Supplementary Material For:

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Title: Gas-Phase Ion-Molecule Reactions Using Regioselectively Generated Radical Cations to Model Oxidative Damage and Probe Radical Sites in Peptides.[†,ξ]

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[†] Dedicated to the memory of Professor Athel Beckwith, an important pioneer of free radical chemistry and a giant in Organic Chemistry in Australia.

[ξ] Part 79 of the series "Gas-Phase Ion Chemistry of Biomolecules".

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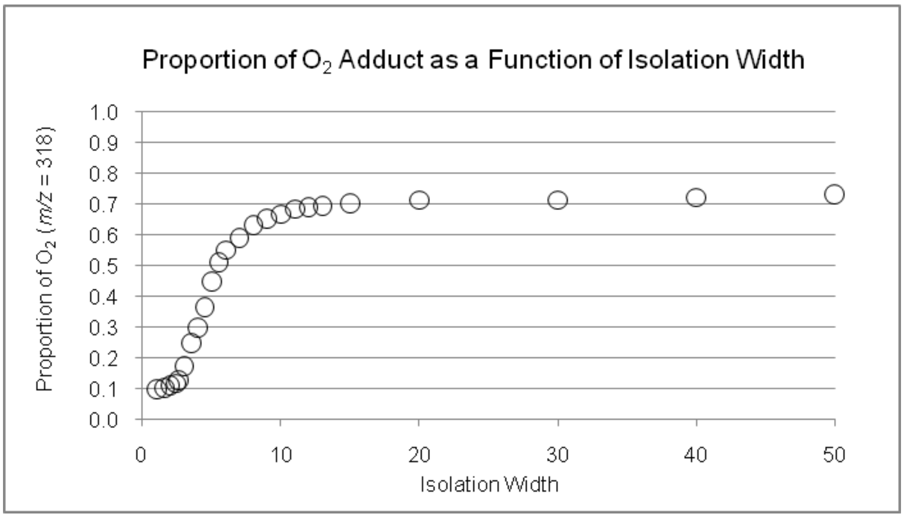
Interrogating the effect of isolation width on the isomerisation of the peptide radical [6+Na]$^+$

A key issue in studying the isomerisation of peptide radicals via intramolecular HAT is the influence of the internal energy of the ion on isomerisation. While it has been recognized that the act of isolating an ion via mass selection in an ion trap can result in dissociation of fragile non covalent cluster ions when small isolation widths are used (see for example, ref. 46), we are not aware of any previous studies that have examined the role of mass selection in isomerisation of the structure of an ion. Thus we have conducted such a study aimed at examining the role of isolation width in the isomerisation of the radical [**6**+Na]$^+$. The significant difference in reactivity between the alanine side-chain radical, [**6**+Na]$^+$, and the C$^\alpha$ radicals, [**4**+Na]$^+$ and [**5**+Na]$^+$ provides a ready means to interrogate the effect of isolation width on the extent of isomerisation of [**6**+Na]$^+$. Figure S1 below shows a plot of the proportion of product ions at *m/z* 318, the O_2 adduct obtained following isolation of the radical ion formed following CID of [**3**+Na]$^+$ for 10 sec using He bath gas seeded with ~1% O_2 as a function of the width used in the isolation of the radical ions at *m/z* 286. From the spectrum shown in Figure 1C it is evident that 10 sec is a sufficient period to ensure consumption of essentially all of the radical [**6**+Na]$^+$. Consequently, the proportion of ions at *m/z* 318 represents the proportion of [**6**+Na]$^+$ remaining following the isolation of the ions at *m/z* 286. This plot in Figure S1 shows that for an isolation width less than 15 significantly more isomerisation occurs as the isolation width is reduced.

Figure S1: Plot of the proportion of ions at *m/z* 318, corresponding to the addition of O_2 following 10 sec reaction time as a function of the isolation width used in the isolation of the radical ions at *m/z* 286. It is clear that significantly more O_2 addition occurs as the isolation width increases, consistent with a narrower isolation width leading to more extensive isomerisation of the reactive [**6** + Na]$^+$ to the non-reactive [**4** + Na]$^+$ and [**5** + Na]$^+$ isomers, which fail to undergo addition of O_2. The scan conditions utilized were:

MS2: parent ion *m/z* 362.2, isolation width = 3.0, NCE = 20, Q = 0.25, t = 30 ms.

MS3: parent ion *m/z* 286.0, isolation width = varied, NCE = 0, Q = 0.25, t = 10,000 ms.

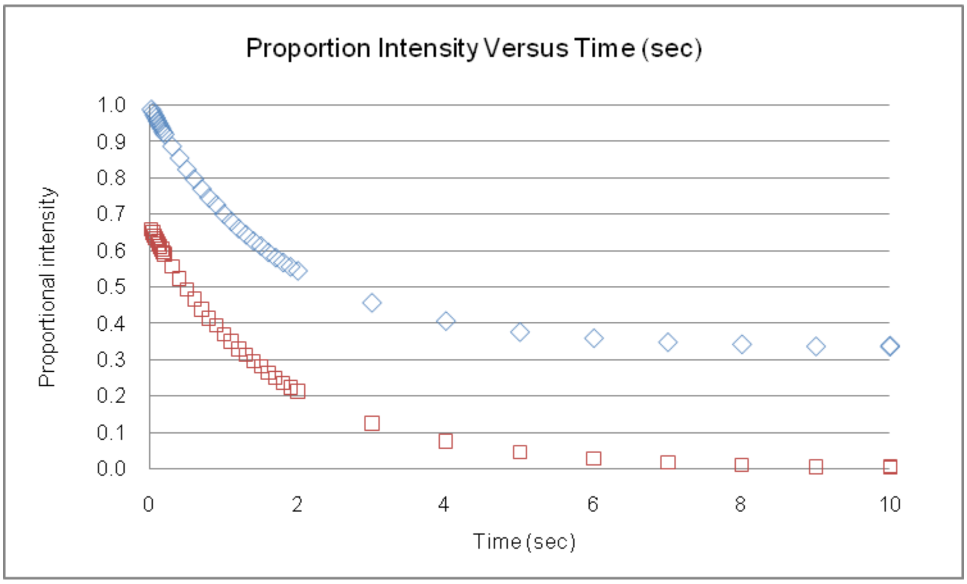


Rate of O_2 addition

Figure S2 below shows the proportion of *m/z* 286 ions as a function of trapping time utilizing 871 ppm O_2 doped He bath gas. This data was generated in a multi-stage MS^3 experiment in which [**6**+Na]$^+$ is isolated and subjected to CID (MS^2) followed by isolation of the resulting radical at *m/z* 286 (isolation width of 5) and trapping (MS^3). The blue diamonds represent the proportional intensity of the *m/z* 286 ions as a sum of *m/z* 286 and *m/z* 318 (the O_2 addition product) as a function of the trapping period in the MS^3 stage. The proportion of *m/z* 286 decays exponentially consistent with pseudo first-order kinetics (O_2 is in vast excess), however it fails to decay to zero due to the isomerisation induced in the isolation step. Consequently, it is necessary to subtract the unreactive portion of the *m/z* 286 ions corresponding to the isomers [**4**+Na]$^+$ and [**5**+Na]$^+$, this corrected decay is shown by the red squares.

The corresponding pseudo first order rate constant is obtained as the negative slope of the plot of the natural log of the adjusted decay curve (red squares). The pseudo first order rate constant may then be readily determined from the negative slope of the lower plot. This was achieved by considering approximately 30 data points over the first 8 sec (approximately 6 half-lives). The experiment is reproducible with reasonable precision, for example the average of four such experiments (not all recorded on the same day) gives an average pseudo first order rate constant of 0.503 sec^{-1} with a standard deviation of 2.8×10^{-2} sec^{-1}. Using the known concentration of O_2 in the cylinder, and taking the $P_{\text{ion trap}}$ as 2.58 ± 20%, an estimate of the absolute rate constant as $2.4 \pm 0.5 \times 10^{-12}$ cm^3 $molecule^{-1}$ sec^{-1} is obtained. Since the average dipole orientation (ADO) collision rate at 298 K is calculated as 5.47×10^{-10} cm^3 $molecule^{-1}$ sec^{-1} (ref. 46) the reaction efficiency corresponds to approximately 0.5%.

Figure S2: Top. The proportion of reactant radical ions (*m/z* 286) as a function of time (blue diamonds) and the proportion of radical ions corrected to account for isomerisation of [**6**+Na]$^+$ to unreactive isomers. Bottom. Natural log of the corrected proportional intensity (red triangles).



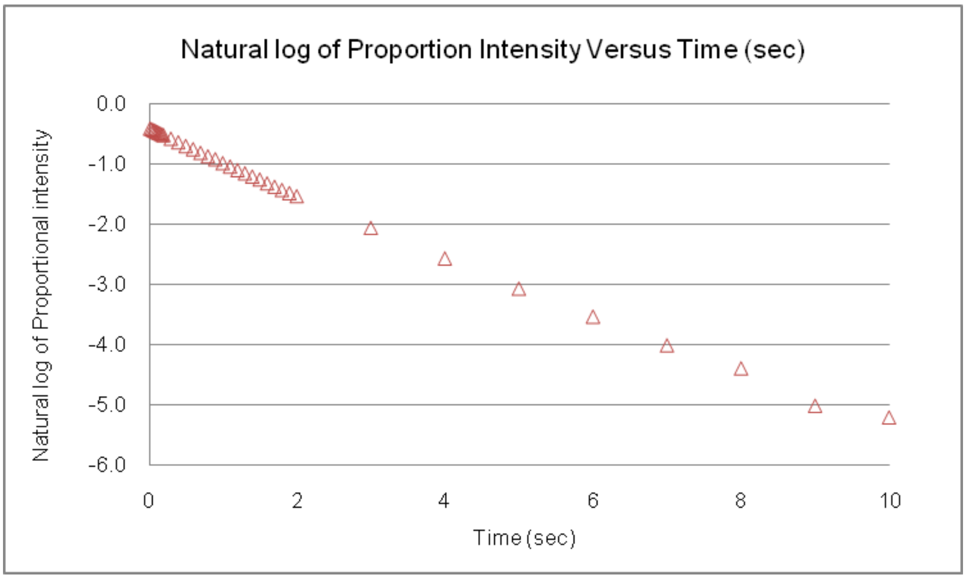


Figure S3: A. Trapping of the radical ion, [**12**+Na]$^+$ (*m/z* 272), clearly shows the addition of both NO$^\bullet$ (*m/z* 302) and NO$_2^\bullet$ (*m/z* 318). B. CID of the nitrite ester, [**25**+Na]$^+$ (*m/z* 318) formed in spectrum A.