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Synthesis of 2-Chloro-2′-Deoxyadenosine (Cladribine) and New Purine Modified Analogues

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Abstract

The efficient two-step synthesis of 2-chloro-2′-deoxyadenosine (cladribine) via the anion glycosylation of purine potassium salt with the glycosyl chloride in binary solvent mixtures is described. A new method for preparation of diprotected 2-chloro-6-fluoropurine 2′-deoxy-β-D-riboside was developed by treatment of the 2,6-dichloropurine precursor with diethylaminosulfur trifluoride (DAST). Novel N6-alkylated cladribine analogue was synthesized by amination of acylated 2,6-dihalogenopurine nucleosides. It was found that a mild hydrolysis reaction of acylated 2-chloro-6-fluoropurine 2′-deoxy-β-D-riboside gave rise to new purine hydroxylated nucleoside.

Introduction

Among a series of known antineoplastic agents belonging to the purine nucleosides, cladribine have found application as the clinical drug for the treatment of hematologic malignances. The cladribine (2-chloro-2′-deoxyadenosine), deaminase-resistant analogue of 2′-deoxyadenosine is used for monotherapy of patients with hairy cell leukemia [1] and treatment of other lymphoid malignances. Its mechanism of significant cytotoxicity and metabolism was widely studied and it has been established that the active metabolite, 5′-triphosphate, inhibits DNA synthesis and ribonucleotide reductase activity [2-3]. Recently, cladribine has also been approved as the oral drug with a promising efficacy and safety profile [4] for the treatment of relapsing multiple sclerosis in adults [5].

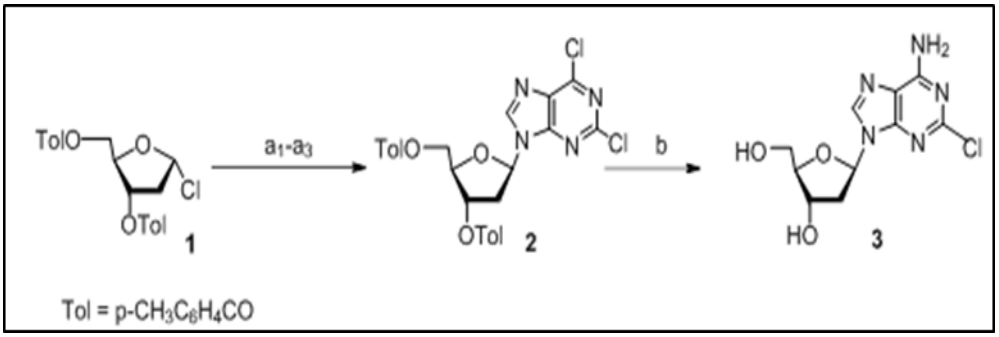
The synthesis of cladribine has widely been investigated in the framework of several approaches a) glycosylation reactions of purine derivatives with sugars [6–9]; b) C2′-deoxygenation of selectively pro-

tected purine nucleoside derivative [10]; c) enzymatic transglycosylation or glycosylation reactions [11–13]. It should be noted, of the known synthetic approaches, the most studied method is derived from glycosylation reactions of purine nucleobase derivative with an activated carbohydrate. The sodium salts of halogenated purines give N9 and N7 glycosyl isomers under the glycosylation and the stereoselectivity varies with heterocyclic bases and the reaction conditions [12, 13]. This challenge was solved via highly stereoselective glycosylation of 6-substituted imidazol-1-yl-2-chloropurines with protected 1-chloro-2-deoxyribose in a mixture of solvents, using Robins' purine salt method, and cladribine was prepared in three steps from the N6-modified purine [14]. A practical and efficient process for the manufacture of cladribine was developed via the Vorbrüggen glycosylation of silylated base with 1-acetoxy 2-deoxyribofuranose derivative [11]. N6-alkylated purine 2′-deoxyribonucleoside analogues display *in vitro* anticancer and antiviral activities [15, 16]. Because of our interest in extending preparation of biologically active nucleosides from sugars, herein we report study of efficient two-step synthetic route to 2-chloro-2′-deoxyadenosine via stereoselective anion glycosylation of purine base with the carbohydrate precursor using readily available reagents and development of simple synthetic approaches to its novel purine modified analogues.

Results and Discussion

Synthesis of cladribine 3 was explored via selective glycosylation of the potassium salt of 2,6-dichloropurine with available 1-α-chlorosugar 1 [17] using various reaction conditions on the key step to increase its regioselectivity (Scheme 1, Table 1). Regioselective coupling of the potassium salt of 2,6-dichloropurine, generated in the presence of potassium *tert*-butoxide in 1,2-dimethoxyethane, with 1-chloro-2-deoxy-3,5-di-O-p-toluoyl-α-D-*erythro*-pentofuranosyl chloride (1) at ambient temperature in a mixture of anhydrous acetonitrile and tetrahydrofuran resulted in formation of 3′,5′-di-O-toluoyl-2,6-dichloropurine-2′-deoxy-β-D-riboside (**2**) as predominant product. ^{1}H NMR data of the crude reaction mixture indicate that glycosylation proceeds with full conversion of the starting 1-α-chlorosugar to a mixture of three nucleosides, giving acylated N9-β-2′-deoxy-β-D-riboside along with the undesired N9-α- and N7-β-isomeric nucleosides as by-products. A ratio of acylated N9-β/N7-β-regioisomers made up 6.2:1 (Table 1, entry 1). Protected N9-2′-deoxy-β-D-nucleoside **2** was prepared in 70% yield after column chromatography on silica gel. The anion glycosylation reaction of 2,6-dichloropurine salt in anhydrous tetrahydrofuran in the presence of 18 crown 6 resulted in the intermediate N9-β-nucleoside **2** in 61% yield after chromatographic separation, N9-β/N7-β-regioisomeric nucleosides being formed in a ratio of 5.2:1 (entry 2). The glycosylation reaction in THF, the solvent with a lower polarity, and in the presence of crown ether as additive for improving solubility of the purine salt gave rise to decrease of the reaction time, yield of the protected N9-β-nucleoside and stereoselectivity compared to the reaction in a binary mixture of solvents (Scheme 1, conditions a_1 and a_2). Next, the glycosylation reaction of the potassium salt of 2,6-dichloropurine in a binary solvent mixture (acetonitrile and 3,4-dihydro-2H-pyran as component with lower polarity) gave the best regioselectivity (ratio of protected N9-β/N7-β-/N9-α-nucleosides – 10:1:1)(entry 3). The protected N9-β-nucleoside 2 was isolated in 67-70% yield after column chromatography on silica gel using mixtures of petroleum ether and ethylacetate. The above results on the anion glycosylation of the 2,6-dichloropurine salt with the 1-α-chlorosugar under tested conditions provide evidence for regio- and stereoselectivity of the reaction depends on rational choice of solvents with different solvation of the purine potassium salt and a minimal anomerization of the starting sugar. Fast anion glycosylation of the purine potassium salt with the 1-α-chlorosugar in binary solvent mixtures improves the stereoselectivity of the heterogeneous reaction to give higher isolated yield of acylated N9-β-nucleoside than the sodium salt glycosylation in acetonitrile [7]. Protected

2′-deoxy-β-D-ribonucleoside of 2,6-dichloropurine 2 was then converted to cladribine 3 in NH_3/MeOH/THF. Selective ammonolysis of 2 occurring with the deprotection gave 2-chloro-2'-deoxyadenosine (3) in 82% yield after column chromatography. The improved approach to cladribine via coupling of the potassium salt of 2,6-dichloropurine with the 1-α-chlorosugar in a mixture of solvents was accomplished using commercially available reagents. It should be noted that overall yields (50-56%) of the target nucleoside are comparable to those for efficient chemical approaches (42-63%) developed from 2-deoxy-D-ribofuranose derivatives [6, 7, 9, 14] and 2-chloroadenosine [10], or the enzymatic method (59%) described from thymidine [13]. However, inherent drawbacks of the studied method are the formation of N-9- and N-7-isomers on the glycosylation step, unlike the enzymatic methods [11-13], and the use of chromatography on two steps in comparison with the cost-efficient and practical four-step procedure via the Vorbrüggen glycosylation of silylated 2-chloroadenine (overall 42%) [6]. When this method is compared with the known synthetic routes derived from 2,6-dichloropurine



Scheme 1. Syntheses of cladribine 3 from 1-α-chlorosugar 1

Syntheses of cladribine 3 from 1-α-chlorosugar 1. Reagents and conditions: a1) chloride 1, K-salt of 2,6-diClPur generated with t-BuOK in 1,2-DME, CH3CN/THF, rt, 4 h, 70% 2; a2) chloride 1, K-salt of 2,6-diClPur generated with t-BuOK in 1,2-DME in the presence of 0.1 equiv 18 crown 6, THF, rt, 2 h, 61% 2 a3) chloride 1, K-salt of 2,6-diClPur generated with t-BuOK in 1,2-DME, CH3CN/3,4-dihydropyran, rt, 240 min, 67 -70%, 2; b) NH3/MeOH, THF (v/v 3.5:1), rt, 24 h, 35-40 0C, 18 h, 82%.

Table 1. Reactions of potassium salt of 2,6-dichloropurine with the chlorosugar 1 under various conditions

Entry	Solvent	Conditions Ratio of heterobase:	Time (min)	Anomeric ratios (N-9-β:N7-β)[a]	Yield (%)[b]
1	MeCN/THF	1.1:1	150 min	6.2:1	70
2	THF[c]	1.1:1	120 min	5.2:1	61
3	MeCN/3,4-DHP	1.1:1	150 min	10.0:1	67-70

[a]Determined by 1H NMR spectroscopy of the reaction mixture in $CDCl_3$

[b]Isolated yield of protected purine N9-β-2′-deoxynucleoside 2 by column chromatography.

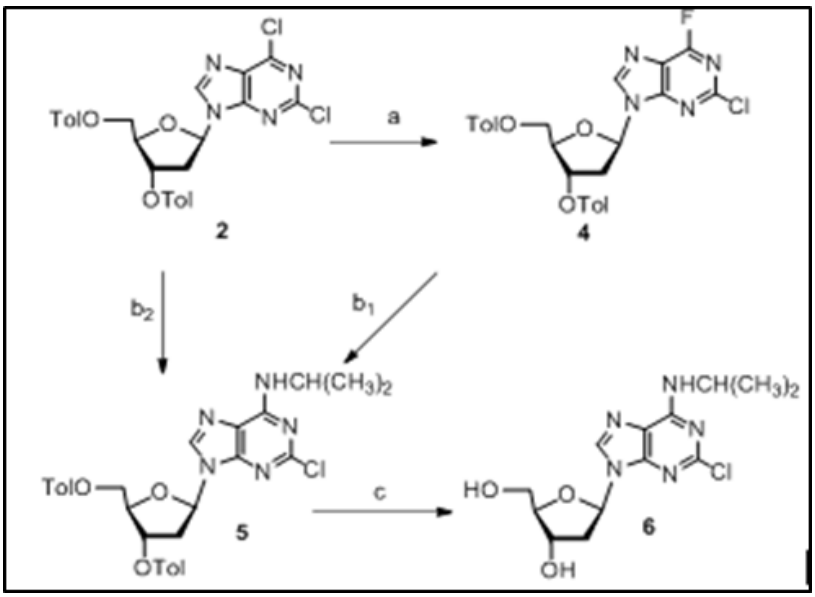
[c]18 Crown 6 (0.1 eqiuv) was used as additive.

and 2-deoxy-D-ribofuranose derivatives it is apparent that cladribine synthesis via the glycosylation of the potassium purine salt in a binary mixture of solvents and ammonolysis under mild reaction conditions (Scheme 1) produced higher overall yield (56%) than the sodium salt method (42%) [7]. Besides, the studied two-step approach is more accessible than the five-step method reported earlier via the stereoselective glycosylation of 2,6-dichloropurine with peracylated 2-deoxy-D-ribofuranose in the presence of gold-containing catalyst [9] and less efficient than Robins' purine salt method [14].

Synthesis of new N6-alkylated derivative of cladribine containing a branched alkyl substituent was investigated from 3′,5′-di-O-p-toluoyl-2′-deoxy-β-D-riboside of 2,6-dichloropurine 2. Several synthetic approaches to biologically active N6-substituted purine nucleosides were described earlier [18] and the most employed methods are based upon introduction of a leaving group (halogen, aryl sulfonate, or O-benzotriazolyl) at the C-6 position of nucleoside derivative followed by a selective nucleophilic S_NAr displacement with the corresponding alkyl amines [15]. Fluorination of O-protected 2,6-dichloropurine 2′-deoxy-β-D-ribonucleoside 2 was performed with an excess of DAST in a mixture of CH_2Cl_2/pyridine under mild heating (Scheme 2).

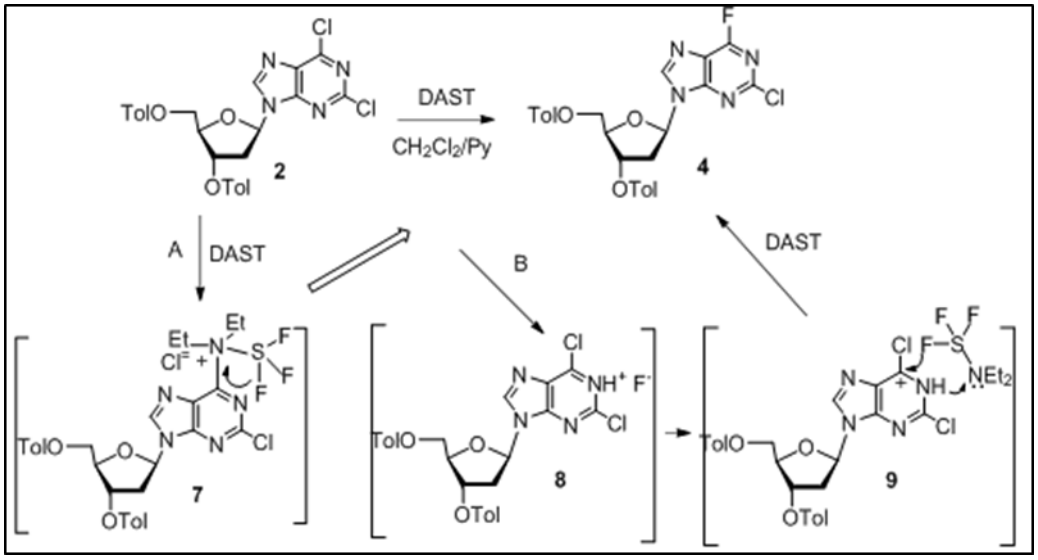
We have found for the first time that such treatment of protected 2,6-dichloropurine 2′-deoxyriboside by the nucleophilic fluorinating reagent gave rise to 2-chloro-6-fluoropurine derivative 4 in 58% yield after chromatography on silica gel. Syntheses of 6-fluorinated purine nucleosides and heterocyclic bases have earlier been reported in several works [19-21]. Selective fluorination reaction of 2 likely to proceed by two pathways A and B via formation of activated intermediates 7, 8, and 9 followed by nucleophilic S_NAr 6-chlorine displacement with DAST in adducts 7 and 9 (Scheme 3). Based on a previous study of synthesis of 6-fluorinated purines and the known method [21] developed by Deng et al., we can conclude that pathway A via formation of intermediate salt 7 is a predominant.

Further, we investigated comparable amination of protected 2-chloro-6-fluoropurine (4) and 2,6-dichloropurine (2) 2′-deoxy-β-D-ribosides with isopropyl amine in similar reaction conditions. Reactions of 2,6-dihalogenated purine derivatives 2 and 4 using fourfold excess of diisopropylamine in 1,2-dimethoxyethane at room temperature resulted in N6-alkylated purine analogue 5 in 81% and 89% yield, respectively, after chromatography on silica gel (Scheme 2). ^{1}H NMR spectral data of N6-isopropyl purine derivative 5 synthesized from 2,6-dichloropurine and 2-chloro-6-fluoropurine derivatives were identical, that unequivocally confirms the assigned structure of new 2-chloro-6-fluoropurine nucleoside precursor 4. Noteworthy, a selective amination of 4 proceeded in higher yield and shorter reaction time than the same transformation of 2,6-dichloropurine derivative 2. Thus, protected 2-chloro-6-fluoropurine 2′-deoxy-β-D-riboside can be utilized as valuable intermediate for preparation of different N6 or C6-substituted purine 2′-deoxyribonucleosides of biological interest via S_NAr selective mild displacement of fluorine atom by various nucleophilic agents. Deprotection of O-acylated N6-isopropyl purine nucleoside 5 with ammonia in methanol afforded novel cladribine analogue 6 in 79% yield after column chromatography on silica gel. Then, hydrolysis reaction of 2-chloro-6-fluoropurine 2′-deoxy-β-D-ribofuranoside 4 was studied under mild basic treatment with sodium hydrocarbonate in acetonitrile (Scheme 4). New purine modified nucleoside 14 was prepared in 65% yield after chromatography on silica gel. A possible mechanism for formation of hydroxylated purine nucleoside 14 via S_NAr selective displacement of fluorine atom in 4 on the first step and subsequent transformations of intermediate 2-chloro-3′,5′-di-O-p-toluoyl-2′-deoxyinosine (10) in the heterobase under mild basic conditions is outlined in Scheme 4. Structures of synthesized novel purine modified nucleosides were confirmed by ^{1}H, ^{13}C, ^{19}F NMR and mass-spectroscopy. Signal of F-6 atom in 2-chloro-6-fluoropurine nucleoside 4



Scheme 2. Synthesis of N6-alkylated cladribine derivative 6

Synthesis of N6-alkylated cladribine derivative 6. a) DAST, CH_2Cl_2/Py, 40-45 ^{0}C, 58%; b_1) 4, $(CH_3)_2CHNH_2$, 1,2-DME, rt, 70 min, 89%; b_2) 2, $(CH_3)_2CHNH_2$, 1,2-DME, rt, 4 h, 81%; c) NH_3/MeOH, rt, 79%.



Scheme 3. Proposed mechanism for fluorination reaction of 2,6-dichloropurine nucleoside 2 with DAST

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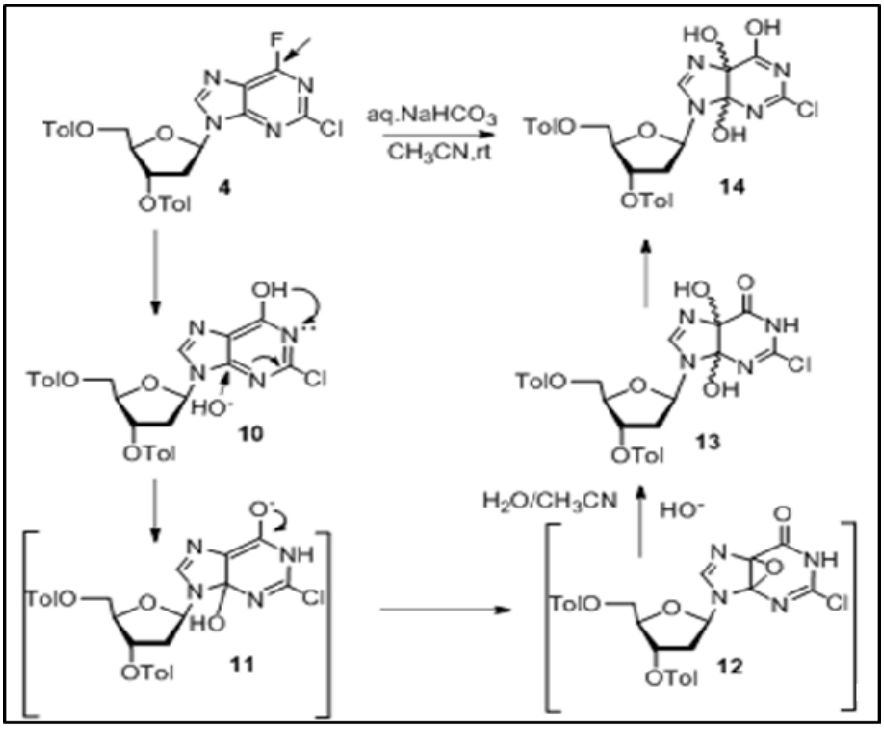

Scheme 4. Synthesis of purine modified 2′-deoxynucleoside 14 from 2-chloro-6-fluoropurine derivative 4

displays as a singlet at - 65.59 ppm in ^{19}F NMR spectrum. Chemical shift of fluorine atom in 2-chloro-6-fluoropurine derivative 4 is in good accordance with ^{19}F NMR spectral data of tri-O-acetylated 2-chloro-6-fluoropurine β-ribonucleoside described earlier [19]. The presence of hydroxy groups at 3.9-4.5 ppm and absence of signal for NH proton (8.1-10.0 ppm) in ^{1}H NMR spectrum and peak of $[M+Na]^+$ in mass-spectrum support the assigned structure of hydroxylated purine nucleoside 14.

Conclusion

In summary, the improved cladribine synthesis was achieved using the 2,6-dichloropurine potassium salt in the coupling reaction with available 1-α-chlorosugar and subsequent mild ammonolysis of the intermediate nucleoside. Solvent effects on stereoselectivity and regioselectivity of the glycosylation procedure were established. A new method to synthesize diprotected 2-chloro-6-fluoropurine 2′-deoxy-β-D-ribofuranoside was developed for the first time from the 2,6-dichloropurine nucloside using nucleophilic fluorinating agent and a plausible mechanism for formation of 2-chloro-6-fluoropurine derivative was proposed by selective nucleophilic S_NAr 6-chlorine displacement in the starting nucleoside with DAST. The efficient synthesis of N6-alkylated cladribine analogue was accomplished via a selective amination of 2-chloro-6-fluoropurine nucleoside derivative. New purine modified nucleoside derivative was prepared by hydrolysis reaction of acylated 2-chloro-6-fluoropurine 2′-deoxy-β-D-ribofuranoside and a possible mechanism for its formation was considered.

Materials 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 following: 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 Cladribine 3, 5 and -2-

*Synthesis of 2,6-dichloro-9-(3′,5′-di-O-p-toluoyl-2′-deoxy-β-D-ribofuranosyl)-9H-purine (**2**)*

a$_1$) Potassium *t*-butoxide (0.022 g, 0.18 mmol) was added to 2,6-dichloropurine (0.036 g, 0.19 mmol) in anhydrous 1,2-dimethoxyethane (3 ml) at 0 ^{0}C and then the resulting solution was stirred for 7 min under cooling and then for 15 min at room temperature and evaporated to dryness. Anhydrous acetonitrile (2.5 ml) and tetrahydrofuran (2.9 ml) were added to the residue and the suspension was stirred under argon at room temperature for 15 min, then the crystalline chloride 1 (0.07 g, 0.18 mmol) was gradually added during 10 min. The reaction mixture was stirred under argon at room temperature for 240 min. Insoluble materials were removed by filtration and the solids were washed with MeCN (5 mL). The solvent was removed under reduced pressure and the residue was chromatographed on silica gel, eluting with toluene/acetone 9:1 to afford β-nucleoside **2** as a white solid (0.068 g, 70%). Mp. 159-162 ^{0}C (EtOH). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.29 (s, 1H, H-8), 7.97, 7.82, 7.28, 7.20 (4d, 8H, 2xTol), 6.55 (t, 1H, $J_{1',2'} = J_{1',2''}$ = 6.4 Hz, H-1′), 5.79 (br.t, 1H, H-3′), 4.80 (dd, 1H, $J_{H\text{-}5',H\text{-}4'}$ = 2.7 Hz, $J_{H5',H5''}$ = 10.5 Hz, H-5′), 4.64-4.69 (m, 2H, H -4′ и H-5′′), 2.94-2.97 (m, 2H, H-2′ and H-2′′), 2.45 (s, 3H, $CH_3C_6H_4CO$) and 2.41 (s, 3H, $CH_3C_6H_4CO$). ^{13}C NMR ($CDCl_3$, 126.76 MHz): δ 166.0 and 165.9 ($COC_6H_4CH_3$), 153.0, 152.2, 151.9, 144.6, 144.4, 143.7, 131.2, 129.8, 129.5, 129.4, 129.3, 126.3, 126.1 (C-6, C-2, C-4, C-5, C-8, 2x$CH_3C_6H_4CO$), 85.3 (C-4′), 83.6 (C-1′), 74.9 (C-3′), 63.7 (C-5′), 38.5 (C-2′), 21.7 and 21.6 (2x$CH_3C_6H_4CO$). HRMS (ESI) calcd for $C_{26}H_{22}Cl_2N_4O_5$ [M + Na]$^+$: 563,0865, found 563,0862.

a$_3$) Potassium *t*-butoxide (0.029 g, 0.24 mmol) was added to 2,6-dichloropurine (0.052 g, 0.28 mmol) in anhydrous 1,2-dimethoxyethane (4 ml) at 0 ^{0}C and then the resulting solution was stirred for 7 min under cooling and then for 15 min at room temperature and evaporated to dryness. Anhydrous acetonitrile (4 ml) and freshly distilled 3,4-dihydropyrane (4.4 ml) were added to the residue and the suspension was stirred under argon at room temperature for 15 min, then the crystalline chloride 1 (0.1 g, 0.26 mmol) was gradually added during 10 min. The reaction mixture was stirred under argon at room temperature for 210 min. Insoluble materials were removed by filtration and the solids were washed with MeCN (20 mL). The solvent was removed under reduced pressure and the residue was chromatographed on silica gel, eluting with EtOAc/petroleum ether 3:1 and 2:1 to afford β-nucleoside **2** a white solid (0.094 g, 67%).

*Synthesis of 2-chloro-2′-deoxyadenosine (**3**)*

To a solution of β-nucleoside 2 (0.3 g, 0.55 mmol) in anhydrous THF (14 mL) was added in 50 ml methanol saturated at 0 ^{0}C with ammonia, the reaction mixture was stirring for 24 h at room temperature, then for 18 h under 35-40 ^{0}C and evaporated. The residue was chromatographed on silica gel using for elution $CHCl_3$, then $CHCl_3$:MeOH-15:1 and 5:1 to afford nucleoside **3** as a white solid (0.13 g, 82%). Mp. >300 ^{0}C (methanol). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.36 (s, 1H, H-8), 7.82 (br. s , 2H, NH_2), 6.27 (t, 1H, $J_{1',2'} = J_{1',2''}$ = 6.4 Hz, H-1′), 5.40 (d, 1H, $J_{3',3\text{-OH}}$ = 4.2, 2′-OH), 5.04 (t, 1H, $J_{5',5\text{-OH}}$ = 5.4, 5′-OH), 4.10 (m, 1H, H-3′), 3.87 (m, 1H, H-4′). 3.53-3.62 (m, 2H, H-5′ and H-5′′), 2.61 (ddd, 1H, H-2′), 2.32 (ddd, 1H, H-2′′). ^{13}C NMR (DMSO-d_6, 126.76 MHz): δ 156.8, 153.3, 150.1, 139.9, 118.2, 87.9, 83.6, 70.7, 61.7, 39.8. HRMS (ESI) calcd for $C_{10}H_{12}ClN_5O_3$: 286.0707 (M+H), found 286.0701.

*Synthesis of Purine Modified Nucleoside Analogues **4, 5, 6** and **14***

*Synthesis of 2-chloro-6-fluoro-9-(3′,5′-di-O-p-toluoyl-2′-deoxy-β-D-ribofuranosyl)-9H-purine (**4**).*

To a solution of 3′,5′-di-O-toluoyl-2,6-dichloropurine-2′-deoxyriboside (**2**) (0.06 g, 0.11 mmol) in anhydrous CH_2Cl_2 (3.7 ml) and pyridine (0.022 ml) was

added dropwise 0.095 ml (0.72 mmol) DAST at room temperature. The reaction mixture was stirred for 30 min at rt and 23 h at 40-45 ^{0}C, then evaporated. The residue was chromatographed on silica gel using for elution mixtures 7:1, 5:1 and 3:1 hexane-EtOAc. Nucleoside 4 (0.034 g, 58%) was prepared as a syrup. Mp. 142-143 ^{0}C (ethylacetate/hexane). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.32 (s, 1H, H-8), 8.02 (d, 2H, Tol), 7.88 (d, 2H, Tol), 7.33 (d, 2H, Tol), 7.25 (d, 2H, Tol), 6.61 (t, 1H, J = 5.9 Hz, J = 6.9 Hz, H-1′), 5.82-5.86 (m, 1H, H-3′), 4.83 (dd, 1H, $J_{H\text{-}5',H\text{-}4'}$ = 6.8 Hz, $J_{H5',H5''}$ = 11.0 Hz, H-5′), 4.67-4.75 (m, 2H, H-4′ and H-5′′), 2.98-3.05 (m, 2H, H-2′ and H-2′′), 2.49 (s, 3H, Tol), 2.45 (s, 3H, Tol). ^{13}C NMR ($CDCl_3$,126.76 MHz): δ 166.1 and 166.0 (C=O, Tol), 159.3 (d, J = 265.0 Hz), 155.9 (d, J = 11.2 Hz), 152.7 (d, J = 16.6 Hz), 144.8, 144.5, 143.5 (d, $J_{C\text{-}8,F\text{-}6}$ ~ 2.0 Hz, C-8), 129.9, 129.6, 129.4, 126.4, 126.2, 119.7, 85.4 (C-4′), 83.7 (C-1′), 74.9 (C-3′), 63.8 (C-5′), 38.6 (C-2′), 21.8 ($CH_3C_6H_5CO$-), 21.7 ($CH_3C_6H_5CO$-). ^{19}F NMR ($CDCl_3$, 470.59 MHz): δ -65.59 (s, F-6). HRMS (ESI) calcd for $C_{26}H_{22}ClFN_4O_5$ [M+Na]$^+$: 547.1160, found 547.1157.

*Synthesis of 2-chloro-6-isopropylamino-9-(3′,5′-di-O-p-toluoyl-2′-deoxy-β-D-ribofuranosyl)-9H-purine (**5**)*

To a solution of 3′,5′-di-O-toluoyl-2-chloro-6-fluoropurine-2′-deoxyriboside (4) (0.018 g, 0.0034 mmol) in anhydrous 1,2-DME (2.0 ml) was added 0.013 ml (0.17 mmol) isopropyl amine at room temperature. The reaction mixture was stirred for 70 min and then evaporated. The residue was chromatographed on a silica gel, using a mixture of 4:1, 2:1, 1:1 and hexane-EtOAc to afford nucleoside 5 (0.017 g, 89%) as a syrup. Mp.72-74 ^{0}C (MeOH). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.02 (d, 2H, Tol), 7.97 (s, 1H, H-8), 7.93 (d, 2H, Tol), 7.33 (d, 2H, Tol), 7.27 (d, 2H, Tol), 6.56 (t, 1H, $J_{1',2'}$= $J_{1',2''}$ = 6.4 Hz, H-1′), 6.02 (br. s, 1H, NH), 5.75-5.83 (m, 1H, H-3′), 4.78 (dd, 1H, $J_{H\text{-}5',H\text{-}4'}$ = 3.2 Hz, $J_{H5',H5''}$ = 11.0 Hz, H-5′), 4.69 (dd, 1H, $J_{H\text{-}5'',H\text{-}4'}$ = 3.3 Hz, H-5′′), 4.63-4.70 (m, 1H, H-4′), 4.52 [br. s, 1H, HNCH(CH_3)$_2$], 2.90-2.93 (m, 2H, H-2′ and H-2′′), 2.49 (s, 3H, Tol), 2.45 (s, 3H, Tol), 1.35 [d, 3H, J = 0.9 Hz, CH(CH_3)$_2$], 1.34 [d, 3H, J = 0.9 Hz, CH(CH_3)$_2$]. ^{13}C NMR ($CDCl_3$,126.76 MHz): δ 166.2 and 166.0 (C=O, Tol), 154.5, 144.6, 144.3, 137.4, 129.9, 129.6, 129.4, 129.3, 126.7, 126.4 (C-6, C-2, C-4, C-8, C-5, $CH_3C_6H_5CO$-), 84.6 (C-4′), 83.2 (C-1′), 75.1 (C-3′), 64.1 (C-5′), 42.8 [CH(CH_3)$_2$], 38.7 (C-2′), 22.8 [CH(CH_3)$_2$], 21.8 ($CH_3C_6H_5CO$), 21.7 ($CH_3C_6H_5CO$). HRMS (ESI): m/z calcd for $C_{29}H_{30}N_5O_5Cl$ [M+Na]$^+$: 586.1883, found 586.1878.

*Synthesis of 2-chloro-6-isopropylamino-9-(2′-deoxy-β-D-ribofuranosyl)-9H-purine (**6**)*

A solution of nucleoside 5 (0.034 g, 0.06 mmol) in MeOH (4 mL) saturated at 0 ^{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-20:1, 15:1 and 8:1 to afford nucleoside 6 (15.6 mg, 79%). Mp.124-126 ^{0}C (MeOH). ^{1}H NMR (CD_3OD, 500 MHz): δ 8.25 (s, 1H, H-8), 6.39 (dd, 1H, $J_{1',2'}$ = $J_{1',2''}$ = 6.2 Hz, H-1′), 4.58-4.61 (m, 1H, H-3′), 4.43 [br. s, 1H, HNCH(CH_3)$_2$], 4.07 (q, 1H, H-4′), 3.87 (dd, 1H, $J_{5',4'}$ = 3.2 Hz, $J_{5',5''}$ = 12.2 Hz, H-5′), 3.76 (dd, 1H, $J_{5'',4'}$ = 3.6 Hz, H-5′′), 2.87 (ddd, 1H, J = 5.5 Hz, J = 6.2 Hz, J = 12.3 Hz, H-2′), 2.42 (ddd, 1H, J = 2.7 Hz, J = 6.2 Hz, H-2′′), 1.33 [br.s, 1H, CH(CH_3)$_2$], 1.31 [br.s, 1H, CH(CH_3)$_2$]. ^{13}C NMR (CD_3OD, 126.76 MHz): δ 155.9, 155.6, 150.6, 120.1, 89.8, 87.0, 72.9, 74.8, 63.6, 43.8, 41.5, 22.7. HRMS (ESI): m/z calcd for $C_{13}H_{18}N_5O_3Cl$ [M+Na]$^+$: 350.0996, found 350.0983.

*Synthesis of 2-chloro-4,5-dihydroxy-3′,5′-di-O-p-toluoyl-2′-deoxyinosine (**14**).*

To a solution of 3′,5′-di-O-toluoyl-2,6-dichloropurine-2′-deoxyriboside (**2**) (0.06 g, 0.11 mmol) in anhydrous CH_2Cl_2 (3.7 ml) and pyridine (0.022 ml) was added dropwise 0.095 ml (0.72 mmol) DAST at room temperature. The reaction mixture was stirred for 30 min and 23 h at 40-45 ^{0}C, then evaporated. To the residue was added 4.0 ml aq. $NaHCO_3$ and acetonitrile (1.2 ml) and reaction mixture was stirred at rt for 24 h. then water phase was extracted with $CHCl_3$(3x20ml), combined organic layer was washed with water and dried over sodium sulfate. The residue was chromatographed on silica gel, using for elution mixtures of 6:1, 5:1 and 3:1 petrolium ether-EtOAc to afford nucleoside 14 (0.023 g, 65%) as oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.03 (s, 1H, H-8), 8.02 (d, 2H, Tol), 7.88 (d, 2H, Tol), 7.33 (d, 2H, Tol), 7.26 (d, 2H, Tol), 6.59 (t, 1H, $J_{1',2'}$= $J_{1',2''}$ = 6.4 Hz, H-1′),

5.77-5.80 (m, 1H, H-3′), 4.75 (dd, 1H, $J_{H\text{-}5',H\text{-}4'}$ = 3.3 Hz, $J_{H5',H5''}$ = 11.0 Hz, H-5′), 4.71 (dd, 1H, $J_{H\text{-}5'',H\text{-}4'}$ = 4.1 Hz, H-5′′), 5.65-5.67 (m, 1H, H-4′), 4.2 (br.s, 1.6H, OH), 3.9 (br.s, 1.4H, OH), 2.91-2.93 (m, 2H, H-2′ and H-2′′), 2.49 (s, 3H, Tol), 2.45 (s, 3H, Tol). ^{13}C NMR ($CDCl_3$, 126.76 MHz): δ 166.2 and 166.0 (C=O, Tol), 154.3, 153.8, 151.1, 144.5, 144.2, 136.2 129.9, 129.7, 129.3, 126.7, 126.4, 118.2, 84.5 (C-4′), 83.1 (C-1′), 75.1 (C-3′), 64.2 (C-5′), 38.6 (C-2′), 21.8 ($CH_3C_6H_5CO$-), 21.7 ($CH_3C_6H_5CO$-). LS (ESI) calcd for $C_{26}H_{25}ClN_4O_8$ [M+Na]$^+$: 578.7, found 578.7.

Conflict of Interest

There are no conflicts to declare

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Supplementary Information

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