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SYNTHESIS OF DERIVATIVES OF PYRAZOLO[3,4-d]PYRIMIDIN-3-YLACETIC ACID AND THEIR NUCLEOSIDES

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3-Cyanomethyl-4,6-dimethylmercaptopyrazolo[3,4-d]pyrimidine was synthesized on the basis of 3-cyanomethyl-4-cyano-5-aminopyrazole. Ribosylation of this product gave 1-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-3-cyanomethyl-4,6-dimethylmercaptopyrazolo[3,4-d]pyrimidine in 63% yield and small amounts of the 1-α and 2-β isomers. A number of derivatives of 6-methylmercaptopyrazolo[3,4-d]pyrimidin-3-ylacetic acid and their 1-ribosides were synthesized. The 4-methylmercapto group was replaced by amino, hydrazino, oxo, N-piperidino, and N-morpholino groups. The nitrile group was saponified in an alkaline medium to carbamoyl and carboxy groups. The corresponding 4-morpholino and 4-piperidino derivatives were obtained by the reaction of 3-cyano-4,6-dimethylmercaptopyrazolo[3,4-d]pyrimidine per-O-acetylated 1-β-D-ribofuranoside with secondary cyclic amines. The high resistance of the 6-methylmercapto group to the action of nucleophilic agents and the higher reactivity of the 4-methylmercapto group as compared with the nitrile group are discussed. Data on the cytotoxic activity of the synthesized compounds were obtained.

The synthesis and chemical transformations of 3-cyano-4,6-dimethylmercaptopyrazolo-[3,4-d]pyrimidine and its 1-riboside, which we previously described in [1, 2], led to a number of compounds that have valuable biological properties. We demonstrated for the first time not only the cytotoxic activity but also the antivirus activity in this series of compounds [3]. High cytotoxic activity was also observed in the case of nucleosides of 4-monosubstituted and 3,4-disubstituted pyrazolo[3,4-d]pyrimidines; the introduction of a substituent in the 3 position led to intensification of the activity [4]. A further study of substituted pyrazolo[3,2-d]pyrimidines and their nucleosides seems of doubtless interest.

In the present paper we describe the synthesis of 3-cyanomethyl-4,6-dimethylmercaptopyrazolo[3,4-d]pyrimidine (IV) and its 1-riboside (VII), as well as their chemical transformations.

We obtained [3,4-d]pyrimidine IV from 3-cyanomethyl-4-cyano-5-aminopyrazole (I) by methods similar to those described in [1, 5]. It may be assumed that pyrazolothiazine II,

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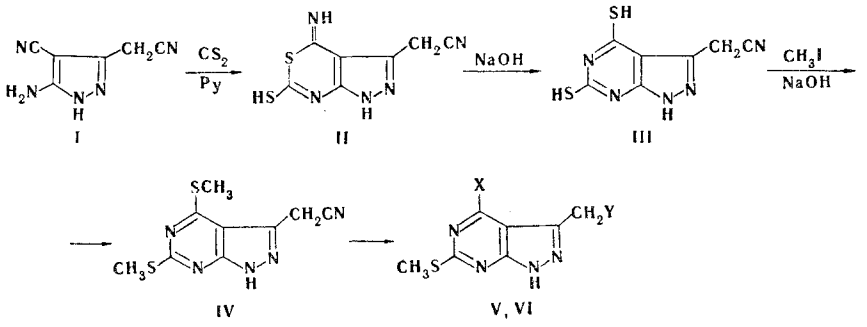
TABLE 1. Data from the ^{13}C NMR Spectra of IV and VII (d_6-DMSO, δ, ppm)

Compound	C_6, C=O	C_4	C_{7a}	C_3	CN	C_{3a}	$C_{1'}$—$C_{5'}$	CH_3	CH_2	SCH_3
IV	169,79	165,63	155,36	136,97	118,02	108,04	—	—	19,26	15,05 12,81
VII	171,27 171,11 170,78 170,68	166,77	154,68	139,05	117,43	109,20	87,47 80,46 73,72 71,48 63,93	21,74 21,58 21,58	19,45	15,21 13,06
Δδ IV—VII	—	−1,14	+0,68	−2,08	+0,59	−1,16	—	—	—	—

which undergoes Dimroth rearrangement in an alkaline medium to give 3-cyanomethyl-4,6-dimercaptopyrazolo[3,4-d]pyrimidine (III), is formed when II is refluxed with carbon disulfide in anhydrous pyridine with subsequent treatment of the reaction product with 1 N HCl. Compound III was methylated without additional purification with methyl iodide in 2 N NaOH to give IV in 87% yield based on starting pyrazole I.

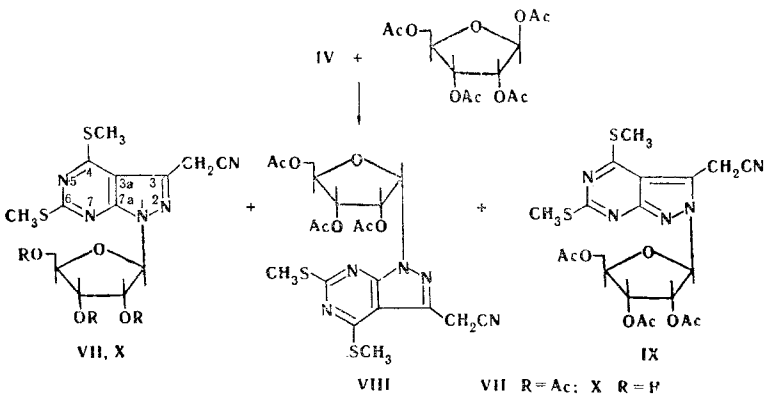
When IV is heated with concentrated ammonium hydroxide in an ampul at 120°C, the nitrile group undergoes saponification to a carboxy group with simultaneous replacement of the methyl mercapto group in the 4 position by an amino group to give ammonium 4-amino-6-methylmercaptopyrazolo[3,4-d]pyrimidin-3-ylacetate (V). The absorption band of a nitrile group (2260 cm^{-1}) that is present in the spectrum of IV vanishes in the IR spectrum of V, and a broad band of a carboxylate ion appears at 1590 cm^{-1}. The corresponding free acid is isolated when an aqueous solution of V is acidified. An absorption band at 1690 cm^{-1} (C=O in the COOH group) appears in the IR spectrum.



V X = NH_2, Y = $COONH_4$; VI X = $NHNH_2$, Y = CN

Replacement of the 4-methylmercapto group by a hydrazino group to give 3-cyanomethyl-4-hydrazino-6-methylmercaptopyrazolo[3, 4-d]pyrimidine (VI) occurs when pyrazolo[3,4-d]-pyrimidine IV is treated with hydrazine hydrate.

1-(2',3',5'-Tri-O-acetyl-β-D-ribofuranosyl)-3-cyanomethyl-4,6-dimethylmercaptopyrazolo-[3,4-d]pyrimidine (VII) was isolated in 63% yield in the glycosylation of IV by fusion with 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose *in vacuo* for 40 min at 170°C in the presence of iodine. In addition, two other nucleosides, identified from the data from the UV and PMR spectra as 1-(2',3',5'-tri-O-acetyl-α-D-ribofuranosyl)-3-cyanomethyl-4,6-dimethylmercapto-



VII, X VIII VII R = Ac; X R = H

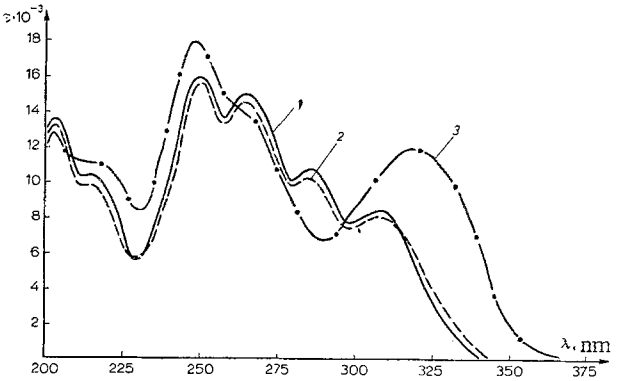


Fig. 1. UV spectra of nucleosides VII, VIII, and IX in ethanol: 1) VII; 2) VIII; 3) IX.

pyrazolo[3,4-d]pyrimidine (VIII) and 2-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-3-cyanomethyl-4,6-dimethylmercaptopyrazolo[3,4-d]pyrimidine (IX), were isolated from the reaction mixture in ∿1% yields after repeated preparative chromatography on silica gel.

The site of addition of the ribose residue to the heteroring in nucleoside VII was established by comparison of its ^{13}C NMR spectrum and the corresponding heterocyclic base IV (Table 1). The weakest-field signals at 169.79 and 165.63 ppm in the spectrum of IV were assigned, respectively, to the C_6 and C_4 atoms, which experience the maximal deshielding effect of the sulfur and nitrogen atoms bonded to them. The signal at 155.36 ppm was assigned to the bridged C_{7a} atom (see structure VII), while the signal at 136.97 ppm was assigned to the C_3 atom. The signals at 118.02 and 108.04 ppm were assigned, in analogy with the literautre data [6], respectively, as signals of the carbon atom in the CN group and of the bridged C_{3a} atom. The signals of the C atoms of the CH_3 and CH_2 atoms were found at strong field at 12-22 ppm.

In the spectrum of nucleoside VII the four signals that are maximally shifted to weak field at 169.8-171.3 ppm were assigned as signals of the C_6 atom and of the carbonyl carbon atoms of the acetyl groups. The signals at 166.77 and 154.68 ppm were assigned to the C_4 and C_{7a} atoms, respectively. In analogy with the above-described data for aglycone IV, the signals at 139.05, 117.43, and 109.20 ppm were assigned, respectively, as signals of the C_3, CN, and C_{3a} atoms. This assignment of the signals is in complete agreement with the data from the ^{13}C NMR spectrum of 1-(β-D-ribofuranosyl)-4-aminopyrazolo[3,4-d]pyrimidine [6].

An analysis of the spectral data shows that when a ribose residue is introduced in heterocycle IV, the signal of the C_{7a} atom is shifted 0.68 ppm to strong field, while the signals of the C_3 and C_{3a} atoms are shifted in this case to weak field to 2.08 and 1.16 ppm, respectively. It is known that [7] when a substituent is introduced in a nitrogen-containing heterocycle the signal of the carbon atom in the ^{13}C NMR spectrum that is in the α position relative to the substituted nitrogen atom is shifted to strong field (an α shift), while the signal of the β-carbon atom is shifted to weak field (a β shift). This makes it possible to conclude that the ribose residue in nucleoside VII is attached to the N_1 atom.

The UV spectra of VII and VIII are extremely similar (Fig. 1). Consequently, the carbon fragments in these nucleosides are attached to the same nitrogen atom of the aglycone, i.e., to the N_1 atom. The anomeric configuration of these compounds is confirmed by a comparison of the chemical shifts of the anomeric protons and the $J_{1',2'}$ spin–spin coupling constants (SSCC). In riboside VII, $J_{1',2'}$ = 3.4 Hz is smaller than in riboside VIII ($J_{1',2'}$ = 5.8 Hz), while the signal of its anomeric proton is located at stronger field as compared with the signal of the anomeric proton of riboside VIII (Table 2). This makes it possible to assign β and α configurations to nucleosides VII and VIII, respectively [8]. In contrast to VII and VIII the UV spectrum of nucleoside IX contains a long-wave absorption maximum at 320 nm (Fig. 1), which, with allowance for the $J_{1',2'}$ value of 3.0 Hz, makes it possible to regard this nucleoside as the 2-β-isomer.

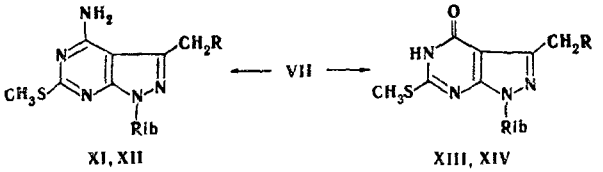
Deacetylation of VII by the action of sodium methoxide in absolute methanol led to 1-(β-D-ribofuranosyl)-3-cyanomethyl-4,6-dimethylmercaptopyrazolo[3,4-d]pyrimidine (X) in 66% yield.

TABLE 2. Data from the PMR Spectra of the Ribosides of Pyrazolo[3,4-d]pyrimidin-3-ylacetic Acid Derivatives VII-XIV and XVI-XX

Compound	Chemical shifts, δ, ppm (SSCC, Hz)							
	$C_H{}^{1'}$ ($J_{1,2}$)	$C_H{}^{2'}$ ($J_{2,3}$)	$C_H{}^{3'}$ ($J_{3,4}$)	$C_H{}^{4'}$–$C_H{}^{5'}$	CH_2	SCH_3	$COCH_3$	Solvent
VII	6,45 (3,4)	5,95 (5,4)	5,76 (5,6)	4,55—4,10	4,07	2,70 2,61	2,13, 2,13 2,08	$CDCl_3$
VIII	6,49 (5,8)	6,02 (5,6)	5,68	4,40—4,10	4,08	2,72 2,63	2,14, 2,08 2,03	$CDCl_3$
IX	6,35 (3,0)	5,90 (5,6)	5,74 (5,6)	4,52—4,28	4,07	2,71 2,64	2,12, 2,08 2,04	$CDCl_3$
X	6,33 (4,0)	4,76 (5,2)	4,52	4,13—3,74	4,24	2,71 2,61	—	CD_3OD
XI	6,24 (4,4)	4,74 (4,8)	4,43 (5,2)	4,06—3,40	3,73	2,50	—	CD_3OD
XII	6,05 (4,8)	4,70—4,18		3,87—3,52	4,40	2,50	—	d_6-DMSO
XIII	6,25 (4,4)	4,70 (5,2)	4,46 (5,4)	4,20—3,40	4,13	2,63	—	CD_3OD
XIV	6,23 (4,0)	4,70 (5,4)	4,44 (5,2)	4,45—3,40	3,82	2,62	—	CD_3OD
XVI[a]	6,35 (4,2)	4,80—4,30		4,10—3,65	—	2,55	—	CD_3OD
XVII	6,32 (4,0)	4,68 (5,0)	4,46 (5,0)	4,16—3,60	4,23	2,51	—	CD_3OD
XVIII[b]	6,27 (4,8)	4,87—4,56		4,30—3,66	—	2,45	—	$CDCl_3$
XIX	6,31 (4,8)	4,83—4,52		4,22—3,40	—	2,51	—	$CDCl_3$
XX	6,31 (4,2)	4,81 (4,8)	4,53 (4,8)	4,18—3,60	3,86	2,51	—	$CDCl_3$

[a]The signals of the protons of the morpholine ring (8H) are found at 3.70-4.10 ppm for XVI, as compared with 3.76 ppm for XVII. [b]The signals of the protons of the piperidine ring are found at 3.7-4.0 and 1.74-1.9 ppm (10H) for XVIII, as compared with 3.40-4.22 and 1.36-1.80 ppm (20H) for XIX, and 3.40-3.98 and 1.45-1.80 ppm (20H) for XX.

We studied the reaction of O-acetylated riboside VII with several nucleophilic reagents. Ammonium 1-(β-D-ribofuranosyl)-4-amino-6-methylmercaptopyrazolo[3,4-d]pyrimidin-3-ylacetate (XI) was obtained when VII was treated with ammonium hydroxide at 120°C. The IR spectrum of XI contains an absorption band at 1580 cm^{-1} (COO^- group) but does not contain the absorption band of a nitrile group that is present in the spectrum of starting riboside VII. We were able to select conditions for the selective replacement of the 4-methylmercapto group by an amino group without involving the nitrile group. 1-(β-D-Ribosuranosyl)-3-cyanomethyl-4-amino-6-methylmercaptopyrazolo[3,4-d]pyrimidine (XII) is formed by treatment of starting riboside VII with a saturated solution of ammonia in absolute methanol in an ampul at 100°C.



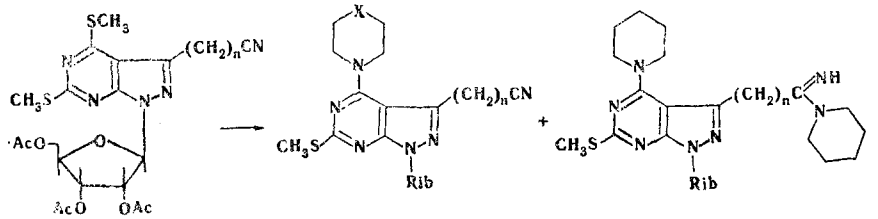
XI, XII XIII, XIV

XI R=$COONH_4$; XII, XIII R=CN; XIV R=$CONH_2$, Rib=β-D-ribofuranosyl

1-(β-D-Ribofuranosyl)-3-cyanomethyl-6-methylmercaptopyrazolo[3,4-d]pyrimidin-4-one (XIII) and 1-(β-D-ribofuranosyl)-3-carbamoylmethyl-6-methylmercaptopyrazolo[3,4-d]pyrimidin-4-one (XIV) were isolated when riboside VII was refluxed in dilute aqueous methanolic alkali. Absorption bands at 2260 cm^{-1} (CN group) and 1697 cm^{-1} (C=O) are observed in the IR spectrum of XIII. The IR spectrum of nucleoside XIV does not contain the absorption band of a nitrile

group but does contain a broad band at 1690 cm^{-1}. Broadening of this band evidently occurs as a consequence of overlapping of the absorption bands of the cyclic and exocyclic carbonyl groups.

A study of the reaction of riboside VII, as well as our previously synthesized nucleoside of XV [1], with secondary cyclic amines showed the possibility of the selective replacement of the methylmercapto group in the 4 position without involving the nitrile group. Thus 1-(β-D-ribofuranosyl)-3-cyano-4-(N-morpholino)-6-methylmercaptopyrazolo[3, 4-d]pyrimidine (XVI) and 1-(β-D-ribofuranosyl)-3-cyanomethyl-4-(N-morpholino)-6-methylmercaptopyrazolo-[3,4-d]pyrimidine (XVII) were obtained, respecitvely, when XV and VII were refluxed in morpholine with subsequent chromatographic purification. 1-(β-D-Ribofuranosyl)-3-cyano-4-(N-piperidino)-6-methylmercaptopyrazolo[3,4-d]pyrimidine (XVIII) is formed when XV is refluxed in piperidine. 1-(β-D-Ribofuranosyl)-3-[C-(N-piperidino)iminomethyl]-4-(N-piperidino)-6-methylmercaptopyrazolo[3,4-d]pyrimidine (XIX) was obtained as the principal product when this reaction was carried out under more severe conditions (heating in an ampul at 150°C); 4-(N-piperidino) derivative XVIII was also isolated at 26% yield. However, only 1-(β-D-ribofuranosyl)-3-[2-(N-piperidino)-2-iminoethyl]-4-(N-piperidino)-6-methylmercaptopyrazolo[3,4-d]pyrimidine (XX) is formed when VII is refluxed in piperidine or when it is heated with piperidine in an ampul at 120-130°C. It is interesting that the heterocyclic bases corresponding to nucleosides VII and XV do not react either with piperidine or morpholine.



VII, XV XVI, XVII, XVIII XIX, XX

VII, XX n=1; XV, XIX n=0; XVI n=0, X=O; XVII n=1, X=O; XVIII n=0, X=CH_2

On the basis of the data of [9-11] we have previously concluded that in nucleoside XV and a number of other trisubstituted pyrazolo[3,4-d]pyrimidines and their nucleosides, of the two 4- and 6-methylmercapto groups the more reactive compound in nucleophilic substitution reactions is the methylmercapto group in the 4 position [2]. Only one of the two methylmercapto groups also participates in the case of derivatives of pyrazolo[3,4-d]-pyrimidin-3-ylacetic acid in nucleophilic substitution reactions. It may be assumed that the more reactive 4-methylmercapto group also participates in all of these transformations.

A comparison of the reactivity of the cyanomethyl group in the 3 position and of the methylmercapto group in the 4 position with respect to the nucleophilic reagents used shows that the 4-methylmercapto group is the more reactive group both in heterocyclic bases and in the corresponding nucleosides. In most cases one can realize the selective replacement of the 4-methylmercapto group without involving the nitrile group. The addition to the nitrile group generally requires more severe reaction conditions.

We have previously shown that the nitrile group in XV with respect to a whole series of nucleophilic reagents such as ammonia, carbon disulfide, hydroxylamine, etc. displays higher reactivity than the methylmercapto group in the 4 position [2]. In the present research we have established that with respect to secondary cyclic amines (piperidine and morpholine) the nitrile group in XV, just as in VII, has lower lability.

The cytotoxic activity of the synthesized compounds was investigated with respect to slowing down of the incorporation of tritium-labeled thymidine in the DNA of cells of the CaOv line of carcinoma of the human ovary in the Laboratory of Cell Pharmacology of the Oncological Science Center of the Academy of Medical Sciences of the USSR (Ya. V. Dobrynin and T. G. Nikolaeva) by the method in [12]. Compounds IV-XX displayed low cytotoxic activity ($CE_{50} > 2.7 \cdot 10^{-4}$ mole/liter) and are considerably inferior in cytotoxic activity to the known 1-(β-D-ribofuranosyl)-4-aminopyrazolo[3,4-d]pyrimidine [3] ($CE_{50} = 3.7 \cdot 10^{-6}$ mole/liter). Compounds IV, VI, and XVI proved to be the most active substances. The introduction of a ribose residue in IV and V did not lead to an increase in activity.

TABLE 3. Derivatives of Pyrazolo[3,4-d]pyrimidin-3-ylacetic Acid and Their Nucleosides

Compound	mp, °C	$[\alpha]_D^{20°}$, (c, chloroform)	UV spectra in ethanol, λ_{max} (log ε)	Found, %			Empirical formula	Calc., %			Yield, %
				C	H	N		C	H	N	
IV	266—268	—	205 (4,06), 212 (4,07), 247 (4,31), 261 (4,21), 278 (4,03), 308 (3,92)	42,7	3,8	27,3	$C_9H_9N_5S_2 \cdot 0{,}25H_2O$	42,3	3,7	27,4	87
V	287—288	—	204 (4,06), 237 (4,43), 270 (4,02)	37,4	4,8	33,3	$C_8H_{12}N_6O_2S$	37,5	4,7	32,8	85
VI	350	—	204 (4,09), 244 (4,43), 275 (4,13)	39,3	4,2	40,1	$C_8H_9N_7S \cdot 0{,}5H_2O$	39,3	4,1	39,8	86
VII	152—153	−43,5 (0,3)	—	47,0	4,6	13,8	$C_{20}H_{23}N_5O_7S_2$	47,1	4,6	13,7	63
X	197—198	−72,3 (0,2)[a]	204 (4,19), 213 (4,11), 251 (4,30), 264 (4,21), 280 (4,05), 308 (3,94)	43,9	4,5	18,2	$C_{14}H_{17}N_5O_4S_2$	43,6	4,5	18,3	66
XI	147—153	54,2° (0,2)[a]	207 (4,13), 244 (4,45), 271 (4,06)	39,7	5,3	—	$C_{13}H_{20}N_6O_6S \cdot 0{,}25H_2O$	39,6	5,3	—	54
XII	219—220	−66,1 (0,2)[a]	205 (4,13), 244 (4,44), 274 (4,10)	40,6	5,4	22,7	$C_{13}H_{18}N_6O_4S \cdot 1{,}5H_2O$	41,1	5,1	22,2	44
XIII	212—213	−62,1 (0,2)[a]	209 (4,06), 272 (3,87)	42,0	4,6	—	$C_{13}H_{18}N_5O_5S \cdot 1{,}5H_2O$	42,2	4,9	—	20
XIV	Oil	−53,9 (0,2)[a]	213 (4,19), 272 (4,00)	41,9	5,0	18,3	$C_{13}H_{17}N_5O_6S \cdot 0{,}75H_2O$	41,7	5,0	18,7	53
XVI	199—201	−52,4 (0,1)[a]	203 (4,18), 244 (4,37), 310 (4,12)	47,1	5,5	19,9	$C_{16}H_{20}N_6O_5S$	47,0	5,0	20,6	61
XVII	133—136	−49,7 (0,3)[a]	203 (4,03), 244 (4,19), 257 (4,20), 287 (4,06)	47,4	5,5	19,3	$C_{17}H_{22}N_6O_5S \cdot 0{,}5H_2O$	47,3	5,4	19,5	87
XVIII	Oil	−75,5 (0,2)	204 (4,21), 241 (4,25), 306 (3,96)	50,3	5,7	21,4	$C_{17}H_{22}N_6O_4S$	50,2	5,5	20,8	49
XIX	Oil	−38,3 (0,3)	205 (4,20), 241 (4,34), 285 (4,06)	50,9	6,8	—	$C_{22}H_{33}N_7O_4S \cdot 1{,}5H_2O$	51,0	7,0	—	45
XX	Oil	−65,1 (0,2)	204 (4,12), 248 (4,29), 257 (4,32), 292 (4,19)	54,1	6,9	19,2	$C_{23}H_{35}N_7O_4S$	54,5	7,0	19,4	57

[a]Methanol.

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EXPERIMENTAL

The PMR spectra were recorded with a JNM-MH-100 spectrometer with tetramethylsilane on the δ scale; the ^{13}C NMR spectra were recorded with a Bruker-WH-360 spectrometer under conditions of complete suppression of the spin—spin coupling of the protons with the carbon atoms. The UV spectra were recorded with a Unicam SP-800 spectrophotometer. The IR spectra of KBr pellets of the compounds were recorded with UR-10 and Perkin—Elmer 283 spectrometers. The mass spectra were recorded with a Varian MAT-311A mass spectrometer. The specific rotation was determined by means of a Perkin-Elmer 241 polarimeter. Analytical TLC was carried out on Silufol UV-254 in chloroform—methanol (99:1) (A), (95:5) (B), (9:1) (C), (4:1) (D), and (2:1) (E) systems. Preparative chromatography was realized in a loose layer of LSL 5/40

silica gel in the same systems. The properties and yields of the synthesized compounds are presented in Table 3.

3-Cyanomethyl-4,6-dimethylmercaptopyrazolo[3,4-d]pyrimidine (IV). A 0.74-g (5 mmole) sample of 3-cyanomethyl-4-cyano-5-aminopyrazole (I) was refluxed in a mixture of 10 ml of anhydrous carbon disulfide and 8 ml of absolute pyridine for 16 h, after which the mixture was cooled, and the resulting precipitate was removed by filtration, washed with water, and transferred to a beaker with 10 ml of concentrated HCl. The acidic mixture was maintained at 20°C for 2 h, and the yellow crystals of pyrazolothiazine II were removed by filtration, washed with water, and dried *in vacuo* over $CaCl_2$. The dried crystals were dissolved in 40 ml of 2 N NaOH, and the insoluble material was removed by filtration. A 2.2-g (15 mmole) sample of CH_3I was added, and the mixture was stirred at 20°C for 2 h. It was then neutralized to pH 6.5-7.0 with glacial acetic acid, and the resulting precipitate was removed by filtration, washed with water, and dried *in vacuo* over P_2O_5 to give 1.1 g of IV. An analytically pure product was obtained by recrystallization from methanol to give a product with R_f 0.12 (in system A); PMR spectrum (d_6-DMSO): 4.27 (CH_2); 2.59 and 2.49 ppm ($2\text{-}SCH_3$).

Ammonium 4-Amino-6-methylmercaptopyrazolo[3,4-d]pyrimidin-3-ylacetate (V). A 0.4-g (1.6 mmole) sample of IV was heated with 12 ml of 25% ammonium hydroxide in an ampul at 120°C for 6 h. The resulting solution was maintained at 0°C for 12 h, and the colorless crystals were removed by filtration, washed with water, and dried *in vacuo* over P_2O_5 at 60°C to give 0.35 g of ammonium salt V. IR spectrum: 1590 cm^{-1} (COO^- group). PMR spectrum (d_6-DMSO): 3.90 (CH_2) and 2.64 ppm (SCH_3). A 0.1-g sample of ammonium salt V was dissolved in 2 ml of water, and two drops of acetic acid were added, during which the corresponding free acid precipitated immediately. It was removed by filtration, washed with water, and dried in vacuo over P_2O_5 to give 0.09 g of 4-amino-6-methylmercaptopyrazolo[3,4-d]pyrimidin-3-ylacetic acid with mp 281-283°C. IR spectrum: 1690 cm^{-1} (C=O in the COOH group).

3-Cyanomethyl-4-hydrazino-6-methylmercaptopyrazolo[3,4-d]pyrimidine (VI). A 1.5-ml sample of hydrazine hydrate was added to a suspension of 0.4 g (1.6 mmole) of IV in 30 ml of ethanol, and the mixture was refluxed for 5 h. It was then filtered and allowed to stand at 0°C for 10 h. The precipitated crystals were removed by filtration, washed with ethanol, and dried *in vacuo* at 60°C to give 0.31 g of VI. An analytically pure product was obtained by recrystallization from methanol—water (2:1). PMR spectrum (d_6-DMSO): 4.34 (CH_2) and 2.42 ppm (SCH_3).

1-(2',3',5'-Tri-O-acetyl-β-D-ribofuranosyl)-3-cyanomethyl-4,6-dimethylmercaptopyrazolo-[3,4-d]pyrimidine (VII), 1-(2',3',5'-Tri-O-acetyl-α-D-ribofuranosyl)-3-cyanomethyl-4,6-dimethylmercaptopyrazolo[3,4-d]pyrimidine (VIII), and 2-(2',3',5'-Tri-O-acetyl-β-D-ribofuranosyl)-3-cyanomethyl-4,6-dimethylmercaptopyrazolo[3,4-d]pyrimidine (IX). A mixture of 0.54 g (2.15 mmole) of pyrazolopyrimidine IV, 0.92 g (2.9 mmole) of 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose, and 0.1 g (0.4 mmole) of iodine was fused at 170°C for 8-10 min, and the resulting homogeneous melt was stirred at this temperature *in vacuo* (10-15 mm of mercury) for 40 min. It was then cooled and treated with 4 ml of chloroform, and the mixture was passed through a layer of silica gel (50 g) with a thickness of 5 cm (elution with chloroform). The eluate was evaporated, 50 ml of methanol was added, and the mixture was refluxed with activated charcoal for 5 min. It was then filtered, and the filtrate was allowed to stand at 0°C for 12 h. The colorless crystals (0.52 g) of riboside VII were removed by filtration, the mothor liquor was evaporated, and the residue was dissolved in chloroform and subjected to preparative chromatography on silica gel in system B to give another 0.17 g of VII with R_f 0.35 (in system A) and R_f 0.76 (in system B). IR spectrum: 2265 (CN group) and 1740 cm^{-1} (C=O). Mass spectrum, m/e: 508 (M^+). The products with R_f 0.41 and 0.39 were subjected to additional fivefold chromatographic separation in system A to give 10 mg of riboside VIII with R_f 0.40 (in system B). IR spectrum: 2265 cm^{-1} (CN) and 1740 cm^{-1} (C=O); $[\alpha]_D^{25}$ +17.4° (c 0.7; chloroform). This procedure also gave 8 mg of riboside IX with R_f 0.37 (in system B). IR spectrum: 2265 (CN) and 1750 cm^{-1} (C=O); $[\alpha]_D^{25}$ −38.4° (s, 0.3; chloroform).

1-(β-D-Ribofuranosyl)-3-cyanomethyl-4,6-dimethylmercaptopyrazolo[3,4-d]pyrimidine (X). A 0.8-ml sample of a 6% solution of sodium methoxide in absolute methanol was added to a solution of 1.2 g (2.35 mmole) of O-acetylated riboside VII in 200 ml of absolute methanol, and the mixture was stirred at 20°C for 1.5 h, after which it was neutralized with Dowex 50 × 2 (the H^+ form), and the ion-exchange resin was removed by filtration. The filtrate

was evaporated, and the residue was recrystallized from methanol with the addition of activated charcoal to give 0.62 g of X. IR spectrum: 2270 cm^{-1} (CN); R_f 0.30 (in system B).

Ammonium 1-(β-D-Ribofuranosyl)-4-amino-6-methylmercaptopyrazolo[3,4-d]pyrimidin-3-ylacetate (XI). As in the synthesis of V, 254 mg (0.5 mmole) of riboside VII was heated in an ampul at 120°C for 6 h in 25% ammonium hydroxide, after which the mixture was evaporated, and chromatography of the residue on silica gel in system E gave 105 mg of ammonium salt XI with R_f 0.20 (in system E). IR spectrum: 1580 cm^{-1} (COO^- group).

1-(β-D-Ribofuranosyl)-3-cyanomethyl-4-amino-6-methylmercaptopyrazolo[3,4-d]pyrimidine (XII). A solution of 280 mg (0.55 mmole) of riboside VII was maintained in an ampul in 15 ml of absolute methanol (saturated with ammonia at 0°C) for 9 h at 100°C and at 20°C for 12 h. It was then evaporated, and the residue was chromatographed on silica gel in system D. The zone with R_f 0.16 was collected and evaporated, and the residue (120 mg) was recrystallized from methanol with the addition of activated charcoal to give 86 mg of XII with R_f 0.19 (in system D). IR spectrum: 2260 cm^{-1} (CN group).

1-(β-D-Ribosuranosyl)-3-cyanomethyl-6-methylmercaptopyrazolo[3,4-d]pyrimidin-4-one (XIII) and 1-(β-D-Ribofuranosyl)-3-carbamoylmethyl-6-methylmercaptopyrazolo[3,4-d]pyrimidin-4-one (XIV). A solution of 220 mg (0.43 mmole) of VII in a mixture of 15 ml of methanol and 15 ml of 0.5 N NaOH was refluxed for 5 h, after which it was cooled and neutralized with Dowex 50 × 2 (the H^+ form). The ion-exchange resin was removed by filtration, the filtrate was evaporated, and the residue was subjected to preparative chromatography on silica gel in system D to give 31 mg of XIII with R_f 0.42 and 83 mg of XIV with R_f 0.19.

1-(β-D-Ribofuranosyl)-3-cyano-4-(N-morpholino)-6-methylmercaptopyrazolo[3,4-d]pyrimidine (XVI). A 200-mg (0.4 mmole) sample of XV was refluxed in 6 ml of morpholine for 5 h, after which the mixture was evaporated, and the residue was subjected to twofold preparative chromatography on silica gel in system B to give 101 mg of XVI with R_f 0.17 (in system B). IR spectrum: 2245 cm^{-1} (CN group).

1-(β-D-Ribofuranosyl)-3-cyanomethyl-4-(N-morpholino)-6-methylmercaptopyrazolo[3,4-d]-pyrimidine (XVII). This compound was similarly obtained by refluxing 200 mg (0.39 mmole) of VII in 5 ml of morpholine for 3 h. Preparative chromatography on silica gel in system D yielded 147 mg of riboside XVII with R_f 0.55 (in system D). IR spectrum: 2260 cm^{-1} (CN group).

1-(β-D-Ribofuranosyl)-3-cyano-4-(N-piperidino)-6-methylmercaptopyrazolo[3,4-d]pyrimidine (XVIII). This compound was similarly obtained by refluxing 248 mg (0.5 mmole) of XV in 4 ml of piperidine for 2 h. Evaporation and preparative chromatography on silica gel in system C yielded 99 mg of XVIII with R_f 0.21 (in system C). IR spectrum: 2245 cm^{-1} (CN group).

1-(β-D-Ribofuranosyl)-3-[C(N-piperidino)iminomethyl]-4-(N-piperidino)-6-methylmercaptopyrazolo[3,4-d]pyrimidine (XIX). A mixture of 0.45 g (0.91 mmole) of XV in 6 ml of piperidine was heated in an ampul at 150°C for 8-9 h, after which the ampul was cooled, the solution was evaporated, and the residue was subjected to fourfold chromatography on silica gel in system C to give 0.21 g of XIX with R_f 0.20 (in system D). Mass spectrum, m/e: 494 (M^+). This procedure also yielded 0.12 g (26%) of XVIII.

1-(β-D-Ribofuranosyl)-3-[2-(N-piperidino)-2-iminoethyl]-4-(N-piperidino)-6-methylmercaptopyrazolo[3,4-d]pyrimidine (XX). This compound was obtained in the same way as XVIII by refluxing 260 mg (0.51 mmole) of riboside VII in 7 ml of piperidine for 7 h. Preparative chromatography on silica gel in system D yielded 147 mg of XX with R_f 0.49 (in system D). The same compound was obtained in 38% yield by heating riboside VII in an ampul at 130°C for 5-6 h.

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