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Synthesis, evaluation of anti-HIV-1 and anti-HCV activity of novel 2′,3′-dideoxy-2′,2′-difluoro-4′-azanucleosides

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ABSTRACT

A series of 2′,3′-dideoxy-2′,2′-difluoro-4′-azanucleosides of both pyrimidine and purine nucleobases were synthesized in an efficient manner starting from commercially available L-pyroglutamic acid via glycosylation of difluorinated pyrrolidine derivative **15**. Several 4′-azanucleosides were prepared as a separable mixture of α- and β-anomers. The 6-chloropurine analogue was obtained as a mixture of N^7 and N^9 regioisomers and their structures were identified based on NOESY and HMBC spectral data. Among the 4′-azanucleosides tested as HIV-1 inhibitors in primary human lymphocytes, four compounds showed modest activity and the 5-fluorouracil analogue (**18d**) was found to be the most active compound (EC_{50} = 36.9 μM) in this series. None of the compounds synthesized in this study demonstrated anti-HCV activity.

1. Introduction

Considerable research efforts have been concentrated to prepare chemically modified nucleoside derivatives as effective anticancer[1] and antiviral agents.[2] Modifications in the carbohydrate moiety of nucleosides have resulted in improved biological properties.[3] In this regard, heteronucleosides, wherein the ring oxygen in the carbohydrate moiety is replaced by sulfur,[4] nitrogen,[5] and more recently selenium,[6] have received much attention for their therapeutic applications. Among the bioactive thionucleosides, two examples are shown in Figure 1: thiarabine (4′-thioaracytosine, **1**) is currently in clinical trials as a potent antitumor agent[7] and lamivudine [(-)-2′,3′-dideoxy-3′-thiacytidine, **2**], in which the 4′-oxygen is present but a sulfur atom was placed at the 3′-position. Lamivudine was approved by the FDA in 1995 for the treatment of HIV infection.[8] More recently, 4′-azanucleosides and 4′-selenonucleosides have shown significant anti-HCV[9] and anticancer[6a] activities, respectively. The synthesis of heterocyclic modified nucleosides has been recently reviewed.[10] Another sugar modification that improves the biological properties of some nucleoside analogues is the introduction of fluorine,[11] a common functionality used in drug discovery efforts.[12]

The presence of fluorine in the carbohydrate moiety of nucleoside offers stabilization of the glycosidic bond. This increases the resistance to metabolic degradation while improving the lipophilicity to cross lipid membranes more effectively. Another important feature that arises from a structure activity relationship (SAR) analysis is the lack of a 3′-hydroxyl group in many of the nucleoside analogues that show anti-HIV activity. It is well known that the incorporation of 3′-deoxynucleosides in a viral DNA prevents chain elongation and terminates cell growth.[13]

Among fluorinated nucleosides with antiviral activity, representative examples are FddC (2′,3′-dideoxy-2′-fluorocytosine, **3**)[14] and FLT (3′-fluoro-3′-deoxythymidine, **4**)[15] which inhibit the HIV reverse transcriptase. In addition, there are two nucleosides fluorinated at the 2′-position of the sugar moiety approved by the FDA for the treatment of cancer: (i) gemcitabine (2′-deoxy-2′,2′-difluorocytidine, **5**), a potent drug against ovarian,[16] pancreatic,[17] and breast[18] cancers, and (ii) clofarabine (2-chloro-2′-deoxy-2′-fluoroarabinoadenosine, **6**) which is used clinically for the treatment of leukemia in children.[19]

Although fluorine substitution in nucleosides and 4′-thionucleosides[11,20] has been extensively studied, few examples of fluorinated 4′-azanucleosides have been described and even less

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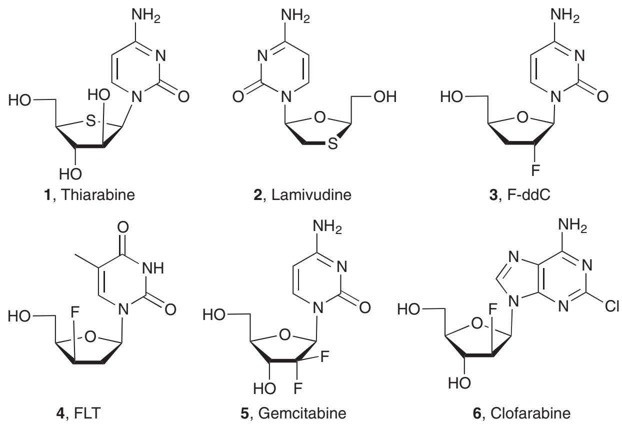


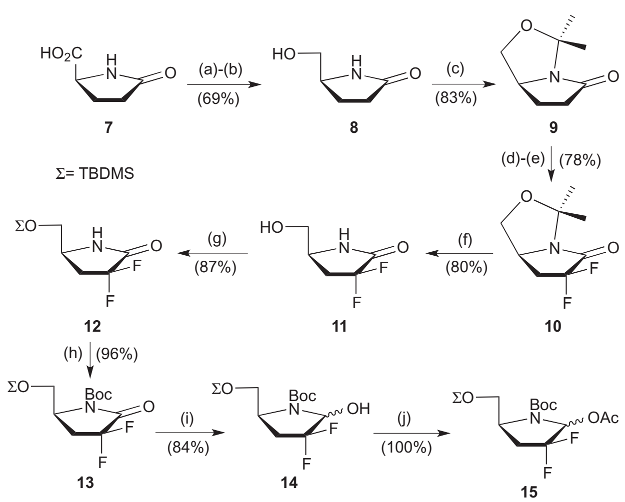
Figure 1. Selected structures of bioactive nucleosides.

biological properties have been reported. Qiu and Qing[21] carried out the preparation of pyrimidine 2′- and 3′-fluoromethyl-4′-azanucleosides, representing the only examples of fluorinated 4′-azanucleosides described to-date. To the best of our knowledge there are no other examples of 4′-azanucleosides in which the 2′,2′-difluoro substituent is directly attached to the pyrrolidine moiety.

Consequently, studies on the synthesis and biological activity of these nucleoside derivatives are worth pursuing. On the basis of the above considerations and our ongoing interest in the preparation and biological evaluation of nucleoside analogues,[22] herein, we report the synthesis and antiviral evaluation of a series of 2′,3′-dideoxy-2′,2′-difluoro-4′-azanucleosides**.**

2. Results and discussion

The synthesis of difluorinated pyrrolidine **15** as a substrate for the glycosylation reaction is outlined in Scheme 1. Commercially available L-pyroglutamic acid (**7**) possesses the correct configuration to furnish 4′-azanucleosides, which mimics the D-configuration of naturally occurring nucleosides. Conversion to L-pyroglutaminol (**8**) was accomplished in two steps via formation of the corresponding methylester from **7**, followed by reduction with $NaBH_4$.[23] Protection with 2,2-dimethoxypropane afforded the bicyclic lactam **9**. Electrophilic difluorination was achieved by the procedure described by Coward and Konas.[24] Treatment of compound **9** with LDA followed by *N*-fluorodibenzenesulfonimide (NFSI) at −78 °C generated a 1.2:1.0 mixture of diastereomers, which was then subjected to the same reaction conditions again to furnish difluorinated product **10** in high overall yield. The acid hydrolysis of hemiaminal ether **10** with AcOH/MeCN/H_2O mixture afforded difluorinated L-pyroglutaminol (**11**) in 80% yield. Protection of the resulting hydroxyl group of **11** with TBDMSCl yielded the silylated compound **12**, which was then treated with Boc_2O under basic conditions to obtain the protected product **13** in high yield. Reduction of lactam **13** using $LiBEt_3H$ in anhydrous THF provided **14** as a 1.9:1 mixture of anomers. Further reaction of **14** with Ac_2O gave **15** in quantitative yield, which was used for glycosylation reactions with silylated nucleobases.

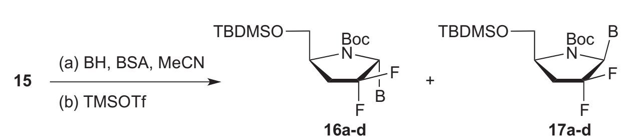


Scheme 1. Synthesis of **15**. Reagents and conditions: (a) 1 equiv $SOCl_2$, MeOH, rt, 4 h; (b) 2 equiv $NaBH_4$, EtOH, rt, 14 h; (c) 2,2-dimethoxypropane (solvent), 0.03 equiv CSA, reflux, 2 h; (d) 1.4 equiv $^{i}Pr_2NH$, 1.2 equiv $^{n}BuLi$, 1.4 equiv NFSI, THF, −78 °C; (e) 1.4 equiv $^{i}Pr_2NH$, 1.2 equiv $^{n}BuLi$, 1.4 equiv NFSI, THF, −78 °C; (f) AcOH/MeCN/H_2O (14:3:3), 90 °C, 14 h; (g) 1.3 equiv TBDMSCl, 1.3 equiv imidazole, 0.1 equiv DMAP, CH_2Cl_2, rt, 30 min; (h) 2 equiv Boc_2O, 1.3 equiv Et_3N, 1.1 equiv DMAP, CH_2Cl_2, rt, 30 min; (i) 1.2 equiv $LiEt_3BH$, THF, −78 °C, 1 h; (j) 15 equiv Ac_2O, 30 equiv Et_3N, CH_2Cl_2, rt, 30 min.

2.1. Synthesis of pyrimidine 4′-azanucleosides

Glycosylation of **15** under Vorbrüggen's conditions[25] with various pyrimidine heterocyclic bases gave α/β mixtures of 4′-azanucleosides in high overall yields (78–85%). The ratio of α/β-anomers was determined by HPLC-MS of the crude reaction mixture (Scheme 2). The poor resolution of the ^{1}H NMR spectra for the TBDMS protecting 4′-azanucleosides hindered the determination of α/β-ratio. Thus, to assign the stereochemistry of the glycosylation products, TBDMS protecting group was removed and the configuration of the anomeric carbon was established by NOESY experiments showing the α-anomer as the major product. This was probably due to the steric hindrance of the bulky silyl protecting group. Similar results were also reported during glycosylation of a Boc-protected proline with pyrimidine base.[21] Correlations between H1′ and H4′ as well as H5′ and H6 were clearly observed in the β-anomers, while correlations between H4′ and H6 of the corresponding nucleobase appeared in the α-anomers.

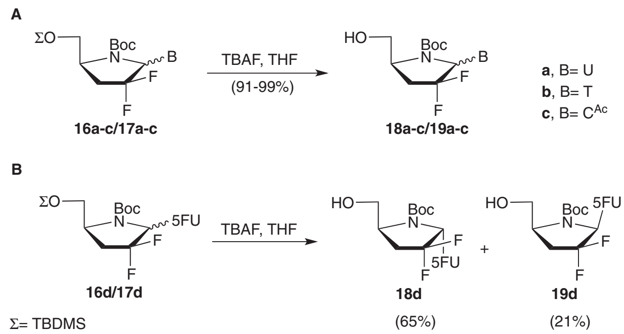
After glycosylation with silylated uracil, the resulting mixture of anomers **16a/17a** had different R_f values, and the products were easily separable by column chromatography. However the other nucleosides **16b–d/17b–d** were isolated as inseparable α/β-anomeric products. Removal of the TBDMS protecting group was accomplished under standard conditions by treating the separated pure anomers (**16a** and **17a**) or the anomeric mixtures **16b–c/17b–c** with TBAF in THF to afford 4′-azanucleosides **18/19** in excellent yields (Panel A, Scheme 3). It is noteworthy that after deblocking the TBDMS, the anomers **18d/19d** had different polarity and were separable by silica gel column chromatography (Panel B, Scheme



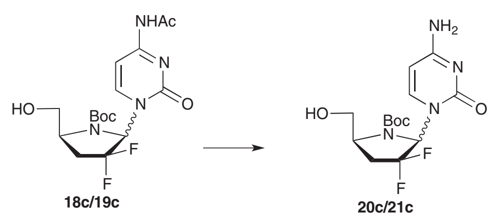
	Yield (%)[a]	Ratio (α:β)
16a+**17a**, B= U	85	2.3:1
16b+**17b**, B= T	78	2.0:1[b]
16c+**17c**, B= C^{Ac}	82	2.0:1
16d+**17d**, B= 5FU	83	2.7:1

[a]Yield of the glycosylation mixture (**16**+**17**)
[b]Ratio calculated by HPLC after TBDMS deprotection

Scheme 2. Glycosylation of **15** with pyrimidines. Reagents and conditions: (a) 1 equiv **15**, 4 equiv base (BH = uracil, thymine, N^4-acetylcytosine, and 5-fluorouracil), 6 equiv BSA, MeCN, 80 °C, 1 h; (b) 2.7 equiv TMSOTf, 0–80 °C, 30 min, 78–85% over two steps.



Scheme 3. Removal of TBDMS protecting group. Reagents and conditions: 1.5 equiv TBAF, THF, 0–25 °C, 1 h.



Scheme 4. Synthesis of **20c/21c**. Reagents and conditions: NH_3 sat-MeOH, rt, 1 h, 82%.

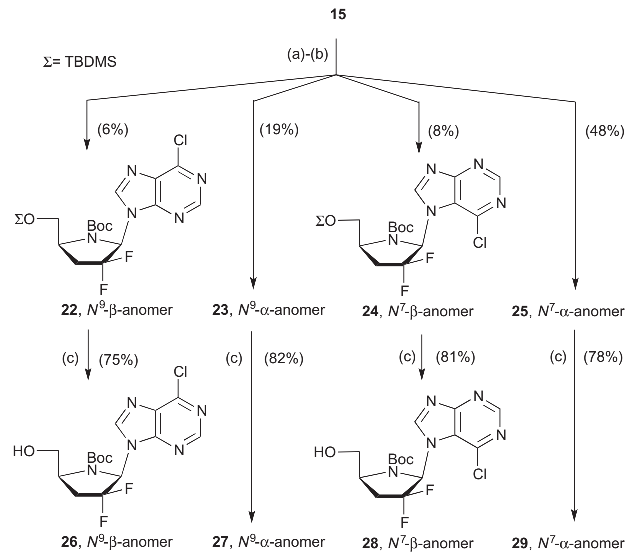
3). After the two step procedure of glycosylation–deprotection, four anomers were isolated as pure compounds (**18a**, **19a**, **18d** and **19d**) while **18b–c/19b–c** were isolated as non separable mixture of α/β-anomeric products.

Next, removal of the acetyl protecting group from the cytidine derivatives **18c/19c** was accomplished by treatment with ammonia affording an anomeric mixture of **20c/21c** (Scheme 4). The deprotection of Boc group from the uracil derivative **18a** was attempted with 2 equiv of TFA in CH_2Cl_2. However, only free uracil was isolated from the reaction mixture showing that despite the stabilization of the glycosidic bond by the two fluorine atoms, the unprotected 4′-azanucleosides are still prone to acid-mediated degradation. Additionally, it has been recently shown that the potent antiviral activity of a series of azanucleoside analogues was not compromised despite the presence of the Boc protecting group.[9]

2.2. Synthesis of purine 4′-azanucleosides

The success with the preparation of the pyrimidine nucleosides together with the few examples of purine 4′-azanucleosides described in the literature[26] encouraged us to try the coupling of **15** with purine nucleobases. It is well know that Vorbrüggen coupling[25] of silylated purine nucleobases typically results in N^7/N^9 isomeric mixtures. In addition, since two anomers may result from each glycosylated regioisomer, four compounds may be present in the crude reaction mixture. As expected, after the coupling of **15** with silylated 6-chloropurine, the TLC of the crude reaction mixture showed the presence of four products. Mass spectrometry analysis indicated the same formula weight for all four products. Interestingly, for this particular case, four possible products had sufficient difference in the R_f values to be separated by silica gel column chromatography (Scheme 5).

To identify the structure of glycosylated products, it was necessary to remove the TBDMS protecting group (TBAF in THF). The structure of **26–29** was established by 2D NMR spectroscopy. NOESY experiments assisted in the assignment of the stereochemical configuration and HMBC experiments allowed us to determine



Scheme 5. Glycosylation of **15** with 6-chloropurine. Reagents and conditions: (a) 1 equiv **15**, 4 equiv 6-chloropurine, 6 equiv BSA, MeCN, 80 °C, 1 h; (b) 2.7 equiv TMSOTf, 0–80 °C, 30 min; (c) 1.5 equiv TBAF, THF, rt, 1 h.

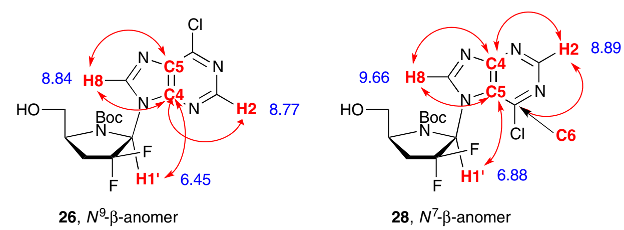
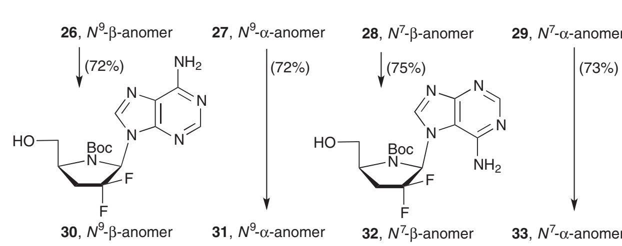


Figure 2. HMBC correlations of N^9 and N^7 regioisomers. In red are correlations mentioned in the text. The ^{1}H NMR values (δ ppm) are in blue.

the attachment of 6-chloropurine via N^7 or N^9 to the pyrrolidine moiety. In the case of nucleoside **26**, H2 and H8 of the 6-chloropurine moiety together with H1′ showed correlation ($^3J_{CH}$) with C4 of the nucleobase (Fig. 2). Correlation of these three hydrogen atoms with the same carbon can only be possible in the N^9 regioisomer. In the case of compound **28** the expected correlations for an N^7 isomer were observed, allowing confirmation of the structure. The same correlations patterns were also observed in the corresponding α-anomers. In addition, the structures were also confirmed by comparing the UV maxima with previously reported data (see experimental section).

The ratio of the four products obtained after glycosylation reaction, was determined by HPLC-MS data on the crude reaction mixture of protected nucleosides **22–25** (**22**:**23**:**24**:**25**, 8:27:12:53). As seen before for the pyrimidine nucleosides, the α-anomers were also the major products with the purine series. Interestingly, de-



Scheme 6. Synthesis of adenine 4′-azanucleosides **30–33**. Reagents and conditions: NH_3 sat-MeOH, 100 °C, 3 h.

spite of higher reaction temperature (80 °C) employed during the glycosylation step, the N^7 product was dominant. This observation, which is unusual for glycosylation of 6-chloropurine base, is likely due to the sterically demanding structure of **15**.

The treatment of 6-chloropurine analogues **26–29** with ammonia furnished the corresponding adenine derivatives **30–33** (Scheme 6) in good yield.

3. Biological evaluation

3.1. Antiviral assays

All azanucleosides were tested against HIV-1_{LAI} using 3′-azido-3′-deoxythymidine (AZT, zidovudine) as a reference in an assay with human peripheral blood mononuclear (PBM) cells. We opted for including all products in the screen to maximize the database despite of the fact that some were isolated as mixture of anomeric products.

A summary of the data expressed as the effective concentration required to inhibit viral replication by 50% (EC_{50}) and 90% (EC_{90}) is shown in the Table 1. The 5-fluorouracil analogues **18d** (EC_{50} = 36.9 μM), **19d** (EC_{50} = 44.5 μM) together with the 6-chloro purine derivatives **27** (EC_{50} = 64.5 μM) and **29** (EC_{50} = 92.3 μM) showed modest activity when compared with the AZT as a control. It is noteworthy that the α-anomers demonstrated better activities in comparison to their β-counterparts. Also, it is of interest to observe modest activity despite the fact that all products were protected with the Boc group.

3.2. Cytotoxicity assays

All compounds were evaluated for their potential cytotoxicity in uninfected phytohemagglutinin stimulated human PBM cells, in lymphocytic CEM cells, and Vero (African green monkey Kidney) cells. The majority of compounds did not show any toxicity except the β-uracil analogue **19a** in Vero cells, **19a**, **18d, 19d**, **27** and **29** in PBM cells, and **26**, **27** and **29** in CEM cells.

3.3. HCV Replicon assays[28]

All compounds were tested at 10 μM in an HCV replicon assay using 2′-C-Me-Cytidine as the positive control. No anti-HCV activity was observed (data not shown).

4. Conclusions

In summary, we have developed an efficient synthesis of novel 2′,3′-dideoxy-2′,2′-difluoro-4′-azanucleosides both as pyrimidine and purine analogues. A high yielding sequence of electrophilic difluorination of L-pyroglutamic acid followed by the coupling of protected pyrrolidine **15** as the glycosyl donor with four pyrimidine and one purine nucleobase was established. The pyrimidine 4′-azanucleosides were obtained as mixtures of α- and β-anomeric products, increasing the breadth of novel nucleoside analogues available for biological screening. The α-anomers were obtained as major products during glycosylation. After glycosylation and TBDMS deprotection, the anomeric mixtures of U (**18a/19a**) and 5-F-U (**18d/19d**) analogues could be easily separated by silica gel column chromatography. However, the anomeric mixtures of T (**18b/19b**) and C (**18c/19c**) analogues could not be separated by silica chromatography. Gratifyingly, glycosylation with 6-chloropurine afforded a separable mixture of four nucleosides arising from the formation of N^7/N^9 glycosylated regioisomers and the corresponding α/β-anomers. These isomers were characterized based on 2D NMR spectroscopy, and constitute the only examples of fluorinated purine 4′-azanucleosides described to date. Further reaction with ammonia of each isomer furnished a direct route for the purine nucleosides **30**–**33**. All fluorinated 4′-azanucleosides synthesized were tested as inhibitors of HIV-1 in PBM cells. The α-5-F-U analogue **18d** was found to be the most active compound (EC_{50} = 36.9 μM) in this series. These compounds did not exhibit anti-HCV activity in a hepatitis C replicon assay probably due to the lack of a 3′-hydroxyl group or mimic for the moiety. The limited examples of fluorinated 4′-azanucleosides described in the literature and the interesting activity found in some of the nucleosides described in this work, warrants further studies with this new class of compounds.

5. Experimental section

All reagents were bought from Aldrich and Acros at highest commercial quality and used without further purification. All non-aqueous reactions were carried out under anhydrous conditions in dry, freshly distilled solvents. THF and CH_2Cl_2 were purified by passage through a bed of activated alumina. Reactions were monitored by TLC carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as visualizing agent and/or acidic aqueous permanganate. Flash chromatography was

Table 1
Effect of analogues against HIV-1_{LAI} in human peripheral blood mononuclear (PBM) cells

Analogue	Base	Anti-HIV-1 activity in PBM cells[a]		Cytotoxicity (IC_{50}, μM)[b]		
		EC_{50}, μM	EC_{90}, μM	PBM cells	CEM cells	VERO cells
AZT	β-T	0.0017	0.0027	>100	14.3	56.0
18a	α-U	>100	>100	>100	>100	>100
19a	β-U	>100	>100	81.4	>100	44.4
18b/19b	α/β-T	>100	>100	>100	>100	>100
18c/19c	α/β-C	>100	>100	>100	>100	>100
18d	α-5-F-U	36.9	75.6	30.5	>100	>100
19d	β-5-F-U	44.5	90.8	28.2	>100	>100
26	N9-β-6-Cl-Pu	>100	>100	>100	57.1	>100
27	N9-α-6-Cl-Pu	64.5	>100	58.1	38.1	>100
28	N7-β-6-Cl-Pu	>100	>100	>100	>100	>100
29	N7-α-6-Cl-Pu	92.3	>100	59.5	55.4	>100
30	N9-β-A	>100	>100	>100	>100	>100
31	N9-α-A	>100	>100	>100	>100	>100
32	N7-β-A	>100	>100	>100	>100	>100
33	N7-α-A	>100	>100	>100	>100	>100

[a] HIV drug susceptibility assay was done as previously describe in Ref. 27.
[b] Cytotoxicity assays in PBM, CEM and Vero cells were done as previously described in Ref. 20d.

performed using silica gel 60 (230–400 mesh). LC-ESI–MS analyses were carried out in a chromatogram with UV detector at 254 nm using Agilent Poroshell column 120 SB, C18, or Mediterranea column (250 × 45 mm) flow 1 mL min^{-1} rt gradient MeCN-H_2O as eluent. Melting points were taken on samples in open capillary tubes and are uncorrected. ^1H, ^{13}C NMR, and DEPT were obtained using Varian Mercury and/or Bruker 300.13, 400.13 or 600.13 MHz for 1H, and 75.5, 100.61 MHz or 150.92 for ^{13}C. The same spectrometers were used for the acquisition of ^1H–^1H homonuclear (COSY and NOESY) and ^1H–^{13}C heteronuclear (HSQC and HMBC) correlations. Optical rotations were recorded on a Jasco P-1010 polarimeter and values are reported as follows: $[\alpha]_\lambda^T$ (*c*: g/100 mL, solvent). High resolution mass spectra (HRMS) were recorded on a VG 7070 HS mass spectrometer under electron spray ionization (ESI) conditions. The *tert*-butyldimethyl silyl protecting group is abbreviated below as TBDMS.

5.1. Synthesis of (*S*)-pyroglutaminol (8)

To a cooled solution of L-pyroglutamic acid (7) (6.0 g, 41.9 mmol) in dry MeOH (80 mL), was added $SOCl_2$ (4.9 g, 41.9 mmol) dropwise with magnetic stirring at room temperature for 2 h. The mixture was concentrated under vacuum to give the methyl ester as clear oil (5.2 g, 80%). This oil (33.2 mmol) was poured in a flask, dissolved in dry EtOH (80 mL) and $NaBH_4$ (2.54 g, 67.0 mmol) was added portionwise. After stirring at room temperature for 2 h, the mixture was acidified with concentrated HCl to pH 1. Solvents were removed under vacuum and the residue subjected to flash chromatography (15% MeOH, CH_2Cl_2) to afford **8** (3.3 g 28.5 mmol). The synthesis of **8** has been previously described.[23]

5.2. Synthesis of (5*S*)-2,2-dimethyl-8-oxo-1-aza-3-oxa-bicyclo[3.3.0]octane (9)

A mixture of compound **8** (3.3 g, 28.7 mmol), CSA (0.68 mmol, 158 mg) and 2,2-dimethoxypropane (DMP; 12 mL) was refluxed for 2 h. The volatiles components (DMP, MeOH) were removed in vacuo. Fresh DMP was added, and the mixture again refluxed for 2 h. This process was repeated a total of three times. After the final evaporation the residue was subjected to flash chromatography (50% AcOEt/hexane) and then distilled under vacuo to afford **9** as a colorless oil (3.67 g, 83%). The synthesis of **9** has been previously described.[24]

5.3. Synthesis of (5*S*)-2,2-dimethyl-7,7-difluoro-8-oxo-1-aza-3-oxa-bicyclo[3.3.0]octane (10)

Diisopropyl amine (2.3 mL, 16.5 mmol) was added to dry THF with magnetic stirring and the solution was cooled to −78 °C. *N*-buthylitium (3.7 mL, 13.9 mmol) was added slowly and the mixture was stirred for 1 h. A solution of **9** (1.8 g, 11.6 mmol) in THF (9 mL) was added slowly. The mixture was stirred for 1 h at −78 °C before the addition of a solution of N-fluorodibenzenesulfonimide (NFSI; 5.19 g, 16.5 mmol) in THF (18 mL), the solution was again stirred for 45 min and then quenched by the addition of saturated NH_4Cl. THF was removed under vacuo and the residue extracted with AcOEt and water. The combined organic layers were dried over Na_2SO_4, filtered and evaporated. The residue was purified by flash chromatography (20–50% AcOEt-hexane) to give the monofluorinated lactam (1.84 g, 92%). The same fluorination procedure was carried out using the previous monofluorinated lactam (1.84 g, 10.6 mmol) as a substrate to get the difluorinated compound **10** (1.72 g, 85%) as a pale yellow oil. The synthesis of **10** has been previously described.[24]

5.4. Synthesis of (5*S*)-3,3-difluoro-5-hydroxymethyl-2-pyrrolidinone (11)

Compound **10** (1.65 g, 8.37 mmol) was stirred in a mixture of acetic acid, acetonitrile and water (14:3:3, v/v) (20 mL). The solution was heated at 90 °C for 14 h. After evaporation of solvents, the residue was purified by flash chromatography (10% MeOH/CH_2Cl_2) to yield pure **11** as a white solid (1.04 g, 80%). The synthesis of **11** has been previously described.[24]

5.5. Synthesis of (5*S*)-5-(*tert*-butyldimethylsilyloxymethyl)-3,3-difluoro-2-pyrrolidinone (12)

To a solution of **11** (960 mg, 6.39 mmol) in dry CH_2Cl_2 (40 mL) at 0 °C was added imidazole (566 mg, 8.31 mmol), DMAP (0.63 mmol, 78 mg) and TBDMSCl (1.25 g, 8.31 mmol). The reaction was stirred at room temperature for 30 min, quenched by the addition of water (300 μL) and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, solvents were evaporated and the residue subjected to flash chromatography (15% AcOEt/hexane) to afford **12** (1.8 g, 87%) as viscous oil. R_f: (20% AcOEt/hexane):0.23. $[\alpha]_D^{20}$ +35 (*c* 0.5, CH_2Cl_2). ^1H NMR ($CDCl_3$, 400.13 MHz): δ 0.05 (s, 6H, Me_2Si), 0.87 (s, 9H, ^{t}Bu), 2.29 (m, 1H, H3), 2.55 (m, 1H, H-3), 3.54 (dd, 1H, CH_2O, J_{HH} 6.2 Hz, J_{HH} 10.3 Hz), 3.67 (dd, 1H, CH_2O, J_{HH} 4.4 Hz, J_{HH} 10.4 Hz), 3.80 (m, 1H, H-5), 7.67 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$, 100.61 MHz): δ 5.4 ($2CH_3$, ^{t}Bu), 18.4 (1C, ^{t}Bu), 26.0 ($3CH_3$, ^{t}Bu), 33.1 (t, C-4, J_{CF} 22.1 Hz), 50.7 (C-5), 65.1 (CH_2O), 118.0 (t, C-3, J_{CF} 249.5 Hz), 166.7 (t, C=O, J_{CF} 31.2 Hz). HRMS (ESI$^+$) Calcd for $C_{11}H_{21}F_2NO_2SiNa$ $[M+Na]^+$ 288.1202, found 288.1203.

5.6. Synthesis of (5*S*)-5-(*tert*-butyldimethylsilyloxymethyl)-*N*-*tert*-butyloxycarbonyl-3,3-difluoro-2-pyrrolidinone (13)

To a solution of **12** (854 mg, 3.21 mmol), in dry CH_2Cl_2 (28 mL), was added Et_3N, (0.6 mL, 4.17 mmol), DMAP (427 mg, 3.53 mmol) and Boc_2O (1.41, 6.42 mmol). The reaction was stirred at room temperature for 30 min. Solvents were concentrated to dryness and the residue purified by column chromatography (10% AcOEt/hexane) to give **13** (1.13 g, 96%). R_f (20% AcOEt/hexane): 0.53. $[\alpha]_D^{20}$ −51 (*c* 0.5, CH_2Cl_2). ^1H NMR ($CDCl_3$, 400.13 MHz): δ 0.02 (s, 6H, Me_2Si), 0.85 (s, 9H, ^{t}Bu), 1.53 (s, 9H, ^{t}Bu), 2.48 (m, 2H, H-4), 3.70 (dd, 1H, CH_2O, J_{HH} 2.5 Hz, J_{HH} 10.3 Hz), 3.83 (dd, 1H, CH_2O, J_{HH} 5.1 Hz, J_{HH} 10.3 Hz), 4.22 (m, 1H, H-5). ^{13}C NMR ($CDCl_3$, 100.61 MHz): δ 5.7 (^{t}Bu), 18.4 (^{t}Bu), 26.0 + 27.8 (^{t}Bu + Boc), 31.0 (t, C-4, J_{CF} 22.1 Hz), 53.4 (C-5), 62.3 (CH_2O), 84.7 (Boc), 116.6 (t, C-3, J_{CF} 251.0 Hz), 149.2 (C=O), 166.7 (t, C=O, J_{CF} 32.2 Hz). MS (ESI$^+$, *m*/*z*) 382 [$(M+NH_4)^+$ 100%]; 388 [$(M+Na)^+$ 10%]. HRMS (ESI$^+$) Calcd for $C_{16}H_{29}F_2NO_4SiNa$ $[M+Na]^+$ 388.1726, found 388.1727.

5.7. Synthesis of (5*S*)-5-(*tert*-butyldimethylsilyloxymethyl)-*N*-*tert*-butyloxycarbonyl-3,3-difluoro-2-hydroxy-pyrrolidine (14) (mixture of anomers)

To a solution of **13** (368 mg, 1.0 mmol) in anhydrous THF (9.3 mL) was slowly added dropwise $LiEt_3BH$ (504 μL, 0.5 mmol) at −78 °C under argon atmosphere. The reaction mixture was stirred 1 h, then additional $LiEt_3BH$ (706 μL, 0.70 mmol) was added. After 1 h, the reaction was quenched with water (4 mL) and the organic solvent was evaporated. The aqueous phase was extracted with CH_2Cl_2 dried over Na_2SO_4, filtered and concentrated under vacuum. The resulting residue was purified by flash chromography (30% AcOEt/hexane) to afford a inseparable mixture of anomers **14** (311 mg, 84%) in 4:1 ratio. R_f (20% AcOEt/hexane): 0.51. Major isomer ^1H NMR ($CDCl_3$, 400.13 MHz): δ 0.11 (s, 6H, Me_2Si), 0.91 (9H, ^{t}Bu), 1.48 (s, 9H, Boc), 2.45 (m, 2H), 3.39 (d, 1H, CH_2O, J_{HH} 10.2 Hz), 3.83 (d, 1H, CH_2O, J_{HH} 10.0 Hz), 4.07 (d, 1H, H-5, J_{HH} 10.0 Hz), 5.16 (t, 1H, H-2, J_{HF} 10.2 Hz). Minor isomer ^1H NMR ($CDCl_3$, 400.13 MHz): δ 0.11 (s, 6H, ^{t}Bu), 0.91 (9H, ^{t}Bu), 1.48 (s, 9H, Boc), 2.45 (m, 2H), 3.51 (d, 1H, CH_2O, J_{HH} 9.8 Hz), 3.69 (m, 1H, CH_2O), 4.21 (m, 1H, H-5), 5.16 (m, 1H, H-2). MS (ESI$^+$, *m*/*z*) 368 [(M+H)$^+$ 10%]; 390 [(M+Na)$^+$ 100%]. HRMS (ESI$^+$) Calcd for $C_{16}H_{31}F_2NO_4SiNa$ [M+Na]$^+$ 390.1883, found 388.1885.

5.8. Synthesis of (5*S*)-2-acetyloxy-5-(*tert*-butyldimethylsilyl oxymethyl)-*N*-*tert*-butyloxycarbonyl-3,3-difluoro-pyrrolidine (15)

(mixture of anomers). To a solution of anomers **14** (300 mg) in CH_2Cl_2 (10 mL) was added Et_3N (3.4 mL, 2.5 mmol), Ac_2O (1.2 mL, 12.0 mmol), and DMAP (catalytic). The solution was allowed to stir for 30 min. Solvents were evaporated and the residue purified by flash column chromatography (20% AcOEt/hexane) to afford **15** (332 mg, 100%). The presence of TBDMS protecting complicates the analysis of the NMR spectra. R_f (20% AcOEt/hexane): 0.64. ^1H NMR ($CDCl_3$, 400.13 MHz): δ 0.11 (s, 6H, Me_2Si), 0.95 (9H, ^{t}Bu), 1.47 (s, 9H, Boc), 2.09 (m, 2H), 3.77 (br s, 1H), 4.01 (br s, 2H), 6.49 (br s, 1H). MS (ESI$^+$, *m*/*z*) 409 [(M+H)$^+$ 10%]; 432 [(M+Na)$^+$ 100%]. HRMS (ESI$^+$) Calcd for $C_{18}H_{33}F_2NO_5SiNa$ [M+Na]$^+$ 432.1988, found 432.2003.

5.9. General procedure for glycosylation of fluorinated pyrrolidine 15 with pyrimidine bases followed by deprotection. Synthesis of nucleosides 18a–d/19a–d

To a stirred solution of **15** (0.3 mmol, 122 mg) and the different bases (1.2 mmol) in dry MeCN was added BSA (0.59 mL, 1.8 mmol). The reaction mixture was stirred at 80 °C for 1 h. This solution was cooled to 0 °C and TMSOTf (0.17 mL, 0.83 mmol) was added dropwise. The solution was heated at 80 °C for 1 h. The reaction was quenched by the addition of Et_3N (0.2 mL). Solvents were evaporated and the resulting residue purified by column chromatography (20–50% AcOEt/hexane) to afford the protected 4′-azanucleosides (**16a–d**/**17a–d**) (78–85%). Then stirred solutions of the nucleoside derivatives in THF (4 mL) were treated with 1.0 M solutions of TBAF in THF (1.5 equiv). Reactions were quenched with water, and after evaporation of solvents the residues purified by flash chromatography (2–5% MeOH/CH_2Cl_2) to afford the pure 4′-azanucleosides (**18a–d**/**19a–d**) as white solids (90–99%).

5.10. 1-[(2*S*,5*S*)-*N*-*tert*-Butyloxycarbonyl-3,3-difluoro-5-(hydro xymethyl)pyrrolidin-2-yl]-uracil (18a)

Sixty-one percentage of yield after glycosylation and 99% yield for TBDMS deprotection. R_f (5% MeOH/CH_2Cl_2): 0.19. mp: 82–85 °C. $[\alpha]_D^{20}$ +18 (*c* 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$. 600.15 MHz, 328 K): δ 1.41 (s, 9H, Boc), 2.55 (m, 2H, H-4), 3.71 (dd, 1H, CH_2O, J_{HH} 6.2 Hz, J_{HH} 11.1 Hz), 3.91 (s, 1H, CH_2O), 4.36 (s, 1H, H-5), 5.75 (s, 1H, H-5B), 6.35 (br s, H-2), 7.09 (1H, H-6B), 9.55 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$. 150.92 MHz, 328 K): δ 28.1 (Boc), 35.0 (C-4), 58.1 (C-5), 63.7 (CH_2O), 70.6 (m, C-2), 83.4 (Boc), 101.6 (C-5B), 124.6 (C-3), 138.1 (C-6B), 150.2 (C-2B), 153.0 (C=O), 162.9 (C-4B). HRMS (ESI$^+$) Calcd for $C_{14}H_{19}F_2N_3O_5Na$ [M+Na]$^+$ 370.1185, found 370.1186.

5.11. 1-[(2*R*,5*S*)-*N*-*tert*-butyloxycarbonyl-3,3-difluoro-5-(hydro xymethyl)pyrrolidin-2-yl]-uracil (19a)

Twenty-four percentage of yield after glycosylation and 98% yield for TBDMS deprotection. R_f (5% MeOH/CH_2Cl_2): 0.21. mp: 73–75 °C. $[\alpha]_D^{20}$ −54 (*c* 0.5, CH_2Cl_2). ^1H NMR (acetone-d_6, 300.13 MHz): δ 1.42 (s, 9H, Boc), 2.63 (m, 2H, H-4), 3.74 (ddd, 1H, CH_2O, J_{HH} 2.4 Hz, J_{HH} 4.2 Hz, J_{HH} 11.4 Hz), 4.10 (tt, 1H, H-5, J_{HH} 2.7 Hz, J_{HH} 8.1 Hz), 4.31 (m, 1H, CH_2O), 4.64 (t, 1H, OH, J_{HH} 4.4 Hz), 5.63 (d, 1H, H-5B, J_{HH} 8.1 Hz), 6.36 (d, 1H, H-2, J_{HH} 13.8 Hz), 8.33 (d, 1H, H-6B, J_{HH} 8.1 Hz), 10.22 (br s, 1H, NH). ^{13}C NMR (acetone-d_6, 75.5 MHz): δ 27.3 (Boc), 33.1 (t, C-4, J_{CF} 22.6 Hz), 54.1 (C-5), 57.1 (CH_2O), 71.8 (m, C-2), 81.5 (Boc), 101.6 (C-5B), 124.5 (t, C-3, J_{CF} 251.8 Hz), 140.1 (C-6B), 150.7 (C-2B), 153.6 (C=O), 162.6 (C-4B). MS (ESI$^+$, *m*/*z*) 348 [(M+H)$^+$ 100%]; 370 [(M+Na)$^+$ 35%]. HRMS (ESI$^+$) Calcd for $C_{14}H_{19}F_2N_3O_5Na$ [M+Na]$^+$ 370.1185, found 370.1190.

5.12. 1-[(2*S*,5*S*)-*N*-*tert*-Butyloxycarbonyl-3,3-difluoro-5-(hydro xymethyl)pyrrolidin-2-yl]-thymine (18b) and 1-[(2*R*,5*S*)-*N*-*tert*-butyloxycarbonyl-3,3-difluoro-5-(hydroxymethyl)pyrrolidin-2-yl]-thymine (19b)

Seventy-eight percentage of yield after glycosylation and 98% yield for TBDMS deprotection. R_f (5% MeOH/CH_2Cl_2): 0.22. α-isomer (**18b**) ^1H NMR ($CDCl_3$, 400.13 MHz): δ 1.38 (s, 9H, Boc), 1.92 (s, 3H, CH_3), 2.54 (m, 2H, H-4), 3.23 (s, 1H, OH), 3.71 (s, 1H, CH_2O), 4.07 (s, 1H, CH_2O), 4.35 (s, 1H, H-5), 6.35 (br s, H-2), 6.83 (1H, H-6B), 9.32 (br s, 1H, NH). β-isomer (**19b**) δ 1.42 (s, 9H, Boc), 1.90 (s, CH_3), 2.54 (m, 2H, H-4), 3.15 (br s, 1H, OH), 3.77 (dd, 1H, CH_2O, J_{HH} 2.8 Hz, J_{HH} 11.2 Hz), 4.10 (q, 1H, H-5, J_{HH} 4.0 Hz), 4.26 (d, 1H, CH_2O, J_{HH} 11.2 Hz), 6.18 (d, 1H, H-2, J_{HF} 13.2 Hz), 7.66 (s, 1H, H-6B), 9.24 (br s, 1H, NH). MS (ESI$^+$, *m*/*z*) 362 [(M+H)$^+$ 25%]; 384 [(M+Na)$^+$ 100%]. HRMS (ESI$^+$) Calcd for $C_{15}H_{21}F_2N_3O_5Na$ [M+Na]$^+$ 384.1341, found 384.1349.

5.13. *N*^{4}-acety1-1-[(2*S*,5*S*)-*N*-*tert*-butyloxycarbonyl-3,3-difluoro-5-(hydroxymethyl)pyrrolidin-2-yl]cytosine (18c) and *N*^{4}-acety1-1-[(2*R*,5*S*)-*N*-*tert*-butyloxycarbonyl-3,3-difluoro-5-(hydroxymethyl)pyrrolidin-2-yl]-cytosine (19c)

Eighty-two percentage of yield after glycosylation and 91% yield for TBDMS deprotection. R_f (70% AcOEt/hexane): 0.11. α-isomer (**18c**): ^1H NMR (MeOH-d_4, 300.13 MHz): δ 1.33 (s, 9H, Boc), 2.21 (s, 3H, CH_3), 2.61 (m, 2H, H-4), 3.58 (t, 1H, CH_2O, J_{HH} 8.7 Hz), 3.91 (s, 1H, CH_2O), 4.33 (m, 1H, H-5), 6.45 (d, 1H, H-2, J_{HF} 12.9 Hz), 7.46 (s, 1H, H-5), 7.92 (s, 1H, H-5). β-isomer (**19c**): δ 1.41 (s, 9H, Boc), 2.20 (s, 3H, CH_3), 2.62 (m, 2H, H-4), 3.66 (d, 1H, CH_2O, J_{HH} 11.4 Hz), 4.07 (s, 1H, H-5), 4.30 (s, 1H, CH_2O), 6.47 (d, 1H, H-2, J_{HF} 12.9 Hz), 7.41 (d, 1H, H-5B, J_{HF} 7.5 Hz), 8.76 (d, 1H, H-6B, J_{HF} 7.5 Hz). HRMS (ESI$^+$) Calcd for $C_{15}H_{22}F_2N_4O_5Na$ [M+Na]$^+$ 411.1450, found 411.1467.

5.14. 1-[(2*S*,5*S*)-*N*-*tert*-Butyloxycarbonyl-3,3-difluoro-5-(hydro xymethyl)pyrrolidin-2-yl]-5-fluorouracil (18d)

Eighty-three percentage of overall yield (α + β) after glycosylation and 65% yield for TBDMS deprotection (pure α-anomer). R_f (5% MeOH/CH_2Cl_2): 0.24. mp: 61-63 °C. $[\alpha]_D^{20}$+57 (c 0.5, CH_2Cl_2). ^1H NMR (acetone-d_6, 300.13 MHz): δ 1.39 (rotamers, 9H, Boc), 2.75 (m, 2H, H-4), 3.55 (t, 1H, CH_2O, J_{HH} 6.2 Hz), 3.98 (d, 1H, CH_2O, J_{HH} 9.6 Hz), 4.43 (s, 1H, H-4), 6.30 (d, 1H, J_{HF} 9.6 Hz), 7.84 (s, 1H, H-6). ^{13}C NMR (acetone-d_6, 75.5 MHz): δ 27.3 (Boc), 33.1 (t, C-4, J_{CF} 23.4 Hz), 54.1 (C-5), 60.7 (CH_2O), 70.9 (m, C-2), 81.5 (Boc), 123.2 (d, C-6B, J_{CF} 36.2 Hz), 124.5 (t, C-3, J_{CF} 249.8 Hz), 142.0 (d, C-5B, J_{CF} 249.1 Hz), 149.2 (C-2B), 156.2, 156.6 (C-4 + C=O). HRMS (ESI$^+$) Calcd for $C_{15}H_{18}F_3N_3O_5Na$ [M+Na]$^+$ 388.1091, found 388.1108.

5.15. 1-[(2*R*,5*S*)-*N*-*tert*-Butyloxycarbonyl-3,3-difluoro-5-(hydro xymethyl)pyrrolidin-2-yl]-5-fluorouracil (19d)

Eighty-three percentage of overall yield (α + β) after glycosylation and 27% yield for TBDMS deprotection (pure β-anomer). R_f

(5% MeOH/CH_2Cl_2): 0.29. mp: 82–84 °C. $[\alpha]_D^{20}$ −57 (*c* 0.5, CH_2Cl_2). ^1H NMR (acetone-d_6, 300.13 MHz): δ 1.44 (s, 9H, Boc), 2.75 (m, 2H, H-4), 3.72 (dd, 1H, CH_2O, J_{HH} 1.5 Hz, J_{HH} 11.4 Hz), 4.12 (tt, 1H, H-5, J_{HH} 2.1 Hz, J_{HH} 8.4 Hz), 4.43 (tt, 1H, CH_2O, J_{HH} 2.7 Hz, J_{HH} 11.4 Hz), 6.33 (d, 1H, H-2, J_{HF} 13.8 Hz), 8.79 (d, 1H, H-6, J_{HF} 7.5 Hz). ^{13}C NMR (acetone-d_6, 125.61 MHz): δ 27.3 (Boc), 33.1 (t, C-4, J_{CF} 23.4 Hz), 54.1 (C-5), 57.1 (CH_2O), 71.8 (m, C-2), 81.5 (Boc), 124.4 (d, C-6B, J_{CF} 36.2 Hz), 124.5 (t, C-3, J_{CF} 251.8 Hz), 142.0 (d, C-5B, J_{CF} 231.8 Hz), 149.3 (C-2B), 156.3, 156.7 (C-4 + C═O). HRMS (ESI$^+$) Calcd for $C_{15}H_{18}F_3N_3O_5Na$ [M+Na]$^+$ 388.1091, found 388.1085.

5.16. 1-[(2*S*,5*S*)-*N*-*tert*-Butyloxycarbonyl-3,3-difluoro-5-(hydroxymethyl)pyrrolidin-2-yl]-cytosine (20c) and 1-[(2*R*,5*S*)-*N*-*tert*-butyloxycarbonyl-3,3-difluoro-5-(hydroxymethyl)pyrrolidin-2-yl]-cytosine (21c)

The mixture of anomers **18c**/**19c** (44 mg, 0.11 mmol) was dissolved in a saturated solution of ammonia in MeOH (3 mL). The reaction was stirred for 2 h at room temperature for 2 h. MeOH was evaporated and the residue subjected to flash chromatography 1–5% MeOH/CH_2Cl_2 to afford the mixture of anomers **20c**/**21c** (32 mg, 82%) as a white solid. R_f (5% MeOH/CH_2Cl_2): 0.38. α-isomer (**20c**) ^1H NMR (MeOH-d_4, 300.13 MHz): δ 1.39 (rotamers, 9H, Boc), 2.59 (m, 2H, H-4), 3.52 (t, 1H, CH_2O, J_{HH} 8.7 Hz), 3.92 (s, 1H, CH_2O), 4.32 (m, 1H, H-5), 5.96 (s, 1H, H-5), 6.45 (d, 1H, H-2, J_{HF} 13.5 Hz), 7.45 (s, 1H, H-6). β-isomer (**21c**) δ 1.39 (rotamers, 9H, Boc), 2.59 (m, 2H, H-4), 3.67 (dd, 1H, CH_2O, J_{HH} 2.1 Hz, J_{HH} 11.4 Hz), 4.05 (tt, 1H, H-5, J_{HH} 2.6 Hz, J_{HH} 7.4), 4.26 (ddd, 1H, CH_2O, J_{HH} 2.3 Hz, J_{HH} 11.4, J_{HH} 11.6), 5.91 (d, 1H, H-5B, J_{HH} 7.5 Hz), 6.40 (d, H-2, J_{HF} 13.5 Hz), 8.29 (d, 1H, H-6B, J_{HH} 9.1 Hz). HRMS (ESI$^+$) Calcd for $C_{14}H_{20}F_2N_4O_4Na$ [M+Na]$^+$ 369.1345, found 369.1348.

5.17. General procedure for the synthesis of purine nucleosides (26–29)

Similar procedure as the described for the synthesis of nucleosides **18a–d**/**19a–d**. After glycosylation and solvents evaporation the residue was purified by column chromatography (20% AcOEt/hexane) to afford the silyl protected isomeric nucleosides **22–25**. Each separated 4′-azanucleoside was treated with 1.0 M solutions of TBAF in THF (1.5 equiv) to give pure **26–29** as white solids (75–82%).

5.18. 9-[(2*R*,5*S*)-*N*-*tert*-Butyloxycarbonyl-3,3-difluoro-5-(hydroxymethyl)pyrrolidin-2-yl]-6-chloropurine (26)

Six percentage of yield after glycosylation and 75% yield for TBDMS deprotection. R_f (10% MeOH/CH_2Cl_2): 0.52. ^1H NMR ($CDCl_3$, 400.13 MHz): δ 1.29 (9H, Boc), 2.64 (m, 1H, H-4), 2.99 (m, 1H, H-4), 3.84 (dd, 1H, CH_2O, J_{HH} 2.8 Hz, J_{HH} 11.6 Hz), 4.24 (t, 1H, H-5, J_{HH} 8.0 Hz), 4.46 (d, 1H, J_{HH} 11.6 Hz), 6.45 (d, 1H, H-2, J_{HF} 12.8 Hz), 8.77 (s, 1H, H-2B), 8.84 (s, 1H, H-8B). ^{13}C NMR ($CDCl_3$, 100.61 MHz): δ 27.9 (Boc), 33.5 (t, C-4, J_{CF} 23.2 Hz), 57.7 (C-5), 61.6 (CH_2O), 72.2 (m, C-2), 83.3 (Boc), 124.5 (t, C-3, J_{CF} 225.9 Hz), 131.71 (C-5B), 144.2 (C-8B), 151.4 (C-4B), 151.6 (C-6B), 152.1 (C-2B), 153.8 (C═O). HRMS (ESI$^+$) Calcd for $C_{15}H_{18}ClF_2N_5O_3Na$ [M+Na]$^+$ 412.0958, found 412.0969.

5.19. 9-[(2*S*,5*S*)-*N*-*tert*-Butyloxycarbonyl-3,3-difluoro-5-(hydroxymethyl)pyrrolidin-2-yl]-6-chloropurine (27)

Ninteen percentage of yield after glycosylation and 82% yield for TBDMS deprotection. R_f (10% MeOH/CH_2Cl_2): 0.52. mp: 64-66 °C. $[\alpha]_D^{20}$ −12 (*c* 0.5, CH_2Cl_2). UV λ_{max} (MeOH) 265 nm (6866 M^{-1} cm^{-1}). ^1H NMR ($CDCl_3$, 300.13 MHz): δ 1.22 (rotamers, 9H, Boc), 2.67 (m, 1H, H-4), 3.17 (m, 1H, H-4), 3.79 (dd, 1H, CH_2O, J_{HH} 5.7 Hz, J_{HH} 10.5 Hz), 4.05 (dd, 1H, CH_2O, J_{HH} 5.7 Hz, J_{HH} 11.1 Hz), 4.65 (s, 1H, H-4), 6.18 (d, 1H, H-2, J_{HF} 10.5 Hz), 8.15 (s, 1H, H-2), 8.77 (s, 1H, H-8). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 27.9 (Boc), 33.5 (t, C-4, J_{CF} 22.7 Hz), 58.9 (C-5), 64.5 (CH_2O), 72.5 (m, C-2), 83.2 (Boc), 124.6 (t, C-3, J_{CF} 254.4 Hz), 132.0 (C-5B), 144.4 (C-8B), 150.9 (C-4B), 151.7 (C-6B), 152.3 (C-2B), 153.2 (C═O). HRMS (ESI$^+$) Calcd for $C_{15}H_{18}ClF_2N_5O_3Na$ [M+Na]$^+$ 412.0958, found 412.0963.

5.20. 7-[(2*R*,5*S*)-*N*-*tert*-Butyloxycarbonyl-3,3-difluoro-5-(hydroxymethyl)pyrrolidin-2-yl]-6-chloropurine (28)

Eight percentage of yield after glycosylation and 81% yield for TBDMS deprotection. R_f (10% MeOH/CH_2Cl_2): 0.50. ^1H NMR ($CDCl_3$, 400.13 MHz): δ 1.31 (9H, Boc), 2.55 (td, 1H, H-4, *J* 7.2, *J* 15.2), 2.89 (m, 1H, H-4), 3.85 (dd, 1H, CH_2O, J_{HH} 2.0 Hz, J_{HH} 11.6 Hz), 4.16 (t, 1H, H-5, J_{HH} 6.2 Hz), 4.69 (d, 1H, J_{HH} 11.6 Hz), 6.88 (d, 1H, H-2, J_{HF} 12.0 Hz), 8.77 (s, 1H, H-2B), 8.84 (s, 1H, H-8B). ^{13}C NMR ($CDCl_3$, 100.61 MHz): δ 27.9 (Boc), 33.5 (t, C-4, J_{CF} 23.6 Hz), 57.4 (C-5), 60.2 (CH_2O), 72.2 (m, C-2), 83.3 (Boc), 122.5 (C-5B), 123.3 (t, C-3, J_{CF} 254.5 Hz), 143.1 (C-4B), 147.4 (C-8B), 152.7 (C-2B), 153.5 (C═O), 161.8 (C-6B). HRMS (ESI$^+$) Calcd for $C_{15}H_{18}ClF_2N_5O_3Na$ [M+Na]$^+$ 412.0958, found 412.0943.

5.21. 7-[(2*S*,5*S*)-*N*-*tert*-Butyloxycarbonyl-3,3-difluoro-5-(hydroxymethyl)pyrrolidin-2-yl]-6-chloropurine (29)

Forty-eight percentage of yield after glycosylation and 78% yield for TBDMS deprotection. R_f (10% MeOH/CH_2Cl_2): 0.47. mp: 76-78 °C. $[\alpha]_D^{20}$ +9 (*c* 0.5, CH_2Cl_2). UV λ_{max} (MeOH) 270 nm (6616 M^{-1} cm^{-1}). ^1H NMR ($CDCl_3$, 400.13 MHz): δ 1.23 (rotamers, 9H, Boc), 2.67 (m, 2H, H-4), 3.28 (br s, 1H, OH), 3.79 (m, 1H, CH_2O), 4.11 (m, 1H, CH_2O), 451 (s, 1H, H-4), 6.83 (d, 1H, H-2, J_{HF} 9.2 Hz), 8.30 (s, 1H, H-8), 8.91 (s, 1H, H-2). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 27.7 (Boc), 33.7 (t, C-4, J_{CF} 22.7 Hz), 57.6 (C-5), 63.0 (CH_2O), 72.4 (m, C-2), 83.7 (Boc), 122.7 (C-5B), 124.2 (t, C-3, J_{CF} 254.4 Hz), 143.2 (C-4B), 144.8 (C-8B), 152.5 (C═O), 152.8 (C-2B), 161.8 (C-6B). HRMS (ESI$^+$) Calcd for $C_{15}H_{18}ClF_2N_5O_3Na$ [M+Na]$^+$ 412.0958, found 412.0970.

5.22. General procedure for the synthesis of adenine nucleosides (30–33)

4′-Azanucleosides **26–29** (40 mg, 0.11 mmol) were treated with a saturated solution of ammonia in MeOH (3 mL) and stirred for 1 h at 100 °C in a sealed tube. The reaction is cooled to room temperature, MeOH evaporated and the residue purified by column chromatography (5–10% MeOH/CH_2Cl_2) to afford nucleosides **30–33** as white solids (72–75%).

5.23. 9-[(2*R*,5*S*)-*N*-*tert*-Butyloxycarbonyl-3,3-difluoro-5-(hydroxymethyl)pyrrolidin-2-yl]-adenine (30)

Seventy-two percentage of yield. R_f (10% MeOH/CH_2Cl_2): 0.31. ^1H NMR (THF-d_8, 400.13 MHz): δ 1.25 (s, 9H, Boc), 2.60 (m, 1H, H-4), 3.01 (m, 1H, H-4), 3.70 (d, 1H, CH_2O, J_{HH} 10.8 Hz), 4.07 (m, 1H, H-5), 4.28 (d, 1H, CH_2O, J_{HH} 10.4 Hz), 4.67 (br s, 1H, OH), 6.40 (d, 1H, H-2, J_{HF} 13.6 Hz), 6.47 (br s, 2H, NH2), 8.14 (s, 1H, H-2 or H-8), 8.39 (s, 1H, H-8 or H-2). ^{13}C NMR (THF-d_8, 100.61 MHz): δ 25.3 (Boc), 31.4 (t, C-4, J_{CF} 11.3 Hz), 57.7 (C-5), 58.9 (CH_2O), 69.0

(m, C-2), 79.2 (Boc), 117.7 (C-5B), 122.5 (t, C-3, J_{CF} 252.3 Hz), 136.8 (C-8B), 148.3 (C-4B), 150.8 (C-2), 151.7 (C-6B), 161.8 (C=O). HRMS (ESI$^+$) Calcd for $C_{15}H_{21}F_2N_6O_3$ [M+H]$^+$ 371.1638, found 371.1642.

5.24. 9-[(2*S*,5*S*)-*N*-*tert*-Butyloxycarbonyl-3,3-difluoro-5-(hydro xymethyl)pyrrolidin-2-yl]-adenine (31)

Seventy-two percentage of yield. R_f (10% MeOH/CH_2Cl_2): 0.29. mp: 105–107 °C. $[\alpha]_D^{20}$ −2 (*c* 0.5, MeOH). UV λ_{max} (MeOH) 260 nm (8975 M^{-1} cm^{-1}). ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ 1.16 (rotamers, 9H, Boc), 2.58 (t, 1H, H-4, J_{HH} 15.2 Hz), 3.15 (m, 1H, H-4), 3.75 (dd, 1H, CH_2O, J_{HH} 6.4 Hz, J_{HH} 10.9 Hz), 4.05 (dd, 1H, CH_2O, J_{HH} 5.2 Hz, J_{HH} 10.9 Hz), 4.63 (s, 1H, H-5), 6.07 (d, 1H, H-2, J_{HF} 9.6 Hz), 6.13 (s, 2H, NH_2), 7.81 (s, 1H, H-2 or H-8), 8.33 (s, 1H, H-8 or H-2). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 27.7 (Boc), 34.3 (t, C-4, J_{CF} 22.5 Hz), 58.5 (C-5), 63.9 (CH_2O), 71.8 (m, C-2), 82.6 (Boc), 119.5 (C-5B), 124.8 (t, C-3, J_{CF} 252.3 Hz), 139.4 (C-8B), 149.3 (C-4B), 153.3, 155.8 (C-2B + C-6B + C=O), 151.7 (C-6B), 161.8 (C=O). HRMS (ESI$^+$) Calcd for $C_{15}H_{21}F_2N_6O_3$ [M+H]$^+$ 371.1638, found 371.1628.

5.25. 7-[(2*R*,5*S*)-*N*-*tert*-Butyloxycarbonyl-3,3-difluoro-5-(hydro xymethyl)pyrrolidin-2-yl]-adenine (32)

Seventy-five percentage of yield. R_f (10% MeOH/CH_2Cl_2): 0.29. mp: 225–227 °C. ^{1}H NMR (THF-d_8, 300.13 MHz): δ 1.29 (s, 9H, Boc), 2.68 (m, 2H, H-4), 3.63 (d, 1H, CH_2O, J_{HH} 11.1 Hz), 4.06 (m, 2H, CH_2O + H-5), 5.38 (t, 1H, OH, J_{HH} 9.6 Hz), 6.75 (d, 1H, H-2, J_{HF} 10.5 Hz), 6.87 (s, 2H, NH_2), 8.24 (s, 1H, H-2 or H-8), 8.86 (s, 1H, H-8 or H-2). ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 28.0 (Boc), 31.4 (t, C-4, J_{CF} 22.3 Hz), 57.4 (C-5), 60.2 (CH_2O), 73.7 (m, C-2), 81.9 (Boc), 111.4 (C-5B), 124.6 (t, C-3, J_{CF} 252.9 Hz), 143.5 (C-8B), 151.6 (C-4B), 152.9 (C-2B), 153.8, 160.4 (C-6B + C=O). HRMS (ESI$^+$) Calcd for $C_{15}H_{21}F_2N_6O_3$ [M+H]$^+$ 371.1638, found 371.1648.

5.26. 7-[(2*S*,5*S*)-*N*-*tert*-Butyloxycarbonyl-3,3-difluoro-5-(hydroxymethyl)pyrrolidin-2-yl]-adenine (33)

Seventy-three percentage of yield. R_f (10% MeOH/CH_2Cl_2): 0.27. mp: 206–208 °C. $[\alpha]_D^{20}$ +29 (c 0.5, MeOH). UV λ_{max} (MeOH) 275 nm (4644 M^{-1} cm^{-1}). ^{1}H NMR (MeOH-d_4, 300.13 MHz) δ 1.56 (rotamers, 9H, Boc), 2.80 (m, 2H, H-4), 3.61 (t, 1H, CH_2O, J_{HH} 9.9 Hz), 4.03 (d, 1H, CH_2O, J_{HH} 9.0 Hz), 4.52 (s, 1H, H-5), 6.68 (d, 1H, H-2, J_{HF} 9.3 Hz), 8.33 (s, 1H, H-2 or H-8), 8.54 (s, 1H, H-8 or H-2). ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 26.6 (Boc), 33.9 (m, C-4), 56.9 (C-5), 60.3 (CH_2O), 72.1 (m, C-2), 82.1 (Boc), 111.4 (C-5B), 124.6 (t, C-3, J_{CF} 256.7 Hz), 142.6 (C-8B), 151.8, 152.1 (C-4B or C=O or C-6B), 152.6 (C-2B), 158.9 (C-4B or C=O or C-6B). HRMS (ESI$^+$) Calcd for $C_{15}H_{21}F_2N_6O_3$ [M+H]$^+$ 371.1638, found 371.1628.

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Supplementary data

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