol and rosuvastatin triol calcium salts to obtain an HPLC chromatogram with retention times;

and determining the relative retention time (RRT) of the triol calcium in the sample by comparing the relative retention time (RRT) of the reference marker to the relative retention time (RRT) of the test sample. Salts in general other than calcium can also be used in this process.

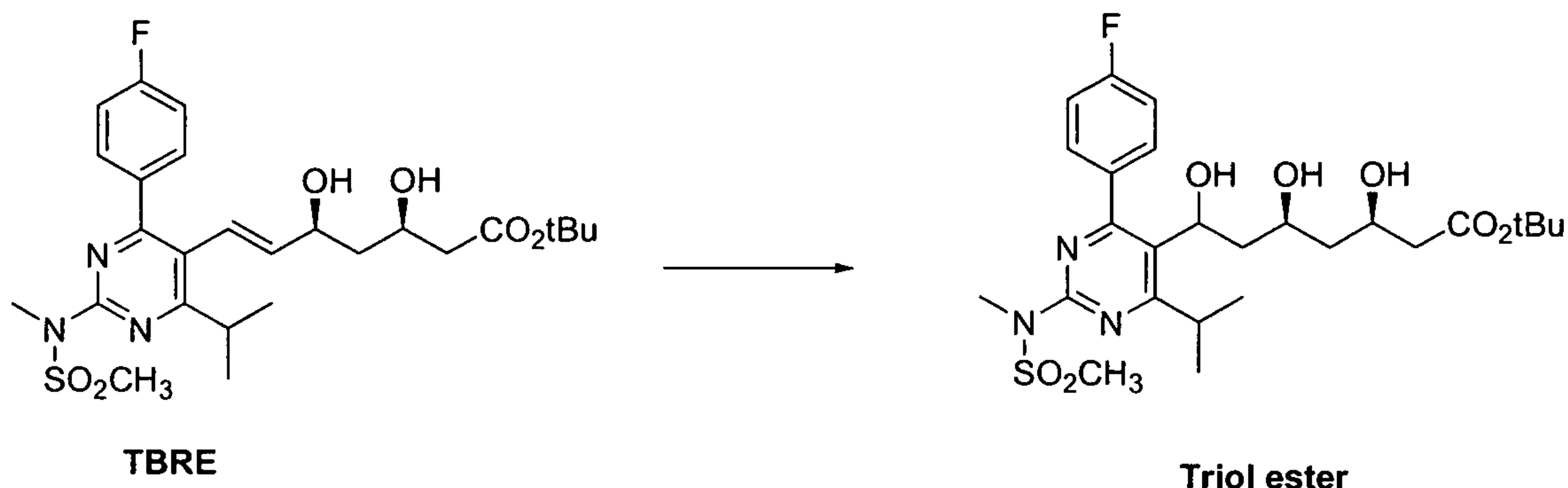
Accordingly in another embodiment, the present invention provides a method of identifying the relative retention time (RRT) of an impurity in a sample of rosuvastatin diol acid comprising measuring by GC or HPLC the relative retention time (RRT) corresponding to rosuvastatin triol acid in a reference marker sample; carrying out GC or HPLC with a test sample comprising of the rosuvastatin diol acid and rosuvastatin triol acid to obtain an HPLC chromatogram with retention times; and determining the relative retention time (RRT) of the triol acid in the sample by comparing the relative retention time (RRT) of the reference marker to the relative retention time (RRT) of the test sample.

Accordingly in another embodiment, the present invention provides a method of determining the relative retention time (RRT) of an impurity in a sample of rosuvastatin diol lactone comprising measuring by GC or HPLC the relative retention time (RRT) corresponding to rosuvastatin triol lactone in a reference marker sample; carrying out GC or HPLC with a test sample comprising of the rosuvastatin diol acid and rosuvastatin triol acid to obtain an HPLC chromatogram with retention times; and determining the relative retention time (RRT) of the triol acid in the sample by comparing the relative retention time (RRT) of the reference marker to the relative retention time (RRT) of the test sample.

Having described the invention with reference to certain preferred embodiments, other embodiments will become apparent to one skilled in the art from consideration of the specification. The invention is further defined by reference to the following examples describing in detail the preparation of the composition and methods of use of the invention. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the scope of the invention.

EXAMPLES

Example 1: Synthesis of Triol Ester

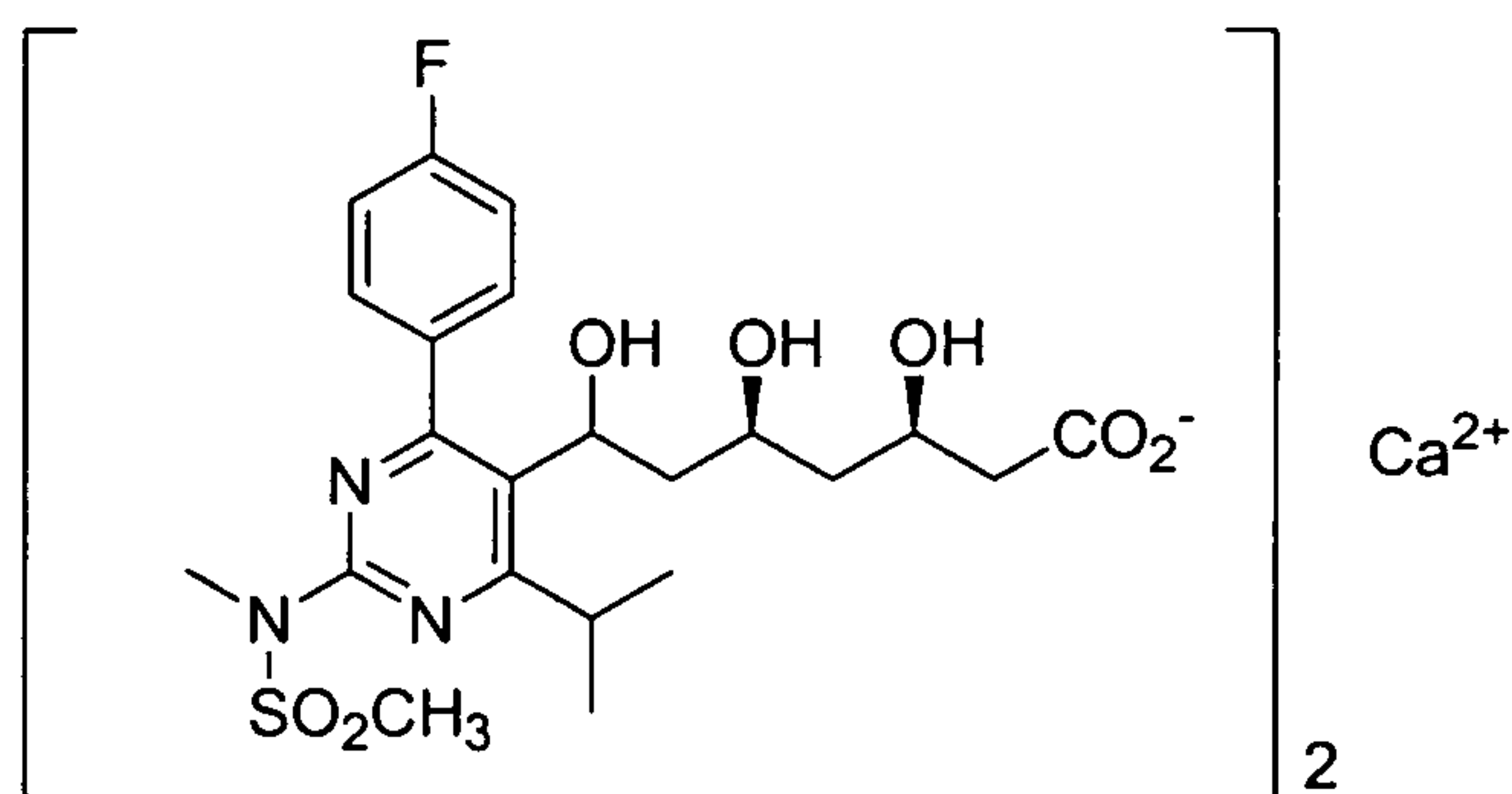


TBRE (10 g) was mixed with 1M solution of borane dimethylsulfide complex in THF (56 ml) in an inert atmosphere. The mixture was stirred for 3 h at 20°C. A solution of NaOH (74 g) in water (5 ml) was slowly added. H₂O₂ (30% in water, 15 ml) was added dropwise, so that the temperature of the mixture was kept below 50°C. The mixture was stirred for 0.5 h. A concentrated solution of ammonium chloride (150 ml) was added, and the precipitate was filtered out. The phases were separated and organic phase was first washed with a concentrated solution of sodium sulfite (40 ml), then with a mixture of water (100 ml) and brine (100 ml), and finally with brine (150 ml). Then an organic solvent was removed at reduced pressure, giving a semi-solid residue, containing triol, non-reacted TBRE and some impurities.

Triol was isolated by two chromatographic separations. First separation was performed on an RP-18 column (RediSep[®] C-18 Reversed Phase Column), using a gradient from 40% to 45% of EtOH in water. The second separation was performed on normal silica column (RediSep[®] Normal Phase Disposable Column), using a gradient from 0% to 1% of EtOH in CH₂Cl₂ as an eluent. Purity 93%. MS (ES⁺): M+H=556 M+Na+= 578

RediSep[®] is manufactured by Teledyne Isco, Inc (Nebraska).

Example 2: Synthesis of Rosu-Ca-triol



2g of material, consisting of TBRE and 2.85% triol-ester (3.7 mmole of the carboxylic group) was suspended in EtOH (10 mL)/water (6 mL) mixture. Saturated NaOH solution (0.35 g, 4.1 mmole) was added dropwise at room temperature and the mixture was stirred for 2 h. The solution was concentrated under vacuum to remove EtOH. Calcium salt was precipitated from the water solution by addition of CaCl_2 (0.41g, 3.7 mmole) at 40°C upon stirring. Stirring was continued for 1 h at room temperature, and the precipitate was filtered, washed with water and dried. The material contained 2.82% Rosu-Ca-triol and 95% Rosu-Ca

Example 3: Synthesis of Rosuvastatin triol acid

Rosu triol Ca (0.5g) is suspended in dichloromethane (5 mL) and HCl (1N in water, 1 mL) is added. After stirring for 15 minutes phases are separated, and organic phase is concentrated in vacuum, giving the residue, which contains mainly the product. It may be additionally purified by column chromatography (silica gel, dichloromethane-methanol mixtures as eluent), giving pure Rosu triol acid.

Example 4: Synthesis of Rosuvastatin triol lactone

Rosu triol Ca (4 g) is dissolved in acetonitrile (40 mL) and HCl (1N in water, 40 mL) is added. The mixture is stirred at room temperature overnight. Acetonitrile and water are removed by distillation at reduced pressure. The residue, containing the product, may be additionally purified by flash chromatography (silica gel, hexane-ethyl acetate mixtures as eluent), giving pure rosu triol lactone.

Example 5: Synthesis of Rosuvastatin triol Calcium

2g of pure triol-ester (3.7 mmol) is suspended in EtOH (10 mL)/water (6 mL) mixture. Saturated NaOH solution (0.35 g, 4.1 mmole) is added dropwise at room temperature and the mixture is stirred for 2 h. The solution is concentrated under vacuum to remove EtOH. Calcium salt is precipitated from the water solution by addition of CaCl_2 (0.41g, 3.7 mmole) at 40°C upon stirring. Stirring is continued for 1 h at room temperature, and the precipitate is filtered, washed with water and dried, giving Rosu-Ca-triol.

MS conditions

Instrument: Bruker Esquir 6000
Source: Positive/ Negative switching ESI
Target mass: 556 Da
Compound stability: 50 %
Trap drive: 100 %
Octopole RF: 195.3 Vpp
Capillary exit: 111.8 V
Drying gas flow rate: 10 L/min
Nebulizer: 60 psig
Drying gas temperature: 365 °C
V cap: 4000 V

HPLC method for Impurity profile of TBRE

Column: C18

Mobile phase: Gradient of Eluent A and Eluent B

Gradient:	Time(min)	Eluent A(%)	Eluent B(%)
	0	100	0
	2	84	16
	23	84	16
	36	10	90
	40	10	90

Eluent A: 60% 0.005M Ammonium Acetate 40% Acetonitrile:Ethanol=2:3

Eluent B: 100% Acetonitrile:Ethanol = 1 : 4

UV detection: 243nm

Run time: 55min

Flow rate: 0.6mL/min

Injection volume: 5µL

Column temperature: 5°C

Discard limit: Less than 0.02%

Sample preparation: 0.5mg/mL

RT of TBRE: about 24.5min

RRT of Triol -TBRE impurity is 0.6 corresponding to the main peak of TBRE.

HPLC method for Impurity profile of ROSU

Column: C18

Mobile phase: Gradient of Eluent A , Eluent B and Eluent C

Gradient:	Time(min)	Eluent A(%)	Eluent B(%)	Eluent C(%)
	0	100	0	0
	15	0	100	0
	20	0	93	7
	30	0	78	22
	40	0	5	95
	45	0	5	95

Eluent A: 60% 0.05% Acetic acid glacial pH 3.5 with Ammonium hydroxide 35%
Acetonitrile 5%Ethanol

Eluent B: 55% 0.05% Acetic acid glacial pH 3.5 with Ammonium hydroxide 45%
Acetonitrile

Eluent C: 100% Ethanol

UV detection: 243nm

Run time: 45min

Flow rate: 0.5mL/min

Injection volume: 10 μ L

Column temperature: 20°C

Discard limit: Less than 0.02%

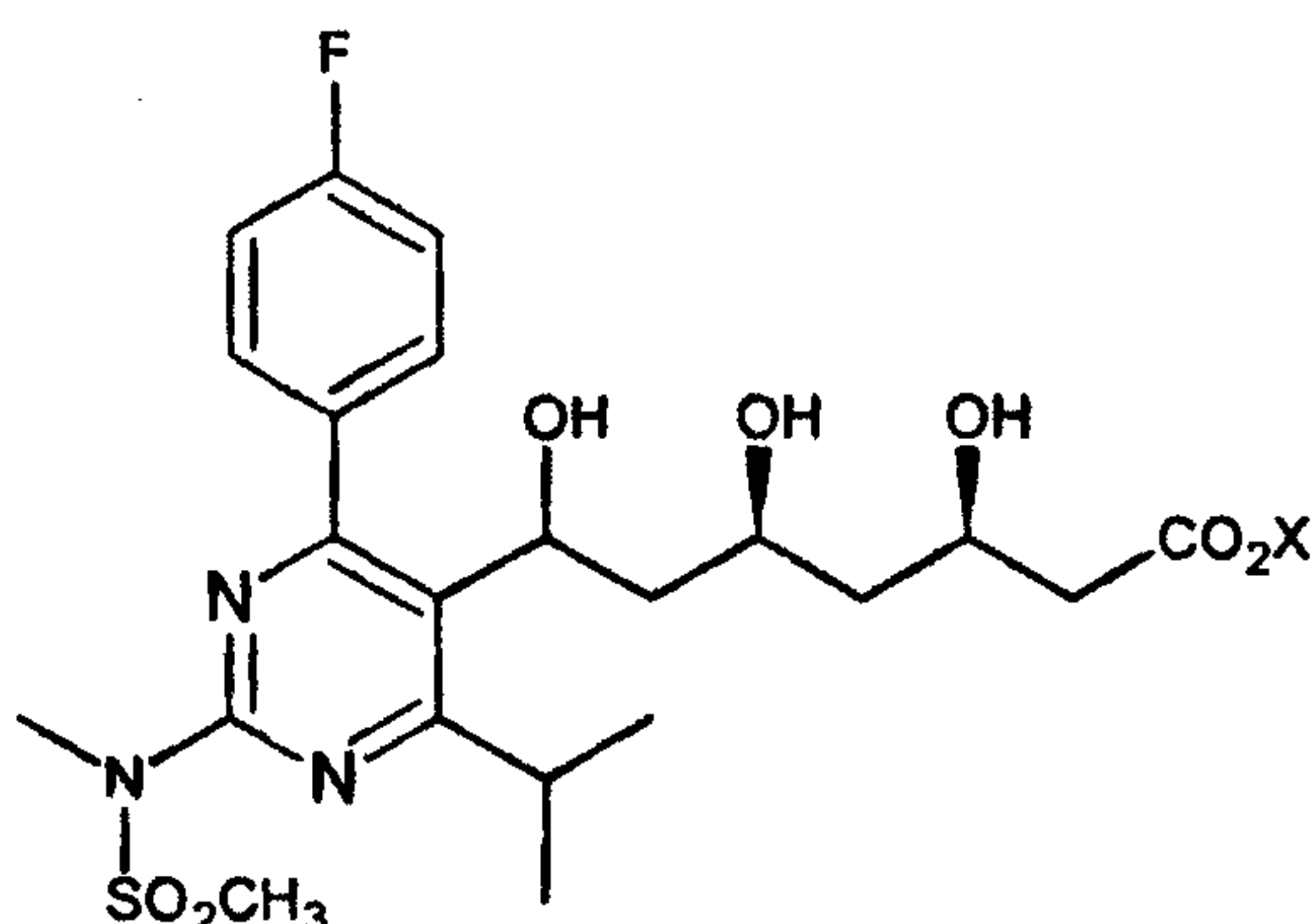
Sample preparation: 0.2mg/mL

RT of ROSU: about 19min

RRT of Triol -ROSU impurity is 0.7 corresponding to the main peak of ROSU.

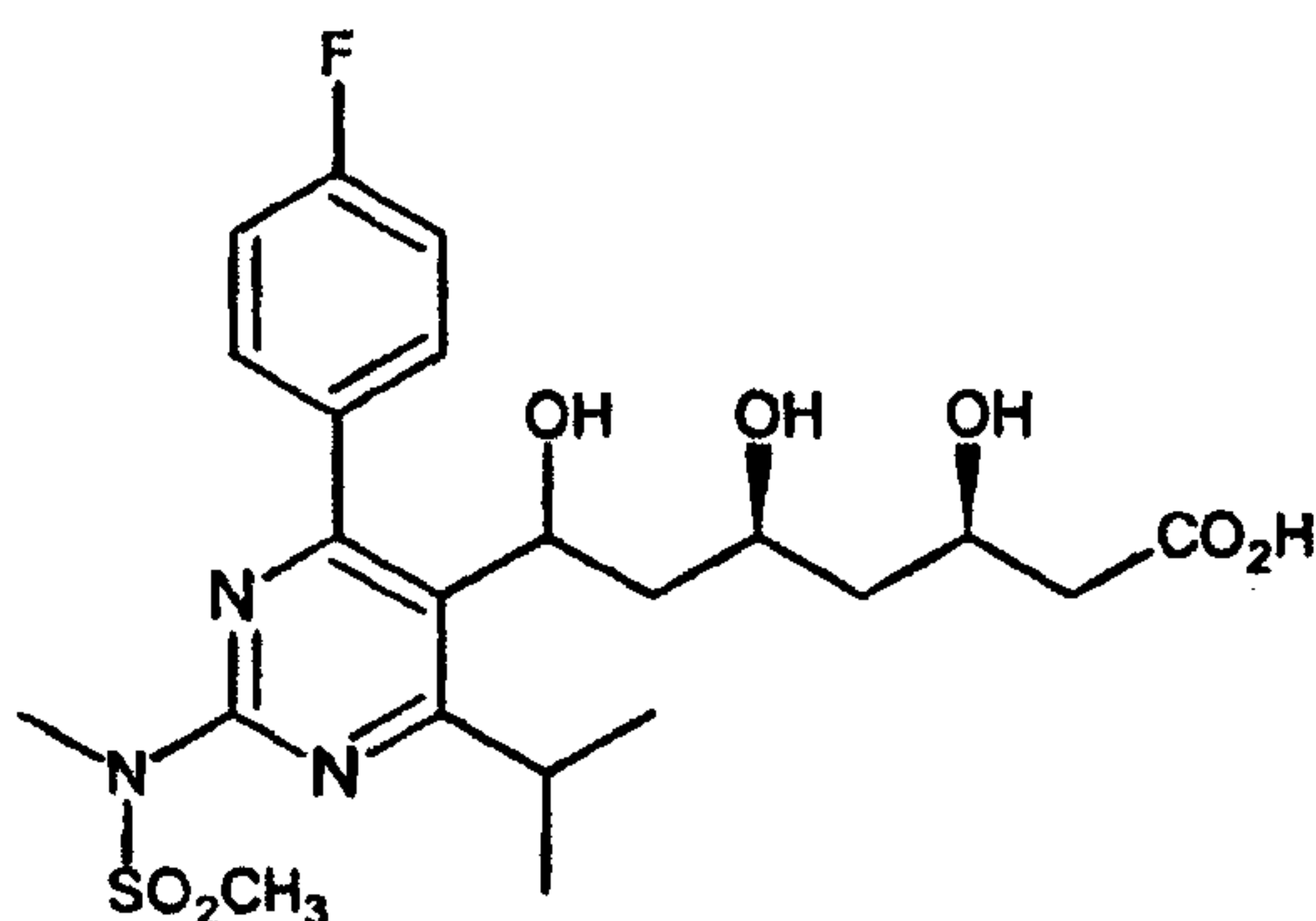
What is claimed is:

1. A rosuvastatin triol having the following structure:

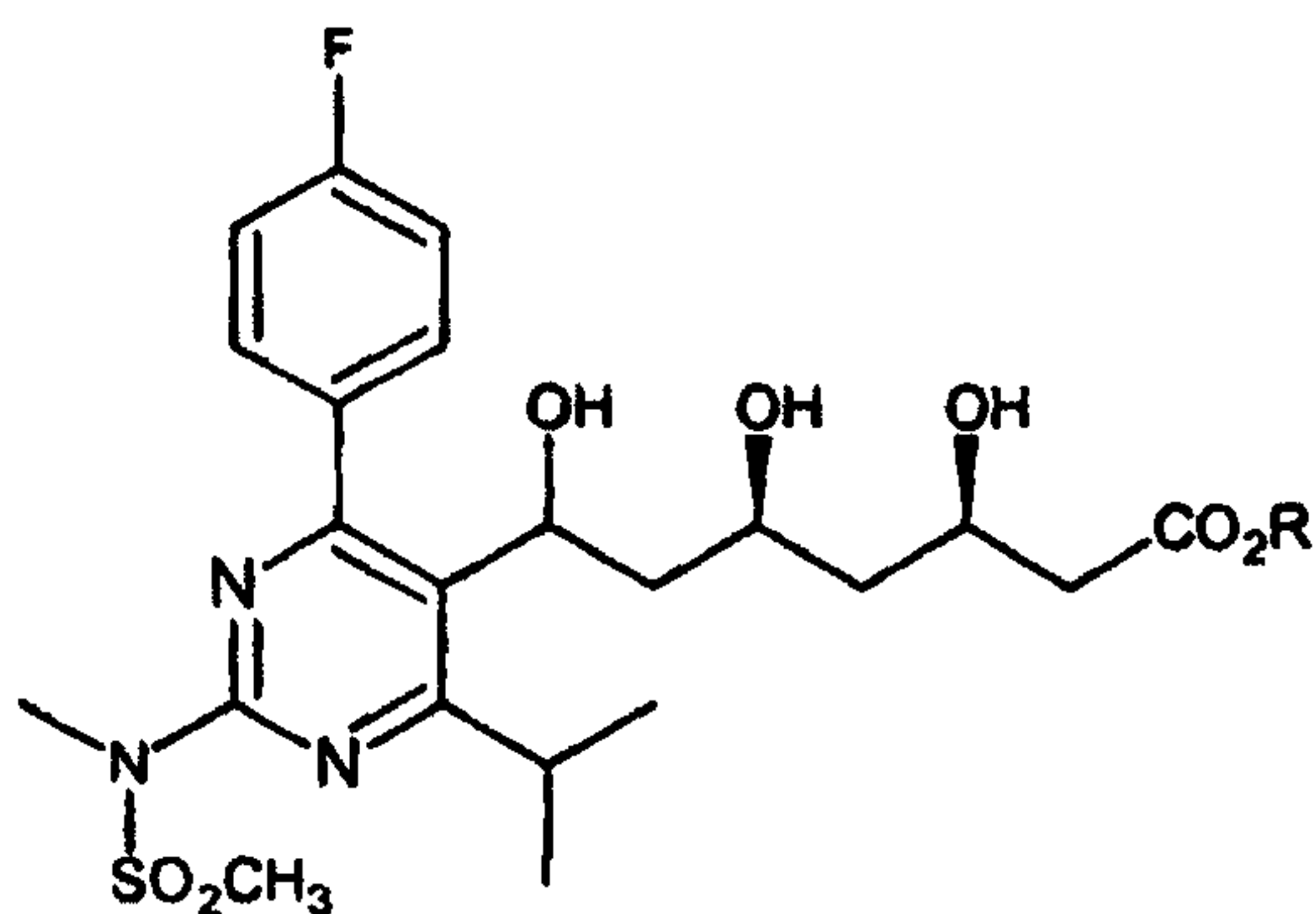


wherein X is a hydrogen, an alkali or alkaline earth metal, or a C₁-C₄ alkyl group.

2. The rosuvastatin triol of claim 1, wherein the rosuvastatin triol is isolated by purification from the reaction mixture.
3. The rosuvastatin triol of claim 1 or 2, wherein the rosuvastatin triol is substantially free from corresponding rosuvastatin diol.
4. The rosuvastatin triol of claim 1 in acid form having the following structure:

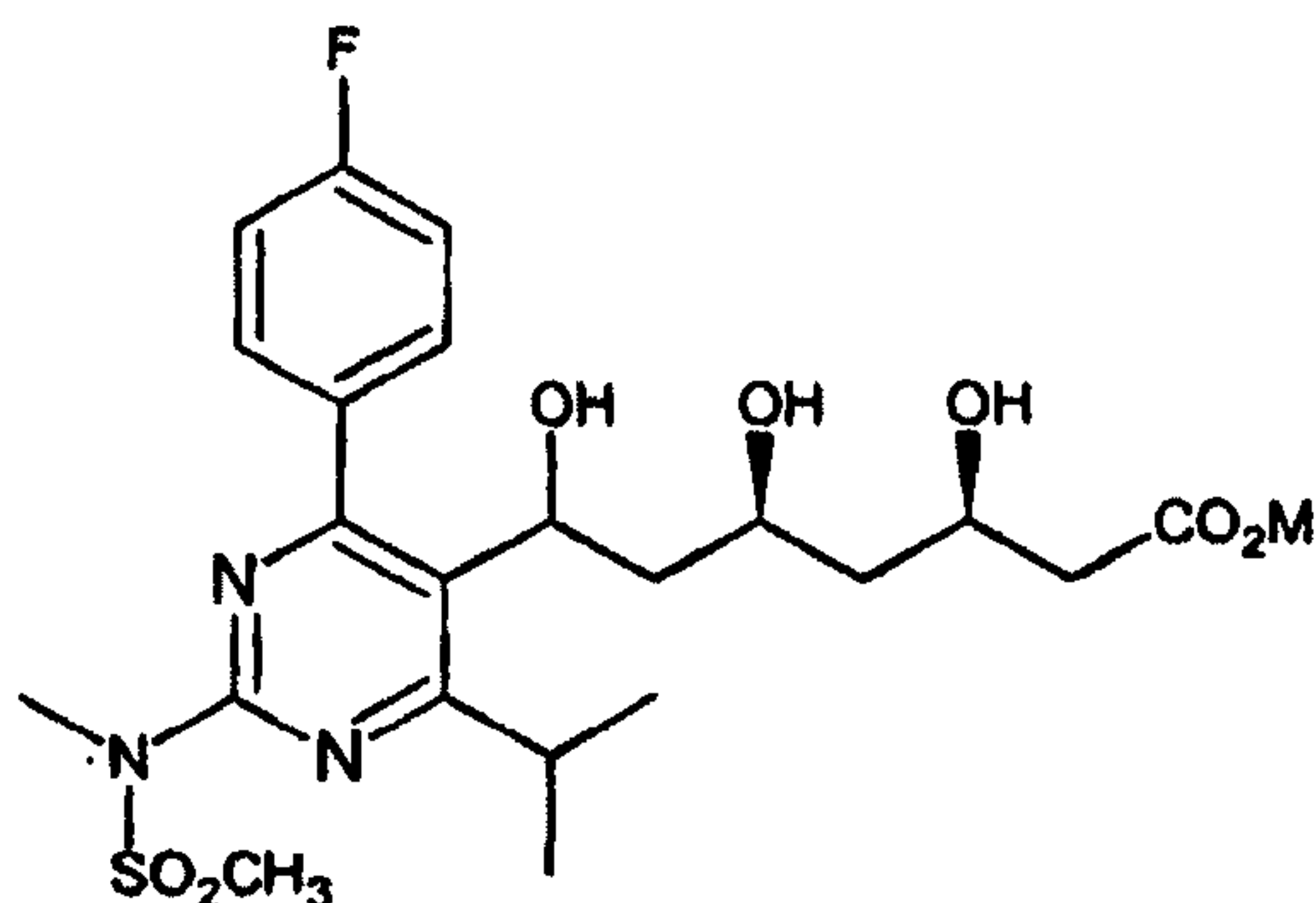


5. The rosuvastatin triol of claim 4, wherein the rosuvastatin triol is isolated by purification from the reaction mixture.
6. The rosuvastatin triol of claim 4 or 5, wherein the rosuvastatin triol is substantially free from corresponding rosuvastatin diol.
7. The rosuvastatin triol of claim 1 in ester form having the following structure:



wherein R represents a C₁₋₄ alkyl.

8. The rosuvastatin triol of claim 7, wherein the rosuvastatin triol ester is isolated by purification from the reaction mixture.
9. The rosuvastatin triol of claim 7 or 8, wherein the rosuvastatin triol ester is substantially free from corresponding rosuvastatin diol ester.
10. The rosuvastatin triol of claim 7, 8, or 9, wherein the ester is a tert-butyl ester.
11. The rosuvastatin triol of claim 1, in salt form having the following structure:

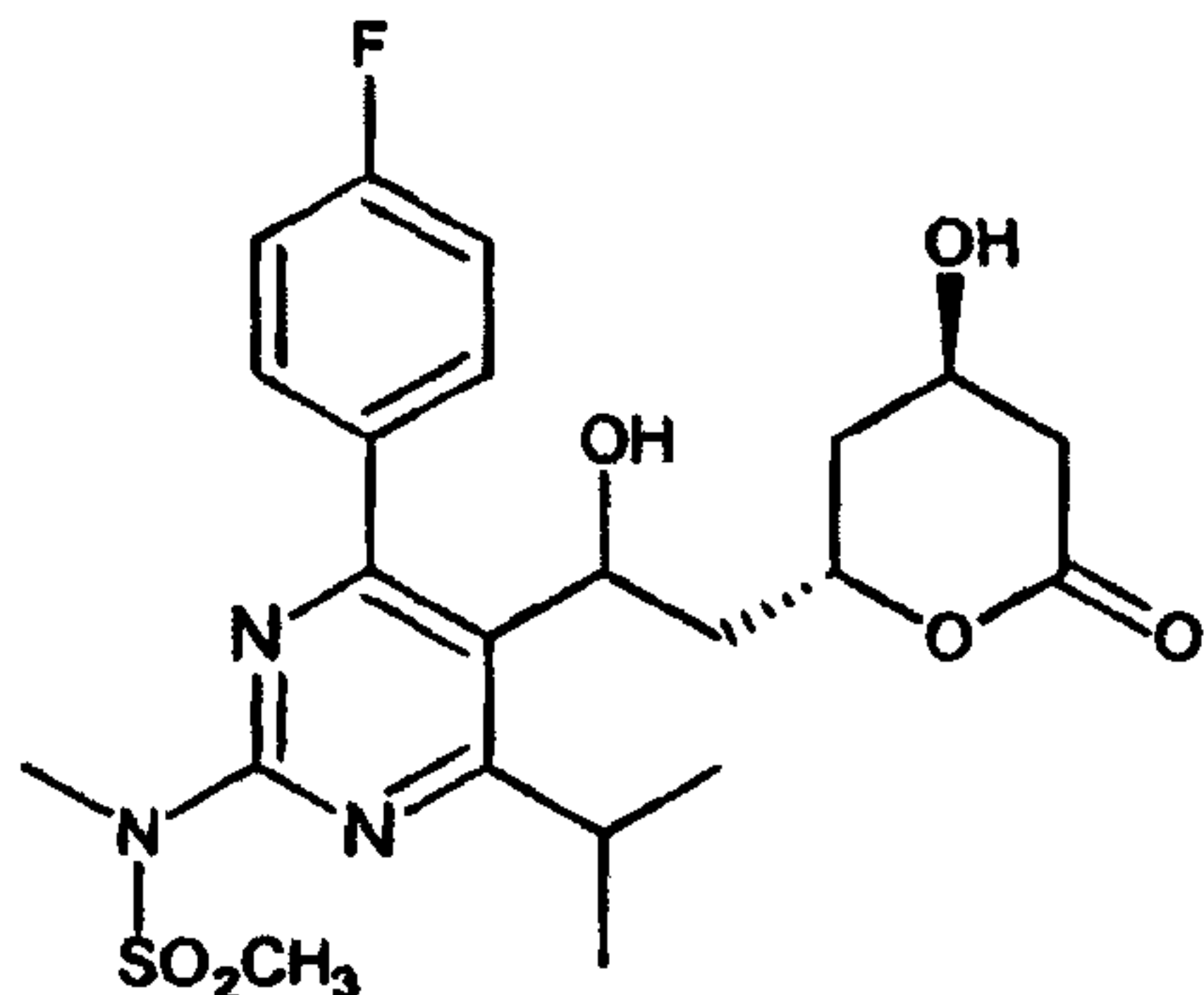


wherein M represents an alkali or alkaline earth metal cation.

12. The rosuvastatin triol of claim 11, wherein the metal cation is an alkaline earth metal with two molecules of rosuvastatin present for each cation.
13. The rosuvastatin triol of claim 12, wherein the metal cation is Ca²⁺.
14. The rosuvastatin triol of claim 11, 12, or 13, wherein the rosuvastatin triol is isolated by purification from the reaction mixture.

15. The rosuvastatin triol of claim 11, 12, 13 or 14, wherein the rosuvastatin triol is substantially free from corresponding rosuvastatin diol.

16. Rosuvastatin triol of claim 1 in lactone form having the following structure:



17. The rosuvastatin triol of claim 16, wherein the rosuvastatin triol lactone is isolated by purification from the reaction mixture.

18. The rosuvastatin triol of claim 16 or 17, wherein the rosuvastatin triol lactone is substantially free from corresponding rosuvastatin diol.

19. The rosuvastatin triol of any one of claims 1-18, wherein the rosuvastatin triol is selected from the group consisting of: rosuvastatin triol in (7S) form; rosuvastatin triol in (7R) form; and rosuvastatin triol racemic form.

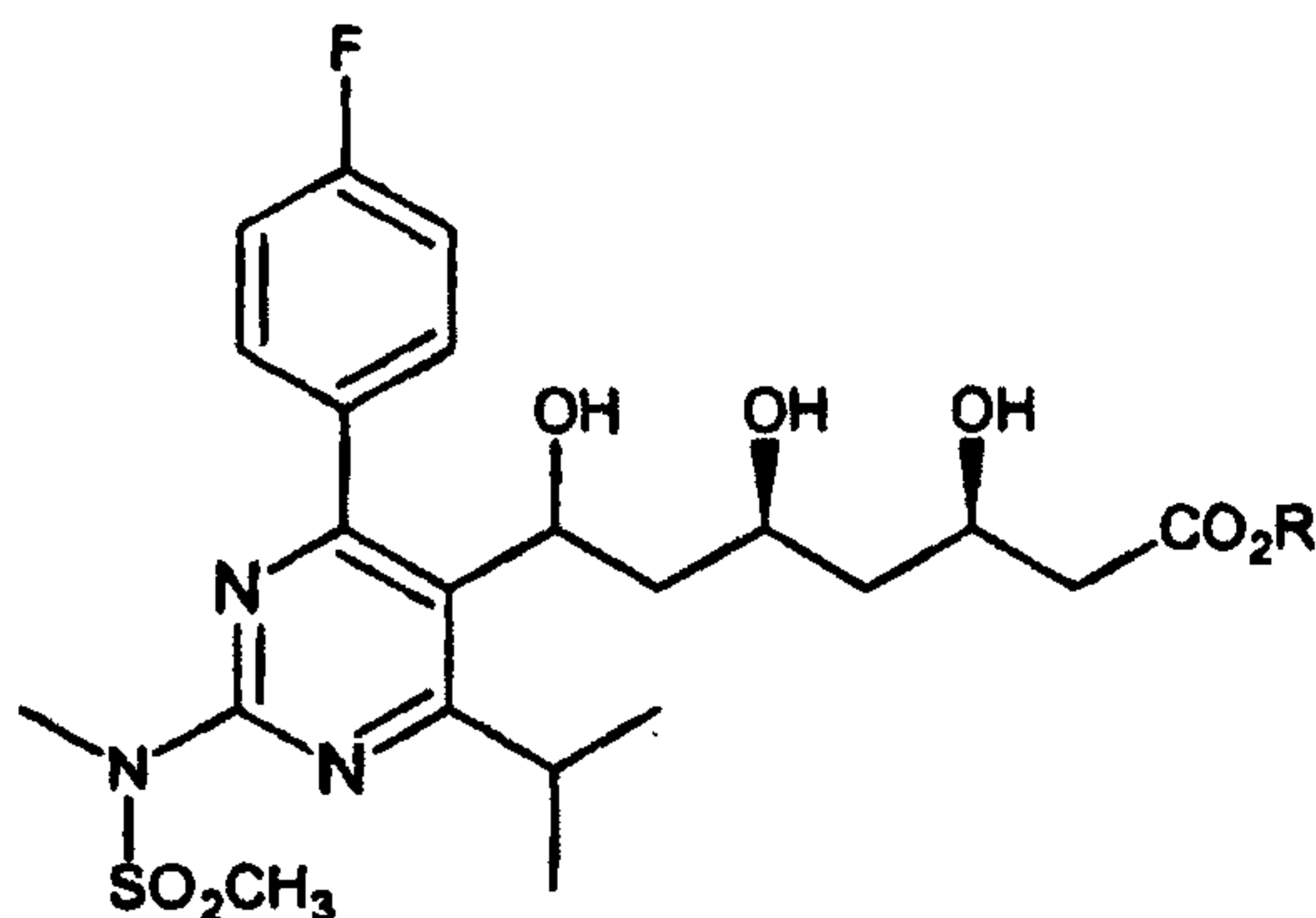
20. A process for preparing the triol of any one of claims 7-10 comprising oxidizing rosuvastatin diol C₁ to C₄ ester to obtain the rosuvastatin triol ester with a hydroxyl group at position 7.

21. The process of claim 20, wherein the process comprises combining rosuvastatin C₁-C₄ ester with a solution of a borane in an organic solvent to obtain a reaction mixture, combining the resulting reaction mixture with a solution of an inorganic base in water, and adding peroxide and recovering the triol ester.

22. The process of claim 21, wherein the borane is a borane complex of dimethylsulfide.

23. The process of claim 22, wherein the borane is a monoalkyl- or dialkyl-borane.

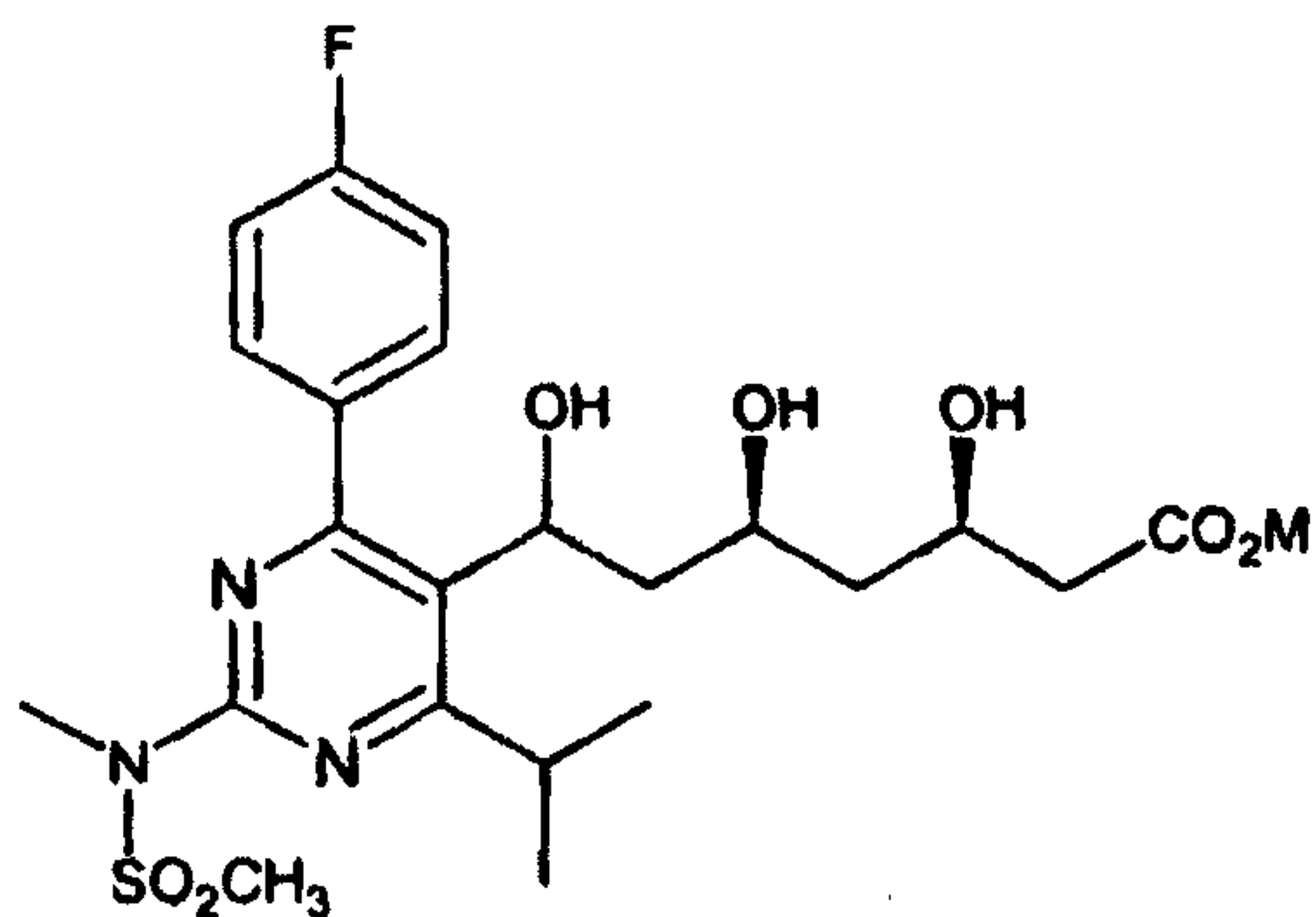
24. The process of claim 21 or 22, wherein the peroxide is H_2O_2 .
25. The process of claim 21 or 22, wherein the peroxide is t-Butyl Hydroperoxide (TBHP) or Magnesium monoperoxyphthalate hexahydrate (MMPP).
26. The process of claim 25, wherein inorganic base is an alkali metal base.
27. The process of claim 26, wherein the base is a hydroxide base.
28. The process of claim 27, wherein the hydroxide base is NaOH, KOH or LiOH.
29. The process of claim 21, wherein the base is NH_4OH .
30. The process of any one of claims 21-29, wherein the organic solvent is a C_3 - C_8 ether.
31. The process of claim 30, wherein the organic solvent is tetrahydrofuran.
32. A process for preparing the triol salt of claim 11 comprising suspending the triol ester of the following formula:



wherein R is a C_1 - C_4 ester, in a mixture of water and an organic solvent, and combining the suspension with a base and a source of calcium ions.

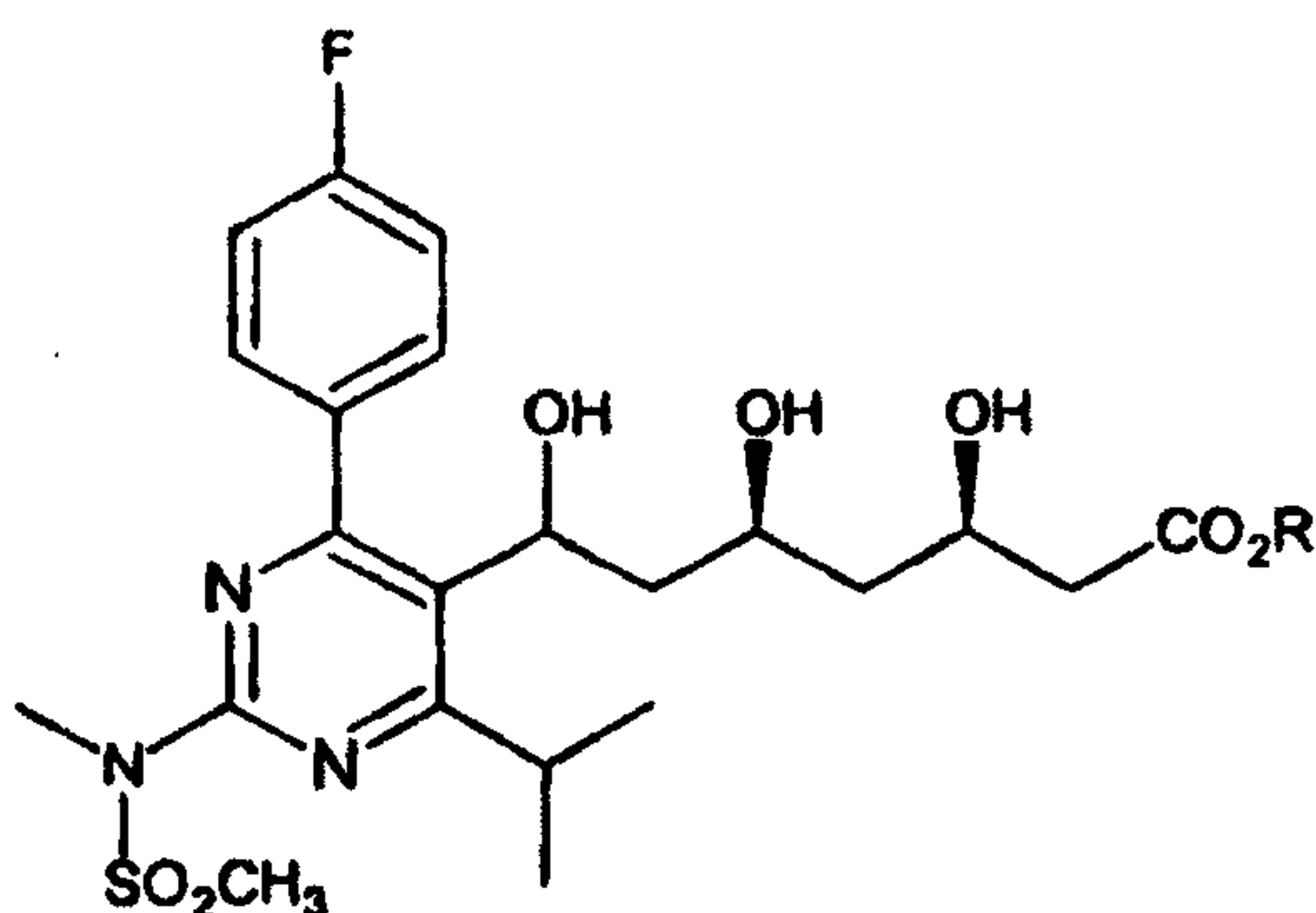
33. The process of claim 32, wherein the organic solvent is a C_1 - C_4 alcohol.
34. The process of claim 32, wherein the organic solvent is ethanol.
35. The process of claim 32, 33 or 34, wherein the source of ions is calcium chloride.

36. A process for preparing the triol acid of claim 4 comprising contacting the rosuvastatin triol salt of the following formula:



with an acid, wherein M is an alkali or alkaline earth metal.

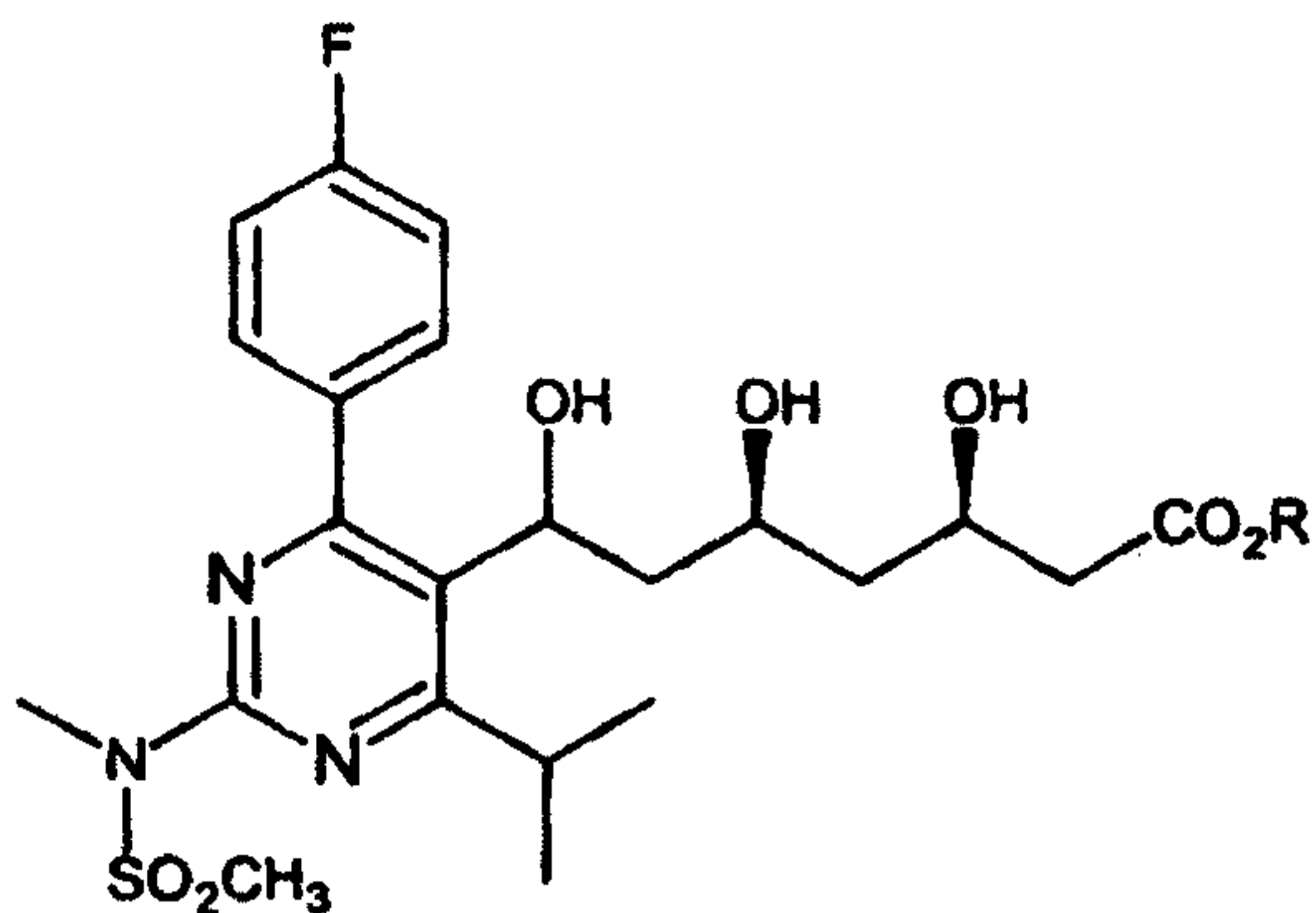
37. The process of claim 36, wherein the acid is hydrochloric or sulfuric acid.
38. The process of claim 36 or 37, wherein rosuvastatin triol salt is combined with an organic solvent.
39. The process of claim 38, wherein the organic solvent is dichloromethane.
40. A process for preparing rosuvastatin triol acid of claim 4 comprising hydrolyzing an ester of the following formula:



with an acid, wherein R is a C₁-C₄ ester.

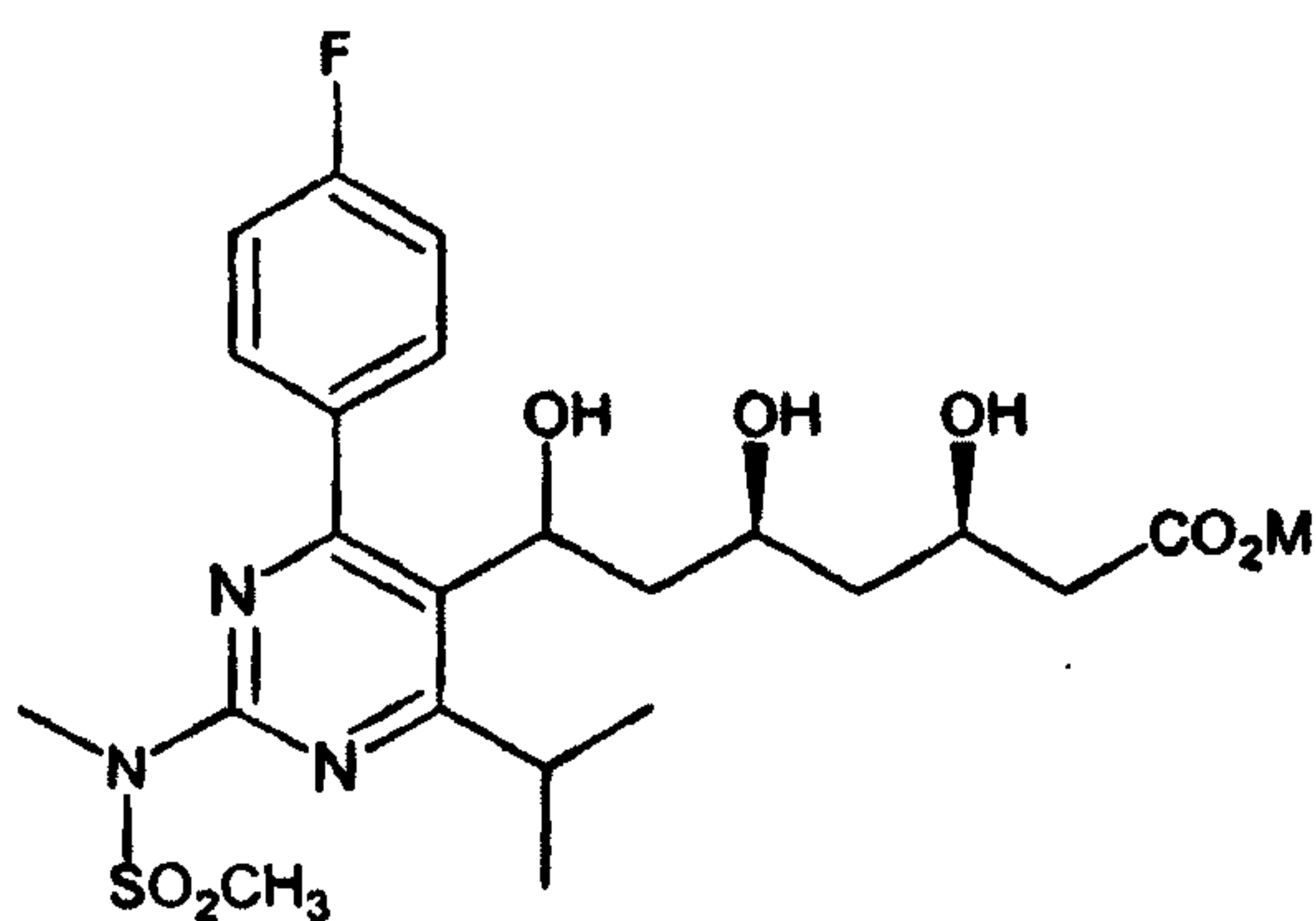
41. The process of claim 40, wherein the acid is HCl or H₂SO₄.
42. A process for preparing the lactone of any one of claims claim 16-19, comprising hydrolyzing the triol ester of the following formula:

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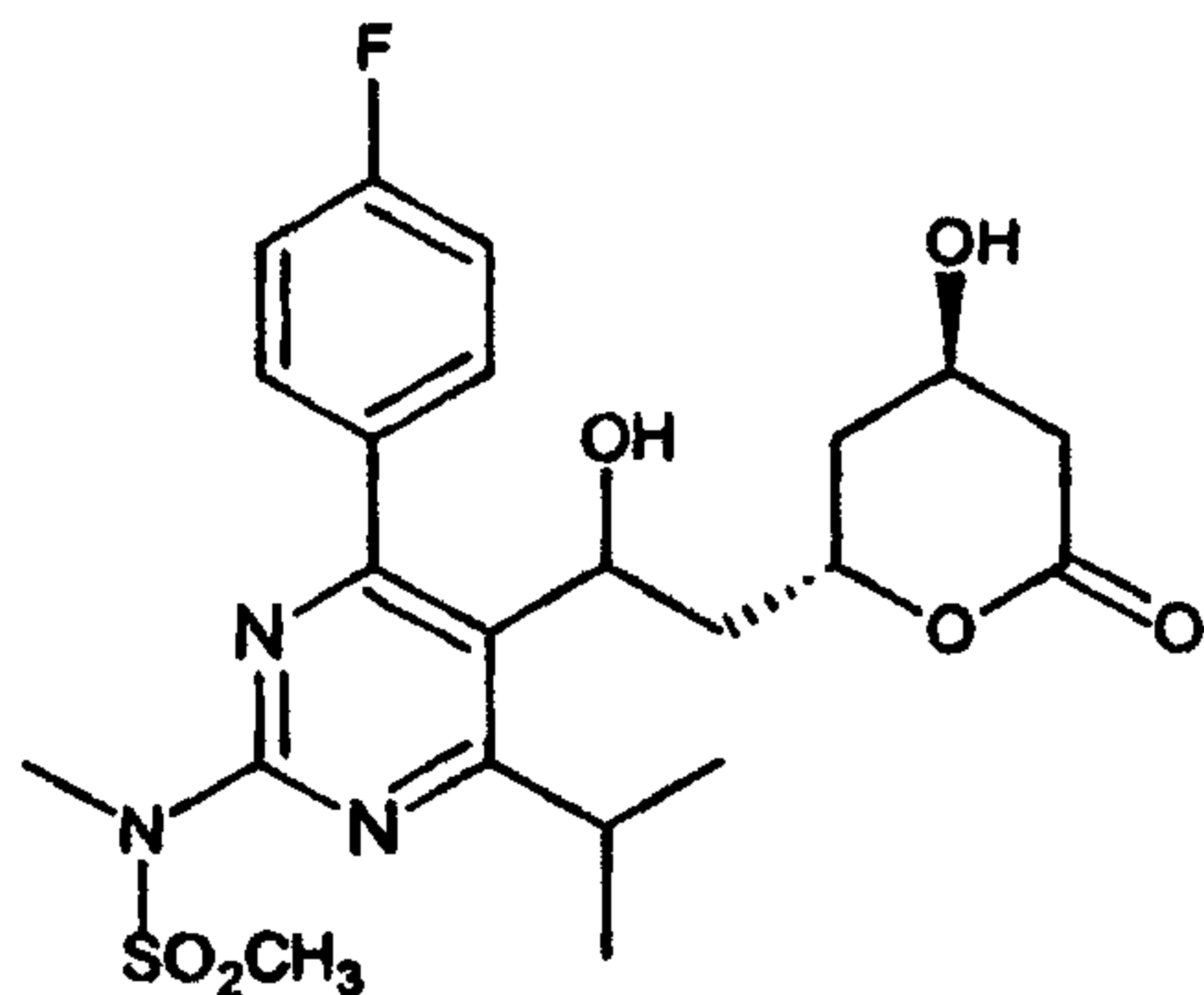


wherein R is a C₁-C₄ ester, and converting the hydrolyzed ester to a lactone.

43. A process for preparing rosuvastatin triol salt of any one of claims 11-15 with the following structure:

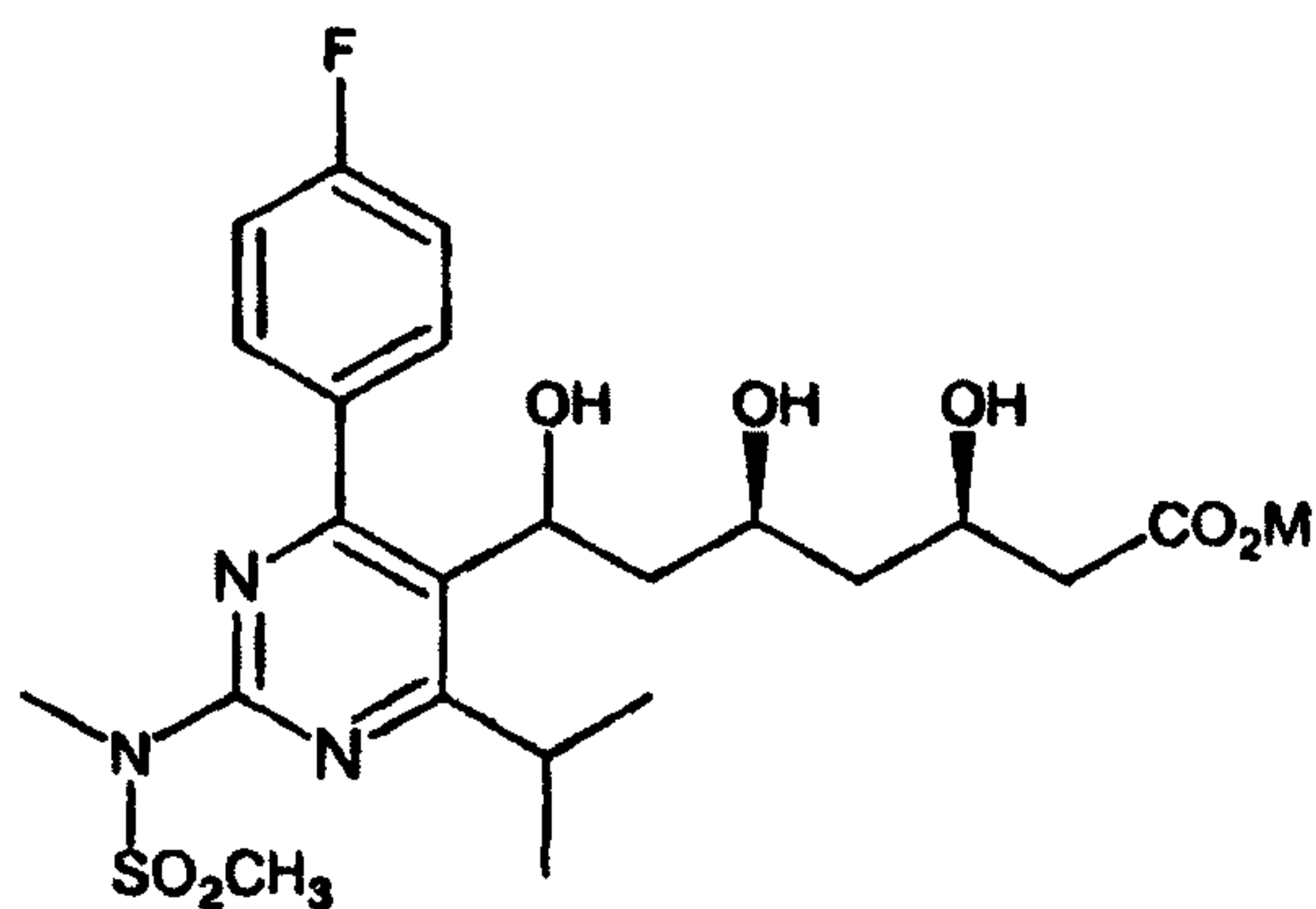


comprising hydrolyzing a lactone having the following structure

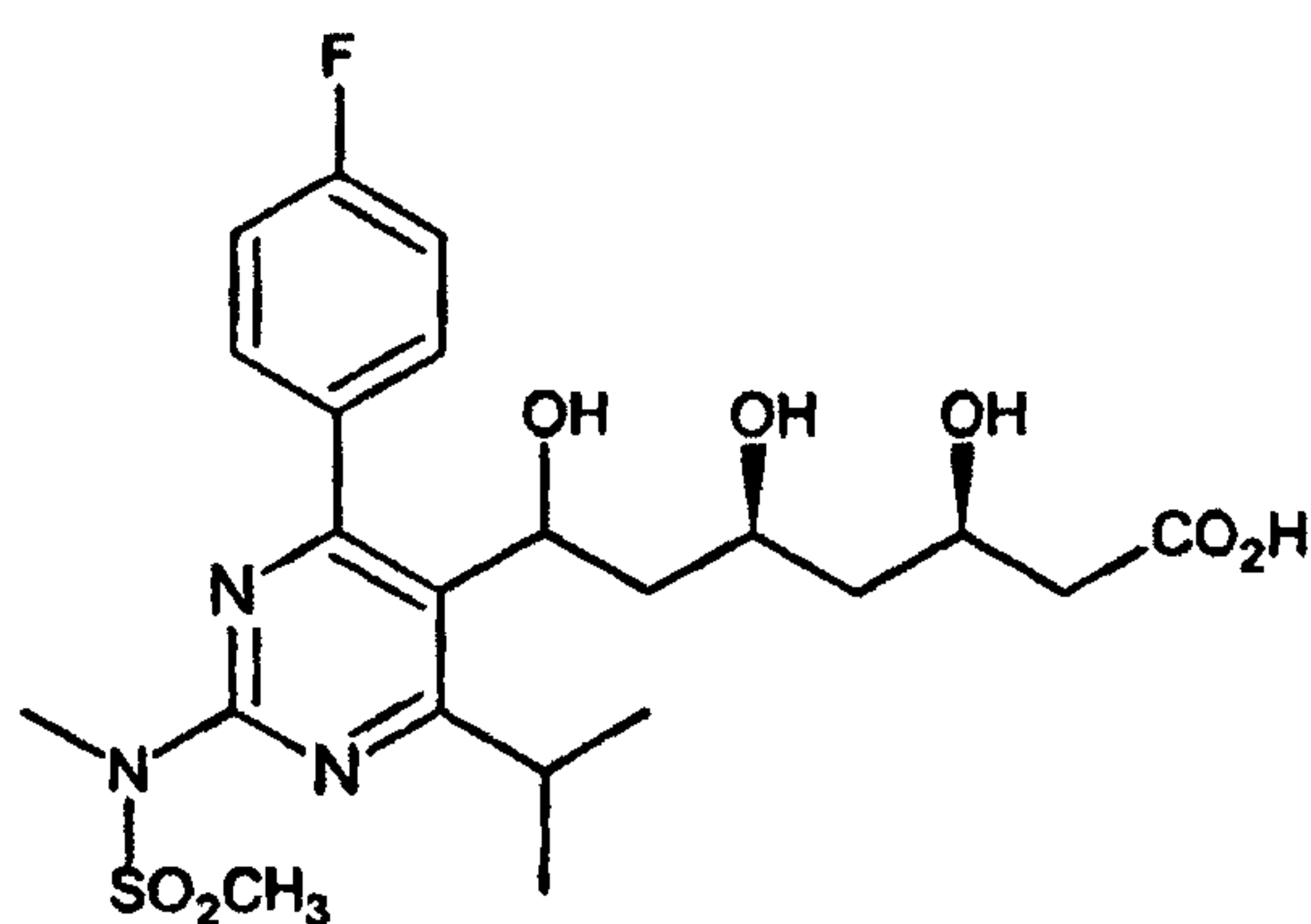


and converting the hydrolyzed lactone to the salt, wherein M is an alkali metal or an alkaline earth metal.

44. A process for preparing a rosuvastatin triol salt of any one of claims 11-15 with the following structure:



wherein M represents an alkali or alkaline earth metal cation, comprising contacting an acid with the following structure



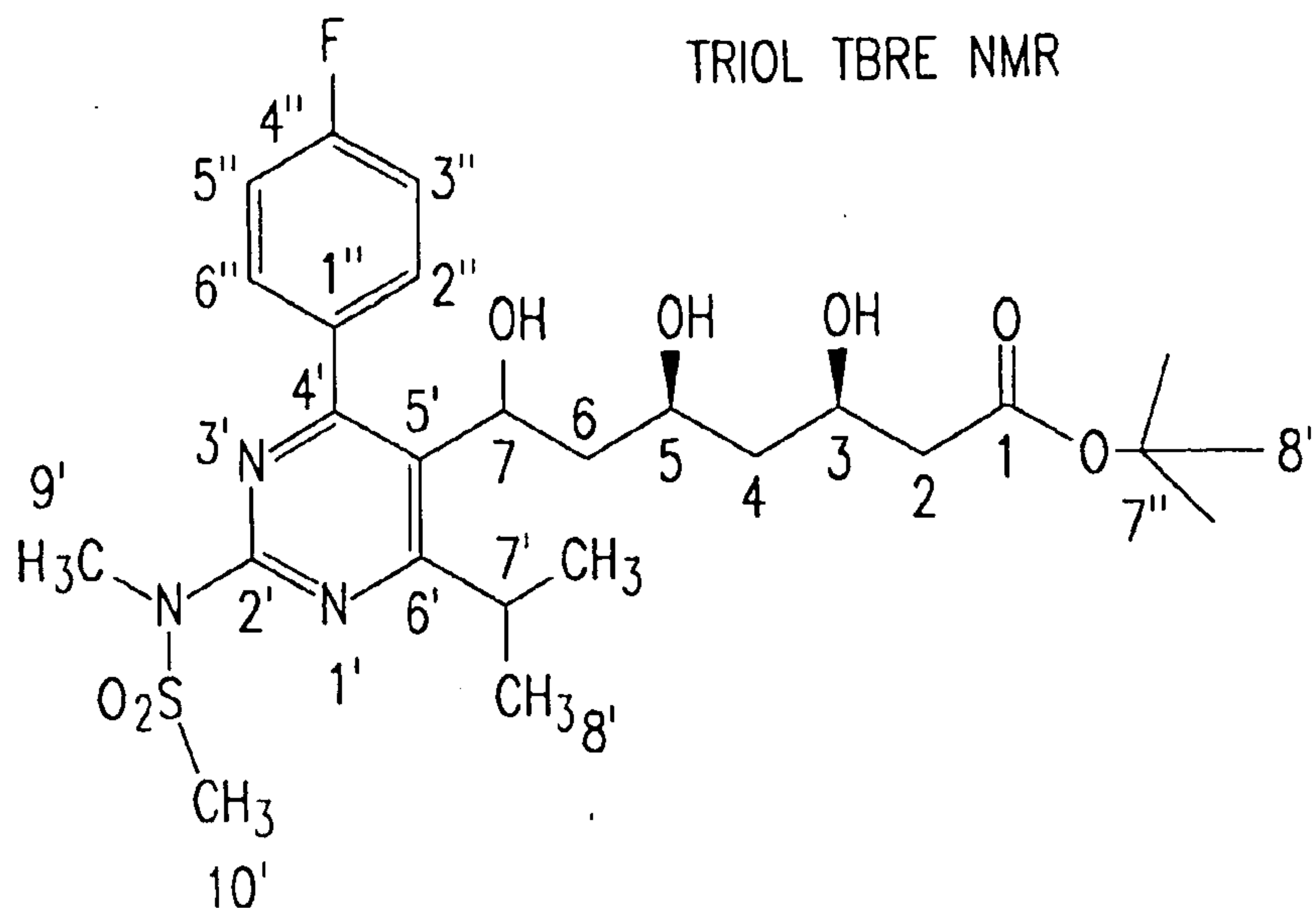
with a base.

45. A process for reducing the amount of impurities present in a pharmaceutical composition of rosuvastatin calcium comprising measuring the amount of rosuvastatin triol calcium in batches of rosuvastatin diol calcium, selecting batches of the rosuvastatin diol calcium with desirable level of the rosuvastatin triol calcium and preparing pharmaceutical compositions with the selected rosuvastatin diol batch.
46. A process for reducing the amount of rosuvastatin triol ester present in a mixture comprising rosuvastatin diol ester and rosuvastatin triol ester comprising measuring the amount of rosuvastatin triol C₁-C₄ ester in batches of rosuvastatin diol C₁-C₄ ester, selecting batches of the rosuvastatin diol C₁-C₄ ester with a desirable level of the triol C₁-C₄ ester and preparing pharmaceutical compositions of rosuvastatin diol calcium with the selected rosuvastatin diol C₁-C₄ ester batch.

47. A method of determining the amount of an impurity in a sample of rosuvastatin diol ester comprising measuring by GC or HPLC the area under a peak corresponding to rosuvastatin triol ester in a reference standard that comprises a known amount of rosuvastatin triol ester; measuring by HPLC or GC the area under a peak corresponding to rosuvastatin triol ester in a sample comprising rosuvastatin triol and rosuvastatin diol esters; and determining the amount of the triol ester in the sample by comparing the area of reference standard with that of the test sample.
48. The method of claim 47, wherein the triol ester is a t-butyl ester.
49. A method of determining the amount of an impurity in a sample of rosuvastatin calcium comprising measuring by GC or HPLC the area under a peak corresponding to rosuvastatin triol calcium in a reference standard comprising a known amount of rosuvastatin triol calcium; measuring by GC or HPLC the area under a peak corresponding to rosuvastatin triol calcium in a sample comprising rosuvastatin triol and rosuvastatin diol calcium salts; and determining the amount of the triol calcium in the sample by comparing the area of reference standard with that of the test sample.
50. A method of identifying the relative retention time (RRT) of an impurity in a sample of rosuvastatin diol ester comprising measuring by GC or HPLC the relative retention time (RRT) corresponding to rosuvastatin triol ester in a reference marker sample; carrying out GC or HPLC with a test sample comprising the rosuvastatin diol ester and rosuvastatin triol ester to obtain an GC or HPLC chromatogram with retention times; and identifying the relative retention time (RRT) of the triol ester in the sample by comparing the relative retention time (RRT) of the reference marker to the relative retention time (RRT) of the test sample.
51. The method of claim 50, wherein the triol ester is a t-butyl ester.
52. A method of identifying the relative retention time (RRT) of an impurity in a sample of rosuvastatin diol calcium comprising measuring by GC or HPLC the relative retention time (RRT) corresponding to rosuvastatin triol calcium in a reference marker sample; carrying out GC or HPLC with a test sample comprising the rosuvastatin diol and

rosuvastatin triol calcium salts to obtain an HPLC chromatogram with retention times; and identifying the relative retention time (RRT) of the triol calcium in the sample by comparing the relative retention time (RRT) of the reference marker to the relative retention time (RRT) of the test sample.

53. Use of a compound according to any one of Claims 1-19 as a reference standard or reference marker for determining the purity of rosuvastatin acid, rosuvastatin ester, rosuvastatin salt, and rosuvastatin lactone.
54. The use, in claim 53, wherein the rosuvastatin salt is the calcium salt.



NUMBER ATOM	¹ H NMR (CDCl ₃)	¹³ C NMR (CDCl ₃)	
	δ	δ	J(Hz)
1		172.17	
2	2.33	42.22	
3	4.19	69.35	
4	1.58, 1.34	40.93	
5	4.21	70.41	
6	2.29, 1.58	42.84	
7	5.31	66.12	
2'		157.23	
4'		165.06	
5'		125.07	
6'		178.04	
7'	3.87	32.42	
8'	1.33	22.29, 22.79	
9'	3.53	33.08	
10'	3.50	42.51	
1''		134.80	3
2'',6''	7.52	131.7	8
3'',5''	7.13	115.24	21
4''		163.24	249
7''		81.95	
8''	1.46	28.09	

FIG. 1

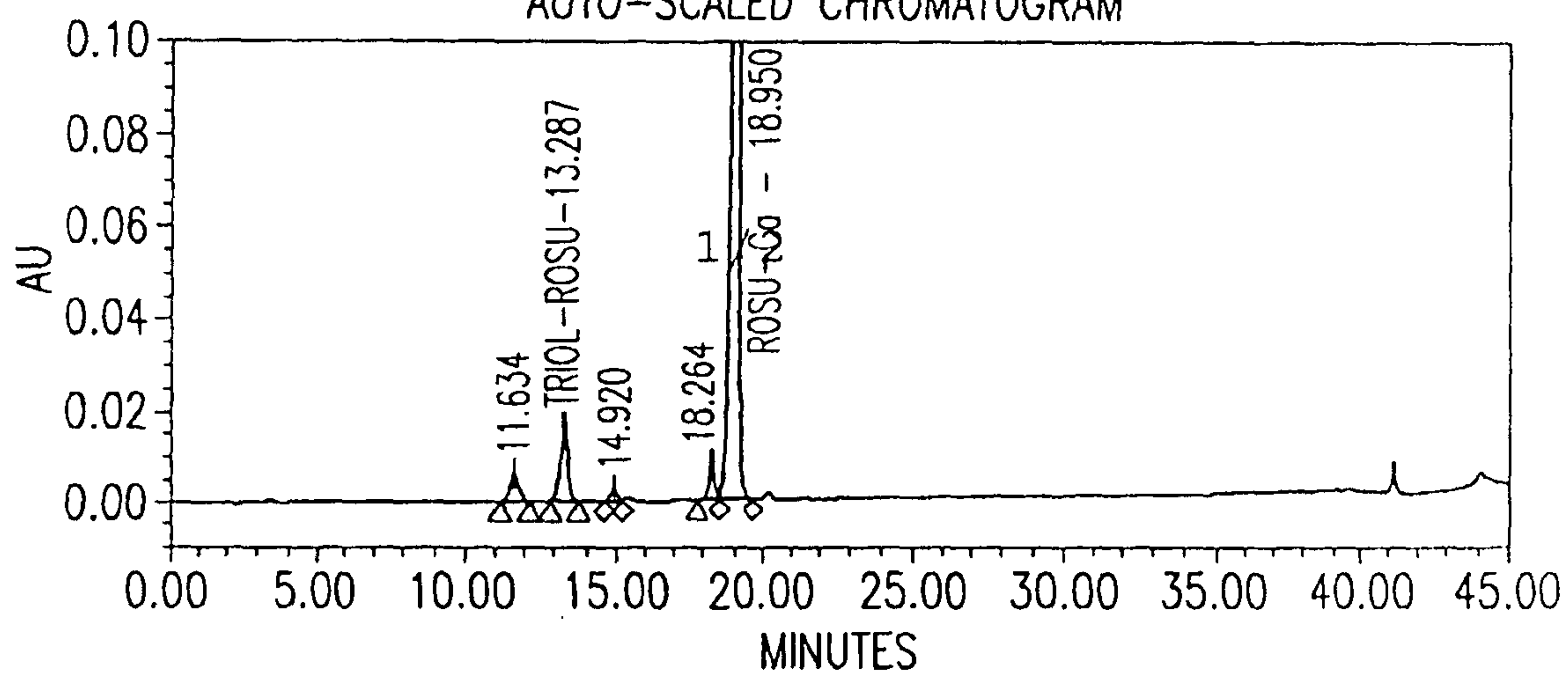
CHROMATOGRAM ILLUSTRATING USE OF ROSUVASTATIN TRIOL
CALCIUM AS A REFERENCE STANDARD (INCLUDING REFERENCE MARKER)

SAMPLE INFORMATION

INJECTION VOLUME: 10.00 μ l
RUN TIME: 45.0 MINUTES

LABEL

AUTO-SCALED CHROMATOGRAM



PEAK RESULTS

	NAME	RT	HEIGHT	AREA	% AREA	USP RESOLUTION
1		11.634	6168	114647	1.20	
2	TRIOL-ROSU	13.287	16643	261465	2.73	3.53
3		14.920	2613	37864	0.40	
4		18.264	8363	113278	1.18	
5	ROSU-Co	18.950	749895	9040213	94.49	2.07

FIG.2

