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Continuous Flow Reduction of Artemisinic Acid Utilizing Multi-Injection Strategies - Closing the Gap Towards a Fully Continuous Synthesis of Antimalarial Drugs

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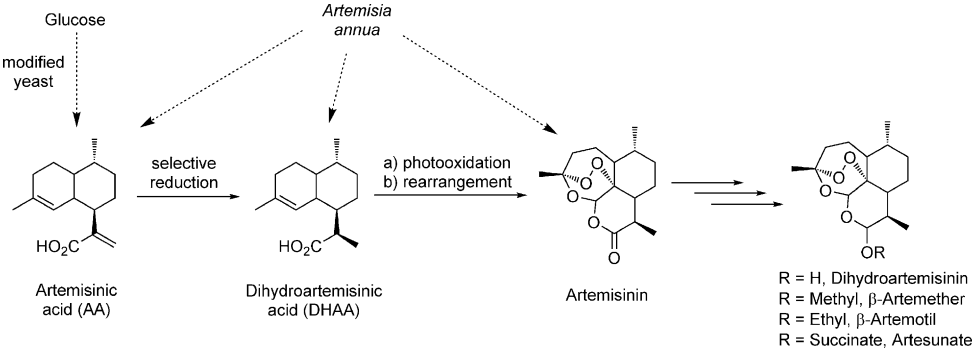
Abstract: One of the rare alternative reagents for the reduction of carbon–carbon double bonds is diimide (HN=NH), which can be generated in situ from hydrazine hydrate ($N_2H_4 \cdot H_2O$) and O_2. Although this selective method is extremely clean and powerful, it is rarely used, as the rate-determining oxidation of hydrazine in the absence of a catalyst is relatively slow using conventional batch protocols. A continuous high-temperature/high-pressure methodology dramatically enhances the initial oxidation step, at the same time allowing for a safe and scalable processing of the hazardous reaction mixture. Simple alkenes can be selectively reduced within 10–20 min at 100–120 °C and 20 bar O_2 pressure. The development of a multi-injection reactor platform for the periodic addition of $N_2H_4 \cdot H_2O$ enables the reduction of less reactive olefins even at lower reaction temperatures. This concept was utilized for the highly selective reduction of artemisinic acid to dihydroartemisinic acid, the precursor molecule for the semisynthesis of the antimalarial drug artemisinin. The industrially relevant reduction was achieved by using four consecutive liquid feeds (of $N_2H_4 \cdot H_2O$) and residence time units resulting in a highly selective reduction within approximately 40 min at 60 °C and 20 bar O_2 pressure, providing dihydroartemisinic acid in $\geq$93% yield and $\geq$95% selectivity.

Introduction

The advantages of continuous-flow processing are increasingly appreciated by a growing number of scientists, from research chemists in academia to process chemists and chemical engineers in pharmaceutical companies. Thus, continuous-flow technologies are nowadays more and more used on a routine basis on laboratory as well as industrial scales for fine chemical production.[1–3] An intriguing recent example of industrial importance is the development of a continuous photochemical reactor for the synthesis of artemisinin from dihydroartemisinic acid.[4]



Scheme 1. Production routes to Artemisinin and its derivatives.

Artemisinin, a sesquiterpene endoperoxide, is the key component of so called artemisinin-based combination therapies (ACTs), which are currently the standard treatment for malaria.[5] The pharmaceutically active ingredient can be extracted in low amounts (typically $\leq$1 wt%) from *Artemisia annua* (sweet wormwood) but unfortunately, environmental and economic factors render the amount of artemisinin obtained to be unpredictable, causing a steadily fluctuating price situation.[6] These issues can be tackled by semisynthetic approaches starting from potential precursor molecules like artemisinic acid (AA) or dihydroartemisinic acid (DHAA), which can be also found in the plant extract (Scheme 1).[7] Furthermore, recent breakthroughs in synthetic biology have shown that glucose can be used to generate amorphadiene or even AA in genetically modified yeast, offering a plant-independent production strategy.[8,9]

The semisynthetic route to artemisinin is based on a biomimetic, photochemical reaction of DHAA which was discovered in the late 1980s.[10] Central in this chemical transformation is an

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ene reaction of dihydroartemisinic acid with singlet oxygen ($^1O_2$). The photooxidation is subsequently followed by a Hock cleavage, addition of triplet oxygen, and a series of spontaneous condensation reactions. Unfortunately, the photochemical generation of singlet oxygen proved to be extremely challenging to scale. In fact, it took industrial scientists more than two decades to overcome these limitation and recently researchers from Sanofi–Aventis were able to adapt this chemical route for industrial artemisinin production.[11] Simultaneously, Seeberger and co-workers showed that the complete reaction sequence can be ultimately performed as a single, fully continuous chemical process.[4] In addition, the same group recently communicated a module-based continuous-flow strategy for the synthesis of dihydroartemisinin, β-arthemeter, β-artemotil, and artesunate from AA, all ingredients of ACT antimalarials.[12]

Since plant extraction, as well as synthetic biology, provides AA, a simple and efficient strategy for the diastereoselective reduction to DHAA is essential for a productive process. Batch hydrogenation in the presence of Wilkinson's catalyst results in quantitative conversion and good diastereoselectivity (94:6) after 19 h at 80 °C at 47 bar hydrogen pressure.[9b] The improved protocol by Sanofi–Aventis provides similar results within 6 h at room temperature and 22 bar using a ruthenium catalyst in the presence of triethylamine.[11] Importantly, the same group recently presented a catalyst-free protocol using diimide (N_2H_2), generated in situ from hydrazine hydrate and O_2.[13] Due to safety reasons, the highest admissible oxygen concentration allowed in the presence of a flammable solvent (*i*PrOH) was 5% O_2 in N_2 (v/v). At 40 °C, full conversion was achieved after 11 h using only 3 equivalents of $N_2H_4{\cdot}H_2O$. The surprisingly high diastereomeric ratio (≥97:3) achieved in this reduction process can be explained mechanistically.[14]

In situ generated diimide has been used as transfer hydrogenation agent for more than one century.[15] Several methods for its generation, such as decarboxylation of dipotassium azodicarboxylate or the thermal decomposition of sulfonylhydrazides, are known.[15] However, from an (atom-)economic point of view, the aerobic oxidation of N_2H_4 is the most attractive source. Since this oxidation is generally very slow, several catalysts, such as Cu[15] or Fe salts,[16,17] guanidine derivatives,[18] flavin-based catalysts,[19] or even visible light,[20] were studied. The generation of diimide in batch by oxidation of hydrazine with O_2 in the absence of a catalyst is also possible, even though significantly longer reaction times in combination with a high excess of hydrazine are required to reduce simple olefins.[21]

Recently, our group reported a catalyst-free continuous-flow protocol for the selective reduction of olefins to alkanes based on the highly efficient in situ generation of N_2H_2 from $N_2H_4{\cdot}H_2O$ and O_2.[22] Herein, we present the application of this methodology towards the stereoselective reduction of artemisinic acid to dihydroartemisinic acid under relatively mild conditions, applying a multi-injection continuous-flow platform.

Results and Discussion

Continuous flow concept

The reduction of olefins is traditionally carried out by metal-catalyzed processes involving hydrogen gas, typically at elevated pressures in dedicated hydrogenation devices. However, in certain cases this strategy is accompanied by severe selectivity problems as several undesired side-reactions, such as hydrogenolysis of protecting groups, reduction of other functionalities, alkene migration or racemization, can occur.[19b] In contrast, in situ generated diimide is an extremely selective reagent for the reduction of unsaturated carbon–carbon bonds.[15] The generation of this highly unstable and reactive azo compound from simple hydrazine hydrate and oxygen/air is of particular interest, as both reagents are readily available and of low cost. In addition, a catalyst-free protocol ultimately results in a virtually workup-free methodology, as only water and nitrogen gas are generated as benign chemical byproducts. However, since this reaction is predominantly carried out in the presence of a catalyst,[15–19] the oxidation of hydrazine is a rather slow and inefficient process under standard batch conditions.[21] Notably, diimide generation using this oxidation protocol results not only in the reduction of olefins; an over-oxidation process of the unstable N_2H_2 intermediate results in the formation of nitrogen and water. Furthermore, a disproportionation can cause the formation of hydrazine and nitrogen. Thus, the diimide precursor has to be usually added in (high) excess to guarantee a quantitative consumption of the unsaturated starting material.

Based on this knowledge, we started to design a continuous-flow reactor for the reduction of olefins using this methodology. Our initial hypothesis was that the typical high surface-to-volume areas in a biphasic reaction mixture in continuous flow should dramatically enhance the oxidation rate of hydrazine hydrate.[2] Furthermore, we assumed that the additional use of a high-temperature/high-pressure protocol (Novel Process Windows)[23] would push the diimide generation to its limits. We thus assembled a two-feed continuous-flow reactor (Figure 1).

As shown in Figure 1, an HPLC pump (P) was used to continuously pump the olefin and hydrazine hydrate dissolved in a proper solvent. Oxygen was delivered from a standard compressed-gas cylinder and the flow controlled by a mass-flow controller (MFC). The two reagent streams were combined in a glass static mixer (GSM) resulting in a segmented flow pat-

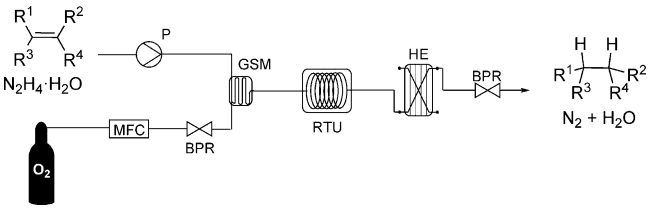


Figure 1. Two-feed continuous-flow setup for the reduction of olefins with diimide.

tern, which was then allowed to pass a heated residence time unit (RTU). For that purpose, perfluoroalkoxy tubing (PFA, i.d. = 0.8 mm, o.d. = 1.6 mm) was used, which has lower gas permeability than, for example, PTFE. Concerning gas permeability, stainless steel would be a more suitable option but, from a chemical point of view, we were concerned that over time iron oxide could be generated in such a corrosive regime. This material was recently shown to catalyze the reduction of nitro and azide groups in the presence of hydrazine and could therefore cause selectivity problems in certain cases.[17b,24] The reaction mixture was finally cooled in a heat exchanger and depressurized by passing a back-pressure-regulating unit (BPR).

Process intensification, scope, and limitations

Initially, the reduction of allylbenzene was studied using the above described continuous-flow setup. Since most protocols involving the generation of diimide from hydrazine and oxygen use polar, protic solvents, such as ethanol, we decided to start the process intensification by screening various alcohols as reaction media. For that purpose, the model substrate (0.1 M) and 5 equivalents of hydrazine hydrate where dissolved in different alcohols (methanol, ethanol, isopropanol, *n*-propanol, *n*-butanol, *n*-pentanol) and pumped at a flow rate of 0.4 mL min^{-1}. The oxygen stream was set at 40 mL$_N$ min^{-1}, resulting in a suitable segmented flow pattern at a back pressure of 20 bar. By using a 10 mL PFA coil, a residence time of 10 min was observed, which appeared to be adequate for comparing different solvents for the chosen model reaction. The assessment of the different alcohols at 80 °C showed a nearly linear trend with respect to the reaction rate (Figure 2). This observation seems to directly relate to the oxygen solubility in this series of alcohols.[25] However, when the same experiments were carried out at a somewhat lower temperature (60 °C) a significant reduction of this phenomenon was observed. Whereas methanol and ethanol resulted in roughly 10% conversion of allylbenzene to propylbenzene, reactions using the higher-boiling homologues showed starting material consumptions between 30% and 40%. Therefore, we assume that, in particular at elevated temperatures, the solvent choice is of crucial importance for enhancing the rate-determining oxidation step.

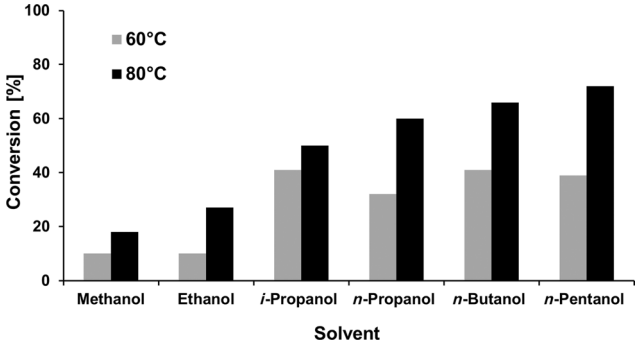
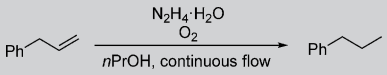


Figure 2. Solvent screening for the continuous reduction of allylbenzene using 5 equivalents of hydrazine hydrate in presence of oxygen.

Since this catalyst-free methodology generates only water and nitrogen as byproducts, finding a convenient workup protocol was one of the ultimate goals of this study. Given the low-boiling nature of some potential target molecules, *n*PrOH (b.p. = 97 °C) was the solvent of choice, being a compromise between reaction rate at higher temperatures and the ability for smooth solvent evaporation. The model reaction was further optimized aiming for quantitative consumption of the starting material (Table 1).

Table 1. Optimization of the reduction of allylbenzene using in situ generated diimide.[a]

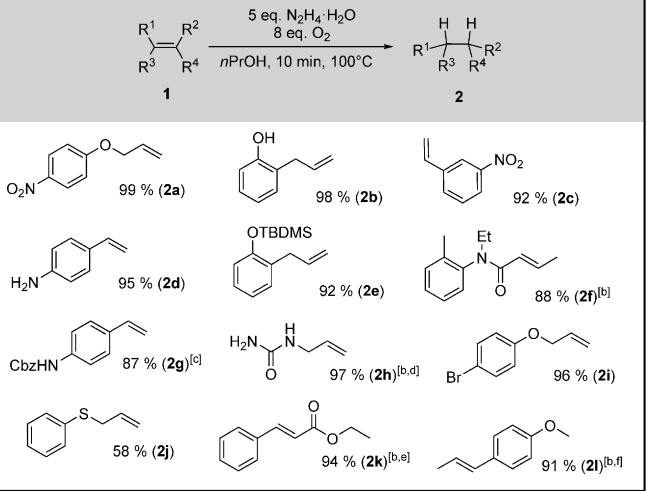


Entry	Concentration [M]	$N_2H_4·H_2O$ [equiv]	Oxidant	T [°C]	Conversion [%][b]
1	0.1	5	O_2	80	60
2	0.1	5	O_2	100	94
3	0.2	5	O_2	100	98
4	0.4	5	O_2	100	>99
5	0.5	5	O_2	100	>99
6	0.5	5	air	100	53
7	0.5	4	O_2	100	>99
8	0.5	3.5	O_2	100	98
9	0.5	3	O_2	100	97
10	0.5	3	O_2	120	98
11	0.5	0	O_2	100	<1
12	0.5	4	N_2	100	8

[a] Conditions: Allylbenzene and hydrazine hydrate in *n*PrOH (2 mL), 0.4 mL min^{-1} liquid flow rate, 40 mL$_N$ min^{-1} gas flow rate, 20 bar back pressure. [b] Conversion was determined as GC peak area percent.

When the reaction temperature was increased to 100 °C, a significantly higher conversion was obtained (Table 1, entry 2). We further noticed that better conversions could be achieved by using a higher olefin concentration (Table 1, entries 3–5). The use of a 0.5 M solution resulted in an oxygen stoichiometry of approximately 8 equivalents. Changing the oxidizing agent to synthetic air (ca. 1.6 equivalents O_2) caused a dramatic reduction in conversion (Table 1, entry 6). Nevertheless, it was possible to reduce the amount of hydrazine hydrate to 4 equivalents, resulting in a selective and quantitative reduction of allylbenzene within just 10 min (Table 1, entry 7). Further attempts to reduce the amount of the diimide precursor gave incomplete reactions, even at higher temperatures, corroborating the need for a relatively high excess of hydrazine owing to over-oxidation and disproportionation of diimide (Table 1, entries 8–10). In the absence of hydrazine hydrate, no conversion was observed at all (Table 1, entry 11). Since a catalytic hydrazine-mediated reduction of double bonds was also reported to proceed under inert conditions,[17] a control experiment using N_2 instead of O_2 was carried out, resulting in a conversion of only 8%. This can be rationalized by small amounts of oxygen dissolved in *n*PrOH. The optimized conditions were further tested for a range of simple olefins to evaluate the

Table 2. Reduction of olefins in continuous flow using hydrazine hydrate and oxygen.[a]



[a] Conditions: Olefin (0.5 M) and hydrazine hydrate (4 equiv) in *n*PrOH, 0.4 mL min^{-1} liquid flow rate, 40 mL$_N$ min^{-1} O_2 flow rate, 20 bar back pressure, 10 mL PFA coil. [b] 5 equivalents of hydrazine hydrate were used. [c] conc. = 0.33 M. [d] *n*PrOH/H_2O (1:1 v/v) as solvent. [e] Reaction was carried out at 120 °C in a 16 mL coil. [f] Reaction was carried out at 120 °C in a 26 mL coil.

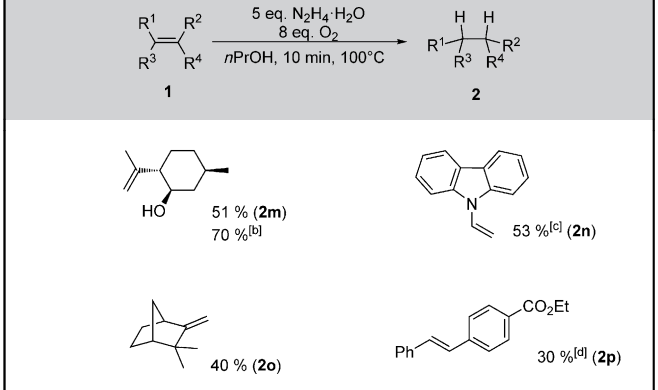
scope and robustness of the continuous methodology (Table 2).

The majority of the chosen simple olefins resulted in quantitative conversion under the optimized reaction conditions (**2a**–**e**, **g**, **i**, **j**). However, in some cases it was necessary to increase the amount of hydrazine hydrate to drive the reaction to completion (**2f**, **h**). For some less reactive examples an increased reaction temperature in combination with a somewhat longer reaction time successfully led to the saturated derivatives (**2k**, **l**). Noteworthy, neither a nitro-reduction (**2c**) nor a deprotection (**2e**, **g**) as side reaction was observed. The only example where a nonselective transfer hydrogenation occurred was by using sulfide **2j**. In this case, oxidation products (sulfoxides and sulfones) of both the olefin and its saturated derivative were observed. We argue that this is not a result of an aerobic oxidation, but more likely due to the formation of hydrogen peroxide during the hydrazine oxidation process. A similar finding was made by Imada and co-workers, who reported that the hydrazine/O_2 system can oxidize sulfides and amines under ambient conditions in the presence of a flavin catalyst.[26] The main limitation of this protocol was the use of aldehydes or ketones as starting materials, as these functionalities immediately undergo hydrazine and azine formation in the presence of hydrazine. It is somewhat surprising that ethyl cinnamate (**2k**) could be selectively reduced, even though the ester moiety is prone to hydrazide formation. In fact, the same reaction in batch under atmospheric conditions (O_2 balloon) resulted mainly in the unreduced hydrazide after 1–2 h at 120 °C. An autoclave experiment resulted in selectivity similar to the continuous experiment under more or less identical conditions (120 °C, 20 min, 10 bar). We can therefore conclude that the oxidation efficiency in the continuous protocol is clearly more a result of the high-temperature/high-pressure concept than of the high surface-to-volume area obtained by the segmented flow pattern.

Efficiency of hydrazine oxidation

The above-described method is limited to olefins of relatively high reactivity. When less reactive substrates, such as menthol (**2m**), were passed through the continuous-flow reactor under optimized conditions only moderate conversions were obtained (Table 3). Even at higher temperatures (120 °C) and a longer residence time (ca. 30 min, 26 mL coil volume) only 70% of the starting material was consumed. Similar reactivates could be observed for the carbazole derivative **2n**, camphene (**2o**) and a stilbene analog (**2p**).

Table 3. Conversion of olefins of lower reactivity in continuous flow using hydrazine hydrate and oxygen.[a]



[a] Conditions: Olefin (0.5 M) and hydrazine hydrate (4 equiv) in *n*PrOH, 0.4 mL min^{-1} liquid flow rate, 40 mL$_N$ min^{-1} O_2 flow rate, 20 bar back pressure, 10 mL PFA coil. Conversions determined as GC-FID peak area percent. [b] Reaction was carried out at 120 °C in a 26 mL coil. [c] Toluene/*n*PrOH (3:7 v/v) was used as solvent. [d] 0.1 M in toluene/*n*PrOH (3:2 v/v).

It appears that the competitive over-oxidation and disproportionation of diimide consumes comparably high amounts of the reactive intermediate resulting in insufficient reduction rates. This would imply that a total reduction of such compounds would theoretically need an enormous excess of hydrazine and therefore also the amount of oxygen gas would have to be increased dramatically. To overcome this severe limitation, a deeper understanding of the initial oxidation step is clearly of great importance.

To get a general idea of the efficiency of the initial hydrazine oxidation and the unwanted (side) reactions of the highly reactive diimide species, we decided to carry out a range of trapping experiments. The underlying idea was that, in a first coil, O_2 and $N_2H_4 \cdot H_2O$ are allowed to react and remaining hydrazine can be subsequently trapped by the addition of benzaldehyde. If unreacted $N_2H_4 \cdot H_2O$ is still in solution after a defined residence time, this will ultimately lead to the formation of the corresponding hydrazone, which subsequently undergoes a condensation to form benzaldehyde azine. Therefore, we

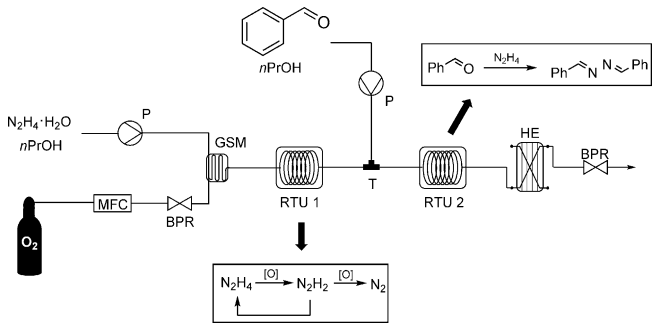


Figure 3. Continuous-flow setup for the hydrazine trapping experiments using benzaldehyde.

modified the original flow set up (Figure 3) by installing a second liquid feed and an additional residence time unit (RTU 2) connected by a T-mixer (T).

Initially, we mounted a 2.5 mL coil for the oxidation step at 100 °C using a hydrazine hydrate concentration of 0.5 M. The liquid flow rate was set at 0.4 mL min^{-1} and the oxygen stream at 40 mL min^{-1}. After passing the heated reaction zone, a 1 M solution of benzaldehyde was added via the mixing unit at a flow rate of 0.2 mL min^{-1} and the mixture passed the second heated zone (100 °C, 6.5 mL PFA coil) before cooling and depressurization. GC-MS analysis of the resulting reaction mixture showed a 38% yield of benzaldehyde and the desired benzaldehyde azine in 52% yield. In addition, we identified an azine formed from benzaldehyde and propanal (10%), which likely resulted from solvent oxidation (Figure 4A). When the same experiment was carried out with a 5.5 mL coil (RTU 1), the majority of hydrazine was consumed as GC analysis showed benzaldehyde as the main analyte (>80%; Figure 4B).

In addition, the same experimental approach was carried out with 3-nitrostyrene instead of benzaldehyde to estimate how much of the remaining hydrazine initializes the reduction of the alkene moiety. The experiment using a 2.5 mL coil resulted in a consumption of only around 10% of the olefin. Since the corresponding benzaldehyde experiment indicated significant amounts of residual hydrazine, it can be concluded that the majority of hydrazine entering the second coil is not contributing to the olefin reduction. This observation could be confirmed in the second example (RTU 1: 5.5 mL), where just trace amounts (ca. 1%) of the reduction product could be detected, although the aldehyde trapping experiments indicated remaining hydrazine. However, the values have to be taken with caution, as all experiments contained small amounts of propanal azine, which was most likely formed in the first coil. In addition, the aldehyde experiments also showed significant amounts of benzaldehyde propanal azine, which is probably a result of propanal hydrazone formed in RTU 1.

Reduction of artemisinic acid

The ultimate target of our studies on the in situ generation of diimide in continuous flow was to establish an efficient protocol for the reduction of artemisinic acid to the artemisinin precursor DHAA. Since the conversion of DHAA to artemisinin, as

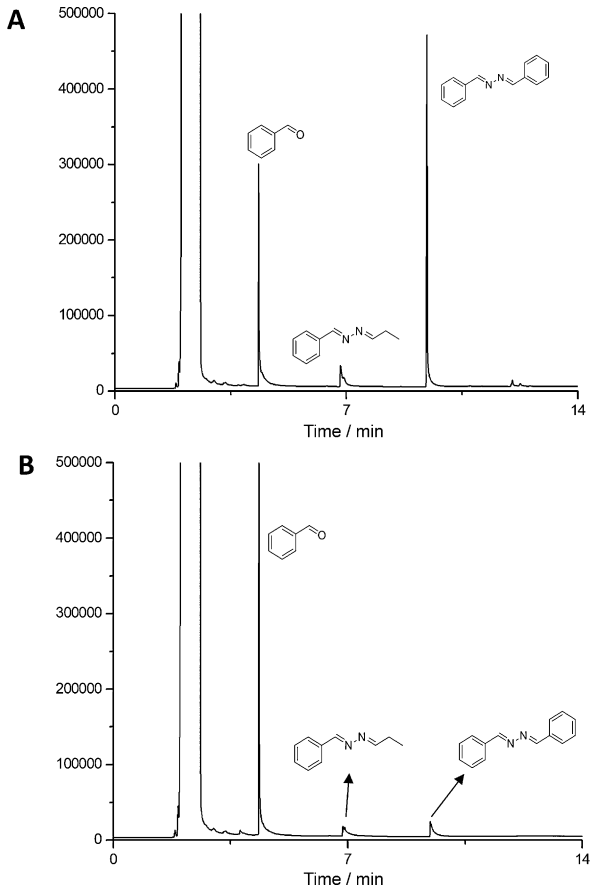


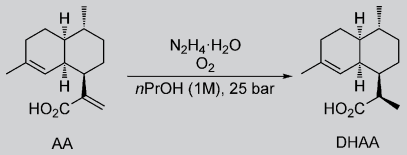
Figure 4. GC-MS chromatograms from the benzaldehyde trapping experiments using a 2.5 (A) or a 5.5 mL coil (B) for the oxidation of hydrazine and diimide oxidation/disproportionation.

well as the subsequent transformation to its pharmaceutically active derivatives, according to a continuous protocol was already reported,[4,12] the selective AA to DHAA reduction can be regarded as the "missing link" towards a fully continuous strategy for the synthesis of such antimalarial drugs. Inspired by the previous work by Sanofi–Aventis,[13] we hypothesized that diimide reduction of AA to DHAA could be dramatically enhanced by our continuous strategy. Safety concerns in batch methods prompted the Sanofi–Aventis team to use 5% O_2 in N_2 at atmospheric pressure, which is most likely the reason for the comparably long reaction time (11 h at 40 °C). The small volumes and channel dimensions in a continuous-flow (micro)-reactor minimize possible flame propagation, leading to an inherently safer processing of such explosive mixtures, even under relatively harsh reaction conditions.[27]

We started our optimization study using a slightly modified protocol (Table 4). The glass static mixer described above was replaced by a simple T-mixer since we realized that a) the flow pattern is similar without the active mixing unit, and b) that the efficiency of the hydrazine oxidation is essentially a result of the elevated temperature/pressure combination and not of the mixing or flow pattern achieved.

By applying similar conditions to those in the previous study, we obtained 82% conversion of AA, as analyzed by

Table 4. Reduction of artemisinic acid in continuous flow.[a]

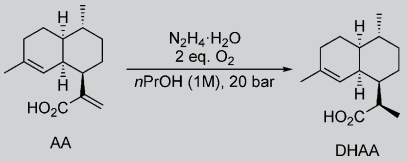


AA DHAA

Entry	N_2H_4 [equiv]	O_2 flow rate [mL min^{-1}]	T [°C]	RTU [mL]	t [min]	Conversion [%][b]
1	4	40 (4 equiv)	100	10	7	82
2	4	20 (2 equiv)	100	10	11	91
3	4	20 (2 equiv)	120	10	11	90
4	4	20 (2 equiv)	100	20	25	92

[a] Conditions: Artemisinic acid (1 mmol) and hydrazine hydrate dissolved in *n*PrOH (1 mL), liquid flow rate 0.4 mL min^{-1}. [b] Determined as HPLC peak area percent at 215 nm; relative response factor of AA/DHAA (4.6:1).

Table 5. Reduction of artemisinic acid in flow using multi-injection of hydrazine hydrate.[a]



AA DHAA

Entry	N_2H_4 [equiv]	T [°C]	RTU [mL]	t [min]	Conversion [%][b]
1	2+2	100	2×10	18	>99(78)[c]
2	2+2	80	2×10	18	>99(79)[c]
3	2+2	60	2×10	18	89
4	3+3	60	2×10	18	95(89)[c]
5	2+2+2	60	3×10	27	96(91)[c]
6	2+1+1	60	3×10	27	97(91)[c]
7	2+1+1	40	3×10	27	80
8	2+1+1	25	3×10	27	67
9	4+0+0	60	3×10	27	83
10	2+1+1+1	60	4×10	37	>99(80)[d]

[a] Conditions: Artemisinic acid (1 mmol) and hydrazine hydrate dissolved in *n*PrOH (1 mL), liquid flow rate 0.4 mL min^{-1}. [b] Determined as HPLC peak area percent at 215 nm; relative response factor of AA/DHAA (4.6:1). [c] Determined by 1H NMR spectroscopy using pyridine as internal standard. [d] Yield of isolated product.

HPLC-UV/Vis (Table 4, entry 1).[28] However, decreasing the oxygen flow to 20 mL min^{-1} resulted in a longer residence time maintaining an excess of the oxidizing agent. Unfortunately, neither an increased residence time nor the use of a higher reaction temperature gave quantitative formation of DHAA (Table 4, entries 2–4).

Taking the previous experiments on oxidation efficiency into consideration we concluded that a multiple injection, mimicking a traditional dropping funnel in batch experiments, could drive the reaction to completion by continuously adding fresh hydrazine hydrate. This strategy was originally used in continuous processes involving highly exothermic reactions to improve thermal control.[29,30] We argued that a multiple injection of hydrazine hydrate would possibly reduce the amount of disproportionation, due to a reduced hydrazine/diimide concentration along the reactor. In addition, this methodology enables the possibility to increase the effective reaction time, since we have already shown that, under the continuous high-temperature/high-pressure conditions, most of the $N_2H_4{\cdot}H_2O$ is consumed within less than 10 min. However, from a technical point of view, this setup is similar to the trapping experiment described above (Figure 3). Since low flow rates for the additional hydrazine feeds were required, syringe pumps were used instead of standard HPLC pumps. The pressure limit of these devices necessitates a maximum back pressure of 20 bar. In addition all further optimization experiments were carried out with a liquid flow rate of 0.4 mL min^{-1} at an AA concentration of approximately 0.8 M and a gas flow rate of 20 mL min^{-1}, corresponding to 2 equivalents of O_2 (Table 5).

The first multi-injection experiment was carried out using 2×2 equivalents of hydrazine hydrate and two identical residence time units (2×10 mL) at 100 °C, resulting in an overall reaction time of approximately 18 min (Table 5, entry 1). Interestingly, we ultimately observed complete consumption of the starting material, whereas a similar experiment with 4 equivalents of $N_2H_4{\cdot}H_2O$ and a 20 mL residence time led to an incomplete reaction (Table 4, entry 4). This result already strongly supports our initial hypotheses on the benefits of the multi-injection approach. However, NMR spectroscopy revealed that the DHAA yield was only 78%. GC-MS analysis after silylation revealed that the reaction was not as selective as the batch protocol,[13] showing a purity of 85% with 7% of the undesired dihydroartemisinic acid diastereomer (DHAA 2) and 8% of the over-reduced tetrahydroartemisinic acid (THAA). We assumed that this selectivity problem is a result of the comparably high reaction temperature. Therefore, a similar experiment was carried out at 80 °C, which unfortunately led to similar values (Table 5, entry 2). A further reduction of the temperature to 60 °C decreased the reaction rate and an even higher excess of hydrazine was not enough to fully consume the substrate (Table 5, entries 3 and 4). However, at this temperature a higher selectivity was obtained according to ^{1}H NMR spectroscopy. Encouraged by this promising result, a third hydrazine feed was added and almost quantitative conversion was obtained (Table 5, entry 5). In addition, the amount of hydrazine could be decreased to an overall value of 4 equivalents (Table 5, entry 6). A further reduction of the reaction temperature caused a significant reduction of the reaction rate (Table 5, entries 7 and 8), indicating that the optimal temperature for high conversions and sufficient selectivity is 60 °C. Importantly, a control experiment also demonstrated that a single hydrazine addition at the beginning results in comparably low conversion (Table 5, entry 9). Finally, by adding a fourth liquid feed, AA could be quantitatively reduced within 37 min at 60 °C using an overall hydrazine stoichiometry of 5 equivalents (2+1+1+1) and just two equivalents of O_2 (Table 5, entry 10, and Figure 5). Isolation by crystallization afforded DHAA in 80% yield. GC-MS analysis confirmed a highly selective reduction with small amounts of THAA (ca. 2%) and DHAA 2 (ca. 1%) as the only byproducts.

Long-run experiments using 8.5 mmol of artemisinic acid resulted in an identical selectivity with slightly lower conversion

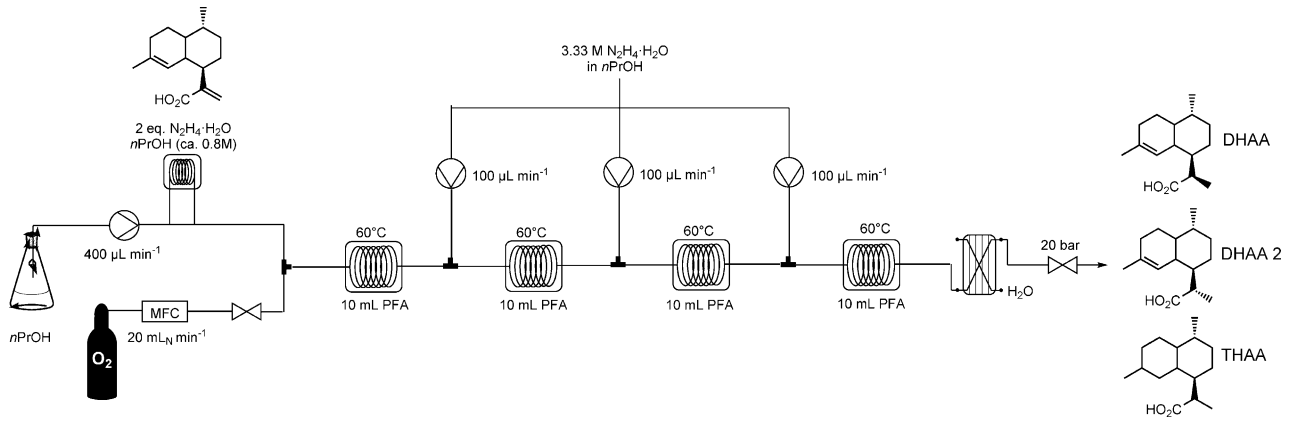


Figure 5. Optimized multi-injection setup for the reduction of artemisinic acid towards in situ generation of diimide from hydrazine hydrate and oxygen.

(ca. 97% according to HPLC). However, on this scale, crystallization afforded the desired artemisinin precursor in significantly higher yield ($\geq$93%) with only 2% of THAA present and a diastereomeric ratio of $\geq$97:3.

A comparison of the continuous methodology with other published batch strategies for the reduction of artemisinic acid demonstrates that the major advantage of this novel protocol is the relatively short reaction time of less than 40 min (Table 6). A more detailed inspection reveals that the diastereomeric ratio in the diimide protocols is slightly higher compared to transition metal-catalyzed hydrogenation procedures. From an economic point of view, it is noteworthy that no precious metal catalyst is required when N_2H_2 is used as reducing agent. Due to the lack of values for isolated yields from the reported procedures, we calculated the space–time-yield based on quantitative reactions for the ruthenium-catalyzed hydrogenation protocol. A comparison of this value shows that the continuous methodology described herein is clearly superior over the batch protocols for the reduction of artemisinic acid.

Due to the relatively clean reaction and the inert nature of the main byproducts (H_2O, N_2), we assume that the crude reaction mixture can be directly converted to the antimalarial artemisinin, since a crude plant extract from *Artemisia annua* could also be processed in the continuous drug synthesis.[4a] In an ideal case, the reduction process could be directly coupled with the photochemical methodology as both transformations require the same gaseous reagent.[4]

Conclusion

The in situ generation of diimide for the transfer hydrogenation of olefins from hydrazine and oxygen can be efficiently carried out in a catalyst-free procedure using a gas–liquid continuous-flow approach. Simple alkenes can be selectively reduced within 10 min in a virtually work-up free procedure.

It could be shown that the oxidation of hydrazine hydrate, a time consuming step under conventional batch conditions, can be dramatically enhanced using this enabling technology. The obtained kinetic information led to the development of a multi-injection principle applying periodic additions of fresh hydrazine hydrate. This methodology enables the possibility for increased effective residence times and can be applied in cases where less reactive olefins need to be reduced.

As an illustrative example, the selective reduction of artemisinic acid yielding the direct precursor molecule for the antimalarial drug artemisinin was successfully accomplished. This industrially relevant reduction was achieved by using four consecutive liquid feeds and residence time units with 2 equivalents of O_2, a total amount of 5 equivalents of $N_2H_4 \cdot H_2O$, and an overall reaction time of approximately 40 min. A compari-

Table 6. Comparison of different strategies for the reduction of artemisinic acid.

	Amyris[a]	Sanofi–Aventis[b]	Sanofi–Aventis[c]	This work
Technology	batch	Batch	batch	continuous flow
Reducing agent	H_2	H_2	N_2H_2	N_2H_2
Catalyst	$[RhCl(PPh_3)_3]$ [0.05 mol%]	$[RuCl_2\{(R)$-dtbm-Segphos$\}](dmf)_2$[e] [0.01 mol%]	–	–
T [°C]	80	25	40	60
P [bar]	47	22	atm.	20
t [h]	19	6	11	ca. 0.6
Yield [%]	quantitative (not isolated)	quantitative (not isolated)	>90	$\geq$93
d.r.	94:6	95:5	$\geq$97:3	$\geq$97:3
Space–time yield [mmol L^{-1} h^{-1}]	0.023	–[d]	0.023	0.56

[a] Data taken from ref. [9b]. [b] Data taken from ref. [10]. [c] Data taken from ref. [13]. [d] Apace–time-yield cannot be calculated as no reactor volume was reported. [e] dtbm = (R)-dtbm-Segphos = (*R*)-(−)-5,5′-bis[di(3,5-di-*tert*-butyl-4-methoxyphenyl)phosphino]-4,4′-bi-1,3-benzodioxole.

son with other published procedures for this reduction shows a >20 fold-higher space–time yield for the continuous process described herein.

Experimental Section

Reduction of simple olefins in a single liquid-injection reactor (Table 2): Feed A consisted of the respective olefin and hydrazine monohydrate dissolved in *n*-propanol, whereas feed B was oxygen (purity 5.0). The liquid stream (0.4 mL min^{-1}) and the gaseous stream (the flow was set at 40 mL min^{-1} (standard conditions) at the instrument, resulting in a calculated flow of 2 mL min^{-1} at a back pressure of 20 bar) were mixed together in a glass static mixer. The resulting segmented flow stream was passed through a PFA reactor coil (0.8 mm inner diameter, 10 mL reactor volume, if not stated otherwise) at 100 °C (if not stated otherwise). Subsequently, the mixture was cooled in a heat exchanger with water as cooling agent. After passing a back pressure regulator (20 bar) the solution was collected. For isolating the title compounds the solvent was evaporated under reduced pressure and the product was dried overnight (workup A). In certain cases, the reaction mixture was concentrated, filtered through a plug of silica, and eluted with copious amounts of $CHCl_3$. The solvent was evaporated under reduced pressure and the product was dried overnight using a desiccator with $CaCl_2$ (workup B).

Reduction of artemisinic acid in a multi-injection flow reactor: Feed A consisted of *n*-propanol, whereas feed B was oxygen (purity 5.0). Artemisinic acid (8.5 mmol, 1.99 g) and hydrazine hydrate (17 mmol, 0.825 μL) were dissolved in *n*PrOH (8.5 mL) resulting in a total volume of approximately 10.2 mL and injected in a sample loop which was connected to feed A via a 6-way valve. The liquid stream (400 μL min^{-1}) and the gaseous stream (40 mL_N min^{-1}) were mixed together in a T-mixer. The resulting segmented flow stream was passed through a PFA reactor coil (0.8 mm inner diameter, 10 mL reactor volume) at 60 °C. After coil 1, a T-mixer connected another feed C adding hydrazine hydrate Feed in *n*PrOH (3.33 M in *n*PrOH) at a flow rate of 100 μL min^{-1}. The combined stream passes another 10 mL of PFA tubing at 60 °C (coil 2). This addition principle is repeated two times (feed D, coil 3; feed E, coil 4) resulting in three additional hydrazine hydrate feeds and an overall reactor volume of 40 mL. Subsequently, the mixture was cooled in a heat exchanger with water as cooling agent. After passing a back-pressure regulator (20 bar) the solution was collected. To isolate the title compounds, the solvent was evaporated under reduced pressure and the product was precipitated by adding water (3–5 mL) and concentrated H_3PO_4 until pH <2 was attained. The solids were filtered and dried overnight, resulting in ≥93% yield of a yellow solid with a purity of ≥95%.

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Keywords: antimalarial drugs · flow chemistry · reactor design · reduction · synthetic methods

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FULL PAPER

Flow Chemistry

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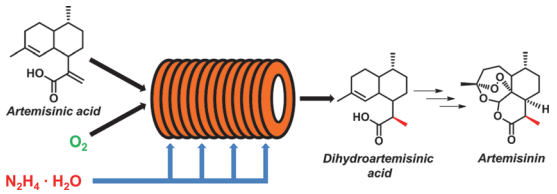
Continuous Flow Reduction of Artemisinic Acid Utilizing Multi-Injection Strategies - Closing the Gap Towards a Fully Continuous Synthesis of Antimalarial Drugs



The missing link for a totally continuous transformation of artemisinic acid to a range of antimalarials is the initial olefin reduction in artemisinic acid. We demonstrate how this transformation can be selectively carried out in continuous flow using in situ generated diimide. Key to the success was the development of a multi-injection reactor for the consecutive addition of the diimide precursor hydrazine hydrate.