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CONVERGENT SYNTHESES AND CYTOSTATIC PROPERTIES OF 2-CHLORO-2'-DEOXY-2'-FLUOROADENOSINE AND ITS N^7-ISOMER

Galina V. Zaitseva[1], Grigorii G. Sivets[1], Zygmunt Kazimierczuk[2], Juhani A. Vilpo[3], and Igor A. Mikhailopulo[1]*

[1]*Institute of Bioorganic Chemistry, Byelorussian Academy of Sciences, 220141 Minsk, Zhodinskaya 5, Byelorussia,* [2]*Department of Biophysics, Institute of Experimental Physics, University of Warsaw, Zwirki i Wigury 93, 02-089 Warsaw, Poland, and* [3]*Tampere University Hospital, Department of Clinical Chemistry, P.O. Box 2000, SF-33521 Tampere, Finland*

Abstract. Glycosylation of trimethylsilylated 2,6-dichloropurine **2** with acetate **1** in anhydrous MeCN was investigated. In the presence of $SnCl_4$, the reaction was regio- and stereoselective affording N^7-β-glycoside **3** (86%). The use of TMS-Tfl instead of $SnCl_4$ afforded a ≈ 9:1 mixture of the N^9-β- and -α-glycosides **5** and **6** (90%, combined). The title nucleosides were tested for their cytotoxicity.

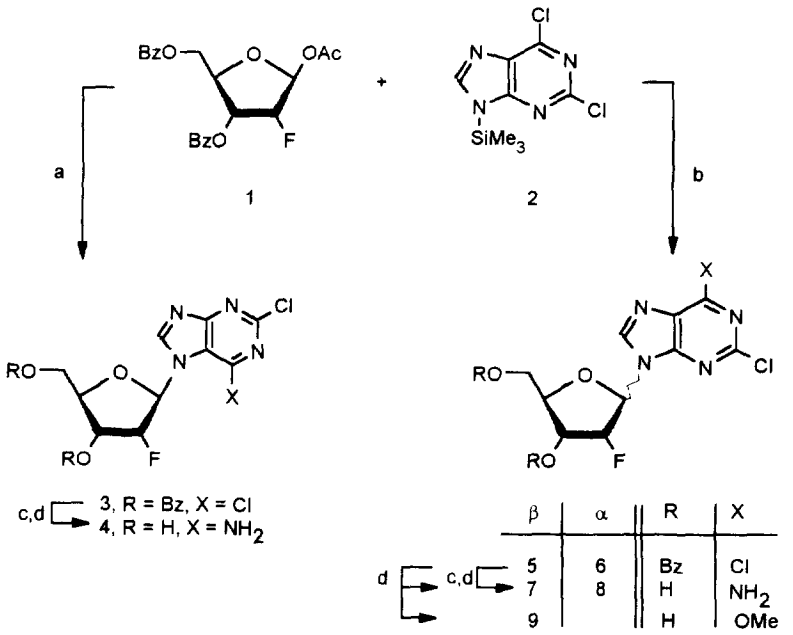
The 2-Chloro-2'-deoxyadenosine (2-CdA, 'Cladribine) is recognized as a potent anticancer and immunosuppressive drug (for reviews, see Refs 1,2) and undergoes extensive clinical trials (*e.g.*, Refs 3). Closely related analogue of 2-CdA, 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine, showed a similar *in vitro* and *in vivo* spectrum of activity (reviewed in Ref. 4). The foregoing, in conjunction with the development of practical synthesis of 1-O-acetyl-3,5-di-O-benzoyl-2-deoxy-2-fluoro-β-D-ribofuranose (**1**)[5], prompted us to synthesize 2-chloro-2'-deoxy-2'-fluoroadenosine (**7**). We describe here the synthesis and cytostatic activity of **7** and its N^7-isomer **4**[6].

Synthesis: The condensation of acetate **1** with trimethylsilylated 2,6-dichloropurine **2** in the presence of tin(IV) chloride in anhydrous acetonitrile at room temperature for 2.5 h proceeded regio- and stereospecifically and the N^7-β-nucleoside **3** was isolated in 86% yield. When tin(IV) chloride is replaced by trimethylsilyl triflate (TMS-Tfl), the reaction led to the formation of the N^9-β-glycoside **5** as principal product along with formation of the N^9-α-anomer **6** (β/α ratio ≈ 9 1 according to the ^{1}H NMR data; 90%, combined) (*cf.* the data in Ref. 7). Transformation of **3** to **4** was effected *via* successive action of saturated solution of ammonia in 1,2-dimethoxyethane at room temperature for 24 h[8] and then methanolic ammonia. After silica gel column chromatography, nucleoside **4**[9] was obtained in 56% combined yield. In a similar manner, the mixture of **5** and **6** was transformed into deblocked anomeric nucleosides and subsequent chromatographical separation gave the corresponding individual compounds **7**[10] and **8**[11] in yields of 72 and 8%, respectively. Treatment of the **5**/**6** mixture with methanolic ammonia at room temperature for 24 h followed by

chromatography afforded nucleosides of 2-chloroadenine **7** (42%) and 2-chloro-6-methoxypurine **9**[12] (39%) (Scheme).

The structures of the compounds were confirmed by ^{1}H NMR, UV, and FAB mass spectra. The ^{1}H NMR data recorded for **7** are in agreement with those reported[7]. The site of glycosylation of 2-chloroadenine was determined by comparison of the ^{1}H NMR and UV spectra of the corresponding compounds with those of the pairs of related adenine N^7- and N^9-glycosides[13]. The most informative features of the ^{1}H NMR spectrum of the α-anomer **8** are *(i)* the 0.36 ppm shift of H-4' resonance signal to a lower field when going from β- to α-anomer (see, *e.g.*, Refs 13,14), and *(ii)* the long-range coupling of H-8 to fluorine of 2.5 Hz exhibited in its ^{1}H NMR spectrum. This coupling is generally indicative of a spatial proximity of the nuclei involved[15] and is not observed in the β-anomers. The structure of the heterocyclic base of **4** was deduced from a comparison of the UV spectral data with those for the N^7-glycosides of the 6-amino-2-chloropurine and its 2-amino-6-chloro isomer[16] which display widely different UV spectra.

Scheme



a) **1**/**2**/$SnCl_4$ (1.0:2.0:3.0, mol), MeCN, 20 °C, 2.5 h (86%); b) **1**/**2**/TMS-Tfl (1.0:1.7:2.55, mol), MeCN, reflux, 20 min (**5**/**6**, ≈ 9:1; 90%, combined); c) saturated at 20 °C ammonia in 1,2-dimethoxyethane, 20 °C, 24 h; d) saturated at 0 °C methanolic ammonia, 20 °C, 24 h [**7** (42%) and **9** (39%); c + d, **4** (56%); **7** (72%) and **8** (8%)]

Cytotoxicity studies: Toxicity of nucleosides **4** and **7** was determined by their effects on protein synthesis in cells in culture. The human leukemia cell lines were obtained from the American Type Culture Collection (Rockville, MD). Test compounds were added to cultures in 96-well microplates containing 2 x 10^4 leukemia cells or 2.5 x 10^4 PHA-stimulated lymphocytes per 200 μL well. Cells were cultured in RPMI 1640 medium containing glutamine (2 mM), penicillin (100 units/mL), streptomycin (100 μg/mL) and fetal calf serum (10%, v/v), in humidified atmosphere containing 5% CO_2 at 37 °C. [U-^{14}C]-L-leucine (specific activity 1.3 mCi/mmol and 0.5 μCi/mL) was added to the cultures for the final 24 h of the 3-day culture period of human leukemia cells and for the 4-day culture period of PHA-stimulated lymphocytes. After incubation, the proteins were precipitated with 0.2 N perchloric acid and collected on glass fiber filters with use of a multiple cell harvester (Wallac, Turku, Finland). The radioactivity incorporated into proteins was measured in a scintillation counter (1410, Wallac, Turku, Finland). The incorporation of [^{14}C]-leucine per cell remains constant during the final 24 h of culture, and a good correlation has been demonstrated between cell number and [^{14}C]-leucine incorporation[17,18].

The toxicities of nucleosides **4** and **7** were first screened at a concentration of 10 μg/mL against four human leukemia/lymphoma lines and against mitogen-stimulated human peripheral blood lymphocytes; no significant toxicity was observed with N^7-β-anomer **4**. In contrast, the N^9-β-anomer **7** was toxic against all lines. Hence, three lower concentrations were also tested. The results are presented in the Table.

Table. Toxicity of Nucleoside **7** Against Malignant and Normal Hematopoietic Cells *in vitro*.

Concentration [μg/mL]	[^{14}C]-Leucine Incorporation (% of control)[a]				
	IM-9[b]	Raji	MOLT-3	U-937	PHA-Ly
10	5.7	2.5	2.6	1.8	4.0
1.0	19.0	9.1	3.3	4.9	17.0
0.1	110	68.0	79.0	70.0	90.0
0.01	108	103	106	99.0	98.0

[a]Mean value of three determinations.
[b]Abbreviations are as follows: IM-9, myeloma cell line; Raji, Burkitt's lymphoma (B-cell); MOLT-3, acute T cell leukemia; U-937, histiocytic lymphoma; PHA-Ly, phytohemagglutinin-stimulated peripheral blood lymphocytes.

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9. Compound **4**. mp 243-244 °C (from ethanol); TLC, Kieselgel 60 F_{254} (Merck, Germany) plates, chloroform-methanol, 10:1 (triple elution), R_f = 0.13; 200 MHz ^{1}N NMR spectrum (DMSO-d_6), δ_{TMS} ppm, *J* Hz: 8.67 (s, 1H, H-8), 7.47 (br.s, 2H, NH_2), 6.42 (dd, 1H, $J_{1',2'}$ = 3.8, $J_{1',F}$ = 14.0, H-1'), 5.80 (d, 1H, $J_{3',OH-3'}$ = 6.0, OH-3'), 5.39 (t, 1H, $J_{5',OH-5'}$ = 4.8, OH-5'), 5.17 (dt, 1H, $J_{2',3'}$ = 5.0, $J_{2',F}$ = 52.0, H-2'), 4.33 (m, 1H, $J_{3',4'}$ = 6.0, $J_{3',F}$ = 15.0, H-3'), 4.04 (m, 1H, H-4'), 3.78 (dd, 1H, $J_{5',4'}$ = 2.5, $J_{5',5''}$ = 12.5, H-5'), 3.62 (dd, 1H, $J_{5'',4'}$ = 3.0, H-5''). UV, (pH 7.0), λ_{max} 216.0 nm (ε 22,150), ≈ 245 sh (ε 5,300), 275.6 (ε 8,700), λ_{min} 234 nm (ε 4,600); (pH 1.0), λ_{max} 215.0 nm (ε 20,000), 274.0 (ε 9,400), λ_{min} 205 nm (ε 17,000); 237 nm (ε 4,300); FAB mass spectrum, *m/z* 304 and 306, $^{35}Cl/^{37}Cl$ ratio ≈ 3:1, $(M + H)^+$.
10. Compound **7**. mp 229-231 °C [from water; (lit.[7]: mp 220-222 °C, from methanol)]; TLC, as above, R_f = 0.36; ^{1}N NMR spectrum (DMSO-d_6), δ_{TMS} ppm, *J* Hz: 8.40 (s, 1H, H-8), 7.92 (br.s, 2H, NH_2), 6.19 (dd, 1H, $J_{1',2'}$ = 2.8, $J_{1',F}$ = 16.8, H-1'), 5.79 (d, 1H, $J_{3',OH-3'}$ = 6.0, OH-3'), 5.38 (ddd, 1H, $J_{2',3'}$ = 4.3, $J_{2',F}$ = 53.2, H-2'), 5.25 (t, 1H, $J_{5',OH-5'}$ = 5.0, OH-5'), 4.44 (ddd, 1H, $J_{3',4'}$ = 6.8, $J_{3',F}$ = 18.5, H-3'), 4.00 (m, 1H, H-4'), 3.78 (dd, 1H, $J_{5',4'}$ = 2.5, $J_{5',5''}$ = 12.3, H-5'), 3.60 (dd, 1H, $J_{5'',4'}$ = 4.0, H-5''). UV, (pH 7.0), λ_{max} 264.0 nm (ε 15,500), λ_{min} 228.0 nm (ε 2,700); (pH 1.0), λ_{max} 265.6 nm (ε 14,700), λ_{min} 229.0 nm (ε 2,600); FAB mass spectrum, *m/z* 304 and 306, $^{35}Cl/^{37}Cl$ ratio ≈ 3:1, $(M + H)^+$.
11. Compound **8**. Lyophilized powder from water; TLC, as above, R_f = 0.31; ^{1}N NMR spectrum (CD_3OD), δ_{TMS} ppm, *J* Hz: 8.28 (d, 1H, $J_{H-8,F}$ = 2.5, H-8), 6.46 (dd, 1H, $J_{1',2'}$ = 4.0, $J_{1',F}$ = 16.5, H-1'), 5.24 (dt, 1H, $J_{2',3'}$ = 4.0, $J_{2',F}$ = 54.0, H-2'), 4.51 (ddd, 1H, $J_{3',4'}$ = 7.0, $J_{3',F}$ = 19.0, H-3'), 4.36 (m, 1H, H-4'), 3.90 (dd, 1H, $J_{5',4'}$ = 2.3, $J_{5',5''}$ = 12.5, H-5'), 3.70 (dd, 1H, $J_{5'',4'}$ = 3.5, H-5''). UV, (pH 7.0), λ_{max} 265.0 nm (ε 11,000), λ_{min} 229.0 nm (ε 2,500); (pH 1.0), λ_{max} 265.0 nm (ε 11,000), λ_{min} 229.0 nm (ε 2,500); FAB mass spectrum, *m/z* 304 and 306, $^{35}Cl/^{37}Cl$ ratio ≈ 3:1, $(M + H)^+$.
12. Compound **9**. mp 177-178 °C (from ethanol)]; TLC, as above, R_f = 0.52; ^{1}N NMR spectrum (CD_3OD), δ_{TMS} ppm, *J* Hz: 8.58 (s, 1H, H-8), 6.33 (dd, 1H, $J_{1',2'}$ = 2.5, $J_{1',F}$ = 16.5, H-1'), 5.39 (ddd, 1H, $J_{2',3'}$ = 4.0, $J_{2',F}$ = 52.5, H-2'), 4.63 (ddd, 1H, $J_{3',4'}$ = 7.0, $J_{3',F}$ = 18.0, H-3'), 4.18 (s, 3H, OMe), 4.14 (m, 1H, H-4'), 3.98 (dd, 1H, $J_{5',4'}$ = 2.5, $J_{5',5''}$ = 12.0, H-5'), 3.80 (dd, 1H, $J_{5'',4'}$ = 3.2, H-5''). UV, (pH 1.0, 7.0, and 11.0), λ_{max} 258.0 nm (ε 10,000), λ_{min} 224.0 nm (ε 2,200); (pH 1.0); FAB mass spectrum, *m/z* 319 and 321, $^{35}Cl/^{37}Cl$ ratio ≈ 3:1, $(M + H)^+$.
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