Supporting Information for

Deamidation of protonated Asparagine-Valine investigated by a combined spectroscopic, guided ion beam, and theoretical study

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Table S1 gives relative energies at the B3LYP, B3P86, and MP2(full) levels of theory for major reaction species for the following decomposition channels of $[AsnVal+H]^+$, $[AsnVal+H-NH_3]^+$, $[AsnVal+H-NH_3-NH_3]^+$, $[AsnVal+H-NH_3-H_2O]^+$, and $[AsnVal+H-NH_3-CO]^+$. Table S2 gives relative energies at the B3LYP, B3P86, and MP2(full) levels of theory for alternate conformations of the furanone product. Figure S1 shows the structures of major reaction species given in Table S1, calculated at the B3LYP/6-311+G(d,p) level of theory. Figure S2 gives the experimental versus theoretical spectral comparison for the lowest energy diketo-morpholine **3.2** structure. Figure S3 gives an expanded comparison of the IRMPD spectrum of $[AsnVal+H - NH_3]^+$ with computed spectra. Figure S4 gives an expanded comparison for the secondary fragment $[AsnVal+H - NH_3 - NH_3]^+$; species formed by cyclization or NH_3 cleavage from Fur **2.3** and by cyclization from Suc **1.1** are included for completeness.

Table S1. Relative Enthalpies (0 K) and Gibbs Free Energies (298 K) of Major Reaction Species for the Decomposition of $[AsnVal+H]^+$ [a]

$[AsnVal+H-NH_3]^+$	B3LYP[b]	B3P86[c]	MP2(full)[c]
TS_{N-FUR}	132.0 (134.2)	138.8 (140.6)	129.4 (129.8)
215 + NH_3	124.0 (82.6)	135.4 (93.6)	137.6 (94.4)
TS_{N-SUC}	171.9 (180.6)	163.7 (172.0)	144.8 (151.8)
$[AsnVal+H-NH_3-NH_3]^+$			
TS_{S-N}	314.4 (274.3)	325.3 (285.2)	322.5 (282.4)
198 + $2NH_3$	185.7 (100.5)	217.7 (132.5)	218.3 (133.1)
$[AsnVal+H-NH_3-H_2O]^+$			
TS_{S-O}	367.3 (332.5)	364.2 (329.4)	360.3 (328.7)
197 + NH_3 + H_2O	179.3 (100.3)	191.6 (112.6)	190.6 (114.8)
$[AsnVal+H-NH_3-CO]^+$			
$TS_{S(FUR)-C}$	362.4 (317.8)	388.5 (343.5)	384.5 (338.1)
187 + NH_3 + CO	232.3 (143.3)	282.6 (193.3)	251.3 (160.6)
$TS_{S(SUC)-C}$	270.6 (229.8)	292.7 (251.6)	265.8 (223.3)
187 + NH_3 + CO	149.8 (66.9)	197.8 (114.5)	167.6 (82.8)

[a] Calculations performed at the stated level of theory using a 6-311+G(2d,2p) basis set with geometries and ZPE corrections calculated at the B3LYP/6-311+G(d,p) level. [b] Single point values given relative to the $[N^1,CO^s,CO^1]$- ttgtgtt conformer at 0 (298) K. [c] Single point values given relative to the $[N^1,CO^s,CO^1]$-gggtgtt conformer at 0 (298) K.

Table S2. Relative Enthalpies (0 K) and Gibbs Free Energies (298 K) of Alternative Conformations of the Furanone **2.3** Structure Formed by Deamidation of $[AsnVal+H]^+$ [a]

Species[b]	B3LYP[c]	B3P86 [d]	MP2(full) [d]
$[N^2,CO^2,N^1]$-t	124.0 (82.6)	135.4 (93.6)	137.6 (94.4)
$[N^2,N^1]$-t	125.1 (82.9)	136.7 (94.1)	139.3 (95.3)
$[N^2,CO^2,N^1]$-g_	124.8 (83.4)	136.3 (94.5)	140.5 (97.3)
$[N^2,CO^2,N^1]$-g_+	128.5 (86.9)	140.3 (98.3)	143.4 (99.9)
$[N^2,N^1]$-g_	130.3 (87.9)	142.3 (99.5)	144.8 (100.6)
$[N^2,N^1]$-g_+	133.0 (88.8)	145.3 (100.6)	147.7 (101.6)
$[N^2,CO^2]$-g_	139.7 (97.9)	151.9 (109.6)	140.0 (96.3)

[a] Calculations performed at the stated level of theory using a 6-311+G(2d,2p) basis set with geometries calculated at the B3LYP/6-311+G(d,p) level. [b] Dihedral angle given indicates the ∠NCCH angle along the backbone, where g_+ structures have their hydrogen oriented towards the carboxylic acid. The long-range (~2.5 Å) $N^2H{\bullet}N^1$ hydrogen bond is included as this interaction orients the N^1 amino group. [c] Single point values including NH_3 given relative to the $[N^1,CO^s,CO^1]$- ttgtgtt conformer at 0 (298) K. [d] Single point values including NH_3 given relative to the $[N^1,CO^s,CO^1]$-gggtgtt conformer at 0 (298) K.

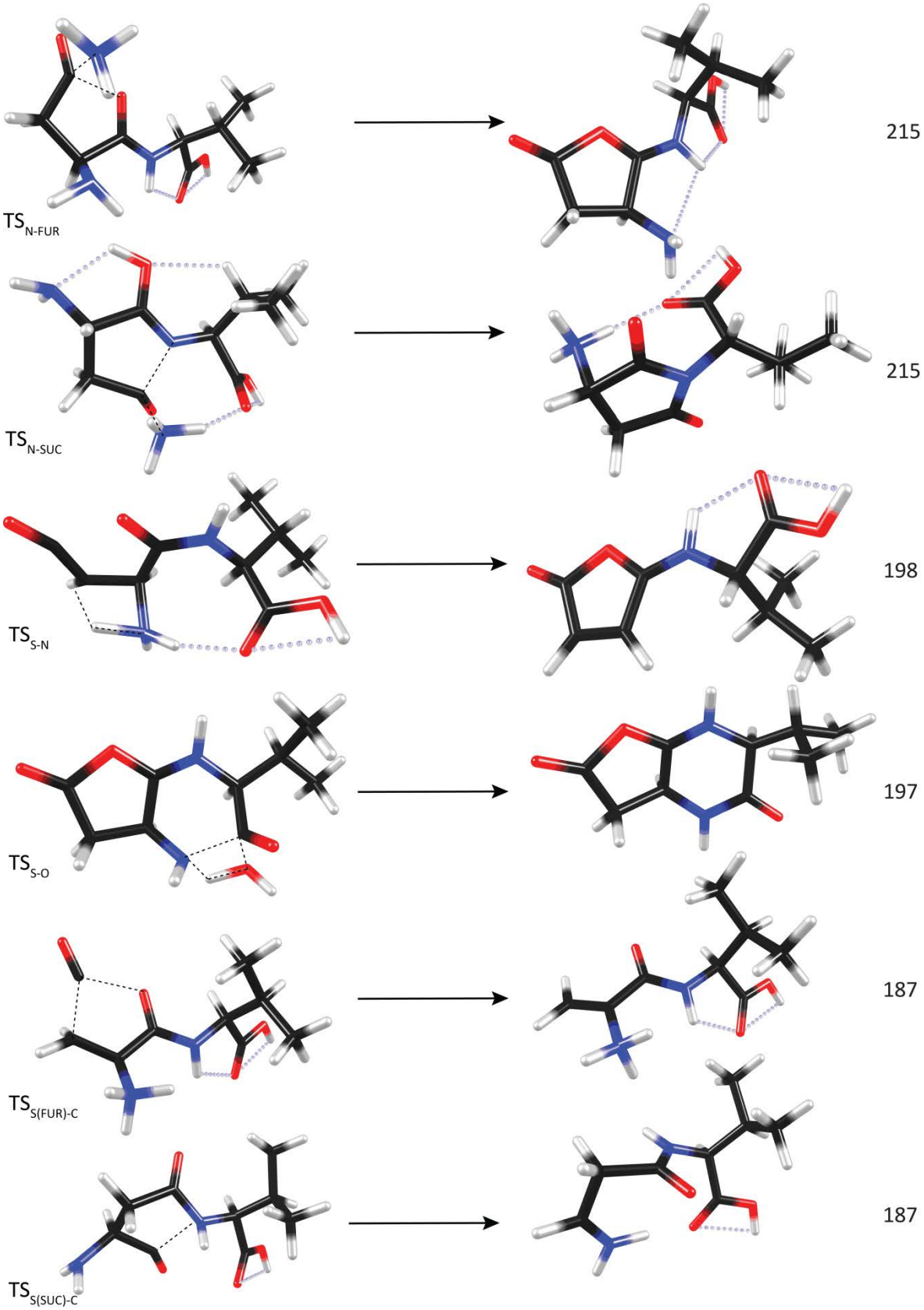

Figure S1. *TS and product structures for the main dissociation pathways from [AsnVal+H]$^+$ calculated at the B3LYP/6-311+G(d,p) level of theory. Hydrogen bonds are indicated by grey dashed lines. Bond rupture and formation is indicated by black dotted lines.*

[AsnVal+H-NH$_3$]$^+$

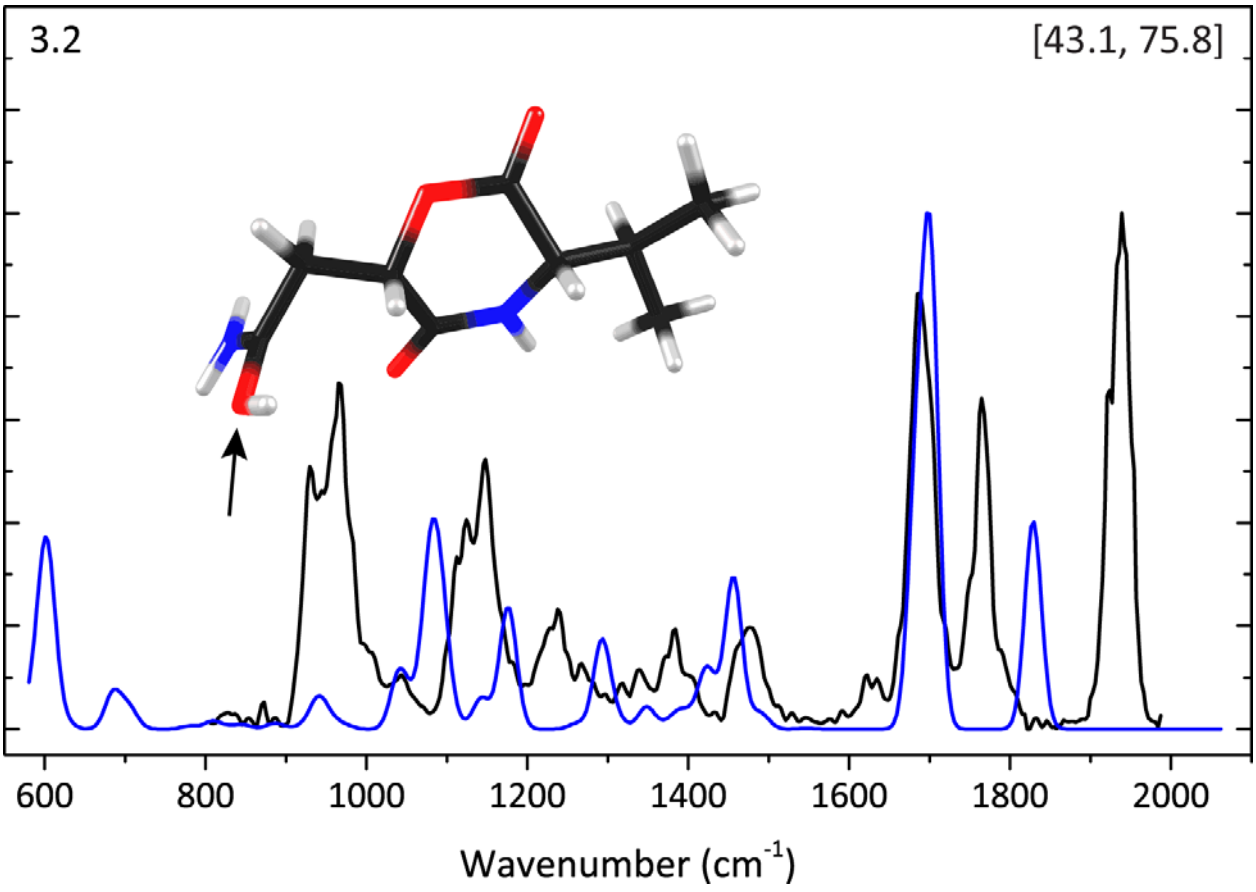

Figure S2. *IRMPD spectrum (black) of [AsnVal+H-NH$_3$]$^+$ compared to the computed spectrum of diketo-morpholine structure* ***3.2****. The protonation site is indicated with an arrow. Energies (kJ/mol, in square brackets) are calculated at the B3LYP/6-311+G(d,p) (first value) and MP2(full)/6-311+G(2d,2p) levels using B3LYP/6-311+G(d,p) geometries (second value).*

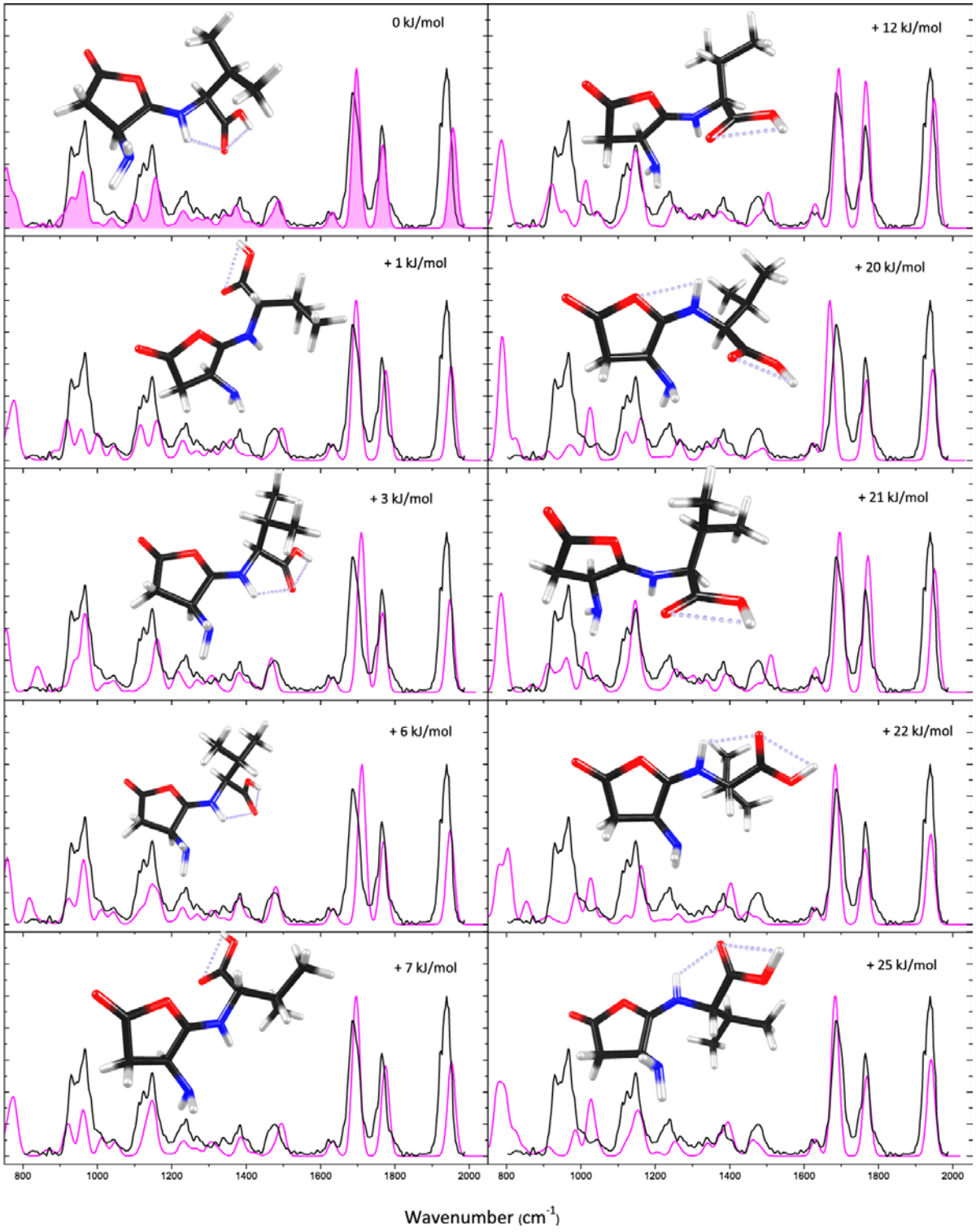

Figure S3. *IRMPD spectrum (black) of [AsnVal+H-NH_3]$^+$ compared to computed higher energy conformers of furanone structure* ***2.3****. Energies (kJ/mol, in square brackets) are calculated at the MP2(full)/6-311+G(2d,2p) level using the B3LYP/6-31++G(d,p) geometry.*

[AsnVal-NH_3-NH_3]

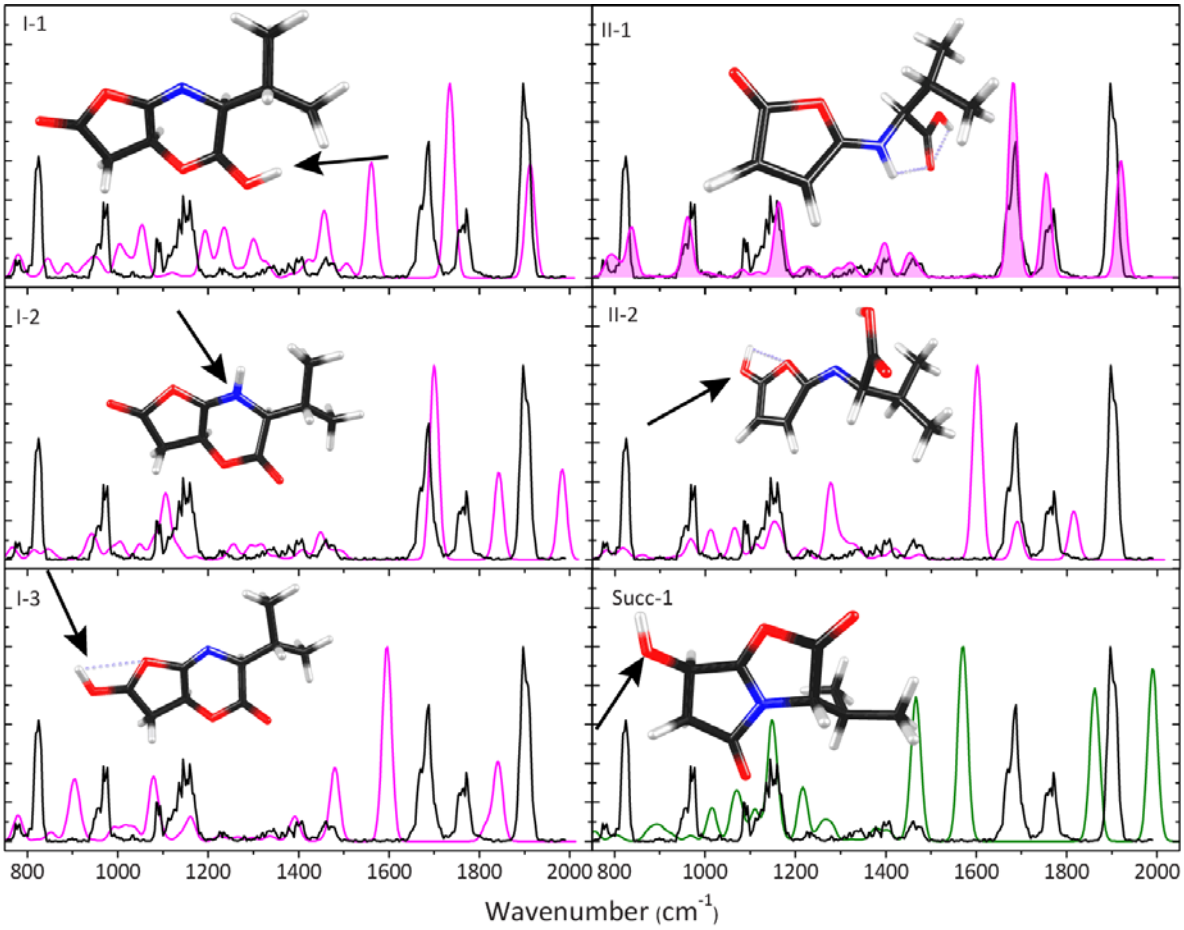

Figure S4. *IRMPD spectra (black) of [AsnVal+H-NH_3-NH_3]$^+$ compared to the computed spectra of NH_3 loss via a cyclization reaction (pathway I) or via NH_3 cleavage pathway (II). The protonation sites are indicated with an arrow. Energies (kJ/mol, in square brackets) are calculated at the B3LYP/6-311+G(d,p) (first value) and MP2(full)/6-311+G(2d,2p) levels using B3LYP/6-311+G(d,p) geometries (second value). To double check, the spectrum of the product from NH_3 loss from the succinimide was also calculated (see bottom right panel); the obvious mismatch reinforces the our structural assignment of [AsnVal+H-NH_3]$^+$ as a furanone ring containing ion.*

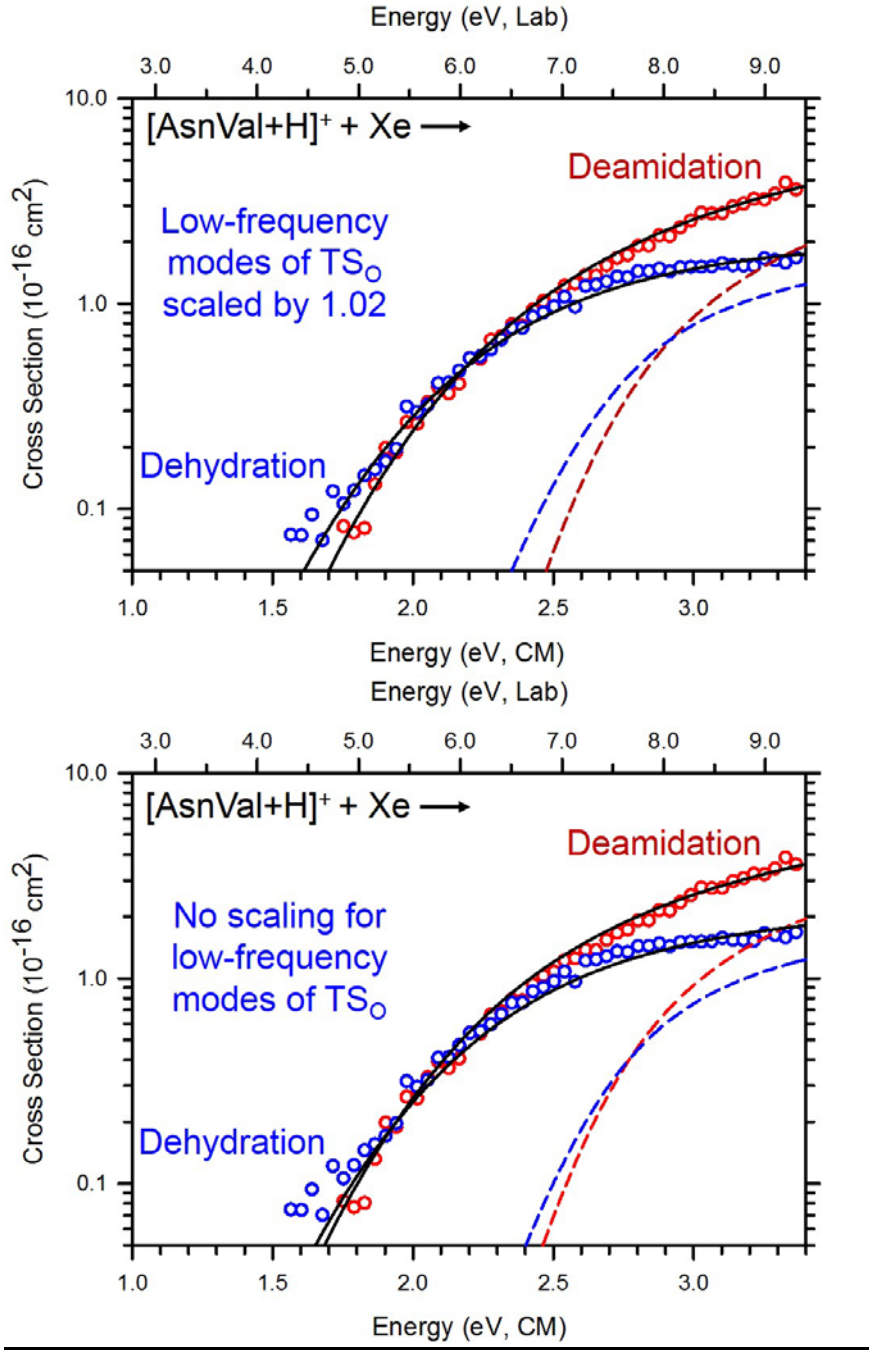

Figure S5: *Modelling of the cross section data with and without the use of frequency scaling. Tightening of the low-frequency* TS_O *modes introduces only subtle differences in the overall fit, but allows the model to more accurately reproduce the data in the threshold region.*