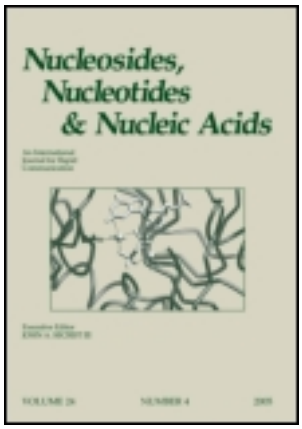

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[a] Biota, Inc., Carlsbad, California, 92008, USA

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Synthesis of Azole Nucleoside 5′-Monophosphate Mimics (P1Ms) and Their Inhibitory Properties of IMP Dehydrogenases[†]

Guangyi Wang,[*] Kandasamy Sakthivel, Vasanthakumar Rajappan, Thomas W. Bruice, Kathleen Tucker, Patrick Fagan, Jennifer L. Brooks, Tiffany Hurd, Janet M. Leeds, and P. Dan Cook

Biota, Inc., Carlsbad, California, USA

ABSTRACT

IMPDH inhibitors have potential antimicrobial, anticancer and immunomodulatory effects. Nucleoside inhibitors of IMPDH exert their inhibitory effects via nucleoside 5′-MPs. Conversion of nucleoside analogs to NMPs by cellular nucleoside kinases is not assured, and usually is inefficient. In order to bypass cellular phosphorylation, a series of azole nucleoside 5′-MP mimics (P1Ms) based on ribavirin, EICAR and bredinin were synthesized and screened against human and *C. albicans* IMP dehydrogenises. P1Ms **8**, **16**, **25**, **28** and **29** demonstrated substantial IMPDH inhibition with K_i values in low micromolar range.

Key Words: Azole nucleoside; Monophosphate mimic; P1M; IMPDH.

[†]In honor and celebration of the 70th birthday of Professor Leroy B. Townsend.

[*]Correspondence: Guangyi Wang, Biota, Inc., Carlsbad, CA 92008, USA; E-mail: g.wang@biota-inc.com.

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INTRODUCTION

Inosine 5′-monophosphate dehydrogenases (IMPDH) catalyze the NAD-dependent oxidation of IMP to XMP, which is the rate-limiting step of the de novo guanine nucleotide biosynthesis. Human IMPDH exists in two isoforms. Type I isoform is constitutively expressed and is the predominant isoform in normal cells. Type 2 isoform is selectively up-regulated in neoplastic and replicating cells and becomes the predominant isoform.[1,2] There is great interest in selective inhibition of the Type 2 isoform for more potent and less toxic cancer therapeutics. Inhibition of IMPDH effectively depletes guanine nucleotide pools in T cells and B cells that primarily depend on the de novo biosynthesis for guanine nucleotides, and thereby results in immunosuppression, which is useful in organ transplantation and for treatment of autoimmune diseases.[3]

Two IMPDH binding sites are known to be of importance for drug targeting, the substrate site (IMP site) and the cofactor site (NAD site). Among the potent inhibitors[4–6] of the NAD site are mycophenolic acid, VX-497 and TAD analogs. Mycophenolate mofetil, an ester prodrug of mycophenolic acid, has been used clinically as an immunosuppressive drug.[7] Among the potent inhibitors[4,10,11] of the IMP site are the 5′-monophosphates of ribavirin, bredinin and EICAR (Figure 1). Ribavirin is a prescription drug for treatment of RSV and HCV infections. Ribavirin 5′-MP (**1**) inhibits IMPDH at sub-micromolar concentrations, which is believed to contribute, at least partially, to the antiviral activities.[8,9] Bredinin (Mizoribine) is used clinically as an immunosuppressant in organ transplantation in Japan. Bredinin 5′-MP (**2**) is the most potent, known IMPDH inhibitor of the IMP site,[10] although bredinin does not show superior inhibitory effects over ribavirin in cell-based assays. EICAR 5′-MP (**3**) is a mechanism-based, irreversible inhibitor of IMPDH, and EICAR has

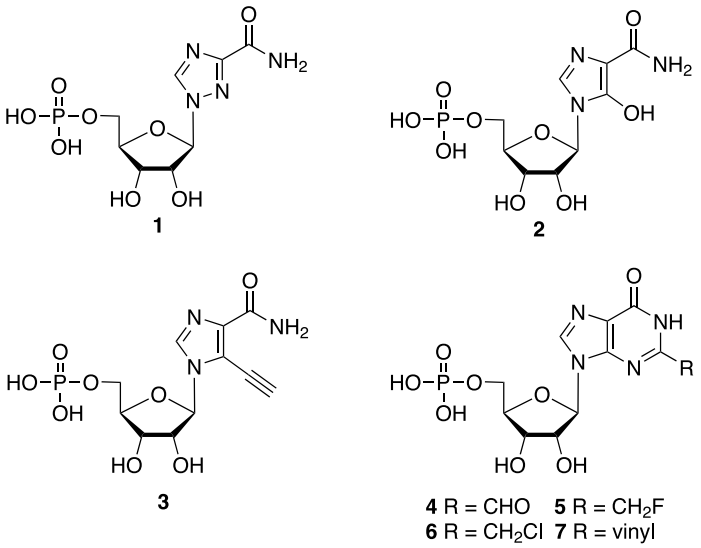


Figure 1. Known IMPDH inhibitors.

broad-spectrum antiviral and anticancer activities in cell-based assays.[11,12] A few 2-substituted inosine 5′-MPs **4–7** have demonstrated potent inhibition of IMPDH with IC_{50} values around one micromolar.[13]

Although potent NMP inhibitors can be identified through enzymatic IMPDH inhibition assays, the NMPs and their parent nucleosides may not be useful drugs. First, efficient cellular phosphorylation of the parent nucleosides to NMP inhibitors is not assured. Thus, the nucleosides are inactive if they fail to be phosphorylated. Second, synthetic NMPs, as negatively charged chemical entities, have difficulty entering cells by passive diffusion. A prodrug or a suitable carrier may help deliver NMPs into cells, but their inherent instability to nucleotidases and phosphatases may regenerate the parent nucleosides. Furthermore, NMPs may be anabolized to NDPs and NTPs, which potentially can interact with unintended cellular targets, causing unpredictable and often deleterious effects. Obviously, it is ideal to keep nucleoside inhibitors at the 5′-MP level for selective and effective IMPDH inhibition.

To enhance the stability of NMPs, a useful approach is to employ phosphate mimics (P1Ms) such as phosphonates, phosphoramidates and phosphorothioates. A desirable nucleoside P1M drug candidate should bind with high affinity and specificity to IMPDH and should have an adequate cellular half-life. So far, very few P1Ms of nucleoside IMPDH inhibitors have been studied. One example is 1-(5,6-dideoxy-β-D-ribo-hexofuranosyl-6-phosphonic acid)-1,2,4-triazole-3-carboxamide.[14] This compound partially retains the inhibitory effect (K_i = 20 μM) of ribavirin 5′-MP. Another P1M is ribavirin 5′-phosphoramidate,[15] which has showed a weak antiviral activity and the

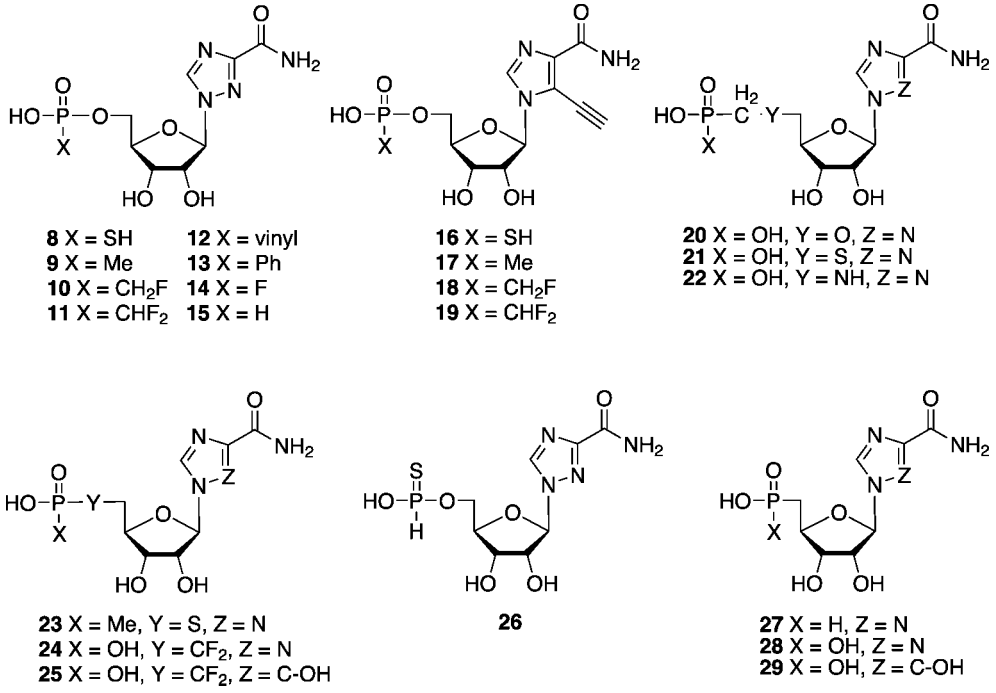


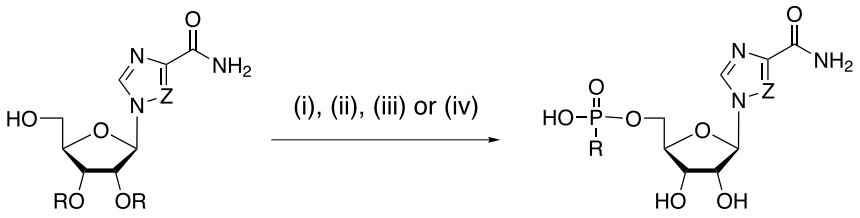
Figure 2. Azole nucleoside 5′-monophosphate mimics (PIMs) synthesized.

inhibition of IMPDH has not been reported. P1Ms of bredinin and EICAR have not been reported in the literature. Thus, the feasibility of P1Ms as IMPDH inhibitors has been explored very little, especially considering the various kinds of known and possible phosphate mimics. It is probable that P1Ms also may have difficulty entering cells. However, a prodrug approach should be well suited for P1Ms. In fact, the combination of phosphonate mimics of acyclic NMPs and a prodrug approach has been used successfully in the newly approved antiviral drugs, adefovir dipivoxil (bis-POM prodrug of PMEA) and tenofovir disoproxil fumarate (bis-POC prodrug of PMPA).[16,17]

As part of our drug discovery program, we have designed and synthesized a series of nucleoside P1Ms as IMPDH inhibitors. For identifying suitable P1M moieties, our program started with known nucleoside inhibitors of IMPDH. When certain promising P1M moieties are identified, they will be extended to other nucleosides, which will be selected based on their inhibitory properties in the catalytic site, but not on phosphorylation by cellular enzymes. In this article we will describe the synthesis and biochemical evaluation of certain azole nucleoside 5′-MP mimics (P1Ms) as shown in Figure 2.

CHEMISTRY

A number of azole nucleoside P1Ms were prepared through direct condensations with phosphorylating reagents, as shown in Scheme 1. Ribavirin and EICAR were reacted with thiophosphoryl chloride, followed by hydrolysis, to give the 5′-phosphorothioate **8** and **16**, respectively, in good yields. Condensations of 2′,3′-*O*-isopropylidene ribavirin[18] with various phosphonic acids ($RP(O)(OH)_2$, R = methyl, fluoromethyl, difluoromethyl, vinyl and phenyl) in the presence of DCC, followed by deprotection using Dowex-H^+, afforded the 5′-*O*-phosphonates **9–13**. By a similar procedure, the EICAR 5′-*O*-phosphonates **17–19** were synthesized. Condensation of



Z = N or C–C≡CH
R = H or Benzoyl
R + R = Isopropylidene

(i) $P(S)Cl_3$, pyridine for **8** and **16**; (ii) a. $R\text{-}P(O)(OH)_2$, DCC, pyridine, b. Dowex-H^+, MeOH for **9-13** and **17-19** or NH_3, water for**14**; (iii) a. Diphenyl H-phosphonate, pyridine, b. TEA/water, then $MeNH_2$ for **15**; (iv) a. 9-Fluorenemethyl H-phosphonothioate, pivaloyl chloride; b. TEA/water, then $MeNH_2$ for **26**

Scheme 1.

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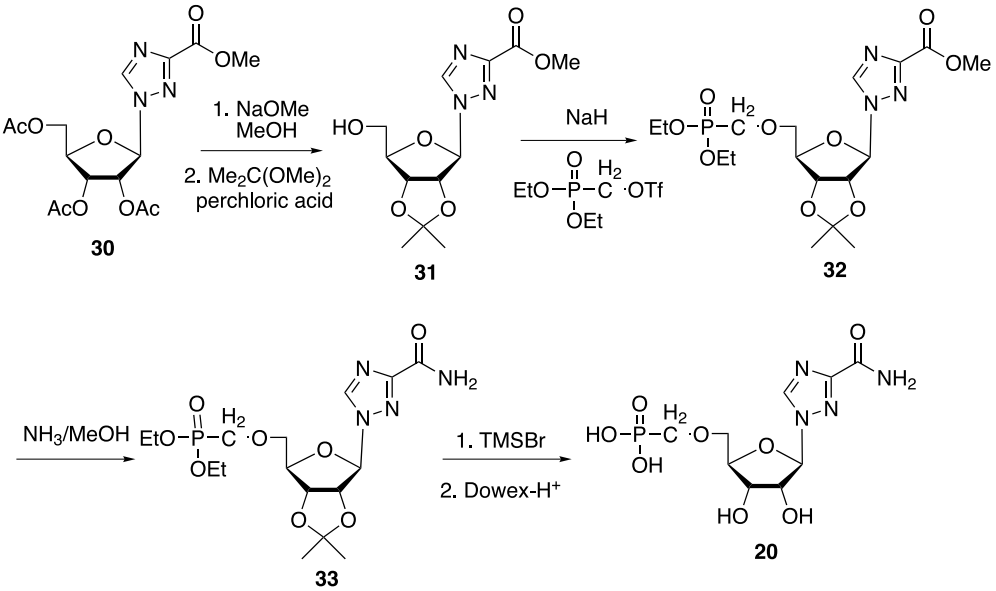
2′,3′-di-*O*-benzoylribavirin with fluorophosphonic acid in the presence of DCC, followed by treatment with aqueous ammonia, afforded the 5′-*O*-phosphofluoridate **14** in good yield. Diphenyl (*H*)-phosphate was condensed with 2′,3′-di-*O*-benzoylribavirin and the resulting intermediate was treated with TEA/water and then with aqueous methylamine to give the 5′-*O*-*H*-phosphonate **15**. Reaction of 2′,3′-di-*O*-benzoylribavirin with 9-fluorenemethyl *H*-phopshonothioate in the presence of trimethylacetyl chloride afforded, after removal of benzoyl groups, the 5′-*H*-phosphonothioate **26**.

Commercially available **30** was subjected to deacetylation with NaOMe in methanol (Scheme 2). Subsequent treatment with dimethoxypropane in the presence of perchloric acid gave the 2′,3′-*O*-isopropylidene derivative **31**, which was condensed with (diethoxyphosphinyl)methyl triflate[19] to yield **32**. Treatment of **32** with methanolic ammonia, followed by deprotection with TMSBr and Dowex-H^+, afforded 5′-*O*-phosphonomethyl ribavirin (**20**).

Ribavirin (**34**) was converted to 2′,3′-isopropylideneribavirin,[18] which was reacted with thiolacetic acid under Mitsunobu conditions to give **35** as shown in Scheme 3. Deacetylation of **35** under oxygen-free conditions yielded the thiol compound **36**. Reaction of **36** with methylphosphonic acid in the presence of DCC, followed by deprotection of isopropylidene, gave **23** (Figure 2). Reaction of **36** with (diethoxyphosphinyl)methyl triflate in the presence of sodium hydride, followed by deprotection using TMSBr and Dowex-H^+ resin, afforded **21**. Compound **37** was prepared by treatment of **34** with iodine in the presence of triphenylphosphine. Treatment of **37** with sodium azide, followed by catalytic hydrogenolysis over palladium, gave **38**. Reaction of **38** with (diethoxyphosphinyl)methyl triflate in the presence of sodium hydride, followed by deprotection with TMSBr, afforded **22**.

The 5′-deoxy-5′-difluoromethylene sugar **39**, prepared according to a published procedure,[20] was condensed with the silylated derivative of **40** in the presence of tin



Scheme 2.

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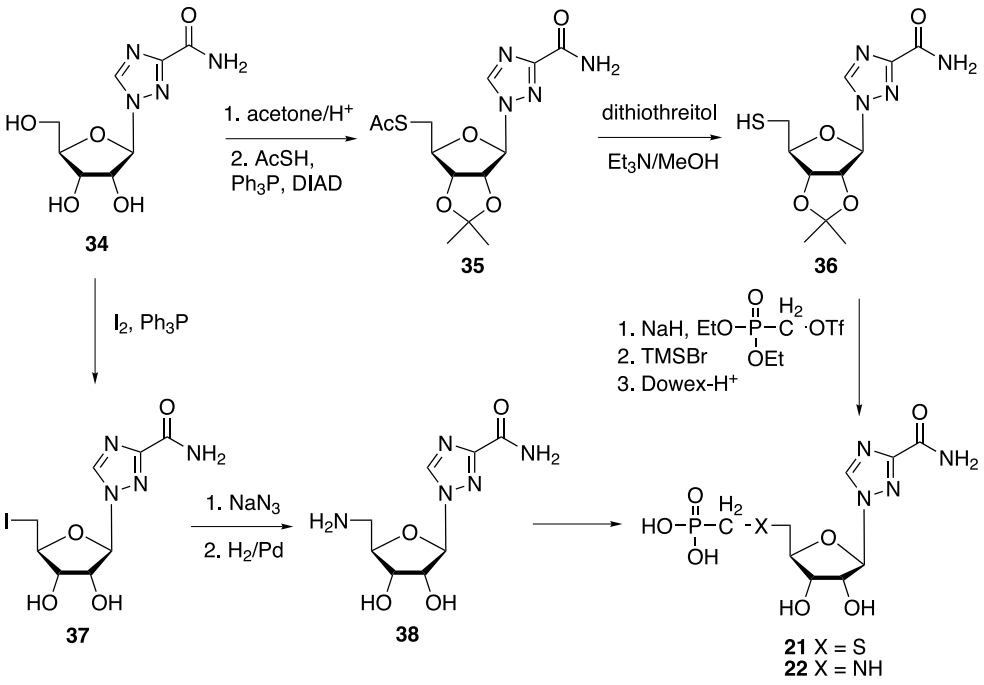
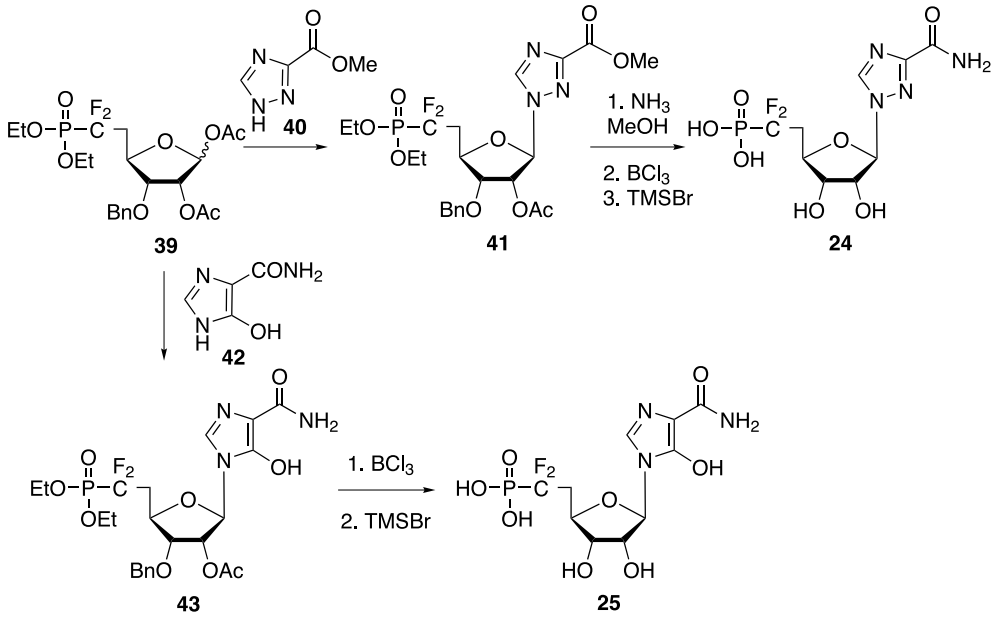


34 35 36 37 38

Scheme 3.



39 40 41 24 42 43 25

Scheme 4.

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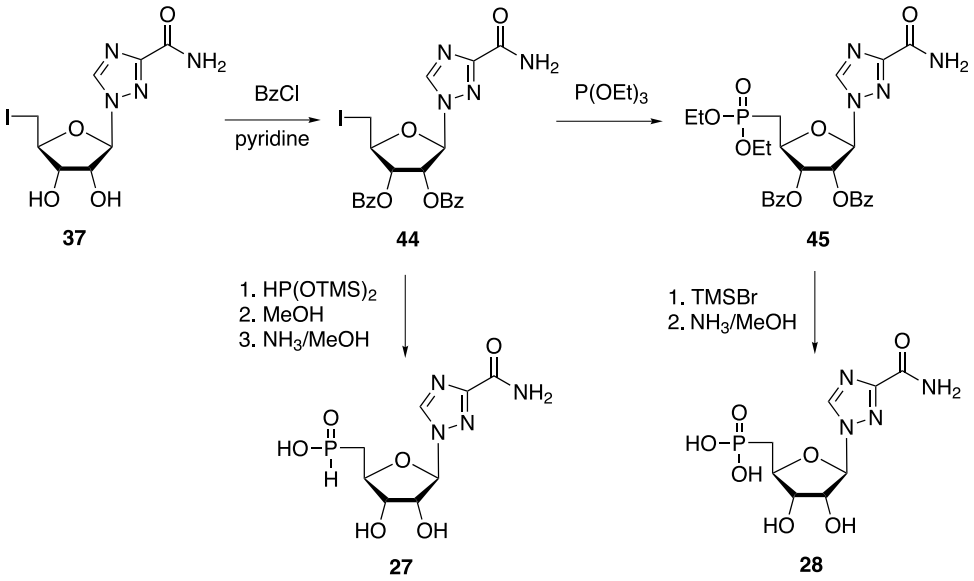

Scheme 5.

(IV) chloride to give **41** (Scheme 4). Treatment of **41** with methanolic ammonia, followed by deprotection using boron trichloride and then bromotrimethylsilane, afforded the 5′-difluoromethylenephosphonate **24**. Similarly, the sugar **39** was condensed with the silylated derivative of **42**[21] in the presence of $TiCl_4$ in nitromethane to give compound **43**. Subsequent deprotection afforded the bredinin 5′-difluoromethylenephosphonate **25**. In a similar manner, compound **29** (Figure 2) was prepared from condensation of the silylated derivative of **42** and 1-*O*-acetyl-5-deoxy-2,3-di-*O*-benzoyl-5-(diethoxyphosphinyl)-D-ribofuranose[22] in the presence of $SnCl_4$ and TMSOTf and subsequent debenzoylation. The condensations of the silylated derivative of **42** with sugars required vigorous reaction conditions (e.g. reflux in acetonitrile) for completion.[23] However, the existence of the phosphonate ester groups did not allow such vigorous conditions owing to their sensitivity to Lewis acid at elevated temperature, thus compounds **25** and **29** were obtained in low to moderate yields.

Scheme 5 shows the synthesis of the 5′-deoxynucleotides **27** and **28**. Compound **44**, prepared by benzoylation of **37**, was subjected to Arbuzov reaction using triethylphosphite at 100°C to give **45**. Subsequent deprotection with bromotrimethylsilane and then with saturated methanolic ammonia yielded **28**. Similarly, compound **44** was reacted with bis(trimethylsilyl)-*H*-phosphinite, followed by deprotection, to afford **27**.

BIOLOGICAL EVALUATION

The assays employed to measure the inhibition of IMPDH activity were a modification of a reported method.[12] Effectiveness of the P1Ms as inhibitors of

IMPDHs was determined by measuring the reduction of NAD, which is coupled to the oxidation of IMP to XMP in the presence or absence of a P1M. This assay measures the activity of IMPDH from several organisms, including human and fungal isoforms. This reaction is monitored at 340 nm using a UV/VIS spectrophotometer or at 474 nm using a fluorometer (excitation wavelength = 344 nm). All of the azole nucleoside P1Ms in Figure 2 were screened first against human IMPDH Type II isozyme and a fungal (*C. albicans*) IMPDH, and initial ''hits'' were then counter-screened against human IMPDH Type I isozyme. Table 1 shows the K_i values of the active azole nucleoside P1Ms. Other P1Ms having K_i values more than 100 μM against Human Type II isozyme were not tested further against Human Type I and *C. albicans* IMPDHs.

As can be seen from Table 1, P1M inhibitors were found for all three IMPDH enzymes tested: human Type I, human Type II and *Candida albicans*. For instance, the phosphonates **25**, **28** and **29** and the phosphorothioate **8** are active against all three IMPDHs, having K_i values in the low micromolar range. The majority of the P1Ms in Figure 2 were significantly less potent than the parent NMP inhibitors, and the inactive P1Ms are not included in Table 1. Evidence was obtained for experimentally significant differences in specificity of inhibition of human and *C. albicans* IMPDHs by P1Ms. For example, compound **16** is about 10 times more active against *C. albicans* IMPDH than human IMPDH. With this small, initial exploratory library of P1Ms, no significant specificity of inhibition of human Type II IMPDH in preference over Type I was observed. Given the small number of the P1Ms and the promising preliminary results, we believe that nucleoside P1M approach has the potential to allow for the discovery of potent and specific IMPDH inhibitors.

Stability of selected azole nucleotide P1Ms was assessed in fetal calf serum at 37°C following a published procedure.[24] The P1Ms in the serum at different time points were analyzed on a reverse phase HPLC (C18). Compared to ribavirin 5′-MP ($t_{1/2}$ = 6 h), ribavirin P1Ms were significantly more stable, with $t_{1/2}$ = 18 h for the phosphorothioate **8**, and $t_{1/2}$ > 24 h for the phosphonates **9**, **24** and **28**.

Table 1. Inhibition of IMPDHs by azole nucleoside P1Ms.

Compound	K_I (μM) human type II	K_I (μM) human type I	K_I (μM) *C. albicans*
8	0.94	2.92	1.34
16	34.2	31.8	3.41
20	85.0	NT	NT
24	27.1	13.9	20.4
25	2.04	3.33	15.5
26	34.7	44.8	10.7
27	1.82	2.44	1.48
29	7.92	9.40	1.98
Ribavirin 5′-MP	0.059	0.098	0.054
Bredinin 5′-MP	0.02	0.057	0.023
EICAR 5′-MP	1.15	NT	1.34

NT = not tested.

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In summary, we have designed and synthesized a series of azole nucleoside 5′-monophosphate mimics (P1Ms), which were evaluated for IMPDH inhibition. The procedures for synthesis of these P1Ms are expected to be applicable to other nucleosides. A few P1Ms showed, although not as potent as the parent NMPs, substantial inhibition of IMPDHs. This, along with the superior stability of P1Ms, especially phosphonate P1Ms, should warrant further exploration of nucleoside P1Ms as inhibitors of IMPDH.

EXPERIMENTAL SECTION

NMR spectra were recorded on a Varian Mercury 300 NMR spectrometer. Tetramethysilane was used as internal reference for ^{1}H NMR, and 85% phosphoric acid was used as external reference for ^{31}P NMR. Mass spectra and purity of compounds were determined on a ThermoFinnigan Deca XP on-line HPLC mass spectrometer. A Phenomenex Luna C18 75 × 2 mm column packed with 3-um particles was used for LC-MS analysis. A 0 to 50% linear gradient (15 min) of acetonitrile in 10 mM dimethylhexylammonium acetate buffer (pH = 7) was used in series with mass spectra detection in the negative ionization mode. The azole nucleoside P1Ms were purified by reverse-phase HPLC with a Luna C18 250 × 21 mm column (Phenomenex) using acetonitrile in triethylammonium acetate (50 mM, pH = 7) as eluent. All the purified azole nucleoside P1Ms of this work were subjected to the LCMS analysis. Amounts of the nucleoside P1M products in this section are based on UV absorptions and calculated based on their H-forms. The P1Ms, as triethylammonium salts, were stored at − 80°C.

1-(5-*O*-Thiophosphoryl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (8). To a suspension of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin, 122 mg. 0.5 mmol) in 2.5 mL of anhydrous pyridine was added proton sponge® [1,8-bis(dimethylamino)naphthalene] (107 mg. 0.5 mmol) at 0–5°C under argon atmosphere, followed by addition of thiophosphoryl chloride (0.1 mL, 1mmol). The mixture was stirred at this temperature for 30 minutes, quenched with 3 mL of 1 M triethylammonium bicarbonate buffer (TEAB) and extracted with chloroform (2 mL). The aqueous layer was subjected to reverse-phase HPLC purification. Collected fractions were lyophilized to give 60 mg of **8** with 100% Purity. ^{1}H NMR (D_2O) δ 8.71 (s, 1H), 5.87 (d, J = 3.5 Hz, 1H), 4.64 (m, 1H), 4.54 (dd, J = 3.75, 4 Hz, 1H), 4.22 (m, 1H), 3.96 (m, 2H); ^{31}P NMR δ 48.89 (s); MS m/z (M^- + 1) 340.25.

1-(5-*O*-Methylphosphonyl-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (9). 1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide[18] (142 mg, 0.5 mmol) was co-evaporated with anhydrous pyridine (3 × 5 mL) under reduced pressure and taken into 5 mL of anhydrous pyridine. To the above solution under argon atmosphere were added dicyclohexylcarbodiimide (DCC, 206 mg, 1.0 mmol) and methyl phosphonic acid (58 mg, 0.6 mmol). The mixture was stirred at 38°C for 36 h, cooled and quenched with water (5 mL). The resulting dicyclohexylurea (DCU) was filtered off and the filtrate was concentrated under reduced pressure and filtered again. After evaporation, the concentrate was co-evaporated with toluene to remove a trace of pyridine. The crude product (105 mg) was dissolved in methanol (5 mL) and Dowex

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50Wx8–100 resin (1 g, pre-washed with water and methanol) was added. The mixture was stirred at 50°C for 2 h, filtered through a short pad of cotton and the resin was thoroughly washed with water. The filtrate was concentrated to yield a viscous residue which was purified on reverse-phase HPLC. The fractions collected were pooled and lyophilized to give 34 mg of **9** with 100% purity. ^{1}H NMR (D_2O) δ 8.62 (s, IH), 5.91 (d, J = 3.7 Hz, 1H), 4.56 (m, 1H), 4.39(m, 1H), 4.20 (m, 1H), 3.92 (m, 2H), 1.11 (d, J = 17.0 Hz, 3H); ^{31}P NMR (D_2O) δ 28.38 (s); MS m/z (M^- + 1) 322.30.

1-[5-*O*-(Fluoromethyl)phosphonyl-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide (10). According to the same procedure as described for **9**, the reaction of 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (142 mg, 0.5 mmol) with (fluoromethyl)phosphonic acid[21] (60 mg, 0.6 mmol) in the presence of DCC (202 mg, 1.0 mmol) at 38°C for 24 h gave, after reverse-phase HPLC purification, 25 mg of **10** with 100% purity. ^{1}H NMR (D_2O) δ 8.59 (s, 1H), 5.87 (d, J = 3.25 Hz, 1H), 4.55 (m, 1H), 4.50 (d, J = 3.75, 1H), 4.38 (dd, J = 4.5, 4 Hz 1H), 4.38 (d, J = 3.5 Hz, 2H), 4.19 (m, 1H), 4.04(m, 1H); ^{31}P NMR (D_2O) δ 15.45-14.97 (d, J = 121.75 Hz); MS m/z (M^- + 1) 340.00.

1-[5-*O*-(Difluoromethyl)phosphonyl-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide (11). Diethyl(difluoromethyl)phosphonate (500 mg, 2.66 mmol) and 0.88 mL (96.66 mmol) bromotrimethylsilane were refluxed in 10 mL of anhydrous methylene chloride for 15 h. Solvent was evaporated and the residue was repeatedly co-evaporated with methanol. The residue (300 mg) was dissolved in 2 mL of anhydrous pyridine to make a (difluoromethyl)phosphonic acid stock solution and stored under argon at −20°C. This was used in the next step.

According to the same procedure as described for **9**, the reaction of 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (142 mg, 0.5 mmol) and (difluoromethyl)phosphonic acid (70 mg, 0.6 mmol) in the presence of DCC (210 mg, 1.0 mmol) at 38°C for 24 h gave, after reverse-phase HPLC purification, 100 mg of **11** with 97.8% purity. ^{1}H NMR (D_2O) δ 8.58 (s, 1H), 5.82 (m, 2H), 4.55 (m, 1H), 4.4(m,1H), 4.38 (dd, J = 4.5, 4 Hz 1H), 4.19 (m, 1H), 4.0 (m, 2H); ^{31}P NMR (D_2O) δ 4.89 (t); MS m/z (M^- + 1) 358.20.

1-(5-*O*-Vinylphosphonyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (12). According to the same procedure as described for **9**, the reaction of 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (142 mg, 0.5 mmol) with vinylphosphonic acid (58 mg, 0.6 mmol) in the presence of DCC (202 mg, 1.0 mmol) at 38°C for 24 h gave, after reverse-phase HPLC purification, 55 mg of **12** with 100% purity. ^{1}H NMR (D_2O) δ 8.59 (s, 1H), 5.82(m, 4H), 4.54 (m, 1H), 4.38 (m, 1H), 4.19 (m, 1H), 3.75(m, 2H); ^{31}P NMR (D_2O) δ 16.23 (s); MS m/z (M^- + 1) 334.30.

1-(5-*O*-(Phenylphosphonyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (13). According to the same procedure as described for **9**, the reaction of 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (142 mg, 0.5 mmol) and phenylphosphonic acid (80 mg, 0.6 mmol) in the presence of DCC (202 mg, 1.0 mmol) at 38°C for 24 h gave, after reverse-phase HPLC purification, 109 mg **13** with 100% purity. ^{1}H NMR (D_2O) δ 8.45 (s, 1H), 7.40 (m, H), 7.28 (m, 1H), 7.19 (m, 2H),

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5.82(d, J = 3.23 Hz, 1H), 5.45(m, 1H), 4.34 (m, 1H), 4.16 (m, 1H), 3.88(m, 1H), 3.76 (m, 1H); ^{31}P NMR (D_2O) δ 15.94 (s); MS m/z (M^- + 1) 384.40.

1-(5-*O*-Fluorophosphoryl-1-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (14). A solution of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (2.4 g, 10.0 mmol) and *tert*-butyldimethylchlorosilane (1.65 g, 11.0 mmol) in anhydrous pyridine (30 mL) was stirred at room temperature overnight. After completion of the reaction, the mixture was poured into saturated sodium bicarbonate solution, extracted with ethyl acetate, dried over sodium sulfate, and concentrated to dryness. The residue was dissolved in pyridine (25 mL) and benzoyl chloride (2.6 mL, 22.0 mmol) was added. The resulting mixture was stirred for 30 min, quenched by adding aqueous sodium bicarbonate and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and concentrated to dryness. Chromatography on silica gel using 2 % methanol in dichloromethane gave 1-(5-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-benzoyl-1-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (3.5 g) which was used as such in the next reaction.

The product from the above reaction (2.8 g, 5.0 mmol) was dissolved in 25 mL of THF and TBAF (1 M in THF, 15 mL) was added. The reaction mixture was stirred at room temperature overnight, quenched with aqueous sodium bicarbonate (100 mL) and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and concentrated to dryness. The residue was purified by chromatography on silica gel using 15% methanol in dichloromethane to give 1-(2,3-Di-*O*-benzoyl-1-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (1.5 g). ^{1}H NMR (DMSO-d_6) δ 8.92 (s, 1H), 7.39–7.91 (m, 12H), 6.55 (d, J = 4.11 Hz, 1H), 6.02 (m, 1H), 5.85 (m, 1H), 5.22 (m, 1H), 4.53 (m, 1H), 3.72 (m, 2H).

According to the same procedure as described for **9**, part of the above product (226 mg, 0.5 mmol) was reacted with fluorophosphonic acid (55 mg, 0.6 mmol) at 38°C for 24 hours. The resulting crude product was treated with 28 % aqueous ammonia (3 mL) for 2 h. After evaporation the crude product was purified on reverse-phase HPLC to give 125 mg of **14** with 100% purity. ^{1}H NMR (D_2O) δ 8.57 (s, 1H), 5.91(d, J = 3.8 Hz, 1H), 4.56(m, 1H), 4.40 (m, 1H), 4.21 (m, 1H), 3.98–4,13(m, 2H). ^{31}P NMR (D_2O) δ − 8.42–0.77 (d, J = 930.23 Hz); MS m/z (M^- + 1) 326.30.

1-(5-*O*-Hydrogenphosphonyl-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (15). According to the same procedure as described for **9**, 1-(2,3-di-*O*-benzoyl-1-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (226 mg, 0.5 mmol) was reacted with diphenyl hydrogen phosphonate (349 mg, 1.5 mmol) at room temperature for 15 min. The reaction mixture was quenched by adding water-triethylamine (1:1, v/v, 5 mL), stirred for 1 min and concentrated. The residue was treated with 50% aqueous methylamine (10 mL) for 1h. After removal of the solvent, the oily residue was purified on reverse-phase HPLC to give 70 mg of **15** with 100% purity. ^{1}H NMR (D_2O) δ 8.62 (s, 1H),7.59 (s, 0.5H), 5.90 (d, J = 3.8 Hz, 1H), 4.45(s, 0.5H), 4.56 (m, 1H), 4.39 (m, 1H), 4.19 (m, 1H), 3.89–4.19 (m, 2H); ^{31}P NMR (D_2O) δ 6.56 (s); MS m/z (M^- + 1) 308.20.

5-Ethynyl-1-(5-*O*-thiophosphono-β-D-ribofuranosyl)imidazole-4-carboxamide (16). According to the same procedure as described for **8**, the reaction of 5-ethynyl-1-

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β-D-ribofuranosylimidazole-4-carboxamide (EICAR, 50 mg, 0.187 mmol) with thiophosphoryl chloride (63 mg, 38 μL, 0.37 mmol) in 1.2 mL of anhydrous pyridine in the presence of proton sponge® (115.5 mg, 0.54 mmol) at 0–5°C under argon atmosphere for 30 min gave, after reverse-phase HPLC purification, 16.7 mg of **16** with 85.4% purity. ^{1}H NMR (D_2O) δ 7.89 (s, 1H), 5.67 (d, J = 4 Hz, 1H), 4.34 (dd, J = 4.5, 4.5 Hz, 1H), 4.21–4.003 (m, 5H); ^{31}P NMR (D_2O) δ 18.05 (S); MS m/z (M^- + 1) 363.30.

5-Ethynyl-1-(5-methylphopsphonyl-β-D-ribofuranosyl)imidazole-4-carboxamide (17). To a stirred suspension of 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (200 mg, 0.75 mmol) in 80 mL of dry acetone at 0°C under argon was added drop-wise 0.02 mL of 70% perchloric acid. The mixture was warmed to room temperature, stirred for 50 min, cooled and carefully neutralized using an equimolar amount of aqueous ammonia. Solvent was evaporated and the residue was purified on a silica gel column with 10% methanol in chloroform to give 160 mg (70%) of 5-ethynyl-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide.[25] This compound was used as such in the next reaction.

According to the same procedure as described for **9**, part of the above product (100 mg, 0.33 mmol) was reacted with methylphosphonic acid (38 mg, 0.39 mmol) in the presence of DCC (161 mg, 0.78 mmol) at 38°C for 36 h and then isopropylidene group was removed using Dowex-H^+ .Purification on reverse-phase HPLC gave 14 mg of **17** with 100% purity. ^{1}H NMR (D_2O) δ 7.95 (s, 1H), 5.81 (d, J = 4.25 Hz), 4.44 (dd, J = 4.25, 4 Hz, 1H), 4.25 (dd, J = 3, 4.5 Hz, 1H), 4.17 (m, 1H), 3.91 (m, 2H), 1.09 (d, J = 17.0 Hz, 3H). ^{31}P NMR (D_2O) δ 28.26 (S); MS m/z (M^- + 1) 345.30.

5-Ethynyl-1-(5-*O*-fluoromethylphosphonyl-β-D-ribofuranosyl)imidazole-4-carboxamide (18). According to the same procedure as described for **9**, 5-ethynyl-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide (60 mg, 0.195 mmol) was reacted with fluoromethylphosphonic acid (26 mg, 0.205 mmol) in the presence of DCC (97 mg, 0.47 mmol) at 38°C for 24 h, and then the isopropylidene group was removed using Dowex-H^+ . Purification on reverse-phase HPLC gave 9.3 mg of **18** with 100% purity . ^{1}H NMR (D_2O) δ 7.94 (s, 1H), 5.8 (d, J = 4.5 Hz), 4.54 (d, J = 3.75 Hz, 1H), 4.45 (dd, J = 4.5, 4.25 Hz, 1H), 4.39 (d, J = 3.5 Hz, 1H), 4.27 (dd, J = 3.75, 4 Hz, 1H), 4.16 (m, 1H), 4.02 (m, 1H). ^{31}P NMR (D_2O) δ 15.52 (d, J = 121.75 Hz); MS m/z (M^- + 1) 363.20.

1-[5-*O*-(Difluoromethyl)phosphonyl-β-D-ribofuranosyl]-5-ethynylimidazole-4-carboxamide (19). According to the same procedure as described for **9**, 5-ethynyl-1-(2,3-*O*-isopropylidene-1-β-D-ribofuranosyl)imidazole-4-carboxamide (60 mg, 0.195 mmol) was reacted with difluoromethyl phosphonate (27 mg, 0.205 mmol) in the presence of DCC (97 mg, 0.47 mmol) at 38°C for 24 h, and then the isopropylidene group was removed using Dowex-H^+. Purification on reverse-phase HPLC gave 14.1 mg of **19** with 100% purity. ^{1}H NMR (D_2O) δ 7.93 (s, br, 1H), 6.00–5.59 (m, 2H), 4.42 (dd, J = 4, 4.25 Hz, 1H), 4.25 (dd, J = 4, 4.25 Hz, 1H), 4.15–4.08 (m, 3H); ^{31}P NMR (D_2O) δ 4.94 (dd, J = 166.25, 167 Hz); MS m/z (M^- + 1) 381.20.

Methyl 1-(2,3-isopropylidene-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate (31). Methyl 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate **30**

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(3.8 g, 10 mmol) was dissolved in anhydrous methanol (50 mL) and sodium methoxide (25 wt.% in methanol, 12 mL) was added. The solution was stirred at room temperature for 6 h and neutralized with Dowex 50WX8-100 ion-exchange resin. The resin was filtered through a short pad of cotton, washed thoroughly with methanol. The methanol solution was concentrated to dryness to give 2.5 g of methyl 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxylate. To a suspension of methyl 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxylate (1.3 g, 0.5 mmol) in dry acetone (20 mL) and dimethoxypropane (18 mL) at 0°C under argon was added dropwise 0.2 mL of 70% perchloric acid. The mixture was warmed to room temperature, stirred for 50 min, cooled with ice and neutralized using an equimolar amount of ammonia. Solvent was evaporated and the residue was loaded on a silica gel column and eluted with 10% methanol in chloroform to give 980 mg of **31**. ^{1}H NMR ($CDCl_3$) δ 8.36 (s, 1H), 6.01 (d, J = 2.4 Hz, 1H), 5.05 (m, 2H), 4.57 (s, br, 1H), 3.47–4.01 (m, 6H), 1.65 (s, 3H), 1.59 (s, 3H)

Methyl 1-[5-*O*-(diethoxyphosphinyl)methyl-2,3-isopropylidene-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate (32). A mixture of methyl 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate (600 mg, 2 mmol) and sodium hydride (100 mg) in anhydrous THF at − 78°C under argon was stirred for 30 minutes, followed by a slow addition of a solution of (diethoxyphosphinyl)methyl triflate (300 mg, 1 mmol) in THF (10 mL). The mixture was brought to room temperature for 1 h, neutralized with acetic acid, and then evaporated. The residue was taken in 20 mL of chloroform and washed with 10 mL water. The chloroform layer was dried over anhydrous magnesium sulfate, filtered, and concentrated to dryness. Reaction product was taken to next step without further purification.

1-[5-*O*-(Dihydroxyphosphinyl)methyl-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide (20). A solution of **32** (400 mg) in 25 mL of methanolic ammonia in a steel vessel was kept at room temperature overnight. Ammonia and methanol were evaporated and the residue was purified on silica gel with 13% methanol in dichloromethane to give 380 mg of the 3-carboxamide derivative **33**. Bromotrimethylsilane (3 mL, 23 mmol) was added to a stirred solution of **33** (350 mg, 0.8 mmol) in acetonitrile (25 mL) and the resulting mixture was stirred at room temperature for 15 h and evaporated to a yellowish syrup, which was dissolved in 5 mL of methanol and concentrated to dryness. This evaporation was repeated three times. The residue was dissolved in 20 mL of methanol and Dowex 50Wx8-100 ion-exchange resin (1 g) was added. The mixture was heated at 50°C for 2 h, filtered, and washed with water thoroughly. The filtrate was concentrated and the residue was purified on reverse-phase HPLC to give 42 mg of **20** with 96.6% purity. ^{1}H NMR (D_2O) δ 8.64 (s, 1H), 5.88 (d, J = 3.8 Hz, 1H), 4.51(m, 1H), 4.36 (m, 1H), 4.17 (m, 1H), 3.45–3.75(m, 4H); ^{31}P NMR (D_2O) δ 15.44 (s); MS m/z (M^- + 1) 338.20.

1-(5-Acetylthio-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (35). Diisopropyl azodicarboxylate (1.53 mL, 7.74 mmol) and triphenylphosphine (2.03 g, 7.74 mmol) were dissolved in anhydrous THF (20 mL) at 0°C. After a white precipitate appeared, 1 g (3.52 mmol) of 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide[18] in 15 mL of anhydrous THF and 0.56 mL (7.74 mmol) of thiolacetic acid in 5 mL of anhydrous THF were added

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simultaneously. The mixture was allowed to warm to room temperature and stirred for 5 h, neutralized with triethylamine, concentrated to dryness, dissolved in ethyl acetate and washed with water. The aqueous layer was extracted with ethyl acetate (2 × 20 mL). The combined organic layer was washed with brine, dried over anhydrous magnesium sulfate, filtered and evaporated. Chromatography on a silica gel column using chloroform/THF (5:1) and then using chloroform/methanol (10:1) afforded 600 mg of **35**. ^{1}H NMR ($CDCl_3$) δ 8.28 (s, 1H), 7.27 (br, s, 1H), 5.94 (br, s, 1H), 5.35 (d, J = 4.75 Hz, 1H), 4.82 (dd, J = 1.75, 5 Hz, 1H), 4.35 (m, 1H), 3.24 (m, 1H), 2.97 (m, 1H), 1.61 (s, 3H), 1.55 (s, 3H), 1.35 (s, 3H).

1-(5-Deoxy-2,3-*O*-isopropylidene-5-thio-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (36). A mixture of methanol and triethylamine (9:1, 7.5 mL) was bubbled with argon at room temperature for 15 minutes and then 200 mg (0.56 mmol) of **35** and 172.7 mg (1.12 mmol) of dithiothreitol were added. The mixture was stirred at room temperature for 5 h. Solvent was evaporated under argon atmosphere and the residue was loaded on a silica gel column. The impurities were eluted using 2% methanol in methylene chloride and then the product using methylene chloride/methanol (30:1). Evaporation of the solvent afforded 130 mg of the 5′-thio nucleoside **36**. ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1H), 7.08 (br, s, 1H), 6.25 (br, s, 1H), 6.05 (d, J = 1 Hz, 1H), 5.40 (dd, J = 1, 5.25 Hz, 1H), 4.95 (m, 1H), 4.35 (m, 1H), 2.69 (m, 3H), 1.58 (s, 3H), 1.37 (s, 3H).

1-(5-Deoxy-5-*S*-phosphonomethyl-5-thio-1-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (21). To a solution of **36** (150 mg, 0.50 mmol) in 5 mL of anhydrous DMF at − 20°C was added 20 mg (0.50 mmol) of 60% sodium hydride in mineral oil, followed by addition of 223 mg (0.74 mmol) of (diethoxyphosphinyl)methyl triflate. The mixture was stirred at this temperature for 1.5 h, concentrated, diluted with ethyl acetate and then washed with brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated to dryness. The residue (156 mg) was dissolved in 15 mL of anhydrous methylene chloride and to this solution was added 1 mL of bromotrimethylsilane and the mixture was stirred at room temperature for 12 h. Solvent was evaporated and the residue was dissolved in 20 mL of methanol/water (1:1). The solution was stirred at 50°C for 3 h and concentrated. Chromatography on reverse phase HPLC afforded 13.5 mg of **21** with 83.6% purity. ^{1}H NMR (D_2O) δ 8.58 (s, 8.58, 1H), 5.89 (d, J = 3.25 Hz, 1H), 4.57 (m, 1H), 4.35 (dd, J = 4.25, 4 Hz, 1H), 4.20 (m, 1H), 2.85 (m, 2H), 2.56 (d, J = 11.5 Hz, 2H). ^{31}P NMR (D_2O) δ 17.64 (d, J = 125 Hz); MS m/z (M^- + 1) 354.28.

1-(5-Deoxy-5-iodo -β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (37). A solution of 1.7 g (6.5 mmol) of triphenylphosphine and 1.52 g (6.0 mmol) of iodine in 10 mL of pyridine was stirred at room temperature for 20 min and then 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide **34** (976 mg, 4.0 mmol) was added. The reaction mixture was stirred at room temperature for 2 h, concentrated to dryness, and co-evaporated with toluene twice. Chromatography on silica gel with methylene chloride/methanol (20:1 to 7.5:1) yielded 1.1 g of **37** which was used in the next step. ^{1}H NMR (DMSO-d_6) δ 8.84 (s, 1H), 7.86 (br, s, 1H), 7.62 (br, s, 1H), 5.87 (d, J = 2.75 Hz, 1H), 5.70 (d, J = 4.75 Hz, 1H), 5.47 (d, J = 4.75 Hz, 1H), 4.45 (dd, J = 3, 4.25 Hz, 1H), 4.17 (dd, J = 4.25, 4.25 Hz, 1H), 4.03 (m, 1H), 3.53 (m, 1H), 3.35 (m, 1H).

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1-(5-Amino-5-deoxy-1-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (38). A reaction mixture of 1.5 g (3.65 mmol) of **37** and 3.65 g (5.35 mmol) of sodium azide in DMF (15 mL) was heated at 90°C for 12 h. After evaporation, the residue was adsorbed on silica gel and loaded onto a silica gel column. The product was eluted using 10% methanol in methylene chloride to afford 1.2 g of 1-(5-azido-5-deoxy-1-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide. ^{1}H NMR (DMSO-d_6) δ 8.84 (s, 1H), 7.83 (br, s, 1H), 7.62 (br, s, 1H), 5.91 (d, J = 2.75 Hz, 1H), 5.69 (d, J = 4.75 Hz, 1H), 5.38 (d, J = 4.75 Hz, 1H), 4.37 (dd, J = 3, 4.25 Hz, 1H), 4.27 (dd, J = 4.25, 4.25 Hz, 1H), 4.06 (m, 1H), 3.53 (m, 2H).

A mixture of the above product and 200 mg of 10% Pd/C (1 g, 3.77 mmol) in 50 mL of methanol was shaken at 30 psi hydrogen for 18 h. The catalyst was filtered and the filtrate was concentrated to dryness to give 600 mg (67%) of **38**. ^{1}H NMR (DMSO-d_6) δ 8.87 (s, 1H), 7.82 (br, s, 1H), 7.61 (br, s, 1H), 5.80 (d, J = 3.5 Hz, 1H), 4.36 (dd, J = 3, 4.25 Hz, 1H), 4.12 (dd, J = 4.25, 4.25 Hz, 1H), 3.88 (m, 1H), 2.68 (m, 2H).

1-(5-Deoxy-5-*N*-phosphonomethylamino-1-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (22). To a solution of the crude **38** (150 mg, 0.62 mmol) in anhydrous DMF and pyridine (1:1, 10 mL) at 0°C and was added 279 mg (0.93 mmol) of (diethoxyphosphinyl)methyl triflate. The mixture was stirred at this temperature for 1.5 h and then concentrated. The residue was taken in ethyl acetate and then washed with brine. The organic phase was dried over $MgSO_4$, filtered and concentrated to give 142 mg of a crude product. The crude product was dissolved in 15 mL of anhydrous methylene chloride and 1 mL (7.5 mmol) of bromotrimethylsilane was added. The mixture was stirred under argon at 40°C for 15 h. Solvent was evaporated and the crude was purified on reverse-phase HPLC to give 13.5 mg of **22** with 100% purity. ^{1}H NMR (D_2O) δ 8.61 (s, 1H), 8.59 (s, 1H), 8.36 (m, 1H), 7.89 (m, 2H), 5.91 (m, 1H), 4.59 (d, J = 10.5 Hz, 2H); ^{31}P NMR (D_2O) δ 7.87 (s); MS m/z (M^- + 1) 337.23.

1-(5-Deoxy-5-methylphosphono-5-thio-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (23). According to the same procedure as described for **9**, the reaction of **36** (100 mg, 0.33 mmol) with methylphosphonic acid (36 mg, 0.37 mmol) in the presence of DCC (137 mg, 0.66 mmol) at 35°C for 24 h and subsequent deprotection of isopropylidene using Dowex-H^+ gave, after purification on reverse-phase HPLC, 6.2 mg of **23** with 100% purity. ^{1}H NMR (D_2O) δ 8.58 (s, 1H), 5.89 (d, J = 3.25 Hz, 1H), 4.63 (m, 1H), 4.58 (m, 1H), 4.36 (dd, J = 4.25, 4.25 Hz, 1H), 4.19 (m, 1H), 2.95 (m, 2H), 1.43 (d, J = 12.5 Hz, 3H); ^{31}P NMR (D_2O) δ 42.66 (s); MS m/z (M^- + 1) 338.40.

Methyl 1-[2-*O*-acetyl-3-*O*-benzyl-5,6-dideoxy-6-(diethoxyphosphinyl)-6,6-difluoro-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxylate (41). Methyl-1,2,4-triazole-4-carboxylate (300 mg, 2.5 mmol) in HMDS (5 mL) was refluxed in the presence of ammonium sulfate (5 mg). Excess HMDS was evaporated under high vacuum. The resulting **40** was dissolved in anhydrous acetonitrile and **39**[19] (1.25 g, 2.5 mmol) was added. After addition of tin (IV) chloride (0.9 mL, 7.5 mmol), the reaction mixture was heated under reflux for 2 h, then cooled to room temperature, diluted with chloroform, filtered through celite, and washed with saturated sodium bicarbonate. The organic layer was dried over anhydrous sodium sulfate and concentrated to dryness. The crude

product was purified on silica gel using 5% methanol in methylene chloride to give 1.1 g of **41**. ^{1}H NMR ($CDCl_3$) δ 8.28 (s, 1H), 7.35 (m, 5H), 5.98 (d, J = 1.7 Hz, 1H), 5.60 (m, 1H), 4.52–4.63 (m, 3H), 4.19–4.37 (m, 5H), 4.00 (s, 3H), 2.22–2.58 (m, 2H), 2.14 (s, 3H), 1.36 (s, 3H); ^{31}P NMR (D_2O) δ 7.48 (t).

1-[5,6-Dideoxy-6-(dihydroxyphosphinyl)-6,6-difluoro-β-D-allofuranosyl]-1,2,4-triazole-3-carboxamide (24). A solution of **41** (1.0 g) and methanolic ammonia saturated at 0°C in a steel vessel was kept at room temperature overnight. Excess ammonia and solvent were evaporated to give 1-[3-O-benzyl-5,6-dideoxy-6-(diethoxyphosphinyl)-6,6-difluoro-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide (725 mg) as a solid crude product. Part of the crude product (505 mg, 1.0 mmol) was dissolved in anhydrous dichloromethane (25 mL) and cooled to − 78°C. Boron trichloride (2 M in dichloromethane, 2.1 mL) was added. The reaction mixture was stirred at room temperature for 1 h. Methanol (10 mL) was added and evaporated and this process was repeated three times. The residue was taken in acetonitrile and DMF (1:1 v/v, 20 mL) and bromotrimethylsilane (2.2 mL, 16.8 mmol) was added. The mixture was stirred under argon atmosphere at room temperature for 40 h. After evaporation, the oily residue was co-evaporated with methanol (3 × 10 mL) and toluene(3 × 10 mL). Purification on reverse-phase HPLC gave 50 mg of **24** with 100% purity. ^{1}H NMR (D_2O) δ 8.55 (s, 1H), 5.88(d, J = 3.52 Hz, 1H), 4.52(m, 1H), 4.41 (m, 1H), 4.31 (m, 1H), 2.31–2.51(m, 2H); ^{31}P NMR (D_2O) δ 5.58 (t); MS m/z (M^- + 1) 358.20.

4-Carbamoyl-1-[2-*O*-acetyl-3-*O*-benzyl-5,6-dideoxy-6-(diethoxyphosphinyl)-6,6-difluoro-β-D-ribofuranosyl]-1,3-imidazolium-5-olate (43). 4-Carbamoylimidazolium-5-olate (127 mg 1.0 mmol) in HMDS (5 mL) and xylene (5 mL) was refluxed in presence of ammonium sulfate (2 mg). Excess HMDS was evaporated under high vacuum. The resulting silylated imidazolium **42** was dissolved in anhydrous nitromethane and **39** (500 mg, 1.0 mmol) was added. After addition of titanium(IV) chloride (0.15 mL, 1.3 mmol), the reaction mixture was stirred at room temperature for 42 h, poured into a suspension of 4 g of sodium carbonate in methanol. The methanol solution was filtered through celite and evaporated. The residue was purified on silica gel using 20% methanol and 0.5% TEA in ethyl acetate to give 280 mg of **43**. ^{1}H NMR ($CDCl_3$) δ 7.84 (s, 1H), 7.32 (m, 5H), 6.24 (bs, 1H), 4.83 (d, J = 11.7 Hz, 1H), 4.6 (m, 1H), 4.45 (d, J = 11.7 Hz, 1H), 4.22(m, 5H), 3.61 (m, 1H), 2.22–2.61 (m, 2H), 1.40 (m, 6H); ^{31}P NMR (D_2O) δ 7.5 (t).

4-Carbamoyl-1-[5,6-dideoxy-6-(dihydroxyphosphinyl)-6,6-difluoro-β-D-ribofuranosyl]-1,3-imidazolium-5-olate (25). Compound **43** (280 mg 0.5 mmol) was subject to the same treatment as **41**, with boron trichloride at room temperature for 2 h and then with bromotrimethylsilane at room temperature for 48 h. Purification on reverse-phase HPLC gave 30 mg of **25** with 90% purity. ^{1}H NMR (D_2O) δ 8.20 (s, 1H), 5.63 (d, J = 3.8 Hz, 1H), 4.48 (m, 1H), 4.35 (m, 1H), 4.18 (m, 1H), 2.22–2.61 (m, 2H); ^{31}P NMR (D_2O) δ 5.14(t); MS m/z (M^- + 1) 373.05.

1-[5-*O*-(*H*-Thiophosphonyl)-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide (26). 1-(2,3-Di-*O*-benozyl-1-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (226 mg. 0.5 mmol, for preparation, see Compound **14**) and 9-fluorenemethyl (*H*)-phosphonothioate (400 mg, 1.5 mmol) were dissolved in 10% pyridine in dichloromethane

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(10 mL). Trimethylacetylchloride (0.07 mL, 0.7 mmol) was added and the mixture was stirred at room temperature for 5 min. Then, triethylamine (10 mL) was added and stirred for further 20 min. Solvent was evaporated and the residue was treated with aqueous methylamine (50%, 5 mL) for 1 h. The solution was concentrated and the residue was purified on reverse-phase HPLC to give 25 mg of **26** with 100% purity (diastereoisomeric mixture 1:1). ^{1}H NMR (D_2O) δ 8.62 (m, 1.5H), 6.64 (s, 0.5H) 5.89(d, J = 3.5 Hz, 1H), 4.56(m, 1H), 4.42 (m, 1H), 4.21 (m, 1H), 3.91–4.13 (m, 2H); ^{31}P NMR (D_2O) δ 56.76 and 56.85 (diastereomers); MS m/z (M^- + 1) 324.03.

1-(2,3-Di-*O*-benzoyl-5-deoxy-5-iodo-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (44). A solution of **37** (1.8 g, 5.0 mmol) was dissolved in anhydrous pyridine (10 mL) was cooled to 0°C and benzoyl chloride (1.3 mL, 11.0 mmol) was added. After 1 h at same temperature, the mixture was poured into saturated sodium bicarbonate and extracted with ethyl acetate. The organic phase was dried over sodium sulfate and evaporated. Purification on silica gel column using 3% methanol in dichloromethane gave 2.1 g of **44**. ($CDCl_3$) 8.46 (s, 1H), 7.94 (m, 4H), 7,58 (m, 2H), 7.37 (m, 4H), 7.02 (s, br, 1H), 6.27 ((d, J = 3.2 Hz, 1H),6.13 (m, 1H),5.85 (m, 2H), 4.64 (m, 1H), 3.50–3.66 (m, 2H).

1-[5-Deoxy-5-(hydroxyl-*H*-phosphinyl)-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide (27). A mixture of ammonium phosphinate (0.4 g. 5.0 mmol) and HMDS (1.07 mL, 5.0 mmol) was heated at 100°C for 2 h under argon. The resulting bis(trimethylsilyl)phosphonite was cooled to 0°C and compound **44** (560 mg, 1.0 mmol) in 20 mL of dichloromethane was added. The reaction mixture was stirred at room temperature overnight, filtered and concentrated. The oily residue was dissolved in 5 mL of dichloromethane and 5 mL of methanol, stirred for 2 h at room temperature and evaporated. Aqueous ammonium (28%, 10 mL) was added to the oily residue and stirred at room temperature for 4 h. The mixture was concentrated to dryness and purified on reverse-phase HPLC to give 20 mg of **27** with 100% purity. ^{1}H NMR (D_2O) δ 8.56 (s, 1H), 7.59 (s, 0.5H), 5.86(d, J = 3.8 Hz, 1H), 4.58(m, 1H), 4.24 (m, 2H), 1.85–2.0(m, 2H); ^{31}P NMR (D_2O) δ − 20.06 (s); MS m/z (M^- + 1) 292.00.

1-[5-Deoxy-5-(dihydroxyphosphinyl)-β-D-ribofuranosyl]-1,2,4-triazole-3-carboxamide (28). Compound **44** (1.6 g) was dissolved in triethylphosphite (5 mL) and heated to 100°C for 50 h. Excess reagent was evaporated to dryness under high vacuum and the residue was adsorbed on a small amount of silica gel. The silica gel bearing the product was loaded on a silica gel column and eluted with 3% methanol in dichloromethane to give 500 mg of **45**. ($CDCl_3$) δ 8.44 (s, 1H), 7.96 (m, 4H), 7.54 (m, 2H), 7.26 (m, 4H) 7.05 (s, br, 1H) 6.25 (d, J = 3.02 Hz 1H) 6.04 (m, 1H), 5.86 (m, 1H), 5.68 (s, br, 1H), 4.95 (m, 1H), 4.08 (m, 4H), 2.01–2.30 (m, 2H) 1.26 (m, 6H). ^{31}P NMR (D_2O) δ 26.35 (s).

Compound **45** (500 mg, 0.9 mmol) was dissolved in DMF and acetonitrile (1:1, 10 mL) and bromotrimethylsilane (0.60 mL, 4.5 mmol) was added. The reaction mixture was stirred at room temperature for 6 h and then concentrated under high vacuum. The residue was co-evaporated with methanol and toluene three times. Aqueous ammonia (28%, 15 mL) was added to the residue and stirred at room temperature for 6 h. After evaporation of aqueous solution, the residue was purified on reverse-phase HPLC to give 50 mg of **28** with 100% purity. ^{1}H NMR (D_2O) δ 8.56 (s, 1H), 5.83(d, J = 3.8 Hz,

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1H), 4.55(m, 1H), 4.25 (m, 2H), 1.91–2.0(m, 2H); ^{31}P NMR (D_2O) δ 20.46 (s); MS *m/z* (M^- + 1) 308.20.

4-Carbamoyl-1-[5-deoxy-5-(dihydroxyphosphinyl)-β-D-ribofuranosyl]-1,3-imidazolium-5-olate (29). A suspension of 4-carbamoyl-1,3-imidazolium-5-olate[21] (188 mg, 1.48 mmol) and sodium sulfate (20 mg) in HMDS (3 mL) and anhydrous xylene (3 mL) was heated under reflux for 3 h and converted to a clear solution. After evaporation of the volatiles, the residue was dried under high vacuum for 30 min, then dissolved in 4 mL of anhydrous 1,2-dichloroethane. Stannic tetrachloride (140 μL, 1.18 mmol) was added, followed by addition of 1-*O*-acetyl-5-deoxy-2,3-di-*O*-benzoyl-5′-(diethoxyphosphinyl)-D-ribofuranose[22] (700 mg, 1.33 mmol) in 1,2-dichloroethane (2 mL) and then trimethylsilyl triflate (85 μL, 0.44 mmol). The resulting mixture was stirred at room temperature under argon for 6 days. After cooling with ice, the stirred mixture was diluted with chloroform and 5% aqueous sodium bicarbonate and then filtered through celite. The organic layer was washed with aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated to dryness. Chromatography on silica gel with 10% MeOH and 4% TEA in methylene chloride gave 310 mg of 4-carbamoyl-1-[5-deoxy-2,3-di-*O*-benzoyl-5-(diethoxyphosphinyl)-β-D-ribofuranosyl]-1,3-imidazolium-5-olate as a colorless foam; ^{1}H NMR δ: 1.32, 1.29 (2t, 6H, J = 7.2 Hz, OCH_2CH_3), 2.53 (m, 2H, H-5′), 4.11 (m, 4H, OCH_2CH_3), 4.70 (m, 1H, H-4′), 5.90 (t, 1H, J = 5.4 Hz, H-2′); 6.09 (t, 1H, J = 5.7 Hz, H-3′), 6.15 (d, 1H, J = 4.8 Hz, H-1′), 7.28–7.96 (m, 10H, Bz), 8.19 (s, 1H); ^{31}P NMR ($CDCl_3$) 27.11; MS (m/z) 586 (M^+ − 1).

A solution of the above product (203 mg, 0.337 mmol) and bromotrimethylsilane (144 μL, 1.1 mmol) in anhydrous acetonitrile (1 mL) was kept at room temperature overnight and then concentrated to dryness. The residue was dissolved in saturated methanolic ammonia and stirred at room temperature for 12 h. After evaporation of volatiles, the residue was subjected to purification on reverse-phase HPLC to yield 21.2 mg of **29** (purity: 94.7%); ^{31}P NMR (D_2O) δ 19.8 (s); MS *m/z* (M^- + 1) 322., 645 (MM^+ − 1).

Assay for Inhibition of IMPDH Activity

Human IMPDH Type I and Type II isozymes and *C. albicans* IMPDH were acquired from the Department of Biochemistry, Brandeis University. The IMPDH assay followed a modification of a reported procedure.[12] Assay buffer conditions: 200 uL-total/reaction (50 mM Tris-HCl, pH 8.0, 100 mM KCl, 3 mM EDTA, 1 mM DTT, 50 uM IMP, 150 uM NAD, 30 nM purified human type I or II IMPDH, or 7.5 nM purified *C. albicans* IMPDH). The P1Ms were tested at various concentrations up to 500 uM final concentration. The assay was performed in duplicate in 96-well plates (Corning). An appropriate volume of assay buffer, containing the substrates IMP and NAD, was pipetted into the plate wells. The P1Ms were added to the reactions at the desired concentrations. The reactions were initiated by addition of the enzymes. The reactions were allowed to proceed for 5 minutes at 25°C. Blank reactions were prepared in parallel with the test reactions in which enzyme was omitted from the reactions, substituted by an appropriate volume of the buffer. The oxidation of inosine 5′-monophosphate (IMP) to xanthosine 5′-monophosphate (XMP) by IMPDH was coupled to the reduction of nicotinamide adenine dinucleotide (NAD). Production of NADH

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was monitored at 340 nm using a UV/VIS microplate spectrophotometer (SpectraMax Plus 384, Molecular Devices Corp.) or at 474 nm (excitation wavelength = 344 nm) using a microplate fluorometer (SpectraMax Gemini XS, Molecular Devices Corp.). Initial velocity data (mA min^{-1}) was collected for each experiment, performed at least two times, and fit to standard equations for % inhibition and Ki for competitive inhibition or irreversible inhibition, where appropriate. The same screening assay format was used for determinations of % inhibition by P1Ms of IMPDH from several organisms–including Human isoforms I and II, and fungal isoforms–except that amounts of different enzymes added were adjusted so as to achieve similar activities.

Serum Stability Assessment

The stability of nucleotide mimics was assessed in fetal calf serum, generally following a published procedure.[25] Fetal calf serum purchased from HyClone Corporation was mixed 1:1 with each compound in Tris-HCl buffer containing $MgCl_2$. The final concentrations of the reaction components were as follows: 50 mM Tris-HCl, pH 7.4, 0.1 mM $MgCl_2$, 500 μM nucleotide mimic, 10% (v/v) fetal calf serum. The reaction mixtures were incubated at 37°C. At appropriate times, aliquots of 25 μl were removed and added to 65 μl ice-cold methanol. These solutions were incubated for at least one hour at − 20°C. After incubation, samples were centrifuged for 20 minutes at high speed in a microcentrifuge. The supernatant was transferred to a clean tube and the extract was concentrated to dryness under vacuum in a LabConco Centrivap Concentrator. The dried extracts were resuspended in water and filtered to remove particulates and then analyzed on reverse phase HPLC. The reverse phase HPLC columns used for the analysis were a Phenomenex C18 Aqua column (3 × 150 mm). The HPLC flow rate was 0.5 ml/min with the following buffer system: 5 mM tetrabutylammonium acetate, 50 mM ammonium phosphate, and an acetonitrile gradient running from 5% up to as high as 60%. The amount of remaining parent compound at each time point was used to determine the half-life of the compound. Time points were only taken through 24 hours so that if greater than 50% of a compound was still intact after 24 hours incubation the half-life was expressed as > 24 hours. Unmodified nucleoside 5′-monophosphates were used as positive controls. Under these conditions unmodified nucleoside 5′-monophosphates had half-lives of approximately three to six hours.

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