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Crystal Structure Analysis

The Absolute Configuration of (+)- and (−)-*erythro*-Mefloquine**

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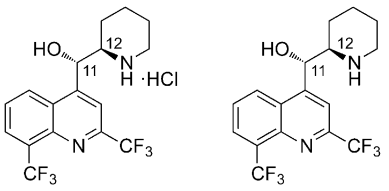
The antimalarial agent *rac-erythro*-mefloquine hydrochloride, established in the 1970s and marketed as Lariam by Roche, is one of the standard medications for malaria chemoprophylaxis and treatment.[1] One major drawback of this drug is the occurrence of dose-related neuropsychiatric adverse effects.[1,2] Indeed, the enantiomer (−)-*erythro*-mefloquine is known to block adenosine receptors in CNS and therefore believed to be responsible for the neuropsychiatric symptoms, while (+)-*erythro*-mefloquine does not.[3] Application of (+)-*erythro*-mefloquine rather than the racemate could therefore result in a better risk to benefit evaluation.

Owing to the different pharmaceutical activity of the *erythro*-mefloquine enantiomers, a number of researchers have attempted to determine the absolute configuration of (+)-*erythro*-mefloquine **1** (Scheme 1) in the past. However,



Scheme 1. The antimalarial agent (+)-*erythro*-mefloquine HCl **1** and its free base **2**.

the results were ambiguous and caused controversy. The first paper by Carroll in 1974 claimed the absolute configuration of (+)-*erythro*-mefloquine as (11*R*,12*S*) based on circular dichroism (CD) and empirical rules.[4] A crystal-structure analysis of (−)-*erythro*-mefloquine HCl using the anomalous signal from single-crystal X-ray diffraction determined the absolute configuration as (11*R*,12*S*), contradicting the aforementioned investigation.[5] A first total synthesis of (+)-*erythro*-mefloquine was based on a L-proline-catalyzed asymmetric direct aldol reaction[6] and supported the CD assignment of Carrol.[4] This version of the aldol reaction is accepted to be reliable but it was not used before for a synthesis similar to that of (+)-*erythro*-mefloquine. In our endeavor to determine the absolute configuration of (+)-*erythro*-mefloquine, we applied residual dipolar coupling (RDC)-enhanced NMR spectroscopy in combination with optical rotatory dispersion (ORD) and circular dichroism (CD) spectroscopy,[7] and confirmed the result of the crystal structure.[5] The approach had been successfully applied in the past for several other flexible natural products[8] and is a standard technique for rigid natural products. However, another total synthesis[9] by the Coltart group confirmed the absolute configuration determined by CD and the previous total synthesis,[4,6] contradicting the X-ray[5] and NMR[7] results. They utilized an asymmetric Darzens reaction, yielding an enantiomerically pure intermediate with an absolute configuration confirmed by crystal-structure analysis. After eight further steps, (+)-*erythro*-mefloquine·HCl was obtained and the absolute configuration was determined as (11*R*,12*S*). A third total synthesis using an enantioselective transferhydrogenation to reduce the ketone presented (+)-*erythro*-mefloquine with (11*R*,12*S*) as the absolute configuration, relying on the assignment of the previous syntheses without checking the optical rotation of the product.[10]

As it is puzzling for a simple albeit important antimalarial agent as mefloquine to have the assignment of (+)-(11*R*,12*S*) four times and of (−)-(11*R*,12*S*) twice, we will show in the present study that the absolute configuration of (+)-*erythro*-mefloquine is indeed (11*S*,12*R*) and of (−)-*erythro*-mefloquine (11*R*,12*S*). Our analysis is based on crystal-structure determinations of the Mosher amides of both enantiomers of mefloquine together with HPLC separations of the diastereomeric Mosher esters to verify the correct starting materials. We indeed obtained the two enantiomeric Mosher amides and determined both crystal structures to ensure the reliability of our results.

(+)-*Erythro*-mefloquine hydrochloride **1** as well as *rac-erythro*-mefloquine hydrochloride (*rac*-**1**) are commercially available. For the (+)-*erythro*-enantiomer we obtained very similar $[\alpha]_D$ values as reported previously.[11] For comparison with reported HPLC data, the hydrochloride was converted into the free base.[12] The synthesis of the Mosher amide derivatives was achieved with (+)-*erythro*-mefloquine as well as the racemate using five equivalents of Hünig's base (iPr$_2$NEt) and (*S*)-MTPA-Cl as well as (*R*)-MTPA-Cl, respectively.[13] With this procedure no difference in reactivity between the hydrochloride and the free base was observed.

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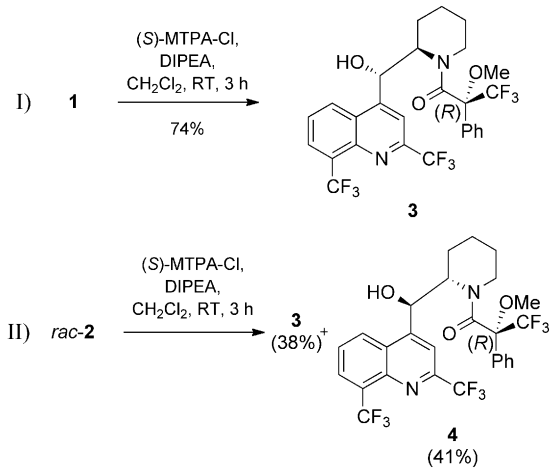
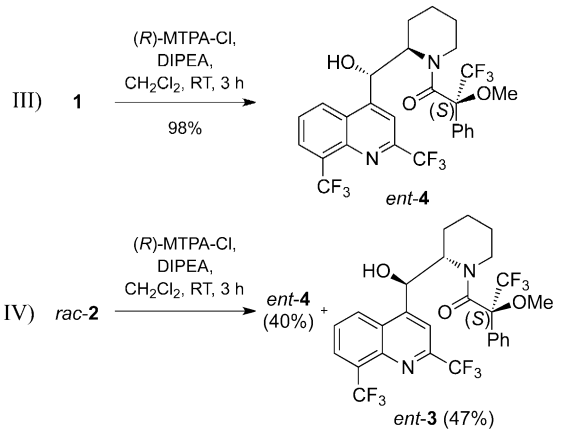
(+)-(11*S*,12*R*)-*Erythro*-mefloquine-(*R*)-Mosher amide (**3**) was obtained in 74% yield by amidation of (+)-*erythro* mefloquine **1** with (*S*)-MTPA-Cl in reaction I. Reaction II was the amidation of the racemic free base *rac*-**2** yielding **3** as well as (+)-(11*R*,12*S*)-*erythro*-mefloquine-(*R*)-Mosher amide (**4**), which could be separated by column chromatography (Scheme 2) and stereochemically assigned by comparison of the R_f-values with **3** obtained from reaction I.



Scheme 2. Synthesis of (+)-(11*S*,12*R*)-*erythro*-mefloquine-(*R*)-Mosher amide (**3**) and (+)-(11*R*,12*S*)-*erythro*-mefloquine-(*R*)-Mosher amide (**4**).

In reaction III we synthesized (−)-(11*S*,12*R*)-*erythro*-mefloquine-(*S*)-Mosher amide (*ent*-**4**) in 98% yield, and in reaction IV we amidated the racemic free base *rac*-**2** yielding (−)-(11*R*,12*S*)-*erythro*-mefloquine-(*S*)-Mosher amide (*ent*-**3**) in 47% and *ent*-**4** in 40% yield (Scheme 3). The identification of *ent*-**3** as enantiomer to **3** was verified through equal retention times by TLC, whereas the retention time of *ent*-**4** was indeed different but identical to the product of reaction III.

The structure, including the absolute configuration of *ent*-**3**, was determined by single-crystal X-ray diffraction[14] (Figure 1, bottom). The conventional IAM (independent



Scheme 3. Synthesis of (−)-(11*S*,12*R*)-*erythro*-mefloquine-(*S*)-Mosher amide (*ent*-**4**) and (−)-(11*R*,12*S*)-*erythro*-mefloquine-(*S*)-Mosher amide (*ent*-**3**).

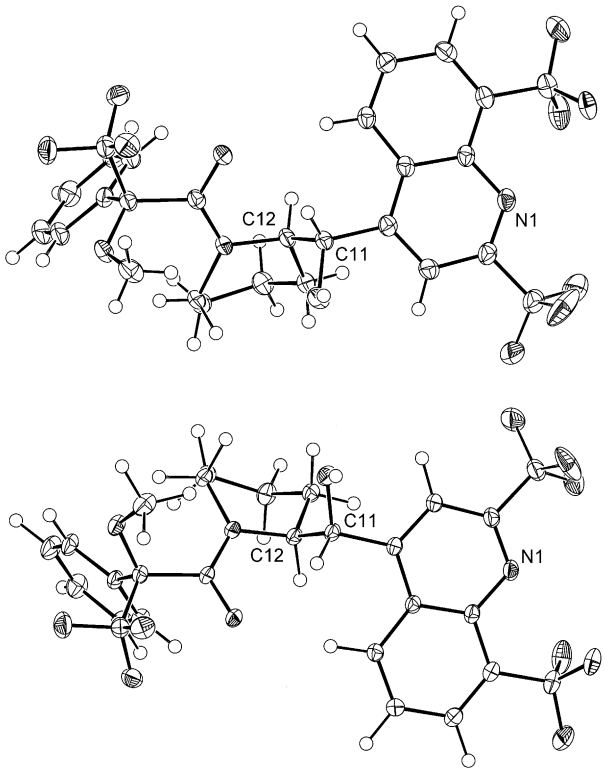


Figure 1. Crystal structures of the enantiomeric pair: (+)-(11*S*,12*R*)-*erythro*-mefloquine-(*R*)-Mosher amide (**3**; top) and (−)-(11*R*,12*S*)-*erythro*-mefloquine-(*S*)-Mosher amide (*ent*-**3**; bottom). Ellipsoids set at 50% probability.

atom model) refinement of the crystal structure was already sufficient to allow an assignment of the absolute configuration based on the anomalous signal of the fluorine atoms. 56% of all Friedel pairs in *ent*-**3** were measured.

To further increase the confidence of our absolute structure determination, an invariom refinement,[15] which better describes the bonding electrons accounting for the asphericity of the electron density distribution, was performed. A locally modified version of the least-squares refinement program XD was used for that purpose.[16]

This procedure was used because invariom refinement reduces the standard uncertainty of all refined parameters in proportion to the improvement of the figures of merit. This also holds for the Flack parameter x and its standard deviation[17] u that measure the reliability of the determination of the absolute configuration. Indeed, after aspherical-atom modeling with scattering factors from the invariom database the Flack parameter for *ent*-**3** changed from $x = -0.02 \pm 0.06$ (IAM) to $x = -0.03 \pm 0.05$ (invariom). According to Flack and Bernardinelli,[18] u should be below 0.05 and x should be close to zero within $2u$ to obtain the absolute configuration with confidence. This is the case for *ent*-**3**. The enantiomeric compound **3** has also been measured and its configuration and conformation in the crystal is enantiomeric (Figure 1, top). The Flack parameter for **3** is $x = 0.00 \pm 0.07$ (IAM) and also verifies the absolute configuration.[19] It should be mentioned that the known absolute configuration of the Mosher acid chloride was reproduced in both crystal structures.

In general, total synthesis is a reliable applied practice for the determination of the absolute configuration of organic molecules. However, in case of mefloquine the absolute configuration of this rather simple molecule could not be determined correctly by total synthesis. To be safe with this statement it is necessary to quantify the reliability of the described results. For crystal structure analyses there are two major pitfalls that can cause a wrong determination. On the one hand it is possible that a crystal of the minor diastereomer was used for X-ray analysis. In our case this is extremely improbable becuase we carried out independent experiments on both enantiomers. (+)-(11*S*,12*R*)-*Erythro*-mefloquine-(*R*)-Mosher amide (**3**) was obtained in reaction I with d.r. > 99:1 (the diastereomer was not detectable on HPLC) and *ent*-**3** was obtained with d.r. = 98:2 in reaction IV. Furthermore, for the respective diastereomers, **4** and *ent*-**4** no single crystal could be obtained. As it is always difficult to obtain a pure crystal of the minor component from a mixture; the chance that in both cases the "wrong" crystal was used for X-ray analysis is much lower than the calculable 0.2‰ (2/98·1/99). On the other hand, the crystal structure analysis itself may be erroneous. However, prior to and after Invariom refinement the Flack parameter and its standard deviation as well as the excellent figures of merit clearly indicate the high reliability of our X-ray analyses. Therefore, this second possible pitfall can also be excluded beyond reasonable doubt.

In conclusion, we have determined the absolute configuration of (+)-*erythro*-mefloquine hydrochloride **1** as (11*S*,12*R*) and confirmed one of the previous assignments[5] and our previous NMR analysis.[7] The three assignments from syntheses[6,9,10] could not be confirmed. For example, in one case,[9] a possible epimerization of the product of the Darzens reaction was not investigated. Likewise in another,[9] only the absolute configuration of an intermediate was determined. We are confident that the absolute configuration of **1** has now been unambiguously determined, closing a topic that was controversial for 40 years. This allows to work out enantioselective syntheses of (+)-*erythro*-mefloquine that might provide improved anti-malaria medication.

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[1] P. Schlagenhauf, M. Adamcova, L. Regep, M. T. Schaerer, H.-G. Rhein, *Malar. J.* **2010**, *9*, 357.
[2] W. R. Taylor, N. J. White, *Drug. Saf.* **2004**, *27*, 25 – 61.
[3] a) C. Mullié, A. Jonet, C. Desgrouas, N. Taudon, P. Sonnet, *Malar. J.* **2012**, *11*, 65; b) J. Shepherd, International patent WO98/39003, **1998**.
[4] F. I. Carroll, J. T. Blackwell, *J. Med. Chem.* **1974**, *17*, 210 – 219.
[5] J. M. Karle, I. L. Karle, *Antimicrob. Agents Chemother.* **2002**, *46*, 1529 – 1534.
[6] Z.-X. Xie, L.-Z. Zhang, X.-J. Ren, S.-Y. Tang, Y. Li, *Chin. J. Chem.* **2008**, *26*, 1272 – 1276.
[7] M. Schmidt, H. Sun, P. Rogne, G. K. E. Scriba, C. Griesinger, L. T. Kuhn, U. M. Reinscheid, *J. Am. Chem. Soc.* **2012**, *134*, 3080 – 3083.
[8] a) U. M. Reinscheid, M. Köck, C. Cychon, V. Schmidts, C. M. Thiele, C. Griesinger, *Eur. J. Org. Chem.* **2010**, 6900 – 6903; b) H. Sun, E. J. d'Auvergne, U. M. Reinscheid, L. Carlos Dias, C. Kleber, Z. Andrade, R. Oliveira Rocha, C. Griesinger, *Chem. Eur. J.* **2011**, *17*, 1811 – 1817; c) H. M. Ge, H. Sun, N. Jiang, Y. H. Qin, H. Dou, T. Yan, Y. Y. Hou, C. Griesinger, R. X. Tan, *Chem. Eur. J.* **2012**, *18*, 5213 – 5221; d) H. Sun, U. M. Reinscheid, E. L. Whitson, E. J. d'Auvergne, C. M. Ireland, A. Navarro-Vázquez, C. Griesinger, *J. Am. Chem. Soc.* **2011**, *133*, 14629 – 14636; e) K. M. Specht, J. Nam, D. M. Ho, N. Berova, R. K. Kondru, D. N. Beratan, P. Wipf, R. A. Pascal, D. Kahne, *J. Am. Chem. Soc.* **2001**, *123*, 8961 – 8966; f) R. K. Kondru, P. Wipf, D. N. Beratan, *Science* **1998**, *282*, 2247 – 2250; g) P. Mukhopadhyay, P. Wipf, D. N. Beratan, *Acc. Chem. Res.* **2009**, *42*, 809 – 819.
[9] J. D. Knight, S. J. Sauer, D. M. Coltart, *Org. Lett.* **2011**, *13*, 3118 – 3121.
[10] W. P. Hems, W. P. Jackson, P. Nightingale, R. Bryant, *Org. Process Res. Dev.* **2012**, *16*, 461 – 463.
[11] for commercially available (+)-*erythro*-mefloquine HCl **1** from SynphaBase AG: $[\alpha]_D^{23} = +32.4$ ($c = 0.5$, MeOH); > 99.5 % *ee*. Literature: $[\alpha]_D^{22} = +33.9$ ($c = 0.28$, MeOH);[4] $[\alpha]_D^{20} = +36$ ($c = 0.6$, MeOH).[6]
[12] Y. Qiu, S. Kitamura, J. K. Guillory, *Pharm. Res.* **1992**, *9*, 1640 – 1643.
[13] (*S*)-MTPA-Cl and (*R*)-MTPA-Cl were obtained from sigma aldrich with ≥ 99 % *ee*.
[14] Refined formula $C_{27}H_{23}F_9N_2O_3$, formula weight 594.47 g mol^{-1}; Crystal size $0.03 \times 0.10 \times 0.11$ mm^3, orthorhombic, $C222_1$, $Z = 8$, $a = 8.6087(3)$, $b = 28.4285(11)$, $c = 21.1151(8)$ Å, $V = 5167.6(3)$ Å^3, $\rho = 1.528$, $\mu = 1.267$ mm, $T = 100$ K, $F(000) = 2432$ e, $\lambda = 1.54178$ Å (CuKα), $2\theta_{max} = 138.38°$, ω- and ϕ-scans with Bruker Smart 6000 detector. 51480 measured reflections, of which 4849 were symmetry independent and 4739 (with $F_O > 3\sigma(F_O)$) used in the refinement against *F*, riding hydrogen atoms and constrained fluorine distances for the disordered trifluoromethyl group, 364 parameters refined, $R_{int} = 3.5\,\%$, $R_1 = 2.33\,\%$, $R_w = 2.43\,\%$, Flack parameter $x = -0.02(6)$, after transfer of the invariom multipole populations (not refined): $R_1 = 1.97\,\%$, Flack parameter $x = -0.03(5)$. CCDC 903889 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Optical rotation of the crystals could not be measured.
[15] B. Dittrich, C. B. Hübschle, M. Messerschmidt, R. Kalinowski, D. Girnt, P. Luger, *Acta Crystallogr. Sect. A* **2005**, *61*, 314 – 320.
[16] A. Volkov, P. Macchi, l. J. Farrugia, C. Gatti, P. Mallinson, T. Richter, T. Koritsánszky, **2006**, XD2006—A computer program Package for Multipole refinement, Topological Analysis of Charge densities and Evaluation of Intermolecular Energies from Experimental or Theoretical Structure Factors.
[17] B. Dittrich, M. Strumpel, M. Schäfer, M. A. Spackman, T. Koritsánszky, *Acta Crystallogr. Sect. A* **2006**, *62*, 217 – 223.
[18] H. D. Flack, G. Bernardinelli, *J. Appl. Crystallogr.* **2000**, *33*, 1143 – 1148.
[19] Refined formula $C_{27}H_{23}F_9N_2O_3$, formula weight 594.47 g mol^{-1}; Crystal size $0.05 \times 0.18 \times 0.23$ mm^3, orthorhombic, $C222_1$, $Z = 8$, $a = 8.624(2)$, $b = 28.410(6)$, $c = 21.114(4)$ Å, $V = 5167(2)$ Å^3, $\rho = 1.528$, $\mu = 1.267$ mm, $T = 100$ K, $F(000) = 2432$ e, $\lambda = 1.54178$ Å (CuKα), $2\theta_{max} = 138.98°$, ω- and ϕ-scans with Bruker Smart 6000 detector. 83378 measured reflections, of which 4848 were symmetry independent (4731 with $F_O^2 > 2\sigma(F_O^2)$) and used in the refinement against F^2, riding hydrogen atoms and constrained fluorine distances for the disordered trifluoromethyl group, 429 parameters refined, $R_{int} = 4.0\,\%$, $R_1 = 2.37\,\%$, $R_w = 6.01\,\%$, Flack parameter $x = -0.00(7)$, Experimental intensities are available on request.

