Preparation of the α and β Anomers of 9-(3,5-Dideoxy-D-*glycero*-pent-4-enofuranosyl)adenine and Their Activity with Leukemia L1210 Cells in Vitro

Leon M. Lerner

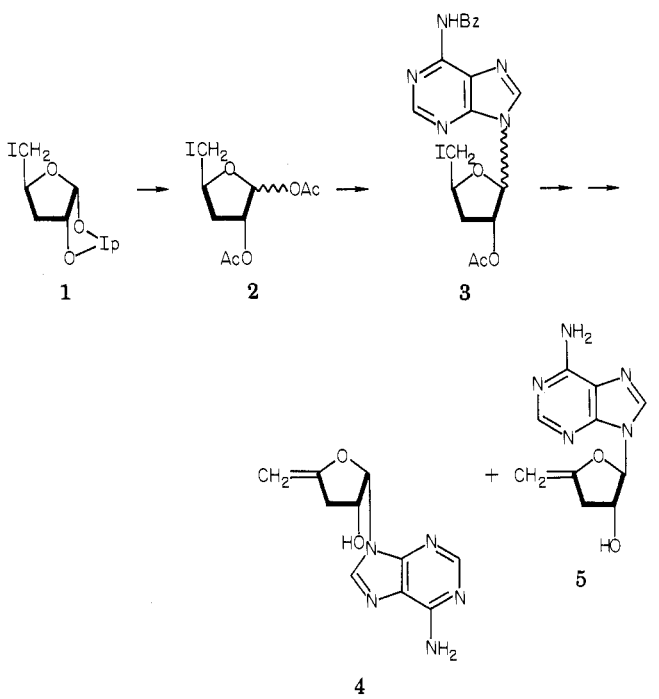
Department of Biochemistry, State University of New York, Downstate Medical Center, Brooklyn, New York 11203.
Received November 20, 1981

A previously reported preparation of 9-(3,5-dideoxy-β-D-*glycero*-pent-4-enofuranosyl)adenine (**5**) was not correct. A new synthesis of **5** is described. 3,5-Dideoxy-5-iodo-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose (**1**) and 3,5-dideoxy-5-iodo-1,2-*O*-isopropylidene-β-L-*threo*-pentofuranose (**6**) were prepared as starting materials. The isopropylidene groups were exchanged for acetyl groups by acetolysis, and the resulting diacetates (**2** and **7**) were coupled with 6-(benzamidochloromercuri)purine by the titanium tetrachloride method. The blocked nucleosides (**3** and **8**) were separated from unreacted sugars by chromatography and were treated with 1,8-diazabicyclo-[5.4.0]undec-7-ene, followed by removal of the blocking groups. The α and β anomers of 9-(3,5-dideoxy-D-*glycero*-pent-4-enofuranosyl)adenine (**4** and **5**, respectively) were obtained from **3** in the ratio of 3:1. Condensation of **7** with the base gave the β-nucleoside **5** in a higher yield; no **4** was detected. When **8** was treated with sodium benzoate in hot DMF and the blocking groups were removed, 9-(5-methyl-2-furyl)adenine (**9**) and 9-(3-deoxy-α-L-*threo*-pentofuranosyl)adenine (**10**) were the products. It was demonstrated that sodium benzoate was solely responsible for formation of the 5-methyl-2-furyl ring and that the first step was formation of the 4′,5′-olefin.

In an earlier paper from this laboratory, a synthesis of 9-(3,5-dideoxy-β-D-*glycero*-pent-4-enofuranosyl)adenine (**5**) was reported.[1] It now turns out that this substance is not **5** at all but is rather the well-known nucleoside antibiotic cordycepin.[2] This was discovered when samples of the supposed new nucleoside were assayed as potential substrates for adenosine deaminase.[3] The nucleoside was nearly as good a substrate as adenosine, which was completely incongruous with the structure it was supposed to have. When the physical data for this compound were checked against the physical data for cordycepin,[4] there was excellent correspondence. In addition, the IR spectrum of the nucleoside was identical with that of an authentic sample of cordycepin,[5] and the mixture melting point determination gave no depression. Once the true identity of this nucleoside was established, it became necessary to begin anew the synthesis of **5**.

The preparation of the starting material, 3,5-dideoxy-5-iodo-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose (**1**, Scheme I) is briefly outlined under Experimental Section. Acetolysis of **1** afforded the diacetate **2** as a syrup, which was condensed with 6-(benzamidochloromercuri)purine by the $TiCl_4$ method.[6,7] The blocked nucleoside **3** was separated from carbohydrate byproducts by silica gel chromatography. It was an anomeric mixture; however, this point was not originally recognized until after the isolation of the deblocked unsaturated nucleosides. Intermediate **3** was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dry DMF. This step was followed, without purification of the unsaturated product, by treatment with NH_4OH in MeOH to remove blocking groups. Ion-exchange chromatography, using the technique of Dekker,[8]

Scheme I

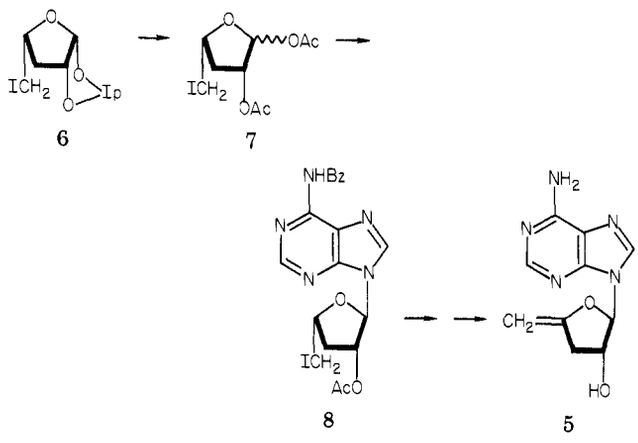


afforded two unsaturated nucleosides in a ratio of approximately 3:1. Surprisingly, the more abundant product was the α-anomer **4**, and the other nucleoside was the desired **5**. This was shown by the NMR spectra for the two nucleosides, which were almost identical in appearance except that the peaks were shifted slightly. The positions for the chemical shifts were typical for α and β anomers of nucleosides.[9] Further support was given by the optical rotations, which was a large positive value for the α-anomer **4** and a large negative value for the β-anomer **5**. The crystalline structure of **4** was somewhat unusual in that it contained 1.25 mol of H_2O of crystallization, which was verified by a quantitative determination. It was expected that the directive effect of the acetoxy group at C-2 would afford the β (trans) nucleoside.[10] It is difficult to reconcile

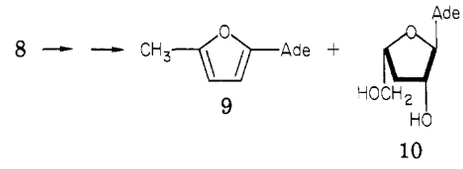
(1) Srivastava, V. K.; Lerner, L. M. *J. Med. Chem.* **1979**, *22*, 24.
(2) A preliminary report of these findings and some of the work reported in this paper were presented at the Gordon Research Conference on Purines, Pyrimidines, and Related Compounds on June 30, 1981.
(3) The discovery that the nucleoside was a substrate for adenosine deaminase was made by Dr. Alan J. Grant. Adenosine deaminase from calf intestinal mucosa was the Type I preparation obtained from Sigma Chemical Co.
(4) Suhadolnik, R. J. "Nucleoside Antibiotics", Wiley-Interscience: New York, 1970; p 52.
(5) Cordycepin was purchased from Sigma Chemical Co.
(6) Baker, B. R.; Schaub, R. E.; Joseph, J. P.; Williams, J. H. *J. Am. Chem. Soc.* **1955**, *77*, 12.
(7) Prokop, J.; Murray, D. H. *J. Pharm. Sci.* **1965**, *54*, 359.
(8) Dekker, C. A. *J. Am. Chem. Soc.* **1965**, *87*, 4027.
(9) Townsend, L. B. in "Synthetic Procedures in Nucleic Acid Chemistry"; Zorbach, W. W.; Tipson, R. S., Eds.; Wiley-Interscience, New York, 1973; Volume 2, p 267.
(10) Baker, B. R. *Chem. Biol. Purines, Ciba Found. Symp.* **1957**, 120.

0022-2623/82/1825-0825$01.25/0

Scheme II



Scheme III



the yield of **4** without advocating a role, either steric or mechanistic, for the terminal iodomethyl group of **2**. The results from the following preparation of **5** appear to support this viewpoint.

A more successful synthesis of **5** began from 3,5-dideoxy-5-iodo-1,2-*O*-isopropylidene-β-L-*threo*-pentofuranose (**6**, Scheme II), whose synthesis is outlined under Experimental Section. The decision to try **6** was based in part on what appeared to be a role of the iodomethyl group in determining the stereochemical outcome of coupling with the base. By placement of the iodomethyl group on the opposite side of the furanose ring, which is also the same side on which the 2-acetoxy group is located, it was thought that perhaps the main product would be **5**. Also, the yield of **5** obtained above was very poor, and even the combined yield of nucleosides **4** and **5** was poor. It was, therefore, desirable to improve the yield of **5** in the coupling step. Acetolysis of **6** gave the diacetate **7**, which was condensed with 6-(benzamidochloromercuri)purine. Silica gel chromatography afforded the blocked nucleoside **8**. Treatment of **8** with DBU in dry DMF and then with NH_4OH in MeOH and chromatography[8] gave only one main product. This substance was identified as **5**. It was notable that the two goals of this synthesis were satisfied. Not only was the α-form **4** not isolated, but there was not even a UV-absorbing peak where **4** was expected to emerge. Furthermore, the yield of **5** was improved tremendously over that obtained before.

In order to obtain further confirmation of the anomeric configuration of **5**, it was envisioned that the intermediate **8** could be converted to 9-(3-deoxy-α-L-*threo*-pentofuranosyl)adenine (**10**, Scheme III). This nucleoside was originally prepared by Prokop and Murray.[7] The anomeric configuration was assumed on the basis of the trans rule[10] and not specifically proved. However, **10** was later prepared from adenosine precursors,[11] where no chemical reactions affected the anomeric position, thereby showing that the configurational designation was indeed correct. Conversion of **8** into **10** would, therefore, be convincing proof of the anomeric configuration. Intermediate **8** was treated with sodium benzoate in hot DMF, and the blocking groups were removed in refluxing methanolic NaOMe. Chromatography on an ion-exchange column[8] yielded two main products from a single UV-absorbing peak. These were separated by fractional crystallization. The main product was identified as 9-(5-methyl-2-furyl)-adenine (**9**), and the product obtained in lower yield was concluded to be **10** from comparison of its physical constants with those reported in the literature.[7]

That **9** was 9-(5-methyl-2-furyl)adenine was originally deduced from its elemental analysis and spectra (NMR and IR). It was proved by comparison to an authentic sample of **9**. The formation of **9** was completely unexpected. It was known that sodium benzoate in hot DMF will cause elimination reactions under certain circumstances,[12] but this should have been limited to the terminal position. At worst, nucleoside **5** was expected as a possible product, since the acetoxy group is considered an unlikely leaving group. In past syntheses of **9**, good leaving groups such as halogeno or tosyl were used in conjunction with strong bases. For example, Wang and Hogenkamp[13] prepared **9** from 9-(3,5-dichloro-2,3,5-trideoxy-β-D-*threo*-pentofuranosyl)adenine in a hot solution of NaOH in aqueous EtOH, and McCarthy et al.[14] prepared **9** from 2′,5′-dideoxy-5′-*S*-ethyl-3′-*O*-(*p*-toluenesulfonyl)-5′-thioadenosine with potassium *tert*-butoxide in Me_2SO. During this latter work, it was demonstrated that elimination occurred first at the 2′,3′-position and then at the terminal position, followed by rearrangement to form **9**. In the present case it appeared more likely that elimination occurred first at the terminal carbons. However, because of the way in which the reaction sequence was carried out, namely, sodium benzoate–DMF followed by NaOMe–MeOH without isolation of an intermediate, it was not known which reagent actually caused the elimination of the acetoxy group and the formation of the furyl ring. Therefore, several experiments were designed to simultaneously determine which reagent was responsible for the formation of the 5-methylfuryl ring and to verify that terminal elimination occurred first.

When **8** was treated with DBU to form the 4′,5′-unsaturated blocked nucleoside and then treated with hot methanolic NaOMe, the reaction mixture turned black, and no products were eluted during chromatography. Intermediate **8** was treated with DBU again, but the crude product was now treated with sodium benzoate in hot DMF. After treatment with NaOMe and chromatography, **9** was the only main product isolated. If **8** was treated with sodium benzoate in hot DMF and the *N*-benzoyl group was displaced by formation of a picrate,[15] removal of the picrate ion with an ion-exchange resin and chromatography again afforded **9**. These experiments clearly demonstrated that sodium benzoate in DMF is solely responsible for aromatization of the sugar ring and that the 4′,5′-unsaturated nucleoside is capable of producing **9**. The latter is good evidence that the 4′,5′-olefin is the immediate product of the reaction sequence from **8** to **9**.

Biological Activity. The activity of **4** and **5** against leukemia L1210 in vitro was determined as the concen-

(11) Nagpal, K. L.; Horwitz, R. A. *J. Org. Chem.* **1971**, *36*, 3743. Robins, M. J.; Jones, R. A.; Mengel, R. *J. Am. Chem. Soc.* **1976**, *98*, 8213.

(12) Lerner, L. M. *J. Org. Chem.* **1972**, *37*, 477 Arzoumanian, H.; Acton, E. M.; Goodman, L. *J. Am. Chem. Soc.* **1964**, *86*, 74.

(13) Wang, Y.; Hogenkamp, H. P. C. *J. Org. Chem.* **1978**, *43*, 998.

(14) McCarthy, Jr., J. R.; Robins, M. J.; Townsend, L. B.; Robins, R. K. *J. Am. Chem. Soc.* **1966**, *88*, 1549.

(15) Parikh, J. R.; Wolff, M. E.; Burger, A. *J. Am. Chem. Soc.* **1957**, *79*, 2778. Wolfom, M. L.; Foster, A. B.; McWain, P.; von Bebenburg, W.; Thompson, A. *J. Org. Chem.* **1961**, *26*, 3095.

tration giving 50% inhibition of growth.[16] For **4**, this concentration was 3.4×10^{-4}M, and for **5** it was 3.2×10^{-4} M. Therefore, the new nucleosides have a very low level of activity.

Experimental Section

Instrumentation and Methods. Melting points were determined on a Kofler hot stage and are corrected values. UV spectra were determined with a Beckman Model 25 spectrophotometer, and IR spectra were recorded on a Perkin-Elmer Model 21 spectrophotometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. NMR spectra were recorded on a Varian T-60A spectrometer with Me_4Si as the internal reference.

TLC was performed on 0.25-mm layers of silica gel GF-254 (E. Merck, Darmstadt). Spots were located with a Mineralight lamp, which produced UV radiation at 254 nm, and by spraying the plates with chromic acid, followed by heating of the plates in an oven at 130 °C. Silica gel (Baker No. 3404, 40–140 mesh) was utilized for column chromatography. Solvent mixtures are expressed as v/v ratios.

All chloroform used in this work contained 0.75% EtOH. Dry DMF was prepared by distillation of benzene at atmospheric pressure, followed by distillation of DMF under reduced pressure and storage over 4A molecular sieves. All moist solutions were dried over anhydrous $MgSO_4$. Evaporations were performed on a rotary evaporator under reduced pressure at a bath temperature of ~40 °C, unless otherwise stated. The new nucleosides were dried overnight in an Abderhalden pistol P_2O_5 under high vacuum. Elemental analyses were performed by the Spang Microanalytical Laboratory, Eagle Harbor, MI.

The tests for I^- in aqueous washings consisted of treatment of a few milliliters of an acidified solution (H_2SO_4) with KIO_3, followed by extraction of this mixture with $CHCl_3$. A positive test was a violet color in the $CHCl_3$ layer.[17]

3,5-Dideoxy-5-iodo-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose (1). D-Xylose was used to prepare 3-deoxy-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose by scaling up the procedure of Nair and co-workers.[18] This compound was tosylated,[19] and the 5-tosylate was converted to **1** with NaI in 2-butanone:[1] mp 75–75.5 °C; $[\alpha]^{24}_D$ −19° (*c* 1.16, $CHCl_3$) (lit.[1] mp 72–73 °C).

3,5-Dideoxy-5-iodo-1,2-*O*-isopropylidene-β-L-*threo*-pentofuranose (6). 3-Deoxy-1,2-*O*-isopropylidene-β-L-*threo*-pentofuranose was prepared by the route of Prokop and Murray.[7] The 3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*erythro*-hex-3-enofuranose used in this preparation was obtained from 1,2:5,6-di-*O*-isopropylidene-3-*O*-(*p*-toluenesulfonyl)-α-D-glucofuranose by reaction with KOH in toluene[20] or from 1,2:5,6-di-*O*-isoproplidene-3-*O*-triflyl-α-D-glucofuranose by reaction with DBU in ether.[21] The latter reaction was scaled up about 30 times over the literature procedure. 3-Deoxy-1,2-*O*-isopropylidene-β-L-*threo*-pentofuranose was tosylated and converted to **6** as previously described.[1]

α and β Anomers of 9-(3,5-Dideoxy-D-*glycero*-pent-4-enofuranosyl)adenine (4 and 5). Compound **1** (5.68 g) was dissolved in a mixture of acetic acid (40 mL) and acetic anhydride (4 mL). The mixture was chilled in an ice bath for a few minutes, and 1.8 mL of concentrated H_2SO_4 was added, dropwise. The solution was stirred for 22 h at room temperature and then poured into 200 mL of ice–water. The mixture was stirred until the ice melted, 50 mL of $CHCl_3$ was added, and stirring was continued for 0.5 h. The $CHCl_3$ layer was separated, and the aqueous layer was extracted with two 50-mL portions of $CHCl_3$. The $CHCl_3$ extracts were combined and washed with H_2O (200 mL), saturated $NaHCO_3$ solution (200 mL), H_2O (200 mL), and dried. The $CHCl_3$ was removed by evaporation, and the syrup was coevaporated with benzene (3 × 10 mL) to remove traces of acetic acid. The clear, light-yellow syrup weighed 6.07 g (92.5% yield). The NMR spectrum clearly showed that the isopropylidene group was gone and had been replaced by acetyl groups. 1,2-Di-*O*-acetyl-3,5-dideoxy-5-iodo-D-*erythro*-pentofuranose (**2**) gave a positive Beilstein test and an ethanolic $AgNO_3$ test in about 5 min at room temperature and immediately upon heating in a steam bath. The first aqueous wash from the workup was concentrated by evaporation and tested for I^-, which was negative.

The entire amount of **2** was dissolved in 1,2-dichloroethane and transferred to a 1-L two-neck reaction flask equipped with a condenser, a take-off adapter, and a drying tube (Drierite). A total of 400 mL of 1,2-dichloroethane was used, and to this solution was added 10.78 g of 6-(benzamidochloromercuri)purine and 10.8 g of Celite-545. Eighty milliliters of solvent was distilled to remove traces of moisture, and 2.65 mL of $TiCl_4$ in 80 mL of fresh 1,2-dichloroethane was added. The mixture was stirred vigorously under reflux for 23 h and then was allowed to cool to room temperature and treated with aqueous $NaHCO_3$ solution (450 mL). After stirring for 2 h, the solids were filtered off on a pad of Celite-545 (suction), and the filter cake was washed with 250 mL of hot solvent. The organic layer was separated and evaporated to a brown foam. The foam was dissolved in 150 mL of $CHCl_3$, washed with 30% KI solution (150 mL) and H_2O (200 mL), and dried. Evaporation gave a dark tan foam (3.81 g), which was dissolved in a minimal amount of benzene and placed on top of a column (28 × 3.7 cm) of silica gel that had been packed with benzene. The column was eluted with 3:1 benzene–ethyl acetate (800 mL) and 1:1 benzene–ethyl acetate (400 mL), which removed some sugar derivatives. Elution with ethyl acetate (400 mL) gave a waxy nucleosidic material, which was not the desired product. The next 700 mL afforded 1.9 g of a hard, white foam (**3**), which gave a positive Beilstein test: TLC R_f 0.19 (EtOAc); UV (MeOH) λ_{max} 280 nm.

The foam (**3**; 1.84 g) was dissolved in 25 mL of dry DMF and treated with DBU (1.22 g) dissolved in 10 mL of DMF. The mixture was stirred at room temperature for 18 h. The DMF was evaported with a vacuum pump (bath temperature 35 °C), the residue was dissolved in MeOH (25 mL), 25 mL of concentrated NH_4OH was added, and the mixture was stirred for 23 h. The solution was evaporated, and the residue was dissolved in hot H_2O. Some insoluble material, which was not a nucleoside, was removed by filtration, and the aqueous solution was applied to a column (35 × 2.4 cm) of Bio-Rad AG1-X2 (200–400 mesh, OH^-) that had been packed in H_2O. The column was eluted with H_2O, and 10-mL fractions were collected. A peak for benzamide and two very minor peaks came off the column. Starting with fraction 71 and continuing through fraction 361, the column was eluted with 30% aqueous MeOH. Two major peaks were obtained, fractions 131–225 and fractions 262–350.

Fractions 131–225 were pooled, the solvents were evaporated, and the residue was crystallized from H_2O in the refrigerator. A yield of 216 mg (4.6% yield from **2**) was obtained in the form of large prisms. The melting-point behavior was somewhat complicated, possibly owing to the fact that **4** had H_2O of crystallization. The crystals underwent a change in state near 155 °C, frosted over, softened, partially melted, resolidified, and began to decompose as the temperature neared 172 °C. Complete melting occurred at 174–177 °C; decomposition continued slowly over the range of heating, while tiny crystals appeared on the cover slip around 195 °C; these melted and decomposed slowly until 270 °C: $[\alpha]^{25}_D$ +80.5° (*c* 1.15, Me_2SO); UV (MeOH) λ_{max} 260 nm (ε 14 840), λ_{min} 229 nm (ε 2280); NMR (Me_2SO-d_6) δ 8.23 and 8.21 (2 s, 1 proton each, H-2, H-8), 7.35 (br s, 2, NH_2), 6.58 (d, 1, $J_{1',2'}$ = 4 Hz, H-1′), 5.78 (d, 1,2′-OH), 4.58 (m, 1, H-2′), 4.33 and 4.03 (2 d, 2, $J_{5a',5b'}$ = 1.5 Hz, H-5a′, H-5b′), 2.95 (complex m, 2, H-3a′, H-3b′). Anal. ($C_{10}H_{11}N_5O_2 \cdot 1.25H_2O$) C, H, N. H_2O: calcd, 8.81; found; 8.71.

Fractions 262–350 were pooled, the solvents were evaporated, and the product (**5**) was crystallized from H_2O in the refrigerator. The crystals (66 mg, 1.5% yield from **2**) were identical in every respect with the product obtained below.

9-(3,5-Dideoxy-β-D-*glycero*-pent-4-enofuranosyl)adenine (**5**). Compound **6** (12.25 g) was acetolyzed in a mixture of acetic

(16) These experiments were performed at the Roswell Park Memorial Institute, Buffalo, N.Y.
(17) Charlot, G. "Colorimetric Determination of Elements"; Elsevier: Amsterdam, 1964; p 268.
(18) Nair, V.; Emanuel, D. J. *J. Am. Chem. Soc.* **1977**, *99*, 1571. Nair, V.; Sinhababu, A. K. *J. Org. Chem.* **1978**, *43*, 5013.
(19) Szabo, P.; Szabo, L. *J. Chem. Soc.* **1965**, 2944.
(20) Chiu, T. M. K.; Watanabe, K. A.; Fox, J. J. *Carbohydr. Res.* **1974**, *32*, 211.
(21) Fletchner, T. W. *Carbohydr. Res.* **1979**, *77*, 262.

acid (200 mL), acetic anhydride (20 mL), and concentrated H_2SO_4 (7.2 mL) as described for the acetolysis of 1. 1,2-Di-*O*-acetyl-3,5-dideoxy-5-iodo-L-*threo*-pentofuranose (**7**) was obtained as an oil (11.1 g, 79% yield). It gave a positive Beilstein test, and a test for I^- performed on the first aqueous wash was negative.

Compound **7** (3.1 g) was dissolved in 1,2-dichloroethane and transferred to a reaction vessel containing 6-(benzamidochloromercuri)purine (5.61 g) and Celite-545 (5.6 g). The final volume of solvent was adjusted to 500 mL, and 80 mL was distilled. A solution of $TiCl_4$ (1.4 mL) in 80 mL of fresh 1,2-dichloroethane was added, and the mixture was stirred under relux for 22 h. The workup was similar to the one described for the preparation of **3**. A yellow foam (4.04 g) was obtained, which was dissolved in a minimal amount of benzene and placed on a column (27 × 3.7 cm) of silica gel that had been packed in benzene. The column was eluted with 3:1 benzene–ethyl acetate (400 mL) and then with 1:1 benzene–ethyl acetate (500 mL). These washings removed sugar derivatives. Elution with ethyl acetate (1200 mL) afforded 2.89 g of a stiff, white foam: TLC R_f 0.25 (EtOAc); UV (EtOH) λ_{max} 281 nm. This substance gave a positive Beilstein test.

The blocked nucleoside was dissolved in DMF (40 mL) and treated with 1.85 of DBU in DMF (10 mL). After 24 h of stirring at room temperature, the DMF was evaporated (pump, 35 °C), and the residue was dissolved in hot H_2O and applied to the top of a column (35 × 2.4 cm) of Bio-Rad AG1-X2 (200–400 mesh, OH^-). Ten-milliliter fractions were collected. Fractions 1–68 were eluted with H_2O, fractions 69–253 were eluted with 30% aqueous MeOH, and fractions 254–430 were eluted with 60% aqueous MeOH. Prior to the main peak in tubes 172–295, there was only a peak for benzamide and a few very small peaks, indicating trace amounts of other UV-absorbing materials. The contents of tubes of 172–295 were combined, and the solvents were evaporated. The product (**5**) was crystallized from H_2O in the refrigerator, giving two crops weighing 537 mg (24% from **7**): mp, softens ~195 °C, melts 203–205 °C (dec); $[\alpha]^{25}_D$ −77.3° (*c* 1.13, Me_2SO); UV (MeOH) λ_{max} 260 (ϵ 14 660), λ_{min} 229 nm (ϵ 3235); NMR (Me_2SO-d_6) δ 8.28 and 8.21 (2 s, 1 proton each, H-2, H-8), 7.35 (br s, 2, NH_2), 6.21 (d, 1, $J_{1',2'}$ = 3 Hz, H-1'), 5.88 (d, 1,2' OH), 4.98 (m, 1, H-2'), 4.30 and 4.03 (both d, 2, $J_{5a',5b'}$ = 1.5 Hz, H-5a', H-5b'), 3.20 and 2.80 (complex multiplets, 1 proton each, H-3a', H-3b'). Anal. ($C_{10}H_{11}N_5O_2$) C, H, N.

9-(5-Methyl-2-furyl)adenine (9) and 9-(3-Deoxy-α-L-*threo*-pentofuranosyl)adenine (10). 6-Benzamido-9-(2-*O*-acetyl-3,5-dideoxy-5-iodo-α-L-*threo*-pentofuranosyl)purine (**8**) was prepared exactly as described in the preceding section. A solution of **8** (788 mg) in DMF (33 mL) to which sodium benzoate (1.4 g) was added was heated in an oil bath at 136 ± 2 °C for 16.5 h. The flask was cooled to room temperature, whereupon a brown solid formed. Acetone (50 mL) was added, the mixture was thoroughly triturated, and the solid material was removed by filtration and washed with acetone (3 × 30 mL). The acetone was evaporated, and the DMF was removed under high vacuum (35 °C). The residue was dissolved in $CHCl_3$ (50 mL), washed with H_2O (100 mL), 10% sodium thiosulfate solution (100 mL), saturated $NaHCO_3$ solution, and H_2O (100 mL), and dried. Evaporation gave a brown syrup (602 mg). TLC (EtOAc) showed two main spots and two minor spots. The product was dissolved in a solution containing 20 mL of MeOH and 2 mL of 1 N methanolic NaOMe, and the mixture was heated under reflux for 2 h. The solution was neutralized with Amberlite CG-120 (H^+) ion-exchange resin, filtered, and evaporated to dryness. To get rid of residual methyl benzoate, several coevaporations with H_2O were performed until no odor of the ester remained. The residue was dissolved in warm MeOH (6 mL), warm H_2O (14 mL) was added, and the somewhat cloudy solution was applied to the top of a column (16.5 × 1.8 cm) of Bio-Rad AG1-X2 (200–400 mesh, OH^-) that had been packed in H_2O. Elution with 30% aqueous MeOH (10-mL fractions) gave only one major peak in tubes 4–37, some of which contained crystals.[22] The contents of these tubes were pooled, and the solvents were evaporated, leaving a white solid. The solid was dissolved in warm MeOH, and a little H_2O was added to near turbidity. Feathery crystals slowly formed as the solution cooled, and further crystallization was achieved overnight in the refrigerator. The crystals were isolated by filtration, washed with cold H_2O, and dried. The yield of **9** was 56 mg (17% yield), mp 237–239 °C. A sample of 9-(5-methyl-2-furyl)adenine, kindly supplied by Dr. Harry P. C. Hogenkamp, also had mp 237–239 °C. The mixture melting point gave no lowering, and the IR spectra of the two samples were identical.

Two more crops of crystals (36 mg, 9% yield) were obtained from the mother liquor. This material was identified as **10**: mp 242–244 °C; $[\alpha]^{25}_D$ −55.3° (*c* 0.872, 1 N HCl) [lit.[7] mp 242.5–243 °C; $[\alpha]^{20}_D$ −51° (*c* 0.815, 1 N HCl)]. The IR spectrum of **10** had the same major peaks as reported in ref 7. Anal. ($C_{10}H_{13}N_5$) C, H, N.

Experiments to Determine the Order of Elimination to Form 9 and the Causative Reagent. Experiment A. To a solution of **8** (401 mg) in DMF (10 mL) was added DBU (300 mg) in 2 mL of DMF. The solution was stirred for 27 h and evaporated (pump), and the residue was dissolved in $CHCl_3$ (30 mL), washed with ice-cold 3% HCl solution (50 mL), saturated $NaHCO_3$ solution (50 mL), and H_2O (50 mL), and dried. Evaporation gave a foam, which was dissolved in DMF (18 mL), sodium benzoate (713 mg) was added, and the flask was heated in an oil bath (~140 °C) for 18 h. The workup was as described above for the preparation of **9** and **10**. Hot NaOMe solution was used to remove the *N*-benzoyl group, and methyl benzoate was removed in vacuo as the H_2O azeotrope. The crude product was chromatographed on a column (15.5 × 1.8 cm) by the Dekker technique. Elution of 10-mL fractions with 30% aqueous MeOH gave a UV-absorbing peak in tubes 4–14. These were pooled, the solvents were evaporated, and the product was crystallized from MeOH–H_2O to afford 26 mg of feathery crystals, mp 238–239 °C. These were identical in every way with previously prepared **9**.

Experiment B. A mixture containing **8** (401 mg), sodium benzoate (713 mg), and DMF (18.5 mL) was heated in an oil bath and worked up as described for the preparation of **9** and **10**, above. The residue obtained after evaporation of the $CHCl_3$ was dissolved in hot EtOH (2 mL), 7 mL of hot 10% ethanolic picric acid was added, and the mixture was heated under reflux for 5 min on a steam bath. During this time (after about 2 min), crystals began to form. The flask was kept at room temperture for 2 h and then in the refrigerator overnight. The yellow crystals were filtered off (suction) and washed with ice-cold EtOH. The picrate (358 mg) was dissolved in warm MeOH (100 mL), H_2O (30 mL) was added, and the stirred solution was treated with Bio-Rad AG1-X8 (100–200 mesh, CO_3^{2-}) ion-exchange resin in small increments until the yellow color was discharged from the solution. The resin was removed on a glass funnel (suction) and washed several times with MeOH. The solvents were evaporated, and the white residue was dissolved in 30% aqueous MeOH and chromatographed on the same size ion-exchange column as in experiment A. The major peak was in tubes 5–12. Evaporation and crystallization (MeOH–H_2O) afforded 33 mg of **9**, mp 238–239 °C, identical in every way to the previous samples of **9**.

Acknowledgment. Part of the work described here was supported by research Grant Ca 13802 from the National Cancer Institute, National Institutes of Health. The author is indebted to Dr. Harry P. C. Hogenkamp for a generous sample of 9-(5-methyl-2-furyl)adenine.

(22) There is a tendency for **9** to crystallize in the effluent tubing. A little heat carefully applied from a hair dryer clears up this problem.