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(71) Applicant (for all designated States except US): **F. HOFFMANN-LA ROCHE AG** [CH/CH]; Grenzacherstrasse 124, CH-4070 Basel (CH).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **TRUSSARDI, René** [CH/CH]; Salinenstrasse 2a, CH-4127 Birsfelden (CH).

(74) Agent: **KJELLSAA-BERGER, Hanny**; Grenzacherstrasse 124, CH-4070 Basel (CH).

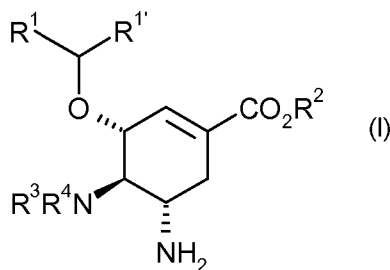
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(54) Title: PROCESS FROM SHIKIMIC ACID TO OSELTAMIVIR PHOSPHATE



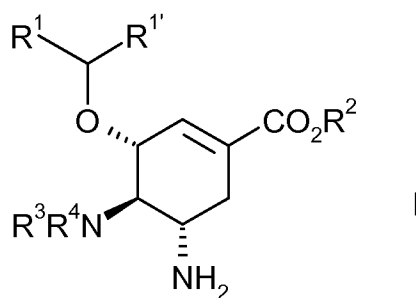
(57) Abstract: The present invention relates to a process for the preparation of a 4,5-diamino shikimic acid derivative of formula I and pharmaceutically acceptable addition salts thereof wherein R^1 , $R^{1'}$ are independent of each other H or alkyl, R^2 is an alkyl and R^3 , R^4 are independent of each other H or an alkanoyl, with the proviso that not both R^3 and R^4 are H. 4,5-diamino shikimic acid derivatives of formula I, especially the (3R,4R,5S)-5- amino-4-acetylamino-3-(1-ethyl-propoxy)-cyclohex-1-ene-carboxylic acid ethyl ester and its pharmaceutically acceptable additional salts are potent inhibitors of viral neuraminidase.



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Case 24218**Process from Shikimic acid to Oseltamivir Phosphate**

The present invention relates to a process for the preparation of a 4,5-diamino shikimic acid derivative of formula



and pharmaceutically acceptable addition salts thereof

5 wherein

R^1 , $R^{1'}$ are independent of each other H or alkyl,

R^2 is an alkyl and

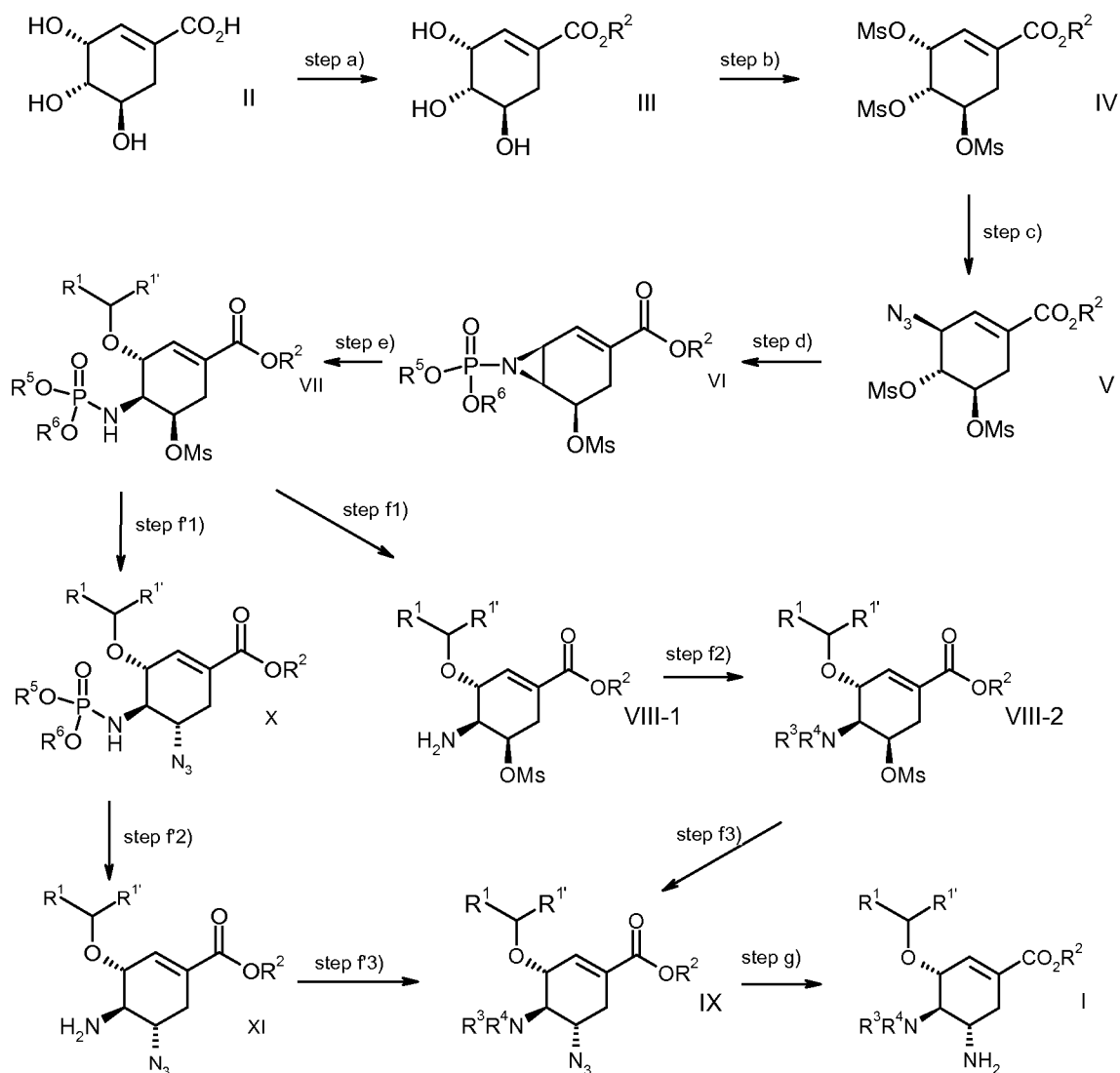
R^3 , R^4 are independent of each other H or an alkanoyl, with the proviso that not both R^3 and R^4 are H.

10 4,5-diamino shikimic acid derivatives of formula I, especially the (3R,4R,5S)-5-amino-4-acetylamino-3-(1-ethyl-propoxy)-cyclohex-1-ene-carboxylic acid ethyl ester and its pharmaceutically acceptable additional salts are potent inhibitors of viral neuraminidase (J. C. Rohloff et al., J. Org. Chem. 63, 1998, 4545-4550; WO 98/07685).

15 The problem at the root of the present invention is to provide a new process for preparing 4,5-diamino shikimic acid derivatives in good quality and yield from a easily obtainable starting material, shikimic acid. Shikimic acid can be easily obtained from biotech processes, e.g. genetic engineering, fermentation (Sunil S. Chandran, Jian Yi, K.M. Draths, Ralph von Daeniken, Wolfgang Weber, and J.W. Frost, Biotechnologie Progress, Vol. 19, No. 3, 2003, 808-814).

20 The problem is solved, according to the present invention, by a process for preparing the compounds of formula I as show in scheme 1:

Scheme 1



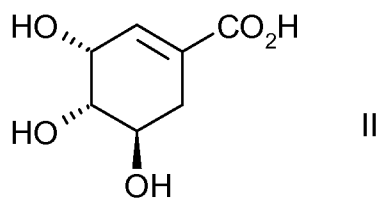
This new process has the advantage that it comprises less steps to reach 4,5-diamino shikimic acid derivatives of formula I comparing with the process known from the art (J.C.Rohloff et al., J.Org.Chem. 63, 1998, 4545-4550; WO 98/07685). In addition the combination of process steps without the isolation and purification of the corresponding intermediates described in Scheme 1 allows to further enhance the efficiency and overall yield of the process

The new process characterized by

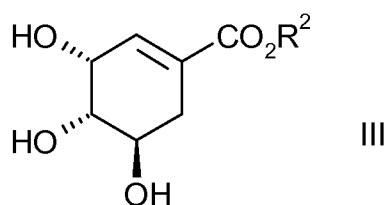
step a)

esterification of shikimic acid of formula

- 3 -



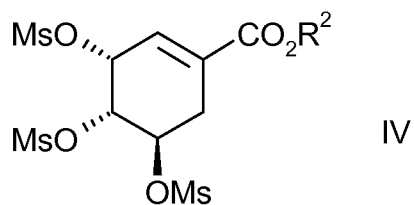
with R^2OH to form a compound of formula



wherein R^2 is as defined above;

5 step b)

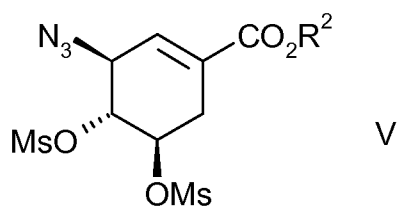
formulation of trimesylate of formula IV by reacting compound of formula III with methanesulfonyl chloride



wherein R^2 is as defined above,

10 step c)

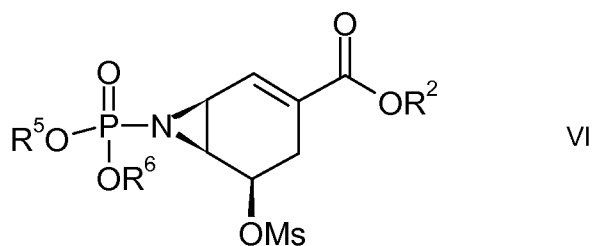
Regioselective S_N2 -substitution of the 3-mesylate-group of the trimesylate IV by an azide reagent to form an azide of formula



wherein R^2 is as defined above,

15 step d)

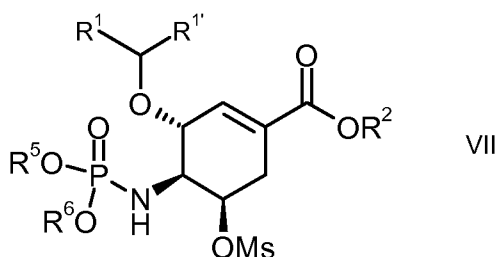
Reduction of the azide of formula V with trialkylphosphite to form aziridine of formula



5 wherein R^2 is as defined above, R^5 and R^6 , independently of each other are C_{1-6} alkyl,

step e)

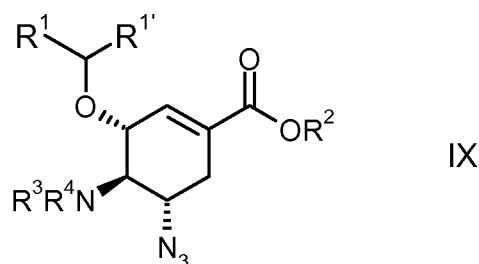
opening of the aziridine of formula VI to form compound of formula



wherein R^1 , $R^{1'}$, R^2 , R^5 and R^6 are as defined above,

10 step f)

transformation of phosphoramidate of formula VII into compound of formula



wherein R^1 , $R^{1'}$, R^2 , R^3 and R^4 are as defined above,

step g)

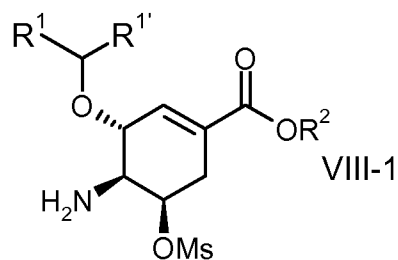
15 reduction of azide of formula IX to form compound of formula I and if necessary, formation of a pharmaceutically acceptable addition salt.

There are two alternative ways for perform step f)

1. step f) comprises the steps of f1), f2) and f3:

step f1)

hydrolysis of phosphoramidate of formula VII to form compound of formula

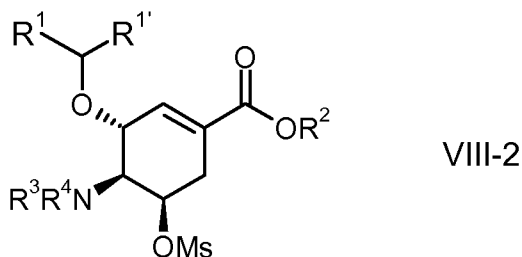


5

wherein R^1 , $R^{1'}$ and R^2 are as defined above,

step f2)

acylation of compound of formula VIII-1 for form compound of formula

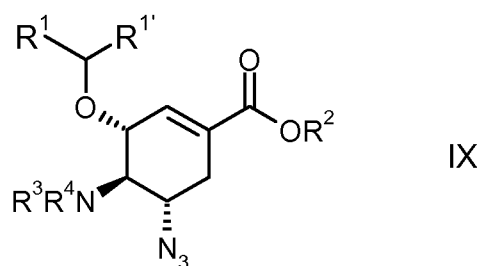


10

wherein R^1 , $R^{1'}$, R^2 , R^3 and R^4 are as defined above,

step f3)

substitution of 5-mesylate-group of compound of formula VIII-2 to an azido to form the compound of formula



15

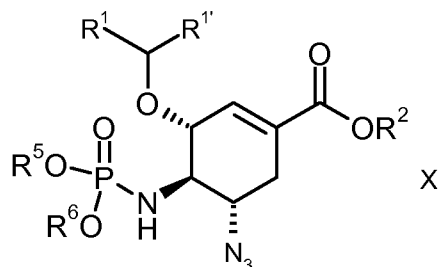
wherein R^1 , $R^{1'}$, R^2 , R^3 and R^4 are as defined above,

or alternatively

2. 1. step f) comprises the steps of f1), f2) and f3):

step f1)

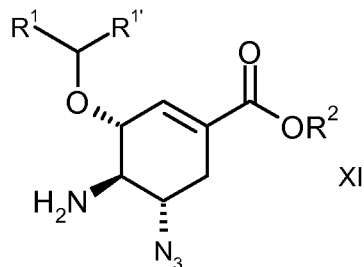
substitution of 5-mesylate-group of compound of formula VII to form the
5 compound of formula



wherein R¹, R^{1'}, R², R³ and R⁴ are as defined above,

step f2)

hydrolysis of compound of formula X to form compound of formula

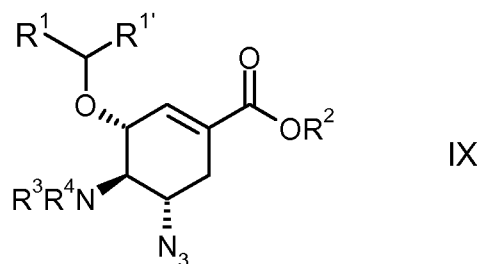


10

wherein R¹, R^{1'} and R² are as defined above,

step f3)

acylation of compound of formula XI to form compound of formula



wherein R^1 , $R^{1'}$, R^2 , R^3 and R^4 are as defined above,

The term alkyl has the meaning of a straight chained or branched alkyl group of 1 to 12 C-atoms, expediently of 1 to 6 C-atoms. Examples of such alkyl groups are methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, tert. butyl, pentyl and its isomers, hexyl and its isomers, heptyl and its isomers, octyl and its isomers, nonyl and its isomers, decyl and its isomers, undecyl and its isomers and dodecyl and its isomers.

This alkyl group can be substituted with one or more substituents as defined in e.g. WO 98/07685. Suitable substituents are C_{1-6} -alkyl (as defined above), C_{1-6} -alkenyl, C_{3-6} -cycloalkyl, hydroxy, C_{1-6} -alkoxy, C_{1-6} -alkoxycarbonyl, F, Cl, Br and I.

10 The term alkyl in R^1 , $R^{1'}$ has the meaning of a straight chained or branched alkyl group of 1 to 12 C-atoms, expediently of 1 to 6 C-atoms.

Preferred meaning for R^1 is ethyl, for $R^{1'}$ is ethyl.

R^2 is a straight chained or branched alkyl group of 1 to 12 C-atoms, expediently of 1 to 6 C-atoms as exemplified above.

15 Preferred meaning for R^2 is ethyl.

R^3 and R^4 have the meaning of alkanoyl groups, more preferably C_{1-6} -alkanoyl such as hexanoyl, pentanoyl, butanoyl (butyryl), propanoyl (propionyl), ethanoyl (acetyl) and methanoyl (formyl).

Preferred meaning for R^3 is acetyl and for R^4 is H.

20 R^5 and R^6 are straight chained or branched alkyl group of 1 to 12 C-atoms, expediently of 1 to 6 C-atoms as exemplified above.

Preferred meaning for R^5 and R^6 are are ethyl.

25 The term "pharmaceutically acceptable acid addition salts" embraces salts with inorganic and organic acids, such as hydrochloric acid, hydrobromic acid, nitric acid, sulfuric acid, phosphoric acid, citric acid, formic acid, fumaric acid, maleic acid, acetic acid, succinic acid, tartaric acid, methanesulfonic acid, p-toluenesulfonic acid, benzenesulfonic acid and the like.

The salt formation is effected with methods which are known per se and which are familiar to any person skilled in the art. Not only salts with inorganic acids, but also salts

with organic acids come into consideration. Hydrochlorides, hydrobromides, sulfates, nitrates, citrates, acetates, maleates, succinates, methan-sulfonates, p-toluenesulfonates and the like are examples of such salts.

- Preferred pharmaceutically acceptable acid addition salt is the 1:1 salt with
5 phosphoric acid which can be formed preferably in ethanolic solution at a temperature of -20°C to 60°C .

Step a)

Step a) comprises esterification of (3R,4S,5R)-3,4,5-trihydroxy-cyclohex-1-enecarboxylic acid of formula II with an alcohol of formula R^2OH .

- 10 Typically, the reaction is performed in an alcohol, preferably ethanol in the presence of a strong acid, such as hydrogen chloride in ethanol, sulfuric acid or in situ preparation of hydrogen chloride with thionyl chloride in ethanol, preferably thionyl chloride in ethanol.

- The reaction temperature mainly depends on the alcohol used, as a rule lies in the
15 range of 60°C to 150°C , preferably 70°C to 100°C .

The reaction is as a rule finished after 1 to 5 hours, preferably 2 to 3 hours.

Step b)

Step b) comprises formulation of trimesylate of formula IV by reacting compound of formula III with methanesulfonyl chloride.

- 20 Typically the reaction is performed in a suspension of compound of formula III, an aprotic organic solvent, preferably ethylacetat, and methanesulfonyl chloride with a tertiary organic base, preferably triethylamine.

The reaction temperature is typically in the range of -20°C to 50°C , preferably 0°C to 5°C .

- 25 Step c)

Step c) comprises regioselective $\text{S}_{\text{N}}2$ -substitution of the 3-mesylate-group of the trimesylate IV by an azide reagent to form an azide of formula V.

The reaction of step c) is typically performed in an inert organic solvent, such as dimethyl sulfoxide or with a water organic solvent mixture, preferably ethylacetate and with a phase transfer catalyst, such as tetrabutylammonium hydrogen sulfate.

5 An azide reagent, preferably sodium azide is added to the reaction mixture at room temperature.

The reaction is as a rule finished after 1 to 30 hours.

Step d)

Step d) comprises reduction of the azide of formula V with trialkylphosphite to form aziridine of formula VI.

10 The reaction of step d) is typically performed in an organic solvent, such as toluene in an inert gas atmosphere. Trialkylphosphite, such as triethylphosphite is added at room temperature.

The reaction is as a rule finished after 1 to 5 hours, preferably 2 to 3 hours.

15 The reaction temperature mainly depends on the organic solvent used, as a rule lies in the range of 50°C to 150°C, preferably 100°C to 120°C.

Step e)

Step e) comprises the opening of the aziridine of formula VI to form compound of formula VII.

20 Typically the reaction is performed in a suspension of compound of formula VI, an alcohol, such as 3-pentanol and boron trifluoride ethyl etherate in an inert gas atmosphere.

The reaction temperature is typically in the range of -20°C to 100°C, preferably 0°C to 5°C.

The reaction is as a rule finished after 10 to 20 hours, preferably 16 hours.

25 Step f)

Step f) can be performed in two alternative ways:

(1) step f) comprises steps f1), f2) and f3).

Step f1) comprises substitution of mesylate-group of compound of formula VII to form the compound of formula VIII-1.

Typically the reaction is performed in a suspension of compound of formula VII, an alcohol, such as ethanol and a strong acid, such as hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, methanesulfonic acid and the like, preferably sulfuric acid in an inert gas atmosphere.

The reaction temperature mainly depends on the alcohol used, as a rule lies in the range of 50°C to 150°C, preferably 80°C to 120°C.

The reaction is as a rule finished after 10 to 20 hours, preferably 16 hours.

Step f2) comprises acetylation of compound of formula VIII-1 for form compound of formula VIII-2.

Acylation can be effected under basic conditions by using acylating agents known to the skilled in the art. Acylating agent can be an acyl halide, a carboxylic acid ester or a carboxylic acid anhydride. Suitable acylating agent preferably is an acetylating agent such as acetylchloride or acetic anhydride. Suitable organic tertiary base such as triethylamine or an aqueous solution with an anorganic base such sodium hydrogen carbonate, potassium carbonate, potassium phosphate in water preferably sodium hydrogen carbonate in water.

Preferably the acylation takes place under basic conditions using a mixture of 0.5 to 2.0 equivalents of acetic anhydride in ethyl acetate.

The temperature is as a rule chosen in the range of -20°C to 100°C.

Step f3) comprises substitution of mesylate-group of compound of formula VIII to an azido to form the compound of formula IX.

The reaction of step f3) is typically performed in an inert organic solvent, such as dimethyl sulfoxide or a solvent mixture with dimethyl sulfoxide and ethanol.

An azide reagent, such as sodium azide is added to the reaction mixture at 20°C to 120°C, preferably at 80°C to 100°C

The reaction is as a rule finished after 10 to 30 hours, preferably 16 hours.

(2) step f) comprises steps f1), f2) and f3).

Step f1) comprises substitution of mesylate-group of compound of formula VII to form the compound of formula X.

The reaction of step f1) is typically performed in an inert organic solvent, such as dimethyl sulfoxide or an solvent mixture with dimethyl sulfoxide and ethanol.

- 5 An azide reagent, such as sodium azide is added to the reaction mixture at 20°C to 120°C, preferably at 80°C to 100°C.

The reaction is as a rule finished after 10 to 30 hours, preferably 20 hours.

Step f2) comprises hydrolysis of compound of formula X to form compound of formula XI.

- 10 Typically the reaction is performed in a suspension of compound of formula X, an alcohol, such as ethanol and a strong acid, such as hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, methanesulfonic acid and the like, preferably sulfuric acid in an inert gas atmosphere.

- 15 The reaction temperature mainly depends on the alcohol used, as a rule lies in the range of 20°C to 150°C, preferably 80°C to 120°C.

The reaction is as a rule finished after 10 to 20 hours, preferably 8 hours at 80°C.

Step f3) comprises acetylation of compound of formula XI to form compound of formula IX.

- 20 Acylation can be effected under basic conditions by using acylating agents known to the skilled in the art. Acylating agent can be an acyl halide, a carboxylic acid ester or a carboxylic acid anhydride. Suitable acylating agent preferably is an acetylating agent such as acetylchloride or acetic anhydride. Suitably organic tertiary base such as triethylamine or an aqueous solution with an anorganic base such as sodium hydrogen carbonate, potassium carbonate, potassium phosphate in water, preferably sodium hydrogen
25 carbonate in water.

Preferably the acylation takes place under basic conditions using a mixture of 0.5 to 2.0 equivalents of acetic anhydride in ethyl acetate.

The temperature is as a rule chosen in the range of -20°C to 100°C, preferably 0 °C to 5 °C.

Step g)

Step g) comprises reduction of azide of formula IX to form compound of formula I and if necessary, formation of a pharmaceutically acceptable addition salt.

Typically the reduction is performed in a polar protic solvent such as alcohols, preferably
5 in aqueous ethanol or aqueous tetrahydrofuran, most preferably in aqueous ethanol.

The reaction temperature mainly depends on the phosphine used but as a rule lies in the range of -20°C to 30°C, preferably between 0 and 25°C.

It can be favorable to perform the reaction at two temperature levels, thereby having the lower temperature range given above for the addition of the phosphine and
10 thereafter having a slightly higher temperature of up to room temperature to bring the reaction to completion.

As a specific embodiment of the present invention it was found that catalytic amounts of an acid present during the conversion suppresses the ester hydrolysis which otherwise takes place to a small extent of some percent and thereby leads to an
15 undesirable impurity.

Suitable acid is a carboxylic acid, expediently acetic acid. The acetic acid is usually added in the form of glacial acetic acid in catalytic quantities of 0.5 mol % to 3.0 mol % relating to the 4-acetylamino-5-azido-shikimic acid derivative of formula (IX).

The process is as a rule finished after 3 h to 6 h.

20 Thereafter work up of the reaction mixture can happen by applying methods known to the skilled in the art. Expediently the reaction mixture is, preferably after stabilization with ≤ 5 mol% acetic acid, concentrated in vacuo.

Though the 4,5-diamino shikimic acid derivative can be isolated e.g. by evaporation and crystallization, it is preferably kept in e.g. an ethanolic solution and then further
25 transformed into the pharmaceutically acceptable addition salt following the methods described in J.C.Rohloff et al., J.Org.Chem. 63, 1998, 4545-4550; WO 98/07685).

The salt formation is effected with methods which are known per se and which are familiar to any person skilled in the art. Not only salts with inorganic acids, but also salts with organic acids come into consideration. Hydrochlorides, hydrobromides, sulphates,

nitrate, citrate, acetate, maleate, succinate, methanesulphonate, p-toluenesulphonate and the like are examples of such salts.

Preferred pharmaceutically acceptable acid addition salt is the 1:1 salt with phosphoric acid which can be formed preferably in ethanolic solution at a temperature of
5 -20°C to 60°C.

The following examples shall illustrate the invention in more detail without limiting it.

Example 1

10 (a) Preparation of (3R,4S,5R)-3,4,5-Trihydroxy-cyclohex-1-enecarboxylic acid ethyl ester (iii)

In a 250 ml four necked round bottom flask equipped with a reflux condenser, magnetic stirrer and an inert gas supply 19.90 g (3R,4S,5R)-3,4,5-Trihydroxy-cyclohex-1-enecarboxylic acid (ii) were suspended in 80 ml ethanol, 4.16 ml thionyl chloride were added in a period of 10 min. The reaction mixture was heated to reflux for 2 hours, the
15 brown solution was cooled to room temperature and evaporated at 40°C/200-10 mbar to yield the crude product 24.39 g as dark brown oil which crystallized over night. The crude product was dissolved in 100 ml ethyl acetate and evaporated at 40°C/200-10 mbar to yield the product 22.56 g as a brown residue.

IR (ATR) 3350, 2982, 1715, 1252, 1097 cm^{-1} ; MS (turbo spray) MNH_4^+ 220.1

20 (b) Preparation of (3R,4S,5R)-3,4,5-Tris-methanesulfonyloxy-cyclohex-1-enecarboxylic acid ethyl ester (iv)

In a 1000 ml two necked round bottom flask equipped with, a thermometer, a mechanic stirrer and an inert gas supply 20.22 g (3R,4S,5R)-3,4,5-Trihydroxy-cyclohex-1-enecarboxylic acid ethyl ester (iii) was suspended in 600 ml ethyl acetate, cooled to 0-
25 5°C, 24.0 ml methanesulfonyl chloride were added. To the dark brown solution 45.0 ml triethylamine were added in a period of 46 min, where by the temperature was kept in the range of 0-5°C. The brown suspension was stirred for 30 min at 0-5°C, filtered over a pre cooled (0-5°C) glass frit filter, the filter cake was washed with total 200 ml ethyl acetate, the combined filtrates were extracted with 400 ml 0.5M H_2SO_4 , the separated organic
30 phase was dried with 100 g anhydrous sodium sulfate, filtered, washed with 200 ml ethyl

acetate and evaporated in a rotary evaporator at 40°C/200-10 mbar to yield the product 42.24 g as a brown resin

IR (ATR) 2943, 1715, 1332, 1251, 1170 cm^{-1} ; MS (ion spray): 454.2 $\text{M}+\text{NH}_4^+$,

5 (c) Preparation of (3R,4S,5R)-3-Azido-4,5-bis-methanesulfonyloxy-cyclohex-1-enecarboxylic acid ethyl ester (v)

In a 5 ml round bottom flask equipped with a magnetic stirrer and an inert gas supply, 436.3 mg (3R,4S,5R)-3,4,5-Tris-methanesulfonyloxy-cyclohex-1-enecarboxylic acid ethyl ester (iv) were dissolved in 0.83 ml dimethyl sulfoxide. To the clear colorless solution 71.5 mg sodium azide were added at r.t., the reaction mixture was stirred for 2.5
10 hour, then 13.7 mg sodium azide were added at r.t. and stirred for another 19 hour at r.t. The reaction mixture was diluted with 5 ml ethyl acetate and extracted three times with a 1M solution of NaHCO_3 . The organic phase was separated dried with anhydrous Na_2SO_4 , filtered and evaporated at 20°C/140-10 mbar to obtain 0.33 g of the crude product as light yellow oil. Purification of the crude product was obtained via a silica column
15 chromatography using a 1/2 mixture of ethyl acetate and n-hexane. The combined fractions were evaporated and dried on a rotary evaporator to obtain 0.27 g (3R,4S,5R)-3-Azido-4,5-bis-methanesulfonyloxy-cyclohex-1-enecarboxylic acid ethyl ester as a colorless oil

IR (ATR) 2942, 2105, 1713, 1660, 1350, 1171 cm^{-1} . MS (turbo spray) $\text{M}+\text{NH}_4^+$
20 401.1

(d) Preparation of (1S,5R,6S)-7-(Diethoxy-phosphoryl)-5-methanesulfonyloxy-7-aza-bicyclo[4.1.0]hept-2-ene-3-carboxylic acid ethyl ester (vi)

In a 10 ml round bottom flask equipped with a magnetic stirrer, a reflux condenser and an inert gas supply, 383.4 mg (3R,4S,5R)-3-Azido-4,5-bis-methanesulfonyloxy-cyclohex-1-enecarboxylic acid ethyl ester (v) were dissolved under argon with stirring in
25 4.0 ml toluene. To the clear solution 191 μl triethyl phosphite were added at r.t.. After 5 minutes were nitrogen was evolved and after 30 minutes stirring at r.t. the light yellow reaction mixture was heated to reflux for 5 hours, then 191 μl triethyl phosphite was added and refluxed for another 2 hours. The reaction mixture was cooled and evaporated
30 at 40°C/60-10 mbar to yield 470 mg of the crude product as a yellow oil. Purification of the crude product was obtained via a silica column chromatography using a 2/1 mixture of toluene and acetone. The combined fractions were evaporated and dried on a rotary evaporator 40°C/250-10 mbar to obtain 87 mg (1S,5R,6S)-7-(Diethoxy-phosphoryl)-5-

methanesulfonyloxy-7-aza-bicyclo[4.1.0]hept-2-ene-3-carboxylic acid ethyl ester as a yellow oil.

IR (ATR) 2984, 1711, 1649, 1356, 1253, 1173, 1019 cm^{-1} . MS (ion spray) MH^+ 398.0

5 **(e) Preparation of (3R,4S,5R)-4-(Diethoxy-phosphorylamino)-3-(1-ethyl-propoxy)-5-methanesulfonyloxy-cyclohex-1-enecarboxylic acid ethyl ester (vii)**

In a 50 ml round bottom flask equipped with a magnetic stirrer and an inert gas supply, 1.94 g (1S,5R,6S)-7-(Diethoxy-phosphoryl)-5-methanesulfonyloxy-7-aza-bicyclo[4.1.0]hept-2-ene-3-carboxylic acid ethyl ester (vi) were dissolved under argon
10 with stirring in 20 ml 3-pentanol. The clear light yellow solution was treated at 0-5°C with 736 μl boron trifluoride ethyl etherate. The reaction mixture was stirred for 16 hours at r.t.. the reaction mixture was diluted with 50 ml ethyl acetate and extracted two times with 70 ml water. The organic phase was separated and dried with 25 g anhydrous Na_2SO_4 , filtered and evaporated in a rotary evaporator at 40°C/70-10 mbar to yield 2.18 g
15 of the crude product as a yellow oil. Purification of the crude product was obtained via a column chromatography using tert-butyl methyl ether. The combined fractions were evaporated and dried on a rotary evaporator at 40°C/250-10 mbar to obtain 1.47 g (3R,4S,5R)-4-(Diethoxy-phosphorylamino)-3-(1-ethyl-propoxy)-5-methanesulfonyloxy-cyclohex-1-enecarboxylic acid ethyl ester as a light yellow solid.

20 IR (ATR) 3236, 1711, 1246, 1024 cm^{-1} . MS (turbo spray) MH^+ 484.2

(f1) Preparation of (3R,4S,5R)-4-amino-3-(1-ethyl-propoxy)-5-methanesulfonyloxy-cyclohex-1-enecarboxylic acid ethyl ester (viii-1)

In a 5 ml round bottom flask equipped with a magnetic stirrer, a reflux condenser and an inert gas supply, 120 mg (3R,4S,5R)-4-(Diethoxy-phosphorylamino)-3-(1-ethyl-propoxy)-5-methanesulfonyloxy-cyclohex-1-enecarboxylic acid ethyl ester (vii) was
25 dissolved in 500 μl ethanol and treated with 120 μl sulphuric acid 96%, the reaction mixture was refluxed for 16 h, cooled to r.t. and diluted with 1.0 ml ethyl acetate and cooled to 05°C. The cooled solution was treated with 100 μl of a 28% aqueous sodium hydroxide solution and with 257 μl of a 25% aqueous ammonium hydroxide solution.
30 The well stirred mixture was diluted with 1.5 ml water, extracted and the organic phase was separated, dried over anhydrous Na_2SO_4 , filtered and evaporated at 20°C/120-10 mbar and dried for 1h at 20°C/<10 mbar to obtain 56 mg (3R,4S,5R)-4-amino-3-(1-

ethyl-propoxy)-5-methanesulfonyloxy-cyclohex-1-enecarboxylic acid ethyl ester as colorless oil.

IR (ATR) 1711, 1655, 1350, 1246, 1171 cm^{-1} . MS (ion spray) MH^+ 350.3

(f2) Preparation of (3R,4S,5R)-4-Acetylamino-3-(1-ethyl-propoxy)-5-methanesulfonyloxy-cyclohex-1-enecarboxylic acid ethyl ester (viii-2)

In a 10 ml round bottom flask equipped with a magnetic stirrer and an inert gas supply, 190 mg (3R,4S,5R)-4-amino-3-(1-ethyl-propoxy)-5-methanesulfonyloxy-cyclohex-1-enecarboxylic acid ethyl ester (vii-1) was dissolved in 1.6 ml ethyl acetate, 1.6 ml of a aqueous 1M sodium hydrogen carbonat solution was added, at room temperature 62 μl acetic anhydride was added, the 2-phase mixture was stirred for 1 hour at room temperature. The mixture was diluted with 2.0 ml ethyl acetate, the organic phase was separated, dried with anhydrous sodium sulfate, filtered and the filter cake was washed with total 3.0 ml ethyl acetate. The filtrate was evaporated at 40°C/200-10mbar to obtain 192 mg (3R,4S,5R)-4-Acetylamino-3-(1-ethyl-propoxy)-5-methanesulfonyloxy-cyclohex-1-enecarboxylic acid ethyl ester as off white crystals

IR (ATR) 3303, 1714, 1648, 1534, 1344, 1251, 1174, 1094, 903 cm^{-1} . MS (ion spray) MH^+ 392.0

(f3) Preparation of (3R,4R,5S)-4-Acetylamino-5-azido-3-(1-ethyl-propoxy)cyclohex-1-enecarboxylic acid ethyl ester (ix)

In a 5.0 ml round bottom flask equipped with a magnetic stirrer, a reflux condenser and an inert gas supply, 340 mg (3R,4S,5R)-4-Acetylamino-3-(1-ethyl-propoxy)-5-methanesulfonyloxy-cyclohex-1-enecarboxylic acid ethyl ester (viii-2) was dissolved in 1.2 ml dimethyl sulfoxide and 1.2 ml ethanol, 113 mg sodium azide was added and the reaction mixture was stirred for 21 hours at 85-90°C. The brown reaction mixture was cooled to r.t., treated with 7.0 ml of a aqueous 1M sodium hydrogen carbonate solution and extracted with 7.0 ml tert-butyl methyl ether. The organic phase was dried with anhydrous Na_2SO_4 , filtered and evaporated at 40°C/250-10mbar to obtain 280 mg of the crude product as brown crystals. 280 mg of the crude product was dissolved in 3.0 ml tert-butyl methyl ether, the brown solution was stirred 16 hours at -20°C, were by a beige suspension was formed. The suspension was filtered over a pre cooled (-20°C) glass filter frite and washed with pre cooled (-20°C) 1.5 ml tert-butyl methyl ether. The white crystals were dried at 40°C/10mbar for 2 hour to obtain 60 mg (3R,4R,5S)-4-

Acetylamino-5-azido-3-(1-ethyl-propoxy)cyclohex-1-enecarboxylic acid ethyl ester as white crystals

IR (ATR) 3265, 2101, 1713, 1659, 1559, 1249, 1078 cm^{-1} . MS (ion spray) MH^+ 339.3

5 (f1) Preparation of (3R,4R,5S)-5-Azido-4-(diethoxy-phosphorylamino)-3-(1-ethyl-propoxy)-cyclohex-1-enecarboxylic acid ethyl ester (x)

In a 5.0 ml round bottom flask equipped with a magnetic stirrer, a reflux condenser and an inert gas supply, 104 mg of (3R,4S,5R)-4-(Diethoxy-phosphorylamino)-3-(1-ethyl-propoxy)-5-methanesulfonyloxy-cyclohex-1-enecarboxylic acid ethyl ester (vii) was dissolved in 0.50 ml ethanol and 0.50 ml dimethyl sulfoxide, 28 mg sodium azide was
10 added and the reaction mixture was stirred at 85-90°C for 20 hours. The brown reaction mixture was cooled to room temperature diluted with 1.0 ml ethyl acetate and extracted with 1.0 ml of a aqueous 1M sodium hydrogen carbonate solution. The separated organic phase was dried with anhydrous Na_2SO_4 , filtered and evaporated at 40°C/120-10mbar to obtain 40 mg of the crude product as brown crystals. 40 mg of the crude product was
15 dissolved in 0.40 ml methylcyclohexane, stirred for 5 hours at room temperature, filtered and washed with 0.15 ml methylcyclohexane, the white crystals were dried at 40°C/10mbar for 2 hours to obtain 14 mg (3R,4R,5S)-5-Azido-4-(diethoxy-phosphorylamino)-3-(1-ethyl-propoxy)-cyclohex-1-enecarboxylic acid ethyl ester as white crystals.

20 IR (ATR) 3205, 2099, 1717, 1660, 1246, 1224, 1025 cm^{-1} . MS (ion spray) $\text{M}+\text{OAc}^-$ 491.3

(f2) Preparation of (3R,4R,5S)-4-Amino-5-azido-3-(1-ethyl-propoxy)-cyclohex-1-enecarboxylic acid ethyl ester (xi)

In a 5.0 ml round bottom flask equipped with a magnetic stirrer, a reflux condenser
25 and an inert gas supply, 300 mg of (3R,4R,5S)-5-Azido-4-(diethoxy-phosphorylamino)-3-(1-ethyl-propoxy)-cyclohex-1-enecarboxylic acid ethyl ester (x) was dissolved in 1.25 ml ethanol and treated with 0.31 ml sulfuric acid 96%. The black solution was stirred for 18 hours at room temperature and 8 hours at 80°C. The black reaction mixture was cooled to room temperature treated with 8.0 ml of a mixture 65 ml ammonium
30 hydroxide 25% in 435 ml brine, and extracted with 9.0 ml ethyl acetate. The separated organic phase was washed with 10 ml water and the water phase was washed back with 9.0 ml ethyl acetate. The comined organic phases were dried with 20 g anhydrous Na_2SO_4 , filtered and evaporated at 40°C/140-10mbar to obtain 175 mg crude product as a dark

brown oil. Purification of the crude product was obtained via a silica column chromatography using ethyl acetate with 1% v/v of aqueous ammonium hydroxide 25%. The combined fraction were evaporated at 40°C/140-10mbar to obtain 97 mg of (3R,4R,5S)-4-Amino-5-azido-3-(1-ethyl-propoxy)-cyclohex-1-enecarboxylic acid ethyl ester as a brown oil.

IR (ATR) 3393, 2098, 1713, 1241, 1070 cm^{-1} . MS (ion spray) MH^+ 297.2

(f3) Preparation of (3R,4R,5S)-4-Acetylamino-5-azido-3-(1-ethyl-propoxy)cyclohex-1-enecarboxylic acid ethyl ester (ix)

In a 5.0 ml round bottom flask equipped with a magnetic stirrer and an inert gas supply, 118.5 mg (3R,4R,5S)-4-Amino-5-azido-3-(1-ethyl-propoxy)-cyclohex-1-enecarboxylic acid ethyl ester (xi) was dissolved in 1.0 ml ethyl acetate. The brown solution was treated with 1.0 ml of a aqueous 1M sodium hydrogen carbonate solution. The mixture was treated with stirring with 45.4 μl acetic anhydride. The mixture was stirred for 1 hour at room temperature. The organic phase was separated and dried with 1g anhydrous Na_2SO_4 , filtered and the filter cake was washed total with 2.0 ml ethyl acetate. The combined filtrate was evaporated at 40°C/240 to 10 mbar to obtain 139 mg crude product as beige solide. The crude solide was suspended in 1.40 ml n-hexane and 0.70 ml tert-butyl methyl ether, stirred for one hour at room temperature, cooled to 0-5°C and stirred for 2 hours at 0-5°C. The suspension was filtered and the filter cake was washed with total 0.70 ml of a precooled (0-5°C) 2/1 solvent mixture of n-hexane and tert-buthyl methyl ether. The filter cake was dried at 45°C/9mbar to obtain 94 mg (3R,4R,5S)-4-Acetylamino-5-azido-3-(1-ethyl-propoxy)cyclohex-1-enecarboxylic acid ethyl ester as white crystals.

IR (NJT) 3269, 2104, 1715, 1660, 1562, 1254 cm^{-1} . MS (turbo spray) MH^+ 339.2.

(g) Preparation of (3R,4R,5S)-4-acetylamino-5-amino-3-(1-ethyl-propoxy)cyclohex-1-enecarboxylic acid ethyl ester (i)

50.0 g (0.147 mol) ethyl (3R, 4R, 5S)-4-acetamido-5-azido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate (ix) were placed in a nitrogen purged 1000 ml glass reactor fitted with a mechanical stirrer, a condenser, and a 250 ml dropping funnel. 300 ml ethanol, 50 ml water and 0.09 g acetic acid were added. To the resulting clear solution 31.4 g (0.155 mol) tributylphosphine dissolved in 150 ml ethanol were continuously added at a temperature of 5 °C (+/- 5°C) over a period of 30 - 90 min. Under slight cooling of the jacket (~ 3°C) the reaction temperature was kept at this temperature. The

feeder was rinsed with 20 ml ethanol. The clear reaction mixture was stirred for additional 90 min at 5 °C (+/-5°C) under slight jacket cooling. Subsequently the temperature was raised within 30 - 60 min to 20 - 25 °C and the solution was stirred for another 3h (nitrogen evolving).

- 5 After the reaction was finished (HPLC control) 0.18 g acetic acid were added to the clear solution. Then the mixture was concentrated under reduced pressure (300 to 50 mbar) at a maximum temperature of 60 °C and a maximum jacket temperature of 75 °C near to dryness. The oily residue (80 – 100 ml) was diluted with 160 ml ethanol, the resulting solution was then again concentrated following the method as mentioned above. The oily
10 residue was dissolved in ethanol up to a volume of 250 ml. The water content of this solution was determined by KF(Karl Fischer) titration of being less than 1.0 % wt. %. Yield: 44.4 g (97 % area by HPLC) of the title product in ethanolic solution.

IR (NJL) 3279, 1720, 1639, 1554, 1252, 1128 cm⁻¹. MS (ion spray) MH⁺ 313.1, MNa⁺ 335.3

- 15 (g1)Preparation of (3R,4R,5S)-4-acetylamino-5-amino-3-(1-ethylpropoxy)cyclohex-1-enecarboxylic acid ethyl ester (i) phosphate (1:1)

- In a dry and nitrogen purged 1000 ml glass reactor fitted with a mechanical stirrer, a condenser, and a 500 ml dropping funnel 17.0 g ortho phosphoric acid (85 % in water) were dissolved in 400 ml ethanol and the resulting clear solution was warmed to 50 - 55
20 °C. Subsequently the 250 ml ethanolic solution obtained from example 1 and containing 0.147 mol of ethyl (3R, 4R, 5S)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate were added under stirring. After fast addition (10-15 min) of two thirds (ca.160 ml) of the total volume of this solution the addition was stopped and the supersaturated clear solution was seeded with 0.2 g of previously obtained ethyl (3R,
25 4R, 5S)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate Phosphate (1:1). Immediately afterwards crystallization commenced. The resulting thick suspension was stirred for 45 - 60 min at 50 – 55 °C. Then the remaining amine solution was slowly added (45 - 60 min) to the suspension at 50 – 55 °C. The feeder was rinsed with 20 ml ethanol. Subsequently the thick suspension was continuously cooled to 12 -
30 20°C in about 4 h (cooling speed = 10 °C/h). To complete the crystallization stirring was continued at 12 – 20 °C for additional 2 ± 1 h. Ethyl (3R, 4R, 5S)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate Phosphate (1:1) was isolated by pressure filtration (0.3 bar nitrogen overpressure, Dacron[®] filter cloth). The crystalline

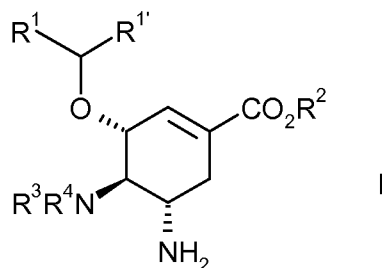
product was washed twice with 240 ml acetone and twice with 300 ml n-heptane at room temperature. Ethyl (3R, 4R, 5S)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate Phosphate (1:1) was dried in vacuo (≈ 20 mbar) at a maximum temperature of 50°C until constant weight.

- 5 Yield: 54 - 55 g (88-91%) of the title product in the form of colorless needles with an assay of = 99 wt. % (sum of impurities < 0.5 wt.%, single impurities $\leq$ 0.1 wt.%).

IR (NJT) 3352, 3164, 2923, 2854, 1724, 1663, 1551, 1463, 1263, 1132 cm^{-1} . MS (ion spray) MH^+ 313.1, MNa^+ 335.3.

Claims

1. Process for the preparation of a 4,5-diamino shikimic acid derivative of formula



and pharmaceutically acceptable addition salts thereof,

5 wherein

R^1 , $R^{1'}$ are independent of each other H or C_{1-6} alkyl,

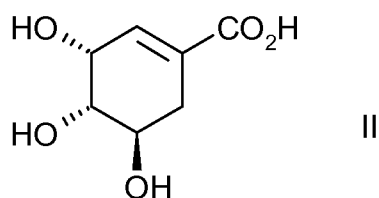
R^2 is an C_{1-6} alkyl and

R^3 , R^4 are independent of each other H or an alkanoyl, with the proviso that not both R^3 and R^4 are H,

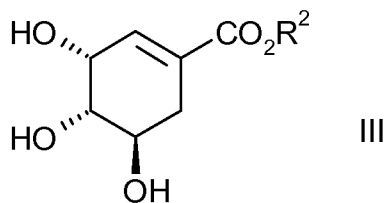
10 characterized in that

step a)

esterification of 5-amino shikimic acid of formula



with R^2OH to form a compound of formula

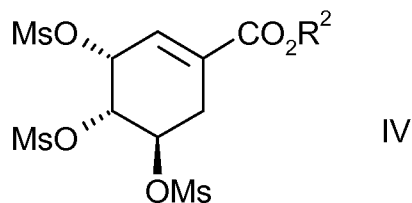


15

wherein R^2 is as defined above;

step b)

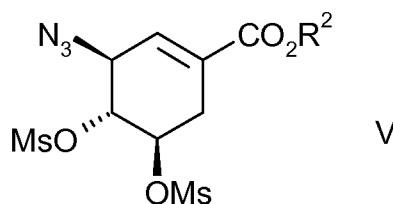
formulation of trimesylate of formula IV by reacting compound of formula III with methanesulfonyl chloride



5 wherein R^2 is as defined above,

step c)

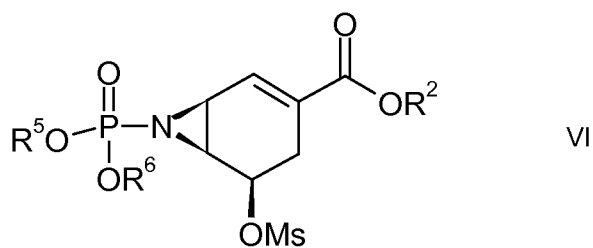
Regioselective S_N2 -substitution of the 3-mesylate-group of the trimesylate IV by an azide reagent to form an azide of formula



10 wherein R^2 is as defined above,

step d)

Reduction of the azide of formula V with trialkylphosphite to form aziridine of formula

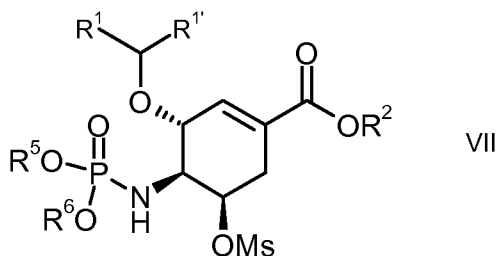


15 wherein R^2 is as defined above, R^5 and R^6 , independently of each other are C_{1-6} alkyl,

step e)

opening of the aziridine of formula VI to form compound of formula

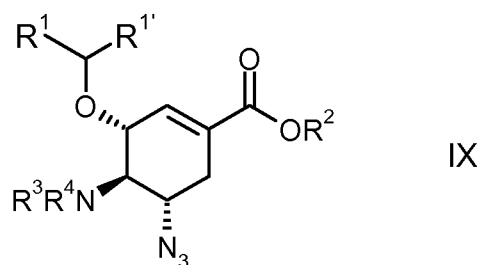
- 23 -



wherein R¹, R^{1'}, R², R⁵ and R⁶ are as defined above,

step f)

transformation of phosphoramidate of formula VII into compound of formula



5

wherein R¹, R^{1'}, R², R³ and R⁴ are as defined above,

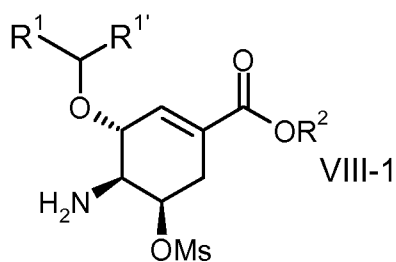
step g)

reduction of azide of formula IX to form compound of formula I and if necessary, formation of a pharmaceutically acceptable addition salt.

10 2. Process of claim 1, wherein step f) comprises the steps,

step f1)

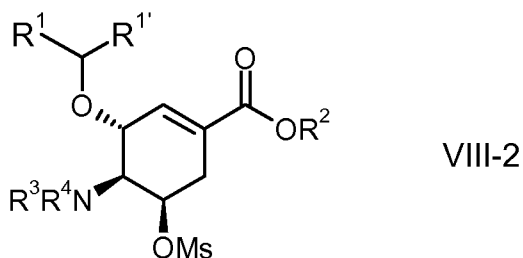
hydrolysis of phosphoramidate of formula VII to form compound of formula



wherein R¹, R^{1'} and R² are as defined above,

15 step f2)

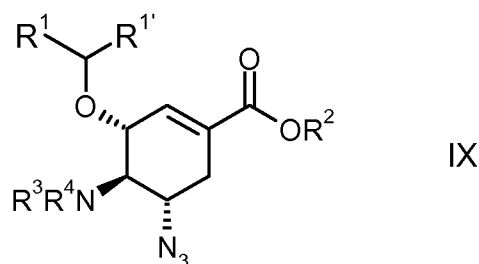
acetylation of compound of formula VIII-1 for form compound of formula



wherein R¹, R¹', R², R³ and R⁴ are as defined above,

step f3)

- 5 substitution of mesylate-group of compound of formula VIII to an azido to form the compound of formula

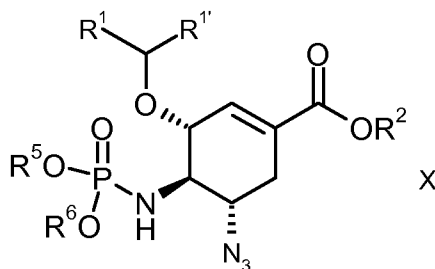


wherein R¹, R¹', R², R³ and R⁴ are as defined above.

3. Process of claim 1, wherein step f) comprises the steps,

- 10 step f¹)

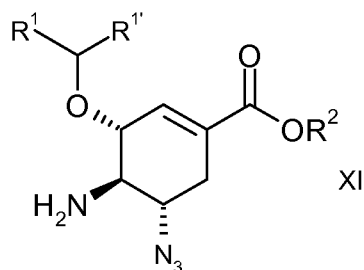
substitution of mesylate-group of compound of formula VII to form the compound of formula



wherein R¹, R¹', R², R³ and R⁴ are as defined above,

- 15 step f²)

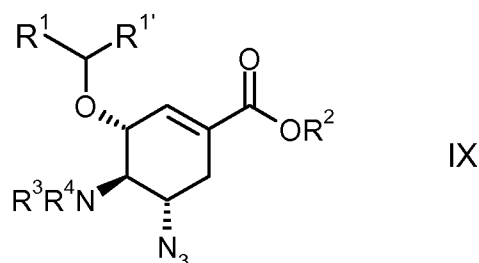
hydrolysis of compound of formula X to form compound of formula



wherein R¹, R^{1'} and R² are as defined above,

step f3)

- 5 acetylation of compound of formula XI to form compound of formula



wherein R¹, R^{1'}, R², R³ and R⁴ are as defined above.

4. Process of claim 1, wherein R¹, R^{1'} and R² are ethyl, R³ is acetyl and R⁴ is hydrogen.

- 10 5. Process of claim 1, wherein R⁵ and R⁶ independently of each other are ethyl.

6. Process of claim 1, wherein the pharmaceutically acceptable acid addition salt is phosphoric acid salt.

- 15 7. Process of claim 1, wherein step a) is performed in an alcohol, preferably ethanol in the presence of a strong acid, preferably thionyl chloride at a reaction temperature range of 60 °C to 150 °C, preferably 70 °C to 100 °C.

8. Process of claim 1, wherein step b) is performed in an aprotic organic solvent, preferably ethylacetat, and methanesulfony chloride with a tertiary organic base, preferably triethylamine at reaction temperature in the range of -20°C to 50°C, preferably 0°C to 5°C.

9. Process of claim 1, wherein step c) is performed in an inert organic solvent, preferably dimethyl sulfoxide or with a water organic solvent mixture, preferably ethylacetate and with a phase transfer catalyst, such as tetrabutylammonium hydrogen sulfate and an azide reagent, preferably sodium azide at room temperature.

5 10. Process of claim 1, wherein step d) is performed in an organic solvent, preferably toluene in an inert gas atmosphere, trialkylphosphite, such as triethylphosphite is added at room temperature, the reaction temperature lies in the range of 50°C to 150°C, preferably 100°C to 120°C.

10 11. Process of claim 1, wherein step e) is performed in an alcohol, such as 3-pentanol and boron trifluoride ethyl etherate in an inert gas atmosphere at a temperature range of -20°C to 100°C, preferably 0°C to 5°C.

12. Process of claim 2, wherein step f1) is performed in an alcohol, such as ethanol and a strong acid, preferably sulfuric acid in an inert gas atmosphere, at a temperature range of 50°C to 150°C, preferably 80°C to 120°C.

15 13. Process of claim 2, wherein step f2) is performed by using acylating agent, preferably under basic conditions using acetic anhydride in ethyl acetate at a temperature range of -20°C to 100°C.

14. Process of claim 2, wherein step f3) is performed in an inert organic solvent, preferably dimethyl sulfoxide or a solvent mixture with dimethyl sulfoxide and ethanol
20 with an azide reagent, preferably sodium azide at 20°C to 120°C, preferably at 80°C to 100°C

15. Process of claim 3, wherein step f1) is performed in an inert organic solvent, preferably dimethyl sulfoxide or an solvent mixture with dimethyl sulfoxide and ethanol and an azide reagent, preferably sodium azide at 20°C to 120°C, preferably at 80°C to
25 100°C.

16. Process of claim 3, wherein step f2) is performed in an alcohol, preferably ethanol and a strong acid, preferably sulfuric acid in an inert gas atmosphere at a temperature range of 20°C to 150°C, preferably 80°C to 120°C.

17. Process of claim 3, wherein step f3) is performed under basic conditions using a
30 mixture of acetic anhydride in ethyl acetate at temperature range of -20°C to 100°C, preferably 0 °C to 5 °C.

18. The invention as hereinbefore described.