The Synthesis of 6-Deazaformycin A

Tony Tite, Nikolaos Lougiakis, Panagiotis Marakos, Nicole Pouli*

Department of Pharmacy, Division of Pharmaceutical Chemistry, University of Athens, Panepistimiopolis Zografou, 15771 Athens, Greece
Fax +30(210)7274747; E-mail: pouli@pharm.uoa.gr

Received 21 July 2009

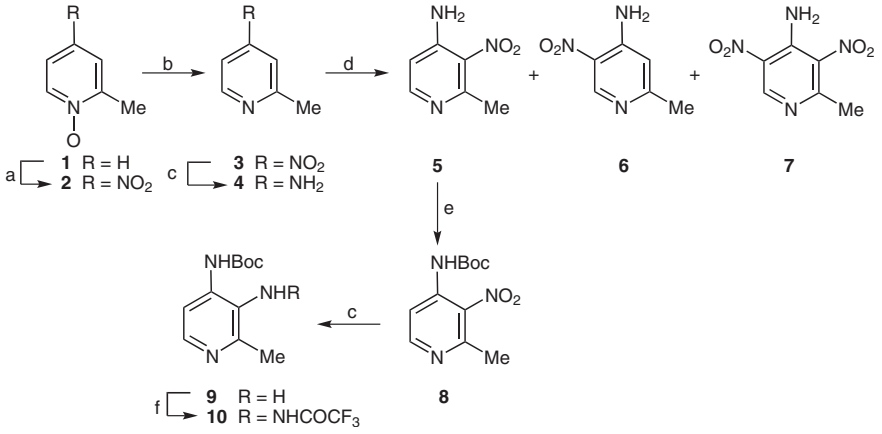
Abstract: The synthesis of the new *C*-nucleoside 6-deazaformycin A was achieved through the condensation of a suitably substituted lithiated 2-picoline with 2,3,5-*tri*-*O*-benzyl-D-ribonolactone, borohydride reduction of the resulting hemiacetals, followed by intramolecular Mitsunobu cyclization of the carbinols, manipulation of the protecting groups, and subsequent ring closure to result in the formation of 7-amino-3-(β-D-ribofuranosyl)pyrazolo[4,3-*b*]pyridine.

Key words: *C*-nucleosides, heterocycles, formycin A, pyrazolo[4,3-*b*]pyridine

The limited use of currently approved nucleosides due to the emergence of poor pharmacokinetic properties, metabolic degradation, cross resistance, and inherent toxicity has stimulated the development of more efficient and stable analogues. The subclass of *C*-nucleosides embodies bioactive natural compounds with an increased hydrolytic and enzymatic stability when compared to the more labile *N*-nucleosides.[1] Among purine-like *C*-nucleosides formycin A (7-amino-3-β-D-ribofuranosyl)-1*H*-pyrazolo[4,3-*d*]pyrimidine), a naturally occurring bioisostere of adenosine isolated from *Nocardia interforma*[2] possesses a broad spectrum of antibiotic activity (antimetabolic, antiviral, antineoplastic, and antiparasitic activities)[3] mainly attributed to its ability to replace adenosine in a variety of enzymatic reactions and affect purine nucleoside and nucleic acid metabolism via triphosphorylation by adenosine kinase.[4] Formycin A is readily deaminated by the catabolic enzyme adenosine deaminase (ADA) to the less active inosine analogue, formycin B, another naturally occuring *C*-nucleoside.[5] The high toxicity associated with the formycins has restricted their use for clinical development, but on the other hand has stimulated interest on the synthesis of structural analogues and their study from both chemical and biological points of view. A considerable number of base-modified analogues has been prepared and tested, including 5-chloro,[6] 5-fluoro,[7] 5-aminoformycin A,[7] 5,7-disubstituted deazaformycin A,[8] and 2- or 3-deoxyformycin A.[8] Some triazolo-*C*-nucleosides related to formycin A have been also prepared and have demonstrated significant cytotoxicity against L1210 and WIL2 leukemia cells in culture.[9]

During the last few years we have been involved in the study of the deazaformycins, which can provide useful informations on the importance of the heteroatoms present on the formycin scaffold, concerning the ability of these molecules to behave as antimetabolites and to possess cytotoxic activity thereof.[10] As a continuation of this ongoing medicinal chemistry effort, we present herein the synthesis of the new isomeric *C*-nucleoside 6-deazaformycin A {7-amino-3-(β-D-ribofuranosyl)-pyrazolo[4,3-*b*]pyridine}.

The synthesis of the target compound was effected through the lithium-mediated coupling of *tert*-butyl-*N*-[3-trifluoroacetamido-2-methylpyridin-4-yl]carbamate (**10**) with the easily accessible 2,3,5-tri-*O*-benzyl-D-ribonolac-

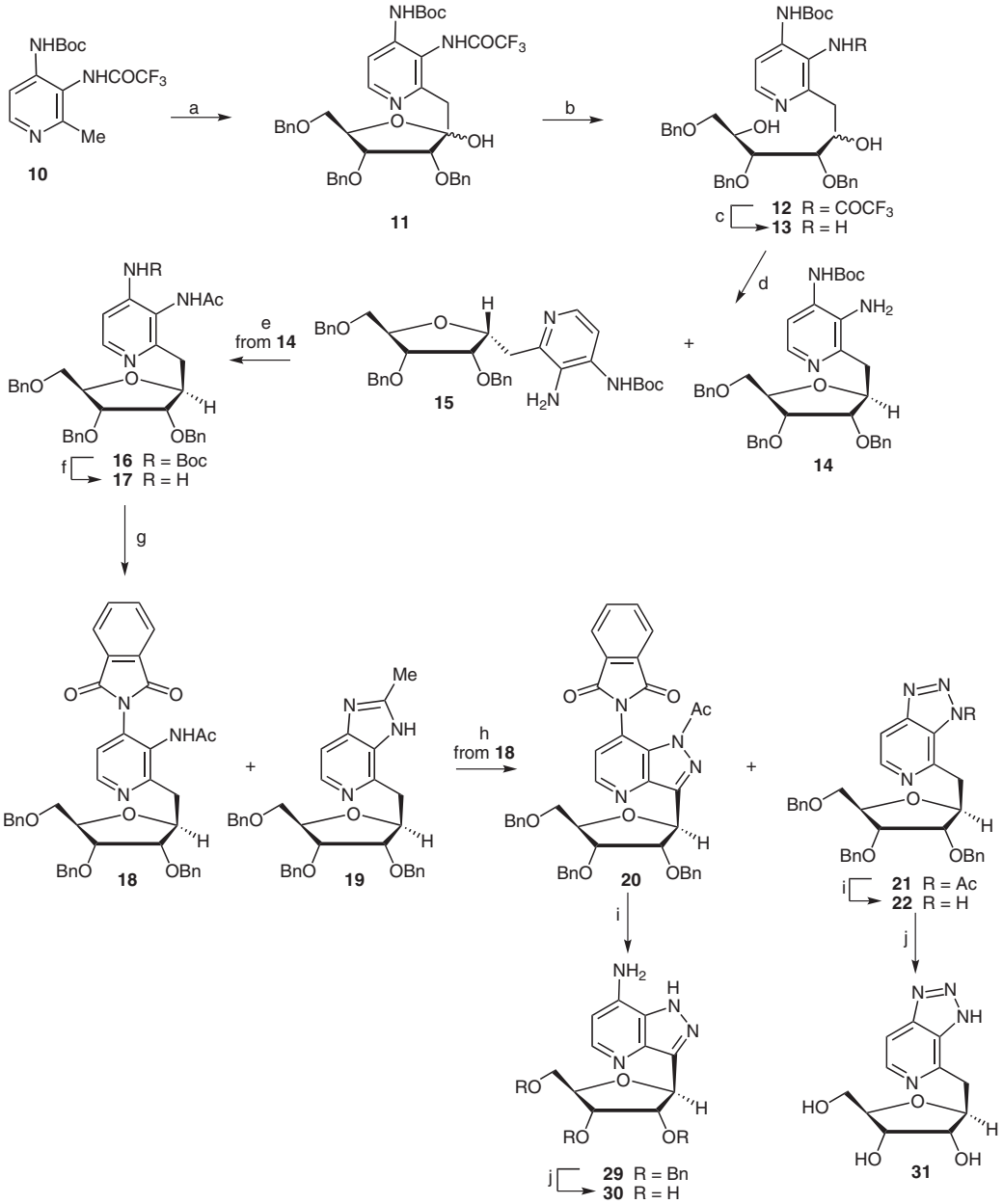


Scheme 1 *Reagents and conditions*: (a) H_2SO_4, HNO_3 (65%), 160 °C, 3 h, 96%; (b) PCl_3, $CHCl_3$, r.t., 18 h, 83%; (c) H_2, 10% Pd/C, EtOH, r.t., 5 h, yield almost quantitative; (d) H_2SO_4, HNO_3 (65%), 65 °C, 3 h, 38% for **5**; (e) THF, NaH (2.5 equiv), 80 min, r.t, then $(Boc)_2O$ (1.1 equiv), 3 h, 88%; (f) $(CF_3CO)_2O$, CH_2Cl_2, r.t., 3 h, 95%.

SYNLETT 2009, No. 18, pp 2927–2930
Advanced online publication: 02.10.2009
DOI: 10.1055/s-0029-1218002; Art ID: D19309ST

tone.[11] The use of the trifluoroacetamide **10** in the lithiation reaction, instead of the corresponding acetamide, was based on our previous observation[10c] concerning the predominance of anion formation on the acetamide methyl rather than on the methyl group of 2-picoline, due to its low acidity. The carbamate **10** was synthesized in seven steps from the commercially available 2-picoline *N*-oxide (**1**, Scheme 1). Compound **1** was nitrated and the resulting nitro-derivative **2** was treated with phosphorus trichloride to provide the picoline **3** which was reduced by catalytic hydrogenation into the previously reported 4-amino-2-picoline **4**.[12] Compound **4** was then nitrated,[13] and the desired nitro isomer **5** was converted into the trifluoroacetamide **10** upon treatment with Boc anhydride, catalytic reduction, and trifluoroacetylation.

The lithiation of the trifluoroacetamide **10** was accomplished by using 4 equivalents of *n*-butyllithium in dry THF at –78 °C. The resulting 2-methylene anion attacks the carbonyl of 2,3,5-tri-*O*-benzyl-D-ribonolactone and results into the anomeric mixture of hemiacetals **11** (Scheme 2). The crude mixture **11** was reduced with sodium borohydride in dry methanol to provide the diastereomeric carbinols **12** (2 steps, 43%). The trifluoroacetyl group of **12** was then selectively cleaved using a saturated potassium carbonate solution and the resulting aminodiols **13** were submitted to Mitsunobu cyclization.[14] The yield



Scheme 2 *Reagents and conditions* (a) *n*-BuLi (4 equiv), 2,3,5-tri-*O*-benzyl-D-ribonolactone, THF, –78 °C, 3 h; (b) $NaBH_4$ (4 equiv), MeOH, r.t., 90 min, 43% (2 steps); (c) K_2CO_3, MeOH, H_2O, 65 °C, 12 h, 64%; (d) Ph_3P (1.7 equiv), THF, DEAD (2.5 equiv), 60 °C, 90 min, 73% for **14**, 14% for **15**; (e) Ac_2O, CH_2Cl_2, r.t., 2 h, 93%; (f) CH_2Cl_2, TFA, r.t., 12 h, 70%; (g) phthalic anhydride (3.5 equiv), Et_3N, THF–toluene, reflux, 36 h, 69% for **18**, 7% for **19**; (h) KOAc (1.5 equiv), Ac_2O (3 equiv), isoamyl nitrite (1.5 equiv), toluene, 100 °C, 15 h, 30% for **20**, 20% for **21**, 43% for **22**; (i) MeOH, NH_3, r.t., 15 min, 96%; (j) CH_2Cl_2, BCl_3, –78 °C, 2 h, 93%.

of this reaction is highly improved by the use of the aminopyridine **13** rather than the trifluoroacetamide **12**. Chromatographic purification of the reaction mixture provided the desired less polar β-epimer **14** in 73% yield,[15] along with a minor amount (14%) of the α-epimer **15**. The stereochemistry was unambiguously determined on the basis of NOE spectroscopic data, where we observed a clear cross peak between H-1′ and H-4′ for **14**; whereas a clear cross peak between H-1′, H-2′, and H-3′ was characteristic in the case of the α-anomer **15**. In order to proceed to the pyrazolopyridine ring formation, the β-anomer **14** was converted to the corresponding acetamide **16**, and the *N*-Boc protecting group was replaced with *N*-phthaloyl, since we had previously found that the carbamate group is not stable under the cyclization reaction conditions.[10b,16] The *N*-phthaloyl derivative **18** was prepared upon refluxing a solution of **17** using triethylamine and excess phthalic anhydride in order to minimize (7%) the inevitable formation of 2-methylimidazolo[4,5-*c*]pyridine **19**,[10b] which is obtained as a byproduct, due to intermolecular nucleophilic attack of the 4-amino group of **17** to the 3-acetamide carbonyl. Treatment of **18** with isoamyl nitrite in the presence of potassium acetate and acetic anhydride in refluxing toluene[17] provided, through rearrangement of the intermediate *N*-nitroso compound, the 1-acetylpyrazolo[4,3-*b*]pyridine **20**, but in rather poor yield (30%). This reaction resulted in the formation of a considerable amount of compound **21**, which was partly deacetylated during the silica gel column purification into **22**. ^{1}H NMR analysis of **22** revealed the presence of the methylene linker as a broad signal which was ascertained through ^{13}C NMR and DEPT experiments (δ = 40.74 ppm corresponding to the methylene group). Mass spectrometric analysis of **22** [MS + 1 = 537; MS – 28 (N_2) = 508], permitted the characterization of the compound as a triazolopyridine *C*-nucleoside (Scheme 2).

In order to confirm this observation we have studied the cyclization on the phthalimide **25** which was easily prepared from **9** (Scheme 3). Heating **25** in toluene provided the 7-phthalimidopyrazolo[4,3-*b*]pyridine (**26**) in poor yield (24%), whereas the acetate of the triazolo[4,5-*c*]-pyridine **27** as well as the corresponding deacetylated derivative **28** were the major products isolated upon purification by column chromatography. These results provided evidence on the difference of reactivity between the 3-acetamido-4-phthalimido-2-picoline and the previously reported isomeric 3-acetamido-2-phtalimido-4-picoline.[10b] It seems that the low acidity of the 2-picoline methyl group enables this alternative cyclization; intramolecular nucleophilic attack of the intermediate diazonium species[18] on the phthalimide carbonyl results in amino group deprotection and, under these conditions, the formation of the 1,2,3-triazolo[4,5-*c*]pyridine ring system is highly favorable.[19]

Both the *N*-phthaloyl and the acetyl groups of compounds **20** and **21** were easily cleaved upon reaction with methanolic ammonia and the resulting tri-*O*-benzyl derivatives **29** and **22** were converted, respectively, to the target 6-deazaformycin A (**30**)[20] and the substituted triazolopyridine **31**.[21]

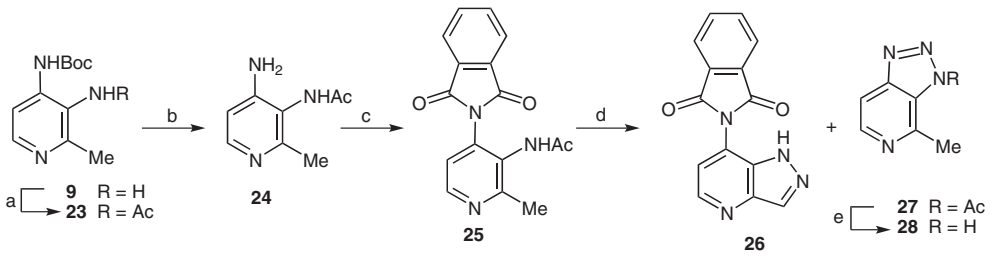
In conclusion, we have developed an efficient method to prepare the novel *C*-nucleoside 6-deazaformycin A. The synthesis was accomplished via the attachment of a protected ribonolactone to a substituted picoline followed by reduction of the resulting hemiacetals. Mitsunobu cyclization of the derived diols furnished stereoselectively the desired β-glycoside which upon ring closure provided the pyrazolo[4,3-*b*]pyridine *C*-nucleoside, along with a considerable amount of a triazolopyridine derivative.

Acknowledgment

This work was supported by a Research Grant provided by the Greek Ministry of Industry, Energy, and Technology (Program ENTER).

References and Notes

(1) Watanabe, K. A. In *Chemistry of Nucleosides and Nucleotides*, Vol. 3; Townsend, L. B., Ed.; Plenum Press: New York, **1994**, 421–535.
(2) (a) Hori, M.; Ito, E.; Takita, T.; Umezawa, H. *J. Antibiot., Ser. A* **1964**, *17*, 96. (b) Koyama, G.; Umezawa, H. *J. Antibiot. Ser. A* **1965**, *18*, 175.
(3) (a) Bzowska, A. In *Modified Nucleosides in Biochemistry, Biotechnology and Medicine*; Herdewijn, P., Ed.; Wiley: New York, **2008**, 473–510. (b) Hidaka, T.; Katayama, K.; Yamashita, K.; Yamashita, T.; Watanabe, K.; Shimazaki, M.; Ohno, M.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1980**, *33*, 303. (c) Rosowsky, A.; Solan, V. C.; Gudas, L. J. *J. Med. Chem.* **1986**, *28*, 1096. (d) Giziewicz, J.; De Clercq, E.; Luczak, M.; Shugar, D. *Biochem. Pharmacol.* **1975**, *24*, 1813. (e) Orozco, M.; Canela, E. I.; Franco, R. *Mol. Pharmacol.* **1989**, *35*, 257.
(4) Malaisse, W. J.; Sener, A.; Gruber, H. E.; Erion, M. D. *Biochem. Med. Metabolic Biol.* **1994**, *53*, 22.



Scheme 3 *Reagents and conditions*: (a) Ac_2O, CH_2Cl_2, r.t., 4 h, 95%; (b) CH_2Cl_2, TFA, r.t, 15 h, 97%; (c) phthalic anhydride (3.5 equiv), toluene–THF, Et_3N, reflux, 12 h, 63%; (d) KOAc (1.5 equiv), Ac_2O (3 equiv), isoamylnitrite (1.5 equiv), toluene, 100 °C, 15 h, 24% for **26**, 21% for **27**, 37% for **28**; (e) MeOH, NH_3, r.t., 15 min, yield almost quantitative.

(5) Ishizuka, M.; Sawa, T.; Koyama, G.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1968**, *21*, 1.
(6) Upadhya, K. G.; Sanghvi, Y. S.; Robins, R. K.; Revankar, G. R.; Ugarkar, B. G. *Nucleic Acids Res.* **1986**, *25*, 1747.
(7) Secrist, J. A. III.; Shortnacy, A. T.; Montgomery, J. A. *J. Med. Chem.* **1985**, *28*, 1740.
(8) Liu, M.-C.; Luo, M.-Z.; Mozdziesz, D. E.; Sartorelli, A. C. *Nucleosides, Nucleotides Nucleic Acids* **2005**, *24*, 45.
(9) Ramasamy, K.; Ugarkar, B. G.; McKernan, P. A.; Robins, R. K.; Revankar, G. R. *J. Med. Chem.* **1986**, *29*, 2231.
(10) (a) Kourafalos, V. N.; Marakos, P.; Pouli, N.; Townsend, L. B. *Synlett* **2002**, 1479. (b) Kourafalos, V. N.; Marakos, P.; Pouli, N.; Townsend, L. B. *J. Org Chem.* **2003**, *68*, 6466. (c) Korouli, S.; Lougiakis, N.; Marakos, P.; Pouli, N. *Synlett* **2008**, 181.
(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) Reich, M. F.; Fabio, P. F.; Lee, V. J.; Kuck, N. A.; Testa, R. T. *J. Med. Chem.* **1989**, *32*, 2474.
(13) (a) Deady, L. W.; Korytsky, O. L.; Rowe, J. E. *Aust. J. Chem.* **1982**, *35*, 2025. (b) Indumathy, M.; Malcolm, R. *J. Heterocycl. Chem.* **1992**, *29*, 359.
(14) Guianvarc'h, D.; Fourrey, J.-L.; Tran Huu Dau, M.-E.; Guerineau, V.; Benhida, R. *J. Org. Chem.* **2002**, *67*, 3724.
(15) **Data for *tert*-Butyl-*N*-[3-amino-2-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosylmethyl)pyridin-4-yl]carbamate (14)**
Oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.55 (s, 9 H, *t*-Bu), 3.00 (dd, 1 H, 2-CH_2, *J* = 7.33, 13.69 Hz), 3.10 (dd, 1 H, 2-CH_2, *J* = 3.91, 13.69 Hz), 3.42 (dd, 1 H, H-5′, $J_{5',4'}$ = 4.40 Hz, $J_{5',5'}$ = 10.27 Hz), 3.45–3.56 (br s, 2 H, D_2O exchange, NH_2), 3.56 (dd, 1 H, H-5′, $J_{5',4'}$ = 2.94 Hz, $J_{5',5'}$ = 10.27 Hz), 3.72 (t, 1 H, H-3′, *J* = 5.87 Hz), 3.92 (t, 1 H, H-2′, *J* = 4.40 Hz), 4.16–4.21 (m, 1 H, H-4′), 4.38–4.68 (m, 7 H, H-1′, 6 × CH_2Ph), 6.91 (s, 1 H, D_2O exchange, NHBoc), 7.23–7.41 (m, 15 H, 3 × C_6H_5), 7.63 (d, 1 H, H-5, $J_{5,6}$ = 5.38 Hz), 8.05 (d, 1 H, H-6, $J_{6,5}$ = 5.38 Hz). ^{13}C NMR (50 MHz $CDCl_3$): δ = 28.40 [(CH_3)$_3$CCONH], 39.34 (CH_2), 69.64 (C-5′), 72.08, 73.41 (CH_2Ph), 77.16 (C-3′), 79.61 (C-2′), 80.52 (C-4′), 81.29 [(CH_3)$_3$*C*CONH], 82.60 (C-1′), 112.73 (C-5), 127.80, 127.88, 128.14, 128.45, 128.54 (tertiary benzylic CH), 130.80 (C-3), 136.62 (C-4), 137.93, 138.09, 138.16 (quaternary benzylic C), 142.27 (C-6), 148.53 (C-2),152.58 (CO).
(16) Kourafalos, V. N.; Marakos, P.; Pouli, N.; Terzis, A.; Townsend, L. B. *Heterocycles* **2002**, *57*, 2335.
(17) Marakos, P.; Pouli, N.; Wise, D.; Townsend, L. B. *Synlett* **1997**, 561.
(18) Chapman, D.; Hurst, J. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2398.
(19) Jones, G. *Adv. Heterocycl. Chem.* **2002**, *83*, 1.
(20) **Data for 7-Amino-(3-β-D-ribofuranosyl)pyrazolo[4,3-*b*]pyridine (30)**
Pale yellow foam. ^{1}H NMR (400 MHz, CD_3OD): δ = 3.82 (dd, 1 H, H-5′, $J_{5',4'}$ = 12.13 Hz, $J_{4',5''}$ = 2.35 Hz), 3.94 (dd, 1 H, H-5′, $J_{5',4'}$ = 12.13 Hz, $J_{4',5''}$ = 2.74 Hz), 4.15–4.19 (m, 1 H, H-4′), 4.28 (dd, 1 H, H-3′, $J_{3',2'}$ = 5.08 Hz, $J_{3',4''}$ = 3.52 Hz), 4.48 (dd, 1 H, H-2′, $J_{2',3'}$ = 5.08 Hz, $J_{2',1''}$ = 7.04 Hz), 5.22 (d, 1 H, H-1′, $J_{1',2''}$ = 7.04 Hz), 6.65 (d, 1 H, H-6, $J_{6,5}$ = 5.86 Hz), 8.07 (d, 1 H, H-5, $J_{5,6}$ = 5.86 Hz). ^{13}C NMR (50 MHz, CD_3OD): δ = 63.28 (C-5′), 73.74 (C-3′), 77.50 (C-2′), 80.80 (C-1′), 87.16 (C-4′), 102.89 (C-6), 129.15 (C-7α), 132.90 (C-3α), 143.15 (C-5), 143.22 (C-3), 146.97 (C-7). Anal. Calcd for $C_{11}H_{14}N_4O_4$: C, 49.62; H, 5.30; N, 21.04. Found: C, 49.81; H, 5.22; N, 20.87.
(21) **Data for 4-[(β-D-Ribofuranosyl)methyl]-3*H*-[1,2,3]triazolo[4,5-*c*]pyridine (31)**
Oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.52–3.63 (m, 2 H, CH_2, H-5′), 3.65–3.74 (m, 2 H, CH_2, H-5′), 3.80–3.86 (m, 1 H, H-4′), 4.02 (t, 1 H, H-3′), 4.17 (t, 1 H, H-2′), 4.30–4.37 (m, 1 H, H-1′), 7.81 (d, 1 H, H-7, $J_{7,6}$ = 6.26 Hz), 8.30 (d, 1 H, H-6, $J_{6,7}$ = 6.26 Hz). ^{13}C NMR (50 MHz $CDCl_3$): δ = 38.49 (CH_2), 62.64 (C-5′), 72.21 (C-3′), 76.64 (C-2′), 83.41 (C-4′), 85.95 (C-1′), 109.07 (C-7), 139.48 (C-6), 142.29 (C-3α), 144.67 (C-7α), 153.13 (C-4). Anal. Calcd for $C_{11}H_{14}N_4O_4$: C, 49.62; H, 5.30; N, 21.04. Found: C, 49.55; H, 5.09; N, 21.18.