

# **Cocaine Hydrochloride Structure in Solution Revealed by** Three Chiroptical Methods

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Structure and flexibility of natural compounds determine their biological activity. In the present study, electronic circular dichroism (ECD), vibrational circular dichroism (VCD), and Raman optical activity (ROA) spectra of cocaine hydrochloride in aqueous solutions were measured and related to the structure with the aid of density functional theory (DFT) computations. Additional measurements in deuterated environment made assignment of vibrational bands easier. The results suggest that the prevalent cocaine conformation in solution differs from that

# 1. Introduction

Cocaine is a tropane alkaloid found in the leaves of *Erythroxy-lum coca*, and is one of the most widely abused drugs. The cocaine molecular skeleton possesses four chiral centers; its four diastereoisomers are known as cocaine, pseudococaine, allococaine and allopseudococaine. Although all of them can be used as local anesthetics, allococaine and allopseudococaine are much less efficient than pseudococaine or the "natural" cocaine,<sup>[11]</sup> the conformations of which are investigated in the present study.

The cocaine tropane ring supports four different conformations: the heterocyclic six-membered ring forms chair or boat conformers, with axial or equatorial position of the *N*-methyl group.<sup>[2]</sup> Such flexibility naturally attracted attention and was examined in a number of studies.<sup>[1-3]</sup> X-ray powder diffraction was used to identify cocaine free base<sup>[4]</sup> and its salt.<sup>[5]</sup> These crystallographic works also indicated that the chair conformation with *N*-methyl group in the equatorial position is preferred.

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adopted in hydrochloride crystal. The spectroscopic results and computational analysis are consistent with X-ray structures of known cocaine-receptor complexes, in which the compound adopts a variety of conformations. All three kinds of chiroptical spectra exhibited significantly greater conformational sensitivity than unpolarized absorption or Raman scattering. The ROA technique provided the largest number of well-resolved bands, bearing rich structural information.

Cocaine conformation in solution was studied by nuclear magnetic resonance spectroscopy, which confirmed the dominance of the chair form.<sup>[3,6]</sup> Stereochemistry of the *N*-methyl group, however, depends on the solvent. The equatorial isomer was observed for cocaine hydrochloride dissolved in D<sub>2</sub>O, whereas the axial isomer was additionally present in deuterated dichloromethane.<sup>[7]</sup> This work focuses on H<sub>2</sub>O/D<sub>2</sub>O solutions, in which the hydrochloride is completely dissociated.

In forensic practice, Raman and infrared (IR) absorption spectroscopies are frequently used to identify cocaine based on its characteristic vibrational bands.<sup>[8]</sup> For example, cocaine hydrochloride has strong Raman signals at ca. 1713 (C=O stretching) and 1203 cm<sup>-1</sup> (C=O-C bending).<sup>[9]</sup> Similarly, in the infrared spectra, 1728 and 1712 cm<sup>-1</sup> bands (C=O stretching of the two carbonyl groups), 1265, 1230 and 1105 cm<sup>-1</sup> (acetate C=O stretching) and those originating from the aromatic ring C=H stretching vibrations were assigned to cocaine.<sup>[9]</sup> An interesting assignment was reported for a cocaine hydrochloride band encountered at ca. 2540 cm<sup>-1</sup> and attributed to N=H stretching, which normally occurs at much higher wavenumbers.<sup>[8c]</sup>

The traditional spectroscopic techniques are versatile and easy to use, but their sensitivity to molecular structure is limited. Chiroptical techniques are much more responsive. In particular, vibrational optical activity (VOA) methods comprising vibrational circular dichroism (VCD, difference in absorption of the left- and right-circularly polarized light) and Raman optical activity (ROA, difference in scattering of the right- and left-circularly polarized light)<sup>[10]</sup> are in general considered to be more reliable for determination of the absolute configuration than the electronic methods.<sup>[11]</sup> The electronic circular dichroism (ECD) provides useful information about the prevalent cocaine conformation as well.

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For cocaine, only the ECD spectroscopy has been used to date, without any backing by quantum-chemical modeling of the spectra.<sup>[12]</sup> Yet today, interpretations of chiroptical spectra are unthinkable without a profound computational analysis and ab initio simulations relating spectral features to the structure.<sup>[13]</sup> In general, quantum-chemical simulations of electronic spectra, such as ECD, are more demanding than of vibrational spectra, which are dependent on the electronic ground-state only. The main goal of the present study is therefore to compare the performance of all three main chiroptical techniques, relate the spectra to the conformation of the cocaine ion, and to provide a solid theoretical basis for the interpretation.

# 2. Results and Discussion

## 2.1. Geometry

Firstly, we inspect the axial and equatorial forms (**A** and **E**, Figure 1). Calculated energies and torsion angles of the lowest energy conformations are listed in Table 1. The lowest energy conformers with respect to other molecular motions are compared. The equatorial methyl position in **E** is clearly more convenient than the axial one, by about 10 kcal mol<sup>-1</sup> for the



**Figure 1.** Cocaine hydrochloride, axial (**A**) and equatorial (**E**) forms of cocaine cation, and definition of the torsion angles ( $\alpha_1 = 4 \ C_{Me}$ -O'-C'<sub>O</sub>-C<sub>2R</sub>,  $\alpha_2 = 4 \ O'$ -C'<sub>O</sub>-C<sub>2R</sub>-C<sub>35</sub>,  $\alpha_4 = 4 \ C_{2R}$ -C<sub>35</sub>-O-C<sub>0</sub>,  $\alpha_5 = 4 \ C_{35}$ -O-C<sub>0</sub>-P<sub>Phe</sub>,  $\alpha_6 = 4 \ O$ -C<sub>0</sub>-P<sub>Phe</sub>-C<sub>sp2</sub>).

Table 1. Relative energies [kcalmol <sup>-1</sup> ] and torsional angles (for DFT, in degrees) of the lowest energy axial and equatorial cocaine conformers.					
	Ε	A			
E <sub>HF</sub>	0	10.3			
E <sub>HF-D</sub>	0	10.5			
E <sub>DFT</sub>	0	9.1			
α <sub>1</sub>	-178	179			
α <sub>2</sub>	98	157			
α <sub>4</sub>	-83	-149			
α <sub>5</sub>	-179	179			
α <sub>6</sub>	0	2			

three computational methods. This is considerably more than 2 kcal mol<sup>-1</sup> obtained earlier with a less advanced AM1 method<sup>[1]</sup> and is consistent with available X-ray experiments either with or without receptors, for which the equatorial form was found exclusively, albeit with various conformations of other molecular parts.<sup>[4–5,14]</sup> The equatorial conformation is stabilized by a hydrogen bond between the NH and carboxyl groups. This is different in the neutral cocaine form (not investigated in the present study) with a more planar arrangement of the nitrogen bonds.<sup>[4]</sup> Thus, both the computational results and experimental data suggest that **E** is practically the only form present in a significant amount in solution.

Next, we investigate all conformers of the equatorial form. The orientations of the methyl ester and benzoyloxy groups described by the torsion angles  $\alpha_i$ , i=1, 2, 4, 5, 6 vary significantly according to the cocaine environment. Examples of five experimental geometries determined by previous studies are summarized in Table 2. Apparently, the  $\alpha_1$  and  $\alpha_5$  angles tend

Table 2. Experimentally observed torsion angles in cocaine.					
	Hydrochloride <sup>[5]</sup>	Free-base <sup>[4]</sup>	1Q72 <sup>[a][15]</sup>	$2AJV^{[a][14a]}$	2PQZ <sup>[a,b][14b]</sup>
α1	-178	173	-144	169	167
α2	140	179	108	102	117
$\alpha_4$	77	-139	-77	-89	-138
$\alpha_5$	179	178	-177	-164	-173
$\alpha_{6}$	3	7	14	13	27
[a] Protein database identifier, http://www.rcsb.org. [b] sp <sup>2</sup> (planar) nitro- gen hybridization.					

to adopt ca. 180°,  $\alpha_6$  is close to 0°, and  $\alpha_2$  and  $\alpha_4$  are rather flexible. This is consistent with the relatively small energy differences among the four lowest energy conformers summarized in Table 3. The calculated values of torsion angles  $\alpha_2$  and  $\alpha_4$  ( $\alpha_1$  and  $\alpha_5$  were always close to 180°) for conformers I and II are consistent with the experimental data (Table 2); conformers III and IV do not occur in the experiment and their relative conformer energies are rather high.

#### 2.2. ECD of Individual Conformers

Calculated absorption and ECD spectra are compared to the experimental data in Figure 2. The computed (B3PW91/6-311 +



**Table 3.** Calculated relative electronic ( $\Delta E$ ) and Gibbs ( $\Delta G$ ) energies [kcal mol<sup>-1</sup>], and  $\alpha_2$  and  $\alpha_4$  DFT torsion angles for four conformers of the cocaine equatorial form.

	$\Delta E_{\rm DFT}$	$\Delta E_{\rm mPW2PLYP}$	$\Delta G_{\rm DFT}$	α2	α4
1	0.0	0.0	0.0	102	-84
П	0.6	0.7	0.9	107	-141
Ш	2.5	2.2	4.5	-70	-87
IV	2.6	2.5	2.4	-66	-147



Figure 2. The four major cocaine conformers (form E), with the intramolecular hydrogen bond plotted by a dashed line, and comparison of the calculated and experimental (in  $D_2O$ ) UV/Vis absorption and ECD spectra. Boltzmann-average for ECD is based on the DFT electronic energies.

+ G\*\*/CPCM) absorption profile reproduces the main experimental features, with a strong band at 236 nm (experimentally at 231 nm), and a smaller band at 256 nm (exp. 275 nm). Correspondence of the ECD bands is not so clear; at the longest wavelengths, unrealistically low intensity is calculated, whereas a broad positive signal was measured around 279 nm. Within 230–350 nm, Boltzmann-averaged computation gives a couplet and only the overall ECD sign is slightly negative, as in experiment, mostly due to the contribution of the second conformer.

Other functionals tried (B3LYP, B86, CAM-B3LYP, and  $\omega$ B97XD, Figure S1 in the Supporting Information) did not lead to a convincing improvement in accuracy. Nevertheless, we may consider the level of agreement of simulated spectra and experiment reasonably good given the TDDFT approximations and the overall accuracy limits.<sup>[16]</sup> Experimental spectra may also be affected by vibrational splitting of the electronic

 $\mathsf{bands}^{\scriptscriptstyle[17]}$  and by interaction with the  $\mathsf{solvent}^{\scriptscriptstyle[18]}$  not explicitly included in the present model.

Analysis of the main orbital contributions (Figure 3) indicates that both the 236 nm and 256 nm bands are dominated by transitions located on the aromatic electronic  $\pi$ -system, with a participation of the ester group. Band positions and UV ab-



Figure 3. Dominant orbital contributions to the longest wavelength absorption bands (B3PW91/6311 + + G\*\*/CPCM calculation for conformer I).

sorption of individual conformers are almost identical. On the other hand, ECD spectra (Figure 2) differ profoundly, and only conformers I and II provide patterns that are somewhat similar to the experimental data (a negative signal at 244 nm and a positive one at 279 nm). The dissymmetry factor (*g*, ratio of ECD/absorption) of conformer II is too high ( $|g| \approx 6 \times 10^{-4}$  around 240 nm) compared with experiment ( $|g| \approx 1 \times 10^{-4}$ ), which is indicative of a conformer mixture that is roughly consistent with the relative conformer energies (Table 3).

#### 2.3. Infrared Absorption and VCD

The computed and experimental IR and VCD spectra are compared in Figure 4. Assignment of the most intense IR and Raman bands is presented in Table 4. Most of the frequencies obtained at the B3PW91 level are closer to experiment than those obtained at the mPW2PLYP level, in spite of the latter method being more sophisticated<sup>[19]</sup> and computationally more expensive. Typically, the B3PW91 computation took less than 3 days of CPU time, and the mPW2PLYP required 30-145 days, according to computational options (numerical vs. analytical frequency calculation). However, the calculated frequencies do not differ much, except for the ND stretching mode. Furthermore, the differences between computed and experimental frequencies are rather minor (typically within 0-20 cm<sup>-1</sup>) and can be explained by intrinsic error of the computational methods, both treating the correlation energy rather incompletely, and by anharmonic interactions<sup>[20]</sup> not included in the modeling.



Figure 4. Calculated IR and VCD spectra of the four lowest energy cocaine conformers (left), and the experiment (right).

Unlike for UV/Vis absorption, conformers III and IV provide quite distinct IR absorption within the C=O stretching region (Figure 4, 1753 and 1795 cm<sup>-1</sup>, experimentally at 1714 cm<sup>-1</sup>). This is due to the internal hydrogen bond (Figure 2); in conformers I and II, the amine hydrogen is bound to the carboxyl oxygen (=O), whereas in conformers III and IV, the ester oxygen (-O-) is involved. Minor differences are also apparent within 1330–1470 cm<sup>-1</sup>, confirming the low conformer ratios for species III and IV. Overall, however, the simulation reproduces the experimental absorption features quite well; frequency differences between the calculation and experiment can be explained by inaccuracy of the functional, continuum solvent model, and by the anharmonic effects.<sup>[21]</sup> Similar to the absorption, only conformer I and partially also II give reasonable VCD spectra compared with the experiment. In particular, the strong negative carbonyl band (in experiment at 1712 cm<sup>-1</sup>) is not predicted for conformers III and IV. Simulated spectra of conformers I and II do reproduce this and other observed VCD features, such as the positive experimental signals at 1394 and 1457 cm<sup>-1</sup>, and to some extent also the 1321 and 1359 cm<sup>-1</sup> bands. The magnitude of the experimental dissymmetry factor (*g*) for the carbonyl band (1712 cm<sup>-1</sup>), for example, is about  $5 \times 10^{-5}$ , which is a value typical for organic compounds. The theory predicts only a slightly smaller  $g \approx 2 \times 10^{-5}$ , mostly due to the overestimated splitting of the C= O stretching bands.

#### 2.4. Raman Scattering and Raman Optical Activity

Calculated and experimental (D<sub>2</sub>O solution) Raman and ROA spectra are plotted in Figure 5. Conformers I–IV provide very similar Raman spectra. The computation reproduces very well the experimental Raman intensities within 200–1830 cm<sup>-1</sup>. The N–D stretching band (at ca. 2378 cm<sup>-1</sup> experimentally, not shown) is difficult to separate from the solvent signal. Unlike for IR absorption in which two bands corresponding to the C= O stretching modes are visible (Figure 4), only one band is apparent in the Raman spectrum, experimentally/calculated at 1716/1753 cm<sup>-1</sup>. The strong Raman scattering calculated at 1649 cm<sup>-1</sup> is due to aromatic ring vibration stretching (Table 5). Within ca. 1100–1390 cm<sup>-1</sup> the computed spectrum mimics the experimental shape well, although a band-to-band comparison is problematic because of the high density of vibrational states. The band assignment is easier below

Colod			N		Calad			יר ער
Calca.		Exp. (D <sub>2</sub> C	)		Calco.		Exp. (D <sub>2</sub> C	))
B3PW91	mPW2PLYP	Ram	IR		B3PW91	mPW2PLYP	Ram	
2372	2489	2383		ν(ND)	940	948	920	aliph. def.
1753	1782	-		ν (CO), Phe	901	906	887	deloc.
1733	1746	1716	1714	ν (CO), Me	855	860	847	aliph. def.
1649	1654	1604		ν (C=C), Phe	795	795	812	aliph. def.
1644	1636		1601	ν (C=C), Phe	781	779	782	aliph. def.
1521	1541	1493		$\nu$ (C=C), $\delta$ (CH) Phe	762	763	753	aliph. def.
1514	1535		1485	$\delta$ (CH),scissor	751	749	736	aliph. def.
1489	1527	1493		$\delta$ (CH),scissor	722	721	713	aliph. def.
1484	1517		1451	$\delta$ (CH),scissor	689	695	682	Phe def.
1479	1509	1456		δ(CH),scissor	626	632	619	Phe def.
1471	1489		1443	$\delta$ (CH),scissor	555	572	557	aliph. def.
1407	1418	1390	1386	δ(CH), ν (C–C)	520	516	520	deloc.
1386	1407	1366		δ(CH), ν (C–C)	484	486	487	deloc.
1309	1323	1321		δ(CH), ν (C–C)	447	443	446	Phe oop def.
1280	1293	1281	1277	$\nu$ (C=C) Phe	418	427	421	deloc.
1220	1236	1229		Me wagging, aliph.	394	403	397	deloc.
1187	1203	1181		δ(CH)	363	371	369	deloc.
1148	1156	1122		aliph. def.	320	322	323	deloc.
1081	1093	1066		aliph. def.	305	309	307	deloc.
1049	1055	1028		Phe def.	244	246	244	Me wag
1015	1018	1004		Phe def.	196	211	193	deloc.
994	999	971		aliph. def.				
[a] For conformer I, v—stretching, $\delta$ —bending, oop—out of plane, in D <sub>2</sub> O.								

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**Figure 5.** Calculated and experimental Raman  $(l^{R} + l^{L})$  and ROA  $(l^{R} - l^{L})$  spectra of cocaine. Atomic and instrumental units are used for the calculated and experimental intensities, respectively.

by various methods	Is [%] of the first and second conform [a]	ners as estimated
	Conformer	
Method	I	
$\Delta G_{\text{DFT}}$	82	16
$[\alpha]_{589}^{20}$	51	49
ECD	75	25
VCD	65	35
ROA	46	54
[a] $\Delta G_{\text{DFT}}$ —based or culated and experin ECD, VCD, and ROA	h Gibbs energies given in Table 3, $[\alpha]_5^2$ mental standard optical rotation, an spectra, see text.	based on cal- on analyses of

1100  $\text{cm}^{-1}$ , where vibrational transitions overlap to a smaller extent.

Unlike for the Raman scattering, computed ROA spectra of the four conformers (lower part of Figure 5) are very different. As for ECD and VCD, conformer I best fits the experimental ROA intensities, although a more quantitative comparison, such as decomposition of the experimental spectrum into calculated subspectra, would be difficult because of computational error. Interestingly, experimental the ROA signal of the C=O stretching region is quite weak, providing a negative signal around 1703 cm<sup>-1</sup>, and no measurable ROA signal was detected for the benzene ring's dominant Raman band at 1604 cm<sup>-1</sup>. This is explicable by the strong solvation of the CO group and dynamical flexibility of the benzoyloxy and methylcarboxylate residues, both of which are only partially captured by the

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CPCM solvent and rigid conformer model.<sup>[22]</sup> The comparison of the experimental and computed ROA curves is also complicated because of the different errors of calculated aromatic and aliphatic stretching and bending vibrations. For example, the dominant C=C benzene stretching mode is calculated (1649 cm<sup>-1</sup>) to be 45 cm<sup>-1</sup> higher than in experiment (1604 cm<sup>-1</sup>), whereas the C-H bending mode (calcd./exp. at 1479/1456 cm<sup>-1</sup>) exhibits a much smaller 23 cm<sup>-1</sup> difference, etc. These effects distort the computed overall shape, mainly within 900–1450 cm<sup>-1</sup>. Below 900 cm<sup>-1</sup> the band correspondence is clearer, similar to the Raman intensity.

#### 2.5. Conformer Equilibrium and the Optical Activity Spectra

All the computational methods favor conformer I, and the effect of conformational averaging is limited. Nevertheless, it brings about subtle changes in spectral intensities pertaining to the optical activity, as documented in Figure 6, in which ECD, VCD, and ROA spectra are plotted for conformer I as well



**Figure 6.** ECD, VCD, and ROA spectra of cocaine simulated with different weighting of conformers: conformer I only, Boltzmann weighting (300 K) with relative conformer energies from the mPW2PLYP and B3PW91 methods, and the experiment (D<sub>2</sub>O).



as a Boltzmann average with the B3PW91m and PW2PLYP energies. Perhaps surprisingly, the strongest effect of averaging is encountered in the ECD spectrum in which the presence of the strong negative experimental signal at 244 nm is explicable only by a conformer mixture. A much smaller effect of the averaging takes place for VCD. ROA spectra are affected more than VCD and less than ECD; for example, the averaging improves the intensity ratio of bands observed at 983 and  $1005 \text{ cm}^{-1}$ . The two methods (mPW2PLYP and B3PW91) provide rather similar results.

Populations of the first and second conformers as obtained by various analyses are summarized in Table 5. Other conformers are not included because the data do not indicate their presence. The estimation from ECD spectra was based on a visual comparison; VCD and ROA spectra were decomposed into conformer subspectra by the method of Lagrange multipliers.<sup>[23]</sup> Prior to the decomposition, the frequency scale-factor was determined as 0.98 by maximizing the similarity<sup>[24]</sup> between the calculated and theoretical curves (Figure S5).

On one hand, one can read Table 5 as a manifestation of the "unreliability" of different spectroscopic methods, because actual conformer ratios significantly vary. On the other hand, they provide a solid and consistent basis for determination of the most probable cocaine structure in solution. One has also to realize that the ECD and optical rotation<sup>[11]</sup> computations are much less reliable than for the vibrational spectra. Given also the complexity of the ROA signal, we can conclude that most probably the population of the first conformer prevails slightly over the second.

#### 2.6. The Effect of Deuteration

In Figure 7, experimental and computed Raman and ROA spectra of cocaine in  $H_2O$  and  $D_2O$  solutions are compared. Apparently, replacing just one cocaine hydrogen atom by deuterium has almost no effect on the Raman spectrum. Much more ex-



Figure 7. Effect of the isotopic substitution: simulated (B3PW91/6-311 + + G\*\*/CPCM) and experimental Raman and ROA spectra of cocaine in H<sub>2</sub>O and D<sub>2</sub>O, and two examples of modes in D<sub>2</sub>O, sensitive to the isotopic substitution. tensive changes appear in ROA. Most interestingly, a strong "+/-" couplet at 982/994 cm<sup>-1</sup> is formed in D<sub>2</sub>O compared with the natural isotopic case. The vibrational normal mode displacements (Figure 7) reveal that these bands are caused by ND/CH vibrations localized at the bicyclic part of the molecule. The NH  $\rightarrow$  ND substitution changes the coupling strength between the N–H(D) and CH bending modes, and stronger coupling in the deuterated species translates to a stronger ROA signal. It is generally accepted that ROA spectroscopy is more sensitive to molecular structure than unpolarized Raman spectroscopy; nevertheless, this is a rather rare example of an isotopic effect so much amplified by a vibrational resonance (coupling of vibrational modes) in the ROA spectrum.

## 3. Conclusions

We studied geometry of cocaine hydrochloride using ECD, VCD, and ROA spectroscopies, complementing the more usual nonpolarized IR absorption, Raman, and UV/Vis absorption methods. The results unambiguously showed that the solution structure was different from the crystal geometry. This conclusion could be made because of a systematic conformer search, ab initio estimation of relative conformer energies, and the comparison of experimental and calculated spectra. All spectroscopic methods indicate a relative rigidity of the cocaine cation in solution, which is mostly present in two conformers differing in rotation of the benzyl residue. The predicted conformational behavior is consistent with previous cocaine complexation studies, in which geometries of the benzyl and methyl residues adapt to the ligand. The chiroptical methods proved to be excellent tools for monitoring the conformation, particularly when coupled with the quantum-chemical spectral simulations. The ROA spectrum provided a larger number of conformer-dependent features compared with ECD and VCD. The TDDFT and DFT simulations agreed reasonably well with experiment, although further improvement of computational accuracy is desirable in the future. This will be especially important for a more detailed tracking of cocaine and other biologically active substances, for example during binding to the receptor.

## **Experimental Section**

#### Spectroscopy

Cocaine hydrochloride ( $\geq$  99.5%, pharmaceutical grade) was purchased from Dr. Kulich Pharma (Czech Republic); the purity was verified by LC-MS and NMR spectra obtained and analyzed in the Forensic Laboratory of Biologically Active Substances, University of Chemistry and Technology, Prague. Specific optical rotation in methanol was measured as  $[\alpha]_{589}^{20} = -66.1^{\circ}$ . For spectra acquisition the compound was dissolved in double-distilled water or deuterium oxide (D<sub>2</sub>O, 99.9% D from ISOSAR GmbH, Germany) to concentrations of 100 gL<sup>-1</sup> (VCD, ROA, IR and Raman) and 0.11 gL<sup>-1</sup> (ECD).

VCD and IR absorption spectra in the 1800–1250 cm<sup>-1</sup> region were measured with a Fourier transform IR (FTIR) IFS 66/S spectrometer equipped with a PMA 37 VCD/IRRAS module (both from Bruker,

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Germany).<sup>[13e]</sup> The sample was placed in an A145 demountable cell (Bruker, Germany) composed of  $CaF_2$  windows separated by a 23  $\mu$ m Teflon spacer. The spectra were recorded at 4 cm<sup>-1</sup> resolution and obtained as average of 7–9 blocks of 2260 scans, each block taking 20 minutes to accumulate. A baseline of pure solvent measured under the same experimental conditions was subtracted.

ECD and UV absorption were measured in a Suprasil quartz cuvette (10  $\mu$ m optical path length, Hellma, Germany) with a J-815 spectropolarimeter (Jasco, Japan), within 195–300 nm, at 100 nm min<sup>-1</sup> scanning speed, 1 s response time, 0.1 nm resolution, and 1 nm bandwidth. Eight accumulations were averaged, and solvent baseline was subtracted. VCD and ECD spectra are plotted as molar circular dichroism,  $\Delta \varepsilon$  IR and UV absorption is given as molar absorption coefficient,  $\varepsilon$ , all in Lmol<sup>-1</sup> cm<sup>-1</sup>.

Prior to the measurement of ROA and Raman scattering, fluorescence from residual impurities was suppressed by photobleaching in the laser beam for 4 hours. The spectra were acquired simultaneously with a ChiralRaman-2X spectrometer (BioTools, Inc., USA) using a 532 nm laser (Opus 2W/MPC6000, Laser Quantum, UK), 7 cm<sup>-1</sup> resolution, fused-silica sample cell (BioTools Inc., USA), and 1.2 s illumination period. A homemade Peltier cell holder set to 15 °C, laser power at the sample was 350 mW, and the accumulation time was about 24 hours. A baseline correction was done using a procedure described elsewhere.<sup>[25]</sup> For presentation of the spectra instrumental intensity units are used.

#### Calculations

The axial and equatorial conformations of the *N*-methyl group (models **A** and **E** in Figure 1) were used to investigate the protonated cocaine (methyl (1*R*,2*R*,3*S*,5*S*)-3-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate) ion. The boat conformation of the six-membered ring was not considered because its energy is bigger by more than 6 kcalmol<sup>-1</sup> than for the chair.<sup>[11]</sup> In addition, the  $\alpha_1$ ,  $\alpha_2$ ,  $\alpha_4$ , and  $\alpha_5$  torsion angles (cf. Figure 1) were systematically varied to generate 36 initial geometry estimates for each model.

The conformer search was conducted by using the Gaussian<sup>[26]</sup> program at the HF/6-31G<sup>\*\*</sup> approximation level including conductorlike continuum solvent model<sup>[27]</sup> (further referred to as CPCM)<sup>[28]</sup> with default parameters for water. Selected lowest energy conformers (<4 kcalmol<sup>-1</sup>) were reoptimized by using the B3PW91<sup>[29]</sup>/ 6-311 + +G<sup>\*\*</sup>/CPCM method. Previously, the B3PW91 functional provided excellent results for ROA simulations.<sup>[30]</sup> For control computation, the empirical dispersion correction of Grimme<sup>[31]</sup> was used (labeled by the "-D" suffix, for example, HF-D). Vibrational frequencies of the lowest energy conformer were also computed at the mPW2PLYP<sup>[19]</sup>/6-311 + +G<sup>\*\*</sup>/CPCM level.

For optimized geometries, energy second derivatives and VCD and ROA polarizability tensors<sup>[10,32]</sup> were calculated by the Gaussian. Back-scattered circularly polarized (SCP) Raman and ROA spectra were obtained as<sup>[33]</sup>  $I(\omega) = \sum_i A_i \{\omega_i [1 - \exp(-\omega_i/(kT))] [4(\omega - \omega_i)^2/\Delta^2 + 1]\}^{-1}$ , where  $A_i$  are calculated intensities for each mode i,  $\omega_i$  is vibrational frequency, k is the Boltzmann constant, and T = 15 °C is temperature. The full width at half maximum was set to  $\Delta = 10$  cm<sup>-1</sup>. IR absorption and VCD spectra are presented using the same Lorentzian bands ( $\Delta = 10$  cm<sup>-1</sup>).

Using time-dependent density functional theory (TDDFT),<sup>[34]</sup> electronic absorption and circular dichroism intensities were calculated for 50 excited electronic states at the same level as for the vibrations, and the spectra were generated by using Gaussian profiles 10 nm wide (FWHM).

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# **Conflict of interest**

The authors declare no conflict of interest.

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