Synthesis of 8-Amino-4-methylthio-6-methyl-2-(β-D-ribofuranosyl)-2,6-dihydro-1,2,3,5,6,7-hexaazaacenaphthylene and an Unusual Reductive Ring-Opening of the 1,2,3,5,6,7-Hexaazaacenaphthylene Ring System

Andrew M. Kawasaki and Leroy B. Townsend*

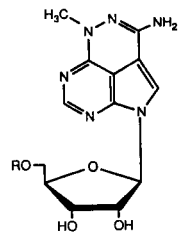
Department of Medicinal Chemistry, College of Pharmacy, and
Department of Chemistry, College of Literature, Sciences and and Arts, The University of Michigan,
Ann Arbor, MI 48109-1065

Received July 20, 1990

The tricyclic nucleoside 8-amino-4-methylthio-6-methyl-2-(β-D-ribofuranosyl)-1,2,3,5,6,7-hexaazaacenaphthylene (**3**) was synthesized from 3-cyano-4,6-*bis*(methylthio)-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**1**). Attempts to synthesize 8-amino-6-methyl-2-(β-D-ribofuranosyl)-1*H*-2,6-dihydro-1,2,3,5,6,7-hexaazaacenaphthylene (**5**) ([an aza analog of 6-amino-4-methyl-8-(β-D-ribofuranosyl)-1,3,4,5,8-pentaazaacenaphthylene (TCN)], which is a potent antitumor agent), by the treatment of **3** with Raney nickel did not afford the desired aza analog of TCN. Instead, it was established that a reductive cleavage of the pyridazine moiety of **3** had occurred to give 4-methylamino-6-methylthio-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-3-carboxamidine (**6**). Assuming that solubility was a problem in the reductive step, the isopropylidene derivative of **3**, 8-amino-6-methyl-4-methylthio-2-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-2,6-dihydro-1,2,3,5,6,7-hexaazaacenaphthylene (**8**), was treated with Raney nickel, only to observe that a similar reductive ring cleavage of **8** had occurred to afford 4-methylamino-6-methylthio-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-3-carboxamidine (**10**) and 4-methylamino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-3-carboxamidine (**11**). Structural assignments for all products were established by physico-chemical procedures.

J. Heterocyclic Chem., **28**, 1 (1991).

The nucleoside 6-amino-4-methyl-8-(β-D-ribofuranosyl)-1,3,4,5,8-pentaazaacenaphthylene [1] (TCN) is a potent antitumor agent and the 5'-phosphate analog [2] (TCN-P) is currently undergoing clinical trials under the auspices of the National Cancer Institute [3]. It is generally thought that TCN is acting as an adenosine analog, [4] but the exact biochemical mechanism for its cytotoxicity has not been elucidated. We were interested in the synthesis of the aza analog of TCN, 8-amino-6-methyl-2-(β-D-ribofuranosyl)-2,6-dihydro-1,2,3,5,6,7-hexaazaacenaphthylene (**5**), for use in a study on the structure-activity relationships involved in the cytotoxicity of TCN.



TCN, R = H
TCN-P, R = PO_3H_2

Results and Discussion.

The nucleoside 8-amino-4-methylthio-6-methyl-2-(β-D-ribofuranosyl)-2,6-dihydro-1,2,3,5,6,7-hexaazaacenaphthylene (**3**) was synthesized from 4,6-*bis*(methylthio)-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (**1**) [5] by a two-step reaction sequence. When the heterocycle and tetra-*O*-acetyl-β-D-ribofuranose were subjected to a high-temperature acid-catalyzed fusion ribosylation, using iodine as catalyst, 3-cyano-4,6-*bis*(methylthio)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine was obtained in about 30% yield on a preparative scale. Treatment of this blocked nucleoside with 1% hydrochloric acid gave **1** in a moderate yield. The reason we used these acidic deprotection conditions was because it had been reported [5] that the usual procedures, basic conditions such as ammonia, sodium methoxide, or sodium hydroxide, when used for the deacetylation resulted in a reaction at the cyano group.

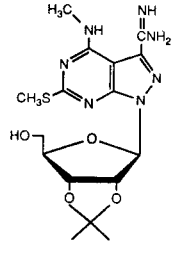
Treatment of **1** with ten equivalents of methylhydrazine afforded a good yield of a compound which was tentatively assigned the structure 3-cyano-4-(1-methylhydrazino)-6-methylthio-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (**2**). If an excess of methylhydrazine was not used, an incomplete reaction occurred and furnished a complex mixture. The nucleophilic displacement of only one of the methylthio groups with the exclusion of a reaction at the exocyclic cyano group was supported by the physicochemical data. The ^{1}H nmr spectrum showed only one signal, a singlet, within the δ 2-3 range which would indicate that only one methylthio function remained and the ir spectrum revealed a strong peak at 2240 cm^{-1}. However, a displacement of the 4-methylthio function by methylhydrazine could have conceivably occurred in two possible ways, *i.e.*, displacement by the substituted nitrogen to give **2** or displacement by the unsubstituted nitrogen to afford the isomeric compound. The ^{1}H nmr spectrum supported the tentative assignment for structure **2**, since the signal assigned to the *N*-methyl function was observed as a singlet at δ 3.35. If the product had possessed the isomeric structure, the signal observed for the *N*-methyl group would

have been coupled to an N–H resonance and would have given rise to a doublet for the *N*-methyl signal. Thus, a selective displacement of the 4-methylthio function of **1** was effected without any involvement of the 3-cyano or 6-methylthio groups.

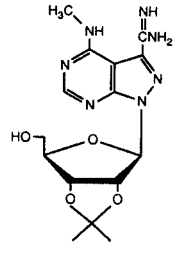
Ring closure of **2** was expected to occur in a facile manner under neutral conditions as previously observed for the ring-opened precursor of TCN. However, it was found that a ring closure of **2** to give 8-amino-6-methyl-4-methylthio-2-(β-D-ribofuranosyl)-2,6-dihydro-1,2,3,5,6,7-hexaazaacenaphythylene (**3**) did not occur under a variety of neutral conditions. The ring closure was finally accomplished, in a high yield, by a reaction of **2** with sodium methoxide which either effected a Neff-type activation [6] of the cyano group or simply acted as a base. The ir spectrum of **3** exhibited the absence of a band within the 2200-2300 cm^{-1} region which indicated the absence of a cyano function. In support of a basic ring system transformation, the uv spectrum of **3**, at *p*H 7, showed a 15 nm bathochromic shift relative to **2**. Also, the ^1H nmr spectrum showed a downfield shift of δ 0.21 for the *N*-methyl signal of **3** relative to the *N*-methyl signal observed for **2**.

With the nucleoside **3** in hand, we assumed that the target compound 8-amino-6-methyl-2-(β-D-ribofuranosyl)-2,6-dihydro-1,2,3,5,6,7-hexaazaacenaphthylene (**5**) would be easily obtained *via* a conventional dethiation reaction with Raney nickel [7]. However, treatment of **3** with Raney nickel under mild conditions resulted in a low yield of a compound which was not the desired product **5**. This unexpected product was subsequently elucidated to be the bicyclic compound 4-methylamino-6-methylthio-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-3-carboxamidine (**6**). This structure assignment was based on physicochemical data including the uv and ^1H nmr spectra and the elemental analysis of **6**. A basic transformation of the ring system was supported by the uv spectrum of **6**, at *p*H 7, which showed a significant hypsochromic shift (about 15 nm) relative to the *p*H 7 spectrum of **3**. Furthermore, the uv spectrum of **6** was similar to that of some closely related substituted pyrazolo[3,4-*d*]pyrimidines which had been previously reported [8,9]. The ^1H nmr spectrum of **6** showed a doublet at δ 2.98 which was assigned to the *N*-methyl protons. This doublet resulted from a coupling with the methylamino proton since upon exchange with deuterium oxide this doublet collapsed to a singlet. To support the fact that the 6-methylthio group remained intact, a singlet (3 H) was observed at δ 2.51. The elemental analysis (C, H, N) also agreed with the calculated values for **6**, as the monohydrate.

It was presumed that this anomalous result might be due to the extreme insolubility of compound **3** in the usual organic solvents. Thus, the Raney nickel reduction was repeated with a more soluble derivative of **3**, 8-amino-6-methyl-4-methylthio-2-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-2,6-dihydro-1,2,3,5,6,7-hexaazaacenaphthylene (**8**), which was synthesized from 3-cyano-4,6-*bis*(methylthio)-1-(2,3-*O*-isopropylidine-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (**4**) [5] *via* the same methodology as used in the synthesis of **3**. Compound **8**, which was very soluble in ethanol, was treated with Raney nickel under mild conditions and again the desired product, 8-amino-6-methyl-2-(2,3-*O*-isopropylidine-β-D-ribofuranosyl)-2,6-dihydro-1,2,3,5,6,7-hexaazaacenaphthylene (**9**), was not obtained. Instead, two major products were isolated from the reaction mixture and subsequently characterized as 4-methylamino-6-methylthio-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-3-carboxamidine (**10**) in a 30% yield and 4-methylamino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-3-carboxamidine (**11**) in 29% yield. Both products were apparently the result of a reductive cleavage of the pyridazine ring of **8**. The physicochemical data for **10** and



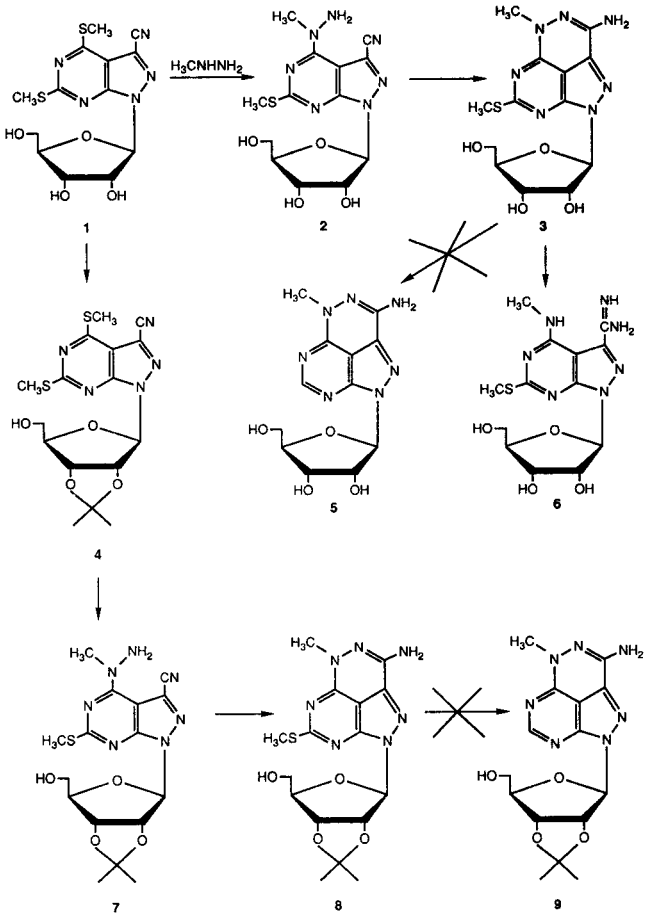
10



11

11 were similar to that of **6**. The uv spectra of **10** and **11** showed a similar hypsochromic shift relative to that of **8** and the ^1H nmr spectra exhibited the same pattern of signals as **6** except that in the spectrum of **11** a singlet was observed at δ 8.31 which was attributed to the aromatic proton. The ^1H nmr spectra for these compounds revealed a doublet appearing at δ 3.09 which was assigned to the *N*-methyl protons. After an exchange with deuterium oxide, this doublet collapsed to a singlet.

This reductive cleavage of the pyridazine ring of **8** was unexpected under the mild conditions used since numerous examples could be cited in the literature where fused pyridazines, [10] *e.g.*, imidazo[4,5-*d*]pyridazines [11,12] or imidazo[4,5-*c*]pyridazines, [13,14] have remained intact under similar conditions employing Raney nickel. After trying various other reducing regents, *e.g.*, tri-*n*-butyltin hydride, [15] Raney cobalt, [16] zinc dust, [17] and deactivated Raney nickel, [18] we have concluded that the requisite 7-aza-TCN can not be prepared by this route due to the unexpected lability of the pyridazine ring of **8** to reductive cleavage under these conditions.



EXPERIMENTAL

General Methods.

Melting points were determined on a Thomas-Hoover Unimelt apparatus and are uncorrected. Rotary evaporations were conducted with a Buchler flash evaporator at less than 50°, unless otherwise specified, using a water aspirator (15 mm Hg) or a vacuum pump (1 mm Hg). Low-pressure chromatography was performed on Instrumentation Specialties Company model 226 absorbance monitor with optical unit (254 nm), model 614 chart recorder, and model 328 fraction collector. A Michel-Miller (Ace Glass) column (4 x 30) which was packed with normal phase silica, EM Reagent Kieselgel 60 (230-400 mesh ASTM), was used as the column unless otherwise specified. Typical flow rates for low-pressure chromatography were 5 ml/minute and 20 ml/fraction were collected. Flash chromatography was performed using normal phase silica, EM Reagent Keisel gel 60 (230-400 mesh ASTM), and open-bed chromatography was performed using normal phase silica, EM Reagent Kieselgel 60 (70-230 mesh ASTM). All eluant systems are stated as volume to volume ratios. Thin layer chromatography (tlc) was accomplished using SilicAR 7GF (250 micrometer layer) on prescored glass plates (2.5 x 8 cm) purchased from Analtech, Inc., Newark, Delaware. Proton nuclear magnetic resonance (^{1}H nmr) spectra were obtained using a Bruker WM 360 (360 MHz) or Bruker WP 270 SY (270 MHz) spectrometers. The nmr spectra were recorded using either deuteriochloroform as solvent and tetramethylsilane as internal standard or dimethyl sulfoxide-d_6 and tetramethylsilane as internal standard. The following abbreviations were used to designate the multiplicity of individual signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, br s = broad singlet. The ir spectra were recorded using a Perkin-Elmer 281 spectrometer. The uv spectra were recorded on a Hewlett-Packard UV 8450 spectrometer. The symbol e is defined as the molar extinction coefficient, and values reported as absorbances indicate relative absorbances at the specified wavelengths λ. Mass spectral data were obtained on a Finnigan Model 4023 GC/MS using electron ionization or chemical ionization. Analytical samples were dried *in vacuo* (vacuum pump) at 78° in the presence of phosphorus pentoxide for at least 12 hours unless otherwise specified. Elemental analyses were obtained from M-H-W Laboratories, P. O. Box 15853, Phoenix, Arizona 85018. Raney nickel was purchased from Aldrich Chemical Co. and was of the W-2 type.

3-Cyano-4-(1-methylhydrazino)-6-methylthio-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (**2**).

The nucleoside **1** (1.0 g, 2.7 mmoles) was dissolved in ethanol (30 ml) and 98% methylhydrazine (2.9 ml, 54.4 mmoles) was added dropwise. The reaction mixture was then heated at reflux temperature for 3 hours. The solvent was evaporated *in vacuo* (water aspirator) to give a white solid which was triturated with ethanol (100 ml) at reflux temperature. The suspension was filtered and the solid was dried at room temperature for 12 hours to give **2** as a white solid (0.62 g, 62%). Compound **2** was recrystallized from methanol at reflux to furnish an analytical sample of **2**, mp 257-258°, Rf 0.4, chloroform/methanol (90/10); ^{1}H nmr (DMSO-d_6): 270 MHz, δ 3.34 (s, 3, NMe), 2.50 (s, 3, SMe), 3.5-6.0 (characteristic pattern of peaks for ribose moiety); CI ms: (methane), m/z 367 (M^+); ir (potassium bromide) 2240 cm^{-1}; uv (methanol): λ max 246 nm (ε 11,700), 302 nm (6,300).

Anal. Calcd. for $C_{13}H_{17}N_7O_4S$: C, 42.49; H, 4.67; N, 26.69. Found: C, 42.32; H, 4.94; N, 26.19.

8-Amino-6-methyl-4-methylthio-2-(β-D-ribofuranosyl)-2,6-dihydro-1,2,3,5,6,7-hexaazaacenaphthylene (**3**).

Compound **2** (1.00 g, 2.7 mmoles) was added to anhydrous methanol (100 ml) to give a white suspension. Powdered anhydrous sodium methoxide (0.147 g, 2.7 mmoles) was then added to the reaction mixture and it was heated at reflux temperature for 20 hours under a nitrogen atmosphere. The reaction mixture was allowed to cool to room temperature and the white precipitate was filtered. The solid was washed with water thrice (3 x 5 ml) at room temperature and dried *in vacuo* (water aspirator) at 50° for 12 hours to give **3** as a white solid (0.97 g, 97%). The analytical sample was prepared by recrystallization from dimethylsulfoxide at 95° followed by washing the recrystallized material with methanol at 20° to give **3** as white crystals, mp 280-281° dec, Rf 0.25, chloroform/methanol (90/10); ^{1}H nmr (DMSO-d_6): 270 MHz, δ 6.73 (br s, 2, NH_2, deuterium oxide exchangeable), 5.91 (d, 1, H-1′, $J_{1'2'}$ = 5.1 Hz), 3.56 (s, 3, NMe), 2.52 (s, 3, SMe), 3.5-6.0 (characteristic pattern of peaks for ribose moiety); uv (ethanol): λ max 264 nm (ε 4,300), 317 nm (2,800); ir (potassium bromide): absence of band in the 2200-2300 cm^{-1} region.

Anal. Calcd. for $C_{13}H_{17}N_7O_4S$: C, 42.49; H, 4.67; N, 26.69. Found: C, 42.51; H, 4.67; N, 26.75.

3-Cyano-4-(1-methylhydrazino)-6-methylthio-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (**7**).

Compound **4** (1.92 g, 4.69 mmoles) was dissolved in ethanol (60 ml) at reflux temperature and 98% methylhydrazine (1.25 ml, 23.4 mmoles) was added dropwise to the reaction mixture. The reaction mixture was heated at reflux temperature for 17 hours then co-evaporated with water *in vacuo* (water aspirator) at 30-50° to give a solid. This solid was dissolved in chloroform/methanol (98/2) (20 ml) and then subjected to low-pressure chromatography using chloroform/methanol (98/2) as eluant. The appropriate fractions were evaporated *in vacuo* (water aspirator) to give a white solid (1.67 g, 87%). An analytical sample was obtained by recrystallization from ethanol to give **7** as a white solid, mp 213°; Rf 0.2, chloroform/methanol (98/2); ^{1}H nmr (deuteriochloroform): 270 MHz, δ 6.50 (d, 1, H-1', $J_{1',2'}$ = 3.1 Hz), 5.24 (dd, 1H, H-2'), 5.05 (dd, 1, H-3'), 4.50 (m, 1H, H-4'), 4.31 (s, 2, NH_2, deuterium oxide exchangeable), 3.9 (m, 1, H-5'), 3.75 (m, 1, H-5'), 3.52 (s, 3, NMe), 2.56 (s, 3, SMe), 1.64 (s, 3, Me), 1.38 (s, 3, Me); uv (methanol): λ max 245 nm (ε 15,200), 304 nm (8,500); ir (potassium bromide): 2230 cm^{-1}.

Anal. Calcd. for $C_{16}H_{21}N_7O_4S$: C, 47.16; H, 5.20; N, 24.07. Found: C, 47.09; H, 5.23; N, 24.25.

3-Carboxamidine-4-methylamino-6-methylthio-1-(β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine Monohydrate (**6**).

Compound **3** (0.50 g, 1.36 mmoles) was dissolved in 2-picoline (50 ml) at 95° and Raney nickel (5 ml) was added as a suspension in picoline over a period of several hours. After heating the reaction mixture for 18 hours, the mixture was filtered through a Celite pad (1 x 5 cm) and the Celite pad was washed with picoline (3 x 60 ml) at 95°. The solvent was evaporated *in vacuo* (vacuum pump) to give a brown solid which was triturated with ether (5 ml). The solid was collected by filtration, washed with ether (5 x 1 ml), air-dired to give a brown solid (0.28 g). This crude product was dissolved in dimethylacetamide (10 ml) at 95° and the turbid solution was filtered. Silica (3 g) was added to the solution and the solvent was evaporated *in vacuo* (vacuum pump) at 55°. The sample which was absorbed to silica was subjected to flash chromatography using normal phase silica and chloroform/methanol (90/10) as eluant. The appropriate fractions were evaporated *in vacuo* (water aspirator) to give a white solid (0.14 g, 31%). The analytical sample was obtained by recrystallization from dimethylacetamide and water to give **6** as a white solid: mp 217-220°; ^{1}H nmr (DMSO-d_6): 270 MHz, δ 7.25 (br s, 1, N-H, deuterium oxide exchangeable), 6.65 (br s, 2, N-H_2, deuterium oxide exchangeable), 6.07 (d, 1, H-1', $J_{1',2'}$ = 4.6 Hz), 2.98 (d, 3, NMe), 2.71 (s, 3, SMe); uv (ethanol): λ max 249 nm (ε 11,700), 288 nm (4,500).

Anal. Calcd. for $C_{13}H_{18}N_7O_4S\ H_2O$: C, 40.30; H, 5.48; N, 25.31. Found: C, 40.51; H, 5.42; N, 25.08.

8-Amino-6-methyl-4-methylthio-2-(2,3-*O*-isopropylidine-β-D-ribofuranosyl)-2,6-dihydro-1,2,3,5,6,7-hexaazaacenaphthylene (**8**).

The nucleoside **7** (0.450 g, 1.10 mmoles) was dissolved in dry methanol (45 ml) at reflux temperature and powdered sodium methoxide (77 mg, 1.1 mmoles) was added to the reaction mixture. The reaction mixture was heated at reflux temperature for 7 hours, cooled to room temperature and Amberlite (IRC-50) carboxylic acid resin (2 mmole) was then added. The reaction mixture had a *p*H of 6 after stirring at room temperature for 12 hours. The suspension was evaporated *in vacuo* (water aspirator) and the resulting solid was triturated with ethanol (50 ml) at reflux temperature. After filtration of the suspension, the solids were washed with ethanol (3 x 10 ml) at 70°. The filtrate and the solutions from the washings were combined and evaporated *in vacuo* (water aspirator) to give a beige solid. This solid was triturated with water (4 ml) at room temperature then collected by filtration. The solid was washed with water (3 x 2 ml) and methanol (3 x 1 ml) at room temperature to give **8** as a solid (398 mg, 88%), mp 140°, Rf 0.3, chloroform/methanol (95/5); ^{1}H nmr (DMSO-d_6): 360 MHz, δ 6.74 (br s, 2, NH_2, deuterium oxide exchangeable), 6.14 (d, 1, H-1', $J_{1',2'}$ = 2.0 Hz), 5.48 (dd, 1, H-2'), 4.96 (dd, 1, H-3'), 4.91 (m, 1, 5'-OH, deuterium oxide exchangeable), 4.16 (m, 1, H-4'), 3.56 (s, 3, NMe), 2.52 (s, 3, SMe), 1.53 (s, 3, Me), 1.34 (s, 3, Me); uv (methanol); λ max 262 nm (ε 14,500), 311 nm (6,800); ir (potassium bromide): no band in the 2200-2300 cm^{-1} region; ms: EI m/z 407 (M^+).

4-Methylamino-6-methylthio-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-3-carboxamidine (**10**) and 4-Methylamino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-3-carboxamidine (**11**).

Compound **8** (180 mg, 0.44 mmole) was dissolved in ethanol (18 ml) at reflux temperature and Raney nickel (2 ml), as an ethanolic suspension, was added over a period of several hours. After heating the reaction mixture for 18 hours, the reaction mixture was filtered through a Celite pad (1 x 5 cm) and the Celite pad was washed with ethanol (2 x 10 ml). The filtrate and washings were combined and evaporated *in vacuo* (water aspirator) to give a yellow oil which was dissolved in chloroform/methanol (93/7) (4 ml). This sample was subjected to low-pressure chromatography using normal phase silica, 2 x 30 cm column, and chloroform/methanol (73/7) as eluant. A flow rate of 1.6 ml/minute was used and 5 ml fractions were collected. Fractions 60-68 were combined and evaporated *in vacuo* (water aspirator) to give **10** as a white solid (55 mg, 30%). Fractions 85-94 were combined and evaporated *in vacuo* (water aspirator) to give **11** as a white solid (46 mg, 29%). An analytical sample was obtained by recrystallization from ethanol to give **10** as white needles, mp 190°, Rf 0.4 chloroform/methanol (97/3); ^{1}H nmr (deuteriochloroform): 270 MHz, δ 11.07 (br d, 1, HN, deuterium oxide exchangeable), 6.87 (br s, 1, HN, deuterium oxide exchangeable), 6.34 (d, 1H, H-1', $J_{1',2'}$ = 3.7 Hz), 5.36 (m, 1, H-2'), 5.10 (m, 1, H-3'), 4.91 (m, 1, 5'-OH, deuterium oxide exchangeable), 4.48 (m, 1, H-4'), 3.85 (m, 2, H-5'), 3.09 (d, 3, NMe), 2.59 (s, 3, SMe), 1.64 (s, 3, Me), 1.38 (s, 3, Me); uv (methanol): λ max 238 nm (ε 7,090), 255 nm (7,300), 297 nm (3,560); (*p*H 1): λ max 240 nm (ε 7,250), 276 nm (4,930); (*p*H 11): λ max 241 nm (ε 8,260), 296 nm (3,950).

Anal. Calcd. for $C_{16}H_{23}N_7O_4S$: C, 46.93; H, 5.67; N, 23.95. Found: C, 46.74; H, 5.84; N, 24.05.

The analytical sample of **11** was obtained by dissolving the chromatographically pure product in ethanol (few drops) at room temperature. Hexane (5 ml) was then added at room temperature to give a white precipitate. The hexane was decanted and the solid was triturated with hexane (2 x 5 ml) at room temperature. After drying the solid, the analytical sample of **11** was obtained as a white solid, mp 128-135°, Rf 0.3, chloroform/methanol (93/7); ^{1}H nmr (deuteriochloroform): 270 MHz, δ 11.24 (br s, 1, NH, deuterium oxide exchangeable), 8.31 (s, 1, C_6-H), 6.85 (br s, 1, NH, deuterium oxide exchangeable), 6.24 (d, 1, H-1', $J_{1',2'}$ = 4.2 H), 5.98 (br s, 1, NH, deuterium oxide exchangeable), 5.3 (br s, 1, 5'-OH, deuterium oxide exchangeable), 5.24 (dd, 1, H-2'), 5.07

(dd, 1, H-3'), 4.50 (m, 1, H-4'), 3.85 (m, 2, H-5'), 3.07 (d, 3, NMe), 1.63 (s, 3, Me), 1.35 (s, 3, Me); uv (methanol): λ max 233 nm (ε 14,800), 292 nm (14,000); (*p*H 1): λ max 224 nm (21,400), 271 nm (14,400); (*p*H 11): λ max 234 nm (16,500), 292 nm (14,000).

Anal. Calcd. for $C_{15}H_{21}N_7O_4$: C, 49.57; H, 5.85; N, 26.98. Found: C, 49.61; H, 5.62; N, 26.99.

Acknowledgement.

This investigation was supported in part by research grants CH-299 and CH-312 from the American Cancer Society and in part by funds from the Department of Health and Human Services Research grant NO1-AI-25739. The authors would also like to thank Warner-Lambert/Parke-Davis for a grant for graduate student research support and Ms. Rae Miller for her expert preparation of the manuscript.

REFERENCES AND NOTES

[1] K. H. Schram and L. B. Townsend, *Tetrahedron Letters*, 4757 (1971).

[2] L. B. Townsend, A. F. Lewis and L. W. Roti Roti, U. S. Patent Appl. No. 804,601 granted 31 October (1978) #4,123,524; *Chem. Abstr.*, **89**, 44130j (1978).

[3] L. G. Feun, N. Savaraj, G. P. Bodey, K. Lu, B. S. Yap, J. A. Ajani, M. A. Burgess, R. S. Benjamin, E. McKelvey and I. Krakoff, *Cancer Res.*, **44**, 3608 (1984).

[4] L. L. Wotring, L. B. Townsend, G. W. Crabtree and R. E. Parks, Jr., *Proc. Am. Assoc. Cancer Res.*, **22**, 257 (1981).

[5] I. A. Korbukh, Y. N. Bulychev and M. N. Preobrazhenskaya, *Chem. Heterocyclic Compd.*, **15**, 1361 (1979).

[6] F. C. Schaeffer and G. A. Peters, *J. Org. Chem.*, **26**, 412 (1961).

[7] H. B. Cottam, G. R. Revankar and R. K. Robins, *Nucleic Acid Res.*, **11**, 871 (1983).

[8] R. A. Earl and L. B. Townsend, *J. Heterocyclic Chem..*, **11**, 1033 (1984).

[9] Y. N. Bulychev, I. A. Korbukh and M. N. Preobrazhenskaya, *Chem. Heterocyclic Compd.*, **2**, 182 (1980).

[10] M. Tišler and B. Stanovnik, Condensed Pyridazines Including Cinnolines and Phthalazines, R. N. Castle, ed, J. Wiley and Sons, New York, 1984, pp 761-1056.

[11] R. N. Castle and W. S. Seese, *J. Org. Chem.*, **23**, 1534 (1985).

[12] S. F. Martin and R. N. Castle, *J. Heterocyclic Chem.*, **6**, 93 (1969).

[13] T. Kuraishi and R. N. Castle, *J. Heterocyclic Chem.*, **1**, 42 (1964).

[14] H. Murakami and R. N. Castle, *J. Heterocyclic Chem.*, **4**, 555 (1967).

[15] C. G. Gutierrez, R. A. Stringham, T. Nitasaka and K. G. Glasscock, *J. Org. Chem.*, **45**, 3393 (1980).

[16] G. Pettit and E. E. Van Tamelen, *Org. React.*, **12**, 356 (1962).

[17] B. Koutek, *Collect. Czech. Chem. Commun.*, **39**, 192 (1974).

[18] H. J. Bestmann and H. Schulz, *Chem. Ber.*, **92**, 530 (1959).