Effects of Branching on the Tautomeric Equilibrium of Amino Acids

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Abstract: Compared to most α-amino acids, *N,N*-dialkylated α-amino acids are very soluble in a wide range of solvents. As a result, *N*-alkylated amino acids are ideal for the study of the effects that substituents have on the tautomeric equilibrium of amino acids. The ratios of zwitterionic and un-ionized tautomers for nine *N,N*-dimethylamino acids [$(CH_3)_2NCH(CR_3)COOH$] were determined by NMR spectroscopy in DMSO-d_6. Compared to dimethylalanine, the tautomeric equilibrium for dimethylamino acids, where R represents alkyl groups, favors the formation of the un-ionized tautomers. For all dimethylamino acids studied, a cyclic intramolecular hydrogen bonded conformer is proposed for the zwitterionic tautomers. For the dimethylamino acid in which R represents hydrogens (dimethylalanine), the zwitterion exists as a stable eclipsed conformer with a very effective hydrogen bond. On the other hand, if R represents alkyl groups, the zwitterionic conformers are not as stable as that of dimethylalanine owing to a distortion of the hydrogen bond which is caused by the presence of the alkyl groups. Since effective solvation of the zwitterions requires that solvent molecules access the region between the charges, zwitterionic conformers in which R represents alkyl groups are less solvated than those where R represents hydrogens. As a result, substituent steric and solvation effects on the stability of the zwitterions play decisive roles on the relative magnitude of the tautomeric equilibrium of amino acids.

Introduction

Owing to the effects that structurally different amino acid residues have on the properties of proteins, various unnatural amino acids are now being synthesized[1] and incorporated into proteins in order to evaluate the bioactive conformations of different peptides.[2] However, a fundamental understanding of the effects that structure variations have on the properties of the individual amino acids is essential in order to interpret and predict correctly the properties of mutant proteins. Owing to the limited solubility of α-amino acids in water and mixed solvents,[3] thorough quantitative structure-activity relationships (QSAR)[4] cannot be accomplished directly. As a result, the behavior of a wide variety of α-amino acids and their derivatives in the condensed phase is still poorly understood.

N,N-Dialkylated α-amino acids are very important compounds in biological chemistry[5] and they are ideal for the study of the effects that substituents and solvents have on the properties of α-amino acids. Compared to most α-amino acids, *N,N*-dialkylated α-amino acids are very soluble in a wide range of solvents.[6] In this paper, the tautomeric equilibria of nine *N,N*-dimethylamino acids in DMSO are determined from NMR spectroscopy and the variations of the magnitude of the equilibria are explained in the context of the substituent and solvation effects on the relative stability of tautomeric species. Since DMSO has been suggested to provide an extremely good model for understanding the factors that affect *in vivo* reactions,[7] a better understanding of the nature of α-amino acids in the biological environment is gained from the results of this research.

Experimental Procedure

N,N-Dimethylamino acids shown in Table 1 were prepared from the corresponding amino acid by reductive methylation,[8] except *N,N*-dimethylglycine (**1**) which was commercially available. Compounds **3**, **4**, and **9** were obtained as colorless solids in quantitative yields. Uncorrected melting points of the amino acids and *N,N*-dimethylamino acids are shown in Table 1.

2-(Dimethylamino)butanoic acid (3): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.17 (br s, 1H), 3.02 (t, J = 6 Hz, 1H), 2.45 (s, 6H), 1.64 (m, J = 7 Hz, 2H), 0.87 (t, J = 7 Hz, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 170.4 (s), 69.9 (d), 41.1 (q), 21.3 (t), 10.5 (q). Anal. Calcd for $C_6H_{13}NO_2$: C, 54.94; H, 9.99; N, 10.68. Found: C, 55.05; H, 10.33; N, 10.56.

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Table 1. Melting Points and Spectral Analyses of α-Substituted α-(Dimethylamino)acetic Acids [$Me_2NCH(R)COOH$] in DMSO (Concentration 0.08 M; Spectra Taken at Ambient Temperature)

no.	R	mp[a]	δ_{Me}[b]	δ_{COO-H}	$\nu_{C=O}$ (cm^{-1})[c]
1	H	180 (245)	2.58	8.88	1638.5(s)
2	CH_3	187 (290)	2.53	8.32	1636.1(s); 1713.4 (w)
3	C_2H_5	180 (306)	2.45	9.17	1634.2(s); 1707.7(w)
4	C_3H_7	187 (298)	2.45	8.30	1633.7(s); 1709.6(w)
5	*i*-C_4H_9	194 (293)	2.39	9.35	1633.5(s); 1709.0(m)
6	*i*-C_3H_7	154 (315)	2.28	11.38	1632.8(s); 1708.0(m)
7	*sec*-C_4H_9	173 (288)	2.29	11.32	1632.5(s); 1708.2(m)
8	*cyclo*-C_6H_{11}	163 (298)	2.27	11.30	1635.5(s); 1707.5(m)
9	*t*-C_4H_9	180 (280)	2.33	11.81	1630.6(m); 1707.8(s)

[a] Values in parentheses indicate the melting point of the corresponding unmethylated amino acid. [b] Chemical shift of the dimethylamino hydrogens. [c] (s) strong absorption band; (w) weak absorption band; (m) medium absorption band.

2-(Dimethylamino)pentanoic acid (4): ^1H NMR (DMSO-d_6, 300 MHz) δ 8.30 (br s, 1H), 3.07 (t, J = 7 Hz, 1H), 2.45 (s, 6H), 1.60–1.53 (m, 2H), 1.35–1.26 (m, 2H), 0.86 (t, J = 7 Hz, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 170.7 (s), 68.1 (d), 41.0 (q), 30.3 (t), 18.9 (t), 13.9 (q). Anal. Calcd for $C_7H_{15}NO_2$: C, 57.90; H, 10.41; N, 9.65. Found: C, 57.81; H, 10.39; N, 9.71.

2-(Dimethylamino)-3,3-dimethylbutanoic acid (9): ^1H NMR (DMSO-d_6, 300 MHz) δ 11.81 (br s, 1H), 2.76 (s, 1H), 2.33 (s, 6H), 0.96 (s, 9H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 171.4 (s), 74.7 (d), 44.5 (q), 34.2 (s), 27.0 (q). Anal. Calcd for $C_8H_{17}NO_2$: C, 60.35; H, 10.76; N, 8.80. Found: C, 60.23; H, 10.95; N, 8.76.

The corresponding amino acid of **8** was prepared from cyclohexylacetic acid.[9] *N,N,N*-Trimethyl betaines of these amino acids were synthesized as outlined in the literature.[10] The hydrochloride salts were obtained by bubbling HCl gas through a solution of each betaine (0.30 mmol) in methanol (10 mL). After evaporation of the solvent, the HCl salts were obtained in quantitative yields. *N,N*-Dimethylamino acid ethyl esters were prepared from the corresponding ethyl ester of the amino acids by reductive methylation. The ethyl esters of the amino acids were prepared from the corresponding amino acid. Each amino acid (25 mmol) was suspended in anhydrous ethanol (60 mL) and HCl gas bubbled into the suspension for 5–10 min. The mixture was then refluxed under an atmosphere of dry N_2 for 24 h. Removal of the solvent in vacu gave the hydrochloride salts of the ester in approximately 90% yield. The HCl salts were neutralized with dilute KOH followed by extraction of the neutral esters with chloroform.

Ambient temperature ^1H NMR spectra were recorded in solutions of DMSO-d_6 on an IBM (Bruker) NR/300 FT-NMR spectrometer. The titration of each *N,N*-dimethylamino acid ethyl ester in DMSO-d_6 (0.71 M) with trifluoromethane sulfonic acid in DMSO-d_6 (0.57 M) was carried out in an NMR tube. After each 25 μL addition of the acid, the chemical shift of the *N,N*-dimethylamino hydrogens was recorded. Infrared spectra of dilute DMSO solutions of the dimethylamino acids were recorded on a Perkin-Elmer FT-IR (1600 model) spectrometer. Spectrophotometric grade DMSO was purchased from Aldrich Chemical Co. and stored over Molecular Sieve (4Å) before being used. Substituted dimethylamino acids were dried in a vacuum oven for 48 h and then transferred to a dry N_2 atmosphere glovebox. Samples of dimethylamino acids were weighed out and dissolved in spectrophotometric grade DMSO in the glovebox before the spectra were taken.

Results and Discussion

Tautomeric Equilibrium for *N,N*-Dimethylamino Acids. Quantum mechanical calculations,[11] electron diffraction analysis,[12] FTMS,[13] IR matrix isolation techniques,[14] and microwave spectroscopy[15] have been used to demonstrate that α-amino acids exist as un-ionized tautomers in the gas phase. Recently, we have shown by FTMS that substituted *N,N*-dimethylamino acids also exist as un-ionized molecules in the gas phase.[16] Amino acids are known to exist as zwitterions in aqueous solution, as well as in the crystalline state. The substitution, however, of two methyl groups for the two hydrogens of α-amino acids causes the intermolecular attraction of amino acids to be reduced and as a result lower melting points for the *N,N*-dimethylamino acids, compared to the unmethylated amino acids, are observed (Table 1). In water, the tautomeric equilibrium for amino acids favors the zwitterions—the equilibrium constant (K_D) is $10^{5.62}$ for glycine.[17] Zwitterions are known to predominate in other polar solvents, such as acetonitrile, whereas the neutral form predominates in nonpolar solvents, such as benzene.[18] In polar protic solvents, the solvation of zwitterions is favored, whereas in nonpolar solvents, the zwitterions are not highly solvated.[19] Compared to the gas phase, zwitterions are favored in most solvents owing to varying degrees of zwitterion/solvent interactions. These interactions include specific and non-specific interactions.[20] The extent of tautomer/solvent and tautomer/tautomer interactions dictates, to a large degree, the relative magnitude of the tautomeric equilibrium constant (K_D) of amino acids.

The ^1H NMR signal of the acidic hydrogen (δ_{COO-H}) for the dimethylamino acids of this study appears as a single signal and the chemical shift varies between 8.3 and 11.30 ppm (Table 1). The range of the chemical shift indicates that the structural features of the dimethylamino acids dictate the magnitude of equilibrium 1.

$$(CH_3)_2NCH(CR_3)COOH \rightleftharpoons (CH_3)_2N^+HCH(CR_3)COO^- \quad (1)$$

The appearance of a single signal for the acidic hydrogen indicates that the proton exchange between the carboxylate and dimethylamino functionalities of the un-ionized and zwitterionic tautomers, respectively, is rapid on the NMR time scale in DMSO. As a result, even though the chemical shifts of these signals give a qualitative description of the distribution of tautomers in solution, they cannot be used to quantify the distribution. Since the neutral dimethylamino acids and the corresponding zwitterions have two different functional groups—the carboxylate and the carboxylic acid—IR spectroscopy was used to determine the relative concentrations of both tautomers for structurally different dimethylamino acids. The sodium salts

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Table 2. Chemical Shift of the Amino Methyl Groups of the Ethyl Ester and Betaine Derivatives of *N,N*-Dimethylamino Acids in DMSO-d_6 and Percent Zwitterionic Tautomer

no.	R	ester	ester·HCl	betaine	betaine·HCl	% zwitterion[a]
1	H	2.23	2.84	3.15	3.24	69
2	CH_3	2.22	2.80	3.11	3.22	66
3	C_2H_5	2.23	2.78	3.08	3.19	50
4	C_3H_7	2.23	2.78	3.08	3.20	51
5	*i*-C_4H_9	2.23	2.78	3.07	3.20	38
6	*i*-C_3H_7	2.20	2.79	3.13	3.24	17
7	*sec*-C_4H_9	2.20	2.80	3.15	3.25	18
8	*cyclo*-C_6H_{11}	2.19	2.79	3.10	3.24	17
9	*t*-C_4H_9	2.31	2.91	3.22	3.33	4

[a] Calculated using eq 3.

of these dimethylamino acids, which reflect the carboxylate functionality of the zwitterions, have a band at 1630.0 ± 1.0 cm^{-1} and the dimethylamino acid hydrochloride salts, which reflect the carboxylic acid functionality of the un-ionized dimethylamino acid, have a band at 1725 ± 2.3 cm^{-1}. Thus, for any particular dimethylamino acid in DMSO, the relative intensities of bands in these regions indicate the relative concentrations of the two tautomers. From Table 1, the $\nu_{C=O}$ stretch of *N,N*-dimethylglycine (**1**), which is known to exist predominantly as the zwitterion tautomer in DMSO,[21] appears at 1638 cm^{-1}. For the other dimethylamino acids, in addition to the band at 1634.7 ± 1.0 cm^{-1}, there is a band at 1710 ± 2.3 cm^{-1} and another near 2800 cm^{-1}. The band near 2800 cm^{-1} indicates the presence of the neutral tautomer.[22] It is obvious from Table 1 that as the number of alkyl groups bonded to the β-carbon of dimethylamino acids increases, the intensity of the band at 1710 ± 2.3 cm^{-1} increases; however, a quantitative assessment of the tautomeric distribution cannot be achieved by this method.

The chemical shifts of the dimethylamino hydrogens of the different dimethylamino acids in DMSO vary between 2.58 and 2.27 ppm (Table 1) and they also qualitatively indicate the tautomeric distributions. In order to establish, however, a quantitative relationship between the chemical shifts of these hydrogens and the tautomeric equilibrium constant, the chemical shifts of the dimethylamino hydrogens of the isolated un-ionized and zwitterionic tautomers must be known. The chemical shifts of the ethyl ester and the ethyl ester hydrochloride derivatives of *N,N*-dimethylglycine have been used to establish these values and in turn used to calculate the percent of neutral *N,N*-dimethylglycine that exists in DMSO.[21] Table 2 shows the chemical shifts for the *N,N*-dimethylamino hydrogens of the derivatives that are used to determine the tautomeric distribution of the dimethylamino acids in this study. The dimethylamino hydrogens' chemical shifts of the ethyl ester and protonated ester derivatives essentially reflect the chemical shifts of the un-ionized tautomer and zwitterion, respectively. In a solution of both neutral and protonated esters, equilibrium 2 will be established and the distribution of these solutes is dictated by the magnitude of the equilibrium constant. A titration of the corresponding dimethylamino acid esters with a strong acid produces various distributions of the neutral and protonated dimethylamino acid esters (eq 2), and a plot of the chemical shifts of the dimethylamino hydrogens versus the concentration of the ratio of the protonated and unprotonated ester establishes the relationship between the chemical shift and the magnitude of equilibrium 2 for the different esters.

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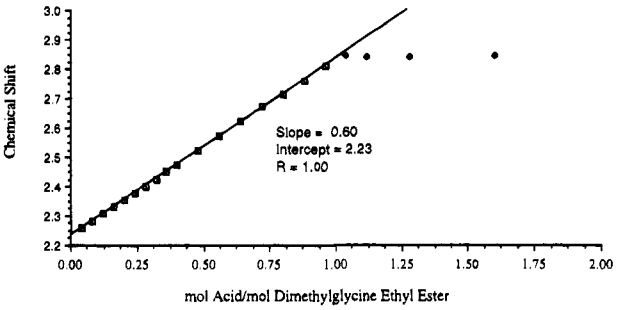

Figure 1. ^1H chemical shifts of the *N,N*-dimethylamino hydrogens of *N,N*-dimethylglycine ethyl ester versus mol of CF_3SO_3H/mol of *N,N*-dimethylglycine ethyl ester; (□) the titration in excess *N,N*-dimethylglycine ester, and (◆) the titration in an excess of acid.

$$H^+ (CH_3)_2NCH(CR_3)COOEt \rightleftharpoons (CH_3)_2N^+HCH(CR_3)COOEt \quad (2)$$

Figure 1 shows one example, the titration of dimethylglycine ethyl ester with trifluoromethanesulfonic acid. Linear relationships are obtained, and the intercepts and the plateaus of these plots are the same as the chemical shifts of the dimethylamino hydrogens of the neutral ester and the HCl ester salts, respectively. These linear relationships between the chemical shifts and the distribution of neutral and protonated ester indicate that the chemical shifts of the dimethylamino hydrogens of the dimethylamino acids give a direct reflection of the distribution of equilibrium tautomers in solution. The equilibria established by the protonation of the dimethylamino acid esters and that established by the protonation of the dimethylamino acid to form the zwitterion are not exactly the same, however. Owing to the presence of the carboxylate anion of the zwitterions, a correction in the chemical shifts of the protonated esters must be made in order to make the two equilibria equivalent. The difference in chemical shifts for the protonated betaine and the betaine represents the effect of the carboxylate anion on the chemical shifts of the dimethylamino hydrogens. Equation 3, which takes into account these factors, was used to calculate the distribution of dimethylamino acid tautomers and the results are shown in Table 2.

$$\text{fraction (zwitterion)} = \delta_{(\text{amino acid})} - \delta_{(\text{ester})}/\{\delta_{(\text{ester·HCl})} - [\delta_{(\text{betaine·HCl})} - \delta_{(\text{betaine})}] - \delta_{(\text{ester})}\} \quad (3)$$

δ is the chemical shift of the dimethyamino hydrogens of different amino acid derivatives. From the results shown, it is obvious that the nature of the substituent influences the tautomeric distribution. There are four distinct categories in which these values fall and the number of alkyl groups that are bonded to the β-carbon appear to dictate the division. This observation may be understood in the context of the effects that substituent and solvation have on the stability of the tautomeric equilibrium species.

Steric Effects on the Stability of the Tautomers. There are two possible modes of proton transfer from the un-ionized dimethylamino acid to form the zwitterionic tautomer—intramolecular and intermolecular proton transfer. An intramolecular proton transfer from an intramolecular hydrogen bonded un-ionized dimethylamino acid to produce an intramolecular hydrogen bonded zwitterionic conformer is entropically favored. It has been proposed that the eclipsed planar hydrogen bonded zwitterionic conformer is the most stable conformer for glycine,[23] alanine,[24] and cysteine[25] in different dielectric fields. The stability of the eclipsed intramolecular hydrogen bonded

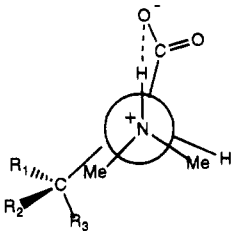

Figure 2. Proposed conformer of *N,N*-dimethylamino acid zwitterions.

conformer is gained primarily from the effective hydrogen bond, which offsets any instability derived from the eclipsed geometry of the hydrogens on the nitrogen and the groups on the α-carbon. Another factor that contributes to the stability of the eclipsed intramolecular hydrogen bonded conformer is the electrostatic interactions between the NH_3^+ and CO_2^- groups.[26] Owing to the stability of the eclipsed conformers of the zwitterions of these amino acids, a similar stable conformation is proposed for the zwitterionic conformer of the *N,N*-dimethylamino acids of this study, which is shown in Figure 2. In DMSO, which is a nonprotic solvent,[27] intramolecular hydrogen bond interaction should play an important role in the stability of the zwitterion.

For equilibrium reactions, such as equilibrium 1, it is known that solvation effects of charged or charge-separated species play a major role in the magnitude of equilibrium constants;[28] and for amino acids, it has been shown that stabilization of the zwitterion plays a decisive role in the magnitude of the tautomeric equilibrium.[29] Thus, the explanation of the distribution variations shown in Table 2 lies in the relative stability of the zwitterionic conformer shown in Figure 2. There are two modes by which alkyl groups influence the stability of species—substituent steric[30] and polarizability effects.[31] The effects that these substituents have on the stability of the zwitterionic tautomers can be understood from the effects that substituents have on the stability of substituted acetate anions ($R-CH_2COO^-$) and mono-substituted trimethylammonium ions ($R-CH_2N^+HMe_2$)—these two ions can be visualized as components of the zwitterions of dimethylamino acids. In solution, acetate anions are destabilized by large bulky groups—mainly by the steric effect[32] —whereas trimethylammonium ions are stabilized by bulky alkyl groups—mainly by substituent polarizability effect.[33] These effects, however, are highly attenuated in solution, and since they oppose each other, they may not contribute substantially to the stability of zwitterions.

Steric effects are known to affect the stability of conformational isomers.[34] Steric effects caused by the different sizes of the groups bonded to the β-carbon of the conformer shown in Figure 2 alter the stability of the zwitterion. For a fairly small group, such as the methyl group ($R_1 = R_2 = R_3 = H$ in Figure 2), the planar eclipsed conformer is the most stable one owing to the effective intramolecular hydrogen bond. The tautomeric distribution for *N,N*-dimethylglycine is similar to that of *N,N*-dimethylalanine ($R_1 = R_2 = R_3 = H$ in Figure 2). Apparently, the stabilities of the intramolecular zwitterionic conformers of *N,N*-dimethylglycine and *N,N*-dimethylalanine are similar in DMSO. In the gas phase, it is known that glycine and alanine have very similar conformations.[35] On the other hand, when $R_1 = R_2 = H$ and R_3 = alkyl (methyl, ethyl, isopropyl) the distribution of tautomers changes dramatically, which indicates that these zwitterions are less stable than those discussed previously. Since these substituents are larger than those previously discussed, the distortion of the planar eclipsed conformation brought about by their sizes affects the overall stability of the conformation. As a result, intramolecular hydrogen bonded zwitterions that have one alkyl group branched at the β-carbon in Figure 2 are less stable than those without branching in that position. The inclusion of the isobutyl substituent (entry 5) in this category truly indicates that branching on the β-carbon and not the γ-carbon is responsible for the stability variations of the intramolecular hydrogen bonded conformer.

Another division in the magnitude of the percent zwitterion shown in Table 2 is observed if $R_1 = H$ and $R_2 = R_3$ = methyl; $R_1 = H$, R_2 = methyl; R_3 = ethyl; and $R_1 = H$ and $R_2 = R_3 = (CH_2)_5$. The distortion of these zwitterionic tautomers, which is brought about by the number and size of the substituents, results in a greater distortion of the intramolecular hydrogen bond. An analogous effect is observed for the distortion of the conformer of phenylalanine which is brought about by the substitution of a methyl group for one hydrogen in the β-position.[36] The last category results for $R_1 = R_2 = R_3 = CH_3$, i.e., *tert*-butyl group. Owing to the substitution of three methyl groups for the hydrogens of *N,N*-dimethylalanine, a much larger space is required to accommodate this substituent. As a result, the planar eclipsed conformer that is proposed for *N,N*-dimethylalanine zwitterion is highly distorted and the effectiveness of the intramolecular hydrogen bond is compromised. Hence, the equilibrium for dimethyl-*tert*-leucine ($R_1 = R_2 = R_3 = CH_3$) favors the un-ionized *N,N*-dimethylamno acid compared to *N,N*-dimethylalanine (and *N,N*-dimethylglycine). Interestingly, a plot of average percent of zwitterionic tautomer for each category versus the number of alkyl groups bonded to the β-carbon results in a linear relationship with a correlation coefficient (*R*) of 0.991. One implication of this observation

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is that there is no real saturation effect on the destability of the hydrogen bonded conformer if there is an increase in the number of alkyl groups bonded to the β-carbon.

The solvation by DMSO of the zwitterions that have different alkyl groups bonded to the β-carbon also plays an important role in their stability. For effective solvation of solutes like zwitterions where the charges are separated from each other by a methylene unit, it was shown that in order to achieve effective solvation, the solvent molecules must gain access between the lines of force of the two charges.[37] Thus, solvation of the zwitterion of dimethyl-*tert*-leucine is less effective by this mode compared to the solvation of the zwitterion of dimethylalanine. Solvation of the zwitterionic tautomers that have hydrogens bonded to the β-carbon is more effective than for zwitterions that have alkyl groups in the same position.

Conclusions. Owing to the greater solubility of dimethylamino acids, compared to amino acids, dimethylamino acids have been used to determine, in general, the effects of β-substitution on the tautomeric equilibrium of amino acids. The magnitude of the tautomeric equilibrium of *N,N*-dimethylamino acids can be used to evaluate the relative stability of the zwitterionic tautomer. There are two factors that contribute to the relative stability of zwitterionic tautomers: steric and solvation effects. Any factor, such as steric, that distorts the eclipsed planar geometry of an intramolecular hydrogen bonded zwitterion to cause crowding about the $C_\alpha-N$ bond will lessen the effectiveness of the hydrogen bond and as a result destabilize the zwitterion, relative to that of *N,N*-dimethylglycine. The tautomeric equilibrium favors the un-ionized dimethylamino acid tautomer for amino acids that have substantial branching on the β-carbon and favors the zwitterionic tautomer for amino acids that have less branching on the β-carbon. The observations reported in this paper are consistent with that made for the effects that substituents have on the magnitude of the tautomeric equilibrium of substituted thiazolidine-4-carboxylic acids.[38] The percentage of 2,2,5,5-tetramethylthiazolidine-4-carboxylic acid that exists in the neutral form is much greater than that of the unsubstituted thiazolidine-4-carboxylic acid in solution.

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