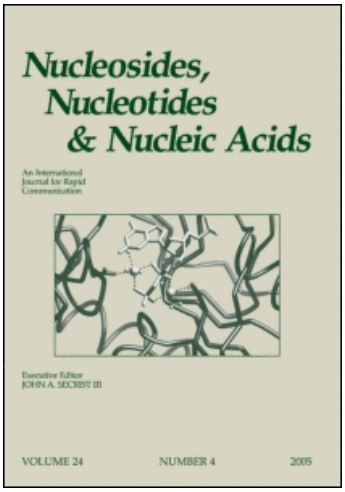

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STUDIES ON GLYCOSIDES OF 3,4,6-TRISUBSTITUTED PYRAZOLO-[3,4-d]PYRIMIDINES. SYNTHESIS OF 2'-DEOXYRIBONUCLEOSIDES.

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ABSTRACT: A synthesis of 4,6-dimethylthio-1-(2-deoxy-β-D-erythro-pentofuranosyl)pyrazolo[3,4-d]pyrimidine-3-carbonitrile (4) is described using the stereospecific sodium salt glycosylation procedure. Condensation of the sodium salt of 4,6-dimethylthiopyrazolo[3,4-d]pyrimidine-3-carbonitrile (1) with 1-chloro-2-deoxy-3,5-di-O-p-toluoyl-α-D-erythro-pentofuranose (2) gave exclusively the corresponding blocked nucleoside (3) with β-anomeric configuration, which on deprotection provided 2'-deoxyriboside 4. Aglycone functional groups transformations of 4 led to related 3,4,6-trisubstituted pyrazolo[3,4-d]pyrimidine-2'-deoxynucleosides. These compounds are devoid of any significant cytotoxic activity in vitro.

INTRODUCTION: Structural resemblance between naturally occuring purine nucleosides and their pyrazolo[3,4-d]pyrimidine analogs prompted a great interest in the preparation of a number of 8-aza-7-deazapurine derivatives to elucidate their biological properties[1-4]. It has been shown that compounds of this group have different types of activity, for example, antiparasitic[5], antitumor[6-10] and antiviral[11]. Pyrazolo-[3,4-d]pyrimidine-4(5H)-one (allopurinol) is a clinically useful agent against gout and related metabolic disorders[12]. Allopurinol and certain its derivatives are inhibitors of xanthine oxidase and purine nucleoside phosphorylase. The inherited deficiency of adenosine deaminase and purine nucleoside phosphorylase play a role in defective immune system function. Therefore, it may be suggested that purine nucleoside derivatives may have a variety effects on the immune system[13].

As compared with D-ribosides of substituted pyrazolo[3,4-d]pyrimidines, little is known about their 2'-deoxy congeners. It was shown

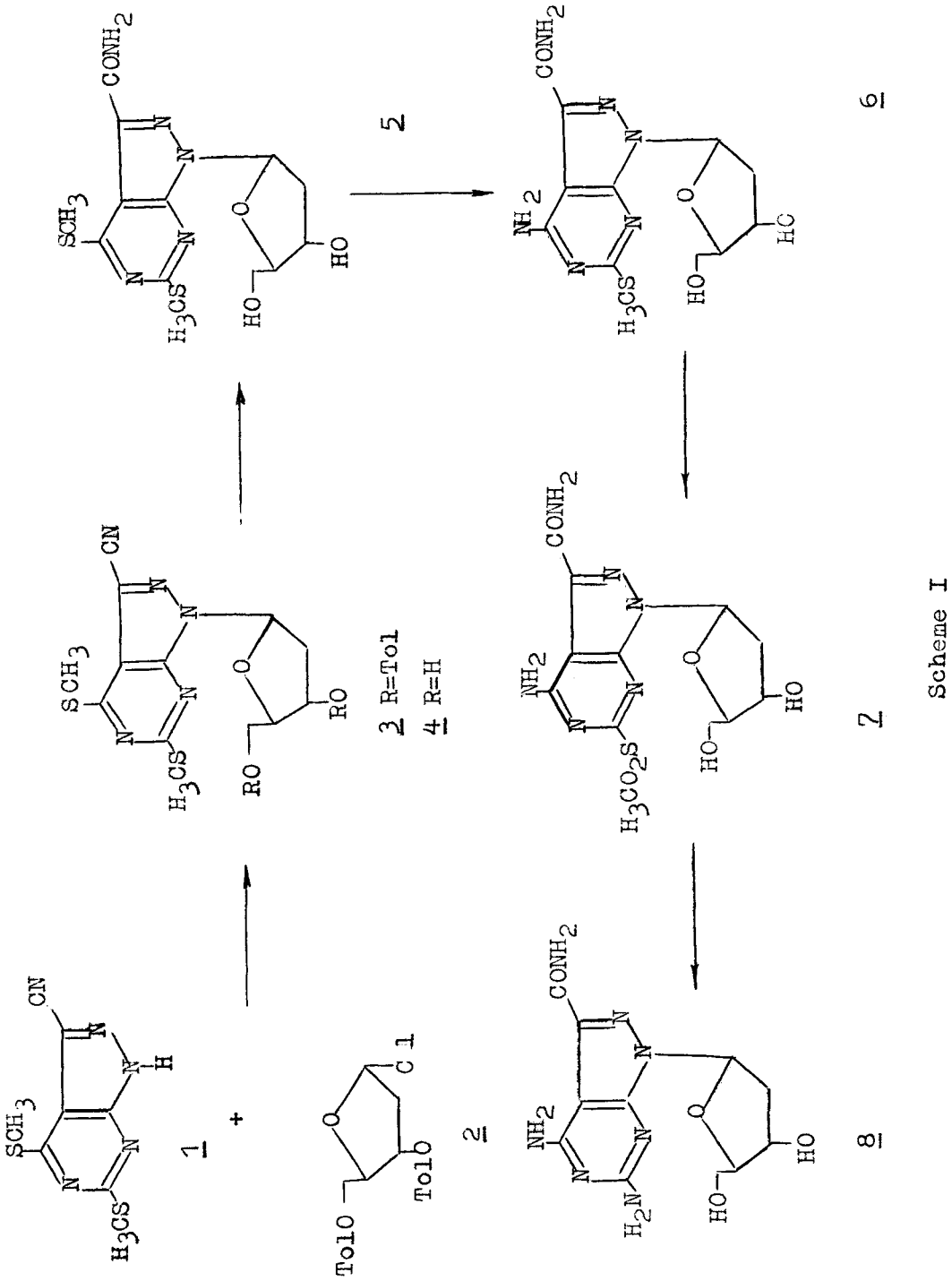
that allopurinol 2'-deoxyriboside mimics 2'-deoxyinosine in oligomers under duplex structure formation[14]. Some other compounds of this type were used as instruments in molecular biological investigations[15].

As a part of a program on the synthesis of analogs of nucleic acids components with potential antitumor properties, D-ribo- and D-arabinofuranosyl nucleosides of 3,4,6-trisubstituted pyrazolo[3,4-d]-pyrimidines were prepared in our laboratory[16,17].

The present paper describes the synthesis of 4,6-dimethylthio-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrazolo[3,4-d]pyrimidin-3-carbonitrile **4** and its subsequent transformations into the other 3,4,6-trisubstituted 2'-deoxynucleosides. The regio- and stereospecific sodium salt glycosylation procedure has been developed for the preparation of purine nucleoside derivatives and related analogs[1]. Utilization of this facile method for the synthesis of monosubstituted pyrazolo[3,4--d]pyrimidine nucleosides[1] as well as pyrrolo[2,3-d]pyrimidine[2] and substituted pyrrole nucleosides[3] has been found to be remarkably successful. Our synthetic pathway involves the direct glycosylation of 3,4,6-trisubstituted pyrazolo[3,4-d]pyrimidine **1** with pentofuranose **2**[18].

RESULTS AND DISCUSSION: The optimal conditions of glycosylation of 3-carbonitrile **1** in our case were reaction of one equivalent of the sodium salt of **1**, generated *in situ* by the treatment with sodium hydride in a mixture of anhydrous acetonitrile and dioxane (1:1, v/v) in a nitrogen atmosphere with one equivalent of the halogenose **2** at 50°C for 2.5 hours. After filtration and subsequent cooling of the reaction, the reaction mixture containing the blocked 2'-deoxynucleoside **3** was crystallized. An additional amount of this compound was obtained by crystallization from ethanol/acetone (1:1, v/v) of the residue remained after evaporation of the filtrate. This reaction gave **3** in 72% total yield. No formation of the α-anomer in this reaction was detected Use of this rather general synthetic procedure for the preparation of 2'-deoxyribonucleosides has now been found to be remarkably successful in the series of trisubstituted pyrazolo[3,4-d]pyrimidines with equal ease in the presence of methylthio and cyano functions.

Attempts to obtain unprotected nucleoside **4** by traditional deacylation of blocked 2'-deoxyribofuranoside **3** with methanolic ammonia or



Scheme I

sodium methoxide in methanol failed. None of these procedures gave the required product **4**. Finally we focused on the alkaline hydrolysis of the compound **3** by 1 N sodium hydroxide in dioxane at pH 9.0. This procedure results in a complex reaction mixture, and isolation of the desired **4** is extremely difficult. 4,6-Dimethylthio-1-(2-deoxy-β-D-erythro-pentofuranosyl)pyrazolo[3,4-d]pyrimidin **4** and -carboxamide (**5**) were isolated by preparative TLC in 61% and 26% yields respectively. The presence of the nitrile function in **3** and **4** was confirmed by IR spectra which revealed a sharp absorption band at 2220 cm^{-1}.

Compound **4** was employed for further chemical transformations (Scheme 1). Unlike the corresponding ribonucleoside, which is readily hydrolyzed to the 3-carbamoyl derivative in ammonium hydroxide and H_2O_2 (3%) in water, the 2'-deoxynucleoside **4** was stable under the same conditions.The 3-carboxamide **5** was obtained by treatment of compound **4** with ammonium hydroxide and H_2O_2 (30%) in a mixture of methanol and dioxane (10:1, v/v) and isolated in 59% yield, 10% of **4** being recovered unchanged after this reaction. Ammonolysis of nucleoside **5** with methanolic ammonia at 80°C led to 4-amino derivative (**6**) in 78% yield. It had been shown previously that under ammonolysis conditions the substitution of the 4-methylthio group occurs preferentially in 4,6-dimethylthiopyrazolo[3,4-d]pyrimidine β-riboside[19]. Conversion of the 6-methylthio group of **6** to an amino group was achieved in two steps. Preliminary oxidation of sulfur atom with 4 equivalents of *m*-chloroperbenzoic acid in methanol went smoothly to give the 6-methylsulfonyl derivative (**7**) in 88% yield. In the ^1H NMR spectrum of **7** in C_5D_5N the SO_2CH_3 protons had a considerable shift of 0.95 ppm as compared with SCH_3 protons of **6**. The 6-methylsulfonyl substituent in **7** proved to be a good leaving group when treated with liquid ammonia at 20°C for 72 hours to form 4,6-diamino-1-(2-deoxy-β-D-erythro-pentofuranosyl)pyrazolo[3,4-d]pyrimidine-3-carboxamide (**8**) in 75% yield.

The anomeric configuration of pyrazolo[3,4-d]pyrimidine 2'-deoxyribonucleoside **4** was assigned as β on the basis of ^1H NMR data. The characteristic pseudotriplet with a peak width of about 14 Hz for the anomeric proton was observed at 6.64 ppm. This pattern is similar to that observed for the anomeric proton of the other β-2'-deoxyribonucleosides[1]. The essentially identical UV absorption spectra of **4** and

corresponding ribonucleoside[16] indicated that the site of glycosylation of **4** is at N1.

The 3,4,6-trisubstituted pyrazolo[3,4-d]pyrimidine derivatives synthesized were tested against human ovarian carcinoma cells culture. The cytotoxicity of analogs was evaluated by inhibition of ^{3}H-thymidine incorporation into DNA of these cells. Only nucleosides **4**, **6**, and **7** were found to be moderately active in this test system (CE_{50} 10-15 μg/ml).

EXPERIMENTAL SECTION: Melting points are uncorrected. ^{1}H NMR spectra were obtained using tetramethylsilane as an internal standard with a Bruker WH-360 spectrometer. Mass spectra (MS) were measured with a Hewlett Packard HP-5985 instrument at 70 eV. Infrared spectra (IR) in KBr were recorded with a Perkin-Elmer 283 spectrophotometer and ultraviolet spectra (UV) were taken on Cary 219 (Varian) spectrophotometer in 95% ethanol. Preparative thin-layer chromatography (TLC) was performed on glass plates with silica gel LL_{254} 5/40 (Chemapol, CSFR) and analytical TLC - on Silufol UV 254 (Kavalier, CSFR). Spots were visualized by UV light.

4,6-Dimethylthio-1-(2-deoxy-3,5-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)pyrazolo[3,4-d]pyrimidine-3-carbonitrile (3). To a stirred solution of **1** (1.31 g, 5.54 mmol) in dry CH_3CN/dioxane (66 ml, 1:1) was added NaH (80% in oil, 0.18g, 5.54 mmol) in small portions and the mixture was stirred at 20°C under nitrogen for 30 min. 1-Chloro-2-deoxy-3,5-di-O-p-toluoyl-α-D-erythro-pentofuranose **2** (2.15 g, 5.54 mmol) was added portionwise with stirring. The reaction mixture was stirred at 50°C for 2.5 h before it was filtered to remove a small amount of insoluble material. The solution was allowed to stand at -5°C overnight and the separated solid was collected by filtration to yield 1.38 g of **3**. The solvent was removed under reduced pressure and the residue was crystallized from ethanol/acetone mixture (1:1) to afford an additional 0.97 g of **3**. After evaporation of the filtrate the oily residue was chromatographed with toluene/acetone (1:1) as the solvent. Total yield of **3** was 2.38 g (72%); mp 141-142°C; IR, ν, cm^{-1}: 2225 (CN); UV, λ_{max}, nm (ε): 247 (41900), 285 (12700), 299 (14200), 317 (10300); ^{1}H NMR ($CDCl_3$) δ: 2.65 (s, 3H, SCH_3), 2.68 (m, 1H, $J_{2',2''}$ 14.4 Hz, C2'-H), 2.72 (s, 3H, SCH_3), 3.43 (m, 1H, C2"-H),

4.53 (m, 1H, C5'-H), 4.59 (m, 1H, C4'-H), 4.66 (m, 1H, C5"-H), 5.88 (m, 1H, $J_{2',3'}$, 3 Hz, $J_{2",3'}$, 6.2 Hz, C3'-H), 6.87 (t, 1H, $J_{1',2'}=J_{1',2"}=6.2$ Hz, C1'-H). Anal. Calcd. for $C_{29}H_{27}N_5O_5S_2$: C, 59.07; H, 4.62; N, 11.88; S, 10,88. Found: C, 58.72; H,4.60; N, 11.60; S, 10.69.

4,6-Dimethylthio-1-(2-deoxy-β-D-erythro-pentofuranosyl)-pyrazolo[3,4-d]pyrimidine-3-carbonitrile (4) and -3-carboxamide (5). To a stirred solution of 3 (0.72 g, 1.22 mmol) in dioxane 12 ml) was added dropwise 1 N aqueous sodium hydroxide until pH 9.0 was reached. After 3 d, TLC monitoring indicated that reaction was complete and Dowex-50 (H^+) resin was added to neutralize the reaction mixture. The resin was removed by filtration and the combined filtrates were evaporated to dryness. The remained solid was chromatographed (4:1, chloroform/methanol) and eluted from plates with ethanol to give 0.27 g (62%) of 4 and 0.12 g (26%) of 5 as crystalline solids.

4: mp 184-186°C; IR, ν, cm^{-1}: 2220 (CN); UV, λ_{max}, nm (ε): 254 (22400), 294 (12000), 316 (8300); ^1H NMR (Me_2SO-d_6) δ: 2.37 (m, 1H, C2'-H), 2.63, 2.72 (2s, 6H, $2SCH_3$), 2.83 (m, 1H, C2"-H), 3.39 (m, 1H, C5'-H), 3.53 (m, 1H, C5"-H), 3.85 (m, 1H, C4'-H), 4.49 (m, 1H, C3'-H), 6.64 (t, 1H, C1'-H). Anal. Calcd. for $C_{13}H_{15}N_5O_3S_2$ 0.5 C_2H_5OH: C, 44.67; H, 4.82, N, 18.60; S, 17.04. Found: C, 44.87; H, 4.71; N, 18,24; S,17.41; MS, m/z: M^+ 353.

5: mp 201-203°C; IR, ν, cm^{-1}: 1675, 3300, 3460; UV, λ_{max}, nm, (ε): 252 (26800), 292 (15600), 309 (11500); ^1H NMR (Me_2SO-d_6) δ: 2.29 (m, 1H, C2'-H), 2.54, 2.62 (2s, 6H, $2SCH_3$), 2.93 (m, 1H, C2"-H), 3.30-3.60 (m, 2H, C5'-H, C5"-H), 3.84 (m, 1H, C4'-H), 4.67 (m, 1H, C3'-H), 6.63 (t, 1H, C1'-H). Anal. Calcd. for $C_{13}H_{17}N_5O_4S$: C, 42.01; H, 4.61; N, 18.84. Found: C, 42.47; H, 4.85; N, 18.57; MS, m/z: M^+ 371.

4,6-Dimethylthio-1-(2-deoxy-β-D-erythro-pentofuranosyl)-pyrazolo[3,4-d]pyrimidine-3-carboxamide (5). To a solution of 4 (0.15 g, 0.40 mmol) in dioxane/methanol (1:10, 11 ml) was added water (2.5 ml) and the reaction mixture was treated with NH_4OH (2 ml) and H_2O_2 (30%, 0.3 ml). The solution was kept at 20°C for 1 h and then evaporated to dryness. The residue was purified by preparative TLC using chloroform/methanol (6:1) as the solvent to yield 0.93g (59%) of 5 as a crystalline solid and 0.015g (10%) of the starting compound 4. ^1H NMR and the other characteristics of nucleoside 5 prepared by this procedure were identical to those described above.

4-Amino-6-methylthio-1-(2-deoxy-β-D-erythro-pentofuranosyl)pyrazolo[3,4-d]pyrimidine-3-carboxamide (6). Compound 5 (0.18 g, 0.48 mmol) was combined with methanolic ammonia (10 ml, saturated at 0°C) and heated in a steel bomb at 80°C for 3 d. After the bomb was cooled the solvent was removed *in vacuo*. The residue was purified by preparative TLC using chloroform/methanol (4:1) as the solvent to give 0.13g (78%) of 6, mp 250-252°C; IR, ν, cm^{-1} :1640, 1680, 3400, 3460; UV, λ_{max}, nm (ε): 251 (23700), 280 (8800), 290 (8000); ^1H NMR (C_5D_5N) δ: 2.55 (s, 3H, SCH_3), 2.69 (m, 1H, C2'-H), 3.31 (m, 1H, C2"-H), 4.14 (m, 1H, C5'-H), 4.18 (m, 1H, C5"-H), 4.63 (m, 1H, C4'-H), 5.19 (m, 1H, C3'-H), 7.14 (t, 1H, C1'-H), 8.58, 8.86, 9.06, 9.65 (4s, 4H, $CONH_2$, NH_2). Anal. Calcd. for $C_{12}H_{16}N_6O_4S \cdot 0.5\ H_2O$: C, 41.25; H, 4.90; N, 24,06. Found: C, 41.21; H, 5.10; N, 23,38. MS, m/z M^+340.

4-Amino-6-methylsulfonyl-1-(2-deoxy-β-D-erythro-pentofuranosyl)pyrazolo[3,4-d]pyrimidine-3-carboxamide (7). To a stirred suspension of 6 (0.12 g. 0.35 mmol) in methanol (2.5 ml) was added m-chloroperbenzoic acid (0.24 g.1.39 mmol) and the mixture was stirred for 1 h at 20°C. The separated solid was collected by filtration, washed thoroughly with chloroform and then dried to give 0.12 g (88%) of title compound 7. A small amount of 7 was purified by preparative TLC with chloroform/methanol (4:1) to afford an analytical sample, mp 190-192°C; IR, ν, cm^{-1}: 1135, 1300 (SO_2); UV, λ_{max}, nm (ε): 244 (8600), 293 (8400); ^1H NMR (C_5D_5N) δ: 2.72 (m, 1H, C'-H), 3.28 (m, 1H, C2"-H), 3.50 (s, 3H, SO_2CH_3), 4.16 (m, 2H, C5'-H, C5"-H), 4.62 (m, 1H, C4'-H), 5.22 (m, 1H, C3'-H), 7.11 (t, 1H, C1'-H), 8.77, 9.27, 9.82, 10.02 (4s, 4H, $CONH_2$, NH_2). Anal. Calcd. for $C_{12}H_{16}N_6O_6S \cdot H_2O$: C, 36.90; H, 4.65. Found: C, 36.40; H 4.56. MS, m/z M^+372.

4,6-Diamino-1-(2-deoxy-β-D-erythro-pentofuranosyl)pyrazolo[3,4-d]pyrimidine-3-carboxamide (8). Nucleoside 7 (0.16 g, 0.43 mmol was combined with liquid ammonia (5 ml) in a steel bomb at 20°C and was kept for 3 d. Then gas was allowed to evaporate, the remained solid was purified by TLC with chloroform/methanol (3:1) as the solvent to afford 0.10 g (75%) of 8. A small amount of this compound was crystallized from methanol for analytical purposes; mp 159-161°C; IR, ν, cm^{-1}: 1670, 1705, 3000-3500; UV, λ_{max}, nm (ε): 232 (17300), 255 (4900), 297 (4200); ^1H NMR (C_5D_5N) δ: 2.56 (m, 1H, $J_{2',2"}$ 13.1 Hz, $J_{2',3'}$,6.7 Hz, C2'-H), 3.24 (m, 1H, $J_{2",3'}$ 3.4 Hz, C2"-H),

4.04 (m, 1H, $J_{5',5''}$ 12 Hz, C5'-H), 4.14 (m, 1H, $J_{4',5''}$ 4.8 Hz, C5"-H), 4.54 (m, 1H, $J_{4',5'}$ 4.0 Hz, C4'-H), 5.10 (m, 1H, $J_{3',4'}$ 3.4 Hz, C3'-H), 6.99 (t, 1H, $J_{1',2'}=J_{1'2''}$ =6.7 Hz, C1'-H), 8.30, 8.77, 9.45 (3s, 6H, $CONH_2$, NH_2). MS, m/z M^+309. Satisfactory elemental analysis couldn't be obtained, because the substance was contaminated by silica gel when eluted from TLC plates with ethanol. It was chromatographically homogeneous, R_f 0.28, chloroform/methanol (3:1).

REFERENCES

1. Kazimierczuk, Z.; Cottam, H.B.; Revankar,G.R.; Robins, R.K. J. Amer. Chem. Soc., (1984), 106, 6379.
2. Ramasamy, K.; Robins, R.K.; Revankar, G.R. Tetrahedron, (1986), 42, 5869.
3. Ramasamy, K.; Robins, R.K.; Revankar, G.R. J. Heterocycl. Chem., (1987), 24, 863.
4. Seela, F.; Steker, H. Heterocycles, (1985), 23, 2521.
5. Cottam, H.B.; Petrie, Ch.R.; McKernan, P.A.; Goebel, R.J.; Dalley, N.K.; Davidson, R.B.; Robins, R.K.; Revankar, G.R. J. Med. Chem., (1984), 27, 1119.
6. Korbukh, I.A; Goryunova, O.V.; Stukalov, Yu.V.; Ivanova T.P.; Dobrynin, Ya.V.; Preobrazhenskaya, M.N. Bioorgan. Chim., (1984), 10, 963.
7. Nelson, D.J.; LaFon, S.W.; Tuttle, J.V.; Miller, N.H.; Miller, R.L.; Krenitsky, T.A.; Elion, G.B.; Berens, R.L., Marr, J.J. J. Biol. Chem., (1979), 254, 11544.
8. Berens, R.L.; Marr, J.J., Nelson, D.J.; LaFon, S.W. Biochem. Pharmacol., (1980), 29, 2397.
9. Panzica, R.P.; Bhat, G.A.; Earl, R.A.; Montero, J.-L.; RotiRoti, L.W.; Townsend, L.B. In Chemistry and Biology of Nucleosides and Nucleotides, Harmon, R.E.; Robins, R.K., Townsend, L.B., Eds., Academic Press, Inc. New York, N.Y., (1978), 121.
10. Bhat, G.A.; Montero, J.-L.; Panzica, R.P.; Wotring, L.L.; Townsend, L.B. J. Med. Chem., (1981), 24, 1165.
11. Ugarkar, B.G.; Cottam, H.B.,McKernan, P.A; Robins, R.K.; Revankar, G.R. J. Med. Chem., (1984), 27, 1026.

12. Robins, R.K.; Srivastava, P.C., Revankar, G.R.; Novinson, T.; Miller, J.P. Lect. Heterocycl. Chem., (1982), 6, 93.
13. Delvin, J.P.; Hargrave, K.D. Tetrahedron, (1989), 45, 4327.
14. Seela, F.; Kaiser, K. Nucleic Acids Res., (1986), 14, 1825.
15. Seela, F.; Driller, H. Helv. Chim. Acta, (1988), 71, 1191.
16. Garaeva, L.D.; Korbukh, I.A.; Dobrynin, Ya.V.; Nikolaeva, T.G.; Preobrazhenskaya, M.N. Chim.-Pharm. Zhurn., (1988), N 7, 798.
17. Garaeva, L.D.; Yartseva,I.V.,Preobrazhenskaya,M.N. Bioorgan. Chim., (1989), 15, 249.
18. Hoffer, M. Chem. Ber., (1960), 131.
19. Korbukh, I.A.; Yakunina, N.G.; Preobrazhenskaya, M.N. Bioorgan. Chim., 1980, 6, 1632.

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