Novel Methylenephosphophosphonate Analogues of Mycophenolic Adenine Dinucleotide. Inhibition of Inosine Monophosphate Dehydrogenase

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Received April 24, 2006

Novel methylenephosphophosphonate analogues of mycophenolic adenine dinucleotide (MAD) have been prepared as potential inhibitors of IMP dehydrogenase. A coupling of the mycophenolic (hydroxymethyl)-phosphonate **6** with the phosphitylated adenosine analogue **11** followed by oxidation and deprotection afforded the phosphophosphonate **8.** A similar coupling between adenosine (hydroxymethyl)phosphonate **10** and phosphitylated mycophenolic alcohol **5** gave the corresponding phosphophosphonate **13**. Both **8** and **13** (K_i = 20−87 nM) were found to be the most potent cofactor type inhibitors of IMP dehydrogenase.

Introduction

Nicotinamide adenine dinucleotide (NAD, Figure 1) serves as a substrate in many biochemical processes, e.g., ribosylation of proteins,[1] cell signaling,[2] DNA repair and recombination, histone deacetylation (by the Sir2 family of NAD-dependent deacetylases),[3,4] and also plays a crucial role as a coenzyme in numerous oxidation−reduction reactions. It was commonly believed at one time that, since the NAD binding domain is conserved among NAD-dependent enzymes, inhibitors that mimic the cofactor would not bind selectively and therefore would cause general toxicity. However, it is now evident that some coenzyme analogues specifically inhibit a single NAD-dependent enzyme and therefore may be of interest as therapeutic agents.[5−9] Some NAD mimics show potential as agents for the treatment of cancer[10,11] and infectious diseases[12] or as immunosuppressants.[13]

NAD-dependent IMPDH[a] emerged in recent years as a major therapeutic target.[14,15] IMPDH controls entry of purines into the guanine nucleotide pool via salvage and de novo purine synthesis pathways. It catalyzes oxidation of IMP to xanthosine monophosphate, which is further converted into guanosine monophosphate (GMP) by GMP synthase. Inhibition of IMPDH leads to decreased GTP concentration, down-regulation of ras and myc oncogenes,[16] and impairment of signal transduction.[17] Human IMPDH is well characterized, and because of its importance in neoplasia, it is an exceptionally attractive target for anticancer drug design.

Four inhibitors of the enzyme (Figure 2), ribavirin, mizoribine, tiazofurin, and mycophenolic acid (in the form of the prodrug mycophenolic mofetil) are currently used in the clinic. Ribavirin and mizoribine (in the form of their corresponding 5′-monophosphates) bind to the enzyme at the substrate site. In contrast, TR and MPA inhibit IMPDH at the cofactor site. As such TR is a poor inhibitor of IMPDH and requires an unusual metabolic activation. In cells, TR is phosphorylated by adenosine kinase and/or 5′-nucleotidase to the mononucleotide (TRMP), which is further coupled with ATP by NMN-adenylyl transferase to form tiazofurin adenine dinucleotide (TAD, Scheme 1). As an analogue of NAD in which TR replaces the nicotinamide riboside moiety of NAD, TAD mimics the natural cofactor but cannot participate in hydride transfer resulting in potent inhibition of IMPDH. TAD inhibits the human enzyme with K_i =

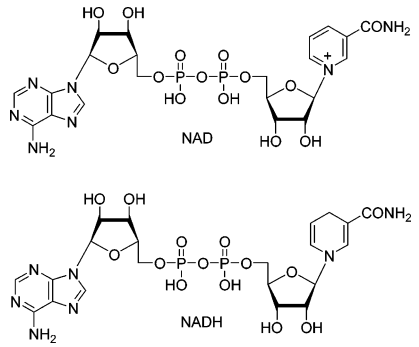

Figure 1.

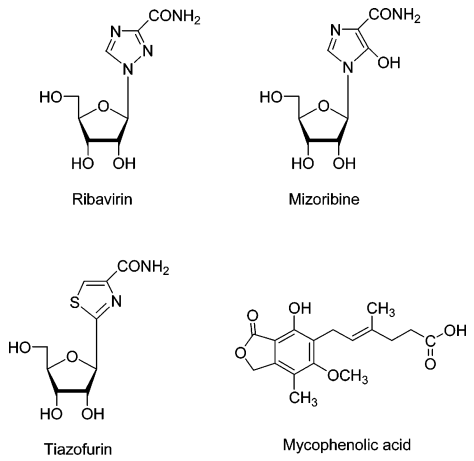

Figure 2.

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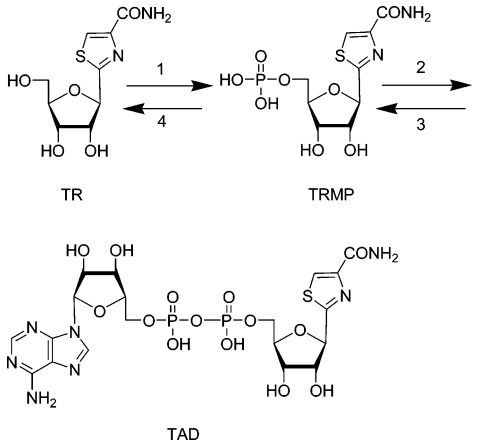
[a] Abbreviations: MAD, mycophenolic adenine dinucleotide; IMPDH, inosine monophosphate dehydrogenase; TR, tiazofurin; MPA, mycophenolic acid; CML, chronic myelogenous leukemia.

10.1021/jm060479r CCC: $33.50
Published on Web 07/18/2006

Scheme 1[a]



[a] Reagents: (1) adenosine kinase; (2) NMN adenylyltransferase; (3) TADase, phosphodiesterase; (4) phosphatases.

110 nM and at this concentration does not affect other cellular dehydrogenases. The K_m for NAD as an IMPDH substrate is 100 μM. The cofactor concentration usually required by other cellular dehydrogenases is in the micromolar range.

MPA is a non-nucleoside and is a specific inhibitor of IMPDH with a K_i of 7−33 nM.[18] It is used as immunosuppressant but is not active against cancer because of extensive glucuronidation of the phenolic function.[19,20] MPA binds at the nicotinamide mononucleotide (NMN) subsite, leaving the adenosine monophosphate (AMP) subsite empty.[21,22] Thus, we synthesized MAD (Figure 3) analogues in which the AMP was attached to the MPA moiety through a methylenebis(phosphonate) linkage.[23,24] The resulting analogues C2-MAD and C4-MAD (Figure 3) retained high specificity and potent inhibitory activity against IMPDH (K_i = 250−520 nM) regardless of number of atoms between the phosphorus of adenosine phosphonate and the aromatic ring of MPA. In contrast to MPA, the C2-MAD analogue was resistant to glucuronidation in vitro under conditions in which MPA was quickly and completely glucuronidated.[25]

Encouraged by the above results, we evaluated the *antileukemic* activity of C2-MAD in SCID mice xenotransplanted with human K562 leukemia cells.[11] We found that C2-MAD at 10, 30, or 60 mg/kg was well tolerated when administered daily for 10 days by the intraperitoneal route. No deaths, no weight loss, and no evidence of behavioral toxicity were observed. At 60 mg/kg, C2-MAD was as potent as TR at 200 mg/kg. TR is approved by FDA as an orphan drug for treatment of patients in blast crisis of CML. Since C2-MAD showed anticancer activity in TR-resistant cell lines, our studies suggest that it may be a promising drug candidate for treatment of CML.

In this paper we present the synthesis of new MAD analogues in which the methylenebis(phosphonate) moiety (P−C−P) is replaced with a methylenephosphophosphonate group (P−O−C−P or P−C−O−P). Thus, the length of the linker of these compounds is one atom longer than that of C2-MAD and one atom shorter than that of C4-MAD.

Results and Discussion

1.Chemical Synthesis of Methylenephosphophosphonate Analogues of C2-MAD. Our original synthesis of P^1,P^2 unsymmetrically disubstituted analogues of methylenebis(phosphonic) acid is laborious and requires HPLC purification of a monosubstituted methylenebis(phosphonate) intermediate and the final disubstituted product.[26] A synthetic route in which fully

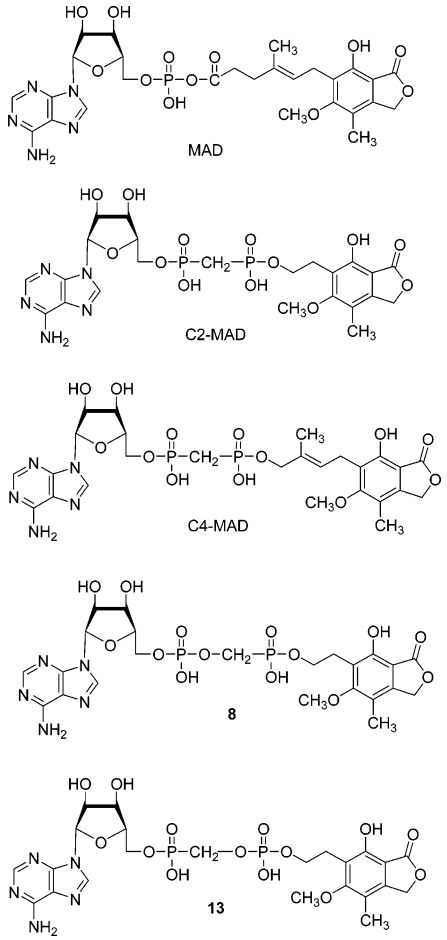
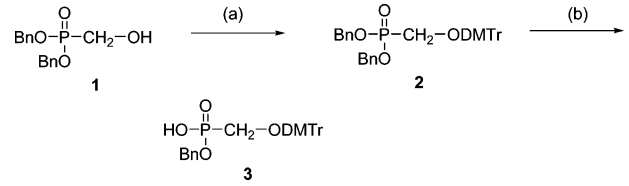


Figure 3.

Scheme 2[a]

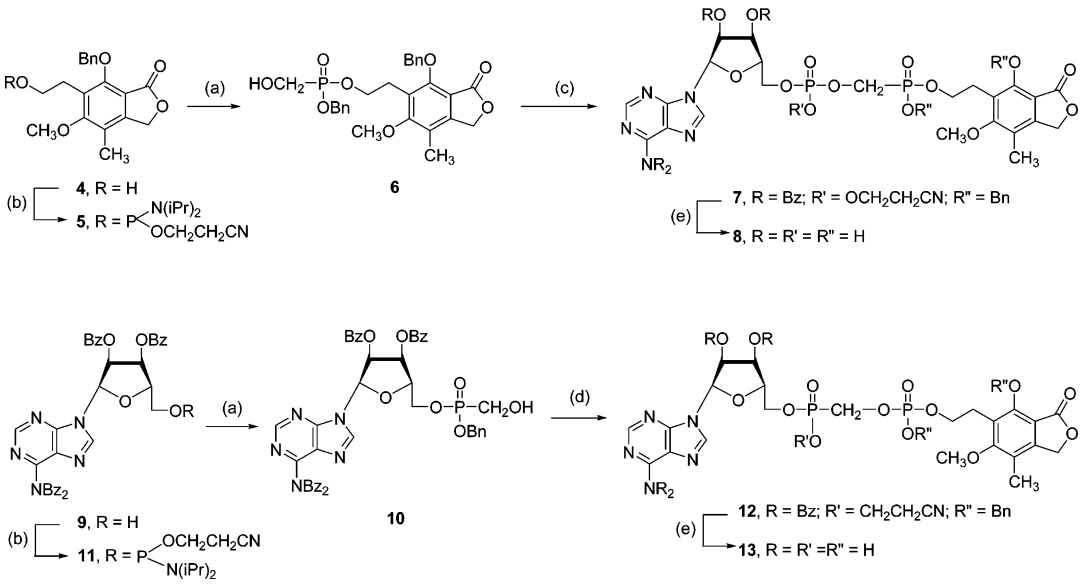


[a] Reaction conditions: (a) pyridine, dimethoxytrityl chloride; (b) DABCO, toluene, reflux, 8 h, then overnight at room temperature.

protected intermediates could be assembled and purified on a silica gel column would be more efficient. The last step, deprotection, would afford a pure final product or a product that requires only HPLC purification. With this in mind we designed a procedure depicted in Schemes 2 and 3, in which new MAD analogues were obtained with an oxygen atom inserted on either side of the methylene group as in **8** and **13**. We expected that insertion of the additional oxygen atom into the C2-MAD linker would result in new analogues with binding affinity similar to that of C2- and C4-MAD. Thus, dibenzyl-(hydroxymethyl)phosphonate (**1**, Scheme 2) was prepared by known reaction of paraformaldehyde with dibenzylphosphite and then protected with dimethoxytrityl chloride to give **2**. Deprotection with 1,4-diazabicyclo[2.2.2]octane (DABCO) in toluene afforded monodebenzylated phosphonate **3** in good yield.

Coupling of **3** with a 7-*O*-benzyl protected C2-mycophenolic alcohol **4**[24] (Scheme 3) in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TIPSCl) followed by detritylation with acetic acid gave the corresponding benzyl protected C2-mycophenolic (hydroxymethyl)phosphonate **6**. Reaction of **6**

Scheme 3[a]



[a] Reaction conditions: (a) **3**, 1-methylimidazole, TPSCl, CH_3CN, overnight at room temperature, 80% acetic acid; (b) **4** or **9**, $[(^iPr)_2N]_2POCH_2CH_2CN$, CH_3CN; (c) **6** and **11**, tetrazole, mCPBA; (d) **10** and **5**, tetrazole, mCPBA; (e) H_2, Pd/C, 8 M ethanolic methylamine, Dowex 50W (Na^+ form).

Table 1. Inhibition of IMPDH by MPA, C2-MAD, and Its Methylenephosphophosphonate Analogues

inhibitors	K_I (type I), nM	K_I (type II), nM
MPA	33 [18]	7 [18]
C2-MAD	330 ± 9	250 ± 11
C4-MAD	520 ± 12	380 ± 7
8	87 ± 18	60 ± 6
13	20 ± 4	37 ± 10

with freshly prepared 2′,3′-*O*-benzoyl-N^6-dibenzoyl-5′-cyanoethylphosphoroamidite (**11**) followed by oxidation with *m*-chloroperbenzoic acid (mCPBA) afforded the fully protected methylenephosphophosphonate analogue of C2-MAD **7** in 70% yield after silica gel column purification. Hydrogenolysis (Pd/C) of **7** with addition of 2 M triethylammonium bicarbonate (TEAB) resulted in debenzylation and removal of the cyanoethyl group. Further treatment of the partially deprotected derivative with a solution of methylamine in EtOH afforded the desired methylenephosphophosphonate analogue **8**.

Similarly, TIPSCl coupling of **3** with the benzoyl protected adenosine **9** followed by detritylation afforded the corresponding 2′,3′-*O*-dibenzoyl-N^6-dibenzoyl-5′-*O*-(hydroxymethyl)phosphonate **10**. Compound **10** was then reacted with freshly prepared 7-*O*-benzyl-C2-mycophenolic-cyanoethyl phosphoroamidite **5** to give the protected methylenephosphophosphonate **12**, which was deprotected in a similar manner as described for **7** to give the desired C2-MAD analogue **13**.

Thus, we developed a new and general procedure for the synthesis of P^1,P^2 unsymmetrically substituted analogues of methylenephosphophosphonates that is more efficient than our original synthesis of the corresponding methylenebis(phosphonate)s. In addition, this new synthetic method can be used for the efficient assembly of methylenephosphophosphonate analogues of other cofactor analogues such as NAD, TAD, SAD,[27] and BAD,[28,29] and analogues of bis(phosphonic) acids used as osteoporosis drugs.

2. Inhibition of Human IMPDH by Methylenephosphophosphonate Analogues of MAD. Our new methylenephosphophosphonate analogues of C2-MAD showed significantly more potent inhibition of IMPDH (Table 1) than the parent methylenebis(phosphonate)s of MAD reported by us earlier.[24] These compounds are also superior to previously reported pyrophosphates such as TAD, BAD, and their analogues.[14] Compound **13** was found to be as potent as an inhibitor of the type I isoform of human IMPDH as MPA and is 10-fold more potent than parent C2-MAD against both isoforms. It is the most potent inhibitor among known cofactor type inhibitors of IMPDH. Compound **8** showed approximately 4-fold better inhibitory activity than its parent C2-MAD.

These results indicate that the characteristic pyrophosphate (−P−O−P−) or bis(phosphonate) (−P−C−P−) geometry of NAD analogues is not critical for preservation of the potent inhibitory activity (or high affinity) of these compounds *against (toward) IMPDH*. Thus, construction of other MAD analogues with phosphorus atoms linked through more than one atom could lead to even higher selectivity toward IMPDH. If other cellular NAD-dependent enzymes are not inhibited significantly by new P−O−C−P analogues, they should show much higher selectivity toward IMPDH than the parent MAD compounds. Vigorous studies are underway in this laboratory to examine this hypothesis and construct other inhibitors with two-atom linkers replacing the pyrophosphate oxygen or bis(phosphonate) carbon.

Experimental Section

General Methods. All commercial reagents (Sigma-Aldrich, Acros) were used as provided unless otherwise indicated. An anhydrous solvent dispensing system (J. C. Meyer) using two packed columns of neutral alumina was used for drying THF, Et_2O, and CH_2Cl_2, while two packed columns of molecular sieves were used to dry DMF. Solvents were dispensed under argon. HPLC was performed on a Dynamax-60A C18-83-221-C column with flow rate of 5 mL/min or Dynamax-300A C18-83-243-C column with a flow rate of 20 mL/min of 0.1 M $Et_3NH_2CO_3$ (TEAB) followed by a linear gradient of 0.1 M TEAB−aqueous MeCN (70%). Nuclear magnetic resonance spectra were recorded on a Varian 500 or 600 MHz with Me_4Si or DDS as the internal standard for ^1H and external H_3PO_4 for ^{31}P. Chemical shifts are reported in ppm, and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), bs (broad singlet), and dd (double doublet). *J* values given for coupling constants are first order and in Hz. High-resolution mass spectra were recorded on a ZAB-EQ (VG analytical spectrometer) using FAB or on an Agilent TOF II TOF/MS instrument equipped with an ESI or APCI interface.

Dimethoxytrityl Benzylhydroxymethylphosphonate (3). Dimethoxytrityl chloride (4.4 g, 13 mmol) was added to the solution of dibenzyl hydroxymethylphosphonate (**1**, 2.53 g, 8.66 mmol) in pyridine (30 mL). The mixture was stirred at room temperature overnight. The reaction was quenched with methanol (1 mL) and evaporated. The residue was chromatographed on a silica gel column using a linear gradient of ethyl acetate in toluene (containing 10% of Et_3N) to give crude **2** ((4.71 g, 91% yield), which was mixed with DABCO (0.89 g, 7.921 mmol) in toluene (60 mL), refluxed for 8 h, and then left at room temp overnight. The crystals that formed were filtered and washed with ethyl acetate followed by petroleum ether to give the DABCO salt of **3** (4.1 g, 84% yield). This salt was converted into the Et_3N salt (3.0 g, 75% overall yield) by passing a solution of **3** (DABCO) in ethanol through a column of Dowex 50, Et_3NH^+ form.

7-Benzyloxy-6-(2-hydroxyethylbenzylhydroxymethylphosphonate)-5-methoxy-4-methylphatalan-1-one (6). Compound **3** (0.72 g, 1.19 mmol), 1-methylimidazole (0.1 mL, 1.19 mmol), and TIPSCl (0.36 g, 1.19 mmol) were premixed in acetonitrile (10 mL), and 7-benzyloxy-6-(2-hydroxyethyl)-5-methoxy-4-methylphatalan-1-one (**4**,[24] 0.21 g, 0.638 mmol) dissolved in acetonitrile (2 mL) was added after 4 min. The mixture was stirred overnight at room temperature and concentrated in vacuo. The residue was purified by column chromatography on silica gel, using a linear gradient of ethyl acetate in toluene (containing 10% of Et_3N), and DMTr derivative was treated with 80% acetic acid (20 mL) for 3 h. Acetic acid was evaporated in vacuo, and the mixture was coevaporated with water (2×), ethanol (2×), and toluene (2×). A further purification by chromatography on a silica gel column using a linear gradient of ethyl acetate in toluene followed by a linear gradient of ethanol in ethyl acetate gave **6** (0.254 g, 78% overall yield).

***P*^{1}-(Cyanoethyl)-*P*^{1}-(*N*^{6},*N*^{6},2′,3′-*O*-tetrabenzoyladenosin-5′-yl)-methylenephospho-*P*^{2}-(benzyl)-*P*^{2}-[7-benzyloxy-6-(ethyl-2-yl)-5-methoxy-4-methylphthalan-1-one]phosphonate (7).** Tetrazole (0.09 g, 1.25 mmol) was added to a mixture of tetrabenzoyladenosine cyanoethyl diisopropylphosphoroamidite **11** [(0.249 g, 0.28 mmol), prepared by phosphitylation of N^6-dibenzoyl, 2′,3′,-*O*-dibenzoyladenosine (**9**)[30] with commercially available 2-cyanoethyl diisopropyl-chlorophosphoramidite] and mycophenolic phosphonate **6** (0.13 g, 0.25 mmol) in acetonitrile (10 mL). After 1 h the reaction mixture was cooled to 0 °C, and mCPBA (0.07 g, 0.4 mmol) was added. Temperature was increased to r.t. and the mixture was stirred for an additional 1 h. The mixture was diluted with ethyl acetate (100 mL), and washed with a saturated solution of sodium bicarbonate (2 × 80 mL). The organic phase was dried over sodium sulfate and concentrated in vacuo. The residue was purified on a silica gel column using a linear gradient of ethyl acetate in toluene followed by a linear gradient of ethanol in ethyl acetate to give compound **7** ((0.2303 g, 70% yield) as a mixture of 4 diastereoisomers.

***P*^{1}-(Adenosin-5′-yl)methylenephospho-*P*^{2}-[7-hydroxy-6-(ethyl-2-yl)-5-methoxy-4-methylphthalan-1-one]phosphonate (8).** Compound **7** was dissolved in a mixture of ethanol (30 mL) and dioxane (20 mL), the 2M TEAB (0.1 mL) and Pd/C (70 mg) were added and the mixture was hydrogenated overnight at r.t. The catalyst was filtered through a Celite pad and the filtrate was concentrated in vacuo. The solid residue was dissolved in 8M ethanolic methylamine (5 mL), and stirred at r.t. for 2 h. The mixture was evaporated, and the residue was purified by HPLC to give the desired product, which was converted into the sodium salt by passing through a column of Dowex 50 (Na^+ form) to give **8** in 64% overall yield (80 mg, 0.1134 mmol). ^{1}H NMR (D_2O) δ 2.05 (s, 3H, CH_3-4), 2.88 (m, 2H, CH_2-6″), 3.82 (s, 3H, OCH_3), 3.98 (m, 2H, CH_2OP), 4.09 (dd, 2H, $J_{H,P}$ = 9.0, 5.0, P−CH_2−OP), 4.24 (ddd, 1H, J_{gem} = 11.7, $J_{H,P}$ = 4.8, $J_{5′b,4′}$ = 2.8, H-5′b), 4.31 (ddd, 1H, J_{gem} = 11.7, $J_{H,P}$ = 4.8, $J_{5′a,4′}$ = 2.6, H-5′a), 4.42 (dq, 1H, $J_{4′,3′}$ = 5.2, $J_{4′,5′}$ = 2.8, 2.6, $J_{H,P}$ = 2.0, H-4′), 4.56 (t, 1H, $J_{3′,4′}$ = 5.2, $J_{3′,2′}$ = 5.1, H-3′), 4.67 (t, 1H, $J_{2′,1′}$ = 5.1, $J_{2′,3′}$ = 5.1, H-2′), 5.15 (s, 2H, H-3″), 6.04 (d, 1H, $J_{1′,2′}$ = 5.1, H-1′), 8.14 (s, 1H, H-2), 8.39 (s, 1H, H-8); ^{13}C NMR (D_2O) δ 11.14 (CH_3-4), 26.20 (d, $J_{C,P}$ = 4, CH_2-6″), 60.72 (dd, $J_{C,P}$ = 161, 7, P−CH_2−OP), 62.03 (OCH_3), 64.82 (d, $J_{C,P}$ = 6, CH_2-5′), 65.34 (d, $J_{C,P}$ = 5, CH_2OP), 70.41 (CH_2-3″), 70.56 (CH-3′), 75.33 (CH-2′), 84.07 (d, $J_{C,P}$ = 9, CH-4′), 87.94 (CH-1′), 107.02 (C-7″a), 116.55 (C-3″a), 118.71 and 118.74 (C-5, C-6″), 139.92 (CH-8), 147.07 (C-4″, C-7″), 148.97 (C-4), 153.04 (CH-2), 155.64 (C-8), 163.50 (C-5″), 173.68 (C-1″); ^{31}P NMR (D_2O) δ 1.91 (m, J_{vic} = 38.0, $J_{P,H}$ = 5.0, 4.8, 2.0 P-phosphate), 16.99 (dp, J_{vic} = 38.0, $J_{P,H}$ = 9.0, P-phosphonate); HR FAB$^+$ calcd (M+H)$^+$ 706.090342, found 706.092567.

***N*^{6}-Dibenzoyl-2′,3′-*O*-dibenzoyl-5′-yl(*O*-benzyl)hydroxymethylphosphonate (10).** Monobenzyl dimethoxytrityloxymethanphosphonate (**3,** 0.82 g, 1.35 mmol), 1-methylimidazole (0.1 mL, 1.35 mmol) and TIPSCl (0.4 g, 1.35 mmol) were premixed in acetonitrile (10 mL). Tetrabenzoyladenosine **(9**, 0.48 g, 0.7 mmol) dissolved in acetonitrile (2 mL) was added after 4 min. The reaction mixture was stirred overnight at r.t. Dimethoxytrityl derivative was obtained by chromatography on silica gel, using a linear gradient of ethyl acetate in toluene (containing 10% of Et_3N) and immediately treated with 80% acetic acid (20 mL) for 3 h. Acetic acid was evaporated and the reaction mixture was coevaporated with water (2x), ethanol (2x), and toluene (2x) to give **10** (0.254 g, 42% yield as a mixture of two diastereoisomers A and B in the ratio of 4:3) after chromatography on a silica gel column using a linear gradient of ethanol in chloroform.

***P*^{1}-(Benzyl)-*P*^{1}-[7-benzyloxy-6-(ethyl-2-yl)-5-methoxy-4-methylphthalan-1-one]methylenephospho-*P*^{2}-(cyanoethyl)-*P*^{2}-(*N*^{6},*N*^{6},2′,3′-*O*-tetrabenzoyladenosin-5′-yl)phosphonate (12).** Tetrazole (0.11 g, 1.55 mmol) was added to the mixture of phosphonate **10** (0.24 g, 0.28 mmol) and mycophenolic phosphoramidite **5** [(0.162 g, 0.315 mmol), prepared by phosphitylation of mycophenolic alcohol **4**[24] with commercially available 2-cyanoethyl diisopropyl-chlorophosphoramidite] in acetonitrile (5 mL). After 1 h the reaction mixture was cooled to 0 °C, and mCPBA (0.2 g, 1.55 mmol) was added. Temperature was increased to r.t. and the mixture was stirred for an additional 1 h. The mixture was diluted with ethyl acetate (100 mL), and washed with saturated solution of sodium bicarbonate (2 × 80 mL). Organic phase was dried over sodium sulfate and concentrated in vacuo. The desired product (0.23 g, 64% yield, a mixture of 4 diastereoisomers) was obtained by chromatography on silica gel using a linear gradient of ethanol in chloroform.

***P*^{1}-[7-Hydroxy-6-(ethyl-2-yl)-5-methoxy-4-methylphthalan-1-one]methylenephospho-*P*^{2}-(adenosin-5′-yl)phosphonate (13).** Compound **12** was dissolved in a mixture of ethanol (15 mL) and dioxane (20 mL), and 2 M TEAB (0.1 mL) and Pd/C (70 mg) were added. The mixture was hydrogenated overnight at room temperature. The catalyst was filtered off on a Celite pad, and the filtrate was concentrated. The obtained solid was dissolved in 8 M ethanolic methylamine (3 mL) and stirred at room temperature for 2 h. The mixture was evaporated, and the residue was purified by HPLC. The triethylammonium salt was converted into the sodium salt by passing through a column of Dowex 50 (Na^+ form) giving 30% overall yield (37.7 mg). ^{1}H NMR (500 MHz, D_2O) δ 2.01 (s, 3H, CH_3-4″), 2.91 (m, 2H, CH_2-6″), 3.80 (s, 3H, OCH_3), 3.92 (q, 2H, $J_{H,P}$ = 8.5, J_{vic} = 7.7, CH_2OP), 4.06 and 4.08 (2 × ddd, 2H, J_{gem} = 14.1, $J_{H,P}$ = 9.0, 5.0, P−CH_2−OP), 4.31 (ddd, 1H, J_{gem} = 11.7, $J_{H,P}$ = 4.8, $J_{5′b,4′}$ = 2.7, H-5′b), 4.35 (ddd, 1H, J_{gem} = 11.7, $J_{H,P}$ = 4.8, $J_{5′a,4′}$ = 2.6, H-5′a), 4.43 (dq, 1H, $J_{4′,3′}$ = 4.3, $J_{4′,5′}$ = 2.7, 2.6, $J_{H,P}$ = 2.0, H-4′), 4.56 (t, 1H, $J_{3′,2′}$ = 5.1, $J_{3′,4′}$ = 4.3, H-3′), 4.66 (t, 1H, $J_{2′,1′}$ = 5.1, $J_{2′,3′}$ = 5.1, H-2′), 5.10 (s, 2H, H-3″), 6.03 (d, 1H, $J_{1′,2′}$ = 5.1, H-1′), 8.20 (s, 1H, H-2), 8.39 (s, 1H, H-8); ^{13}C NMR (125.8 MHz, D_2O) δ 11.09 (CH_3-4), 25.66 (d, $J_{C,P}$ = 7, CH_2-6″), 60.26 (dd, $J_{C,P}$ = 163, 7, P−CH_2−OP), 61.90 (OCH_3), 64.64 (d, $J_{C,P}$ = 5, CH_2-5′), 65.38 (d, $J_{C,P}$ = 6, CH_2OP), 70.19 (CH_2-3″), 70.56 (CH-3′), 75.53 (CH-2′), 84.24 (d, $J_{C,P}$ = 8, CH-4′), 88.06 (CH-1′), 107.04 (C-7″a), 115.41 (C-3″a), 118.63 and 118.81 (C-5, C-6″), 139.88 (CH-8), 147.03 (C-4″, C-7″), 148.82 (C-4), 153.01 (CH-2), 155.63 (C-8), 163.49 (C-5″), 173.84 (C-1″); ^{31}P NMR (202.5 MHz, D_2O) δ 1.90 (dtt, J_{vic} = 39.6, $J_{P,H}$ = 9.0, 4.8, P-phosphate), 17.61 (m, J_{vic} = 39.6, $J_{P,H}$ = 8.5, 5.0, 3.3, P-phosphonate); HR FAB$^+$ calcd (M + H)$^+$ 706.090 342, found 706.093 022.

Enzyme Assays. Human type 1 and type 2 IMPDH was expressed and purified as previously described.[31,32] Inhibition assays were performed as previously described.[33] Briefly, assays were set up in duplicate using two different concentrations of IMPDH type 1 (87 and 155 nM) and type 2 (33 and 66 nM) and varying concentrations of inhibitor. IMPDH and inhibitors were added to 1 mL of reaction buffer (50 mM Tris, pH 8.0, 100 mM KCL, 1 mM DTT, 100 μM IMP, 100 μM NAD) at 25 °C, mixed gently while the production of NADH was monitored by following changes in absorbance at 340 nm on a Hitachi U-2000 spectrophotometer. Steady-state velocities were used to determine K_i(app) values by fitting the velocities vs inhibitor concentration to a simple binding model with Dynafit.[34]

Acknowledgment. This research was supported by USDOD ARMY Grant W81XWH-05-01-0216 and by the Center for Drug Design in the Academic Health Center of the University of Minnesota. We thank Drs. Takashi Tsuji and Kunisuke Izawa of Ajinomoto Co., Inc., Japan, for a gift of MPA.

Supporting Information Available: ^{1}H, ^{13}C, ^{31}P NMR spectra and HPLC profiles of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM060479R