A Comparison of the AM1 and PM3 Semiempirical Models for Evaluating Model Compounds Relevant to Catalysis by Serine Proteases†

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Abstract: We present the results of semiempirical molecular orbital calculations employing the AM1 and PM3 molecular models. The compounds studied are related to components of the active site of serine proteases. Our goal of this work was to determine which of the parameter sets is most appropriate for the study of amide and ester catalysis by serine proteases. We found that AM1 and PM3 were very similar in terms of calculated heats of formation and proton affinities. PM3 was much better, however, at reproducing hydrogen bond geometries.

Introduction

One of the challenges in computational chemistry is to develop accurate solutions to the Schrödinger equation for large molecular systems. Semiempirical molecular orbital methods are one approach to meeting this challenge, and the methods developed in the Dewar group (and offspring) have improved the state of the art considerably in recent years. In particular, MINDO/3,[1] MNDO,[2] AM1,[3] and most recently, PM3[4] have been important landmarks in ever improving methodologies for semiempirical calculations. These have been applied with considerable success to many problems of chemical interest.

The basic feature of semiempirical quantum mechanical methods is to neglect many of the electron repulsion integrals, which are the bottleneck of ab initio calculations. One then must adjust a number of empirical parameters for other terms in the Hamiltonian in order to compensate for such approximations in neglecting electron–electron repulsion. In the Dewar approach, these parameters are adjusted to fit experiment for a wide variety of molecules and properties. This empirical adjustment can, in some cases, make the method reproduce reality better than ab initio methods involving single-determinant wave functions because correlation effects are included in the parameterization.

Nonetheless, one of the problems with semiempirical approaches is that, by adjusting parameters to fit some experimental properties, others may be poorly represented. For example, hydrogen bonding has been poorly represented in MINDO/3 and MNDO, because hydrogen-bonded systems were not included in the parameterization set. In the most recent parameterization from the Dewar group, AM1, some hydrogen-bonded structures and energies were included in the parameterization and the representation of hydrogen bonding did improve. Another related parameterization, PM3, also considered some hydrogen-bonded properties in its development. Because there is some controversy over whether PM3 and AM1 differ in a significant way,[5,6] it is of interest to compare the properties of each for hydrogen-bonded and ion pair complexes not considered in the parameterization process. It is also of interest to compare the predicted properties of each to the results of higher level ab initio calculations. We undertook these calculations not only for the sake of comparing the AM1 and PM3 molecular models, but also to determine which model would best represent model compounds pertinent to the serine protease catalyzed hydrolysis of amides and esters. We found that AM1 and PM3 gave similar heats of formation for the various compounds tested, but PM3 was slightly better. PM3 was superior, though, with regard to hydrogen bond geometries in the models. Overall, we found AM1 to give unsatisfactory geometries for the model hydrogen-bonded complexes, and further studies on the actual reaction pathway catalyzed by serine proteases were performed using PM3 (accompanying paper).

Methods

The calculations reported below were carried out using either the AM1[3] or PM3[4] molecular models as implemented within a modified version of the MOPAC program.[7] We began this study by calculating heats of formation, proton affinities, and hydrogen bond strengths using the AM1 molecular model to assess its ability to reproduce various hydrogen-bonding properties of relevant models for the serine protease catalyzed reaction. Because AM1 did not satisfactorily reproduce the structural features of the hydrogen bonds in the model structures, we investigated these same model systems using the new PM3 model, when it became available. For all of the calculations, the geometries of the models were fully optimized to determine the heats of formation.

Two starting structures were used for the model compound calculations: structures with idealized geometries and conformations derived from the crystal structure of trypsin. The structures with idealized geometries were generated by specifying ideal internal coordinates for the molecules and complexes (e.g., bond lengths, bond angles, and hydrogen bond distances for the complexes). Various manipulations were necessary to prepare the components of the crystal structure for these calculations, and they are described in the accompanying paper. The compounds and complexes of interest were taken from the coordinates of the completely optimized, truncated active site model of trypsin.

We also investigated the tetrahedral complexes formed from hydroxide and methoxide attack of *N*-methylacetamide. Idealized geometries were used, and the effect of hydrogen bonding to the oxyanion of the complex was determined by addition of two water molecules. The reaction coordinate method[8] was used to generate the complex, with the distance between the nucleophilic oxygen and the carbonyl carbon of NMA specified as the reaction coordinate. The resulting tetrahedral complex was then fully optimized.

In order to evaluate the errors of the semiempirical method, 6-31G*/MP2 calculations with the GAUSSIAN 86 package[9] were performed using the following approach: The optimized PM3 structures of methanol, methanolate, H_δ-histidine, and protonated histidine were used as starting points for a complete geometry optimization on the 6-31G* level.

† Abbreviations used: HID = H_δ-methylimidazole, HIE = H_ϵ-methylimidazole, HIP = protonated methylimidazole, NMA = *N*-methylacetamide.

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(1) Bingham, R. C.; Dewar, M. J. S.; Lo, D. H. *J. Am. Chem. Soc.* **1975**, *97*, 1285.
(2) Dewar, M. J. S.; Thiel, W. *J. Am. Chem. Soc.* **1977**, *99*, 4899.
(3) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
(4) Stewart, J. J. P. *J. Comput. Chem.* **1989**, *10*, 209–220.
(5) Dewar, M. J. S.; Healy, E. F.; Holder, A. J.; Yuan, Y. C. *J. Comput. Chem.* **1990**, *11*, 541.
(6) Stewart, J. J. P. *J. Comput. Chem.* **1990**, *11*, 543.
(7) Merz, K. M., Jr.; Besler, B. H. QCPE Program No. 589. *QCPE Bull.* **1990**, *10*, 15.
(8) Dewar, M. J. S.; Kirschner, S. J. *J. Am. Chem. Soc.* **1971**, *93*, 4290.
(9) GAUSSIAN 86: Frisch, M. J.; Binkley, J. S.; Schlegel, H. B.; Raghavachari, K.; Melius, C. F.; Martin, R. L.; Stewart, J. J. P.; Bobrowicz, F. W.; Rohlfing, C. M.; Kahn, L. R.; Defrees, D. J.; Seeger, R.; Whiteside, R. A.; Rox, D. J.; Fleuder, E. M.; Pople, J. A. Carnegie-Mellon Quantum Chemistry Publishing Unit Pittsburgh, PA, 1984.

0002-7863/91/1513-8922$02.50/0

Table I. Calculated Heats of Formation of Reference Compounds (kcal/mol) Using Different Molecular Models and Different Starting Structures

	derivation of starting structures and molecular model			
	idealized geometries		crystal structure	
compound	AM1	PM3	AM1	PM3
CH_3OH	−57.0	−51.9	−57.0	−51.9
CH_3O^-	−38.5	−37.9	−38.5	−37.9
CH_3COOH	−103.0	−101.9	−103.0	−102.0
CH_3COO^-	−115.4	−119.7	−115.4	−119.6
HID	42.3	21.2	42.4	21.2
HIE	43.0	21.8	43.0	21.8
HIP^+	185.7	166.8	185.7	166.6
NMA	−46.6	−51.2	−47.3	−51.1
CH_3NH_2	−7.4	−5.2	−7.4	−5.2
H_2O	−59.2	−53.4		
OH^-	−14.1	−17.5		

The resulting structures were then aligned to give the hydrogen-bonding distance of the corresponding PM3 complexes. Single-point 6-31G*//MP2 calculations were then performed on those structures, and the hydrogen-bonding distances were varied in 0.1-Å steps in order to screen the potential surface. Resulting hydrogen bond energies, minimum distances, and the energy difference for the proton-transfer step were compared to the PM3 results.

Results and Discussion

We have performed semiempirical molecular orbital calculations of model compounds relevant to trypsin-catalyzed hydrolysis of amides and esters using the AM1 and PM3 molecular models. We began doing model calculations relevant to trypsin catalysis using the AM1 molecular model. We used AM1 because it was reported that AM1 reproduces experimental hydrogen bond energetics[3,10] and proton affinities.[11] However, it was also noted that the structural features of hydrogen bonds are not well reproduced by AM1.[10,12] We also found some poor geometries for hydrogen-bonded complexes using AM1 and tested the PM3 model when it became available. We had two main objectives in performing the model compound calculations: to determine how well the models reproduce hydrogen bond properties of complexes relevant to serine protease catalyzed amide hydrolysis and to determine which molecular model to employ.

First, we calculated the heats of formation for the reference compounds listed in Table I. Two different starting structures were used for each of the complexes: one with idealized geometry and the other with the orientations in the trypsin crystal structure. The calculated enthalpies for the different starting structures are very similar when the same molecular models are compared, suggesting that the structures optimize to the same local minimum geometry with the different molecular models.

On the request of a reviewer, we have included the atomic charges (Table II) for some of the compounds in Table I, on the belief that the PM3 charges may be anomalous. There are some significant differences in the charges; for example, the charge on the amide hydrogen is quite low in magnitude for PM3. We found no evidence, however, of severely aberrant charges in any of the model compounds (even those not given in Table II). We note that Mulliken populations are only a rough measure of charge distributions. We also should stress the general problem with semiempirical molecular orbital methods; i.e., fitting some properties better with parameter adjustment may lead to less satisfactory properties elsewhere. This issue has been amply discussed in refs 5 and 6.

From the calculated heats of formation, the proton affinities for methylimidazole, methanol, and acetic acid were determined. These values and the corresponding experimental values are given in Table III. The experimental heat of formation for protons (367.2 kcal/mol)[13] was used in determining the proton affinities. The calculated proton affinities of methoxide and acetate using AM1 agree with the earlier values calculated by Dewar and Dieter[11] (Table III). But, these values determined using AM1 were all higher than the experimental values. PM3, however, yielded proton affinities in fairly good agreement with experiment. The proton affinities determined from the different starting structures were extremely close, suggesting again that all converge to the same local minimum state.

Table II. Atomic Charges for Some of the Model Compounds from Table I with the Crystal Structure Geometries

compound	atom	AM1	PM3
CH_3OH[a]	C	−0.0730	0.0697
	H	0.0978	0.0409
	H	0.0532	0.0086
	H	0.0527	0.0083
	O	−0.3264	−0.3084
	H	0.1957	0.1809
CH_3COO^-[b]	C	−0.2680	−0.1761
	H	0.0452	0.0130
	H	0.0470	0.0169
	H	0.0461	0.0149
	C	0.3212	0.4205
	O	−0.5969	−0.6459
	O	−0.5946	−0.6433
HID[c]	C	−0.1494	−0.0168
	H	0.0982	0.0551
	H	0.0818	0.0399
	H	0.0857	0.0413
	C	−0.1178	−0.3033
	N	−0.2054	0.3145
	H	0.2487	0.0700
	C	−0.1043	−0.2573
	H	0.1962	0.1621
	N	−0.1401	−0.1162
	C	−0.1715	−0.1315
	H	0.1780	0.1421
NMA[d]	C	−0.2429	−0.1437
	H	0.1007	0.0650
	H	0.1174	0.0722
	H	0.0940	0.0549
	C	0.3009	0.2345
	O	−0.3708	−0.3583
	N	−0.3919	−0.0433
	H	0.2203	0.0615
	C	−0.0750	−0.0880
	H	0.1176	0.0739
	H	0.0650	0.0268
	H	0.0646	0.0445

[a] Dipole moment: AM1, 1.622; PM3, 1.489. [b] Dipole moment: AM1, 3.818; PM3, 4.017. [c] Dipole moment: AM1, 3.685; PM3, 3.922. [d] Dipole moment: AM1, 3.512; PM3, 3.171.

We then evaluated the properties of hydrogen bonds between pairs of molecules relevant to the catalytic process (Table IV). Both semiempirical models gave qualitatively reasonable hydrogen bond energies, but we did see differences between the molecular models and different starting structures. The models differ significantly, however, with regard to hydrogen bond geometries. Figure 1 gives hydrogen bond distances and angles for some of the complexes from Table IV. Using these models, the dependence of the starting geometries is considered for each molecular model. Then, the AM1 and PM3 models are compared.

In the case of the negatively charged complex (CH_3COO^-–HID), the resulting hydrogen-bonding geometries were nearly independent of the starting configuration. Use of AM1 resulted in double coordination of the hydrogen bond to both of the acetate oxygens. Models with PM3, however, showed only a single hydrogen bond with a shorter distance and closer to linear hydrogen bond angle compared to AM1 (d_1 and θ_1 of Figure 1). In fact, d_1 with PM3 was very close to the value of minimized trypsin (1.76 Å) (see accompanying paper).

(10) Dannenberg, J. J. *J. Phys. Chem.* **1988**, *92*, 6869.
(11) Dewar, M. J. S.; Dieter, K. M. *J. Am. Chem. Soc.* **1986**, *108*, 8075.
(12) Williams, I. H. *J. Am. Chem. Soc.* **1987**, *109*, 6299.
(13) Stull, D. R.; Prophet, J. *JANAF Thermochemical Tables*; NSRDS-NBS37.

Table III. Calculated Proton Affinities[a] (kcal/mol) of Model Compounds Using Different Molecular Models and Different Starting Structures

	derivation of starting structures and molecular model					
	idealized geometries		crystal structure		previous work	
reaction	AM1	PM3	AM1	PM3	AM1	exptl
$CH_3OH \rightarrow CH_3O^- + H^+$	385.7	381.2	385.7	381.2	385.4	376.8[16] 379.2[11] 381.4[11]
$CH_3COOH \rightarrow CH_3COO^- + H^+$	354.8	349.4	354.8	349.6	354.2	342.0[17] 348.5[11] 345.2[11]
$HIP^+ \rightarrow HID + H^+$	223.8	221.6	223.9	221.8		220.0[14]
$HIP^+ \rightarrow HIE + H^+$	224.5	222.2	224.5	222.4		

[a] The experimental value (367.2 kcal/mol) for ΔH_f of the proton was used.[13]

Table IV. Calculated Heats of Formation[a] and Relative Stabilities[b] of Hydrogen-Bonded Complexes of the Reference Compounds Using Different Starting Geometries and Different Molecular Models

	derivation of starting structures and molecular model							
	idealized geometries				crystal structure			
	AM1		PM3		AM1		PM3	
complex	ΔH_f	$\Delta\Delta H$	ΔH_f	$\Delta\Delta H$	ΔH_f	$\Delta\Delta H$	ΔH_f	$\Delta\Delta H$
$CH_3COO^-\cdots HID$	−96.2	−23.1	−123.3	−24.8	−95.1	−22.1	−123.3	−24.9
$CH_3COO^-\cdots HIP^+$	NS	NS	−67.4	−114.3	NS	NS	−62.3	−109.3
$CH_3OH\cdots HID$	−17.5	−2.8	−33.9	−3.2	−16.2	−1.6	−33.0	−2.3
$NMA\cdots 1H_2O$	−110.5	−4.7	−107.2	−2.6	−110.5	−4.7	−108.2	−3.7
$NMA\cdots 2H_2O$	−174.3	−9.3	−166.0	−8.0	−179.2	−14.2	−164.2	−6.3

[a] NS = not stable. [b] $\Delta\Delta H = \Delta H_f$(A–B complex) − ΔH_f(A) − ΔH_f(B).

Table V. Calculated Heats of Formation (kcal/mol) and Relative Stabilities[a] of Tetrahedral Intermediates from Nucleophilic Addition to NMA Using Different Molecular Models

	AM1		PM3	
compound	ΔH_f	$\Delta\Delta H$	ΔH_f	$\Delta\Delta H$
CH_3O^-–NMA	−100.8	−15.7	−105.8	−16.7
OH^-–NMA	−107.2	−46.5	−115.8	−47.1
CH_3O^-–$NMA\cdots 2H_2O$	−249.1	−29.9	−242.3	−29.7
OH^-–$NMA\cdots 2H_2O$	−255.7	−30.1	−253.4	−30.8

[a] $\Delta\Delta H = \Delta H_f$(A–B complex) − ΔH_f(A) − ΔH_f(B) or $\Delta\Delta H = \Delta H_f$(A–B complex$\cdots 2H_2O$) − ΔH_f(A–B complex) − $2\Delta H_f(H_2O)$.

The corresponding neutral complex (CH_3COO^-–HIP^+) was not stable with AM1; proton abstraction occurred, leaving both components neutral. With PM3, short hydrogen bond distances were found with both starting structures but the model derived from the crystal structure gave a better hydrogen-bonding distance of nearly the same length as in minimized trypsin (1.60 versus 1.65 Å).

We then investigated methylimidazole complexes with methanol. Again, a much better hydrogen-bonding angle was achieved with the crystal structure using AM1 but the geometry was better with ideal geometries using PM3. In comparing AM1 and PM3, the starting geometry was more important in determining the hydrogen-bonding geometry than the molecular model, although three of the four hydrogen bonds were long and kinked. We must emphasize that the idealized geometries are the real test of the method and the starting conformations derived from the crystal structure may hide flaws, although they are ultimately more relevant for our further studies of catalysis described in the accompanying paper.

Finally, in the complex between *N*-methylacetamide and two water molecules, with AM1 the two starting structures gave comparable hydrogen-bonding distances but the angles were better with the coordinates derived from the crystal structure. The best of the PM3 models was much better than AM1 in mimicking the oxyanion hole hydrogen bond distances found in minimized trypsin (d_1 = 1.84 Å and d_2 = 1.90 Å in trypsin).

We then investigated tetrahedral intermediates from methoxide and hydroxide attack of *N*-methylacetamide. The energy of the adducts was also computed after adding two water molecules to evaluate the importance of the waters in stabilizing the oxyanion. These adducts were all constructed from ideal geometries. The

Table VI. Energies from ab Initio Calculations of Reference Compounds and Complexes Using Two Different Basis Sets

	total energies (au)		relative energies (kcal/mol)		
	6-31G*	MP2	6-31G*	MP2	PM3
CH_3OH	−115.035 417 9	−115.344 941 1			
HID	−263.855 343 9	−264.688 450 7	0.00	0.00	0.00
$CH_3OH\cdots HID$ (r = 1.82)	−378.899 435 4	−380.046 853 6	−5.44	−8.44	−3.24
$CH_3\cdots HID$ (r = 1.92)	−378.900 815 2	−380.047 704 7	−6.30	−8.98	
$CH_3OH\cdots HID$ (r = 2.02)	−378.901 404 8	−380.047 839 5	−6.67	−9.06	
$CH_3OH\cdots HID$ (r = 2.12)	−378.901 473 3	−380.047 511 0	−6.72	−8.85	
$CH_3OH\cdots HID$ (r = 2.22)	−378.901 212 8	−380.046 898 3	−6.55	−8.47	
$CH_3O^-\cdots HIP$ (r = 1.59)	−378.808 058 8	−379.961 039 3	51.86	45.37	
$CH_3O^-\cdots HIP$ (r = 1.69)	−378.811 107 7	−379.962 648 4	49.95	44.36	42.28
$CH_3O^-\cdots HIP$ (r = 1.79)	−378.811 785 1	−379.962 118 3	49.52	44.69	
$CH_3OH^-\cdots HIP$ (r = 1.89)	−378.810 764 9	−379.960 077 6	50.16	45.97	
Minimum Values Calculated from a Parabolic Fit					
$CH_3OH\cdots HID$ (r = 2.10/2.02)			−6.80	−9.07	
$CH_3O^-\cdots HIP$ (r = 1.78/1.73)			49.46	44.28	
proton transfer energies			56.26	53.35	45.52

	Starting From Idealized Geometries		Starting from Crystal Structure	
CH_3COO^-...HID				
AM1	d_1=2.02	θ_1=152.4°	d_1=1.97	θ_2=165.8°
	d_2=2.12	θ_1=143.3°	d_2=2.20	θ_2=128.7°
PM3	d_1=1.72	θ_1=173.4°	d_1=1.72	θ_2=174.2°
	d_2=2.56	θ_1=129.1°	d_2=2.58	θ_2=128.8°
CH_3COO^-...HIP^+				
AM1	Not Stable		Not Stable	
PM3	d_1=1.69	θ_1=148.5°	d_1=1.60	θ_2=174.3°
	d_2=2.88	θ_1=99.1°	d_2=2.40	θ_2=112.9°
CH_3OH...HID				
AM1	d_1=2.73	θ_1=123.9°	d_1=2.66	θ_2=157.7°
PM3	d_2=1.82	θ_1=175.6°	d_2=2.49	θ_2=155.2°
NMA...2 Water Molecules				
AM1	d_1=2.13	θ_1=156.6°	d_1=2.30	θ_2=135.4°
	d_2=2.11	θ_1=175.5°	d_2=2.19	θ_2=104.0°
PM3	d_1=2.75	θ_1=105.2°	d_1=1.81	θ_2=178.1°
	d_2=2.49	θ_1=158.9°	d_2=1.81	θ_2=166.2°

Figure 1. Geometric properties of hydrogen bonds in fully optimized complexes from different starting structures and different molecular models.

heats of formation and changes in enthalpy compared to the reactants are presented in Table V. AM1 and PM3 both gave similar relative energies for all of the complexes. The complexes were stabilized substantially over the uncomplexed components. The relative stability of the hydroxide–NMA complex was more favorable than that of the methoxide–NMA complex (e.g., $\Delta(\Delta\Delta H)$). The addition of the two water molecules stabilized all of the complexes by approximately 30 kcal/mol. As discussed above for the hydrogen-bonded complexes, the hydrogen bonds between the oxyanion of the tetrahedral intermediate and the waters were much better with PM3 than AM1 (for the methoxide–NMA adduct, d_1 = 2.08 Å, d_2 = 2.06 Å, θ_1 = 108.1°, and θ_2 = 115.2° with AM1 and d_1 = 1.74 Å, d_2 = 1.74 Å, θ_1 = 158.9°, and θ_2 = 160.0° with PM3).

To get an error estimate of this semiempirical approach, we performed comparative calculations at the 6-31G*/MP2 level for the hydrogen-bonded complex of methylimidazole–methanol and for the complex after proton transfer (methanolate + protonated methylimidazole). The results are given in Table VI. The hydrogen bond distances for the uncharged complex are longer in the ab initio calculations (2.10 Å at the SCF level and 2.01 Å at the MP2 level) than the PM3 result (1.82 Å), and the hydrogen bond energy is significantly underestimated by PM3. The geometrical differences for the ion pair complex are smaller (1.79 Å at the SCF level, 1.75 Å at the MP2 level, and 1.69 Å for PM3) as well as the difference in hydrogen bond energies. The relative energy corresponding to the proton transfer is in a comparable range for semiempirical and ab initio calculations and approximately 20 kcal/mol larger than the 4-31G value previously reported.[14]

Conclusions

We calculated similar proton affinities for the model compounds using AM1 and PM3, although PM3 better reproduced the experimental values. PM3 produced much better hydrogen bond geometries than AM1, however, yielding geometries that were very similar to those found in the trypsin crystal structure. A major technical problem with using AM1 to study trypsin catalysis is that the complex between the positively charged histidine mimic and acetate (for Asp) was not stable. Instead of maintaining the charged states, the acetate abstracted a proton from the imidazole group. In the gas phase it is clear that the neutral structures are more stable than the ion pair, but the presence and size of the barrier between them is not definitively known. Thus, we cannot rule out that AM1 is more "correct" in not finding the His^+Asp^- ion pair model. However, the fact that the ion pair is not stable with AM1 is especially undesirable since the active site Asp of serine proteases is not observed to be protonated experimentally.[15] Hence, PM3 was the better molecular model for this system where hydrogen bonding is clearly important and where the process of interest is occurring in the enzyme active site as opposed to the gas phase. The comparison to high-level ab initio calculations showed that the relative energies for the catalytically important proton-transfer reaction are in a comparable range. In the following paper, we present PM3 calculations on serine protease catalysis of amides and esters.

Acknowledgment. We are grateful for research support from the NIH (Grant GM-29072), the NSF (Grant DMB-87-14775, and DARPA (Grant N00014-86-K-0757). S.S. thanks the Deutsche Forschungsgemeinschaft for a postdoctoral fellowship.

Registry No. HID, 88054-16-4; HIE, 822-36-6; HIP^+, 24378-15-2; NMA, 79-16-3; MeOH, 67-56-1; MeO^-, 3315-60-4; AcOH, 64-19-7; AcO^-, 71-50-1; $MeNH_2$, 74-89-5; H_2O, 7732-18-5; HO^-, 14280-30-9; serine protease, 37259-58-8.

(14) Kollman, P. A.; Hayes, D. M. *J. Am. Chem. Soc.* **1981**, *103*, 2955.
(15) Kossiakoff, A. A.; Spencer, S. A. *Biochemistry* **1981**, *20*, 6462–6474. Bachovchin, W. W. *Biochemistry* **1986**, *25*, 7751.
(16) McIver, R. T., Jr.; Miller, J. S. *J. Am. Chem. Soc.* **1974**, *96*, 4323.
(17) Yamdagni, R.; Kebarle, P. *J. Am. Chem. Soc.* **1973**, *95*, 4050.