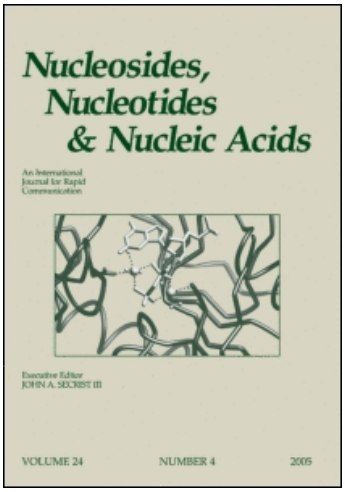

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SYNTHESIS AND BIOLOGICAL PROPERTIES OF 9-(2,4-DIHYDROXYBUTYL)ADENINE AND GUANINE: NEW ANALOGUES OF 9-(2,3-DIHYDROXYPROPYL)ADENINE (DHPA) AND 9-(2-HYDROXYETHOXYMETHYL)GUANINE (ACYCLOVIR)

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ABSTRACT: New analogues of antiviral agents 9-(2,3-dihydroxypropyl)adenine (DHPA, 1a) and 9-(2-hydroxyethoxymethyl) guanine (acyclovir, 1b) - compounds 1c and 1d were prepared and their biological activity was investigated. Racemic 1,2,4-butanetriol (2) was converted to the corresponding benzylidene derivative (3a) by acetalation with benzaldehyde and triethyl orthoformate. Acetal 3a and p-toluenesulfonyl chloride in pyridine gave the corresponding p-toluenesulfonate 3b. Alkylation of adenine 5a via sodium salt of 5a with 3b in dimethylformamide or in the presence of tetra-n-butylammonium fluoride in tetrahydrofuran gave intermediate 6a. Reaction of 2-amino-6-chloropurine (5b) with 3b effected by K_2CO_3 in dimethylsulfoxide gave compound 6b and a smaller amount of 7-alkylated product 7. A similar transformation catalyzed by tetra-n-butylammonium fluoride afforded only intermediate 5b. Acid-catalyzed deprotection (hydrolysis) of 6b and 6a gave the title compounds 1c and 1d. The S-enantiomer of 1c was deaminated with adenosine deaminase. Our results argue against the presence of a methyl group-binding site of adenosine deaminase. Compounds 1c and 1d exhibited little or no activity in antiviral assays with several DNA and RNA viruses.

The synthesis and biological studies of analogues of antiviral agents DHPA (1a) and acyclovir (1b) have been a subject of several recent investigations [1,2]. Compounds 1a and 1b are considered to be nucleoside analogues where the

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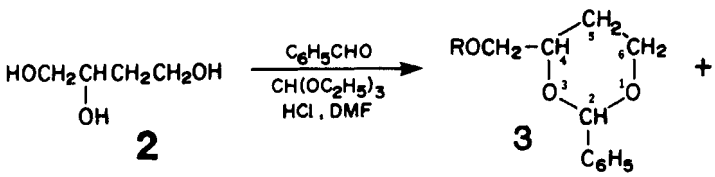
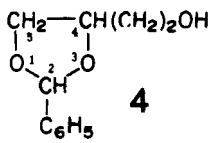
former resembles the "bottom" C_1'--C_2'--C_3' portion and the latter the "top" C_1'--O--C_4'--C_5' part of the ribofuranose in adenosine and guanosine, respectively. A view has also been expressed, based on an X-ray study, that the hydroxymethyl function of 1a could mimic the same moiety in adenosine[3]. It was also shown[4] that replacement of oxygen -O- with the -CHOH- function in inorganic pyrophosphate led to a derivative with biological activity. In that context, compound 1d and 1c can be considered "hydroxymethylidene" analogues of acyclovir (1b) and the respective adenine derivative 1e. In addition, diol 1c may also be viewed as a homologue of DHPA (1a). A recent preliminary report[5] described briefly 4'-O-benzyl derivatives of 1c and 1d and 2'-keto analogues 1f and 1g. These circumstances have prompted us to report on our studies of the synthesis and biological activity of compounds 1c and 1d.

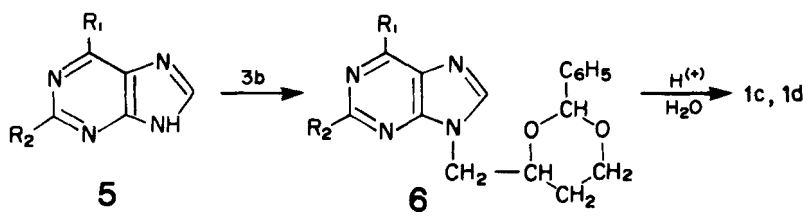
Synthesis. Our approach to analogues 1c and 1d is based on alkylation of a suitable heterocyclic base with a protected derivative of 1,2,4-butanetriol (2). The synthesis commenced with the known[6] benzylidene derivative 3a which was obtained by an improved procedure. Acetalation of racemic triol 2 with benzaldehyde in the presence of triethyl orthoformate and HCl in dimethylformamide[7] (DMF) gave 3a in 50 - 70% yield depending on the work up of the reaction mixture (Scheme 1). The retractive index and boiling point of 3a agreed with the described values[6]. The only signal reported previously in the NMR spectrum of 3a was H_2. Our NMR studies showed that the product is a



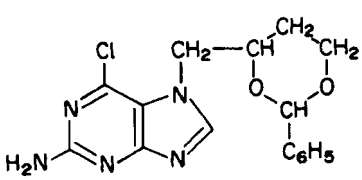


3a : R = H
3b : R = p-$CH_3C_6H_4SO_2$

Scheme 1



5a, 6a : $R_1 = NH_2$, $R_2 = H$
5b, 6b : $R_1 = Cl$, $R_2 = NH_2$



Scheme 2

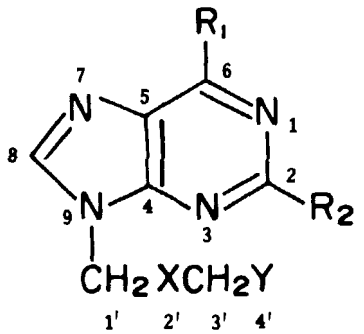
mixture consisting of ca. 90% of 3a and 10% of the dioxolane derivative 4. This is in agreement with the literature data[6] (90 - 95% of 3a) based on methylation, deacetalation and subsequent periodate oxidation. Thus, in addition to a sharp singlet at δ 5.49 belonging to the H_2 of compound 3a (cis isomer[8]) two singlets of the H_2 protons at δ 5.89 and 5.75 belonging to cis and trans isomers[8] of dioxolane 4 were observed. The assignment of relevant signals of 3a was made possible by deuterium exchange and spin-decoupling experiments.

Purification of crude 3a was achieved previously via the corresponding p-phenylazobenzoyl derivative[6] in only 26% yield. Fortunately, we were able to avoid this time-consuming and inefficient procedure. Thus, a mixture of acetals 3a and 4 (9:1) was reacted with p-toluenesulfonyl chloride in pyridine to give the desired p-toluenesulfonate 3b which crystallized from ether free from dioxolane isomer (NMR) in almost 80% yield. The NMR spectrum of 3b (dioxane moiety) was strikingly different from that of 3a. Thus, the signals of H_4, H_6 and 4-CH_2 appeared as a cluster of multiplets at δ 4.08 and the differentiation of H_5 protons (multiplet at δ 1.59) became much less pronounced.

Compound 3b was used for alkylation of the appropriate bases, adenine (5a) and 2-amino-6-chloropurine (5b). The latter derivative was used as a precursor of the guanine residue because previous attempts to alkylate guanine or N-acetylguanine directly gave poor results[9]. Two methods were examined: (1) alkylation with 3b via alkali metal

salts [10,11] of base 5a or 5b and (ii) alkylation catalyzed by fluoride ion in THF[12]. The former method was superior in both cases. Thus, reaction of the sodium salt of 5a with 3b in DMF at 115 - 125°C for 40 min. gave intermediate 6a in 57% yield whereas refluxing of both components with tetra-n-butylammonium fluoride (TBAF) in tetrahydrofuran (THF) for 12 h gave 6a in only 16% yield. In the case of 5b milder conditions for alkylation were sought because of the presence of a reactive chlorine atom. Thus, compound 5b was smoothly alkylated with 3b in the presence of K_2CO_3 in DMSO[13] at 80 - 100°C for 1.5 h to give intermediate 6b in 49% yield accompanied by the N^7-alkylated product, 7 (11% yield). Again, although the reaction mediated by TBAF in THF (reflux for 15 h) did not lead to any significant alkylation at N-7, it was less effective affording, derivative 6b, in only 19% yield after an extensive chromatographic purification. An attempt to alkylate 5b with p-toluenesulfonate 3b using the hexamethyldisilazane - $Hg(CN)_2$ method[14] was not successful probably because of a lower reactivity of 3b under such conditions. The latter compound was recovered unchanged in almost quantitative yield from the reaction mixture.

The structures of 6a, 6b and 7 were confirmed by spectral (NMR and UV) data. The pattern of NMR signals of the dioxane portion of 6a, 6b and 7 was strikingly similar to that of the p-toluenesulfonate 3b. Thus, H_4, H_6 and 4-CH_2 of the dioxane portion appeared at δ 4.2 - 4.3 as clusters of multiplets and two H_2 protons as a multiplet



1

1a: R_1 = NH_2, R_2 = H, X = CHOH, Y = OH

1b: R_1 = OH, R_2 = NH_2, X = O, Y = CH_2OH

1c: R_1 = NH_2, R_2 = H, X = CHOH, Y = CH_2OH

1d: R_1 = OH, R_2 = NH_2, X = CHOH, Y = CH_2OH

1e: R_1 = NH_2, R_2 = H, X = O, Y = CH_2OH

1f: R_1 = NH_2, R_2 = H, X = CO, Y = CH_2OH

1g: R_1 = OH, R_2 = NH_2, X = CO, Y = CH_2OH

1h: R_1 = NH_2, R_2 = H, X = CH_2, Y = CH_2OH

1i: R_1 = OH, R_2 = H, X = CHOH, Y = CH_2OH

1j: R_1 = NH_2, R_2 = H, X = CHOH, Y = H

at ca. δ 1.7. As expected from an analogy with the corresponding 9-alkyl-6-chloropurines[13], the N^7-isomer 7 exhibited a bathochromic shift of the long-wavelength UV absorption maximum relative to the N^9-isomer 6b.

Deacetalation of both intermediates 6a and 6b was accomplished by acid hydrolysis. Compound 6a was hydrolyzed in 80% acetic acid at 100°C for 1 h to give 9-(2,4-dihydroxybutyl)adenine (1c) in 80% yield. It is of interest that the deblocking of 6a was accompanied by a partial acetylation as indicated by thin-layer chromatography (TLC). Ammonolysis of the crude product led to compound 1c. More vigorous conditions were used for the hydrolysis of 6b in order to remove simultaneously the 6-chloro function[15]. Thus, intermediate 6b was refluxed in 0.1 M HCl for 5 h to give guanine derivative 1d in 60% yield. The structures of 1c and 1d were confirmed by UV spectra which corresponded to 9-methyladenine[16] and 9-methylguanine[17], respectively.

Biological Activity. Compound 1e was a substrate for adenosine deaminase[18]. By contrast, the corresponding methylene analogue 1h and S-DHPA (1a) were not deaminated but they acted as weak inhibitors [19,20]. In vivo, however, deamination of 1a was observed[21]. It was therefore of interest to examine the behavior of compound 1c toward adenosine deaminase. We have found that one enantiomer of the racemic mixture 1c was readily deaminated (5×10^{-3} M 1c, 1 mg enzyme/mL, 37°C, 24 h)[22] to the corresponding hypoxanthine derivative 1i as shown by UV

spectra. The circular dichroism (CD) spectrum of the unchanged enantiomer of 1c exhibited a negative Cotton effect at 260 nm indicating that the S-isomer was deaminated whereas the R-enantiomer remained unchanged. The CD spectrum of a related (lower homologue) R-isomer of DHPA (1a) also exhibited a negative Cotton effect[11] at 258 nm. We can thus conclude that replacement of the ether oxygen function of 1e with the -CHOH- moiety of a definite (S) configuration is compatible with substrate requirements of adenosine deaminase. Previously[23], inhibition of adenosine deaminase with S-(2-hydroxypropyl)adenine (1j) was interpreted in terms of a hypothetical relationship of the 2'-hydroxy group to a similar function of adenosine. In order to explain a tighter binding of the S-isomer of 1j relative to that of 9-(2-hydroxyethyl)adenine, a methyl group-binding site of adenosine deaminase was proposed[23]. Our results have led to an alternative and simpler explanation. Thus, attachment of a hydroxymethyl group to the methyl function of 1j afforded an active substrate (compound 1c). Previous substitutions of this methyl function invariably caused a loss of inhibitory potency[23]. In no case was substrate activity observed. It is also important to recognize that the (5') hydroxymethyl group of nucleosides (adenosine) is necessary for maintaining a good substrate activity[18,24]. It is then likely that 1j is a "shortened" analogue of 1c. In such a case the 2'-hydroxy group of 1j resembles a similar function of 1c. The methyl-binding site of adenosine deaminase need not be postu-

lated and compound 1j can be viewed as an analogue of the "top" of the ribofuranose portion of adenosine.

Compounds 1c and 1d were tested as potential antiviral agents against the following viruses in cell cultures: Influenza A, Parainfluenza 3, Coxsackie A-21, Equine Rhinovirus, Herpes Simplex 1 (HSV 1), Herpes Simplex 2 (HSV 2), Vaccinia and Vesicular Stomatitis Virus (VSV). Protection of cells from viral cytopathology was investigated with HSV-1, HSV-2 and VSV. Compound 1c was also tested in virus cultures of Rift Valley Fever, Venezuelan Equine Encephalitis, Pichinde, Yellow Fever, Sandfly Fever and Japanese Encephalitis. The only measurable activity was found in VSV assay where compound 1d decreased the viral cytopathogenicity by 50% at 2.8×10^{-4} M. The results with 1c argue against the view[3] that antiviral activity of DHPA can be explained in terms of analogy with the "top" ribofuranose portion of adenosine. It is also obvious that replacement of the ether oxygen of acyclovir (1b) with a CHOH function does not lead to an active compound in contrast to the situation found in substrates for adenosine deaminase 1c and 1e. Interestingly, 9-(3,4-dihydroxybutyl)guanine which is isomeric with 1d is an active antiviral agent.[25]

Experimental Section

General Procedures. All solvents and starting materials were of the highest available purity or they were purified as specified. Dimethylformamide (DMF) and dimethylsulfoxide (DMSO) were stored over Linde molecular sieves (4A).

Tetrahydrofuran (THF) was distilled from sodium and it was kept over a sodium ribbon. Thin-layer chromatography (TLC) was performed on 6 x 2 cm precoated aluminum sheets of silica gel 60 F_{254} (Merck) in the following solvents: S_1 - benzene - ethyl acetate, 9:1; S_2 - benzene - ethyl acetate, 4:1; S_3 - dichloromethane - methanol, 9:1; S_4 - dichloromethane - methanol, 95:5 and S_5 - 2-propanol - NH_4OH - water, 7:1:2. For preparative TLC loose layers[26] of silica gel, Kieselgel 60 (70 - 400 mesh ASTM, Merck) containing 1% of fluorescent indicator (Lumilux Grun ZS Super) or 2 mm thick, 20 x 20 cm, precoated silica gel GF layers (Analtech) were used. Kieselgel 60 without indicator was used for column chromatography. Paper electrophoresis was performed at 15°C on a flat plate (Savant). Melting points were determined by using a Thomas-Hoover apparatus and they are uncorrected. UV spectra were obtained with a Beckman Model 25 or Perkin Elmer Lambda 5 spectrophotometer. NMR spectra were determined with an FX-100 Fourier transform NMR spectrometer (JEOL). Tetramethylsilane was used as an internal reference. Circular dichroism (CD) spectra were run by using a JASCO optical rotatory dispersion recorder, Model ORD/UV-5 in a CD modification SS-10 (Sproul Scientific).

4-Hydroxymethyl-2-phenyl-1,3-dioxane (3a). 1,2,4-Butanetriol (2, 7.07 g, 0.067 mol) was made anhydrous by evaporation with DMF (15 mL) in vacuo (oil pump) at room temperature (rotary evaporator, Dry Ice condenser). The residue was dissolved in DMF (20 mL), triethyl orthoformate (12.6

mL, 0.08 mol), benzaldehyde (8.1 mol, 0.08 mol) and 6 M HCl in DMF (0.02 mL, 0.012 mol) were added with magnetic stirring. A mildly exothermic reaction ensued and the clear solution was kept for 17 h at room temperature. TLC (S_1) showed one major UV-absorbing and $HClO_4$-positive spot accompanied by a faster moving impurity. Another portion of 6 M HCl in DMF (0.4 mL, 0.024 mol) was added and the solution was kept for 7 h at room temperature. Ammonia in methanol (saturated at 0°C, 10 mL) was then added with stirring and the mixture was evaporated. The residue was partitioned between benzene (100 mL) and water (2 x 30 mL), the organic layer was dried ($MgSO_4$) and evaporated (finally at 60°C and 0.05 mm Hg). The crude product was chromatographed on a silica gel column (70 g, 8 x 5 cm) in benzene (600 mL) followed by solvent S_1 (400 mL) and S_2 (1 L). The fractions containing 3a were pooled and evaporated to give a pale yellow oil, uniform on TLC (S_1 and S_2), 7.5 g (58%), n_D^{21} 1.5353. Distillation gave 6.73 g (52%) of 3a, bp. 130-132°C/0.05 mm Hg, n_D^{22} 1.5360; lit.[6] bp.146°C/0.2 mm Hg, n_D^{26} 1.5356. NMR ($CDCl_3$) δ, 7.37 (m, 5, C_6H_5),5.49 (s, 1, H_2), 4.25 (q, 1, H_6), 3.90 (m, 2, H_4 + H_6), 3.58 (t, 2, 4-CH_2), 2.59 (t, 1, OH), 1.78 (sextet, 1, H_5), 1.36 (apparent d of d, 1, H_5). This product contained 9% of compound 4: δ, 5.89 (s, H_2, cis-isomer), 5.75 (s, H_2, trans isomer), 2.06 (m, 4-CH_2). Mass spectrum (m/e) 194 (M).

2-Phenyl-4-(p-toluenesulfonyloxy)methyl-1,3-dioxane (3b). A mixture of compound 3a (1.94 g, 0.01 mol), p-toluenesulfonyl chloride (2.1 g, 0.011 mol) and pyridine (5 ml) was

magnetically stirred for 3 h at room temperature. TLC (S_1) showed a single UV-absorbing and $HClO_4$-positive spot. Methanol (2 mL) was added and the solution was evaporated. The residue was partitioned between dichloromethane (100 mL) and saturated aqueous $NaHCO_3$ (50 mL). The organic portion was washed with water (50 mL), it was dried ($MgSO_4$) and evaporated. The resultant sirup was crystallized from ether (25 mL, 0°C) to give compound 3b, 6.16 g (77%), mp. 75 - 76°C, homogeneous on TLC (S_1). NMR ($CDCl_3$) δ, 7.78 and 7.27 (two d, p-toluenesulfonyl overlapped with 7.34, m, C_6H_5, total 9 protons), 5.44 (s,1,H_2), 4.08 (cluster of m, 5, H_4 + H_6 + 4-CH_2), 2.40 (s, 3,CH_3), 1.59 (m, 2,H_5). Mass spectrum (m/e) 348 (M). Anal. Calcd. for $C_{18}H_{20}O_5S$: C,62.05; H,5.79; S,9.20. Found: C,62.05; H,5.84; S,9.20. Compound 3b is of limited stability; it decomposes during storage to give benzaldehyde and p-toluenesulfonic acid.

Acetal 3a which was purified only by distillation (72% yield, n_D^{23} 1.5345, content of isomer 4 was 11%) gave 54% yield of tosylate 3b, mp. 76 - 78°C, homogeneous on TLC (S_1).

9-(2,4-O-Benzylidene-2,4-dihydroxy)butyladenine (6a). A. Alkylation of Adenine (5a) with p-Toluenesulfonate 3b Using NaH in DMF. A mixture of adenine (5a, 2.35 g, 17.4 mmol), NaH (50% oil dispersion, 0.84 g, 17.4 mmol) and DMF (100 mL) was magnetically stirred for 1 h at room temperature and 30 min. at 50°C. After cooling compound 3b (5.22 g, 15 mmol) was added and the mixture was heated at 115 -

125°C for 40 min. The clear red-brown solution was evaporated and the solid residue was extracted with dichloromethane (400 mL) in a Soxhlet apparatus for 7 h. TLC (S_1) showed disappearance of 3b and (S_3) formation of a new major UV absorbing spot. The solvent was evaporated, the solid residue was mixed with silica gel (10 g), dichloromethane (500 mL) was added and the suspension was put on the top of the silica gel column (50 g, 19 x 2.5 cm). Elution started with dichloromethane (600 mL) and it was continued with 1% methanol in dichloromethane (12 L). The major UV-absorbing fraction was evaporated and the solid residue was washed with ether (10 mL) to give compound 6a, 2.68 g (57%), homogeneous on TLC (S_1), mp. 191 - 192°C, UV (ethanol) max 261 nm (ε 14 100), NMR ($CDCl_3$) δ, 8.38 (s, 1, H_8-purine), 7.94 (s, 1, H_2-purine), 7.39 (s, 5, C_6H_5), 6.08 (broad s, 2, NH_2), 5.47 (s, 1, H_2), 4.30 (cluster of m, 5, H_4 + H_6 + exocyclic CH_2), 1.70 (m, 2, H_5). Mass spectrum (m/e) 311 (M). Anal. Calcd. for $C_{16}H_{17}N_5O_2$: C, 61.72; H, 5.50; N, 22.50. Found: C, 61.71; H, 5.59; N, 22.28.

B. Using Tetra-n-butylammonium Fluoride (TBAF) in THF.

A mixture of adenine (5a 0.16 g, 1.2 mmol), compound 3b (0.35 g, 1 mmol) and 1 M TBAF in THF (5 mL, 5 mmol) was refluxed with magnetic stirring in THF (10 mL) for 5 h. A white precipitate of adenine p-toluenesulfonate was filtered off, it was washed with THF (2 mL) and dried, 0.25 g (0.69 mmol, i.e. 30% conversion to 6a). This product was combined with the filtrate, triethylamine (0.14 mL, 1 mmol) was added and the reflux was continued for 7 h, 0.2 g (0.55

mmol) of adenine toluenesulfonate was obtained (45% conversion). The filtrate was evaporated, the residue was partitioned between saturated aqueous $NaHCO_3$ (10 mL) and dichloromethane (10 mL), the organic layer was washed with water (10 mL) and dried ($MgSO_4$). The solvent was evaporated and the residue was stirred with Dowex 50 WX4 (200 - 400 mesh, 2 g wet weight, K^+ form) in 50% ethanol (20 mL) for 1 h at room temperature. The resin was filtered off, it was washed with ethanol (20 mL), the filtrate was evaporated and the resultant sirup was dissolved in dichloromethane (5 mL). The insoluble precipitate was filtered off, the filtrate was evaporated and the residue was chromatographed on a single silica gel layer (Analtech) in solvent S_3. The major UV absorbing band was eluted with the same solvent, the eluate was evaporated and the resultant sirup was stirred with water (5 mL) to give compound 6a, 51 mg (16%), mp. 188 - 191°C, identical /mp., TLC (S_3), NMR and mass spectra/ with the product prepared by method A.

9-(2,4-O-Benzylidene-2,4-dihydroxy)butyl-2-amino-6-chloropurine (6b). A. Alkylation of 2-Amino-6-chloropurine (5b) with p-Toluenesulfonate 3b Using K_2CO_3 in DMSO. A mixture of 2-amino-6-chloropurine (5b, 0.45 g, 2.7 mmol), compound 3b (0.95 g, 2.7 mmol) and K_2CO_3 (freshly dried, 0.41 g, 3 mmol) in DMSO (20 mL) was heated at 80 - 100°C (bath temperature) for 1.5 h with magnetic stirring. The reaction was followed by TLC in solvents S_1 (disappearance of 3b) and S_3 (formation of 6b). After cooling, the solution was evaporated (oil pump), the residue was washed with

dichloromethane (3 x 25 mL), the insoluble material was filtered off and the filtrate was evaporated. The crude product was chromatographed on a single loose layer of silica gel (35 x 15 cm) in solvent S_4. After the first development, the top 1/2 of the layer was removed up to the solvent front, the layer was restored with a fresh silica gel and the chromatography was continued in the same solvent (total of 5 developments). The two major UV-absorbing bands were eluted with solvent S_3. The slower moving zone afforded, after evaporation of the eluate, compound 7 (0.1 g, 11%), homogeneous on TLC (S_4), mp. 183 - 185°C, UV (ethanol) max 321 and 221 nm (ε 5 300 and 23 500), shoulder 254 nm (ε 3 700). NMR (CD_3SOCD_3) δ, 8.33 (s, 1, H_2-purine), 7.33 (s, 5, C_6H_5), 5.50 (s, 1, H_2), ca. 4.1 (poorly resolved m, 5, H_4 + H_6 + 4-CH_2), 1.64 (poorly resolved m, 2, H_5). Anal. Calcd for $C_{16}H_{17}ClN_5O_2$: C, 55.41; H, 4.94; Cl, 10.22; N, 20.20. Found: C, 55.37; H, 5.06; Cl, 10.46; N, 20.25.

The faster moving band afforded a sirup which crystallized after addition of ether (15 mL) to give compound 6b (0.46% g, 49%), homogeneous on TLC (S_4), mp. 148-150°C, UV (ethanol) max 310, 248 and 223 nm (ε 7 500, 5 900 and 27 900). NMR ($CDCl_3$) δ, 7.89 (s, 1, H_2-purine), 7.38 (m, 5, C_6H_5), 5.46 (s, 1, H_2), 5.27 (broad s, 2, NH_2), 4.23 (cluster of m, 5, H_4 + H_6+ 4-CH_2), 1.68 (m, 2, H_5). Anal. Calcd. for $C_{16}H_{17}ClN_5O_2$: C, 55.41; H, 4.94; Cl, 10.22; N, 20.20. Found: C, 55.37; H, 4.83; Cl, 10.41; N, 20.16.

B. Using TBAF in THF. The procedure for synthesis of

compound 6a (method B) was followed with some modifications. A mixture of 2-amino-6-chloropurine (5b, 0.17 g,1 mmol), compound 3b (0.3 g, 0.9 mmol) and TBAF (5 mmol) was refluxed in THF (15 mL) for 15 h. The clear solution was evaporated and the residue was stirred with Dowex 50 (Na^+, 5 g, wet weight) in 50% ethanol (25 mL) for 1 h. The resin was filtered off, it was washed with ethanol (10 mL) and dichloromethane (10 mL). The filtrate was evaporated and the crude product was chromatographed on a single layer of silica gel (Analtech) in solvent S_3. The major UV absorbing band of 6b was rechromatographed in solvent S_4 (developed three times) and then again in S_3. The relevant band was worked up as described in Method A to give compound 6b (66 mg, 19%), homogeneous on TLC (S_4), in solvent S_3 a trace of faster moving contaminant was detected, mp. 147 - 149°C, identical mp., NMR, TLC (S_3) and mass spectra with a sample prepared by method A.

Attempted Alkylation of 2-Amino-6-chloropurine (5b) with p-Toluenesulfonate 3b Using Hexamethyldisilazane - $Hg(CN)_2$ Method. The general procedure[14] was followed on a 0.9 mmol scale of 5b and 3b. The mixture was evaporated, the residue was washed with dichloromethane, the solids were filtered off and the filtrate was evaporated to give compound 3b (97%) identical (TLC, S_1) with an authentic sample.

9-(2,4-Dihydroxybutyl)adenine (1c). A solution of compound 6a (1.56 g, 5 mmol) in 80% acetic acid (20 mL) was heated on a steam bath for 1 h. TLC (S_3) showed a complete disappearance of 6a. The clear solution was diluted with water

(50 mL), it was extracted with petroleum ether (3 x 20mL), the organic phase was washed with water (20 mL) and the combined aqueous portions were evaporated to give a solid which was crystallized from 90% ethanol (60 mL), 0.74 g (66%) of 1c, mp.228 - 229°C. Recrystallization afforded 0.53 g (48%), mp.229 - 230°C. UV (0.01 M Na_2HPO_4,pH7) max 259 nm (ε 14 300), (0.01 M HCl) 259 nm (ε 14 000). NMR (CD_3SOCD_3 + D_2O) δ, 8.16 (s, 1, H_8-adenine), 8.08 (s, 1, H_2-adenine), 4.15 (m, 1) and 3.54 (apparent t, 4, H_1', H_2' and H_4'), 1.54 (m, 2, H_3'). Mass spectrum (m/e) 223 (M). Anal. Calcd for $C_9H_{13}N_5O_2$. $1/8H_2O$: C, 47.93; H, 5.92; N, 31.06. Found: C,48.10; H, 6.03; N,30.94. Compound 1c obtained by evaporation of the combined mother liquors was contaminated (TLC in S_3) by a faster moving component, possibly an O-acetyl derivative. This product was stirred with conc. NH_4OH (60 mL) for 16 h at room temperature to give a TLC (S_3) homogeneous compound which was crystallized as described above to give 0.39 g (35%, total yield 83%) of 1c, mp. 228 - 229°C.

9-(2,4-Dihydroxybutyl)guanine (1d). A solution of compound 6b (0.32 g, 0.92 mmol) was refluxed in 0.1 M HCl (15 mL) for 5 h. The mixture was evaporated and the residue was co-evaporated with water (10 mL) whereupon it was stirred with Dowex 1 (acetate, 6 g, wet weight) in water (20 mL) for 1 h. The resin was filtered off, it was thoroughly washed with water (1 L) and the filtrate was evaporated. The residue was crystallized from water (7mL) to give 0.15g (63%) of 1d, homogeneous on TLC (S_5), mp. 247 - 248°C, UV max

(0.01 M Na_2HPO_4,pH 7) 250 nm (ε 12 700), shoulder 270 nm (ε 9 300), (0.01 M HCl) 277 and 254 nm (ε 8 000 and 12 100). NMR (CD_3SOCD_3 + D_2O) δ, 7.65 (s, 1, H_2-purine), 3.92 and 3.54 (two m partly overlapped with HDO signal, H_1', H_2' and H_4'), 1.52 (poorly resolved m, 2, H_3'). Mass spectrum (m/e) 239 (M). Anal. Calcd. for $C_9H_{13}N_5O_3.1.25\ H_2O$: C,41.29; H,5.97; N,26.76. Found: C, 41.56; H, 5.73; N, 26.79.

Deamination of 9-(2,4-Dihydroxybutyl)adenine (1c) with Adenosine Deaminase. A solution of compound 1c (0.5 mg, 2.2μmol) and adenosine deaminase (Type II, Sigma, 0.4 mg) in 0.05 M Na_2HPO_4 (0.4 mL, pH 7.6) was incubated at 37°C for 24 h. The whole mixture was applied on a strip of Whatman 3 MM paper which was developed in solvent S_5. The spots of product 1i (UV max 249 nm) and 1c (faster moving, UV max 257 nm) were eluted with 0.01 M HCl (3 mL each). UV spectrophotometry of the eluates established that deamination of one enantiomer of 1c was essentially quantitative (the ratio of 1i and 1c was 0.94). In another experiment (1 mg of 1c), the products were separated by paper electrophoresis on Whatman 3 MM paper (10 V/cm, 4h) in 1 M acetic acid and products 1i and 1c were eluted with water. The mobility of deaminated product 1i was 0.18 of compound 1c. CD spectrum of the eluate containing unreacted 1c exhibited a negative Cotton effect at 260 nm.

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