Reaction Rate Measurements. Diazo Ketones. Hydrolysis.—The substance (1 mmole) was dissolved in 10 ml. of dioxane and placed into an erlenmeyer flask equipped with a side arm (leading to a gas buret) and a separatory funnel with a pressure-equalizing connection. Sulfuric acid (9.5 ml. of 14% w./w., 13 mmoles, final concentration 3 *N*) was run in within 1–2 sec., with rapid magnetic stirring. The temperature of the solution was kept constant within ±1°, and the evolved N_2 was plotted against time.

Thiosulfate.—The reaction was run as in the hydrolysis experiments, but 10 mmoles of $Na_2S_2O_3 \cdot 5H_2O$/mmole of diazo group, dissolved in 3 ml. of water, was added prior to the addition of the acid. The very fine precipitate of thiosulfate dissolved immediately on addition of the acid. The evolved N_2 was measured as a function of time.

Bromo Ketones. Hydrolysis.—Since no Br^- could be detected on reaction with water, the hydrolysis was run in the presence of NaOH. The substance (0.5 mmole for difunctional compounds or 1 mmole) was dissolved in 10 ml. of tetrahydrofuran, and 10 ml. of 0.1 *N* NaOH and 20 ml. of water were added. Six to eight identical samples were prepared and were kept in the same constant temperature bath, being titrated with 0.1 *N* HCl against methyl orange after suitable time intervals.

Thiosulfate.—The substance (0.5 or 1 mmole) in 5 ml. of acetone and 10 ml. of 0.1 *N* $Na_2S_2O_3$ was kept at constant temperature, and each sample was titrated with standard iodine solution using starch indicator.

Mesyloxy Ketones. Hydrolysis was measured in the same way as in the case of the bromo ketones.

Thiosulfate was measured as with bromo ketones, but acetone or acetic acid was used as the solvent.

Calculations.—All the reaction rate data fitted a first-order plot well. The rate constants were calculated from half-times, according to the equation $k = (\ln 2)/t_{1/2}$.

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3′-Deoxynucleosides. II. Purine 3′-Deoxynucleosides

EDWARD WALTON, FREDERICK W. HOLLY, GEORGE E. BOXER, RUTH F. NUTT, AND SUSAN R. JENKINS

Merck Sharp and Dohme Research Laboratories, Rahway, New Jersey

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The 9-(3-deoxy-β-D-ribofuranosides) of adenine, 2,6-diaminopurine, purine, purine-6-thiol, 6-methylaminopurine, 6-ethylaminopurine, and 6-dimethylaminopurine have been synthesized in order to compare their properties in certain biological systems.

3′-Deoxyadenosine (cordycepin),[1] an inhibitor of the growth of KB cells in culture,[1] *B. subtilis*,[2] an avian tubercle bacillus,[2] and Ehrlich ascites carcinoma[3] in mice, has more recently been shown[4] to be a potent inhibitor of RNA synthesis.

In a recent communication[5] we reported a brief description of a synthesis of 3′-deoxyadenosine. This synthetic scheme was designed to permit the synthesis of large amounts of 3′-deoxyadenosine as well as to supply a synthetic approach to analogs of 3′-deoxyadenosine which might have interesting biological properties.

The present paper describes the detailed experimental procedure for this synthesis of 3′-deoxyadenosine as well as the synthesis of 3′-deoxyadenosine-8-C^{14} by minor modifications of these procedures. In addition, six new related purine 3′-deoxynucleosides have been prepared to permit a comparison of some of their biological properties with those of cordycepin.

It is known[6a,b] that 3′-deoxyadenosine is metabolized *via* deamination to 3′-deoxyinosine. 3′-Deoxyinosine is not an inhibitor of cell growth nor is it an inhibitor of RNA synthesis. It seemed desirable, therefore, to synthesize compounds which would not be inactivated by deamination[6c] and which might retain the inhibitory properties of 3′-deoxyadenosine. It has been reported[6a,7] that, although 2′deoxyadenosine is deaminated by adenosine deaminase, 6-N-methyl-2′-deoxyadenosine is not a substrate for this enzyme; moreover, it is actually an inhibitor of adenosine deaminase. On the other hand, it has been demonstrated that 6-methylaminopurine is demethylaminated to hypoxanthine and methylamine by both bacterial[8a] and mammalian[8b,c] cells, and that 6-dimethylaminopurine is resistant to a similar enzymatic degradation. Information concerning the stability of 6-ethylaminopurine in these systems does not appear to be available. In the light of these findings, 6-methylamino-, 6-dimethylamino-, and 6-ethylamino-9-(3-deoxy-β-D-ribofuranosyl)purine (**4**, **5**, and **6**) were synthesized. Both the 6-N-methyl and 6-N-ethyl derivatives retain a proton on the 6-nitrogen of the adenine moiety, a desirable feature if the biological activity of 3′-deoxyadenosine depends on hydrogen bonding with enzymes at this position. The similarity of the dimethyl derivative

(1) E. A. Kaczka, E. L. Dulaney, C. O. Gitterman, H. B. Woodruff, and K. Folkers, *Biochem. Biophys. Res. Commun.*, **14**, 452 (1964).

(2) K. G. Cunningham, S. A. Hutchinson, W. Manson, and F. S. Spring, *J. Chem. Soc.*, 2299 (1951).

(3) D. V. Jagger, N. M. Kredich, and A. J. Gaurino, *Cancer Res.*, **21**, 216 (1961).

(4) (a) H. Klenow and S. Frederickson, Abstracts, the VIth International Congress of Biochemistry, New York, N. Y., July 1964, p. 66; (b) H. T. Shigeura and C. N. Gordon, *J. Biol. Chem.*, **240**, 806 (1965).

(5) E. Walton, R. F. Nutt, S. R. Jenkins, and F. W. Holly, *J. Am. Chem. Soc.*, **86**, 2952 (1964).

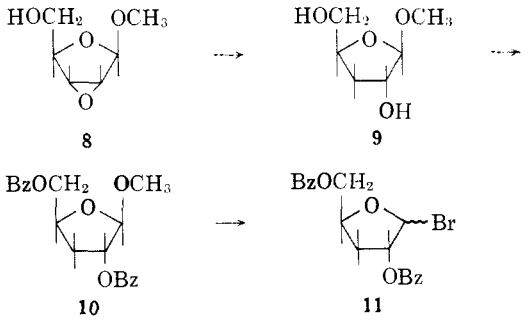
(6) (a) H. Klenow, *Biochem. Biophys. Acta*, **76**, 347 (1963). (b) G. Weinbaum, J. C. Cory, R. J. Suhadolnik, P. Meyers, and M. A. Rich, ref. 4a, Abstract No. IV-195, p. 343. (c) Studies of the effects of structural modifications on the activity of variously substituted purines as inhibitors and substrates of adenosine deaminase have been reported. See, for example, H. J. Schaeffer and R. Vince, *J. Med. Chem.*, **8**, 33 (1965), as well as G. A. LePage and I. G. Junga, *Cancer Res.*, **25**, 46 (1965).

(7) A. Coddington and M. Bagger-Sorensen, *Biochim. Biophys. Acta*, **72**, 598 (1963).

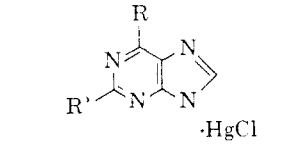
(8) (a) C. H. Reny, *J. Biol. Chem.*, **236**, 2999 (1961); (b) D. E. Duggan and E. Titus, *Biochim. Biophys. Acta*, **55**, 273 (1962); (c) C. E. Jamison, J. W. Huff, and M. P. Gordon, *Cancer Res.*, **22**, 1256 (1962).

(**5**) to the antibiotic puromycin should be noted. Not only the dimethylamino group in the 6-position of purine, but also the altered functionality of the 3-carbon of the sugar moiety are features in common. To test the essentiality of the amino function to the activity of cordycepin, 9-(3-deoxy-β-D-ribofuranosyl)-purine (**7**), a deoxynebularine, was also synthesized. Two additional 3′-deoxynucleosides, 2,6-diamino-9-(3-deoxy-β-D-ribofuranosyl)purine (**2**) and 9-(3-deoxy-β-D-ribofuranosyl)purine-6-thiol (**3**), were synthesized because of the known antitumor properties of the respective purine bases.

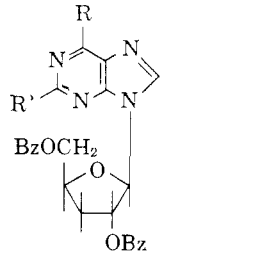
For the synthesis of these purine 3′-deoxynucleosides, 2,5-di-O-benzoyl-3-deoxy-D-ribofuranosyl bromide (**11**) was coupled with the appropriate chloromercuripurine. The preparation of the bromo sugar utilized methyl 2,3-anhydro-β-D-ribofuranoside (**8**)[9] as the starting material. Stereospecific reduction of the epoxide function in methyl 2,3-anhydro-β-D-ribofuranoside (**8**) by hydrogenation in ethanol over a Raney nickel catalyst at 80° and 2.8 kg./cm.² gave almost exclusively the 3-deoxyribose derivative, methyl 3-deoxy-β-D-ribofuranoside (**9**), characterized in part by its n.m.r. spectrum. The reduction could not be accomplished using platinum as the catalyst nor was Raney nickel effective at room temperature. Hydrogenolysis avoids the alternative[9] epoxide ring opening using ethyl mercaptan, a process requiring an extra step as well as using large amounts of Raney nickel for desulfurization. Benzoylation of the methyl glycoside **9** in pyridine with benzoyl chloride gave crystalline methyl 2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranoside (**10**) in good yield. The benzoylated methyl glycoside **10** was converted to 2,5-di-O-benzoyl-3-deoxy-D-ribofuranosyl bromide (**11**) by treatment with 10 parts of a 16% solution of HBr in acetic acid at 25° for 20 min. After removal of the solvents, the product was obtained as a clear, practically colorless oil whose n.m.r. spectrum no longer showed a band characteristic of the O-methyl protons.



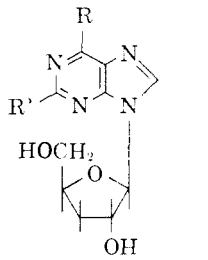
For the synthesis of 3′-deoxyadenosine (**1**) and 2,6-diamino-9-(3-deoxy-β-D-ribofuranosyl)purine (**2**), the bromo sugar **11** was coupled[10] with chloromercuri-6-benzamidopurine (**12**) and chloromercuri-2,6-dibenzamidopurine (**13**)[11] in dry xylene. The intermediate coupling products were obtained as amorphous solids containing exactly 1 equiv. of mercuric halide (*i.e.*, HgBrCl). The products were freed of mercuric salts by extraction of a chloroform[12] solution with 30% aqueous KI. The benzoyl blocking groups were removed in boiling methanol containing sodium methoxide. The methyl benzoate formed in this reaction was easily removed by extraction of a water solution of the products with chloroform. The 3′-deoxynucleosides **1** and **2** were purified by crystallization from water.



12, R = NHBz; R′ = H
13, R = R′ = NHBz
14, R = Cl; R′ = H
15, R = N(CH₃)Bz; R′ = H



16, R = NHBz; R′ = H
17, R = R′ = NHBz
18, R = Cl; R′ = H
19, R = SH; R′ = H
20, R = R′ = H
21, R = N(CH₃)Bz; R′ = H
22, R = NHCH₃; R′ = H



1, R = NH₂; R′ = H
2, R = R′ = NH₂
3, R = SH; R′ = H
4, R = NHCH₃; R′ = H
5, R = N(CH₃)₂; R′ = H
6, R = NHC₂H₅; R′ = H
7, R = R′ = H

For the preparation of the 6-mercapto- (**3**), 6-methylamino- (**4**), 6-dimethylamino- (**5**), and 6-ethylaminopurine 3′-deoxynucleosides (**6**), the intermediate 6-chloro-9-(2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)-purine (**18**) was synthesized from chloromercuri-6-chloropurine (**14**)[13] and the bromo sugar **11**. Treatment of the chloro intermediate **18** with thiourea in boiling ethanol gave the mercapto derivative **19** from which the benzoyl blocking groups were removed as before with sodium methoxide in methanol.

The 6-N-methylated compounds **4** and **5** as well as the 6-N-ethyl compound **6** were synthesized in one step from the chloro intermediate **18** in reactions with methylamine, dimethylamine, and ethylamine. This process not only introduces the appropriately substituted amino function but also removes the benzoyl protective groups from the sugar portion as the corresponding benzamides. The benzamides were removed by extraction of water solutions of the products with benzene.

(9) Prepared from xylose in 7 steps according to the very lucid directions of C. D. Anderson, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **80**, 5247 (1958).

(10) For a general review of the chloromercuri procedure see J. A. Montgomery and H. J. Thomas, "Advances in Carbohydrate Chemistry," Vol. 17, M. L. Wolfrom, Ed., Academic Press Inc., New York, N. Y., 1962, p. 301.

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

(12) Surprisingly, when ethyl acetate was substituted for chloroform, the mercuric halides were not removed from the product but remained in the organic layer.

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

For the synthesis of larger amounts of 6-methylamino-9-(3-deoxy-β-D-ribofuranosyl)purine (**4**), an alternative method was used. 6-(N-Methylbenzamido)purine, obtained by fusion of 6-methylaminopurine with benzoic anhydride, was converted into its chloromercuri derivative **15** which was glycosidated with the bromo sugar **11**. Thin layer chromatography of the coupling product on alumina revealed that two sugar-substituted purines had been produced. These compounds were separated by alumina chromatography as noncrystalline glasses and their infrared and ultraviolet absorption spectra as well as elemental and group analyses and chromatographic behavior indicated that one was the expected 6-(N-methylbenzamido)-9-(2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)purine (**21**). The other product was 6-methylamino-9-(2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)purine (**22**), produced by acidic[14] cleavage of the N-benzoyl group during the coupling reaction. Both intermediate products **21** and **22**, when debenzoylated in methanolic sodium methoxide, gave 6-methylamino-9-(3-deoxy-β-D-ribofuranosyl)purine (**4**), identical with that obtained from the 6-chloropurine (**18**). This result confirms the structures of the intermediates by showing that the 3-deoxy-β-D-ribofuranosyl moiety is attached to the same position of the purine nucleus in both **21** and **22**. Although the above method for synthesizing the 6-methylamino compound (**4**) is longer than that starting with 6-chloropurine, the over-all yield of product was better and the intermediates were more easily purified.

Hydrogenolysis of the intermediate chloropurine nucleoside **18** in the presence of a palladium catalyst yielded the dechloro product **20**. Removal of the benzoyl blocking groups with sodium methoxide in methanol gave 3′-deoxynebularine (**7**). In all cases, the final purine 3′-deoxynucleosides were purified by recrystallization from water. All of these new purine 3′-deoxynucleosides were tested[15] as inhibitors of the growth of KB and chick fibroblast cells and as inhibitors of the incorporation of C^{14}-labeled uridine into the RNA of these cells.

Experimental[16]

Methyl 3-Deoxy-β-D-ribofuranoside (**9**).—A solution of 15 g. (1.103 moles) of methyl 2,3-anhydro-β-D-ribofuranoside (**8**)[9] in 750 ml. of ethanol was shaken with 1 tablespoonful of Raney nickel[17] catalyst at 80° in hydrogen at 2.8 kg./cm.^{2}. After 6 hr. the uptake of hydrogen had ceased. The mixture was filtered and the catalyst was washed with ethanol. The combined filtrate and washing were concentrated. Toluene (15 ml.) was distilled twice from the residual oil in order to remove last traces of ethanol. Most of the residual toluene was removed at about 60° (<1 mm.). The product (15.8 g.) was obtained as a colorless oil. A small sample was filtered and dried at 56° (<1 mm.) for several hours for analysis: $[\alpha]_D$ −91°; $[\alpha]_{578}$ −88° (*c* 1.05, $CHCl_3$)[18]; λ_{max}^{neat} 2.92 (OH), 3.52 (OCH_3), no band at 11.6 μ (epoxide). The n.m.r. spectrum shows O-methyl protons, τ 6.16 (C-1 proton), 5.22 (singlet).

Anal. Calcd. for $C_6H_{12}O_4$: C, 48.64; H, 8.16. Found: C, 48.51; H, 8.18.

Methyl 2,5-Di-O-benzoyl-3-deoxy-β-D-ribofuranoside (**10**).—A solution of 5.0 g. (0.034 mole) of methyl 3-deoxy-β-D-ribofuranoside (**9**) in 125 ml. of dry (BaO) pyridine was cooled in an ice bath and treated dropwise while being stirred with 11.7 ml. (0.161 mole) of benzoyl chloride. During the addition a solid (pyridine hydrochloride) precipitated. The mixture was stirred at 5° for 1 hr. and kept at 25° for 16 hr. It was cooled and stirred while 125 ml. of water was added, and the mixture was concentrated to about 50 ml. The concentrate was diluted with 125 ml. of chloroform and washed with six 45-ml. portions of 1 *N* $NaHCO_3$ and one 50-ml. portion of water. The dried ($MgSO_4$) chloroform layer was concentrated to 11.8 g. of an oil which crystallized on standing. Recrystallization of the solid from 110 ml. of hexane gave 8.1 g. (67%) of **10**: m.p. 80–81°; $[\alpha]_D$ −32°; $[\alpha]_{546}$ −40° (*c* 1.7, $CHCl_3$); λ_{max}^{MeOH} 281 mμ (ε 1510), 273 (1880), 229 (25,600).

Anal. Calcd. for $C_{20}H_{20}O_6$: C, 67.40; H, 5.66. Found: C, 67.38; H, 5.74.

2,5-Di-O-benzoyl-3-deoxy-D-ribofuranosyl Bromide (**11**).—A solution of 6.0 g. (16.8 mmoles) of methyl 2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranoside (**10**) in 30 ml. of acetic acid was treated at 10° with 2 ml. of acetyl bromide. Thirty milliliters of a freshly prepared (33%, w./w.) solution of HBr in acetic acid was added, and the solution was kept at 10° for 20 min. T.l.c. on alumina with benzene–chloroform (1:1) showed spots (developed with I_2 vapor) of R_f 0.26 (halosugar), 0.62 (by-product), and 0.68 (starting material). Disappearance of the spot of R_f 0.68 after 2 to 3 min. indicated that the reaction was complete.

The pale yellow solution was concentrated at 30°. The residual bromo sugar was freed of last traces of HBr and acetic acid by the successive removal of five 20-ml. portions of dry toluene. The residual oil was used directly in the next step.

6-Benzamido-9-(2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)-purine (**16**).—About 100 ml. of xylene was distilled from a suspension of 2.66 g. (5.62 mmoles) of chloromercuri-6-benzamidopurine (**12**)[11] in 220 ml. of xylene in order to remove the last traces of water. The suspension was cooled to room temperature and stirred while a solution of 2.12 g. (5.62 mmoles) of 3-deoxy-2,5-di-O-benzoyl-D-ribofuranosyl bromide in 25 ml. of dry xylene was added. The character of the suspended material changed from a fine white particulate solid to a flocculant slurry. The mixture was stirred and refluxed for 30 min. during which time most of the suspended solid dissolved. The reaction mixture was filtered while hot to remove 0.6 g. of solid. The filtrate, when partially cooled, was diluted with 400 ml. of petroleum ether. After being cooled at 5° for 30 min., the mixture was filtered, and the solid was washed with two 50-ml. portions of petroleum ether. Testing a small sample with H_2S showed that the amorphous solid (3.8 g.) contained Hg^{+2}. T.l.c. on silica in ethyl acetate–acetone (1:1) showed two ultraviolet-absorbing spots of R_f 0.7 (weak) and 0.95 (strong).

Anal. Calcd. for $C_{31}H_{25}BrClHgN_5O_6$: C, 42.33; H, 2.86; Cl, 4.03; N, 7.96. Found: C, 42.93; H, 2.78; Cl, 3.92; N, 8.30.

The solid was dissolved in 100 ml. of chloroform and washed with three 30-ml. portions of 30% KI solution and two 30-ml. portions of water. The dried $CHCl_3$ solution was concentrated to a glass (2.9 g.). T.l.c. on silica in ethyl acetate–acetone (1:1) showed ultraviolet-absorbing spots, R_f 0.9 (strong, product) and 0.43 (weak, 6-benzamidopurine).

A 400-mg. portion of the product was chromatographed on a short column of silica in ethyl acetate–acetone (4:1). Combina- of appropriate fractions (homogeneous on t.l.c.) gave, after concentration, 300 mg. (69%) of amorphous **16**: $[\alpha]_D$ −49° (*c* 1, $CHCl_3$); λ_{max}^{EtOH} 281 mμ (ε 23,300), 264 (15,200), 231 (42,500).

(14) The acid may be HBr not completely removed after the preparation of the bromo sugar or that produced by elimination from the bromo sugar during the high-temperature coupling reaction with concurrent formation of the corresponding glycal.

(15) C. O. Gitterman, R. W. Burg, G. E. Boxer, D. J. Meltz, and J. E. Hitt, *J. Med. Chem.*, **8**, 664 (1965).

(16) N.m.r. spectra (60 Mc.) were determined in $CDCl_3$ solution (*c* ~5) by Dr. N. R. Trenner and Mr. B. Arison using a Varian Associates Model 4300B spectrometer with TMS as an internal standard. Microanalyses were performed by Mr. R. N. Boos and his associates, and the ultraviolet spectral measurements were done by Mr. E. A. MacMullin and his associates. All melting points were determined on a micro hot stage and are corrected. T.l.c. stands for thin layer chromatography. Solvent concentrations were carried out at reduced pressure on a rotary evaporator.

(17) Raney nickel similar to W-3 described by A. A. Pavlic and H. Adkins, *J. Am. Chem. Soc.*, **68**, 1471 (1946).

(18) Although the analytical values for the product are excellent, its n.m.r. spectrum indicated the presence of about 10% of an isomeric product. This impurity is most likely the 2-deoxy isomer. For this reason the rotational values are reported here with some reservation. The product was further characterized by conversion of its 2,5-di-O-acetyl derivative into 3-deoxy-D-ribose *p*-nitrophenylosazone, m.p. 255° dec., by the method described by C. D. Anderson, L. Goodman, and B. R. Baker, *ibid.*, **81**, 298 (1959).

Anal. Calcd. for $C_{31}H_{25}N_5O_8$: C, 66.06; H, 4.47; N, 12.43. Found: C, 65.52; H, 4.71; N, 12.37.

3′-Deoxyadenosine (Cordycepin) (1).—A solution of 2.0 g. (3.4 mmoles) of 6-benzamido-9-(2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)purine (**16**) in 30 ml. of dry methanol was treated with a solution prepared from 100 mg. (4.4 mmoles) of Na and 30 ml. of dry methanol, and the mixture was refluxed for 3 hr. Examination of the ultraviolet spectrum of small samples withdrawn every 30 min. indicated that the reaction was complete after 1 hr. The methanol solution was concentrated, and the residue was dissolved in 36 ml. of water. The pH was adjusted to 6.5–7.0 by the dropwise addition of acetic acid. The aqueous solution was washed with three 10-ml. portions of $CHCl_3$ to remove methyl benzoate, and the aqueous phase was concentrated to about 15 ml. The product crystallized during concentration. After being kept at 0° for 30 min., the product (512 mg., m.p. 224–225°) was removed and dried at reduced pressure at 25° over P_2O_5. The filtrate was concentrated to dryness and the residue when recrystallized from 4 ml. of water gave a second crop of product (83 mg., m.p. 224–226°). The total yield was 595 mg. (70%). Recrystallization of the combined crops from 12 ml. of water gave 511 mg. (60%) of 3′-deoxyadenosine (cordycepin), m.p. 224–225° with a transition at 200–205°. T.l.c. on cellulose in water shows only one ultraviolet-absorbing spot of R_f 0.42, $[\alpha]_D$ −44°, $[\alpha]_{578}$ −46° (*c* 0.5, H_2O), $\lambda_{max}^{H_2O}$ 260 mμ (ε 14,200), $\lambda_{max}^{pH\ 1}$ 258 mμ (ε 13,950), $\lambda_{max}^{pH\ 11}$ 260 mμ (ε 14,350).

Anal. Calcd. for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.22; N, 27.88. Found: C, 48.05; H, 5.08; N, 27.41.

2,6-Dibenzamido-9-(2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)purine (17).—In a manner similar to that described for the synthesis of **16**, 5.01 g. (8.43 mmoles) of chloromercuri-2,6-dibenzamidopurine (**13**)[11] was condensed with 3 g. (8.43 mmoles) of 2,5-di-O-benzoyl-3-deoxy-D-ribofuranosyl bromide. The condensation product (6.6 g.) was obtained as a complex with mercuric halide.

Anal. Calcd. for $C_{38}H_{30}BrClHgN_6O_7$: C, 45.70; H, 3.03; Cl, 3.55; N, 8.42. Found: C, 45.67; H, 3.03; Cl, 3.92; N, 8.13.

After removal of the mercuric halide, 4.4 g. (77%) of **17** was obtained as a glass. An analytical sample prepared by trituration with ether was dried at 80° at reduced pressure for 2.5 hr.; $[\alpha]_D$ +38° (*c* 1, $CHCl_3$); λ_{max}^{EtOH} 292 mμ (ε 18,700), 232 (47,800); λ_{sh}^{EtOH} 280 mμ (ε 12,300), 272 (22,300). T.l.c. on silica in ethyl acetate–acetone (1:1) showed only one ultraviolet-absorbing spot at R_f 0.80.

Anal. Calcd. for $C_{38}H_{30}N_6O_7$: C, 66.85; H, 4.43; N, 12.31. Found: C, 66.50; H, 4.37; N, 12.05.

2,6-Diamino-9-(3-deoxy-β-D-ribofuranosyl)purine (2).—As described in the synthesis of **1**, 1 g. (1.4 mmoles) of 2,6-dibenzamido-9-(2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)purine (**7**) was deacylated with 4.2 mmoles of sodium methoxide in methanol. After crystallization from 3 ml. of water, the product (304 mg., m.p. about 120°) was still contaminated with sodium acetate as indicated by the presence of a small amount of high-melting material. Recrystallization of 290 mg. of the product from 5 ml. of water gave 199 mg. (m.p. 120–123°) which still contained a small amount of high-melting impurity. The combined filtrates and washings were treated with a solution of 200 mg. of picric acid dissolved in 5 ml. of hot water. The mixture was quickly cooled to 10° and after 2 hr. the picrate was filtered and washed with 10 ml. of cold water. The dried picrate (166 mg.) in 25 ml. of water at about 70° was treated portionwise with a total of 1.6 g. of 1R-45 (OH^-) resin. The resin was filtered and washed with three 10-ml. portions of hot water. The colorless filtrate and washes were concentrated to 2 ml. After being kept at 5° for 18 hr., the product (23.5 mg., m.p. 120–121°) was removed, combined with 178 mg. of product from above, and recrystallized from 6 ml. of water. The dried **2** (178 mg., 46%) melted at 120–121°. T.l.c. in water on cellulose showed one ultraviolet-absorbing spot of R_f 0.46; $[\alpha]_D$ −47°; $[\alpha]_{546}$ −58° (*c* 1, H_2O); $\lambda_{max}^{H_2O}$ 280 mμ (ε 9709), 256 (8850); $\lambda_{max}^{0.1\ N\ HCl}$ 293 mμ (ε 9500), 253 (10,700), 270 (20,750), 215 (49, 955); $\lambda_{max}^{0.1\ N\ NaOH}$ 280 mμ (ε 9590), 256 (8630).

Anal. Calcd. for $C_{10}H_{14}N_6O_3$: C, 45.11; H, 5.30; N, 31.57. Found: C, 45.13; H, 5.01; N, 31.42.

6-Chloro-9-(2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)-purine (18).—As in the synthesis of **16**, 6.55 g. (16.8 mmoles) of chloromercuri-6-chloropurine (**14**)[13] was condensed with 6 g. (16.8 mmoles) of 2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl bromide. The crude product (5.2 g.) was obtained as a glass. About 3.0 g. of product was purified by chromatography on a short alumina column in benzene–chloroform (1:99). Collection of fractions on the basis of homogeneity on t.l.c. gave a total of 2.8 g. (61%) of **18** as a glass. T.l.c. on alumina in benzene–chloroform (1:9) showed one ultraviolet-absorbing spot of R_f 0.55; λ_{max}^{EtOH} 281 mμ (ε 2730), 265 (9960), 232 (28,000).

Anal. Calcd. for $C_{24}H_{19}ClN_4O_5$: C, 60.19; H, 4.00; Cl, 7.40; N, 11.70. Found: C, 59.53; H, 3.79; Cl, 7.71; N, 11.55.

9-(2,5-Di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)purine-6-thiol (19).—A suspension of 830 mg. (1.7 mmoles) of 6-chloro-9-(2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)purine (**16**) and 184 mg. (2.4 mmoles) of thiourea in 26 ml. of ethanol was refluxed for 40 min. After 5 min. a clear, colorless solution was obtained which became yellow in 15 min. and shortly thereafter colorless crystals of the product were deposited. The mixture was cooled to 25° and filtered. The solid was washed with three 5-ml. portions of ethanol and two 10-ml. portions of ether. The dried product (**19**) (420 mg., 52%) melted at 239–242° dec. (stage preheated to 150°); λ_{max}^{EtOH} 324 mμ (ε 23,500), 285 (4860), 278 (3960), 230 (33,600).

Anal. Calcd. for $C_{24}H_{20}N_4O_5S$: C, 60.50; H, 4.23; N, 11.76; S, 6.66. Found: C, 60.21; H, 4.39; N, 11.60; S, 7.02.

9-(3-Deoxy-β-D-ribofuranosyl)purine-6-thiol (3).—As in the synthesis of **1**, 367 mg. (0.77 mmole) of 9-(2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)purine-6-thiol (**19**) was deacylated with 0.85 mmole of sodium methoxide in methanol. After crystallization from water, the crude product (163 mg., 79%) melted at 202–206° dec. Recrystallization of 86.6 mg. of this material gave 73.4 mg. (67%) of **3**; m.p. 203–206°; $\lambda_{max}^{pH\ 4}$ 322 mμ (ε 23,000), 223 (9140); $\lambda_{max}^{pH\ 11}$ 311 mμ (ε 21,200), 233 (14,140); $[\alpha]_D$ −41°; $[\alpha]_{578}$ −44° (*c* 0.5, H_2O plus 1 equiv. of NaOH).

Anal. Calcd. for $C_{10}H_{12}N_4O_3S$: C, 44.78; H, 4.51; N, 20.89; S, 11.95. Found: C, 44.82; H, 4.69; N, 20.93; S, 12.28.

6-(N-Methylbenzamido)purine.—A mixture of 3 g. (0.02 mole) of 6-methylaminopurine and 11.3 g. (0.05 mole) of benzoic anhydride was heated to 205–210° for 20 min. A clear melt was obtained at 190°. After being cooled to about 100°, 30 ml. of ethanol was added, and the solution was refluxed for 45 min. The solution was concentrated and the residual oil in 25 ml. of ethyl acetate yielded 1 g. of crystalline solid. Concentration of the filtrate gave an oil which was chromatographed on a short column of silica gel. Elution with ethyl acetate removed some fast running impurities and the product (2.9 g.) was eluted with ethyl acetate–acetone (1:1). Dissolution of the product (3.9 g.) in 200 ml. of ethyl acetate followed by concentration to 20 ml. gave 3.45 g. (69%) of 6-(N-methylbenzamido)purine, m.p. 187.5–189°, λ_{max}^{EtOH} 282.5 mμ (ε 11,800).

Anal. Calcd. for $C_{13}H_{11}N_5O$: C, 61.65; H, 4.38; N, 27.66. Found: C, 61.75; H, 4.11; N, 27.70.

Chloromercuri-6-(N-methylbenzamido)purine (15).—A solution of 4.69 g. (18.5 mmoles) of 6-(N-methylbenzamido)purine and 5.02 g. (18.5 mmoles) of $HgCl_2$ in 76 ml. of ethanol–water (1:1) was stirred and heated (70°) while 7.4 ml. (18.5 mmoles) of 10% NaOH was added dropwise at a rate which did not allow a permanent yellow color to develop. The mixture was cooled and the colorless product was filtered and washed with water until the washings gave a negative test for chloride ion. Washing was continued with two 60-ml. portions of ethanol and two 120-ml. portions of ether. After being dried over P_2O_5 at 25° and reduced pressure the product (**15**) weighed 8.4 g. (93%).

Anal. Calcd. for $C_{13}H_{10}ClHgN_3O$: C, 31.97; H, 2.06; N, 14.35. Found: C, 32.15; H, 2.09; N, 14.18.

6-Methylamino-9-(3-deoxy-β-D-ribofuranosyl)purine (4). From 6-Chloropurine.—A mixture of 1 g. (0.08 mmole) of 6-chloro-9-(2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)purine (**18**) and 8 g. of methylamine in 25 g. of dry methanol was heated for 10 hr. at 100° in a sealed tube. The solution was concentrated to dryness, and the residue was dissolved in 25 ml. of water. The water solution was washed with two 5-ml. portions of benzene. The aqueous layer was stirred for 2.5 hr. with 3.5 g. of moist Dowex 2-X8 (CO_3^{-2}) resin, during which time the pH of the solution rose from 7 to 9. The resin was removed and washed with three 15-ml. portions of water. The filtrate and washings were concentrated to 5 ml., and, after being kept at 5° for 16 hr., the product (141 mg., m.p. 185–193°) was removed. A second crop (78 mg., m.p. 185–193°) was obtained from the filtrate. The two crops were combined and recrystallized from 2.5 ml. of water, 154 mg. (28%) of **4**, m.p. 196.5–198°, was obtained. The physical properties of this product were identical

with those obtained for the product synthesized from 6-methylaminopurine.

From 6-Methylaminopurine.—In a manner similar to that used for the synthesis of **16**, 60 g. (0.134 mole) of chloromercuri-6-(N-methylbenzamido)purine (**15**) was condensed with 2,5-di-O-benzoyl-3-deoxy-D-ribofuranosyl bromide [from 48 g. (0.135 mole) of methyl 2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranoside]. The crude product (52.2 g.) was combined with 31.3 g. of similar material from a second coupling reaction, wherein 40 g. (0.082 mole) of chloromercuri-6-(N-methylbenzamido)purine was used.

The crude 6-(N-methylbenzamido)-9-(2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)purine (80 g.) was chromatographed on a short column of 3000 g. of acid-washed alumina. The progress of the chromatographic separation was followed by t.l.c. on alumina in $CHCl_3$. Elution of the column with benzene removed a fast moving by-product (R_f 0.65). Further elution with chloroform–benzene (9:1) gave fractions containing varying amounts of two materials (R_f 0.55 and 0.39). By pooling fractions and rechromatographing some mixed fractions there was finally obtained 37 g. (31%) of 6-(N-methylbenzamido)-9-(2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)purine (**21**) as a glass. T.l.c. on alumina in chloroform showed only one spot of R_f 0.55; λ_{max}^{Nujol} 5.8 (–COO–), 6.0 (–CON–), no band at 3.0 μ (–NH); λ_{max}^{EtOH} 281 mμ (ε 14,000), 228 (37,000); $[\alpha]_D$ −62°; $[\alpha]_{578}$ −66° (*c* 1, $CHCl_3$). For analysis a sample was dried to constant weight at 100° to remove occluded chloroform.

Anal. Calcd. for $C_{32}H_{27}N_5O_6$: C, 66.54; H, 4.71; N, 12.13. Found: C, 66.37; H, 4.69; N, 11.88.

From the chromatography there was also obtained 13 g. (13%) of 6-methylamino-9-(2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)-purine (**22**) as a glass. T.l.c. on alumina in chloroform showed a single spot of R_f 0.39; λ_{max}^{Nujol} 3.02 (–NH), 5.8 (–COO–), and no band at 6.0 μ (–CON–); λ_{max}^{EtOH} 266 mμ (ε 17,000), 231 (29,400), 225 (25,200); λ_{infl}^{EtOH} 270 mμ (ε 16,600); $[\alpha]_D$ −57°; $[\alpha]_{578}$ −60° (*c* 1, $CHCl_3$).

Anal. Calcd. for $C_{25}H_{23}N_5O_5$: C, 63.41; H, 4.90; N, 14.79. Found: C, 63.11; H, 4.76; N, 14.23.

In addition 9.9 g. (9%) of a mixture of equal amounts of the blocked products **21** and **22** was obtained. The over-all yield of coupling products was 53%.

As in the synthesis of **1**, 36 g. (0.062 mole) of 6-(N-methylbenzamido)-9-(2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)purine (**21**) was debenzoylated in methanol containing 0.07 mole of sodium methoxide. The crude product (16 g., m.p. 195–198°) was recrystallized from 215 ml. of water and 10.3 g. (61%) of 6-methylamino-9-(3-deoxy-β-D-ribofuranosyl)purine (**4**), m.p. 197–199°, was obtained; $[\alpha]_D$ −44°, $[\alpha]_{578}$ −46° (*c* 1, H_2O), $\lambda_{max}^{pH\ 1}$ 263 mμ (ε 16,300), $\lambda_{max}^{pH\ 7}$ 267 mμ (ε 16,100), $\lambda_{max}^{pH\ 13}$ 266 mμ (ε 16,400).

Anal. Calcd. for $C_{11}H_{15}N_5O_3$: C, 49.80; H, 5.70; N, 26.40. Found: C, 49.76; H, 5.30; N, 26.62.

In a similar fashion 11.4 g. (0.024 mole) of 6-methylamino-9-(2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)purine (**22**) was debenzoylated in methanol with sodium methoxide. Recrystallization of the crude product (5.5 g., m.p. 197–199°) from 90 ml. of water gave 3.1 g. (48%) of **4**, m.p. 198–199°. The 9.9 g. (0.019 mole) of mixed **21** and **22** gave, after treatment with methanolic sodium methoxide, an additional 3.3 g. (65%) of **4**, m.p. 196–197°.

The combined filtrates and washings from the recrystallizations of **4** were concentrated to 200 ml. and treated with 9.5 g. of picric acid in 200 ml. of hot water. The yellow solid (9.9 g.) obtained, when recrystallized from 400 ml. of methanol, gave 5 g. of the picrate (m.p. 210–260° dec.) of **4**.

Anal. Calcd. for $C_{17}H_{18}N_8O_{10}$: C, 41.30; H, 3.67; N, 22.67. Found: C, 41.60; H, 3.80; N, 22.95.

Removal of the picric acid in aqueous solution with Dowex 2-X8 (CO_3^{-2}) gave after recrystallization an additional 2.1 g. of **4**, m.p. 197–198°. The over-all yield of **4** from the chloromercuripurine (**15**) was 35%.

6-Dimethylamino-9-(3-deoxy-β-D-ribofuranosyl)purine (5).—A suspension of 0.9 g. (1.88 mmoles) of 6-chloro-9-(2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)purine (**18**) in 25 ml. of methanol containing 7.6 g. of dimethylamine was treated as in the preparation of **4**. Two crops of crude product (159 mg., m.p. 199–201°, and 122 mg., m.p. 199–200°) were obtained. The two crops were combined and recrystallized from 10 ml. of water, and pure **5** (220 mg., 42%, m.p. 199–200°) was obtained. T.l.c. on cellulose in water showed one ultraviolet-absorbing spot of R_f 0.68; $[\alpha]_D$ −37°; $[\alpha]_{578}$ −39° (*c* 0.5, H_2O); λ_{max}^{H2O} 276 mμ (ε 18,300), 214 (16,000); $\lambda_{max}^{pH\ 13}$ 276 mμ (ε 17,900); $\lambda_{max}^{pH\ 1}$ 268 mμ (ε 18,100), 210 (17,300).

Anal. Calcd. for $C_{12}H_{17}N_5O_3$: C, 51.60; H, 6.14; N, 25.08. Found: C, 51.45; H, 5.95; N, 24.81.

6-Ethylamino-9-(3-deoxy-β-D-ribofuranosyl)purine (6).—A suspension of 2.0 g. (4.17 mmoles) of 6-chloro-9-(2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)purine (**18**) in 30 ml. of ethanol containing 12 ml. of ethylamine was treated as in the preparation of **4**. The crude product (400 mg., m.p. 165–167°) crystallized from water. Concentration of the mother liquors gave a second crop (200 mg., m.p. 164–167°). Combination and recrystallization of these crops from 4 ml. of water gave 442 mg. (38%) of **6**, m.p. 171–173°. T.l.c. on cellulose in water showed a single spot at R_f 0.69, $[\alpha]_D$ −39°, $[\alpha]_{578}$ −42° (*c* 1, H_2O), $\lambda_{max}^{pH\ 1}$ 263 mμ (ε 16,800), $\lambda_{max}^{pH\ 7}$ 268 mμ (ε 16,700), $\lambda_{max}^{pH\ 13}$ 268 mμ (ε 16,900).

Anal. Calcd. for $C_{18}H_{17}N_5O_3$: C, 51.60; H, 6.14; N, 25.08. Found: C, 51.96; H, 6.07; N, 24.81.

Treatment of all of the filtrates from the above recrystallizations with 0.58 g. of picric acid in 35 ml. of water gave 600 mg. of 9-(3-deoxy-β-D-ribofuranosyl)-6-ethylaminopurine picrate. Recrystallization from methanol gave 500 mg. of pure picrate.

Anal. Calcd. for $C_{18}H_{20}N_8O_{10}$: C, 42.52; H, 3.97; N, 22.04. Found: C, 42.16; H, 3.80; N, 21.74.

The picrate (487 mg.) was added portionwise to a suspension of 5 g. of Dowex 2-X8 (CO_3^{-2}) in 25 ml. of water. The resin was filtered and washed, and the filtrate was concentrated to 2 ml. An additional 173 mg. (total yield 53%) of product, m.p. 171–174°, was obtained.

9-(2,5-Di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)purine (20).—A solution of 1 g. (2.08 mmoles) of 6-chloro-9-(2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)purine (**18**) in 17 ml. of dioxane with 100 mg. (2.5 mmoles) of MgO and 0.5 g. of 5% Pd–charcoal catalyst was shaken for 98 hr. in an atmosphere of hydrogen at 25°. The mixture was filtered and concentrated to an oil (0.95 g.). The oil was chromatographed on a short alumina column in benzene–chloroform (1:4). Several fractions showing only one spot at R_f 0.33 (t.l.c. on alumina in the same solvent mixture as above) were pooled and gave 400 mg. of the desired product as a glass.

Those fractions containing starting material (R_f 0.62) were combined and rehydrogenated as above. After 7 hr., the product was worked up as before. A second crop (150 mg.) of product was obtained by chromatography. The total yield of **20** was 550 mg. (60%); λ_{infl}^{EtOH} 280 mμ (ε 2350); λ_{max}^{EtOH} 263 mμ (ε 8170), 231 (28,400); $[\alpha]_D$ −30°; $[\alpha]_{578}$ −33° (*c* 1, $CHCl_3$).

Anal. Calcd. for $C_{24}H_{20}N_4O_5$: C, 64.86; H, 4.54; N, 12.61. Found: C, 64.95; H, 4.50; N, 12.56.

9-(3-Deoxy-β-D-ribofuranosyl)purine (7).—As in the synthesis of **1**, 410 mg. (0.93 mmole) of 9-(2,5-di-O-benzoyl-3-deoxy-β-D-ribofuranosyl)purine (**20**) was debenzoylated with 1 mmole of sodium methoxide in methanol. Crystallization of the crude product from water gave two crops of product (82 mg., m.p. 198–200°, and 37 mg., m.p. 196–200°). Combination and recrystallization of these fractions from 1 ml. of water gave 86 mg. (37%) of **7**, m.p. 198–199°. T.l.c. on cellulose showed only one ultraviolet-absorbing spot at R_f 0.89, $[\alpha]_D$ −29°, $[\alpha]_{578}$ −31° (*c* 0.52, H_2O), $\lambda_{max}^{pH\ 7}$ 263 mμ (ε 7630), $\lambda_{max}^{pH\ 4}$ 262.5 mμ (ε 7370), $\lambda_{max}^{pH\ 1)}$ 263 mμ (ε 7520).

Anal. Calcd. for $C_{10}H_{12}N_4O_3$: C, 50.84; H, 5.12; N, 23.72. Found: C, 50.80; H, 4.86; N, 23.30.