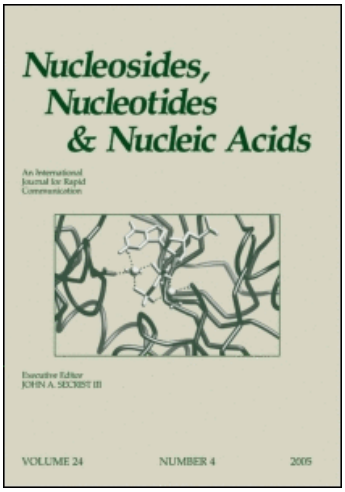

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2-Chloro-2'-deoxyadenosine: Synthesis and Antileukemic Activity of 8-Substituted Derivatives

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2-CHLORO-2'-DEOXYADENOSINE: SYNTHESIS AND ANTILEUKEMIC ACTIVITY OF 8-SUBSTITUTED DERIVATIVES

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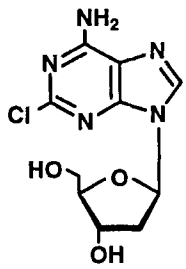
ABSTRACT: A series of 8-substituted 2-chloro-2'-deoxyadenosine (2-CdA, **1**) derivatives were prepared as potential anticancer agents. They were synthesized stereoselectively by the anion glycosylation of 2,6,8-trichloropurine or obtained by nucleophilic displacement reactions on 8-bromo-2-chloro-2'-deoxyadenosine (**3**). Within the 8-substituted CdA derivatives the 8-thioxo compound **11** was cytotoxic to several leukemia cell lines.

INTRODUCTION

The 2'-deoxyadenosine analogue 2-chloro-2'-deoxyadenosine (**1**) (2-CdA, Cladribine, Leustatin) has found clinical application for the treatment of lymphoid and immunoaggressive diseases[1,2]. Recently, we have reported on the synthesis and antileukemic activity of base-modified derivatives of 2-chloro-2'-deoxyadenosine[3]. It was observed that nucleosides having the same substituent pattern as **1** but containing the 1-deaza-, 3-deaza-, 7-deaza- or 8-aza-7-deazapurine moiety were inactive against a number of leukemic cell lines. The replacement of the 6-amino group of **1** by a secondary amino function also decreased cytotoxic activity[3]. The sugar modified 2-chloroadenine 2'-deoxy-4'-thioribofuranoside[4] as well as the 2'-deoxy-2'-fluoroarabinofuranoside[5,6] show also cytotoxicity to several human tumor cells.

Previously, we have observed that the 8-bromo derivative **3** shows cytotoxicity[3] comparable to that of 2-bromo-2'-deoxyadenosine (**2**)[7] in leukemia cell lines. In the following we report on the synthesis and properties of other 2-chloro-2'-deoxy-adenosine derivatives carrying various substituents at position 8.



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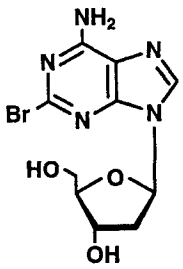

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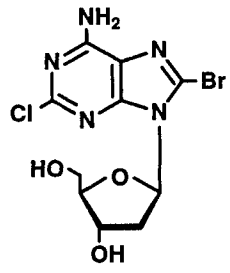

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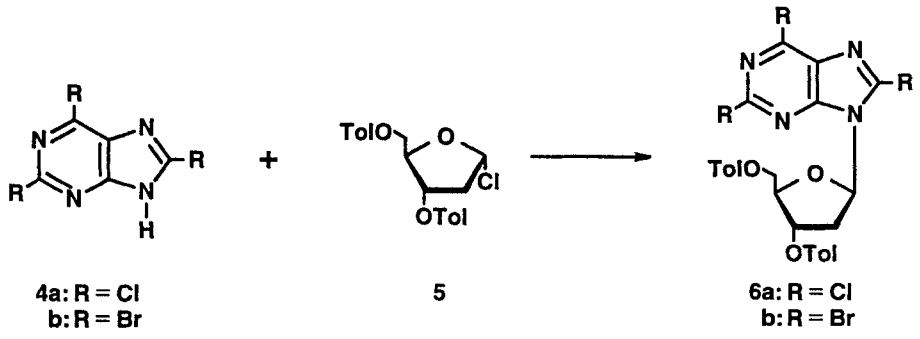
RESULTS AND DISCUSSION

Chemistry. - Glycosylation of the anions of 2,6,8-trichloropurine (**4a**) or 2,6,8-tribromopurine (**4b**) with the halogenose **5** results in the protected N^9-nucleosides **6a** (56%) and **6b** (59%), respectively. As the reaction proceeds stereoselectively no α-D anomers are formed. Also N^7-glycosylation products were not detected. The latter may result from the steric or electronic influence of the bulky 8-substituent. In addition, it cannot be excluded that the N^7-glycosides are too labile at their N-glycosylic bonds and are hydrolysed during the work-up procedure. Compounds **6a** and **6b** were deblocked under subsequent displacement of halo substituents.

Reaction of **6a** and **6b** with methanolic ammonia at room temperature gave 8-amino-2,6-dichloropurine or 8-amino-2,6-dibromopurine deoxyribonucleosides **7a** and **7b**, respectively. However, at elevated temperature the second halogen displacement takes place at position 6 yielding the 6,8-diamino-2-halopurine deoxynucleosides **8a** and **8b**. Also in the case of the N^9-tetrahydropyranyl derivatives the first displacement occurs at C-8 followed by the reaction at C-6[8]. The structure of the reaction was confirmed by the catalytic hydrogenation of **8a** to give 8-amino-2'-deoxyadenosine. This was hydrolyzed in 1 M HCl at elevated temperature to give 6,8-diaminoadenine which was compared with an authentical sample[9]. Triaminonucleosides are not formed under these conditions. The halogen displacement on this 9-substituted 2,6,8-halogenopurines is analogous to the reaction on 7-substituted compounds. Thus halogen displacement on 7-methyl-2,6,8-trichloropurine with ammonia furnishes 2-chloro-6,8-diamino-7-methylpurine[10].

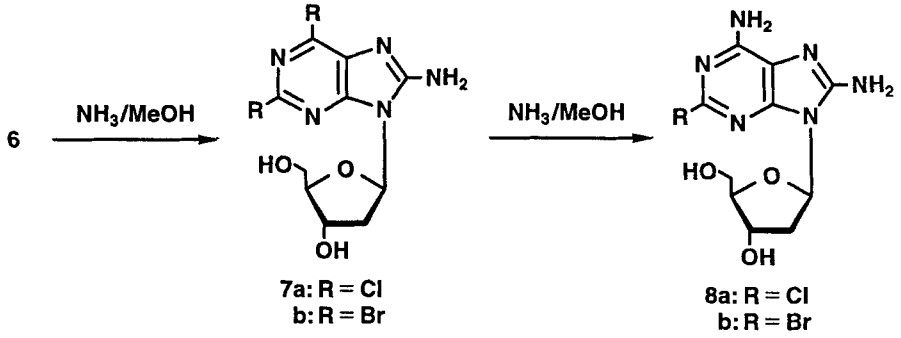
The order of displacement reactions is altered in the case of the 2,6,8-trihalogenopurine base. Here, the 6-substituent is displaced at first followed by that of position 8 and then by the 2-substituent[11-13]. In some cases small amounts of 6-substituted derivatives were found together with the compound already displaced at position 8.



4a: R = Cl
b: R = Br

5

6a: R = Cl
b: R = Br



7a: R = Cl
b: R = Br

8a: R = Cl
b: R = Br

The 8-substituted derivatives **10-12** were obtained by nucleophilic displacement performed on compound **3**. Treatment with sodium methoxide or ethoxide provides compounds **10a** and **10b**, respectively. However, when using the bulky potassium tert-butoxide as a base the 8,5'-cyclo derivative **9** is formed. A similar observation was made on 7-deazapurine nucleosides[14].

The introduction of sulphur into position 8 of the purine ring was realized by reaction of **3** with sodium hydrogen sulphide providing compound **11**. The latter reagent has been shown to be more useful than thiourea. According to the ^{1}H NMR spectrum the structure of **11** is represented by the thiooxo compound and not by the tautomeric thiol. The thioalkyl derivatives **12a** and **12b** were obtained by reaction of **11** with methyl or ethyl iodide in slightly alkaline medium.

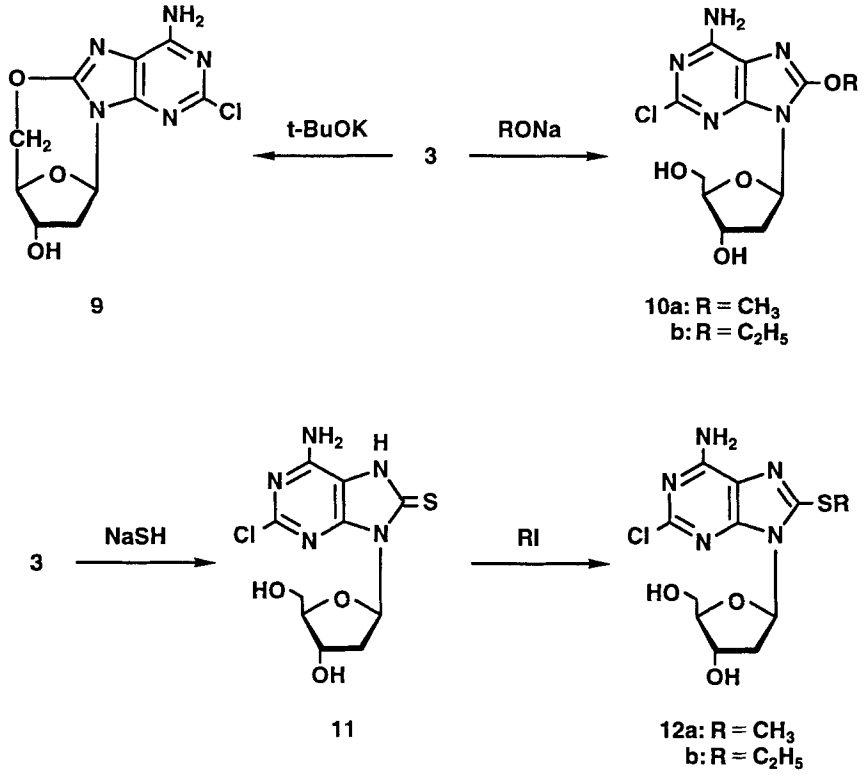
The new derivatives of compound **1** were characterized in detail. The ^{13}C NMR chemical shifts are summarized in Table 1. According to the substituent pattern of the

TABLE 1. ^{13}C NMR Chemical Shifts of Purine 2'-Deoxyribonucleosides in DMSO-d_6 at 23°C[a].

Comp.	C-2[b]	C-4	C-5	C-6[b]	C-8	CH_3/CH_2
1	153.0	150.0	118.1	156.8	139.8	-
2[15]	144.1	149.9	118.5	156.6	139.6	-
7a	145.6	156.2[b]	131.1[b]	139.8	154.2[b]	-
7b	135.4	155.8[b]	131.3[b]	134.2	152.8[b]	-
8a	148.3	151.7[b]	115.9[b]	153.3	150.0[b]	-
8b	139.1	151.5[b]	116.1	153.1	149.9[b]	-
9	151.8	148.0[b]	112.9	155.6	153.5	-
10a	149.1	148.1	112.1	153.3	152.8	57.3
10b	150.5	149.5	113.6	154.7	153.6	14.3/66.3
11	148.2	149.3[b]	106.8	152.1	167.5	-
12a	151.6	151.9	118.5	155.0	149.8	14.6
12b	151.6	151.7	118.6	155.2	148.7	14.9/26.7

	C-1'	C-2'	C-3'	C-4'	C-5'
1	83.5	DMSO	70.7	87.9	61.6
2[15]	83.5	39.3	70.7	87.7	61.6
7a	83.6	37.6	71.1	87.8	61.4
7b	83.5	37.6	71.2	87.9	61.4
8a	83.0	37.6	71.3	87.6	61.6
8b	83.0	37.5	71.3	87.6	61.5
9	82.3	43.4	70.7	87.5	75.0
1a	82.2	36.5	70.9	87.7	62.0
10b	82.1	36.5	70.9	87.6	62.0
11	84.8	36.6	71.3	88.1	62.2
12a	84.4	37.1	71.1	88.2	62.1
12b	84.5	37.1	71.1	88.2	62.1

[a]Assignment from gated-decoupled spectra. [b]Tentative.



8-substituted derivatives the number of ^{1}H, ^{13}C couplings is limited. This results in tentative assignments of various ^{13}C NMR signals.

Biological Evaluation. - Compounds **7a** - **12b** were tested against several leukemia cell lines. From the series of 8-modified CdA-derivatives only a few show cytotoxicity. Table 2 shows data of the cytotoxic compounds **10a** and **11**. The ID_{50}-value was exceeded with the high test dose (10 μg/ml) of compound **10a** only with the most sensitive cell line or U-937. Similarly, this dose was close to the ID_{50} of the IM-9 and Raji lines, when compound **11** was tested. The Molt-3 and the U-937 lines were more sensitive, but the ID_{50} was not obtained with PHA-stimulated lyphocytes (Table 2). The toxicity of compounds **10a** and **11** is significantly weaker than that of 8-bromo CdA, CdA or 2-bromo-2'-deoxyadenosine. The higher toxicity of CdA vs 2-bromo-2'-deoxyadenosine is in contrast to earlier reports. Quite similar toxicities against human cell lines have been recorded with the 2-halo-2'-deoxyadenosines, if tested in the same experiment[16].

TABLE 2. Toxicity Data of 2-Chloro-2'-deoxyadenosine Derivatives (10 μg/ml) against Malignant and Normal Hematopoietic Cells in vitro.

Compound	^{14}C-leucine incorporation (% of control)[a]				
	IM-9[b]	Raji	MOLT-3	U-937	PHA-Ly
10a	104	79	72	28	110
11	46	57	4.5	4.5	72

[a]Determined as described. 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 phytohemagglutin-stimulated peripherial blood lymphocytes.

It has been observed that 8-substituted adenine 2',3'-dideoxynucleosides are remarkably stable with respect to hydrolytic cleavage[17]. All of the 8-substituted compounds described in the manuscript are resistant towards cleavage by mammalian or *E. coli* purine nucleoside phosphorylase[18] which may be an advantage from the viewpoint of pharmacological action.

EXPERIMENTAL SECTION

General. Elemental analyses were performed by Mikroanalytisches Labor Beller (Göttingen, Germany). NMR-Spectra were measured on a AC 250 spectrometer (Bruker, Germany). Chemical shifts are in ppm relative to TMS as internal standard. UV-spectra were recorded on a U3200 spectrometer (Hitachi, Japan). Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (Merck, Germany) and preparative TLC (layer: 2 mm). Column chromatography was performed on silica gel 60 (Merck, Germany).

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 x 10^4 cells per a 200 μl well (or 2.5 x

10^5 peripheral blood lymphocytes, stimulated with phytohemagglutin. Cells were cultured in RPMI 1640, medium containing fetal calf serum (10%), in humidified atmosphere containing 5% CO_2 at 37°C. [^{14}C] L-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-day culture period. After incubation the proteins were precipitated with 0.2 N $HClO_4$ and collected on 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). The incorporation of [^{14}C] leucine per cell remain constant during the final 24h of culture. A good correlation between cell number and [^{14}C] leucine incorporation has been demonstrated[19,20].

9-[2-Deoxy-(3,5-di-O-p-toluoyl)-ß-D-*erythro*-pentofuranosyl]-2,6,8-trichloropurine (6a).

A solution of compound **4a**[21] (1.11 g, 5.0 mmol) in MeCN (50 ml) was treated with NaH (280 mg, 5.8 mmol, 50% in oil) at 50°C under stirring. The reaction was brought to room temperature and the stirring was continued for 15 min. The halogenose **5**[22] (1.95 g, 5.0 mmol) was added at room temperature. After 20 min the reaction mixture was filtered through Celite, the solvent was evaporated to give an oil and chromatographed on a silica gel 60 column (3 x 25 cm) with toluene-acetone (9:1) as eluent. The nucleoside-containing fractions were pooled and evaporated. The residue was dissolved in cold MeOH and upon reduction of the volume a colourless powder (1.61 g, 56%) was obtained. M.p. 147-149°C. TLC (silica gel, toluene-acetone 9:1): R_f 0.56. ^{1}H-NMR (DMSO-d_6): 2.33 and 2.38 (2s, 2 CH_3), 2.80 and 3.50 (2m, $H_{a,b}$-2'), 4.60 (m, 4'-H and 5'-H), 5.97 (q, 3'-H), 6.49 (t, J = 5.3 Hz, 1'-H), 7.0 - 8.0 (arom. H). Anal. calcd. for $C_{26}H_{21}N_4O_5Cl_3$ (575.8): C 54.23, H 3.68, N 9.73. Found: C 54.20, H 3.78, N 9.60.

9-[2-Deoxy-(3,5-di-O-p-toluoyl)-ß-D-*erythro*-pentofuranosyl]-2,6,8-tribromo-purine (6b).

Compound **6b** was prepared from **4b**[23] (1.78 g, 5.0 mmol) as described for **6a**. Crystallization from MeOH gave a white powder (2.1 g, 59%). M.p. 131°C. TLC (silica gel, toluene-acetone 9:1): R_f 0.60. ^{1}H-NMR (DMSO-d_6): 2.34 and 2.38 (2s, 2CH_3), 2.80 and 2.55 (2m, $H_{a,b}$-2'), 4.60 (2m, H-4'and H-5'), 5.95 (q, H-3'); 6.55 (pt, J = 5.4 Hz, 1'-H), 7.0 - 8.0 (arom. H). Anal. calcd. for $C_{26}H_{21}Br_3N_4O_5$ (709.2): C 44.03, H 2.98, N 7.90. Found: C 44.18, H 3.07, N 7.77.

8-Amino-9-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-2,6-dichloropurine (7a).

A suspension of **6a** (700 mg, 1.2 mmol) in methanolic ammonia (50 ml, saturated at

0°C) was stirred for 3 days at room temperature. The reaction mixture was evaporated to dryness and the residue chromatographed on a silica gel 60 column (3 x 15 cm) with chloroform (200 ml) and chloroform-methanol (9:1, 500 ml). The nucleoside containing fractions were pooled, evaporated, and the residue crystallized from EtOH-EtOAc (1:2) to yield a white powder (240 mg, 61%). M.p. 180°C (decomp.). TLC (silica gel, CH_2Cl_2-MeOH, 9:1): R_f 0.40. UV (H_2O): 221 (14900), 265 (7200), 300 (12000). ^{1}H-NMR (DMSO-d_6): 2.10 and 2.65 (2m, $H_{a,b}$-2'), 3.65 (m, H-5'), 3.90 (q, H-4'), 4.35 (bs, H-3'), 5.36 (d, OH-3'), 5.52 (t, OH-5'), 6.33 (pt, J = 6.1 Hz, H-1'), 7.79 (s, NH_2). Anal. calcd. for $C_{10}H_{11}Cl_2N_5O_3$ (320.1): C 37.52, H 3.46, N 21.88. Found: C 37.68, H 3.55, N 21.82.

8-Amino-9-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-2,6-dibromopurine (7b). Compound **6b** (800 mg, 1.2 mmol) was treated as described for **7a**. Crystallization from EtOH/EtOAc (1:2) afforded colourless crystals (280 mg, 62%) with m.p. 190°C (decomp.). TLC (silica gel, CH_2Cl_2-MeOH 9:1): R_f 0.45. UV (H_2O): 222 (15400), 267 (7400), 302 (11500). ^{1}H-NMR (DMSO-d_6): 2.10 and 2.63 (2m, $H_{a,b}$-2'), 3.65 (m, H-5'), 3.91 (bs, H-4'), 4.41 (bs, H-3'), 5.38 and 5.52 (2bs, OH-3'and OH-5'), 6.32 (pt, J = 7.1 Hz, H-1'), 7.80 (s, NH_2). Anal. calcd. for $C_{10}H_{11}Br_2N_5O_3$ (409.04): C 29.36, H 2.71, N 17.12. Found: C 29.49, H 2.77, N 17.22.

2-Chloro-9-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-6,8-diaminopurine (8a). The solution of **6a** (700 mg, 1.1 mmol) in methanolic ammonia (60 ml, saturated at 0°C) was heated in a steel vessel for 12 h at 100°C. The light yellow solution was evaporated, the residue applied on 4 preparative silica gel plates (20 x 20 cm, layer 2 mm) and developed in CH_2Cl_2-MeOH 9:1. From the main zone compound **8a** was isolated and colourless needles (215 mg, 59%) were obtained from a small volume of EtOH. M.p. 152-155°C. TLC (silica gel, CH_2Cl_2-MeOH 9:1): R_f 0.18. UV(H_2O): 280 (14600). ^{1}H-NMR (DMSO-d_6): 2.05 and 2.65 (2m, $H_{a,b}$-2'), 3.60 (bs, H-5'), 3.87 (bs, H-4'), 4.50 (d,H-3'), 5.30 and 5.40 (2bs, OH-3'and OH-5'), 6.24 (pt, J= 6.05 Hz, H-1'), 6.68 and 6.93 (2s, NH_2-6 and NH_2-8). Anal. calcd. for $C_{10}H_{13}ClN_6O_3$ (300.7): C 39.94, H 4.36, N 27.95. Found: C 40.05, H 4.42, N 27.76.

2-Bromo-9-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-6,8-diaminopurine (8b). A suspension of **6b** (800 mg, 1.2 mmol) in methanolic ammonia (60 ml) was heated in a steel vessel at 80°C overnight. Work-up was as described for **8a**. Colourless

powder (260 mg, 67%) from EtOH-EtOAc (1:1). M.p. 121-123°C. TLC (silica gel, CH_2Cl_2-MeOH 9:1): R_f 0.18. UV(H_2O): 281 (14500). ^{1}H-NMR (DMSO-d_6): 1.80 and 2.40 (2m, $H_{a,b}$-2'), 3.40 (bs, H-5'), 3.63 (bs, H-4'), 4.16 (bs, H-3'), 5.06 and 5.14 (2bs, OH-3' and OH-5'), 5.99 (pt, J = 6.0 Hz, H-1',), 6.46 and 6.69 (2s, NH_2-6 and NH_2-8). Anal. calcd. for $C_{10}H_{13}BrN_6O_3$ (345.2): C 34.80, H 3.80, N 24.35. Found: C 34.65, H 3.88, N 24.52.

6-Amino-2-chloro-9-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-8-methoxy-9H-purine (10a).

A suspension of compound **3** (365 mg, 1.0 mmol) in MeOH (30 ml) was treated with NaOMe (1N in MeOH, 3 ml, 3.0 mmol). The reaction mixture was stirred under reflux for 2h. Then it was cooled and neutralized with AcOH (0.12 ml). Evaporation to dryness and crystallization from MeOH-water (1:1) gives colourless needles (227 mg, 72%). M.p. 210-212°C. TLC (silica gel, CH_2Cl_2-MeOH, 9:1): R_f 0.57. UV (water): 268 (15400). ^{1}H-NMR (DMSO-d_6): 2.12 and 2.91 (2m, $H_{a,b}$-2'), 3.44 and 3.56 (2m, H-5'), 3.80 (q, H-4'), 4.05 (s, OCH_3), 4.47 (bs, H-3'), 4.79 (t, OH-5'), 5.24 (d, OH-3') 6.14 (t, J = 7.1 Hz, H-1'), 7.30 (s, NH_2). Anal. calcd. for $C_{11}H_{14}ClN_5O_4$ (315.7): C 41.84, H 4.47, N 22.18. Found: C 41.96, H 4.44, N 22.31.

6-Amino-2-chloro-9-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-8-ethoxy-9H-purine (10b).

Compound **10b** was prepared as described for **10a**. Colourless needles (225 mg, 68%) from EtOH-water (1:1). M.p. 226-228°C. TLC (silica gel, CH_2Cl_2-MeOH 9:1): R_f 0.60. UV (water) 268 (15500). ^{1}H-NMR (DMSO-d_6): 1.41 and 4.42 (t and q, OC_2H_5), 2.12 and 2.92 (2m, $H_{a,b}$-2'), 3.45 and 3.57 (2m, H-5'), 3.77 (bs, H-4'), 4.22 (bs, H-3'), 4.79 (t, OH-5'), 5.25 (d, OH-3'), 6.15 (t, J = 7.0 Hz, H-1'), 7.37 (s, NH_2). Anal. calcd. for $C_{12}H_{16}ClN_5O_4$ (329.7): C 43.71, H 4.89, N 21.24. Found: C 43.82, H 4.82, N 21.33.

6-Amino-2-chloro-8,5'-O-cyclo-9-ß-D-(2-deoxy-*erythro*-pentofuranosyl)-9H-purine (9).

The suspension of **3**[3] (365 mg, 1.0 mmol) in tert-BuOH (35 ml) was treated with tert-BuOK (235 mg, 3.0 mmol). The reaction mixture was stirred at 70°C for 24 h. Then it was evaporated to dryness and chromatographed on a silica gel 60 column (3 x 15 cm) with chloroform (200 ml) and chloroform-methanol (9:1, 500 ml). The nucleoside containing fractions were evaporated to dryness and crystallized from

water to give colourless needles (141 mg, 50%). M.p. 172-175°C. TLC (silica gel, CH_2Cl_2-MeOH 9:1): R_f 0.52. UV (water) 266 (12900). ^{1}H-NMR (DMSO-d_6): 2.42 (d, $H_{a,b}$-2'), 4.07 and 4.60 (2m, H-5'), 4.47 (bs, H-3'), 5.35 (s, OH-3'), 6.40 (d, J = 2.8 Hz, H-1'), 7.53 (s, NH_2). Anal. calcd. for $C_{10}H_{10}ClN_5O_3$ (283.7): C 42.34, H 3.55, N 24.69. Found: C 42.19, H 3.50, N 24.52.

6-Amino-2-chloro-9-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-8-thioxo-9H-purine (11).

The mixture of **3** (440 mg, 1.25 mmol) and NaSH x H_2O (400 mg) in ethanol (96%, 40 ml) was stirred under reflux for 2 h. The pale yellow solution was adsorbed on a silica gel and placed on the top of a silica gel 60 column. The elution was performed with chloroform (100 ml) and chloroform-methanol (9:1, 600 ml). The nucleoside containing fractions were evaporated to dryness and the residue crystallized from ethanol-water (4:1) to yield white powder (245 mg, 62%). M.p. higher than 200°C (decomp.). TLC (silica gel, $CHCl_3$-MeOH 9:1): R_f 0.20. UV (water): 233 (18300), 310 (28000). ^{1}H-NMR (DMSO-d_6): 2.10 (2m, H_{ab}-2'), 3.50 and 3.65 (2m, H-5'), 3.81 (bs. H-4'), 4.43 (bs, H-3'), 4.78 (t, OH-5'), 5.26 (OH-3'), 6.67 (pt, J = 7.2 Hz, H-1'), 7.31 (bs, NH_2), 12.56 (bs, N-H). Anal. calcd. for $C_{10}H_{12}ClN_5O_3S$ (317.8): C 37.80, H 3.81, N 22.04. Found: C 37.66, H 3.73, N 21.85.

6-Amino-2-chloro-9-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-8-methylthio-9H-purine (12a).

To a solution of **11** (220 mg, 0.7 mmol) in 0.2 M K_2CO_3 (10 ml) methyl iodide (0,1 ml, 230 mg, 1.6 mmol) was added under stirring. The stirring was continued for 8h and the solution was stored at 4°C overnight. The white precipitate was filtered and crystallized from MeOH-water (1:1) to give colourless needles (155 mg, 67%). M.p. higher than 250°C (decomp.). TLC (silica gel, $CHCl_3$-MeOH 9:1): R_f 0.36. UV (water): 284 (21800). ^{1}H-NMR (DMSO-d_6): 2.12 and 2.95 (2m, $H_{a,b}$-2'), 2.69 (s, SCH_3), 3.48 and 3,62 (2m, H-5'), 3.82 (bs, H-4'), 4.40 (bs, H-3'), 4.87 (t, OH-5'), 5.32 (d, OH-3'), 6.14 (pt, J = 7.0 Hz, H-1'), 7.66 (s, NH_2). Anal. calcd. for $C_{11}H_{14}ClN_5O_3S$ (331.8): C 39.82, H 4.25, N 21.11. Found: C 39.68, H 4.17, N 20.94.

2-Amino-2-chloro-9-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-8-ethylthio-9H-purine (12b).

Compound **12b** was prepared from **11** (220 mg, 0.7 mmol) as described for **12a** but using ethyl iodide (0.1 ml, 195 mg, 1.25 mmol). Colourless needles (175 mg, 72%).

M.p. 169-171°C. TLC (silica gel, $CHCl_3$-MeOH 9:1): R_f 0.40. UV (water): 285 (21300). ^{1}H-NMR (DMSO-d_6): 1.34 and 3.26 (t and q, SC_2H_5), 2.12 and 3.00 (2m, $H_{a,b}$-2'), 3.48 and 3.62 (2m, H-5'), 3.83 (bs, H-4'), 3.41 (bs, H-3'), 4.88 (t, OH-5'), 5.32 (d, OH-3'), 6.17 (pt, J = 7.0 Hz, H-1'), 7.69 (s, NH_2). Anal. calcd. for $C_{12}H_{16}ClN_5O_3S$ (345.8): C 41.68, H 4.66, N 20.25. Found: C 41.51, H 4.58, N 20.12.

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