229. Cyclisation of 7-Deazaxanthine-9-propionic Acid to an Active-Site-Directed, Irreversibly Acting Inhibitor of Xanthine Oxidase

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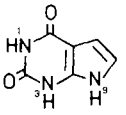
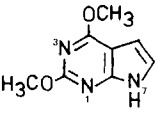
Phase-transfer alkylation of 2,4-dimethoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine (**2**) with ethyl 3-bromopropionate leads regioselectively to ethyl 2,3-dimethoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine-7-propionate (**3a**). After saponification and ether cleavage, the functionalised 7-deazaxanthine **4b** was obtained. Reaction of **4b** with H_2O-soluble carbodiimide resulted in an intramolecular acylation at N(3) with formation of the tricyclic compound **5**. Compound **5** – bearing a reactive lactam ring – is an active-site-directed, irreversibly acting inhibitor of xanthine oxidase from cow's milk.

Introduction. – The 7-deazaxanthine (= 1,3-dihydro-7*H*-pyrrolo[2,3-*d*]pyrimidin-2,4(1*H*,3*H*)-dione; **1**) – a synthetic isostere of xanthine – is a potent competitive inhibitor of xanthine oxidase ($\mathbf{K_i}$ = 0.025 mM) [1]. It is a product of the enzymatic oxidation of 7-deazahypoxanthine which can be considered to be a prodrug for the enzyme.

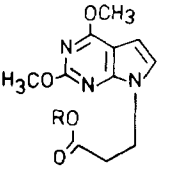
Recently, we were able to show that 7-deazahypoxanthine-9-propionic acid undergoes an intramolecular acylation at N(3) in the presence of H_2O-soluble carbodiimide (purine numbering). The cyclisation is followed by hydroxylation at C(2) and opening of the pyrimidine ring with formation of a pyrrolo[1,2-*a*]pyrimidine [2]. This reaction cascade has been discussed as a nonenzymatic model reaction for the oxidation of hypoxanthine and 7-deazahypoxanthine catalysed by xanthine oxidase [2].



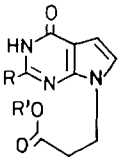
1[a])



2[b])

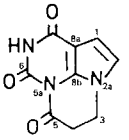


3a[b]) R = C_2H_5
3b[b]) R = H



4[b])

	R	R'
4a	OCH_3	H
b	OH	H
c	OH	C_2H_5



5

[a]) *Purine numbering.* [b]) Systematic numbering.

We have also described a completely analogous reaction sequence (intramolecular acylation→hydroxylation→pyrimidine-ring opening) for the propionic-acid derivatives of allopurinol (= 1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one) [3] as well as of hypoxanthine and 6-mercaptopurine [4].

We now report on the synthesis and reversible intramolecular acylation of 7-deazaxanthine-9-propionic acid to the tricyclic compound **5**. This is structurally analogous to the intermediates which we have hitherto postulated for the above-mentioned ring-transformation reactions, but it does not undergo pyrimidine-ring opening. Compound **5** shows extraordinary inhibitory properties towards xanthine oxidase.

Synthesis and Intramolecular Acylation of 7-Deazaxanthine-9-propionic Acid (4b). – Phase-transfer alkylation of the chromophore **2** with ethyl 3-bromopropionate in a bi-phasic mixture of benzene and 50% aq. NaOH soln. in the presence of Bu_4NHSO_4 as catalyst leads regioselectively to the ester **3a** in 85% yield. From this, the acid **3b** can be obtained by saponification.

In the ^{13}C-NMR spectra of **3a, b** an upfield shift of the C(6) signals (2.6 ppm) compared to **2** and a downfield shift of the C(7a) signals (1.6 ppm) indicates N(7) alkylation *(Table)* [5]. In the ^1H-coupled ^{13}C-NMR spectrum, the C(6) signal of the acid **3b** shows a characteristic *tdd* multiplicity due to ^1J (C(6),*H*–C(6)) = 188.2 Hz, ^2J (C(6),*H*–C(5)) = 7.7 Hz, and ^3J(C(6),CH_2) = 3.9 Hz.

If the acid **3b** is refluxed for 6 h in 1,4-dioxane/0.5N HCl, only the 4-MeO group is cleaved, and the acid **4a** is obtained selectively. Its ^1H- and ^{13}C-NMR spectrum shows only the signal for the 2-MeO group *(Table)*. Even a higher concentration of HCl does not give the acid **4b** but leads to unidentified coloured products within a few minutes. The acid **4a** can also be obtained from **3b** by a nucleophilic displacement reaction of the 4-MeO group by refluxing in 2N aq. NaOH soln. (3 h; TLC control). A substitution of the 2-MeO group does not take place, even under more vigorous conditions. This is due to the anion formation at N(1) of **4a** which prevents it from the attack by hydroxide. Therefore, we cleaved both MeO groups of **3b** by refluxing in 3M HBr/AcOH for 30 min under N_2. The functionalised 7-deazaxanthine **4b** is isolated in 87% yield after hydrophobic chromatography. The ester **4c** is obtained from **3a** by the same route.

Reaction of the acid **4b** with *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride (EDCI) in H_2O (pH 6) results in the precipitation of **5** within a few minutes. Its constitution is proved by its elemental analysis, by an upfield shift (^1H-NMR, 0.4 ppm; ^{13}C-NMR, 1.6 ppm) of the CH_2 group in the α-position of the acyl-CO group, and by the lack of the HN(3) and the CO_2H signals in the ^1H-NMR spectrum. In addition, the structure of compound **5** is confirmed by its ^1H-coupled ^{13}C-NMR spectrum [J(C(2),*H*–C(2)) = 192.9 Hz; J (C(6),*H*–C(1)) = 8 Hz; J(C(1),*H*–C(1)) = 177.6 Hz; J(C(1),*H*–C(2)) = 8 Hz; *t* for C(5) with J(C(5),H–C(4)) = 6.5 Hz].

A bathochromic shift in the UV spectrum of **5** (λ_{max} 293 nm) compared with that of **4b** allows the monitoring of the reaction kinetics by UV *(Fig. 1)*. An isosbestic point between the UV maxima of educt and product indicates a simple reaction mechanism of **4b**→**5** in the presence of a high excess of EDCI.

Compound **5** proves to be relatively labile since it is rapidly hydrolysed in H_2O with formation of **4b** (TLC control). Repeated addition of EDCI to the reaction mixture results in a reconstitution of **5**. *Fig. 2* shows four complete cycles of formation and hydrolysis of the tricycle in the presence of 2 mol-equiv. of EDCI by a continuous

Table. *^{13}C-NMR Chemical Shifts of 7-Deazaxanthine Derivatives in (D_6)Me_2SO*[a])

	C(2)	C(4)	C(4a)	C(5)	C(6)	C(7a)	CH_3O–C(4)	CH_3O–C(2)	CH_2N	CH_2COO	CH_2COO	CH_3CH_2O	CH_3CH_2O
1	151.1	159.9	98.6	102.9	116.5	138.9							
2	161.0	163.4	99.6	97.7	122.4	154.4	52.9	53.6					
3a	161.0	163.6	99.9	97.8	125.1	152.8	53.3	54.0	39.7	33.9	170.6	60.0	13.9
3b	161.1	163.6	100.0	97.7	125.0	152.8	53.3	54.0	40.0	34.3	172.1		
4a	154.8	158.9	102.7	101.4	121.7	147.2		54.3	40.4	34.5	172.0		
4c	150.8	159.2	98.5	102.7	119.4	138.5			40.6	34.2	170.0	59.8	13.6
4b	151.2	159.7	99.0	102.9	119.8	138.8			39.7	34.5	172.0		
	C(6)	C(8)	C(8a)	C(1)	C(2)	C(8b)			C(3)	C(4)	C(5)		
5[b])	145.9	158.0	98.6	103.3	119.8	136.1			40.3	32.9	162.8		

[a]) δ Values given in ppm relative to Me_4Si as internal standard. Systematic numbering.

[b]) δ Values of corresponding C-atoms of both heterocyclic systems are listed in the same column.

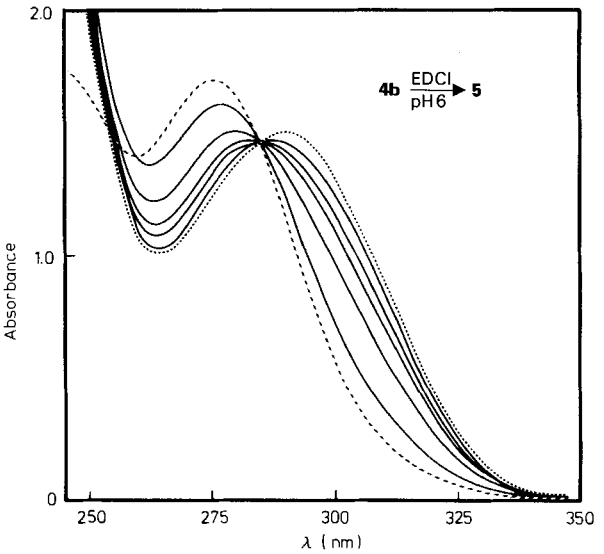


Fig. 1. *Serial overlay of UV spectra during the intramolecular cyclisation of the acid* **4b** *in* H_2O *at 20°*. The reaction mixture (1 ml) contained **4b** (243 μM) and *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride (EDCI; 1 mg, 5.2 mM). **4b** (274 nm); **5** (292 nm).

monitoring of the absorbance at the absorption maximum of the acid **4b** (λ_{max} 274 nm). *Fig. 2* demonstrates the complete reversibility of the intramolecular acylation and excludes an N→O acyl rearrangement of the activated ester moiety of **4b.**

In addition, the half-life values ($\tau_{1/2}$) of both reactions can be estimated from the graph: the apparent $\tau_{1/2}$ value for the formation of **5** amounts to 4.5 min and that for its decomposition to 23 min (25°). This difference in half-life values allows the preparative isolation of compound **5** under the reaction conditions employed *(Fig. 2)*. An increase of the EDCI concentration by a factor of 25 does not significantly affect the $\tau_{1/2}$ value of the cyclisation reaction (4.3 min) but retards the hydrolysis of the tricycle so that one can proceed from an equilibrium between **4b** and **5**.

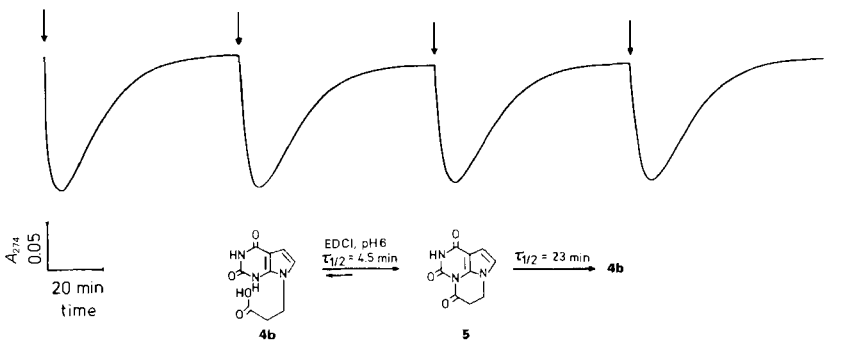


Fig. 2. A_{274}*/time plot of four cycles of the reversible intramolecular cyclisation of* **4b** (100 μM) *in the presence of 2 mol-equiv. of EDCI* (200 μM) *in* H_2O (1 ml) *at 35°*. EDCI (200 μM) is added every 80 min at the points indicated by an arrow.

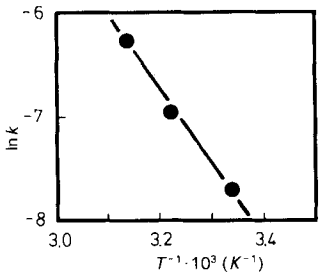


Fig. 3. Arrhenius *plot of the lactam-ring opening of* **5** (40 μM) *in* H_2O

The lability of the lactam ring in **5** is also reflected by the relatively low activation energy necessary for the ring opening. From an *Arrhenius* plot *(Fig. 3)* of the hydrolysis of compound **5**, an E_a value of 24 kJ · mol^{-1} can be taken.

The intramolecular cyclisation of the acid **4b** implies a comparison with the corresponding intramolecular acylation of *N*-propionic acid derivatives of hypoxanthine, 7-deazahypoxanthine, and allopurinol [2–4]. In these cases, the high reactivity at C(2) which is due to the paraquinoid structure of the pyrimidine ring after the acylation enforces a nucleophilic attack of a H_2O molecule at this C-atom; the hydroxylation is then followed by an opening of the pyrimidine ring. In the present case, only the first reaction step, *i.e.* the intramolecular acylation of the acid **4b** with formation of the tricyclic-ring system **5**, is possible – a ring system which we hitherto have postulated as an intermediate of the above-described ring transformations.

The spontaneous hydroxylation of 7-deazahypoxanthine-9-propionic acid as well as of its isosteres at C(2) after intramolecular acylation has been discussed as a nonenzymatic model reaction for the first step of the enzymatic oxidation of hypoxanthine by xanthine oxidase [2]. The reason for this is the fact that experiments with $^{18}O_2$ and $H_2{}^{18}O$ have unambiguously shown that also the enzymatic oxidation of the natural substrate starts with a hydroxylation at C(2) [6] [7]. Therefore, in both cases, intermediates can be assumed which show a structural analogy in the pyrimidine ring. To obtain the essential electrophilicity at C(2), necessary for a hydroxylation at this position, *Bergmann* and *Levene* postulated a tautomeric shift of the H-atom at N(1) to N(3) by an acceptor group at the active site of xanthine oxidase [8]. Extending this model, very recently *Robins et al.* proposed the coordination of the non-binding electron pairs of N(3) and N(9) of hypoxanthine at an electron acceptor, in detail at a reduced pterin-Mo(VI) enzyme cofactor [9]. This leads obviously to an enhanced electrophilicity at C(2) and enables a hydroxylation at this position. A similar effect can also be produced by other electron acceptors, *e.g.* by an acyl group. After hydroxylation and subsequent oxidation at C(2) under reduction of Mo(VI) to Mo(IV), the bond between N(3) and the electron acceptor is destabilised because of the additional electron-withdrawing effect of the C(2) = 0 group. This allows a simple release of the product xanthine from the active center of xanthine oxidase. From the results obtained until now, it cannot be decided what particular electron acceptor is involved at the active site. Nevertheless, our findings strongly suggest that a mechanism of a type as discussed above occurs during hypoxanthine oxidation.

Irreversible Inhibition of Xanthine Oxidase.– Preliminary experiments showed that the tricyclic compound **5** has extraordinary inhibitory properties towards xanthine oxi-

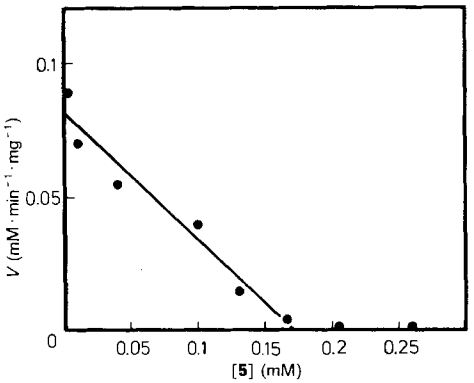


Fig. 4. *Activity* V [mM · min^{-1} · mg^{-1}] *of xanthine oxidase as a function of the concentration of* **5**

dase. In order to quantify the enzyme deactivation, xanthine oxidase (50 μg/ml) is incubated with varying amounts of **5** (0–260 nmol/ml) in H_2O at 35° for 40–60 min. During this time, the opening of the lactam ring is monitored by continuous measurement of A_{274}. Subsequent addition of hypoxanthine (69 μM) allows the determination of the residual activity V [mM · min^{-1} · mg^{-1}] of the enzyme by measuring the uric-acid production at 293 nm according to *Kalckar* [10]. *Fig. 4* shows the xanthine-oxidase activity in H_2O as a function of the concentration of **5**. The graph shows a linear decrease of the activity of the enzyme between 0 and 170 nmol of **5**/ml. Above this concentration, no residual activity can be observed. A blank experiment without inhibitor clearly demonstrates that the deactivation of xanthine oxidase is not due to a heat effect by incubation at 35°.

A 4-fold increase of the substrate concentration (280 μM), after complete inactivation of the enzyme, does not result in a partial reactivation so that one can proceed from a quasi-irreversible inhibition of xanthine oxidase by compound **5** – a fact which is underlined by the linearity of the graph in *Fig. 4* [11].

The reactivity of the lactam ring of **5** implies that the inactivation of the enzyme may be due to the formation of a covalent complex between the heterocycle and the protein.

In **5** the reactive lactam ring is annellated to the pyrrolo[2,3-*d*]-pyrimidine *via* non-essential positions with regard to its binding to xanthine oxidase. This has recently been proved by using all conceivable monomethyl isomers of 7-deazahypoxanthine in a kinetic model study for a probing of the regiochemical requirements of the active center [1]. These results make it likely that the tricycle **5** is active-site directed through the 7-deazaxanthine moiety of the molecule.

In order to confirm whether or not chemical modification of xanthine oxidase by compound **5** is occurring at the catalytic center, a protection experiment was run: For this purpose, compound **5** (44 μM and 165 μM, respectively) and hypoxanthine as substrate (69 μM) are incubated simultaneously with xanthine oxidase (50 μg/ml) at 35°. The remaining enzyme activities, measured by the method of *Kalckar*, amount to 95 and 32%, respectively. Since the nominal values of residual activity are 73 and 0% *(Fig. 4)*, the conclusion can be drawn that a significant protection of the catalytic site from covalent modification by compound **5** can be provided by equal amounts of substrate. This means that the reaction of **5** with xanthine oxidase is active-site directed and may allow further elucidation of the structure of the catalytic center.

Experimental Part

General. The 2,4-dimethoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine (**2**) was prepared according to [12]; *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide (EDCI) was purchased from *Sigma Chemical Co.* (St. Louis, USA). TLC: silica gel *Sil G-25* UV_{254} plates (*Macherey-Nagel & Co.*, Düren, FRG); solvent systems: $CHCl_3$ (A), $CHCl_3$/MeOH 95:5 (B), $CHCl_3$/MeOH 9:1 (C), $CHCl_3$/MeOH 85:15 (D), $CHCl_3$/MeOH 8:2 (E), 0.25 M LiCl (F). Column chromatography: silica gel *60* (230–400 mesh ASTM, *Merck*, Darmstadt, FRG) and *Amberlite-XAD-4* resin (*Serva*, Heidelberg, FRG); the columns were connected to an *Uvicord-S* detector and an *UltroRac* fractions collector (*LKB Instruments*, Bromma, Sweden). M.p.: *Linström* apparatus (*Wagner & Munz*, FRG); not corrected. UV spectra (λ_{max} (ε) in nm): *Uvikon 810* spectrometer (*Kontron*, Switzerland); reaction and enzyme kinetics were performed on a *Varian SuperScan 3* spectrometer (*Varian*, Australia) equipped with thermostatted quartz cuvettes. NMR spectra: *Bruker WM 250* spectrometer; δ's are relative to Me_4Si as internal standard for ^1H and ^{13}C (memory size: 16 K; digital resolution: ^1H, 0.6 Hz; ^{13}C, 2 Hz; ^{13}C (^1H-coupled): 2000 Hz; digital resolution: 0.25 Hz). Elemental analyses were performed by *Mikroanalytisches Labor Beller* (Göttingen, FRG).

*Ethyl 2,4-Dimethoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine-7-propionate* (**3a**). Compound **2** (500 mg, 2.79 mmol) suspended in benzene/ethylene glycol dimethyl ether 1:1 (*v/v*; 20 ml) was added to a soln. of Bu_4NHSO_4 (85 mg, 0.25 mmol) in 50% aq. NaOH soln. (20 ml) and the mixture agitated with a vibromixer for 5 min. Thereupon, ethyl 3-bromopropionate (3.5 ml, 5.1 g, 28.1 mmol) was added, and mixing was continued for 30 min. After separation of the org. layer and repeated extraction of the aq. layer with benzene, the combined org. layers were washed with H_2O, dried over Na_2SO_4, and evaporated to dryness. The crude product was adsorbed on silica gel (5 g), applied to the top of a silica-gel column (3.5 × 40 cm) and chromatographed. $CHCl_3$ eluted a main zone from which 660 mg (85%) of amorphous **3a** was obtained. TLC (silica gel, A): R_f 0.25. UV (MeOH): 260 (5800), 274 (6300). ^1H-NMR ($(D_6)Me_2SO$): 1.11 (*t*, *J* = 7, CH_3CH_2O); 2.87 (*t*, *J* = 7, $CH_2C=O$); 3.91 (*s*, CH_3O); 3.99 (*s*, CH_3O); 4.0 (*q*, *J* = 7, CH_3CH_2O); 4.35 (*t*, *J* = 7; CH_2N); 6.37 (*d*, *J* = 3.5, H–C(5)); 7.17 (*d*, *J* = 3.5, H–C(6)). Anal. calc. for $C_{13}H_{17}N_3O_4$:C 55.90, H 6.14, N 15.05; found: C 55.77, H 6.18, N 15.03.

*2,4-Dimethoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine-7-propionic Acid* (**3b**). Ester **3a** (1 g, 3.58 mmol) was dissolved in EtOH/1N NaOH 1:1 (40 ml) and stirred for 30 min at r.t. After dilution with H_2O (50 ml), the soln. was neutralised by addition of *Amberlite IR-120* (H^+ form) at a glass electrode. The ion-exchange resin was filtered off and washed with H_2O/EtOH. After evaporation, the residue was dissolved in H_2O (10 ml), and **3b** was crystallised by addition of AcOH: 798 mg (88%) of colourless needles, m.p. 134–135° (H_2O) TLC (silica gel, C): R_f 0.32 TLC (silica gel, D): R_f 0.61. UV (MeOH): 272 (7100), 260 (6600), 220 (27600). ^1H-NMR ($(D_6)Me_2SO$): 2.82 (*t*, *J* = 7, $CH_2C=O$); 3.93 (*s*, CH_3O); 4.0 (*s*, CH_3O); 4.36 (*t*, *J* = 7, CH_2N); 6.38 (*d*, *J* = 3.5, H–C(5)); 7.18 (*d*, *J* = 3.5, H–C(6)). Anal. calc. for $C_{11}H_{13}N_4O_3$: C 52.39, H 5.57, N 16.66; found: C 52.55, H 5.30, N 16.52.

*4,7-Dihydro-2-methoxy-4-oxo-*3H*-pyrrolo[2,3-*d*]pyrimidin-7-propionic Acid* (**4a**). Acid **3b** (150 mg, 0.59 mmol) in 50 ml of 1,4-dioxane/15 ml of 0.5N HCl was refluxed for 6 h. Thereupon, the soln. was neutralised by addition of solid NH_4HCO_3, filtered, and evaporated. After repeated evaporation from aq. MeOH, the residue was dissolved in 0.1N HCl (250 ml) and desalted by hydrophobic chromatography on *Amberlite-XAD* (1.5 × 25 cm). After washing with 0.1N HCl (250 ml) and H_2O, elution with 60% aq. MeOH afforded 1 main zone from which 80 mg (58%) of crystalline **4a** were obtained, m.p. 230–232° (dec., H_2O) TLC (silica gel, D): R_f 0.42. UV (MeOH): 270 (7300), 257 (9100), 217 (22600). ^1H-NMR ((D_6) Me_2SO): 2.76 (*t*, *J* = 7, $CH_2C=O$); 3.92 (*s*, CH_3O); 4.24 (*t*, *J* = 7, CH_2N); 6.31 (*d*, *J* = 4, H–C(5)); 6.91 (*d*, *J* = 4, H–C(6)); 11.7 (*s*, NH). Anal. calc. for $C_{10}H_{11}N_3O_4$: C 50.63, H 4.67, N 17.72; found: C 50.49, H 4.59, N 17.75.

*2,3,4,7-Tetrahydro-2,4-dioxo-1*H*-pyrrolo[2,3-*d*]pyrimidin-7-propionic Acid* (**4b**). Acid **3b** (200 mg, 0.79 mmol) was dissolved in 3M HBr/AcOH (10 ml) and refluxed for 30 min under N_2. After cooling and dilution with H_2O (50 ml), the pH was adjusted to 4–5 by adding solid NH_4HCO_3, the mixture filtered, and the soln. evaporated. After repeated evaporation from aq. MeOH, the residue was dissolved in 2N HCl (250 ml) and desalted as described for **4a**. Elution with 15% aq. MeOH afforded 1 main zone from which 154 mg (87%) of crystalline **4b** was obtained, m.p. 268–270° (H_2O) TLC (silica gel, F): R_f 0.76. UV (MeOH/1N NaOH 1:1): 285 (7300), 255 (10500). ^1H-NMR ($(D_6)Me_2SO$): 2.66 (*t*, *J* = 7, $CH_2C=O$); 4.15 (*t*, *J* = 7, CH_2N); 6.24 (*d*, *J* = 4, H–C(5)); 6.68 (*d*, *J* = 4, H–C(6)); 10.54 (*s*, H–N(1)); 11.56 (*s*, H–N(3)); 12.11 (*s*, CO_2H). Anal. calc. for $C_9H_9N_3O_4$: C 48.43, H 4.06, N 18.83; found: C 48.34, H 4.22, N 18.95.

*Ethyl 2,3,4,7-Tetrahydro-2,4-dioxo-1*H*-pyrrolo[2,3-*d*]pyrimidin-7-propionate* (**4c**). Ester **3a** (600 mg, 2.15 mmol) was dissolved in 3M HBr/AcOH (30 ml) and refluxed for 1.5 h under N_2. After cooling and dilution with H_2O the pH was adjusted to 4–5 by addition of solid NH_4HCO_3. After evaporation, the residue was taken up in MeOH, filtered, and chromatographed on silica gel (2.5 × 45 cm). Elution with $CHCl_3$MeOH 95:5 gave 1 main zone. Concentration of the soln. to 5 ml afforded 305 mg (55%) of **4c** as colourless needles, m.p. 208–212° (MeOH)

TLC (silica gel, B): R_f 0.2 TLC (silica gel, C): R_f 0.4 TLC (silica gel, E): R_f 0.76. UV (0.01N NaOH): 285 (7100), 255 (9800). ^{1}H-NMR ($(D_6)Me_2SO$): 1.14 (*t*, *J* = 7, CH_3CH_2O); 2.74 (*t*, *J* = 7, $CH_2C=O$); 4.04 (*q*, *J* = 7, CH_3CH_2O); 4.18 (*t*, *J* = 7, CH_2N); 6.24 (*d*, *J* = 3.5, H–C(5)); 6.67 (*d*, *J* = 3.5, H–C(6)); 10.55 (*s*, H–N(1)); 11.57 (*s*, H–N(3)). Anal. calc. for $C_{11}H_{13}N_3O_4$: C 52.58, H 5.21, N 16.73; found: C 52.58, H 5.20, N 16.75.

2aH,3H,5aH,6H-2a,5a,7-Triazaacenaphtylene-5,6,8(4H,7H)-trione (**5**). Acid **4b** (100 mg, 0.45 mmol) was dissolved in H_2O (25 ml), and EDCI (300 mg, 1.56 mmol) was added in 1 portion. After shaking the mixture for *ca.* 45 min at r.t., the precipitate was filtered off, washed with cold H_2O, and dried: 85 mg (92%) of crystalline **5**, m.p. 258–260°. UV (H_2O): 293 (5000). ^{1}H-NMR ($(D_6)Me_2SO$): 3.04 (*t*, *J* = 7, H–C(3)); 4.25 (*t*, *J* = 7, H–C(4)); 6.37 (*d*, *J* = 3.5, H–C(1)); 6.84 (*d*, *J* = 3.5, H–C(2)); 10.9 (*s*, H–N(7)). Anal. calc. for $C_9H_7N_3O_3$: C 52.69, H 3.44, N 20.48; found: C 52.78, H 3.52, N 20.48.

Enzyme Kinetics. The enzyme-inhibition experiments were performed at 35° in thermostatted 1-ml quartz cuvettes (1-cm path length). The reaction mixtures contained per ml of H_2O (pH 6) 0.05 mg of xanthine oxidase from cow's milk (EC 1.2.3.2) and **5** at 7 different concentrations ranging from 9.2 to 260 μM. After incubation for 40–60 min, the residual activity *V* [$mM \cdot min^{-1} \cdot mg$ of $protein^{-1}$] was determined by addition of hypoxanthine as substrate (69 μM). The oxidation was followed at 293 nm (uric-acid production) according to the method of *Kalckar* [10]. Reaction rates were calculated by using the molar absorption coefficient at that wavelength (ε_{293} for uric acid: 12500 $M^{-1}cm^{-1}$).

The protection experiments were carried out according to the following protocol: hypoxanthine (69 μM) was incubated together with **5** (44 μM or 165 μM, resp.). The oxidations were started by addition of 0.05 mg of xanthine oxidase and the enzyme activities determined as described above. The activity of a blank experiment without inhibitor (0.082 $mM \cdot min^{-1} \cdot mg^{-1}$) was set as 100%.

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