

20. Synthesis of 2'-Deoxyisoinosine and Related 2'-Deoxyribonucleosides

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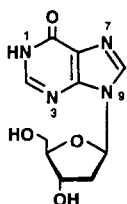
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(18.X.93)

Various 2-substituted purine and pyrrolo[2,3-*d*]pyrimidine 2'-deoxyribonucleosides with methylthio (**13a**), chloro (**13b**), methoxy (**9b**), and oxo (**2**, **3**) substituents at C(2) are prepared. They are obtained either *via* stereoselective nucleobase-anion glycosylation or by base transformation. A three-step synthesis of the unknown 2'-deoxyisoinosine (**2**) from 2'-deoxyguanosine (**15**) is described. Compound **2** as well as its 7-deazapurine derivative **3** exhibit strong fluorescence.

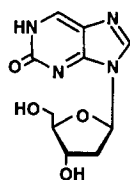
The 2'-deoxyinosine (**1**) is widely used as ambiguous nucleoside moiety in oligonucleotides as it forms base pairs with all four conventional nucleosides within double-stranded oligonucleotides [1]. The isomeric 2'-deoxyisoinosine (**2**) is unknown as well as congeners such as the pyrrolo[2,3-*d*]pyrimidine derivative **3**. Only a preliminary report on the enzymatic synthesis of **2** appeared [2]. The synthesis of the ribonucleoside isoinosine was reported [3]. The 2-oxopurine base is an electrophile adding easily nucleophiles at the 6-position [4]. The polarized character of the molecule led to unusual alkylation products during benzylation. Also covalent adducts can be formed [5].

The 2-substituted purine nucleosides, such as 2-aminopurine 2'-deoxyribonucleoside **4**, possess strong fluorescent properties [6]. Such fluorescent purine nucleosides are used to detect nucleic acids in extremely low concentration, and for the purpose of sequencing, compound **4** found wide application in nucleic-acid chemistry [7]. In the following, we report on a three-step synthesis of the fluorescent 2'-deoxyisoinosine (**2**). Related 2-substituted purine and pyrrolo[2,3-*d*]pyrimidine (= 7-deazapurine) 2'-deoxyribonucleosides are also prepared.

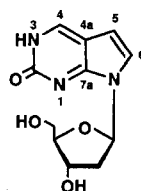


1

purine numbering

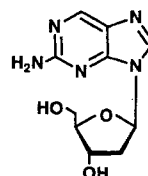


2



3

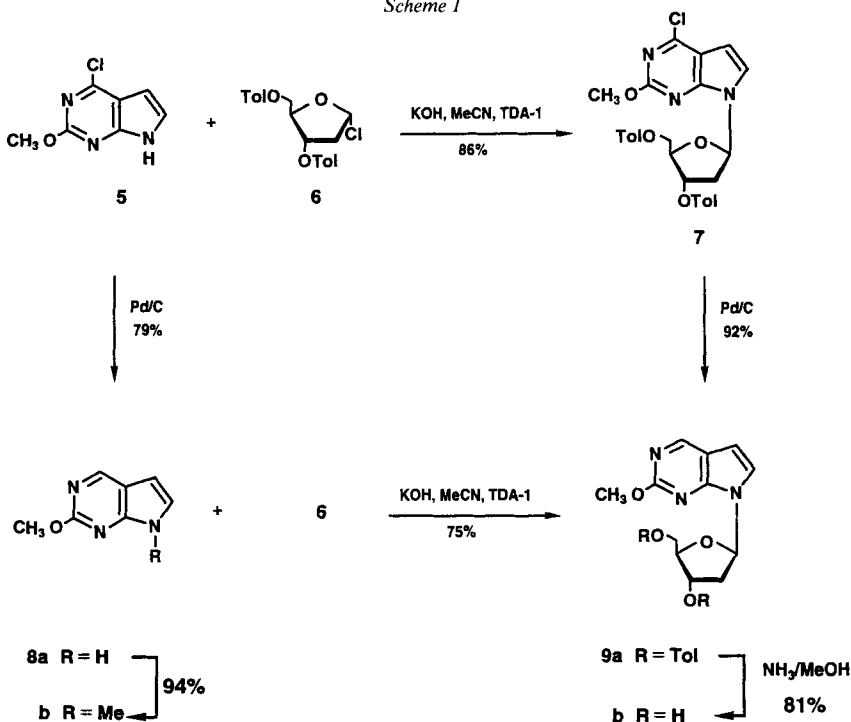
systematic numbering



4

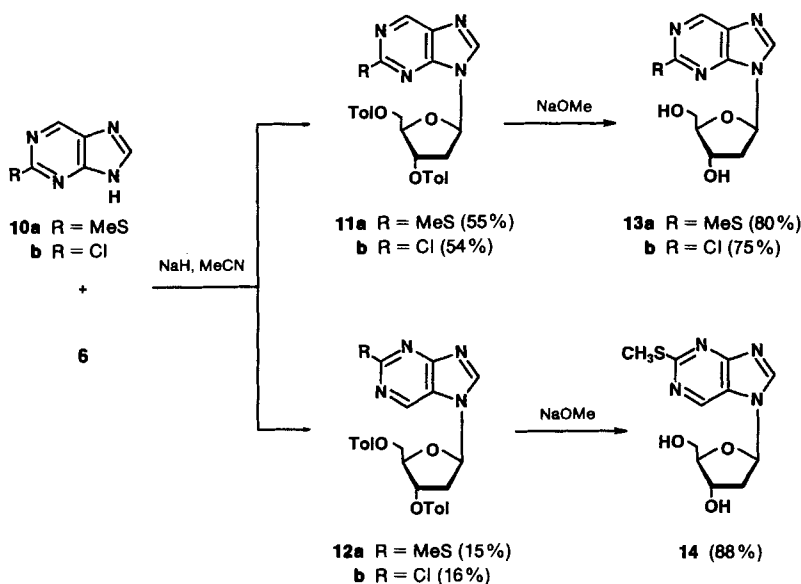
Results and Discussion. – *2-Substituted Pyrrolo[2,3-d]pyrimidine 2'-Deoxyribonucleosides.* The synthesis of 2'-deoxyisinosine (**2**) or 2-substituted congeners was accomplished by two different routes: *i*) glycosylation of a suitable base precursor and conversion of the protected intermediate into the 2-oxo derivative and *ii*) the transformation of a naturally occurring nucleoside into the corresponding 2-oxo compound. For the synthesis of 7-deaza-2'-deoxyisinosine (**3**), route *i* was chosen, because a naturally occurring precursor molecule was not available. As starting material, 4-chloro-2-methoxy-pyrrolo[2,3-d]pyrimidine (**5**) [8] was used. It was already glycosylated under liquid-liquid phase-transfer conditions [9] but was now reacted with halogenose **6** in MeCN in the presence of tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) and powdered KOH [10] (Scheme 1). Under these conditions, the reaction was stereoselective (\rightarrow **7**), and the glycosylation yield was increased from 67 [9] to 86%. The 4-chloro substituent was removed by catalytic hydrogenation affording **9a**. Compound **9a** was also prepared by glycosylation of **8a** with halogenose **6** in 75%. The base **8a** was obtained by catalytic hydrogenation of **5**. To compare the regiospecificity of the glycosylation and alkylation, the anion of **8a** was treated with MeI yielding **8b** in 94%. The overall yield for the preparation of **9a** was 79% employing **5** for glycosylation, while only 59% were obtained when **8a** was glycosylated. Next, **9a** was deblocked with methanolic ammonia furnishing **9b**. Treatment of **9b** with 2N NaOH under reflux conditions afforded crystalline 7-deaza-2'-deoxyisinosine (**3**). As the nucleophilic displacement of the MeO group was slow in aqueous NaOH, the reaction rate was enhanced by addition of DMSO, a method which was already used in other cases [11].

Scheme 1



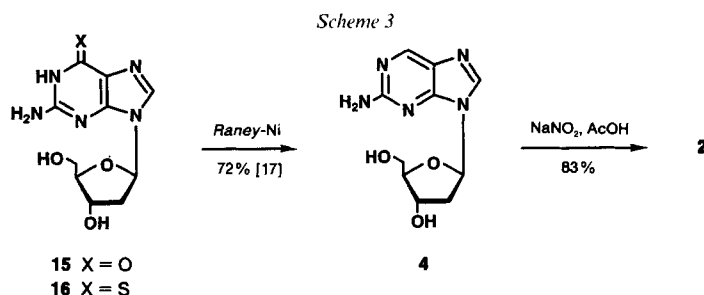
2-Substituted Purine 2'-Deoxyribonucleosides Including 2'-Deoxyisoinosine (2). For the synthesis of **2** and related compounds, a convergent synthesis was considered at first. The 2-(methylthio)-9H-purine (**10a**) [12] and 2-chloro-9H-purine (**10b**) [13] were employed in nucleobase-anion glycosylation (Scheme 2). For this purpose, **10a** was obtained by condensation of 4,5-diamino-2-(methylthio)pyrimidine and triethyl orthoformate [12]. The corresponding chloropurine **10b** was synthesized from 2,6-dichloropurine and Pd/C catalyst according to a procedure of Breshraes *et al.* [13]. The anions of **10a** and **10b** were treated with the halogenose **6** furnishing the β -D-nucleosides **11a,b** and **12a,b** exclusively. These mixtures of N^7 - and N^9 -glycosylation products were separated chromatographically. The overall glycosylation yield was 70% in both cases, the N^9/N^7 ratio 3.6:1. Deblocking of **11a** and **12a** furnished the (methylthio)nucleosides **13a** and **14**, respectively. The N^9 -isomer **13a** crystallized, and the N^7 -compound **14** was isolated as a foam. In the case of the 2-chloro compounds, only N^9 -isomer **11b** was deprotected yielding crystalline **13b**. Efforts to convert the 2-(methylthio)- or the 2-chloronucleosides **13a** or **13b** into 2'-deoxyisoinosine (**2**) failed. Even oxidation of **13a** to a sulfoxide intermediate and nucleophilic displacement with NaOH did not give **2** in appreciable amounts. The 2-chloro substituent was also resistant to substitution by OH^- . Also nucleophilic photosubstitution in H_2O , which was already employed in the case of 2'-deoxyisoguanosine [14], was not successful. As a consequence, we chose another route.

Scheme 2



From guanosine, 2-aminopurine ribonucleoside can be obtained *via* the 6-thioxo compound followed by desulfurization [15]. For the preparation of the thionucleoside, phosphorous pentasulfide or Lawesson's reagent is employed. These reagents fail in the case of 2'-deoxyguanosine (**15**). Recently, two new protocols for the sulfurization of **15** were published [16] [17]. We selected the approach of Kung and Jones [17] who use a

pyridinium salt as an intermediate. This was treated with NaHS to give **16** in 65% yield. Compound **16** was then desulfurized with *Raney*-Ni catalyst affording crystalline 2-aminopurine 2'-deoxyribonucleoside **4** in 42% overall yield (Scheme 3). As the reaction can be easily adapted to larger scale, this protocol is the method of choice for the preparation of **4**. Compound **4** was then treated with NaNO₂ in the presence of AcOH/H₂O, using similar conditions as described for the synthesis of 2'-deoxyisoguanosine [18]. Crystalline 2'-deoxyisoinosine (**2**) was isolated upon chromatographic purification.



Compared to 2'-deoxyinosine (**1**) showing a half-life of glycosylic-bond hydrolysis of 27 min (0.1N HCl, 30°, 249 nm), the corresponding 2'-deoxyisoinosine (**2**) is slightly less stable ($t_{1/2}$ = 24 min, 0.1N HCl, 30°, 314 nm) in acid.

Spectral Properties of 2-Substituted Purine and 7-Deazapurine 2'-Deoxyribonucleosides. The structures of the various 2-substituted 2'-deoxyribonucleosides and their synthetic precursors were established by NMR spectroscopy. The site of sugar attachment of the purine nucleosides obtained by glycosylation was determined by ¹H-NMR NOE difference spectroscopy.

In the case of the N⁷-isomer **14**, a NOE at H-C(6) and H-C(8) was observed upon irradiation of the anomeric proton (Table 1). The NOE at H-C(6) was absent in the case of the N⁹-compounds. The H-C(6) signal was assigned from its NOE observed upon irradiation of NH. This experiment also indicated that a high population of molecules is existing in the lactim form with the proton at N(3). The NOE's at H-C(4') or H_x-C(2') obtained after irradiation of H-C(1') confirmed the β-D-configuration [19]. The NOE's occurring on the OH groups were due to an energy transfer *via* H₂O molecules.

Table 1. NOE Data of Purine and Pyrrolo[2,3-d]pyrimidine 2'-Deoxyribonucleosides^{a)b)}

	Irradiated proton	NOE
2	H-C(8)	H-C(1') (4.2%), H _β -C(2') (3.2%)
	H-C(1')	H-C(8) (4.8%), H _x -C(2') (7.4%), H-C(4') (1.2%)
3	H-C(6)	NH (2.2%), H-C(7) (3.6%), OH-C(3') (2.0%), H-C(5') (2.1%)
	NH	H-C(6) (12.3%), H-C(8) (3.0%), H-C(7) (3.1%), H-C(3') (12.9%)
13a	H-C(8)	H-C(1') (5.7%), OH-C(5') (1.4%), H-C(3') (1.5%), H _β -C(2') (3.8%)
	H-C(1')	H-C(8) (5.8%), H-C(4') (2.1%), H _x -C(2') (7.1%)
13b	H-C(8)	H-C(1') (5.3%), H-C(3') (3.0%), H _β -C(2') (4.3%)
14	H-C(1')	H-C(8) (8.3%), H-C(6) (4.1%), H-C(4') (2.2%), H _x -C(2') (6.7%)
	H-C(6)	H-C(1') (3.7%), OH-C(5') (2.0%)
	H-C(8)	H-C(1') (7.3%)

^{a)} Measured in (D₆)DMSO at 23°.

^{b)} Purine numbering.

The ^{13}C -NMR chemical shift of the 2-substituted nucleosides and their precursors are summarized in Table 2. The signals were assigned using gated-decoupled ^{13}C -NMR spectra (Table 3) as well as ^1H , ^{13}C -correlation spectra. The N^7 - vs. N^9 -regioisomers, e.g. **14** and **13a**, showed the typical up- and downfield shifts (10 ppm) of C(4) and C(5), respectively, induced by the substitution of the N-atom in α -position [20]. The coupling constants of C(1') vs. C(4') [21] were used for their assignment. It appeared that between the toluoylated and the deprotected nucleosides, a change of the sugar signal order was observed.

It was reported that 2-aminopurine 2'-deoxyribonucleoside can replace 2'-deoxyadenosine in oligomers [22] as 2'-deoxyadenosine substitute. This replacement makes also

Table 2. ^{13}C -NMR Chemical Shifts of Purine and Pyrrolo[2,3-d]pyrimidine 2'-Deoxyribofuranosides^{a)}

	C(2) ^{b)} C(2) ^{c)}	C(6) ^{b)} C(4) ^{c)}	C(5) ^{b)} C(4a) ^{c)}	– C(5) ^{c)}	C(8) ^{b)} C(6) ^{c)}	C(4) ^{b)} C(7a) ^{c)}	Me
2	156.1	139.4	123.6	–	145.5	158.8	–
3	155.7	140.0	108.1	101.4	126.6	158.1	–
4	160.5	149.4	127.2	–	140.7	152.8	–
5	160.6	153.8	111.9	98.8	126.1	151.4	54.6
7	160.6	152.7	113.1	100.2	126.8	152.0	55.7
8a	161.5	150.8	113.9	99.7	125.3	153.4	54.3
b	161.5	150.8	114.2	99.1	129.6	152.4	54.3
9a	161.5	151.6	115.0	101.1	126.0	152.6	54.6
b	161.4	151.3	114.7	100.7	125.7	152.5	54.4
11a	165.3	148.8	131.7	–	144.9	151.7	14.2
b	153.0	150.4	133.8	–	146.5	152.6	–
12a	165.0	147.5	121.5	–	142.8	161.9	13.9
b	153.4	149.1	123.7	–	144.2	162.8	–
13a	164.9	148.5	131.5	–	144.4	151.8	14.1
b	152.8	150.1	133.7	–	146.3	152.6	–
14	164.6	142.8	121.4	–	147.6	161.9	14.0
16	153.1	175.2	128.5	–	138.3	147.5	–

	C(1')	C(2')	C(3')	C(4')	C(5')	MeC ₆ H ₄
2	82.9	^{d)}	70.9	87.9	61.7	–
3	81.9	^{d)}	70.9	87.1	61.9	–
4	82.6	^{d)}	70.8	87.7	61.8	–
7	84.2	36.0	74.7	81.3	64.0	21.3
9a	83.3	35.8	75.2	81.1	64.3	21.3
b	82.6	39.4	71.1	87.4	62.1	–
11a	84.0	^{d)}	74.6	81.6	64.0	21.3
b	84.1	^{d)}	74.7	82.0	64.0	21.2
12a	86.1	^{d)}	74.7	82.1	64.0	21.3
b	86.4	^{d)}	74.6	82.3	64.0	21.2
13a	83.5	^{d)}	70.7	88.0	61.7	–
b	83.8	^{d)}	70.5	88.2	61.4	–
14	86.1	^{d)}	70.4	88.1	61.3	–
16	82.8	^{d)}	70.7	87.8	61.7	–

^{a)} Measured in (D₆)DMSO.

^{b)} Purine numbering.

^{c)} Systematic numbering.

^{d)} Superimposed by DMSO.

Table 3. $J(C,H)$ Coupling Constants [Hz] of Purine and Pyrrolo[2,3-*d*]pyrimidine 2'-Deoxyribonucleosides^{a)}

	2 (=O)	3 (=O)	4 (NH ₂)	7	8a (OMe)	13a	13b	14
$J(C(2),H-C(6))$	6.8	6.6	11.8	–	12	12.1	13.8	12.1
$J(C(2),MeO)$	–	–	–	4.4	4.7	–	–	–
$J(C(2),MeS)$	–	–	–	–	–	4.8	–	4.4
$J(C(6),H-C(6))$	183.7	183.0	179.7	–	150.8	184.9	188.4	189.4
$J(C(5),H-C(6))$	13.0	<i>m</i>	11.7	<i>m</i>	<i>m</i>	11.6	11.8	8.8
$J(C(5),H-C(8))$	2.3	<i>m</i>	6.2	<i>m</i>	<i>m</i>	6.3	6.1	3.7
$J(C(7),H-C(7))$	–	180.0	–	181.2	179.0	–	–	–
$J(C(7),H-C(8))$	–	6.8	–	6.9	5.8	–	–	–
$J(C(7),H-C(6))$	–	2.1	–	–	4.9	–	–	–
$J(C(8),H-C(8))$	214.9	190.0	213.3	190.8	153.5	214.7	215.9	212.4
$J(C(8),H-C(7))$	–	6.3	–	7.4	7.9	–	–	–
$J(C(8),NH)$	–	–	–	–	4.3	–	–	–
$J(C(8),H-C(1'))$	3.9	4.8	4.2	4.2	–	4.2	4.0	3.1
$J(C(4))$	<i>m</i>	<i>m</i>	<i>m</i>	–	<i>m</i>	<i>m</i>	<i>m</i>	<i>m</i>
$J(C(1'),H-C(1'))$	163.3	164.4	167.4	166.2	–	166.5	163.3	166.6
$J(C(2'),H-C(2'))$	^{c)}	^{c)}	138.8	135.2	–	^{c)}	^{c)}	^{c)}
$J(C(3'),H-C(3'))$	149.6	149.4	149.4	158.7	–	150.0	149.4	149.5
$J(C(4'),H-C(4'))$	147.1	147.2	147.5	152.5	–	148.1	148.3	147.6
$J(C(5'),H-C(5'))$	140.0	140.2	140.4	149.2	–	139.9	140.4	140.1

a) From ¹³C-NMR spectra measured in (D₆)DMSO at 23°.

b) Purine numbering.

c) Superimposed by DMSO.

oligonucleotides fluorescent [23] and causes mutagenic events during replication of DNA [24]. Compound **4** was also recently employed for one molecule sequencing of DNA [25]. We already showed that the 2-aminopyrrolo[2,3-*d*]pyrimidine 2'-deoxyribonucleoside is fluorescent [26]. It is also very stable against acids and bases, and the fluorescence is quenched if it is incorporated into oligonucleotides [27]. As the fluorescence labelling of oligonucleotides is now commonly used to detect DNA in small quantities, fluorescent nucleosides are of decisive importance [28]. Table 4 summarizes the excitation and emission spectra of 2'-deoxyisoinosine (**2**) and its pyrrolo[2,3-*d*]pyrimidine derivative **3** as well as of the 2-amino and 2-methoxy compounds **4** and **9b**, respectively. It can be seen that 2-oxo compounds are strongly fluorescent showing nearly the same excitation and emission maxima as 2-aminopurine 2'-deoxyribonucleoside. As 2-oxopurine nucleosides are structurally related to pyrimidinone nucleosides [29] but contain a different proton donor-acceptor pattern, their incorporation into oligonucleotides is of considerable interest.

Table 4. Excitation and Emission Maxima Taken from the Fluorescence Spectra of 2-Substituted Purine and Pyrrolo[2,3-*d*]pyrimidine 2'-Deoxyribonucleosides Measured in H₂O

	Group at C(2)	Excitation [nm]	Emission [nm]
2	C=O	315	380
3	C=O	330	430
4	NH ₂	305	370
9b	CH ₃ O	295	415

We thank Dr. H. Rosemeyer for the NOE spectra. Financial support by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie is gratefully acknowledged.

Experimental Part

General. See [30]. Solvent systems: $\text{CHCl}_3/\text{MeOH}$ 9:1 (*A*), cyclohexane/ AcOEt 3:2 (*B*), $\text{CHCl}_3/\text{MeOH}$ 4:1 (*C*), $\text{H}_2\text{O}/i\text{-PrOH}$ 9:1 (*D*), cyclohexane/ AcOEt 1:1 (*E*), $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1 (*F*), cyclohexane/ AcOEt 4:6 (*G*), $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 4:1 (*H*), $i\text{-PrOH}/\text{H}_2\text{O}/\text{conc. aq. NH}_3$ soln. 7:2:1 (*I*). Flash chromatography (FC): at 0.3 bar using a *Uvicord-II* detector (*LKB*, Sweden).

4-Chloro-7-(2-deoxy-3,5-di-O-(4-toluoyl)- β -D-erythro-pentofuranosyl)-2-methoxy-7H-pyrrolo[2,3-d]pyrimidine (7). Powdered KOH (600 mg, 8.91 mmol), TDA-1 (100 μl , 0.31 mmol), and **5** (500 mg, 2.72 mmol) were added to anh. MeCN (40 ml) within 5 min. After additional 5 min, **6** (1.16 g, 2.99 mmol) was added and the mixture stirred for 15 min. Insoluble material was filtered off, the residue washed with MeCN, and the soln. evaporated. The residue was purified by FC (column 4×5 cm, CHCl_3). Colorless crystals (1.26 g, 86%) from MeOH. M.p. 118° ([9]; 115°).

2-Methoxy-7H-pyrrolo[2,3-d]pyrimidine (8a). To a soln. of **5** (1.40 g, 7.83 mmol) [9] in MeOH (50 ml), conc. aq. NH_3 soln. (1 ml) was added. The mixture was hydrogenated in presence of 10% Pd/C (500 mg) for 4 h (normal pressure, r.t.). The catalyst was filtered off and the filtrate evaporated. Recrystallization from H_2O gave colorless needles (900 mg, 79%). M.p. 180° . TLC (*A*): R_f 0.8. UV (MeOH): 220 (27500), 293 (4400). $^1\text{H-NMR}$ ((D_6) DMSO): 3.90 (s, MeO); 6.47 (dd, $J = 1.8, 3.5$, H-C(5)); 7.30 (dd, $J = 2.3, 3.5$, H-C(6)); 8.75 (s, H-C(4)); 11.85 (br. s, NH). Anal. calc. for $\text{C}_7\text{H}_7\text{N}_3\text{O}$ (149.2): C 56.35, H 4.73, N 28.16; found: C 56.39, H 4.74, N 28.20.

2-Methoxy-7-methyl-7H-pyrrolo[2,3-d]pyrimidine (8b). As described for **7**, with KOH (700 mg, 12.5 mmol), TDA-1 (100 μl , 0.31 mmol), **8a** (400 mg, 2.68 mmol), MeCN (40 ml; within 15 min), and MeI (300 μl , 4.65 mmol; 1 h stirring at r.t.). The residue was adsorbed on silica gel and purified by FC (column 4×5 cm, CHCl_3). Recrystallization from CHCl_3 gave **8b** as colorless crystals. M.p. 83° (dec.). TLC (*B*): R_f 0.3. UV (MeOH): 224 (27200), 278 (4600). $^1\text{H-NMR}$ ((D_6) DMSO): 3.71 (s, MeN); 3.93 (s, MeO); 6.50 (d, $J = 3.6$, H-C(5)); 7.34 (d, $J = 3.6$, H-C(6)); 8.73 (s, H-C(4)). Anal. calc. for $\text{C}_8\text{H}_9\text{N}_3\text{O}$ (163.2): C 58.89, H 5.55, N 25.75; found: C 58.95, H 5.55, N 25.91.

7-[2-Deoxy-3,5-di-O-(4-toluoyl)- β -D-erythro-pentofuranosyl]-2-methoxy-7H-pyrrolo[2,3-d]pyrimidine (9a). *Method A:* As described for **7**, with KOH (500 mg, 8.91 mmol), TDA-1 (100 μl , 0.31 mmol), **8a** (310 mg, 2.08 mmol), MeCN (40 ml; within 15 min), and **6** (800 mg, 2.04 mmol; 15 min stirring). FC (column 3×15 cm, CHCl_3) and recrystallization from MeOH yielded colorless needles (780 mg, 75%).

Method B: Compound **7** (1.00 g, 1.87 mmol) was dissolved in MeOH (50 ml). After addition of MeOH saturated with NH_3 at 0° (1.2 ml), the mixture was hydrogenated in presence of 10% Pd/C (250 mg) for 30 min (normal pressure, r.t.). The catalyst was filtered off, the filtrate evaporated, and the residue adsorbed at silica gel (2 g). FC (column 4×5 cm, CHCl_3) and recrystallization from MeOH gave colorless needles (860 mg, 92%). M.p. $106\text{--}108^\circ$ (softening at 60°). TLC (*B*): R_f 0.4. UV (MeOH): 226 (41400), 240 (33000). $^1\text{H-NMR}$ ((D_6) DMSO): 2.37 (s, 2 arom. Me); 2.71 (m, $\text{H}_\alpha\text{-C}(2'')$); 3.18 (m, $\text{H}_\beta\text{-C}(2'')$); 3.96 (s, MeO); 4.58 (m, H-C(4')), 2 H-C(5''); 5.80 (m, H-C(3'')); 6.60 (d, $J = 3.8$, H-C(5)); 6.66 (m, H-C(1'')); 7.33, 7.90 (m, 8 arom. H); 7.58 (d, $J = 3.8$, H-C(6)); 8.79 (s, H-C(4)). Anal. calc. for $\text{C}_{28}\text{H}_{27}\text{N}_3\text{O}_6$ (501.5): C 67.05, H 5.43, N 8.38; found: C 67.25, H 5.46, N 8.53.

7-(2-Deoxy- β -D-erythro-pentofuranosyl)-2-methoxy-7H-pyrrolo[2,3-d]pyrimidine (9b). A soln. of **9a** (1.00 g, 2.00 mmol) in MeOH saturated with NH_3 at 0° (50 ml) was stirred at r.t. After 24 h, the turbid soln. became clear, the solvent was evaporated, and the residue adsorbed at silica gel (2 g). FC (column 2×20 cm, *A*) and recrystallization from MeOH gave colorless needles (430 mg, 81%). M.p. 171° . TLC (*C*): R_f 0.7. UV (MeOH): 224 (30700), 290 (5900). $^1\text{H-NMR}$ ((D_6) DMSO): 2.00 (m, $\text{H}_\alpha\text{-C}(2'')$); 2.56 (m, $\text{H}_\beta\text{-C}(2'')$); 3.54 (m, 2 H-C(5'')); 3.83 (m, H-C(4'')); 3.93 (s, MeO); 4.38 (m, H-C(3'')); 4.95 (t, $J = 6.3$, OH-C(5'')); 5.26 (d, $J = 3.1$, OH-C(3'')); 6.55 (m, H-C(1'')); 6.58 (d, $J = 4.7$, H-C(5)); 7.60 (d, $J = 4.7$, H-C(6)); 8.78 (s, H-C(4)). Anal. calc. for $\text{C}_{12}\text{H}_{15}\text{N}_3\text{O}_4$ (265.3): C 54.34, H 5.70, N 15.84; found: C 54.32, H 5.72, N 15.67.

7-(2-Deoxy- β -D-erythro-pentofuranosyl)-7H-pyrrolo[2,3-d]pyrimidin-2(3H)-one (3). A soln. of **9b** (250 mg, 0.94 mmol) in 2N NaOH (10 ml) and DMSO (0.1 ml) was heated under reflux for 5 h. The soln. was cooled, neutralized with AcOH, and evaporated. The residue was co-evaporated with toluene and dissolved in H_2O (100 ml). Insoluble material was filtered off and the mixture applied to *Amberlite XAD-4* (column 2×15 cm, *D*). From the main zone, a colorless residue was obtained which was recrystallized from H_2O : colorless needles (200 mg, 85%). M.p. 248° . TLC (*C*): R_f 0.2. UV (MeOH): 260 (2700), 337 (2900). UV (H_2O): 260 (3100), 330 (2900). $^1\text{H-NMR}$ ((D_6) DMSO): 2.10 (m, $\text{H}_\alpha\text{-C}(2'')$); 2.37 (m, $\text{H}_\beta\text{-C}(2'')$); 3.52 (m, 2 H-C(5'')); 3.80 (m, H-C(4'')); 4.31 (m, H-C(3'')); 4.99 (br. s, OH-C(5'')); 5.27 (br. s, OH-C(3'')); 6.33 (m, H-C(5), H-C(1'')); 7.34 (d, $J = 4.0$, H-C(6)); 8.21 (s, H-C(4)); 11.71 (br. s, NH). Anal. calc. for $\text{C}_{11}\text{H}_{13}\text{N}_3\text{O}_4$ (251.2): C 52.60, H 5.22, N 16.73; found: C 52.40, H 5.33, N 16.58.

2-(*Methylthio*)-9H-purine (**10a**). A mixture of 4,5-diamino-2-(methylthio)pyrimidine (1.0 g, 6.4 mmol), triethyl orthoformate (12 ml, 6.4 mmol), and TsOH (100 mg, 72.2 mmol) was refluxed for 40 min. The light yellow mixture was cooled and filtered and the residue washed with Et₂O: chromatographically pure solid (0.95 g, 89%). M.p. 253–255° ([12]: 250–255°). ¹H-NMR ((D₆)DMSO): 8.94 (s, H-C(6)); 8.47 (s, H-C(8)); 2.54 (s, MeS).

9-[2-Deoxy-3,5-di-O-(4-toluoyl)-β-D-erythro-pentofuranosyl]-2-(methylthio)-9H-purine (**11a**) and 7-[2-Deoxy-3,5-di-O-(4-toluoyl)-β-D-erythro-pentofuranosyl]-2-(methylthio)-7H-purine (**12a**). At r.t., **10a** (1.66 g, 10 mmol) was suspended in MeCN (110 ml) and treated with NaH (500 mg, 10 mmol; 50% in oil) under stirring. After 15 min, **6** (3.95 g, 10.1 mmol) was added in portions and stirring continued for 20 min. The mixture was filtered through *Celite*, the filtrate evaporated, and the oil chromatographed (silica gel 60H, column 5 × 20 cm, cyclohexane/AcOEt 100:0 → 2:3 (3.0 l)). The fast migrating zone gave a residue which was crystallized from MeOH. Colorless needles of **11a** (2.85 g, 55%). M.p. 91–93°. TLC (*E*): *R*_f 0.5. ¹H-NMR ((D₆)DMSO): 2.34, 2.39, 2.60 (3s, 3 Me); 2.83, 3.40 (2m, 2 H-C(2')); 4.55 (m, H-C(4')), 2 H-C(5')); 5.88 (br. s, H-C(3')); 6.58 ('r', *J* = 6.6, H-C(1')); 7.3–8.0 (arom. H); 8.64, 9.00 (2s, H-C(6), H-C(8)). Anal. calc. for C₂₇H₂₆N₄O₅S (518.6): C 62.53, H 5.05, N 10.80; found: C 62.66, H 5.15, N 10.81.

From the slow migrating zone, **12a** was isolated as solid foam (800 mg, 15%). ¹H-NMR ((D₆)DMSO): 2.35, 2.39, 2.52 (3s, 3 Me); 2.85, 3.05 (2m, 2 H-C(2')); 4.60 (m, H-C(4')), 2 H-C(5')); 5.70 (br. s, H-C(3')); 6.60 ('r', *J* = 6.4, H-C(1')); 7.2–8.0 (arom. H); 8.83, 9.10 (2s, H-C(6), H-C(8)). Anal. calc. for C₂₇H₂₆N₄O₅S (518.6): C 62.53, H 5.05, N 10.80; found: C 62.62, H 5.01, N 10.88.

9-(2-Deoxy-β-D-erythro-pentofuranosyl)-2-(methylthio)-9H-purine (**13a**). The suspension of **11a** (2.4 g, 4.6 mmol) in MeOH (100 ml) was stirred with 1M NaOMe in MeOH (10 ml) for 2 h. The mixture was neutralized with AcOH and chromatographed (silica gel, column 3 × 20 cm, CH₂Cl₂ (200 ml) and CH₂Cl₂/MeOH 9:1). Crystallization from EtOH gave colorless needles (1.04 g, 80%). M.p. 180°. TLC (*F*): *R*_f 0.4. UV (H₂O): 230 (17200), 260 (9900), 303 (7400). ¹H-NMR ((D₆)DMSO): 2.35 (m, H_β-C(2')); 2.80 (m, H_β-C(2')); 2.58 (s, MeS); 3.53, 3.60 (2m, 2 H-C(5')); 3.87 (q, H-C(4')); 4.45 (m, H-C(3')); 4.88 (t, OH-C(5')); 5.33 (d, OH-C(3')); 6.58 ('r', *J* = 6.8, H-C(1')); 8.64 (s, H-C(8)); 8.97 (s, H-C(6)). Anal. calc. for C₁₁H₁₄N₄O₅S (282.3): C 46.80, H 5.00, N 19.85; found: C 46.92, H 5.06, N 19.84.

7-(2-Deoxy-β-D-erythro-pentofuranosyl)-2-(methylthio)-7H-purine (**14**). A soln. of **12a** (650 mg, 1.25 mmol) in MeOH was stirred with 1M NaOMe in MeOH (3 ml) at r.t. for 1 h. The mixture was neutralized with AcOH and evaporated. The residue was chromatographed (silica gel, column 3 × 15 cm, CH₂Cl₂ (200 ml) and CH₂Cl₂/MeOH 9:1): solid foam (310 mg, 88%). TLC (*A*): *R*_f 0.3. UV (H₂O): 239 (22000), 313 (4400). ¹H-NMR ((D₆)DMSO): 2.35 (m, H_β-C(2')); 2.60 (m, H_β-C(2')); 2.53 (s, MeS); 3.57 (br. s, 2 H-C(5')); 3.89 (br. s, H-C(4')); 4.39 (br. s, H-C(3')); 5.04 (t, OH-C(5')); 5.37 (q, OH-C(3')); 6.39 ('r', *J* = 6.0, H-C(1')); 8.80, 9.18 (2s, H-C(6), H-C(8)). Anal. calc. for C₁₁H₁₄N₄O₅S (282.3): C 46.80, H 5.00, N 19.85; found: C 46.92, H 5.06, N 19.84.

2-Chloro-9-[2-deoxy-3,5-di-O-(4-toluoyl)-β-D-erythro-pentofuranosyl]-9H-purine (**11b**) and 2-Chloro-7-[2-deoxy-3,5-di-O-(4-toluoyl)-β-D-erythro-pentofuranosyl]-7H-purine (**12b**). As described for **11a/12a**, with 2-chloro-9H-purine [**13**] (**10b**; 925 mg, 6 mmol), MeCN (90 ml), NaH (350 mg, 7 mmol; 50% in oil; 20 min at r.t.), and **6** (2.45 g, 6.24 mmol; 30 min). Chromatography (silica gel, column 5 × 22 cm, cyclohexane/AcOEt 100:0 → 2:3 (3 l)) gave first **11b** as a solid foam (1.63 g, 54%). TLC (*E*): *R*_f 0.5. ¹H-NMR ((D₆)DMSO): 2.41, 2.45 (2s, 2 Me); 2.90, 3.35 (2m, 2 H-C(2')); 4.70 (m, H-C(4')), 2 H-C(5')); 5.87 (br. s, H-C(3')); 6.66 ('r', *J* = 6.6, H-C(1')); 7.3–8.1 (arom. H); 8.91, 9.17 (2s, H-C(6), H-C(8)). Anal. calc. for C₂₆H₂₃ClN₄O₅ (506.9): C 61.60, H 4.57, N 11.05; found: C 61.67, H 4.71, N 11.01.

The slow migrating zone gave **12b** as solid foam (480 mg, 16%). TLC (*E*): *R*_f 0.4. ¹H-NMR ((D₆)DMSO): 2.51, 2.61 (2s, 2 Me); 3.05, 3.20 (2m, 2 H-C(2')); 4.75 (m, H-C(4')), H-C(5')); 5.82 (q, H-C(3')); 6.76 ('r', *J* = 6.4, H-C(1')); 7.3–8.1 (arom. H); 9.12, 9.32 (2s, H-C(6), H-C(8)). Anal. calc. for C₂₆H₂₃ClN₄O₅ (506.9): C 61.60, H 4.57, N 11.05; found: C 61.67, H 4.71, N 11.01.

2-Chloro-9-(2-deoxy-β-D-erythro-pentofuranosyl)-9H-purine (**13b**). As described for **13a**, with **11b** (1.52 g, 3 mmol), MeOH (80 ml), and 1N NaOMe (3 ml; 1.5 h). Crystallization from EtOH/AcOEt gave a white powder (610 mg, 75%). TLC (*F*): *R*_f 0.4. UV (H₂O): 272 (8000). ¹H-NMR ((D₆)DMSO): 2.60 (m, H_β-C(2')); 2.95 (m, H_β-C(2')); 3.75, 3.85 (2m, 2 H-C(5')); 4.12 (br. s, H-C(4')); 4.66 (br. s, H-C(3')); 5.14 (t, OH-C(5')); 5.57 (q, OH-C(3')); 6.64 ('r', *J* = 6.2, H-C(1')); 9.07 (s, H-C(8)); 9.33 (s, H-C(6)). Anal. calc. for C₁₀H₁₁ClN₄O₅ (270.7): C 44.37, H 4.10, N 20.70; found: C 44.29, H 4.07, N 20.64.

2-Amino-9-(2-deoxy-β-D-erythro-pentofuranosyl)-6-mercapto-9H-purine (**16**). According to [17] using the following amounts: 2'-Deoxyguanosine (**15**; 1.06 g, 4 mmol), pyridine (80 ml), trifluoroacetic anhydride (4.6 ml, 32 mmol), NaSH (6.8 g, 120 mmol) suspended in anh. DMF (120 ml). The reaction was monitored by TLC (*H*). The crude product was purified by FC (column 8 × 5 cm, CH₂Cl₂/MeOH 95:5, CH₂Cl₂/MeOH 4:1). Colorless needles (0.74 g, 65%) from H₂O. M.p. 190–191°. TLC (*H*): *R*_f 0.5. UV (MeOH): 345 (35900), 258 (10500). UV

(H₂O): 342 (38400), 260 (11700). ¹H-NMR ((D₆)DMSO): 2.32, 2.03 (2*m*, 2 H–C(2′)); 3.12 (*m*, H–C(4′)); 3.36, 3.30 (2*m*, 2 H–C(5′)); 4.15 (br., H–C(3′)); 4.70 (br., OH–C(5′)); 5.06 (br., OH–C(3′)); 5.91 (*t*, *J* = 6.75, H–C(1′)); 6.58 (br., NH₂); 7.90 (*s*, H–C(8)). Anal. calc. for C₁₀H₁₃N₅O₃S (283.3): C 42.39, H 4.62, N 24.72, S 11.31; found: C 42.34, H 4.65, N 24.78, S 11.37.

2-Amino-9-(2-deoxy-β-D-erythro-pentofuranosyl)-9H-purine (**4**) was prepared as described [17]. Colorless crystals from MeOH/Et₂O. M.p. 153–155° ([18]: 157–159°). TLC (*I*): R_f 0.9. ¹H-NMR ((D₆)DMSO): 2.63, 2.23 (2*m*, 2 H–C(2′)); 3.83 (*m*, H–C(4′)); 3.53 (*m*, 2 H–C(5′)); 4.37 (br., H–C(3′)); 5.00 (br., OH–C(5′)); 5.32 (br., OH–C(3′)); 6.26 (*t*, *J* = 6.76, H–C(1′)); 6.54 (br., NH₂); 8.28 (*s*, H–C(8)); 8.58 (*s*, H–C(6)). Anal. calc. for C₁₀H₁₃N₅O₃ (251.2): C 47.81, H 5.21, N 27.88; found: C 47.89, H 5.18, N 27.85.

9-(2-Deoxy-β-D-erythro-pentofuranosyl)-9H-purin-2(3H)-one (= 2′-Deoxyisoinosine; **2**). To a soln. of **4** (0.48 g, 1.92 mmol) and NaNO₂ (0.6 g, 8.6 mmol) in H₂O (12 ml), AcOH (0.88 ml) was added dropwise at 50° while stirring (TLC (*I*) monitoring). After 30 min, the mixture was neutralized with NH₃/H₂O. The soln. was separated on *Serdolit AR-4* (column 20 × 3 cm, washing with H₂O (removal of inorg. salts), then H₂O/i-PrOH 9:1) and **2** recrystallized from MeOH/Et₂O: colorless powder (400 mg, 83%). TLC (*I*): R_f 0.7. UV (MeOH): 322 (4200), 244 (2900). UV (H₂O): 314 (4600), 242 (2900). ¹H-NMR ((D₆)DMSO): 2.29 (*m*, H_a–C(2′)); 2.66 (*m*, H_b–C(2′)); 3.66, 3.58 (2*m*, 2 H–C(5′)); 3.91 (*m*, H–C(4′)); 4.44 (*m*, H–C(3′)); 6.23 (*t*, *J* = 6.7, H–C(1′)); 8.43 (*s*, H–C(8)); 8.51 (*s*, H–C(6)). Anal. calc. for C₁₀H₁₂N₄O₄ (252.2): C 47.63, H 4.80, N 22.22; found: C 47.55, H 4.98, N 22.16.

REFERENCES

- [1] M. D. Topal, J. R. Fresco, *Nature (London)* **1976**, 263, 289.
- [2] R. Cardinaud, *J. Chromatogr.* **1978**, 154, 345.
- [3] A. Holy, *Collect. Czech. Chem. Commun.* **1979**, 44, 2846.
- [4] A. Albert, W. L. F. Amarego, in 'Advances in Heterocyclic Chemistry', Academic Press, New York, 1965, Vol. 4, pp. 1–42.
- [5] A. Gogoll, L. Gundersen, F. Rise, M. Valli, *Heterocycles* **1993**, 36, 231.
- [6] D. C. Ward, E. Reich, L. Stryer, *J. Biol. Chem.* **1969**, 244, 1228.
- [7] A. Graslund, F. Claessens, L. W. McLaughlin, P. B. Lycksell, U. Larsson, R. Rigler, in 'Structure, Dynamics and Function of Biomolecules, Springer Series in Biophysics', Eds. A. Ehrenberg, R. Rigler, A. Graslund, and L. Nilsson, Springer Verlag, Berlin, 1987, Vol. 1, p. 201.
- [8] F. Seela, U. Liman, *Liebigs Ann. Chem.* **1984**, 273.
- [9] F. Seela, H. Driller, U. Liman, *Liebigs Ann. Chem.* **1985**, 312.
- [10] H. Winkler, F. Seela, *J. Org. Chem.* **1983**, 48, 319; F. Seela, B. Westermann, U. Bindig, *J. Chem. Soc., Perkin Trans. 1* **1988**, 697.
- [11] D. Martin, H. G. Hanthall, 'Dimethylsulfoxid', Akademie Verlag, Berlin, 1971.
- [12] A. Albert, D. J. Brown, *J. Chem. Soc.* **1954**, 2060.
- [13] A. G. Beaman, R. K. Robins, *J. Appl. Chem.* **1962**, 432; S. R. Breshears, S. S. Wang, S. G. Becholt, B. E. Christensen, *J. Am. Chem. Soc.* **1959**, 81, 3789.
- [14] Z. Kazimierzczuk, R. Mertens, W. Kawczynski, F. Seela, *Helv. Chim. Acta* **1991**, 74, 1742.
- [15] J. J. Fox, I. Wempen, A. Hampton, I. L. Doerr, *J. Am. Chem. Soc.* **1958**, 80, 1669.
- [16] T. R. Waters, B. A. Connolly, *Nucleos. Nucleot.* **1992**, 11, 985.
- [17] P.-P. Kung, R. A. Jones, *Tetrahedron Lett.* **1991**, 32, 3919.
- [18] F. Seela, B. Gabler, Z. Kazimierzczuk, *Collect. Czech. Chem. Commun.* **1993**, 58, 170.
- [19] H. Rosemeyer, G. Toth, F. Seela, *Nucleos. Nucleot.* **1989**, 8, 587.
- [20] Z. Kazimierzczuk, F. Seela, *Liebigs Ann. Chem.* **1990**, 647.
- [21] F. Seela, W. Bussmann, *Nucleos. Nucleot.* **1985**, 4, 391.
- [22] R. C. Pless, M. J. Bessman, *Biochemistry* **1983**, 22, 4905.
- [23] C. R. Guest, R. A. Hochstrasser, L. C. Sowers, D. P. Millar, *Biochemistry* **1991**, 30, 3271.
- [24] S. M. Watanabe, M. F. Goodman, *Proc. Natl. Acad. Sci. U.S.A.* **1981**, 78, 2864.
- [25] T. M. Nordlund, S. Andersson, L. Nilsson, R. Rigler, A. Gräslund, L. W. McLaughlin, *Biochemistry* **1989**, 28, 9095.
- [26] F. Seela, H. Steker, *Liebigs Ann. Chem.* **1984**, 1719.
- [27] F. Seela, U. Engelke, *Liebigs Ann. Chem.* **1985**, 1175.
- [28] J. M. Prober, G. L. Trainor, R. J. Dam, F. W. Hobbs, C. W. Robertson, R. J. Zagursky, A. J. Cocuzza, M. A. Jensen, K. Baumeister, *Science* **1987**, 238, 336.
- [29] P. Strazewski, C. Tamm, *Angew. Chem.* **1990**, 102, 37.
- [30] S. Kazimierzczuk, U. Bindig, F. Seela, *Helv. Chim. Acta* **1989**, 72, 1527.