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Reference:

Kohout Michal, Vandenbussche Jelle, Roller Alexander, Tuma Jiri, Bogaerts Jonathan, Bultinck Patrick, Herrebout Wouter, Lindner Wolfgang.- Absolute configuration of the antimalarial erythro-mefloquine Vibrational Circular Dichroism and X-ray diffraction studies of mefloquine and its thiourea derivative

Journal of materials chemistry C : materials for optical and electronic devices - ISSN 2050-7534 - (2016), p. 1-5

Full text (Publishers DOI): http://dx.doi.org/doi:10.1039/C6RA19367F

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

M. Kohout*[a], J. Vandenbussche,[b] A. Roller[c], J. Tůma[a], J. Bogaerts[d], P. Bultinck[b], W. Herrebout*[d], and W. Lindner[e]

[a.] *Department of Organic Chemistry, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague, Czech Republic*
[b.] *Department of Inorganic and Physical Chemistry, Ghent University, Krijgslaan 281 (S3), 9000 Ghent, Belgium*
[c.] *Institute of Inorganic Chemistry, University of Vienna, Währinger Strasse 42, 1090 Vienna, Austria*
[d.] *Department of Chemistry, University of Antwerp, Groenenborgerlaan 171 (G.V.023), 2020 Antwerp, Belgium*
[e.] *Institute of Analytical Chemistry, University of Vienna, Währinger Strasse 38, 1090 Vienna, Austria*

Electronic supplementary information

This Electronic supplementary information provides details on enantioseparation of (*rac*)-*erythro*-mefloquine, the subsequent synthesis of a heavy atom labelled mefloquine derivatives and the crystallographic analysis of the labelled products **3** and **4**. Details on the VCD measurements and the methods that were used to compare the measured and calculated spectra are provided as well.

1. Chromatographic separation of *erythro*-mefloquine enantiomers

The separation of (*rac*)-*erythro*-mefloquine was performed on an in-house made, zwitterion ion exchange column, whose selector was of Chiralpak ZWIX (+) type. (+)-Mefloquine was harvested as the first eluting enantiomer and (-)-mefloquine as the second eluting enantiomer (Figure ESI-1).

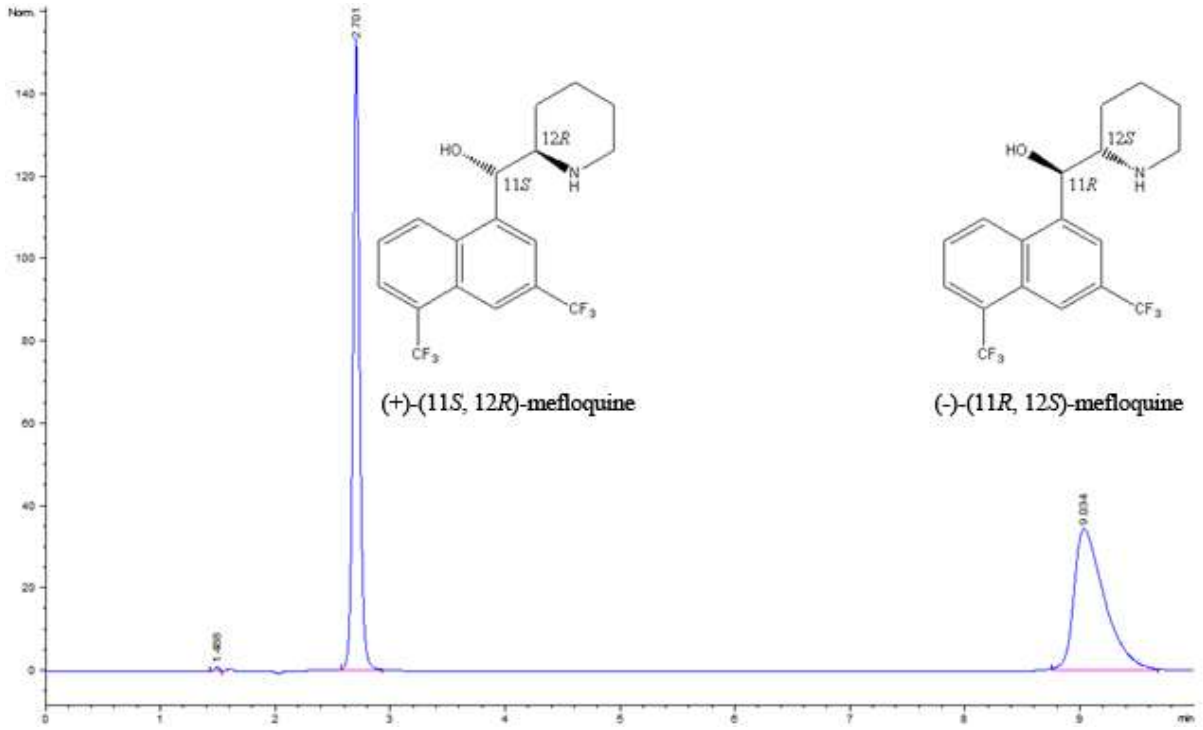

Figure ESI-1. Separation of (*rac*)-*erythro*-mefloquine using a ZWIX-type column (150×4 mm ID, 5 μm) with acetonitrile/methanol/water 49/49/2 as a mobile phase containing 50 mM formic acid and 25 mM ammonia at 20 °C, flow 1 ml/min, temperature 20°C.

2. Synthesis and characterization of heavy atom labelled compounds

Structures of products were confirmed by ^{1}H and ^{13}C NMR spectroscopy (Agilent 400-MR DDR2 spectrometer), deuterochloroform was used as a solvent and the signals of the solvent served as an internal standard, *J* values being given in Hz. The mass spectrometry data were obtained with LTQ Orbitrap Velos (Thermo Scientific) under ESI+ ionization.

*(11S, 12R)-N-(N-Allylthiocarbamoyl) mefloquine (**3**)*

To a solution of (+)-mefloquine (0.22 g, 0.58 mmol) in dry THF (30 ml) in the inert argon atmosphere, allyl isothiocyanate (65 μl, 0.66 mmol) was added and the reaction mixture was stirred over night. The solvent was removed and the crude product was purified by column chromatography (eluent hexane/ethyl acetate, 1:1). It was obtained 0.22 g (79%) of a white solid, m.p. = 135-143 °C, decomposition. $a_D^{20} = -22.2^\circ$ (21 °C, methanol). ^{1}H NMR (δ, 400 MHz, $CDCl_3$): 1.17 (m, 1 H, CH_2), 1.36 (m, 1 H, CH_2), 1.59 (m, 1 H, CH_2), 1.69 (m, 1 H, CH_2), 1.74-1.89 (m, 2×1 H, different CH_2), 2.74 (bs,

1 H, OH), 3.74 (m, 2 H, CH_2), 4.39 (m, 2 H, $\underline{CH_2}CH=CH_2$), 5.22-5.32 (m, 2 H, $CH_2CH=\underline{CH_2}$), 5.49 (m, 1H, H-12), 5.69 (t, 1 H, *J* = 3.8 Hz, urea H), 5.94-6.04 (m, 1 H, $\underline{CH}=CH_2$), 6.30 (d, 1 H, *J* = 3.9 Hz, H-11), 7.66 (t, 1 H, *J* = 7.8 Hz, H-6), 8.12 (d, 1 H, *J* = 7.1 Hz, H-5), 8.16 (s, 1 H, H-3), 9.20 (d, 1 H, *J* = 8.6 Hz, H-7). ^{13}C NMR (δ, 100.6 MHz, $CDCl_3$): 182.5 (C=S), 151.2 (Cq), 148.1 (Cq), 143.5 (Cq), 133.8 ($\underline{CH}=CH_2$), 129.3 (CH), 129.2 (CH), 128.7 (Cq), 127.2 (CH), 126.5 (Cq), 124.4 (CF_3), 121.4 (CF_3), 117.5 ($\underline{CH_2}=CH$), 115.9 (CH), 72.4 (CH), 60.1 (CH), 48.7 (CH_2), 43.4 (CH_2), 23.1 (CH_2), 22.4 (CH_2), 18.3 (CH_2). HRMS (ESI+) for $C_{21}H_{21}F_6N_3OS$ calculated 477.1310; found [$M+H^+$] 478.1384.

Analogously, modification of (-)-mefloquine provided (11*R*, 12*S*)-*N-(N-allylthiocarbamoyl) mefloquine* (**4**) in 86% yield, m.p. 136-144 °C, decomposition. $a_D^{20} = +25.5^{\circ}$ (20 °C, methanol). ^{1}H NMR (δ, 400 MHz, $CDCl_3$): 1.17 (m, 1 H, CH_2), 1.35 (m, 1 H, CH_2), 1.59 (m, 1 H, CH_2), 1.70 (m, 1 H, CH_2), 1.74-1.88 (m, 2×1 H, different CH_2), 2.91 (bs, 1 H, OH), 3.74 (m, 2 H, CH_2), 4.39 (m, 2 H, $\underline{CH_2}CH=CH_2$), 5.22-5.32 (m, 2 H, $CH_2CH=\underline{CH_2}$), 5.49 (m, 1H, H-12), 5.69 (t, 1 H, *J* = 3.8 Hz, urea H), 5.92-6.03 (m, 1 H, $\underline{CH}=CH_2$), 6.30 (d, 1 H, *J* = 3.9 Hz, H-11), 7.66 (t, 1 H, *J* = 7.8 Hz, H-6), 8.12 (d, 1 H, *J* = 7.1 Hz, H-5), 8.16 (s, 1 H, H-3), 9.20 (d, 1 H, *J* = 8.6 Hz, H-7). ^{13}C NMR (δ, 100.6 MHz, $CDCl_3$): 182.5 (C=S), 151.2 (Cq), 148.1 (Cq), 143.5 (Cq), 133.8 ($\underline{CH}=CH_2$), 129.3 (CH), 129.1 (CH), 128.7 (Cq), 127.2 (CH), 126.5 (Cq), 124.4 (CF_3), 121.4 (CF_3), 117.5 ($\underline{CH_2}=CH$), 115.9 (CH), 72.4 (CH), 60.1 (CH), 48.7 (CH_2), 43.4 (CH_2), 23.1 (CH_2), 22.4 (CH_2), 18.3 (CH_2). HRMS(ESI+) for $C_{21}H_{21}F_6N_3OS$ calculated 477.1310; found [$M+H^+$] 478.1383.

It is worth noting that the sign of optical rotation of the mefloquine enantiomers changed upon derivatization: the specific rotations for (+)-*erythro*-mefloquine **1** and (-)-*erythro*-mefloquine **2** were $a_D^{20} = +33.4^{\circ}$ and $a_D^{20} = -34.1^{\circ}$, respectively. The values for the corresponding thiourea compounds (-)-enantiomer **3** and (+)-enantiomer **4** were found to be $a_D^{20} = -22.2^{\circ}$ and $a_D^{20} = +25.5^{\circ}$, respectively.

3. VCD measurements and spectra analysis

IR and VCD spectra were recorded on a BioTools dual-PEM Chiral*IR*-2X spectrometer. The PEMs were optimized for 1400 cm^{-1} and a resolution of 4 cm^{-1} was used throughout. The path length of the cell equipped with BaF_2 windows is 100 µm. (+)-*erythro*-Mefloquine and *N*-(*N*-allylthiocarbamoyl) mefloquine spectra were recorded as solutions of ca. 4.5 and 5.2 mg in 0.1 mL $CDCl_3$, respectively. For the first, a baseline correction was accomplished using the VCD spectrum of the solvent, while for the second, both enantiomers were available and measured separately allowing for a virtual racemate subtraction.

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 can be considered as a technique offering 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. Since enantiomers have identical VCD spectra apart from an opposite sign, the elucidation of the unknown AC of a compound comes down to verifying for which AC the simulated VCD bands agree in sign with the measured ones. Moreover, IR and VCD spectral bands

occur at the same frequencies and, therefore, both are used in coherence to ensure that patterns are being matched correctly between experiment and calculation.

Both routines evaluate the agreement between the measured and computed VCD spectra using a normalized similarity measure. In the first routine, this is the Carbó-similarity index:

$$R_{\mathrm{calc,exp}} = \frac{I_{\mathrm{calc,exp}}}{\sqrt{I_{\mathrm{calc,calc}} \cdot I_{\mathrm{exp,exp}}}}$$

, where the overlap integral is defined as:

$$I_{\mathrm{calc,exp}} = \int_{\nu_1}^{\nu_2} s_{\mathrm{calc}}(\nu) . s_{\mathrm{exp}}(\nu) d\nu$$

and likewise for $I_{\mathrm{calc,calc}}$ and $I_{\mathrm{exp,exp}}$. $s_{\mathrm{calc}}(\nu)$ represents the calculated spectrum and $s_{\mathrm{exp}}(\nu)$, the experimental one. These spectra were triangularly weighted (L=15cm^{-1}) to correct for residual bad alignment after frequency scaling the calculated spectrum. We refer to reference[1] and the reference therein for further details. The similarity is evaluated over a user-defined wavenumber range $[\nu_1, \nu_2]$. The similarity between IR spectra will be labeled with an 'IR' superscript; in case of VCD spectra the 'VCD' superscript is added. $R^{\mathrm{VCD}}_{\mathrm{calc,exp}}$ lies within the interval [−1, +1]. A similarity of +1 means that the spectra are identical, a similarity of -1 means that the spectra are identical, though with an opposite sign. Next, the statistical approach by Vandenbussche et al. relies on the comparison of the $R^{\mathrm{VCD}}_{\mathrm{calc,exp}}$-value with normally distributed $R^{\mathrm{VCD}}_{\mathrm{x,exp}}$-data, where x represents a collection of 25000 randomly generated VCD spectra. From this distribution the significance is derived as the probability that a random spectrum yields a worse (Carbó-based) agreement with the experiment than the simulated VCD spectrum. This probability is referred to as the robustness P of the AC assignment.

The second – CompareVOA – routine employs an experimental database of similarity values with independently validated correct and incorrect VCD-based AC assignments. The obtained similarity is projected into the database, yielding a weighted Euclidean distance measure that scales between 0 and 100%. This value has been termed the Confidence Level (CL) of the AC assignment and reflects the degree in which successful VCD based AC-assignments have been performed in the past for the same similarity as the one obtained for the current analysis.

The neighborhood similarity indices, the CL and the robustness obtained using the approaches described above are summarized in Table ESI-1. The second routine, CompareVOA, yields a CL of 99% and shows that past VCD based AC assignments in the database for the same level of similarity were found to be correct. The similarity measure $R^{\mathrm{VCD}}_{\mathrm{calc,exp}}$ derived using the former approach is high (59.43%). The robustness P equals 99.58%.

Table ESI-1

Neighbourhood similarities, Confidence Level (CL) and Robustness (P) for the assignment of the absolute configuration of (+)-*erythro*-mefloquine and *N*-(*N*-allylthiocarbamoyl) mefloquine. The numerical data were obtained using a 1650-1000 cm^{-1} spectral range.

Compound	AC	$R^{\mathrm{IR}}_{\mathrm{calc,exp}}$ (%)	$R^{\mathrm{VCD}}_{\mathrm{calc,exp}}$ (%)	P (%)	CL (%)
(+)-*erythro*-mefloquine	(11*S*, 12*R*)	85.68	60.35	99.89	99
(-)-*N*-(*N*-allylthiocarbamoyl) mefloquine	(11*S*, 12*R*)	92.93	44.14	99.03	79

The robustness of the obtained agreement for compound **3** is also visualized using randomization plots in Figure ESI-2. On the horizontal axis for each plot, the similarity is given between a random spectrum x and the theoretically inferred one: $R^{\mathrm{VCD}}_{\mathrm{x,calc}}$. On the vertical axis, on the other hand, the similarity is plotted between the same random spectrum x and the experimental VCD spectrum ($R^{\mathrm{VCD}}_{\mathrm{x,exp}}$). The more robust the VCD based AC assignment, the less random spectra (blue dots) will outperform the agreement between the calculated and experimental spectra (red diamond). Also, the more the data is distributed on a straight diagonal line, the more robust the assignment becomes. Based on this plot, it is clear that the AC assignment matching (11*S*,12*R*) is very robust.

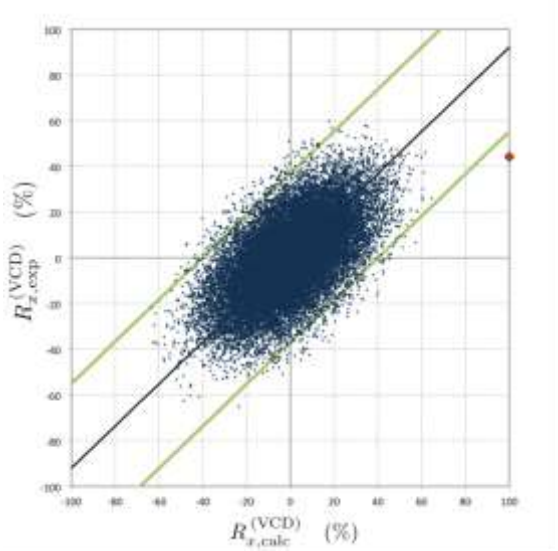

Figure ESI-2. $\boldsymbol{R^{\mathrm{VCD}}_{\mathrm{x,exp}}}$ versus $\boldsymbol{R^{\mathrm{VCD}}_{\mathrm{x,calc}}}$ scatter diagram for the measured (-)-*erythro*-*N*-(*N*-allylthiocarbamoyl) mefloquine (**3**) derivative spectrum and the computed one for the (11S,12R) absolute configuration. Blue datapoints represent random spectra. The red datapoint represents the calculated spectrum which has a 100% similarity with respect to itself and a similarity of R (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.

4. ECD measurements and spectra analysis

UV and ECD spectra were recorded on Jasco J-810 (Jasco, Japan) spectropolarimeter in methanol as a solvent. Theoretical spectra were obtained using Time Dependent Density Functional Theory (TD-DFT) at the CAM-B3LYP/6-31G* level. Spectra were obtained for the different conformations considered and a Boltzmann weighted spectrum was calculated afterwards.

5. XRD measurements

The X-ray intensity data were measured on a Bruker D8-Venture equipped with multilayer monochromator, Cu K/a INCOATEC micro focus sealed tube and KryoflexII cooling device. The samples were uploaded to the CCDC. The CCDC-Codes are listed in Table ESI-2. The structures were solved by direct methods and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were inserted in calculated positions and refined with a riding model respectively as rotating systems or in case of possible short interactions refined under dfix restraints. The following software was used: Frame integration, *Bruker SAINT software package*[2] using a narrow-frame algorithm, Absorption correction, *SADABS*[3] , structure solution, *SHELXS-97*[4], refinement, *SHELXL-2013*[5], *OLEX2*[6], *SHELXLE*[7], molecular diagrams, *OLEX2*[6]. Experimental data can be found in Table ESI-2. Crystal data, data collection parameters, and structure refinement details are given in Tables ESI-3-6. Molecular Structures in "Ortep View" are presented in Figures ESI-3-4.
Both structures detect a short interaction B-Alerts at identical positions (PLAT414_ALERT_2_B Short Intra D-H..H-X). This interaction seems to be enforced by geometry.

Table ESI-2. Experimental parameters and CCDC-Codes.

Sample	Temp	Detector Distance	Time/ Frame	#Frames	Frame width	CCDC
	[K]	[mm]	[s]		[°]	
3	100 (2)	45	64	2691	0.4	1480524
4	100 (2)	45	24	2724	0.4	1480523

In the following part, the structures and crystallographic data for the thiourea derivatives of (+)-*erythro*-mefloquine and (-)-*erythro*-mefloquine are presented.

(11*S*, 12*R*)-(-)-*erythro*-*N*-(*N*-allylthiocarbamoyl) mefloquine (3)

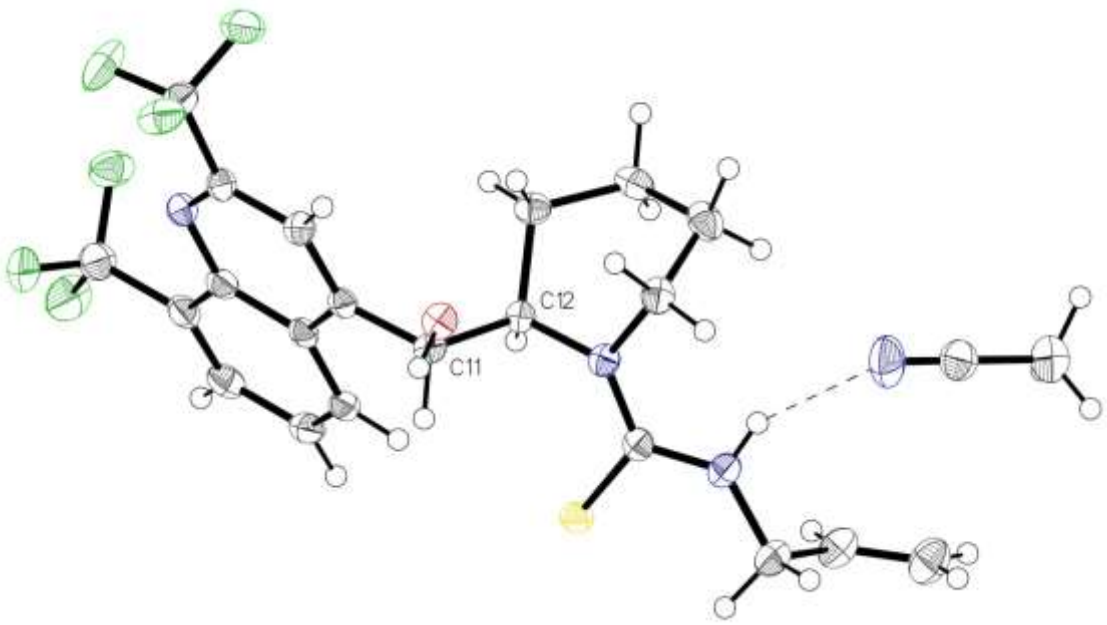

Figure ESI-3. Asymmetric Unit of **3**, drawn with 50% displacement ellipsoids. According to the resulting chiral spacegroup $P2_12_12_1$, the absolute configuration can be determined as (11*S*,12*R*) (Flack = 0.015(13), Hooft = 0.001(12)).

Table ESI-3. Sample and crystal data of **3**.

Chemical formula	$C_{23}H_{24}F_6N_4OS$	**Crystal system**	orthorhombic	
Formula weight [g/mol]	518.52	**Space group**	$P2_12_12_1$	
Temperature [K]	100	**Z**	4	
Measurement method	\Φ and \ω scans	**Volume [Å^3]**	2397.94(9)	
Radiation (Wavelength [Å])	CuKα (λ = 1.54178)	**Unit cell dimensions [Å] and [°]**	5.76580(10)	90
Crystal size / [mm^3]	0.099 × 0.036 × 0.019		15.8201(4)	90
Crystal habit	clear colorless stick		26.2887(6)	90
Density (calculated) / [g/cm^3]	1.436	**Absorption coefficient / [mm^{-1}]**	1.841	
Abs. correction Tmin	0.839	**Abs. correction Tmax**	0.966	
Abs. correction type	numerical	**F(000) [e$^-$]**	1072	

Table ESI-4. Data collection and structure refinement of **3**.

Index ranges	$-6 \le h \le 6$, $-18 \le k \le 19$, $-31 \le l \le 31$	**Theta range for data collection [°]**	6.52 to 136.478	
Reflections number	14932	**Data / restraints / parameters**	4356/4/333	
Refinement method	Least squares	**Final R indices**	all data	R1 = 0.0414, wR2 = 0.0836
Function minimized	$\Sigma\, w(F_o^2 - F_c^2)^2$		$I>2\sigma(I)$	R1 = 0.0356, wR2 = 0.0809
Goodness-of-fit on F^2	1.04	**Weighting scheme**	$w=1/[\sigma^2(F_o^2)+(0.0268P)^2+1.0944P]$	
Largest diff. peak and hole [e Å^{-3}]	0.27/-0.18		where $P=(F_o^2+2F_c^2)/3$	

(11*R*,12*S*)-(+)-*erythro*-*N*-(*N*-allylthiocarbamoyl) mefloquine (4)

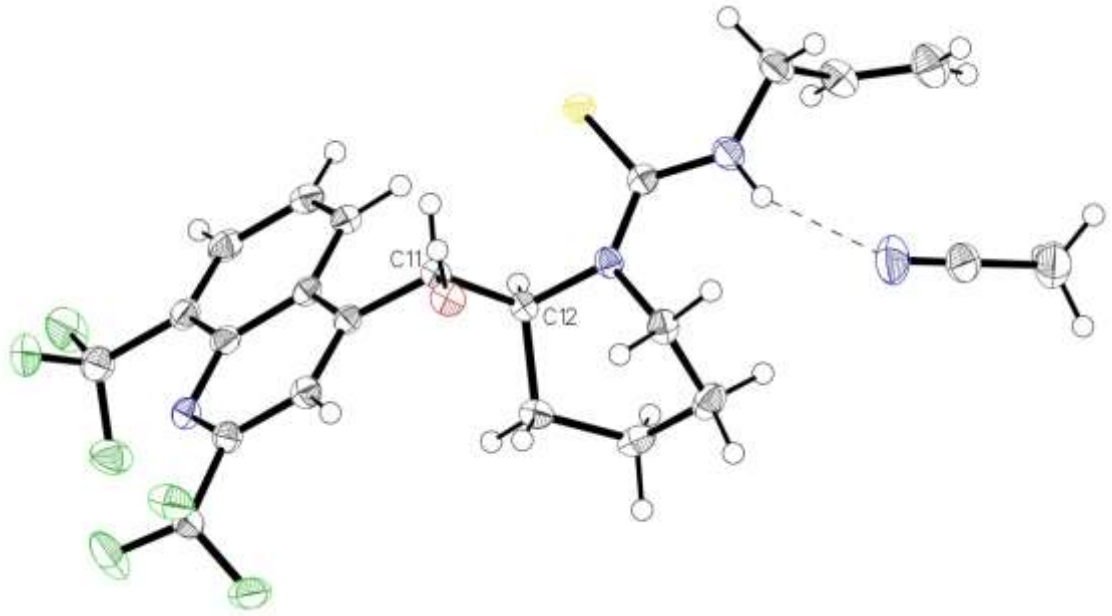

Figure ESI-4. Asymmetric Unit of **4**, drawn with 50% displacement ellipsoids. According to the resulting chiral spacegroup $P2_12_12_1$, the absolute configuration can be determined as (11*R*,12*S*) (Flack = -0.001(4), Hooft = 0.001(4)).

Table ESI-5. Sample and crystal data of **4**.

Chemical formula	$C_{23}H_{24}F_6N_4OS$	**Crystal system**	orthorhombic	
Formula weight [g/mol]	518.52	**Space group**	$P2_12_12_1$	
Temperature [K]	100	**Z**	4	
Measurement method	\Φ and \ω scans	**Volume [Å^3]**	2395.35(9)	
Radiation (Wavelength [Å])	CuKα (λ = 1.54178)	**Unit cell dimensions [Å] and [°]**	5.75560(10)	90
Crystal size / [mm^3]	0.227 × 0.051 × 0.019		15.8271(4)	90
Crystal habit	clear colorless plate		26.2952(6)	90
Density (calculated) / [g/cm^3]	1.438	**Absorption coefficient / [mm^{-1}]**	1.843	
Abs. correction Tmin	0.680	**Abs. correction Tmax**	0.966	
Abs. correction type	multi-scan	**F(000) [e$^-$]**	1072	

Table ESI-6. Data collection and structure refinement of **4**.

Index ranges	$-6 \le h \le 6$, $-18 \le k \le 19$, $-31 \le l \le 31$	**Theta range for data collection [°]**	6.518 to 136.412	
Reflections number	15524	**Data / restraints / parameters**	4381/4/333	
Refinement method	Least squares	**Final R indices**	all data	R1 = 0.0273, wR2 = 0.0700
Function minimized	$\Sigma\, w(F_o^2 - F_c^2)^2$		I>2σ(I)	R1 = 0.0265, wR2 = 0.0696
Goodness-of-fit on F^2	1.064	**Weighting scheme**	$w=1/[\sigma^2(F_o^2)+(0.0435P)^2+0.4801P]$	
Largest diff. peak and hole [e Å^{-3}]	0.27/-0.15		where $P=(F_o^2+2F_c^2)/3$	

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