The Synthesis of a New Pyrazolo[3,4-*c*]pyridine C-Nucleoside, Structurally Related to Formycin B

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Abstract: The first preparation of the 4-deaza analogue of formycin B is described, via the reaction of 3-acetamido-2-methoxy-4-methylpyridine with a suitably protected ribonolactone and subsequent ring closure to result in the 3-substituted pyrazolo[3,4-*c*]pyridine riboside **12**.

Key words: lithiation, ring closure, heterocycles, C-nucleosides, pyrazolopyridine

Nucleoside analogues have been extensively investigated as antitumor and antiviral agents. The research towards the preparation of novel nucleosides has been focused in the development of synthetic methods that will allow access to analogues with potential bioactivity, through alterations of the sugar part or of the heterocyclic base.[1] Among these modified derivatives, C-nucleosides, which contain a carbon-to-carbon ribosidic linkage, instead of the carbon-to-nitrogen bond of the naturally occurring N-nucleosides, are included**.** This structural feature imparts some unusual physicochemical and biochemical properties to this class of compounds, mainly derived from their remarkable stability to both chemical and enzymatic cleavage. A number of purine-like C-nucleosides, both naturally occurring or synthetic, such as the antibiotics formycin A,[2] formycin B,[3] oxoformycin,[4] and pyrrolosine[5] (Figure), have shown interesting biological activity, attributed to their ability to mimic isosteric N-nucleosides and substitute for them in enzymatic reactions.[6]

As a continuation of our ongoing efforts towards the design and synthesis of C-nucleoside antibiotics[7] we present here the preparation of 3-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*c*]pyridine-7(6*H*)-one (**12**), which can be viewed as a singly modified (4-deaza) formycin B and thus provide a probe for studies on the importance of the 4-nitrogen of this molecule, concerning its ability to behave as antimetabolite and the potential to possess cytotoxicity or antiviral activity thereof.

For the preparation of the target nucleoside we have applied a method previously developed by us[8] in which the attachment of a suitably substituted pyridine at the anomeric position of an appropriately protected ribose is followed by an elaboration of the pyrazolo[3,4-*c*]pyridine ring system.

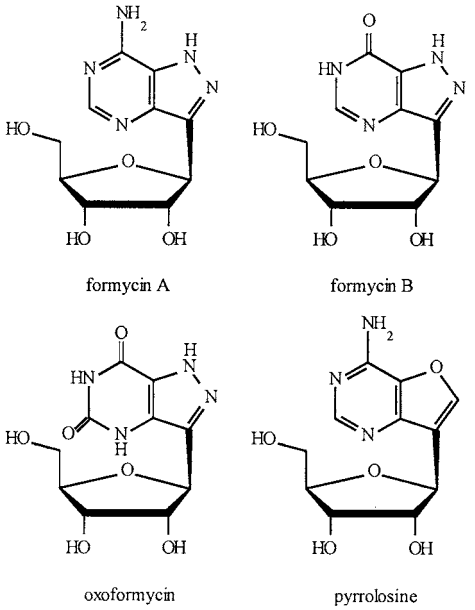

Figure Structures of purine-like C-nucleosides.

In our study we used 3-acetamido-2-methoxy-4-methylpyridine[9] (**1**) as starting material (Scheme), which was lithiated using *n*-butyllithium in dry THF.[10] The resulting 4-methylene anion attacks the carbonyl of the easily accessible D-ribonolactone **2**[11] to provide a 1:7, α-D/ β-D[12] anomeric mixture of the hemiacetals **3**, in a 63% isolated yield.[13,14] From this reaction, a less polar compound was also isolated (6%), which was unambiguously identified as **4** by the use of 1D and 2D NMR experiments.[15–17] Through the optimization of the reaction conditions, we have concluded that the percentage of this by-product (**4**) can be minimized, if the temperature during the anion formation doesn't exceed –40 °C.

The anomeric mixture of the hemiacetals **3** was then treated with boron trifluoride diethyletherate in dichloro-

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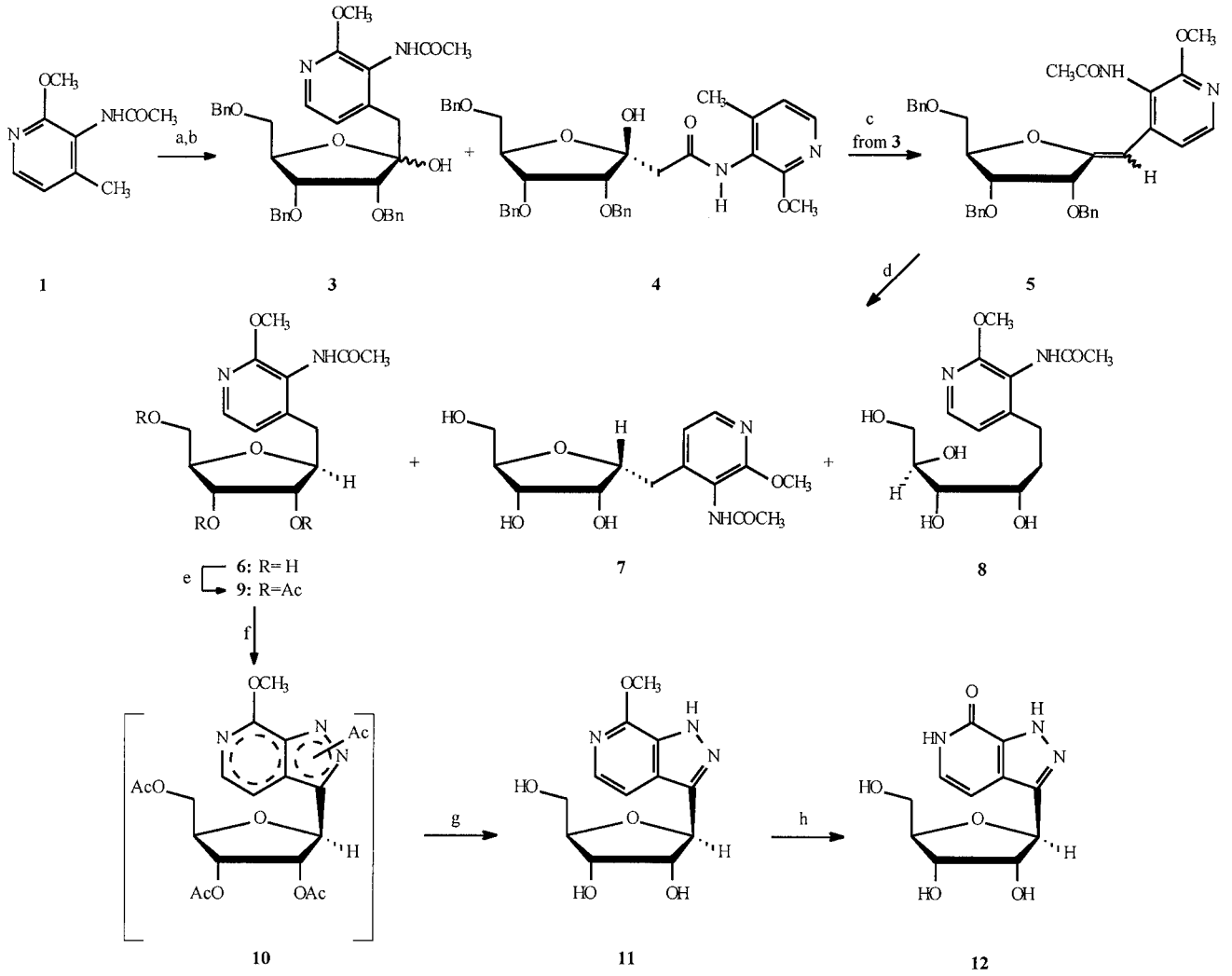

Scheme a) *n*-BuLi, THF, –78 °C to –40 °C, b) 2,3,5-tri-*O*-benzylribonolactone (**2**), THF, –78 °C, c) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0–5 °C, d) H_2, Pd/C, EtOH, e) Ac_2O, pyridine, r.t., f) AcOK, Ac_2O, isoamylnitrite, benzene, 90 °C, g) NH_3–MeOH, r.t., h) HCl/MeOH, r.t.

methane solution to result almost quantitatively in the *E*/*Z* mixture **5**,[18] which was subjected to catalytic hydrogenation, to give the deprotected derivatives **6** and **7** (86%, 1/2 ratio), along with a small amount (12%) of the open–chain product **8**. Although the lability of aromatic C-glycosides towards hydrogenolysis of the C–O bond at the benzylic position is well established,[19] in this case the existence of the methylene linker allows the almost exclusive formation of the desired C-glycosides **6** and **7**.[20] Both anomers were separated by column chromatography and their structure was unambiguously determined by the use of nOe experiments. The less polar β-anomer showed a clear cross-peak between the aromatic 5-H and the 5′-H, while for the more polar α-anomer, we observed strong couplings between the 1′-H with both 2′-H and 3′-H. The β-anomer (**6**) was subsequently acetylated to provide **9**, which was then refluxed in benzene with isoamyl nitrite, in the presence of acetic anhydride[8] to result, through the rearrangement of the intermediate *N*-nitroso compound, in a mixture of the 1- and 2-acetylpyrazolo[3,4-*c*]pyridines **10** (85%). The acetyl groups were easily removed by treatment with methanolic ammonia to afford quantitatively the corresponding deprotected derivative **11**. This nucleoside was finally treated with an ethanolic saturated HCl solution at room temperature to provide a 90% yield of the target compound **12**.[21]

In conclusion, we have developed an efficient method for preparing C-nucleosides through the incorporation of an appropriately substituted picoline at the anomeric position of sugars, followed by the transformation of the pyridine ring into the purine-like heterocyclic base. The application of this methodology allowed us to accomplish the first preparation of the 4-deazaformycin B.

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References

(1) (a) Revankar, G. R.; Robins, R. K. In *Chemistry of Nucleosides and Nucleotides*, Vol. 2; Townsend, L. B., Ed.; Plenum Press: New York, **1994**, 161–398. (b) Srivastava, P. C.; Robins, R. K.; Meyer, R. B. In *Chemistry of Nucleosides and Nucleotides*, Vol. 3; Townsend, L. B., Ed.; Plenum Press: New York, **1994**, 421–535. (c) Perigaud, C.; Gosselin, G.; Imbach, J. L. *Nucleosides Nucleotides* **1992**, *11*, 903. (d) De Clercq, E. *Nucleosides Nucleotides* **1994**, *12*, 1271.

(2) (a) Hori, M.; Ito, E.; Takida, T.; Koyama, Y.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1964**, *17A*, 96. (b) Long, R. A.; Lewis, A. F.; Robins, R. K.; Townsend, L. B. *J. Org. Chem.* **1971**, *36*, 2443. (c) Lewis, A. F.; Townsend, L. B. *J. Am. Chem. Soc.* **1982**, *104*, 1073. (d) Orozco, M.; Canela, E. I.; Franco, R. *Mol. Pharmacol.* **1989**, *35*, 257.

(3) (a) Robins, R. K.; Townsend, L. B.; Cassidy, F.; Gersrter, F. G.; Lewis, A. T.; Miller, R. L. *J. Heterocycl. Chem.* **1966**, *3*, 110. (b) Koyama, G.; Umezawa, H. *Antibiot. Ser. A* **1965**, *18*, 175.

(4) (a) Ishizuka, M.; Sawa, T.; Hori, S.; Takayama, T.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1968**, *21*, 5. (b) Sheen, M. R.; Martin, F. H.; Parks, H. F. Jr. *Mol. Pharmacol.* **1970**, *6*, 255.

(5) Otter, B. A.; Patil, S. A.; Klein, R. S. *Abstract CARB 046, the 4th Chemical Congress of North America and the 202nd American Chemical Society Meeting*; ACS: New York, **August 1991**.

(6) Ward, D. C.; Fuller, W.; Reich, E. *Proc. Natl. Acad. Sci. U.S.A.* **1969**, *62*, 581.

(7) (a) Gudmundsson, K. S.; Drach, J. C.; Townsend, L. B. *J. Org. Chem.* **1997**, *62*, 3453. (b) Walker, J. A. I. I.; Liu, W.; Wise, D. S.; Drach, J. C.; Townsend, L. B. *J. Med. Chem.* **1998**, *41*, 1236.

(8) Marakos, P.; Pouli, N.; Wise, S. D.; Townsend, L. B. *Synlett* **1997**, 561.

(9) Chapman, D.; Hurst, J. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2398.

(10) Beumel, O. F. Jr.; Smith, W. N.; Rubalka, B. *Synthesis* **1974**, 43.

(11) (a) Cousineau, T. J.; Secrist, J. A. III *J. Org. Chem.* **1979**, *44*, 4351. (b) Timpe, W.; Dax, K.; Wolf, N.; Weidmann, H. *Carbohydr. Res.* **1975**, *69*, 51.

(12) For hemiacetals **3** the prefix α refers to the position of the glycosidic OH group relative to the configuration at the reference C-atom (C-4′ in **3**; i.e. the methylpyridinyl moiety is in the β-position). For C-glycosides (no glycosidic OH present), the prefix α-D refers to the alkyl (or aryl) position relative to the reference C-atom.

(13) Optimized procedure for the preparation of the hemiacetals **3**: To a solution of **1** (0.5 g, 2.72 mmol) in dry THF (30 ml) at –78 °C was added under argon *n*-BuLi (4.3 ml, 6.95 mmol, 1.6 M solution in hexanes). The resulting light yellow solution was stirred at –78 °C for 15 min and the temperature then raised to –40 °C for 1 h. The orange-colored solution was then cooled to –78 °C, a solution of the D-ribonolactone **2** (1.4 g, 3.34 mmol) in dry THF (10 ml) was added dropwise and the resulting mixture was stirred at –78 °C for 1 h and at –40 °C for an additional 5 h. A saturated ammonium chloride solution was then added to the reaction mixture to quench the excess *n*-BuLi. The solvent was vacuum-evaporated, water was added to the residue and this was extracted with dichloromethane. The organic extracts were dried (Na_2SO_4) and concentrated to dryness to give an oil, which was purified by flash chromatography (silica gel 20 × 2 cm) using a mixture of cyclohexane–ethyl acetate, 7:3 v/v as the eluent to give the isomeric hemiacetals **3** (63%), together with **4** (6%).

(14) The configuration at C-1′ was assigned on the basis of nOe spectral data. In the case of the β-D anomer clear correlation peaks between the OH-1′ and the H-2′, H-3′ and H-5′ were observed.

(15) In the HMBC spectrum of **4**, a strong correlation peak between the 5-aromatic H and the carbon of the methyl group (^3J coupling) is evident. The methyl group protons correlate also with the aromatic carbons 3,5 (^3J coupling) and 4 (^2J coupling). The methylene group protons possess a strong correlation with both the anomeric carbon and the carbonyl. NOESY data provided evidence for the β-conformation, since we observed correlation peaks between the methylene protons attached at the anomeric position with H-4′.

(16) Data of 3-[(1-hydroxy-2,3,5-tri--benzyl)-β-D-ribofurano-syl)acetylamino]-2-methoxy-4-methylpyridine(**4**): Colorless oil. ^{1}-NMR (400 MHz, $CDCl_3$) δ 1.95 (s, 3 H, 4-CH_3), 2.78 (m, 2 H, $COCH_2$), 3.38 (m, 2 H, H-5′), 3.73 (d, 1 H, $J_{2',3'}$ = 4.56 Hz, H-2′), 3.85 (m, 1 H, H-3′), 3.99 (s, 3 H, OCH_3), 4.29 (m, 1 H, H-4′), 4.32–4.68 (m, 7 H, 3 × CH_2-Ph, OH), 6.70 (d, 1 H, $J_{5,6}$ = 4.98 Hz, H-5), 7.2–7.4 (m, 15 H, 3 × C_6H_5), 7.84 (d, 1 H, $J_{6,5}$ = 4.98 Hz, H-6), 7.94 (br s, 1 H, D_2O exchangeable, NH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 18.0 (4-CH_3), 41.2 ($COCH_2$), 53.3 (CH_3O), 69.3 (C-5′), 72.3 (CH_2-Ph), 72.6 (CH_2-Ph), 73.3 (CH_2-Ph), 76.9 (C-3′), 78.9 (C-2′), 80.4 (C-4′), 105.0 (C-1′), 118.9 (C-5), 119.1 (C-3), 127.7 [CH(Ph)], 128.0 [CH(Ph)], 128.2 [CH(Ph)], 128.3 [CH(Ph)], 128.6 [CH(Ph)], 136.7 [C(Ph)], 136.9 [C(Ph)], 137.6 [C(Ph)], 145.6 (C-4), 146.0 (C-6), 158.4 (C-2), 169.2 (C=O). Anal. Calcd. For $C_{35}H_{38}N_2O_7$: C: 70.21, H: 6.40, N: 4.68. Found: C: 70.10, H: 6.62, N: 4.63.

(17) The excess *n*-butyllithium (two equivalents) required for the lithiation of **1**, induces the formation of an anion on the acetamide's methyl group, which probably attacks the ribonolactone to provide **4**.

(18) The use of acid labile protecting groups for the 5-OH of the lactone component (e.g. TBDMS) should be avoided, since the corresponding 1′,5′-anhydro derivative is obtained from this reaction as the major product, irrespective of the reaction conditions or of the Lewis acid used for catalysis.

(19) Krohn, K.; Heidi, H.; Wielkens, K. *J. Med. Chem.* **1992**, *35*, 511.

(20) Preparation of **6**: To a solution of the anomers **3** (0.5 g, 0.84 mmol) in dry CH_2Cl_2 (20 ml) at 0 °C was added under argon $BF_3{\cdot}Et_2O$ (0.22 ml, 1.68 mmol). The solution was stirred at 5–10 °C for 5 h and then, was neutralized with a saturated $NaHCO_3$ solution. The mixture was extracted with CH_2Cl_2 and the combined organic extracts were dried (Na_2SO_4) and concentrated to dryness. The residue was purified by flash chromatography (silica gel, 18 × 1 cm) using EtOAc as the eluent to give 0.46 g (95%) of an inseparable *E*/*Z* mixture of **5**. This mixture was dissolved in absolute EtOH (20 mL) and hydrogenated (10% Pd/C, 90 mg) at 50 psi for 5 h. The catalyst was filtered off, washed with EtOH, the solvent was vacuum-evaporated and the residue was purified by column chromatography (CH_2Cl_2–MeOH, 97:3, silica gel, 18 × 1 cm), to give **6**, together with the corresponding α-anomer(**7**). 3-Acetamido-2-methoxy-4-[(β-D-ribofuranosyl)methyl]py-ridine(**6**): White foam. 250 mg (29%). ^{1} NMR (400 MHz, $CDCl_3$) δ 1.98 (s, 3 H, $COCH_3$), 2.53 (dd, 1 H, J = 9.15 Hz, 14.64 Hz, $pyCH_2$), 2.78 (dd, 1 H, J = 4.03 Hz, 14.64 Hz, $pyCH_2$), 3.36 (d, 1 H, $J_{4',5'}$ = 5.12 Hz, $J_{5',5'}$ = 11.71 Hz, H-5′), 3.41 (d, 1 H, $J_{4',5'}$ = 4.03 Hz, $J_{5',5'}$ = 11.71 Hz, H-5′), 3.59 (m, 2 H, H-2′, H-4′), 3.81 (m, 5 H, H-1′, H-3′, OCH_3), 4.88 (m, 1 H, OH, D_2O exchangeable), 5.07 (m, 2 H, 2 × OH, D_2O

exchangeable), 6.96 (d, 1 H, $J_{5,6}$ = 5.12 Hz, H-5), 7.93 (d, 1 H, $J_{6,5}$ = 5.12 Hz, H-6), 9.21 (br s, 1 H, NHAc, D_2O exchangeable). ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.6 (CH_3-CO), 36.0 ($pyCH_2$), 53.6 (OCH_3), 61.6 (C-5′), 70.8 (C-3′), 74.62 (C-2′), 81.45 (C-4′), 84.32 (C-1′), 118.7 (C-5), 120.1 (C-3), 143.9 (C-6), 147.6 (C-4), 159.7 (C-2), 169.0 (C=O). Anal. Calcd. for $C_{14}H_{20}N_2O_6$: C: 53.84, H: 6.45, N: 8.97. Found: C: 53.69, H: 6.17, N: 8.76. 3-Acetamido-2-methoxy-4-[(α-D-ribofuranosyl)methyl]pyridine(**7**): Mp: 187 °C (MeOH). 490 mg (57%). ^{1} NMR (400 MHz, $CDCl_3$) δ 2.02 (s, 3 H, $COCH_3$), 2.72 (dd, 1 H, J = 8.05 Hz, 14.27 Hz, $pyCH_2$), 2.74 (dd, 1 H, J = 5.12 Hz, 14.27 Hz, $pyCH_2$), 3.32 (d, 1 H, $J_{4',5'}$ = 5.13 Hz, $J_{5',5'}$ = 12.08 Hz, H-5′), 3.48 (d, 1 H, $J_{4',5'}$ = 2.20 Hz, $J_{5',5'}$ = 12.08 Hz, H-5′), 3.67 (m, 1 H, H-4′), 3.78 (m, 1 H, H-2′), 3.81 (s, 3 H, OCH_3), 3.88 (m, 1 H, H-3′), 4.04 (m, 1 H, H-1′), 4.59 (m, 1 H, OH, D_2O exchangeable), 4.84 (m, 2 H, 2 × OH, D_2O exchangeable), 6.98 (d, 1 H, $J_{5,6}$ = 5.12 Hz, H-5), 7.92 (d, 1 H, $J_{6,5}$ = 5.12 Hz, H-6), 9.28 (br s, 1 H, NHAc, D_2O exchangeable). ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.7 (CH_3CO), 31.3 ($pyCH_2$), 53.3 (OCH_3), 61.7 (C-5′), 71.9 (C-3′), 72.1 (C-2′), 79.1 (C-4′), 81.9 (C-1′), 118.7 (C-5), 120.0 (C-3), 143.5 (C-6), 148.0 (C-4), 159.5 (C-2), 168.5 (C=O). Anal. Calcd. for $C_{14}H_{20}N_2O_6$: C: 53.84, H: 6.45, N: 8.97. Found: C: 53.55, H: 6.39, N: 9.11.

(21) Data of **12**: Yield: 90%. Mp: 253 °C (dec.) (EtOH). UV (CH_3OH) λ_{max}(nm) ($\varepsilon \times 10^{-3}$): 302 (8.17), 251 (6.62). ^{1} NMR (400 MHz, DMSO-d_6) δ 3.48 (m, 2 H, H-5′), 3.81 (m, 1 H, H-4′), 3.95 (m, 1 H, H-3′), 4.12 (m, 1 H, H-2′), 4.81 (d, 1 H, $J_{1',2'}$ = 6.95 Hz, H-1′), 4.82 (br s, 1 H, D_2O exchangeable, OH-5′), 4.89 (br. s., 1 H, D_2O exchangeable, OH-3′), 4.98 (br. s., 1 H, D_2O exchangeable, OH-2′), 6.68 (d, 1 H, $J_{4,5}$ = 6.22 Hz, H-4), 6.88 (d, 1 H, $J_{5,4}$ = 6.22 Hz, H-5), 11.2 (br s, 1 H, D_2O exchangeable, NH-6), 13.8 (br. s., 1 H, D_2O exchangeable, NH-1). ^{13}C NMR (50 MHz, DMSO-d_6) δ 62.2 (C-5′), 71.5 (C-3′), 75.1 (C-2′), 79.5 (C-1′), 85.5 (C-4′), 99.3 (C-4), 123.4 (C-3α), 132.8 (C-7α), 125.4 (C-5), 145.8 (C-3), 154.3 (C-7). Anal. Calcd. for $C_{11}H_{13}N_3O_5$: C: 49.44, H: 4.90, N: 15.72. Found: C: 49.60, H: 5.12, N: 15.51.