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SYNTHESIS OF 3-CYANO-4,6-BIS(METHYLTHIO)PYRAZOLO[3,4-d]PYRIMIDINE 1-RIBOSIDE

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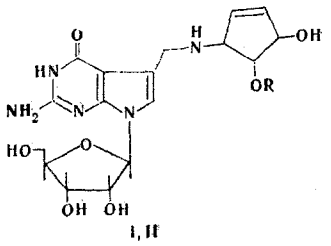
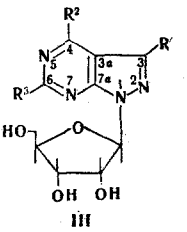
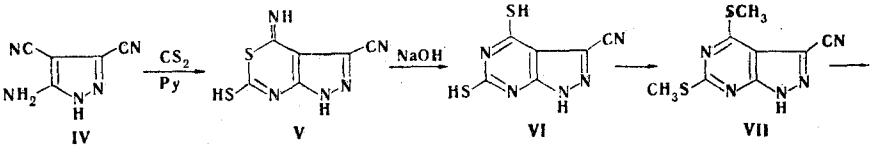
3-Cyano-4,6-bis(methylthio)pyrazolo[3,4-d]pyrimidine was synthesized by cyclization of 3,4-dicyano-5-aminopyrazole with CS_2 in pyridine with subsequent Dimroth rearrangement and methylation of the resulting 3-cyano-4,6-dimercaptopyrazolo[3,4-d]-pyrimidine with methyl iodide. Glycosylation of the product by fusion with 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose in the presence of iodine gave 1-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-3-cyano-4,6-bis(methylthio)pyrazolo[3,4-d]pyrimidine, the deacetylation of which with a 1% solution of hydrogen chloride in methanol led to 3-cyano-4,6-bis(methylthio)pyrazolo[3,4-d]pyrimidine 1-riboside. The structures of the compounds were established by IR, UV, circular dichroism, PMR, and ^{13}C NMR spectroscopy.

The high biological activity of toyocamycin and sangivamycin, which are pyrrolopyrimidine analogs of adenosine that contain substituents in the pyrrole ring, is well-known [1]. Considerably less study has been devoted to guanosine analogs with substituents in the five-membered ring. In the 7-desazaguanosine series nucleosides Q (I) and Q* (II) belong to this class [2, 3]. The preparation of pyrazolopyrimidine analogs of purine nucleosides with the general formula III that contain substituents in both the pyrazole and pyrimidine rings and are simultaneously analogs of toyocamycin (or sangivamycin) and guanosine seems of interest.

In the present paper we describe the synthesis of 3-cyano-4,6-bis(methylthio)pyrazolo-[3,4-d]pyrimidine (VI) and its 1-riboside (X), which are key compounds for the preparation of trisubstituted pyrazolo[3,4-d]pyrimidines and their ribosides with the general formula III.







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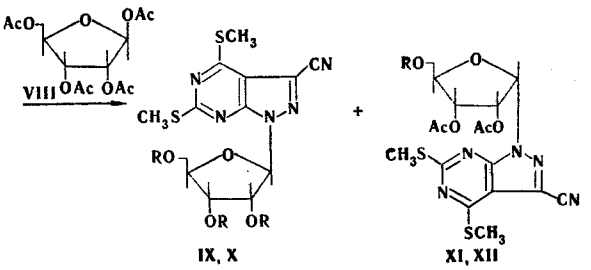

I, X, XI R=H; II R= mannosyl and galactosyl; IX, XII R=Ac

3-Cyano-4,6-dimercaptopyrazolo[3,4-d]pyrimidine was obtained from 3,4-dicyano-5-aminopyrazole (IV) [4] by a method similar to that used to synthesize 4,6-dimercaptopyrazolo[3,4-d]pyrimidine from 3-amino-4-cyanopyrazole [5]. Pyrazolothiazine V, which undergoes Dimroth rearrangement on treatment with alkali to give 3-cyano-4,6-dimercaptopyrazolo[3,4-d]pyrimidine (VI), is formed by refluxing (for 36 h) IV with CS_2 in anhydrous pyridine and subsequent treatment of the reaction product with 1 N HCl. Compound V was obtained in 88% yield based on the dicyanoaminopyrazole. Methylation of VI with excess methyl iodide in 2 N NaOH gave 3-cyano-4,6-bis(methylthio)pyrazolo[3,4-d]pyrimidine (VII) in 82% yield; we used the latter compound for the conversion to nucleosides.

The glycosylation of VII was accomplished by fusion with 1,2,3,5-tetra-O-acetyl-β-dirobofuranose (VIII) in the presence of iodine in vacuo at 170°C for 45 min; as a result, 1-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-3-cyano-4,6-dimethylthiopyrazolo[3,4-d]pyrimidine (IX) was obtained in 77% yield. 1-(2',3'-Di-O-acetyl-α-D-ribofuranosyl)-3-cyano-4,6-bis(methylthio)pyrazolo[3,4-d]pyrmidine (XI), which, after treatment with acetic anhydride in pyridine, gave 1-(2',3',5'-tri-O-acetyl-α-D-ribofuranosyl)-3-cyano-4,6-bis(methylthio)-pyrazolo[3,4-d]pyrimidine (XII), was also isolated from the reaction mixture in ∿ 1% yield by preparative chromatography on silica gel.

A decrease in the temperature and (or) fusion time lowers the yield of the desired product. Pronounced resinification of the reaction products occurs in the case of more prolonged glycosylation and at higher temperatures. The use of bis(p-nitrophenyl) phosphate as the glycosylation catalyst leads to a decrease in the yields of the nucleosides. Riboside IX was also obtained in 36% yield by glycosylation of substituted pyrazolopyrimidine VII with tetraacetylribofuranose VIII by the trimethylsilyl method [6] in 1,2-dichloroethane in the presence of $SnCl_4$.

It should be noted that 2-nucleosides were not obtained in a single case, while 1-α anomer XI was isolated in only very small amounts; this constitutes evidence for the high stereospecificity and positional specificity of the glycosylation of 3-cyano-4,6-bis(methylthio)pyrazolo[3,4-d]pyrimidine VII. At the same time, it is known that the glycosylation of monosubstituted pyrazolo[3,4-d]pyrimidines [for example, 5-acetamido- and 4-methylthiopyrazolo[3,4-d]pyrimidines) by the fusion method leads to mixtures of nucleosides that are positional isomers and anomers [7, 8].

The deacetylation of IX by the action of a 1% solution of hydrogen chloride in methanol [9] led to 1-(β-D-ribofuranosyl)-3-cyano-4,6-bis(methylthio)pyrazolo[3,4-d]pyrimidine (X) in 68% yield. The deacetylation of riboside IX with a saturated (at 0°C) solution of ammonia in methanol gives riboside X in low yield and is accompanied by the formation of a large number of side products. The O-acetyl groups cannot be removed by the action of a solution of sodium methoxide in absolute methanol without conversion of the cyano group to an imino ester group.

1-(2',3'-O-Isopropylidene-β-D-ribofuranosyl)-3-cyano-4,6-bis(methylthio)pyrazolo[3,4-d]pyrimidine (XIII) was synthesized in 55% yield by the action of absolute acetone on deacetylated riboside X in the presence of p-toluenesulfonic acid and ethyl orthoformate [10].

The site of addition of the ribose residue to the heterocyclic base in the synthesized nucleosides was established by comparison of the ^{13}C NMR spectra of O-acetylated riboside IX and the corresponding heterocyclic base VII (Table 1). The spectrum of aglycone VII was recorded under conditions in which it is deprotonated by an equivalent of lithium methoxide.

TABLE 1. Data from the ^{13}C NMR Spectra of VII and IX (in d_6-DMSO) (δ, ppm)

Compound	C_4 and C_6	C_{7a}	C_3, C_{3a}, CN	SCH_3	>C=O	$-CH_3(OCOCH_3)$
VII	165,4 163,0	160,6	118,0 115,7 112,3	15,16 12,83	—	—
IX	172,36 166,57	153,37	119,17 113,39 110,98	15,08 12,74	170,93 170,60 170,34	21,26 27,28 27,28

TABLE 2. Data from the PMR Spectra of 3,4,6-Trisubstituted Pyrazolo[3,4-d]pyrimidine in $CDCl_3$

Compound	Chemical shift, δ, ppm (SSCC, Hz)						
	$C^{1'}H$ ($J_{1,2}$)	$C^{2'}H$ ($J_{2,3}$)	$C^{3'}H$ ($J_{3,4}$)	$C^{4'}H$	$C^{5'}H$	SCH_3	$COCH_3$ or $C(CH_3)_2$
IX	6,51 (3,6)	5,95 (5,2)	5,74 (5,6)	4,6—4,06		2,69 2,62	2,13 2,10 2,10
X*	6,23 (4,4)	4,63 (4,6)	4,00 (5,2)	3,76—3,30		2,70 2,64	—
XI	6,80 (5,6)	5,64 (7,2)	5,36 (4,0)	4,82	3,89	2,66 2,54	2,02 1,76
XII	6,84 (6,0)	5,64 (6,8)	5,29 (4,4)	4,98	4,36	2,58 2,68	2,12 2,07 1,79
XIII	6,50 (2,8)	5,18 (6,0)	4,98 (2,0)	4,44—3,78		2,65 2,58	1,58 1,33

*In d_6-DMSO.

The weakest-field signals in the spectrum of VII at 165.4 and 163.0 ppm were assigned to the C_4 and C_6 atoms, which experience the maximum deshielding effect of the sulfur and nitrogen atoms bonded to them. The next strong-field signal at 160.6 ppm was assigned to the bridging C_{7a} atom located between the two nitrogen atoms of the pyrimidine ring. The signals in the spectrum of nucleoside IX that are maximally shifted to weak field and are located at 166-172 ppm were assigned as signals of the C_4 and C_6 atoms, as well as signals of the C=O atoms of the acetyl groups. The next strong-field signal at 153.37 ppm was assigned by analogy to the C_{7a} atom. It follows from this that when a substituent (a ribose residue) is introduced in the heteroring of VII, the signal of the C_{7a} atom is shifted 7.23 ppm to strong field. According to the well-known principle [11], this is possible only when the substituent in the nitrogen-containing heteroring is attached to the nitrogen atom adjacent to the C atom, the signal of which in the ^{13}C NMR spectrum underwent a strong-field shift (α shift), i.e., the N_1 atom in this case.

The identical character of the UV spectra of O-acetylated ribosides IX and XII makes it possible to conclude that the carbohydrate residue in XII is also attached to the N_1 atom.

The configuration of the glycoside bond of IX and XII was determined by PMR and circular dichroism (CD) spectroscopy. The opposite signs of the Cotton effects in the CD spectra of these compounds at 242-245, 262-265, and 291 nm indicate their different anomeric configurations.

The signal of the anomeric protons is shifted 0.33 pm to weak field in the PMR spectrum of XII as compared with IX and has a large spin—spin coupling constant, viz., $J_{1',2'}$ = 6.0 Hz ($J_{1',2'}$ = 3.6 Hz for IX); this constitutes evidence for an α configuration for XII and a β configuration for IX. The characteristic (for α anomers [12]) strong-field 0.31 ppm shift of the signals of the protons of the 2'-O-acetyl group is observed in the PMR spectrum of XII. The β configuration of IX was also confirmed by the data from the PMR spectrum of isopropylidene derivative XIII. The difference (Δδ) in the chemical shifts of the signals of the protons of the CH_3 groups of the isopropylidene grouping viz.,

TABLE 3. Properties of 3,4,6-Trisubstituted Pyrazolo[3,4-d]pyrimidines and Their Ribosides

Compound	mp, °C	$[\alpha]_D^{20}$ (c; solvent)	UV spectra in ethanol, λ_{max}, nm	Found, %				Empirical formula	Calculated, %				Yield, %
				C	H	N	S		C	H	N	S	
V	>360	—	—	42,6	3,4	—	20,8	$C_6H_3N_5S_2 \cdot C_5H_5N \cdot H_2O$	43,0	3,3	—	20,9	91,0
VI	>360	—	204 (4,10), 257 (4,05), 262 (4,05), 300 (4,04)	42,9	3,5	27,1	20,8	$C_6H_3N_5S_2 \cdot C_5H_5N \cdot H_2O$	43,0	3,3	27,4	20,9	97,0
VII	234—235	—	202 (4,20), 249 (4,41), 288 (4,09)	40,6	3,0	29,4	26,9	$C_8H_7N_5S_2$	40,5	3,5	29,5	27,0	82,0
IX	Oil	−42,4° (0,25; $CHCl_3$)	205 (4,27), 254 (4,42), 296 (4,19), 322 (4,04)	44,1	4,3	13,2	11,9	$C_{19}H_{21}N_5O_7S_2 \cdot H_2O$	44,4	4,5	13,8	12,5	77,3
X	171—173	−56,8° (0,50; CH_3OH)	203 (4,19), 253 (4,32), 293 (4,07), 315 (3,89)	42,3	4,3	19,2	17,4	$C_{13}H_{15}N_5O_4S_2$	42,3	4,1	19,0	17,4	68,0
XIII	Foam	−79,5° (0,66; $CHCl_3$)	203 (4,14), 252 (4,32), 293 (4,08), 315 (3,92)	—	—	17,2	—	$C_{16}H_{19}N_5O_4S_2$	—	—	17,1		55,2

0.25 ppm, makes it possible to conclude that the glycoside bond of ribosides IX and XII has a β configuration, in conformity with the rule in [13].

The signals of the protons attached to the C_5' atom is shifted 0.47 ppm to strong field in the PMR spectrum of diacetylated riboside XI as compared with triacetylated riboside XII, and this constitutes evidence for the absence of an acetyl group attached to the 5'-O atom in XI.

The properties of the synthesized compounds and data from their PMR spectra are presented in Tables 2 and 3.

The authors thank A. I. Chernyshev for recording and participating in the discussion of the ^{13}C NMR spectra.

EXPERIMENTAL

The PMR spectra of the compounds were recorded with a JNM-MH-100 spectrometer with tetramethylsilane (TMS) as the internal standard. The ^{13}C NMR spectra were recorded with a Brücker WH-90 pulse spectrometer at 22.63 MHz under conditions of complete suppression of the spin–spin coupling of the protons with the carbon atoms and also with incomplete decoupling of the protons with TMS as the internal standard. The UV spectra were recorded with a Unicam SP-800 recording spectrophotometer. The IR spectra of KBr pellets of the compounds were recorded with a UR-10 spectrometer. The mass spectra were recorded with a Varian MAT-311A mass spectrometer. The circular dichroism (CD) spectra were recorded with a Mark III Jobin Ivon dichrograph. The specific rotation was determined with a Perkin–Elmer 241 polarimeter. Analytical thin-layer chromatography (TLC) was carried out on Silufol UV-254 in chloroform–methanol system [99:1 (A), 95:5 (b), and 9:1 (C)]. Preparative chromatography was accomplished in a loose layer of LSL 5/40 silica gel (Czechoslovakian SSR) in the same systems and also with a Jobin Ivon Prep-100 preparative chromatograph on Merck H-60 silica gel.

3-Cyano-4-imino-6-mercaptopyrazolo[3,4-d]-1,3,6-thiazine (V). A 10.0-g (75 mmole) sample of 3,4-dicyano-5-aminopyrazole (IV) was refluxed for 60 h in a mixture of 100 ml of absolute pyridine and 150 ml of anhydrous CS_2, after which the mixture was cooled, and the resulting precipitate was removed by filtration, washed with water, and transferred to a beaker containing 200 ml of concentrated HCl. The acidic mixture was allowed to stand at 20°C for 2 h, and the resulting yellow crystals were removed by filtration, washed with water and acetone, and dried in vacuo over $CaCl_2$ for 12 h to give 14.3 g of V.

3-Cyano-4,6-dimercaptopyrazolo[3,4-d]pyrimidine (VI). A solution of 14.3 g (68.5 mmole) of pyrazolothiazine V in 250 ml of 6 N NaOH was maintained at 20°C for 10 h, after which it was neutralized to pH 6.0-6.5 with dilute acetic acid. The resulting yellow precipitate was removed by filtration, washed with cold water and acetone, and dried in vacuo over P_2O_5 to give 13.9 g of VI. An analytically pure product was obtained by recrystallization from water. IR spectrum: 2245 cm^{-1} (CN group).

3-Cyano-4,6-bis(methylthio)pyrazolo[3,4-d]pyrimidine (VII). A 7.1-g (50 mmole) sample of CH_3I was added to a solution of 8.0 g (38.5 mmole) of VI in 100 ml of 2 N NaOH, and the mixture was stirred at 20°C for 2 h. It was then neutralized to pH 6.5-7.0 with dilute acetic acid, and the resulting precipitate was removed by filtration, washed with water and acetone, and dried in vacuo over P_2O_5 to give 7.46 g of VII. An analytically pure product with R_f 0.15 (TLC in system A), was obtained by recrystallization from ethanol. IR spectrum: 2245 cm^{-1} (CN group). Mass spectrum (m/e): 237 (M^+).

1-(2',3',5'-Tri-O-acetyl-β-D-ribofuranosyl)-3-cyano-4,6-bis(methylthio)pyrazolo[3,4-d]pyrimidine (IX) and 1-(2',3'-Di-O-acetyl-α-D-ribofuranosyl)-3-cyano-4,6-bis(methylthio)-pyrazolo[3,4-d]pyrimidine (XI). A) A mixture of 3.0 g (1.27 mmole) of pyrazolopyrimidine VII, 6.0 g of VIII, and 0.6 g (0.21 mmole) of I_2 was fused at 170°C for 10 min, and the resulting melt was stirred at this temperature in vacuo [10-15 mm (mercury column)] for 45 min. It was then cooled and treated with 30 ml of chloroform, and the chloroform solution was chromatographed through a layer of silica gel (50 g) with a thickness of cm (elution with chloroform). The eluate was concentrated in vacuo to a volume of 40 ml, and the resulting mixture was separated with a preparative chromatograph with a 50 by 5 cm column filled with silica gel under a pressure of 10 atm with elution with chloroform (the elution pressure was 3 atm). The fractions containing the product with R_f 0.37 (TLC in system A) were combined and evaporated to give 4.05 g of individual IX. The fractions containing mixtures of products were subjected to additional separation. The overall yield of IX was 4.85 g. IR spectrum: 2245 (CN) and 1740 cm^{-1} (C=O). CD spectrum (c 0.003; ethanol): 245 ([θ] − 6200), 265 (0), 291 (−3700), and 320 nm (−1900). Workup of the fractions containing a product with R_f 0.13 (TLC in system A) also yielded 55 mg (0.88%) of XI. IR spectrum: 2245 (CN) and 1740 cm^{-1} (C=O). UV spectrum in ethanol, λ_{max}(log ε): 204 (4.27), 253 (4.40), 293 (4.15), and 316 nm (3.98).

B) A mixture of 237 mg (1.0 mmole) of VII, 330 mg (1.1 mmole) of ribofuranose VIII, 0.1 ml (0.8 mmole) of trimethylchlorosilane, 0.2 ml (0.8 mmole) of hexamethyldisilazane, and 0.16 ml of $SnCl_4$ was refluxed with stirring for 5 h in the absence of moisture in 6 ml of 1,2-dichloroethane, after which it was maintained at 20°C for 12 h. Chloroform (4 ml) was added, and the solution was washed with 10 ml of water, 5 ml of 1% $NaHCO_3$ solution, and 10 ml of water. The organic phase was separated, dried over $MgSO_4$, and filtered. The filtrate was evaporated, and the residue was dissolved in 3 ml of chloroform and subjected to preparative chromatography on silica gel in system B to give 160 mg (36%) of IX, which was identical to O-acetylated riboside IX obtained by method A. The product had R_f 0.47 (TLC in system B) and 0.37 (TLC in system A). Workup of the reaction mixture also yielded 76 mg (32%) of starting VII.

1-(β-D-Ribofuranosyl)-3-cyano-4,6-bis(methylthio)pyrazolo[3,4-d]pyrimidine (X). A solution of 0.45 g (0.88 mmole) of riboside IX in 15 ml of a 1% solution of dry hydrogen chloride in absolute methanol was maintained at 20°C for 48 h in a sealed flask, after which it was neutralized to pH 7.0 with Ag_2CO_3. The precipitated AgCl was removed by filtration, and the filtrate was evaporated to give 0.22 g of X. An analytically pure product was obtained by recrystallization from ethanol and had R_f 0.54 (TLC in system C).

1-(2',3',5'-Tri-O-acetyl-α-D-ribofuranosyl)-3-cyano-4,6-bis(methylthio)pyrazolo[3,4-d]pyrimidine (XII). A 2-ml sample of acetic anhydride was added to a solution of 45 mg (0.1 mmole) of XI in 2 ml of absolute pyridine, and the mixture was maintained at 20°C for 24 h, after which it was evaporated to dryness, and the residue was dissolved in 1 ml of chloroform. Preparative chromatography of the chloroform solution on silica gel in system B yielded 39 mg (79.5%) of XII in the form of a yellow oil with R_f 0.36 (TLC in system A) and $[\alpha]_D^{20}$ + 48.7° (c 1.12, $CHCl_3$). IR spectrum: 2245 (CN) and 1740 cm^{-1} (C=O). UV spectrum in ethanol, λ_{max} (log ε): 206 (4.11), 253 (4.32), 293 (4.08), and 316 nm (3.92). Mass spectrum (m/e): 495 (M^+), 259, and 237. CD spectrum (c 0.004; ethanol): 242 ([θ] + 7900), 262 (−5000), 291 (+4400), and 320 nm (−3600).

1-(2',3'-O-Isopropylidene-β-D-ribofuranosyl)-3-cyano-4,6-bis(methylthio)pyrazolo[3,4-d]pyrimidine (XIII). A 0.3-ml sample of ethyl orthoformate was added to a suspension of 183 g (0.5 mmole) of riboside X in 3 ml of absolute acetone containing 25 mg (0.16 mmole) of p-toluenesulfonic acid, and the mixture was stirred for 2 h (at 20°C) until riboside X dissolved completely. The mixture was neutralized with $NaHCO_3$, stirred for 20 min, and filtered. The filtrate was evaporated, and the residue was subjected to preparative chromatography on silica gel in system B to give 110 mg of XIII with R_f 0.64 (TLC in system B). IR spectrum: 2245 cm^{-1} (CN). Mass spectrum (m/e): 409 (M^+), 394 ($M^+ - CH_3$), 379 ($M^+ - 2CH_3$), and 237.

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