A Theoretical Investigation of Phosphonamidates and Sulfonamides as Protease Transition State Isosteres

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The conformations and electrostatic potentials of phosphonamides, phosphonamidates and sulfonamides have been compared to the tetrahedral intermediate for base-catalyzed amide hydrolysis. The wide variation in inhibition by these similar compounds is explained through differences in electrostatic effects.

Introduction

Proteases catalyze the hydrolysis of amide bonds in both peptides and proteins.[1] The active sites of these enzymes are complementary to the transition state for amide hydrolysis; the transition state is bound more tightly than the substrate. Transition state isosteres which are bound more tightly than substrates are potential inhibitors of the enzyme.[2] Many such isosteres have been developed as inhibitors of various proteases,[3] particularly for the HIV-1 protease.[4] We have investigated two isosteres, phosphonamidate and sulfonamide. Two major factors were considered; how faithfully do these groups reproduce the transition state or tetrahedral intermediate geometry? Do they reproduce the transition state electrostatic potential? The first property ensures that the inhibitor will fit into the binding site, while the second ensures that the inhibitor will interact with the enzyme in a fashion similar to the transition state. Both phosphonamidates and sulfonamides possess the required tetrahedral structure of the transition state; however, phosphonamidates work well as inhibitors[5] while sulfonamides rarely perform well.[6,7] For example, the phosphonamidate inhibitor, **1**, of thermolysin has a K_i of 9.1 nM;[5c] the corresponding sulfonamide, **2**, only has a K_i of 419 μM (Figure 1).[7a] In addition, the sulfonamide version, **4**, of the HIV-1 protease inhibitor **3** (IC_{50} = 93 μM)[5j] shows no inhibition at 10 μM (Figure 1).[7a] We have investigated the differences between the inhibition capabilities of sulfonamides and phosphonamidates through ab initio calculations of model systems for the transition state and comparisons to phosphonamidate, sulfonamide, and phosphonamide. Some of these systems have been calculated previously.[8−10] The conformational potential energy surfaces were investigated for *N*-methylmethanesulfonamide[8] and for a similar tetrahedral intermediate.[9] Teraishi et al. performed calculations on *N*-methyl-

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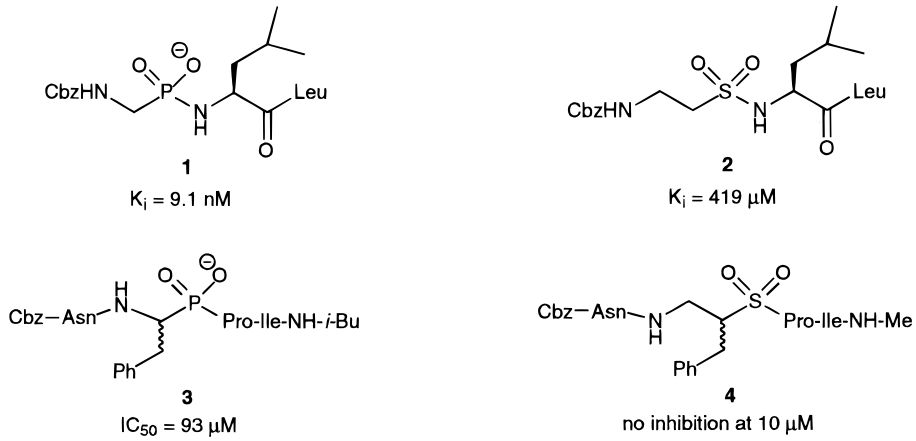

Figure 1. Examples of phosphonamidate and sulfonamide inhibitors.

methanephosphonamidate and the corresponding tetrahedral intermediate and concluded that phosphonamidate is a good analogue of the intermediate.[10] However, these studies only reported one conformer of each structure.

At first glance, it appears that the difference in their performances can be attributed to charge; phosphonamidate and the transition state are negatively charged and sulfonamide is neutral. However, there are many examples of inhibitors which contain a neutral isostere, so charge may not necessarily be the only reason for the differences in inhibition. The hydroxyethylene isoteres of HIV protease inhibitors provide good examples of this charge difference. In this paper, possible explanations for the better performance of phosphonamidates as transition state mimics will be explored. First, phosphonamidates may be more structurally similar to the tetrahedral intermediate. To investigate this possibility, the conformational potential energy surfaces of model systems were investigated. Second, if both phosphonamidate and sulfonamide are structurally different from the tetrahedral intermediate, phosphonamidate may more easily assume a binding conformation than sulfonamide. Calculations were performed on possible binding conformations, and the results were compared to the preferred binding conformation of inhibitors as determined from X-ray crystal structures. Finally, phosphonamidates may have partial atomic charges that more closely resemble those of the transition state. Two compounds with similar charges will form hydrogen bonds of similar strengths. The atomic charges and electrostatic potentials of the model systems were calculated and compared.

Methodology

GAUSSIAN92[11a] and GAUSSIAN94[11b] programs were used to perform calculations at the RHF/6-31+G* level of theory. The geometries of all conformers were fully optimized with conventional Gaussian 92 default convergence criteria. For all nonconstrained structures, frequency calculations were performed in order to determine the nature of stationary points and to obtain zero-point energies (ZPE). ZPE corrections were scaled by 0.9. The CHELPG charges were calculated with the 6-31+G* basis set, and the electrostatic potentials were calculated with the SPARTAN program.[12] The structurally similar tetrahedral intermediate was used as a model of the transition state for attack of hydroxide on an amide. Only conformers with a syn hydroxy group were optimized, since previous calculations on a similar system showed that anti conformers were 4−9 kcal/mol higher in energy.[9] The Protein Data Bank was searched for X-ray crystal structures of proteases containing bound transition state isostere inhibitors. The dihedral angles presented in this paper were obtained from the crystal structures of the inhibitors.

Results and Discussion

Conformational Potential Energy Surface. The stereoisomers and conformers of these systems are defined in Figure 2. Phosphonamidate and sulfonamide each possess a stereogenic center at the nitrogen. Each conformation will exist as two enantiomers; inversion at nitrogen (**A** to **B**, Figure 2) converts one series of diastereomers to the other. The tetrahedral intermediate and the phosphonamide possess two stereogenic centers, at carbon and nitrogen, and at the phosphorus and nitrogen, respectively. Each conformation will have one enantiomer and two diastereomers. One diastereomeric series can be converted to the other by inversion of the nitrogen (**A** to **B**), and the other diastereomer is obtained by proton transfer from one oxygen to the other (**A** to **D**). Nitrogen inversion of the proton-transfer structure (**D** to **C**) gives the enantiomer of the original conformer (**C** and **A**). Thus, for each structure of the tetrahedral intermediate or phosphonamide, there is a corresponding diastereomer. Due to the similarities in energy of the diastereomers, only the set of diastereomers containing the global minimum of the tetrahedral intermediate and the corresponding set of phosphonamide conformers are reported in this paper (**C**, Figure 2).

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(12) SPARTAN version 4.0, 1995, Wavefunction, Inc., 18401 Von Karman Ave., #370, Irvine, CA 92715.

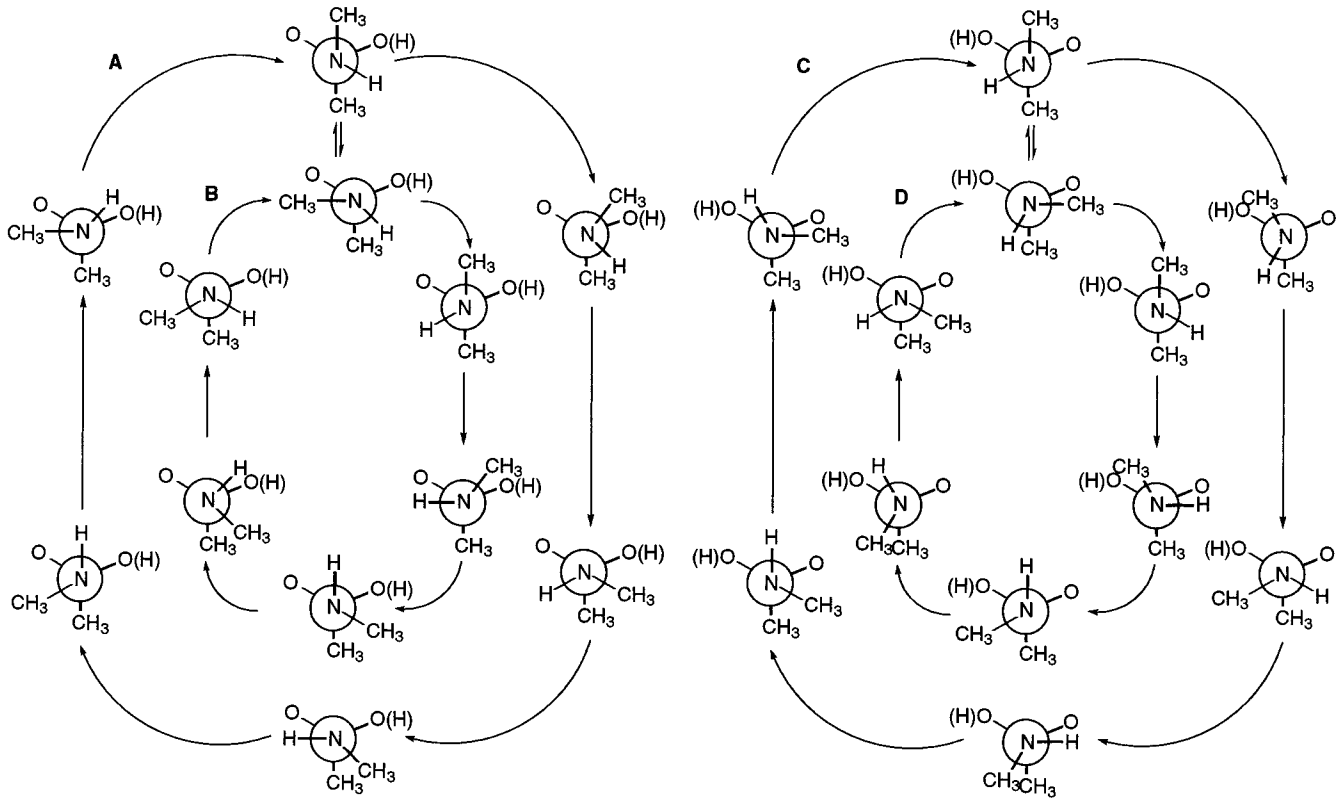
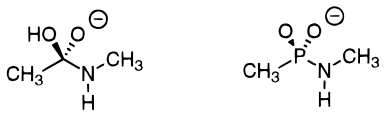
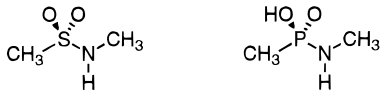


Figure 2. Diagrams depict various possible conformations of the model systems and the rotational transition states between them. The structures in the two diagrams are mirror images of each other which means that the structures in cycles **A** and **C** are enantiomers, and the structures in cycles **B** and **D** are enantiomers. The structures in **A** and **C** are diastereomers of those in **B** and **D**.

All minima and transition states of the conformational potential energy surfaces of the tetrahedral intermediate model **TI** (Figure 3), *N*-methylmethanephosphonamidate **P** (Figure 4), *N*-methylmethanesulfonamide **S** (Figure 5), and *N*-methylmethanephosphonamide **POH** (Figure 6) were located. A phosphonamide is included in these calculations, since it can be formed by a proton transfer between the enzyme and phosphonamidate; in some cases, a phosphonamide may be the actual active form of the inhibitor. As mentioned above, the conformational spaces of *N*-methylmethanesulfonamide[8] and a similar tetrahedral intermediate[9] have been explored previously at the RHF/6-31G* and the RHF/6-31+G* levels of theory, respectively, and one conformer of *N*-methylmethanephosphonamidate and the tetrahedral intermediate were calculated and compared at the MP2/6-31+G* level of theory.[10]



TI **P**



S **POH**

As expected, calculations on the tetrahedral intermediate model system resulted in three ground-state conformers, all of which are staggered. These minima can be interconverted via rotational transition structures, all of which are eclipsed. The global minimum **TI2** has the two methyl groups anti to each other, reducing steric interactions. There are also attractive Me−O^- interactions in these gas-phase calculations. Rotation through **TI3** gives the second minimum **TI4**, which has both the *N*-methyl group and NH gauche to the *C*-methyl group. Transition structure **TI5** leads to the third minimum **TI6** which has the NH anti to a methyl group. Conversion back to **TI2** is achieved via rotation through **TI1**. **T14** has gauche Me−Me repulsion and lacks Me−O^- attraction; it is the highest energy conformer.

The phosphonamidate and sulfonamide conformational potential energy surfaces (PES's) are quite similar to each other but differ significantly from the tetrahedral intermediate PES. Only two minima, which were separated by rotational transition structures, were located for **P** and **S**. The global minima, **P1** and **S1**, have the hydrogen and methyl group of the nitrogen eclipsed with the oxygens and methyl groups approximately at right angles. This structure corresponds to a low-lying transition structure on the **TI** PES. Rotation through **P3** or **S3** gives the other minimum, **P4** or **S4**, which is similar to the **TI4** gauche Me−Me minimum. Passage through the transition state, **P5** or **S5**, gives back the global minimum, **P1** or **S1**. The two rotational transition structures of phosphonamidate and sulfonamide correspond to similar transition structures of the tetrahedral intermediate; however, one of the minima of **P** and **S** corresponds to the third transition structure of **TI**. Only one of the **TI** minima is also a minimum for **P** and **S**. The other two **TI** minima are not even stationary points on the **P** and **S** potential energy surfaces.

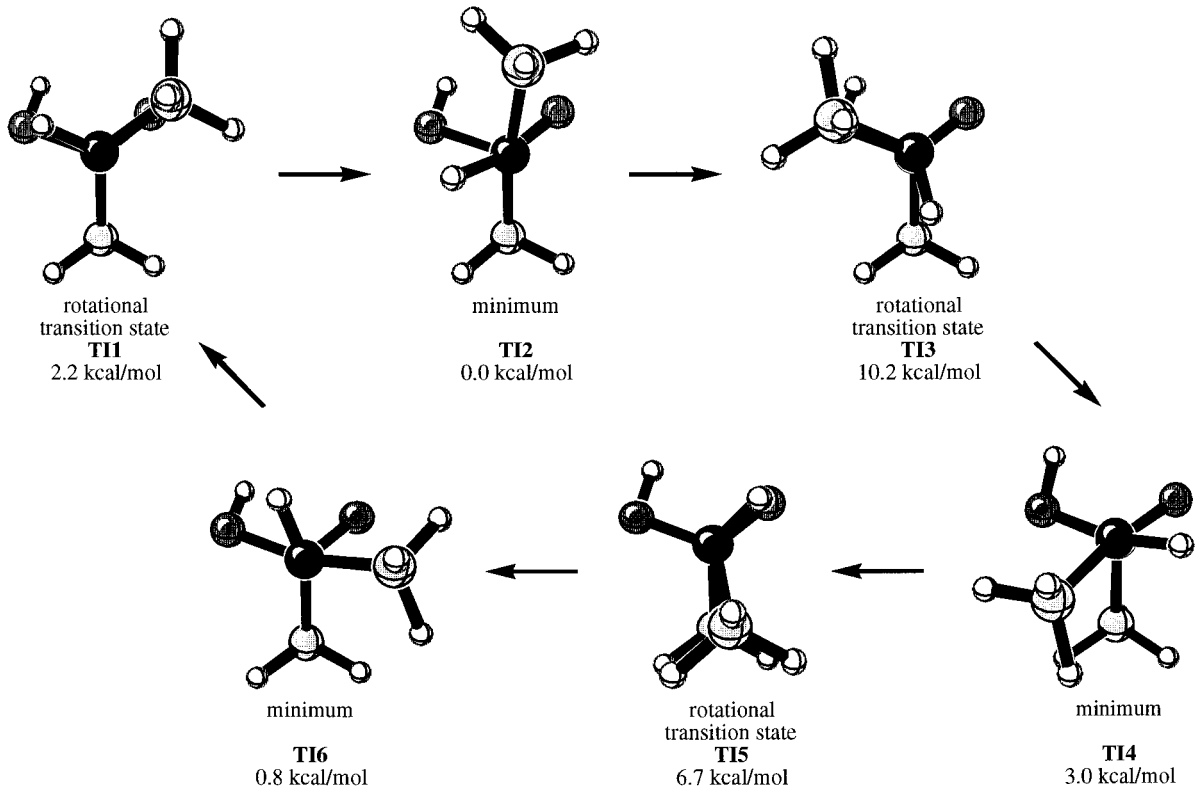


Figure 3. Optimized RHF/6-31+G* geometries of the conformational minima and transition states of the tetrahedral intermediate. The perspective shown here is the view along the N−C bond.

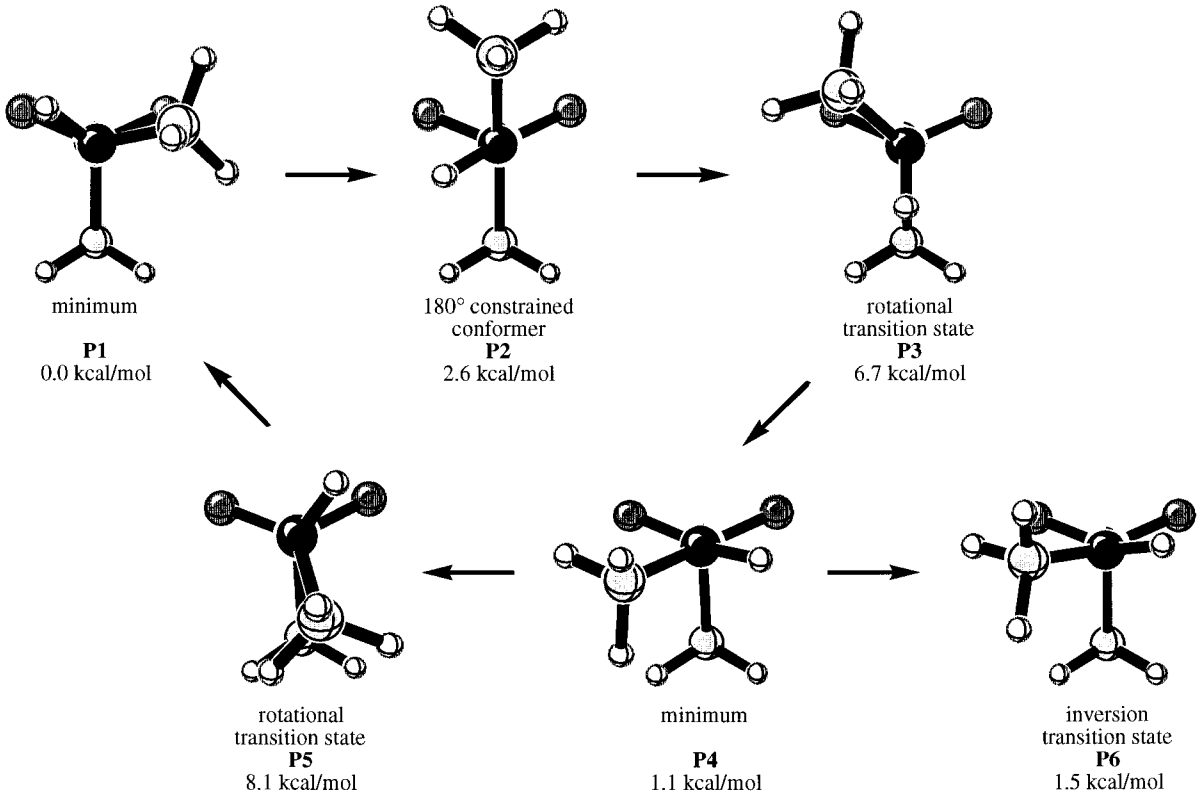


Figure 4. Optimized RHF/6-31+G* geometries of the conformational minima and transition states of *N*-methylmethanephosphonamidate. The perspective shown here is the view along the N−P bond.

The eclipsed minimum, **P1** or **S1**, is favored over the staggered minimum, **P4** or **S4**, despite steric interference. In **P1** and **S1**, the nitrogen lone pair is further away from the oxygen lone pairs and this reduces unfavorable electrostatic interactions present in **P4** and **S4.** Plots of the electrostatic potentials display the expected increased electron density near the oxygens in **P4** and **S4** compared to **P1** and **S1** (Figure 7).

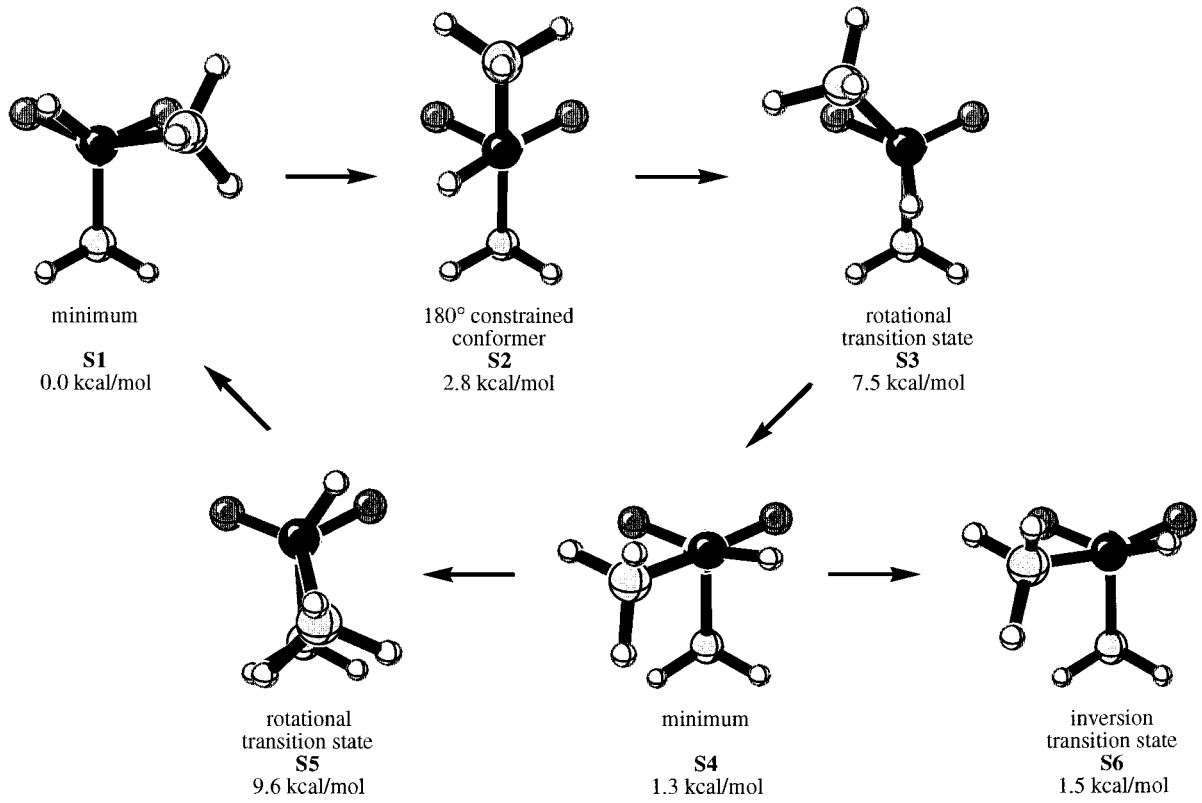


Figure 5. Optimized RHF/6-31+G* geometries of the conformational minima and transition states of *N*-methylmethanesulfonamide. The perspective shown here is the view along the N−S bond.

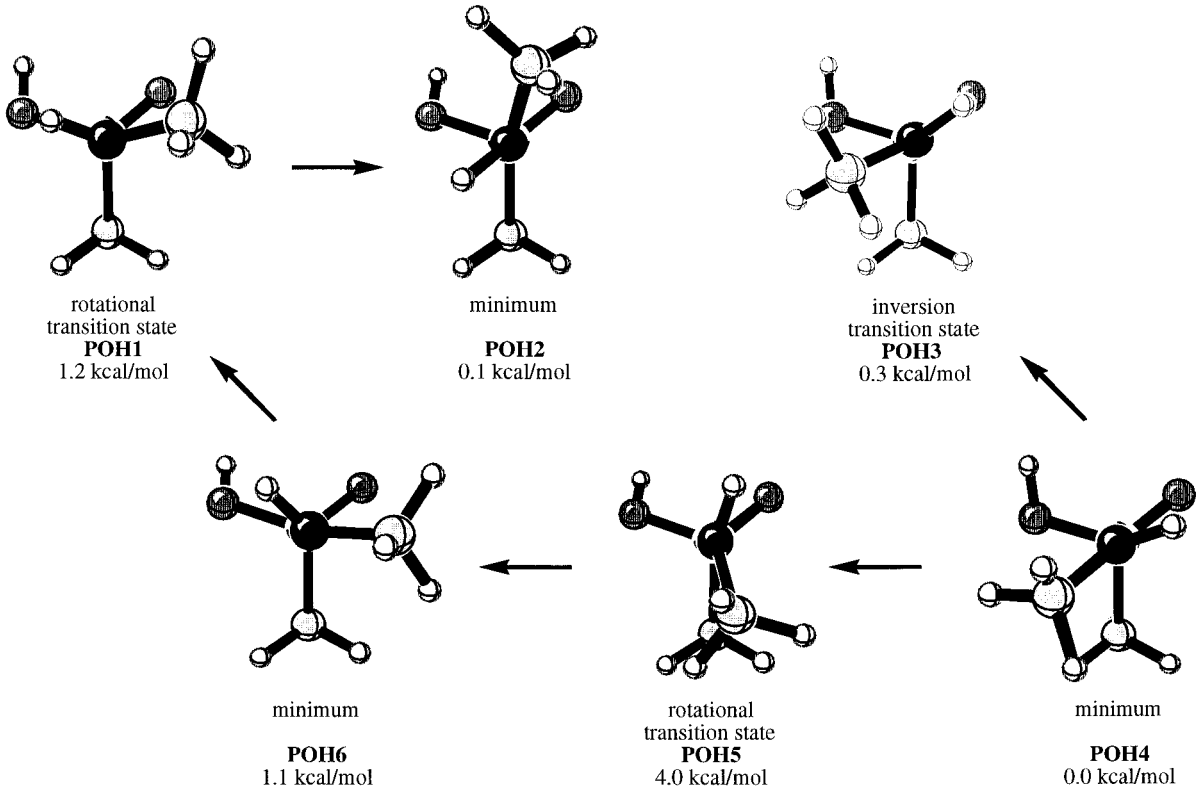


Figure 6. Optimized RHF/6-31+G* geometries of the conformational minima and transition states of *N*-methylmethanephosphonamide. The perspective shown here is the view along the N−P bond.

Very low energy inversion barriers, **P6** and **S6**, were also located for phosphonamidate and sulfonamide. This indicates that the amine undergoes rapid interconversion between the two possible minima (**P1** to **P4**, **S1** to **S4**). In fact, with the inclusion of zero-point energies, the inversion barrier completely disappears for both molecules (Table 1). Previous calculations on amides and sulfonamides have shown that the planarity of the amino

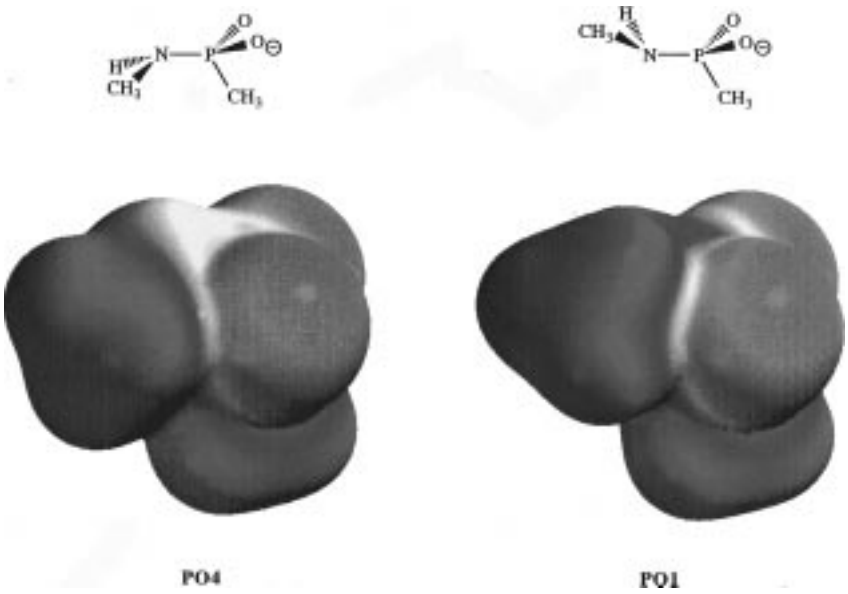


Figure 7. RHF/6-31+G* electrostatic potentials of the two minima of phosphoamidate.

Table 1. Relative Energies, with and without Zero-Point Energies, for the Conformations of the Tetrahedral Intermediate, Phosphonamidate, Sulfonamide, and Phosphonamide (RHF/6-31+G*)

conformation	relative energy (kcal/mol)	relative energy with zpe (kcal/mol)
tetrahedral intermediate		
rot. transition state **TI1**	2.2	2.1
minimum **TI2**	0.0	0.0
rot. transition state **TI3**	10.2	9.9
minimum **TI4**	3.0	3.0
rot. transition state **TI5**	6.7	6.6
minimum **TI6**	0.8	0.9
N-methylmethanephosphonamidate		
minimum **P1**	0.0	0.0
constrained conformer **P2**	2.7	N/A
rot. transition state **P3**	6.7	6.4
minimum **P4**	1.1	1.0
rot. transition state **P5**	8.1	8.1
inv. transition state **P6**	1.5	0.9
N-methylmethanesulfonamide		
minimum **S1**	0.0	0.0
constrained conformer **S2**	2.8	N/A
rot. transition state **S3**	7.5	7.0
minimum **S4**	1.3	1.1
rot. transition state **S5**	9.6	9.6
inv. transition state **S6**	1.5	0.8
N-methylmethanephosphonamide		
rot. transition state **POH1**	1.2	0.9
minimum **POH2**	0.1	0.1
inv. transition state **POH3**	0.3	0.0
minimum **POH4**	0.0	0.0
rot. transition state **POH5**	4.0	4.0
minimum **POH6**	1.1	1.1

group is dependent upon the basis set.[13] In agreement with experimental evidence, calculations on formamide show that the ZPE of the amino group out-of-plane bend is higher in energy than the difference between planar and nonplanar geometries indicating a planar average structure.[13a] Thus, phosphonamidate and sulfonamide are probably planar in actuality and the pyramidalization observed in these calculations is an artifact of the basis set.

The PES of phosphonamide, shown in Figure 6, is similar to that of the tetrahedral intermediate. Three staggered minima and two eclipsed rotational transition structures were located, but a rotational transition state between **POH2** and **POH4** was not found. The lowest-lying minima, **POH4** and **POH2**, have the *N*-methyl group and *N*-hydrogen gauche to the other methyl group and the two methyl groups anti to each other, respectively. A very low lying inversion transition structure **POH3** was also located. Not only does the inversion barrier disappear upon addition of zero-point energies but it becomes as favorable as two minima **POH4** and **POH2**.

The pathway from **POH2** to **POH4** most likely involves inversion to the diastereomeric potential surface, rotation on this surface, and inversion through the diastereomeric inversion transition state to **POH4**. The total barrier for this process is estimated to be ~2 kcal/mol. Attempts to confirm this theory via IRC calculations on the inversion transition state **POH3** were unsuccessful due to the flatness of the potential energy surface about this structure.

Despite the differences between the phosphonamidate and sulfonamide PES's and the tetrahedral intermediate PES, the results indicate that conformation and geometry are not important in distinguishing between P, S, and TI. As can be seen, Figure 8 shows that the tetrahedral intermediate conformational energies are actually not very different from those of the sulfonamide, phosphonamidate, or phosphonamide. The results indicate that conformation and geometry are not important in distinguishing between phosphonamidate and sulfonamide as protease inhibitors. While the maxima and minima are not in exactly the same places for the four models, the relative energy trends are similar. Comparing phosphonamidate and sulfonamide shows that the only major difference between the potential energy surfaces of the two compounds is that sulfonamide has slightly higher rotational barriers. A comparison of the geometrical parameters given in Table 2 shows that the geometries of the sulfonamide conformers, rather than those of

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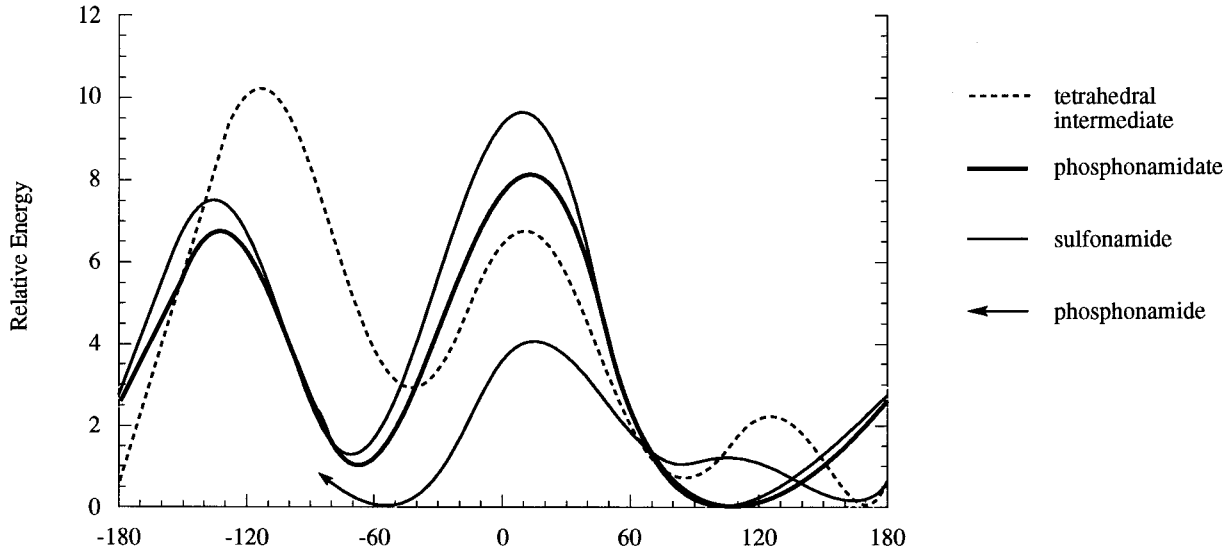
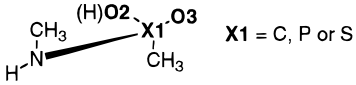


Figure 8. Graph of the rotational potential energy surfaces of the tetrahedral intermediate model, *N*-methylmethanephosphonamidate, *N*-methylmethanesulfonamide, and *N*-methylmethanephosphonamide at the RHF/6-31+G* level of theory. The phosphonamide curve does not cover the whole 360°, since no rotational transition state could be located between two of the minima. The graph for the enantiomeric conformers would have a low-lying area in the −180° to −120° range.

Table 2. RHF/6-31+G* Optimized Geometrical Parameters for Selected Conformations of the Tetrahedral Intermediate, Phosphonamidate, Sulfonamide, and Phosphonamide



parameter	tetrahedral intermediate	*N*-methylmethane-phosphonamidate	*N*-methylmethane-sulfonamide	*N*-methylmethane-phosphonamide
conformer 1	**TI1**	**P1**	**S1**	**POH1**
X1−O2 (Å)	1.469	1.491	1.433	1.607
X1−O3 (Å)	1.296	1.490	1.433	1.464
X1−N (Å)	1.503	1.724	1.645	1.662
O2−X1−O3 (deg)	110.9	122.8	120.6	114.3
O2−X1−N (deg)	103.8	105.5	106.0	102.1
O3−X1−N (deg)	114.9	108.8	109.7	115.2
CH_3−N−X1−CH_3 (deg)	124.2	102.2	98.0	102.5
N−X1−O3−O2 (deg)	−117.3	−123.8	−123.5	−117.8
conformer 2	**TI2**	**P2**	**S2**	**POH2**
X1−O2 (Å)	1.480	1.494	1.437	1.614
X1−O3 (Å)	1.296	1.487	1.431	1.464
X1−N (Å)	1.482	1.735	1.657	1.656
O2−X1−O3 (deg)	110.3	121.1	118.8	111.4
O2−X1−N (deg)	105.8	109.1	110.6	107.6
O3−X1−N (deg)	114.0	107.6	107.3	113.2
CH_3−N−X1−CH_3 (deg)	170.6	180.0	180.0	164.0
N−X1−O3−O2 (deg)	−118.9	−126.3	−126.3	−121.4
conformer 4	**TI4**	**P4**	**S4**	**POH4**
X1−O2 (Å)	1.450	1.488	1.430	1.604
X1−O3 (Å)	1.298	1.488	1.431	1.462
X1−N (Å)	1.490	1.711	1.628	1.654
O2−X1−O3 (deg)	112.8	124.0	121.6	115.4
O2−X1−N (deg)	105.0	107.2	107.4	104.8
O3−X1−N (deg)	109.9	105.9	106.0	110.8
CH_3−N−X1−CH_3 (deg)	−43.3	−66.9	−70.0	−49.9
N−X1−O3−O2 (deg)	−116.8	−123.7	−122.8	−122.1

phosphonamidate, better approximate the geometries of the tetrahedral intermediate conformers. While phosphonamide best approximates the tetrahedral intermediate, the greatest amount of similarity between geometrical parameters exists between the sulfonamide conformers and the phosphonamidate conformers.

Conformation of Binding. In peptide and protein substrates, the backbone dihedral angle ω is approximately 180°,[14] which is analogous to an anti arrangement of the peptide bond. One would assume that the tetrahedral intermediate is bound in a similar conformation. This corresponds to an anti arrangement of the two backbone chains or, in the case of our model systems, of the two methyl groups. This is the global minimum (**TI2**)

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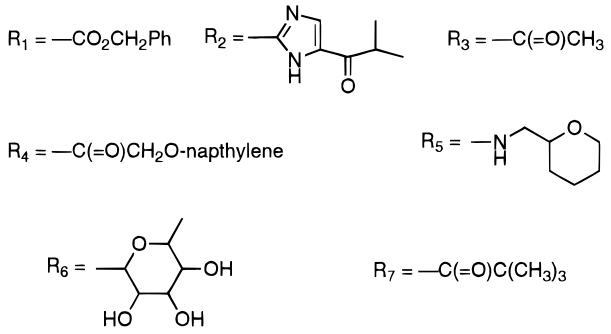
(15) Bartlett, P. A.; Marlowe, C. K. *Science* **1987**, 569−571.
(16) Komiyama, T.; Suda, H.; Aoyagi, T.; Takeuchi, T.; Umezawa, H.; Fujimoto, K.; Umezawa, S. *Arch. Biochem. Biophys.* **1975**, *171*, 727−731.

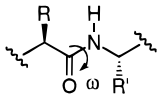
Table 3. Crystal Structures of Proteases with a Bound Transition State Isostere. The Reported Dihedral Angle Was Determined from the PDB Crystal Structure[a]



enzyme/inhibitor	K_i	TS isostere	backbone dihedral angle of TS isostere
HIV-1 protease			
Ala-Ala-Phe*-Phe-Val--Val-OCH_3	1 nM[4d]	hydroxyethylene	122.8
Val-Ser-Gln-Asn-Leu*-Val-Ile-Val-OH	<1 nM[4b]	hydroxyethylene	133.3
R_1-Val-Phe*-CH_2-CH(CH_2Ph)-R_2	0.6 nM[4h]	hydroxyethylene	139.9
R_3-Thr-Ile-Nle*-Nle-Gln-Arg-NH_2	0.78 μM[4a]	-CH_2NH-	142.1
R_4-Trp-Phe*-Val-Ile-R_5		dihydroethylene	145.5
Ala-Ala-Phe*-Gly-Val-Val-OCH_3	18 nM[5f]	hydroxyethylene	147.9
R_3-Ser-Leu-Asn-Phe*-CH_2-Pro-Ile-Val-OCH_3	0.24 nM[4c]	hydroxyethylamine	151.2
R_3-Ala-Phe*-Gly-Val-Val-OCH_3	118 nM[4e]	hydroxyethylene	168.9
thermolysin			
R_1-Gly*-Leu-Leu	9.1 nM[5c]	phosphonamidate	137.1
R_1-Gly*-Leu-Leu	9,000 nM[15]	phosphonate	146.8
R_6-O_3P*-Leu-Trp	28 nM[16]	phosphoramidon	147.9
R_1-Phe*-Leu-Ala	0.068 nM[5f]	phosphonamidate	158.2
Gly-Phe*-Ile-Ile		phosphonamidate	158.5
renin			
R_7-His-Pro-Phe-His-Leu*-Leu-Tyr-Tyr-Ser-NH_2		hydroxyethylene	166.6

[a] * indicates peptide bond that has been replaced with a transition state analogue. Nle = amino acid with $(CH_2)_3CH_3$ side chain.

on the tetrahedral intermediate potential energy surface, where the methyl−methyl dihedral angle is 170.6°.



To be good inhibitors, phosphonamide, phosphonamidate, and sulfonamide should all readily assume a ∼180° binding conformation. This conformer is a low-lying minimum on the phosphonamide surface, although the methyl−methyl dihedral angle is now 164.0°. An ∼180° conformer is neither a transition state nor a minimum on the potential energy surfaces of both phosphonamidate and sulfonamide. Since an ideal transition state isostere should bind in this conformation, a conformer with the methyl groups constrained to 180° was calculated for both phosphonamidate and sulfonamide (Tables 1 and 2, Figures 4 and 5). The 180° conformers of phosphonamidate, **P2**, and sulfonamide, **S2**, are only 2.7 and 2.8 kcal/mol higher in energy than the global minima, respectively. Thus, this conformation is energetically accessible for both of these molecules and should not be a factor in the better performance of phosphonamidates than sulfonamides as inhibitors.

Information obtained from the Protein Data Bank (Table 3) further supports the conclusion that possible binding conformations do not explain the difference in inhibition between phosphonamidates and sulfonamides. While the inhibitors in Table 3 show no correlation between K_i and the dihedral angle (ω) of the reduced peptide bond, they do provide a range of dihedral angles over which a transition state isostere binds to a protease enzyme. Table 3 shows that this range is from 120° to 170°, which corresponds to energetically low-lying areas of the potential energy surfaces of all four model systems (Figure 8). In particular, the surfaces of phosphonamidate and sulfonamide are almost identical in this area, and both should assume reasonable binding conformations with only a small cost in energy.

Interestingly, three of the crystal structures examined contain phosphonamidate based inhibitors (Table 3, thermolysin). The dihedral angles for these bound inhibitors are larger than the 102.2° angle of the global minimum of the model system but smaller than the 170.6° angle of the tetrahedral intermediate conformer. A balance between assuming a higher energy conformation and achieving stronger bonds to residues or metals in the active site may determine the dihedral angle with which the inhibitor binds to the enzyme.

Charge and Electrostatic Potential. The results from the ChelpG charge calculations (Table 4) provide a clearer explanation of the better performance of phosphonamidate as an inhibitor. The partial atomic charges of the oxygens and nitrogen of the tetrahedral intermediate are very similar to those of the phosphonamidate but differ from those of the sulfonamide and phosphonamide. Phosphonamidate will be able to form hydrogen bonds and electrostatic interactions with the enzyme that are as strong as those of the intermediate, making it a good inhibitor. The atomic charges of sulfonamide are so dissimilar to those of the tetrahedral intermediate that

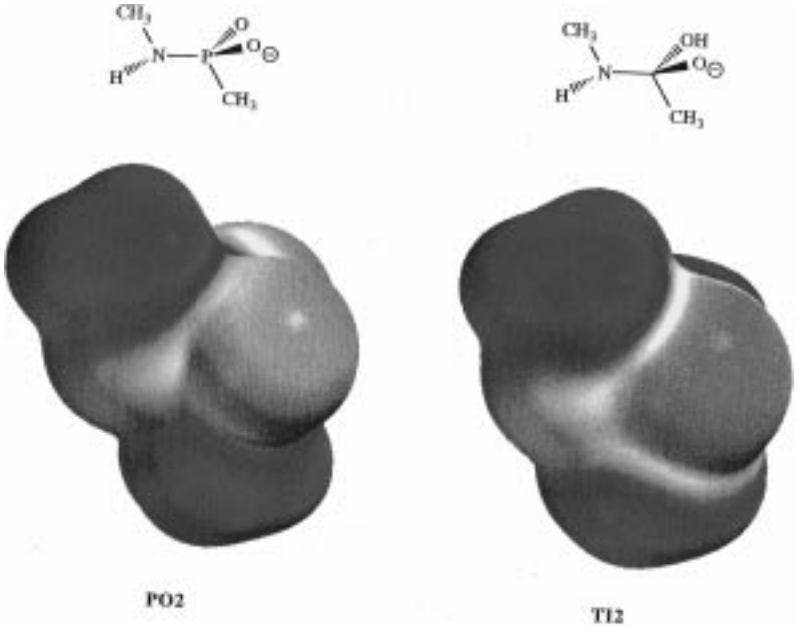


Figure 9. RHF/6-31+G* electrostatic potentials of phosphonamide **PO2** and the tetrahedral intermediate **TI2** graphed over the range of −166 to −44 kcal/mol.

Table 4. RHF/6-31+G* ChelpG Charges for the Tetrahedral Intermediate, Phosphonamidate, Sulfonamide, and Phosphonamide

atom	tetrahedral intermediate	*N*-methylmethane-phosphonamidate	*N*-methylmethane-sulfonamide	*N*-methylmethane-phosphonamidate
conformer 1	**TI1**	**P1**	**S1**	**POH1**
A1	1.64	1.72	1.46	1.51
O2	−0.97	−1.02	−0.68	−0.79
O3	−1.14	−1.00	−0.66	−0.84
N	−1.11	−1.01	−0.82	−0.86
conformer 2	**TI2**	**P2**	**S2**	**POH2**
A1	1.40	1.63	1.32	1.49
O2	−0.93	−0.99	−0.65	−0.78
O3	−1.07	−0.95	−0.60	−0.81
N	−1.03	−0.98	−0.77	−0.86
conformer 3	**TI4**	**P4**	**S4**	**POH4**
A1	1.27	1.57	1.33	1.34
O2	−0.86	−0.96	−0.62	−0.73
O3	−1.11	−0.99	−0.66	−0.82
N	−1.02	−0.91	−0.69	−0.85

it will be unable to effectively reproduce the binding interactions of the intermediate, making it a poor inhibitor. For the same reason, phosphonamide should also not perform well as an inhibitor.

This discrepancy between sulfonamide and phosphonamidate with respect to the tetrahedral intermediate can be further demonstrated by examining the electrostatic potentials (Figure 9). The potential energy ranges were −166 to −56 kcal/mol for **TI2**, −46 to 66 kcal/mol for **S2**, −158 to −44 kcal/mol for **P2**, and −60 to 76 kcal/mol for **POH2**. Figure 9 compares phosphonamidate and the tetrahedral intermediate over the range of −166 to −45 kcal/mol. As can be observed, the electrostatic potential of **P2** closely resembles that of **TI2**, indicating that this isostere should be able to duplicate the electrostatic interactions of the intermediate with the enzyme. Upon first observation, sulfonamide also appears to resemble the electrostatic potential of the tetrahedral intermediate; however, when the potentials are plotted over the range of −100 to 0 kcal/mol (Figure 10), the electrostatic potential of **S2** is vastly different from that of **T2** and **P2**.

Conclusion

Both phosphonamidate and sulfonamide possess the required tetrahedral geometry of a transition state isostere for protease inhibitors, although the conformational minima differ from that of the tetrahedral intermediate. Despite their similarities, phosphonamidates are much more potent inhibitors than sulfonamides. Our calculations show that the conformations and geometries of these molecules do not account for this difference. Rather, the difference in charges and consequently the electrostatic potential makes phosphonamidate a better inhibitor than sulfonamide. The charges and electrostatic potential of phosphonamidate are nearly identical with those of the tetrahedral intermediate, but those of sulfonamide are not. Phosphonamidate will be able to mimic interactions, such as hydrogen bonding and electrostatic that occur between the tetrahedral intermediate and the enzyme. The reasonable agreement between the geometries of phosphonamide and the tetrahedral intermediate indicate that phosphonamide esters could be good inhibitors. Phosphonamide charges do not repro-

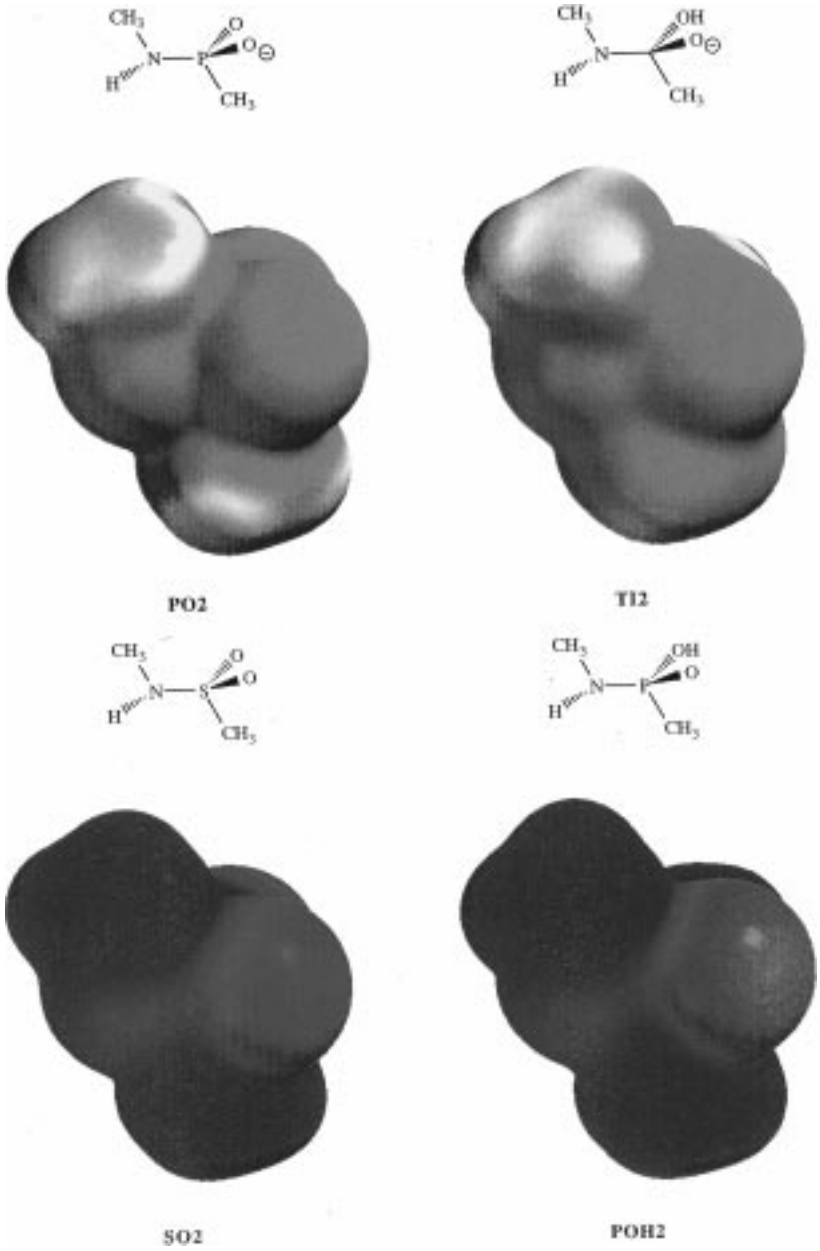


Figure 10. RHF/6-31+G* electrostatic potentials of phosphonamidate **PO2**, the tetrahedral intermediate **TI2**, sulfonamide **SO2**, and phosphonamide **POH2** graphed over the range of −100 to −0 kcal/mol.

duce those of the tetrahedral intermediate very closely, but they do reproduce them better than sulfonamide. Thus, phosphonamide and its esters should be better inhibitors than sulfonamides, but worse than phosphonamidates.

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