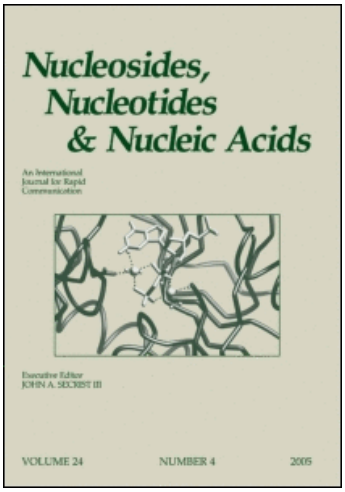

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

ACYCLIC NUCLEOSIDE/NUCLEOTIDE ANALOGUES WITH AN IMIDAZOLE RING SKELETON

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Online publication date: 31 July 2001

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(8), 1599–1614 (2001)

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ABSTRACT

Syntheses of a few acyclic nucleoside and acyclic nucleoside phosphonate analogues containing an imidazole ring have been reported. These analogues include methyl 1-(2-hydroxyethoxymethyl)imidazole-4, 5-dicarbo-xylate (**1**), 4,5-dicarbamoyl-1-(2-hydroxyethoxymethyl)imidazole (**2**), 4,5-dicyano-1-(2-hydroxyethoxymethyl)imidazole (**4**), Methyl 1-(2-bromoethoxymethyl)imidazole-4,5-dicarboxylate (**7**), 4,5-dicyano-(2-bromoethoxymethyl)imidazole (**8**), and Methyl 1-(2-phosphonomethoxyethyl)imidazole (**10**). Also reported are a few potential prodrugs of the above compounds, including the acetyl derivatives **5** and **6** (of **1** and **4**, respectively), and the diethyl phosphonate ester **9** (of **10**). In addition, the corresponding benzyl-protected precursors **11** and **12** (of **1** and **4**, respectively), along with their common hydrolysis product, 1-(2-benzyloxy-ethoxymethyl)-4,5-imidazoledicarboxylic acid (**3**), are reported. Another potential prodrug included in the list is 1-(2-acetoxyethyl)-4,5-dicyanoimidazole (**15**). The compounds were screened for *in vitro* antiviral activity against a wide variety of herpes and respiratory viruses. The most active compound was the phosphonate analogue **9** which exhibited an anti-measles virus activity with an EC_{50} of < 2.5 μg/mL and an SI value of > 176.

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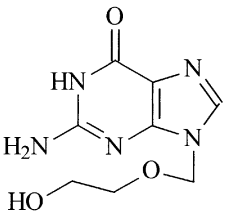
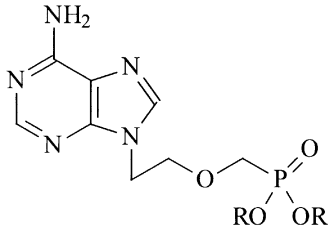
INTRODUCTION

Acyclic nucleoside[1–9] and acyclic nucleotide[10–25] analogues have played important roles as powerful antiviral and anticancer agents in modern medicine[1–26]. These are the analogues of natural nucleosides or nucleotides whose pentafuranosyl sugar ring has been replaced with an acyclic moiety that mimics part or whole of the natural sugar or sugar phosphate. Included in this category are 9-(2-hydroxyethoxymethyl)guanine (acyclovir, ACV)[1] and 9-[2-(phosphono-methoxy)ethyl]adenine (PMEA)[10–11], among host of other nucleoside/nucleotide analogues discovered in recent years[1–26].



ACV



PMEA (R =H)

Acyclovir is highly effective for treatment against herpes simplex viruses (HSV-1 and HSV-2)[7], varicella-zoster virus (VZV)[6–8], and also shows good activity against Epstein-Barr virus (EBV)[26]. PMEA[10–11] and its guanine counterpart PMEG[17–18] are two of the most potent, broad spectrum acyclic nucleotide analogues reported to date[11,19–20]. The presence of a phosphonate group, instead of a phosphate moiety as present in natural nucleotides, renders PMEA less prone to *in vivo* chemical or enzymatic hydrolysis, thus increasing its therapeutic life time and efficacy. It has also been discovered that the antiviral efficacy further increases if the compound is administered as a prodrug, for example, as a phosphonate ester (*e.g.* Bis-POM-PMEA where R=$(CH_3)_3CCO_2CH_2$-) that has a better chance of penetration through the cell membrane, but undergoes facile hydrolysis in the cell interior to form the free acid[10]. Most, if not all, acyclic nucleoside/nucleotide analogues require *in vivo* conversion into their triphosphate derivatives by kinases so as to be incorporated into viral genome, and subsequently cause nucleic acid chain termination[17]. In this regard, phosphonate derivatives offer a distinct advantage over other conventional nucleoside analogues in that they are able to bypass the crucial first step of phosphorylation, the lack of which has often been found to be the underlying cause of therapeutic failure of many otherwise promising nucleoside analogues.

In spite of extensive research performed on acyclic purine and pyrimidine nucleoside/nucleotide analogues in recent years, relatively less is known on the same analogues containing 5-membered heterocyclic ring

counterparts. This is surprising in view of the fact that a number of imidazole, pyrazole, and triazole nucleoside analogues have long been documented as potent antiviral, antibacterial, and antitumor agents[27–31]. These include, but are not limited to, bredinin[32], a potent nucleoside antibiotic containing an imidazole ring, and ribavirin[29,33–36], a broad spectrum antiviral nucleoside possessing a triazole nucleus. We report here the synthesis of a few acyclic nucleoside and nucleoside phosphonate analogues of imidazole, which contain the acyclic sugar moiety of ACV or PMEA.

RESULTS AND DISCUSSION

Chemistry

The analogues reported herein include methyl 1-(2-hydroxyethoxymethyl)imidazole-4,5-dicarboxylate (**1**), 4,5-dicarbamoyl-1-(2-hydroxy ethoxymethyl)imidazole (**2**), 4,5-dicyano-1-(2-hydroxyethoxymethyl)imida zole (**4**), Methyl 1-(2-bromoethoxymethyl)imidazole-4,5-dicarboxylate (**7**), 4,5-dicyano-(2-bromoethoxymethyl)imidazole (**8**), and Methyl 1-(2-phosphonomethoxyethyl)imidazole (**10**). Also reported are a few potential prodrugs of the above compounds, including the acetyl derivatives **5** and **6** (of **1** and **4**, respectively), and the diethyl phosphonate ester **9** (of **10**). In addition, the corresponding benzyl-protected precursors **11** and **12** (of **1** and **4**, respectively), along with their common hydrolysis product, the dicarboxylic acid **3**, are reported. Another potential prodrug included in the list is 4,5-dicyano-1-(2-acetoxyethyl)imidazole (**15**). Most of the chemistry employed in here is either simple or well documented in the literature[37].

Syntheses of **1**, **2**, **5**, **7**, **9**, **10**, and **11** are outlined in Scheme I, while those of **4**, **6**, **12** and **15** are given in Scheme II. Synthesis of compound **3**, obtained from both 4,5-dicarbomethoxy- and 4,5-dicyanoimidazole precursors, is outlined in both schemes I and II. The acyclic nucleoside **1** was prepared by sequential reactions involving alkylation of methyl 4,5-imidazoledicarboxylate (**13**)[38] with 2-benzyloxyethoxymethyl chloride[39] in the presence of triethylamine or potassium carbonate in dimethylformamide (to form **11**), followed by catalytic hydrogenation over palladiun-charcoal in methanol. The acyclic nucleoside **2** was synthesized by treatment of **5** with ammonium hydroxide or methanolic ammonia. Compound **5**, in turn, was prepared by sequential silylation and alkylation of **13**, using bis(trimethylsilyl)trifluoroacetamide (BTMSTFA)[40] in pyridine and 2-acetoxyethoxymethyl bromide[41], respectively. The acyclic nucleoside **4** was prepared by hydrolysis of **6**, using an ion-exchange resin (H^+ form) as shown in Scheme II. Compound **6** was prepared from 4,5-dicyanoimidazole (**14**)[40], using an analogous procedure employed for **5**. The acyclic nucleoside phosphonate analogue **10** was synthesized by sequential reactions involving base-catalyzed

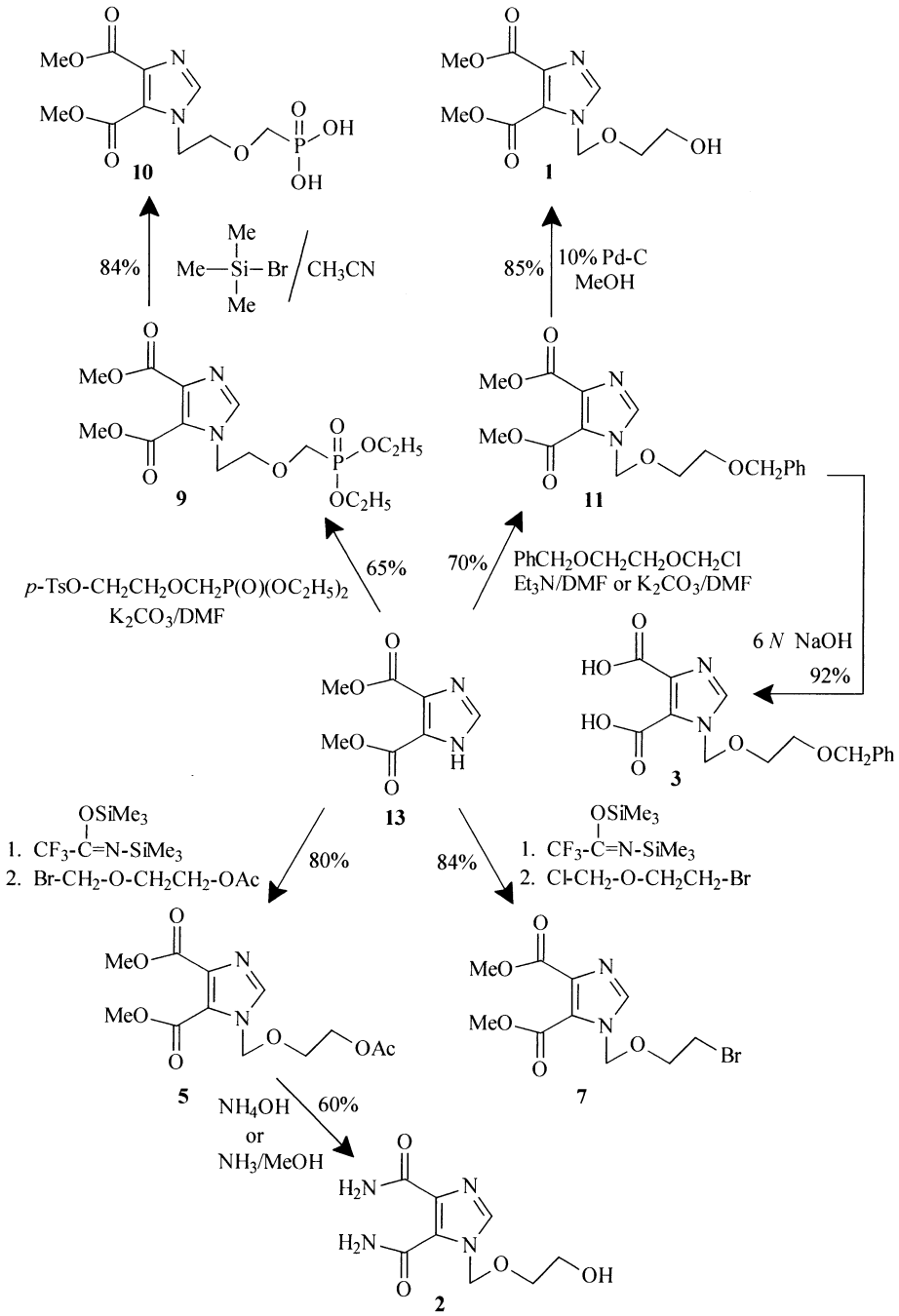
condensation of **13** with diethyl (2-*p*-toluenesulfonyloxyethoxymethanephos phonate[42] (to form **9**), followed by dealkylation of the phosphonate ester groups using bromotrimethylsilane in acetonitrile. The acyclic nucleoside **3** containing a 4,5-dicarboxyimidazole nucleus was prepared by saponification of either the corresponding diester precursor **11** or the dinitrile precursor **12**. The acyclic nucleosides **7** and **8** were prepared by condensation of the silylated derivatives of the respective imidazole precursors **13** and **14** with 2-bromoethoxymethyl chloride[43]. Compound **15** was prepared by alkylation of **14** with 2-acetoxyethyl bromide[40] in the presence of potassium carbonate in dimethylformamide. The percent yields shown for each reaction in Schemes I and II are for the pure products obtained either by recrystallization or by chromatography. All products were characterized by spectroscopic and by either microanaytical or high resolution mass spectral analyses.

Biological Screening

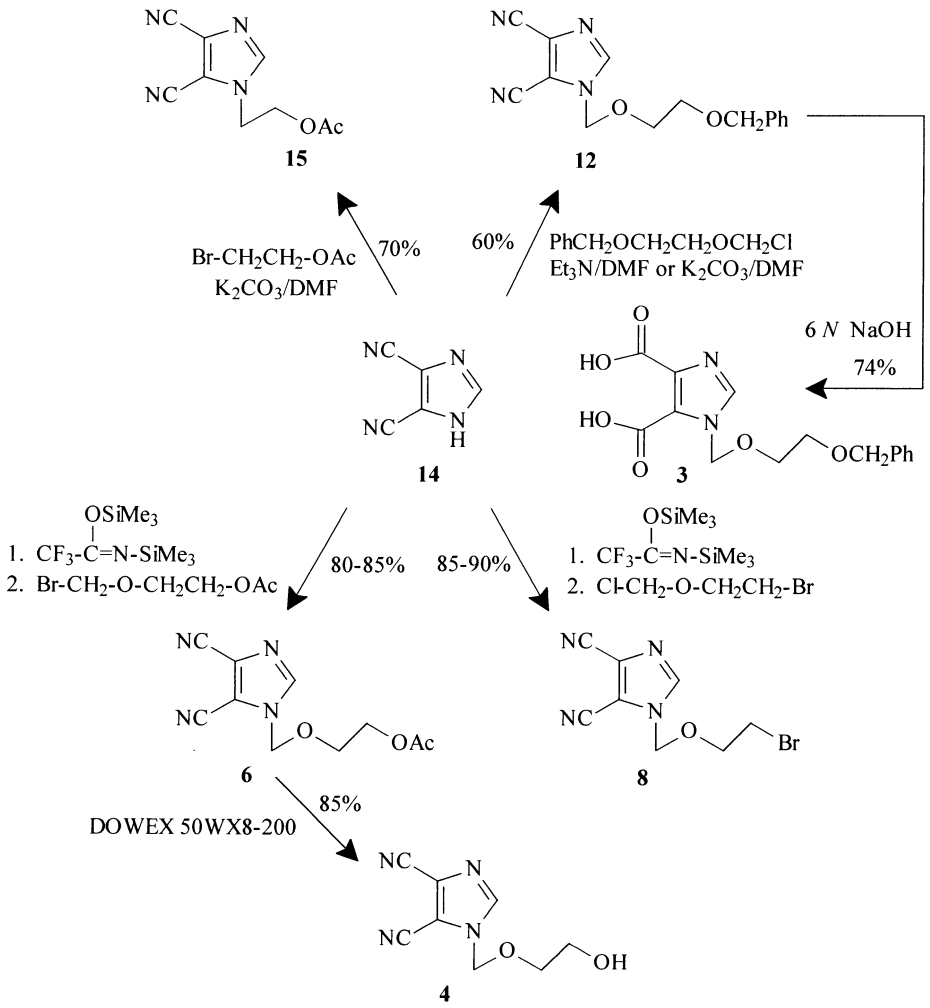
A majority of compounds of Schemes I and II were screened for *in vitro* antiviral activity against a wide variety of herpes and respiratory viruses under the auspices of the antiviral drug screening program of the National Institute of Allergy and Infectious Diseases (NIAID)[44]. While the herpes family of viruses included HSV-1, HSV-2, HCMV, MCMV, VZV, and EBV, the respiratory viruses screened against were adenovirus type I, measles virus, parainfluenza virus type 3, rhinovirus type 2, influenza A (H1N1), influenza A (H3N2), and influenza B. A number of the tested compounds failed to show either activity or adequate selectivity over toxicity against most of the viruses, with the exception of compound **9** which exhibited an EC_{50} of $< 2.5\,\mu g/mL$, an IC_{50} of $440\,\mu g/mL$, and a selectivity index (SI) of > 176 against the measles virus in a Cytopathic Effect Inhibition (CPE) assay[45]. The compound tested positive in a Neutral Red (NR) uptake assay[45] as well with an EC_{50} of $2.5\,\mu g/mL$, an IC_{50} of $59\,\mu g/mL$, and a selectivity index (SI) of 24. Compound **10**, which is anticipated to possess a similar activity profile as its likely prodrug precursor **9**, remains yet to be screened. Compounds **9** and **10** are the target imidazole analogues of PMEA described under Introduction.

EXPERIMENTAL

The ^1H and ^{13}C nmr spectra were recorded on a General Electric QE-300 nmr spectrometer operating at 300 MHz for ^1H and 75 MHz for ^{13}C. The data are reported in the following format: Chemical shift (all relative to Me_4Si), multiplicity (s = singlet, d = doublet, dt = double triplet, dd = double doublet, t = triplet, q = quartet, m = multiplet, b = broad, coupling constants, integration and assignment). Evaporations were done under reduced pressure



Scheme I. Synthesis of acyclic nucleoside and nucleoside phosphonate analogues.



Scheme II. Synthesis of acyclic nucleoside analogues **3, 4**, **6**, **8**, **12**, and **15**.

on a rotary evaporator. Thin layer chromatography was performed on Merck Kieselgel 60 F_{254} (0.2 mm thickness). Elemental microanalyses were performed by Atlantic Microlab, Inc., Norcross, Georgia. Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Dry solvents were prepared as follows: methanol was distilled from calcium hydride and was stored over molecular sieves (type 3Å); dimethylformamide was dried over calcium oxide and then distilled under reduced pressure from calcium hydride, and was subsequently stored over molecular sieves (type 3Å). All starting materials were purchased from Aldrich Chemical Co. All solvents were reagent grade and were purchased

from VWR Scientific. All yields reported are for pure, dry compounds that require no further purification for use in other reactions.

Methyl 1-(2-Hydroxyethoxymethyl)-4,5-imidazoledicarboxylate(1)

Methyl 1-(2-benzyloxyethoxymethyl)-4,5-imidazoledicarboxylate(**11**) (1.74 g, 5 mmol) was dissolved in a mixture of 20 mL of methanol and 5 mL of glacial acetic acid, and the solution was transferred to a hydrogenation bottle. Palladium on activated carbon (10% (1.74 g) was added, and the bottle shaken in a Parr Hydrogenation apparatus at 50 psi for 48 h. The reaction mixture was filtered through a pad of Celite.™ A TLC analysis showed that the reaction was complete. The filtrate was evaporated to dryness. The residue was purified by silica gel flash chromatography, eluting first with chloroform and then with chloroform-methanol (20 : 1) to give **1** as a pure waxy product (semi-solid) (1.1 g, 85%). Rf 0.37 ($CHCl_3$-MeOH, 10 : 1). ^{1}H-NMR(DMSO-d_6) δ 8.12 (s, 1H, imidazole), 5.57 (s, 2H, NCH_2O), 4.70 (brs, 1H, OH, exchangeable with D_2O), 3.83 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.40 (m, 4H, OCH_2CH_2OH); ^{13}C-NMR (DMSO-d_6) δ 52.05, 52.69, 59.75, 70.44, 75.84, 136.27, 140.85, 140.97, 160.05, 162.73.

Anal: Calcd. for $C_{10}H_{14}N_2O_6$: C, 46.49; H, 5.47; N, 10.85. Found: C, 46.52; H, 5.52; N, 10.69.

4,5-Dicarbamoyl-1-(2-Hydroxyethoxymethyl)imidazole (2)

A solution of methyl 2-acetoxyethoxymethyl-4,5-imidazoledicarboxylate (50 mg) in 5 mL of 30% aqueous ammonia was stirred overnight. The solvent was removed *in vacuo*, the residue was purified by silica gel column chromatography to give a waxy solid (23 mg, 60%), Rf 0.20, $CHCl_3$: Methanol (20 : 1). ^{1}H NMR ($CDCl_3$) δ 10.59 (brs, 1H, NH, exchangeable with D_2O), 8.09 (s, 1H, imidazole), 8.03 (brs, 1H, NH, exchangeable with D_2O), 7.85 (brs, 1H, NH, exchangeable with D_2O), 7.65 (brs, 1H, NH, exchangeable with D_2O), 5.85 (s, 2H, NCH_2O), 3.2–3.5 (m, 4H, OCH_2-CH_2OH); FABMS m/z: 229 [MH^+].

Anal: HRMS (FAB): Calcd for $C_8H_{14}N_4O_4$ 229.0937, Found; 229.0948.

1-(2-Benzyloxyethoxymethyl)-4,5-imidazoledicarboxylic Acid (3)

Method A: A mixture of 1-(2-benzyloxyethoxymethyl)-4,5-dicyanoimidazole (**13**) (50 mg, 0.18 mmol) and 6N aq. sodium hydroxide (5 mL) was refluxed for 24 h. The reaction mixture was neutralized with 1 N hydrochloric acid, filtered and the solid was washed with water to give a crude product, which was recrystallized from water to obtain pure **3** (43 mg, 74%), mp $>$ 250 °C. Rf 0.55 ($CHCl_3$-MeOH, 2 : 1).

^{1}H-NMR (DMSO-d_6) δ 9.02 (s, 1H, imidazole), 7.24–7.35 (m, 5H, Ph), 5.91 (s, 2H, NCH_2O), 4.44 (s, 2H, $PhCH_2$), 3.75 (t, 2H, J = 4.8 Hz, OCH_2-CH_2OCH_2Ph), 3.53 (t, 2H, J = 4.8 Hz, $OCH_2CH_2OCH_2Ph$).

Anal: HRMS (FAB): Calcd for $C_{15}H_{17}N_2O_6$ 321.1086. Found: 321.1075.

Method B: Compound **3** was also obtained in 92% yield by hydrolysis of methyl 1-(2-benzyloxyethoxymethyl)-4,5-imidazoledicarboxylate (**11**), using the procedure described under Method A above.

1-(2-Hydroxyethoxymethyl)-4,5-dicyanoimidazole (4)

A mixture of 1-(2-acetoxyethoxymethyl)-4,5-dicyanoimidazole (**6**) (0.5 g, 2.14 mmol) and Dowex 50WX8-200 ion-exchange resin (H^+ form, pre-washed with ethanol) (0.5 g) in 10 mL of ethanol was refluxed with stirring for 24 h until the reaction was complete. The mixture was filtered, the resin washed with ethanol and the combined filtrate was concentrated *in vacuo*. The residue was purified by silica gel flash chromatography, eluting with chloroform-methanol (20 : 1) to give **4** as a colourless liquid 0.35 g (85%). Rf 0.37 ($CHCl_3$-MeOH, 10 : 1). ^{1}H-NMR(DMSO-d_6) δ 8.09 (s, 1H, imidazole), 5.41 (s, 2H, NCH_2O), 4.65 (brs, 1H, OH, exchangeable with D_2O), 3.50 (m, 4H, OCH_2CH_2OH); FABMS m/z: 193 [MH^+].

Anal: HRMS (FAB): Calcd for $C_8H_9N_4O_2$ 193.0726. Found: 193.0730.

Methyl 1-(2-Acetoxyethoxymethyl)-4,5-imidazoledicarboxylate (5)

A mixture of methyl 4,5-imidazoledicarboxylate (1.84 g, 10 mmol), bis(trimethylsilyl)- trifluoroacetamide (BTMSTFA) (10.3 g, 40 mmol) and pyridine (5 mL, 60 mmol) in 30 mL of dry acetonitrile was stirred for 12 h at room temperature. The solvent and excess reagents were evaporated *in vacuo* under anhydrous conditions, and the residue coevaporated with dry acetonitrile and toluene (3 × 10 mL) was kept in vacuum until a syrup was formed, and then dissolved in 30 mL of dry acetonitrile. To the solution was added 2-acetoxyethoxymethyl bromide(2.94 g, 15 mmol). The mixture was stirred for 24 h at room temperature, and the reaction was quenched with methanol (20 mL) and stirred for 2 h. The solvents were evaporated and the residue was purified by column chromatography eluting with chloroform to give **5** as a pure colourless liquid (2.4 g, 80%). ^{1}H-NMR ($CDCl_3$) δ 7.76 (s, 1H, imidazole), 5.64 (s, 2H, NCH_2O), 3.95 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 4.19 (t, 2H, J = 6.0 Hz, CH_2OAc), 3.74 (t, 2H, J = 6.0 Hz, OCH_2), 2.10 (s, 3H, Ac); FABMS m/z: 301 [MH^+].

Anal: HRMS (FAB): Calcd for $C_{12}H_{17}N_2O_7$ 301.1036. Found: 301.1019.

1-(2-Acetoxyethoxymethyl)-4,5-dicyanoimidazole (6)

A mixture of 4,5-dicyanoimidazole (1.2 g, 10 mmol), bis(trimethylsilyl)-trifluoroacetamide (BTMSTFA) (10.3 g, 40 mmol) and pyridine (5 mL, 60 mmol) in 30 mL of dry acetonitrile was stirred for 12 h at room temperature. The solvent and excess reagents were evaporated *in vacuo* under anhydrous conditions, and the residue coevaporated with dry acetonitrile and toluene (3×10 mL) was kept in vacuum until a syrup was formed, and then dissolved in 30 mL of dry acetonitrile. To the solution was added 2-acetoxyethoxymethyl bromide(2.94 g, 15 mmol). The mixture was stirred for 24 h at room temperature, and the reaction was quenched with methanol (20 mL) and stirred for 2 h. The solvents were evaporated and the residue was purified by column chromatography eluting with chloroform to give **6** as a pure colourless liquid (2.0 g, 85%). Rf 0.55 ($CHCl_3$-MeOH, 10:1). ^{1}H-NMR ($CDCl_3$) δ 7.85 (s, 1H, imidazole), 5.45 (s, 2H, OCH_2N), 4.25 (t, 2H, J = 4.8 Hz, $AcOCH_2$), 3.81 (t, 2H, J = 4.8 Hz, OCH_2), 2.10 (s, 3H, Ac); FABMS m/z: 235 [MH^+].

Anal: HRMS (FAB): Calcd for $C_{10}H_{11}N_4O_3$ 235.0831. Found: 235.0836.

Methyl 1-(2-Bromoethoxymethyl)-4,5-imidazoledicarboxylate (7)

A mixture of methyl 4,5-imidazoledicarboxylate (1.84 g, 10 mmol), bis(trimethylsilyl)-trifluoroacetamide (BTMSTFA) (10.3 g, 40 mmol) and pyridine (5 mL, 60 mmol) in 30 mL of dry acetonitrile was stirred for 12 h at room temperature. The solvent and the excess reagents were evaporated *in vacuo* under anhydrous conditions, and the residue coevaporated with dry acetonitrile and toluene (3×10 mL) was kept in vacuum until a syrup was formed, and then dissolved in 30 mL of dry acetonitrile. To the solution was added 2-bromoethoxymethyl chloride (2.58 g, 15 mmol). The mixture was stirred for 24 h at room temperature, and the reaction was quenched with methanol (20 mL) and stirred for 2 h. The solvents were evaporated and the residue was purified by column chromatography eluting with chloroform to give **7** as a pure colorless liquid (2.7 g, 84%). ^{1}H-NMR ($CDCl_3$) δ 7.78 (s, 1H, imidazole), 5.68 (s, 2H, NCH_2O), 3.95 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 3.81 (t, 2H, J = 6.0 Hz, OCH_2), 3.42 (t, 2H, J = 6.0 Hz, CH_2Br); FABMS m/z: 321, 323 [MH^+].

Anal: HRMS(FAB): Calcd for $C_{10}H_{14}N_2O_5{}^{79}Br$ 321.0086. Found: 321.0089; Calcd for $C_{10}H_{14}N_2O_5{}^{81}Br$ 323.0066. Found: 323.0070.

1-(2-Bromoethoxymethyl)-4,5-dicyanoimidazole (8)

A mixture of 4,5-dicyanoimidazole (1.2 g, 10 mmol), bis(trimethylsilyl)-trifluoro-acetamide (BTMSTFA) (10.3 g, 40 mmol) and pyridine (5 mL,

60 mmol) in 30 mL of dry acetonitrile was stirred for 12 h at room temperature. The solvent and the excess reagents were evaporated *in vacuo* under anhydrous conditions, and the residue coevaporated with dry acetonitrile and toluene (3×10 mL) was kept in vacuum until a syrup was formed, and then dissolved in 30 mL of dry acetonitrile. To the solution was added 2-bromoethoxymethyl chloride (2.58 g, 15 mmol). The mixture was stirred for 24 h at room temperature, and the reaction was quenched with methanol (20 mL) and stirred for 2 h. The solvents were evaporated and the residue was purified by column chromatography eluting with chloroform to give **8** as a pure colorless liquid (2.2 g, 87%). Rf 0.59, Chloroform:Methanol (10 : 1). ^{1}H-NMR ($CDCl_3$) δ 7.90 (s, 1H, imidazole), 5.57 (s, 2H, NCH_2O), 3.93 (t, 2H, J = 5.4 Hz, OCH_2), 3.49 (t, 2H, J = 5.4 Hz, CH_2Br); FABMS *m/z*: 254, 256 [MH^+].

Anal: HRMS (FAB): Calcd for $C_8H_8N_4O^{79}Br$ 254.9880. Found: 254.9883; Calcd for $C_8H_8N_4O^{81}Br$ 256.9861. Found: 256.9858.

Methyl 1-(2-diethoxyphosphonylmethoxyethyl)-4,5-imidazoledicarboxylate (9)

A mixture of methyl imidazole-4,5-dicarboxylate (1.0 g, 5.4 mmol) and potassium carbonate (0.75g, 5.4 mmol) in 40 mL of anhydrous DMF was stirred at 100 °C for 3 h, then a solution of diethyl *2-p*-toluenesulfonyloxyethoxymethanephosphonate (2.75 g, 7.5 mmol) in 5 mL of dry DMF was to the above mixture and continued to be stirred for 48 h at 100 °C. The reaction mixture was evaporated *in vacuo*. The residue dissolved in chloroform and washed with 0.1 M hydrochloric acid and water. After drying over $MgSO_4$, the chloroform solution was evaporated to dryness *in vacuo*. The resulting residue was purified by column chromatography, eluted with chloroform to give **9** as a colorless liquid (1.33 g, 65%). Rf 0.74, Chloroform:Methanol (10 : 1). ^{1}H NMR ($CDCl_3$) δ 7.67 (s, 1H, imidazole), 4.44 (t, 2H, J = 4.8 Hz, H-1′), 4.10 (dq, 4H, $J_{P\text{-}OCH} = 8.1$ Hz, J = 6.9 Hz, CH_2CH_3), 3.92 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.88 (t, 2H, J = 4.8 Hz, H-2′), 3.75 (d, 2H, $J_{P\text{-}CH} = 8.1$ Hz, OCH_2P), 1.31 (t, 6H, J = 6.9 Hz, CH_2CH_3); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 16.40 (s, CH_3), 16.48 (s, CH_3), 46.79 (s, C-1′), 52.17 (s, OCH_3), 52.40 (s, OCH_3), 62.33 (s, OCH_2CH_3), 62.41 (s, OCH_2CH_3), 65.17 (d, $J_{P,C} = 166.4$ Hz, OCH_2P), 71.50 (d, $J_{P,C\text{-}2'} = 9.9$ Hz, C-2′), 137.08 (C-4 or 5), 139.81 (C-5 or 4), 140.63 (C-2), 160.44 (C=O), 162.74 (C=O); FABMS *m/z*: 379 [MH^+]; Anal: Calcd for $C_{14}H_{23}N_2O_8PH_2O$: C, 42.64; H, 6.39; N, 7.10. Found: C, 42.29; H, 6.26; N, 6.84.; HRMS(FAB): Calcd for $C_{14}H_{24}N_2O_8P$ 379.1270, Found 379.1268.

Compound **9** was also prepared from pure diethyl 2-bromoethoxymethanephosphonate according to the above procedure.

Methyl 1-(2-Phosphonylmethoxyethyl)-4,5-imidazoledicarboxylate (10)

To a solution of methyl 1-(2-diethoxyphosphonylmethoxyethyl)-4,5-imidazoledicarboxylate (**9**) (0.48 g, 1.3 mmol) in 10 mL of dry acetonitrile was added bromotrimethylsilane (0.40 g, 2.6 mmol), and the mixture was stirred for 24 h at room temperature. The reaction mixture was evaporated to dryness, the residue was codistilled with acetonitrile (3×20 mL), mixed with water (30 mL), and adjusted to pH 8 with triethylamine. The mixture was allowed to stand for 1 h and then evaporated *in vacuo* and the residue codistilled with methanol (2×20 mL), and then applied to a silica gel column. The column was eluted first with chloroform and then with chloroform-methanol (10 : 1). Evaporation of appropriate fractions afforded **10** as a colorless liquid (350 mg, 84%). Rf 0.13, Chloroform:Methanol:30% Ammonium Hydroxide (2:1:0.3).

^{1}H NMR (DMSO-d_6) δ 7.98 (s, 1H, imidazole), 5.64 (brs, 2H, OH, exchangeable with D_2O), 4.34 (t, 2H, J = 4.8 Hz, H-1′), 3.81 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.77 (t, 2H, J = 4.8 Hz, H-2′), 3.55 (d, 2H, $J_{P\text{-}CH} = 8.4$ Hz, OCH_2P); ^{13}C NMR (DMSO-d_6, 75.5 MHz) δ 46.41 (s, C-1′), 52.51 (s, OCH_3), 53.07 (s, OCH_3), 66.36 (d, $J_{P,C} = 160.2$ Hz, OCH_2P), 71.09 (d, $J_{P,C\text{-}2'} = 10.2$ Hz, C-2′), 135.32 (C-4 or 5), 141.43 (C-5 or 4), 141.43 (C-2), 160.45 (C=O), 162.95 (C=O); FABMS *m/z*: 323 [MH^+].

Anal: HRMS(FAB): Calcd for $C_{10}H_{16}N_2O_8P$ 323.0644. Found: 323.0649.

Methyl 1-(2-Benzyloxyethoxymethyl)-4,5-Imidazoledicarboxylate (11)

A mixture of methyl 4,5-imidazoledicarboxylate (0.92 g, 5 mmol) and K_2CO_3 (0.7 g, 5 mmol) was stirred for 1 h at 80 °C. Then a solution of 1-benzyloxy-2-chloromethoxyethane (1.5 g, 7.5 mmol) in 20 mL of DMF was dropwise added, and the mixture was continued to stir for 24 h at 90 °C. The reaction mixture was evaporated under reduced pressure, and the residue was dissolved in 30 mL of water and then neutralized with 2M hydrochloric acid. The solution was extracted with chloroform. The extract was evaporated *in vacuo*, and the residue was purified by silica gel column chromatography, eluting with chloroform to give **11** as a colourless liquid, 1.22 g (70%); Rf 0.31 (chloroform-methanol (30:1)).

^{1}H-NMR ($CDCl_3$) δ 7.72 (s, 1H, imidazole), 7.24–7.35 (m, 5H, Ph), 5.66 (s, 2H, NCH_2O), 4.52 (s, 2H, $PhCH_2$), 3.92 (s, 3H, OCH_3), 3.91 (s, 2H, OCH_3), 3.63 (m, 4H, OCH_2CH_2O); ^{13}C NMR ($CDCl_3$) δ (52.16, 53.10, 69.06, 69.71, 73.25, 76.44, 127.58, 127.58, 127.66, 127.66, 128.32, 128.32, 137.65, 139.38, 139.47, 160.21, 162.45.

Anal: Calcd for $C_{17}H_{20}N_2O_6$: C, 58.61; H, 5.79; N, 8.04. Found: C, 58.81; H, 5.88; N, 7.77.

1-(2-Benzyloxyethoxymethyl)-4,5-dicyanoimidazole (12)

A mixture of 4,5-dicyanoimidazole (0.6 g, 5 mmol) and K_2CO_3 (0.7 g, 5 mmol) was stirred for 1 h at 80 °C. Then a solution of 1-benzyloxy-2-chloromethoxyethane (1.5 g, 7.5 mmol) in 20 mL of DMF was dropwise added and continue to stirred for 24 h at 90 °C. The mixture was evaporated under reduced pressure, and the residue was dissolved in 30 mL of water and neutralized with 2M hydrochloric acid. The solution was extracted with chloroform. The extract was evaporated *in vacuo*, and the residue was purified by column chromatography, eluting with chloroform to give a colourless liquid, 0.85 g (60%); Rf 0.44 (chloroform-methanol (30 : 1)). ^{1}H NMR ($CDCl_3$) δ 7.79 (s, 1H, imidazole), 7.26-7.39 (m, 5H, Ph), 5.53 (s, 2H, NCH_2O), 4.51 (s, 2H, $PhCH_2$), 3.64 (m, 4H, OCH_2CH_2O); FABMS *m/z*: 283 [MH^+].

Anal: HRMS(FAB): Calcd for $C_{15}H_{15}N_4O_2$ 283.1195. Found: 283.1197.

1-(2-Acetoxyethyl)-4,5-dicyanoimidazole (15)

A mixture of 4,5-dicyanoimidazole (0.6 g, 5 mmol) and K_2CO_3 (0.7 g, 5 mmol) was stirred for 1 h at 80 °C. Then a solution of 2-bromoethyl acetate (1.25 g, 7.53 mmol) in 20 mL of DMF was drop wise added, and the mixture was continued to stir for 24 h at 90 °C. The reaction mixture was evaporated under reduced pressure, and the residue was dissolved in 30 mL of water and then neutralized with 2M hydrochloric acid. The solution was extracted with chloroform. The extract was evaporated *in vacuo*, and the residue was purified by silica gel column chromatography, eluting with chloroform to give **15** as a colourless liquid, 0.71 g (70%), which crystallized upon standing, mp 92–93 °C. Rf 0.62 ($CHCl_3$-MeOH, 10 : 1). ^{1}H NMR ($CDCl_3$) δ 8.25 (s, 1H, imidazole), 4.67 (t, 2H, J = 4.8 Hz, $AcOCH_2$), 4.49 (t, 2H, J = 4.8 Hz, CH_2N), 2.05 (s, 3H, Ac); MS (EI) m/z: 204, 145, 144 (100), 132.

Anal: Calcd for $C_9H_8N_4O_2$: C, 52.94; H, 3.95; N, 27.44. Found: C, 52.78; H, 3.95; N, 27.28.

Biological Screening Protocols

The Cytopathic Effect Inhibition (CPE) Assay. The CPE (virus effects on cell morphology) inhibition assay used in this study was done as described by Barnard et al[45]. Compounds, using a 1/2 log dilution scheme, were serially diluted in a 96-well plate containing confluent cell monolayers (1×10^4 cells/well) with MEM without serum as diluent. Virus in MEM supplemented with 2% FBS (+20 mM $MgCl_2$ for rhinovirus; no FBS was included in the influenza virus assays, instead 0.2% trypsin was included to

cleave hemagglutinin of virus to achieve a better attachment) was added within five minutes of drug addition in an equal volume to drug to the appropriate wells at a multiplicity of infection (MOI) = 0.001–0.01, depending on the virus. The plates were incubated at 37 °C (33 °C for rhinovirus) for 4–7 days until 100% CPE was detected in the virus infected, untreated, control cells by light microscopy.

The positive control drugs that were employed are as follows: For Measles Virus, Parainfluenza Type 3 Virus and Respiratory syncytial virus and Influenza viruses, the positive control drug is ribavirin, a 5-membered heterocyclic nucleoside analog of guanine. For rhinovirus, the positive control drug was pirodavir, a compound that binds in the protein "canyons" of the virus to prevent uncoating of the protein capsid surrounding the RNA of the virus.

The compounds and the positive control drugs were assayed for virus inhibition in quadruplicate and for cytotoxicity in duplicate. For each compound, two wells were set aside as uninfected, untreated cell controls per test and four wells per test received virus only and represented controls for virus replication. Changes due to viral cytopathic effect were graded on a scale of 1–4, grade 4 representing a scenario in which the entire (100%) monolayer in a well showed viral cytopathic effect. For all CPE-based assays, the 50% effective concentration (EC_{50}) was calculated by regression analysis using the means of the CPE ratings at each concentration of compound.

Morphological changes due to compound cytotoxicity were graded on a scale of 0–5; grade 5 was defined as 100% cytotoxicity. The 50% cytotoxic dose (IC_{50}) was calculated by regression analysis. After determining the EC_{50} and IC_{50} values, a selective index (SI) for each compound was then calculated by using the formula, $SI = IC_{50}/EC_{50}$.

Neutral Red (NR) Uptake Assay. This was done as described by Barnard et al[45]. Medium was removed from each well of each plate used for the CPE inhibition assay and 0.2 mL of NR (0.034% in physiological saline) was added to the wells of the plates and incubated for 2 h at 37 °C in the dark. The NR solution was then removed from the wells and each plate was rinsed two times with phosphate-buffered saline (pH 7.4). Equal volumes (0.1 mL) of absolute ethanol and Sorenson citrate buffer (0.1 M sodium citrate, 0.1 M HCl, pH 4.2) were mixed together and added to the wells of each plate. The plates were incubated in the dark for 30 min at room temperature to solubilize the dye. The plates were then gently mixed on a 96-well plate adapted vortexer for 1 min. Absorbance at 540 nm and 450 nm was read with a microplate reader (Bio-Tek EL 1309; Bio-Tek Instruments, Inc., Winooski, VT). Absorbance values were expressed as percent of untreated controls and EC_{50} and IC_{50} values were calculated by regression analysis.

ACKNOWLEDGMENTS

This research was supported by a grant from the National Institutes of Health (# 1RO1CA71079). Mass spectral data were run at the Michigan State University Mass Spectrometry Facility which was supported, in part, by a grant (DRR-00480) from the Biotechnology Research Technology Program, National Center for Research Resources, National Institutes of Health. The authors gratefully acknowledge Dr. Chris Tseng of NIAID, Dr. Earl Kern of the University of Alabama, Birmingham, and Drs. Robert Sidwell, Dale Barnard, and D. Smee of the Utah State University, Logan. For all the antiviral screening results reported in this paper, and described in reference 44.

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Received July 5, 2000
Accepted February 23, 2001