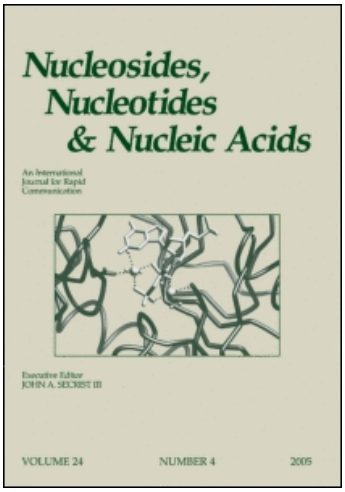

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Nicolai E. Poopeiko[a]; Natalia B. Khripach[a]; Zygmunt Kazimierczuk[b]; Jan Balzarini[c]; Erik De Clercq[a]; Igor A. Mikhailopulo[a]
[a] Institute of Bioorganic Chemistry, Byelorussian Academy of Sciences, Minsk, Byelorussia [b] Institute of Experimental Physics, University of Warsaw, Warsaw, Poland [c] Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

To cite this Article Poopeiko, Nicolai E. , Khripach, Natalia B. , Kazimierczuk, Zygmunt , Balzarini, Jan , De Clercq, Erik and Mikhailopulo, Igor A.(1997) 'Synthesis and Biological Evaluation of 2',3'-Dideoxy-3'-Fluororibofuranosyl Purine Nucleosides', Nucleosides, Nucleotides and Nucleic Acids, 16: 7, 1083 — 1086
To link to this Article: DOI: 10.1080/07328319708006137
URL: http://dx.doi.org/10.1080/07328319708006137

NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 1083-1086 (1997)

SYNTHESIS AND BIOLOGICAL EVALUATION OF 2',3'-DIDEOXY-3'-FLUORORIBOFURANOSYL PURINE NUCLEOSIDES

Nicolai E. Poopeiko[1], Natalia B. Khripach[1], Zygmunt Kazimierczuk[2], Jan Balzarini[3], Erik De Clercq[3], and Igor A. Mikhailopulo[1]*

[1]*Institute of Bioorganic Chemistry, Byelorussian Academy of Sciences, 220141 Minsk, Byelorussia,* [2]*Institute of Experimental Physics, University of Warsaw, 02-089 Warsaw, Poland, and* [3]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium*

ABSTRACT: Synthesis of 9-(2,3-dideoxy-3-fluoro-β-D-ribofuranosyl)-2-chloroadenine (**7**b) and -2-chloro-6-methoxypurine (**9**b), as well as the α-D-anomer **7**a of the former and its N^7 isomer **10**a is reported. Among the compounds synthesized, only the β-D-anomer **7**b displays moderate cytotoxic activity.

The 2-chloro-2'-deoxyadenosine (2-CdA, Cladribine) is established as a highly effective agent in the treatment of hematologic malignancies. It is an adenosine deaminase-resistant analogue of 2'-deoxyadenosine metabolizing to its 5'-triphosphate (2Cl-dATP) which interferes with several cellular processes (for a review, see Ref. 1). Amongst these, 2Cl-dATP was shown to be a good substrate for human DNA polymerases that leads to incorporation of 2Cl-dAMP into growing DNA strands resulting in the reduction of strand elongation and subsequently chain termination. On the other hand, it was shown in previous studies that the replacement of the 3'-hydroxyl group of natural dNTP's by a fluorine atom results in potent chain terminators of DNA biosynthesis[2]. It was, therefore, of interest to synthesize the 3'-deoxy-3'-fluoro *ribo* derivative of cladribine and to evaluate its biological activity.

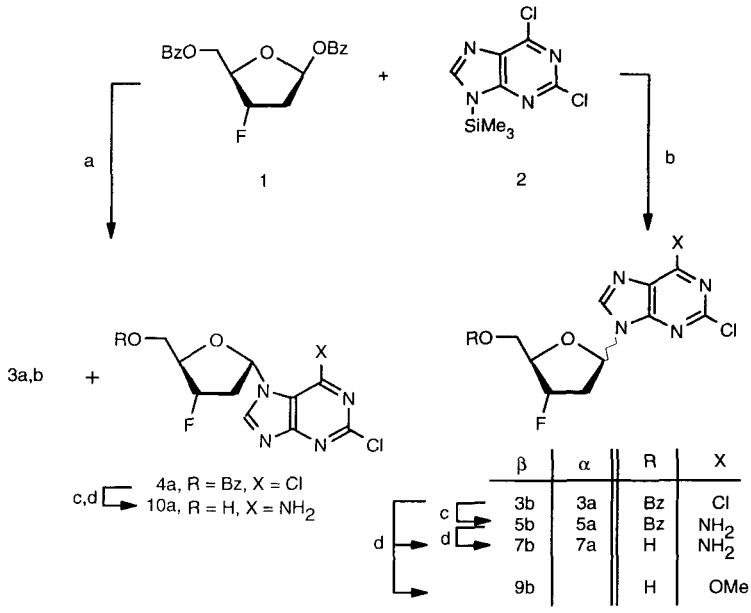
The condensation of 1,5-di-*O*-benzoyl-2,3-dideoxy-3-fluoro-β-D-*erythro*-pentofuranose[3] (**1**) with silylated 2,6-dichloropurine (**2**) in the presence of excess $SnCl_4$ in acetonitrile at r.t., followed by chromatography afforded a mixture of the blocked N^9-β-D- and -α-D-anomers **3**b,a (~2:5; 29%) and the N^7-α-D-nucleoside **4**a (66%). The use of TMS-Tfl instead of $SnCl_4$ and refluxing dichloromethane as solvent gave a ca. 4:5 mixture of the 5'-*O*-benzoylated N^9-β-D- and -α-D-nucleosides **3**a,b (93%). Treatment of

this mixture with a saturated solution of ammonia in 1,2-dimethoxyethane[4] furnished a chromatographically unresolved mixture of **5**a,b as an oil. Standard debenzoylation of the latter with saturated methanolic ammonia, followed by chromatography, gave **7**b (33%) and **7**a (44%). The N^7-α-D-anomer **10**a was prepared from **4**a in a similar way *via* successive amination and then debenzoylation. Amination and deblocking of the ~4:5 mixture of the N^9-β-D- and -α-D-nucleosides **3**a,b with saturated methanolic ammonia, followed by chromatography, afforded **7**b (17%), **7**a (20%) and **9**b (15%).

The structures of the compounds were confirmed by ^{1}H and ^{13}C NMR, and UV spectra. The site of glycosylation of the 2-chloroadenine derivative was unequivocally established by comparison of the ^{13}C NMR[5,6] and UV[7,8] spectra of the corresponding compounds with those of the pairs of related adenine N^7- and N^9-glycosides. The regioisomeric structure and the anomeric configuration were deduced from (*i*) the differences between the chemical shifts of the base carbon atoms, (*ii*) the $^3J_{C5,H8}$ of 11-12 Hz and $^3J_{C4,H8}$ of 4.0-4.5 Hz in the case of N^9-regioisomers and *vice versa* in the case of N^7-glycoside **10**a, and (*iii*) the long-range couplings of C-8 to fluorine of 7.5 Hz and 4.3 Hz that exhibit in ^{13}C NMR spectra of the α-anomers **7**a and **10**a, respectively. The latter couplings are generally indicative of a spatial proximity of the nuclei involved[9] and are not observed in the β-D-anomers **7**b and **9**b.

Inspection of $^3J_{H,H}$ values of the furanose rings of **7**b and **9**b as well as a comparison of splitting patterns for H-1', H-2', H-2", and H-3' with those of computer-generated for hypothetical 2-deoxy-β-D-*erythro*-pentofuranose moieties of nucleosides[10] point clearly to a high preference for the S sugar conformation. This conclusion is consistent with the $^3J_{C5',F}$ values of ca. 11 Hz, as expected[11] for a dihedral angle of ~180° between these atoms in the S region near E_3 conformer. Subsequently, we performed a series of calculations using the least-squares minimization program PSEUROT (version 6.2) in order to calculate the conformational parameters for the most populated S conformer. The geometry of the minor N conformer was constrained (P_N = 18°; ψ_m = 36°) during PSEUROT analyses. In the case of the β-D-anomers **7**b and **9**b the experimental sets of coupling constants were compatible with a high preference for the S conformation (>97%; rms <0.5 Hz), *viz.*, near $_3T^2$ conformer. A similar PSEUROT analysis of the α-D-anomers **7**a and **10**a indicated that the pentofuranose moiety of these nucleosides also exists practically as a single S conformer. This is consistent with the $^3J_{C5',F}$ values of ca. 11 Hz. Hence, the conformational behavior of the 2,3-dideoxy-3-fluoro-D-*erythro*-pentofuranosyl moieties of both α- and β-anomers results essentially from the gauche effect of the F3'-C3'-C4'-O4' fragment.

The ability of compounds **7**a, **7**b, and **9**b to inhibit the proliferation of murine leukemia cells (L1210/C8), murine mammary carcinoma cells (FM3A), and human T-



a) **1**/**2**/$SnCl_4$ (1.0:1.26:3.0, mol), MeCN, 20 °C, 18 h (**3**a,b, ~5:2, 29%, combined; **4**a, 66%); b) **1**/**2**/TMS-Tfl (1.0:1.26:2.0, mol), CH_2Cl_2, reflux, 1 h (**3**a,b, ~5:4, 93%, combined); c) saturated at 20 °C ammonia in 1,2-dimethoxyethane, 20 °C, 72 h (**5**a,b, ~5:4, 95%, combined); d) saturated at 0 °C methanolic ammonia, 20 °C, 24-48 h [**9**b (15%), **7**b (17%) and **7**a (20%); c + d, **7**b (33%) and **7**a (44%); c + d, **10**a (78%)].

Scheme

lymphocyte cells (Molt4/C8 and CEM/0) was investigated: only the β-D-anomer **7**b displayed moderate toxicity against all cell lines (IC_{50}, μg/mL, 54.5, 87.0, 2.45 and 110, respectively).

Compounds **7**a, **7**b, and **9**b were further evaluated for their inhibitory effect on HIV-1 and HIV-2-induced cytopathicity in human T-lymphocyte (CEM/0) cells: none of them are active at subtoxic concentrations.

The test compounds **7**a, **7**b, and **9**b were also evaluated for their inhibitory effects on the replication of HSV-1 (KOS, TK^- B2006 and TK^- VMW1837), HSV-2 (G), and vaccinia virus in E_6SM cells, vesicular stomatitis virus in E_6SM and HeLa cells, parainfluenza-3 virus, reovirus-1, Sindbis virus, and Semliki forest virus in Vero cell cultures, Coxsackie virus B4 in Vero and HeLa cell cultures, and polio virus-1 in HeLa cell cultures, as well as for their inhibitory effects on proliferation of the above cells.

None of the compounds proved inhibitory to virus replication or cell viability or proliferation at subtoxic concentrations.

Acknowledgments

I.A.M. is deeply grateful to the Alexander von Humboldt-Stiftung (Bonn - Bad-Godesberg, Germany) and the INTAS (grant 93-1500) for partial financial support of this work. Work at the Rega Institute was supported by the Biomedical Research Programme of the European Commission and grants from the Belgian Nationaal Fonds voor Wetenschappelijk Onderzoek (NFWO) and Geconcerteerde Onderzoeksacties (GOA).

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