phosphate, pH 12. Due to the pH of the column after acid washing, concentration, and pH of the sodium phosphate, the fractions containing L-[4-^{11}C]aspartic acid were isotonic and at pH 7.4. The solution was sterilized by passage through a 0.22-μm Millipore filter and transferred into a sterile, pyrogen-free vial. If [4-^{11}C]oxaloacetic acid was desired, L-glutamic acid was omitted from the reaction mixture, and the second enzyme column, glutamic/oxaloacetic acid transaminase, was deleted from the system.[22] [4-^{11}C]Oxaloacetic acid and unreacted substrates were collected in a vial with 1 mL of 0.9 M $NaHCO_3$, and the solution was first acidified with 2 N HCl, stirred for 3 min to expel unreacted $^{11}CO_2$, and then neutralized (pH 7.4) by the addition of 2 N NaOH. The solution was made isotonic and finally passed through a 0.22-μm pore filter into a sterile, pyrogen-free vial. The production of [4-^{11}C]oxaloacetic acid and L-[4-^{11}C]aspartic acid was completed within 15–25 min after cyclotron production of $^{11}CO_2$. The actual product yields were 20–25 mCi of L-[4-^{11}C]-aspartic acid and 30–35 mCi of [4-^{11}C]oxaloacetic acid, with a specific activity of 3.5–5.0 Ci/mmol at the time of injection.

Verification of Radiochemical Properties. The radiochemical purity and specific activity of the L-[4-^{11}C]aspartic acid preparation were verified by using the *o*-phthaldialdehyde (OPT) precolumn fluorescence derivatization procedure as previously described for ^{13}N-labeled L-amino acids.[3] The strongly fluorescent amino acid–OPT product was separated with reversed-phase HPLC[23] (Beckman Model 334, Ultrasphere ODS, 5 μm, 4.6 × 150 mm column; 55% 100 mM potassium phosphate, pH 7.4, and 45% MeOH; flow rate 1.0 mL/min; Varian Fluorichrom fluorescence detector; Ortec Model 406A radioactivity detector; retention time for L-[4-^{11}C]aspartate–OPT complex, 2.5 min). The absence of $^{11}CO_2$ in the final ^{11}C-labeled oxaloacetic acid preparation was verified by direct analysis of radioactive components of the mixture by using reversed-phase HPLC, under similar chromatographic conditions as described above, except that 98% 10 mM potassium phosphate, pH 2.5/2% methanol was used as a solvent: retention time for [4-^{11}C]oxaloacetic acid, 2.5 min; for $^{11}CO_2$, 6.0 min. The specific activity of [4-^{11}C]oxaloacetate was calculated from that obtained for L-[4-^{11}C]aspartic acid.

Myocardial Uptake of ^{11}C-Labeled Substrates. For each experiment the ^{11}C-labeled compounds (20–30 μCi/0.2 mL) were injected into the left anterior descending coronary artery of open-chest instrumented dogs. The venous and arterial blood samples for ^{11}C metabolite determination were taken from the left anterior coronary vein and left atrial appendage, respectively. The myocardial activity was recorded for 20 min; the time–activity curve was corrected for physical decay and plotted on semilogarithmic paper, and the numerical values were printed out at 0.1-s intervals. The residue fraction of ^{11}C activity retained in myocardium was determined with a graphic extrapolation of the third slow clearance phase (*C*) back to the time of the maximal peak (*A*) representing the total amount of activity injected. The residue fraction was computed as the ratio of *C*/*A*. The half-times of the components of the clearance curve were calculated from the slopes (Figure 1). All experimental protocols for the single pass uptake technique have been reported in detail previously,[24] and the technique has been used with ^{13}N-labeled L-amino acids.[3] This method has also been previously employed with other radiolabeled compounds and validated for studies in the brain[25,26] and in the heart.[24,27,28] Inhibition of myocardial transaminases with aminooxyacetic acid was produced as previously described.[13] Tomographic images were obtained with the UCLA positron emission computed axial tomograph, ECAT,[29] as described previously,[30] following intravenous injections of 5 mCi of L-[4-^{11}C]aspartic acid in mongrel dogs or rhesus monkeys. Imaging was begun 5 min after injection.

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(22) The enzymatic synthesis were performed using a remote, semiautomated system as described for the preparation of ^{13}N-labeled L-amino acids.[3]

(23) Roth, M. *Anal. Chem.* **1971**, *43*, 880.
(24) Schelbert, H. R.; Phelps, M. E.; Huang, S. C.; MacDonald, N. S.; Hansen, H.; Selin, C.; Kuhl, D. E. *Circulation* **1981**, *63*, 1259.
(25) Raichle, M. E.; Eichling, J. O.; Straatmann, M. G.; Welch, M. J.; Larson, K. B.; Ter-Pogossian, M. M. *Am. J. Physiol.* **1976**, *230*, 543.
(26) Phelps, M. E.; Hoffman, E. J.; Raybaud, C. *Stroke* **1977**, *8*, 694.
(27) Poe, N. D. *J. Nucl. Med.* **1972**, *13*, 557.
(28) Metzger, J. M.; Ph.D. Thesis, Washington University, St. Louis, MO, 1972.
(29) Phelps, M. E.; Hoffman, E. J.; Huang, S. C.; Kuhl, D. E. *J. Nucl. Med.* **1978**, *19*, 635.
(30) Schelbert, H. R.; Phelps, M. E.; Hoffman, E. J.; Huang, S. C.; Selin, C. E.; Kuhl, D. E. *Am. J. Cardiol.* **1979**, *43*, 209.

1-β-D-Arabinofuranosyl-1*H*-imidazo[4,5-*c*]pyridine (*ara*-3-Deazaadenine)

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The arabino isomer of 3-deazaadenosine was prepared and evaluated for biological activity. It is a mediocre inhibitor of 5-adenosyl-L-homocysteine hydrolase. It was only slightly cytotoxic and slightly inhibitory to the growth of herpes simples type 1 virus in L929 cells.

S-Adenosyl-L-homocysteine (AdoHcy) is the product of the biological methylation reactions in which *S*-adenosylmethionine (AdoMet) serves as the methyl donor, and *S*-adenosyl-L-homocysteine hydrolase (EC 3.3.1.1) catalyzes the hydrolysis of AdoHcy in eukaryotes to adenosine and L-homocysteine. AdoHcy is a potent inhibitor of biological methylations and the AdoMet/AdoHcy ratio is thought to be important in cellular control

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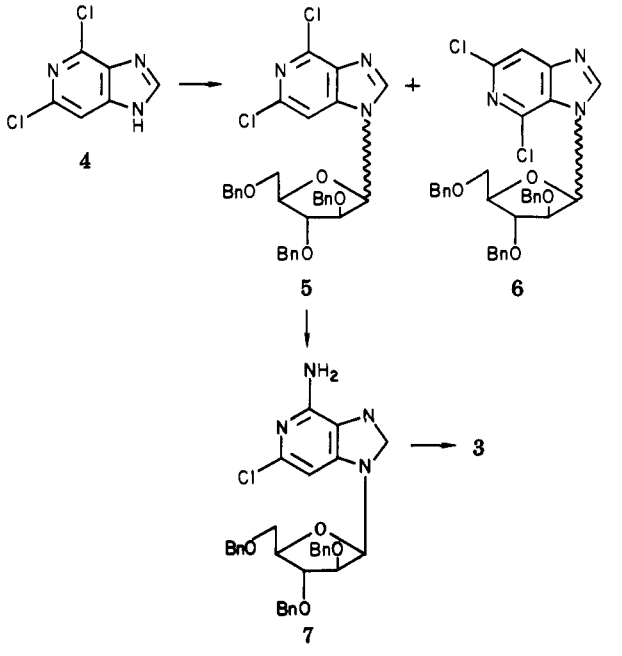
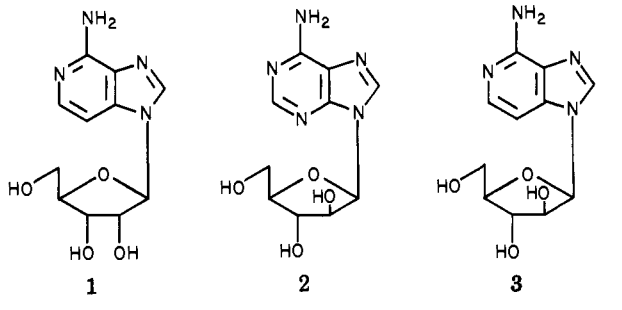
Scheme I



of these methylations.[1]

3-Deazaadenosine (1-β-D-ribofuranosyl-1*H*-imidazo[4,5-*c*]pyridine, **1**) is an alternative substrate and potent com-



petitive inhibitor of AdoHcy hydrolase[2] that has shown activity against herpes simplex type 1[3] and oncogenic DNA viruses.[4,5] Since this compound is neither deaminated by adenosine deaminase[6] nor phosphorylated by adenosine kinase,[7] its activity is thought to result from its ultimate effects on the methylation reaction via AdoHCy hydrolase.

The recent finding that the well-known antiviral agent 1-β-D-arabinofuranosyladenine (*ara*-A, **2**) inactivates AdoHcy hydrolase[8,9] caused us to undertake the preparation of the arabino analogue of 3-deazaadenosine (1-β-D-arabinofuranosyl-1*H*-imidazo[4,5-*c*]pyridine, **3**).

The best procedure for the preparation of 3-deazaadenosine (1)—fusion of tetra-*O*-acetyl-β-D-ribofuranose with 4,6-dichloroimidazo[4,5-*c*]pyridine (4) followed by amination with concomitant deacetylation and then catalytic hydrodechlorination[10] —had to be modified for the preparation of **3** (Scheme I), since fusion with tetra-*O*-acetyl-D-arabinofuranose would give predominately the α- and not the desired β-arabino nucleoside.[11] Consequently, 4 was allowed to react with 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride in refluxing 1,2-dichloroethane in the presence of molecular sieve, a modification of the procedure[12] used for the preparation of 2,6-dichloro-9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)purine (containing a trace of α-anomer[13]). In this case, NMR and HPLC data indicated that four nucleosides were formed. The UV spectra of these nucleosides obtained by stop-flow technique from the HPLC showed that the first two nucleosides eluted (μPorasil, $CHCl_3$) were 4,6-dichloro-3-substituted-imidazo[4,5-*c*]pyridines (**6**), while the latter pair were the desired 1-substituted compounds (**5**). Comparison with previous data indicated that the order of elution was 3-β, 3-α, 1-α, 1-β, that the N-1 to N-3 ratio was 4.4, that the β/α ratio was 5.8, and that 70% of the nucleoside mixture was the desired 1-β isomer. The mixture was resolved by silica gel chromatography and 4,6-dichloro-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-1*H*-imidazo-[4,5-*c*]pyridine (β-**5**) suitable for amination was obtained in 34–37% yield. Treatment of β-**5** with ethanolic ammonia at 140 °C for 4 days gave a 50% yield of the 4-amino-6-chloroimidazo[4,5-*c*]pyridine **7**. Hydrodechlorination and debenzylation with 30% Pd/C took place very slowly in ethanol. Addition of an equivalent of HCl caused the reduction to proceed in 48 h at 40–50 psi. The desired product, 4-amino-1-β-D-arabinofuranosylimidazo[4,5-*c*]pyridine (**3**) adhered strongly to the catalyst and had to be eluted with boiling water. Recrystallization from water gave analytically pure product in about 50% yield. The alcoholic reduction solution contained a mixture of partially debenzylated nucleosides (MS and TLC) that obviously adhered less tightly to the catalyst.

Biologic Evaluations. *ara*-3-deazaadenine inhibited AdoHcy hydrolase only at relatively high levels ($I_{50} \simeq 0.1$ mM vs. 3-deazaadenosine I_{50} of 0.004 mM^2) and was marginally active against herpes simplex virus type 1 grown in L929 cells having a virus rating of 0.8, and an MED_{50} of 189 μg/mL compares to *ara*-A in the same experiment with a virus rating of 3.7 and an MED_{50} of 0.8 μg/mL.[14] It was only slightly toxic to the L929 cells at the highest dose level tested (1 mM). Since the activity of 3-deazaadenosine appears to depend on its potent inhibition of AdoHcy hydrolase, the minimal antiviral activity of **3** is not surprising.

Experimental Section

All evaporations were carried out in vacuo with a rotary evaporator. Analytical samples were normally dried in vacuo over P_2O_5 at room temperature for 16 h. Analtech precoated (250 μm) silica gel G(F) plates were used for TLC analyses; the spots were detected by irradiation with a Mineralight and by charring after

(1) Cantoni, G. L.; Richards, H. H.; Chiang, P. K. In "Transmethylation"; Usdin, E.; Borchardt, R. T.; Creveling, E. R., Eds.; Elsevier: New York, 1979; pp 154–164.
(2) Guranowski, A.; Montgomery, J. A.; Cantoni, G. L.; Chiang, P. K. *Biochemistry* **1981**, *20*, 110.
(3) Bodner, A. J.; Cantoni, G. L.; Chiang, P. K. *Biochem. Biophys. Res. Commun.* **1981**, *98*, 476.
(4) Bader, J. P.; Brown, N. R.; Chiang, P. K.; Cantoni, G. L. *Virology* **1978**, *89*, 494.
(5) Chiang, P. K.; Cantoni, G. L.; Bader, J. P.; Shannon, W. M.; Thomas, H. J.; Montgomery, J. A. *Biochem. Biophys. Res. Commun.* **1978**, *82*, 417.
(6) Chiang, P. K.; Richards, H. H.; Cantoni, G. L. *Mol. Pharmacol.* **1977**, *13*, 939.
(7) Miller, R. L.; Adamczyk, D. L.; Miller, W. H.; Koszalka, G. W.; Rideout, J. L.; Beachman III, L. M.; Chao, E. Y.; Haggerty, J. J.; Krenitsky, T. A.; Elion, G. B. *J. Biol. Chem.* **1979**, *254*, 2346.
(8) Chiang, P. K.; Guranowski, A; Segall, J. E. *Archiv. Biochem. Biophys.* **1981**, *207*, 175.
(9) Hershfield, M. S. *J. Biol. Chem.* **1979**, *254*, 22.
(10) Montgomery, J. A.; Shortnacy, A. T.; Clayton, S. D. *J. Heterocycl. Chem.* **1977**, *14*, 195.
(11) Montgomery, J. A.; Shortnacy, A. T.; Arnett, G.; Shannon, W. M. *J. Med. Chem.* **1977**, *20*, 401.
(12) Keller, F.; Botviniek, I. J.; Bunker, J. E. *J. Org. Chem.* **1967**, *32*, 1644.
(13) Montgomery, J. A.; Hewson, K. *J. Med. Chem.* **1969**, *12*, 498.
(14) Shannon, W. M.; Shortnacy, A.; Arnett, G.; Montgomery, J. A. *J. Med. Chem.* **1974**, *17*, 361.

spraying with saturated $(NH_4)_2SO_4$. Compounds containing amino groups were also detected with ninhydrin spray. All analytical samples were essentially TLC homogeneous. Melting points were determined with a Mel-Temp apparatus and are not corrected. The UV absorption spectra were determined in 0.1 N HCl, pH 7 buffer, and 0.1 N NaOH with a Cary 17 spectrophotometer. The ^1H NMR spectra were determined with a Varian XL-100-15 spectrometer in Me_2SO-d_6 with tetramethylsilane as an internal reference: chemical shifts quoted in the case of multiplets are measured from the approximate center. The high-pressure liquid chromatographic analysis was carried out with a Waters Associates ALC-242 chromatography with an M-6000 pump and equipped with a μPorasil column (0.25 in. × 30 cm) using $CHCl_3$ (1% EtOH) as the solvent. The stop–flow UV spectra were determined with a Beckman 25 UV spectrophotometer interfaced to the chromatograph. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within 0.4% of the theoretical values.

4-Amino-1-β-D-arabinofuranosylimidazo[4,5-*c*]pyridine (3). A solution of 4-amino-6-chloro-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)imidazo[4,5-*c*]pyridine (29.8 g, 52.2 mmol) in 1.3 L of EtOH containing 1 equiv of HCl and 5 g of 30% Pd/C catalyst was hydrogenated at 40–50 psi for about 48 h. The catalyst was removed by filtration and extracted with boiling water, which was cooled, neutralized, and evaporated to dryness. The residue was recrystallized from water with charcoal treatment: yield 6.6 g (46.8%); mp 286–288 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1 and 7, 262 nm (10.5); at pH 13, 264 nm (10.9); NMR (Me_2SO-d_6) δ 3.5 (broad, H_2O), 3.7 (m, $H_{4'}$, and 2 $H_{5'}$), 4.15 (m, $H_{2'}$ and $H_{3'}$), 5.6 (broad, OH), 6.13 (d, $J_{1',2'}$ = 4 Hz, $H_{1'}$ and NH_2), 6.85 (d, H_7), 7.65 (d, H_6), 8.2 (s, H_2). Anal. ($C_{11}H_{14}N_4O_4 \cdot 0.33H_2O$) C, H, N.

4,6-Dichloro-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)imidazo[4,5-*c*]pyridine (β-5). To a suspension of 54 mg (0.287 mmol) of 4,6-dichloroimidazo[4,5-*c*]pyridine (4) and 0.5 g of 4Å molecular sieves in 10 mL of dry 1,2-dichloroethane was added a solution of 2,3,5-tri-*O*-benzylarabinofuranosyl chloride (0.288 mmol from 164 mg of 1-*p*-nitrobenzoyl-2,3,5-tri-*O*-benzylarabinofuranose) in 15 mL of dry 1,2-dichloroethane and an additional 0.5 g of molecular sieve. The mixture was refluxed overnight with stirring. The solid removed by filtration was washed with chloroform, and the combined organic solvents were washed with saturated sodium bicarbonate followed by water. The solution was dried over $MgSO_4$, filtered, and evaporated to dryness: yield 181.3 mg. This material (127.7 mg) was shown by HPLC with stop–flow UV scans to be a mixture of two 1-substituted (5) and two 3-substituted (6) imidazo[4,5-*c*]pyridines and was resolved by chromatography on silica gel plates (Brinkmann) developed three times with cyclohexane–ethyl acetate (3:1). Elution of the major band with ethyl acetate gave the 1-β isomer (β-5) as a glass (63 mg, 37%): UV (95% EtOH) λ_{max} ($\epsilon \times 10^{-3}$) 208 (54.6), 252 (sh), 258 (7.03), 273 (5.67), 281 nm (sh); NMR ($CDCl_3$) δ 3.65 (m, 2 $H_{5'}$), 4.25 (m, $H_{2'}$, $H_{3'}$, $H_{4'}$, O_2-CH_2), 4.6 (m, O_3-CH_2, O_5-CH_2), 5.98 (d, $J_{1',2'}$ = 4 Hz, $H_{1'}$), 7.0 and 7.3 (2 m, Ph and H_7), 8.25 (s, H_2).

Repetition of the reaction using 37.4 g (0.199 mol) of 4 and 113.3 g (0.2 mol) of 1-*p*-nitrobenzoyl-2,3,5-tri-*O*-benzylarabinofuranose followed by chromatography on a silica gel column (Mallinkrodt 7, 2.25 × 41 in.) with cyclohexane–ethyl acetate (3:1) twice gave 39.4 g (33.5%) of material that was used directly in the next step.

4-Amino-6-chloro-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)imidazo[4,5-*c*]pyridine (7). A solution of 5 g (8.5 mmol) of 4,6-dichloro-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)imidazo[4,5-*c*]pyridine in 50 mL of ethanol saturated with anhydrous ammonia (0 °C) was heated in a stainless-steel bomb at 140 °C for 4 days. The residue from evaporation of the reaction mixture was dissolved in hot ethanol, and the solution was treated with charcoal and filtered through Celite. The solid that crystallized from the chilled solution was removed by filtration, washed with EtOH, and dried in vacuo: yield 2.6 g (54%); mp 115–116 °C. Recrystallization from EtOH gave 2.3 g (48%): mp 116–117 °C; NMR ($CDCl_3$) δ 3.7 (m, 2 $H_{5'}$), 4.2 (m, $H_{2'}$, $H_{3'}$, $H_{4'}$ and O_2-CH_2), 4.57 (2 s, O_3-CH_2 and O_5-CH_2), 5.45 (br s, NH_2), 5.98 (d, $J_{1',2'}$ = 4 Hz, $H_{1'}$), 6.65 (s, H_7), 6.9 and 7.3 (2 m, phenyl), 8.4 (s, H_2). Anal. ($C_{32}H_{31}ClN_4O_4 \cdot 0.5C_2H_5OH$) C, H, N.

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Syntheses and Diuretic Activity of 1,2-Dihydro-2-(3-pyridyl)-3*H*-pyrido[2,3-*d*]pyrimidin-4-one and Related Compounds

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The title compound, 5, was prepared and found to be a potent diuretic in the rat. At 27 mg/kg, urine output was 250% of the saline control, and the excretion of electrolytes was similar to the hydrochlorothiazide control. At 80 mg/kg, the potassium excretion was the same as the saline control, and the sodium and chloride excretions more than doubled. Several analogues were prepared and tested. Some show diuretic activity.

This paper reports our efforts to synthesize a potassium-sparing diuretic using a pyrido[2,3-*d*]pyrimidine as the lead compound. Some potassium-sparing diuretics, such as triamterene, are derivatives of nitrogen-containing heterocyclic compounds. They usually are not of sufficient natriuretic potency when used alone and frequently must be delivered in conjunction with other diuretic agents in order to augment natriuresis and reduce potassium loss.[1]

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