

Full Paper

## Symmetrically and Unsymmetrically Bridged Methylenebis(allopurinols): Synthesis of Dimeric Potential Anti-Gout Drugs

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**Abstract:** Liquid-liquid phase transfer alkylation of 4-methoxy-pyrazolo[3,4-*d*]-pyrimidine (**1a**) with a dichloromethane/dibromomethane mixture (3:1, v/v) gave the regioisomeric methylenebis(heterocycles) **3a–5a**. These were converted by dilute aqueous sodium hydroxide containing dimethylsulfoxide (DMSO) at concentrations between 0 and 60 vol-% into the methylenebis(allopurinols) **3b–5b** by nucleophilic S<sub>N</sub>Ar reactions at C(4). The effect of DMSO on the reaction kinetics was investigated.

**Keywords:** Allopurinol, dimethylsulfoxide, dimeric drugs.

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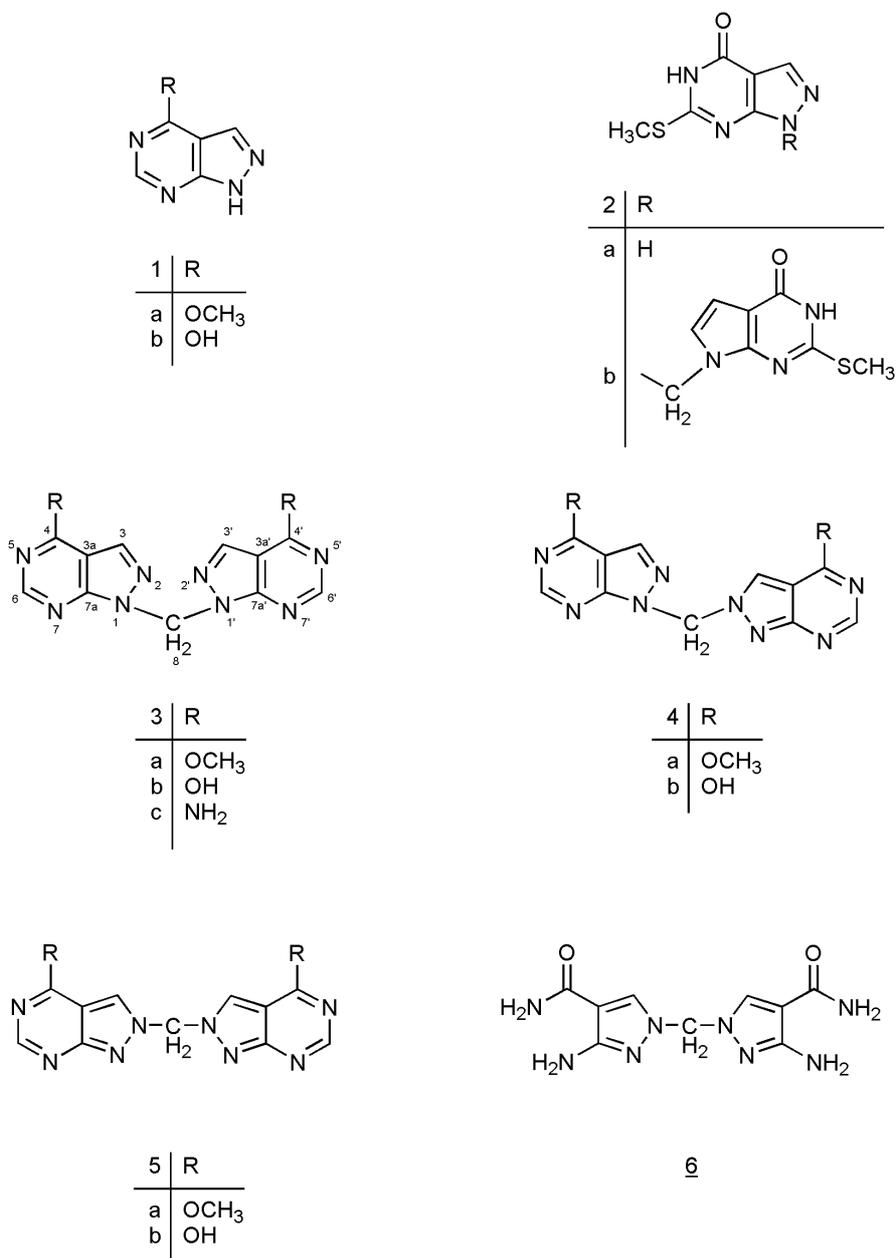
### Introduction

1,5-Dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidine = 8-aza-7-deazahypoxanthine (allopurinol, **1b**, Figure 1; IUPAC numbering is used throughout the manuscript) [1,2] is a progressive inhibitor of xanthine oxidase with alloxanthine – a 6-oxo derivative of **1b** – being 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 of its 4-amino analogue is augmented by their effects on pyrimidine and purine biosynthesis [5,6].

We have previously reported on the inhibitor properties of pyrrolo[2,3-*d*]pyrimidines (*i.e.* 7-deazapurines) towards xanthine oxidase (from cow's milk) [7]. In this context we now disclose that 2-methylthio-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (**2a**) [8,9] is also a moderately active competitive inhibitor of xanthine oxidase, with an inhibition constant ( $K_i$ ) of 120  $\mu\text{M}$ . A corresponding dimeric inhibitor was obtained when two 4-methoxy-2-methylthio-7*H*-pyrrolo[2,3-*d*]pyrimidine molecules were linked by a methylene bridge *via* liquid-liquid phase-transfer-catalyzed alkylation of the latter using dichloromethane, followed by hydrolysis of the methoxy groups with dilute hydrochloric acid ( $\rightarrow$  **2b**) [10]. The methylenebis(heterocycle) **2b** was also tested as a xanthine oxidase inhibitor and found to exhibit a  $K_i$  value of 6  $\mu\text{M}$ , which is 20-fold more potent than the corresponding  $K_i$  of the monomeric heterocycle **2a**.

In this paper we now report the synthesis of methylenebis(allopurinols) **3b–5b** applying nucleophilic substitution reactions ( $S_NAr$ ) on the corresponding 4-methoxy precursors **3a–5a**, as well as the influence of dimethylsulfoxide on the reaction kinetics.

**Figure 1.** Chemical structures of novel methylenebis(heterocycles).



## Results and Discussion

4-Methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (**1a**) [1,11,12] has been proven as a versatile heterocycle for the synthesis of alkylated [13] and glycosylated [14] pyrazolo[3,4-*d*]pyrimidines, while allopurinol has not [15]. In phase-transfer-catalyzed reactions this lactam-protected chromophore can be alkylated exclusively at N(1) and N(2), while reactions on the pyrimidine ring do not take place.

The methoxy group can subsequently undergo S<sub>N</sub>Ar nucleophilic displacement reactions, which are particularly advantageous for the synthesis of N(1)- and N(2)-glycosylated allopurinol nucleosides [13,16,17]. Cleavage of the methoxy function with acid always bears the risk of concomitant N-glycosylic bond cleavage. In a number of methylation and glycosylation reactions of pyrrolo[2,3-*d*]pyrimidines under liquid-liquid phase-transfer catalysis conditions with dichloromethane as solvent we observed a regioselective bridging of two chromophore moieties via N(7) by a methylene bridge [10]. This reaction is then always favored when the alkyl halide is almost inert.

In contrast to this, the reaction of the heterocycle **1a** with dichloromethane proceeded very slowly with formation of three reaction products. However, when dibromomethane was added to the reaction mixture compound **1a** had disappeared completely with 60 min. Silica gel chromatography separated three methylenebis(4-methoxy-pyrazolo[3,4-*d*]pyrimidines) in a 8:16:1 ratio. Comparison of their <sup>1</sup>H- and <sup>13</sup>C-NMR spectra with those of **1a**, as well as with those of its N(1)- and N(2)-methylated derivatives, revealed that the fastest migrating product is the N(1)-N(1) bridged compound **3a**, as only one set of NMR signals could be observed. The second zone contained the unsymmetrically bridged compound **4a**. In this case, the NMR spectra showed two sets of signals – one for the N(1)- and a second one for the N(2)-alkylated chromophore. The slowest migrating zone contained the N(2)-N(2) bridged compound **5a** in low yield. This may be due to the low reactivity of the 2-nitrogen compared to N(1).

**Table 1.** [<sup>1</sup>H-<sup>13</sup>C] Coupling constants of compounds **3a–5a**<sup>a</sup>.

Compound		<b>3a</b>	<b>4a</b> <sup>b</sup>	<b>5a</b>
C-6	H-C(6)	205.5	204.1/205.6	204.1
C-3	H-C(3)	195.9	195.9/196.2	197.6
C-3	CH <sub>2</sub>	-	3.0	3.0
C-3a	H-C(3)	10.1	7.2/10.4	8.0
C-7a	CH <sub>2</sub>	n. r.	-	-
C-7a	H-C(6)	n. r.	-	12.8
C-7a	H-C(3)	n. r.	-	6.8
<u>CH</u> <sub>2</sub>	<u>CH</u> <sub>2</sub>	155.4	157.3	156.0
CH <sub>2</sub>	H-C(3)	-	-	~ 2

a) Measured in DMSO-D<sub>6</sub> at room temperature.

b) The first value refers to the N(2)-alkylated and the second to the N(1)-alkylated chromophore of compound **4a**.

For unequivocal structural proof proton-coupled  $^{13}\text{C}$ -NMR spectra of the three regioisomers **3a–5a** were obtained (Table 1). While C(7a) of compound **3a** appears as a complex multiplet, due to three  $^3J(\text{C,H})$  couplings with H-C(6), H-C(3) and  $\text{CH}_2$ , the corresponding C(7a) of compound **5a** shows only two  $^3J(\text{C,H})$  couplings with H-C(6) and H-C(3) (12.8 and 6.8 Hz, respectively). Correspondingly, the  $\text{CH}_2$  groups of both isomers exhibit different coupling patterns: the multiplicity of  $\text{CH}_2$  of **3a** is a triplet ( $^1J(\text{C,H}) = 155.4$  Hz), while that of **5a** shows a triplet of triplets due to an additional  $^3J(\text{C,H})$  coupling with both H-C(3) atoms. In addition, the C(3) signal of the isomer **5a** exhibits an additional fine splitting compared with that of **3a**, due to a long range coupling ( $^3J(\text{C,H})$ ) with its methylene group. All these data confirmed the proposed structures of compounds **3a** and **5a** [18].

In the gated-decoupled  $^{13}\text{C}$ -NMR spectrum of the unsymmetrically bridged isomer **4a** the coupling patterns of both chromophore moieties behave additively. A positive indicator for the correct structure is the fact that only the C(3) signal of the N(2)-alkylated moiety shows an additional fine splitting due to a  $^3J(\text{C,H})$  coupling with  $\text{CH}_2$  (3 Hz) while the corresponding C(3) signal of the N(1)-alkylated chromophore moiety exhibits only a doublet ( $^1J(\text{C,H}) = 196.2$  Hz).

Nucleophilic displacement of the 4-methoxy groups of the methylenebis(4-methoxy-pyrazolo[3,4-*d*]pyrimidines) **3a–5a** by 1N NaOH/MeOH (1:1, v/v) gave the corresponding methylenebis(allopurinols) **3b–5b** (Figure 1). Their structures were confirmed by UV- and  $^1\text{H}$ -NMR spectroscopy as well as by elemental analyses. The different UV spectra of educts and products allow a simple quantitative spectrophotometric monitoring and the determination of pseudo first-order kinetic constants (half life  $\tau$  and  $k$ ) of the  $\text{S}_{\text{N}}\text{Ar}$  reactions (Table 2). As can be seen from Table 2 the reaction rates of  $\text{S}_{\text{N}}\text{Ar}$  reactions of the methylenebis(4-methoxy-pyrazolo[3,4-*d*]pyrimidines) **3a–5a** are significantly faster than those of the N(1)- and N(2)-methylated compounds. In case of the N(2)-N(2)-bridged derivative the half life is one order of magnitude higher than for the N(2)-methylated chromophore.

**Table 2.** Pseudo first order kinetic data of nucleophilic  $\text{OCH}_3$  group displacement by hydroxyl of 4-methoxy-pyrazolo[3,4-*d*]pyrimidine derivatives.

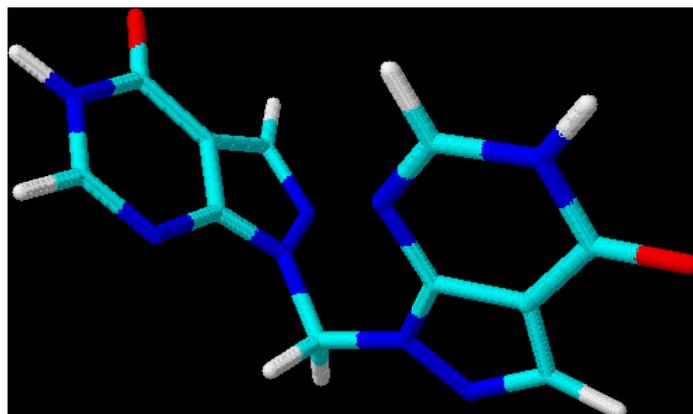
Reaction conditions	1N NaOH/MeOH (1:1, v/v)		0.5 N NaOH			
	half life $\tau$ [min]	$k$ [ $\text{min}^{-1} \times 10^3$ ]	half life $\tau$ [min]	$k$ [ $\text{min}^{-1} \times 10^3$ ]	$\lambda^{\text{a}}$ [nm]	$m^{\text{b}}$
N(1)-methylated <b>1a</b>	305	2.28	95	7.3	273	8.8
N(2)-methylated <b>1a</b>	200	3.57	134	5.2	283	7.9
<b>3a</b>	145	4.81	90	7.7	273	8.8
<b>4a</b>	48	14.4	-	-	273	7.3
<b>5a</b>	20	34.6	-	-	283	7.9

a) Wavelength for spectrophotometric monitoring of the nucleophilic displacement reactions.

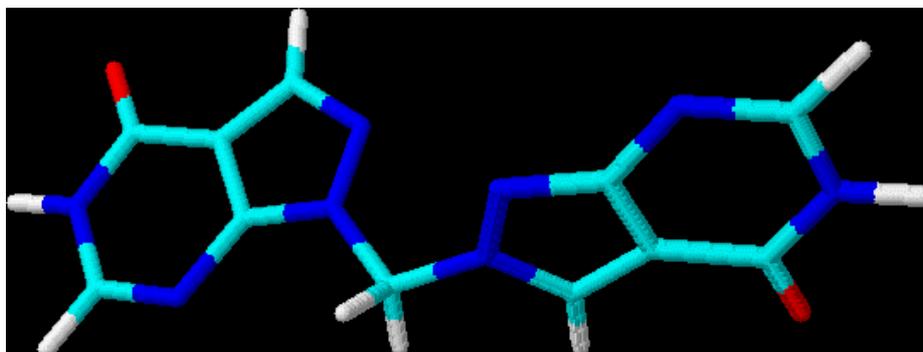
b)  $m$  = sensitivity of the substrates to the dimethylsulfoxide content in the varying solvent compositions (1N NaOH/DMSO) according to equation 1 (correlation coefficients of linear regression,  $r^2$ , better than 0.99).

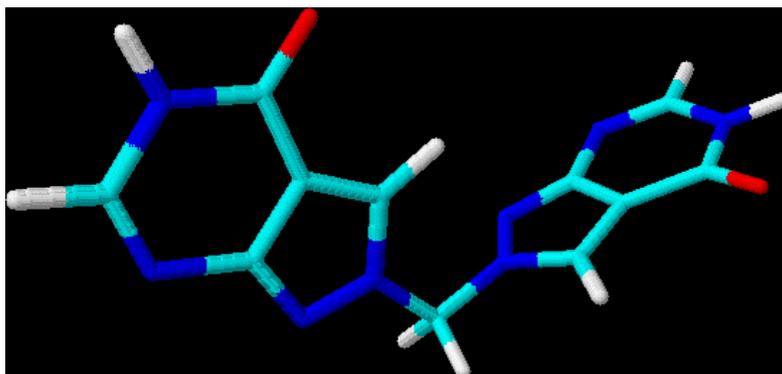
This leads to the assumption that a strong interaction exists between both chromophore moieties, probably caused by an orbital overlap of the two heterocyclic  $\pi$ -electron systems separated by the non-conjugating methylene group (homoconjugation). In this context it is interesting to see that for all three regioisomeric methylenebis(allopurinols) 3D-optimized molecular models [19] display structures with heterocyclic planes which are perpendicular to each other (Figures 2-4, Table 3); the heterocycles are not stacked one upon the other, as it has been shown for ethylenebis- or higher alkyl analogues [20,21]. A mutual (-M) – effect of one chromophore on the other leads to an increase in electrophilicity at C(4), compared with the methylated heterocycles and, therefore, to a higher reaction rate for displacement reactions by hydroxyl. Even a weaker nucleophile such as ammonia (25 % aq.  $\text{NH}_3$ ) is able to substitute the 4-methoxy group of compound **3a**, yielding compound **3c**. If the nucleophilic displacement reaction of the methoxy group by hydroxyl on compound **5a** was extended for more than 1 h, an opening of the pyrimidine rings at C(6) was observed, and small amounts of compound **6** were formed [22]. If the nucleophilic displacement reactions of **3a–5a** are performed in a methanol-free medium (0.5 N NaOH), all reaction rates are enhanced (Table 3) which points to a weaker solvation of the reaction intermediates by methanol-water mixtures compared to pure water.

**Figure 2.** 3D-Optimized structure of compound **3b** [19].



**Figure 3.** 3D-Optimized structure of compound **4b** [19].

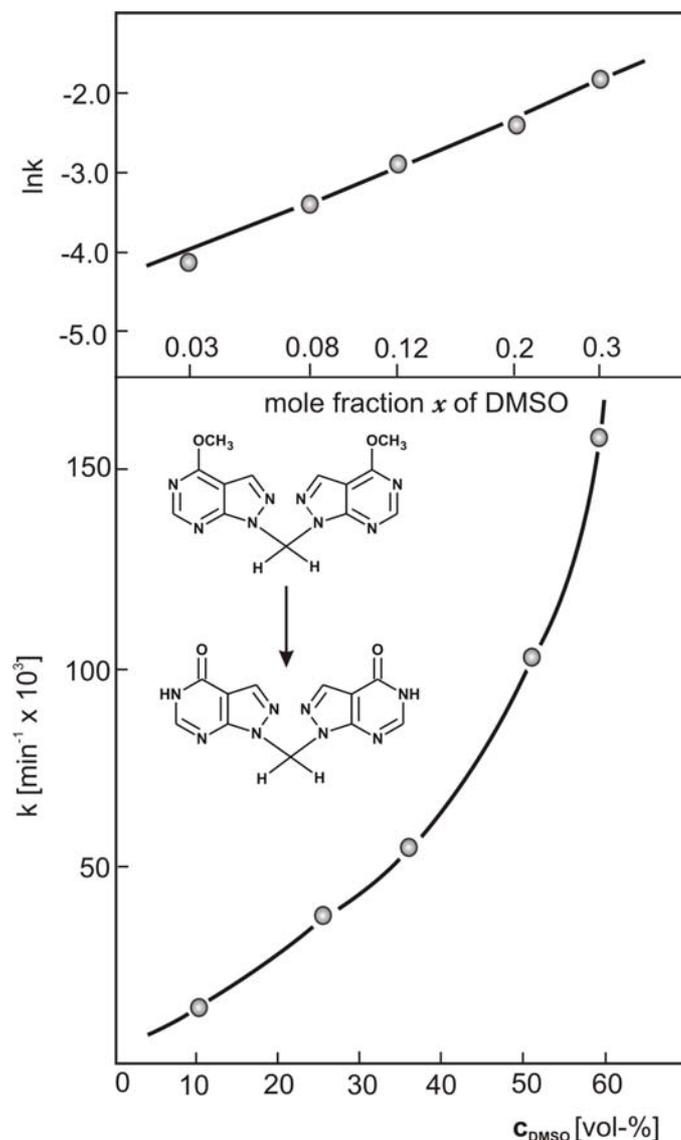


**Figure 4.** 3D-Optimized structure of compound **5b** [19].**Table 3.** Selected torsion and bond angles as well as inter-moiety N-N distances (Å) of compounds **3b–5b**.

Compound	torsion and bond angles [deg]; inter-moiety N-N distances (Å)
<b>3b</b> [1,1'-methylenebis(allopurinol)]	N(2)-N(1)-C(8)-N(1'), -79.3
	N(2')-N(1')-C(8)-N(1), -22.2
	C(7a)-N(1)-C(8)-N(1'), -90.4
	C(7a')-N(1')-C(8)-N(1'), -41.4
	N(1)-C(8)-N(1'), 114.2
	N(2) - N(2'), 4.1
<b>4b</b> [1,2'-methylenebis(allopurinol)]	N(2)-N(1)-C(8)-N(2'), -14.8
	N(1')-N(2')-C(8)-N(1), -59.2
	C(7a)-N(1)-C(8)-N(2'), 165.8
	C(3')-N(2')-C(8)-N(1), 122.0
	N(1)-C(8)-N(1'), 115.9
	N(2) - N(1'), 3.2
<b>5b</b> [2,2'-methylenebis(allopurinol)]	N(1)-N(2)-C(8)-N(2'), 177.8
	N(1')-N(2')-C(8)-N(2), 94.1
	C(3)-N(2)-C(8)-N(2'), -2.1
	C(3')-N(2')-C(8)-N(2), -86.4
	N(2)-C(8)-N(2), 112.4
	N(1) - N(1'), 4.5

It has been postulated that any bimolecular reaction of a small anion passing through a larger polarizable transition state will be accelerated considerably upon changing from protic to aprotic solvents [23]. Based on this hypothesis, the rate of an ester saponification has been reported to be drastically effected by the solvent composition, e.g. when aqueous alcohol is substituted by aqueous dimethylsulfoxide [24-29].

**Figure 5.** Pseudo first-order rate constant  $k$  as well as  $\ln k$  versus DMSO concentration for the reaction of **3a**  $\rightarrow$  **3b**.



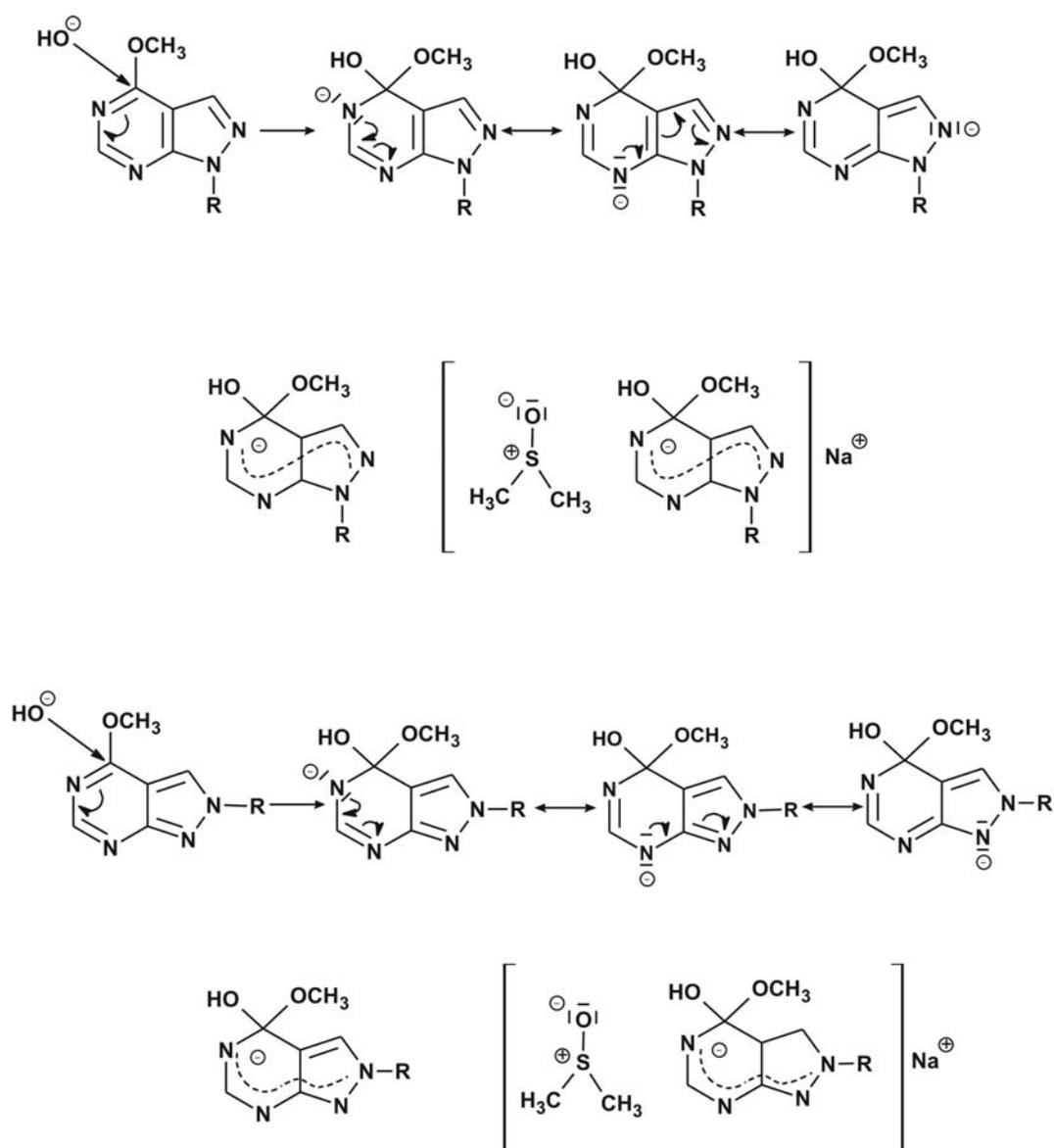
This prompted us to measure the influence of the DMSO concentration on the reaction rates of nucleophilic displacements on compounds **3a–5a** in 1N NaOH/DMSO mixtures (Figure 5). This figure using **3a** as an example how the reaction rate (rate constant  $k$  for pseudo first-order reactions) increases with the DMSO concentration. Analogous results were obtained for **4a** and **5a** (data not shown). Two reasons have been reported [23] for such hydrolysis rate enhancements in water - DMSO - sodium hydroxide mixtures: (i) with increasing DMSO concentrations the activity of the  $\text{OH}^-$  ion is enhanced; at a mole fraction  $x_{\text{DMSO}}$  of 0.35 the thermodynamic excess functions are maximal, and with increasing DMSO content the solvation of  $\text{OH}^-$  is reduced. (ii) At lower DMSO concentrations the solvent lowers the energy of the reaction intermediate by specific solvation. This leads consequently to a rate enhancement. Because in our case the mole fraction of DMSO ranges between 0 and 0.3 (0 – 60 vol-%), the second effect should be predominant. For all reactions measured under pseudo first-order conditions linear  $\ln k$  versus  $x_{\text{DMSO}}$  plots were obtained following the relation:

$$\ln k = m x_{\text{DMSO}} + \ln k_0 \quad (1)$$

where  $m$  = sensitivity of the substrates to the dimethylsulfoxide content in the varying solvent compositions;  $\ln k$  = natural logarithm of the pseudo first-order rate constant;  $\ln k_0$  = natural logarithm of the pseudo first-order rate constant in a DMSO-free medium and  $x_{\text{DMSO}}$  = mole fraction of dimethylsulfoxide).

The sensitivity values,  $m$ , are listed in Table 2. As can be seen, all  $S_{\text{N}}\text{Ar}$  reactions performed show almost the same sensitivity toward the dimethylsulfoxide concentration in the solvent composition (7.9–8.8). There is no significant difference between the N(1)- and N(2)-alkylated heterocycles. This becomes understandable under the assumption that in all cases similar reaction intermediates of the *Meisenheimer* complex type are formed. This is likely because the  $\text{OCH}_3$  group is a rather weak leaving group. Both intermediates can be formulated by three mesomeric *Lewis* structures which are probably energetically equivalent as their negative charges are located on the nitrogen atoms (Scheme 1).

**Scheme 1.** Mesomeric structures of the *Meisenheimer* complexes formed as intermediates during the nucleophilic displacement reactions of **3a** and **5a** with  $\text{OH}^-$  and its solvation by dimethylsulfoxide.



This implies that for the nucleophilic displacement reactions of the methoxy groups of the N(1)- as well as for the N(2)-alkylated 4-methoxy-pyrazolo[3,4-*d*]pyrimidines the intermediates with a tetrahedral C(4) are energetically lowered by the same amount through solvation by dimethylsulfoxide (Scheme 1).

## Conclusions

Liquid-liquid phase transfer alkylation of 4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (**1a**) with a mixture of dichloromethane/dibromomethane afforded the N(1)-N(1)-, N(1)-N(2)-, and N(2)-N(2) methylene-bridged dimeric heterocycles **3a–5a** which are not stacked, but rather form distorted tetraedra at the central carbon. The three regioisomers were transformed into the corresponding methylenebis(allopurinols) **3b–5b** by nucleophilic S<sub>N</sub>Ar reactions with dilute aqueous sodium hydroxide containing dimethylsulfoxide at concentrations ranging between 0 and 60 vol-%. These products represent a structural type of dimeric drug analogous to methylenebis(4-methoxy-2-methylthio-7*H*-pyrrolo[2,3-*d*]pyrimidine) (**2b**) which is a 20-fold stronger competitive inhibitor of xanthine oxidase than the corresponding monomeric heterocycle **2a**. The various biomedical properties of the methylenebis(allopurinols) will be investigated and the results published later.

## Experimental Section

### General

All chemicals were purchased from Aldrich, Sigma or Fluka (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany). Solvents were of laboratory grade and were distilled before use. Thin layer chromatography (TL): glass sheets, silica gel 60 F<sub>254</sub>, 0.2 mm layer (Macherey-Nagel & Co, Düren, Germany). Column flash chromatography (FC): silica gel 60 (Merck, Germany) at 0.5 bar (4 x 10<sup>4</sup> Pa); sample collection with an UltroRac II fraction collector (LKB Instrumnets, Bromma, Sweden). Melting points (uncorrected): Linström apparatus (Wagner & Munz), Germany). UV spectra and reaction kinetics were measured in MT-4 cuvettes (1 mL) on a SuperScan 3 (Varian, Australia) or a Shimadzu U-210 (Shimadzu, Japan) spectrophotometers. The temperature was controlled with a Lauda R20-K thermostat, connected to a R40/2 digital thermometer (MGW Lauda, Germany). NMR Spectra were measured on AC-250 and AMX-500 spectrometers (Bruker, Karlsruhe, Germany) operating at 250.13/500.14 MHz (<sup>1</sup>H) and 62.896/125.700 MHz (<sup>13</sup>C), respectively. Chemical shifts (δ values) are in parts per million relative to tetramethylsilane used as internal standard. Elemental analyses were performed by Mikroanalytisches Laboratorium Beller (Göttingen, Germany). Inhibition constants, *K<sub>i</sub>*, were taken from Dixon plots, measured according to [7].

### Methylenebis(4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidines) **3a-c** – General Procedure

*Caution! Use a well ventilated hood.* 4-Methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (500 mg, 3.33 mmol), suspended in dichloromethane/dibromomethane (3:1, v/v, 20 mL), was added to a solution of tetrabutylammonium hydrogen sulfate (700 mg, 2.5 mmol) in 50 % aq. sodium hydroxide (20 mL) and agitated with a vibromixer for 1 h at room temperature. 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 (calcium chloride) and evaporated to dryness. Crystallization from methanol gave a mixture of **3a–5a** (305 mg, 61% yield). For isolation of the three regioisomers the mixture (500 mg) was dissolved in chloroform/methanol (9:1, v/v, 10 mL), applied to a silica gel column (silica gel 60H, 6 x 30 cm) and chromatographed with the same solvent mixture as eluent. Three zones were separated.

*1,1'-Methylenebis(4-methoxy-1H-pyrazolo[3,4-d]pyrimidine (3a)*. The fastest migrating zone afforded compound **3a** as colorless needles (155 mg, 32 % yield) with a melting point of 202-204 °C upon evaporation of the solvent and crystallization from methanol. TLC (silica gel, chloroform/methanol, 9:1, v/v):  $R_f = 0.92$ . UV (chloroform/methanol, 1:1, v/v):  $\lambda_{max} = 247$  nm ( $\epsilon = 16.600$ );  $^1\text{H-NMR}$  ( $(\text{CD}_3)_2\text{SO}$ )  $\delta$  8.68 (1H, s, H-C(6)); 8.03 (1H, s, H-C(3)); 6.99 (2H, s,  $\text{CH}_2$ ); 4.13 (3H, s,  $\text{OCH}_3$ );  $^{13}\text{C-NMR}$  ( $(\text{CD}_3)_2\text{SO}$ )  $\delta$  164.1 (C-4); 156.2 (C-6); 155.9 (C-7a); 133.2 (C-3); 103.0 (C-3a); 55.9 ( $\text{CH}_2$ ); 54.2 ( $\text{OCH}_3$ ); EI MS (70 eV): m/e 312 (52 %,  $\text{M}^+$ ), 163 (100 %,  $\text{M}^+$  - chromophore); Anal. Calcd. for  $\text{C}_{13}\text{H}_{12}\text{N}_8\text{O}_2$ : C, 49.99; H, 3.87; N, 35.88. Found: C, 49.84; H, 4.01; N, 35.99.

*4-Methoxy-1-[(4-methoxy-2H-pyrazolo[3,4-d]pyrimidin-2-yl)methyl]-1H-pyrazolo[3,4-d]pyrimidine (4a)*. The second zone afforded compound **4a** as colorless needles (309 mg, 64 % yield) with a melting point of 211-214 °C upon evaporation of the solvent and crystallization from methanol. TLC (silica gel, chloroform/methanol, 9:1, v/v):  $R_f = 0.68$ ; UV (chloroform/methanol, 1:1, v/v):  $\lambda_{max} = 251, 262$  nm ( $\epsilon = 15.100, 14.300$ );  $^1\text{H-NMR}$  ( $(\text{CD}_3)_2\text{SO}$ )  $\delta$  8.63 (1H, s, H-C(6), N(1)-alkylated moiety); 8.27 (1H, s, H-C(6), N(2)-alkylated moiety); 8.59 (1H, s, H-C(3), N(2)-alkylated moiety); 8.07 (1H, s, H-C(3), N(1)-alkylated moiety); 6.89 (2H, s,  $\text{CH}_2$ ); 4.13 (3H, s,  $\text{OCH}_3$ , N(1)-alkylated moiety); 4.08 (3H, s,  $\text{OCH}_3$ , N(2)-alkylated moiety);  $^{13}\text{C-NMR}$  ( $(\text{CD}_3)_2\text{SO}$ )  $\delta$  165.6 (C-4, N(2)-alkylated moiety); 164.2 (C-4, N(1)-alkylated moiety); 161.8 (C-7a, N(2)-alkylated moiety); 156.4 (C-6, N(2)-alkylated moiety); 156.2 (C-6, N(1)-alkylated moiety); 156.0 (C-7a, N(1)-alkylated moiety); 133.8 (C-3, N(1)-alkylated moiety); 123.7 (C-3, N(2)-alkylated moiety); 104.3 (C-3a, N(2)-alkylated moiety); 103.4 (C-3a, N(1)-alkylated moiety); 62.2 ( $\text{CH}_2$ ); 54.3 ( $\text{OCH}_3$ , N(1)-alkylated moiety); 54.0 ( $\text{OCH}_3$ , N(2)-alkylated moiety); EI MS (70 eV): m/e 312 (100 %,  $\text{M}^+$ ), 163 (57 %,  $\text{M}^+$  - chromophore); Anal. Calcd. for  $\text{C}_{13}\text{H}_{12}\text{N}_8\text{O}_2$ : C, 49.99; H, 3.87; N, 35.88. Found: C, 50.10; H, 4.01; N, 35.92.

*2,2'-Methylenebis(4-methoxy-2H-pyrazolo[3,4-d]pyrimidine) (5a)*. From the slowest migrating zone compound **5a** (18.6 mg, 4 % yield) was obtained as colorless needles upon evaporation of the solvent and crystallization of the residue from 1,4-dioxane; melting point 202-204 °C. TLC (silica gel, chloroform/methanol, 9:1, v/v):  $R_f = 0.45$ ; UV (chloroform/methanol, 9:1, v/v):  $\lambda_{max} = 262$  nm ( $\epsilon = 18.500$ );  $^1\text{H-NMR}$  ( $(\text{CD}_3)_2\text{SO}$ )  $\delta$  8.45 (1H, s, H-C(6)); 8.59 (1H, s, H-C(3)); 6.79 (2H, s,  $\text{CH}_2$ ); 4.08 (3H, s,  $\text{OCH}_3$ );  $^{13}\text{C-NMR}$  ( $(\text{CD}_3)_2\text{SO}$ )  $\delta$  165.9 (C-4); 161.2 (C-7a); 156.3 (C-6); 125.8 (C-3); 104.1 (C-3a); 64.7 ( $\text{CH}_2$ ); 54.0 ( $\text{OCH}_3$ ); EI MS (70 eV) m/e 312 (45 %,  $\text{M}^+$ ), 163 (100 %,  $\text{M}^+$  - chromophore); Anal. Calcd. for  $\text{C}_{13}\text{H}_{12}\text{N}_8\text{O}_2$ : C, 49.99; H, 3.87; N, 35.88. Found: C, 49.74; H, 3.97; N, 35.61.

*Methylenebis(allopurinols) 3b–5b – General Procedure*

Compounds **3a–5a** (**3a**, **4a**: 500 mg, 1.65 mmol, each; **5a**: 70 mg, 0.26 mmol) were each dissolved in 2N sodium hydroxide/MeOH (1:1, v/v, 20 mL) and refluxed for 1 h. In the cases of compounds **3b** and **4b** acidification of the reaction mixtures with 6N HCl afforded the corresponding methylenebis(allopurinols) directly as colorless solids; in the case of **5b** further chromatographic purification proved to be necessary.

*1,1'-Methylenebis(1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one) (3b)*. Compound **3b** (440 mg, 95 % yield); m.p. > 320 °C. TLC (silica gel, 0.25 M LiCl):  $R_f = 0.75$ ; UV (1N sodium hydroxide):  $\lambda_{\max} = 272$  nm ( $\epsilon = 19.500$ );  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}/\text{NaOD}$ ):  $\delta$  6.71 (s,  $\text{CH}_2$ ); 8.02 (s, H-C(3)); 8.25 (s, H-C(6)); Anal. Calcd. for  $\text{C}_{11}\text{H}_8\text{N}_8\text{O}_2$ : C, 46.48; H, 2.84; N, 39.42. Found: C, 46.31; H, 2.98; N, 39.49.

*1-[(4-Oxo-4,5-dihydro-2H-pyrazolo[3,4-d]pyrimidin-2-yl)methyl]-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (4b)*. Compound **4b** (423 mg, 91 % yield); m.p. > 320 °C; TLC (silica gel, 0.25 M LiCl):  $R_f = 0.70$ ; UV (1N sodium hydroxide):  $\lambda_{\max} = 273, 281$  (sh) nm ( $\epsilon = 20.500, 18.700$ );  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}/\text{NaOD}$ ):  $\delta$  6.71 (2H, s,  $\text{CH}_2$ ); 8.08 (1H, s, H-C(3), N(1)-alkylated moiety); 8.14 (1H, s, H-C(3), N(2)-alkylated moiety); 8.26 (1H, s, H-C(6), N(1)-alkylated moiety); 8.43 (1H, s, H-C(6), N(2)-alkylated moiety); Anal. Calcd. for  $\text{C}_{11}\text{H}_8\text{N}_8\text{O}_2$ : C, 46.48; H, 2.84; N, 39.42. Found: 46.39; H, 2.90; N, 39.42.

*2,2'-Methylenebis(2,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one) (5b)*. The reaction mixture was chromatographed on a Dowex<sup>®</sup> 1x2 ion exchange resin column (1.5 x 30 cm). After washing with water (1 L) compound **5b** (62 mg, 96 % yield) was eluted with aq. acetic acid (10 %). M.p. > 320 °C; TLC (silica gel, 0.25 M LiCl):  $R_f = 0.77$ ; UV (1N sodium hydroxide):  $\lambda_{\max} = 283$  nm ( $\epsilon = 19.500$ );  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}/\text{NaOD}$ ):  $\delta$  8.06 (2H, br. s, H-C(6) and H-C(3)); 5.91 (2H,  $\text{CH}_2$ ); Anal. Calcd. for  $\text{C}_{11}\text{H}_8\text{N}_8\text{O}_2$ : C, 46.48; H, 2.84; N, 39.42. Found: C, 46.33; H, 2.96; N, 39.31.

*1,1'-Methylenebis(1H-pyrazolo[3,4-d]pyrimidin-4-amine) (3c)*

1,1'-Methylenebis(4-methoxy-1H-pyrazolo[3,4-d]pyrimidine (**3a**, 100 mg, 0.33 mmol) was dissolved in concentrated aq. ammonia (20 mL) and stirred for 60 min at room temperature. After evaporation of the solvent the residue was taken up in water and chromatographed on Amberlite<sup>®</sup> XAD, type 4 (Serva, Germany). Methanol/water (1:4, v/v) eluted one main peak from which compound **3c** (70 mg, 75 % yield) was isolated as colorless solid. M.p.: 88–89 °C; TLC (silica gel, chloroform/methanol, 9:1, v/v):  $R_f = 0.4$ ; UV (chloroform/methanol, 1:1, v/v):  $\lambda_{\max} = 260, 276$  nm ( $\epsilon = 16.800, 18.700$ );  $^1\text{H-NMR}$  ( $(\text{CD}_3)_2\text{SO}$ )  $\delta$  8.25 (1H, s, H-C(6)); 8.09 (1H, s, H-C(3)); 7.77 and 7.26 (2H, br,  $\text{NH}_2$ ); 6.69 (2H, s,  $\text{CH}_2$ );  $^{13}\text{C-NMR}$  ( $(\text{CD}_3)_2\text{SO}$ )  $\delta$  54.8 ( $\text{CH}_2$ ); 99.9 (C-3a); 133.8 (C-3); 154.1 (C-7a); 156.5 (C-6); 158.0 (C-4); Anal. Calcd. for  $\text{C}_{11}\text{H}_{10}\text{N}_{10}$ : C, 46.80; H, 3.57; N, 49.62. Found: C, 46.72, H, 3.65; N, 49.50.

## References and Notes

1. Robins, R. K. Potential Purine Antagonists. I. Synthesis of Some 4,6-Substituted Pyrazolo[3,4-*d*]pyrimidines. *J. Am. Chem. Soc.* **1956**, *78*, 784-790.
2. Schmidt, P.; Druey, J. Heilmittelchemische Untersuchungen in der heterocyclischen Reihe. 14. Mitteilung. Pyrazolo[3,4-*d*]pyrimidine. *Helv. Chim. Acta* **1956**, *39*, 986-991.
3. Massey, V.; Komai, H.; Palmer, G.; Elion, G. B. On the Mechanism of Inactivation of Xanthine Oxidase by Allopurinol and Other Pyrazolo[3,4-*d*]pyrimidines. *J. Biol. Chem.* **1970**, *245*, 2837-2844.
4. Elion, G. B.; Callahan, S., Nathan, H.; Bieber, S.; Rundles, R. W.; Hitchings, G. H. Potentiation by Inhibition of Drug Degradation: 6-Substituted Purines and Xanthine Oxidase. *Biochem. Pharmacol.* **1963**, *12*, 85-93.
5. Fyfe, J. A. ; Miller, R. L. ; Krenitsky, T. A. Kinetic Properties and Inhibition of Orotidine 5'-Phosphate Decarboxylase. *J. Biol. Chem.* **1973**, *248*, 3801-3809.
6. Nelson, D. J.; LaFon, S. W.; Tuttle, J. V.; Miller, W. H.; Miller, R. L.; Krenitsky, T. A.; Elion, G. B.; Berens, R. L.; Marr, J. J. Allopurinol Ribonucleosides as an Antileishmanial Agent. *J. Biol. Chem.* **1979**, *254*, 11544-11549.
7. Rosemeyer, H.; Seela, F. Methylated 7-Deazahypoxanthines as Regiochemical Probes of Xanthine Oxidase. *Eur. J. Biochem.* **1983**, *134*, 513-515.
8. Seela, F.; Richter, R. Aminomethylation of 3,7-Dihydropyrrolo[2,3-*d*]pyrimidines at C-5. A Method for the Synthesis of Aglycon Analogues of Nucleoside "Q". *Chem. Ber.* **1978**, *111*, 2925-2930.
9. Davoll, J. Pyrrolo[2,3-*d*]pyrimidines. *J. Chem. Soc.* **1960**, 131-138.
10. Seela, F.; Menkhoff, S. 4,4'-Dimethoxy-2,2'-bis(methylthio)-7,7'-methylendi(7*H*-pyrrolo[2,3-*d*]pyrimidin) – Phasentransferkatalysierte Aglyconverbrückung in Dichlormethan. *Liebigs Ann. Chem.* **1982**, 1405-1408.
11. Robins, R. K. Potential Purine Antagonists. IX. Further Studies of Some 4,6-Disubstituted Pyrazolo[3,4-*d*]pyrimidines. *J. Am. Chem. Soc.* **1956**, *79*, 6407-6415.
12. Lichtenthaler, F. W.; Cuny, E. The Ribonucleosides of Allopurinol. *Chem. Ber.* **1981**, *114*, 1610-1623.
13. Rosemeyer H.; Kaiser, K.; Seela, F. Spontaneous Hydroxylation of a Cyclization Intermediate of Allopurinol. *J. Org. Chem.* **1985**, *50*, 1847.
14. Seela, F.; Steker, H. Synthesis of 2'-Deoxyribonucleosides of Allopurinol By Phase-Transfer Glycosylation. *Tetrahedron Lett.* **1984**, *25*, 5017-5018.
15. Bergmann, F.; Frank, A.; Neiman, Z. Studies on the Chemical Reactivity and the Physical Properties of Allopurinol (Pyrazolo[3,4-*d*]pyrimidin-4-one) and Related Compounds. *J. Chem. Soc. Perkin Tans. 1* **1979**, 2795-2802.
16. Seela, F.; Steker, H. Facile Synthesis of 2'-Deoxyribofuranosides of Allopurinol and 4-Amino-1*H*-pyrazolo[3,4-*d*]pyrimidine Via Phase-Transfer Glycosylation. *Helv. Chim. Acta* **1985**, *68*, 563-570.
17. Cheng, C. C.; Robins, R. K. Potential Purine Antagonists. Synthesis of 1-Alkyl- and 1-Aryl-4-substituted Pyrazolo[3,4-*d*]pyrimidines. *J. Org. Chem.* **1956**, *21*, 1240-1256.

18. Uzawa, J.; Uramoto, M. Assignment of Indirect  $^{13}\text{C}$ ,  $^1\text{H}$  Couplings in the  $^{13}\text{C}$ - NMR Spectra of Some Purine and Pyrimidine Nucleosides and Their Analogues by Long-Range Selective  $^1\text{H}$  Decoupling. *Org. Magn. Res.* 1979, 12, 612-615.
19. Advanced Chemistry Development, Toronto. <http://www.acdlabs.com>. ChemSketch/3D Viewer, version 10.0.
20. Itahara, T. Stacking Conformation of 9-[ $\omega$ (Thymin-1-yl)alkyl]adenine in Aqueous Solution. *Nucleos. Nucleot. Nucl. Acids* 2003, 22, 309-317.
21. Itahara, T. NMR Study of Stacking Interactions Between Adenine and Xanthine Rings. *J. Chem. Soc. Perkin Trans. 2* 1998, 1455-1462.
22. Compound **6**: Brownish solid.  $^1\text{H}$ -NMR ( $(\text{CD}_3)_2\text{SO}$ )  $\delta$  8.06 (2H, s, H-C(3)); 5.91 (2H, s,  $\text{CH}_2$ ); ~ 5.5 (br,  $\text{NH}_2$ );  $^{13}\text{C}$ -NMR ( $(\text{CD}_3)_2\text{SO}$ )  $\delta$  64.1 ( $\text{CH}_2$ ); 99.7 (C-4); 134.1 (C-3); 156.7 (C-5); 164.8 (C=O).
23. Martin, D.; Hauthal, H. G. *Dimethylsulfoxid*; Akademie-Verlag: Berlin, 1971; pp 213-225.
24. Roberts, D. D. Alkaline Hydrolysis of Ethyl Benzoate in Aqueous Dimethyl Sulfoxide. *J. Org. Chem.* 1964, 29, 2039-2040.
25. Roberts, D. D. Solvent Effects in Quantitative Structure-Reactivity Correlations of Esters. *J. Org. Chem.* 1964, 29, 2714-2717.
26. Roberts, D. D. Solvent Effects. II. The Influence of Aqueous Dimethyl Sulfoxide on Ester Saponification Reactions. *J. Org. Chem.* 1965, 30, 3516-3520.
27. Roberts, D. D. Solvent Effects. III. The Influence of Aqueous Dimethyl Sulfoxide on Alkyl Benzoate Ester Saponification Reactions. *J. Org. Chem.* 1966, 31, 4037.
28. Tommila, E.; Palenius, I. The Influence of the Solvent on Reaction Velocity XXV. Dependence of the Substituent Effect on Solvent Composition in the Alkaline Hydrolysis of Benzoic Esters in Dimethyl Sulfoxid – Water Mixtures. *Acta Chem. Scand.* 1963, 17, 1980-1984.
29. Tommila, E.; Murto, M.-L. The Influence of the Solvent on Reaction Velocity XXIII. The Alkaline Hydrolysis of Ethyl Acetate in Dimethyl Sulfoxide – Water Mixtures. *Acta Chem. Scand.* 1963, 17, 1947-1956.

*Sample availability:* Available from the corresponding author.