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Cofactor-type inhibitors of inosine monophosphate dehydrogenase via modular approach: Targeting the pyrophosphate binding sub-domain

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ABSTRACT

Cofactor-type inhibitors of inosine monophosphate dehydrogenase (IMPDH) that target the nicotinamide adenine dinucleotide (NAD) binding domain of the enzyme are modular in nature. They interact with the three sub-sites of the cofactor binding domain; the nicotinamide monophosphate (NMN) binding sub-site (N sub-site), the adenosine monophosphate (AMP) binding sub-site (A sub-site), and the pyrophosphate binding sub-site (P sub-site or P-groove). Mycophenolic acid (MPA) shows high affinity to the N sub-site of human IMPDH mimicking NMN binding. We found that the attachment of adenosine to the MPA through variety of linkers afforded numerous mycophenolic adenine dinucleotide (MAD) analogues that inhibit the two isoforms of the human enzyme in low nanomolar to low micromolar range. An analogue **4**, in which 2-ethyladenosine is attached to the mycophenolic alcohol moiety through the difluoromethylenebis(phosphonate) linker, was found to be a potent inhibitor of *h*IMPDH1 (K_i = 5 nM), and one of the most potent, sub-micromolar inhibitor of leukemia K562 cells proliferation (IC_{50} = 0.45 μM). Compound **4** was as potent as Gleevec (IC_{50} = 0.56 μM) heralded as a 'magic bullet' against chronic myelogenous leukemia (CML). MAD analogues **7** and **8** containing an extended ethylenebis(phosphonate) linkage showed low nanomolar inhibition of IMPDH and low micromolar inhibition of K562 cells proliferation. Some novel MAD analogues described herein containing linkers of different length and geometry were found to inhibit IMPDH with K_i's lower than 100 nM. Thus, such linkers can be used for connection of other molecular fragments with high affinity to the N- and A-sub-site of IMPDH.

1. Introduction

IMPDH, a NAD dependent enzyme, has emerged in recent years as a major therapeutic target.[1,2] It controls de novo synthesis of purine nucleotides catalyzing oxidation of inosine 5′-monophosphate (IMP) to xanthosine 5′-monophosphate (XMP) which is further converted into guanosine 5′-monophosphate (GMP) by GMP synthase. Two isoforms of human IMPDH are known,[3] both contain 514 amino acids and share 84% of amino acid sequences.[4,5] The *h*IMPDH1 was identified as an *anti*-angiogenic drug target,[6] and mycophenolic acid (MPA), a nanomolar inhibitor of *h*IMPDH, was found to block tumor-induced angiogenesis in vivo. The *h*IMPDH2 is selectively up-regulated in neoplastic cells and activated lymphocytes and emerges as the dominant form.[7] (e.g., 15–42 fold elevated in human myelogenous leukemia compared to normal leukocytes.)[8] It is therefore believed that IMPDH is a *key* enzyme in neoplasia and is one of the most sensitive targets for cancer chemotherapy.

NAD-based inhibitors of IMPDH that target the cofactor binding site of the enzyme can interact with the three sub-sites of the cofactor binding domain (Fig. 1); the nicotinamide monophosphate (NMN) binding sub-site (N sub-site), the adenosine monophosphate (AMP) binding sub-site (A sub-site), and the pyrophosphate binding sub-site (P sub-site or P-groove). Thus, such inhibitors are modular in nature, and usually consist of two fragments interacting with the N and A binding sub-sites connected via an appropriate linker (accommodated in the P-groove).

Abbreviations: AMP, adenosine 5′-monophosphate; IMP, inosine 5′-monophosphate; IMPDH, inosine monophosphate dehydrogenase; NAD, nicotinamide adenine dinucleotide; NMN, nicotinamide mononucleotide; TR, tiazofurin; TAD, thiazole-4-carboxamide adenine dinucleotide; MPA, mycophenolic acid; MAD, mycophenolic adenine dinucleotide; CML, chronic myelogenous leukemia; AICAR, 5-aminoimidazole-4-carboxamide riboside; PMB, *p*-methoxybenzyl; DIC, *N,N*-disopropylcarbodiimide; Mycophenolic alcohol, 7-(hydroxy)-6-(2-ethyleneyl)-5-methoxy-4-methyl-phthalan-1-one with protected or unprotected 7 hydroxyl group.

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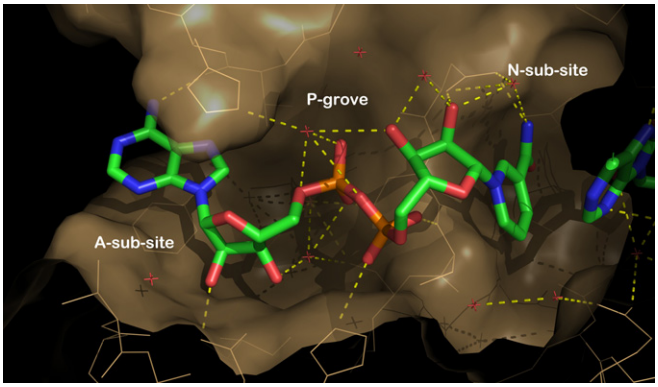


Figure 1. The modular nature of the cofactor binding domain of NAD-dependent IMPDH (PDB entry 1NFb).

Four potent and selective inhibitors of IMPDH are used in the clinic. Two of them, ribavirin and mizoribine, are phosphorylated in the cell to the corresponding 5′-monophosphates that interact with the substrate (IMP) domain of IMPDH. Ribavirin is a broad spectrum antiviral drug currently used for treatment of hepatitis C infections and mizoribine found an application in Japan as an immunosuppressant. The third, tiazofurin (TR, Fig. 2), a C-nucleoside prodrug used for treatment of chronic myelogenous leukemia (CML) in blast crisis, is metabolically converted into thiazole-4-carboxamide adenine dinucleotide (TAD), which as a NAD analogue interacts with all three N, A, and P sub-sites of NAD-dependent IMPDH. TAD is a selective and potent inhibitor of IMPDH1 and IMPDH2 (K_i = 110 nM, Table 1).

Finally, mycophenolic acid (MPA, Fig. 3) used in the clinic as an immunosuppressant, binds to the N sub-site of IMPDH and is one of the most potent inhibitors of *h*IMPDH2 (K_i = 7 nM).

In the last decade we and others have shown clearly that the cofactor binding domain, although conserved in variety NAD-dependent enzymes, is diversified enough to be a valuable target for therapeutic intervention with selective cofactor-type inhibitors.[9] For example, we used MPA and its fragments (that bind exclusively at the N sub-site) for construction of mycophenolic (see abbreviations) adenine dinucleotides (MAD) analogues (such as C2-MAD, Table 1) by attachment of adenosine to the mycophenolic alcohol moiety through methylenebis(phosphonate) linker (Fig. 3).[10] The crystal structure of the C2-MAD/*h*IMPDH2 complex showed that indeed the MPA moiety was bound at the N sub-site, and adenosine was located at the A sub-site mimicking closely interactions of NAD and TAD with the protein.[11] In this article we focus on IMPDH inhibitors with modified pyrophosphate moieties and their interactions with the P-groove of the enzyme.

2. Design of inhibitors and their biological activity

We reported earlier that the analogue of TAD **1** (Table 1) containing a 2-ethyl group at the adenine ring is a low nM inhibitor of

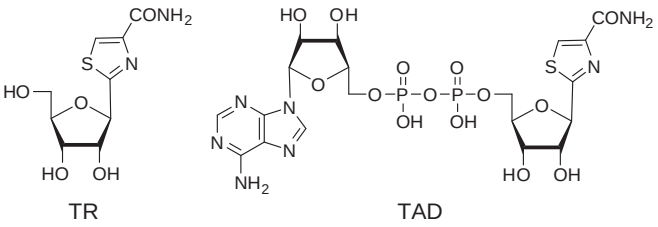


Figure 2. Tiazofurin (TR), thiazole-4-carboxamide adnine dinucleotide (TAD).

Table 1
Inhibition of IMPDH type I and type II, as well as inhibition of K562 cells proliferation by TAD and MAD analogues

Inhibitors	IMPDH type I K_i (nM)	IMPDH type II K_i (nM)	K562 cells proliferation IC_{50} (μM)[a]
TAD	110	110	3.7[b]
MPA	33	7	7.7
C2-MAD	330	250	5.7
1	1	14	ND
2	13 ± 1.9[c]	30 ± 2.7	4.7
3	16	38	1.0
4	5 ± 1.1	24 ± 2.1	0.45
5	87	60	118.4
6	20	37	71.3
7	95 ± 11.7	84 ± 4.3	10.5
8	99 ± 4.9	73 ± 3.4	4.0
9	5550 ± 350	3770 ± 240	>100
10	1100 ± 80	770 ± 42	>100
11	349	167	>100
12	255 ± 7.8	83 ± 2.0	>100
13	23,600	18,800	>100
14	14,200	6600	>100
15	94 ± 5.4	39 ± 3.7	>100
16	7900 ± 600	1900 ± 330	>100
17	70	44	>100
18	77	34	>100

[a] The values of IC_{50} for inhibition of K562 cells proliferation are averages from three experiments.
[b] This IC_{50} value is based on degradation of TAD which releases TR that enters cells to be metabolized back to TAD.
[c] Standard deviations are reported for new compounds only.

*h*IMPDH1 (K_i = 1.0 nM) and *h*IMPDH2 (K_i = 14 nM).[12] However, as a pyrophosphate analogue, compound **1** is metabolically unstable and cannot penetrate cells membrane. Thus, we report herein that the replacement of the natural pyrophosphate (P-O-P) moiety of **1** with the isosteric and isopolar difluoromethylenebis(phosphonate) (P-CF_2-P) group afforded TAD analogue **2** (Fig. 4). It still shows (Table 1) low nM inhibition of *h*IMPDH1 (K_i = 13 nM) and *h*IMPDH2 (K_i = 30 nM) and, as expected, inhibits proliferation of leukemia K562 cells (IC_{50} = 4.7 μM). Earlier, we also prepared the mycophenolic alcohol 2-ethyladenosine dinucleotide analogue **3** containing a methylenebis(phosphonate) group as the replacement for the pyrophosphate linkage (Fig. 4).[12] Compound **3** showed 20-fold more potent inhibitory activity (K_i = 16 nM) against *h*IMPDH1 than the parent C2-MAD (K_i = 330 nM). We have now converted **3** into its difluoromethylenebis(phosphonate) analogue **4**. As expected, we found an improvement in enzyme inhibitory activity (K_i = 5 nM, *h*IMPDH1) and consequently a potent inhibition of K562 cells growth (IC_{50} = 0.45 μM). In fact, analogue **4** is the most potent anti-proliferating agent among IMPDH inhibitors (Table 1); it is 17-fold more potent than MPA showing the inhibitory activity similar to that of Gleevec (imatinib) (IC_{50} = 0.56 μM), the *Bcr-abl* thyrosine kinase inhibitor, used in the clinic for treatment of CML.[13]

Thus, indeed linking potent N sub-site binders, such as TR or MPA with high affinity A sub-site binders (such as 2-ethyladenosine) through the bis(phosphonate) (P-CF_2-P or P-CH_2-P) moiety afforded potent inhibitors of IMPDH.

However, as we found earlier, linkers that are not as close to the natural architecture of the pyrophosphate group or its methylenebis(phosphonate) P-C-P analogue are also of interest. We reported the synthesis of phosphonophosphate analogues of C2-MAD (**5** and **6**, Fig. 5), in which an oxygen atom was inserted on either side of the methylene group of C2-MAD.[14] Both analogues **5** and **6** showed more potent inhibition of *h*IMPDH1 (K_i = 20 nM and K_i = 87 nM, respectively) than the parent methylenebis(phosphonate) C2-MAD (K_i = 330 nM). A brief examination of the P-groove region, as shown in Figure 1, indicates that the P sub-site is large and interactions of the enzyme with the phosphate groups of the cofactor

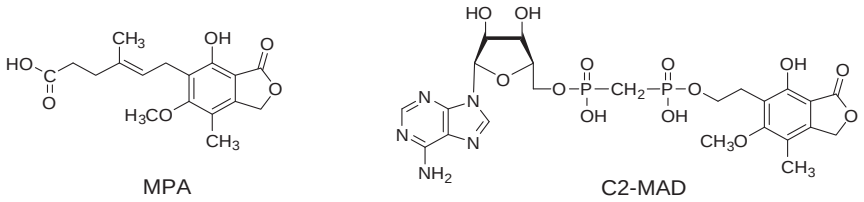


Figure 3. Mycophenolic acid (MPA) and mycophenolic adenine dinucleotide derivative (C2-MAD).

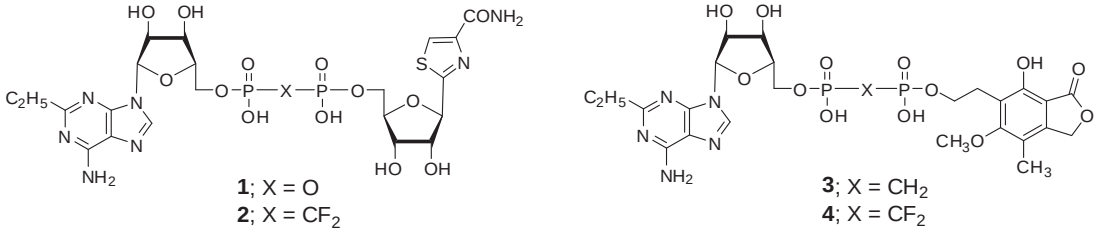


Figure 4. The 2-ethyl substituted analogues of TAD and MAD.

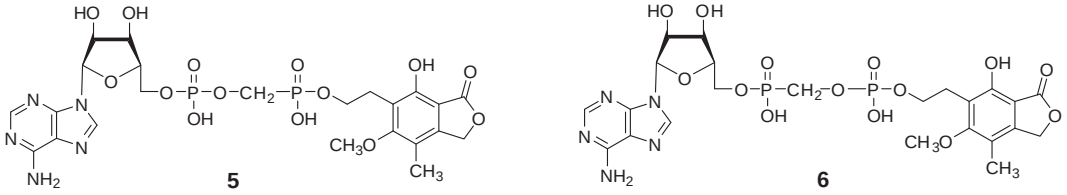


Figure 5. Phosphonophosphate analogues of MAD.

analogue are weak, maintained mainly by water molecules (represented by red stars). Thus, we became interested in further examination of this region.

Since compounds **5** and **6** were poorly active in vitro, likely due to their mixed phospho-phosphonate character, we prepared herein ethylenebis(phosphonate) analogue **7** and its 2-ethyl substituted derivative **8** (Fig. 6) that are strictly phosphonate derivatives that consequently should be able to penetrate cells membrane well.

Compound **7** inhibited *h*IMPDH1 (K_i = 95 nM) and *h*IMPDH2 (K_i = 84 nM) as potently as **5**, and as expected showed 10-fold more potent anti-proliferative activity against K562 cells (IC_{50} = 10.5 μM). The ethyl analogue **8** showed similar nanomolar inhibitory activity against IMPDH and exhibited even more potent anti-proliferative activity (IC_{50} = 4.0 μM). The low nanomolar inhibitory activity of compounds **7** and **8** against IMPDH indicates high affinity of both mycophenolic alcohol and adenosine fragments to their corresponding N and A sub-sites. Indeed, we found that mycophenolic alcohol ethylenebis(phosphonate) **9** (without the adenosine moiety) showed 50-fold less potent activity against IMPDH than that of **7** (Table 1). The replacement of adenosine of **7** by mycophenolic alcohol moiety afforded 10-fold less potent inhibitor **10** (Fig. 7). Apparently aromatic mycophenolic moiety of **10** shows some affinity for the adenine sub-site or interacts favorably with other parts of the cofactor binding domain.

Recently, we reported the synthesis of bis(sulfonamide) isosters of our bis(phosphonate) MAD analogues.[15] We found that the replacement of the two phosphorus atoms of C2-MAD with geometrically similar (tetrahedral) sulfur atoms afforded sulfonamide **11** with a very similar nanomolar inhibitory activity against IMPDH to that of the parent bis(phosphonate). Now we examined the importance of the preservation of the geometry of the pyrophosphate linker by preparation of the phosphonoacetamide derivative of C2-MAD **12** (Fig. 8) in which one of the two phosphorus atoms was replaced by non-isosteric (planar) carboxyamido group. We found that compound **12** (K_i = 83 nM) was two-fold more potent inhibitor of *h*IMPDH2 than the corresponding bis(sulfonamide) analogue **11** (K_i = 167 nM).

Unexpectedly, the bis(sulfonamide) analogue of TAD **13** as well as their phosphonoacetamide derivative **14** (Fig. 9) showed significantly decreased inhibition of IMPDH as compared to the corresponding MAD analogues (Table 1). Nevertheless, preservation of the tetrahedral geometry was not important in this case either,

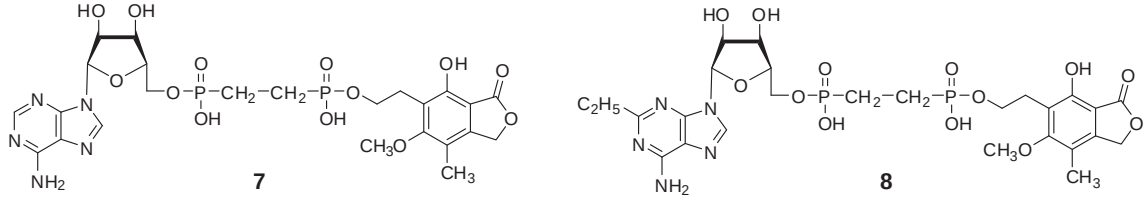


Figure 6. Ethylenebis(phosphonate) analogues of MAD **7** and **8**.

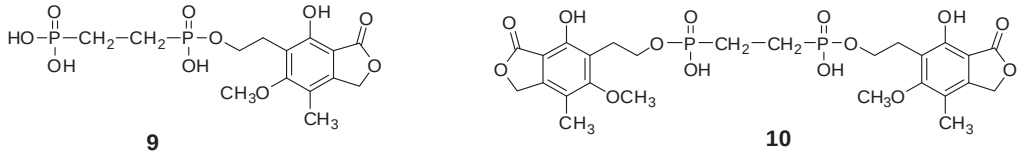


Figure 7. Mycophenolic alcohol etylenebis(phosphonate) **9** and its symmetrical analogue **10**.

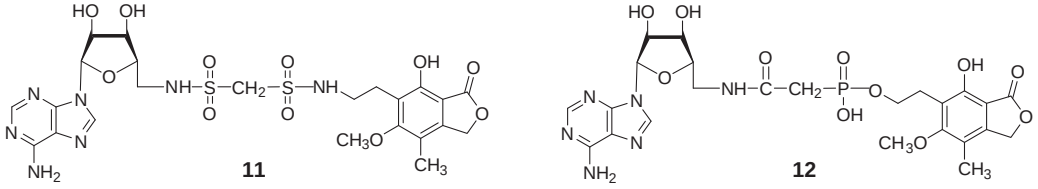


Figure 8. Bis(sulfonamide) analogue of C2-MAD **11** and phosphonoacetamide derivative **12**.

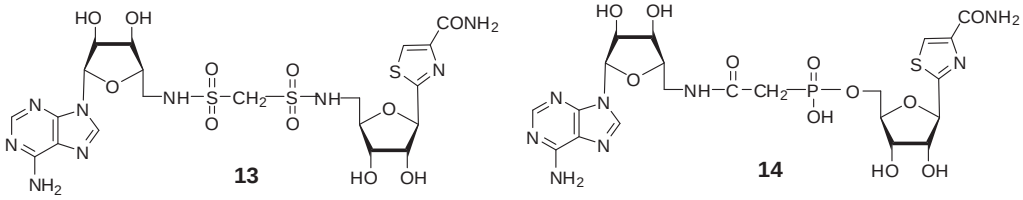


Figure 9. Bis(sulfonamide) analogue of TAD **13** and phosphonoacetamide derivative **14**.

since phosphonoacetamide analogue of TAD **14** (K_i = 6.6 μM) was three-fold more potent against *h*IMPDH2 than the corresponding bis(sulfonamide) derivative TAD **13** (K_i = 18.8 μM).

Next, we decided to check whether or not the presence of two phosphorus atoms (or their structural equivalents such as sulfonyl group) is crucial in the design of IMPDH inhibitors. Thus we synthesized mycophenolic alcohol ethylenephosphonate-5′-thioadenosine derivative **15** and the corresponding analogue **16** (Fig. 10) with the sulfur atom of the linker attached to the position 8 of the adenine ring, containing only one phosphorus group. Again we found a potent inhibitory activity of analogue **15** against *h*IMPDH1 (K_i = 94 nM) and *h*IMPDH2 (K_i = 39 nM). However, moving of the linker from the 5′-position of the sugar moiety to the carbon eight of the base resulted in weakly active compound **16** against the isoform 1 (K_i = 7.9 μM) and the isoform 2 (K_i = 1.9 μM) (Table 1). The significant decrease of the enzymatic activity of **16** is likely due to structural differences between isomers **15** and **16** and/or due to conformational change of **16** from *anti*- to *syn*-conformation.

Finally, we recently reported triazole-linked mycophenolic adenine inhibitors of IMPDH.[16] Compounds such as **17** and **18** (Fig. 11) showed low nanomolar inhibition of the two isoforms of the human enzyme with K_i's in the range of 34–77 nM (Table 1), indicating that the presence of phosphorous group is not required for the potent activity against human IMPDH isoforms.

All these results indicate that P-groove of the NAD binding domain is quite promiscuous and can accommodate variety of linkers of different length and geometry.

3. Chemistry

The synthesis of difluoromethylenebis(phosphonate) analogues of TAD **2** and MAD **4** is depicted in Scheme 1. The starting material, 2′,3′-*O*-isopropylidene-2-ethylinosine [**19**, prepared from protected 5-aminoimidazole-4-carboxamide riboside (AICAR)][17] was converted into the adenosine analogue **20** by chlorination of **19** at the 6-position with $P(O)Cl_3$ followed by aminolysis. The synthesis of the key bis(phosphonate) intermediate **22** was accomplished by coupling of 2′,3′-*O*-isopropylidene-5′-tosyl-2-ethyladenosine (**21**) with difluoromethylenebis(phosphonic) acid in a similar manner as was reported by Poulter and co-workers for coupling of 5′-tosyl adenosine.[18] However, we prepared the starting difluoromethylenebis(phosphonic) acid differently, that is, by convenient and efficient electrophilic fluorination of tetraisopropyl methylenebis(phosphonate) with *N*-fluorobenzenesulfonimide[19] followed by hydrolysis.

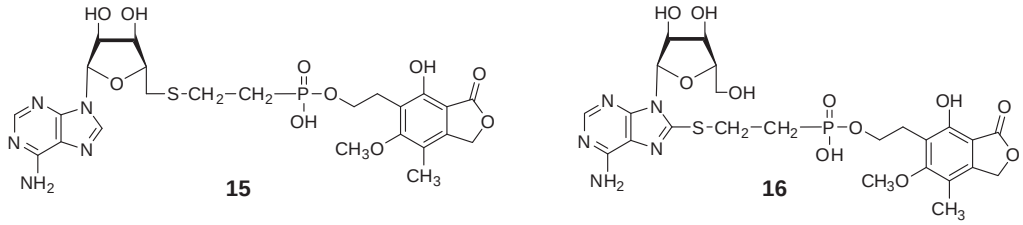


Figure 10. Mycophenolic alcohol ethylenephosphonate-5′-thioadenosine **15** and the 8-substituted derivative **16**.

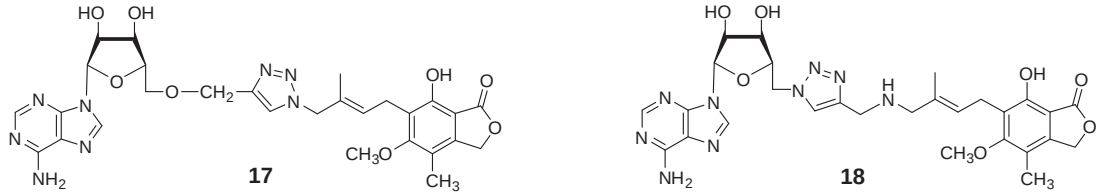
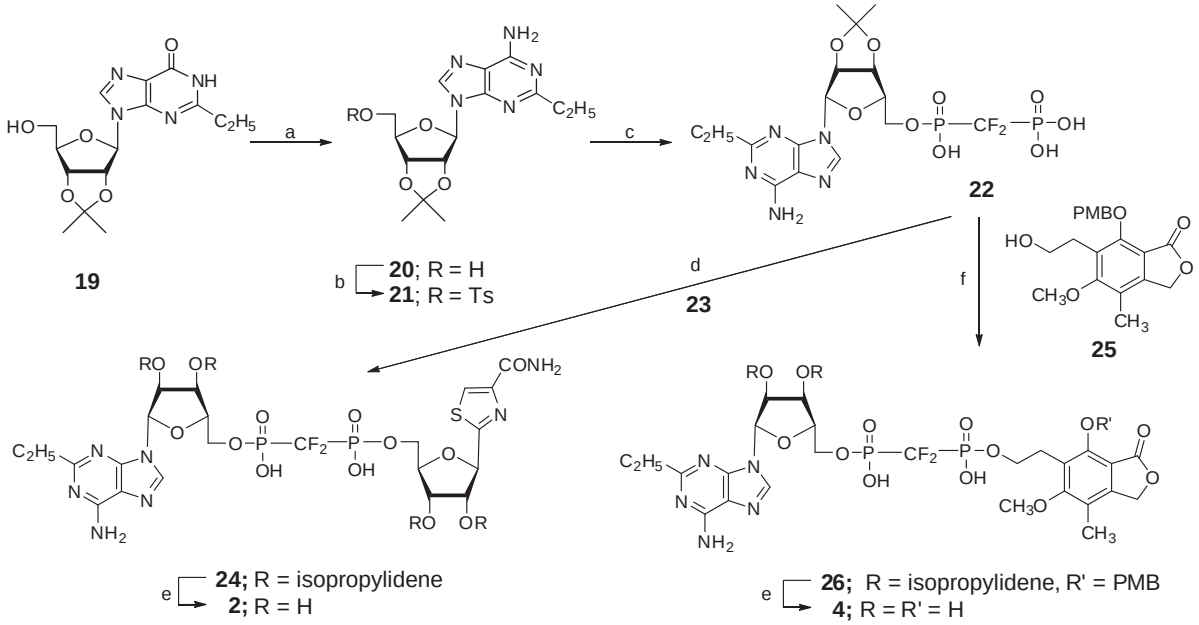


Figure 11. Triazole-linked MAD analogues **17** and **18**.



Scheme 1. Synthesis of tiazofurin 2-ethyladenosine–, and mycophenolic alcohol 2-ethyladenosine difluoro-methylenebis(phosphonate)s **2** and **4**, respectively. Reagents and conditions: (a) (i) Ac_2O, Py, (ii) Et_4NCl, *N*,*N*-dimethylaniline, $POCl_3$, MeCN, 80 °C 2 h, (iii) 2 M NH_3 *i*-PrOH, 80 °C, overnight; (b) TsCl, Py, −25 °C; (c) tris-tetrabutylammonium difluoromethylenebis(phosphonate), MeCN, rt, 24 h; (d) (i) DIC, Py, rt, overnight, (ii) **23**, 62 °C, 70 h, (iii) H_2O/TEA (9:1), overnight, rt; (e) 50% HCOOH, overnight, rt; (f) (i) DIC, Py, rt, overnight, (ii) 25, 62 °C, 75 h, (iii) H_2O/TEA (9:1), 27 h, 55–65 °C.

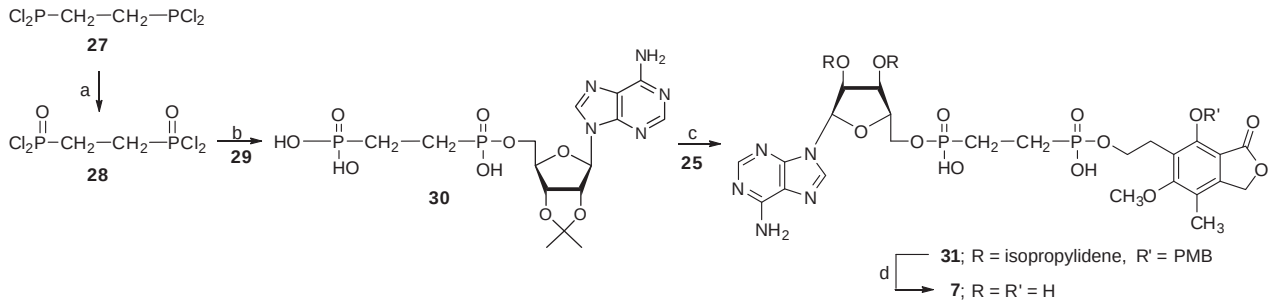
N,*N*-Disopropylcarbodiimide (DIC) promoted coupling of 2′3′-*O*-isopropylidene-2-ethyladenosin-5′-yl-difluoromethylenebis (phosphonate) **22** with 2′,3′-*O*-isopropylidenetiazofurin[20] (**23**) afforded the protected difluoromethylenebis(phosphonate) analogue of TAD **24**, which without purification was deisopropylidenated by treatment with aq HCOOH to give the desired tiazofurin 2-ethyladenosine difluoromethylenebis(phosphonate) **2**. Similarly, the reaction of **22** with *p*-methoxybenzyl (PMB) protected mycophenolic alcohol derivative **25** (prepared from C2-mycophenolic alcohol reported earlier by us)[10] gave the protected mycophenolic alcohol 2-ethyladenosine bis(phosphonate) analogue **26**. Further treatment of **26** with formic acid resulted in deprotection of both *p*-methoxybenzyl and isopropylidene protecting groups to give the desired mycophenolic alcohol 2-ethyladenosin-5′-yl difluoromethylenebis(phosphonate) derivative **4** in moderate yield.

The synthesis of MAD analogues containing a linker extended by one carbon atom is illustrated in the next two schemes. First, we prepared ethylenebis(phosphonic dichloride) (**28**) by DMSO oxidation of commercially available 1,2-bis(dichlorophosphino)ethane (**27**) which gave **28** as a white solid in quantitative yield (Scheme 2). We found this new method much more superior to the published procedure[21] via chlorination of the corresponding ethylenebis(phosphonate)s with PCl_5.

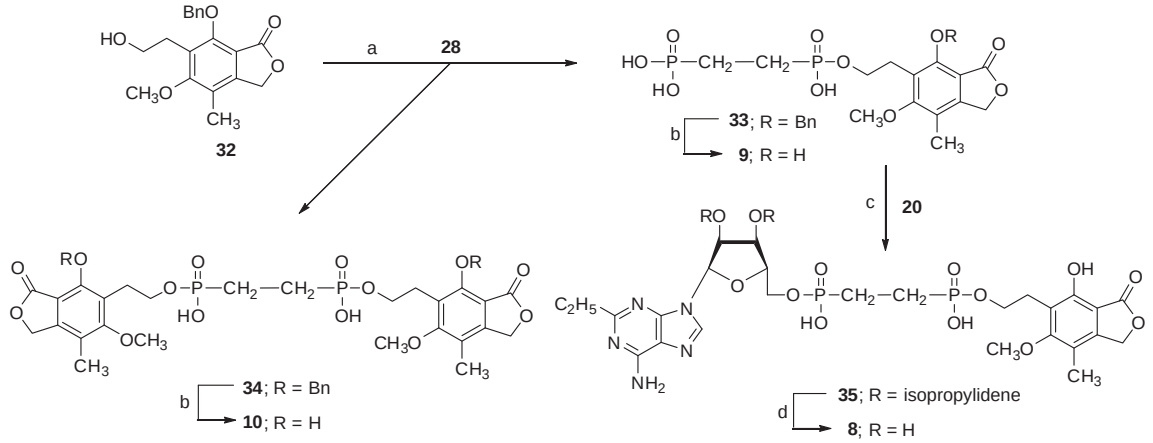
Then, in conditions of Yoshikawa phosphorylation compound **28** reacted with a commercially available 2′,3′-*O*-isopropylideneadenosine **29** to give the adenosin-5′-yl-ethylenebis(phosphonic acid) **30** as a major product. DIC coupling of **30** with PMB protected mycophenolic alcohol **25** afforded MAD analogue **31**, which under treatment with 50% HCOOH was converted into the desired mycophenolic alcohol adenosine ethylenebis(phosphonate) **7**.

The synthesis of ethylenebis(phosphonate) MAD analogue **8** was performed in a similar manner (Scheme 3). Briefly, Yoshikawa phosphorylation of benzyl protected mycophenolic alcohol **32**[10] with ethenebis(phosphonic dichloride) (**28**) in the presence of proton sponge and at the elevated temperature afforded the corresponding mycophenolic alcohol ethylenebis(phosphonic acid) **33** as a major product (73% yield) together with disubstituted ethylenebis(phosphonate) **34** as a minor product (9%). Hydrogenolysis of **33** and **34** gave the debenzylated compounds **9** and **10**, respectively. Finally, DIC coupling of **9** with 2′,3′-*O*-isopropylidene-2-ethyladenosine **20** afforded the protected MAD analogue **35**, which under treatment with 50% HCOOH was converted into the desired mycophenolic alcohol 2-ethyladenosine ethylenebis(phosphonate) **8**.

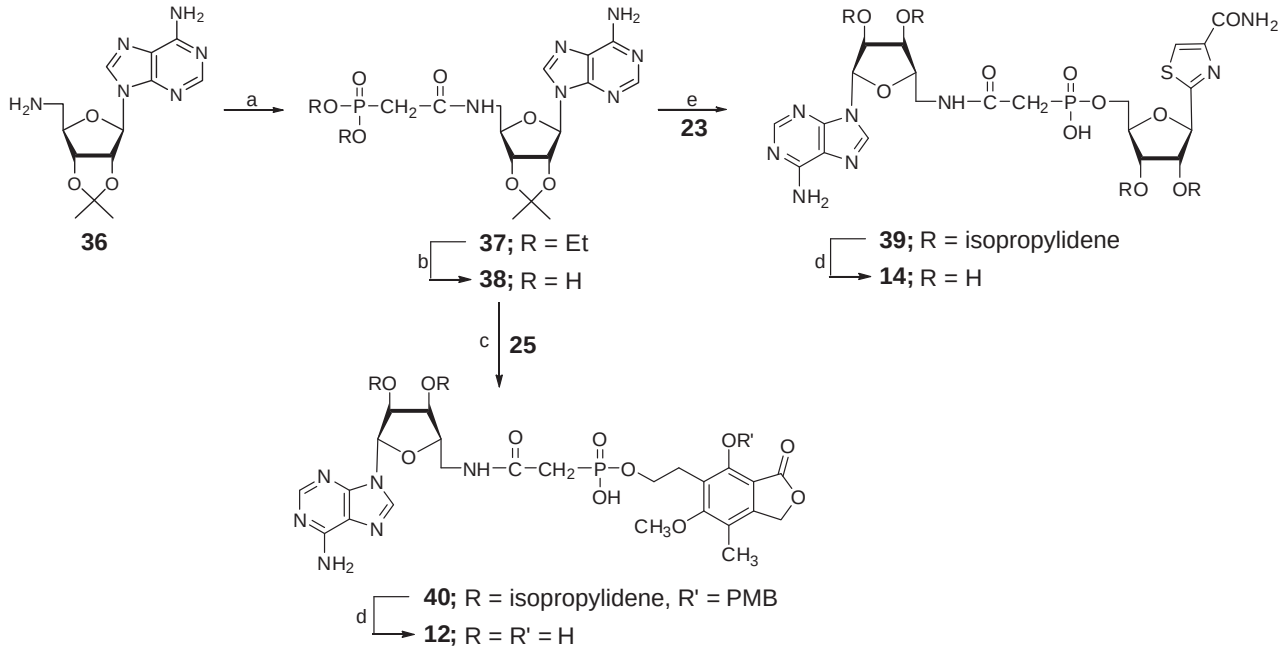
Next, we prepared phosphonoacetamido MAD analogue **12** and TAD analogue **14** that contain non-isosteric carbonyl group as shown in Scheme 4. The key intermediate for this synthesis is 2′,3′-*O*-isopropylideneadenosin-5′-deoxy-5′-*N*-acetamidophosphonic acid (**38**), which was prepared by coupling of 2′,3′-*O*-isopropylidene-5′-aminoadenosine (**36**)[22] with diethyl (phosphono)acetic acid followed by a selective deprotection of the phosphonate ethyl groups. We found that in the presence of *N*-hydroxybenzotriazole (as the carboxylic group activator) the yield of the intermediate **37** was much higher than that reported.[23] Coupling of the phosphonic acid



Scheme 2. Synthesis of mycophenolic alcohol adenosine ethylenebis(phosphonate) analogue **7**. Reagents and conditions: (a) DMSO, benzene, 30 min, 0 °C; (b) (i) **29**, $PO(OEt)_3$, overnight, 50 °C, (ii) 48 h, 72 °C, (iii) TEAB, overnight, rt; (c) **25**, DIC, Py, 72 h, 60 °C; (d) 50% HCOOH, overnight, rt.



Scheme 3. Synthesis of mycophenolic alcohol 2-ethyladenosine ethylenebis(phosphonate) analogue **8**. Reagents and conditions: (a) **28**, 1,8-diaminonaphtalene, $PO(OMe)_3$, 72 h, 80 °C; (b) $HCOONH_4$, Pd EnCAT 40 (Aldrich), DMF/MeOH (1:5), overnight, reflux; (c) (i) **20**, DIC, Py, 1 h, rt, (ii) 65 °C, 48 h; (d) 50% HCOOH, overnight, rt.



Scheme 4. Synthesis mycophenolic alcohol (adenosine-5′-*N*-acetamido)phosphonate **12** and tiazofurin-5′-yl-(adenosine-5′-*N*-acetamido)phosphonate **14**. Reagents and conditions: (a) HOBt, diethyl-phosphonoacetic acid, DIC, DMF, 20 h, rt; (b) TMS-Br, 2,6-lutidine, MeCN, overnight, rt; (c) **25**, DIC, Py, 48 h, 65 °C; (d) 50% HCOOH, overnight, rt; (e) **23**, DIC, Py, 72 h, 65 °C.

38 in the presence of DIC with 2′,3′-*O*-isopropylidenetiazofurin (**23**) afforded after deisopropylidenation the desired tiazofurin-5′-yl-(adenosine-5′-*N*-acetamido)phosphonate (**14**). In a similar manner coupling of the PMB protected mycophenolic alcohol **25** with the

intermediate **38** gave after deprotection the corresponding mycophenolic alcohol (adenosine-5′-*N*-acetamido)phosphonate **12**.

Finally, we synthesized MAD analogues **15** and **16** containing only one phosphorus atom in the linker. Thus, diethyl 2′,3′-*O*-isopropylideneadenosine-5′-thioethylenephosphonate (**42**) was prepared by *S*-alkylation (Scheme 5) of 2′,3′-*O*-isopropylidene-5′-mercaptoadenosine (formed in situ from **41**) with commercially available diethyl 2-bromoethylphosphonate.

The 2′,3′-*O*-isopropylideneadenosine-5′-thioethylenephosphonic acid (**43**) was obtained by treatment of **42** with trimethylsilyl bromide. MAD analogue **15** was prepared by coupling of **43** with protected mycophenolic alcohol **25** in the presence of DIC to give **44**, which was deisopropylidenated with formic acid.

Analogously, 8-thioethylenephosphonate analogue (MAD **16**) was synthesized from 2′,3′-*O*-isopropylidene-8-mercaptoadenosine (**46**),[24] as shown in Scheme 6.

Briefly, *S*-alkylation of **46** with diethyl 2-bromoethylphosphonate afforded phosphonate **47** in high yield, which was hydrolyzed into the free acid **48**. Then, the standard *t*butyldimethylsilyl protection gave the 5′-silyl derivative **49**. Further coupling with mycophenolic alcohol **25** afforded the protected phosphonate **50**. Treatment of **50** with formic acid resulted in the removal of all three protecting groups to give the desired final product **16**.

4. Conclusions

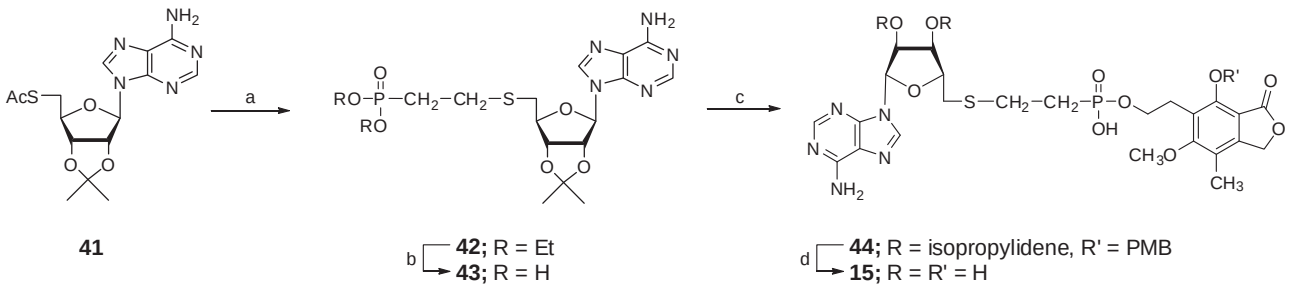
In summary, we synthesized novel cofactor-type inhibitors of IMPDH in which the pyrophosphate group that connects nicotinamide riboside and adenosine moiety in the natural NAD is modified or replaced by other linkers. For construction of our inhibitors we selected tiazofurin and mycophenolic moiety, the binding fragments with high affinity to the N sub-site of the cofactor binding domain, used by us and others for preparation of IMPDH inhibitors such as TAD and MAD analogues. Adenosine and 2-ethyladenosine were used to secure binding at the A sub-site of the enzyme. We found as expected that the replacement of the P-O-P group by its isosteric and isoelectronic P-CF_2-P moiety afforded potent inhibitors of IMPDH. The most potent compound was MAD analogue **4** containing 2-ethyladenosine. It was as potent (IC_{50} = 0.45 μM) against leukemia K562 cells proliferation as Gleevec (IC_{50} = 0.56 μM) used in the clinic as anti-CML drug. MAD analogue **3** containing methylenebis(phosphonate) linker (P-CH_2-P) was only slightly less potent as the enzyme inhibitor and the anti-proliferative agent (IC_{50} = 1.0 μM). An extension of the length of the linker by one carbon atom as in ethylenebis(phosphonate) (P-CH_2-CH_2-P) afforded MAD analogues **7** and **8**, both 2-5-fold less potent against IMPDH isoforms (but still in a low nanomolar range). They were 10-fold less active as proliferation agents, as compared with MAD analogue **3**. We also found that the replacement of the methylenebis(phosphonate) group by a geometrically similar (tetrahedral) methylenebis(sulfonyl) moiety resulted in MAD analogue **11** and TAD analogue **13**. On the other hand replacement of the only one phosphorus atom by a geometrically different (planar) carbonyl group afforded phosphonoacetamide derivatives MAD **12** and TAD **14**. Both MAD analogues **11** and **12** showed enzymatic activity in the nanomolar range. Interestingly, the non-isosteric phosphonoacetamide analogue **12** (K_i = 0.083 μM) was two-fold more potent than isosteric sulfonamide **11** (K_i = 0.167 μM). This indicates that preservation of tetrahedral geometry of the pyrophosphate bridge is apparently not that crucial. For unknown reasons, the corresponding sulfonamide TAD analogue **13** and phosphonoamide TAD analogue **14** showed less potent enzymatic activity (6.6–23.6 μM). Again the phosphonoamide analogue **14** was more potent than isosteric sulfonamide **13**. Finally, compound **15** containing only one phosphonate atom in the linker was found to be a potent inhibitor of *h*IMPDH1 (K_i = 94 nM) and *h*IMPDH2 (K_i = 39 nM). Recently we reported that triazole-linked MAD analogues, such as **17** and **18**, that do not contain phosphorus atoms, also showed potent inhibition of IMPDH. Taken together, it became clear that the pyrophosphate binding sub-domain (P-groove) of IMPDH is much more promiscuous that it was originally anticipated. Thus, our studies described herein would encourage future attempts of constructing novel inhibitors by using molecular fragments that fit to N and A sub-domain and linkers that not necessarily resemble well the natural pyrophosphate.

Although, almost all new compounds **1**–**16** showed a potent nanomolar inhibition of IMPDH, many, that is, compound **9**–**16** were not active as anti-proliferative agents in vitro. It was believed, that negatively charged bis(phosphonate)s could not be able to cross cells membranes efficiently and we faced such criticism frequently in the past. In contrast, we found herein that bis(phosphonates) showed potent anti-proliferative activity in vitro, whereas neither uncharged sulfonamides **11** and **13** nor negatively charged phosphonoacetamides **12** and **14**, or phosphonate analogue **15** showed such activity. Since sulfonamides, phosphonoacetic analogues, and phosphonate nucleosides are well established pharmacophores present in numerous drugs, we are focusing now on possible explanation of such bizarre behavior of modified NAD analogues reported in this work.

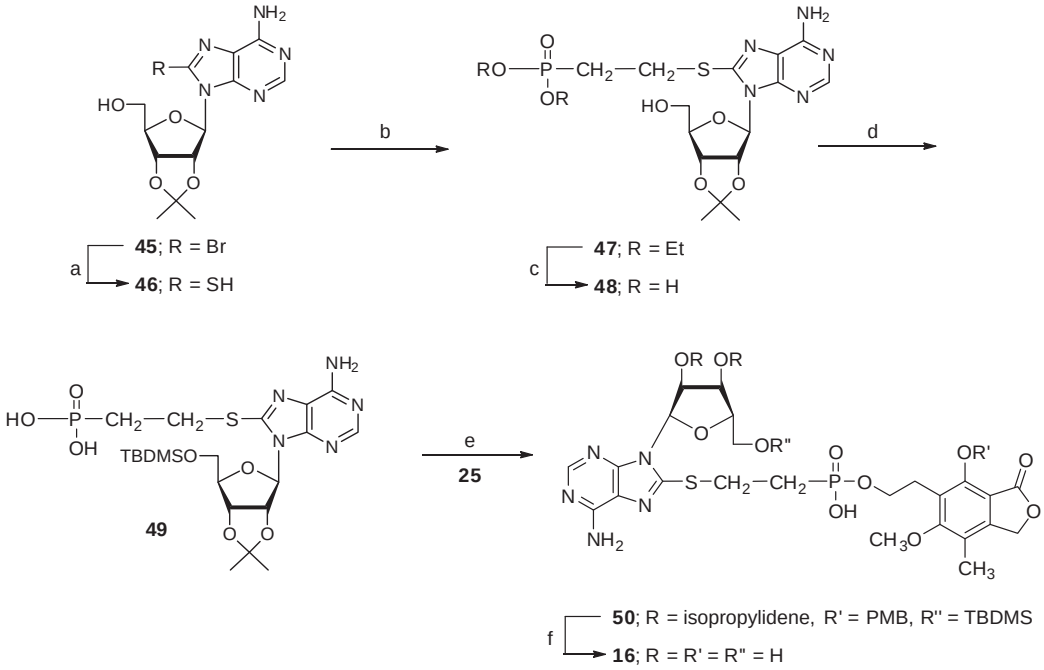
5. Experimental section

5.1. General methods

All commercial reagents (Sigma-Aldrich, Alfa Aesar) 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. Analytical HPLC was performed on a Varian Microsorb column (C18, 5 μ, 4.6 × 250 mm) with a flow rate of 0.5 mL/min while preparative HPLC was performed on a Varian Dynamax column (C18, 8 μ, 41.4 × 250 mm) with a



Scheme 5. Synthesis of 5′-thioethylenephosphonate analogue of MAD. Reagents and conditions: (a) diethyl 2-bromoethylphosphonate, MeONa, MeOH, overnight, −20 °C → rt; (b) TMS-Br, 2,6-lutidine, MeCN, overnight, rt; (c) **25**, DIC, Py, 24 h, 55 °C; (d) 50% HCOOH, overnight, rt.



Scheme 6. Synthesis of 8-thioethylenephosphonate analogue of MAD. Reagents and conditions: (a) NaSH[24]; (b) diethyl 2-bromoethylphosphonate, K_2CO_3, DMF, overnight, rt; (c) TMS-Br, 2,6-lutidine, MeCN, overnight, rt; (d) TBDMS-Cl, imidazole, DMF, overnight, rt; (e) **25**, DIC, Py, 48 h, 58 °C; (f) 50% HCOOH, overnight, rt.

flow rate of 40 mL/min. An isocratic or linear gradient of 0.1 M triethylammonium bicarbonate (TEAB) and aq MeCN (70%) were used. Flash chromatography was performed using Teledyne ISCO CombiFlash Rf equipped with Teledyne ISCO RediSep Rf flash column silica cartridges (www.isco.com/combiflash) with the indicated solvent system. Nuclear magnetic resonance spectra were recorded on a Varian 600 MHz with Me_4Si, DDS or signals from residual solvent 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), br s (broad singlet), and dd (double doublet). Values given for coupling constants are first order. High resolution mass spectra were recorded on an Agilent TOF II TOF/MS instrument equipped with either an ESI or APCI interface. All reactions were performed under an inert atmosphere of dry Ar in oven dried (150 °C) glassware. The purity of compounds was ⩾95%, and it was determined by HPLC using above mentioned analytical column, and conditions described in the Supplementary data.

5.2. 2′,3′-*O*-Isopropylidene-2-ethylinosine (19)

It was prepared from 2′,3′-*O*-isopropylidene-AICAR according to the old procedure[17] to give **19** as a white crystals, mp 225–6 °C, (lit. 212 °C). ^1H NMR ($CDCl_3$) δ 12.80 (br s, 1H), 7.81 (s, 1H), 5.84 (d, J = 4.94 Hz, 1H), 5.29 (d, J = 10.99 Hz, 1H), 5.18 (t, J = 5.44 Hz, 1H), 5.08 (dd, J = 5.89, 1.12 Hz, 1H), 4.52–4.50 (m, 1H), 3.97 (d, J = 12.29 Hz, 1H), 3.78 (t, J = 11.78 Hz, 1H), 2.88 (q, J = 7.69 Hz, 2H), 1.65 (s, 3H), 1.42 (t, J = 7.69 Hz, 3H), 1.39 (s, 3H).

5.3. 2′,3′-*O*-Isopropylidene-2-ethyladenosine (20)

Compound **19** (2.86 g, 8.5 mmol) was dissolved in a mixture of Ac_2O (12 mL) and pyridine (8 mL). After stirring for 3 h at rt, the mixture was diluted with toluene and evaporated to dryness. The residue was dissolved in EtOH and toluene, evaporated in vacuo, and then co-evaporated with dry xylene. To the viscous 5′-*O*-acetyl derivative tetraethylammonium chloride (2.82 g, 17 mmol) was added and the mixture was dissolved in dry MeCN (20 mL). Next, *N*,*N*-dimethylaniline (1.1 mL, 8.5 mmol) was added followed by dropwise addition of $POCl_3$ (4.8 mL, 51 mmol). The resulting mixture was heated at 80 °C for 2 h (preheated bath). The mixture was cooled, diluted with CH_2Cl_2, and poured into a stirred mixture of ice and saturated $NaHCO_3$ (150 mL). After 10 min of stirring, CH_2Cl_2 (200 mL) was added, and vigorous stirring was continued until ice melted. The aqueous fraction was separated and extracted with CH_2Cl_2. The combined extracts were dried over $MgSO_4$, filtered, and evaporated to dryness leaving partially crystallizing oil. The crude 6-chloro derivative was purified by flash chromatography on a silica gel with EtOAc/$CHCl_3$ (0–40%) as an eluent to give the crystalline compound, which was dissolved in 2 M NH_3 in *i*-PrOH (20 mL) and heated at 80 °C overnight. After evaporation of solvents the residue was dissolved in a mixture 1,4-dioxane/concd NH_4OH (1:1, 40 mL) and stirred at 50 °C overnight. Solvents were evaporated and the residue was purified by flash chromatography on a silica gel with MeOH/CH_2Cl_2 (0–10%) as the eluent to give a final compound **20** as a foam (2.1 g 74%). Mp 159–159.5 °C. ^1H NMR ($CDCl_3$) δ 7.76 (s, 1H), 6.82 (d, J = 11.81 Hz, 1H), 5.82 (d, J = 4.97 Hz, 1H), 5.68 (br s, 2H), 5.23 (t, J = 5.33 Hz, 1H), 5.11 (d, J = 5.70 Hz, 1H), 4.54 (s, 1H), 3.99 (d, J = 12.73 Hz, 1H), 3.79 (t, J = 12.34 Hz, 1H), 2.80 (q, J = 7.60 Hz, 2H), 1.65 (s, 3H), 1.39 (s, 3H), 1.33 (t, J = 7.60 Hz, 3H). HRMS calcd for $C_{15}H_{22}N_5O_4$ 336.1666 $(M+H)^+$, found 336.1681.

5.4. 2′,3′-*O*-Isopropylidene-5′-O-p-tosyl-2-ethyladenosine (21)

Compound **20** (335 mg, 1 mmol) was dissolved in dry pyridine (6 mL). The mixture was cooled to −25 °C, TsCl (310 mg, 1.61 mmol) was added in small portions, and after 30 min of stirring the mixture was left in a freezer overnight. It was then poured into a vigorously stirred mixture of 2 N HCl (25 mL) with ice and EtOAc, and when ice had melted, the two layers were separated. The inorganic phase was extracted with EtOAc. Organic fractions were combined, washed with H_2O, dried over $MgSO_4$, filtered, and evaporated to give the crude product which was quickly

re-crystallized from EtOAc. The crystals were washed with hexane and dried under vacuum to give yellow, unstable product which was used in the next step without further purification (386 mg, 79%). ^{1}H NMR ($CDCl_3$) δ 7.72 (s, 1H), 7.57 (d, J = 8.25 Hz, 2H), 7.12 (d, J = 7.96 Hz, 2H), 6.00 (d, J = 1.14 Hz, 1H), 5.36 (dd, J = 6.25, 1.31 Hz, 1H), 5.12 (dd, J = 6.16, 2.91 Hz, 1H), 4.31 (dd, J = 10.54, 4.57 Hz, 1H), 4.47 (s, 1H), 2.77 (q, J = 7.69 Hz, 2H), 4.25 (dd, J = 10.52, 7.18 Hz, 1H), 2.38 (s, 3H), 1.59 (s, 3H), 1.37 (s, 3H), 1.29 (t, J = 7.58 Hz, 3H).

5.5. 2′,3′-*O*-Isopropylidene-2-ethyladenosin-5′-yl-difluoromethylenebis(phosphonate) (22)

A mixture of **21** (385 mg, 0.786 mmol) and tris-tetrabutylammonium difluoromethylenebis(phosphonate) (760 mg; 0.786 mmol) in dry MeCN (2 mL) was stirred 24 h at rt, concentrated, and the residue was dissolved in water (2 mL), and this solution was then passed through a column of Dowex 50WX8-200 (Na^+ form). After elution with water, fractions containing product were collected, lyophilized, and purified by preparative HPLC with 70% MeCN/0.1 M TEAB (5-100 linear gradient) to give **22** as triethylammonium salt (356 mg, 72%). ^{1}H NMR (D_2O) δ 8.33 (s, 1H), 6.19 (d, J = 3.20 Hz, 1H), 5.32 (dd, J = 6.03, 3.26 Hz, 1H), 5.13 (dd, J = 6.06, 2.13 Hz, 1H), 4.54–4.50 (m, 1H), 4.16–4.05 (m, 1H), 3.07 (q, J = 7.33 Hz, 12H), 2.71 (q, J = 7.63, Hz, 2H), 1.55 (s, 3H), 1.33 (s, 3H), 1.19 (t, J = 7.63 Hz, 3H), 1.15 (t, J = 7.33 Hz, 18H). ^{31}P NMR (D_2O) δ 5.15 (J_{P1P2} = 55.8, J_{P1F} = 83.6 Hz), 3.93 (J_{P1P2} = 55.8, J_{P2F} = 82.2 Hz). HRMS calcd for $C_{16}H_{22}F_2N_5O_9P_2$ 528.0866 $(M-H)^-$, found 528.0887.

5.6. Tiazofurin-5′-yl-2-ethyladenosin-5′-yl-difluromethylene bis(phosphonate) (2)

To the solution of **22** (67 mg, 0.22 mmol) in dry pyridine (2 mL), DIC (172 μL, 1.11 mmol) was added, and the mixture was stirred overnight at rt. Next, 2′,3′-*O*-isopropylidenetiazofurin (**23**)[20] (156 mg, 0.22 mmol) was added, and stirring was continued for 70 h at 62 °C. After cooling to rt, a mixture of water (0.9 mL) and TEA (0.1 mL) was added, and the reaction was kept at rt overnight. After evaporation the intermediate **24** was obtained as a solid. It was dissolved in 50% HCOOH (5 mL) and stirred overnight at rt, solvents were evaporated, and the residue was co-evaporated with a mixture of EtOH and water. The crude product **24** was purified by preparative HPLC with 70% MeCN/0.1 M TEAB (10–15 linear gradient). Fractions containing product were evaporated. The residue was dissolved in water and then passed through a column of Dowex 50 WX8-200 (Na^+ form). After elution with water, UV active fractions were collected, and lyophilized to give deprotected **2** (sodium salt) as a white powder (32 mg, 20%). ^{1}H NMR (D_2O) δ 8.28 (s, 1H), 7.83 (s, 1H), 5.96 (d, J = 5.62 Hz, 1H), 4.92 (d, J = 5.65 Hz, 1H), 4.54 (t, J = 5.35 Hz, 1H), 4.38 (dd, J = 4.83, 3.99 Hz, 1H), 4.20–4.17 (m, 2H), 4.17–4.12 (m, 2H), 4.12–4.06 (m, 4H), 2.60 (q, J = 7.67 Hz, 2H), 1.13 (t, J = 7.67 Hz, 3H). ^{31}P NMR (D_2O) δ 4.96 (J_{P1P2} = 51.5 Hz, J_{P1F} = 82.5), 4.62 (J_{P1P2} = 51.5, J_{P2F} = 84.3 Hz). HRMS calcd for $C_{22}H_{28}F_2N_7O_{13}P_2S$ 730.0915 $(M-H)^-$, found 730.0926.

5.7. 7-(*p*-Methoxybenzyl)-6-(2-hydroxyethyl)-5-methoxy-4-methyl-phthalan-1-one (25)

To the solution of 7-hydroxy-6-(2-hydroxyethyl)-5-methoxy-4-methyl-phthalan-1-one (1.94 g, 8.186 mmol) in 1 N TBAF in THF (17 mL) *p*-methoxybenzyl chloride (1.26 mL, 8.89 mmol) was added and the mixture was stirred at rt overnight, then another portion of *p*-methoxybenzyl chloride (0.3 mL) was added, and stirring was continued at rt. When reaction was complete the mixture was diluted with satd $NaHCO_3$, extracted with EtOAc, extracts were dried over $MgSO_4$, filtered, and evaporated leaving semi crystalline product which was purified by crystallization from EtOH (1.8 g). The mother liquor was concentrated and purified by flash chromatography on a silica gel with MeOH/$CHCl_3$ (0–2%) to give additional (1 g) of crystalline **25** (total 2.8 g, 73%). ^{1}H NMR ($CDCl_3$) δ 7.43–7.40 (m, 2H), 6.91–6.88 (m, 2H), 5.25 (s, 2H), 5.17 (s, 2H), 3.81 (s, 3H), 3.79 (s, 3H), 3.70 (q, J = 6.27 Hz, 2H), 2.89 (t, J = 6.47 Hz, 2H), 2.20 (s, 3H), 1.83 (t, J = 5.50 Hz, 1H). HRMS calcd for $C_{20}H_{22}NaO_6$ 381.1308 $(M+Na)^+$, found 381.1301.

5.8. Mycophenolic 2-ethyladenosin-5′-yl-difluoromethylenebis (phosphonate) (4)

To a solution of **22** (190 mg, 0.27 mmol) in dry pyridine (2 mL), DIC (250 μL, 1.6 mmol) was added, and the resulting mixture was left overnight at rt. After addition of protected mycophenolic alcohol **25** (96 mg, 0.27 mmol) stirring was continued for 75 h at 62 °C. After cooling to rt, a mixture of water (0.9 mL) and TEA (0.1 mL) was added, and the reaction was kept at 55–65 °C for 27 h. The mixture was diluted with toluene and EtOH, evaporated, and co-evaporated twice. The intermediate **26** was dissolved in 50% HCOOH (5 mL) and stirred overnight at rt, solvents were evaporated, and the residue was co-evaporated with EtOH and water. The crude product was purified by preparative HPLC with 70% MeCN/0.1 M TEAB (20–30 linear gradient). Fractions containing the desired product were evaporated. The residue was dissolved in water and then passed through a column of Dowex 50 WX8-200 (Na^+ form). After elution with water, UV active fractions were collected and lyophilized to give the deprotected **4** (sodium salt) as a white powder (76 mg, 37%). ^{1}H NMR (D_2O) δ 8.08 (s, 1H), 5.81 (d, J = 4.70 Hz, 1H), 4.88–4.79 (m, 2H), 4.40 (t, J = 4.87, Hz, 1H), 4.30 (t, J = 4.82 Hz, 1H), 4.18–4.07 (m, 3H), 3.93–3.83 (m, 2H), 3.57 (s, 3H), 2.76–2.60 (m, 2H), 2.52 (q, J = 7.69 Hz, 2H), 1.79 (s, 3H), 1.07 (t, J = 7.69 Hz, 3H). ^{31}P NMR (D_2O) δ 5.11 (J_{P1P2} = 75.0, J_{P1F} = 84.0 Hz), 4.95 (J_{P1P2} = 75.0, J_{P2F} = 86.5 Hz). HRMS calcd for $C_{25}H_{30}F_2N_5O_{13}P_2$ 708.1289 $(M-H)^-$, found 708.1300.

5.9. Ethylenebis(phosphonic dichloride) (28)

To a solution of commercially available 1,2-bis(dichlorophosphino) ethane (**27**, 3 mL, 20 mM) in benzene (160 mL), a solution of DMSO (3.2 mL) in benzene (160 mL) was added over 30 min under cooling in ice. ^{31}P NMR showed a complete conversion of **27** (δ 191.0) into the desired product **28** (δ 41.5). The reaction mixture was concentrated in vacuo to give **28** as a white solid (5.27 g, 100%). ^{31}P NMR (C_6D_6) δ 41.5 (s).

5.10. 2′,3′-*O*-Isopropylideneadenosin-5′-yl-ethylenebis (phosphonate) (30)

To a suspension of tetrachloride **28** (1.06 g, 4 mmol) in triethylphosphate (25 mL), a suspension of 2′,3′-*O*-isopropylidene adenosine **29** (614 mg, 2 mmol) in triethylphosphate (25 mL) was added. The resulting mixture was stirred at 50 °C overnight, and then at 72 °C for 2 days, cooled, and added to a solution of TEAB (0.5 M, 20 mL, cooled with ice-water), and stirred at rt overnight. This mixture was extracted with EtOAc, the aqueous layer was concentrated, lyophilized, and purified by preparative HPLC with 70% MeCN/0.1 M TEAB (10–30 linear gradient) to give **30** as a foam (350 mg, 26%).^{1}H NMR (D_2O) δ 8.25 (s, 1H), 8.14 (s, 1H), 6.15 (d, J = 2.76 Hz, 1H), 5.34 (dd, J = 6.07, 2.78 Hz, 1H), 5.05 (dd, J = 6.06, 1.94 Hz, 1H), 4.57–4.54 (m, 1H), 3.93–3.86 (m, 2H), 3.07 (q, J = 7.33 Hz, 6H), 1.67–1.56 (m, 2H), 1.54 (s, 3H), 1.45–1.34 (m, 2H), 1.34 (s, 3H), 1.15 (t, J = 7.33 Hz, 9H). ^{31}P NMR (D_2O) δ 30.26 (d, J = 73.12 Hz), 22.86 (d, J = 70.80 Hz). HRMS calcd for $C_{15}H_{22}N_5O_9P_2$ 478.0898 $(M-H)^-$, found 478.0932.

5.11. Mycophenolic adenosin-5′-yl-ethylenebis(phosphonate) (7)

The ethylenebis(phosphonate) **30** (185 mg, 0.275 mmol) and **25** (108 mg, 0.303 mmol) were dissolved in dry pyridine (2 mL). To the resulting mixture DIC (220 μL, 1.375 mmol) was added, reaction was stirred at 60 °C for 72 h, and concentrated to dryness. The crude intermediate **31** was dissolved in 50% HCOOH (5 mL) and stirred overnight at rt, solvents were evaporated, and the residue was purified by preparative HPLC with 70% MeCN/0.1 M TEAB (15–20 linear gradient). Fractions containing the product were pooled, evaporated in vacuo, the residue was dissolved in water, and then passed through a column of Dowex 50 WX8-200 (Na^+ form). After elution with water, UV active fractions were collected and lyophilized to give **7** as a white powder (60 mg, 31%). ^{1}H NMR (D_2O) 8.12 (s, 1H), 7.88 (s, 1H), 5.83 (d, J = 5.44 Hz, 1H), 4.82 (s, 2H), 4.40 (t, J = 5.25, 5.25 Hz, 1H), 4.30–4.26 (m, 1H), 4.19–4.16 (m, 1H), 3.97–3.90 (m, 2H), 3.60–3.53 (m, 2H), 3.49 (s, 3H), 2.61–2.51 (m, 2H), 1.72 (s, 3H), 1.66–1.56 (m, 4H). ^{31}P NMR (D_2O) δ 28.50 (d, J = 75.51 Hz), 27.37 (d, J = 75.52). HRMS calcd for $C_{24}H_{30}N_5O_{13}P_2$ 658.1321 $(M-H)^-$, found 658.1339.

5.12. 7-Benzyl-mycophenolic ethylenebis(phosphonic acid) (33) and disubstituted analogue (34)

A mixture of ethylenebis(phosphonic dichloride) **28** (530 mg, 2 mmol) and **32** (330 mg, 1 mmol), with addition of the proton sponge, 1,8-dimethylaminonaphtalene, (210 mg, 1 mmol) in trimethylphosphate (5 mL) was stirred at 80 °C for 72 h. The reaction mixture was cooled, poured on iced water (100 mL), made slightly basic with TEA, and when ice melted, it was extracted with EtOAc. The organic fractions were discarded, and water phase was lyophilized to thick oil. The crude mixture was purified by preparative HPLC with 70% MeCN/0.1 M TEAB (25–100 linear gradient) to give benzyl protected monosubstituted bis(phosphonate) **33** as a major product and benzyl protected disubstituted bis(phosphonate) **34** as a minor product. Compound **33** was dissolved in MeOH, and then passed through a column of Dowex 50 WX8-200 (H^+ form). After elution with MeOH, the UV active fractions were concentrated to give **33** as a white, crystalline powder (360 mg, 73%). ^{1}H NMR (CD_3OD) δ 7.49 (d, J = 7.47 Hz, 2H), 7.37 (t, J = 7.42 Hz, 2H), 7.34 (t, J = 7.20 Hz, 1H), 5.30 (s, 2H), 5.28 (s, 2H), 4.10 (q, J = 6.79 Hz, 2H), 3.82 (s, 3H), 3.00 (t, J = 6.91 Hz, 2H), 2.23 (s, 3H), 1.87–1.76 (m, 2H), 1.75–1.63 (m, 2H). ^{31}P NMR (CD_3OD) δ 30.32 (d, J = 79.21 Hz), 28.14 (d, J = 79.21 Hz). HRMS calcd for $C_{21}H_{25}O_{10}P_2$ 499.0928 $(M-H)^-$, found 499.0976. Compound **34** was converted into free acid form as described for **33** to give a white powder (75 mg, 9%). ^{1}H NMR (DMSO-d_6) δ 7.46 (d, J = 7.28 Hz, 4H), 7.35 (t, J = 7.36 Hz, 4H), 7.30 (t, J = 7.22 Hz, 2H), 5.27 (s, 4H), 5.16 (s, 4H), 3.94–3.85 (m, 2H), 3.71 (s, 6H), 2.86 (t, J = 7.12 Hz, 4H), 2.11 (s, 6H), 1.52–1.42 (m, 4H). ^{31}P NMR (DMSO-D_6) δ 26.76 (s). HRMS calcd for $C_{40}H_{43}O_{14}P_2$ 528.0866 $(M-H)^-$, found 528.0887.

5.13. Mycophenolic ethylenebis(phosphonic acid) (9)

A solution of phosphonate **33** (300 mg, 0.6 mmol) in DMF (10 mL) was diluted with MeOH (50 mL) and ammonium formate (1 g) and Pd EnCat 40 from Aldrich (200 mg) was added. The mixture was refluxed overnight, filtered, and filtrate was concentrated into a thick oil which was purified by preparative HPLC with 70% MeCN/0.1 M TEAB (10–30 linear gradient) to give **9** as a foam (290 mg, 79%, triethylammonium salt). ^{1}H NMR (D_2O) δ 5.10 (s, 2H), 3.88 (q, J = 6.68 Hz, 2H), 3.73 (s, 3H), 2.90 (t, J = 6.77 Hz, 2H), 2.01 (s, 3H), 1.51 (m, 2H), 1.37 (m, 2H); ^{31}P NMR (D_2O) δ 28.87 (d, J = 73.33 Hz), 25.11 (d, J = 73.24 Hz). HRMS calcd for $C_{14}H_{19}O_{10}P_2$ 409.0459 $(M-H)^-$, found 409.0487.

5.14. Di-(mycophenolic) ethylenebis(phosphonic acid) (10)

It was prepared from **34** as described for **9**. ^{1}H NMR (D_2O) δ 5.18 (s, 4H), 3.86 (q, J = 6.60 Hz, 4H), 3.77 (s, 6H), 2.92 (t, J = 6.80 Hz, 4H), 2.02 (s, 6H), 1.47 (m, 4H). ^{31}P NMR (D_2O) δ 26.37 (s). HRMS calcd for $C_{26}H_{31}O_{14}P_2$ 629.1195 $(M-H)^-$, found 629.1224.

5.15. Mycophenolic 2-ethyladenosin-5′-yl-ethylenebis(phosphonate) (8)

Bisphosphonate **9** (99.1 mg, 0.194 mmol) and **20** were dissolved in dry pyridine (2 mL). To the resulting solution, DIC (150 μL, 0.97 mmol) was added. The mixture was stirred at rt for 1 h and then at 65 °C for 48 h, cooled to rt, evaporated, and co-evaporated with EtOH to give the crude intermediate **35**, which was dissolved in 50% HCOOH and stirred vigorously overnight at rt, evaporated, co-evaporated with EtOH, and dissolved in small amount of MeOH. The product was purified by preparative HPLC with 70% MeCN/0.01 M TEAB (15–30 linear gradient). Fractions containing the product were pooled, evaporated to dryness, co-evaporated with H_2O and EtOH, dissolved in small amount of H_2O, and passed through small column of Dowex 50 WX8-200 (Na^+). Fractions containing the desired product were combined and lyophilized to give **8** as a white powder (50 mg, 38%). ^{1}H NMR (D_2O) δ 8.14 (s, 1H), 5.93 (d, J = 5.48 Hz, 1H), 4.92–4.84 (m, 2H), 4.47 (t, J = 5.26 Hz, 1H), 4.38–4.34 (m, 1H), 4.27–4.24 (m, 1H), 4.03–3.96 (m, 2H), 3.73–3.63 (m, 2H), 3.55 (s, 3H), 2.62 (dd, J = 8.48, 7.50 Hz, 2H), 2.56 (q, J = 7.69 Hz, 2H), 1.78 (s, 3H), 1.73–1.62 (m, 4H), 1.10 (t, J = 7.68 Hz, 3H). ^{31}P NMR (D_2O) δ 28.42 (d, J = 75.40 Hz), 27.41 (d, J = 75.61 Hz). HRMS calcd for $C_{26}H_{34}N_5O_{13}P_2$ 686.1634 $(M-H)^-$, found 686.1662.

5.16. Diethyl (2′,3′-*O*-isopropylideneadenosine-5′-*N*-acetamido) phosphonate (37)[23]

To the solution of HOBt (135 mg, 1 mmol), 2′,3′-*O*-isopropylidene-5′-amino-adenosine[22] **36** (305 mg, 1 mmol), and diethylphosphonoacetic acid (170 μL, 1 mmol) in DMF (2 mL), DIC (320 μL, 2 mmol) was added dropwise and the mixture was stirred vigorously at rt for 20 h. After filtration, the solvent was evaporated and the residue was co-evaporated with EtOH (3 × 10 mL). It was then dissolved in CH_2Cl_2 (15 mL) and the organic phase was washed with H_2O (20 mL), dried over $MgSO_4$, filtered, and evaporated to dryness. The resulting oil was purified by flash chromatography with MeOH/CH_2Cl_2 (0–10%) as the eluent to give **37** (410 mg, 85%). ^{1}H NMR ($CDCl_3$) δ 8.40 (s, 1H), 7.95 (s, 1H), 8.31 (d, J = 6.47 Hz, 1H), 5.88 (d, J = 4.53 Hz, 1H), 5.76 (s, 2H), 5.38 (dd, J = 5.92, 4.90 Hz, 1H), 4.87 (dd, J = 6.27, 2.20 Hz, 1H), 4.50–4.43 (m, 1H), 4.20–4.15 (m, 2H), 4.15–4.10 (m, 4H), 4.08 (ddd, J = 14.16, 8.40, 3.51 Hz, 1H), 3.38–3.32 (m, 1H), 2.97 (qd, J = 21.07, 14.49 Hz, 2H), 1.61 (s, 3H), 1.35 (s, 3H), 1.30 (q, J = 7.02 Hz, 6H). ^{31}P NMR ($CDCl_3$) δ 23.03 (s). MS calcd for $C_{16}H_{26}N_6O_7P$ 446.17 $(M+H)^+$, found 446.18.

5.17. 2′,3′-*O*-Isopropylideneadenosine-5′-*N*-acetamidophosphonic acid (38)

To the solution of **37** (355 mg, 0.73 mmol) in dry MeCN (10 mL), 2,6-lutidine (460 μL, 3.65 mmol) was added followed by TMS-Br (500 μL, 3.65 mmol). The mixture was stirred overnight at rt, evaporated, diluted with EtOH and evaporated. The residue was purified by preparative HPLC with 0.01 M TEAB/70% MeCN (10–30 linear gradient) to give triethylammonium salt of **38** (420 mg, 91%). ^{1}H NMR (D_2O) δ 8.18 (s, 1H), 8.15 (s, 1H), 6.10 (d, J = 3.12 Hz, 1H), 5.41 (dd, J = 6.43, 3.16 Hz, 1H), 4.95 (dd, J = 6.41, 3.16 Hz, 1H), 4.35 (dt, J = 5.78, 5.59, 3.34 Hz, 1H), 3.43 (dd,

J = 14.40, 6.21 Hz, 1H), 3.33 (dd, J = 14.40, 5.13 Hz, 1H), 3.07 (q, J = 7.33 Hz, 6H), 2.60–2.51 (m, 2H), 1.52 (s, 3H), 1.31 (s, 3H), 1.15 (t, J = 7.33 Hz, 9H). ^{31}P NMR (D_2O) δ 14.78 (s). HRMS calcd for $C_{15}H_{20}N_6O_7P$ 427.1137 $(M-H)^-$, found 427.1209.

5.18 Mycophenolic (adenosine-5′-*N*-acetamido)phosphonate (12)

To the phosphonate **38** (85 mg, 0.0135 mmol) the protected mycophenolic alcohol **25** (49 mg, 0.0135) was added followed by dry pyridine (2 mL). To the stirred solution DIC (105 μL, 0.675 mmol) was added and a mixture was stirred at 65 °C for 2 days. After cooling to rt the mixture was evaporated and the crude intermediate **40** was deprotected by treatment with 50% HCOOH at rt overnight. After evaporation, and co-evaporation with a mixture of EtOH and water the residue was purified by preparative HPLC with 0.01 M TEAB/70% MeCN (15–30 linear gradient), dissolved in small amount of H_2O, and passed through a column of Dowex 50 WX8-200 (Na^+). Fractions containing product were combined and lyophilized to give **12** as a white powder (26 mg, 32%). ^{1}H NMR (D_2O) δ 8.27 (s, 1H), 8.10 (s, 1H), 5.92 (d, J = 6.46 Hz, 1H), 5.06 (q, J = 15.35 Hz, 2H), 4.73–4.69 (m, 1H), 4.38–4.34 (m, 1H), 4.26 (dd, J = 5.32, 3.56 Hz, 1H), 4.05–3.99 (m, 2H), 3.85 (dd, J = 14.65, 5.88 Hz, 1H), 3.78 (s, 3H), 3.58–3.51 (m, 1H), 2.91–2.78 (m, 4H), 2.02 (s, 3H). ^{31}P NMR (D_2O) δ 17.01 (s). HRMS calcd for $C_{24}H_{28}N_6O_{11}P$ 607.1559 $(M-H)^-$, found 607.1578.

5.19. Tiazofurin-5′-yl-(adenosine-5′-*N*-acetamido)phosphonate (14)

To the phosphonate **38** (85 mg, 0.0135 mmol) 2′,3′-*O*-isopropylidenetiazofurine **23** (45 mg, 0.0149 mmol) was added followed by dry pyridine (2 mL). To the stirred solution DIC (105 μL, 0.675 mmol) was added and the mixture was stirred at 65 °C for 72 h. After cooling to rt, the mixture was evaporated, the crude intermediate **39** was dissolved in 50% HCOOH (5 mL), and the solution was stirred overnight at rt. Solvents were evaporated, the residue co-evaporated with EtOH and water and purified by preparative HPLC with 0.01 M TEAB/70% MeCN (3–15 linear gradient) to give an oil, which was dissolved in small amount of H_2O and passed through a column of Dowex 50 WX8-200 (Na^+). Fractions containing product were combined and lyophilized to give **14** as a white powder (60 mg, 70%). ^{1}H NMR (D_2O) δ 8.13 (s, 1H), 8.06 (s, 1H), 7.82 (s, 1H), 5.81 (d, J = 6.14 Hz, 1H), 4.84 (d, J = 5.88 Hz, 1H), 4.58 (t, J = 5.76 Hz, 1H), 4.02–3.90 (m, 2H), 4.09 (t, J = 3.68 Hz, 2H), 4.18–4.11 (m, 3H), 3.60 (dd, J = 14.65, 5.58 Hz, 1H), 3.35 (ddd, J = 14.54, 3.08, 1.04 Hz, 1H), 2.69 (dd, J = 20.51, 7.68 Hz, 1H). ^{31}P NMR (D_2O) δ 17.13 (s). HRMS calcd for $C_{21}H_{26}N_8O_{11}PS$ 629.1185 $(M-H)^-$, found 629.1217.

5.20. 2′,3′-*O*-Isopropylideneadenosine-5′-thioethylenephosphonic acid (43)

2′,3′-*O*-Isopropylidene-5′-*S*-acetyladenosine (**41**, 0.5 g, 1.37 mmol)[25] was dissolved in MeOH (10 mL), the solution was degassed with Ar, cooled to −20 °C, and diethyl 2-bromoethylphosphonate (380 μL, 2.05 mmol) was added followed by MeONa (165 mg). The mixture was stirred overnight, evaporated, dissolved in EtOAc (100 mL), washed with H_2O, dried over $MgSO_4$, filtered, and evaporated to give the intermediate **42** as a foam (0.53 g, 79%). ^{1}H NMR ($CDCl_3$) δ 8.35 (s, 1H), 7.90 (s, 1H), 6.06 (d, J = 2.10 Hz, 1H), 5.74 (s, 2H), 5.50 (dd, J = 6.40, 2.11 Hz, 1H), 5.06 (dd, J = 6.39, 3.32 Hz, 1H), 4.40–4.34 (m, 1H), 2.89 (dd, J = 13.68, 7.41 Hz, 1H), 2.81 (dd, J = 13.70, 6.32 Hz, 1H), 2.78–2.72 (m, 2H), 2.02–1.94 (m, 2H), 1.63–1.59 (m, 3H), 1.39 (s, 3H), 1.29 (dt, J = 7.06, 2.27 Hz, 6H). ^{31}P NMR ($CDCl_3$) δ 29.21 (s). To the solution of **42** (0.52 g, 1.08 mmol) in dry MeCN (10 mL) 2,6-lutidine (0.64 mL, 5.4 mmol) was added followed by TMS-Br (0.73 mL, 5.4 mmol). The mixture was stirred overnight at rt, evaporated, diluted with EtOH, and evaporated. The crude product was purified by preparative HPLC with 70% MeCN/0.1 M TEAB (25–50 linear gradient) to give **43** (410 mg, 71% triethylammonium salt). ^{1}H NMR (D_2O) δ 8.36 (s, 1H), 8.29 (s, 1H), 6.30 (d, J = 2.79 Hz, 1H), 5.62 (dd, J = 6.48, 2.82 Hz, 1H), 5.18 (dd, J = 6.49, 3.10 Hz, 1H), 4.56 (dt, J = 6.91, 3.10 Hz, 1H), 3.25 (q, J = 7.34 Hz, 6H), 2.92 (d, J = 6.72 Hz, 1H), 2.77 (dd, J = 15.41, 7.48 Hz, 2H), 1.90–1.82 (m, 2H), 1.71 (s, 3H), 1.49 (s, 3H), 1.33 (t, J = 7.33, Hz, 9H). ^{31}P NMR (D_2O) δ 22.61 (s). HRMS calcd for $C_{15}H_{21}N_5O_6PS$ 430.0956 $(M-H)^-$, found 430.0983.

5.21. Mycophenolic ethylenephosphonate-5′-thioadenosine (15)

To the solution of **25** (86 mg, 0.24 mmol) and phosphonate **43** in dry pyridine (2 mL) DIC (186 μL, 1.2 mmol) was added. The mixture was heated at 55 °C for 24 h, cooled to rt, evaporated, and co-evaporated with EtOH to give the intermediate **44** which was dissolved in 50% HCOOH (8 mL), stirred at rt overnight, evaporated, co-evaporated with EtOH, and purified by preparative HPLC with 70% MeCN/0.1 M TEAB (20–50 linear gradient). The desired compound **15** was obtained as a triethylammonium salt. It was dissolved in water and passed through a small column of Dowex 50 WX8-200 (Na^+). Fractions containing product were combined and lyophilized to give **15** as a white powder (70 mg, 48%). ^{1}H NMR (D_2O) δ 8.33 (s, 1H), 8.14 (s, 1H), 6.04 (d, J = 4.77 Hz, 1H), 5.17 (s, 2H), 4.73 (t, J = 5.06, 1H), 4.36 (t, J = 5.29 Hz, 1H), 4.32 (dd, J = 10.93, 5.03 Hz, 1H), 3.99–3.89 (m, 2H), 3.81 (s, 3H), 3.02 (dd, J = 14.42, 4.45 Hz, 1H), 2.94 (dd, J = 14.42, 6.36 Hz, 1H), 2.90–2.80 (m, 2H), 2.61–2.51 (m, 2H), 2.05 (s, 3H), 1.79 (ddd, J = 17.02, 11.02, 6.00 Hz, 2H). ^{31}P NMR (D_2O) δ 25.18 (s). HRMS calcd for $C_{24}H_{29}N_5O_{10}PS$ 610.1378 $(M-H)^-$, found 610.1419.

5.22. 2′,3′-*O*-Isopropylideneadenosine-8-thioethylenephosphonic acid (48)

To a suspension of K_2CO_3 (140 mg, 1.02 mmol) in DMF (2 mL) 2′,3′-*O*-isopropylidene-8-mercaptoadenosine (**46**) (prepared from **45** as described[24] (288 mg, 0.85 mmol) was added followed by diethyl 2-bromoethylphopsphonate (187 mg, 1.02 mmol). The mixture was stirred overnight at rt, evaporated, dissolved in EtOAc (100 mL), washed with H_2O, dried, and evaporated to give intermediate **47** as a foam. ^{1}H NMR ($CDCl_3$) δ 8.23 (s, 1H), 6.55 (dd, J = 11.81, 1.27 Hz, 1H), 5.95 (d, J = 5.27 Hz, 1H), 5.53 (s, 2H), 5.21 (t, J = 5.46 Hz, 1H), 5.06 (dd, J = 5.62, 0.60 Hz, 1H), 4.51 (s, 1H), 4.20–4.07 (m, 4H), 3.98–3.93 (m, 1H), 3.77 (dt, J = 12.89, 1.68 Hz, 1H), 3.59–3.41 (m, 2H), 2.34 (ddd, J = 18.31, 8.58, 7.73 Hz, 2H), 1.67 (s, 3H), 1.37 (s, 3H), 1.35 (t, J = 7.08 Hz, 6H). To a solution of **47** (0.428 g, 0.85 mmol) in dry MeCN (10 mL) 2,6-lutidine (0.5 mL, 4.25 mmol) was added followed by TMS-Br (0.572 mL, 4.25 mmol). The mixture was stirred overnight at rt, evaporated, diluted with EtOH and evaporated again. The crude product was purified by preparative HPLC with 70% MeCN/0.1 M TEAB (25-50 linear gradient) to give **48** as a foam (360 mg, 77% triethylammonium salt). ^{1}H NMR (D_2O) δ 8.10 (s, 1H), 6.22–6.13 (m, 1H), 5.48–5.40 (m, 1H), 5.18–5.08 (m, 1H), 4.47–4.38 (m, 1H), 3.88 (dd, J = 12.51, 3.49 Hz, 1H), 3.83 (dd, J = 12.48, 4.75 Hz, 1H), 3.53–3.40 (m, 1H), 3.21 (q, J = 7.33 Hz, 4H), 2.04 (td, J = 17.01, 8.46 Hz, 2H), 1.70 (s, 3H), 1.45 (s, 3H), 1.30 (t, J = 7.33 Hz, 6H)). ^{31}P NMR (D_2O) δ 20.57 (s). HRMS calcd for $C_{15}H_{21}N_5O_7PS$ 446.0905 $(M-H)^-$, found 446.0979.

5.23. Mycophenolic ethylenephosphonate-8-thioadenosine (16)

To the solution of dried **48** in DMF (5 mL) imidazole (103 mg, 1.53 mmol) was added followed by TBDMS-Cl (122 mg, 0.81 mmol). The mixture was stirred at rt overnight, evaporated, and crude product was purified by HPLC with 70% MeCN/0.1 M TEAB (20–50 linear gradient) to give the intermediate **49** as a foam (not stable—partially decomposing into substrate) which was used immediately in the next step. The carefully dried **49** (65 mg, 0.1 mmol) and **25** (36 mg, 0.1 mmol) were dissolved in dry pyridine (1 mL), followed by addition of DIC (80 μL, 0.5 mmol). The mixture was stirred at rt for a few minutes and then heated at 58 °C for 48 h. The crude intermediate **50** was dissolved in 50% HCOOH (8 mL) and stirred at rt overnight, evaporated, co-evaporated with EtOH. It was then purified by preparative HPLC with 70% MeCN/0.1 M TEAB (20–50 linear gradient), dissolved in water, and passed through a small column of Dowex 50 WX8-200 (Na^+). Fractions containing product were combined and lyophilized to give **16** as a white powder (22 mg, 35%). ^{1}H NMR (D_2O) δ 8.15 (s, 1H), 6.06 (d, J = 7.12 Hz, 1H), 4.99 (dd, J = 7.03, 5.52 Hz, 1H), 4.97 (s, 2H), 4.50 (dd, J = 5.45, 2.41 Hz, 1H), 4.35 (q, J = 2.65 Hz, 1H), 4.04 (q, J = 6.40 Hz, 2H), 4.00 (dd, J = 12.92, 2.52 Hz, 1H), 3.91 (dd, J = 12.90, 3.21 Hz, 1H), 3.82 (s, 3H), 3.07–3.00 (m, 2H), 2.94 (t, J = 6.34 Hz, 2H), 2.03 (s, 3H), 1.95–1.87 (m, 2H). ^{31}P NMR (D_2O) δ 24.28 (s). HRMS calcd for $C_{24}H_{29}N_5O_{11}PS$ 626.1378 $(M-H)^-$, found 626.1327.

5.24. Enzyme assays and cytotoxicity

5.24.1. IMPDH

Human IMPDH type I and type II were expressed and purified as previously described[26,27] with our recent modifications.[16] Inhibition assays were performed as previously described.[28] Briefly, assays were set up in duplicate using two different concentrations of IMPDH type I (87 and 155 nM) and type II (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 versus inhibitor concentration to a simple binding model with Dynafit.[29]

5.25. Cytotoxicity of MAD analogues to human myelogenous leukemia K562 cells

Logarithmically growing K562 cells in RPMI 1640 medium supplemented with 10% fetal bovine serum were plated in 96-well plates at a density of 800 cells/0.1 mL and incubated at 37 °C in an atmosphere of air and 5% CO_2. Twenty-four hours later, various concentrations of the compounds were added in 3 μL volume, mixed and further incubated for 72 h. At the end of the incubation period, 20 μL of cellTiter 96® reagent (Promega, Madison, WI) containing tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt; MTS] was added and incubated at 37 °C in an atmosphere of air and 5% CO_2 and then the color developed was read at 490 nm using SpectraMax software in a microplate spectrophotometer (Molecular Devices Corp., Sunnyvale, CA) and the data were analyzed by GraphPad Prism 4 software (GraphPad Software, San Diego, CA) as described earlier.[30]

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Supplementary data

Supplementary data (table of HPLC purity of compounds **2**–**16**. This material is available free of charge via the Internet) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.01.042.

References and notes

1. Pankiewicz, K. W.; Goldstein, B. M. In *Inosine Monophosphate Dehydrogenase: A Major Therapeutic Target*; Oxford University Press: Washinghton, 2003; Vol. 839,
2. Hedstrom, L. *Chem. Rev.* **2009**, *109*, 2903.
3. Natsumeda, Y.; Ohno, S.; Kawasaki, H.; Konno, Y.; Weber, G.; Suzuki, K. *J. Biol. Chem.* **1990**, *265*, 5292.
4. Collart, F. R.; Huberman, E. *J. Biol. Chem.* **1988**, *263*, 15769.
5. Hager, P. W.; Collart, F. R.; Huberman, E.; Mitchell, B. S. *Biochem. Pharmacol.* **1995**, *49*, 1323.
6. Chong, C. R.; Qian, D. Z.; Pan, F.; Wei, Y.; Pili, R.; Sullivan, D. J., Jr.; Liu, J. O. *J. Med. Chem.* **2006**, *49*, 2677.
7. Collart, F. R.; Chubb, C. B.; Mirkin, B. L.; Huberman, E. *Cancer Res.* **1992**, *52*, 5826.
8. Balzarini, J.; De Clercq, E. *Biochem. J.* **1992**, *287*, 785.
9. Chen, L.; Petrelli, R.; Gao, G.; Bonnac, L.; Yu, J. S.; Bennett, E. M.; Pankiewicz, K. W. *Curr. Med. Chem.* **2008**, *15*, 650.
10. Pankiewicz, K. W.; Lesiak-Watanabe, K. B.; Watanabe, K. A.; Patterson, S. E.; Jayaram, H. N.; Yalowitz, J. A.; Miller, M. D.; Seidman, M.; Majumdar, A.; Prehna, G.; Goldstein, B. M. *J. Med. Chem.* **2002**, *45*, 703.
11. Pankiewicz, K. W.; Patterson, S. E.; Black, P. L.; Jayaram, H. N.; Risal, D.; Goldstein, B. M.; Stuyver, L. J.; Schinazi, R. F. *Curr. Med. Chem.* **2004**, *11*, 887.
12. Chen, L.; Gao, G.; Felczak, K.; Bonnac, L.; Patterson, S. E.; Wilson, D.; Bennett, E. M.; Jayaram, H. N.; Hedstrom, L.; Pankiewicz, K. W. *J. Med. Chem.* **2007**, *50*, 5743.
13. Radujkovic, A.; Schad, M.; Topaly, J.; Veldwijk, M. R.; Laufs, S.; Schultheis, B. S.; Jauch, A.; Melo, J. V.; Fruehauf, S.; Zeller, W. J. *Leukemia* **2005**, *19*, 1198.
14. Rejman, D.; Olesiak, M.; Chen, L.; Patterson, S. E.; Wilson, D.; Jayaram, H. N.; Hedstrom, L.; Pankiewicz, K. W. *J. Med. Chem.* **2006**, *49*, 5018.
15. Chen, L.; Petrelli, R.; Olesiak, M.; Wilson, D. J.; Labello, N. P.; Pankiewicz, K. W. *Bioorg. Med. Chem.* **2008**, *16*, 7462.
16. Chen, L.; Wilson, D. J.; Xu, Y.; Aldrich, C. C.; Felczak, K.; Sham, Y. Y.; Pankiewicz, K. W. *J. Med. Chem.* **2009**, *53*, 4768.
17. Yamazaki, A.; Kumashiro, I.; Takenishi, T. *J. Org. Chem.* **1967**, *32*, 3258.
18. Davisson, J.; Davis, D. R.; Dixit, V. M.; Poulter, C. D. *J. Org. Chem.* **1987**, *52*, 1794.
19. Boyle, N. A. *Org. Lett.* **2006**, *8*, 187.
20. Gebeyehu, G.; Marquez, V. E.; Kelley, J. A.; Cooney, D. A.; Jayaram, H. N.; Johns, D. G. *J. Med. Chem.* **1983**, *26*, 922.
21. Kosolapoff, G. M.; Brown, J. D. *J. Chem. Soc. (C)* **1966**, 757.
22. Townsend, A. P.; Roth, S.; Williams, H. E. L.; Stylianou, E.; Thomas, N. R. *Org. Lett.* **2009**, *11*, 2976.
23. Manfredini, S.; Solaroli, N.; Angusti, A.; Nalin, F.; Durini, E.; Vertuani, S.; Pricl, S.; Ferrone, M.; Spadari, S.; Focher, F.; Verri, A.; De Clercq, E.; Balzarini, J. *Antiviral Chem. Chemother.* **2003**, *14*, 183.
24. Ji, C.; Kuo, W.; Chi, C.; Ming Jyh, C.; Lee Tai, L. *Nucleosides Nucleotides* **1993**, *12*, 941.
25. Marc, P.; Goran, P.; Elmar, W. *Eur. J. Org. Chem.* **2000**, *3*, 549.
26. Farazi, T.; Leichman, J.; Harris, T.; Cahoon, M.; Hedstrom, L. *J. Biol. Chem.* **1997**, *272*, 961.
27. Mortimer, S. E.; Hedstrom, L. *Biochem. J.* **2005**, *390*, 41.
28. Umejiego, N. N.; Li, C.; Riera, T.; Hedstrom, L.; Striepen, B. *J. Biol. Chem.* **2004**, *279*, 40320.
29. Kuzmic, P. *Anal. Biochem.* **1996**, *237*, 260.
30. Zhang, Y.; Guo, L.; Roeske, R. W.; Antony, A. C.; Jayaram, H. N. *Anal. Biochem.* **2004**, *332*, 168.