*J. Med. Chem.* **1998,** *41,* 618−622

# Synthesis of a Methylenebis(phosphonate) Analogue of Mycophenolic Adenine Dinucleotide: A Glucuronidation-Resistant MAD Analogue of NAD

Krystyna Lesiak,[†] Kyoichi A. Watanabe,[†] Alokes Majumdar,[‡] James Powell,[‡] Michael Seidman,[‡] Kristen Vanderveen,[§] Barry M. Goldstein,[§] and Krzysztof W. Pankiewicz*,[†]

*Division of Medicinal Chemistry and Division of Biology, Codon Pharmaceuticals, Inc., 200 Perry Parkway, Gaithersburg, Maryland 20877, and University of Rochester Medical Center, Rochester, New York 14642*

*Received October 15, 1997*

Mycophenolic alcohol (MPAlc), obtained by reduction of the carboxylic group of mycophenolic acid (MPA), was coupled with 2′,3′-*O*-isopropylideneadenosine 5′-methylenebis(phosphonate) (**4**) in the presence of diisopropylcarbodiimide (DIC) to give $P^1$-(2′,3′-*O*-isopropylideneadenosin-5′-yl)-$P^2$-(mycophenolic alcohol-6′-yl)methylenebis(phosphonate) (**8**) in 32% yield. Deisopropylidenation of **8** with $CF_3COOH/H_2O$ afforded the methylenebis(phosphonate) analogue **3** of mycophenolic adenine dinucleotide (MAD). Compound **3**, $\beta$-methylene-MAD, was found to be a potent inhibitor of inosine monophosphate dehydrogenase (IMPDH) type II ($K_i = 0.3\ \mu M$) as well as an inhibitor of growth of K562 cells ($IC_{50} = 1.5\ \mu M$). In contrast to MPA and mycophenolic alcohol, $\beta$-methylene-MAD was not converted into the glucuronide when incubated with uridine 5′-diphosphoglucuronyltransferase.

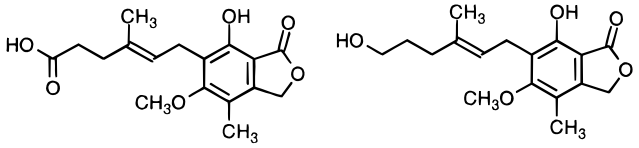
## Introduction

Inosine monophosphate dehydrogenase (IMPDH) catalyzes the NAD-dependent conversion of inosine 5′-monophosphate (IMP) to xanthine monophosphate, which is the rate-limiting step in guanine nucleotide biosynthesis.[1] There is an increased production of guanine nucleotides in rapidly proliferating cells,[2] while concentrations are reduced in differentiating and nonproliferating cells. Two forms of the enzyme are found in mammalian cells, each encoded by distinct cDNAs.[3] Type I is expressed constitutively,[3−5] while the levels of type II are markedly increased in tumor cells and activated lymphocytes. Conversely, when tumor cells are induced to differentiate, transcripts of type II decline to below those of type I. IMPDH has been recognized as a therapeutic target for many years, and there has been a considerable effort applied to the development of specific inhibitors.[1]

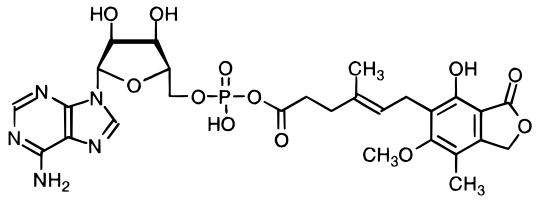
Mycophenolic acid (MPA; Chart 1) is the most potent and specific inhibitor of IMPDH.[6] MPA inhibits IMPDH with greater specificity against the type II isoform ($K_i = 6-10$ nM), which is the dominant form in cancer cells, than type I expressed in normal cells[5] ($K_i = 33-37$ nM). Although it would appear to be a promising candidate for cancer chemotherapy, it is inactive against tumors. This is because it is quickly converted (via its phenolic function) to the inactive $\beta$-glucuronide after administration. As much as 90% of the drug circulates in this form.[7] Unfortunately, derivatives of MPA with a protected or modified phenolic group are inactive.[8] The compound has been used with some success in the prodrug form, mycophenolate mofetil, as an immunosuppressant because it inhibits proliferation of B and T lymphocytes.[6]

The binding of MPA to the cofactor site on IMPDH resembles the binding of the nicotinamide mononucleotide (NMN) moiety of NAD, the natural cofactor. The carboxyl group is positioned at the space occupied by the phosphoryl group of NMN.[9] We were interested in measuring the activity of an MPA derivative which would more closely resemble the entire NAD molecule. Attachment of AMP to the carboxyl group of MPA would provide a mycophenolic adenine mixed anhydride (**1**; Chart 1), which should be a good mimic of NAD.

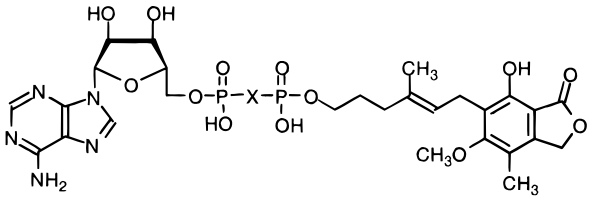
**Chart 1**



MPA MPAlc



**1** Mixed anhydride



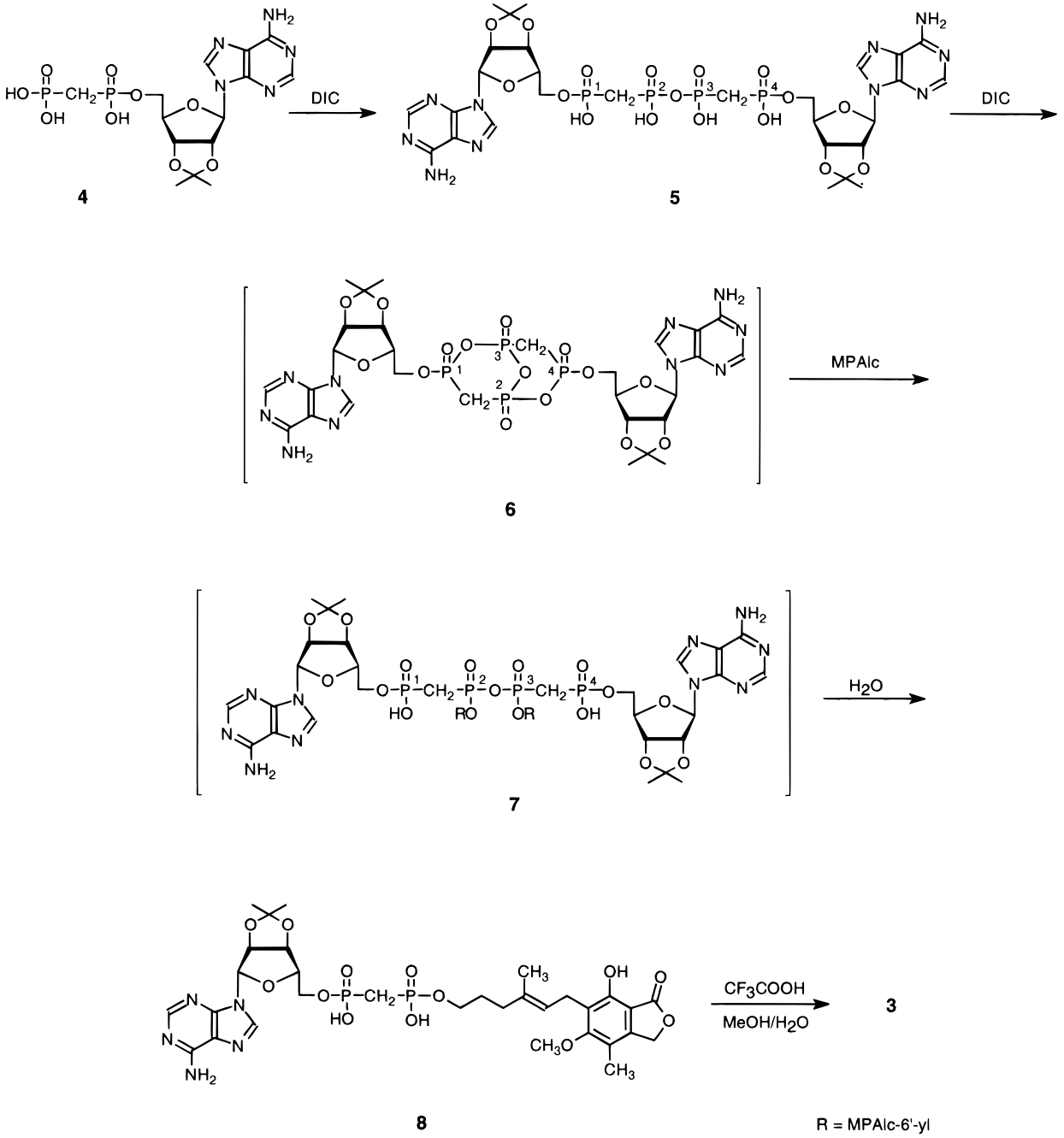
**2** Mycophenolic adenine dinucleotide (MAD, X=O)

**3** β-Methylene-MAD (X=$CH_2$)

[†] Division of Medicinal Chemistry.
[‡] Division of Biology.
[§] University of Rochester Medical Center.

S0022-2623(97)00705-X CCC: $15.00 
Published on Web 01/24/1998

**Scheme 1**




Compound **1** would be expected to have a much better binding capability than MPA alone due to additional binding at the adenosine subsite of IMPDH.[10] As a mixed anhydride, however, this compound would not be stable in water solution.

On the other hand, reduction of the carboxylic group of MPA into the corresponding mycophenolic alcohol (MPAlc)[11] and further coupling with ADP would give mycophenolic adenine dinucleotide (MAD, **2**, X = O). Although chemically stable, MAD as a pyrophosphate would not be expected to penetrate cell membranes efficiently and would be vulnerable to cleavage by phosphodiesterases. In contrast, a similar coupling of MPAlc (via its 6′-hydroxyl function) with adenosine 5′-methylenebis(phosphonate)[12] should produce a metabolically stable analogue of MAD (**3**, $\beta$-methylene-MAD, X = $CH_2$). The reduction of the carboxylate function of MPA to the alcohol was reported to decrease inhibition of IMPDH 50-fold as a result of lost hydrogen bonding between the carboxylic group of MPA and serine-276 of IMPDH.[9] Synthesis of the $\beta$-methylene-MAD offered the opportunity to ask if the addition of the adenosine pyrophosphate to the alcohol function of MPAlc would influence the activity against IMPDH. We were also interested in asking if the new compound would be sensitive to glucuronidation as are MPA and MPAlc.

## Chemical Synthesis

Recently, we developed a new and efficient method for preparation of methylenebis(phosphonate) analogues of $P^1$,$P^2$-disubstituted pyrophosphates of biological interest.[13] We found that treatment of 2′,3′-*O*-isopropylidene nucleoside 5′-methylenebis(phosphonate)s in general and the adenosine derivative **4** (Scheme 1) in

particular with diisopropylcarbodiimide (DIC) led to the formation of $P^1$,$P^4$-diadenosin-5′-yl tetraphosphonate **5**, which upon further dehydration with DIC was converted into an active intermediate (**6**) having the structure of bicyclic trisanhydride.[13] In reactions with nucleosides, carbohydrates, and alcohols, the bicyclic intermediate **6** gave the corresponding $P^2$,$P^3$-disubstituted $P^1$,$P^4$-diadenosin-5′-yl tetraphosphonates **7** which upon hydrolysis and deprotection afforded the desired $P^1$,$P^2$-disubstituted methylenebis(phosphonate)s.[13] Herein we report that the reaction of **6** with MPAlc afforded after hydrolysis with water the desired $\beta$-methylene-MAD derivative **8** in 32% yield. Deisopropylidenation of **8** with a mixture of $CF_3COOH-H_2O$ (8:2, v/v) gave the final product **3** in 78% yield.

The rather low yield of the coupling reaction was most probably due to the lack of protection of the 7-phenol group of MPAlc which also may react with the intermediate **6**. If both the 6′-hydroxyl group and the 7-phenol function react with **6**, then formation of a variety of polymers should be expected. Indeed, the HPLC profile of the products of the coupling reaction consists of a peak of **8** ($t_R$ = 52 min) as well as of a number of poorly separated peaks with longer retention times. The assignment of the structure of **8** was confirmed by $^1$H and $^{31}$P NMR (see Experimental Section). The resonance signal of the 6′$CH_2$ of the MPAlc moiety in the proton NMR of **8** at 3.79 ppm was recorded as a quartet ($J_{H-P}$ = 6.3 Hz, $J_{H-H}$ = 6.3 Hz) showing the coupling with the phosphorus atom. Heteronuclear shift correlation experiments also confirmed the phosphorus−6′$CH_2$ coupling.

## Biochemical Properties

**Enzyme Inhibition.** The new MAD analogue was assayed for inhibitory activity against human IMPDH type II. $IC_{50}$ values were measured in the presence of 100 $\mu$M NAD, 50 $\mu$M IMP, 100 nM Tris-HCl, 100 mM KCl, 3 mM EDTA, and 25 nM enzyme at pH 8.0. The formation of NADH was monitored as described previously.[14] We found that the MAD analogue **3** was a potent inhibitor of IMPDH type II with $K_i$ = 0.3 $\mu$M.

**Stability of MAD.** The premise for the synthesis of $\beta$-methylene-MAD was that it would be resistant to phosphodiesterases in serum and cells. We tested this by incubating the compound with purified phosphodiesterase and also in the calf serum used in our experiments with cultured cells. We found that the compound was stable after incubation for 16 h at 37 °C in either condition. Neither formation of MPAlc nor formation of adenosine was detected. These data indicated that the compound was stable as expected.

**Resistance to Glucuronidation.** We incubated $\beta$-methylene-MAD, MPA, and MPAlc with uridine 5′-diphosphoglucuronyltransferase (UDPGT) and uridine 5′-diphosphoglucuronic acid at 30 °C for 2 h. We found extensive conversion of both MPA and MPAlc to the 7-*O*-glucuronide derivative (see Supporting Information). This interpretation was confirmed by enzymatic hydrolysis of the glucuronide with $\beta$-glucuronidase which afforded the starting MPA and MPAlc. In contrast no formation of the corresponding glucuronide of $\beta$-methylene-MAD was observed in similar conditions.

**Activity of $\beta$-Methylene-MAD as an Antiproliferative and Differentiation Inducer.** The activity of the compound as an antiproliferative and inducer of differentiation in human erythroleukemia K562 cells was also measured. We found that growth was inhibited with an $IC_{50}$ = 1.5 $\mu$M. The extent of cell differentiation was assessed by determining the fraction of cells expressing hemoglobin (benzidine-positive cells) following a 5-day incubation with $\beta$-methylene-MAD. Concentrations of 5 and 10 $\mu$M **3** converted 84.3% and 91.0% of cells, respectively, into benzidine-positive cells. MPAlc ($IC_{50}$ = 1.5 $\mu$M) converted 37.8% and 58.4% of cells at 5 and 10 $\mu$M, respectively. These data indicated that MAD had the same antiproliferative activity as the parent MPAlc and was somewhat more active as a differentiating agent. MPA, in comparison, had an $IC_{50}$ = 0.08 $\mu$M, with 85% benzidine-positive cells at 1 $\mu$M.

## Discussion

These results demonstrate that the $\beta$-methylene-MAD construct is an effective inhibitor of IMPDH. Since the compound was completely resistant to phosphodiesterase cleavage, the biological activity of the compound indicates that it was taken up into K562 cells, as expected for a pyrophosphate analogue with $\beta$-methylene substitution.

The approximately 19-fold lower antiproliferative activity of **3** and MPAlc versus MPA may be due to the absence of a carboxyl or carboxyl analogue precisely positioned as in MPA. Derivation of MPAlc produced a side chain, linking MPA with the adenosine diphosphate moiety, that is two atoms longer compared to that in the ideal mixed anhydride **1**. Synthesis of a derivative with a shortened linker will be of considerable interest.

The most important observation is that $\beta$-methylene-MAD was resistant to glucuronidation in vitro. Although, it is known that glucuronidation efficacy by UDPGT may differ depending on incubation conditions and that significant species differences exist in substrate specificity and reaction rate in vivo, our results indicated that, in contrast to MPA, our $\beta$-methylene-MAD would not be prone to glucuronidation in vivo.

There have been many efforts to eliminate inactivation of MPA by glucuronidation by modification of the phenolic group. These attempts have failed to produce an active compound. Recently, derivatives of the side chain were described. A methoxymethyl group on the side chain α to the carboxyl group had the effect of reducing glucuronidation by 50% without any loss of antiproliferative activity. This is an encouraging result although the reduction in glucuronidation activity is probably not sufficient to be of pharmacologic value. This suggestion that appropriate side chain modifications could yield compounds that would resist glucuronidation with retention of activity is consistent with our own data on the $\beta$-methylene-MAD derivative. Since MAD analogue **3** is not a subject for metabolic deactivation via glucuronidation, it, and other analogues like it, may exibit potent anticancer activity in vivo. Further studies on synthesis and biological activity of other MAD derivatives are in progress.

## Experimental Section

**General Methods. HPLC Analysis**. Preparative HPLC was performed on a Dynamax-300A C18-83-243-C column with a flow rate of 20 mL/min of 0.1 M triethylammonium bicarbon-

ate (TEAB) followed by a linear gradient of 0.1 M TEAB−aqueous MeCN (70%). Analytical HPLC was performed on a Waters 2690 separations module equipped with a Waters 996 diode array detector (Waters Chromatography, Milford, MA). Samples were chromatographed on a 4.6-mm × 250-mm i.d. Vydac Protein $C_4$ column. Elution was achieved using a linear gradient of 1−20% B in 20 min: buffer A, 20 mM sodium phosphate, pH 5; buffer B, acetonitrile; flow rate, 1 mL $min^{-1}$. Detection was at 254 nm.

**NMR.** Nuclear magnetic resonance spectra were recorded on a JEOL Eclipse EX-270 instrument. Chemical shifts are reported in ppm ($\delta$), and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), bs (broad singlet), and dd (double doublet). Values given for coupling constants are first-order.

***P*$^1$-(2′,3′-*O*-Isopropylideneadenosin-5′-yl)-*P*$^2$-(mycophenolic alcohol-6′-yl)methylenebis(phosphonate) (8).** To a solution of **4** (120 mg, 0.17 mmol) in pyridine (1 mL) was added DIC (156 mL, 1 mmol), and the mixture was left (approximately for 6 h) at room temperature until intermediate **6** was formed (multisignal resonances in $^{31}$P NMR).[13] MPAlc[11] (61 mg, 0.2 mmol) was then added, and the reaction mixture was heated at 55−60 °C for 14 h and at 70 °C for 6 h. At that time $^{31}$P NMR of the reaction mixture showed two broad signals at 8 and 25 ppm, characteristic for the presence of intermediate **7**. Then a mixture of water (200 $\mu$L) and $Et_3N$ (100 $\mu$L) was added, and the reaction mixture was kept at 75 °C for 30 h. HPLC purification afforded compound **8** (52 mg, 32.0%) as the triethylammonium salt. $^1$H NMR ($D_2O$) $\delta$: 1.24 (t, 18H, $Et_3N$), 1.37 and 1.61 (s, 3H each, isopropylidene), 1.63 [m, 2H, $CH_2$5′ (MPAlc)], 1.67 (s, 3H, $CH_3$), 1.93 (s, 3H, $CH_3$), 1.95 [m, 2H, $CH_2$4′ (MPAlc)], 2.07 (t, 2H, P-$CH_2$-P, $J$ = 19.9 Hz), 3.09 [d, 1H, $CH_2$1′ (MPAlc)], 3.16 (q, 12H, $Et_3N$), 3.60 (s, 3H, $OCH_3$), 3.79 [q, 2H, 6′$CH_2$ (MPAlc), $J_{H-P}$ = 6.3 Hz, $J_{H-H}$ = 6.3 Hz], 4.05 [m, 2H, H5′,5″ (Ado)], 4.52 [m, 1H, H4′ (Ado)], 4.94 [dd, 1H, CH1′ (MPAlc), $J$ = 6.0, 6.5 Hz], 5.13 (s, 2H, $CH_2$3 (MPAlc)], 5.15 [dd, 1H, H3′ (Ado), $J_{3',4'}$ = 1.7 Hz, $J_{2',3'}$ = 6.0 Hz], 5.20 [dd, 1H, H2′ (Ado), $J_{1'-2'}$ = 3.0 Hz], 6.00 [d, 1H, H1′ (Ado)], 8.03 and 8.29 [2 1H singlets, H2, H8 (Ado)]. $^{31}$P NMR ($D_2O$) $\delta$: 17.70 and 18.02 (AB system, $J$ = 11.4 Hz).

***β*-Methylene-MAD (3).** Compound **8** (24 mg, 0.025 mmol, as the triethylammonium salt) was dissolved in a mixture of methanol (1 mL) and water (1 mL) containing $CF_3COOH$ (0.5 mL). The mixture was kept at room temperature for 1 h and heated at 50 °C for 30 min. Methanol was removed in vacuo; the mixture was diluted with water (2 mL), neutralized with concentrated ammonia, and concentrated. The residue was chromatographed on a HPLC column to give **3** as the triethylammonium salt. This compound was converted into the sodium salt of **3** (14 mg, 78%) by passing through a column of Dowex 50WX8/$Na^+$ form. $^1$H NMR ($D_2O$) $\delta$: 1.64 [m, 2H, $CH_2$5′ (MPAlc)], 1.73 (s, 3H, $CH_3$), 2.01 [m, 2H, $CH_2$4′ (MPAlc)], 2.07 (s, 3H, $CH_3$), 2.18 (t, 2H, P-$CH_2$-P, $J$ = 20.0 Hz), 3.21 [d, 2H, $CH_2$1′ (MPAlc), $J$ = 6.9 Hz], 3.68 (s, 3H, $OCH_3$), 3.85 [q, 2H, $CH_2$6′ (MPAlc), $J$ = 6.3 Hz], 4.19 [m, 2H, H5′,5″ (Ado)], 4.33 [m, 1H, H4′ (Ado)], 4.51 [dd, 1H, H3′ (Ado), $J_{3',4'}$ = 4.2 Hz, $J_{2',3'}$ = 5.1 Hz], 4.67 [dd, 1H, H2′ (Ado), $J_{1',2'}$ = 5.2 Hz], 4.93 [m, 1H, CH2′ (MPAlc)], 5.26 [s, 2H, $CH_2$3 (MPAlc)], 5.99 [d, 1H, H1′ (Ado)], 8.13 and 8.49 (2 1H singlets, H2, H8). $^{31}$P NMR ($D_2O$) $\delta$: 17.65 and 18.35 (AB system, $J$ = 11.5 Hz). HRMS (FAB, $M^{2-}$ + H): calcd for $C_{28}H_{36}N_5O_{13}P_2$ 712.1752, found: 712.1785.

**Stability of *β*-Methylene-MAD to Pyrophosphatase and Serum.** $\beta$-Methylene-MAD (0.05 mg) was dissolved in a total volume of 250 $\mu$L containing 10 mM Tris (pH 8), 10 mM $MgCl_2$, and 0.1 unit of pyrophosphatase from *Crotalus durissus* (Sigma Chemicals, St. Louis, MO). A second sample of 0.05 mg of $\beta$-methylene-MAD was dissolved in 250 $\mu$L of 10% fetal calf serum (Gibco, Gaithersburg, MD). Control consisted of 0.05 mg of $\beta$-methylene-MAD dissolved in 250 $\mu$L of $H_2O$. Each sample was incubated at 37 °C for 16 h. The samples were then transferred to ULTRAFREE-MC centrifugal filters containing a 5000 MW cutoff regenerated cellulose membrane (Millipore Corp., Bedford, MA) and were centrifuged at 10 000 rpm for 45 min. The filtrates were transferred to autosampler vials and analyzed by HPLC as described above.

**Studies of Glucuronidation of MPA, MPAlc, and *β*-Methylene-MAD.** A solution of MPA (1 mmol), uridine 5′-diphosphoglucuronyltransferase (UDP glucuronate $\beta$-*O*-glucuronosyltransferase, EC 2.4.1.17; Sigma, type III, lyophilized crude microsomal preparation from bovine liver, 0.01 U), uridine 5′-diphosphoglucuronic acid (UDPGA; Sigma, trisodium salt, 10 mmol), in HEPES (50 mmol) containing $MgCl_2$ (5 mmol) and CHAPS (0.5 mg/mL), pH 7.4 (100 $\mu$L total volume), was incubated at 30 °C for 2 h. The reaction was quenched by addition of SDS (1% final concentration) and heating at 95 °C for 5 min. Proteins and other high-molecular-weight materials were removed by filtration through 5000 MW cutoff membranes (ULTRAFREE-MC centrifugal filters). The filtrate was analyzed by HPLC showing (see Supporting Information) 69% conversion of MPA into 7-*O*-glucuronide. The UV spectrum of MPA showed a maximum at 305 nm (which shifted with increasing pH). The maximum was changed to 294 nm (not dependent on pH) after formation of the 7-*O*-glucuronide.

Under similar conditions, MPAlc was completely glucuronidated (see Supporting Information). The similar reaction of compound **3** did not produce any glucuronide (see Supporting Information).

**Enzymatic Hydrolysis of 7-*O*-Glucuronide of MPA and 7-*O*-Glucuronide of MPAlc.** A sample (25 $\mu$L of the filtrate described above) containing 69% of 7-*O*-glucuronide of MPA and 31% of MPA was treated with $\beta$-glucuronidase (EC 3.2.1.31, glucurase, 25 U; Sigma) in 0.1 M acetate buffer, pH 5 (3 h, 37 °C, 50 $\mu$L total volume). The mixture was diluted with 50 mL of water, filtered through ULTRAFREE-MC membranes, and analyzed by HPLC as described above. The glucuronide was completely hydrolyzed to MPA. Using the same conditions, it was determined that the enzymatically prepared 7-*O*-glucuronide of MPAlc was also completely hydrolyzed to MPAlc. As expected, no reaction was observed when MPA or MPAlc was incubated with glucurase.

**Cells, Growth, and Differentiation Assay.** Human erythroleukemia K562 cells were grown and assayed for proliferation and differentiation exactly as described in our previous publication.[16]

**Supporting Information Available:** HPLC profiles of the reaction mixtures of glucuronidation of MPA, MPAlc, and $\beta$-methylene-MAD (2 pages). Ordering information is given on any current masthead page.

## References

(1) NAD Analogues. Part 13. For part 12, see: Pankiewicz, K. W. NAD Analogues Designed as Potential Anticancer Agents. Quest for Selective Inhibition of Inosine Monophosphate Dehydrogenase (IMPDH). *Pharmacol. Ther.* **1997**, *75*, 1−12.

(2) (a) Jackson, R. C.; Weber, G.; Morris, H. P. IMP Dehydrogenase: An Enzyme Linked with Proliferation and malignancy. *Nature* **1975**, *256*, 331−333. (b) Weber, G.; Prajda, N.; Jackson, R. C. *Adv. Enzyme Regul.* **1986**, *14*, 3.

(3) Natsumeda, Y.; Ohno, S.; Kawasaki, H.; Konno, Y.; Weber, G.; Suzuki, K. Two Distinct cDNAs for Human IMP Dehydrogenase. *J. Biol. Chem.* **1990**, *265*, 5292−5295.

(4) Sherley, J. L. Guanine Nucleotide Biosynthesis Is Regulated by the Cellular p53 Concentration. *J. Biol. Chem.* **1991**, *266*, 24815−24828.

(5) Carr, S. F.; Papp, E.; Wu, J. C.; Natsumeda, Y. Characterization of Human Type I and Type II IMP Dehydrogenase. *J. Biol. Chem.* **1993**, *268*, 27286−27290.

(6) Wu, J. C. Mycophenolate Mofetil: Molecular Mechanism of Action. In *Perspectives in Drug Discovery and Design*; Wyvratt, M. J., Sigal, N. H., Eds.; ESCOM Science Publishers: Leiden, 1994; Vol. 2, pp 185−204.

(7) Franklin, T. J.; Jacobs, V.; Jones, G.; Ple, P.; Bruneau, P. Glucuronidation Associated with Intrinsic Resistance to Mycophenolic Acid in Human Colorectal Carcinoma Cells. *Cancer Res.* **1996**, *56*, 984−987.

(8) Anderson, W. K.; Lee, J.; Swann, R. T.; Boehm, T. L. In *Advances in New Drug Development*; Kim, B.-K., Lee, E. B., Kim, C.-H., Han, Y. N., Eds.; The Pharmaceutical Society of Korea: Seoul, 1991; pp 8−17.

(9) Sintchak, M. D.; Fleming, M. A.; Futer, O.; Raybuck, S. A.; Chambers, S. P.; Caron, P. R.; Murcko, M. A.; Wilson, K. P. Structure and Mechanism of Inosine Monophosphate Dehydrogenase in Complex with the Immunosuppressant Mycophenolic Acid. *Cell* **1996**, *85*, 921−930.
(10) Hedstrom, L.; Wang, C. C. Mycophenolic Acid and Thiazole Adenine Dinucleotide Inhibition of *Tritrichomonas foetus* Inosine 5′-Monophosphate Dehydrogenase: Implications on Enzyme Mechanism. *Biochemistry* **1990**, *29*, 849−854.
(11) Jones, D. F.; Mills, S. D. Preparation and Antitumor Properties of Analogues and Derivatives of Mycophenolic Acid. *J. Med. Chem.* **1971**, *14*, 305−311.
(12) Davisson, V. J.; Davis, D. R.; Dixit, V. M.; Poulter, C. D. Synthesis of Nucleotide 5′-Diphosphates from 5′-O-Tosyl Nucleosides. *J. Org. Chem.* **1987**, *52*, 1794−1801.
(13) Pankiewicz, K. W.; Lesiak, K.; Watanabe, K. A. Efficient Synthesis of Methylenebis(phosphonate) Analogues of $P^1,P^2$-Disubstituted Pyrophosphates of Biological Interest. A Novel Plausible Mechanism. *J. Am. Chem. Soc.* **1997**, *119*, 3691−3695.
(14) Zatorski, A.; Watanabe, K. A.; Carr, S. F.; Goldstein, B. M.; Pankiewicz, K. W. Chemical Synthesis of Benzamide Adenine Dinucleotide: Inhibition of Inosine Monophosphate Dehydrogenase (Types I and II). *J. Med. Chem.* **1996**, *39*, 2422−2426.
(15) Lesiak, K.; Watanabe, K. A.; Majumdar, A.; Seidman, M.; Vanderveen, K.; Goldstein, B. M.; Pankiewicz, K. W. Synthesis of Nonhydrolyzable Analogues of Thiazole-4-carboxamide and Benzamide Adenine Dinucleotide Containing Fluorine Atom at the C2′ of Adenine Nucleoside: Induction of K562 Differentiation and Inosine Monophosphate Dehydrogenase Inhibitory Activity. *J. Med. Chem.* **1997**, *40*, 2533−2538.
(16) Pankiewicz, K. W.; Lesiak, K.; Zatorski, A.; Goldstein, B. M.; Carr, S. F.; Sochacki, M.; Majumdar, A.; Seidman, M.; Watanabe, K. A. The Practical Synthesis of a Methylenebis(phosphonate) Analogue of Benzamide Adenine Dinucleotide: Inhibition of Human Inosine Monophosphate Dehydrogenase (Type I and II). *J. Med. Chem.* **1997**, *40*, 1287−1291.

JM970705K