Me_2SO. The solution was filtered through a 0.22-μm Millipore filter, and the sterilized solution was injected via a lateral tail vein into the ether-anesthetized animals. The animals were then anesthetized with ether and killed by cervical fracture at various time intervals. Blood samples were obtained immediately from the beating hearts by cardiac puncture. Organs were excised, rinsed with saline solution, blotted dry, and placed in tared vials and weighed. The radioactive contents of the tissues were determined with an auto-gamma counter. Samples of the injected radioactive solution were also assayed as standards to calculate the percent injected dose per gram of tissue values. The thyroid glands were not weighed directly, but the weights were calculated in the usual manner[22] by multiplying the animal weight by 7.5 mg/100 g. Imaging studies were performed in a male dog (30 kg) with a Phogamma camera equipped with a medium energy collimator. The dog was injected intravenously with a 1% Me_2SO–saline solution (5 mL) of ^{123}I-labeled **4**. Counts (300–500 k) were accumulated at time intervals ranging from 2 to 43 min to obtain a series of sequential images (Figure 1).

(*E*)-Triphenyl(1-borono-1-penten-5-yl)phosphonium Iodide (3). A solution of (*E*)-(5-iodo-1-penten-1-yl)boronic acid (1; 1.1 g, 5 mmol) and triphenylphosphine (1.3 g, 5 mmol) in acetone (5 mL) was refluxed for 16 h. The (*E*)-triphenyl(1-borono-1-penten-5-yl)phosphonium iodide (3) separated from the reaction solution as a crystalline precipitate and was collected by filtration and washed with acetone to give 1.43 g (57%) of pure **3**: mp 185–187 °C; NMR (Me_2SO-d_6) δ 7.3–8.4 (m, 15 H, triphenyl), 5.3 and 6.3 (2 d, 1 H each, vinyl), 1.68, 2.3, and 3.5 [3 m, 2 H each, $(CH_2)_3$]. Anal. Calcd for $C_{23}H_{25}O_2BIP$: C, 55.01; H, 5.02; B, 2.15; I, 25.27; P, 6.17. Found: C, 54.95, H, 5.03; B, 2.38; I, 25.37; P, 5.96.

(*E*)-Triphenyl(1-iodo-1-penten-5-yl)phosphonium Iodide (4). Method A. A solution of chloramine-T (450 mg, 1.6 mmol) in 15 mL of 50% aqueous tetrahydrofuran (THF) was added to a stirred solution of (3; 502 mg, 1 mmol) and NaI (150 mg, 1 mmol) in 50% aqueous THF (15 mL) protected from light. The solution was stirred at room temperature for 30 min in the dark, diluted with $CHCl_3$, and washed with H_2O. The $CHCl_3$ layer was separated and washed thoroughly with 10% aqueous $Na_2S_2O_5$, followed by H_2O. The $CHCl_3$ portion was dried (Na_2SO_4), and the solvent was evaporated under vacuum. The syrupy residue was treated with acetone (10 mL) containing NaI (150 mg, 1 mmol). A crystalline product separated, which was collected by filtration, washed with acetone, and recrystallized from $CHCl_3$–petroleum ether to yield 288 mg (49%) of **4**: mp 199–200 °C; NMR ($CDCl_3$) 7.40–8.26 (m, 15 H, triphenyl), 6.13–6.51 (m, 2 H, vinyl), 3.38–4.09 (m, 2 H, Ph_3PCH_2), 2.7–2.79 (m, 2 H, $CH_2CH{=}CHI$), 1.29–2.24 (m, 2 H $CH_2CH_2CH_2$). Anal. Calcd for $C_{23}H_{23}I_2P$: C, 47.44; H, 4.11; P, 5.10. Found: C, 47.28; H, 3.97; P, 5.30.

Method B. A solution of (*E*)-1,5-diiodo-1-pentene (**2**; 644 mg, 2 mmol) and triphenylphosphine (525 mg, 2 mmol) in acetone (4 mL) was refluxed for 18 h. The crystalline (*E*)-triphenyl-1-iodo-1-penten-5-yl)phosphonium iodide (4) was collected by filtration and washed with acetone to give 779 mg (67%) of pure **4**, mp 199–200 °C. Compound **4** obtained by method B was identical (mp, TLC, and NMR) with a sample of **4** prepared by method A.

(*E*)-Triphenyl(1-[^{123}I]iodo-1-penten-5-yl)phosphonium Iodide ([^{123}I]4). A solution of (*E*)-triphenyl(1-borono-1-penten-5-yl)phosphonium iodide (**3**; 12.5 mg, 25 μmol) and $Na^{123}I$ (6.0 mCi, 3.8 mg, 25 μmol) in 50% aqueous THF (3 mL) was treated with chloramine-T (7 mg, 25 μmol) for 30 min in the dark. The solution was partitioned between $CHCl_3$ (15 mL) and H_2O (15 mL). The $CHCl_3$ layer was washed with 10% aqueous $Na_2S_2O_5$ followed by H_2O, dried (Na_2SO_4), and evaporated under vacuum. The syrupy residue was treated with acetone (5 mL) containing NaI (150 mg), and the acetone was evaporated. The residue was partitioned between H_2O (5 mL) and $CHCl_3$ (5 mL). The $CHCl_3$ portion was evaporated and purified by silica gel column chromatography by elution with $CHCl_3$, followed by 30% acetone in $CHCl_3$, to yield 1.2 mCi (~25%) of [^{123}I]**4** with a specific activity of 194 mCi/mmol. The [^{123}I]**4** cochromatographed with a cold authentic sample of **4**. Iodine-125-labeled **4** was similarly prepared in 40% yield with a specific activity of 1180 mCi/mmol.

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Registry No. 1, 84928-70-1; 2, 84928-71-2; 3, 90047-60-2; 4, 90047-61-3; [^{123}I]-4, 90047-62-4; triphenylphosphine, 603-35-0; [^{125}I]-4, 90132-05-1.

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Isomeric *N*-Methyl-7-deazaguanines: Synthesis, Structural Assignment, and Inhibitory Activity on Xanthine Oxidase[1]

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The *N*-methyl isomers of 2-amino-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one[1] (**2a**) have been synthesized regiospecifically and their structures assigned. The 3-methyl compound **3** was obtained by alkylation of the parent chromophore **2a** with dimethyl sulfate, and the 1-methyl isomer **5b** was obtained by condensation of ethyl 2-cyano-4,4-diethoxybutyrate with *N*-methylguanidine and subsequent cyclization. Methylation of 2-amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**7b**), however, with methyl iodide in the presence of 50% NaOH, by phase-transfer techniques, followed by the replacement of halide by hydroxyl, yielded the 7-methyl compound **2b**. The *N*-methyl isomers of **2a** were all found to be inhibitors of xanthine oxidase from cow's milk. While the 3-methyl isomer **3** exhibits a K_i of 40 μM, the 7- and 1-isomers show K_i values of 4.5 and 3 μM, respectively.

Syntheses of methylated nucleobases are undertaken in our laboratory in order to study biological and pharmacological activity. Furthermore, they help to evaluate routes for the regioselective synthesis of methylated derivatives of naturally occuring nucleosides, such as 7-deazaguanosine (**1a**)[2] and the recently discovered cadeguomycin (**1b**).[3] *N*-Methyl derivatives of guanine can serve as potent inhibitors and regiospecific probes of nu-

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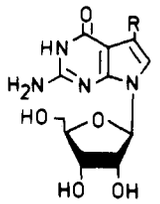
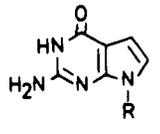


	R
1a	H
b	CO_2H

cleobase converting enzymes, like xanthine oxidase.[4] It has been reported that 9-arylguanines may be useful therapeutic agents in the treatment of gout.[5] On the other hand, methylation of nucleobases, like guanine, incorporated in DNA causes mutagenic events that may eventually lead to cancer.

Recently, we evaluated the essential binding positions of xanthine oxidase by using all the conceivable *N*-methyl isomers of 7-deazahypoxanthine as regiospecific probes for the active site.[6] We could show that they are oxidized always at C-2 without being affected in the five-membered ring. This behavior is similar to that of pyrazolo[3,4-*d*]-pyrimidines. The latter, e.g., allopurinol,[7] act as progressive inhibitors of xanthine oxidase.[8] The promising results of our study prompted us to prepare *N*-methyl derivatives of 7-deazaguanine which might possibly be used to control serum urate concentration.

The synthesis and assignment of the *N*-methyl isomers of **2a** are not simple tasks, since the direct methylation of



	R
2a	H
b	CH_3

7-deazaguanine does not lead to all conceivable methyl isomers. Therefore, regioselective routes for the unambiguous synthesis had to be developed. In this paper we report on the synthesis of the 1-, 3-, and 9-methyl isomers of 2-amino-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (**2a**) and their inhibitory activity toward xanthine oxidase.

Results and Discussion

Chemistry. 1-Methyl-7-deazaguanine. The methylation of 7-deazaguanine [2-amino-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (**2a**)], a parent aglycon of the naturally occurring 7-deazapurine nucleosides queousine[9] and cadeguomycin,[3] with dimethyl sulfate in aqueous sodium hydroxide leads to one main product and a variety of byproducts, as shown by TLC (silica gel, B). This main product becomes obtainable in high yield (79%)

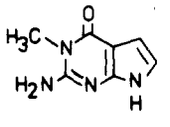
Table I. ^1H NMR Chemical Shifts of 7-Deazaguanosine, Its Aglycon **2a**, and the Isomeric Methyl-7-deazaguanines in Me_2SO-d_6 Solution[a]

no.	NCH_3/ OCH_3	5-H	6-H	NH_2	ring NH
1a[b]		6.56 (d, 3.7)	7.04 (d, 3.7		
2a		6.20 (m)	6.60 (m)	6.06	10.25, 10.97
5b	3.68	6.50 (d, 3)	7.00 (d, 3)	8.77	12.6
3	3.38	6.22 (m)	6.63 (m)	6.53	10.85
2b	3.53	6.25 (d, 4)	6.68 (d, 4)	6.20	10.0
7a	3.96	6.23 (m)	6.87 (m)	6.03	11.03

[a] δ values given in parts per million relative to Me_4Si as internal standard. Multiplicity and coupling constants (in hertz) are in parentheses. [b] In D_2O.

if a sixfold excess of the alkylating agent is used, and it can be isolated in crystalline form. The ^1H NMR spectrum (Table I) and the elemental analysis reveal that the substance is a N-methylated product. Alkylation of the exocyclic amino group can be excluded, since the ^1H NMR spectrum shows only a singlet for the methyl group at 3.38 ppm and another broader singlet of two exchangable protons at 6.3 ppm. Furthermore, an authentic sample of the O-methylated **7a**[10] exhibits a higher mobility on TLC (silica gel, B).

This isomer was shown to be 1-methyl-7-deazaguanine by an unambiguous synthesis starting from the 2-methylthio derivative of 1-methyl-7-deazahypoxanthine whose structure had been established recently.[11] The transformation of the methylthio into an amino group was accomplished by the nucleophilic displacement of the former in **4b** by the anion of acetamide. Prior to this, the pyrrolo nitrogen was protected to avoid anion formation in the pyrrole ring. This was done by alkylation under phase-transfer conditions with chloromethoxymethane. The resulting compound **4c** was then kept in a melt of acetamide/sodium hydride under nitrogen, yielding the 2-acetamido derivative **4d**, whose structure was confirmed by the ^1H NMR signals at 2.16 and 7.6 ppm for the acetamido group. The methoxymethyl and the acetyl pro-



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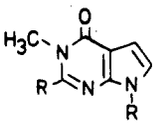

	R^2	R^7
4a	H	H
4b	CH_3S	H
4c	CH_3S	CH_2OCH_3
4d	NHAc	CH_2OCH_3
4e	NH_2	CH_2OCH_3
4f	NHAc	CH_2OH

tective groups had to be removed in order to generate 1-methyl-7-deazaguanine (**3**). Cleavage of the latter was accomplished with concentrated aqueous ammonia. Since the solubility of **4e** in aprotic solvents was low, the sequence of deprotection was reversed, and the methoxymethyl group was removed first from compound **4d**. Regular cleavage with acid was difficult as had already been noticed by Goto et al. during the synthesis of

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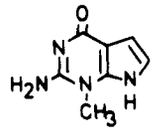
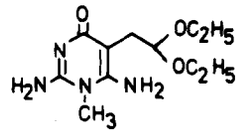
queousine.[9] This is due to the low nucleophilicity of the pyrrolo nitrogen. The problem is overcome by employing boron trichloride in dichloromethane. This reaction proceeds slowly but leads to complete conversion within 17 h at −20 °C. However, when we inspected the reaction product by ^{1}H NMR the compound still revealed a methylene signal at 5.40 ppm. The mass spectrum of the molecule displayed a molecular weight of 236, which is in accord with the unexpected structure **4f**. The hydroxymethyl group is removed by aqueous ammonia, and this leads to 1-methyl-7-deazaguanine (**3**). Its chromatographic behavior is identical with that of the main product of the methylation. Moreover, both exhibit identical ^{1}H and ^{13}C NMR spectra. Consequently, the compound obtained by methylation of 7-deazaguanine was structure **3**, as already suggested.

3-Methyl-7-deazaguanine. Traube and Winter[12] have reported that the condensation of *N*-methylthiourea and ethyl cyanoacetate gave 4-amino-2-mercapto-3-methylpyrimidin-6-one, regioselectively. This reaction was also observed with the condensation of *N*-methylthiourea and 2-cyano-4,4-diethoxybutyronitrile, which led to the formation of 1-methyl-7-deazaadenine-2-thione (4-amino-2-mercapto-1-methyl-1*H*-pyrrolo[2,3-*d*]pyrimidine) exclusively.[13] This regioselectivity prompted us to condense *N*-methylguanidine with 2-cyano-4,4-diethoxybutyrate. Again, only one product was formed, which was obtained as a crystalline solid after careful neutralization. Because of the solubility of the material in water, isolation of the pyrimidine **6** causes loss of material. Therefore, it was cyclized directly to the corresponding pyrrolo[2,3-*d*]pyrimidine in 64% yield as its hydrochloride.



5a

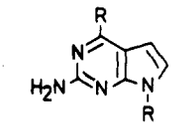


6

5b • HCl

Inspecting the literature we found that condensation of methylguanidine with ethyl 2-cyano-4,4-diethoxybutyrate should lead to pyrrolo[2,3-*d*]pyrimidines with an exocyclic amino group.[14] However, careful examination of the ^{1}H NMR spectrum of our condensation product shows that the methyl group is bound to a pyrimidine nitrogen and definitely not in an exocyclic position. If this were the case, the methyl group signal would be split into a doublet by the amino proton. Instead singlets are observed for both groups. Therefore, we assume the anticytokinines of Iwamura et al.[14] are not 2-methylamino but 3-methyl-7-deazaguanines (1-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidines). Since the methyl-7-deazaguanine obtained by cyclization was different from the product of methylation, and the latter was assigned to the N-1 isomer, this compound has to be formulated as **5b** and its precursor pyrimidine as **6**.

9-Methyl-7-deazaguanine. 2-Amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**7b**)[15] is a good starting material for the synthesis of 9-methyl-7-deazaguanine, since it does not form an anion in the pyrimidine ring and the 4-chloro



	R^7	R^4
7a	H	OCH_3
7b	H	Cl
7c	CH_3	Cl
7d	CH_3	OCH_3
7e	CH_2OCH_3	Cl
7f	CH_2OCH_3	OH
7g	CH_3	SCH_2CH_2OH

substituent is replacable by a hydroxyl function. Phase-transfer catalysis was used for the methylation and led to the regiospecific formation of compound **7c**. To prove its structure, we boiled it in sodium methoxide/methanol to afford **7d**, identical in all respects with 2-amino-4-methoxy-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (**7d**), which was synthesized recently by another route.[15] When compound **5d** was treated with thiocresolate in HMPA under nitrogen, rapid cleavage of the ether occurred, and 9-methyl-7-deazaguanine [2-amino-3,7-dihydro-7-methyl-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (**2b**)] could be obtained in crystalline form.

This reaction sequence enables the synthesis of open-chain nucleoside analogues as the recently reported acycloguanosine derivatives.[15]

To show the scope of this reaction, we alkylated **7b** with chloromethyl methyl ether and obtained crystalline **7e** in 60% yield. Its structure was assigned according to NMR data. To exchange the 4-chloro substituent for a hydroxyl group, we chose a sequence without isolation of intermediates. Compound **7e** was treated with mercaptoethanol, followed by alkaline hydrolysis. The reaction product **7f** was isolated as a crystalline solid in 49% yield.

By the same route the 4-chloro-7-methyl compound **7c** was converted into the 4-mercaptoethanol derivative **7g**, which was isolated as a crystalline solid. Nucleophilic displacement of the 4-substituent by a hydroxyl group resulted in the formation of **2b**.

As can be seen from Table II, the differences in the UV spectra of compounds **2b**, **3**, and **5** are small under acidic and neutral conditions and, hence, cannot be used to identify the different isomers. At pH 13, however, compound **5** shows the strongest bathochromic shift, as expected for N-1 alkylated pyrrolo[2,3-*d*]pyrimidines, and so allows the identification of this isomer without having the others for comparison.

Another difficulty for the unequivocal identification of this compounds is the almost identical chromatographic behavior of the 1- and 9-methyl isomers of 7-deazaguanine, which exhibit almost identical mobilities on TLC. The position of methylation may, however, be derived from the chemical shifts of the heterocyclic ring NH in the ^{1}H NMR spectra (Table I). 7-Deazaguanine (**2a**) possesses two exchangable protons bound to the heterocycle, one around 11 ppm and another close to 10 ppm bound to the pyrimidine moiety. Replacement of either of these by methylation causes the disappearance of the corresponding signal. Methylation of the pyrrolo[2,3-*d*]pyrimidine at the N-1 (purine N-3) alters the tautomeric structure of **2a** and, therefore, causes gross changes of all ^{1}H NMR signals. Finally, identification of either isomer from a mixture is

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Table II. UV Data of 7-Deazaguanosine (1a), 7-Deazaguanine (2a), and Isomeric *N*-Methyl-2-amino-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-ones

	0.1 N HCl		sodium phosphate buffer, pH 7.0		0.1 N NaOH	
no.	λ_{max}, nm	λ_{min}, nm	λ_{max}, nm	λ_{min}, nm	λ_{max}, nm	λ_{min}, nm
1a	260	238	260 (280)	236	263	237
2a	258	234	256 (280)	238	260 (280)	237
2b	265	242	264 (280)	241	267	245
3	262	239	260 (280)	235	260 (280)	235
5a	259	237	270	244	277	251

Table III. Inhibition Constants of Various Pyrrolo[2,3-*d*]pyrimidines on Xanthine Oxidase

no.	K_i, μM	mode of inhibn
2a	3	competitive
5a	4.5	competitive
3	40	competitive
2b	3	competitive

easily achieved by using the signals of the *N*-methyl groups, which are characteristic for each isomer.

Enzyme Kinetics. Recently we were able to show that 7-deazahypoxanthine is oxidized by cow's milk xanthine oxidase exclusively at carbon 2.[6] The resulting 7-deazaxanthine acts as a competitive inhibitor of the enzymatic reaction with a K_i of 25 μM.[6] Substituting the 2-OH of 7-deazaxanthine by an amino group, as in 7-deazaguanine (**2a**), results in an eightfold stronger inhibition of the oxidation of hypoxanthine [K_i = 3 μM (Table III)].

The blocking of the particular sites of 7-deazaguanine by methyl groups enables the probing of the regiochemical requirements of the active center. Table III shows the K_i values of the 3-, 1-, and 9-methyl isomers of 7-deazaguanine. It can be seen that blocking of the 9-position of **2a** by a methyl group does not affect the affinity toward the active site of xanthine oxidase. This is in agreement with the finding that methylation of the 9-position of guanine[16] does not alter the binding significantly.[17] Also, the 3-methyl compound **3** shows a low K_i (4.5 μM), while the 1-methyl isomer exhibits a 13-fold increased K_i value (40 μM), indicating that a substitution at N-1 lowers the binding to the active site significantly.

The findings confirm the idea of the structural requirements of hypoxanthine oxidation, which has been drawn from the kinetic properties of all the monomethyl isomers of 7-deazahypoxanthine. Here, the 1-methyl isomer does not bind at all to the active site, while all the other isomers are bound whether as substrates or as inhibitors.[6]

As shown by Baker and Wood[5] in the guanine series, large hydrophobic substituents at position 9 increase the binding strongly, which should enable us to design more potent inhibitors on the basis of 7-deazaguanins by the introduction of such substituents at N-9.

Experimental Section

Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were measured in Me_2SO-d_6 or $CDCl_3$ (internal Me_4Si) with a Bruker WM 250 spectrometer (Germany) and chemical shifts are reported in δ values. UV spectra were recorded on an Uvicon 810 (Kontron, Switzerland) or a Varian Super Scan 3 spectrophotometer (Varian, Australia) equipped with thermostatted quartz cuvettes. Mass spectra were obtained on a Varian MAT 311 A spectrograph. Melting points were determined with a Linström apparatus (Wagner and Munz, Germany) and are not corrected. Microanalyses were performed by Mikroanalytisches Labor Beller (Göttingen, Germany). Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the calculated value. Thin-layer chromatography (TLC) was carried out with silica gel plates, Sil G-25 UV_{254} (Macherey and Nagel, Germany). Column chromatography was performed on silica gel (Merck, Germany, 230–400 mesh ASTM) in the following solvent systems: A, $CHCl_3$/MeOH (95:5); B, $CHCl_3$/MeOH (9:1); C, $CHCl_3$/MeOH (4:1); D, $CHCl_3$/MeOH (7:3).

Enzyme Kinetics. Xanthine oxydase (EC 1.2.3.2) was purchased from Boehringer (Mannheim, Germany); the stock suspension contained 10 mg of protein/mL. Inhibition constants, K_i, were determined at 25 °C in thermostatted 1-mL quartz cuvettes (1-cm path length). The reaction mixtures contained per milliliter of Sørensen phosphate buffer (0.07 M, pH 7.5, saturated with oxygen) 0.05 mg of xanthine oxydase and the particular inhibitor at six different concentrations, ranging from 3 to 140 μM (**2a**, 8.6–85.6 μM; **3**, 8.3–137.3 μM; **5a**, 5.4–110.3 μM; **2b**, 3.1–105.9 μM). The reactions were started by the addition of hypoxanthine as substrate at concentrations ranging from 5.5 to 11.4 μM. Oxidation was followed at 293 nm (uric acid production) according to Kalckar.[18] Reaction rates were calculated by using the molar absorption coefficient at that wavelength (ϵ_{293} for uric acid in phosphate buffer, pH 7.5, 12500 M^{-1} cm^{-1}). K_i values were obtained from Dixon plots,[19] and the lines of best fit were calculated by the least-square method (correlation coefficients, r^2, were in the range 0.95–0.999).

2-Amino-1,7-dihydro-3-methyl-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (3). A solution of 2-amino-1,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (**2a**;[20] 0.5 g, 3.3 mmol) in aqueous sodium hydroxide (0.1 M, 33 mL) is stirred at room temperature with dimethyl sulfate (2.1 g, 16.7 mmol) for 1.5 h. The reaction mixture is then evaporated in vacuo. The residue is taken up with a large volume of methanol/water, heated, and filtered hot. The filtrate is evaporated to dryness, dissolved in methanol (5 mL), and adsorbed on silica gel (10 g). Methanol is evaporated, and the silica gel is suspended in chloroform/methanol (9:1, 15 mL), which is applied to the top of a silica gel column (40 × 5 cm) and eluted with solvent B. The eluate is collected, and the fractions are monitored by TLC. The main zone contains **3**, and after the solvent is partially removed in vacuo, 360 mg (79%) of colorless needles is obtained, which decompose at 280 °C ($CHCl_3$): TLC (B) R_f 0.26; UV (MeOH) λ_{max} 276, 260, 215 nm (ε 8200, 10800, 18300). Anal. ($C_7H_8N_4O$) C, H, N.

3,7-Dihydro-7-(methoxymethyl)-3-methyl-2-(methylthio)-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (4c). To a suspension of 3,7-dihydro-3-methyl-2-(methylthio)-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one[11] (**4b**; 1.0 g, 5.0 mmol) in dichloromethane/dimethoxyethane (25 mL each) is added 50% aqueous sodium hydroxide (50 mL) and benzyltriethylammonium chloride (0.4 g, 2 mmol). This mixture is stirred with a vibromixer for 5 min, chloromethyl methyl ether (500 mg, 6.25 mmol) is added, and the reaction is continued for 1 h by vigorous agitation with a vibromixer. Thereupon the organic layer is separated, and the aqueous layer is diluted with water (about 50 mL) and extracted with dichloromethane (50 mL) twice. The combined organic extracts are dried over $MgSO_4$. On evaporation, colorless needles of 4c (740 mg, 60%) are obtained: mp 98–99 °C; TLC (B) R_f 0.74; UV (MeOH) λ_{max} 292, 270 nm (ε 11400, 10600); ^{1}H NMR ($CDCl_3$) δ 2.67 (s, SCH_3), 3.37 (s, NCH_3), 3.63 (s, OCH_3), 5.50 (s, NCH_2O), 6.70 (d, J = 3 Hz, 5-H), 6.92 (d, J = 3 Hz, 6-H); ^{13}C NMR (Me_2SO-d_6) δ 14.69 (SCH_3), 29.43 (NCH_3), 56.10 (OCH_3), 74.51 (NCH_2O), 102.36 (C-5), 103.36 (C-4a), 123.26 (C-6), 146.73 (C-2), 157.08 (C-7a), 158.14 (C-4). Anal. ($C_{10}H_{13}N_3O_2S$) C, H, N, S.

2-Acetamido-3,7-dihydro-7-(methoxymethyl)-3-methyl-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (4d). A mixture of freshly sublimated acetamide (5.0 g) and sodium hydride (200 mg, 4.2 mmol) with 20% paraffin is brought to a clear melt under nitrogen atmosphere. After the mixture is cooled, **4c** (500 mg, 2.1 mmol) is added, and the mass is melted again and stirred at 120 °C for 2 h, whereupon it is cooled and triturated with 1 N acetic acid

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(4.3 mL) and water (20 mL). The solid is dissolved by vigorous shaking, and the aqueous solution is extracted with chloroform until it shows no more UV absorption. The combined extracts are dried over $MgSO_4$, and the solvent is removed in vacuo. The residue is dissolved in dichloromethane and chromatographed on a silica gel column (22 × 2.5 cm) with solvent B. After evaporation, the main zone affords **4d** as a solid residue, which is crystallized from methanol to yield colorless crystals (440 mg, 80%): mp 139 °C; TLC (B) R_f 0.54; UV (MeOH) λ_{max} 287, 265 nm (ϵ 9200, 8800); ^{1}H NMR (Me_2SO-d_6) δ 2.16 (s, CH_3CO), 3.24 (s, NCH_3), 3.43 (s, OCH_3), 5.43 ((s, NCH_2O), 6.90 (d, J = 3 Hz, 5-H), 7.16 (broad, NH), 7.47 (d, J = 3 Hz, 6-H); ^{13}C NMR (Me_2SO-d_6) δ 22.61 (CH_3CO), 30.13 (NCH_3), 55.65 (OCH_3), 74.52 (NCH_2O), 102.20 (C-5), 124.19 (C-6), 145.58, 145.84 (C-7a, C-4), 170.25 (CO). Anal. ($C_{11}H_{14}N_4O_3$) C, H, N.

Preparation of Compound 3 by Deprotection of Compound 4d. A solution of **4d** (175 mg, 0.7 mmol) in dry dichloromethane (10 mL) is cooled to about −20 °C and a 1 M solution of BCl_3 in dichloromethane (7 mL), which had also been cooled, is added. The mixture is left at this temperature for 17 h while the procedure is followed by TLC, in case of incomplete cleavage, another 7 mL of the BCl_3 solution is added. When the reaction is complete, the mixture is allowed to warm up to room temperature, and methanol (10 mL) is carefully added. After 30 min of storing at room temperature, the solvent is evaporated in vacuo, and the residue is evaporated several times with methanol. The solid residue is taken up in methanol (10 mL), and concentrated aqueous ammonia (15 mL) is added. The solution is left for 17 h at room temperature and evaporated in vacuo. The crystalline residue is recrystallized from water/methanol to yield colorless needles (95 mg, 83%) that are identical in all respects with compound **3** prepared by methylation.

2-Amino-1,7-dihydro-1-methyl-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one Hydrochloride (5b). To methylguanidine hydrochloride (3.8 g, 35 mmol) in methanol (30 mL) is added a 1 M methanolic solution of sodium methylate (35 mL). Precipitated sodium chloride is filtered off, and another portion of sodium methylate (40 mL) and ethyl 2-cyano-4,4-diethoxybutyrate (8.0 g, 35 mmol) is added. The solution is refluxed for about 4 h. Thereupon the methanol is evaporated, and the yellowish residue is dissolved in water (60 mL) and acidified with 2 N hydrochloric acid (40 mL). The mixture is stirred for 4 h at room temperature and then filtered from a white precipitate. The filtrate is evaporated in vacuo, and the residue is crystallized from water, affording **5b** as colorless crystals (4.4 g, 64%), which decompose at 280 °C: TLC (D) R_f 0.1; UV (MeOH) λ_{max} 266 nm (ϵ 9900). Anal. ($C_7H_9ClN_4O$) C, H, N.

2-Amino-4-chloro-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (7c). To the chloro compound **7b**[15] (1.0 g, 5.9 mmol) suspended in dichloromethane (15 mL) and dimethoxyethane (5 mL) is added benzyltriethylammonium chloride (75 mg, 0.27 mmol). After addition of 50% aqueous sodium hydroxide (15 mL), the mixture is agitated with a vibromixer for 30 min, during which methyliodide (0.38 mL, 850 mg, 6 mmol) is added twice. After a further 20 min of stirring, the organic phase is separated, and the solvent is evaporated in vacuo. Recrystallization of the residue from chloroform yields 820 mg (77%) of **7c** as pale yellow crystals: mp 170 °C; TLC R_f 0.41; UV (MeOH) λ_{max} 317, 263 (sh), 236 nm (ϵ 5800, 3700, 30 200); ^{1}H NMR (Me_2SO-d_6) δ 3.67 (s, NCH_3), 6.33 (d, J = 3.5 Hz, 5-H), 6.67 (br s, NH_2), 7.17 (d, J = 3.5 Hz, 6-H); ^{13}C NMR (Me_2SO-d_6) δ 30.66 (NCH_3), 98.11 (C-5), 108.69 (C-4a), 127.37 (C-6), 150.33 (C-7a), 152.50 (C-4), 159.40 (C-2). Anal. ($C_7H_7ClN_4$) C, H, Cl, N.

2-Amino-4-methoxy-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (7d). Chloro compound **7c** (1.0 g, 5.5 mmol) dissolved in 1 M sodium methylate (about 75 mL) is held under reflux for 24 h. The mixture is neutralized with acetic acid, and the solvent is removed in vacuo. The residue is then taken up with water, whereby **7d** starts to crystallize; it is filtered off and recrystallized from chloroform to afford **7d** (730 mg, 75%) as colorless crystals, mp 134 °C (lit.[15] 133 °C) TLC (A) R_f 0.67.

2-Amino-3,7-dihydro-7-methyl-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (2b). Compound **7d** (300 mg, 1.67 mmol) and sodium 4-methylthiophenolate (490 mg, 3.4 mmol) are dissolved in toluene (5 mL) and *N,N*-hexamethylphosphoric acid triamide (5 mL). The solution is heated under dry nitrogen for about 4 h, cooled, and evaporated in vacuo. The oily residue is taken up in methanol (5 mL), silica gel is added (ca. 5 g), and after evaporation, the residue is suspended in dichloromethane/methanol (95:5, 10 mL) and applied to the head of a silica gel column (30 × 1.5 cm). Elution with dichloromethane/methanol (95:5) affords one main zone, which on concentration of the solvent yields 195 mg (71%) of crystalline **2b**: TLC (B) R_f 0.57; UV (MeOH) λ_{max} 224, 263, 281 (sh) nm (ϵ 19 700, 11 400, 8100). Anal. ($C_7H_8N_4O$) C, H, N.

2-Amino-4-chloro-7-(methoxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (7e). 2-Amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**7b**;[15] 0.5 g, 2.9 mmol) is suspended in dichloromethane (20 mL) and 1,2-dimethoxyethane (5 mL). Benzyltriethylammonium chloride (50 mg, 0.18 mmol) and 50% aqueous sodium hydroxide (15 mL) are added, and the mixture is stirred for 5 min with a vibromixer. Chloromethyl methyl ether (0.18 mL, 2.6 mmol) is dropped into the stirred solution, and agitation is continued for another 15 min. The organic layer is separated, and the aqueous layer is extracted twice with dichloromethane. The combined organic extracts are shaken with water, separated, dried over sodium sulfate, and evaporated in vacuo to leave a crystalline residue. This is recrystallized from toluene to yield **7e** (380 mg, 60%) of yellowish crystals: mp 126 °C; TLC (A) R_f 0.63; UV (MeOH) λ_{max} 234, 263 (sh), 316 nm (ϵ 29 100, 5700, 6500); ^{1}H NMR (Me_2SO-d_6) δ 3.22 (CH_3), 5.36 (CH_2), 6.37 (d, J = 4 Hz, 5-H), 6.68 (NH_2), 7.27 (d, J = 4 Hz, 6-H); ^{13}C NMR (Me_2SO-d_6) δ 55.73 (CH_3), 74.20 (CH_2), 99.28 (C-5), 108.61 (C-4a), 126.24 (C-6), 151.25 (C-7a), 154.16 (C-2), 159.52 (C-4). Anal. ($C_8H_9N_4OCl$) C, H, N, Cl.

2-Amino-3,7-dihydro-7-(methoxymethyl)-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (7f). To a solution of **7e** (1.7 g, 7.99 mmol) in absolute methanol (150 mL) are added 1 M sodium methylate (31 mL) and mercaptoethanol (3.1 mL). The solution is heated under reflux for 24 h and then evaporated to about half the volume: 1 M aqueous sodium hydroxide (75 mL) is added, and the mixture is boiled again for 4.5 h. After neutralization with acetic acid, the solvent is removed in vacuo, leaving a crystalline residue. After recrystallization from water, there are obtained colorless crystals (760 mg, 49%), which melt at 220 °C under decomposition: TLC (B) R_f 0.20; UV (MeOH) λ_{max} 216, 259, 284 (sh) nm (ϵ 21 200, 12 200, 8100); ^{1}H NMR (Me_2SO-d_6) δ 3.24 (CH_3), 5.31 (CH_2), 6.37 (NH_2), 6.40 (d, J = 5 Hz, 5-H), 6.94 (d, J = 4 Hz, 6-H); ^{13}C NMR (Me_2SO-d_6) δ 55.44 (CH_3), 74.14 (CH_2), 100.02 (C-4a), 101.82 (C-5), 120.23 (C-6), 150.93 (C-7a), 152.64 (C-2), 158.58 (C-4). Anal. ($C_8H_{10}N_4O_2$) C, H, N.

2-Amino-4-[(2-hydroxyethyl)thio]-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (7g). A solution of compound **7c** (500 mg, 2.7 mmol) in dry methanol (150 mL), mercaptoethanol (0.9 g), and 1 M sodium methylate (9 mL) are combined. After heating for 8 h under reflux, the mixture is neutralized with acetic acid, and the solvent is evaporated in vacuo. Recrystallization from water affords **7g** (350 mg, 58%) as pale yellow crystals: mp 111 °C; TLC (A) R_f 0.24; UV (MeOH) λ_{max} 318, 235 nm (ϵ 9400, 31 700); ^{1}H NMR (Me_2SO-d_6) δ 2.83 (t, SCH_2), 3.37 (m, CH_2OH), 3.62 (s, NCH_3), 4.93 (br, OH), 6.05 (d, J = 3 Hz, 5-H), 6.98 (d, J = 3 Hz, 6-H); ^{13}C NMR (Me_2SO-d_6) δ 30.31 (SCH_3), 30.40 (NCH_3), 60.42 (OCH_2), 97.76 (C-5), 108.22 (C-4a), 125.19 (C-6), 151.21 (C-7a), 159.23 (C-2), 159.87 (C-4). Anal. ($C_9H_{12}N_4OS$) C, H, N, S.

2-Amino-3,7-dihydro-7-methyl-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (2b). A solution of **7g** (0.5 g, 2.2 mmol) in methanol (30 mL) and 1 N sodium hydroxide (50 mL) is heated for 3 h under reflux. After neutralization with acetic acid, the solvent is evaporated in vacuo, and the residue is taken up with water and filtrated; after partial evaporation, colorless crystals (220 mg, 61%) are obtained, which do not melt until 350 °C: TLC (B) R_f 0.57; identical in all respects with **2b** prepared by ether cleavage.

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