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Facile Stoichiometric Reductions in Flow: An Example of Artemisinin

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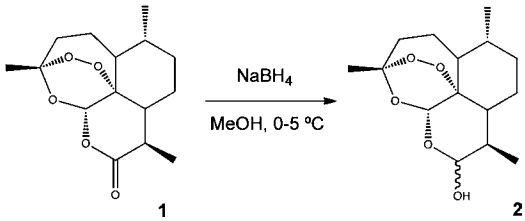
ABSTRACT: Stoichiometric reduction of artemisinin to dihydroartemisinin (DHA) has been successfully transferred from batch to continuous flow conditions with a significant increase in productivity and an increase in selectivity. The DHA space-time-yield of up to 1.6 kg h^{-1} L^{-1} was attained which represents a 42 times increase in throughput compared to that of conventional batch process.

1. INTRODUCTION

Stoichiometric reductions are ubiquitous in organic synthesis due to their synthetic utility and high selectivity. Many reductions are performed at low temperatures for the reasons of safety and maintaining selectivity in the highly exothermic reactions. They also involve handling of solids. Both factors complicate scale-up of reductions. In this respect flow chemistry offers potential significant advantages for the generic class of stoichiometric reductions. Here we present an example of a typical stoichiometric reduction of a current drug precursor transferred into a flow process with a significant gain in productivity and selectivity.

The routes to the active ingredients in the artemisinin combination therapies (ACTs) for the treatment of *Plasmodium falciparum* malaria, e.g., artesunate is via reduction of artemisinin **1** into dihydroartemisinin (DHA) **2** using sodium borohydride in methanol or ethanol, see Scheme 1.[1−8] This synthetic

Scheme 1. Reaction scheme of artemisinin reduction using conventional batch protocol



protocol involves batch reaction at low temperatures (0−5 °C) with a suspension of $NaBH_4$, followed by a multistep workup procedure.[1−5,7,8] Diisobutylaluminium hydride (DIBAL-H)[9−11] is another reductant reported in the literature, requiring dry dichloromethane as solvent, lower reaction temperature −78 °C and with a smaller yield of **2**. Both protocols require long reaction times (0.75−3 h) at low temperatures due to the exothermic nature of the reaction. Scale-up of this reaction is problematic for four reasons: (*i*) artemisinin is pyrophoric due to the presence of a highly unstable endoperoxide function, (*ii*) batch protocol requires low temperatures to avoid thermal runaway, (*iii*) batch reaction with suspended solids is not easily scalable due to high sensitivity to variations in the feedstocks and the physical form of the reactants (size of particles, crystal allotrope, etc.), and (*iv*) the multistep workup procedure required to obtain a stable formulation of DHA. These factors were assembled by authors from heuristic data on end-user experiences with scaling up artemisinin reduction in UK, India, and China, the earlier project on artemisinin derivatisation sponsored by Medicines for Malara Ventures,[12] and the material safety data sheets information. On the basis of our earlier studies of flow processes[13−15] we undertook to develop a flow protocol for the synthesis of dihydroartemisinin, which is reported in this paper.

2. EXPERIMENTAL SECTION

Reduction of Artemisinin 1 with $NaBH_4$. Artemisinin (200 mg, 0.71 mmol) was suspended in methanol (10 mL) under moderate stirring speed and cooled in an ice−water bath to ∼4 °C. Sodium borohydride (67 mg, 1.77 mmol, 2.5 equiv) was added in portions to the suspension over a period of 5 min. The reaction mixture was stirred vigorously under N_2 until TLC showed no **1** left in the reaction mixture (∼90 min). Then the reaction mixture was neutralised (pH 5−6) with 50% v/v of a mixture of acetic acid/methanol (added by portion, 50 μL each time). The reaction mixture was evaporated to dryness under vacuum (at 40 °C). This standard procedure was developed by Buzzi et al.[12]

Workup Procedure. Dry residue was extracted using ethyl acetate 2, three times (10 mL each time) for transferring the product completely (monitored by TLC) into ethyl acetate. The combined ethyl acetate extracts were dried with Na_2SO_4 (for 6 h), filtered, and evaporated to dryness to give a white flakelike product.

Reduction of Artemisinin with $LiAlH(O^tBu)_3$. A 1 M solution of $LiAlH(O^tBu)_3$ in THF (2.2 mL) was added dropwise (with a syringe) to a solution of **1** (200 mg, 0.71 mmol) in dry THF (5 mL) stirred under N_2 at ∼3 and 40 °C. After two hours of reaction time the reaction was quenched to pH 5−6 with 20%v/v acetic acid solution in THF. Ethyl acetate and distilled water were added into the mixture, and the phases were separated. The aqueous layer was extracted with ethyl acetate several times. The combined ethyl acetate extracts were

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Table 1. Summary of results of reduction of artemisinin under batch reaction conditions

entry	reductant	mol equiv/−	T/°C	t/min	solvent	conversion of **1**/%[a]	yield of **2**/%[b]
1	$NaBH_4$	2.5	4	90	MeOH	96	87
2		3	4	100		98	90
3[c]		3	4	100		97	89
4	$LiAlH(O^tBu)_3$	3	3	120	THF	65	42
5		3	40	60		81	67
6	RedAl	3	3	10	Toluene	93	46
7	$LiBHEt_3$	1	2	10	THF	96	0
8		2	2	10		100	75
9		2.5	2	10		100	87
10		3	2	10		100	94
11[d]		3	2	10		100	92
12		2	18	5		100	80
13		2.5	18	5		100	81
14		3	19	5		100	83
15[e]		3	20	15		100	75
16		3	20	5		100	46

[a]X - conversion determined by HPLC. [b]isolated yield determined by HPLC (the total amount of α- and β-dihydroartemisinin epimers), ratio of α-**2** to β-**2** was ~40%/60% for fresh sample by H NMR. [c]Synthesis with 1 g of substrate **1** [d]200 mg of **1** in 5 mL THF. [e]Synthesis with 1.412 g of substrate **1**.

dried with Na_2SO_4 (for 6 h), filtered, and evaporated to dryness under reduced pressure to give a white residue.

Reduction of Artemsinin with Red Al. Red-Al (≥65 wt % in toluene, 0.65 mL) was added dropwise (with a syringe) to a solution of **1** (200 mg, 0.71 mmol) in dry toluene (20 mL) stirred at ~3 °C under N_2. The solution was stirred at 3 °C for 10 min, and then a 0.01 M aqueous NaOH solution was added dropwise to stop the reaction. This was followed by the addition of ethyl acetate. The phases were separated, and the aqueous phase was back-extracted with ethyl acetate. The combined organic layer was dried over Na_2SO_4 (for 6 h), filtered, and evaporated to dryness under reduced pressure to give a combination of oily residue and white residue.

Reduction of 1 with $LiBHEt_3$. $LiBHEt_3$ solution (1 M in THF) was added (with a syringe) dropwise to the solution of **1** in dry THF (200 mg, 0.71 mmol; 20 mL) stirred under N_2 (reactions were performed at ~2 °C and ~19 °C). The stirring was continued until TLC showed no **1** left in the reaction mixture (5−10 min). The temperature of the reaction mixture was maintained constant by either using water bath or ice−water bath. An acetic acid solution in THF (20% v/v) was added by portions (100 or 50 μL each time) to quench the reaction mixture to pH 5−6. The reaction mixture was vacuum evaporated to dryness. Ethyl acetate and distilled water were used to extract the product from the dry residue. The two phases were separated, and the aqueous layer was extracted with ethyl acetate twice. The combined ethyl acetate extracts were dried with Na_2SO_4 (for 6 h), filtered, and evaporated to dryness under reduced pressure to give a white flake-like product.

Flow Synthesis Procedure. The reactor was an XXL-ST-03 by The Little Things Factory GmbH, with reactor internal volume of 3 mL and incorporating an internal cross-flow heat exchanger. Solution of artemisinin was pumped with a Kontron 42 HPLC pump. The $LiBHEt_3$ solution was prepared under inert atmosphere and pumped employing a Knauer 100 HPLC pump. After the reaction a Y-shaped micromixer was used to quench the reaction with an acetic acid solution. The pH of the solution was controlled in the collected aliquots.

(a) **Analytical Protocols.** TLC, eluents: CH_2CL_2 + MeOH (v/v = 20/0.5). Developing agent: phosphomolybdic acid.

(b) H NMR, Bruker ICONNMR. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 0.94 (d, J = 6.8 Hz, 6H, CH_3), 1.24 (s, 3H, CH_3), 1.65 (dd, J_1 = 3.41 Hz, J_2 = 13.23 Hz, 2H) 1.8−1.9 (m, 4H), 2−2.1 (m, 1H, H), 2.33 (m, 1H), 4.73 (d, J =9.2 Hz, 1H), 5.26 (t, J =3.2 Hz, 1H), 5.37 (s, 1H), 5.58 (s 1H).

(c) HPLC, Shimadzu Prominence instrument equipped with ELSD-ET (low temperature−evaporative light-scattering detector), cell temperature 40 °C, polarity +, response time 1.5 s; column: Thermo hypersil-keystone, 250 mm × 4.6 mm, 5 μm, BETASIL C18, column oven temperature 45 °C. Mobile phase: acetonitrile/H_2O (65/35, %v/v, flow rate 0.8 mL min^{-1}. Sample: ~2 mg in 1 mL acetonitrile each time, 20 μL injection volume.

(d) LC−MS, Dionex 3000RS UHPLC coupled with Bruker MaXis Q-TOF mass spectrometer, A Sigma Ascentis Express column (C18, 150 mm × 2.1 mm, 2.7 μm) was used. Mobile phases consisted of A (water with 0.1% formic acid) and B (as acetonitrile with 0.1% formic acid). A gradient of 30% B to 100% B in 15 min was employed with flow rate at 0.2 mL min^{-1}, UV was set at 220 nm. Mass spectrometer was operated in electrospray positive mode with a scan range 50−2000 m/z. Source conditions are: end plate offset at −500 V; capillary at −4500 V; nebulizer gas (N_2) at 1.6 bar; dry gas (N_2) at 8 L min^{-1}; dry temperature at 180 °C. Ion tranfer conditions as, ion funnel RF at 200 Vpp; multiple RF at 200 Vpp; quadruple low mass set at 55 m/z; collision energy at 5.0 ev; collision RF at 600 Vpp; ion cooler RF at 50−350 Vpp; transfer time set at 121 μs; prepulse storage time set at 1 μs. Calibration was done with sodium formate (10 mM) through a loop injection of 20 μL of standard solution at the beginning of each run.

3. RESULTS AND DISCUSSION

Batch experiments were carried out first to screen the best reducing agents for the continuous-flow processes. Due to the

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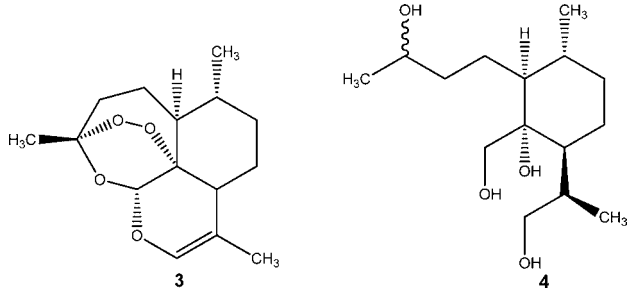
labile nature of the peroxy bond in **1** and the requirement of the continuous-flow experiments, i.e. high solubility of a reducing agent, only three candidates were selected on the basis of the literature,[16] namely, lithium tri-*tert*-butoxyaluminum hydride ($LiAlH(O^tBu)_3$), sodium bis(2-methoxyethoxy)aluminum hydride ($NaAlH_2(OCH_2CH_2OMe)_2$, Red-Al), and lithium triethylborohydride ($LiBHEt_3$).

We first examined the published protocol of $NaBH_4$ reduction of **1**.[12] The highest yield (90%) of **2** was obtained at 4 °C after 100 min (Table 1, entry 2). $LiAlH(O^tBu)_3$ was reported to be able to reduce a peroxy ester into the corresponding alcohol without breaking the peroxy bond.[16] A 1 M solution of $LiAlH(O^tBu)_3$ in THF was used in this study. The best result obtained for $LiAlH(O^tBu)_3$ reduction was 67% yield of **2** within 60 min (entry 5, 40 °C, 81% conversion of **1**). Reaction of **1** with Red-Al was found to be fast at 3 °C: disappearance of **1** was confirmed by TLC after 10 min. The yield of **2** was only 46% by HPLC (93% conversion of **1** by HPLC, entry 6). The mass imbalance was most likely caused by the formation of byproduct. These byproducts (Scheme 2) were identified by H NMR and LC−MS analysis.

Scheme 2. Identified byproducts in the reduction of 1 to 2



The two byproducts (**3** and **4**) have been well-documented for the process of reducing **1** to **2**. The glycal **3** (anhydrodihydroartemisinin, MS: *m*/*z* (% intensiy) 267.1588 [MH^+] (calcd 267.1591); 284.1855 [MNH_4^+] (calcd 284.1856))[17,18] is the dehydration product of **2**. By-product **4** (MS: *m*/*z* (% intensiy) 297.2046 [MH^+] (calcd 297.2036) is a product of fragmentation of **2** under reductive conditions.[5,18] The two side reductions may be attributed to bad mixing (uneven distribution of reactants and distribution of residence time) and poor temperature control in the batch reactor. Another byproduct (trace amount) with a molecular mass of 143.1073 (MS calculated formula: $C_8H_{15}O_2$) was also detected by LC−MS. This byproduct has previously been identified by us during the accelerated stressing of **1** and is yet to be structurally characterised.

$LiBHEt_3$ was found to be more effective in terms of reaction time and yield. The substrate was found to be fully consumed after 10 min of reaction at 2 °C. The yield of **2** was found to depend on the molar equivalent of $LiBHEt_3$ (entries 7−10). The product **2** could be produced in 94% yield by using 3 mol equiv of $LiBHEt_3$. The tolerance of synthesis of **2** with $LiBHEt_3$ to temperature was also examined. Lower yield of **2** at ∼20 °C was obtained after 5 min reaction (entries 15). Apart from the workup procedure (described in ESI), a direct water precipitation method was also tested for quenching the reaction (entry 16). However, only 46% yield of **2** was recovered after the workup. Among the reductants examined, $LiBHEt_3$ demonstrated the advantages of excellent reducing power and high chemoselectivity. Further advantages of using $LiBHEt_3$ for the synthesis of **2** are short reaction time and tolerance to reaction temperature.

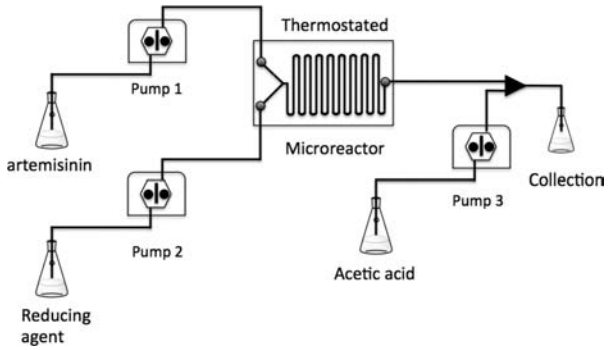


Figure 1. Schematic diagram of the continuous flow rig for stoichiometric reductions.

Experiments under flow conditions were performed in a rig shown schematically in Figure 1. Results are summarized in Table 2. Flow experiments showed very high conversion and selectivity under all experimental conditions tested. The residence times as low as 30 s were enough to produce **2** quantitatively when THF was employed as a solvent. This represents a reduction of at least 1 order of magnitude in reaction time compared to batch experiments. The reaction was performed at room temperature, thus reducing the overall energy intensity compared to the traditional reduction protocols, requiring cooling.

Table 2. Summary of results of reduction of artemisinin under flow conditions

entry	solvent	residence time/min	*T*/°C	conversion of **1**/%[a]	yield of **2**/%[b]
1	THF	2	5	99	98
2		1	5	99	98
3		2	25	99	98
4		0.5	25	99	98
5		1	25	99	98
6		1	15	99	97
7		1	0	98	95
8	2-MeTHF	0.5	25	97	93
9		0.5	5	96	95
10		0.33	25	97	94
11		0.33	5	97	94

[a]Calculated from NMR and HPLC data. [b]Yield means the total amount of α- and β-dihydroartemisinin epimers, **2**, was confirmed by MS and H NMR; yields were determined by HPLC, α-**2**/β-**2** ≈ 45%/55% by H NMR. Reaction conditions: artemisinin 0.033 M, $LiBHEt_3$ 0.1 M, acetic acid 20%.

The replacement of traditional solvents by less toxic and more environmentally benign solvents is a key principle of green chemistry.[19] We studied the replacement of THF by a biomass-derived alternative solvent.[20] 2-Methyltetrahydrofuran is synthesized in two steps from 2-furaldehyde, a chemical obtained from agricultural waste.[21] It has Lewis base properties and polarity between that of THF and dimethyl ether. $LiBHEt_3$ showed a good solubility in MeTHF, and the reaction was conducted in flow (Table 2, entries 8−11). Conversion was found to be slightly lower than that with THF (compare entries

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8 and 4). Traces of artemisinin were found under these conditions by HPLC and NMR, even though conversion was found to be very high (>96%). An even lower residence time of 20 s (entries 10, 11) showed similar degrees of conversion and selectivity, indicating fast kinetics of the reaction. A small amount of side products from rearrangement or over-reduction (<1%, **3** and **4**) was found by HPLC. Therefore, one can see that, in comparison with batch reactors, the well-controlled reaction conditions in the microflow reactors can significantly reduce the possibility of forming byprodcuts.

On the basis of the reaction data we evaluated basic reaction mass metrics of the benchmark batch reaction and of the flow reaction using Me-THF solvent. Atom economy is better for the process using sodium borohydride due to its lower mass, i.e., 0.89 against 0.73 for the flow process using $LiBHEt_3$. However, energy intensity and the life cycle data are required for more detailed comparison. Full life cycle assessment of the new process as well as further optimisation of the flow protocol (solvent replacements and new reducing agents) are underway.

4. CONCLUSIONS

We demonstrated a new flow protocol for stoichiometric reductions using an example reaction of reduction of artemisinin to dihydroartemisinin.[22] DHA was obtained in high yields using $LiBHEt_3$ at room temperature. Short residence time and full conversion attained result in high overall productivity ~1.60 kg h^{-1} L^{-1}. A biomass-derived solvent, Me-THF, was successfully employed to substitute THF in this protocol. The developed flow protocol for reduction using stoichiometric reducing agents is likely to have broader applicability in organic synthesis as well as synthesis of ligands.

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Notes

The authors declare no competing financial interest.

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