

Decomposition of Protonated Threonine, Its Stereoisomers, and Its Homologues in the Gas Phase: Evidence for Internal Backside Displacement

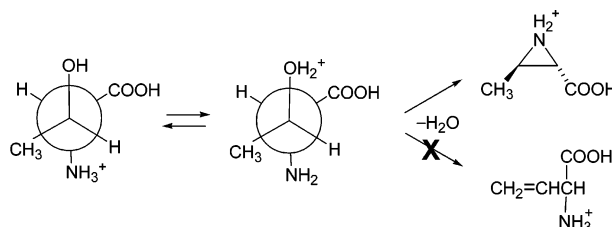
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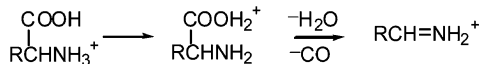
ABSTRACT



Protonated threonine and its *allo* diastereomer exhibit different proportions of collisionally activated dissociation (CAD) product ions. *N*-Methylation attenuates these differences. Water loss from protonated *allo*-threonine gives protonated *trans*-3-methylaziridinecarboxylic acid via an internal S_N2 pathway, rather than protonated vinylglycine.

Protonated amino acids readily expel water in the gas phase. In most cases, loss of water from the carboxylic group is accompanied by CO loss,¹ producing an iminium ion,² as Scheme 1 depicts. In a few instances (notably threonine³), loss of water from the side chain predominates.

Scheme 1. Dissociation of a Protonated Amino Acid



Threonine has two asymmetric carbons. The diastereomer not involved in protein biosynthesis is found in a variety of naturally occurring cyclic peptides.⁴ This fact prompts the question as to whether the two diastereomeric amino acids

can be differentiated by mass spectrometry. This letter reports the collisionally activated dissociation (CAD) spectra of the conjugate acid ions from threonine, its *allo* diastereomer, and their *N*-methyl homologues.

N-Methylthreonine has also been found in peptides,⁵ but the *N*-methyl *allo* diastereomer has yet to be reported. As molecules incorporating this latter isomer may occur in nature, we describe its preparation (starting from the amino acid, using a method previously reported⁶) and its CAD. The *threo* and *allo* isomers exhibit different fragmentation pat-

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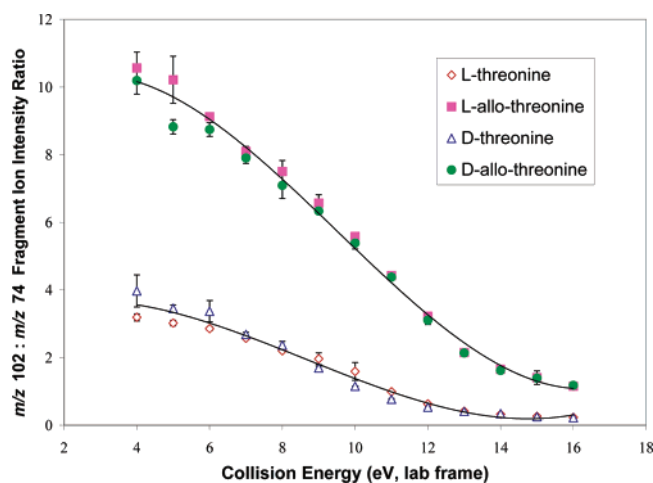


Figure 1. Ratios of H₂O loss to H₂O + CO loss from D- and L-threonine (open symbols) and their *allo*-diastereomers (solid symbols) as a function of collision energy of *m/z* 120 parent ions.

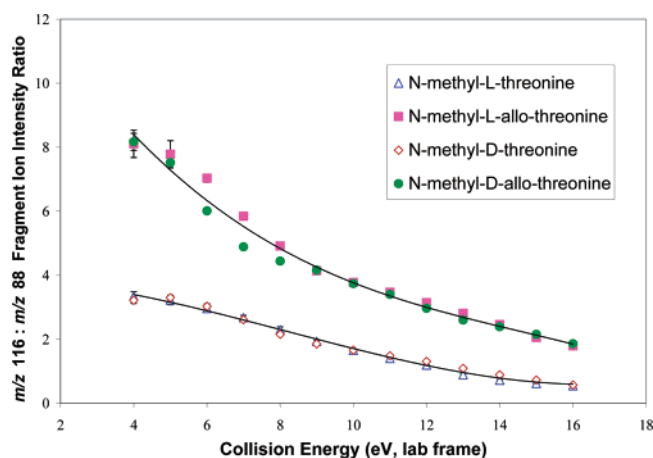
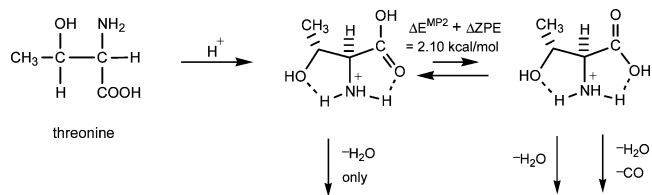


Figure 2. Ratios of H₂O loss to H₂O + CO loss for D- and L-*N*-methylthreonine (open symbols) and their *allo*-diastereomers (solid symbols) as a function of energy of *m/z* 134 parent ions.

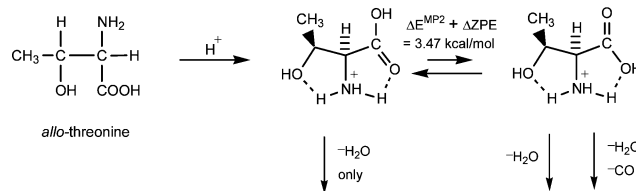
function of collision energy for all four stereoisomers. Figure 2 displays the same type of plot for all four stereoisomers of *N*-methylthreonine. Given the pronounced increase of single-water loss in the *allo* relative to *threo*, the first question concerns whether that dissociation represents elimination only from the side chain. This was assessed by labeling the *allo* with both carboxylic oxygens replaced by ¹⁸O (in the same manner as doubly ¹⁸O-labeled *threo* has been prepared³). The CAD spectrum of labeled *allo* shows that loss of a single water comes exclusively from the side chain, while loss of H₂O plus CO comes exclusively from the carboxyl group (consistent with Scheme 1).

As will be discussed below, additional experimental data corroborate the S_N2 mechanism shown in Scheme 4. Before presenting that evidence, though, an alternative hypothesis should be weighed; namely, that the difference between diastereomers does not result from competing transition states

Scheme 5. Rotamers of Protonated Threonine and Their Dissociation Products



Scheme 6. Rotamers of Protonated *allo*-Threonine and Their Dissociation Products



but instead reflects the rotameric distribution of the protonated amino acids prior to dissociation. The effect of methylation tests this second hypothesis. Schemes 5 and 6 portray these equilibria, including ab initio enthalpy differences.⁸ The most favored structures have the NH₃⁺ hydrogen-bonded to both the side chain oxygen and the carbonyl oxygen. Only side-chain water loss can take place from this geometry. Less favored rotamers have the NH₃⁺ hydrogen-bonded to the side-chain oxygen and to the carboxylic OH group. Loss of water from either position can occur from this geometry.

N-Methylation increases the enthalpy difference ($\Delta\Delta H$) between *allo* and *threo* isomers. The calculated $\Delta\Delta H$ for the amino acids is 1.37 kcal mol⁻¹ (the difference between $\Delta E^{\text{MP2}} + \Delta ZPE$ in Scheme 6 and $\Delta E^{\text{MP2}} + \Delta ZPE$ in Scheme 5). The corresponding difference for the *N*-methyl amino acids is $\Delta\Delta H = 1.66$ kcal mol⁻¹. If the rotameric equilibrium by itself determined the dissociation pattern, one would have expected *N*-methylation to increase the proportion of *allo* side-chain water loss relative to *threo*. Comparison of Figures 1 and 2 demonstrates that the exact opposite result is obtained.

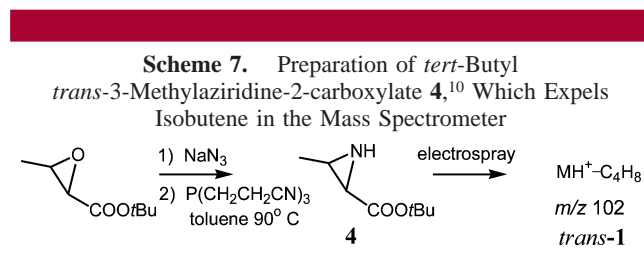
The observed consequences of *N*-methylation do agree with expectation, if the rate-limiting step for side-chain water loss corresponds to the pathways depicted in Scheme 4. The question then arises whether that reaction operates via the internal S_N2 pathway or via the internal E2. O'Hair and Reid⁸ have argued in favor of the former, on the basis of their deuterium labeling experiments. MS/MS of the ions from skimmer-induced side-chain water loss substantiates their interpretation.

As Table 1 summarizes, CAD of the ions from water loss (*m/z* 102 from the threonines; *m/z* 116 from the *N*-methyl derivatives) shows significant differences between the ions

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originating from *threo* and *allo* isomers. Such an outcome is consistent with the S_N2 pathway. The stereospecificity of backside displacement predicts that the *threo* ought to yield a cis aziridine, while the *allo* should give a trans aziridine.

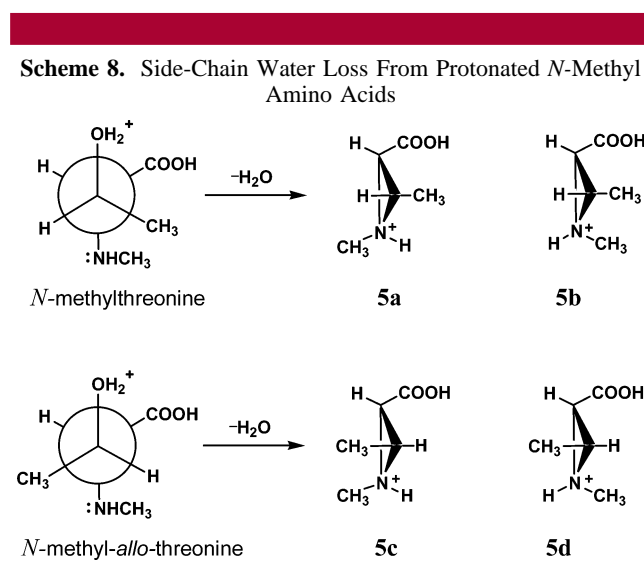
To test this stereochemical assignment, the authentic trans aziridine ion was synthesized from the epoxide of *tert*-butyl *trans*-crotonate using established methods,¹⁰ as Scheme 7



outlines. Because of the known lability of free aziridinecarboxylic acids, *tert*-butyl ester **4** was prepared. Under electrospray conditions, the aziridine ester exhibits not only the protonated parent ion (*m/z* 158) but also an intense, skimmer-induced *m/z* 102 peak, which comes from isobutene loss from the protonated parent via the McLafferty rearrangement.

The CAD fragmentation pattern of the *m/z* 102 ion from **4** is the same as that of the *m/z* 102 ion derived from *allo*-threonine. By contrast, protonated vinylglycine **2** (an ion that would have resulted from the E2 pathway), gives a very different CAD pattern, as Table 1 summarizes. The other *m/z* 102 ions listed in Table 1 also give decomposition patterns that differ greatly from those of the protonated aziridinecarboxylic acids. Table 1 does not include protonated azetidine-3-carboxylic acid, which primarily loses the elements of CH₂=NH and water to give *m/z* 55.

As noted above, N-methylation attenuates the difference between diastereomers. This is consistent with aziridine formation, as outlined in Scheme 8. N-Alkylation introduces an additional stereogenic center (the protonated, methylated nitrogen) into the three-membered ring. S_N2 displacement



of side-chain water from *N*-methylthreonine can produce the two protonated aziridines **5a** and **5b**. The latter ion has all substituents cis and is therefore likely to be formed much less often than is **5a**. By contrast, both of the protonated aziridines from *N*-methyl-*allo*-threonine, **5c** and **5d**, have one cis and two trans substituents on the ring, as does ion **5a**.

The difference between protonated threonine and protonated *allo*-threonine can be ascribed to the steric hindrance introduced by placing two substituents cis on the developing ring during displacement of side-chain water. In the *N*-methyl homologues, **5a**, **5c**, and **5d** all have nearly the same degree of steric hindrance. While the torsional strain in the aziridine rings does not fully develop in the S_N2 transition state, it does tend to diminish the difference between *threo* and *allo* isomers. If ions **5a**, **5c**, and **5d** all form at the same rate, then (on the basis of a naïve statistical argument) side-chain water loss in the *N*-methyl-*allo* should be twice as abundant as in the *N*-methyl-*threo*. The ratio of the two curves in Figure 2 ranges from 2.3 (at low collision energies) to 2.9, not far from this prediction.

The distinctions described above hinge on the stereochemistry of backside displacement, thus enlarging the repertoire of stereospecific dissociations by which diastereomers can be differentiated using MS/MS. Methods previously reported from this laboratory¹¹ for discriminating acyclic diastereomers have relied on syn eliminations via four-membered transition states. Are there other mechanisms by which MS/MS can tell acyclic stereoisomers apart? Preliminary negative ion data suggest there might be.

Anions from threonine display the same CAD patterns for both diastereomers. By contrast, MS/MS of negative ions from isoleucine do exhibit a statistically significant difference. The isoleucine and *allo*-isoleucine *M* – 1 anions display different extents of dissociation via CO + H₂O loss, relative to the parent ion (although the *M* + 1 positive ions show no differences). No variations are to be found among the competing fragmentation pathways themselves.

The example of isoleucine illustrates a less robust way to differentiate between diastereomers than the competition among pathways described above for the *M* + 1 positive ions of the threonines. Current efforts seek methodologies for dipeptides and other modified amino acids.

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Supporting Information Available: Synthesis and spectra of *N*-methylthreonines and of ester **4**; tabulated DFT and ab initio energies. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(9) Geometries were optimized at the B3LYP and MP2(FC) levels using the 6-31G** basis. Computed stabilities include DFT zero-point energies.

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