Gas-phase structure and reactivity of the keto tautomer of the deoxyguanosine radical cation†

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Guanine radical cations are formed upon oxidation of DNA. Deoxyguanosine (dG) is used as a model, and the gas-phase infrared (IR) spectroscopic signature and gas-phase unimolecular and bimolecular chemistry of its radical cation, dG⁺⁺, A, which is formed via direct electrospray ionisation (ESI/MS) of a methanolic solution of Cu(NO3)2 and dG, are examined. Quantum chemistry calculations have been carried out on 28 isomers and comparisons between their calculated IR spectra and the experimentally-measured spectra suggest that A exists as the ground-state keto tautomer. Collision-induced dissociation (CID) of A proceeds via cleavage of the glycosidic bond, while its ion–molecule reactions with amine bases occur via a number of pathways including hydrogen-atom abstraction, proton transfer and adduct formation. A hidden channel, involving isomerisation of the radical cation via adduct formation, is revealed through the use of two stages of CID, with the final stage of CID showing the loss of CH2O as a major fragmentation pathway from the reformed radical cation, dG⁺⁺. Quantum chemistry calculations on the unimolecular and bimolecular reactivity are also consistent with A being present as a ground-state keto tautomer.

Introduction

Reports on the carcinogenic effects of ionising radiation date back to as early as 1902, and Marie Curie and her daughter Irene are believed to have succumbed to complications from radiation-induced leukemia.1 Early research on the effects of ionising radiation on DNA2 predicted Watson and Crick’s revelation of its structure,3 which provided an important molecular foundation for all subsequent research.4 It is now widely recognised that oxidative damage to DNA, through nucleobase modifications and the formation of strand breaks, can cause mutagenesis, cancer and is involved in aging.5

Extensive research focusing on a molecular understanding of the effects of ionising radiation on DNA and its consequences6 has revealed that the holes produced during the initial ionising event in DNA transfer mostly to the guanine (G) sites, which have the lowest ionisation energy (IE) of the four DNA bases.7 Thus, the electron-loss centre generated in duplex DNA ultimately ends up at guanine sites via hole migration through the DNA duplex. On loss of one electron, the resultant DNA base radical cation has a greatly increased acidity. For example, work on DNA models has demonstrated that the acidity of deoxyguanosine is enhanced in aqueous solution by around 5.6 orders of magnitude (5.6 pKₐ units) upon oxidation.7 Thus, proton-coupled electron and hole transfer becomes an important feature in the radiation damage process.6 One proposed pathway to strand breakage in DNA proceeds via species resulting from deprotonation of the guanine radical cation, which trigger specific hydrogen-atom abstraction reactions from the sugar moiety,8 thereby causing the heterolytic elimination of the phosphate–ester bond.9

Infrared (IR) spectroscopy integrated to tandem mass spectrometry has emerged as an important tool to characterise the structure of mass-selected and trapped molecular ions.10 The highly intense and tuneable IR beam delivered by Free Electron Lasers (FEL)11,12 is particularly well suited since it provides access...
to the so-called IR fingerprint region. As a result, isobaric ions bearing distinct functional groups can be clearly distinguished. Thus, the tautomers of protonated pyrimidic bases (thymine, cytosine, and uracil) have been characterised.\textsuperscript{13} It turns out that the CO stretching region around 1800 cm\textsuperscript{-1} is the most diagnostically useful part of the IR spectrum, since it is characteristic of the keto form. Although the spectral assignment in the 1500–1800 cm\textsuperscript{-1} region is more challenging, it also appears to be very informative for the structural assignment of tautomers.

We have recently shown that mass-spectrometry-based gas-phase studies that combine the use of IR spectroscopy, ion–molecule reactions and computational chemistry provide a powerful way of relating the structure of radical cations of relevance to biological damage to their fundamental chemistry.\textsuperscript{14,15} For example, we have used IR spectroscopy to show that the radical cation of 9-methylguanine (9MG) has the same ground-state keto tautomer found in DNA, and discovered via the use of ion–molecule reactions that its N–H acidity is enhanced by \textasciitilde 470 kJ mol\textsuperscript{-1} in the gas phase.\textsuperscript{15} As noted in our previous paper,\textsuperscript{15} a key motivation for our studies is that Steenken has emphasised that the local environment experienced by nucleobases within DNA is different to model systems in bulk water, and suggested that gas-phase acidity data may be more appropriate to characterise the equilibrium position of the proton in oxidised GC base pairs.\textsuperscript{6c,7a}

Given that sugar radicals have been implicated as products arising from ionisation at guanine sites, we examine here the gas-phase IR spectrum, and the bimolecular and unimolecular chemistry of the deoxyguanosine radical cation, dG\textsuperscript{**}. In previous work, we showed that electrospray ionisation (ESI) of a solution of dG and Cu(NO\textsubscript{3})\textsubscript{2}, coupled with multistage collision-induced dissociation (CID) in an ion-trap mass spectrometer, resulted in isomeric dG\textsuperscript{**} species A and B, which could be distinguished by their different fragmentation behaviour under CID (Scheme 1).\textsuperscript{16b} Thus, the major fragment ion formed from A arises from cleavage of the glycosidic bond. In contrast, formaldehyde loss is the major fragmentation channel for B. We speculated that the difference in the unimolecular chemistry was due to the nucleobase moiety G, existing in different tautomeric forms in A and B.\textsuperscript{16b}

Here we use gas-phase IR spectroscopy, ion–molecule reactions and quantum chemistry calculations to interrogate the structure and reactivity of species A, which is formed via direct ESI/MS. Compared with the radical cation of 9MG,\textsuperscript{15} the presence of the sugar moiety greatly increases the number of isomers whose structures, energies and IR spectra need to be considered by quantum chemistry calculations. Apart from the keto and enol tautomers, there are the syn and anti conformational isomers associated with rotations around the C–N glycosidic bond.\textsuperscript{15} In addition, distonic ions can be formed via H-atom transfer from the sugar to the nucleobase radical cation.

**Methods**

Reagents were used as supplied: Cu(NO\textsubscript{3})\textsubscript{2} (Ajax chemicals, 99%) and deoxyguanosine (Sigma, 99%). Complexes were prepared by mixing 2 : 1 mM solutions of the nucleoside: Cu(NO\textsubscript{3})\textsubscript{2}, dissolved in 3 : 1 methanol:water, immediately before infusing the reaction mixture into the mass spectrometer.

**Infrared spectroscopy**

IR spectroscopy in the 1000–2000 cm\textsuperscript{-1} spectral range was carried out using the FEL of CLIO\textsuperscript{12} coupled to a hybrid FT-ICR tandem mass spectrometer.\textsuperscript{18} Hybrid stands for the fact that prior to the transfer to the ICR cell, electrosprayed ions can be mass-selected in a quadrupole and accumulated in a linear hexapole pressurised with Ar (\textasciitilde 10\textsuperscript{-7} mbar) allowing for CID and/or thermalisation of the ions. As described previously, dG\textsuperscript{**} species A can be observed directly by ESI.\textsuperscript{16} Following mass-selection, the isolated dG\textsuperscript{**} (species A) was irradiated with the FEL IR beam for 250 or 500 ms. On the basis of previous results,\textsuperscript{18} it is important to emphasise that the mass-selected radical ions are subjected to multiple collisions with argon, thus ensuring an efficient thermalisation prior to the pulsed extraction towards the ICR cell.

Upon resonant vibrational excitation, dissociation of the m/z 267 radical ions was monitored via the formation of the diagnostic m/z 151 fragment peak ions. The abundance of this photofragment and its corresponding precursor were recorded as a function of the IR wavelength in order to derive the IR action spectra, where the IR multiple-photon dissociation (IRMPD) efficiency is plotted against the photon energy, which was varied stepwise.

**Mass spectrometry**

All mass spectrometry experiments were carried out using a commercially available Finnigan-LTQ-FT (Thermo, Bremen,
Germany) mass spectrometer equipped with ESI source described in detail elsewhere. The procedure used to generate dG\(^{+}\) species A was as described above. The sample was introduced into the mass spectrometer at 5.0 \(\mu\)l min\(^{-1}\) via ESI. Typical ESI conditions used were: spray voltage, 3.3–5.0 kV; capillary temperature, 250 °C; nitrogen sheath pressure, 8–40 (arbitrary units). The capillary voltage and the tube lens offset were tuned to maximise the desired peak. The injection time was set using the automatic gain control function. The LTQ-FT mass spectrometer consists of (i) a linear ion trap (LTQ); (ii) ion-transfer optics; and (iii) an FT-ICR mass analyser. For the tandem mass spectrometry (MS/MS) experiments, dG\(^{+}\) species A produced via ESI was trapped in the LTQ and subjected to CID at an He bath gas pressure of ca. \(2 \times 10^{-5}\) Torr. CID was carried out by mass selecting the desired ions with a 1.5–6 \(m/z\) units window and subjecting them to the following typical conditions: normalised collision energy between 16% and 40%, which determines the translational kinetic energy of the ions; activation \((Q)\), 0.25–0.35, which assigns the radio-frequency (RF) amplitude used to fragment ions, and activation time of 30 ms that is the time set to excite the ions via CID. The high resolution of the FT-ICR mass spectrometer was used to confirm the charge states of the mass-selected precursor ions. For high-resolution mass analysis, the ions were transferred via the ion optics transfer region (\(2 \times 10^{-7}\) Torr) into an FT-ICR cell at a pressure below \(1.5 \times 10^{-9}\) Torr.

**Ion–molecule reactions**

The mass spectrometry instrument described above has been modified to allow ion–molecule reactions (IMRs) to take place in the LTQ.\(^{21}\) dG\(^{+}\) species A was trapped in the LTQ and subjected to IMRs with the desired neutral reagents: diisopropylethylamine ([iPr\(_2\)NEt], triethylamine (Et\(_3\)N), and diisopropylamine ([iPr\(_2\)NH]). IMRs between the mass selected dG\(^{+}\) species A and the various bases were carried out with reaction times varying between 30 and 1000 ms. Branching ratios of the products were calculated by integrating the intensities under the appropriate peaks.

**Computational methods**

Standard density functional theory calculations were carried out with Gaussian 09.\(^{22}\) Geometries were optimised at the B3-LYP/6-31+G(d,p) level. Improved single-point energies were obtained using the M06-2X/6-311+G(3df,2p) protocol.\(^{23}\) Zero-point vibrational energies and thermal corrections for enthalpies at 298 K were obtained using scaled B3-LYP harmonic vibrational frequencies.\(^{24}\) Unless otherwise noted, energies in the text refer to free energies at 298 K. The vibrational frequencies (B3-LYP) used for the simulated IR spectra were scaled according to literature recommendations.\(^{25}\) A scaling factor of 0.98 was applied to the calculated B3-LYP/6-31+G(d,p) harmonic frequencies in the 1000–2000 \(cm^{-1}\) region. Each calculated band was convoluted assuming a Gaussian shape with full width at half maximum of 20 \(cm^{-1}\).

**Results and discussion**

**IR spectroscopy of dG\(^{+}\) species A directly formed via ESI/MS on copper(n) solutions**

The gas-phase deoxyguanosine radical cations, dG\(^{+}\) A, formed via electrospray ionisation of a methanolic solution of a mixture of copper nitrate and dG,\(^{16}\) were mass selected and stored in the cell of a Fourier transform ion cyclotron resonance mass spectrometer, where they were subjected to infrared radiation. The resulting IR multiple-photon dissociation (IRMPD) spectrum shown in Fig. 1a was obtained. Table S1 (ESI\(^{\dagger}\)) lists the experimental band positions.

The IR spectrum is dominated by an unresolved feature with two maxima at \(\sim 1595\) and \(\sim 1650\) \(cm^{-1}\). The band observed at \(\sim 1750\) \(cm^{-1}\) when dG\(^{+}\) ions are formed in the source region is characteristic of a C=O stretching mode. Two other intense bands are observed at \(\sim 1110\) and \(\sim 1372\) \(cm^{-1}\). Finally, weaker features are observed at \(\sim 1200\), \(\sim 1275\), and \(\sim 1511\) \(cm^{-1}\).

The experimental IR spectrum of A is compared with the spectra calculated (at the B3-LYP/6-31+G(d,p) level) for a number of different tautomers lying within 50 kJ \(mol^{-1}\) in free energy (at the M06-2X/6-311+G(3df,2p) level) of the lowest-energy isomer 1a, together with some higher-energy tautomers such as one proposed in our previous study.\(^{16}\) A total of 28 tautomers of dG\(^{+}\) were examined theoretically in this way, and their structures and IR spectra are given in Fig. S1–S3 (ESI\(^{\dagger}\)). An examination of the calculated IR spectra (Fig. 1 and Fig. S3, ESI\(^{\dagger}\)) shows that the IR spectrum is very sensitive to the nature of the tautomers. The experimental spectrum (Fig. 1a) nicely matches the spectrum calculated for the lowest-energy keto tautomer 1a (Fig. 1b). On this basis, band assignments are proposed in Table S1 (ESI\(^{\dagger}\)). As mentioned
above, the band observed at \( \sim 1750 \text{ cm}^{-1} \) is a signature of a keto isomer, and the corresponding C–O stretching mode is predicted at 1748 cm\(^{-1} \) for the lowest energy isomer 1a. A very good agreement between theory and experiment can also be found for the lower energy bands, which are assigned to the ring deformation (N3–C4 stretching) (1a: 1653 cm\(^{-1} \), ext: 1640 cm\(^{-1} \)), NH\(_2\) scissoring (1a: 1606 cm\(^{-1} \), ext: 1595 cm\(^{-1} \)), guanine six-membered-ring breathing (1a: 1510 cm\(^{-1} \), ext: 1511 cm\(^{-1} \)), multiply-coupled C–H/N–H bending (1a: 1353, 1355, and 1398 cm\(^{-1} \), ext: 1372 cm\(^{-1} \)) and multiply-coupled guanine/sugar ring breathing (1a: 1076, 1084, 1089, 1099, and 1152 cm\(^{-1} \), ext: 1110 cm\(^{-1} \)) modes. Thus we assign species A as the keto isomer 1a.

It is interesting to compare our results on the IR spectroscopic identification of the keto tautomer radical cation of dG with results for the parent neutral.\(^2^6\) Although the keto tautomer of dG is predicted to be more stable than the enol tautomer by 3.8 kJ mol\(^{-1} \), gas-phase IR spectroscopy of laser-desorbed dG only identified the enol tautomer,\(^2^6a,b\) which has been ascribed to a short excited-state lifetime of the keto form in the two-photon ionization event required to generate the IR spectrum.\(^2^6c\) In contrast, hydration of laser-desorbed dG by one or two water molecules, results in the keto form being observed in the gas-phase IR spectrum.\(^2^6c\) Thus the way the dG radical cation or neutral is transferred to the gas phase appears to play a key role as to which tautomer is formed. Although the exact mechanism by which dG\(^+\) A is formed during the ESI process, which transfers species from solution to the gas phase, remains unknown, our assignment of this species as the keto form in the gas phase is consistent with the following known solution-phase chemistry of dG: (i) NMR studies have demonstrated that it exists largely as the keto tautomer in its neutral form;\(^2^7\) and (ii) upon binding of Cu\(^{2+}\), the keto tautomer is maintained.\(^2^8\)

### Ion–molecule reactions of dG\(^+\) A

The acidity of dG\(^+\) A was estimated via IMRs with neutral reference bases B with known proton affinities.\(^3^1\) Five types of reactions were observed: proton transfer (PT, eqn (1)), electron transfer (ET, eqn (2)), hydrogen-atom abstraction (HAA, eqn (3)), adduct formation (AddF, eqn (4)) and complexation-induced fragmentation (CplxFr, eqn (5)).

\[
\begin{align*}
\text{dG}^+ + B & \rightarrow (\text{dG} - H)^+ + (B + H)^+ \quad \text{PT} \\
\text{dG}^+ + \text{B}^+ & \rightarrow (\text{dG} + \text{H})^+ + (\text{B} - H)^+ \quad \text{ET} \\
(\text{dG} + \text{H})^+ + (\text{B} - H)^+ & \rightarrow (\text{dG} + \text{B})^+ \quad \text{HAA} \\
(\text{dG} + \text{B})^+ & \rightarrow [(\text{dG} + \text{B})^+ + \text{X}]^+ \quad \text{AddF} \\
(\text{dG} + (\text{B} - \text{X}))^+ & \rightarrow \text{CplxFr} \quad \text{CplxFr}
\end{align*}
\]

Table 1 provides a summary of the branching ratios (BRs) for these reactions, and a sample spectrum of the IMR of dG\(^+\) with iPr\(_2\)NEt at 100 ms reaction time is shown in Fig. 2. We found that during the IMRs with the bases, the reactions did not go to completion and that the dG\(^+\) at m/z 267 was still observed even at very long reaction times (e.g., 10,000 ms). We attribute this observation to the complexation-induced isomerisation reaction of the dG\(^+\) radical cation, which produces a different isomer(s), which we call species C. We base this conclusion on the CID spectrum of C, which is formed in the following sequential MS\(^2\) reactions: (i) the IMR between dG\(^+\) A and a base produces the adduct [dG + B]\(^+\) (MS\(^3\)); (ii) isolation of this adduct followed by CID results in loss of the base and generation of dG\(^+\) C (MS\(^4\)); (iii) isolation of dG\(^+\) C allows its CID spectrum to be collected in an MS\(^4\) experiment.

As an example, the MS\(^4\) spectrum of dG\(^+\) C is shown in Fig. S4a (ESI), where isomerisation has taken place through the reaction with iPr\(_2\)NET, and the resultant new species C fragments via the major loss of formaldehyde.\(^\S\) In contrast, C is less reactive than A in IMRs with bases, and does not undergo reactions via eqn (1)–(3). Thus it appears that in IMRs of dG\(^+\) A can not only react directly via eqn (1)–(3), but it can also undergo isomerisation. Such reactions have been observed in the gas phase before and Bohme has classed them as proton-transport-catalysed reactions.\(^3^2\) Of particular relevance to the potential tautomers that might be formed in these reactions are previous observations of proton-transport-catalysed reactions that isomerise the keto form of amide radical cations to their enol forms.\(^3^3\)

Theoretical modelling of the ion–molecule reactions of the keto and enol tautomers is summarised in Fig. S5 (ESI*). Note that the calculated M06-2X/6-311+G(3df,2p) proton affinities (PAs) of the bases are slightly underestimated by the theory (by 4–8 kJ mol\(^{-1} \)) while the IEs are slightly overestimated. Nevertheless, the experimental IMR results are consistent with the proton affinities calculated for the keto tautomer.

The PA for the (9MG – HN\(_3\))^+ radical is 968.9 kJ mol\(^{-1} \) at the 100 ms reaction time is shown in Fig. 2. We found that during the IMRs with the bases, the reactions did not go to completion and that the dG\(^+\) at m/z 267 was still observed even at very long reaction times (e.g., 10,000 ms). We attribute this observation to the complexation-induced isomerisation reaction of the dG\(^+\) radical cation, which produces a different isomer(s), which we call species C. We base this conclusion on the CID spectrum of C, which is formed in the following sequential MS\(^2\) reactions: (i) the IMR between dG\(^+\) A and a base produces the adduct [dG + B]\(^+\) (MS\(^3\)); (ii) isolation of this adduct followed by CID results in loss of the base and generation of dG\(^+\) C (MS\(^4\)); (iii) isolation of dG\(^+\) C allows its CID spectrum to be collected in an MS\(^4\) experiment.

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smaller than that of (1a − H)*, shows a branching ratio for eqn (1) < 1%. The most acidic proton of 1a is the C2N–H of the amino group, the proton that would be involved in a guanine–cytosine pairing, consistent with the results observed for 9MG**.15

The electron-transfer reaction (eqn (2)) depends on the IE of the bases versus the electron affinity (EA) of the dG* radical cation. The calculated IEs (Fig. S5, ESI†) for iPr2NET, Et3N and iPr2NH are 705, 744 and 756 kJ mol\(^{-1}\), respectively, whereas the EA of 1a is calculated to be 712 kJ mol\(^{-1}\). In this case also, theory is consistent with the experimental results, where the branching ratio for the iPr2NET is the highest (18%, Table 1) and that of iPr2NH is the lowest (0%, Table 1). Our theoretical results are also consistent with the gas-phase IE of dG, which has been bracketed as 718 kJ mol\(^{-1}\) from DFT calculations of 730 kJ mol\(^{-1}\). The gas-phase acidity of neutral dG has been recently determined to 996 kJ mol\(^{-1}\) \cite{ref. 39, 19}, thus in the gas phase, ionisation of dG* is generally exothermic, with reaction energies ranging from −3 kJ mol\(^{-1}\) for Et3N to −31 kJ mol\(^{-1}\) for iPr2NET. The relatively small energies for these proton-transfer (eqn (1)) or hydrogen-atom-abstraction (eqn (3)) reactions suggest that either class of reaction might be involved as an intermediate step for the observed isomerisation of A to C. In any case, the calculated thermochemical quantities and the experimental branching ratios, as well as the very observation of C, indicates a complex set of processes induced by IMRs.

Finally, it is worth considering the results of these gas-phase reactions within the context of the proposed biological chemistry of one-electron-oxidised guanine residues within DNA. It has been proposed that oxidised guanine residues have a significantly enhanced acidity, which allows them to undergo proton transfer to the adjacent cytosine residues. Our experimental results suggest that the PA for the (dG−H)* radical 1a (978–996 kJ mol\(^{-1}\)) is close to that of iPr2NET (994.3 kJ mol\(^{-1}\)). The gas-phase acidity of neutral dG has been recently determined to be 1409 kJ mol\(^{-1}\) \cite{ref. 39, 19, 35, 36}. Thus in the gas phase, ionisation of dG enhances its acidity by ~415 kJ mol\(^{-1}\), somewhat less than what we found for 9MG (~470 kJ mol\(^{-1}\)), but still a significant enhancement of acidity. Indeed, the enhanced acidity of dG* is close to the PA of dC (988.4 kJ mol\(^{-1}\)) \cite{ref. 39, 19, 35, 36} and this suggests that proton transfer within the ionised base pair may become viable. As noted in the introduction, a mechanism proposed for strand breaking in ionised DNA involves hydrogen-atom abstraction from the sugar moiety induced by deprotonation of the guanine radical cations.8 Our observation of a hydrogen-atom abstraction channel for dG* suggests that ionised guanine sites within DNA may indeed be cable of inducing hydrogen-atom abstraction reactions.
A mechanism for the formation of m/z 151 in the unimolecular fragmentation of dG** species A

As noted in the introduction, the way in which the various dG** species fragment depends on the way that they are generated (Scheme 1). The main dissociation channel in the low-energy CID spectrum of A is the cleavage of the Nglycosidic bond to give the radical cation of the guanine base at m/z 151,18 (Fig. 3b, ESI†) which contrasts with the series of fragment ions observed in the El mass spectrum39 and upon REMPI.40 The IR spectroscopy and IMR results suggest that A exists as the keto tautomer (1a). Thus we have used DFT calculations to examine the energetics for loss of C5H8O3 (observed in the CID spectrum (1a–1c)) to give the radical cation of the guanine base at m/z 151,18 (Fig. 3a, ESI†). Fragmentation is triggered by N–C bond cleavage (1b–1c†) to yield the glycine moiety with concerted ring closure (1c–1d†) to yield the cyclised sugar (1d, 1e). In contrast, loss of CH2O is triggered by hydrogen-atom abstraction from the dissociated sugar moiety with concerted ring closure (1e–1f†) to yield the cyclised sugar (1f). The free energy barrier for C5H8O3 loss is 108 kJ mol−1, which is slightly lower than the barrier for loss of CH2O (110 kJ mol−1), consistent with C5H8O3 being the main fragmentation channel.

Fig. 3 M06-2X/6-311+G(3df,2p) schematic free energy profile (kJ mol−1) associated with the fragmentation reactions of 1a via (a) loss of the cyclised sugar C5H8O3 (red), or (b) loss of CH2O (blue).

Conclusions

The gas-phase structure and reactivity of dG** species A has been investigated by a combination of IR spectroscopy, mass spectrometry experiments involving both ion–molecule reactions and CID, and computational quantum chemistry calculations. This powerful combination has allowed us to assign A as the ground-state keto form. It undergoes a number of reactions with amines, including hydrogen-atom abstraction, proton transfer, electron transfer and adduct formation. Thus, the PA of [dG-H]° radical has been experimentally bracketed as 994.3 kJ mol−1 > PA [dG-H]+ > 981.8 kJ mol−1, consistent with our calculated value of 993.5 kJ mol−1 for removal of the N1 proton from the keto form of dG**. Similarly, the electron affinity of the dG** radical cation (A) has been bracketed as 756 kJ mol−1 > electron affinity A > 705 kJ mol−1, consistent with our calculated value of 712 kJ mol−1. Theoretical calculations on the unimolecular dissociation of the keto form (1a) of dG** suggest that the favoured pathway involves cleavage of the glycosidic bond.

On a final note, the dG** species B and C remain to be fully characterised. A technical challenge is that they need to be formed in a series of multistage mass spectrometry experiments. Nonetheless, preliminary results suggest that B and C consist of isomer(s) that are different to A, and that they are less reactive than A in ion–molecule reactions.

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