The Synthesis of the New C-Nucleoside 6-Deazaformycin B

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Abstract: The synthesis of the 6-deaza analogue of formycin B is described, through the condensation of lithiated 4-methoxy-2-methyl-3-trifluoroacetamidopyridine with a suitably protected ribonolactone, dehydration of the resulting hemiacetal, reduction and subsequent ring closure followed by protecting group manipulation.

Key words: C-nucleosides, heterocycles, pyrazolo[4,3-*b*]pyridine, lithiation, ring closure

Nucleosides and nucleotides are involved, either by themselves or in combination with other molecules, in almost all cell activities, including catalysis, transfer of energy and mediation of hormone signals. Structural modifications of naturally occurring nucleosides have been used by medicinal chemists to provide new compounds, which can serve as valuable tools in structure–activity studies.[1] A great number of the synthesized molecules display antibiotic,[2] antifungal[3] and antineoplastic properties[4] and, most importantly, the majority of antiviral drugs currently in clinical use are nucleoside analogues.[5] A very simple alteration of the purine or pyrimidine base of nucleosides is the replacement of the nitrogen, linking the heterocyclic part to the sugar, with a methine unit. The resulting C-nucleosides show interesting biological activity and they are also endowed with stability towards chemical or enzymatic cleavage.[6]

C-Nucleoside analogues of cytidine (1-deazacytidine),[7] guanosine (9-deazaguanosine),[8] adenosine (9-deazaadenosine, Figure 1)[9] and inosine (9-deazainosine, Figure 1)[10] have been synthesized and studied. In fact, 9-deazainosine is active against *Trypanosoma brucei* subsp. experimental infections and against *Pneumonocystis carinii* pneumonias in rats,[11] while 9-deazaadenosine displays potent antineoplastic activity against a variety of human tumor cell lines.[12]

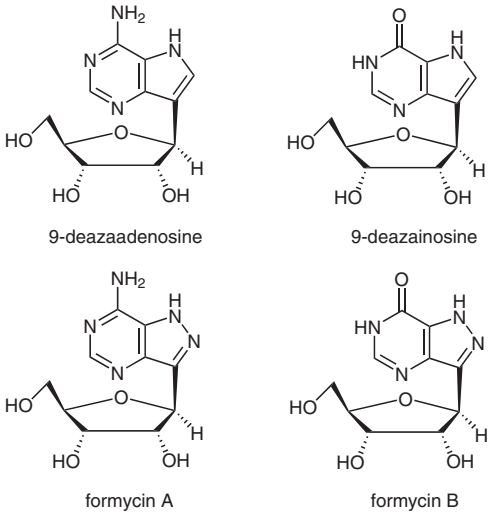
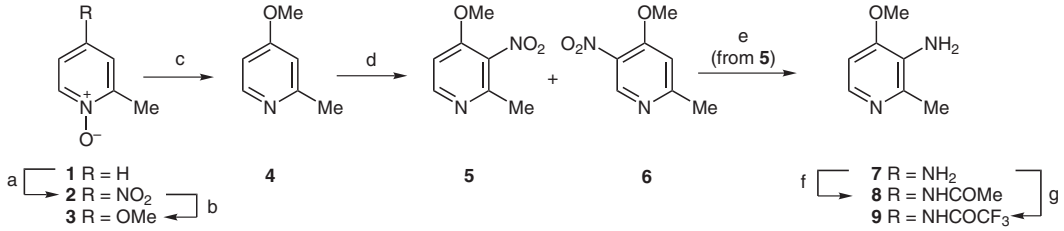


Figure 1 Structures of C-nucleosides

We are involved in the synthesis of purine-like C-nucleosides bearing structural similarity with the natural antibiotics formycin A and formycin B (Figure 1), which mimic the isosteric adenosine and inosine molecules, respectively, and substitute for them in many enzymatic reactions.[13] In view of our interest towards the importance of the 4- or the 6-nitrogen of these molecules, concerning their ability to behave as antimetabolites, we have previously accomplished the synthesis of 4-deazaformycin A[14] and 4-deazaformycin B.[15] As a continuation of this ongoing research effort we present here the synthesis of 1,4-dihydro-3-(β-D-ribofuranosyl)-7*H*-pyrazolo[4,3-*b*]pyridin-7-one (6-deazaformycin B).



Scheme 1 *Reagents and conditions*: (a) H_2SO_4, HNO_3 (65%), 160 °C, 3 h; (b) MeONa, MeOH, 80 °C, 2 h; (c) Fe, AcOH, 130 °C, 2 h; (d) H_2SO_4, HNO_3 (65%), 65 °C, 12 h; (e) H_2, 10% Pd/C, EtOH, r.t., 4 h.; (f) Ac_2O, CH_2Cl_2, r.t., 10 h; (g) $(CF_3CO)_2O$, CH_2Cl_2, r.t., 1 h.

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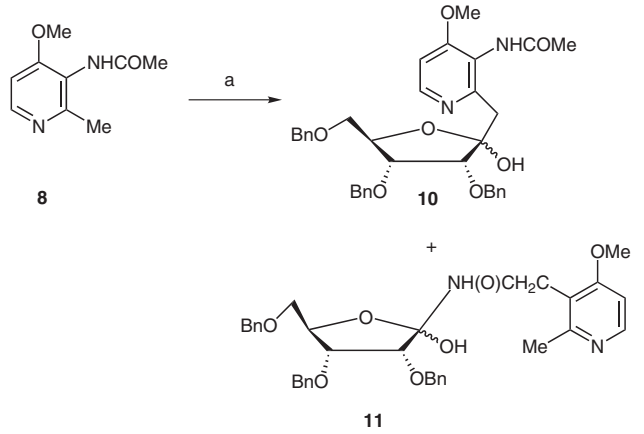
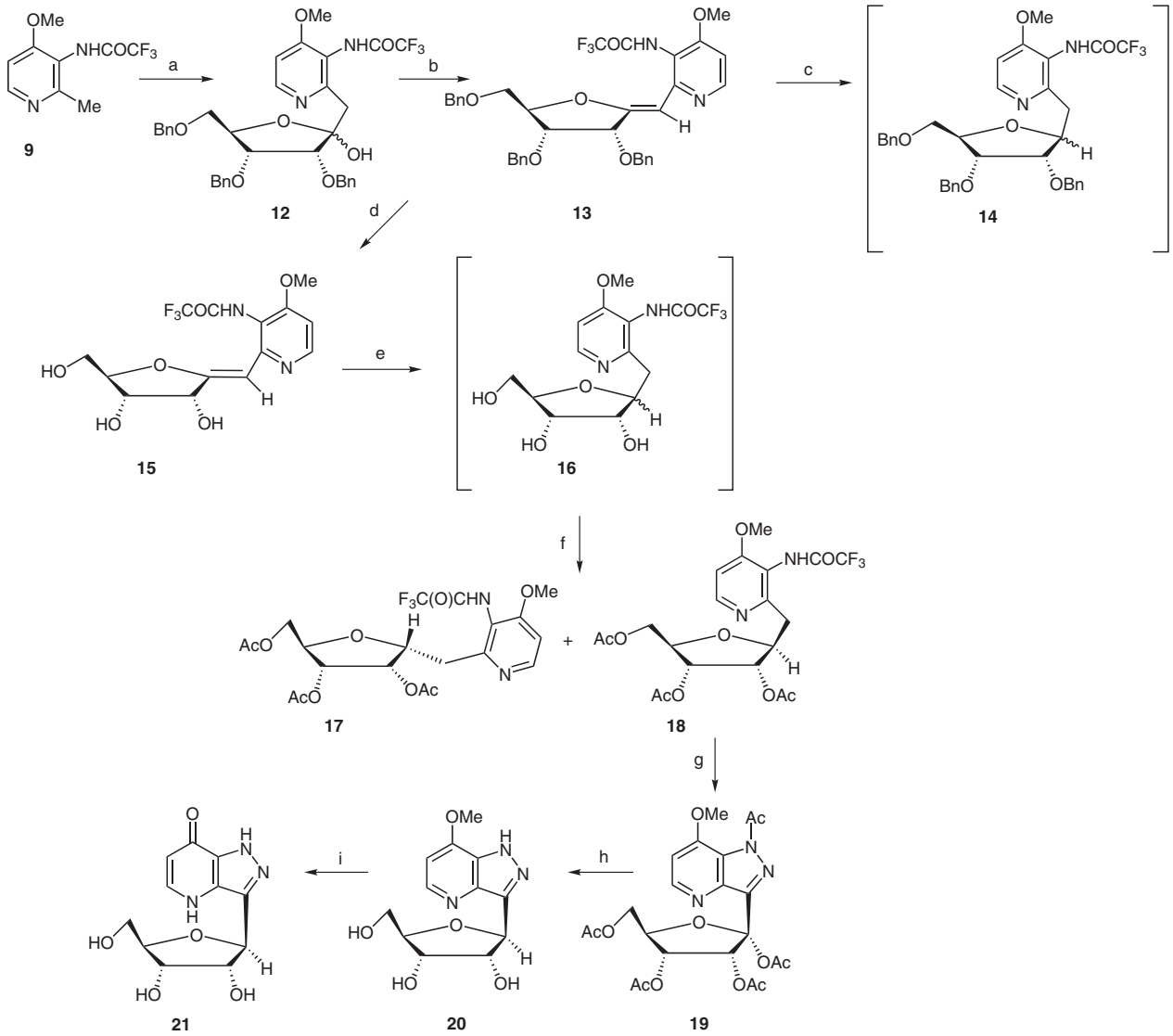


Scheme 2 *Reagents and conditions*: (a) (i) *n*-BuLi (2 equiv), THF, –78 °C → –55 °C, 1 h; (ii) 2,3,5-tri-*O*-benzyl-D-ribonolactone, THF, –78 °C → –20 °C, 3 h.

For the synthesis of the target derivative we used 2-picoline *N*-oxide (**1**), which was easily converted into 4-methoxy-2-picoline (**4**, Scheme 1).[16]

Compound **4** was nitrated and upon separation of the mixture of the resulting nitro compounds, the desired isomer **5** was reduced to provide the aminopicoline **7**. This compound has been previously reported in the literature,[17] as a side product of the ammonolysis of 3-methoxy-2-acylfuran, although its structure was not unambiguously confirmed. The aminopicoline **7** was then converted into the corresponding acetamide **8**, which was lithiated using *n*-butyllithium in anhydrous THF and the resulting anion was allowed to attack the carbonyl of the easily accessible 2,3,5-tri-*O*-benzyl-D-ribonolactone.[18] However, this reaction provided the expected anomeric mixture of hemiacetals **10** (Scheme 2) in only 13% yield, while the mixture of compounds **11** was obtained as the major product (35% yield); this is indicative of anion formation on the acetamide's methyl, which then attacks the ribonolactone.



Scheme 3 *Reagents and conditions*: (a) (i) *n*-BuLi (2 equiv), THF, –78 °C → –45 °C, 1 h; (ii) 2,3,5-tri-*O*-benzyl-D-ribonolactone, THF, –78 °C → –20 °C, 3 h; (b) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C, 2 h; (c) H_2, 10% Pd/C, EtOH, r.t., 24 h; (d) BCl_3, CH_2Cl_2, –78 °C, 1 h; (e) H_2, 10% Pd/C, EtOH, r.t., 5 h; (f) Ac_2O, Et_3N, CH_2Cl_2, r.t., 12 h; (g) AcOK, Ac_2O, isoamyl nitrite, C_6H_6, reflux, 12 h; (h) NH_3–MeOH, r.t., 10 h; (i) NaI, $(Me)_3SiCl$, MeCN, 80 °C, 4 h.

Consequently, we decided to use the trifluoroacetamide **9**,[19] which was easily prepared by treatment of **7** with trifluoroacetic anhydride. The lithium-mediated anion of **9** was reasonably stable at –45 °C and upon reaction with 2,3,5-tri-*O*-benzyl-D-ribonolactone provided the mixture of hemiacetals **12** in 62% yield (Scheme 3). This mixture was treated with $BF_3{\cdot}OEt_2$ in CH_2Cl_2 solution and provided almost quantitatively the olefin **13**.[20] Unfortunately, even if the ^{1}H NMR spectrum of the isolated compound corresponded to only one isomer, the NOE spectral data did not provide clear evidence concerning the exact stereochemistry of the olefin **13**. The olefin was submitted to catalytic hydrogenation over Pd/C, to result in an anomeric mixture of compounds **14**, while no reductive debenzylation took place. Only the major and more polar component of the abovementioned mixture **14** could be isolated pure by column chromatography and according to NOE experiments, proved to be the α-anomer, since we observed correlation peaks between H-1′ and H-3′, as well as between H-1′ and H-5′. Due to the extremely low yield of the desired β-anomer, we decided to cleave the benzyl groups of the olefin **13** and then reduce the resulting compound. For this purpose, a CH_2Cl_2 solution of **13** was treated with boron trichloride at –78 °C to provide exclusively the *Z*-isomer of the olefin **15**.[21] The stereochemistry was unambiguously determined on the basis of NOE data, where a strong correlation peak between the olefinic proton and H-2′ was obvious.

Catalytic hydrogenation of compound **15** over Pd/C provided the mixture of anomers **16** in a 1:1.5 ratio, as estimated by ^{1}H NMR. The chromatographic separation of the mixture proved to be very difficult at this point, therefore both anomers were acetylated and the resulting acetates were separated by column chromatography and identified. The configuration at C-1′ was assigned on the basis of NOE experiments, where we observed clear correlation peaks between H-1′ and H-3′ for **17** (α-anomer)[22] and between H-4′ and H-1′ for compound **18** (β-anomer).[23] The anomer **18** was then treated with isoamyl nitrite in benzene at reflux in the presence of acetic anhydride and potassium acetate,[24] to result upon rearrangement of the intermediate *N*-nitroso compound in the 1-acetylpyrazolopyridine **19**. The acetyl groups of **19** were easily cleaved upon treatment with methanolic ammonia and the resulting C-nucleoside **20** was converted into the target 6-deazaformycin B (**21**)[25] by reaction with trimethylsilyl chloride in the presence of sodium iodide.[26]

In conclusion, we have prepared 6-deazaformycin B using an efficient method, through the attachment of a protected ribonolactone to a suitably substituted picoline, cyclization of the intermediate to elaborate the pyrazolo[4,3-*b*]pyridine ring system, followed by cleavage of the protective groups.

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(19) Data for *N*-(4-Methoxy-2-methylpyridin-3-yl)trifluoroacetamide (**9**). Yield: 96%; mp 164–165 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ = 2.31 (s, 3 H, CH_3), 3.86 (s, 3 H, CH_3O), 7.05 (d, $J_{5,6}$ = 5.80 Hz, 1 H, H-5), 8.35 (d, $J_{6,5}$ = 5.80 Hz, 1 H, H-6), 10.93 (br s, D_2O exch., 1 H, NH). ^{13}C NMR (DMSO-d_6): δ = 20.30 (CH_3), 56.18 (CH_3O), 106.00 (C-5), 107.50, 113.22, 118.96, 124.69 (CF_3CO), 118.23 (C-3), 149.93 (C-6), 154.15, 154.88, 155.60, 156.31 (CF_3CO), 156.09 (C-2), 160.89 (C-4). Anal. Calcd for $C_9H_9F_3N_2O_2$: C, 46.16; H, 3.87; N, 11.96. Found: C, 45.87; H, 3.76; N, 12.13.
(20) Data for *N*-[4-Methoxy-2-(2,3,5-tri-*O*-benzyl-D-ribofuranosylidene)methylpyridin-3-yl]trifluoroacetamide (**13**). Yield: 71%; mp 146–151 °C (EtOH). ^{1}H NMR ($CDCl_3$): δ = 3.56 (m, 1 H, H-5′), 3.74 (m, 1 H, H-5′), 3.89 (s, 3 H, CH_3O),

3.97 (m, 1 H, H-3′), 4.40 (m, 2 H, CH_2), 4.43 (m, 1 H, H-2′), 4.47 (m, 2 H, CH_2), 4.56 (m, 2 H, CH_2), 4.70 (m, 1 H, H-4′), 5.85 (s, 1 H, =CH), 6.79 (d, $J_{5,6}$ = 5.62 Hz, 1 H, H-5), 7.18–7.42 (m, 15 H, PhH), 8.45 (d, $J_{6,5}$ = 5.62 Hz, 1 H, H-6), 8.79 (br s, D_2O exch., 1 H, NH). ^{13}C NMR ($CDCl_3$): δ = 56.10 (CH_3O), 68.52 (C-5′), 71.28 (CH_2), 72.27 (CH_2), 72.52 (=CH), 72.99 (C-2′), 73.20 (CH_2), 75.80 (C-3′), 76.89 (C-1′), 85.18 (C-4′), 105.81 (C-5), 107.52, 113.25, 118.99, 124.73 (*C*F_3CO), 118.15 (C-3), 127.61, 127.81, 128.10, 128.16, 128.22, 128.31, 128.41, 128.51, 128.56, 137.04, 137.47 (CPh), 147.07 (C-6), 150.04 (C-2), 153.80, 154.52, 155.26, 155.99 (CF_3*C*O), 163.17 (C-4). Anal. Calcd for $C_{35}H_{33}F_3N_2O_6$: C, 66.24; H, 5.24; N, 4.41. Found: C, 66.02; H, 5.31; N, 4.29.

(21) Data for (*Z*)-*N*-[4-Methoxy-2-(D-ribofuranosylidene)methylpyridin-3-yl]trifluoroacetamide (**15**): yield: 86%; mp >250 °C (dec.; EtOH). ^1H NMR (DMSO-d_6): δ = 3.63 (m, 2 H, H-5′), 4.05 (s, 3 H, CH_3O), 4.07 (m, 1 H, H-3′), 4.53 (m, 1 H, H-4′), 4.81 (m, 1 H, H-2′), 5.28–5.46 (br s, D_2O exch., 2 H, 2 × OH), 5.51 (s, 1 H, =CH), 5.77–5.84 (br s, D_2O exch., 1 H, OH), 7.45 (d, $J_{5,6}$ = 7.09 Hz, 1 H, H-5), 8.17 (br s, D_2O exch., 1 H, NH), 8.64 (d, $J_{6,5}$ = 7.09 Hz, 1 H, H-6). ^{13}C NMR (DMSO-d_6): δ = 57.52 (CH_3O), 60.33 (C-5′), 69.71 (C-3′), 73.46 (C-2′), 74.07 (C-1′), 84.71 (=CH), 93.15 (C-4′), 104.41 (C-5), 107.45, 113.18, 118.91, 124.65 (*C*F_3CO), 116.28 (C-3), 143.80 (C-6), 148.17 (C-2), 153.94, 154.65, 155.40, 156.12 (CF_3*C*O), 166.92 (C-4). Anal. Calcd for $C_{14}H_{15}F_3N_2O_6$: C, 46.16; H, 4.15; N, 7.69. Found: C, 46.22; H, 3.96; N, 7.51.

(22) Data for *N*-[4-Methoxy-2-(2,3,5-tri-*O*-acetyl-α-D-ribofuranosyl)methylpyridin-3-yl]trifluoroacetamide (**17**). Yield: 51%; light yellow oil; $[\alpha]_D$ +33.1° (*c* = 0.124, CH_2Cl_2). ^1H NMR ($CDCl_3$): δ = 2.03 (s, 3 H, CH_3CO), 2.05 (s, 3 H, CH_3CO), 2.12 (s, 3 H, CH_3CO), 2.88 (m, 1 H, CH_2), 3.17 (m, 1 H, CH_2), 3.90 (s, 3 H, CH_3O), 4.03 (m, 1 H, H-5′), 4.24 (m, 1 H, H-5′), 4.30 (m, 1 H, H-4′), 4.54 (m, 1 H, H-1′), 5.26 (m, 1 H, H-3′), 5.55 (m, 1 H, H-2′), 6.81 (d, $J_{5,6}$ = 5.86 Hz, 1 H, H-5), 8.38 (d, $J_{6,5}$ = 5.86 Hz, 1 H, H-6), 8.84 (br s, D_2O exch., 1 H, NH). ^{13}C NMR ($CDCl_3$): δ = 20.57 (2 × *C*H_3CO), 20.77 (*C*H_3CO), 35.49 (CH_2), 56.22 (CH_3O), 63.77 (C-5′), 72.17 (C-3′), 72.69 (C-2′), 77.57 (C-4′), 80.48 (C-1′), 106.35 (C-5), 106.51, 112.24, 117.97, 123.71 (*C*F_3CO), 119.35 (C-3), 150.32 (C-6), 154.98 (C-2), 154.18, 154.91, 155.63, 156.35 (CF_3*C*O), 160.92 (C-4), 168.98 (CH_3*C*O), 169.82 (CH_3*C*O), 170.69 (CH_3*C*O). Anal. Calcd for $C_{20}H_{23}F_3N_2O_9$: C, 48.78; H, 4.71; N, 5.69. Found: C, 48.63; H, 4.67; N, 5.52.

(23) Data for *N*-[4-Methoxy-2-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)methylpyridin-3-yl]trifluoroacetamide (**18**): yield: 34%; light yellow oil; $[\alpha]_D$ –30.2° (*c* = 0.227, CH_2Cl_2). ^1H NMR ($CDCl_3$): δ = 1.98 (s, 3 H, CH_3CO), 2.03 (s, 3 H, CH_3CO), 2.07 (s, 3 H, CH_3CO), 3.03 (m, 1 H, CH_2), 3.22 (m, 1 H, CH_2), 3.89 (s, 3 H, CH_3O), 4.00 (m, 1 H, H-5′), 4.10 (m, 1 H, H-5′), 4.33 (m, 1 H, H-4′), 4.37 (m, 1 H, H-1′), 4.89 (m, 1 H, H-2′), 4.96 (m, 1 H, H-3′), 6.82 (d, $J_{5,6}$ = 5.86 Hz, 1 H, H-5), 8.40 (d, $J_{6,5}$ = 5.86 Hz, 1 H, H-6), 8.71 (br s, D_2O exch., 1 H, NH). ^{13}C NMR ($CDCl_3$): δ = 20.49 (*C*H_3CO), 20.58 (*C*H_3CO), 20.64 (*C*H_3CO), 36.77 (CH_2), 56.21 (CH_3O), 63.56 (C-5′), 71.32 (C-2′), 72.47 (C-3′), 80.79 (C-1′), 80.98 (C-4′), 106.46 (C-5), 106.60, 112.33, 118.07, 123.82 (*C*F_3CO), 119.98 (C-3), 150.35 (C-6), 154.40 (C-2), 154.51, 155.22, 155.98, 156.64 (CF_3*C*O), 161.11 (C-4), 169.87 (2 × CH_3*C*O), 170.62 (CH_3*C*O). Anal. Calcd for $C_{20}H_{23}F_3N_2O_9$: C, 48.78; H, 4.71; N, 5.69. Found: C, 48.92; H, 4.64; N, 5.76.

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(25) Data for 1,4-Dihydro-3-(β-D-ribofuranosyl)-7*H*-pyrazolo[4,3-*b*]pyridin-7-one(**21**): yield: 78.9%; mp 200 °C (dec.; EtOH); $[\alpha]_D$ –43.0° (*c* = 0.330, MeOH). ^1H NMR (DMSO-d_6): δ = 3.61 (m, 2 H, H-5′), 3.86 (m, 1 H, H-4′), 3.94 (m, 1 H, H-3′), 4.11 (m, 1 H, H-2′), 4.87 (m, 1 H, H-1′), 4.93 (br s, D_2O exch., 1 H, OH-3′), 5.11 (br s, D_2O exch., 1 H, OH-2′), 5.76 (br s, D_2O exch., 1 H, OH-5′) 5.88 (d, $J_{6,5}$ = 7.14 Hz, 1 H, H-6), 7.59 (d, $J_{5,6}$ = 7.14 Hz, 1 H, H-5), 11.66 (br s, D_2O exch., 1 H, NH-4), 13.46 (br s, D_2O exch., 1 H, NH-1). ^{13}C NMR (DMSO-d_6): δ = 61.27 (C-5′), 71.58 (C-3′), 75.33 (C-2′), 79.09 (C-1′), 84.71 (C-4′), 109.10 (C-6), 126.00 (C-3a), 133.50 (C-7a), 137.23 (C-5), 138.20 (C-3), 168.20 (C-7). Anal. Calcd for $C_{11}H_{13}N_3O_5$: C, 49.44; H, 4.90; N, 15.72. Found: C, 49.27; H, 4.73; N, 15.59.

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