Supporting Information

Proton-driven Amide Bond Cleavage Pathways of Gas-Phase Peptide Ions Lacking Mobile Protons

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Figure S1. The structure of the lowest energy CS → SB transition structure of $[GGR+H]^+$ determined at the B3LYP/6-31+G(d,p) level of theory.

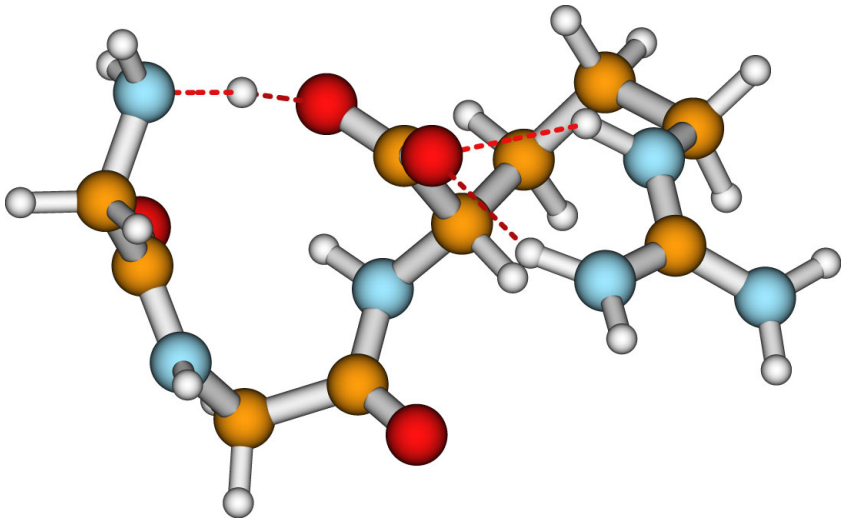
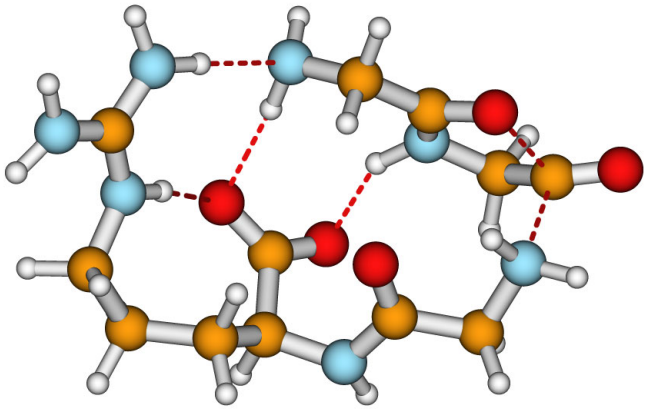


Figure S2. Structures and relative energy of the lowest energy b_n-y_m TSs for $[GGGR+H]^+$ determined at the B3LYP/6-31+G(d,p) level of theory with the relative energies corrected for zero-point vibration energy (ZPE) using B3LYP/6-31G(d) level of theory. (a) The Salt-bridge b_2-y_2 Transition Structure; *(b)* The Anhydride b_3-y_1 Transition Structure.

(a)

TS Salt-bridge b_2-y_2
47.8 kcal mol^{-1}



(b)

TS Anhydride b_3-y_1
41.2 kcal mol^{-1}

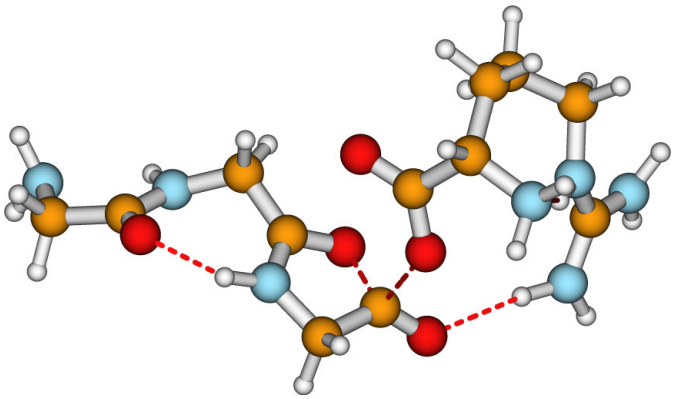
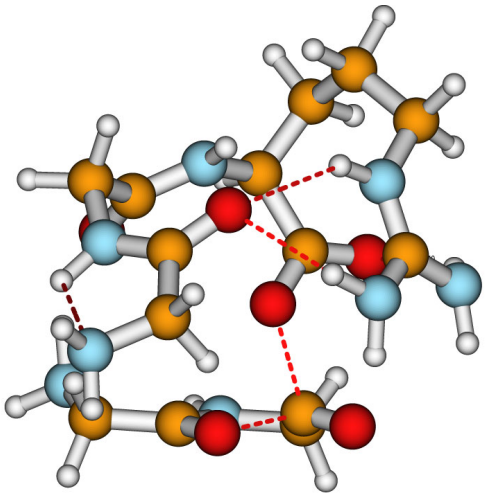


Figure S3. Structures and relative energy of the lowest energy b_n-y_m TSs for $[GGGGR+H]^+$ determined at the B3LYP/6-31+G(d,p) level of theory with the relative energies corrected for zero-point vibration energy (ZPE) using B3LYP/6-31G(d) level of theory. (a) The Anhydride b_2-y_3 Transition Structure; *(b)* The Salt-bridge b_3-y_2 Transition Structure; (c) The Anhydride b_4-y_1 Transition Structure.

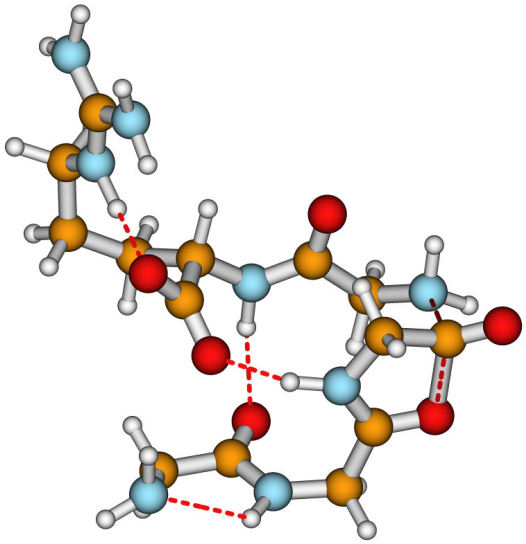
(a)

TS Anhydride b_2-y_3
46.4 kcal mol^{-1}



(b)

TS Salt-bridge b_3-y_2
44.8 kcal mol^{-1}



(c)

TS Anhydride b_4-y_1
42.7 kcal mol^{-1}

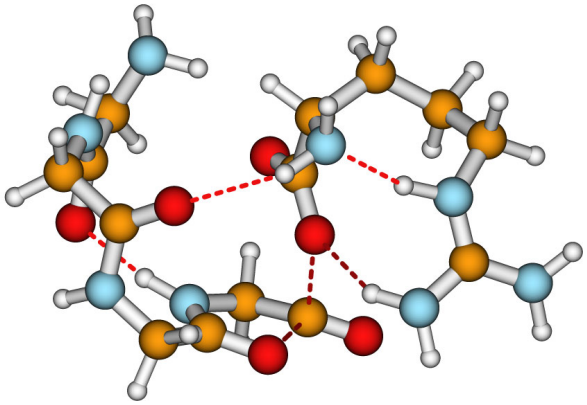


Table S1. Total and relative energies of the various of $[GGR+H]^+$ determined at the B3LYP/6-31+G(d,p) level of theory with the relative energies corrected for zero-point vibration energy (ZPE) using B3LYP/6-31G(d) level of theory.

Structure	E_{Total} + ZPE /H	E_{rel}/ kcal mol^{-1}	ΔG_{298}/ kcal mol^{-1}	ΔS_{298}/ cal K^{-1} mol^{-1}
CS Global Minimum	-1022.732645	0.0	0.0	0.0
CS N-term. Prot.	-1022,691145	26.1	27.9	-8.5
CS G(1)-G(2) amide N Prot.	-1022.669234	39.8	40.5	-4.2
CS G(1)-R amide N Prot.	-1022.68833	40.1	40.0	-2.3
CS G(1)-G(2) amide O Prot.	-1022.67566	22.0	22.6	-3.9
CS G(1)-R amide O Prot.	-1022.705649	16.9	17.1	-1.9
SB N-term. Prot.	-1022.714126	11.6	13.5	-9.5
SB G(1)-G(2) amide N Prot.	-1022.670681	38.9	39.1	-1.7
SB G(1)-R amide N Prot.	-1022.684293	30.3	30.2	-0.1
SB G(1)-G(2) amide O Prot.	-1022.696598	22.6	23.4	-4.4
SB G(1)-R amide O Prot.	-1022.702927	18.7	19.2	-3.4
Imine enol G(1)-G(2)	-1022.714666	11.3	10.3	3.5
Imine enol G(2)-R	-1022.713844	11.8	12.0	-1.3

Table S2. MALDI-TOF/TOF peak intensities of sequence ions and neutral losses from the MS/MS of $[G_nR+H]^+$ peptides.

Protonated System	Assignment	Relative Intensity
GGR	$[GGR+H-NH_3]^+$	57
	b_2	32
	a_2/ R (87)	7
	y_1	100
	y_1^*	82
	y_2^*	21
GGGR	$[G_3R+H -NH_3]^+$	82
	$[G_3R+H-2NH_3]^+$	21
	b_2	31
	a_2 / R (87)	1
	y_3	5
	y_3^*	56
	y_2	21
	y_2^*	100
	b_3	77
	y_1	92
	y_1^*	82
GGGGR	$[G_4R+H -NH_3]^+$	100
	b_2	13
	a_2 / R	4
	y_3	21
	y_3^*	68
	b_3	30
	y_2	13
	y_2^*	70
	b_4	38
	b_4°	28
	a_4	15
	y_1	64
	y_1*	56
	y_4	9
	y_4^*	26

Table S3. Ion trap (LCQ, Thermo San Diego, (ACN:H2O 1:1 0.1% Formic Acid)) peak intensities for MS/MS of $[G_nR+H]^+$ peptides.

Protonated System	Assignment	Relative Intensity
GGR	$[GGR+H-H_2O-NH_3]^+$	100
	$[GGR+H-NH_3]^+$	59
	y_1	89
	y_1^*	28
	b_2	6
GGGR	$[G_3R+H-H_2O-NH_3]^+$	40
	$[G_3R+H-NH_3]^+$	100
	y_3^*	6
	b_3	11
	y_1	15
	y_1^*	10
	b_2	3.5
	y_2	2.5
	y_2^*	16
GGGGR	$[G_3R+H-2NH_3]^+$	28
	$[G_3R+H-H_2O-NH_3]^+$	31
	$[G_3R+H-NH_3]^+$	100
	y_4	1
	y_4^*	3
	b_4	16.5
	a_4	4
	a_4^*	3
	y_1	8
	y_1^*	6
	b_3	6
	y_2	2
	y_2^*	6
	b_2	1
	y_3	2.5
	y_3^*	13

Table S4. FT-ICR (Bruker 9.4 T FT-ICR, (ACN:H2O 1:1 0.1% Formic Acid)) QCID peak intensities of sequence ions and neutral losses from the MS/MS of $[G_nR+H]^+$ peptides.

Protonated System	Assignment	Relative Intensity
GGR	$[GGR+H-H_2O-NH_3]^+$	100
	$[GGR+H-NH_3]^+$	32
	y_2^*	14
	y_1	97
	y_1^*	47
GGGR	$[G_3R+H-H_2O-NH_3]^+$	100
	$[G_3R+H-2NH_3]^+$	42
	$[G_3R+H-NH_3]^+$	83
	$[G_3R+H-H_2O-2NH_3]^+$	48
	y_3^*	56
	y_1	89
	y_1^*	86
	y_2^*	85
GGGGR	$[G_3R+H-2NH_3]^+$	67
	$[G_3R+H-H_2O-NH_3]^+$	78
	$[G_3R+H-NH_3]^+$	100
	$[G_3R+H-H_2O-2NH_3]^+$	43
	y_4	5
	y_4^*	27
	y_4^{**}	12
	$y_4^{\circ *}$	11
	b_4	45
	a_4	18
	a_4^*	11
	y_1	46
	y_1^*	40
	b_3	6
	y_2	6
	y_2^*	43
	b_2	1
	y_3	9
	y_3^*	80
	y_3^{**}	15

Table S5. Total and relative energies of the various minima and transition structures of $[GGG+H]^+$ determined at the B3LYP/6-31+G(d,p) level of theory with the relative energies corrected for zero-point vibration energy (ZPE) using B3LYP/6-31G(d) level of theory.

Structure	E_{Total} + ZPE Corr./ H	E_{rel}/ kcal mol^{-1}
CS Global Minimum	-700.683445	0.0
TS classical[7] b_2-y_1	**-700.636209**	**29.6**
TS Imine enol b_2-y_1	-700.614794	43.1

This demonstrates that the imine enol mechanism is much less favorable for systems which do not contain arginine, where the normally invoked b_n-y_m mechanism still prevails. The salt-bridge structures collapsed to form charge-solvated structures during the TS optimizations, indicating there non-applicability of this mechanism is these systems too.

Full citation for Reference 19:

Gaussian 03, Revision C.02, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, Jr., J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; and Pople, J. A.; Gaussian, Inc., Wallingford CT, **2004**.

Full citation for Reference 17:

Case, D. A.; Pearlman, D. A.; Caldwell, J. W.; Cheatham III, T. E.; Ross, W. S.; Simmerling, C. L.; Darden, T. A.; Merz, K., M.; Stanton, R. V.; Cheng, A. L.; Vincent, J. J.; Crowley, M.; Tsui, V.; Radmer, R. J.; Duan, Y.; Pitera, J.; Massova, I. G.; Seibel, G. L.; Singh, U. C.; Weiner, P. K.; Kollmann, P. A. In; AMBER 99, University of California: San Francisco, 1999.