Structure of the Pb$^{2+}$–deprotonated dGMP complex in the gas phase: a combined MS-MS/IRMPD spectroscopy/ion mobility study†

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The structure of the Pb$^{2+}$–deprotonated 2′-deoxyguanosine-5′-monophosphate (dGMP) complex, generated in the gas phase by electrospray ionization, was examined by combining tandem mass spectrometry, mid-infrared multiple-photon dissociation (IRMPD) spectroscopy and ion mobility. In the gas phase, the main binding site of Pb$^{2+}$ onto deprotonated dGMP is the deprotonated phosphate group, but the question is whether an additional stabilization of the metallic complex can occur via participation of the carbonyl group of guanine. Such macrochelates indeed correspond to the most stable structures according to theoretical calculations. A multiplexed experimental approach was used to characterize the gas-phase conformation of the metallic complex and hence determine the binding mode of Pb$^{2+}$ with [dGMP]$^-$: MS/MS analysis, observation of characteristic bands by IRMPD spectroscopy, and measurement of the ion mobility collision cross section suggest that gaseous [Pb(dGMP)-H]$^+$ complexes adopt a macrochelate folded structure, which consequently differs strongly from the zwitterionic forms postulated in solution from potentiometric studies.

1. Introduction

Since its discovery in the beginning of the 20th century, mass spectrometry has become one of the most powerful analytical methods, especially with the advent of soft ionization methods, which allow intact biomolecules to be easily ionized and transferred into the gas phase. Structural characterization of biopolymers such as peptides, oligosaccharides and oligonucleotides is generally achieved using tandem mass spectrometry methods (MS/MS) based upon the activation of precursor ions by collisions with a buffer gas. Collision induced dissociation (CID) experiments indeed provide invaluable data regarding the primary sequence of these biomolecules. More recently, alternate activation methods such as electron capture dissociation (ECD),1 electron transfer dissociation (ETD),1,2 electron detachment dissociation (EDD)3 or electron photodetachment dissociation (EPD),4 have demonstrated the ability to provide complementary sequence information in comparison to CID, due to different dissociation mechanisms, thereby leading to increased peptide sequence coverage.

Although particularly useful, these methods provide little information about the spatial arrangement of ions in the gas phase. To address this question, several instrumental approaches have emerged during the last decade. Coupled with mass spectrometry, these setups offer value-added data that cannot be obtained by mass spectrometry alone. Among those approaches, ion mobility spectrometry (IMS) is an analytical technique used to separate and characterize ionized molecules in the gas phase based on their mobility through a buffer gas, under the influence of an electric field. It allows isomer separation, and provides global insight into conformation of ions through the measurement of their collision cross section (CCS) in the buffer gas.5–10 A complementary and strongly structure-sensitive technique is infrared (IR) multiple photon dissociation (IR-MPD).11,12 This spectroscopic technique is often termed “action spectroscopy” because resonant IR absorption cannot be directly probed due to the low ion density within a mass spectrometer. With the use of intense and tunable infrared sources such as free electron lasers, ion fragmentation can be induced by a multiple-photon absorption process, and fragment ions can be mass analyzed as a function of the photon energy. Detailed analysis of spectral features can yield information on the local molecular arrangement and the intramolecular H-bond network. In many cases, results from one of the above techniques alone are not sufficient to unambiguously assign a structure to a complex molecular

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Electrospray mass spectra were recorded on an Applied Biosystems/MDS Sciex API2000 triple-quadrupole instrument fitted with a "turboionspray" ion source. Solutions of lead nitrate–dGMP were introduced in the source using direct infusion with a syringe pump at a flow rate of 5 μl min⁻¹. Ionization of the samples was achieved by applying a voltage of 4.0 kV on the sprayer probe, and by the use of a nebulizing gas (GAS1, air) surrounding the sprayer probe, intersected by a heated gas (GAS2, air) at an angle of approximately 90°. The operating pressure of GAS1 and GAS2 was adjusted to 2.1 bar, while that of the curtain gas (N₂) was set to 1.4 bar. The temperature of GAS2 was set at 373 K. The difference in potentials between the orifice plate and the skimmer (cone voltage, CV) ranged from 20 to 80 V for the experiments performed here.

MS/MS spectra were systematically recorded at various collision energies ranging from 0 to 30 eV in the laboratory frame (corresponding to the difference between the potentials of Q0 and Q2). The CAD parameter controlling the pressure of N₂ introduced into Q2 was set to its minimum value in order to limit multiple ion–molecule collisions.

Unless otherwise noted, mass to charge ratios mentioned throughout this paper refer to peaks including the most abundant lead isotope (²⁰⁸Pb).

(c) IRMPD spectroscopy experiments

The present IRMPD study was performed using a Fourier transform-ion cyclotron resonance (FT-ICR) instrument into which pulsed IR light is coupled.¹⁷ This particular experimental setup has already been described in detail elsewhere.²⁷ The most important feature of the present study is the quadrupole–hexapole interface between the electrospray source and the ICR cell. The bias voltage and RF amplitude of the quadrupole are adjusted to selectively transmit [Pb(dGMP)-H]⁺ complexes. Mass-selected ions are then trapped in an ~5 cm long hexapole ion trap, contained within a collision cell, where ions are collisionally cooled using a flow of high-purity argon buffer gas. Ions are pulse-extracted from the hexapole toward the ICR cell where mass-selection of the complexes is performed. Mass-selected ions are irradiated with IR light, after which the resulting ions are mass-analyzed. [Pb(dGMP)-H]⁺ complexes were prepared using an electrospray ion (ESI) source by direct infusion of equimolar lead nitrate–dGMP mixtures (10⁻⁴ M, water–methanol 50/50 v/v) at a flow rate of 3 μl min⁻¹, a spray voltage of 3500 V, and a capillary temperature of 473 K.

IR spectroscopy was performed using the CLIO (centre laser infrarouge d’Orsay) free electron laser (FEL), which produces pulsed, tunable IR light covering the 100–2500 cm⁻¹ wavenumber range.²⁸ The light is produced in an 8 μs long pulse train, the macropulse, of IR laser pulses a few picoseconds in duration, the micropulses. The macropulse repetition rate is 25 Hz while that of the micropulse is 62.5 MHz. Using an electron energy of 45 MeV for the FEL, IRMPD spectra could be recorded over the 1000–2000 cm⁻¹ range.

If the IR light is in resonance with an IR active vibrational mode of the molecular ions stored in the ICR cell, IR photons can be absorbed and the sequential absorption of multiple IR photons can lead to fragmentation of the mass-selected ions. This photo-fragmentation, which is the result of a multiple photon absorption process, is often termed infrared multiple

2 Methodology

(a) Materials

Lead nitrate (Pb(NO₃)₂) and dGMP used in this work were research grade products from commercial sources (Sigma-Aldrich, Saint Quentin Fallavier, France) and were used without further purification. Stock aqueous solutions of lead nitrate and the nucleotide were prepared at 10⁻² M by using Milli-Q purified water (resulting pH 5.5). Lead nitrate–dGMP mixtures were diluted at various concentrations and ratios, in a water–methanol mixture (50/50 v/v), prior to their introduction into the electrospray sources.

(b) Tandem-mass spectrometry experiments

Electrospray mass spectra were recorded on an Applied Biosystems/MDS Sciex API2000 triple-quadrupole instrument fitted with a...
 photon dissociation (IRMPD). In the present work, only one or two macropulses were sufficient to achieve high photo-fragmentation yields (irradiation time 50–100 ms). At each wavelength, the mass spectrum is the Fourier transform of a time-domain transient averaged 5 times. The IRMPD spectrum is obtained by plotting the photofragmentation yield $R = \frac{\ln[I_{\text{parent}}/I_{\text{fragment}}]}{I_{\text{parent}} + \sum I_{\text{fragment}}}$ where $I_{\text{parent}}$ and $I_{\text{fragment}}$ are the integrated intensities of the precursor and fragment ions, respectively, as a function of the frequency of the IR radiation.\cite{28} A recent study\cite{29} has demonstrated that this data treatment allows for a better comparison between IRMPD intensities and calculated infrared absorption spectra while providing better spectral resolution than other analysis methods.

(d) Ion mobility experiments

Ion mobility spectrometry-mass spectrometry (IMS-MS) measurements were performed on a custom-built ion mobility spectrometer first described elsewhere.\cite{31} Briefly, a 1 m-long drift tube is inserted between the electrospray ion source and the ion transfer region of a commercial Q-TOF mass spectrometer (microToFQ, Bruker-Daltonics, Bremen, Germany). Ions formed in the ESI source are trapped and accumulated in an hourglass-shaped funnel ion trap. Short ion bunches (800 μs) are injected into the drift tube at a repetition rate of ~5 Hz. A helium pressure of 12.8 Torr is maintained in the drift tube, and the temperature of the entire setup is maintained at 296 K, the regulated temperature of the experimental room. Drift fields ranging from 300 to 1000 V m\(^{-1}\) can be used for measurements.

Ions exiting the drift tube are collected via a series of three 3 ion-funnels and guided through the Q-TOF instrument. Time of flight (TOF) mass spectra can be recorded as a function of the drift time to yield high resolution IMS-MS measurements, which allow accurate comparison between the drift times of species with different m/z ratios. When the mass spectrum of the sample solution is uncontagged and the ion of interest can be efficiently mass-selected in the q-TOF quadrupole (which is the case in the present study), arrival time distributions can be recorded directly from the dynode detector by turning off the push–pull voltages of the orthogonal TOF. Although mass-resolution is lower in the latter mode, it offers much higher sensitivity.

The determination of the collision cross section (CCS) for an ion relies on the measurement of its IMS drift time $t_d$ as a function of the drift voltage. Under experimental conditions, the movement of an ion of mass $M$ and charge $q$ in the drift tube falls in the so-called low-field ion mobility regime.\cite{32} The drift time across the tube depends on the buffer gas mass $m$, temperature $T$, and pressure $p$, and on the applied electric field $E$, and is related to the collision cross section $\Omega$ (more rigorously the rotationally-averaged momentum transfer cross section in ion-buffer gas collisions) through$^{24}$

$$t_d = \frac{16}{3} \sqrt{\frac{\mu}{2 \pi k_B T q E}} \frac{\rho L}{\Omega}$$

where $\mu = \frac{Mm}{m + M}$ is the reduced mass, and $k_B$ is the Boltzmann constant.

The time that is actually measured in an IMS experiment is $t_{\text{mes}} = t_d + \Delta t$, $t_d$ being the time necessary for an ion to travel from the end of the drift region to the detector. The most accurate way to measure $\Omega$ is then to plot $t_{\text{mes}}$ against the inverse voltage and to perform a linear regression on the recorded data (see ESI$^\dagger$). The value of $\Omega$ is finally extracted from the slope, the intercept being $t_0$.

In the present case, trapping voltages are adjusted to optimize the [Pb(dGMP)-H]$^+$ ion signal intensity. [Pb(dGMP)-H]$^+$ ions are selected at m/z 554 ± 5 using the quadrupole mass filter. Selected ions are injected with a very low energy (2 eV) into the collision cell [gas flow 20% in Bruker control software] and their arrival time is monitored directly from the dynode on a scope. The drift field is varied from 400 to 850 V m\(^{-1}\) in steps of 50 V m\(^{-1}\). The [Pb(dGMP)-H]$^+$ ion arrival time is measured repeatedly (up to 4 times per drift field value, see Section S5 of the ESI$^\dagger$).

(e) Theoretical calculations

Molecular orbital calculations were carried out using the B3LYP density functional, as implemented in the Gaussian-03 set of programs.\cite{33} B3LYP combines the non-local correlation functional of Lee, Yang and Parr,\cite{34} with the Becke's three-parameter non-local hybrid exchange functional.\cite{35} In the first step, the different structures were optimized using the dp-polarized 6-31G(d,p) basis set, without any symmetry constraint. For Pb we used the “Stuttgart” quasi-relativistic pseudo-potential developed by Küchle et al.\cite{36} This particular 78-electron effective core potential (ECP) employs a (4s, 4p, 1d)/[2s, 2p, 1d] basis set with a (3,1) contraction scheme for s and p functions that can be used directly in conjunction with the standard 6-31G(d,p) Pople basis set describing C, N, O, P and H atoms. Harmonic vibrational frequencies were estimated at this level to classify the stationary points as local minima or saddle points, and to estimate the zero-point vibrational energy (ZPE) corrections. Provided the use of an appropriate scaling factor, hybrid DFT methods such as B3LYP have been shown to outperform other DFT methods as well as traditional \textit{ab initio} approaches to describe both position and relative intensities$^{38}$ of IR bands. With regard to band positions, a scaling factor of 0.96 was chosen on the basis of the overall good agreement between experimental and computed frequencies for a large set of molecules.\cite{39} Finally, for ease of comparison with the experimental spectrum, calculated spectra were convoluted with a 15 cm\(^{-1}\) fwhm Gaussian function. Relative energies were refined at the 6-31+G(2df,2p) level using a self-developed 6-311+G(2df) basis set for Pb. We demonstrated that this basis set, in combination with the B3LYP functional, provides a good compromise between accuracy and computational cost for energy calculations.\cite{40} Throughout this paper relative free energies are expressed in kJ mol\(^{-1}\). For the sake of simplicity, Pb-basis sets will be referred to as 6-31G(d,p) and 6-311+G(2df,2p) basis sets. Detailed geometries of all structures mentioned in this paper are available from authors upon request.

In order to compare the results of the calculations with IMS experimental results, theoretical collision cross sections (CCSs)
were also calculated for candidate structures using an exact hard-sphere scattering (EHSS) model and the projection approximation (PA). Because lead and phosphorus were not originally parameterized in the model, and because the trajectory method developed by Mesleh et al. requires additional parameters more difficult to evaluate than hard-sphere radii, only PA and EHSS approaches were used in this study. Although those methods are not as accurate as the trajectory method or the projected superposition approximation, they provide approximate CCSs that prove to be very useful in comparing families of conformations. Hard-sphere radii had to be adjusted: from the literature atomic radii of P and Pb$^{2+}$ are, respectively, 110 and 120 pm, both comparable to Si radius (115 pm). As a result, Si parameters (from M.F. Jarrold’s MOBCAL program) are taken to model both P and Pb$^{2+}$. The variability in CCSs arising from this hypothesis was evaluated by carrying out calculations with hard sphere radii of P and Pb$^{2+}$ varied by $\pm$20% of the tabulated value for Si. Resulting CCSs increase by $\sim 2\text{--}7\text{Å}^2$ (respectively decrease by $\sim 2\text{--}4\text{Å}^2$) with an expected larger effect for unfolded structures. However, those differences are consistent among different structures and methods, and the CCS gap between folded and unfolded structures remains large and significant. The “Si approximation” can therefore be considered reasonable.

3 Results/discussion

3.1 Mass spectrometry

During sample preparation, dGMP is dissolved in purified water with pH 5.5. At this pH value, mononucleotides (XMP) are singly deprotonated and interaction with the Pb$^{2+}$ cation results in the formation of [Pb(XMP)-H]$^+$ complexes. Logically, electrospraying a 1 : 1 mixture ($10^{-4}$ M) of lead nitrate and dGMP results in the formation of abundant [Pb(dGMP)-H]$^+$ complexes ($m/z$ 554). However, regardless of the interface conditions, this species does not correspond to the base peak, due to competitive interaction between the metal and the solvents. At low CV values (below 40 V), the most prominent ions are indeed lead hydroxide PbOH$^+$ ($m/z$ 225) and PbOCH$_3^+$ ($m/z$ 239). As the CV is increased, these two ions quickly disappear and a slow decay of the lead–nucleotide complex is observed. Finally, at high cone voltage (CV > 120 V), the electrospray spectrum is dominated by Pb$^+$ ($m/z$ 208) and PbH$^+$ ions ($m/z$ 209). Other species such as protonated guanine ($m/z$ 152) and protonated dGMP ($m/z$ 348) are also detected with significant abundance. No doubly charged ions were detected during these experiments.

The MS/MS study was initiated by first recording the MS/MS spectrum of [Pb(dGMP)-H]$^+$, in order to characterize its fragment ions (Fig. 1). We then observed that increasing the CV parameter allows fragment ions of the [Pb(dGMP)-H]$^+$ complex to also be detected in the ESI spectrum (in-source fragmentation). So, we opted for a CV value of 40 V to induce in-source fragmentation. Then, each fragment ion thus generated was selected individually by the first quadrupole to record its MS/MS spectrum. The whole set of MS/MS data led to the dissociation pattern summarized in Scheme 2.

The CID spectrum obtained for [Pb(dGMP)-H]$^+$ at 25 eV (laboratory frame) is given in Fig. 1.

Starting from the [Pb(dGMP)-H]$^+$ precursor ion, three main processes are observed. The first one (A) corresponds to dehydration associated with the formation of the $m/z$ 536 ion, which further expels the nucleobase (GH) to generate the $m/z$ 385 species. This particular sequence has already been observed for other [Pb(XMP)-H]$^+$ ions (XMP = UMP, 24 CMP, 23 dCMP 23), and also for Cat$^+$–TMP complexes (Cat = Li, Na, Cs). The latter species then dissociates according to two processes, associated
either with the loss of the sugar moiety (-C₅H₆O) and the formation of [PbHPO₄]⁺ (m/z 303), or to the elimination of PbHPO₄ giving rise to C₅H₅O⁺ (m/z 81). The latter fragment is very likely a sugar-derived species (Scheme 2) as deduced previously from deuterium exchange experiments.⁵⁰ These two fragment ions strongly suggest that the metallic center interacts with the phosphate group. The loss of PbHPO₄ is also observed from the m/z 536 ion, leading to the m/z 232 ion which can be assigned to (C₅H₅O-G)⁺ as it further dissociates by loss of neutral guanine to generate C₅H₅O⁺ (m/z 81).

The second dissociation channel (B) experienced by the initial complex is associated with the formation of the protonated nucleobase (GH₂⁺) detected at m/z 152. Its intensity in the MS/MS spectra is rather weak. Note that direct elimination of neutral guanine is not observed, whereas it is detected in significant abundance for the [Pb(GMP)-H]⁺ complex (see Fig. S1 of the ESI†). As already shown in a previous study,²³ direct elimination of an either neutral or protonated nucleobase is more pronounced in the case of ribo-mononucleotides, suggesting that the 2'-hydroxyl group might play an important role in these fragmentation processes.

These two first dissociation pathways indicate the interaction of the Pb²⁺ center with the phosphate moiety of dGMP. These findings are not in agreement with the structure of the complexes formed in solution between Pb²⁺ and GMP. Indeed, the potentiometric studies carried out by Sigel and co-workers⁵¹,⁵² suggest that Pb²⁺ ions should mostly interact with the guanine moiety. On the other hand, the third dissociation route (C) leading ultimately to the [PbG]⁺ ion (m/z 358) indicates that within the complex, Pb²⁺ also interacts with the nucleobase. To complement MS/MS data, quantum chemical calculations as well as complementary experimental approaches (ion mobility and IRMPD spectroscopy) have been carried out.

### 3.2 Theoretical calculations

Mononucleotides are flexible molecules, and consequently performing a complete conformational survey is challenging. However, based on (i) our previous studies on pyrimidic nucleobases¹⁹ and mononucleotides,²³,²⁴ which have shown the strong affinity of lead for carbonyl groups, and (ii) the fact that the phosphate group is at least singly deprotonated under our experimental conditions, we limited the number of starting geometries. Zwitterionic forms (with the metal located away from the deprotonated phosphate group) were considered because they have been postulated as the main forms in solution for Pb²⁺-[GMP-H]⁺ complexes, according to potentiometric studies.⁵¹,⁵² In addition, we also considered the possibility of a doubly-deprotonated phosphate group because elimination of neutral [Pb(HPO₄)]⁻ is observed experimentally.

Representative structures obtained for [Pb(dGMP)-H]⁺ complexes are presented in Fig. 2, whereas their relative free energies are summarized in Table 1. The various forms are labeled according to the syn or anti orientation of the nucleobase. A detailed description of all the forms investigated (coordination mode, ring puckering, base orientation, internal hydrogen bonding) is provided in Section S2 of the ESI.† Relative free energies of all forms were refined at the B3LYP/6-311+G(2df,2p) level of theory (except dGMPA10 and dGMPS1, which did not converge at this particular level). A slight variation in relative free energies is observed as the level of calculation is increased. One noticeable effect is the narrowing in the energy gap for the three most stable species.

We located stable (positive eigenvalue) zwitterionic forms both with anti (dGMPA1) and syn oriented (dGMPS1) nucleobases. However, these forms are located at about 370 kJ mol⁻¹ above the global minimum (Table 1), and consequently are not likely to be generated in the gas phase. We also optimized a series of structures for which the metal interacts with a doubly deprotonated phosphate group and the guanine residue is protonated. For anti forms (dGMPA2-7), we considered three distinct protonation sites, N7, O6 and N3 (Scheme 1). Comparison between dGMPA2 and dGMPA3 shows that interaction with the carbonyl group of the guanine residue (dGMPA3) does not provide significant stabilization. Examination of Table 1 indicates that the energy order follows the relative proton affinity order of these three sites.⁵³-⁵⁵ N7-protonated forms being more stable (∼58 kJ mol⁻¹) for anti structures. Consequently, for syn structures only protonation at N7 was considered (dGMPS2).

All doubly-deprotonated forms correspond to minima on the potential energy surface and are considerably more stable than the zwitterions. N7-protonated forms are 280–300 kJ mol⁻¹ lower in relative free energies than zwitterions, but still 74–120 kJ mol⁻¹ above the global minimum (dGMPS10).

With our sample preparation conditions, dGMP should be singly deprotonated in water, and we therefore concentrated our efforts on structures involving singly deprotonated nucleotides. These structures can be classified into two groups. The first group includes forms in which the metallic center only interacts with the phosphate moiety (dGMPAS12 and dGMPS3-4). These forms are found to be more stable than the zwitterionic structures, suggesting that unlike in solution, the metal exhibits a much stronger affinity for the singly-deprotonated phosphate group than for the nucleobase. Like dCMP,²³ the most favorable coordination scheme is characterized by a metal interacting with two oxygen atoms of the phosphate group and the O3’H hydroxyl...
group (dGMPA11-12/dGMPS4). The other binding mode (dGMPA10) involving the phosphate hydroxyl group appears much less favorable (Table 1). This finding is consistent with previous studies,¹⁹,²² which demonstrated the poor affinity of lead for OH groups in the gas phase.

The second group of structures, denoted as macrochelates, is characterized by the metal chelating the phosphate moiety and the guanine residue. Unlike doubly deprotonated forms (dGMPA2), additional interaction with the nucleobase results in strong structural stabilization. Different types of macrochelates were obtained. The most stable coordination mode is characterized by a tetradequate interaction involving two oxygens of the phosphate group, and N7 and the carbonyl group of the guanine residue. Both syn and anti forms were obtained, although optimization of the former turned out to be easier. They exhibit very similar stabilities, the global minimum being dGMPS10. The latter form is characterized by a O4′-C1′-N9-C4 torsional angle of −66.5°, and Pb···N7 and Pb···O6 distances equal to 2.487 Å and 2.694 Å, respectively. By comparison, these geometrical parameters, for the anti macrochelate dGMPA13, are equal to 139.3°, 2.467 Å and 2.669 Å, respectively. The two additional interactions with the guanine residue provide

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**Fig. 2** Representative optimized structures of the [Pb(dGMP)-H]+ complex. Relative free energies calculated at the B3LYP/6-31+G(2df,2p)//B3LYP/6-31G(d,p) level are given in kJ mol⁻¹.
Table 1: Relative energies and structural features of the different structures optimized for the [Pb(dGMP)-H]+ complex

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<th>Structure</th>
<th>$\Delta E_{298}^a$</th>
<th>$\Delta_{298}G_{1}^b$</th>
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\(a\) By convention, the \(\text{syn}\) conformation (S) corresponds to a \(\text{C4}–\text{N9}–\text{C1}–\text{O4}'\) torsional angle ranging from 0° to ±90°. Otherwise, the conformation is \(\text{anti} (A)\). \(b\) Obtained at the B3LYP/6-31G(d,p) level, including ZPE or thermal corrections. \(c\) Obtained at the B3LYP/6-311+G(2df,2p)/B3LYP/6-31G(d,p) level, including ZPE or thermal corrections.

additional important stabilization, macrochelates being at least 126 kJ mol\(^{-1}\) more stable than the unfolded structures of the first group (dGMP54). Other binding schemes were optimized, notably involving N3 (dGMP66) or the amino group (dGMP55), but they appear sensibly less stable. Interaction with a single phosphate oxygen (dGMP66 and dGMP58) also results in less stable forms. Within macrochelates, interaction with the carbonyl group is more pronounced in the case of the tridentate binding scheme (dGMP58) than for the tetradentate coordination mode (dGMPA13/dGMP59/dGMP10), as attested by the more important bond lengthening (1.263 Å and 1.247 Å, respectively), compared to the value typically obtained at this level of calculation for free carbonyls (1.210 Å). Interaction with the nucleobase is also characterized by a small distortion of the purine moiety from planarity (typical values for the N7C5C4C6 torsional angle being in the range of 165°–166°). This distortion effect is less pronounced (178°) when the metal interacts with N3 (dGMP66 and dGMP7). Finally, interaction with the endocyclic O4' oxygen (dGMP66/dGMP7) atom has also been considered, but has no positive effect on the stability of the complex.

In summary, from calculations three near iso-energetic structures are found to be particularly stable: dGMPA13, dGMP59 and dGMP10. These three forms correspond to macrochelates involving tetradentate coordination of Pb\(^{2+}\), and are therefore very different from those present in solution (zwitterions).

3.3 IRMPD spectroscopy

In order to gain more insight, we performed IRMPD spectroscopy experiments. IRMPD spectroscopy has been widely applied to characterize the structure of metal-cationized complexes, and notably of metal/DNA block species. In the current work, IRMPD spectra have been recorded in the 1000–1900 cm\(^{-1}\) energy range. Upon resonance with an infrared active mode of the mass-selected [Pb(dGMP)-H]+ complex, two photo-fragments are observed, a particularly intense dehydration (m/z 536) together with combined elimination of water plus phosphoric acid (m/z 438). The fragmentation scheme was similar to [Pb(dCMP)-H]+.\(^2\)\(^3\)\(^4\) The experimental optical spectrum presently reported was obtained by considering the two photo-fragments.

The IRMPD spectrum of the [Pb(dGMP)-H]+ ion is shown in Fig. 3a. The assignment of the IRMPD spectrum is based on its comparison with spectra computed for various low-energy isomers. The vibrational bands computed for nearly degenerate forms are summarized in Table 2. In making these comparisons, one should keep in mind that the calculated IR intensities, which assume single photon absorption, often do not correspond well with the multiple photon spectrum, because of the complex nature of the IRMPD process.\(^2\)^9\(^7\)\(^0\)

The IRMPD spectrum of the [Pb(dGMP)-H]+ complex features a very broad band (fwhm \(\sim 120 \text{ cm}^{-1}\)) centered at 1080 cm\(^{-1}\). As already established from the comparison between the IRMPD spectra of [Pb(dMP)-H]+ and [Pb(Urd)-H]+ complexes,\(^2\)\(^4\) (UMP and Urd stand for Uridine-5'-monophosphate and Uridine, respectively), this broad feature corresponds to IR-active modes of the phosphate group. A shoulder is observed at around 1110 cm\(^{-1}\), suggesting that this feature corresponds to the convolution of at least two IR-active modes, because with this particular experimental setup, isolated IR-active vibrational modes generally give rise to IRMPD bands of 10–20 cm\(^{-1}\) width.\(^2\)\(^7\) This is also consistent with the numerous vibrational modes (notably P–O and C’–O’ stretches, P–O–H bending mode) computed in this energy range (Table 2). Two smaller but significant bands are
detected at about 1180 and 1325 cm\(^{-1}\). A weak band is also detected at about 1390 cm\(^{-1}\). Finally, the high-energy region above 1500 cm\(^{-1}\) is characterized by three strong and sharp partially coalescing features, detected at 1586, 1625 and 1670 cm\(^{-1}\). As the guanine residue possesses a carbonyl group, the C=O stretching mode constitutes an excellent infrared diagnostic to determine whether the metallic center interacts with this particular group. Fig. 3e and f show the DFT-calculated IR absorption spectrum of dGMPA12 and dGMPA2, in which the metal solely interacts with the phosphate group. These two forms exhibit strong transitions at 1774 and 1768 cm\(^{-1}\), respectively, corresponding to the free C6—O6 stretching mode. Experimentally, there is no signal above 1700 cm\(^{-1}\), unlike that observed for the [Pb(UMP)-H]\(^+\) complex. In addition, these two spectra do not account for the strong experimental feature measured at 1670 cm\(^{-1}\). Consequently, gas-phase structures characterized by the lead atom interacting
Table 2 Experimental and computed IR vibrational bands of the [Pb(dGMP)-H]⁺ complex

<table>
<thead>
<tr>
<th>Wavenumbers</th>
<th>DFT-computed intensities (km mol⁻¹)</th>
<th>Vibrational modea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp.</td>
<td>Calc. dGMPS10</td>
<td>dGMPS10</td>
</tr>
<tr>
<td>1000</td>
<td>981 43</td>
<td>δP–O–H</td>
</tr>
<tr>
<td></td>
<td>986 90</td>
<td>δP–O–H + δC’–O–H</td>
</tr>
<tr>
<td></td>
<td>1098 169</td>
<td>δP–O</td>
</tr>
<tr>
<td>1025–1150</td>
<td>1024 101</td>
<td>νP–O + δP–O–H</td>
</tr>
<tr>
<td></td>
<td>1024 227</td>
<td>νP–O</td>
</tr>
<tr>
<td></td>
<td>1062 220</td>
<td>νC₅’–O4’</td>
</tr>
<tr>
<td></td>
<td>1062 183</td>
<td>νC₃’–O3’</td>
</tr>
<tr>
<td></td>
<td>1070 78</td>
<td>νP–O</td>
</tr>
<tr>
<td></td>
<td>1078 445</td>
<td>νP–O</td>
</tr>
<tr>
<td></td>
<td>1082 53</td>
<td>νC₃’–C₄’</td>
</tr>
<tr>
<td></td>
<td>1083 354</td>
<td>νC₃’–O3’</td>
</tr>
<tr>
<td></td>
<td>1096 174</td>
<td>νC₃’–O4’</td>
</tr>
<tr>
<td></td>
<td>1101 39</td>
<td>νC₃’–O3’</td>
</tr>
<tr>
<td>1165–1210</td>
<td>1160 130</td>
<td>δC₂H₆ + δC₈H</td>
</tr>
<tr>
<td></td>
<td>1175 135</td>
<td>ν C₁’–N9 (guanine ring breathing)</td>
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<tr>
<td></td>
<td>1184 56</td>
<td>δC’–O’–H + δC’–C’–H</td>
</tr>
<tr>
<td></td>
<td>1195 80</td>
<td>δC’–O’–H + δC’–C’–H</td>
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<td></td>
<td>1205 101</td>
<td>δC’–O’–H + δC’–C’–H</td>
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<td>1300–1340</td>
<td>1297 27</td>
<td>δC’–C’–H</td>
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<td></td>
<td>1300 32</td>
<td>δC’–C’–H + δN1H</td>
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<td></td>
<td>1301 41</td>
<td>δC’–C’–H + δN1H</td>
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<tr>
<td></td>
<td>1304 73</td>
<td>δC’–C’–H + δN1H</td>
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<tr>
<td></td>
<td>1318 24</td>
<td>δC’–C’–H</td>
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<td></td>
<td>1343 70</td>
<td>νC₃N–N7</td>
</tr>
<tr>
<td>1390</td>
<td>1380 59</td>
<td>νC₂–N1 + νC₄≡N9</td>
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<tr>
<td></td>
<td>1381 59</td>
<td>νC₂–N1 + νC₄≡N9</td>
</tr>
<tr>
<td></td>
<td>1397 33</td>
<td>δC’–C’–O3’–H</td>
</tr>
<tr>
<td>1586</td>
<td>1570 202</td>
<td>νC₄–C₅</td>
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<tr>
<td></td>
<td>1574 178</td>
<td>νC₄–C₅</td>
</tr>
<tr>
<td>1625</td>
<td>1621 1091</td>
<td>δNH₂ scissoring</td>
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<tr>
<td></td>
<td>1621 1042</td>
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<tr>
<td>1670</td>
<td>1670 615</td>
<td>νC₆≡O6</td>
</tr>
<tr>
<td></td>
<td>1674 609</td>
<td>νC₆≡O6</td>
</tr>
</tbody>
</table>

a Bond multiplicity as described by the NBO analysis.

solely with the phosphate group (including zwitterionic forms, see ESI,† Section S4) can be reasonably ruled out. This also suggests that such structures, postulated in solution for the Pb²⁺/[GMP·H]⁺ system, are not stable in the gas phase. Conversely, very good agreement is observed between the experimental spectrum and the IR spectrum of the global minimum dGMPS10 as well as low energy forms dGMPS9 and dGMPS13. The spectrum of dGMPS10 exhibits three strong IR active modes lying in the 1000–1100 cm⁻¹ energy range, which may account for the very broad band observed experimentally. Table 2 indicates that the broad feature centered at 1080 cm⁻¹ may correspond to combined C’–O’ and P–O stretching modes, as well as P–O–H bending mode. One particularly important finding is the excellent agreement in terms of position between the experimental signal and the computed frequencies above 1500 cm⁻¹. First, the sharp signal detected at 1586 cm⁻¹ may be attributed to the C₄–C₅ stretching mode. According to Table 2, the band at 1625 cm⁻¹ may correspond to the NH₂ scissoring mode. Finally, the band observed at high energy (1670 cm⁻¹), is consistent with the C₆≡O6 stretching mode. This CO stretch is therefore red-shifted by ~100 cm⁻¹ with respect to dGMPA2 or dGMPA12, and is a signature of an interaction taking place between the metal and the guanine carbonyl group, resulting in C₆≡O6 bond lengthening. However, this red-shift is markedly less pronounced than those observed for [Pb(UMP)·H]⁺ and [Pb(dCMP)·H]⁺ ions (>150 cm⁻¹), suggesting a weaker Pb/C=O interaction with the guanine residue. Accordingly, the computed bond length for the interacting C=O is shorter for guanine (1.247 Å) than for cytosine (1.275 Å) or uracil (1.277 Å). This weaker interaction partly explains why the IRMPD signature of the [Pb(dGMP)·H]⁺ complex above 1400 wavenumbers differs from those recorded for [Pb(dCMP)·H]⁺, [Pb(CMP)·H]⁺, or [Pb(UMP)·H]⁺, the C=O stretch of the interacting carbonyl group being located between 1556 and 1586 cm⁻¹ for the latter three. Note also that the IRMPD spectrum of [Pb(dGMP)·H]⁺ is devoid of any strong feature at around 1480 cm⁻¹, which was notably characteristic of the presence of a tautomer for [Pb(UMP)·H]⁺.²⁴

The three remaining experimental signals are also in agreement with computed vibrational modes of the three most stable forms (dGMPS10, dGMPS9, and dGMPS13). Experimental bands detected at around 1180 and 1325 cm⁻¹ may be attributed to combined C’–O’–H and C’–C’–H bending modes and combined C–N–H and C’–C–H bending modes, respectively. Finally, the weak feature detected at approximately 1390 cm⁻¹ may also be interpreted (Table 2). Several modes expected at 1025 cm⁻¹ and between 1400 and 1550 wavenumbers are not observed experimentally. It has been previously observed that small absorptions may be missing in IRMPD spectra.⁷¹–⁷⁴ Possible reasons lie in the multiple photon nature of IRMPD and the requisite efficiency of intramolecular vibrational redistribution.

Interestingly, macrochelates such as dGMPS10 (Fig. 3c), dGMPS9 (Fig. 3d), or dGMPS13 (Fig. 3a), differing by the nucleobase orientation but sharing the same binding scheme and the same ring puckering, lead to very good agreement with the experimental trace. Computed spectra for these forms are indeed very similar. Consequently, such slight structural differences can be difficult to distinguish by IRMPD and the formation in the gas phase of a mixture of several macrochelate forms appears very likely.

Fig. S4 (ESI†) displays the computed spectrum of dGMPA3, which is a macrochelate form exhibiting a doubly deprotonated phosphate group. This spectrum differs from the experimental trace in several aspects and notably in the high energy region, where the computed energy gap between the two most active modes (30 cm⁻¹) does not reproduce the gap observed experimentally (45 cm⁻¹). Additionally, one would expect bands with comparable intensities at 1080 and 1280 cm⁻¹. So, doubly deprotonated phosphate macrochelates present a poorer agreement with the experimental IRMPD spectrum, and can be reasonably ruled out.

The structure of gaseous deprotonated mononucleotides has been investigated by spectroscopy techniques.⁷⁵–⁷⁷ More particularly,
Nei et al. recently recorded the IRMPD spectrum of $(dGMP-H)^-$ from 600 to 1800 cm$^{-1}$. Comparison with DFT calculations points to a mixture of three syn forms, the ribose moiety adopting a C3'-endo conformation. These structures appear to be rather compact due to the formation of an intramolecular hydrogen bond between the 2-amino group and the deprotonated phosphate moiety. Interestingly, syn and anti forms could be differentiated thanks to several experimental bands. Such a distinction could not be achieved in the present study. One noticeable difference is the guanine C=O stretch, observed at 1715 cm$^{-1}$ for $(dGMP)^+$. This mode is red-shifted by 45 cm$^{-1}$ in the presence of Pb$^{2+}$, further supporting the interaction of the metal with the guanine carbonyl group. Finally, as for $(dGMP)^+$, the comparison between IRMPD and DFT-computed spectra supports the formation of a complex adopting a C3'-endo ribose conformation in the gas phase.

In summary, IRMPD experiments strongly suggest that gaseous [Pb(dGMP-H)]$^+$ ions adopt a macrochelate structure, in which Pb$^{2+}$ interacts with both the phosphate group and the guanine residue moiety. This is in excellent agreement with the three most stable forms obtained from DFT calculations ([dGMP10, dGMP13, and dGMP9]).

### 3.4 Cross section measurements by ion mobility (IMS-MS)

The geometry of representative structures found by quantum chemistry calculations was extracted for both unfolded and macrochelate forms. The collision cross sections calculated for the mono-dispersity and compactness derived from IRMPD spectra. The expected cross section gap is greater than the experimental uncertainty such that macrochelates and extended conformations can be differentiated experimentally (see ESI,† Fig. S5).

Ion mobility measurements were therefore performed to discriminate between the two conformational families. The arrival time distribution of [Pb(dGMP-H)]$^+$ is shown in Fig. S5 of the ESI.† The width of this highly dominant peak is consistent with a single family of conformations drifting through the tube. The average CCS corresponding to this single feature is calculated, as explained in the experimental section, from evolution of ion drift time with $1/E$ via linear regression (correlation coefficient is 0.9997, standard error on slope is less than 0.5%). The derived average CCS of [Pb(dGMP-H)]$^+$ is determined to be 99.6 Å$^2$ (the calibration curve is provided in the ESI,† Section S5). The main sources of experimental uncertainties leading to an error in $\Omega$ are temperature readout (~1%) and pressure readout and fluctuations (~3–4%), such that it is safe to consider $\Omega_{exp} = 100 \pm 5$ Å$^2$, which is more compact than expected based on the predicted values (Table 3). Interestingly, the structure of deprotonated dGMP has also been investigated by ion mobility and the experimental CCS (103±1 Å$^2$) determined is the same within the uncertainty in the present measurements as for the [Pb(dGMP-H)]$^+$ complex. Such a value is again consistent with a compact form in the gas phase, and was assigned by comparison with molecular mechanics and later confirmed by IRMPD spectroscopy,77 to the ribose being twisted into a C3'-endo conformation and guanine in syn orientation, which allows the establishment of a hydrogen bond between the amino group and the deprotonated phosphate of the (dGMP-H)$^-$ anion. The most stable forms found for the metal complex, macrochelates, present the same type of ribose twist. Remarkably enough, despite the addition of lead the average CCS has the same magnitude as deprotonated dGMP. In the most stable structures, guanine and phosphate establish each a bidentate interaction to tetracoordinated lead, which is most likely responsible for the compactness of the structure.

The global conclusion from ion mobility experiments on [Pb(dGMP-H)]$^+$ is twofold: one single family of conformations is experimentally observed, and those conformations are compact. CCS values obtained for macrochelates (folded structures) are in better agreement with the experimental value than unfolded forms. The mono-dispersion and compactness derived from mobility experiments suggest that only macrochelate structures are populated in the gas phase, i.e. conformations where guanine folds to maximize coordination to Pb$^{2+}$. Syn and anti conformations cannot necessarily be differentiated by their CCSs. This observation is therefore in agreement with the analysis of IRMPD spectra.

### 4. Conclusions/perspectives

The present study nicely illustrates how complementary experimental techniques can provide very detailed information about the structure of gaseous ions generated by electrospray...
Pb$^{2+}$ ions simultaneously interact with the deprotonated phosphate group and the nucleobase, both IRMPD spectroscopy and ion mobility experiments indeed point to the gas-phase formation of macrochelates, in which Pb$^{2+}$ ions simultaneously interact with the deprotonated phosphate group and the guanine residue. More precisely, ion mobility data indicate that a single family of structures is generated in the gas phase, and the IRMPD spectrum is in excellent agreement with macrochelates in which the metal, in addition to the phosphate group is coordinated to both the N7 and O6 positions of guanine. This mode of binding does not prevent the complexes from expelling the intact nucleobase under CID conditions. As already mentioned in previous studies, elimination of the nucleobase does not necessarily mean a lack of interaction between the metal and the nucleobase moiety. Additional experiments are currently in progress, which examine larger oligonucleotides. Finally, the present study also demonstrates that the structure of [Pb(dGMP)-H]$^+$ in the gas phase differs from those deduced from potentiometric studies.

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