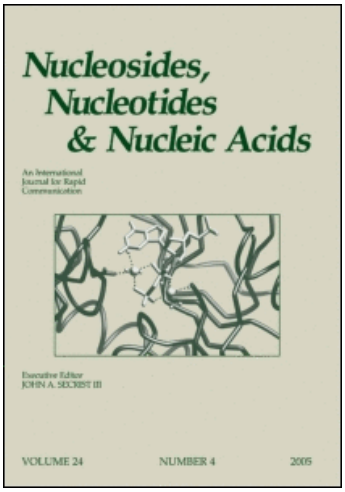


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Biological Activities of 2-Functionalized Purine Nucleosides

Vasu Nair[abc]; Bindu Bera[b]; Earl R. Kern[d]
[a] Department of Pharmaceutical and Biomedical Sciences, University of Georgia, Georgia, Athens, USA [b] Department of Chemistry, University of Iowa, Iowa City, Iowa, USA [c] Department of Pharmaceutical and Biomedical Sciences, Room 320A, R. C. Wilson PH, University of Georgia, Athens, GA, USA [d] Department of Pediatrics, University of Alabama at Birmingham, Birmingham, Alabama, USA

Online publication date: 04 February 2003

To cite this Article Nair, Vasu , Bera, Bindu and Kern, Earl R.(2003) 'Synthesis and Biological Activities of 2-Functionalized Purine Nucleosides', Nucleosides, Nucleotides and Nucleic Acids, 22: 2, 115 — 127
To link to this Article: DOI: 10.1081/NCN-120019498
URL: http://dx.doi.org/10.1081/NCN-120019498

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, No. 2, pp. 115–127, 2003

Synthesis and Biological Activities of 2-Functionalized Purine Nucleosides

Vasu Nair,[1,2,*] Bindu Bera,[2] and Earl R. Kern[3]

[1]Department of Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, Georgia, USA
[2]Department of Chemistry, University of Iowa, Iowa City, Iowa, USA
[3]Department of Pediatrics, University of Alabama at Birmingham, Birmingham, Alabama, USA

ABSTRACT

Novel purine nucleosides functionalized at the 2-position have been prepared using new applications of synthetic methodology. The target molecules were designed as potential inhibitors (as their monophosphates) of the enzyme, inosine monophosphate dehydrogenase (IMPDH), and representative inhibition data are presented. Antiviral data of the compounds are discussed.

Key Words: Functionalized purine nucleosides; IMPDH; Antiviral activity.

*Correspondence: Vasu Nair, Department of Pharmaceutical and Biomedical Sciences, Room 320A, R. C. Wilson PH, University of Georgia, Athens, GA, 30602, USA; E-mail: vnair@rx.uga.edu.

DOI: 10.1081/NCN-120019498
1525-7770 (Print); 1532-2335 (Online)

www.dekker.com

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

INTRODUCTION

Inosine monophosphate dehydrogenase (IMPDH) is an NAD-dependent enzyme that catalyzes the oxidative conversion of inosine 5′-monophosphate (IMP) to xanthosine 5′-monophosphate (XMP). XMP is subsequently converted to guanosine 5′-monophosphate (GMP) by the enzyme, GMP synthase.[1–6] IMP is also converted in two biosynthetic steps to adenosine 5′-monophosphate (AMP).[1–6] Thus, because of its position at the branching point in the *de novo* synthesis of purine nucleotides, IMPDH has been viewed as an important target for chemotherapeutic agents and its inhibition manifests itself in a number of biological responses including anticancer, immunosuppressive and antiviral activities. While there are differences among mammalian IMPDHs as well as bacterial or parasite enzymes, there is nevertheless a substantial degree of sequence conservation.[5]

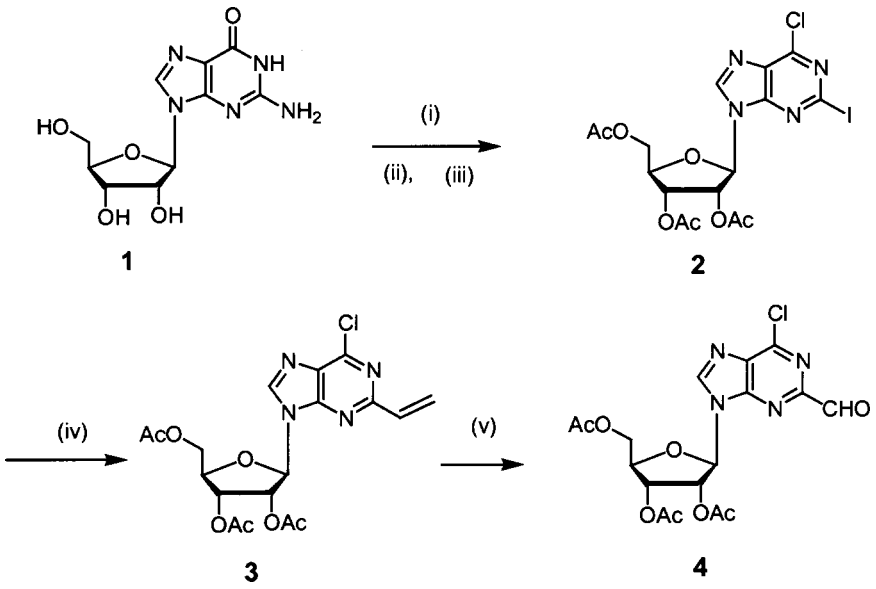
Virus-infected cells have an increased demand for purine nucleotides which are needed for viral RNA or DNA synthesis,[5] and this renders the enzyme, IMPDH, as a sensitive target for antiviral chemotherapy. This, together with information on the antiviral activity of inhibitors of IMPDH such as ribavirin, mizoribine, and 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (EICAR),[7–9] led us to the design of purine nucleosides with unsaturation at carbon-2 as potential antiviral agents, with particular emphasis on activity against orthopoxviruses. The design also took into account the mechanism of action of IMPDH which involves interaction of the enzyme and coenzyme (NAD^+) complex at the 2-position of IMP.[1,2] It has been shown that IMPDH contains a cysteine residue at the active site that attacks the 2-position of substrate IMP and forms a covalent complex (E-IMP). This intermediate (E-IMP) forms XMP by transferring hydride to NAD^+ and then undergoing hydration at the 2-position which is followed by ejection of the enzyme. In support of our design was the observation that 2-vinylinosine synthesized earlier by Nair and coworkers[10] had broad-spectrum activity against exotic RNA viruses.[11] In the molecular design, 2-ethynyl and substituted ethynyl analogs were chosen as unsaturated analogs of 2-vinylinosine. 2-(2-Fluorovinyl) analogs were designed to increase the electrophilic character of the double bond of 2-vinylinosine for enhanced nucleophilic addition of the sulfhydryl group of Cys-331 of IMPDH at the fluorovinyl group. The cyclopropyl group was introduced at the 2-position because many of the addition reactions of the strained cyclopropane ring parallel those of alkenes.[12]

RESULTS AND DISCUSSION

Chemistry

6-Chloro-2-iodo-tri-O-acetylpurine riboside (**2**) was synthesized from commercially available guanosine (**1**) (Sch. 1) in 3 steps as previously described.[10] Compound **2** was cross-coupled with tri-n-butyl(vinyl) stannane[10] in the presence of catalytic amounts of $PdCl_2$ $(CH_3CN)_2$ in DMF to give the 6-chloro-2-vinylpurine **3** in 92% yield. Compound **3** was converted to the aldehyde **4** (68%) using OsO_4, N-methylmorpholine-N-oxide, $NaIO_4$ in dichloromethane and water. Compounds

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

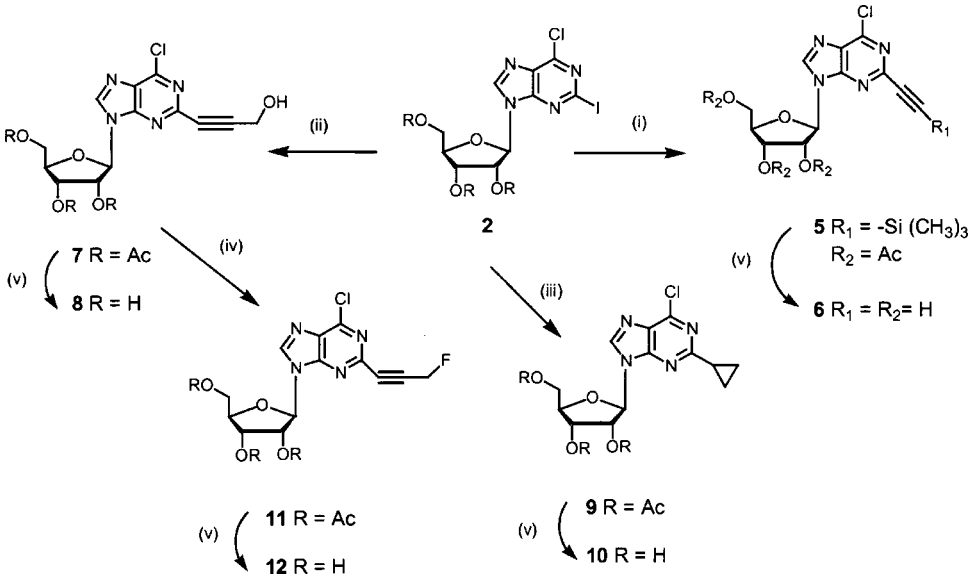


Scheme 1. Reagents and Conditions: (i) Ac_2O, Et_3N, DMAP, CH_3CN, rt, 0.5 h; (ii) $POCl_3$, N,N-diethylaniline, 70°C, 1 h; (iii) n-pentyl nitrite, CH_2I_2, 60°C, 16 h; (iv) tributylvinylstannane, $PdCl_2(CH_3CN)_2$, DMF, 95°C, 4 h; (v) OsO_4, N-methylmorpholine N-oxide, $NaIO_4$, H_2O/DCM, rt, 24 h.

2 and **4** served as the starting materials for the synthesis of the 2- and 2, 6-subsititued purine nucleosides.

Coupling of 6-chloro-2-iodopurine ribonucleoside (2) with Terminal Alkynes. Thorand and Krause[13] reported an improvement of the methodology of the palladium-catalyzed coupling of terminal alkynes with aryl halides reported by Sonogashira et al.[14]. Application of this improved method to the alkyne cross-coupling reactions of this work resulted in short reaction times and good to excellent yields of product. Thus, when compound **2** was coupled with (trimethylsilyl) acetylene or propargyl alcohol in the presence of $PdCl_2(PPh_3)_2$, CuI, and triethylamine in THF at 45–50°C for 1 h, compounds **5** and **7** were produced in 96% and 65% yields, respectively (Sch. 2).

Synthesis of 2-cyclopropylpurine Ribonucleoside Using the Suzuki-Miyaura Coupling. Previously known methods[15] of coupling (e.g., the Stille coupling) of tributylstannylcyclopropane were unsuccessful with intermediate **2**. Also, all reported coupling procedures involving the use of cyclopropylboronic acid derivatives (i.e., *c*-$PrB(OR)_2$) normally require high temperatures, long reaction times and/or large amounts of Pd catalyst.[16] Recently, it was reported that dihydroboration of propargyl bromide with 9-BBN-H (9-borabicyclo[3.3.1]nonane) followed by treatment of the adduct with aqueous NaOH affords the hydroxy(cyclopropyl)-9-BBN complex, which undergoes efficient palladium-catalyzed cross-coupling (Suzuki-Miyaura) to produce a variety of aryl and vinyl cyclopropanes.[17] We applied this methodology using intermediate **2** and produced 6-chloro-2-cyclopropyl-purine



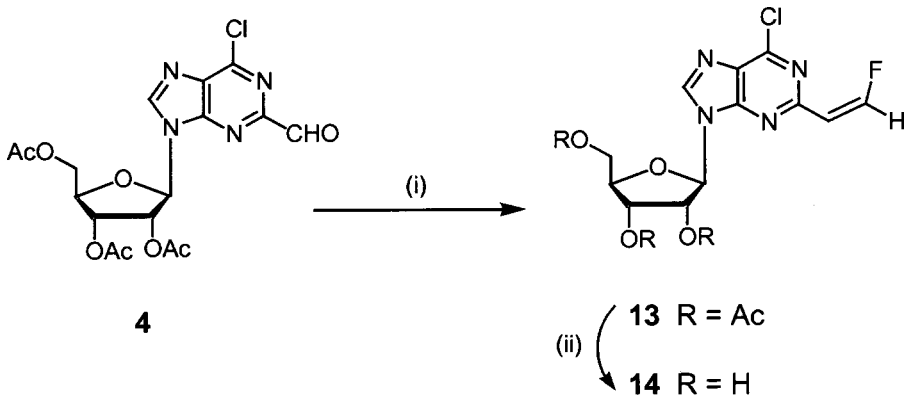
Scheme 2. Reagents and Conditions: (i) $PdCl_2(PPh_3)_2$, (TMS)acetylene, CuI, TEA, 45–50°C; (ii) propargyl alcohol, $PdCl_2(PPh_3)_2$, CuI, TEA, 45–50°C; (iii) (a) 9-BBN-H, propargyl bromide, THF, reflux, (b) 3N aq NaOH, r.t., (c) $Pd(PPh_3)_4$, THF, reflux; (iv) DAST, CH_2Cl_2, −78°C, r.t, 2 h; (v) NH_3/MeOH, rt, 3 h.

ribonucleoside (**9**) in 47% yield (Sch. 2). To our knowledge this is the first application of the methodology in nucleoside chemistry.

Synthesis of 2(2-fluorovinyl) Purine Ribonucleosides. The Wittig-type olefination procedure described by Burton and coworkers[18a] is an efficient method for the synthesis of fluoroalkenes (terminal HF-alkenes) from aldehydes. A modification of this approach described by Kataoka and Tsuboi[18b] was used for the synthesis of compound **14**. In this method, the monofluorinated ylid generated from the reaction of excess tributylphosphine and trichlorofluoromethane, was treated with aldehyde **4** and the vinylphosphonium salt produced was hydrolyzed with aqueous NaOH. Only the *Z*-isomer of **13** was isolated (Sch. 3). This is the first application of the methodology in nucleoside synthesis.

Hydrolytic Dechlorination of 6-chloropurine Nucleosides with Adenosine Deaminase. For deprotection of 6-chloropurine nucleosides to the corresponding inosine derivatives, chemical methods using 2N NaOH in dioxane[19a] and thioethanol and sodium methoxide in methanol[19b] failed. We resorted then to milder enzymatic conditions for deprotection. Adenosine deaminase catalyzes the hydrolytic deamination of adenosine and 2′-deoxyadenosine to inosine and 2′-deoxyinosine and the enzyme has been used previously in synthetic conversions.[20] Further, the hydrolytic dechlorination of 6-chloropurine nucleosides by adenosine deaminase in the synthesis of dideoxynucleosides has been reported previously by Nair and Sells.[21] Thus, compounds **6**, **8, 10**, **12** and **14** were cleanly converted to their inosine derivatives **15**,

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

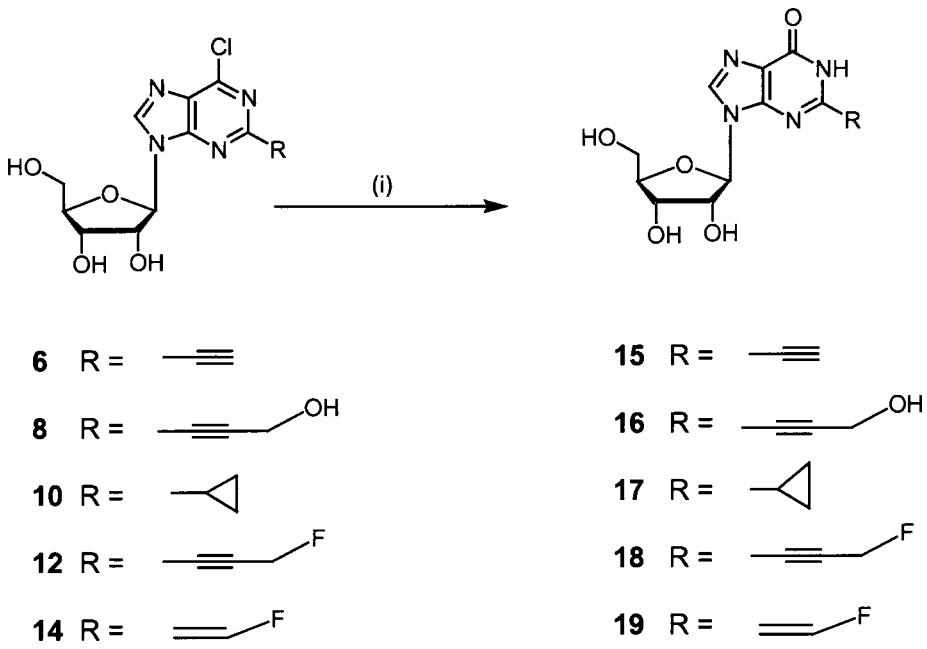


Scheme 3. Reagents and Conditions: (i) (a) Bu_3P, $CFCl_3$, CH_2Cl_2, 0°C- rt, 20 h, (b) 10% NaOH, rt, 24 h; (ii) NH_3/MeOH.

16, **17**, **18** and **19** using adenosine deaminase in 0.1 M phosphate buffer (pH 7.4) at 25°C for 2–4 days (Sch. 4).

Enzymology

Representative examples of the 2- and 2,6-disubstituted purine nucleosides (as their monophosphates) were examined for their ability to inhibit IMPDH (from



Scheme 4. Reagents and conditions: adenosine deaminase, 0.1 M phosphate buffer, 25°C.

E. coli B3 strain[22]). The inhibitions followed a two-step mechanism:

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E.I \xrightarrow{k_{inact}} E - I$$

where $K_i = k_1/k_{-1}$ is the apparent dissociation constant and k_{inact} is the rate constant of inactivation of IMPDH.[22] The data were fitted into the equation:

$$\ln(V_t/V_o) = -k_{obs}t$$

where V_t is the activity at time t and V_o is the activity at time $t = 0$. The k_{obs} values for inactivation of *E. coli* IMPDH were then fitted into the equation:

$$k_{obs} = k_{intact}[I]/(K_i + [I])$$

For example, the values of k_{inact} and K_i obtained for the monophosphate of the monosubstituted compound, **15**, was $0.013\,sec^{-1}$ and $4.25\,\mu M$, respectively. For the monophosphate of the 2,6-disubstituted compound, **6**, k_{inact} and K_i values were $0.03\,sec^{-1}$ and $4.70\,\mu M$. For the standard purine nucleotide inhibitor, 6-chloropurine ribonucleoside monophosphate, these values were $0.08\,sec^{-1}$ and $62.0\,\mu M$.

Antiviral Activity

Antiviral evaluations of selected compounds against the vaccinia virus (in vitro) [23–26] were carried out as previously described.[26] 6-Chloro-2-ethynylpurine ribonucleoside (**6**) was active but toxic against the vaccinia virus: $IC_{50} > 0.8\,\mu g/mL$; CC_{50} $3.0\,\mu g/mL$. Interestingly, 2-ethynylinosine (**15**) ($IC_{50} > 20\,\mu g/mL$; CC_{50} $66.5\,\mu g/mL$) was less active than 2-vinylinosine ($IC_{50}13\,\mu g/mL$; CC_{50} $\sim 100\,\mu g/mL$). 6-Chloro-2-(3-fluoro-propynyl)purine riboside (**12**) and 2-(3-fluoropropynyl)inosine (**18**) had comparable activity to 2-ethynylinosine (**15**). Both 2-(2-fluorovinyl) inosine (**19**) and 6-chloro-2-(2-fluorovinyl)purine riboside (**14**) also showed some activity towards the vaccinia virus in HFF cells (SI < 4.5 and < 3.7). The other compounds in the series were inactive. Interestingly, 2-vinylinosine shows broad-spectrum antiviral activity (IC_{50} in Vero cells): IC_{50} $13\,\mu g/mL$; CC_{50} $100\,\mu g/mL$; JEV ($3.2\,\mu g/mL$); PIC ($2.5\,\mu g/mL$); PT ($2.7\,\mu g/mL$); RVF ($24\,\mu g/mL$); VEE ($7.7\,\mu g/mL$); YF ($7.7\,\mu g/mL$).[11]

SUMMARY

In summary, new applications of synthetic methodologies in nucleoside chemistry are described for the preparation of 2-functionalized purine ribonucleosides in good to excellent yields. Representative compounds, as their monophosphates, chosen to examine the inhibitory effect toward IMPDH showed that the compounds were strong inhibitors of the enzyme. In vitro antiviral evaluation against the vaccinia virus (orthopoxvirus) showed some activity with the most active compound in the series ($IC_{50} > 0.8\mu g/mL$) also being the most toxic. The mechanism of the antiviral activity appears to be associated with the ability of the cellularly produced monophosphates to be inhibitors of IMPDH.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

EXPERIMENTAL

Melting points reported are uncorrected and were determined on an Electrothermal Engineering Ltd. melting point apparatus. Nuclear Magnetic resonance spectra were recorded on Bruker Model AC300 and WM 360 systems. Chemical shifts for ^{1}H and ^{13}C NMR spectra used $CDCl_3$, CD_3OD, or D_2O as reference. Ultraviolet spectra were recorded on a Varian Cary Model 3 spectrophotometer. Flash chromatography used 230–400 mesh silica gel. HPLC separations were carried out on a Beckman-Coulter instrument with C-18 reversed-phase columns. Purity criteria came from HPLC, ^{13}C NMR spectra and quantitative UV data (ε values). Adenosine deaminase (type X from calf spleen) was purchased from Sigma.

General Procedure for Deprotection of 2-substituted-6-chloro-9-(β-D-ribofuranosyl)purines. A mixture of 2-substituted-6-chloro-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl) purines (0.50 mmol) in methanolic ammonia (3–5 mL) was stirred for 2–4 h at room temperature. After the reaction was complete, the reaction mixture was neutralized and the solvent was removed under reduced pressure. The crude residue was initially purified over silica gel and further purified by HPLC (MeOH-H_2O) to give 2-substituted-6-chloro-9-(β-D-ribofuranosyl)purines.

General Procedure for Phosphorylation. To a mixture of the target nucleoside (0.17 mmol) and anhydrous triethyl phosphate (0.03 mL) at 0°C was added anhydrous $POCl_3$ (0.1 mL).[22] The reaction mixture was stirred at 0°C for 6 h and neutralized with $NaHCO_3$. Ether was added and the resulting precipitate was separated by centrifugation. The white precipitate was dissolved in water and was purified by HPLC on a C_{18} column with water-methanol. ^{31}P NMR (D_2O) δ Approx 1.20.

6-Chloro-2-formyl-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (4). A mixture of compound **2** (0.924 g, 1.72 mmol), $Pd(CH_3CN)_2Cl_2$ (0.02 g, 0.08 mmol), and tributyl-(vinyl)stannane (2.5 mL, 8.56 mmol) in anhydrous DMF (5 mL) was heated at 90–95°C for 4 h. The reaction mixture was worked up by adding DCM (20 mL) and water (3 × 10 mL). The organic layer was collected and dried over anhydrous Na_2SO_4 and concentrated to dryness and the crude residue was purified on silica gel (45–50% EtOAc/hexanes) to afford 6-chloro-2-vinyl-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (**3**) as an oil (0.694 g, 92%). ^{1}H NMR ($CDCl_3$) δ 2.04 (s, 3H), 2.08 (s, 3H), 2.14 (s, 3H), 4.41 (m, 1H), 4.44 (m, 2H), 5.78 (m, 2H), 5.99 (t, J = 4.9 Hz, Hz, 1H), 6.13 (d, J = 4.3 Hz, 1H), 6.72 (m, 1H), 6.85 (m, 1H), 8.17 (s, 1H); ^{13}C NMR ($CDCl_3$)δ 20.4, 20.5, 20.8, 62.7, 70.2, 73.2, 80.2, 87.2, 124.9, 131.4, 135.2, 143.7, 151.3, 152.4, 159.5, 169.3, 169.4, 169.5. To a solution of compound **3** (4.38 g, 9.98 mmol), OsO_4 (0.51 g, 0.20 mmol), N-methylmorpholine N-oxide (1.28 g, 10.97 mmol) in DCM (45 mL) was added a saturated aqueous solution of $NaIO_4$ (4.27 g, 19.95 mmol). The reaction mixture was stirred at room temperature for 24 h. After the reaction was complete, the organic layer was separated and washed with water (3 × 20 mL) and dried (Na_2SO_4). On concentration, the crude residue was purified over silica gel (40% EtOAc/ hexanes) to afford a white solid (compound **4**, 2.99 g) in 68% yield. Mp. 62–63°C; ^{1}H NMR ($CDCl_3$) δ 2.05 (s, 3H), 2.08 (s, 3H), 2.14 (s, 3H), 4.41 (m, 1H), 4.49 (m, 2H), 5.59 (m, 1H), 5.82 (m, 1H), 6.35

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

(d, J = 5.4 Hz, 1H), 8.49 (s, 1H), 10.08 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 20.3, 20.5, 20.6, 62.9, 70.6, 73.4, 80.9, 86.6, 133.6, 146.0, 151.6, 152.5, 153.4, 169.4, 169.5, 170.1, 188.8; HRMS (FAB) calcd for $C_{17}H_{18}ClN_4O_8$ $[M + H]^+$ 441.0815, found 441.0813.

2-Ethynyl-6-chloro-9-(β-D-ribofuranosyl)purine (6). To a mixture of compound **2** (0.306 g, 0.57 mmol), $PdCl_2(PPh_3)_2$ (7.8 mg, 0.011 mmol), CuI (4.3 mg, 0.23 mmol), and triethylamine (0.12 mL, 0.85 mmol) was added anhydrous THF (5 mL). To this mixture, trimethylsilylacetylene (0.08 mL, 0.54 mmol) in THF (5 mL) was added dropwise over a period of 1 h at room temperature. After the addition was over, the reaction mixture was heated at 45–50°C for 2 h. Solvent was then evaporated to dryness under reduced pressure. The crude material was purified over silica gel and the product was eluted with 50–55% ethyl acetate/hexanes to yield 0.278 g (96%) of 2-(2-trimethylsilylethynyl)-6-chloro-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (**5**) as a syrup. ^{1}H NMR ($CDCl_3$) δ 0.25 (s, 9H), 2.08 (s, 3H), 2.13 (s, 3H), 2.17 (s, 3H), 4.35–4.49 (m, 3H), 5.51 (m, 1H), 5.58 (m, 1H), 6.18 (d, J = 5.6 Hz, Hz, 1H), 8.20 (s, 1H).; ^{13}C NMR ($CDCl_3$) δ −0.54, −0.52, −0.48, 20.3, 20.5, 20.8, 62.9, 70.7, 73.1, 80.9, 85.9, 95.2, 101.6, 131.1, 143.7, 145.5, 151.3, 151.4, 169.3, 169.5, 170.1. Compound **5** was deprotected and purified by HPLC (35% $MeOH/H_2O$) using the general procedure described above to give **6** as a white solid in 40% yield. Mp. 191–192°C (decomp); UV (MeOH) λ_{max} 277(ε 11,614), 228 (ε 17,631); ^{1}H NMR (CD_3OD) δ 3.79–3.89 (m, 3H), 4.15 (m, 1H), 4.36 (t, J = 4.6 Hz, 1H), 4.66 (t, J = 5.0 Hz, 1H), 6.11 (d, J = 4.9 Hz, 1H), 8.89 (s, 1H); ^{13}C NMR (CD_3OD) δ 62.6, 71.7, 76.0, 78.2, 82.0, 87.4, 90.9, 132.7, 145.8, 147.9, 151.2, 152.6; HRMS (FAB) calcd for $C_{12}H_{12}ClN_4O_4$ $[M + H]^+$ 311.0549, found 311.0553.

2-Ethynyl-9-(β-D-ribofuranosyl)hypoxanthine (15). To a solution of compound **6** (26.4 mg) in 0.1M phosphate buffer (pH 7.4, 4 mL), was added 100 units of adenosine deaminase. The enzymatic reaction was left for 2 days at room temperature and monitored using UV spectrophotometry. A bathochromic shift in λ_{max} from 277 nm to 293 nm indicated completion of the conversion. The enzyme was filtered using an Amicon ultrafiltration device with YM-10 membrane and the product was purified by HPLC. Compound **15** was eluted with 20% $MeOH/H_2O$ (17.1 mg, 70%). Mp. 224–228°C; UV (MeOH) λ_{max} 293(ε 12,069), 260 (ε 10,918); ^{1}H NMR (CD_3OD) δ 3.69 (dd, J = 3.3, 12.3 Hz, 1H), 3.78 (dd, J = 3.0, 12.4 Hz, 1H), 4.08 (m, 2H), 4.25 (m, 1H), 4.52 (t, J = 5.2 Hz, 1H), 5.91 (d, J = 5.4 Hz, 1H), 8.30 (s, 1H); ^{13}C NMR (CD_3OD) δ 62.8, 71.9, 76.1, 76.8, 83.2, 87.4, 90.6, 126.5, 139.4, 141.8, 149.1, 158.6; HRMS (FAB) calcd for $C_{12}H_{13}N_4O_5$ $[M + H]^+$ 293.0888, found 293.0896.

6-Chloro-2-(3-hydroxypropynyl)-9-(β-D-ribofuranosyl)purine (8). A mixture of compound **2** (1.00 g, 1.85 mmol), $PdCl_2(PPh_3)_2$ (25.6 mg, 0.037 mmol), CuI (14.1 mg, 0.074 mmol), and triethylamine (1.28 mL, 9.25 mmol) in anhydrous THF (10 mL) was prepared. To this reaction mixture, propargyl alcohol (0.11 mL, 1.93 mmol) in THF (5 mL) was added dropwise at room temperature for a period of 1 h. The reaction mixture was then heated at 50–55°C for 2 h. Solvent was removed under reduced pressure and the crude product was purified over silica gel (65% EtOAc/ hexanes) to yield 1.198 g (65%) of 6-chloro-2-(3-hydroxypropynyl)-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (**7**) as a syrup. ^{1}H NMR ($CDCl_3$) δ

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

2.05 (s, 3H), 2.11 (s, 3H), 2.14 (s, 3H), 4.44–4.52 (m, 5H), 5.64 (t, J = 3.6 Hz, 1H), 5.86 (t, J = 6.5 Hz, 1H), 6.20 (d, J = 5.7 Hz, 1H), 8.28 (s, 1H).; ^{13}C NMR ($CDCl_3$) δ 20.3, 20.5, 20.7, 51.0, 63.0, 70.7, 73.0, 80.7, 83.5, 86.6, 87.5, 131.5, 144.4, 145.3, 151.1, 151.3, 169.3, 169.6, 170.5. Compound **7** was deprotected and purified by HPLC (40% MeOH/H_2O) using the general procedure described above to give **8** as a white solid in 45% yield. Mp. 174–175°C; UV (MeOH) λ_{max} 279 (ε 12,570), 229 (ε 17,952); ^{1}H NMR (CD_3OD) δ 3.79 (dd, J = 2.2, 11.5 Hz, 1H), 3.89 (dd, J = 2.8, 12.0 Hz, 1H), 4.14 (d, J = 3.5 Hz, 1H), 4.35 (t, J = 4.6 Hz, 1H), 4.44 (s, 2H), 4.66 (t, J = 5.0 Hz, 1H), 6.10 (d, J = 5.0 Hz, 1H), 8.85 (s, 1H); ^{13}C NMR (CD_3OD) δ 50.9, 62.6, 71.8, 75.9, 83.3, 87.4, 88.5, 91.0, 132.4, 146.4, 147.8, 151.3, 152.7; HRMS (FAB) calcd for $C_{13}H_{14}ClN_4O_5$ $[M + H]^+$ 341.0654, found 341.0642.

2-(3-Hydroxypropynyl)-9-(β-D-ribofuranosyl)hypoxanthine (16). To a solution of compound **8** (12.9 mg) in 0.1M phosphate buffer (3 mL) was added 60 units of adenosine deaminase and the enzymatic reaction was left at room temperature for 2 days. A UV bathochromic shift in λ_{max} from 279 nm to 292 nm indicated the completion of reaction which was worked up and purified as described for compound **15** to give **16** in 77% yield. Mp. 197°C; UV (MeOH) λ_{max} 295 (ε 12,158), 260 (ε 10,807); ^{1}H NMR (CD_3OD)δ 3.70 (m, 1H), 3.87 (m, 1H), 4.15 (m, 1H), 4.28 (m, 1H), 4.38 (m, 2H), 4.75 (m, 1H), 5.84 (d, J = 6.5 Hz, 1H), 8.02 (s, 1H); ^{13}C NMR (CD_3OD) δ 51.0, 63.8, 72.9, 75.2, 84.4, 84.8, 88.2, 91.5, 132.7, 141.5, 147.5, 150.3, 168.4; HRMS (FAB) calcd for $C_{13}H_{15}N_4O_6$ $[M + H]^+$ 323.0993, found 323.0993.

6-Chloro-2-cyclopropyl-9-(β-D-ribofuranosyl)purine (10). A solution of 9-BBN (1.49 g, 6.12 mmol) and propargyl bromide (0.27 mL, 3.06 mmol) in anhydrous THF (7 mL) was heated under reflux for 2 h and 3M aqueous NaOH (3 mL) was added and the mixture was stirred for 1 h at room temperature. To this solution was added compound **2** (1.49 g, 2.76 mmol) and $Pd(PPh_3)_4$ (0.091 g, 0.08 mmol) in anhydrous THF (10 mL) and the reaction mixture refluxed for 16 h. It was quenched with water (5 mL) and concentrated to dryness under reduced pressure. The crude product was purified on silica gel and 6-chloro-2-cyclopropyl-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine **(9)** eluted with 35% EtOAc/ hexanes to give a pale yellow hygroscopic solid (0.58 g, 47%). ^{1}H NMR ($CDCl_3$) δ 1.12 (m, 2H), 1.16 (m, 2H), 2.05 (s, 3H), 2.09 (s, 3H), 2.12 (s, 3H), 2.32 (m, 1H), 4.31 (m, 1H), 4.42 (m, 2H), 5.71 (m, 1H), 5.95 (m, 1H), 6.08 (d, J = 4.3 Hz, 1H), 8.18 (s, 1H).; ^{13}C NMR ($CDCl_3$) δ 11.0, 11.1, 18.3, 20.4, 20.5, 20.7, 63.1, 70.4, 73.0, 79.8, 86.8, 131.0, 140.9, 150.1, 163.9, 166.7, 169.3, 169.5, 170.4. Compound **10** was deprotected and purified by HPLC (55% MeOH/H_2O) using the general procedure described above to give **10** as a white solid in 37% yield. Mp. 15–151°C; UV (MeOH) λ_{max} 277 (ε 11,372), 252 (ε 7,793); ^{1}H NMR (CD_3OD) δ 1.17 (m, 4H), 2.27 (m, 1H), 3.78 (m, 1H), 3.86 (m, 1H), 4.14 (m, 1H), 4.36 (m, 1H), 4.72 (m, 1H), 6.06 (d, J = 5.5 Hz, 1H), 8.65 (s, 1H); ^{13}C NMR (CD_3OD) δ 11.7, 11.8, 18.7. 62.9, 72.1, 75.6, 87.5, 90.8, 130.7, 146.1, 151.3, 153.2, 168.5; HRMS (FAB) calcd for $C_{13}H_{15}ClN_4O_4$ $[M + H]^+$ 327.0862, found 327.0874.

2-Cyclopropyl-9-(β-D-ribofuranosyl)hypoxanthine (17). To a solution of compound **10** (12 mg) in 0.1M phosphate buffer (2 mL), was added 125 units of adenosine deaminase and the enzymatic reaction was left at 37°C for 7 days when

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

completion of the conversion was indicated by the disappearance of the absorbance at 277 nm. The reaction mixture was worked up and purified as described for **15** to give compound **17** in 62% yield. Mp. 161–162°C; UV (MeOH) λ_{max} 255 (ε 11,372); ^{1}H NMR (CD_3OD) δ 1.07 (m, 2H), 1.09 (m, 2H), 1.99 (m, 1H), 3.73 (m, 1H), 3.81 (m, 1H), 4.07 (m, 1H), 4.30 (m, 1H), 4.62 (m, 1H), 5.91 (d, J = 5.5 Hz, 1H), 8.17 (s, 1H); ^{13}C NMR (CD_3OD) δ 10.5, 10.6, 14.8, 63.0, 72.0, 75.7, 87.0, 90.1, 123.4, 140.3, 150.9, 160.2, 163.0; HRMS (FAB) calcd for $C_{13}H_{16}N_4O_5$ $[M+H]^+$ 309.1201, found 309.1204.

6-Chloro-2[(*Z*)-2-fluorovinyl]-9-(β-D-ribofuranosyl)purine (14). To a solution of tributylphosphine (6.7 mL, 27.16 mmol) in anhydrous DCM (15 mL) at 0°C under nitrogen, was added trichlorofluoromethane (0.9 mL, 10.18 mmol). This mixture was stirred at 0°C for 1 h, and then at room temperature for 3 h. Compound **4** (2.99 g, 6.79 mmol) in anhydrous DCM (20 mL) was then added and the mixture was stirred for 18 h at room temperature followed by addition of 10% NaOH (12 mL). After further stirring at room temperature for 20 h, the reaction mixture was neutralized and worked up by separating the organic layer and washing with water (3 × 15 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to dryness. Purification over silica gel (40% EtOAc/ hexanes) gave (*Z*)-6-chloro-2(2-fluorovinyl)-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (**13**) as a syrup (1.43 g, 46%). ^{1}H NMR ($CDCl_3$) δ 2.04 (s, 3H), 2.07 (s, 3H), 2.12 (s, 3H), 4.33 (m, 1H), 4.34 (m, 2H), 5.75 (t, J = 5.6 Hz, 1H), 5.97 (m, 1.5H), 6.09 (m, 1.5H), 6.75 (dd, J = 5.7, 78.8 Hz, 0.5H), 6.97 (dd, J = 5.6, 78.9 Hz, 0.5H), 8.12 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 20.3, 20.4, 20.6, 63.0, 70.3, 73.3, 80.3, 87.8, 110.3 (d, $J_{F,C}$ = 4.3 Hz), 130.4, 144.2, 150.9, 151.2, 153.4 (d, $J_{F,C}$ = 285.6 Hz), 156.1, 169.3, 169.4, 170.3. Compound **13** was deprotected and purified by HPLC (45% $MeOH/H_2O$) using the general procedure described above to give **14** as a white solid in 48% yield. Mp. 176–177°C; UV (MeOH) λ_{max} 274 (ε 10,582), 228 (ε 19,007);^{1}H NMR (CD_3OD) δ 3.79 (m, 1H), 3.86 (m, 1H), 4.14 (m, 1H), 4.40 (m, 1H), 4.79 (m, 1H), 5.97 (dd, J = 5.5, 39.0 Hz, 0.5H), 6.06 (dd, J = 5.5, 39.1 Hz, 0.5H), 6.11 (d, J = 5.1 Hz, 1H), 6.93 (dd, J = 79.2, 5.5 Hz, 0.5H), 7.15 (dd, J = 79.2, 5.5 Hz, 0.5H), 8.78 (s, 1H); ^{19}F NMR (CD_3OD) δ −108.52 (dd, J = 38.2, 79.9 Hz, 1F); ^{13}C NMR (CD_3OD)δ 62.9, 72.0, 75.6, 87.4, 90.9, 111.0 (d, $J_{F,C}$ = 4.4 Hz), 131.2, 147.5, 151.0, 152.9, 155.0 (d, $J_{F,C}$ = 266.8 Hz), 157.1; HRMS (FAB) calcd for $C_{12}H_{12}ClN_4O_4$ $[M+H]^+$ 331.0611, found 331.0609.

2-[(Z)-2-Fluorovinyl]-9-(β-D-ribofuranosyl)hypoxanthine (19). To a solution of compound **14** (20 mg) in 0.1M phosphate buffer (2 mL) was added 125 units of adenosine deaminase. After 3 days at room temperature, disappearance of the UV λ_{max} at 272 nm indicated completion of the conversion. The reaction mixture was worked up and purified as described for compound **15** to give **19** in 58% yield. Mp. 216–217°C (decomp); UV (MeOH) λ_{max} 299 (ε 6,871), 253 (ε 7,863); ^{1}H NMR (CD_3OD) δ 3.74 (m, 1H), 3.83 (m, 1H), 4.12 (m, 1H), 4.34 (m, 1H), 4.68 (m, 1H), 5.76 (dd, J = 5.6, 39.2 Hz, 0.5H), 5.86 (dd, J = 5.6, 39.2 Hz, 0.5H), 5.97 (d, J = 5.7 Hz, 1H), 6.93 (d, J = 5.5, 79.8 Hz, 0.5H), 7.15 (dd, J = 5.5, 79.8 Hz, 0.5H), 8.23 (s, 1H); ^{19}F NMR (CD_3OD): δ −105.23 (dd, J = 39.4, 76.2 Hz, 1F).; ^{13}C NMR (CD_3OD) δ 63.1, 72.1, 75.8, 87.4, 90.6, 106.0 (d, $J_{F,C}$ = 4.3 Hz), 124.5, 141.6, 149.9, 152.2,

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

156.2 (d, $J_{F,C} = 283.4$ Hz), 157.8; HRMS (FAB) calcd for $C_{12}H_{13}FN_4O_5$ $[M + H]^+$ 313.0950, found 313.0945.

6-Chloro-2-(3-fluoropropyn-1-yl)-9-(β-D-ribofuranosyl)purine (12). To a solution of compound **7** (0.249 g, 0.53 mmol) in DCM (15 mL) at −78°C was added DAST (0.352 g, 2.66 mmol) and the reaction mixture was stirred while the temperature raised over 2 h from −78°C to room temperature. The reaction was quenched at −15°C with methanol and then concentrated to dryness under reduced pressure. Purification over silica gel gave 6-chloro-2-(3-fluoropropyn-1-yl)-9-(2, 3, 5-tri-O-acetyl-β-D-ribofuranosyl)purine (**11**) as a pale yellow solid (0.11 g, 44%). Mp. 131–132°C; ^{1}H NMR ($CDCl_3$) δ 2.06 (s, 3H), 2.14 (s, 3H), 2.15 (s, 3H), 4.40 (m, 2H), 4.46(m, 1H), 5.20 (d, J = 47.1 Hz, 2H), 5.55 (m, 1H), 5.78 (m, 1H), 6.27 (d, J = 5.7 Hz, Hz, 1H), 8.34 (s, 1H).; ^{13}C NMR ($CDCl_3$) δ 20.2, 20.4, 20.7, 62.8, 70.4 (d, J = 169 Hz), 70.6, 73.1, 78.7, 80.8, 83.8, 86.0, 131.7, 144.2, 144.7, 151.3, 151.4, 169.3, 169.5, 170.1. Compound **11** was deprotected following the general procedure described above and purified by HPLC to give **12** as a pale yellow solid in 44% yield. Mp. 131–132°C; UV (MeOH) λ_{max} 270 (ε 11,560), 252 (ε 7,621); ^{1}H NMR (CD_3OD) δ 3.75 (m, 1H), 3.87 (m, 1H), 4.12 (m, 1H), 4.31 (m, 1H), 4.61 (m, 1H), 5.22 (d, J = 47.1 Hz, 2H), 6.06 (d, J = 4.9 Hz, 1H), 8.84 (s, 1H); ^{13}C NMR (CD_3OD) δ 62.5, 71.5 (d, J = 165 Hz), 71.6, 76.0, 82.3, 82.5, 87.4, 90.0, 132.7, 145.5, 148.0, 151.3, 152.6; HRMS (FAB) calcd for $C_{13}H_{13}ClFN_4O_4$ $[M + H]^+$ 343.0611, found 343.0685.

2-(3-Fluoropropyn-1-yl)-9-(β-D-ribofuranosyl)hypoxanthine (18). To compound **12** (20 mg) in 0.1M phosphate buffer (2 mL) was added 50 units of adenosine deaminase. The enzymatic reaction was left at room temperature for 2 days when disappearance of the UV peak at 270 nm indicated completion of the reaction. The reaction mixture was worked up and purified as described for **15** to give compound **18** in 74% yield. Mp. 162–163°C (decomp); UV (MeOH) λ_{max} 301 (ε 12,421), 252 (ε 10, 276); ^{1}H NMR (CD_3OD) δ 3.76 (dd, J = 3.1, 12.4 Hz, 1H), 3.86 (dd, J = 2.7, 12.4 Hz, 1H), 4.13 (m, 1H), 4.31 (m, 1H), 4.63 (t, J = 5.3 Hz, 1H), 5.25 (d, J = 47.0 Hz, Hz, 2H), 5.95 (d, J = 5.6 Hz, 1H), 8.29 (s, 1H).; ^{13}C NMR (CD_3OD) δ 63.0, 71.4 (d, J = 165 Hz), 72.1, 75.9, 83.7, 84.7, 87.6, 90.8, 126.5, 141.4, 149.4, 161.2, 170.4; HRMS (FAB) calcd for $C_{12}H_{13}N_4O_5$ $[M + H]^+$ 325.0950, found 325.0936.

Inhibition Studies with Inosine Monophosphate Dehydrogenase (IMPDH). Inhibition studies with bacterial IMPDH was carried out as described previously by us.[22]

Virus Pools, Media, Cell Cultures and Assay Methodology. The vaccinia virus strain, the media, and cell cultures used, and the assay methods for determining efficacy and toxicity were essentially those previously reported.[26]

ACKNOWLEDGMENTS

The project described was supported by Grant Numbers AI 48495 (Nair) and AI-85347 (Kern) from the National Institutes of Health. Its contents are solely the

responsibility of the authors and do not necessarily represent the official views of the NIH. We thank K. Keith, S. Pal and R. Kamboj for technical assistance.

REFERENCES

1. Sintchak, M.D.; Nimmesgern, E. Immunopharmacology **2000**, *47*, 163–184.
2. Hedstrom, L. Curr. Med. Chem. **1999**, *6*, 545–560.
3. Franchetti, P.; Grifantini, M. Curr. Med. Chem. **1999**, *6*, 599–614.
4. Goldstein, B.M.; Colby, T.D. Curr. Med. Chem. **1999**, *6*, 519–536.
5. Pankiewicz, K.W. Exp. Opin. Ther. Patents **1999**, *9*, 55–65.
6. Zimmermann, A.G.; Gu, J-J.; Laliberte, J.; Mitchell, B.S. Prog. Nucl. Acid Res. Mol. Biol. **1998**, *61*, 181–209.
7. (a) Smith, R.A.; Kirkpatrick, W. Eds. *Ribavirin, A Broad Spectrum Antiviral Agent* ; Academic Press: New York, 1980; (b) De Clercq, E.; Cools, M.; Balzarini, J.; Snoeck, R.; Andrei, G.; Hosoya, M.; Shigeta, S.; Ueda, T.; Minakawa, M.; Mastuda, A. Antimicrob. Agents Chemother. **1991**, *35*, 679–684; (c) Rankin, J.T. Jr.; Eppe, S.B.; Antczak, J.B.; Joklik, W.K. Virology **1989**, *168*, 147–158; (d) Sidwell, R.W.; Huffmann, J.H.; Khare, G.P.; Allen, L.B.; Witkowski, J.T.; Robins, R.K. Science, **1972**, *177*, 705–706.
8. Shuto, S.; Obara, T.; Toriya, M.; Hosoya, M.; Snoeck, R.; Andrie, G.; Balzarini, J.; De Clercq, E. J. Med. Chem. **1992**, *35*, 324–331.
9. (a) Mizuno, K.; Tsujino, M.; Takada, M.; Hayashi, M.; Atsumi, K. J. Antibiotics **1974**, *27*, 775–782; (b) Sakaguchi, K.; Tsujino, M.; Yoshizawa, M.; Mizuno, K.; Hayano, K. Cancer Res. **1975**, *35*, 1643–1648; (c) Shiraki, K.; Ishibashi, M.; Okuno, T.; Kokado, Y.; Takahara, S.; Yamanishi, K.; Sonoda, T.; Takahashi, M. Transplant. Proc. **1990**, *22*, 1682–1685.
10. (a) Nair, V.; Turner, G.A.; Chamberlain, S.D. J. Am. Chem. Soc. **1987**, *109*, 7223–7224; (b) Nair, V.; Turner, G.A.; Buenger, G.S.; Chamberlain, S.D. J. Org. Chem. **1988**, *53*, 3051-3057.
11. Nair, V.; Ussery, M.A. Antiviral Res. **1992**, *19*, 173–178.
12. Suckling, C.J. Angew. Chem. Int. Ed. **1988**, *27*, 537–552.
13. Thorand, S.; Krause, N. J. Org. Chem. **1998**, *63*, 8551–8553.
14. Sonogashira, K.; Tohda, Y.; Hagihara, N. Tetrahedron Lett. **1975**, *50*, 4467–4470.
15. Peters, D.; Hornfeldt, A.B.; Gronowitz, S. J. Heterocycl. Chem. **1991**, *28*, 1629–1631.
16. (a) Charette, A.B.; Giroux, A. J. Org. Chem. **1996**, *61*, 8718–8719; (b) Hildebrand, J.P.; Marsden, S.P. Synlett **1996**, *9*, 893–894; (c) Wang, X.-Z.; Deng, M.-Z. J. Chem. Soc. Perkin Trans. **1996**, *22*, 2663–2664; (d) Charette, A.B.; De Freitas-Gil, R.P. Tetrahedron Lett. **1997**, *38*, 2809–2812; (e) Zhou, S.-M.; Yan, Y.-L.; Deng, M.-Z. Synlett **1998**, 198–200; (f) Luithle, J.E.A.; Pietruszka, J. J. Org. Chem. **1999**, *64*, 8287–8297.
17. Soderquist, J.A.; Huertas, R.; Leon-Colon, G. Tetrahedron Lett. **2000**, *41*, 4251–4255.
18. (a) Cox, D.G.; Gurusamy, N.; Burton, D.J. J. Am. Chem. Soc. **1985**, *107*, 2811–2812; (b) Kataoka, K.; Tsuboi, S. Synthesis, **2000**, 452–456.

19. (a) Hanna, N.B.; Ramasamy, K.; Robins, R.K.; Revankar, G.R. J. Heterocyclic Chem. **1988**, *25*, 1899–1903; (b) Wang, P.; Gullen, B.; Newton, M.G.; Cheng, Y-C.; Schinazi, R.F.; Chu, C.K. J. Med. Chem. **1999**, *42*, 3390–3399.
20. Ferrero, M.; Gotor, V. Chem. Rev. **2000**, *100*, 4319–4347.
21. Nair, V.; Sells, T.B. Synlett **1991**, 753–754.
22. Pal, S.; Bera, B.; Nair, V. Bioorg. Med. Chem. **2002**, *10*, 3615–3618.
23. Smee, D.F.; Huggins, J.W. Antiviral Res. **1998**, *37*, A89.
24. Neyts, J.; DeClercq, E.J. Med. Virol. **1993**, *41*, 242–246.
25. De Clercq, E. Clin. Microbiol. Rev. **2001**, *14*, 382–397.
26. Kern, E.R.; Hartline, C.; Harden, E.; Keith, K.; Rodriguez, N.; Beadle, J.R.; Hostetler, K.Y. Antimicrob. Agents Chemother. **2002**, *46*, 991–995.

Received September 6, 2002
Accepted December 9, 2002

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016