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Trapped in Misbelief for Almost 40 Years: Selective Synthesis of the Four Stereoisomers of Mefloquine

Nina Schützenmeister, Michael Müller, Uwe M. Reinscheid, Christian Griesinger,* and Andrei Leonov*[a]

Abstract: Here we report the synthesis of all four stereoisomers of mefloquine. Mefloquine (Lariam) is an important anti-malaria drug that is applied as a racemate of the erythro form. However, the (−)-isomer induces psychosis, while the (+)-enantiomer does not have this undesired side effect. There are six syntheses of which five lead to the wrong enantiomer without the authors of these syntheses noting that they had synthesized the wrong compound. At the same time physical chemistry investigations had assigned the absolute configuration correctly and the last enantioselective synthesis that took these results into account delivered the correct absolute configuration. Since various synthetic approaches failed to provide the correct stereoisomers in previous syntheses, we submit here a synthetic approach with a domino Sonogashira-6π-electrocyclisation as key step that confirmed synthetically the correct absolute configuration of all four isomers.

Keywords: domino reactions · drug design · mefloquine · structure elucidation · total synthesis

Introduction

Treatment of malaria is still dominated by conventional small organic drug molecules, among which the family of artemisinin derivatives represents a breakthrough in treatment and prophylaxis.[1] To slow down the development of resistance and to increase the potency, artemisinin-based combination therapies (ACTs) were introduced.[2] One of the current combinations recommended by the World Health Organization (WHO) for the treatment of uncomplicated *Plasmodium falciparum* malaria consists of artesunate and mefloquine.[3] The latter drug molecule has a long history in malaria treatment and prophylaxis, and targets the heme polymerisation of the parasites.[4] Mefloquine alone has also demonstrated further interesting biological activities: it was active against mycobacteria,[5] *Schistosoma* spp.,[6] and was successfully used to treat progressive multifocal leukoencephalopathy caused by the John Cunningham virus (JC virus).[7] Moreover, the synthesis of pentafluorosulfanyl analogues led to improved activities.[8]

Mefloquine is a small molecule with a relative molecular mass (M_r) of 378.31 Da and two independent chiral centres, and therefore mefloquine has four stereoisomers. The antimalarial agent mefloquine hydrochloride marketed as Lariam by Roche is a racemic compound and contains two of these isomers, namely, (+)- and (−)-*erythro*-mefloquine. One major drawback of this drug is the occurrence of dose-related neuropsychiatric and gastrointestinal adverse effects.[4a,d] Indeed, the enantiomer (−)-*erythro*-mefloquine is known to be a potent blocker of adenosine receptors in the central nervous system (CNS) and is therefore believed to be responsible for the neuropsychiatric symptoms, whereas (+)-*erythro*-mefloquine is not.[9] Application of (+)-*erythro*-mefloquine rather than the racemate could therefore have a better risk-to-benefit ratio.

Despite its status as an established anti-malaria drug, the absolute stereochemistry of mefloquine had been undecided in the literature for almost 40 years until very recently. Furthermore, the first assignment of (+)-*erythro*-mefloquine as (11*R*,12*S*)[10] was designated "tentatively", but subsequent citations removed this important demand for further research.[11] The first X-ray crystal structure by Karle and Karle in 2002 determined the stereochemistry of (+)-*erythro*-mefloquine as (11*S*,12*R*),[12a] which is supported by a recent crystal structure of both enantiomers by Sonnet et al.[12b] Also, residual dipolar coupling (RDC)-enhanced NMR spectroscopy in combination with optical rotatory dispersion (ORD) and electronic circular dichroism (ECD) spectroscopy[13] confirmed the result of the crystal structure in 2012. Furthermore, an X-ray crystal structure analysis of the Mosher amides of (+)-*erythro*- and (−)-*erythro*-mefloquine led to an absolute configuration of (+)-*erythro*-mefloquine as (11*S*,12*R*).[14] Importantly, all past published syntheses[15] determined the stereochemistry of (+)-*erythro*-mefloquine as (11*R*,12*S*), which is opposite to the mentioned

[a] Dr. N. Schützenmeister, Dr. M. Müller, Prof. Dr. U. M. Reinscheid, Prof. Dr. C. Griesinger, Dr. A. Leonov
Max-Planck-Institute of Biophysical Chemistry
Department of NMR-Based Structural Biology
Am Fassberg 11, 37077 Göttingen (Germany)
Fax: (+49) 551 201-2202
E-mail: cigr@nmr.mpibpc.mpg.de
anle@nmr.mpibpc.mpg.de

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physical chemistry techniques. With regard to the fact that all published syntheses of this important drug did not deliver the correct enantiomer so far, we developed an asymmetric total synthesis for the four isomers of mefloquine·HCl and confirmed analytically that the chosen reactions now provide the correct absolute configuration synthetically. While preparing this manuscript, Hall and co-workers published the first synthetic approach, which delivered the correct stereochemical outcome.[16]

Results and Discussion

The main strategy of our synthesis was the use of the predefined configuration of one chiral centre. Analysis of the mefloquine structure revealed that fixing the configuration of C-12 (Figure 1) is preferred over the centre of C-11. In this case optically pure derivatives of (*S*)- and (*R*)-pipecolinic acid, such as aldehydes **2** and *ent*-**2**, can be used as starting materials. With the defined configuration of the stereogenic centre of C-12, the construction of the stereogenic centre at C-11 can be done in the next step. Finally, the structural core of the quinoline moiety[17] should be synthesised in such a manner that the configuration at C-12 is retained. Thus, a reliable approach to the stereoisomers of mefloquine is achieved by separation of diastereomers followed by the determination of configuration at C-11, and comparison of the optical rotation value of the final product with the reference to the individual enantiomers.

With this overview in mind, the retrosynthetic analysis of **1a** is based on an Li-mediated alkyne addition to construct the stereogenic centre on C-11 and a domino Sonogashira-6π-electrocyclisation reaction as key step for the establishment of the quinoline moiety (Figure 2).[18]

The synthesis of the literature-known[19] imidoyl iodide building block **3** is inspired by the work of Sadighi et al.[20] The preparation starts with the trifluoroacetylation of commercially available 2-trifluoromethylaniline **5** with trifluoroacetic acid anhydride and a catalytic amount of 4-dimethylaminopyridine (DMAP) in CH_2Cl_2 using pyridine as base to yield the trifluoroacetamide **6** in quantitative yield. Trifluoroacetamide **6** is subsequently converted into the trifluoroacetimidoyl iodide **3** using PPh_3 and I_2 in toluene and iPr_2NEt as base (Figure 3).

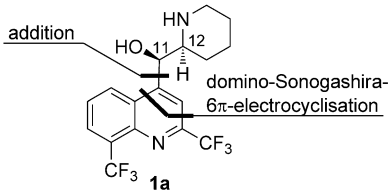

Figure 2. Retrosynthetic analysis of **1a**.

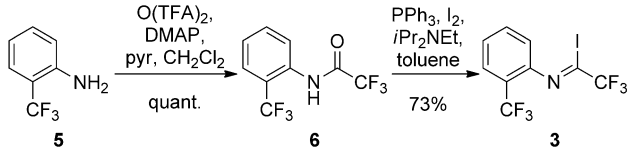

Figure 3. Synthesis of iodide **3**.

The starting point of the synthesis of the alkyne building block **12** for the domino process to build (−)-*erythro*- and (+)-*threo*-mefloquine **1** is the enantio-enriched aldehyde (*S*)-1-*N*-Boc-piperidine-2-al **2** or (*R*)-1-*N*-Boc-piperidine-2-al *ent*-**2** (Boc = *tert*-butyloxycarbonyl).[21] Since the stereochemical integrity of the starting materials and products of each step during the synthesis must be ensured, we decided to check the optical purity of the starting aldehydes and later of **9** and **1a**/**1b**.

The common method for this, namely, the measurement of the optical rotation value proved to be unreliable owing to the large variation in the literature data.[22] Therefore, the enantiomeric ratio of **2** (*ent*-**2**) was determined by means of analytical chiral HPLC after reduction and benzoyl (Bz) protection to benzoate **7** in 88% yield (84% for *ent*-**7**) (Figure 4). We obtained an enantiomeric ratio of 95:5 for **7** and 96:4 for *ent*-**7**.

Addition of lithiated TMS-acetylene **4** to aldehyde **2** followed by tetramethylsilane (TMS) deprotection yielded the Felkin–Anh product **9** in 62% yield (65% for *ent*-**9**) as well as the minor diastereomer **8**, the *anti*-Felkin–Anh product, in 5% yield (4% for *ent*-**8**).[23] To determine the relative stereochemistry of C-1′ and C-2 in the adducts **8** and **9** as well as their enantiomers, the two alcohols *ent*-**8** and *ent*-**9** were

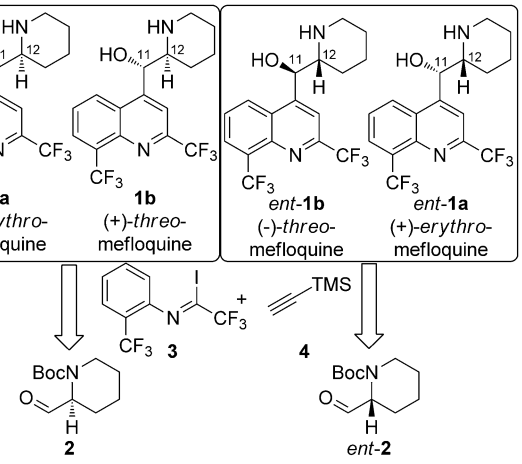

Figure 1. Structures of individual mefloquine isomers and retrosynthetic analysis.

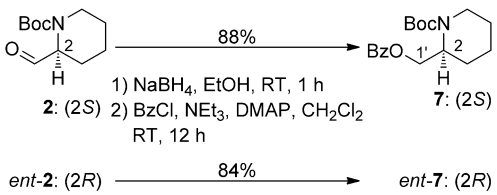

Figure 4. Synthesis of benzoates **7** and *ent*-**7**.

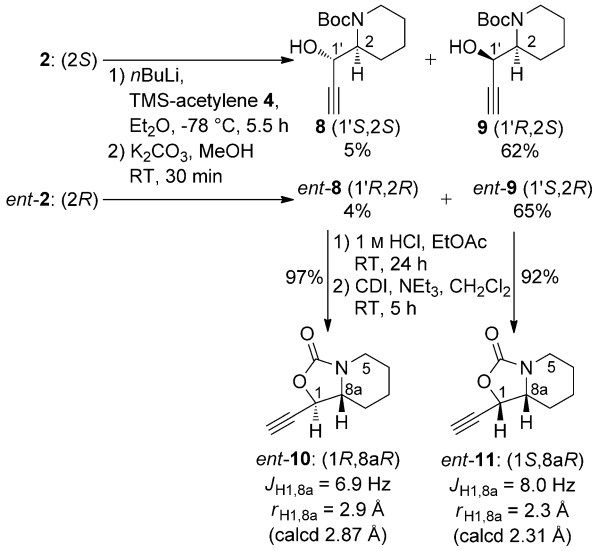

Figure 5. Synthesis of alcohols **8**, *ent*-**8**, **9** and *ent*-**9** and determination of the relative stereochemistry of C-1.

transferred into their corresponding conformationally rigid oxazolidinone derivatives[24] *ent*-**10** and *ent*-**11**, respectively (Figure 5).

The configuration of the stereogenic centre at C-1 in *ent*-**10** and *ent*-**11** has been determined by the measurement of the ^{1}H coupling constants of the 1-H atom to 8a-H. Additionally, a 2D NOESY experiment with suppression of zero-quantum coherence according to Thrippleton and Keeler[25] was carried out, which permits a reliable detection of weak nuclear Overhauser effect (NOE) enhancements. The larger vicinal coupling constant value between 8a-H and 1-H indicated the *cis* orientation of both protons in relation to the five-membered ring in *ent*-**11**. For the oxazolidinone *ent*-**10** a smaller coupling constant J(1-H,8a-H) was measured. Interproton distances between 1-H and 8a-H from 2D NOE measurements using well-resolved resonances of diastereotopic methylene protons at C-5 as a reference were in good agreement with values calculated by ACD/3D Viewer program (for *ent*-**10**: $r_{H1,8a}$ = 2.9 Å (calcd 2.87 Å); for *ent*-**11**: $r_{H1,8a}$ = 2.3 Å (calcd 2.31 Å). For the spectra, see Figures S43 and S46 in the Supporting Information. The important observed NOE signals are marked with red arrows to clarify the interpretation of the spectrum). Thus, the configuration of compound *ent*-**10** was established as (1*R*,8a*R*) and of compound *ent*-**11** as (1*S*,8a*R*). Since the configuration of stereogenic centres C-1 and C-8a is retained in the conversion of *ent*-**8** to *ent*-**10** or *ent*-**9** to *ent*-**11**, respectively, which included an acidic removal of the Boc group followed by ring closure with *N*,*N*′-carbonyldiimidazole, the configurations of the compounds *ent*-**8**, *ent*-**9** and their enantiomers **8** and **9** have been assigned as depicted in Figure 5.

The previously formed major isomers of alcohol **9** and *ent*-**9**, respectively, were benzoyl-protected with BzCl, NEt_3 and DMAP in quantitative yield to give the building block **12** (*ent*-**12**) for the domino reaction (Figure 6). The protec-

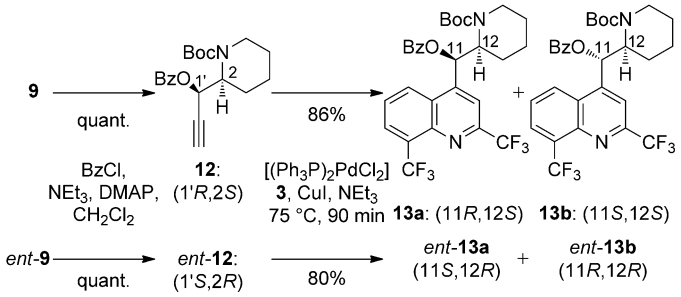

Figure 6. Benzoylation and domino reactions.

tion of the alcohol was necessary for the success of the domino reaction, because the alcohol showed decomposition under the reaction conditions. The enantiomeric ratios (e.r.) of the benzoates **12** (e.r. = 95:5) and *ent*-**12** (e.r. = 94:6) determined by means of chiral HPLC were almost identical to the values of the corresponding benzoates **7** and *ent*-**7**, which indicates that no racemisation at C-2 took place during the addition of the lithiated species of **4** to aldehyde **2** (*ent*-**2**) followed by benzoylation. The domino Sonogashira-6π-electrocyclisation reaction was carried out under standard Sonogashira-coupling conditions with 5 mol% [Pd(PPh_3)$_2$Cl$_2$] and 5 mol% CuI in NEt_3 at 75 °C to yield the domino product in 86% yield as an inseparable mixture of the protected *erythro*-mefloquine **13a** and its *threo*-isomer **13b**. The enantiomeric benzoate *ent*-**12** afforded the mixture of *ent*-**13a** and *ent*-**13b** in an analogous manner in 80% yield (Figure 6).

The isomerisation of the stereogenic centre at C-11 during the domino process can be explained by the occurrence of the base-catalysed propargyl–allenyl isomerisation of the Sonogashira product **14** to a planar allene intermediate **15**, which was previously proposed by Gao et al. for quinolone.[26] The allene intermediate **15** undergoes a 6π-electrocyclisation to intermediate **16**, which is followed by tautomerisation to give **13a** and **13b** in a ratio of 1:3.6 for **13a**/**13b** (*ent*-**13a**/*ent*-**13b** = 3.6:1) determined by NMR spectroscopy (Figure 7).

Final deprotection of the Bz and Boc protecting groups of **13** gave the free base (+)-*threo*-mefloquine **1b** in 62% yield, (−)-*threo*-mefloquine *ent*-**1b** in 65% yield, (−)-*erythro*-mefloquine **1a** in 17% yield and (+)-*erythro*-mefloquine *ent*-**1a** in 18% yield over two steps (Figure 8). The diastereomers **1a** and **1b** (*ent*-**1a** and *ent*-**1b**) could be separated by means of column chromatography. The *erythro* and *threo* isomers were assigned by comparison of the TLC R_f value with commercially available *rac*-*erythro*-mefloquine **1a**/*ent*-**1a** and *rac*-*threo*-mefloquine **1b**/*ent*-**1b**. The e.r. of the final products **1a** (e.r. = 96:4), **1b** (e.r. = 96:4), *ent*-**1a** (e.r. = 94:6) and *ent*-**1b** (e.r. = 93:7) has also been checked by means of chiral HPLC[27] and did not change significantly during the synthetic pathway relative to **7** (*ent*-**7**) as well as **12** (*ent*-**12**).

Therefore, one can assume that no isomerisation of the stereogenic centre at C-12 has taken place. Additionally, chiral HPLC retention times for **1a** and *ent*-**1a** obtained by

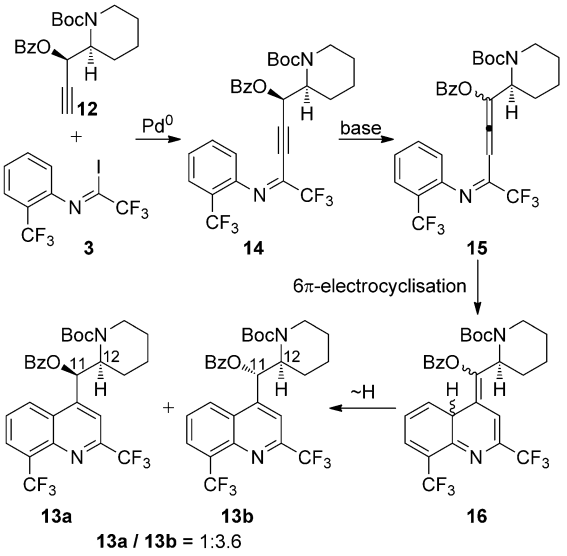

Figure 7. Proposed mechanism of the domino reaction.

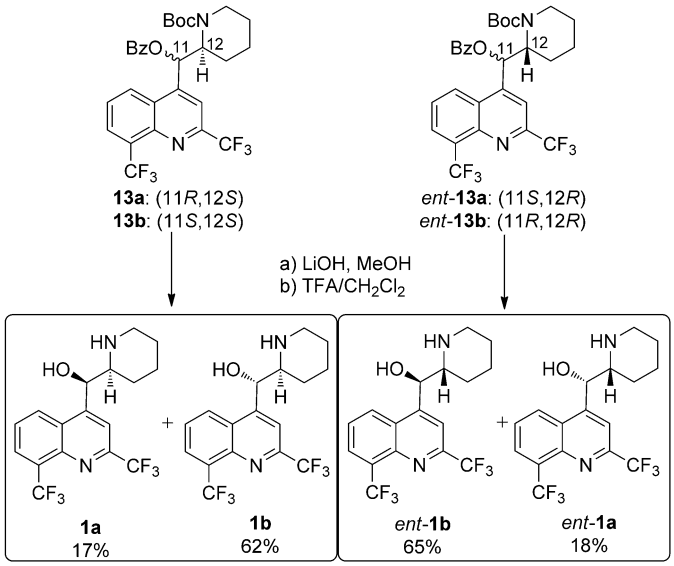

Figure 8. Bz and Boc deprotection.

an internal standard method with commercial available racemic *erythro*-mefloquine (Lariam) as a reference (with racemic *threo*-mefloquine for **1b** and *ent*-**1b**) were identical to values of the corresponding peaks of the racemic compound. Other physical analysis data (NMR spectroscopy, UV, IR, and HRMS) were found to be in agreement with structures of the synthesised products. Finally, optical rotation measurements were carried out for hydrochlorides of **1a**, *ent*-**1a**, **1b** and *ent*-**1b**. The good agreement of the experimental optical rotation with reported values[10] clearly confirmed the configuration of all four mefloquine isomers.[28]

Conclusion

In conclusion, we have reported a five-step total synthesis of all four stereoisomers of mefloquine. In contrast to previous syntheses,[15] excepting the most recent synthesis,[16] we have obtained all four possible stereoisomers of mefloquine, including (−)-(11*R*,12*S*)-*erythro*-mefloquine **1a** and (+)-(11*S*,12*R*)-*erythro*-mefloquine *ent*-**1a**. This constitutes a synthetic approach that synthesises all four stereoisomers of mefloquine correctly and supports the recent enantioselective synthesis[16] according to a different synthetic pathway.

Acknowledgements

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[28] Compound **1a**·HCl: $[\alpha]_D^{23} = -32.83°$ ($c = 0.4$, MeOH), literature[10] for (−)-*erythro*-mefloquine·HCl: $[\alpha]_D^{25} = -33.0°$ ($c = 0.306$, MeOH); *ent*-**1a**·HCl: $[\alpha]_D^{23} = +26.01°$ ($c = 0.4$, MeOH), literature[10] for (+)-*erythro*-mefloquine·HCl: $[\alpha]_D^{22} = +33.90°$ ($c = 0.208$, MeOH); **1b**·HCl: $[\alpha]_D^{23} = +51.85°$ ($c = 0.4$, MeOH), literature[10] for (+)-*threo*-mefloquine·HCl: $[\alpha]_D^{24} = +55.27°$ ($c = 0.431$, MeOH); *ent*-**1b**·HCl: $[\alpha]_D^{24} = -50.49°$ ($c = 0.4$, MeOH), literature[10] for (−)-*threo*-mefloquine·HCl: $[\alpha]_D^{25} = -53.37°$ ($c = 0.308$, MeOH).

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