172. Base-Modified Nucleosides Related to 2-Chloro-2′-deoxyadenosine

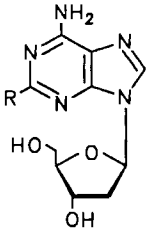
by **Zygmunt Kazimierczuk**[a]), **Juhani A. Vilpo**[b]), and **Frank Seela**[c])*

[a]) Department of Biophysics, University of Warsaw, Zwirki i Wigury 93, PL-02089 Warsaw
[b]) Department of Clinical Chemistry, University of Tampere, SF-33520 Tampere
[c]) Laboratorium für Organische und Bioorganische Chemie, Institut für Chemie, Universität Osnabrück, Barbarastr. 7, D-4500 Osnabrück

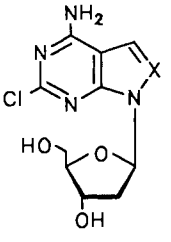
(27.VII.92)

Derivatives of 2-chloro-2′-deoxyadenosine (**1a**) containing secondary 6-NH_2 groups (**5a–c**) or a 8-Br substituent (**9**) were synthesized. They were tested together with ring-modified congeners containing a pyrrolo[2,3-*b*]pyridine, pyrrolo[3,2-*c*]pyridine, or pyrazolo[3,4-*d*]pyrimidine ring system as inhibitors of various leukemic cell lines. Only the 8-Br derivatives **9** showed inhibitory activity, whereas the base-modified congeners were not active. Compound **1a** was protonated at a $pK_a = 1.4$ (2′-deoxyadenosine at $pK_a = 3.8$). Protonation occurred at N(7) and not at N(1) as observed for dA.

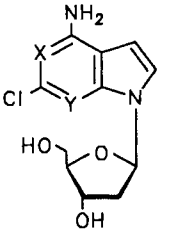
Introduction. – The naturally occurring 2′-deoxyadenosine is toxic to certain lymphoid cells, if adenosine deaminase is absent or inhibited [1]. The adenosine deaminases resistant 2-chloro- and 2-bromo-2′-deoxyadenosines, **1a** and **1b**, respectively, are much more cytotoxic to T-lymphoblastic, B-lymphoblastic, myeloblastic, and melanoma cells then dA [2–6]. A potent activity of **1a** against human monocytes was observed as well [7]. Currently, compound **1a** is used for the treatment of several chronic lymphoid malignances as hairy cell leukemia, cutaneous T-cell lymphoma, and acute leukemia [8] [9].



1a R = Cl
b R = Br



2a X = C
b X = N



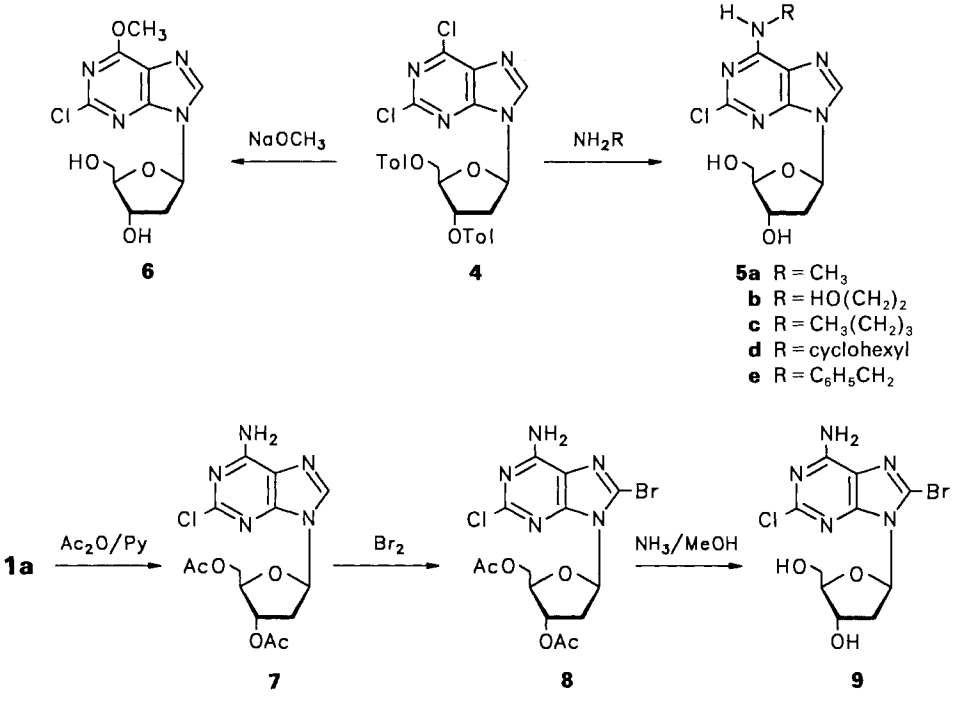
3a X = C, Y = N
b X = N, Y = C

Compounds **1a** and **1b** have been prepared by enzymatic and chemical methods. Enzymatic synthesis was achieved by transfer of the deoxyribose moiety from thymidine to 2-haloadenines catalyzed by transdeoxyribosylase [2] [3]. The chemical synthesis of **1** was realized at first by the fusion method, and it was shown that the 2′-deoxyribonucleoside **1a** was two magnitudes more active against leukemia L-1210 cell as the ribonucleoside [10]. Compounds **1a** and **1b** were obtained stereospecifically using the sodium

salts of 2,6-dihalogenopurines and a 2′-deoxy-sugar chloride, followed by ammonolysis [11] [12]. We have extended this work to compounds such as **2a, b** and **3a, b** containing a pyrrolo[2,3-*b*]pyridine, pyrrolo[3,4-*c*]pyridine, pyrrolo[3,4-*d*]pyrimidine, and pyrazolo-[3,4-*d*]pyrimidine ring system instead of the purine moiety [13–15]. Now, derivatives of **1a** with alkyl or aryl residues at the 6-amino group or Br substituents at C(8) will be described, and the activity of base-modified compounds against leukemic cell lines will be studied.

Results and Discussion. – As synthetic intermediate, the dichloro nucleoside **4** was chosen [11]. The 6-Cl substituent was displaced by various amino groups (*Scheme*, **5a–c**).

Scheme



In the case of the reaction with sterically hindered amines **5e** and **5d**, MeONa/MeOH was added to complete the removal of the *p*-toluoyl protecting groups. Compound **4** was also converted into **6** by the action of MeONa. For introduction of an 8-Br substituent, compound **1a** was protected selectively at C(3′)–OH and C(5′)–OH yielding **7**. The bromination of **7** was then carried out in the mixture of dioxane/AcONa buffer with Br_2 to afford **8**. Treatment of **7** with *N*-bromosuccinimide or *N*-bromoacetamide gave low yields. Compound **8** was deacylated with NH_3 affording crystalline **9**.

Nucleosides were characterized by elemental analyses, ^{1}H- and ^{13}C-NMR spectra (*Table 1*). The ^{13}C-NMR chemical shifts were assigned from gated-decoupled spectra (*Table 2*). However, assignment of C(2) *vs.* C(6) was difficult in the case of the amino

Table 1. *^{13}C-NMR Chemical Shifts of Purine 2′-Deoxyribonucleosides in (D_6)DMSO at 23°*

Compound	C(2)	C(4)	C(5)	C(6)	C(8)	CH_3/CH_2
1a [14]	153.0	150.0	118.1	156.8	139.8	–
1b [14]	144.1	149.9	118.5	156.6	139.6	
5a	153.2	148.9	118.6	155.5	139.5	27.1
5b	153.2	149.3	118.7	155.3	139.7	59.4/45.2
5c	153.3	149.2	118.6	155.2	139.5	13.7/19.6
5d	153.2	149.3	118.4	154.2	139.4	
5e	153.2	149.5	118.7	155.0	139.2	43.2
6	151.4	152.6	120.3	160.8	142.7	54.9
7	153.2	150.2	118.2	156.9	139.9	
8	153.0	150.7	118.4	155.6	126.7	
9	153.0	150.9	118.9	155.9	127.0	
	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	
1a	83.5	DMSO	70.7	88.2	63.3	
1b	83.5	DMSO	71.1	88.1	62.0	
5a	83.5	DMSO	70.7	89.1	61.6	
5b	83.7	DMSO	70.8	88.0	61.7	
5c	83.7	DMSO	70.8	88.0	61.7	
5d	83.6	DMSO	70.7	88.0	61.6	
5e	83.7	DMSO	70.7	88.0	61.6	
6	83.8	DMSO	70.5	88.0	61.4	54.9
7	83.4	35.4	74.2	81.7	63.5	
8	85.4	33.7	73.7	81.6	63.1	
9	86.2	36.7	71.1	88.4	62.1	

Table 2. *J(C,H) Values* [Hz] *of Purine 2′-Deoxyribonucleosides* [a])

	1a	**1b**	**6**	**9**
J(C(4),H–C(1′))	*m*	*m*	*m*	5.0
J(C(5),H–C(8))	11.3	11.1	12.5	–
J(C(5),NH_2–C(6))	3.8	4.4	–	–
J(C(6),CH_3O–C(6))	–	–	3.8	–
J(C(8),H–C(8))	214	214	216	–
J(C(8),H–C(1′))	3.8	4.0	3.8	3.8
J(C(1′),H–C(1′))	166	166	168	165
J(C(3′),H–C(3′))	150	149	149	150
J(C(4′),H–C(4′))	147	147	149	149
J(C(5′),H–C(5′))	140	140	140	138

[a]) Data taken from measurements in (D_6)DMSO at 25°.

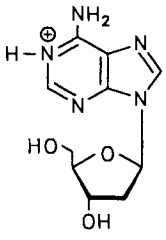
Table 3. *NOE Data* [%] *of Purine 2′-Deoxyribonucleosides upon Irradiation of H–C(8)* [a])

	1a	**dA** [17]
H–C(2′) + H–C(3′)	5.6	2.7
H–C(1′)	4.7	6.0

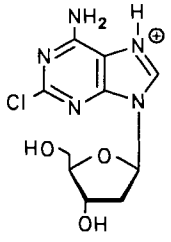
[a]) Measured in (D_6)DMSO at 25°.

compounds. On the other hand, the MeO derivative **6** gave a *quadruplet* for C(6) which made the assignments of this compound unambiguously. By using the increments of amino *vs.* MeO groups [16], the assignment of **1a** and the derivatives **5a–e** were established. To get information about the '*syn*'/'*anti*'-ratio of the nucleobase around the *N*-glycosylic bond, NOE spectra were measured [17]. *Table 3* summarizes the NOE value of H–C(1′) and the sum of H–C(2′) and H–C(3′) upon irradiation of H–C(8). According to that, a '*syn*'-population is favored in the case of **1a**, similar to 2′-deoxyadenosine. As the 8-Br compound **9** shows almost identical coupling constants between C(8) and the anomeric proton as the compounds **1a** or **1b** '*syn*'-orientiation is also most likely for this derivative.

Adenosine and 2′-deoxyadenosine are protonated at N(1) in H_2O [18] with a $pK_a = 3.8$ for deoxyadenosine [19]. We have determined the pK_a value of **1a** as 1.4. However, it was not clear whether N(1), as reported for 2′-deoxyadenosine, is still the first protonation site. Therefore, ^{15}N-NMR spectra have been measured [20]. The first measurements were carried out in DMSO in the absence of acid. Five signals appeared which showed similar shifts as 2′-deoxyadenosine (*Table 4*). INEPT Spectra confirmed the assignment. The main differences between **1a** and the parent 2′-deoxyadenosine appeared at N(1) and at the exocyclic amino group. According to *Table 4*, the ribonucleoside showed almost identical ^{15}N-NMR spectra as the 2′-deoxyribonucleoside apart from the *ca.* 4-ppm downfield shift of the ribonucleoside at N(9).



$pK = 3.8$



$pK = 1.4$

Table 4. *^{15}N-NMR Chemical Shifts*[a]) *and ^{15}N,^{1}H Coupling Constants from Gated-Decoupled Spectra Measured in (D_6)DMSO at 25°*

	N(1)	N(3)	N(7)	N(9)	NH_2
2′-Deoxyadenosine [20]	145.1	157.8	140.4	207.6	299.1
Adenosine [20]	145.0	158.3	140.0	211.4	299.3
1a	149.3	159.9	138.9	205.6	293.6
2-Chloroadenosine	149.2	160.1	141.3	209.3	293.0

Coupling constants [Hz]

	J(N(1),H–N(C(6)))	*J*(N(1),H–C(2))	*J*(N(3),H–C(2))	*J*(N(7),H–C(8))	*J*(N(9),H–C(8))	*J*(NH_2)
1a	*t*, 5	–	–	*d*, 10	*d*, 5	*t*, 91
dA	–	*d*, 19	*d*, 15	*d*, 12	*d*, 10	*t*, 88

[a]) Relative to $MeNO_2$.

Stepwise addition of CF_3COOH to a DMSO solution of **1a** led to precipitation of the nucleobase. Apparently, traces of H_2O hydrolyzed the molecule at the *N*-glycosylic bond. This was circumvented using 2-chloroadenosine which is much more stable under these conditions. The *Figure* shows the result of CF_3COOH titration. Only, N(7) was shifted downfield, whereas small upfield shifts occurred for the amino group and N(9). The consumption of more than 1 equiv. of acid was surprising and may be the result of the basicity of DMSO. This experiment confirmed N(7) as protonation site. The same can be expected for the 2′-deoxynucleoside **1a**.

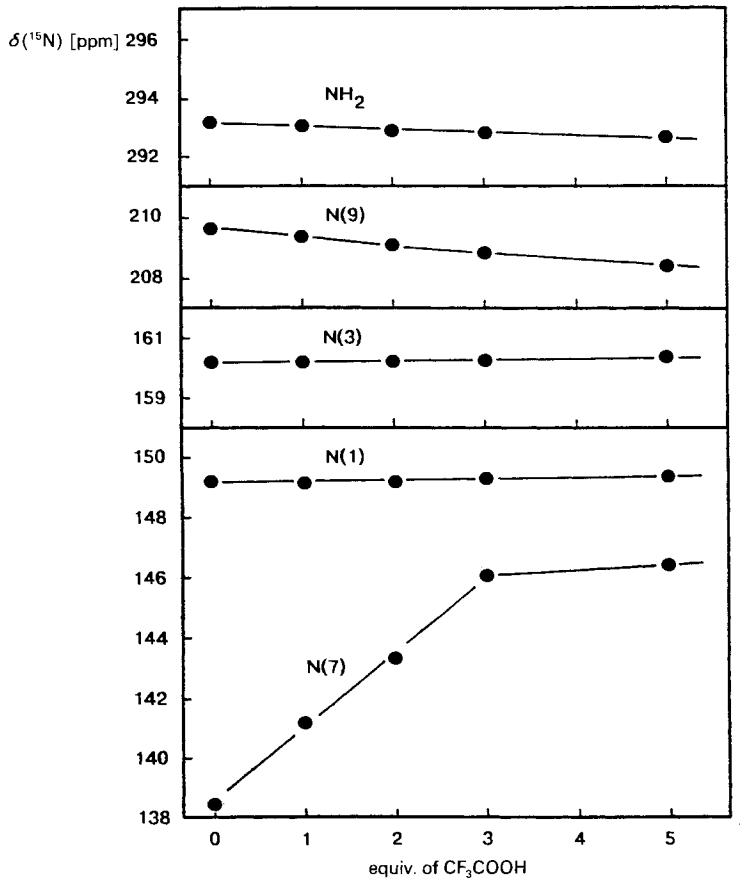


Figure. *^{15}N-NMR Chemical shifts of 2′-chloroadenosine in (D_6)DMSO as a function of CF_3COOH concentration*

It has been observed that **1a** as well as its ribonucleoside are much more resistant against adenosine deaminase than the parent 2′-deoxyadenosine. For the enzymatic deamination, a mechanism has been proposed requiring the addition of a H_2O molecule to the N(1)=C(6) bond of the adenine moiety as the first reaction step [22]. Enzymatic H_2O addition on **1a** may be more difficult as in the case of dA, as the bulky 2-Cl substituent may interfere during the binding at the active centre of the enzyme.

Compound **1a** is phosphorylated to the nucleoside monophosphate by deoxycytidine kinase and then further converted into the triphosphate [3] [4] [23]. Triphosphates are incorporated into DNA-developing strand breaks [24] [25]. The DNA damage is followed by accelerated consumption of NAD for poly(ADP-ribose) synthesis [26]. These effects provide a decline in ATP levels and cell death. Compound **1a** acts also as an inhibitor of ribonucleotide reductase, decreasing deoxynucleotides concentration within the cells [4].

Table 5. *Inhibitory Activity of 2-Halopurine 2'-Deoxyribonucleosides and Congeners*

Compound	ID_{50} Values [μg/ml]				
	MOLT-3	U-937	K-562	IM-9	PHA-ly
1a	0.017	0.020	0.045	0.012	0.029
2a	> 100	> 100	> 100	> 100	> 100
2b	> 100	> 100	> 100	> 100	> 100
3a [28]	> 100	> 100	> 100	> 100	> 100
3b	> 100	> 100	> 100	> 100	> 100
5a	–	–	–	–	18
5b	> 100	27	> 100	> 100	> 100
5c	> 10	3.1	> 10	> 10	> 10
9	0.93	0.35	10	2.6	6.6

[a]) ID_{50} is the concentration of compounds which caused a 50% decrease in L-[^{14}C]leucine incorporation by the cells in the culture. MOLT-3: acute T cell leukemia; U-937: histiocytic lymphoma; K-562: chronic myelogenous leukemia; IM-9: human myeloma (ATCC No.: CCL 159); PHA-ly: phytohemagglutinin-stimulated peripheral blood lymphocytes.

Cytotoxicity studies on compounds indicated in *Table 5* allow a correlation of these properties with nucleoside structure. The replacement of the H-atom at the exocyclic amino group by bulky residues (**5a–c**) decreases the cytotoxic activity by a factor more than 100 against leukemia cell lines. On the other hand, substitution of H–C(8) by Br reduced the activity only by a factor of *ca.* 10. The 7-deazapurine analogues **2a, b** as well as the 1,7- or 3,7-dideazapurine derivatives **3a, b** are not active at concentrations lower than 100 μg/ml. Compound **1a** is not phosphorylated by deoxythymidine kinases (TK1 and TK2) but is phosphorylated by deoxycytidine kinase, as it occurs with a few other purine 2'-deoxyribonucleosides, arabinonucleosides, and 2'-fluoronucleosides [27] [28]. As phosphorylation is a crucial step required for cellular activity of these nucleosides, the low activity of the derivatives shown in *Table 5* implies that base-modified derivatives of **1a**, containing an altered nitrogen pattern or bulky substituents at the exocyclic amino group, are not substrates for this enzyme.

We thank Dr. *H. Rosemeyer* for the ^{15}N-NMR spectra. Financial support is gratefully acknowledged by *Polish Committee of Scientific Research* (*Z. K.*) and the *Deutsche Forschungsgemeinschaft* (*F. S.*).

Experimental Part

General. See [14]. 2-Chloroadenosine was a commercial product of *Sigma* (St. Louis, USA). ^{15}N-NMR Spectra were measured at a *Bruker AMX-500* spectrometer with $MeNO_2$ as standard.

Cytotoxicity Tests. Toxicity of the compounds was determined by their effects on protein synthesis (^{14}C-L-leucine incorporation). The cell lines were obtained from the *American Type Culture Collection*. Test compounds were added to triplicate cultures in 96-well microplates containing 2×10^4 cells per a 200-µl well (or 10^5 peripheral blood lymphocytes, stimulated with phytohemagglutinin). Cells were cultured in *RPMI 1640*, medium-containing fetal calf serum (10%), in humidified atmosphere containing 5% CO_2 at 37°. L-[^{14}C]leucine (specific activity 1.3 mCi/mmol and 0.5 µCi/ml) was added to the culture for the final 24 h of the 4-d culture period. After incubation, the proteins were precipitated with 0.2N $HClO_4$ and collected on a glass fibre filters using a multiple cell harvester (*Wallac*, Finland). The radioactivity incorporated into proteins was measured in a scintillation counter (*LKB-Wallac*; *1410*, Finland).

*2-Chloro-9-(2'-deoxy-β-*D-erythro-*pentofuranosyl)-6-(methylamino)-9*H*-purine* (**5a**). The suspension of **4** (1.08 g, 2 mmol) [11] in MeCN/MeOH (1:5, 30 ml) was stirred at r.t. for 2 d. The mixture was evaporated to dryness and the residue chromatographed on a silica-gel column (2 × 25 cm) with CH_2Cl_2/MeOH 9:1 to yield colorless needles from H_2O (430 mg, 72%). M.p. 163–165°. TLC (CH_2Cl_2/MeOH, 9:1): R_f 0.35. UV (H_2O): 270 (16800). ^{1}H-NMR ((D_6)DMSO): 2.30, 2.65 (2*m*, 2 H–C(2')); 2.92 (*d*, *J* = 4.1, CH_3N); 3.55 (*m*, 2 H–C(5')); 3.86 (*m*, H–C(4')); 4.39 (*m*, H–C(3')); 4.96 (*t*, *J* = 5.3, HO–C(5')); 5.32 (*d*, *J* = 4.0, HO–C(3')); 6.27 ('*t*', *J* = 6.8, H–C(1')); 8.26 (*d*, *J* = 4.2, NH); 8.35 (*s*, H–C(8)). Anal. calc. for $C_{11}H_{14}ClN_5O_3$ (299.7): C 44.08, H 4.71, N 23.37; found: C 43.96, H 4.90, N 23.24.

*2-Chloro-9-(2'-deoxy-β-*D-erythro-*pentofuranosyl)-6-[(2-hydroxyethyl)amino]-9*H*-purine* (**5b**). As described for **5a**, (2-hydroxyethyl)amine (915 mg, 15 mmol) was reacted with **4** (810 mg, 1.5 mmol) in MeOH (25 ml). Colorless needles (310 mg, 63%) from AcOEt/acetone. M.p. 167–168°. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.15. UV (H_2O): 271 (18700). ^{1}H-NMR ((D_6)DMSO): 2.30, 2.65 (2*m*, H–C(2')); 3.56 (*m*, H–C(5'), H–CH_2(N)); 3.86 (*q*, H–C(4')); 4.39 (br. *s*, H–C(3')); 4.76 (*t*, *J* = 4.6, CH_2CH_2OH); 4.95 (*t*, *J* = 5.5, HO–C(5')); 5.30 (*d*, *J* = 4.1, OH–C(3')); 6.27 (*t*, *J* = 6.7, H–C(1')); 8.13 (br. *s*, H–N(6)); 8.35 (*s*, H–C(8)). Anal. calc. for $C_{12}H_{16}ClN_5O_4$ (329.7): C 43.71, H 4.89, N 21.24; found: C 43.59, H 4.80, N 21.09.

*6-(Butylamino)-2-chloro-9-(2'-deoxy-β-*D-erythro-*pentofuranosyl)-9*H*-purine* (**5c**). Analogously to **5a**, **4** (540 mg, 1 mmol) in MeOH (15 ml) was treated with $BuNH_2$ (730 mg, 10 mmol) at 37°. Purification on 20 × 20 cm prep. silica-gel plates with CH_2Cl_2/MeOH 9:1 afforded a colorless solid (210 mg, 61%) which crystallized as needles (H_2O). M.p. 159°. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.50. UV (H_2O): 271 (18800). ^{1}H-NMR ((D_6)DMSO): 0.88, 1.31, 1.56, 3.40 (*m*, aliph. H); 2.27, 2.63 (2*m*, H–C(2')); 3.56 (*m*, H–C(5')); 3.86 (br. *s*, H–C(4')); 4.40 (br. *s*, H–C(3')); 4.95 (*t*, *J* = 5.3, HO–C(5')); 5.30 (*d*, *J* = 3.8, HO–C(3')); 6.27 ('*t*', *J* = 6.7, H–C(1')); 8.30 (br. *s*, H–N(6)); 8.34 (*s*, H–C(8)). Anal. calc. for $C_{14}H_{20}ClN_5O_3$ (341.8): C 49.20, H 5.90, N 24.49; found: C 49.16, H 5.85, N 24.30.

*2-Chloro-6-(cyclohexylamino)-9-(2'-deoxy-β-*D-erythro-*pentofuranosyl)-9*H*-purine* (**5d**). Cyclohexylamine (1.0 g, 10 mmol) was added to a stirred soln. of **4** (1.08 g, 2 mmol) in MeOH (30 ml). The stirring was continued for 2 d at r.t. The mixture was treated with MeONa (3 ml, 1M in MeOH) and the stirring was continued for another day. The mixture was evaporated to an oil and purified by FC on a silica-gel column (2.5 × 25 cm) with CH_3Cl/MeOH 9:1. The nucleoside-containing fractions were evaporated to dryness, and the residue gave colorless crystals (460 mg, 63%) from AcOEt. M.p. 160–162°. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.63. UV (H_2O): 273 (19600). ^{1}H-NMR ((D_6)DMSO): 1.0–2.0 (*m*, cyclohexyl H); 2.25, 2.60 (2*m*, H–C(2')); 3.55 (*m*, H–C(5')); 3.86 (*q*, H–C(4')); 4.38 (br. *s*, H–C(3')); 4.97 (*t*, *J* = 5.6, HO–C(3')); 5.32 (*d*, *J* = 4.2, HO–C(5')); 6.26 ('*t*', *J* = 6.6, H–C(1')); 8.15 (*d*, *J* = 8.4, H–N(6)); 8.34 (*s*, H–C(8)). Anal. calc. for $C_{16}H_{22}ClN_5O_3$ (367.8): C 52.24, H 6.03, N 19.04; found: C 52.11, H 5.96, N 18.88.

*6-(Benzylamino)-2-chloro-(2'-deoxy-β-*D-erythro-*pentofuranosyl)-9*H*-purine* (**5e**). As described for **5d**, $PhCH_2NH_2$ (1.07 g, 10 mmol) was reacted with **4** (920 mg, 1.7 mmol) in MeOH (30 ml). Purification was accomplished on a 3 × 24 cm column using AcOEt (200 ml) and then AcOEt/MeOH 9:1. Colorless crystals (390 mg, 61%) from EtOH/H_2O. M.p. 147°. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.60. UV (H_2O): 273 (21600). ^{1}H-NMR ((D_6)DMSO): 2.30, 2.60 (2*m*, H–C(2')); 3.53 (*m*, H–C(5')); 3.85 (*q*, H–C(4')); 4.39 (br. *s*, H–C(3')); 4.64 (*d*, *J* = 5.7, CH_2); 4.96 (*t*, *J* = 5.9, HO–C(5')); 5.33 (*d*, *J* = 4.2, HO–C(3')); 6.27 ('*t*', *J* = 6.6, H–C(1')); 7.25–7.32 (H–C(phenyl)); 8.37 (*s*, H–C(8)); 8.89 (*t*, *J* = 4.8, H–N(6)). Anal. calc. for $C_{17}H_{18}ClN_5O_3$ (375.8): C 54.33, H 4.83, N 18.64; found: C 54.27, H 4.85, N 18.54.

*2-Chloro-9-(2'-deoxy-β-*D-erythro-*pentofuranosyl)-6-methoxy-9*H*-purine* (**6**). The suspension of **4** (810 mg, 1.5 mmol) in MeOH (30 ml) was treated with MeONa/MeOH (4.5 ml, 1M soln.) and stirred 3 h at r.t. A clear soln.

was formed after 30 min. The mixture was evaporated and the residue chromatographed on a silica gel *60H* (column: 3 × 22 cm) with AcOEt (200 ml) and then AcOEt/MeOH 9:1. The nucleoside-containing fractions were pooled, evaporated, and the residue crystallized. Colorless crystals (290 mg, 64%) from AcOEt. M.p. 141–142°. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.54. UV (H_2O): 258 (12300). ^{1}H-NMR ((D$_6$)DMSO): 2.35, 2.70 (2*m*, H–C(2′)); 3.56 (*m*, H–C(5′)); 3.85 (*q*, H–C(4′)); 4.10 (*s*, MeO); 4.42 (br. *s*, H–C(3′)); 4.94 (*t*, *J* = 5.5, HO–C(5′)); 5.36 (*d*, *J* = 4.3, HO–C(3′)); 6.35 ('*t*', *J* = 6.6, H–C(1′)); 8.60 (*s*, H–C(8)). Anal. calc. for $C_{11}H_{13}ClN_4O$ (300.7): C 43.94, H 4.36, N 18.63; found: C 44.08, H 4.45, N 18.63.

*6-Amino-2-chloro-9-(2′-deoxy-3′,5′-di-*O*-acetyl-β-*D-erythro-*pentofuranosyl)-9*H*-purine* (**7**). The stirred suspension of **1a** (850 mg, 3 mmol) in pyridine (10 ml) was treated with Ac_2O (10 ml), and the starting material was dissolved within 30 min. After 3 h, the mixture was evaporated to an oil and then co-evaporated three times with toluene/EtOH. The oily residue was dried (high vacuum) and crystallized from EtOH to yield **7** (1.03 g, 92%). M.p. 173–175°. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.52. UV (MeOH/H_2O): 264 (15300). ^{1}H-NMR ((D$_6$)DMSO): 1.82, 2.01 (2*s*, 2 Ac); 2.55, 3.00 (2*m*, H–C(2′)); 4.25 (*m*, H–C(4′), H–C(5′)); 5.37 (*m*, H–C(3′)); 6.29 ('*t*', *J* = 6.5, H–C(1′)); 7.85 (s, NH_2); 8.37 (*s*, H–C(8)). Anal. calc. for $C_{14}H_{16}ClN_5O_5$ (369.8): C 45.48, H 4.06, N 18.94; found: C 45.61, H 4.12, N 18.90.

*6-Amino-8-bromo-2-chloro-9-(2′-deoxy-3′,5′-di-*O*-acetyl-β-*D-erythro-*pentofuranosyl)-9*H*-purine* (**8**). A soln. of **7** (400 mg, 1.08 mmol) in dioxane (16 ml) and aq. AcONa (pH 4.7, 0.5M, 4 ml) was stirred, and a soln. of Br_2 (240 mg, 1.5 mmol) in dioxane was added within 15 min. The stirring was continued for another 15 min (TLC control). The mixture was diluted with $CHCl_3$ (50 ml) and extracted with H_2O (50 ml), sat. $NaHCO_3$ (50 ml), 1% $Na_2S_2O_4$ soln. (50 ml), and H_2O (2 × 50 ml). The org. phase was dried (Na_2SO_4) and evaporated to dryness. The residue was crystallized from EtOH to give colorless crystals of **8** (370 mg, 76%). M.p. 163–164°. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.65. UV (MeOH/H_2O 1:1): 269 (17500). ^{1}H-NMR ((D$_6$)DMSO): 1.95, 2.09 (2*s*, 2 Ac); 2.55, 3.45 (2*m*, H–C(2′)); 4.17 (*m*, H–C(5′)); 4.34 (*m*, H–C(4′)); 5.33 (*q*, H–C(3′)); 6.29 ('*t*', *J* = 6.8, H–C(1′)); 7.96 (*s*, NH_2). Anal. calc. for $C_{14}H_{15}BrClN_5O_5$ (448.7): C 37.48, H 3.37, N 15.61; found: C 37.63, H 3.43, N 15.66.

*6-Amino-8-bromo-2-chloro-9-(2′-deoxy-β-*D-erythro-*pentofuranosyl)-9*H*-purine* (= *8-Bromo-2-chloro-2′-deoxyadenosine*; **9**). To the soln. of **8** (300 mg, 0.67 mmol) in MeOH (10 ml), NH_3/MeOH (10 ml, sat., at 0°) was added. The mixture was stirred overnight at 4°. The light-yellow, chromatographically pure crystals were formed (192 mg, 79%). An anal. sample was crystallized from EtOH. M.p. 190° (dec.). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.57. UV (H_2O): 269 (16300). ^{1}H-NMR ((D$_6$)DMSO): 2.20, 3.15 (2*m*, H–C(2′)); 3.45, 3.62 (2*m*, H–C(5′)); 3.82 (*q*, H–C(4′)); 4.45 (br. *s*, H–C(3′)); 4.85 (*t*, *J* = 6.2, HO–C(5′)); 5.35 (*d*, *J* = 4.2, HO–C(3′)); 6.23 ('*t*', *J* = 7.1, H–C(1′)); 7.99 (*s*, NH_2). Anal. calc. for $C_{10}H_{11}BrClN_5O_3$ (364.6): C 32.94, H 3.04, N 19.21; found: C 33.12, H 3.11, N 19.22.

REFERENCES

[1] A. Cohen, R. Hirschhorn, S.D. Horowitz, A. Rubinstein, S.H. Polmar, R. Hong, D.W. Martin, Jr., *Proc. Natl. Acad. Sci. U.S.A.* **1978,** *75,* 472.

[2] M.-C. Huang, K. Hatfield, A.W. Roetker, J.A. Montgomery, R.L. Blakley, *Biochem. Pharmacol.* **1981,** *30,* 2663.

[3] D.A. Carson, D.B. Wasson, J. Kaye, B. Ullman, D.W. Martin, R.K. Robins, J.A. Montgomery, *Proc. Natl. Acad. Sci. U.S.A.* **1980,** *77,* 6865.

[4] R.L. Blakley, M.-C. Huang, R.A. Ashmun, R. Koob, in 'Purine and Pyrimidine Metabolism in Man', Eds. W.L. Nyhan, L.F. Thomson, and R.W.E. Watts, Plenum Publishing Corp., New York, 1983, Vol. 5, pp. 589.

[5] T.L. Avery, J.E. Rehy, W.C. Lumm, F.C. Harwood, V.M. Santana, R.L. Blakley, *Cancer Res.* **1989,** *49,* 4972.

[6] P.G. Parsons, E.P.W. Bowman, R.L. Blakley, *Biochem. Pharmacol.* **1986,** *35,* 4025.

[7] C.J. Carrera, Ch. Terai, M. Lotz, J.G. Curd, L.D. Piro, E. Beutler, D.A. Carson, *J. Clin. Invest.* **1990,** *86,* 1480.

[8] L.D. Piro, C.J. Carrera, D.A. Carson, E. Beutler, *New Engl. J. Med.* **1990,** *322,* 1117.

[9] V.M. Santana, J. Mirro, F.C. Harwood, J. Cherrie, M. Schnell, D. Kalwinsky, R.L. Blakley, *J. Clin. Oncol.* **1991,** *9,* 416.

[10] L.F. Christensen, A.D. Broom, M.J. Robins, A. Bloch, *J. Med. Chem.* **1972,** *15,* 735.

[11] Z. Kazimierczuk, H.B. Cottam, G.R. Revankar, R.K. Robins, *J. Am. Chem. Soc.* **1984,** *106,* 6379.

[12] Z. Kazimierczuk, J. Vilpo, C. Hildebrand, G.E. Wright, *J. Med. Chem.* **1990,** *33,* 1683.

[13] F. Seela, W. Bourgeois, *Synthesis* **1988,** 938.
[14] Z. Kazimierczuk, R. Mertens, W. Kawczynski, F. Seela, *Helv. Chim. Acta* **1991,** *74,* 1742.
[15] F. Seela, H. Steker, H. Driller, U. Bindig, *Liebigs Ann. Chem.* **1987,** 15.
[16] E. Breitmaier, W. Voelter, '^{13}C-NMR Spectroscopy', in 'Monographs in Modern Chemistry', Ed. F. Kohler, Verlag Chemie, Weinheim, 1978, Vol. 5, pp. 80.
[17] H. Rosemeyer, G. Toth, B. Golankiewicz, Z. Kazimierczuk, W. Bourgeois, U. Kretschmer, H.-P. Muth, F. Seela, *J. Org. Chem.* **1990,** *55,* 5784.
[18] C. D. Jardetzky, O. Jardetzky, *J. Am. Chem. Soc.* **1960,** *82,* 222.
[19] J. J. Christensen, J. H. Rytting, R. M. Izatt, *Biochemistry* **1970,** *9,* 4907.
[20] H. Rosemeyer, F. Seela, *Helv. Chim. Acta* **1988,** *71,* 1573.
[21] R. Gumbiowski, Thesis, Osnabrück, 1991.
[22] R. Wolfenden, D. F. Wentworth, G. N. Mitchell, *Biochemistry* **1977,** *16,* 5071.
[23] J. Griffig, R. Koob, R. L. Blakley, *Cancer Res.* **1989,** *49,* 6923.
[24] L. Brox, A. Ng, E. Pollock, A. Blech, *Cancer Res.* **1984,** *44,* 934.
[25] S. Seto, C. J. Carrera, M. Kubota, D. B. Wasson, D. A. Carson, *J. Clin. Invest.* **1985,** *75,* 377.
[26] D. A. Carson, C. J. Carrera, D. B. Wasson, H. Yamanaka, *Adv. Enz. Reg.* **1988,** *27,* 397.
[27] S. Eriksson, B. Kierdaszuk, B. Munch-Petersen, B. Öberg, N. G. Johansson, *Biochem. Biophys. Res. Commun.* **1991,** *176,* 586.
[28] T. A. Krenitsky, J. V. Tuttle, G. W. Koszalka, I. S. Chen, L. M. Beacham III, J. L. Rideout, G. B. Elion, *J. Biol. Chem.* **1976,** *251,* 4055.