(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 September 2006 (14.09.2006)

PCT

(10) International Publication Number
WO 2006/095359 A1

(51) International Patent Classification[7]: C07H 19/073

(21) International Application Number:
PCT/IN2005/000160

(22) International Filing Date: 19 May 2005 (19.05.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
240/CHE/2005 10 March 2005 (10.03.2005) IN

(71) Applicant *(for all designated States except US)*: **SMS PHARMACEUTICALS LIMITED** [IN/IN]; 417, Nilgiri, Aditya Enclave, Ameerpet, Hyderabad 500 038 (IN).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **POTLURI, Ramesh, Babu** [IN/IN]; 417, Nilgiri, Aditya Enclave, Ameerpet, Hyderabad 500 038 (IN). **VENKATA SUBRAMANIAN, Hariharakrishnan** [IN/IN]; 417, Nilgiri, Aditya Enclave, Ameerpet, Hyderabad 500 038 (IN). **BETINI, Ramesh** [IN/IN]; 417, Nilgiri, Aditya Enclave, Ameerpet, Hyderabad 500 038 (IN). **GUNTURU, Satyanarayana** [IN/IN]; 417, Nilgiri, Aditya Enclave, Ameerpet, Hyderabad 500 038 (IN).

(74) Agent: **VISHWANATH, Ramesh, B.**; Bldg # 48 SRT, 1st Floor, Prakash Nagar, Begumpet, Hyderabad 500 016 (IN).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2006/095359 A1

(54) **Title:** SYNTHESIS OF 2-DEOXY-2, 2-DI FLUORO-D-RIBO FURANOSE-3, 5 DI(4-METHY/4-NITRO-CHLORO)BENZOATE AND ITS CONVERSION TO GEMCITABINE HYDROCHLORIDE THEREOF

(57) **Abstract:** The invention describes the synthesis of hereto unreported 2-deoxy-2,2-difluoro-D-ribofuranose-3,5-di-(aroyl) derivative of formula-Vb, where in R=;CH_3,CI,NO_2 and its conversion to Gemcitabine HCl of formula-I, an anti cancer product.

DESCRIPTION OF THE INVENTION:

Field of invention:

The invention relates to the filed of chemistry and more particularly relates to the synthesis of 2-deoxy-2,2-di fluoro-D-ribo furanose – 3,5 di (4-methyl / 4-nitro/4-chloro) benzoate and its conversion to Gemcitabine. HCl thereof.

References Cited

US Pat.No 4526988

US Pat.No 4808614

US Pat No 5233608

US Pat No 5945547

EP Pat No 306190

Other References

Synthesis 565-570 1992

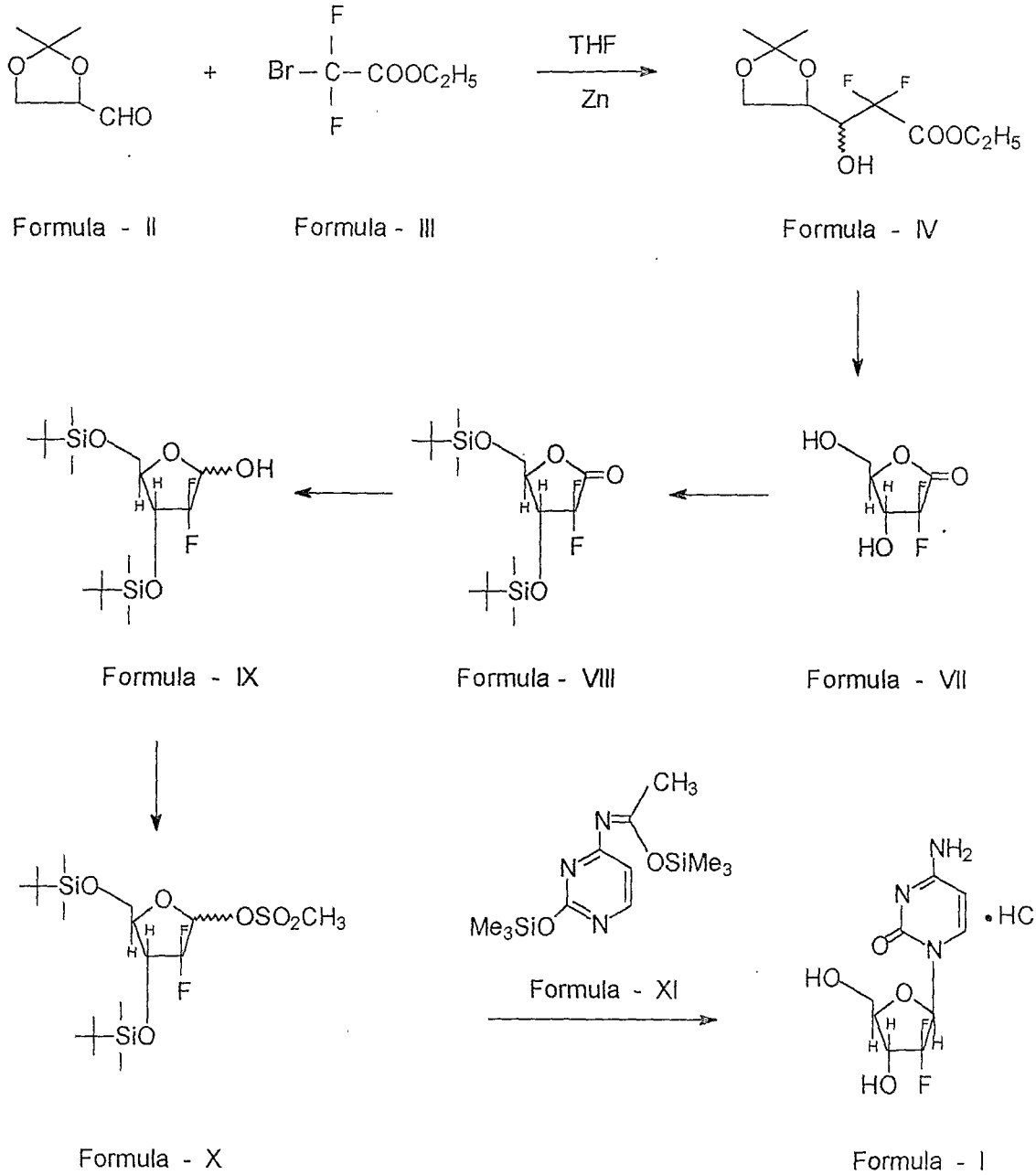
BACKGROUND OF THE INVENTION:

Furanosyl nucleosides like Cytarabine and Zalcitabine have been used for the treatment of acute mylogenous leukemia. Introducing fluorine into nucleosides has also proved to be useful procedure for modifying the biological activity of these compounds. Gemcitabine, a 2'-deoxy-2',2'-difluoro nucleoside has proved to be highly active against cancer and has been used for treating several types of tumors.

The synthesis of Gemcitabine has been described in many patents.The basic chemistry approach has remained same,except variations in the protecting groups and improvements in the purification methodology.

The synthesis as described in US Pat Nos 4526988 and 4808614 is given in

scheme-I



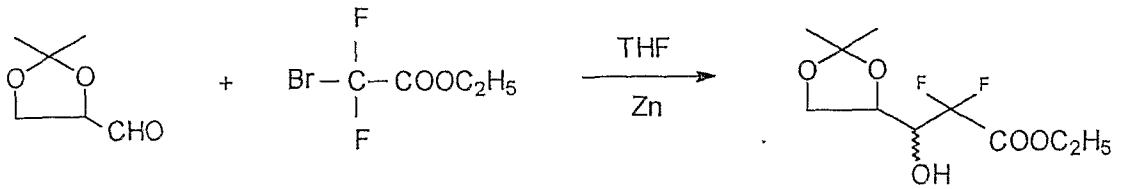
Scheme-I

In this process, (R)-2,3-O-isopropylidene carboxaldehyde was treated with ethyl bromo difluoro acetate under Reformatsky conditions to yield the product of formula IV, which contained R and S isomers in the ratio 3:1 and this mixture

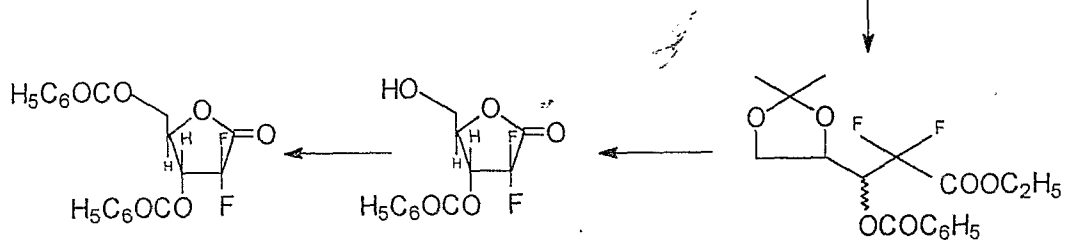
was purified by column chromatography to get the pure R-isomer. This product was hydrolytically cyclized using ion-exchange resin to give 2-deoxy-2,2-difluoro-1-oxo ribose of formula VII, which was converted into bis-t-butyl dimethyl silylated derivative and purified by column chromatography to get a product of formula VIII. The lactone of formula VIII was reduced with DIBAL to give lactol of formula IX, which was again purified by column chromatography.

Reaction of this product with methane sulfonyl chloride in presence of a base yielded the product of formula X, which was coupled with bis silylated cytosine of formula XI and the coupled product was deprotected and purified by preparative HPLC to get Gemcitabine HCl product of formula I

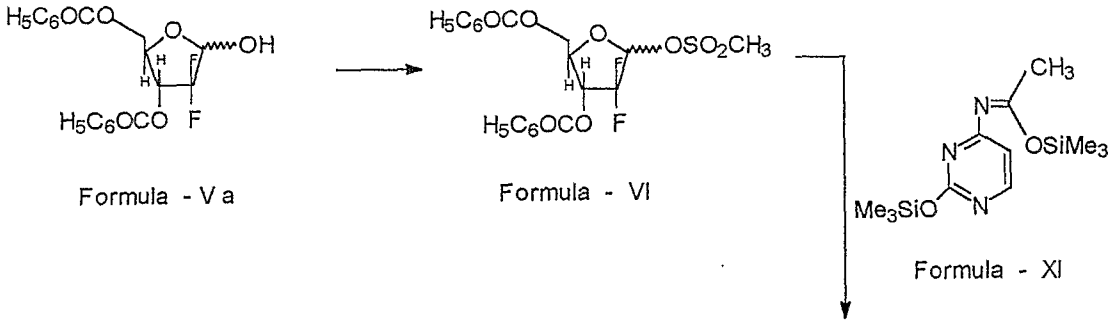
The process, as described in US Pat No. 5223608, is depicted in scheme II



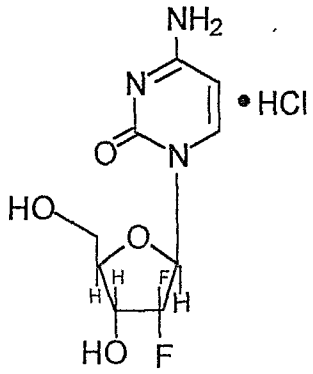
Formula - II Formula - III Formula - IV



Formula - V Formula - XIII Formula - XII



Formula - V a Formula - VI Formula - XI



β - anomer

Scheme - II

The method followed in US Pat No 5223608 is similar to the one described in scheme-I upto the preparation of the product of formula -IV This was first benzoylated without separation of R&S isomers to give a product of formula XII, which was subjected to hydrolytic cyclization to give a product of formula XIII. This product was benzoylated and purified by crystallization, preferably, from a mixture of dichloromethane and hexane/heptane or isopropyl alcohol/hexane to give a product of formula V viz. 2-deoxy-2,2-difuluoro-D-erythreo pentafuranose-1-ulose-3,5-dibenzoate in about 95% purity.

The product of formula V was reduced to give lactol derivative of formula Va and then mesylated to give a product of formula VI. The mesylate, of formula VI, was coupled with bissilyl acetyl cytosine to give the protected nucleoside, which was hydrolyzed, converted into hydrochloride salt and crystallized to give Gemcitabine HCl of greater than 99% purity.

These are the two major approaches for the synthesis of Gemcitabine HCl. The method described in US Pat Nos 4526988 and 4808614 adopt laborious chromatographic purifications in different stages which make the process industrially unviable. The process described in US Pat.No.5223608 takes care of these shortcomings. In the latter procedure also, there are a few shortcomings , which are listed below

a) isolation of 2-deoxy-2,2,-difluoro-D-erythropentafurano-1-ulose-3,5-dibenzoate

b) reduction of the lactone of formula V to the lactol of formula Va

c) isolation of the 1:1 α/β anomers and further purification

The crude product of formula V containing the erythro and threo

isomers was crystallized, preferably, from a mixture of dichloromethane and hexane. When this method was followed, there was large variation in the proportion of erythro and threo for different batches and it was tedious to get a product mixture containing 95% rich in erythrose isomer

The reduction of formula V to the lactol of formula Va was carried out using DIBAL or tri-tertiary butoxy lithium aluminum hydride. Both these reagents are expensive and difficult to handle.

The crude Gemcitabine HCl was isolated as 1:1 α and β anomer mixture in aqueous isopropyl alcohol and subsequently crystallized from aqueous acetone to get the product of formula I in greater than 99% purity by HPLC.

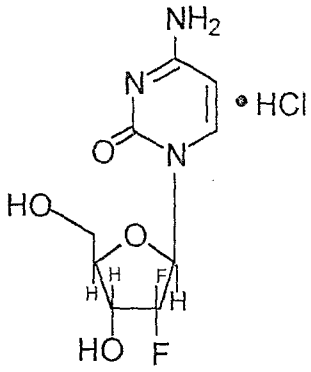
Object of the invention :

One of the objective of the present invention is to prepare a novel derivative similar to di benzoate of fomula-V Viz diaroyl derivative of formula Vb , which can be isolated and easily purified .Another objective is to convert the lactone derivative of formula Vb to lactol derivative by using a reagent, which is cheaper and easier to handle.

Yet another objective of the invention is to develop a methodology , which results in Gemcitabine hydrochloride of extra high purity.

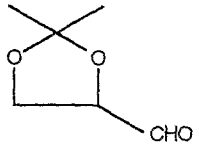
Summary of the invention :

The invention describes a new process for the manufacture of high pure Gemcitabine hydrochloride of formula I



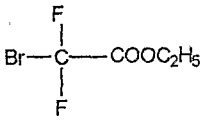
Formula-I

by the reaction of (R)2,3-o-isopropylidene glyceraldehyde of formula II



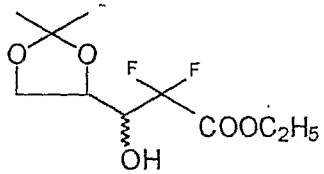
Formula - II

with ethyl bromo difluoro acetate of formula III



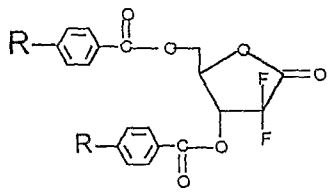
Formula - III

to give a product of formula IV,



Formula - IV

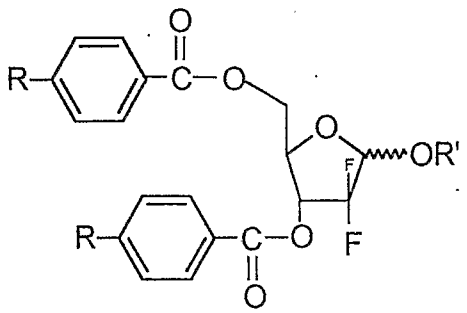
which is hydrolytically cyclized, converted into a diaroyl derivative and purified to give a product of formula Vb



Formula - Vb

where R is CH_3,Cl,NO_2

Product of formula Vb is reduced and converted into a mesylate of formula VIb



Formula - VI b

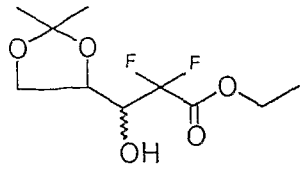
R is CH_3.Cl, NO_2

R' is CH_3SO_2, $C_6H_5SO_2$, 4-CH_3—$C_6H_4SO_2$ etc

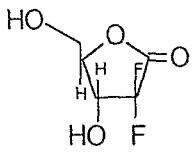
which is coupled with bis trimethylsilyl acetyl cytosine of formula XI, under Verburggen condition, hydrolyzed and isolated to give product of formula I in 95% purity. This is further purified to yielded a product of extra high purity of >99.8%.

Detailed description of the invention :

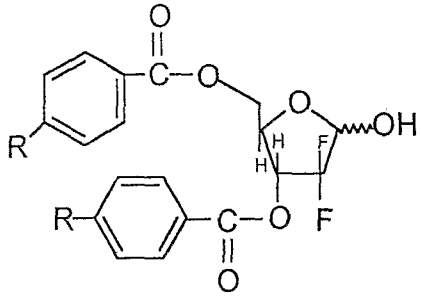
The method adopted in this invention is represented in the Scheme -III



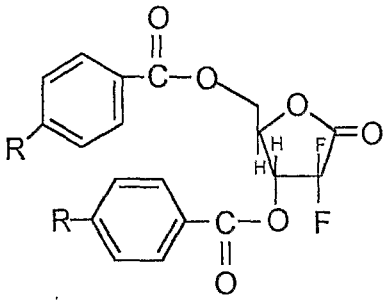
IV



VII

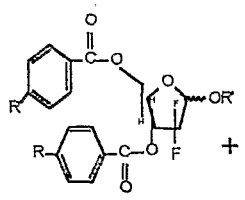


VIa



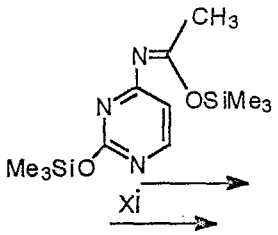
V b

a) R=CH_3, b) R=Cl; c) R=NO_2

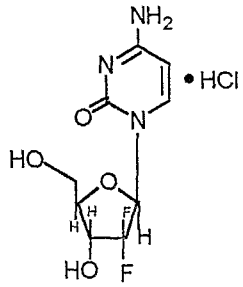


VIb

a) R=CH_3, b) R=Cl, c) R=NO_2



Xi



I

a) R'=CH_3SO_2, b) R'=$C_6H_5SO_2$, c) R'=$CH_3C_6H_4SO_2$, d) R'=$^4ClC_6H_4SO_2$

Scheme-III

Ethyl 2,2-difluoro-3-hydroxy-3-(2,2-dimethyl dioxalan-4-yl) acetate was hydrolytically cyclised to give a product of formula - VII. This reaction was carried out in acetone, acetonitrile, etc containing water and an acid like trifluoroacetic acid, sulphuric acid, benzene sulphonic acid, p-toluene sulphonic acid etc. The reaction was preferably conducted in aqueous acetone or acetonitrile using acid catalyst at a temperature of 60 – 80^{O} C. After the maintenance for about 4 – 5 hours, the reaction mixture was subjected to distillation along with simultaneous addition of toluene and this was continued until the mass temperature was ~ 110^{O} C. The progress of the reaction was monitored by IR. As the reaction progressed, the >C=O stretching vibration for the starting material at 1760 Cm^{-1} disappeared and >C=O stretching vibration for the product appeared at 1812 Cm^{-1}.

On completion, the reaction mixture was concentrated under reduced pressure, the residue obtained was dissolved in ethyl acetate, washed with ice-cold water, dried over anhydrous sodium sulphate and filtered. The filtrate was used for the next stage.

The product of formula - VII was reacted with an aroyl chloride like p-toluoyl chloride, p-chloro benzoyl chloride, p-nitro benzoyl chloride etc in the presence of an organic base like pyridine, picolines etc. The reaction was preferably conducted in the presence of a promoter like 4-dimethyl amino pyridine. The reaction was conducted more preferably at a temperature of 60 – 70^{O} C and the progress of the reaction was followed by TLC. On completion, the reaction was worked up and the residue obtained was purified using a mixture di chloro methane, di chloro ethane, chloroform, etc and

hexane, heptane, petroleum ether, cyclo hexane etc. The purity of the product (HPLC) was not acceptable. The product was a mixture of 80 – 85% of erythro, 15 – 20% threo .Since the purification using a mixture of dichloro methane, di chloro ethane, chloroform etc and hexane, heptane, petroleum ether, cyclo hexane etc. did not yield a product of acceptable quality, it was decided to try out other solvent systems. The solvent systems tried were mixture of benzene, toluene, xylene, fluoro benzene etc and hexane, petroleum ether, heptane, cyclohexane etc. It was preferable to use a mixture of toluene or fluoro benzene with hexane, heptane, cyclohexane etc. The residue after aroylation, with the respective aroyl chloride, was dissolved in about 6 to 8 volumes of the aromatic solvent and subjected to azeotropic distillation. It was preferably concentrated completely and then the residue was again dissolved in 4 to 6 volumes of the aromatic solvent. The solution was warmed to 60 – 80^{O} C and with stirring was diluted with an equal volume of the alkane VIZ petroleum ether, hexane, heptane, cyclohexane etc. On cooling the diaroyl derivative precipitated in good yield and excellent purity (>99% by HPLC). The yield of the diaroyl derivative was about 25%.

Earlier reports describe the reduction of lactone derivative of formula - Vb to the corresponding lactol derivative of formula VIa using either bis--tert-butoxy lithium aluminium hydride or DIBAL. Since these reagents are expensive and difficult to handle, another objective of the invention was to utilize a reagent, which is cheaper and easier to handle. Vitride i.e Sodium bis(2-methoxy ethoxy) aluminium hydride, as a 65% w/w solution in toluene, is commercially available. This is a versatile reagent, which is cheap and easy

to handle. It was decided to try the conversion of the product of formula Vb to the product of formula VIa using vitride.The reaction was tried in solvents like 1,2 dimethoxy ethane, diethylene glycol dimethyl ether, 1,4-dioxane etc. It was preferable to use 1,2 di methoxy ethane, di ethylene glycol di methyl ether, THF, etc. The mole ratio of the substrate to that of the reagent was preferably

1:0.5 – 0.75 moles. The reaction was preferably conducted at -10^{O} to +20^{O}C. The progress of the reaction mixture was monitored by HPLC(Zorbax CN hexane and isopropylakcohol 92:8 at λ 254). The product showed up as two peaks.

The product obtained as described above was converted into a product of formula - Vib by reacting with aliphatic/aromatic sulphonyl chloride. This reaction was conducted preferably in a solvent like dichloro methane dichloroethane, ethylacetate etc using triethylamine or diisopropylethylamine or N-methyl morpholine as the acid scavenger. The progress of the reaction was monitored by HPLC(Zorbax CN hexane:isopropyl alcohol 92:8 λ 254). The product showed up as two peaks. On completion, the reaction mixture was worked up and the product obtained was used for coupling with bis silyl acetyl cytosine.

Acetyl cytosine was converted into bissilyl acetyl cytosine by treatment with hexa methyl disilazane before it was subjected to coupling with product of product of VI b. The reaction was conducted preferably in the presence of trimethyl silyltri fluoro methane sulfonate. Several other reagents like $SnCl_4$, $ZnCl_2$ etc. were also tried but the yields were not very good. The coupling reaction was tried in several solvents like benzene, toluene, xylene, fluoro

benzene, methylenechloride,dichloro ethane, ethylacetate, acetonitrile, etc. It was preferable to conduct the reaction in toluene, fluoro benzene, dichloroethane, ethyl acetate, acetonitrile, etc.It was more preferably to conduct the reaction in toluene,fluoro benzene,ethylacetate etc. It was preferable to conduct the reaction at a temperature of 60 - 80° C for a duration of 10 to 20 hours. On completion of the reaction, the product was deprotected by treatment with methanol containing ammonia, mono methyl amine, di methyl amine, etc. The deprotected product was converted into hydrochloride and selectively crystallized to give a product rich in >95% of β-anomer . This was further purified to give a product of >99.8%HPLC purity.

The product, obtained on coupling of bis silyl acetyl cytosine and the di aroyl derivative of formula VIb, was preferably deprotected in a solvent like methanol, ethanol, isopropanol, etc. in the presence of base like ammonia, mono methyl amine, dimethyl amine etc at a temperature of 0 – 30° C during a period of 15 – 20 hours. On completion, the alcoholic solution was concentrated under reduced pressure, and dissolved in 2 to 4 volumes of water. The aqueous solution was washed preferably with an organic solvent like ethyl acetate, hexane, etc. Sub -sequently the aqueous layer was diluted with 3 - 4 volumes of isopropyl alcohol and concentrated. This process was preferably repeated 2 to 4 times. The residue obtained was again dissolved in 3 to 6 volumes of iso propyl alcohol. The residue was more preferably dissolved in 4 to 6 volumes of iso propyl alcohol, warmed to 60 – 80° C and hydrochloric acid, 0.5 volume to that of the residue, was added. The reaction mixture was preferably stirred at 65 – 75° C for 30 - 60 minutes, allowed to cool to room temperature under stirring and was stirred further

for 10 to 16 hours. Then the reaction mixture was cooled to about 0 – 5° C and preferably stirred for 2 to 4 hours. The precipitated solid was filtered, washed with acetone and dried. The sample was ~ 95% rich in the β-anomer. The yield at this step was ~ 15% starting the diaroyl derivative Vb.

The solid, obtained above, was further purified by first stirring with water. The solid was taken in 1 to 2 volumes of water and stirred preferably for 1 – 3 hours at 15 - 30° C, filtered, washed with acetone and dried. Dissolving in water and precipitating with a solvet like acetonitrile or isopropyl alcohol or acetone further purified the product. The solid was preferably dissolved in 3 to 6 volumes of water. The solid was more preferably dissolved in 4 to 6 volumes of water, heated to about 60 – 70° C
and filtered. The filterate was diluted with one of the organic solvent mentioned earlier. The ratio of water to that of the organic solvent was preferably 1:8 to 12. The ratio of water to organic solvent was more preferably 1:9 to 11. After dilution, the mixture was stirred at 60 to 70° C for 2 to 4 hours, slowly cooled to about 20 to 25° C and further cooled to 0 – 5° C. The precipitated solid was filtered, washed with acetone and dried. The overall yield, after two purifications was

The purity of Gemcitabine HCl was 99.8 to 99.9 % by HPLC

The $[\alpha]_D^{25}$ = +48 (C=1.0, D_2O) and mp 288 – 289°C

Thus the major achievements of the invention are :

a) synthesis of new intermediates for the preparation Gemcitabine
b) utilizing a new reducing agent for the reduction of 3,5-di aroyl-2-deoxy-2,2-di fluoro-D-ribofurose

c) Verbruggen coupling in a solvent like toluene, fluoro benzene, ethyl acetate, etc by which a class I solvent like di chloro ethane, can be avoided.

d) a new method of purification for Gemcitabine hydrochloride to get an ultra pure compound.

The following examples illustrate the specific aspects of the invention. It should not be misconstrued in limiting the scope of the invention..

Example 1: Preparation of 2-deoxy-2,2-difluoro-D-erythro pento furano-1-ulose-3,5-diaroyl derivative (Vb)

Ethyl-2,2-difluoro-3-(2,2-dimethyl dioxalan-4-yl) propionate was prepared by the methods described in the literature. The product was rich in R-isomer by about 75% and this product was used in the following reactions

Ethyl-2,2-difluoro-3-(2,2dimethyl dioxalaln-4-yl) propionate 100gms, was charged into a 2 lit four-necked round bottom flask fitted with a stirrer, condenser, dropping funnel and a stopper. Acetonitrile, 1 lit, trifluoroacetic acid 5.0ml and DM water 28ml were also charged into the flask and the reaction mixture was stirred to reflux. The reaction mixture was stirred at reflux temperature for about 3 hours. Then acetonitrile was slowly distilled from the reaction mixture. Simultaneously toluene was charged dropwise into the RB flask at the rate acetonitrile was being distilled off. This process was continued until the mass temperature rose to about 110°C and the reaction mixture was stirred at 110 °C for about 15minutes. Later toluene was distilled off completely under reduced pressure at about 60 °C and the residue was used for the next reaction.

The residue, obtained above, was dissolved in ethyl acetate, 500ml and transferred into a 2 lit four-necked flask fitted with a stirrer, condenser carrying

a guard tube, an addition funnel carrying a nitrogen bubbling system and a stopper. 4-Dimethyl amino pyridine 10.3gms and pyridine 86ml were charged into the flask. Aroyl chloride, 0.83 mole was dissolved in 500 ml ethyl acetate and charged into the addition funnel. The reaction mixture was warmed to about 60 °C to 65°C under stirring and the solution of aroyl chloride was added into the flask dropwise in about 3 hours. After the addition, the reaction mixture was stirred at 60 °C to 65 °C for about 3 hours and then cooled to 25 °C to 30 °C and filtered through a bed of hyflow. Then the hyflow bed was washed with 200 ml ethyl acetate. The combained extract was successively washed with 300 ml each of 10%hydrochloric acid,10% sodium bicarbonate,saturated sodiumchloride solution and finally dried over anhydrous sodium sulphate .The ethylacetate solution was filtered and concentrated under reduced pressure. The residue was dissolved in 150 ml toluene and transferred into a three-necked round bottom flask fitted with a stirrer, addition funnel and a thermometer socket. Hexane, 300ml, was charged into the addition funnel and this was added dropwise into the stirred toluene solution. After the addition, the solution was stirred at 25 °C to 30 °C for about 1 hours, when solid started precipitating out. The reaction mixture was then cooled to about 10 °C to 15 °C and stirred at that temperature for about one hour. The solid was filtered, washed with a mixture of dichloromethane at 60 °C to yield 60.0gms of title compound having the following characteristics.

A) Aroyl= Vb, R= CH_3

^1H NMR [$CDCl_3$] δ 2.30 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 4.65 (s, 1H, CH) 5.10 (m, 1H, CH) 5.9 (m, 2H, CH_2) 7.18 (2H, d, J=8Hz, aromatic) 7.22 (2H ,d,J=8Hz,aromatic) 7.75 (2H,d,J=8Hz, aromatic) 7.85 (2H,d,J=8Hz, aromatic)

IR (KBr): 1830,1736,1711,1609,1408,1332,1273,1136 Cm^{-1}

MASS:M+405

MP: 123-125^{0}C, $[\alpha]^{25}_D$ =+ 47^0 [c=1.0, $CHCl_3$]

B) Aroyl= Vb,R=NO_2

MP: 115-117^{0}C , , $[\alpha]^{25}_D$ = +46.5^0 [c=1.0, $CHCl_3$]

^{1}H NMR [$CDCl_3$] δ 2.50 (s, 3H, CH_3), 2.65 (s, 3H, CH_3), 4.9 (s, 1H, CH) 5.10 (m, 1H, CH) 6.3 (m, 2H, CH_2) 7.2 (2H,d,J=8Hz aromatic) 7.25 (2H,d,J=8Hz, aromatic) 8.2 (2H,d.J=8Hz, aromatic) 8.4 (2H ,d,J=8Hz, aromatic)

IR(KBr): 1839,1744,1720,1615,1412,1339,1281,1140 Cm^{-1}

MASS :M+ 469

Example – 2: Preparation of 2-deoxy-2,2-difluoro-D-ribo furanose-3,5-diaroyl derivative(VIa)

In a 2 lit four-necked round bottom flask with a stirrer, condenser carrying a guard tube, addition funnel carrying a nitrogen inlet system and thermometer socket, was taken 2-deoxy-2,2-difluoro-D-erythro penta furanose-1-ulose-3,5-diaroyl derevative 100gms, and THF 1 lit. The solution was cooled to about –20°C under stirring. Vitride 60ml (65% w/w in toluene) was taken in the addition funnel and added dropwise into the flask at such a rate so to maintain the mass temperature at -20 °C. After the addition, the reactor mixture was stirred at -20 °C for 1 hour. TLC (Mobile phase-Ethyl acetate: Pet. Ether: 2:8) was checked for completion of the reaction. HPLC analysis in zarboax CN using hexane + isopropyl alcohol 92ml + 8ml indicated the product as two peaks. On completion the reaction mixture was allowed to warm upto 0 °C and treated with methanol, 15ml. Later 10% aqueous hydrochloric acid, 300ml, was added into the reaction mass and stirred for

30min. The organic layer was separated. The aqueous layer was extracted with ethyl acetate 500ml. The combined organic layer was washed with 10% $NaHCO_3$ solution, 300ml. The organic layer was separated and then washed with saturated sodium chloride solution, 300ml. Finally organic layer was separated, dried over anhydrousNa_2SO_4, filtered and concentrated under reduced pressure at 50°Cgave 100 gms crude compound. This was used in the next stage to prepare the product of formula VIa

Example –3: Preparation of 2-deoxy-2,2-difluoro-D-Ribofuranose-3,5-diaroyl-1-methyl/aryl sulfonate(VIb)

In a 2 lit four-necked round bottom flask fitted with a stirrer, condenser, addition funnel and a thermometer socket, was added the lactol, 100gm, prepared as given in example 2, and toluene, 1 lit. The solution was cooled to 0 °C under stirring and triethyl amine 54ml was added. The solution was stirred at 0 °, methane sulphonyl/arylsulphonyl chloride 0.28 mol was added dropwise maintaining the mass temperature at 0 °C. Later the reaction mass was allowed to warm upto 20 °C to 25 °C and stirred further for 12-14hours. The reaction mixture was analyzed byTLC (Mobile phase-Ethyl acetate: Pet. Ether: 2:8). It was also analyzed by HPLC in zarboax CN using hexane + isopropyl alcohol 92ml + 8ml which indicated the product as two peaks. The reaction mixture was washed with 5% aqueous sodium bicarbonate solution, 325ml, saturated sodium chloride solution 325ml and separated. The toluene extract was dried over anhydrous sodium sulphate, filtered and then concentrated to give 100 gms of the title product. This was used for the Verbruggen protocol.

Example – 4: Preparation of 2'-Deoxy-2',2'-difluoro cytidine(I)

In a 5 lit three-necked round bottom flask fitted with a stirrer, condenser and a stopper, acetyl cytosine 97.36gms, toluene 2lit, hexamethyl disilazane 169ml and trimethyl silyl chloride 5ml were charged and the reaction mixture was heated to reflux under stirring. The reaction mixture slowly attained clarity and from that point the mixture was stirred at reflux for another 3 hours. Then toluene was completely distilled off and fresh toluene 1250ml, was charged into the flask and the reaction mixture was cooled to 20 °C to 25 °C. The reaction system was maintained under nitrogen atmosphere and trimethyl silyl trifluoromethane sulfonate, 113ml, was added. Then 2-deoxy-2,2-difluoro-D-ribofuranose-3,5-diaroyl, -1-methane/ aryl sulfonate, 100gm, was also added into the flask. The reaction mixture was heated to 70 °C to 75 °C and maintained for 12-14 hours. Subsequently the reaction mixture was stirred under reflux for 2 hours. The progress of the reaction was monitored by TLC (dichloromethane: methanol 9:1). The reaction mixture was cooled to 20 °C to 25 °C and 5% aqueous hydrochloric acid, 600ml, was added dropwise in about 30minutes. After the addition, the reaction mixture was stirred for 15minutes and allowed the layers to separate. The aqueous layer was washed with toluene 2000ml and the combined toluene 9 extract was washed with a saturated solution of sodium chloride, 300ml. Then the organic layer was separated and dried with anhydrous sodium sulphate. The fluoro benzene solution was filtered and concentrated under reduced pressure at 50 °C. the residue was dissolved in 1.5lit methanol and cooled to about 0 °C to 5 °C. Ammonia gas was bubbled into the methanolic solution for about 8 hours. Then the solution was brought to about 20 °C to 25 °C and ammonia gas was bubbled for further 8 hours. The progress of the reaction reaction was monitored by TLC for completion. The methanolic solution was treated with carbon, 15gms and filtered. The filtrate was concentrated under reduced pressure

at 45 °C. The residue was dissolved in about 400ml water and the aqueous solution was washed first with 100ml ethyl acetate and then with 100ml hexane . The ethyl acetate and hexane extracts were separately washed with 50ml each of water and the water extracts were combined with the main aqueous solution. The aqueous extract was stirred with carbon, 10gm, and filtered through a bed of hyflow.The hyflow bed was washed with water, 50ml. The combined aqueous solution was then concentrated. The residue was dissolved in 1500ml isopropyl alcohol and then the solution was subjected to distillation. The addition of isopropyl alcohol and distillation was repeated two more times by adding 750ml of alcohol each time. Finally isopropyl alcohol, 700ml, was added to the residue and the solution was warmed to about 70 °C. To the warm aqueous isopropyl alcohol, concentrated hydrochloric acid 75ml was added dropwise in about 30min. The solution was stirred at 70 °C to 75 °C for about 30min, then cooled to RT and stirred for 12 hours. Later the solution was cooled to 0 °C to 5 °C, stirred at that temperature for 3 hours, the solid formed was filtered, washed with acetone and dried to give 10.0 gm of product, which was >95% rich in the β-anomer and showed $[\alpha]_D^{25}$ = +46° (c=1.0, D_2O)

Example – 5: Purification of 2'-deoxy-2',2'-difluoro cytidine(I)

Method A: About 50gms of the product of about 95% rich in β-anomer was stirred with 50ml DM water at 30 °C for about 30minutes in a 250ml three-necked round bottom flask. The solid material was filtered, washed with acetone and pressed dry to give40.0 gm of product.

The solid obtained above was taken in about 188 ml water in a 250 ml three-necked round bottom flask fitted with a stirrer, condenser and a stopper. The slurry

was heated to 70 °C to 75 °C in order to dissolve the solid: The solution was filtered to remove any insoluble material and the filtrate was transferred into a 3 lit three-necked flask fitted with a stirrer, addition funnel and stopper. The aqueous solution was stirred at 25 °C to 30 °C and acetone2.3 lit, was added dropwise from the addition funnel. After the completion of addition the slurry was stirred at 25 °C to 30 °C for 1 hour and the solid was filtered. It was washed with acetone and dried at 60 °C to give 36.0 gm of very pure product.

HPLC 99.9%, $[\alpha]_D^{25} = +47^0$ (c=1.0, D_2O)

Method B: About 50gms of the product of about 95% rich in β-anomer, was stirred with 50ml DM water at 30 °C for about 30minutes in a 250ml three-necked round bottom flask. The solid material was filtered, washed with acetone and pressed dry to give gms of product

The solid obtained above was taken in about 190 ml of water in a 250 ml three-necked round bottom flask fitted with a stirrer, condenser and a stopper. The slurry was heated to about 70 °C to 75 °C in order to dissolve the solid. The solution was filtered to remove any insoluble material and the filtrate was transferred into a 3lit three-necked round bottom flask fitted with a stirrer, addition funnel and stopper. The aqueous solution was stirred at 25 °C to 30 °C and 3 lit acetonitrile was added dropwise from the addition funnel. After the completion of addition the slurry was stirred at 25 °C to 30 °C and the solid was filtered, washed with acetone, pressed dry and dried 60 °C to give 36 gm of very pure product.

HPLC 99.92%, $[\alpha]_D^{25} = +47^0$ (c= 1.0, D_2O)

Method C: About 50gms of the product about 95% rich β-anomer was stirred with

50ml DM water at 30 °C for about 30minutes in a 250ml three-necked round bottom flask. The solid material was filtered, washed with acetone and pressed dry to give 40 gm of product.

The solid obtained above was later dissolved in about 188 ml water in a 250 ml three-necked round bottom flask fitted with a stirrer, condenser and a stopper. The slurry was heated to about 70 °C to 75 °C in order to dissolve the solid. The solution was filtered to remove any insoluble material and the filtrate was transferred into a 3 lit three necked round bottom flask fitted with a stirrer, addition funnel and stopper. The aqueous solution was stirred at 25 °C to 30 °C and isopropyl alcohol 2.3 lits was added dropwise from the addition funnel. After completion of addition, the slurry was stirred at 25 °C to 30 °C for one hour and the solid was filtered, washed with first with isopropyl alcohol, then with acetone and dried at 60 °C to give 36 gms of very pure product.

HPLC 99.92%, $[\alpha]_D^{25} = +47^0$ (c= 1.0, D_2O)

CLAIMS

1. A process for the synthesis of hereto unreported novel intermediates VIZ 2-deoxy-2,2-difluoro-D-ribofuranose-3,5-di (aroyl) derivative of formula - VbI, where is R=CH_3,Cl,NO_2 as described earlier, and its conversion to gemcitabine HCl of formual - I.
2. A claim, as claimed in claim 1, wherein the dibenzoate of formula - Vb is prepared by reacting 2-deoxy-2,2-di fluoro-1-oxo ribose of formula -VII with aroyl chloride in an organic solvent in the presence of an organic base as an acid scavenging agent and a promoter at a temperature of 0 – 100^{O} C.
3. A claim, as claimed in claim 2, wherein the acid chloride used are 4-methyl benzoyl chloride,4-nitro benzoyl chloride and 4-chloro benzoyl chloride.
4. A claim, as claimed in claim 2, wherein the reaction is conducted in ethyl acetate, isopropyl acetate, butyl acetate.
5. A claim, as claimed in claim 2, wherein the organic base used is pyridine, α-picoline, β-picoline, γ-picoline a mixture of picolines etc.
6. A claim, as claimed in claim 2, wherein the promoter used is 4-dimethyl amino pyridine.
7. A claim, as claimed in claim2, wherein the reaction is conducted at a temperature of 50 – 70^{O} C, preferably 60 – 65^{O} C.
8. A claim, as claimed in claim 2, wherein the aroyl derivative of formula - Vb, where R is as given earlier, is isolated in pure form (98 – 99 % HPLC) by crystallizing from a mixture of toluene/hexane, or heptane or petroleum ether.

9. A claim, as claimed in claim 1, wherein the aroyl derivative of formula - Vb, where R is as described earlier, is converted to gemcitabine HCl of formula - I by

a) reducing the product of formula - Vb, to give a product of formula - VIa, where R is as described earlier,

b) converting the product of formula - VIa to a product of formula - VIb, were R is as described earlier

c) coupling the product of formula - VIb with disilylated product of formula - XI

d) hydrolyzing and

e) purifying

10. A claim, as claimed in claim 9a, wherein the reduction is carried out using VITRIDE in toluene, THF, dioxane, monoglyme, diglyme, etc at -30 to +30^{O} C.

11. A claim, as claimed in claim 9b, wherein product of formula - VIa is converted to a product of formula - VIb, by reacting with alkyl or aryl sulphonyl chloride wherein alkyl/aryl sulphonyl chloride is methane sulphonyl chloride,benzene sulphonyl chloride.p-toluene sulphonyl chloride in dichloro methane or ethyl acetate in the presence of a acid scavenging agent like tri ethyl amine or diiso propyl ethyl amine or N-methyl morpholine at 0 – 50^{O} C.

12. A claim, as claimed in claim 9c, wherein the product of formula - VIb is coupled with the silylated product of formula - XI in a solvent like toluene, fluoro benzene di chloro ethane, ethylacetate and acetonitrile using tri methyl silyl tri fluoro methane sulfonate at 60 – 65^{O} C for

a duration of 8 to 24 hours.

13. A claim, as claimed in claim 9d, wherein the product formed after coupling of products of formula - VIb and XI respectively was treated with ammonia or dimethyl amine or diethylamine in anhydrous alcohols like methanol, ethanol, I-propanol or 2-propanol at $0-5^{\circ}$ C.

14. A claim, as claimed in claim 9d, wherein the hydrolyzed product was purified by,

a) isolation of the crude gemcitabin HCl in aqueous isopropanolic hydrochloric acid

b) leaching the crude product with water

c) crystallizing the water leached product in aqueous organic solvents like ethanol, 2-propanol or acetonitrile or acetone.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN 2005/000160

A. CLASSIFICATION OF SUBJECT MATTER
IPC[7]: C07H 19/073
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)
IPC[7]: C07H 19/073

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO:WPI, STN: REG,CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	T. S. Chou et al , Stereospecific Synthesis of 2-Deoxy-2,2-difluororibonolactone and Ist Use in the Preparation of 2-Deoxy-2,2-difluoro-ß-D-ribofuranosyl Pyrimidine Nucleosides: The Key Role of Selective Crystallization . Synthesis. June 1992. Pages 565-70.	1-14
	--	
A	EP 0719788 A2 (ELI LILLY AND COMPANY) 3 July 1996 (03.07.1996) *claims 1-10.*	1-14

☐ 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
31 August 2005 (31.08.2005)	14 September 2005 (14.09.2005)
Name and mailing address of the ISA/ AT **Austrian Patent Office** Dresdner Straße 87, A-1200 Vienna Facsimile No. +43 / 1 / 534 24 / 535	Authorized officer BÖHM K. Telephone No. +43 / 1 / 534 24 / 519

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/IN 2005/000160

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
A		none	
EP A 719788		GR T3 3030265T	1999-08-31
		NO A 972679	1997-06-11
		BG A 101591	1998-03-31
		BG B1 62738	2000-06-30
		US A 5808048	1998-09-15
		WO A1 9618637	1996-06-20