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Quantum-Cascade Laser-Based Vibrational Circular Dichroism

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Supporting Information

ABSTRACT: Vibrational circular dichroism (VCD) spectra were recorded with a tunable external-cavity quantum-cascade laser (QCL). In comparison with standard thermal light sources in the IR, QCLs provide orders of magnitude more power and are therefore promising for VCD studies in strongly absorbing solvents. The brightness of this novel light source is demonstrated with VCD and IR absorption measurements of a number of compounds, including proline in water.

Most biological and many pharmaceutically active molecules are chiral. Optical methods provide a direct means of distinguishing the enantiomers of a chiral molecule in solution and have become a valuable tool in determining the stereochemistry of chiral molecules. The differential response to left and right circularly polarized light in absorption [i.e., circular dichroism (CD)] is particularly useful for determining the absolute configuration of a chiral molecule.[1] CD in the visible and UV parts of the electromagnetic spectrum is known as electronic CD (ECD). While ECD often requires only small concentrations of a chiral analyte and has proven its use in the analysis of DNA and protein secondary structure, it requires a suitable chromophore and generally offers only one or two broad difference bands for stereochemical analysis. In contrast, vibrational circular dichroism (VCD) provides a characteristic band pattern with contributions from up to $3N - 6$ vibrational modes.[2−4] VCD has therefore become one of the key methods for the determination of the absolute configuration of pharmaceutically active chiral compounds and natural products.[3−5] Dramatic advances in the accurate computation of VCD spectra have facilitated the unambiguous identification of the chiral enantiomers.[6] Vibrational transitions can be calculated with higher accuracy and at significantly lower computational cost than is possible for electronic transitions, where one has to consider excited states.

A VCD spectrum can be interpreted as a linear combination of the spectral contributions of all of the conformers that are populated under the given experimental conditions. This makes VCD not only a tool for conformational analysis of small molecules[5,7] but also a sensitive probe for secondary structure in biomolecules.[8] One difficulty in VCD spectroscopy, however, is the strong IR absorption by most solvents. Traditional VCD instrumentation therefore limits the study of (bio)molecules to selected spectral regions and solvents.

Inter-sub-band quantum-cascade lasers (QCLs), which were first demonstrated in 1994,[9] represent the only practical laser source available in the mid-IR (MIR) spectral range. QCLs provide at least 10^4 times more power at a given wavelength in the MIR than a thermal IR light source such as an incandescent wire or a silicon carbide rod. QCLs are technically less demanding than the use of nonlinear optical schemes based on frequency conversion with high-peak-power, short-pulse lasers which have recently been applied to vibrational optical activity measurements.[10,11] Another advantage is that because of the high power levels, QCLs permit the use of IR detectors that do not require N_2 cooling. Moreover, continuous-wave (cw) operation of the QCL does not suffer from optical saturation effects that may complicate measurements with pulsed sources. In conjunction with an external cavity, it is possible to tune the wavelength of a QCL within a window of ∼8% around the central wavelength, which in commercial systems currently lies anywhere between 800 and 2500 cm^{-1}. The resulting spectral range of ∼100 to 200 cm^{-1} is considerably (10-fold) smaller than that of a typical FT-IR measurement. Recent developments involving the combination of multiple QCLs have extended the coverage beyond 600 cm^{-1}. However, a spectral range of ∼100 cm^{-1} may be sufficient to cover important difference bands, such as characteristic bands in the fingerprint region of small molecules. Even traditional VCD measurements are often restricted to a narrow spectral window around spectral features of interest, such as the prominent amide I (1700−1600 cm^{-1}) and amide II (1600−1450 cm^{-1}) bands in peptides and proteins.[12]

Here, we present VCD spectra recorded with a cw tunable MIR laser. We show that QCLs are useful for VCD spectroscopy. The spectral brightness and resolution of QCLs surpass those of traditional FT-IR-based spectrometers, permitting measurements in strongly absorbing (aqueous) solutions.

For three chiral compounds, the metal-(−)-sparteine complex Ni(sp)Cl_2 (**1**), limonene (**2**), and proline (**3**) (Figure 1), we examined the spectral region between 1320 cm^{-1} (7.6 μm) and 1220 cm^{-1} (8.2 μm) and compared the QCL-based measurements with those from a commercial Fourier transform (FT) VCD spectrometer and with the results of calculations. The setup is shown in Figure 2. The polarization of the external-cavity QCL (Daylight Solutions) was modulated between left- and right-circular at 37 kHz with the help of a ZnSe photoelastic modulator (Hinds PEM 80) centered at 1270 cm^{-1}. To minimize interference effects at the modulation frequency, the modulator was tilted, allowing the beam reflected inside the PEM to be separated from the transmitted one and blocked by an aperture. The light was passed through the sample cell and a chopper and then directed via a ZnSe lens onto a thermoelectrically cooled photovoltaic detector (VIGO System S.A. PVMI-3TE-10.6) with a detectivity (D^*) of ∼3×10^9 cm $Hz^{1/2}$/W. The circular intensity difference was recorded with lock-in amplifiers.

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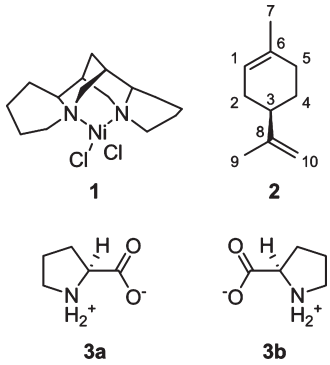

Figure 1. Structures of Ni(sp)Cl_2 (**1**), (*R*)-(+)-limonene (**2**), L-proline (**3a**), and D-proline (**3b**).

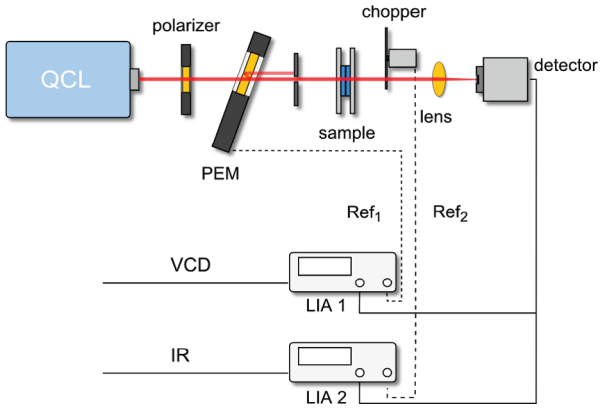

Figure 2. Setup used for VCD measurements, consisting of a quantum-cascade laser (QCL), a polarizer, a photoelastic modulator (PEM), a liquid cell, an optical chopper, a lens, and an IR detector. Two lock-in amplifiers were used to extract the VCD and IR signals.

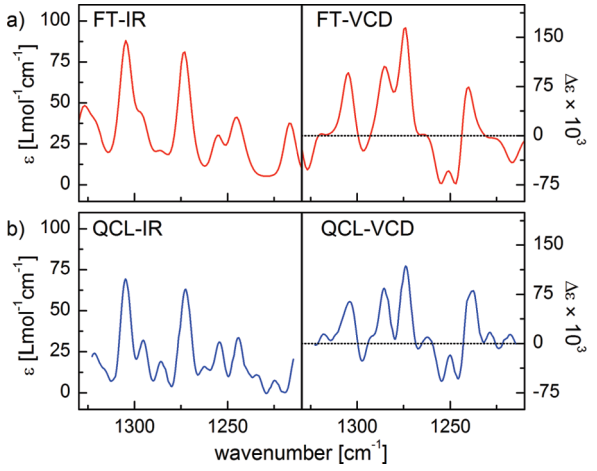

Figure 3. Spectra of a 200 mM solution of **1** in $CDCl_3$. (a) FT-IR and FT-VCD spectra recorded with a resolution of 4 cm^{-1} using a 100 μm path length BaF_2 cell. (b) QCL measurements recorded in a 1000 μm KBr cell with a resolution of 4 cm^{-1} after Fourier filtering.

The nicke-(−)-sparteine complex **1** was synthesized as described in the literature.[13] The open-shell complex **1** exhibits strong VCD signals yet has a molar absorptivity comparable to those of

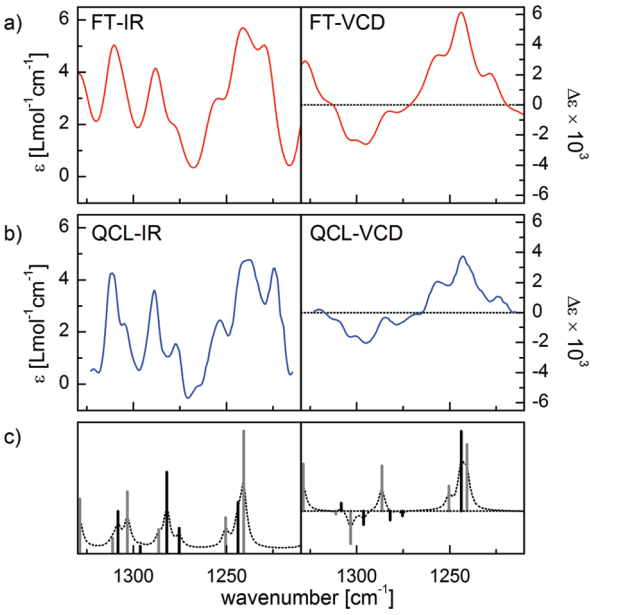

Figure 4. Spectra of a 3 M solution of **2** in $CDCl_3$. (a) FT-IR and FT-VCD spectra recorded in a 100 μm path length BaF_2 cell. (b) QCL measurements recorded in a 1000 μm KBr cell. All of the spectra are shown with a resolution of 4 cm^{-1}. (c) Theoretically predicted, Boltzmann-weighted dipolar strengths (left) and rotational strengths (right) calculated at the B3PW91/cc-pVTZ level for the two most abundant conformers (43:57, respectively; gray and black vertical lines; arb. units).

typical organic and inorganic molecules.[14] This has been shown to be due to vibronic coupling of low-lying magnetic-dipole-allowed d−d transitions to the ground state, resulting in a VCD enhancement for all modes.[15] VCD and IR spectra recorded with the QCL-based setup (Figure 2) and a lamp-based FT instrument are shown in Figure 3. All of the bands observed in the FT measurements (Figure 3a) are clearly reproduced in the QCL spectra (Figure 3b), where additional spectral features can also be seen. The QCL data were recorded with a resolution of 0.5 cm^{-1} and processed with a 4 cm^{-1} Fourier filter in order to suppress higher-frequency spectral components that we attribute to interference effects.

Nonpolar terpenoids, such as pinene and limonene, are small and rigid and show few intra- and intermolecular interactions, permitting accurate computation of their chiroptical properties using high-level ab initio methods.[16−18] These compounds do not have low-lying transitions and show weaker VCD, typically by a factor of 10^{-4} to 10^{-3} relative to the corresponding IR transitions.[2,4,19]

In Figure 4, we compare the FT-IR and QCL vibrational spectra of **2**. The relative IR and VCD intensities, computed at the B3PW91/cc-pVTZ level using Gaussian03,[20] are shown for comparison. The broad band around ~1308 cm^{-1} in the FT-IR spectrum appears as a doublet (1311 and 1305 cm^{-1}) in the QCL IR spectrum. It corresponds to the in-phase wagging modes of the hydrogens at C1, C3, C4, and C5 (see Figure 1 for atom numbering) with contributions from both conformers. The C−H twisting modes at ~1240 cm^{-1} are resolved with the QCL in the VCD spectrum.

In contrast to nonpolar organic solvent/solute systems, aqueous solutions not only are strongly absorbing but also give rise to more complex solvation characteristics. Aqueous VCD studies are computationally and experimentally demanding. High concentrations of, for example, proteins and peptides are often necessary in order to allow for the use of short-path-length

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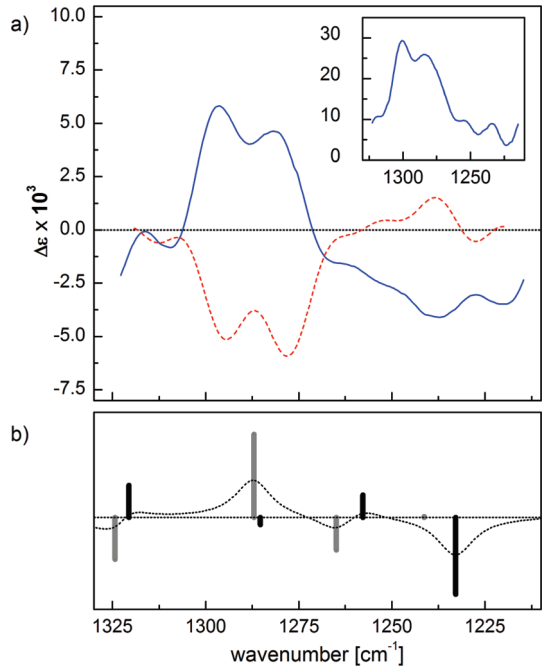

Figure 5. Spectra of aqueous 3 M solutions of **3**. (a) QCL-VCD spectra of **3a** (solid blue) and **3b** (dashed red) in a 100 μm CaF_2 cell. Spectra were background-corrected using a 1:1 (3 M) mixture of the two enantiomers. Inset: **3a** in water corrected with a pure water background. (b) Relative VCD intensities computed at the B3LYP/COSMO/6-31++G** level for two ring-puckering conformers of **3a** (1:1, gray and black vertical lines, arb. units).

sample cells. This can lead to aggregation problems.[12] Nevertheless, some aqueous VCD studies have been reported with ≤25 μm cells.[21−23]

In Figure 5 we show QCL-VCD spectra of the amino acid **3** between 1320 and 1220 cm^{-1} taken in a 100 μm sample cell. The molar absorptivity of water in this spectral range is ∼4.5 L mol^{-1} cm^{-1}.[24] The samples used for the spectra in Figure 5 had an optical density of up to 3.5, of which 2.5 was due to water. Nevertheless, satisfactory spectra of **3a** and **3b** were observed, albeit with a slight asymmetry. While a racemic mixture (3 M in water) was used for the background correction in Figure 5a, pure water was used in the spectrum shown in the inset. Here the strong absorbance of both the solvent and the solute complicated the background subtraction and was responsible for the observed offset.

For the assignment of the modes, we performed calculations using the B3LYP/COSMO/6-31++G** model, which was used previously to compute the Raman optical activity spectrum of **3** in aqueous solution.[25] Two dominant ring-puckering conformers were identified and found to be equally populated according to NMR data.[25] On the basis of the results of the DFT calculations (Figure 5b), the dominant positive band at 1295 cm^{-1} and the feature at 1286 cm^{-1} are most probably explained by methine bending vibrations of the two ring-puckering conformers, with the corresponding modes appearing with opposite sign in the VCD spectrum. A prominent positive band for the methine bending mode of **3a** had previously been observed at 1275 cm^{-1} in D_2O solution.[26] The red shift of ∼20 cm^{-1} relative to the results shown here is most likely caused by H/D exchange at the amino group.

In summary, VCD spectra of **1** and **2** were recorded using a tunable quantum-cascade laser and found to be in good agreement with conventional FT-VCD measurements. Although QCLs are limited in their spectral range, they offer a number of distinct advantages. They can be tuned with high resolution, which permits close-lying bands to be resolved and facilitates comparison with calculations. The significantly higher output power of a QCL permits measurements in longer-path-length cells. This should be particularly promising for chiroptical spectroscopies that scale linearly with path length, such as vibrational circular birefringence.[18,27] The spectral brightness of a QCL (>10^4 relative to conventional FT light sources) allows measurements in strongly absorbing solvents. We have presented spectra of aqueous solutions of **3** that were measured in 100 μm cells with optical densities of up to 3.5. These results demonstrate the viability of QCLs for VCD spectroscopy of biomolecules in spectral regions that are difficult to study because of significant absorption by water, such as the amide III region (1400−1100 cm^{-1}).

■ ASSOCIATED CONTENT

Supporting Information. Methods and complete ref 20 (as SI ref 3). This material is available free of charge via the Internet at http://pubs.acs.org.

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