

Intrinsic Structure and Electronic Spectrum of Deprotonated Biliverdin: Cryogenic Ion Spectroscopy and Ion Mobility

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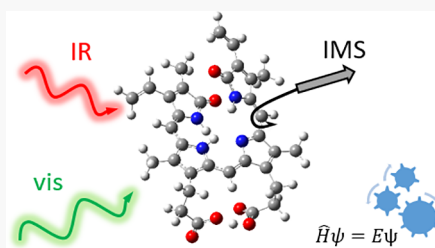


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ABSTRACT: We investigated the structural and spectroscopic properties of singly deprotonated biliverdin anions *in vacuo*, using a combination of cryogenic ion spectroscopy, ion mobility spectrometry, and density functional theory. The ion mobility results show that at least two conformers are populated, with the dominant conformer at 75–90% relative abundance. The vibrational NH stretching signatures are sensitive to the tetrapyrrole structure, and they indicate that the tetrapyrrole system is in a helical conformation, consistent with simulated ion mobility collision cross sections. The vibrational spectrum in the fingerprint region of this singly deprotonated species shows that the two propionate groups share the remaining acidic proton. The S_1 band of the electronic spectrum *in vacuo* is broad, despite ion trap temperatures of 20 K during ion preparation, with a congested Franck–Condon envelope showing partially resolved vibrational features. The vertical transition exhibits a small solvatochromic red shift (-320 cm^{-1}) in aqueous solution.



INTRODUCTION

Biliverdin is a linear tetrapyrrole (see Figure 1), a class of pigments found in many organisms. In humans, biliverdin occurs as an intermediate in the metabolism of heme,¹ highlighting the structural kinship with porphine-type molecules, although the pyrrole rings do not form a porphine macrocycle in biliverdin. In some organisms, biliverdin is produced directly, rather than as a breakdown product. For example, biliverdin is the chromophore of the light-sensing apparatus in many bacteriophytochromes.^{1–4} Binding to a cysteine in bacteriophytochromes enables the use of biliverdin as a genetically encoded (and engineered) red fluorescent biomarker.^{2,3,5,6} The fluorescence peak of biliverdin occurs in the 700–750 nm range,⁷ which is in the “first near-infrared window” of biological tissue (650–950 nm), where absorption by most tissue components is small, and chromophore emission is well separated from the typical yellow autofluorescence of tissue.⁸ Fluorescent biomarkers in this window allow deeper tissue penetration and thus enable better microscopic imaging than markers emitting at shorter wavelengths.

Out of the several position isomers of biliverdin,⁷ biliverdin IX α is the one most readily encountered in bacteriophytochromes,³ and for the remainder of this work, we restrict our discussion to this form of biliverdin, referring to it as BV. As a linear tetrapyrrole, BV can undergo larger conformational changes than its porphine relatives. In most solvents, the main conformation of BV is helical,⁹ with all NH groups pointing toward the helix axis and all methine bridge CH groups pointing away from it (ZZZ_{sss} conformer family, see Figure 1

and rotating structure animations in Supporting Information). Other conformers have been found in solution,^{10,11} and BV typically deviates significantly from the helical structure in protein environments.² As the absorption and fluorescence properties of BV depend on its conformation and chemical environment,^{2,12} the conformational flexibility of BV (similar to other tetrapyrroles, such as bilirubin) provides avenues to the engineering of the photophysical properties of this chromophore that are not available for porphine-based chromophores. An important step toward rational engineering of BV for applications is a detailed understanding of its photophysics, including the *intrinsic* molecular properties in the absence of a chemical environment. Intrinsic properties of interest include both molecular structure and the electronic spectrum. Since one approach to the engineering of a chromophore is based on varying the chromophore conformation *in silico* to obtain desired properties, good experimental benchmarks for computational methods to be applied to a molecule of this complexity would be valuable. However, these are difficult to obtain from solution data. In particular, solvatochromic shifts are difficult to capture computationally, because polarizable continuum models do

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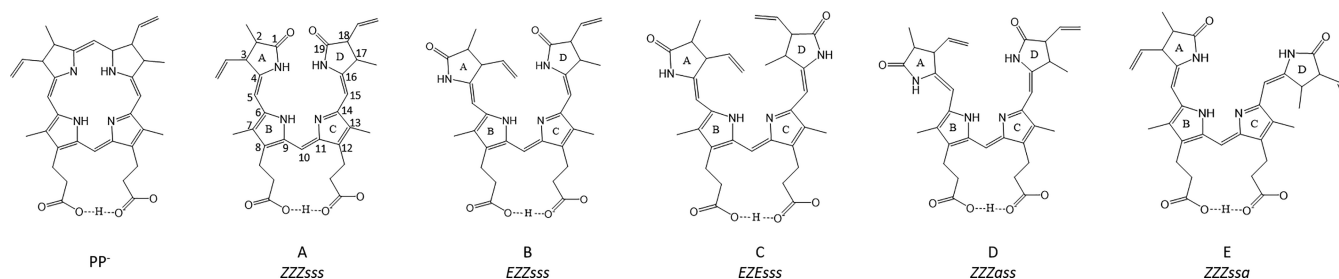


Figure 1. Examples of conformer families of singly deprotonated biliverdin IXa, BV^- , with the remaining acidic proton shared between the two carboxylate groups on the propionate groups. Numbering the carbon atoms in the conjugated system as shown in the helical structure A, the abbreviated Z/E and *syn/anti* labels refer to the double and single bonds of atoms 5, 10, and 15 in sequence. For example, the full conformational label of structure C (EZEss) would be C5-E, C10-Z, C15-E, C5-*syn*, C10-*syn*, and C15-*syn*. Shown on the left is singly deprotonated protoporphyrin IX, PP^- .

not generally have the necessary accuracy, and explicit solvation is computationally expensive.

Experiments on mass selected ionic chromophores *in vacuo* are an ideal tool to investigate these intrinsic properties, without the influence of a protein or solvent environment, and they can be used as direct benchmarks for the evaluation of computations.^{13–24} In this work, we report on the vibrational and electronic spectroscopy of cryogenically prepared, singly deprotonated BV ($[BV - H]^-$, abbreviated BV^-) *in vacuo*. We employ a messenger tagging approach, using BV^- ions in complexes with weakly bound N_2 molecules, prepared in a cryogenic ion trap. Since the conformation of an isolated molecule with this much conformational freedom is certainly not clear *a priori*, we use ion mobility spectrometry to narrow down the large range of possible structures. We interpret the experimental data in terms of density functional theory (DFT) and time-dependent DFT (TDDFT).

EXPERIMENTAL METHODS

The electronic and vibrational spectroscopy experiments were performed with a cryogenic ion photodissociation spectrometer at JILA, described previously in detail.²⁵ In brief, we electrosprayed 0.5 mM solutions of BV (Santa Cruz Biotechnology) in 3:1 methanol-water (adjusted to pH $\sim$ 12 by addition of KOH) into a desolvation capillary held at 25 °C. The ions exited the desolvation capillary and passed through a series of skimmers and radiofrequency (RF) octopole guides into a high-vacuum chamber, where they were stored and cooled for 50 ms in a 3D Paul trap held at 20 K. During storing and cooling, complexes of deprotonated BV ions with N_2 molecules formed in three-body collisions. After ejection into the acceleration region of a Wiley–McLaren time-of-flight mass spectrometer, target ions of the composition $BV^- \cdot N_2$ were mass-selected using a pulsed interleaving comb mass gate²⁶ and irradiated with the output of a pulsed optical parametric converter with 5–7 ns pulse duration and bandwidths of 2 cm^{-1} (1000–3800 cm^{-1}) and 5 cm^{-1} (12500–22000 cm^{-1}), respectively. Absorption of a single photon by the $BV^- \cdot N_2$ target complexes lead to the loss of the weakly bound N_2 molecule. We used a two-stage reflectron to monitor the intensity of fragment ions produced by the loss of the N_2 messenger tag, with 16 laser shots averaged for each data point. The light source was fired every other experimental cycle of the mass spectrometer (which operated at 20 Hz repetition rate) to allow subtraction of background signals from unimolecular decay of the target ions due to collision-induced dissociation. Fragment ion signals were normalized to the photon fluence to generate photodissociation spectra. For each spectral region, several spectra were acquired on different days to ensure reproducibility and were averaged to improve the signal-to-noise ratio.

The absorption spectrum of an aqueous solution of BV (0.48 mM) was acquired using an Agilent Cary 5000 spectrophotometer. The pH of the solution for those measurements was adjusted to pH 9

(different from the solutions used for ESI) by dropwise addition of aqueous KOH (50 mM).

Ion mobility data were obtained at KIT with a commercial instrument that combines a trapped ion mobility spectrometer with a time-of-flight mass spectrometer (timsTOF, Bruker). In brief, the ions are generated in a similar fashion, by electrospray ionization using 0.5 mmol/L $H_2O/MeOH$ 1:1 solutions of BV and protoporphyrin IX (PP, Sigma-Aldrich). Typical spray conditions were 3.5 kV needle potential and 500 V at a transfer capillary held at 100 °C. For ion mobility measurements, the ions are guided by a combination of RF and DC electrical fields through a counterflow of 2–3 mbar of nitrogen gas (held at 25 °C). Based on the measured arrival time distribution of the respective ionic species, we determined its collision cross section, $^{TIMS}CCS_{N_2}$, with the implemented calibration procedure using Agilent Tunemix. We used the Tunemix calibration parameters of Stow et al.²⁷ and operated the instrument at the highest achievable resolution (“custom mode”, $CCS/\Delta CCS \approx 200$) by applying a long ramp time (500 ms) and small mobility window (0.1 $cm^2/(Vs)$). For further details of trapped ion mobility spectrometry (TIMS), see ref 28.

COMPUTATIONAL METHODS

As will be shown below, comparison of the infrared photodissociation spectra of N_2 -tagged BV^- with those of singly deprotonated protoporphyrin IX, $[PP - H]^-$ (PP^-), shows that the propionic acid tails in BV^- and in PP^- adopt very similar structures (see **Results and Discussion**), with the intact and the deprotonated carboxylic groups sharing the remaining proton.²⁴ This restricts the conformational space of BV^- . We used a structural searching algorithm developed in-house similar to that described by Rijs and co-workers.²⁹ With the restriction that rings B and C were in a *Zs* configuration sharing the remaining proton of the propionate groups, we generated 100 structures by assigning random values to dihedral angles controlling the orientation of rings A and D. These structures were used as starting structures for geometry optimization at the PM6 level of theory.³⁰ Duplicate structures were discarded, and the remaining 65 structures were optimized using DFT with the CAM-B3LYP functional³¹ and cc-pVDZ basis sets³² for all atoms, followed by another discarding of duplicate structures. The resulting structures were then compared energetically, and those more than 1 eV higher than the lowest energy structure were discarded. Additional conformers were added to the list of structures resulting from the randomized search after comparison of calculated infrared spectra with the experimental spectrum, using targeted modifications, specifically the relative orientation of rings A and D and the propionic acid chains being either on the same side or opposite sides of the plane defined by rings B and C. For the final 23 unique structures, infrared spectra were predicted within the harmonic approximation at the same level of theory as the final geometry optimization.

Since the shared proton shuttle vibration is strongly anharmonic, and an amplitude analysis of the harmonic approximation calculation shows it contributing to the pattern of the OCO and CO stretching

vibrations, we performed another set of calculations predicting the infrared spectra of selected conformers where the shared proton was given an artificially high mass (200 u). The vibrational modes between 1500 and 2000 cm^{-1} reported here were calculated with this “heavy shared proton” approximation to avoid unphysical contributions of the shared proton to the CO stretching region, while modes outside this range are reported with the original proton mass. Finally, vertical excitation calculations were performed using TDDFT³³ at the same level of theory as the geometry optimizations to compare with the experimental electronic spectrum. These calculations were performed using Gaussian 16.³⁴

Ion mobility measurements can be regarded as a method to assign or rule out candidate structures, complementary to, for example, infrared spectroscopy. The procedure is as follows: Based on the DFT-optimized structures, the collision cross section $\text{TheorCCS}_{\text{N}_2}$ for each conformer is modeled by performing trajectory method calculations with the ImoS 1.09 package.^{35,36} In short, this method calculates the CCS of an ion by simulating the scattering of a large number of nitrogen molecules on the respective ion. The scattering angle distribution thus obtained is numerically integrated over a sufficiently large number of relative orientations. The ion–nitrogen interaction potential is approximated by the superposition of Lennard-Jones potentials and charge-induced dipole interactions based on partial charges. To this end, the partial charge distribution of each conformer has to be calculated. We did this by a Mulliken population analysis based on the DFT electron density distribution using the BP86 functional.^{37–40} For each conformer, a total of 9×10^6 trajectories was run, sufficient to converge $\text{TheorCCS}_{\text{N}_2}$ to within 0.5%. Nevertheless, one cannot expect that calculated and measured CCS match exactly. Therefore, we used the PP^- ion as a reference point, since it has only one reasonable conformer (see Figure 1). The calculated $\text{TheorCCS}_{\text{N}_2}$ of PP^- is 258.1 $\text{\AA}^2$, ca. 5% larger than the experimental value $\text{TIMS}_{\text{CCS}_{\text{N}_2}}$ of 246.1 $\text{\AA}^2$. Based on this ratio, we determine a correction factor of 0.953 and scale the calculated CCS of the various BV^- conformers with this factor before comparing with the experimental CCS (see Table 1).

RESULTS AND DISCUSSION

The structural flexibility of BV^- presents a challenge for structural identification, both in the condensed phase^{2,10–12} and *in vacuo*, as both the tetrapyrrole ring system and the propionic acid tails can in principle take many conformations. The structure of deprotonated PP can serve as a guide for determining the conformation of the propionate groups. Figure 2 shows a comparison of the infrared spectrum of messenger tagged PP^- reported in earlier work²⁴ and that of messenger tagged BV^- in the region 1150–1850 cm^{-1} , which encodes many of the conformational characteristics of the propionate groups. The two spectra show striking similarities, suggesting that the propionic acid tails in both molecules adopt a similar structural motif, even though the tetrapyrrole system of BV lacks the structural constraints imposed by the macrocyclic nature of PP. This means that one of the two carboxylate groups has been deprotonated, and the remaining proton is shared between the two.²⁴ This is a reasonable assumption, since the shared proton motif serves to lower the energy of the molecule by ca. 0.5 eV, based on DFT calculations for PP^- ,²⁴ which were performed at the same level of theory as used in the present work. With this structural motif in mind, we can restrict the number of conformers to only those structures that have rings B and C and the methine bridge connecting them in roughly the same *Zs* conformation as in PP^- , since a deviation from this local structure would disrupt the shared proton motif.

Table 1. Calculated Collision Cross Sections, Energies, and S_1 Transitions of Selected Conformers^a

conformer ^b	family ^c	collision cross section $\text{TheorCCS}_{\text{N}_2}$ ^d ($\text{\AA}^2$)	energy ^e (meV)	S_1 vertical transition ^f (cm^{-1})
1	ZZZsss (A)	241.0	9	17824
2	EZEsss (C)	242.6	425	19114
3	EZZsss (B)	243.2	186	18953
4	ZZZsss (A)	244.1	109	17904
5	EZZssa	246.0	320	19437
6	ZZZass (D)	246.1	73	18715
7	ZZZass (D)	246.2	155	17582
8	ZZEssa	246.3	254	19598
9	ZZEssa	246.8	393	18630
10	ZZZssa (E)	247.0	150	18228
11	ZZZass (D)	248.7	0	18146

^aConformers given here are those with the 10 lowest calculated collision cross sections (see text). We judge the four lowest of these to be compatible with the measured collision cross section of the main conformer of BV^- . The sole exception is the lowest energy conformer found in our calculations (last row), whose calculated collision cross section is not compatible with the experimental value. ^bSee also Figure 3. ^cSee Figure 1 for nomenclature; letters in parentheses refer to the families shown there. ^dThe experimental collision cross sections for BV^- are $(243.5 \pm 2) \text{\AA}^2$ and $(244.9 \pm 2) \text{\AA}^2$. Note that the errors comprise both statistical ($\pm 1 \text{\AA}^2$) and systematic errors ($\pm 1 \text{\AA}^2$, due to uncertainties in temperature and calibration accuracy). However, we refer for the structure assignment to the experimental CCS of PP^- (by introducing the scaling factor of 0.953), that is, we focus on CCS differences. Their uncertainty is smaller, well below $\pm 1 \text{\AA}^2$ since the BV^- and PP^- data are always obtained in one run, under exactly the same instrumental conditions. Estimated errors for the calculated values are $\pm 1 \text{\AA}^2$. ^eZero-point corrected, using the CAM-B3LYP functional and cc-pVDZ basis sets for all atoms. ^fTDDFT, using the CAM-B3LYP functional and cc-pVDZ basis sets for all atoms.

As discussed above, the fingerprint region of the infrared spectrum establishes a *Zs* configuration for rings B and C and the connecting methine bridge, due to the shared-proton motif. However, this region of the infrared spectrum provides very little information on the configurations of rings A and D of the tetrapyrrole system. We therefore used ion mobility experiments to investigate the structural motif of the tetrapyrrole system of the molecule in more detail, considering only structures with the shared proton motif for the propionic acid chains in our interpretation. Figure 3 shows the comparison of experimental ion mobility CCS for PP^- (as an internal calibrant) and BV^- , together with calculated CCS for conformers listed in Table 1. The experimental CCS of BV^- is at the lower end of the range of calculated values. The ion mobility trace of BV^- shows two contributions, where the dominant conformation has an average cross section of 243.5 $\text{\AA}^2$. Ion mobility data for BV^- were taken twice (on different days) under conditions that do not heat the ions particularly strongly. Given the high pressure (2.5–3 mbar) and low electric drift field ($<50 \text{ V/cm}$), any additional collisional heating due to the electric field is small compared to energy transfer in diffusional motion. As a consequence, the ions are

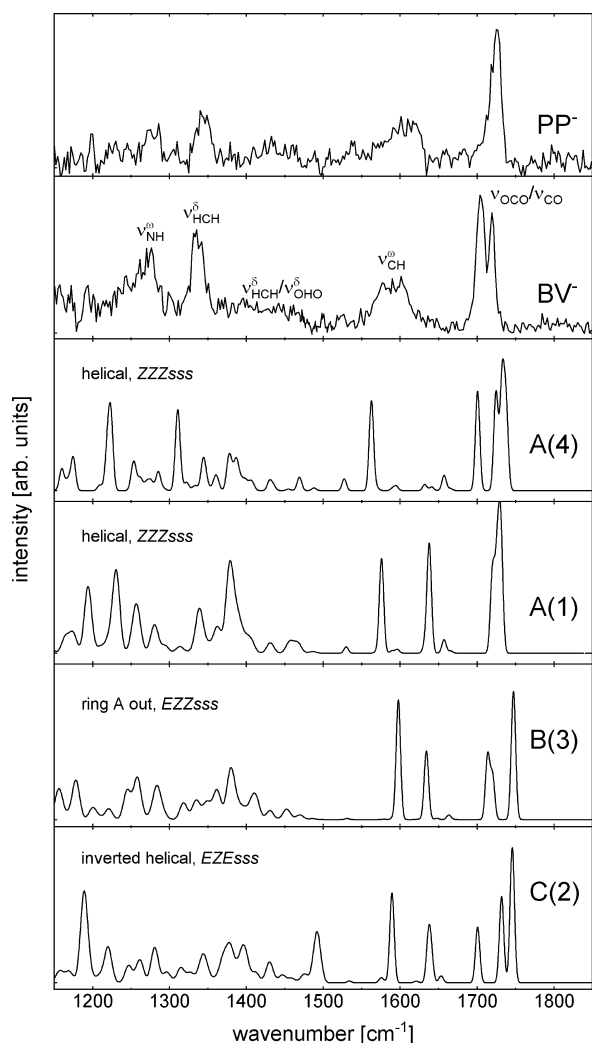


Figure 2. Comparison of the infrared photodissociation spectra of $\text{PP}^-\cdot\text{N}_2$ (top trace, data taken from ref 24) and $\text{BV}^-\cdot\text{N}_2$ (second from top). The four lowest traces are simulated infrared spectra of conformers consistent with the measured ion mobility collision cross section (harmonic frequencies scaled by 0.950, as used earlier²⁴ for PP^-). Conformer families are labeled as in Figure 1, and numbers in parentheses give the isomer number as listed in Table 1. See text for labels of vibrational features and for the structural differences between conformers 1 and 4.

effectively at room temperature during the ion mobility measurement. Both times, we observed a shoulder toward larger cross sections with a relative abundance of 10–25%. This is consistent with NMR experiments by Franski et al., who found two conformers in aqueous solution at room temperature, with the dominant conformer being a helical ZZZsss structure, the other attributed to a slightly unfolded ZZZssa conformer (structure E in Figure 1). Unfortunately, the multitude of possible conformers with CCS compatible with this minor component prevents us from assigning its structure *in vacuo*, and we will focus solely on the dominant conformer. We note that despite the slight differences of the ion mobility traces in the two different experimental runs, both the vibrational and electronic spectra of $\text{BV}^-\cdot\text{N}_2$ in different experimental runs proved to be identical within the signal-to-noise ratio of the experiment, indicating that at least the dominant conformer of BV^- is robust.

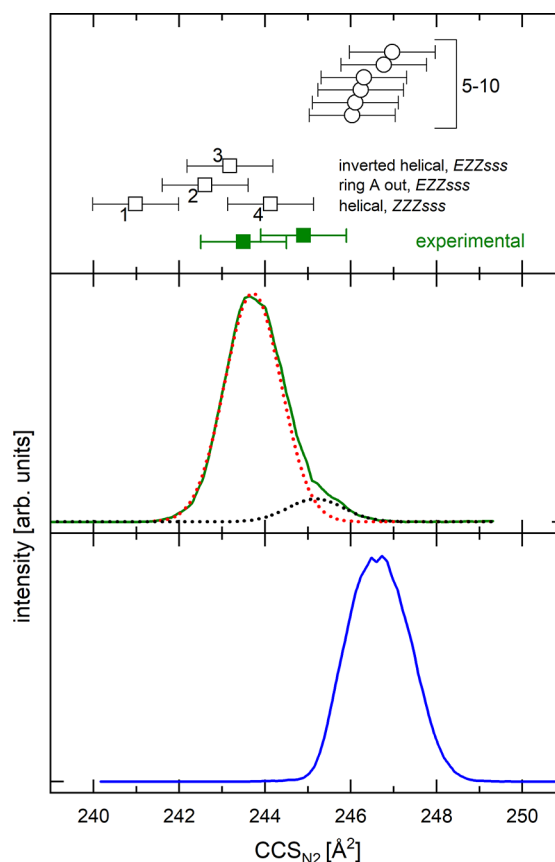


Figure 3. Ion mobility data and analysis. Full lines are averaged ion mobility curves from two independent runs for BV^- (center) and PP^- (bottom). Filled squares in the top panel represent the experimental average cross sections of the two contributions for BV^- , while open squares are calculated CCS for several candidates of the dominant conformer of BV^- . Data shown as open circles belong to other structures in this range of calculated CCS. The numbers in the top panel refer to the data given in Table 1. The dotted lines in the experimental BV^- data are Gaussian fits representing the two observed contributions (see text).

Data for selected conformers are summarized in Table 1. We judge that four conformers have calculated CCS that are compatible with the average experimental CCS of the main conformer (see rotating structure images conveying a clearer 3D impression in Supporting Information). These structures belong to conformer families A–C, as shown in Figure 1. Two helical conformers (family A) are in this group of likely candidate structures. Their main structural difference is the distance of the carboxylate groups from the CH group of the methine bridge connecting rings B and C (195 pm vs 225 pm between the H atom and the nearest O atom, respectively). Their zero-point corrected energies differ by ca. 100 meV, and the lower energy conformer of the two is nearly isoenergetic with the lowest energy structure we found in our search (structure 11 in Table 1). These two helical conformers may interconvert easily in the room-temperature ion mobility experiments. The predicted infrared spectra of the four candidate structures are all somewhat compatible with the experimental spectrum in the fingerprint region (see Figure 2), but we judge the helical conformer 4 (ZZZsss, structure family A) to provide the best match to the calculated mobility CCS. In addition, the infrared spectrum of conformer 4 is most consistent with the experimentally observed single dominant

peak at 1590 cm^{-1} and the two strong signatures at 1275 and 1337 cm^{-1} . While there is no perfect agreement between computational and experimental infrared data, they suggest, together with the ion mobility data and the prior identification of the main conformer in solution as helical,^{9,11} that the dominant conformer of BV^- in *vacuo* is also helical. We will further substantiate this assignment through our analysis of the infrared spectrum in the NH stretching region (see below).

The structural identification of the shared proton motif allows us to perform an approximate assignment of some of the prominent features in the infrared spectrum of $\text{BV}^- \cdot \text{N}_2$, and we use the helical conformer 4 (ZZZsss) for our description. The partially resolved doublet at 1704 and 1719 cm^{-1} in the spectrum of $\text{BV}^- \cdot \text{N}_2$ contains the CO stretching vibrations of the carbonyl groups of rings A and D (ν_{CO}) as well as the signatures of the antisymmetric OCO stretching vibrations of the carboxylate groups (ν_{OCO}). The dominant mode in the broad feature around 1590 cm^{-1} is composed of CC stretching and CH wagging motions of the methine bridge between rings B and C (ν_{CH}). In both $\text{PP}^- \cdot \text{N}_2$ and $\text{BV}^- \cdot \text{N}_2$ spectra, the broad feature around 1450 cm^{-1} contains the signature of the OHO bending motion of the shared proton between the carboxylate groups (ν_{OHO}^δ), although this mode is calculated to be weaker in most conformers for BV^- , where the methyl group bending vibrations carry most of the oscillator strength in this region. The peaks at 1337 and 1275 cm^{-1} carry the signatures of HCH bending motions delocalized over the molecule (ν_{HCH}^δ), strongly mixed with NH wagging motions on the rings, particularly for the peak at 1275 cm^{-1} (ν_{NH}^ρ).

As mentioned above, the infrared spectrum in the fingerprint region and the ion mobility data strongly suggest that the dominant conformer of BV^- in *vacuo* is helical. The mid-infrared region contains the signatures of the NH groups on rings A, B, and D, which encode structural information on the tetrapyrrole system. Figure 4 shows the CH stretching and NH stretching regions of the infrared spectrum of $\text{BV}^- \cdot \text{N}_2$ and its comparison with simulated spectra. The experimental spectrum shows a series of congested features between 2800 and 3150 cm^{-1} which can be generally assigned to CH stretching motions (and Fermi resonances with HCH bending mode overtones). At higher frequencies, there is a feature at 3444 cm^{-1} and a weak, broadened doublet at 3405 and 3391 cm^{-1} . For the helical structures 1 and 4, the two highest frequency features have a calculated splitting of ca. 30 cm^{-1} , roughly compatible with the observed difference of the highest experimentally observed frequency feature and the upper component of the doublet. The calculated spectra for structures 2 and 3 are not compatible with the experimental spectrum in this frequency range. The NH stretching vibrations are quite sensitive to the interactions of the NH groups with their immediate surroundings. In particular, the N atom on ring C, which does not have a proton in BV^- , interacts strongly with the NH group on ring B (NH_B), providing a low-lying proton transfer channel. Based on harmonic calculations, we would expect to observe the NH_B oscillator to give rise to a strong feature between 3140 and 3400 cm^{-1} for all calculated structures that are compatible with the collision cross section, but such a feature is not experimentally observed. This is reminiscent of a problem encountered in the excited-state infrared spectrum of methyl anthranilate,⁴¹ where a low-lying proton-transfer channel will result in a strong red shift and broadening of the NH stretching mode, diluting its oscillator strength over many transitions and

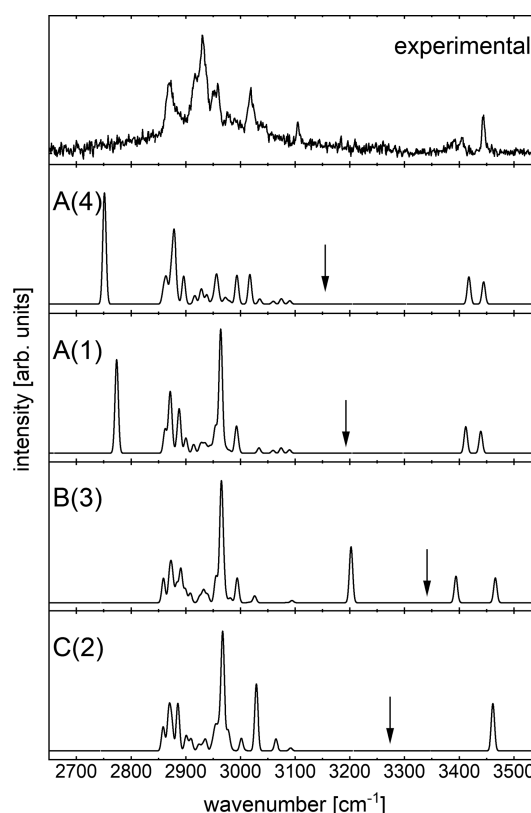


Figure 4. Comparison of the infrared photodissociation spectrum of $\text{BV}^- \cdot \text{N}_2$ in the mid-infrared (top trace) with computational predictions. The four lower traces are simulated infrared spectra of conformers consistent with the measured ion mobility collision cross section, with conformer families as labeled in Figure 1, and numbers in parentheses giving the conformer number as listed in Table 1. For the simulated spectra, the NH_B vibration was treated anharmonically (see text), while the remainder of (harmonic) frequencies were scaled by 0.942 in this region to match the highest energy NH stretching vibration for the helical conformers. Arrows reflect the predicted NH_B features in the scaled harmonic approximation.

making it difficult to observe experimentally. Similar effects have been found in other H-bonded systems with low-lying proton-transfer channels, for example, $\text{F}^- \cdot \text{H}_2\text{O}$.⁴² Calculating single point energies along the NH_B vibrational coordinate for fixed $(\text{N}-\text{H})_\text{B}$ distances, but allowing relaxation of the angular orientation of the NH_B bond, shows that the mode is indeed quite anharmonic (see Supporting Information). Fitting the resulting potential energy curve to a Morse potential, we estimate a frequency of ca. 2750 cm^{-1} for conformer 4 and ca. 2770 cm^{-1} for conformer 1, placing the NH_B signature even below the congested CH stretching region. As a result, we should only expect two NH stretching signatures above the CH stretching region. We assign the peak at 3444 cm^{-1} to a largely free NH stretching mode localized on ring A. The broad doublet around 3400 cm^{-1} is likely due to helical configurations with small differences in the relative orientations of ring D relative to ring C, leading to a broadening and splitting of the NH stretching signature, which would result from weak, but non-negligible interactions with the N atom on ring C. This corroborates our assignment of the dominant conformer belonging to the helical conformational family. Figure 5 shows several views of conformer 4 (see Supporting Information for a rotating model).

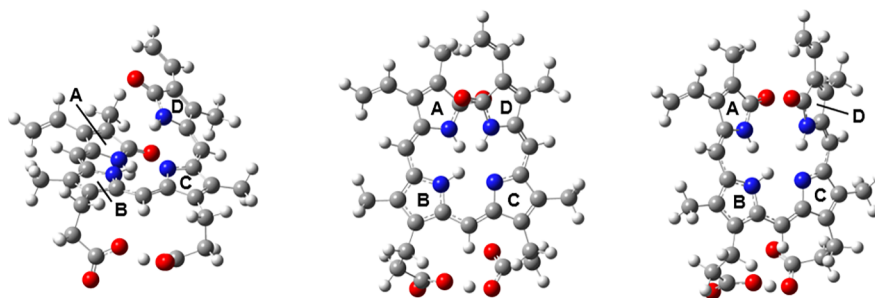


Figure 5. Views of the helical conformer 4, with the rings labeled as in Figure 1. The center view has rings B and C approximately in the plane of drawing, and the helix axis approximately perpendicular to it. Ring D is above the plane of drawing, ring A below. In the left view, the helix axis is tilted upward from the center view, while it is tilted to the right in the right-hand side view.

The electronic spectrum of $\text{BV}^-\cdot\text{N}_2$ is shown in Figure 6. The vertical excitation energy of the S_1 state (Q-band) is

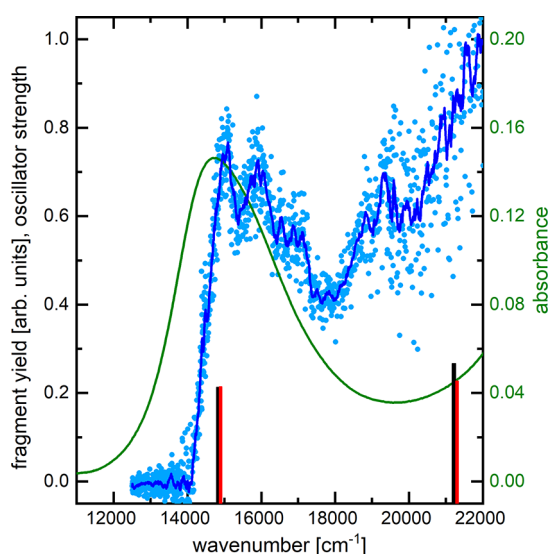


Figure 6. Electronic spectrum of $\text{BV}^-\cdot\text{N}_2$ (blue data points, the blue curve is a 15-point gliding average, arbitrary units) in comparison with the absorption spectrum of BV in aqueous solution at pH 9 (0.48 mM, green curve, right scale) and TDDFT predictions (left scale), red-shifted by 2980 cm^{-1} , for conformer 1 (black columns) and conformer 4 (red columns).

marked by a peak at $(15040 \pm 100) \text{ cm}^{-1}$, with a sharp onset at $(14100 \pm 100) \text{ cm}^{-1}$ and a partially resolved shoulder at $(14500 \pm 100) \text{ cm}^{-1}$. Given the sharpness of the onset, we tentatively assign the band origin to the vertical transition energy, that is, at 15040 cm^{-1} , and the lower energy shoulder to a hot band. On the high-energy side of the Franck–Condon envelope, we observe broad features at $(15900 \pm 100) \text{ cm}^{-1}$, $(16800 \pm 100) \text{ cm}^{-1}$, and $(19360 \pm 100) \text{ cm}^{-1}$. Toward higher energies, the photodissociation cross section continues to rise all the way to the edge of the spectral range probed in our experiment. The TDDFT calculations on the four conformers that are compatible with the experimental collision cross section (structures 1–4, see Table 1) overestimate the S_1 state vertical excitation energy on average by ca. 3400 cm^{-1} , but are surprisingly insensitive to the structure of the tetrapyrrole system, falling within an interval of 1300 cm^{-1} . The calculated S_2 vertical excitation energies (Soret band) are on average 6700 cm^{-1} higher than for the S_1 state, again with very similar values for the relevant structures. This under-

estimates the splitting between S_1 and S_2 , which is experimentally found in solution to be of the order of 1.5 eV (13000 cm^{-1}).¹² The vertical excitation energies for the two helical conformers we consider to be the most likely structures are included in Figure 6, shifted by -2980 cm^{-1} to match the experimental S_1 transition energy for conformer 4. We note that earlier TDDFT calculations by Gonzalez and co-workers¹² without the use of range-corrected functionals, but with a polarizable continuum model to account for solvation, predicted the vertical excitation energies for the S_1 band at significantly lower energies (14046 cm^{-1}).

Given the large energy difference between the calculated S_1 and S_2 values, the features above the S_1 vertical transition must be part of the Franck–Condon envelope of the S_1 state. Also included in Figure 6 is the comparison with a UV–vis spectrum of BV in aqueous solution. The deprotonation state is pH dependent, and with the known pK_a values⁴³ of BV ($\text{pK}_{a1} = 3.9$; $\text{pK}_{a2} = 5.3$), the singly deprotonated molecule should be dominant at pH 9, which was used here. The vertical transition under these conditions is at $(14720 \pm 50) \text{ cm}^{-1}$, corresponding to a solvatochromic shift of -320 cm^{-1} , suggesting as expected that this transition has only little charge-transfer character, if any.

SUMMARY AND CONCLUSIONS

We have investigated the structure and the infrared and electronic spectroscopy of BV^- using a combination of cryogenic ion spectroscopy, ion mobility mass spectrometry, and quantum chemical calculations. Ion mobility data show that BV^- at room temperature takes (at least) two conformers, and we can assign the dominant species to be helical (ZZZ_{ss}). The S_1 state has its vertical transition energy at 15040 cm^{-1} , which is likely also the S_1 band origin. The solvatochromic shift of the S_1 state of BV^- in aqueous solution is small (only -320 cm^{-1}). The energy of the vertical transition to the S_1 state is overestimated by ca. 3000 cm^{-1} in our TDDFT calculations, while the splitting between S_1 and S_2 vertical transition energies is underestimated.

The flexibility of BV^- makes its structural characterization *in vacuo* a difficult task. The interpretation of the infrared spectrum is made challenging by two significantly anharmonic parts of the potential energy surface: one for the shared proton between the propionate groups and the other for the NH_B oscillator. Only the combination of the techniques used here and the comparison with the infrared spectrum of PP^- ultimately enable us to assign the dominant structure of BV^- *in vacuo* to the helical conformer family, highlighting the need for several angles of investigation for a molecule of this

complexity. Our electronic spectroscopy data provide new benchmarks for the computational treatment of linear tetrapyrroles, which can be used to aid the design of new structural environments for this chromophore.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/jacs.1c08701>.

Anharmonic NH_B stretching potential energy surface; coordinates for structures 1–4 (PDF)

Rotating structure animations (PPT)

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Notes

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