Pyrrolopyrimidine Nucleosides VII. A Study on Electrophilic and Nucleophilic Substitution at Position Six of Certain Pyrrolo[2,3-*d*]pyrimidine Nucleosides (1)

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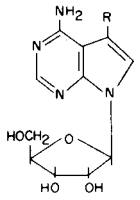
A study involving the reactivity of the pyrrolo[2,3-*d*]pyrimidine ring system at position 6 with another exocyclic group (CN or $\overset{O}{\overset{\|}{C}}-NH_2$) already residing at C5 has established that hydrogen and bromine are susceptible to electrophilic and acid-catalyzed nucleophilic substitution, respectively. In one instance a strong nucleophile (hydrazine) gave nucleophilic substitution at position 6 which was followed by a reaction with the *o*-nitrile group to afford the tricyclic nucleoside 4,5-diamino-8-(β-D-ribofuranosyl)pyrazolo[3′,4′:5,4]pyrrolo[2,3-*d*]pyrimidine (**4**).

The isolation, characterization and chemical synthesis (4-6) of the pyrrolo[2,3-*d*]pyrimidine nucleoside antibiotics tubercidin (**1a**), toyocamycin (**1b**), and sangivamycin (**1c**) has created considerable interest in this area. We have been involved in studies designed to probe not only the chemical character and reactivity of the parent heterocyclic ring system (pyrrolo[2,3-*d*]pyrimidine) but also several derivatives including certain nucleosides.



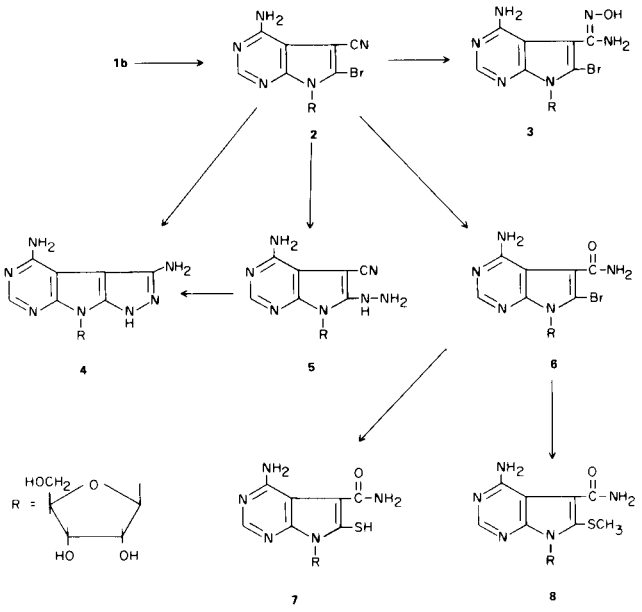
1a, R = H (Tubercidin)
b, R = CN (Toyocamycin)
c, R = $\overset{O}{\overset{\|}{C}}-NH_2$ (Sangivamycin)

The nucleophilic displacement of a chloro group from the pyrimidine moiety (C4) of a pyrrolo[2,3-*d*]pyrimidine nucleoside has been established (7). Electrophilic substitution has been observed to occur in the pyrrole moiety (C-5) of 4-substituted pyrrolo[2,3-*d*]pyrimidines (8) and 4-substituted 7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidines (9). This prompted us to extend our investigation to 4,5-disubstituted 7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidines where the six position would be predicted to be susceptible to electrophilic substitution but very resistant to nucleophilic attack. Pyrrole and condensed pyrrole systems are notoriously poor substrates for nucleophilic displacement (10,11).

Treatment of toyocamycin (**1b**) with bromine water at ambient temperatures gave 6-bromotoyocamycin (**2**) in good yield. The structure of **2** was confirmed by elemental analysis and the absence of the H6 singlet in the pmr spectrum. However, treatment of **1b** with other electrophilic reagents (nitronium tetrafluoborate, tetranitromethane, nitric acid, DMF-phosphorus oxychloride, trimethylamine-SO_3 complex and chlorosulfonic acid) gave either no reaction or extensive decomposition with the formation of highly colored products under more strenuous conditions.

In an investigation on the ease of bromide displacement at position 6 by a nucleophile, treatment of **2** with the excellent nucleophilic agents hydroxylamine and hydrazine produced two products, **3** and **4** respectively. However, the infrared spectra of **3** and **4** lacked the characteristic absorption at 2250 cm^{-1} for a nitrile group. Elemental analysis of **3** confirmed the presence of bromine and the structure of the product was established as 4-amino-6-bromo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamidoxime (**3**). We presumed **4** to be 4-amino-6-bromo-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]-pyrimidine-5-carboxamidrazone from the attack of hydrazine on the nitrile group at C-5. However, elemental anal-

ysis established that bromine was not present. The ana-



lytical and spectral data were consistent for the formation of the tricyclic nucleoside 4,5-diamino-8-(β-D-ribofuranosyl)pyrazolo[3′,4′:5,4]pyrrolo[2,3-*d*]pyrimidine (**4**) (12a) and careful monitoring of the reaction of **2** with hydrazine permitted the isolation of a small amount of the intermediate 4-amino-5-cyano-6-hydrazino-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (**5**, 6-hydrazinotoyocamycin). The structure was assigned on the basis of an absorption band at 2230 cm^{-1} (C≡N) in the ir spectrum and satisfactory elemental analysis. A conversion of **5** into the tricyclic nucleoside **4** in near quantitative yield was effected by heating in ethanol at reflux temperature.

Whereas hydrazine was found to displace bromine at position 6 and then ring close to **4**, hydroxylamine attacked the nitrile group only and displacement of the bromo group was not achieved even under forcing conditions. In order to eliminate the alternate site (5-cyano) for nucleophilic attack, the reactive nitrile group was converted to the less reactive carboxamide group. Treatment of **2** with 30% hydrogen peroxide in concentrated ammonium hydroxide gave a good yield of 4-amino-6-bromo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (**6**, 6-bromosangivamycin) and subsequent attempts to displace the bromo group from **6** with thiourea in ethanol gave only glycosidic cleavage products with the predominant product being 4-amino-5-cyano-6-mercaptopyrrolo-[2,3-*d*]pyrimidine. There was no nucleoside recovered even when displacement was attempted with thiourea in a *p*H 6 buffer. This would suggest that a significant factor in the mechanism of this glycosidic hydrolysis may be the close proximity of the sugar to the positively charged isothiouronium salt intermediate at position six or a possible tricyclic intermediate (12b). This is of considerable interest since pyrrolo[2,3-*d*]pyrimidine nucleosides have been reported (13) to be unusually stable toward acidic cleavage of the glycosidic bond. Nucleophilic displacement of the bromo group by sulfur was accomplished without appreciable glycosidic cleavage by treatment of **6** at 100° in a sealed vessel with aqueous methanol saturated with hydrogen sulfide to give 6-thiosangivamycin (**7**) in fair yield. This is the first example to our knowledge of an acid catalyzed nucleophilic substitution in a pyrrole or condensed pyrrole system, although the technique has been applied to substituted pyrimidines and purines (14). Similarly, treatment of **6** in a sealed vessel at 80° with methanol containing excess methanethiol and one drop of anhydrous formic acid gave 6-methylthiosangivamycin (**8**). The structure of **8** was confirmed by the pmr spectrum, which displayed a singlet at δ 2.6 (3 protons, SCH_3). Investigation of the application of acid-catalyzed nucleophilic substitution to other heterocyclic systems is in progress.

EXPERIMENTAL

Melting points were determined with a Thomas Hoover Unimelt and are uncorrected. Ultraviolet spectra were determined with a DK-2 spectrophotometer, ir spectra with a Beckman IR-5A, and pmr spectra with a high resolution Varian A-60 spectrometer (chemical shifts were measured at δ, ppm, from tetramethylsilane).

Elemental analyses were performed by Heterocyclic Chemical Corp., Harrisonville, Mo. and M-H-W Laboratories, Garden City, Mich.

4-Amino-6-bromo-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (**2**).

Red bromine water was added in small portions, to one g. of 4-amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**1b**, toyocamycin)(15) until the yellow color no longer disappeared after a period of 12 to 18 hours. The pale yellow solid was collected by filtration (780 mg.) and recrystallized from aqueous ethanol to afford an analytically pure product as white needles, m.p. 245° dec. (61% yield). The product was identical in all respects to an authentic sample of **2** prepared by an unambiguous procedure (6). An additional quantity of product and unreacted starting material could be recovered by neutralization of the filtrate followed by concentration until crystallization occurred; uv: λ max (*p*H 1) 281 nm (17,400), 231 nm (16,300); λ max (ethanol) 284 nm (18,300), λ max (*p*H 11) 283 nm (17,800); ir: 2200 cm^{-1} (C≡N).

Anal. Calcd. for $C_{12}H_{12}BrN_5O_4$: C, 38.94; H, 3.27; N, 18.91; Br, 21.58. Found: C, 39.10; H, 3.43; N, 19.18; Br, 21.24

4-Amino-6-bromo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamidoxime (**3**).

6-Bromotoyocamycin (**2**, 500 mg.) was added to a solution of 500 mg. of hydroxylamine in 200 ml. of absolute ethanol and this mixture was heated at reflux temperature for 18 hours. The solvent was removed *in vacuo* and the resulting amorphous pale yellow

solid was dissolved in a minimum (30 ml.) amount of methanol at room temperature. Frequent scratching of the sides of the vessel induced crystallization within minutes. The analytical sample was recrystallized from an ethyl acetate-ethanol mixture to furnish 410 mg. (75%) of **3**; m.p. 179° dec.; uv: λ max (*p*H 1) 278 nm (16,100), λ max (ethanol) 284 nm (14,500), λ max (*p*H 11) 280 nm (14,900).

Anal. Calcd. for $C_{12}H_{15}BrN_6O_5$: C, 35.75; H, 3.75; N, 20.84. Found: C, 35.55; H, 3.58; N, 20.61.

4,5-Diamino-8-(β-D-ribofuranosyl)pyrazolo[3′,4′:5,4]pyrrolo[2,3-*d*]pyrimidine (**4**).

A mixture of 6-bromotoyocamycin (**2**, 500 mg.) and 125 ml. of absolute ethanol containing 3.0 ml. of anhydrous hydrazine was heated at reflux temperature for 18 hours while being protected from moisture. The solvent was removed *in vacuo* and the resulting amorphous white solid was dissolved in 20 ml. of 25% aqueous methanol. Crystallization of the product was induced by frequent scratching of the sides of the vessel and the product was collected by filtration to afford 345 mg. (80%) of **4**, m.p. 257-258° dec.; uv: λ max (*p*H 1) 296 nm (8,400) 252 nm (15,800), λ max (ethanol) 295 nm (8,400) 250 nm (17,400), λ max (*p*H 11) 293 nm (8,900) 255 nm (14,300); ir: no C≡N stretch in the 2200 cm^{-1} area.

Anal. Calcd. for $C_{12}H_{15}N_7O_4$: C, 44.86; H, 4.71; N, 30.52. Found: C, 44.68; H, 4.66; N, 30.68.

4-Amino-5-cyano-6-hydrazino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (**5**).

Absolute ethanol (100 ml.) containing 3 ml. of 97% hydrazine and 750 mg. of 6-bromotoyocamycin (**2**) was heated at reflux temperature until all the solid had dissolved (10 minutes). The reaction was then cooled to 40° and monitored with thin layer chromatography until all the starting material had reacted (*ca.* 45 minutes). The solvent was removed *in vacuo* (below 20°) with the successive removal of two 50 ml. portions of a 1:1 methanol/toluene mixture *in vacuo* (below 20°) to furnish a pale yellow residue. This residue was dissolved in a minimum amount of a 1:1 ethanol/ethyl acetate mixture at 30° and subsequent cooling to 5° for 18 hours afforded a pale yellow solid, (420 mg.) which was collected by filtration (65%). The solid was air dried to yield **5**, 214-215° dec., uv: λ max (*p*H 1) 289 nm (13,500) 227 nm (9,700), λ max (ethanol) 295 nm (16,800) 222 nm (22,200), λ max (*p*H 11) 277 nm (14,000).

Anal. Calcd. for $C_{12}H_{15}N_7O_4$: C, 44.86; H, 4.71; N, 30.54. Found: C, 44.34; H, 4.83; N, 31.17.

4-Amino-6-bromo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (**6**).

To 1.64 g. of 4-amino-6-bromo-5-cyano-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (**2**) in 50 ml. of concentrated ammonium hydroxide was added 5 ml. of 30% hydrogen peroxide and the mixture stirred for 2 hours at room temperature.

The solution was allowed to stand for 18 hours at 5° and the white solid (1.16 g.) which had separated from solution was collected by filtration and washed well with water (65% yield). A small sample was recrystallized from aqueous ethanol to furnish colorless needles, m.p. 221°; uv: λ max (*p*H 1) 280 nm (16,000), λ max (ethanol) 285 nm (15,500), λ max (*p*H 11) 283 nm (15,200); ir: no C≡N stretch in the 2200 cm^{-1} area.

Anal. Calcd. for $C_{12}H_{14}BrN_5O_5$: C, 37.13; H, 3.63; N, 18.04. Found: C, 37.05; H, 3.66; N, 18.17.

4-Amino-6-mercapto-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (**7**).

6-Bromosangivamycin (**6**, 0.2 g.) was suspended in 100 ml. of methanol which had been previously saturated with hydrogen sulfide at -20°. Water (5 ml.) was added to this mixture which was then heated in a sealed vessel at 100° for 18 hours. The resultant solution was evaporated to dryness *in vacuo*. The residue was triturated three times with hot benzene (100 ml.), the benzene was then decanted and the white solid recrystallized from aqueous methanol. Colorless fine needles separated after cooling 18 hours at room temperature to yield 128 mg. (67%) of **7**, m.p. 284°; uv: λ max (*p*H 1) 325 nm (8,500) λ max (ethanol) 327 nm (8,200), λ max (*p*H 11) 328 nm (11,600) 248 nm (8,200).

Anal. Calcd. for $C_{12}H_{15}N_5O_5S$ 1.5 H_2O: C, 39.23; H, 4.92; N, 19.01. Found: C, 39.49; H, 4.83; N, 18.84.

4-Amino-6-methylthio-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (**8**).

One drop of anhydrous formic acid was added to a solution of 6-bromosangivamycin (**6**, 0.2 g.) in absolute methanol (100 ml.) containing an excess of methanethiol (5 ml.). The mixture was heated for 48 hours in a sealed vessel at 80° and the cooled reaction mixture was then evaporated to dryness *in vacuo*. The white solid was triturated with hot benzene (3 x 100 ml.) and the solvent was decanted and discarded. The resultant white solid was extracted with methanol and the insoluble solid was removed by filtration. The product crystallized after a volume reduction of the filtrate to less than 30 ml. and sufficient cyclohexane added to the hot solution to effect a cloud point. The reaction mixture was allowed to stand for 18 hours at 5° to furnish 110 mg. of white crystalline product (57%), m.p. 200°. The analytical sample was recrystallized from aqueous methanol: uv: λ max (*p*H 1) 290 nm (10,300), λ max (ethanol) 298 nm (10,000), λ max (*p*H 11) 296 nm (10,700) 235 nm (8,900).

Anal. Calcd. for $C_{13}H_{17}N_5O_5S$: C, 43.94; H, 4.82; N, 19.71. Found: C, 43.70; H, 5.03; N, 19.98.

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REFERENCES

(1) This research supported by Research Contract # PH 43-65-1041 with the Cancer Chemotherapy National Service Center, National Institutes of Health, Public Service.

(2) National Aeronautics and Space Administration Fellow, 1965-1968.

(3a) Author to whom correspondence should be addressed; (b) The authors wish to acknowledge the support of Professor John Spikes and the NIH Biomedical Sciences support (grant # FR 07092 #) for the procurement of Vengicide in sufficient quantity for this invesitigation.

(4) K. Ohkuma, *J. Antibiot.*, 13A, 361 (1960).

(5) R. L. Tolman, R. K. Robins and L. B. Townsend, *J. Am. Chem. Soc.*, 90, 524 (1968).

(6) R. L. Tolman, R. K. Robins and L. B. Townsend, *ibid.*, 91, 2102 (1969) and references cited therein.

(7) J. F. Gerster, B. Carpenter, R. K. Robins and L. B. Townsend, *J. Med. Chem.*, 10, 326 (1967).

(8) J. F. Gerster, B. C. Hinshaw, R. K. Robins and L. B. Town-

send, *J. Heterocyclic Chem.*, 6, 207 (1969).

(9) B. C. Hinshaw, J. F. Gerster, R. K. Robins and L. B. Townsend, *ibid.*, 6, 215 (1969).

(10 A. H. Corwin, "Heterocyclic Compounds", R. C. Elderfield, ed., Wiley, New York, N. Y., 1950, pp. 300-317.

(11) K. Schofield, "Hetero-aromatic Nitrogen Compounds", Plenum Press, New York, N. Y., 1967, p. 60.

(12a) A preliminary report of the synthesis and mechanism of formation of the tricyclic nucleoside **4** has appeared: R. L. Tolman and L. B. Townsend, *Tetrahedron Letters*, 4815 (1968); (b) C. Temple Jr., C. L. Kussner and J. A. Montgomery, *J. Org. Chem.*, 34, 316 (1969).

(13) G. Acs. E. Reich, and M. Mori, *Proc. Nat. Acad. Sci. USA*, 52, 493 (1964).

(14) A. Giner-Sorolla and A. Bendich, *J. Am. Chem. Soc.*, 80, 3932 (1958).

(15) Purchased from Koninklijke Nederlandsche Gist-en Spiritusfabriek, N. V., Royal Netherlands Fermentation Industries, Ltd., Delft, Holland, under the name of vengicide.