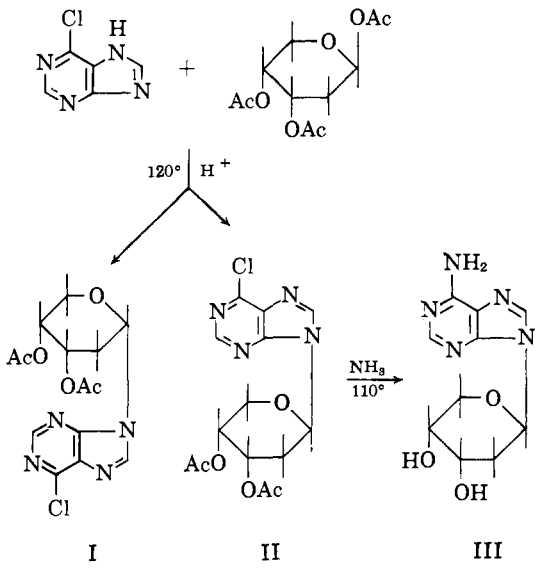
furanosyl)purine, m.p. 155–156°, which shows $[\alpha]^{25}D$ +61.1° (H_2O), $\lambda_{max}^{H_2O}$ 263.5 mμ (ε 10,000), infrared bands 9.22 and 12.25 μ (lit.[13] m.p. 150–152°, $[\alpha]^{25}D$ +60.0° (H_2O), $\lambda_{max}^{H_2O}$ 264 mμ (ε 8869)). The concentrated filtrate gave 0.6 g. of 6-chloro-9-(2′-deoxy-β-D-ribofuranosyl)purine which was recrystallized from a small volume of ethyl acetate to yield 0.45 g. of colorless needles,[12] m.p. 144–145°, $[\alpha]^{25}D$ −10.8° (methanol), infrared bands at 8.77 and 10.75 μ, $\lambda_{max}^{H_2O}$ 264 mμ (ε 10,000) (lit.[13] m.p. 142–145°, $[\alpha]^{26}D$ −11.0° (methanol), $\lambda_{max}^{H_2O}$ 264 mμ (ε 8930)). These α- and β-anomers were readily distinguished on the basis of characteristic infrared bands.[13]

Similar acid-catalyzed fusion of purine[14] (2.77 g.) and 1,3,5-tri-*O*-acetyl-2-deoxy-D-ribofuranose[10] (12 g.) at 135–145° (30 min.) followed by deacetylation with methanolic ammonia gave a sirup which deposited crystals of crude nucleoside (1.7 g., mostly β-anomer), m.p. 171–174°, from methanol. The methanolic filtrate gave an additional 1.1 g. of crystalline nucleoside, m.p. 120–130° (largely α-anomer). The identification of these products as the α- and β-anomers of 9-(2′-deoxy-D-ribofuranosyl)purine was readily made in each case after an additional recrystallization by comparison of data recorded for these compounds prepared by another route.[13]

This general synthetic procedure has been found to be applicable equally well to the preparation of purine



I II III

2′-deoxy-D-ribopyranosides. This represents the first example of the preparation of a purine pyranosyl nucleoside by the fusion procedure.

6-Chloropurine (1.54 g.) and 1,3,4-tri-*O*-acetyl-2-deoxy-β-D-ribopyranose[15,16] (2.60 g.) were thoroughly mixed and heated at 115–120° (oil bath) until a light yellow melt was obtained. Then *p*-toluenesulfonic acid (20 mg.) was added and the contents were heated at 120° *in vacuo* for 15 min. The residue was dissolved in 125 ml. of warm ethyl acetate and the solution was filtered to remove unreacted 6-chloropurine (0.20 g.). The ethyl acetate solution, after washing, was finally concentrated to a tan sirup. This sirup was dissolved in 50 ml. of absolute ethanol which, upon cooling, deposited 1.28 g. of a crystalline anomeric mixture of 6-chloro-9-(3′,4′-di-*O*-acetyl-2′-deoxy-α- and -β-D-ribopyranosyl)purines,[12] m.p. 178–184°. The ultraviolet absorption, $\lambda_{max}^{methanol}$ 263.5 mμ (ε 9900) is indicative of 9-substitution.[17,18] Separation of anomers was accomplished by fractional crystallization from absolute ethanol to give 0.88 g. of the more ethanol-insoluble isomer[12] (I), m.p. 206–207°, $\lambda_{max}^{pH\ 1}$ 263 mμ (ε 8890), $\lambda_{max}^{pH\ 11}$ 264 mμ (ε 10700), $[\alpha]^{26}D$ +22.4° (*c* 0.75, acetone). The ethanolic filtrates were combined and evaporated to dryness. The solid residue was crystallized several times from methanol to yield 0.18 g. of white needles[12] (II), m.p. 149–150°, $[\alpha]^{26}D$ −28.3° (*c* 1.0, ethyl acetate).

The assignment of I as 6-chloro-9-(3′,4′-di-*O*-acetyl-2′-deoxy-α-D-ribopyranosyl)purine and II as 6-chloro-9-(3′,4′-di-*O*-acetyl-2′-deoxy-β-D-ribopyranosyl)purine is tentative as far as the anomeric configuration is concerned. Proton magnetic resonance spectra of I and II in $CDCl_3$ show clearly two acetylmethyl groups at δ 1.95 and 2.1, respectively. The presence of two protons at C-2 is indicated by the fact that the C-1 proton is split into two doublets in the δ 5.7–5.9 region. Treatment of 6-chloro-9-(3′,4′-di-*O*-acetyl-2′-deoxy-β-D-ribopyranosyl)purine (II, 400 mg.) with methanolic ammonia at 110° gave 210 mg. of crystalline 6-amino-9-(2′-deoxy-β-D-ribopyranosyl)purine (III). Recrystallization from methanol and water gave 120 mg. of pure[12] III, m.p. 266–267°, $\lambda_{max}^{pH\ 1}$ 256 mμ (ε 17100), $\lambda_{max}^{pH\ 13}$ 258.5 mμ (ε 17100), $[\alpha]^{26}D$ −17.0° (*c* 0.6, water), R_f 0.26, R_{ad} 0.56 (*n*-butyl alcohol–water 86:14). Compound III was shown to be identical with a product assigned the structure 9-(2′-deoxy-β-D-ribopyranosyl)-adenine recently prepared[19] by the mercury salt procedure (lit.[19] m.p. 262–264°, $\lambda_{max}^{pH\ 1}$ 256 mμ (ε 16,600), $\lambda_{max}^{pH\ 13}$ 259.5 mμ (ε 16,400), $[\alpha]^{20}D$ −17.8° (*c* 0.58, water), R_f 0.27, R_{ad} 0.60 (*n*-butyl alcohol–water 86:14). Acidic hydrolysis of III revealed the presence of adenine and 2-deoxy-D-ribose which were identified by paper chromatography in several solvent systems.

The direct attachment of the 2-deoxy-D-ribopyranosyl and 2-deoxy-D-ribofuranosyl functions to various purines and other related heterocycles by this simple procedure is presently under investigation in our laboratory.

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(19) H. Zinner and E. Wittenburg, *Chem. Ber.*, **95**, 1866 (1962).

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The Direct Utilization of Glycals for the Preparation of Purine Deoxynucleosides[1]

Sir:

We wish to report the first recorded synthesis of a heterocyclic nucleoside by direct utilization of a glycal in an acid-catalyzed fusion reaction. This simple procedure avoids the necessity of synthesis of the usual 2-deoxy halosugar (often prepared from the glycal) and would appear to compete favorably with other known synthetic methods. The suggestion for the use of glycals in a direct alkylation of the purine ring was first made by Robins, *et al.*,[2] in a model study with

(1) Supported by research grants CY-4008(C4) and CA 04008-06 from the National Cancer Institute of the National Institutes of Health, Public Health Service.
(2) R. K. Robins, E. F. Godefroi, E. C. Taylor, L. R. Lewis, and A. Jackson, *J. Am. Chem. Soc.*, **83**, 2574 (1961).

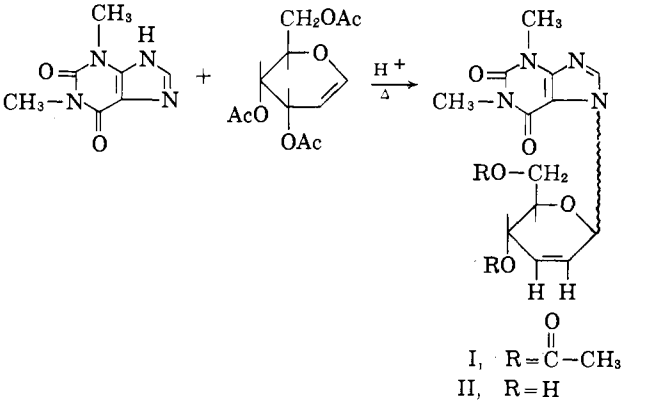
2,3-dihydropyran and 2,3-dihydrofuran.[3,4] However, earlier attempts to employ the use of various glycals under similar reaction conditions as for 2,3-dihydropyran[2] involving the use of ethyl acetate as a solvent did not result in nucleoside formation.

The recent fusion procedures[5] successfully applied to nucleoside syntheses suggested the possibility of the direct reaction of the requisite purine and glycal in the absence of a solvent, since it appeared quite possible that a similar resonance-stabilized carbonium ion at C-1 might be involved in the alkylation process. This reaction now has been shown to occur with 6-chloropurine and 3,4-di-*O*-acetyl-D-arabinal. A suspension of 6.16 g. of 6-chloropurine[6] and 8.80 g. of 3,4-di-*O*-acetyl-D-arabinal[7] was heated to 120° in the presence of 50 mg. of *p*-toluenesulfonic acid in a manner similar to that described[5] for the fusion of 6-chloropurine and 1,3,4-tri-*O*-acetyl-2-deoxy-β-D-ribopyranose. After a similar isolation procedure,[5] 1.02 g. of crystalline product was isolated, m.p. 175–185°. Examination revealed that this material was an anomeric mixture of 6-chloro-9-(3′,4′-di-*O*-acetyl-2′-deoxy-α- and -β-D-ribopyranosyl)purine. Fractional crystallization from absolute ethanol gave 0.9 g. of 6-chloro-9-(3′,4′-di-*O*-acetyl-2′-deoxy-α-D-ribopyranosyl)purine, m.p. 205–207°, $[\alpha]^{26}D$ +22.5° (*c* 0.75, acetone). *Anal.* Calcd. for $C_{14}H_{15}ClN_4O_5$: C, 47.4; H, 4.23; N, 15.8. Found: C, 47.3; H, 4.26; N, 15.9. The ultraviolet, infrared, and proton magnetic resonance spectra and paper chromatography established the fact that this compound was identical with that anomer prepared[5] by fusion of 6-chloropurine and 1,3,4-tri-*O*-acetyl-2-deoxy-β-D-ribopyranose. The presence of 6-chloro-9-(3′,4′-di-*O*-acetyl-2′-deoxy-β-D-ribopyranosyl)purine[5] in the ethanolic filtrates was also confirmed by paper chromatography in two systems.

In an effort to study the scope of this reaction, 3,4,6-tri-*O*-acetyl-D-glucal[8] (7.5 g.) and theophylline (5.0 g.) were similarly fused *in vacuo* in the presence of 50 mg. of *p*-toluenesulfonic acid. During this process, however, a copious evolution of acetic acid was noted. From the reaction mixture was isolated, after recrystallization from ethanol, 5.0 g. of a crystalline nucleoside (presumably an anomeric mixture, I), m.p. 102–104°, $[\alpha]^{25}D$ +128° (*c* 1.0, ethanol). The ultraviolet absorption spectra, $\lambda_{max}^{pH\ 1}$ 273 mμ (ε 7800) and $\lambda_{max}^{pH\ 11}$ 231, 273 mμ (ε 4300, 9000), indicated 7-substitution.[9] *Anal.* Calcd. for $C_{17}H_{20}N_4O_7$: C, 52.0; H, 5.10; N, 14.3. Found: C, 51.7; H, 5.24; N, 14.4.

Proton magnetic resonance spectra in deuteriochloroform (TMS internal standard) showed the presence of only two acetylmethyl groups at δ 2.02 and 2.15, respectively. The two vinyl protons occur in the region δ 6.1–6.3.

On this basis and on the analogy of a similar acid-catalyzed reaction of 3,4,6-tri-*O*-acetyl-D-glucal and *p*-nitrophenol as the aglycone,[10] the structure of I is tentatively assigned as 7-(4′,6′-di-*O*-acetyl-2′,3′-didehydro-2′,3′-dideoxy-D-glucopyranosyl)theophylline. Additional evidence for this structure was obtained by mild acid hydrolysis of I which gave theophylline and a carbohydrate residue identified as 4,6-di-*O*-acetyl-2,3-didehydro-2,3-dideoxy-D-*erythro*-hexose (di-*O*-acetylpseudo-D-glucal) by comparison with an authentic sample[11] as judged by paper chromatography in two different solvent systems. No other products were detected in the hydrolysate. Deacetylation of I (1.0 g.) with methanolic ammonia gave 0.52 g. of 7-(2′,3′-didehydro-2′,3′-dideoxy-D-glucopyranosyl)theophylline (II), m.p. 197–198°. *Anal.* Calcd. for $C_{13}H_{16}N_4O_5$: C, 50.6; H, 5.2; N, 18.2. Found: C, 50.3; H, 5.6; N, 17.9. Such unsaturated nucleosides should prove most interesting synthetic intermediates for further work. Additional current interest in 2′,3′-unsaturated nucleosides stems from the fact that such compounds have been postulated as possible biochemical intermediates in the enzymatic conversion of various purine and pyrimidine ribonucleotides to the corresponding deoxyribonucleotides.[12,13] The application of this procedure for the preparation of unusual nucleosides *via* the use of additional glycals and the detailed study of the structure of resulting nucleoside derivatives are problems under active investigation in our laboratory.



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The Specific Chemical Synthesis of γ-P^{32} Labeled Adenosine 5′-Triphosphate

Sir:

Nucleoside 5′-triphosphates labeled specifically with P^{32} in the β- or γ-position constitute a valuable tool for the elucidation of the mechanism of many biological reactions. Successful syntheses of these compounds have invariably been enzymatic in nature[1] and frequently are restricted to adenosine polyphosphates by enzyme specificities. As yet, chemical attempts have led only to products with nonspecific labeling.[2,3] We now describe an entirely specific chemical synthesis of ATP-γ-P^{32} which may be extended to any nucleoside 5′-triphosphate and to δ-P^{32} nucleoside 5′-tetraphosphates.

(1) See, *e.g.*, A. Kornberg, S. G. Kornberg, and E. S. Simms, *Biochim. Biophys. Acta*, **20**, 215 (1956); G. Pfleiderer, *ibid.*, **47**, 389 (1961).

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(3) A mixed chemical and enzymatic method has been used by R. Tanaka [*J. Biochem.* (Tokyo), **47**, 207 (1960)] for the synthesis of ATP-β-P^{32}.