JCTC Journal of Chemical Theory and Computation

The ω, ϕ, and ψ Space of *N*-Hydroxy-*N*-methylacetamide and *N*-Acetyl-*N*′-hydroxy-*N*′-methylamide of Alanine and Their Boron Isosteres

Alpeshkumar K. Malde, Santosh A. Khedkar, and Evans C. Coutinho*

Department of Pharmaceutical Chemistry, Bombay College of Pharmacy, Kalina, Santacruz (E), Mumbai 400 098, India

Received September 28, 2005

Abstract: The conformational space of *N*-hydroxy-*N*-methylacetamide [$CH_3-CO-N(OH)CH_3$, NMAOH] and its boron isostere [$CH_3-CO-B(OH)CH_3$, BMAOH] has been studied by quantum chemical methods. The potential energy surface of NMAOH and BMAOH has been built at the HF, B3LYP, and MP2 levels of theory with the 6-31+G* basis set. The minima and transition states for rotations about various torsional angles have been located, and the energy barriers have been estimated. The global minimum energy structure of both peptides exhibits an intramolecular hydrogen bond between the carbonyl oxygen and the hydroxyl group, imparting a conformational rigidity to the peptides. The *omega* rotation barrier is lower in the boron isostere than in NMAOH. The difference in the rotation barrier has been attributed to second-order orbital interactions, like negative hyperconjugation, as revealed by NBO calculations. In contrast, the rotation barrier around the torsion angle *tau* (torsion governing rotation about the N−O and B−O bonds) is relatively higher in the boron analogue. This difference is due to the double bond character in the B−O bond as opposed to the N−O bond which has the character of a single bond. As an extension, *N*-acetyl-*N*′-hydroxy-*N*′-methylamide of alanine (Ala-NOH) and its boron isostere (Ala-BOH) have been adopted as model peptides to study the conformational preferences about the ϕ and ψ torsion angles. The study reveals a strong preference for a Type I beta turn as well as inclinations for a left-handed alpha helix, for positive *phi* torsions, and for extended *psi* conformations for Ala-NOH; Ala-BOH, on the other hand, shows a leaning toward positive *phi* and extended *psi*, with no preference for any regular secondary structure motifs. The replacement of nitrogen by boron changes the electronic and conformational properties of the peptide, extending greater flexibility around the *omega* angle, a strong preference for positive *phi* values, and a shift in the site of nucleophilic attack from the carbonyl group to boron.

Introduction

Peptides and proteins are one of the important classes of biomolecules. The conformations of peptides and protein are crucial determinants of their biological effects. The values of the three backbone torsion angles—*omega* (ω), *phi* (ϕ), and *psi* (ψ)—dictate the secondary structure of peptides.[1] Most natural peptides adopt ω with 180° (trans), and occasionally, ω assumes 0° (cis) for peptides with the Xxx-Pro and Xxx-Gly motifs.[2] The ϕ and ψ values in natural peptides and proteins are restricted to the allowed regions of the Ramachandran space.[1] Peptides form an important area of therapeutics[3], e.g. insulin, substance P, growth hormone, thyrotropin releasing hormone, gastric inhibitory polypeptide, gastrin, neurokinins, bradykinin, etc. have important therapeutic applications. There are certain advantages with peptide

* Corresponding author phone: +91-22-26670871; fax: +91-22-26670816; e-mail: evans@bcpindia.org.

10.1021/ct050242v CCC: $33.50
Published on Web 01/05/2006

therapeutics. Hormones and neurotransmitter peptides are very potent and consequently administered in very small doses, besides exhibiting a high selectivity and specificity in binding to their target. However, there are also complexities in using and developing peptides as therapeutics. The oral delivery of peptides is restricted due to degradation at the "scissile" amide bond. Imparting potency, specificity, and selectivity for peptides designed from natural analogues for certain biological end-points still remains a challenge. Issues such as proteolytic stability, potency, specificity, and selectivity can be addressed by modification of the amide bond and/or isosteric/bioisosteric replacement of the backbone atoms of the peptide. *N*-methylation;[4] *N*-hydroxylation;[5] replacement of the amide bond[6,7] by sulfonamide, phosphonamidate, and carbamate; isosteric replacement of the carbonyl carbon with boron (peptide boronic acid[8,9]); and isosteric replacement of the alpha carbon with boron (ammonia-carboxyboranes[10−12]) have been reported in the literature as techniques to explore new peptide conformations and to design "druglike," proteolytically stable molecules. *N*-Hydroxylation of peptides has been used to impart conformational rigidity through formation of an intramolecular hydrogen bond with the CO group.[5] This imparts an ability to chelate metal ions for specific binding to proteins containing metals in the active site.

We had reported for the first time a boron isostere of the amide nitrogen in peptides and studied the ω, ϕ, and ψ preferences by ab initio and density functional methods.[13,14] These molecules were designed as plausible serine protease inhibitors. The replacement of nitrogen with boron leads to two new characteristics: a preference for the ω angle for 90°, in contrast to 180° or 0° for natural peptides, and second, conformations that lie in the "disallowed regions" (positive ϕ angles) of the Ramachandran plot. These peptides also exhibit greater flexibility around the ω angle.

In this paper, we look at the ω, ϕ, and ψ preferences of an *N*-hydroxy peptide and its boron isostere, by ab initio and density functional methods. *N*-Methylacetamide (NMA, **I**) has been extensively studied, both experimentally and theoretically, as a model for the peptide backbone. In a like manner, *N*-methyl-*N*-hydroxyacetamide (NMAOH, **II**) is a good model to study *N*-hydroxy peptides and acetylmethylhydroxyborane (BMAOH, **III**) an analogous model for the boron isostere. In addition, *N*-acetyl-*N*′-hydroxy-*N*′-methylamide of alanine (Ala-NOH, **IV**) and its boron isostere (Ala-BOH, **V**) have been adopted as models to study the ϕ and ψ distribution of such peptides. The hypersurfaces of NMAOH (**II**) and BMAOH (**III**), with its associated ground and transition states, and the corresponding ground states of Ala-NOH (**IV**) and Ala-BOH (**V**) have been mapped by ab initio Hartree−Fock (HF), density functional, and post-HF methods. Second-order orbital interactions by Natural Bond Orbitals (NBO) method were also carried out to understand the fundamental differences in the structures of the *N*-hydroxy peptides and their boron isosteres.

Computational Details

Ab initio molecular orbital[15] and density functional theory[16] calculations have been carried out using the Gaussian03W[17]

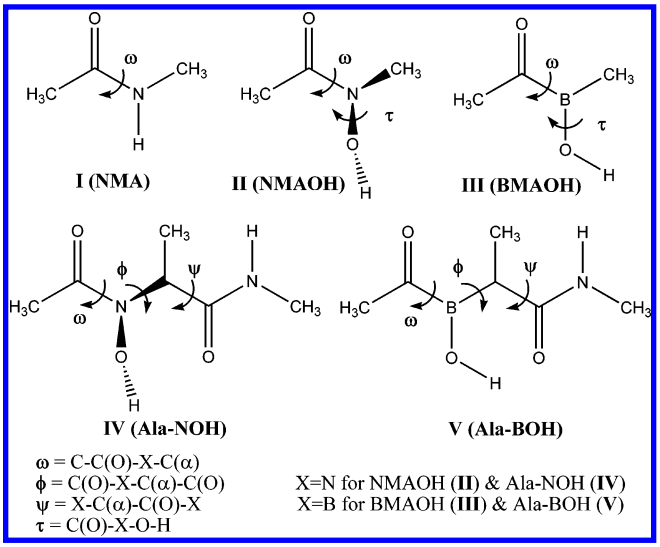
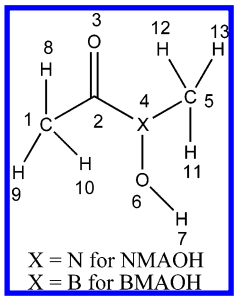


Figure 1. Structures of NMA, NMAOH, and Ala-NOH and their boron counterparts.

Chart 1



(revision C.01) package running on a Pentium III processor with 512 MB RAM. The stability of all wave functions was checked at the HF,[18] Becke's three parameter exchange functional, and the gradient corrected functional of Lee, Yang, and Paar (B3LYP),[19−21] second-order Møller−Plesset MP2 (full)[22,23] level of theory using the 6-31+G* basis set.

The atom labels for NMAOH (**II**) and BMAOH (**III**) are listed in Chart 1, and the two torsion angles, ω and τ, are defined as shown in Figure 1. In NMAOH, the hydroxylamine moiety can adopt two conformations around the N−O bond. In the first, the two lone pairs of electrons of O are *syn-clinal* and in the second, *anti-clinal* with respect to the lone pairs of electrons on N. This has been observed from a conformational search of hydroxylamine by ab initio calculations. These initial two conformations around the N−O bond in NMAOH were chosen, and for each arrangement of τ, a scan in increments of 30° of the ω torsion angle was carried out at the HF/6-31+G* level of theory. Conformations with an ω value of 30° and 210° were found to be the lowest in energy. Now, for each of these two conformations with ω values of 30° and 210°, respectively, a τ scan in increments of 30° was run at the HF/6-31+G* level of theory. The minima saddle points for rotation about the ω torsion and saddle points for rotation about the τ torsion rotation were thus identified. All these conformations were further optimized at the B3LYP and MP2 levels of theory with the same basis set, and the conformations were confirmed by frequency calculations, which returned one imaginary frequency for

Table 1. Relative Energies (kcal/mol) of Various Minima and Transition States on PES of NMAOH (**II**) at the HF, B3LYP, and MP2 Levels of Theory with the 6-31+G* Basis Set[c]

				HF/6-31+G*	B3LYP/6-31+G*	MP2(full)/6-31+G*		
		NIMAG	PG	rel.[a]	rel.[a]	rel.[a]	ω^b	τ^b
minima	GM	0	C_1	0.0	0.0	0.0	32	10
	LM	0	C_1	1.8	1.1	0.4	202	120
ω rotation transition state (TS)	ωTS1/ωTS1′	1	C_1	13.6	15.6	12.6	125/−125	123/−123
	ωTS2/ωTS2′	1	C_1	13.5	15.3	13.0	135/−135	−60/60
	ωTS3/ωTS3′	1	C_1	14.1	15.8	13.3	36/−36	−105/105
	ωTS4/ωTS4′	1	C_1	21.1	21.7	20.3	−39/39	−74/74
τ rotation TS	τTS1	1	C_1	13.2	12.2	12.2	39	−146
	τTS2	1	C_1	8.5	6.5	7.3	−165	7
	τTS3	1	C_1	6.5	6.2	6.3	−159	−147
pyramidal inversion TS	PyTS	1	C_s	2.0	0.0	0.4	0	0

[a] Relative energy in kcal/mol. [b] Torsion angle in degrees. [c] NIMAG = number of imaginary frequency, PG = point group, GM = global minimum, LM = local minimum.

each transition state and all positive frequencies for each ground state.

A similar strategy was adopted for probing the conformational space of BMAOH. The hydroxylborane moiety has a planar conformation, and the resulting τ angles in BMAOH are either 0° or 180°. The two BMAOH conformations with τ values of 0° and 180° were then examined by an ω scan in increments of 30° at the HF/6-31+G* level of theory. Two conformations with ω values of 0° and 180° were identified as the lowest in energy. These two conformations with ω values of 0° and 180° were then evaluated by a τ scan in increments of 30° at the HF/6-31+G* level of theory. The minima, saddle points for rotation around the ω angle, and saddle points for rotation about the τ angle were thus located. All these structures were further optimized at the B3LYP and MP2 levels of theory using the 6-31+G* basis set and confirmed by frequency calculations.

The NBO[24−26] analysis was carried out on the minimum energy structures of NMAOH (**II**) and BMAOH (**III**), optimized at the MP2(full)/6-31+G* level, to quantitatively estimate the second-order interactions as $E_{ij} = -2F_{ij}/\Delta E_{ij}$, where E_{ij} is the energy of the second-order interaction; $\Delta E_{ij} = E_i - E_j$ is the energy difference between the interacting molecular orbitals i and j; and F_{ij} is the Fock matrix element for the interaction between orbitals i and j. The "atomic partial charges" of the global minimum of NMAOH (**II**) and BMAOH (**III**), optimized at the MP2(full)/6-31+G* level, were calculated using Natural Population Analysis (NPA) as implemented in NBO and additionally by the 'ESP fit' method formulated by Merz, Singh, and Kollman.[27]

For Ala-NOH (**IV**), the minima in the ω and τ space was searched starting with two different conformations for ω and τ as identified previously for NMAOH (**II**). This corresponds to structures with $\omega = 32°$; $\tau = 10°$ and $\omega = 202°$; $\tau = 120°$. For each (ω, τ) pair, 144 conformations were generated with 30° increments of the ϕ, ψ dihedrals. Each conformation was geometry optimized first at the HF/3-21G level of theory with "constraints" on the initial ϕ, ψ angles. A Ramachandran plot of the 144 conformations was constructed, and conformations within 5.0 kcal/mol of the global minimum were identified. These low-energy conformations were further optimized without constraints at the B3LYP/6-31+G* level of theory. A similar study was carried out for Ala-BOH (**V**) with the starting (ω, τ) pairs of (0°, 0°) and (153°, 180°).

Results and Discussion

All wave functions for molecules **II**−**V** were found to be stable under the perturbations considered at the HF, B3LYP, and MP2 levels of theory.

Potential Energy Surface (PES) of NMAOH (II). For NMAOH (**II**) besides the global minimum (GM), there is also a local minimum (LM) within 2.0 kcal/mol of the GM. For each structure, several transition states (TS) for rotation about the ω angle exists. The geometries of these TS depend on the state of the pyramidal amide nitrogen, i.e. the lone pair of electrons on nitrogen may either be directed downward, which we label as 'pyramidal up', or the lone pair of electrons on nitrogen may be positioned upward, which we call as 'pyramidal down'. This is further complicated by the orientation of the two lone pairs of electrons on the hydroxyl oxygen relative to the lone pair on the amide nitrogen. In all, eight transition states can be identified for 'ω rotation' taking into consideration all positions of the lone pair of electrons on the amide nitrogen and hydroxyl oxygen atoms.

Further, proceeding from the GM and LM structures three TS corresponding to rotation about the τ angle have been identified. Last, there also exists a third type of TS for inversion of the pyramidal state of nitrogen leading to a planar arrangement of the amide nitrogen. In summation, a total of 14 TS have been identified on the potential energy surface of NMAOH (**II**). The relative energies of the minima and TS at the HF, B3LYP, and MP2 levels of theory are listed in Table 1 (The absolute values have been provided in the Supporting Information Table 1A.) The geometries of the minima and TS have been pictorially depicted in Figure 2, and the geometrical data (bond lengths, bond angles, and torsion angles) are given in Table 3.

All structures, except the transition state for inversion of the nitrogen (PyTS), exhibit C_1 symmetry; PyTS has a C_s symmetry. At the HF/6-31+G* level of theory the ranking of the global minimum and local minimum are inverse of that observed at the B3LYP and MP2 levels of theory. It appears that the consideration of electron correlation in both

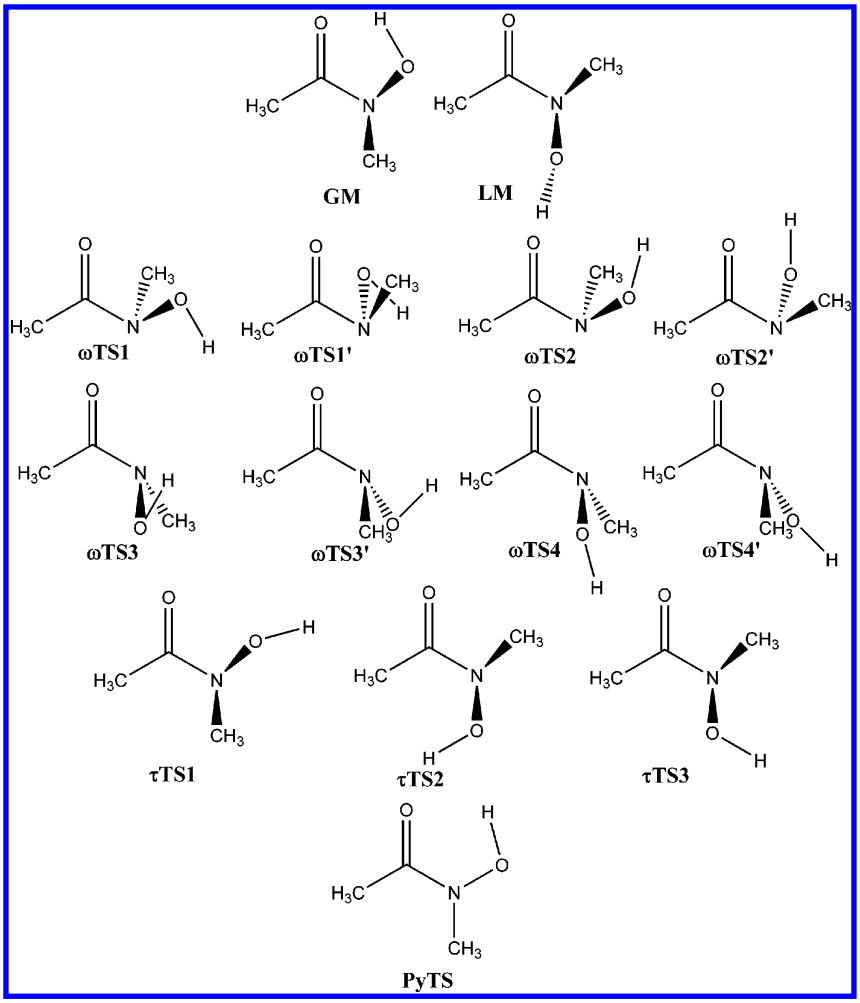


Figure 2. Ground and transition stets of NMAOH.

Table 2. Relative Energies (kcal/mol) of Various Minima and Transition States on PES of BMAOH (**III**) at the HF, B3LYP, and MP2 Levels of Theory Using the 6-31+G* Basis Set[c]

				HF/6-31+G*	B3LYP/6-31+G*	MP2(full)/6-31+G*		
		NIMAG	PG	rel.[a]	rel.[a]	rel.[a]	ω[b]	τ[b]
minima	GM	0	C_s	0.0	0.0	0.0	0	0
	LM	0	C_1	1.9	2.7	2.8	153/−153	180
ω rotation TS	ωTS1	1	C_s	4.3	4.7	4.8	180	0
	ωTS2	1	C_s	6.1	6.5	6.6	0	180
τ rotation TS	τTS	1	C_1	12.5	14.0	15.1	99/−99	86

[a] Relative energy in kcal/mol. [b] Torsion angle in degrees. [c] NIMAG = number of imaginary frequency, PG = point group, GM = global minimum, LM = local minimum.

the B3LYP and MP2 methods resolves this position. The GM is characterized by an intramolecular hydrogen bond between the CO and OH groups forming a five-membered cyclic structure. The O−O distance is 2.566 Å, and the H-bond angle (O−H···O) is 120.4°. Although there is a very small difference in the energies of the GM and LM at the MP2 level, the rotation barrier to interconversion is significant as seen in the energy of the corresponding TS. The rotation barrier to the inversion of nitrogen (PyTS) is negligible (0.4 kcal/mol) at the MP2 level of theory. In the LM the N−O lone pair of electrons is *anti-clinal* exactly like the global minimum of hydroxylamine.

There is a small increase of about 0.08−0.1 Å in the C(O)−N bond length in the transition states for rotation about the ω angle compared to the two ground states (GM and LM). Aubry et al.[28] have reported the crystal structure of a small *N*-hydroxy unnatural peptide ^{t}BuCO-Ψ[CO−N(OH)]-Gly-NH^{i}Pr. The reported structure has a close resemblance to the LM of NMAOH around the *N*-hydoxy amide region. The bond lengths and angles of NMAOH at the MP2(full)/6-31+G* level of theory are close to those in the crystal structure around the *N*-hydroxy amide region (Table 3).

PES of BMAOH (III). The potential energy surface of BMAOH is characterized by two minima—the global minimum (GM) and a local minimum (LM); two transition states for rotation about the ω angle ωTS1 and ωTS2—one arising from the GM and the second from the LM; and one transition state for rotation about the τ angle (τTS). As boron adopts a planar structure (not pyramidal as N in NMAOH), there is no transition state for inversion of boron. The conformations

Table 3. Bond Length (Å) and Bond Angles (deg) of NMAOH (**II**) Optimized at the MP2(full)/6-31+G* Level[a]

parameter	GM	LM	ωTS1	ωTS2	ωTS3	ωTS4	τTS1	τTS2	τTS3	PyTS
CC (1,2)	1.506	1.505	1.498	1.492	1.506	1.512	1.515	1.513	1.504	1.507
CO (2,3)	1.245	1.232 (1.249)	1.218	1.223	1.219	1.217	1.227	1.238	1.235	1.251
CN (2,4)	1.366	1.395 (1.396)	1.475	1.469	1.466	1.462	1.394	1.372	1.387	1.348
NC (4,5)	1.449	1.457 (1.452)	1.466	1.462	1.469	1.455	1.454	1.448	1.449	1.439
NO (4,6)	1.416	1.428 (1.407)	1.464	1.447	1.466	1.442	1.433	1.430	1.441	1.405
OH (6,7)	0.992	0.977	0.977	0.987	0.977	0.986	0.977	0.976	0.977	0.994
CCO (1,2,3)	123.3	123.6	125.4	125.7	123.6	122.7	122.7	122.4	123.9	123.2
CCN (1,2,4)	117.3	116.4	112.5	113.1	118.3	118.5	114.7	116.7	115.9	117.8
CNC (2,4,5)	125.9	118.4	109.5	111.8	114.7	115.1	121.4	121.7	119.6	132.7
CNO (2,4,6)	113.7	112.9	103.5	103.5	101.8	105.6	110.5	120.5	112.3	115.7
CNO (5,4,6)	109.4	110.7	105.7	108.6	105.5	109.3	112.3	108.6	113.9	111.6
NOH (4,6,7)	101.1	103.6	101.4	105.5	101.7	107.1	104.8	106.2	104.5	100.5
CCNC (ω) (1,2,4,5)	31.5	−158.0 (−163.6)	125.5	134.7	36.4	−39.1	38.7	−164.9	−158.6	0.0
CCNO (1,2,4,6)	171.3	−26.3	−122.2	−108.6	−77.0	81.5	173.4	−21.7	−21.1	180.0
CNOH (τ) (2,4,6,7)	9.5	119.4 (119.0)	122.7	−59.1	−105.2	−73.8	−145.9	7.4	−146.7	0.0
OCNC (3,2,4,5)	−151.3	25.4	−54.3	−46.8	−145.7	140.9	−145.7	18.8	25.4	180.0

[a] The values in the parentheses are from the crystal structure of an *N*-hydroxy peptide ^{t}BuCO-Ψ[CO−N(OH)]-Gly-NH^{i}Pr.[28]

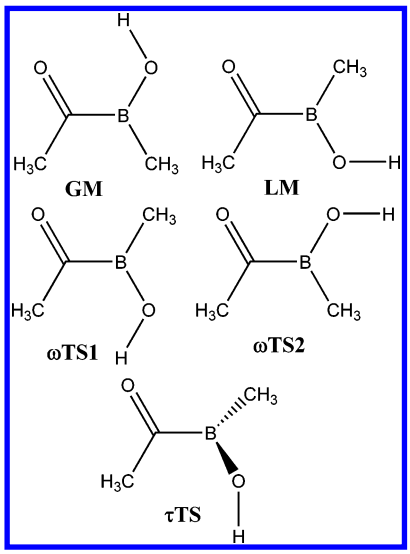


Figure 3. Ground and transition states of BMAOH.

Table 4. Bond Length (Å) and Bond Angles (deg) of BMAOH Optimized at the MP2(full)/6-31+G* Level

parameter	GM	LM	ωTS1	ωTS2	τTS
CC (1,2)	1.503	1.508	1.515	1.513	1.502
CO (2,3)	1.246	1.244	1.241	1.240	1.247
CB (2,4)	1.626	1.608	1.621	1.620	1.593
BC (4,5)	1.564	1.566	1.559	1.575	1.566
BO (4,6)	1.356	1.369	1.369	1.360	1.387
OH (6,7)	0.984	0.974	0.974	0.974	0.968
CCO (1,2,3)	120.8	121.0	120.0	120.2	121.9
CCB (1,2,4)	122.8	120.6	121.1	119.8	126.4
CBC (2,4,5)	124.8	121.1	120.9	122.1	122.0
CBO (2,4,6)	113.6	114.0	119.6	114.1	116.4
CBO (5,4,6)	121.5	124.9	119.5	123.8	121.6
BOH (4,6,7)	108.3	113.3	114.4	113.0	122.6
CCBC (ω) (1,2,4,5)	0.0	−152.7	180.0	0.0	99.1/−99.1
CCBO (1,2,4,6)	180.0	−27.8	0.0	180.0	79.8
CBOH (τ) (2,4,6,7)	0.0	−178.1	0.0	180.0	86.3
OCBC (3,2,4,5)	180.0	−28.9	0.0	180.0	83.3

of the ground and TS of BMAOH are shown in Figure 3, and the relative energies at the HF, B3LYP, and MP2(full) levels of theory with the 6-31+G* basis set are given in Table 2 (The absolute values have been provided in the Supporting Information Table 2A.) The LM and the structure corresponding to the transition state for τ rotation (τTS) exhibit a C_1 symmetry, while the remaining three structures; namely the GM and transition states for ω rotation (ωTS1 and ωTS2) exhibit a C_s symmetry. In the ground-state structures, the ω and τ values in the GM are 0° and 0°, while in the LM they are 150° and 180°, respectively. In ωTS1 and ωTS2, the ω and τ angles have values of 0° and 180° and 180° and 0°, respectively. In the case of τTS, the values of both these torsion angles are 90°.

The geometric parameters of the minima and all transition states of BMAOH at the MP2(full)/6-31+G* level of theory are given in Table 4. In the GM, the −OH group is intramolecularly hydrogen bonded to the CO as is the case with NMAOH; the O−O distance is 2.727 Å, and the H-bond angle (O−H···O) is 117.0°. The hydrogen bond energy, in the case of BMAOH, is roughly estimated as the difference between the LM and GM structures, i.e. ∼2.9 kcal/mol. The changes in the bond lengths from the ground to the TS are relatively small. Some geometric parameters for alkylboranes, arylboranes, and borane complexes have been reported, but there are no experimental data for acylboranes such as BMA[14] and BMAOH. We had earlier reported the geometry of an acylborane BMA, the boron isostere of NMA (**I**), at the QCISD/6-31G* level of theory.[14] The B−O bond length in BMAOH is found to be 1.36 Å (GM) and 1.37 Å (LM) at the MP2(full)/6-31+G* level of theory. The B−O bond length, reported in the literature for a range of organic and inorganic boron containing molecules, is 1.34−1.42 Å (average 1.38 Å) with trigonal planar geometry and 1.39−1.52 Å (average 1.48 Å) for tetrahedral geometry.[29,30] The B−O bond length for BMAOH calculated in this study is in the range of the experimental values. The NBO calculations on the minimum energy structure of BMAOH at the MP2-(full)/6-31+G* level of theory reveal a double bond character for the B−O bond, and the second B−O bond has an

occupancy of 1.99 (~2.0) electrons being contributed from one of the lone pairs of electrons of oxygen. In alkylboranes, the B−C (aliphatic carbon) bond length is about 1.590 Å as in for e.g. dimethylborane,[31] 1.596 Å in dimesityborane,[32] 1.570 Å in ditripylborane,[33] and 1.571 Å in BMA.[14] The B4−C5 bond length in BMAOH is 1.564 Å, which comes near to the experimental value for the aliphatic carbon−boron bond.

Rotation Barrier in NMAOH and BMAOH. The barrier to rotation about the ω angle in the natural peptide is 16.0−25.0 kcal/mol,[34] while that for the boron isostere is about 5.0 kcal/mol.[14] The boron analogues are thus relatively more flexible than the natural peptides. In case of *N*-hydroxy peptides and the corresponding boron isosteres, there are two rotation barriers governed by the ω and τ angle. In the example of NMAOH, the ω rotation barrier is relatively higher (12.6−20.3 kcal/mol) than the τ rotation barrier (6.3−12.2 kcal/mol). In BMAOH, the τ rotation barrier is comparatively higher (15.1 kcal/mol) than the ω rotation barrier (4.8−6.6 kcal/mol). The relative higher τ rotation barrier in boron peptides is a consequence of the B−O double bond character as revealed by NBO calculations.

The rotation barrier in amide systems (like peptides, urea, guanidine, etc.) has been attributed to delocalization of the lone pair of electrons on nitrogen onto the C−N bond as explained by the classical resonance model.[35] This imparts a partial double bond character to the C−N bond. But recent experimental and theoretical studies[36−40] tell a different tale. The electron delocalization in the amide system has been attributed to second-order orbital interactions namely, $n_O \rightarrow \sigma^*_{C-N}$ (delocalization from lone pairs on carbonyl oxygen into the sigma antibonding orbital of the C−N bond i.e. negative hyperconjugation) and $n_N \rightarrow \pi^*_{C-O}$ (delocalization from the lone pair on amide nitrogen to the pi antibonding orbital of the carbonyl group). The energy $E^{(2)}$ associated with negative hyperconjugation i.e. $n_O \rightarrow \sigma^*_{C-N}$ is 29.1 kcal/mol (occupancy of n_O is 1.902 and σ^*_{C-N} is 0.063) and that with $n_N \rightarrow \pi^*_{C-O}$ is 64.1 kcal/mol (occupancy of n_N is 1.772 and π^*_{C-O} is 0.214) for the global minimum of NMAOH at the MP2(full)/6-31+G* level. In case of BMAOH, the energy associated with negative hyperconjugation i.e. $n_O \rightarrow \sigma^*_{C-B}$ is only 11.7 kcal/mol (occupancy of n_O is 1.936 and σ^*_{C-B} is 0.040), indicating that the C−B bond delocalization is insignificant, as a result of which the rotation barrier in boron amides is very small. Thus, in BMAOH, the C−B bond has an essentially single bond character, while the C−N bond in NMAOH has a larger double bond character. The boron peptides are thus far more flexible than the *N*-hydroxy peptides. There is also a $n_O \rightarrow \sigma^*_{O-H}$ interaction i.e. delocalization of the lone pair of electrons on the carbonyl oxygen into the sigma antibonding orbital of the O−H bond which is observed in the global minimum energy structures of both NMAOH and BMAOH but absent in the local minimum structure which affirms the presence of an intramolecular hydrogen bond between CO and OH in the GM of both molecules.

Partial Atomic Charges of NMAOH and BMAOH. The "natural charges" derived from NPA for the global minimum energy structure of NMAOH (**II**) and BMAOH (**III**) are given in Table 5. Replacement of nitrogen by boron decreases the positive charge on the carbonyl oxygen and increases the negative charge on C5 methyl carbon. In BMAOH (**III**), the boron atom has a much greater positive charge than the carbonyl carbon (1.056 vs 0.344). The site for nucleophilic attack in case of NMAOH (**II**) is normally the carbonyl carbon. In BMAOH (**III**), a nucleophile will be drawn toward boron rather than the carbonyl group. This preference for boron as the site for nucleophilic attack is also evident in the 'ESP fitted charges', even though the partial charges differences are of a smaller magnitude. This was the basis of our hypothesis, used to design boron peptides[13] as potential inhibitors of the enzyme serine protease. Figure 4 shows the plausible mechanism by which the boron peptide can act as k_{cat} inhibitor of serine protease. The hydroxyl group of serine in the active site is the nucleophile which attacks the carbonyl carbon of the amide of the substrate peptide, leading to a final hydrolysis of the substrate. When the boron peptide is present in the active site, the hydroxyl group of serine preferentially attacks boron instead of the carbonyl carbon and forms a tetrahedral covalent complex leading to irreversible inhibition of the enzyme. The inhibitors of serine protease could have a potential application in therapeutics.

Table 5. Partial Atomic Charges of NMAOH (**II**) and BMAOH (**III**) Calculated Using NPA and the 'ESP Fit' as per Merz−Singh−Kollman Scheme at the MP2(full)/6-31+G* Level

		natural charges		esp fitted charges	
atom	atom no.	NMAOH (**II**)	BMAOH (**III**)	NMAOH (**II**)	BMAOH (**III**)
C	1	−0.735	−0.737	−0.513	−0.379
C	2	0.818	0.344	0.833	0.484
O	3	−0.753	−0.653	−0.677	−0.580
X	4	−0.279	1.056	−0.263	0.652
C	5	−0.408	−1.059	−0.131	−0.556
O	6	−0.628	−0.972	−0.495	−0.736
H	7	0.542	0.544	0.452	0.426
H	8	0.266	0.249	0.150	0.133
H	9	0.248	0.241	0.164	0.096
H	10	0.246	0.241	0.154	0.096
H	11	0.243	0.254	0.152	0.157
H	12	0.221	0.245	0.065	0.102
H	13	0.217	0.245	0.109	0.102

Conformations of Ala-NOH (IV). The preferred ω and τ angles in NMAOH (**II**) were fixed for Ala-NOH (**IV**), and the (ϕ, ψ) space of Ala-NOH was scrutinized (Table 6). With an *omega* value of 30° and a *tau* value of 10°, the global minimum corresponds to a structure with $\phi = -85°$ and $\psi = -30°$ (Figure 5a). These values are close to the values for a residue at the $i+1$ position in a Type I β-turn ($\phi = -60°$, $\psi = -30°$). The local minimum within 5.0 kcal/mol of the GM has $\phi = 60°$ and $\psi = 50°$ (Figure 5b). These are values of a left-handed alpha helix ($\phi = 57°$, $\psi = 47°$). Both minima display a regular secondary structure motif, which falls in the "allowed regions" of the Ramachandran map. The GM and LM structures are distinguished by two intramolecular hydrogen bonds (Figure 4, parts a and b, respectively)), one between the carbonyl oxygen and the

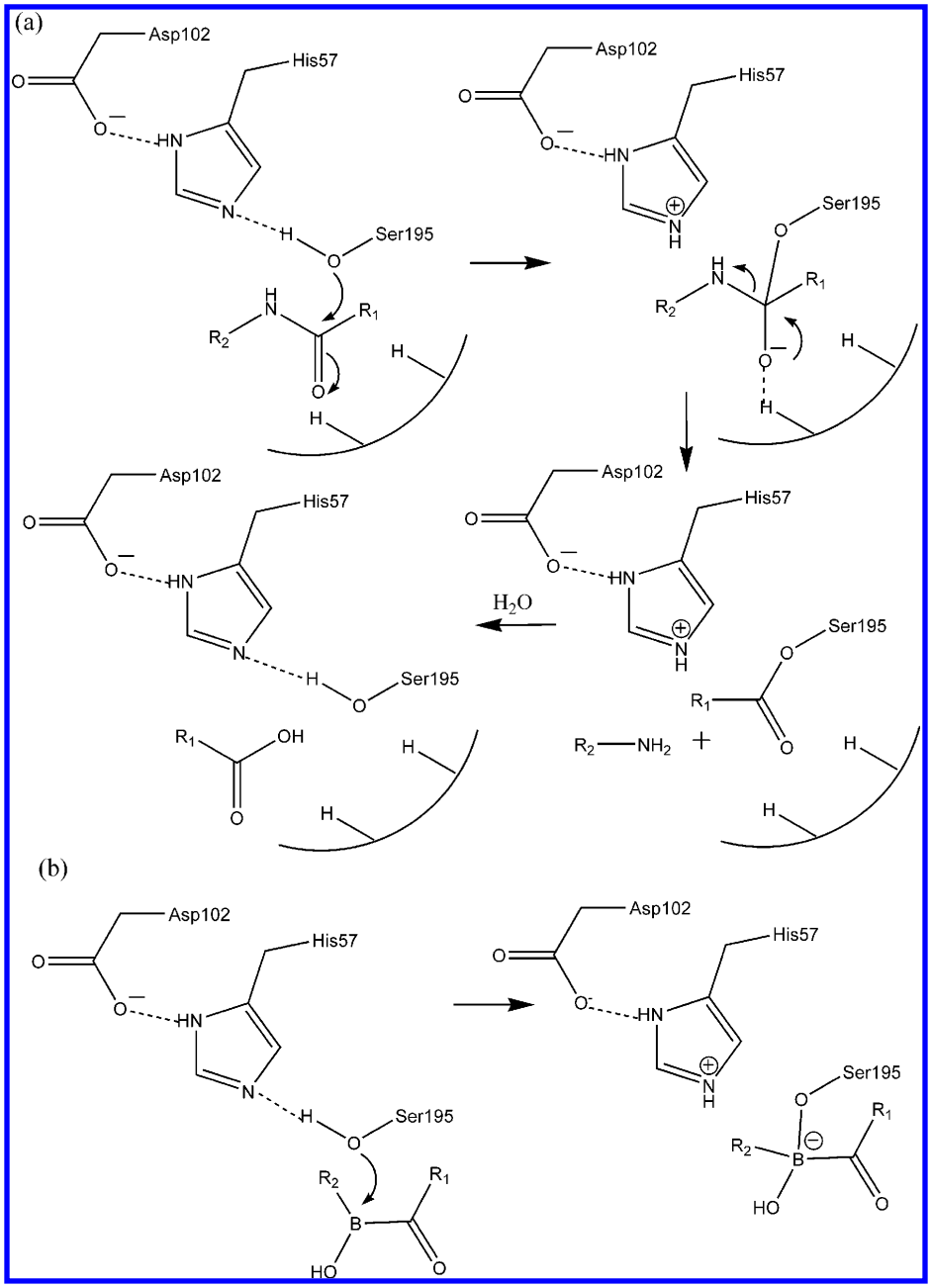


Figure 4. (a) Mechanism of normal substrate hydrolysis by serine protease. (b) Tetrahedral complex of boron peptide with the active site serine.

N-hydroxyl OH, forming a five-membered ring, and the second is found between the *N*-hydroxyl oxygen and the amide NH, figuring a six-membered ring. With an ω angle of 200° and a τ angle of 120°, there is only one favored structure for Ala-NOH (**IV**) with $\phi = -90°$ and $\psi = 140°$. This structure is characterized by only one intramolecular hydrogen bond (Figure 5c) between the *N*-hydoxyl OH and the carbonyl oxygen outlining a six-membered ring. Thus, all the preferred conformations of Ala-NOH are characterized by the presence of one or two intramolecular hydrogen bonds and are conformationally rigid.

Conformations of Ala-BOH (V). In a similar manner, the ϕ, ψ preferences of Ala-BOH (**V**) were investigated, and the results are shown in Table 6. With an *omega* value of 0°, there are two conformations observed within 5.0 kcal/mol of the global minimum energy conformer. The global minimum corresponds to a structure with $\phi = 50°$ and $\psi = -150°$ (Figure 6a), while the local minimum relates to a structure with $\phi = -60°$ and $\psi = 150°$ (Figure 6b). The global minimum shows a strong preference for a positive ϕ value, and ψ in both structures adopts an extended state. The two structures exhibit an intramolecular hydrogen bond (Figure 6a,b) like the one seen in the GM of BMAOH i.e. the preceding carbonyl oxygen and the hydroxyl group on boron are locked, forming a five-membered ring. With an ω angle of 150° and a τ value of 180°, the only structure energetically favored is with $\phi = -160°$ and $\psi = 140°$. The structure is characterized by an intramolecular hydrogen bond (Figure 6c) between the hydroxyl group and the succeeding carbonyl oxygen forming a six-membered ring. Thus, all favored conformations of Ala-BOH exhibit at least one intramolecular hydrogen bond.

Table 6. Conformations and Energies of Ala-NOH (**IV**) and Ala-BOH (**V**)[a]

ω	τ	ϕ	ψ	rel. E (kcal/mol)
		Ala-NOH		
30°	30°	−85°	−30°	0.0
		60°	50°	3.83
200°	120°	−90°	140°	0.0
		Ala-BOH		
0°	0°	50°	−150°	0.0
		−60°	150°	0.13
150°	180°	−160°	140°	0.0

[a] The ω, ϕ, and ψ space of *N*-hydroxy-*N*-methylacetamide and *N*-acetyl-*N*′-hydroxy-*N*′-methylamide of alanine and their boron isosteres.

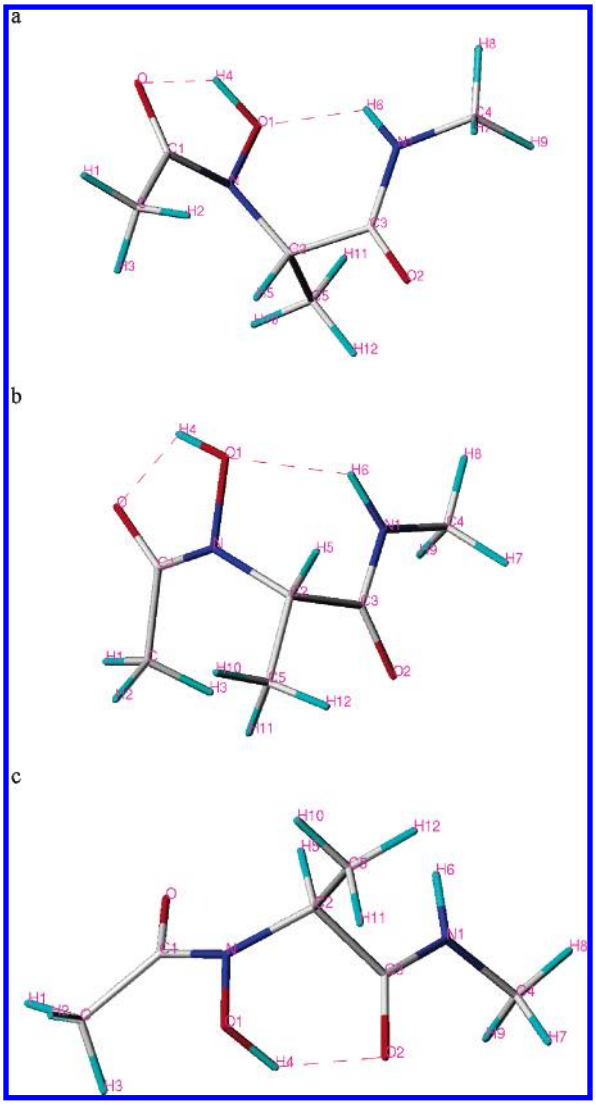


Figure 5. Preferred conformations of Ala-NOH: (a) $\omega = 30°$, $\Phi = -85°$, $\psi = -30°$, (b) $\omega = 30°$, $\Phi = 60°$, $\psi = 50°$, and (c) $\omega = 200°$, $\Phi = -90°$, $\psi = 140°$.

Conclusions

In previous papers[13,14] we had designed a boron isostere of an amino acid by replacement of the amide nitrogen with boron, with the intention of developing an inhibitor of the enzyme serine protease. The synthetic feasibility of such a molecule is a big challenge. As a result, we have modified

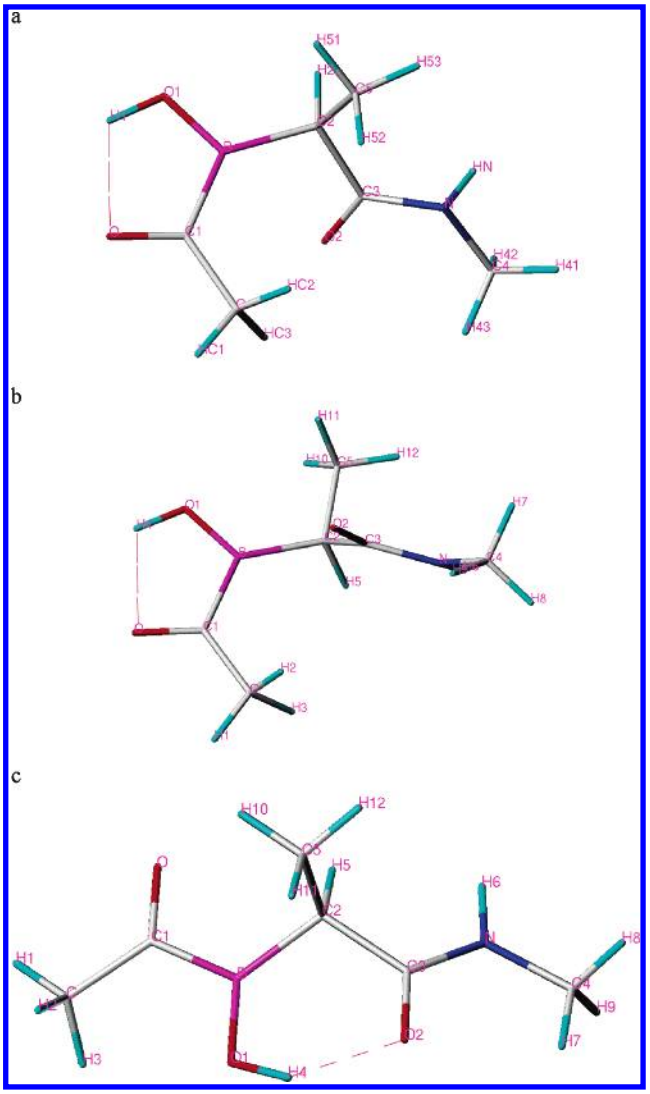


Figure 6. Preferred conformations of Ala-BOH: (a) $\omega = 0°$, $\Phi = 50°$, $\psi = -150°$, (b) $\omega = 0°$, $\Phi = -60°$, $\psi = 150°$, and (c) $\omega = 150°$, $\Phi = -160°$, $\psi = 140°$.

the molecule by replacing B−H with B−OH, which should make it relatively easy to synthesize; and this also has an analogy with *N*-hydroxy amides which are well-known. The conformational space of *N*-hydroxy peptides and their boron isosteres has been the focus of investigation in this paper. The minimum in the *omega* torsion space of such molecules has been identified using *N*-hydroxy-*N*-methylacetamide (NMAOH) and acetylmethylhydroxyborane (BMAOH) as model peptides. The ground and various transition states have been calculated at the HF, B3LYP, and MP2(full) levels of theory with the 6-31+G* basis set. The *omega* rotation barrier is 12.6−20.3 kcal/mol for the *N*-hydroxy peptide (NMAOH) and 4.8−6.6 kcal/mol for its corresponding boron isostere, BMAOH. The difference in the rotation barriers has been attributed to second-order orbital interactions, mainly negative hyperconjugation. The global minimum energy conformation of both molecules exhibits an intramolecular hydrogen bond between the carbonyl oxygen and the hydroxyl group which confers some rigidity to the conformation. The barrier for rotation about the torsion angle τ i.e. rotation about N−O and B−O bonds is 6.3−12.2 kcal/mol

for the *N*-hydroxy peptide and is 15.1 kcal/mol for the boron isostere. The elevated value for the boron isostere has been attributed to the single bond character of the N−O bond against the double bond character of the B−O bond. The replacement of nitrogen by boron also significantly changes the charge distribution in these molecules. A relatively greater positive charge on the boron atom over the carbonyl carbon makes boron the preferential site of attack by a nucleophile in boron peptides, which otherwise occurs on the carbonyl carbon in the natural peptides. This observation can be potentially exploited for the design of serine protease inhibitors. It would be interesting to study the transition state barrier for hydrolysis at the carbonyl carbon versus the boron, which is the next step in the study. The minimum energy structures of NMAOH and BMAOH were then used to study the ϕ and ψ preferences in *N*-acetyl-*N*′-hydroxy-*N*′-methylamide of alanine (Ala-NOH) and its boron isostere (Ala-BOH). Ala-NOH demonstrates conformations with Type-I beta turn, left-handed α-helix, positive ϕ values and extended ψ states. Ala-BOH, on the other hand, favors conformations with positive ϕ and extended ψ values. In previous work on natural peptides and their boron isosteres, we had noticed a much lower barrier to rotation about the ω angle and a unique preference for positive *phi* values in the boron analogues. The boron isosteres of *N*-hydroxy peptide also show a similar tendency. In conclusion, the replacement of nitrogen by boron in natural and N-hydroxy peptides causes a significant change in the conformational space and electronic properties, and these features can be profitably exploited to design peptides with specific geometries and chemical attributes.

Acknowledgment. This work was supported by the Department of Science and Technology, New Delhi through their FIST program (SR/FST/LS1-163/2003). A.K.M. thanks the University Grants Commission, New Delhi and the Council of Scientific and Industrial Research, New Delhi, and S.A.K. thanks the Lady Tata Memorial Trust, Mumbai for financial support.

Supporting Information Available: Absolute energy values (au) of conformations of NMAOH (**II**) and BMAOH (**III**) (Tables 1A and 2A, respectively) and XYZ coordinates of the global minimum of structures **II** and **III** optimized at the MP2(full)/6-31+G* level of theory and of structures **IV** and **V** optimized at the B3LYP/6-31+G* level of theory (Tables 7−10). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ramachandran, G. N.; Sasisekharan, V. Conformation of Polypeptides and Proteins. *Adv. Protein Chem.* **1968**, *28*, 283−437.

(2) Hagler, T. A.; Leiserowitz, L.; Tuval, M. Experimental and Theoretical Studies of the Barrier to Rotation about N−Cα and Cα-C′ Bonds (ϕ and ψ) in Amides and Peptides. *J. Am. Chem. Soc.* **1976**, *98*, 4600−4612.

(3) Hruby, V. J. Designing Peptide Receptor Agonists and Antagonists. *Nat. Rev. Drug Discovery* **2002**, *1*, 847−858.

(4) Vogen, S. M.; Paczkowski, N. J.; Kirnarsky, L.; Short, A.; Whitmore, J. B.; Sherman, S. A.; Taylor, S. M.; Sanderson, S. D. Differential Activities of Decapeptide Agonists of Human C5a: The Conformational Effects of Backbone *N*-Methylation. *Int. Immunopharmacol.* **2001**, *12*, 2151−62.

(5) Ye, Y.; Liu, M.; Kao, J. L.; Marshall, G. R. Peptide-bond Modification for Metal Coordination: Peptides Containing Two Hydroxamate Groups. *Biopolymers* **2003**, *71*, 489−515.

(6) Fischer, P. M. The design, synthesis and application of stereochemical and directional peptide isomers: a critical review. *Curr. Protein Pept. Sci.* **2003**, *4*, 339−356.

(7) Baldauf, C.; Günther, R.; Hofmann, H. J. Conformational Properties of Sulphonamido Peptides. *J. Mol. Struct. (THEOCHEM)* **2004**, *675*, 19−28.

(8) Kettner, C. A.; Shenvi, A. B. Inhibition of Serine Protease Leukocyte Elastase, Pancreatic Elastase, Cathepsin G, and Chymotrypsin by Peptide Boronic Acids. *J. Biol. Chem.* **1984**, *259*, 15106−15114.

(9) Kettner, C. A.; Bone, R.; Agard, D. A.; Bachovchin, W. W. Kinetic Properties of the Binding of α-Lytic Protease to Peptide Boronic Acids. *Biochemistry* **1988**, *27*, 7682−7688.

(10) Spielvogel, B. F.; Wojnowich, L.; Das, M. K.; McPhail, A. T.; Hargrave, K. D. Boron Analogues of Amino Acids. Synthesis and Biological Activity of Boron Analogues of Betaine. *J. Am. Chem. Soc.* **1976**, *98*, 5702−5703.

(11) Spielvogel, B. F.; Das, M. K.; McPhail, A. T.; Onam, K. D.; Hall, I. H. Boron Analogues of the alpha-Amino Acids. Synthesis, X-Ray Crystal Structure, and Biological Activity of Ammonia-Carboxyborane, the Boron Analogue of Glycine. *J. Am. Chem. Soc.* **1980**, *102*, 6343−6344.

(12) Miller, M. C.; Sood, A.; Spielvogel, B. F.; Hall, I. H. Synthesis and Antitumor Activity of Boronated Dipeptides containing Aromatic Amino Acids. *Anticancer Res.* **1997**, *5A*, 3299−3306.

(13) Datar, P. A.; Coutinho, E. C. The ϕ, ψ Space of Boron Isosteres of Amino Acids: An *Ab Initio* Study. *J. Theor. Comput. Chem.* **2004**, *3*, 189−202.

(14) Malde, A. K.; Khedkar, S. A.; Coutinho, E. C.; Saran A. Geometry, Transition States, and Vibrational Spectra of Boron Isostere of *N*-methylacetamide by *Ab Initio* Calculations. *Int. J. Quantum Chem.* **2005**, *102*, 734−742.

(15) Hehre, W. J.; Random, L.; Schleyer, P. V. R.; Pople, J. A. In *Ab Initio Molecular Orbital Theory;* Wiley: New York, 1985.

(16) Parr, R. G.; Yang, W. In *Density Functional Theory of Atoms and Molecules;* O.U.P.: New York, 1989.

(17) *Gaussian 03, Revision C.01*; Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.;

Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.;. Pople, J. A. Gaussian, Inc.: Wallingford, CT, 2004.

(18) Roothan, C. C. New Developments in Molecular Orbital Theory. *Rev. Mod. Phys.* **1951**, *23*, 69−89.

(19) Becke, A. D. Density functional thermochemistry. III. The role of exact exchange. *J. Chem. Phys.* **1993**, *98*, 5648−5652.

(20) Lee, C.; Yang, W.; Parr, R. G. Development of the Colle-Salvetti Correlation-Energy Formula into a Functional of the Electron Density. *Phys. Rev.* **1988**, *37B*, 785−789.

(21) Perdew, J. P.; Wang, Y. Accurate and simple analytic representation of the electron-gas correlation energy. *Phys. Rev.* **1992**, *45B*, 13244−13249.

(22) Møller, C.; Plesset, M. S. Note on an Approximation Treatment for Many-Electron Systems. *Phys. Rev.* **1934**, *46*, 618−622.

(23) Martin-Head, G.; Pople, J. A.; Frisch, M. J. MP2 Energy Evaluation by Direct Methods. *Chem. Phys. Lett.* **1988**, *153*, 503−506.

(24) Glendening, E. D.; Reed, A. E.; Carpenter, J. E.; Weinhold, F. NBO Version 3.1.

(25) Reed, A. E.; Weinstock, R. B.; Weinhold, F. Natural Population Analysis. *J. Chem. Phys.* **1985**, *83*, 735−746.

(26) Reed, A. E.; Weinhold, F.; Curtiss, L. A. Intermolecular interactions from a natural bond orbital, donor−acceptor viewpoint. *Chem. Rev.* **1988**, *88*, 899−926.

(27) Singh, U. C.; Kollman, P. A. An Approach to Computing Electrostatic Charges for Molecules. *J. Comput. Chem.* **1984**, *5*, 129−145.

(28) Aubry, A.; Dupont, V.; Marraud, M. ^{t}BuCO-Ψ[CO−N(OH)]-Gly-NH^{i}Pr. *Acta Crystallogr., Sect. C*: *Cryst. Struct. Commun*. **1995**, *51*, 1577−1579.

(29) Filatov, S.; Shepelev, Y.; Bubnova, R.; Sennova, N.; Egorysheva, A. V.; Kargin, Y. F. The study of $Bi_3B_5O_{12}$: synthesis, crystal structure and thermal expansion of oxoborate $Bi_3B_5O_{12}$. *J. Solid State Chem.* **2004**, *177*, 515−522.

(30) Shishkov, I. F.; Khristenko, L. V.; Rudakov, F. M.; Vilkov, L. V.; Karlov, S. S.; Zaitseva, G. S.; Samdal, S. The molecular structure of boratrane determined by gas electron diffraction and quantum mechanical calculations. *J. Mol. Struct.* **2002**, *641*, 199−205.

(31) Vijay, A.; Sathyanarayana, D. N. Effects of Basis Set and Electron Correlation on the Structure and Vibrational Spectra of Diborane. *J. Mol. Struct.* **1995**, *351*, 215−229.

(32) Entwistle, C. D.; Marder, T. B.; Smith, P. S.; Howard, A. K.; Fox, M. A.; Mason, S. A. Dimesitylborane monomer−dimer equilibrium in solution, and the solid-state structure of the dimer by single-crystal neutron and X-ray diffraction. *J. Organomet. Chem.* **2003**, *680*, 165−172.

(33) Bartlett, R. A.; Rasikadis, H. V.; Olmstead, M. M.; Power, P. P.; Weese, K. J. Synthesis of the Monomeric $HBtrip_2$ (trip − 2,4,6-iso-$Pr_3C_6H_2$) and the X-ray Crystal Structures of $[HBMes_2]_2$ (Mes = 2, 4, 6,-$Me_3C_6H_2$) and $HBtrip_2$. *Organometallics* **1990**, *9*, 146−150.

(34) Villani, V.; Alagona, G.; Ghio, C. Ab Initio Studies on *N*-Methylacetamide. *Mol. Eng.* **1999**, *8*, 135−153.

(35) Pauling, L. *In The Nature of Chemical Bond*; Cornell University Press: Ithaca, 1960.

(36) Bharatam, P. V.; Iqbal, P.; Malde, A.; Tiwari, R. Electron Delocalization in Aminoguanidines: A Computational Study. *J. Phys. Chem.* **2004**, *108*, 10509−10517.

(37) Bharatam, P. V.; Moudgil, R.; Kaur, D. Electron Delocalization in Isocyanates, Formamides, and Ureas: Importance of Orbital Interactions. *J. Phys. Chem.* **2003**, *107*, 1627−1634.

(38) Glendening, E. D.; Hrabal, J. A., II. Resonance in Formamide and Its Chalcogen Replacement Analogues: A Natural Population Analysis/Natural Resonance Theory Viewpoint. *J. Am. Chem. Soc.* **1997**, *119*, 12940−12946.

(39) Lauvergnat, D.; Hiberty, P. C. Role of Conjugation in the Stabilities and Rotational Barriers of Formamide and Thioformamide. An ab initio Valence-Bond Study. *J. Am. Chem. Soc.* **1997**, *119*, 9478−9482.

(40) Wiberg, K. B.; Rush, D. J. Solvent Effects on the Thioamide Rotational Barrier: An Experimental and Theoretical Study. *J. Am. Chem. Soc.* **2001**, *123*, 2038−2046.

CT050242V