Anti-Malaria Drugs

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Continuous-Flow Synthesis of the Anti-Malaria Drug Artemisinin**

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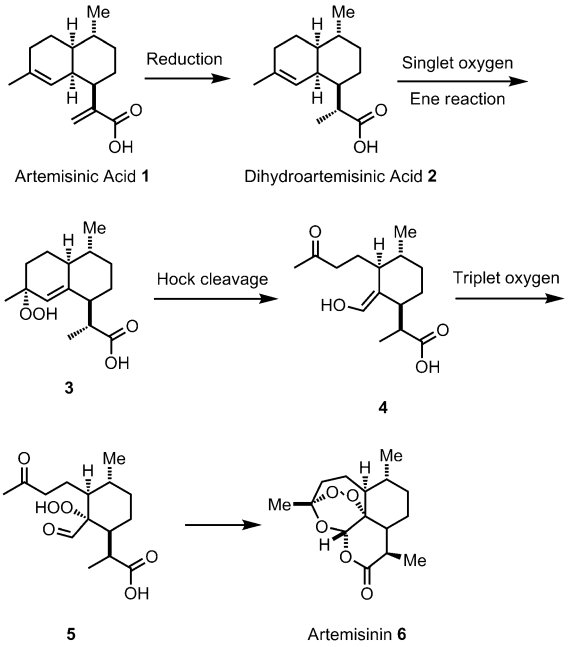
Malaria remains a major global health problem, killing about one million people each year, with the protozoan parasite *Plasmodium falciparum* responsible for this most deadly illness. The sesquiterpene endoperoxide artemisinin is currently the most effective treatment against multi-drug resistant Plasmodium species, and artemisinin combination treatments (ACTs) are now first-line drugs, as recommended by the WHO.[1] The compound is extracted from the plant *Artemisia annua* (sweet wormwood), now cultivated in many countries for this purpose.[2] However, reliance on cultivated *A. annua* restricts the supply of the drug and elevates costs for the patients.

The total synthesis of artemisinin[3] is too laborious to be considered a viable alternative for supplying the highly cost-sensitive market. Artemisinic acid, a much-less complex molecular precursor, can be extracted from the same plant in higher yields, or produced in engineered yeast.[4] Therefore, artemisinic acid is an ideal starting point for synthetically producing artemisinin. Still, the conversion of artemisinic acid to artemisinin has proven a formidable challenge for chemists, since a high-yielding, scalable, and low-cost process for constructing a highly complex molecule is needed.

Herein we report a continuous-flow conversion of dihydroartemisinic acid, derived from artemisinic acid by hydrogenation or produced by fermentation in engineered yeast,[5] into artemisinin. Central to the reaction is a continuous photochemical transformation involving a singlet-oxygen-induced ene reaction and the addition of triplet oxygen, which triggers the reaction cascade that incorporates the essential endoperoxide group. This technically simple, efficient, and inexpensive synthesis is readily scalable by virtue of the continuous-flow process, and does not require isolation and purification of intermediates. Combined with the production of artemisinic acid in engineered yeast,[4] access to the much-needed malaria drug is now possible by semi-synthesis rather than isolation from plants, ensuring a steady supply of artemisinin at greatly reduced cost.

Artemisinic acid (**1**, Scheme 1), a bicyclic molecule, lacks the structural complexity that imparts anti-malarial activity to artemisinin (**6**, Scheme 1) a sesquiterpene endoperoxide with a dense array of functional groups.[6] A strain of *Saccharomyces cerevisiae* has been engineered to produce large quantities of artemisinic acid, making **1** an attractive starting point for chemical synthesis. Transformation of artemisinic acid to artemisinin involves a series of challenging steps (Scheme 1). The reaction sequence starts with the reduction of artemisinic acid to the corresponding dihydroartemisinic acid.



Scheme 1. Reaction sequence for the synthesis of artemisinin from artemisinic acid.

Reaction between **2** and singlet oxygen gives rise to the formation of tertiary allylic hydroperoxide **3**. This compound is treated with trifluoroacetic acid to promote the Hock cleavage of the hydroperoxide, followed by migration of the allyl functional group and opening of the ring to enol **4**. The highly reactive enol **4** reacts with triplet oxygen to generate hydroperoxide **5**. Finally, a series of condensation reactions generate the three missing rings of artemisinin. Several prior synthetic endeavors were partially successful but relied on strategies that were too technically complex to be viable for

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large-scale production in conventional batch reactors while meeting stringent cost targets.[7]

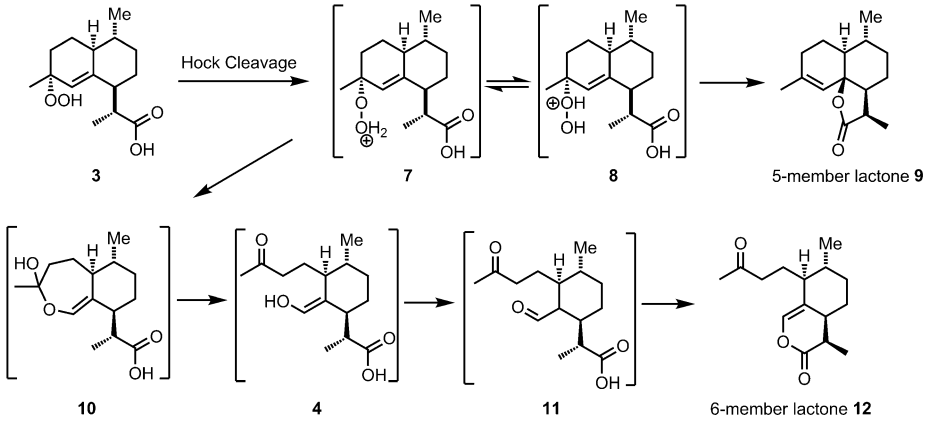
One crucial step in the transformation requires singlet oxygen ($^1O_2$), a photochemically generated, highly reactive molecule that must be prepared in situ. In conventional batch systems, widespread use of $^1O_2$ is prohibited by the low rate of mass transfer of oxygen gas into the solution, as well as the need for specialized equipment to produce the reagent. Additionally, the use of photochemistry for singlet-oxygen generation is restricted in these systems because light can penetrate only a limited distance through the solution due to absorption by the photosensitizer, as described by the Beer–Lambert law. This problem is exacerbated as the size of the reaction vessel is increased, greatly diminishing conversion rates and yields.[8]

Recently, we established that photoinduced singlet-oxygen generation can be achieved on a preparative scale in a continuous-flow reactor adapted by simply wrapping the tubing that contains the reactant flow around a lamp that is surrounded by a cooled immersion well.[9] Illumination of the entire solution is highly efficient because the tubing has a constant, narrow diameter, and is positioned close to the light source. Increasingly large quantities of product are made by extending the duration of the reaction rather than changing to a larger vessel.[10] Additional hallmarks of flow chemistry are excellent control over reaction parameters, such as residence time, temperature, mass transfer, and mixing. It has been shown already that artemisinic acid (**1**) can be efficiently reduced to dihydroartemisinic acid (**2**) in a large-scale batch reaction.[11] Mindful of the need to build a simple, scalable, and inexpensive process for the conversion of dihydroartemisinic acid (**2**) into artemisinin (**6**), the use of light, which is relatively inexpensive and environmentally benign, for this transformation in a continuous-flow reactor was extremely appealing.

Scheme 1 shows the reaction sequence employed for the transformation. Our challenge was to convert **2** into artemisinin (**6**) by a three-step reaction sequence of photochemically induced oxidation with singlet oxygen,[12] acid-mediated cleavage of the oxygen–oxygen bond (Hock cleavage),[13] and oxidation with triplet oxygen.[14] Initially, each step was investigated individually. Ultimately, the reaction sequence was performed as a single continuous-flow chemical process that did not require purification and work-up of intermediates.

First, photooxidation of **2** to tertiary allylic hydroperoxide **3** was explored in a continuous-flow system fabricated in-house.[9b] The 20 mL volume device consisted of fluorinated ethylene propylene (FEP) tubing wrapped around a Schenk photochemical reactor containing a 450 W medium-pressure mercury lamp that was cooled to 25 °C. Numerous combinations of solvents, equivalents of oxygen and gas pressures, as well as flow rates and, consequently, residence times, were screened to identify optimal conditions. To prevent fires fed by oxygen, a non-flammable, halogenated solvent was required, and on a large scale, dichloromethane, the least-toxic halogenated solvent, was preferred. A solution of **2** in dichloromethane (2.5 mL min^{-1}) was added by a Vapourtec R2C + pump and oxygen gas (5 mL min^{-1}) was delivered by a mass-flow controller connected to a gas cylinder. The solution of **2** and the oxygen gas were mixed using an ethylene tetrafluoroethylene (ETFE) T-mixer. Tetraphenylporphyrin (TPP) was used as photosensitizer owing to its high quantum yield and high stability against photo-bleaching.[15] Under these conditions, 1.5 mmol of **3** was produced per minute, in 91 % conversion and 75 % yield.

Next, the conversion of **3** into **6** by a Hock cleavage and triplet-oxygen addition was examined, at first, in a batch reactor. Hock cleavage is the heterolytic cleavage of the oxygen–oxygen bond of hydroperoxides as catalyzed by Brønsted and Lewis acids (**9**, Scheme 2).



Scheme 2. Formation of five-membered lactone **9** and six-membered lactone **12** during Hock cleavage.

Treatment of tertiary allylic hydroperoxide **3** with an acid can protonate either the terminal (**7**) or the external (**8**) oxygen of the hydroperoxide. In external case, the hydroperoxide becomes an excellent leaving group that can be displaced by the carboxylic acid functional group, generating the undesired five-membered lactone **9**. If the terminal oxygen is protonated, migration of the allylic group is favored leading to the formation of hemiketal **10**. Under acidic conditions, the hemiketal can be opened to give the corresponding enol **4**, which is an intermediate in the formation of artemisinin. The highly sensitive enol **4** can be converted, under acidic conditions, into aldehyde **11**, which condenses to the six-membered lactone **12**. To suppress the formation of **9**, the carboxylic acid of dihydroartemisinic acid can be converted into an ester or a mixed anhydride, requiring additional (earlier) steps. Unfortunately, use of the masked carboxylic acid considerably increases the formation of the six-membered lactone byproduct (**12**, Scheme 2).

Different Brønsted and Lewis acids were tested, such as camphorsulfonic acid, copper(II) trifluoromethanesulfonate, DOWEX, *p*-toluenesulfonic acid, and trifluoroacetic acid in various solvents to find the most efficient conditions for converting **3** into desired intermediate **4**. Trifluoroacetic acid (TFA) performed best to induce the desired solvent-dependent Hock cleavage. The final synthetic transformation from **4** via intermediate **5** to artemisinin **6** requires an oxidation with triplet oxygen, which triggers a series of condensation reactions[16] that generate three rings and incorporates the endoperoxide functional group.

The Hock cleavage was conducted sequentially with triplet-oxygen oxidation, by treating **3** (obtained after the photo-transformation of **2** in the flow reactor) with 0.5 equivalents of TFA at 0 °C while bubbling oxygen for 2 h, and gave a 50 % yield of **6** after purification by chromatography.

The same reaction was then adapted to the continuous-flow reactor as a move towards combining all three steps in a single continuous-flow process. The ratio of **6** to the undesired lactone byproducts was found to be highly dependent on the solvent and the reaction temperature. Optimal results were obtained when a 42 mL reactor was used with the solution of **3** in dichloromethane added at a flow rate of 2.5 mL min^{-1}, oxygen at 5.0 mL min^{-1}, and TFA in dichloromethane at 0.5 mL min^{-1}. The first portion of the reactor (32 mL) was maintained at room temperature while the last portion (10 mL) was heated to 60 °C to push the reaction to completion. Under these conditions the desired product **6** and the undesired five-membered lactone byproduct **9** were produced at a ratio of 5.3:1.0 in favor of **6**. Subsequent purification by chromatography yielded 46 % of artemisinin from dihydroartemisinic acid in this sequential continuous-flow reaction.

A single, fully integrated continuous-flow synthesis to convert **2** into **6** required a reactor that would receive as input, **2**, oxygen gas, and photosensitizer. The reactor would introduce TFA at the appropriate time, allowing for the photochemical step, as well as for transformations without light, to continuously produce artemisinin. For this purpose, a commercially available continuous-flow system that included HPLC pumps was combined with our in-house photochemical flow set-up, as well as an additional mixer and thermal reactor (Figure 1).

The reactor is composed of: A) Reservoir for the solution of dihydroartemisinic acid and TPP in CH_2Cl_2, B) HPLC pump, C) mass-flow controller to control the flow rate of oxygen, D) manometer, E) oxygen tank, F) check valve, G) ETFE T-mixer, H) FEP tubing, I) quartz immersion well connected to a cooling system, J) Pyrex filter, K) connection to the medium-pressure Hg lamp (450 W), L) reservoir for the TFA solution, M) acid-resistant HPLC pump, N) PTFE thermal reactor, O) back-pressure regulator, and P) collection flask. In this reactor, a solution of **2** and the photosensitizer TPP in dichloromethane were mixed at a flow rate of 2.5 mL min^{-1} with a stream of oxygen gas (7.5 mL min^{-1}), and passed through the photoreactor. The residence time in the reactor is approximately 2.0 min.

Using the acid-resistant pump from the Vapourtec RC2+ unit, a solution of TFA in dichloromethane was added at a flow rate of 0.5 mL min^{-1} to the outlet stream of the photoreactor, that contained mainly **3**, to induce the acid-catalyzed Hock cleavage. The acid can be added at the beginning or just after the completion of the singlet-oxygen reaction, however, better results were obtained when the acid was added after the photooxidation, because protonation of TPP reduces its reactivity.

Hock cleavage took place in a PTFE reactor (26 mL volume total, with 16 mL maintained at room temperature and 10 mL heated at 60 °C). A residence time of approximately 2.5 min was required for the Hock cleavage, oxidation with triplet oxygen, and further condensation. After a total residence time of 4.5 min a product stream, comprising mainly **6** was obtained. Purification by chromatography followed and yielded 39 % of **6** from dihydroartemisinic acid (**2**).

Based on this result, we calculate that this particular set-up could produce 200 g of artemisinin per day. Estimating that roughly 225 million doses (number of cases of malaria estimated by the WHO; in 2009 the number of ACT treatment courses procured was 158 million)[1] of the anti-malarial medication are needed per year, approximately 1500 efficient, simple, and productive photoreactors could meet this demand. Further optimization may result in even higher yields and productivities, but even at this time, the process disclosed herein is capable of meeting the ever-growing demand for low-cost artemisinin to treat malaria victims around the world.

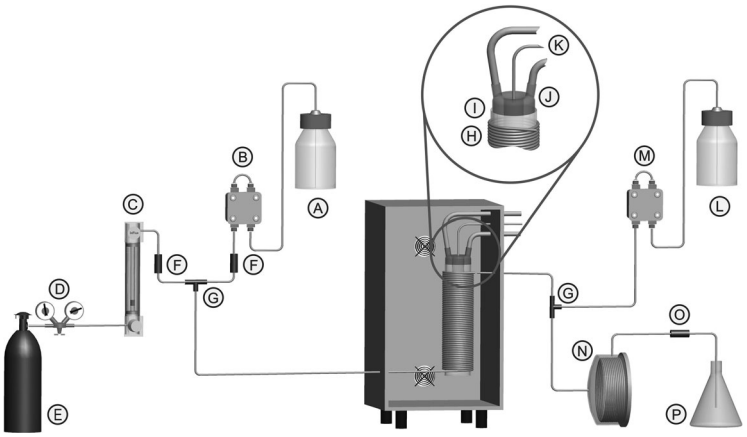


Figure 1. Continuous flow set-up for the conversion of dihydroartesiminic acid (**2**) into artemisinin (**6**). See text for details.

Experimental Section

Optimized Reaction Conditions for Continuous Flow Synthesis of Artemisinin

A solution of dihydroartemisinic acid (**2**, Supporting Information Scheme SI-4; 2.95 g, 5.0 mmol) and tetraphenylporphyrin (TPP, 15 mg, 0.025 mmol) in dichloromethane (CH_2Cl_2; total volume of the solution: 25 mL, volumetric flask), and a solution of trifluoroacetic acid (TFA, 1.9 mL, 25 mmol) in CH_2Cl_2 (18 mL) were prepared. The lamp was turned on 30 min prior to the beginning of the experiment and the second section of the PTFE reactor

was heated to 60 °C. The entire reactor was flushed with pure CH_2Cl_2 (pump 1: 2.5 mL min^{-1}, pump 2: 0.5 mL min^{-1}), and oxygen (7.5 mL min^{-1}, 11.5 bar) for 10 min. The solution of reagents (**2** and TPP in CH_2Cl_2) was then injected at a flow rate of 2.5 mL min^{-1} and the flow rate of oxygen was adjusted to 7.5 mL min^{-1} (11.5 bar). The solution of TFA was injected at a flow rate of 0.5 mL min^{-1} after the solution exits the photoreactor but prior to entering the first PTFE reactor. The crude product was collected in a flask containing a saturated aqueous solution of $NaHCO_3$. The resulting biphasic mixture was stirred at room temperature until the green color disappeared. The phases were separated and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Purification over silica gel (5–20% EtOAc in cyclohexane) afforded artemisinin **6** (1.36 g, 39%) as an off-white solid. Further purification by recrystallization in cyclohexane afforded white needles. Mp = 153–154 °C. $[\alpha] = +66.3°$ ($c = 0.97$, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.86$ (s, 1H), 3.40 (dq, $J =$ 7.3, 5.4 Hz, 1H), 2.47–2.39 (m, 1H), 2.08–1.98 (m, 2H), 1.91–1.86 (m, 1H), 1.81–1.74 (m, 2H), 1.51–1.34 (m, 3H), 1.45 (s, 3H), 1.21 (d, $J =$ 7.3 Hz, 3H), 1.11–1.04 (m, 2H), 1.00 ppm (d, $J = 6.0$ Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 172.2$, 105.5, 93.9, 79.6, 50.2, 45.1, 37.7, 36.1, 33.8, 33.0, 25.4, 25.0, 23.6, 19.9, 12.7 ppm. IR (film): $\tilde{\nu} =$ 2960, 2933, 2860, 1731, 1112, 991 cm^{-1}. HRMS calcd for $C_{15}H_{22}O_5$ [M^+] 282.1467, found 282.1463. MS (EI) m/z 282 (1) [M^+], 250 (5), 192 (70), 150 (40), 55 (63), 43 (100). In agreement with published data.[17]

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