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The Distal Effect of Electron-Withdrawing Groups and Hydrogen Bonding on the Stability of Peptide Enolates

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Abstract: Relative gas-phase carbon acidities have been computed for a series of acetamides, diketopiperazines, and linear dipeptides. The results show that *N*-electron-withdrawing substituents, protonation, and hydrogen bonding at amide nitrogen in these systems increase the acidity of both a C−H proton adjacent to the amide carbonyl and that of one proximal to the amide nitrogen. There is a good correlation between the magnitudes of the increases at the two positions, but the extent of the increase for the distal C−H adjacent to the carbonyl is greater than that for the proximal C−H, in most cases by a factor of about two. The effects on the stability of the distal enolate are shown to result from predominantly inductive affects. The size of these effects is such that protonation and hydrogen bonding at nitrogen increase the acidity of the distal C−H to almost the same extent as seen for the analogous interactions at the carbonyl oxygen. The effect is also seen in solution, where the computed aqueous pK_a values are greater for the C−H adjacent to the amide carbonyl, by up to 13 units, and where preliminary experimental studies have shown that *N*-acetylation of an amide increases the rate of hydrogen−deuterium exchange via formation of the corresponding distal enolate by more than 3 orders of magnitude above the rates of exchange via the proximal enolate, of the nonacetylated amide and of diisopropylketone. The results also indicate that hydrogen bonding to amide nitrogen could be as important as bonding to oxygen in enzyme-catalyzed cleavage of α-C−H bonds.

Introduction

The stereochemical integrity of α-amino acids and peptides and their derivatives is directly related to the acidity of the α-CH protons, while formation of the corresponding enolates is often a key step in the synthesis[1,2] and biochemical transformations[3−11] of compounds of these types. In this context there have been few reports on quantitative studies of the α-carbon acidities of amino acids and peptides. Amyes and Richard and co-workers developed convenient ^{1}H NMR spectroscopic methods to provide reliable estimates of carbon acid pK_a's in water[12,13] and applied these to investigate the formation and stability of amino acid[14−18] and peptide[19] enolates. More recently, we examined the performance of several computational procedures in calculating general gas-phase and aqueous acidities. A proton exchange procedure that combines high level *ab initio* gas-phase energies with solvation energies from a continuum solvent model was found to perform particularly well in predicting the pK_a's of a range of α-carbonyl carbon acids, including ketones, esters, acetamides and small peptides.[20,21] We have now exploited this approach to examine the effects of amide nitrogen substituents on the stability of peptide enolates in both the gas and aqueous phases, with a view to better understanding factors that affect the formation of these species and how those might be used to manipulate chemical synthesis. We have also studied the effects of hydrogen bonding at the amide nitrogen and the oxygen on the stability of the enolates, as well as the more extreme case

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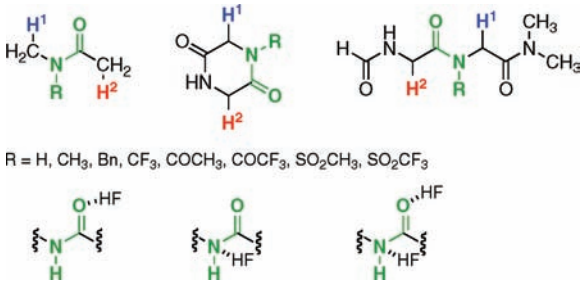


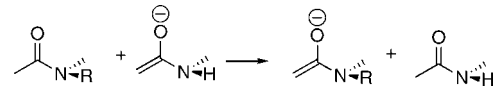
Figure 1. Carbon acids (top) and the three hydrogen-bonded derivatives (bottom) of the parent systems (R = H) investigated in this study.

of protonation, because of the possible relevance to enzyme-catalyzed processes such as epimerization of amino acid residues in peptides.

Normally amides and other carboxylic acid derivatives exhibit much weaker carbon acidities than ketones,[13,22,23] mainly as a result of the effects of the various carbonyl carbon substituents to attenuate resonance stabilization of the corresponding enolates. However, underlying this resonance effect is an opposing inductive electron-withdrawing effect of the carbonyl carbon substituents to more effectively stabilize the enolates. Several recent studies have examined the contributions of resonance and inductive effects to the gas-phase acidities of acetamides, methyl acetate, and acetyl fluoride, compared to that of acetone.[24,25] They estimate that inductive effects provide a 3−5 and 5−8 kcal mol^{-1} relative stabilization to the enolates of acetamides and esters, respectively. More notably, acetyl fluoride is more acidic than acetone, indicating that in this case the inductive effect dominates and that there is a direct correlation between the magnitude of the inductive effect and the electronegativity of the carbonyl carbon substituent. In a related vein, a resonance effect has been traditionally invoked for rationalizing the increased carbon acidity of carboxylic acids over alcohols, but there is growing theoretical evidence that inductive effects are in fact predominantly responsible for this enhancement.[26−29] In the present work we have therefore delineated the resonance and inductive effects.

Accordingly, the gas-phase carbon acidities ($-\Delta G_{gas}$) have been calculated for the protons designated H^1 and H^2 in the series of *N*-substituted and hydrogen-bonded acetamides, diketopiperazines, and dipeptides illustrated in Figure 1, as well as of protonated analogues of the hydrogen-bonded species. The substituents were selected from those commonly exploited for *N*-protection in synthetic applications.[30,31] Aqueous pK_a values were also computed for the series of acetamides and diketopiperazines. Resonance effects were investigated by measuring *inter alia* the barriers to rotation about the N−C(O) bond of the acetamides and the C−C(O) bond of the corresponding enolates,

Scheme 1



while the magnitude of the inductive contribution to the acidity of the *N*-substituted acetamides has been estimated using the approach of Rablen and Bentrup,[24] by calculating the free energy of the reaction illustrated in Scheme 1, at the torsional transition state where amide conjugation is eliminated or strongly attenuated.

Through these studies, we have observed a notable distal effect, whereby *N*-electron-withdrawing substituents and protonation and hydrogen bonding to amide nitrogen result in a substantially larger increase in the gas-phase acidity ($-\Delta G_{gas}$) of an α-CH proton adjacent to the amide carbonyl (Figure 1, protons designated C−H^2), compared to that of one proximal to the amide nitrogen (Figure 1, protons designated C−H^1), by 5−18 kcal mol^{-1}. This corresponds with computed aqueous pK_a values that are lower by 3−13 units and the effect on the stability of the distal enolates is shown to result predominantly from inductive contributions. The $-\Delta G_{gas}$ and pK_a calculations have been confirmed through preliminary, experimental kinetic studies in the condensed phase, where we have observed, for example, that *N*-acetylation of glycylsarcosine anhydride (**1**, also shown in Figure 1 as the diketopiperazine with R = CH_3) increases the rate of hydrogen−deuterium exchange *via* formation of the corresponding distal enolate, by more than 3 orders of magnitude above the rates of exchange *via* the proximal enolate, of the nonacetylated diketopiperazine **1** and of the methine hydrogens of diisopropylketone.

Theoretical and Experimental Procedures

Theoretical. Standard *ab initio* molecular orbital theory and density functional theory calculations were performed with Gaussian 03.[32] For the linear dipeptides, the conformational search was carried out using the energy-directed tree search algorithm.[33] Optimized structures and harmonic frequencies were determined at the BMK/6-31+G(d) level, and scale factors[34] for BMK/6-31+G(d,p) vibrational frequencies have been used with G3MP2(+) electronic energies on the same geometries for the free energy calculations. The G3MP2(+) composite procedure is a modified version of G3MP2[35] in which calculations with 6-31G(d) have been replaced with those derived with the 6-31+G(d) basis set, so as to allow for an improved description of anionic species. This abbreviated G3MP2(+)//BMK procedure delivers reliable gas-phase acid dissociation free energies.[20,21]

Solvent effects were examined through the computation of aqueous pK_a values using a proton exchange scheme (Scheme 2) that combines G3MP2(+)//BMK gas-phase free energies (ΔG^*_{gas}) with B3LYP/6-31+G(d) solvation free energies (ΔG^*_{solv}) obtained through the CPCM model[36,37] utilizing UAKS cavities.

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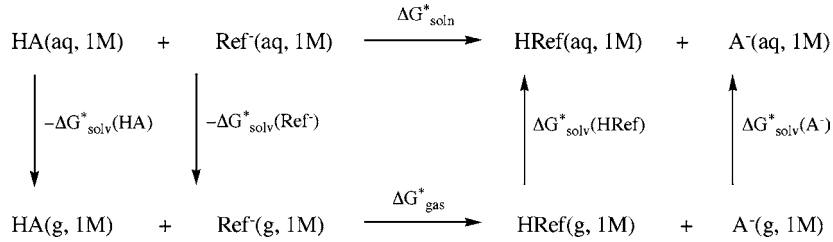
Scheme 2



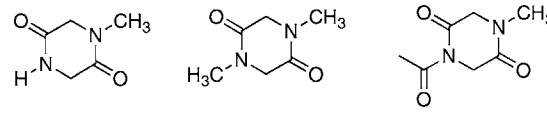
All geometries of the studied species have been optimized in the presence of solvent unless otherwise noted. This approach provides estimates of aqueous pK_a values, with a reliability of 1−2 pK_a units in most cases.[20,21]

The pK_a of the acid (HA) is obtained via eq 1 where HRef refers to the reference acid and the superscript * denotes that the quantities are calculated at the standard state of 1 mol dm^{-3}. The success of this approach depends heavily on the choice of reference acid, with best results expected if HRef is structurally similar to HA, since the errors incurred by the continuum solvent model are then likely to be very similar and significant error cancellation is expected. For these reasons, NH_2COCH_3 (experimental methyl pK_a = 28.4[22]) and $MeCONHCH_2CONH_2$ (experimental methylene pK_a = 23.9[19]) were used as reference carbon acids for the pK_a calculations for the acetamides and diketopiperazines, while $H_3N^+CH_2CO_2Me$ (experimental methylene pK_a = 21[15]) was used for the protonated analogues.

$$pK_a(HA) = \frac{\Delta G^*_{soln}}{RT\ln(10)} + pK_a(HRef) \quad (1)$$

$$\Delta G^*_{soln} = \Delta G^*_{gas} + \Delta G^*_{solv}(HRef) + \Delta G^*_{solv}(A^-) - \Delta G^*_{solv}(Ref^-) - \Delta G^*_{solv}(HA)$$

Experimental Section. Glycylsarcosine anhydride (**1**) and *N*-acetylglycylsarcosine anhydride (**3**) were prepared using literature procedures[38,39] while sarcosine anhydride (**2**) and diisopropylketone were obtained from commercial sources. Experimental condensed phase acidity was investigated by incubating a sample of *N*-acetylglycylsarcosine anhydride (**3**) (0.088 M) in d_6-acetone containing diisopropylketone (0.088 M) and triethylamine (0.11 M), at 308 K for 56 days, during which time the mixture was periodically examined using ^{1}H NMR spectroscopy. Singlet resonances at δ 2.47 and 4.22 ppm, corresponding to the acetyl and sarcosyl methylene groups, respectively, decreased in intensity with time, being initially replaced by triplet resonances at δ 2.46 (J = 3 Hz) and 4.21 (J = 3 Hz) ppm for the corresponding monodeuterated analogues, before further deuteration. Integration of the spectra was used to monitor the changes as a function of time, from which pseudo-first-order rate constants of 2.4×10^{-7} and 1.1×10^{-5} s^{-1} were calculated for hydrogen−deuterium exchange of the methyl and methylene group protons. There was no evidence of exchange of any of the other protons of the diketopiperazine **3** or of the diisopropylketone hydrogens and, under analogous conditions, the diketopiperazines **1** and **2** were also inert. The limit for detection of hydrogen−deuterium exchange was estimated to be <5%, corresponding to a pseudo-first-order rate constant of $<5.2 \times 10^{-9}$ s^{-1}.



1 **2** **3**

Results and Discussion

Gas-Phase Acidities. The calculated gas-phase carbon acidities ($-\Delta G_{gas}$) are summarized in Tables S1, S2, and S4 in the Supporting Information, and the relative values are illustrated in Figure 2. Diketopiperazines were studied because they are often used in synthesis,[38,40,41] and the inherent symmetry of the parent system (R = H) allows for direct comparison of changes to the acidity of the C−H^1 and C−H^2 protons. With these species, *N*-electron-withdrawing substituents, and protonation and hydrogen bonding at nitrogen, increase the gas-phase acidity at both positions, and there is a good correlation between the magnitudes of the increases at the two positions. That is, a larger increase in the acidity of C−H^1 generally corresponds with a larger effect on C−H^2. Figure 2 shows that the magnitude of the increase on the more remote C−H^2 is substantially greater than that on the adjacent C−H^1, in most cases by a factor of ~2. This is discussed in more detail below. The magnitude of this distal effect is such that protonation and hydrogen bonding at nitrogen increase the acidity of C−H^2 to almost the same extent as seen for the analogous interactions at oxygen. The trends observed with the acetamides are the same as those seen with the diketopiperazines, apart from minor perturbations that are mostly due to conformational changes.

The parent linear dipeptide shown in Figure 1 (R = H) was used to model the interior of a peptide. Initially, *N*-formylglycylglycinamide was investigated but in that case intramolecular hydrogen bonding was found to have a significant influence on the stability of the *N*-terminal glycyl enolate (Figure 3, Table S3). Effects of this type potentially mask substituent effects, so attempts were made to locate an extended conformation of the enolate lacking the hydrogen bonding, but these were not successful. In principle, larger peptides could have been used but the high computational cost prohibited such an approach. Instead, the di-*N*-methylated glycylglycinamide was used, where the methyl groups prevent the intramolecular stabilization (Figure 3, Table S4). In this parent system the difference between the acidity at the two α-carbons is 2 kcal mol^{-1}. Due to side reactions occurring on deprotonation, it was not feasible

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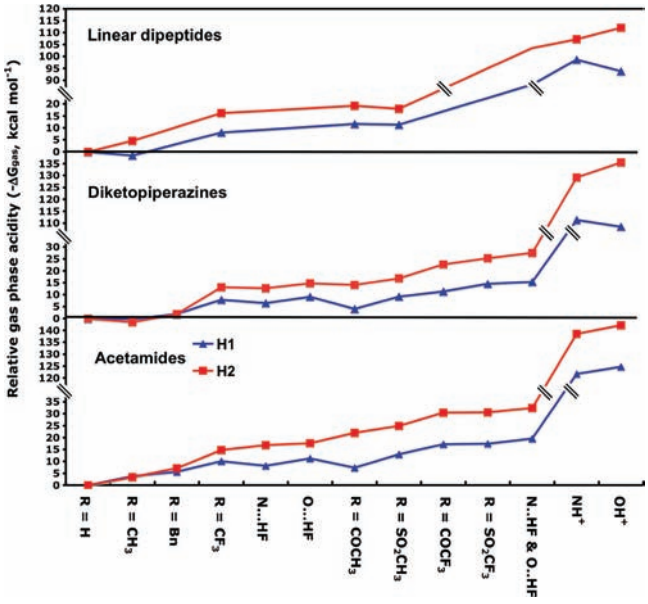

Figure 2. Relative G3MP2(+)//BMK gas-phase carbon acidities (negative Gibbs free energies) of *N*-substituted, hydrogen-bonded, and protonated acetamides, diketopiperazines, and linear dipeptides.

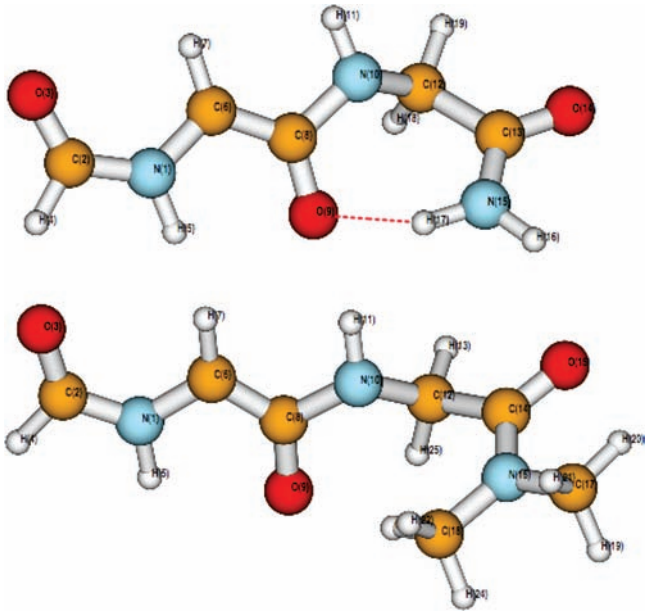

Figure 3. Equilibrium geometries of the *N*-terminal glycyl enolates of *N*-formylglycylglycinamide (top) and the di-*N*-methylated analogue used as the parent system in this work (bottom).

to calculate the acidity of the *N*-trifluoromethanesulfonyl, *N*-trifluoroacetyl, and hydrogen-bonded derivatives of the dimethylated glycylglycinamide. Otherwise, the trends in gas-phase acidity in the linear dipeptides parallel those observed with the diketopiperazines and acetamides.

Inductive and Resonance Contributions. The electron-withdrawing *N*-substituents and protonation and hydrogen bonding at amide nitrogen are likely to affect the acidity of the C−H[1] protons by decreasing the electron density on the amide nitrogen, thereby increasing the extent of inductive stabilization of the corresponding carbanions. Presumably their main effect on the acidity of the C−H[2] protons also results from their decreasing the electron density on nitrogen but with the consequence that, in this case, the stability of the corresponding

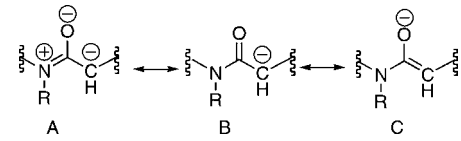

Figure 4. *N*-Electron-withdrawing substituents and protonation and hydrogen bonding at amide nitrogen stabilize the carbanion **B** through resonance by reducing the importance of contributor **A**, thereby increasing the role of contributor **C**.

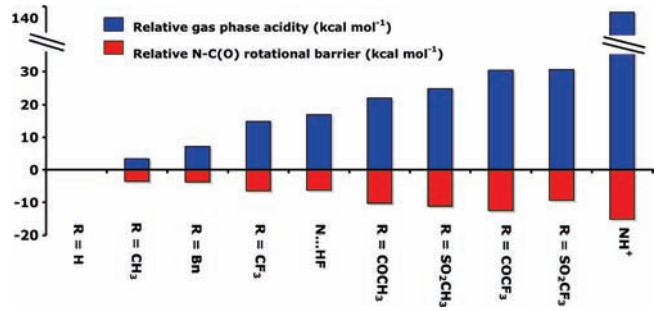

Figure 5. Inverse correlation between relative gas-phase acidity and amide N−C(O) rotational barrier (kcal mol^{-1}) in *N*-substituted, hydrogen-bonded, and protonated *N*-methylacetamides at 298 K.

enolates is increased through a combination of inductive and resonance effects. With respect to the latter and as referred to above, the α-carbonyl carbon acidity of an amide is affected by the ability of the nitrogen to conjugate with the carbonyl group (Figure 4, resonance contributor A) and therefore reduce the extent of resonance stabilization of the enolate (Figure 4, resonance contributor C). The substituents and hydrogen bonding and protonation decrease the conjugating ability of the nitrogen, thereby reducing the importance of contributor A and increasing that of contributor C.

Consistent with this explanation, the acetamides generally show an inverse correlation of their relative calculated gas-phase acidities with the C(α)−C(O) bond lengths in the corresponding enolates, and a direct correlation with the N−C(O) bond lengths in the amides and enolates (Table S7). Further, when the rotational barriers were calculated to measure the energy required to disrupt the resonance contributions, the results showed an inverse correlation between the calculated relative gas-phase acidities and the acetamide N−C(O) rotational barriers (Figure 5), and a direct correlation with the corresponding enolate C−C(O) rotational barriers (Table S8). With the *N*-protonated acetamide, in which case it is logical to assume that resonance is completely attenuated, the calculated N−C(O) rotational barrier is 15 kcal mol^{-1} lower than that of *N*-methylacetamide. This correlates well with the experimentally determined rotational barrier of *N*,*N*-dimethylacetamide which is about 16 kcal mol^{-1}.[42,43] The 3−4 kcal mol^{-1} lowering of the N−C(O) barrier in the methyl- and benzyl-substituted acetamides is presumably a consequence of the effect of the steric bulk of these groups on the extent of amide conjugation.

It follows that, while resonance is important, at most it contributes about 15 kcal mol^{-1} to the effects of the *N*-substituents, and hydrogen bonding and protonation, on the increases in gas-phase acidity of the distal C−H[2] protons shown

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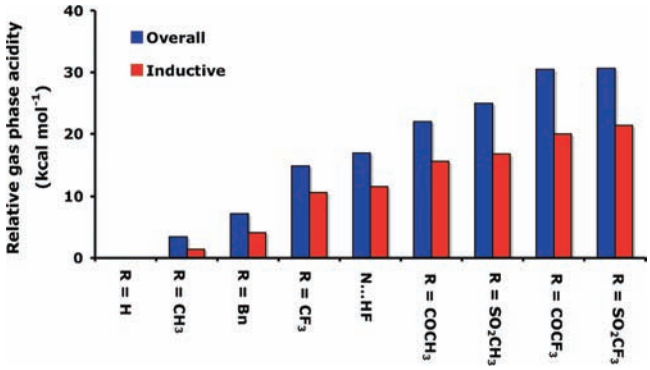

Figure 6. Comparison of the inductive contributions and overall increases in the relative gas-phase acidities of the C−H[2] protons in a series of *N*-substituted and hydrogen-bonded acetamides.

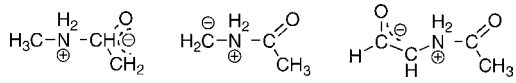

Figure 7. Negatively charged regions in the proximal anions and distal enolate of *N*-protonated systems separated from the electron deficient nitrogen by only one bond.

in Figure 2. The remainder, which is more than 120 kcal mol^{-1} for the *N*-protonated acetamide, is a reflection of the inductive contributions. These were independently assessed for the *N*-substituted and hydrogen-bonded acetamides, using the method of Rablen and Bentrup[24] referred to in the Introduction (Scheme 1), and the results are illustrated in Figure 6. It was not possible to use this approach to independently assess the inductive effect for the *N*-protonated amide due to elimination of the torsional enolate to form the ketene. Even so, it is clear from the results for the other systems that at the distal position, inductive effects dominate the resonance contributions, to increase the acidity of the C−H[2] protons by decreasing the electron density on amide nitrogen.

Whereas the distal enolate stability is affected by a combination of resonance and inductive effects, the stability of the proximal anion is determined only by the inductive component. This largely accounts for the generally 2-fold lower magnitude of the effect at the proximal position. The exceptions to the ratio of the distal to proximal effects being approximately two occur only in the *N*-protonated cases where resonance is completely eliminated and the inductive contributions to the stability far outweigh those due to resonance. In these cases, the effect at the distal position is only 10−15% larger. Qualitatively it makes sense that the distal and proximal effects are of similar magnitude under these circumstances, because inductive effects depend strongly upon the distance between interacting centers and in these systems, the electron deficient nitrogen is separated from the negatively charged region of each of the anion by only one bond (Figure 7).

Computed Aqueous pK_a Values. The relative and absolute calculated aqueous pK_a values for the acetamides and diketopiperazines are summarized in Tables S5 and S6 in the Supporting Information, the relative values are illustrated in Figure 8, and the absolute values for the diketopiperazines are given in Table 1. While there are no experimental values for the pK_a's of most of the species examined in this work, it is worth noting that this calculated pK_a of 29.7 for the α-carbonyl C−H of *N*,*N*-dimethylacetamide is in excellent agreement with the experimental value of 29.4.[22] The glycyl α-C−H protons of *N*-acetylglycinamide have an experimentally determined pK_a of 23.9.[19] As a further comparison, using the methodology described above, this pK_a was computed to be 24.9. This discrepancy is very small in the context that the effect of the *N*-electron-withdrawing substituents and hydrogen bonding is to decrease the pK_a values by up to 14 units. Even though the errors may be higher with the protonated systems,[21] where the solvation terms are substantially larger, they are expected to be very much less than the calculated effects of up to 19 pK_a units in those cases. In this regard it is interesting to observe that protonation of acetone has been estimated to decrease its α-carbonyl C−H pK_a by about 15 units,[44] whereas the analogous effects calculated in the present work for *O*-protonation of *N*-methylacetamide and the parent diketopiperazine, glycylglycine anhydride, are 17.2 and 11.2, respectively.

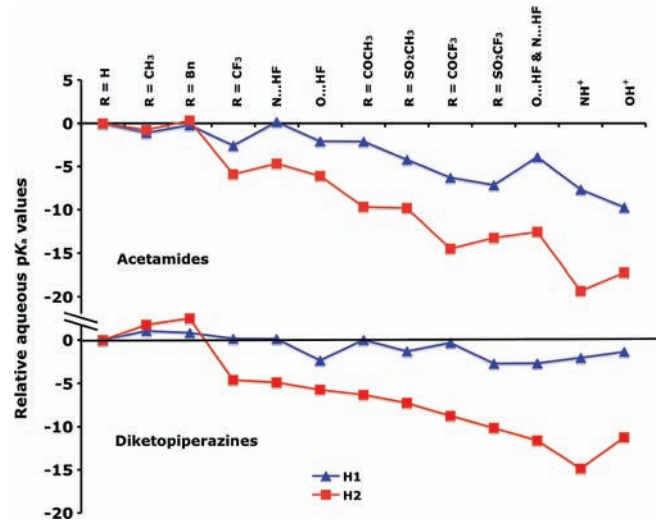

Figure 8. Relative computed aqueous (CPCM-UAKS) pK_a values of *N*-substituted, hydrogen-bonded, and protonated acetamides and diketopiperazines.

Table 1. Calculated Aqueous pK_a Values of C−H Protons at 298 K for the Diketopiperazines Illustrated in Figure 1[a]

	pK_a	
	CH[1]	CH[2]
R = H	24.0	24.0
R = CH_3	25.1	25.8
R = Bn	24.9	26.6
R = CF_3	24.2	19.4
R = H, N···HF	24.2	19.1
R = H, O···HF	21.7	18.3
R = $COCH_3$	24.1	17.7
R = SO_2CH_3	22.8	16.8
R = $COCF_3$	23.7	15.3
R = SO_2CF_3	21.3	13.9
R = H, N···HF, and O···HF	21.4	12.4
R = H, NH^+	22.0	9.2
R = H, OH^+	22.7	12.8

[a] For details, see Supporting Information.

The trends in aqueous acidity for the acetamides and diketopiperazines parallel those observed in the gas phase. The distal effect on the acidity of the C−H[2] protons is retained in solution where the *N*-electron-withdrawing substituents, and hydrogen bonding and protonation at amide nitrogen decrease the computed aqueous pK_a values by from 4 to 19. The effect on C−H[2] is greater than that on C−H[1] by about 3 to 13 pK_a units.

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Experimental Validation. To investigate the potential for application of the effects discussed above, and particularly the selective increase in the acidity of the distal hydrogens, we have also carried out some preliminary experimental studies. Initially we considered examining base-catalyzed hydrogen−deuterium exchange reactions of *N*-acyltrialkylammonium salts[45] in water, but facile competing hydrolysis reactions complicated the interpretation of the results. By comparison, with triethylamine as the base in d_6-acetone as the solvent, there was no decomposition of the diketopiperazines **1**−**3**, and in the case of *N*-acetylglycylsarcosine anhydride (**3**), the acetyl methyl and sarcosyl methylene groups underwent hydrogen−deuterium exchange with the acetone in a pseudo-first-order manner. There was no evidence of exchange of the other diketopiperazine hydrogens. This method does not provide a good indication of the relative acidity of acetone, so diisopropylketone was also investigated under analogous conditions and in competitive experiments with the diketopiperazines **1**−**3**. With this compound there was also no indication of hydrogen−deuterium exchange under these conditions. Accordingly, pseudo-first-order rate constants of 2.4×10^{-7} and 1.1×10^{-5} s^{-1} were measured for reaction of the acetyl methyl and sarcosyl methylene groups of the diketopiperazine **3**, respectively, while the corresponding values for the other protons of the diketopiperazine **3** or of any of the hydrogens of the diketopiperazines **1** and **2** and diisopropylketone were found to be less than 5.2×10^{-9} s^{-1}.

The acetyl methyl and sarcosyl methylene protons of the diketopiperazine **3** constitute distal hydrogens ($C-H^2$) of an *N*-acylated amide. For this methyl group there are no directly comparable proximal hydrogens ($C-H^1$), but the proximal analogue of the sarcosyl methylene is the glycyl methylene of the same compound **3**. The effect of acylation is also directly reflected in the comparative reactivity of the diketopiperazines **1**, **2**, and **3**. On this basis, the rate constant of 1.1×10^{-5} s^{-1} for hydrogen−deuterium exchange of the sarcosyl methylene protons, *via* formation of the corresponding distal enolate, corresponds to an increase of at least 3 orders of magnitude over the rate constant of less than 5.2×10^{-9} s^{-1} for either exchange of the glycyl methylene hydrogens *via* the proximal enolate, of the nonacetylated diketopiperazine **1**, or of the hydrogens of diisopropylketone. That is, the calculated effect of an *N*-electron-withdrawing acetyl group to selectively increase the gas-phase acidity and aqueous pK_a of an amide distal hydrogen is mirrored in these condensed phase experiments, where the distal hydrogens of the *N*-acetylated amide are also demonstrated to be much more reactive than the α-methine protons of diisopropylketone.

Relevance to Enzyme Catalysis. Biochemical catalysis of some reactions of amino acids and peptides, such as epimerization, depends on the capacity of enzymes to facilitate removal of their substrates' α-C−H protons.[3−5,8,10,11] One way in which Nature has managed to overcome the large thermodynamic barrier associated with this process is through the use of the pyridoxal phosphate cofactor, in which case the formation of an imine linkage between the cofactor and the substrate greatly acidifies the α-proton, since the anionic intermediate is stabilized by resonance. There are also cofactor-independent enzymes that readily catalyze α-proton abstractions at physiological pH. Examples include proline,[46,47] aspartate, and glutamate racemases[48] as well as peptide isomerases.[3−5,10,11] Within a peptide, the pK_a of an α-C−H proton is around 24, which translates to a thermodynamic barrier for deprotonation of about 22 kcal mol^{-1} (assuming a pK_a of 8 for the base catalyzing the proton abstraction), whereas the k_{cat} values for peptide isomerases are around 10^2 to 10^3 s^{-1}, corresponding to kinetic barriers of about 13−15 kcal mol^{-1}.[3] In such cases, the formation of a strong hydrogen bond (Figure 9, left) or metal ion coordination to the oxygen of the adjacent amide carbonyl appears to be a general strategy for enzyme catalysis. Specifically, the formation of short, strong, low barrier (single-well) hydrogen bonds to the carbonyl oxygen adjacent to the α-C−H proton has been proposed to provide 10−20 kcal mol^{-1} stabilization to the enolate intermediate (*cf.* 3−7 kcal mol^{-1} for typical hydrogen bonds).[49,50] However, the existence of such hydrogen bonds in condensed phases and in enzyme active sites has been questioned,[51,52] as the strength of these bonds diminishes rapidly in moderately polar environments. Recently, there have also been reports supporting the existence of π-oriented hydrogen bonding to the carbonyl oxygen, rather than lone-pair oriented hydrogen bonding, such that the enolate is preferentially stabilized compared to the neutral substrate.[53−55]

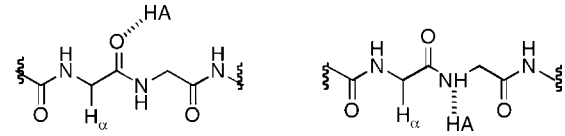

Figure 9. Hydrogen bonding to both amide nitrogen (right) and amide oxygen (left) increases the acidity of an α-CH proton adjacent to that amide carbonyl in a peptide and could be employed by enzymes for this purpose.

It is difficult to probe such systems experimentally, since analysis of enzyme-bound reaction intermediates is generally impractical; however, all the results discussed above indicate that hydrogen bonding to either the amide nitrogen (Figure 9, right) or oxygen would have a similar effect. Further, as shown in Figures 2 and 8, simultaneous hydrogen bonding to both nitrogen and oxygen produces an additive effect, increasing the gas-phase acidity of the $C-H^2$ protons in the diketopiperazine by ∼28 kcal mol^{-1} and decreasing the aqueous pK_a by ∼12. In view of this very substantial reduction in thermodynamic barrier, it therefore seems likely that enzymes exploit hydrogen bonding to nitrogen as an alternative or in addition to hydrogen bonding to oxygen as a way to cleave the normally very stable α-C−H bonds.

Conclusion

Our results therefore show that *N*-electron-withdrawing substituents and hydrogen bonding and protonation at amide nitrogen selectively increase the acidity of the distal C−H

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protons adjacent to the amide carbonyl and that the major cause of this is the combination of inductive and resonance stabilization of the corresponding enolates. Analogous effects are seen in computed gas phase and calculated aqueous pK_a values, as well as in preliminary condensed phase experiments, where the magnitude of the effects appears to be sufficient for synthetic applications. The results also indicate that hydrogen bonding to amide nitrogen as well as oxygen could contribute to enzyme-catalyzed reactions involving α-C−H bond cleavage of amino acids and peptides.

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Supporting Information Available: Full computational results, including Gaussian archive entries for BMK/6-31+G(d) optimized geometries and complete refs 10 and 32. This material is available free of charge via the Internet at http://pubs.acs.org.

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