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Total Synthesis and Biological Investigations of (−)-Artemisinin - The Antimalarial Activity of Artemisinin is not Stereospecific

Johannes Krieger[a], Toni Smeilus[a], Marcel Kaiser[b,c], Ean-Jeong Seo[d], Thomas Efferth[d], and Athanassios Giannis*[a]

Abstract: Here, we describe an efficient and diversity oriented entry to both (−)-artemisinin (**1**) and to its natural antipode (+)-artemisinin starting from commercially and readily available *S*-(+)- and *R*-(−)-citronellene respectively. Subsequently, we have answered a still open question, i.e. specificity of artemisinins action. Using a drug sensitive *Plasmodium falc.* NF54 strain we showed that the antimalarial activity of artemisinin is not stereospecific. Our straightforward and biomimetic approach to this natural endoperoxide allows synthesis of artemisinin derivatives not accessible applying current methods and may help to address the problem of emerging resistance of *Plasmodium falciparum* towards artemisinin.

Artemisinin and its derivatives dihydroartemisinin, artemether, and artesunate belong to the most important antimalarial drugs.[1,2] In 2015, Youyou Tu was awarded the Nobel Prize in Physiology or Medicine for the discovery of antimalarial activity of artemisinin.[3] Whereas these drugs are almost nontoxic to normal cells, several studies have confirmed their potent anticancer activity.[4-6] Recently, it was shown that artemisinins are ligands of the mammalian protein gephyrin[7] and that the mechanism of action of these molecules depends on the enhancement of GABA-A receptor signaling, leading to the regeneration of pancreatic *β*-cells from *α*-cells in a ROS-independent way. Therefore, artemisinins may also find application in the therapy of diabetes mellitus. In addition, Lisewski *et al.*[8] reported that *Plasmodium falciparum* antigen EXP1, a membrane glutathione *S*-transferase, is potently inhibited by artesunate in a competitive mode. Despite all that, the exact nature of the mechanism, the molecular target as well as the question of stereospecific action of artemisinins remains elusive and are still under debate.[9,10] Here we show that antimalarial activity of artemisinin is not stereospecific and also a general and straightforward approach to this natural endoperoxide is presented that allows synthesis of artemisinin derivatives not accessible applying current methods. Several proteins have been reported to be the targets of this sesquiterpene lactone. Recently, identification of a plethora of artemisinin-binding proteins of *Plasmodium falciparum* was reported by Wang *et al.*.[11,12] Similar studies were also carried out by Ismail *et al.*.[13] Therefore, the hypothesis that artemisinin interacts with a specific protein of *Plasmodium falciparum* is questionable as described by Fügi *et al.*.[14] On the other hand, according to investigations of O'Neill *et al.* artemisinin activity against this parasite is not stereospecific.[15] To unequivocally answer this question, investigation of the biological activity of (−)-artemisinin (**1**) (i.e. the antipode of natural (+)-artemisinin) is absolutely necessary.

Although quite a few[16,17] innovative and remarkable synthetic[18-21] and semisynthetic[22-24] methods for the synthesis of (+)-artemisinin have appeared in the literature over the last four decades, its antipode (−)-artemisinin (**1**) is still unknown and its antimalarial potency is in question.

Here, we report an efficient entry to both (−)-artemisinin (**1**) and (+)-artemisinin starting from commercially and readily available *S*-(+)- and *R*-(−)-citronellene (**5**)[25,26] respectively. In addition, antimalarial activity of (−)-artemisinin against the drug sensitive *Plasmodium falc.* NF54 strain is investigated.

With the aim of the development of a diversity-oriented synthesis, our retrosynthetic analysis focussed on the late stage generation of (−)-9-desmethyl artemisinin (**15**), enabling access to other non-natural artemisinin analogues. As shown in Figure 1, an intramolecular *Diels-Alder* reaction of triene (**3**) presented the key role in the formation of the artemisinic ester framework **2**. The stereo configuration at the stereogenic centers 5a and 8a (corresponding to C-atoms marked by an asterisk in structure (**2**)) would be influenced by the chirality of citronellene, by the general principles governing the intramolecular *Diels-Alder* reaction[27] and the diastereoselectivity of the reduction of the *α,β*-unsaturated esters **11** and **12** (Scheme 2).

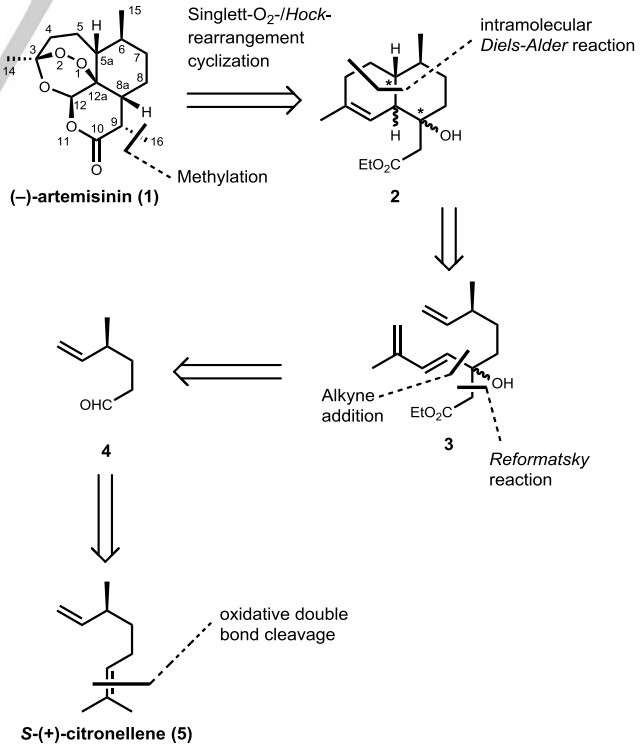

Figure 1. Retrosynthetic analysis of (−)-artemisinin (**1**).

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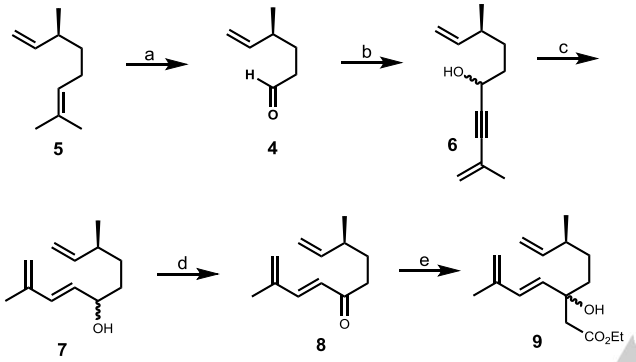
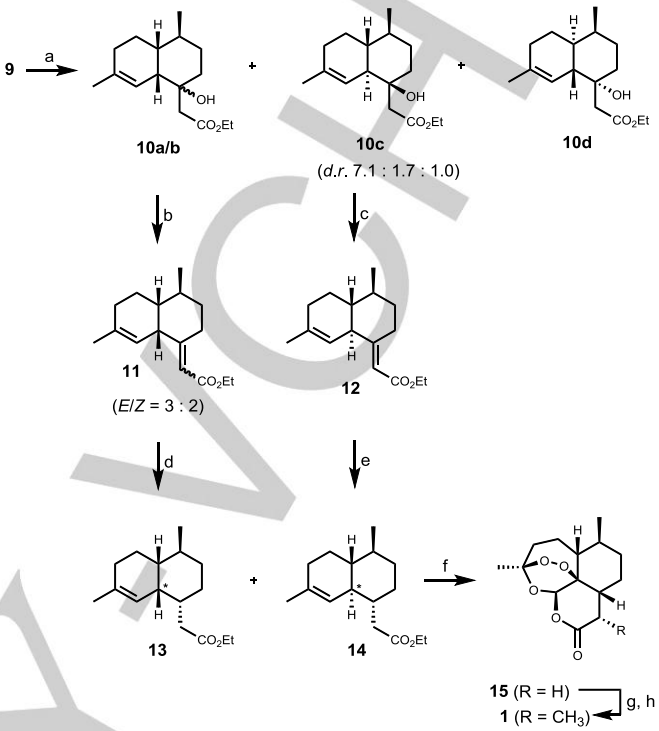
Epoxidation of (*S*)-(+)-citronellene (**5**) using *m*-CPBA, followed by oxidative cleavage of the intermediate epoxide using H_5IO_6 afforded aldehyde **4**. Addition of the organolithium species formed by treatment of 2-methylbut-1-en-3-yne with *n*-BuLi afforded propargylic alcohol **6** with 74 % yield over three steps. Subsequent treatment of derivative **6** with $LiAlH_4$ in THF gained allylic alcohol **7** in quantitative yield. This alcohol was readily oxidized by BAIB/TEMPO system, delivering ketone **8** in 86 % yield. *Reformatsky* reaction of the latter with activated Zn/ethyl bromoacetate in toluene yielded triene **9** (Scheme 1), which was subjected to a thermal *Diels-Alder* reaction (190 °C, toluene). Through this method, artemisinic acid derivatives **10a/b,c,d** (*d.r.* = 7.1:1.7:1.0) were obtained with the total yield of 91 %. The stereochemistry of these isomers was confirmed by NOE experiments (see experimental part).



Scheme 1. Synthesis of the cyclization precursor 9. Reaction conditions: (a) i) *m*-CPBA, NaOAc, DCM, 0 °C, 6 h; ii) H_5IO_6, Et_2O/THF, 0 °C, 5 h; b) 2-methylbut-1-en-3-yne, *n*-BuLi, THF, −78 °C to rt, (74 % over three steps); (c) $LiAlH_4$, THF, rt, 16 h, quant.; (d) BAIB, TEMPO, DCM, rt, 16 h, 86 %; (e) $BrCH_2COOEt$, Zn, Toluene, 90 °C, 30 min, 86 %.

Alcohols **10a/b** and **10c** were treated with *Martin* Sulfurane[28] and afforded amorphadienes **11** and **12** in excellent yields. Compound **12** was obtained as pure *E*-isomer, whereas derivative **11** was isolated as *E*:*Z* mixture = 3:2. Reduction of the *cis*-fused *α,β*-unsaturated esters with $NiCl_2$/$NaBH_4$ in methanol delivered derivative **13** in nearly quantitative yield and acceptable diastereoselectivity (*d.r.* = 2.3 : 1). Fortunately, *Birch*-reduction of the *trans*-fused *α,β*-unsaturated ester **12** yielded derivative **14** with the desired (*R*)-stereochemistry at the newly generated stereogenic center. Exposure of a solution of **13** and **14** in dichloromethane containing catalytic amount of methylene blue to sunlight and oxygen followed by treatment of the resulting intermediate hydroperoxide with catalytic amount of trifluoroacetic acid as described[29] afforded 9-desmethyl-(−)-artemisinin (**15**) in 32 % yield. These demonstrates that the stereochemistry at positions labelled by an asterisk in derivatives **13** and **14** does not have any influence on the stereochemistry at position 12a of the obtained artemisinin derivative: In both cases exclusive formation of **15** was observed. Methylation of the later with LDA/MeI followed by inversion of the stereo configuration at C9 with DBU, gave **1**, the antipode of the natural product.



Scheme 2. Synthesis of (−)-artemisinin (1). Reaction conditions: (a) Toluene, 190 °C, 30 h; 91 %, (*d.r.* = 7.1:1.7:1.0); (b) *Martin* Sulfurane, DCM, 0 °C, 30 min, 94 %; (c) *Martin* Sulfurane, DCM, 0 °C, 30 min, 84 %; (d) $NiCl_2 \cdot 6\ H_2O$, $NaBH_4$, MeOH, 0 °C, 97 %; (e) Li, NH_3, EtOH, −76 °C, 76 %; (f) O_2, Methylene blue, light , DCM, −30 °C; then O_2, cat. TFA, DCM, 0 °C to rt, 2 d, 32 %; (g) LDA, THF, MeI, −78 °C, 75 %; (h) DBU, DCM, rt, 16 h, quant..

Subsequently, we checked the antimalarial activity of (−)-artemisinin (**1**) against the drug sensitive *Plasmodium falc.* NF54 strain. Interestingly, both (−)-artemisinin (**1**) as well as (+)-artemisinin showed similar potency (Table 1).

The majority of drugs used in therapy act stereospecific and there are only a few exceptions.[30,31] The identical antimalarial activity of both artemisinin antipodes adds another exception to this rule. On the other hand, the question of pharmacological action of (−)-artemisinin (**1**) on mammalian targets remains open. In this context, we checked the cytotoxicity of both artemisinins towards L-6 cells, a primary cell line derived from rat skeletal myoblasts (Table 1) as well as towards sensitive CCRF-CEM cell line and multidrug-resistant CEM/ADR500 and compared the results to the cytotoxicity of (+)-artemisinin.[32] Although there are differences in the cytotoxicity in L-6 cells, their cytotoxicities in CCRF-CEM cells and in CEM/ADR5000 cells are almost similar.

Table 1. Antiplasmodial activitiy and cytotoxicitiy (IC50 in µM) of the natural (+)-artemisinin (**Qinghaosu, Sigma 65932)** and its synthetic enantiomer **1**.

Compound	*Plasmodium falc.*	Cytotoxicity		
	NF54	L6	CCRF-CEM	CEM/ADR500
(+)-artemisinin	0.009 ± 0.001	>350	36.90 ± 6.90	26.90 ± 4.40
(−)-artemisinin (**1**)	0.011 ± 0.005	188 ± 21	55.54 ± 7.00	45.41 ± 8.06

To sum up, this new artemisinin synthesis uses readily available starting materials, robust transformations along with high yields in a minimum of reaction steps, delivering the (1´)-hydroxy artemisinic ester derivatives **10a-d** in an overall yield of 50 % over seven steps with the possibility of performing the reactions in 10 g-scale. Moreover, it is important to emphasize that about 90 % of the cyclization products **10a,b,c** (Scheme 2) could be transformed to the desired artemisinin and its 9-desmethyl analogue.

Our convergent approach to artemisinin enables not only access to both of its antipodes, but also paves the way for the introduction of diverse substituents at different positions in the artemisinin framework. For example, various alkyl or allyl groups at position 9 could be introduced whereas using different alkene-alkynes, variations at position 3 of the natural product could be established. Other possibilities using slight modifications in our approach are shown in Figure 2.

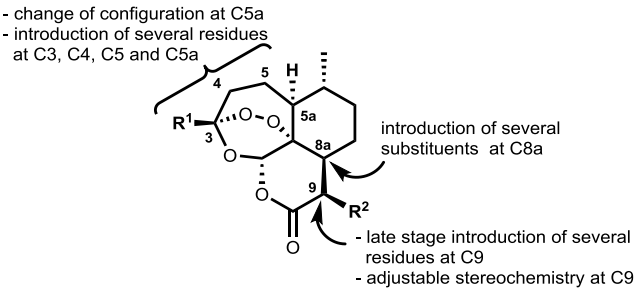


Figure 2. Possibilities of derivatization of the (+)-artemisinin skeleton offered by this synthesis.

In several Southeast Asian countries emergence of *Plasmodium falciparum* strains with reduced susceptibility to artemisinins and artemisinin combination therapy associated drugs have been reported in the last years, resulting in increased rates of treatment failures.[33] Therefore, alternatives are urgently needed and we believe that our results will facilitate the development of such options. The presented method opens up the possibility of stereospecific synthesis of new artemisinin derivatives not accessible by existing methods. Access to enantiomeric forms/analogs could lead to artemisinin analogs with more favorable pharmacokinetic properties, a liability of current artemisinin analogs. It will be also of interest to investigate the activity of (−)-artemisinin agains other parasites like *Schistosoma mansoni*, *Trypanosoma cruzi and T. brucei*. Eventually, our synthetic (−)-artemisinin (**1**) can be used to answer the question of the stereospecificity of (+)-artemisinin action on mammalian cells and proteins like gephyrin.[7]

Experimental Section

Experimental Details.

Keywords: chirality, enantiomeric drugs, ene reaction, malaria, trioxane

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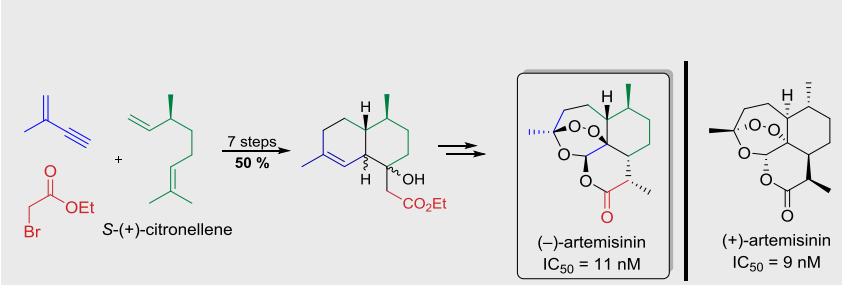

*J. Krieger, T. Smeilus, M. Kaiser, E.-J. Seo, T. Effert, A. Giannis**

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The Antimalarial Activity of Artemisinin is not Stereospecific

Mirror, mirror on the wall, who is most active one of all? Both (−)- and (+)-artemisinin have been synthesized biomimetically and their activity tested against *Plasmodium falc.* Both have been proved as equipotent.