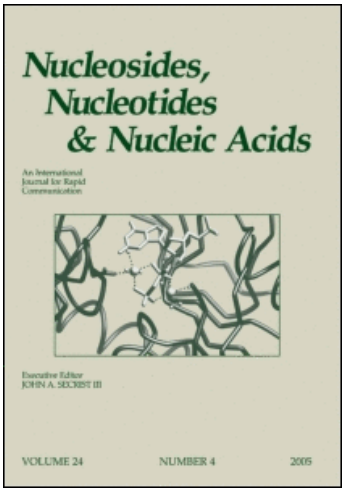

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Conversion of Tubercidin to Toyocamycin: Some Properties of Tubercidin Derivatives (Nucleosides and Nucleotides. 47)[1]

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CONVERSION OF TUBERCIDIN TO TOYOCAMYCIN: SOME PROPERTIES OF TUBERCIDIN DERIVATIVES (NUCLEOSIDES AND NUCLEOTIDES. 47)[1]

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ABSTRACT

A facile method of conversion of tubercidin to the 5-methyl derivative and toyocamycin is described. The NMR and CD spectra of 5- and 6-substituted tubercidins are presented. These data show that the 6-substituted tubercidins are in the syn-conformations in solution.

In a previous paper we have reported that treatment of tubercidin with thiocyanogen chloride gave the 5-thiocyanate.[2] Conversion of the 5-thiocyanate to the 6-cyano and 6-propyl tubercidins has also been described. In the continuing study of synthesis of tubercidin analogs, the present paper describes further electrophilic reactions of tubercidin, namely the introduction of a carbon unit and nitro group at position 5. Some conformational properties of 5 and 6-substituted tubercidins as studied by NMR and CD spectra are discussed.

Among reagents used for the introduction of a carbon unit into the position 3 of indoles, such as the Mannich reagent,[3] dithiolanylating agents[4], arylsulfonyl isocyanates,[5] and a combination of triphenylphosphine and thiocyanogen[6], the Mannich reagent seemed to be quite efficient in preliminary investigations. Treatment of tubercidin (1) with an immonium salt prepared from formaldehyde and morpholine in dimethylformamide gave the 5-morpholinomethyl derivative (2) in 33% isolated yield. When the reaction mixture was further treated with acetyl chloride the tri-O-acetate (3) was isola-

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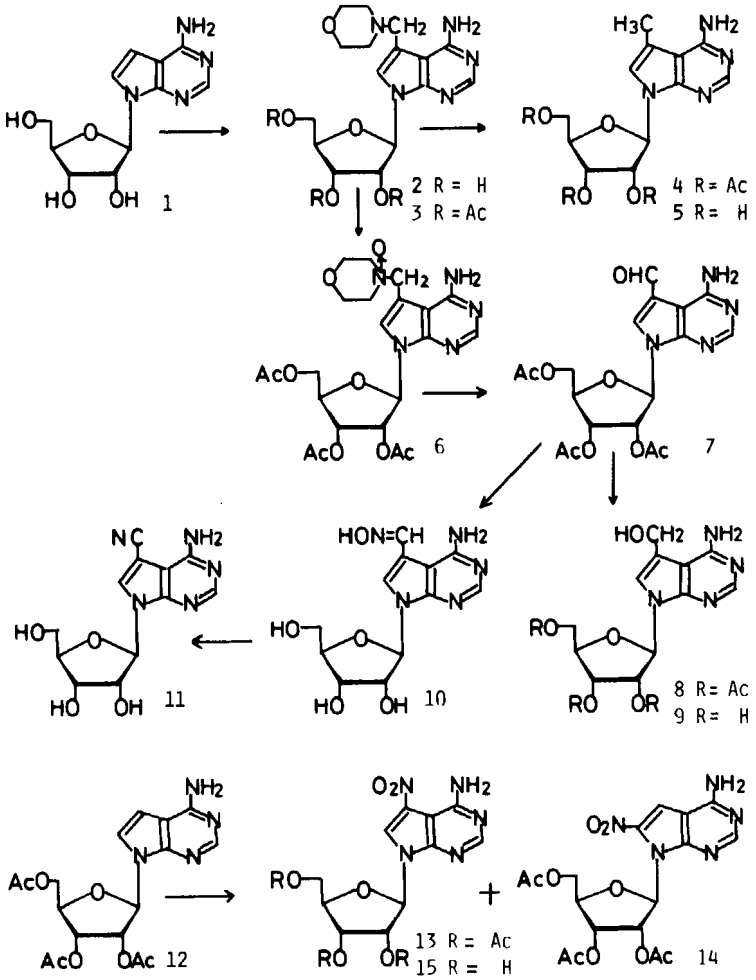
ted in 58% yield. The NMR spectra of 3 clearly showed that the morpholinomethyl group was on position 5 of 1.

Hydrogenation of 3 over a palladium catalyst afforded the 5-methyl derivative (4) which was deacetylated to furnish 5-methyltubercidin (5).[7] Compound 5 has been synthesized by a multistep conversion of a pyrrolopyrimidine base.[7]

Conversion of 3 to toyocamycin was then undertaken. Treatment of 3 with peracetic acid gave the oxido-morpholinomethyl derivative (6). In the peracid treatment of adenine derivatives the formation of N_1-oxides was the general course. In the present case N-oxidation of the aliphatic amine moiety took precedence to that of the pyrimidine portion, which was evident by no substantial change in the UV spectra of the pro-



duct. Treatment of 6 with acetic acid gave rise to the Polonovski reaction[8] to afford the tubercidin 5-carboxaldehyde (7) in a crystalline form. The presence of the formyl group in 7 was evidenced by the detection of a proton signal at 9.75 ppm in the NMR spectra. Reduction of 7 with sodium borohydride gave the 5-hydroxymethyl derivative 8, together with the deacetylated derivative 9. Treatment of 7 with hydroxylamine in aqueous ethanol at 70° overnight followed by deacetylation afforded the aldoxime (10) in a crystalline form. Treatment of 7 with hydroxylamine and successive treatment of the product with acetic acid-acetic anhydride gave, after deacetylation, 5-cyanotubercidin, known as toyocamycin. The physical properties (UV, IR and NMR spectra) of 5-cyanotubercidin (11) were completely identical with those of the natural product. This reaction sequence is the first example of the conversion of tubercidin to toyocamycin.

In order to introduce a nitrogen function at position 5 or 6 of tubercidin, nitration was studied with various nitrating systems. Treatment of 2',3',5'-tri-O-acetyltubercidin (12[2]) with fuming nitric acid-sulfuric acid in methylene chloride gave two products which were separable on a silica gel column. One of the products, isolated in a crystalline form, was assigned as the 5-nitro derivative (13) on the basis of its NMR spectrum. The other product was determined to be 6-nitro derivative (14). Base catalyzed deacetylation of 13 resulted in decomposition of the product. Deacetylation by acid catalysis at room temperature gave 5-nitrotubercidin (15). All attempt at deacetylation of 14 was unsuccessful. The use of nitronium tetrafluoroborate for the nitration did not improve the yields or change the ratio of the products. It is to be noted that halogenation of tubercidin gives 5- or 6-derivatives by selecting the reaction conditions.[9] Thiocyanation of 1 always gives the 5-thiocyanate.[2]

Physical Properties of 5-(and 6-)Substituted Tubercidins.

One of the structural features of nucleosides related to their biological activities is the conformation around the glycosylic linkage. For the estimation of anti-syn conformations of nucleosides in solution NMR and CD spectra have been used. It has been generally recognized that the usual purine

nucleosides prefer to possess anti conformations in solution, whereas 8-substituted purine nucleosides tend to possess syn conformations.[10] The differences in the conformations are reflected in the chemical shifts of the 2'-protons of the sugar moiety. In the syn nucleosides deshielding anisotropy of the N_3-electrons has been observed for the 2'-proton. In Table I the selected chemical shift values of tubercidin derivatives prepared in this and previous[2] works are summarized. As is evident the 2'-protons of all 6-substituted tubercidins appeared at the downfield as compared to those of 5-substituted derivatives. This is due to the similar deshielding effect of the N_1-electrons (equivalent to the N_3-electrons of purines) of the base moiety in the syn form of these derivatives. The downfield shifts of the anomeric protons of 20 and 21 are due to the deshielding effects of the respective 6-carbonyl groups.

The signs of CD bands at the main absorption region have also been used for the estimation of anti-syn conformations. In the purine nucleosides the anti forms (2-, 6- and 2,6-disubstituted purines) show negative Cotton bands while those in the syn forms (8-substituted purines) show positive bands[11], in general.

The CD spectral data of 5- and 6-substituted tubercidins are summarized in Table II. All 5-substituted derivatives showed negative CD bands at the main absorption region which reflect that these are in the anti conformations. In the case of 6-substituted derivatives the 6-carboxamide and 6-carboxylic acid (20 and 21) showed a reversal of the sign as expected. However, the 6-cyano and 6-propyl derivatives (18 and 19) exhibited negative CD bands at the main absorption region. This would mean that these substituents are less bulky as compared with the 6-carboxylates, thus causing some differences in the torsion angle of the glycosylic linkages. Since it is confirmed that these also exist in the syn form by NMR measurements, the anti-syn preference cannot be simply concluded from the sign of the CD bands. As the molar ellipticities of all tubercidin derivatives are rather small there would be frequent interconversion of anti-syn equilibrium.

EXPERIMENTAL

UV spectra were measured on a Shimadzu UV-300 spectrophotometer. IR spectra were taken on a Hitachi 215 spectro-

Table I. Characteristic NMR Chemical Shifts of 5- and 6-Substituted Tubercidins.

5-Substituent		H-2	H-5	H-6	H-1'	H-2'	H-3'
-H	(1)	8.04	6.58	7.33	5.98	4.42	4.09
$-SCH_3$	(16)	8.10	-	7.60	6.01	4.39	4.08
$-SO_2CH_3$	(17)	8.23	-	8.26	6.11	4.40	4.14
$-CH_2N(C_2H_4)_2O$	(2)	8.01	-	7.28	5.95	4.38	4.06
$-CH_3$	(5)	8.00	-	7.07	5.96	4.36	4.06
$-CH_2OH$	(9)	8.05	-	7.26	5.98	4.37	4.06
-CHN=OH	(10)	8.14	-	7.72	6.00	4.36	4.07
-CN	(11)	8.22	-	8.44	6.06	4.38	4.10
$-NO_2$	(15)	8.24	-	8.92	6.13	4.37	4.14
6-Substituent							
$-n-C_3H_7$	(18)	7.95	6.34	-	5.67	4.96	4.13
-CN	(19)	8.20	7.56	-	5.97	4.79	4.13
$-CONH_2$	(20)	8.07	7.07	-	6.50	4.90	4.17
$-CO_2H$	(21)	8.10	7.49	-	6.79	4.97	4.17

NMR spectra were taken in DMSO-d_6. For the chemical shifts of other protons and coupling constants, see Experimental.

Table II. Molar Ellipticities of 5- and 6-Substituted Tubercidins.

5-Substituent		nm (molar ellipticity, θ)
$-SCH_3$	(16)	238 (0), 274 (-2400), 321 (0)
$-SO_2CH_3$	(17)	221 (0), 227 (+1200), 233 (0), 238 (-1500), 248 (-1700), 266 (-3600), 283 (-2200), 288 (-800), 303 (0)
$-NO_2$	(15)	231 (0), 248 (-1900), 255 (-1600), 273 (-3000), 308 (0), 325 (+300), 350 (0)
$-CH_3$	(5)	231 (-6200), 245 (-2200), 259 (-4400), 265 (-3800), 275 (-4100), 311 (0)
-CN	(11)	226 (-1500), 233 (-500), 271 (-4100), 296 (0)
6-Substituent		
$-n-C_3H_7$	(18)	222 (-2600), 229.5 (0), 233 (+500), 239 (0), 276 (-3400), 310 (0)
-CN	(19)	218 (+1900), 225 (0), 236 (-1800), 247 (0), 253 (+300), 261 (0), 291 (-1200), 319 (0)
$-CONH_2$	(20)	220 (-4300), 228 (-12500), 249 (0), 268.5 (+5400), 280 (+3300), 320 (0)
$-CO_2H$	(21)	229 (-900), 232 (0), 240 (+3100), 248.5 (0), 252 (-1100), 259.5 (0), 282 (+3100), 295 (+2700), 305 (+1000), 315 (0)

photometer. Mass spectra were measured on a JEOL JMS-D 300 mass spectrometer. NMR spectra were taken on a JEOL JNM-FX 100 FT spectrometer with tetramethylsilane as an internal standard. CD spectra were measured on a JASCO J-40 spectropolarimeter in H_2O at room temperature. Melting points were measured on a Yanagimoto MP-3 micromelting point apparatus and were uncorrected.

5-(1-Morpholinomethyl)tubercidin (2)----A formaldehyde solution (37%, 45.7 mL) was slowly added to 48.6 mL of morpholine under stirring. After the exothermic reaction was completed, compound 1 (5 g) was added to the solution, which was kept overnight at 90°C. The mixture was evaporated with added water and the evaporation with water was repeated several times. The final residue was adsorbed onto 60 g of silica gel and the mixture was dried in vacuo. The residue was placed on top of a silica gel column (90 g, 25x4.8 cm) with $CHCl_3$. The column was washed with $CHCl_3$-MeOH (100:2) and the product was eluted with 4-8% MeOH in $CHCl_3$. The product was purified through a silica gel column (25x4.8 cm) by the same solvent to give 2.25 g of 2 as a powder. A part of the powder was crystallized from EtOH: mp 123-126°C; UV (H_2O) λmax 275nm (ε, 9800), λmin 246 (ε,3300) and (0.1 N HCl) 273 (ε, 11000), λmin 248 (ε, 4000); NMR (DMSO-d_6)δ8.01 (s, 1, H-2), 7.9-7.5 (broad s, 2, NH_2), 7.28 (broad s, 1, H-6), 5.95 (d, 1, H-1', J= 6.3 Hz), 5.4-5.1 (m, 3, HOx3), 4.38 (ddd, 1, H-2'), 4.06 (m, 1, H-3'), 3.57 (broad s, 8, H-5', NCH_2-5, CH_2OCH_2), 2.53 (broad, CH_2NCH_2, buried in DMSO). Anal. Calcd for $C_{16}H_{23}N_5O_5 \cdot EtOH$: C, 52.54; H, 7.10; N, 17.02. Found: C, 52.43; H, 6.87; N, 17.04.

2',3',5'-Tri-O-acetyl-5-(1-morpholinomethyl)tubercidin (3) ----Paraformaldehyde (740 mg) was suspended in 30 mL of dimethylformamide and morpholine (2.16 mL) was added to the mixture, which was stirred for 1.5 h at 60°C. To the mixture was added 3 g of 1 and the reaction was kept at 70°C for 5.5 h. After removal of the solvent in vacuo the residue was taken up in 50 mL of 50% AcOH and kept at 60°C for 1.5 h. The solvent was removed in vacuo and the residue was dried and added to a mixture of AcOH (20 mL) and acetyl chloride (8 mL). After 1 h, the solvent was evaporated and the residue was patitioned between $CHCl_3$ and

H_2O, and neutralized by the addition of $NaHCO_3$. The organic layer was dried over Na_2SO_4 and then evaporated. The residue was taken up in $CHCl_3$ and applied to a silica gel column (15x 4.8 cm). After washing the column with $CHCl_3$ the product was eluted with $CHCl_3$-MeOH (100:1) to give 3.2 g (58%) of 3 as a powder. This was used for the next step without purification.

2',3',5'-Tri-O-acetyl-5-methyltubercidin (4)----Compound 3 (1 g) in 50 mL of 30% EtOH was hydrogenated over 10% Pd-C (400 mg) under gentle reflux and atmospheric pressure for 3 days. The catalyst was removed, the filtrate was concentrated, and the residue was taken up in AcOH-AcCl (10 mL-1 mL). After 1 h, the solvent was removed and the residue was crystallized from EtOH to give 0.76 g (92%) of 4: mp 188-191°C (sintered at 152°C); UV (H_2O) λmax 278nm, λmin 247 and (0.1 N HCl) λmax 281nm, λmin 252. Anal. Calcd for $C_{18}H_{19}N_4O_7 \cdot 1/2$ EtOH: C, 53.52; H, 5.20; N, 13.14. Found: C, 53.26; H, 5.42; N, 13.38.

5-Methyltubercidin (5)----Hydrogenation of 2 (500 mg) in 25 mL of 50% EtOH over Pd-C (150 mg) for 2 days under reflux and crystallization of the product from H_2O gave 238 mg (62%) of 5: mp 265-268°C (lit.[7] 262-263°C, dec.); UV (H_2O) λmax 280 nm (ε, 13200), λmin 242 (ε, 4600) and (0.1 N HCl) λmax 283 (ε, 12900), λmin 253 (ε, 5600) and (0.1 N NaOH) λmax 281 (ε, 12900), λmin 242 (ε, 4900); NMR (DMSO-d_6) δ 8.00 (s, 1, H-2), 7.07 (broad s, 1, H-6), 6.59 (broad s, 2, NH_2), 5.96 (d, 1, H-1', J= 6.4 Hz), 5.37-4.89 (m, 3, HOx3), 4.36 (ddd, 1, H-2'), 4.06 ddd, 1, H-3'), 3.87 (dt, 1, H-4'), 3.56 (broad d, 2, H-5'), 2.34 (broad s, 3, Me-5). Anal. Calcd for $C_{12}H_{16}N_4O_4 \cdot 1/4\ H_2O$: C, 50.61; H, 5.84; N, 19.68; Found: C, 50.80; H, 5.77; N, 19.66.

2',3',5'-Tri-O-acetyl-5-(1-oxido-1-morpholinomethyl)-tubercidin (6)----To a solution of 3 (1.86 g) in 20 mL of AcOH was added 0.43 mL of 30% H_2O_2 and the mixture was heated at 60°C for 17 h. Water was added to the mixture and the solvent was removed in vacuo. Evaporation with H_2O was repeated several times and the residue was taken up in $CHCl_3$, which was applied to a column of silica gel (15x3.8 cm). Elution with $CHCl_3$-MeOH (100:8, 100:16) gave, after evaporation of the solvent, 1.22 g (64%) of 6 as a foam: UV (H_2O) λmax 272nm, λmin 246 and (0.1 N HCl) λmax 272, λmin 229; NMR ($CDCl_3$) δ 8.28 (s,

1, H-2), 7.11 (s, 1, H-6), 6.42 (d, 1, H-1', J= 6.3 Hz), 5.66 (dd, 1, H-2'), 5.48 (m, 1, H-3'), 4.51 (broad s, 2, H-5'), 4.37 (broad s, 4, $-CH_2OCH_2-$), 4.29 (m, 1, H-4'), 3.83 (m, 2, CH_2-5), 3.23 (m, 4, $-CH_2N(O)CH_2-$), 2.15, 2.12, 2.02 (s, 3 each, Acx3).

2',3',5'-Tri-O-acetyl-5-formyltubercidin (7)----A solution of 6 (1.16 g) in 50 mL of AcOH was heated at 120°C for 6 h with an addition of a small volume of AcCl. After evaporation of the solvent the residue was partitioned between H_2O (containing $NaHCO_3$) and $CHCl_3$. The organic layer was separated and applied to a silica gel column (10x2.8 cm). Elution of the column with $CHCl_3$-MeOH (100:6) gave a product which was crystallized from EtOH to give 0.98 g (quant.) of 7: mp 159-162°C; UV (MeOH) λmax 319nm (ε, 5600), 278 (ε, 12300), 259 (sh, ε, 9900), 242 (ε, 9700), λmin 248 (ε, 8700), 224 (ε, 6800); NMR ($CDCl_3$) δ 9.75 (s, 1, CHO-5), 8.33 (s, 1, H-2), 7.83 (s, 1, H-6), 6.37 (d, 1, H-1', J= 5.1 Hz), 5.73 (dd, 1, H-2'), 5.56 (m, 1, H-3'), 4.42 (broad s, 3, H-4',5'), 2.15, 2.08 (s, 6 and 3, Acx3). The protons of the amino group were undetected. Anal. Calcd for $C_{18}H_{20}N_4O_4$: C, 51.43; H, 4.80; N, 13.33. Found: C, 51.26; H, 4.72; N, 13.18.

5-Hydroxymethyltubercidin (9)----To a solution of 7 (0.5g) in 25 mL of 80% EtOH was added $NaBH_4$ (40 mg). After 1 h, the solution was neutralized with AcOH and the solvent was evaporated, and the residue was partitioned between $CHCl_3$ and H_2O. The organic layer was applied to a column of silica gel (12x 1.8 cm). Elution of the column with $CHCl_3$-MeOH (100:6-12) gave 8 in the first fraction (211 mg, 42% as a foam). The later fraction was concentrated and combined with the aqueous layer of the partition and treated with 1 N NaOH for 1 h. After neutralization, the solution was passed through a column of XAD-4 (28x4.8 cm). The eluate was concentrated and the residue was crystallized from aqueous EtOH to give 163 mg (46%) of 9: mp 237-238°C; UV (H_2O) λmax 273nm (ε, 10300), λmin 244 (ε, 3600) and (0.1 N HCl) λmax 276 (ε, 9500), λmin 251 (ε, 4400) and (0.1 N NaOH) λmax 273 (ε, 10100), λmin 245 (ε, 3800); NMR (DMSO-d_6) δ 8.05 (s, 1, H-2), 7.26 (s, 1, H-6), 6.93 (broad s, 2, NH_2), 5.98 (d, 1, H-1', J= 6.3 Hz), 5.73 (t, 1, $HOCH_2$-5, J= 5.1 Hz),

5.28-5.04 (m, 3, HOx3), 4.58 (d, 2, $\underline{HO}CH_2$-5), 4.37 (ddd, 1, H-2'), 4.06 (ddd, 1, H-3'), 3.88 (m, 1, H-4'), 3.55 (m, 2, H-5'). Anal. Calcd for $C_{12}H_{16}N_4O_5$: C, 48.64; H, 5.44; N, 18.91. Found: C, 48.53; H, 5.37; N, 18.94.

5-Formyltubercidin Oxime (10)----To a solution of 7 (0.4 g) in 10 mL of 50% EtOH was added 73 mg of hydroxylamine hydrochloride and 86 mg of AcONa, and the solution was kept at 70°C overnight. After evaporation of the solvent the residue was partitioned between $CHCl_3$ and H_2O. The organic layer was concentrated and the residue was obtained as a powder (450 mg, quant.). This was dissolved in 20 mL of MeOH containing 230 mg of NaOMe and stirred for 4 h at room temperature. The solution was neutralized with Dowex-50 (H^+) resin, the resin was filtered, and the filtrate was concentrated. The residue was crystallized from EtOH to give 239 mg (81%) of 10: mp 197.5-198.5°C; UV (H_2O)λmax 280nm (ε, 16000), 263 (sh,ε, 12300), λmin 240 (ε, 8600) and (0.1 N HCl) λmax 285 (ε, 10500), 263 (ε,10600), 238 (ε, 14100) and (0.1 N NaOH) λmax 288 (ε, 16200), 270 (sh, ε, 14100), λmin 248 (ε, 10000); NMR (DMSO-d_6) δ 10.99 (s, 1, =NOH), 8.14 (s, 1, H-2), 8.08 (s, 1, -N=CH-5), 7.72 (s, 1, H-6), 8.40 and 7.00 (broad s each, 2, NH_2), 6.00 d, 1, H-1', J=6.1 Hz), 5.40-5.06 (m, 3, HOx3), 4.36 (ddd, 1, H-2'), 4.07 (ddd, 1, H-3'), 3.90 (dt, 1, H-4'), 3.58 (m, 2, H-5'). Anal. Calcd for $C_{12}H_{15}N_5O_5 \cdot 3/4\ H_2O$: C, 44.65; H, 5.15; N, 21.70. Found: C, 44.75; H, 5.09; N, 21.69.

Toyocamycin (11)----Compound 7 (200 mg) was treated with 73 mg of hydroxylamine hydrochloride and 86 mg of AcONa in 50% EtOH for 2 h at 60°C to give the oxime as described in the previous section. This was dissolved in a mixture of AcOH (3 mL) and Ac_2O (3 mL) with 100 mg of AcONa, and the mixture was heated for 8 h under reflux. The solvent was removed in vacuo, and the residue was partitioned between $CHCl_3$ and H_2O containing $NaHCO_3$. The organic layer was concentrated, and the residue was taken up in EtOH saturated with NH_3 and kept overnight at room temperature. After evaporation of the solvent the residue was applied to a preparative thin layer chromatogram developed with $CHCl_3$-MeOH (100:6). The appropriate band was excised and the product was extracted with MeOH. The concentrate was crys-

tallized from H_2O to give 42 mg (30%) of 11: mp 210-212°C; UV (H_2O) λmax 290nm (sh), 279, 273 (sh), 232, 208, λmin 248, 233 and (0.1 N HCl) λmax 275, 235, λmin 250, 215; NMR (DMSO-d_6) δ 8.44 (s, 1, H-6), 8.22 (s, 1, H-2), 6.91 (broad s, 2, NH_2), 6.06 (d, 1, H-1', J= 5.6 Hz), 5.46 (d, 1, HO), 5.22 (m, 2, HOx2), 4.38 (ddd, 1, H-2'), 4.10 (ddd, 1, H-3'), 3.95 (m, 1, H-4'), 3.62 (broad s, 2, H-5', partially buried in the signal of H_2O); IR (KBr) 2230 cm^{-1} (-CN). Anal. Calcd for $C_{12}H_{13}N_5O_4$: C, 49.48; H, 4.50; N, 24.05. Found: C, 49.40; H, 4.58; N, 23.86.

2',3',5'-Tri-O-acetyl-5-nitrotubercidin (13)----A. Compound 12[2] (7 g) was dissolved in 120 mL of CH_2Cl_2 in an ice-bath. A mixture of 9.5 mL of H_2SO_4 and 9.5 mL of fuming HNO_3 was added dropwise to the solution under vigorous stirring for 5 min. After 20 min in the bath, the stirring was continued at room temperature for 20 min. The mixture was poured into an ice cold $NaHCO_3$ solution, and the separated organic layer was dried over Na_2SO_4 and concentrated. This was applied to a column of silica gel (200 g, 30x4.8 cm). Elution of the column with $CHCl_3$-MeOH (100:1 and 100:2) gave a product which was crystallized from EtOH to give 2.83 g (36%) of 13: mp 182.5-184°C; UV (MeOH) λmax 360nm (ε, 4500), 291 (sh, ε, 7700), 275 (ε, 11000), 254 (ε, 11300), 236 (sh, ε, 9500), λmin 310 (ε, 1900), 264 (ε, 10600), 224 (ε, 7400); NMR ($CDCl_3$) δ 8.32 (broad s, 2, H-2,6), 6.43 (d, 1, H-1', J= 4.4 Hz), 5.67 (dd, 1, H-2'), 5.51 (dd, 1, H-3'), 4.43 (m, 3, H-4',5'), 2.24, 2.13, 2.11 (s each, 9, Acx3); MS (m/z) 437 (M^+), 259 (sugar$^+$), 180 (B+2$^+$), 179 (B+1$^+$). Anal. Calcd for $C_{17}H_{19}N_5O_9$: C, 46.68; H, 4.38; N, 16.01. Found: C, 46.46; H, 4.35; N, 15.92.

B. Nitromium tetrafluoroborate (1.57 g) was suspended in 50 mL of CH_2Cl_2 in an ice bath and 12 (4.63 g) was added to the mixture. The mixture was stirred overnight at room temperature. After the work-up as in the previous section, 1.38 g (27%) of 13 was obtained.

2',3',5'-Tri-O-acetyl-6-nitrotubercidin (14)----The later eluate from the column for the separation of 13 was concentrated to leave 1.37 g (18%) of 14 as an amorphous powder: UV (H_2O) λmax 380nm, 246, λmin 300, 222 and (0.1 N HCl) λmax 345, 218, λmin 290; NMR ($CDCl_3$) δ 8.42 (s, 1, H-2), 7.50 (s, 1, H-5),

6.88 (d, 1, H-1', J= 3.7 Hz), 6.18 (dd, 1, H-2'), 5.91 (broad m, 3, H-3', NH_2), 4.65-4.18 (m, 3, H-4',5'), 2.15, 2.11, 2.06 (s each, 9, Acx3); MS (m/z) 437 (M^+), 395 ($M-Ac^+$), 259 ($sugar^+$), 180 ($B+2^+$), 179 ($B+1^+$).

5-Nitrotubercidin (15)----Compound 13 (600 mg) was taken in 10 mL of EtOH and 10 mL of 0.1 N HCl, and the solution was stirred for 2 days at room temperature. After neutralization, the solution was concentrated and the residue was taken in a small volume of H_2O. The separated crystals were collected to to give 88 mg (21%) of 15. Recrystallization from H_2O gave a pure sample: mp 232°C (dec.); UV (H_2O)λmax 365nm (ε, 3900), 290 (sh, ε, 6800), 275 (ε, 9100), 257 (ε, 8750), 237 (sh, ε, 7300), λmin 317 (ε, 2000), 264 (ε, 8650), 225 (ε, 6200) and (0.1N HCl) λmax 333 (ε, 4800), 260 (ε, 10700), λmin 298 (ε, 3400), 234 (ε, 6300); NMR (DMSO-d_6) δ 8.92 (s, 1, H-6), 8.24 (s, 1, H-2), 7.55 (broad d, 2, NH_2), 6.13 (d, 1, H-1', J= 4.9 Hz), 5.55 (d, 1, HO), 5.22 (t, 1, HO), 5.20 (d, 1, HO), 4.37 (ddd, 1, H-2'), 4.14 (ddd, 1, H-3'), 3.98 (m, 1, H-4'), 3.70 (m, 2, H-5'). Anal. Calcd for $C_{11}H_{13}N_5O_6$: C, 42.44; H, 4.21; N, 22.50. Found: C, 42.62; H, 4.17; N, 22.63.

REFERENCES

1. A preliminary account of this work has appeared: S. Watanabe and T. Ueda, Nucleic Acids Res. Symposium Series, No. 8, s21 (1980). For part 46, see: K. Miura, S. Tsuda, T. Ueda, F. Harada, and N. Kato, Biochim. Biophys. Acta, in press (1983).
2. S. Watanabe and T. Ueda, Nucleosides and Nucleotides, 1, 191 (1982).
3. For example see: S. Swaminatan and K. Narashiman, Chem. Ber., 99, 889 (1966).
4. K. Hiratani, T. Nakai, and M. Okawara, Bull. Chem. Soc. Japan, 46, 3510 (1973).
5. M. Seefelder, Chem. Ber., 96, 324 (1963).
6. Y. Tamura, T. Kawadaki, M. Adachi, M. Tanio, and Y. Kita, Tetrahedron Letters, 4417 (1977).
7. T. Kondo, T. Ohgi, and T. Goto, Agr. Biol. Chem., 41, 1501 (1977).

8. V. Sunjic,F. Kajfez, D. Kolbah, H. Hofman, and M. Stromar, Tetrahedron Letters, 3209 (1973).
9. D. E. Bergstrom and A. J. Brattesani, Nucleic Acids Res., 8, 6231 (1980).
10. a) M. P. Schweizer and R. K. Robins, The Jerusalem Symposia on Quantum Chemistry and Biochemistry, 5, 311 (1973); b) P. A. Hart and J. P. Davis, The Jerusalem Symposia on Quantum Chemistry and Biochemistry, 5, 297(1973); c) A. Matsuda, Y. Nomoto, and T. Ueda, Chem. Pharm. Bull.(Tokyo), 27, 183 (1979).
11. a) D. W. Miles, R. K. Robins, and H. Eyring, Proc. Nat. Acad. Sci. US, 57, 1138 (1967); b) M. Ikehara and S. Yamada, Chem. Pharm. Bull. (Tokyo), 19, 104 (1971).