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## Process For The Preparation Of Clofarabine

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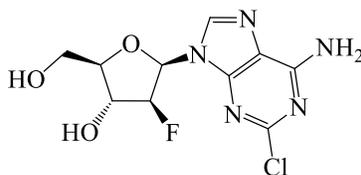


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## Process for the preparation of Clofarabine

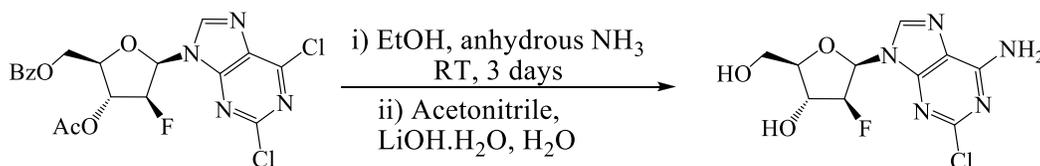
The present invention provides a process for the preparation of Clofarabine represented by the following structural formula-1.



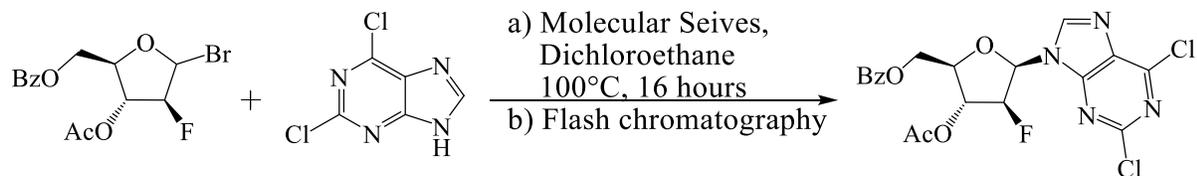
Formula-1

2-Chloro-9-(2'-deoxy-2'-fluoro- $\beta$ -D-arabinofuranosyl)-9H-purine-6-amine, commonly known as Clofarabine is a purine nucleoside metabolic inhibitor. The drug has been approved both in US and Europe for the treatment of relapsed or refractory acute lymphoblastic leukaemia.

Various synthetic routes are available for the synthesis of Clofarabine. US5034518 patent first discloses Clofarabine and process for its preparation. The disclosed process involves the reaction of 2,6-dichloro-9-(3-O-acetyl-5-O-benzyl-2-deoxy-2-fluoro- $\beta$ -D-arabinofuranosyl)-9H-purine with anhydrous ammonia in ethanol at room temperature. However, after three days maintenance of the reaction mixture at RT, two major products were present in the reaction mixture, the required 2-Chloro-9-(2-deoxy-2-fluoro-beta-D-arabinofuranosyl)-9H-purin-6-amine along with its 5'-benzoyl analog. The said mixture upon treatment with lithium hydroxide hydrate produces the required product in 42% yield.

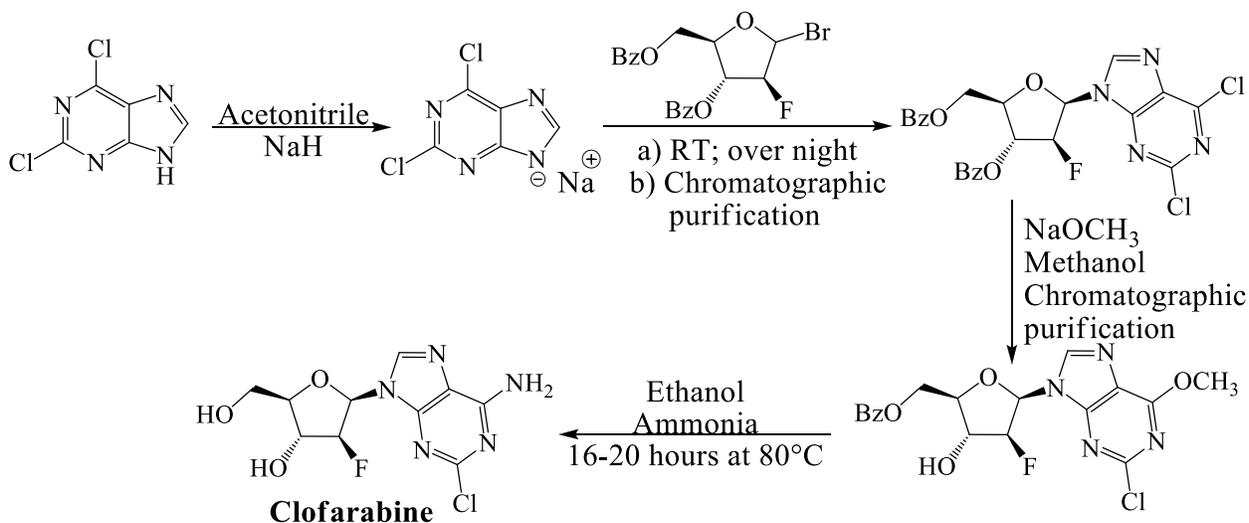


In the above process, the starting material was prepared by the process disclosed in Journal of Medicinal Chemistry, 1986, Vol. 29, No. 11, page: 2389-2392. The disclosed process involve the reaction of 3-acetyl-5-benzoyl-2-deoxy-2-fluoroarabinofuranosylbromide with 2,6-dichloropurine in dichloroethane in presence of molecular sieves at 100°C for 16 hours provides 2,6-Dichloro-9-(3-O-acetyl-5-O-benzyl-2-deoxy-2-fluoro- $\beta$ -D-arabinofuranosyl)-9H-purine. The obtained compound was purified by flash column chromatography to give the required product in an overall yield of 32%.



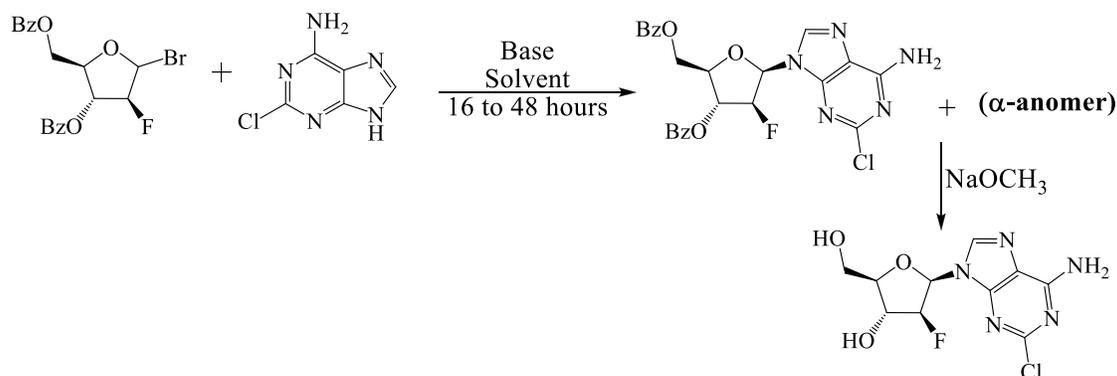
The above disclosed route of synthesis has so many disadvantages. The reaction procedure involves the usage of toxic, highly flammable and carcinogenic solvents such as dichloroethane as reaction solvent and requires higher temperatures and longer time for completion of the reaction. It involves column chromatography technique for purification of the reaction intermediate which is not suggestible. Further the above process provides the required product in very low yields.

Later US6949640 B2 patent (herein after referred as '640' patent) discloses the preparation of Clofarabine by the reaction of 2-deoxy-2-fluoro-3,5-di-O-benzoyl-[alpha]-D-arabinofuranosyl bromide with 2,6-dichloropurine in presence of NaH at room temperature. The reaction takes place over night and the obtained compound contains mixture of  $\alpha$  and  $\beta$  isomers, which are separated by chromatography. The obtained  $\beta$ -anomer was further treated with sodium methoxide in methanol followed by chromatography purification to provide 2-Chloro-9-(2-deoxy-2-fluoro-[beta]-D-arabinofuranosyl)-6-methoxy-9H-purine. Finally the obtained methoxy compound was treated with anhydrous ammonia in ethanol at 80°C for 16-20 hours to provide Clofarabine.



The above process involves the usage of NaH as a base for the reaction of bromo compound with 2,6-dichloropurine. Since, NaH is a pyrophoric base its use in the laboratory is not advisable in safety point of view. The process disclosed in '640' patent involves longer reaction times and requires column chromatography technique at various stages for purification of the reaction products, which makes the process tedious and uneconomic.

US6680382 patent discloses a process for the preparation of Clofarabine by the reaction of 2-deoxy-2-fluoro-3,5-di-O-benzoyl-[alpha]-D-arabinofuranosyl bromide with chloroadenine in presence of KOBt in various solvents provides mixture of  $\alpha$ ,  $\beta$ -anomers of 2-chloro-9-(3,5-O-dibenzoyl-2-deoxy-2-fluoro-[beta]-D-arabinofuranosyl)adenine. Further the obtained compound was purified from different solvents and provide pure  $\beta$ -anomer which was further converted into Clofarabine by treating it with sodium methoxide. This process also involves longer reaction times and provides the product with less purity.



All the prior-reported processes for the preparation of Clofarabine by reaction of 3-acetyl-5-benzoyl-2-deoxy-2-fluoroarabinofuranosylbromide/2-deoxy-2-fluoro-3,5-di-O-benzoyl-[alpha]-D-arabinofuranosyl bromide with 2,6-dichloropurine/chloroadenine requires longer reaction times, higher temperatures and unsafe solvents or bases and leads to the formation of corresponding  $\alpha$ ,  $\beta$ -anomer mixture. Further the yields and quality of the product is also very low.

The present inventors overcomes the major problems encountered in the prior-art by substituting the strong base like NaH with alkali metal carbonates during the condensation of 2-deoxy-2-fluoro-3,5-di-O-benzoyl-[alpha]-D-arabinofuranosyl bromide with 2,6-dichloropurine facilitates the completion of the reaction within 5-7 hrs with excellent yield and purity of the

final product. Apart from this, the formation of  $\alpha$ -anomer is reduced to minimum levels to provide highly pure required  $\beta$ -anomer in high yields which also enhances the purity and yield of final clofarabine.

### **Advantages of the present invention:**

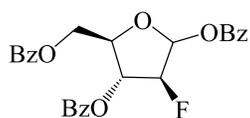
- Avoiding pyrophoric bases by adopting simple and mild bases such as alkali metal carbonates.
- Lesser reaction times
- Avoiding column chromatography technique for the purification of the reaction products.
- The present invention provides highly pure Clofarabine with enhanced yields.

The term “suitable solvent” used in the present invention refers to “hydrocarbon solvents” such as n-pentane, n-hexane, n-heptane, cyclohexane, methyl cyclohexane, cycloheptane, pet ether, toluene, xylene and the like; “ether solvents” such as dimethyl ether, diethyl ether, diisopropyl ether, methyl tert-butyl ether, ethyl tert-butyl ether, di-tert-butyl ether, dimethoxy methane, 1,2-dimethoxyethane, diglyme, 1,4-dioxane, tetrahydrofuran, 2-methyl tetrahydrofuran and the like; “ester solvents” such as methyl acetate, ethyl acetate, n-propyl acetate, isopropyl acetate, n-butyl acetate, iso-butyl acetate, tert-butyl acetate, diethyl carbonate and the like; “polar-aprotic solvents” such as dimethylacetamide (DMAc), N,N-dimethylformamide (DMF), dimethylsulfoxide (DMSO), N-methyl-2-pyrrolidone (NMP), hexamethylphosphoramide (HMPA) and the like; “nitrile solvents” such as acetonitrile, propionitrile, butyronitrile, isobutyronitrile and like; “chloro solvents” such as dichloromethane, dichloroethane, chloroform, carbon tetrachloride and the like; “ketone solvents” such as acetone, methyl ethyl ketone, diethyl ketone, methyl isopropyl ketone, methyl isobutyl ketone and the like; “alcohol solvents” such as methanol, ethanol, n-propanol, isopropanol, n-butanol, iso-butanol, tert-butanol, 2-pentanol, ethylene glycol, diethylene glycol, propylene glycol, 2-ethyl hexanol, benzyl alcohol and the like; “polar solvents” such as water; acetic acid or mixtures thereof.

The term “suitable base” used in the present invention refers to inorganic bases selected from “alkali metal carbonates” such as sodium carbonate, potassium carbonate, lithium carbonate, cesium carbonate and the like; “alkali metal bicarbonates” such as sodium bicarbonate, potassium bicarbonate, lithium bicarbonate, cesium bicarbonate and the like; “alkali metal hydroxides” such as sodium hydroxide, potassium hydroxide, lithium hydroxide and the like; “alkali metal alkoxides” such as sodium methoxide, sodium ethoxide, potassium methoxide, potassium ethoxide, sodium tert.butoxide, potassium tert.butoxide and the like; “alkali metal amides” such as sodium amide, potassium amide, lithium amide, lithium diisopropyl amide (LDA) and the like; “alkali metal phosphates” such as disodium hydrogen phosphate, dipotassiumhydrogen phosphate; and “organic bases” selected from but not limited to methyl amine, ethyl amine, diisopropyl amine, diisopropylethyl amine (DIPEA), diisobutylamine, triethylamine, tert.butyl amine, pyridine, 4-dimethylaminopyridine (DMAP), N-methyl morpholine (NMM), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), 1,5-diazabicyclo[4.3.0]non-5-ene (DBN), 1,4-diazabicyclo[2.2.2]octane (DABCO), imidazole; or mixtures thereof.

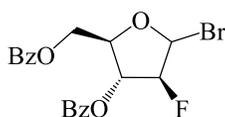
The first aspect of the present invention provides a process for the preparation of pure Clofarabine compound of formula-1, comprising of;

- a) Reacting the 2-Deoxy-2-fluoro-1,2,5-tri-O-benzoyl-D-ribofuranose compound of formula-2



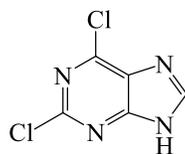
Formula-2

with a suitable brominating agent in a suitable solvent to provide 2-Deoxy-1- $\alpha$ -bromo-2- $\beta$ -fluoro-3,5-di-O-benzoyl-D-ribofuranose compound of formula-3,



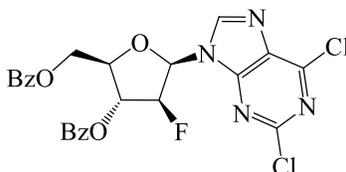
Formula-3

- b) reacting the compound of formula-3 with 2,6-dichloropurine compound of formula-4



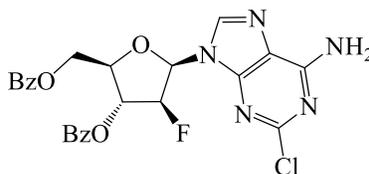
Formula-4

in presence of suitable inorganic base in a suitable solvent to provide 2,6-dichloro-9-(3,5-di-O-benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl)-9H-purine compound of formula-5,



Formula-5

- c) amination of compound of formula-5 with a solution of ammonia in dimethoxyethane in a suitable solvent to provide 6-amino-2-chloro-9-(2'-deoxy-2'-fluoro-3',5'-di-O-benzoyl-β-D-arabino furanosyl)-9H-purine compound of formula-6



Formula-6

- d) debenzoylation of compound of formula-6 by treating it with a suitable base in a suitable solvent to provide 2-chloro-9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-9H-purine-6-amine (Clofarabine) compound of formula-1,
- e) purifying the compound of formula-1 by recrystallizing it from a suitable solvent to provide pure Clofarabine compound of formula-1.

Wherein, in step-a) the suitable brominating agent is selected from hydrobromic acid optionally in combination with acetic acid, bromine, N-bromosuccinimide (NBS), phosphorous tribromide and the like; the suitable solvent is selected from chloro solvents, acetic acid, alcohol solvents, polar solvents, hydrocarbon solvents, nitrile solvents, ether solvents, ester solvents, polar-aprotic solvents or their mixtures;

in step-b) the suitable inorganic base is selected from alkali metal carbonates, alkali metal

hydroxides, alkali metal bicarbonates and the like; the suitable solvent is selected from nitrile solvents, polar-aprotic solvents, chloro solvents, hydrocarbon solvents, ester solvents, ketone solvents, ether solvents or their mixtures;

in step-c) the suitable solvent is selected from chloro solvents, acetic acid, alcohol solvents, polar solvents, hydrocarbon solvents, nitrile solvents, ether solvents, ester solvents, polar-aprotic solvents or their mixtures;

in step-d) the suitable base is selected from organic or inorganic base, preferably alkali metal alkoxides; the suitable solvent is selected from chloro solvents, acetic acid, alcohol solvents, polar solvents, hydrocarbon solvents, nitrile solvents, ether solvents, ester solvents, polar-aprotic solvents or their mixtures;

in step-e) the suitable solvent is selected from alcohol solvents, preferably methanol.

A preferred embodiment of the present invention provides a process for the preparation of pure Clofarabine compound of formula-1, comprising of;

- a) Reacting the 2-Deoxy-2-fluoro-1,2,5-tri-O-benzoyl-D-ribofuranose compound of formula-2 with hydrobromic acid in acetic acid in presence of dichloromethane solvent to provide 2-Deoxy-1- $\alpha$ -bromo-2- $\beta$ -fluoro-3,5-di-O-benzoyl-D-ribofuranose compound of formula-3,
- b) reacting the 2-Deoxy-1- $\alpha$ -bromo-2- $\beta$ -fluoro-3,5-di-O-benzoyl-D-ribofuranose compound of formula-3 with 2,6-dichloropurine compound of formula-4 in presence of cesium carbonate in acetonitrile to provide 2,6-dichloro-9-(3,5-di-O-benzoyl-2-deoxy-2-fluoro-D-arabino furanosyl)-9H-purine compound of formula-5,
- c) amination of compound of formula-5 with a solution of ammonia in dimethoxyethane in presence of dichloromethane solvent to provide 6-amino-2-chloro-9-(2'-deoxy-2'-fluoro-3',5'-di-O-benzoyl- $\beta$ -D-arabinofuranosyl)-9H-purine compound of formula-6,
- d) debenzoylation of compound of formula-6 by treating it with sodium methoxide in methanol to provide 2-chloro-9-(2'-deoxy-2'-fluoro- $\beta$ -D-arabinofuranosyl)-9H-purine-6-amine compound of formula-1,
- e) purifying the compound of formula-1 by recrystallizing it from methanol to provide pure Clofarabine compound of formula-1.

The 2-Deoxy-2-fluoro-1,2,5-tri-O-benzoyl-D-ribofuranose compound of formula-2 utilized in the present invention can be synthesized by any of the processes known in the art. For example it can be synthesized by the process described in *J.Org.Chem*, 1985, 50, 3644-47.

The 2,6-dichloropurine can be obtained from any commercial sources or it can be synthesized by any of the processes known in the art such as described in WO2003048161A1, *Org. Proc. Res. Dev.*, 2004, 8 (6), 962–963.

The second aspect of the present invention provides a process for the preparation of 2,6-dichloro-9-(3,5-di-O-benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl)-9H-purine compound of formula-5, comprising of reacting the 2-Deoxy-1- $\alpha$ -bromo-2- $\beta$ -fluoro-3,5-di-O-benzoyl-D-ribofuranose compound of formula-3 with 2,6-dichloropurine compound of formula-4 in presence of a suitable inorganic base in a suitable solvent to provide 2,6-dichloro-9-(3,5-di-O-benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl)-9H-purine compound of formula-5.

Wherein, the suitable inorganic base is selected from alkali metal carbonates, alkali metal hydroxides, alkali metal bicarbonates and the like, preferably cesium carbonate; the suitable solvent is selected from nitrile solvents, polar-aprotic solvents, chloro solvents, hydrocarbon solvents, ester solvents, ketone solvents, ether solvents or their mixtures.

The reaction of 2-Deoxy-1- $\alpha$ -bromo-2- $\beta$ -fluoro-3,5-di-O-benzoyl-D-ribofuranose compound of formula-3 with 2,6-dichloropurine compound of formula-4 is preferably carried out in presence of alkali metal carbonates, more preferably cesium carbonate.

In the above reaction, the inorganic base is used in an amount ranging from 0.7 to 2.5 mole ratio, preferably 0.8 to 2.2 mole ratio, more preferably 1.0 to 2.0 mole ratio per one mole of compound of formula-3.

In the present invention, the reaction of 2-Deoxy-1- $\alpha$ -bromo-2- $\beta$ -fluoro-3,5-di-O-benzoyl-D-ribofuranose compound of formula-3 with 2,6-dichloropurine compound of formula-4 is carried out at a temperature of 20-35°C, preferably at a temperature of 25-30°C.

A preferred embodiment of the present invention provides a process for the preparation of 2,6-dichloro-9-(3,5-di-O-benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl)-9H-purine compound of formula-5, comprising of reacting the 2-Deoxy-1- $\alpha$ -bromo-2- $\beta$ -fluoro-3,5-di-O-benzoyl-D-

ribofuranose compound of formula-3 with 2,6-dichloropurine compound of formula-4 in presence of cesium carbonate in acetonitrile to provide 2,6-dichloro-9-(3,5-di-O-benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl)-9H-purine compound of formula-5.

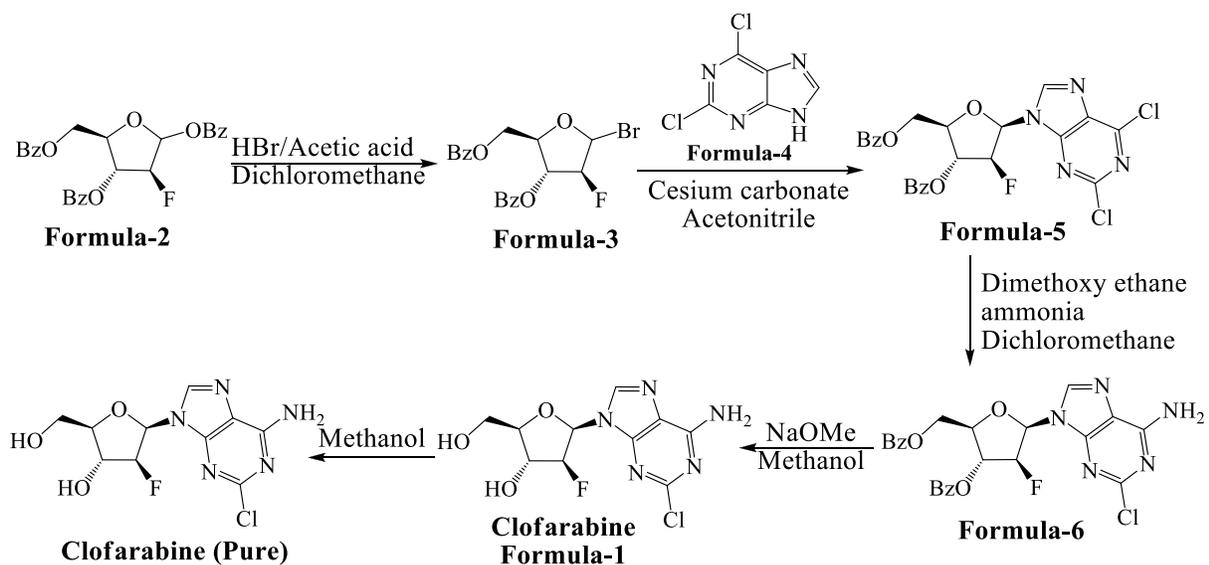
Clofarabine obtained by the process of the present invention is having a purity of greater than 98%, preferably greater than 99%, more preferably greater than 99.5%, most preferably greater than 99.9% by HPLC.

Clofarabine obtained by the process of the present invention was analyzed by HPLC under the following conditions;

Apparatus: A liquid chromatographic system is to be equipped with variable wavelength UV-detector; Column: Akzonobel Kromasil 100-5C18 250×4.6 mm, 5 µm or equivalent; Flow rate: 1.2 mL/minute; Wave length: 263 nm; Injection volume: 5 µL; Column temperature: 30°C; Run time: 50 minutes; Mobile phase-A: Transfer accurately 1 ml of orthophosphoric acid (85%) in 1000 ml of milli-Q-water, filter the solution through 0.22 µm nylon membrane filter paper. Mobile phase-B: Acetonitrile: water (90:10 v/v); Diluent: Methanol; Elution: Gradient.

Clofarabine compound of formula-1 of the present invention can be further micronized or milled to get the desired particle size to achieve desired solubility profile based on different forms of pharmaceutical composition requirements. Techniques that may be used for particle size reduction include, but not limited to ball mills, roller and hammer mills and jet mills. Milling or micronization may be performed before drying or after the completion of drying of the product.

The present invention is schematically represented as follows.



### **Examples:**

#### **Example-1: Preparation of 2-Deoxy-1- $\alpha$ -bromo-2- $\beta$ -fluoro-3,5-di-O-benzoyl-D-ribofuranose (Formula-3)**

A mixture of 2-Deoxy-2-fluoro-1,2,5-tri-O-benzoyl-D-ribofuranose compound of formula-2 (100 gm) and dichloromethane (400 ml) was stirred for 10 min at 25-30°C. A solution of hydrobromic acid in acetic acid (200 ml) was added to the reaction mixture at 25-30°C and stirred for 3 hrs at the same temperature. Cooled the reaction mixture to 10-15°C, aqueous sodium bicarbonate solution was slowly added to the reaction mixture at 15-20°C and stirred for 10 min at the same temperature. Both the organic and aqueous layers were separated and the organic layer was washed with aqueous sodium bicarbonate solution. Distilled off the solvent completely from the organic layer under reduced pressure and co-distilled with n-heptane. To the obtained compound, n-heptane (300 ml) was added and stirred for 5 min. Cooled the reaction mixture to 20-25°C and stirred for 90 min at the same temperature. Filtered the solid, washed with n-heptane and dried to get the title compound.

Yield: 82.5 gm; Purity by HPLC: 98.81%; Ribofuranose impurity: 0.11%.

#### **Example-2: Preparation of 2,6-dichloro-9-(3,5-di-O-benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl)-9H-purine (Formula-5)**

A mixture of 2,6-dichloropurine compound of formula-4 (34.4 gm) and acetonitrile (1000 ml) was stirred for 10 min at 25-30°C. Cesium carbonate (71.2 gm) was added to the reaction mixture at 25-30°C under nitrogen atmosphere and stirred for 90 min at the same temperature. 2-Deoxy-1- $\alpha$ -bromo-2- $\beta$ -fluoro-3,5-di-O-benzoyl-D-ribofuranose compound of formula-3 (77 gm) was added to the reaction mixture at 25-30°C and stirred for 6 hrs at the same temperature under nitrogen atmosphere. Filtered the reaction mixture and distilled off the solvent completely from the filtrate under reduced pressure. Dichloromethane was added to the obtained compound at 25-30°C and stirred for 15 min at the same temperature. Filtered the reaction mixture, water was added to the filtrate and stirred for 10 min. Both the organic and aqueous layers were separated and the organic layer was washed with water. Distilled off the solvent completely from the organic layer and co-distilled with methanol. To the obtained compound, methanol (385 ml) was added at 25-30°C. Heated the reaction mixture to 50-55°C and stirred for

90 min at the same temperature. Cooled the reaction mixture to 20-25°C and stirred for 90 min at the same temperature. Filtered the solid, washed with methanol and suck dried. Dichloromethane (77 ml) was added to the obtained wet solid at 20-25°C and stirred for 10 min at the same temperature. Methanol (308 ml) was added to the reaction mixture at 20-25°C and stirred for 60 min at the same temperature. Filtered the precipitated solid, washed with methanol and dried to get the title compound.

Yield: 64.2 gm.

Purity by HPLC: 94.42%; Dichloropurine impurity: Not detected; Bromo impurity: 2.2%.

### **Example-3: Preparation of 6-amino-2-chloro-9-(2'-deoxy-2'-fluoro-3',5'-di-O-benzoyl-β-D-arabinofuranosyl)-9H-purine (Formula-6)**

A mixture of 2,6-dichloro-9-(3,5-di-O-benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl)-9H-purine compound of formula-5 (60 gm) and dichloromethane (120 ml) was stirred for 10 min at 25-30°C. Slowly added a solution of ammonia in dimethoxyethane (900 ml) lot wise to the reaction mixture at 25-30°C and stirred for 12 hrs at the same temperature. Dichloromethane (360 ml) and followed by water (180 ml) were added to the reaction mixture and stirred for 15 min at the same temperature. Both the organic and aqueous layers were separated and the organic layer was washed with water. Distilled off the solvent completely from the organic layer under reduced pressure. Methanol (300 ml) was added to the obtained compound at 25-30°C and stirred for 90 min at the same temperature. Filtered the solid and washed with methanol. To the obtained wet solid, methanol (300 ml) was added at 25-30°C. Heated the reaction mixture to 55-60°C and stirred for 60 min at the same temperature. Cooled the reaction mixture to 25-30°C and stirred for 45 min at the same temperature. Filtered the solid, washed with methanol and dried to get the title compound.

Yield: 48.0 gm.

### **Example-4: Preparation of Clofarabine**

A mixture of 6-amino-2-chloro-9-(2'-deoxy-2'-fluoro-3',5'-di-O-benzoyl-β-D-arabinofuranosyl)-9H-purine compound of formula-6 (48 gm) and methanol (240 ml) was stirred for 10 min at 25-30°C. Sodium methoxide solution (6 ml) was added to the reaction mixture at

25-30°C and stirred for 3 hrs at the same temperature. Acetic acid (1.3 ml) was added to the reaction mixture, cooled the reaction mixture to 0-5°C and stirred for 90 min at the same temperature. Filtered the precipitated solid, washed with methanol. Methanol (180 ml) was added to the obtained solid, heated the reaction mixture to 55-60°C and stirred for 60 min at the same temperature. Cooled the reaction mixture to 25-30°C and stirred for 45 min at the same temperature. Filtered the precipitated solid, washed with methanol and dried to get the title compound.

Yield: 24.0 gm.

Purity by HPLC: 99.91%;  $\alpha$ -anomer impurity: 0.01%; Dichloropurine impurity: Not detected; Chloroadenine impurity: Not detected; Clofarabine monobenzoate impurity: 0.09%.

#### **Example-5: Purification of Clofarabine**

A mixture of Clofarabine (20 gm) and methanol (1200 ml) was heated to 55-60°C and stirred for 45 min at the same temperature. Activated carbon (0.2 gm) was added to the reaction mixture at 55-60°C and stirred for 15 min at the same temperature. Filtered the reaction mixture through hyflow bed and distilled off the solvent completely from the filtrate. Methanol (120 ml) was added to the obtained compound, heated the reaction mixture to 55-60°C and stirred for 60 min at the same temperature. Slowly cooled the reaction mixture to 25-30°C and stirred for 90 min at the same temperature. Filtered the precipitated solid, washed with methanol and dried to get the title compound.

Yield: 16.5 gm; Specific optical rotation: (+) 53.47° (C= 1% in methanol); Water content: 0.09%; Purity by HPLC: 99.95%;  $\alpha$ -anomer impurity: 0.01%; Dichloropurine impurity: Not detected; Chloroadenine impurity: Not detected; Clofarabine monobenzoate impurity: 0.02%; Clofarabine dibenzoate impurity: Not detected; Chloro impurity: 0.01%.