tivity of a single dose administered orally 6 hr after infection. All animals were observed for 14 days, deaths were recorded daily, and the CD_{50} values were calculated by the method of Reed and Muench.[6]

Methyl 2-[(2-Ethoxycarbonylethyl)ethylamino]-6-methylnicotinate (1a). A solution containing 337.6 g (1.82 mol) of methyl 2-chloro-6-methylnicotinate[7] and 571.3 g (3.94 mol) of ethyl 3-ethylaminopropionate[8] in 1 l. of *o*-dichlorobenzene was heated under reflux for 6 hr. The solvent was removed by vacuum distillation. The fraction distilling at 163–165° (0.5 mm) amounted to 129.4 g (44%) of **1a**: ir (film) 5.74 (aliphatic ester C=O), 5.80 μ (aromatic ester C=O). Anal. ($C_{15}H_{22}N_2O_4$) C, H, N.

Ethyl 1-Ethyl-1,2-dihydro-4-hydroxy-7-methyl-1,8-naphthyridine-3-carboxylate (2a). To a solution of sodium ethoxide prepared from 10.1 g (0.44 g-atom) of sodium in 1 l. of ethanol was added 129.4 g (0.44 mol) of **1a**. The reaction mixture was heated under reflux for 10 min and then cooled in ice. The insoluble material was collected on a filter and triturated with 20% aqueous acetic acid solution. There was obtained 81.5 g of product (yield 71%), mp 75–80°. Recrystallization from petroleum ether gave an analytical sample: mp 69–71°; ir (KBr) 5.95 (ester C=O), 3.5–4.3 μ (chelated OH); ^{1}H NMR ($CDCl_3$) δ 7.98 (d, 1, arom), 6.48 (d, 1, arom). Anal. ($C_{14}H_{18}N_2O_3$) C, H, N.

The hydrochloride salt of **2a** was prepared in 95% yield by adding an ethereal solution of hydrogen chloride to the free base in ethyl acetate. The analytical sample (mp 162–164°) was obtained by recrystallization from acetonitrile: ^{1}H NMR ($CDCl_3$) δ 4.60 (s, 2, CH_2), 12.12 (s, 1, OH). Anal. ($C_{14}H_{19}N_2ClO_3$) C, H, N, Cl.

1-Ethyl-2,3-dihydro-7-methyl-1,8-naphthyridin-4(1*H*)-one Hydrochloride (3). To 200 ml of 2% sodium hydroxide solution were added 0.2 g of **2a** and a few milliliters of ethanol to aid solubility. The solution was stirred at room temperature for 4 hr and extracted with ether (2 × 50 ml). The ether extracts were combined, dried over magnesium sulfate, and filtered. The filtrate was evaporated to dryness. The oily residue was dissolved in a few milliliters of dry ether and acidified with ethereal hydrogen chloride. Recrystallization of the salt from ethyl acetate gave a product with mp 179–184°: ir (KBr) 5.88 μ (aromatic ketone). Anal. ($C_{11}H_{15}ClN_2O$) C, H, N.

1-Ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic Acid (Nalidixic Acid). To 40 ml of a 20% sodium hydroxide solution was added 0.2 g of **2a** and a few milliliters of ethanol to aid solubility. The solution was heated for 5 min at 40–50°, acidified with glacial acetic acid, and cooled to room temperature. Recrystallization of the resulting precipitate from ethanol gave nalidixic acid, mp 225–230°. No depression of melting point was observed on admixture with an authentic sample. Anal. ($C_{12}H_{12}N_2O_3$) C, H, N.

Ethyl 1-Ethyl-1,2,3,4-tetrahydro-3,7-dimethyl-4-oxo-1,8-naphthyridine-3-carboxylate Hydrochloride (4a). To a solution of sodium ethoxide prepared from 0.35 g (0.015 g-atom) of sodium in 75 ml of ethanol was added 3.9 g (0.015 mol) of **2a** in 40 ml of ethanol. The precipitate which formed was collected and dissolved in 75 ml of *N,N*-dimethylformamide. To this solution was added 2.1 g (0.015 mol) of iodomethane. The mixture was stirred for 5 min, diluted with 150 ml of water, and extracted with ether (2 × 50 ml). The ether layer was dried over magnesium sulfate, filtered, and acidified with an ethereal hydrogen chloride solution. Ethyl acetate was added to initiate crystallization. The solid was recrystallized from ethyl acetate to give 0.7 g of product: mp 117–120°; ir (KBr) 5.73 (ester C=O), 5.84 μ (aromatic ketone). Anal. ($C_{15}H_{21}ClN_2O_3$) C, H, N.

Ethyl 3-Carbethoxy-1-ethyl-1,2,3,4-tetrahydro-7-methyl-4-oxo-1,8-naphthyridine-3-acetate Hydrochloride (5). To a solution of sodium ethoxide prepared from 0.09 g (0.004 g-atom) of sodium in 50 ml of ethanol was added 1.0 g (0.004 mol) of **2a** in 50 ml of ethanol. The sodium salt thus formed was collected, dried, and dissolved in 50 ml of *N,N*-dimethylformamide. To this solution was added 0.6 g (0.004 mol) of ethyl bromoacetate. The reaction mixture was stirred for 10 min, 150 ml of water was added, and the mixture was extracted with ether (2 × 100 ml). The ether layer was dried over magnesium sulfate, filtered, and diluted with 20 ml of ethyl acetate. The oil which initially separated crystallized on cooling. Recrystallization from ethyl acetate gave 0.6 g of product, mp 150–153°. Anal. ($C_{18}H_{25}ClN_2O_5$) C, H, N.

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Synthesis of Tritium- and Deuterium-Labeled 9-β-D-Arabinofuranosyladenine and the Tritium-Labeled 5′-Monophosphate Ester with Increased Metabolic Stability

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Preparation of both a 5′-deuterium and a 5′-tritium-labeled 9-β-D-arabinofuranosyladenine (**6a** and **6b**) by reduction of the protected 5′-aldehyde **4** is described. Conversion of **6b** to the 5′-tritium-labeled 5′-monophosphate **7b** was effected directly with a phosphoryl chloride–formic acid reagent. The product **7b** exhibited consistently higher blood levels of nonvolatile tritium than the 2-labeled compound when tested in dogs.

Pharmacological studies on the nucleoside antiviral agent, 9-β-D-arabinofuranosyladenine (**1**),[1-4] and the 5′-phosphate (**7a**)[1] require a stable tritium-labeled derivative that is metabolically resistant to tritium removal in vivo by the various enzymic processes of exchange and oxidation. While an exchange reaction with tritium oxide[5] or more elegant catalytic exchange procedures[6] are available for labeling respectively the C-8[5] and C-2[6] positions of adenine nucleosides, neither process provides a derivative of **1** or **7a** that will retain high levels of its label in a wide range of test

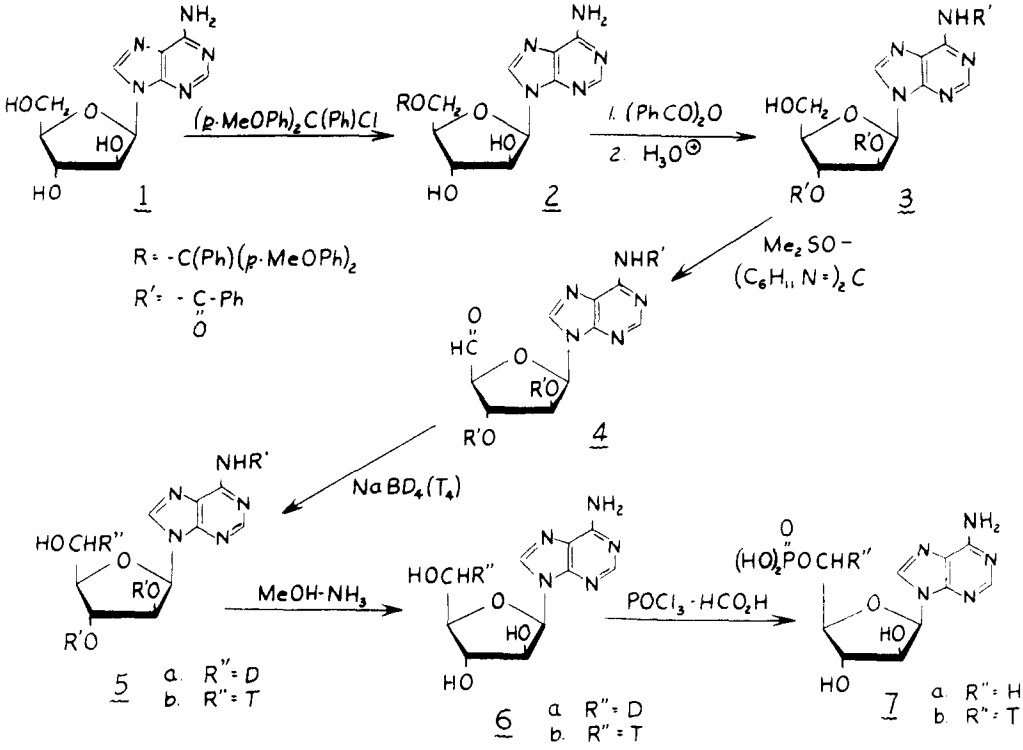
animals.[7-9] A C-8 tritium-labeled adenosine is readily exchanged in boiling water.[5] A C-2 labeled 1, shown to be quite stable chemically,[9] becomes incorporated in the body water both in vitro[7,10] and in vivo[7-9] where, especially in the rat and dog,[9] a high percentage of the label has been found to be tritiated water. As an alternative to both 1 and 7a labeled at C-2, the synthesis of derivatives with an aliphatic C-^{3}H bonded label at the 5′ position in the sugar moiety was developed.

Nucleoside 5′-aldehydes, shown to be useful synthetic intermediates[11] that are conveniently prepared from their respective 2′,3′-*O*-acetal protected precursors by oxidation[11] or via a photolytic route,[12] are good sources for 5′-labeled compounds and have been employed for synthesis of both 5′-deuterated[12] and 5′-tritiated[13,14] ribonucleosides. The requisite intermediate 3 for synthesis of the analogous β-D-arabino-5′-aldehyde 4 was prepared (see Scheme I). By reacting 1 with an excess of chlorobis(4-methoxyphenyl)-phenylmethane[15] in anhydrous *N,N*-dimethylformamide, the 5′-*O*-[bis(4-methoxyphenyl)phenylmethyl] derivative 2 was obtained. Subsequent benzoylation of 2 with excess benzoic anhydride in boiling pyridine cleanly afforded the tribenzoylated derivative of 2 without the complications that were found with the use of aroyl chlorides.[15,16] Subsequent removal of the 5′-O protecting group with aqueous formic acid gave, in 58% yield from 2, crystalline 3, identified by NMR spectroscopy and elemental analysis. Oxidation of 3 with methyl sulfoxide–dicyclohexylcarbodiimide[11] yielded the intermediate aldehyde 4, which without isolation was reduced with the appropriately labeled sodium borohydride reagent to furnish **5a** and **5b**. To demonstrate that no epimerization[17] at C-4′ of 4 had occurred under the conditions of reduction, the product **5a** was shown to be of a structure identical with 3 by melting point and NMR spectrum (see Experimental Section). The proton NMR spectrum for **5a** was like that of 3, except that the H-5′,5′a signals (an AB portion of an ABX system for 3) were replaced by a four-line multiplet that integrated for one proton, and the H-4′ signal (a six-line pattern for 3) narrowed to a four-line multiplet with the loss of one H-4′–H-5′ coupling. After column chromatography **5a** and **5b** afforded, upon treatment with methanolic ammonia, pure **6a** and **6b**, respectively, identical with authentic, nonlabeled 1 by melting point and uv spectroscopy.

Conversion of the tritium-labeled **6b** to the 5′-phosphate **7b** was carried out using a modification of the formic acid–phosphoryl chloride procedure[18] reported for the direct phosphorylation of unblocked nucleosides. By maintaining dry conditions, in the cold, a good yield of 7 in 96% isotopic purity could be obtained upon work-up on charcoal and subsequent crystallization of the free acid ester **7b**. Additional labeled product of >98% purity was obtained via anion exchange chromatography of the mother liquors.

Studies of the tritium levels in the plasma of two beagle dogs, each dosed intravenously with 10 mg kg^{-1} of the 5′-labeled derivative **7b** and the 2-labeled compound, respectively, revealed that the label in **7b** was removed at a rate that is significantly lower than that observed for the 2-labeled derivative. Whereas (see Table I) the ratio of volatile to nonvolatile tritium for the 2-labeled compound reached 1:1 in 0.5 hr, ca. 4 hr was required for the plasma level of tritium to reach the ca. 1:1 ratio when **7b** was employed. The increased stability of the 5′-label provides a more reliable indicator for in vivo studies of the metabolism of 7, and it is especially useful in determining the fate of the sugar moiety. Detailed studies[9b] will be reported in the future regarding the metabolism of 7 in laboratory animals and in man.

Scheme I



Experimental Section

General Methods. Evaporations were conducted at 40° in vacuo unless otherwise indicated. Melting points were determined using capillary tubes in a Hoover "Unimelt" and are uncorrected. Spectral determinations were carried out using the following instruments: (1) for 90-MHz NMR spectroscopy, a Bruker WH-90 pulsed Fourier-transform spectrometer; chemical shifts for ca. 2.5% solutions are reported on the δ scale (ppm) downfield from tetramethylsilane (internal standard); (2) a Cary-11 spectrophotometer for uv determinations; (3) a Digilab FTS-14 pulsed Fou-

Table I. Plasma Levels in Two Beagle Dogs Following 10 mg/kg Iv Doses of **7b**[a]

Time after dosing, hr	Tritium label in 2 position of purine ring			Tritium label in 5′ position of arabinose		
	T, μg/ml	NV, μg/ml	^3H_2O, %	T, μg/ml	NV, μg/ml	^3H_2O, %
0.083	10.1	8.5	16	10.8	9.2	14
0.25	8.5	6.4	25	9.8	8.4	14
0.50	7.0	3.5	50	9.0	7.6	16
1.0	6.0	2.3	62	8.1	6.8	16
2.0	5.2	0.62	88	6.7	4.1	39
4.0	4.8	0.10	98	5.7	2.6	54
8.0	4.6	0.05	99	4.7	1.1	77
24.0	4.4	0.05	99	4.5	0.8	82

[a]Drug concentrations are expressed in terms of ara-A equivalents. T refers to total tritium by direct liquid scintillation counting; NV refers to nonvolatile tritium following evaporation of the sample to dryness, oxygen-flask combustion, and liquid scintillation counting. Percent ^3H_2O calculated from 100(T − NV)/T.

rier-transform spectrophotometer for ir measurements; (4) a Perkin-Elmer 141 spectropolarimeter for optical rotations. Chromatography was carried out using silica gel 60 (Merck). Thin-layer chromatography (TLC) was carried out with silica gel 60-F plates (Merck, 0.25-mm thickness) using either solvent A, 9:1 ethyl acetate–methanol; B, 1:1 chloroform–ethyl acetate; or C, 45:45:10 chloroform–ethyl acetate–methanol. TLC on cellulose was carried out on Quanta Q5WF plates using solvent D, 65:25:10 2-propanol–water–ammonium hydroxide.

9-[5-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-β-D-arabinofuranosyl]adenine (2). To a suspension 29.0 g (0.113 mol) of 1 (dried at 100° in vacuo) in 900 ml of dimethylformamide and 110 ml of pyridine was added 40.9 g (0.121 mol) of chlorobis(4-methoxyphenyl)phenylmethane. The mixture was stirred with exclusion of moisture for 3 days, at which time the reaction was terminated by the addition of 50 ml of water, followed by stirring for 1 hr. The solvents were evaporated in vacuo at 50°, leaving a slurry, which was poured into 1500 ml of cold saturated sodium hydrogen carbonate solution. The resulting flocculent precipitate was removed by suction filtration, washed with 2 × 200 ml of water, and air-dried overnight.

The resulting semidry cake was extracted with 3 × 250 ml of hot ethyl acetate, and the combined, dried ($MgSO_4$) extracts were concentrated to ca. 200 ml. Warming and addition of benzene to the cloud point produced, upon slow cooling, white crystals that were isolated by suction filtration, washed with 2 × 25 ml of cold ethyl acetate, and dried in vacuo to yield, in two crops, 16.75 g (26%) of **2** that was homogeneous by TLC and NMR. Additional **2** was obtained by rapidly chromatographing the residue from evaporation of the mother liquors over a 6 × 20 cm column of silica gel using 300 ml of ethyl acetate, followed by successive, 300-ml elutions with 5 and 10% (v/v) methanolic ethyl acetate. By such processing, the total yield of **2** was 43.77 g (68%), homogeneous by TLC and NMR. An analytical sample was crystallized from ethyl acetate: R_f 0.60 (solvent A); mp 149–151° dec; $[\alpha]^{23}D$ −22° (*c* 1, methanol); λ_{max}^{MeOH} 236 nm (ε 24,300), 257.5 (17,100); λ_{max}^{KBr} 3360, 1644, 1606, 1516, 1257, and 1039 cm^{-1}; NMR data (acetone-d_6, 10% D_2O) 3.34, 3.52 (2, AB of ABX, H-5′,5′a, $J_{5',5'a}$ = 16.0 Hz, $J_{4,5'}$ = 6.1 Hz, $J_{4',5'a}$ = 4.0 Hz), 3.76 (6, s, OCH_3), 4.16 (1, m, X of ABX, width 14 Hz, H-4′), 4.33 (1, t, $J_{2',3'} = J_{3',4'}$ = 3.6 Hz, H-3′), 4.40 (1, t, H-2′), 6.47 (1, d, $J_{1',2'}$ = 4.1 Hz, H-1′), 6.80–6.89, 7.21–7.55 (13, m, aryl-H), and 8.18 ppm (2, s, H-2, H-8).

Anal. Calcd for $C_{31}H_{31}N_5O_6$ (569.6): C, 65.37; H, 5.49; N, 12.29. Found: C, 65.00; H, 5.67; N, 12.04.

***N*-Benzoyl-9-[2,3-di-*O*-benzoyl-β-D-arabinofuranosyl]adenine (3). A. Benzoylation of 2.** A solution of 16 g (28.1 mmol) of **2** and 25.5 g (113 mmol) of benzoic anhydride in 300 ml of pyridine was heated under reflux, with protection from moisture, for 2.5 hr, at which time TLC examination (solvent B) revealed complete replacement of **2** (R_f 0.22) by a zone (R_f 0.65), along with some presumed products of 5′-*O*-(triarylmethyl) cleavage and unreacted benzoic anhydride (R_f 0.87–0.91). Excess benzoic anhydride was decomposed by addition of 20 ml of methanol, followed by a 15-min reflux period. The solvents were removed in vacuo, and 3 × 100 ml of toluene was distilled from the residue, yielding a yellow gum. A solution of the gum in 500 ml of chloroform was washed successively with 2 × 100 ml of saturated sodium hydrogen carbonate and 1 × 100 ml of water, dried ($MgSO_4$), and evaporated to give a glassy residue upon drying in vacuo: yield 29 g (containing some methyl benzoate); R_f 0.65; λ_{max}^{MeOH} A_{231}/A_{276} 2.96; λ_{max}^{KBr} 1722 cm^{-1} (ν C=O, aryl ester, no aroyl anhydride C=O band).

B. Acid Hydrolysis of the 5′-*O*-[Bis(4-methoxyphenyl)-phenylmethyl] Protective Group of Benzoylated 2. The crude benzoylated product from the foregoing was treated with 530 ml of 2:2:1 (v/v) water–90% formic acid–tetrahydrofuran. Dissolution of the gum was effected by warming the mixture to 50°, and the resulting solution was stirred for 45 min at ambient temperature. The acidic solution was neutralized with 50% aqueous sodium hydroxide (ice cooling), and the products were partitioned into 3 × 200 ml of chloroform. The combined extracts were washed with 2 × 200 ml of water, dried ($MgSO_4$), and evaporated to yield a glassy residue that by TLC (solvent B) revealed two major products, **3** (R_f 0.45) and presumed triarylmethyl cleavage products (R_f 0.95).

Crystallization of the crude product was effected by pouring 310 ml of boiling absolute ethanol onto the glass, which dissolved and immediately deposited crystals. Cooling to 25° for 10 hr gave, upon filtering and washing with cold ethanol, followed by drying in vacuo, 9.88 g (57%) of **3**: homogeneous by TLC and NMR; mp 186–188°. An analytical sample was prepared by recrystallization from 4:1 ethanol–acetone: mp 187.5–188°; $[\alpha]^{23}D$ −97.6° (*c* 1, chloroform); λ_{max}^{MeOH} 232 nm (ε 39,400), 279 (22,000); λ_{max}^{KBr} 1725, 1704, 1618, 1585, 1450, 1260–1280, and 710 cm^{-1}; NMR data (chloroform-*d*) 4.04, 4.18 (2, AB of ABX, $J_{5',5'a}$ = 12.5 Hz, $J_{4',5'}$ = 2.5 Hz, $J_{4',5'a}$ = 3.1 Hz, H-5′,5′a), 4.38 (1, m, width 10.6 Hz, X of ABX, H-4′), 5.99–6.16 (2, m, H-2′, H-3′), 6.79 (1, d, $J_{1',2'}$ = 5.0 Hz, H-1′), 7.14–7.68, 7.88–8.14 (15–16, m, aryl-H), 8.76, 8.32 (2, s, H-2, H-8), and 9.02 ppm (1, s, NH).

Anal. Calcd for $C_{31}H_{25}N_5O_7$ (579.6): C, 64.24; H, 4.35; N, 12.09. Found: C, 64.34; H, 4.40; N, 12.37.

9-β-D-Arabinofuranosyladenine-5′-*d* (6a). A solution of 600 mg (1.04 mmol) of **3** and 650 mg (3.16 mmol) of dicyclohexylcarbodiimide in 5 ml of dry methyl sulfoxide was treated with 0.04 ml (0.47 mmol) of dichloroacetic acid. The mixture was stirred, under dry conditions, for 1.5 hr, at which time **3** (R_f 0.80) was shown by TLC (solvent C) to have been converted to the aldehyde **4** (R_f 0.83, positive to alkaline Purpald spray reagent[19]). The reaction was terminated by addition of 250 mg (2.0 mmol) of oxalic acid dihydrate in 5 ml of ethanol.

The precipitated solids were removed by filtration and washed with 2 × 3 ml of ethanol. The acidic filtrate was carefully neutralized (pH 7.5) with a few drops of concentrated aqueous sodium hydroxide, and 30 mg (0.72 mmol) of sodium borohydride-d_4 was added, portionwise, with stirring, to the solution. After 10 min, the excess reagent was decomposed by addition of glacial acetic acid (final pH 6.5), and the solvents were removed in vacuo at 40–60°.

The crude product was extracted with 2 × 20 ml of hot chloroform and applied to a 0.9 × 60 cm column of silica gel and eluted with solvent B. After a forerun containing *N,N′*-dicyclohexylurea, pure **5a** was obtained and crystallized as for **3**: yield 456 mg (76%); mp 187–188°; NMR data (chloroform-*d*) revealed a spectrum as for **3**, but with H-5′,5′a appearing as a four-line multiplet (integral 1 proton) and H-4′ as a narrower multiplet (width 7.6 Hz).

The product from the foregoing was suspended in 100 ml of absolute methanol and saturated with gaseous ammonia at 0°. The resulting solution was allowed to stand at 25° for 26 hr, at which time the methanol was evaporated, and the residue was triturated in 10 ml of water to give a crystalline mass. Filtration of the crystals, washing with 2 × 5 ml of methanol, and drying at 60° in vacuo

yielded 206 mg (72% from 3) of **5a** as the monohydrate: mp 251–252° (lit.[20] for the nondeuterated analog 1 = 257–257.5°); $\lambda_{max}^{0.1\ N\ HCl}$ 257 nm (ε 15,000).

9-β-D-Arabinofuranosyladenine-5′-*t* (6b). As for **6a**, 400 mg (0.69 mmol) of **3** was oxidized, and the neutralized solution (pH 7.5) of the aldehyde was reduced with 13 mg (ca. 0.3 mmol) of sodium borohydride-t_4 (50 mCi as ^3H). An additional 20 mg of unlabeled sodium borohydride was added to ensure complete reduction of **4**. Processing as for **5a** gave, after chromatography, pure **5b** (330 mg, 83%), which was hydrolyzed to yield 152 mg (80% from **3**) of **6b** as the hydrate, identical (R_f 0.85) with authentic 1 by chromatography on cellulose (solvent D): $\lambda_{max}^{0.1\ N\ HCl}$ 257 nm (ε 15,200).

Radiochemical assay indicated 73.98 μCi mg^{-1} of ^3H; zone analysis by TLC indicated 99.7% of the activity at R_f 0.8–0.9.

9-β-D-Arabinofuranosyladenine-5′-*t* 5′-Phosphate (7b). Pyridine (10 ml) was added dropwise, under dry conditions, over a period of 0.5 hr, to a stirred mixture of 20 ml of acetonitrile, 10 ml of phosphoryl chloride, and 2.65 ml of 98% formic acid, maintained at 10–14° with aid of an ice–salt bath. To this mixture, cooled to 3°, was added, in one portion with stirring, a mixture of 1.6 g of crude **6b** and 3.4 g of dry 1 (19.4 mmol, total), whereby the temperature rose to 8° in 5 minutes. The mixture was stirred at 3–5° for 1 hr and poured onto 100 ml of ice cubes, and 15 ml of 50% aqueous sodium hydroxide was added over a period of 0.75 hr in such a manner as to keep the pH ca. 1.5. The solution was then slurried with a mixture of 75 g of Darco G-60 and 37 g of hyflo-Supercel in 100 ml of water and then filtered with suction to near dryness. The charcoal–Supercel slurry was washed in the Büchner funnel successively with 600 ml of water (wash A, 0 ODU, 7.6 μCi), 400 ml of 1:1 water–ethanol containing 2% ammonium hydroxide [wash B, 225,000 ODU (260 nm, pH 1), 29.64 mCi], and 300 ml of additional eluent solution (wash C, 11,470 ODU, 0.866 mCi). The solvent was evaporated from wash B to give a solid that was dissolved in 20 ml of water and adjusted to pH 1.5 with concentrated hydrochloric acid to yield, upon filtering and drying the crystalline deposit at 60° in vacuo, 4.16 g of **7**. Dissolution of the product in 15 ml of water with aid of ammonium hydroxide (pH 7.5) and crystallization at pH 1.5 furnished, upon drying, 3.64 g (55% based on 5.0 g of 1) of **7**: $\lambda_{max}^{pH\ 7}$ 259 nm ($E_{1\ cm}^{1\%}$ 401); $[\alpha]^{23}D$ +12.1° (c 1, water, pH 7). High-pressure ion-exchange chromatography (0.21 × 60 cm Aminex A-7 resin, 0.15 ml min^{-1}, 2000 psi, 0.1 *M* disodium hydrogen phosphate, adjusted to pH 6.67 with 0.2 *M* phosphoric acid, 254-nm detection) analyzed the product as >99.2% **7**. TLC analysis revealed upon radioassay of a sectioned plate (R_f 0.60, system D) >96% of **7**, with ca. 2% of 1 (R_f 0.85) and ca. 2% (R_f 0.45) of presumed polyphosphorylated **7**: specific activity, 5.01 μCi mg^{-1}.

The mother liquors from the foregoing crystallizations were combined, adjusted to pH 7.5 with ammonium hydroxide, and applied in 500 ml of water to a 2.5 × 80 cm column of DEAE-Sephadex (HCO_3^-). Elution was carried out with a linear gradient of 0.005–0.25 *M* triethylammonium bicarbonate, 20-ml fractions being collected. Compound **7b** appeared at a buffer strength of 0.16–0.20 *M* (17,800 ODU), cleanly separated from neutral and monoanionic impurities. The appropriate fractions were combined and concentrated to ca. 50 ml. Decationization was effected over a 1 × 6 cm column of Dowex-50 X8 (H^+) resin by eluting with 500 ml of water. Lyophilization of the eluate, followed by drying the solid at 100° in vacuo, gave 426 mg of **7b**: $[\alpha]^{23}D$ +16° (c 1, water, pH 7); $\lambda_{max}^{pH\ 7}$ 259 nm (ε, 15,550, $E_{1\ cm}^{1\%}$ 426) [lit.[21] $[\alpha]^{25}D$ +17.7° (c 1, water, pH 7); $\lambda_{max}^{pH\ 7}$ 259 nm ($E_{1\ cm}^{1\%}$ 418)]. Radioassay of a sectioned TLC plate (system D) showed >98% of the activity at R_f 0.60: specific activity 5.06 μCi mg^{-1}; NMR data (9:1 pyridine-d_5–water-d_2) 4.68 (1, m, width 18 Hz, H-4′), 4.84–5.04 (2, m, H-5′,5′a), 5.16 (2, m, width 12 Hz, H-2′, H-3′), 6.90 (1, d, $J_{1',2'}$ = 5 Hz, H-1′), 8.48, 8.91 (1, 1, s, H-2, H-8).

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