**ω-Hydroxypyridoxol N-Oxide.**—Oxidation of ω-hydroxypyridoxol with *m*-CPBA in ethanol as described above afforded the title compound, mp 160° dec, in 75% yield.

*Anal.* Calcd for $C_8H_{11}NO_5$: C, 47.76; H, 5.51; N, 6.96. Found: C, 47.80; H, 5.44; N, 7.02.

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**Registry No.**—**1**, 61-67-6; **2**, 42253-78-1; **3**, 42253-79-2; **4**, 42253-80-5; **6**, 42253-81-6; **7a**, 20885-15-8; **7b** hydrochloride, 42253-82-7; **7c**, 29712-70-7; **7c** *N*-oxide, 42253-83-8; **7d** hydrochloride, 42253-84-9; **8**, 42253-85-0; **8** bis(*p*-toluidine) Schiff base, 42253-86-1; **9**, 42253-87-2; **13**, 1136-52-3; **14**, 42253-89-4; **15**, 42253-90-7; pyridoxal hydrochloride, 65-22-5.

# Nucleotide Synthesis. IV.[1] Phosphorylated 3′-Amino-3′-deoxythymidine and 5′-Amino-5′-deoxythymidine and Derivatives[2,3]

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3′-Amino-3′-deoxythymidine 5′-phosphate (**10**) and 5′-amino-5′-deoxythymidine 3′-phosphate (**18**) were prepared. Compounds **10** and **18** are analogs of deoxythymidine 5′- and 3′-phosphates in which the 3′- and 5′-hydroxyl groups are replaced by amino groups. The synthetic sequence leading to **10** and **18** involved the synthesis of 3′-azido-3′-deoxythymidine (**5**) and 5′-azido-5′-deoxythymidine (**15**) by different multistep pathways. Phosphorylation of **5** and **15**, followed by removal of the protecting groups, gave nucleotides **9** and **17** which contained azide groups in the 3′ and 5′ positions, respectively. The azide group was unaffected by these transformations. Catalytic reduction of the azide groups of **9** and **17** gave the title compounds **10** and **18** in good yield. Moreover, **10** and **18** formed crystalline inner salts, **11** and **19**, respectively, which facilitated purification and characterization. In addition, **10** was converted into 3′-chloroacetamido-, 3′-*N*-(*O*-ethylcarbamoyl)-, and 3′-heptafluorobutyramido-3′-deoxythymidine 5′-phosphates (**12**, **13**, and **14**, respectively) and **18** was converted into 5′-acetamido-, 5′-chloroacetamido-, and 5′-*N*-(*O*-ethylcarbamoyl)-5′-deoxythymidine 3′-phosphates (**20**, **21**, and **22**, respectively); these derivatives were candidate active-site-directed inhibitors of a nuclear exoribonuclease isolated from nuclei of mammalian cells.

The presence of 3′-amino-3′-deoxy-β-D-ribofuranose moiety in the antibiotic puromycin[4] has stimulated considerable interest in other amino sugar nucleosides and nucleotides as pharmacological agents. Furthermore, several types of 3′-deoxy or 3′-amino-3′-deoxy nucleoside and nucleotide analogs have been reported to inhibit the synthesis of nucleic acid and, at least in some cases, the inhibition is due to incorporation of a nucleoside which cannot support further chain elongation. Thus, 3′-deoxyadenosine has been shown to inhibit the synthesis of both DNA and RNA in Ehrlich ascites tumor cells.[5,6] Other studies[6,7] have demonstrated *in vitro* inhibition of RNA synthesis by 3′-deoxyadenosine 5′-triphosphate, catalyzed by RNA polymerase. Also described was inhibition due to incorporation of 3′-deoxyadenosine at the 3′ terminus of the growing RNA molecule. The absence of 3′-hydroxyl function in this position prohibits further chain elongation. 3′-Amino-3′-deoxyadenosine[8] and 3′-deoxyguanosine[9] appear to function in a similar manner.

The synthesis of 3′-amino-3′-deoxythymidine 5′-phosphate (**10**), 5′-amino-5′-deoxythymidine 3′-phosphate (**18**), and derivatives containing a haloacetamido inactivating group was undertaken in these laboratories as part of a program devoted to the design of candidate active-site-directed inhibitors of a nuclear exoribonuclease. The exoribonuclease, isolated from the nuclei of mammalian cells, selectively degrades single-stranded, newly synthesized *m*-RNA from the 3′ end, liberating nucleotides with a 5′ phosphate group.[10] The enzyme has affinity for mononucleotides and oligonucleotides, but not for uncharged nucleosides. In addition, the enzyme is present in relatively large amounts in neoplastic tissues, relative to most normal tissues,[11] thus enhancing the importance of the acquisition of selective inhibitors of nuclear exoribonuclease in studying normal and abnormal nucleic acid metabolism. This paper describes the synthesis, purification, and characterization of these novel nucleotides; detailed biochemical results will be described elsewhere.

The key concept underlying the successful preparation of compounds **10** and **18** was the use of stable

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(2) A preliminary account of a portion of this work has appeared: R. P. Glinski, M. S. Khan, R. L. Kalamas, C. L. Stevens, and M. B. Sporn, *Chem. Commun.*, 915 (1970).

(3) This investigation was supported by NIH Contract PH 43-66-929.

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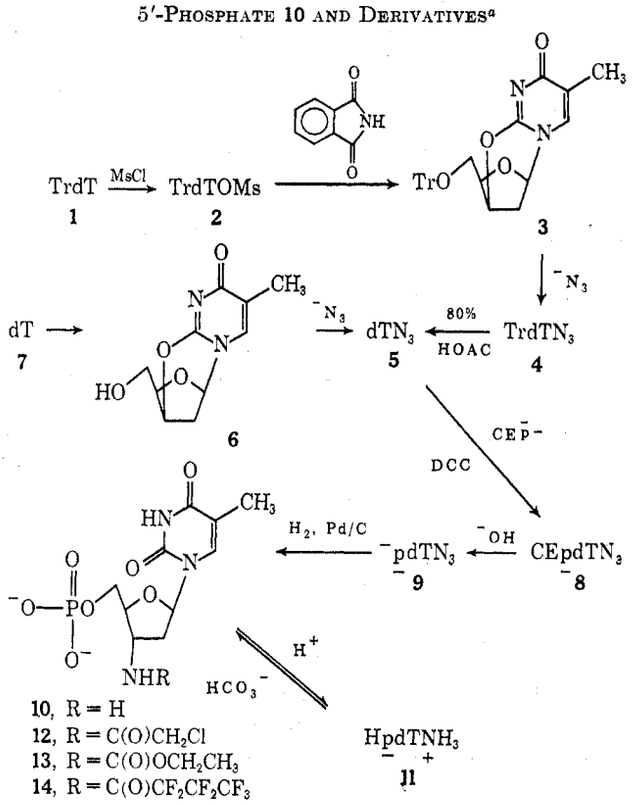
azidodeoxy nucleosides, instead of amino or protected aminodeoxy nucleosides, in the phosphorylation reaction. This approach had been used first in the synthesis of D-ribofuranosylamine 5′-phosphate.[12] Since the appearance of our preliminary communication[2] describing the synthesis of nucleotides **10** and **11**, other groups[13] have used this approach successfully to synthesize derivatives of phosphorylated 2′-amino-2′-deoxyuridine and poly(2′-amino-2′-deoxyuridylic acid). Another recent report[14] describes the synthesis of poly(2′-azido-2′-deoxyuridylic acid). Finally, Letsinger and Mungall[15] have prepared short oligonucleotides containing phosphoramidate linkages derived from 5′-amino-5′-deoxythymidine; this synthesis, however, did not require the use of an azido nucleoside in the phosphorylation reaction. Briefly, in the present case, the azido nucleosides were converted into azido nucleotides, which, in turn were deblocked and reduced catalytically to afford the desired amino nucleotides. The azido group was stable to the phosphorylating conditions employed, and the basic (1 *N* sodium hydroxide at 100°) and the mild acidic conditions used for the removal of the protecting groups.

The key intermediate, 5′-*O*-trityl-2,3′-anhydrothymidine (**3**, Scheme I), in the synthesis of 3′-amino-3′-deoxythymidine 5′-phosphate (**10**) is available from thymidine *via* a three-step reaction sequence.[16] In our hands, however, the literature procedure proved to be unsuitable for large-scale synthesis and was modified. Thus, 5′-*O*-trityl-3′-*O*-mesylthymidine (**2**), prepared from 5′-*O*-tritylthymidine (**1**),[17] was allowed to react with potassium phthalimide in dimethylformamide–water mixtures at 90° to give **3** in 77% yield. Treatment of **3** with sodium azide in a mixture of dimethylformamide–water under reflux for 11 hr gave crude 3′-azido-3′-deoxy-5′-*O*-tritylthymidine (**4**). Crude **4** could be used as such for the preparation of **5**. A sample, however, was obtained in crystalline form for analysis after purification by column chromatography over silica gel. The 5′-*O*-trityl group of **4** was removed with 80% acetic acid at 100° in 1.75 hr. Crystallization from 2-propanol gave pure 3′-azido-3′-deoxythymidine (**5**) in 71% yield. Compound **5** had been prepared earlier by a similar route[18] without isolation or characterization of various intermediates. While this work was in progress, a brief communication appeared[19] reporting the synthesis of 2,3′-anhydrothymidine (**6**) directly from thymidine using 2-chloro-1,1,2-trifluorotriethylamine reagent.[20] The direct synthesis of **6** from thymidine provided a better alternate route for the preparation of **5**. The conversion of **6** into **5** was performed in a similar manner to the conversion of **3** into **5** in yields of 40–60%. Samples of **5** prepared by both routes were identical in all respects.

Phosphorylation of **5** in anhydrous pyridine containing *N*,*N*′-dicyclohexylcarbodiimide using β-cyanoethyl phosphate reagent[21] gave 3′-azido-3′-deoxythymidine 5′-(β-cyanoethyl phosphate) sodium salt (**8**) in essentially quantitative yield. This product was sufficiently pure for further transformations. For characterization, however, a small sample was purified by preparative paper chromatography and fractional precipitation. The β-cyanoethyl group of **8** was removed in 1 *N* NaOH at 100° for 1.5 hr to give 3′-azido-3′-deoxythymidine 5′-phosphate disodium salt (**9**). Crude **9** was purified by large-scale preparative paper chromatography and fractional precipitation from methanol–2-propanol mixtures. Catalytic reduction of the azide group of compound **9** in the presence of 10% Pd/C in water solution gave 3′-amino-3′-deoxythymidine 5′-phosphate disodium salt (**10**). Crude **10** was readily purified by conversion into the crystalline inner salt **11** with trifluoroacetic acid. Treatment of **11** with mild base regenerated pure **10**.

Compound **10**, interesting in itself as an analog of deoxythymidine 5′-phosphate, served as a starting material for the synthesis of various candidate active-site-directed inhibitors of the nuclear exoribonuclease. A number of inactivating groups were incorporated into the 3′ position of **10** for evaluation of biological activity. Thus, **10**, on reaction with chloroacetic anhydride in methanol–water mixtures gave 3′-chloro-

SCHEME I
SYNTHETIC PATHWAY TO 3′-AMINO-3′-DEOXYTHYMIDINE 5′-PHOSPHATE **10** AND DERIVATIVES[a]




10, R = H
12, R = C(O)$CH_2Cl$
13, R = C(O)$OCH_2CH_3$
14, R = C(O)$CF_2CF_2CF_3$

[a] Abbreviated formulas are as follow: dT is thymidine; Tr to the left of dT in TrdT refers to a 5′-*O*-trityl group; OMs to the right of dT as in TrdTOMs refers to 3′-*O*-mesylate substitution; CEp refers to 2-cyanoethyl phosphate.

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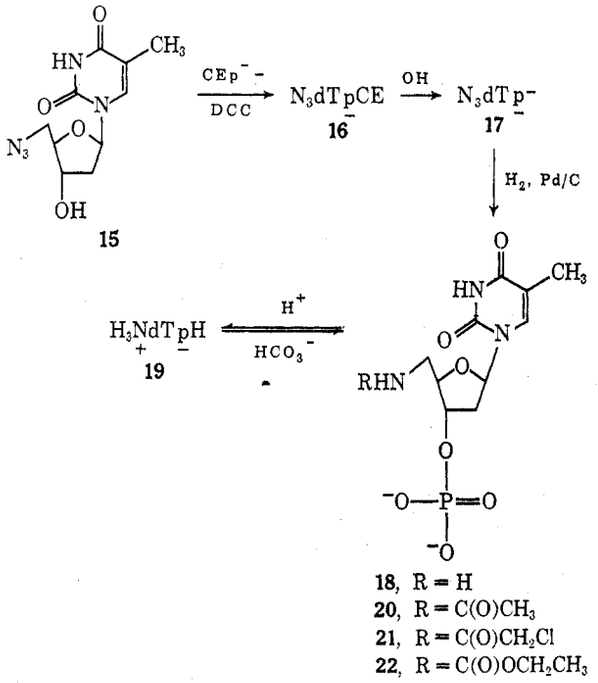
acetamido-3′-deoxythymidine 5′-phosphate disodium salt (12). In a similar manner, 3′-*N*-(*O*-ethylcarbamoyl)-3′-deoxythymidine 5′-phosphate disodium salt (13) was prepared from 10 on reaction with ethyl chloroformate. Finally, reaction of 10 with heptafluorobutyryl–imidazole reagent in pyridine gave 3′-heptafluorobutyramido-3′-deoxythymidine 5′-phosphate (14). For these reactions, isolated 10 (prepared from crystalline inner salt 11) or compound 10 generated *in situ* from 11 was utilized. Compounds 12, 13, and 14 were purified for characterization and analysis by preparative paper chromatography and fractional precipitation.

The experience accumulated during the preparation of derivatives of 10 prompted us to direct our attention to the companion syntheses of the corresponding 5′-substituted derivatives (Scheme II). The starting

SCHEME II

SYNTHETIC PATHWAY TO 5′-AMINO-5′-DEOXYTHYMIDINE 3′-PHOSPHATE (18) AND DERIVATIVES




material for these syntheses, 5′-azido-5′-deoxythymidine (15), available *via* a six-step reaction sequence from thymidine,[22] was phosphorylated, as was described earlier for the 3′-substituted isomer, with β-cyanoethyl phosphate reagent[21] in pyridine in the presence of *N*,*N*′-dicyclohexylcarbodiimide to give 5′-azido-5′-deoxythymidine 3′-(β-cyanoethyl phosphate) pyridinium salt (16). Compound 16 was not purified, but was used as such in the next reaction. Compound 16 was allowed to stand in 1 *N* NaOH at room temperature for 45 min to cleave the β-cyanoethyl ester group to give 5′-azido-5′-deoxythymidine 3′-phosphate (17). Compound 17 was purified for analysis and characterization by preparative paper chromatography and fractional precipitation. Hydrogenation of 17 in water solution in the presence of 10% Pd/C gave 5′-amino-5′-deoxythymidine 3′-phosphate dilithium salt (18). As was the case for the corresponding 3′-amino analog, 18 afforded a crystalline inner salt (19) on treatment with trifluoroacetic acid, facilitating purification and characterization. Compound 19 regenerated 18 on treatment with mild base. Treatment of 18, or 18 generated *in situ* from inner salt 19, with the appropriate reagents in a manner similar to that described earlier for the corresponding 3′ isomers gave 5′-acetamido-5′-deoxythymidine 3′-phosphate (20), 5′-chloroacetamido-5′-deoxythymidine 3′-phosphate (21), and 5′-*N*-(*O*-ethylcarbamoyl)-5′-deoxythymidine 3′-phosphate (22). Compounds 20, 21, and 22 were purified by preparative paper chromatography and fractional precipitation for characterization purposes.

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## Experimental Section

Paper chromatography (Table I) was by the descending technique using the following solvent systems: A, 1-butanol–acetic acid–water (5:2:3, v/v); B, 2-propanol–water–concentrated $NH_4OH$ (7:2:1, v/v); C, 2-propanol–aqueous 1% ammonium sulfate (5:2, v/v); and D, ethanol–aqueous 1% ammonium acetate (5:2, v/v). The nucleotides were detected with ultraviolet light. Analytical pc[23] was performed using Whatman No. 1 paper. Whatman 3 MM paper and solvent system A were used for the large-scale ppc (1–2 g of compound per 20 sheets) as outlined earlier.[24]

TABLE I

PAPER CHROMATOGRAPHY OF NUCLEOTIDE DERIVATIVES

| Compd | $R_f$'s in systems A | B | C | D |
|---|---|---|---|---|
| 8 | 0.49 | 0.47 | 0.54 | 0.70 |
| 9 | 0.42 | 0.24 | 0.38 | 0.53 |
| 10 and 11 | 0.22 | 0.08 | 0.13 | 0.30 |
| 12 | 0.38 | 0.09[a] | 0.37 | 0.50 |
| 13 | 0.41 | 0.21 | 0.34 | 0.53 |
| 14 | 0.71 | 0.61 | 0.65 | 0.73 |
| 17 | 0.41 | 0.16 | 0.36 | 0.54 |
| 18 and 19 | 0.20 | 0.08 | 0.15 | 0.27 |
| 20 | 0.31 | 0.18 | 0.38 | 0.45 |
| 21 | 0.32 | 0.16 | 0.31 | 0.47 |
| 22 | 0.43 | 0.23 | 0.41 | 0.51 |

[a] Streaking in this system due to decomposition.

Thin layer chromatography (tlc) was performed using Eastman chromatogram sheets 6060 (silica gel) impregnated with a fluorescent indicator unless stated otherwise. Other tlc plastic sheets used were Eastman chromatogram sheets 6064 (cellulose) impregnated with a fluorescent indicator. The spots were visualized with uv light and compounds containing a trityl group were detected by spraying with aqueous 5% $HClO_4$ acid and heating at 80° for 5 min. The following tlc systems were used: A, diethyl ether; B, ethyl acetate–EtOH–tetrahydrofuran (1:1:1-v/v); C ethyl acetate; D, $CHCl_3$–1% $NH_4OH$ in $CH_3OH$ (3:2, v/v); E, 2-propanol–water–concentrated $NH_4OH$ (7:2:1, v/v).

Fractions from the fractional precipitations were collected by centrifugation. The residual pellets were washed with anhydrous diethyl ether and dried in a gentle anhydrous $N_2$ stream and *in vacuo* at room temperature.

Uv spectra (distilled water) were recorded using a Hitachi-Coleman 124 spectrophotometer. Ir spectra were recorded using a KBr disk on a Perkin-Elmer Model 237B spectrophotometer. Nmr spectra were recorded using a Varian T-60 spectrometer. Determinations of $pK_a$ and molecular weight were performed according to procedures outlined earlier,[24] unless

(23) The abbreviations used are ppc, pc, and tlc for preparative paper chromatography, paper chromatography, and thin layer chromatography, respectively. The ion exchange resin AGC-244H⁺ (strong acid) was purchased from J. T. Baker Chemical Company, Phillipsburg, N. J.

(24) R. P. Glinski, A. B. Ash, C. L. Stevens, and M. B. Sporn, *J. Org. Chem.* **36**, 245 (1971).

indicated otherwise. Elemental analyses were performed by Midwest Microlab, Inc., Indianapolis, Ind. Pyridine was distilled and stored over Linde molecular sieves (type 4A).

**5′-*O*-Trityl-2,3′-anhydrothymidine (3).**—The starting material, 5′-*O*-trityl-3′-*O*-mesylthymidine (2), was prepared according to the literature procedure (Michelson and Todd, 1955)[17] without modification from thymidine by a two-step reaction sequence. Compound 2 (27 g) was added to a solution of potassium phthalimide (45 g) in dimethylformamide (410 ml) and water (120 ml). The mixture was heated to 95° with stirring over a period of 20 min. The reaction mixture was cooled to room temperature and the course of the reaction was monitored by tlc using tlc system A. Tlc indicated that the reaction was complete. The solution was poured onto an ice–water mixture (4 l.) and the resulting heterogeneous mixture was stirred for 30 min. The product was removed by filtration and washed well with water. The wet precipitate was dissolved in hot 2-propanol (1 l.) and the solution was seeded. After standing at room temperature for 5 days, the resulting crystalline product was removed by filtration and dried to give 4.4 g of 3 with mp 224–227° (softening at 150°). An additional 16.1 g of 3 with mp 229–232° (softening at 150°) was obtained by concentrating the 2-propanol mother liquor to dryness *in vacuo* and stirring the resulting residue under anhydrous diethyl ether overnight. Thus, the total yield was 20.5 g (91.4%). Tlc (tlc system A) indicated the presence of a trace amount of phthalimide. The solid was heated under reflux on a steam bath in 2-propanol for 2 hr. The heterogeneous mixture was allowed to cool. The solid was removed by filtration, yield 12.26 g of 3 with mp 228–233° (no softening at 150°). The mother liquor was concentrated *in vacuo* to *ca.* 200-ml volume. Additional 3 which resulted was removed by filtration, yield 5.68 g of pure 3 with mp 231–233° (no softening at 150°). The previous 12.26-g crop was dissolved in $CHCl_3$ (700 ml) and the solution was filtered, yield 830 mg of impurity with mp <300°. The $CHCl_3$ mother liquor was concentrated *in vacuo* to *ca.* 100-ml volume, diethyl ether was added to the point of turbidity, and seeds of 3 were added, yield 11.46 g of pure 3 with mp 230–233° (no softening at 150°). The total yield of pure 3, therefore, was 17.14 g (77%). The physical constants of 3 were in agreement with the literature values.[16]

**2,3′-Anhydrothymidine (6).**—Thymidine (55 g), dissolved in dimethylformamide (280 ml, which had been purified by passage through acid-washed alumina), was added to 75 g of 2-chloro-1,-1,2-trifluorotriethylamine.[20] The mixture was heated at 70° (oil bath temperature) for 30 min. The reaction mixture was poured into acetone (700 ml) and the resulting crude crystalline product (24 g) was removed by filtration and air dried. The crude product was recrystallized from a mixture of dimethylformamide–acetone to give 20.5 g (40%) of product 6 with mp 242–243°. The physical properties of compound 6 were in agreement with those recorded in the literature.[19]

**3′-Azido-3′-deoxy-5′-*O*-tritylthymidine (4).**—5′-*O*-Trityl-2,-3′-anhydrothymidine (3, 22 g) was dissolved in dimethylformamide (220 ml). Sodium azide (11 g) and water (30 ml) were added with stirring. The resulting homogeneous solution was heated under reflux for 11 hr. The course of the reaction was followed by tlc using tlc system C. The reaction was essentially complete after 11 hr and only a very faint trace of starting material remained. The dimethylformamide was removed *in vacuo* at room temperature. Water (2 l.) was added and the heterogeneous mixture was stirred in room temperature for 3 hr. The solid was removed by filtration and washed well with water. The solid was dried *in vacuo* over $P_2O_5$ for 16 hr to give 23.2 g (97%) of crude 4. Compound 4 was purified by column chromatography using silica gel (600 g). The column was eluted with benzene (3 l.) to remove a minor yellow impurity. A second minor yellow impurity was eluted from the column with 8 l. of 50% chloroform–benzene. Elution of the column with 8 l. of chloroform and 1 l. of 50% chloroform–diethyl ether gave 13.5 g of relatively pure product (71%). Tlc (tlc system C) indicated that the solid was one spot. Tlc (tlc system A) indicated the presence of two minor impurities. A small sample was purified for analysis by crystallization and recrystallization from chloroform–*n*-pentane mixtures and dried at 60° ($5 \times 10^{-3}$ mm) over $P_2O_5$: mp 104–105°; uv max (ethanol) 265 mμ (ε 8800), 250/260 (0.55), 260/270 (1.00), 270/280 (1.76).

*Anal.* Calcd for $C_{29}H_{27}N_5O_4 \cdot 1.25H_2O$: C, 65.46; H, 5.59; N, 13.16. Found: C, 65.48; H, 5.13; N, 12.92.

The analysis was repeated after block drying at the melting point by the analyst.

*Anal.* Calcd for $C_{29}H_{27}N_5O_4$: C, 68.36; H 5.34; N 13.74; O, 12.56. Found: C, 67.70; H 5.35; N 13.53; O 12.34.

**3′-Azido-3′-deoxythymidine (5).** A.—5′-*O*-Trityl-2,3′-anhydrothymidine (3, 146 g) was dissolved in dimethylformamide (1500 ml) containing sodium azide (75 g) and water (225 ml). The mixture was refluxed for 13 hr and was allowed to stand at room temperature for 10 hr after cooling. Tlc (tlc system A) showed only trace amounts of starting material remaining and a major faster migrating spot corresponding to product. The reaction mixture was poured into ice–water (6 l.) with stirring. The resulting precipitate was collected by filtration. The wet solid was dissolved in chloroform (1.5 l.), and the aqueous layer was separated. The chloroform was dried ($Na_2SO_4$) and removed *in vacuo* to afford 220 g of brown syrup which, by tlc, was mostly 3′-azido-3′-deoxy-5′-*O*-tritylthymidine (4). Compound 4 was dissolved in aqueous 80% acetic acid (1500 ml) and the mixture was heated for 1.75 hr on a steam bath with stirring. Tlc (tlc system A) showed that no starting material remained. The mixture (heterogenous owing to the presence of trityl alcohol) was poured into cold water (1.5 l.) and the insoluble trityl alcohol was removed by filtration. The filtrate was concentrated *in vacuo* to *ca.* 750-ml volume. The solution was extracted twice with *n*-pentane (250 ml). The aqueous layer was lyophilized and the residue was dissolved in water (250 ml). The solution was seeded with crystalline 3′-azido-3′-deoxythymidine (5) prepared earlier and the solution was allowed to stand at room temperature for 1 day, followed by 2 days at 1°. The resulting crystalline product (35 g) was removed by filtration. The mother liquor was extracted ten times with ether (200 ml). The ether extracts were combined and concentrated *in vacuo* to a syrup. The syrup was dissolved in a minimum amount of 2-propanol and the solution was seeded. More crystalline product (6 g) resulted. The combined 41 g of crystalline product was recrystallized from a minimum amount of hot 2-propanol to give 25 g (30% for two steps) of product 5 with mp 118–120°. The mother liquor of the 2-propanol recrystallization was concentrated *in vacuo* to a syrup. The syrup was dissolved in water (125 ml) and the solution was seeded to give an additional 2.4 g (3%), mp 115–120°, of 5. A small sample was recrystallized twice from water for analysis: mp 120–122°; uv max 266 mμ (ε 10,200), uv min 234 mμ (ε 2600), 250/260 (0.66), 260/270 (0.96), 270/280 (1.55).

*Anal.* Calcd for $C_{10}H_{13}N_5O_4$: C, 44.91; H, 4.90; N, 26.20. Found: C, 44.92; H, 4.82; N, 26.50.

In several earlier experiments, 5 with mp 105–106° (bubbling and softening at 100°) was obtained by crystallization from dilute, hot diethyl ether solution. A mixture melting point of this material with 5 (mp 120–122°) was 120–122° and the uv spectra were identical. A sample of the lower melting form was dried at 110° ($5 \times 10^{-3}$ mm) for 30 min for analysis, mp 105–106° (no softening at 100°).

*Anal.* Calcd for $C_{10}H_{13}N_5O_4$: C, 44.91; H, 4.90; N, 26.20; O, 23.95. Found: C, 44.83; H, 4.96; N, 25.98; O 23.94.

B.—2,3′-Anhydrothymidine (6, 5 g), lithium azide (2.2 g), and $NH_4Cl$ (300 mg) were heated in dimethylformamide at 123–129° (oil bath temperature) for 17 hr. Tlc (tlc system D) showed no starting material (6) and a major spot corresponding to product 5, contaminated with a minor amount of slower migrating impurities. The reaction mixture was poured into water (300 ml) and the aqueous solution was allowed to cool. Product 5 was extracted from the aqueous solution with ethyl acetate (12 × 150 ml) to give, after removal of the ethyl acetate *in vacuo*, 5.57 g of brown syrup. The syrup was dissolved in a minimum amount of acetone and the acetone solution was applied to a silica gel (60–200 mesh, 100 g, 250 ml) column (40 × 300 mm) which had been preequilibrated in diethyl ether. The column was eluted with diethyl ether and 250-ml fractions were collected. Fractions 3–6 were combined and concentrated *in vacuo* to give 4.55 g of 5 as a semisolid. Crystallization of the semisolid from a mixture of acetone, diethyl ether, and *n*-pentane gave 3.02 g of pure 5 with mp 121–122°. The mother liquor was purified again by column chromatography to give additional 5 (780 mg) as a syrup, which, on crystallization gave 245 mg of 5 with mp 119–121°. The total yield was 3.27 g (55%). Compound 5, prepared from 6, was identical in all respects with 5 prepared from 3.

**3′-Azido-3′-deoxythymidine 5′-(β-Cyanoethyl Phosphate) Sodium Salt (8).**—3′-Azido-3′-deoxythymidine (5, 5 g, 18.7 (mequiv) was dissolved in pyridine (100 ml) and *N*,*N*′-dicyclohexylcarbodiimide (38.5 g, 187 mequiv in 77 ml of pyridine) was added with stirring. β-Cyanoethyl phosphate pyridinium

salt[21] (23.4 mequiv in 140 ml of pyridine) was added dropwise over a period of 1 hr. The course of the reaction was monitored by pc (system A) after 16 hr. Starting material, or an impurity migrating the same as starting material, was evident in addition to product. The reaction mixture was concentrated *in vacuo* to *ca.* 150-ml volume and additional *N,N'*-dicyclohexylcarbodiimide (38.5 g, 187 mequiv in 77 ml of pyridine) was added. Additional β-cyanoethyl phosphate pyridinium salt (17.5 mequiv in 105 ml of pyridine) was added dropwise over a period of 90 min. The solution was allowed to stand at room temperature overnight. Again, a spot migrating the same as starting material was evident by pc in addition to product. The paper strips were sprayed with molybdate reagent to detect the presence of phosphorus. The spot with a $R_f$ corresponding to starting material (**5**) gave a positive test for phosphorus, indicating that this material was not **5**. The solution was diluted with water (200 ml), frozen, and lyophilized. Water (1 l.) was added to the residue and the heterogeneous mixture was stirred for 3 days at room temperature. The precipitate (*N,N'*-dicyclohexylurea) was removed by filtration and washed well with water. Tlc (tlc system C) indicated that the *N,N'*-dicyclohexylurea contained a small amount of product in addition to the spot which had an $R_f$ corresponding to starting material (**5**). The filtrate was concentrated *in vacuo* to a small volume and lyophilized to afford 9.2 g of crude **8**, contaminated with a small amount of *N,N'*-dicyclohexylurea. A sample of **8** (1 g) was purified by ppc using pc system A to give 640 mg of homogeneous **8**. A sample of this material (240 mg) was purified further by fractional precipitation from methanol–ethanol and methanol–ethanol–2-propanol mixtures for analysis. Seven fractions were collected. Fraction 5 (45 mg) was dissolved in a minimum amount of water and the solution was passed through a Dowex 50 ($Na^+$) column (3 ml). The column was eluted with water (20 ml). The eluate was concentrated *in vacuo* and the residue was azeotroped with water. The residue was redissolved in water and lyophilized to give *ca.* 25 mg of a hygroscopic solid: ir (KBr) 4.0 (C≡N), 4.72 μ ($N_3$); uv max 267 mμ (ε 10,200), uv min 234 mμ (ε 2600), 250/260 (0.66), 260/270 (0.96), 270/280 (1.57).

*Anal.* Calcd for $C_{13}H_{16}N_6O_7P\cdot Na\cdot 1.5H_2O$: C, 34.75; H, 4.26; N, 18.71; P, 6.89. Found: C, 35.17; H, 4.09; N, 18.55; P, 6.37.

**3′-Azido-3′-deoxythymidine 5′-Phosphate Disodium Salt (9).**—Crude **8** (1 g) was dissolved in 1 *N* sodium hydroxide (33 ml). The solution was heated in an oil bath with stirring at 100° (bath temperature) for 1.5 hr. Tlc (tlc system D) indicated that the reaction was complete. The reaction mixture was cooled to room temperature and applied to a Dowex 50 ($Li^+$) column (180 ml). The column was eluted with water (300 ml). The eluate was lyophilized to afford 997 mg of compound **9** (dilithium salt). The product was purified by ppc (20 sheets of 3 MM paper) using pc system A. The product was eluted off the paper with 10% acetic acid (2 l.). The last traces of acetic acid were removed by repetitive lyophilization. The extremely hygroscopic residue was dissolved in a minimum amount of water, the solution was applied to a Dowex 50 ($H^+$) column (30 ml), and the column was eluted with water (200 ml). The eluate was lyophilized to give 337 mg (45%) of **9** (free acid) as a nonhygroscopic solid, uv max 267 mμ (ε 9200 as a dihydrate), uv min 234 mμ (ε 2470). Free acid **9** was converted into disodium salt by neutralization (pH 7–7.5) of an aqueous solution of **8** with 1 *N* NaOH: yield 300 mg (81%) after lyophilization; uv max 267 mμ (ε 9800 as a dihydrate), uv min 234 mμ (ε 2600). Compound **9** was purified by fractional precipitation from methanol–2-propanol solutions. Three fractions were collected. Fraction 1 was insoluble in methanol while fractions 2 and 3 were insoluble in methanol–2-propanol mixtures and appeared to be crystalline. A sample of fractions 2 and 3 (187 mg after azeotroping with water and lyophilization) was sent for analysis: uv max 267 mμ (ε 10,200 as a dihydrate), uv min 234 mμ (ε 2600), 250/260 (0.66), 260/270 (0.98), 270/280 (1.59).

*Anal.* Calcd for $C_{10}H_{12}N_5O_7P\cdot 2Na\cdot 2H_2O$: C, 28.11; H, 3.78; N, 16.39; P, 7.25. Found: C, 28.34; H, 3.87; N, 16.33; P, 7.02.

**3′-Amino-3′-deoxythymidine 5′-Phosphate Disodium Salt (10).**—3′-Azido-3′-deoxythymidine 5′-phosphate (**9**, 100 mg, analytically pure) was hydrogenated in the presence of 10% Pd/C (30 mg) in water solution (1 ml) for 3 hr at room temperature. Processing of the reaction mixture in the usual manner afforded a hygroscopic white solid. Attempts to purify the solid by fractional precipitation failed because of the formation of oils. A glass resulted on combination and evaporation of the various solutions from the fractional precipitation. The glass was dissolved in a minimum amount of water, and 2-propanol was added to the point of turbidity. The mixture was centrifuged and a small amount of oil deposited. The supernatant was concentrated *in vacuo* to afford a gum. The gum was dissolved in water and the solution was lyophilized to afford a hygroscopic solid: uv max 267 mμ (ε 9400) uv min 234 mμ (ε 2500), 250/260 (0.68), 260/270 (1.00), 270/280 (1.65).

*Anal.* Calcd for $C_{10}N_{14}N_3O_7PNa_2\cdot 3H_2O$: C, 28.65; H, 4.80; N, 10.02; P, 7.39. Found: C, 29.13; H, 4.79; N, 9.63; P, 7.13.

*Anal.* Calcd for $C_{10}H_{14}N_3O_7PNa_2\cdot 1H_2O$: C, 31.34; H, 4.21. Found (after block drying by the analyst at 160°): C, 31.48; H, 4.13.

**3′-Amino-3′-deoxythymidine 5′-Phosphate Inner Salt (11).**—3′-Azido-3′-deoxythymidine 5′-phosphate disodium salt dihydrate (**9**, 1.05 g, ε 8000 as a dihydrate, purified by ppc only) was dissolved in water (15 ml) containing 10% Pd/C (300 mg) and the mixture was stirred under hydrogen at atmospheric pressure for *ca.* 4 hr. Tlc (cellulose sheets, pc system B) showed the absence of starting material (**9**) and one spot corresponding to 3′-amino-3′-deoxythymidine 5′-phosphate disodium salt (**10**). The catalyst was removed by filtration using a Celite bed and was washed thoroughly with water. The combined filtrate and washings were lyophilized to afford 880 mg (86%) of extremely hygroscopic product **10**. Compound **10** was dissolved in water (3 ml) and trifluoroacetic acid (0.47 ml) was added. 2-Propanol was added to the point of turbidity and the solution was seeded with crystalline inner salt **11**. After standing at room temperature for several hours and at 1° for 16 hr, the crystalline material which resulted was removed by filtration and air dried to afford 400 mg of **11** with mp 219–220° dec. A sample was dried at room temperature ($5 \times 10^{-3}$ mm) and 110° ($5 \times 10^{-3}$ mm) for 16 and 3 hr, respectively, for analysis: uv max 265 mμ (ε 10,800, calcd per mol wt 359.3 based on first analysis), uv min 233 mμ (ε 2700), 250/260 (0.82), 260/270 (1.00), 270/280 (1.69).

*Anal.* Calcd for $C_{10}H_{16}N_3O_7P\cdot H_2O\cdot 0.33\ (CH_3)_2CHOH$: C, 36.77; H, 5.83; N, 11.70; P, 8.62. Found: C, 36.51; H, 5.68; N, 11.84; P, 8.56.

*Anal.* Calcd for $C_{10}H_{16}N_3O_7P$: C, 37.39; H, 5.02; N, 13.08; P 9.64. Found (after block drying by the analyst at 170°): C, 37.14; H, 5.47; N, 12.86; P, 9.10.

**3′-Chloroacetamido-3′-deoxythymidine 5′-Phosphate Disodium Salt (12).**—3′-Amino-3′-deoxythymidine 5′-phosphate disodium salt (**10**, 200 mg) was dissolved in water (1 ml), and methanol (2 ml) was added. The mixture was stirred at 0° and chloroacetic anhydride (100 mg) was added portionwise as a solid. After stirring at 0° for 10 min, all of the chloroacetic anhydride dissolved. The course of the reaction was monitored by tlc (pc system A, cellulose tlc plates); tlc showed that the reaction was essentially complete. The reaction mixture was diluted with water and streaked directly on six sheets of 3 MM paper and **12** was purified by ppc (pc system A). Processing in the usual manner yielded 173 mg of homogeneous **12**. The product was combined with an earlier preparation of **12** (total wt 269 mg) and the solid was dissolved in a minimum amount of water. The solution was applied to a Dowex 50 ($Na^+$) column (15 ml). The column was eluted with water and the effluent was lyophilized to afford 174 mg of compound **12**. An analytically pure sample was obtained by fractional precipitation from water–methanol–2-propanol mixtures. Four fractions were collected. The fourth fraction, a gum, was solidified by trituration under 2-propanol. The solid was removed by centrifugation and was washed with diethyl ether to give 90 mg of **12**. The solid was dissolved in water (5 ml) and the solution was concentrated *in vacuo* three times. Finally, the residue was dissolved in water and the solution was lyophilized to afford 80 mg of analytically pure **12**: uv max 267 mμ (ε 9700), uv min 237 mμ (ε 2500), 250/260 (0.65), 260/270 (0.95), 270/280 (1.51).

*Anal.* Calcd for $C_{12}H_{15}ClN_3O_8PNa_2\cdot 2.5H_2O$: C, 29.61; H, 4.14; Cl, 7.28; N, 8.63; P, 6.36. Found: C, 29.90; H, 4.16; Cl, 6.95; N, 8.91; P, 6.35.

**3′-*N*-(*O*-Ethylcarbamoyl)-3′-deoxythymidine 5′-Phosphate Disodium Salt (13).**—3′-Amino-3′-deoxythymidine 5′-phosphate inner salt (**11**, 300 mg) was dissolved in a mixture of methanol (2 ml) and water (2 ml) containing $Na_2CO_3$ (290 mg). The mixture was stirred at 0° and ethyl chloroformate (130 mg, 1.2 mmol) was added dropwise. The reaction mixture was stirred at 0° for 10 min and the course of the reaction was monitored by tlc (pc

system A, cellulose tlc sheets). Tlc indicated that the reaction was complete. The solution was neutralized (pH 7) with 0.1 *N* HCl and was streaked directly onto five sheets of 3 MM paper for purification by ppc (pc system A): yield, 299 mg; uv max 267 mμ (ε 8200), as a dihydrate), uv min 234 mμ (ε 1400), 250/260 (0.66), 260/270 (0.95), 270/280 (1.51). The solid was purified by fractional precipitation for analysis using water–methanol–ethanol–2-propanol mixtures. The fractions were collected on the addition of 2-propanol. Fraction 4 (120 mg) was dissolved in water and the solution was concentrated *in vacuo*. This procedure was repeated five times using 5-ml portions of water. The residue was dissolved in a small amount of water and lyophilized to afford 100 mg of analytically pure **13**: uv max 267 mμ (ε 9900), uv min 234 mμ (ε 2600), 250/260 (0.66), 260/270 (0.94), 270/280 (1.49); nmr ($D_2O$) δ 1.14 [t, 3, $J$ = 3.5 Hz, C-(O)$OCH_2CH_3$], 1.84 (s, 3, C-3 $CH_3$), 2.33 (t, 2, $J$ = 3.0 Hz, C-2′ $H_2$), 3.95 [m, 6, C(O)$OCH_2CH_3$, C-3′ H, C-4′ H, C-5′ $H_2$], *ca.* 4.75 (broad s, 1, OH), 6.18 (t, 1, $J$ = 3.0 Hz, C-1′ H), 7.67 (s, 1, C-2 H).

*Anal.* Calcd for $C_{13}H_{18}N_3O_9PNa_2 \cdot 3.5H_2O$: C, 31.21; H, 5.04; N, 8.40; P, 6.19. Found: C, 31.53; H, 5.38; N, 8.46; P, 5.92.

*Anal.* Calcd for $C_{13}H_{13}N_3O_9PNa_2$: C, 35.71; H, 4.15; N, 9.61; P, 7.08. Found (after block drying by the analyst at 150°): C, 36.11; H, 4.60; N, 8.99; P, 7.04; weight loss, 10.71%.

**3′-Heptafluorobutyramido-3′-deoxythymidine 5′-Phosphate (14).**—3′-Amino-3′-deoxythymidine 5′-phosphate inner salt (**11**, 300 mg) was suspended in anhydrous pyridine (3 ml), and heptafluorobutyrylimidazole (1.18 g) was added in portions [210 (177 μl), 210 (177 μl), 450 (400 μl), and 340 mg (300 μl)] over a period of several hours with magnetic stirring. The solution was still heterogeneous at the end of this time. The course of the reaction was monitored after each addition by tlc. After the last addition, tlc (cellulose tlc sheets, pc system D) indicated that the reaction was essentially complete. Water (20 ml) was added and the solution was lyophilized to yield a semisolid. The semisolid was dissolved in water. The water solution was applied to an AGC-244 ($H^+$)[23] column (*ca.* 10 ml, *ca.* 40 mequiv) and the column was eluted with water (*ca.* 200 ml) until the effluent no longer absorbed uv light at 254 mμ. The effluent was lyophilized to give 710 mg of crude **14** (free acid) as a solid. The solid was dissolved in water (2 ml), and methanol (1 ml) was added. The solution was clarified by centrifugation. The supernatant was lyophilized to afford a solid. The solid was purified by ppc in pc system A on 12 sheets to give 550 mg of homogeneous **14**. The product was dissolved in water and the water solution was passed through a Dowex 50 ($H^+$) column (10 ml), eluting with water. The effluent was lyophilized. The resulting solid (*ca.* 400 mg) was dissolved in minimum water and the solution was clarified by centrifugation. The pellet was discarded and the supernatant was diluted with methanol (2 ml). The solution was clarified by centrifugation and the pellet was discarded. The supernatant was diluted with water and the solution was lyophilized to afford 290 mg (62%) of pure **14**: uv max 267 mμ (ε 9500), uv min 234 mμ (ε 3100), 250/270 (0.69), 260/270 (0.97), 270/280 (1.57).

*Anal.* Calcd for $C_{14}H_{15}F_7N_3O_8P \cdot 2H_2O$: C, 30.39; H, 3.46; N, 7.59; P, 5.60. Found: C, 30.36; H, 2.98; N, 7.02; P, 5.26.

**5′-Azido-5′-deoxythymidine 3′-Phosphate Dilithium Salt (17).**—*N*,*N*′-Dicyclohexylcarbodiimide (6.3 g, 24 mmol) in 42 ml of pyridine and β-cyanoethyl phosphate[21] (1.30 g, 8 mmol) in 35 ml of pyridine were added to a solution of 5′-azido-5′-deoxythymidine (**15**, 1.056 g, 5 mmol) in anhydrous pyridine (10 ml). The solution was stirred at room temperature for 2 days. Tlc (tlc solvent system D) indicated that the reaction was complete. The product (**16**) migrated slower than starting material (**15**). Water (3 ml) was added and the mixture was allowed to stand at room temperature for 1 hr. Additional water (100 ml) was added. The resulting precipitate was removed by filtration and washed well with water. The washings and filtrate were combined and lyophilized to afford a solid. The solid was dissolved in water (50 ml) and the solution was clarified by filtration. The filtrate was lyophilized to give 1.8 g of intermediate **16** as an amorphous solid. The solid was dissolved in 1 *N* NaOH (50 ml) and the solution was allowed to stand at room temperature for 45 min. Tlc indicated that intermediate **16** had been converted into the product (**17**). The reaction mixture was applied to a Dowex 50 ($H^+$) column (175 ml) and the column was eluted with water until the eluate was neutral. The pH of the eluate was adjusted to 7.5 by the dropwise addition of 1 *N* LiOH solution. The solution was lyophilized to give 2.1 g of crude **17**. The ir (KBr) of crude **17** showed a strong azide absorption at 4.8 μ. Pc indicated that the product was relatively pure. Compound **17** was purified by ppc using 20 3 MM sheets and pc system D to give 720 mg of homogeneous **17**. The product was purified further by fractional precipitation from methanol–water mixtures. Ten fractions were collected. Fraction 9 was dissolved in water (4 ml) and lyophilized to give 100 mg. A portion of fraction 9 was dried at room temperature for 16 hr, and at 110° for 1 hr for analysis: uv max 266 mμ (ε 10,200), uv min 234 mμ (ε 2,600), 250/260 (0.67), 260/270 (0.96), 270/280 (1.55).

*Anal.* Calcd for $C_{10}H_{12}N_5O_7PLi_2 \cdot 2H_2O$: C, 30.40; H, 4.08; N, 17.72; P, 7.85. Found: C, 30.35; H, 4.00; N, 17.68; P, 8.01.

**5′-Amino-5′-deoxythymidine 3′-Phosphate Dilithium Salt (18).**—Compound **17** (200 mg), dissolved in water (15 ml), was stirred magnetically under hydrogen at atmospheric pressure at room temperature in the presence of 10% Pd/C (100 mg) for 2.5 hr. Tlc (tlc system E) indicated that the reduction was complete. The product (**18**) migrated slower than starting material (**17**). The catalyst was removed by filtration using a Celite bed and was washed thoroughly with water. The filtrate and washings were combined and lyophilized to yield 170 mg of **18**. The ir spectra (KBr) of the product showed the absence of an azide band at 4.8 μ which had been present in the starting material (**17**). The product was purified by fractional precipitation from water–ethanol mixtures. Five fractions were collected. Fraction 4 was dissolved in water (3 ml) and the solution was lyophilized to yield 50 mg of pure **18**. A sample was dried at room temperature ($5 \times 10^{-3}$ mm) for 12 hr for analysis: uv max 266 mμ (ε 9600), uv min 234 mμ (ε 2600) 250/260 (0.69), 260/270 (1.03), 270/280 (1.68).

*Anal.* Calcd for $C_{10}H_{14}N_3O_7PLi_2 \cdot 2H_2O$: C, 32.54; H, 4.92; N, 11.38; P, 8.38. Found: C, 32.32; H, 5.16; N, 11.14; P, 8.10.

**5′-Amino-5′-deoxythymidine 3′-Phosphate Inner Salt (19).**—5′-Amino-5′-deoxythymidine 3′-phosphate dilithium salt (**18**, 100 mg) was dissolved in water (3 ml), and trifluoroacetic acid (89 mg) was added with stirring. When the addition was complete, 2-propanol was added dropwise to the point of turbidity. A small amount of water was added, dropwise, to obtain a clear solution. The solution was allowed to stand at room temperature for 24 hr. The resulting crystalline product (**19**, 80 mg) was removed by filtration and air dried. Two recrystallizations from water–2-propanol mixtures afforded 70 mg of **19** with mp 213–215° dec. A sample was dried at room temperature ($5 \times 10^{-3}$ mm) for 14 hr for analysis: uv max 265 mμ (ε 10,300), uv min 234 mμ (ε 2600), 250/260 (0.68), 260/270 (1.02), 270/280 (1.75).

*Anal.* Calcd for $C_{10}H_{16}N_3O_7P \cdot 2H_2O$: C, 33.62; H, 5.64; N, 11.76; P, 8.67. Found: C, 33.65; H, 5.49; N, 11.89; P, 8.69.

**5′-Acetamido-5′-deoxythymidine 3′-Phosphate Dilithium Salt (20).**—5′-Amino-5′-deoxythymidine 3′-phosphate dilithium salt (**18**, 444 mg) was dissolved in a mixture of methanol (5 ml) and water (5 ml) and the solution was cooled to 0°. Acetic anhydride (140 mg) was added dropwise, with magnetic stirring, over a period of 10 min. After the addition was complete, the reaction mixture was stirred for an additional 20 min at 0°. The course of the reaction was followed by tlc (cellulose tlc sheets, pc system A). The reaction mixture migrated as a single spot ($R_f$ 0.65), different from starting material ($R_f$ 0.42). The solution was concentrated *in vacuo* to a small volume. Water (5 ml) was added and the solution was lyophilized to give 520 mg of crude **20**. Crude **20** was purified by ppc (11 sheets of 3 MM paper, pc system A) to afford 360 mg of homogeneous solid. The solid was dissolved in water (2 ml) and the solution was applied to a Dowex 50 ($H^+$) column (10 ml). The column was eluted with water. The course of the elution was monitored by uv at 254 mμ. The pH of the eluate was adjusted to 7.5 (pH paper) by the dropwise addition of LiOH solution. The effluent was lyophilized to yield 310 mg of **20** as a dilithium salt. Compound **20** was purified further by fractional precipitation from water–ethanol–2-propanol mixtures. Five fractions were collected. Fraction 4 was dissolved in water (15 ml) and the solution was concentrated *in vacuo* (repeated three times). The residue was dissolved in water again and the solution was lyophilized to afford 130 mg of pure **20**: uv max 266 mμ (ε 10,100), uv min 234 mμ (ε 2500), 250/260 (0.67), 260/270 (0.98), 270/280 (1.53).

*Anal.* Calcd for $C_{12}H_{16}N_3O_8PLi_2 \cdot 2H_2O$: C, 35.05; H, 4.90; N, 10.22; P, 7.53. Found: C, 35.11; H, 4.87; N, 10.15; P, 7.37.

**5'-Chloroacetamido-5'-deoxythymidine 3'-Phosphate Disodium Salt (21).**—5'-Amino-5'-deoxythymidine 3'-phosphate inner salt (**19**, 385 mg) and $Na_2CO_3$ (297 mg) were dissolved in a mixture of methanol (5 ml) and water (5 ml) and the solution was stirred at 0°. Chloroacetic anhydride (342 mg) was added. As the reaction progressed, the chloroacetic anhydride dissolved in the reaction media. After stirring at 0° for 20 min, tlc (cellulose sheets, pc system A) indicated that the reaction was complete (product $R_f$ 0.47, starting material $R_f$ 0.26). The solution was concentrated *in vacuo* to a small volume. Water was added and the solution was lyophilized to give 920 mg of crude **21**. Compound **21** was purified by ppc (18 sheets of 3 MM paper, pc system A) to afford 580 mg of homogeneous solid with a low $\epsilon$ value: uv max 267 m$\mu$ ($\epsilon$ 5600) as a dihydrate, uv min 234 m$\mu$ ($\epsilon$ 2300). The solid was extracted with three portions of 2-propanol (10 ml). The 2-propanol extracts were discarded. The residue (560 mg, $\epsilon$ 5800) was extracted with three portions of ethanol (10 ml). The ethanol extract had no significant uv absorption and was discarded. The residue (300 mg, $\epsilon$ 8100) was purified for analysis by fractional precipitation from water–ethanol mixtures. Five fractions were collected. All five fractions were contaminated with a slower migrating impurity by tlc (cellulose tlc sheets, pc system A). The supernatants from the five fractions were combined and concentrated *in vacuo*. The residue (100 mg, $\epsilon$ 9200) was homogeneous by tlc. The solid was fractionally precipitated from water–2-propanol mixtures. Two fractions were collected. Fraction 2 (55 mg) was dissolved in water (10 ml) and the solution was concentrated *in vacuo* (repeated four times). The residue was dissolved in water again and the solution was lyophilized to give 50 mg of pure **21**: uv max 267 m$\mu$ ($\epsilon$ 9700), uv min 234 m$\mu$ ($\epsilon$ 2500), 250/260 (0.66), 260/270 (0.97), 270/280 (1.53).

*Anal.* Calcd for $C_{12}H_{15}ClN_3O_8PNa_2 \cdot 2H_2O$: C, 30.17; H, 4.00; N, 8.79; P, 6.48; Cl, 7.42. Found: C, 30.18; H, 3.83; N, 8.44; P, 6.45; Cl, 6.78.

**5'-*N*-(*O*-Ethylcarbamoyl)-5'-deoxythymidine 3'-Phosphate Disodium Salt (22).**—5'-Amino-5'-deoxythymidine 3'-phosphate inner salt (**19**, 300 mg) and $Na_2CO_3$ (310 mg) were dissolved in a mixture of methanol (4 ml) and water (4 ml) and the solution was stirred at 0°. Ethyl chloroformate (135 mg) was added dropwise over a period of 5 min. The reaction mixture was stirred for an additional 10 min at 0°. The course of the reaction was monitored by tlc (cellulose tlc sheets, pc system A) and showed one spot ($R_f$ 0.70), different from starting material ($R_f$ 0.47). The reaction mixture was neutralized with 0.3 *N* HCl solution and concentrated *in vacuo* to a small volume. Water was added and the solution was lyophilized to give 500 mg of crude **22**. The product was purified by ppc (pc system A, 12 sheets of 3 MM paper) to afford 250 mg of homogeneous **22**. Compound **22** was purified further by fractional precipitation from water–2-propanol mixtures. Five fractions were collected. Fraction 2 was dissolved in water (10 ml) and the solution was concentrated *in vacuo* (repeated three times). The residue was dissolved in water again and lyophilized to give 40 mg of pure **22**: uv max 267 m$\mu$ ($\epsilon$ 9700), uv min 234 m$\mu$ ($\epsilon$ 2500), 250/260 (0.67/270 (0.98), 270/280 (1.62).

*Anal.* Calcd for $C_{13}H_{18}N_3O_9PNa_2 \cdot 2H_2O$: C, 32.98; H, 4.69; N, 8.88; P, 6.55. Found: C, 33.27; H, 4.91; N, 9.04; P, 6.33.

**Registry No.**—**2**, 42214-24-4; **3**, 25442-42-6; **4**, 29706-84-1; **5**, 30516-87-1; **6**, 15981-92-7; **8**, 30516-88-2; **9**, 29706-87-4; **10**, 29706-88-5; **11**, 42214-32-4; **12**, 42214-33-5; **13**, 42214-34-6; **14**, 42214-35-7; **15**, 19316-85-9; **16**, 29912-68-3; **17**, 42214-38-0; **18**, 29706-89-6; **19**, 42319-49-3; **20**, 42214-40-4; **21**, 42214-41-5; **22**, 42214-42-6.

# General Methods of Synthesis of Indole Alkaloids. XII. Syntheses of *dl*-18,19-Dihydroantirhine and Methyl Demethylilludinate[1,2]

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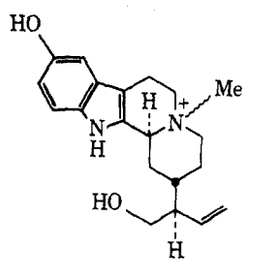
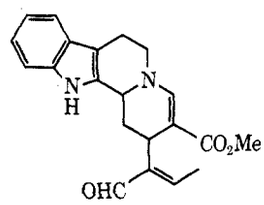
Methyl 4-carbomethoxymethylnicotinate was prepared from 4-methylnicotinic acid and condensed with acetaldehyde and with 3,3-dimethylcyclopentanone. Esterification, reduction, and oxidation of the first condensation product led to 4-(1-hydroxy-2-butyl)nicotinic lactone and thence in three steps to a derivative of antirhine, while esterification and cyclization of the second condensation product afforded an illudinine derivative.

The two-reaction sequence of partial hydrogenation of 1-alkyl-3-acylpyridinium salts and cyclization of the resultant 2-piperideines has constituted the backbone of alkaloid synthesis of a large variety of structure types.[3] This scheme of synthesis now has been exploited for the construction of a base structurally representative of the hunterburnine $\alpha$- and $\beta$-metho salts (**1**),[4] vallesiachotamine (**2**),[5] and antirhine (**3**),[6] while intermediates on route to this base have been utilized for the synthesis of a derivative of illudinine (**4**).[7]




1

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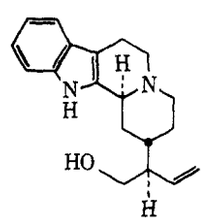


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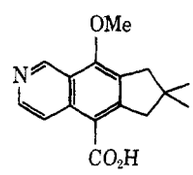


4

(1) Dedicated to Professor Edgar Lederer on the occasion of his 65th birthday.

(2) (a) This investigation was supported by the U. S. Public Health Service. (b) Part XI: E. Wenkert and G. D. Reynolds, *Syn. Commun.*, **3**, 241 (1973).

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