Purine Nucleosides. XXV. The Synthesis of Certain Derivatives of 5′-Amino-5′-deoxy- and 5′-Amino-2′,5′-dideoxy-β-D-ribofuranosylpurines as Purine Nucleotide Analogs[1]

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The 2′,3′-*O*-isopropylidene-5′-amino-5′-deoxy nucleoside derivatives of adenosine (**2a**) and guanosine (**2b**) were isolated and characterized for the first time. The synthesis of 5′-amino-2′,5′-dideoxyadenosine (**11**) has now been accomplished by a similar route. Treatment of the 5′-amino purine nucleosides **2a**, **2b**, and **11** with $MeSO_2Cl$ provided the corresponding 5′-*N*-methanesulfonylamino purine nucleoside derivatives. Appropriate deblocking yielded 5′-*N*-methanesulfonylamino-5′-deoxyadenosine (**6a**), 5′-*N*-methanesulfonylamino-5′-deoxyguanosine (**6b**), and 5′-*N*-methanesulfonylamino-2′,5′-dideoxyadenosine (**12**), respectively. Similar reaction of the requisite 5′-amino purine nucleosides **2a**, **2b**, and **11** with ethyl chlorocarbonate followed by appropriate deblocking procedures gave 5′-*N*-ethoxycarbonylamino-5′-deoxyadenosine (**5a**), 5′-*N*-ethoxycarbonylamino-5′-deoxyguanosine (**5b**), and 5′-*N*-ethoxycarbonylamino-2′,5′-dideoxyadenosine (**10**), respectively. The rationale for consideration of these nucleoside derivatives as 5′-nucleotide analogs is presented and discussed.

A major problem inherent in the utilization of analogs of nucleic acid constituents in chemotherapy is the fact that the nucleotide is often the biologically active derivative at the molecular level within the cell. The base or nucleoside must be converted to the nucleotide intracellularly for effective action. Resistance to drug response often develops when the required enzymes fail to perform this "lethal synthesis" of the nucleotide.

Attempts to employ the nucleotide derivatives directly as chemotherapeutic agents have been generally unsuccessful due to enzymatic cleavage to the heterocyclic base or nucleoside during transport or by failure of the highly polar nucleotides to cross cellular membranes. These problems have been reviewed in detail.[2,3] An approach to this problem has been the simulation of a nucleotide (*i.e.*, 5′-adenylic acid) by adenin-9-ylalkanoic acids[2] in which a more weakly ionized carboxylic acid was employed for binding at the phosphoric acid site.

Such an approach has been partially successful in *isolated* enzyme systems since adenin-9-ylvaleric acid inhibits both lactic dehydrogenase and glutamic dehydrogenase[2] although uracil-1-valeric acid failed to mimic 2′-deoxyuridylate and did not inhibit thymidylate synthetase.[4] The inhibitory activity of 5-(6-mercaptopurin-9-ylvaleramide)salicylic acid was found to be about one-fourth that of 6-thioinosinic acid against succinoadenylate kinosynthetase.[5]

Since one of the major considerations in the design of any drug is its transport characteristics, nucleotide analogs which would be polar but nonionic were chosen for the present investigation. Adenosine 5′-phosphate is presumed to possess a proton at N^1 and an anionic oxygen on the phosphate group (an assumption which has been confirmed in the solid state by X-ray analysis[6]). Apparently it is this ionic structure which prevents passage of the nucleotide through the cellular membrane.[7] Thus nucleosides which possess un-ionized groups which could simulate 5′-phosphate binding but which would still enter the cell as potent inhibitors pose excellent candidates for chemotherapy. Baker states[8] that "...such inhibitors would not need activation for inhibition of enzymes operating on nucleotides and thus would not be subject to the type of drug resistance resulting by mutational loss of a kinase or pyrophosphorylase."

Indeed, nucleocidin, a potent nucleoside antibiotic of recently proposed structure 5′-*O*-sulfamoyl-4′-*C*-fluoroadenosine,[9] could be envisaged as such an un-ionized nucleotide analog which lends support to this concept.

In the present study derivatives of 5′-amino-5′-deoxy-β-D-ribofuranosylpurines were selected for investigation. It was hoped that the 5′-amino function would give added stability against both chemical and enzymatic hydrolysis of the group selected as a simulator of the 5′-phosphate function. The syntheses of 5′-amino-5′-deoxyadenosine and 5′-amino-5′-deoxyguanosine have been reported by Jahn.[10] In the present study, N^6-formyl-2′,3′-*O*-isopropylidene-5′-azido-5′-deoxyadenosine (**1a**) was prepared essentially by the method of Jahn[10] and isolated crystalline and characterized for the first time. The desired 2′,3′-*O*-isopropylidene-5′-amino-5′-deoxyadenosine (**2a**) intermediate was obtained crystalline by deformylation of **1a** in methanolic NH_3 followed by catalytic reduction of the 5′-azido group (see Scheme I). Treatment of **2a** with methanesulfonyl chloride in pyridine gave 2′,3′-*O*-isopropylidene-5′-*N*-methanesulfonylamino-5′-deoxyadenosine (**4a**). Removal of the isopropylidene group was achieved with 50% aqueous formic acid to yield 5′-*N*-methanesulfonylamino-5′-deoxyadenosine (**6a**).

The method of Jahn[10] was utilized to introduce the 5′-amino function into the guanosine molecule except that DMF proved to be superior to DMSO as a solvent for introduction of the azide group. The 2′,3′-*O*-isopropylidene-5′-azido-5′-deoxyguanosine **1b** was sepa-

(1) This work was supported by Research Grant CA-08109 from the National Cancer Institute of the National Institutes of Health and by Research Grant GB-5446 from the Molecular Biology Division of the National Science Foundation.

(2) B. R. Baker and H. S. Sachdev, *J. Pharm. Sci.*, **52**, 933 (1963).

(3) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Enzymic Active-Site," John Wiley and Sons, Inc., New York, N. Y., 1967, Chapters 5 and 6.

(4) B. R. Baker and G. B. Chheda, *J. Pharm. Sci.*, **54**, 25 (1965).

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(6) J. Kraut, *Acta Cryst.*, **16**, 79 (1963).

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(8) B. R. Baker and P. M. Tanna, *J. Pharm. Sci.*, **54**, 1774 (1965).

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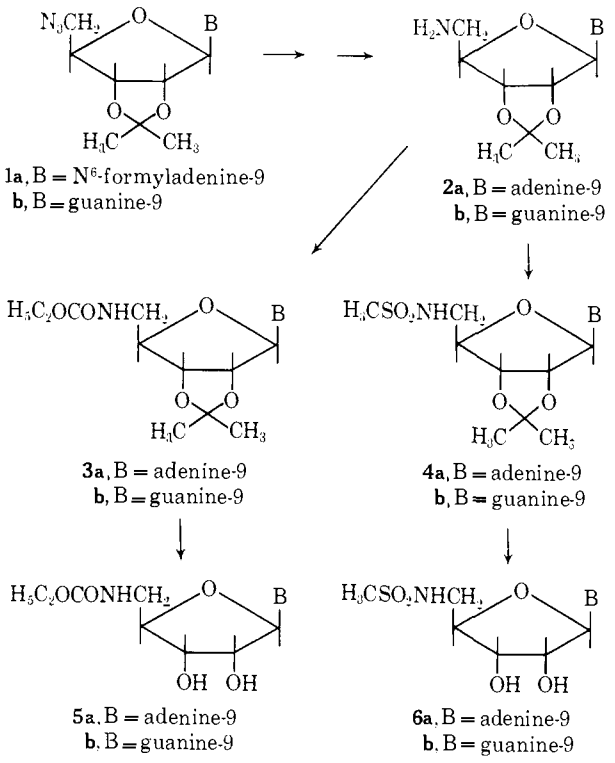
SCHEME I



rated from 2',3'-*O*-isopropylideneguanosine $N^3 \rightarrow 5'$-cyclonucleoside[11] by fractional crystallization of the mixture from water. By this procedure **1b** was obtained crystalline and was adequately characterized for the first time. Reduction of **1b** provided crystalline 2',3'-*O*-isopropylidene-5'-amino-5'-deoxyguanosine (**2b**) which served as the starting material for the 5'-amino-5'-deoxyguanosine nucleotide analogs. Treatment of **2b** with methanesulfonyl chloride in chloroform–pyridine provided 2',3'-*O*-isopropylidene-5'-*N*-methanesulfonylamino-5'-deoxyguanosine (**4b**). Removal of the isopropylidene blocking group with 50% aqueous formic acid readily gave 5'-*N*-methanesulfonylamino-5'-deoxyguanosine (**6b**).

Preliminary antiviral activity exhibited by 5'-*N*-methanesulfonylamino-5'-deoxyadenosine[12] (**6a**) prompted us to prepare 5'-*N*-methanesulfonylamino-2',5'-dideoxyadenosine (**12**) as a nucleotide analog of 2'-deoxyadenosine 5'-phosphate. For this purpose 5'-amino-2',5'-dideoxyadenosine (**11**) was required. 5'-*O*-*p*-Toluenesulfonyl-2'-deoxyadenosine (**7**) was prepared by the method of Robins, *et al.*,[13] and purified by crystallization from EtOAc and EtOH. 5'-*O*-*p*-Toluenesulfonyl-2'-deoxyadenosine (**7**) was then treated with acetic–formic anhydride to yield 3'-*O*,N^6-diformyl-5'-*O*-*p*-toluenesulfonyl-2'-deoxyadenosine (**8**) which was obtained as a crystalline solid (see Scheme II). Treatment of **8** with NaN_3 in DMF gave a good yield of 3'-*O*,N^6-diformyl-5'-azido-2',5'-dideoxyadenosine (**9**). The formyl groups were then removed with methanolic NH_3 and the 5'-azido group was reduced with 5% Pd–C and H_2 to give an excellent yield of 5'-amino-2',5'-dideoxyadenosine (**11**). Under carefully controlled conditions in pyridine–NEt_3, methanesulfonyl chloride reacted selectively with the 5'-amino group of **11** to give an 85% yield of 5'-*N*-methanesulfonylamino-2',5'-dideoxyadenosine (**12**).

The proposal of Baker and Tanna[8] that the 5'-carbamoyloxy group can simulate phosphate binding and the synthesis by Mertes and Coats[14] of several (3'→5')-carbonate analogs of dinucleotides stimulated interest in the ethoxycarbonylamino group as a second simulator for the 5'-phosphate. Treatment of 2',3'-*O*-isopropylidene-5'-amino-5'-deoxyadenosine (**2a**) with ethyl chlorocarbonate gave an excellent yield of 2',3'-*O*-isopropylidene-5'-*N*-ethoxycarbonylamino-5'-deoxyadenosine (**3a**). Removal of the isopropylidene group gave 5'-*N*-ethoxycarbonylamino-5'-deoxyadenosine (**5a**). Similarly 2',3'-*O*-isopropylidene-5'-amino-5'-deoxyguanosine (**2b**) and ethyl chlorocarbonate gave 2',3'-*O*-isopropylidene-5'-*N*-ethoxycarbonylamino-5'-deoxyguanosine (**3b**). Removal of the isopropylidene group gave 5'-*N*-ethoxycarbonylamino-5'-deoxyguanosine (**5b**).

Treatment of 5'-amino-2',5'-dideoxyadenosine (**11**) with ethyl chlorocarbonate in pyridine under carefully controlled conditions gave selective reaction at the 5'-amino group to yield 5'-*N*-ethoxycarbonylamino-2',5'-dideoxyadenosine (**10**).

Preliminary antiviral activity has been assayed in African green monkey kidney cells BSC_1 *in vitro* used for the growth of Herpes simplex virus.[15] 5'-*N*-Methanesulfonylamino-5'-deoxyadenosine (**6a**) has been shown[12] to possess superior antiviral activity to 5'-amino-5'-deoxyadenosine[16] in this system. 5'-*N*-Methanesulfonylamino-5'-deoxyadenosine was not toxic to host cells at 2.5 μmoles/l. and at this concentration exhibited 73% inhibition of virus growth.[12] Several of these nucleotide analogs exhibited significant antibacterial activity (see Table I).

TABLE I

EFFECT OF NUCLEOTIDE ANALOGS ON THE GROWTH OF *Escherichia coli*[a]

Compound	50% growth inhib., *M*
5'-*N*-Ethoxycarbonylamino-5'-deoxyadenosine (**5a**)	4.5×10^{-5}
5'-*N*-Methanesulfonylamino-5'-deoxyadenosine (**6a**)	4.4×10^{-4}
5'-*N*-Methanesulfonylamino-2',5'-dideoxyadenosine (**12**)	4.2×10^{-4}

[a] The other nucleotide analogs reported in the present study were without effect at 10^{-3} *M*. These preliminary results were kindly supplied by Dr. A. Bloch, Roswell Park Memorial Institute, Buffalo, N. Y.

Experimental Section[17]

Melting points were determined on a Fischer-Johns block and are uncorrected. Spectral data were obtained on Beckman DK-2 (uv), Beckman IR-5A (ir), or Varian A-60 (nmr) instruments. Evaporations were accomplished using a Buchler rotating evaporator under reduced (aspirator or oil vacuum pump) pressure

(11) R. E. Holmes and R. K. Robins, *J. Org. Chem.*, **28**, 3483 (1963).

(12) A. R. Diwan, R. K. Robins, and W. H. Prusoff, *Experientia*, **25**, 98 (1969).

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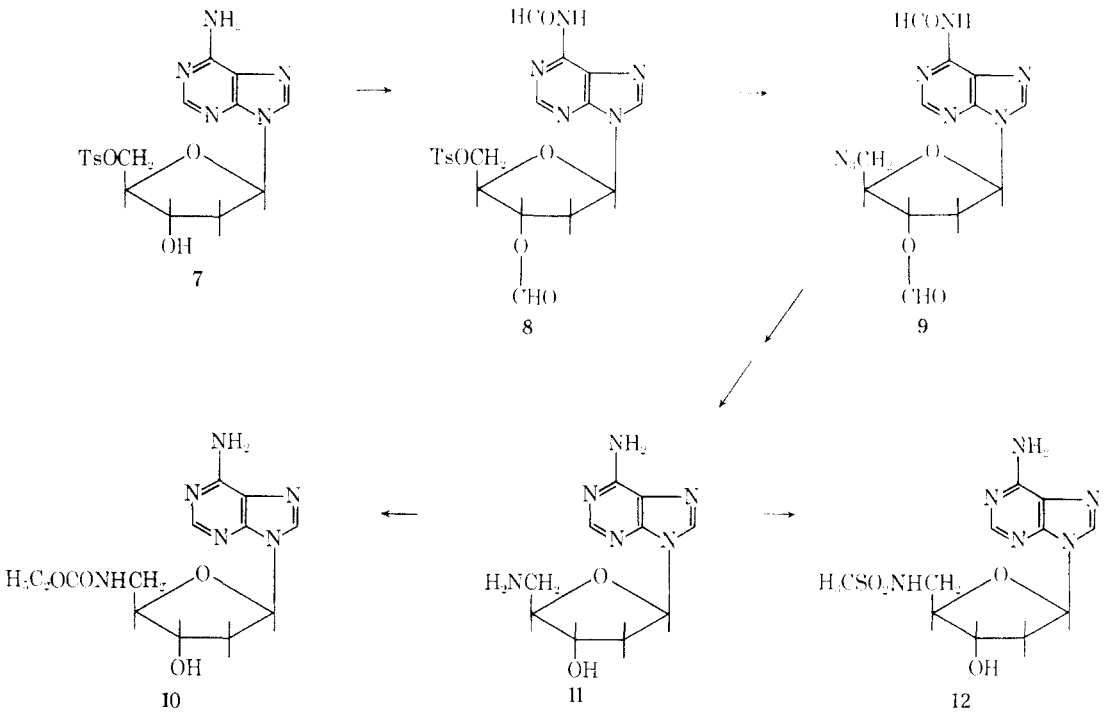
(15) A. Diwan, C. N. Gowdy, R. K. Robins, and W. H. Prusoff, *J. Gen. Virol.*, **3**, 393 (1968).

(16) T. Neilson, W. V. Ruyle, R. L. Bugianesi, K. H. Boswell, and T. Y. Shen, Abstracts, 154th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1967, P 29.

(17) Spectral data (uv) are given for compounds **2a** and **1b**. Spectra not given are directly comparable to these for the respective nucleoside series.

SCHEME II



unless otherwise stated. Hydrogenations were effected using a Parr apparatus at designated H_2 pressure and catalyst. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

N^6-Formyl-2′,3′-*O*-isopropylidene-5′-azido-5′-deoxyadenosine (1a).—To a solution of N^6-formyl-5′-*O*-tosyl-2′,3′-*O*-isopropylideneadenosine[10] (32.0 g, 0.065 mol) in 250 ml of DMSO was added 15.8 g (0.243 mol) of NaN_3 and the resulting mixture was stirred at 65–70° for 30 min. The reaction mixture was then poured into 800 ml of $CHCl_3$–H_2O (1:1). The layers were separated and the aqueous phase was extracted with three 75-ml portions of $CHCl_3$. The combined organic phase was washed with three 75-ml portions of H_2O and dried ($MgSO_4$). The mixture was filtered and the filtrate was evaporated to dryness. The resulting white foam was dissolved in $CHCl_3$–MeOH and again evaporated to give a white solid. Recrystallization of this product from EtOH gave 20 g (85%) of crystals, mp 101–104°. A small sample for analysis was twice recrystallized from EtOH to give needles, mp 105–107°. *Anal.* ($C_{14}H_{16}N_8O_4$) C, H, N.

2′,3′-*O*-Isopropylidene-5′-amino-5′-deoxyadenosine (2a).—To 200 ml of MeOH presaturated with NH_3 at −10° was added 14 g (0.039 mol) of **1a** and the resulting solution was allowed to stand for 18 hr at room temperature in a sealed vessel. This solution was evaporated and the resulting syrup was dissolved in 45 ml of MeOH, filtered, and cooled for 8–10 days at 0°. The crystalline product (10.7 g, 83%) was filtered, dissolved in 150 ml of EtOH–H_2O (1:1), and hydrogenated at 2.4g kg/cm^3 for 3 hr in the presence of 3 g of 5% Pd–C. The catalyst was removed by filtration and the filtrate was evaporated to dryness to give 9.33 g (95%) of crude product. This material was recrystallized from EtOAc–petroleum ether (60–90°) to give needles of **2a**: mp 203–207°; uv max (pH 1) 256 mμ (ε 15,700), (pH 11) 259 mμ (ε 15,900). *Anal.* ($C_{13}H_{18}N_6O_3$) C, H, N.

2′,3′-*O*-Isopropylidene-5′-*N*-methanesulfonylamino-5′-deoxyadenosine (4a).—A stirred solution of 4.0 g (0.013 mol) of **2a** in 80 ml of pyridine (dried over KOH) was cooled in an ice bath and a solution of 1.1 ml (0.014 mol) of MsCl in 40 ml of $CHCl_3$–pyridine (1:1) was added dropwise over a period of 1 hr. The resulting orange solution was stirred at ice-bath temperature for an additional 5 hr and was poured onto ice (300 g) and $NaHCO_3$ (1.2 g). The aqueous layer was separated and washed with three 50-ml portions of $CHCl_3$. The combined organic phase was washed with ice–water, dried ($MgSO_4$), filtered, and then evaporated. The residue was dissolved in 200 ml of EtOH and this solution was evaporated to yield a tan solid (1.5 g, 33%) which was recrystallized from EtOAc–EtOH to give crystals of **4a**, mp 250–251°. *Anal.* ($C_{14}H_{20}N_6O_5S$) C, H, N.

5′-*N*-Methanesulfonylamino-5′-deoxyadenosine (6a).—A solution of 0.92 g (0.0024 mol) of **4a** in 20 ml of 50% aqueous HCO_2H was allowed to stand for 6 days at room temperature. The solvents were evaporated and EtOH was added to the resulting clear syrup. Precipitation of a white solid occurred upon stirring and the mixture was evaporated to dryness. The resulting solid was recrystallized from EtOH–EtOAc (2:1) to give 0.8 g (97%) of needles of **6a**, sinters at 150–170°. *Anal.* ($C_{11}H_{16}N_6O_5S$) C, H, N.

2′,3′-*O*-Isopropylidene-5′-*N*-ethoxycarbonylamino-5′-deoxyadenosine (3a).—A solution of 1.4 ml (0.014 mol) of ethyl chlorocarbonate in 40 ml of THF was added dropwise over a period of 45 min to a magnetically stirred solution of 4.0 g (0.013 mol) of **2a** in 150 ml of dry THF and 2.2 ml of Et_3N at room temperature. Et_3NH^+ Cl^- was removed by filtration and the filtrate was evaporated to dryness. The resulting solid foam was dissolved in 35 ml of $CHCl_3$ and this solution was added dropwise to 1 l. of cyclohexane which was stirred vigorously. The resulting precipitate (4.77 g, 97%) was collected by filtration and a small sample for analysis was passed through a small column of neutral alumina using EtOAc–EtOH (9:1). *Anal.* ($C_{16}H_{22}N_6O_5$) C, H, N.

5′-*N*-Ethoxycarbonylamino-5′-deoxyadenosine (5a).—A solution of 0.8 g (0.002 mol) of **3a** in 16 ml of 50% aqueous HCO_2H was allowed to stand for 24 hr at room temperature. The solution was evaporated to dryness and the residue was coevaporated twice using EtOH. The resulting solid was recrystallized from EtOH–EtOAc (1:2) to give 0.64 g (90%) of **5a**, mp 100–112°. *Anal.* ($C_{13}H_{18}N_6O_5$) C, H, N.

2′,3′-*O*-Isopropylidene-5′-azido-5′-deoxyguanosine (1b).—A mixture of 54.0 g (0.113 mol) of 5′-*O*-tosyl-2′,3′-*O*-isopropylideneguanosine[18] and 54 g (0.83 mol) of NaN_3 in 540 ml of DMF was stirred for 2.5 hr at 95–100°. The hot mixture was filtered and the filtrate was evaporated to dryness. The resulting mixture (31 g) was treated with 500 ml of H_2O at 65–70° for 15 min and then filtered while hot. The insoluble product, **1b**, was treated again with hot H_2O if necessary as judged by tlc. This separation gave 16.3 g (42%) of the H_2O-insoluble **1b** and 12.2 g (36%) of 2′,3′-*O*-isopropylideneguanosine $N^3 \rightarrow 5'$-cyclonucleoside[11] which was obtained by cooling the aqueous filtrate to room temperature and filtering immediately. A small sample of **1b** was recrystallized from 95% EtOH to give material with mp 240–244° dec; ir (KBr) 2175 cm^{-1} (azide); uv max (pH 1) 256 mμ (ε 13,000), (pH 11) 258 mμ (ε 13,000), (MeOH) 253 mμ (ε 15,200). *Anal.* ($C_{13}H_{16}N_8O_4$) C, H, N.

(18) E. J. Reist, P. A. Hart, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 1557 (1961).

2′,3′-*O*-Isopropylidene-5′-amino-5′-deoxyguanosine (2b).—A solution of 5.7 g (0.016 mol) of **1b** in 110 ml of DMF and 55 ml of H_2O was hydrogenated for 16 hr at 2.8 kg/cm² in the presence of 0.6 g of 10% Pd-C. The reaction mixture was filtered using a Celite pad and the filtrate was evaporated. The resulting oil was treated with 30 ml of absolute EtOH and the product which crystallized was collected to give 4.47 g (85%) of **2b**. A small sample of this material was recrystallized from EtOH–EtOAc to give needles of **2b**, mp 260° dec. *Anal.* ($C_{13}H_{18}N_6O_4 \cdot 0.5H_2O$) C, H, N.

2′,3′-*O*-Isopropylidene-5′-*N*-methanesulfonylamino-5′-deoxyguanosine (4b).—A solution of 0.90 ml (0.012 mol) of MsCl in 50 ml of $CHCl_3$ was added dropwise over a period of 1.5 hr to a stirred solution of 2.0 g (0.006 mol) of **2b** in 100 ml of dry pyridine–$CHCl_3$ (1:1) which was cooled in an ice bath. The reaction was stirred an additional 2 hr and then 4 ml of H_2O was added. Stirring was continued for 0.5 hr, the solution was evaporated to dryness, and the residue coevaporated with two 50-ml portions of EtOH. The resulting solid was dissolved in 25 ml of MeOH and 5 g of dry silica gel was added. This mixture was evaporated to dryness and applied to the top of a dry packed column (3 × 50 cm, 150 g) of silica gel. Elution was begun with 500 ml of EtOAc–MeOH (4:1) followed by 1 l. of EtOAc–MeOH (3:1). The first 350 ml of eluate contained contaminants and was discarded. The following 300 ml contained pure product (as judged by tlc) and was evaporated to dryness. The residue was recrystallized from MeOH to give crystals (0.9 g, 37%) of **4b**, mp 246–247°. *Anal.* ($C_{14}H_{20}N_6O_6S \cdot 0.5H_2O$) C, H, N.

5′-*N*-Methanesulfonylamino-5′-deoxyguanosine (6b).—A solution of 0.8 g (0.002 mol) of **4b** in 20 ml of 50% aqueous HCO_2H was allowed to stand for 20 hr at room temperature. It was then evaporated to dryness and the residue was crystallized from MeOH–H_2O to give 0.3 g (40%) of **6b**. A small sample was recrystallized from H_2O to give crystals of **6b**, mp 210–212°.

The sample for analysis was dried *in vacuo* over refluxing PhMe; however, the presence of 3 H_2O protons (1.5 mol of H_2O) was observed in the integrated nmr spectrum of **6b** determined in absolute DMSO-d_6. *Anal.* ($C_{11}H_{16}N_6O_6S \cdot 1.5H_2O$) C, N; H: calcd, 4.94; found, 4.39.

2′,3′-*O*-Isopropylidene-5′-*N*-ethoxycarbonylamino-5′-deoxyguanosine (3b).—A solution of 0.35 ml (0.0037 mol) of ethyl chlorocarbonate in 10 ml of dry THF was added dropwise over a period of 1 hr to a stirred suspension of 1.0 g (0.003 mol) of **2b** in 50 ml of dry THF and 5 ml of Et_3N at room temperature. The mixture was filtered after an additional 5 min of stirring. The filtrate was evaporated to dryness and the resulting residue was dissolved in 5 ml of $CHCl_3$. This solution was added dropwise to 150 ml of cyclohexane which was stirred rapidly. The yield of the resulting precipitate was 0.86 g. A 0.35-g portion of this material was purified on a silica gel column (1.2 × 22 cm, 10 g) by elution with $CHCl_3$–Me_2CO (1:1). The residue obtained from evaporation of the fractions containing **3b** was crystallized from $CHCl_3$–Me_2CO to give 0.14 g (29% yield) of pure **3b**, mp 237–237.5°. *Anal.* ($C_{16}H_{22}N_6O_6$) C, H, N.

5′-*N*-Ethoxycarbonylamino-5′-deoxyguanosine (5b).—A solution of 0.10 g (0.00025 mol) of **3b** in 2 ml of 50% aqueous HCO_2H was allowed to stand for 26 hr at room temperature and was then evaporated to dryness. The residue was triturated with 2 ml of MeOH and filtered. The solid was recrystallized from MeOH–H_2O–*i*-PrOH to give 0.05 g (56%) of needles of **5b**, mp 277–278°. *Anal.* ($C_{13}H_{18}N_6O_6$) C, H, N.

5′-*O*-*p*-Toluenesulfonyl-2′-deoxyadenosine[13] (7).—A solution of 20 g (0.08 mol) of 2′-deoxyadenosine was treated with TsCl in pyridine as previously described. However, it was found that the product can be obtained in crystalline form by dissolving the resulting solid foam in 60 ml of EtOAc–EtOH (4:1) at room temperature and cooling this solution at 1° for 24–48 hr. The crystalline product (15 g, 46%) was collected and washed with a small volume of cold EtOAc–EtOH (4:1). Additional product can be obtained by column chromatographic purification of the mother liquor as previously described.

3′-*O*,*N*⁶-Diformyl-5′-*O*-tosyl-2′-deoxyadenosine (8).—A solution of 15.0 g (0.037 mol) of **7**[13] in 200 ml of acetic–formic anhydride was allowed to stand for 60 hr at room temperature while protected from moisture. The solution was evaporated to dryness and the residue was dissolved in 30 ml of EtOAc–MeOH and again evaporated to dryness. The resulting white solid foam was dissolved in 30 ml of EtOAc–MeOH (2:1) and cooled at 1° to effect crystallization. The solid (14.0 g, 82%) was filtered and washed with a small volume of the precooled solvent mixture. A small sample was recrystallized from $CHCl_3$–MeOH (1:1) to give needles of **8**, mp 126.5–128° dec, uv max (MeOH) 272 mμ (ε 19,000). *Anal.* ($C_{19}H_{19}N_5O_7S$) C, H, N.

3′-*O*,*N*⁶-Diformyl-5′-azido-2′,5′-dideoxyadenosine (9).—To a solution of 10 g (0.022 mol) of **8** in 100 ml of DMF was added 5.0 g (0.077 mol) of NaN_3 and the mixture was stirred for 3 hr at 60–65°. It was filtered and the filtrate was evaporated to dryness. The residue was extracted with two 100-ml portions of boiling $CHCl_3$ and this combined extract was cooled, extracted with H_2O, filtered through anhydrous Na_2SO_4, and evaporated to dryness. This solid foam was dissolved in 100 ml of boiling $CHCl_3$ and concentrated slowly on a rotating evaporator. The product began crystallizing from solution and the mixture was cooled at 1° after concentration to about 40 ml. The product was collected by filtration to yield 6.64 g (92%) of crystalline **9**. Recrystallization of this material from $CHCl_3$ or EtOAc gave crystals of **9**, mp 146.5–147.5°, ir (KBr) band at 2215 cm^{-1} (azide), uv max (MeOH) 272 mμ (ε 20,000). *Anal.* ($C_{12}H_{12}N_8O_4$) C, H, N.

5′-Amino-2′,5′-dideoxyadenosine (11).—To a vigorously stirred solution of 300 ml of MeOH presaturated with NH_3 at −10° was added 10 g (0.03 mol) of **9** and stirring was continued until solution was complete. The flask was then sealed and allowed to stand overnight at room temperature. The solution was evaporated to dryness and the residual oil was dissolved in 75 ml of EtOH. This solution was evaporated to dryness and the remaining oil was triturated with three 70-ml portions of absolute Et_2O and then dissolved in 50 ml of hot EtOAc, filtered, concentrated to 25 ml, and cooled at −18° for several days. Crystallization occurred slowly and 9.03 g (109%) of large colorless crystals of 5′-azido-2′,5′-dideoxyadenosine solvate was collected. This product contained EtOAc of solvation as revealed by nmr and ir spectroscopy which was not removed by drying *in vacuo* at a temperature below mp 74–76°; ir (KBr) 1760 (EtOAc), 2210 cm^{-1} (azide); uv max (MeOH) 259 mμ.

This product was dissolved in 180 ml of EtOH–H_2O (1:1) and hydrogenated at 2.46 kg/cm² for 5 hr in the presence of 2 g of 5% Pd-C. The catalyst was removed by filtration and the filtrate was evaporated to give 7.14 g (95% over-all from **9**) of crude **11**. This material was crystallized from absolute EtOH to give 6.12 g (82% from **9**) of pure **11**: mp 185–187° dec; uv max (pH 1) 256 mμ (ε 12,400), (pH 11) 259 mμ (ε 12,800). *Anal.* ($C_{10}H_{14}N_6O_2$) C, H, N.

5′-*N*-Methanesulfonylamino-2′,5′-dideoxyadenosine (12).—A solution of 0.5 g (0.002 mol) of **11** in 25 ml of hot dry pyridine was cooled in an ice bath and 1 ml of Et_3N was added. A solution of 0.17 ml (0.0022 mol) of MsCl in 5 ml of dry pyridine was then added dropwise over a period of 15 min with vigorous stirring. The solution was allowed to stir while warming to room temperature over a period of 4 hr. A saturated H_2O solution of $NaHCO_3$ (2.5 ml) was added and the resulting mixture was filtered. The filtrate was evaporated to dryness, 25 ml of EtOH–H_2O (20:5) was added, and this solution was evaporated to dryness. The resulting solid was crystallized from 15 ml of MeOH–H_2O using Norit to yield 0.56 g (85%) of colorless needles. A small sample of **12** was recrystallized from EtOH for analysis: mp 229.5–231°; uv max (pH 1) 256 mμ (ε 15,300), (pH 11) 258 mμ (ε 15,800); nmr (DMSO-d_6) δ 3.00 (s, 3, $NHSO_2CH_3$), 7.81 (t, 1, 5′-$CH_2NHSO_2CH_3$), 5.55 (d, 1, 3′-O*H*) plus the remainder of a usual β-2′-deoxynucleoside spectrum; nmr (DMSO-d_6, D_2O) peaks at δ 7.81 and 5.55 missing. *Anal.* ($C_{11}H_{16}N_6O_4S$) C, H, N.

5′-*N*-Ethoxycarbonylamino-2′,5′-dideoxyadenosine (10).—A hot solution of 1.0 g (0.004 mol) of **11** in 50 ml of anhydrous pyridine was cooled in an ice bath and 2 ml of Et_3N was added. A solution of 0.42 ml (0.0044 mol) of EtO_2CCl in 5 ml of dry pyridine was then added dropwise over a period of 20 min with vigorous stirring. The solution was allowed to stir while warming to room temperature over a period of 2 hr. Saturated aqueous $NaHCO_3$ (5 ml) was added, the mixture was filtered, and the filtrate was evaporated to dryness. The resulting solid foam was dissolved in a small volume of EtOH–Me_2CO and applied to a dry packed column (1 × 16 in) of silica gel. The column was washed with 500 ml of $CHCl_3$–Me_2CO (8:2) and this wash was discarded. Elution with EtOH was begun and the first 50 ml containing faster migrating spots plus product was discarded. The following 200 ml of eluate was chromatographically homogeneous (tlc) and was pooled and evaporated to give a light yellow

foam. This material was dissolved in 8 ml of hot H_2O, treated with Norit, filtered, and cooled at 2° for several days. The colorless crystals of **10** (0.6 g, 47%) which separated were collected by filtration. A second crop (0.3 g, 23%) raised the yield to 70%. A small sample was recrystallized from H_2O for analysis: mp 180.5–181.5°; uv max (pH 1) 256 mμ (ε 14,700), (pH 11) 259 mμ (ε 14,700); ir (KBr) 1704 cm^{-1} ($NHCO_2Et$); nmr (DMSO-d_6) δ 1.16 (t, 3, J = 7 Hz, $-COOCH_2CH_3$), 4.04 (q, 2, J = 7 Hz, $-CO_2CH_2CH_3$), 7.54 (m, 1, 5′-CH_2NHCO_2Et), 5.44 (m, 1, 3′-OH) plus remainder of β-2′-deoxynucleoside spectrum; nmr (DMSO-d_6, D_2O) peaks at δ 7.54 and 5.44 missing. *Anal.* ($C_{24}H_{34}N_6O_4$) C, H, N.

Dihydrofolate Reductase from *Trypanosoma equiperdum*.[1] II. Inhibition by 2,4-Diaminopyrimidines and Related Heterocycles

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A number of 2,4-diaminopyrimidines and related heterocyclic compounds have been evaluated as inhibitors of dihydrofolate reductase obtained from *Trypanosoma equiperdum*, chicken liver, and rat liver. 2,4-Diaminopyrimidine itself (at 10^{-4} M) was not effective as an inhibitor of dihydrofolate reduction in all three systems studied but 5-aryl derivatives of 2,4-diaminopyrimidine were good inhibitors (ID_{50} = 10^{-8} to 10^{-6} M) of this enzymatic reaction. 2,4-Diamino-5-benzylpyrimidines and 2,4-diamino-5-aryloxypyrimidines were considerably more effective as inhibitors of the trypanosomal enzyme system than of the mammalian and avian systems. Although 2,4-diamino-6-phenyl-*s*-triazine was not active as an inhibitor of the enzymes studied, related 4,6-diamino-1,2-dihydro-*s*-triazines were potent inhibitors of the reductases. 2,4-Diamino-6,7-diphenylpteridine was found to be approximately tenfold more effective as an inhibitor of the three reductase systems than was 2,4-diamino-6,7-dimethylpteridine; 2-amino-6,7-diphenylpteridine and 4-amino-6,7-diphenylpteridine were not effective as inhibitors of these enzymes. 2,4,7-Triamino-6-arylpteridines bearing an *ortho* substituent in the 6-aryl moiety were found to be 10–100-fold more potent as inhibitors of the reductase systems than were the corresponding *para*-substituted derivatives. The 2-amino-4-hydroxypteridine derivatives biopterin, xanthopterin, and isoxanthopterin were found to be effective neither as substrates nor as inhibitors of the trypanosomal reductase.

We have described recently[2] the isolation of dihydrofolate reductase from the protozoan *Trypanosoma equiperdum* and have shown that the pattern of susceptibility of this trypanosomal enzyme system to inhibition by several diamino heterocycles is different from those observed for reductases isolated from bacterial and mammalian sources. The extensive studies carried out by Burchall and Hitchings[3] and by Baker and his colleagues[4] on the structural requirements for inhibition of dihydrofolate reductase from different sources have established that seemingly small changes in chemical structure can produce marked alterations in the ability of an agent to inhibit a particular reductase. We have initiated a similar comparative study of the relationship between the chemical structure of various 2,4-diaminopyrimidines and related heterocyclic systems, and their ability to inhibit dihydrofolate reductases from *T. equiperdum*, chicken liver, and rat liver. It was hoped that such a study would provide information which might prove useful in the design of new agents for use in the chemotherapy of trypanosomiases and other protozoal diseases.

Experimental Section

Initially dihydrofolate reductase from *T. equiperdum* was prepared exactly as outlined in ref 1; in later studies acetone powders, prepared from 3–6 × 10^{10} trypanosomes, were extracted with 7 ml of pH 7.0 phosphate buffer (0.1 M) and the extracts were centrifuged for 40° at 100,000g and 4° in a Beckman Model L refrigerated centrifuge; the resulting supernatant solutions served as the source of enzyme. This modified procedure for obtaining trypanosomal enzyme was introduced because of the high loss of enzyme activity encountered during the dialysis step employed in our original procedure. It yielded an enzyme preparation which did not differ significantly, with respect to sensitivity to various inhibitors, from the enzyme preparation made by the previous procedure. In this connection, it is worth pointing out that Schrecker and Huennekens[5] found that dihydrofolate reductase in crude extracts of chicken liver and this enzyme after partial purification showed similar sensitivity to the diaminopteridine inhibitor, aminopterin. Acetone powders[6] were prepared from sections of chicken liver, the powders were extracted as for the trypanosomal preparation, the extracts were dialyzed overnight against 100 vol of pH 5.5 buffer (0.01 M), and the dialyzed material was used for enzymatic assays. Acetone powders were prepared also from sections of rat liver and were extracted and dialyzed as described for the chicken liver preparations. A similar preparation[7] of (dihydro) folate reductase, obtained by high-speed centrifugation of a rat liver homogenate, was used by Hampshire and her colleagues[8] for evaluating the inhibitory potency of a series of 2,4-diamino-5-arylazopyrimidines.

Reduced nicotinamide–adenine dinucleotide phosphate (NADPH) was purchased from P. L. Biochemicals, Inc., Milwaukee, Wis.; folic acid was purchased from Calbiochem, Los Angeles, Calif. Dihydrofolate was prepared by the method of Futterman[9] and also by the procedure of Friedkin and his colleagues.[10] No differences were observed between the rates of enzymatic reduction of dihydrofolate prepared by the different methods. References to syntheses of pteridines carried out in this laboratory are given in the tables.

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