Pyridopyrimidines. 7. Ribonucleosides Structurally Related to the Antitumor Antibiotic Sangivamycin

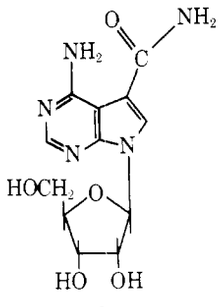
Gary L. Anderson and Arthur D. Broom*

Department of Biopharmaceutical Sciences, College of Pharmacy, University of Utah, Salt Lake City, Utah 84112

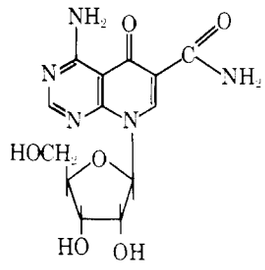
Received July 2, 1976

The synthesis and characterization of some new pyrido[2,3-*d*]pyrimidine ribonucleosides structurally related to the pyrrolo[2,3-*d*]pyrimidine antibiotic sangivamycin are reported. These include the N-8 ribonucleosides of 6-carboxamido-2,4-diamino-5-oxopyrido[2,3-*d*]pyrimidine, the isomeric 5-carboxamido-7-oxo derivative, and 4-amino-5-carboxamido-7-oxopyrido[2,3-*d*]pyrimidine.

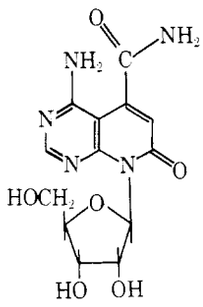
The potent antileukemic activity[1] of the pyrrolo[2,3-*d*]-pyrimidine nucleoside antibiotic sangivamycin (1) prompted the synthesis of the pyrido[2,3-*d*]pyrimidine nucleoside **2**.[2]



1



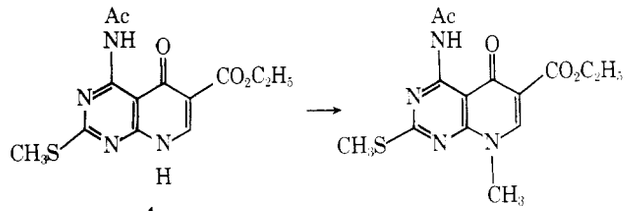
2



3

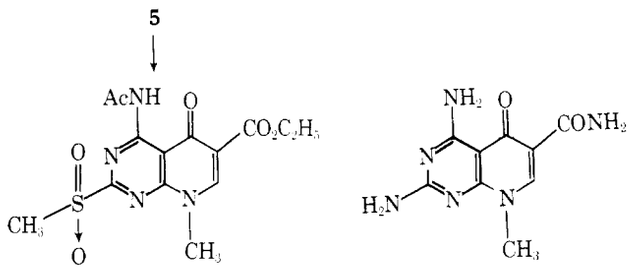
This compound, which may be regarded as a simple homologue of sangivamycin, was also found to have confirmed antileukemic activity.[3] These findings have led to a further investigation into the synthesis of ribonucleosides of pyrido[2,3-*d*]pyrimidines bearing the carboxamide group in either the 5 or 6 position. The present report describes the synthesis and characterization of a number of such nucleosides, the prototype molecule in this study being the 5-carboxamido-7-oxo isomer **3**.

The initial goal of this study was to extend the earlier work[2] to the synthesis of 6-carboxamido-2,4-diamino-5-oxo-8-(β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine. This reaction required a good leaving group in the 2 position of the pyridopyrimidine; it was decided that the initial exploration would be carried out using readily accessible bases by oxidation of the methylthio group followed by nucleophilic displacement. Alkylation of 4-acetamido-6-carbethoxy-2-methylthio-5-oxopyrido[2,3-*d*]pyrimidine (**4**)[2] with methyl iodide gave the 8-methyl derivative **5**. The position of alkylation is supported by the similarity of the UV spectrum of **5** to that of the previously reported[2] N-8 nucleoside of **4**. It has previously been shown that substitution of an alkyl group for hydrogen on the nitrogen of a potentially tautomeric heteroaromatic system results in a substantial downfield shift of the ^{1}H NMR signal for an adjacent proton.[4] A downfield shift of the C-7 H resonance of 0.35 ppm in **5** relative to that of **4** confirms N-8 alkylation.



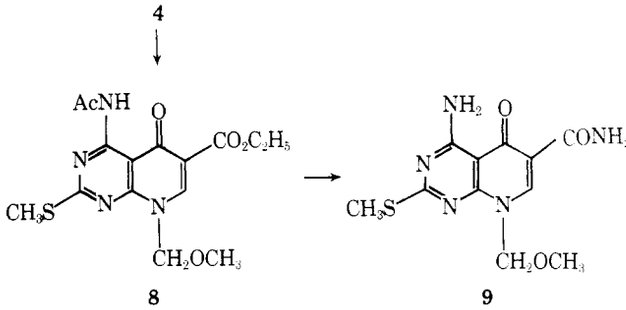
4 5

Oxidation of **5** with *m*-chloroperbenzoic acid yielded 4-acetamido-6-carbethoxy-8-methyl-2-methylsulfonyl-5-oxopyrido[2,3-*d*]pyrimidine (**6**). It has been shown that the methyl group will be shifted about 0.5 ppm downfield when a methylthio is oxidized to the methylsulfinyl and about 1.0 ppm when oxidized to the methylsulfonyl[5] in the pyridazine series. The shift of over 1 ppm from the methylthio of **5** is indicative of oxidation to the methylsulfonyl of (**6**). Treatment of **6** with liquid ammonia did not give the desired diamino carboxamide compound **7** as judged by the ^{1}H NMR spectrum of the product, which still had signals attributed to the ethyl ester. This was somewhat surprising in view of the previous conversion of the ester to the amide in the nucleoside series under identical conditions.[2]



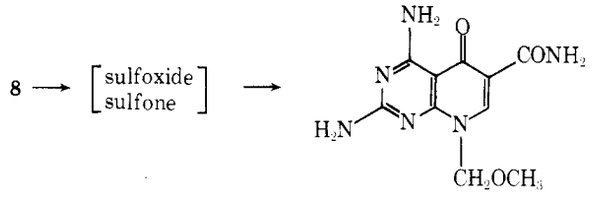
6 7

The replacement of the N-8 methyl with an N-8 methoxymethyl group did lead to the desired reaction. Compound **4** was alkylated with α-chloromethyl ether to give 4-acetamido-6-carbethoxy-8-methoxymethyl-2-methylthio-5-oxopyrido[2,3-*d*]pyrimidine (**8**).



8 9

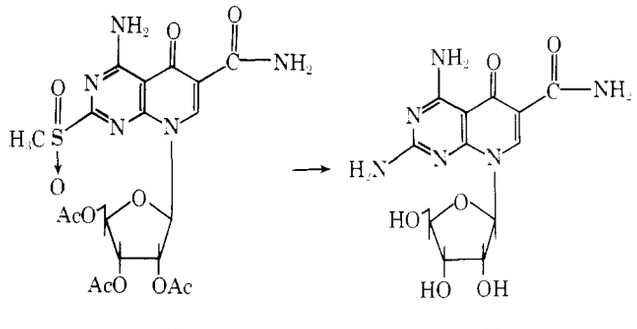
Alkylation at N-8 was supported by the similarity of the UV spectra and the downfield shift of C-7 H (0.63 ppm) as described previously. Upon treatment of **8** with liquid ammonia a good yield of 4-amino-6-carboxamido-8-methoxymethyl-2-methylthio-5-oxopyrido[2,3-*d*]pyrimidine (**9**) was obtained. Oxidation of **8** with *m*-chloroperbenzoic acid gave a mixture of two compounds, which by ^{1}H NMR spectroscopy appeared to be the corresponding sulfoxide and sulfone. This mixture was obtained even when the oxidizing agent was used in excess. The mixture was treated with liquid ammonia to give a good yield of 6-carboxamido-2,4-diamino-8-methoxymethyl-5-oxopyrido[2,3-*d*]pyrimidine (**10**). The difference



10

in reactivity of the esters of **6** and the oxidation products of **8** toward liquid ammonia may be explained by the electron-withdrawing effect of the methoxymethyl group which decreases electron density at the carbonyl carbon atom resulting in enhancement of reactivity, as compared to the electron-donating effect of the methyl group.[6] The ^{1}H NMR spectra of **9** and **10** were similar except that the methylthio signal at δ 2.53 of **9** was replaced by a broad singlet at δ 6.80 for the 2-amino group of **10**. These reactions demonstrated that the methylthio group in the 2 position could be easily converted to a group which would undergo nucleophilic displacement under conditions which would not disrupt the nucleoside.

This procedure was extended to the ribonucleoside series using 4-amino-6-carboxamido-2-methylthio-5-oxo-8-(β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine.[2] Acetylation gave the tri-*O*-acetyl derivative **11** which underwent *m*-chloroperbenzoic acid oxidation smoothly to give the tri-*O*-acetyl-2-methylsulfonyl derivative **12**. The ^{1}H NMR signal for the methylsulfonyl group appeared at δ 3.29, 0.66 ppm downfield from the methylthio group of **11**; this value is intermediate between the 0.5 and 1.0 ppm downfield shifts predicted for methylsulfinyl and methylsulfonyl, respectively.[5] The presence of the methylsulfonyl group was confirmed by the observation of a molecular ion at *m/e* 541 in the mass spectrum of **12**. Nucleophilic displacement of the methylsulfonyl group and deblocking of the sugar of **12** was accomplished with liquid ammonia to give compound **13**. The amino group at the 2

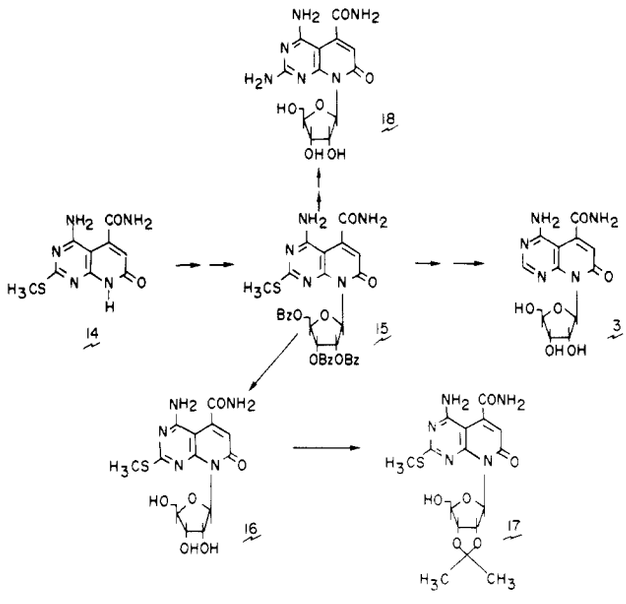


12 **13**

position was confirmed by the signal in the ^{1}H NMR spectrum at δ 6.78.

The synthesis of the sangivamycin homologue **3** required attachment of a β-D-ribofuranosyl moiety to the 8 position of 4-amino-5-carboxamido-2-methylthio-7-oxopyrido[2,3-*d*]-pyrimidine (**14**). The use of trimethylsilyl derivatives of nitrogen heterocycles circumvented difficulties associated with the low solubility and high melting point of **14**. Reaction (Scheme I) of **14** with hexamethyldisilazane gave the tris-

Scheme I



(trimethylsilyl) derivative which, when treated with freshly prepared 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide in dry toluene in the presence of a mercuric bromide–mercuric oxide catalyst,[7] gave a complex mixture from which 4-amino-5-carboxamido-2-methylthio-7-oxo-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (**15**) was isolated by a chromatographic procedure. Treatment of **15** with methanolic sodium methoxide gave 4-amino-5-carboxamido-2-methylthio-7-oxo-8-(β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (**16**). Assignment of the β configuration was made by conversion of **16** to the 2′,3′-*O*-isopropylidene derivative **17**. The small coupling constant for the anomeric proton ($J_{1,2}$ < 1.0 Hz) permits assignment of β configuration.[8] Further support lies in the difference in the chemical shifts; $\Delta\delta$ of the methyl groups on the isopropylidene is 0.20, within the accepted range for β nucleosides of $0.18 \leq \Delta\delta \leq 0.23$.[9] The mass spectrum of **17** exhibited a signal at 423 amu corresponding to the molecular ion. The site of alkylation was established as N-8 by UV and ^{1}H NMR spectroscopy. The UV spectra of the nucleoside **16** and the starting pyridopyrimidine **14** were very similar at pH 1 and 7. The ^{1}H NMR spectra of **14** and **16** revealed almost identical chemical shifts for all the base protons. In contrast, the spectrum of 4-amino-7-benzyloxy-5-carboxamido-2-methylthiopyrido[2,3-*d*]pyrimidine,[10] an example of the lactim tautomer which would have resulted from O-7 alkylation, shows a signal for C-6 H at δ 6.87, 0.60 ppm downfield for C-6 H of **14** and **16**.

The blocked nucleoside **15** was treated (Scheme I) with Raney nickel followed by treatment with methanolic sodium methoxide to give the sangivamycin analogue, 4-amino-5-carboxamido-7-oxo-8-(β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (**3**). The ^{1}H NMR spectrum of **3** revealed the loss of the signal due to the protons of the 2-methylthio function and the presence of two one-proton singlets corresponding to C-2 H (δ 8.33) and C-6 H (δ 6.45).

Finally, oxidation of the blocked nucleoside **15** with *m*-chloroperbenzoic acid followed by treatment with liquid ammonia and deblocking with methanolic methoxide afforded (Scheme I) the 5-carboxamido-2,4-diamino-7-oxo-8-(β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (**18**).

Experimental Section

The ^1H NMR spectra were recorded on a Jeol C-60H spectrometer with tetramethylsilane or DSS as an internal standard. Chemical shifts are expressed as δ, parts per million, from the standard. Ultraviolet spectra were obtained on a Cary Model 15 spectrophotometer. Mass spectra were recorded on a LKB-GC MS Model 9000S at 70 keV. Only the molecular ion and first major fragments are reported. Elemental analyses were performed by Het-Chem-Co, Harrisonville, Mo. Melting points were determined on a Thomas-Hoover Unimelt and are uncorrected. All analytical samples were dried in the presence of P_2O_5 in vacuo.

Thin layer chromatography was performed on 5 × 20 cm plates of Mallinckrodt SilicAR TLC-7GF (250-nm thickness). Solvent systems employed were (1) $CHCl_3$–MeOH (19:1), (2) EtOAc–*n*-PrOH–H_2O (4:1:2, upper layer), and (3) 1,2-dimethoxyethane–MeOH–NH_4OH (12:1:1).

4-Acetamido-6-carbethoxy-8-methyl-2-methylthio-5-oxopyrido[2,3-*d*]pyrimidine (5). 4-Acetamido-6-carbethoxy-2-methylthio-5-oxopyrido[2,3-*d*]pyrimidine (4,[2] 1.49 g, 4.64 mmol) was dissolved in DMF (50 ml). Anhydrous potassium carbonate (700 mg) and methyl iodide (720 mg, 5 mmol) were added. The suspension was stirred at 25 °C for 3 h, then filtered. The filtrate was evaporated in vacuo and coevaporated with toluene and ethanol to yield a yellow solid. This was dissolved in boiling ethanol, filtered through charcoal–Celite, and cooled to 5 °C. Filtration afforded 1.31 g (84%) of **5**: mp 191 °C; UV (pH 1) 281 nm (ϵ 44 600); (pH 7) 281 (45 000); (pH 11) 279 (42 800); ^1H NMR δ 2.55 (s, 3 H, SCH_3), 2.50 (s, 3 H, CH_3CO), 3.73 (s, 3 H, NCH_3), 8.65 (s, 1 H, C-7 H).

Anal. Calcd for $C_{14}H_{16}N_4O_4S$: C, 49.99; H, 4.79; N, 16.66. Found: C, 49.80; H, 4.85; N, 16.76.

4-Acetamido-6-carbethoxy-8-methyl-2-methylsulfonyl-5-oxopyrido[2,3-*d*]pyrimidine (6). To a suspension of **5** (336 mg, 1 mmol) in EtOAc (50 ml) was added a solution of *m*-chloroperbenzoic acid (600 mg, 3 mmol) in EtOAc (10 ml). The solvent was evaporated in vacuo after 4 h. The solid was dissolved in chloroform (100 ml) and extracted twice with 10% aqueous sodium bicarbonate (40 ml). The chloroform layer was dried over sodium sulfate and evaporated to yield a white solid. Recrystallization twice from ethanol–water afforded 106 mg (28%) of **6**: mp 171–173 °C; UV (pH 1) 269 nm (ϵ 41 600), 311 (14 000); (pH 7) 268 (43 100), 311 (14 200); (pH 11) 266 (60 000), 307 (12 900); ^1H NMR δ 3.80 (s, 3 H, SO_2CH_3), 2.47 (s, 3 H, CH_3CO), 3.90 (s, 3 H, NCH_3), 8.80 (s, 1 H, C-7 H).

Anal. Calcd for $C_{14}H_{16}N_4O_6S \cdot 0.5H_2O$: C, 44.56; H, 4.54; N, 14.85. Found: C, 44.59; H, 4.68; N, 14.83.

4-Acetamido-6-carbethoxy-8-methoxymethyl-2-methylthio-5-oxopyrido[2,3-*d*]pyrimidine (8). To a suspension of compound **4** (645 mg, 2 mmol) and K_2CO_3 (800 mg) in DMF (10 ml) was added methyl α-chloromethyl ether[11] (0.17 ml, 2.22 mmol). After stirring for 1 h at room temperature, the mixture was filtered and washed with DMF. The filtrate was evaporated in vacuo to give a white solid. This was recrystallized from EtOH to yield 530 mg (72%) of **8**: mp 154–156 °C; UV (pH 1) 275 nm (ϵ 45 100); (pH 7) 276 (44 700), 274 (42 800); ^1H NMR δ 2.65 (s, 3 H, SCH_3), 2.57 (s, 3 H, CH_3CO), 3.40 (s, 3 H, CH_3O), 5.80 (s, 2 H, OCH_2), 8.93 (s, 1 H, C-7 H).

Anal. Calcd for $C_{15}H_{18}N_4O_5S \cdot 0.5H_2O$: C, 48.04; H, 5.10; N, 14.92. Found: C, 47.81; H, 4.94; N, 15.15.

4-Amino-6-carboxamido-8-methoxymethyl-2-methylthio-5-oxopyrido[2,3-*d*]pyrimidine (9). Compound **8** (3.75 g, 10 mmol) was treated with liquid ammonia (60 ml) in a glass-lined bomb for 48 h at room temperature. Evaporation of the ammonia gave a solid which was recrystallized from DMF to yield 2.35 g (77%) of **9**. Dissolution in hot DMF, then addition of H_2O to the cloud point and cooling afforded an analytical sample: mp 286–288 °C; UV (pH 1) 275 nm (ϵ 40 000); (pH 7) 274 (43 400); (pH 11) 274 (43400); ^1H NMR δ 2.53 (s, 3 H, SCH_3), 7.63, 8.92 (br s, 2 H, NH_2, $J_{HNH} \sim 4$ Hz), 8.42, 9.42 (br s, 2 H, $CONH_2$, $J_{HNH} \sim 4$ Hz), 3.37 (s, 3 H, CH_3O), 5.70 (s, 2 H, OCH_2), 8.80 (s, 1 H, C-7 H).

Anal. Calcd for $C_{11}H_{13}N_5O_3S \cdot 0.5H_2O$: C, 43.38; H, 4.64; N, 23.01. Found: C, 43.61; H, 4.68; N, 23.27.

6-Carboxamido-2,4-diamino-8-methoxymethyl-5-oxopyrido-[2,3-*d*]pyrimidine (10). To compound **8** (2.75 g, 7.33 mmol) in $CHCl_3$ (150 ml) at 5 °C was added *m*-chloroperbenzoic acid (3.75 g, 18.7 mmol) and the solution was stirred for 3 h. The solvent was removed in vacuo and the residue triturated with Et_2O (50 ml), then filtered to give a white solid. This solid was treated with liquid ammonia (70 ml) in a glass-lined bomb at room temperature for 72 h. After evaporation of the ammonia, the solid was recrystallized from DMF–H_2O to give 1.35 g (68%) of **10**. One further recrystallization from DMF–H_2O afforded an analytical sample: mp 290–291 °C; UV (pH 1) 265 nm (ϵ 45 000); (pH 7) 265 (35 500); (pH 11) 265 (36 900); ^1H NMR δ 6.80 (br s, 2 H, 2-NH_2), 7.60, 9.03 (br d, 2 H, 4-NH_2, $J_{HNH} \sim 4$ Hz), 8.59, 9.22 (br d, 2 H, $CONH_2$, $J_{HNH} \sim 4$ Hz), 3.32 (s, 3 H, CH_3O), 5.70 (s, 2 H, OCH_2), 8.59 (s, 1 H, C-7 H).

Anal. Calcd for $C_{10}H_{12}N_6O_3 \cdot 0.5H_2O$: C, 43.95; H, 4.79; N, 30.76. Found C, 44.29; H, 4.71; N, 30.55.

4-Amino-6-carboxamido-2-methylthio-5-oxo-8-(2,3,5-tri-*O*-acetylribofuranosyl)pyrido[2,3-*d*]pyrimidine (11). 4-Amino-6-carboxamido-2-methylthio-5-oxo-8-(β-D-ribofuranosyl)-pyrido[2,3-*d*]pyrimidine (2.5 g, 6.2 mmol)[2] was stirred in pyridine (35 ml)–acetic anhydride (60 ml) for 48 h at 40 °C. The solution was evaporated in vacuo and coevaporated three times each with toluene and ethanol. The residue was dissolved in hot ethanol (300 ml)–chloroform (100 ml), and treated with charcoal. Filtration and evaporation to ≈250 ml gave a white precipitate which was filtered to give 3.03 g (93%) of **11**: mp 229–232 °C dec; MS *m/e* 509 (M^+); UV (pH 1) 275 nm (ϵ 40 000); (pH 7) 275 (40 000); (pH 11) 275 (41 000); ^1H NMR δ 8.77 (s, 1 H, C-7 H), 6.57 (d, 1 H, C-1′H, $J_{1'2'}$ = 3.0 Hz), 2.43 (s, 3 H, SCH_3).

Anal. Calcd for $C_{20}H_{23}N_5O_9S \cdot H_2O$: C, 45.54; H, 4.78; N, 13.28. Found: C, 45.19; H, 4.79; N, 12.96.

4-Amino-6-carboxamido-2-methylsulfonyl-5-oxo-8-(2,3,5-tri-*O*-acetylribofuranosyl)pyrido[2,3-*d*]pyrimidine (12). To a suspension of **11** (527 mg, 1 mmol) in 1,2-dimethoxyethane (50 ml) was added *m*-chloroperbenzoic acid (500 mg, 2.5 mmol). After 15 min a clear solution resulted. After 8 h the solvent was removed in vacuo to give a white powder which was recrystallized from ethanol to yield 475 mg (88%) of **12**. One further recrystallization from ethanol afforded **12** as white needles: mp 225–226 °C dec; MS *m/e* 541 (M^+); UV (pH 1) 275 nm (ϵ 18 400); (pH 7) 275 (18 100); (pH 11) 268 (36 900); ^1H NMR (s, 1 H, C-7 H), 6.51 (d, 1 H, C-1′H, $J_{1'2'}$ = 2.3 Hz), 3.29 (s, 3 H, SO_2CH_3).

Anal. Calcd for $C_{20}H_{23}N_5O_{11}S$: C, 44.36; H, 4.28; N, 12.93. Found: C, 44.41; H, 4.57; N, 12.76.

6-Carboxamido-2,4-diamino-5-oxo-8-(β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (13). Compound **12** (5.47 g, 10 mmol) was treated with liquid ammonia (90 ml) in a glass-lined bomb for 36 h at room temperature. The ammonia was evaporated to give a white solid. The solid was dissolved in boiling H_2O, filtered through a charcoal pad, and cooled to 5 °C. The white, gelatinous precipitate was filtered to give 2.4 g (68%) of **13**. One further recrystallization from H_2O afforded an analytical sample: mp 265 °C dec; UV (pH 1) 266 nm (ϵ 45 000); (pH 7) 252 (27 700), 267 (35 800); (pH 11) 252 (27 400), 267 (35 800); ^1H NMR δ 8.66 (s, 1 H, C-7 H), 6.46 (d, 1 H, C-1′ H, $J_{1'2'}$ = 4.2 Hz).

Anal. Calcd for $C_{13}H_{16}N_6O_6$: C, 44.32; H, 4.58; N, 23.85. Found: C, 44.51; H, 4.78; N, 23.68.

4-Amino-5-carboxamido-2-methylthio-7-oxo-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (15). Compound **14** (6.02 g, 24 mmol) was refluxed in toluene (250 ml) with hexamethyldisilazane (15 ml) and a few crystals of ammonium sulfate for 18 h. The clear solution was filtered through a sintered-glass funnel using a vacuum pump equipped with a manostat to prevent foaming of filtrate. The solvent was removed in vacuo to a yellow solid (hygroscopic!). Mass spectrum indicates that three trimethylsilyl groups are present [*m/e* 467 (M^+)]. This was dissolved in anhydrous benzene (150 ml) containing $HgBr_2$ (7.5 g) and HgO (7.5 g). To this suspension was added 2,3,5-tri-*O*-benzoylribofuranosyl bromide [prepared from 1-acetyl-2,3,5-tri-*O*-benzoylribofuranose (15.2 g, 30 mmol)][12] in benzene. The mixture was refluxed for 10 h, cooled, and filtered. The filtrate was evaporated to give an oily solid. This was dissolved in $CHCl_3$ (300 ml) and extracted with 15% aqueous KI (3 × 200 ml), H_2O (200 ml), then saturated $NaHCO_3$ (200 ml) and dried over $MgSO_4$. Evaporation of the solvent gave a red oil which was dissolved in $CHCl_3$ (20 ml) and applied to a silica gel column (460 g). Elution was with $CHCl_3$ (1000 ml), then MeOH in $CHCl_3$, 0.5% (820 ml), 1% (800 ml), 2% (800 ml), 3% (1000 ml), and 4.5% (1000 ml). Fractions of 20 ml were collected. Fractions 251–286 were evaporated to dryness to give 6.57 g (39%) of **15**. Preceding this compound off the column was a complex mixture of compounds which was not investigated.

4-Amino-5-carboxamido-2-methylthio-7-oxo-8-(β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (16). To MeOH (40 ml), in which Na (300 mg) was previously dissolved, was added **15** (850 mg, 1.22 mmol). The solution was stirred at room temperature for 4 h. After neutralization with HOAc, the solvent was removed in vacuo. The residue was coevaporated with H_2O–EtOH (1:1) (20 ml) three times to give a solid. This recrystallized from H_2O to give 347 mg (74%) of **16**: mp 248 °C (effervescence); MS *m/e* 743 (M^+) for penta-Me_3Si derivative; UV (pH 1) 258 nm (ϵ 15 300), 331 (14 400); (pH 7) 264 (16 600), 336 (14 600); (pH 11) 264 (16 600), 336 (14 600); ^1H NMR

δ 6.31 (s, 1 H, C-6 H), 6.82 (d, 1 H, C-1′ H, $J_{1'2'}$ = 3.5 Hz), 2.53 (s, 3 H, SCH_3).

Anal. Calcd for $C_{14}H_{17}N_5O_6S$: C, 43.99; H, 4.45; N, 18.30. Found: C, 43.68; H, 4.63; N, 18.41.

4-Amino-5-carboxamido-2-methylthio-7-oxo-8-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (17). To DMF (4 ml) was added 1 drop of concentrated HCl and 0.6 ml of dimethoxypropane. After stirring for 1 h, **16** (60 mg) was added and the suspension stirred for 1 h. Ammonium hydroxide solution was added until pH 8 was obtained. The solution was evaporated in vacuo to give a white solid. The solid was dissolved in MeOH (3 ml)–H_2O (3 ml). Removal of MeOH in vacuo and filtration gave 44 mg (80%) of **17**: MS *m/e* 423 (M^+); ^1H NMR δ 6.35 (s, 1 H, C-6 H), 7.02 (s, 1 H, C-1′ H, $J_{1'2'}$ < 1.0 Hz), 1.32, 1.52 [2 s, 6 H, $C(CH_3)_2$], 2.50 (s, 3 H, SCH_3).

4-Amino-5-carboxamido-7-oxo-8-(β-D-ribofuranosyl)pyrido-[2,3-*d*]pyrimidine (3). Compound **15** (1.0 g, 1.44 mmol) was refluxed in EtOH (100 ml) containing Raney nickel (3 g) for 12 h. Additional Raney nickel (3 g) was added and reflux continued for an additional 24 h. The reaction mixture was filtered while hot through Celite and the nickel washed with an additional 100 ml of hot EtOH. Evaporation of the filtrate to dryness gave 797 mg of oily solid which was dissolved in MeOH (100 ml). MEOH (10 ml), in which Na (100 mg) was previously dissolved, was added and the solution was stirred at room temperature overnight. H_2O (30 ml) was added, the pH was adjusted to 7 with Dowex 50-X8 (H^+), and the solution was filtered. Evaporation, followed by coevaporation with EtOH–H_2O three times, gave a white solid. Recrystallization from H_2O gave 209 mg (43%) of **3**: mp 240 °C dec; MS *m/e* 697 (M^+) for penta-Me_3Si derivative; UV (pH 1) 297 nm (ε 9500), 313 (sh, 7800); (pH 7) 250 (11 800), 322 (9000); (pH 11) 250 (11 100), 322 (8300); ^1H NMR δ 6.45 (s, 1 H, C-6 H), 8.33 (s, 1 H, C-24), 6.87 (d, 1 H, C-1′ H, $J_{1'2'}$ = 3.4 Hz), 7.50 (br s, 2 H, 4-NH_2), 8.30 8.60 (2 br s, 2 H, $CONH_2$).

Anal. Calcd for $C_{13}H_{15}N_5O_6$: C, 46.29; H, 4.48; N, 20.76. Found: C, 46.58; H, 4.78; N, 20.48.

5-Carboxamido-2,4-diamino-7-oxo-8-(β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (18). Compound **15** (795 mg, 1.0 mmol) was dissolved in $CHCl_3$ (50 ml) containing m-chloroperbenzoic acid (400 mg, 2 mmol). After stirring for 3 h, the solvent was removed in vacuo. The solid was triturated with Et_2O and filtered. The white powder was treated with liquid NH_3 (30 ml) in a glass bomb for 18 h. Evaporation of the ammonia gave an oily solid which was dissolved in MeOH (50 ml) in which Na (23 mg) was previously dissolved. After stirring for 3 h at room temperature, the pH was adjusted to 7 with Dowex 50-X8 (H^+). The resin was removed by filtration and the filtrate evaporated in vacuo, then coevaporated three times with EtOH–H_2O. The residue was triturated with $CHCl_3$ (50 ml) and filtered. The solid was dissolved in H_2O–EtOH by heating. Cooling gave a precipitate, which was filtered and washed with EtOH and Et_2O to give 195 mg (55%) of **18**: mp 192 °C dec; UV (pH 1) 300 nm (ε 13 500), 327 (13 500); (pH 7) 342 (14 600); (pH 11) 342 (14 600); ^1H NMR δ 5.93 (s, 1 H, C-6 H), 6.77 (d, 1 H, C-1′ H, $J_{1'2'}$ = 3.0 Hz).

Anal. Calcd for $C_{13}H_{16}N_6O_6 \cdot 2H_2O$: C, 40.21; H, 5.19; N, 21.64. Found: C, 39.99; H, 5.31; N, 21.57.

Acknowledgment. This research was supported by Research Grant CA 12823 from the National Cancer Institute, NIH. One of us (G.L.A.) wishes to express his appreciation to the American Foundation for Pharmaceutical Education for the Albert H. Diebold Memorial Fellowship, 1974–1976. The authors are grateful to Dr. James A. McCloskey and Ms. Ute Krahmer for performing the mass spectral determinations.

Registry No.—**3**, 61140-07-6; **4**, 36707-44-5; **5**, 61140-08-7; **6**, 61140-09-8; **8**, 61140-10-1; **8** sulfoxide, 61140-11-2; **8** sulfone, 61140-12-3; **9**, 61140-13-4; **10**, 61140-14-5; **11**, 61140-15-6; **12**, 61140-16-7; **13**, 61140-17-8; **14**, 61129-19-9; **15**, 61140-18-9; **16**, 61140-19-0; **17**, 61140-20-3; **18**, 61140-21-4; methyl α-chloromethyl ether, 107-30-2; 4-amino-6-carboxamido-2-methylthio-5-oxo-8-(β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine, 36707-04-7; 2,3,5-tri-*O*-benzoylribofuranosyl bromide, 16205-60-0.

References and Notes

(1) J. A. Cairns et al., *Cancer Chemother. Rep.*, **51**, 197 (1967).
(2) B. H. Rizkalla and A. D. Broom, *J. Org. Chem.*, **37**, 3980 (1972).
(3) Antileukemic activity data (L1210 mouse tumor) are available from Drug Development Branch, National Cancer Institute, by citing NSC 177223.
(4) A. D. Broom and R. K. Robins, *J. Org. Chem.*, **34**, 1025 (1969).
(5) T. Sega, A. Pollack, B. Stanovnik, and M. Tisler, *J. Org. Chem.*, **38**, 3307 (1973).
(6) A. L. J. Beckwith in "The Chemistry of Amides", J. Zabicky, Ed., Wiley-Interscience, New York, N.Y., 1970, p 73.
(7) E. Wittenburg, *Chem. Ber.*, **101**, 1095 (1968).
(8) R. U. Lemieux and D. R. Lineback, *Annu. Rev. Biochem.*, **32**, 155 (1963).
(9) J. L. Imbach, J. L. Barascut, B. L. Kam, B. Rayner, C. Tamby, and C. Tapiero, *J. Heterocycl. Chem.*, **10**, 1069 (1973).
(10) G. L. Anderson, J. L. Shim, and A. D. Broom, *J. Org. Chem.*, preceding paper in this issue.
(11) Methyl α-chloromethyl ether has been reported to be carcinogenic and should be handled with extreme caution.
(12) J. D. Stevens, R. K. Ness, and H. G. Hetcher, Jr., *J. Org. Chem.*, **33**, 1806 (1968).

Synthesis and Stereochemistry of 3-Hydroxy-5-methylproline, a New Naturally Occurring Imino Acid

Anthony B. Mauger* and Oswald A. Stuart

Research Foundation of the Washington Hospital Center, Washington, D.C. 20010

Edward Katz and Kaarin T. Mason

Department of Microbiology, Georgetown University Schools of Medicine and Dentistry, Washington, D.C. 20007

Received September 3, 1976

3-Hydroxy-5-methylproline was synthesized via Dieckmann cyclization of methyl *N*-methoxycarbonyl-3-methoxycarbonylmethylaminobutyrate (**6**) to *N*,2-dimethoxycarbonyl-5-methylpyrrolid-3-one (**8**). Reduction of the latter to *N*,2-dimethoxycarbonyl-5-methylpyrrolidin-3-ol (**9**) and subsequent hydrolysis afforded a mixture (**10**) of the four diastereoisomers (**1–4**) of 3-hydroxy-5-methylproline, which were separated by ion-exchange chromatography. From ^1H NMR data and epimerization studies, the relative stereochemistry of these stereoisomers was established. The NMR study also revealed conformational differences between the various isomers. Isomer **1**, which was reported earlier to correspond with a component of the peptide antibiotic actinomycin Z_1, has 2,3-trans-2,5-cis stereochemistry and a C_3-exo, C_4-endo ("twist") conformation.

A preliminary communication[1] reported the identification of 3-hydroxy-5-methylproline as a component of the peptide antibiotic, actinomycin Z_1, and the same imino acid has also been identified in some members of an actinomycin complex from *Micromonospora floridensis* NRRL8020.[2] A synthesis of the four racemic diastereoisomers (racemates of **1–4**, Figure 1) and an investigation of their stereochemistry are described here.