Discovery of Phosphonic Diamide Prodrugs and Their Use for the Oral Delivery of a Series of Fructose 1,6-Bisphosphatase Inhibitors

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Like most phosphonic acids, the recently discovered potent and selective thiazole phosphonic acid inhibitors of fructose 1,6-bisphosphatase (FBPase) exhibited low oral bioavailability (OBAV) and therefore required a prodrug to achieve oral efficacy. Syntheses of known phosphonate prodrugs did not afford the desired OBAV; hence, a new class of prodrugs was sought. Phosphonic diamides derived from amino acid esters were discovered as viable prodrugs, which met our preset goals: excellent aqueous stability over a wide pH range, benign byproducts (amino acids and low molecular weight alcohols), and most importantly good OBAV leading to robust oral glucose lowering effects. These desirable properties of phosphonic diamides represent significant improvements over existing prodrug classes. Optimization of the diamide prodrugs of phosphonic acid **2a** (MB05032) led to the identification of diamide **8** (MB06322), the first reported orally efficacious FBPase inhibitor.

Introduction

We recently reported the discovery of a series of AMP-mimetic phosphonic acids that function as potent inhibitors of fructose 1,6-bisphosphatase (FBPase[a]), a rate controlling enzyme in mammalian glucose biosynthesis.[1–3] In particular, **2a** (Figure 1), a 16 nM inhibitor of human FBPase, displays potent glucose-lowering effects upon intraperitoneal (ip) dosing in animal models of type 2 diabetes mellitus (T2DM).[3] These agents, like other phosphonic acids that are emerging as important chemotypes with application across a range of therapeutic areas,[4] exhibit low oral bioavailability (OBAV) and therefore require a suitable prodrug to achieve sufficient oral efficacy.

To enhance the OBAV of phosphonic acids, we and other investigators have pursued various prodrug strategies,[5,6] including acyloxyalkyl esters,[7] *p*-acetoxybenzyl esters,[8] *p*-methoxybenzyl esters,[9] SATE esters,[10] and amidate aryl esters.[11,12] Recently, cyclic 1-aryl-1,3-propanyl (HepDirect) esters capable of selective activation in the liver, and therefore achieving delivery of phosphonate- and phosphate-containing drugs to the liver, were described.[13] These prodrug classes vary widely with respect to chemical stability, rate of conversion, and byproducts generated following prodrug cleavage. Despite these advances, there remains a large need for new phosphonate prodrug strategies with high chemical stability, efficient oral absorption, and conversion to the active drug in vivo with production of only benign byproducts.

A conceptually attractive starting point for design of a new type of prodrug was the aryl phosphoramidate approach

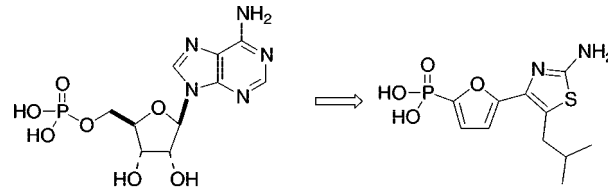

Figure 1. Thiazole phosphonate (**2a**) as an AMP mimic.

developed by McGuigan,[11,12] which has been extensively explored for nucleotides. Despite numerous reports of improved cellular penetration with these prodrugs, there are few examples of in vivo application, particularly where oral bioavailability has been measured. A notable exception is the {9-[(*R*)-2-(phosphonomethoxy)propyl]adenine} (tenofovir) prodrug GS-7340, which was reported to display 17% OBAV in the dog.[14] However, these prodrugs generate an equivalent of phenol, which presents a potential toxicity concern. In addition, they contain an asymmetric center at phosphorus, which complicates the synthesis of stereoisomerically pure prodrug on kilogram scale. We envisioned that a phosphonic diamide derived from two amino acid esters would have the potential to alleviate these two concerns. The resulting phosphonic diamide would be nonstereogenic at phosphorus, and the byproducts of cleavage would be benign: an amino acid and an alcohol.

Analogous phosphoric diamide prodrugs of 3′-azido-3′-deoxythymidine (AZT) monophosphate were prepared by McGuigan, et al. and shown to inhibit HIV-1 in a cellular assay, albeit at least 10-fold more weakly than AZT itself;[15] however, there have been no further reports on the application of this prodrug type to nucleoside monophosphates. As for application to phosphonates, Serafinowska et al. prepared a glycine methyl ester phosphonic diamide of a 9-[2-(phosphonomethoxy)ethyl]-adenine (PMEA) analogue, which failed to produce detectable drug levels in the blood after oral administration to mice;[16] this appears to be the only existing literature report of in vivo evaluation of a diamide prodrug deriving from amino acids. Kern et al. prepared diamides of PMEA and 9-[2-(phospho-

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[a] Abbreviations: FBPase, fructose 1,6-bisphosphatase; OBAV, oral bioavailability; T2DM, type 2 diabetes mellitus; AZT, 3′-azido-3′-deoxythymidine; PMEA, 9-[2-(phosphonomethoxy)ethyl]adenine; DCMC, *N*,*N*-dicyclohexyl-4-morpholine carboxamidine.

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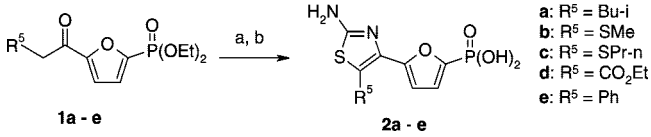
Table 1. OBAV of **2a** and Its Prodrugs (**3**−**23**)

entry	compd	R	OBAV, %
1	**2a**		2
2	**3**		11
3	**4**		13
4	**5**	CH_2CO_2Et	26
5	**6**	CH_2CO_2Bn	17
6	**7**	$CH_2CO_2{}^tBu$	9
7	**8**	(*S*)-CH(Me)CO_2Et	22
8	**9**	(*S*)-CH(Me)CO_2Bn	11
9	**10**	(*S*)-CH(Me)CO_2-neopentyl	18
10	**11**	(*R*,*S*)-CH(Me)CO_2Et	28
11	**12**	(*S*)-CH(Me)$CO_2{}^iPr$	31
12	**13**	(*S*)-CH(Me)$CO_2{}^nPr$	16
13	**14**	(*S*)-CH(Me)$CO_2{}^tBu$	10
14	**15**	(*R*)-CH(Me)CO_2Me	22
15	**16**	(*S*)-CH(CH_2SBn)CO_2Et	5
16	**17**	(*S*)-CH($CH_2{}^iPr$)CO_2Et	3
17	**18**	(*S*)-CH(^iPr)CO_2Et	7
18	**19**	(*S*)-CH(Bn)CO_2Et	3
19	**20**	(*S*)-CH(Et)CO_2Et	8
20	**21**	C$(Me)_2CO_2Et$	47
21	**22**	C$(CH_2CH_2CH_2CH_2)CO_2Et$	27
22	**23**	^iBu	0

Scheme 1[a]



[a] Conditions and reagents: (a) (i) $CuBr_2$, EtOAc, 80 °C; (ii) thiourea, EtOH, 80 °C; (b) TMSBr, CH_2Cl_2.

nomethoxy)ethyl]-N^6-(cyclopropyl)-2-aminoadenine using L-alanine butyl ester, and both derivatives improved cellular antiviral activity over their corresponding parent phosphonic acids. However, characterization was limited to in vitro evaluations.[15,17] These reports of cellular activities of diamide prodrugs provided encouragement that diamide prodrugs can be converted to the active metabolite intracellularly, but at the outset there was no literature evidence of their in vivo utility.

Herein, we report the discovery of phosphonic diamides as prodrugs for the oral delivery of several phosphonate FBPase inhibitors that demonstrated glucose-lowering effects after intravenous administration. This new type of prodrug offers characteristics of high OBAV without issues associated with byproduct toxicity and stereogenicity at phosphorus. Structure−activity relationships generated from these diamides led to the identification of **8** (MB06322, Table 1), the first potent and selective FBPase inhibitor with oral glucose-lowering activity in rodent models of T2DM.

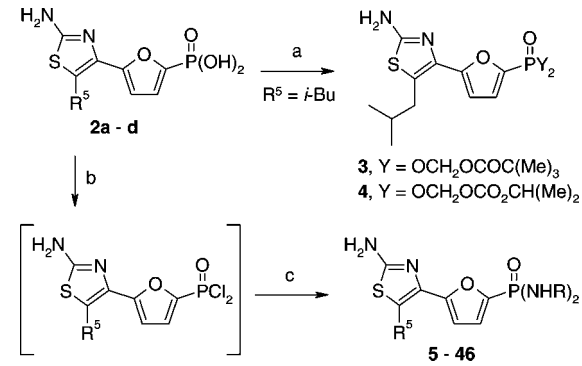
Results and Discussion

Synthesis of Phosphonic Diamides. The thiazole phosphonate FBPase inhibitors **2a** and **2e** were reported previously,[3] while phosphonates **2b**−**d** were prepared analogously as described in Scheme 1.

The bis[(pivaloyloxy)methyl] (bis(POM)) and bis[(isopropyloxycarbonyloxy)methyl] (bis(POC)) ester prodrugs of phosphonic acid **2a** were synthesized using standard alkylation conditions (Scheme 2). Thus, treatment of **2a** with either iodomethyl pivalate or iodomethyl isopropyl carbonate in the presence of *N*,*N*-dicyclohexyl-4-morpholine carboxamidine (DCMC) gave the corresponding bis(POM) (**4**) and bis(POC) (**5**) esters, respectively.

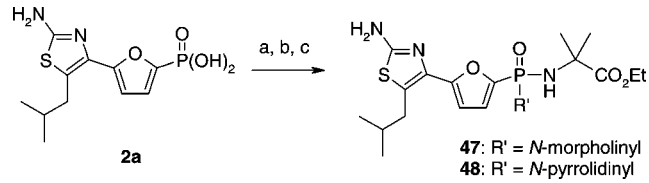
The diamide prodrugs were prepared via the phosphonic dichloride, which was generated by treatment of the phosphonic

Scheme 2[a]



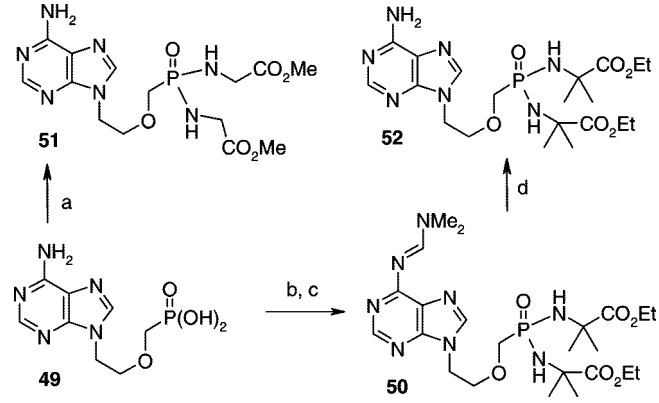
[a] Conditions and reagents: (a) $ICH_2OCOC(Me)_3$ or $ICH_2OCO_2CH(Me)_2$, DCMC, DMF; (b) thionyl chloride, pyridine, $(CH_2Cl)_2$; (c) RNH_2, Hunig's base, CH_2Cl_2.

Scheme 3[a]



[a] Conditions and reagents: (a) thionyl chloride, pyridine, $(CH_2Cl_2)_2$; (b) morpholine or pyrrolidine; (c) 2-Me-Ala-OEt, Hunig's base, CH_2Cl_2.

Scheme 4[a]



[a] Conditions and reagents: (a) PPh_3, (-S-2-pyridyl)$_2$, pyridine, TEA, HCl·Gly-OMe; (b) oxalyl chloride, DMF, $(CH_2Cl)_2$; (c) 2-Me-Ala-OEt, Hunig's base, CH_2Cl_2; (d) AcOH, ^iPrOH.

acid **2a** with thionyl chloride in the presence of a catalytic amount of anhydrous pyridine; the phosphonic dichloride was then reacted with amines in the presence of Hunig's base to give the desired diamides. Diamide prodrugs **47**−**48**, derived from two different amines (mixed diamides), were similarly prepared from the phosphonic dichloride via sequential addition of amines as shown in Scheme 3.

Two diamide prodrugs of PMEA (**51**, **52**) were also prepared for comparison purposes. The synthesis of PMEA diamides proved to be more challenging, presumably because of the poor solubility of PMEA in organic solvents. For diamide **51**, the phosphonic dichloride method described in Scheme 2 for diamides **5**−**46** did not produce any detectable PMEA diamide. Thus, the Mukaiyama coupling method was explored as an alternative, as it has been used for the synthesis of phosphoric acid diesters and phosphoramidates (Scheme 4).[18,19] Treatment of PMEA in pyridine with the Mukaiyama reagent (preformed from triphenylphosphine and 2,2′-dipyridyl disulfide) followed

by glycine ethyl ester gave the desired diamide **51** in 54% yield. In contrast, the coupling of PMEA with 2-methylalanine ethyl ester under the Mukaiyama conditions did not give any desired product, presumably because of steric hindrance. Fortunately, optimization of the initial dichloride coupling method with concomitant protection of the 6-amino group was successful in synthesizing the sterically hindered diamide. Thus, the PMEA dichloride was generated using oxalyl chloride in the presence of 1.2 equiv of DMF, which concurrently protected the 6-amino group as the dimethylformamidine and improved solubility of the resulting PMEA dichloride. Subsequent coupling with 2-methylalanine ethyl ester in the presence of Hunig's base gave compound **50**, which is the amidine-protected form of diamide **52**; removal of the dimethylformamidine group using acetic acid in 2-propanol produced diamide **52** in 16% yield over the three-step sequence.

OBAV of Phosphonic Diamides. To rapidly evaluate the OBAV of a large number of phosphonic diamides, an efficient screening assay was desired. Pharmacokinetic analysis of the thiazole phosphonic acids (**2a**−**e**) revealed that they are primarily cleared via the kidney; therefore, an OBAV screen based on urinary recovery of the parent phosphonic acid following oral administration was established to assess the potential of the corresponding phosphonic diamides as oral prodrugs. Thus, urine was collected for 24 h from rats dosed orally with diamides at 10 mg/kg, and the total amount of parent phosphonic acid excreted was quantified. Comparison to dose-normalized urinary recovery following an intravenous dose of the phosphonic acid allowed estimation of the OBAV for each compound. For comparison, the commonly used bis(POM) and bis(POC) phosphonate prodrugs **3** and **4** were also evaluated; results for these prodrugs of **2a** are summarized in Table 1. The results for a particular prodrug represent a composite of oral absorption and activation (conversion to the active drug) of the prodrug.

As expected, the parent phosphonic acid **2a** displayed very low OBAV (2%), while the bis(POM) and bis(POC) prodrugs **3** and **4** showed only modest improvement (11% and 13% OBAV, respectively) over **2a**. On the other hand, the glycine diamide **5** showed quite acceptable OBAV of 26%. Other diamides derived from the bulkier esters of glycine, such as benzyl (**6**) and *tert*-butyl (**7**), showed lower OBAV. Interestingly, the diamides from L-alanine (**8**) and racemic alanine (**11**) ethyl esters showed comparable OBAV at 22% and 28%, respectively, and that derived from L-alanine isopropyl ester (**12**) also performed well (31%). However, diamides from other L-alanine alkyl esters (**9**, **10**, **13**, **14**) gave slightly lower OBAV (11−18%) compared to the simple ethyl or isopropyl esters. Increasing lipophilicity via substitution of the methyl group of L-alanine (**16**−**20**) gave poor OBAV (2−8%). Conversely, substitution of the α-position of the amino acid, as in 2-methylalanine ethyl ester (**21**) and 1-aminocyclopentanecarboxylate ethyl ester (**22**) led to diamides with very good OBAV (47% and 27%, respectively). To test whether the amino acid ester is required, an analogous derivative was prepared using isobutylamine; this diamide (**23**) did not give any detectable levels of the parent drug.

Encouraged by these results, diamide prodrugs **24**−**36** of a second FBPase inhibitor **2c** were prepared. The OBAV data for these diamides are summarized in Table 2. The α-disubstituted amino acid esters **25** and **28** gave the highest OBAV (31−49%), followed by the smaller alkyl esters of L-alanine (**24**, **34**, and **35**; 17−19%).

Table 2. OBAV of Compound **2c** Diamides (**24**−**36**)

compd	R	OBAV, %
24	(*S*)-CH(Me)CO_2Et	17
25	C(Me)$_2CO_2$Et	31
26	(*S*)-CH(CH_2O^tBu)CO_2Me	9
27	(*S*)-CH(^{i}Pr)CO_2Et	6
28	C($CH_2CH_2CH_2CH_2$)CO_2Et	49
29	(*S*)-CH(Et)CO_2Et	6
30	(*S*)-CH(^{n}Pr)CO_2Et	6
31	(*S*)-CH(cyclohexyl)CO_2Et	1
32	(*S*)-CH(Me)$CO_2{}^t$Bu	4
33	(*S*)-CH(Me)$CO_2{}^n$Bu	2
34	(*S*)-CH(Me)$CO_2{}^i$Pr	17
35	(*S*)-CH(Me)$CO_2{}^n$Pr	19
36	(*S*)-CH(Me)CO_2-cyclopentyl	12

Table 3. OBAV of Diamides (**37**−**46**) of Various FBPase Inhibitors

entry	compd	R^5	R	OBAV, %
1	**37**	SMe	C(Me)$_2CO_2$Et	45
2	**38**	SMe	(*S*)-CH(Me)CO_2Et	23
3	**39**	CO_2Et	(*S*)-CH(Me)CO_2Et	30
4	**40**	CO_2Et	(*S*)-CH(Et)CO_2Et	5
5	**41**	CO_2Et	(*S*)-CH(^{n}Pr)CO_2Et	4
6	**42**	CO_2Et	(*S*)-CH(^{i}Pr)CO_2Et	6
7	**43**	CO_2Et	C$(CH_2)_4CO_2$Et	5
8	**44**	Ph	(*S*)-CH(Me)CO_2Et	31
9	**45**	Ph	(*S*)-CH(Me)$CO_2{}^i$Pr	23
10	**46**	Ph	C(Me)$_2CO_2$Et	30

The scope of this new phosphonate prodrug type was further tested with three other thiazole phosphonate FBPase inhibitors (**2b**, **d**, **e**), which have diverse substituents at the C5-position; OBAV data are shown in Table 3.

As evident from Table 3, phosphonic diamides prepared from other thiazole phosphonate FBPase inhibitors containing varied C5-substituents also showed acceptable OBAV. In all cases, the diamides derived from alanine ethyl ester and 2-methylalanine ethyl ester exhibited the highest OBAV (20−45%), as was observed with diamides of **2a**. As before, the highest OBAV results were obtained with prodrugs derived from 2-methylalanine (**37**, **46**; 30−45%) and smaller alkyl esters of L-alanine (**38**, **39**, **44**, **45**; 23−31%).

Phosphonic diamides derived from simple glycine esters may afford good OBAV (compound **5**), but the corresponding benzyl (**6**) and *tert*-butyl (**7**) esters gave reduced OBAV. This may be due to the slower hydrolysis of the bulkier esters by esterase enzymes. Phosphonic diamides derived from simple alkyl (methyl, ethyl, propyl) esters of alanine tend to give consistently good OBAV (20−30%). It is also noteworthy that the stereochemistry of the amino acids had no effect on OBAV, as diamides derived from L-, D- and D,L-alanine all gave similar OBAV results. This observation is in stark contrast to other phosphoramidate prodrugs of antiviral compounds. McGuigan et al. reported that the phosphoramidate prodrugs of [(1*R*)-4-[2-amino-6-(cyclopropylamino)purin-9-yl]-1-cyclopent-2-enyl]-methanol (abacavir) showed a clear preference for L-amino acids: the abacavir prodrug from L-alanine methyl ester is 50-fold more potent than the corresponding prodrug from D-alanine methyl ester in a cellular anti-HIV assay.[20] Given that the phosphonic diamides and the aryl phosphoramidates are believed to share a similar mechanism of activation (both prodrugs generate a monoamidate intermediate), it is intriguing that the phosphoramidase responsible for the P−N bond cleavage appears to be sensitive to the stereochemical configuration of the amino acid in one case but not the other. Similar to glycine diamides, bulkier esters such as benzyl and isobutyl esters of L-alanine led to diamides with 2-fold lower OBAV compared

Table 4. Physical Property Comparison of PMEA and Compound **2a** Diamides[a]

compd	AA ester[b]	MW	HB-D[c]	HB-A[d]	TPSA[e]	RB[f]	cLogP	OBAV,[g] %
5	Gly	444.44	4	10	183.83	12	2.68	26
51	Gly	415.34	4	13	182.39	13	−1.93	2
21	MeAla	528.60	4	10	183.83	14	5.11	47
52	MeAla	499.50	4	13	182.39	15	0.50	4

[a] ADME Boxes version 3.5 (Pharma Algorithm, Toronto, Canada) was used. [b] AA ester denotes amino acid ester. Gly is glycine ethyl ester. MeAla is 2-methylalanine ethyl ester. [c] Hydrogen bond donors. [d] Hydrogen bond acceptors. [e] Total polar surface area (Å^2). [f] Rotatable bonds. [g] Experimental values based on urinary recovery assay.

with the corresponding ethyl ester. Substitution on the α-methyl group of alanine was also explored but led to a significant decrease in OBAV, which may be attributed to either decreased oral absorption or being poor substrates of the enzyme that is responsible for cleavage of the final P−N bond. The phosphonic diamides derived from 2-methylalanine ethyl ester (**21**, **25**, **37**, **46**) gave consistently higher OBAV (30−50%) than diamides derived from either glycine or alanine ethyl esters.

Upon the observation that many diamides of **2a**−**e** are effective prodrugs for thiazole phosphonate FBPase inhibitors (achieving moderate to good OBAV of 20−40%), it was logical to evaluate the OBAV of PMEA diamides, especially in light of the poor results obtained by Serafinowska et al.[16] Thus, the glycine methyl ester and 2-methylalanine ethyl ester prodrugs **51** and **52** were prepared and tested in the above-described OBAV screen; both showed OBAV of <5%. In an attempt to elucidate the reasons for this observation, physical parameters for two diamide prodrugs of **2a** and PMEA were calculated (Table 4).

Considerable efforts have been directed toward understanding the factors that affect the OBAV of pharmacological agents.[21–24] For example, Lipinski's "rule of five" is derived from the observation that molecular weight, number of hydrogen bond donor and acceptors, and log *P* have shown a correlation with OBAV.[21] Subsequent studies by Veber et al. suggest that total polar surface area and the rigidity of a molecule (measured by number of rotatable bonds) are also important factors that influence OBAV.[25] Compared to the corresponding diamides of **2a**, diamides **51** and **52** appear to have similar molecular properties in most categories except log *P*, wherein PMEA diamides **51** and **52** appear to be considerably more polar than their **2a** counterparts; this may be a major contributor to their greatly reduced OBAV. It is therefore logical to hypothesize that the phosphonic diamide prodrug approach may also be applicable to other phosphonic acids with similar lipophilicity as the thiazole FBPase inhibitors, and for more hydrophilic scaffolds such as PMEA further optimization to increase lipophilicity (e.g., exploring more lipophilic amino acid esters) would achieve acceptable OBAV.

The mechanism of activation for these diamide prodrugs was proposed to involve two sequential enzymatic reactions (Scheme 5).[3] The initial esterase-mediated step is quite rapid, but the hydrolysis of the final P−N bond of an intermediate (**53**) to give the parent phosphonic acid **2a** occurs much more slowly. Consequently, for certain diamide prodrugs the intermediate **53** was formed in significant amount. Nevertheless, it is clear that the in vivo conversion rate is sufficient to produce efficacy in animal models. The phosphoramidase enzyme responsible for this hydrolytic step was identified and will be reported in due course.[26]

Additional supporting evidence for the proposed mechanism of activation is provided by mixed diamides **47** and **48**, in which one amino acid ester is replaced by morpholine and pyrrolidine. Interestingly, these displayed high OBAV of 32% and 37%, respectively. The similar OBAV displayed by diamides **47** and **48** compared to that of diamide **21**, derived from two 2-methylalanine units, indicates that only one amino acid ester is required. Presumably, the one amino acid ester in diamides **47** and **48** is sufficient to trigger the process of activation; the second amine merely functions as a leaving group. In contrast, diamide **23** derived from two isobutylamine units did not produce any detectable parent phosphonic acid **2a**. This result highlights the requirement that at least one amino acid ester be present to initiate the conversion to the parent phosphonic acid.

Scheme 5. Diamide Prodrug Activation Pathway[3]

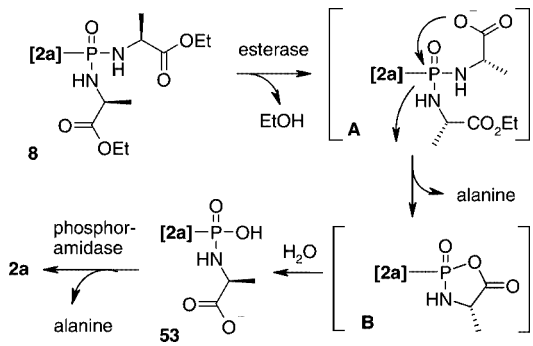


Table 5. Oral Glucose-Lowering Effects in Fasted SD Rats

compd	OBAV, %	glucose-lowering, %	time,[a] h
5	26	51	2
8	22	59	2
11	28	52	2
12	31	63	2
15	22	26	2
21	47	45	2
22	27	20	2
25	31	51	2
37	45	80	2
38	23	38	2
39	30	12	3
45	23	19	5
46	30	25	2

[a] Time of maximum glucose lowering.

Oral Glucose-Lowering Effects. OBAV determination by measuring the urinary recovery of the parent phosphonic acid was a useful tool in rapidly assessing a composite of oral absorption and in vivo prodrug conversion. However, an assay with a pharmacological end point is needed to assess whether drug levels achieved by these diamide prodrugs were sufficient for efficacy. In addition to inhibiting human FBPase, the thiazole phosphonates **2a**−**e** are potent inhibitors of the rat FBPase, and when administered intravenously, compounds **2a**−**e** all lowered plasma glucose levels in 18 h fasted normal Sprague−Dawley (SD) rats (>50% at doses of ≤10 mg/kg).[27] This efficacy assay was selected on the basis of the fact that glycogen storage should be largely depleted following an 18 h fast in a normal rat, and therefore, the majority of endogenous glucose production is the result of gluconeogenesis, providing maximum sensitivity to the effects of a gluconeogenesis inhibitor. Therefore, compounds that passed the urinary OBAV screen (>20%) were evaluated for their ability to reduce plasma glucose levels following oral dosing in SD rats; the results are shown in Table 5. Most of the diamides with >20% OBAV elicited a significant decrease (>40%) in fasting plasma glucose levels in this model at a dose of 30 mg/kg. On the other hand, several diamides (**15**, **22**, **39**,

45, and **46**) displayed weak efficacy (<30%) despite good OBAV (22−30%) demonstrated in the urinary excretion assay.

Finally, to gauge the utility of the diamide prodrugs in delivering phosphonic acids to human patients, the cellular uptake and prodrug activation of diamide **8** were studied in primary human hepatocyte cultures.[26] Diamide **8** was rapidly taken up into human hepatocytes with maximal levels of approximately 7 nmol per million cells after approximately 60 min of incubation time. Following maximal cell penetration, the intracellular diamide **8** concentration decreased with a half-life of approximately 16 min. Intracellular concentrations of the **2a** monophosphoramidate were highest after approximately 20 min of incubation time and then decreased with a slower half-life of approximately 47 min.

Furthermore, to assess the degree of variability in humans, diamide **8** was subjected to metabolism studies using 12 different aliquots of liver S9 fractions from pooled or individual male or female human subjects.[26] Good conversion rates of diamide **8** to **2a** were observed in five individual male and five individual female liver S9 fractions, which compare favorably to the rates obtained in the pooled male and female S9 fractions (15 donors/pool, 0.004 to 0.005 (nmol/min/mg protein). These results suggest that it is likely that low variability can be expected in humans with regard to conversion of diamide prodrugs to the parent phosphonic acids.

Conclusions

In order to achieve oral bioavailability for a series of potent and selective thiazole phosphonic acid inhibitors of FBPase, a new class of prodrugs was sought with the objective of eliminating issues associated with known classes related to lack of stability, byproduct toxicity, and stereogenicity at phosphorus. These goals were achieved with phosphonic diamide prodrugs derived from amino acid esters, which exhibit excellent aqueous stability over a wide pH range (t_{90} > 7 days at pH 3.0−7.4),[1] generate only the byproducts of an amino acid and a low molecular weight alcohol, and most importantly are able to deliver these phosphonate FBPase inhibitors orally to produce robust glucose-lowering effects. These desirable properties of phosphonic diamides represent significant improvements over existing phosphonate prodrug classes. Optimization of the diamide prodrugs of **2a** led to the identification of diamide **8**, the first reported orally efficacious FBPase inhibitor.

Experimental Section

General Methods. Compounds **1a**, **1e**, **2a**, **2e**, **3**, **5**, **8**, **21**, and **53** were prepared according to reported procedures.[3] 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. Microanalyses were performed by Robertson Microlit Laboratories, Inc., Madison, NJ. Low resolution mass spectra were obtained from Mass Consortium Corp., San Diego, CA.

[5-(2-Methylsulfanylacetyl)furan-2-yl]phosphonic Acid Diethyl Ester (1b). A solution of 2-bromoacetyl-5-diethylphosphonofuran[3] (3.54 g, 10.9 mmol) in anhydrous ethanol (20 mL) was treated with sodium thiomethoxide (764 mg, 10.9 mmol) at room temperature under nitrogen. After 18 h, the reaction mixture was concentrated under reduced pressure and the residue was diluted with saturated ammonium chloride (10 mL) and water (50 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were dried ($MgSO_4$) and filtered, and the filtrate was concentrated under reduced pressure to give a yellow oil. The crude oil was purified by flash chromatography (SiO_2, 4 cm × 10 cm, 10% EtOAc−hexane) to give compound **1b** as a clear oil (2.30 g, 72%). ^{1}H NMR ($CDCl_3$): δ 7.26−7.17 (m, 2H), 4.30−4.10 (m, 4H), 3.62 (s, 2H), 2.14 (s, 3H), 1.35 (t, 6H, J = 7.2 Hz).

[5-(2-Propylsulfanylacetyl)furan-2-yl]phosphonic Acid Diethyl Ester (1c). The compound was prepared in a similar manner as compound **1b**. Dark-red oil. ^{1}H NMR ($CDCl_3$): δ 7.19 (m, 2H), 4.22 (m, 4H), 3.52 (s, 2H), 2.87 (t, 2H, J = 6.6 Hz), 1.61 (m, 2H), 1.39 (m, 6H), 0.97 (t, 3H, J = 6.6 Hz).

3-[5-(Diethoxyphosphoryl)furan-2-yl]-3-oxopropionic Acid Ethyl Ester (1d). A solution of (5-acetylfuran-2-yl)phosphonic acid diethyl ester[3] (3.0 g, 12 mmol) in anhydrous THF (5 mL) was added to a suspension of diethyl carbonate (7.5 g, 63 mmol) and sodium hydride (1.0 g, 42 mmol) in anhydrous THF (10 mL) under nitrogen. The resulting reaction mixture was then heated to reflux for 1.5 h. The cooled reaction mixture was neutralized with acetic acid (2.6 mL) and evaporated to dryness. The residue was diluted with water (20 mL) and extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine (50 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a brown oil. The crude oil was purified by flash chromatography (SiO_2, 2 cm × 15 cm, 60% EtOAc−hexane) to give compound **1d** as a yellow oil (0.5 g, 13%). ^{1}H NMR ($CDCl_3$): δ 7.26 (m, 1H), 7.22 (m, 1H), 4.23 (m, 6H), 3.91 (s, 2H), 1.33 (m, 9H).

2-Amino-5-methylsulfanyl-4-[2-(5-diethylphosphono)furanyl]thiazole (2b). A solution of [5-(2-methylsulfanylacetyl)furan-2-yl]phosphonic acid diethyl ester (**1b**, 542 mg, 1.86 mmol) in anhydrous carbon tetrachloride (10 mL) was treated with *N*-bromosuccinimide (396 mg, 2.23 mmol) and stirred at room temperature under nitrogen. After 1.5 h, thiourea (283 mg, 3.72 mmol) and anhydrous ethanol (5 mL) were added to the reaction solution, and the resulting reaction mixture was heated to reflux for 2 h. The cooled reaction solution was evaporated to dryness, and the resulting yellow solid was suspended in saturated sodium bicarbonate (10 mL) and water (50 mL). The suspension was sonicated for 20 min, and the resulting yellow solid was collected via filtration (washed with water, 3 × 10 mL). The yellow solid was then purified by flash chromatography (SiO_2, 3 cm × 10 cm, 50% EtOAc−hexane) to give [5-(2-amino-5-methylsulfanylthiazol-4-yl)furan-2-yl]phosphonic acid diethyl ester as a yellow solid (434 mg, 67%). ^{1}H NMR ($CDCl_3$): δ 7.75 (bs, 2H), 7.24 (m, 1H), 7.10 (m, 1H), 4.15 (m, 4H), 2.35 (s, 3H), 1.35 (m, 6H).

A solution of the above [5-(2-amino-5-methylsulfanylthiazol-4-yl)furan-2-yl]phosphonic acid diethyl ester (165 mg, 0.474 mmol) in anhydrous dichloromethane (5 mL) was treated with TMSBr (835 mg, 4.74 mmol) at room temperature for 16 h. The reaction mixture was evaporated to dryness, and the residue was stirred with water (1 mL) at room temperature for 1 h. The resulting solid was collected via filtration (washed with water, 3 × 5 mL; acetone, 1 × 5 mL) and dried under vacuum to give **2b** as a yellow powder solid (110 mg, 79%), mp 220−224 °C. ^{1}H NMR (D_2O): δ 6.86 (m, 1H), 6.52 (m, 1H), 2.21 (s, 3H). Anal. ($C_8H_9N_2O_4PS_2 \cdot 0.08H_2O$) C, H, N, H_2O.

2-Amino-4-[2-(5-phosphono)furanyl]-5-propylsulfanylthiazole (2c). This compound was prepared in a similar manner as compound **2b**. Tan powder solid, mp 184−187 °C. ^{1}H NMR (DMSO-d_6): δ 7.42 (bs, 2H), 6.99 (m, 1H), 6.92 (m, 1H), 2.70 (t, 2H, J = 7.0 Hz), 1.52 (m, 2H), 0.89 (t, 3H, J = 7.2 Hz). Anal. ($C_{10}H_{13}N_2O_4PS_2 \cdot 0.1H_2O$) C, H, N, H_2O.

2-Amino-4-[2-(5-phosphono)furanyl]-5-ethoxycarbonylthiazole (2d). A solution of 3-[5-(diethoxyphosphoryl)furan-2-yl]-3-oxopropionic acid ethyl ester (**1d**, 630 mg, 1.98 mmol) in anhydrous ethanol (5 mL) was treated with copper(II) bromide (884 mg, 3.96 mmol) and stirred at room temperature under nitrogen. After 18 h, the reaction mixture was filtered through a Celite pad (washed with EtOAc, 3 × 5 mL), and the filtrate was evaporated to dryness. The crude material was purified by flash chromatography (SiO_2, 2 cm

× 15 cm, 50% EtOAc−hexane) to give 2-bromo-3-[5-(diethoxyphosphoryl)furan-2-yl]-3-oxopropionic acid ethyl ester as a clear oil (470 mg).

The above bromoketone (470 mg, 1.18 mmol) in anhydrous ethanol (6 mL) was treated with thiourea (180 mg, 2.37 mmol), and the resulting reaction mixture was heated to reflux for 2 h. The cooled reaction solution was evaporated to dryness, and the residue was treated with saturated sodium bicarbonate (10 mL) and water (20 mL) and extracted with EtOAc (3 × 30 mL). The combined extracts were dried ($MgSO_4$) and filtered, and the filtrate was concentrated under reduced pressure to give a yellow solid. The crude material was purified by flash chromatography (SiO_2, 2 cm × 15 cm, 50%, 65%, 80% EtOAc−hexane, gradient elution) to give 2-amino-4-[5-(diethoxyphosphoryl)furan-2-yl]thiazole-5-carboxylic acid ethyl ester as a white solid (175 mg, 40%).

A solution of the above 2-amino-4-[5-(diethoxyphosphoryl)furan-2-yl]thiazole-5-carboxylic acid ethyl ester (75 mg, 0.20 mmol) in anhydrous dichloromethane (5 mL) was treated with TMSBr (302 mg, 2.00 mmol) at room temperature for 16 h. The reaction mixture was evaporated to dryness, and the residue was stirred with water (1 mL) at room temperature for 1 h. The resulting solid was collected via filtration (washed with water, 3 × 5 mL; acetone, 1 × 5 mL) and dried under vacuum to give **2d** as a white solid (55 mg, 86%), mp 248−250 °C. ^{1}H NMR (DMSO-d_6): δ 7.99 (bs, 2H), 7.51 (m, 1H), 6.98 (m, 1H), 4.15 (q, 2H, J = 7.8 Hz), 1.21 (t, 3H, J = 7.8 Hz). Anal. ($C_{10}H_{11}N_2O_6PS \cdot 0.1HBr$) C, H, N.

2-Amino-4-[2-(5-bis(isopropyloxycarbonyloxymethylphosphono)furanyl]-5-isobutylthiazole (4). A suspension of **2a** (1.0 g, 3.31 mmol) in anhydrous DMF (20 mL) was treated with *N*,*N*-dicyclohexyl-4-morpholine carboxamidine (2.9 g, 9.88 mmol) followed by iodomethyl isopropyl carbonate (1.78 mL, 7.29 mmol), and the resulting mixture was stirred at room temperature under nitrogen for 24 h. The reaction solution was evaporated to dryness and the residue was purified by flash chromatography (SiO_2, 2 cm × 15 cm, 33% EtOAc−hexane) to give compound **4** as a yellow solid (140 mg, 8%), mp 109−111 °C. ^{1}H NMR ($CDCl_3$): δ 7.27 (m, 1H), 6.62 (m, 1H), 5.76 (d, 4H, J = 12.8 Hz), 5.06 (bs, 2H), 4.89 (p, 2H, J = 6.3 Hz), 2.82 (d, 2H, J = 7.0 Hz), 1.88 (p, 1H, J = 7.0 Hz), 1.28 (d, 6H, J = 6.2 Hz), 1.27 (d, 6H, J = 6.2 Hz), 0.98 (d, 6H, J = 7.0 Hz). Anal. ($C_{21}H_{31}N_2O_{10}PS$) C, H, N.

2-Amino-4-{2-[5-(*N*,*N*′-bis((*S*)-1-ethoxycarbonyl)ethyl)phosphonamido]furanyl}-5-methylsulfanylthiazole (38). A suspension of **2b** (500 mg, 1.71 mmol) in anhydrous 1,2-dichloromethane (10 mL) was treated with a solution of pyridine in dichloromethane (0.5 M, 0.34 mL) followed by thionyl chloride (0.62 mL, 8.55 mmol), and the resulting mixture was heated to reflux under nitrogen for 1 h. The cooled reaction mixture was evaporated to give a dark-orange semisolid, which was dissolved in anhydrous dichloroethane (9 mL) and cooled to 0 °C under nitrogen and treated with a solution of L-alanine ethyl ester in anhydrous dichloromethane (1 M, 10.26 mL). The resulting reaction solution was stirred at room temperature for 18 h, diluted with dichloromethane (50 mL), and treated with pH 7 phosphate buffer (50 mL). 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, 3 cm × 10 cm, 80%, 100% EtOAc−hexane and 5% ethanol in EtOAc, gradient elution) to give compound **38** as a beige powder solid (306.5 mg, 37%), mp 94−95 °C. ^{1}H NMR ($CDCl_3$): δ 8.12 (bs, 2H), 7.22 (m, 1H), 7.03 (m, 1H), 4.30−3.90 (m, 6H), 3.60−3.38 (m, 2H), 2.32 (s, 3H), 1.41−1.18 (m, 12H). Anal. ($C_{18}H_{27}N_4O_6PS_2$) C, H, N.

Compounds **6**, **7**, **9**−**20**, **22**−**37**, and **39**−**46** were prepared in a similar manner as compound **38**.

2-Amino-4-{2-[5-(*N*,*N*′-bis(benzyloxycarbonylmethyl)phosphonodiamido]furanyl}-5-isobutylthiazole (6). Foam. ^{1}H NMR ($CDCl_3$): δ 7.32 (s, 10H), 7.09 (m, 1H), 6.44 (m, 1H), 5.13 (s, 4H), 4.00−3.60 (m, 6H), 2.74 (d, 2H, J = 6.8 Hz), 1.81 (p, 1H, J = 6.8 Hz), 0.94 (d, 6H, J = 6.8 Hz). Anal. ($C_{29}H_{33}N_4O_6PS$) C, H, N.

2-Amino-4-{2-[5-(*N*,*N*′-bis((*tert*-butoxycarbonyl)methyl)phosphonamido]furanyl}-5-isobutylthiazole (7). Foam. ^{1}H NMR ($CDCl_3$): δ 7.14 (m, 1H), 6.52 (m, 1H), 6.17 (bs, 2H), 3.82−3.40 (m, 6H), 2.75 (d, 2H, J = 6.6 Hz), 1.82 (p, 1H, J = 6.6 Hz), 1.43 (s, 18H), 0.97 (d, 6H, J = 6.6 Hz). Anal. ($C_{23}H_{37}N_4O_6PS \cdot 0.15CH_2Cl_2$) C, H, N.

2-Amino-4-[2-(5-*N*,*N*′-bis((*S*)-1-(1-benzyloxycarbonyl)ethyl)phosphonoamido)furanyl]-5-isobutylthiazole (9). Foam. ^{1}H NMR ($CDCl_3$): δ 7.32−7.26 (m, 10H), 7.07 (m, 1H), 6.58 (m, 1H), 5.14−5.06 (m, 4H), 4.21−3.98 (m, 2H), 3.60−3.40 (m, 2H), 2.74 (d, 2H, J = 7.0 Hz), 1.83 (p, 1H, J = 7.0 Hz), 1.35 (m, 6H), 0.95 (d, 6H, J = 7.0 Hz). Anal. ($C_{31}H_{37}N_4O_6PS$) C, H, N.

2-Amino-4-[2-{5-(*N*,*N*′-bis((*S*)-1-(1-neopentoxycarbonyl)ethyl)-phosphonamido)}furanyl]-5-isobutylthiazole (10). Foam. ^{1}H NMR (DMSO-d_6): δ 6.90 (m, 3H), 6.52 (m, 1H), 5.00 (m, 2H), 3.98−3.60 (m, 6H), 2.79 (d, 2H, J = 7.0 Hz), 1.79 (p, 1H, J = 7.0 Hz), 1.26 (d, 6H, J = 7.0 Hz), 0.86 (m, 24H). Anal. ($C_{27}H_{45}N_4O_6PS \cdot 0.1H_2O$) C, H, N.

2-Amino-4-{2-[5-(*N*,*N*′-bis((*R*,*S*)-1-(1-ethoxycoarbonyl)ethyl)phosphonamido)]furanyl}-5-isobutylthiazole (11). Mp 143−146 °C. ^{1}H NMR ($CDCl_3$): δ 6.94 (m, 1H), 6.92 (bs, 2H), 6.53 (m, 1H), 5.00 (m, 2H), 4.02 (m, 4H), 3.84 (m, 2H), 2.79 (d, 2H, J = 7.0 Hz), 1.78 (p, 1H, J = 7.0 Hz), 1.25 (d, 6H, J = 7.0 Hz), 1.36 (m, 6H), 0.92 (d, 6H, J = 7.0 Hz). Anal. ($C_{21}H_{33}N_4O_6PS$) C, H, N.

2-Amino-4-[2-{5-(*N*,*N*′-bis((*S*)-1-(1-isopropoxycarbonyl)ethyl)-phosphonamido)}furanyl]-5-isobutylthiazole (12). Foam. ^{1}H NMR (DMSO-d_6): δ 6.92 (m, 1H), 6.89 (bs, 2H), 6.54 (m, 1H), 4.81 (m, 4H), 3.78 (m, 2H), 2.78 (d, 2H, J = 7.0 Hz), 1.79 (p, 1H, J = 7.0 Hz), 1.22 (d, 6H, J = 7.0 Hz), 1.15 (m, 12H), 0.89 (d, 6H, J = 7.0 Hz). Anal. ($C_{23}H_{37}N_4O_6PS$) C, H, N.

2-Amino-4-[2-{5-(*N*,*N*′-bis((*S*)-1-(1-propoxycarbonyl)ethyl)phosphonamido)}furanyl]-5-isobutylthiazole (13). Foam. ^{1}H NMR (DMSO-d_6): δ 6.94 (m, 3H), 6.51 (m, 1H), 4.98 (m, 2H), 3.91 (m, 6H), 2.80 (d, 2H, J = 7.0 Hz), 1.78 (p, 1H, J = 7.0 Hz), 1.51 (m, 4H), 1.24 (d, 6H, J = 7.0 Hz), 0.86 (d, 6H, J = 7.0 Hz), 0.82 (m, 6H). Anal. ($C_{23}H_{37}N_4O_6PS \cdot 0.1CH_2Cl_2$) C, H, N.

2-Amino-4-[2-{5-(*N*,*N*′-bis((*S*)-1-(1-isobutoxycarbonyl)ethyl)phosphonamido)}furanyl]-5-isobutylthiazole (14). Foam. ^{1}H NMR (DMSO-d_6): δ 6.98 (m, 1H), 6.89 (bs, 2H), 6.51 (m, 1H), 5.00 (m, 2H), 3.98−3.68 (m, 6H), 2.79 (d, 2H, J = 7.0 Hz), 1.79 (m, 3H), 1.24 (d, 6H, J = 7.0 Hz), 0.86 (m, 18H). Anal. ($C_{25}H_{41}N_4O_6PS$) C, H, N.

2-Amino-4-{2-[5-(*N*,*N*′-bis((*R*)-1-methoxycarbonyl)ethyl)phosphonamido]furanyl}-5-isobutylthiazole (15). Foam. ^{1}H NMR ($CDCl_3$): δ 7.12 (m, 1H), 6.61 (m, 1H), 5.71 (bs, 2H), 4.08 (m, 2H), 3.72 (s, 3H), 3.65 (s, 3H), 3.60 (m, 2H), 2.79 (d, 2H, J = 6.6 Hz), 1.83 (p, 1H, J = 6.6 Hz), 1.41 (d, 3H, J = 6.6 Hz), 1.38 (d, 3H, J = 6.6 Hz), 0.98 (d, 6H, J = 6.6 Hz). Anal. ($C_{19}H_{29}N_4O_6PS \cdot 0.6CH_2Cl_2$) C, H, N.

2-Amino-4-{2-[5-(*N*,*N*′-bis((*R*)-1-ethoxycarbonyl-2-benzylthio)-ethyl)phosphonamido]furanyl}-5-isobutylthiazole (16). Foam. ^{1}H NMR ($CDCl_3$): δ 7.32−7.23 (m, 10H), 7.10 (m, 1H), 6.52 (m, 1H), 6.31 (bs, 2H), 4.40−3.50 (m, 12H), 2.95−2.60 (m, 6H), 1.90 (m, 1H), 1.29−1.14 (m, 6H), 0.95 (d, 6H, J = 6.6 Hz). Anal. ($C_{35}H_{45}N_4O_6PS_3 \cdot 0.4$ toluene) C, H, N.

2-Amino-4-{2-[5-(*N*,*N*′-bis((*S*)-1-ethoxycarbonyl-3-methyl)butyl)phosphonamido]-furanyl}-5-isobutylthiazole (17). Foam. ^{1}H NMR ($CDCl_3$): δ 7.05 (m, 1H), 6.50 (m, 1H), 6.38 (bs, 2H), 4.22−3.80 (m, 6H), 3.25 (m, 2H), 2.78 (d, 2H, J = 6.8 Hz), 1.90−0.82 (m, 31H). Anal. ($C_{27}H_{45}N_4O_6PS$) C, H, N.

2-Amino-4-{2-[5-(*N*,*N*′-bis((*S*)-1-ethoxycarbonyl-2-methyl)propyl)phosphonamido]furanyl}-5-isobutylthiazole (18). Foam. ^{1}H NMR ($CDCl_3$): δ 7.08 (m, 1H), 6.52 (m, 1H), 6.18 (bs, 2H), 4.22−3.70 (m, 6H), 3.41−3.21 (m, 2H), 2.76 (d, 2H, J = 7.0 Hz), 2.20−1.80 (m, 3H), 1.30−0.80 (m, 24H). Anal. ($C_{25}H_{41}N_4O_6PS$) C, H, N.

2-Amino-4-{2-[5-(*N*,*N*′-bis((*S*)-1-ethoxycarbonyl-2-phenyl)ethyl)-phosphonamido]furanyl}-5-isobutylthiazole (19). Foam. ^{1}H NMR ($CDCl_3$): δ 7.30−7.00 (m, 10H), 6.85 (m, 1H), 6.61 (bs, 2H), 6.42 (m, 1H), 4.30−3.98 (m, 6H), 3.39−2.91 (m, 6H), 2.68 (d, 2H, J =

6.8 Hz), 1.81 (p, 1H, J = 6.8 Hz), 1.25−1.02 (m, 6H), 0.93 (d, 6H, J = 6.8 Hz). Anal. ($C_{33}H_{41}N_4O_6PS \cdot 0.15CH_2Cl_2$) C, H, N.

2-Amino-4-[2-{5-(*N*,*N*′-bis((*S*)-1-(1-ethoxycarbonyl)propyl)-phosphonamido)}furanyl]-5-isobutylthiazole (20). Foam. ^{1}H NMR (DMSO-d_6): δ 6.95 (m, 3H), 6.51 (m, 1H), 4.80 (m, 2H), 4.02 (m, 4H), 3.64 (m, 2H), 2.79 (d, 2H, J = 7.0 Hz), 1.81−1.50 (m, 5H), 1.12 (m, 6H), 0.89 (d, 6H, J = 7.0 Hz), 0.80 (m, 6H). Anal. ($C_{23}H_{37}N_4O_6PS$) C, H, N.

2-Amino-4-[2-{5-(1-ethoxycarbonyl)cyclopentyl)phosphonamido)}furanyl]-5-isobutylthiazole (22). Foam. ^{1}H NMR (DMSO-d_6): δ 6.90 (m, 3H), 6.48 (m, 1H), 4.70 (d, 2H, J = 11.0 Hz), 4.00 (q, 4H, J = 7.0 Hz), 2.82 (d, 2H, J = 7.0 Hz), 2.20−1.40 (m, 17H), 1.12 (t, 6H, J = 7.0 Hz), 0.92 (d, 6H, J = 7.0 Hz). Anal. ($C_{27}H_{41}N_4O_6PS$) C, H, N.

2-Amino-4-{2-[5-(*N*,*N*′-bis(1-isobutyl)phosphonamido)]furanyl}-5-isobutylthiazole (23). Foam. ^{1}H NMR ($CDCl_3$): δ 7.09 (m, 1H), 6.61 (m, 1H), 5.70 (bs, 2H), 2.83−2.53 (m, 6H), 1.87 (p, 1H, J = 6.8 Hz), 1.68 (p, 2H, J = 6.3 Hz), 0.98 (d, 6H, J = 6.8 Hz), 0.90 (d, 12H, J = 6.3 Hz). Anal. ($C_{19}H_{33}N_4O_2PS$) C, H, N.

2-Amino-4-{2-[5-(*N*,*N*′-(1-(*S*)-ethoxycarbonyl)ethyl)phosphonamido]furanyl}-5-propylsulfanylthiazole (24). Foam. ^{1}H NMR ($CDCl_3$): δ 8.20 (bs, 2H), 7.26 (m, 1H), 7.04 (m, 1H), 4.30−3.90 (m, 6H), 3.60−3.30 (m, 2H), 2.68 (t, 2H, J = 7.4 Hz), 1.59 (m, 2H), 1.43−1.16 (m, 12H), 0.94 (t, 3H, J = 7.4 Hz). Anal. ($C_{20}H_{31}N_4O_6PS_2$) C, H, N.

2-Amino-4-{2-[5-(*N*,*N*′-(1-methyl-1-ethoxycarbonyl)ethyl)-phosphonamido]furanyl}-5-propylsulfanylthiazole (25). Mp 110−115 °C. ^{1}H NMR ($CDCl_3$): δ 8.30 (bs, 2H), 7.25 (m, 1H), 7.05 (m, 1H), 4.22 (q, 4H, J = 7.2 Hz), 3.90 (d, 2H, J = 10.8 Hz), 2.69 (t, 2H, J = 7.0 Hz), 1.65 (s, 6H), 1.42 (s, 6H), 1.30 (t, 6H, J = 7.2 Hz), 0.95 (t, 3H, J = 7.0 Hz). Anal. ($C_{22}H_{35}N_4O_6PS_2 \cdot 0.4HCl \cdot 0.5Et_2O$) C, H, N.

2-Amino-4-{2-[5-(*N*,*N*′-bis(*S*)-1-methoxycarbonyl-2-(*tert*-butoxy)-ethyl)phosphonamido]furanyl}-5-isobutylthiazole (26). Foam. ^{1}H NMR (DMSO-d_6): δ 7.37 (bs, 2H), 6.99 (m, 1H), 6.90 (m, 1H), 4.81 (m, 2H), 3.89 (m, 2H), 3.60−3.30 (m, 10H), 2.71 (t, 2H, J = 7.0 Hz), 1.52 (m, 2H), 1.05 (m, 18H), 0.86 (t, 3H, J = 7.0 Hz). Anal. ($C_{26}H_{43}N_4O_8PS_2$) C, H, N.

2-Amino-4-[2-{5-(*N*,*N*′-bis(*S*)-1-ethoxycarbonyl-2-methylpropyl-phosphonamido}furanyl]-5-isobutylthiazole (27). Foam. ^{1}H NMR (DMSO-d_6): δ 7.39 (bs, 2H), 6.95 (m, 2H), 4.71 (m, 2H), 4.03 (m, 4H), 3.53 (m, 2H), 2.71 (t, 2H, J = 7.0 Hz), 1.92 (m, 2H), 1.55 (m, 2H), 1.20−0.76 (m, 21H). Anal. ($C_{24}H_{39}N_4O_6PS_2$) C, H, N.

2-Amino-4-[2-{5-(*N*,*N*′-bis(1-ethoxycarbonyl)cyclopentyl)phosphonamido}furanyl]-5-propylsulfanylthiazole (28). Foam. ^{1}H NMR ($CDCl_3$): δ 8.37 (bs, 2H), 7.28 (m, 1H), 7.05 (m, 1H), 4.18 (q, 4H, J = 7.2 Hz), 3.65 (d, 2H, J = 13.2 Hz), 2.66 (t, 2H, J = 7.4 Hz), 2.40−1.50 (m, 18H), 1.25 (m, 6H), 0.92 (t, 3H, J = 7.4 Hz). Anal. ($C_{26}H_{39}N_4O_6PS_2$) C, H, N.

2-Amino-4-[2-{5-(*N*,*N*′-bis(*S*)-1-ethoxycarbonyl)propylphosphonamido}furanyl]-5-propylsulfanylthiazole (29). Foam. ^{1}H NMR (DMSO-d_6): δ 7.37 (bs, 2H), 6.94 (m, 2H), 4.82 (m, 2H), 4.00 (m, 4H), 3.63 (m, 2H), 2.71 (t, 2H, J = 7.0 Hz), 1.62−0.75 (m, 21H). Anal. ($C_{22}H_{35}N_4O_6PS_2$) C, H, N.

2-Amino-4-[2-{5-(*N*,*N*′-bis((*S*)-1-ethoxycarbonyl)butyl)phosphonamido)}furanyl]-5-propylsulfanylthiazole (30). Foam. ^{1}H NMR (DMSO-d_6): δ 7.36 (bs, 2H), 6.97 (m, 2H), 4.80 (m, 2H), 3.99 (m, 4H), 3.70 (m, 2H), 2.71 (t, 2H, J = 7.0 Hz), 1.52−0.70 (m, 25H). Anal. ($C_{24}H_{39}N_4O_6PS_2$) C, H, N.

2-Amino-4-[2-{5-(*N*,*N*′-bis((*S*)-1-ethoxycarbonyl)cyclohexanylmethyl)phosphonamido)furanyl]-5-propylsulfanylthiazole (31). Foam. ^{1}H NMR (DMSO-d_6): δ 7.35 (bs, 2H), 6.91 (m, 2H), 4.68 (m, 2H), 4.13−3.90 (m, 6H), 3.43 (m, 2H), 2.71 (t, 2H, J = 7.0 Hz), 1.71−0.82 (m, 31H). Anal. ($C_{30}H_{47}N_4O_6PS_2$) C, H, N.

2-Amino-4-[2-{5-(*N*,*N*′-bis((*S*)-1-*tert*-butoxycarbonyl)ethyl)phosphonamido)}furanyl]-5-propylsulfanylthiazole (32). Foam. ^{1}H NMR (DMSO-d_6): δ 7.40 (bs, 2H), 6.98 (m, 2H), 4.79 (m, 2H), 3.70 (m, 2H), 2.71 (t, 2H, J = 7.0 Hz), 1.60−1.30 (m, 20H), 1.20 (d, 6H, J = 7.4 Hz), 0.89 (t, 3H, J = 7.0 Hz). Anal. ($C_{24}H_{39}N_4O_6PS_2$) C, H, N.

2-Amino-4-[2-{5-(*N*,*N*′-bis((*S*)-1-*n*-butoxycarbonyl)ethyl)phosphonamido)}furanyl]-5-propylsulfanylthiazole (33). Foam. ^{1}H NMR (DMSO-d_6): δ 7.38 (bs, 2H), 6.99 (m, 2H), 4.99 (m, 2H), 3.99−3.78 (m, 6H), 2.71 (t, 2H, J = 7.0 Hz), 1.60−0.82 (m, 25H). Anal. ($C_{24}H_{39}N_4O_6PS_2$) C, H, N.

2-Amino-4-[2-{5-(*N*,*N*′-bis((*S*)-1-isopropoxycarbonyl)ethyl)phosphonamido)}furanyl]-5-propylsulfanylthiazole (34). Foam. ^{1}H NMR (DMSO-d_6): δ 7.37 (bs, 2H), 6.97 (m, 2H), 5.00−4.79 (m, 4H), 3.79 (m, 2H), 2.71 (t, 2H, J = 7.0 Hz), 1.51 (m, 2H), 1.23−1.09 (m, 18H), 0.89 (t, 3H, J = 7.0 Hz). Anal. ($C_{22}H_{35}N_4O_6PS_2$) C, H, N.

2-Amino-4-[2-{5-(*N*,*N*′-bis((*S*)-1-*n*-propoxycarbonyl)ethyl)phosphonamido)}furanyl]-5-propylsulfanylthiazole (35). Foam. ^{1}H NMR (DMSO-d_6): δ 7.39 (bs, 2H), 6.96 (m, 2H), 5.00 (m, 2H), 3.99−3.78 (m, 6H), 2.70 (t, 2H, J = 7.0 Hz), 1.60−1.42 (m, 6H), 1.25 (m, 6H), 0.97−0.78 (m, 9H). Anal. ($C_{22}H_{35}N_4O_6PS_2$) C, H, N.

2-Amino-4-[2-{5-(*N*,*N*′-bis((*S*)-1-cycloheptoxycarbonyl)ethyl)phosphonamido)}furanyl]-5-propylsulfanylthiazole (36). Foam. ^{1}H NMR (DMSO-d_6): δ 7.38 (bs, 2H), 6.94 (m, 2H), 5.02−4.81 (m, 4H), 3.79 (m, 2H), 2.71 (t, 2H, J = 7.0 Hz), 1.88−1.40 (m, 18H), 1.22 (m, 6H), 0.90 (t, 3H, J = 7.0 Hz). Anal. ($C_{26}H_{39}N_4O_6PS_2$) C, H, N.

2-Amino-4-{2-[5-(*N*,*N*′-bis(1-methyl-1-ethoxycarbonyl)ethyl)phosphonamido]furanyl}-5-methylsulfanylthiazole (37). Foam. ^{1}H NMR ($CDCl_3$): δ 7.25 (m, 3H), 7.08 (m, 1H), 4.21 (q, 4H, J = 7.2 Hz), 3.99 (d, 2H, J = 12.2 Hz), 2.36 (s, 3H), 1.65 (s, 6H), 1.45 (s, 6H), 1.24 (t, 6H, J = 7.2 Hz). Anal. ($C_{20}H_{31}N_4O_6PS_2 \cdot 0.5H_2O$) C, H, N.

2-Amino-4-[2-(5-({*N*,*N*′-(1-(*S*)-ethoxycarbonyl)ethyl}phosphono)furanyl]-5-ethoxycarbonylthiazole (39). Foam. ^{1}H NMR (DMSO-d_6): δ 7.97 (bs, 2H), 7.47 (m, 1H), 6.99 (m, 1H), 5.20−4.97 (m, 2H), 4.27−3.77 (m, 8H), 1.30−1.12 (m, 15H). Anal. ($C_{20}H_{29}N_4O_8PS$) C, H, N.

2-Amino-4-[2-(5-({*N*,*N*′-(1-(*S*)-ethoxycarbonyl)propyl}phosphono)furanyl]-5-ethoxycarbonylthiazole (40). Foam. ^{1}H NMR (DMSO-d_6): δ 7.96 (bs, 2H), 7.48 (m, 1H), 6.98 (m, 1H), 5.02−4.82 (m, 2H), 4.17 (q, 2H, J = 7.0 Hz), 4.15−3.95 (m, 4H), 3.62 (m, 2H), 1.59 (m, 4H), 1.26−1.04 (m, 9H), 0.84−0.75 (m, 6H). Anal. ($C_{22}H_{33}N_4O_8PS$) C, H, N.

2-Amino-4-[2-(5-({*N*,*N*′-(1-(*S*)-ethoxycarbonyl)butyl}phosphono)furanyl]-5-ethoxycarbonylthiazole (41). Foam. ^{1}H NMR (DMSO-d_6): δ 7.95 (bs, 2H), 7.47 (m, 1H), 6.97 (m, 1H), 5.02−4.79 (m, 2H), 4.17 (q, 2H, J = 7.0 Hz), 4.15−3.92 (m, 4H), 3.66 (m, 2H), 1.61−0.73 (m, 23H). Anal. ($C_{24}H_{37}N_4O_8PS$) C, H, N.

2-Amino-4-[2-{5-(*N*,*N*′-bis(*S*)-1-ethoxycarbonyl-2-methylpropyl)-phosphonamido}furanyl]-5-ethoxycarbonylthiazole (42). Foam. ^{1}H NMR (DMSO-d_6): δ 7.95 (bs, 2H), 7.46 (m, 1H), 6.96 (m, 1H), 4.90−4.62 (m, 2H), 4.22−3.84 (m, 6H), 3.52 (m, 2H), 1.91 (m, 2H), 1.29−0.65 (m, 21H). Anal. ($C_{24}H_{37}N_4O_8PS$) C, H, N.

2-Amino-4-[2-(5-({*N*,*N*′-(1-(*S*)-ethoxycarbonyl)cyclopentyl}phosphono)furanyl]-5-ethoxycarbonylthiazole (43). Foam. ^{1}H NMR (DMSO-d_6): δ 7.97 (bs, 2H), 7.50 (m, 1H), 6.96 (m, 1H), 4.75 (d, 2H, J = 10.6 Hz), 4.18 (q, 2H, J = 7.0 Hz), 3.99 (q, 4H, J = 7.0 Hz), 2.20−1.44 (m, 16H), 1.22 (t, 3H, J = 7.0 Hz), 1.12 (t, 6H, J = 7.0 Hz). Anal. ($C_{26}H_{37}N_4O_8PS$) C, H, N.

2-Amino-4-{2-[5-(*N*,*N*′-(1-(*S*)-ethoxycarbonyl)ethyl)phosphonamido]furanyl}-5-phenylthiazole (44). Foam. ^{1}H NMR ($CDCl_3$): δ 7.41 (m, 7H), 6.98 (m, 1H), 6.31 (m, 1H), 4.21−3.80 (m, 6H), 3.60−3.39 (m, 2H), 1.40−1.18 (m, 12H). Anal. ($C_{23}H_{29}N_4O_6PS$) C, H, N.

2-Amino-4-{2-[5-(*N*,*N*′-(1-(*S*)-isopropoxycarbonyl)ethyl)phosphonamido]furanyl}-5-phenylthiazole (45). Foam. ^{1}H NMR ($CDCl_3$): δ 7.39 (m, 7H), 6.99 (m, 1H), 6.34 (m, 1H), 5.05−4.92 (m, 2H), 4.01−3.79 (m, 2H), 3.60−3.33 (m, 2H), 1.38−1.15 (m, 18H). Anal. ($C_{25}H_{33}N_4O_6PS \cdot 0.2$ hexane) C, H, N.

2-Amino-4-{2-[5-(*N*,*N*′-(1-methyl-1-ethoxycarbonyl)ethyl)phosphonamido]-furanyl}-5-phenylthiazole (46). Foam. ^{1}H NMR ($CDCl_3$): δ 7.42 (m, 7H), 6.99 (m, 1H), 6.33 (m, 1H), 4.22−4.02 (m, 6H), 1.58 (s, 6H), 1.41 (s, 6H), 1.24 (t, 6H, J = 7.0 Hz). Anal. ($C_{25}H_{33}N_4O_6PS$) C, H, N.

2-Amino-4-{2-[5-(*N*-morpholino-*N*′-(1-methyl-1-ethoxycarbonyl)ethyl)phosphonamido]furanyl}-5-isobutylthiazole (47). A suspension of **2a** (500 mg, 1.65 mmol) in anhydrous 1,2-dichloromethane (10 mL) was treated with anhydrous pyridine (0.013 mL, 0.165 mmol) followed by thionyl chloride (0.62 mL, 8.55 mmol), and the resulting mixture was heated to reflux under nitrogen for 2 h. The cooled reaction mixture was evaporated to give a dark-orange semisolid, which was dissolved in anhydrous dichloroethane (10 mL) and cooled to 0 °C under nitrogen and treated with a solution of morpholine (144 mg, 1.65 mmol) and *N*,*N*-diisopropylethylamine (1.14 mL, 3.30 mmol) in anhydrous dichloromethane (5 mL). The resulting reaction solution was stirred at room temperature for 1 h and then cooled back to 0 °C and treated with a solution of 2-methylalanine ethyl ester (434 mg, 3.30 mmol) and DMAP (606 mg, 4.95 mmol) in anhydrous dichloromethane (5 mL). The resulting reaction mixture was stirred at room temperature for 18 h and diluted with dichloromethane (30 mL) and pH 7 phosphate buffer (50 mL). 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, 3 cm × 10 cm, 80%, 100% EtOAc−hexane and 5% ethanol in EtOAc, gradient elution) to give compound **47** as an off-white solid (160 mg, 20%), mp 182−183 °C. ^{1}H NMR ($CDCl_3$): δ 7.03 (m, 1H), 6.58 (m, 1H), 4.79 (bs, 2H), 4.18 (q, 2H, J = 7.0 Hz), 3.80 (d, 1H, J = 11.0 Hz), 3.60 (m, 4H), 3.20 (m, 4H), 2.78 (d, 2H, J = 6.8 Hz), 1.85 (m, 1H), 1.61 (s, 3H), 1.58 (s, 3H), 1.25 (t, 3H, J = 7.0 Hz), 0.98 (d, 6H, J = 7.0 Hz). Anal. ($C_{21}H_{33}N_4O_5PS$) C, H, N.

2-Amino-4-{2-[5-(*N*-pyrrolidino-*N*′-(1-methyl-1-ethoxycarbonyl)ethyl)phosphonamido]furanyl}-5-isobutylthiazole (48). 48 was prepared in a similar manner as compound **47**, mp 189−190 °C. ^{1}H NMR ($CDCl_3$): δ 6.99 (m, 1H), 6.69 (bs, 2H), 6.48 (m, 1H), 4.18 (q, 2H, J = 7.0 Hz), 3.79 (d, 1H, J = 11.0 Hz), 3.19 (m, 4H), 2.75 (d, 2H, J = 6.8 Hz), 1.80 (m, 5H), 1.59 (s, 3H), 1.48 (s, 3H), 1.21 (t, 3H, J = 7.0 Hz), 0.97 (d, 6H, J = 7.0 Hz). Anal. ($C_{21}H_{33}N_4O_4PS$) C, H, N.

9-(Bis(*N*,*N*′-(methoxycarbonylmethyl)phosphonamido)methoxyethyl)adenine (51). A suspension of PMEA (49, 273 mg, 1 mmol) and glycine methyl ester hydrogenchloride salt in anhydrous pyridine (2 mL) was treated with triethylamine (0.42 mL, 3 mmol) followed by addition of a freshly prepared solution of triphenylphosphine (1.05 g, 4 mmol) and 2,2′-dithiodipyridine (881 mg, 4 mmol) in anhydrous pyridine (2 mL). The resulting reaction solution was heated to 90 °C under nitrogen. After 24 h, the cooled reaction solution was evaporated under reduced pressure and the residue was purified by flash chromatography using an ISCO Combiflash system (40 g silica column, 1−10% MeOH−CH_2Cl_2 gradient elution) to give compound **51** as a yellow solid (210 mg, 51%). ^{1}H NMR ($CDCl_3$): δ 8.38 (s, 1H), 8.28 (s, 1H), 7.63 (bs, 2H), 4.49 (t, 2H, J = 4.8 Hz), 3.99 (t, 2H, J = 4.8 Hz), 3.90−3.67 (m, 14H). $[MH]^+$ calcd for $C_{14}H_{22}N_7O_6P$: 416. Found: 416. Anal. ($C_{14}H_{22}N_7O_6P$) C, H, N.

9-(Bis(*N*,*N*′-((1-methyl-1-ethoxycarbonyl)ethyl)phosphonamido)-methoxyethyl)adenine (52). A suspension of PMEA (49, 600 mg, 2.2 mmol) in anhydrous 1,2-dichloroethane (10 mL) was treated with oxalyl chloride (0.77 mL, 8.8 mmol) followed by anhydrous *N*,*N*-dimethylformamide (0.2 mL, 2.6 mmol). The resulting mixture was heated to reflux under nitrogen, and after 3 h the mixture became a clear orange-brown solution. The cooled reaction solution was evaporated under reduced pressure, and the resulting yellow solid was dissolved in anhydrous dichloromethane (10 mL) under nitrogen, cooled to 0 °C, and treated with *N*,*N*-diisopropylethylamine (1.46 mL, 11 mmol) followed by 2-methylalanine ethyl ester (1.15 g, 8.8 mmol). The resulting reaction solution was stirred at room temperature for 24 h. The reaction solution was diluted with dichloromethane (20 mL) and washed with saturated ammonium chloride (2 × 20 mL), dried ($MgSO_4$), and evaporated. The residue was treated with ethanol−acetic acid (9:1, 10 mL) and heated to reflux. After 24 h, the cooled reaction solution was evaporated and the residue was purified by flash chromatography using an ISCO Combiflash system (40 g silica column, 1−10% MeOH−CH_2Cl_2 gradient elution) to give compound **52** as a yellow solid (180 mg, 16%). ^{1}H NMR ($CDCl_3$): δ 8.30 (s, 1H), 8.06 (s, 1H), 6.30 (bs, 2H), 4.41 (t, 2H, J = 4.8 Hz), 4.15 (m, 4H), 3.88 (t, 2H, J = 4.8 Hz), 3.67−3.63 (m, 4H), 1.55 (s, 6H), 1.46 (s, 6H), 1.24 (t, 6H, J = 7.2 Hz). $[MH]^+$ calcd for $C_{20}H_{34}N_7O_6P$: 500.9. Found: 500.9. Anal. ($C_{20}H_{34}N_7O_6P$) C, H, N.

Activation of Diamide 8 in Human Hepatocytes. Cryopreserved human hepatocytes (male) were purchased from Celsis In Vitro Technologies. The cells were thawed and suspended in Krebs bicarbonate buffer (1.3 million cells/mL) at 37 °C under a 95% oxygen/5% carbon dioxide atmosphere. After a 10-min preincubation, diamide **8** was added to a final concentration of 100 μM. Aliquots (200 μL) were removed at 0, 15, 30, 60, and 90 min and then centrifuged in a microcentrifuge at 14000*g* for 20 s at room temperature. The cell pellet was extracted by sonication with 300 μL methanol at 4 °C. The methanol-extracted cell samples were clarified by centrifugation and the resulting supernatants analyzed by HPLC for **8**, **2a**, and **53** content by comparison to authentic standards. HPLC analysis was accomplished with a chromatographic system (1100 series, Agilent Technologies Inc.) equipped with a C18 column (Ultrasphere ODS, 4 mm × 150 mm, 15 μm, Beckman Inc.) and guard cartridge (Alltech Inc.). The column was equilibrated with buffer A, 20 mM potassium phosphate, pH 6.2. Chromatographic separation was accomplished in buffer A with a linear gradient of 0−80% acetonitrile over 20 min at a flow rate of 1.5 mL/min and at 40 °C. The UV absorbance was monitored at 300 nm.

Estimation of Oral Bioavailability of Diamide Prodrugs in Rats Based on Urinary Excretion of Phosphonic Acid. Prodrugs were dissolved in 10% ethanol/90% polyethlene glycol (MW 400) and administered by oral gavage to 18 h fasted male Sprague−Dawley rats (220−250 g; n = 3−4/group) at doses ranging from 10 to 50 mg/kg. The rats were subsequently placed in metabolic cages, and urine was collected for 24 h. In a separate study, 24 h urinary recovery was determined following intravenous (tail vein) administration of the corresponding phosphonic acid at a dose of 10 mg/kg. Phosphonic acids were dissolved in water, and the solution was adjusted to neutrality with sodium hydroxide. The quantity of phosphonic acid excreted into urine was determined by HPLC as described above. The OBAV percentage of the prodrugs was estimated by comparison of the percent recovery of dose of phosphonic acid in urine 24 h following oral administration of the prodrug to that recovered in urine 24 h after intravenous administration of the corresponding phosphonic acid.

Glucose Lowering in Fasted Rats after Intravenous or Oral Administration of Phosphonic Acids or Prodrugs Thereof. Glucose lowering was assessed in 18 h fasted male Sprague−Dawley rats (250−300 g; n = 3/4/group). Phosphonic acids were formulated and administered intravenously at a dose of 10 mg/kg as described above. Prodrugs were dissolved in 10% ethanol/90% polyethlene glycol and administered at a dose of 30 mg/kg by gavage. Blood samples were taken via a small incision in the tail vein immediately prior to dosing and at 1 h intervals thereafter. Blood glucose was analyzed by means of a HemoCue glucose analyzer (HemoCue Inc., Mission Viejo, CA). The maximum percent glucose lowering achieved relative to control animals dosed with saline via the appropriate route was calculated. The nadir of glucose lowering was typically within 1 h after intravenous administration of phosphonic acid or at 2−3 h after oral administration of prodrug.

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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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