standard TLC plate (20 × 6 cm). An analogous reaction, using ^{127}I, was conducted with all reagents and solvents at 3 times higher concentration. The contents of this reaction were applied to the second half of the same TLC plate. The plate was developed for about 10 cm (CH_3OH–$CHCl_3$, 15:35) and then air-dried in a hood. The TLC plate was cut in half and the ^{127}I side used to mark (visualized under UV light, TLC R_f 0.41, CH_3OH–$CHCl_3$, 15:35) the expected band for the ^{125}I amine on the remaining half. The zone corresponding to the ^{125}I amine, so identified, was scrapped into a 10-mL vial containing CH_3OH (5 mL). The mixture was vortexed for 1 min and then centrifuged. The organic layer, containing the labeled amine, was removed with a pipet and retained. This procedure was repeated twice more, the organic layers were combined, and the solvent was removed under a stream of nitrogen. The combined CH_3OH extracts was found to contain 60% of the originally added $Na^{125}I$.

The ^{125}I amine was dissolved in aqueous acetic acid (100 μL, AcOH–H_2O, 2:8, v/v). After cooling (ice bath), $NaNO_2$ (0.3 μg, 4.3 nmol) in water (2 μL) was added and the reaction mixture allowed to stand for 5 min, before the addition of sodium azide (0.3 μg, 4.6 nmol) in water (2 μL). After being allowed to stand an additional 5 min, the entire reaction mixture was applied to a standard TLC plate (20 × 6 cm). The plate was developed for about 10 cm (CH_3OH–$CHCl_3$, 15:35) and then air-dried in a hood. The ^{125}I azide (TLC R_f 0.52, CH_3OH–$CHCl_3$, 15:35) was isolated in the same manner as its precursor amine. A portion of the product so obtained was diluted with 12 μg of the fully characterized unlabeled **28** and subjected to the usual reversed-phase HPLC analysis. Of the applied radioactivity, 87% was recovered in the eluate and 99.6% of the recovered radioactivity resided in the UV-absorbing peak corresponding to the iodo azide **28** (t_R = 29.4 min).

Biology. Inhibition of U46619 Activity. Platelet aggregation was studied by the turbidometric method of Born[42] at 37 °C over a 3-min time course. Citrated human platelet-rich plasma from normal, healthy donors who denied receiving any medication for 10 days was purchased from a commercial blood bank. The plasma was centrifuged at 164*g* to remove any remaining red blood cells and maintained at 25 °C until the experiments were performed. The antagonists were dissolved in 50% ethanol/normal saline and then added as 10-μL aliquots per 1 mL of platelet-rich plasma to give the specified concentration 3 min before being challenged with U46619 (3 μM). Inhibition was measured as a percentage of control bloods that had not received any drug. The ED_{50} values were estimated from three to four concentrations of drug that gave inhibition in the range of 10–90%.

Dark and Light Inhibition Studies of Aromatic Azides. 13-Azaprostanoic acid, or one of the three derivatives listed in Table II, was added to platelet-rich plasma at the minimum concentration necessary to produce 100% inhibition of 3 μM U46619-induced aggregation. The incubates were photolyzed (30 min) with a HBD100W OSRAM mercury light. This extended photolysis time was necessary because of the high UV absorption by the plasma. The platelets were then centrifuged and the platelet pellet resuspended in Tris·HCl buffer (pH 7.4). Platelet aggregation of the resuspended cells was induced by addition of ADP (10 μM) or U46619 (0.5 μM). The results are presented in Table II, where the values were calculated by the following relationship: percent inhibition relative to 13-azaprostanoic acid = [1 − (residual platelet activity with given azide/the residual platelet activity with 13-azaprostanoic acid)] × 100.

Acknowledgment. This work was supported by the National Institutes of Health (HL 24530). U46619 was kindly provided by Dr. Gordon L. Bundy of the Upjohn Co., Kalamazoo, MI. The investigators acknowledge the Research Resources Center at the University of Illinois at Chicago for providing in part the instrumentation used in this study and Lolita Strebel for technical assistance in the aggregation studies.

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Notes

Synthesis of 5-β-D-Ribofuranosylnicotinamide and Its *N*-Methyl Derivative. The Isosteric and Isoelectronic Analogues of Nicotinamide Nucleoside[1]

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The pyridine *C*-nucleosides 5-β-D-ribofuranosylnicotinamide and its *N*-methylpyridinium derivative (**1** and **2**), which are isosteric and isoelectronic, respectively, to nicotinamide nucleoside were synthesized. Condensation of 3-bromo-5-lithiopyridine with 2,4:3,5-di-*O*-benzylidene-D-aldehydoribose (**7**) afforded an allo/altro mixture of the corresponding bromopyridine derivatives, which were converted into nicotinamide *C*-nucleoside precursors **10**. Mesylation of the hydroxyl group of **10** followed by acid hydrolysis of the product afforded the anomeric nicotinamide *C*-nucleosides. The β anomer **1** was separated and treated with MeI to give **2**.

The pyridine *C*-nucleosides **1** and **2** (Chart I), which are isosteric and isoelectronic, respectively, to nicotinamide nucleoside, may be converted biologically into the corresponding NAD analogues **3** and **4** and exert interesting biological activities. The noncharged NAD isostere **3** may inhibit the NAD-dependent enzyme IMP-dehydrogenase and may induce cytotoxicity by blocking the de novo GMP synthesis. The isoelectronic analogue of NAD (**4**) should be capable of participating in the same enzymic oxidoreduction process(es) as NAD. Both **3** and **4** certainly cannot serve as ADP-ribose donors and may inhibit ADP-ribosylation, which is important in protein synthesis regulation[2-4] and in the DNA repair process.[5-8] Actually, the

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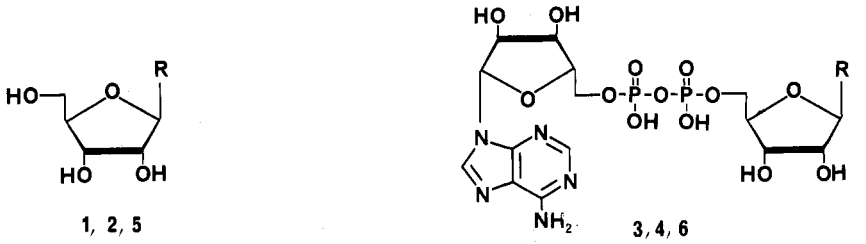
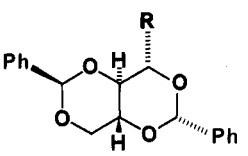
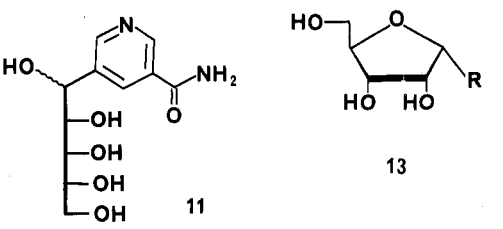
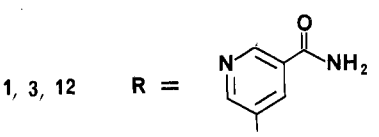
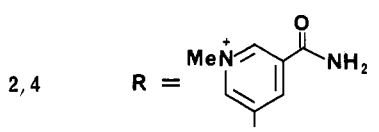
Chart I

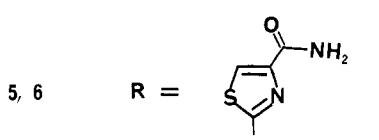


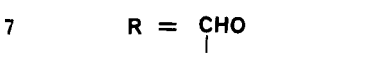


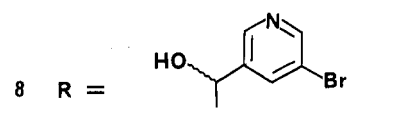


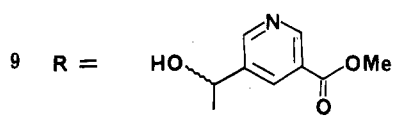


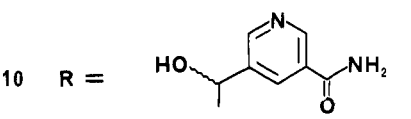


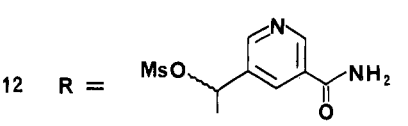












synthetic *C*-nucleoside tiazofurin (**5**)[9-14] is converted into the NAD analogue TAD (**6**) and inhibits IMP-dehydrogenase,[13,15] causing a profound depletion of GMP and accumulation of IMP.[12,15-17] TAD has been synthesized[13,18] and is found to be a much more potent inhibitor of IMP-dehydrogenase than tiazofurin.[19] We report herein the synthesis and preliminary biological results of the pyridine *C*-nucleosides **1** and **2**.

Condensation of 2,4:3,5-di-*O*-benzylidene-D-aldehydoribose (**7**)[20] with 3-bromo-5-lithiopyridine afforded a mixture of allo/altro (45:55) isomers of the bromopyridine derivative **8** in 57% yield as microcrystals.

Lithiation of the mixture **8** followed by carboxylation with CO_2 and esterification of the product with CH_2N_2 led to an isomeric mixture of esters **9** in 65% yield.

Treatment of the mixture **9** with NH_3/MeOH afforded epimeric nicotinamide derivatives **10** in 87% yield as a glass.

All attempts at debenzylidenation[21-23] of **10** resulted in

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the formation of the pentahydroxy derivative **11** instead of the ribofuranosyl structures (**1** and its α-isomer, **13**).[24] Mesylation of **10** to **12** followed by acid hydrolysis, however, afforded a mixture of **1** and **13**. Separation of these α,β isomers was readily achieved on silica gel plates. Treatment of **1** with a large excess of MeI in 1:1 MeOH/Me_2CO gave **2**.

Preliminary studies[25] of **1** against four tumor cells, P-815, CCRF-CEM, F-Molt-3, and HL-60, in vitro show 0.50% inhibition of growth at concentrations of 16.0, 13.0, 15.0, and 24.0 μg/mL, respectively, indicating that **1** is about 1–2 log orders less active than tiazofurin (**5**). The isoelectronic analogue **2**,[26] as expected, was found to be inactive against these cells.

Experimental Section

General Methods. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL FT90Q spectrometer using Me_2SO-d_6 or $CDCl_3$ as the solvents and Me_4Si as the internal standard. Chemical shifts are reported in parts per million (δ). Apparent shapes of signals are described as s (singlet), d (doublet), t (triplet), q (quartet), dd (double doublet), m (multiplet), and br s (broad singlet). Values given for coupling constants are first order. TLC was performed on Uniplates (Analtech Co., Newark, DE) and column chromatography on Woelm silica gel (70–230 mesh). Microanalyses were performed by Galbraith Laboratories, Inc., or by M.H.W. Laboratories. Mass spectral data were collected at Rockefeller University, Mass Spectrometric Biotechnology Resource, by F. A. Bencsath.

5-(2,4:3,5-Di-*O*-benzylidene-D-pentitol-1-yl)-3-bromopyridine (8). To a solution of 3,5-dibromopyridine (1.45 g, 6.12 mmol) in dry Et_2O (50 mL) was slowly added (ca. 10 min) a solution of *n*-BuLi (2.35 mL, 2.6 M solution in *n*-hexane, 6.12 mmol) below −50 °C under argon atmosphere. After addition was completed, the reaction mixture was further stirred for 15 min. The mixture was then cooled to −78 °C, and a solution of 2,4:3,5-di-*O*-benzylidene-D-aldehydoribose (**7**) (500 mg, 1.53 mM) in THF (5 mL) was added dropwise, and then the reaction mixture was allowed to warm slowly to room temperature. Water (50 mL) was added to the reaction mixture. The organic layer was separated, washed with brine (30 mL × 3), dried over Na_2SO_4, and then concentrated in vacuo. The residue was chromatographed on a column of silica gel (20 g) using first CH_2Cl_2 and then 10% Et_2O in CH_2Cl_2 as the eluent, to give an allo/altro mixture of **26** (420 mg, 57%): mp 170–174 °C; ^{1}H NMR ($CDCl_3$) δ 3.8–4.4 (5 H, m, H-2′,3′,4′,5′,5″), 5.0 (1 H, m, H-1′), 5.60, 5.64, 5.71, 5.74 (2 H, 4 s, benzylidene), 7.38 (10 H, s, phenyl), 7.98 (1 H, m, H-2), 8.58 (2 H, m, H-4,6); IR (KBr) γ 3500 cm^{-1} (OH), 1460 (aromatic); UV (MeOH) λ_{max} 271.5 nm (ϵ 2850); MS, *m/e* 483 (M^+, 100), 107 (PhCHO, 80).

Anal. ($C_{24}H_{22}BrNO_5$) C, H, N.

Methyl 5-(2,4:3,5-Di-*O*-benzylidene-D-pentitol-1-yl)nicotinate (9). To a solution of **8** (200 mg, 0.43 mmol) in a mixture of HMPA (0.5 mL) and Et_2O (5 mL) was added a solution of *n*-BuLi (2 mL of 2.5 M solution in *n*-hexane, 5 mmol) under argon atmosphere at −78 °C. After the addition, the mixture was stirred at −78 °C for 15 min. A large excess of solid carbon dioxide was added, and the mixture was allowed to warm to room temperature. The mixture was acidified by addition of 1 N HCl to pH 4, and the organic layer was washed with brine (3 × 5 mL) and dried over Na_2SO_4. After removal of Na_2SO_4 by filtration, the filtrate was cooled to 0 °C and treated with a large excess of ethereal diazomethane. Excess diazomethane was then decomposed by addition of CH_3COOH. The mixture was concentrated in vacuo, and the residue was chromatographed on a silica gel column using *n*-hexane/EtOAc (7:3) as the eluent. Methyl 5-(2,4:3,5-di-*O*-benzylidene-D-pentitol-1-yl)nicotinate was obtained as a mixture of allo/altro epimers (121 mg, 65%): mp 175–178 °C; ^{1}H NMR ($CDCl_3$) δ 3.5–4.4 (5 H, m, H-2′,3′,4′,5′,5″), 3.92, 3.94 (3 H, 2 s, Me ester), 5.11 (1 H, m, H-1′), 5.58, 5.65, 5.71, 5.75 (2 H, 4 s, benzylidene), 7.37, 7.38 (10 H, 2 s, Ph), 8.42, 8.83, 9.13 (3 H, 3 s, pyridine); IR (KBr) γ 1745 cm^{-1} (ester); UV (MeOH) λ_{max} 266.5 nm (ϵ 3080); MS, *m/e* 464 (MH^+, 100), 358 (M^+ − PhCHO, 34), 107 (PhCHO, 80).

5-(2,4:3,5-Di-*O*-benzylidene-D-pentitol-1-yl)nicotinamide (10). An allo/altro epimeric mixture of **9** (220 mg, 0.48 mmol) was saturated with methanolic ammonia (4 mL) containing a catalytic amount of NaH (ca. 2 mg), and the mixture was stirred at room temperature for 20 h. Solvent was removed in vacuo, and the residue was chromatographed on a silica gel column using $CHCl_3$/EtOH (95:5) to give **10** as an allo/altro mixture (177 mg, 87%): ^{1}H NMR (Me_2SO-d_6) δ 3.5–4.3 (5 H, m, H-2′,3′,4′,5′,5″), 5.00 (1 H, m, H-1′), 5.64, 5.76, 5.77, 5.89 (2 H, 4 s, benzylidene), 7.39 (10 H, s, phenyl), 7.50, 8.21 (4 H, 2 br s, $CONH_2$), 8.29, 8.69, 8.95 (3 H, 3 m, pyridine); IR (KBr) γ 1690 cm^{-1} (carboxamide); UV (MeOH) λ_{max} 266.5 nm (ϵ 2690); MS, *m/e* 449 (MH^+, 71), 343 (MH^+ − PhCHO, 73), 237 (MH^+ − 2 × PhCHO, 8), 107 ($PhCHOH^+$, 100).

Anal. ($C_{25}H_{24}N_2O_4 \cdot {}^1/_2H_2O$) C, H, N.

5-(2,4:3,5-Di-*O*-benzylidene-1-*O*-mesyl-D-pentitol-1-yl)nicotinamide (12). A solution of **10** (600 mg, 1.4 mmol) in CH_2Cl_2 (40 mL) containing Et_3N (150 mL) and a catalytic amount of dimethylaminopyridine was treated with mesyl chloride at room temperature. The reaction mixture was stirred for 30 min and evaporated in vacuo. The residue was chromatographed on a column of silica gel using CH_2Cl_2/MeOH (95:5 v/v) to give **12** (600 mg, 85%) as a foam: ^{1}H NMR ($CDCl_3$) δ 2.93, 2.95 (3 H, 2 s, Ms), 3.52–4.60 (5 H, m, H-2′,3′,4′,5′,5″), 5.52, 5.64, 5.66, 5.79 (2 H, 4 s, benzylidene), 5.97 (1 H, d, H-1′, $J_{1',2'}$ = 2.7 Hz), 7.40 (10 H, s, Ph), 8.26, 8.79, 9.03 (3 H, 3 m, pyridine); UV (MeOH) λ_{max} 262.5 nm (ϵ 2420); MS, *m/e* 527 (MH^+, 100), 107 ($PhCHOH^+$, 60).

5-β-D- and 5-α-D-Ribofuranosylnicotinamide (1 and 13). An allo/altro mixture of **12** (48 mg, 0.19 mmol) was dissolved in a mixture of CF_3COOH and CH_3Cl (4:1, v/v), and the mixture was stirred for 15 min at room temperature. Water (8 mL) was added, and the aqueous layer was washed five times with Et_2O (equal volumes of each). After evaporation of the aqueous solution, the residue was chromatographed on a silica gel column using $CHCl_3$/MeOH (9:1, v/v) as the eluent. 5-β-D-Ribofuranosylnicotinamide (**1**) was eluted first from the column and obtained as a foam, which was crystallized from 2-propanol/EtOH: mp 176–178 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.92 (1 H, d, H-4, spacing 1.92 Hz), 8.70 (1 H, d, H-2, spacing 1.92 Hz), 8.16 (1 H, m, H-2), 4.68 (1 H, d, H-1′, $J_{1',2'}$ = 7.4 Hz), 3.06–3.16 (5 H, m, H-2′,3′,4′,5′,5″); MS, *m/e* 255 (MH^+, 100); UV (MeOH) λ_{max} 269.5 nm (ϵ 2380).

Anal. ($C_{11}H_{14}N_2O_5 \cdot {}^1/_2H_2O$) C, H, N.

The α isomer **13** mp 210–212 °C (recrystallized from methanol), was then eluted from the column: ^{1}H NMR (Me_2SO-d_6) δ 8.89 (1 H, br s, H-2), 8.59 (1 H, br s, H-6), 8.14 (1 H, br s, H-4), 5.08 (1 H, br s, H-1′), 4.12–3.17 (5 H, m, H-2′,3′,4′,5′,5″); MS, *m/e* 255 (MH^+, 100); UV (MeOH) λ_{max} 269.5 nm (ϵ 2390).

5-β-D-Ribofuranosyl-3-(aminocarbonyl)-1-methylpyridinium Iodide (2). A mixture of **1** (25 mg, 0.1 mmol), MeI (1 mL), MeOH (1 mL), and Me_2CO (1 mL) was heated at reflux for 5 h. After concentration of the mixture in vacuo, the residue was dissolved in water (5 mL) and the aqueous solution extracted with Et_2O (5 mL × 3). The aqueous solution was concentrated in vacuo, and the residue was dissolved in a minimal amount of MeOH. Upon dilution of the methanol solution with Et_2O (50

(24) It was reported that treatment of a 3-(1,2:4,5-di-*O*-isopropylidene-1,2,3,4,5-pentahydroxypentyl)pyrrazole with acid only afforded the corresponding pentahydroxy derivative. Reacetonation of the pentahydroxy derivative to a mixture of 2,3:4,5- and 1,2:4,5-di-*O*-isopropylidenates followed by mesylation and acid hydrolysis of the mesylates yielded a mixture of the furanosyl and 3-*O*-mesylpentyl derivatives, which were separated. Buchanan, J. G.; Chacon-Fuentes, M. E.; Stobie, A.; Wightman, R. H. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2561.

(25) We are indebted to Drs. J. H. Burchenal and B. Mehta of this institute for these preliminary data.

(26) Unlike a negatively charged molecule such as a nucleotide, a positively charged compound such as **2** may readily penetrate the cell membrane. It is well-known that rhodamin 123, a positively charged dye, can stain mitochondria in living cells. Johnson, L. V.; Walsh, M. L.; Chen, L. B. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 990.

mL), **2** precipitated as a powder, which was collected by filtration (28 mg, 71%): UV (MeOH λ_{max} 277.0 nm (ϵ 3150); ^{1}H NMR (Me_2SO-d_6) δ 9.33 (1 H, s, H-2), 9.06 (1 H, s, H-6), 8.84 (1 H, m, H-4), 4.85 (1 H, d, H-1′, $J_{1'2'}$ = 6.70 Hz), 4.47 (3 H, s, Me), 3.93–3.22 (5 H, m, H-2′,3′,4′,5′,5″); MS, *m/e* 269 (M – iodide, 26), 255, [(M – CH_3I)H^+, 100].

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Reactive 5′-Substituted 2′,5′-Dideoxyuridine Derivatives as Potential Inhibitors of Nucleotide Biosynthesis

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5′-(Bromoacetamido)-2′,5′-dideoxyuridine (**3**) and derivatives (**8**, **10**, **12**, and **14**) substituted at the 5-position with bromo, iodo, fluoro, and ethyl groups have been synthesized as potential inhibitors of enzymes that metabolize pyrimidine nucleosides. Also prepared were 2′,5′-dideoxyuridine derivatives (**4–6**) substituted at the 5′-position with 2-bromopropionamido, iodoacetamido, and 4-(fluorosulfonyl)benzamido groups. Compounds **3**, **5**, **8**, **12**, and **14** were examined for effect on macromolecular synthesis in L1210 leukemia cells in culture and compared with 5′-(bromoacetamido)-5′-deoxythymidine (**1**, BAT), a compound with demonstrated cytotoxicity and activity in vivo against P388 murine leukemia. Compounds **3**, **8**, **12**, and **14** inhibited DNA synthesis without significant inhibition of RNA synthesis, and protein synthesis was affected less than DNA synthesis. Compounds **3**, **5**, **6**, **8**, **10**, **12**, and **14** were cytotoxic to H.Ep.-2 and L1210 cells in culture, and **3**, **5**, **8**, and **12** showed activity in the P388 mouse leukemia screen.

In the course of synthesizing and evaluating nucleosides containing reactive groups attached at C-5′ that can act as irreversible enzyme inhibitors,[1–6] major emphasis has been placed on the preparation of nucleosides closely related to 5′-(bromoacetamido)-5′-deoxythymidine (**1**, BAT), a compound that showed significant activity (71% ILS) in the P388 mouse leukemia screen.[6] A study of the effects of BAT on macromolecular synthesis in L1210 cells in culture showed that BAT did not inhibit intermediary metabolism of dThd to dTMP, dTDP, and dTTP but did selectively inhibit incorporation of pyrimidine nucleoside precursors into DNA.[4] BAT is also an effective inhibitor of thymidylate synthase from L1210 cells.[5] The bromoacetamide group of BAT has been replaced with a variety of other reactive groups,[6] but none of the 13 thymidine analogues prepared had better in vivo activity than BAT. The present paper describes the synthesis and evaluation of 2′,5′-dideoxyuridines with H, Et, Br, F, and I at the 5-position of the pyrimidine ring and several reactive groups, principally bromoacetamide, at the 5′-position of the sugar.

5′-Amino-2′,5′-dideoxyuridine (**2**) was prepared via the corresponding tosylate and azide[7] and selectively bromoacetylated on the amino group with 4-nitrophenyl bromoacetate[8] to give a 93% yield of the bromoacetamide **3**. A similar reaction of **2** with 4-nitrophenyl 2-bromopropionate[6] gave an 89% yield of the bromopropionamide **4**. The iodoacetamide **5** was synthesized by reaction of **2** with the activated ester *N*-(iodoacetoxy)succinimide.[9] The reported use of 4-(fluorosulfonyl)benzoyl derivatives of adenosine[10] and guanosine[11] as affinity labels suggested the preparation of the (fluorosulfonyl)benzamide **6**. Reaction of **2** with 4-(fluorosulfonyl)benzoyl chloride in the presence of *N*,*N*-diisopropylethylamine gave an 85% yield of **6**. Direct bromination of **2** in acetic acid gave **7**·HBr, which was converted with aqueous NH_4OH to the free amine **7** in 52% yield. This procedure is an improvement over a previously described conversion of **2** to **7** via the 5-mercuriacetate.[12] Reaction of **7** with 4-nitrophenyl bromoacetate by the usual procedure gave an 85% yield of the bromoacetamide **8**. The corresponding 5′-amino-2′,5′-dideoxy-5-iodouridine (**9**) was prepared from **2** via the 5′-mercuriacetate[12] and converted in 93% yield to the bromoacetamide **10**. A similar reaction of 5′-amino-2′,5′-dideoxy-5-fluorouridine[13] (**11**) with 4-nitrophenyl bromoacetate gave a 63% yield of **12**. 5′-Amino-2′,5′-dideoxy-5-ethyluridine (**13**) was prepared from 2′-deoxy-5-ethyluridine[14] via the 5′-tosyl and 5′-azido intermediates[15] and treated with 4-nitrophenyl bromoacetate to give a 94% yield of the bromoamide **14**.

Biological and Biochemical Data. The cytotoxicities of these reactive nucleosides to H.Ep.-2[16] and L1210[17] cells in culture were determined (Table I) and compared with

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