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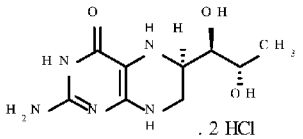
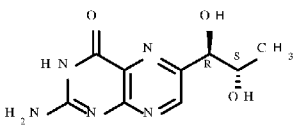
(54) **Title**: NOVEL PROCESS FOR THE PREPARATION OF SAPROPTERIN DIHYDROCHLORIDE AND ITS KEY INTERMEDIATE, L-BIOPTERIN



(1)



(2)

(57) **Abstract**: The present invention relates to a novel process for the preparation of Sapropterin dihydrochloride of formula (1) and its key intermediate L-*erythro*-biopterin of formula (2). The present process is a simple and economically viable process for commercial production of Sapropterin dihydrochloride of formula (1) and its key intermediate L-biopterin of formula (2).

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NOVEL PROCESS FOR THE PREPARATION OF SAPROPTERIN DIHYDROCHLORIDE AND ITS KEY INTERMEDIATE, L-BIOPTERIN

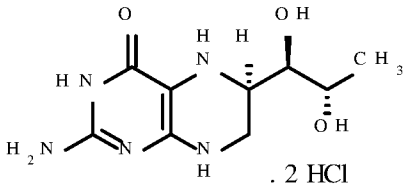
Field of invention

The present invention relates to a novel process for the preparation of Sapropterin dihydrochloride of formula (1) and its key intermediate L-erythro-biopterin of formula (2) (hereinafter referred as L-biopterin).

Background of the invention

Sapropterin commonly known as tetrahydrobiopterin (THB or BH4) developed by BioMarin and marketed as Sapropterin dihydrochloride under the brand name of KUVAN®. It is indicated for the treatment of phenylketonuria (PKU) and tetrahydrobiopterin deficiencies.

Sapropterin dihydrochloride is chemically known as (6R)-2-amino-6-[(1R, 2S)-1, 2-dihydroxypropyl]-5,6,7,8-tetrahydro-4(1H)-pteridinone dihydrochloride and structurally represented as below.



(1)

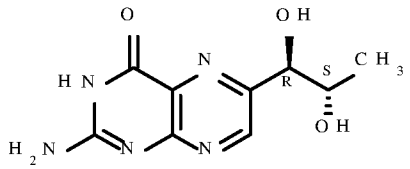
Sapropterin dihydrochloride

Due to its vital role in the conversion of L-tyrosine into L-DOPA, which is the precursor for dopamine, a deficiency in tetrahydrobiopterin can cause severe neurological disorders unrelated to toxic build-up of L-phenylalanine; dopamine is a crucial neurotransmitter, and is the precursor of norepinephrine and epinephrine. Thus, a deficiency of tetrahydrobiopterin can result in phenylketonuria (PKU) from L-phenylalanine concentrations or hyperphenylalaninemia (HPA), as well as monoamine and nitric oxide

neurotransmitter deficiency or chemical imbalance. The chronic presence of PKU can result in severe brain damage, including symptoms of mental retardation, speech impediments like stuttering, slurring, seizures or convulsions and behavioural abnormalities.

In an article published in Bio Chem J 347 (1): 1–16, tetrahydrobiopterin is reported to be biosynthesized from guanosine triphosphate (GTP) by three chemical reactions mediated by the enzymes GTP cyclohydrolase I (GTPCH), 6-pyruvoyltetrahydropterin synthase (PTPS), and sepiapterin reductase (SR).

Preparation of Sapropterin is reported with a mixture of R & S isomers in Helv. Chim. Acta, 60, 1977, 211-214, by catalytic reduction of L-biopterin of formula (2). Similar process with slight modifications is also published in Hel. Chim. Acta, 61, 1978, 2731-2738.



(2)

In another publication reported in Helv. Chim. Acta, 62, 1979, 2577-2580, separation of the diastereomers (6R) and (6S)-5,6,7,8-tetrahydro-L-biopterin is reported by fractional crystallization of corresponding tetraacetyl derivative followed by hydrolysis using aq. HCl.

In another process published in Heterocycles, 23(12), 1985, 3115-3120, Sapropterin dihydrochloride of formula (1) is prepared by catalytic hydrogenation of L-biopterin of formula (2) in the presence of PtO2 under 1atm hydrogen pressure in 0.1 M potassium phosphate buffer at pH 11.8 for 18hr followed by filtration and recrystallization from 8M HCl. With slight modifications in the above reaction conditions like using platinum black, aq. base solutions like tetraethylammonium hydroxide or triethylamine etc. under 100 Kg/cm2 hydrogen pressure / 0° C / pH 12.0 / 1000 rpm / 20h/3N HCl-EtOH with 85% yield is disclosed in US4713454.

In another process disclosed in US4595752, L-biopterin of formula (2) is catalytically reduced in the presence of platinum oxide in aq. base / acid solutions like (10% aq. potassium carbonate, aq. sodium carbonate, aq. potassium acetate and 0.1 N aq. HCl) under bubbling of hydrogen gas for 5-30hr at room temperature followed by filtration and isolated as HCl salt of formula (1) using aq. HCl and ethanol to obtain Sapropterin dihydrochloride.

In another approach disclosed in WO2005049614, racemic isomers of Sapropterin dihydrochloride are prepared from L-neopterin.

In another process disclosed in WO2009088979, the diacetyl biopterin is hydrolysed in the presence of aq. diethyl amine-n-butanol mixture at 40°C for 16hr at pH >11.5 followed by hydrogenation in the presence of platinum black using 50 bar hydrogen pressure at 25°C. Product of formula (1) isolated as HCl salt from ethanol or butanol.

In another process disclosed in US20130197222, Sapropterin dihydrochloride of formula (1) is prepared starting from condensation of crotonoic acid.

The process for preparation of key intermediate, L-biopterin of formula (2) is cited in the following references.

In an article published in J. Am. Chem. Soc., 1955, 77, 3167-3168, L-biopterin of formula (2) is reported to be first isolated from human urine. The melting point reported to be 250-280°C. In another article published in J. Am. Chem. Soc., 1956, 78, 5868-5871, L-biopterin of formula (2) is prepared starting from L-rhamnose. A slight modification in the reaction conditions mentioned above is disclosed in US3505329.

In the article published in Helv. Chim. Acta, 1969, 52, 1225-1228, L-biopterin of formula (2) along with 7-biopterin is synthesized by condensing 2, 4, 5-triamino-6-oxo-1, 6-dihydropyrimidine dihydrochloride with (1-benzyl-1-phenyl-hydrazino)-5-desoxy-L-ribulose followed by oxidation of the tetrahydro derivative.

Later in the year 1974, in an article, J. Am. Chem. Soc., 1974, 96, 6781-6782, L-biopterin is reported to be prepared starting from L-rhamnose.

In another approach published in Bull. Chem. Soc. Jpn., 1975, 48(12), 3767-3768, L-biopterin of formula (2) is prepared from 2, 4, 5-triamino-6-hydroxypyrimidine dihydrochloride is reacted with hydrazone derivative in aq. methanol at reflux temperature.

In another process disclosed in US5043446 (1989), L-biopterin process is claimed to be synthesized starting from D-ribose. Similar approach with slight variations in the process, later published in Liebigs Ann. Chem., 1989, 1267-1269.

In another approach published in Agric. Biol. Chem., 1989, 53, 2095-2100, L-biopterin is synthesized starting from (S)-ethyl lactate. Prior to this publication the methodology is claimed by the same authors in JP01-221380 (1989).

In another approach disclosed in US5037981 (1990), L-biopterin is synthesized from 2-methylfuran.

In the article, Synthesis, 1992, 303-308, L-biopterin is synthesized from (4S)-4(3β-Acetoxy-5-androsten-17β-ylcarbonyloxy)-2-pentynol.

In the approach published in J. Org. Chem., 1996, 61, 8698-8700, L-biopterin is synthesized from L-tartaric acid.

In the patent US7361759 (2005), L-biopterin of formula (2) is made from L-rhamnose diethyl mercaptal.

US20120157671 application discloses the preparation of compound of formula (4a) is by reacting D-ribose of formula (3) with acetone in the presence of sulphuric acid at room temperature followed by neutralization with sodium carbonate and concentrated under vacuum.

However in spite of having the choice of variety of methods for preparation of Sapropterin dihydrochloride of formula (1) and L-biopterin of formula (2), there is still a need to develop commercially viable process for large scale operations since, in general, the processes reported for preparation of Sapropterin dihydrochloride of formula (1) & its key intermediate, L-biopterin have the following disadvantages:

1. Majority of the processes demand highly expensive reagents like neopterin, crotonic acid, L-Rhamnose, sepiapterin reductase enzyme, bistriphenylphosphine copper (I) borohydride etc.,

2. Highly explosive reagents like sodium azide, lithium perchlorate and diazomethane used in the processes.

3. Purification methods like column chromatography, flask chromatography and preparative HPLC are used in majority of the processes. Such type of operations are not suitable on plant scale operation as they require large volumes of solvent and time consuming.

4. In one of the processes, crystallization from 8M HCl would yield very poor recovery of Sapropterin dihydrochloride as the product is highly soluble is aq. HCl medium.

5. Most of the processes require very high pressures (100 Kg/cm2) of hydrogen for hydrogenation of L-biopterin of formula (2).

6. The processes cited, require toxic reagents like diphenyl disulfide, pyridine, 1,3-dithiane, 2-mercaptoethanol, Jone's reagent, thionyl chloride etc.,

7. In one of the processes, the reported purity of Sapropterin dihydrochloride of formula (1) is only 98.5% and its enantiomeric purity is 99.2%. These purities are inadequate for drug formulation applications and need multiple purification steps to attain high purity.

8. In one of the processes, for protection of D-ribose, cyclohexanone is used. During de-protection at a later stage, degradation of the product is observed.

9. The reported yields are very low and not reproducible.

10. Cryogenic conditions are required in some of the processes.

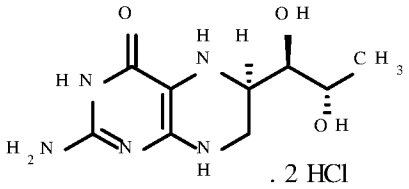
11. In most of the processes, during isolation of Sapropterin dihydrochloride and L-biopterin, very cumbersome work-up procedures are applied.

Keeping in view of the drawbacks associated with the processes disclosed in the literature for the preparation of Sapropterin dihydrochloride of formula (1) and L-biopterin of formula (2), the inventors of the present invention have developed a simple and

economically viable process for commercial production of Sapropterin dihydrochloride of formula (1) and its key intermediate L-biopterin of formula (2).

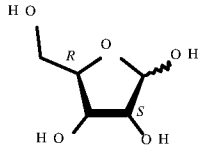
Summary of the invention:

One aspect of the present invention provides an improved process for the preparation of Sapropterin dihydrochloride of formula (1) comprising the steps of:



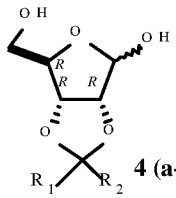
(1)

i) reacting of D-ribose of formula (3)



3

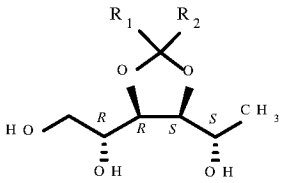
with a ketone (a-h) in the presence of acid catalyst to get cyclic ketal derivative of formula (4 a-h),



4 (a-h)

wherein R1 & R2 together or independently H, C1-C6 alkyl or C3-C6 cycloalkyl

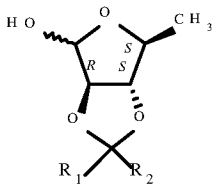
ii) methylation of compound of formula (4 a-h) with methyl magnesium halide solution in tetrahydrofuran to get compound of formula (5 a-h) as white to off-white crystalline solid,



5 (a-h)

wherein R1 & R2 together or independently H, C1-C6 alkyl or C3-C6 cycloalkyl

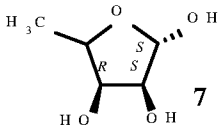
iii) oxidative degradation of compound of formula (5 a-h) in the presence of sodium periodate in biphasic medium containing water-organic solvent to get compound of formula (6 a-h) as an oily residue,



6 (a-h)

where R1 & R2 together or independently H, C1-C6 alkyl or C3-C6 cycloalkyl

iv) hydrolysis of compound of formula (6 a-h) in the presence of an acid catalyst in water medium to get compound of formula (7),



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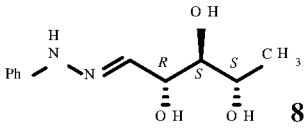
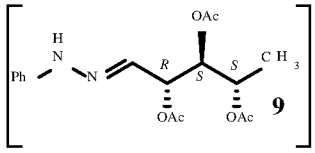
v) condensation of compound of formula (7) in water medium with phenylhydrazine in the presence of an acid catalyst to get compound of formula (8) as yellow crystalline solid,



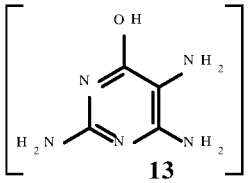
8

vi) acylation of compound of formula (8) with acetic anhydride in an organic solvent in the presence of N, N-dimethylaminopyridine to get compound of formula (9) as crystalline orange solid,

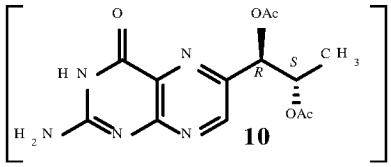


alternatively ethyl acetate solution containing compound of formula (9) is utilized in the next stage without isolation of the product,

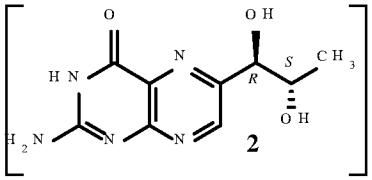
vii) triacetyl derivative of formula (9) is condensed with 4-hydroxy-2,5,6-triaminopyrimidine of formula (13)



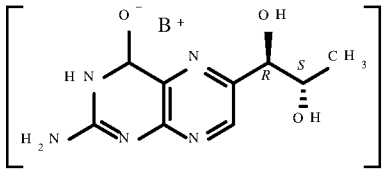
in the presence of an in-situ generated salt quaternary ammonium perchlorate in water medium followed by oxidative degradation using 35% aq. hydrogen peroxide solution to get diacetyl biopterin of formula (10) as orange colour solid,



viii) hydrolysis of diacetyl biopterin of formula (10) in the presence of aq. acid or aq. base solution to get L-biopterin of formula (2) as dark brown solid,



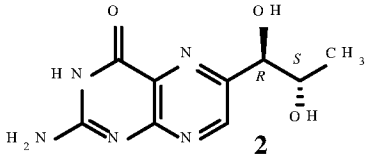
ix) treating of compound of formula (2) with a base in an organic solvent to get the salt of biopterin of formula (11 a-f),



11 (a-f)

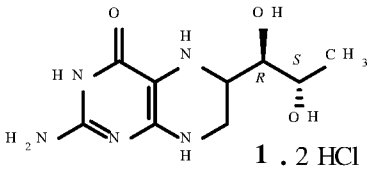
where B= inorganic metal salt like sodium, potassium, lithium, ammonium, salt or organic amine salt like triethyl amine, N, N-diisopropylethyl amine , tetraethylammonium hydroxide, tetrabutyl ammonium hydroxide, diethyl amine, dimethyl amine salt

x) neutralization of salt of formula (11 a-f) with an acid to get pure L-biopterin of formula (2) in >98% purity by HPLC,



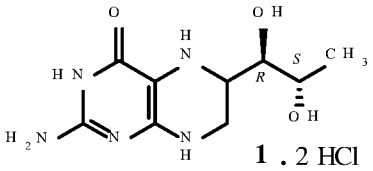
2

xi) hydrogenation of L-biopterin of formula (2) using hydrogen in the presence of a metal catalyst in aq. base solution followed by acidification and distillation of solvent mixture to get technical grade Sapropterin dihydrochloride of formula (1) as off-white solid,



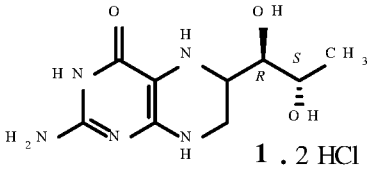
1 . 2 HCl

xii) Alternatively hydrogenation of salt of biopterin of formula (11 a-f)) in the presence of hydrogen using metal catalyst in aqueous medium containing quaternary ammonium salt followed by acidification and distillation with organic solvent mixture to get Sapropterin dihydrochloride of formula (1) as off-white solid,



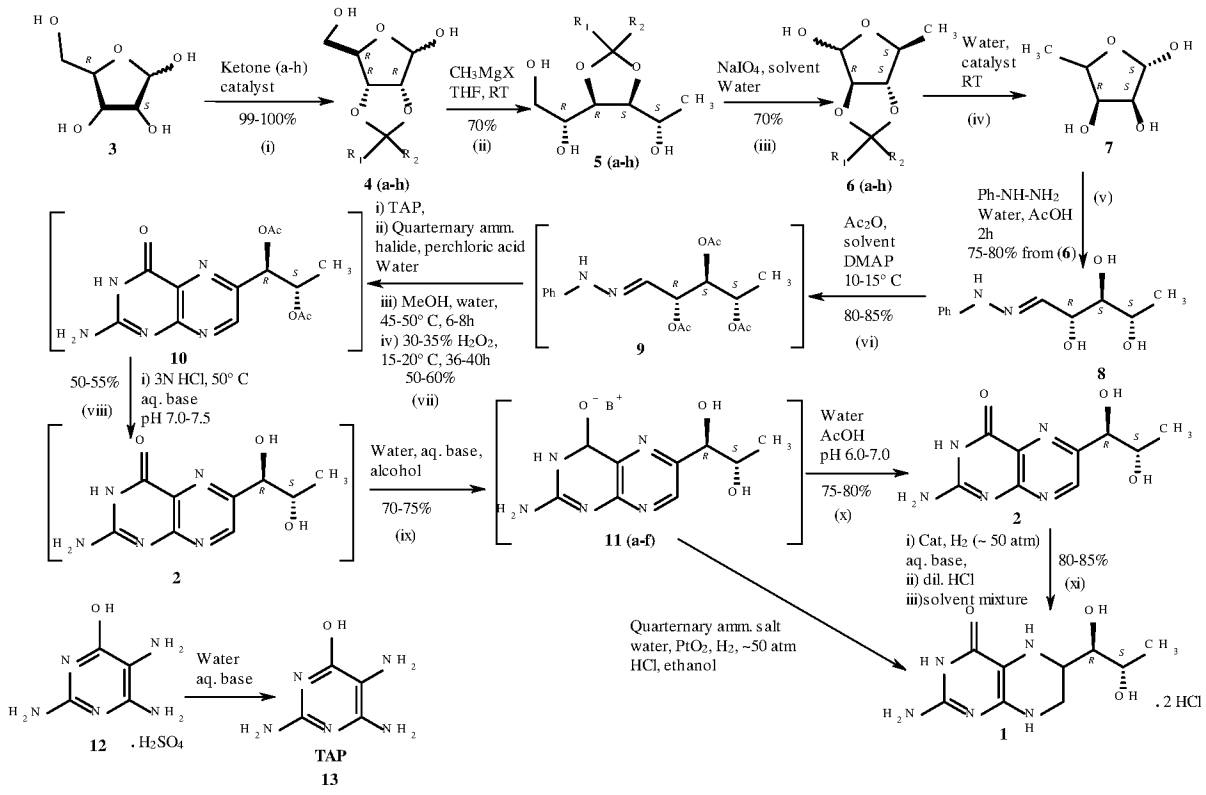
1 . 2 HCl

xiii) recrystallization of Sapropterin dihydrochloride of formula (1) with an organic solvent to get pure compound of formula (1).



1 . 2 HCl

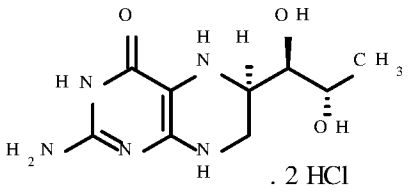
The present invention is shown in below scheme-1



Scheme-1

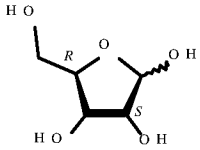
Detalied description of the invention:

One embodiment of the present invention provides an improved process for the preparation of Sapropterin dihydrochloride of formula (1) comprising the steps of:



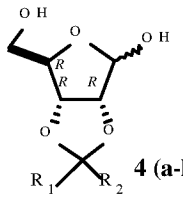
(1)

i) reacting of D-ribose of formula (3)



3

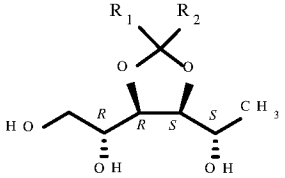
with a ketone (a-h) in the presence of acid catalyst to get cyclic ketal derivative of formula (4 a-h),



4 (a-h)

wherein R1 & R2 together or independently H, C1-C6 alkyl or C3-C6 cycloalkyl

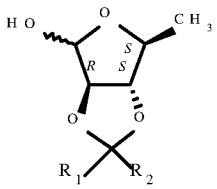
ii) methylation of compound of formula (4 a-h) with methyl magnesium halide solution in tetrahydrofuran to get compound of formula (5 a-h) as white to off-white crystalline solid,



5 (a-h)

wherein R1 & R2 together or independently H, C1-C6 alkyl or C3-C6 cycloalkyl

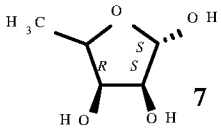
iii) oxidative degradation of compound of formula (5 a-h) in the presence of sodium periodate in biphasic medium containing water-organic solvent to get compound of formula (6 a-h) as an oily residue,



6 (a-h)

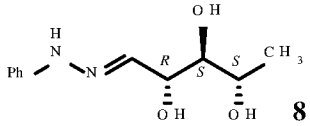
where R1 & R2 together or independently H, C1-C6 alkyl or C3-C6 cycloalkyl

iv) hydrolysis of compound of formula (6 a-h) in the presence of an acid catalyst in water medium to get compound of formula (7),



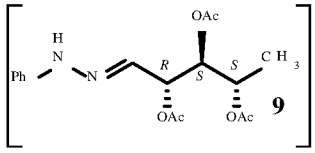
7

v) condensation of compound of formula (7) in water medium with phenylhydrazine in the presence of an acid catalyst to get compound of formula (8) as yellow crystalline solid,



8

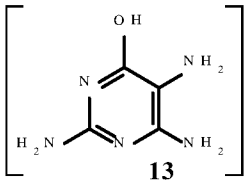
vi) acylation of compound of formula (8) with acetic anhydride in an organic solvent in the presence of N, N-dimethylaminopyridine to get compound of formula (9) as crystalline orange solid,



9

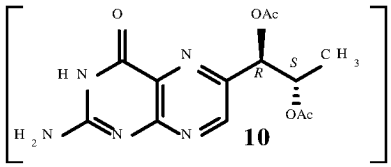
alternatively ethyl acetate solution containing compound of formula (9) is utilized in the next stage without isolation of the product,

vii) triacetyl derivative of formula (9) is condensed with 4-hydroxy-2,5,6-triaminopyrimidine of formula (13)



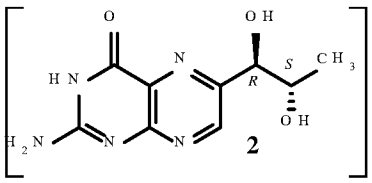
13

in the presence of an in-situ generated salt quaternary ammonium perchlorate in water medium followed by oxidative degradation using 35% aq. hydrogen peroxide solution to get diacetyl biopterin of formula (10) as orange colour solid,



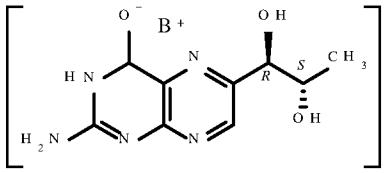
10

viii) hydrolysis of diacetyl biopterin of formula (10) in the presence of aq. acid or aq. base solution to get L-biopterin of formula (2) as dark brown solid,



2

ix) treating of compound of formula (2) with a base in an organic solvent to get the salt of biopterin of formula (11 a-f),

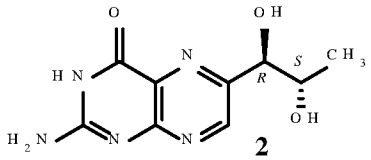


11 (a-f)

where B= inorganic metal salt like sodium, potassium, lithium, ammonium, salt or organic amine salt like triethyl amine, N, N-diisopropylethyl amine ,

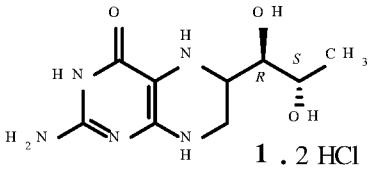
tetraethylammonium hydroxide, tetrabutyl ammonium hydroxide, diethyl amine, dimethyl amine salt

x) neutralization of salt of formula (11 a-f) with an acid to get pure L-biopterin of formula (2) in >98% purity by HPLC,



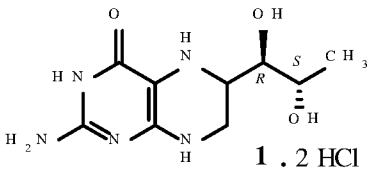
2

xi) hydrogenation of L-biopterin of formula (2) using hydrogen in the presence of a metal catalyst in aq. base solution followed by acidification and distillation of solvent mixture to get technical grade Sapropterin dihydrochloride of formula (1) as off-white solid,



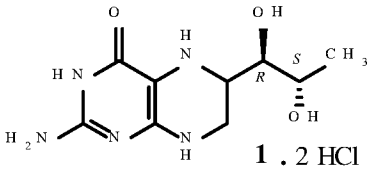
1 . 2 HCl

xii) Alternatively hydrogenation of salt of biopterin of formula (11 a-f)) in the presence of hydrogen using metal catalyst in aqueous medium containing quaternary ammonium salt followed by acidification and distillation with organic solvent mixture to get Sapropterin dihydrochloride of formula (1) as off-white solid,



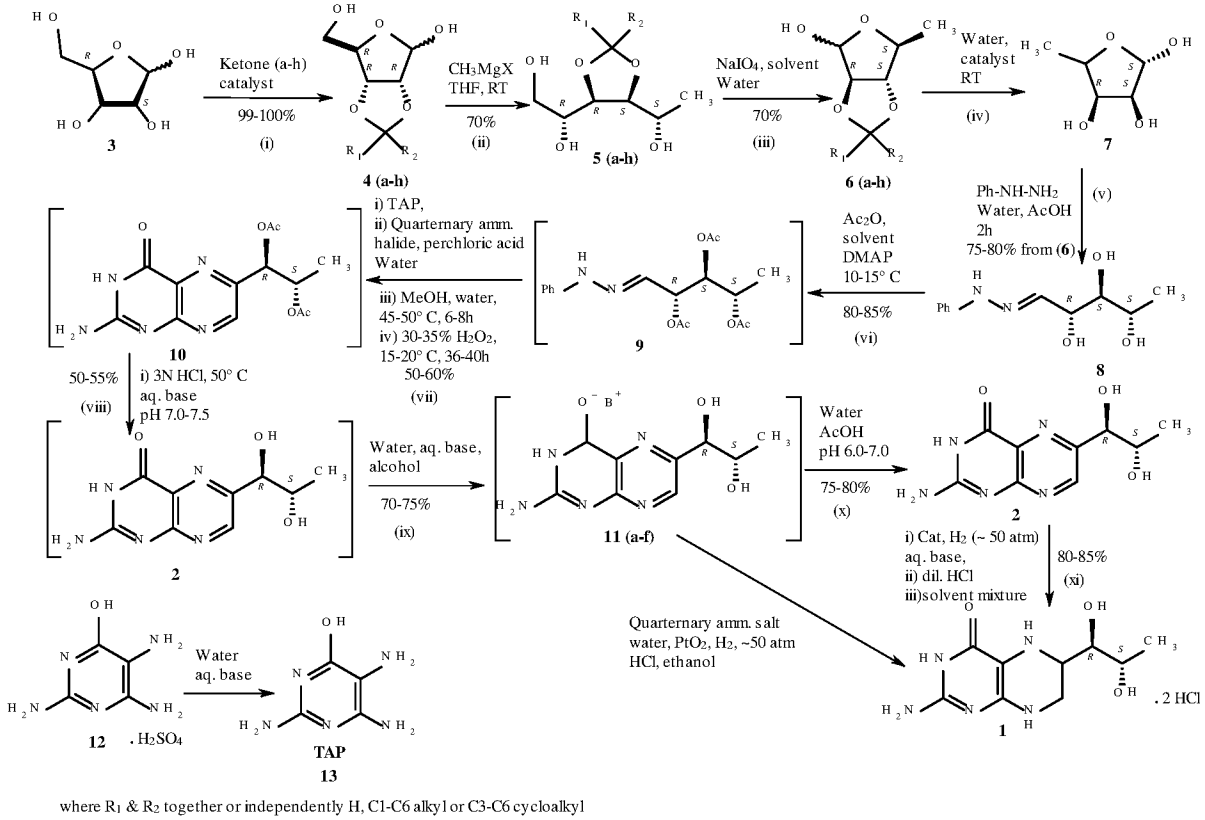
1 . 2 HCl

xiii) recrystallization of Sapropterin dihydrochloride of formula (1) with an organic solvent to get pure compound of formula (1).



1 . 2 HCl

The present invention is shown in below scheme-1



where R_1 & R_2 together or independently H, C1-C6 alkyl or C3-C6 cycloalkyl

Scheme-1

According to the present invention, D-ribose of formula (3) is reacted with a ketone (a-h) selected from acetone (for 4a), methylethyl ketone (for 4b), methylisobutyl ketone (for 4c), diethyl ketone (for 4d), 1-cyclopropaylethanone (for 4e), 1-cyclobutylethanone (for 4f), 1-cyclopentylethanone (for 4g) or 1-cyclohexylethanone (for 4h) preferably acetone in the presence of an acid catalyst like p-toluene sulfonic acid monohydrate, methane sulfonic acid, pyridinium p-toluene sulfonate preferably p-toluene sulfonic acid monohydrate at temperature ranging from 10-40° C and preferably at 20-25° for 4-8 hours. Later the reaction mixture is neutralized with a base selected preferably from potassium carbonate, sodium bicarbonate, sodium carbonate preferably sodium carbonate

and filtered. The filtrate is evaporated under vacuum to get oily residue of compound of formula (4 a) with having >95% GC purity and 1, 2 isomer in quantitative yield.

Compound of formula (4 a-h) is methylated using Grignard reagent selected from methyl magnesium iodide, methyl magnesium bromide, methyl magnesium chloride preferably 3M methyl magnesium chloride in tetrahydrofuran at -30 to 50° C most preferably at 30-40°C for 5-15 hours and most preferably for 10-12 hours. After completion of the reaction, the mass is diluted with an organic solvent selected from ethyl acetate, methyl acetate, toluene, isopropyl ether, methylene chloride or chloroform preferably ethyl acetate followed by quenching with aq. ammonium chloride solution. The organic layer is evaporated to dryness under vacuum to get an oily product of compound of formula (5 a-h). The oily residue, for example of compound of formula (5a) is dissolved in organic solvent selected from ethyl acetate, methyl acetate, toluene, isopropyl ether, methylene chloride or chloroform preferably ethyl acetate and cooled to 0-5°C followed by addition of an non-polar organic solvent selected from cyclohexane, hexane, n-hexane, n-heptane, isopropyl ether, methyl t-butyl ether etc. preferably cyclohexane to get derivative of formula (5 a) (yield is >95% & purity by GC is >98%)

Compound for formula (5 a-h) is subjected to oxidative degradation using sodium periodate in biphasic medium containing water and an organic solvent selected from methyl t-butyl ether, isopropyl ether, diethyl ether preferably methyl t-butyl ether at 0-40° C preferably at 25-30° C for 1-3 hours. After completion of reaction, the organic layer is separated and washed with aq. base selected from sodium bicarbonate, sodium carbonate, potassium bicarbonate or potassium carbonate preferably sodium bicarbonate and the resulting organic layer is concentrated in vacuum to get oily product of formula (6 a-h) (yield 90-95% & purity >98% by GC).

Compound of formula (6 a-h) is subjected to de-protection in a solvent selected from water, methanol, ethanol, isopropanol preferably water in the presence of a catalyst

selected from, p-toluene sulfonic acid monohydrate, hydrochloric acid, sulphuric acid or acid resin like amberlite IR118H, amberlyst 15H, amberjet 1200H, IR120H, hydrochloric acid preferably amberlite IR 120H for 10-30 hours at 15-40° C preferably at 25-30° C. After completion of reaction, the catalyst is filtered and the resulting ketone by-product is distilled off completely under vacuum and the remaining aq. solution containing compound of formula (7) is used in the next stage without further isolation & purification (purity is >95% by TLC).

The aq. solution of compound of formula (7) is condensed with phenyl hydrazine in the presence of weak acid catalyst like acetic acid at 10-35° C preferably at 25-30°C for 1-4 hours. After completion of reaction, the product of formula (8) is filtered and washed with an organic solvent selected from hexane, heptane, cyclohexane, methyl t-butyl ether, isopropyl ether etc., preferably isopropyl ether and dried the wet product in a vacuum oven at 20-60°C preferably at 45-55 C (yield 75-80% & purity by HPLC is >99.5%).

In the next stage, 1 mole equivalent of compound of formula (8) is acylated with acetic anhydride in the presence of 0.02 moles of N, N-dimethyl amino pyridine in an organic solvent selected from ethyl acetate, toluene, isopropyl ether, chloroform, and methylene chloride etc., preferably ethyl acetate 5-35°C preferably at 10-15°C for 2-6 hours. After completion of reaction, the reaction mixture is treated with aq. base solution selected from sodium carbonate, sodium bicarbonate, potassium carbonate or potassium bicarbonate preferably sodium bicarbonate and followed by distillation of the solvent to get compound of formula (9). The oily residue of formula (9) is further triturated in isopropyl ether at room temperature to get highly pure orange solid of formula (9) (yield 80-85% & purity by HPLC is >95%). Alternatively, the compound of formula (9) present in organic solvent is directly utilized in next stage without isolation of formula (9).

Compound of formula (9) is reacted with 4-hydroxy-2,5,6-triaminopyrimidine of formula (13) which was generated in-situ from 4-hydroxy-2,5,6-triaminopyrimidine sulphate of formula (12) using aq. base solution selected from aq. ammonia, aq. sodium carbonate, aq. sodium hydroxide, aq. potassium hydroxide preferably aq. ammonia in an organic solvent mixture-water medium in the presence of a salt prepared from a mixture of

quaternary ammonium halide-perchloric acid 35-50°C preferably 45-50°C for 4-8 hours. The organic solvent mixture is selected from ethyl acetate-methanol or toluene-methanol or isopropyl ether-methanol or chloroform-methanol or methylene chloride-methanol preferably ethyl acetate-methanol. After completion of reaction the reaction mixture is cooled and subjected to oxidative degradation with aq. hydrogen peroxide at 5-25°C preferably 15-20°C and filtered (yield 55-60% & its purity by HPLC is >90%) to get compound of formula (10). Alternatively, the wet solid can be directly utilized in the next step without drying.

Diacetyl biopterin of formula (10) is then hydrolyzed in the presence of aq. acid or base solution selected from aq. hydrochloric acid, aq. sodium hydroxide, aq. potassium hydroxide or aq. ammonia preferably aq. hydrochloric acid at 25-55°C preferably 40-50°C for 2-6 hours. The solution is cooled to room temperature and the pH of the solution is adjusted to 6.0-8.0 preferably 6.5-7.0 with aq. ammonia solution. The resulting biopterin of formula (2) is filtered and dried in a hot air oven at below 60°C. The crude L-biopterin of formula (2) is dissolved in aq. base solution selected from aq. potassium hydroxide (for 11a), aq. sodium hydroxide (for 11b), aq. lithium hydroxide (for 11c), aq. ammonia solution (for 11d) or in organic base selected from triethylamine (for 11e), N, N-diisopropylethyl amine (for 11f) preferably aq. potassium hydroxide and precipitated the resulting salt with an organic solvent selected from methanol, ethanol or isopropyl alcohol preferably methanol. The isolated wet potassium salt of biopterin of formula (11 a) is dissolved in water and the pH of the aq. solution adjusted to 5.0-8.0 preferably 6.5-7.0 with acetic acid followed by filtration and drying to get highly pure crystalline L-biopterin of formula (2) having >98% HPLC purity. The isolated overall yields are 55-60% from compound of formula (8). Alternatively, the wet salt of formula (11 a-f) can be dried and characterized by 1HNMR, 13C NMR, FTIR & Mass spectra.

L-biopterin of formula (2) is dissolved in aq. base solution selected from aq. triethylamine (TEA), aq. N,N-diisopropylethylamine (DIPEA), aq. diethyl amine (DEA), aq. sodium hydroxide, aq. potassium hydroxide, aq. potassium carbonate, aq. ammonium hydroxide or aq. tetraethyl ammonium hydroxide (aq. TEAH) preferably aq. TEAH,

clarified the solution using charcoal followed by filtration and hydrogenated the filtrate in the presence of catalyst selected from platinum oxide, ruthenium oxide, palladium on carbon (5 or 10% wet) using hydrogen pressure ranging from 10-60 bar preferably 50-55 bar for 15-30 hours. After completion of reaction, the catalyst is recovered by filtration and the pH of the filtrate is adjusted to <1.0 with aq. hydrochloric acid solution followed by distillation of water completely under vacuum to get wet Sapropterin dihydrochloride of formula (1). Traces of water present in the wet product of formula (1) is azeotropically removed. The resulting wet product is triturated in methanol 40-60° C preferably 50-55° C followed by filtration to get off-white crystalline product of formula (1) having >98.0 % HPLC purity. The product of formula (1) is further purified by trituration from a mixture of organic solvents selected from 1-pentanol-methanol, 1-pentanol-ethanol, 1-pentanol-isopropanol, methanol, ethanol, isopropanol preferably 1-pentanol-methanol mixture to get pharmaceutically acceptable grade of Sapropterin dihydrochloride of formula (1) having > 99.9% HPLC purity and the product of formula (1) is free from all genotoxic impurities.

Alternatively salt of biopterin of formula (11 a-f) is directly converted to Sapropterin dihydrochloride of formula (1) by hydrogenating salt of biopterin of formula (11 a-f) in the presence of quaternary ammonium salt under hydrogen pressure of about 50kg/cm-2. The quaternary ammonium salt is selected from tetraethyl ammonium hydroxide, tetra butyl ammonium hydroxide, benzyl triethylammonium chloride, benzyltrimethylammonium chloride, tetraethyl ammonium chloride etc. preferably tetraethyl ammonium hydroxide.

Advantages of the present process:

1. Present process does not require expensive, toxic and corrosive reagents.
2. Present process does not require cryogenic conditions.
3. Present process avoids explosive reagents like diazomethane, lithium perchlorate etc.,
4. Present process avoids fractional crystallization and chromatographic purification steps.

5. L-biopterin of formula (2) obtained by this process is highly pure (>98% by HPLC) and suitable for making Sapropterin dihydrochloride of formula (1).

6. Sapropterin dihydrochloride of formula (1) obtained by this process is highly pure (>99.9% by HPLC) and suitable for drug formulations.

7. This process exemplifies a process of making Sapropterin dihydrochloride of formula (1) employing commercially available metal oxides.

8. The procedure can be easily scaled up to multi-kilogram level.

9. The catalysts used in the reaction can be easily recovered and regenerated for recycling.

10. The present process provides an efficient purification method to eliminate the impurities from the drug substance of formula (1)

11. The present process produces Sapropterin dihydrochloride of formula (1) free from genotoxic impurities rendering the product directly suitable for therapeutic use.

The following examples are provided for illustration purpose only and are not intended to limit the scope of invention.

Experimental Section:

Example-1: Preparation of (6R)-2-amino-6-[(1R, 2S)-1, 2-dihydroxypropyl]-5,6,7,8-tetrahydro-4(1H)-pteridinone dihydrochloride of formula (1):

Step (i): Preparation of 2, 3-O-isopropylidene-D-ribose of formula (4a)

Into a 5L, 4 necked round-bottomed flask equipped with a mechanical stirrer, a thermometer socket, and a condenser, were charged acetone (3.0 L), D-ribose (300.0 gm, 2.0 mole) and p-toluene sulfonic acid (11.5 gm). The solution was stirred and maintained at 20-25°C for 2.5-3.0hrs. After completion of reaction, the reaction mixture was neutralized with aq. base solution and filtered. The filtrate was evaporated to dryness to get 375.0 gm (98.8% by theory) of 2, 3-O-isopropylidene-D-ribose of formula (4a) as light brown colour oily residue. Purity: >95% by GC.

Step (ii): Preparation of 1-deoxy-3, 4-O-isopropylidene-D-allitol of formula (5a)

Into a 5L, 4 necked round-bottomed flask equipped with a mechanical stirrer, a thermometer socket, and a condenser, was charged, 2.0L, 3M methyl magnesium chloride and cooled to 10° C. To this stirred solution, a solution of 200gm of 2,3-O-isopropylidene-D-ribose of formula (4a) dissolved in 200 mL tetrahydrofuran was added. After completion of reaction, the reaction mixture was quenched with ammonium chloride, extracted with ethyl acetate and separated. The solvent was evaporated to dryness under vacuum to get 185gm of 1-deoxy-3, 4-O-isopropylidene-D-allitol of formula (5a) as dark brown colour oily residue. The crude product was purified by crystallization from ethyl acetate/hexane mixture to get 130g (60% by theory) as white crystalline solid. Purity: >98% by GC.

IR (λ Cm-1, KBr disc): 3317.64, 2993.69-2976.90, 2926.08, 2873.26 (m) -CH3, 1074.35; 1 HNMR (400 MHz, DMSO-d6, [illegible] (ppm): 1.22 (t, CH3, J=6.8Hz, 3H), 1.148 (s, CH3, 3H), 1.290 (s,CH3), 3.415-3.357 (m, CH, 1H), 3.652-3.571 (m, CH2, 2H), 3.812-3.803 (d, 2 X CH, 2H), 4.00-3.969 (q, CH, 1H), 4.504-4.476 (t, OH,1H), 4.504-4.476 (d, OH, 1H), 5.381-5.371 (d, OH, 1H): 13 CNMR (100 MHz, DMSO-d6, (ppm): 20.59, 25.35, 27.73, 63.18, 64.61, 69.77, 76.82, 81.40, 107.31; Mass: 206.42 [M], 205.41 [M-1]. DSC (° C): 77.58° C

Step (iii): Preparation of 5-deoxy-2, 3-O-isopropylidene-D-ribose of formula (6a)

Into a 5L 4 necked round-bottomed flask equipped with a mechanical stirrer, a thermometer socket, and a condenser, were charged, 1.6 L of water and 270 gm of sodium meta periodate. The solution was cooled to 10-20°C. To the stirred solution, a solution of 200 gm of 1-deoxy-3, 4-O-isopropylidene-D-allitol of formula (5a) dissolved in 1.4 L of isopropyl ether at 25°C. After addition, the reaction mixture was maintained at 25-30° C for 1-2h. After completion of reaction, the layers were separated and the organic layer was washed with water, aq. sodium bicarbonate and separated. The excess solvent was removed by distillation under vacuum to get 145 gm (85.4% by theory) of 5-

deoxy-2, 3-O-isopropylidene-D-ribose of formula (6a) as yellow oil. Purity: >98% by GC.

Step (iv & v): Preparation of 5-deoxy-L-ribose phenyl hydrazone of formula (8)

a) Step (iv): Preparation of 5-deoxy-L-ribose of formula (7)

Into a 2L 4 necked round-bottomed flask equipped with a mechanical stirrer, a thermometer socket, and a condenser, were charged 600ml of water and 200gm of 5-deoxy-2, 3-O-isopropylidene-D-ribose of formula (6a). To the stirred reaction mixture, 180gm of resin was charged and stirred for 8-10 h at 10-15° C. After completion of reaction, the resin was recovered and the filtrate was clarified by activated charcoal and filtered. The filtrate was distilled off under vacuum and the resulting 5-deoxy-L-ribose of formula (7) present water was directly used in the next step without further isolation and purification. The purity of 5-deoxy-L-ribose of formula (7) present in water was above 95% by TLC.

b) Step (v): Preparation of 5-deoxy-L-ribose phenyl hydrazone of formula (8)

Into a 2L 4 necked round-bottomed flask equipped with a mechanical stirrer, a thermometer socket, and a condenser, were charged the above aq. solution of 5-deoxy-L-ribose of formula (7), 5.0 mL of acetic acid. To the stirred solution, 125g of phenyl hydrazine was charged and stirred the reaction mixture for 1-2h at 25-35° C. After completion of reaction, the reaction product was filtered and washed with isopropyl ether. The wet product was dried to get 190g (73.9% by theory) of 5-deoxy-L-ribose phenyl hydrazone of formula (8) as yellow colour crystalline powder. Purity: >99.0% by HPLC.

Step (VI toX): Preparation of L-erythro-biopterin of formula (2)

a) Step (vi): Preparation of triacetoxy-5-deoxy-L-ribose phenylhydrazone of formula (9)

Into a 10L 4 necked round-bottomed flask equipped with a mechanical stirrer, a thermometer socket, and a guard tube, were charged 5L of ethyl acetate, 500g of 5-deoxy-L-ribose phenyl hydrazone of formula (8) and 54gm of 4-dimethylaminopyridine. The reaction mixture was cooled to 25-30° C and was added 730gm of acetic anhydride drop wise. The reaction mixture was maintained under stirring for 2-3h. After completion

of reaction, the reaction mixture was washed with water, aq. sodium carbonate and water, and separated. The organic layer was used in the next stage without further isolation and purification.

b) Step (vii): Preparation of 1,2-diacetyl-biopterin of formula (10)

Into a 20L 4 necked round-bottomed flask equipped with a mechanical stirrer, a thermometer socket, addition funnel, and a condenser, were charged, the above organic layer containing triacetoxy-5-deoxy-phenyl hydrazone of formula (9) obtained in step (vi), 3.0 L methanol and 4-hydroxy-2,5,6-triaminopyrimidine base (generated from 600 gm of corresponding sulphate salt) and salt (generated from 350 gm of tetra butyl ammonium bromide and 154g of 70% perchloric acid) and 5.3L water under stirring and heated and maintained at 35-40°C for 6-8h. The reaction mixture was then cooled to 20-25°C and added 1.0 Kg 35% aq. hydrogen peroxide drop wise. The reaction mixture was maintained for 36-40h under stirring at 25-30°C and resulting product was filtered under suction. The wet product was washed with water and utilized in the next step without further purification.

c) Step (viii): Preparation of L-*erythro* biopterin of formula (2)

Into a 10L 4-necked round-bottomed flask equipped with a mechanical stirrer, condenser, thermometer socket, and addition funnel, were charged 1.35 L of aq. potassium hydroxide and the above wet product obtained from step (vii). The reaction mixture was heated to 45-50° C and maintained form 2-3h and filtered. The pH of the filtrate was adjusted to neutral and the resulting product was filtered and dried to get 205 g of crude L-erythro-biopterin of formula (2) as dark brown solid. Purity: >90% by HPLC

d) Step (ix): Preparation of potassium salt of L-*erythro* biopterin of formula (11a)

Into a 10L 4 necked round-bottomed flask equipped with a mechanical stirrer, thermometer socket, and a glass stopper, were charged 650 mL water followed by 110g of potassium hydroxide and dissolved under stirring. The potassium hydroxide solution was cooled to 25-30° C and the above crude L-erythro-biopterin of formula (2) was charged under stirring. The resulting solution was then clarified using activated carbon and filtered. The potassium salt was regenerated from the solution by the addition of 8.5L

of isopropyl alcohol. The resulting salt was filtered and washed with isopropyl alcohol. The wet product of formula (11a) was utilized in the next step without further purification.

e) Step (x): Preparation of pure L-erythro biopterin of formula (2) from potassium salt of L-erythro biopterin of formula (2)

Into a 5L 4 necked round-bottomed flask equipped with mechanical stirrer, thermometer socket, and addition funnel, were charged 3.2 L of water and the above wet potassium salt of formula (11a). The reaction mixture was stirred to dissolve completely. The resulting solution was clarified using activated carbon and filtered. The pH of the filtrate was adjusted to 6.0-7.0 to get pure L-erythro-biopterin of formula (2). The product was filtered and washed with water followed by isopropyl alcohol followed by isopropyl ether to get 130g of highly pure L-erythro biopterin of formula (2) with > 98% HPLC purity

Appearance: pale brown coloured solid.

1H NMR (3N DCl) δ(ppm): 1.569-1.585(d, 3H), 4.596-4.657(p, 1H), 5.325-5.337(d, 1H), 9.355(s, 1H); Mass: 238.29(M+1), 239.22(M+2).

Step (xi): Preparation of Sapropterin dihydrochloride of formula (1)

Into a 5L 4 necked round-bottomed flask equipped with mechanical stirrer, and thermometer socket, were charged 1.8L of water, 250g of L-erythro-biopterin of formula (2) followed by 800mL of 20% aq. potassium carbonate solution under stirring. The solution was then added 90g of platinum oxide catalyst. The reaction mixture was then transferred into an autoclave and pressurized with 40 bar hydrogen gas and hydrogenated at room temperature for 24-30h under stirring. After completion of reaction, the catalyst was filtered off and the pH of the filtrate was acidified with concentrated hydrochloric acid. The water was evaporated under vacuum and the resulting crude Sapropterin dihydrochloride of formula (1) was isolated as pale yellow colour solid by addition of isopropanol/1-pentanol mixture. The product was dried in a vacuum oven to get 250g of crude Sapropterin dihydrochloride of formula (1).

Step (xii): Purification of Sapropterin dihydrochloride of formula (1)

Into a 2L 4 necked round-bottomed flask equipped with a mechanical stirrer, thermometer socket, and reflux condenser, were charged 1L water and 250g of Sapropterin dihydrochloride of formula (1). The contents were stirred to dissolve completely. The clear solution was treated with activated charcoal and filtered. The filtrate was distilled off completely under vacuum to afford pale yellow solid. The product was isolated from isopropanol/1-pentanol mixture to get 225.0 g (90%) pure Sapropterin dihydrochloride of formula (1) as pale yellow to off-white solid. HPLC purity is >99.9%.

Example 2: Preparation of triacetoxy-5-deoxy-L-ribose phenylhydrazone of formula (9)

Into a 10L 4 necked round-bottomed flask equipped with a mechanical stirrer, a thermometer socket, and a guard tube, were charged 50mL of ethyl acetate, 5.0g of 5-deoxy-L-ribose phenyl hydrazone of formula (8) and 0.54g of N, N-dimethylamino pyridine. The reaction mixture was cooled to 15-20°C and was added 7.2gm of acetic anhydride drop wise. The reaction mixture was maintained under stirring for 6-8h. After completion of reaction, the reaction mixture was washed with water, aq. sodium carbonate and water, and separated. The organic layer was distilled under reduced pressure and product was isolated from n-hexane to get 6.2g of triacetoxy-5-deoxy-L-ribose phenylhydrazone of formula (9) 79.4% yield.

Appearance: Orange coloured solid.

Melting point: 70-75°C.

1HNMR (CDCl3): 1.275-1.291(d, 3H), 2.039(s, 3H), 2.085-2.095(d, 6H), 5.083-5.144(m, 1H), 5.390-5.416(t, 1H), 5.589-5.619(t, 1H), 6.849-6.886(t, 1H), 6.922-6.937(t, 1H), 6.966-6.987(d, 2H), 7.221-7.242(d, 2H), 7.563(s, 1H(D2O exchangeable).

13CNMR (CDCl3): 15.325, 20.816-21.053, 68.482, 71.717, 73.043, 112.759, 120.510, 129.212, 132.105, 144.049, 169.496, 169.948.

Example 3: Preparation of potassium salt of L-erythro biopterin of formula (11)

Into a 1.0L 4 necked round-bottomed flask equipped with a mechanical stirrer, thermometer socket, and a glass stopper, were charged 75 mL water followed by 3.7g of potassium hydroxide and dissolved under stirring. The potassium hydroxide solution was cooled to 25-30° C and 15.0g of crude L-erythro-biopterin of formula (2) was charged under stirring. The resulting solution was then clarified using activated carbon and filtered. The potassium salt was regenerated from the solution by the addition of 500mL of ethanol. The resulting salt was filtered and washed with ethanol and dried to get 9.1g of potassium salt of L-erythro biopterin of formula (11) with 52.3% yield. HPLC <98%

Appearance: Brown coloured solid.

1H NMR (D2O): 1.187-1.203(d, 3H), 4.158-4.220(p, 1H), 4.731-4.745(d, 1H), 8.623(s, 1H).

13C NMR (D2O): 18.198, 70.645, 76.703, 128.811, 147.875, 149.410, 156.504, 164.774, 173.731.

Mass: 276.23(M+1), 277.21(M+2), 238.29(M-K+1); DSC (° C): 313.12°

Example 4: Preparation of Sapropterin dihydrochloride of formula (1)

Into a 5L 4 necked round-bottomed flask equipped with mechanical stirrer, and thermometer socket, were charged 1.8L of water, 250g of L-erythro-biopterin of formula (2) followed by 800ml of 20% aq. potassium hydroxide solution under stirring. The solution was then added 90gm of platinum oxide catalyst. The reaction mixture was then transferred into an autoclave and pressurized with 50 bar hydrogen gas and hydrogenated at room temperature for 24-30h under stirring. After completion of reaction, the catalyst was recovered by filtration and the filtrate was acidified with concentrated hydrochloric acid. The water was evaporated under vacuum and the resulting crude Sapropterin dihydrochloride of formula (1) was isolated as pale yellow colour solid by addition of ethanol-1-pentanol mixture. The product was dried in a vacuum oven to get 250g of crude Sapropterin dihydrochloride of formula (1).

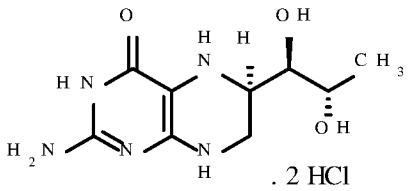
Example 5: Purification of Sapropterin dihydrochloride of formula (1)

Into a 2L 4 necked round-bottomed flask equipped with a mechanical stirrer, thermometer socket, and reflux condenser, were charged 1L water and 250g of Sapropterin dihydrochloride of formula (1). The contents were stirred to dissolve completely and the clear solution was treated with activated charcoal and filtered. The filtrate was distilled off completely under vacuum to afford pale yellow solid. The product 225.0 g (90%) was isolated ethanol-1-pentanol mixture as pure Sapropterin dihydrochloride of formula (1) as pale yellow to off-white solid. HPLC purity is >99.9%.

CLAIMS

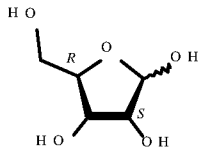
We claim:

1. An improved process for the preparation of Sapropterin dihydrochloride of formula (1) comprising the steps of:



(1)

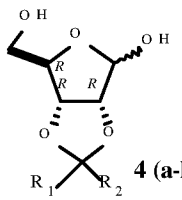
i) reacting of D-ribose of formula (3)



3

with a ketone (a-h) in the presence of acid catalyst to get cyclic ketal derivative of

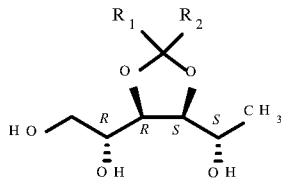
formula (4 a-h),



4 (a-h)

wherein R1 & R2 together or independently H, C1-C6 alkyl or C3-C6 cycloalkyl

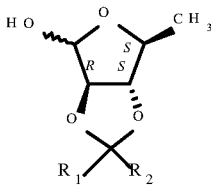
ii) methylation of compound of formula (4 a-h) with methyl magnesium halide solution in tetrahydrofuran to get compound of formula (5 a-h),



5 (a-h)

wherein R1 & R2 together or independently H, C1-C6 alkyl or C3-C6 cycloalkyl

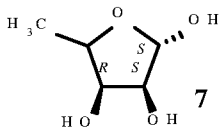
iii) oxidative degradation of compound of formula (5 a-h) in the presence of sodium periodate in biphasic medium containing water-organic solvent to get compound of formula (6 a-h),



where R1 & R2 together or independently H, C1-C6 alkyl or C3-C6 cycloalkyl

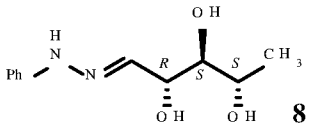
6 (a-h)

iv) hydrolysis of compound of formula (6 a-h) in the presence of an acid catalyst in water medium to get compound of formula (7),



7

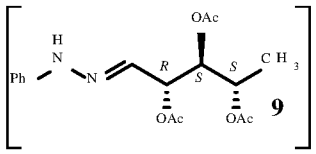
v) condensation of compound of formula (7) in water medium with phenylhydrazine in the presence of an acid catalyst to get compound of formula (8),



8

vi) acylation of compound of formula (8) with acetic anhydride in an organic solvent in

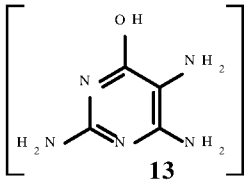
the presence of N, N-dimethylaminopyridine to get compound of formula (9),



9

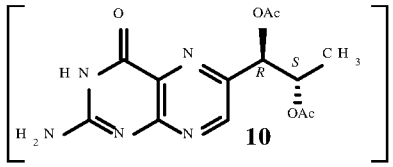
alternatively ethyl acetate solution containing compound of formula (9) is utilized in the next stage without isolation of the product,

vii) triacetyl derivative of formula (9) is condensed with 4-hydroxy-2,5,6-triaminopyrimidine of formula (13)



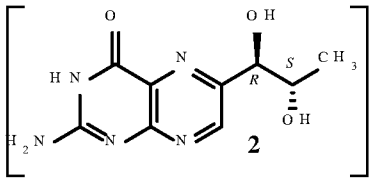
13

in the presence of an in-situ generated salt quaternary ammonium perchlorate in water medium followed by oxidative degradation using 35% aq. hydrogen peroxide solution to get diacetyl biopterin of formula (10),



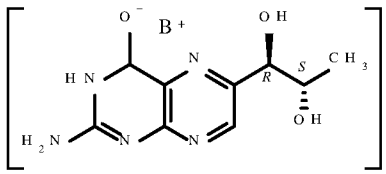
10

viii) hydrolysis of diacetyl biopterin of formula (10) in the presence of aq. acid or aq. base solution to get L-biopterin of formula (2),



2

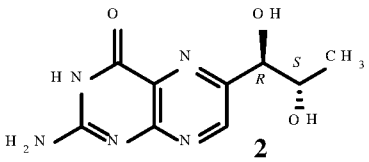
ix) treating of compound of formula (2) with a base in an organic solvent to get the salt of biopterin of formula (11 a-f),



11 (a-f)

where B= inorganic metal salt like sodium, potassium, lithium, ammonium, salt or organic amine salt like triethyl amine, N, N-diisopropylethyl amine , tetraethylammonium hydroxide, tetrabutyl ammonium hydroxide, diethyl amine, dimethyl amine salt

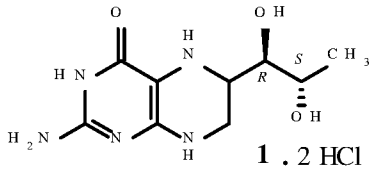
x) neutralization of salt of formula (11 a-f) with an acid to get pure L-biopterin of formula (2) in >98% purity by HPLC,



2

xi) hydrogenation of L-biopterin of formula (2) using hydrogen in the presence of a metal catalyst in aq. base solution followed by acidification and

distillation of solvent mixture to get Sapropterin dihydrochloride of formula (1).



1 . 2 HCl

2. The process according to claim 1, wherein in step (i)

a) the ketone employed in the condensation of D-ribose of formula (3) is selected from acetone, methylethyl ketone, methylisobutyl ketone, diethyl ketone, 1-cyclopropylethanone, 1-cyclobutylethanone, 1-cyclopentylethanone or 1-cyclohexylethanone,

b) the acid catalyst employed in the condensation of D-ribose of formula (3) with acetone is selected from p-toluene sulfonic acid monohydrate, methane sulfonic acid, pyridinium p-toluene sulfonate.

3. The process according to claim 1, wherein in step (ii) Grignard reagent used for methylation of compound of formula (4) is selected from methyl magnesium chloride in THF, methyl magnesium bromide in THF, methyl magnesium iodide in THF;

4. The process according to claim 1, wherein in step (iii) the organic solvent employed for oxidative degradation is selected from methyl t-butyl ether, isopropyl ether, diethyl ether.

5. The process according to claim 1, wherein in step (iv) the catalyst employed for de-protection is selected from p-toluene sulfonic acid, hydrochloric acid, sulphuric acid or acid resin selected from amberlite IR118H, amberlyst 15H, amberjet 1200 H, amberlite IR120H.

6. The process according to claim 1, wherein in step (v) the catalyst employed in the condensation reaction compounds of formula (7) with phenyl hydrazine is acetic acid.

7. The process according to claim 1, wherein in step (vi) the solvent employed for acetylation of compound of formula (8) is selected from ethyl acetate, toluene, isopropyl ether, chloroform, methylene chloride.
8. The process according to claim 1, wherein in step (viii) reagent employed for hydrolysis of diacetyl biopterin of formula (10) is selected from aq. hydrochloric acid, aq. sodium hydroxide, aq. potassium hydroxide, aq. ammonia solution.
9. The process according to claim 1, wherein in step (ix)

a) the aq. base employed to dissolve crude wet L-biopterin of formula (2) is selected from aq. ammonia, aq. potassium hydroxide, aq. sodium hydroxide, aq. lithium hydroxide or in organic base solutions selected from aq. triethylamine, aq. N,N-diisopropyl ethyl amine, aq. tetraethylammonium hydroxide, aq. tetrabutyl ammonium hydroxide, aq. diethyl amine, aq. dimethyl amine,

b) the organic solvent employed to precipitate the salt of L-biopterin of formula (11) is selected from methanol, ethanol, 2-propanol, 1-propanol.

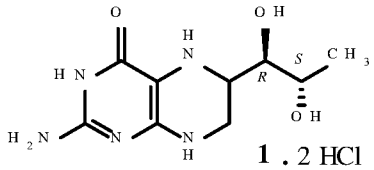
10. The process according to claim 1, wherein in step (xi)

a) the solvent medium used for hydrogenation of L-biopterin of formula (2) in step (xi) is selected from aq. potassium hydroxide, aq. triethylamine, aq. sodium hydroxide, aq. potassium carbonate, aq. sodium carbonate, aq. tetraethyl ammonium hydroxide, aq. tertiary butyl ammonium hydroxide, aq. N, N-diisopropyl ethyl amine, aq. diethyl amine.

b) the catalyst employed in the hydrogenation of L-biopterin of formula (2) is selected from platinum oxide, ruthenium oxide, palladium on carbon (5 or 10% wet) preferably platinum oxide.

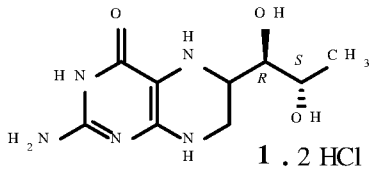
11. An improved process for the preparation of Sapropterin dihydrochloride comprising the steps of:
    i) hydrogenation of salt of biopterin of formula (11 a-f)) in the presence of hydrogen using metal catalyst in aqueous medium containing quaternary

ammonium salt followed by acidification and distillation with organic solvent mixture to get Sapropterin dihydrochloride of formula (1),



1 . 2 HCl

ii) recrystallization of Sapropterin dihydrochloride of formula (1) with an organic solvent to get pure compound of formula (1).



1 . 2 HCl

12. The process according to claim 11, wherein in step (i) the catalyst employed in the hydrogenation of salt of L-biopterin of formula (11) is selected from platinum oxide, ruthenium oxide, palladium on carbon (5 or 10% wet) preferably platinum oxide.

13. The process according to claim 11, wherein in step (ii) the organic solvent employed to precipitate Sapropterin dihydrochloride of formula (1) is 1-pentanol-methanol, 1-pentanol-ethanol, 1-pentanol-isopropanol, methanol, ethanol, isopropanol.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2015/050071

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D475/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, CHEM ABS Data, BEILSTEIN Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 043 446 A (KIKUCHI HARUHIKO [JP] ET AL) 27 August 1991 (1991-08-27) cited in the application reaction scheme see passage bridging columns 4 and 5	1-13

X	EP 2 628 740 A1 (INNOPHARMAX INC [TW]) 21 August 2013 (2013-08-21) cited in the application	11-13
A	claims; examples	1-10

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 December 2015	07/01/2016

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer
Schmid, Arnold

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IN2015/050071

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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