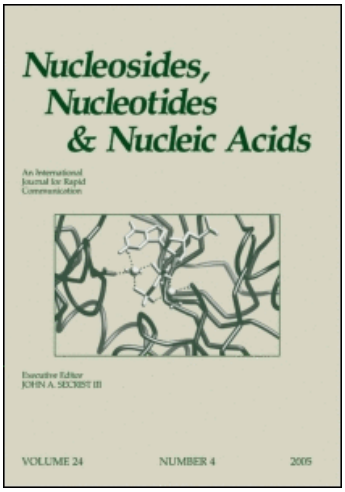

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EFFICIENT SYNTHESES OF CLOFARABINE AND GEMCITABINE FROM 2-DEOXYRIBONOLACTONE

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□ *The development of a new methodology to achieve electrophilic fluorination of triisopropylsilyl-protected 2-deoxyribonolactone has been employed to synthesize clofarabine and gemcitabine with improved synthetic efficiency versus prior synthetic methods. These studies highlight the versatility of this new methodology to obtain medically relevant 2′-fluoronucleosides.*

Keywords Diastereoselective; electrophilic fluorination; 2-deoxyribonolactone

INTRODUCTION

Nucleosides bearing fluorine or fluorinated substituents within the carbohydrate moiety are of value in biochemical research and therapeutic treatment.[1–4] The presence of fluorine functional groups alters the physical and chemical properties of nucleosides with respect to their nonfluorinated counterparts and can cause increased resistance to metabolic decomposition.[3] Some best selling prescription drugs are fluorinated nucleosides,[1] such as the prodrug clofarabine (**1**, 2-chloro-2′-deoxy-2′-*arabino*-fluoroadenosine, Figure 1), a purine nucleoside antimetabolite used clinically to treat pediatric patients with relapsed or refractory acute lymphocytic leukemia.[5] Clofarabine metabolites deplete 2-deoxynucleotide pools within cells by inhibiting ribonucleotide reductase. This, in turn, restricts DNA synthesis, although it exhibits toxicity by other mechanisms as well.[5] Another well-known drug, Gemcitabine (**2**, Gemzar, 2′-deoxy-2′,2′-difluorocytidine, Figure 1; Eli Lilly, Indianapolis, IN, USA), is a *gem*-difluoronucleoside, that is widely used in the clinical setting to treat ovarian,[6] pancreatic,[7] and breast cancers[8] with annual sales well in excess of 1 billion dollars a year.[9] Gemcitabine metabolites interfere directly with DNA replication[10,11] and

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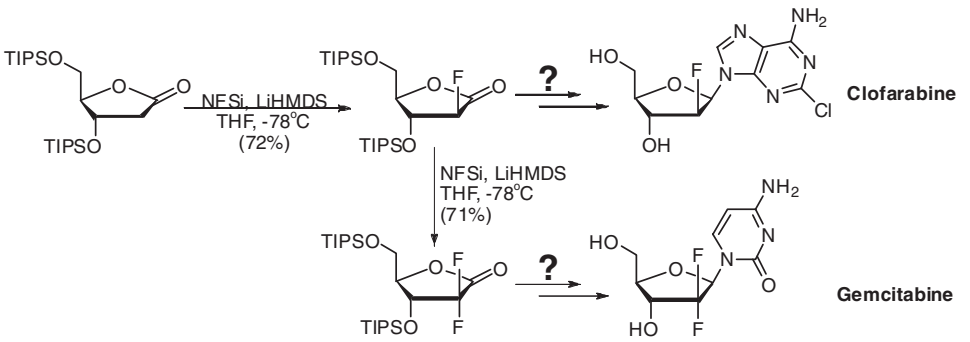


SCHEME 1 General strategy for synthesis of gemcitabine and clofarabine.

inhibit ribonucleotide reductase.[10,11] Despite their importance as cancer drugs current procedures to synthesize clofarabine and gemcitabine are still of limited efficiency.

We recently developed highly efficient syntheses of silyl-protected 2-deoxy-2-fluoro (*arabino* and *gem*-difluoro)-furanoses from 2-deoxyribonolactone (Scheme 1) using a diastereoselective electrophilic fluorination methodology.[12] It is important to note that the sugars are precursors for synthesis of 2′-deoxy-2′-fluoronucleosides. We wondered if we could successfully synthesize clofarabine and gemcitabine (Figure 1) by use of these methods as shown in Scheme 1. We herein describe new methods to synthesize clofarabine and gemcitabine via routes utilizing electrophilic fluorination of protected 2-deoxyribonolactone. These syntheses provide substantially improved yields versus other reported methods. These studies demonstrate the efficiency of electrophilic fluorination methodologies to construct commercially important 2′-fluoro-substituted nucleosides.

RESULTS AND DISCUSSION

Previous Syntheses of Clofarabine

Clofarabine was first synthesized by Montgomery and coworkers (Scheme 2).[13] The synthetic route depends on a protected *arabino*-fluororiboside

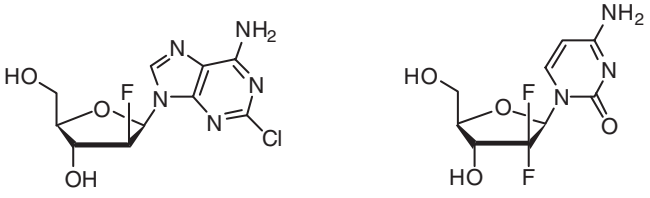
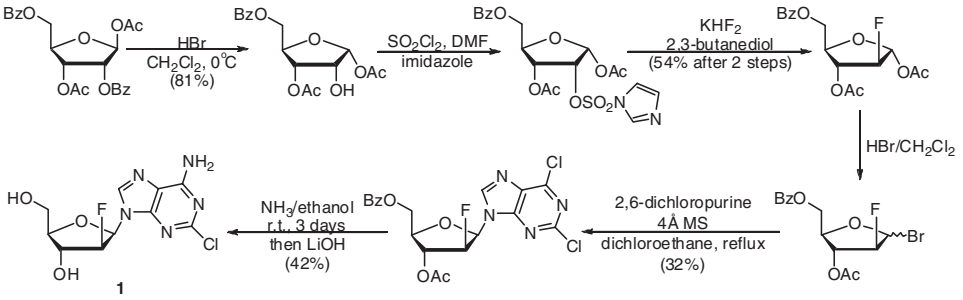


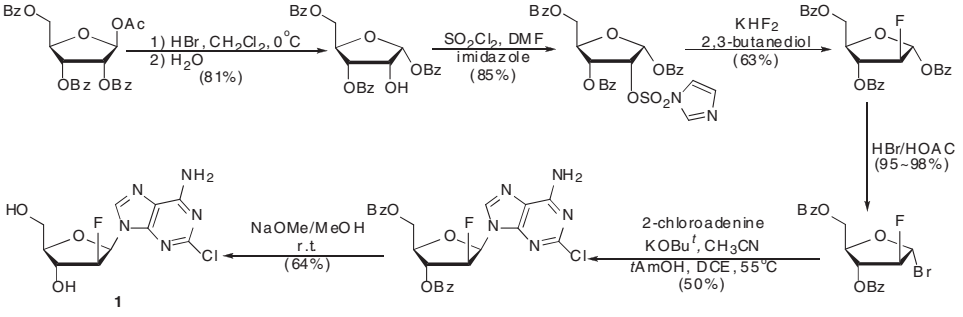
FIGURE 1 Structures of clofarabine and gemcitabine.



SCHEME 2 Montgomery's synthesis of clofarabine.

precursor that is coupled to 2,6-dichloropurine. Installation of the 6-amino group occurred concomittantly with removal of ester protecting groups by ethanolic ammonia and base. The key protected *arabino*-fluororibofuranose was obtained by conversion of a 2-*ribo*-hydroxyl group to a 2-*arabino*-fluoro group by nucleophilic fluoride. The use of nucleophilic fluoride to obtain 2-*arabino*-fluororibose and 2′-*arabino*-fluoronucleosides has been the conventional method of synthesis for these compounds for several decades.[14] Clofarabine was prepared in 6 steps in 6% overall yield.

Recently, ILEX Products Inc. (San Antonio, TX, USA) reported an improved synthesis of clofarabine (Scheme 3).[15] The newer method utilizes an appropriate stereochemically pure (α-anomer) 2-deoxy-2-fluoro-1-bromosugar (Scheme 3) obtained by nucleophilic fluorination chemistry from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-α-D-ribofuranose.[15,16] The base conjugation step to form the nucleoside is achieved via a preformed 2-chloro-6-amino purine and proceeds with excellent stereoselectivity ($\beta/\alpha = 21/1$). Deprotection completed the synthesis in 6 steps 14% overall yield starting from a fully protected ribose starting material.[15]



SCHEME 3 ILEX's synthesis of clofarabine.

A New Efficient Synthesis of Clofarabine From 2-Deoxyribonolactone

Improved access to silyl-protected 2-deoxy-2-*arabino*-fluorofuranose[12] led us to wonder if we could construct clofarabine in a succinct manner. Starting from TIPS-protected 2-deoxyribonolactone **3**, diastereoselective electrophilic fluorination of **3** with LiHMDS with the presence of NFSi at −78°C produced only the *arabino*-isomer **4** in 72% isolated yield as reported (Scheme 4).[12] Reduction of **4** with DIBAL-H provided lactol **5** in 91% yield.[12] **5** was activated with methanesulfonylchloride and triethylamine, which formed only the α-chloro sugar **6** in quantitative yield.[12] **6** was coupled with 2,6-dichloropurine[17] by analogy to the method of Montgomery.[13] The coupling provided both β and α isomers with a 3.5 to 1 ratio favoring the desired β-isomer. The mixture of protected nucleosides **7** (α and β) was then submitted to the next step without purification. Amination of **7** with ammonia in isopropanol in a sealed tube at 105°C afforded the desired 6-amino-2-chloropurine nucleoside **8**. At this point the β-anomer and α-anomer were separately purified by silica gel chromatography. Yield of the stereochemically pure silyl-protected β clofarabine after coupling and amination steps was 65% whereas the yield of the α isomer was 16%, which was fully characterized, but we did not deprotect it. Removal of the protecting groups from the β isomer occurred in 90% yield to provide clofarabine as an off-white solid.[13,15] The synthesis of clofarabine from TIPS-protected lactone was 6 steps with overall yield of 38% (Scheme 4). Table 1 shows our method requires the same number of steps as prior methods, but has a significant improvement in overall yield (14% vs. 38%).

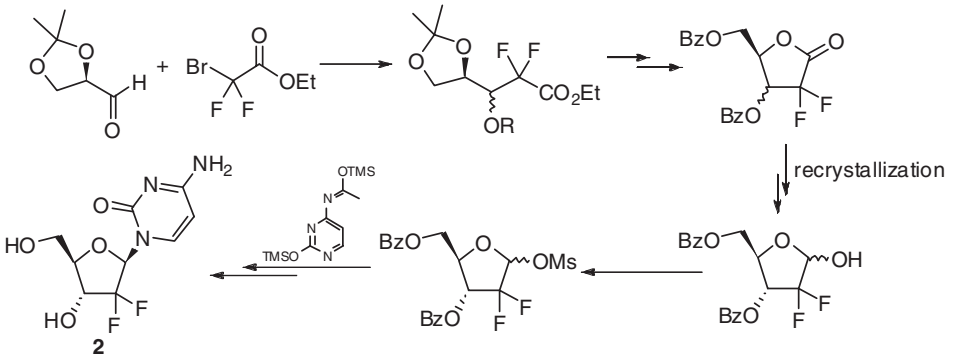
Previous Syntheses of Gemcitabine

Synthetic procedures to produce gemcitabine were developed by Chou and coworkers for commercial production of the nucleoside and are adapted from Hertel's method.[18] The method of Chou involves coupling of ethyl bromodifluoroacetate and isopropylidene glyceraldehyde to obtain the important precursor a protected *gem*-difluorolactone that can be used for nucleoside synthesis.[19] The route has the pitfall that it is not diastereoselective

TABLE 1 Comparison of three different clofarabine syntheses

Method	Steps	Total yield (%)
Montgomery	6[a]	6
ILEX	6[a]	14
Sauve	6[b]	38

[a]From acyl-protected ribofuranose.
[b]From silyl-protected 2-deoxyribonolactone.

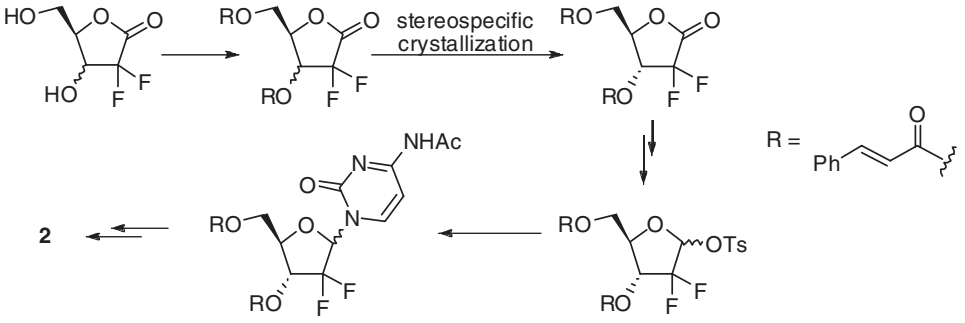


SCHEME 4 Eli Lilly' synthesis of gemcitabine.

and depends on crystallization to obtain the preferred isomer (Scheme 5). Shen's group reported a slightly improved method for synthesis of gemcitabine which was quite similar to the Chou method, differing mostly in the use of a different protection scheme.[20] The Shen method requires 10 steps and still requires crystallization step for isomer purification, with an overall yield of 10% (starting from isopropylidene glyceraldehyde, Scheme 6).

A New Efficient Synthesis of Gemcitabine

We recently reported that *arabino*-fluorolactone **4** could be fluorinated with NFSi at −78°C to access the corresponding *gem*-difluorolactone.[12] 2-deoxy-2,2-difluororibonolactone **9** was obtained in an overall yield of 51% yield from the initial TIPS-protected 2-deoxy-ribonolactone (Schemes 4 and 7).[12] Lactol **10** was obtained via DIBAL-H reduction of **9** in 91% yield. We then prepared 1-mesylate **11**.[12] Coupling of **11** with bis(trimethylsilyl)cytosine yielded nucleoside **12** in both α and β configurations (Scheme 7). Integration of 1′-hydrogen on NMR spectrum indicated that the stereochemistry ratio of coupling was $\alpha/\beta = 1{:}1$, similar to that reported by other workers for this step.[19,20] Subsequent deprotection and

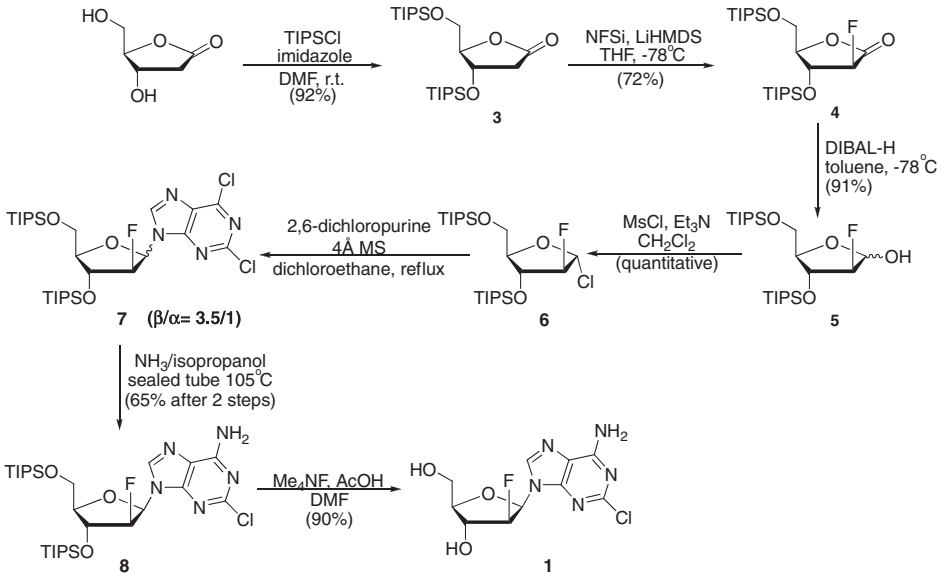


SCHEME 5 Shen's synthesis of gemcitabine.

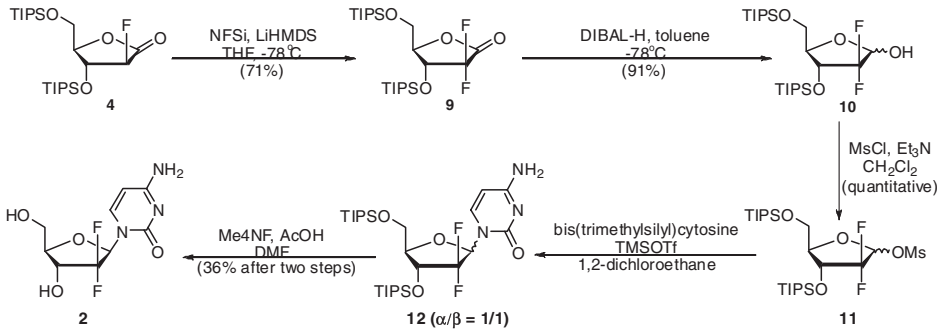
TABLE 2 Comparison of three different gemcitabine syntheses

Method	Steps	Total yield (%)
Chou	8[a]	5.8
Shen	10[a]	10
Sauve	6[b]	17

[a]From isopropylidene glyceraldehyde.
[b]From silyl-protected 2-deoxyribonolactone.



SCHEME 6 Stereoselective synthesis of clofarabine.



SCHEME 7 Stereoselective synthesis of gemcitabine.

HPLC purification provided both isomers of gemcitabine (**2**) with the β isomer in 36% yield and the α isomer in 42% from the mesylate. The synthesis of β-gemcitabine was completed in 6 steps with 17% overall yield (starting from fully protected 2-deoxyribonolactone). This new synthesis exceeds the methods of Chou and Shen for selectivity, brevity and yield (Table 2).

CONCLUSION

TIPS-protected 2-deoxyribonolactone is a versatile precursor to 2-deoxy-2-fluororibonolactones and furanoses via electrophilic fluorination.[12] Here we demonstrated that electrophilic fluorination of TIPS-protected 2-deoxyribonolactone can be used to synthesize the commercially important fluorinated nucleosides gemcitabine and clofarabine with greater efficiency as compared with prior published methods. The TIPS groups do not appear to present an impediment to nucleoside coupling steps, which still occur with desirable stereochemical yield. Furthermore, the TIPS groups are readily removed after nucleoside formation, to obtain the desired nucleosides in high yield. These results demonstrate improved synthetic access to 2′-fluoronucleosides by methods integrating electrophilic fluorination of TIPS-protected 2-deoxyribonolactone.

EXPERIMENTAL

General

^{1}H and ^{13}C NMR spectra were obtained using either a Bruker 400 MHz AMX or a Bruker Avance DMX 500 MHz spectrometer (Bruker Biospin, Rhinstetten, Germany). Mass spectra were recorded either on a PerSeptive (PerSpective Biosystems, Inc., Framingham, MA, USA) Voyager DE STR MALDI-TOF mass spectrometer (PerSpective Biosystems, Inc., Framingham, MA, USA) or Agilent 6520 Q-TOF mass spectrometer (Agilent Technologies, Inc., Wilmington, DE, USA). HPLC analyses were performed on a Hitachi elite lachrom system equipped with Diode array detector using C_{18} columns (Hitachi High Technologies, Tokyo, Japan). Compounds **3** to **6**, **9** to **11** were synthesized as described before.

2,6-dichloro-9-(2-deoxy-2-fluoro-3,5-di-*O*-(triisopropylsilyl)-D-arabino furanosyl)-9*H*-purine (7). To a solution of **6** (36 mg, 0.075 mmol) in 1.1 mL of 1,2-dichloroethane were added 10 mg of 4 Å molecular sieves and 2,6-dichloropurine (21.5 mg, 0.113 mmol). The reaction mixture was refluxed at 100°C overnight. Solvent was then removed in vacuo, the residue was redissolved in $CHCl_3$ and filtered, the filtrate was concentrated under reduced pressure. NMR of reaction mixture indicates the formation of both α and β isomers with a ratio of 1/3.5 (α/β). The crude product was used for the next step without further purification.

6-amino-2-chloro-9-(2-deoxy-2-fluoro-3,5-di-*O*-(triisopropylsilyl)-β-D-arabinofuranosyl)-9*H*-purine (8). **7** (75 mg, 0.118 mmol) was dissolved in 4 mL of ammonia in isopropanol solution (2 M), the reaction was carried out in a sealed tube at 105°C overnight. The reaction mixture was then cooled to room temperature and concentrated under reduced pressure. Column chromatography (hexanes: ethyl acetate 3:1) afforded **8** (47 mg, 0.076 mmol, 65%) as a colorless oil, and its α-anomer (11.5 mg, 0.019 mmol, 16%) as white solid. **8** ^{1}H NMR ($CDCl_3$, 500 MHz), δ ppm: 0.96 (stack, 42H), 3.95 (m, 2H), 4.00 (q, $J = 4.5$ Hz, 1H), 4.75 (dd, $J = 3.1$, 18 Hz, 1H), 5.00 (dd, $J = 2.8$, 52 Hz, 1H), 6.08 (s, broad, 2H), 6.42 (dd, $J = 3.5$, 19.8 Hz, 1H), 8.00 (d, $J = 2.8$ Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$), δ ppm: 11.9, 12.1, 17.89, 17.91, 62.0, 75.0, 75.2, 82.7, 82.8, 85.7, 95.0, 96.6, 117.9, 140.6, 150.8, 154.3, 156.0. HRMS (ESI): calcd. for $C_{28}H_{51}ClFN_5O_3Si_2$: 615.3203; Found: 615.3203. **α-anomer** ^{1}H NMR ($CDCl_3$, 500 MHz), δ ppm: 1.01 (stack, 42H), 3.80 (dd, $J = 1.1$, 7.4 Hz, 1H), 3.87 (ddd, $J = 2.1$, 5.0, 10.5 Hz, 1H), 4.48 (t, $J = 5.2$ Hz, 1H), 4.71 (d, $J = 15.3$ Hz, 1H), 5.35 (d, $J = 49.5$ Hz, 1H), 5.77 (s, broad, 2H), 6.34 (d, $J = 15.9$ Hz, 1H), 8.06 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$), δ ppm: 11.88, 11.91, 17.7, 17.8, 17.88, 17.90, 62.6, 75.6, 75.8, 88.2, 88.5, 99.2, 100.7, 118.6, 139.6, 150.6, 154.3, 155.8. HRMS (ESI): calcd. for $C_{28}H_{51}ClFN_5O_3Si_2$: 615.3203; Found: 615.3204.

6-amino-2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-9*H*-purine (Clofarabine, 1). **8** (18.5 mg, 0.03 mmol) was dissolved in 1 mL of DMF, to this solution were added acetic acid (7.2 mg, 0.12 mmol) and tetramethylammonium fluoride (11 mg, 0.12 mmol). The reaction was allowed to stir at room temperature overnight. Solvent was removed in vacuo and column chromatography (ethyl acetate:methanol 20:1) afforded **1** (8.2 mg, 0.027 mmol, 90%) as a pale yellow foam. ^{1}H NMR (DMSO, 500 MHz), δ ppm: 3.63 (m, 2H), 3.84 (q, $J = 4.9$ Hz, 1H), 4.42 (dt, $J = 4.9$, 19 Hz, 1H), 5.23 (dt, $J = 4.3$, 53 Hz, 1H), 6.31 (dd, $J = 4.6$, 13.7 Hz, 1H), 7.88 (s, broad, 2H), 8.27 (d, $J = 1.8$ Hz, 1H). ^{13}C NMR (125 MHz, DMSO), δ ppm: 60.3, 72.3, 72.5, 81.3, 81.5, 83.4, 83.5, 84.5, 96.1, 117.3, 140.0, 150.2, 153.3, 156.8. HRMS (ESI): calcd. for $C_{11}H_{11}ClFN_5O_3$: 303.0534; Found: 303.0540.

2′-deoxy-2′,2′-difluoro-3′,5′-di-*O*-(triisopropylsilyl)cytidine (12). Freshly prepared bis(trimethylsilyl)cytosine (75 mg, 0.03 mmol) was dissolved in 1 mL of 1,2-dichloroethane, to this solution was added TMSOTf (6.7 mg, 0.03 mmol). The mixture was stirred at room temperature for 30 mins before **11** (10 mg, 0.018 mmol) was added, and the reaction was refluxed overnight. The reaction mixture was cooled to room temperature and solvent was removed under reduced pressure. NMR spectrum of reaction mixture indicates the formation of both α and β isomers with a ratio of 1/1. The crude product **12** was used for the next step without further purification.

2′-deoxy-2′,2′-difluorocytidine (Gemcitabine, 2). To a solution of **12** (10 mg, 0.017 mmol) in 1 mL of DMF were added acetic acid (5.2 mg,

0.087 mmol) and tetramethylammonnium fluoride (8 mg, 0.087 mmol). The reaction was stirred at room temperature overnight, then was concentrated and purified by HPLC (solvent was 20 mM ammonium acetate, compounds were eluted at a flow rate of 2 mL/min) to afford **2** (β-isomer, $t_R = 16.4$ minutes, 1.7 mg, 0.006 mmol, 36%) and α-isomer ($t_R = 13.2$ minutes, 1.9 mg, 0.007 mmol, 42%). **2** ^{1}H NMR (D_2O, 500 MHz), δ ppm: 3.86 (dd, $J = 5.0$, 12.9 Hz, 1H), 4.01 (dd, $J = 1.6$, 12.9 Hz, 1H), 4.13 (m, 1H), 4.40 (m, 1H), 6.25 (t, $J = 8.8$ Hz, 1H), 6.30 (d, $J = 8.0$ Hz, 1H), 8.03 (d, $J = 8.0$ Hz, 1H). ^{13}C NMR (125 MHz, D_2O), δ ppm: 59.0, 68.2, 68.4, 68.6, 80.4, 80.5, 83.4, 83.5, 83.6, 83.7, 94.5, 120.7, 123.0, 125.3, 140.8, 154.7, 165.6. HRMS (ESI): calcd. for $C_9H_{11}F_2N_3O_4$: 263.0718; Found:263.0719. **α-anomer** ^{1}H NMR (D_2O, 500 MHz), δ ppm: 3.82 (dd, $J = 5.0$, 12.9 Hz, 1H), 3.95 (dd, $J = 1.6$, 12.9 Hz, 1H), 4.44 (m, 1H), 4.57 (m, 1H), 6.29 (d, $J = 8.0$ Hz, 1H), 6.38 (dd, $J = 5.9$, 8.8 Hz, 1H), 7.93 (d, $J = 7.9$ Hz, 1H). ^{13}C NMR (125 MHz, D_2O), δ ppm: 60.8, 70.4, 70.6, 70.8, 84.5, 84.7, 85.6, 85.7, 85.8, 85.9, 96.1, 123.4, 144.5, 149.5, 160.6. HRMS (ESI): calcd. for $C_9H_{11}F_2N_3O_4$: 263.0718; Found:263.0721.

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