Dimethyl 1-(Dimethoxyphosphinyl)ethenyl Phosphate (8). A. Stoichiometric Reaction. To 8.0 mL (0.10 mol) of 1 under nitrogen at 10 °C was added dropwise 11.8 mL (0.10 mol) of **5** at a rate such that the temperature did not exceed 15 °C. No chloromethane evolution was detected. The mixture was allowed to warm to ambient temperature. Kugelrohr distillation yielded 9.38 g (72%): bp 97–110 °C (0.1mm); n^{20}_D 1.4393 (lit.[3] n^{20}_D 1.4400); ^{1}H NMR ($CDCl_3$) δ 3.80 (6, d, J = 12 Hz, CH_3), 3.83 (6, d, J = 12 Hz, CH_3), 5.56–6.15 (2, m, $=CH_2$); IR (neat) 1630 (C=C) cm^{-1}.

B. With Two Equivalents of 1 in Pentane. To 4.7 mL (0.059 mol) of 1 in 50 mL of pentane at 0 °C was added dropwise, at 0–5 °C, a solution of 6.97 mL (0.059 mol) of **5** in 10 mL of pentane. No chloromethane evolution was observed and an insoluble layer separated. The pentane supernate was removed (pipette) and run into a solution of 5.40 mL of aniline in 100 mL of ether. Workup of the ether solution afforded 4.90 g of chlorobenzanilide. The pentane-insoluble layer was allowed to warm slowly. At 17 °C, copious gas evolution started. Isolated was 7.19 g (93.6%) of 8.

Diethyl (2-Chloroacetyl)phosphonate (4). To 2.0 mL (0.025 mol) of freshly distilled 1 was added dropwise 6.0 mL (0.026 mol) of diethyl trimethylsilyl phosphite at a rate such that the temperature did not exceed 30 °C. Trimethylchlorosilane was removed in vacuo; 5.74 g. Kugelrohr distillation yielded 2.0 g (37%); bp 110–120 °C (0.1 mm) before decomposition started. The product cannot be distilled conventionally because it decomposes: ^{1}H NMR ($CDCl_3$) δ 1.37 (6, t, J = 7 Hz, OCH_2CH_3, enol tautomer), 1.40 (6, t, J = 7 Hz, OCH_2CH_3, keto tautomer), 4.28 (4, d of g, J_{HH} = 7 Hz, J_{PH} = 7 Hz, OCH_2CH_3, both tautomers), 4.60 (2, d, J_{PH} = 6 Hz), $ClCH_2$), 6.20 (1, d, J_{PH} = 6 Hz), ClCH=), 9.9 (1, br, HOC=C).

Diisopropyl (2-Chloroacetyl)phosphonate (9a). To 8.0 mL (0.10 mol) of 1 was added dropwise 25.0 mL (0.10 mol) of triisopropyl phosphite at a rate such that the temperature stayed between 27 and 30 °C and then stirred for 1.0 h. Kugelrohr distillation yielded 9.26 g (38%), bp 80–100 °C (0.1 mm), and 4.39 g (18%), bp 105–120 °C (0.1 mm). The latter contained some 2:1 adduct. The first fraction was redistilled (Kugelrohr) to furnish 5.23 g, bp 80–100 °C (0.2 mm). The product cannot be distilled conventionally because it decomposes: ^{1}H NMR ($CDCl_3$) δ 1.41 (12, d, J = 6 Hz, CH_3), 5.12 (2, d, J_{PH} = 1.8 Hz), $ClCH_2$), 5.30 (m, 2, $OCH(CH_3)_2$); IR (neat) 3600–3100 (OH, enol tautomer), 1720 (C=O), 1260 (P=O) cm^{-1}. Anal. Calcd for $C_8H_{16}ClPO_4$: C, 39.60; H, 6.65; Cl, 14.61; P, 12.77. Found: C, 39.84; H, 6.67; Cl, 14.73; P, 12.70.

Diisopropyl (2-Chloropropionyl)phosphonate (9b). Same as the procedure for **9a** described above. Kugelrohr distillation yielded 5.65 g (43%), bp 70–75 °C (0.2 mm), and 2.90 g (22%), bp 80–84 °C (0.2 mm): ^{1}H NMR ($CDCl_3$) δ 1.38 (12, d, J = 6 Hz, CH_3), 1.67 (3, d, J = 7.5 Hz, CH_3CCl), 4.3–5.0 (3, m, ClCH and $OCH(CH_3)_2$); IR (neat) 3600–3100 (OH, enol tautomer), 1720 (C=O), 1260 (P=O) cm^{-1}. Anal. Calcd for $C_9H_{18}ClO_4P$: C, 42.12; H, 7.07. Found: C, 42.15; H, 7.27.

Dimethyl (2-Methoxyacetyl)phosphonate (12). To 6.0 mL (0.066 mol) of methoxyacetyl chloride in 75 mL of THF at −78 °C was added dropwise a solution of 9.0 mL (0.076 mol) of **5** in 30 mL of THF. The mixture was allowed to warm to ambient temperature. Distillation yielded 6.47 g (54%): bp 68–70 °C (0.55 mm); ^{1}H NMR ($CDCl_3$) δ 3.44 (3, s, CH_3OC), 3.87 (6, d, J_{PH} = 11 Hz), P(=O)OCH_3), 4.44 (2, d, J_{PH} = 1.5 Hz, OCH_2C=O); IR (neat) 1700 (C=O) cm^{-1}; HRMS (70 eV) calcd for $C_5H_{11}O_5P$ m/e 182.0344; found m/e 182.0344.

Spontaneous Hydroxylation of a Cyclization Intermediate of Allopurinol

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Phase-transfer methylation of 4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (**2b**) leads to a 2:1 mixture of the N-1 and the N-2 methylated chromophores (**3**, **4**). Both were found to be converted to the corresponding methylated allopurinol derivatives (**5**, **6**) by nucleophilic displacement of the 4-methoxy groups in dilute aqueous sodium hydroxide. Alkylation of **2b** with ethyl 3-bromopropionate using the phase-transfer technique yielded—after deesterification—1-(2-carboxyethyl)-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (**7b**) regioselectively. Via this intermediate the N-1-functionalized allopurinol **8** and its 4-amino analogue **7c** could be obtained by acidic cleavage of the 4-methoxy group of **7b** or a nucleophilic displacement reaction by either dilute aqueous sodium hydroxide or concentrated aqueous ammonia. Reaction of the acid **8** with water-soluble carbodiimide results in an intramolecular cyclization, and subsequent water addition produces the tricyclic intermediate **11**. This compound undergoes spontaneous ring opening of the pyrimidine system to give its acyclic oxo tautomer **12**. In dilute alkaline medium, deformylation occurs to give pyrazolo[1,5-*a*]pyrimidine **13**. The reaction sequence is discussed as a nonenzymatic model reaction for the hydroxylation of hypoxanthine and allopurinol by xanthine oxidase.

Allopurinol[1,2] (1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one, 1) has been found to act as a progressive inhibitor of xanthine oxidase with alloxanthine—a 6-oxo derivative of **1**—as the actual inhibitor.[3] This has led to a clinical application in the treatment of gout and related metabolic disorders.[4] The value of allopurinol as well as of its 4-amino analogue is augmented by their effects on pyrimidine and purine biosynthesis.[5,6]

In the course of our investigations on modified nucleosides[7] and polymer-linked nucleoside antimetabolites,[8] our

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interest is focused on the development of regioselective methods of glycosylation or alkylation of pyrazolo[3,4-*d*]pyrimidines, especially of allopurinol.[9]

The high reactivity of $NADH^+$ toward a nucleophilic attack in the paraquinoid nicotinamide moiety[10,11] led us to the idea that acylation of N-7 of allopurinol should result in the formation of a molecule with a high reactivity toward nucleophiles at C-6. Since a regioselective acylation of allopurinol is difficult to achieve we decided to synthesize a 1-(2-carboxyethyl) derivative of allopurinol which can be cyclized via N-7 in a carbodiimide-mediated reaction to form a stable six-membered ring. The resulting tricyclic compound is expected to possess a high electrophilicity at position 6. The covalent addition of a water molecule would serve as a model reaction for the first step of xanthine oxidase catalyzed oxidation reactions.

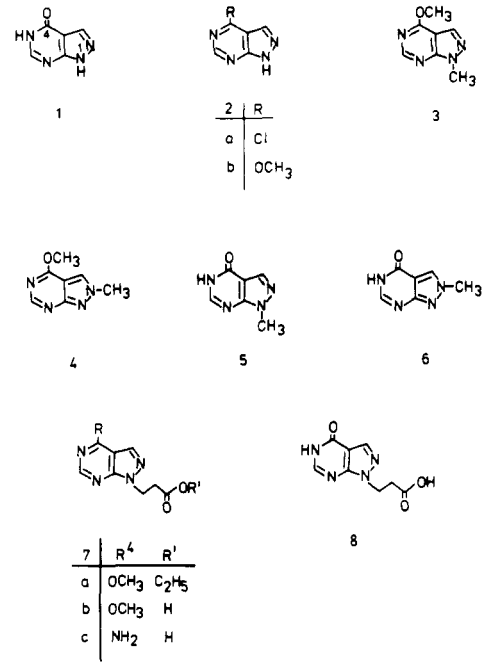
Results and Discussion

Regioselective Phase-Transfer Alkylation of the Allopurinol Precursor 2b. Earlier findings have demonstrated that allopurinol (1) is not the appropriate starting material for N-1 alkylation[12] or glycosylation. To diminish the nucleophilicity of the pyrimidine moiety the lactam system was protected by methylation. According to already published procedures the conversion of 1 by treatment with phosphorus oxytrichloride yielded the 4-chloro compound **2a**. Nucleophilic displacement of the halogen by sodium methoxide furnished the methoxy derivative **2b**.[1,13,14]

From our results of alkylation and glycosylation of pyrrolo[2,3-*d*]pyrimidines it was concluded that anion formation and utilization of phase-transfer techniques increase regioselectivity in favor of substitution at the five-membered ring.[15] This prompted us to apply these reaction conditions now on pyrazolo[3,4-*d*]pyrimidines like the heterocyclic chromophore **2b**. Due to its p*K* value for deprotonation of 10.5, anion formation should be even easier than on pyrrolo[2,3-*d*]pyrimidines; however, charge distribution between N-1 and N-2 may result in a lower selectivity of the reaction in pyrazolo[3,4-*d*]pyrimidines compared to pyrrolo[2,3-*d*]pyrimidines.

Alkylation of the chromophore **2b** with methyl iodide was carried out under phase-transfer conditions in a biphasic reaction mixture of benzene and 50% aqueous sodium hydroxide in the presence of tetrabutylammonium hydrogen sulfate as catalyst. Methylation took place under thorough mixing with a vibromixer and was complete after 30 min at room temperature. TLC monitoring indicated the formation of two main reaction products which were separated by silica gel chromatography to yield a faster migrating material (41%) and a slower migrating byproduct (24%).

Methylation of **2b** in the absence of a phase-transfer catalyst required more vigorous reaction conditions (50 °C, 4 h) and was less selective; several byproducts could be detected (TLC, A). The main product obtained from the phase-transfer methylation was identical in all respects



with the N-1 methyl isomer **3**. This has already been described by Cheng and Robins[16] but was synthesized by a different procedure.

For the structural assignment of the unknown isomer the ^{13}C NMR spectrum was measured and compared with those of the N-1 methyl isomer **3** and the parent chromophore **2b**. As Table I shows, methylation of N-1 (compound **3**) results in almost unchanged chemical shifts of nearly all of the chromophore signals, except C-7a was shifted upfield. This shielding is similar to that observed for N-7 methylated pyrrolo[2,3-*d*]pyrimidines.[17] In contrast to this finding the ^{13}C NMR spectrum of the slower migrating isomer shows a significant upfield shift of C-3 (6.4 ppm) and a downfield shift of C-7a (4.8 ppm) compared to the parent compound **2b**. As demonstrated for other heterocycles the upfield shift of a carbon atom in the α-position of an alkylated nitrogen can be used as an unequivocal method of structural proof; β-carbons are shifted downfield under these conditions.[18] Therefore the slower migrating methylation product was assigned to be compound **4**. This assignment was supported by proton-coupled ^{13}C NMR spectra (Table II). While carbon 3 of isomer **3** shows only a $^1J(CH)$ coupling with H-3, an additional $^3J(CH)$ coupling of C-3 with the protons of the methyl group was observed in the spectrum of **4**, indicating N-2 alkylation. Corresponding results are found for C-7a. While the spectrum of compound **3** shows a complex nonresolved multiplet for C-7a due to three $^3J(CH)$ couplings with H-3, H-6, and CH_3, the C-7a signal of **4** exhibits only coupling constants of 13.4 Hz (H-6) and 7.3 Hz (H-3).

The results of phase-transfer methylation of **2b** encouraged us to evaluate regioselective routes for glycosylation[9] and for the synthesis of N-1 carboxyethylated allopurinol derivatives which should be of pharmacological interest due to the carboxylic group which can be coupled to polymer supports, e.g., soluble dextranes.

The alkylation of the chromophore **2b** with an eightfold excess of ethyl 3-bromopropionate was carried out by using

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Table I. ^{13}C Chemical Shifts in ^{1}H-Decoupled NMR Spectra of Pyrazolo[3,4-*d*]- and Pyrazolo[1,5-*a*]pyrimidines[a]

compd	C-3	C-3a	C-4	C-6	C-7a	CH_2N	$CH_2C{=}O$	C=O	CH_2-ester	CH_3-ester	OCH_3	NCH_3
1	134.1	105.6	157.9	147.5	154.4							
2b	131.1	101.2	163.2	154.6	156.1						53.6	
3	130.3	101.5	163.1	154.6	154.1						53.7	33.5
4	124.7	102.1	164.4	154.3	160.9						53.5	38.5
5	133.5	106.2	156.7	147.7	152.2							33.4
6	129.2	107.9	159.5	146.2	158.2							38.3
7a	130.7	101.7	163.1	154.6	154.1	42.4	33.2	169.9	59.7	13.5	53.8	
7b	130.7	101.7	163.2	154.7	154.0	42.6	33.2	171.5			53.8	
7c	131.6	99.9	157.8	155.4	152.8	42.1	33.4	171.5				
8	134.0	105.6	156.9	147.5	151.6	42.6	33.2	171.3				

compd	C-2	C-3	C=O (imide)	C=O (formyl)	C-3a	C-7	C-6	C=O (lactam)
12[b]	138.3	98.2	162.2	163.1	142.7	42.5	29.7	165.7
13[b]	137.6	99.6	164.3		140.9	42.5	29.9	165.4

[a] Values are reported relative to tetramethylsilane. [b] δ values of corresponding carbons in the pyrazolo[3,4-*d*]- and pyrazolo[1,5-*a*]pyrimidine system are listed in the same column.

Table II. Fine Splitting Pattern[a] of Pyrazolo[3,4-*d*]- and Pyrazolo[1,5-*a*]pyrimidines and ^{1}H–^{13}C Coupling Constants in $(CD_3)_2SO$

		3		**4**		**7b**		**12**	
C	H	splittings	*J* (Hz)	splittings	*J* (Hz)	splittings	*J* (Hz)	splittings	*J* (Hz)
3	3	d	195.7	q, d	197.5	d	195.8		
	1′				3.2				
	2							d	5.9
3a	3	d	10.1	d	8.0	d	10.6		
6	6	d	204.7	d	203.3	d	205.0		
7a	6	m	nr	d, d	13.4	t, d, d	12.4		
	3		nr		7.3		3.7		
	1′		nr				1.6		
4	OCH_3	m	nr	m	nr	m	nr		
1′	1′	q	140.8	m	nr	m	nr		
formyl	formyl							d	206.0
2	2							d	190.8

[a] t, d, d: triplet of doublets of doublets; q, d: quadruplet of doublets; m: multiplet; nr: not resolved

phase-transfer catalysis techniques as described for the methylation. The concentration of the catalyst was 75 mol % relative to **2b** in order to assure a rapid transfer of the chromophore with tetrabutylammonium as counterion into the organic layer (benzene). The alkylation proceeded significantly slower than methylation, requiring 60 min for complete reaction. TLC monitoring indicated the formation of one predominant alkylation product. After chromatography, the ester **7a** was obtained in 59% yield. Deesterification of **7a** in EtOH/1 N NaOH (1:1) yielded the acid **7b** in 74% yield.

For an unequivocal structural proof we measured the proton-coupled ^{13}C NMR spectrum of **7b** (Table II). A positive indicator of N-1 alkylation is the fine splitting of the C-7a signal. This carbon shows three $^{3}J(CH)$ couplings: 1, ^{3}J(C-7a/H-6) = 12.4 Hz; 2, ^{3}J(C-7a/H-3) = 3.7 Hz; 3, ^{3}J(C-7a/CH_2N) = 1.6 Hz. The complex pattern of the C-7a signal appears as a double pseudoquintet since two signals coincide because of the incomplete resolution (0.5 Hz). Furthermore, the coupling patterns of C-3, C-3a, and C-6 (Table II) reveal N-1 alkylation.[19]

The fact that the phase-transfer alkylation of **2b** with ethyl 3-bromopropionate leads predominantly to the N-1 alkylated product **7a** whereas methylation yields a 2:1-mixture of the N-1 and N-2 methylated compounds may be due to the lower reactivity of the bromo ester compared to methyl iodide. This is also supported by the shorter reaction time in the case of methylation. Alkylation with the bromo ester leads then in a regioselective way to the thermodynamically most stable N-1 alkylated product.

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In order to obtain 1-(2-carboxyethyl)allopurinol (**8**), acidic and alkaline cleavage of the methoxy group of **7b** was employed. Hydrochloric acid (0.5 N)/*p*-dioxane cleaved the ether function to give compound **8** in 73% yield. However, these reaction conditions could not be applied to *N*-glycosides of 4-methoxyallopurinol since hydrolysis of the N-glycosylic bond took place.[9] We were therefore required to look for alkaline conditions for displacement of the methoxy group.

As has been observed by Lichtenthaler and Cuny,[14] dimethylated allopurinols are labile under alkaline conditions. They undergo opening of the pyrimidine ring to give pyrazolo derivatives. When we employed alkaline conditions (1 N NaOH/MeOH, 1:1) to the monomethyl isomers **3** and **4**, a quantitative conversion to single reaction products was observed. Monitoring of the kinetics by UV spectroscopy (273 nm for **3**; 283 nm for **4**) revealed that the reaction followed first-order kinetics and was complete within 60 h (**3**, $\tau_{1/2}$ = 305 min, $k = 2.28 \times 10^{-3}$ min^{-1}) and 16 h (**4**, $\tau_{1/2}$ = 200 min, $k = 3.57 \times 10^{-3}$ min^{-1}) at room temperature. Preparative-scale experiments resulted in the isolation of the methylated allopurinols **5** and **6** which had been isolated previously from the methylation reaction of allopurinol.[12,14]

Structural proof was provided by ^{13}C and ^{1}H NMR spectra (Table I) which correspond to the data obtained by Lichtenthaler[14] and Bergmann.[12] The monomethyl isomers **5** and **6** are stable in alkaline medium, which is in contrast to the N-1/N-5 or the N-2/N-5 dimethylated allopurinol which suffer ring opening of the pyrimidine moiety. These findings can be explained by the fact that—in contrast to the dimethylated allopurinols—**5** and **6** can form inert anions in alkaline medium.

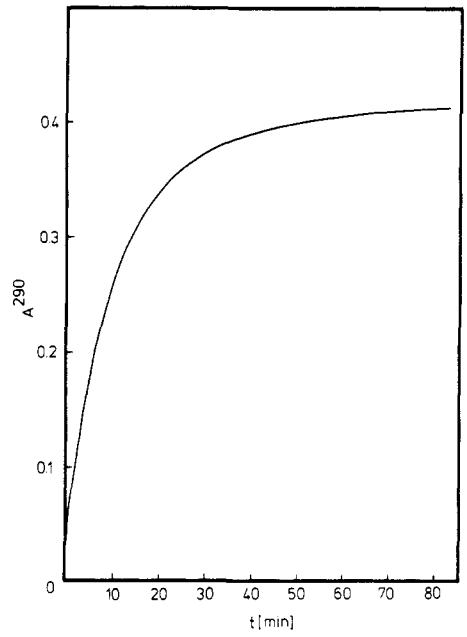
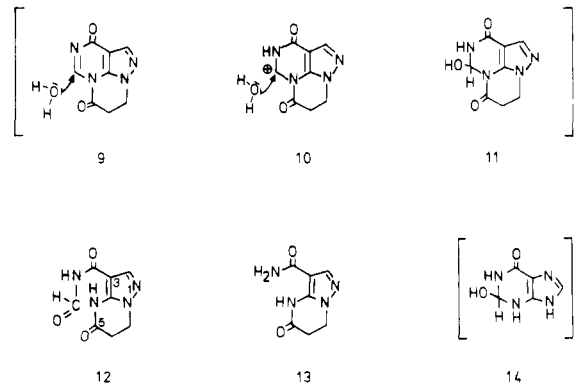


Figure 1. A_{290}–time plot of the reaction of the acid 8 (232 μM) with *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride (EDC, 1 mg) in water/*p*-dioxane (1:1, v/v) at room temperature.

The acid **7b** was now converted under similar conditions as already described for the methyl isomer **3**. Stirring of **7b** in 1 N NaOH for 48 h at room temperature resulted in nucleophilic displacement of the 4-methoxy group and the formation of compound **8** in 74% yield. The yield of this reaction was the same as that obtained for acidic cleavage of **7b** with 0.5 N hydrochloric acid/*p*-dioxane at elevated temperature. The C-4 position of **7b** is highly activated toward nucleophilic attack since treatment of **7b** with an even weaker nucleophile, e.g., concentrated aqueous ammonia (60 h, room temperature) resulted in a complete conversion of **7b** into the acid **7c** in 70% yield.

These nucleophilic displacement reactions of 4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine derivatives offer a simple route for the synthesis of nucleosides of allopurinol as well as of 4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidine via a common intermediate since the acid-labile N-glycosylic bond is stable during the deprotection of the nucleobase in alkaline medium.[9]

Intramolecular Cyclization of 1-(2-Carboxyethyl)-allopurinol (8) and Spontaneous Hydroxylation. Reaction of the acid **8** with *N*-[3-(dimethylamino)-propyl]-*N'*-ethylcarbodiimide hydrochloride (EDC) in water/*p*-dioxane (1:1, pH 5–6) results in the precipitation of a crystalline product which was later identified as **12**. The elemental analysis of this compound was found to be identical with that of the starting material **8** but the ^{13}C and ^{1}H NMR spectra (Table I) as well as the UV spectrum were altered. Figure 1 shows the kinetics of the carbodiimide-mediated reaction of the acid **8** which was followed spectrophotometrically at 290 nm. From the A_{290}–time plot, the half-life of the reaction was estimated to be approximately 5 min. However, the reaction does not show simple first-order kinetics, implying a more complex reaction mechanism. A feasible route would involve the intramolecular cyclization of the activated ester of the acid **8** with the 7-nitrogen of the chromophore and subsequent hydroxylation resulting in the formation of the intermediate **11**. In the pyrimidine ring (formula scheme) the molecule possesses a structure which is analogous to the proposed intermediate of the xanthine oxidase catalyzed oxidation of hypoxanthine. But whereas in the latter case a coupled transfer of a proton and two electrons to a Mo^{VI}=S group of the enzyme occurs,[20,21] in our case oxidation at C-6, e.g., by $K_2Cr_2O_7$, could not be observed. Instead a spontaneous ring opening of the pyrimidine system is favored which leads to the acyclic oxo tautomer **12**. This compound could be isolated in a crystalline state.



This mechanism is underlined by the fact that $NADH^+$ undergoes rapid nucleophilic addition reactions in the nicotinamide moiety.[10,11] The reaction occurs on the reduced state of the molecule having then a paraquinoide structure. Since the direct product of intramolecular cyclization, **9**, possesses a paraquinoid structure it is feasible that hydroxylation of **9** occurs at carbon 6 under formation of **11**. In order to confirm this reaction sequence and its end product **12**, the NMR and mass spectra were taken. The mass spectrum of the pyrazolo[1,5-*a*]pyrimidine **12** shows a stepwise fragmentation pattern which is characteristic for the formylated carboxamide side chain; the *m/e* values are as follows: 180 (M^+ – CO), 163 (M^+ – $HCONH_2$), and 135 (M^+ – HCONHCO – H).

The ^{13}C NMR spectrum of **12** shows the signal of the formyl carbon at 163.1 ppm, which—in the proton-coupled spectrum—appears as a doublet with a coupling constant of 204 Hz due to the $^{1}J(CH)$ coupling with the formyl proton.[22] In addition the C-7a signal (pyrazolo[1,5-*a*]-pyrimidine numbering) reveals a significant upfield shift (8.9 ppm) compared to the acid **8** since it is the carbon in α-position of the acylation site. The C-3 signal is also significantly upfield shifted (7.4 ppm) and appears as a doublet in the proton-coupled ^{13}C NMR spectrum due to the $^{2}J(CH)$ coupling (5.9 Hz) with H-3 (H-2) (Table II). In the ^{1}H NMR spectrum the $^{3}J(HH)$ coupling (8.5 Hz) of the formyl proton at 9.15 ppm with the imide proton can be observed; correspondingly the imide proton appears as a doublet with the same coupling constant at 11.38 ppm. The lactam NH of the pyrimidine ring appears as a singlet at 9.83 ppm. H-D exchange results in a complete disappearance of both NH signals whereas the formyl proton now reveals a singlet.

In order to confirm the structure of **12**, deformylation was carried out in concentrated aqueous ammonia. The reaction was found to be complete within 1 h at room temperature and led to the pyrazolo[1,5-*a*]pyrimidine derivative **13** which could be obtained crystalline. In the ^{1}H NMR spectrum the signals of both methylene groups were almost unchanged compared to **12**, indicating that

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no cleavage of the lactam ring had occurred. The primary amide group reveals two singlets at 7.18 and 7.62 ppm, a fact which may be interpreted by its restricted rotation probably due to an intramolecular hydrogen bond with the lactam NH of the pyrimidine ring. Again the mass spectrum exhibits a characteristic fragmentation pattern of the carboxamide group with m/e values of 163 ($M^+ - NH_2 - H$), 152 ($M^+ - CO$), and 135 ($M^+ - H_2NCO - H$). All these findings confirm that the reaction sequence results in a conversion of a pyrazolo[3,4-*d*]pyrimidine system to a pyrazolo[1,5-*a*]pyrimidine ring.[23]

The spontaneous nonenzymatic hydroxylation of the cyclic intermediate **9** may to some extent serve as a model reaction for the first oxidation step of xanthine oxidase. This enzyme oxidizes hypoxanthine at C-2, yielding xanthine which then undergoes further oxidation at C-8 with the formation of uric acid. In contrast to this hydroxypurine, the corresponding pyrazolo[3,4-*d*]pyrimidines like compound **1** or 7-deazahypoxanthine[24] are exclusively oxidized at C-6 (**1**) or C-2, respectively. By using $^{18}O_2$ in the xanthine oxidase catalyzed hypoxanthine oxidation it has been shown that all the ^{18}O label ends up in $H_2{}^{18}O_2$ and not in the uric acid product. Complementary experiments with $H_2{}^{18}O$ show that water is the source of the hydroxyl group introduced into the product.[25,26] These results imply that the enzyme-catalyzed oxidation of hypoxanthine at C-2 or of allopurinol at C-6 may start with a hydroxylation similar to that of the nonenzymatic hydroxylation of the cyclic intermediate **9**.

The intramolecular acylation of N-7 of the allopurinol derivative **8** generates the paraquinoid intermediate **9**. This or its charged structure **10** are highly reactive and enforce hydroxylation which additionally is facilitated by the electron-withdrawing effect of the carbonyl group of the new lactam ring. The result of this reaction sequence is compound **11** which possesses a sp^3-carbon at C-6, a structure which—in the pyrimidine ring—is analogous to the postulated hypoxanthine oxidation intermediate **14** formed at the active site of xanthine oxidase.[26,27]

In conclusion our findings underline the hypothesis that formation of a paraquinoid structure of the pyrimidine ring is a requirement of the enzymatic hypoxanthine and allopurinol oxidation.[28,29]

Experimental Section

Melting points were determined on a Linström apparatus (Wagner-Munz, West Germany) and are not corrected. Reaction kinetics were assayed on a SuperScan 3 spectrophotometer (Varian, Australia). δ values in NMR spectra are relative to Me_4Si as internal standard for ^1H and ^{13}C nuclei. TLC was performed on silica gel SIL G-25 UV_{254} plates (Macherey-Nagel, West Germany) with solvent systems (A) $CHCl_3$–MeOH (95:5), (B) $CHCl_3$–MeOH (99:1), (C) $CHCl_3$–MeOH (9:1), (D) 0.25 M LiCl. Silica gel 60, 230–400-mesh ASTM (Merck, West Germany), was used for column chromatography with eluants as indicated; ion exchange chromatography was performed on Dowex 1×2, acetate form (Serva, West Germany). The columns were connected to a Uvicord III UV detector and a MultiRac fraction collector (LKB Instruments, Sweden). The pK_a value was determined UV spectrophotometrically at 286 nm in Teorell–Stenhagen buffer.

Allopurinol was purchased from Sigma Chemical Co. (St. Louis, MO) and xanthine oxidase from cow's milk (EC 1.2.3.2) from Boehringer (Mannheim, West Germany).

1-[2-(Ethoxycarbonyl)ethyl]-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (7a). The chromophore **2b**[1,14] (500 mg, 3.33 mmol) suspended in benzene (20 mL) was added to a solution of tetrabutylammonium hydrogen sulfate (700 mg, 2.5 mmol) in 50% aqueous sodium hydroxide (20 mL) and agitated with a vibromixer until all solid material had dissolved. Thereupon ethyl 3-bromopropionate (3.5 mL, 5.1 g, 28.1 mmol) was added and mixing was continued for 60 min. The biphasic mixture was then poured into chloroform (200 mL) containing glacial acetic acid (30 mL). After filtration of the precipitated sodium acetate, the organic layer was washed twice with water, dried over sodium sulfate, and evaporated to dryness. The residue was dissolved in solvent A (10 mL), applied to a silica gel column (30 × 6 cm), and chromatographed (A). From the main peak 495 mg (59%) of pure **7a** was obtained as colorless foam after evaporation and lyophilization from *p*-dioxane: TLC (B) R_f 0.16; TLC (A) R_f 0.76; UV (MeOH) λ_{max} 246 nm (ϵ 7100), 268 (ϵ 4300); ^1H NMR ($(CD_3)_2SO$) δ 1.06 (3 H, t, CH_3-ester; J = 7.1 Hz), 2.95 (2 H, t, $CH_2C{=}O$, J = 6.6 Hz), 3.97 (2 H, q, CH_2-ester, J = 7.1 Hz), 4.09 (3 H, s, OCH_3), 4.62 (2 H, t, CH_2N, J = 6.7 Hz), 8.22 (1 H, s, H-3), 8.59 (1 H, s, H-6). Anal. Calcd for $C_{11}H_{14}N_4O_3$: C 52.79; H, 5.64; N, 22.39. Found: C, 53.76; H, 5.71; N, 22.16.

1-(2-Carboxyethyl)-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (7b). The ester **7a** (460 mg, 1.83 mmol) was dissolved in ethanol–1 N NaOH (1:1, v/v, 40 mL) and stirred for 30 min at room temperature. After dilution with water (50 mL), the solution was neutralized by addition of Amberlit IR-120, H^+ form (glass electrode). The ion-exchange resin was filtered off and thoroughly washed with ethanol–water (1:1). After evaporation to a volume of about 5 mL and addition of a few drops of glacial acetic acid, 300 mg (74%) of **5b** were obtained as colorless needles: mp 197–200 °C; TLC (D) R_f 0.47; TLC (C) R_f 0.33; UV (MeOH) λ_{max} 246 nm (ϵ 6700), 267 (ϵ 4100); ^1H NMR ($(CD_3)_2SO$) δ 2.89 (2 H, t, $CH_2C{=}O$, J = 7.0 Hz), 4.11 (3 H, s, OCH_3), 4.61 (2 H, t, CH_2N, J = 7.0 Hz), 8.23 (1 H, s, H-3), 8.60 (1 H, s, H-6). Anal. Calcd for $C_9H_{10}N_4O_3$: C, 48.65; H, 4.54; N, 25.22. Found: C, 48.79; H, 4.68; N, 24.92.

1,5-Dihydro-1-(2-carboxyethyl)-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (8). Method 1. The acid **7b** (350 mg, 1.58 mmol) was dissolved in 50 mL of *p*-dioxane/15 mL of 0.5 N HCl and refluxed for 5 h. After dilution with water (50 mL), the reaction mixture was neutralized by addition of solid NH_4HCO_3 and evaporated to dryness. After repeated evaporation from aqueous methanol the residue was dissolved in water (10 mL) and **8** was crystallized by addition of glacial acetic acid (1 mL) as colorless needles; 240 mg (73%): mp 262–265 °C; TLC (D) R_f 0.77; UV (H_2O) λ_{max} 252 nm (ϵ 5900); ^1H NMR ($(CD_3)_2SO$) δ 2.84 (2 H, t, $CH_2C{=}O$, J = 7.0 Hz), 4.47 (2 H, t, CH_2N, J = 7.0 Hz), 8.05 and 8.08 (1 H each, s, H-3 and H-6). Anal. Calcd for $C_8H_8N_4O_3$: C, 46.15; H, 3.87; N, 26.91. Found: C, 46.16; H, 3.93; N, 26.91.

Method 2. The acid **7b** (100 mg, 0.46 mmol) was dissolved in 1 N NaOH/MeOH (1:1, v/v, 10 mL) and was stirred for 48 h at room temperature (TLC monitoring). The reaction mixture was applied to a Dowex 1×2 column (acetate form). After extensive washing with water, 10% aqueous acetic acid eluted a main peak from which 72 mg (74%) of pure **8** could be obtained upon evaporation of the solvent. The isolated material was identical with that prepared by method 1 in all respects.

4-Amino-1-(2-carboxyethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (7c). The acid **7b** (50 mg, 0.23 mmol) was dissolved in concentrated aqueous ammonia (10 mL) and stirred for 60 h at room temperature. After evaporation of the solvent the residue was taken up in water and chromatographed on Amberlit XAD, type 4, (Serva, West Germany). Methanol–water (1:4, v/v) eluted one main peak from which 33 mg (70%) of crystalline **7c** was obtained upon evaporation of the solvent to a small volume: mp 252–257 °C; TLC (D) R_f 0.64; UV (MeOH) λ_{max} 260, 277 nm (ϵ 8500, 9400); ^1H NMR ($(CD_3)_2SO$) δ 2.72 (2 H, t, $CH_2C{=}O$, J = 6.9 Hz), 4.38 (2 H, t, CH_2N, J = 6.9 Hz),7.64 (2 H, broad, NH_2), 8.12 and 8.07 (1 H each, s, H-6 and H-3). Anal. Calcd for $C_8H_9N_5O_2$: C, 46.37; H, 4.38; N, 33.80. Found: C, 46.25; H, 4.43; N, 33.92.

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***N*-Formyl-5-oxo-4,5,6,7-tetrahydro-4*H*-pyrazolo[1,5-*a*]-pyrimidine-3-carboxamide (12).** The acid **8** (100 mg, 0.48 mmol) was dissolved in water/*p*-dioxane (1:1, v/v, 20 mL) and *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride (300 mg, 1.56 mmol) was added. After stirring for 12 h at room temperature, 68 mg (68%) of **12** precipitated as colorless needles: mp 278–285 °C. TLC (C) R_f 0.35; UV (MeOH λ_{max} 270 nm (ϵ 12 100); ^{1}H NMR ($(CD_3)_2SO$) δ 2.89 (2 H, t, $CH_2C{=}O$, J = 7.1 Hz), 4.31 (2 H, t, CH_2N, J = 7.2 Hz), 8.09 (1 H, s, H-2), 9.15 (1 H, d, $HC{=}O$, J = 8.5 Hz), 9.83 (1 H, s, lactam NH), 11.38 (1 H, d, imide NH, J = 8.5 Hz); MS (70 eV), m/e (relative intensity) 208 (100, M^+), 180 (83.5, M^+ – CO), 163 (56.5, M^+ – HCONH – H), 135 (82, M^+ – HCONHCO – H). Anal. Calcd for $C_8H_8N_4O_3$: C, 46.15; H, 3.87; N, 26.91. Found: C, 46.18; H, 4.02; N, 26.61.

5-Oxo-4,5,6,7-tetrahydro-4*H*-pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (13). Compound **12** (60 mg, 0.29 mmol) was dissolved in concentrated aqueous ammonia (5 mL) and stirred for 60 min at room temperature. After evaporation of the solvent compound **13** was crystallized from methanol (42 mg, 79%): mp 270–272 °C; TLC (C) R_f 0.15; UV (pH 2) λ_{max} 255 nm (ϵ 12 600); UV (pH 7) λ_{max} 255 nm (ϵ 12 800); UV (pH 10) λ_{max} 280 nm (ϵ 11 800); ^{1}H NMR ($(CD_3)_2SO$) δ 2.86 (2 H, t, $CH_2C{=}O$, J = 7.1 Hz), 4.27 (2 H, t, CH_2N, J = 7.1 Hz), 7.18 and 7.62 (2 H, NH_2), 7.80 (1 H, s, H-2), 9.33 (1 H, s, NH); MS (70 eV), m/e (relative intensity) 180 (100, M^+), 163 (74, M^+ – NH_3), 152 (61, M^+ – CO), 135 (82.5, M^+ – $H_2NC{=}O$ – H), 109 (50.5, 4-pyrazolecarboxamide). Anal. Calcd for $C_7H_8N_4O_2$: C, 46.66; H, 4.48; N, 31.10. Found: C, 46.54; H, 4.43; N, 30.92.

Phase-Transfer Methylation of 4-Methoxy-1*H*-pyrazolo-[3,4-*d*]pyrimidine (2b) with Methyl Iodide. The chromophore **2b** (250 mg, 1.65 mmol) suspended in benzene (20 mL) was added to a solution of tetrabutylammonium hydrogen sulfate (350 mg, 1.03 mmol) in 50% aqueous sodium hydroxide (20 mL) and agitated with a vibromixer until the solid material had dissolved. Thereupon methyl iodide (400 μL, 6.6 mmol) was added and mixing was continued for 30 min at room temperature. The reaction mixture was then poured into chloroform (200 mL) containing glacial acetic acid (30 mL). After filtration of the precipitated sodium acetate, the organic layer was washed twice with water, dried over sodium sulfate, and evaporated to dryness. The residue was dissolved in solvent A (10 mL), applied to a silica gel column (30 × 6 cm), and chromatographed (A). Column chromatography separated two methyl isomers.

4-Methoxy-1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (3). The faster migrating zone afforded the 1-methyl isomer upon evaporation of the solvent in 41% yield (110 mg): mp 104–105 °C (lit.[16] mp 105–106 °C); UV (MeOH) λ_{max} 247 nm (ϵ 6900), 267 sh (ϵ 4200) (lit. λ_{max} 247 nm); TLC (C) R_f 0.9; ^{1}H NMR ($(CD_3)_2SO$) δ 8.55 (1 H, s, H-6), 8.15 (1 H, s, H-3), 4.08 (3 H, s, OCH_3), 3.98 (3H, s, CH_3). The material obtained was identical in all respects with that described by Cheng and Robins.[16]

4-Methoxy-2-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (4). From the slower migrating zone 65 mg (24%) of the 2-methyl isomer was obtained upon evaporation of the solvent: mp 166–169 °C; TLC (C) R_f 0.56; UV (MeOH) λ_{max} 259 nm (ϵ 6500); ^{1}H NMR ($(CD_3)_2SO$) δ 8.59 and 8.51 (1 H each, s, H-3 and H-6), 4.14 (3 H, s, OCH_3), 4.06 (3 H, s, CH_3). Anal. Calcd for $C_7H_8N_4O$: C, 51.21; H, 4.91; N, 34.13. Found: C, 51.29; H, 4.99; N, 34.10.

1,5-Dihydro-1-methyl-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (5). A sample of compound **3** (50 mg, 0.30 mmol) was dissolved in 1 N NaOH/MeOH (1:1, v/v, 5 mL) and stirred at room temperature for 60 h. Desalting was accomplished by ion-exchange chromatography on Dowex 1×2 acetate form (30 × 2.5 cm). Dilute acetic acid (0.5 N) eluted one main peak from which compound **5** (35 mg, 77%) could be obtained upon evaporation of the solvent to a small volume; mp 295–305 °C (lit.[16,12] mp >300 °C); TLC (D) R_f 0.67; UV (1 N NaOH/MeOH, 1:1, v/v) λ_{max} 272 nm (lit.[12] 270 nm); UV (pH 7) λ_{max} 251 nm (lit.[12] 249 nm); ^{1}H NMR ($(CD_3)_2SO$) δ 3.89 (3 H, s, CH_3), 8.06 and 8.02 (1 H each, H-6 and H-3); MS (70 eV), m/e (relative intensity) 150 (100 M^+), 122 (62.5, M^+ – H – HCN), 80 (42.5, M^+ – HCNO – HCN), 68 (52, M^+ – pyrazole moiety), 43 (37, HNCO).

2,5-Dihydro-2-methyl-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (6). Compound **6** was prepared from 10 mg (0.06 mmol) of **4** as described for **5**, except duration of hydrolysis was only 16 h; yield 8 mg (87%): mp >300 °C (lit.[30] mp >300 °C); TLC (D) R_f 0.61; UV (pH 13) λ_{max} 281 nm (lit.[12] 280 nm); UV (pH 7) λ_{max} 257 nm (lit.[12] 255 nm); UV (pH 2) λ_{max} 257 nm (lit.[12] 257 nm); ^{1}H NMR ($(CD_3)_2SO$) δ 4.00 (3 H, s, CH_3), 6.47 (s, OH), 7.90 (1 H, d, H-6, J = 3.4 Hz), 8.45 (1 H, s, H-3); MS (70 eV), m/e (relative intensity) 150 (100, M^+), 44 (80, H + HNCO), 43 (73, HNCO).

Rate Constants of Nucleophilic Displacement Reactions on the Isomers 3 and 4. The reaction mixtures contained per milliliter of solvent (1 N NaOH/MeOH, 1:1, v/v) the isomers **3** and **4**, respectively (**3**, 24 mM; **4**, 22 mM). The reactions were followed spectrophotometrically at 273 nm (**3**) or at 283 nm (**4**); k values were calculated according to $k = (1/t)[\ln (A_0 - A_\infty/A_t - A_\infty)]$.

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