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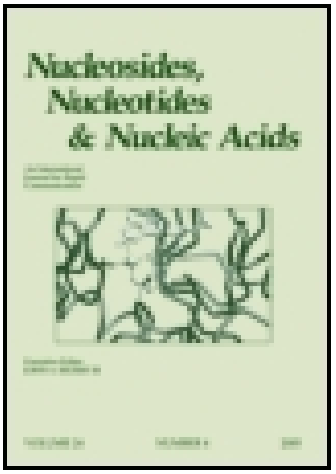

Nucleosides, Nucleotides and Nucleic Acids

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A simple and efficient synthesis of puromycin, 2,2′-anhydro-pyrimidine nucleosides, cytidines and 2′,3′-anhydroadenosine from 3′,5′-O-sulfinyl Xylo-nucleosides

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A SIMPLE AND EFFICIENT SYNTHESIS OF PUROMYCIN, 2,2′-ANHYDRO-PYRIMIDINE NUCLEOSIDES, CYTIDINES AND 2′,3′-ANHYDROADENOSINE FROM 3′,5′-*O*-SULFINYL *XYLO*-NUCLEOSIDES

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□ *Synthesis of antibiotics, puromycin and 3′-amino-3′-deoxy-N^6,N^6-dimethyladenosine* ***11*** *was achieved by utilizing the cyclic sulfite* ***6a*** *of the* xylo-*3′,5′-dihydroxy group as a new protective group. The key synthetic step is the deprotection of the sulfite moiety through the intramolecular cyclization of 2-α-carbamate* ***7****. In a similar manner, 2,2′-anhydro-pyrimidine nucleosides* ***15****,* ribo-*cytidines* ***17*** *and 2′,3′-anhydroadenosine* ***14*** *were prepared in high yields from the corresponding sulfites* ***4****,* ***5****, and* ***6b****, respectively.*

Keywords Puromycin; 2,2′-Anhydro-pyrimidine Nucleoside; 3′,5′-*O*-sulfinyl *xylo*-nucleosides

INTRODUCTION

There has been considerable interest in nucleosides modified on the sugar moiety as potential antiviral, anticancer, and biosynthetic inhibitor agents; therefore, a variety of new effective protective groups for hydroxyl groups have been devised to design nucleoside analogue.[1] In the

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preliminary report, we exploited a new protective group, the cyclic sulfite, which was not only an efficient protective group for 3′,5′-*O*-xylohydroxy groups, but also a potent leaving group. The deprotection itself was the key synthetic step of our nucleoside synthesis. The cyclic sulfite protective group was eliminated either by an intramolecular nucleophilic attack with 2′-carbamoyl group (synthesis of **8**) or with hydroxy group (synthesis of **15**). In this article we demonstrate the effectiveness of this protecting group in the syntheses of the puromycin and 2,2′-anhydro pyrimidines **15**.

The family of 3′-amino-3′-deoxyadenosines is known to possess strong antibacterial, anticancer, and biosynthetic inhibitory properties.[2] One of the most important members of the family is puromycin, a metabolite of *Streptomyces alboniger*, which was first isolated by Porter and coworkers in 1952.[3] Puromycin has been used extensively for elucidation of the mechanism of protein biosynthesis.[2] It has close structural similarity to the aminoacyl end of aminoacyl-tRNA.[4] Consequently, it is a strong acceptor for the peptidyl tRNA site of the ribosome. Puromycin inhibits the growth of Gram-positive bacteria and various animal and insect cells. In addition, the nucleoside moiety of puromycin, 3′-amino-3′-deoxy-N^6,N^6-dimethyladenosine **11**, showed activity against *Trypanosoma equiperdum*.[5] Even though a number of synthetic routes to puromycin and its analogues have been reported, most of them have problems owing to numerous synthetic steps and low overall yields.[6] Recently, Robins[6a] and Strazewski[6c] reported the synthesis of puromycin and its analogues via 3′-amino-3′-deoxyadenosine starting from D-*ribo*-adenosine. However, their syntheses still had problems in 3′-regioselectivity[6c] and a usage of a pyrophoric reagent, bromodimethylborane,[6a] and an inaccessible reagent, *N*,*N*′-*bis*[(dimethylamino)methylen]hydrazine (BDMAMH) dihydrochloride.[6a,7] In this article, we have challenged to solve these critical problems and developed a novel synthesis of puromycin using 3′,5′-*O*-sulfinylxyloadenosine with a reasonably safe procedure.

In our previous paper, we communicated the efficacy of this protective group, 3′,5′-*O*-sulfinyl, in the synthesis of xylo-nucleosides, 2,2′-anhydro-pyrimidine nucleosides and a puromycin intermediate (3′-amino-3′-deoxyadenosine).[8] However, in the puromycin synthesis via 3′-amino-3′-deoxyadenosine, it is inevitable to use (BDMAMH)dihydrochloride, a N^6-methylation[6a,7] reagent rather difficult to obtain. Therefore, we studied a new short-step synthesis of puromycin and **11** from easily available N^6,N^6-dimethyladenosine **3a**. Herein, we report on the details of their synthesis and the synthesis of **15** and **14** by applying a novel and efficient *ribo*-stereoselective and 3′-regioselective rearrangement of 3′,5′-*O*-sulfinyl *xylo*-nucleosides **4**, **5**, **6**, and **7**, respectively, prepared from D-xylose. Also, we presented a new type of stereospecific hydroxylation via 2,2′-anhydrocytidines 16 resulting in a formation of *ribo*-cytidines **17**.

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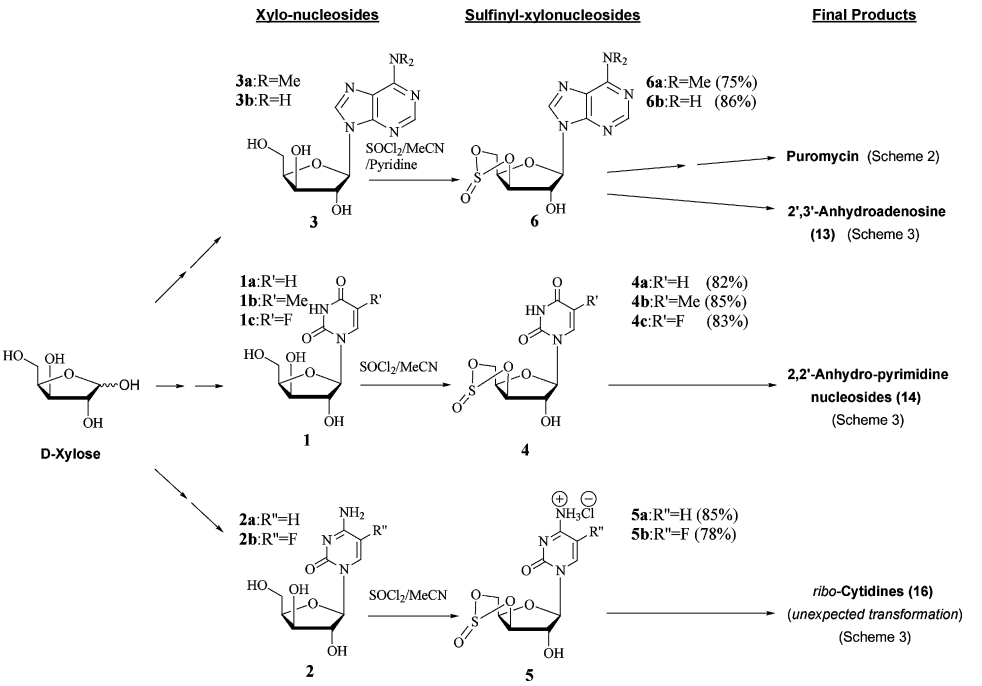
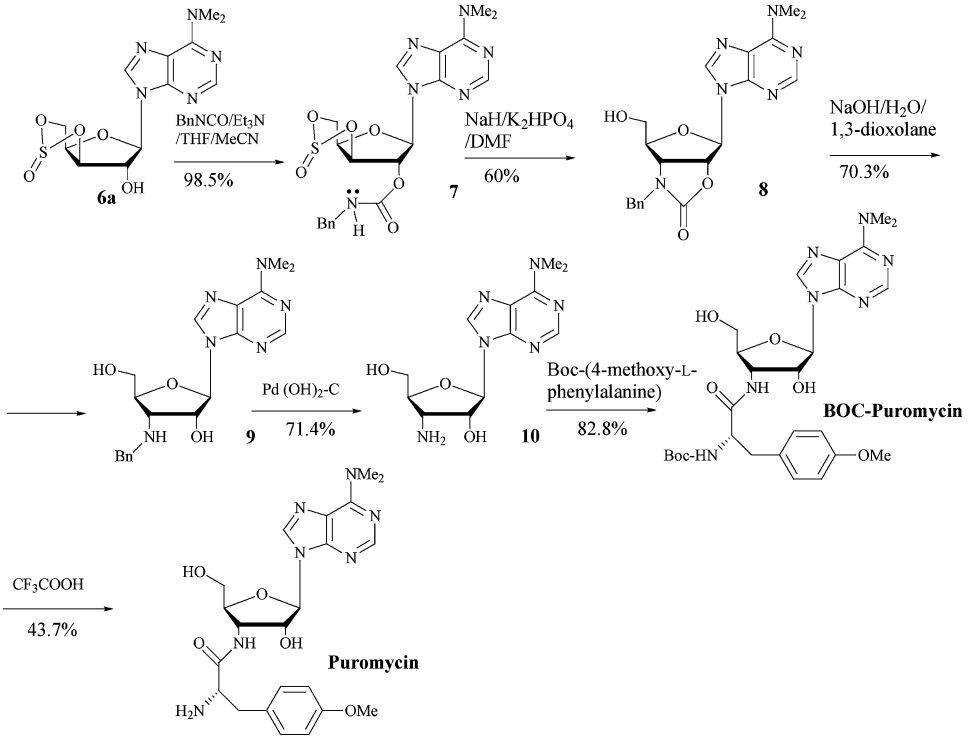
RESULTS AND DISCUSSION

*Synthesis of 3′,5′-O-Sulfinyl Xylonucleosides (**4**, **5**, and **6**) and Property of the 3′,5′-O-Sulfinyl Group.* β-D-Xylofuranosyl nucleosides **1**, **2**, and **3** were prepared from commercially available inexpensive D-xylose according to the literature.[9,10] N^6,N^6-Dimethyl-xyloadenosine **3a** was obtained from *N*,*N*-dimethyladenine[11] and xylofuranoside. The synthesis of 3′,5′-*O*-sulfinyl xylo-nucleosides **4**, **5**, and **6** is shown in Scheme 1. Reactions of **1**, **2**, and **3** with thionyl chloride in dry acetonitrile afforded crystalline 3′,5′-*O*-cyclosulfinyl derivatives **4**, **5**, and **6** in 77.9–85.3% yields[12] without chromatographic purification. Their infrared spectra showed the sharp bands corresponding to the $\nu_{S=O}$ of the alkylated sulfinate ester at 1180–1257 cm^{-1}. Their structures were determined based on NMR and FAB-MS spectral analyses. The 3′,5′-*O*-sulfinyl group can be a good leaving group with an assistance of a nucleophilic attack by the 2′-carbamoyl or 2′-hydroxyl group from the α-side of 3′-position affording 3′-substituted *ribo*-nucleosides in a stereo-and regioselective way.

Synthesis of 3′-amino-3′-deoxy-N^6,N^6*-Imethyladenosine (11) and Puromycin.* The synthesis of **11** and puromycin is shown in Scheme 2. Treatment of the cylosulfinyl xyloadenosine **6a** with benzylisocyanate at room temperature gave the carbamate **7**, keeping the sulfinyl group and the amino group intact. Subsequent treatment of **7** with NaH in dry DMF at –30°C for 20 h gave 3′-cyclization product **8**[6a] in 60% yield after silica gel column





chromatography with epoxide **9** similar to **13** as a minor product (less than 10% yield) detected by thin-layer chromatography. The epoxide **9** would be formed via **6a** possibly derived from the hydrolysis of **7**. This minor product was also reported by Robins.[6] Following hydrolytic decabonylation ($NaOH/H_2O$/1,3-dioxolane) of **8** gave 3′-benzylamine derivative **10** (70.3%). Hydrogenolysis of **10**[6a] with $Pd(OH)_2$-C (Pearlman's catalyst) as a catalyst afforded **11** (71.4%), a key intermediate for puromycin. Completely different results were reported for this hydrogenolysis in literature. Robins[6a] reported that the reaction was highly efficient (reaction time: 1.5 h), whereas Strazewski[6c] commented that it was an inefficient and problematic procedure. In our hands, the hydrogenolysis was completed in 16.0 h with the yield mentioned above. Finally, puromycin was prepared from **11** according to the literature.[6a] Condensation of BOC-(4-methoxy-L-phenyalanine) with **11** gave the protected aminoacyl-aminonucleoside. The removal of BOC from the aminonucleoside with trifluoroacetic acid completed our practical puromycin synthesis.

*Synthesis of 2,2′-Anhydro-Pyrimidine Nucleosides (**15**) and Their Transformation into* ribo*-cytidines (**17**).* The synthetic route to 2,2′-Anhydro-Pyrimidine nucleosides **15** is outlined in Scheme 3. Treatment of cyclosulfinyluridines **4a**, **4c** and cyclosulfinylthymidine **4b** with sodium hydrogencarbonate in dry DMF at 90°C for 5 h gave 2,2′-anhydronucleosides **15a**, **15c**, and **15b**[13]

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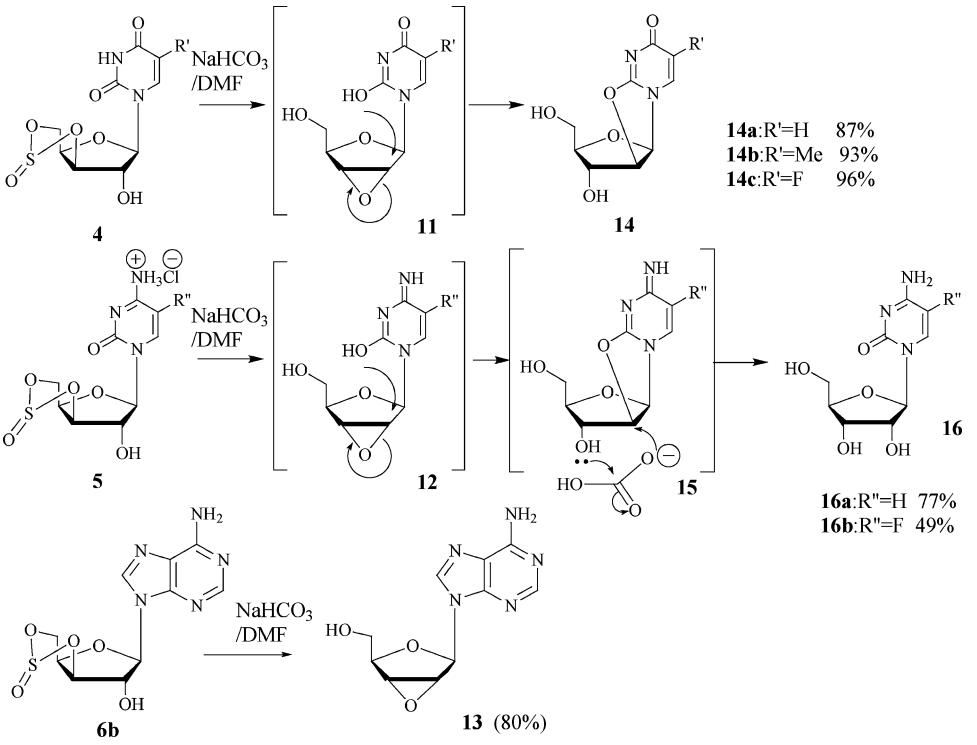

in **87**.3–95.9% yields, respectively. The reaction proceeded in a high yield irrespective of the electronic nature of substituents at the 5-position.

Cyclosulfinylcytidines **5a** and **5b**, obtained by the same procedure as **4**, were stable at room temperature. Surprisingly, they were transformed into *ribo*-cytidines **17a** and **17b** in 76.5 and 49.2% yields, respectively. In contrast to the synthesis of **15**, neither the formation of 2,2′-anhydrocytidines nor arabino-cytidines was observed. In order to confirm the reaction mechanisms, we carried out the reaction using an independently prepared **16a**. The reaction of **16a** under the same reaction conditions as for the preparation of **17a** directly from 5 afforded **17a** in 80.9% yield. Consequently, the results support the assumption that the reactions proceed via 2,2′-anhydrocytidines **16**. It is well known that the hydrolysis of **16** gives arabinocytidine (ara-C).[12a,14] Similar to this, cytidines were probably formed via the intermediate **16** incorporated with a carbonate in dry DMF as shown in Scheme 3.

*Synthesis of 2′,3′-Anhydroadenosine (**14**).* The base-catalyzed reaction of **6b** afforded **14** in a yield of 83.0% (Scheme 3). The 2′,3′-oxirane structure is evidently prepared by the substitution of the cyclosulfinyl group at the 3′-position with the 2′-hydroxy group from the α-side. The results also indicate the presence of the 2′,3′-oxirane intermediates **12** and **13** derived from 3′,5′-sulfinyl pyrimidine nucleosides **4** and **5**, respectively.

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CONCLUSION

In conclusion, we have established an efficient synthesis of puromycin, 3′-amino-3′-deoxy-N^6,N^6-dimethyladenosine, 2,2′-anhydro-pyrimidine nucleosides, and 2′,3′-anhydroadenosine from 3′,5′-*O*-sulfinyl *xylo*-nucleosides in a novel *ribo*-stereoselective and 3′-regioselective manner.

EXPERIMENTAL SECTION

Materials

Reagents (extra pure grade) except the mentioned below were purchased from Wako Pure Chemical Industries, Ltd., and were used without purification. Thionyl chloride was purchased from Tokyo Kasei Kogyo Co., Ltd. 2,2′-anhydrouridine (authentic sample of **15a**), cytidinew (authentic sample of **17a**), 2,2′-anhydrocytidine hydrochloride (authentic sample of **16**), BOC-4(methoxy-L-phenylalanine), $Pd(OH)_2$-C (Pearlman's catalyst), and DCC were purchased from Sigma-Aldrich Co. β-D-Xylofuranosyl nucleosides (**1a-c**, **2a**,**b**, and **3a**,**b**) were synthesized from commercially available D-xylose via 2,3,5-tri-*O*-benzoyl-β-D-xylofuranosyl-nucleosides according to the literature.[10]

General Methods

Melting points were determined with Yamato Melting Point Apparatus Model MP-21. ^{1}H-NMR(400 MHz) and ^{13}C-NMR(126.5 MHz) spectra were recorded on JEOL LA 400 and GSX500 spectrometers, respectively. DMSO-d^6 or D_2O was used as solvent and Me_4Si or 3-(trimethylsilyl)propionic-*2,2,3,3-d*^{4} acid sodium salt (TSP) as an internal standard. Chemical shifts are reported as δ, ppm. IR spectra were recorded on a JSACO FT/IR-410 spectrometer as a KBr-pellet. High-resolution mass spectra were performed on a JEOL JMX-HX110. Column chromatography was performed on silica gel (230–400 mesh). Thin-layer chromatography (TLC) was performed on silica gel (Merck, TLC grade 7749 with gypsum binder).

Synthesis of β-D-Xylofuranosyl Nucleosides[15]

9-(2,3,5-Tri-O-benzoyl-β-D-xylofuranosyl)-N^6,N^6*-dimethyladenine, Which, was Used to Synthesize (**3a**).* Oil; ν_{max}(KBr)/cm^{-1} 1726, 1598, 1264 and 1094; ^{1}H NMR (DMSO-d^6; Me_4Si) δ_H 3.39(6H, m, N–Me_2), 4.77 (2H, m, 5′-H), 5.06 (1H, pseudo-quartet, J = 4.9 Hz, 4′-H), 6.05 (1H, d, J = 4.9 Hz, 1′-H), 6.47 (2H, br s, 2′-H and 1′-H), 7.69–7.45 (9H, m, ArH), 8.08–7.94 (7H, m, 8-H and ArH), 8.39 (1H, d, J = 3.1 Hz, 2-H); ^{13}C NMR (DMSO-d^6; Me_4Si) δ_C 61.9, 75.64, 77.73, 78.48, 78.75, 79.01, 86.9, 119.5, 128.36, 128.43, 128.47, 128.52, 128.60, 128.63, 128.96, 129.01,

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129.13, 129.20, 129.27, 129.33, 129.56, 133.2, 133.5, 133.7, 149.9, 151.8, 154.2, 134.3, 134.5, 135.2; *m/z* (EI) 608([M+H]$^+$, 21%), 445(9), 201(10), 134(9), 105(100).

*1-β-*D*-Xylofuranosyluracil (**1a**).* mp 153°C; ^{1}H NMR (DMSO-*d*^{6}; Me_4Si) δ_H 3.66 (1H, m), 3.73N (1H, m), 3.94 (2H, d, J = 20.8 Hz, 2-H), 4.09 (1H, dd, J= 9.0 and 5.7 Hz), 4.72 (1H, s, OH), 5.40 (1H, d, J = 3.4 Hz, OH), 5.61 (1H, d, J = 7.9 Hz, 5-H), 5.66 (1H, s, OH), 5.73 (1H, d, J = 4.0 Hz, 1′-H), 7.76 (1H, d, J = 8.2Hz, 6-H), 11.25 (1H, br s, NH); ^{13}C NMR (DMSO-*d*^{6}; Me_4Si) δ_C 59.0, 74.5, 80.6, 83.7, 90.8, 100.8, 141.3, 150.5, 133.2; *m/z* (FAB) 245.0760 ([M+H]$^+$, $C_9H_{13}N_2O_6$ requires *m/z*: 245.0774).

*1-β-*D*-Xylofuranosylthymine (**1b**).* mp 158°C; ^{1}H NMR (DMSO-*d*^{6}; Me_4Si) δ_H 1.76 (3H, d, J = 0.6 Hz, Me), 3.71 (2H, m), 3.96 (2H, d, J = 14.3 Hz), 4.07 (1H, m), 4.74 (1H, br s, OH), 5.39 (1H, d, J = 2.4 Hz, OH), 5.68 (1H, d, J = 1.8 Hz, OH), 5.72 (1H, d, J = 4.3 Hz, 1′-H), 7.66 (1H, d, J = 0.9 Hz, 6-H), 11.28 (1H, br s, NH); ^{13}C NMR (DMSO-*d*^{6}; Me_4Si) δ_C 12.4, 59.2, 74.8, 80.6, 83.2, 90.4, 108.4, 137.1, 150.6, 133.8; *m/z* (FAB) 259.0938 ([M+H]$^+$, $C_{10}H_{15}N_2O_6$ requires *m/z*: 259.0930).

*1-(β-*D*-Xylofuranosyl)-5-fluorouracil (**1c**).* Oil; ^{1}H NMR (DMSO-*d*^{6}; Me_4Si) δ_H 3.71 (2H, m), 3.93 (1H, br s), 3.99 (1H, br s), 4.09 (1H, m), 4.78 (1H, br s, OH), 5.50 (1H, br s, OH), 5.65 (1H, t, J 1.4 Hz, OH), 5.77 (1H, br s, 1′-H), 8.00 (1H, d, J = 7.6 Hz, 6-H), 1135 (1H, br s, NH); ^{13}C NMR (DMSO-*d*^{6}; Me_4Si) δ_C 59.0, 74.4, 80.4, 83.8, 90.9, 125.6 (d, J_{cp} = 35.2Hz), 139.4 (d, J_{cp} = 229.7 Hz), 149.0, 157.0 (d, J_{cp} = 25.9 Hz); *m/z* (FAB) 263.0702 ([M+H]$^+$, $C_9H_{12}N_2O_6F$ requires *m/z*: 263.0679).

*1-β-*D*-Xylofuranosylcytosine (**2a**).* mp 229°C; ^{1}H NMR (DMSO-*d*^{6}; Me_4Si) δ_H 3.65 (1H, quintet, J = 5.7 Hz), 3.73(1H, quintet, J = 5.6 Hz), 3.87 (1H, t, J= 3.5 Hz), 3.90 (1H, d, J = 4.0 Hz), 4.08 (1H, m), 4.69 (1H, t, J= 5.6 Hz, OH), 5.28 (1H, d, J = 3.7 Hz, OH), 5.63 (2H, m, 1′-H and OH), 5.68 (1H, d, J= 7.3 Hz, 5-H), 7.00 (1H, br s, NH), 7.10 (1H, br s, NH), 7.70 (1H, d, J = 7.3 Hz, 6-H); ^{13}C NMR (DMSO-*d*^{6}; Me_4Si) δ_C 59.2, 74.9, 80.8, 83.5, 92.1, 93.0, 142.1, 155.3, 135.7; *m/z* (FAB) 244.0946 ([M+H]$^+$, $C_9H_{14}N_3O_5$ requires *m/z*: 244.0933).

*1-(β-*D*-Xylofuranosyl)-5-fluorocytosine (**2b**).* mp 219°C; ^{1}H NMR (DMSO-*d*^{6}; Me_4Si) δ_H 3.68 (1H, quintet, J = 5.5 Hz), 3.74 (1H, quintet, J = 5.5 Hz), 3.89 (1H, br s), 3.92 (1H, br s), 4.09 (1H, m), 4.76 (1H, t, J = 5.5 Hz, OH), 5.37 (1H, br s), 5.62 (1H, s, OH), 5.70 (1H, d, J = 3.7 Hz, 1′-H), 7.47 (1H, br s, NH), 7.68 (1H, br s, NH), 7.85 (1H, d, J = 7.3 Hz, 6-H); ^{13}C NMR (DMSO-*d*^{6}; Me_4Si) δ_C 59.2, 74.8, 80.6, 83.7, 91.9, 126.4 (d, J_{cp} = 33.1Hz), 135.5 (d, J_{cp} = 240 Hz), 153.4, 157.3 (d, J_{cp} = 13.5 Hz); *m/z* (FAB) 262.0847 ([M+H]$^+$, $C_9H_{13}N_3O_5F$ requires *m/z*: 262.0839).

*9-(β-*D*-Xylofuranosyl)-*N^6,N^6*-dimethyladenine (**3a**).* Oil; ^{1}H NMR (DMSO-*d*^{6}; Me_4Si) δ_H 3.50 (6H, m, N–Me_2), 3.70 (1H, m, 5′-H), 3.82 (1H, m, 5″H), 4.09 (1H, m, 1′-H), 4.21 (1H, m, 4′-H), 4.34 (1H, s, 2′-H), 4.85 (1H, br s, 5′-OH), 5.94 (3H, br s, 1′-H, 2′-OH, and 3′-OH), 8.23 (1H, s, 8-H), 8.29 (1H,

s, 2-H); ^{13}C NMR (DMSO-d^6; Me_4Si) δ_C 39.0, 59.4, 75.3, 80.9, 83.5, 89.7, 119.4, 138.5, 149.4, 151.5, 154.2; *m/z* (EI) 296([M+H]$^+$, 84%), 206(12), 192(13), 134(100), 157(57).

*9-β-*D*-Xylofuranosyladenine (**3b**).* mp 152°C; (lit.[16] mp 154–156°C) ^{1}H NMR (DMSO-d^6; Me_4Si) δ_H 3.69 (1H, dd, J = 113 and 6.1 Hz, 5′-H), 3.79 (1H, dd, J = 11.5 and 4.7 Hz, 5″-H), 4.07 (1H, br s, 1′-H), 4.18 (1H, m, 4′-H), 4.35 (1H, s, 2′-H), 4.81 (1H, br s, OH), 5.88 (2H, br s, OH), 5.90 (1H, d, J = 1.8 Hz, 1′-H), 7.35 (2H, br s, NH_2), 8.18 (1H, s, 8-H), 8.29 (1H, s, 2-H); *m/z* (EI) 268([M+H]$^+$, 100%), 89 (50), 77 (40); (lit.[16] ^{1}H NMR (DMSO-d^6; Me_4Si) δ_H 3.64 (1H, dd, J = 12.0 and 5.5 Hz, 5′-H), 3.82 (1H, dd, J = 12.0 and 5.5 Hz, 5″-H), 4.06 (1H, dd, J = 3.5 and 1.5 Hz, 1′-H), 4.20 (1H, m, 4′-H), 4.34 (1H, m, 2′-H), 4.73 (1H, t, J = 5.5 Hz, 5′-OH), 5.9 (2H, br s, 2′-OH and 3′-OH), 5.90 (1H, d, J = 2.0 Hz, 1′-H), 7.34 (2H, br s, NH_2), 8.13 (1H, s, 8-H), 8.28 (1H, s, 2-H).

*Synthesis of 3′,5′-*O*-Sulfinyl Xylo-Nucleosides*

*1-(3,5-*O*-Sulfinyl-β-*D*-xylofuranosyl)uracil (**4a**).* To a solution of thionyl chloride (7.80 g, 65.6 mmol) in acetonitrile (41 mL) was added 1-(β-D-xylofuranosyl)uracil **1a** (4.00 g, 13.4 mmol) with vigorous stirring, and then the temperature of the reaction mixture was maintained at 10°C. After stirring for an additional 3 h, the mixture was poured into a suspension of sodium bicarbonate (22.0 g) in water (100 mL) and extracted with ethyl acetate (2 × 200 mL). The combined extracts were dried over anhydrous magnesium sulfate and evaporated to dryness in vacuum. Recrystallization from 4-methyl-2-pentanone gave 3.90 g (82.0% yield) of **4a** as white crystals; mp 185°C; ν_{max}(KBr)/cm^{-1} 3271, 3139, 3043, 1371, 1471, 1415, 1273, 1210 (RO-SO-OR), 1191, 1113, 1089, 1010, 833, 768, and 715; ^{1}H NMR (DMSO-d^6; Me_4Si) δ_H 4.21 (1H, d, J = 4.3 Hz), 4.34 (1H, d, J = 13.4 Hz), 4.39 (1H, br s), 4.71 (1H, d, J = 2.4 Hz), 4.91 (1H, dd, J = 13.4 and 1.8 Hz), 5.71 (1H, dd, J = 8.2 and 1.5 Hz, 1H), 5.73 (1H, s, 2′-OH), 6.27 (1H, d, J = 4.3 Hz, 1′-H), 7.61(1H, d, J = 8.2Hz, 6-H), 11.41 (1H, s, NH); ^{13}C NMR (DMSO-d^6; Me_4Si) δ_C 55.8, 70.5, 73.1, 78.7, 91.0, 101.5, 139.4, 150.4, 133.1; *m/z* (FAB) 291.0295 ([M+H]$^+$, $C_9H_{11}N_2O_7S$ requires *m/z*: 291.0287).

*1-(3,5-*O*-Sulfinyl-β-*D*-xylofuranosyl)thymine (**4b**).* Similar to the synthesis of **4a**, **4b** was obtained from the reaction of **1b** (4.23 g, 13.4 mmol) and thionyl chloride (7.80 g, 65.6 mmol) in acetonitrile (41 mL). Recrystallization from 4-methyl-2-pentanone gave 4.19 g (84.6% yield) of **4b** as white crystals; mp 176°C; ν_{max}(KBr)/cm^{-1} 3245, 3087, 2962, 1700, 1470, 1401, 1268, 1205 (RO-SO-OR), 1104, 997, 839, and 795; ^{1}H NMR ($CDCl_3$; Me_4Si) δ_H 1.94 (3H, d, J = 0.9 Hz); 4.33 (1H, d, J = 13.1 Hz), 4.45 (1H, d, J = 2.2 Hz), 4.51 (1H, d, J = 1.8 Hz), 4.95 (1H, d, J = 2.4 Hz), 5.09 (1H, dd, J = 13.1 and 13 Hz), 5.81 (1H, s, 2′-OH), 5.96 (1H, d, J = 3.4 Hz, 1′-H), 7.76 (1H, d, J = 1.2Hz, 6-H), 10.67 (1H, s, NH); ^{13}C NMR ($CDCl_3$; Me_4Si) δ_C12.6,

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55.7, 69.8, 75.2, 79.7, 93.2, 110.2, 135.7, 150.8, 134.8; *m/z* (FAB) 305.0437 ($[M+H]^+$, $C_{10}H_{13}N_2O_7S$ requires *m/z*: 305.0443).

*1-(3,5-O-Sulfinyl-β-D-xylofuranosyl)-5-fluorouracil (**4c**).* In a similar manner of the synthesis for **4a**, **4c** was obtained from the reaction of **1c** (4.30 g, 13.4 mmol) and thionyl chloride (7.80 g, 65.6 mmol) in acetonitrile (41 mL). Recrystallization from 4-methyl-2-pentanone gave 4.19 g (82.9% yield) of **4c** as white crystals; mp 215°C; ν_{max}(KBr)/cm^{-1} 3432, 3088, 1712, 1468, 1380, 1332, 1257 (RO-SO-OR), 1155, 1096, 1069, 1038, 1006, 887, 840, and 714; ^{1}H NMR (DMSO-d^6; Me_4Si) δ_H 4.27 (1H, d, J = 3.7 Hz), 4.39 (1H, d, J = 13.7 Hz), 4.42 (1H, m), 4.71 (1H, d, J = 2.5 Hz), 4.92 (1H, dd, J = 13.1 and 1.8 Hz), 5.68 (1H, s, 2′-OH), 6.32 (1H, d, J = 4.3 Hz, 1′-H), 7.74 N(1H, d, J = 7.3 Hz, 6-H), 11.97 (1H, s, NH); ^{13}C NMR (DMSO-d^6; Me_4Si) δ_C 55.8, 70.3, 73.5, 78.4, 91.1, 123.6 N(d J_{cp} = 35.2Hz), 139.7 (d J_{cp} = 230.7 Hz), 148.8, 156.9(d, J_{cp} = 26.9 Hz); *m/z* (FAB) 309.0191 ($[M+H]^+$, $C_9H_{10}N_2O_7FS$ requires *m/z*: 309.0193).

*1-(3,5-O-Sulfinyl-β-D-xylofuranosyl)cytosine Hydrochloride (**5a**).* To a solution of thionyl chloride (7.80 g, 65.6 mmol) in acetonitrile (41 mL) and 1,3-dioxolane (20 mL) was added 1-(β-D-xylofuranosyl)cytosine **2a** (3.99 g, 13.4 mmol) with vigorous stirring, and then the temperature of the reaction mixture was maintained at 25°C. After stirring for an additional 5 h, the solid precipitated was collected by filtration, washed twice with acetonitrile, and dried to give 4.56 g (85.3% yield based on **2a**) of **5a** as white crystals, which contained some amount of free base; mp 178°C; ν_{max}(KBr)/cm^{-1} 3377, 1731, 1377, 1543, 1399, 1331, 1275, 1186 (RO-SO-OR), 1119, 1036, 1007, 956, 904, 881, 853, 834, 788, 762, and 731; ^{1}H NMR (D_2O; TSP) δ_H 4.01–5.09 (5H + HDO, m), 5.80 (0.14H, s, 1′-H), 5.82 (0.86H, s, 1′-H), 6.21 (0.14H, pseudo-d, J = 8.0 Hz, 5-H), 6.26 (0.86H, dd, J = 8.0 and 1.8 Hz, 5-H), 8.10 (0.14H, pseudo-d, J = 7.9 Hz, 6-H), 8.13 (0.86H, dd, J = 7.9 and 1.5 Hz, 6-H); ^{13}C NMR (D_2O; TSP) δ_C 56.8, 60.5, 70.8, 75.2, 76.1, 79.5, 81.0, 85.5, 93.1, 93.3, 94.9, 95.2, 144.5, 145.6, 149.0, 149.1, 130.1; *m/z* (FAB) 290.0475 ($[M+H-HCl]^+$, $C_9H_{12}N_3O_6S$ requires *m/z*: 290.0447). The compound was pure enough for the next step.

*1-(3,5-O-Sulfinyl-β-D-xylofuranosyl)-5-fluorocytosine Hydrochloride (**5b**).* Similar to the synthesis of **5a**, **5b** was obtained as white crystals from the reaction of **2b** (4.28 g, 13.4 mmol) and thionyl chloride (7.80 g, 65.6 mmol) in acetonitrile (41 mL) and 1,3-dioxolane (20 mL); yield: 4.39 g (77.9% based on **2b**). The product contained some amount of free base; mp 172°C ν_{max}(KBr)/cm^{-1} 3311, 2767, 1731, 1385, 1344, 1558, 1402, 1350, 1276, 1189 (RO-SO-OR), 1114, 1084, 1057, 1032, 1004, 959, 930, 913, 893, 878, 852, 834, 795, 778, and 742; ^{1}H NMR (D_2O; TSP) δ_H 4.02–5.10 (5H + HDO, m), 5.78 (1H, br s, 1-H), 8.10 (0.24H, d, J = 6.4 Hz, 6-H), 8.28 (0.76H, d, J = 6.2Hz, 6-H); ^{13}C NMR (D_2O; TSP) δ_C 56.8, 60.5, 70.7, 75.2, 76.3, 79.5, 81.1, 85.7, 93.3, 93.5, 128.7, 129.0, 129.9, 130.2, 135.8, 137.7, 148.8, 149.1,

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154.8; *m/z* (FAB) 308.0361 ([M+H − HCl]$^+$, $C_9H_{11}N_3O_6FS$ requires *m/z*: 308.0353). The compound was pure enough for the next step.

*9-(3,5-*O*-Sulfinyl-β-*D*-xylofuranosyl)-*N^6,N^6*-dimethyladenine (**6a**).* Thionyl chloride (3.57 g, 30.0 mmol) was added to **3a** (2.95 g, 10.0 mmol) in acetonitrile (10.0 mL) and pyridine (2.37 g, 30.0 mmol), and the resulting solution was stirred for 4 h at 25°C. After stirring for an additional 4 h, the mixture was poured into a suspension of sodium bicarbonate (10.1 g) in water (60 mL) and extracted with ethyl acetate (2 × 50 mL). The combined extracts were dried over magnesium sulfate and evaporated to dryness in vacuum. Recrystallization from (4-methyl-2-pentanone/diisopropyl ether) gave 2.56 g (75.0% yield) of **6a** as white crystals; mp 180°C; ν_{max}(KBr)/cm^{-1} 3140, 2952, 1308, 1482, 1349, 1299, 1227, 1181 (RO-SO-OR), 1082, 998, and 836; ^{1}H NMR (DMSO-*d*^{6}; Me_4Si) δ_H 3.42(6H, br s, N–Me_2), 4.35 (1H, d, J = 13.4 Hz, 5′-H), 4.50 (1H, s, 5″-H), 4.58 (1H, d, J = 4.0 Hz, 2′-H), 4.84 (1H, d, J = 2.4 Hz, 1′-H), 4.96 (1H, dd, J = 13.1 and 1.2Hz, 4′-H), 6.09 (1H, s, 1′-H), 6.45 (1H, d, J = 4.0 Hz, OH), 8.13 (1H, s, 8-H), 8.25 (1H, s, 2-H); ^{13}C NMR (DMSO-*d*^{6}; Me_4Si) δ_C 55.8, 70.7, 73.2, 78.64, 78.74, 78.90, 89.8, 119.2, 136.4, 149.8, 152.4, 154.2; *m/z* (EI) 342([M+H]$^+$, 81%), 307(23), 289(13).

*9-(3,5-*O*-Sulfinyl-β-*D*-xylofuranosyl)adenine (**6b**).* Thionyl chloride (7.80 g, 49.2 mmol) was added to **3b** (4.38 g, 13.4 mmol) in acetonitrile (13.4 mL) and pyridine (3.89 g, 49.2 mmol), and the resulting solution was stirred for 4 h at 25°C. After being stirred for an additional 4 h, the mixture was poured into a suspension of sodium bicarbonate (13.5 g) in water (75 mL) and pyridine (25 mL) and extracted with ethyl acetate (2 × 5 mL). The combined extracts were dried over anhydrous magnesium sulfate and evaporated to dryness in vacuum. Recrystallization from 4-methyl-2-pentanone gave 4.43 g (86.2% yield) of **6b** as white crystals; mp 229°C; ν_{max}(KBr)/cm^{-1} 3396, 3334, 3194, 2907, 1355, 1307, 1578, 1488, 1427, 1330, 1248, 1225, 1186 (RO-SO-OR), 1109, 1092, 1069, 1015, 970, 910, 886, 841, 797, 736, and 714; ^{1}H NMR (DMSO-*d*^{6}; Me_4Si) δ_H 4.36 (1H, d, J = 13.1Hz, 5′-H), 4.51 (1H, d, J = 1.8 Hz, 5″-H), 4.63 (1H, d, J = 2.8 Hz, 2′-H), 4.87 (1H, d, J = 2.4 Hz, 1′-H), 4.98 (1H, dd, J = 13.3 and 1.9 Hz, 4′-H), 6.09 (1H, br s, 1′-H), 6.48 (1H, d, J = 4.0 Hz, OH), 7.37 (2H, br s, NH_2), 8.15 (1H, s, 8-H), 8.22 (1H, s, 2-H); ^{13}C NMR (DMSO-*d*^{6}; Me_4Si) δ_C 55.8, 70.8, 73.1, 78.7, 89.8, 118.7, 137.7, 149.0, 152.7, 155.9; *m/z* (FAB) 314.0537 ([M+H]$^+$, $C_{10}H_{12}N_5O_5S$ requires *m/z*: 314.0559).

Synthesis of 3′-Amino-3′-Deoxy-N^6,N^6-Dimethyladenosine (11) and Puromycin

*9-[2-*O*-(*N*-Benzylcarbamoyl)-3,5-*O*-sulfinyl-β-*D*-xylofuranosyl]-*N^6,N^6*-dimethyl-adenine (**7**).* Benzylisocyanate (2.66 g, 20.0 mmol) and Et_3N (1.51 g, 15 mmol) were added to the solution of **6a** (3.41 g, 10.0 mmol) in THF (50 mL) and MeCN (50 mL). The resulting solution was stirred

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for 21 h at 25°C. EtOH (99%, 10 mL) was added, and stirring was continued for 30 min. Volatiles were evaporated, and the residue was recrystallized (4-methyl-2-pentanone/diisopropyl ether) to give 4.67 g (98.5% yield) of **7** as white crystals; mp 140°C; ν_{max}(KBr)/cm^{-1} 3228, 3044, 2946, 1749, 1301, 1429, 1287, 1229, 1199 (RO-SO-OR), 1133, 1092, 1043, and 840; ^{1}H NMR (DMSO-d^6; Me_4Si) δ_H 3.46 (6H, br s, NMe_2), 4.24 (2H, m, ArCH_2N), 4.35 (1H, d, J = 13.2 Hz, 5′-H), 4.45 (1H, d, J = 1.7 Hz, 5″-H), 4.96 (1H, dd, J = 13.4 and 2.0 Hz, 4′-H), 5.11 (1H, d, J = 2.4 Hz, 1′-H), 5.44 (1H, s, 2′-H), 6.28 (1H, d, J = 1.5 Hz, 1′-H), 7.30 (5H, m, ArH), 8.20 (1H, s, 2-H), 8.26 (2H, m, NH and 8-H); ^{13}C NMR (DMSO-d^6; Me_4Si) δ_C 42.9, 43.9, 55.4, 69.1, 73.0, 80.4, 86.8, 119.0, 126.87, 126.91, 127.1, 128.10, 128.22, 136.4, 138.9, 149.8, 152.2, 154.1, 154.4; *m/z* (EI) 475([M+H]$^+$, 78%), 312(20), 241(14).

*9-[3-(Benzylamino)-3-*N*,2-O-carbonyl-3-deoxy-β-*D*-ribofuranosyl]-*N^6,N^6*-dimethyladenine (**8**).* To a suspension of NaH (50% in mineral oil; 112 mg, 2.32 mmol, rinsed with n-hexane) in DMF (20 mL) were added crystalline **7** (0.95 g, 2.00 mmol) and dipotassium hydrogenphosphate (0.48 g, 2.78 mmol) at –30°C. The resulting mixture was stirred for 20 h. After stirring for 1 h with NH_4Cl (0.64 g, 12 mmol), the mixture was filtered (with a layer of through Celite). Volatiles were evaporated, and the residue was purified on silica gel column chromatography eluting with chloroform-methanol (15:1, v/v) to give 0.49 g (60.0% yield based on **7**) of **8** as an oil. Although the compound contained a small amount of impurities, it was pure enough for further synthetic use; *m/z* (EI) 411([M+H]$^+$).

*9-(3-Amino-3-deoxy-β-*D*-ribofuranosyl)-*N^6,N^6*-dimethyladenine (3′-Amino-3′-deoxy-*N^6,N^6*-dimethlyladenosine) (**11**).* To an aqueous solution of NaOH (1 M, 30 mL) was added **8** (0.82 g, 2.0 mmol based on **7**) in 1,3-dioxolane (30 mL), and the solution was stirred for 96 h at ambient temperature. After the solution was neutralized with HCl solution (1 M), volatiles were evaporated. The residue was extracted with chloroform (40 mL × 2) and concentrated to dryness in vacuum. The resulting syrup was purified by silica gel column chromatography eluting with chloroform-methanol (15:1, v/v) to give 0.54 g (70.3% yield) of **10** as an oil; ^{1}H NMR (DMSO-d^6; Me_4Si) 3.36–3.56 (12H, m), 3.71–3.83 (2H, m, 5′-H), 3.93–3.97 (1H, m), 4.54 (1H, dd, J = 4.9 and 1.5 Hz), 6.02 (1H, d, J = 3.4 Hz, 1′-H), 7.28–7.36 (5H, m, ArH), 8.24 (1H, s, 2-H), 8.34 (1H, s, 1H, 8-H); *m/z* (EI) 385([M+H]$^+$).

The benzylaminocompound **10** (0.38 g, 1.0 mmol), NH_4HCO_2 (0.43 g, 5.1 mmol), and Pd(OH)$_2$-C (0.07 g) were heated at reflux in H_2O/MeOH (1/10 v/v) for 13.0 h. After filtration of the residue, volatiles were evaporated. The resulting syrup was purified by silica gel column chromatography eluting with chloroform-methanol (4:1, v/v) to give 0.21 g (71.4% yield) of **11** as white crystals; mp 213°C, (lit.[6a] 213–215.5°C); ν_{max}(KBr)/cm^{-1} 3398 and 3330, 2914, 2362, 2341, 1305, 1037; ^{1}H NMR (DMSO-d^6; Me_4Si) δ_H 3.1–3.7 (12H, m), 3.75–3.79 (2H, m, 5′-H), 4.28 (1H, q, J = 3.4 Hz),

5.97 (1H, d, J = 2.4 Hz, 1′-H), 8.22 (1H, s, 2-H), 8.41 (1H, s, 8-H); ^{13}C NMR (DMSO-d^6; Me_4Si) δ_C 52.3, 60.9, 74.7, 79.2, 85.3, 89.1, 119.6, 138.0, 149.6, 152.0, 154.2, (lit.[6a]) ^{13}C NMR (DMSO-d^6; Me_4Si) δ_C 52.3, 60.9, 75.0, 85.5, 89.3, 119.8, 138.2, 149.7, 152.0, 154.5); *m/z* (EI) 295 ($[M+H]^+$).

Puromycin. Synthesis of Puromycin was carried out according to the literature.[6a] DCC (146 mg, 0.708 mmol) was added to a cold (0°C) solution of **11** (199 mg, 0.676 mmol), BOC-(4-methoxy-L-phenylalanine) (206 mg, 0.70 mmol) and *N*-hydroxysuccinimide (82.6 mg, 0.718 mmol) in dried DMF (8 mL). The resulting solution was stirred for 30 min in an ice-water bath and then for additional 24 h at 25°C. Dicyclohexylurea was filtered off and washed with EtOAc (17 mL), and the combined filtrate and washing were evaporated to dryness. The residue was purified by silica gel column chromatography eluting with chloroform-methanol (48:2, v/v) to give 320 mg (82.8% yield) of BOC-Puromycin.

The BOC-Puromycin (236 mg, 413 mmol) was stirred with trifluoroacetic acid (2 mL) for 10 min at 20°C, then MeCN (60 mL) was added and evaporated in vacuum. The residue was dissolved into chloroform (20 mL), and the solution was poured into a suspension of sodium hydrogen carbonate (1.0 g) in water (20 mL). The organic layer was dried over magnesium sulfate and evaporated to dryness in vacuum. The resulting solid was purified by silica gel column chromatography eluting with chloroform-methanol (9:1, v/v) to give Puromycin (85 mg, 43.7% deprotection yield) as foam; ν_{max}(KBr)/cm^{-1} 3352, 2925, 1740, 1369, 1597, 1513, 1425, 1249, and 1034; ^{1}H NMR ($CDCl_3$; Me_4Si) δ_H 1.26(2H, br s, NH_2), 2.61–2.70 (1H, m), 2.80 (1H, dd, J = 13.8 and 7.9 Hz), 3.39–3.63 (8H, m, 5′-H and NMe_2), 3.72–3.82 (4H, m), 3.99 (1H, dd, J = 13.1 and 2.1 Hz), 4.33–4.51 (2H, m, 1′-H and 4′-H), 4.69 (1H, dd, J = 5.8 and 3.7 Hz, OH), 5.87 (1H, d, J = 3.3 Hz, OH), 5.98 (1H, d, J = 1.8 Hz, 1′-H), 6.83 (2H, dd, J = 8.8 and 2.4 Hz, ArH), 7.11 (2H, dd, J = 8.8 and 2.1 Hz, ArH), 8.00 (1H, d, J = 6.7 Hz, NH), 8.13 (1H, s, 2-H), 8.17 (1H, s, 8-H), (lit.[6a] [1]H NMR (DMSO-d^6; Me_4Si) δ_H 1.73 (2H, br s), 2.5–2.6 (1H, m), 2.91 (1H, dd, J = 13.4 and 4.9 Hz), 3.35–3.62 (8H, m), 3.62–3.78(4H, m), 3.9–4.0(1H, m), 4.4–4.5(2H, m), 5.15(1H, t, J = 5.4 Hz), 5.98(1H, d, J = 1.8 Hz), 6.15 (1H, d, J = 4.3 Hz), 6.84 (2H, d, J = 8.5 Hz), 7.15 (2H, d, J = 8.5 Hz), 8.05 (1H, s), 8.24 (1H, s), 8.45 (1H, s,), peaks at 1.73, 5.15, 6.15 and 8.05 exchanged with D_2O.); [13]C NMR ($CDCl_3$; Me_4Si) 40.4, 50.9, 55.5, 56.9, 62.2, 74.2, 81.8, 85.1, 91.4, 114.2, 114.4, 121.2, 130.4, 130.6, 137.5, 148.9, 151.8, 155.1, 158.8, 175.2, 176.0, (lit[6a]) ^{13}C NMR (DMSO-d^6; Me_4Si) δ_C 50.1, 55.2, 56.3, 61.0, 73.3, 83.6, 89.7, 113.9, 119.8, 130.5, 130.6, 138.2, 149.8, 152.1, 154.5, 158.0, 175.2, 177.8); *m/z* (EI) 472($[M+H]^+$).

*Synthesis of 2,2′-Anhydro-Pyrimidine Nucleosides (**15**), cytidines (**17**) and 2′,3′-Anhydroadenosine (**14**).* **6b** (0.63 g, 2.0 mmol) and sodium bicarbonate (0.76 g, 9.0 mmol) were heated in DMF (40 mL) at 110°C for 1h and then

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cooled. The solid precipitated was filtrated off and washed with 5 mL of DMF. The filtrate and washing were combined and concentrated to dryness in vacuum. Recrystallization from ethanol gave 0.40 g (80.3% yield) of 2′,3′-anhydroadenosine; mp 181°C; ν_{max}(KBr)/cm^{-1} 1385, 1308, 1340, 1303 and 1097; ^{1}H NMR (DMSO-d^6; Me_4Si) δ_H 3.55 (2H, m, 5′-H), 4.20 (2H, m, 1′-H and 4′-H), 4.45 (1H, d, J = 2.7 Hz, 2′-H), 5.46 (1H, br s, OH), 6.22 (1H, s, 1′-H), 7.31 (2H, br s, NH_2), 8.18 (1H, s, 8-H), 8.34 (1H, s, 2-H); ^{13}C NMR (DMSO-d^6; Me_4Si) δ_C 57.7, 58.7, 79.1, 81.1, 82.0, 139.5, 149.1, 152.6, 156.0; *m/z* (EI) 250 ([M+H]$^+$, 100%), 89 M(57), 77 (50).

*2,2′-O-Anhydro-1-(β-D-arabinofuranosyl)uracil (**15a**).* **4a** (1.45 g, 5.0 mmol) and sodium bicarbonate (1.89 g, 22.5 mmol) were heated in DMF (100 mL) at 90°C for 5 h and then cooled. The solid precipitated was filtrate off and washed with 10 mL of DMF. The filtrate and washing were combined and concentrated to dryness in vacuum. The resulting syrup was purified by silica gel column chromatography eluting with chloroform-methanol (4:1, v/v) to give **15a** (0.99g, 87.3%). Further recrystallization from 2-propanol-ethanol (5:1, v/v) gave white crystals; mp 246°C, (lit.[17] 244–247°C). Its ^{1}H– and ^{13}C-NMR spectra were identical with those of the authentic sample (Sigma-Aldrich).

*2,2′-Anhydro-1-β-D-arabinofuranosylthymine (**15b**).* Similar to the synthesis of **15a**, **15b** was obtained from the reaction of **4b** (1.52 g, 5.0 mmol) and sodium bicarbonate (1.89 g, 22.5 mmol) in DMF (100 mL); yield: 1.12 g (93.3%). Recrystallization from 2-propanol-ethanol (5:1, v/v) gave white crystals; mp 218°C; ^{1}H NMR (DMSO-d^6; Me_4Si) δ_H 1.80 (3H, s, Me), 3.17 (1H, m), 3.26 (1H, m), 4.06 (1H, t, J = 4.9 Hz), 4.37 (1H, d, J = 3.4 Hz), 4.95 (1H, t, J = 5.2Hz, OH), 5.18 (1H, d, J = 5.8 Hz, 2′-H), 5.87 (1H, d, J = 4.3 Hz, OH), 6.29 (1H, d, J = 5.8 Hz, 1′-H), 7.74 (1H, d, J = 1.2Hz, 6-H); (lit.[13] ^{1}H NMR (250 MHz; DMSO-d^6; Me_4Si) δ_H 1.79(3H, d, J = 0.9 Hz), 3.22 (2H, m), 4.06 (1H, m), 4.37 (1H, br s), 4.97 (1H, t, J = 5.31Hz), 5.15 (1H, d, J = 5.75 Hz), 5.88 (1H, d, J = 4.52Hz), 6.29 (1H, d, J = 5.75 Hz), 7.75 (1H, d, J = 1.33 Hz)); ^{13}C NMR (DMSO-d^6; Me_4Si) δ_C 13.6, 60.9, 74.8, 88.6, 89.2, 90.2, 113.7, 132.3, 159.4, 171.7; *m/z* (FAB) 241.0819 ([M+H]$^+$, $C_{10}H_{13}N_2O_5$ requires *m/z*: 241.0824).

*2,2′-O-Anhydro-1-(β-D-arabinofuranosyl)-5-fluorouracil (**15c**).* Similar to the synthesis of **15a**, **15c** was obtained from the reaction of **4c** (1.54 g, 5.0 mmol) and sodium bicarbonate(1.89 g, 22.5 mmol) in DMF (100 mL); yield: 1.17 g (95.9%). Recrystallization from 2-propanol-ethanol (5:1, v/v) gave white crystals; mp 190°C; ^{1}H NMR (DMSO-d^6; Me_4Si) δ_H 3.24 (1H, m), 3.26 (1H, m), 4.12 (1H, m), 4.40 (1H, br s), 4.97 (1H, t, J = 4.9 Hz, OH), 5.26 (1H, d, J = 5.8 Hz, 2′-H), 5.93 (1H, br s, OH), 6.31 (1H, d, J = 5.8 Hz, 1′-H), 8.26 (1H, d, J = 4.6 Hz, 6-H); ^{13}C NMR (DMSO-d^6; Me_4Si) δ_C 60.8, 75.0, 89.75, 89.82, 90.8, 121.4(d, J_{cp} = 37.2Hz), 145.3 (d, J_{cp} = 248 Hz), 155.4 (d, J_{cp} = 256 Hz), 157.4; *m/z* (FAB) 245.0581 ([M+H]$^+$, $C_9H_{10}N_2O_5F$ requires *m/z*: 245.0574).

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*Cytidine (**17a**) from (**5a**).* The hydrochloride **5a** containing some amount of free base (1.63 g, 5.0 mmol based on **5a**) and sodium bicarbonate (2.31 g, 27.5 mmol) were heated in DMF (100 mL) at 105°C for 3 h and cooled. The solid precipitated was filtrated off and washed with 10 mL of DMF. The filtrate and washing were combined and concentrated to dryness in vacuum. The resulting syrup was purified by silica gel column chromatography eluting with chloroform-methanol (4:1, v/v) to give cytidine **17a** (0.93 g, 76.5%). Recrystallization from methanol gave white crystals; mp 213°C; ^{1}H NMR (DMSO-d^6; Me_4Si) δ_H 3.53 (1H, m), 3.65 (1H, m), 3.81 (1H, m), 3.93 (2H, m), 4.97 (1H, d, J = 4.6 Hz), 5.03 (1H, t, J = 5.2 Hz), 5.27 (1H, d, J = 4.6 Hz), 5.71 (1H, d, J = 7.6 Hz), 5.76 (1H, d, J = 3.7 Hz), 7.10 (1H, br s), 7.13 (1H, br s), 7.84 (1H, d, J = 7.6 Hz); ^{13}C NMR (DMSO-d^6; Me_4Si) δ_C 60.6, 69.4, 74.0, 84.1, 89.2, 93.8, 141.5, 155.4, 135.5; *m/z* (FAB) 244.0953 ($[M+H]^+$, $C_9H_{14}N_3O_5$ requires *m/z*: 244.0933). [Authentic sample **17a**; mp 213°C; ^{1}H NMR (DMSO-d^6; Me_4Si) δ_H 3.54 (1H, m), 3.65 (1H, m), 3.81 (1H, m), 3.92 (2H, m), 4.98 (1H, d, J = 4.9 Hz, OH), 5.04 (1H, t, J = 5.2Hz, OH), 5.27 (1H, d, J = 5.2Hz, OH), 5.71 (1H, d, J = 7.3 Hz, 5-H), 5.76 (1H, d, J = 3.7 Hz, 1′-H), 7.11 (1H, br s, NH), 7.17 (1H, br s, NH), 7.84 (1H, d, J = 7.3 Hz, 6-H); ^{13}C NMR (DMSO-d^6; Me_4Si) δ_C 60.6, 69.4, 74.0, 84.0, 89.2, 93.8, 141.5, 155.4, 135.6]

*Cytidine (**17a**) from (**16a**).* Hydrochloride **16a** (Sigma-Aldrich) (0.785 g, 3.0 mmol) and sodium bicarbonate (0.88 g, 10.5 mmol) were heated in DMF (60 mL) at 105°C for 3 h and cooled. The solid thus precipitated was filtrated and washed with DMF (10 mL). The filtrate and washing were combined and concentrated to dryness in vacuum. The resulting syrup was purified by silica gel column chromatography eluting with chloroform-methanol (2:1, v/v) to give **17a** (0.59 g, 80.9%) whose spectral data were identical with those of the authentic sample (Sigma-Aldrich).

*5-Fluorocytidine (**17b**).* The hydrochloride **5b** containing some amount of free base (0.462 g, 1.5 mmol based on **5b**) and sodium bicarbonate (0.693 g, 8.25 mmol) were heated in DMF (30 mL) at 105°C for 3 h. The solid precipitated after cooling was filtrated and washed with DMF (5 mL). The filtrate and washing were combined and concentrated to dryness in vacuum. The resulting syrup was purified by silica gel column chromatography eluting with chloroform-methanol (2:1, v/v) to give **17b** (0.18 g, 49.2%) as an oil; ^{1}H NMR (DMSO-d^6; Me_4Si) δ_H 3.57 (1H, m), 3.68 (1H, m), 3.82 (1H, m), 3.95 (2H, m), 4.99 (1H, d, J = 5.5 Hz, OH), 5.20 (1H, d, J = 4.9 Hz, OH), 5.33 (1H, d, J = 5.2 Hz, OH), 5.71 (1H, dd, J = 3.7 and 1.9 Hz, 1′-H), 7.49 (1H, br s, NH), 7.76 (1H, br s, NH), 8.20 (1H, d, J = 7.3 Hz, 1H, 6-H); ^{13}C NMR (DMSO-d^6; Me_4Si) δ_C 60.2, 69.0, 74.3, 84.1, 89.3, 125.7 (d, J_{cp} = 32.1 Hz), 136.1 (d, J_{cp} = 240 Hz), 153.6, 157.4 (d, J_{cp} = 13.4 Hz); *m/z* (FAB) 262.0826 ($[M+H]^+$ $C_9H_{13}N_3O_5F$ requires *m/z*: 262.0839).

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