Gas-Phase Acidities and Proton Affinities of Amino Acid Analogs from the Extended Kinetic Method

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Gas-Phase Acidities and Proton Affinities of Amino Acid Analogs from the Extended Kinetic Method

A thesis submitted in partial fulfillment of the requirement for the degree of Bachelor of Science in Chemistry from the College of William and Mary

by

Ian Kilby Webb

Accepted for ________________________________
(Honors, High Honors, Highest Honors)

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Director

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Williamsburg, Virginia
April 23, 2008
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Abstract

The gas-phase acidities and proton affinities of analogs of protein amino acids were investigated. The gas-phase acidities of the lysine homologues were measured by the extended kinetic method in a quadrupole ion trap mass spectrometer. Deprotonation entropy changes were also measured. For ornithine, 2,4-diaminobutyric acid, and 2,3-diaminopropionic acid, the gas-phase acidities measured were 1416 ± 17 kJ/mol, 1420 ± 8 kJ/mol, and 1405 ± 24 kJ/mol. Their changes in entropy were measured to be -19 J/mol K, 1 J/mol k, and -24 J/mol K, respectively.

The gas-phase acidities and entropies of two structural analogs of arginine, citrulline and canavanine, were measured by the extended kinetic method. The gas-phase acidity of citrulline was measured to be 1366 ± 11 kJ/mol, and the gas-phase acidity of canavanine was measured to be 1401 ± 13 kJ/mol. The proton affinity and protonation entropy change for citrulline was measured as well. The proton affinity was determined to be 984 ± 11 kJ/mol with an entropy change of -6 J/mol K.

The proton affinity and protonation entropy change of L-BMAA, a structural analogue of alanine and 2,3-diaminopropionic acid, was measured by the extended kinetic method to be 960 ± 7 kJ/mol. The entropy for the protonation reaction was found to be -4 J/mol K.

Hybrid density functional theory calculations were performed on the compounds examined. Energy-optimized geometries were examined for structural trends and theoretical predictions for the gas-phase acidities and proton affinities were made. The experimental and theoretical determinations reveal the effects of structural changes on gas-phase thermochemical properties.
Chapter I – Introduction

1.1 Protein and Non-Protein Amino Acids

The study of amino acids is an important field of biochemistry because the structural diversity of amino acid side chains directly affects protein function. The primary structure of proteins consists of amide-bonded protein amino acids (PAA). These peptide sequences are folded into structurally functional forms to make biologically active proteins. These structures are formed by various methods of covalent and non-covalent interactions, including hydrogen bonds, disulfide linkages, Van der Waals forces, and electrostatic interactions. The intrinsic properties of the amino acid side chains are believed to be one of the factors directing protein folding\(^1\). Catalytic protein active sites are also defined by the chemistry of side chains present in the binding pocket. Different substrates can be targeted by the enzyme depending on the substrate hydrophobicity or hydrophilicity, substrate charge, and substrate size. Studying intrinsic acid/base properties of amino acids is essential in predicting both protein folding behavior and enzyme active-site reactivity.

The twenty PAAAs are the only amino acids coded for by human DNA. However, hundreds of amino acids not coded for by the genome, known as non-protein amino acids (NPAA), are ubiquitous throughout nature. They not only serve similar structural and enzymatic purposes to PAAAs, but also are found in metabolism and nitrogen fixation mechanisms and help in defense from predators. L-BMAA, a NPAA structural analog of L-alanine, has been studied for its neurotoxicity. It has been found in high concentrations in ALDS and Parkinson’s disease patients in Guam and a small population in North America.\(^2\)
Many NPAAs are structurally analogous to the twenty PAAs. Gas-phase acid base properties of structural analogs of lysine, arginine, and alanine were examined in this study. The thermochemical properties of PAAs are well known in the literature, so it is expedient to conduct a comparative study between the properties of PAAs and NPAAs. Studying how small changes in side chain structure affect these properties results in a better understanding of the intrinsic properties and biological functions of the amino acids.

1.2 Gas-Phase Acidity Measurement Methods

Foundational thermochemical properties of organic molecules are vitally important the understanding of the chemical behavior of these molecules. These properties direct the stability, conformational chemistry, and reactivity of these molecules. An effective way to measure these properties is to measure them in the gas phase. The gas phase is effective for multiple reasons: gas-phase kinetics are readily understood, the absence of a solution matrix ensures that the properties measured are intrinsic to the analyte under study, and mass spectrometry can be used to measure these properties. Since gas-phase kinetics are readily understood, developing and utilizing thermochemical experiments using kinetics and equilibrium properties is useful, as these experiments can be modeled and predicted well by theoretical calculations. The absence of a solution matrix negates any effects that the polarity, volatility, etc. of the solvent would have on the thermochemical properties of a particular molecule. This allows measurements to be a product only of the intrinsic chemical properties of the analyte molecule itself. The ability to use mass spectrometry provides many benefits to a thermodynamic gas-phase study. First, with a selection of ionization sources and sample
introductions, a variety of volatile and non-volatile compounds can be introduced into the
gas phase via soft ionization methods such as electrospray ionization (ESI) and matrix-
assisted laser desorption ionization (MALDI). Measuring acidities in a mass
spectrometer is made possible by the ability to leak neutral reagents into a flow tube or
ion trap as well as the ability to regulate temperature. More recently, tandem mass
spectrometers have been utilized to use collision induced dissociation (CID) to determine
gas-phase acidities via the kinetic method. Four methods have been used to measure gas-
phase acidities with mass spectrometry: ion/molecule equilibrium determinations,
ion/molecule reaction bracketing, thermochemical cycles, and Cooks’ kinetic method.

The first thermochemical measurement method, ion/molecule equilibrium
constant determination (IMEC), relies on the ability to reach chemical equilibrium
between a reference compound and the analyte under study inside the mass spectrometer.
The negative reference ion and neutral analyte are introduced into the mass spectrometer.
If using such an ion cyclotron resonance (ICR) system, the mixture of analyte and
reference is allowed to establish the following equilibrium:

\[
\frac{k_f}{k_r} B^- + AH \quad \rightleftharpoons \quad A^- + BH ,
\]

where B denotes the reference acid. \(^{3,4,5}\) Thus, the equilibrium consists of two competing
deprotonation reactions; the forward reaction being the deprotonation of the analyte, and
the reverse reaction being the deprotonation of the reference acid. Since equilibrium is
assumed at the point when the rate constants are equal, the expression

\[
K = \frac{[A^-][BH]}{[B^-][BH]} = \frac{K_f}{K_r}
\]
can be used to describe an equilibrium constant for the reaction. The equilibrium constant, then, relies on the measurement of the concentrations of each of the four species. The partial pressures of the two neutral reagents at equilibrium, measured by an ion gauge, provide the concentration for these two species. The ratio of the partial pressure of reference to analyte concentration can then be multiplied by the ratio of the abundance in the mass spectrum of the analyte anion to the reference anion. This product is the equilibrium constant of the reaction. Flowing afterglow, guided ion beam, and high-pressure systems can be made by equilibrium method measurements by measuring the rate constants for the forward reaction and reverse reaction as separate experiments. The ratio of the forward to reverse reaction rate constant at equilibrium will yield the equilibrium constant for the reaction. An expression for the enthalpy of deprotonation (gas-phase acidity) can be derived from the measured equilibrium constant where temperature is defined by the Van’t Hoff equation and entropy is either calculated or found experimentally by a Van’t Hoff experiment.

\[
\ln \left( \frac{K_2}{K_1} \right) = -\frac{\Delta H_{\text{acid}}}{R} \left( \frac{1}{T_2} - \frac{1}{T_1} \right)
- RT \ln(K) = \Delta G_{\text{acid}} = \Delta H_{\text{acid}} - T\Delta S_{\text{acid}}
\]

\[
\ln(K) = -\frac{\Delta H_{\text{acid}}}{RT} + \frac{\Delta S_{\text{acid}}}{R}
\]

Several values for the equilibrium constant are determined by using several reference compounds and different ratios of neutral reagent gases. This way, the mean of the free energies can be used to determine the gas-phase acidity. The validity and accuracy of IMEC has been determined through other thermochemical studies.
Equilibrium studies have several noticeable advantages. Although several reference acids can be used to add more experimental trials, the free energy of deprotonation only requires one reaction to be measured. This is a distinct advantage in both time and expenses compared with the many references required by the bracketing method, which will be discussed below. Another advantage is that IMEC is well understood and repeatable. IMEC fails when equilibrium cannot be established or if reactant concentrations are not well known.

Ion/molecule reaction bracketing experiments (IMRB) can be used in place of IMEC if equilibrium conditions between a neutral analyte under study and a reference acid cannot be reached. The theory behind the bracketing method is fairly simple. In IMRB, a neutral species is reacted with a reference anion, most commonly by introducing the reference and leaked neutral into a collision cell (quadrupole two in a tandem system or ion trap) or into a flow tube. The reference anion has a known gas-phase acidity, and if the proton is transferred from the neutral analyte to the reference base, the resulting mass spectrum will show the analyte anion, indicating that the following exothermic reaction has taken place.

\[
B^- + AH \rightarrow A^- + BH
\]

If the proton does not transfer, the reaction is endothermic. In this way, the analyte anion acidity can be bracketed by a range of reference acids, eventually between the most acidic reference that undergoes the exothermic reaction and the least acidic reference that does not. The Gibbs free energy of deprotonation can then be assigned a value, with experimental error assigned to cover a range including each of the bracketing references.
From the Gibbs free energy, the gas-phase acidity, an enthalpy value, can be calculated from the definition

$$\Delta H_{acid} = \Delta G_{acid} + T\Delta S_{acid}$$

where entropy is calculated from statistical mechanics.

The bracketing method has several advantages over IMRE experiments, due to the fact that sometimes an equilibrium constant determination cannot be made. An important example of such is if the concentration of the analyte is not able to be determined.\textsuperscript{6,7} This makes using the equilibrium constant expression ineffective. However, this does not affect bracketing measurements, as only the products of the neutral-anion reaction are analyzed. A possible problem with this method is that it is a qualitative method for free energy determination, which may make the assignment of experimental error difficult. Also, a wide range of compounds must be reacted with the analyte to find the smallest bracket range, which can possibly be expensive and time consuming depending on the types of references needed to react with the analytes.

Another method for determination of gas-phase acidities is the use of thermochemical cycle. A thermochemical cycle is a way of calculating the gas-phase acidity by measuring and including other parameters. The thermochemical cycle for gas-phase acidity can be shown by the following

\begin{align*}
\text{HF} & \xrightarrow{\Delta_e H^0[H\text{HF}]} \text{H}^+ + \text{F}^- \\
\Delta_{acid} H^0[H\text{HF}] & \xrightarrow{IE(H^+)} \text{H}^+ + \text{F}^- \quad - EA(F^-)
\end{align*}

\textbf{Figure 1 – Diagram of a Thermochemical Cycle}
From this cycle, it can be seen that the determination of the gas-phase acidity of an acid (in this example, hydrofluoric acid) can be expressed as a function of the bond dissociation energy of the acid and the ionization energies of the hydrogen and fluorine radicals. The ionization energy of hydrogen is a well known value in the literature, so it does not need to be measured for each experiment. The electron affinity can be measured using photoelectron or photodetachment negative ion spectroscopy, measuring the wavenumber, and thus, energy, at which electrons are most likely to detach from the anion. Also, the kinetic method has been used as well to determine the electron affinity of organic radicals. The bond dissociation energy can be also measured by photoionization spectroscopy or photoionization mass spectrometry and by equilibrium methods, similar to the one detailed above. The equation used to determine the acidity is given as

\[ \Delta H_{\text{acid}}(R - H) = D(R - H) - EA(R^+) + IE(H) \]

The sum of the bond dissociation energy of the acidic hydrogen and the hydrogen’s ionization energy minus the electron affinity from the deprotonated radical gives the gas-phase acidity of the molecule.

Using a thermochemical cycle presents the distinct advantage that it is not a direct measurement of the acidity. If the acidity cannot be directly measured, then this method is desirable. Another advantage is that any of the thermochemical properties in the cycle can be determined by measuring the three other properties. For example, a cycle can be used to measure the bond dissociation energy if the activation energy and acidity are known, or the activation energy can be measured if the bond dissociation energy and acidity are known. The major disadvantage of this method also comes from the fact that
this is not a direct measurement. By having to measure the other properties used in the cycle, error is propagated from each measurement, not just in the measurement of acidity itself as in the other methods.

A more recent method developed for thermodynamic studies is R. Graham Cooks’ kinetic method. In the kinetic method, the analyte and reference acid are added to a basicified matrix. In the matrix, a negatively charged, proton-bound dimer of the two compounds can form. This ion, existing in solution, is often transferred to the gas phase by a soft ionization source, commonly, ESI. Then, an inert gas, such as argon or helium, can then be used to collide with the dimer in the collision cell of a tandem mass spectrometer. This immediately presents advantages over the above methods. Since the deprotonation reaction already occurs in solution, there is no need to leak in any reagents. For the above methods, the reagents had to be leaked in as a gas, which means that they were limited by their volatility. With the kinetic method, nonvolatile analytes and references can be used. Therefore, thermodynamic measurements can be made on larger molecules, such as biologically relevant species, like the chemical amino acid analogs considered in this study. A detailed description of the kinetic method is given in the Experimental Section.
Chapter II – Experimental

2.1 Kinetic Method Proton Affinity and Gas-Phase Acidity Determinations

2.1.1 Experimental Procedure

Solutions of the analyte compounds and references were prepared with a concentration of $\approx 5 \times 10^{-4}$ M. For positive ion solutions, a 49.5:49.5 methanol/water solvent was used, with an added 1% acetic acid to encourage the formation of positive proton-bound dimer complexes. Negative ion solutions, excluding canavanine, were solvated in solutions of 79.5:19.5 methanol/water with 1% ammonium hydroxide, to encourage negative proton-bound dimer complex formation. Canavanine was dissolved in a 99% methanol, 1% ammonium hydroxide solvent.

Solutions were introduced into the mass spectrometer via a 500 µL Hamilton Gastight® syringe by an automated syringe pump at a flow rate of 20 µL/min. The instrument used for data collection was a Finnigan LCQ DECA quadrupole ion trap mass spectrometer, equipped with an electrospray ionization source. The heated capillary was maintained at a temperature of 125° C. The voltage of the source and offsets of the focusing lenses were set by the LCQ Tune™ software that served as the user interface to the mass spectrometer. The target heterodimer mass/charge was input into the software, and the voltage and lenses were “tuned” to provide optimal ion counts. The procedures for both positive and negative ions were the same; however, the software was used to set the mass spectrometer in either positive or negative ion mode, for proton affinity and gas-
phase acidity studies, respectively. The nitrogen sheath gas flow rate was set to 20 arbitrary units.

Figure 2 – Cartoon of ESI-Quadrupole Ion Trap Mass Spectrometer

The resulting mass spectra were reviewed to determine whether or not a peak existed for the \([A-H^+-B]\) species. Since the ion trap has MS\(^n\) capabilities, the heterodimer peak was then isolated in the mass spectrometer and retuned in MS/MS mode if the ion count was low or the isolation was poor. MS\(^n\) refers to the ability of the ion trap to repeatedly scan in tandem the same population of ions after sequential isolations and activations. The isolated dimer peak was then activated by helium gas, with activation
amplitudes scanned from 0-100% (of activation voltage), causing collision induced
dissociation (CID). The ion counts of the resulting products, A and B, protonated in
proton affinity studies and deprotonated in gas-phase acidity studies, were recorded for
every 2% increase in activation amplitude. The ratios of B/A for each of the 51 data
points were imported into a Microsoft® Excel for extended kinetic method analysis.

Figure 3 – Sample Mass Spectrum
Figure 4 – Sample Isolation Spectrum

Figure 5 – Sample Fragmentation Spectrum
2.1.2 Using Cooks’ Kinetic Method

The gas-phase acidity of ornithine, 2,4-diaminobutyric acid, 2,3-diaminopropionic acid, canavanine, and citrulline, and the proton affinity of citrulline and L-BMAA were determined by Cooks’ kinetic method. The kinetic method was chosen because it allows for the measurement of non-volatile species. The enthalpies of deprotonation and protonation are related by the kinetic method to the rate constants of the two dissociation channels of a proton-bound heterodimer of the analyte ion A and several reference ions \( B_i \).\(^{13}\) The dissociation channels for gas-phase acidity are described by the following scheme:

\[
A^- + B_i H \xrightleftharpoons[k_A]{k_{A}} [A HB_i] \xrightarrow[k_{Bi}]{k_{Bi}} AH + B_i^- 
\]

The proton affinity is given by:

\[
AH^+ + B_i \xrightleftharpoons[k_A]{k_{A}} [A HB_i] \xrightarrow[k_{Bi}]{k_{Bi}} A + B_i H^+ 
\]

The rate constants are related to enthalpy by the following simple kinetic method equations:

\[
\ln \left( \frac{k_A}{k_{Bi}} \right) = \frac{\Delta(\Delta G)}{RT} = \frac{\Delta(\Delta H)}{RT} = \frac{\Delta PA}{RT}
\]

In the simple kinetic method, the entropy term is disregarded. The \( \Delta (\Delta H) \) term represents the change in enthalpy between both dissociation channels, which is shown to be equal to the difference in proton affinity. The kinetic method assumes that the ratio of the abundance of reference to analyte, measurable by the mass spectrometer, is equal to the ratio of dissociation constants if there is no secondary fragmentation.\(^{14}\) This gives the following equation:

\[
\ln \left( \frac{I_A}{I_B} \right) \approx \ln \left( \frac{k_A}{k_{Bi}} \right) = \frac{\Delta PA}{RT}
\]
The temperature term is not the temperature of the ion trap itself, but an effective
temperature of the reaction system. The assumption that entropy is negligible does not
hold when reference ions are structurally dissimilar to analyte ions, which was often the
case with this experiment. Fenselau\textsuperscript{15} and Wesdemiotis\textsuperscript{16} proposed an extended kinetic
method where the entropy term is included in the kinetic method equation. Armentrout\textsuperscript{17}
proposed the following procedure for kinetic method experiments: plot \( \ln(I_A/I_B) \) vs \( \Delta H_{B_i} - \Delta H_{avg} \), the difference in enthalpy between each reference and the average enthalpy of
all references, obtaining the slope of the plot, \(-1/RT_{eff}\), and the intercept, \([\Delta H(A) - \\
\Delta H(B_{i,avg})]/RT_{eff} - \Delta S/R\). The point where the lines plotted by the first kinetic method
plot converge is called the isothermal point, and the enthalpy can be taken from this
point. The x-value of this point corresponds to the difference between the average acidity
of the reference acids and observable gas-phase acidity. The y-value corresponds to the
entropy term. The negative intercept is plotted against the slope to create the second
kinetic method plot. The slope of kinetic method plot two, \( \Delta H(A) - \Delta H(B_{i,avg}) \), gives
either the proton affinity or gas-phase acidity, and the intercept of the plot, \( \Delta S/R \), gives
the entropy.

2.1.3 Orthogonal Distance Regression (ODR)

As described by Armentrout,\textsuperscript{18} the estimation of experimental error is made by
forcing a single isothermal point in the first kinetic method plot. The isothermal point
exists when the equilibrium constant \( K = \frac{k_B}{k_A} \approx \frac{B_i}{A} \) is the same for all effective
temperatures. Since the extended kinetic method gas-phase acidity or proton affinity as
well as the entropy is extracted from the isothermal point, the error in this point is the
experimental error. With the ODR analysis, the user specifies the number of best fit lines, $n$, and the number of energies, $m$, that are forced to intersect at the calculated isothermal point. A Monte Carlo analysis in a user-specified range of uncertainty in reference values and ion abundance measurements generates random error to account for the error in these measurements. The range of error used in these experiments was ± 8 kJ/mol for PA and ± 0.05 in the natural log of the fragmentation ratio. The error derived from these analyses represents a 95% confidence interval. The final ODR analysis gives values for enthalpy and entropy that, if there is a sharp isothermal point, is the same as the extended kinetic method value.

2.2 Computational Methods

Energy-minimized structural conformations of the NPAAs under study were found by using a GMMX search algorithm in PCModel. These conformers were used as starting structures for ab initio Hartree-Fock and hybrid B3LYP density functional theory (DFT) calculations$^{19,20}$ to find the lowest energy structures, using Gaussian98.\textsuperscript{21} The structures were subjected to a sequence of RFH/3-21 G, B3LYP/3-21 G, 6-31+G*, and 6-311++G** levels of theory. Vibrational frequencies and optimized geometries were calculated at the B3LYP/6-31+G* level of theory. Zero point energies and thermal corrections to enthalpy were acquired from the unscaled vibrations. Single-point energies were calculated at the 6-311++G** level. The single-point electronic energies for the neutral and ionic structures were added to their respective enthalpy corrections, which take into account zero-point energies, the integrated heat capacity, and the pressure-volume work term to give the enthalpies of the structures at room temperature.
Proton-affinities were predicted at 298K by the isodesmic reaction with the reference ethylenediamine (PA = 951.6 kJ/mol).\textsuperscript{22}

\[ A + \text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_3^+ \rightarrow \text{AH}^+ + \text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2 \]

Likewise, gas-phase acidities were found by the isodesmic reaction with the reference acetic acid (\(\Delta H_{\text{acid}} = 1456\) kJ/mol).\textsuperscript{23}

\[ A + \text{OAc}^- \rightarrow A^- + \text{HOAc} \]

If more than one protonation or deprotonation site was present for the NPAA, separate calculations were run for each of the isomers. The lowest energy optimized geometries were examined to predict protonation and deprotonation sites and any structural changes upon proton transfer.

**Chapter III – Results and Discussion**

3.1 The Lysine Series: Ornithine, 2,4-Diaminobutyric Acid, 2,3-Diaminopropionic Acid

The proton affinities of the lysine series have been previously measured by the Poutsma group.\textsuperscript{24} Lysine is interesting both for its biological relevance and its gas-phase chemistry. Lysine’s role in proteins is important as its positively-charged side chain at a neutral pH gives it hydrophilic properties. These properties cause proteins to fold in such a way that lysine residues are generally found on the outside of globular proteins. Lysine is also important in stabilizing negatively charged substrates at enzyme active sites. Trypsin peptide hydrolysis targets lysine and arginine residues, so lysine has important proteomics implications as well. Ornithine, one of the structural homologs of lysine, is an important urea-cycle intermediate.\textsuperscript{25}
The proton affinities of the lysine series was shown to closely correspond to their respective α,ω-diamines. The proton affinity of these diamines has been shown to largely reflect upon their ability to form hydrogen bonds between the α and ω amino groups.\textsuperscript{36} Hydrogen bonding for the lysine series was exhibited by density functional theory calculations by the Poutsma group. The change in conformation from extended to constricted was shown to yield a large decrease in entropy upon protonation of the lysine homologs. Another important trend found was that proton affinity decreased significantly with chain length size for 2,4-diaminobutyric acid and 2,3-diaminopropionic acid. The gas-phase acidities of the lysine homologs were measured to discover whether these trends would carry over to the anionic case.

![Neutral Structures of 2,3-Diaminopropionic Acid, 2,4-Diaminobutyric Acid, Ornithine, and Lysine](image)

**Figure 6— Neutral Structures of 2,3-Diaminopropionic Acid, 2,4-Diaminobutyric Acid, Ornithine, and Lysine**
3.1.1 Ornithine

Reference acids for ornithine were chosen based on previous reference acids used for lysine by the Poutsma group. The reference acids and their gas-phase acidities are listed in Table 1:

<table>
<thead>
<tr>
<th>Reference Acid</th>
<th>Gas-Phase Acidity (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Fluorobenzoic acid</td>
<td>1410 ± 8.8</td>
</tr>
<tr>
<td>3-Hydroxybenzoic acid</td>
<td>1417 ± 8.8</td>
</tr>
<tr>
<td>2,5-Dimethylbenzoic acid</td>
<td>1420 ± 8.8</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>1423 ± 9.2</td>
</tr>
<tr>
<td>p-Toluic acid</td>
<td>1425 ± 8.8</td>
</tr>
<tr>
<td>Phenylacetic acid</td>
<td>1429 ± 8.8</td>
</tr>
</tbody>
</table>

Table 1 – Ornithine Reference Acids

Ornithine and reference acids were solvated in solutions of 79.5:19.5 methanol/water with 1% ammonium hydroxide at a concentration of $5 \times 10^{-4}$ M. The ornithine-reference acid fragmentation spectra were taken as described in the procedure in steps of activation amplitude of 2% from 2% to 100%. Using Armentrout’s procedure, a plot of $\ln(I_B/I_A)$ vs $\Delta H_B - \Delta H_{B_{avg}}$ was made for several activation amplitudes. In the workup of the data, the slope and intercept are calculated for all activation amplitudes. Activation amplitude is plotted against the effective temperature, found from the slope of the first plot, $-R/T_{eff}$. 

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Figure 7 – Kinetic Method Plot 1: Ornithine

Figure 7, an illustration of the first kinetic method plot, shows a selection of three representative activation amplitudes from the entire scanned amplitude range. Each of the regression lines represents a different activation energy. From the x-axis, it can be seen that the gas-phase acidity for each reference compound is represented by a horizontally-equivalent set of three points, corresponding to some value +/- x from the average acidity of all reference acids. By the kinetic method prescription, the lines intersect at the isothermal point, where the fragmentation ratio remains constant for all effective temperatures. With the average of the acidities being 1421 kJ/mol, and the isothermal point being at -9 kJ/mol, an estimate of the extended kinetic method measurement for the gas-phase acidity of ornithine is 1412 kJ/mol.
Using Armentrout’s treatment, the negative intercepts for each of the activation amplitudes in kinetic method plot one were plotted against all of the slopes.

![KM Plot 2](image)

**Figure 8 – Kinetic Method Plot 2: Ornithine**

The $R^2$ value shows the regression line is a good fit for the data, so the slope and intercept of the trend line will accurately reflect the actual slope and intercept of the data. From the extended kinetic method, the sum of the slope of kinetic method plot two and the average of the reference acidities is equal to the gas-phase acidity of ornithine. This was measured to be 1411 kJ/mol. The deprotonation entropy change can also be found by the extended treatment, by multiplying the intercept of plot 2 by the gas constant, giving a value of -19 J/mol K. This shows evidence that upon deprotonation, the structure of ornithine becomes more constricted, which is typical for such gas-phase
processes. The range of activation amplitudes chosen for kinetic method plot one is taken from the plot of effective temperatures versus activation amplitude. Values were taken where the change in temperature with respect to increasing activation energy remained constant.

\[ T_{\text{eff}} \text{ vs AA} \]

![Graph of \[ T_{\text{eff}} \text{ vs AA} \]](image)

**Figure 9 – Effective Temperature Plot: Ornithine**

The best estimate from this range is from 16\% to 34\% activation amplitude. Beyond 34\%, the points level off, with the effective temperatures no longer increasing at a constant rate with the increase in activation amplitude. One explanation for this is that beyond 34\% activation amplitude parent heterodimer is completely fragmented. Another explanation is that after a certain number of collisions the parent ion might be cooled by
transferring energy back to the helium collision gas. Many of the other effective temperature plots also exhibit this behavior.

The error bars were obtained using ODR analysis. Using the six references above with the described effective temperature range, the regression lines for kinetic method plot one were forced to intersect at one isothermal point. The plotted points are the same points used in the extended kinetic method plot one shown above. The Monte Carlo statistical analysis from this plot gave a 95% confidence interval of ± 20 kJ/mol for the gas-phase acidity measurement. Forcing one isothermal point provides a kinetic method plot two $R^2$ value of 1, showing a perfect correlation of the trend line to slope and intercept. Thus, the final measured values for enthalpy and entropy are extracted from the ODR analysis to be 1410 ± 20 kJ/mol and -19 J/mol K, respectively.

KM Plot 1

![Figure 10 – ODR Kinetic Method Plot 1: Ornithine](image-url)
The gas-phase acidity of lysine was measured previously by the extended kinetic method.\textsuperscript{25} Its measured value of 1416 ± 17 kJ/mol overlaps the gas-phase acidity of ornithine, taking experimental error into consideration. Thus, the removal of one methylene group from the lysine side chain was shown to have no effect on the measured gas-phase acidity. This was found to be true for proton affinity as well, with the proton affinity of lysine being 1006.5 ± 7.2 kJ/mol and the proton affinity of ornithine being 1001.1 ± 6.6 kJ/mol. The positive ion entropy changes, however, were much larger, measured to be -77 J/mol K and -52 J/mol K for lysine and ornithine, respectively, in contrast to the much smaller respective negative ion entropy changes of -9 J/mol K and -19
The large entropy changes for the positive ions were due to cyclization by intramolecular hydrogen bonding between the two amino groups upon protonation. The disparity between positive and negative ion entropies is most likely due to a cyclic, N-terminus to side chain amine-bound, neutral lysine transition state\textsuperscript{27} for lysine, and for the neutral and deprotonated structures found by density functional theory for ornithine both being fairly extended.

Theoretical calculations were performed using the hybrid DFT B3LYP method, with the same basis sets used as described above for single-point energies and optimized geometries. Using the isodesmic reaction with acetic acid, the gas-phase acidity of ornithine was calculated to be 1421 kJ/mol, in considerable agreement with the measured value within experimental error.

![Neutral Ornithine and Deprotonated Ornithine](image)

**Figure 12 – Geometrically Optimized Neutral and Deprotonated Ornithine Structures**

The change in structure upon deprotonation helps explain the decrease in entropy of ornithine. Where the neutral structure is free to rotate all bonds both on the side chain and at the N- and C-terminal ends, the deprotonated structure appears more constricted. There is clear evidence of hydrogen bonding between N-terminal amino hydrogen and the
deprotonated carboxylate oxygen. Decreasing the degrees of freedom of the molecule has the obvious result of lowering its total entropy. Neither of the side chains take part in an intramolecular hydrogen bonding network, and thus do not contribute to the measured entropy change. Since neither side chain is conformationally constricted, it is obvious as to why the entropy change for ornithine deprotonation is much less than for its protonation.

3.1.2 2,4-Diaminobutyric Acid

Reference acids for 2,4-diaminobutyric acid (DABA) were chosen from a modified ornithine reference acid set. They are displayed with their acidities in Table 2:

<table>
<thead>
<tr>
<th>Reference Acid</th>
<th>Gas-Phase Acidity (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Fluorobenzoic acid</td>
<td>1406 ± 8.8</td>
</tr>
<tr>
<td>4-Fluorobenzoic acid</td>
<td>1410 ± 8.8</td>
</tr>
<tr>
<td>3-Hydroxybenzoic acid</td>
<td>1417 ± 8.8</td>
</tr>
<tr>
<td>m-Toluic acid</td>
<td>1425 ± 8.8</td>
</tr>
<tr>
<td>Phenylacetic acid</td>
<td>1429 ± 8.8</td>
</tr>
</tbody>
</table>

Table 2 – DABA Reference Acids

DABA-reference binary solutions were made in 79.5:19.5 methanol/water with 1% ammonium hydroxide at a concentration of $5 \times 10^{-4}$ M. Again, the activation amplitude for the isolated heterodimer peak was scanned from 2% to 100%. The fragmentation ratios at each of the amplitudes were recorded and kinetic method plots one and two were made for representative activation amplitudes, found using the constant-slope effective temperature method detailed above.
\[ \ln(A_i/A) \text{ vs } GA_i - GA_{avg} \]

Figure 13 – Kinetic Method Plot 1 – DABA

\[ y = 2.1354x + 0.0495 \]
\[ R^2 = 0.6224 \]

Figure 14 – Kinetic Method Plot 2 – DABA
Figure 15 – Effective Temperature Plot – DABA

The constant slope range from the effective temperature plot is from activation amplitude 18% to 46%. Plotting the two extreme points and a middle point gives the above kinetic method plot one. There appears to be an isothermal point at $x \approx 1$ kJ/mol, which would give an estimate of the extended kinetic method gas-phase acidity of 1418 kJ/mol. However, the isothermal point is somewhat ambiguous, as it appears all three trend lines may not cross in the same place. An ODR analysis is especially important for DABA because the isothermal point ambiguity is removed with the forcing of all three lines to intersect at one point. The $R^2$ value in kinetic method plot two is lower than optimal, showing a poor correlation between the linear best-fit line and the calculated slopes and intercepts for the data points. The sum of the slope of plot two and the average gas-phase acidity of the references gives 1420 kcal/mol as the gas-phase acidity for DABA. Multiplying the intercept of plot two by the gas constant gives an entropy change
of approximately 0 J/mol K. The absence of a true isothermal point in plot one coupled with poor correlation between a linear best fit line and the plot two data necessitate an ODR analysis of the data. The apparent lack of entropy warrants further discussion, and can be more readily discussed upon comparing the optimized DFT geometries.

**Figure 16 – ODR Kinetic Method Plot 1: DABA**

The first ODR kinetic method plot shows the characteristic single isothermal point. The experimental gas-phase acidity of DABA with Monte Carlo-generated error is found to be 1420 ± 8 kJ/mol. The entropy is 1 J/mol K. The ODR analysis numbers match closely with the numbers found from the extended kinetic method workup. The gas-phase acidity of DABA was measured to be the same as for ornithine and lysine, within the ODR-calculated experimental error. This measurement continues to provide evidence that removing side chain methylene groups has little or no effect on the thermodynamic
stability of the conjugate bases. This is not true for the positive ion case, where the proton affinity of DABA is $975.8 \pm 7.4$ kJ/mol, more than 25 kJ/mol less than the proton affinity of ornithine. It is thus shown that the two side chain methylene groups that were removed have no effect on the electron withdrawing or donating with respect to the carboxylic acid group. Perhaps this is indicative that the presence of the amino groups affects the gas-phase acidity more than their relative distance from the C-terminus. There is a significant difference between the measured entropy change in DABA from the other lysine homologs measured, as well as for the DABA positive ion case, where the entropy change was measured previously to be -36 J/mol K. This suggests that whereas for lysine and ornithine the deprotonated structures were more constrained, the DABA neutral and anion were both constrained. The protonated DABA structure is much more constrained than the neutral, accounting for the entropy-change difference. This is elucidated by DFT calculations.

Optimized geometries and single-point energies were calculated for DABA with hybrid DFT for comparison with the experimental values and other lysine homologs. Using the described basis sets and the isodesmic reaction with acetic acid, DFT predicts the gas-phase acidity of DABA to be 1413 kcal/mol, in agreement with the experimental value within assigned experimental error.
The DFT optimized geometries are helpful in understanding why the deprotonation entropy of DABA might be lower than is expected, considering the larger value found for ornithine. Examination of the neutral structure reveals that there may be hydrogen bonding present between the two amino groups. The DABA side chain amine serves as the hydrogen bond donor, while the terminal amine serves as the hydrogen bonding acceptor. Therefore, the neutral structure is already conformationally constrained. The deprotonated form shows two hydrogen bonding possibilities, one of which is the terminal amine donating to one of the carboxylate oxygens, which would account for much less negative entropy than would the hydrogen bonding of the neutral species. However, the side chain also donates to the other carboxylate oxygen, with the resulting optimized geometry very similar entropically to the neutral molecule. In both structures, the side chains are rotationally restricted.
3.1.3 2,3-Diaminopropionic Acid

Reference acids chosen for 2,3-diaminopropionic acid (DAPA) are a subset of the previously listed references, detailed below:

<table>
<thead>
<tr>
<th>Reference Acid</th>
<th>Gas-Phase Acidity (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Fluorobenzoic acid</td>
<td>1406 ± 8.8</td>
</tr>
<tr>
<td>3-Hydroxybenzoic acid</td>
<td>1417 ± 8.8</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>1423 ± 9.2</td>
</tr>
<tr>
<td>Phenylacetic acid</td>
<td>1429 ± 8.8</td>
</tr>
</tbody>
</table>

Table 3 – DAPA Reference Acids

DAPA and the reference acids were at a concentration of 5x10^{-4} M, with a 79.5:19.5 methanol/water mixture solvent acidified by 1% NH₄OH. Fragmentation ratios of the collision activated analyte-reference dimers were recorded for scanned activation amplitudes from 2-100%. The natural logs of the fragmentation ratios were plotted against the enthalpy differences between each reference and the average, resulting in the following plot:
The lines appear to cross at approximately -12 kJ/mol, giving an extended kinetic method apparent gas-phase acidity of 1407 kJ/mol. However, there is not a clear isothermal point here, so this approximation may not be valid for DAPA. The negative intercepts from plot one for each scanned amplitude were plotted against the slopes, giving the second kinetic method plot, as described by Armentrout.

**Figure 18 – DAPA Kinetic Method Plot 1**
As with DABA, the second kinetic method plot for DAPA shows poor correlation between the trend line and the set of data points. Using the extended kinetic method treatment, the gas-phase acidity extracted from the slope of the trend line is measured to be 1409 kJ/mol. The intercept yields an entropy of -18 J/mol K. The range of data points used was again picked from the effective temperature plot where the most constant change in effective temperature as a function of activation amplitude was found.
Therefore, the chosen activation amplitude range for analysis was from 18% to 46%. To provide for the magnitude of the experimental error, an ODR analysis was performed for DAPA, giving the following plots after forcing an isothermal point.
Figure 21 – ODR Kinetic Method Plot 1 - DAPA

The first kinetic method plot after ODR analysis shows that the lines cross before what is expected from the extended kinetic method plot, meaning that the apparent gas-phase acidity was overestimated from the data. The gas-phase acidity of DAPA within the Monte Carlo confidence interval is $1405 \pm 24$ kJ/mol. The entropy is $-24$ J/mol K. The gas-phase acidity of DAPA is slightly lower than the rest of the lysine series, but there is not the characteristic difference as seen with the proton affinities. The proton affinity of DAPA was measured to be $950.2 \pm 7.2$ kJ/mol, about lower 25 kJ/mol than DABA. The difference in gas-phase acidities is about 15 kJ/mol. The trend in the gas-phase acidities of the lysine homologs is thus much less pronounced than is the case for the proton affinities. The entropy change found for DAPA is significantly similar to the
entropy change found for ornithine, and likewise, is much less than the entropy change upon protonation, previously measured to be -49 J/mol K, for the same reasons given for ornithine.

Figure 22 – Optimized Neutral and Deprotonated DAPA Geometries

Using B3LYP with the same basis sets as above for optimization, frequency, and single-point energy calculations, the gas-phase acidity was found to be 1415 kJ/mol using the isodesmic reaction with acetic acid. This prediction falls within the range of experimental error from the measured value. Therefore, the optimized geometries can be examined as reliable illustrations of the molecules in the gas phase. Similar to ornithine, the neutral form of the molecule appears to be less constricted than the deprotonated form, decreasing the entropy of the molecule. The deprotonated form has hydrogen bonding which prevents the N-terminus and the C-terminus from rotating about their bonds. The N-terminal hydrogen is donated to one of the C-terminal oxygens through hydrogen bonding.

The compiled results for the lysine series are compiled in the following table:
<table>
<thead>
<tr>
<th></th>
<th>Experimental Gas-Phase Acidity (kJ/mol)</th>
<th>Experimental ∆S (J/mol K)</th>
<th>Hybrid DFT Gas-Phase Acidity (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>1416 ± 7</td>
<td>-9</td>
<td>1415</td>
</tr>
<tr>
<td>Ornithine</td>
<td>1410 ± 20</td>
<td>-19</td>
<td>1421</td>
</tr>
<tr>
<td>DABA</td>
<td>1420 ± 8</td>
<td>1</td>
<td>1413</td>
</tr>
<tr>
<td>DAPA</td>
<td>1405 ± 24</td>
<td>-24</td>
<td>1415</td>
</tr>
</tbody>
</table>

**Table 4 – Lysine Series Results**

The evident decrease in proton affinity between ornithine and DABA and between DABA and DAPA was not apparent for the gas-phase acidities, especially if DAPA’s full 95% confidence interval is considered. With this consideration, the gas-phase acidity is shown to be almost constant for all of the lysine homologs. To see if this difference in trends was true for all twenty amino acids, the previously measured range of proton affinity and acidity values were compared. The proton affinities ranged from 886 kJ/mol (glycine) to 1018 kJ/mol (arginine), while the gas-phase acidities ranged from 1434 kJ/mol (glycine) to 1345 (aspartic acid). The proton affinity range was 132 kJ/mol while the gas-phase acidity range was 89 kJ/mol. The proton affinity range is larger, but still not indicative of the lack of enthalpy differences found for the gas-phase acidities of the lysine homologs. The entropy changes for deprotonation of the lysine homologs were found to be much less than the entropy changes for protonation.

### 3.2 Arginine Analogs: Canavanine and Citrulline

Arginine exhibits chemical properties and roles in proteins similar to lysine and is the most basic PAA. Canavanine, a structural analog of arginine, has been extensively studied for its ability to be incorporated into a protein in place of arginine. This has led some researchers to believe that canavanine can be used for cancer treatment. Citrulline, another analog of arginine, is used by the kidneys to synthesize arginine.
The proton affinity of canavanine has been measured by the extended kinetic method.\textsuperscript{35} The structural change between arginine and canavanine, the side-chain methylene to oxygen substitution, was shown to decrease proton affinity. The proton affinity of arginine was measured to be $1034 \pm 18$ kJ/mol and the proton affinity of canavanine was measured to be $1001 \pm 9$ kJ/mol. The effects of substituting oxygen for a guanidinium amino group on proton affinity as well as the gas-phase acidities of citrulline and canavanine are investigated in this study. These experiments are complimentary to the lysine homolog studies. In that case, the effects of side-chain length on proton affinity and gas-phase acidity were investigated, in contrast to altering the functional groups on the amino acid side chain.

![Neutral Structures of Arginine, Citrulline, and Canavanine](image)

**Figure 23 – Neutral Structures of Arginine, Citrulline, and Canavanine**

### 3.2.1 Canavanine

The following reference acids were used for measuring the gas-phase acidity of canavanine\textsuperscript{36}:
<table>
<thead>
<tr>
<th>Reference Acid</th>
<th>Gas-Phase Acidity (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Nitrophenol</td>
<td>1400 ± 11</td>
</tr>
<tr>
<td>2-Chlorobenzoic acid</td>
<td>1402 ± 8.8</td>
</tr>
<tr>
<td>3-Fluorobenzoic acid</td>
<td>1406 ± 8.8</td>
</tr>
<tr>
<td>4-Fluorobenzoic acid</td>
<td>1410 ± 8.8</td>
</tr>
<tr>
<td>3-Hydroxybenzoic acid</td>
<td>1417 ± 8.8</td>
</tr>
</tbody>
</table>

**Table 5 – Canavanine Reference Acids**

Binary solutions of canavanine and the reference acids were solvated by 1% NH₄OH, 99% methanol solutions to a concentration of 5x10⁻⁴ M. The canavanine-reference heterodimers were isolated in the mass spectrometer, and fragmented by collision induced dissociation with the helium buffer gas. These activation amplitudes were scanned from 2% to 100%, as with the lysine series. The reference/analyte fragmentation ratios were recorded, and the natural logarithms of these ratios were plotted against the gas-phase acidity differences between the average of the reference acidities and each reference.
Figure 24 – Kinetic Method Plot 1: Canavanine

Again, three representative activation amplitudes were chosen from the data set. As with the lysine series, all the points were used in the range of activation amplitudes used, not only the three picked for this illustration. Again, the isothermal point is used to determine an approximation for the extended kinetic method gas-phase acidity. The three best fit lines appear to cross at about -7 kJ/mol. The average gas-phase acidity of the reference acids is 1407 kJ/mol, giving an apparent gas-phase acidity of 1400 kJ/mol.

The negative intercepts of the data from the first kinetic method plot were plotted against the slopes.
Figure 25 – Kinetic Method Plot 2: Canavanine

The $R^2$ value is close to unity, showing a close correlation between the fit line and the data points. Using the extended kinetic method treatment, the slope of the best-fit line from kinetic method plot two is added to the average of the references’ gas-phase acidities. The extended kinetic method gas-phase acidity of canavanine was measured to be 1399 kJ/mol, as compared to the apparent value of 1400 kJ/mol. By multiplying the intercept by the gas constant, the entropy change for the deprotonation of canavanine is -13 J/mol K. The range of activation amplitudes used in the kinetic method plots, 30%-84%, was again found by using the constant slope portion of the effective temperature plot. The activation amplitude range 14%-24% represents another constant slope range of the plot, though the slope is larger than for 30%-84%. The extended kinetic method gas-phase acidity of canavanine found over this activation amplitude interval was also
1399 kJ/mol, no different than the value found for the next constant slope range of the effective temperature plot.

\[ T_{\text{eff}} \text{ vs AA} \]

**Figure 26 – Effective Temperature Plot: Canavanine**

An ODR analysis with Monte Carlo statistical analysis was performed on the canavanine data. By forcing an isothermal point, the following ODR plots were generated:
Figure 27 – ODR Kinetic Method Plot 1: Canavanine

The fit lines from the first ODR plot appear to intersect again at about -7, so the extended kinetic method plot one lines intersected at a sharp isothermal point. The gas-phase acidity of canavanine with experimental error and its entropy change can be extracted from ODR plot 2. The Monte Carlo statistical analysis gave a standard error of ± 13 kJ/mol. Thus, the gas-phase acidity of canavanine with a 95% confidence interval is 1401 ± 13 kJ/mol. The entropy change for the deprotonation of canavanine was -8 J/mol K. The measured gas-phase acidity of arginine was 1381 ± 9 kJ/mol\(^2\). The substitution of the methylene group alpha to the guanidinium group with oxygen decreases the gas-phase acidity.
The gas-phase acidity of canavanine was predicted theoretically by B3LYP calculations, as with the lysine series\textsuperscript{37}. Using the isodesmic reaction with acetic acid, the gas-phase acidity prediction was 1420 kJ/mol. The hybrid DFT result is high, slightly above the range of the standard error for canavanine. This is probably indicative that the GMMX search algorithm did not find the true minimum energy structure for the canavanine anion. However, the optimized geometries are still helpful in explaining the decrease in entropy upon deprotonation. The lowest energy deprotonated structure appears to have a side chain amino group hydrogen bonded to the N-terminal amino group. This becomes the favored conformation because of another apparent hydrogen bond, this one between the N-terminus and C-terminal oxygen. Since the N-terminus is not allowed to freely rotate, the side chain is able to hydrogen bond to it, constraining the structure.
3.2.2 Citrulline

3.2.2.1 The Gas-Phase Acidity of Citrulline

Binary solutions of citrulline and reference acids were made up of 79.5:19.5 methanol/water with 1% ammonium hydroxide at a concentration of 5x10^{-4} M. The reference acids used with citrulline were the following\textsuperscript{38,39}:

<table>
<thead>
<tr>
<th>Reference Acid</th>
<th>Gas-Phase Acidity (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Hydroxybenzoic acid</td>
<td>1362 ± 9.2</td>
</tr>
<tr>
<td>Dichloroacetic acid</td>
<td>1369 ± 8.8</td>
</tr>
<tr>
<td>4-Nitrophenol</td>
<td>1372 ± 8.8</td>
</tr>
<tr>
<td>4-Nitrobenzoic acid</td>
<td>1373 ± 9.2</td>
</tr>
<tr>
<td>3-Nitrobenzoic acid</td>
<td>1377 ± 8.8</td>
</tr>
</tbody>
</table>

Table 6 – Citrulline Reference Acids

Activation amplitudes were scanned from 2% to 100% and the fragmentation ratios of reference to citrulline were used to develop the following plots:
Figure 29 – Kinetic Method Plot 1: Citrulline Acidity

Figure 30 – Kinetic Method Plot 2: Citrulline Acidity
Figure 31 – Effective Temperature Plot: Citrulline Acidity

The effective temperature plot was used to find a range of activation amplitudes over which the slope of the plot remained constant to make the two kinetic method plots. Three activation amplitudes were chosen from this range in the depiction of the kinetic method plot one (Fig 29). Here, the isothermal point does not exist, as the three lines do not cross at the same point. A good estimate for where this point should be is -5 kJ/mol, giving an enthalpy measurement of 1366 kJ/mol. The negative intercepts of plot one were plotted against the slopes, giving kinetic method plot two, which exhibits a strong linear correlation. Multiplying the intercept by the gas constant gives an extended kinetic method entropy change of -9 J/mol K. The slope of this plot is -5 kJ/mol, giving an extended kinetic method enthalpy measurement of 1366 kJ/mol, as is expected from the estimated isothermal point. The Monte Carlo statistical error analysis as well as the ODR
analysis results for enthalpy and entropy, forcing the isothermal point in kinetic method plot one, gave the following results:

**Figure 32 – ODR Kinetic Method Plot 1: Citrulline Acidity**

The ODR plot one has an isothermal point of -5 kJ/mol, as was estimated earlier, meaning that the ODR and extended kinetic method values would be close. The ODR-obtained gas-phase acidity is 1366 kJ/mol, the same as the extended kinetic method version. Standard error in the acidity measurement was ± 11 kJ/mol. The entropy change for citrulline deprotonation was -8 J/mol K.

The experimental gas-phase acidities of the studied arginine analogs are 1401 kJ/mol for canavanine, 1381 kJ/mol for arginine, and 1366 kJ/mol for citrulline. The side chain methylene substitution for oxygen in canavanine was shown to make canavanine less acidic than arginine, while substituting oxygen for a side chain imine group in
citrulline was shown to make citrulline more acidic than arginine (refer to Figure 23, pg. 38).

The B3LYP prediction for the gas-phase acidity of citrulline, using the isodesmic reaction with acetic acid, is 1377 kJ/mol. This prediction lies at the top of the standard error from the measured value for gas-phase acidity. The optimized geometries first can be used to explain the negative entropy change, as the deprotonated is more constricted than the neutral structure. While there is hydrogen bonding between the first side chain amino group and the N-terminus for the neutral structure, the second side chain amino group is hydrogen bonded to the C-terminus carboxylate oxygen. A longer portion of the side chain is constricted for the deprotonated structure, suggesting a decrease in disorder, and therefore, in entropy. These geometries are also useful in predicting why citrulline is much more acidic than canavanine. The formal negative charge on the citrulline C-terminus is stabilized by hydrogen bonding, while this is not the case for canavanine.
The stability of the conjugate base is indicative of the strength of the parent acid, and since deprotonated citrulline’s carboxylate is more stable, it is a stronger acid.

<table>
<thead>
<tr>
<th></th>
<th>Experimental Gas-Phase Acidity (kJ/mol)</th>
<th>Experimental ΔS (J/mol K)</th>
<th>Hybrid DFT Gas-Phase Acidity (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrulline</td>
<td>1366 ± 11</td>
<td>-8</td>
<td>1377</td>
</tr>
<tr>
<td>Arginine</td>
<td>1381 ± 9</td>
<td>-</td>
<td>1387</td>
</tr>
<tr>
<td>Canavanine</td>
<td>1401 ± 13</td>
<td>-8</td>
<td>1420</td>
</tr>
</tbody>
</table>

**Table 7 – Arginine Homologs: Negative Ions**

### 3.2.3.2 The Proton Affinity of Citrulline

Citrulline’s proton affinity was also measured and compared with those of arginine and canavanine. Solutions for positive ions were made in 49.5:49.5 methanol/water with 1% acetic acid. The following reference bases were used with citrulline:

<table>
<thead>
<tr>
<th>Reference Base</th>
<th>Proton Affinity (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Methylpiperidine</td>
<td>971.1</td>
</tr>
<tr>
<td>Triallylamine</td>
<td>972.3</td>
</tr>
<tr>
<td>Tris(2-methylallyl)amine</td>
<td>980.2</td>
</tr>
<tr>
<td>Triethylamine</td>
<td>981.8</td>
</tr>
<tr>
<td>4,4-Dimethyl-2-imidazoline</td>
<td>988.1</td>
</tr>
</tbody>
</table>

**Table 8 – Citrulline Reference Bases**

Proton-bound positive ion heterodimers of citrulline and reference bases were collisionally activated by scanning activation amplitude from 2% to 100%. The natural logarithms of the resulting ratio of reference base to citrulline were plotted against the difference in proton affinity between each reference base and the average of all references. The negative intercepts of these plots were plotted against the slopes, in the
same was as the negative ion case. Activation amplitude was plotted against temperature to determine a suitable range of activation amplitudes to use for data analysis.

\[
\ln(A_{iH^+/AH^+}) \text{ vs } PA_i - PA_{avg}
\]

![Graph showing \(\ln(A_{iH^+/AH^+})\) vs \(PA_i - PA_{avg}\)](image)

**Figure 34 – Kinetic Method Plot 1: Positive Citrulline**

**KM Plot 2**

![Graph showing \(y = 5.6177x - 0.9437\) with \(R^2 = 0.8614\)](image)

**Figure 35 – Kinetic Method Plot 2: Positive Citrulline**
Figure 36 – Effective Temperature Plot: Positive Citrulline

The slope in the effective temperature plot remains constant from activation amplitude 28% to 68%, so the corresponding data points were used in the analysis. There is no isothermal point in the first kinetic method plot. Thus, the citrulline proton affinity cannot be estimated by the isothermal point. The $R^2$ value in the second kinetic method plot shows good correlation of the data to a linear best fit line. By adding the slope of the best fit line to the average proton affinity of the reference compounds, the proton affinity of citrulline is measured to be 984 kJ/mol. The product of the intercept and the gas constant gives a protonation entropy change for citrulline of -8 J/mol K.

ODR analysis yields the following plots:
By definition, the first ODR kinetic method plot gives a single isothermal point for positive citrulline at approximately 5 kJ/mol. Using the slope and intercept of the second ODR kinetic method plot as well as the Monte Carlo statistical analysis, the proton affinity of citrulline was measured to be 984 ± 11 kJ/mol with an entropy change of -6 J/mol K. Both values are in close agreement with the extended kinetic method results. The proton affinities of arginine and canavanine have been previously measured\textsuperscript{36} to be 1051 and 1001 kJ/mol, respectively. Citrulline is thus the most acidic and least basic of the three structures in the gas phase. This is likely due to the fact that one of the basic sites of the molecule, a guanidinium imine, is replaced with oxygen. Theoretical predictions of positive citrulline are still in the process of being completed.
3.3 Alanine Analogs: L-BMAA

L-β-methylamino alanine (BMAA) is a structural analog of L-alanine, a hydrophobic PAA, as well as of β-alanine, an NPAA. Its biological relevance is its previously mentioned neurotoxicity. BMAA is naturally produced by cyanobacteria found in every ecosystem, and high levels of BMAA can accumulate in human tissues and pass through the blood-brain barrier. The proton affinity of BMAA is an important property as BMAA is not only structurally similar to α and β-alanine, but it is also structurally related to ethylenediamine and DAPA, both of which have measured proton affinities. By measuring the proton affinity of BMAA and comparing it with its analogs, the effects of both the addition of the amino functional group and the methyl group at the end of the side chain upon proton affinity can be studied.

Figure 38 – Neutral Structures of β-Alanine and L-β-Methylamino Alanine

The proton affinity of β-methylamino alanine (BMAA) was measured by using the following reference bases:22
<table>
<thead>
<tr>
<th>Reference Base</th>
<th>Proton Affinity (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethylamine</td>
<td>952.4</td>
</tr>
<tr>
<td>Piperidine</td>
<td>954</td>
</tr>
<tr>
<td>4-Tert-butyl-pyridine</td>
<td>957.7</td>
</tr>
<tr>
<td>2,6-Lutidine</td>
<td>963</td>
</tr>
<tr>
<td>1-Methylpyrrolidine</td>
<td>965.6</td>
</tr>
</tbody>
</table>

**Table 9 – BMAA Reference Bases**

BMAA and reference bases were made into solutions at a concentration of $5 \times 10^{-4}$ M in 49.5:49.5 methanol/water with 1% acetic acid. The proton-bound heterodimers were activating by scanning amplitude from 2% to 100%, and the natural logarithms of the resulting fragmentation ratios of reference base to BMAA were plotted against the differences in each reference base proton affinity and the average of all proton affinities.
$\ln(A_iH^+/AH^+) \text{ vs } PA_i - PA_{avg}$

**Figure 39 – Kinetic Method Plot 1: BMAA**

**Figure 40 – Kinetic Method Plot 2: BMAA**
Effective temperature was plotted against activation amplitude, and the range 18% to 72% was chosen for analysis. The isothermal point for kinetic method plot one (Fig. 39) appears to be at 1.5 kJ/mol, giving an estimate for proton affinity of 960 kJ/mol. The negative intercepts of the plot one data were plotted against their corresponding slopes, showing a strongly linear correlation. Using the slope from kinetic method plot two, the proton affinity of BMAA is measured to be 961 kJ/mol. Multiplying the intercept by the gas constant gives an entropy change of -8 J/mol K. The experimental data were analyzed by ODR with a Monte Carlo statistical analysis.
Figure 42 – ODR Kinetic Method Plot 1: BMAA

The ODR plot one isothermal point is shifted upward in energy from the kinetic method plot, estimated to be about 2 kJ/mol. The proton affinity and entropy change are given by the second ODR plot, with the standard error provided by the Monte Carlo analysis. The proton affinity of BMAA was measured to be 960 ± 7 kJ/mol, with a protonation entropy change of -4 J/mol K. Alanine has a known proton affinity of 902 kJ/mol, while β-alanine has a measured proton affinity\(^{40}\) of 927 kJ/mol. The added proton of BMAA is due to the second amino group in the side chain not present in the other two compounds. The second protonation site makes BMAA a much more basic compound than either of the two other alanine analogs. The proton affinities of ethylenediamine and DAPA are 952 kJ/mol\(^{22}\) and 950.2 ± 7.2 kJ/mol,\(^{24}\) respectively. Therefore, the carboxylic acid group has very little effect on the proton affinity difference
between ethylenediamine and DAPA. The addition of the methyl group to one of the amino groups gives BMAA its extra proton affinity, which is to be expected from the conversion of a secondary amine to a tertiary amine.

Hybrid DFT calculations on BMAA were performed at the levels of theory mentioned above. Using the isodesmic reaction with ethylenediamine, the proton affinity of BMAA was predicted to be 972 kJ/mol, a value higher than the measured proton affinity of BMAA but not far above standard error. The optimized geometries of neutral and protonated BMAA are presented as follows:

Figure 43 – Optimized Neutral and Protonated BMAA Structures

The lowest energy structure of BMAA presented here shows side-chain protonation. There was found another minimized structure found that exhibited N-terminal protonation. Both of these structures predict a proton affinity of 972 kJ/mol. Predicting two active protonation sites reinforces the claim that the two amino groups contribute to BMAA’s added basicity over the other alanine analogs. The small entropy change is also predicted by a long hydrogen bond between the side-chain amine and the N-terminus.
Chapter IV – Conclusions

The gas-phase acidities of the lysine homologs was measured to determine whether there was a trend of gas-phase acidity corresponding to the side-chain length, as exists for proton affinity.\textsuperscript{24} Lysine and ornithine have the same proton affinity, but proton affinity decreases as more methylene groups are removed from the side chain. No such trend was found for gas-phase acidity. The acidities of ornithine, DABA, and DAPA were measured to be 1410 ± 20, 1420 ± 8, and 1405 ± 24 kJ/mol. Another interesting result was the lack of deprotonation entropy change for DABA, although this was predicted by hybrid DFT. The lowest energy neutral and deprotonated structures of DABA showed similar hydrogen bonding networks, showing the same amount of conformational restriction. The B3LYP calculations gave predictions for gas-phase acidity of 1421, 1413, and 1415 kJ/mol for ornithine, DABA, and DAPA, respectively. Each of these predictions was found to lie within the experimental error of the measured acidities.

The gas-phase acidity of the arginine analogs citrulline and canavanine was also measured. Citrulline had a measured gas-phase acidity of 1366 ± 11 kJ/mol while canavanine was measured to be 1415 ± 13 kJ/mol. Citrulline is the most acidic of the arginine analogs, while canavanine is less acidic than canavanine. Again, B3LYP was used to predict the acidity difference by showing a hydrogen bonding stabilization of the citrulline carboxylate. B3LYP predicted the gas-phase acidities to be 1377 kJ/mol for citrulline and 1420 kJ/mol for canavanine. The density functional theory predictions laid within standard error of the measured values. The proton affinity of canavanine was also measured and compared to the proton affinities of arginine and canavanine. This value
was measured to be 984 ± 11 kJ/mol, making citrulline less basic than the other arginine analogs.

The proton affinity of L-BMAA was measured to be 960 ± 7 kJ/mol, in contrast to a value previously measured by the Poutsma lab⁴¹ of 972 ± 4 kJ/mol that was measured by selectively choosing activation amplitudes between 2% and 100%. The old value is closer to the prediction of 972 kJ/mol by B3LYP. The proton affinity of BMAA shows that it is more basic than α– or β–alanine. The two amino groups of BMAA are both predicted to protonate, providing for its extra basicity.
References


37 Canavanine neutral calculations were performed by Tullo, E.


41 The proton affinity of L-BMAA was measured by Wind, J.