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An efficient large-scale synthesis of gemcitabine employing a crystalline 2,2-difluoro-α-ribofuranosyl bromide

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ABSTRACT

An efficient large-scale synthesis of gemcitabine was achieved without chromatography or fractional crystallization. The key steps include stereospecific conversion of a novel β -ribofuranosyl phosphate into a highly crystalline α -ribofuranosyl bromide and coupling of the α -ribofuranosyl bromide and trime-thylsilyl cytosine to produce a β -nucleoside. *p*-Phenylbenzoyl group was introduced for the protection of one of hydroxy groups in order to enhance the crystallinity of intermediates. Continuous removal of trimethylsilyl bromide, generated during the coupling reaction, by distillation from the reaction medium substantially enhanced the β -selectivity of the crucial coupling reaction.

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1. Introduction

Gemcitabine (2'-deoxy-2',2'-difluorocytidine, **1**), the active pharmaceutical ingredient of Gemzar^{®,1} is a nucleoside antimetabolite² that exhibits antitumor activity. It has shown that activity against a number of human solid tumors including lung, bladder, pancreatic, and non-small cell lung cancers.³ Gemcitabine is converted intracellularly into active 5'-diphosphate and 5'-triphosphate nucleosides by nucleoside kinases. The cytotoxicity of gemcitabine was attributed to the combined action of the diphosphate and triphosphate nucleosides, which lead to inhibition of DNA synthesis.⁴



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The first synthesis of gemcitabine (1) was accomplished by Hertel and co-workers through coupling of tert-butyldimethylsilyl (TBDMS)-protected difluororibofuranosyl methanesulfonate 2 and trimethylsilyl (TMS) cytosine 3 followed by removal of protecting groups.⁵ Chou and co-workers reported a large-scale synthesis of 1 by coupling of benzoyl (Bz)-protected difluororibofuranosyl methanesulfonate (OMs) **4** and **3**.⁶ Although there have been reports on the synthesis of **1** by using benzoyl-protected difluororibofuranoses bearing other leaving groups, such as trifluoromethanesulfonate, p-toluenesulfonate, and iodide at C-1,⁷ the procedure employing the coupling of the benzoyl-protected methanesulfonate **4** and the trimethylsilylated cytosine **3** appears to be better than others.^{6,8} Nevertheless, because the stereoselectivity in the coupling reaction of **4** and **3** is not quite satisfactory,^{6,8} there has been an effort to enhance the β -selectivity of the coupling reaction by employing the pure α -anomer of **4** instead of the anomeric mixture, **4**.^{8c} However, the isolation of the pure α -anomer of **4** requires preparation of the α -anomer enriched anomeric mixture of **4** at around $-80 \degree C$ followed by fractional crystallization of the α-anomer out of the anomeric mixture.⁹ Therefore, there still remains a need for development of a more efficient large-scale synthesis of 1. In this paper, we reported the preparation of stable crystalline *p*-phenylbenzoyl (PhBz)-protected¹⁰ difluororibofuranosyl α -bromide **5** and a highly stereospecific large-scale synthesis of 1 by the coupling of 5 and 3.





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2. Results and discussion

Synthesis started with Reformatsky reaction of (R)-2,3-O-isopropylideneglyceraldehyde (6)¹¹ and ethyl bromodifluoroacetate (7)¹² according to the Hertel's prodecure.⁵ Thus, coupling of **6** and **7** in the presence of activated Zn in THF followed by distillation of the reaction mixture provided a diastereomeric mixture (3:1) of pentonic acid ester **8** as a colorless liquid in 57% yield with an excess of the desired *erythro*-isomer. Without separation of the diastereomeric mixture, the hydroxy group of the compound **8** was protected with the *p*-phenylbenzoyl group, which was introduced first in a prostaglandin synthesis for the preparation of a more crystalline, more readily separable intermediate than other esters.⁹ Thus, reaction of **8** with *p*-phenylbenzoyl chloride in the presence of triethylamine afforded PhBz-protected difluoroester **9** as a diastereomeric mixture in 98% yield as shown in Scheme 1. After **10** in 70% yield, of which HPLC analysis indicated that it contained only a trace (around 0.1%) of the *threo*-isomer. The present procedure for the separation of the *erythro*-isomer **10** from diastereomeric mixture by employing the PhBz-protecting group is much more efficient and practical for the large-scale synthesis than previous methods^{5,6} because it does not require chromatography or fractional crystallization. In the Hertel's procedure,⁵ the desired *erythro*-isomer was separated by column chromatography from the product mixture of the Reformatsky reaction, whereas Chou and co-workers⁶ obtained the pure *erythro*-isomer only in 26% yield by fractional crystallization in the later lactone stage.

Removal of the isopropylidene group of **10** and simultaneous lactonization of the resulting hydroxy carboxylic acid under acidic condition gave ribonolactone **11** as shown in Scheme 2. Benzoylation of the lactone **11**, without purification, provided fully protected ribonolactone **12** in 72% yield in two steps from **10** after recrystallization of the crude product. In this stage, HPLC analysis indicated that the protected lactone **12** did not contain even a trace amount of its *threo*-isomer. Reduction of **12** with lithium tri-*tert*-butoxyaluminohydride provided 2-deoxy-2,2-difluororibofuranose **13** as a yellow syrup. Without purification of **13**, phosphorylation of the anomeric hydroxy group of the lactol **13** with diphenyl-chlorophosphate afforded the anomeric mixture of desired β -ribofuranosyl diphenyl phosphate **14** and a small amount of its α -anomer (α/β =1:10.8). Recrystallization of the anomeric mixture of **14** and its anomer from isopropanol/water (3:1) gave pure β -



Scheme 1. Synthesis of potassium erythro-pentonate 10.



Scheme 2. Synthesis of crystalline α-ribofuranosyl bromide 5.

hydrolysis of the diastereomeric ethyl ester **9** with K_2CO_3 in THF/ MeOH/H₂O at room temperature, when the volume of the reaction mixture was reduced to one third by evaporation of the solvent, solid potassium *erythro*-pentonate **10** precipitated while the *threo*isomer of **10** stayed in solution. Filtration followed by washing the residue with Et₂O afforded the pure potassium *erythro*-pentonate phosphate **14** in 77% yield in two steps and HPLC analysis indicated that it contained less than 2% of the α -anomer. Treatment of the β -phosphate **14** with 30% hydrogen bromide in acetic acid at room temperature afforded a mixture of solid α - and β -ribofuranosyl bromides with a large excess α -anomer **5** (α/β =10.8:1). Recrystallization of the anomeric mixture of the ribosyl bromides

from isopropanol provided pure α -ribofuranosyl bromide **5** in 82% yield and HPLC analysis indicated that it contained less than 0.3% of the β -anomer. We, however, found that purification of the crude phosphate **14** by recrystallization was unnecessary: bromination of the crude β -ribosyl phosphate **14** containing a small amount of its α -anomer provided the crystalline α -bromide **5** in almost same purity and yield as the bromination of pure **14**. The *p*-phenyl-benzoyl-protecting group at 0-3 of **5** was found to be essential for the easy isolation of **5** from its anomeric mixture by recrystallization: we found that the ribofuranosyl bromide possessing the benzoyl-protecting group at both 0-3 and 0-5 positions was liquid.

Then, stage was set for coupling of α -ribosyl bromide **5** with trimethylsilyl cytosine 3. However, the reaction of 5 and 3 was not stereoselective at all, providing a mixture of almost an equal amount of β -nucleoside **15** and its α -anomer. Previously, Hertel and co-workers reported that the β/α ratio was 1:4 in the coupling reaction of a ribosyl mesylate and the cytosine⁵ while Chou and coworkers obtained almost an equal amount of the desired β-nucleoside and its α -anomer.⁶ The formation of the mixture of α - and β -nucleosides from the pure α -bromide **5** would be explained by assuming the S_N1-type reaction involving an oxocarbenium ion intermediate and/or the S_N2-type reaction of a mixture of the α bromide and the β -bromide, which might be generated by the anomerization of α -bromide **5** during reaction. The anomerization of the α -bromide **5** would be facilitated in the presence of bromide sources, such as trimethylsilvl bromide (TMSBr) generated during the reaction. We indeed observed formation of a small amount of the β -anomer of **5**, when the pure α -bromide **5** was treated with TMSBr. Because it is known that the β -glycosyl halide is generally less stable but more reactive than its α -anomer,¹³ even a small amount of the β -bromide in the present work would generate a substantial amount of the α -nucleoside. Based on the above result and consideration, we envisioned that the β -selectivity would be enhanced by the S_N2 -like displacement of the α -bromide 5 with the nucleophilic base 3 if the anomerization of 5 is suppressed by removing TMSBr as soon as it is generated and at same time if the concentration of the oxocarbenium ion is kept as low as possible by using nonpolar solvents. Removal of TMSBr in the present work was carried out by continuous distillation of a mixture of TMSBr and heptane as a carrier in a high boiling solvent with simultaneous addition of heptane. Thus, the reaction of 5 and 3 was conducted in octane/diphenyl ether (2:1, v/v) while adding heptane in a dropwise manner (around 300 mL per g of the bromide 5) and at same time carrying out distillation by maintaining the reaction temperature at 140–150 °C. The β -selectivity of the reaction, indeed, increased substantially and a mixture of the β-nucleoside 15 and its α -anomer was obtained in 92% yield, with an excess of the β anomer ($\beta/\alpha=5.5:1$) as shown in Scheme 3. The β/α ratio in the coupling reaction of 5 and 3 employing the present protocol varied from 4 to 8 depending on the equivalent of cytosine and the scale of the reaction, whereas the β/α ratio without distilling off TMSBr during reaction remained 1.3-1.4. On the other hand, the reaction in the distillation mode but without addition of the heptane carrier was not satisfactory, exhibiting the poor β -selectivity. Finally, the mixture of 15 and its α -anomer, without separation, was treated with ammonia in methanol provided a crude mixture of gemcitabine **1** and its α -anomer. Isolation of pure β -nucleoside **1** as either hemihydrate form or dihydrate form was possible just by extraction, evaporation, and crystallization from water. Cooling down the warmed aqueous solution of the mixture of **1** and its anomer with stirring to room temperature afforded pure gemcitabine **1** as a hemihydrate form in 65% yield in two steps from 5. Cooling the aqueous solution without stirring gave **1** as a dihydrate form. We have, thus, found that the gemcitabine hemihydrate is also a stable crystalline compound while the gemcitabine dihydrate was known previously.⁵ Both gemcitabine hemihydrate and dihydrate were found to contain less than 0.02% of its α -anomer based on HPLC analysis and their water contents were not changed on standing or under humid condition in the prolonged time. Overall yield of the present procedure (20%) from known compound 8 is substantially higher than those of Hertel's method $(6\%)^5$ and Chou's procedure (9%).⁶

3. Conclusion

A highly efficient method for the large-scale synthesis of gemcitabine has been developed. The pure erythro-isomer of potassium salt of the pentonic acid, 10, was readily isolated from the mixture of *erythro-* and *threo-*isomers by a filtration. The highly crystalline α -ribofuranosyl bromide **5** was obtained by the reaction of β ribofuranosyl diphenyl phosphate 14 with HBr and readily purified by recrystallization. The stereospecific coupling of an α-ribofuranosyl bromide 5 and trimethylsilyl cytosine 3 was possible by conducting the reaction in the nonpolar solvent and by suppressing the anomerization of 5. Thus, the coupling was carried out in octane/diphenyl ether by continuously removing TMSBr generated during the reaction. Introduction of a *p*-phenylbenzoyl group as the hydroxy-protecting group was essential for the easy purification of compounds **5** and **10**. The stable solid and crystalline intermediates, higher overall yield, and stereospecific reactions including the crucial coupling reaction of 5 and 3 make the present procedure more efficient and practical than previous ones in the large-scale synthesis of gemcitabine.

4. Experimental

4.1. General methods

All reagents were purchased from commercial suppliers and used without further purification unless otherwise noted. Solvents were purified and dried by standard methods prior to use. Thinlayer chromatography (TLC) was performed using silica gel 60 F₂₅₄ precoated plates (0.25 mm thickness) with a fluorescent indicator to monitor the reactions. Visualization on TLC was achieved by UV light (254 nm) and a typical TLC indication solution (cerium sulfate/ molybdic acid solution). Melting points are uncorrected. Optical rotations were determined at 20 °C with an automatic polarimeter.



Scheme 3. Synthesis of gemcitabine 1 by coupling of 5 and 3.

¹H NMR and ¹³C NMR spectra were recorded at 300 MHz spectrometer (300 MHz for ¹H, 75 MHz for ¹³C). Chemical shifts are reported as δ values in parts per million relative to tetramethylsilane as an internal standard and *J* values are given in hertz (Hz).

4.1.1. Ethyl 2-deoxy-2,2-difluoro-p-erythro, p-threo-pentonate (8). To a stirred suspension of zinc (13 g, 198 mmol) and dibromoethane (0.51 mL) in dry THF (26 mL) was added chlorotrimethylsilane (0.76 mL) at 40 °C. After being stirred for 10 min at 40 °C, the reaction mixture was heated to 60 °C. A solution of (R)-2,3-O-isopropylideneglyceraldehyde 6 (30.8 g, 236 mmol) and ethyl bromodifluoroacetate 7 (25.5 mL, 198 mmol) in THF (39 mL) was added dropwise to the above solution and the resulting mixture was heated to reflux for 30 min and then cooled down to room temperature. After sequential addition of ice (260 g), Et₂O (65 mL), and aqueous 1 N HCl (260 mL) to the reaction mixture, it was stirred until the ice was completely melted. The resulting mixture was extracted with Et_2O (3×90 mL). The combined extracts were washed successively with brine, saturated aqueous NaHCO₃, dried over MgSO₄, and concentrated in vacuo. The residue was distilled at 130–134 °C/10 Torr to afford the compound **8** (28.9 g, 57%, *erythro*/ threo=3:1,) as a colorless liquid, ¹H NMR (300 MHz, CDCl₃) δ 1.34–1.47 (m, 9H), 2.18 (s, 1H), 2.93 (br s, 1H), 3.7–4.4 (m, 5H); ¹³C NMR (75 MHz, CDCl₃) δ 13.3, 13.4, 24.5, 24.8, 25.7, 30.4, 62.6, 62.8, 65.1, 65.8, 70.4, 70.8, 71.2, 71.5, 71.8, 72.9, 109.2, 109.8, 110.1, 113.5, 116.9, 162.6.

4.1.2. Ethyl 2-deoxy-2,2-difluoro-3-O-(p-phenylbenzoyl)-D-erythro, *p*-threo-pentonate (9). A solution of compound 8 (50.0 g. 196 mmol), p-phenylbenzoyl chloride (51.1 g, 235 mmol), and triethylamine (42 mL, 301 mmol) in CH₂Cl₂ (500 mL) was stirred for 6 h at room temperature and then the reaction was guenched with aqueous 1 N HCl (360 mL). The organic layer was separated, washed successively with water, saturated aqueous NaHCO₃, and brine, dried over MgSO₄, and concentrated in vacuo to afford compound **9** (83.7 g, 98%) as a bright yellowish liquid, $[\alpha]_{D}^{20}$ +8.85 (*c* 1.0, MeOH); ¹H NMR (300 MHz, CDCl₃) δ 1.25–1.74 (m, 9H), 4.11–4.19 (m, 2H), 4.30–4.36 (m, 2H), 4.56–4.58 (m, 2H), 5.78 (ddd, 1H×1/3, J=5.9, 7.7, 16.9 Hz), 5.95 (ddd, 1H×2/3, *J*=5.7, 13.0, 12.8 Hz), 7.42–7.53 (m, 3H), 7.63-7.73 (m, 4H), 8.15-8.17 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 13.8, 14.1, 25.1, 25.3, 26.0, 26.1, 53.5, 63.3, 63.4, 65.6, 65.8, 70.6, 70.9, 71.1, 71.2, 71.5, 71.8, 72.4, 72.5, 109.5, 110.1, 110.2, 112.8, 112.9, 116.2, 116.3, 127.0, 127.2, 127.2, 127.4, 127.5, 128.3, 129.0, 130.6, 139.6, 139.7, 146.5, 146.6, 161.7, 162.1, 162.5, 164.5, 164.7. HRMS (ESI) m/z calcd for C₂₃H₂₄F₂O₆Na [M+Na]⁺, 457.1439; found, 457.1440.

4.1.3. Potassium 2-deoxy-2,2-difluoro-3-O-(p-phenylbenzoyl)-D-erythropentonate (10). Aqueous 1.0 M K₂CO₃ (750 mL) solution was added to a solution of compound 9 (83.8 g, 193 mmol) in THF/MeOH (2:3, 1.5 L). The reaction mixture was stirred for 30 min at room temperature and evaporated until the volume is reduced to one third. During evaporation white solids started to precipitate. The solid collected by filtration was washed with Et₂O and dried to afford pure *erythro*-compound **10** (60.1 g, 70%) as a white solid, $[\alpha]_D^{20}$ +43.51 (*c* 1.0, MeOH); ¹H NMR (300 MHz, DMSO- d_6) δ 1.07 (s, 3H), 1.22 (s, 3H), 4.00 (t, 1H, J=7.6 Hz), 4.11 (t, 1H, J=7.5 Hz), 4.49 (t, 1H, J=6.7 Hz), 5.88 (dd, 1H, J=6.5, 21.2 Hz), 7.38-7.54 (m, 3H), 7.75 (d, 2H, J=7.1 Hz), 7.85 (d, 2H, J=8.5 Hz), 8.07 (d, 2H, J=8.4 Hz); ¹³C NMR (75 MHz, CDCl₃) δ 25.1, 26.0, 63.6, 63.7, 71.0, 71.3, 71.6, 73.3, 73.4, 107.8, 111.1, 114.5, 117.9, 127.0, 127.1, 128.1, 128.5, 129.1, 130.1, 138.8, 145.1, 162.9, 163.2, 163.5, 164.6. HRMS (ESI) m/z calcd for C₂₁H₁₉F₂KO₆K [M+K]⁺, 483.1770; found, 483.1772.

4.1.4. 5-O-Benzoyl-2-deoxy-2,2-difluoro-3-O-(*p*-phenylbenzoyl)-*p*ribonolactone (**12**). To a suspension of compound **10** (38.8 g, 87.3 mmol) in acetonitrile (232 mL) was added aqueous 12 N HCl solution (9.2 mL). The reaction mixture was heated to reflux for 6 h, cooled down to room temperature, diluted with toluene (464 mL), and concentrated in vacuo to dryness. The residue was dissolved in EtOAc (232 mL) and insoluble KCl was filtered off. The filtrate was concentrated in vacuo to afford ribonolactone **11** (30.1 g) as foamy solid. This crude ribonolactone **11** was used for the next step without further purification. A solution of **11** (30.1 g. 86.4 mmol) and benzovl chloride (15 mL, 129 mmol) in the presence of pyridine (14 mL, 173 mmol) in EtOAc (375 mL) was stirred at room temperature for 6 h and then the reaction was guenched with agueous 1 N HCl solution (210 mL). The organic layer was separated, washed successively with H₂O and saturated aqueous NaHCO₃, dried over MgSO₄, and concentrated in vacuo to give a cream-color solid. The solid was recrystallized from Et₂O/hexane (300 mL, 5:1) to afford compound 12 (28.4 g, 72% in two steps) as a white solid, mp 130–131 °C; $[\alpha]_D^{20}$ +69.7 (c 1.0, DMF); IR (KBr) 1820, 1723, 1268, 1216, 1113, 743, 714, 690 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 4.69-4.76 (m, 2H), 5.00-5.03 (m, 1H), 5.74-5.81 (m, 1H), 7.42-7.71 (m, 10H), 8.01-8.04 (m, 2H), 8.11-8.14 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 62.4, 69.3, 69.7, 69.9, 78.5, 78.6, 108.2, 111.6, 111.7, 115.1, 126.2, 127.4, 127.5, 128.7, 128.8, 129.2, 129.9, 130.9, 133.9, 139.6, 147.4, 162.2, 162.7, 163.1, 164.6, 165.8. HRMS (ESI) m/z calcd for C₂₅H₁₈F₂O₆Na [M+Na]⁺, 475.0969; found, 475.0967.

5-O-benzoyl-2-deoxy-2,2-difluoro-3-O-(p-phenyl-4.1.5 Dinhenvl *benzoyl*)- β -*D*-*ribofuranosyl phosphate* (**14**). To a stirred solution of lithium tri-tert-butoxyaluminohydride (338 g, 1.33 mol) in THF (4.0 L) was added the ribonolactone 12 (500 g, 1.11 mol) in THF (2.0 L) at $-40 \degree$ C. The reaction mixture was slowly warmed up to room temperature, and stirred for further 2 h at room temperature. Upon the completion of the reaction, aqueous 1 N HCl (5.5 L) solution was added to the reaction mixture to quench the excess lithium tri-tert-butoxyaluminohydride. The resulting mixture was extracted with Et₂O (5.5 L) and the extract was washed successively with H₂O, saturated aqueous NaHCO₃, dried over MgSO₄, and concentrated to afford 2-deoxy-2,2-difluororibofuranose **13** (461 g) as a vellowish syrup, which was used for the next step without further purification. To a solution of 13 (461 g, 1.01 mol) and triethylamine (168 mL, 1.20 mol) in toluene (3.7 L) was added a solution of diphenylchlorophosphate (311 mL, 1.50 mol) in toluene (930 mL). The reaction mixture was stirred for 4 h at room temperature and then quenched with aqueous 1 N HCl solution (1.2 L). The organic layer was washed successively with H₂O and saturated aqueous NaHCO₃, dried over MgSO₄, and concentrated in vacuo to give a mixture of α - and β -ribofuranosyl phosphates (α/β =1:10.8) as a solid. Recrystallization of the solid mixture from isopropanol/ water (3:1, v/v) afforded pure β -phosphate **14** (584 g, two steps 77%) as a white crystal, mp 101–103 °C; $[\alpha]_D^{20}$ +13.0 (*c* 1.0, DMF); IR (KBr) 1740. 1721, 1490, 1292, 1272, 1190, 997, 963, 744, 715 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 4.44–4.65 (m, 3H), 5.87–5.97 (m, 1H), 6.06 (t, 1H, J=6.1 Hz), 7.18-7.37 (m, 16H), 7.61-7.69 (m, 4H), 8.00 (d, 2H, J=8.4 Hz), 8.12 (d, 2H, J=8.4 Hz); ¹³C NMR (75 MHz, CDCl₃) δ 63.7, 69.8, 70.0, 70.1, 70.3, 79.2, 79.3, 97.6, 97.7, 98.0, 98.1, 98.2, 98.5, 120.0, 120.1, 120.2, 120.3, 125.7, 125.9, 126.7, 127.27, 127.29, 127.6, 128.4, 128.5, 129.1, 129.3, 129.8, 129.9, 130.7, 133.3, 139.6, 146.8, 150.0, 150.05, 150.09, 150.1, 164.8, 165.8. HRMS (ESI) m/z calcd for C₃₇H₂₉F₂O₉PNa [M+Na]⁺, 709.1415; found, 709.1418.

4.1.6. 5-O-Benzoyl-2-deoxy-2,2-difluoro-3-O-(*p*-phenylbenzoyl)- α *p*-ribofuranosyl bromide (**5**). A solution of β -phosphate **14** (500 g, 0.728 mol) in 30% HBr in acetic acid (1.8 L) was stirred for 6 h at room temperature. The reaction mixture was diluted with CH₂Cl₂ (8.7 L) and poured onto ice. The organic layer was separated, washed successively with H₂O and saturated aqueous NaHCO₃, dried over MgSO₄, and concentrated to give a mixture of α - and β ribofuranosyl bromides (α/β =10.8:1) as a solid. Recrystallization of the mixture from isopropanol afforded pure α -bromide **5** (308 g, 82%) as a white crystal, mp 111–112 °C; $[\alpha]_D^{20}$ +140.6 (*c* 1.0, DMF); IR (KBr) 1725. 1607, 1263, 1119, 1084, 860, 748, 709 cm⁻¹; ¹H NMR (300 MHz, CDC1₃) δ 4.68–4.87 (m, 3H), 5.60 (dd, 1H, *J*=4.5, 4.2 Hz), 6.54 (d, 1H, *J*=9.0 Hz), 7.41–7.73 (m, 10H), 8.05 (d, 2H, *J*=9.0 Hz), 8.19 (d, 2H, *J*=8.4 Hz); ¹³C NMR (75 MHz, CDCl₃) δ 62.2, 71.0, 71.2, 71.4, 71.7, 82.9, 84.7, 85.0, 85.2, 85.6, 118.4, 121.7, 122.0, 125.3, 126.9, 127.1, 127.3, 127.6, 127.7, 128.5, 128.78, 128.84, 129.1, 129.3, 129.5, 129.6, 129.8, 130.7, 133.4, 139.2, 139.6, 146.7, 164.9, 165.9. HRMS (ESI) *m/z* calcd for C₂₅H₁₉BrF₂O₅Na [M+Na]⁺, 539.0282; found, 539.0284.

4.1.7. 1-(5'-O-Benzoyl-2'-deoxy-2',2'-difluoro-3-O-(p-phenylbenzoyl)-p-ribofuranosyl)-4-aminopyrimidin-2-one (15). A mixture of cytosine (951 g, 8.56 mol), hexamethyldisilazane (5.4 L, 25.9 mol), and ammonium sulfate (5.4 g, 0.041 mol) was heated to reflux for 1.5 h. The resulting solution was diluted with toluene (5.14 L) and refluxed for further 1 h. To the reaction mixture, which was cooled down to 80 °C, a solution of the α -bromide **5** (300 g, 0.58 mol) in octane (3.44 L) and diphenyl ether (1.72 L) was added. Then, a mixture of diphenyl ether (860 mL) and heptane (85.7 L) was added dropwise to the reaction mixture and at same time the solvent and TMSBr were continuously removed by slow distillation by maintaining the reaction temperature at 140–150 °C for 10 h. After addition of heptane (4.30 L) at once to the reaction mixture, water (1.03 L) was added at 80 °C. The resulting mixture was cooled down and stirred for 1 h at room temperature. The precipitated solid was collected by filtration and washed with heptane. The solid, which contained α - and β -nucleosides and unreacted cvtosine, was mixed with CH₂Cl₂/MeOH (7.72 L, 5:1) and the mixture was refluxed for 1 h. After filtration of the mixture, the residue was washed with CH₂Cl₂/MeOH (3.60 L, 5:1). The combined filtrate was concentrated in vacuo to afford a mixture of desired β -nucleoside **15** and its α -anomer (292 g, 92%, β/α =5.5:1) as a white solid. This anomeric mixture was used for the next step without further purification. For the identification of β -nucleoside **15**, the solid anomeric mixture was recrystallized from MeOH to afford pure βnucleoside **15**, mp 252–253 °C; $[\alpha]_D^{20}$ –5.4 (*c* 0.5, MeOH); ¹H NMR (300 MHz, DMSO-*d*₆) δ 3.33 (s, 1H), 4.67–4.78 (m, 3H), 5.78 (d, 2H, J=7.5 Hz), 5.84 (s, 1H), 6.39 (s, 1H), 7.46-7.55 (m, 6H), 7.76 (d, 2H, J=7.5 Hz), 7.87 (d, 2H, J=8.3 Hz), 7.96 (d, 2H, J=7.4 Hz), 8.11 (d, 2H, J=8.2 Hz); ¹³C NMR (75 MHz, CDCl₃) δ 64.0, 72.0, 72.3, 72.6, 76.2, 95.6, 122.4, 125.9, 127.4, 127.6, 129.2, 129.3, 129.7, 129.7, 129.8, 130.9, 134.2, 139.2, 142.5, 146.2, 155.0, 164.8, 166.0, 166.4. HRMS (ESI) m/z calcd for C₂₉H₂₃F₂N₃O₆Na [M+Na]⁺, 570.1453; found, 570.1451.

4.1.8. 2'-Deoxy-2',2'-difluorocytidine (**1**). To a stirred solution of 2 N NH₃ in MeOH (8.12 L) was added the mixture of β -nucleoside **15** and its α -anomer (290 g, 0.53 mol, β/α =5.5:1). The reaction mixture was stirred at room temperature for 12 h and concentrated in vacuo. The residue was dissolved in water (3.24 L) and EtOAc (2.14 L) with stirring and the organic phase was extracted with water (1.07 L). The combined aqueous extract was washed with Et₂O (1.07 L) and concentrated to dryness. The residue was mixed with water (670 mL) and stirred by heating to 50 °C. The solution was cooled down to room temperature and stirred for further 2 h.

The precipitated solid was collected by filtration, washed with cold water (150 mL), and dried to afford pure gemcitabine **1** (98.9 g, 71%) as a white crystalline hemihydrate form, mp 198–202 °C; $[\alpha]_D^{20}$ +76.4 (*c* 1.0, MeOH); ¹H NMR (300 MHz, DMSO-*d*₆) δ 3.57–3.64 (m, 1H), 3.73–3.81 (m, 2H), 4.10–4.15 (m, 1H), 5.18 (t, 1H, *J*=5.4 Hz), 5.77 (t, 1H, *J*=7.5 Hz), 6.12 (t, 1H, *J*=8.3 Hz), 6.21 (d, 1H, *J*=6.4 Hz), 7.35 (d, 1H, *J*=6.8 Hz), 7.67 (d, 1H, *J*=7.5 Hz); ¹³C NMR (75 MHz, DMSO-*d*₆) δ 58.9, 68.4, 68.7, 69.0, 80.3, 80.4, 83.1, 83.5, 83.9, 94.7, 119.6, 123.0, 126.5, 140.8, 154.8, 165.6. Anal. Calcd for C₉H₁₁N₃O₄F₂·0.5H₂O: C, 39.51; H, 4.48; N, 14.36. Found: C, 39.58; H, 4.45; N, 15.07.

When the solution was cooled without stirring, filtration of the precipitated solid, washing the solid with cold water, and drying provided pure gemcitabine **1** as a white crystalline dihydrate form, mp 220–224 °C; $[\alpha]_D^{20}$ +71.1 (*c* 1.0, MeOH); ¹H NMR (300 MHz, DMSO-*d*₆) δ 3.57–3.64 (m, 1H), 3.73–3.81 (m, 2H), 4.10–4.15 (m, 1H), 5.18 (t, 1H, *J*=5.4 Hz), 5.77 (t, 1H, *J*=7.5 Hz), 6.12 (t, 1H, *J*=8.3 Hz), 6.21 (d, 1H, *J*=6.4 Hz), 7.35 (d, 1H, *J*=6.8 Hz), 7.67 (d, 1H, *J*=7.5 Hz); ¹³C NMR (75 MHz, DMSO-*d*₆) δ 58.9, 68.4, 68.7, 69.0, 80.4, 80.4, 83.1, 83.5, 83.9, 94.7, 119.6, 123.0, 126.4, 140.8, 154.8, 165.6. Anal. Calcd for C₉H₁₁N₃O₄F₂·2H₂O: C, 37.12; H, 4.88; N, 14.43. Found: C, 36.78; H, 4.98; N, 14.01.

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