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Phosphatase Inhibitors—III. Benzylaminophosphonic Acids as Potent Inhibitors of Human Prostatic Acid Phosphatase

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Abstract—Further investigation of the structural requirements of a series of benzylphosphonic acid inhibitors of human prostatic acid phosphatase has led to the highly potent series of α-aminobenzylphosphonic acids. The α-benzylaminobenzylphosphonic acid, with an $IC_{50}=4$ nM, exhibited a 3500-fold improvement in potency over the carbon analogue, α-phenylethyl. The enhanced potency may be due to a combination of four favorable interactions including those with the phosphate binding region, the presence the hydrophobic moieties of the benzylamino and phenylphosphonic acid, and a rigid conformer produced by an internal salt bridge between the phosphonate and the α-amino group. Replacement of the phosphonic acid moiety with a phosphinic or carboxylic acid as well as deletion of the benzyl substitution on the α-amino group led to great reductions in potency. Copyright © 1996 Elsevier Science Ltd

Introduction

Protein phosphorylation is known to be important in the regulation of cellular processes through transduction of extracellular signals. The mitogenic action of growth factors such as insulin, epidermal growth factor, and skeletal growth factor involves receptors linked to protein tyrosine phosphorylation and is physiologically antagonized by protein tyrosylphosphatases.[1,2] Specific protein phosphatase inhibitors could be an effective way to prolong or potentiate the action of endogenous growth factors. For example, vanadate, a nonspecific inhibitor of phosphatases, potentiates the action of insulin and bone growth factors.[3,4] Fluoride, a specific inhibitor of bone tyrosyl phosphatase, has been shown to potentiate the action of bone growth factors.[4]

Few specific inhibitors of tyrosyl phosphatases are known, but recently, halomethylphenyl phosphate analogues have been reported as mechanism-based suicide inhibitors acting through a quinone methide intermediate. Depending upon substituents, these inhibitors may exhibit selectivity for prostatic acid phosphatase,[5,6] suggesting that the active sites of both enzymes may share a similar environment. More recently still, Moran et al. utilized combinatorial chemistry to successfully synthesize a library of PTP1B inhibitors.[7]

Prostatic acid phosphatase, a high molecular weight phosphatase, is one of the most widely studied phosphatases since it is used as a marker for prostatic cancer and is synthesized under androgen induction in the prostate. Generally, acid phosphatases are rather nonspecific with regard to synthetic substrates. While not a tyrosine phosphate phosphatase, the prostatic enzyme is capable of utilizing aryl and tyrosylphosphate as substrate and thus has been used as a model to study aryl phosphatases.[8]

We recently reported the inhibitory activity of a series of α-substituted benzylphosphonic acids against human prostatic acid phosphatase.[9] Hydrophobic contributions to inhibitor potency were observed to be optimum for α-(phenylethyl)-benzylphosphonic acid **11**, having an $IC_{50}=14$ μM. Limited studies concerning conformationally more rigid analogues suggested that conformation may be important for optimal interaction near the phosphate binding region of the phosphatase. We report further studies with a series of α-amino-substituted benzylphosphonic acids that are several 1000-fold more potent as inhibitors of human prostatic acid phosphatase.

Chemistry

The reaction of substituted imines with a dialkyl phosphite yields the phosphonodiesters **1**, while reaction with hypophosphorous acid gives the phosphinic acid **3**. Hydrolysis of the phosphono diesters **1** using either bromotrimethylsilane or refluxing in hydrochloric acid gives the phosphonic acids **2** (Scheme 1). A 'one pot' synthesis of phosphonic acids **4** is accomplished when the imine is treated with di(4-methylbenzyl)phosphite.[10,11] The resulting ester readily hydrolyses to the acid by the addition of formic acid and heat (Scheme 2). Ethyl α-bromophenylacetate was reacted with benzylamine under phase transfer catalytic conditions to give the ester **5**. The ester was then hydrolysed in base. Neutralization with aqueous hydrochloric acid to pH 7 yielded

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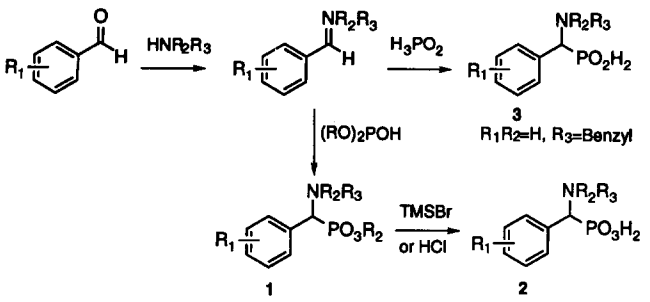


Scheme 1.

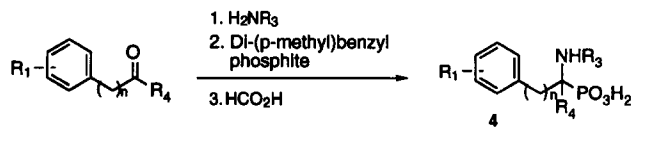
the product, α-benzylaminophenylacetic acid, **6** (Scheme 3).

Diethyl α-aminophenylmethylphosphonate was benzoylated in dimethylformamide with triethylamine smoothly affording the amide ester **8** that was hydrolysed with bromotrimethylsilane to give α-(*N*-benzoyl)aminophenylmethyl phosphonic acid **9**. Acidic hydrolysis of diethyl α-aminophenylmethylphosphonate gave the primary amine **12**.[12,13]

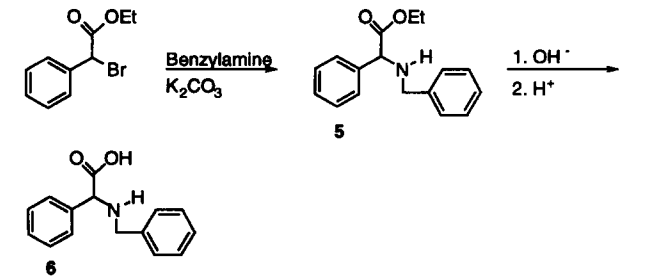
Molecular modeling

Studies were initiated to determine the basis for the 3500-fold enhancement of inhibitory potency of the benzylamine analogue **2A**, over the phenethyl analogue **11**. Since the enzyme assay was run at acidic pH, compound **2A** was built in SYBYL14 as both the neutral and zwitterionic structures and compared with **11**.

All three compounds were minimized using MAXIMIN and searched (CSEARCH) using the energy option to find the low energy conformers that overlapped. (That is, energies were recorded for each conformer generated.) These conformers were optimized using PM3 in MOPAC.[15] A second search



Scheme 2.



Scheme 3.

was performed on the C-phenyl and C-N(C) bonds (highlighted) using both energy and charge. This resulted in 670 conformers for compound **2A**-zwitterion, 577 conformers for **2A**-neutral, and 688 conformers for compound **11**. Single point energies (PM3) were calculated for each conformer from SEARCH. A 3-D plot (X = bond 1, Y = PM3 energy, and Z = bond 2) of the data results in a surface that shows a very deep energy well for the zwitterionic compound, which did not exist for the other compounds. This indicated the formation of an internal salt bridge resulting in a rigid structure with few low energy conformations. Figures 1 and 2 show energy surfaces and the preferred conformer of **2A**.

This strong conformational preference may be responsible for the increased potency of **2A**. To test this hypothesis, methyl-substituted compounds, **A**, **J**, and **N**, were studied as models to determine if they may be predicted to disrupt the proposed binding conformation. The phenyl ring of the benzyl substituent was removed leaving three rotatable bonds. Each compound was subjected to another SEARCH using all three rotatable bonds. PM3 energies for all the conformers from the SEARCHes were calculated. The lowest energy conformers (PM3 energy) are listed in Table 1.

Figure 3 shows the low energy conformer of each compound found by the method described above. Structure **A**, the mono N-methyl analogue of **2A** forms an internal salt bridge that results in the torsion angle between the benzylic carbon, the carbon α to the PO_3, the N and the bondable-H (i.e. hydrogen not involved in the internal H-bond) on the N to be −31.4° (328.6°). Compound **J**, or the *N*,*N*-dimethylamino analogue of **2J**, is also capable of forming an internal salt bridge, but where the N-hydrogen is replaced by a methyl group thus presenting potential steric interactions at the binding site. In structure **N**, the α-methyl, N-methyl analogue of **4N**, the rotatable bond of interest is equal to 161.6°, a difference of 167° from compound **A**. An internal salt bridge is again formed but the bondable-H and the methyl (benzyl) moiety are not superimposable with the corresponding functionality in **2J** or **2A** as shown in Figure 4, which depicts all the conformers within 1 kcal/mol of the lowest found. As can be seen, the shapes of the low energy conformers of **A**, **J**, or **N** are not conserved.

The low energy conformers of the models of compounds **A**, **J**, and **N** were optimized using PM3 in SPARTAN.[16] Figures 5 and 6 show the electron density maps of the model compounds. The surfaces are color coded by electrostatic potential. As can be seen, compounds **J** and **N** do not present the same shape/surface to the receptor as does compound **A**.

Discussion

We have previously described a series of α-substituted benzylphosphonic acids that owed their affinity to the

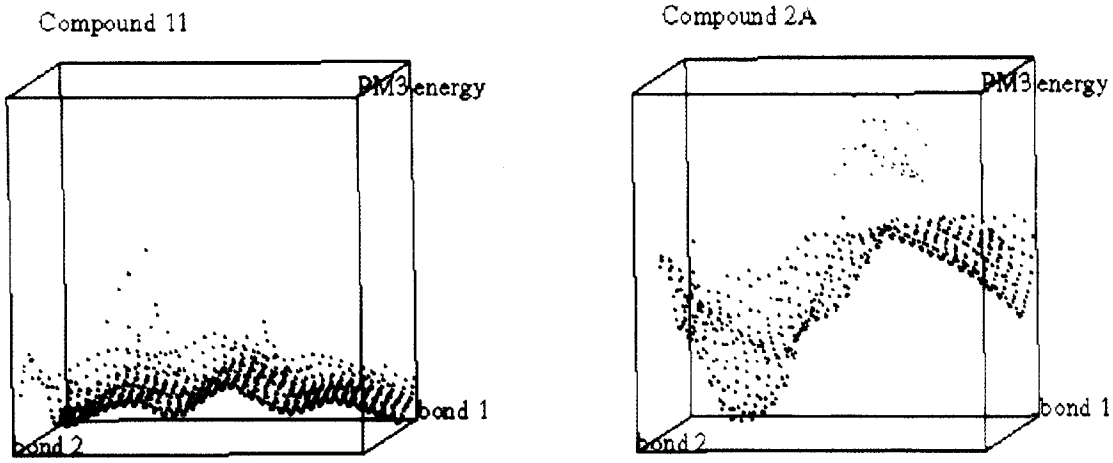


Figure 1.

enzyme active site due to high hydrophobic character.[9] Potency and hydrophobic contribution were maximized at about α-phenylethyl in size and aromatic substitution had no significant effect. These studies suggested that a limited hydrophobic area adjacent to the phosphate binding region existed along with considerable bulk tolerance beyond the hydrophobic area. Further investigation of the structural requirements at the α or benzylic carbon position of the benzylphosphonic acids has led to the highly potent series of α-aminobenzylphosphonic acids (Table 2). The α-benzylamino substituted analogue, **2A**, with an $IC_{50}=4$ nM, exhibited a nearly 3500-fold improvement in potency over the carbon analogue, α-phenylethyl **11**. Replacement of the phosphonic acid moiety with a phosphinic **3**, or carboxylic **6**, deletion of the benzyl substitution on the α-amino **12**, or reduction of the cationic nature of the α-N such as α-benzamido **9** and α-phenyl **2F**, led to great reductions in potency. The optimal distance from the phosphorus atom to the phenyl of the benzylphosphonic acid is one carbon atom ($n=0$) as evidenced by the reduced potency of the phenylethyl compound **4M** (78 nM) vs **2A** (4 nM). Potency was optimized when R_3 was cyclopentyl or benzyl (**2H** or **2A**) suggesting that the presence of these lipophilic ring systems is an important component to affinity. Acyclic groups at R_3 (**2B** and **4I**) resulted in a 10- to 20-fold reduction in potency when compared with **2A**.

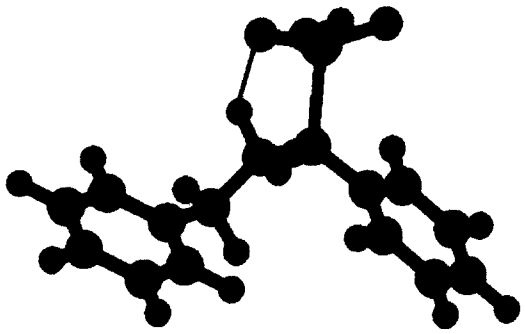
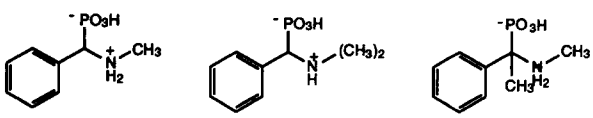


Figure 2.

Based upon the modeling studies, the N-benzyl, N-methyl analogue **2J** and the α-methyl, N-benzyl analogue **4N** were prepared and evaluated as inhibitors. As predicted by the model studies, unfavorable steric interactions induced by zwitterion-induced conformations resulting in a considerable reduction in potency of **2J** and **4N** (4600-fold) when compared with **2A**. This information also suggests that rigid conformers as synthetic targets involving either of these centers are not likely to lead to potent inhibition due to steric interactions.

Recently, X-ray crystallographic information[8,17] concerning both rat prostatic acid phosphatase and a tyrosine phosphate phosphatase together with point

Table 1.



Compound A Compound J Compound N

Compound	No conformers from search	No conformers low energy	RB3	RB4	RB5	PM3 energy
A	854	18	90	120	60	−136.31
J	319	32	90	120	90	−129.89
N	394	40	40	80	60	−131.58

mutation studies[18] identify two key acidic residues that appear to participate in general acid–base catalysis of phosphate ester hydrolysis. In protein tyrosine phosphatases, acidic residues D356 and E290 appear to be in close proximity to the phosphate ether oxygen of the substrate and may be involved as a general acid-base catalysts. Prostatic acid phosphatase contains a D256 that is also in close proximity to the substrate binding site. It is possible that the internal salt bridge ammonium group of the potent inhibitor **2A** may be in an ideal location to strongly contribute to an ionic binding interaction between the inhibitor and the aspartate residue of the prostatic acid phosphatase. In addition, the salt bridge may be involved in directing the hydrophobic N-benzyl group to its binding site by virtue of its rigid conformer structure.

Overall, the enhanced potency of α-benzylaminobenzylphosphonic acid **2A** appears to be due to the combination of a four-point favorable interaction including the phosphate binding region, the presence of two hydrophobic moieties, and a rigid conformer produced by an internal salt bridge which may also participate in an

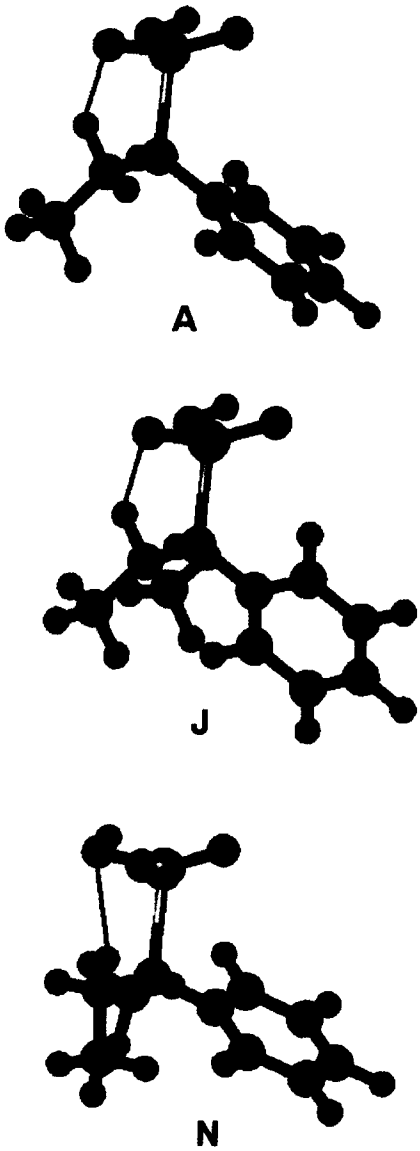


Figure 3.

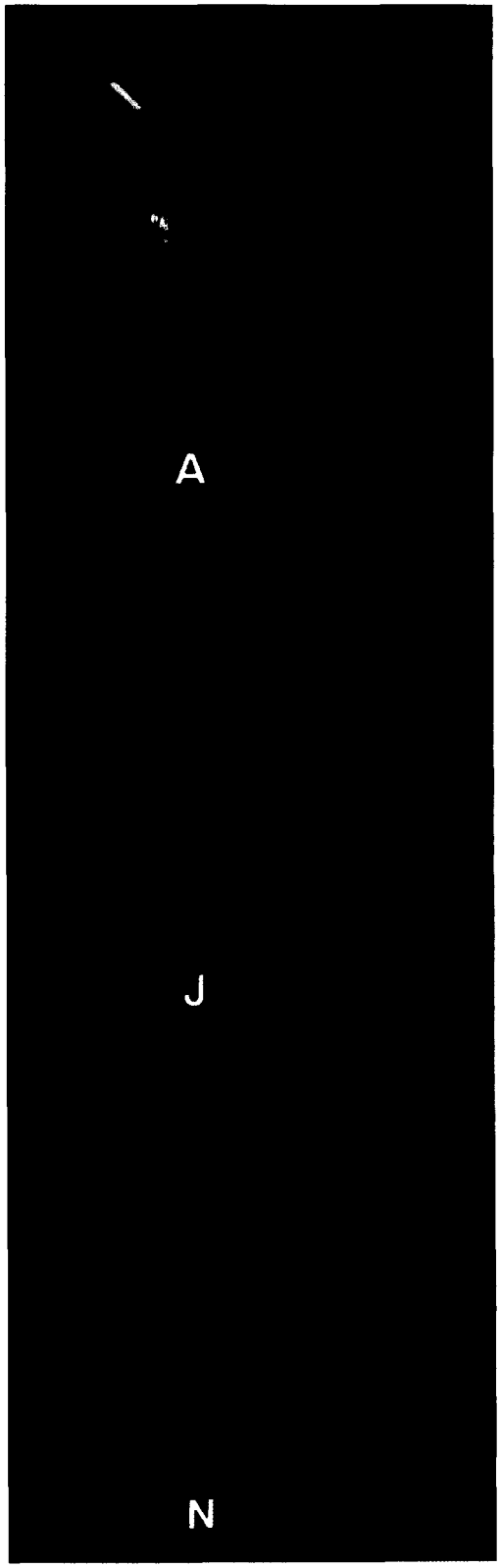


Figure 4.

ionic binding with an aspartate residue present in the active site of the phosphatase.

Experimental

All melting points were taken on a Mel-Temp II open capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were taken on a General Electric QE-300 spectrometer. Signals are reported in parts per million using tetramethylsilane as an internal standard. Mass spectra (DCI) were obtained with a Finnigan MAT Incos 50 single quadrupole mass spectrometer. For column chromatography, silica gel 60 (Merck) was used. Microanalytical results are indicated with atomic symbols and are within $\pm 0.4\%$ of theoretical values.

Tyrosyl phosphatase assay

Inhibition of tyrosyl protein phosphatase was evaluated by incubating an aliquot of purified human prostatic tyrosyl protein acid phosphatase (Sigma Chemical Co.) with radiolabelled phosphotyrosine. The radiolabelled substrate, [^{14}C]phosphotyrosine, is separated from the product, [^{14}C]tyrosine by ion exchange chromatography and the production of radiolabelled tyrosine is quantified. Test compounds were incubated in the presence of tyrosyl acid phosphatase and radiolabelled substrate ([^{14}C]phosphotyrosine, (NEN Dupont Custom Synthesis)) plus cold *O*-phospho-L-tyrosine (10 μM) (Sigma Chemical Company) in a 50 mM sodium acetate buffer (pH 5.5) for 30 min at 37 °C. The reaction was stopped by placing the assay on ice and

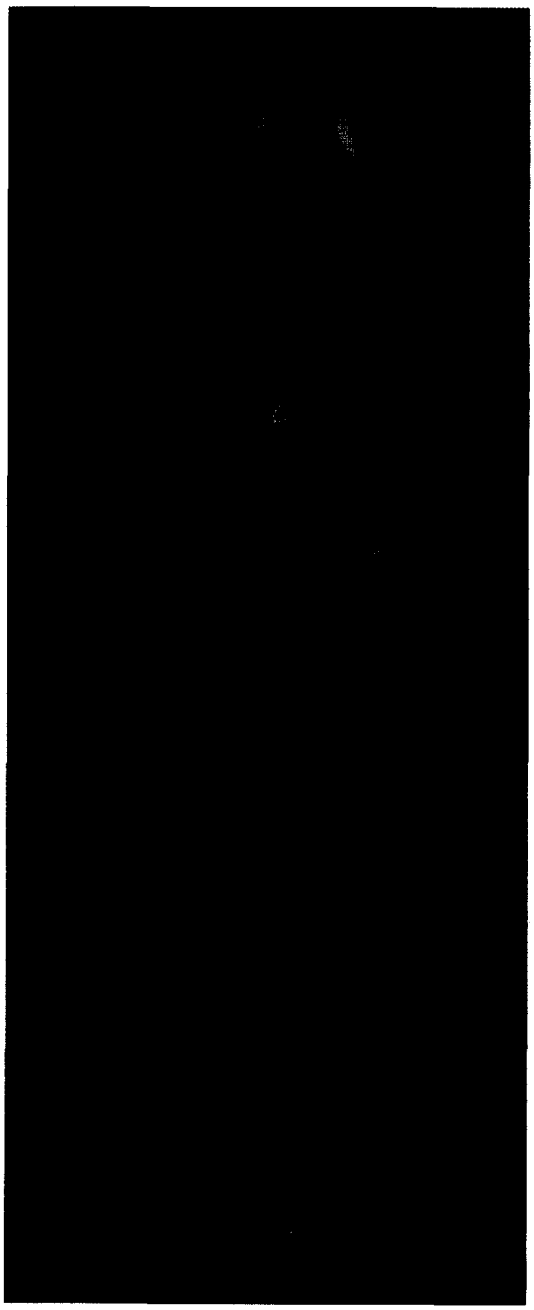


Figure 5.

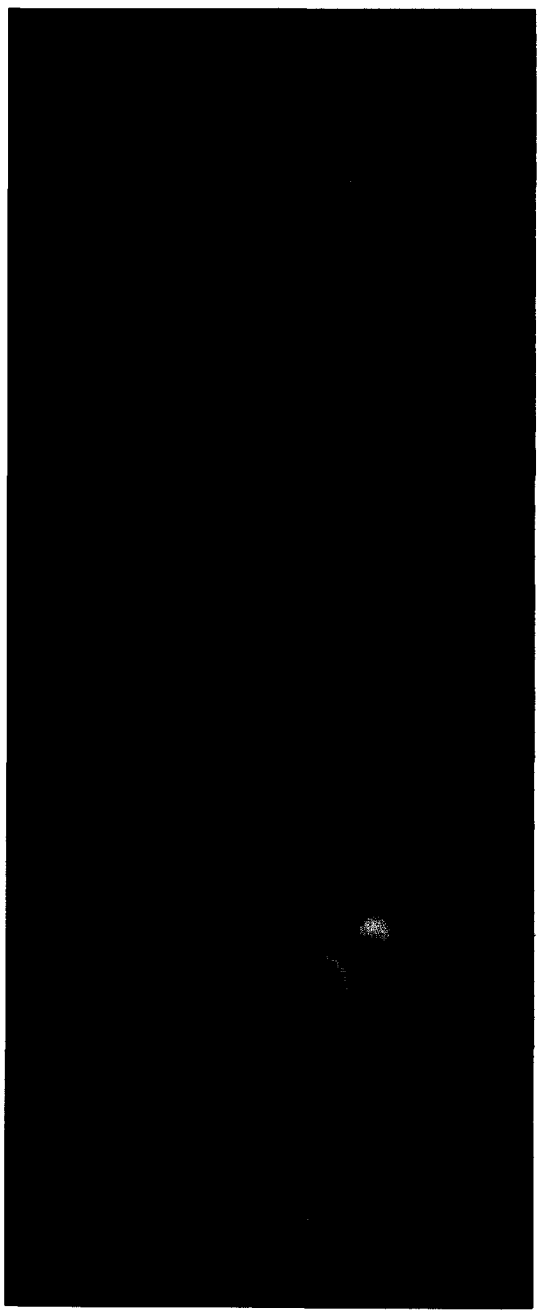


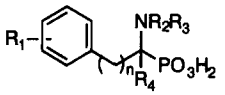
Figure 6.

the addition of a 100 μL aliquot of of an enzyme inhibitor solution (1.1 mM sodium orthovanadate, Sigma Chemical Co.; 0.55 M sodium fluoride, Sigma Chemical Co.). The incubation mixture was passed through an ion exchange column [(Ag 1-x8) (Bio-Rad Laboratories)] and washed with 2.5 mL of distilled deionized water. The total column effluent containing the radiolabelled product [^{14}C]tyrosine) was collected and quantified by liquid scintillation spectroscopy. The test compound IC_{50} or the concentration of test compound necessary to inhibit 50% of the dephosphorylation was calculated using a quantal dose-response calculation and is reported as an average of at least duplicate determinations using several inhibitor concentrations.

General method for the synthesis of diethyl esters 1

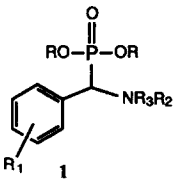
The desired aldehyde was dissolved in anhydrous ether. To this mixture was added molecular sieves and one molar equivalent of the appropriate amine. This mixture was allowed to stand at room temperature for 2–4 h, filtered, and concd in vacuo to generally give the imine intermediate as a clear, colorless oil. The crude imine was heated to 120 °C for 3 h with one molar equivalent of either diethylphosphite or diisopropylphosphite. The phosphonodiester was purified by silica gel column chromatography eluted with ethyl acetate:hexane, 1:1, to give the ester products as oils unless otherwise noted. Yields and molecular ions are listed in Table 3.

Table 2.



Compound	*n*	R_1	R_2	R_3	R_4	Acid	IC_{50} (nM)
2A	0	H	H	benzyl	H	PO_3H_2	4
2B	0	H	H	propargyl	H	PO_3H_2	402
2C	0	H	H	4-Cl-benzyl	H	PO_3H_2	27.7
2D	0	H	H	3-CH_3-benzyl	H	PO_3H_2	3.9
2E	0	H	H	3-CF_3-benzyl	H	PO_3H_2	4.9
2F	0	H	H	3-CF_3-Phenyl	H	PO_3H_2	>1000
2G	0	H	H	cyclohexylmethyl	H	PO_3H_2	>1000
2H	0	H	H	cyclopentyl	H	PO_3H_2	10.7
2I	0	H	H	cycloheptyl	H	PO_3H_2	3100
2J	0	H	CH_3	benzyl	H	PO_3H_2	>1000
2K	0	H	H	phenylethyl	H	PO_3H_2	170
2L	0	2,3-benzo	H	benzyl	H	PO_3H_2	8.6
2M	0	3,4-benzo	H	benzyl	H	PO_3H_2	48
2N	0	2-CF_3	H	benzyl	H	PO_3H_2	270
2O	0	H	H	4-OCH_3-benzyl	H	PO_3H_2	14.7
2P	0	2-OH	H	benzyl	H	PO_3H_2	3.9
2Q	0	2-OCH_3	H	benzyl	H	PO_3H_2	54.9
2R	0	H	H	cyclohexyl	H	PO_3H_2	800
2S	0	3-CF_3	H	benzyl	H	PO_3H_2	27.7
3	0	H	H	benzyl	H	PO_3H_2	534
4A	0	4-propoxy	H	benzyl	H	PO_3H_2	20
4B	0	4-phenoxy	H	(3,4-OCH_2)-benzyl	H	PO_3H_2	50
4C	0	3-phenoxy	H	benzyl	H	PO_3H_2	18.1
4D	0	4-phenoxy	H	benzyl	H	PO_3H_2	18.9
4E	0	H	H	4-CO_2CH_3-benzyl	H	PO_3H_2	329
4F	0	H	H	4-CO_2H-benzyl	H	PO_3H_2	384
4G	0	H	H	(3,4-OCH_2)-benzyl	H	PO_3H_2	24.6
4H	0	2-OH_5-NO_2	H	benzyl	H	PO_3H_2	193
4I	0	H	H	*n*-butyl	H	PO_3H_2	226
4J	0	H	H	cyclopropyl	H	PO_3H_2	1500
4K	0	H	H	cyclobutyl	H	PO_3H_2	>1000
4L	0	H	H	3-OCH_3-benzyl	H	PO_3H_2	5.4
4M	1	H	H	benzyl	H	PO_3H_2	78.1
4N	0	H	H	benzyl	CH_3	PO_3H_2	18,500
6	0	H	H	benzyl	H	CO_2H	>5000
9	0	H	H	benzoyl	H	PO_3H_2	>1000
11	0	H	H	H	PhEt	PO_3H_2	14,000
12	0	H	H	H	H	PO_3H_2	>1000
Na_3VO_4							269

Table 3. Physical properties of phosphonate ester intermediates



1

Compound	R_1	R_2,R_3	R	Yield (%)	MH^+	Mp (°C)
1A	H	H,benzyl	ethyl	80	334	oil
1B	H	H,propargyl	ethyl	27	282	oil
1C	H	H,4-Cl-benzyl	ethyl	98	368	oil
1D	H	H,3-CH_3-benzyl	ethyl	46	348	oil
1E	H	H,3-CF_3-benzyl	ethyl	83	402	oil
1F	H	H,3-CF_3-Phenyl	ethyl	94	388	102–104
1G	H	H,cyclohexylmethyl	ethyl	77	340	oil
1H	H	H,cyclopentyl	ethyl	26	312	40–42
1I	H	H,cycloheptyl	ethyl	47	340	oil
1J	H	CH_3,benzyl	*i*-propyl	24	376	oil
1K	H	H,phenylethyl	ethyl	51	348	38–41
1L	2,3-benzo	H,benzyl	ethyl	57	384	oil
1M	3,4-benzo	H,benzyl	ethyl	52	246[a]	oil
1N	2-CF_3	H,benzyl	ethyl	58	402	oil
1O	H	H,4-OCH_3-benzyl	ethyl	89	364	oil
1P	2-OH	H,benzyl	ethyl	55	350	279
1Q	2-OCH_3	H,benzyl	ethyl	41	362	oil
1R	H	H,cyclohexyl	ethyl	16	326	oil
1S	3-CF_3	H,benzyl	ethyl	72	402	oil

[a]M-PO_3Et_2.

Synthesis of compounds 2B–R

The diethyl ester (**1**) was dissolved in either methylenechloride or acetonitrile and bromotrimethylsilane (5 M equiv) was added. The resultant mixture was allowed to stand at ambient temperature for 3–20 h. The mixture was then evapd in vacuo, redissolved in methanol and propylene oxide was added. After 30 min, the mixture was evapd in vacuo, to give the crude acid as product. The products were then recrystallized from the solvents shown in Table 4.

***N*-Benzylamino-(3-trifluoromethyl)phenylmethylphosphonic acid (2S).** The ester **1S** (2.00 g, 4.98 mmol) was refluxed in concd HCl (25 mL) for 8 h. The mixture was then concd in vacuo. Addition of methanol and propylene oxide yielded a crude solid which was filtered and recrystallized from ethanol:water to give 1.31 g (76%). ^1H NMR (trifluoroacetic acid-*d*): δ 4.99 (d, 1H, PCH), 4.40 (s, 2H, NCH_2).

***N*-Benzylaminophenylmethylphosphinic acid (3).** Benzaldehyde (2.00 mL, 19.7 mmol) was dissolved in ethanol (100 mL). Benzylamine (2.15 mL, 19.7 mmol) and 2.36 g concd hypophosphorous acid (obtained by evapn of the commercially available 50% aq soln at 40–50 °C at 3–5 mm). This was stirred at reflux for 2 h, cooled, and filtered to give 2.55 g (49.6%) of product. An analytical sample was prepared by recrystallization from methanol. ^1H NMR (acetic acid-d_4): δ 7.45 (m, 10H), 4.64 (d, 1H, PCH), 4.23 (s, 2H, NCH_2).

***N*-(4-Carboxybenzyl)aminophenylmethylphosphonic acid (4F).** The ester **4E** (2.00 g, 5.89 mmol) was refluxed in 1 N aq sodium hydroxide for 2 h. The mixture was then cooled in an ice-water bath and acidified to a pH of 5 with 1 N HCl. The resulting solid was filtered and recrystallized from ethanol:water to give 1.10 g (58%) of product. ^1H NMR (trifluoroacetic acid-*d*): δ 4.96 (d, 1H, PCH), 4.55–4.37 (m, 2H, NCH_2).

Ethyl α-(*N*-benzyl)aminophenylacetate (5). Ethyl α-bromophenylacetate (2.00 mL, 11.4 mmol) was dissolved in acetonitrile and benzylamine (1.25 mL, 11.4 mmol), potassium carbonate (4.74 g, 34.3 mmol), and benzyltriethylammonium bromide (380 mg) were added. This mixture was stirred at reflux for 16 h, poured into water and extracted with ether. The organic layer was dried over magnesium sulfate, concd, and purified on a silica gel column by elution with ethyl acetate:hexane (1:1) to give the product (1.55 g, 50%) as an oil. ^1H NMR ($CDCl_3$): δ 7.30 (m, 10H), 4.38 (s, 1H, benzyl methine), 4.15 (m, 2H, OCH_2), 3.73 (s, 2H, NCH_2), 1.18 (t, 3H, CH_3) MS, 270 (MH^+).

***N*-Benzylaminophenylacetic acid (6).** Ethyl α-(*N*-benzyl)aminophenylacetate (1.50 g, 5.57 mmol) was dissolved in methanol (30 mL) and 30 mL of 20% aq potassium hydroxide. This mixture was stirred for 1.5 h at ambient temperature and refluxed for 30 min. After the mixture was concd in vacuo, the pH was adjusted with 1 N HCl to pH 7 yielding 1.12 g (83%) of crude solid product. An analytical sample was prepared by dissolving the solid in 1 N KOH, extracting with ether

Table 4. Analytical and physical properties of phosphonic acids

Compound	Mp (°C)	MH^+	Formula	Anal.	Recrystyn solvent
2B	241–242	226	$C_{10}H_{12}NO_3P$	C,H,N	acetone/H_2O
2C	248–249	230[a]	$C_{14}H_{15}ClNO_3P$	C,H,N	HOAc/H_2O
2D	228–230	292	$C_{15}H_{18}NO_3P$	C,H,N	HOAc/H_2O
2E	233–234	346	$C_{15}H_{15}F_3NO_3P$	C,H,N	HOAc/H_2O
2F	95–96	332	$C_{14}H_{13}F_3NO_3P \cdot 0.25H_2O$, $0.25C_2H_5OH$	C,H,N	EtOH/H_2O
2G	225–227	284	$C_{14}H_{22}NO_3P$	C,H,N	HOAc/EtOH
2H	246–248	174[a]	$C_{12}H_{18}NO_3P \cdot 0.25H_2O$	C,H,N	HOAc/EtOH
2I	223–225	284	$C_{14}H_{22}NO_3P \cdot 0.25\ H_2O$	C,H,N	HOAc/EtOH
2J	209–211	292	$C_{15}H_{18}NO_3P$	C,H,N	EtOH/Et_2O
2K	255–258	292	$C_{15}H_{18}NO_3P$	C,H,N	HOAc/EtOH
2L	245	328	$C_{18}H_{18}NO_3P \cdot 0.5H_2O$	C,H,N	EtOH/H_2O
2M	206–209	246[a]	$C_{18}H_{18}NO_3P \cdot HOAc$	C,H,N	HOAc/H_2O
2N	191–194	346	$C_{15}H_{15}F_3NO_3P \cdot H_2O$	C,H,N	MeOH/H_2O
2O	241–244	226[a]	$C_{15}H_{18}NO_4P$	C,H,N	EtOH/H_2O
2P	198	212[a]	$C_{14}H_{16}NO_3P$	C,H,N	EtOH/H_2O
2Q	175–177	308	$C_{15}H_{18}NO_4P$	C,H,N	i-PrOH/ETOAc
2R	225–227	284	$C_{14}H_{22}NO_3P$	C,H,N	HOAc/EtOH
2S	246–248	346	$C_{15}H_{15}F_3NO_3P$	C,H,N	EtOH/H_2O
3	227–228	196[b]	$C_{14}H_{16}NO_2P$	C,H,N	MeOH
4A	155–159	332	$C_{18}H_{24}NO_4P$	C,H,N	EtOH/H_2O
4B	207–212	332[a]	$C_{19}H_{24}NO_6P$	C,H,N	HOAc/H_2O
4C	212–220	288[a]	$C_{20}H_{20}NO_4P$	C,H,N	HOAc/H_2O
4D	224–227	288[a]	$C_{20}H_{20}NO_4P$	C,H,N	HOAc/H_2O
4E	232–236	254	$C_{16}H_{18}NO_5P \cdot 0.25H_2O$	C,H,N	EtOH/H_2O
4F	254–257	240[a]	$C_{15}H_{16}NO_5P$	C,H,N	EtOH/H_2O
4G	221–229	240[a]	$C_{15}H_{16}NO_5P$	C,H,N	EtOH/H_2O
4H	233–235	257[a]	$C_{14}H_{15}N_2O_6P$	C,H,N	EtOH/H_2O
4I	240–243	244	$C_{11}H_{18}NO_3P$	C,H,N	EtOH/H_2O
4J	245–247	228	$C_{10}H_{14}NO_3P$	C,H,N	EtOH/H_2O
4K	253–255	242	$C_{11}H_{16}NO_3P$	C,H,N	HOAc/EtOH
4L	209–214	308	$C_{15}H_{18}NO_4P \cdot 0.5H_2O$	C,H,N	MeOH/H_2O
4M	225–227	210[a]	$C_{15}H_{18}NO_3P$	C,H,N	HOAc/H_2O
4N	185–187	210[a]	$C_{15}H_{18}NO_3P$	C,H,N	HOAc
6	220–221	242	$C_{15}H_{15}NO_2 \cdot 0.1H_2O$	C,H,N	H_2O
9	226–228	292	$C_{14}H_{14}NO_4P \cdot C_6H_{13}N$	C,H,N	H_2O

[a]M—PO_3H_2.
[b]M—PO_2H_2.

and neutralizing the aqueous phase with 1 N HCl to a pH of 7 and filtering the solid, mp 220–221 °C; ^1H NMR (D_2O/NaOD): δ 7.40 (M, 10H), 4.17 (s, 1H, benzyl methine), 3.70 (q, 2H, NCH_2); MS, 242 (MH^+).

Diethyl (*N*-benzoyl)aminophenylmethyl phosphonate (8). Diethyl aminophenylmethylphosphonate hydrochloride,[12,13] compound **7** (1.73 g, 6.51 mmol) was dissolved in dimethylformamide (50 mL). Triethylamine (1.81 mL, 13.2 mmol) was added and the resultant mixture cooled in an ice-water bath. Benzoyl chloride (0.80 mL, 6.89 mmol) was added dropwise and the bath was removed. After stirring at room temperature for 1 h, the mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with satd aq sodium bicarbonate, water, and dried over magnesium sulfate. Evapn of the solvent, in vacuo, gave 1.55 g (69%) of **8** as a clear colorless oil which was used without further purification. MS, 348 (MH^+).

***N*-Benzoylaminophenylmethylphosphonic acid (9).** The ester **8**, or diethyl *N*-benzoylaminophenylmethylphosphonate (1.40 g, 4.02 mmol) was dissolved in methylenechloride (50 mL). Bromotrimethylsilane (2.65 mL, 20.1 mmol) was added and the mixture allowed to stand at room temperature for 16 h. Methanol (20 mL) was added and the mixture was stirred for an additional hour. The mixture was then evapd in vacuo and the crude oil obtained was dissolved in tetrahydrofuran. Cyclohexylamine (0.46 mL, 4.02 mmol) was added. Addition of ether yielded 800 mg (51%) of the product, **9**, mp 226–228 °C. ^1H NMR (DMSO-d_6): δ 4.95 (dd, 1H, PCH); MS, 292 (MH^+); Anal. ($C_{20}H_{27}N_2O_4P \cdot 1/4H_2O$) C,H,N.

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