Journal of New Developments in Chemistry
Open Access - Peer-Reviewed - ISSN 2377-2549

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Research Article

Syntheses of Clofarabine and Related C2′-β-fluorinated Nucleoside Analogues

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Published citation: Grigorii Sivets, Aleksey Sivets (2022) **Syntheses of Clofarabine and Related C2′-β-fluorinated Nucleoside Analogues**. *Journal of New Developments in Chemistry* - 3(4):15-29. https://doi.org/10.14302/issn.2377-2549.jndc-22-4277

Article DOI: 10.14302/issn.2377-2549.jndc-22-4277

Abstract

A multistep synthesis of 2-chloro-9-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl) adenine (clofarabine) is described from methyl β-D-ribofuranoside. A new improved method for preparation of 1,2-diacetyl D-ribofuranose derivative was developed via acetolysis of tri-O-pivaloylated D-ribofuranoside and a plausible mechanism of the reaction was proposed. Synthesis of 3′,5′-di-O-pivaloyl-2,6-dichloropurine β-D-riboside along with isomeric 2′,5′-di-O-pivaloyl nucleoside was carried out by stereoselective glycosylation reaction of 2,6-dichloropurine with peracylated D-ribofuranose followed by regioselective 2′-O-deacetylation of protected β-ribonucleoside with different bases. Mild C2′-β-fluorination of the purine 3′,5′-di-O-pivaloyl ribonucleoside with an excess of diethylaminosulfur trifluoride afforded protected 2,6-dichloropurine 2′-fluoro β-D-arabinoside as the key intermediate. An efficient route to clofarabine was also investigated using anion glycosylation of 2-chloroadenine potassium salt with the 1-α-bromide and potassium *tert*-butoxide in a binary solvent mixture, chromatography for separation of a mixture of anomeric nucleosides (a β/α ratio of 3.0:1) and deacylation of benzoylated 2′-fluoro β-nucleoside. A novel N6-isopentyl clofarabine analogue was synthesized by a direct alkylation of the parent nucleoside.

Keywords:
Anticancer purine nucleoside; selectively protected D-ribofuranose derivatives; glycosyl bromide; stereoselective glycosylation; fluorination

Article Information	
Academic Editor	Loai Aljerf, Department of Life Sciences, Faculty of Dentistry, University of Damascu.
Received Date	5 August 2022
Accepted Date	1 September 2022

Article Information (continued)

Published Date	5 September 2022
Online	5 September 2022
Publication	Volume 3; Issue 4; Pages 15–29
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Introduction

Cytotoxic agents relating to a class of modified nucleosides are widely used as effective therapeutics for treatment of cancer [1], [2], [3], [4]. Nucleoside antimetabolites as mimics of natural purine or pyrimidine counterparts are capable of disrupting normal DNA synthesis after incorporation in DNA or by promoting apoptosis, exert their cytotoxic effects and different mechanisms of biological action [3]. Pharmacological studies and clinical trials of synthetic purine nucleosides led to discovery of several analogues as antitumor drugs [2], [5]. Among a series of known purine arabinonucleosides with anticancer activities (Figure 1), fludarabine and clofarabine have found applications as clinical drugs for the treatment of hematologic malignances. Clofarabine (2-chloro-2′-fluoro-2′-deoxyarabinofuranosyl adenine, **3**), potent cytostatic agent with increased stability of the glycosidic bond, is currently used as the antileukemic drug in therapy of pediatric patients with refractory and relapsed lymphoblastic leukemia [6], [7].

Multidirectional mechanism of cytotoxic action of clofarabine, that is not a substrate of adenosine deaminase, includes inhibition of synthesis of DNA and RNA, DNA polymerases, ribonucleotide reductase, and direct induction of cell apoptosis [7], [8]. Clofarabine was shown to display *in vitro* activity against HIV-1 strain and possess dual inhibitory anti-HIV-1 mechanism in virus replication [9].

Various chemical and chemoenzymatic methods were explored for synthesis of anticancer nucleosides [2]. The synthetic routes to purine C2′-β-fluorinated nucleosides and clofarabine associated with this class have been elaborated using different 2-deoxy-2-fluoro-D-arabinofuranose derivatives, acyclic C2-fluorinated dithioacetal precursors and selectively protected ribonucleosides [10], [11], [12], [13], [14]. Bauta *et al* has developed practical and cost-effective synthesis of clofarabine via the stereoselective coupling reaction of 2-chloroadenine with the 1-bromosugar in the presence of potassium *tert*-butoxide followed by deprotection of the intermediate benzoylated β-nucleoside without exploiting chromatography [11]. Cen and Sauve used silyl protected 2-deoxy-2-fluoro-D-arabinofuranose derivative, prepared by electrophilic fluorination of TIPS-protected 2-deoxyribonolactone, for efficient synthesis of clofarabine in six steps via coupling 2,6-dichloropurine with 1-α-chlorosugar [12]. Chemoenzymatic approaches to synthesis of clofarabine were described by Mikhailopulo *et al* utilizing benzoylated 2-deoxy-2-fluoro-D-arabinofuranose and its 1-α-phosphate derivative [14]. Stereospecific formation of N-β-glycosidic bond on the condensation step of the sugar phosphate with 2-chloroadenine and

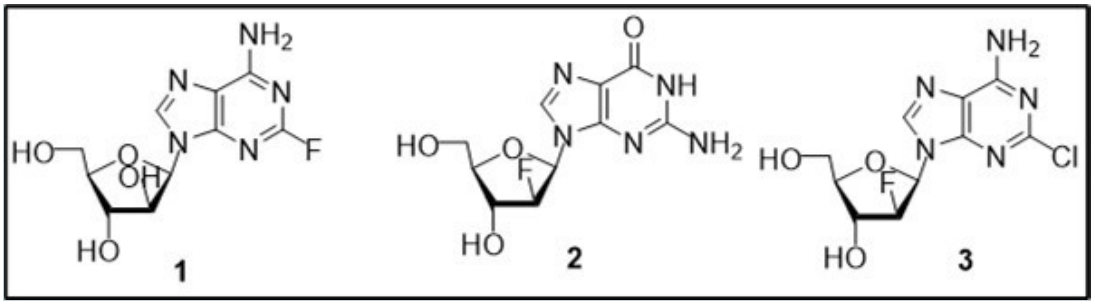


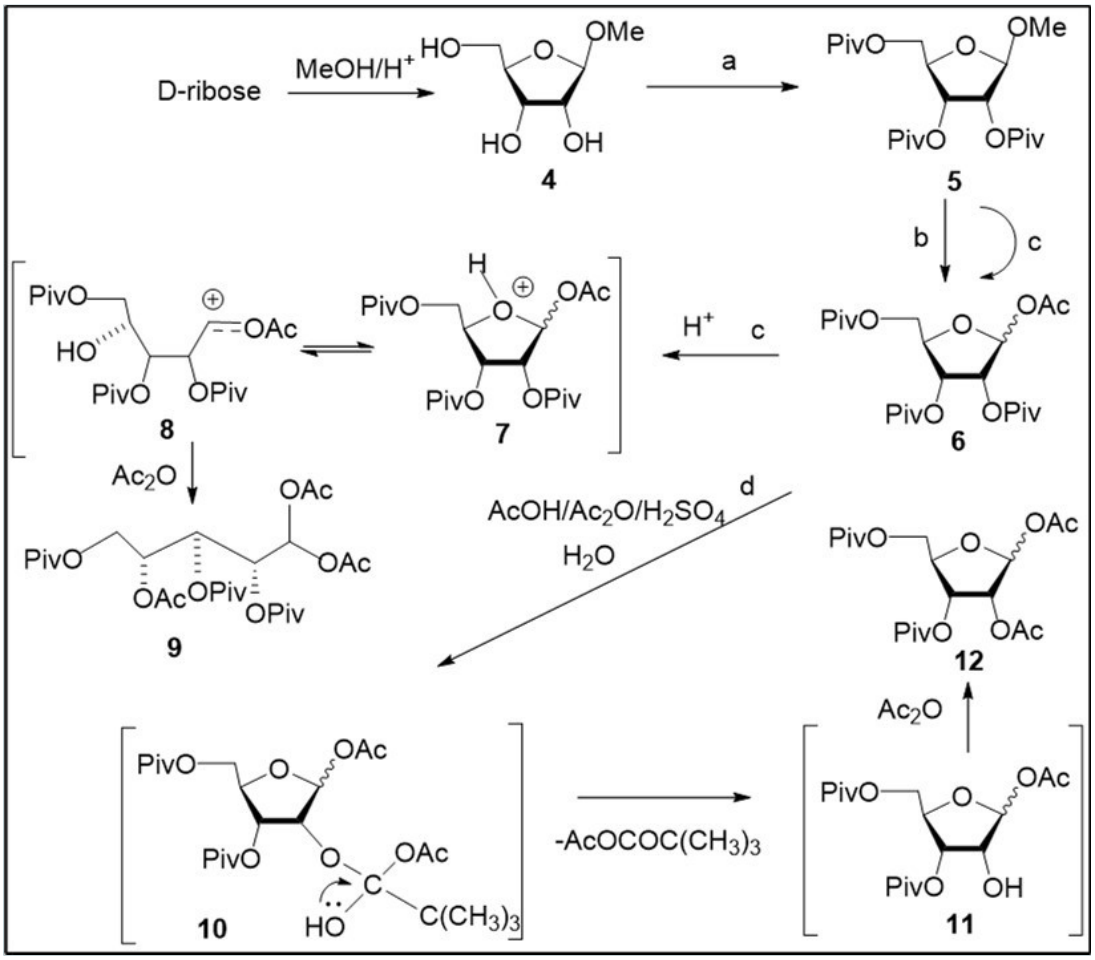
Figure 1. Structures of anticancer fluorinated purine nucleosides

other purine bases catalysed by the recombinant *E.coli* nucleoside phosphorylase is a distinct feature of enzymatic methodologies studied [14], [15], [16] towards purine 2′-deoxy-2′-fluoro-β-D-arabinonucleosides compared to the known chemical methods [10], [11], [17]. Another interesting synthetic route to purine 2′-deoxy-2′-fluoro-β-D-arabinonucleosides was developed by direct introduction of the C2′-β-fluorine function via nucleophilic fluorination of C2′-α-hydroxyl activated derivatives of 3′,5′-diprotected ribonucleosides with different fluorinating agents [18], [19], [20], [21].

As part of our efforts on synthesis of biologically active purine nucleosides from sugars, herein we report a study of synthetic approaches to clofarabine using regio- and stereoselective transformations of pivaloylated D-ribose derivatives and nucleosides, N-glycosylation reactions of purine bases with available carbohydrate precursors, and novel purine modified analogues.

Results and Discussion

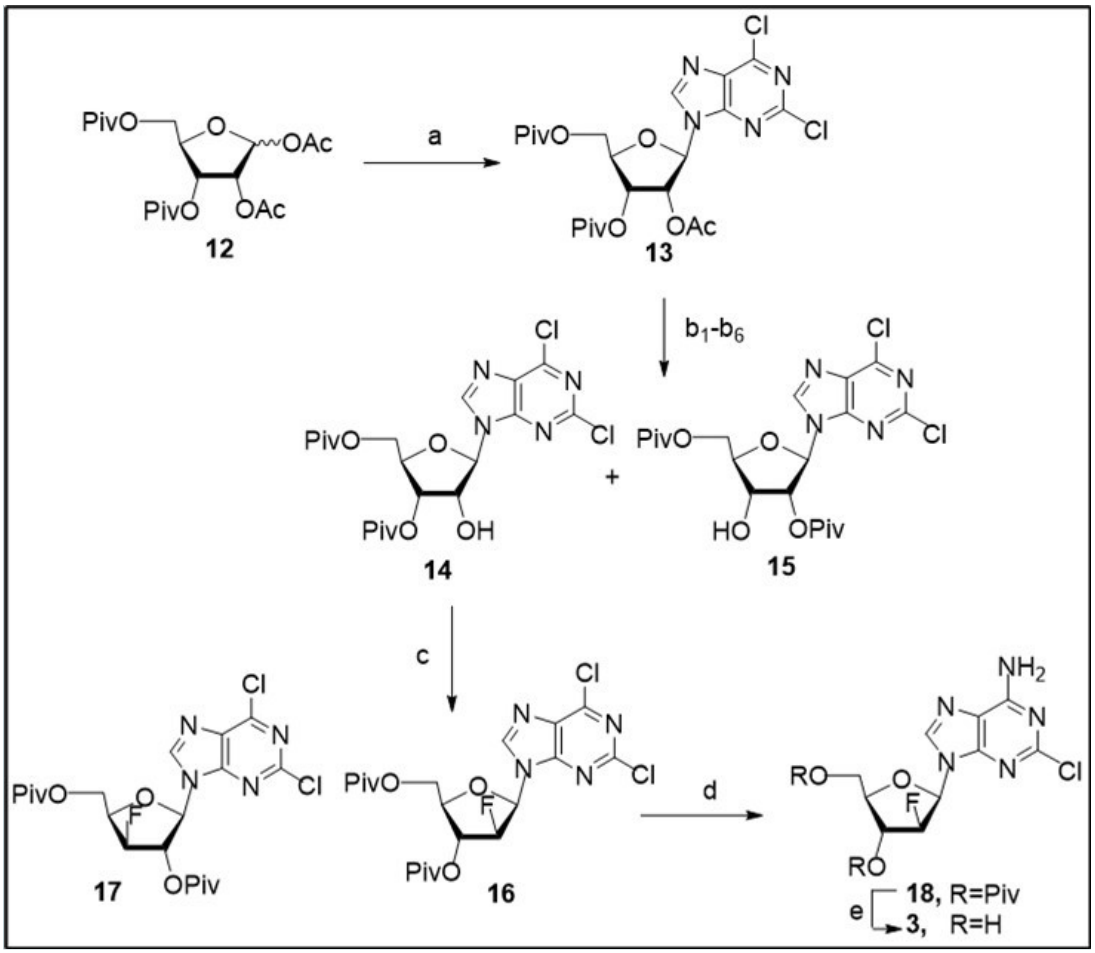
Synthesis of clofarabine was studied using two synthetic routes from known carbohydrate derivatives. In the first approach, we have employed peracylated D-ribofuranose **12** prepared by acetolysis reaction of methyl 2,3,5-O-pivaloylated β-D-ribofuranoside (**5**) taking into account our previous investigations in this field of 2́-β-fluoro-substituted adenine nucleosides [21] (Scheme 1). However, we failed to reproduce our synthetic procedure [21] reported for preparation of the diacetate **12** through acetolysis of acylated methyl β-D-riboside in a mixture of $Ac_2O/AcOH/H_2SO_4$ (5.2% vol sulphuric acid) at room temperature. 1-O-Acetate **6** along with the target 1,2-diacetyl D-ribofuranose derivative **12** (21-45%), or only monoacetyl derivative, were prepared in a series of experiments (Scheme 1, conditions b).



Scheme 1. Study of acetolysis reaction of pivaloylated methyl D-ribofuranoside 5 and proposed mechanism for the formation of the diacetate 12. Reagents and conditions: a) ref. 21, 89%; b) AcOH, Ac_2O, H_2SO_4 (12.6:1.6 :1.0, vol), 5.2% H_2SO_4, rt, 3-4 h, 6, 20-41%; 12, 21-45%; c) AcOH, Ac_2O, 9.6% H_2SO_4 (8.0:1.3:1.0, vol), 24 h, rt, 6, 68%; 9, 10%; d) AcOH, Ac_2O, H_2SO_4 (8.05:1.0:1.1, ratio), 10.0% H_2SO_4 rt, 5 h, H_2O, 90 min, 12, 72%;

Further, we have undertaken a study of acetolysis of **5** varying various reaction conditions in order to prepare **12** with high yield. Acetolysis (conditions c) in a mixture of reagents - AcOH, Ac_2O, H_2SO_4 (8.0:1.0:1.1) with increased content of sulphuric acid (9.6% vol) gave rise to the 1-O-acetyl derivative **6** as a mixture of β/α-anomers (68%) and acyclic product **9** (10%). Mechanism of the formation of by-product **9** via protonation of the monoacetate **6** and generation of open furanose derivative **8** is outlined in Scheme 1. 1-O-acetate **6** was prepared from methyl ribofuranoside derivative **5** without formation of the diacetate **12**, using anhydrous conditions for acetolysis reaction explored earlier for preparation of 1,2-di-O-acetyl-3,5-di-O-pivaloyl-L-arabinofuranoses from pivaloylated methyl L-arabinofuranoside [22]. As a result of studying acetolysis reaction we have found optimal conditions to obtain **12** with high yield (72%, conditions d) after chromatography on silica gel.

Carrying out acetolysis of **5** in the presence of a small quantity of water resulted in the target diacetate with a high yield. The proposed mechanism of the formation of **12** is shown in Scheme 1. We propose that synthetic route to the target acylated D-ribofuranose derivative includes formation of the 1-O-acetate **6** on the first step of acetolysis ($Ac_2O/AcOH/H_2SO_4$) followed by generation, in the presence of water, intermediate D-ribofuranose derivatives **10** and **11**, which lead to exchange of the 2-O-pivaloyl group in the starting riboside by the 2-O-acetyl group after acetylation at 2-hydroxyl group in selectively acylated ribofuranose **11** with acetic anhydride. It is important to note that the mechanism under above consideration for the acetolysis reaction of D-glycoside **5** with the formation of the 1,2-diacetate **12** differs from that suggested earlier in our previous work [22] for preparation of isomeric 1,2-di-O-acetyl-3,5-di-O-pivaloyl-L-arabinofuranoses via intermediate 1,2-pivaloyloxonium ion from protected L-arabinoside under anhydrous conditions.



Scheme 2. Synthesis of clofarabine 3 from the diacetate 12. Reagents and conditions: a) MeCN, silylated 2,6-diClPurine, TMSOTf, rt, 96%; b_{1-6}) deacylation of 13, solvent/base, a mixture of nucleosides 14 and 15, 51-69% (Table 1); c) 14/15, DAST, CH_2Cl_2, Py, $0^0 \rightarrow$rt, 59% for 16, 2-7% for 17; d) NH_3/1,2-DME, rt, 18 h, 96%; e) MeONa/MeOH, rt, 74%.

Stereoselective glycosylation of silylated 2,6-dichloropurine with the 1,2-diacetate **12** in acetonitrile in the presence of TMSOTf gave fully O-acylated β-D-riboside of 2,6-dichloropurine **13** in 96% yield (Scheme 2).

Selective removal of 2′-O-acetyl group in protected nucleoside **13** was explored under different conditions which are summarized in Table 1 (entries 1-6). Mild removal of 2′-O-acetyl group in nucleoside **5** with the known method, with an excess of $NaHCO_3$ in methanol [10], gave rise to a mixture of 3′,5′-di-O-pivaloyl (**14**) and 2′,5′-di-O-pivaloyl (**15**) 2,6-dichloropurine β-D-ribosides, which were inseparable by column chromatography on silica gel, in an overall 51% yield. The deacetylation of **13** under the mild basic conditions was accompanied by migration the 3′-O-pivaloyl group of **14** from 3′-position to 2′-position with the formation of the 2′-O-pivaloylate **15** (a ratio of **14**/**15** – 1.7/2.0:1 according to ^{1}H NMR data of the crude reaction mixtures). We failed to isolate individual acylated nucleosides **14** and **15** from a mixture using chromatography on silica gel or crystallization. Second known approach with application of dibutylthin oxide (Bu_2SnO) in methanol [23], [24] was tested for selective removal of the 2́-O-acetyl group in nucleoside **13**. Mixtures of 3′,5′-di-O-pivaloyl (**14**) and 2′,5′-di-O-pivaloyl (**15**) 2,6-dichloropurine ribosides were prepared in 55% and 69% yields, respectively, using 1.1 or 2.1 equiv of Bu_2SnO in anhydrous methanol (entries 2-3), the best ratio of isomeric ribosides being achieved under refluxing of **13** with 2.1 equiv of Bu_2SnO. Novel method for selective deacetylation of nucleoside **13** was explored using NaOCN under various conditions (solvent and excess of inorganic salt, entries 4-6). Best yield of a mixture of nucleosides **14**/**15** and regioselectivity of the deacylation reaction (a ratio of **14**/**15** –2.2:1) was prepared with 1.9 equiv of sodium cyanate in anhydrous methanol at room temperature (entry 5). The regioselective deacetylation of **13** with $NaHCO_3$, NaCNO or Bu_2SnO in methanol was accompanied by migration the 3′-O-pivaloyl group of 3′,5′-diprotected nucleoside **14** with the formation of the 2′-O-pivaloylate **15** (Table 1). Making use 4.3-fold excess of $NaHCO_3$ or 1.9 equiv of NaCNO in anhydrous methanol, mixtures of **14**/**15** were prepared in 51% and 56% yields, respectively. The use of a large excess of $NaHCO_3$ in methanol (entry 1) produced less yield of a mixture of isomeric nucleosides **14** and **15** than that isolated after selective deprotection of the 2′-O-acetate **13** with NaCNO (entry 5). These findings may be explained by different solubility of inorganic sodium salts at room temperature that is the important factor in development of efficient method for cleavage of 2′-O-acetyl group. The increase of the reaction time in control experiment with an excess of NaCNO in anhydrous methanol resulted in the formation of by-products (according to TLC data and ^{1}H NMR data of the reaction mixture) and reducing overall yield of isomeric nucleosides. Fast removal of 2′-O-acetyl protecting group in the intermediate nucleoside 13 (entry 5) likely proceeds by a transesterification mechanism via generation of methoxide anion from sodium cyanate in anhydrous methanol with a gradual release of HOCN.

Further, introduction of the C2′-β-fluorine atom via the nucleophilic replacement of an activated C2′-α-hydroxyl of 3′,5′-di-O-pivaloyl ribonucleoside **14** containing bulky directing groups with a fluoride anion was studied on the next step. The treatment of a mixture of selectively blocked ribonucleosides **14** and **15** (a ratio - 2.3:1 after column chromatography on silica gel) with an excess of DAST in CH_2Cl_2/pyridine at room temperature afforded protected 2′-β-fluoro nucleoside (**16**, 59%). Isomeric 3′-β-fluoro (**17**, 2-7%) nucleoside was also isolated as a by-product under the fluorination step of mixtures of nucleosides **14** and **15** after column chromatography on silica gel. New 2,6-dichloropurine 3′,5′-di-O-pivaloyl β-D-2′-fluoroarabinonucleoside **16** was prepared as the key intermediate for synthesis of different purine modified 2′-β-F-nucleosides. Analysis of conformational peculiarities of selectively protected 2,6-dichloropurine ribonucleoside **14** based upon its ^{1}H NMR spectral data ($J_{1',2'}$ = 6.25, $J_{2',3'}$ = 5.6, $J_{3',4'}$ = 3.27 Hz) shows that the pentofuranose ring in this nucleoside takes a C-2′-endo conformation, S-type, as in the case of the adenine analogue [21] studied earlier in the DAST reaction which leads to adenine 2′-β-fluoro-nucleoside under reflux in moderate yield. However, despite the population of *S*-type conformation in *N*/*S* pseudorotational equilibrium being predominant in solution for the both 3′,5′-di-O-acyl purine ribonucleosides, there are some differences in the spatial organization of 3′,5′-di-O-pivaloyl-2,6-dichloropurine nucleoside and the corresponding adenosine derivative, their C2′-α-hydroxyl activated intermediates forming with DAST during the course of fluorination reactions. Selective amination of 2,6-dichloropurine derivative **16** with ammonia in 1,2-dimethoxyethane followed by the removal of acyl groups in 3′,5′-di-O-pivaloyl 2′-β-fluoro nucleoside of 2-chloroadenine

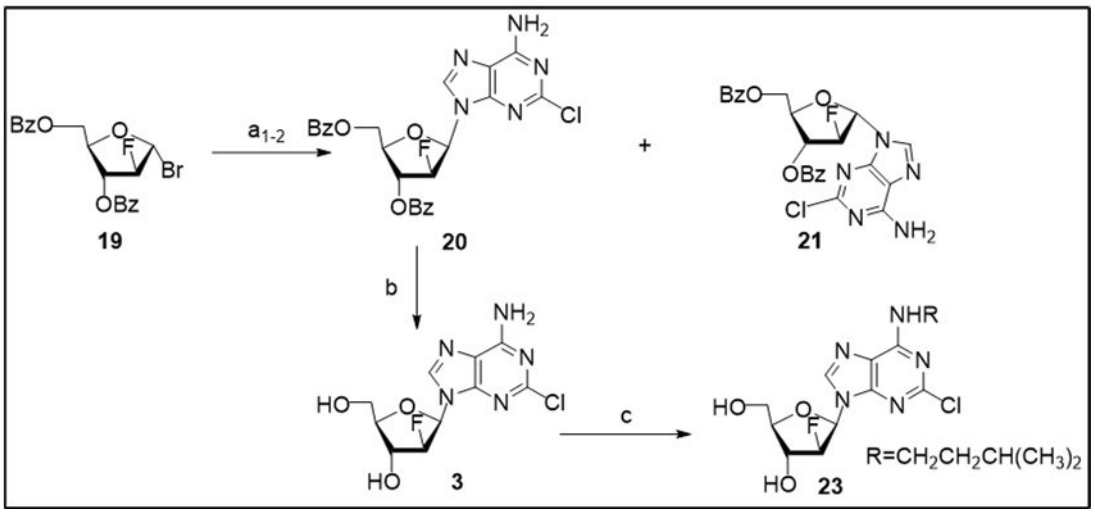
Table 1. Study of regioselective deacetylation of 2′-O-Ac-2′,3′-di-O-pivaloyl ribonucleoside of 2,6-dichloropurine 13 under various conditions.

Entry	Reaction conditions	Ratio of reagent: nucleoside 13	Reaction time (h)	Ratios of isomeric nucleosides 14 and 15[a]	Rate of conversion of 2′-O-acetate 13	Yield[b] (%)
1	$NaHCO_3$/MeOH	4.3:1	1.8 h	1.80:1.0	73	51
2	Bu_2SnO/MeOH	1.1:1	7.2 h	2.00:1.0	71	55
3	Bu_2SnO/MeOH	2.1:1	4h50 min	2.44:1.0	85	69
4	NaOCN/MeOH	1.2:1	2h20 min	2.00:1.0	75	66[c]
5	NaOCN/MeOH	1.9:1	42 min	2.20:1.0	81	67
6	NaOCN/MeOH/THF	1.9:1	46 min	2.20:1.0	79	60

[a] Determined by ^{1}H NMR spectroscopy of the reaction mixtures after treatment in $CDCl_3$;
[b] Isolated yield of mixtures of isomeric nucleosides **14** and **15** by column chromatography.
[c] Yield of isomeric nucleosides from ^{1}H NMR spectrum of the reaction mixture after treatment

18 with sodium methoxide in methanol resulted in the target nucleoside **3** in 71% combined yield on two steps after column chromatography on silica gel. Thus, synthesis of clofarabine was carried out in seven steps from D-ribose in moderate overall yield exploiting the stereoselective glycosylation of 2,6-dichloropurine with available peracylated D-ribose, selective 2′-O-deacetylation of the intermediate tri-O-acylated nucleoside, and the DAST fluorination of 3′,5′-di-O-pivaloyl-2,6-dichloropurine ribonucleoside.

Next, a short approach to clofarabine (Scheme 3) was investigated from the glycosyl bromide **19** prepared by mild bromination of available perbenzoylated 2-deoxy-2-fluoro-α-D-arabinofuranose with TMSBr [17] taking into account the cost-effective synthesis reported earlier [11]. Anion glycosylation of 2-chloroadenine salt, generated in the presence of potassium *tert*-butoxide and anhydrous potassium



Scheme 3. Synthesis of clofarabine 3 and its N6-alkylated analogue 23 from the glycosyl bromide 19. Reagents and conditions: a_1) bromide 19, K-salt of 2-ClAde generated with t-BuOK in 1,2-DME in the presence of 1.6 equiv anhydrous KBr, CH_3CN, rt, 18 h, 45-48% 20, 16%, 21; a_2) bromide 19, K-salt of 2-ClAde generated with t-BuOK in 1,2-DME in the presence of 1.5 equiv anhydrous KBr, CH_3CN/CH_2Cl_2 (2:1), rt, 55 h, 55% 20, 18% 21; b) 20, NH_3/MeOH, rt, 78%; c) 3, t-BuOK, DMSO, $(CH_3)_2CHCH_2CH_2OMs$ (22), 89-90 ^{0}C, 90 min, 23, 73% taking into account of recovery of the starting nucleoside.

bromide as additive in 1,2-dimethoxyethane, with the 1-α-bromide **19** in acetonitrile resulted in a mixture of N9-β-anomer **20** (48%) and its α-anomer **21** (16%) which were separated by column chromatography on silica gel. Formation of protected β- and α-nucleosides (β/α ratio - 3:1) along with by-product (7-8%) was observed in this experiment according to ^{1}H NMR data of the crude reaction mixture in $CDCl_3$ (Scheme 2, conditions a_1).

The coupling of **19** with 2-chloroadenine salt, produced by treatment with t-BuOK in the presence of potassium bromide, in a mixture of CH_3CN/CH_2Cl_2 (2:1) (Scheme 2, a_2) at room temperature gave rise to β-(55%) and α-(18%) protected nucleosides after column chromatography on silica gel with a similar β/α ratio and higher overall yield in comparison to the previous reaction conditions. No increase of anomeric stereoselectivity of the glycosylation reaction of 2-chloroadenine salt was observed in a mixture of CH_3CN/CH_2Cl_2 (1:1) in the presence of calcium hydride as additive and conversion of bromide was 86% for 36 h in this experiment. Synthesis of anomeric benzoylated nucleosides by anion glycosylation of 2-chloroadenine with 1-α-bromide involves competitive S_N2 and S_N1-reaction pathways, the latter occurs via generation of an oxonium ion intermediate. Formation of α-glycosylated product **21** in CH_3CN/CH_2Cl_2 with potassium bromide as additive is likely to proceed via a 3,5-benzoxonium ion [25] deriving from the 1-α-bromide, and a subsequent nucleophilic α-attack of the nucleobase at C1.

Characteristics of the studied glycosylation reactions of the potassium salt of 2-chloroadenine, generated using t-BuOK in 1,2-dimethoxyethane, with 1-α-bromosugar are: i) the anion glycosylation reaction with a small excess of the heterobase proceeds in heterogeneous phase in acetonitrile or a mixture of solvents with different polarities at room temperature with a full conversion of bromosugar to nucleoside products; ii) KBr as additive results in a little increase of β/α-anomeric selectivity towards protected N9-β-nucleoside, and iii) good isolated yield of intermediate benzoylated β-2′F-arabinonucleoside is achieved after selective glycosylation in a binary solvent system and separation of N9-β- and α-glycosylated products by column chromatography on silica gel in detected conditions. Furthermore, based on our previous results on efficient synthesis of benzoylated 2-amino-6-chloropurine 2′-fluoro-β-arabinonucleoside from the bromide **19** [17] and the above findings tested for isomeric 2-chloroadenine nucleoside **20**, we may conclude that a better solubility of the modified purine base in organic solvents provides a higher β/α-stereoselectivity of heterogeneous glycosylation reaction of the purine potassium salt in a binary solvent mixture.

Deprotection of benzoylated 2′-fluoro β-nucleoside **20** with ammonia in methanol yielded clofarabine **3** in 78% yield after crystallization. The target nucleoside was prepared in 35-43% overall yields using column chromatography. Synthesis of N6-substituted clofarabine derivative **14** was carried out by a direct alkylation of **3** with 1-methanesulfonyloxy-3-methylbutane (**22**) in anhydrous dimethylsulfoxide in the presence of *t*-BuOK at 89-90^{0}C. The use of bulky alkylating agent prepared by mesylation of isoamyl alcohol with mesyl chloride resulted in a selective reaction on an exocyclic N6-amino group of the starting nucleoside under studied conditions to give new N6-isopentyl clofarabine analogue **23** in 73% yield after column chromatography on silica gel.

Conclusion

In summary, multistep synthesis of clofarabine and C2′-β-fluorinated purine modified nucleosides was accomplished using regio- and stereoselective reactions of O-pivaloylated D-ribose derivatives and mild fluorination of 3′,5′-di-O-pivaloyl-2,6-dichloropurine riboside containing bulky directing groups with DAST on the key step. Optimized method for preparation of the 1,2-diacetyl D-ribofuranose derivative was developed via acetolysis of tri-O-pivaloylated D-ribofuranose. The intermediate selectively protected purine nucleoside was obtained by stereoselective glycosylation reaction of the 1,2-diacetate with 2,6-dichloropurine followed by regioselective 2′-O-deacetylation of fully protected β-ribonucleoside with different deacylating reagents. The efficient two-step synthetic route to clofarabine via selective anion glycosylation of 2-chloroadenine with 3,5-di-O-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide in a mixture of solvents is described under mild reaction conditions. N6-isopentyl clofarabine derivative with potential biological activity was prepared by a direct alkylation of the parent nucleoside with 1-methanesulfonyloxy-3-methylbutane in the presence of t-BuOK.

Material and Methods

Column chromatography was performed on silica gel 60 H (70-230 mesh; Merck, Darmstadt, Germany), and thin-layer chromatography (TLC) on Merck silica gel aluminum 60 F_{254} precoated plates. The anhydrous solvents were distilled over CaH_2, P_2O_5 or magnesium prior to the use. All commercially available reagents were used without further purification. ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded in $CDCl_3$, CD_3OD and DMSO-d_6 with a Bruker Avance-500-DRX spectrometer at 500.13, 126.76 and 470.59 MHz, respectively. ^{1}H and ^{13}C NMR chemical shifts (δ, ppm) are relative to internal chloroform peak (7.26 ppm for ^{1}H and 77.0 for ^{13}C NMR). Chemical shifts are also reported downfield from internal $SiMe_4$ (^{1}H) or external $CFCl_3$ (^{19}F). Splitting patterns were reported as follows: s: singlet, d: doublet, t: triplet, m: multiplet. *J* values are reported in Hz. Melting points were determined on a Boetius apparatus and were uncorrected. High resolution mass spectra (HRMS) were recorded on an Agilent Q-TOF 6550 Instrument (USA) using ESI (electrospray ionization).

Experimental Procedures

Synthesis of 1-O-acetyl-2,3,5-tri-O-pivaloyl-D-ribofuranose (6) and 1,1,4-tri-O-acetyl-2,3,5-tri-O-pivaloyl-D-ribose hydrate (9)

Methyl D-ribofuranoside **5** (0.6 g, 1.44 mmol) was coevaporated with anhydrous toluene (2x20 ml), dissolved in a mixture of CH_3COOH (4.35 ml), Ac_2O (0.52 ml) and H_2SO_4 (0.29 ml), prepared previously by adding H_2SO_4 to a mixture of CH_3COOH and Ac_2O and stirring the solution for 7 min. The reaction mixture was stirred at ambient temperature for 4 h. Then Ac_2O (0.2 ml) was added to it and the reaction mixture was stirred for 60 min at room temperature and H_2SO_4 (0.25 ml) was added to the prepared solution. After stirring at rt for 24 h, the prepared solution was diluted with ethyl acetate (45 ml), washed with cooled water (4x25 ml) and then dried over anhydrous Na_2SO_4, evaporated to dryness. The residue was chromatographed on a silica gel, using mixtures of 6:1, 5: 1 and 3:1 petroleum ether-EtOAc to afford a mixture of 1-O-acetyl derivatives **6** (0.436 g, 68%, α/β =1.2:1) as a syrup and acyclic product **9** (0.07 g 10%) as a syrup.

Compound **6**, β-anomer. ^{1}H NMR ($CDCI_3$): δ 6.11 (br.s, lH, $J_{1,2}$<1.0 Hz, H-1), 5.34 (dd, lH, H-2), 5.25 (dd, 1H, $J_{3,4}$=1.6, H-3), 4.39-4.45 (m, lH, H-4), 4.26 (dd, lH, $J_{5,5'}$ =12.2, $J_{5,4}$=3.2, H-5), 4.23 (dd, lH, $J_{5',4}$ = 4.5, H-5'), for α- and β-anomers: 2.09 and 2.08 (2s, CH_3CO), 1.24, 1.23, 1.22, 1.20 and 1.19 [5s, $(CH_3)_3$-CO].

Compound **6**, α-anomer. ^{1}H NMR ($CDCI_3$): δ 6.46 (d, lH, $J_{1,2}$ =3.8 Hz, H-l), 5.24 (dd, lH, H-2), 5.35 (dd, lH, 4.1, H-3), 4.32-4.38 (m, lH, H-4), 4.29 (dd, lH, $J_{5,5'}$ = 12.4, $J_{5,4}$ = 4.6, H-5), 4.18 (dd, lH, $J_{5',4}$ = 3.5, H-5'), for α- and β-anomers: 2.09 and 2.08 (2s, CH_3CO), 1.24, 1.23, 1.22, 1.20 and 1.19 [5s, $(CH_3)_3$-CO]. ^{13}C NMR ($CDCl_3$): δ 178.08, 177.93, 177.54, 177.20, 176.87, 176.80 [(C=O, 6x$COC(CH_3)_3$], 169.56 and 169.19 (C=O, $COCH_3$), 98.16, 79.60, 74.07, 70.11, 63.07 (C-1, C-2, C-3, C-4, C-5, alpha-anomer), 94.23, 83.01, 70.57, 70.22, 63.49 (C-1, C-2, C-3, C-4, C-5, beta-anomer), 38.86 [2x$COC(CH_3)_3$], 27.25, 27.18, 27.15 and 27.09 [4x$COC(\mathbf{C}H_3)_3$], 21.08 and 20.99 (2x$CO\mathit{CH}_3$). HRMS (EI): m/z calcd for $C_{22}H_{36}O_9(M+Na)^+$: 467.2252, found 467.2254.

Acyclic product **9**, ^{1}H NMR ($CDCl_3$): δ 7.08 (d, lH, $J_{1,2}$ =5.7 Hz, H-l), 5.43-5.49 (m, 2H, H-2 and H-3), 5.34-5.39 (m, lH, H-4), 4.42 (dd, lH, $J_{5,5'}$ = 11.6, $J_{5,4}$ = 2.3, H-5), 4.10 (dd, lH, $J_{5',4}$ = 6.3, H-5'), 2.16, 2.14, and 2.09 (3s, CH_3CO), 1.26, 1.25 and 1.22 (3s, 3x3H, 3x$COC(CH_3)_3$]. ^{13}C NMR ($CDCl_3$): δ 177.86, 176.81 and 176.58 [(C=O, 3x$COC(CH_3)_3$], 169.72 and 168.22 (C=O, 2x$COCH_3$), 85.82, 69.58, 69.35, 68.07, 61.58 (C-1, C-2, C-3, C-4, C-5), 38.04, 38.97 and 38.83 [3x$COC(CH_3)_3$], 27.05, 27.03 and 26.98 [3x$COC(\mathbf{C}H_3)_3$], 20.74 and 20.59 (2x$CO\mathit{CH}_3$). HRMS (EI): m/z calcd for $C_{26}H_{42}O_{12}(M+Na)^+$: 569.2569, found 569.2574.

Synthesis of 1,2-di-O-acetyl-3,5-di-O-pivaloyl-α,β-D-ribofuranose (12)

Methy-2,3,5-tri-O-pivaloyl-β-D-ribofuranose **5** (0.387 g, 0.929 mmol) was coevaporated with anhydrous toluene (2x20 ml), dissolved in a mixture of CH_3COOH (3.7 ml), Ac_2O (0.32 ml) and H_2SO_4 (0.35 ml), prepared previously by adding H_2SO_4 to a mixture of CH_3COOH and Ac_2O and stirring the solution for 7 min. The reaction mixture was stirred for 3 h at ambient temperature and for 2 h at 30 ^{0}C. Then H_2O (0.043 ml) was added and the reaction mixture was stirred for 90 min at room temperature and

diluted with ethyl acetate (45 ml), the prepared organic phase was washed with cooled water (4x40 ml) and then dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was chromatographed on a silica gel, using a mixture of 8:1, 6:1 and 5:1 petroleum ether-EtOAc to afford the diacetate **12** (0.27 g, 72%, β/α=1.3:1) as a syrup.

1,2-diacetate **12**, β-anomer. ^{1}H NMR ($CDCI_3$): δ 6.17 (d, lH, $J_{1,2}$ =1.4, H-1), 5.40 (dd, lH, $J_{2,3}$= 4.9, H-2), 5.31 (dd, H, $J_{3,4}$= 1.3 Hz, H-3), 4.36-4.40 (m, lH, H-4), 4.24 (dd, $J_{5,5'}$ = 12.1, $J_{5,4}$=3.5, H-5), 4.27 (dd, 1H, $J_{5',4}$=4.7, H-5'), for α- and β-anomers: 2.154, 2.15, 2.14, and 2.09 (4s, CH_3CO), 1.29, 1.28, 1.27, and 1.24 [4s, $(CH_3)_3$-CO].

1,2-diacetate **12**, α-anomer.^{1}H NMR ($CDCI_3$): δ 6.43 (d, lH, $J_{1,2}$ =4.4, H-1), 5.30 (dd, lH, $J_{2,3}$= 6.3, H-2), 5.30 (dd, lH, $J_{3,4}$=1.7, H-3), 4.41-4.49 (m, lH, $J_{4,5}$= 3.1, H-4), 4.24 (dd, lH, $J_{5,5'}$=12.2, $J_{5,4}$=3.0, H-5), 4.19 (dd, 1H, $J_{5',4}$=3.0, H-5').

^{13}C NMR ($CDCl_3$): δ 178.06, 177.92, 177.50, 177.13 [(C=O, 4x$COC(CH_3)_3$], 169.74 and 169.26 (C=O, $COCH_3$), 98.37, 80.15, 74.27, 70.37, 63.43 (C-1, C-2, C-3, C-4, C-5, beta-anomer), 94.12, 82.43, 70.53, 69.69, 63.43 (C-1, C-2, C-3, C-4, C-5, alpha-anomer), 38.86 [2x$COC(CH_3)_3$], 27.21, 27.17, 27.02 and 27.00 [4x$COC(\mathbf{C}H_3)_3$], 21.09, 21.05, 20.55 and 20.29 (4x$CO\mathit{C}H_3$). HRMS (EI): m/z calcd for $C_{19}H_{30}O_9(M+Na)^+$: 425.1782, found 425.1780.

Synthesis of 2,6-dichloro-9-(2'-O-acetyl-3',5'-di-O-pivaloyl-β-D-ribofuranosyl)-9H-purine (13)

To a mixture of the diacetate **12** (0.35 g, 0.87 mmol) and silylated derivative of 2,6-dichloropurine, prepared by conventional procedure from 0.164 g (0.87 mmol) of 2,6-dichloropurine, in anhydrous CH_3CN (5 ml), TMSOTf (0.2 ml, 1.2 mmol) was added and then the reaction mixture was stirred for 150 min at room temperature and poured into 5% aq $NaHCO_3$. The mixture was extracted with $CHCl_3$ (3x50 ml), combined extracts were washed with water, dried and evaporated. The residue was chromatographed on silica gel, eluting with mixtures of 3:1, 2:1 and 1:1 hexane-EtOAc to afford the nucleoside **13** (0.44 g, 96%) as syrup. ^{1}H NMR ($CDCl_3$): δ 8.29 (s, 1H, H-8), 6.20 (d, 1H, $J_{1',2'}$ = 6.2 Hz, H-1′), 5.76 (t, 1H, H-2′), 5.52 (dd, 1H, H-3′), 4.44-4.47 (m, 1H, H-4′), 4.43 (dd, 1H, H-5′), 4.35 (dd, 1H, H-5″), 2.06 (s, 3H, CH_3CO), 1.28 and 1.24 (2s, 2x9H, 2x$COC(CH_3)_3$]. ^{13}C NMR ($CDCl_3$): δ 178.0 and 177.2 [(C=O, 2x$COC(CH_3)_3$], 169.37 (C=O, $COCH_3$), 153.6, 152.7, 152.5, 143.7, 131.5 (C-2, C-6, C-4, C-8, C-5), 86.4 (C-1′), 81.5 (C-3′), 73.5 (C-4′), 70.4 (C-2′), 63.3 (C-5′), 39.07 and 39.01 [2x$COC(CH_3)_3$], 27.35 and 27.2 [2x$COC(CH_3)_3$], 20.4 ($CO\mathit{C}H_3$). HRMS (EI): m/z calcd for $C_{22}H_{28}Cl_2N_4O_7$ $(M+Na)^+$: 553.1233, found 553.1228.

Synthesis of 2,6-dichloro-9-(3',5'-di-O-pivaloyl-β-D-ribofuranosyl)-9H-purine (14) and 2,6-dichloro-9-(2',5'-di-O-pivaloyl-β-D-ribofuranosyl)-9H-purine (15)

Method 1

To a solution of protected nucleoside **13** (0.233 g, 0.438 mmol) in anhydrous methanol (12 mL) was added solid anhydrous $NaHCO_3$ (0.16 g, 1.9 mmol) and the prepared solution was stirred for 110 min at room temperature, then the reaction mixture was neutralized with acetic acid and evaporated, coevaporated with anhydrous benzol (2 x 10 ml). The residue was chromatographed on silica gel, using a mixture of 3:1, 2:1 and 1:1 hexane-EtOAc to afford the starting nucleoside 0.062 g (27%) and a mixture of nucleosides **14** and **15** (0.11 g, 51%) as a syrup.

^{1}H NMR of a mixture of **14** and **15** ($CDCl_3$): δ 8.31 (s, 1H, H-8, compound **14**), 8.27 (s, 0.37H, H-8, compound **15**), 6.16 (d, 0.37H, $J_{1',2'}$ = 4.2 Hz, H-1′), 6.00 (d, 1H, $J_{1',2'}$ = 6.25 Hz, H-1′), 5.55 (dd, 0.37H, H-2′), 5.32 (dd, 1H, $J_{3',4'}$ = 3.27 Hz, H-3′), 4.97 (m, 1H, $J_{2',3'}$ = 5.6 Hz, H-2′), 4.72 (m, 0.37H, H-3′), 4.48-4.51 (m, 1H, H-4′), 4.37 - 4.67 (m, 2H-5′, H-5′ and H-5″, H-4′), 1.31, 1.26, 1.21 and 1.20 (4s, 24.6H, 4x$COC(CH_3)_3$]. ^{13}C NMR ($CDCl_3$): δ 178.3, 177.96, 177.92 and 177.68 [(C=O, 4x$COC(CH_3)_3$], 154.9, 152.5, 152.4, 152.3, 144.1, 131.4; 89.2, 83.5, 73.8, 72.2 (C-1′, C-3′, C-4′, C-2′), 87.3, 82.5, 75.7, 70.1 (C-1′, C-3′, C-4′, C-2′), 63.11 (C-5′), 39.1 and 38.9 [4x$COC(CH_3)_3$], 27.21 and 27.14 [4x$COC(\mathbf{C}H_3)_3$]. HRMS (EI): m/z calcd for $C_{20}H_{26}Cl_2N_4O_6(M+Na)^+$: 511.1127, found 511.1227.

Method 2

To a solution of protected nucleoside **13** (0.02 g, 0.038 mmol) in anhydrous methanol (1.2 mL) was added Bu_2SnO (0.008 g, 0.0402 mmol) and the prepared solution was stirred under reflux for 7h 15 min, then the reaction mixture was evaporated. The residue was dissolved with EtOAc (35 ml), the organic layer was washed with water (2x10 ml) and dried over anhydrous Na_2SO_4, and then evaporated to dryness. The residue was chromatographed on silica gel, eluting with mixtures of 3:1, 2:1 and 1:1 hexane-EtOAc to afford a mixture of nucleosides **14** and **15** (0.010 g, 55%) as a syrup.

Method 3

To a solution of protected nucleoside **13** (0.022 g, 0.041 mmol) in anhydrous methanol (2 mL) was added Bu_2SnO (0.022 g, 0.0884 mmol) and the prepared solution was stirred under reflux for 4h 50 min, then the reaction mixture was evaporated. The residue was dissolved with EtOAc (30 ml), the organic layer was washed with water (2x10 ml) and dried over anhydrous Na_2SO_4, and then evaporated to dryness. The residue was chromatographed on silica gel, eluting with mixtures of 4:1, 3:1 and 2.5:1 petroleum ether-EtOAc to afford the starting nucleoside (0.003 g, 14 %) and a mixture of nucleosides **14** and **15** (0.012 g, 69%) as a syrup with recovery of the starting nucleoside.

Method 4

To a solution of protected nucleoside **13** (0.021 g, 0.039 mmol) in anhydrous methanol (4 mL) was added NaOCN (0.003 g, 0.046 mmol) and the prepared solution was stirred for 140 min at room temperature. Then the reaction mixture was diluted with EtOAc (30 ml), the organic layer was washed with water (2x10 ml) and dried over anhydrous Na_2SO_4, evaporated to dryness. The residue (0.02 g) contained the starting nucleoside (25%) and a mixture of nucleosides 14 and 15 (66%) according to ^1H NMR data of the reaction mixture measured in $CDCl_3$.

Method 5

To a solution of protected nucleoside **13** (0.052 g, 0.0978 mmol) in anhydrous methanol (3 mL) was added NaOCN (0.006 g, 0.092 mmol) and the prepared solution was stirred for 22 min at room temperature, then sodium salt (0.006 g, 0.092 mmol) was added to the prepared solution. The reaction mixture was stirred for 20 min and acetic acid (0.1 ml) was added, then evaporated, coevaporated with anhydrous toluene. The residue was dissolved with EtOAc (30 ml), the organic layer was washed with water (10 ml) and dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was chromatographed on silica gel, eluting with mixtures of 3:1, 2:1 and 1:1 hexane-EtOAc to afford the starting nucleoside (0.009 g, 17%) and a mixture of nucleosides **14** and **15** (0.027 g, 67%) as a syrup with recovery of the starting nucleoside.

Method 6

To a solution of protected nucleoside 13 (0.043 g, 0.081 mmol) in anhydrous methanol (2.9 mL) was added NaOCN (0.01 g, 0.154 mmol) and the prepared solution was stirred for 30 min at room temperature, then anhydrous THF (1.8 ml) was added. The reaction mixture was stirred for 15 min and acetic acid (0.1 ml) was added, then evaporated, coevaporated with anhydrous toluene. The residue was chromatographed on silica gel, eluting with mixtures of 3:1, 2:1 and 1:1 hexane-EtOAc to afford the starting nucleoside (0.008 g, 19%), a mixture of nucleosides **14** and **15** (0.019 g, 60%) as a syrup with recovery of the starting nucleoside.

Synthesis of 2,6-dichloro-9-(3',5'-di-O-pivaloyl-2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-9H-purine (16) and 2,6-dichloro-9-(2',5'-di-O-pivaloyl-3'-deoxy-3'-fluoro-β-D-xylofuranosyl)-9H-purine (17)

To a solution of a mixture of nucleosides **14** and **15** (0.12 g, 0.245 mmol, a ratio - 2.3:1) in anhydrous CH_2Cl_2 (4.5 ml) and pyridine (0.06 ml) was added 0.16 ml (1.22 mmol) DAST at 0 ^0C. The reaction mixture was stirred for 60 min under cooling and then for 18 h at 25-30^0C. The residue prepared after treatment of reaction mixture was chromatographed on silica gel, using a mixture of 8:1, 5:1 and 3:1 hexane-EtOAc to afford protected 2´-β-fluoro nucleoside **16** (0.049 g, 59%) as a syrup and 3´-β-fluoro nucleoside **17** (0.003 g, 7%) as a syrup.

2′-β-fluoro nucleoside **16**. ^{1}H NMR ($CDCl_3$): δ 8.37 (d, 1H, $J_{H\text{-}8,\ F\text{-}2'}$ = 2.9 Hz, H-8), 6.48 (dd, 1H, $J_{1',2'}$ = 3.8 Hz, $J_{1',F\text{-}2'}$ = 21.7 Hz H-1′), 5.37 (dd, 1H, $J_{3',2'}$ = 2.7 Hz, $J_{3',F\text{-}2'}$ = 17.6 Hz, H-3′), 5.19 (dd, 1H, $J_{2',F\text{-}2'}$ = 50.3 Hz, H-2′), 4.53 (dd, 1H, H-5′), 4.41 (dd, 1H, H-5″), 4.23-4.27 (m, 1H, H-4′). 1.29 and 1.24 (2s, 2x9H, 2xCOC$(CH_3)_3$]. ^{13}C NMR ($CDCl_3$): δ 178.2 and 177.2 [(C=O, 2xCOC$(CH_3)_3$], 153.4, 152.5, 152.3, 130.7 (C-2, C-6, C-4, C-5), 145.2 (d, $J_{C\text{-}8,F\text{-}2'}$ = 5.9 Hz, C-8), 92.7 (d, $J_{C\text{-}2',F\text{-}2'}$ =193.4 Hz, C-2′), 83.9 (d, $J_{C\text{-}1',F\text{-}2'}$ =16.9 Hz, C-1′), 81.8 (C-4′), 75.9 (d, $J_{C\text{-}3',F\text{-}2'}$=20.3 Hz, C-3′), 62.6 (C-5′), 39.0 and 38.9 [2xCOC$(CH_3)_3$], 27.27 and 27.1 [2xCOC(**C**$H_3)_3$]. ^{19}F NMR ($CDCl_3$): δ -197.5 (dt, F-2′). HRMS (EI): m/z calcd for $C_{20}H_{25}Cl_2FN_4O_5$ $(M+Na)^+$: 513.1084, found 553.1064.

3′-β-fluoro nucleoside **17**. ^{1}H NMR ($CDCl_3$): δ 8.36 (s, 1H, H-8), 6.26 (d, 1H, $J_{1',2'}$ = 1.9 Hz, H-1′), 5.52 (dd, 1H, $J_{2',F\text{-}3'}$ = 15.2 Hz, H-2′), 5.22 (dd, 1H, $J_{3',4'}$ = 1.9 Hz, $J_{3',F\text{-}3'}$ = 49.3 Hz, H-3′), 4.43-4.54 (m, 3H, H-4′, H-5′ and H-5″), 1.32 and 1.28 (2s, 2x9H, 2xCOC$(CH_3)_3$]. ^{13}C NMR ($CDCl_3$): δ 178.0 and 176.6 [(C=O, 2xCOC$(CH_3)_3$], 153.5, 152.6, 152.2, 143.4, 143.3, 130.8 (C-2, C-6, C-4, C-5), 93.2 (d, $J_{C\text{-}3',F\text{-}3'}$ = 183.5 Hz, C-3′), 87.6 (C-1′), 80.4 (d, $J_{C\text{-}4',F\text{-}3'}$ = 20.9 Hz, C-4′), 79.7 (d, $J_{C\text{-}2',F\text{-}3'}$ = 31.9 Hz, C-2′), 60.53 (d, $J_{C\text{-}5',F\text{-}3'}$ = 10.0 Hz C-5′), 38.85 and 38.81 [2xCOC$(CH_3)_3$], 27.1 and 26.9 [2xCOC(**C**$H_3)_3$]. ^{19}F NMR ($CDCl_3$): δ -200.9 (ddd, F-3′). HRMS (EI): m/z calcd for $C_{20}H_{25}Cl_2FN_4O_5$ $(M+Na)^+$: 513.1084, found 553.1063.

Synthesis of 6-amino-2-chloro-9-(3',5'-di-O-pivaloyl-2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-9H-purine (18)

A solution of nucleoside **16** (0.05 g, 0.1 mmol) in 1,2-dimethoxyethane (4 mL) saturated at 0°C with ammonia was kept for 18 h at room temperature and then evaporated. The residue was chromatographed on silica gel, eluting with $CHCl_3$, then $CHCl_3$:MeOH - 30:1 to afford nucleoside **18** (0.046 g, 96%) as an amorphous powder. ^{1}H NMR ($CDCl_3$): δ 8.03 (d, 1H, $J_{H\text{-}8,\ F\text{-}2'}$ = 3.1 Hz, H-8), 6.40 (dd, 1H, $J_{1',2'}$ = 2.7 Hz, $J_{1',F\text{-}2'}$ = 22.3 Hz, H-1′), 6.24 (br.s, 2H, NH_2), 5.34 (dd, 1H, $J_{3',2'}$ = 2.9 Hz, $J_{3',F\text{-}2'}$ = 17.9 Hz, H-3′), 5.17 (dd, 1H, $J_{2',F\text{-}2'}$ = 49.6 Hz, H-2′), 4.48 (dd, 1H, $J_{4',5'}$ = 5.5 Hz, $J_{5',5''}$ = 12.0 Hz, H-5′), 4.40 (dd, 1H, $J_{4',5''}$ = 3.7 Hz, H-5″), 4.15-4.18 (m, 1H, H-4′). ^{13}C NMR ($CDCl_3$): δ 178.2 and 177.3 [(C=O, 2xCOC$(CH_3)_3$], 156.3, 154.5, 150.7, 118.0 (C-2, C-6, C-4, C-5), 140.4 (d, $J_{C\text{-}8,F\text{-}2'}$ = 6.9 Hz, C-8), 92.9 (d, $J_{C\text{-}2',F\text{-}2'}$ = 194.4 Hz, C-2′), 83.4 (d, $J_{C\text{-}1',F\text{-}2'}$=16.9 Hz, C-1′), 81.26 (C-4′), 76.1 (d, $J_{C\text{-}3',F\text{-}2'}$ = 30.9 Hz, C-3′), 62.8 (C-5′), 39.0 and 38.9 [2xCOC$(CH_3)_3$], 27.27 and 27.1 [2xCOC(**C**$H_3)_3$]. ^{19}F NMR ($CDCl_3$): δ -197.56 (dt, F-2′). HRMS (EI): m/z calcd for $C_{20}H_{27}ClFN_5O_5$ $(M+Na)^+$: 494.1582, found 494.1570.

Synthesis of 6-amino-2-chloro-9-(3',5'-di-O-benzoyl-2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-9H-purine (20) and its α-anomer (21)

Method a_1

To 2-chloroadenine (0.056 g, 0.32 mmol) in anhydrous 1,2-dimethoxyethane (12 ml) was added (0.038 g, 0.32 mmol) potassium *tert*-butoxide and the resulting mixture was stirred for 40 min under argon at room temperature and then anhydrous potassium bromide (0.058 g, 0.48 mmol) was added, and then the suspension was stirred for 10 min and evaporated to dryness, coevaporated with anhydrous MeCN. Anhydrous MeCN (5 ml) was added to the residue and a suspension was stirred under argon at room temperature for 10 min, and then a solution of bromosugar **19** (0.116 g, 0.28 mmol) in MeCN (6.0 mL) was added dropwise at 0°C to the prepared potassium salt of the purine for 40 min. The reaction mixture was stirred under argon at room temperature for 18 h. Insoluble materials were removed by filtration and the solids were washed with CH_2Cl_2 (50 mL). The combined filtrate and washings were evaporated. The residue was chromatographed on silica gel, eluting with chloroform, $CHCl_3$/EtOAc 7:1 and $CHCl_3$/EtOAc/MeOH 7:1:0.1 to afford protected β-nucleoside **20** (0.063 g, 45%) as a foam and α-nucleoside **21** as a foam (0.022 g, 16%).

β-nucleoside **20**. ^{1}H NMR ($CDCl_3$): δ 8.07-8.11 (2m, 10H, Bz), 8.05 (d, 1H, $J_{H\text{-}8,\ F\text{-}2'}$ = 2.7 Hz, H-8), 7.44-7.67 (4m, 6H, Bz), 6.57 (dd, 1H, $J_{1',2'}$ = 2.2 Hz, $J_{1',F\text{-}2'}$ = 23 Hz, H-1′), 6.40 (br.s, 2H, NH_2), 5.74 (dd, 1H, $J_{3',2'}$ = 2.3 Hz, $J_{3',F\text{-}2'}$ = 17.2 Hz, H-3′), 5.36 (dd, 1H, $J_{2',F\text{-}2'}$ = 49.8 Hz, H-2′), 4.81 (dd, 1H, H-5′), 4.78 (dd, 1H, H-5″), 4.53-4.57 (m, 1H, H-4′). ^{13}C NMR ($CDCl_3$): δ 166.28 and 165.33 (C_6H_5CO), 156.39 (C-2), 154.44 (C-4), 150.75 (C-6), 140.41 (d, $J_{C\text{-}8,F\text{-}2'}$ = 6.5 Hz, C-8), 134.3, 133.5, 130.1, 129.9, 129.3, 129.4, 128.9, 128.7 (2Ph), 117.94 (C-5), 92.81 (d, $J_{C\text{-}2',F\text{-}2'}$ =191.48 Hz, C-2′), 83.62 (d, $J_{C\text{-}1',F\text{-}2'}$ = 17.0

Hz, C-1′), 81.32 (C-4′), 76.89 (d, $J_{C\text{-}3',F\text{-}2'}$ = 30.9 Hz, C-3′), 63.44 (C-5′). ^{19}F NMR ($CDCl_3$): δ -198.0 (dt, F-2′). HRMS (EI): m/z calcd for $C_{24}H_{19}ClFN_5O_5$ $(M+Na)^+$: 534.0956, found 534.0951.

α-nucleoside **21**. ^{1}H NMR ($CDCl_3$): δ 8.09-8.11 (m, 2H, Bz), 8.00 (s, 1H, H-8), 7.25-7.78 (m, 8H, Bz), 6.43 (d, 1H, $J_{1',2'}$ < 1.0 Hz, $J_{1',F\text{-}2'}$ = 14.4 Hz, H-1′), 6.25 (br.s, 2H, NH_2), 6.16 (d, 1H, $J_{2',F\text{-}2'}$ = 49.0 Hz, H-2′), 5.79 (dm, 1H, $J_{3',F\text{-}2'}$ = 17.0 Hz, H-3′), 4.94-4.97 (m, 1H, H-4′), 4.70 (dd, 1H, H-5′), 4.68 (dd, 1H, H-5″). ^{13}C NMR ($CDCl_3$): δ 166.27 and 165.15 (C_6H_5CO), 156.33 (C-2), 154.66 (C-4), 150.49 (C-6), 139.18 (s, C-8), 134.1, 133.4, 129.9, 129.8, 129.3, 128.7, 128.6, 128.0 (2Ph), 119.1 (C-5), 96.6 (d, $J_{C\text{-}2',F\text{-}2'}$=188.5 Hz, C-2′), 89.49 (d, $J_{C\text{-}1',F\text{-}2'}$ = 36.0 Hz, C-1′), 81.3 (C-4′), 76.98 (d, $J_{C\text{-}3',F\text{-}2'}$ = 29.9 Hz C-3′), 63.48 (C-5′). ^{19}F NMR ($CDCl_3$): δ -188.0 (dt, F-2′). HRMS (ESI): m/z calcd for $C_{24}H_{19}ClFN_5O_5$ $(M+Na)^+$: 534.0956, found 534.0949.

Method a_2

To 2-chloroadenine (0.069 g, 0.39 mmol) in anhydrous 1,2-dimethoxyethane (16 ml) was added (0.045 g, 0.39 mmol) potassium *tert*-butoxide and the resulting mixture was stirred for 40 min under argon at room temperature and then anhydrous potassium bromide (0.075 g, 0.58 mmol) was added and then the suspension was stirred for 10 min and evaporated to dryness. To a residue was added anhydrous MeCN (4.8 ml) and a suspension was stirred under argon at room temperature for 10 min, then anhydrous CH_2Cl_2 (2.4 ml) and a solution of bromide **19** (0.147 g, 0.33 mmol) in a mixture of MeCN/CH_2Cl_2 (8.4 mL, 2:1) was added dropwise at 0^{0}C to the prepared potassium salt of the purine. The reaction mixture was stirred under argon at room temperature for 55 h. Insoluble materials were removed by filtration and the solids were washed with CH_2Cl_2 (35mL). The combined filtrate and washings were evaporated. The residue was chromatographed on silica gel, eluting with chloroform, $CHCl_3$/EtOAc 7:1 and $CHCl_3$/EtOAc/MeOH - 7:1:0.1 to afford protected β-nucleoside **20** (0.098 g, 55%) as a foam and α-nucleoside **21** (0.03 g, 17%) as a foam.

Synthesis of 6-amino-2-chloro-9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-9H-purine (3)

Method a

A solution of nucleoside **20** (0.29 g, 0.57 mmol) in MeOH (14 mL) saturated at 0°C with ammonia was kept for 18 h at room temperature and then evaporated. To the residue was added water (14 ml) and the aqueous phase was extracted with EtOAc (3x14 ml), aqueous phase was evaporated and coevaporated with methanol. The residue was washed with ether (8 ml), crystallized from methanol to give nucleoside **3** (0.134 g, 78%). Mp. 230-231^{0}C. ^{1}H NMR (DMSO-d_6): δ 8.22 (d, 1H, $J_{H\text{-}8,\ F\text{-}2'}$ = 2.0 Hz, H-8), 7.84 (br.s, 2H, NH_2), 6.28 (dd, 1H, $J_{1',2'}$ = 4.4 Hz, $J_{1',F\text{-}2'}$ = 14.1 Hz, H-1′), 5.92 (d, 1H, $J_{3',3'\text{-}OH}$ = 5.2 Hz, 3′-OH), 5.23 (dt, 1H, $J_{2',F\text{-}2'}$ = 52.6 Hz, $J_{2',3'}$ = 4.2 Hz, H-2′), 5.04 (t, 1H, $J_{5',5\text{-}OH}$ = 5.7 Hz, 5′-OH), 4.40 (dq, 1H, $J_{3',F\text{-}2'}$ = 18.8 Hz, H-3′), 3.8 (m, 1H, H-4′), 3.62 (m, 1H, $J_{5',4'}$ = 4.7 Hz, $J_{5',5''}$ = 12.0 Hz, H-5′), 3.57 (dd, 1H, $J_{5'',4'}$ = 5.5 Hz, H-5″). ^{13}C NMR (DMSO-d_6): δ 157.3, 153.8, 150.7, 140.52 (d, $J_{C\text{-}8,F\text{-}2'}$ = 2.99 Hz, C-8), 117.9, 95.88 (d, $J_{C\text{-}2',F\text{-}2'}$ = 192.1 Hz, C-2′), 84.1 (d, $J_{C\text{-}4',F\text{-}2'}$ = 4.6 Hz, C-4′), 82.0 (d, $J_{C\text{-}1',F\text{-}2'}$ = 17.0 Hz, C-1′), 73.1 (d, $J_{C\text{-}3',F\text{-}2'}$ = 23.0 Hz, C-3′), 60.9 (C-5′). ^{19}F NMR ((DMSO-d_6): δ -198.38 (dt, F-2′). HRMS (ESI): m/z calcd for $C_{10}H_{11}N_5O_3FCl(M+H)^+$: 304.0613, found 304.0597.

Method b

To a solution of protected nucleoside **18** (0.046 g, 0.097 mmol) in anhydrous methanol (0.7 mL) was added 0.35 ml 0.22 M sodium methoxide in methanol and the prepared solution was stirred for 18 h at room temperature, then the reaction mixture was neutralized with acetic acid and evaporated, coevaporated with a mixture of toluene:ethanol - 1:1 (20 ml). The residue was chromatographed on silica gel using for elution $CHCl_3$, then $CHCl_3$:MeOH-20:1 and 7:1 to afford nucleoside **3** as a white solid (0.022 g, 74%).

Synthesis of 1-methanesulfonyloxy-3-methylbutane (22) from 3-methyl-butanol

To solution 3-methyl-butanol (2.0 ml, 1.62 g, 18.36 mmol) was added dropwise 18 ml 1M Li-tri-*sec*-borohydride in THF at 0 ^{0}C, the prepared solution was stirring for 30 min at room temperature, then 2.14 ml (27.54 mmol) mesyl chloride was added at 0 ^{0}C. The reaction mixture was stirred for 20 h at room temperature and poured out into water/ice (60 ml), the water phase was extracted with chloroform

(3 x100 ml). The organic extracts were washed with 1% aq. H_2O_2, water, dried over sodium sulfate, and evaporated. The residue was chromatographed on silica gel, using mixtures of 8:1, 5:1 and 3:1 hexane-EtOAc. 3-Methyl-butanol mesylate **22** was prepared as colorless syrup (2.1 g, 70%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.31 [t, 2H, $(CH_3)_2CHCH_2CH_2OMs$], 3.05 (s, 3H, $-OSO_2CH_3$), 1.78 - 1.87 [m, 1H, $(CH_3)_2CHCH_2CH_2OMs$], 1.68 (q, 2H, $(CH_3)_2CHCH_2CH_2OMs$], 0.99 (d, 6H, $(CH_3)_2CHCH_2CH_2OMs$]. ^{13}C NMR (125 MHz, $CDCl_3$) δ: 68.66 ($-CH_2OMs$), 37.43 [$(CH_3)_2CHCH_2CH_2$-], 37.43 (OSO_2CH_3), 25.51 [$(CH_3)_2CH$-)], 22.27 [$(CH_3)_2CH$-].

Synthesis of 2-chloro-6-isopentylamino-9-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-9H-purine (23)

To a solution of clofarabine **3** (0.132 g, 0.435 mmol) in anhydrous DMSO (5.2 mL) was added potassium *tert*-butoxide (0.06 g, 0.52 mmol) and the prepared solution was stirred for 15 min at room temperature, then mesyl derivative of 3-methyl-1-butanol (**22**) (0.27 ml, 0.231 g, 1.56 mmol) was added. The reaction mixture was stirred for 15 min at room temperature, and then 90 min at 89-90 ^{0}C, and, after cooling, a brown solution was diluted with water (3 ml) and the aqueous phase was extracted with $CHCl_3$ (3x40 ml). The combined organic extracts were evaporated, coevaporated with toluene (3x7ml). The residue was chromatographed on silica gel, eluting with $CHCl_3$, then $CHCl_3$:petroleum ether: MeOH - 30:6:1 and 6:4:1 to afford N6-isopentyl nucleoside **23** (0.05 g, 73%) as an amorphous powder taking into account the recovery of the starting nucleoside **3** (0.08 g). Mp.184-186 ^{0}C. ^{1}H NMR (CD_3OD): δ 8.24 (d, 1H, $J_{H\text{-}8,\ F\text{-}2'}$ = 1.9 Hz, H-8), 6.41 (dd, 1H, $J_{1',2'}$ = 4.2 Hz, $J_{1',F\text{-}2'}$ = 15.4 Hz, H-1′), 5.16 (ddd, 1H, $J_{2',F\text{-}2'}$ = 52.2 Hz, $J_{2',3'}$ = 4.0 Hz, H-2′), 4.51 (ddd, 1H, $J_{3',4'}$ = 3.3Hz, $J_{3',F\text{-}2'}$ = 18.2 Hz, H-3′), 3.97 (m, 1H, H-4′), 3.85 (ddd, 1H, $J_{5',4'}$ = 3.8 Hz, $J_{5',F\text{-}2'}$ = 0.8 Hz, $J_{5',5''}$ = 12.1 Hz, H-5′), 3.80 (dd, 1H, $J_{5'',4'}$ = 5.1 Hz, H-5″), 3.58-3.62 [m, 2H, $HNCH_2CH_2CH(CH_3)_2$], 1.7-1.78 [m, 1H, $CH_2CH_2CH(CH_3)_2$], 1.57-1.61 [m, 2H, $CH_2CH_2CH(CH_3)_2$], 1.01 [br.s, 3H, $CH(CH_3)_2$], 1.34 [br.s, 3H, $CH(CH_3)_2$]. ^{13}C NMR (CD_3OD): δ 155.2, 154.5, 149.2, 117.5, (C-6, C-2, C-4, C-5), 139.78 (br.s, $J_{C\text{-}8,F\text{-}2'}$ <2.0 Hz, C-8), 95.5 (d, $J_{C\text{-}2',F\text{-}2'}$ = 191.5 Hz, C-2′), 84.19 (d, $J_{C\text{-}4',F\text{-}2'}$ = 3.7 Hz, C-4′), 82.9 (d, $J_{C\text{-}1',F\text{-}2'}$ = 17.0 Hz, C-1′), 73.3 (d, $J_{C\text{-}3',F\text{-}2}$=24.3 Hz, C-3′), 60.7 (C-5′), 38.55 and 37.95 [$CH_2CH_2CH(CH_3)_2$], 25.5 [$CH_2CH_2CH(CH_3)_2$], 21.5 [$CH_2CH_2CH(CH_3)_2$]. ^{19}F NMR (CD_3OD): δ -199.5 (dt, F-2′). HRMS (ESI): m/z calcd for $C_{15}H_{21}N_5O_3FCl$ $(M+Na)^+$: 396.1215, found 396.1199.

Competing Interests

There are no conflicts to declare

Funding

This study was supported by grant from FOI «Chemical processes, reagents and technologies, bioregulators and bioorgchemistry», s/p «Chemical foundations of life activity processes» (Bioorgchemisrty 2.3.2.2) and partially with grant (Biologically active compounds 2.18).

References

1. Guinan M, Benckendorff C, Smith M, Miller G. Recent Advances in the Chemical Synthesis and Evaluation of Anticancer Nucleoside Analogues. *Molecules*. 2020;25:1–25. https://doi.org/10.3390/molecules25092050
2. Shelton J, Lu X, Hollenbaugh J, Cho J, Amblard F, et al. Metabolism, Biochemical Actions, and Chemical Synthesis of Anticancer Nucleosides, Nucleotides, and Base Analogs. *Chem. Rev*. 2016;116:14379–14455. https://doi.org/10.1021/acs.chemrev.6b00209
3. Parker W. Enzymology of Purine and Pyrimidine Antimetabolites Used in the Treatment of Cancer. *Chem Rev*. 2009;109:2880–2893. https://doi.org/10.1021/cr900028p
4. Robak T, Korycka A, Lech-Maranda E, Robak P. Current status of older and new purine nucleoside analogues in the treatment of lymphoproliferative diseases. *Molecules*. 2009;14:1183–1226. https://doi.org/10.3390/molecules14031183
5. Ewald B, Sampath D, Plunkett W. Nucleoside analogs: molecular mechanisms signing cell death. *Oncogene*. 2008;27:6522–6637.
6. Bonate P, Arhoud L, Cantrell W, Stephenson K, Secrist J III, Weitman S. Discovery and development of clofarabine: a nucleoside analogue for treating cancer. *Nature. Rev. Drug Discovery*. 2006;5:855–863. https://doi.org/10.1038/nrd2055

7. Majda K, Lubecka K, Kaufman-Szymczyk A, Fabianowska-Majewska K. Clofarabine (2-Chloro-2′-Fluoro-2′-deoxyarabinofuranosyladenine) - Biochemical Aspects of Anticancer Activity. *Acta Pol. Pharm. Drug. Res*. 2011;68:459–466.
8. Zhenchuk A, Lotfi K, Juliousson G, Albertioni F. Mechanism of anti-cancer action and pharmacology of clofarabine. *Biochem. Pharmacol*. 2009;78:1531–1359.
9. Daly M, Roth M, Bonnac L, Maldonado J, Xie J, et al. Dual anti-HIV mechanism of clofarabine. *Retrovirology*. 2016;13:1–12. https://doi.org/10.1186/s12977-016-0254-0
10. Montgomery J, Shortnacy A, Clayton S, Riordan J, Secrist J III. Synthesis and Biological Activity of 2′-Fluoro-2-halo Derivatives of 9-β-D-Arabinofuranosyladenine. *J. Med. Chem*. 1992;35:397–401. https://doi.org/10.1021/jm00080a029
11. Bauta W, Schulmeir B, Burke B, Puente J, Cantrell W, et al. A New Process for Antineoplastic Agent Clofarabine. *Org. Process Res. & Develop*. 2004;8:889–896. https://doi.org/10.1021/op049884n
12. Cen Y, Sauve A. Efficient Synthesis of clofarabine and gemcitabine from 2-deoxyribonolactone. *Nucleosides, Nucleotides and Nucleic Acids*. 2010;29:113–122.
13. Dostie S, Prévost M, Mochirian P, Tanveer K, Andrella N, et al. Diastereoselective Synthesis of C2′-Fluorinated Nucleoside Analogues Using an Acyclic approach. *J Org. Chem*. 2016;81:10769–10790. https://doi.org/10.1021/acs.joc.6b01845
14. Izawa K, Takamatsu S, Katayama S, Hirose N, Kozai S, et al. An Industrial Process for Synthesizing Lodenosine (FddA). *Nucleos Nucleot. Nucleic Acids*. 2003;22:507–517. https://doi.org/10.1081/ncn-120021951
15. Fateev I, Antonov K, Konstantinova I, Muravyova T, Seela F, et al. The chemoenzymatic synthesis of clofarabine and related 2-deoxyfluoroarabinofuranosyl nucleosides: the electronic and stereochemical factors determining substrate recognition by *E.coli* nucleoside phosphorylases. *Beilstein J. Org. Chem*. 2014;10:1657–1669.
16. Yamada K, Matsumoto N, Haykawa H. Stereoselective Synthesis of 2-Deoxy-2-Fluoroarabinofuranosyl-α-1-Phosphate and Its Application to the Synthesis of 2-Deoxy-2-Fuoroarabinofuranosyl Purine Nucleosides by a Chem-Enzymatic Method. *Nucleosides, Nucleotides Nucleic Acids*. 2009;28:1117–1130. https://doi.org/10.1080/15257770903396741
17. Sivets G. Syntheses of 2-deoxy-2-fluoro-β-D-arabinofuranosyl purine nucleosides via selective glycosylation reactions of potassium salts of purine derivatives with the glycosyl bromide. *Tetrahedron Lett*. 2016;57:268–271. https://doi.org/10.1016/j.tetlet.2015.11.091
18. Pankiewicz K, Krzeminski J, Watanabe K. Synthesis of 2′-β-Fluoro-and 3′-α-Fluoro-Substituted Guanine Nucleosides. Effects of Sugar Conformational Shifts on Nucleophilic Displacement of the 2′-Hydroxy and 3′-Hydroxy Group with DAST. *J. Org. Chem*. 1992;57:7315–7321.
19. Pankiewicz K, Watanabe K. Synthesis of 2′-β-fluoro-substituted nucleosides by a direct approach. *J. Fluor. Chem*. 1993;64:15–36.
20. Maruyama T, Takamatsu S, Kozai S, Satoh Y, Izawa K. Synthesis of 9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)adenine Bearing a Selectively Removable Protecting Group. *Chem. Pharm. Bull*. 1999;47:966–970. https://doi.org/10.1248/cpb.47.966
21. Sivets G, Kalinichenko E, Mikhailopulo I. Synthesis of C2′-β-Fluoro-substituted Adenine Nucleosides Via Pivaloyl Derivatives of Adenosine and 3'-Deoxyadenosine. *Lett. Org. Chem*. 2006;3:402–408.
22. Sivets G. Regio- and stereoselective syntheses of L-pentose derivatives from L-arabinose. *Tetrahedron*. 2018;74:920–931. https://doi.org/10.1016/j.tet.2017.12.045
23. Shao-Min W, Hong-Min L. Synthesis of Androrapholide Glucopyranoside and Selective Cleavage of O-Acetyl Groups in Sugar Moiety. *Chinese J. Chem*. 2008;26:343–347.
24. Zhang Q, He P, Zhou G, Gu Y, Fu T, et al. Synthesis and biological evaluation of 2,6-dichloropurine bicyclonucleosides containing a triazolyl-carbohydrate moiety. *Carbohydr. Res*. 2013;382:65–70. https://doi.org/10.1016/j.carres.2013.10.004
25. Howell G, Brodfuehrer P, Brundidge S, Benigni D, Sapino J. Antiviral Nucleosides. A Stereospecific, Total Synthesis of 2′-Fluoro-2′-deoxy-β-D-arabinofuranosyl Nucleosides. *J. Org. Chem*. 1988;53:85–88. https://doi.org/10.1021/jo00236a017

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