Peptide Conformations

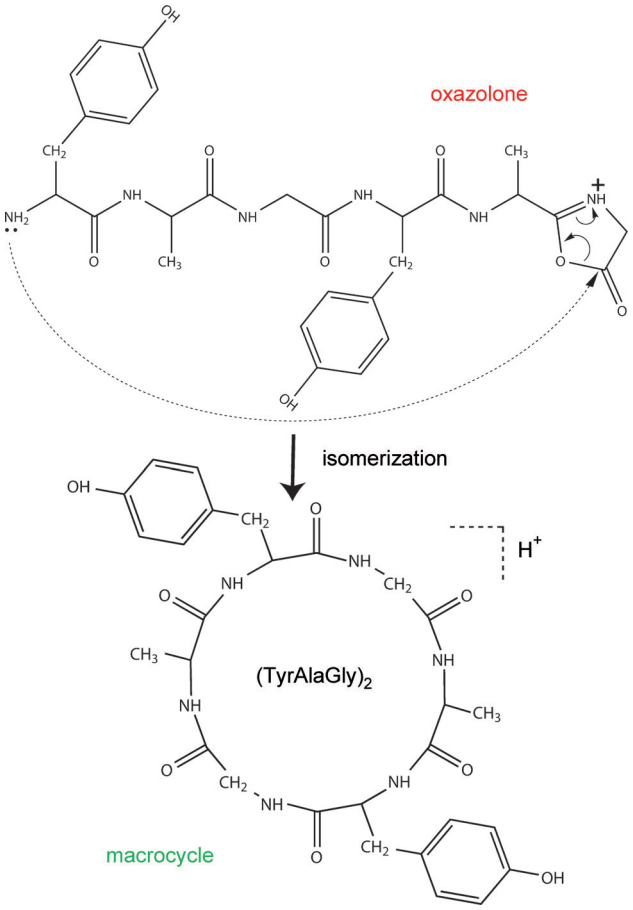
DOI: 10.1002/anie.201202405

Defying Entropy: Forming Large Head-to-Tail Macrocycles in the Gas Phase**

*Marcus Tirado and Nicolas C. Polfer**

In solution, the synthesis of head-to-tail macrocyclic peptides is notoriously challenging for smaller peptides due to trans amide bonds, and synthetic strategies frequently involve the insertion of proline or D-amino acids.[1] Conversely, cyclization of larger peptides is often straightforward, provided that competing reactions can be minimized.[2] In recent years, it was discovered that macrocyclic peptides can also be formed in the gas phase, during the fragmentation chemistry of protonated peptides inside mass spectrometers.[3] Collision-induced dissociation (CID) of peptide ions leads to vibrationally excited ions that are labile to amide bond cleavage. The resulting fragment ions that contain the N terminus are referred to as b_n ions (where n is the number of residues from the N terminus).[4] Two distinct chemical structures have been confirmed for b ions: 1) linear structures terminated by a five-membered oxazolone ring on their C-terminal side[5] and 2) head-to-tail macrocycles (Scheme 1)—low-energy pathways have been found that allow interconversion between both forms.[3] Direct evidence for these structures has come from infrared multiple photon dissociation (IRMPD) spectroscopy measurements,[6,7] and in particular the diagnostic CO stretch modes of oxazolone ring structures, located in the background-free 1770–1950 cm^{-1} region of the mid-IR spectrum.[8–13]

IR evidence has shown that the smaller b_2 fragments generally prefer to adopt oxazolone structures,[14–17] with some exceptions for basic histidine- and arginine-containing sequences.[18,19] The propensity for head-to-tail macrocycle formation is, however, enhanced for mid-sized to larger b fragments, as complementary IRMPD spectroscopy, gas-phase H/D exchange and dissociation studies have shown.[10,11,20–24] This raises the question how much larger macrocyclic structures can become in the gas phase, given the elevated internal energies of the ions during these reactions, and hence correspondingly non-negligible entropic barriers. The question is also timely, as the macrocyclization of b ions is intimately linked to permutation processes in peptide fragmentation, as in subsequent re-arrangement processes, the macrocycle may not re-open where it was put together, and thus result in non-direct/scrambled/permuted sequence ions,[3,25] potentially complicating sequence analysis in mass spectrometry-based proteomics.[26–28]



Scheme 1. Oxazolone and macrocycle structures for the example of b_6 ions with the sequence motif $(TyrAlaGly)_2$.

Here, we have chosen the sequence motif GlyAlaTyr, and the related motifs TyrAlaGly and AlaTyrGly, to study the formation of larger macrocycles in the gas phase. A series of peptides, including $(GlyAlaTyr)_2$ProGly, $(GlyAlaTyr)_3$ProGly, $(GlyAlaTyr)_4$ProGly, $(GlyAlaTyr)_5$ProGly, cyclo(TyrAlaGlyTyrAlaGly), and other sequence variants, were made by solid-phase synthesis, as described previously.[29] The presence of proline (i.e., Pro) in these motifs yields efficient amide bond cleavage on its N-terminal side, and thus abundant b_6, b_9, and b_{12} CID product ions. As in previous experiments, the peptides were ionized by electro-

[*] M. Tirado, Prof. N. C. Polfer
Department of Chemistry, University of Florida
Buckman Drive, P.O. Box 117200, Gainesville, FL 32611 (USA)
E-mail: polfer@chem.ufl.edu

[**] This work was supported by the U.S. National Science Foundation under grant number CHE-0845450. Travel support to the Netherlands for M.T. was provided by the NSF-PIRE (grant number OISE-0730072). The FELIX staff, in particular Dr. J. Oomens, J. Grzetic, Dr. B. Redlich, and Dr. A. F. G. van der Meer, are gratefully acknowledged for their assistance. Dr. J. van Maarseveen and J. Rutters from the University of Amsterdam are also thanked for their help in the synthesis of cyclic peptides.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201202405.

spray ionization (ESI) and fragmented by "nozzle-skimmer" dissociation in the ESI source, followed by IR spectroscopic interrogation with the free electron laser (FELIX) in the Penning trap of a Fourier transform ion cyclotron resonance (FTICR) mass spectrometer.[10,11] The IRMPD spectrum was obtained by recording the IRMPD yield as a function of the FELIX wavelength. Note that this yield is defined here as yield $= -\ln[1-(\Sigma Int_{Photofragments}/\Sigma Int_{All\_Ions})]$, and that it is normalized with the relative laser power at each wavelength step.

Figure 1 depicts the IRMPD spectra for the b_4 and b_6 ions for the various sequence motifs. In all cases for b_4, sizeable bands are observed in the high-frequency region from 1770–

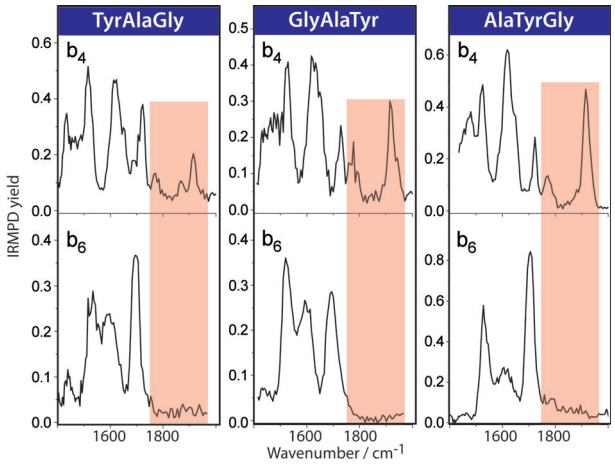


Figure 1. Top: IRMPD spectra of the b_4 ions with the sequence motifs a) TyrAlaGly, b) GlyAlaTyr, and c) AlaTyrGly. Bottom: IRMPD spectra of the b_6 ions with the sequence motifs a) TyrAlaGly, b) GlyAlaTyr, and c) AlaTyrGly. The CID product were made from the precursor ions $(TyrAlaGly)_2ProGly$, $(GlyAlaTyr)_2ProGly$, and $(AlaTyrGly)_2ProGly$, respectively. The spectral region associated with oxazolone band(s) is colored in light red.

1950 cm^{-1}, which are assigned to the diagnostic oxazolone CO stretch modes. The high-frequency band at 1915 cm^{-1} is consistent with oxazolone structures protonated at the oxazolone ring N, whereas the lower-frequency band at 1775 cm^{-1} is consistent with oxazolone structures protonated at the N-terminus.[10] On the other hand, for the slightly larger b_6 ions, there is no evidence for oxazolone bands, suggesting that exclusively head-to-tail macrocycles are formed. A more definite proof for this assignment is shown in Figure 2, where the IRMPD spectrum for the sequence motif TyrAlaGly b_6 matches closely with the corresponding spectrum of the synthetically made cyclic peptide, cyclo(TyrAlaGlyTyrAlaGly)H^+. Conversely, in a control experiment, the b_6 ion made from the N-terminally acetylated variant of TyrAlaGly exhibits an intense band at 1915 cm^{-1}. If any of the b_6 ions with sequence motif TyrAlaGly adopted an oxazolone structure, this would be visible by the presence of a diagnostic band in the 1770–1950 cm^{-1} region of the spectrum. The same rationale applies to the b_6 fragments of the sequence variants TyrAlaGly and GlyAlaTyr, implying that the size effect, upon going from b_4 to b_6, is critical in promoting head-to-tail

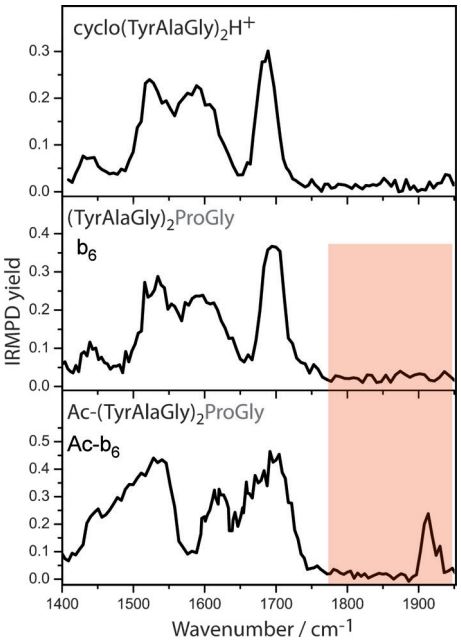


Figure 2. Comparison of IRMPD spectra of cyclo(TyrAlaGlyTyrAlaGly)H^+, b_6 ion with sequence motif TyrAlaGly, and b_6 ion from the N-terminally acetylated peptide with sequence motif TyrAlaGly. The two CID products were made from the precursor ions $(TyrAlaGly)_2ProGly$ and Ac-$(TyrAlaGly)_2ProGly$, respectively. The spectral region associated with oxazolone band(s) is colored in light red.

cyclization. Sustained off-resonance irradiation collision-induced dissociation (SORI CID) for these b_6 ions in the ICR cell are also consistent with sequence permutation processes (see Figure S1 and Table S2 in the Supporting Information).

Figure 3 shows a series of b ions with the sequence motif GlyAlaTyr from b_4 up to b_{12}. As a control experiment, the b_{14} ion for the N-terminally acetylated version of the peptide completes the series. Strikingly, none of the intermediate fragment ions, b_6, b_9, nor b_{12}, show any evidence for oxazolone structures. The spectral region between 1760–1960 cm^{-1} is magnified to visualize the presence/absence of these bands more clearly. Note that the photodissociation at the oxazolone CO stretch mode is more challenging in larger b ions, given the presence of merely one IR oscillator in a larger molecule. Nonetheless, the presence of a weak, yet reproducible band for the control acetylated b_{14} ion (Ac-b_{14}) confirms that this band can be detected in molecules of this size. Under identical conditions, photodissociation of the smaller b ions, b_9 and b_{12}, would be expected to give rise to bands in the 1770–1950 cm^{-1} region, if in fact oxazolone structures were present. These results strongly indicate the presence of head-to-tail macrocycles for all of the larger b ions, b_6, b_9 and b_{12}, as opposed to oxazolone structures for the b_4 ion, yet again confirming a size effect in the formation of macrocycle structures. The IRMPD spectrum for the still larger b_{15} ion (see Figure S3 in the Supporting Information) also lacked a diagnostic oxazolone band in the high-frequency region, but this result is less definite, as no control experiment on N-acetylated b_n ions, where $n > 14$, could be carried out successfully.

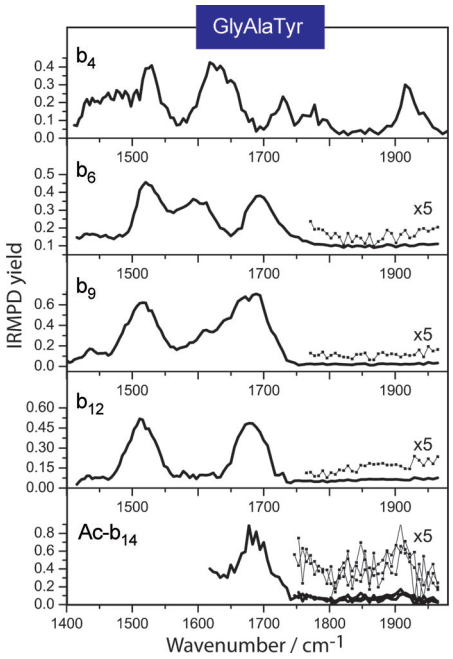


Figure 3. IRMPD spectra of a series of b ions with the sequence motif GlyAlaTyr: b_4, b_6, b_9, b_{12}, and N-acetylated b_{14}. The CID products were made from the precursor ions $(GlyAlaTyr)_2ProGly$, $(GlyAlaTyr)_2ProGly$, $(GlyAlaTyr)_3ProGly$, $(GlyAlaTyr)_4ProGly$, and $Ac\text{-}(GlyAlaTyr)_5ProGly$, respectively.

It appears that there are similarities in the trends for head-to-tail cyclization in solution and in the gas phase. While smaller peptide lengths discriminate against head-to-tail cyclization, no such problems are encountered for larger chains. One key difference between the chemistry in both phases is that the fragmentation chemistry in the gas phase takes place at much elevated temperatures (about 600–800 K), suggesting that entropic barriers could impede macrocyclization in larger systems. The results presented here indicate that these barriers do not pose a challenge for head-to-tail macrocyclization, at least for systems up to 12 residues in length. These findings are also consistent with a number of previous studies. Quantum-chemical calculations had indicated that macrocycles are enthalpically favored over oxazolone structures.[8] A number of IR studies on peptide systems with diverse amino acid sequences (e.g., YGGFL, YAGFL, GGGGG, and QWFGLM) have now confirmed that the formation of mid-sized macrocycles in the gas phase is prolific.[8–12,30] Finally, the general trend indicating a higher propensity for forming macrocycles as a function of peptide length appears to be validated with these results.[10,21,28]

While the current results do not offer insights into the chemistry of b ions exceeding twelve, and possibly fifteen, residues, in practical terms, in "bottom-up" proteomics studies, the range from b_6–b_{12} ions constitutes a substantial sub-set of CID product ions, as the most abundant enzymatically cleaved peptide precursors occur in the size range from 12–16 residues.[28] The current results lend credence to the claim that head-to-tail cyclization in b ions is highly prevalent in low-energy CID of peptides.

Received: March 27, 2012
Published online: May 22, 2012

Keywords: dissociation · IR spectroscopy · isomerization · mass spectrometry · peptides

[1] U. Schmidt, J. Langner, *J. Pept. Res.* **1997**, *49*, 67.
[2] C. J. White, A. K. Yudin, *Nat. Chem.* **2011**, *3*, 509.
[3] A. G. Harrison, A. B. Young, B. Bleiholder, S. Suhai, B. Paizs, *J. Am. Chem. Soc.* **2006**, *128*, 10364.
[4] K. Biemann, *Biomed. Environ. Mass Spectrom.* **1988**, *16*, 99.
[5] T. Yalcin, I. G. Csizmadia, M. B. Peterson, A. G. Harrison, *J. Am. Soc. Mass Spectrom.* **1996**, *7*, 233.
[6] T. D. Fridgen, *Mass Spectrom. Rev.* **2009**, *28*, 586.
[7] N. C. Polfer, *Chem. Soc. Rev.* **2011**, *40*, 2211.
[8] N. C. Polfer, J. Oomens, S. Suhai, B. Paizs, *J. Am. Chem. Soc.* **2007**, *129*, 5887.
[9] U. Erlekam, B. Bythell, D. Scuderi, M. J. Van Stipdonk, B. Paizs, P. Maitre, *J. Am. Chem. Soc.* **2009**, *131*, 11503.
[10] X. Chen, L. Yu, J. D. Steill, J. Oomens, N. C. Polfer, *J. Am. Chem. Soc.* **2009**, *131*, 18272.
[11] X. Chen, J. D. Steill, J. Oomens, N. C. Polfer, *J. Am. Soc. Mass Spectrom.* **2010**, *21*, 1313.
[12] A. Gucinski, A. Somogyi, J. Chamot-Rooke, V. H. Wysocki, *J. Am. Soc. Mass Spectrom.* **2010**, *21*, 1329.
[13] T. N. Wassermann, O. V. Boyarkin, B. Paizs, T. R. Rizzo, *J. Am. Soc. Mass Spectrom.* **2012**, DOI: 10.1007s13361.
[14] S. Yoon, J. Chamot-Rooke, B. Perkins, A. E. Hilderbrand, J. Poutsma, V. H. Wysocki, *J. Am. Chem. Soc.* **2008**, *130*, 17644.
[15] J. Oomens, S. Young, S. Molesworth, M. J. van Stipdonk, *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 334.
[16] B. Bythell, A. Somogyi, B. Paizs, *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 618.
[17] D. Wang, K. Gulyuz, C. Stedwell, N. C. Polfer, *J. Am. Soc. Mass Spectrom.* **2011**, *22*, 1197.
[18] B. Perkins, J. Chamot-Rooke, S. Yoon, A. Gucinski, A. Somogyi, V. H. Wysocki, *J. Am. Chem. Soc.* **2009**, *131*, 17528.
[19] S. Zou, J. Oomens, N. C. Polfer, *Int. J. Mass Spectrom.* **2012**, 12, 316–318.
[20] A. Somogyi, *J. Am. Soc. Mass Spectrom.* **2008**, *19*, 1771.
[21] S. Molesworth, S. Osburn, M. J. van Stipdonk, *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 2174.
[22] A. G. Harrison, *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 2248.
[23] A. Fattahi, B. Zekavat, T. Solouki, *J. Am. Soc. Mass Spectrom.* **2010**, *21*, 358.
[24] X. Li, Y. Huang, P. B. O'Connor, C. Lin, *J. Am. Soc. Mass Spectrom.* **2011**, *22*, 245.
[25] C. Bleiholder, S. Osburn, T. Williams, S. Suhai, M. J. van Stipdonk, A. G. Harrison, B. Paizs, *J. Am. Chem. Soc.* **2008**, *130*, 17774.
[26] I. S. Saminathan, X. S. Wang, Y. Guo, O. Krakovska, S. Voisin, A. C. Hopkinson, K. W. M. Siu, *J. Am. Soc. Mass Spectrom.* **2010**, *21*, 2085.
[27] A. A. Goloborodko, M. V. Gorshkhov, D. M. Good, R. A. Zubarev, *J. Am. Soc. Mass Spectrom.* **2011**, *22*, 1121.
[28] L. Yu, Y. Tan, Y. Tsai, D. R. Goodlett, N. C. Polfer, *J. Proteome Res.* **2011**, *10*, 2409.
[29] M. Tirado, J. Rutters, X. Chen, A. Yeung, J. van Maarseveen, J. R. Eyler, G. Berden, J. Oomens, N. C. Polfer, *J. Am. Soc. Mass Spectrom.* **2012**, *23*, 475.
[30] X. Chen, M. Tirado, J. D. Steill, J. Oomens, N. C. Polfer, *J. Mass Spectrom.* **2011**, *46*, 1011.

