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

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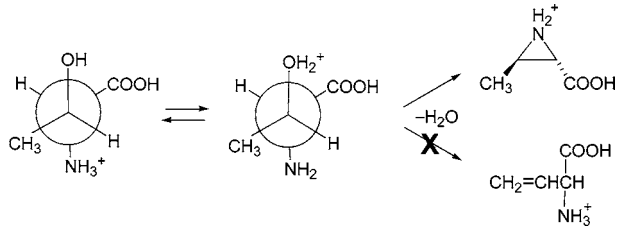
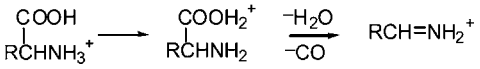
ABSTRACT



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 threonine[3]), 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 reported[6]) and its CAD. The *threo* and *allo* isomers exhibit different fragmentation pat-

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(1) Kulik, W.; Heerma, W. *Biomed. Environ. Mass Spectrom.* **1988**, *15*, 419−427.
(2) Dookeran, N. N.; Yalcin, Y.; Harrison, A. G. *J. Mass Spectrom.* **1996**, *31*, 500−508.
(3) Van Dongen, W. D.; de Koster, C. G.; Heerma, W.; Haverkamp, J. *Rapid Commun. Mass Spectrom.* **1995**, *9*, 845−850.
(4) (a) Bender, C. L.; Alarcón-Chaidez, F.; Gross, D. C. *Microbiol. Mol. Biol. Rev.* **1999**, *63*, 266−292. (b) Matsunaga, S.; Fusetani, R. *Curr. Org. Chem.* **2003**, *7*, 945−966.
(5) Aurelio, L.; Box, J. S.; Brownlee, R. T. C.; Hughes, A. B.; Sleebs, M. M. *J. Org. Chem.* **2003**, *68*, 2652−2667.

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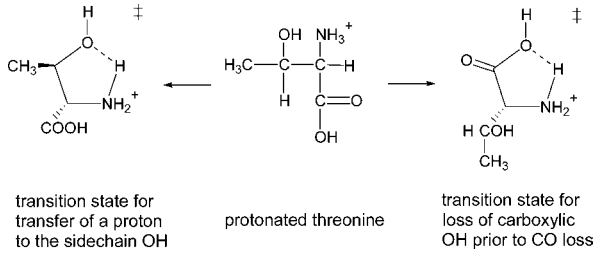
Table 1. Proportions of Neutrals Lost in MS/MS (12 eV Collision Energy) for Selected Parent Ions (Relative to Loss of H_2O + CO)

		fragment ions from loss of:				
neutral precursor	*m/z* of parent	H_2O	NH_3	$2H_2O$	CO	CO + $2H_2O$
threonine	120	0.58 ± 0.10		0.20 ± 0.03		0.12 ± 0.01
	102	0.85 ± 0.05			0.05 ± 0.04	
allo-threonine	120	3.19 ± 0.14		1.34 ± 0.12		0.28 ± 0.03
	102	2.08 ± 0.22			0.32 ± 0.07	
N-methylthreonine	134	1.24 ± 0.12		0.56 ± 0.06		0.21 ± 0.04
	116	0.49 ± 0.15			0.09 ± 0.03	
N-methyl-*allo*-threonine	134	3.05 ± 0.17		0.81 ± 0.06		0.35 ± 0.06
	116	0.54 ± 0.14			0.48 ± 0.09	
vinylglycine	102		0.33 ± 0.05			
1-aminocyclo-propane-1-COOH	102	0.37 ± 0.07				
aziridine ester **4**	102	2.12 ± 0.10			0.35 ± 0.01	
azetidine-2-COOH	102	0.02 ± 0.00				
homoserine	120	0.49 ± 0.03		0.04 ± 0.00		0.11 ± 0.00
	102	1.7 ± 0.5			3.3 ± 0.8	

terns, significantly expanding the list of acyclic stereoisomers that mass spectrometry can distinguish.[7] Three different hypotheses have been explored to account for this difference.

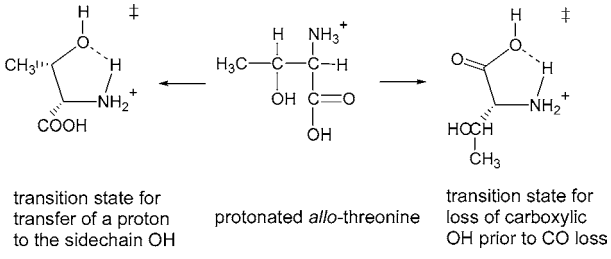
The first hypothesis looks to the two five-membered cyclic transition states drawn in Scheme 2. If they corresponded to

Scheme 2. Transition States for H_2O Loss from ThreonineH$^+$



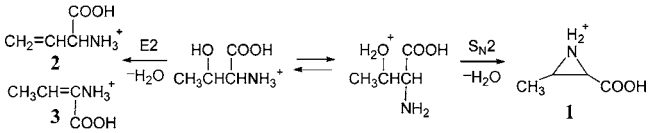
the rate-limiting steps for the two most prominent pathways, then competition between them would predict measurable differences between diastereomers. Assuming that the acidic proton resides on the nitrogen to begin with, varying the relative stereochemistry of the two asymmetric centers should affect the likelihood of loss of side chain OH. As Scheme 3 depicts, the transition state for the *allo* puts the two substituents cis to one another in the five-membered ring. For steric reasons, then, the aforementioned hypothesis predicts that loss of a single water ought to be more favorable for the *threo* than for the *allo*.

Scheme 3. H_2O Loss Transition States for *allo*-ThreonineH$^+$



The experiments described below do not confirm that prediction: the *allo* isomer displays the greater proportion of single water loss. O'Hair and Reid have reported deuterium labeling studies that suggest that the rate-limiting step comes after proton transfer to the side chain oxygen.[8] Scheme 4 illustrates two plausible pathways.

Scheme 4. Alternative Mechanisms for Side-Chain Water Loss



One pathway, originally proposed by O'Hair and Reid, proceeds via internal S_N2 displacement to give protonated aziridinecarboxylic acid **1**. This mechanism implies that diastereomeric threonines ought to produce isomeric ions: *threo* should yield *cis*-**1,** and *allo* should yield *trans*-**1**. An alternative pathway would involve internal E2 elimination via five-membered cyclic transition states to yield protonated vinylglycine **2** or protonated α-aminocrotonic acid **3**, which MP2 calculations[9] predict to be 1.9 kcal mol^{-1} and 6.2 kcal mol^{-1} ((*Z*)-isomer), respectively, more stable than *trans*-**1**.

The dissociation patterns of the protonated amino acids were studied using an orthogonal quadrupole/time-of-flight mass spectrometer with electrospray sample introduction. Table 1 summarizes the abundance of other neutral losses relative to the dissociation in Scheme 1 (which all α-amino acids have in common). The threonine diastereomers show quantitative differences in fragment ion abundances. These patterns vary with the collision energy. The ratios of the two most intense fragment ions are plotted in Figure 1 as a

(6) (a) Aurelio, L.; Brownlee, R. T. C.; Hughes, A. B.; Sleebs, B. E. *Aust. J. Chem.* **2000**, *53*, 425−433. (b) Aurelio, L.; Brownlee, R. T. C.; Hughes, A. B. *Org. Lett.* **2003**, *4*, 3767−3769.

(7) *Applications of Mass Spectrometry to Organic Stereochemistry*; Splitter, J. S., Turečk, F., Ed.; VCH: New York, 1994.

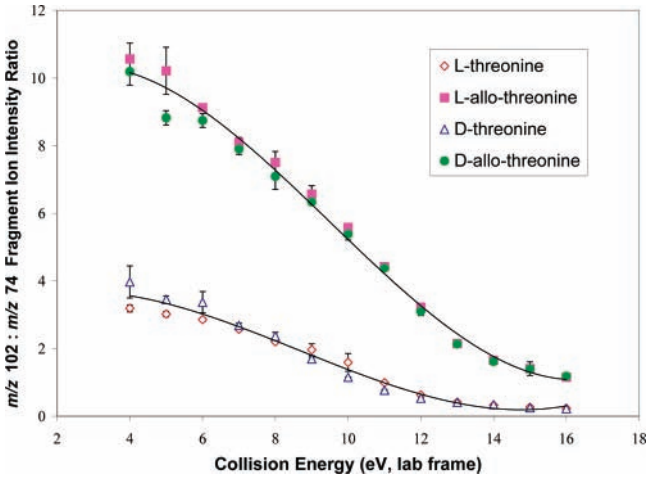


Figure 1. Ratios of H_2O loss to H_2O + CO loss from D- and L-threonine (open symbols) and their *allo*-diastereomers (solid symbols) as a function of collision energy of *m*/*z* 120 parent ions.

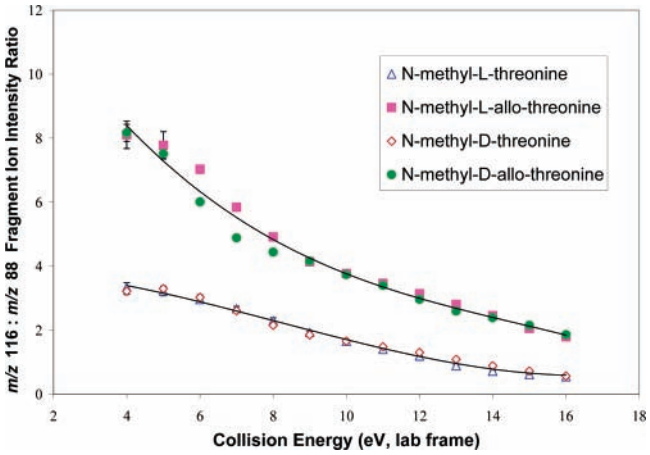
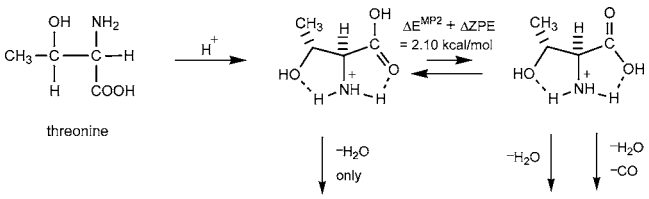


Figure 2. Ratios of H_2O loss to H_2O + CO loss for D- and L-*N*-methylthreonine (open symbols) and their *allo*-diastereomers (solid symbols) as a function of energy of *m*/*z* 134 parent ions.

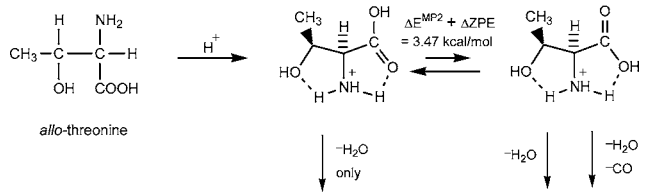
function of collision energy for all four stereoisomers. Figure 2 displays the same type of plot for all four stereoisomers of *N*-methylthreonine. Given the pronounced increase of single-water loss in the *allo* relative to *threo*, the first question concerns whether that dissociation represents elimination only from the side chain. This was assessed by labeling the *allo* with both carboxylic oxygens replaced by ^{18}O (in the same manner as doubly ^{18}O-labeled *threo* has been prepared[3]). The CAD spectrum of labeled *allo* shows that loss of a single water comes exclusively from the side chain, while loss of H_2O 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 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 NH_3^+ 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 NH_3^+ 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 $\Delta E^{MP2} + \Delta ZPE$ in Scheme 6 and $\Delta E^{MP2} + \Delta ZPE$ 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 Reid[8] 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.

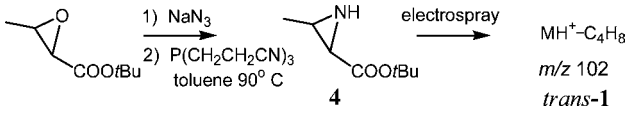
As Table 1 summarizes, CAD of the ions from water loss (*m*/*z* 102 from the threonines; *m*/*z* 116 from the *N*-methyl derivatives) shows significant differences between the ions

(8) (a) O'Hair, R. A. J.; Reid, G. E. *Rapid Commun. Mass Spectrom.* **1998**, *12*, 999−1002. (b) Reid, G. E.; Simpson, R. J.; O'Hair, R. A. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 1047−1060.

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.

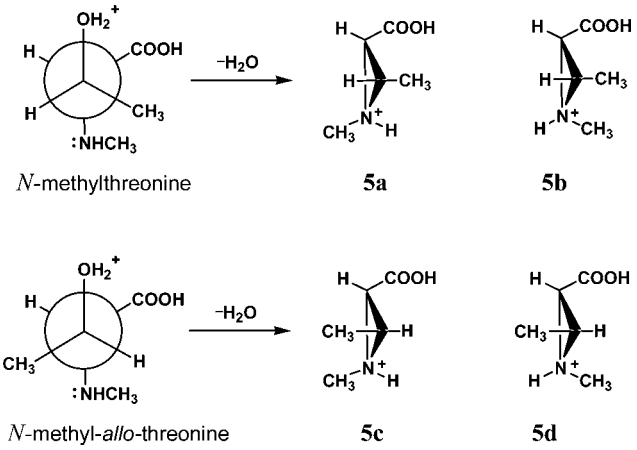
Scheme 7. Preparation of *tert*-Butyl *trans*-3-Methylaziridine-2-carboxylate **4**,[10] Which Expels Isobutene in the Mass Spectrometer



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 $CH_2{=}NH$ 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**.

Scheme 8. Side-Chain Water Loss From Protonated *N*-Methyl Amino Acids



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 laboratory[11] 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 + H_2O 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.

Acknowledgment. This work was supported by NSF Grant CHE0316515.

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.

(10) Legters, J.; Thijs, L.; Zwanesburg, B. *Recl. Trav. Chim. Pays-Bas* **1992**, *111*, 1−15. The authors are grateful to Prof. Francois Mathey for suggesting tris(2-cyanoethyl)phosphine in place of triphenylphosphine.

(11) (a) Zhang, K.; Bouchonnet, S.; Serafin, S. V.; Morton, T. H. *Int. J. Mass Spectrom.* **2003**, *227*, 175−189. (b) Morizur, J.-P.; Taphanel, M.-H.; Mayer, P. S.; Morton, T. H. *J. Org. Chem.* **2000**, *65*, 381−387. (c) Taphanel, M.-H.; Morizur, J.-P.; Leblanc, D.; Borchardt, D.; Morton, T. H. *Anal. Chem.* **1997**, *69*, 4191−4196.