= 0.60 and 0.90, in *n*-butyl-alcohol-water (86:14). Compound IVb had R_f 0.60 in this solvent system. The substance (VIb) having R_f 0.90 showed an ultraviolet absorption maximum at 275 mμ in water, which shifted to 318 mμ on addition of alkali. Acidification gave a maximum at 308 mμ. The sirup was taken up in 50 ml. of 1 *N* hydrochloric acid and allowed to stand overnight at room temperature. The solution was concentrated *in vacuo* to dryness, the residue was taken up in 30 ml. of water and applied to a column of Dowex 50 (H^+ 2.2 × 15 cm.) resin, and washed with water. The washings were collected in 70-ml. fractions. Fractions 2 and 3, having an absorption maximum at 260 mμ were combined and concentrated *in vacuo* to dryness. The residual semisolid was taken up in ethanol, the insoluble material was removed by filtration, and the filtrate was concentrated *in vacuo*, whereupon crystals formed, m.p. 163–169°. Recrystallization from ethyl acetate gave 0.1 g. of 3-methyluracil (VIII), m.p. 177–179° (lit.[6] m.p. 174–175°); mixture melting point with authentic material (m.p. 177–178°) was 177–179°. The ultraviolet absorption properties are identical to those reported.[7] The infrared spectrum was also identical with that of an authentic sample.

The column was then eluted with 1 *N* hydrochloric acid and fractions containing IIIb were obtained. The presence of trace amounts of 3-methylcytosine (IXb) was observed in the fractions collected after IIIb was eluted, although the separation was not complete.

The Reaction of IVb in Acetic Anhydride and/or Acetic Acid.—Compound IVb (*ca.* 30 mg. each) was dissolved in 5 ml. of acetic anhydride, or acetic acid, or acetic anhydride–acetic acid (2:1) and refluxed for 20 hr. An aliquot of each reaction solution was applied to paper chromatography (*n*-butyl alcohol–water, 86:14). Only the reaction of IVb in acetic acid–acetic anhydride showed the presence of a spot at R_f 0.9, along with the R_f 0.60 spot of the starting material. This reaction solution was concentrated to dryness and the residue was dissolved in 3 ml. of 1 *N* hydrochloric acid and allowed to stand for 2 hr. at 45°. An aliquot of the solution was examined by paper electrophoresis (pH 5.0, 0.1 *M* ammonium acetate, 800 v. for 2 hr.). Three spots were obtained migrating at −1.0, −5.9, and −13.5 cm. (VIII, IIIb, and IXb, respectively, as identified by spectral determination). From the spectral calculations *ca.* 45% of IVb was shown to be converted to VIII and IXb in the ratio of 3:1.

Acetylation of 3-Methylcytosine (IXb) Followed by Acid Hydrolysis.—3-Methylcytosine hydrochloride[8a] (0.5 g.) and anhydrous sodium acetate (0.2 g.) were suspended in 3.0 ml. of acetic anhydride and shaken for 4 hr. or refluxed for 1 hr. After cooling, the precipitate was removed by filtration, and the filtrate was concentrated *in vacuo* to a sirup (VIb). This sirup showed a single spot at R_f 0.9 in *n*-butyl alcohol–water (86:14) paper chromatography. The ultraviolet absorption maximum in water was at 275 mμ. On addition of 1 drop of 30% sodium hydroxide in the 3-ml. cuvette, the maximum shifted to 318 mμ, and acidification of the solution showed a new maximum at 308 mμ. The sirup was dissolved in 10 ml. of 1 *N* hydrochloric acid and the solution was allowed to stand overnight at room temperature. The solution was concentrated *in vacuo* to dryness, the residue was dissolved in ethanol at room temprature, the insoluble material was separated by filtration, and the filtrate was concentrated *in vacuo* to a solid mass (0.2 g.), which showed the characteristic ultraviolet absorption spectra for VIII.[7] The alcohol-insoluble material was treated with boiling ethanol and separated from a small amount of insoluble material. The ethanol solution was concentrated to dryness to give 0.4 g. of a solid. Paper electrophoretic examination of this solid showed the presence of 3-methylcytosine along with a large amount of 3-methyluracil. The ratio of IXb to VIII was 1:9.

Reaction of 3-Methylcytosine with Acetic Anhydride–Acetic Acid.—The hydrochloride salt of 3-methylcytosine (IXb, 0.1 g.) and anhydrous sodium acetate (0.5 g.) was suspended in acetic anhydride (3.0 ml.) and acetic acid (2.0 ml.), and refluxed for 20 hr. The solution was concentrated *in vacuo* to a small volume, treated with ethanol, and evaporated to a sirup. The sirup was dissolved in 5 ml. of 1 *N* hydrochloric acid and kept for 18 hr. at room temperature. After concentration *in vacuo* to a solid mass, this amorphous solid was dissolved in 25 ml. of water. An aliquot of the solution was examined by paper electrophoresis (pH 5.0, 0.1 *M* ammonium acetate, 800 v., 90 min.). Three spots were obtained migrating −0.2, −4.2, and −12.0 cm. Each spot was excised, eluted with 40 ml. of water, and examined spectrophotometrically. From the comparison of the migration of authentic materials and ultraviolet absorption spectra, the spots were characterized as 3-methyluracil (−0.2), N^4-methylcytosine (−4.2), and 3-methylcytosine (−12.0 cm.).

The ratio of formation of 3-methyluracil, 3-methylcytosine, and N^4-methylcytosine was approximately 1.6:1.0:1.0, respectively. These data show that ~30% of 3-methylcytosine was converted to N^4-methylcytosine (IIIb). The water solution of the acid hydrolysate was further applied to a column of Dowex 50 (H^+ form, 2.5 × 12 cm.), washed with water, and eluted with 0.5 *N* hydrochloric acid. From the water washings, fractions containing 3-methyluracil were obtained. With 0.5 *N* hydrochloric acid, fractions containing N^4-methylcytosine, which was eluted first, and 3-methylcytosine were obtained, although the separation of the latter two was not complete.

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Nucleosides. XXI. Synthesis of Some 3′-Substituted 2′,3′-Dideoxyribonucleosides of Thymine and 5-Methylcytosine[1]

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The disulfide of 3′-deoxy-3′-mercaptothymidine (VI) was synthesized by reaction of anhydronucleoside II with potassium thiobenzoate in dimethylformamide followed by removal of the protecting groups. Potassium phthalimide in dimethylformamide was shown to be a useful reagent for the conversion of a 3′-*O*-mesylthymidine (I, R = trityl) to the 3′-deoxy-3′-phthalimido derivative (VIII). This latter reaction also proceeds *via* anhydronucleoside II. Removal of the protecting groups from VIII yielded 3′-amino-3′-deoxythymidine (X). Detritylation of VIII followed by acetylation yielded XII which was thiated to the 4-thionucleoside and converted to the 3′-amino-3′-deoxy derivative (XV) of 5-methyl-2′-deoxycytidine. Under certain conditions, the 4-amino group of cytosine nucleosides was readily exchanged with *n*-butylamine to produce 4-*n*-butylamino nucleoside derivatives.

It was demonstrated in a previous study[2] that under acid-catalyzed conditions di-*O*-mesylthymidine (I, R = mesyl) is converted directly in refluxing *N*,*N*-dimethylformamide containing sodium benzoate to di-*O*-benzoylthymidine (III, R = benzoyl). This reaction was

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(2) Paper XVI in this series: J. J. Fox and N. C. Miller, *J. Org. Chem.*, **28**, 936 (1963).

shown to proceed *via* the 2,3′-anhydronucleoside[3,4] intermediate (II, R = mesyl). It was further demonstrated that II (R = mesyl) also is converted easily to III with the same reagent and reaction conditions only when benzoic acid was added to the reaction mixture. It was suggested,[2] therefore, that under acid-catalyzed conditions other appropriate nucleophiles might be introduced into the "down" configuration of the sugar moiety of anhydronucleosides (*i.e.*, II) (or their sulfonyloxy precursors, *i.e.*, I) of 1-β-D-aldosylpyrimidines bearing a 2-carbonyl function in the aglycon. The present study deals with the successful application of this rationale to the syntheses of 3′-deoxy-3′-mercaptothymidine and 3′-amino-3′-deoxythymidine as part of our program in the synthesis of nucleosides of potential biochemical interest. Preliminary reports have appeared.[5]

Reaction of II (R = trityl) with potassium thiobenzoate in dimethylformamide at reflux temperature for two hr. gave intractable material from which no definable product could be isolated. When this reaction was repeated with the addition of approximately 1 equiv. of benzoic acid, crude 5′-*O*-trityl-3′-*S*-thiobenzoate (IV) was obtained as an amorphous powder. The ultraviolet absorption spectrum of IV was similar to that for III (R = trityl)[2] as would be expected if thiobenzoate ion attacked the 3′-position of anhydronucleoside II. Detritylation of IV with acid yielded the crystalline thiobenzoate (V). Saponification of V with dilute alkali at room temperature for 1 day afforded the crystalline disulfide of 3′-deoxy-3′-mercaptothymidine (VI). Disulfide VI also was obtained by brief, hot alkaline hydrolysis of V followed by titration of the reaction solution with iodine. After the consumption of iodine ceased (∽1 molar equiv. consumed), the disulfide VI crystallized from the acidified reaction solution. A molecular weight determination of VI was consistent with the disulfide structure. Attempts to isolate the thiol of VI by saponification of V were unsuccessful. The disulfide VI also was obtained by alkaline treatment of IV to afford crude VII which was probably a mixture of the 3′-thiol derivative and its disulfide. Detritylation of VII yielded the disulfide VI (see Fig. 1).

The disulfide VI exhibited an ultraviolet absorption spectrum similar to that for thymidine[6] showing that the sulfur atoms in VI were not located in the aglycon.[7] That the thiobenzoate group in IV (and thereby in V) is in the ribo (or "down") configuration is established by virtue of its synthesis from anhydronucleoside II and by analogy with the conversion of II to III under similar reaction conditions with a similar type nucleophile (benzoate).[2] Compound VI, therefore, is the 3′-deoxy-3′-mercapto analog of thymidine.

Reaction of I (R = trityl) with potassium phthalimide in dimethylformamide for 10 hr. at reflux afforded VIII as an amorphous powder. Treatment of this powder with methylamine in methanol at ∽105° for 20 hr. yielded XI as a sirup which was detritylated with acid to yield crystalline X in ∽35% over-all yield from I. As expected, the ultraviolet absorption spectrum of X was similar to that for thymidine but differed from that for isocytidine[8] again showing that the nucleophile (phthalimido ion) had entered the sugar moiety (in VIII) and not the aglycon. An alternate synthesis of X was achieved by detritylation of VIII to afford crystalline IX which was deacylated to X. This latter procedure gave poorer yields of X. Treatment of X with acetic anhydride in water yielded the *N*-acetyl derivative (Xa).

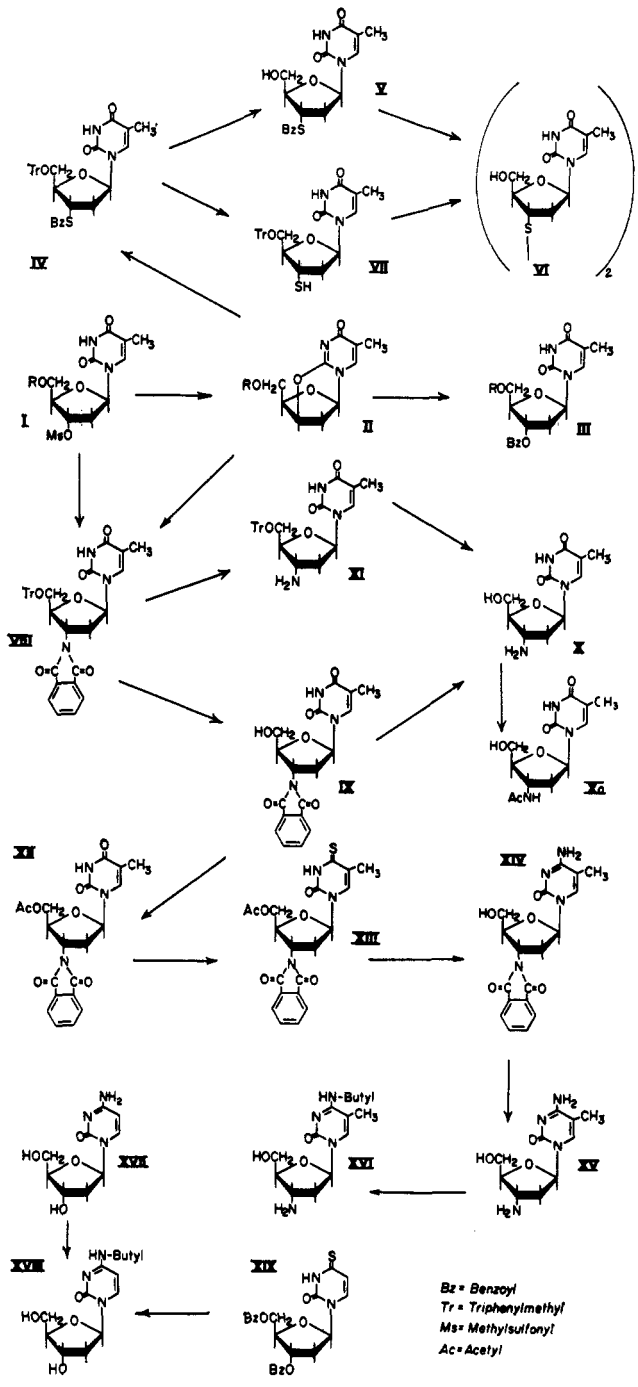

Figure 1.

(3) Although the term "cyclonucleoside" has been employed for naming this class of compounds, the term "anhydronucleoside"[4] is more in keeping with carbohydrate nomenclature.

(4) J. J. Fox and I. Wempen, *Advan. Carbohydrate Chem.*, **14**, 283 (1959).

(5) J. J. Fox and N. C. Miller, Abstracts of the 144th National Meeting of American Chemical Society, Los Angeles, Calif., April, 1963, p. 4C; N. Miller and J. J. Fox, Abstracts of 145th National Meeting of American Chemical Society, New York, N. Y., Sept., 1963, p. 21D.

(6) J. J. Fox and D. Shugar, *Biochim. Biophys. Acta*, **9**, 369 (1952).

(7) Location of the sulfur atom on position 2 or 4 of the aglycon would markedly alter the ultraviolet spectrum from that for thymidine; [see D. Shugar and J. J. Fox, *Bull. soc. chim. Belges*, **61**, 293 (1952), and G. B. Elion, W. S. Ide, and G. H. Hitchings, *J. Am. Chem. Soc.*, **68**, 2137 (1946)].

(8) D. M. Brown, D. B. Parihar, A. R. Todd, and S. Varadarajan, *J. Chem. Soc.*, 3028 (1958).

As in the case of the reaction of I → III,[2] the conversion of I to VIII proceeded *via* the anhydronucleoside II. Short-term treatment of I with potassium phthalimide in dimethylformamide (120° for 10 min.) gave a high yield (~80–90%) of II. Moreover, when II was treated with potassium phthalimide in dimethylformamide with added phthalimide (10 hr., reflux), amorphous VIII was obtained which was also converted to IX. Thus it is established that the phthalimido group entered by attack at C-3′. By analogy with the synthesis of VI from II described above and the synthesis of III from II reported previously,[2] the amino group in X (and the phthalimido group in IX and VIII) must be in the "down" (ribo) configuration and X is, therefore, 3′-amino-3′-deoxythymidine, that is 1-(3′-amino-2′,3′-dideoxy-β-D-ribofuranosyl)thymine. The adenine derivative of this sugar moiety has been synthesized by Lee, *et al.*,[9] by a different route.

Compound IX served as starting material for the synthesis of the 3′-amino-3′-deoxy analog of 5-methyl-2′-deoxycytidine. Acetylation of IX yielded the blocked nucleoside (XII). Thiation of XII with phosphorus pentasulfide in pyridine yielded a yellow glass (XIII) which was heated with alcoholic ammonia to form the cytosine derivative (XIV) as a sirup. Spectral examination of the sirup (absence of absorption at 330 mμ) showed that the 4-thio group had been replaced. This sirup was not purified but was treated directly with *n*-butylamine in methanol at ~105° for 20 hr. XV was obtained in ~40% over-all yield from XII. A chromatographic examination (1-butanol–water, 86:14) of the mother liquor showed that a second major ultraviolet absorbing spot (R_f 0.87) was formed along with some fluorescent material. The ultraviolet absorbing spot (R_f 0.87) like XV (R_f 0.12) also gave a 5-methylcytidine-like spectrum.[10] This chromatographic behavior is generally similar to that shown by 5-fluoro-2′-deoxycytidine (R_f 0.29) and its *N*-*n*-butyl derivative (R_f 0.85).[11] It is highly likely, therefore, that the faster migrating component obtained by treatment of sirup XIV with butylamine in methanol at ~105° for 20 hr. is the *N*-*n*-butyl derivative (XVI) of XV. Attempts to separate and crystallize XVI were unsuccessful. However, support for the structural assignment to XVI was obtained by treatment of the closely related 2′-deoxycytidine (XVII) with *n*-butylamine in methanol in a manner similar to that used in the synthesis of XV and XVI. Only a trace of XVIII was obtained. However, when 1 equiv. of ammonium acetate was added to this reaction (impure XIV should contain this salt) the proportion of XVIII formed increased to about one-third. When the hydrochloride salt of XVII was employed in this reaction, the yield of XVIII isolated as the picrate salt was increased to 90%. For comparative purposes, XVIII was prepared from XIX[11,12] by refluxing the latter in methanolic *n*-butylamine. It is thus evident that cytosine-type nucleosides can undergo amine exchange which may provide a simpler route for the synthesis of *N*-substituted cytosine nucleosides.

This amine-exchange reaction with nucleosides has its counterpart in pyrimidine chemistry. Whitehead and Traverso[13] have shown that certain 4- or 6-aminopyrimidines will exchange with amine hydrochlorides under rigorous conditions (165–170° for several hours) to yield 4- or 6-*N*-substituted aminopyrimidines. Curran and Angier[14] recently have demonstrated that certain 4- and 6-aminopyrimidines will undergo a similar amine-exchange reaction when heated with alkylammonium acetates.

Experimental[15]

Disulfide of 3′-Deoxy-3′-mercaptothymidine (VI).—2,3′-Anhydro-1-(5′-*O*-trityl-2′-deoxy-β-D-lyxosyl)thymine[2] (II, R = trityl; 4.65 g.) was refluxed under nitrogen for 1.5 hr. in 500 ml. of dimethylformamide with 10.5 g. of potassium thiobenzoate and 1.22 g. of benzoic acid. The solvent was removed *in vacuo* and the residue treated with 2 l. of water. After filtration, the amorphous tan solid (crude IV) was washed well with water. The tan solid was dissolved in 100 ml. of 95% ethanol, treated with 3 ml. of 10 *N* sodium hydroxide, refluxed for about 5 min., and allowed to remain at room temperature overnight. Upon neutralization of the reaction solution with acetic acid, precipitation of an amorphous solid occurred. The volume of the reaction mixture was reduced *in vacuo* to about 30 ml. and water was added to complete precipitation. The solids were collected on a filter and washed well with water. The residue was dissolved in chloroform and dried over sodium sulfate. After filtration, the filtrate was concentrated to about 50 ml., to which 150 ml. of ether was added with stirring to prevent the formation of a gum. Detritylation of crude VII was effected by bubbling hydrogen chloride into the cooled ether–chloroform mixture to saturation during which time precipitation occurred. After 40 min. in the ice bath, solvents were removed *in vacuo* and most of the remaining hydrogen chloride removed azeotropically with benzene. Trituration of the sirupy brown residue several times with ether extracted the triphenylcarbinol leaving an acidic brown sirup. This sirup was dissolved in ethanol and neutralized with triethylamine after which crystallization occurred (1.0 g.), m.p. 230–235°. A second crop gave 0.2 g. with a similar melting point. Average yields in this over-all reaction from II were ~45%. Recrystallization from 70% ethanol gave minute crystals, m.p. 239–241.5°. For analytical purposes, further purification was achieved by dissolving the solid in dilute sodium hydroxide followed by careful neutralization with dilute acetic acid to turbidity. After several hours, precipitation of VI was completed, m.p. 245.5–246.5°, $[\alpha]^{23}D$ −7 ± 2° (*c* 0.61, 0.1 *N* sodium hydroxide). Ultraviolet absorption properties follow: at pH 1–7, maximum at 267 mμ (ϵ_{max} 10,900), minimum at 234 mμ (ϵ_{min} 2700); in 0.1 *N* sodium hydroxide, maximum at 261.5 mμ (ϵ_{max} 8100), minimum at 244 mμ (ϵ_{min} 5200).

Anal. Calcd. for $C_{20}H_{26}N_4O_8S_2$: C, 46.68; H, 5.09; N, 10.89; S, 12.46; mol. wt., 515. Found: C, 46.34; H, 5.78; N, 10.77, 10.84; S, 12.32; mol. wt., 510 ± 50.[16]

3′-Deoxy-3′-thio-*S*-benzoylthymidine (V).—The amorphous tan solid (IV), obtained above from 0.005 mole of II (R = trityl), was dissolved in 10 ml. of chloroform and the stirred solution treated with ether (100 ml.). The stirred mixture was saturated with hydrogen chloride with cooling and the mixture was allowed to remain for 1 hr. at ~5°. Solvents were removed *in vacuo* (bath temperature, 35–40°); benzene was added and removed three times leaving a thin brown sirup which still contained traces of hydrogen chloride. Cyclohexane (100 ml.) was added and the resulting solution was separated by filtration from a small amount of insolubles. The yellow filtrate was evaporated

(9) W. W. Lee, A. Benitez, C. D. Anderson, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **83**, 1906 (1961).

(10) J. J. Fox, D. Van Praag, I. Wempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich, and G. B. Brown, *ibid.*, **81**, 178 (1959).

(11) I. Wempen, R. Duschinsky, L. Kaplan, and J. J. Fox, *ibid.*, **83**, 4755 (1961).

(12) The authors are indebted to Miss I. Wempen for a sample of XIX.

(13) C. W. Whitehead and J. J. Traverso, *J. Am. Chem. Soc.*, **82**, 3971 (1960).

(14) W. V. Curran and R. B. Angier, *J. Org. Chem.*, **28**, 2672 (1963).

(15) All melting points were taken on a Thomas–Hoover capillary melting point apparatus and are corrected. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., and by Spang Microanalytical Laboratory, Ann Arbor, Mich.

(16) The authors are indebted to Dr. A. Motchane of Hoffmann–LaRoche, Inc., Nutley, N. J., for the determination of this molecular weight. The thermoosmotic method used here depends on efficient equilibration of solvent and solution. Since the solvent used (*N*,*N*-dimethylformamide) has a low vapor pressure, equilibration is slow and the variations are, therefore, large.

to a thin sirup which was treated with a *minimal* amount of ethanol to cause solution. The solution was treated with cyclohexane to turbidity. Crystallization occurred as needles, which were collected on a filter and washed with cyclohexane to yield 0.7 g., m.p. 90°, with resolidification at ~120°, and final melting at 170–172°. One recrystallization from ethanol gave pure V, m.p. 179–180°, $[\alpha]^{23}D$ +14° (*c* 0.29, ethanol). Ultraviolet absorption properties in ethanol follow: maxima at 267.5 and 246.5 mμ, minima at 251 and 224 mμ, shoulder at 240 mμ.

Anal. Calcd. for $C_{17}H_{18}N_2O_5S$: C, 56.34; H, 5.01; N, 7.73; S, 8.85. Found: C, 56.80; H, 4.96; N, 7.61; S, 8.71.

Synthesis of VI from V.—Compound V (100 mg.) was treated with 10 ml. of 0.1 *N* sodium hydroxide and allowed to remain overnight with stirring at room temperature. The reaction solution was treated with dilute acetic acid until precipitation began. After cooling to complete crystallization, the product was collected, 65 mg., m.p. 242–244°. A mixture melting point with VI obtained above did not show any depression.

Compound V also may be converted to VI by saponification followed by titration with iodine. V (100 mg.) was treated with 5 ml. of water and 9 ml. of 0.1 *N* sodium hydroxide. The mixture was warmed (with stirring) until solution occurred. The solution was titrated immediately with an aqueous iodine solution, ~0.5 *N* (sodium iodide–iodine, 2:1 by weight), whereupon approximately 0.75 equiv. of iodine was consumed. (The low consumption of iodine is probably due to the fact that some disulfide formation had occurred in the prior alkaline de-esterification.) The resulting clear solution was treated with dilute acetic acid until turbidity was reached. The product, 54 mg., m.p. 235–240°, was purified in a manner similar to that described above. The recovery was almost quantitative, yielding product with m.p. 244–246°.

3′-Deoxy-3′-phthalimidothymidine (IX).—Compound I (R = trityl, 2.4 g.)[17] was refluxed for 10 hr. in 300 ml. of dimethylformamide with 3.7 g. of potassium phthalimide. Solvents were removed *in vacuo* and the residual brown gum was extracted with chloroform (500 ml.). The insolubles were filtered and discarded and the chloroform filtrate was washed twice with 250-ml. portions 0.1 *N* sodium hydroxide. Sodium chloride was added to the chloroform–water mixture to facilitate separation of the emulsion into distinct layers. The chloroform layer was finally washed with water three times and concentrated to a thick yellow gum (compound VIII). Crude VIII was refluxed for 10 min. with 100 ml. of ethanol and 5 drops of concentrated hydrochloric acid. Upon removal of solvent precipitation of a tan granular solid began. The residue was triturated with ether and the solid collected, 1 g., softening at 180° and melting at 235–240°. Treatment with ethanol removed the color and gave 0.65 g. of a colorless solid, m.p. 265–266°. Recrystallization from ethanol gave m.p. 269–269.5°, $[\alpha]^{23}D$ −45° (*c* 0.40, dimethylformamide). Ultraviolet absorption properties in ethanol follow: maxima at 266 and 242 mμ, minima at 248 and 239 mμ, shoulder at 302 mμ.

Anal. Calcd. for $C_{18}H_{17}N_3O_6$: C, 58.21; H, 4.61; N, 11.31. Found: C, 57.95; H, 4.90; N, 11.31.

Isolation of Intermediate II (R = trityl).—Compound I (R = trityl, 1.12 g.) in 200 ml. of dimethylformamide and 1.85 g. of potassium phthalimide was heated with stirring to 120°. When this temperature was reached, the reaction was cooled to precipitate salts. After filtration, the filtrate was evaporated to dryness, the residue extracted with chloroform, and the chloroform extract evaporated to a thin yellow sirup. The sirup was triturated with ether and stirred. The mixture was cooled and the crystals separated, 0.75 g., m.p.231–232°. An additional 0.1 g. (same melting point) was obtained from the mother liquor. The ultraviolet spectrum and melting point properties were identical with those for anhydronucleoside II (R = trityl).[2]

3′-Amino-3′-deoxythymidine (X).—5′-*O*-Trityl-3′-*O*-mesylthymidine (I, R = trityl; 5.2 g.) was refluxed for 12 hr. with 10 g. of potassium phthalimide in 500 ml. of dimethylformamide. Crude VIII (5.3 g.), obtained by the same procedure as described above, was treated at 105° with 17 ml. of methylamine[18] in 100 ml. of methanol for 20 hr. The amber reaction mixture was evaporated to a semicrystalline residue and extracted with water. The aqueous extracts were discarded and the oily residue was treated with 100 ml. of ethanol and enough concentrated hydrochloric acid to neutralize the residual methylamine. Four drops of acid were then added; the solution was refluxed for 8 min. The acidic solution was concentrated *in vacuo* after which crystallization began. Benzene was added and removed *in vacuo* a few times to remove water and as much acid as possible. The crystalline residue was triturated with ethanol and ether. The reddish crystals (hydrochloride of X), 1.2 g., m.p. 250° dec. (efferv.), gave a thymidine-like ultraviolet absorption spectrum. The hydrochloride of X was absorbed on Dowex 50 (H^+), washed with water, and eluted with 1 *N* ammonium hydroxide. The eluates were evaporated to dryness, whereupon colorless crystals, 0.7 g., m.p. 180–182°, separated. Pure material was obtained by dissolving a sample in methanol followed by concentration of the solution to a smaller volume, whereupon crystallization began. The mixture was allowed to remain at room temperature until crystallization was complete. The sample was collected and washed with ethanol, m.p. 187–187.5°, $[\alpha]^{23}D$ +20° (*c* 0.64, water). Ultraviolet absorption properties follow: in 0.1 *N* hydrochloric acid, maximum at 265 mμ (ϵ_{max} 9400), minimum at 233 mμ (ϵ_{min} 2300); at pH 7.53, maximum at 266.5 mμ (ϵ_{max} 9300), minimum at 233 mμ (ϵ_{min} 1900); in 0.1 *N* sodium hydroxide, maximum at 266.5 mμ (ϵ_{max} 7400), minimum at 244 mμ (ϵ_{min} 4400).

Anal. Calcd. for $C_{10}H_{15}N_3O_4$: C, 49.78; H, 6.26; N, 17.41. Found: C, 49.85; H, 6.09; N, 17.20.

3′-Acetylamino-3′-deoxythymidine (Xa).—X (500 mg.) was added to 3 ml. of water and treated with 0.4 ml. of acetic anhydride. The stirred solution formed a precipitate. After 10 min., the precipitate (460 mg., m.p. 108–116°) was removed. The mother liquor yielded an additional 90 mg. The combined crops were dissolved in a minimal amount of hot water. Upon cooling, a precipitate formed which was dried at 60° *in vacuo* for 4 days, m.p. 183–184°, with some softening at 115°, $[\alpha]^{23}D$ +23° (*c* 0.77, water). Ultraviolet properties in water follow: maximum at 266 mμ, minimum at 234.5 mμ.

Anal. Calcd. for $C_{12}H_{17}N_3O_5 \cdot H_2O$: C, 47.83; H, 6.35; N, 13.95. Found: C, 47.27; H, 6.33; N, 14.25.

5′-*O*-Acetyl-3′-deoxy-3′-phthalimidothymidine (XII).—Compound IX (2.1 g.) was treated with 40 ml. of anhydrous pyridine and 6 ml. of acetic anhydride and allowed to remain at room temperature overnight. Solvents were evaporated *in vacuo* and the thick sirup was triturated repeatedly with ether. A white granular solid was obtained, 1.9 g., m.p. 230–237°. After two recrystallizations from ethanol (with treatment with charcoal), pure material was obtained, m.p. 238–239°, $[\alpha]^{23}D$ −40° (*c* 0.39, dimethylformamide). Ultraviolet properties in ethanol follow: maxima at 265 and 242 mμ, minima at 248 and 239 mμ.

Anal. Calcd. for $C_{20}H_{19}N_3O_7$: C, 58.11; H, 4.63; N, 10.16. Found: C, 57.78; H, 4.95; N, 9.79.

3′-Amino-2′,3′-dideoxy-5-methylcytidine (XV).—Crystalline XII was heated with 11 ml. of reagent grade pyridine and 0.9 g. of "reactive" phosphorus pentasulfide.[19] When reflux temperature was reached, 0.02 ml. of water was added and the reaction mixture refluxed for 2.5 hr. The brown reaction mixture was cooled and decanted from a thick green oil, and the decantate was evaporated to about 3 ml. and treated with water. The insolubles were filtered and washed well with water. The solids were extracted with chloroform and filtered. The chloroform filtrate was dried with sodium sulfate and evaporated to a thin yellow sirup which contained some residual pyridine. The residual pyridine was removed azeotropically by codistillation *in vacuo* with water. The sirup was taken to dryness (yellow glass, 950 mg., XIII) by codistillation with benzene. Methanolic ammonia, 100 ml., was added to glass XIII and the solution was heated in a bomb at 105° for 22 hr. The amber sirup, after evaporation of solvents, was treated with methanol and filtered from some insoluble material. The solution was evaporated to dryness *in vacuo* and the residual brown sirup was treated with 50 ml. of methanol containing 10 ml. of *n*-butylamine in a bomb for 20 hr. at 105°. After removal of solvents the sirupy residue was triturated with ether, which gave a yellow hygroscopic flocculent solid. This solid was dissolved in water; the solution was extracted three times with chloroform. The aqueous fraction was concentrated to a slightly yellow amorphous solid which was treated with 10 ml. of methanol and allowed to remain at room temperature for a few days. Clusters of needles (XV) were obtained, 260 mg., m.p. 200–208° dec. (efferv.). This solid showed only one spot by paper chromatography (1-

(17) A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, 816 (1955).

(18) L. Goldman and J. W. Marsico, *J. Med. Chem.*, **6**, 413 (1963).

(19) Obtained from Monsanto Chemical Co., St. Louis, Mo.

butanol–water, 86:14). The mother liquor from this solid was saved for later examination. Crude XV was then absorbed on Dowex 50 (H^+), washed with water, and then eluted with 1 *N* ammonium hydroxide. The basic eluates were combined and concentrated to dryness. Ethanol was added and removed several times. The colorless sirup was treated with a few milliliters of ethanol and allowed to remain at room temperature overnight. XV was obtained as colorless needle clusters, 200 mg. (40% from XII), m.p. 229–230° with yellowing at 217°, $[\alpha]^{23}D$ +47° (*c* 0.35, water). Ultraviolet properties follow: in 0.1 *N* hydrochloric acid, maximum at 285 mμ (ϵ_{max} 11,900), minimum at 243.5 mμ (ϵ_{min} 1200); at pH 7.53, maximum at 276 mμ (ϵ_{max} 8300), minimum at 254 mμ (ϵ_{min} 5100), shoulder at 240 mμ (ϵ 6700); in 0.1 *N* sodium hydroxide, maximum at 277.5 mμ (ϵ_{max} 8200), minimum at 254 mμ (ϵ_{min} 4600), shoulder at 240 mμ (ϵ_{sh} 6300).

Anal. Calcd. for $C_{10}H_{16}N_4O_3$: C, 49.98; H, 6.71; N, 23.32. Found: C, 50.43; H, 6.86; N, 22.90.

The methanolic mother liquor from crude XV was examined chromatographically in two solvent systems (ascending method, Whatman No. 1 paper, 1-butanol–water, 86:14, and 1-butanol–ammonium hydroxide (1 *N*), 86:14), each of which showed two ultraviolet absorbing spots along with some fluorescence. The lower spot corresponded to XV. The upper spot (assumed to be the 4-*N*-*n*-butyl derivative, XVI) was excised and showed a cytidine-like spectrum. A crystalline sample of XVI could not be isolated.

Picrate of 4-*N*-*n*-Butyl-2′-deoxycytidine (XVIII) from 2′-Deoxycytidine (XVII).—The hydrochloride salt of 2′-deoxycytidine (XVII, 1.0 g.) and 12 ml. of *n*-butylamine in 60 ml. of methanol was heated in a sealed tube at 105° for 20 hr. The tube was cooled and opened, and the contents were evaporated to dryness. The residue was partitioned between water and chloroform, the organic layer was discarded, and the aqueous layer was concentrated to dryness. The residue was examined by paper chromatography (1-butanol–water, 86:14, ascending system, Whatman No. 1 paper). The major spot (R_f 0.83) corresponded to that for *N*-*n*-butyl-2′-deoxycytidine (see below). A trace spot corresponding to 2′-deoxycytidine (R_f 0.25) was also present. The residue was then dried azeotropically with benzene. The residue was dissolved in ethanol and treated with a saturated solution of picric acid in methanol. Concentration of the solution to a small volume and addition of water yielded the crystalline picrate (1.65 g.) which was filtered and washed with water, cold alcohol, and ether. One recrystallization from 95% ethanol gave pure material, m.p. 165–166° dec., which was not depressed when admixed with an authentic sample of picrate of XVIII.

When 2′-deoxycytidine was treated with *n*-butylamine in a manner similar to that described above, only a faint trace spot of the *N*-*n*-butyl derivative (XVIII) was detected chromatographically.

Treatment of 2′-deoxycytidine with *n*-butylamine in methanol plus 1 equiv. of ammonium acetate in a sealed tube at 105° for 20 hr. yielded a mixture of products which by chromatographic examination showed two spots corresponding to starting material XVII and product XVIII in the proportion of 2:1, respectively.

Picrate of 4-*N*-*n*-Butyl-2′-deoxycytidine (XVIII) from XIX.—XIX[11] (0.8 g.) was refluxed in 40 ml. of methanol containing 6 ml. of *n*-butylamine for 1 day. The solution was concentrated to dryness and fractionated between chloroform and water. The almost colorless aqueous layer was concentrated to dryness and the residue azeotroped with benzene. The residue was dissolved in ethanol and treated with alcoholic picric acid. The picrate salt crystallized (0.4 g.), m.p. 157–159°. After recrystallization from ethanol, needle clusters were obtained, m.p. 165–166° dec.

Anal. Calcd. for $C_{19}H_{24}N_6O_{11}$: C, 44.54; H, 4.72; N, 16.39. Found: C, 44.90; H, 4.69; N, 16.38.

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The Anomeric 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-D-glucopyranoses

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1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α- and -β-D-glucopyranose (I and VII) were prepared by several different procedures. The evidence of nuclear magnetic resonance, as well as route of synthesis, indicate that the isomer having m.p. 218–219° and $[\alpha]D$ +9.1° (chloroform) is the α-D anomer (I), and the isomer having m.p. 167.0–167.5° and $[\alpha]D$ +50° (chloroform) is the β-D anomer (VII), contrary to predictions based on the Hudson rules of rotation. The relative difference between the specific rotations of I and VII increases at shorter wave lengths. The corresponding 2-acetamido analogs (III and IX) of I and VII give plain rotatory dispersion curves in agreement with the Hudson rules at all wave lengths between 300 and 700 mμ.

Conflicting reports exist in the literature for the physical constants of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranose (I) and its β-D anomer (VII). A compound of unspecified anomeric configuration was reported by Kent[1,2] to have m.p. 159–160° and $[\alpha]D$ +73° (chloroform), while Lloyd and associates[3,4] give m.p. 166–167°, $[\alpha]D$ +47.9° (chloroform) for a compound described as I. It has been suggested by Wang and Tai[5] that Lloyd's product[3] is in fact the β-D anomer (VII), and the Chinese workers describe a product, m.p. 214–215°, $[\alpha]D$ +12° (chloroform), which they consider to have the structure I; this assignment would involve a violation of Hudson's empirical rule[6] that the more dextrorotatory isomer of a pair of anomeric D sugar derivatives has the α-D-configuration.

This work describes the preparation of compounds I and VII by various independent routes (Chart I). The homogeneity of the product has been rigorously established with a thin layer chromatographic technique by which the anomers are well differentiated. The structures of the products are defined by the route of synthesis and are supported by nuclear magnetic resonance data. All evidence indicates that 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranose (I) has m.p. 218–219° and $[\alpha]D$ +9.1° (chloroform), and the β-D anomer (VII) has m.p. 167.0–167.5° and $[\alpha]D$ +50° (chloroform). This direct contradiction[5] of Hudson's rule[6] holds true over a range of observed wave lengths.

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(3) P. F. Lloyd and M. Stacey, *Tetrahedron*, **9**, 116 (1960).
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