

16. Synthesis of 6-Substituted 1-Deazapurine 2'-Deoxyribonucleosides

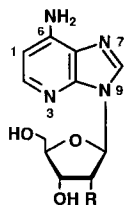
by Thomas Wenzel and Frank Seela*

Laboratorium für Organische und Bioorganische Chemie, Institut für Chemie, Universität Osnabrück,
Barbarastr. 7, D-49069 Osnabrück

(4.X.95)

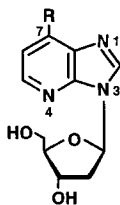
The synthesis of 6-substituted 1-deazapurine 2'-deoxyribonucleosides is described. Glycosylation of the 1-deazapurine (imidazo[4,5-*b*]pyridine) anions with the α -D-halogenose **5** gives stereoselectively *N*⁷- and *N*⁹-regioisomers. ¹H-NMR NOE and ¹³C-NMR spectroscopy are used for unambiguous assignment of isomers, and ¹⁵N-NMR chemical shifts are correlated with σ_{para} Hammett constants and point charges.

Introduction. – The 1-deazapurine nucleosides, such as 1-deaza-2'-deoxyadenosine (**1a**) [1] [2] (purine numbering is used throughout the *General Part*) or the ribonucleoside **1b** [3] [4] can form *Hoogsteen* base pairs within oligonucleotide duplexes [2] [5]. The synthesis of 1-deazapurine deoxyribonucleosides can be performed by *i*) deoxygenation of ribonucleosides [1], *ii*) the microbial transglycosylation of imidazo[4,5-*b*]pyridine bases [6] [7], and *iii*) the stereoselective glycosylation of the nucleobases with sugar halides [8] [9]. In the following, we report on the nucleobase-anion glycosylation of 6-substituted 1-deazapurines and their conversion into the nucleosides **2a–d** or **3a–d**. These reactions should give information on steric and electronic effects of 6-substituents on the distribution of regioisomers. The formation of compounds **2a** and **2c** leads to immediate precursors for the synthesis of 1-deaza-2'-deoxyadenosine (**1a**). The assignment of regioisomeric 1-deazapurine nucleosides by UV or usual ¹H-NMR spectroscopy was shown to be problematic and led to the revision of structures [10] [11]. We now present the unambiguous assignment of regioisomers on the basis of ¹H-NOE and ¹³C-NMR spectroscopy. Also the glycosylic-bond stability of regioisomers will be investigated, and a correlation of ¹⁵N-NMR chemical shifts with point charges and *Hammett* constants will be undertaken.



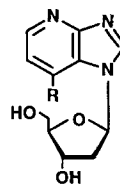
(purine numbering)

1a R = H
b R = OH



(systematic numbering)

2a R = NO₂
b R = H
c R = Cl
d R = MeO



3a R = NO₂
b R = H
c R = Cl
d R = MeO

Results and Discussion. – *Synthesis.* The glycosylation of the nucleobases **4a–c** was performed under conditions developed in our laboratory for the stereoselective synthesis of 2'-deoxyribonucleosides [12] [13] (*Scheme 1*). For this purpose, the bases were dissolved in MeCN and treated with 2-deoxy-3,5-di-*O*-(4-toluoyl)- α -D-erythro-pentofuranosyl chloride (**5**) in the presence of powdered KOH (5 equiv.) and *TDA-1* as catalyst.

Scheme 1

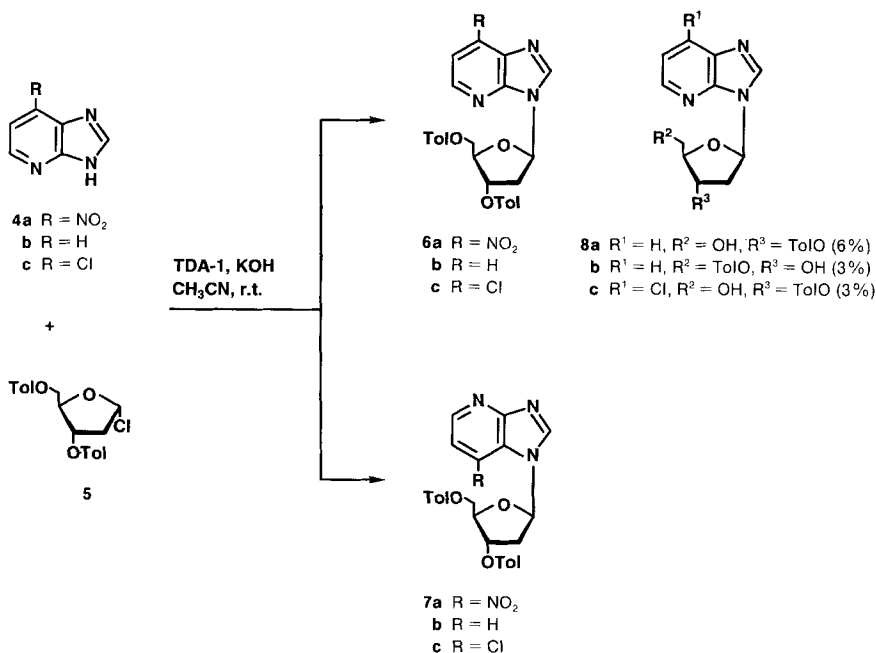


Table 1. Yields^{a)} of Regioisomeric 1-Deazapurine N⁹- and N⁷-Nucleosides during Nucleobase-Anion Glycosylation of **4a–c** with the Halogenose **5**

	Products		Total yield [%]
	N ⁹ -Isomer [%]	N ⁷ -Isomer [%]	
4a ^{b)}	48 (6a)	6 (7a)	54
a ^{c)}	57 (6a)	16 (7a)	73
b ^{c)}	48 (6b)	29 (7b)	77
c ^{c)}	67 (6c)	–	67

^{a)} Determined after FC purification. ^{b)} At 0°; 3 h. ^{c)} At r.t., 20 min.

Table 1 shows the distribution of regioisomers of the various glycosylation experiments and their yields. At room temperature, two glycosylation products, **6a/7a** and **6b/7b**, were formed, in the case of the bases **4a** and **4b**, respectively, and only one, **6c**, in the case of **4c** (*Scheme 1*). The faster-migrating zones on TLC were identified as the N⁹-regioisomers **6a–c** (see below) and separated by flash chromatography (FC). Small quantities (3–6%) of the partially deprotected nucleosides **8a–c** were obtained from the glycosylation of

4b and **4c**. As the m.p. of compound **6a** did not agree with literature data (see *Exper. Part*), a full set of analytical data was collected which confirmed the structure already reported [8].

When the glycosylation of **4a** was carried out at 0° – conditions which had already been used by *Cristalli et al.* [8], also the two regioisomers **6a** and **7a** were formed, a finding which is different from studies described earlier [8] [9]. The total yield decreased from 73% (25°) to 54%. The *N*⁹/*N*⁷-isomer ratio was 8:1 at low temperature compared to 3.6:1 at 25°. However, the highest yield of isomer **6a** was observed at 25°. In the presence of KOH instead of NaH (*cf.* [8] [9]), only marginal changes in the glycosylation products were observed.

Thus, the 6-unsubstituted base **4b** [14] yielded significantly higher amounts of *N*⁷-isomer than **4a** (R = NO₂) and **4c** (R = Cl; see *Table 1*). Apparently, the bulky 6-NO₂ or 6-Cl substituents hinder the glycosylation at N(7) and direct the reaction towards N(9). The more favorable *N*⁹/*N*⁷-isomer ratio obtained on glycosylation of **4a** at lower temperature can be explained by the kinetic control of the glycosylation with N(9) as the most reactive site.

Regarding the synthesis of nucleoside **1a**, the 6-chloro glycosylation product **6c** seems to be superior to the 6-nitro derivative **6a**. However, the nucleophilic displacement of the Cl-atom requires hydrazine for displacement, and a second step is necessary to convert the hydrazino into the amino group which makes this route more laborious. Indeed, hydrogenation of nitro compound **2a** (NH₂NH₂/Raney-Ni catalyst, room temperature) proceeded smoothly to 1-deaza-2'-deoxyadenosine (**1a**) in high yield [15].

Detoluoylation of the compounds **6a–c** and **7a–c** was performed in NH₃/MeOH. In the case of **6a**, the NO₂ group was partially displaced by a MeO group [8] yielding 19% of the methoxy derivative **2d** and 68% of nitro derivative **2a**. On deblocking of the *N*⁷-isomer **7a**, the 6-methoxy nucleoside **3d** was the main product (36%), and the 6-nitro compound **3a** was isolated in only 16% yield. Apparently, the *ipso*-substitution is favored when a bulky residue is attached at N(7). The *ipso*-substitution of the NO₂ group was circumvented when 1,4-dioxane/conc. aqueous NH₃ 2:1 was used instead of NH₃/MeOH. The low yield (37%) of compound **3a** can be attributed to hydrolytic decomposition due to its very labile *N*-glycosylic bond.

It was of interest to compare the half-life values of the 1-deazapurine *N*⁷- and *N*⁹-(2'-deoxyribofuranosides) **1a** and **2a–d**, and **3a–d**, respectively, in aqueous 0.1N or 0.01N HCl at 20°. Data are shown in *Table 2*. The half-life values were determined

Table 2. Half-Life Values (*t*_{1/2}) of Proton-Catalyzed Hydrolysis of the Glycosylic Bond of 1-Deazapurine 2'-Deoxyribofuranosides^{a)}

	<i>t</i> _{1/2} [min] 0.1N HCl	<i>t</i> _{1/2} [min] 0.01N HCl	λ [nm]
1a	4	63	287
dA	160	> 1000	258
2a	1	12	302
b	27	317	275
c	45	638	281
d	3	39	271
3a	^{b)}	1	290
b	3	35	270
d	^{b)}	4	270

^{a)} Determined UV-spectrophotometrically at 20°. ^{b)} Too labile for determination.

UV-spectrophotometrically [16]. Similarly to purine nucleosides, the N^7 -regioisomers are always more labile than the N^9 -compounds. Compared to 2'-deoxyadenosine (dA), the 1-deaza-2'-deoxyadenosine (**1a**) is very labile at the N -glycosylic bond, a factor which plays a role in oligonucleotide synthesis [2].

Structural Assignment and NMR Spectra. Modifications in previous assignments of regioisomeric 1-deazapurine nucleosides were necessary [8–11], due to the methods used for identification. Neither UV spectroscopy nor ^1H -NMR spectra give sufficient information for the assignment of regioisomeric 1-deazapurine nucleosides. Unequivocal identification of the glycosylation site as well as of the anomeric configuration of all nucleosides described herein was made by ^1H -NOE difference spectroscopy (Table 3).

Table 3. ^1H -NMR 1D-NOE Data of 1-Deazapurine 2'-Deoxyribofuranosides^{a)b)}

	Irradiated proton	Observed NOE's [%]
1a	H–C(1')	H–C(8) (7.8), H _α –C(2') (6.7), H–C(4') (2.2), OH–C(3') (1.5)
	H–C(8)	H _β –C(2') (2.4), H–C(3') (0.9), OH–C(5') (1.0)
2a	H–C(1')	H–C(8) (4.6), H _α –C(2') (6.9), H–C(4') (1.8), OH–C(3') (1.2)
	H–C(8)	H–C(1') (4.4), H _β –C(2') (3.4), H–C(3') (1.4), OH–C(5') (1.3)
2b	H–C(1')	H–C(8) (6.1), H _α –C(2') (6.4), H–C(4') (2.5), OH–C(3') (1.6)
	H–C(8)	H–C(1') (5.5), H _β –C(2') (3.5), H–C(3') (1.0), OH–C(5') (1.2)
2c	H–C(1')	H–C(8) (5.9), H _α –C(2') (7.0), H–C(4') (2.4), OH–C(3') (1.6)
	H–C(8)	H–C(1') (5.6), H _β –C(2') (4.0), H–C(3') (1.7), OH–C(5') (1.2)
2d	H–C(1')	H–C(8) (6.5), H _α –C(2') (6.6), H–C(4') (2.6), OH–C(3') (1.4)
	H–C(8)	H–C(1') (6.9), H _β –C(2') (3.9), H–C(3') (1.3), OH–C(5') (1.1)
3a	H–C(1')	H–C(8) (1.1), H _α –C(2') (5.3), H–C(4') (2.2), OH–C(3') (1.7)
	H–C(8)	H–C(1') (1.5), H _β –C(2') (6.0), H–C(3') (2.5), OH–C(5') (1.5)
3b	H–C(1')	H–C(8) (6.8), H–C(6) (5.2), H _α –C(2') (7.2), H–C(4') (2.5), OH–C(3') (1.2)
	H–C(8)	H–C(1') (6.0), H _β –C(2') (3.3), H–C(3') (1.2), OH–C(5') (1.0)
3d	H–C(1')	H–C(8) (2.2), H _α –C(2') (6.4), H–C(4') (2.0), OH–C(3') (0.8), MeO (1.5)
	H–C(8)	H–C(1') (2.5), H _β –C(2') (4.1), H–C(3') (1.6), OH–C(5') (1.3)

^{a)} Spectra are measured in (D₆)DMSO at 23°. ^{b)} Purine numbering.

All N^7 - and N^9 -isomers give NOE's on H–C(8) upon irradiation of H–C(1'). As no NOE's are observed on H–C(2), the presence of N^3 -regioisomers can be excluded. They were observed in the case of ribonucleosides prepared by the *Vorbrüggen* glycosylation of **4a** [9]. The NOE's on the 6-substituents – the MeO group of **3d** and H–C(6) of **3b** – confirm N^7 -glycosylation in these cases. As the methoxy compounds were formed by *ipso*-substitution of nitro compounds, the assignment is also unequivocal for the nitro compounds **2a** and **3a**. The NOE's on H_α–C(2') and H–C(4') upon irradiation of H–C(1') confirm β -D-configuration in all cases [17].

As only a few ^{13}C -NMR data exist of 1-deazapurine nucleosides [7] [15] [18], the ^{13}C -NMR spectra were measured (Table 4). The assignment was performed on the basis of [^1H , ^{13}C] gated-decoupled NMR spectra (Table 5) as well as 2D ^1H , ^{13}C -correlation spectra.

The most straightforward assignment is made for C(1) and the bridgehead atoms C(4) and C(5) which show couplings to H–C(1) and H–C(8) for C(5) (*dd*) and couplings to H–C(2), H–C(8), and H–C(1') for C(4) (*ptd*), respectively. The change of the glycosylation position from N(9) to N(7) results in a significant downfield shift of C(4) (10–15 ppm) and a similar upfield shift of C(5) which is in line with observations on regioisomeric purine N^9 - vs. N^7 -nucleosides [19]. The chemical shifts of C(2) shows minor changes compared to C(1) within the series of 6-substituted 1-deazapurine derivatives (**1a**, **2a–d**, and **3a–d**).

Table 4. ^{13}C -NMR Chemical Shifts δ [ppm] of 1-Deazapurine 2'-Deoxyribonucleosides^{a)}

Comp.	C(2) ^{b)} C(8) ^{c)}	C(3a) ^{b)} C(4) ^{c)}	C(5) ^{b)} C(2) ^{c)}	C(6) ^{b)} C(1) ^{c)}	C(7) ^{b)} C(6) ^{c)}	C(7a) ^{b)} C(5) ^{c)}	CO
1a	139.4	146.4	144.2	102.3	147.2	123.6	
2a	147.8	143.7	144.8	112.1	150.0	127.2	
b	143.9	146.3	143.7	118.9	127.9	135.6	
c	144.6	147.1	144.6	118.7	133.3	133.0	
d	141.5	148.1	144.6	102.3	157.5	125.4	
3a	147.2	160.4	144.5	112.3	141.8	115.6	
b	144.5	156.3	144.4	118.1	120.0	125.2	
d	142.4	157.6	145.8	101.4	153.1	115.0	
4a	148.0	156.9	144.9	111.5	140.0	118.7	
b	143.8	150.9	143.6	117.6	123.9	130.6	
6a	148.4	144.2	144.9	112.4	149.9	127.4	165.4, 165.5
b	144.3	146.3	144.0	118.8	127.8	135.7	165.3, 165.5
c	145.1	147.1	144.8	119.1	133.5	133.3	165.4, 165.6
7a	146.6	160.0	145.0	112.6	142.1	115.6	165.3, 165.5
b	144.7	156.4	144.5	118.2	120.0	125.0	165.4, 165.6
8a	144.1	146.3	143.9	118.8	128.1	135.8	165.4
b	144.0	146.4	143.8	118.7	127.8	135.6	165.6
c	144.6	147.0	143.9	118.8	133.4	133.0	165.2
	C(1')	C(2')	C(3')	C(4')	C(5')	Me	
1a	84.5	^{d)}	71.4	88.1	62.1	–	
2a	84.1	^{d)}	70.5	88.1	61.5	–	
b	83.6	^{d)}	71.0	88.1	61.9	–	
c	84.0	^{d)}	70.7	88.0	61.6	–	
d	83.9	^{d)}	71.0	88.0	62.0	–	
3a	87.4	40.9	69.4	87.8	60.6	–	
b	85.2	40.3	70.6	87.8	61.5	–	
d	86.0	41.1	70.2	87.7	61.3	–	
6a	84.6	35.7	74.9	82.0	64.0	21.2, 21.3	
b	84.0	35.3	75.0	81.6	64.1	21.2, 21.3	
c	84.4	35.5	75.0	81.8	64.1	21.2, 21.3	
7a	87.3	37.2	74.2	82.0	63.7	21.2, 21.3	
b	85.4	36.3	69.6	81.7	64.1	21.2, 21.3	
8a	84.1	36.6	76.0	85.3	61.9	21.3	
b	83.5	38.6	70.7	84.1	64.4	21.2	
c	84.1	36.1	75.6	85.3	61.5	21.1	

^{a)} In (D₆)DMSO relative to SiMe₄. ^{b)} Systematic numbering. ^{c)} Purine numbering. ^{d)} Superimposed by (D₆)DMSO.

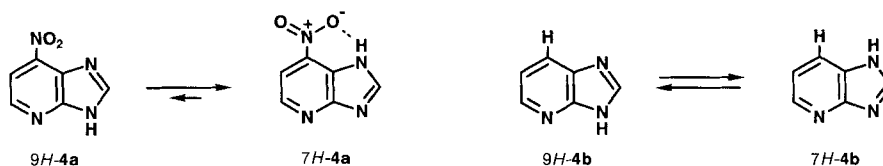
The chemical shifts of the bridgehead C-atoms of the *N*⁷- and *N*⁹-nucleosides allow the assignment of the tautomeric forms of the bases. For this purpose, the ^{13}C -NMR chemical-shift differences of these C-atoms of **2a**, **b** and **3a**, **b** were compared to those of the nucleobases **4a**, **b** in (D₆)DMSO. Good agreement was found using either C(4) or C(5) for the calculation. According to that, the preferred tautomeric form of nitroimidazo[4,5-*b*]pyridine **4a** is the 7*H*-tautomer 7*H*-**4a** (75%; see Scheme 2). Almost equal quantities of the 7*H*- and 9*H*-tautomers are determined for the imidazo[4,5-*b*]pyridine **4b**. The contribution of a 3*H*-tautomer is neglected, as its formation is very unlikely. The higher

Table 5. ^1H , ^{13}C -Coupling Constants [Hz] of 1-Deazapurine 2'-Deoxyribonucleosides^{a)}b)

Coupling atoms	$J(\text{C},\text{H})$				
	1a	2a	2b	2c	2d
C(2)/H-C(2)	210.0	214.6	212.2	213.8	212.6
H-C(1')	3.8	4.2	3.8	3.8	3.8
C(3a)/H-C(2), H-C(5)	$m^c)$	m	m	m	m
C(5)/H-C(5)	184.5	186.8	183.1	186.1	178.6
C(6)/H-C(6)	174.9	171.7	164.0	169.3	163.5
H-C(5)	10.4	10.1	8.9	9.5	8.8
C(7)/H-C(7)	–	–	164.9	–	–
H-C(5)	3.5	3.7	3.8	3.6	3.8
H-C(6)	8.8	9.4	11.3	9.9	8.8
C(7)/H-C(2)	5.7	5.9	6.0	6.2	6.2
H-C(6)	10.7	12.1	10.1	11.3	11.3
C(1')/H-C(1')	167.9	168.0	168.2	166.4	167.3
C(2')/H-C(2')	$d)$	$d)$	$d)$	$d)$	$d)$
C(3')/H-C(3')	149.7	148.3	159.7	149.6	149.7
C(4')/H-C(4')	149.1	148.2	149.8	147.2	147.2
C(5')/H-C(5')	140.9	140.1	141.6	139.0	139.6

^{a)} Data taken from measurements in (D_6)DMSO at 23°. ^{b)} Systematic numbering. ^{c)} m = Multiplett. ^{d)} Superimposed by (D_6)DMSO.

Scheme 2



percentage of the 7H-tautomer in the case of 4a can be attributed to a H-bond to the NO_2 group which was already suggested for 7-nitro-1H-benzimidazole [20].

As 6-substituted 1-deazapurine 2'-deoxyribonucleosides (see 1a and 2a–d) were available in sufficient quantities, the ^{15}N -NMR spectra were measured in (D_6)DMSO (Table 6). The assignment of the ^{15}N signals was made on the basis of [^1H , ^{15}N]-coupled spectra (Table 7).

Table 6. ^{15}N -NMR Chemical Shifts δ [ppm] of 1-Deazapurine 2'-Deoxyribofuranosides^{a)}b)

	N(1) ^{c)} N(6) ^{d)}	N(3) ^{c)} N(4) ^{d)}	N(7) ^{c)} N(1) ^{d)}	N(9) ^{c)} N(3) ^{d)}	NH_2	NO_2
1a	–	–150.1	–142.4	–210.5	–313.7	–
dA	–145.1	–157.8	–140.4	–207.6	–299.1	–
2a	–	–95.2	–142.5	–204.5	–	–13.2
b	–	–112.4	–137.6	–209.5	–	–
c	–	–114.7	–140.7	–205.9	–	–
d	–	–131.9	–142.9	–209.2	–	–

^{a)} Measured in (D_6)DMSO relative to nitromethane at 23°. ^{b)} Assignment from ^1H , ^{15}N -coupled spectra. ^{c)} Purine numbering. ^{d)} Systematic numbering.

Table 7. Multiplicities and Coupling Constants J [Hz] of Several 1-Deazapurine 2'-Deoxyribofuranosides^{a)} ^{b)}

	N(1) H–C(2)	N(3) H–C(2)	N(7) H–C(8)	N(9) H–C(1'), H–C(8)	NH ₂ H
1a		<i>d</i> (<i>J</i> = 10.1)	<i>d</i> (<i>J</i> = 15.2)	<i>dd</i> (<i>J</i> = 4.0, 9.0)	<i>t</i> (<i>J</i> = 90.0)
dA	<i>d</i> (<i>J</i> = 17.0)	<i>d</i> (<i>J</i> = 15.0)	<i>d</i> (<i>J</i> = 12.0)	<i>d</i> (<i>J</i> = 10.0)	<i>t</i> (<i>J</i> = 88.0)
2b		<i>d</i> (<i>J</i> = 10.9)	<i>d</i> (<i>J</i> = 13.2)	<i>d</i> (<i>J</i> = 6.5)	–
c		<i>d</i> (<i>J</i> = 11.0)	<i>d</i> (<i>J</i> = 13.7)	<i>d</i> (<i>J</i> = 7.0)	–

^{a)} Measured in (D₆)DMSO relative to nitromethane at 23°. ^{b)} Purine numbering.

The imidazole N-atoms of **1a** show chemical shifts very similar to those of 2'-deoxyadenosine (dA) [21]. N(3) can be assigned according to the coupling with H–C(2), whereas N(7) shows a coupling with H–C(8). The exocyclic NH₂ group of **1a** and dA exhibits a *t*. It can be seen from Table 6 that the exocyclic NH₂ group of **1a** is shifted upfield compared to the parent dA. This is in line with an increase of the electron density within the π -system of the 1-deazapurine system over that of purines. A strong shift is observed on the δ of N(3) when the 6-substituent changes (Table 6).

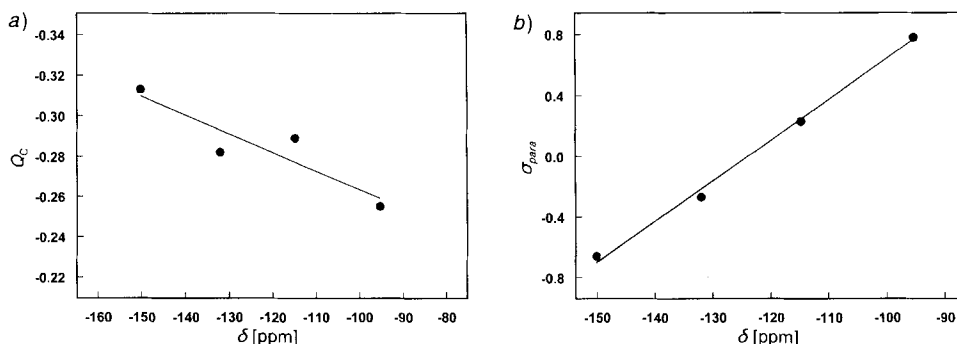


Fig. a) Correlation of electron charges Q_C (N(3)) with ^{15}N -NMR chemical shifts at N(3) for **1a**, **2a**, **2b**, and **2d**;
b) correlation of Hammett constants σ_{para} (N(3)) with N(3) ^{15}N -NMR chemical shifts for **1a**, **2a**, **2b**, and **2d**

The ^{15}N -NMR chemical shifts of the nucleosides **1b**, **2a**, **2c**, and **2d** revealed an almost linear dependence on the point-charges at position N(3) which were calculated using Alchemy III [22–24] (Fig. a). A linear correlation ($r^2 = 0.99$) was also found in the graph of the ^{15}N -NMR chemical shifts of N(3) vs. the σ_{para} Hammett constants (Fig. b). These results support the assignment of ^{15}N -NMR chemical shifts of the 6-substituted 1-deazapurine 2'-deoxyribonucleosides **1a** and **2a–d**.

We thank Dr. H. Rosemeyer for helpful discussions and the Deutsche Forschungsgemeinschaft for financial support.

Experimental Part

General. See [2]. Flash chromatography (FC): at 0.5 bar on silica gel 60H (Merck, Darmstadt, Germany). Thin-layer chromatography (TLC): glass plates coated with a 0.25-mm layer of silica gel G-25 with fluorescent indicator UV 254 (Macherey-Nagel, Germany). Solvent systems for flash chromatography and TLC: light petroleum ether/AcOEt 3:7 (*A*), light petroleum ether/AcOEt 1:1 (*B*), light petroleum ether/AcOEt 7:3 (*C*), CHCl₃/MeOH/NH₃ 88:10:2 (*D*), and CHCl₃/MeOH/NH₃ 78:20:2 (*E*).

7-Nitro-1H-imidazo[4,5-b]pyridine (4a) was prepared as described [25]. ¹H-NMR ((D₆)DMSO): 8.05 (*d*, *J* = 5.3, H–C(6)); 8.73 (*d*, *J* = 5.4, H–C(5)); 9.00 (*s*, H–C(2)); 13.50 (*s*, NH).

Glycosylation of 4a with 2-Deoxy-3,5-di-O-(4-toluoyl)-α-D-erythro-pentofuranosyl Chloride (5). *Method A:* Powdered KOH (1.4 g, 25.0 mmol) and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1; 100 μl, 0.24 mmol) were added to a soln. of **4a** (800 mg, 4.9 mmol) in dry MeCN (100 ml) at r.t. with stirring. After 15 min, **5** [26] (2.1 g, 5.4 mmol) was added in portions, and stirring was continued for another 20 min at r.t. Insoluble material was filtered off and the solvent evaporated. The oily residue was chromatographed (silica gel 60H, column 30 × 3 cm, *B*).

Method B: As described for *Method A*, except that the reaction temp. was 0° and the reaction time 3 h.

3-[2-Deoxy-3,5-di-O-(4-toluoyl)-β-D-erythro-pentofuranosyl]-7-nitro-3H-imidazo[4,5-b]pyridine (6a): From the faster migrating zone (solvent *B*), **6a** was obtained as a colourless foam (*Method A*: 1.43 g, 57%; *Method B*: 1.21 g, 48%). Colorless needles. M.p. 123° (MeOH); [8]: 69–71°. TLC (*B*): *R*_f 0.5. ¹H-NMR ((D₆)DMSO): 2.35, 2.39 (2*s*, 2 Me); 2.87 (*m*, H_α–C(2′)); 3.45 (*m*, H_β–C(2′)); 4.55–4.68 (*m*, H–C(4′), 2 H–C(5′)); 5.85 (*m*, H–C(3′)); 6.74 (*t*′, *J* = 6.7, H–C(1′)); 7.25–7.37 (2*d*, *J* = 7.9, arom. H); 7.79–7.96 (2*d*, *J* = 7.7, arom. H); 8.01 (*d*, *J* = 5.3, H–C(6)); 8.61 (*d*, *J* = 5.2, H–C(5)); 9.02 (*s*, H–C(2)). Anal. calc. for C₂₇H₂₄N₄O₇ (516.5): C 62.79, H 4.68, N 10.85; found: C 62.91, H 4.65, N 10.79.

1-[2-Deoxy-3,5-di-O-(4-toluoyl)-β-D-erythro-pentofuranosyl]-7-nitro-1H-imidazo[4,5-b]pyridine (7a): The slower migrating zone (solvent *A*) yielded **7a** as a light yellow foam (*Method A*: 400 mg, 16%; *Method B*: 150 mg, 6%). TLC (*A*): *R*_f 0.3. ¹H-NMR ((D₆)DMSO): 2.35, 2.39 (2*s*, 2 Me); 2.95 (*m*, H_α–C(2′)); 3.24 (*m*, H_β–C(2′)); 4.43–4.67 (*m*, H–C(4′), 2 H–C(5′)); 5.62 (*m*, H–C(3′)); 6.60 (*t*′, *J* = 5.8, H–C(1′)); 7.23–7.37 (2*d*, *J* = 8.0, arom. H); 7.62 (*d*, *J* = 8.0, arom. H); 7.84 (*d*, *J* = 5.2, H–C(6)); 7.91 (*d*, *J* = 8.0, arom. H); 8.65 (*d*, *J* = 5.4, H–C(5)); 9.07 (*s*, H–C(2)). Anal. calc. for C₂₇H₂₄N₄O₇ (516.5): C 62.79, H 4.68, N 10.85; found: C 62.73, H 4.74, N 10.87.

3-(2-Deoxy-β-D-erythro-pentofuranosyl)-7-nitro-3H-imidazo[4,5-b]pyridine (2a) and 3-(2-Deoxy-β-D-erythro-pentofuranosyl)-7-methoxy-3H-imidazo[4,5-b]pyridine (2d) were prepared as described [8].

2a: Pale yellow crystals. M.p. 132–133° (acetone). UV (MeOH): 309 (5800). ¹H-NMR ((D₆)DMSO): 2.43 (*m*, H_α–C(2′)); 2.79 (*m*, H_β–C(2′)); 3.52–3.70 (*m*, 2 H–C(5′)); 3.93 (*d*, *J* = 3.6, H–C(4′)); 4.48 (*m*, H–C(3′)); 5.02 (*t*′, *J* = 5.4, OH–C(5′)); 5.40 (*d*, *J* = 4.1, OH–C(3′)); 6.59 (*t*′, *J* = 6.5, H–C(1′)); 8.02 (*d*, *J* = 5.3, H–C(6)); 8.68 (*d*, *J* = 5.3, H–C(5)); 9.04 (*s*, H–C(2)).

2d: UV (MeOH): 253 (10300). ¹H-NMR ((D₆)DMSO): 2.30 (*m*, H_α–C(2′)); 2.75 (*m*, H_β–C(2′)); 3.55 (*m*, 1 H–C(5′)); 3.63 (*m*, 1 H–C(5′)); 3.90 (*m*, H–C(4′)); 4.07 (*s*, MeO); 4.44 (*m*, H–C(3′)); 5.18 (*t*′, *J* = 5.8, OH–C(5′)); 5.30 (*d*, *J* = 4.0, OH–C(3′)); 6.48 (*t*′, *J* = 7.0, H–C(1′)); 6.88 (*d*, *J* = 5.5, H–C(6)); 8.21 (*d*, *J* = 5.5, H–C(5)); 8.49 (*s*, H–C(2)).

1-(2-Deoxy-β-D-erythro-pentofuranosyl)-7-nitro-1H-imidazo[4,5-b]pyridine (3a). *Method A:* A soln. of **7a** (800 mg, 1.55 mmol) in 1,4-dioxane (50 ml) was treated with 25% aq. ammonia (25 ml) and stirred for 72 h at 50°. The soln. was evaporated and the residue adsorbed on silica gel and submitted to FC (silica gel, column 15 × 3 cm, *E*). The main zone was evaporated and crystallized from acetone: yellow crystals of **3a** (160 mg, 37%).

Method B: A soln. of **7a** (800 mg, 1.55 mmol) in MeOH (60 ml; sat. with ammonia at 0°) was stirred for 48 h at r.t. After evaporation, the product was purified by FC (silica gel, column 20 × 3 cm, *E*). The faster migrating zone yielded **3a** as yellow solid, which was crystallized from acetone (70 mg, 16%). M.p. 166°. TLC (*E*): *R*_f 0.4. UV (MeOH): 287 (3300), 319 (3400). ¹H-NMR ((D₆)DMSO): 2.45 (*m*, H_α–C(2′)); 2.68 (*m*, H_β–C(2′)); 3.48 (*m*, 2 H–C(5′)); 3.84 (*d*, *J* = 3.0, H–C(4′)); 4.35 (*m*, H–C(3′)); 4.87 (*s*, OH–C(5′)); 5.30 (*d*, *J* = 3.8, OH–C(3′)); 6.42 (*t*′, *J* = 4.9, H–C(1′)); 7.89 (*d*, *J* = 4.8, H–C(6)); 8.68 (*d*, *J* = 4.9, H–C(5)); 9.12 (*s*, H–C(2)). Anal. calc. for C₁₁H₁₂N₄O₅ (280.24): C 47.15, H 4.32, N 19.99; found: C 47.19, H 4.56, N 19.98.

1-(2-Deoxy-β-D-erythro-pentofuranosyl)-7-methoxy-1H-imidazo[4,5-b]pyridine (3d): The slower migrating zone (see **3a**, *Method B*) yielded **3d** as a colorless solid which was crystallized from acetone (150 mg, 36%). M.p. 148–149°. TLC (*E*): *R*_f 0.35. UV (MeOH): 258 (sh, 8300), 266 (8500). ¹H-NMR ((D₆)DMSO): 2.37 (*m*, H_α–C(2′)); 2.62 (*m*, H_β–C(2′)); 3.59 (*m*, 2 H–C(5′)); 3.89 (*m*, H–C(4′)); 4.01 (*s*, MeO); 4.37 (*m*, H–C(3′)); 5.03 (*s*, OH–C(5′)); 5.36 (*d*, *J* = 3.6, OH–C(3′)); 6.62 (*t*′, *J* = 6.5, H–C(1′)); 6.96 (*d*, *J* = 5.5, H–C(6)); 8.31 (*d*, *J* = 5.2, H–C(5)); 8.69 (*s*, H–C(2)). Anal. calc. for C₁₂H₁₅N₃O₄ (265.3): C 54.33, H 5.70, N 15.84; found: C 54.34, H 5.70, N 15.89.

1*H*-Imidazo[4,5-*b*]pyridine (**4b**) was prepared as described [14]. ¹H-NMR ((D₆)DMSO): 7.22 (*dd*, *J* = 4.8, 7.7, H-C(6)); 8.02 (*d*, *J* = 5.2, H-C(7)); 8.35 (*d*, *J* = 5.0, H-C(5)); 8.45 (*s*, H-C(2)); 12.99 (*s*, NH).

Glycosylation of 4b with 5. A soln. of **4b** (800 mg, 6.7 mmol) in anh. MeCN (150 ml) containing powdered KOH (1.9 g, 33.9 mmol) and TDA-1 (100 μ l, 0.3 mmol) was stirred for 10 min at r.t. Then **5** (2.9 g, 7.5 mmol) was added, and stirring was continued for 20 min. Insoluble material was filtered off and the filtrate evaporated. The resulting oil was chromatographed (silica gel 60H, column 20 $\times$ 3 cm, *A*).

3-[2-Deoxy-3,5-di-O-(4-toluoyl)- β -D-erythro-pentofuranosyl]-3*H*-imidazo[4,5-*b*]pyridine (**6b**): From the fast migrating zone, **6b** was obtained as a colorless foam (1.52 g, 48%). TLC (*A*): *R_f* 0.4. ¹H-NMR ((D₆)DMSO): 2.36, 2.39 (2*s*, 2 Me); 2.78 (*m*, H _{α} -C(2')); 3.47 (*m*, H _{β} -C(2')); 4.55–4.70 (*m*, H-C(4'), 2 H-C(5')); 5.84 (*m*, H-C(3')); 6.69 (*t'*, *J* = 7.0, H-C(1')); 7.27–7.38 (*m*, H-C(6), arom. H); 7.85–7.97 (*m*, arom. H); 8.13 (*d*, *J* = 8.0, H-C(7)); 8.35 (*d*, *J* = 3.8, H-C(5)); 8.69 (*s*, H-C(2)). Anal. calc. for C₂₇H₂₅N₃O₅ (471.5): C 68.78, H 5.34, N 8.91; found: C 68.84, H 5.37, N 8.92.

3-[2-Deoxy-3-O-(4-toluoyl)- β -D-erythro-pentofuranosyl]-3*H*-imidazo[4,5-*b*]pyridine (**8a**): Zone II yielded **8a** (140 mg, 6%) as colorless foam. M.p. 163° (colorless crystals, acetone). TLC (*A*): *R_f* 0.2. ¹H-NMR ((D₆)DMSO): 2.40 (*s*, Me); 2.66 (*ddd*, *J* = 3.2, 5.7, 13.8, H _{α} -C(2')); 3.15 (*m*, H _{β} -C(2')); 3.71 (*d*, *J* = 4.0, 2 H-C(5')); 4.26 (*m*, H-C(4')); 5.37 (*t*, *J* = 5.5, OH-C(5')); 5.64 (*m*, H-C(3')); 6.64 (*dd*, *J* = 5.9, 8.6, H-C(1')); 7.32–7.39 (*m*, H-C(6), arom. H); 7.95 (*m*, arom. H); 8.15 (*d*, *J* = 7.8, H-C(7)); 8.38 (*d*, *J* = 4.4, H-C(5)); 8.73 (*s*, H-C(2)). Anal. calc. for C₁₉H₁₉N₃O₄ (353.4): C 64.58, H 5.42, N 11.89; found: C 64.71, H 5.48, N 11.90.

1-[2-Deoxy-3,5-di-O-(4-toluoyl)- β -D-erythro-pentofuranosyl]-1*H*-imidazo[4,5-*b*]pyridine (**7b**): Elution with AcOEt gave **7b/8b** which were separated by FC (column 20 $\times$ 3 cm, *D*). The faster migrating zone yielded **7b** (920 mg, 29%) as colorless foam. TLC (*D*): *R_f* 0.7. ¹H-NMR ((D₆)DMSO): 2.36, 2.39 (2*s*, 2 Me); 2.81 (*m*, H _{α} -C(2')); 3.08 (*m*, H _{β} -C(2')); 4.59 (*m*, H-C(4'), 2 H-C(5')); 5.73 (*m*, H-C(3')); 6.61 (*t'*, *J* = 6.9, H-C(1')); 7.17 (*dd*, *J* = 4.7, 7.9, H-C(6)); 7.28–7.38 (*m*, arom. H); 7.81–7.99 (2*d*, *J* = 7.9, arom. H); 8.17 (*d*, *J* = 8.0, H-C(7)); 8.43 (*d*, *J* = 4.3, H-C(5)); 8.74 (*s*, H-C(2)). Anal. calc. for C₂₇H₂₅N₃O₅ (471.5): C 68.78, H 5.34, N 8.91; found: C 68.62, H 5.39, N 8.98.

3-[2-Deoxy-5-O-(4-toluoyl)- β -D-erythro-pentofuranosyl]-3*H*-imidazo[4,5-*b*]pyridine (**8b**): From the slower migrating zone (see **7b**), **8b** (70 mg, 3%) was isolated as a colorless foam. TLC (*D*): *R_f* 0.6. ¹H-NMR ((D₆)DMSO): 2.36 (*s*, Me); 2.39 (*m*, H _{α} -C(2')); 3.01 (*m*, H _{β} -C(2')); 4.16 (*m*, H-C(4')); 4.41 (*dd*, *J* = 5.8, 11.7, 1 H-C(5')); 4.53 (*dd*, *J* = 4.2, 11.7, 1 H-C(5')); 4.66 (*m*, H-C(3')); 5.57 (*d*, *J* = 4.0, OH-C(3')); 6.55 (*t'*, *J* = 6.6, H-C(1')); 7.26–7.33 (*m*, H-C(6), arom. H); 7.80 (*d*, *J* = 8.0, arom. H); 8.10 (*d*, *J* = 8.0, H-C(7)); 8.35 (*d*, *J* = 4.5, H-C(5)); 8.63 (*s*, H-C(2)). Anal. calc. for C₁₉H₁₉N₃O₄ (353.4): C 64.58, H 5.42, N 11.89; found: C 64.67, H 5.43, N 11.82.

3-(2-Deoxy- β -D-erythro-pentofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (**2b**). A soln. of **6b** (800 mg, 1.7 mmol) in MeOH (60 ml), saturated with ammonia at 0°, was stirred at r.t. for 36 h. The mixture was evaporated and the residue chromatographed (silica gel 60H, column 15 $\times$ 3 cm, *D*): **2b** (360 mg, 90%) as colorless foam. Crystallization from acetone afforded colorless needles. M.p. 144°. TLC (*D*): *R_f* 0.4. UV (MeOH): 246 (4900), 282 (8200), 287 (sh, 6400). ¹H-NMR ((D₆)DMSO): 2.33 (*m*, H _{α} -C(2')); 2.80 (*m*, H _{β} -C(2')); 3.56 (*m*, 1 H-C(5')); 3.65 (*m*, 1 H-C(5')); 3.92 (*m*, H-C(4')); 4.46 (*m*, H-C(3')); 5.10 (*t*, *J* = 5.8, OH-C(5')); 5.33 (*d*, *J* = 4.5, OH-C(3')); 6.53 (*t'*, *J* = 6.8, H-C(1')); 7.31 (*dd*, *J* = 3.5, 8.0, H-C(6)); 8.11 (*d*, *J* = 8.0, H-C(7)); 8.36 (*d*, *J* = 5.0, H-C(5)); 8.68 (*s*, H-C(2)). Anal. calc. for C₁₁H₁₃N₃O₃ (235.2): C 56.16, H 5.57, N 17.86; found: C 56.09, H 5.58, N 17.87.

1-(2-Deoxy- β -D-erythro-pentofuranosyl)-1*H*-imidazo[4,5-*b*]pyridine (**3b**). As described for **3a** (see *Method B*), with **7b** (600 mg, 1.3 mmol) in MeOH (50 ml; sat. with NH₃; 16 h). Crystallization from acetone, gave **3b** (220 mg, 72%). Colorless crystals. M.p. 142°. TLC (*D*): *R_f* 0.2. UV (MeOH): 250 (4100), 282 (10000), 287 (sh, 8200). ¹H-NMR ((D₆)DMSO): 2.30 (*m*, H _{α} -C(2')); 2.60 (*m*, H _{β} -C(2')); 3.57 (*m*, 2 H-C(5')); 3.88 (*m*, H-C(4')); 4.40 (*m*, H-C(3')); 5.01 (*t*, *J* = 5.1, OH-C(5')); 5.37 (*d*, *J* = 3.9, OH-C(3')); 6.38 (*t'*, *J* = 6.8, H-C(1')); 7.31 (*dd*, *J* = 4.7, 8.1, H-C(6)); 8.20 (*d*, *J* = 7.2, H-C(7)); 8.42 (*d*, *J* = 3.8, H-C(5)); 8.72 (*s*, H-C(2)). Anal. calc. for C₁₁H₁₃N₃O₃ (235.2): C 56.16, H 5.57, N 17.86; found: C 56.22, H 5.59, N 17.97.

Glycosylation of 7-Chloro-3*H*-imidazo[4,5-*b*]pyridine (4c) with 5. As described for **4b**, with **4c** (400 mg, 2.6 mmol), MeCN (50 ml), KOH (730 mg, 13.0 mmol), TDA-1 (40 μ l, 0.15 mmol; 15 min), and **5** (1.1 g, 2.83 mmol; 20 min). Chromatography on silica gel 60H (column 10 $\times$ 3 cm, *C*).

7-Chloro-3-[2-deoxy-3,5-di-O-(4-toluoyl)- β -D-erythro-pentofuranosyl]-3*H*-imidazo[4,5-*b*]pyridine (**6c**): The main zone yielded **6c** as colorless foam (880 mg, 67%). Crystallization from MeOH afforded colorless plates. M.p. 67–68°. TLC (*C*): *R_f* 0.3. ¹H-NMR ((D₆)DMSO): 2.36, 2.40 (*s*, 2 Me); 2.80 (*m*, H _{α} -C(2')); 3.47 (*m*, H _{β} -C(2')); 4.54–4.67 (*m*, H-C(4'), 2 H-C(5')); 5.85 (*m*, H-C(3')); 6.67 (*t'*, *J* = 7.0, H-C(1')); 7.27–7.38 (2*d*,

$J = 8.0$, arom. H); 7.49 (d , $J = 5.3$, H–C(6)); 7.82–7.97 ($2d$, $J = 7.9$, arom. H); 8.29 (d , $J = 5.3$, H–C(5)); 8.78 (s , H–C(2)). Anal. calc. for $C_{27}H_{24}ClN_3O_5$ (506.0): C 64.10, H 4.78, N 8.30; found: C 64.25, H 4.90, N 8.31.

7-Chloro-3-[2-deoxy-3-O-(4-toluoyl)- β -D-erythro-pentofuranosyl]-3H-imidazo[4,5-b]pyridine (**8c**): The slower migrating zone yielded 30 mg (3%) of **8c**. Colorless foam. TLC (A): R_f 0.3. 1H -NMR ((D_6) DMSO): 2.40 (s , Me); 2.71 (m , H_x –C(2')); 3.13 (m , H_β –C(2')); 3.70 (m , 2 H–C(5')); 4.27 (m , H–C(4')); 5.26 (t , $J = 5.4$, OH–C(5')); 5.62 (m , H–C(3')); 6.62 (t' , $J = 7.0$, H–C(1')); 7.37 (d , $J = 8.0$, arom. H); 7.51 (d , $J = 5.2$, H–C(6)); 7.95 (d , $J = 8.0$, arom. H); 8.35 (d , $J = 5.2$, H–C(5)); 8.83 (s , H–C(2)). Anal. calc. for $C_{19}H_{18}ClN_3O_4$ (387.8): C 58.84, H 4.68, N 10.83; found: C 58.92, H 4.59, N 10.79.

7-Chloro-3-(2-deoxy- β -D-erythro-pentofuranosyl)-3H-imidazo[4,5-b]pyridine (**2c**). As described for **2b**, with **6c** (600 mg, 1.19 mmol) in MeOH (50 ml; sat. with NH_3 ; 24 h). Crystallization from acetone gave **2c** (280 mg, 88%). Colorless plates. M.p. 149°. TLC (D): R_f 0.2. UV (MeOH): 253 (7600), 278 (8100), 284 (sh, 7200). 1H -NMR ((D_6) DMSO): 2.34 (ddd , $J = 3.4$, 6.2, 13.2, H_x –C(2')); 2.76 (m , H_β –C(2')); 3.47–3.67 (m , 2 H–C(5')); 3.89 (dd , $J = 4.3$, 7.4, H–C(4')); 4.44 (m , H–C(3')); 5.03 (t , $J = 5.6$, OH–C(5')); 5.35 (d , $J = 4.1$, OH–C(3')); 6.50 (t' , $J = 6.7$, H–C(1')); 7.46 (d , $J = 4.8$, H–C(6)); 8.31 (d , $J = 4.9$, H–C(5)); 8.77 (s , H–C(2)). Anal. calc. for $C_{11}H_{12}ClN_3O_3$ (269.7): C 48.99, H 4.49, N 15.58; found: C 49.04, H 4.48, N 15.58.

REFERENCES

- [1] T. Sugawara, T. Ishikura, T. Itoh, Y. Mizuno, *Nucleosides Nucleotides* **1982**, *1*, 239.
- [2] F. Seela, T. Wenzel, *Helv. Chim. Acta* **1994**, *77*, 1485.
- [3] T. Itoh, T. Sugawara, Y. Mizuno, *Heterocycles* **1982**, *17*, 305.
- [4] T. Itoh, T. Sugawara, Y. Mizuno, *Nucleosides Nucleotides* **1982**, *1*, 179.
- [5] M. Ikehara, T. Fukui, S. Uesugi, *J. Biochem.* **1974**, *76*, 107.
- [6] I. A. Mikhailopoulou, A. I. Zinchenko, S. B. Bokut, N. V. Dudchik, V. N. Baraj, E. N. Kalinichenko, H. Rosemeyer, F. Seela, *Biotechnol. Lett.* **1992**, *14*, 885.
- [7] D. Betbeder, D. W. Hutchinson, A. O'L. Richards, *Nucleic Acids Res.* **1989**, *17*, 4217.
- [8] G. Cristalli, S. Vittori, A. Eleuteri, M. Grifantini, R. Volpini, G. Lupidi, L. Capolongo, E. Pesenti, *J. Med. Chem.* **1991**, *34*, 2226.
- [9] T. A. Devlin, E. Lacosaz-Rouanet, D. Vo, D. J. Jebaratnam, *Tetrahedron Lett.* **1995**, *36*, 1601.
- [10] S. K. Chatterji, M. M. Dhar, N. Anand, M. L. Dhar, *J. Sci. Ind. Res. (India)* **1960**, *19c*, 35.
- [11] Y. Mizuno, M. Ikehara, T. Itoh, K. Saito, *Chem. Pharm. Bul.* **1963**, *11*, 265.
- [12] H.-D. Winkeler, F. Seela, *J. Org. Chem.* **1983**, *48*, 3119.
- [13] F. Seela, B. Westermann, U. Bindig, *J. Chem. Soc., Perkin Trans. 1* **1988**, 697.
- [14] I. Antonini, G. Cristalli, P. Franchetti, M. Grifantini, S. Martelli, F. Petrelli, *J. Pharm. Sci.* **1984**, *73*, 366.
- [15] F. Seela, T. Wenzel, *Heterocycles* **1993**, *36*, 237.
- [16] F. Seela, K. Kaiser, *Helv. Chim. Acta* **1988**, *71*, 1813.
- [17] H. Rosemeyer, G. Toth, F. Seela *Nucleosides Nucleotides* **1989**, *8*, 587.
- [18] F. Seela, T. Wenzel, H. Debelak, *Nucleosides Nucleotides* **1995**, *14*, 957.
- [19] F. Seela, H. Winter, *Nucleosides Nucleotides* **1995**, *14*, 129.
- [20] J. L. Rabinowitz, E. C. Wagner, *J. Am. Chem. Soc.* **1951**, *73*, 3030.
- [21] H. Rosemeyer, F. Seela, *Helv. Chim. Acta* **1988**, *71*, 1573.
- [22] J. Gasteiger, M. Marsili, *Tetrahedron* **1980**, *36*, 3219.
- [23] J. Gasteiger, M. Marsili, *Org. Magn. Reson.* **1981**, *15*, 353.
- [24] 'Alchemy III, Release Version 1992', Tripos Co., St. Louis, USA.
- [25] G. Cristalli, P. Franchetti, M. Grifantini, S. Vittori, T. Bordoni, C. Geroni, *J. Med. Chem.* **1987**, *30*, 1686.
- [26] M. Hoffer, *Chem. Ber.* **1960**, *93*, 2777.