

Excellence in Chemistry Research

Announcing our new flagship journal

- Gold Open Access
- Publishing charges waived
- Preprints welcome
- Edited by active scientists



Meet the Editors of *ChemistryEurope*



Luisa De Cola

Università degli Studi
di Milano Statale, Italy



Ive Hermans

University of
Wisconsin-Madison, USA



Ken Tanaka

Tokyo Institute of
Technology, Japan

Structure of Zinc and Nickel Histidine Complexes in Solution Revealed by Molecular Dynamics and Raman Optical Activity

Jana Hudecová,^{*,[a, b]} Josef Kapitán,^[b] Martin Dračínský,^[a] Pavel Michal,^[b] Václav Profant,^[c] and Petr Bour^{*,[a]}

Abstract: The histidine residue has an exceptional affinity for metals, but solution structure of its complexes are difficult to study. For zinc and nickel complexes, Raman and Raman optical activity (ROA) spectroscopy methods to investigate the link between spectral shapes and the geometry were used. The spectra were recorded and interpreted on the basis of ionic equilibria, molecular dynamics, ab initio molecular dynamics, and density functional theory. For zwitterionic histidine the dominant tautomer was determined by the

decomposition of experimental spectra into calculated subspectra. An octahedral structure was found to prevail for the ZnHis₂ complex in solution, in contrast to a tetrahedral arrangement in the crystal phase. The solution geometry of NiHis₂ is more similar to the octahedral structure found by X-ray. The Raman and ROA structural determinations of metal complexes are dependent on extensive computations, but reveal unique information about the studied systems.

Introduction

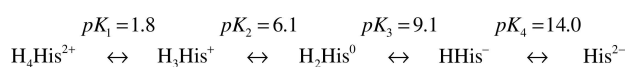
Histidine is the most potent metal binder of all natural amino acids. About 50% of all enzymes contain histidine residue at their active sites.^[1] The affinity for the metal is very much supported by the imidazole ring containing two nitrogen atoms. In monomeric histidine the amine and carboxyl groups can also participate in the binding.^[2] In aqueous solutions, conformational and metal-binding studies of histidine are complicated by its ability to form many protonated/deprotonated states including tautomeric forms of the ring. The binding affinity thus strongly depends on pH and other environmental factors. For example, different tautomeric histidine forms were encountered in the same protein.^[3]

In the present study we used computational chemistry, Raman and Raman optical activity (ROA) spectroscopy to understand histidine and its binding modes in zinc and nickel complexes. The power of optical spectroscopies and methods exploring optical activity for the characterization of histidine

behavior has already been recognized.^[4] However, approximate solvent models allowed for a limited reproduction of experimental data. Better results were obtained by combination of quantum and molecular mechanics (QM/MM) with a more explicit inclusion of water molecules in the computations.^[5] We use this approach together with a decomposition of experimental spectra into calculated subspectra^[6] to determine a dominant tautomer of monomeric zwitterionic histidine in solution. The QM/MM methodology based on classical molecular dynamics (MD) cannot be used for the metal complexes, because of the lack of reliable force fields describing the histidine-metal interaction. Therefore, ab initio MD (AIMD) with density functional theory (DFT) potential is applied to model the geometry and its variation in time. As shown below, this approach allows us to interpret the main spectral features and even determine preferred structures of the complexes.

The monomeric molecule can exist in five possible protonation states^[2] where the charge varies from 2+ to 2− (Scheme 1).

The neutral H₂His⁰ and mononegative HHis[−] further form two tautomers, where either the nitrogen closer (N_δ) or farther (N_ε) from the CH₂ linkage is protonated. Soluble metal complexes are not stable in extreme acidic or basic environments. Therefore, the H₃His⁺ and H₂His⁰ forms dominant within pH 3.9–7.6 are most relevant for the complexation. They exist in equilibrium with histidine bound to the metal (Figure 1).



Scheme 1. Protonation constants and the five possible forms of histidine.

[a] Dr. J. Hudecová, Prof. M. Dračínský, Prof. P. Bour
Institute of Organic Chemistry and Biochemistry Academy of Sciences
Flemingovo náměstí 2, 16000 Prague (Czech Republic)
E-mail: hudecova@optics.upol.cz
bour@uochb.cas.cz

[b] Dr. J. Hudecová, Dr. J. Kapitán, P. Michal
Department of Optics
Palacký University
17. listopadu 12, 771 46 Olomouc (Czech Republic)

[c] Dr. V. Profant
Faculty of Mathematics and Physics
Charles University
Ke Karlovu 5, 121 16 Prague (Czech Republic)

Supporting information for this article is available on the WWW under
<https://doi.org/10.1002/chem.202202045>

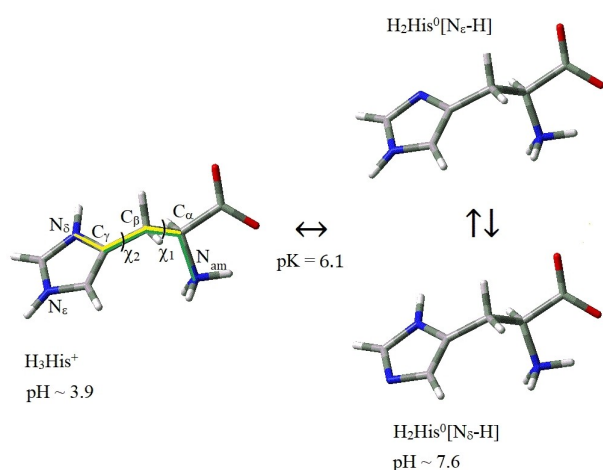


Figure 1. L-histidine forms prevailing at pH 3.9 (H_3His^+) and pH 7.6 ($\text{H}_2\text{His}^0[\text{N}_\delta\text{-H}]$ and $\text{H}_2\text{His}^0[\text{N}_\epsilon\text{-H}]$ tautomers); characteristic torsion angles χ_1 ($\text{N}_{\text{am}}\text{-C}_\alpha\text{-C}_\beta\text{-C}_\gamma$) and χ_2 ($\text{C}_\alpha\text{-C}_\beta\text{-C}_\gamma\text{-N}_\delta$) are indicated for H_3His^+ .

The populations of the tautomers are difficult to determine. A 4:1 $\epsilon:\delta$ ratio was suggested on the basis of NMR spectroscopy.^[7] Even higher prevalence of the $\text{N}_\epsilon\text{-H}$ form was predicted theoretically, with a stabilization energy of ~ 3 kcal/mol.^[8] The effect of the tautomerization on FTIR spectra was discussed as well, with a limited success.^[8] A novel conformational sampling methodology was used to interpret Raman and ROA spectra.^[5] Although this led to reasonable spectra simulations, the tautomeric ratio could not be determined.

Both nitrogens of the imidazole ring can coordinate to a metal ion. In metalloproteins, N_ϵ -binding prevails.^[9] Both nitrogens also participate in hydrogen bonding.^[10] The Ni^{2+} ion prefers an octahedral arrangement of the ligands in proteins, whereas in smaller complexes approximately planar square geometry is about equally probable.^[9a] Zinc as Zn^{2+} prefers a tetrahedral coordination in metalloproteins and an octahedral arrangement in small-molecule crystals.^[9a] Note that large metalloproteins can stabilize coordination geometries that would be unstable with smaller ligands.

Binding to the metal changes conformation of the histidine, presumably restricts its flexibility, and affects protonation equilibria. X-ray studies revealed that the amino and carboxyl groups can also coordinate to the metal and complexes are formed in 1:2 metal/histidine ratios.^[11] This ratio is also consistent with our data obtained for the solutions. In crystalline NiHis_2 the nickel atom lies on a two-fold axis and is octahedrally coordinated to the amino nitrogen, imidazole N_δ , and carboxyl oxygen of each histidine.^[12] For ZnHis_2 crystals the coordination bonds form a distorted tetrahedron, connecting Zn^{2+} with amino and imidazole δ -nitrogens.^[11]

Apart from hoping to contribute to the knowledge of the histidine-metal interactions, we also performed the present study to broaden the application span of ROA. The spectroscopy detects a small difference in the scattering of right and left circularly polarized light, and as such it was found convenient not only to detect the absolute configuration,^[13] but

also the conformation and small structural changes in small molecules, peptides, proteins, saccharides and nucleic acids.^[14] For biologically-oriented assays, ROA offers a flexible technique suitable for the natural aqueous environment, large scale of pH and temperatures. However, studies of metal-containing molecules often suffer from resonance, where samples absorbing the excitation light may decompose or produce other unwanted effects.^[15] The interpretation of the spectra based on computer simulations is complicated by system polarity, molecular flexibility, absence of reliable MD force fields, and the complexity of metal electronic systems.

Fortunately, the formation of zinc and nickel complexes is clearly visible in the ROA spectra, where the histidine bands typically become more intense and sharper. Also the effects of the resonance are limited. Zn does not absorb the excitation light (532 nm) significantly, and for Ni absorption of the light does not prevent spectral interpretation in a pre-resonance approximation. To build the computational and experimental methodology, we analyze Raman and ROA spectra of H_3His^+ , both tautomeric forms of H_2His^0 , and the ZnHis_2 and NiHis_2 complexes. It appears that ROA is clearly able to detect the change of histidine conformation upon complexation, although some spectral changes can be attributed to the direct interaction with the metal. The spectroscopy also indicates structural differences between the two metals and between the crystal and solution structures.

Due to the fast ($\sim$ fs) optical response of a molecule during Raman/ROA scattering measured spectra are sums of spectra of individual species in the sample.^[16] This is different in NMR, where the time scale is longer and often an average signal is observed.^[17] If “everything goes well”, we can fit an experimental spectrum with theoretical spectrum (S_t) composed of calculated subspectra of individual conformers (S_i),

$$S_t = \sum_i c_i S_i \quad (1)$$

Conformer populations are then equal to the fitting coefficients c_i , obeying the obvious conditions $\sum_i c_i = 1$ and $0 \leq c_i \leq 1$. In real life, the results are often hampered by inaccuracy of the calculations or even by unknown structure of the conformers. Yet for model dipeptides we could calibrate the Raman/ROA spectra decomposition against NMR, achieving an accuracy in conformer population of $\sim 5\%$.^[6a] Only slightly worse results were obtained for more polar and more flexible molecules.^[6b,14e] Also in the present study this method with high probability reveals conformer/tautomer ratios of histidine and structure of the histidine metal complexes.

Results and Discussion

The Experimental Spectra: Raman and ROA spectra of H_3His^+ , H_2His^0 , ZnHis_2 and NiHis_2 are summarized in Figure 2. The data for H_2His^0 are consistent with a previous report,^[4] so are the differences in Raman spectra of H_3His^+ and H_2His^0 .^[18] Note that no form of histidine can be obtained in a pure state. For example, at pH 7.6 the sample contains about 94% of H_2His^0 ,

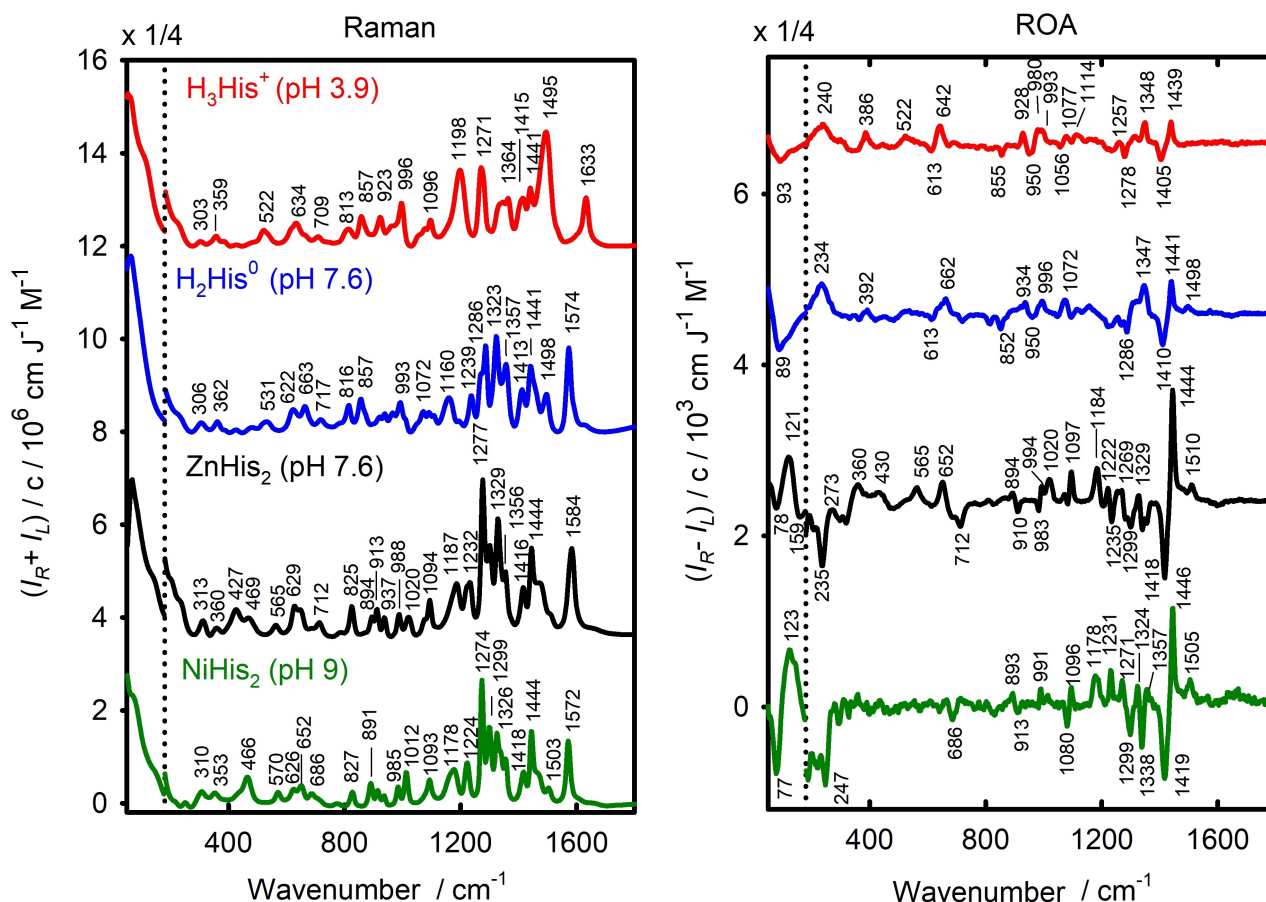


Figure 2. Experimental Raman ($I_R + I_L$) and ROA ($I_R - I_L$) spectra of H_3His^+ , H_2His^0 , and $ZnHis_2$ and $NiHis_2$ complexes, L-enantiomers. The spectra were normalized to the histidine concentration, system pH is indicated, region below 180 cm^{-1} is plotted in a different scale for better visibility.

but also 3% of $HHis^-$ and 3% of H_3His^+ , which explains the small Raman peak at 1633 cm^{-1} visible also for H_2His^0 . The purity of H_3His^+ at pH 3.9 is higher, ~99%. More Raman spectra for pH values within 1–13 can be seen in Figure S1; an analysis (Figure S2, ref. [19]) of this titration provided spectra of other histidine forms and protonation constants ($pK_1=1.8$, $pK_2=6.1$, $pK_3=9.1$) that agree well with previously published values ($pK_1=1.8$, $pK_2=6.0$, $pK_3=9.2$).^[20] Similar titrations and analyses were performed for the complexes, indicating their stability and purity at given pH (Figures S3–S4).

For H_2His^0 , the 1574 cm^{-1} Raman band indicates the ϵ -tautomer.^[2] Other, less specific markers of this form are bands at 1286 and 1323 cm^{-1} . A simultaneous presence of the δ -tautomer may be deduced from the 1357 cm^{-1} band and the 1268 cm^{-1} and 1588 cm^{-1} shoulders. In the complexes, apart from other changes, the position of the C=C stretching Raman band shifts to 1584 cm^{-1} for $ZnHis_2$ and 1572 cm^{-1} for $NiHis_2$, and a new band appears at $1277/1274\text{ cm}^{-1}$. This means that the metal is bound through N_δ and N_ϵ is protonated,^[21] as in crystalline $ZnHis_2$ ^[11] and $NiHis_2$.^[12]

Also the histidine ROA spectra are consistent with previous reports.^[4,5] In addition, low-frequency ROA is observed, such as the strong negative bands at $93/89\text{ cm}^{-1}$ for H_3His^+/H_2His^0 . Similar low-frequency vibrational optical activity has been

observed, for example, for polyaniline^[22] and a nitrile^[23] molecule; in the latter case it was assigned to a motion of whole molecules.

For the metal complexes, we can immediately see that the ROA signal is on average stronger and the bands are better resolved than for bare histidine. For example, the negative signal of free histidines splits into three (Zn, $78(-)$, $121(+)$, $159(-)\text{ cm}^{-1}$) or two (Ni, $77(-)$, $123(+)\text{ cm}^{-1}$) bands, and the signal around 240 cm^{-1} changes sign. The most striking is perhaps the about $4\times$ increase of intensity of the signal around 1430 cm^{-1} , forming the $1418(-)/1444(+)$ (Zn) and $1419(-)/1446(+)$ (Ni) couplets in the complexes. The similarity of Raman and ROA spectra of the zinc and nickel samples suggests that the geometries of the histidine ligands might be similar, too. On the other hand, within $300\text{--}800\text{ cm}^{-1}$ the nickel complex almost does not provide measurable signal, while ROA of the zinc one is well-developed, and smaller differences exist across the entire region of wavenumbers. A more detailed vibrational assignment is provided in Table S1.

Histidine MD: To understand the observations in terms of the structure, we performed a set of simulations. For H_3His^+ and two tautomers of H_2His^0 it was possible to explore the whole conformational space spanned by the χ_1 and χ_2 torsion angles. The dependence of the free energy on the angles as

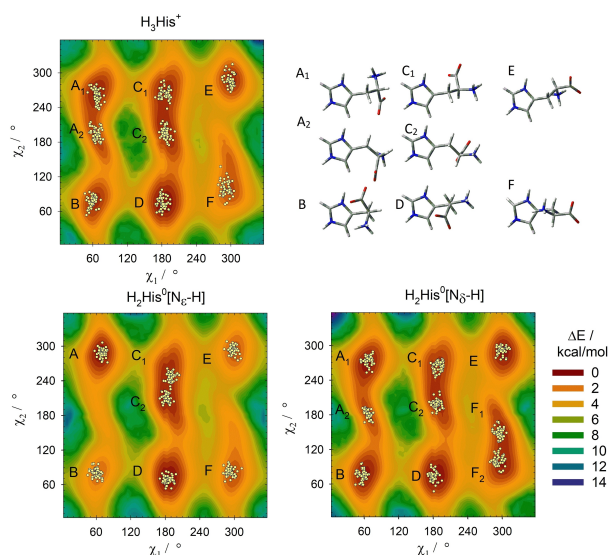


Figure 3. Dependence of the free energy on the χ_1 and χ_2 angles for protonated (H_3His^+) and zwitterionic ($\text{H}_2\text{His}^0[\text{N}_\delta\text{-H}]$, $\text{H}_2\text{His}^0[\text{N}_\epsilon\text{-H}]$) histidine, as obtained by WHAM-MD. The dots indicate clusters used in spectra averaging, examples of the conformer classes (A–F) are shown for H_3His^+ (cf. Figure S5 for H_2His^0).

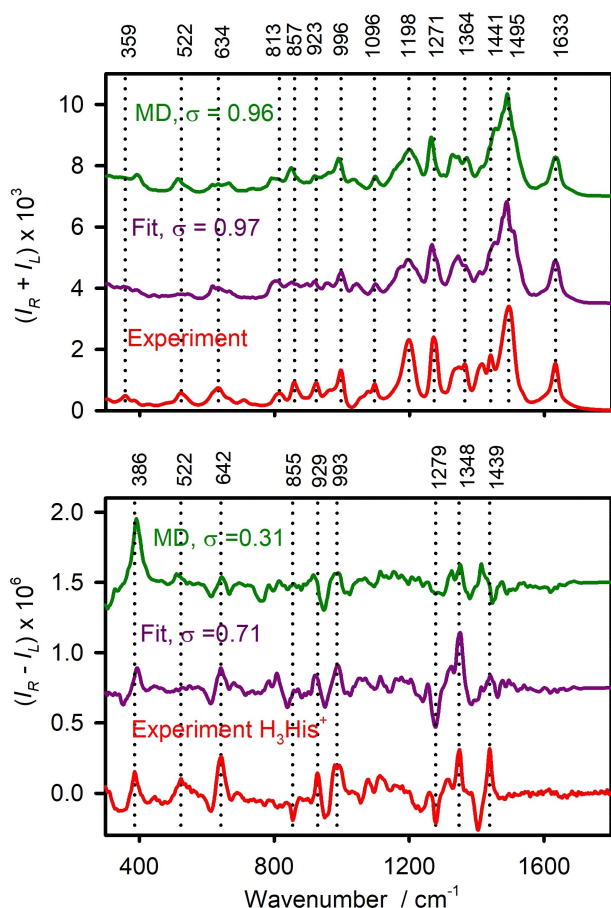


Figure 4. Simulated and experimental Raman and ROA spectra of H_3His^+ . Computed wavenumbers are scaled as $0.968 \times \omega + 18.2$, the spectra are normalized to Raman integral intensities, and the “similarity index” σ to the experiment is indicated.

obtained by the WHAM method is shown in Figure 3, and is consistent with previous theoretical works.^[4,5,8] For all forms at least 7 local energy minima are predicted, their coordinates together with single-point B3LYP/6-31++G**/PCM geometries for H_3His^+ and H_2His^0 tautomers are listed in Table S2. The B3LYP results are in a rough agreement with MD; based on the previous experience we consider the MD structures to be more reliable, because of the strong interaction of the solute with the water molecules.^[24]

Calculated H_3His^+ Spectra: In Figure 4 the MD-averaged spectra and a fit from calculated conformers A–F are compared to Raman and ROA experiment. For Raman, the theory matches the experiment almost perfectly. Bigger discrepancies appear for ROA, which reflects the bigger sensitivity of this spectroscopy to molecular conformation and computational inaccuracies. Yet also here the principal character of the experimental shape is reproduced, such as the 386 cm^{-1} positive band, the w-shape features around 950 cm^{-1} and within $1279\text{--}1370\text{ cm}^{-1}$. Very problematic is the region around 1439 cm^{-1} where one of the four strongest positive experimental bands is not reproduced, even for the fitted combination of the conformers. We explain this by an error of the MD force field and a high density of different vibrational transitions in this region (CH, NH bending, C=O, C=N, and C=C stretching), frequencies of which are reproduced by DFT with different precision.

The fitted curves are obviously closer to the experiment than the plain average MD results. Spectra of individual conformers are plotted in Figure S6, conformer populations are listed in Table 1. The correct conformer averaging is crucial to interpret the experiment. For example, the resultant ROA spectrum is about two times weaker (in terms of average ROA/Raman ratios) than the spectra of individual conformers, already obtained as averages of 50 clusters. As expected, the DFT conformer populations are quite unrealistic, due to the overestimation of the effect of intramolecular hydrogen bonds and inadequacy of the PCM solvent model for the histidine-water interactions.^[6a,25] The Raman populations only approximately follow the ROA ones; we consider the ROA data more reliable, as they are more sensitive to geometry differences. Because of the previous experience^[6,26] and a slight dependence of the results on the interval of wavenumbers used for the decomposition, we estimate the error of experimental conformer populations within 5–15%. This appears sufficient to say that

Table 1. Relative conformer populations (in %) of H_3His^+ , from DFT (B3LYP/6-31++G**/PCM), WHAM-MD, and Raman and ROA spectra fit.

Conformer ^[a]	DFT	MD	Raman	ROA
A ₁	0	15	4	18
A ₂	–	5	21	9
B	2	9	32	15
C ₁	98	17	0	4
C ₂	–	13	0	0
D	0	26	11	14
E	0	8	16	36
F	0	6	16	4

^[a] Definition in Figure 3 and Table S2.

the MD simulation overestimates the occurrence of the C_1 , C_2 and D conformers, while it underestimates the population of E .

H_2His^0 Tautomers: For the zwitterionic histidine, calculated spectra of both tautomers, a fit, and the experimental spectra are plotted in Figure 5. Currently, we do not have a reliable theoretical method to determine relative energies of the ϵ and

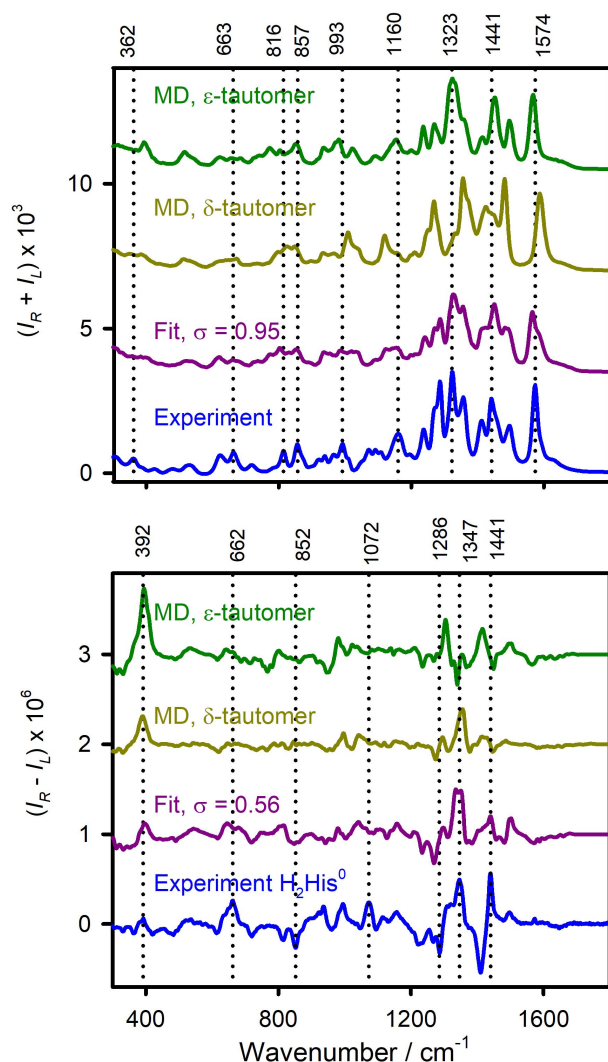


Figure 5. H_2His^0 Raman and ROA spectra, simulation from MD snapshots for both tautomers, a fit, and experiment.

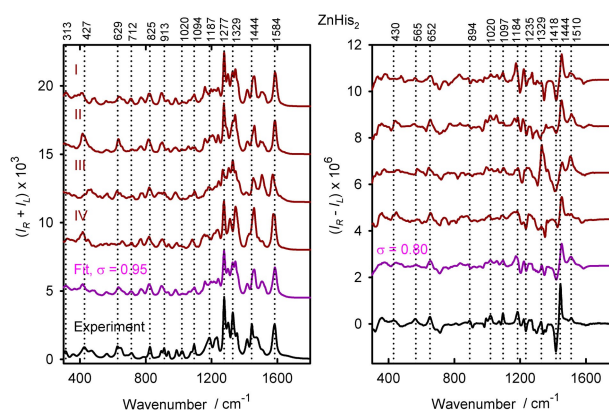


Figure 6. Calculated Raman and ROA spectra of four $ZnHis_2$ geometries (Figure 8), a fit, and the experiment.

δ tautomers. The B3LYP/6-311++G**/PCM calculation predicts that the ϵ -tautomer is favored a lot, by 3 kcal/mol, which about agrees with previous data, although the 4:1 ϵ : δ ratio corresponding to a smaller energy difference was deduced from experiment.^[7–8] The decomposition of the experimental spectra into subspectra of all calculated H_2His^0 forms (Figures S7 and S8, Table 2, definition of the conformers in Table S2.) indicates a more even distribution, from about 2:1 (Raman) to 2:3 (ROA). The spectra reflect the mixture; the three Raman bands within 1400–1500 cm^{-1} are better reproduced by the ϵ form, but the region around 1300 cm^{-1} is better explained by an ϵ and δ combination, etc. In ROA, a bigger presence of δ is indicated, for example, by the relatively low intensity of the 381 cm^{-1} band. The combined spectroscopic/computational method thus reveals the potential to determine the tautomer ratio in solution, in spite of the relatively large uncertainty of the current decomposition.

$ZnHis_2$ Structure is Octahedral: The spectra of different $ZnHis_2$ structures are compared with the fit and the experiment in Figure 6. Overall, the modeling seems to be more realistic than for the free histidine forms, presumably because of the limited flexibility of the complex. The more numerous bands and higher ROA signal observed in the complex as opposed to free histidine (Figure 2) are reproduced, with mostly correct signs and relative intensities of the ROA bands if the four structures are combined to a fit. Visually, structure III gives ROA

Table 2. Relative conformer and ϵ and δ tautomer populations (%) of H_2His^0 , from Raman and ROA spectra fit.

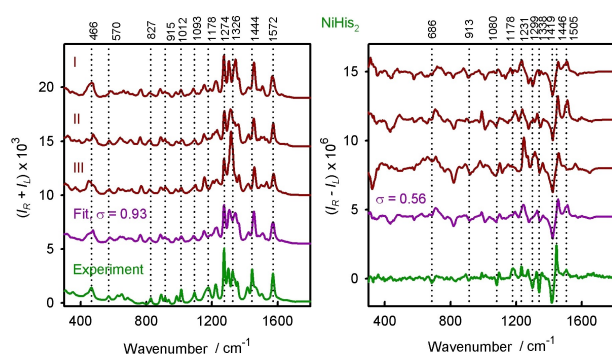
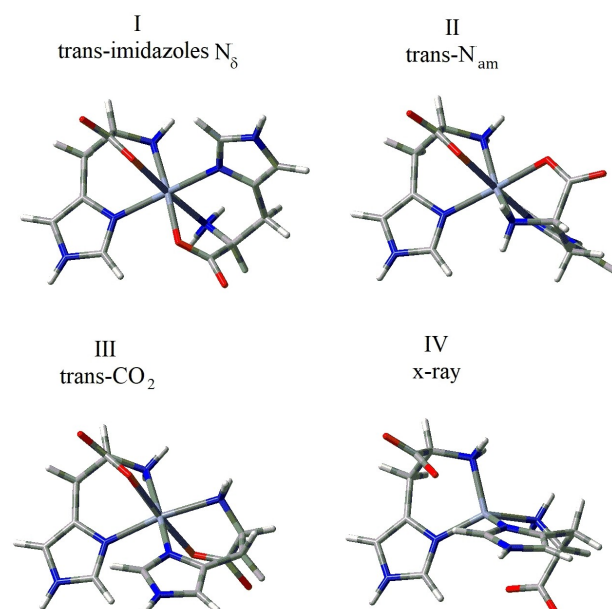
Conformer	Raman	ROA	Conformer	Raman	ROA
ϵ			δ		
A	10	21	A ₁	9	0
B	14	4	A ₂	6	14
C ₁	10	10	B	5	0
C ₂	14	5	C ₁	0	3
D	0	0	C ₂	8	0
E	16	1	D	0	1
F	0	0	E	1	21
–	–	–	F ₁	6	10
–	–	–	F ₂	0	9
Sum	64	41		36	59

Table 3. ZnHis₂ and NiHis₂ conformer populations as obtained by the fit of the experimental spectra with the simulated curves.

Conformer ZnHis ₂	Raman	ROA	Conformer NiHis ₂	Raman	ROA
I	53	40	I ^[a]	55	27
II	22	21	II	45	47
III	22	18	III	0	26
IV ^[a]	3	21	IV ^[b]	–	–

^[a] crystal geometry, ^[b] unstable during AIMD.

spectra very different from the experiment, with a strong positive band at 1329 cm^{−1} spectral region. This is reflected in its low percentage obtained by the decomposition (Table 3). We can thus see that the tetrahedral conformation found in the crystal (IV) is rather a minor form of the complex in solution,

**Figure 7.** Calculated Raman and ROA spectra of three NiHis₂ geometries (Figure 8), a fit, and the experiment.**Figure 8.** Starting AIMD geometries for the MHis₂ complexes, I–III are octahedral structures named according to the groups in approximate trans positions, and IV is a tetrahedral geometry. Crystal structure corresponds to IV for ZnHis₂ and to I for NiHis₂.

where the octahedral structure I prevails. The most prominent ROA feature is the 1418(−)/1444(+) couplet. Its intensity is at least three times higher than for other bands, and it originates in CH₂ scissoring combined with NH₂ twist, and C=O stretching.

The NiHis₂ Complex: For NiHis₂, the measurement was complicated by a relatively intense absorption at 532 nm (full absorption and ECD spectra are plotted in Figure S9) and a lower power of the excitation radiation had to be used. The absorption, resonance of the excitation energy with electronic transitions of the complex, also made it more difficult to average the spectra simulated for individual MD snapshots. In this case clusters where the laser excitation frequency was too close to an electronic transition energy gave extremely high Raman and ROA intensities and were omitted from the calculations. We thus model a pre-resonance situation,^[15c] where the excitation frequency is close, but not equal to an electronic energy. The simulated spectra, a fit and the experimental spectra are plotted in Figure 7. During AIMD, only the octahedral structures (I–III, Figure 8) were stable. According to the Raman fit predicting 55% of conformer I (Table 3), the crystal “x-ray” geometry^[12] is preferred in the solution, while the ROA spectra decomposition gives 27% of this form. This instability of the decomposition results may be related to error of the simulated intensities calculated in the pre-resonance approximation.^[27] Unlike for the zinc complex, the populations also more depend on the spectral interval where the spectra decomposition is done (by default 300–1800 cm^{−1} was used), again indicating larger errors in calculated intensities.

Other Complex Geometries: The 2:1 histidine/metal ratio is consistent with the experimental and theoretical data, and with a titration analysis where the ratio was changed in the experiment. The spectra clearly show that histidine changes its conformation upon binding. Computationally, we explored also 1:1 ratios with bidental metal binding (involving combinations of the amine, carboxyl and ring nitrogens); resultant spectra, however, did not indicate that such complexes would be present in the measured samples. We cannot exclude presence of larger complex aggregates, as the Raman and ROA technique does not capture weak long-range interactions.^[28] However, their presence is harder to deal with theoretically and was not investigated here.

Conclusion

We have recorded and analyzed Raman and ROA spectra of two histidine forms, and histidine zinc and nickel complexes, to get an insight into the geometry and broaden the application span of the vibrational optical activity. The monomeric histidine was found to be quite flexible, many conformers needed to be included to reproduce the experiment by calculations, and ROA bands were relatively weak and broad. The complexation with the metals provided stronger and better resolved bands. The computations suffered from the limited precision of the simulated spectral intensities, nevertheless they were accurate enough to indicate prevalent forms of the studied compounds in the solutions. For H_2His^+ they indicated a significant drawback of the MD Amber03/TIP3P force field, predicting an unreasonable distribution of the conformers. For H_2His^0 a more even distribution between the ϵ and δ -tautomers was found than in previous studies. The combined Raman/ROA/AIMD/DFT methodology also provided important information on the conformer distribution of the metal complexes; in particular the most abundant ZnHis_2 conformer in the solution was found to differ from the crystal structure. In spite of the computational effort that needs to be conducted for the interpretation we find that the Raman and ROA spectra provide extremely useful information about the solution geometry that cannot be obtained by other means.

Experimental Section

Sample Preparation: Commercial chemicals were used (L-His: Fluka 53320, D-His: Sigma-Aldrich H3751, ZnCl_2 : Sigma-Aldrich 229997, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$: Sigma-Aldrich 223387, 1 N NaOH: Sigma H9892-100 ml, 1 N HCl: Sigma S2770-100 ml), pH was measured with a Thermo Orion™ microglass electrode. Titration experiments with histidine were carried out by mixing two 0.2 M His solutions, in 0.4 M HCl and in 0.4 M NaOH. For the complexes, 0.2 M histidine and 0.1 M Zn^{2+} or Ni^{2+} concentrations were used. For Zn^{2+} , pH was adjusted directly by 0.4 M HCl or 0.2 M NaOH; at $\text{pH} > 7.7$ the samples became milky and were not measurable. For Ni^{2+} , to avoid preliminary precipitation, 60 μL of dissolved HCl or NaOH was added to 200 μL of the His/ Ni^{2+} mixture ($\text{pH} \sim 4.3$); pH values within 1.9–12.5 could thus be achieved. The samples were measured immediately after preparation.

The protonation states of histidine and its complexes at a given pH were estimated using the ChemEQL program^[29] and complex stability constants from ref. [30]. According to ChemEQL, the desired ZnHis_2 complex is favored within $\text{pH} 8\text{--}11$, NiHis_2 is present for $\text{pH} > 8$. This was confirmed by Raman spectra of both complexes, reasonably stable at $\text{pH} > 8$. The H_2His^0 and H_3His^+ forms were obtained by dissolving 0.2 M histidine in deionized water or 0.2 M HCl, respectively. A factor analysis^[31] of Raman spectra combined with acido-basic relations^[19] confirmed that at $\text{pH} 7.6$ 94% of histidine was present as H_2His^0 and at $\text{pH} 3.9$ 99% was present as H_3His^+ .

Spectra Measurement: Raman and ROA spectra were acquired on an ROA spectrometer developed at Palacký University Olomouc.^[23] H_2His^0 , H_3His^+ and the zinc complexes were measured at 25 °C in a rectangular fused silica cell ($\sim 70 \mu\text{L}$ volume). The spectral intensities are given in units of detected photoelectrons per frequency interval and per excitation energy (cm^{-1}). The backscattering geometry and scattered circular polarization (SCP) modulation was used, with 532 nm laser excitation, 6 cm^{-1} resolution, 675 mW laser

power at the sample, and 14 h (H_2His^0 , H_3His^+) and 25 h (ZnHis_2) acquisition times. For the nickel samples the laser power was 162 mW and acquisition time 14.6 h. For the ECD-Raman correction, absorption was measured within 240–800 nm by a Jasco V-760 spectrometer, and ECD was measured within 300–800 nm using a J-815 (ECD) spectrometer (Figure S9). Raw ROA spectra of the NiHis_2 complex were corrected for the ECD-Raman effect,^[27,32] Raman signal of water was subtracted and baseline corrected.^[33] Idealized ROA spectra obtained as an average “(D-L)/2” from both enantiomers are presented, original spectra are provided in the Supporting Information (Figure S10).

Molecular Dynamics (MD) and DFT: MD was performed within the Amber14 program.^[34] Histidine and 262 water molecules were placed in a $(20 \text{ \AA})^3$ cubic box; for H_3His^+ , the Cl^- counterion was added, 1 fs integration step, the Amber03 (TIP3P for water) force field, NVT ensemble and temperature of 300 K were applied. For a control, free MD was run. For a better exploration of the molecular conformational space, constrained MD (weighted histogram analysis method; WHAM)^[35] was performed, where the χ_1 and χ_2 angles were changed from -180° to 180° in 36° increments. For simplicity, we classify the conformations – minima of the free energy – by bold letters, A with $(\chi_1, \chi_2) \sim (60^\circ, -90^\circ)$, B $(60^\circ, 90^\circ)$, C $(180^\circ, -90^\circ)$, D $(180^\circ, 90^\circ)$, E $(-60^\circ, -90^\circ)$ and F $(-60^\circ, 90^\circ)$. In some cases also sub-conformers were distinguished; conformer populations were obtained by integration over relevant coordinate areas. For each conformer class, 50 snapshots were selected during a restrained MD and their spectra averaged. In the snapshots, only water molecules hydrogen-bonded and closer than 3.6 Å to the histidine were kept for the spectral computations.

At the DFT level, the snapshots/clusters were partially optimized in the vibrational normal mode coordinates,^[36] modes below 200 cm^{-1} were fixed. The Gaussian program^[37] was used for the quantum mechanics performed using the B3LYP/6-31++G** (solvent)/6-31G(solvent) method, distant solvent was simulated by the PCM^[38] model. After the optimization, Raman and ROA intensities were calculated at the same level. For spectra generation, water polarizabilities were set to zero, and Lorentzian peaks with 10 cm^{-1} full width at half maximum were used.

Ab Initio Molecular Dynamics (AIMD): AIMD was performed for the ZnHis_2 and NiHis_2 complexes within the Castep program,^[39] using the generalized gradient approximation (GGA) functional PBE,^[40] an NVT ensemble, temperature of 298 K, Langevin thermostat, 0.5 fs integration time step, ultrasoft pseudopotentials,^[41] $(12.4 \text{ \AA})^3$ box containing the complex and water molecules, and plane wave cutoff energy of 300 eV. Integrals were taken over the Brillouin zone using a single k-point sampling grid.^[42] The atomic positions were optimized by energy minimization prior to the MD runs. AIMD allowed us to run short simulations only. Therefore several starting geometries (Figure 8) were formed beforehand, and $\sim 6 \text{ ps}$ simulations were performed for each, to investigate the stability, determine possible structures of the complex, its flexibility, structure of the first solvation sphere, and to generate about 50 clusters processed for the spectra computations as before. The starting geometries and selected structural parameters are provided in Tables S3–S5. We also experimented with 1:1 His/Zn complexes; however, no indication was found that these would be needed to explain the experimental observations indicating the 2:1 ratio.

Comparison to Experiment and Spectral Decomposition: For the decomposition (Eq. (1)) we maximized the “similarity index” σ between theoretical S_t and experimental S_e spectra,^[43]

$$\sigma = \int S_e S_t d\omega \left(\int S_e^2 d\omega \int S_t^2 d\omega \right)^{-1/2} \quad (2)$$

The integration comprised 300–1800 cm⁻¹, calculated frequencies were scaled as $\omega' = a\omega + b$, where $a = 0.968$ and $b = 18.2$ cm⁻¹.

Acknowledgements

The work was supported by the Grant Agency (22-04669S, 22-15374S) and Ministry of Education (CZ.02.1.01/0.0/0.0/16_019/0000729 and e-INFRA CZ LM2018140) of the Czech Republic. We thank David Kopečný (Palacký University) for the help with ECD measurement.

Conflict of Interest

K, JH and PM pursue commercialization of the ROA spectrometer as employees of Palacký University Olomouc in cooperation with ZEBR and Meopta - optika companies. The other authors declare no conflict of interests.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: density functional calculations · histidine · metal complexes · molecular dynamics · Raman optical activity · Raman spectroscopy

- [1] L. Huo, A. J. Fielding, Y. Chen, T. Li, H. Iwaki, J. P. Hosler, L. Chen, Y. Hasegawa, L. Que, Jr., A. Liu, *Biochemistry* **2012**, *51*, 5811–5821.
- [2] J. G. Mesu, T. Visser, F. Soulimani, E. E. van Faassen, P. de Peinder, A. M. Beale, B. M. Weckhuysen, *Inorg. Chem.* **2006**, *45*, 1960–1971.
- [3] J. A. Vila, Y. A. Arnautovaa, Y. Vorobjev, H. A. Scheragaa, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 5602–5607.
- [4] E. Deplazes, W. vanBronswijk, F. Zhu, L. D. Barron, S. Ma, L. A. Nafie, K. J. Jalkanen, *Theor. Chem. Acc.* **2008**, *119*, 155–176.
- [5] S. Cardamone, B. A. Caine, E. Blanch, M. G. Lizio, P. L. A. Popelier, *Phys. Chem. Chem. Phys.* **2016**, *18*, 27377–27389.
- [6] a) M. Buděšínský, P. Daněček, L. Bednárová, J. Kapitán, V. Baumruk, P. Bouř, *J. Phys. Chem. A* **2008**, *112*, 8633–8640; b) J. Jungwirth, J. Šebestík, M. Šafařík, J. Kapitán, P. Bouř, *J. Phys. Chem. B* **2017**, *121*, 8956–8964.
- [7] W. F. Reynolds, I. R. Peat, M. H. Freedman, J. R. Lyster, *J. Am. Chem. Soc.* **1973**, *95*, 328–331.
- [8] F. Pflüger, B. Hernández, M. Ghomi, *J. Phys. Chem. B* **2010**, *114*, 9072–9083.
- [9] a) L. Rulišek, J. Vondrášek, *J. Inorg. Biochem.* **1998**, *71*, 115–127; b) L. Rulišek, Z. Havlas, *J. Am. Chem. Soc.* **2000**, *122*, 10428–10439.
- [10] R. N. V. Krishna Deepak, R. Sankararamakrishnan, *Biochemistry* **2016**, *55*, 3774–3783.
- [11] M. M. Harding, S. J. Coel, *Acta Crystallogr.* **1963**, *16*, 643–650.
- [12] K. A. Fraser, M. M. Harding, *J. Chem. Soc. A* **1967**, 415–420.
- [13] a) J. Haesler, I. Schindelholz, E. Riguete, C. G. Bochet, W. Hug, *Nature* **2007**, *446*, 526–529; b) X. Li, K. H. Hopmann, J. Hudecová, J. Isaksson, J. Novotná, W. Stensen, V. Andrushchenko, M. Urbanová, J. S. Svendsen, P. Bouř, K. Ruud, *J. Phys. Chem. A* **2013**, *117*, 1721–1736.
- [14] a) E. W. Blanch, I. H. McColl, L. Hecht, K. Nielsen, L. D. Barron, *Vib. Spectrosc.* **2004**, *35*, 87–92; b) L. D. Barron, E. W. Blanch, L. Hecht, *Adv. Protein Chem.* **2002**, *62*, 51–90; c) J. Kessler, J. Kapitán, P. Bouř, *J. Phys. Chem. Lett.* **2015**, *6*, 3314–3319; d) C. Shen, M. Srebro-Hooper, T. Weymuth, F. Krausbeck, J. T. L. Navarrete, F. J. Ramirez, B. Nieto-Ortega, J. Casado, M. Reiher, J. Autschbach, J. Crassous, *Chem. Eur. J.* **2018**, *24*, 15067–15079; e) V. Palivec, V. Kopecký, P. Jungwirth, P. Bouř, J. Kaminský, H. Martinez-Seara, *Phys. Chem. Chem. Phys.* **2020**, *22*, 1983–1993.
- [15] a) S. Luber, M. Reiher, *ChemPhysChem* **2010**, *11*, 1876–1887; b) S. Luber, M. Reiher, *Chem. Phys.* **2008**, *346*, 212–223; c) G. Zając, P. Bouř, *J. Phys. Chem. B* **2022**, *126*, 355–367.
- [16] L. D. Barron, *Molecular Light Scattering and Optical Activity*, Cambridge University Press, Cambridge, UK, **2004**.
- [17] B. Dolenský, J. Kroulík, V. Král, J. L. Sessler, H. Dvořáková, P. Bouř, M. Bernátková, C. Bucher, V. Lynch, *J. Am. Chem. Soc.* **2004**, *126*, 13714–13722.
- [18] a) S. Martusevičius, G. Niaura, Z. Talaikyte, V. Razumas, *Vib. Spectrosc.* **1996**, *10*, 271–280; b) J. G. Mesu, T. Visser, F. Soulimani, B. M. Weckhuysen, *Vib. Spectrosc.* **2005**, *39*, 114–125.
- [19] J. Palacký, P. Mojžeš, J. Bok, *J. Raman Spectrosc.* **2011**, *42*, 1528–1539.
- [20] a) R. M. Smith, A. E. Martell, *Critical stability constants*, Plenum Press, New York-London, **1975**; b) N. N. Vlasova, L. P. Golovkova, *Colloid J.* **2004**, *66*, 657–662.
- [21] T. Miura, T. Satoh, A. Hori-i, H. Takeuchi, *J. Raman Spectrosc.* **1998**, *29*, 41–47.
- [22] S. Yamamoto, S. Ishiro, J. Kessler, P. Bouř, *Phys. Chem. Chem. Phys.* **2021**, *23*, 26501–26509.
- [23] P. Michal, R. Čelechovský, M. Dudka, J. Kapitán, M. Vůjtek, M. Berešová, J. Šebestík, K. Thangavel, P. Bouř, *J. Phys. Chem. B* **2019**, *123*, 2147–2156.
- [24] a) P. Bouř, D. Michalík, J. Kapitán, *J. Chem. Phys.* **2005**, *122*, 144501; b) K. H. Hopmann, K. Ruud, M. Pecul, A. Kudelski, M. Dračinský, P. Bouř, *J. Phys. Chem. B* **2011**, *115*, 4128–4137; c) J. Hudecová, J. Horníček, M. Buděšínský, J. Šebestík, M. Šafařík, G. Zhang, T. A. Keiderling, P. Bouř, *ChemPhysChem* **2012**, *13*, 2748–2760.
- [25] P. Bouř, T. A. Keiderling, *J. Chem. Phys.* **2003**, *119*, 11253–11262.
- [26] J. Šugar, P. Bouř, *J. Raman Spectrosc.* **2016**, *47*, 1298–1303.
- [27] T. Wu, J. Kapitán, P. Bouř, *J. Phys. Chem. Lett.* **2022**, *13*, 3873–3877.
- [28] S. Yamamoto, X. Li, K. Ruud, P. Bouř, *J. Chem. Theory Comput.* **2012**, *8*, 977–985.
- [29] B. Müller, *Eawag: Swiss Federal Institute of Aquatic Science and Technology Kastanienbaum*, **2015**.
- [30] Y. Altun, F. Koseoglu, *J. Solution Chem.* **2005**, *34*, 213–231.
- [31] E. R. Malinowski, *Factor Analysis in Chemistry*, 2 ed., Wiley, New York, **1991**.
- [32] T. Wu, G. Li, J. Kapitán, J. Kessler, Y. Xu, P. Bouř, *Angew. Chem. Int. Ed.* **2020**, *59*, 21895–21898.
- [33] P. Eilers, H. Boelens, in *Leiden University Medical Centre report*, **2005**.
- [34] D. A. Pearlman, D. A. Case, J. W. Caldwell, W. S. Ross, T. E. Cheatham, S. Debolt, D. M. Ferguson, G. Seibel, P. A. Kollman, *Comp. Phys. Commun.* **1995**, *91*, 1–41.
- [35] S. Kumar, B. Bouzida, R. H. Swendsen, P. A. Kollman, J. M. Rosenberg, *J. Comput. Chem.* **1992**, *13*, 1011–1021.
- [36] P. Bouř, T. A. Keiderling, *J. Chem. Phys.* **2002**, *117*, 4126–4132.
- [37] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, D. J. Fox, Gaussian, Inc., Wallingford, CT, **2016**.
- [38] G. Scalmani, M. J. Frisch, *J. Chem. Phys.* **2010**, *132*, 114110.
- [39] S. J. Clark, M. D. Segall, C. J. Pickard, P. J. Hasnip, M. J. Probert, K. Refson, M. C. Payne, *Z. Kristallogr.* **2005**, *220*, 567–570.
- [40] J. P. Perdew, K. Burke, M. Ernzerhof, *Phys. Rev. Lett.* **1996**, *77*, 3865–3868.
- [41] D. Vanderbilt, *Phys. Rev. B* **1990**, *41*, 7892–7895.
- [42] H. J. Monkhorst, J. D. Pack, *Phys. Rev. B* **1976**, *13*, 5188–5192.
- [43] P. L. Polavarapu, C. L. Covington, *Chirality* **2014**, *26*, 539–552.

Manuscript received: June 30, 2022
Accepted manuscript online: July 25, 2022
Version of record online: August 22, 2022