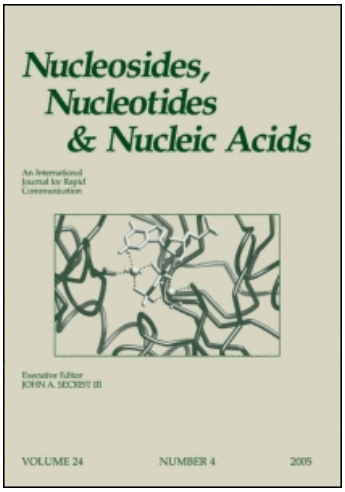

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N^4-(3-Methyl-2-Butenyl)-2-(Methylthio)Tubercidin and Its α-Anomer - 7-Deazapurine Nucleosides with Potential Anticytokinin Activity

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N^4-(3-METHYL-2-BUTENYL)-2-(METHYLTHIO)TUBERCIDIN AND ITS α-ANOMER - 7-DEAZAPURINE NUCLEOSIDES WITH POTENTIAL ANTICYTOKININ ACTIVITY

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ABSTRACT - Phase-transfer catalysis of pyrrolo[2,3-d]pyrimidine 4a with the halogenose 5 yields the anomers 6a and 7a. Deprotection with boron trichloride gives the chloro nucleosides 6b and 7b, which are converted into the potential anticytokinin 2 and its α-anomer 3.

Cytokinins are a group of plant hormones that promote growth and participate in cell differenciation at a minimum concentration of 10^{-7} M[1]. Most plants require cytokinins, but can grow without those from exogenous sources due to their own production. Naturally occuring cytokinins are N^6-(Δ^2-isopentenyl)adenines which can be further modified in the side chain. 9-β-D-Ribofuranosides like 1a or its 2-methylthio-derivative 1b with cytokinin activity are located adjacent to the 3'-site of the anticodon of certain tRNAs[2]. Their function in tRNA is still unknown, but it is believed that they are able to modulate codon/anticodon recognition during translation of the genetic message[3].

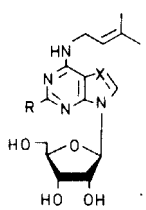
In the course of screening of candidate cytokinins it became evident that some of them possess anticytokinin activity. Especially nucleosides derived from pyrrolo[2,3-d]pyrimidines bearing a 2-methylthio group were highly active[4]. The parent nucleoside 1c has already been prepared semi-synthetically from tubercidin[5]. The synthesis of the methylthio nucleoside 2 and its α-anomer 3 can now be approached

by regio selective phase-transfer glycosylation[6] of the aglycon 4a by the halogenose 5.

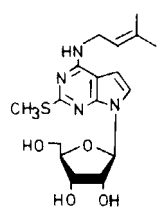
RESULTS AND DISCUSSION

The synthesis of nucleoside 2 and its α-anomer 3 can be performed on several routes. The most direct approach, glycosylation of the aglycon 4b, had to be dismissed because of problems encountered with the removal of the protecting groups. Both methods of deprotection, catalytic hydrogenation or cleavage with boron trichloride affect the substituents of the aglycon. We, therefore, decided to glycosylate the chloro compound 4a. The 2',3',5'-benzoyl protected nucleoside of 4a has already been prepared as an intermediate, but has not been converted into the free nucleoside[7].

The reaction was performed by phase-transfer catalysis in a biphasic mixture containing dichloromethane/tetrahydrofuran as organic layer, 50% aqueous sodium hydroxide as aqueous phase and tetrabutylammonium bisulfate as catalyst. By this means the chromophore 4a is converted into its anion which can displace the bromine substituent of halogenose 5 by S_N2 reaction. Two main products with very similiar mobilities were isolated after twofold chromatography on silica gel in a total yield of 63%. They showed almost identical UV spectra as being expected for anomers.



1a: X = N; R = H
b: X = N; R = CH_3S
c: X = CH; R = H



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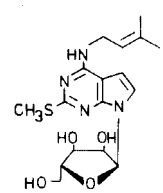

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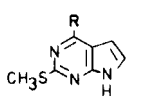
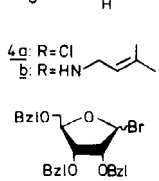


4a: R = Cl
b: R = HN



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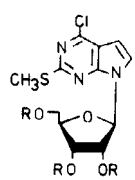
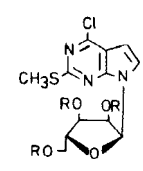


6a: R = Bzl
b: R = H



7a: R = Bzl
b: R = H

Their proton NMR spectra with a chemical shift for the 1'-H of 6.67 ppm for the faster migrating zone and 6.30 ppm for the slower migrating, reveal that the former consists of the α- and the latter of the β-anomer.

Next the benzyl protecting groups were removed using boron trichloride in dichloromethane as reagent[8]. After excessive boron trichloride had been destroyed by methanol and aqueous ammonia the chloro nucleosides 6b and 7b were isolated and could be obtained crystalline. Their UV spectra were similiar to that of the aglycon 4a indicating that the latter was not altered. The chromatographic mobilities (silica gel, CH_2Cl_2/MeOH, 4:1) of nucleosides 6b and 7b turned out to be reversed refered to the benzyl protected compounds. For easy distinction the melting points can be used, exhibiting 76°C for the α-anomer and 191-194°C for the β-anomer.

We then introduced the Δ^2-isopentenylamino group by exchanging the 4-chloro substituent of 6b or 7b by 3-methyl-2-butenylamine. After chromatographic separation the nucleosides 2 and 3 were obtained as yellowish solids, exhibiting UV maxima at 287 and 237 nm with absorbances nearly identical to that of the aglycon 4b.

The intact Δ^2-isopentenyl moiety of the anomeric nucleosides 2 and 3 is indicated both by ^1H and ^{13}C NMR spectroscopy (Table 1 and 2). Typically the triplet of the olefinic methine proton is observed at 5.33 ppm, the methylene group protons at 4.07 and a triplet of the alkylamino group at 7.58 ppm. Therefore isomerisation or hydroxylation of the side chain can be excluded. Comparison of the ^{13}C NMR spectra of the chloro nucleosides 6b and 7b as well as the Δ^2-isopentenyl nucleosides 2 and 3 with that of their aglycons shows them to suffer a downfield shift for the signal of C-6, a finding underlining that the D-ribofuranosyl moiety is attached to N-7 as has been found for tubercidin. Assignment of the nucleosides to their anomeric lines follows directly from the signals of the sugar moiety. As shown in table 2 the C-1'-signals of the β-anomers are generally more downfield shifted than those of the α-anomers and thus similar to tubercidin.

Both, the methylthio group and the Δ^2-isopentenyl chain sterically shield N-3, which has been definitely shown in the case of the corresponding purine nucleosides by X-ray crystallography[9]. This shielding interferes with hydrogen bonding during Watson-Crick base pair formation. Since in compound 2 N-7 is also missing, no Hoogsteen base pairs can

Table 1. Proton NMR Spectral Data of Pyrrolo[2,3-d]pyrimidine Nucleosides[a]

compd	6-H	5-H	1'-H	2'-H	3'-H	4'-H	5'-H	SCH_3	NH	CH_2	CH=	CH_3
7b	7.83 (d,4)	6.54 (d,4)	6.52 (d,7)	4.36 - 4.09			3.52 (m)	2.58				
6b	7.80 (d,4)	6.65 (d,4)	6.12 (d,6)	4.42 (t,6)	4.14 (m)	3.93 (m)	3.6 (m)	2.58				
4a	7.53 (m)	6.55 (m)						2.58				
3	7.38 (d,4)	6.53 (d,4)	6.45 (d,5)	4.3 - 3.9			3.53 (m)	2.50	7.58 (t,6)	4.07 (m)	5.33 (t,6)	1.75
2	7.20 (d,4)	6.57 (d,4)	5.98 (d,6)	4.38 (m)	4.05 (m)	3.85 (t,3)	3.56 (m)	2.50	7.64 (t,6)	4.05 (m)	5.26 (t,6)	1.71
4b	6.93 (m)	6.54 (m)						2.48	7.52 (t,6)	4.07 (m)	5.33 (t,8)	1.74
Tu[b]	7.31 (d,4)	6.57 (d,4)	5.97 (d,6)	4.42 (m)	4.06 (m)	3.88 (m)	3.55 (m)		7.04			

[a] All spectra were recorded in Me_2SO-d_6 with TMS as an internal standard. Chemical shifts are given in δ values; coupling constants in parentheses are in hertz. [b] Tu = Tubercidin; Chemical shifts of OH not given.

TABLE 2. Carbon-13 NMR Data of Pyrrolo[2,3-d]pyrimidine Nucleosides [a]

compd	C-2	C-4	C-4a	C-5	C-6	C-7a	C-1'	C-2'	C-3'	C-4'	C-5'
4a	162.8	152.8	113.3	99.0	126.7	150.5					
7b[c]	163.1	152.2	113.7	98.4	130.2	150.4	85.0	70.9	70.7	84.0	61.6
6b	163.5	152.5	114.0	99.9	127.3	150.5	87.3	74.1	70.5	85.3	61.6
4b	163.6	155.4	99.7	98.8	119.3	150.9					
3[c]	162.4	155.5	99.8	98.3	123.2	150.5	83.9	70.9	70.6	83.2	61.6
2	162.9	155.5	100.4	99.6	120.4	150.6	87.0	73.6	70.4	84.6	61.7
Tu[b]	151.5	157.5	103.1	99.5	122.3	149.9	87.6	73.7	70.8	85.1	61.9

	CH_3S	CH_2	CH=	C=	CH_3	CH_3
4a	13.7					
7b	13.7					
6b	13.7					
4b	13.3	36.7	122.2	133.1	25.3	17.7
3	13.2	37.8	122.2	133.3	25.2	17.6
2	13.2	37.8	122.0	133.3	25.1	17.5

[a] All spectra were recorded in Me_2SO-d_6 with TMS as an internal standard. Chemical shifts are given in δ values. [b] Tu = Tubercidin. [c] C-1'/C-4' and C-2'/C-3' might be reversed.

be formed. These features should alter the conformation of multistranded nucleic acid structures formed from polynucleotide chains having the nucleoside 2 incorporated.

EXPERIMENTAL

Melting points were determined on a Berl apparatus (Wagner & Munz, Germany) and are not corrected. Elemental analyses were performed by Mikroanalytisches Labor Beller (Göttingen, Germany). ^{1}H and ^{13}C NMR spectra were recorded on Varian EM 390 or Bruker WM 250 spectrometers; δ-values are in parts per million relative to tetramethylsilane as internal reference. UV spectra were measured on an Uvicon 810 (Kontron, Switzerland) or Shimadzu UV-210A spectrophotometer (Shimadzu, Japan), respectively. Thin-layer chromatography (TLC) was carried out using silica gel F 254 plates (Woelm, Germany), visualisation being achieved by irradiation at 254 nm. Column chromatography was performed on silica gel 60 (Merck, Germany, 230-400 mesh ASTM); solvent systems: A, CH_2Cl_2; B, CH_2Cl_2- MeOH (98:2); C, $CHCl_3$- MeOH (4:1).

Phase-Transfer Glycosylation of 4-Chloro-2-(methylthio)-7H-pyrrolo-[2,3-d]pyrimidine (4a) with 2,3,5-Tri-O-benzyl-D-ribofuranosyl bromide (5).

Dry hydrogen bromide was passed into a solution of 2,3,5-tri-O-benzyl-1-O-(p-nitrobenzoyl)-D-ribofuranose[10] (3.5 g, 6.2 mmol) in dichloromethane (20 mL) until no further precipitation of p-nitrobenzoic acid could be observed. The colorless acid was filtered off and washed with a small volume of cold dichloromethane; the combined filtrates containing the halo sugar 5 were used directly in the next step.

The chloro compound 4a[11] (1.24 g, 6.2 mmol) suspended in dichloromethane/tetrahydrofuran (15 mL each) was added to a solution of tetrabutylammonium hydrogensulfate (0.4 g, 1 mmol) in 50% aqueous sodium hydroxide (30 mL) and agitated with a vibromixer until all solid material had dissolved. Thereupon the freshly prepared solution of the halogenose 5 was added and mixing was continued for 30 min. The organic layer was diluted to about 200 mL with dichloromethane, separated, washed with water, dried over magnesium sulfate and concentrated in vacuo to a volume of about 10 mL. This solution was applied to a silica gel column (45 x 5 cm) and chromatographed with dichloromethane. A total of 2.35 g (63%) of a yellowish syrup was obtained. In order to

achieve full separation of the anomers repeated chromatography was necessary.

4-Chloro-2-(methylthio)-7-(2',3',5'-tri-O-benzyl-α-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidine (7a).

The faster migrating zone afforded the α-anomer 7a (1.25 g, 33%) as a yellowish syrup. - TLC (solvent A) R_f 0.71; UV (MeOH) λ_{max} 310 nm (ε 6600), 281 (ε 6700), 252 (ε 24000). - ^{1}H NMR (Me_2SO-d_6) δ 2.53 (s, SCH_3), 3.50 (m, 5'-H), 4.1 - 4.7 (m, 2', 3', 4'-H), 4.46 (d, benzyl-CH_2), 6.40 (d, 5-H, J=5Hz), 6.67 (d, 1'-H, J=6Hz), 7.3 (m, 3 phenyl), 7.73 (d, 6-H, J=5Hz). - ^{13}C NMR (Me_2SO-d_6) δ 13.8 (SCH_3), 70.1 (C-5'), 72.0 and 72.4 (3 benzyl-CH_2), 77.1 (C-3'), 77.4 (C-2'), 81.7 (C-4'), 82.5 (C-1'), 98.8 (C-5), 113.9 (C-4a), 126.4 - 129.4 and 137.4 - 138.1 (3 phenyl), 127.9 (C-6), 150.7 (C-7a), 152.0 (C-4), 163.1 (C-2).

4-Chloro-2-(methylthio)-7-(2',3',5'-tri-O-benzyl-β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidine (6a).

The slower migrating zone contained the β-anomer 6a (1.1 g, 29%) as a yellowish, viscous oil. - TLC (solvent A) R_f 0.64; UV (MeOH) λ_{max} 310 nm (ε 7300), 281 (ε 8100), 252 (ε 24500). - ^{1}H NMR (Me_2SO-d_6) δ 2.57 (s, SCH_3), 3.67 (m, 5'-H), 4.1 - 4.7 (m, 2', 3', 4'-H), 4.53 and 4.65 (3 benzyl-CH_2), 6.30 (d, 1'-H, J=5Hz), 6.42 (d, 5-H, J=3Hz), 7.33 (m, 3 phenyl and 6-H). - ^{13}C NMR (Me_2SO-d_6) δ 13.5 (SCH_3), 69.4 (C-5'), 71.2, 71.4, 72.4 (3 benzyl-CH_2), 76.3 (C-3'), 79.5 (C-2'), 81.3 (C-4'), 86.4 (C-1'), 99.8 (C-5), 114.1 (C-4a), 126.8 (C-6), 127.3 - 128.2 and 137.6 - 138.0 (3 phenyl, 150.8 (C-7a), 151.7 (C-4), 163.7 (C-2).

Anal. calcd. for $C_{33}H_{32}ClN_3O_4S$: C 65.82, H 5.36, Cl 5.88, N 6.98, S 5.33. Found α-anomer 7a: C 65.96, H 5.41, Cl 6.15, N 6.91, S 5.33; β-anomer 6a: C 65.76, H 5.42, Cl 6.14, N 6.92, S 5.56.

4-Chloro-2-(methylthio)-7-(α-D-ribofuranosyl)-7H-pyrrolo[2,3-d]-pyrimidine (7b).

The protected nucleoside 7a (0.6 g, 1 mmol) was dissolved in dichloromethane (20 mL) and cooled to -78°C (dry ice/acetone). A cooled solution of boron trichloride in dichloromethane (1M, 5 mL) was added and the mixture was kept at this temperature for about 4 h. Methanol was then carefully added and the solution was allowed to stand at room temparature for 1 h. After the solvent had been evaporated in vacuo, the dark reddish residue was taken up in dichloromethane/water

(25 mL/100 mL). The organic layer was discarded, the aqueous layer was neutralized with aqeous ammonia and evaporated in vacuo. The colorless residue was crystallized from water affording 205 mg (62%) of colorless needles, mp. 76°C. - TLC (solvent C) R_f 0.71; UV (MeOH) λ_{max} 310 nm (ε 6500), 274 (ε 10000), 251 (ε 25200).

4-Chloro-2-(methylthio)-7-(β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]-pyrimidine (6b).

The β-anomer 6a (0.56 g, 0.93 mmol) in dichloromethane (20 mL) was treated the same as described for the α-anomer. Recrystallization of the solid residue from water afforded compound 6b as colorless needles 207 mg (67%), mp. 191-194°C. - TLC (solvent C) R_f 0.76; UV (MeOH) λ_{max} 310 nm (ε 6300), 275 (ε 6300), 251 (ε 22400). - Anal. calcd. for $C_{12}H_{14}ClN_3O_4S$: C 43.44, H 4.25, N 12.67, S 9.66. Found α-anomer 7b: C 43.43, H 4.24, N 12.61, S 9.80; β-anomer 6b: C 43.57, H 4.30, N 12.60, S 9.89.

4-(3-Methyl-2-butenylamino)-2-(methylthio)-7-(α-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidine (3).

A solution of the α-anomer 7b (300 mg, 0.9 mmol) and 3-methyl-2-butenylamine hydrochloride[12] (180 mg, 1.5 mmol) in 1-butanol (10 mL) and triethylamine (1.5 mL) was refluxed for 24 h. The solvent was removed in vacuo and the tan residue was dissolved in dichloromethane/methanol (9:1, 10 mL) which were applied to a silica gel column (20 x 2 cm) and eluted with solvent B. After evaporation of the solvent compound 3 was obtained as a yellowish, amorphous solid 360 mg (88%). - TLC (solvent C) R_f 0.67; UV (MeOH) λ_{max} 287 nm (ε 16000), 236 (ε 25800).

4-(3-Methyl-2-butenylamino)-2-(methylthio)-7-(β-D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidine (2).

A solution of the β-anomer 6b (330 mg, 1.0 mmol) and 3-methyl-2-butenylamine hydrochloride (180 mg, 1.5 mmol) in 1-butanol (12 mL) and triethylamine (1.5 mL) was refluxed for 20 h. The solution was then treated as described for the α-anomer, yielding 320 mg (85%) of compound 2 as a colorless, amorphous solid. - TLC (solvent C) R_f 0.69; UV (MeOH) λ_{max} 287 nm (ε 17200), 237 (ε 26100). - Anal. calcd. for $C_{17}H_{24}N_4O_4S$: C 53.67, H 6.36, N 14.73, S 8.43. Found α-anomer 3: C 53.55, H 6.32, N 14.82, S 8.55; β-anomer 2: C 53.83, H 6.40, N 14.56, S 8.63.

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