The Synthesis of Analogs of the Aminonucleoside from Puromycin[1]: 3′-Amino-3′-deoxyinosine and 2,3′-Diamino-3′-deoxyadenosine[2]

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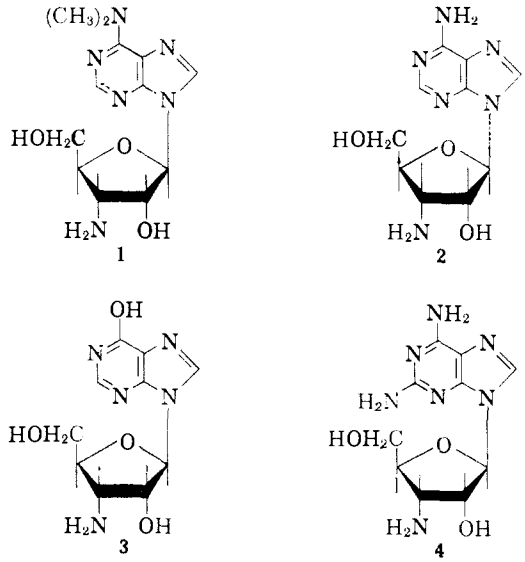
3′-Deoxy-3′-phthalimidoadenosine (**6**), obtained by the action of methanolic diisopropylamine on 6-benzamido-3′-phthalimidonucleoside (**5**), was deaminated to give 3′-deoxy-3′-phthalimidoinosine (**7**). Removal of the phthaloyl group with methanolic butylamine gave 3′-amino-3′-deoxyinosine (**3**). 2,6-Dibenzamido-3′-phthalimidonucleoside **(10),** shown to be a β-nucleoside by p.m.r. measurements, and methanolic butylamine gave 3′-amino-2-benzamido-3′-deoxyadenosine (**11**) which was converted to 2,3′-diamino-3′-deoxyadenosine (**4**) by treatment with sodium methoxide.

Because of the trypanocidal[3] and tumor-inhibiting[4] properties of the aminonucleoside (**1**)[5] from puromycin,[1,6,7] it was desired to synthesize analogs in which substituents on the purine moiety were varied. We have reported[8] the beneficial replacement of the 6-dimethylamino group of **1** by various monoalkylamino and dialkylamino groups.

The *in vivo* trypanocidal activities of puromycin and its aminonucleoside are reversed by a number of purines, including adenine and 2,6-diaminopurine,[3] and both respiration and growth of *Trypanosoma cruzi*[9] are inhibited by several purines, including 2,6-diaminopurine. 3′-Amino-3′-deoxyadenosine (**2**),[10] recently isolated from culture filtrates of *Helminthosporium* sp.,[11] is more active than the puromycin aminonucleoside against a transplanted mammary adenocarcinoma of the C_3H mouse, but it is more toxic; in tissue culture it is 20 times more toxic. However, it is only one-half as active as **1** against *Trypanosoma equiperdum* in the mouse.[12] The growth of two yeasts is inhibited by **2**.[11] It seemed desirable, therefore, to synthesize the 3′-amino-3′-deoxy derivatives of other purine nucleosides, especially of those occurring in nucleic acid.[13] We are describing here the syntheses of 3′-amino-3′-deoxyinosine (**3**) and 2,3′-diamino-3′-deoxyadenosine (**4**).



Synthesis of 3′-amino-3′-deoxyinosine (**3**)[14] was accomplished by reaction of 3′-deoxy-3′-phthalimidoadenosine (**6**)[10a] with nitrous acid to produce 3′-deoxy-3′-phthalimidoinosine (**7**) which was dephthaloylated with methanolic butylamine.[8]

3′-Deoxy-3′-phthalimidoadenosine (**6**) was obtained[10a] from the 6-benzamido-3′-phthalimidonucleoside dibenzoate **5** by sodium methoxide catalyzed methanolysis followed by cyclization of the intermediate phthalamic acid in refluxing acetic acid and *N,N*-dimethylformamide. An alternate route to **6** was developed which made use of the selective removal of blocking groups by methanolic diisopropylamine (a hindered amine).

We have observed[8b] that when the 6-chloro-3′-phthalimidonucleoside dibenzoate **8**[8] was allowed to react with methanolic diisopropylamine at 150°, O-benzoates were removed, chloride was displaced by methoxide, but the phthalimido group remained intact, and the 6-methoxy-3′-phthalimidonucleoside **9** was obtained.

The action of methanolic diisopropylamine is contrasted with the finding[8] that O-benzoates are removed by the action of methanolic solutions of unhindered secondary amines and the phthalimido group is opened

(1) Stylomycin®.

(2) Presented in part before the Division of Medicinal Chemistry, 133rd National Meeting of the American Chemical Society, San Francisco, Calif., April 13-18, 1958.

(3) R. I. Hewitt, A. R. Gumble, W. S. Wallace, and J. H. Williams, *Antibiot. Chemotherapy*, **4**, 1222 (1954).

(4) P. L. Bennett, S. L. Halliday, J. J. Oleson, and J. H. Williams, "Antibiotics Annual 1954-1955", Medical Encyclopedia, Inc., New York, N. Y., 1954, pp. 766-769.

(5) B. R. Baker, J. P. Joseph, and J. H. Williams, *J. Am. Chem. Soc.*, **76**, 2838 (1954).

(6) J. N. Porter, R. I. Hewitt, C. W. Hesseltine, G. Krupka, J. A. Lowery, W. S. Wallace, N. Bohonos, and J. H. Williams, *Antibiot. Chemotherapy*, **2**, 409 (1952).

(7) P. W. Fryth, C. W. Waller, B. L. Hutchings, and J. H. Williams, *J. Am. Chem. Soc.*, **80**, 2736 (1958).

(8) (a) L. Goldman, J. W. Marsico, and R. B. Angier, *ibid.*, **78**, 4173 (1956); (b) L. Goldman and J. W. Marsico, *J. Med. Chem.*, **6**, 413 (1963).

(9) M. Nakamura and M. Jumes, *Exptl. Parasitol.*, **2**, 37 (1954).

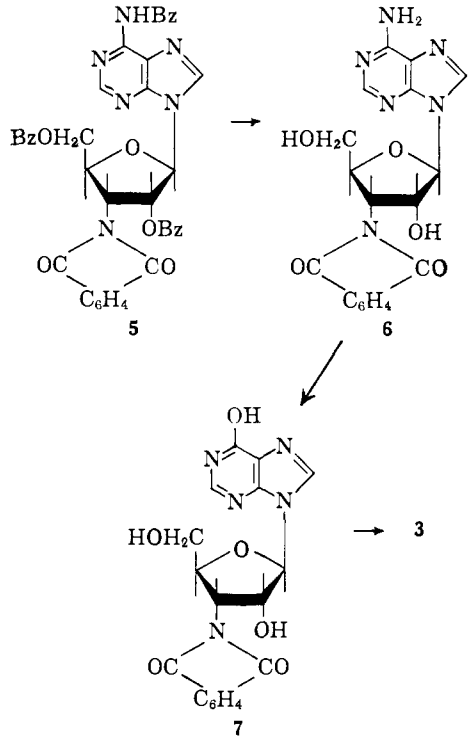
(10) (a) B. R. Baker, R. E. Schaub, and H. M. Kissman, *J. Am. Chem. Soc.*, **77**, 5911 (1955); (b) E. J. Reist and B. R. Baker, *J. Org. Chem.*, **23**, 1083 (1958).

(11) N. N. Gerber and H. L. Lechevalier, *ibid.*, **27**, 1731 (1962).

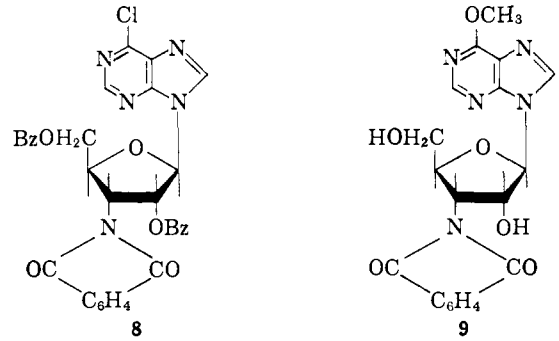
(12) R. I. Hewitt, A. R. Gumble, and H. Steinman, unpublished.

(13) 3′-Amino-3′-deoxyuridine and 3′-amino-3′-deoxyguanosine were synthesized and found to be biologically inactive [H. M. Kissman, A. S. Hoffman, and M. J. Weiss, *J. Med. Chem.*, **6**, 497 (1963)].

(14) 3′-Amino-3′-deoxyinosine (**3**) was obtained by selective diazotization of 3′-amino-3′-deoxyadenosine (**2**) at pH < 3 and by the action of Takadiastase on **2** [N. N. Gerber, Abstracts of Papers, 142nd National Meeting, American Chemical Society, September 9-14 (1962), p. 51Q].



to produce 3′-deoxy-3′-(*o*-N,N-disubstituted carbamoyl)benzamidonucleosides.



The lability of the 6-acetamido group of a nucleoside to attack by methanolic ammonia[15] led us to expect that the 6-benzamido group of **5** would be cleaved by the action of methanolic diisopropylamine. This, indeed, proved to be the case. Thus, when **5** was refluxed for 17 hr. with this reagent the N- and O-benzoyl groups were removed to yield 3′-deoxy-3′-phthalimidoadenosine (6) in 43% yield. 3′-Deoxy-3′-phthalimidoadenosine (**6**) was allowed to react with nitrous acid at 75–80° for 15 min.[16] but 84% of starting material was recovered. However, when 6 was allowed to react with excess nitrous acid at room temperature[17] for 18 hr., a smooth conversion to 3′-deoxy-3′-phthalimidoinosine (7) was realized. Reaction of **7** with methanolic butylamine gave 88% of 3′-amino-3′-deoxyinosine (**3**).

The 2,6-diamino-3′-phthalimidonucleoside tetrabenzoate (**10**), required for the synthesis of 2,3′-diamino-3′-deoxyadenosine (**4**), was obtained by the general procedure of Davoll and Lowy.[15] Condensation of chloromercuri-2,6-dibenzamidopurine[15] with 2,5-di-O-benzoyl-3-deoxy-3-phthalimido-β-D-ribofuranosyl chloride[18] in refluxing xylene gave 78% of nucleoside **10**, which was of sufficient purity to be carried through the remaining transformations.

In the proton magnetic resonance spectrum of **10**, a signal from the proton at C-1′ was observed at 3.52 τ as a singlet ($J_{H_1'-H_2'} \leq 0.5$ c.p.s.). Using a Karplus type equation[19] the dihedral angle between the H-(C-2′)-(C-1′) and (C-2′)-(C-1′)-H planes was calculated to fall in the range of 80 to 100°[20] and hence the C-1′ hydrogen is *trans* to the C-2′ hydrogen and **10** is a β-nucleoside.[21]

The formation of the β-anomeric nucleoside **10**, in which the ribose moiety is attached to the 9-position of the purine, is analogous to the formation of 2,6-diamino-9-β-D-ribofuranosyl-9H-purine by condensation of chloromercuri-2,6-dibenzamidopurine with 2,3,5-tri-O-acetyl-D-ribofuranosyl chloride and removal of blocking groups.[15] Furthermore, condensation of a 2-acyloxy-1-chlorosugar with a chloromercuripurine gives, as the major product, a nucleoside where the purine is *trans* to the 2-acyloxy group.[22] The formation of the anomeric nucleoside has been noted in several instances,[8,10a,23] but it was always the minor product.

Reaction of **10** with refluxing methanolic butylamine for 8.5 hr. gave a monobenzoyl derivative (**11**) of 2,3′-diamino-3′-deoxyadenosine (**4**) and a small amount of **4**. Increasing the reflux time to 17 hr. gave no substantial change in the ratio of products. The assignment of the 2-benzamido structure **11** to the monobenzoyl derivative was based on the observed greater hydrolytic lability of N-6-acyl over N-2-acyl groups in purine nucleosides.[24] Furthermore, the N-6-benzoyl group of 5 was hydrolyzed with methanolic butylamine to produce **2**,[10b] and methanolic diisopropylamine to produce **6** (*vide supra*).

Refluxing *N* methanolic sodium methoxide effected removal of the N-2-benzoyl group of **11**, giving a 23% yield of pure 2,3′-diamino-3′-deoxyadenosine (**4**). The ultraviolet absorption spectrum of **4** was in excellent agreement with that of 2,6-diamino-9-β-D-ribofuranosyl-9H-purine,[25] thus supporting the assignment of the attachment of the sugar to the 9-position of the purine.[26]

3′-Amino-3′-deoxyinosine (**3**), at 250 mg./kg., 3′-amino-2-benzamido-3′-deoxyadenosine (**11**) at 150 mg./kg., and 2,3′-diamino-3′-deoxyadenosine (**4**) at 125 mg./kg., when tested sequentially[27] against sar-

(15) J. Davoll and B. A. Lowy, *J. Am. Chem. Soc.*, **73**, 1650 (1951).

(16) This procedure was used by R. Kuhn and K. Henkel, *Z. physiol. Chem.*, **269**, 41 (1941), for conversion of 5′-deoxy-5′-methylthioadenosine to 5′-deoxy-5′-methylthioinosine.

(17) This procedure was used[15] for conversion of 2-acetamidoadenosine to guanosine (obtained after deacetylation).

(18) B. R. Baker, J. P. Joseph, and R. E. Schaub, *J. Am. Chem. Soc.*, **77**, 5905 (1955).

(19) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

(20) *Cf.* plots by C. D. Jardetzky, *J. Am. Chem. Soc.*, **82**, 229 (1960), and H. Conroy, *Advan. Org. Chem.*, **2**, 311 (1960), for theoretical and observed values.

(21) For a discussion of the use of p.m.r. spectra for assignment of anomeric configuration of ribofuranoses see L. Goldman and J. W. Marsico, ref. 8b.

(22) The "C_1-C_2-*trans* rule" of B. R. Baker and R. E. Schaub, *J. Am. Chem. Soc.*, **77**, 2396 (1955).

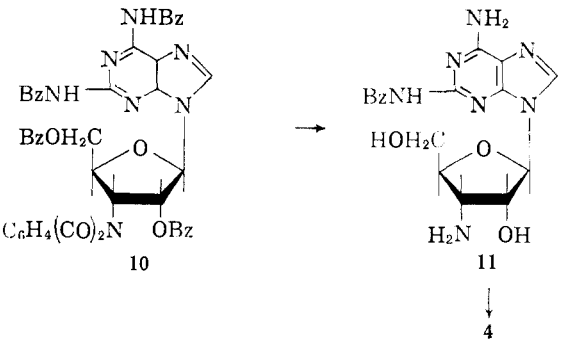
(23) H. M. Kissman and B. R. Baker, *ibid.*, **79**, 5534 (1957).

(24) 2-Acetamidonucleosides were obtained by the action of methanolic ammonia at 0° on 2,6-diacetamido-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-9H-purine and 2,6-diacetamido-9-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-9H-purine.[15]

(25) Reported[15]: $\lambda_{max}^{pH\ 6.8}$ 215 mμ (ε 25,200), 255 mμ (ε 9,450), and 280 mμ (ε 10,000).

(26) *Cf.* J. M. Gulland and F. Story, *J. Chem. Soc.*, 692 (1938), and references cited for application of ultraviolet spectral data to assignment of carbohydrate moieties of purine nucleosides to the 7- or 9-positions of the purine ring.

(27) E. H. Dearborn, *Acta Unio Intern. Contra Cancrum*, **15**, 76 (1959); A. W. Vogel and J. D. Haynes, *Cancer Chemotherapy Rept.*, **22**, 23 (1962).



coma 180, 6C3HED lymphosarcoma, and 72j mammary adenocarcinoma in the C_3H mouse, were inactive. These compounds were inactive against *Trypanosoma equiperdum* in mice when tested at 4 times the active dose level of the puromycin aminonucleoside. 3'-Amino-3'-deoxyinosine (**3**) did not inhibit the phosphorolysis of inosine by rat liver nucleoside phosphorylase,[28] but instead apparently underwent phosphorolysis at about one-fifth the rate of inosine.[29]

Experimental

All melting points are corrected. The ultraviolet absorption spectra were determined by means of a Cary recording spectrophotometer and the infrared spectra were measured in potassium bromide discs by means of a Perkin–Elmer spectrophotometer (Model 21). Circular paper chromatography[30] was carried out on Whatman No. 2 paper and fluorescent spots were observed under an ultraviolet lamp (G. W. Gates and Co., Inc., Long Island, N. Y.).

3'-Deoxy-3'-phthalimidoadenosine (6).—A mixture of 1.00 g. (0.00141 mole) of 6-benzamido-9-(2,5-di-O-benzoyl-3-deoxy-3-phthalimido-β-D-ribofuranosyl)-9H-purine (**5**),[20] 2 ml. of diisopropylamine, and 20 ml. of anhydrous methanol was refluxed for 17 hr. The resulting reddish brown solution was evaporated to dryness *in vacuo*, the tan glassy residue was triturated twice with anhydrous ether, and the ether was decanted. The residue, 0.611 g., was then dissolved by heating in 6 ml. of water and, on standing, 0.241 g. (43%) of tan crystals of **6** were deposited, m.p. 224–226° dec. A mixture melting point with authentic **6**, m.p. 227–230° dec., was 224–226° dec. Comparison of infrared spectra showed identity. Baker, *et al.*,[10a] give m.p. 228–230° for 6.

3'-Deoxy-3'-phthalimidoinosine (7).—A hot solution of 0.332 g. (0.000837 mole) of 3'-deoxy-3'-phthalimidoadenosine (**6**) in 15 ml. of water and 4 ml. of glacial acetic acid was cooled to room temperature. To the solution 0.800 g. (0.0116 mole) of sodium nitrite was added and the solution allowed to stand at room temperature overnight. The mixture was chilled by means of an ice bath and the nearly colorless crystals of **7** were removed by filtration, washed with water, and dried at 100°. The yield of product, m.p. 254–256° dec., was 0.200 g. Further chilling of the mother liquor yielded an additional 0.062 g., giving a total of 0.262 g. (76% as a hydrate). Recrystallization of the first crop from aqueous acetic acid gave 0.114 g. of nearly colorless crystals, m.p. 247° dec., $[\alpha]^{25}D$ −151° (*c* 0.93, pyridine); $\lambda_{max}^{0.1\ N\ HCl}$ 242 mμ (ε 19,900); λ_{max}^{MeOH} 241 mμ (ε 19,400); $\lambda_{max}^{0.1\ N\ NaOH}$ 252 mμ (ε 17,000).

Anal. Calcd. for $C_{18}H_{15}N_5O_6 \cdot 0.9H_2O$: C, 52.4; H, 4.10; N, 17.0; H_2O, 3.93. Found: C, 52.7; H, 4.09; N, 16.7; H_2O (K.F.), 4.03.

Reaction of 0.396 g. of **6** with sodium nitrite in aqueous acetic acid at 75–80° for 15 min. gave a clear solution from which 0.332 g. (84%) of **6** was recovered.

3'-Amino-3'-deoxyinosine (3).—A mixture of 0.500 g. (0.00121 mole) of 3'-deoxy-3'-phthalimidoinosine (**7**), 1.0 ml. (0.74 g., 0.011 mole) of butylamine, and 20 ml. of anhydrous methanol was refluxed for 18.5 hr. during which time solution resulted and after about 1.5–2 hr. of reflux, colorless crystals separated. The cream-colored crystals were removed by filtration, washed with methanol, and air-dried. The air-dried **3**, m.p. 253–255° dec., weighed 0.268 g. Concentration of the mother liquor gave an additional 0.020 g., m.p. 251–252° dec., yielding a total of 0.288 g. (89%). Recrystallization of the first crop from 12 ml. of water (using Norit) gave 0.144 g. of colorless crystals of **3**, m.p. 248–249° dec. (the melting point behavior depended on the rate of heating); $[\alpha]^{25}D$ −21.9° (*c* 0.20, water); $\lambda_{max}^{0.1\ N\ HCl}$ 248 mμ (ε 12,100); $\lambda_{max}^{pH\ 7}$ 248 mμ (ε 12,600); $\lambda_{max}^{0.1\ N\ NaOH}$ 251 mμ (ε 13,600).

Anal. Calcd. for $C_{10}H_{13}N_5O_4$: C, 44.9; H, 4.90; N, 26.2. Found: C, 45.0; H, 4.93; N, 26.2.

2,6-Dibenzamido-9-(3-deoxy-3-phthalimido-β-D-ribofuranosyl)-9H-purine Dibenzoate (10).—Pulverized chloromercuri-2,6-dibenzamidopurine[15] (70.7 g., 0.119 mole) was suspended in 900 ml. of reagent grade xylene and refluxed with stirring into a Dean and Stark trap to remove any water present. To the hot suspension was added 400 ml. of a hot xylene solution of 54.7 g. (0.107 mole) of 2,5-di-O-benzoyl-3-deoxy-3-phthalimido-β-D-ribofuranosyl chloride.[18] Refluxing and stirring were continued for an additional 4.5 hr. and the hot suspension, containing an insoluble gum, was filtered. The insoluble gum was washed well with several portions of hot chloroform. The combined filtrate and washings were washed with 350 ml. of 30% potassium iodide in two portions, followed by 200 ml. of water. The separated organic phase was evaporated to dryness *in vacuo* to a tan glass which was dissolved in ethyl acetate and filtered to remove some solid. The filtrate, on evaporation *in vacuo*, gave 93.0 g. (78%) of crude **10** as a tan glass, $[\alpha]^{25}D$ −17.8° (*c* 2.4, $CHCl_3$).

Anal. Calcd. for $C_{46}H_{33}N_5O_8$: C, 66.7; H, 4.02; N, 11.8. Found: C, 65.9; H, 4.51; N, 10.1.

3'-Amino-2-benzamido-3'-deoxyadenosine (11).—A mixture of 4.00 g. (0.00484 mole) of 2,6-dibenzamido-9-(3-deoxy-3-phthalimido-β-D-ribofuranosyl)-9H-purine dibenzoate (**10**), 75 ml. of anhydrous methanol, and 7.5 ml. of butylamine was refluxed for 8.5 hr. The resulting dark red solution was evaporated to dryness *in vacuo* to give a crystalline residue from which aqueous ethanol was distilled several times to remove methyl benzoate. The residual tan crystals were extracted by trituration with four 75-ml. portions of hot heptane, leaving 1.55 g. of crude **11** as a tan solid. A solution of 1.54 g. of crude **11** in 60 ml. of methanol–water (2:1) was passed through a column containing 20 g. of IRC-50 (H^+) resin. The column was washed with 1 l. of methanol–water (2:1) and the effluent collected in 2 equal fractions, the last containing little ultraviolet absorbing material. The column was then eluted with 750 ml. of methanol–2 *N* ammonium hydroxide (2:1), and 3 fractions were collected. The pooled ammonia fractions were evaporated to dryness *in vacuo* to give 1.20 g. of tan crystals, m.p. 155–160° with previous sintering. Recrystallization from aqueous ethanol gave 0.491 g. (26%) of **11** as tan crystals, m.p. 144–157°. Chromatography[30] on Whatman No. 2 paper in butanol–acetic acid–water (2:1:1.4) gave, when examined under ultraviolet light, a yellow fluorescing spot (R_f 0.78) corresponding to **11**, and a trace of a blue fluorescing spot (R_f 0.63) corresponding to 2,3'-diamino-3'-dexoyadenosine (**4**).

A sample (0.291 g.), recrystallized three times from aqueous *N*,*N*-dimethylformamide (Norit), gave **11** as nearly colorless crystals, m.p. 172.5–174°; $[\alpha]^{25}D$ +37.6° (*c* 0.72, MeOH); $\lambda_{max}^{0.1\ N\ HCl}$ 258 mμ (ε 13,300), 272 mμ (ε 22,000), λ_{infl} 290 mμ (ε 15,900); λ_{max}^{MeOH} 235 mμ (ε 24,200), 271 mμ (ε 15,800); $\lambda_{max}^{0.1\ N\ NaOH}$ 270 mμ (ε 16,200). The melting point was found to vary considerably with the amount of hydration.

Anal. Calcd. for $C_{17}H_{17}N_7O_4 \cdot 0.25H_2O$: C, 52.4; H, 5.04; N, 25.2. Found: C, 52.3, 52.1; H, 5.07, 5.40; N, 25.2.

A 12 g. sample of **10** was refluxed with methanolic butylamine for 17 hr. to give 4.86 g. (87%) of crude **11**. Chromatography as above showed this material to consist of **11** (R_f 0.74) as the main component and a lesser amount of **4** (R_f 0.61). Two additional unidentified components (light blue fluorescence with R_f 0.51 and yellow fluorescence with R_f 0.90) were present in small amount. This material was used in the next step.

2,3'-Diamino-3'-deoxyadenosine (4).—A solution of 4.00 g. (0.0104 mole) of crude 3'-amino-2-benzamido-3'-deoxyadenosine (**11**) in 40 ml. of methanol containing 1.0 ml. of *N* methanolic sodium methoxide was refluxed for 21.5 hr., 3.0 ml. of *N* metha-

(28) H. M. Kalckar, *J. Biol. Chem.*, **167**, 477 (1947).

(29) The apparent phosphorolysis of 3'-amino-3'-deoxyinosine (**3**), as measured by ultimate formation of uric acid, is also explainable by assuming conversion of **3** to inosine by a deaminase contaminant of the enzyme preparation, with subsequent phosphorolysis of the inosine.

(30) K. V. Giri and N. A. N. Rao, *Nature*, **169**, 923 (1952).

nolic sodium methoxide being added in portions to maintain an alkaline pH. The resulting dark red-brown solution was evaporated *in vacuo* to a tan glassy residue which was crystallized by trituration with a small amount of water. The water was removed *in vacuo* and the residue was triturated with absolute ethanol and filtered to yield 1.76 g. of crude **4** as tan crystals, m.p. 200–209° dec. From the mother liquor an additional 0.706 g. was obtained, giving a total of 2.47 g. (85%). Recrystallizations from N,N-dimethylformamide gave colorless crystals, m.p. 234–236° dec.; $[\alpha]^{25}D$ −28.9° (*c* 1.04, H_2O); $\lambda_{max}^{pH\ 6.8}$ 215 mμ (ε 23,800), 256 mμ (ε 9,200), 279 mμ (ε 9,750).

Anal. Calcd. for $C_{10}H_{15}N_7O_3$: C, 42.7; H, 5.38; N, 34.9. Found: C, 43.1, 43.0; H, 5.65, 5.63; N, 34.7.

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Synthesis and Reactions of 3′-Amino-3′-deoxyribosides of 6-Chloropurine

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Blocked 6-chloro-3′-aminonucleosides (**3**, **14**) were synthesized and found to be excellent intermediates for the preparation of analogs of the puromycin aminonucleoside (**7**). Chloride was displaced from **3** and **14** by primary and secondary amines in methanol with simultaneous removal of the O-benzoyl groups. Primary amines removed the N-phthaloyl group of **3**, whereas secondary amines opened the N-phthaloyl group to produce N,N,N′-trisubstituted phthalamides. Primary amines cleaved the latter phthalamides to produce unblocked 3′-amino-3′-deoxynucleosides. Diisopropylamine failed to displace chloride from **3** and failed to open the phthalimide function. Several analogs of the puromycin aminonucleoside were found to possess enhanced trypanocidal activity. The application of p.m.r. spectral measurements to determination of anomeric configuration in ribofuranoses is discussed.

9-(3-Amino-3-deoxy-β-D-ribofuranosyl)-6-dimethylamino-9H-purine (**7**),[1] the aminonucleoside from puromycin,[2-4] has trypanocidal[5,6] and tumor-inhibiting[7] properties in experimental animals. It was desirable, therefore, to synthesize structural variants of **7** in order to determine the relation of structure to biological activity.[8] Analogs of **7** have been synthesized in which the methylthio group was substituted for hydrogen at C-2,[9] amino was substituted for dimethylamino,[10] the aminosugar was varied,[11] and pyrimidines were substituted for the purine moiety.[12]

This paper is concerned with the synthesis of analogs of **7** by nucleophilic displacements on 6-chloronucleosides **3** and **14** by amines[8] and methoxide. 3′-Amino-3′-deoxyinosine,[13,14] 2,3′-diamino-3′-deoxyadenosine,[14,15] 3′-amino-3′-deoxyguanosine,[15] and 3′-amino-3′-deoxycrotonoside[16] were synthesized by other paths.

Of the several routes available for the synthesis of the desired 6-substituted aminonucleoside analogs of **7**, the condensation of the chloromercuri (and/or bismercury) derivative of purine, bearing the desired 6-substituent, with a suitably blocked aminosugar may be mentioned. Some limitations of this route, the one by which the aminonucleoside from puromycin has been synthesized,[16] are the following: (1) for each analog the condensation of a specifically substituted purine with an aminosugar is required; (2) each purine may require a number of steps for its synthesis; (3) the specifically substituted purine must orient the aminosugar to the 9-position; (4) the attachment of purine to aminosugar must be β; and (5) the 6-substituent must survive the rigorous condensation conditions.

To obviate these difficulties it was decided to synthesize, as an intermediate, a nucleoside bearing a chlorine atom in the 6-position since it was expected that the chlorine atom could be displaced by a wide variety of nucleophilic reagents to produce the desired analogs.[17]

Following the procedure of Brown and Weliky[18] for the synthesis of 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-6-chloro-9H-purine, according to the general method of Davoll and Lowy,[19] a mixture of chloromercuri-6-chloro-

(1) B. R. Baker, J. P. Joseph, and J. H. Williams, *J. Am. Chem. Soc.*, **76**, 2838 (1954).

(2) Stylomycin®.

(3) J. N. Porter, R. I. Hewitt, C. W. Hesseltine, G. Krupka, J. A. Lowery, W. S. Wallace, N. Bohonos, and J. H. Williams, *Antibiot. Chemotherapy*, **2**, 409 (1952).

(4) P. W. Fryth, C. W. Waller, B. L. Hutchings, and J. H. Williams, *J. Am. Chem. Soc.*, **80**, 2736 (1958).

(5) R. I. Hewitt, A. R. Gumble, W. S. Wallace, and J. H. Williams, *Antibiot. Chemotherapy*, **4**, 1222 (1954).

(6) E. J. Tobie and B. Highman, *Am. J. Trop. Med.*, **5**, 504 (1956).

(7) P. L. Bennett, S. L. Halliday, J. J. Oleson, and J. H. Williams, "Antibiotics Annual 1954–1955," Medical Encyclopedia, Inc., New York, N. Y., 1955, pp. 766–769.

(8) For a preliminary account of some of the material described in this paper see L. Goldman J. W. Marsico, and R. B. Angier, *J. Am. Chem. Soc.*, **78**, 4173 (1956).

(9) B. R. Baker, J. P. Joseph, and R. E. Schaub, *ibid.*, **77**, 5905 (1955).

(10) B. R. Baker, R. E. Schaub, and H. M. Kissman, *ibid.*, **77**, 5911 (1955).

(11) (a) B. R. Baker, J. P. Joseph, R. E. Schaub, and J. H. Williams, *J. Org. Chem.*, **19**, 1786 (1954); (b) F. J. McEvoy, B. R. Baker, and M. J. Weiss, *J. Am. Chem. Soc.*, **82**, 209 (1960), and references cited therein.

(12) H. M. Kissman and M. J. Weiss, *ibid.*, **80**, 2575 (1958).

(13) L. Goldman, J. W. Marsico and M. J. Weiss, Abstracts of Papers, 133rd National Meeting of the American Chemical Society, San Francisco, Calif., April 1958, p. 23M.

(14) L. Goldman, J. W. Marsico, and M. J. Weiss, *J. Med. Chem.*, **6**, 410 (1963).

(15) H. M. Kissman, A. S. Hoffman, and M. J. Weiss, *ibid.*, **6**, 407 (1963).

(16) B. R. Baker, R. E. Schaub, J. P. Joseph, and J. H. Williams, *J. Am. Chem. Soc.*, **76**, 4044 (1954).

(17) When this investigation was initiated the displacement of halide from a halonucleoside by an amine had not been previously reported. The only displacements described were by ammonia; *cf.* E. Fischer and B. Helferich, *Ber.*, **47**, 210 (1914); J. Davoll and B. A. Lowy, *J. Am. Chem. Soc.*, **73**, 1650 (1951); see also ref. 18.

(18) G. B. Brown and V. S. Weliky, *J. Biol. Chem.*, **204**, 1019 (1953).

(19) J. Davoll and B. A. Lowy, *J. Am. Chem. Soc.*, **74**, 1563 (1952).