Regioselectivity of the Mannich Reaction on Pyrrolo[2,3-*d*]pyrimidine Nucleosides Related to 7-Deaza-2′-deoxyadenosine or 7-Deaza-2′-deoxyguanosine

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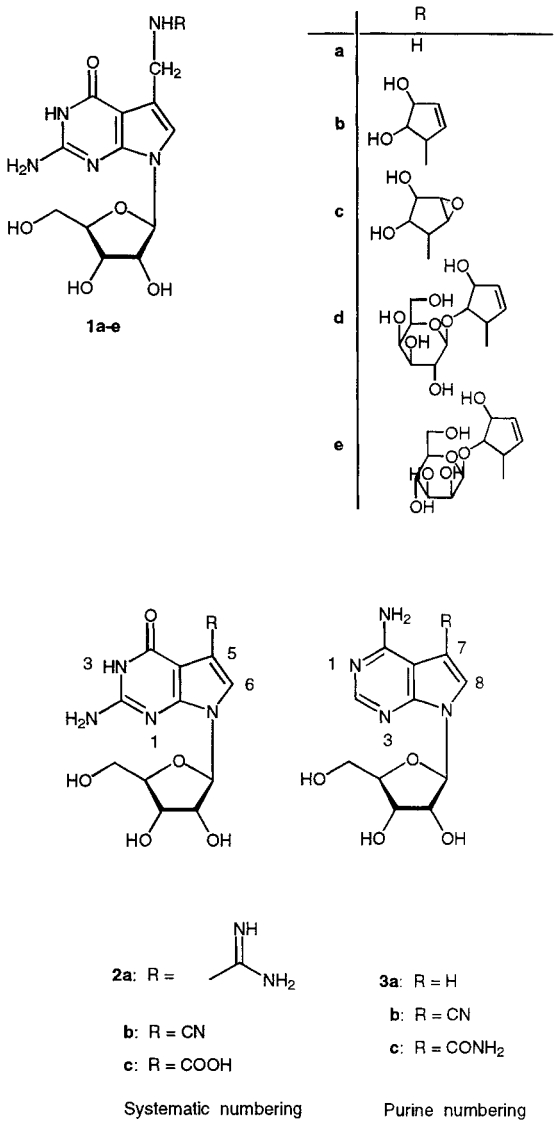
Received 27 March 1997

Mannich reactions were performed on 7-deazapurine 2′-deoxyribonucleosides and the regioselectivity was studied. 7-Deaza-2′-deoxyadenosine (2′-deoxytubercidin, **4a**) furnished the 7-substituted Mannich base **5a**. The side chain was introduced in the 8-position when 7-deaza-2′-deoxyguanosine was used (Mannich base **7a**). The regioselectivity changed back from position 8 to 7 when the reaction was performed on 4-methoxy-2-methylthio-7*H*-pyrrolo[2,3-*d*]pyrimidine 2′-deoxyribonucleoside (**12**). Thus a 7-substituted Mannich product of 7-deaza-2′-deoxyguanosine could be obtained after demethylation and oxidation of the methylthio group followed by displacement of the oxidized 2-substituent with ammonia.

The posttranscriptional modification of ribonucleic acids, in particular of a tRNA, generates a variety of modified nucleosides.[1] Among them is queuosine (2-amino-5-[(4,5-dihydroxycyclopent-2-en-1-yl)aminomethyl]-1,7-dihydro-7-β-D-ribofuranosyl-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one) (**1b**),[2-6] containing a 7-deazaguanine moiety as heterocyclic base (purine numbering is used throughout the discussion section). This structural modification is unique for naturally occurring nucleic acid components as it represents the only example in which the purine system shows an altered nitrogen pattern. Besides queuosine (**1b**) other 7-deazaguanine ribonucleosides **1c–e** have been found in tRNA.[1] They are either biosynthetic precursors, such as preQ$_1$ (**1a**), preQ$_0$ (**2b**), glycosides **1d–e**[1] or as in the case of archaeosine (**2a**) have been isolated from tRNA of archaebacteria.[7] Contrary to the 7-deazaguanine moiety, the 7-deazaadenine base has not yet been found in nucleic acids. Nevertheless, 7-deazaadenine nucleosides, such as tubercidin (**3a**), toyocamycin (**3b**), and sangivamycin (**3c**)[8,9] and also another guanine derivative such as (cadeguomycin) (**2c**)[10] have been isolated as fermentation products formed by microorganisms.

As most of the naturally occurring 7-deazapurine ribonucleosides carry substituents at the 7-position and many of them which are found in tRNA represent Mannich bases, it is of interest to transfer this structural motif from RNA to DNA and to investigate the effect on DNA stability and/or structure. Moreover, the Mannich base can be used to introduce reporter groups into the DNA molecule in a sterically favorable position. The present manuscript describes the application of the Mannich reaction to the synthesis of 7-deazapurine 2′-deoxyribonucleosides carrying an alkylaminoalkylidene side chain.

The Mannich bases of 7-deazapurine nucleosides can be synthesized either by (i) the glycosylation of a suitable nucleobase precursor carrying already the Mannich side chain or (ii) by the Mannich reaction performed on a 7-deazapurine nucleoside. As the solubility of the Mannich bases is low in MeCN, a solvent which is used for the stereoselective glycosylation, it was decided to investigate the reaction on nucleosides. It was already reported



that the ribonucleoside tubercidin (**3a**) yields a 7-substituted Mannich base when an aqueous formaldehyde solution and morpholine are used.[11] These conditions when applied to 7-deaza-2′-deoxyadenosine (2′-deoxytubercidin, **4a**) did not yield the required reaction product. However, when the Mannich reaction was performed with HOAc as a solvent component (condition I) the reaction took place (12 h at 60 °C) and compound **5a** was isolated in 43% yield (Scheme 1). The position of the side chain was assigned by NMR spectroscopy (see below) and was found to be C-7. The 7-regioselectivity of the Mannich reaction is retained when other 6-sub-

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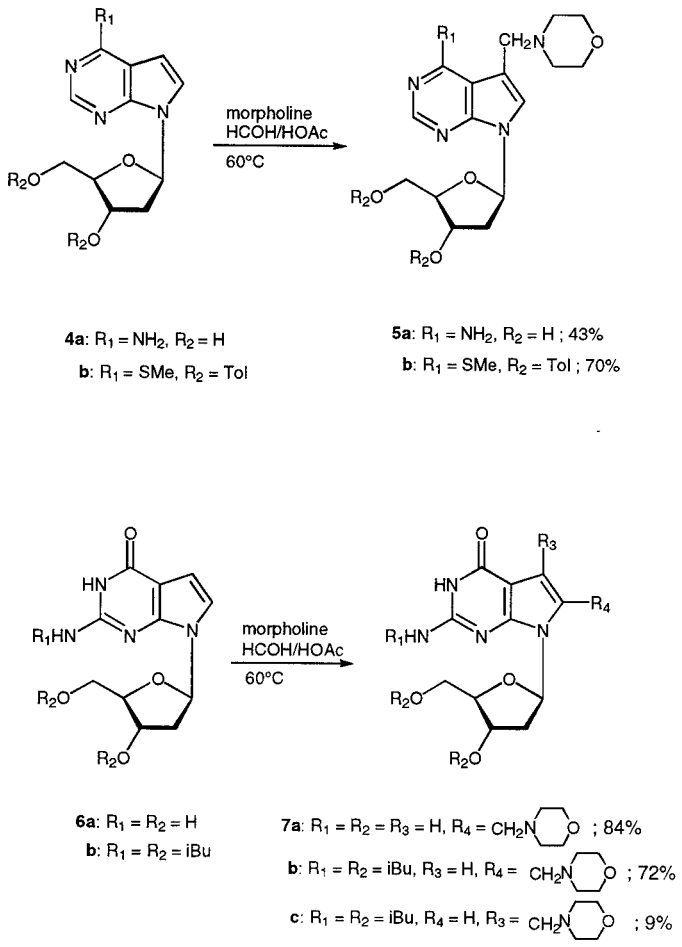
stituted 7-deazaadenine nucleosides are used. Thus, the sugar protected 6-methylthionucleoside **4b** gave the Mannich base **5b** with the side chain located at C-7 (Scheme 1).

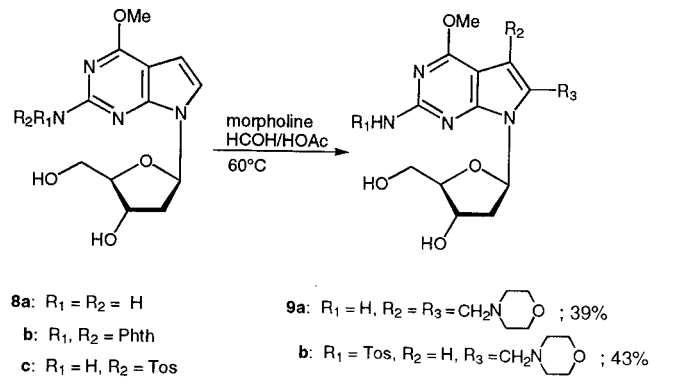
Earlier, it has been found that the Mannich reaction, when performed on 7-deazahypoxanthine, leads to a product carrying the side chain at C-7.[12] Later, it was reported from our laboratory that 7-deazaguanine forms a Mannich base with the substituent at C-8.[13] The change in the regioselectivity was explained by the influence of the electron-donating properties of the 2-amino group stabilizing the σ-complex during the electrophilic attack at C-8.[13] This stabilizing effect is decreased when the electron density is reduced on the amino group. Consequently, other laboratories have performed the Mannich reaction on acylated 7-deazaguanine bases and have obtained Mannich products with the side chain at C-7.[14] However, mixtures of regioisomers were formed in most of the cases and also 7,8-bis-substituted derivatives were isolated.[14] As it was not clear whether the results found on the heterocycles can be transferred to the nucleosides, the Mannich reaction was applied to 7-deaza-2′-deoxyguanosine (**6a**). When acetic acid was used as solvent (condition I) decomposition of the starting material took place. Therefore, the amount of acid was reduced and the temperature was strictly controlled (60 °C) (condition II). In this case the Mannich base **7a** was isolated in 85% yield. According to NMR-data (see Tables 1, 2) the side chain is located in the 8-position (Scheme 2) which is the same as found for 7-deazaguanine.[13] As the regioselectivity changed from position 8 to position 7 when 7-deazaguanine was acylated at the 2-amino group,[14] the Mannich reaction was now performed on the triisobutyrylated derivative **6b**. Condition I (acetic acid as solvent) furnished two reaction products; the C-8 substituted **7b** (72% yield) together with the 7-substituted derivative **7c** (9%) (Scheme 1). The selectivity change from position 7 (acylated base) to position 8 (acylated nucleoside) might be due to a change of the reaction mechanism. The unsubstituted pyrrole system can give an *N*-Mannich base which is not possible in the case of the *N*-glycosylated derivative.[14]



Scheme 1

In order to increase the regioselectivity towards 7-substitution, other 7-deaza-2′-deoxyguanosine analogues were employed. At first the Mannich reaction performed on the 2-amino-6-methoxynucleoside **8a** in acetic acid (condition I) caused decomposition. Then, condition II was employed. However, in this case a bis-Mannich product **9a** was formed (39% yield). The monosubstituted compound carrying the side chain in the 7-position could not be detected (Scheme 2). In order to reduce the reactivity of compound **8a** the electron density of the heterocycle was reduced by preparing the *N*-phthaloyl derivative **8b**. Phthaloyl derivatives of regular nucleosides have already been synthesized by Hata[15] and were also studied by Pfleiderer to be used as protecting groups in oligonucleotide synthesis.[16] In our case, compound **8a** was treated with phthaloyl dichloride under the condition of transient protection[16,17] and the phthaloyl derivative **8b** was isolated in 51% yield. Unfortunately, the aminoalkylation on compound **8b** did not lead to a Mannich base. Instead, the phthaloyl protecting group was partially hydrolyzed.

Next, the tosyl group was examined and compound **8c** was prepared as described for the phthaloyl derivative **8b**. In this case the Mannich product **9b** was formed from **8c** under weakly acidic conditions (condition II). Nevertheless, the side chain was also located in position 8 (nucleoside **9b**) (Scheme 2). From these experiments it is apparent that 7-deaza-2′-deoxyguanosine derivatives cannot be used for the introduction of a Mannich side chain in the 7-position of 7-deazapurines.



Scheme 2

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In order to overcome this problem, other synthetic precursor molecules were used. Earlier it was shown that 7-deazapurines give a product with the Mannich base moiety at C-7 when 2-methylthiopyrrolo[2,3-*d*]pyrimidines are used.[18] As a 2-methylthio group can be converted later into an amino group,[19] the 6-methoxy-2-methylthio-7-deazapurine 2′-deoxynucleoside **12** was employed as the starting material. The nucleoside **12** has been previously synthesized in our laboratory using liquid-liquid phase-transfer glycosylation.[20] As the yield of this protocol was relatively low (21%) the glycosylation was now performed in MeCN in the presence of TDA-1 (tris[2-(2-methoxyethoxy)ethyl]amine) and KOH furnishing the β-D-nucleoside **12** in 69% yield, similar to the reaction performed in the presence of NaH.[21] Then, the Mannich reaction was carried out on compound **12** in acetic acid (condition I). The reaction proceeded smoothly and a Mannich base with the side chain located in the position-7 was formed in 95% yield (crude product). Conversion of the 4-methoxy group of **13** into an oxo group occurred upon treatment with NaI/trimethylsilyl chloride in absolute MeCN. Next, the methylthio nucleoside **14** was treated with 3-chloroperbenzoic acid (MCPBA) in CH_2Cl_2 followed by NH_3 in dioxane. Finally it was deblocked on the sugar moiety with methanolic ammonia giving the Mannich base **15** in 69% yield (Scheme 3).

The assignment of the position of the Mannich side chain was accomplished by NOE difference spectroscopy in combination with ^{13}C NMR spectroscopy as well as ^1H NMR spectra. In all cases where the side chain is attached to the 7-position a NOE on H-8 is observed upon irradiation of the anomeric proton. Irradiation of H-1′ in the case of the Mannich bases **7a** and **7b** with the side chain in 8-position did not lead to a NOE of a pyrrole proton. In this case of NOE on the methylene group of the side chain was observed (Table 1). ^{13}C NMR chemical shifts were used to confirm the NOE experiments (Table 2). According to Table 2, the side chain shifts the C-7 or C-8 signal of the 7-deazaadenine or the 7-deazaguanine moiety to lower field (about 10 ppm) depending on the position of substitution. On the other hand, ^1H NMR spectra of the Mannich bases with side chain at C-7 or C-8 show a singlet for H-8 around $\delta = 7$ or a singlet for H-7 around $\delta = 6.5$, whereas the starting materials show two doublets for H-8 ($\delta = \sim 7$) and H-7 ($\delta = \sim 6.5$).

Table 1. ^1H NMR NOE Data of Compounds **5a**, **5b**, **7a**, **7b**, **7c** and **13**[a]

Product	Proton Irradiated	NOE observed
5a	H-C(1′)	H-C(8) (3.4%), H_α-C(2′) (5.8%), H-C(4′) (2.8%), OH-C(3′) (0.8%)
5b	H-C(1′)	H-C(8) (0.7%), H_α-C(2′) (2.6%), H-C(4′) (1.3%)
7a	H-C(1′)	H_α-C(2′) (7.4%), CH_2N (2.9%), H-C(4′) (1.6%)
7b	H-C(1′)	H_α-C(2′) (6.2%), CH_2N (4.4%), H-C(4′) (3.5%)
7c	H-C(1′)	H-C(8) (1.3%), H_α-C(2′) (2.8%), H-C(4′) (1.9%)
13	H-C(1′)	H-C(8) (1.1%), H_α-C(2′) (8.6%)

[a] Purine numbering. Measured in DMSO-d_6 at 23°C.

Table 2. ^{13}C NMR Chemical Shifts of 7-Deazapurine Nucleosides[a]

Product	C(2)[b] C(2)[c]	C(4)[b] C(6)[c]	C(4a)[b] C(5)[c]	C(5)[b] C(7)[c]	C(6)[b] C(8)[c]	C(7a)[b] C(4)[c]	OCH_2	NCH_2	OCH_3	SCH_3	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	CH_3	CO
4a	151.6	157.5	102.9	99.6	121.6	149.6					83.3	–[f]	71.1	87.3	62.1		
4b	150.5	160.9	116.1	99.8	125.9	148.1				11.4	83.6	36.1	75.1	81.3	64.3		
5a	151.8	158.3	102.4	111.5	120.6	150.6	66.2	54.6/52.9			83.0	–[f]	71.2	87.3	62.2		
5b	150.3	161.7	115.6	112.1	124.1	148.7	66.1	53.7/52.9		12.0	83.0	36.3	75.1	81.3	64.1		
6a	152.5	158.5	100.0	102.1	116.7	150.5					82.2	–[f]	70.8	86.9	61.9		
7a	152.0	158.5	99.7	103.6	127.6	151.0	66.2	54.4/52.8			83.9	–[f]	71.0	87.0	62.0		
7b	146.0	156.2	104.1	104.3	130.7	148.2	66.2	54.1/52.7			83.9	–[f]	74.2	80.4	63.5		
8a	159.4	163.0	97.3	98.9	119.5	154.2			52.5		82.4	–[f]	70.9	86.9	62.0		
8b	144.9	163.1	104.6	99.2	123.9	151.8			54.2		83.2	–[f]	70.9	87.5	61.8		166.0
8c	151.2[d]	162.5	100.4	99.1	122.4	152.2[d]			53.6		82.5	–[f]	70.9	87.2	61.9	20.9	
9a	158.3	163.5	98.4	110.0	128.6	153.0	66.3	51.6/50.9			85.0	–[f]	71.5	87.5	62.4		
9b	151.0	162.0	99.9	100.5	133.6	153.4	66.1	52.8	53.6		83.7	–[f]	70.5	86.8	61.7	20.9	
10	161.8[d]	162.2[d]	101.3	98.1	122.7	153.5			53.3	13.6							
12	161.7[d]	163.0[d]	102.1	99.2	123.0	152.3			53.3	13.5	83.5	36.0	74.6	80.9	64.0	20.9	165.1/165.3
13	162.4[d]	162.9[d]	102.0	111.4	121.5	152.4	66.1	54.8/52.6	53.6	13.6	83.2	36.1	75.0	81.0	64.1	21.1	165.4/165.2
14[e]	158.1	161.4	104.8	113.8	120.0	149.1	66.3	55.2/52.7		13.7	84.2	37.0	75.4	81.8	64.6	21.8	166.6/166.5
15	152.5	159.0	99.4	115.4	114.3	150.4	66.2	53.0/52.7			82.0	–[f]	70.9	86.8	62.0		

[a] Measured in DMSO-d_6.
[b] Systematic numbering.
[c] Purine numbering.
[d] Tentative.
[e] Measured in DMSO-d_6/0.4 M aq NH_4OAc.
[f] Superimposed by DMSO.

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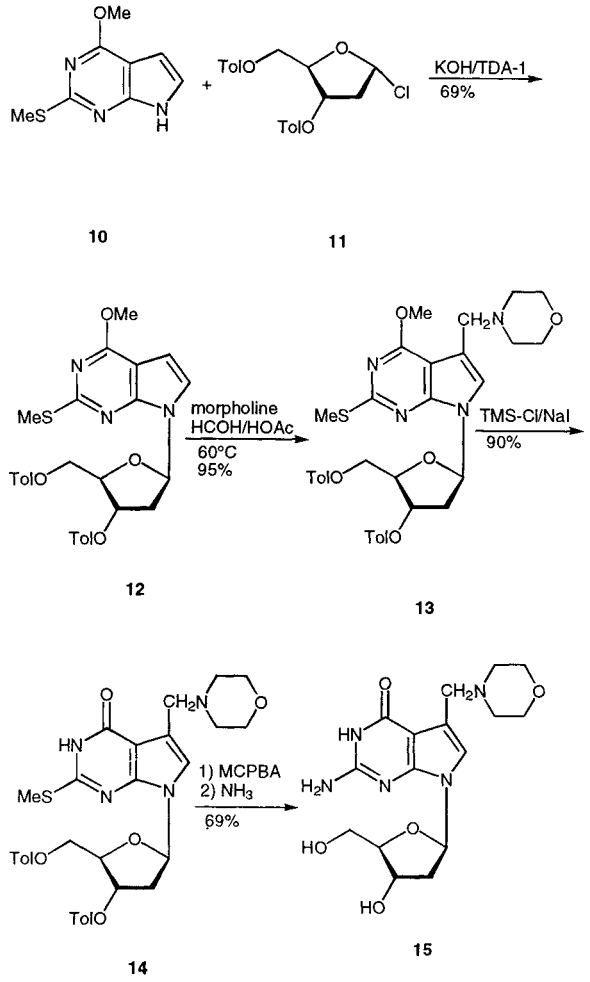

Scheme 3

From the experiments described above it can be concluded that a Mannich base side chain can be introduced into the 7-position of 7-deaza-2′-deoxyadenosine directly on the unprotected nucleoside (**4a**→**5a**). In the case of 7-deaza-2′-deoxyguanosine the nucleoside yields a Mannich base carrying the side chain in the 8-position (**7a,b**). The 7-substituted Mannich base of 7-deaza-2′-deoxyguanosine (**6a**) is obtained when the reaction is performed on 2-methylthio derivatives as precursor compound (**12**→**15**). This route is considered to be the most efficient protocol for the synthesis of queuosine (**1b**) and derivatives. Transformation of the tertiary amino groups of **5a** or **15** into other Mannich compounds can be accomplished after quarternization.[14b,22] Furthermore, the synthetic or enzymatic incorporation of Mannich bases derived from compounds **4a** or **6a** into nucleic acids allows the introduction of reporter groups in a favorable position of the DNA molecule.[23,24]

TLC was done on TLC aluminum sheets silica gel 60 F_{254} (0.2 mm, Merck, Germany). Flash chromatography (FC) was carried out at 0.5 bar (silica gel 60, Merck, Germany). Solvent systems for TLC and FC: cyclohexane/EtOAc (2:1, A), CH_2Cl_2/MeOH (95:5, B), CH_2Cl_2/MeOH (9:1, C), CH_2Cl_2/MeOH (4:1, D), *i*-PrOH/H_2O/concd aq NH_3 (7:2:1, E). UV Spectra: Hitachi-150-20 spectrometer (Hitachi, Japan). NMR spectra were measured on AC-250 and AMX-500 spectrometers (Bruker Germany); δ values are in ppm downfield from internal TMS (^1H, ^{13}C). Elemental analyses were performed by Mikroanalytisches Laboratorium Beller, Göttingen, Germany.

4-Amino-7-[2-deoxy-β-D-*erythro*-pentofuranosyl]-5-(1-morpholinomethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine [2′-Deoxy-5-(1-morpholinomethyl)tubercidin, 5a]:
Formaldehyde (37% in H_2O, 0.6 mL, 8 mmol) was slowly added to morpholine (0.6 mL, 7 mmol) under stirring. After the exothermic reaction had ceased, HOAc (0.6 mL) was added to the solution and the stirring continued for 5 min. Compound **4a**[25] (100 mg, 0.4 mmol) was added and the reaction mixture was kept for 12 h at 60 °C. The solution was evaporated and coevaporated several times with toluene/MeOH (50 mL each, 1:1). The residue was dissolved in CH_2Cl_2 and subjected to FC (column, 3 × 8 cm, eluent C) yielding a colorless foam (60 mg, 43%); TLC (silica gel, eluent D): R_f 0.6.

UV (MeOH): λ_{max} = 273 nm (ε = 8800).

^1H NMR (DMSO-d_6): δ = 2.15 (m, 1H, H-2′), 2.46 (m, 5H, H-2′, CH_2NCH_2), 3.56 (m, 8H, H-5′, CH_2N, CH_2OCH_2), 3.81 (m, 1H, H-4′), 4.33 (m, 1H, H-3′), 5.06 (t, 1H, J = 5.2 Hz, 5′-OH), 5.22 (d, 1H, J = 3.7 Hz, 3′-OH), 6.46 (t, 1H, J = 7.0 Hz, H-1′), 7.30 (s, 1H, H-8), 7.50 (br, 2H, NH_2), 8.04 (s, 1H, H-2).

$C_{16}H_{23}N_5O_4$	calc.	C 55.00	H 6.64	N 20.04
(349.4)	found	55.30	6.40	20.24

7-[2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-β-D-*erythro*-pentofuranosyl]-4-methylthio-5-(1-morpholinomethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (5b):
To a solution of aq HOAc (80%, 40 mL) containing aq formaldehyde (37%, 0.87 mL, 11.7 mmol), and morpholine (1.0 mL, 11.5 mmol) was added compound **4b**[26] (500 mg, 0.97 mmol) under stirring at 60 °C. The stirring was continued for 5 d and the mixture was diluted with H_2O (80 mL). The precipitate formed after neutralization with 25% NH_4OH was collected by filtration and washed with H_2O. The dried crude product was submitted to FC (column 4 × 8 cm, eluent B). The main zone was collected and evaporated to give a colorless foam (420 mg, 70%); TLC (silica gel, eluent B): R_f 0.4.

UV (MeOH): λ_{max} = 230, 297 nm (ε = 36700, 10600).

^1H NMR (DMSO-d_6): δ = 2.36 (br, 4H, CH_2NCH_2), 2.40, 2.42 (2s, 6H, 2 CH_3), 2.62 (s, 3H, CH_3S), 2.71, 3.06 (2m, 2H, H-2′), 3.52 (m, 6H, CH_2N, CH_2OCH_2), 4.52 (m, 2H, H-5′), 4.66 (m, 1H, H-4′), 5.76 (m, 1H, H-3′), 6.75 (t, 1H, J = 6.5 Hz, H-1′), 7.48 (s, 1H, H-6), 7.3–8.0 (m, 8H_{arom}), 8.59 (s, 1H, H-2).

$C_{33}H_{36}N_4O_6S$	calc.	C 64.27	H 5.88	N 9.08
(616.7)	found	64.10	5.86	8.99

2-Amino-7-[2-deoxy-β-D-*erythro*-pentofuranosyl]-6-(1-morpholinomethyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (7a):
An aq solution of formaldehyde (37%, 3.5 mL, 47.0 mmol) was added to morpholine (4 mL, 45.9 mmol) under stirring. After the exothermic reaction was over compound **6a**[27] (500 mg, 1.9 mmol) and aq HOAc (80%, 0.4 mL) were added to the solution. The stirring was continued for 8 h at 60 °C. The solution was evaporated and coevaporated with MeOH (3 × 20 mL). The residue was dissolved in MeOH and absorbed on silica gel (4 g). The silica gel mass was evaporated to dryness and subjected to FC (column 4 × 10 cm, eluent C) yielding a colorless foam (580 mg, 84%); TLC (silica gel, eluent D): R_f 0.7.

UV (MeOH): λ_{max} = 263 nm (ε = 15500).

^1H NMR (DMSO-d_6): δ = 1.96 (m, 1H, H-2′), 2.32 (2m, 5H, H-2′, CH_2NCH_2), 3.52 (m, 8H, H-5′, CH_2N, CH_2OCH_2), 3.75 (m, 1H, H-4′), 4.33 (m, 1H, H-3′), 6.04 (br, 2H, NH_2), 6.15 (s, 1H, H-5), 6.35 (t, 1H, J = 6.7 Hz, H-1′).

$C_{16}H_{23}N_5O_5$	calc.	C 52.60	H 6.34	N 19.17
(365.4)	found	52.68	6.33	19.07

7-[2-Deoxy-3,5-di-*O*-isobutyryl-β-D-*erythro*-pentofuranosyl]-2-(isobutyrylamino)-6-(1-morpholinomethyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (7b) and 7-[2-Deoxy-3,5-di-*O*-isobutyryl-β-D-*erythro*-pentofuranosyl]-2-(isobutyrylamino)-5-(1-morpholinomethyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (7c):

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As described for **5b**, compound **6b**[28] (460 mg, 0.97 mmol) was treated with aq formaldehyde (37%, 0.87 mL, 11.7 mmol), morpholine (1.0 mL, 11.5 mmol) and aq HOAc (80%, 2 mL) at 60°C for 30 h. The dried crude product was submitted to FC (column 4 × 8 cm, B). The fast migrating zone was collected and evaporated to give a colorless foam **7b** (400 mg, 72%); TLC (silica gel, eluent B): R_f 0.7.

UV (MeOH): λ_{max} = 271, 299 nm (ε = 14400, 13200).

^{1}H NMR (DMSO-d_6): δ = 1.16 (m, 18H, 6 CH_3), 2.20 (m, 1H, H-2'), 2.34 (m, 4H, CH_2NCH_2), 2.54, 2.61, 2.78 (3q, 3H, J = 7.1, 7.0, 6.9 Hz, 3CH), 3.56 (m, 6H, CH_2N, CH_2OCH_2), 4.12, 4.30, 4.40 (3m, 3H, H-5', H-4'), 5.38 (m, 1H, H-3'), 6.40 (s, 1H, H-5), 6.51 (t, 1H, J = 7.3 Hz, H-1'), 11.05, 11.84 (2s, 2H, 2 NH).

$C_{28}H_{41}N_5O_8$	calc.	C	58.42	H	7.18
(575.7)	found		58.60		7.10

From the slow migrating zone, compound **7c** was isolated as solid foam (50 mg, 9%); TLC (silica gel, B): R_f 0.4.

^{1}H NMR (DMSO-d_6): δ = 1.0 (m, 18H, 6CH_3), 2.20 (m, 1H, H-2'), 2.30 (m, 4H, CH_2NCH_2), 2.45, 2.56, 2.75 (3q, 3H, J = 7.0 Hz, 3CH), 3.50 (m, 4H, CH_2OCH_2), 3.67 (s, 2H, CH_2N), 4.15 (m, 2H, H-5'), 4.22 (m, 1H, H-4'), 5.31 (m, 1H, H-3'), 6.45 (t, 1H, J = 7.3 Hz, H-1'), 7.14 (s, 1H, H-6), 11.42, 11.77 (2s, 2H, 2 NH).

2-Amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5,6-bis-(1-morpholinomethyl)-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine (9a):

As described for **7a**, compound **8a**[27] (300 mg, 1.1 mmol) was treated with aq formaldehyde (37%, 2.5 mL, 33.6 mmol), morpholine (2.9 mL, 33.4 mmol) and aq HOAc (80%, 0.2 mL) at 60°C for 20 h. FC yielded a colorless foam (205 mg, 39%); TLC (silica gel, C): R_f 0.5.

UV (MeOH): λ_{max} = 228, 264, 288 nm (ε = 32600, 12700, 9800).

^{1}H NMR (DMSO-d_6): δ = 2.04 (m, 1H, H-2'), 2.33 (m, 4H, CH_2NCH_2), 3.00 (m, 1H, H-2'), 3.55 (m, 8H, H-5', CH_2N, CH_2OCH_2), 3.85 (m, 1H, H-4'), 3.93 (s, 3H, CH_3O), 4.42 (m, 1H, H-3'), 5.87 (s, 2H, NH_2), 6.41 (t, 1H, J = 6.4 Hz, H-1').

$C_{22}H_{34}N_6O_6$	calc.	C	55.21	H	7.16 N 17.56
(478.5)	found		54.83		7.23 17.07

7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-4-methoxy-2-*N*,*N*-phthaloylamino-7*H*-pyrrolo[2,3-*d*]pyrimidine (8b):

Compound **8a** (1.0 g, 3.57 mmol) was dried by coevaporation with anhyd pyridine and then suspended in anhyd pyridine (30 mL). $ClSiMe_3$ (1.2 mL, 9.0 mmol) was then added at r.t. After stirring for 15 min the mixture was treated with phthaloyl dichloride (0.77 mL, 5.4 mmol) and maintained at r.t. for 3 h. H_2O (5 mL) was added to the mixture and the stirring was continued for 30 min. The mixture was poured into aq $NaHCO_3$ (5%, 150 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), filtered and evaporated. The residue was submitted to FC (column 4 × 10 cm, eluent B) and the compound present in the main zone was isolated by evaporation of the solvent furnishing a colorless powder (750 mg, 51%); TLC (silica gel, eluent C): R_f 0.6.

UV (MeOH): λ_{max} = 228, 280 nm (ε = 26000, 12500).

^{1}H NMR (DMSO-d_6): δ = 2.27, 2.55 (2m, 2H, H-2'), 3.55 (2m, 2H, H-5'), 3.85 (m, 1H, H-4'), 4.07 (s, 3H, CH_3O), 4.37 (br, 1H, H-3'), 4.92 (t, 1H, J = 5.4 Hz, 5'-OH), 5.27 (d, 1H, J = 4.2 Hz, 3'-OH), 6.57 (t, 1H, J = 7.6 Hz, H-1'), 6.72 (d, 1H, J = 3.5 Hz, H-5), 7.81 (d, 1H, J = 3.6 Hz, H-6), 7.9–8.0 (m, 4H_{arom}).

$C_{20}H_{18}N_4O_6$	calc.	C	58.54	H	4.42 N 13.65
(410.4)	found		58.45		4.64 13.61

7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-4-methoxy-2-tosylamino-7*H*-pyrrolo[2,3-*d*]pyrimidine (8c):

As described for **8b**, compound **8a** (1.0 g, 3.57 mmol) was treated with $ClSiMe_3$ (1.2 mL, 9.0 mmol) and TosCl (1.0 g, 5.2 mmol) in anhyd pyridine (30 mL). FC (eluent B) gave a white powder (807 mg, 52%); TLC (silica gel, eluent C): R_f 0.4.

UV (MeOH): λ_{max} = 223, 269 nm (ε = 29300, 11200).

^{1}H NMR (DMSO-d_6): δ = 2.13 (m, 1H, H-2'), 2.36 (s, 3H, CH_3), 3.53 (m, 2H, H-5'), 3.86 (br, 1H, H-4'), 3.89 (s, 3H, CH_3O), 4.35 (br, 1H, H-3'), 4.87 (br, 1H, 5'-OH), 5.31 (d, 1H, J = 2.3 Hz, 3'-OH), 6.40 (d, 1H, J = 2.9 Hz, H-5), 6.45 (br, 1H, H-1'), 7.39 (m, 3H, H-6, H_{arom}), 7.91 (d, 2H, J = 7.0 Hz, H_{arom}), 11.28 (s, 1H, NH).

$C_{19}H_{22}N_4O_6S$	calc.	C	52.53	H	5.10 N 12.90
(434.5)	found		52.48		5.31 12.85

7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-4-methoxy-6-(1-morpholinomethyl)-2-tosylamino-7*H*-pyrrolo[2,3-*d*]pyrimidine (9b):

As described for **7a**, compound **8c** (200 mg, 0.46 mmol) was treated with aq formaldehyde (37%, 1.5 mL, 20.6 mmol), morpholine (1.8 mL, 20.7 mmol) and aq HOAc (80%, 0.2 mL) overnight at 60°C. FC (eluent B) yielded a colorless foam (105 mg, 43%); TLC (silica gel, eluent C): R_f 0.7.

UV (MeOH): λ_{max} = 226, 274 nm (ε = 32500, 15200).

^{1}H NMR (DMSO-d_6): δ = 1.94 (m, 1H, H-2'), 2.34 (br, 7H, CH_3, CH_2NCH_2), 3.53 (m, 8H, H-5', CH_2N, CH_2OCH_2), 3.75 (m, 1H, H-4'), 3.84 (s, CH_3O), 4.40 (br, 1H, H-3'), 4.84 (br, 1H, 5'-OH), 5.22 (m, 1H, 3'-OH), 6.31 (s, 1H, H-5), 6.44 (t, 1H, J = 6.5 Hz, H-1'), 7.38, 7.87 (2d, 4H, J = 7.7, 7.6 Hz, H_{arom}).

$C_{24}H_{31}N_5O_7S$	calc.	C	54.02	H	5.86 N 13.13
(533.6)	found		54.15		6.03 12.94

7-[2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-β-D-*erythro*-pentofuranosyl]-4-methoxy-2-methylthio-7*H*-pyrrolo[2,3-*d*]pyrimidine (12):

To a suspension of **10**[29] (2.0 g, 10.2 mmol) in anhyd MeCN (100 mL) were added powdered KOH (85%, 2.3 g), and TDA-1 (0.39 mL, 1.2 mmol) under stirring at r.t. After stirring for 5 min, **11**[30] (4.3 g, 11.1 mmol) was added and the stirring was continued for another 15 min. Insoluble material was filtered, washed with MeCN, and the filtrate was evaporated. The residue was purified by FC (column 5 × 10 cm, eluent A). The content of the main zone was crystallized from MeOH furnishing a colorless solid (3.9 g, 69%).[21]

7-[2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-β-D-*erythro*-pentofuranosyl]-4-methoxy-2-methylthio-5-(1-morpholinomethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (13):

As described for **5b**, compound **12** (750 mg, 1.37 mmol) was treated with aq formaldehyde (37%, 2.1 mL, 28.2 mmol), morpholine (2.5 mL, 28.7 mmol) and aq HOAc (80%, 80 mL) at 60°C overnight. The crude product (840 mg, 95%) can be directly used for the next step. An analytical sample was prepared by FC (eluent B) yielding a colorless foam; TLC (silica gel, eluent C): R_f 0.6.

UV (MeOH): λ_{max} = 240, 282 (ε = 49100, 15700).

^{1}H NMR (DMSO-d_6): δ = 2.33 (br, 4H, CH_2NCH_2), 2.39, 2.42 (2s, 6H, 2 CH_3), 2.58 (s, 3H, CH_3S), 2.70, 3.06 (2m, 2H, H-2'), 3.50 (m, 6H, CH_2N, CH_2OCH_2), 4.02 (s, 3H, CH_3O), 4.50 (m, 2H, H-5'), 4.63 (m, 1H, H-4'), 5.76 (m, 1H, H-3'), 6.66 (t, 1H, J = 6.5 Hz, H-1'), 7.24 (s, 1H, H-6), 7.3–8.0 (m, 8H_{arom}).

$C_{34}H_{38}N_4O_7S$	calc.	C	63.14	H	5.92 N 8.66
(646.8)	found		63.29		6.11 8.55

7-[2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-β-D-*erythro*-pentofuranosyl]-2-methylthio-5-(1-morpholinomethyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidine-4-one (14):

A solution of **13** (730 mg, 1.13 mmol) in anhyd MeCN (30 mL) was treated with NaI (730 mg, 4.9 mmol) and $ClSiMe_3$ (0.7 mL, 5.5 mmol) under stirring at r.t. The resulting suspension was stirred for 45 min at r.t. and evaporated to dryness. The residue was supplied to FC (column 3 × 6 cm, eluent B) yielding a colorless foam (645 mg, 90%); TLC (silica gel, eluent C): R_f 0.5.

UV (MeOH): λ_{max} = 222, 272 (ε = 47700, 9700).

^{1}H NMR (DMSO-d_6): δ = 2.36 (s, 6H, 2 CH_3), 2.38 (br, 4H, CH_2NCH_2), 2.54 (s, 3H, CH_3), 2.64, 3.00 (2m, 2H, H-2'), 3.49 (m, 4H, CH_2OCH_2), 3.58 (m, 2H, CH_2N); 4.49 (m, 2H, H-5'), 4.60 (m, 1H, H-4'), 5.74 (m, 1H, H-3'), 6.56 (t, 1H, J = 7.0 Hz, H-1'), 7.00 (s, 1H, H-6), 7.3–8.0 (m, 8H_{arom}).

$C_{33}H_{36}N_4O_7S$	calc.	C	62.64	H	5.73 N 8.85
(632.7)	found		62.48		5.66 8.57

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2-Amino-7-[2-deoxy-β-D-*erythro*-pentofuranosyl]-5-(1-morpholinomethyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (15):
To a solution of compound **14** (730 mg, 1.15 mmol) in CH_2Cl_2 (60 mL) was added MCPBA (50–60%, 500 mg, ~1.7 mmol) at 0°C. After 30 min the ice bath was removed and stirring was continued at r.t. for 1 h. The mixture was diluted with CH_2Cl_2 (60 mL) and the organic layer was washed with sat. aq $NaHCO_3$ (2 × 50 mL) and brine (2 × 30 mL), and dried ($NaSO_4$). The solution was evaporated and chromatographed through a short column of silica gel (3 × 4 cm, eluent C) and concentrated. The intermediate sulfoxide was suspended in sat. dioxane/NH_3 (20 mL) and heated overnight in a Parr Bomb (stainless steel) at 140°C. The mixture was cooled and concentrated. The residue was suspended in sat. MeOH/NH_3 (20 mL) and heated in a Parr Bomb at 140°C for 4 h, cooled and concentrated to dryness. The residue was dissolved in H_2O (40 mL) and applied to a Serdolit AR-4 column (4 × 10 cm). The column was washed first with H_2O (removal of salts), then with *i*-PrOH/H_2O (1:9). The product was obtained from the *i*-PrOH/H_2O fraction by evaporation and precipitation from CH_2Cl_2 yielding light yellow solid (290 mg, 69%); TLC (silica gel, eluent E): R_f 0.6.

UV (MeOH): λ_{max} = 264 nm (ε = 11100).

^{1}H NMR (DMSO-d_6): δ = 2.03, 2.30 (2 m, 2 H, H-2′), 2.39 (m, 4 H, CH_2NCH_2), 3.54 (m, 8 H, H-5′, CH_2N, CH_2OCH_2), 3.72 (m, 1 H, H-4′), 4.26 (m, 1 H, H-3′), 4.87 (br, 1 H, 5′-OH), 5.18 (br, 1 H, 3′-OH), 6.20 (br, 3 H, H-1′, NH_2), 6.74 (s, 1 H, H-6), 10.38 (br, 1 H, NH).

$C_{16}H_{23}N_5O_5$	calc.	C	52.60	H	6.34	N 19.17
(365.4)	found		52.54		6.19	19.10

We thank Dr. Natalya Ramzaeva for helpful discussions and Dr. Helmut Rosemeyer for the measurements of NOE spectra. Financial support by the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (BMBF) is gratefully acknowledged.

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