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Absolute configuration of the antimalarial *erythro*-mefloquine – Vibrational Circular Dichroism and X-ray diffraction studies of mefloquine and its thiourea derivative

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The long-standing discussion of the absolute configuration of *erythro*-mefloquine is revisited, showcasing the strength of a combination of experimental and calculated Vibrational Circular Dichroism spectroscopy. The resulting assignment of one of the most important antimalarials is subsequently confirmed by Electronic Circular Dichroism spectroscopy and X-ray diffraction-based structure elucidation of thiourea derivatives, adding to the amount of evidence on the absolute configuration that had long remained a matter of debate.

Malaria remains one of the most devastating diseases in the world, yearly responsible for many hundreds of thousands of deaths as a consequence of nearly 200 million infections.¹ Moreover, about 40% of the world's population lives in areas where malaria is transmitted with several hundreds of millions who live in non-endemic areas visiting endemic areas.² Most of the 500000 deaths in 2013 concern children living in Africa where a child dies every minute from malaria. Although some elementary precautions help to reduce the risk, antimalarial medicines can also be used to prevent malaria or as treatment of those already infected. Moreover, the recent outbreak of Ebola in Africa strengthens the pressing necessity of malaria prevention as both deadly diseases share the same initial symptoms. Establishing that a person is a victim of ebola requires ruling out malaria.³

As no effective vaccines are still available, antimalarial drugs are the most important tools for combatting clinical illness and for

preventing disease transmission.² One of the most effective and affordable drugs used in both prevention and as standard medication in malaria chemoprophylaxis and treatment is *rac*-*erythro*-mefloquine hydrochloride, commercialized as Lariam.⁴ This molecule (see figure 1) is known to be chiral and both (+)-*erythro*-mefloquine and (-)-*erythro*-mefloquine have been investigated. Their stereochemistry not only influences the antimalarial activity^{5,6} but also results in different side effects for the respective enantiomers.^{7,8} While (+)-*erythro*-mefloquine was found to be more active against various malaria species,⁹ (-)-*erythro*-mefloquine shows longer plasma half-life than the (+)-enantiomer as well as higher affinity to adenosine receptors leading to various neuropsychiatric adverse effects.⁷ Despite the above mentioned impact of malaria on the lives of billions, the ensuing necessity for proper treatment medication and the detailed information on differences in activity and side effects of (+)- and (-)-*erythro*-mefloquine^{9,10}, the assignment of the absolute configuration (AC) has been quite problematic for a long time. The first assignment was based on electronic circular dichroism (ECD) data, and the authors tentatively assigned the AC of the (+)-enantiomer as (11*R*, 12*S*).¹¹ This assignment was later disproved by anomalous X-ray diffraction (XRD) of (-)-mefloquine hydrochloride for which the AC (11*R*, 12*S*) was derived and hence infers the (+)-enantiomer has the (11*S*, 12*R*) configuration.¹² It should be noted that Karle et al.¹³ used X-ray diffraction (XRD) to establish the AC already in 1991. Later, do Prado et al.¹⁴ and Pitaluga et al.¹⁵ used a combination of X-ray powder diffraction and infrared spectroscopy to unambiguously determine the crystal structure of the molecule. The resulting stereochemistry is in line with that from two different approaches using residual dipolar coupling enhanced NMR spectroscopy in combination with optical rotational dispersion,¹⁶ and by XRD analysis of Mosher amides.¹⁷ The experimental data reported in Refs ⁹ and ¹⁰ in turn contradict data derived from stereoselective total syntheses that, until very recently, confirmed the results of the ECD measurement.¹⁸⁻²¹ The most recent stereoselective approaches based on Pd-catalyzed enantioselective borylative alkene isomerization, domino-Sonogashira-6π-electrocyclization and Heck coupling followed by Sharpless

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dihydroxylation not only provided stereoselectively all four stereoisomers of mefloquine but also established the AC of (+)-*erythro*-mefloquine as (11*S*, 12*R*) and (-)-*erythro*-mefloquine as (11*R*, 12*S*).²²⁻²⁴ Zhou et al.²⁵ confirmed recently via a unique stereospecific system approach the correctness of the AC assigned by physico-chemical methods. His comment about the “unbelievable erroneous assignment by all previous five asymmetric syntheses” only underline the difficulties which can arise in such cases. To follow the concept of complementary methodologies to assign the AC of complex molecules is indeed of high scientific level.

Again in 2013, the AC was investigated using ECD,²⁶ establishing the AC of the two enantiomers as well as their biological activity, thus confirming the importance of the stereochemistry in the design of mefloquine analogues that have fewer adverse effects. Clearly, such ambiguity in determining the AC of a crystalline organic compound but that many physical as well as asymmetric synthetic methods is puzzling, in particular, for an important and widely used drug. Although the debate now seems to have been settled, we here set out to establish the AC of both *erythro*-enantiomers using Vibrational Circular Dichroism (VCD) to show, for the first time, the unique strength of this method for establishing the AC of the present molecules. To independently confirm the VCD based results, heavy atom labeled mefloquine derivatives were synthesized (see ESI). The applied enantiopure thiourea label allows for effective crystallization and AC determination using XRD.

Figure 1. Structures of (+)-*erythro*-mefloquine (1), (-)-*erythro*-mefloquine (2), (-)-*erythro*-*N*-(*N*-allylthiocarbamoyl) mefloquine (3) and (+)-*erythro*-*N*-(*N*-allylthiocarbamoyl) mefloquine (4).

As the previously repeatedly used ECD method, VCD is a chiroptical spectroscopy, relying on the difference in absorption between left and right handed circular polarized infrared (IR) radiation. As a consequence, VCD offers the structural specificity of solution-based IR spectroscopy combined with stereochemical sensitivity. In general, a VCD analysis requires (1) the measurement of both IR and VCD spectra of a sample in solution with unknown AC and (2) a quantum chemical simulation of these spectra for a specific AC. Due to the differential nature of VCD spectra, both positive and negative bands can occur. This means that for a specific wavenumber, if the (+) enantiomer preferentially absorbs the right circularly polarized light, the (-) enantiomer preferentially absorbs the left circularly polarized light. The resulting spectra, however, are not so easily linked to a specific AC, hence the need for quantum chemically computed spectra. These can be computed for any AC and by carefully establishing the agreement between every computed spectrum with an experimental one, the AC of the experimental sample(s) is obtained.^{27,28} As reported above, ECD has been used

previously but did not allow to conclusively establish the AC. One of the main advantages of VCD as opposed to ECD is that vibrational transitions are studied of which there are many more than the electronic transitions that underlie ECD.^{27,28} This therefore results in a more information rich set of data, reducing the risk of erroneous AC assignments. Moreover, the calculation of VCD spectra to compare experimental data is computationally less demanding than the calculation of ECD spectra, again showing an important advantage of the VCD method.²⁹

It is important to note that mefloquine and its thiourea-derivative each contain 2 chiral centers, and hence, for a complete VCD analysis, this would require the simulation of the VCD spectra of all diastereomers. Obviously, the elucidation of the molecular AC of the sample can then only be achieved when the computed VCD spectra of the diastereomers are sufficiently different. Fortunately, however, it is known that mefloquine in Lariam has the *erythro*-configuration and²² thus, the AC either corresponds to (11*S*, 12*R*) or (11*R*, 12*S*). One of the issues that needs to be dealt with when modelling VCD spectra is that the resulting spectra depend significantly on the molecular conformation. As the present molecules are expected to have a fairly complicated potential energy surface (PES), it is of prime importance that all significant molecular geometries are properly accounted for. This is done by conformational analysis in which all important (local) minima on the PES are identified. For all these, the IR and VCD spectra are computed leading to a molecular IR and VCD spectrum as an enthalpy based Boltzmann average over all conformations and their spectra. As exploring the PES with *ab initio* methods from the start is computationally too demanding, a first conformational analysis is performed at the molecular mechanics level. Three different software packages were used with the molecular mechanics force fields given between brackets: HyperChem³⁰ (mm-³¹), Conflex³²⁻³⁵ (MMFF94s³⁶) and Spartan³⁷ (MMFF94³⁸ and SYBYL³⁹) each with their specific algorithms for PES exploration. The resulting minimum energy structures were then gathered and redundancies eliminated. The remaining conformations were further optimized using the B3LYP functional and the 6-31G* basis set. Solvent-solute interactions were accounted for using a Polarizable Continuum solvent Model (PCM).⁴⁰ Geometry optimizations and calculations of the IR and VCD spectral data were performed using Gaussian09.⁴¹ The Boltzmann weighted IR and VCD spectra for mefloquine and *N*-(*N*-allylthiocarbamoyl) mefloquine were obtained taking into account 42 and 23 unique conformations, respectively.

The experimentally obtained VCD spectra for (+)-*erythro*-mefloquine and the calculated one for the (11*S*, 12*R*)-configuration are shown in Figure 2. Bands are labeled by performing the manual assignment of the bands in coherence with the IR spectra. Even though a few significant features including, amongst others, the regions near 1075, 1150 and 1450 cm⁻¹ are less reproduced, Figure 2 in general shows a good agreement between the spectra. Based on the visual agreement, the AC of (+)-*erythro*-mefloquine can clearly be assigned as (11*S*, 12*R*).

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Figure 2. Experimental VCD spectrum for (+)-erythro-mefloquine (upper panel) and the B3LYP/PCM/6-31G* computed spectrum for the (11*S*, 12*R*)-absolute configuration (lower panel). The spectrum has been frequency scaled with a factor $\sigma=0.978$ which yields a maximal similarity between the computed and experimental IR spectra.

The differences between experiment and theory in Figure 2 are believed to be due to the appearance of a strong intramolecular O–H⋯N hydrogen bond influencing both the vibrational frequencies and the IR and VCD intensities of the modes involved. To rationalize the effect of this interaction, additional calculations were performed using larger basis sets including aug-cc-pVTZ, using the B3PW91 functional, and using Grimme D3 empirical corrections for dispersion.⁴³ Correcting the B3LYP/6-31G* data for dispersion interactions slightly strengthens the intramolecular hydrogen bond, the differences in N⋯H interatomic distances being in the order of 0.03 Å, and red shifts the modes of interest by 15 to 20 cm^{−1}. Although the use of dispersion corrections significantly improves the agreement between experiment and theory in the region near 1150 cm^{−1}, it negatively affects the general agreement and the numerical descriptors used to quantify the level of agreement. The calculations using the larger basis sets and/or the B3PW91 functional confirmed the assignment reported above, but did not further improve agreement between the experiment and theory.

To avoid personal bias in manual assignments, it is recommended to establish the agreement between experimental and theoretical spectra using numerical methods. To this end we used the statistical validation approach by Vandebussche et al.⁴² where the reader is referred to this work for full technical details.

Figure 3. R_{VCD}^{exp} versus R_{VCD}^{calc} scatter diagram for the measured (+)-erythro-mefloquine spectrum and the computed (11*S*, 12*R*)-mefloquine spectrum. Blue datapoints represent random spectra. The red datapoint represents the quantum chemical spectrum which has a 100% similarity with respect to itself and a similarity of $R=61.59\%$ (VCD) with respect to the experiment. The black line is obtained by orthogonal regression. The area between the green lines contains 99% of the datapoints.

Figure 3 shows the resulting plot which is to be interpreted as follows. A very large number of spectra are computationally generated although only one (the red dot in Figure 3) originates from a quantum chemical and therefore physically meaningful calculation. The others (the blue dots) are quasi random spectra. In the ordinate the similarity between the computational spectra and the quantum chemical one is presented, where 100% means perfect similarity, -100% means perfect similarity with the quantum chemical spectrum of the enantiomer and 0% means complete dissimilarity. On the abscissa, the similarity between each computational spectrum and the experimental spectrum is represented. The key to a good assignment is that the quantum chemical spectrum (the red dot) must have a high similarity to the experiment (here 59%) and the blue ones must have a poorer similarity to the experimental spectrum and appear as an elongated point cloud around the bisector.

More specifically, the poorer the similarity with the quantum chemical spectrum, the poorer the agreement with the experiment. This is clearly the case here. The cloud of blue dots stretches as a fairly narrow elongated cloud along the bisector. As per reference 34, the assignment can be considered very trustworthy.

To assess the results derived above, experimental UV and ECD spectra for (+)-erythro-mefloquine were recorded in methanol and theoretical spectra for the (11*S*, 12*R*)-absolute configuration were calculated at the CAM-B3LYP/PCM/6-31G* level. The spectra obtained are summarized in Figure 4. It can be seen that in general the transitions in the UV and their corresponding features in ECD are predicted to appear at a wavelength slightly lower than those observed experimentally. The general agreement between experiment and theory, however, nicely confirms the assignment based on VCD.

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Figure 4. Experimental Ultraviolet and Electronic Circular Dichroism spectra for (+)-erythro-mefloquine (solid line in black) and the CAM-B3LYP/PCM/6-31G* computed spectra for the (11S, 12R)-absolute configuration (dashed line in red).

The experimental and calculated spectra for **3** obtained using the B3LYP/PCM/6-31G* method are shown in Figure 5. A manual AC assignment reveals very good agreement with the (11S, 12R)-configuration, major discrepancies between experiment and theory being almost exclusively related to differences in relative intensity. The numerical, unbiased analysis (see ESI) supports the claim that the analyzed compound has the (11S, 12R)-configuration. Only few random VCD spectra are able to outperform our quantum chemical spectrum at reproducing the experimental one. That some spectra can be slightly better is to be expected for a normal distribution and given the fact that no quantum chemical calculation can be expected to fully and exactly reproduce experiment due to inherent deficiencies in describing all intricate aspects of nature. The plot therefore reflects that assigning compound **3** to (11S, 12R) is very robust, which is consistent with results obtained with XRD.

Figure 5. Experimental VCD spectrum for thiourea derivative **3** (lower panel) and the computed spectrum for the (11S, 12R)-absolute configuration (upper panel).

Even though the AC of erythro-mefloquine seems to have converged^{14, 16, 22-26} and our VCD results support these, given the fact that previous works repeatedly contradicted each other concerning the AC of (+)-erythro-mefloquine we decided to again independently repeat single crystal XRD studies using a specifically prepared set of heavier atom containing molecules. Our above VCD based assignment agrees with previous XRD work^{12, 14} but the reliability of the XRD data is improved thanks to considering molecules **3** and **4** which were prepared from (+)-mefloquine **1** and (-)-mefloquine **2**, respectively. Their advantage is the presence of a heavier atom and thus better scatterer of the X-rays. Moreover, the X-ray based conformations may depend on the environment of the molecule although this does not alter the AC. By performing own XRD studies, we could control all steps in our work.

As mentioned above (Figure 1), the sign of specific rotation changed upon derivatization of (+)-mefloquine **1** and (-)-mefloquine **2** to **3** and **4**. The AC, obtained from XRD analysis (Figure 6), was determined as (11S, 12R) by two independent parameter tests, Flack = 0.015(13), Hooft = 0.001(12) for the (-)-thiourea derivative **3** and as (11R, 12S) by Flack = -0.001(4), Hooft = 0.001(4) for the (+)-thiourea derivative **4**. Given the reported change in sign of the optical rotation, we may conclude that our VCD based assignment for (+)-erythro-mefloquine is correct and the AC of this important molecule is again established using two independent techniques.

Figure 6. XRD structures of (-)-erythro-N-(N-allylthiocarbamoyl) mefloquine (**3**) (CCDC:1480524) with the absolute configuration assigned as (11S, 12R) and (+)-erythro-N-(N-allylthiocarbamoyl) mefloquine (**4**) (CCDC: 1480523) with absolute configuration (11R, 12S). Acetonitrile is a crystal solvent; mirror plane is assigned as m.

The determination of the absolute configuration of (+)-erythro-mefloquine and its enantiomer, as a racemic mixture constituting an important and highly effective antimalarial, has been used to showcase the added value of Vibrational Circular Dichroism (VCD) spectroscopy. The absolute configuration of both enantiomers was assigned on the basis of thorough statistical analysis of the VCD spectra and then independently confirmed by Electronic Circular Dichroism and single crystal X-ray diffraction of heavy atom labeled derivatives.

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