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Machines vs Malaria: A Flow-Based Preparation of the Drug Candidate OZ439

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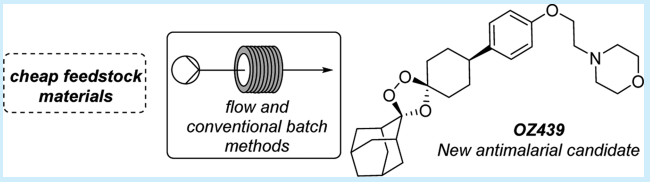
Supporting Information

ABSTRACT: An efficient preparation of the antimalarial drug candidate OZ439, which was obtained by integrating a machine-assisted approach with batch processes, is reported. This approach allows a rapid and cost-effective production of the key intermediates that were readily elaborated into the target molecule.



Even in our highly developed society, malaria still represents a threat to humankind.[1] The statistics for 2012 indicate that this infection caused the deaths of more than half a million people, with a further quarter billion being infected in most cases by *Plasmodium falciparum*. Despite considerable research efforts, effective antimalarial chemotherapy is still needed.[2] While efficacy, pharmacokinetics, and pharmacodynamics are key issues to address, during the search for new potential antimalarial treatments the primary criterion is related to the cost of goods.[3] New antimalarial agents therefore must be available at low cost in order to match the economies mostly afflicted by this disease. Drug resistance and the widespread use of artemisinin and its derivatives (**1a**–**d**, Figure 1) are important additional issues that need to be addressed.[4,5] Climate change is also a problem, causing malaria to spread to more heavily populated regions of the world.[6]

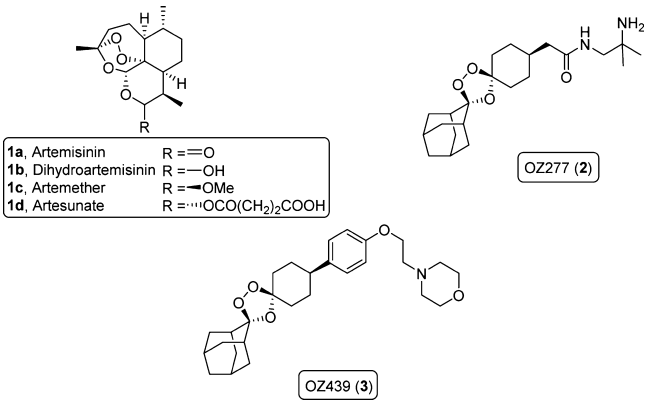


Figure 1. Artemisinin and its derivatives (**1a**–**d**), OZ277 (**2**), and OZ439 (**3**).

Recent investigations[7] have led to the identification of new types of antimalarial agents containing a trioxolane unit as main structural feature, with compound OZ277 (**2**) as one of the most promising hits arising from this research.[2a,8] Further studies directed to improve the pharmacokinetic and pharmacodynamic properties resulted in the identification of the more potent analogue OZ439 (**3**).[2a,9] The important property of **3** is that it provides a single oral dose treatment for the total eradication of the parasite in humans; it is currently undergoing phase IIa trials after having successfully completed phase I clinical trials.[2a,10]

Despite the promising activity displayed by the drug candidate, its preparation is not without problems. For example, the reported synthesis of this compound relies on the use of commercially available but relatively expensive 4-(4-hydroxyphenyl)cyclohexan-1-one (**4**) and makes use of large quantities of pentane, which is an undesirable solvent for the preparation of the key trioxolane motif.[11] Additionally, the morpholine functionality is introduced using a potentially genotoxic material in the last synthetic step (Scheme 1a).

Enabling technologies, in particular flow chemistry, provide benefits and opportunities to devise new strategies for molecular assembly. Additionally, the flexibility of continuous processing of material is highly amenable to later scale-up, thereby making this an attractive approach. By integrating new technologies with traditional batch methods, we aimed to overcome some of the synthesis issues associated with the preparation of OZ439. This could, in principle, pave the way for low cost manufacturing in the countries where malaria is prevalent. To this end, we have devised a route integrating the advantages of batch- and continuous-processing methods (Scheme 1b).[12,13]

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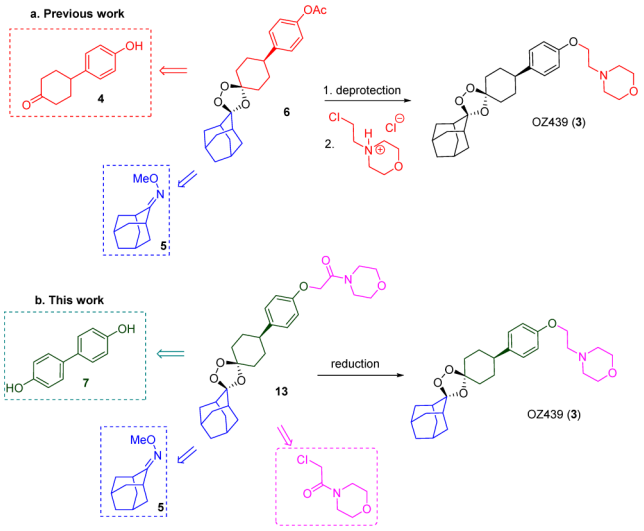
Scheme 1. (a) Previous Preparation of OZ439; (b) This Work

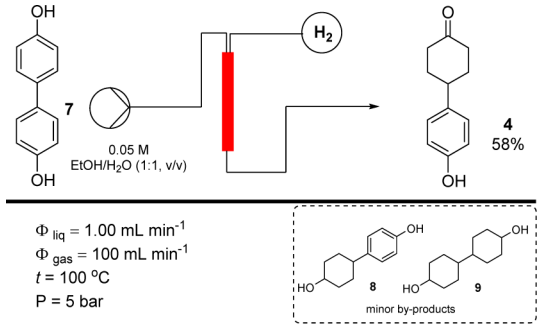


To achieve of our initial aim, we envisaged a new preparation of the intermediate trioxolane **6**[7,9] which would take advantage of the use of enabling methods. Key transformations in this new strategy would be the selective reduction of readily available biphenol **7** to *selectively* afford 4-(4-hydroxyphenyl)cyclohexan-1-one (**4**) and subsequent reaction of this ketone with the *O*-methyl oxime **5** by co-ozonolysis. We would then install the amide substituent (to furnish **13**) and eventually reveal the final product, OZ439 (**3**), via an amide reduction process.

Previous work from our group has shown the extreme flexibility of a small footprint platform (HEL FlowCAT)[14,15] for liquid/gas reactions under trickle heterogeneous catalysis conditions. Our approach for the selective reduction of **7** to **4** started with the screening of relevant parameters, including catalyst, temperature, gas feed, liquid feed, and substrate concentration as well as solvent, which are all conveniently achieved on this machinery.[16]

Catalyst screening allowed us to identify Pd/C 20 mol % as a suitable material for the transformation. Using other metal catalysts (i.e., Rh and Pt) or lowering the loading of Pd (5, 10, or 15 mol %) reduced the selectivity as well as the conversion. Similarly, negative results were obtained when moving from Pd/C to Pd/Al_2O_3. We also found that although the use of EtOH as solvent gave the best results in terms of conversion, the use of $EtOH/H_2O$ (1:1, v/v) as solvent mixture yielded the best result in terms of selectivity. The concentration was set at 0.05 M (equating to a throughput of 3 mmol h^{-1}) to avoid any precipitation of starting material **7** or product **4**. Temperature was another important factor to consider in the optimization because higher or lower temperatures either reduced the selectivity or decreased the conversion of **7** to **4**, thereby increasing the amount of the otherwise minor byproducts **8** and **9** (Scheme 2). Similar behavior was observed for the amount of H_2 fed into the system, with flow rates above and below 0.1 L min^{-1} giving disruption in terms of both selectivity and conversion. Under the optimized conditions, a solution of **7** in $EtOH/H_2O$ (1:1 v/v, 0.05 M) was passed through a packed bed reactor (3 mL internal volume) containing the heterogeneous catalyst (Pd/C, 20 mol %) at 1.00 mL min^{-1}, feeding the reactor with 0.1 L min^{-1} of H_2, while keeping the pressure of the whole system at 5 bar. With this protocol, we

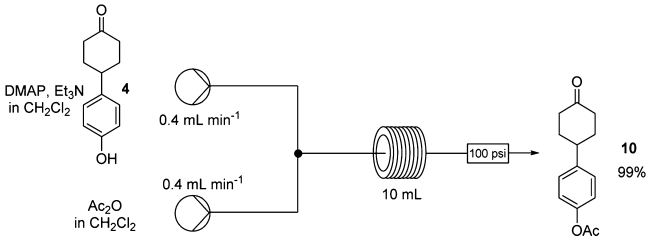
Scheme 2. Selective Continuous Hydrogenation of 4,4′-Biphenol 7 To Prepare 4



were able to quickly isolate pure material in an appreciable yield of 58% after recrystallization from the reaction mixture (>95% purity as determined by NMR). By simply extending the reaction run time, we successfully delivered around 2 g of compound **4** in 5 h, with this reactor setup. This result clearly highlights the advantages of continuous processing compared to the batch protocol.[17]

With an optimized protocol for preparation of ketone **4** in hand, we then developed a method for its continuous acetylation. In a very straightforward manner, we were able to perform the desired transformation in quantitative yield. Suitable conditions were soon found whereby a solution of phenol **4** in CH_2Cl_2 was combined at a T-piece with a stream of acetic anhydride (1.5 equiv), DMAP (5 mol %), and Et_3N (4.5 equiv) in CH_2Cl_2 (combined flow rate 0.8 mL min^{-1}) and reacted in a 10 mL PFA coil at rt (Scheme 3). Simple filtration through silica and evaporation of the reactor output gave **10**, which could be used in the next stage without a need for further purification.[16]

Scheme 3. Flow Acetylation of 4-(4-Hydroxyphenyl)cyclohexan-1-one 4 to afford 10



Next, we commenced an investigation of the Griesbaum oxidation reaction under flow conditions.[18] The co-ozonolysis reaction between ketones and alkylated oximes had been reported in 1997 by Griesbaum et al. as an efficient methodology for the preparation of 1,2,4-trioxolane.[19,20] We began by adapting a flow set up which had been previously proven to effectively afford the continuous ozonolysis of olefins.[21] The investigation of processing conditions for the reaction was carried out using 4-phenylcyclohexanone **11** as a commercially available model substrate.[15] A solution containing the oxime **5** and the ketone **11** (X = H) in EtOAc was reacted with a stream of ozone. Both the liquid and gas feed were adjusted to maximize the reaction yield.[16] Experiments conducted with an excess of ketone counterpart partner afforded the reaction product in moderate yield while an excess of oxime with respect to the ketone afforded a great

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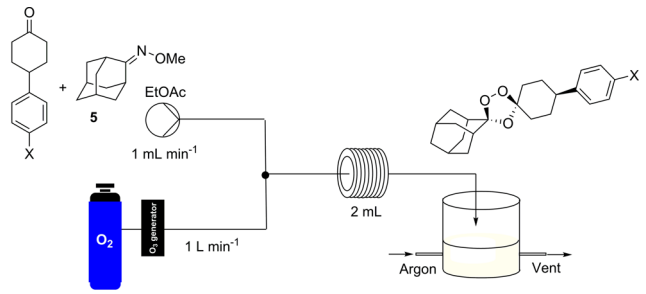
improvement of reaction yield, leading to isolation of 76% of the desired compound **12** after purification (Scheme 4). The

Scheme 4. Flow Setup for the Griesbaum Co-ozonolysis



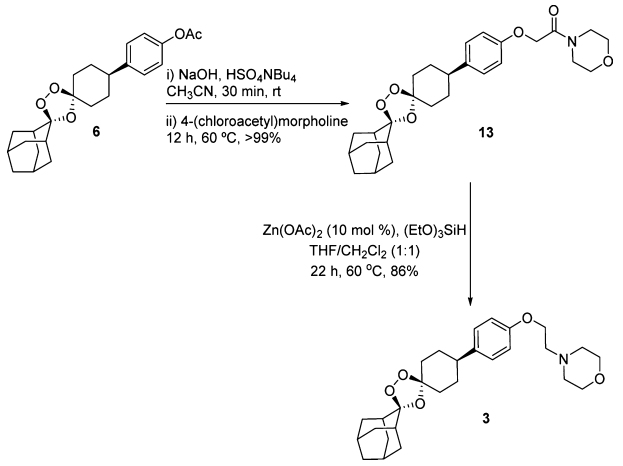
continuous process was run for 45 min without issue. Unfortunately, under longer reaction times blockages occurred due to the slight evaporation of the solvent. This was easily avoided by decreasing the concentration of oxime **5** (from 0.4 to 0.2 M) and ketone **11** (from 0.2 to 0.1 M) while increasing both the liquid and gaseous flow rates (1 mL min^{-1} and 1 L min^{-1}, respectively) in order to keep an identical throughput for the reaction system.

Under these diluted conditions we were able to continuously run the reaction without any clogging issue for over 3 h, isolating 78% of the model product with a calculated throughput of 1.6 g·h^{-1} (equating to 38.4 g·d^{-1}).

We then assessed the suitability of this particular reactor setup (Scheme 4) for the flow co-ozonolysis between **5** and **10**. Pleasingly, the reaction proceeded nicely and allowed the isolation of the desired 1,2,4-trioxolane **6** in 70% yield (9:1 cis–trans selectivity toward the desired product). This yield equates to a production of 1.9 g·h^{-1} (45.6 g·d^{-1}). The procedure clearly surpasses limitations and safety issues associated with operating with ozone in batch mode,[20] in particular, by avoiding the use of pentane as solvent by replacing it with ethyl acetate. Additionally, this approach ensures continuous production of the desired material in good yield. Once an efficient synthesis of this key fragment **6** was assured, the compound was subjected to reaction conditions previously reported in the literature for the cleavage of the acetyl group. While the formed phenoxide anion can be reacted with 4-(2-chloroethyl)morpholine affording OZ439 (**3**) in excellent yield,[16] we wanted to examine whether an alternative route that would avoid the use of the genotoxic agent 4-(2-chloroethyl)morpholine was feasible (Scheme 5).

We designed a simple alternative route to avoid the use of this material that consisted of two steps: the generation of the amide intermediate **13** and its reduction to produce the API OZ439 (**3**). We began with the preparation of **13** via a straightforward nucleophilic substitution of the *in situ* deprotected phenolate in the presence of 4-(chloroacetyl)-morphine. This product (**13**) was then subjected to a selective reduction of the amide moiety. We noticed a remarkable sensitivity associated with the trioxolane ring of **13**, as most reducing reagents (i.e., BH_3·THF complex, BH_3·SMe_2 complex, $NaBH_4$, $LiAlH_4$, and $Tf_2O/NaBH_4$) resulted in both reduction of the amide group and cleavage of the trioxolane moiety. To our delight, however, using a Zn-catalyzed amide reduction in

Scheme 5. Synthesis of OZ439 via Reduction of Amide 13



the presence of triethoxysilane,[22] we found that OZ439 (**3**) could be obtained in 86% isolated yield without disruption of the trioxolane system.

In summary, we have developed a more robust and efficient protocol for the synthesis of the antimalarial drug candidate OZ439 (**3**), using a machine-assisted protocol. Flow technologies were successfully applied to implement three key transformations (i.e., selective partial hydrogenation, acetylation reaction of the phenol group, and Griesbaum co-ozonolysis) surpassing limitations previously observed for batch process while affording a very attractive outcome in terms of continuous processing. The new route avoids the use of the genotoxic 4-(2-chloroethyl)morpholine. The development of a fully continuous synthesis of OZ439 (**3**) is currently under investigation in our laboratories.

■ ASSOCIATED CONTENT

Supporting Information

Characterization data for all compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01307.

■ AUTHOR INFORMATION

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Author Contributions

‖S.-H.L. and A.G. contributed equally to this work.

Notes

The authors declare no competing financial interest.

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