Fructose-1,6-bisphosphatase Inhibitors. 1. Purine Phosphonic Acids as Novel AMP Mimics

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Inhibition of FBPase is considered a promising way to reduce hepatic gluconeogenesis and therefore could be a potential approach to treat type 2 diabetes. Herein we report the discovery of a series of purine phosphonic acids as AMP mimics targeting the AMP site of FBPase, which was achieved using a structure-guided drug design approach. These non-nucleotide purine analogues inhibit FBPase in a similar manner and with similar potency as AMP. More importantly, several purine analogues exhibited potent cellular and in vivo glucose-lowering activities, thus achieving proof-of-concept for inhibiting FBPase as a drug discovery target. For example, compounds **4.11** and **4.13** are as equipotent as AMP with regard to FBPase inhibition. Furthermore, compound **4.11** inhibited glucose production in primary rat hepatocytes and significantly lowered blood glucose levels in fasted rats.

Type 2 diabetes mellitus (T2DM[a]) is an increasingly widespread disease, approaching epidemic proportions worldwide.[1] Hyperglycemia is a hallmark feature of T2DM, and there is a strong correlation between fasting blood glucose levels and increased endogenous glucose production (EGP).[2] It has also been established that the gluconeogenesis (GNG, a metabolic pathway through which glucose is synthesized from noncarbohydrate carbon substrates such as pyruvate, lactate, glycerol, and glucogenic amino acids) component of EGP is responsible for the excess hepatic production of glucose in T2DM.[3−5] Therefore, key regulatory enzymes of the GNG pathway are often explored as potential drug discovery targets. Inhibitors of fructose-1,6-bisphosphatase (FBPase), a rate-limiting enzyme of the GNG pathway, consequently represent a new class of potential drug candidates for the treatment of T2DM.[6] Over the past two decades, various classes of FBPase inhibitors have been reported.[7−12] However, no suitable drug candidates were identified until recently when we described compound **1**, a potent and selective FBPase inhibitor,[13] which was discovered as a novel 5′-adenosinemonophosphate (AMP) mimic (Figure 1) using a structure-guided drug design approach.[14,15]

Here we report the design, synthesis, and evaluation of purine phosphonic acids as FBPase inhibitors. These phosphonate analogues function as AMP mimetics that bind to the AMP site of FBPase, leading to potent inhibition of the enzyme. The structure−activity relationships (SAR) established with these purine analogues proved instrumental to the discovery of other series of heterocyclic phosphonic acids. Further optimization of these novel AMP mimetics culminated in the discovery of orally bioavailable FBPase inhibitors to be studied in the clinic as a potential treatment for T2DM.[16]

Inhibitor Design. X-ray crystallographic studies of human FBPase complexed with AMP[17] indicated that the phosphate group forms a constellation of hydrogen bond interactions that are essential for binding affinity. Consequently, our initial efforts to design FBPase-specific AMP mimics focused on the 5′-phosphate group. The first challenge was the identification of a phosphate surrogate that retained most of the associated binding affinity while exhibiting good stability in biological fluids.

Phosphonates are structurally and electronically very similar to phosphates and have been successfully applied as phosphate mimics.[18] However, it was surprising to see that the direct 5′-phosphonate AMP analogue **A** (Figure 2) exhibited a more than 2000-fold loss in inhibitory potency,[19,20] suggesting that the discovery of a suitable phosphate mimic is challenging and might require novel design approaches. Supported by crystallography and computational chemistry, we utilized a structure-guided drug design approach in our search for novel AMP mimics as FBPase inhibitors. Careful examination of the FBPase−AMP complex revealed that the phosphate binding site might be accessed from the 8-position of the purine base. Accordingly, we sought a suitable linker to connect a phosphate mimic to that position.

Synthesis of 8-Substituted Purine Analogues. The adenosine analogues with a carboxylic acid linked to the C8-positon of the purine were prepared as shown in Scheme 1. Stille coupling of ethyl (*E*)-3-(tributylstannanyl)-acrylate (**3**) with 8-bromoadenosine gave compound **4** in 72% yield, and subsequent saponification of the ethyl ester gave analogue **1.1** in 94% yield. Direct hydrogenation of compound **1.1** gave analogue **1.2** in 85% yield.

The adenosine and purine analogues with either an aminoalkyl or an amide linker were prepared as shown in Scheme 2. Direct substitution of either 8-bromoadenosines or 8-bromoadenines with primary amines gave analogues **1.3**−**1.9** and **2.1**−**2.4** (7−85% yields). The amide-linked adenine analogue **2.6** was prepared using a four-step sequence. Carbonylation of 8-bromo-9-phenethyl-adenine (**5a**) gave the corresponding methyl ester **6** in 73% yield. Saponification of the ester to its corresponding

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[a] Abbreviations: FBPase, fructose 1,6-bisphosphatase; OBAV, oral bioavailability; T2DM, type 2 diabetes mellitus; EGP, endogenous glucose production; GNG, gluconeogenesis; AMP, 5′-adenosinemonophosphate; ZMP, 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside-5-monophosphate.

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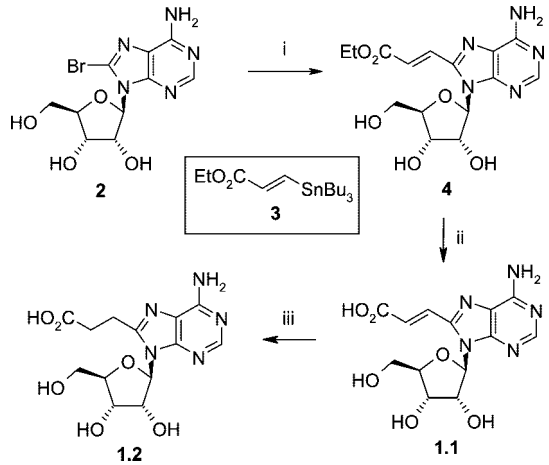
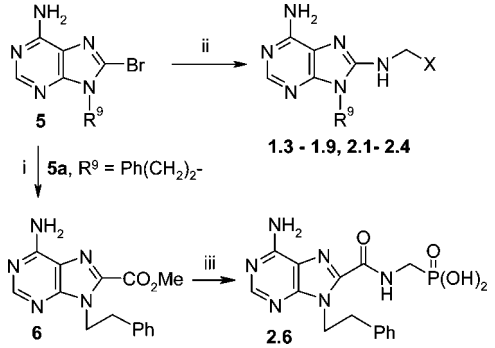
Scheme 1[a]



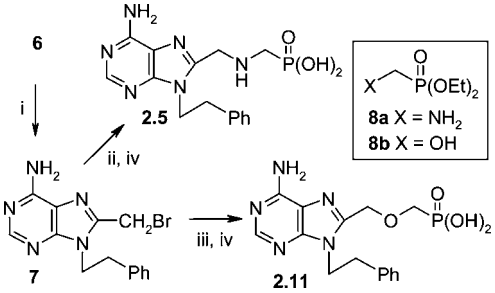
[a] Conditions and reagents: (i) **3**, $Pd(PPh_3)_4$, DMF, 80 °C; (ii) NaOH, THF-MeOH-H_2O; (iii) H_2, Pd−C, NaOH (aq).

Scheme 2[a]



[a] Conditions and reagents: (i) CO (50 psi), $Pd(PPh_3)_4$, MeOH, 80 °C; (ii) H_2NCH_2X, NaOH, EtOH-H_2O; (iii) NaOH, THF-EtOH-H_2O; **8a**, EDCI, HOBt, DMF; TMSBr, MeCN.

Scheme 3[a]

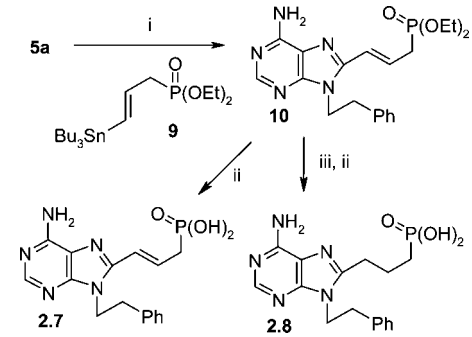


[a] Conditions and reagents: (i) $LiAlH_4$, THF; PBr_3, CH_2Cl_2; (ii) **8a**, Et_3N, DMF; (iii) **8b**, NaH, DMF; (iv) TMSBr, MeCN.

acid, followed by EDCI coupling with diethyl aminomethylphosphonate (**8a**) and final TMSBr-mediated removal of the phosphonate diester, gave compound **2.6** in 14% yield over the three steps.

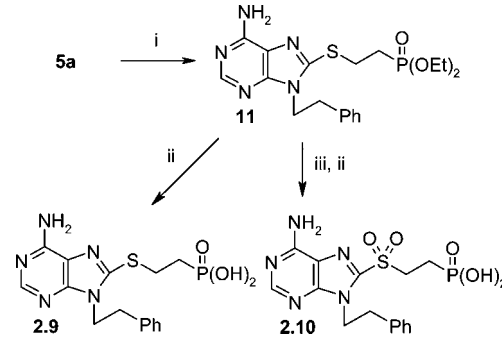
Compound **6** was also used to prepare methylaminomethyl adenine analogue **2.5** and methoxymethyl adenine analogue **2.11**, as shown in Scheme 3. Reduction of ester **6** to its corresponding alcohol, followed by conversion to the corresponding bromide, gave compound **7** in 49% yield over the two steps. Substitution of bromide **7** with diethyl aminomethylphosphonate (**8a**), followed by deprotection of the phosphonate ester, gave analogue **2.5** in 45% yield over the two steps. Alternatively, substitution of bromide **7** with diethyl hydroxymethylphosphonate (**8b**), followed by deprotection of the phosphonate ester, gave analogue **2.11** in 21% yield over the two steps.

Scheme 4[a]



[a] Conditions and reagents: (i) **9**, $Pd(PPh_3)_4$, DMF; (ii) TMSBr, MeCN; (iii) H_2, Pd−C, MeOH.

Scheme 5[a]



[a] Conditions and reagents: (i) Na_2S, DMSO-H_2O; $H_2C{=}CH\text{-}PO(OEt)_2$, DMSO; (ii) TMSBr, MeCN; (iii) *m*-CPBA, CH_2Cl_2.

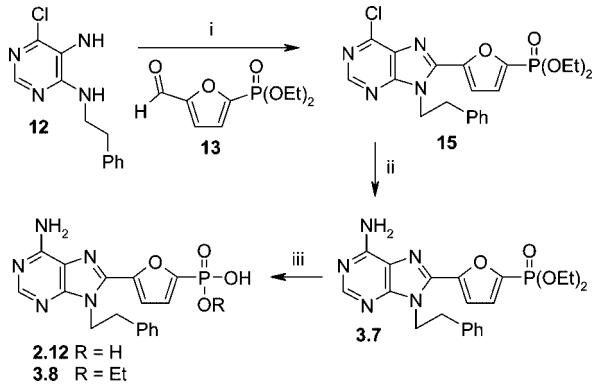
The carbon linked analogues **2.7** and **2.8** were readily prepared from 8-bromo-9-phenethyladenine (**5a**), as depicted in Scheme 4. Stille coupling of diethyl ((*E*)-3-tributylstannyl-allyl)-phosphonate (**9**) with **5a** gave compound **10** in 72% yield. Standard TMSBr-mediated removal of the phosphonic acid diethyl ester gave analogue **2.7** in 59% yield. Hydrogenation of compound **10**, followed by TMSBr-mediated removal of the phosphonate diester, gave analogue **2.8** in 31% yield over the two steps.

Two sulfur-containing linker analogues **2.9** and **2.10** were also prepared from **5a** (Scheme 5). Treatment of **5a** with sodium sulfide followed by reaction with diethyl vinylphosphonate gave compound **11** in 51% yield over the two steps. Compound **11** was deprotected using TMSBr to give analogue **2.9** in 66% yield. Oxidation of **11** to its corresponding sulfone, followed by TMSBr deprotection of the phosphonate ester, gave analogue **2.10** in 27% yield over the two steps.

The 2,5-furandiyl linker analogues were readily prepared from 5-diethylphosphono-2-furaldehyde (**13**)[19] using a $FeCl_3$-promoted cyclization reaction,[21] as shown in Scheme 6. Thus, treatment of compound **12** with 5-diethylphosphono-furan-2-aldehyde (**13**) under the $FeCl_3$-promoted purine formation reaction conditions gave compound **15** in 90% yield. Amination with ammonia, followed by deprotection of the phosphonate diester with excess TMSBr, gave analogue **2.12** in 77% yield over the two steps. Partial deprotection of compound **3.7** using only two equivalents of TMSBr gave the monoester analogue **3.8** in 13% yield.

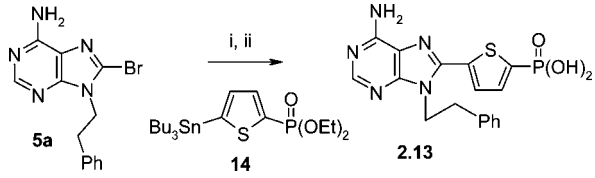
The thiophene-linked analogue **2.13** was prepared from compound **5a** via a two-step sequence depicted in Scheme 7. Stille coupling of compound **5a** with diethyl 5-tri-*n*-butylstannyl-

Scheme 6[a]



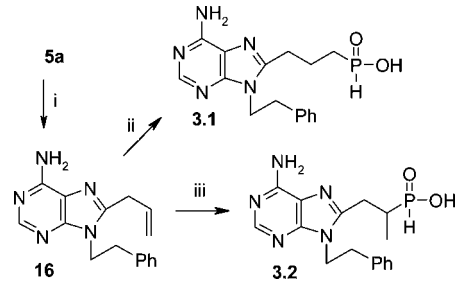
[a] Conditions and reagents: (i) **13**, $FeCl_3-SiO_2$, DMSO, 80 °C; (ii) NH_3, DMSO-THF; (iii) TMSBr, MeCN.

Scheme 7[a]



[a] Conditions and reagents: (i) **14**, $Pd(PPh_3)_4$, DMF, 85 °C; (ii) TMSBr, MeCN.

Scheme 8[a]



[a] Conditions and reagents: (i) $Bu_3SnCH_2CH{=}CH_2$, $Pd(PPh_3)_4$, DMF, 80 °C; (ii) NaH_2PO_2, AIBN, MeOH, 110 °C, sealed tube; (iii) NaH_2PO_2, AIBN, H_2SO_4, EtOH, 80 °C.

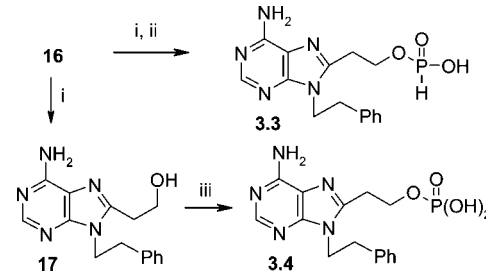
thiophen-2-yl-phosphonate (**14**), followed by the standard TMSBr-mediated removal of the phosphonate diester, gave compound **2.13** in 48% yields over the two steps.

Two H-phosphinate analogues were also prepared as potential AMP mimics (Scheme 8). Stille coupling of **5a** with allyltributyl-stannane gave 8-allyl-9-phenethyladenine (**16**) in 15% yield. Hydrophosphorylation of **16** under two different radical reaction conditions gave analogues **3.1** and **3.2** in 2.1% and 4.9% yields, respectively.

The 8-allyl-9-phenethyladenine (**16**) was also used to prepare H-phosphinate and phosphate purine analogues **3.3** and **3.4** as shown in Scheme 9. Ozonolysis of **16**, followed by sodium borohydride reduction of the resulting aldehyde, gave 8-hydroxyethyl-9-phenethyladenine (**17**) in 25% yield over the two steps. Compound **17** was converted to analogue **3.3** using Froehler's procedure.[22] Alternatively, compound **17** was used to prepare the phosphate analogue **3.4** using Johns' procedure.[23]

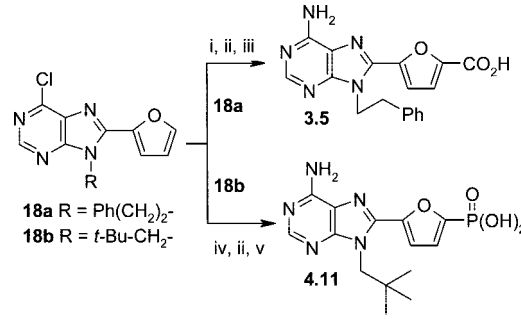
The carboxylate analogue **3.5** was prepared from compound **18a**[21] via a three-step procedure shown in Scheme 10. Thus, selective lithiation of the 5-position of the furan group using LDA at −78 °C, followed by quenching of the resulting anion with ethyl chloroformate, gave ethyl 5-(6-chloro-9-phenethyl-

Scheme 9[a]



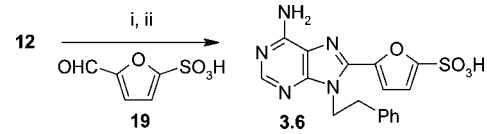
[a] Conditions and reagents: (i) O_3, CH_2Cl_2, −78 °C; $NaBH_4$, EtOH. (ii) PCl_3, Et_3N, imidazole, CH_3CN. (iii) $Et_2NP(O\text{-}t\text{Bu})_2$, tetrazole, CH_2Cl_2; *m*-CPBA, CH_2Cl_2; HCl.

Scheme 10[a]



[a] Conditions and reagents: (i) LDA, THF, −78 °C; $ClCO_2Et$. (ii) NH_3, DMSO-THF. (iii) NaOH, THF-EtOH-H_2O. (iv) LDA, THF, −78 °C; $ClPO(OEt)_2$. (v) TMSBr, MeCN.

Scheme 11[a]



[a] Conditions and reagents: (i) **19**, $FeCl_3-SiO_2$, DMSO, 80 °C; (ii) NH_3, DMSO-THF.

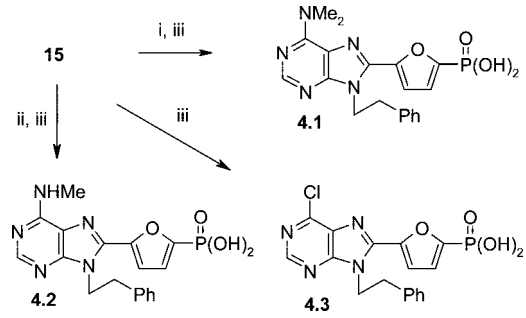
9*H*-purin-8-yl)-furan-2-carboxylate (61%), which was reacted with ammonia and saponified to give analogue **3.5** in 10% yield over the two steps. Similarly, analogue **4.11** was prepared from compound **18b** in three steps with an overall yield of 55% (Scheme 10).

The sulfonate analogue **3.6** was prepared as shown in Scheme 11. Treatment of **12** with 5-formyl-2-furansulfonic acid (**19**) under the $FeCl_3$-promoted purine formation reaction conditions gave 5-(6-chloro-9-phenethyl-9*H*-purin-8-yl)-furan-2-sulfonic acid, which was converted to **3.6** via an amination reaction (27% over the two steps).

The C6 purine phosphonate analogues **4.1**−**4.3** were readily prepared from compound **15** using the sequence shown in Scheme 12. Treatment of compound **15** with *N*,*N*-dimethylamine in DMSO, followed by TMSBr-mediated deprotection, gave analogue **4.1** (84% yield over the two steps), while reaction of **15** with *N*-methylamine in DMSO, followed by deprotection, gave analogue **4.2** (33% yield over the two steps). The C6-chloro analogue **4.3** was obtained in 43% yield directly from **15** via TMSBr-mediated removal of the diethyl phosphonate ester.

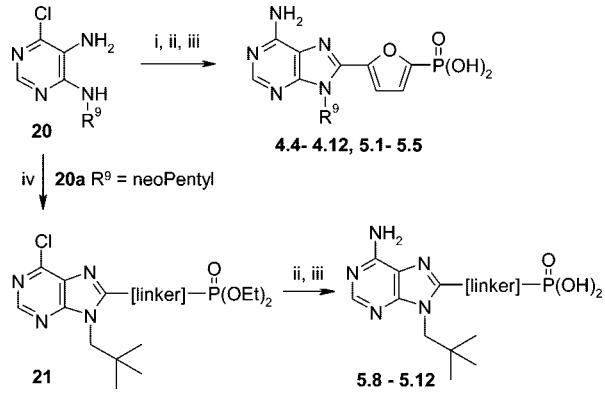
To rapidly explore the N^9-SAR of the furan-linked phosphonate analogues, an efficient four-step sequence was developed (Scheme 13). Compounds **20**, which were readily prepared using our previously reported procedure,[21] were cyclized with alde-

Scheme 12[a]



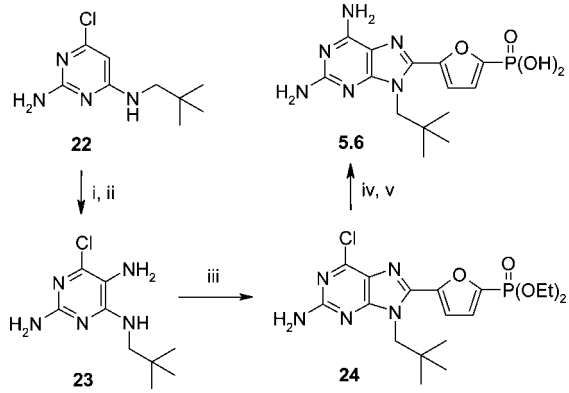
[a] Conditions and reagents: (i) Me_2NH, DMSO; (ii) $MeNH_2$, DMSO; (iii) TMSBr, CH_2Cl_2.

Scheme 13[a]



[a] Conditions and reagents: (i) **13**, $FeCl_3-SiO_2$, DMSO, 80 °C; (ii) NH_3, DMSO-THF; (iii) TMSBr, MeCN; (iv) OHC-[linker]-$PO(OEt)_2$, $FeCl_3-SiO_2$, DMSO, 80 °C.

Scheme 14[a]

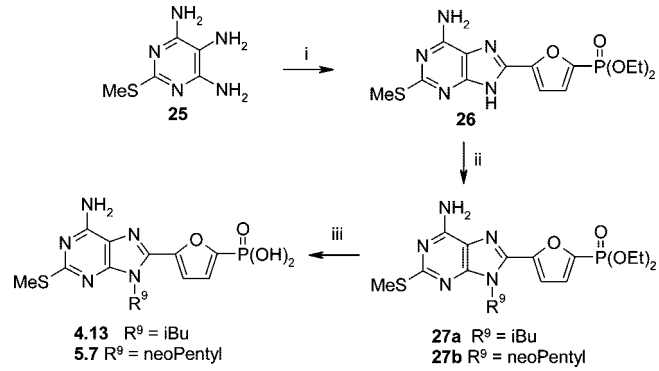


[a] Conditions and reagents: (i) 4-Cl-$C_6H_4-N_2PF_6$, EtOH-H_2O; (ii) Zn, AcOH; (iii) **13**, $FeCl_3-SiO_2$, DMSO, 100 °C; (iv) NH_3, DMSO-THF; (v) TMSBr, CH_2Cl_2.

hyde **13** in the presence of $FeCl_3-SiO_2$; subsequent amination with ammonia in DMSO, followed by TMSBr-mediated deprotection of the phosphonate ester, gave analogues **4.4**−**4.12** and **5.1**−**5.5**. Alternatively, compound **20a** was cyclized with various aryl aldehydes (OHC-[linker]-$PO(OEt)_2$, see Table 5 for list of linker groups) to prepare analogues **5.8**−**5.12**.

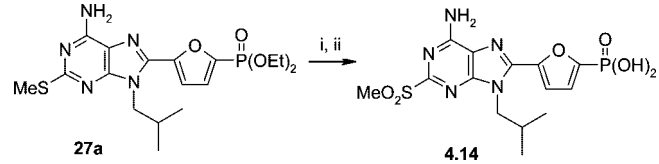
The 2-aminoadenine phosphonic acid analogue **5.6** was prepared as shown in Scheme 14. Pyrimidine **22** (prepared via direct substitution of 2-amino-4,6-dichloropyrimidine with neopentylamine) was converted to the corresponding 5-amino derivative **23** using a two-step sequence: treatment with 4-chlorophenyldiazonium hexafluorophosphate salt to give a 5-(4-chlorophenyldiazo) derivative and reduction of the diazo group

Scheme 15[a]



[a] Conditions and reagents: (i) **13**, $FeCl_3-SiO_2$, DMSO, 80 °C; (ii) NaH, DMF, 0 °C, *i*Bu-I or neoPentyl-I; (iii) TMSBr, MeCN.

Scheme 16[a]



[a] Conditions and reagents: (i) oxone, MeOH-H_2O; (ii) TMSBr, MeCN.

to the corresponding amine using zinc in acetic acid (30% yield over the two steps). Purine ring formation was accomplished using the $FeCl_3$-promoted cyclization with aldehyde **13** to give compound **24**. Amination of **24** using ammonia followed by deprotection of the phosphonate diethyl ester gave analogue **5.6**.

The 2-(methylthio)adenine analogues **4.13** and **5.7** were prepared as shown in Scheme 15. Treatment of pyrimidine **25** with aldehyde **13** under the $FeCl_3-SiO_2$ promoted cyclization reaction conditions gave compound **26** in 66% yield. Alkylation of compound **26** with either isobutyl iodide or neopentyl iodide in the presence of sodium hydride gave compounds **27a** and **27b**, respectively. Deprotection of the phosphonate diethyl esters **27a** and **27b** gave analogues **4.13** and **5.7**, respectively.

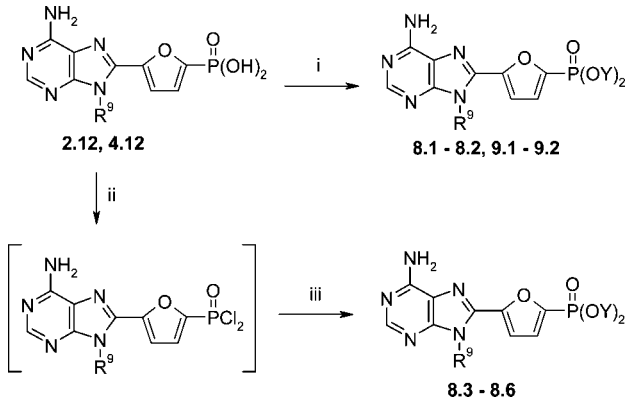
Alternatively, compound **27a** was oxidized to its corresponding C2-methanesulfonyl derivative, and subsequent TMSBr-mediated removal of the diethyl phosphonate ester gave analogue **4.14** in 42% yield over the two steps, as shown in Scheme 16.

Various prodrugs of the two lead purine FBPase inhibitors **2.12** and **4.11** were prepared as shown in Scheme 17 (Table 8 and 9). Prodrugs of the acyloxyalkyl ester type were prepared using the reported procedure[13] via alkylation reactions with the suitable electrophiles (**8.1**−**8.2**, **9.1**−**9.2**), while other ester prodrugs (**8.3**−**8.6**) were prepared via the phosphonic dichloride.[24]

Inhibition of Human Liver FBPase by 8-Substituted Purine Analogues. Working under the assumption that the phosphate and the ribosyl groups are important for AMP to function as an FBPase inhibitor, adenosine was selected as the initial scaffold for lead generation and various acidic groups were linked to the 8-position of the purine base in order to find a suitable phosphate mimic (Table 1).

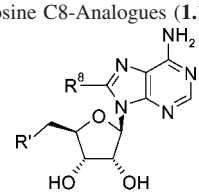
Initially, carboxylic acids with various 2- to 4-atom linkers were explored. Analogues **1.1**−**1.4** showed little inhibition of FBPase (Table 1), which may be due to the poor mimicry of AMP by these carboxylate analogues both electronically and geometrically. Next, we examined phosphonic acids because they more closely resemble a phosphate. The 2- and 4-atom

Scheme 17[a]



[a] Conditions and reagents: (i) I-Y, Hunig's base, MeCN; (ii) thionyl chloride; (iii) HO-Y, Hunig's base, DMF.

Table 1. SAR of Adenosine C8-Analogues (**1.1**−**1.9**)[a]



compd	R^8	R′	IC_{50} (μM)
AMP			1
1.1	−CH=CHCO$_2$H	OH	≫100[b]
1.2	−(CH$_2$)$_2$CO$_2$H	OH	≫100[b]
1.3	−NHCH$_2$CO$_2$H	OH	≫100[b]
1.4	−NH(CH$_2$)$_2$CO$_2$H	OH	≫100[b]
1.5	−NHCH$_2$PO$_3$H$_2$	OH	≫100[b]
1.6	−NH(CH$_2$)$_2$PO$_3$H$_2$	OH	100
1.7	−NH(CH$_2$)$_3$PO$_3$H$_2$	OH	≫100[b]
1.8	−NH(CH$_2$)$_2$OPO$_3$H$_2$	OH	140
1.9	−NH(CH$_2$)$_2$PO$_3$H$_2$	H	100

[a] Inhibition of human liver FBPase is reported as IC_{50} values. [b] <25% inhibition at 100 μM.

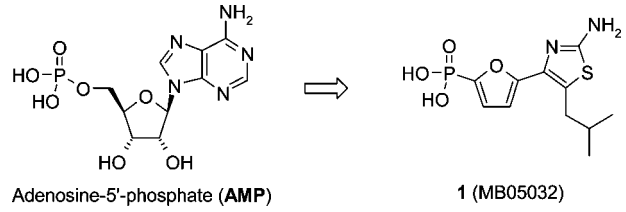


Figure 1. Compound **1** as a novel AMP mimic.

linked analogues **1.5** and **1.7** (Table 1) were inactive, but the 3-atom linked analogue **1.6** showed moderate inhibition of FBPase, with an IC_{50} of 100 μM. This represented a significant observation given that the AMP analogue wherein the phosphate was replaced with $CH_2PO_3^{2-}$ (compound **A**, Figure 2) showed an IC_{50} of 2000 μM.[19] The 20-fold enhancement in potency suggested that the 8-position enabled better alignment of the phosphonic acid in the phosphate-binding pocket of the AMP site compared to the 5′-position. The 4-atom linked phosphate analogue **1.8** showed FBPase inhibition comparable to the 3-atom linked phosphonate analogue **1.6**. This observation suggests that electronic effects are also important because computer modeling studies indicate a 3-atom linker provides the optimum distance.[19] Removing the 5′-hydroxyl group of analogue **1.6** gave analogue **1.9**, which retained similar potency, suggesting that this hydroxyl group is not critically involved in binding to FBPase. Although the inhibitory activity of analogue

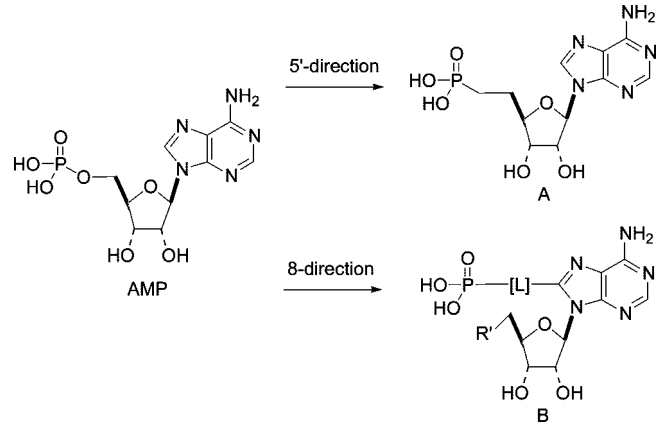


Figure 2. Phosphonates that might mimic AMP binding interactions to FBPase.

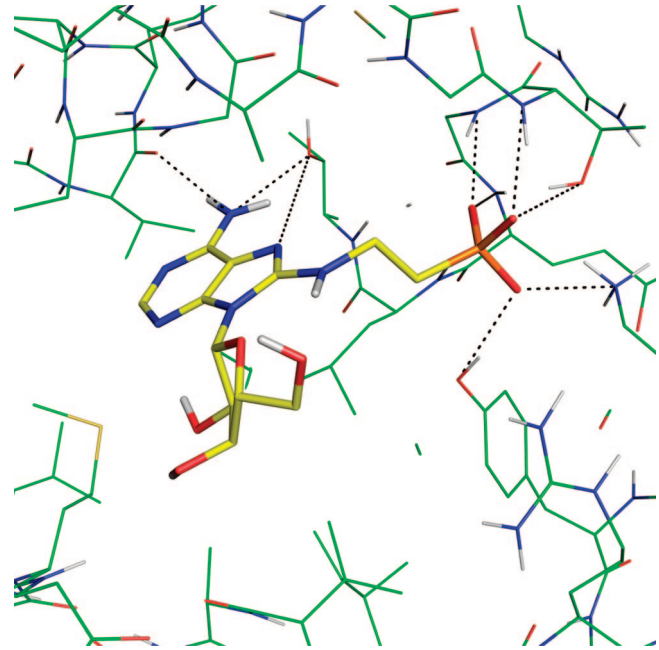
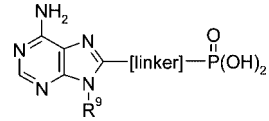


Figure 3. X-ray structural analysis of **1.6** bound to FBPase.

1.6 was still weak, this discovery provided encouragement to further pursue this scaffold using our structure-guided drug discovery approach. Thus, an X-ray crystal structure of compound **1.6** bound to FBPase was obtained (Figure 3). Detailed analysis of the interactions between compound **1.6** and FBPase provided key insights into the binding mode of this compound.

Compound **1.6** does indeed bind to the AMP site of FBPase. Analysis of the structural information showed that the adenine base formed three hydrogen bonds in the same manner as AMP. More importantly, the phosphonate group linked via the 8-position of the adenine serves as an excellent mimic of the binding interactions between the phosphate of AMP and FBPase. Six strong hydrogen bonds are formed between the phosphonate group and the enzyme just as with the phosphate group of AMP. An important finding was the lack of hydrogen bond interactions between the ribose moiety and the enzyme. This was not unexpected, given precedent from inhibitors of purine nucleoside phosphorylase wherein the ribose is not essential for binding.[25−27] Because the polar hydroxyl groups of the ribose moiety do not enhance binding affinity but require higher desolvation energy, the presence of the ribosyl group likely contributed to the moderate activity of compound **1.6**. It was therefore logical

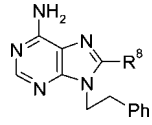
Table 2. Linker SAR of Phosphonate Analogues[a]



compd	[linker]	R^9	IC_{50}, μM
2.1	$-NH(CH_2)_2-$	H	>100[b]
2.2	$-NH(CH_2)_2-$	Bn	91
2.3	$-NH(CH_2)_2-$	$Ph(CH_2)_2-$	100
2.4	$-NH(CH_2)_2-$	2-naphthyl-CH_2-	35
2.5	$-CH_2NHCH_2-$	$Ph(CH_2)_2-$	>500
2.6	$-CONHCH_2-$	$Ph(CH_2)_2-$	100
2.7	$-(CH_2)_3-$	$Ph(CH_2)_2-$	100
2.8	$-CH{=}CHCH_2-$	$Ph(CH_2)_2-$	100
2.9	$-S(CH_2)_2-$	$Ph(CH_2)_2-$	145
2.10	$-SO_2(CH_2)_2-$	$Ph(CH_2)_2-$	>1000
2.11	$-CH_2OCH_2-$	$Ph(CH_2)_2-$	23
2.12	−2,5-furanyl−	$Ph(CH_2)_2-$	5
2.13	−2,5-thienyl−	$Ph(CH_2)_2-$	48

[a] Inhibition of human liver FBPase is reported as IC_{50} values. [b] <25% inhibition at 100 μM.

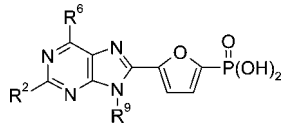
Table 3. SAR of Various Phosphate Mimics (**3.1**−**3.8**)



compd	R^8	IC_{50}, μM[a]
2.12	−2,5-furanyl-$PO(OH)_2$	5
3.1	$-(CH_2)_3$-PO(H)(OH)	>1000
3.2	$-CH_2CH(Me)$-PO(H)(OH	>1000
3.3	$-(CH_2)_2$-OPO(H)(OH)	>1000
3.4	$-(CH_2)_2$-$OPO(OH)_2$	40
3.5	−2,5-furanyl-CO_2H	>100[b]
3.6	−2,5-furanyl-SO_3H	150
3.7	−2,5-furanyl-PO(OEt)(OH)	>100[b]
3.8	−2,5-furanyl-$PO(OEt)_2$	>100[b]

[a] Inhibition of human liver FBPase is reported as IC_{50} values. [b] <25% inhibition at 100 μM.

Table 4. SAR of Furan-Linked Purine Phosphonate Analogues[a]



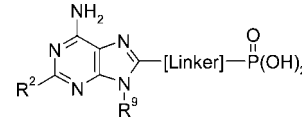
compd	R^2	R^6	R^9	IC_{50} (μM)
2.12	H	$-NH_2$	$-(CH_2)_2Ph$	5
4.1	H	$-N(Me)_2$	$-(CH_2)_2Ph$	250
4.2	H	−NHMe	$-(CH_2)_2Ph$	50
4.3	H	Cl	$-(CH_2)_2Ph$	50
4.4	H	$-NH_2$	$-CH_2CH(Ph)_2$	70
4.5	H	$-NH_2$	$-(CH_2)_2$(cyclohexyl)	1.4
4.6	H	$-NH_2$	$-CH_2$(2-naphthyl)	3.3
4.7	H	$-NH_2$	cyclopropyl	1.5
4.8	H	$-NH_2$	cyclopentyl	2.0
4.9	H	$-NH_2$	Et	1.8
4.10	H	$-NH_2$	isobutyl	1.5
4.11	H	$-NH_2$	neopentyl	0.8
4.12	H	$-NH_2$	adamantyl	>10[b]
4.13	−SMe	$-NH_2$	isobutyl	0.7
4.14	$-SO_2Me$	$-NH_2$	isobutyl	28

[a] Inhibition of human liver FBPase is reported as IC_{50} values. [b] <25% inhibition at 10 μM.

to replace the ribosyl group with a simple hydrophobic group, which would simplify the scaffold and facilitate rapid exploration of SAR.

The N^9-benzyl analogue **2.2** (Table 2) was prepared and showed potency equal to compound **1.6**, confirming our theory that the ribose moiety was not important for binding. A brief

Table 5. SAR from the Fine-Tuning of Compound **4.11**[a]



compd	R^2	R^9	linker	IC_{50} (μM)
4.11	H	neopentyl	2,5-furanyl	0.8
5.1	H	$-CH_2C(Me)_2CH_2NMe_2$	2,5-furanyl	>10[b]
5.2	H	$-CH_2C(Me)_2CH_2OH$	2,5-furanyl	4.5
5.3	H	$-CH_2C(Me)_2CH_2Cl$	2,5-furanyl	0.9
5.4	H	$-(CH_2)_2CMe_3$	2,5-furanyl	1.6
5.5	H	$-CH(Me)CMe_3$	2,5-furanyl	5
5.6	$-NH_2$	$-CH_2CMe_3$	2,5-furanyl	5.5
5.7	−SMe	$-CH_2CMe_3$	2,5-furanyl	1.1
5.8	H	$-CH_2CMe_3$	1-Me-2,5-pyrrolyl	>10[b]
5.9	H	$-CH_2CMe_3$	2,5-(1-Me)imidazolyl	>10[b]
5.10	H	$-CH_2CMe_3$	3,5-furanyl	>10[b]
5.11	H	$-CH_2CMe_3$	$-CH_2$-(1,2-imidazolyl)-	>100[c]
5.12	H	$-CH_2CMe_3$	2,5-(3,4-di-Cl)furanyl	13

[a] Inhibition of human liver FBPase is reported as IC_{50} values. [b] <25% inhibition at 10 μM. [c] <25% inhibition at 100 μM.

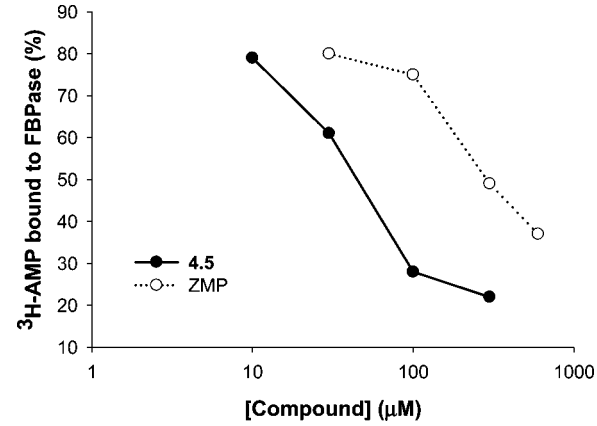


Figure 4. ^{3}H-labeled AMP Displacement Assay.

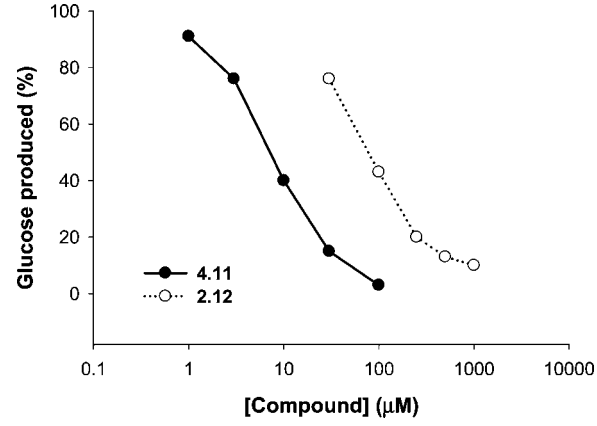


Figure 5. Hepatocyte Glucose Production.

SAR exploration of R^9 did not lead to significant improvement in potency (compounds **2.1**−**2.4**, Table 2), but the SAR did indicate that a lipophilic group is preferred at N^9 position of the new scaffold because analogue **2.1** was much weaker. To improve potency in this series, other 3-atom linking groups were explored. Moving the amino group into the middle of the 3-atom linker completely abolished activity (compound **2.5**), while introducing a carbonyl group (compound **2.6**), thus converting the amine to an amide, restored the original activity. These findings suggest that a basic amine functionality, which is likely

Table 6. Cellular Activity of Purine Phosphonic Acids

compd	hl IC_{50}, μM[a]	rl IC_{50}, μM[b]	rat EC_{50}, μM[c]
2.12	5	40	90
4.5	1.4	20	90
4.8	2	>20	24
4.10	1.5	7	18
4.11	0.8	1.25	4.5
4.13	0.7	>20	>25
5.2	4.5	>20	50
5.3	0.9	>2	7.5
5.7	1.1	4	8.5

[a] hl IC_{50} denotes human liver IC_{50} values. [b] rl IC_{50} denotes rat liver IC_{50} values. [c] Rat EC_{50} denotes inhibition of glucose production in primary rat hepatocytes.

to be protonated at physiological pH, is poorly tolerated in the linker. The three-carbon linker analogue **2.7** gave the same activity as compound **2.3**, while addition of conformational restriction via a *trans* double bond (compound **2.8**) did not improve potency. The sulfide analogue **2.9** showed a slight loss in potency, while the corresponding sulfone analogue **2.10** was completely inactive.

A significant improvement in potency was observed when oxygen-containing linker analogues were prepared. The methyleneoxymethylene-linked analogue **2.11** showed 4-fold improvement over compound **2.3**. Further improvement in potency was achieved by introducing conformational restriction in furan analogue **2.12**, leading to a 20-fold improvement over **2.3**. It is interesting to note that the corresponding thienyl analogue **2.13** is approximately 10-fold weaker than compound **2.12**, suggesting that the furan oxygen (and the ether oxygen of **2.11**) may be involved in either electrostatic or hydrogen-bonding interactions with the enzyme.

Although compound **2.12** represented a major advance in achieving inhibitory potency similar to AMP, there were reasons to reinvestigate other phosphate mimics (Table 3). First, a phosphonic acid is highly charged at physiologic pH and will likely require a suitable prodrug for oral activity. Second, the initial attempts with other phosphate mimics such as carboxylic acids were carried out on the adenosine scaffold, and the new SAR generated from compounds **2.1**−**2.13** might improve the chance of discovering a new phosphate mimic. Whereas two H-phosphinates (compounds **3.1** and **3.2**) and an H-phosphonate (compound **3.3**) were inactive, the 3-atom linked phosphate analogue **3.4** showed modest inhibition of FBPase, indicating that a single charge is not sufficient to mimic the phosphate binding interactions. Armed with the precedent that potent AMPDA inhibitors were obtained with a carboxylic acid group mimicking the interactions of the phosphate,[28] the carboxylate analogue of the new phosphonate lead **2.12** was prepared with the hope that improved interactions with other parts of the molecule might overcome the deficiencies of a carboxylic acid as a phosphate mimic in this series. Unfortunately, compound **3.5** proved inactive, likely due to its inability to attain the required six hydrogen bonds formed by the phosphate group of AMP. Because a sulfonate group has three oxygen atoms and can theoretically form six hydrogen bonds, analogue **3.6** was prepared. Compound **3.6** did inhibit FBPase (IC_{50} of 150 μM), albeit more weakly than **2.12**. The mono- and diethyl esters of **2.12** also proved to be inactive, again likely due to their inability to form the required six hydrogen bonds.

Having determined that a phosphonate group linked via the 8-position of the purine base is a suitable phosphate mimic, optimization of the new lead **2.12** was carried out at the C2-, C6-, and N^9-positions using the furan as the linking group (Table 4). SAR at the C6-position (compounds **4.1**−**4.3**) indicates that the unsubstituted amino group is optimal for potency because monomethylation of the 6-amino group, double-methylation, and replacement of the amino group with a chloro all led to significant loss in potency. This observation is also consistent with X-ray crystallography studies, which suggest a hydrogen bond donating amino group is required at this position. Conversely, N^9 SAR (compounds **4.4**−**4.12**) is more permissive. Various lipophilic groups are tolerated, but there appears to be a limit for the size of R^9 groups. For example, small alkyl and cycloalkyl groups are good replacements of the phenethyl group. On the other hand, larger R^9 analogues such as **4.4** and **4.12** are significantly less potent than compound **2.12**. The SAR at the C2-position was also explored through the syntheses of two analogues. Introducing a methylthio group (compound **4.13**) produced a slight increase in potency, but a methylsulfonyl group (compound **4.14**) led to >10-fold decrease in potency. The initial round of optimization of R^2, R^6, and R^9 resulted in potent FBPase inhibitors such as compound **4.11**, which is 5-fold more potent than compound **2.12**. Subsequently, an effort to fine-tune compound **4.11** was undertaken to determine if further improvements in potency were attainable.

Introduction of polar groups at the end of the neopentyl group in compound **4.11** (compounds **5.1** and **5.2**) led to significant losses in potency, while addition of a chloro group (compound **5.3**) proved to be without effect on potency (Table 5). Extending the neopentyl group by adding one methylene unit (**5.4**) reduced the potency slightly, but methyl branching (**5.5**) led to a 5-fold loss in potency. The C2-amino analogue proved 5-fold weaker than compound **4.11**, but the addition of a thiomethyl group at the 2-position did not produce the 2-fold enhancement in potency observed for compound **4.13** compared to compound **4.10**. Even though various linking groups were explored in the early SAR exploration, it was desirable to check other heterocyclic linkers with the optimized neopentyl group as R^9. All heterocycles explored, which included pyrrole, imidazole, 3,5-furanyl, and 3,4-dichloro-2,5-furanyl, led to significant losses in potency, indicating that the 2,5-furanyl linker is optimum. Despite extensive optimization, the most potent FBPase inhibitors in this purine series were all around 1 μM, equipotent to the natural regulator, AMP. It was important at this point to evaluate whether these novel AMP mimics could produce the biological effects expected of an inhibitor binding to the AMP site of FBPase.

In Vitro Evaluations of Purine Phosphonate FBPase Inhibitors. Given the major structural differences between these new purine analogues and AMP, it was important to determine whether these compounds were able to compete with AMP at the allosteric site. A displacement assay was used to evaluate the ability of compound **4.5** to displace ^{3}H-labeled AMP bound to FBPase (Figure 4); 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside-5-monophosphate (ZMP, an AMP analogue known to bind to the AMP site of FBPase)[29] was used as a positive control. Compound **4.5** displaced AMP from human liver FBPase in a dose-dependent manner similar to ZMP, indicating that the newly discovered purine analogue **4.5** does indeed bind to the same site on the enzyme as AMP. As expected, compound **4.5**, an 8-fold more potent FBPase inhibitor than ZMP (IC_{50} = 12 μM), had a lower ED_{50} for AMP displacement (35 μM) than ZMP (250 μM).

The cellular efficacy of these novel AMP mimetics was assessed in a glucose production assay using freshly isolated primary rat hepatocytes.[16] Both compound **2.12** and **4.11** inhibited glucose production in primary rat hepatocytes in a

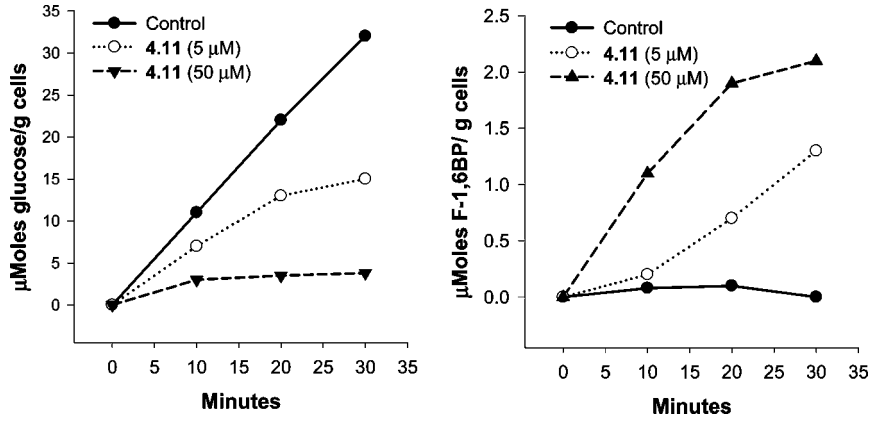


Figure 6. Hepatocyte glucose production and FBPase substrate levels.

Table 7. Selectivity of Compounds **2.12** and **4.11**[a]

enzymes	compd **2.12**	compd **4.11**
FBPase	$IC_{50} = 5.0\ \mu M$	$IC_{50} = 0.8\ \mu M$
adenosine kinase	$IC_{50} \gg 100\ \mu M$	$IC_{50} \gg 100\ \mu M$
adenylate kinase	$IC_{50} \gg 500\ \mu M$	$IC_{50} \gg 500\ \mu M$
AMP deaminase	$IC_{50} = 6.7\ \mu M$	$IC_{50} = 390\ \mu M$
glycogen phosphorylase	$EC_{50} \gg 250\ \mu M$	$EC_{50} \gg 100\ \mu M$
phosphofructokinase	$EC_{50} \gg 200\ \mu M$	$EC_{50} \gg 100\ \mu M$

[a] Inhibition of enzymes is reported as IC_{50}, while activation of enzymes is reported as EC_{50}.

dose-dependent manner with EC_{50} values of 5 and 90 μM, respectively (Figure 5).

To better understand results from our planned studies in rats, purine analogues with IC_{50} values below 5 μM were assayed against rat liver FBPase. In addition, cellular efficacy in rat hepatocytes was determined in a glucose production assay. These data are summarized in Table 6.

In general, there is a variable rightward shift in potency from the human to the rat enzyme for these compounds, as is also observed for the natural ligand AMP. For example, compound **4.13** is a potent inhibitor of human FBPase ($IC_{50} = 0.7\ \mu M$) but it is more than 20-fold weaker against the rat enzyme; conversely, compound **4.11** was shifted by <2-fold. The rat liver IC_{50} value does not correlate well to the EC_{50} in hepatocytes, which suggests that these phosphonic acids may have different degrees of cell penetration. The fact that some of these phosphonate FBPase inhibitors are able to inhibit glucose production in rat hepatocytes indicates that they are cell permeable to some extent. It is believed that organic anion transporter proteins (OATP)[30] may recognize a subset of FBPase inhibitors facilitating cell penetration because the highly charged nature of these phosphonic acids is expected to restrict their passive diffusion. Most likely, some phosphonates are simply better OATP substrates than others, which may be the reason for the poor correlation between rat liver FBPase IC_{50} and rat hepatocyte EC_{50}.

To study the mechanism of the observed inhibition of glucose production in rat hepatocytes by compound **4.11**, glucose and fructose-1,6-bisphosphate levels were measured in freshly isolated rat hepatocytes. Compound **4.11** inhibited glucose production in a dose-dependent manner while at the same time the concentration of fructose-l,6-bisphosphate, the substrate of FBPase, increased (Figure 6A,B). These data indicate that the site of action of compound **4.11** is in the last four steps of the gluconeogenic pathway and is consistent with the inhibition of FBPase.

Selectivity toward Enzymes with an AMP Site. To determine the selectivity of the purine series of novel AMP mimics, effects of FBPase inhibitors **2.12** and **4.11** on five key AMP binding enzymes were measured.[16] The results are summarized in Table 7.

Compound **2.12** is a relatively weak inhibitor of FBPase. However, it displays excellent selectivity against most of these AMP-binding enzymes except for AMP deaminase (AMPDA), which it inhibits with an IC_{50} of 6.7 μM. We had hoped that further improving the FBPase inhibitory potency of compounds in this series would increase selectivity against AMPDA. Indeed, the more potent compound **4.11** (hlFBPase $IC_{50} = 0.8\ \mu M$) maintained excellent selectivity toward most AMP-binding enzymes and exhibited >480-fold selectivity against AMPDA ($IC_{50} = 390\ \mu M$).

In Vivo Glucose Lowering Effects. To evaluate whether the observed cellular activity displayed by these newly discovered AMP mimics could be extended to in vivo glucose lowering activities, compound **4.11** was tested in overnight-fasted Sprague−Dawley (SD) rats. After intraperitoneal (ip) administration of compound **4.11** at a dose of 20 mg/kg, glucose levels as well as drug and fructose-1,6-bisphosphate concentrations were measured in order to establish a pharmacokinetic−pharmacodynamic relationship. Drug levels achieved in this study were 38.5 ± 7 μM in plasma and 51.3 ± 10 nmol/g in the liver, respectively, one hour after compound administration. Compound **4.11** lowered blood glucose levels by 65% (from 82 ± 3 mg/dL for control animals to 28 ± 9.9 mg/dL for drug treated animals) within one hour (Figure 7, left), while a 10-fold elevation of fructose-1,6-bisphosphate levels was observed in the livers of animals in the drug-treated group (Figure 7, right). These observations indicated that the mechanism of action for the glucose lowering effects displayed by compound **4.11** involves blocking hepatic glucose production via inhibition of FBPase in the GNG pathway. Thus, the glucose lowering activity elicited by compound **4.11** established proof of concept for FBPase inhibition as a potential approach to lowering blood glucose levels in T2DM.

As expected, oral administration of compounds **2.12** and **4.11** (up to 300 mg/kg) did not produce significant glucose lowering effects in fasted SD rats, most likely because of their low oral bioavailability (OBAV, measured using a urinary excretion assay reported recently[24]) of <5%. To achieve acceptable OBAV and potentially to improve cell penetration, a survey of known phosphonate prodrugs was conducted using compound **2.12**. These prodrugs were first screened in the cellular glucose production assay (Table 8). Efficacy in the cellular assay provides a composite picture of both cell penetration and prodrug conversion to the active FBPase inhibitor, which should assist

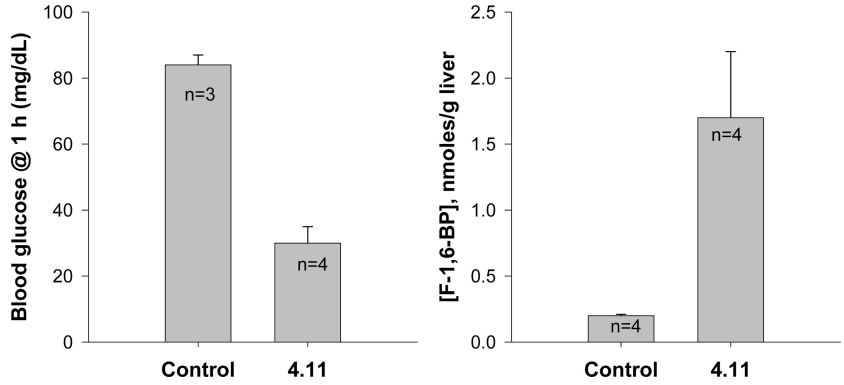
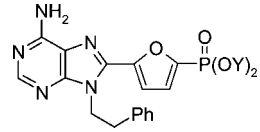


Figure 7. Glucose-lowering and target engagement in rats.

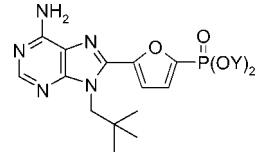
Table 8. Cellular EC_{50} of Compound **2.12** Prodrugs



compd	Y =	EC_{50}[a], μM
2.12	H	90
8.1	$-CH_2OC(O)i$-Pr	25
8.2	3-phthalidyl	75
8.3	$-CH_2$-(3-NC-4-MeO)Ph	>100
8.4	$-(CH_2)_2SS(CH_2)_2$-OH	>500
8.5	$-(CH_2)_2SS(CH_2)_2-$	250
8.6	(3-AcO)-cyclohexyl	400

[a] EC_{50} denotes inhibition of glucose production in primary rat hepatocytes.

Table 9. Evaluations of Prodrugs of Compound **4.11**



compd	Y =	EC_{50}[a], μM	OBAV[b]
4.11	H	4.5	1.6
9.1	$-CH_2OC(O)$Pr-*i*	3	8.1
9.2	$-CH_2OC(O)$Bu-*t*	NT	5.3

[a] EC_{50} denotes inhibition of glucose production in primary rat hepatocytes. [b] OBAV, oral bioavailability measured by the urinary excretion assay.

us in selecting prodrugs that are readily converted to the active phosphonate FBPase inhibitors within hepatocytes.

The classical acyloxyalkyl ester prodrug **8.1** showed 3.6-fold improvement in EC_{50}, suggesting an enhancement of cell penetration. The more stable 3-phthalidyl[31] prodrug **8.2** also showed a slight improvement in EC_{50}. This is presumably due to slower intracellular conversion of prodrug **8.2** to the active drug **2.12**. On the other hand, all other phosphonate prodrugs (**8.3**−**8.6**) did not enhance cellular EC_{50}, suggesting no improvement in cell penetration or very slow conversion to the active FBPase inhibitor **2.12**. Thus, acyloxyalkyl ester prodrugs were selected for further evaluation, using the most promising phosphonate FBPase inhibitor, compound **4.11**, as the active drug.

The isobutyryloxymethyl prodrug **9.1** showed a slight improvement in cellular EC_{50}, but more importantly it showed a 4-fold enhancement in OBAV compared to compound **4.11** (Table 9). On the other hand, pivaloyloxymethyl prodrug **9.2** had a lower OBAV than compound **9.1**. Prodrug **9.2** exhibited very poor solubility compared to compound **9.1**, which may explain the difference in OBAV between the two prodrugs. Compound **9.1** was tested for oral efficacy, but unfortunately no statistically significant glucose-lowering was observed after oral administration (50 mg/kg). The levels of compound **4.11** in liver at this oral dose of compound **9.1** were 3.5 and 2.0 nmol/g (at 1 and 2 h postdose, respectively), 15- and 25-fold lower than the levels achieved following ip dosing of compound **4.11**.

Conclusion

To discover novel FBPase inhibitors, a series of purine analogues was designed as AMP mimics using a structure-guided drug design approach. This approach led to the discovery of a series of purine phosphonic acids that mimic AMP and its effects on FBPase activity following binding to the FBPase allosteric binding site. Further lead optimization produced purine analogues such as compounds **4.11**, **4.13**, and **5.7**, which are essentially equipotent with AMP with regard to inhibition of human liver FBPase. This represents a significant advance in the field: these compounds are non-nucleotides because they lack a phosphate group and a ribosyl moiety, yet they inhibit FBPase in the same manner as AMP. Evaluation of these compounds further demonstrated that they are capable of inhibiting glucose production in hepatocytes and lowering blood glucose levels in fasted rats. The lead compound identified, **4.11**, is a potent inhibitor of both human and rat liver FBPase and has excellent selectivity toward five other AMP-binding enzymes. Moreover, compound **4.11** elicited profound glucose-lowering (65%) in fasted SD rats after intraperitoneal administration, validating the approach of FBPase inhibition to lower blood glucose levels. Compound **9.1** (a prodrug of compound **4.11**) showed a 4-fold higher OBAV than compound **4.11**. The structure−activity relationships established with these purine analogues became the basis for the discovery of other series of heterocyclic phosphonic acids as highly potent and selective FBPase inhibitors, which will be reported in future publications.

Experimental Section

General Methods. Compounds **1.5**, **1.6**, **1.7**, **2.1**, **4.5**, **4.10**, and **13** were prepared according to reported procedures.[19] Glassware for moisture-sensitive reactions was flame-dried and cooled to room temperature under vacuum, and all reactions were carried out under a nitrogen atmosphere. Anhydrous solvents were purchased and used directly. TLC was performed on Merck Kieselgel 60 F_{254} plates, and flash chromatography was performed on 230−240 mesh EM Science Silica Gel 60. Melting points were recorded on a Thomas−Hoover capillary melting point apparatus and are uncorrected. Proton NMR spectra were obtained on a Varian Gemini-200 operating at 200 MHz and recorded in δ with tetramethylsilane (δ 0.00) as reference line internal standard. Purities of final compounds were determined by microanalyses (which were performed by Robertson Microlit Laboratories, Inc., Madison, NJ) and

are >95% except for compounds **3.1**, **3.2**, **4.7**, and **5.11** (purities of these four compounds were determined by reverse-phase HPLC and are >95% based on UV detection at 280 nm). Low resolution mass spectra were obtained from Mass Consortium Corp., San Diego, CA.

(*E*)-3-[6-Amino-9-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-9*H*-purin-8-yl]-acrylic acid (1.1). A solution of 8-bromoadenosine (3.54 g, 10.9 mmol) in anhydrous DMF was treated with ethyl (*E*)-3-tributylstanyl-acrylic acid (1.413 g, 3.22 mmol) and $Pd(PPh_3)_4$ (125 mg, 0.11 mmol) and heated to 85 °C under nitrogen. After 15 h, the cooled reaction solution was evaporated and the residue was purified by flash chromatography (SiO_2, 2 cm × 15 cm, 1−10% MeOH-CH_2Cl_2) to give ethyl (*E*)-3-[6-amino-9-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-9*H*-purin-8-yl]-acrylate (**4**) as a clear film (298 mg, 72%).

A solution of compound **4** (108 mg, 0.296 mmol) in methanol (1.0 mL) and THF (1.0 mL) was treated with sodium hydroxide (1*N*, 0.6 mL) at room temperature under nitrogen. After 18 h, the reaction mixture was concentrated under reduced pressure to remove organic solvents and the residue was treated with hydrogen chloride (1 N, 0.6 mL). The resulting solid was collected through filtration (washed with water, 2 × 5 mL) and dried under vacuum to give compound **1.1** as a white solid (94 mg, 94%); mp >250 °C. ^{1}H NMR (DMSO-d_6): δ 8.16 (s, 1H), 7.73 (d, 1H, J = 15.5 Hz), 7.65 (bs, 2H), 6.90 (d, 1H, J = 15.5 Hz), 6.01 (d, 2H, J = 7.0 Hz), 5.65 (m, 1H), 5.48 (d, 1H, J = 7.0 Hz), 5.32 (d, 1H, J = 4.8 Hz), 4.88 (m, 1H), 4.20−3.50 (m, 4H). Anal. ($C_{13}H_{15}N_5O_6$) C, H, N.

3-[6-Amino-9-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-9*H*-purin-8-yl]-propionic acid (1.2). A solution of compound **1.1** (47 mg, 0.139 mmol) in aqueous sodium hydroxide (0.05 N, 2.8 mL) was treated with palladium on carbon (10%) and stirred under 1 atm of hydrogen at room temperature. After 17 h, the reaction mixture was filtered through a celite pad (washed with water, 3 × 5 mL). The filtrate was acidified with HCl (1 N) to pH = 2−3, and the resulting solid was collected through filtration (washed with water, 2 × 5 mL) and dried under vacuum to give compound **1.2** as a white solid (40 mg, 85%); mp > 250 °C. ^{1}H NMR (DMSO-d_6): δ 8.09 (s, 1H), 7.27 (bs, 2H), 5.95 (m, 1H), 5.81 (d, 1H, J = 7 Hz), 5.44 (m, 1H), 5.26 (m, 1H), 4.91 (m, 1H), 4.20−3.48 (m, 4H), 3.12 (t, 2H, J = 7.7 Hz), 2.81 (t, 2H, J = 7.7 Hz). Anal. ($C_{13}H_{17}N_5O_6$) C, H, N.

[6-Amino-9-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-9*H*-purin-8-ylamino]-acetic acid (1.3). A suspension of 8-bromoadenosine (158 mg, 0.456 mmol), glycine (343 mg, 4.56mmol), and potassium carbonate (945 mg, 6.84 mmol) in H_2O (2 mL) was heated to reflux under nitrogen. After 24 h, the cooled reaction solution was subjected to HPLC purification (C18 reverse phase column, methanol-0.1% aqueous acetic acid as the mobile phases) to give compound **1.3** as a white solid (13.7 mg, 9%); mp 205 °C (decomp). ^{1}H NMR (DMSO-d_3 + D_2O): δ 7.86 (s, 1H), 5.85 (d, 1H, J = 7.1 Hz), 4.68 (m, 1H), 4.11 (m, 1H), 3.93 (m, 1H), 3.80−3.40 (m, 4H). Anal. ($C_{12}H_{16}N_6O_6 \cdot 0.75H_2O$) C, H, N.

3-[6-Amino-9-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-9*H*-purin-8-ylamino]-propionic acid (1.4). This compound was prepared in a similar manner as compound **1.3** from 8-bromoadenosine and 3-aminopropionic acid to give compound **1.4** as a white solid (15 mg, 7.4% yield); mp 209−210 °C. ^{1}H NMR (DMSO-d_6 + D_2O): δ 7.89 (s, 1H), 5.83 (d, 1H, J = 7.1 Hz), 4.59 (m, 1H), 4.09 (m, 1H), 3.91 (m, 1H), 3.80−3.40 (m, 4H), 2.57 (t, 2H, J = 7.0 Hz). Anal. ($C_{13}H_{18}N_6O_6 \cdot 1H_2O$) C, H, N.

3-[6-Amino-9-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-9*H*-purin-8-ylamino]-propionic acid (1.8). This compound was prepared in a similar manner as compound **1.3** from 8-bromoadenosine and mono-(2-amino-ethyl)-phosphate to give compound **1.8** as a white solid (35 mg, 14%); mp 164−166 °C. ^{1}H NMR (D_2O): δ 8.13 (s, 1H), 6.03 (m, 1H), 4.40−3.51 (m, 9H). Anal. ($C_{12}H_{19}N_6O_8P \cdot 0.7H_2O \cdot 0.1AcOH$) C, H, N.

3-[6-Amino-9-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-9*H*-purin-8-ylamino]-propionic acid (1.9). This compound was prepared in a similar manner as compound **1.3** from 8-bromoadenosine and (2-amino-ethyl)-phosphonic acid to give compound **1.9** as a light-yellow solid (25 mg, 17.5%); mp 184−187 °C. ^{1}H NMR (D_2O): δ 8.12 (s, 1H), 5.77 (d, 1H, J = 7.0 Hz), 5.06 (t, 1H, J = 6.8 Hz), 4.23−4.05 (m, 2H), 3.70−3.52 (m, 2H), 2.10−1.91 (m, 2H), 1.89 (d, 3H, J = 7.0 Hz). Anal. ($C_{12}H_{19}N_6O_6P \cdot 1.4H_2O \cdot 0.1AcOH$) C, H, N.

[2-(6-Amino-9-benzyl-9*H*-purin-8-ylamino)-ethyl]-phosphonic acid (2.2). A suspension of 8-bromo-9-benzyladenine (118 mg, 0.388 mmol), (2-amino-ethyl)-phosphonic acid (971 mg, 7.76 mmol), tetra-*n*-butyl ammonium iodide (143 mg, 0.388 mmol), and sodium hydroxide (326 mg, 8.15 mmol) in EtOH-H_2O (1:1, 5 mL) was heated to reflux under nitrogen. After 18 h, the reaction solution was subjected to preparative HPLC purification (C18 reverse phase column, MeOH-1% aqueous AcOH as the mobile phases) to give compound **2.2** as a white solid (115 mg, 85%); mp 248−250 °C. ^{1}H NMR (D_2O): δ 7.80 (s, 1H), 7.20−6.90 (m, 5H), 5.01 (s, 2H), 3.31 (m, 2H), 1.51 (m, 2H). HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{14}H_{17}N_6O_3P$, 349.1178; found, 349.1180.

[2-(6-Amino-9-phenethyl-9*H*-purin-8-ylamino)-ethyl]-phosphonic acid (2.3). This compound was prepared in a similar manner as compound **2.2** from 8-bromo-9-phenethyladenine (**5a**, 55 mg, 0.173 mmol) to give compound **2.3** as a white solid (25 mg, 40% yield); mp 159−160 °C. ^{1}H NMR (DMSO-d_6): δ 7.70 (s, 1H), 7.09 (m, 3H), 6.85 (m, 2H), 4.11 (t, 2H, J = 7.0 Hz), 3.37 (m, 2H), 2.95 (t, 2H, J = 7.0 Hz), 1.61 (m, 2H). Anal. ($C_{15}H_{19}N_6O_3P \cdot 1.25H_2O$) C, H, N.

[2-(6-Amino-9-naphthalen-2-ylmethyl-9*H*-purin-8-ylamino)-ethyl]-phosphonic acid (2.4). This compound was prepared in a similar manner as compound **2.2** from 8-bromo-9-naphthyl-methyl-adenine (63 mg, 0.178 mmol) to give compound **2.4** as a white solid (22 mg, 31% yield); mp 189−191 °C. ^{1}H NMR (D_2O): δ 7.26 (s, 1H), 7.15−6.60 (m, 7H), 4.47 (s, 2H), 3.01 (m, 2H), 1.22 (m, 2H). Anal. ($C_{18}H_{19}N_6O_3P \cdot 1.5H_2O$) C, H, N.

{[(6-Amino-9-phenethyl-9*H*-purin-8-ylmethyl)-amino]-methyl}-phosphonic acid (2.5). A solution of 8-bromo-9-phenethyladenine (**5a**, 1.622 g, 5.1 mmol), triethylamine (3.50 mL, mmol), and $Pd(PPh_3)_4$ (589 mg, 0.51 mmol) in anhydrous DMF (15 mL) and methanol (30 mL) was put under carbon monoxide (50 psi) atmosphere and heated to 90 °C. After 70 h, the cooled reaction solution was evaporated and the residue was purified by flash chromatography (SiO_2, 5 cm × 15 cm, 1−10% MeOH-CH_2Cl_2) to give methyl 6-amino-9-phenethyl-9*H*-purine-8-carboxylate (**6**) as a yellow solid (1.107 g, 73%).

A solution of compound **6** (1.107 g, 3.72 mmol) in anhydrous THF (35 mL) was cooled to 0 °C under nitrogen and treated with $LiAlH_4$ (284 mg, 7.48 mmol), and the resulting reaction mixture was stirred at room temperature for 30 h. The reaction mixture was cooled to 0 °C, quenched with NaF (2 g), and partitioned between water (50 mL) and dichloromethane (50 mL). The layers were separated, and the aqueous layer was extracted with dichloromethane (3 × 25 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, 5 cm × 15 cm, 1−10% MeOH-CH_2Cl_2) to give 8-hydroxymethyl-9-phenethyladenine as a yellow solid (639 mg, 64%).

A suspension of 8-hydroxymethyl-9-phenethyladenine (43.5 mg, 0.162 mmol) in anhydrous dichloromethane (1 mL) was cooled to 0 °C and treated with a solution of PBr_3 in dichloromethane (1 N, 0.16 mL). The resulting reaction mixture was stirred at room temperature for 24 h. The reaction was quenched with saturated sodium bicarbonate (20 mL) and extracted with dichloromethane (3 × 15 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to give 8-bromomethyl-9-phenethyladenine (**7**) as a white solid (40.8 mg, 76%).

A solution of diethyl aminomethyl-phosphonate (**8a**, 147 mg, 1.23 mmol) in anhydrous DMF (0.6 mL) was treated with a solution of compound **7** (40.8 mg, 0.123 mmol) in anhydrous DMF (0.6 mL) at room temperature under nitrogen. After 3 h, the reaction

solution was evaporated to dryness and the residue was purified by flash chromatography (SiO_2, 1 cm × 15 cm, 1−10% MeOH-CH_2Cl_2) to give diethyl {[(6-amino-9-phenethyl-9*H*-purin-8-yl-methyl)-amino]-methyl}-phosphonate as a clear film (23 mg, 51%).

A solution of diethyl {[(6-amino-9-phenethyl-9*H*-purin-8-yl-methyl)-amino]-methyl}-phosphonate (23 mg, 0.062 mmol) in anhydrous acetonitrile (0.6 mL) was treated with TMSBr (0.082 mL, 0.62 mmol) at room temperature. After 5 h, the reaction mixture was evaporated and the residue was treated with acetonitrile (1 mL) and water (1 mL) and evaporated to give a white solid. The crude material was purified by preparative HPLC to give compound **2.5** as a white solid (20.1 mg, 89%); mp 224 °C (decomp). ^{1}H NMR (D_2O + NaOD): δ 7.89 (s, 1H), 6.97 (m, 3H), 6.67 (m, 2H), 4.22 (t, 2H, J = 6.8 Hz), 3.29 (s, 2H), 2.86 (t, 2H, J = 6.8 Hz), 2.28 (d, 2H, J = 13 Hz). Anal. ($C_{15}H_{19}N_6O_3P\cdot 0.75H_2O$) C, H, N.

{[(6-Amino-9-phenethyl-9*H*-purine-8-carbonyl)-amino]-methyl}-phosphonic Acid (2.6). A suspension of compound **6** (130 mg, 0.438 mmol) in THF-MeOH-H_2O (3:2:1, 4 mL) was treated with an aqueous solution of sodium hydroxide (1M, 0.53 mL) and stirred at room temperature for 1.5 h. The reaction solution was evaporated to dryness, and the resulting yellow solid was dissolved in anhydrous DMF (4 mL). The resulting DMF solution was treated with diethyl aminomethyl-phosphonic acid (**8a**, 110 mg, 0.657 mmol), HOBt (89 mg, 0.657 mmol), and EDCI (109 mg, 0.569 mmol). The resulting solution was stirred at room temperature for 72 h. The reaction solution was diluted with saturated sodium bicarbonate (5 mL) and water (10 mL) and extracted with dichloromethane (3 × 20 mL). The combined organic extracts were dried ($MgSO_4$), filtered, evaporated, and the residue was purified by preparative TLC (SiO_2, 100% EtOAc) to give diethyl {[(6-amino-9-phenethyl-9*H*-purine-8-carbonyl)-amino]-methyl}-phosphonate as a white solid (35 mg, 19%).

A solution of diethyl {[(6-amino-9-phenethyl-9*H*-purine-8-carbonyl)-amino]-methyl}-phosphonate (35 mg, 0.081 mmol) in anhydrous acetonitrile (1 mL) was treated with TMSBr (0.11 mL, 0.81 mmol) at room temperature. After 24 h, the reaction solution was evaporated to dryness and the residue was treated with acetone (5 mL) and water (0.5 mL) at room temperature for 2 h. The resulting solid was collected through filtration (washed with H_2O, acetone) and dried to give compound **2.6** as a light-yellow solid (22 mg, 72%); mp > 250 °C. ^{1}H NMR (D_2O): δ 8.02 (s, 1H), 7.12 (m, 3H), 6.83 (m, 1H), 4.73 (t, 2H, J = 7.0 Hz), 3.23 (d, 2H, J = 12.9 Hz), 3.02 (t, 2H, J = 6.9 Hz). Anal. ($C_{15}H_{17}N_6O_4P\cdot 0.5H_2O\cdot 0.3$acetone) C, H, N.

[(*E*)-3-(6-Amino-9-phenethyl-9*H*-purin-8-yl)-allyl]-phosphonic Acid (2.7). A solution of 8-bromo-9-phenethyladenine (**5a**, 0.341 g, 1.07 mmol), diethyl ((*E*)-3-tributylstannanyl-allyl)-phosphonate (**9**, 1.413 g, 3.22 mmol), and $Pd(PPh_3)_4$ (125 mg, 0.11 mmol) in anhydrous DMF (5 mL) was heated to 85 °C under nitrogen. After 15 h, the cooled reaction solution was evaporated and the residue was purified by flash chromatography (SiO_2, 2 cm × 15 cm, 1−10% MeOH-CH_2Cl_2) to give diethyl [(*E*)-3-(6-amino-9-phenethyl-9*H*-purin-8-yl)-allyl]-phosphonate (**10**) as a clear film (298 mg, 72%).

A solution of compound **10** (137 mg, 0.354 mmol) in anhydrous acetonitrile (3.5 mL) was treated with TMSBr (0.47 mL, 3.56 mmol) at room temperature. After 18 h, the reaction solution was evaporated to dryness and the residue was treated with acetonitrile (4 mL) and water (1 mL) at room temperature for 1 h. The resulting solid was collected via filtration (washed with water, 3 × 5 mL) and dried to give **2.7** as a white solid (75 mg, 59%); mp > 255 °C. ^{1}H NMR ($CDCl_3$): δ 8.10 (s, 1H), 7.39−6.55 (m, 7H), 4.40 (t, 2H, J = 6.9 Hz), 3.00 (t, 2H, J = 6.9 Hz), 2.75−2.60 (m, 2H). Anal. ($C_{16}H_{18}N_5O_3P\cdot 0.5H_2O$) C, H, N.

[3-(6-Amino-9-phenethyl-9*H*-purin-8-yl)-propyl]-phosphonic Acid (2.8). A solution of compound **10** (36 mg, 0.1 mmol) in anhydrous methanol was treated with palladium on carbon (4 mg) and shaken under 50 psi of hydrogen for 7 h. The reaction mixture was filtered through a celite pad (washed with MeOH, 3 × 5 mL) and the filtrate was evaporated to dryness. The crude material was purified by flash chromatography (SiO_2, 1 cm × 10 cm, 1−10% MeOH-CH_2Cl_2, gradient elution) to give dimethyl [3-(6-amino-9-phenethyl-9*H*-purin-8-yl)-propyl]-phosphonate as a clear film (30.4 mg, 75%).

A solution of dimethyl [3-(6-mino-9-phenethyl-9*H*-purin-8-yl)-propyl]-phosphonate (30.4 mg, 0.076 mmol) in anhydrous acetonitrile (1 mL) was treated with TMSBr (0.1 mL, 0.76 mmol) at room temperature. After 24 h, the reaction solution was evaporated to dryness and the residue was purified by preparative HPLC purification (C18 reverse phase column, MeOH-1% aqueous AcOH as the mobile phases) to give **2.8** as a white solid (13.7 mg, 41%); mp 254 °C (decomp). ^{1}H NMR (DMSO-d_6): δ 8.12 (s, 1H), 7.30−7.05 (m, 5H), 4.29 (t, 2H, J = 7.0 Hz), 3.03 (t, 2H, J = 6.9 Hz), 2.62 (t, 2H, J = 6.9 Hz), 1.81 (m, 2H), 1.52 (m, 2H). Anal. ($C_{16}H_{20}N_5O_3P\cdot 0.5H_2O$) C, H, N.

[2-(6-Amino-9-phenethyl-9*H*-purin-8-ylsulfanyl)-ethyl]-phosphonic Acid (2.9). A suspension of 8-bromo-9-phenethyladenine (**5a**, 1.5 g, 4.714 mmol) and potassium sulfide (1.6 g, 14.14 mmol) in anhydrous EtOH (16 mL) was treated to reflux under nitrogen. After 3 h, the cooled reaction mixture was filtered through a silica gel plug (washed with MeOH) and the filtrate was evaporated to give 8-mercapto-9-phenethyladenine which was used for the next step.

A solution of the above-generated 8-mercapto-9-phenethyladenine in anhydrous DMF (10 mL) was treated with potassium carbonate (0.78 g, 5.657 mmol) followed by diethyl (2-bromoethyl)-phosphonate (1.07 g, 5.657 mmol), and the resulting mixture was stirred at room temperature under nitrogen for 18 h. The reaction mixture was diluted with dichloromethane (20 mL) and water (20 mL), and the pH of the aqueous phase was adjusted to ca. 4. The layers were separated, and the aqueous phase was extracted with dichloromethane (3 × 15 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, 4 cm × 10 cm, 1−10% MeOH-CH_2Cl_2, gradient elution) to give diethyl [2-(6-amino-9-phenethyl-9*H*-purin-8-ylsulfanyl)-ethyl]-phosphonate (**11**) as a yellow solid (1.05 g, 51%).

A solution of compound **11** (61 mg, 0.139 mmol) in anhydrous acetonitrile (1 mL) was treated with TMSBr (0.18 mL, 1.34 mmol) at room temperature. After 18 h, the reaction solution was evaporated to dryness and the residue was treated with water (1 mL) and methanol (4 mL) at room temperature for 1 h. The resulting solid was collected via filtration (washed with water, 3 × 5 mL) and dried to give **2.9** as a white solid (35 mg, 66%); mp 256−257 °C. ^{1}H NMR (DMSO-d_6 + D_2O + NaOD): δ 7.69 (s, 1H), 7.30−7.05 (m, 5H), 4.20−1.62 (m, 8H). Anal. ($C_{15}H_{18}N_5O_3PS\cdot 1H_2O$) C, H, N.

[2-(6-Amino-9-phenethyl-9*H*-purine-8-sulfonyl)-ethyl]-phosphonic Acid (2.10). A solution of diethyl [2-(6-amino-9-phenethyl-9*H*-purin-8-ylsulfanyl)-ethyl]-phosphonate (224 mg, 0.515 mmol) in dichloromethane (2.5 mL) was treated with *m*-CPBA (50−60%, 356 mg, 1.03−1.24 mmol) at room temperature. After 3 h, the reaction solution was diluted with saturated sodium bicarbonate (15 mL) and extracted with dichloromethane (3 × 15 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, 3 cm × 10 cm, 1−10% MeOH-CH_2Cl_2, gradient elution) to give diethyl [2-(6-amino-9-phenethyl-9*H*-purine-8-sulfonyl)-ethyl]-phosphonate as a yellow solid (193 mg, 80%).

A solution of diethyl [2-(6-amino-9-phenethyl-9*H*-purine-8-sulfonyl)-ethyl]-phosphonate (118 mg, 0.253 mmol) in anhydrous acetonitrile (1.3 mL) was treated with TMSBr (0.33 mL, 2.53 mmol) at room temperature. After 18 h, the reaction solution was evaporated to dryness and the residue was treated with water (1 mL) and methanol (4 mL) at room temperature for 1 h. The resulting solid was collected via filtration (washed with water, 3 × 5 mL) and dried to give **2.10** as a white solid (35 mg, 34%); mp 198−199 °C. ^{1}H NMR ($CDCl_3$): δ 8.45 (s, 1H), 7.16 (m, 1H), 7.02 (m, 1H), 4.26 (m, 2H), 3.30 (m, 2H), 3.02 (m, 2H), 1.94 (m, 2H). Anal. ($C_{15}H_{18}N_5O_5PS$) C, H, N.

(6-Amino-9-phenethyl-9*H*-purin-8-ylmethoxymethyl)-phosphonic Acid (2.11). A solution of diethyl hydroxymethyl-phosphonate (**8b**, 0.33 mL, 2.24 mmol) in anhydrous DMF (2.3 mL) was cooled to 0 °C under nitrogen, treated with sodium hydride (90 mg, 2.25

mmol), and stirred at room temperature. After 1 h, the reaction solution was cooled back to 0 °C and treated with 8-bromomethyl-9-phenethyladenine (**7**, 82.4 mg, 0.248 mmol). After 1 h, the reaction was quenched with saturated sodium bicarbonate (3 mL) and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, 1 cm × 15 cm, 1−10% MeOH-CH_2Cl_2, gradient elution) to give diethyl (6-amino-9-phenethyl-9*H*-purin-8-ylmethoxymethyl)-phosphonate as a clear film (23.1 mg, 22%).

A solution of diethyl (6-amino-9-phenethyl-9*H*-purin-8-ylmethoxymethyl)-phosphonate (23.1 mg, 0.055 mmol) in anhydrous acetonitrile (0.6 mL) was treated with TMSBr (0.044 mL, 0.62 mmol) at room temperature. After 22 h, the reaction mixture was evaporated and the residue was treated with acetonitrile (1 mL) and water (1 mL) and evaporated to give a white solid. The crude material was purified by preparative HPLC to give compound **2.11** as a white solid (18.9 mg, 94%); mp 252 °C (decomp). ^{1}H NMR (D_2O + NaOD): δ 7.91 (s, 1H), 7.01 (m, 1H), 6.70 (m, 1H), 4.30 (t, 2H, J = 7.0 Hz), 4.07 (s, 2H), 3.20 (d, 2H, J = 9.0 Hz), 2.91 (t, 2H, J = 7.0 Hz). Anal. ($C_{15}H_{18}N_5O_4P\cdot0.75H_2O$) C, H, N.

[5-(6-Amino-9-phenethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonic Acid (2.12). A solution of 5-amino-4-chloro-6-(*N*-phenethylamino)pyrimidine (**12**,[21] 128 mg, 0.516 mmol) and diethyl 5-formyl-furan-2-phosphonate (**13**,[19] 180 mg, 0.77 mmol) in anhydrous DMSO (6 mL) was treated with $FeCl_3-SiO_2$ (20% wt %, 628 mg, 0.77 mmol). The resulting reaction mixture was heated at 80 °C for 6 h. The cooled reaction mixture was diluted with EtOAc (60 mL) and filtered through a silica gel plug (SiO_2, 5 cm × 5 cm) and washed with EtOAc (3 × 20 mL). The filtrate was evaporated to dryness, and the residue was purified by flash chromatography (SiO_2, 2 cm × 10 cm, 60, 80% EtOAc-hexane, gradient elution) to give diethyl [5-(6-chloro-9-phenethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonate as white crystals (214.5 mg, 90%).

A solution of 6-chloro-N^9-phenethyl-8-(2-(5-diethylphosphono)-furanyl)-purine (1.79 g, 3.89 mmol) in DMSO-THF (1:1, 20 mL) cooled at −78 °C was treated with liquid ammonia (ca. 7 mL) in a sealed tube. The reaction solution was stirred at room temperature for 18 h. The reaction solution was diluted with EtOAc (150 mL) and washed with water (5 × 100 mL), dried ($MgSO_4$), and evaporated to dryness and the resulting oil was purified by flash chromatography (SiO_2, 3.5 cm × 15 cm, 5% MeOH-CH_2Cl_2) to give diethyl [5-(6-amino-9-phenethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonate (**3.7**) as a white solid (1.38 mg, 81%). ^{1}H NMR ($CDCl_3$): δ 8.72 (s, 1H), 7.49−7.18 (m, 7H), 4.91 (t, 2H, J = 7.0 Hz), 4.38−4.15 (m, 4H), 3.18 (t, 2H, J = 7.0 Hz). Anal. ($C_{21}H_{24}N_5O_4P\cdot0.25H_2O$) C, H, N.

A solution of compound **3.7** (727 mg, 1.65 mmol) in anhydrous acetonitrile (16 mL) was treated with TMSBr (2.18 mL, 16.49 mmol) at room temperature. After 18 h, the reaction mixture was evaporated to dryness and the residue was treated with water at room temperature for 1 h. The resulting white solid was collected via filtration (washed with water, 10 mL, MeCN, 10 mL) and dried under vacuum to give compound **2.12** as a white solid (634 mg, 95%); mp 242−244 °C. ^{1}H NMR (D_2O + NaOD): δ 7.80 (s, 1H), 6.91−6.64 (m, 7H), 4.61 (t, 2H, J = 7.0 Hz), 2.93 (t, 2H, J = 7.0 Hz). Anal. ($C_{17}H_{16}N_5O_4P\cdot1.37H_2O$) C, H, N.

[5-(6-Amino-9-phenethyl-9*H*-purin-8-yl)-thiophen-2-yl]-phosphonic Acid (2.13). A solution of 8-bromo-9-phenethyladenine (**5a**, 214 mg, 0.625 mmol), diethyl 5-tri-*n*-butylstannyl-thiophen-2-yl-phosphonate (**14**, 1.592 g, 3.13 mmol), and $Pd(PPh_3)_4$ (72.2 mg, 0.0625 mmol) in anhydrous DMF (6 mL) was heated to 85 °C under nitrogen. After 16 h, the cooled reaction solution was evaporated and the residue was purified by flash chromatography (SiO_2, 2 cm × 15 cm, 1−10% MeOH-CH_2Cl_2) to give diethyl [5-(6-amino-9-phenethyl-9*H*-purin-8-yl)-thiophen-2-yl]-phosphonate as a clear film (192 mg, 67%).

A solution of diethyl [5-(6-amino-9-phenethyl-9*H*-purin-8-yl)-thiophen-2-yl]-phosphonate (112 mg, 0.245 mmol) in anhydrous acetonitrile (2.5 mL) was treated with TMSBr (0.32 mL, 2.42 mmol) at room temperature. After 5.5 h, the reaction mixture was evaporated and the residue was treated with acetonitrile (2 mL) and water (0.5 mL). After stirring at room temperature for 1 h, the resulting solid was collected via filtration (washed with H_2O, acetonitrile) and dried to give compound **2.13** as a white solid (70.6 mg, 72%); mp > 260 °C. ^{1}H NMR (DMSO-d_6): δ 8.21 (s, 1H), 7.61−7.06 (m, 7H), 4.63 (t, 2H, J = 6.8 Hz), 3.08 (t, 2H, J = 6.8 Hz). Anal. ($C_{17}H_{16}N_5O_3PS\cdot0.5H_2O$) C, H, N.

[3-(6-Amino-9-phenethyl-9*H*-purin-8-yl)-propyl]-phosphinic Acid (3.1). A solution of **5a** (923 mg, 2.90 mmol) in anhydrous DMF (9 mL) was treated with $Pd(PPh_3)_4$ (168 mg, 0.145 mmol) and allyltributyltin (1.12 mL, 3.63 mmol) under nitrogen. The resulting mixture was heated to 95 °C for 3.5 h. The cooled reaction mixture was evaporated to give a dark oil−solid mixture, which was triturated with hexane (3 × 10 mL). The residue was purified by flash chromatography (SiO_2, 3 cm × 15 cm, 3−5% MeOH-CH_2Cl_2, gradient elution) to give 8-allyl-9-phenethyl-9*H*-purin-6-ylamine (**16**) as a yellow solid (121 mg, 15%); mp 178−180 °C.

In a glass tube, a solution of compound **16** (47 mg, 0.168 mmol) in methanol (3 mL) was treated with NaH_2PO_2 (44 mg, 0.51 mmol) and AIBN (10 mg) and the reaction vessel was sealed and heated at 95 °C for 5 h. The cooled reaction solution was treated with additional reagents (NaH_2PO_2, 10 mg; AIBN, 5 mg; MeOH, 1 mL), sealed, and heated at 100 °C for 20 h. Another 5 mg of AIBN was added, and the reaction vessel was sealed and heated at 110 °C for 46 h. The cooled reaction solution was subjected to preparative HPLC (C18 reverse phase column, 35−82% MeOH-0.1 M aqueous AcOH gradient) to give compound **3.1** as a white solid (1.2 mg, 2.1%). ^{1}H NMR (D_2O): δ 8.22 (s, 1H), 7.32−6.95 (m, 5H), 6.91 (d, 1H, J = 503 Hz), 4.48 (t, 2H, J = 7.0 Hz), 3.12 (t, 2H, J = 7.0 Hz), 2.49 (t, 2H, J = 7.0 Hz), 1.91−1.41 (m, 4H). HRMS (*m*/*z*): 346.1419, calcd $[M + H]^+$: 346.1433.

[2-(6-Amino-9-phenethyl-9*H*-purin-8-yl)-1-methyl-ethyl]-phosphinic Acid (3.2). A solution of 8-allyl-9-phenethyl-9*H*-purin-6-ylamine (56 mg, 0.20 mmol) in ethanol (10 mL) was treated with NaH_2PO_2-H_2O (53 mg, 0.60 mmol) and AIBN (10 mg), and the pH was adjusted to 2.5 by adding two drops of H_2SO_4. The reaction mixture was heated to reflux for 18 h. The cooled reaction solution was subjected to preparative HPLC (C18 reverse phase column, 50% MeOH-0.1 M aqueous AcOH) to give compound **3.2** as a white solid (3.4 mg, 4.9%). ^{1}H NMR (D_2O): δ 8.26 (s, 1H), 7.28−6.87 (m, 5H), 6.74 (d, 1H, J = 507 Hz), 4.51 (t, 2H, J = 7.0 Hz), 3.14 (t, 2H, J = 7.0 Hz), 2.85−1.82 (m, 3H), 0.95 (dd, 3H, J_1 = 7.0 Hz, J_2 = 17.5 Hz). HRMS (*m*/*z*): 346.1438, calcd $[M + H]^+$: 346.1433.

[2-(6-Amino-9-phenethyl-9*H*-purin-8-yl)-ethoxy]-phosphinic Acid (3.3). A solution of 8-allyl-9-phenethyl-9*H*-purin-6-ylamine (942 mg, 3.3 mmol) in dichloromethane (7 mL) and methanol (1 mL) was treated with a drop of Sudan III, cooled to −78 °C, and bubbled with ozone gas until the color changed from red to yellow. The reaction solution was flushed with nitrogen, quenched with dimethyl sulfide (3 mL), and warmed to room temperature. After 2 h, the reaction solution was evaporated to dryness and the crude aldehyde was used for the next step without purification.

A solution of the above crude aldehyde in ethanol (8 mL) was cooled to 0 °C and treated with $NaBH_4$ (68 mg, 1.80 mmol). After 2.5 h, the reaction was quenched with 3% HCl and adjusted the pH to 7. The reaction solution was extracted with EtOAc (3 × 15 mL), and the combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to dryness. The material was purified by flash chromatography (SiO_2, 3 cm × 10 cm, 1−5% MeOH-CH_2Cl_2, gradient elution) to give 8-hydroxyethyl-9-phenethyl-9*H*-purin-6-ylamine (**17**) as a yellow solid (234 mg, 25% over two steps).

Compound **17** (50 mg, 0.176 mmol) was subjected to Froehler's procedure[22] and preparative HPLC (C18 reverse phase column, 30−90% MeCN-0.1 M aqueous AcOH) to give compound **3.3** as a white solid (23 mg, 34%); mp 188−189 °C. ^{1}H NMR (DMSO-d_6-D_2O): δ 8.07 (s, 1H), 7.20−6.95 (m, 5H), 6.43 (d, 1H, J = 598 Hz), 4.33 (t, 2H, J = 7.1 Hz), 3.00 (t, 2H, J = 7.0 Hz), 2.68 (t, 2H, J = 7.0 Hz). Anal. ($C_{15}H_{18}N_5O_3P\cdot0.5AcOH\cdot0.33H_2O$) C, H, N.

Phosphoric Acid Mono-[2-(6-amino-9-phenethyl-9*H*-purin-8-yl)-ethyl] Ester (3.4). Compound **17** (100 mg, 0.353 mmol) was subjected to Johns' procedure[23] and preparative HPLC (C18 reverse

phase column, 30−90% MeCN-0.1 M aqueous TFA) to give compound **3.4** as a white solid (22 mg, 14%). ^{1}H NMR (DMSO-d_6-D_2O): δ 7.83 (s, 1H), 7.23−7.01 (m, 5H), 6.61−5.36 (m, 4H), 4.33 (t, 2H, J = 7.0 Hz), 2.96 (t, 2H, J = 7.0 Hz). Anal. ($C_{15}H_{18}N_5O_4P\cdot 0.25MeCN\cdot 0.5TFA$) C, H, N.

5-(6-Amino-9-phenethyl-9*H*-purin-8-yl)-furan-2-carboxylic Acid (3.5). A solution of 6-chloro-8-furan-2-yl-9-phenethyl-9*H*-purine[21] (**18a**, 746 mg, 2.30 mmol) in anhydrous THF (20 mL) was cooled to −78 °C and treated with a solution of LDA in THF (1.5 M, 3.4 mL) under nitrogen. After 3 h, ethyl chloroformate (0.55 mL) was added and stirred at −78 °C for another 0.5 h. The reaction solution was warmed to room temperature, quenched with saturated sodium bicarbonate (100 mL), and extracted with dichloromethane (3 × 100 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to give a brown solid, which was purified by flash chromatography (SiO_2, 3 cm × 10 cm, 20% EtOAc-hexane) to give ethyl 5-(6-chloro-9-phenethyl-9*H*-purin-8-yl)-furan-2-carboxylate as a yellow solid (555 mg, 61%); mp 171−172 °C. Anal. ($C_{20}H_{17}N_4O_3Cl$) C, H, N.

A solution of ethyl 5-(6-chloro-9-phenethyl-9*H*-purin-8-yl)-furan-2-carboxylate (50.3 mg, 0.127 mmol) in DMSO-THF (1:1, 1.2 mL) cooled at −78 °C was treated with liquid ammonia (ca. 1 mL) in a sealed tube. The reaction solution was stirred at room temperature for 14 h. The reaction solution was diluted with saturated sodium bicarbonate (15 mL) and extracted with dichloromethane (3 × 15 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to give a brown solid, which was purified by flash chromatography (SiO_2, 1 cm × 15 cm, 100% EtOAc) to give ethyl 5-(6-amino-9-phenethyl-9*H*-purin-8-yl)-furan-2-carboxylate as a yellow solid (16.8 mg, 35%).

A solution of ethyl 5-(6-amino-9-phenethyl-9*H*-purin-8-yl)-furan-2-carboxylate (38.6 mg, 0.102 mmol) in ethanol-THF (1:3, 2 mL) was treated with sodium hydroxide (1 N, 0.15 mL) at room temperature under nitrogen. After 3 h, the organic solvents were evaporated and the residue was quenched with HCl (1 N, 0.15 mL). The resulting mixture was stored in a refrigerator overnight. The resulting solid was collected via filtration (washed with water, 3 × 5 mL) and dried under vacuum to give compound **3.5** as a white solid (9.8 mg, 29%); mp > 250 °C. ^{1}H NMR (DMSO-d_6): δ 8.20 (s, 1H), 7.47−7.11 (m, 7H), 4.66 (t, 2H, J = 7.0 Hz), 3.07 (t, 2H, J = 7.0 Hz). Anal. ($C_{18}H_{15}N_5O_3\cdot H_2O$) C, H, N.

5-(6-Amino-9-phenethyl-9*H*-purin-8-yl)-furan-2-sulfonic Acid (3.6). A solution of 5-amino-4-chloro-6-phenethylaminopyrimidine (100 mg, 0.412 mmol) and 2-formyl-furan-5-sulfonic acid sodium salt in anhydrous DMSO (4 mL) was treated with $FeCl_3$-SiO_2 (20%, 667 mg), and the resulting reaction mixture was heated at 80 °C for 6 h. The cooled reaction mixture was filtered through a celite pad (washed with 10% MeOH-CH_2Cl_2, 3 × 20 mL), and the filtrate was evaporated to give 5-(6-chloro-9-phenethyl-9*H*-purin-8-yl)-furan-2-sulfonic acid as a yellow oil, which was used for the next step without purification.

A solution of 5-(6-chloro-9-phenethyl-9*H*-purin-8-yl)-furan-2-sulfonic acid (55 mg, 0.136 mmol) in DMSO (1 mL) cooled at −78 °C was treated with liquid ammonia (ca. 2 mL) in a sealed tube. The reaction solution was stirred at room temperature for 18 h. The reaction solution was evaporated to give a yellow oil, which was purified by preparative HPLC (C18 column, 10−90% MeOH-0.1 N aqueous AcOH) to give compound **3.6** as a white solid (14 mg, 27%); mp >250 °C. ^{1}H NMR (D_2O + NaOD): δ 7.82 (s, 1H), 6.84−6.50 (m, 7H), 4.40 (t, 2H, J = 7.0 Hz), 2.73 (t, 2H, J = 7.0 Hz). Anal. ($C_{17}H_{15}N_5O_4PS$) C, H, N.

Diethyl [5-(6-Amino-9-phenethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonate (3.7). See synthesis of compound **2.12**.

[5-(6-Amino-9-phenethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonic Acid Monoethyl Ester (3.8). A solution of diethyl [5-(6-amino-9-phenethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonate (**3.7**, 76 mg, 0.172 mmol) in anhydrous acetonitrile (2 mL) was treated with TMSBr (0.136 mL, 1.03 mmol) at room temperature. After 16 h, another 0.086 mL of TMSBr was added and the reaction was stirred at room temperature for another 2 h. The reaction mixture was evaporated to dryness, and the residue was treated with acetonitrile (1 mL) and water (0.5 mL) at room temperature for 4 h. The resulting suspension was filtered (washed with water, 10 mL, MeCN, 10 mL) and the filtrate was evaporated to give a yellow solid, which was purified by preparative HPLC (C18 column, 10−90% MeOH-0.1 N aqueous AcOH) to give compound **3.8** as a white solid (9.5 mg, 13%); mp 257−258 °C. ^{1}H NMR (D_2O + NaOD): δ 8.18 (s, 1H), 7.21−7.01 (m, 7H), 4.99−4.70 (m, 2H), 3.95 (t, 2H, J = 7.0 Hz), 3.13 (t, 2H, J = 7.0 Hz). Anal. ($C_{19}H_{20}N_5O_4P\cdot 0.5H_2O$) C, H, N.

[5-(6-Dimethylamino-9-phenethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonic Acid (4.1). A solution of 6-chloro-N^9-phenethyl-8-(2-(5-diethylphosphono)furanyl)-purine (369 mg, 0.8 mmol) in DMF-THF (1:1, 4 mL) was treated with dimethylamine (2 mL, 4 mmol) at room temperature for 1 h. The reaction solution was quenched with saturated sodium bicarbonate (25 mL) and extracted with dichloromethane (3 × 25 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to give a brown solid, which was purified by flash chromatography (SiO_2, 2 cm × 10 cm, 3−5% MeOH-CH_2Cl_2, gradient elution) to give diethyl [5-(6-*N*,*N*-dimethylamino-9-phenethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonate as a light-brown solid (358 mg, 95%).

A solution of diethyl [5-(6-*N*,*N*-dimethylamino-9-phenethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonate (358 mg, 0.764 mmol) in anhydrous acetonitrile (7.5 mL) was treated with TMSBr (1.0 mL, 7.6 mmol) at room temperature. After 18 h, the reaction mixture was evaporated to dryness and the residue was treated with acetonitrile (8 mL) and water (3 mL) at room temperature for 1 h. The resulting white solid was collected via filtration (washed with water, 5 mL, MeCN, 5 mL) and dried under vacuum to give compound **4.1** as a white solid (279 mg, 88%); mp > 250 °C. ^{1}H NMR (D_2O + NaOD): δ 7.66 (s, 1H), 6.91−6.60 (m, 7H), 4.44 (t, 2H, J = 7.0 Hz), 3.11 (s, 6H), 2.83 (t, 2H, J = 7.0 Hz). Anal. ($C_{19}H_{20}N_5O_4P$) C, H, N.

[5-(6-Methylamino-9-phenethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonic Acid (4.2). A solution of 6-chloro-N^9-phenethyl-8-(2-(5-diethylphosphono)furanyl)-purine (206 mg, 0.45 mmol) in DMSO-THF (1:1, 2 mL) was treated with methylamine hydrogenchloride (152 mg, 2.25 mmol) and triethylamine (0.31 mL, 2.22 mmol). The reaction mixture was heated to 70 °C for 17 h. The reaction solution was quenched with saturated sodium bicarbonate (15 mL) and extracted with dichloromethane (3 × 15 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to give a brown oil, which was purified by flash chromatography (SiO_2, 2 × 10 cm, 3−5% MeOH-CH_2Cl_2, gradient elution) to give diethyl [5-(6-*N*-methylamino-9-phenethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonate as a yellow solid (92 mg, 38%).

A solution of diethyl [5-(6-*N*-methylamino-9-phenethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonate (76.5 mg, 0.168 mmol) in anhydrous acetonitrile (2 mL) was treated with TMSBr (0.22 mL, 1.68 mmol) at room temperature. After 18 h, the reaction mixture was evaporated to dryness and the residue was treated with acetonitrile (3 mL) and water (2 mL) at room temperature for 1 h. The resulting white solid was collected via filtration (washed with water, 5 mL, MeCN, 5 mL) and dried under vacuum to give compound **4.2** as a white solid (59.4 mg, 88%); mp 241−242 °C. ^{1}H NMR (D_2O + NaOD): δ 7.65 (s, 1H), 6.84−6.62 (m, 7H), 4.39 (t, 2H, J = 7.0 Hz), 2.79 (t, 2H, J = 7.0 Hz), 2.71 (s, 3H). Anal. ($C_{18}H_{18}N_5O_4P\cdot H_2O$) C, H, N.

[5-(6-Chloro-9-phenethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonic Acid (4.3). A solution of 6-chloro-N^9-phenethyl-8-(2-(5-diethylphosphono)furanyl)-purine (206 mg, 0.45 mmol) (4500 mg, 9.80 mmol) in anhydrous acetonitrile (20 mL) was treated with TMSBr (31 mL) at room temperature. After 18 h, the reaction solution was evaporated to dryness and the residue was treated with acetonitrile (10 mL) and water (5 mL) at room temperature for 1 h. The resulting solid was collected via filtration (washed with water, 5 mL and acetonitrile, 5 mL) and dried under vacuum to give compound **4.3** as a beige powder solid (1970 mg, 43%). ^{1}H NMR (DMSO-d_6): 8.74 (s, 1H), 7.49−7.13 (m, 7H), 4.83 (t, 2H, J = 7.0 Hz), 3.12 (t, 2H, J = 7.0 Hz). Anal. ($C_{17}H_{14}N_4O_4PCl\cdot 2H_2O\cdot 0.28HBr$) C, H, N.

{5-[6-Amino-9-(2,2-diphenyl-ethyl)-9*H*-purin-8-yl]-furan-2-yl}-phosphonic Acid (4.4). This compound was prepared in a similar manner as compound **2.12** from 5-amino-4-chloro-6-[*N*-(2,2-diphenyl)ethylamino]pyrimidine to give compound **4.4** as a yellow solid; mp > 220 °C. ^{1}H NMR (D_2O): δ 7.89 (s, 1H), 7.20−6.72 (m, 13H), 5.03 (d, 2H, J = 7.0 Hz). Anal. ($C_{23}H_{20}N_5O_4P\cdot 0.25H_2O$) C, H, N.

[5-(6-Amino-9-naphthalen-2-ylmethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonic Acid (4.6). This compound was prepared in a similar manner as compound **2.12** from 5-amino-4-chloro-6-(*N*-2-naphthylmethylamino)pyrimidine to give compound **4.6** as a white solid; mp 255−256 °C. ^{1}H NMR (D_2O): δ 7.89 (s, 1H), 7.62−6.49 (m, 9H), 5.55 (s, 2H). Anal. ($C_{20}H_{16}N_5O_4P\cdot 0.25H_2O$) C, H, N.

[5-(6-Amino-9-cyclopropyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonic Acid (4.7). This compound was prepared in a similar manner as compound **2.12** from 5-amino-4-chloro-6-(*N*-cyclopropylamino)pyrimidine to give compound **4.7** as a yellow solid; mp 278−285 °C. ^{1}H NMR (D_2O): δ 8.02 (s, 1H), 7.22 (m, 1H), 6.72 (m, 1H), 3.35 (m, 1H), 1.21 (m, 2H), 0.91 (m, 2H). MS *m*/*z* 322, calcd [M + H]$^+$ 322.

[5-(6-Amino-9-cyclopentyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonic Acid (4.8). This compound was prepared in a similar manner as compound **2.12** from 5-amino-4-chloro-6-(*N*-cyclopentylamino)pyrimidine to give compound **4.8** as a yellow solid; mp 220 °C (decomp). ^{1}H NMR (D_2O): δ 7.92 (s, 1H), 6.89 (m, 1H), 6.72 (m, 1H), 4.93 (m, 1H), 2.15−1.48 (m, 8H). Anal. ($C_{14}H_{16}N_5O_4P\cdot 1.25H_2O$) C, H, N.

[5-(6-Amino-9-ethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonic Acid (4.9). This compound was prepared in a similar manner as compound **2.12** from 5-amino-4-chloro-6-(*N*-ethylamino)pyrimidine to give compound **4.9** as a white solid; mp > 230 °C. ^{1}H NMR (D_2O): δ 8.09 (s, 1H), 7.12 (m, 1H), 6.80 (m, 1H), 4.41 (q, 2H, J = 7.0 Hz), 1.37 (t, 3H, J = 7.0 Hz). Anal. ($C_{11}H_{12}N_5O_4P\cdot 1H_2O$) C, H, N.

{5-[6-Amino-9-(2,2-dimethyl-propyl)-9*H*-purin-8-yl]-furan-2-yl}-phosphonic Acid (4.11). A solution of 6-chloro-9-neopentyl-8-(2-furanyl)adenine[21] (**18b**, 3.3 g, 11.3 mmol) in anhydrous THF (120 mL) was cooled to −78 °C under nitrogen and treated with LDA (17 mmol, 1.5 equiv) dropwise. The resulting reaction solution was stirred at −78 °C for 1 h and then treated with diethyl chlorophosphate (17 mmol, 1.5 equiv), and the reaction was continued at −78 °C for another hour. The reaction was quenched with saturated ammonium chloride (50 mL), and the phases were separated. The aqueous phase was extracted with ethyl acetate (3 × 50 mL), and the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a dark-red oil. The crude material was purified by flash chromatography on silica gel (5 cm × 15 cm, 40% EtOAc-hexane) to give 6-chloro-9-neopentyl-8-[2-(5-diethylphosphono)-furanyl]adenine as a red oil (4.4 g, 90%). ^{1}H NMR ($CDCl_3$): δ 8.70 (s, 1H), 7.36 (m, 1H), 7.27 (m, 1H), 4.57 (s, 2H), 4.18 (m, 4H), 1.35 (m, 6H), 0.86 (s, 9H). [MH]$^+$: 428 (as expected).

A solution of 6-chloro-9-neopentyl-8-[2-(5-diethylphosphono)-furanyl]adenine (4.4 g, 10.2 mmol) in THF (10 mL) and DMSO (10 mL) was cooled to −78 °C and treated with liquid ammonia (5 mL) in a pressure tube. The closed pressure tube was stirred at 25 °C for 12 h. The reaction was cooled to −78 °C, and the pressure tube was opened to air and let warmed to 25 °C slowly with stirring. The reaction solution was evaporated under reduced pressure, and the residue was dissolved in ethyl acetate (100 mL) and washed with saturated sodium bicarbonate (30 mL), water (2 × 100 mL), and brine (20 mL) and dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a brown solid. The crude material was purified by flash chromatography on silica gel (5 cm × 15 cm, 10% MeOH-EtOAc) to give 6-amino-9-neopentyl-8-[2-(5-diethylphosphono)furanyl]adenine as a white solid (2.6 g, 63%). ^{1}H NMR (DMSO-d_6): δ 8.17 (s, 1H), 7.45 (bs, 2H), 7.38 (m, 1H), 7.24 (m, 1H), 4.36 (s, 2H), 4.11 (m, 4H), 1.26 (t, 6H, J = 6.9 Hz), 0.77 (s, 9H). [MH]$^+$: 408 (as expected).

A solution of 6-amino-9-neopentyl-8-[2-(5-diethylphosphono)-furanyl]adenine (2.6 g, 6.4 mmol) in anhydrous acetonitrile (50 mL) was treated with trimethylsilyl bromide (64 mmol, 10 equiv) at 25 °C. After 12 h, the reaction was evaporated to dryness under reduced pressure and the residue was treated with water (12 mL) and stirred at 25 °C for 2 h. The resulting solid was collected via filtration (washed with water, 3 × 10 mL) and dried under vacuum at 50 °C for 12 h to give **4.11** as a yellow solid (2.2 g, 97%). ^{1}H NMR (DMSO-d_6): δ 8.16 (s, 1H), 7.57 (bs, 2H), 7.12 (m, 1H), 7.01 (m, 1H), 4.39 (s, 2H), 0.75 (s, 9H). [MH]$^+$: 352 (as expected). Anal. ($C_{14}H_{18}N_5O_4P$) C, H, N.

[5-(9-Adamantan-1-ylmethyl-6-amino-9*H*-purin-8-yl)-furan-2-yl]-phosphonic Acid (4.12). This compound was prepared in a similar manner as compound **2.12** from 5-amino-4-chloro-6-(*N*-admantylamino)pyrimidine to give compound **4.12** as a yellow solid; mp 262 °C (decomp). ^{1}H NMR (DMSO-d_6 + D_2O): δ 8.01 (s, 1H), 7.02 (m, 1H), 6.65 (m, 1H), 4.21 (s, 2H), 2.87−1.28 (m, 15H). Anal. ($C_{20}H_{24}N_5O_4P\cdot 0.5H_2O\cdot 0.25MeOH$) C, H, N.

[5-(6-Amino-9-isobutyl-2-methylsulfanyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonic Acid (4.13). A solution of 2-methylthio-4,5,6-triaminopyrimidine (250 mg, 1.462 mmol) and 5-diethylphosphono-2-furaldehyde (**13**, 407 mg, 1.754 mmol) in anhydrous DMSO (10 mL) was treated with $FeCl_3$-SiO_2 (20%, 1.78 g, 2.193 mmol), and the resulting reaction mixture was heated at 80 °C. After 18 h, the cooled reaction mixture was diluted with EtOAc (100 mL) and filtered (washed with EtOAc, 3 × 15 mL). The filtrate was evaporated, and the residue was purified by flash chromatography (SiO_2, 3 cm × 15 cm, 3−5% MeOH-CH_2Cl_2, gradient elution) to give 6-amino-2-methylthio-8-(2-(5-diethylphosphono)furanyl)purine (**26**) as a yellow solid (369 mg, 66%).

A solution of compound **26** (30 mg, 0.078 mmol) in anhydrous DMF (1 mL) was cooled to 0 °C and treated with sodium hydride (60%, 4.7 mg, 0.117 mmol) under nitrogen. After stirring at room temperature for 1 h, isobutyliodide (36 mg, 0.235 mmol) was added and the reaction solution was stirred at room temperature for another 2 h. The reaction was quenched with saturated ammonium chloride (15 mL) and extracted with dichloromethane (3 × 15 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to dryness. The resulting yellow gum was purified by preparative TLC to give diethyl [5-(6-amino-9-isobutyl-2-methylsulfanyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonate (**27a**) as a yellow solid (30 mg, 87%).

A solution of compound **27a** (30 mg, 0.068 mmol) in anhydrous acetonitrile (1 mL) was treated with TMSBr (0.134 mL, 1.02 mmol) at room temperature. After 18 h, the reaction solution was evaporated to dryness and the residue was diluted with dichloromethane (5 mL) and water (1 mL). After stirring at room temperature for 20 min, dichloromethane was evaporated and the resulting solid was collected via filtration (washed with water, 3 × 5 mL, EtOAc, 3 × 1 mL) and dried under vacuum to give compound **4.13** as a white solid; mp 218−220 °C. ^{1}H NMR ($CDCl_3$): δ 6.97 (m, 1H), 6.73 (m, 1H), 4.07 (d, 2H, J = 7.0 Hz), 2.40 (s, 3H), 2.02 (m, 1H), 0.71 (d, 6H, J = 7.0 Hz). Anal. ($C_{14}H_{18}N_5O_4PS\cdot 0.25HBr\cdot 0.25EtOAc$) C, H, N.

[5-(6-Amino-9-isobutyl-2-methanesulfonyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonic Acid (4.14). A solution of diethyl [5-(6-amino-9-isobutyl-2-methylsulfanyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonate (35 mg, 0.08 mmol) in methanol (0.5 mL) was cooled to 0 °C and treated with a solution of oxone (80 mg, 0.13 mmol) in acetate buffer (pH = 4.2, 0.5 mL). The resulting suspension was stirred at room temperature for 3 h. The reaction was quenched with saturated sodium bicarbonate (15 mL) and extracted with EtOAc (3 × 15 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to dryness. The resulting yellow gum was purified by preparative TLC to give diethyl [5-(6-amino-9-isobutyl-2-methylsulfonyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonate as a white solid (30 mg, 80%).

A solution of diethyl [5-(6-amino-9-isobutyl-2-methylsulfonyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonate (51 mg, 0.11 mmol) in anhydrous acetonitrile (1.1 mL) was treated with TMSBr (0.21 mL, 1.6 mmol) at room temperature. After 18 h, the reaction solution was evaporated to dryness and the residue was diluted with dichloromethane (5 mL) and water (1 mL). After stirring at room

temperature for 1 h, dichloromethane was evaporated and the resulting solid was collected via filtration (washed with water, 3 × 5 mL, EtOAc, 3 × 1 mL) and dried under vacuum to give compound **4.14** as a white solid (24 mg, 53%);mp 245 °C (decomp). ^{1}H NMR (CD_3OD): δ 7.34 (m, 1H), 7.25 (m, 1H), 4.49 (d, 2H, J = 7.0 Hz), 3.35 (s, 3H), 2.23 (m, 1H), 0.95 (d, 6H, J = 7.0 Hz). Anal. ($C_{14}H_{18}N_5O_6PS \cdot 0.5H_2O$) C, H, N.

{5-[6-Amino-9-(3-dimethylamino-2,2-dimethyl-propyl)-9*H*-purin-8-yl]-furan-2-yl}-phosphonic Acid (5.1). This compound was prepared in a similar manner as compound **2.12** from 5-amino-4-chloro-6-[*N*-(2,2-dimethyl-2-dimethylaminomethyl)-ethylamino]-pyrimidine to give compound **5.1** as a yellow solid; mp > 220 °C. ^{1}H NMR (D_2O): δ 7.89 (s, 1H), 6.98 (m, 1H), 6.71 (m, 1H), 4.31 (s, 2H), 2.18 (s, 2H), 2.09 (s, 6H), 0.61 (s, 6H). Anal. ($C_{16}H_{23}N_6O_4P \cdot 1.3H_2O \cdot 0.5HBr \cdot 1AcOH$) C, H, N.

{5-[6-Amino-9-(3-hydroxy-2,2-dimethyl-propyl)-9*H*-purin-8-yl]-furan-2-yl}-phosphonic Acid (5.2). This compound was prepared in a similar manner as compound **2.12** from 5-amino-4-chloro-6-[*N*-(2,2-dimethyl-2-hydroxymethyl)ethylamino]-pyrimidine to give compound **5.2** as a white solid; mp > 220 °C. ^{1}H NMR (D_2O): δ 8.01 (s, 1H), 7.03 (m, 1H), 6.71 (m, 1H), 4.36 (s, 2H), 3.08 (s, 2H), 0.62 (s, 6H). Anal. ($C_{14}H_{18}N_5O_5P \cdot 0.25H_2O$) C, H, N.

{5-[6-Amino-9-(3-chloro-2,2-dimethyl-propyl)-9*H*-purin-8-yl]-furan-2-yl}-phosphonic Acid (5.3). This compound was prepared in a similar manner as compound **2.12** from 5-amino-4-chloro-6-[*N*-(2,2-dimethyl-2-chloromethyl)-ethylamino]-pyrimidine to give compound **5.3** as a yellow solid; mp > 220 °C. ^{1}H NMR (D_2O): δ 7.99 (s, 1H), 7.02 (m, 1H), 6.70 (m, 1H), 4.41 (s, 2H), 3.31 (s, 2H), 0.69 (s, 6H). Anal. ($C_{16}H_{23}N_6O_4P \cdot 0.23CH_2Cl_2$) C, H, N.

{5-[6-Amino-9-(3,3-dimethyl-butyl)-9*H*-purin-8-yl]-furan-2-yl}-phosphonic Acid (5.4). This compound was prepared in a similar manner as compound **2.12** from 5-amino-4-chloro-6-(*N*-(3,3-dimethyl-butyl-amino)pyrimidine to give compound **5.4** as a white solid; mp > 220 °C. ^{1}H NMR (D_2O + NaOD): δ 8.01 (s, 1H), 6.98 (m, 1H), 6.75 (m, 1H), 4.25 (t, 2H, J = 7.0 Hz), 1.51 (t, 2H, J = 6.9 Hz), 0.91 (s, 9H). Anal. ($C_{15}H_{20}N_5O_4P \cdot 1.25H_2O \cdot 0.13AcOEt$) C, H, N.

{5-[6-Amino-9-(1,2,2-trimethyl-propyl)-9*H*-purin-8-yl]-furan-2-yl}-phosphonic Acid (5.5). This compound was prepared in a similar manner as compound **2.12** from 5-amino-4-chloro-6-(*N*-(1,2,2-trimethyl-propyl-amino)-pyrimidine to give compound **5.5** as a white solid; mp > 250 °C. ^{1}H NMR (D_2O + NaOD): δ 8.08 (s, 1H), 7.01 (m, 1H), 6.71 (m, 1H), 4.99 (q, 1H, J = 7.0 Hz), 1.73 (d, 3H, J = 7.0 Hz), 0.79 (s, 9H). Anal. ($C_{15}H_{20}N_5O_4P \cdot 0.67H_2O$) C, H, N.

{5-[2,6-Diamino-9-(2,2-dimethyl-propyl)-9*H*-purin-8-yl]-furan-2-yl}-phosphonic Acid (5.6). A solution of 2-amino-4,6-dichloro-pyrimidine (947 mg, 5.77 mmol), neopentylamine (529 mg, 6.063 mmol), and triethylamine (1.61 mL, 11.54 mmol) in *n*-butanol (20 mL) was stirred at 110 °C for 12 h. The cooled reaction solution was evaporated to dryness, and the residue was partitioned between dichloromethane (30 mL) and saturated sodium bicarbonate (30 mL). The layers were separated, and the aqueous phase was extracted with dichloromethane (3 × 20 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated. The residue was purified by flash chromatography (SiO_2, 3 cm × 15 cm, 10, 30, 50% EtOAc-hexane, gradient elution) to give 2-amino-4-chloro-6-*N*-neopentylaminopyrimidine (**22**) as a yellow solid (1.23 g, 99%).

A mixture of compound **22** (922 mg, 4.294 mmol), $NaOAc \cdot 3H_2O$ (8.18 g, 60.12 mmol), acetic acid (21 mL), and 4-chlorobenzene-diazonium hexafluorophosphate (1.41 g, 4.94 mmol) in water (20 mL) was stirred at 25 °C for 12 h. The resulting yellow solid was collected via filtration (washed with water, 3 × 40 mL) and dried under vacuum. The yellow solid was treated with zinc dust (2.81 g, 42.94 mmol) and acetic acid (1.36 mL) in EtOH-H_2O (1:1, 20 mL) at 80 °C for 4 h. The cooled reaction mixture was filtered through a celite pad (washed with dichloromethane, 3 × 25 mL). The filtrate was diluted with saturated sodium bicarbonate (50 mL), and the layers were separated. The aqueous layer was extracted with dichloromethane (3 × 30 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated. The residue was purified by flash chromatography (SiO_2, 4 cm × 10 cm, 50% EtOAc-hexane) to give 4-chloro-2,5-diamino-6-*N*-neopentylaminopyrimidine (**23**) as a yellow solid (292 mg, 30% for the two steps).

Compound **23** was subjected to the reaction sequences used for the preparation of compound **2.12** to give compound **5.6** as a yellow solid; mp 240 °C (decomp). ^{1}H NMR (D_2O): δ 6.82 (m, 1H), 6.60 (m, 1H), 4.12 (s, 2H), 0.61 (s, 9H). Anal. ($C_{14}H_{19}N_6O_4P \cdot 2.2HBr \cdot 0.5$acetone) C, H, N.

{5-[6-Amino-9-(2,2-dimethyl-propyl)-2-methylsulfanyl-9*H*-purin-8-yl]-furan-2-yl}-phosphonic Acid (5.7). Compound **5.7** was prepared in a similar manner as compound **4.13** from compound **26** and neopentyl iodide to give compound **5.7** as a yellow solid; mp > 250 °C. ^{1}H NMR ($CDCl_3$): δ 7.07 (m, 1H), 6.78 (m, 1H), 4.39 (s, 2H), 2.53 (s, 3H), 0.76 (s, 9H). Anal. ($C_{15}H_{20}N_5O_4PS \cdot 0.2HBr \cdot 0.2$toluene) C, H, N.

{5-[6-Amino-9-(2,2-dimethyl-propyl)-9*H*-purin-8-yl]-1-methyl-1*H*-pyrrol-2-yl}-phosphonic Acid (5.8). A solution of *N*-methylpyrrole (1 g, 12.34 mmol) in anhydrous THF (30 mL) was treated with TMEDA (1.57 g, 13.58 mmol) and *n*-BuLi (2.5 N hexane, 5.43 mL) at room temperature. After 40 min, the reaction solution was added into a solution of diethyl chlorophosphate (3.6 mL) in THF (30 mL) and cooled at 0 °C under nitrogen. The resulting reaction solution was stirred at room temperature for 1 h and quenched with saturated sodium bicarbonate (30 mL). Most of THF was removed under vacuum, and the residue was extracted, followed by thionyl chloride (0.62 mL, 8.55 mmol), and the resulting mixture was extracted with EtOAc (3 × 30 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, 3 cm × 10 cm, 50, 80, 100% EtOAc-hexane, gradient elution) to give *N*-methyl-2-diethylphosphonopyrrole as an oil (1.25 g, 47%).

A solution of *N*-methyl-2-diethylphosphonopyrrole (500 mg, 2.3 mmol) in anhydrous THF (11 mL) was cooled to −78 °C under nitrogen and treated with LDA (1.5 N, 2.3 mL). After 40 min, methyl formate (0.43 mL, 6.9 mmol) was added and the reaction solution was stirred at −78 °C for another 1.5 h. The reaction was quenched with saturated sodium bicarbonate (15 mL) and extracted with dichloromethane (3 × 20 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, 3 cm × 10 cm, 50, 80, 100% EtOAc-hexane, gradient elution) to give *N*-methyl-2-diethylphosphono-5-formylpyrrole as an oil (140 mg, 25%).

N-Methyl-2-diethylphosphono-5-formylpyrrole was subjected to the reaction sequences used for the preparation of compound **2.12** to give compound **5.8** as an off-white solid; mp >240 °C. ^{1}H NMR (D_2O + NaOD): δ 8.09 (s, 1H), 6.40 (m, 2H), 4.11 (s, 2H), 3.74 (s, 3H), 0.59 (s, 9H). Anal. ($C_{15}H_{21}N_6O_3P \cdot 0.3HBr \cdot 0.1MePh \cdot 1H_2O$) C, H, N.

{5-[6-Amino-9-(2,2-dimethyl-propyl)-9*H*-purin-8-yl]-1-methyl-1*H*-imidazol-2-yl}-phosphonic Acid (5.9). A solution of 1-methylimidazole (3 g, 36.6 mmol) in anhydrous THF (100 mL) was cooled to −78 °C under nitrogen and treated with TMEDA (10 mL), followed by *n*-BuLi (2.5 N hexane, 16 mL). After 0.5 h, the reaction solution was added to a solution of diethyl chlorophosphate (10 mL) in anhydrous THF (100 mL) and cooled at −78 °C under nitrogen. After 1 h, the reaction was quenched with saturated sodium bicarbonate (50 mL) and warmed to room temperature. The layers were separated, and the aqueous phase was extracted with dichloromethane (2 × 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to give a brown−orange sludge, which was purified by flash chromatography (SiO_2, 5 cm × 10 cm, 50, 80, 100% EtOAc-hexane) to give 1-methyl-2-diethylphosphonoimidazole as an oil (1.5 g, 19%).

A solution of 1-methyl-2-diethylphosphonoimidazole (1500 mg, 6.9 mmol) in anhydrous THF (35 mL) was cooled to −78 °C under nitrogen and treated with LDA (1.5N, 5 mL). After 30 min, methyl formate (1.24 g, 20.7 mmol) was added and the reaction solution was stirred at −78 °C for another 1 h. The reaction was quenched with saturated sodium bicarbonate (30 mL) and extracted with dichloromethane (3 × 20 mL). The combined organic extracts were

dried ($MgSO_4$), filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, 3 cm × 10 cm, 50, 80, 100% EtOAc-hexane, gradient elution) to give 1-methyl-2-diethylphosphono-5-formylimidazole as an oil (550 mg, 32%).

1-Methyl-2-diethylphosphono-5-formylimidazole was subjected to the reaction sequences used for the preparation of compound **2.12** to give compound **5.9** as a yellow solid; mp 240 °C (decomp). ^{1}H NMR (D_2O + NaOD): δ 8.04 (s, 1H), 7.39 (s, 1H), 4.01 (s, 2H), 3.75 (s, 3H), 0.51 (s, 9H). Anal. ($C_{14}H_{20}N_7O_3P\cdot 1.5HOAc\cdot 0.25H_2O$) C, H, N.

{5-[6-Amino-9-(2,2-dimethyl-propyl)-9*H*-purin-8-yl]-furan-3-yl}-phosphonic Acid (5.10). A solution of 3-bromofuran (2 g, 13.6 mmol) in anhydrous ether (28 mL) was cooled to −78 °C under nitrogen and treated with *n*-BuLi (2.5 N hexane, 5.5 mL). After 0.5 h, the reaction solution was treated with diethyl chlorophosphate (3.9 mL, 27.2 mmol) dropwise at −78 °C under nitrogen. After 2 h, the reaction was quenched with water (30 mL) and warmed to room temperature. The layers were separated, and the aqueous phase was extracted with dichloromethane (2 × 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to give a brown−orange sludge, which was purified by flash chromatography (SiO_2, 5 cm × 10 cm, 50, 80, 100% EtOAc-hexane) to give 3-diethylphosphonofuran as an oil (1.3 g, 47%).

A solution of 3-diethylphosphonofuran (1200 mg, 5.88 mmol) in anhydrous THF (58 mL) was cooled to −78 °C under nitrogen and treated with LDA (1.5N, 3.92 mL). After 30 min, methyl formate (1.1 mL, 17.7 mmol) was added and the reaction solution was stirred at −78 °C for another 1.5 h. The reaction was quenched with saturated sodium bicarbonate (30 mL) and extracted with dichloromethane (3 × 20 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, 3 cm × 10 cm, 50, 80, 100% EtOAc-hexane, gradient elution) to give 3-diethylphosphono-5-formylfuran as an oil (880 mg, 65%).

3-Diethylphosphono-5-formylfuran was subjected to the reaction sequences used for the preparation of compound **2.12** to give compound **5.10** as a yellow solid; mp > 240 °C. ^{1}H NMR (D_2O + NaOD): δ 8.12 (s, 1H), 7.59 (m, 1H), 6.64 (m, 1H), 4.24 (s, 2H), 0.59 (s, 9H). Anal. ($C_{14}H_{18}N_5O_4P\cdot 0.75H_2O$) C, H, N.

{1-[6-Amino-9-(2,2-dimethyl-propyl)-9*H*-purin-8-ylmethyl]-1*H*-imidazol-2-yl}-phosphonic Acid (5.11). A solution of 1-benzylimidazole (5.1 g, 32.24 mmol) in anhydrous THF (80 mL) was treated with LDA (1.5M-THF, 23.6 mL) under nitrogen at −78 °C for 1 h, followed by addition of diethyl chlorophosphate (9.2 mL, 64 mmol), and stirred for another 2 h. The reaction was quenched with saturated sodium bicarbonate (50 mL) and warmed to room temperature. THF was removed under vacuum, and the residue was extracted with EtOAc (3 × 40 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, 3 cm × 10 cm, 50, 80, 100% EtOAc-hexane, gradient elution) to give 1-benzyl-2-diethylphosphonoimidazole as an oil (880 mg, 65%).

A solution of 1-benzyl-2-diethylphosphonoimidazole (95 mg, 0.323 mmol) in EtOH (5 mL) was treated with palladium on carbon (10%, 10 mg) at 25 °C under 1 atm of hydrogen for 19 h. The reaction mixture was filtered through a celite pad (washed with EtOH, 3 × 10 mL), and the filtrate was evaporated to dryness. The resulting white solid was confirmed to be 2-diethylphosphonoimidazole (65 mg, 98%). MS (*m*/*z*): 227, calcd for $[M + Na]^+$ 227. ^{1}H NMR ($CDCl_3$): δ 7.30−7.20 (m, 2H), 4.25−4.05 (m, 4H), 1.28 (t, 6H, J = 6.9 Hz).

A solution of 2-diethylphosphonoimidazole (40 mg, 0.197 mmol) in anhydrous acetonitrile (1 mL) was treated with Hunig's base (0.034 mL, 0.197 mmol) at room temperature under nitrogen. After 0.5 h, a solution of 6-chloro-8-bromomethyl-9-neopentyl-9*H*-purine (25 mg, 0.079 mmol) in acetonitrile (1 mL) was added and the resulting reaction solution was heated to 50 °C. After 2 h, the reaction solution was evaporated to dryness and the residue was purified by preparative TLC to give diethyl {1-[6-chloro-9-(2,2-dimethyl-propyl)-9*H*-purin-8-ylmethyl]-1*H*-imidazol-2-yl}-phosphonate as a yellow solid (19 mg, 55%).

A solution of diethyl {1-[6-chloro-9-(2,2-dimethyl-propyl)-9*H*-purin-8-ylmethyl]-1*H*-imidazol-2-yl}-phosphonate (54 mg, 0.123 mmol) in DMSO-THF (1:1, 2 mL) cooled at −78 °C was treated with liquid ammonia (ca. 2 mL) in a sealed tube. The reaction solution was stirred at room temperature for 18 h. The reaction solution was diluted with EtOAc (15 mL) and washed with water (5 × 20 mL), dried ($MgSO_4$), and evaporated to dryness, and the resulting oil was purified by preparative TLC to give diethyl {1-[6-amino-9-(2,2-dimethyl-propyl)-9*H*-purin-8-ylmethyl]-1*H*-imidazol-2-yl}-phosphonate as a yellow solid (26 mg, 50%).

A solution of diethyl {1-[6-amino-9-(2,2-dimethyl-propyl)-9*H*-purin-8-ylmethyl]-1*H*-imidazol-2-yl}-phosphonate (35 mg, 0.083 mmol) in anhydrous acetonitrile (1.6 mL) was treated with TMSBr (0.16 mL, 1.25 mmol) at room temperature. After 18 h, the reaction mixture was evaporated to dryness, and the residue was treated with water at room temperature for 1 h. The resulting white solid was collected via filtration (washed with water, 5 mL, MeCN, 5 mL) and dried under vacuum to give compound **5.11** as a white solid; mp >250 °C. ^{1}H NMR (D_2O + NaOD): δ 8.06 (s, 1H), 6.93 (m, 2H), 5.82 (s, 2H), 4.05 (s, 2H), 0.90 (s, 9H). MS (*m*/*z*): 366, calcd for $[M + H]^+$, 366. HPLC purity (UV detection at 280 nm) >95%.

{5-[6-Amino-9-(2,2-dimethyl-propyl)-9*H*-purin-8-yl]-3,4-dichloro-furan-2-yl}-phosphonic Acid (5.12). A solution of 3,4-dichloro-2-furoic acid (3 g, 16.57 mmol) in anhydrous diethyl ether (165 mL) was treated with LDA (1.5 M THF, 33 mL, 49.72 mmol) at −78 °C under nitrogen. The reaction solution was stirred at −78 °C for 45 min and then treated with diethyl chlorophosphate (7 mL, 49.72 mmol) at −78 °C and stirred for another 2 h. The reaction was quenched with saturated ammonium chloride (30 mL), and the layers were separated. The aqueous layer was extracted with diethyl ether (2 × 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to give 5-diethylphosphono-3,4-dichloro-2-furoic acid as a yellow foam (6.5 g, crude material).

A solution of 5-diethylphosphono-3,4-dichloro-2-furoic acid (16.57 mmol) in anhydrous dichloromethane (40 mL) was cooled to 0 °C and treated with oxalyl chloride (20 mL), followed by DMF (1.6 mmol). After stirring at 25 °C for 1 h, the reaction solution was evaporated and the residue was dissolved in anhydrous THF (20 mL), cooled to 0 °C, and treated with a solution of 4-chloro-5-amino-6-neopentylaminopyrimidine (4.2 g, 19.8 mmol) and pyridine (4 mL) in THF (60 mL). After stirring at 25 °C for 16 h, the reaction solution was evaporated to dryness and the residue was partitioned between EtOAc (50 mL) and water (40 mL). The layers were separated, and the organic phase was dried ($MgSO_4$), filtered, and evaporated to dryness. The crude material was purified by flash chromatography (SiO_2, 5 cm × 15 cm, 30%, 50% EtOAc-hexane, gradient elution) to give diethyl {3,4-dichloro-5-[4-chloro-6-(2,2-dimethyl-propylamino)-pyrimidin-5-ylcarbamoyl]-furan-2-yl}-phosphonate as a yellow solid (2.2 g, 26% for the two steps).

A solution of diethyl {3,4-dichloro-5-[4-chloro-6-(2,2-dimethyl-propylamino)-pyrimidin-5-ylcarbamoyl]-furan-2-yl}-phosphonate (50 mg, 0.1 mmol) in dichloroethane (2 mL) was treated with silicone tetrachloride (1 M CH_2Cl_2, 0.24 mL, 0.24 mmol) and triethylamine (34 μL, 0.24 mmol) and heated at 45 °C for 18 h. The cooled reaction mixture was quenched with saturated sodium bicarbonate (15 mL) and extracted with dichloromethane (3 × 20 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to dryness. The crude material was purified by preparative TLC (SiO_2, 50% EtOAc-hexane) to give diethyl {3,4-dichloro-5-[6-chloro-9-(2,2-dimethyl-propyl)-9*H*-purin-8-yl]-furan-2-yl}-phosphonate (20 mg, 41%) as a yellow solid.

A solution of diethyl {3,4-dichloro-5-[6-chloro-9-(2,2-dimethyl-propyl)-9*H*-purin-8-yl]-furan-2-yl}-phosphonate (40 mg, 0.08 mmol) in DMSO-THF (1:1, 1 mL) cooled at −78 °C was treated with liquid ammonia (ca. 2 mL) in a sealed tube. After stirring at room temperature for 18 h, the reaction solution was evaporated to dryness and the resulting oil was purified by preparative TLC (70% EtOAc-hexane) to give diethyl {3,4-dichloro-5-[6-amino-9-(2,2-dimethyl-propyl)-9*H*-purin-8-yl]-furan-2-yl}-phosphonate (25 mg, 65%) as a white solid.

A solution of diethyl {3,4-dichloro-5-[6-amino-9-(2,2-dimethyl-propyl)-9*H*-purin-8-yl]-furan-2-yl}-phosphonate (25 mg, 0.052 mmol) in anhydrous acetonitrile (1 mL) was treated with TMSBr (0.1 mL, 0.78 mmol) at room temperature. After 18 h, the reaction mixture was evaporated to dryness and the residue was treated with water at room temperature for 1 h. The resulting white solid was collected via filtration (washed with water, 5 mL, EtOAc, 5 mL) and dried under vacuum to give compound **5.12** as a white solid (13 mg, 60%); mp > 250. ^{1}H NMR (D_2O + NaOD): δ 8.05 (s, 1H), 4.25 (s, 2H), 0.6 (s, 9H). Anal. ($C_{14}H_{16}N_5O_4PC1_2 \cdot 0.5H_2O \cdot 0.15$EtOAc) C, H, N.

Isobutyric Acid [5-(6-Amino-9-phenethyl-9*H*-purin-8-yl)-furan-2-yl]-isobutyryloxymethoxy-phosphinoyloxymethyl Ester (8.1). A solution of 8-(2-(5-phosphono)furanyl)-N^9-phenethyladenine (2.12, 832 mg, 2.16 mmol) and *N*,*N*,*N*-diisopropylethylamine (1.396 g, 10.8 mmol) in acetonitrile (20 mL) was treated with isobutyric acid iodomethyl ester (1.97 g, 8.64 mmol) at 0 °C under nitrogen. After 24 h, another 326 mg of isobutyric acid iodomethyl ester was added and the reaction solution was stirred at 0 °C for another 24 h. The reaction solution was diluted with dichloromethane (200 mL), water (100 mL), and saturated sodium bicarbonate (100 mL). The layers were separated, and the aqueous phase was extracted with dichloromethane (3 × 150 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to dryness. The resulting yellow gum was purified by preparative HPLC (C18, ODS-AQ-S5 YMC column, 30−90% MeCN-0.5% aqueous AcOH) to give compound **8.1** as a yellow foam (190 mg, 16%). ^{1}H NMR ($CDCl_3$): δ 8.42 (s, 1H), 7.30−7.02 (m, 7H), 6.06 (bs, 2H), 5.79 (d, 4H, J = 20 Hz), 4.78 (t, 2H, J = 7.0 Hz), 3.13 (t, 2H, J = 7.0 Hz), 2.53 (p, 2H, J = 6.9 Hz), 1.10 (d, 6H, J = 6.9 Hz), 1.12 (d, 6H, J = 6.9 Hz). Anal. ($C_{27}H_{32}N_5O_8P$) C, H, N.

[5-(6-Amino-9-phenethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonic Acid Bis-(4,5,6-trimethoxy-3-*oxo*-1,3-dihydro-isobenzofuran-1-yl) Ester (8.2). Compound **8.2** was synthesized in a similar manner as compound **8.1** from compound **2.12** (227 mg, 0.589 mmol) using 3-bromo-5,6,7-trimethoxyphthalide as the alkylating reagent to give compound **8.2** as a white solid (14 mg, 3%) after preparative HPLC purification; mp 155−160 °C. ^{1}H NMR ($CDCl_3$): δ 8.32 (s, 1H), 7.30−6.45 (m, 11H), 5.81 (bs, 2H), 4.69 (t, 2H, J = 7.0 Hz), 4.16 (s, 6H), 3.99 (s, 6H), 3.91 (s, 6H), 3.12 (t, 2H, J = 7.0 Hz). Anal. ($C_{39}H_{36}N_5O_{14}P$ + H_2O) C, H, N.

[5-(6-Amino-9-phenethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonic Acid Bis-(3-cyano-4-methoxy-benzyl) Ester (8.3). A suspension of 8-(2-(5-phosphono)furanyl)-N^9-phenethyladenine (300 mg, 0.74 mmol) in thionyl chloride (1.5 mL) was warmed at refluxing 1 h. The cooled reaction mixture was evaporated to dryness. The residue was dissolved in anhydrous dichloromethane (1.5 mL) and added to a solution of the 3-cyano-4-methoxybenzyl alcohol (302 mg, 1.86 mmol) and pyridine (0.15 mL, 1.86 mmol) in dichloromethane (0.5 mL). After stirring at room temperature for 4 h, the reaction solution was quenched with water and neutralized to pH = 7 and extracted with dichloromethane (3 × 20 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to dryness. The resulting yellow gum was purified by preparative TLC to give [5-(6-chloro-9-phenethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonic acid bis-(3-cyano-4-methoxy-benzyl) ester as a yellow solid (270 mg, 52%).

A solution of [5-(6-chloro-9-phenethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonic acid bis-(3-cyano-4-methoxy-benzyl) ester (300 mg, 0.43 mmol) in DMSO-THF (1:10, 2.2 mL) was treated with liquid ammonia (ca. 3 mL) in a sealed tube at −78 °C. The resulting reaction solution was stirred at room temperature for 2 h. The reaction solution was evaporated to dryness, and the residue was purified by preparative TLC to give compound **8.3** as a sticky solid (59 mg, 33%). ^{1}H NMR ($CDCl_3$): δ 8.40 (s, 1H), 7.60−6.85 (m, 13H), 5.81 (bs, 2H), 5.20−4.95 (m, 2H), 4.65 (t, 2H, J = 6.9 Hz), 3.92 (s, 6H), 3.08 (t, 2H, J = 6.9 Hz). Anal. ($C_{35}H_{30}N_7O_6P$ + 1.5 H_2O) C, H, N.

[5-(6-Amino-9-phenethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphonic Acid Bis-[2-(2-hydroxy-ethyldisulfanyl)-ethyl] Ester (8.4). This compound was prepared in a similar manner as compound **8.3** from 8-(2-(5-phosphono)furanyl)-N^9-phenethyladenine (100 mg, 0.25 mmol) and 2-(2-hydroxy-ethyldisulfanyl)-ethanol (78 mg, 0.5 mmol) to give compound **8.4** as a yellow solid (20 mg, 23 and 21% yields for the two steps). ^{1}H NMR ($CDCl_3$): δ 8.34 (s, 1H), 7.38−6.92 (m, 7H), 6.31 (bs, 2H), 4.71 (t, 2H, J = 7.0 Hz), 4.51−4.35 (m, 4H), 3.89 (t, 4H, J = 6.8 Hz), 3.18 (t, 2H, J = 7.0 Hz), 3.06−2.81 (m, 8H). Anal. ($C_{25}H_{32}N_5O_6S_4P$ + 0.5DMSO + 1.5H_2O) C, H, N.

6-Amino-8-(3′,4′-thia-nonacyclic)phosphonofuranyl-9-phenethyl Purine (8.5). This compound was prepared in a similar manner as compound **8.3** from 8-(2-(5-phosphono)furanyl)-N^9-phenethyladenine (500 mg, 1.24 mmol) and 2-(2-hydroxy-ethyldisulfanyl)-ethanol (190 mg, 1.24 mmol) to give compound **8.5** as a yellow solid (70 mg, 17 and 42% yields for the two steps). ^{1}H NMR ($CDCl_3$): δ 8.39 (s, 1H), 7.30−7.05 (m, 7H), 5.73 (bs, 2H), 4.81 (t, 2H, J = 7.0 Hz), 3.16 (t, 4H, J = 6.8 Hz), 3.01−2.61 (m, 8H). Anal. ($C_{21}H_{22}N_5O_4S_2P$ + DMSO) C, H, N.

Acetic Acid 3-{(3-Acetoxy-cyclohexyloxy)-[5-(6-amino-9-phenethyl-9*H*-purin-8-yl)-furan-2-yl]-phosphinoyloxy}-cyclohexyl Ester (8.6). This compound was prepared in a similar manner as compound **8.3** from 8-(2-(5-phosphono)furanyl)-N^9-phenethyladenine (200 mg, 0.5 mmol) and acetic acid 3-hydroxy-cyclohexyl ester (200 mg, 1.25 mmol) to give compound **8.6** as a yellow solid (53 mg, 41 and 43% yields for the two steps). ^{1}H NMR ($CDCl_3$): δ 8.37 (s, 1H), 7.28−7.02 (m, 7H), 6.34 (bs, 2H), 5.16−4.40 (m, 6H), 3.15 (t, 4H, J = 6.8 Hz), 2.01 (s, 6H), 1.99−1.20 (m, 16H). Anal. ($C_{33}H_{40}N_5O_8P$ + 0.75H_2O) C, H, N.

Isobutyric Acid {5-[6-Amino-9-(2,2-dimethyl-propyl)-9*H*-purin-8-yl]-furan-2-yl}-isobutyryloxymethoxy-phosphinoyloxymethyl Ester (9.1). Compound **9.1** was prepared in a similar manner as compound **8.1** from compound **4.11** (1.176 g, 3.35 mmol) to give compound **9.1** as a yellow oil (260 mg, 14%). ^{1}H NMR ($CDCl_3$): δ 8.36 (s, 1H), 7.35−7.17 (m, 2H), 6.03 (bs, 2H), 5.83−5.72 (m, 4H), 4.50 (s, 2H), 2.53 (p, 2H, J = 6.8 Hz), 1.17−1.10 (m, 12H), 0.88 (s, 9H). Anal. ($C_{24}H_{34}N_5O_8P$) C, H, N.

2,2-Dimethyl-propionic Acid {5-[6-Amino-9-(2,2-dimethyl-propyl)-9*H*-purin-8-yl]-furan-2-yl}-(2,2-dimethyl-propionyloxymethoxy)-phosphinoyloxymethyl Ester (9.2). Compound **9.2** was prepared in a similar manner as compound **8.1** from compound **4.11** (867 mg, 2.454 mmol) to give compound **9.2** as a yellow foam (95 mg, 6.5%). ^{1}H NMR ($CDCl_3$): δ 8.35 (s, 1H), 7.34−7.20 (m, 2H), 5.90−5.75 (m, 6H), 4.51 (s, 2H), 1.26−1.16 (m, 18H), 0.86 (s, 9H). Anal. ($C_{26}H_{38}N_5O_8P$ + 0.2EtOAc) C, H. N.

Diethyl 5-Tri-*n*-butylstannyl-thiophen-2-yl-phosphonate (14). A solution of 2-thienyl lithium in THF (1M, 4.5 mL) was added dropwisely to a cooled solution of diethyl chlorophosphate (500 mg, 4.5 mmol) in anhydrous THF (45 mL) at −78 °C under nitrogen. After stirring at −78 °C for 45 min, the reaction solution was warmed to room temperature and quenched with saturated ammonium chloride solution (20 mL). The resulting mixture was extracted with dichloromethane (3 × 30 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to give a yellow oil. The crude material was purified by flash chromatography (SiO_2, 3 cm × 15 cm, 55% EtOAc-hexane) to give diethyl thiophen-2-yl-phosphonate as a yellow oil (0.465 g, 46%).

A solution of diethyl thiophen-2-yl-phosphonate (941.3 mg, 4.27 mmol) in anhydrous THF (20 mL) was cooled to −78 °C under nitrogen and treated with a solution of *n*-butyl lithium in hexane (1.6 M, 3.2 mL). After 1 h, the reaction solution was treated with tributylstannyl chloride (1.39 mL, 5.12 mmol) and stirred at −78 °C for another 2 h. The reaction was quenched with water (75 mL) and extracted with dichloromethane (3 × 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to give a yellow oil. The crude material was purified by flash chromatography (SiO_2, 5 cm × 15 cm, 25% EtOAc-hexane) to give compound **14** as a yellow oil (1.785 g, 82%).

Biology Methods. Purification of FBPase. The pET3a expression vector containing the human liver fructose-1,6-bisphosphatase gene was a kind gift from Dr. Simon J. Pilkis (Stony Brook, NY). The enzyme was expressed in *Escherichia coli* and purified to homogeneity as described previously.[17]

Enzyme Assays. FBPase activity and its inhibition were measured spectrophotometrically in reactions that coupled the production of fructose-6-phosphate to the reduction of $NADP^+$.[32] AMP-activated protein kinase (rat liver, Upstate Biotechnology, Rochester, NY) was assayed using the "SAMS" peptide substrate according to the supplier's instructions. Adenosine kinase (human recombinant) was obtained, purified, and assayed as described.[33] AMP deaminase (porcine heart) was purified and assayed as described.[25] Glycogen phosphorylase (rabbit muscle), phosphofructokinase (rabbit liver), and adenylate kinase (rabbit muscle) were obtained from Sigma Chemical Company (St. Louis, MO) and assayed as described.[34−36]

Gluconeogenesis Inhibition in Rat Hepatocytes. Hepatocytes were prepared from 24 h fasted, male Sprague−Dawley or freely feeding, male ZDF rats (Genetics Models Inc., Indianapolis, IN or Charles River Laborabories, Indianopolis, IN) according to the procedure of Berry and Friend[37] as modified by Groen et al.[38] Hepatocytes (10−60 mg/mL) were preincubated with each compound in suspension culture for 15−30 min prior to a 30−60 min incubation with gluconeogenic substrate (dihydroxyacetone was used as the substrate, which feeds into the GNG pathway at a step just prior to FBPase). Reactions were terminated by centrifugation. Glucose in cell supernatants was assayed by means of a glucose oxidase kit (Sigma Chemical Company, St. Louis, MO).

Determination of Fructose-1,6-bisphosphate Levels. Snap-frozen liver or hepatocyte samples were extracted in 10% perchloric acid, neutralized, and then analyzed for fructose-1,6-bisphosphate levels by means of an enzyme-coupled spectrophotometric assay as previously described.[39] Coupling enzymes for these determinations were obtained from Roche Diagnostics (Indianapolis, IN) and Boehringer Mannheim (Ridgefield, CT).

Glucose Lowering in Sprague−Dawley Rats. Rats were housed under standard vivarium conditions (12 h light/dark cycle) with free access to Purina 5008 (ZDF rats) or standard chow (Sprague−Dawley rats) and water unless otherwise indicated. All studies were conducted in accordance with the Institutional Animal Care and Use Committee guidelines. Prior to each study, rats (∼250 g) were fasted overnight and then administered test compound or vehicle (PEG-400). Blood samples were obtained from the tail vein at baseline and at regular time intervals for up to 3 h thereafter. Blood glucose was determined by means of a HemoCue analyzer (HemoCue Inc., Mission Viejo, CA).

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Supporting Information Available: Elemental analysis data for all final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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