Supporting Information

Continuous Synthesis of Artemisinin-Derived Medicines

Kerry Gilmore, Daniel Kopetzki, Ju Weon Lee, Zoltán Horváth, D. Tyler McQuade, Andreas Seidel-Morgenstern, Peter H. Seeberger*

Corresponding Author: Peter H. Seeberger
Email: peter.seeberger@mpikg.mpg.de

Table of Content:

General :

Modules 1-3: All commercially available compounds and solvents (Acros, Aldrich, Fluka, Alfa Aesar and Merck) were used without purification. Lithium chloride was obtained from Grüssing (99%). Critically, the sodium borohydride ($NaBH_4$) was purchased from Merck (fine granular for synthesis). $NaBH_4$ purchased from Sigma-Aldrich, either the powdered or granular 10-40 mesh, did not yield satisfactory results (module 2). Tetrahydrofuran (THF) and methanol were dried using a JC Meyer Solvent System. The cotton used in module 2 was obtained from Lilibe Cosmetics. Dihydroartemisinic acid (**2**) was obtained from Honseabio, China. The Vapourtec R-2+ model pump was used for the continuous flow synthesis. Oxygen (Air Liquide, 99.995%, H_2O <3.0 ppm//mol; ALPHAGAZ™ 1 O_2; Werk DEF 2 Krefeld-Gellep) was delivered through a check valve from an oxygen gas tank. Gas pressure was regulated to 15 bar and the flow adjusted to 5 mL/min with a gas-flow controller (Influx, SV1B5-AI05, range 5-90 cm^3/min). Pressure was measured at the exit of the pump head with a built-in sensor. The LED module used in module 1 emits at 420 nm (OSA Opto Lights, 72 W electrical power, cooled by a fan, emission area 2.5 x 2.5 cm^2), connected to electronics for supplying a constant current to the LED module (OSA Opto Lights) and a power supply (Manson HCS-3202). Glass Omnifit columns (6.6 x 150 mm) were used in module 2 with 6.0 mm OD 40 µm PTFE frit filters in both the fixed and adjustable end caps. The proton signal of residual non-deuterated solvent (δ 7.26 ppm for $CHCl_3$) was used as an internal reference for ^1H spectra and measured on a 400 MHz

Bruker NMR. Data are reported as follows: chemical shift in parts per million (δ, ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, m = multiplet, dt = doublet of triplets, dq = doublet of quartets, ddd = doublet of doublet of doublets), coupling constant reported in Hertz (Hz) and integration. Column chromatography was performed using Fluka technical grade silica gel (230-400 mesh). Analytical thin layer chromatography (TLC) was performed on Kieselgel 60 F254 glass plates precoated with a 0.25 mm thickness of silica gel. The TLC plates were visualized by heating after staining with a solution of cerium-ammonium-molybdate (CAM), which was prepared by dissolving 40 g of ammonium molybdate and 1.6 g of cerium(IV) sulfate in 800 mL of dilute sulfuric acid (1:9 with water, v/v).

Module 4: α-Artesunate was purchased from TCI Deutschland GmbH (> 98%, Eschborn, Germany) and dissolved in ethanol (HPLC grade, Merck, Germany). Deionized water (Milli-Q A10, Millipore, MA, U.S.A.) and acetonitrile (HPLC grade, VWR, Germany) to be used as the analytical HPLC mobile phase. Formic acid, used as an additive in the analytical HPLC mobile phase, was purchased from Bernd Kraft (ACS grade, Duisburg, Germany). Ethyl acetate (HPLC grade, Merck, Germany), ethanol (HPLC grade, Merck, Germany), and *n*-hexane (HPLC grade, VWR, Germany) were used as the mobile phase of gradient elution chromatography. Ethyl acetate and n-hexane were used for recrystallization of α-artesunate. For the filtration of the diluted reaction mixture and the recrystallized α-artesunate, a glass filter (10~16 μm, Robu, Germany) was used. A HPLC unit (Agilent 1100, Agilent Technologies Inc., CA, U.S.A.) was used for concentration analysis with a Kinetex C-18 column (5 μm, 4.6 X 250 mm, Phenomenex Inc., Aschaffenburg, Germany). The UV signals were acquired by the ChemStation software (Agilent Technologies Inc., CA, U.S.A.). A Dionex HPLC unit, which consists of a P580 four-channel pump, a TCC-100 column oven, and an UVD-340U detector, was used to study the behavior of multicolumn preparative chromatography with a representative column (Phenomenex Luna-Prep Silica, 10 μm, 4.6 X 250 mm).

The crystallization experiments were performed in a jacketed 100 mL glass vessel equipped with a Pt-100-element, which was connected to a thermostat (RP845, Lauda Proline, Germany). A simple magnetic stirrer bar was used for agitation. The liquid phase composition of the slurry was continuously controlled during the crystallization process by means of Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR, ReactIR45m device from Mettler Toledo, Switzerland). Acetonitrile (HiperSolv CHROMANORM HPLC) purchased from

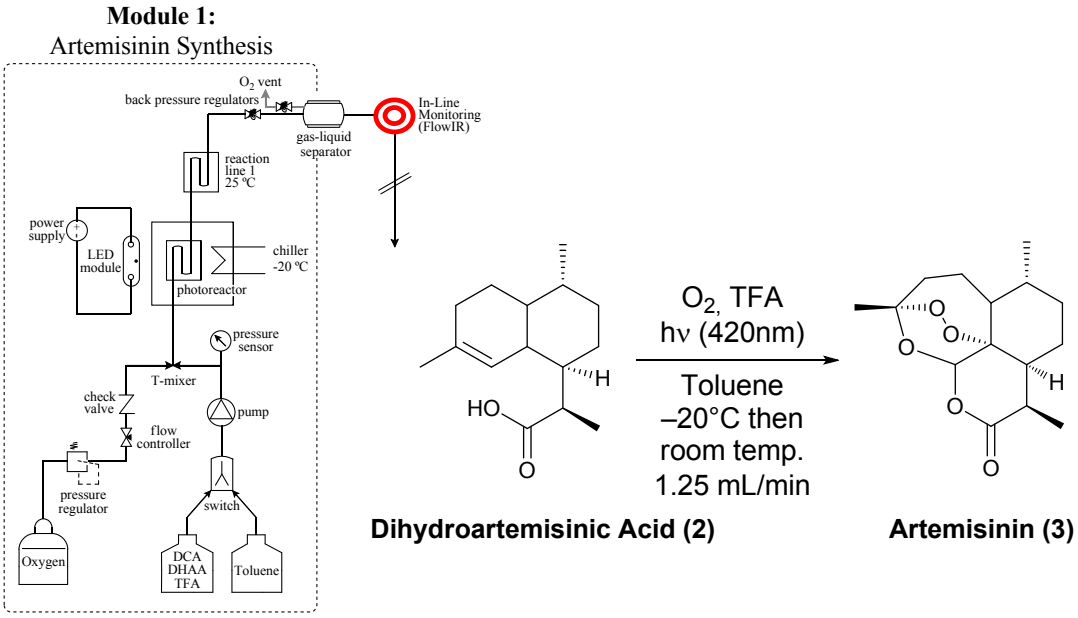
VWR GmbH, Germany and deionized water purified by Milli-Q Advantage A10 system (Millipore Corporation, Billerica, MA, U.S.A.).

The full author list for reference 3 from the text can be found below.

[1] a) S. Mascia, P. L. Heider, H. Zhang, R. Lakerveld, B. Benyahia, P. I. Barton, R. D. Braatz, C. L. Cooney, J. M. B. Evans, T. F. Jamison, K. F. Jensen, A. S. Myerson, B. L. Trout, *Angew. Chem.* **2013**, *125*, 12585-12589; *Angew. Chem. Int. Ed.* **2013**, *52*, 12359-12363. b) P. L. Heider, S. C. Born, S. Basak, B. Benyahia, R. Lakerveld, H. Zhang, R. Hogan, L. Buchbinder, A. Wolfe, S. Mascia, J. M. B. Evans, T. F. Jamison, and K. F. Jensen, *Org. Process Res. Dev.*, 2014, **18**, 402.

Figure S1: Module 1 of the overall process for the continuous production of artemisinin **3** from dihydroartemisinic acid **2**.

Module 1: Artemisinin (3) Synthesis from Dihydroartemisinic Acid (2).



The flow reactor setup for the synthesis of artemisinin **3** (Figure S1) consists of a feed solution (see below) of dihydroartemisinic acid (DHAA, **2**), trifluoroacetic acid (TFA) and the photosensitizer 9,10-dicyanoanthracene (DCA), an HPLC pump (Vapourtec, R2+ unit) downstream to switch valve (to switch between the starting material solution and eluent), an ETFE T-mixer (IDEX Health and Science, P-632) for mixing the feed solution and oxygen, a mass flow controller (Influx, SV1B5-AI05, allowing control of the oxygen flow rate from 5-90 cm^3/min) connected to a manometer fixed on an oxygen tank, thus generating a steady oxygen flow of 5 mL/min, a check valve (IDEX Health and Science, inline check-valve CV-3010) between the mass flow controller and the mixer, a photochemical reactor comprising the mixer and a tubing inlet, consisting of multiple loops of FEP tubing (7 mL, IDEX Health & Science, fluorinated ethylene polymer 1520, natural color, outside diameter (OD) 1/16 in and inside diameter (ID) 0.030 in) wrapped tightly around a transparent body (glass plate, size 9.0 x 14.0 cm^2) which is irradiated by the LED module emitting at 420 nm. The wrapped FEP tubing was irradiated directly by the LED module, which was installed in a distance of 3 cm in front of the transparent body. For maximum efficiency, the tubing was irradiated in a tray made of stainless

steel to reflect through passing light onto the photochemical reactor, which was immersed in this tray, filled with an ethylene glycol//water bath (3//2 v/v) cooled to -20 °C with the help of an immersion cooler (Huber, TC100E-F-NR). The LED was held above the tubing by a sheet of cardboard acting as a top for the tray, the underside of which was wrapped in aluminum foil. The top of the wrapped tubing was 1 cm beneath the surface of the liquid. After leaving the photochemical reactor the solution was passed through a reactor with 10 mL volume (inner diameter 0.03 inch, FEP tubing), kept at room temperature and then 30 mL (inner diameter 0.06 inch, FEP tubing), kept at room temperature. A back-pressure regulator (BPR) of 8 bar (Vapourtec) was installed after the tubing outlet in order to increase the internal pressure of the system. A piece of 32 cm FEP tubing connected the BPR to a homemade Teflon gas/liquid separator based on Jensen's[1,2] design, bearing widened channels (1 mm x 10 mm x 30 mm (h x w x l)) sandwiching a PTFE membrane (Whatman). A manual BPR controlled the pressure across the membrane, allowing for separation of the organic phase from the oxygen. A piece of 32 cm FEP tubing connected the separator to a FlowIR unit (Mettler Toledo). The reaction was monitored at 1033 cm^{-1} (Figure S2). A final piece of 32 cm FEP tubing connected the FlowIR unit to an empty collection vessel. Artemisinin **3** can be obtained at this point following basic work-up either by column chromatography or recrystallization in 69% yield, as has been shown previously.[3] It can also be utilized directly in module 2.

The feed was a solution of DHAA (**2**) at a concentration of 0.5 M, TFA at a concentration of 0.25M and the photosensitizer DCA at a concentration of 2.5m M in toluene (29.5 g DHAA (**2**), 7.13 g TFA and 143 mg DCA, with a total volume of 250 mL as determined by volumetric flask). The feed was introduced at a flow rate of 1.25 mL/min and the oxygen flow adjusted to 5 mL/min.

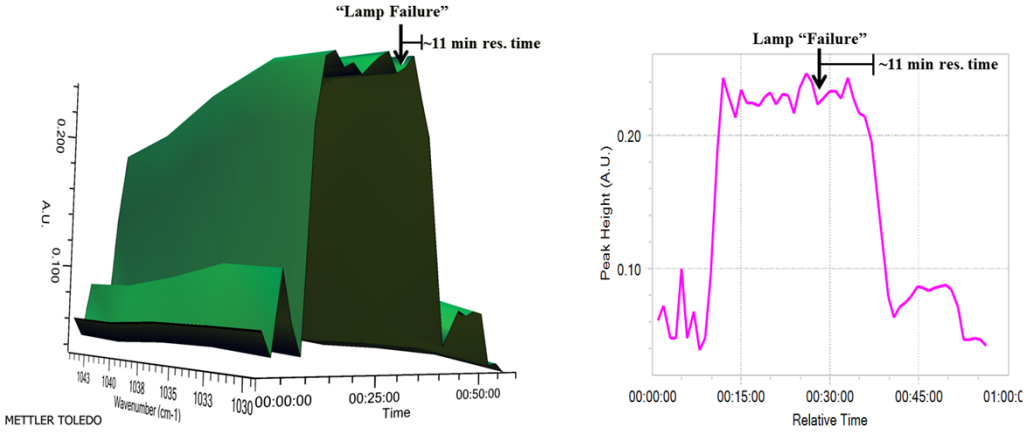
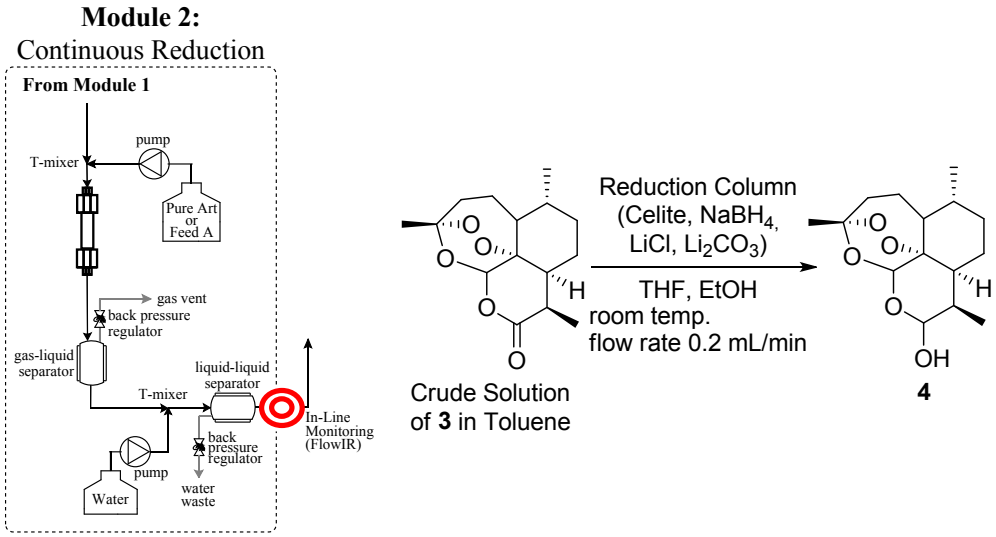


Figure S2: The reaction exiting module 1 was monitored using a FlowIR (Mettler Toledo), observing at 1033 cm^{-1}. To simulate an equipment error, the lamp was turned off during the reaction, resulting in immediate cessation of the monitored peak (following the ~11 minute residence time).

Module 2: Reduction of Artemisinin (3) Exiting Module 1 to Dihydroartemisinin (4).



A reduction column was prepared withwith the adjustable length end cap at the inlet. The column packing consisted of a mixture of four components ground in a mortar-pestle: 500 mg Celite®535, 500 mg of $NaBH_4$ (Merck, fine granular for synthesis), 500 mg of Li_2CO_3, and 380 mg of LiCl. At the outlet end of the column was 1 cm packed cotton (Lilibe Cosmetics) followed by a fixed length end cap. The material in the column was loosely packed by tapping the column on the bench top until no change in the height of the material was observed. The column was further packed, following incorporation into the system described below, by flowing dry THF at 2.5 mL/min for approximately four minutes until no gas was observed following the BPR. The average column volume of 2.9 mL was determined by dividing the differences in wet and dry masses of the reduction column by the density of THF (0.889 g/ml).

The column was incorporated into a flow system driven by an HPLC pump (Vapourtec, R2+ unit) with a switch valve (to switch between the starting material solution and eluent). A 32 cm piece of FEP tubing (IDEX Health & Science, fluorinated ethylene polymer 1520, natural color, outside diameter (OD) 1/16 in and inside diameter (ID) 0.030 in) connected the in-line pressure monitor of the Vapourtec unit following the HPLC pump to the reduction column. The

direction of flow through the reduction column was vertical from top to bottom. A second identical 32 cm piece of FEP tubing was used to connect the outlet of the reduction column to a BPR (8 bar, Vapourtec) followed by a 50 cm piece of FEP tubing to a homemade Teflon gas/liquid separator based on Jensen's[1,2] design, bearing widened channels (1 mm x 10 mm x 30 mm (h x w x l)) sandwiching a PTFE membrane (Whatman). A manual BPR controlled the pressure across the membrane, allowing for separation of the organic phase from the gas evolved in the reduction column. A piece of 32 cm FEP tubing connecting the separator to T-mixer mixed the organic phase with distilled water (added using an HPLC pump, Knauer, Smartline pump 100). The resulting biphasic stream entered a 1 mL loop of 1/16" OD FEP tubing, which was connected to a homemade Teflon liquid/liquid separator based on Jensen's[1] design, bearing widened channels (1 mm x 10 mm x 30 mm (h x w x l)) sandwiching a PTFE membrane (Whatman). A manual BPR controlled the pressure across the membrane, allowing for separation of the aqueous and organic phases. A 32 cm piece of FEP tubing bore the organic phase from the separator to the FlowIR (Mettler Toledo) unit. The reaction was monitored at either 1671 or 988 cm^{-1} (Figure S3). Following the FlowIR unit, a 32 cm piece of FEP tubing deposited the solution into an empty collection flask. The resulting solution can either be purified to yield dihydroartemisinin **4** (not done in this work) or utilized directly in module 3.

Typical Experiment: The starting material solution was prepared as follows: to the crude solution of Artemisinin **3** (3.4 mL), prepared as described above in module 1, was added 0.37 mL of ethanol. This was passed through the 2.9 mL reduction column at a flow rate of 0.2 mL/min at room temperature. The average pressure of the system was approximately 8-9 bar, however fluctuations between 2-25 bar have been observed (Figure S4). Following addition of the starting material solution, the reduction column was flushed with approximately two column volumes of THF (6 mL). The exiting biphasic (gas/liquid) stream fed into the homemade separator to remove the gas. The organic phase was then mixed with distilled water (flow rate 0.2 mL/min) and, following a 1 mL reactor, separated using a second homemade extractor. The organic phase then entered the FlowIR unit and was subsequently collected in an empty collection flask. The solution was used without modification in the third module.

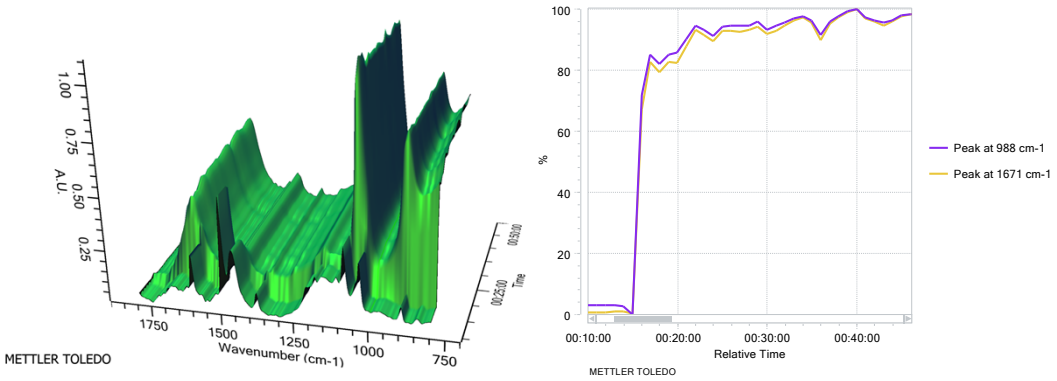


Figure S3: The reaction exiting module 2 was achieved using Mettler Toledo's FlowIR, monitoring at both 988 cm^{-1} and 1671 cm^{-1}.

Column Optimization: Several experiments were run varying both the additives and alcohol cosolvent to determine the most stable conditions, as determined by observed pressure. Each run proceeded as follows: to 3.4 mL of crude artemisinin solution (from module 1) was added 0.37 mL of MeOH. This was passed through one of four reduction columns comprised of either 500 mg $NaBH_4$, 500 mg Celite® (Blue Line), 500 mg $NaBH_4$, 500 mg Celite®, 500 mg Li_2CO_3 (Green Line), 500 mg $NaBH_4$, 500 mg Celite®, 380 mg LiCl (Pink Line), or 500 mg $NaBH_4$, 500 mg Celite®, 500 mg Li_2CO_3, and 380 mg LiCl (Black Line). The column was run vertically from top to bottom at 0.2 mL/min and, prior to use, was packed as described above. A 32 cm piece of FEP tubing connected the column to an 8 bar BPR and a second 32 cm piece of FEP tubing connected the BPR to an empty collection flask.

As can be seen in Figure S4, only when both Li_2CO_3 and LiCl are added to the column was the column considered stable (the pressure did not exceed 30 bar). When the alcohol cosolvent was exchanged for ethanol (Red Line), the pressure was further stabilized. These conditions (ethanol cosolvent, column composition 500 mg $NaBH_4$, 500 mg Celite®, 500 mg Li_2CO_3, and 380 mg LiCl) were then utilized as standard in module 2.

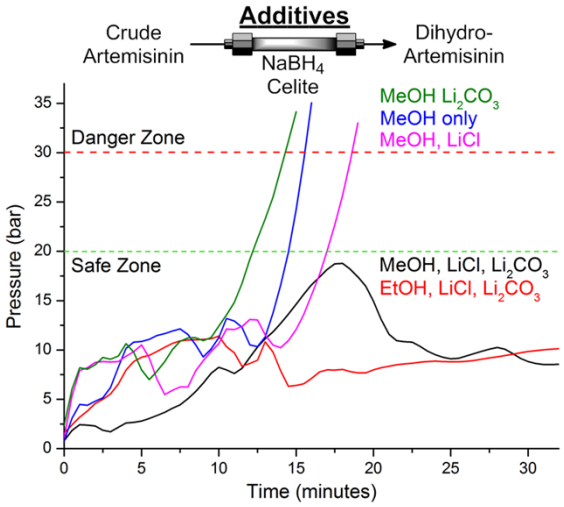
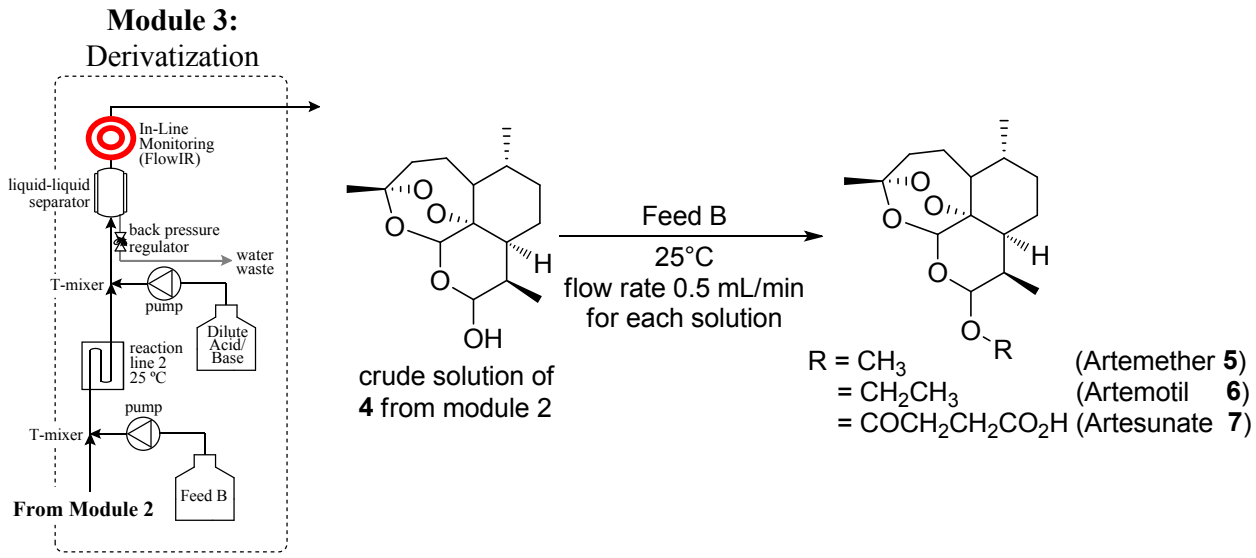


Figure S4: System pressure observed during reduction of crude artemisinin **3** as a function of cosolvent and solid additives in the Celite®/$NaBH_4$ column.

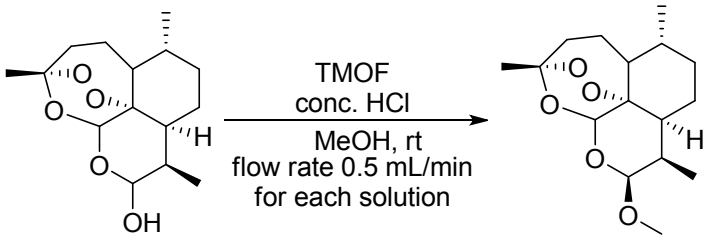
Module 3: Derivatization of Dihydroartemisinin 4 to Either β-Artemether 5, β-Artemotil 6, or α-Artesunate 7.



The organic phase exiting module 2 was incorporated into a flow system driven at 0.5 mL/min by an HPLC pump (Vapourtec, R2+ unit) with a switch valve (to switch between the starting material solution and eluent). A 32 cm piece of FEP tubing (IDEX Health & Science, fluorinated ethylene polymer 1520, natural color, outside diameter (OD) 1/16 in and inside

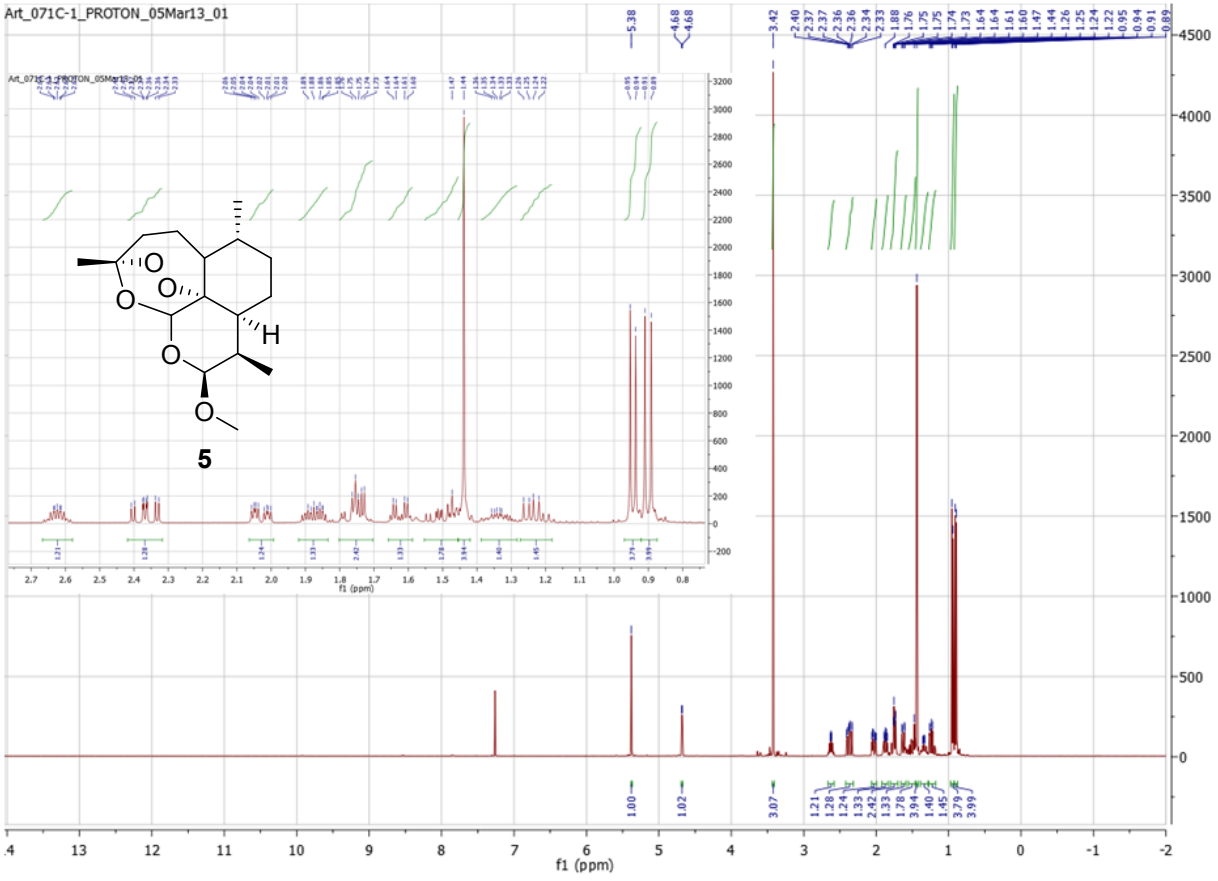
diameter (ID) 0.030 in) connected the in-line pressure monitor of the Vapourtec unit following the HPLC pump to an ETFE T-mixer, where the organic phase was mixed with the Feed B solution (driven at 0.5 mL/min by the second HPLC pump of the Vapourtec, R2+ unit with a switch valve to switch between the starting material solution and eluent) and connected to the T-mixer via an identical 32 cm piece of tubing. The solution leaving the T-mixer entered reactor line 2, consisting of 25 mL of tubing (IDEX Health & Science, fluorinated ethylene polymer 1520, natural color, OD 1/16", ID 0.030"). Following reactor line 2 was an 8 bar BPR and a 32 cm piece of FEP tubing connecting the BPR to an ETFE T-mixer. Here, the solution was mixed with either dilute aqueous $NaHCO_3$ (Artemether **5**/Artemotil **6** synthesis) or 1 M HCl_{aq} (Artesunate **7** synthesis), driven by an HPLC pump (Knauer, Smartline pump 100) at 0.3 mL/min. The T-mixer was connected to 1 mL of FEP tubing (1/16" OD and ID 0.03") and the biphasic system was fed into a homemade Teflon liquid/liquid separator based on Jensen's[2] design, bearing widened channels (1 mm x 10 mm x 30 mm (h x w x l)) sandwiching a PTFE membrane (Whatman). A manual BPR controlled the pressure across the membrane, allowing for separation of the aqueous and organic phases. A 32 cm piece of FEP tubing bore the organic phase from the separator to the FlowIR (Mettler Toledo) unit. Following the FlowIR unit, a 32 cm piece of FEP tubing deposited the solution into an empty collection flask. The resulting solution can either be purified manually (as done for Artemether **5** and Artemotil **6**) or utilized directly in module 4 (as done with Artesunate **7**).

Conversion of Crude Dihydroartemisinin (4) Exiting Module 2 to β-Artemether (5):



Typical Experiment: The organic phase from module 2 (2.1 mL) was pumped at a flow rate of 0.5 mL/min into the T-mixer where it met Feed B_{AM} (2 mL methanol, 1 mL trimethyl orthoformate (TMOF), 0.45 mL conc. HCl) also pumped at a flow rate of 0.5 mL/min. Following

reactor 2, the solution was mixed with dilute $NaHCO_3$, pumped at 0.3 mL/min. The biphasic system was separated using the homemade liquid/liquid separator and the organic phase collected in an empty flask. The organic phase was then concentrated *in vacuo* to a white solid. Purification was achieved by column chromatography over silica gel (5% - 20% EtOAc, in cyclohexane) providing pure β-artemether **5** as a white solid (61 mg), with 25% yield over the three modular steps. ^{1}H NMR ($CDCl_3$): 5.38 (s, 1H), 4.68 (d, J = 4 Hz, 1H), 3.42 (s, 3H), 2.63 (m, 1H), 2.37 (ddd, J = 16, 12, 4 Hz, 1H), 2.02 (ddd, J = 16, 4, 4 Hz, 1H), 1.88 (m, 1H), 1.76 (m, 2H), 1.64 (m, 1H), 1.49 (m, 2H), 1.44 (s, 3H), 1.34 (m, 1H), 1.24 (m, 1H), 0.96 (d, J = 8 Hz, 3H), 0.92 (m, 1H), 0.9 (d, J = 8 Hz, 3H). This spectrum is in accordance with that reported in the literature.[4]



Combining Modules 2 + 3 for the Continuous Production of Artemether (5) from Crude Artemisinin (3) with Varying Hydride Sources:

The effect of both hydride source and work up conditions on the resulting epimeric ratio of β:α artemether following treatment with Feed B in batch was examined. The ratio was

determined following basic work-up and solvent removal using ^{1}H NMR of the crude reaction mixture comparing the doublets at 4.65 ppm (β-artemether **5**, J = 4 Hz) and 4.32 ppm (α-artemether, J = 8 Hz).[4]

Three reaction vials (A, B, C) were prepared by dissolving 20 mg of pure artemisinin **3** in 200 µL toluene in a 1 mL vial, to which was added 200 µL of a 1M $LiEt_3BH$ in THF solution. These solutions were mixed for 10 minutes. To vial A, 160 µL of Feed B_{AM} (450 µL conc. HCl, 2 mL MeOH, 1 mL TMOF) was added and allowed to stir for 30 minutes. The resulting solution was quenched with 2 mL aqueous $NaHCO_3$ and extracted with DCM (3 x 3 mL). The combined organic layers were dried via rotary evaporation and ^{1}H NMR revealed an approximate 50:50 ratio of epimers (Entry 1, Table S1). To vial B, 200 µL distilled water was added following reduction and allowed to stir for 5 minutes. The organic layer was decanted and added to a clean vial, where the subsequent derivatization/work up steps were identical to A. ^{1}H NMR again revealed an approximate 50:50 ratio of epimers (Entry 2). However, when ethanolamine was added to the aqueous wash (Entry 3), the epimeric ratio increased to those obtained in literature (5:1).[5] The ethanolamine was presumably required to remove the trialkylborane.

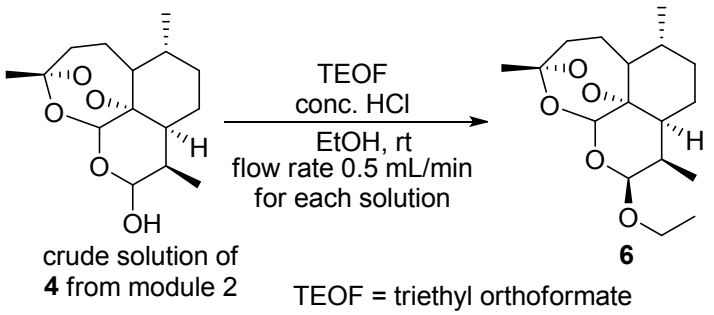
For the $NaBH_4$ column (vials D, E, F, Entries 4-6), an approximate 1" column was prepared fresh in a 150 mm Disposable Glass Pasteur Pipette (VWR) by packing with the same ratio of materials as described above over a small wad of cotton for each entry. The artemisinin solution (20 mg pure artemisinin **3** in 200 µL toluene) was added and flowed through the column using a pipet bulb. To vial D was added 160 µL Feed B_{AM} and afterafter 10 minutes was worked up as described above. The resulting epimeric ratio, while better than that obtained with $LiEt_3BH$ (Entries 4 vs 1), was still below that of standard batch conditions. However, when the post-column solution was treated with distilled water (Entry 5), the resulting β:α mixture of artemether was the desired 5:1. The same results were obtained with the ethanolamine/water solution (Entry 6).

Table S1: The effects of hydride source and intermediate wash (red dashed box) on artemether epimer ratios from artemisinin **3** in batch.

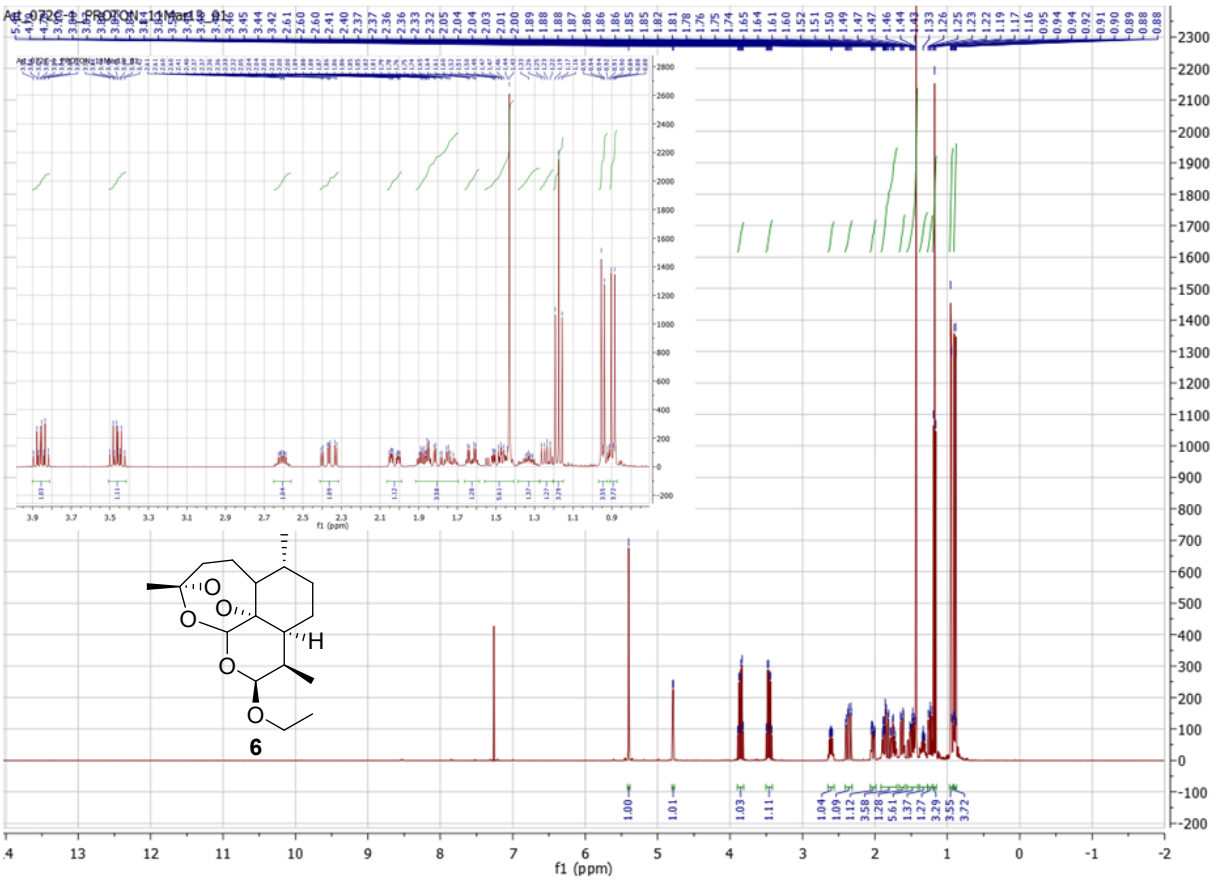
Entry	Hydride Source	Intermediate Wash	β:α
1	Superhydride® ($LiHEt_3$)	none	50:50
2	Superhydride® ($LiHEt_3$)	H_2O	50:50
3	Superhydride® ($LiHEt_3$)	H_2O/ethanolamine (3/1, v/v)	80:20

4	$NaBH_4$ Column	none	75:25
5	$NaBH_4$ Column	H_2O	81:19
6	$NaBH_4$ Column	H_2O/ethanolamine (3/1, v/v)	82:18

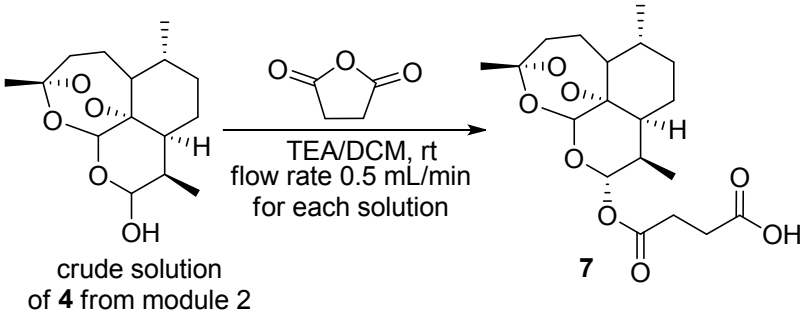
Conversion of Crude Dihydroartemisinin (4) Exiting Module 2 to β-Arteether (Artemotil, 6):



Typical Experiment: The organic phase from module 2 (2.1 mL) was pumped at a flow rate of 0.5 mL/min into the T-mixer where it met Feed B_{AE} (2 mL ethanol, 1 mL triethyl orthoformate (TEOF), 0.45 mL conc. HCl) also pumped at a flow rate of 0.5 mL/min. Following reactor 2 (25 mL), the solution was mixed with dilute aqueous $NaHCO_3$, pumped at 0.3 mL/min. The biphasic system was separated using the homemade liquid/liquid separator and the organic phase collected in an empty flask. The organic phase was then concentrated *in vacuo* to a white solid. Purification was achieved by column chromatography over silica gel (5% - 20% EtOAc, in cyclohexane) providing pure β-arteether **6** as a white solid (70 mg) with a 23% yield over the three modular steps. ^{1}H NMR ($CDCl_3$): 5.40 (s, 1H), 4.79 (d, J = <4 Hz, 1H), 3.86 (dq, J = 12, 8, 8 Hz, 1H), 3.47 (dq, J = 8, 8, 4 Hz, 1H), 2.59 (m, 1H), 2.34 (ddd, J = 12, 12, 4 Hz, 1H), 2.01 (ddd, J = 16, 4, 4 Hz, 1H), 1.80 (m, 3H), 1.61 (ddd, J = 12, 8, 4 Hz, 1H), 1.45 (m, 2H), 1.41 (s, 3H), 1.31 (m, 1H), 1.22 (dd, J = 12, 8 Hz, 1H), 1.16 (t, J = 8 Hz, 3H), 0.93 (d, J = 8 Hz, 3H), 0.90 (m, 1H), 0.88 (d, J = 8 Hz, 3H). This spectrum is in accordance with that reported in the literature.[6]

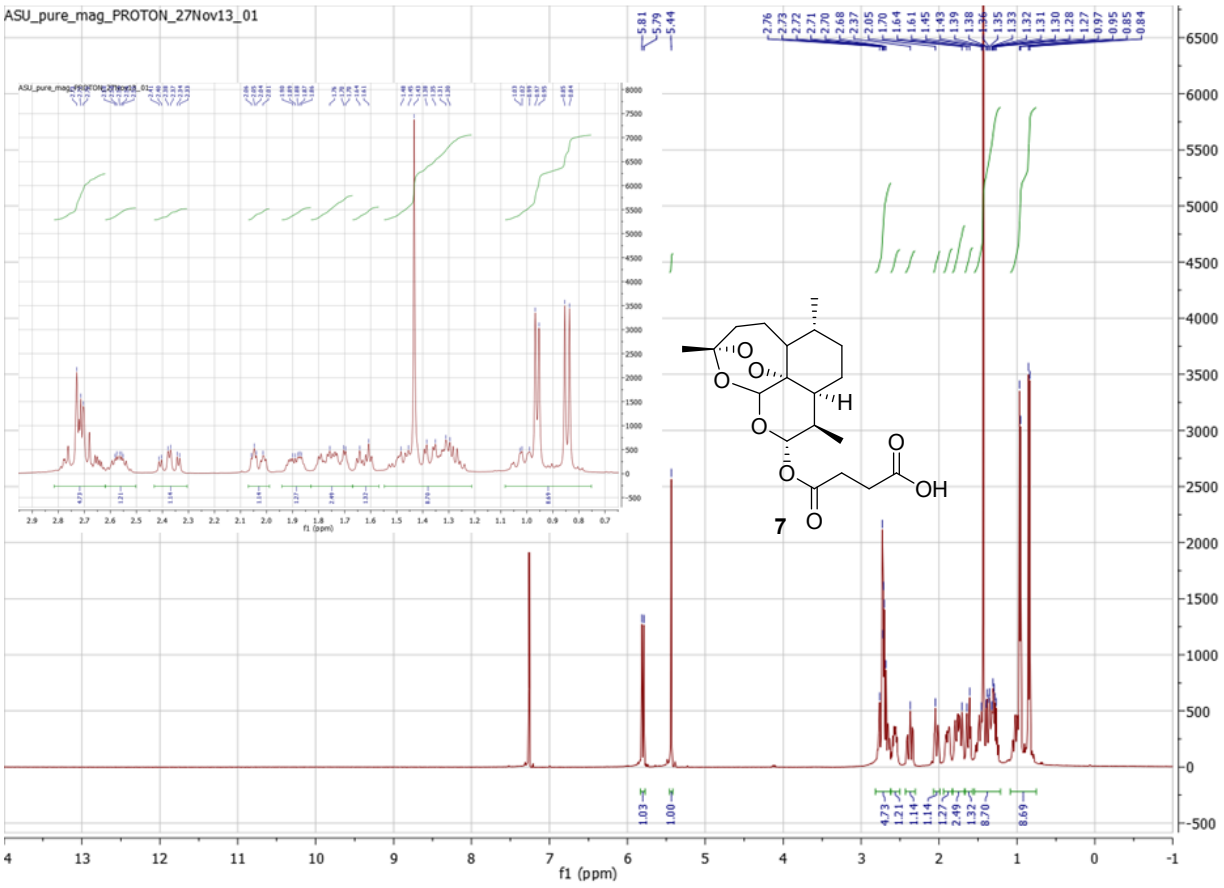


Conversion of Crude Dihydroartemisinin (4) Exiting Module 2 to α-Artesunate (7):



Typical Experiment: The organic phase from module 2 (2.1 mL) was pumped at a flow rate of 0.5 mL/min into the T-mixer where it met Feed B_{AS} (0.525 g succinic anhydride dissolved in 0.73 mL triethylamine and 3.3 mL of DCM) also pumped at a flow rate of 0.5 mL/min. Following

reactor 2 (25 mL), the solution was mixed with 1M HCl, pumped at 0.3 mL/min. The biphasic system was separated using the homemade liquid/liquid separator and the organic phase collected in an empty flask. The organic phase was then concentrated *in vacuo* to a white solid. Purification was achieved by column chromatography over silica gel (5% - 40% EtOAc, in cyclohexane) providing pure α-artesunate **7** as a white solid (146 mg) with a 28% yield over the three modular steps. ^{1}H NMR ($CDCl_3$): 5.80 (d, J = 8 Hz, 1H), 5.44 (s, 1H), 2.71 (m, 4H), 2.56 (m, 1H), 2.37 (td, J = 16, 4 Hz, 1H), 2.04 (dt, J = 16, 4 Hz, 1H), 1.87 (m, 1H), 1.75 (m, 2H), 1.62 (dt, J = 12, 4 Hz, 1H), 1.43 (s, 3H), 1.49-1.27 (m, 4H), 1.02 (m, 1H), 0.96 (d, J = 8 Hz, 3H), 0.85 (d, J = 8 Hz, 3H). This spectrum is in accordance with that reported in the literature.[7,8]



Monitoring the Formation of α-Artesunate Exiting Module 3:

The formation of α-artesunate **7** was utilized to showcase the monitoring of module 3. The FlowIR unit (Mettler Toledo) was inserted following the separation of the aqueous layer. The reaction can be monitored at either 1566 or 1398 cm^{-1} (Figure S5).

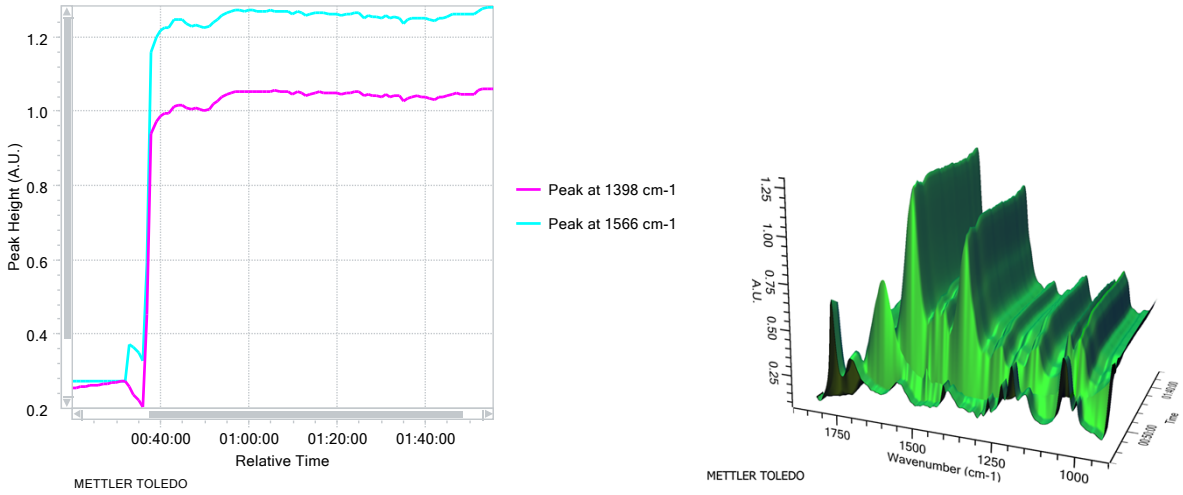


Figure S5: Monitoring of the formation of α-Artesunate **7** from crude DHA using FlowIR (Mettler Toldedo) can be achieved at either 1398 or 1596 cm^{-1}.

Module 4: Continuous Purification of Artesunate 7 Exiting Module 3.

HPLC Analysis of α–Artesunate: The mobile phase composition was water/acetonitrile/formic acid = 40/60/0.1 (v/v/v) and the flow-rate was fixed to 0.5 mL/min. The column temperature was 25ºC. The injection volume was fixed to 1 µL. The wavelength of UV detector was 220 nm. For the identification and calibration of artesunate peaks in the HPLC chromatograms, three standard solutions of artesunate were prepared (6.56, 10.43, and 37.61 g/L). Figure S6 shows the linearity between peak areas and injected amounts.

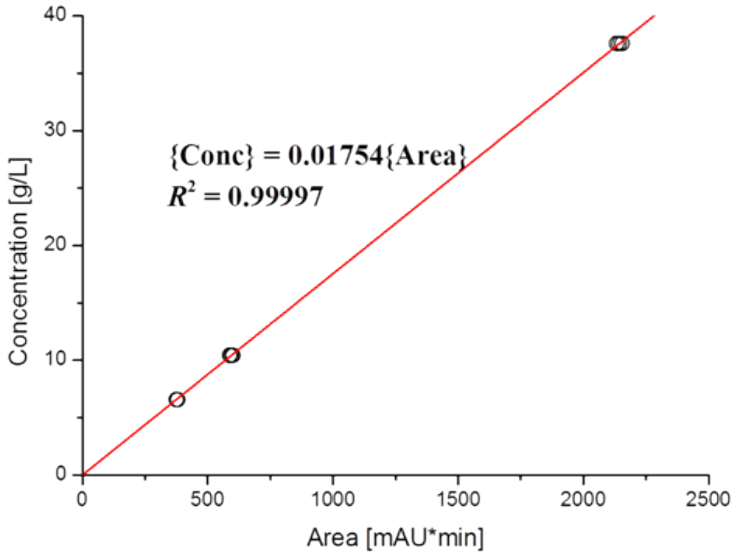


Figure S6: Calibration curve of α-artesunate **7** (UV detector, 220 nm).

Dilution and Filtration of the Reactor Effluent: The solvent composition of the reaction mixture is mainly toluene/dichloromethane = 50/50 (v/v), so that normal-phase chromatography (nonpolar solvent with polar adsorbent) is suitable to directly connect the reactor outlet to the inlet of multicolumn chromatography for a continuous production.[9] The concentration of α-artesunate **7** in the reaction mixture was 20.4 g/L.

Since many polar substances and solvents were used in the reaction steps and still contained in the reactor effluent, the reaction mixture is too strong to hold artesunate on a polar adsorbent. To weaken the solvent strength of the reaction mixture, *n*-hexane was mixed to the reaction mixture (9/1), diluting **7** to 1.33 g/L. After mixing with *n*-hexane, polar substances were immediately precipitated and subsequently removed by filtration (Figure S7). The recovery yield

of artesunate in this step was 65.2% based on peak areas of HPLC analysis. This diluted and filtered mixture was used as the feed of the subsequent chromatographic separation.

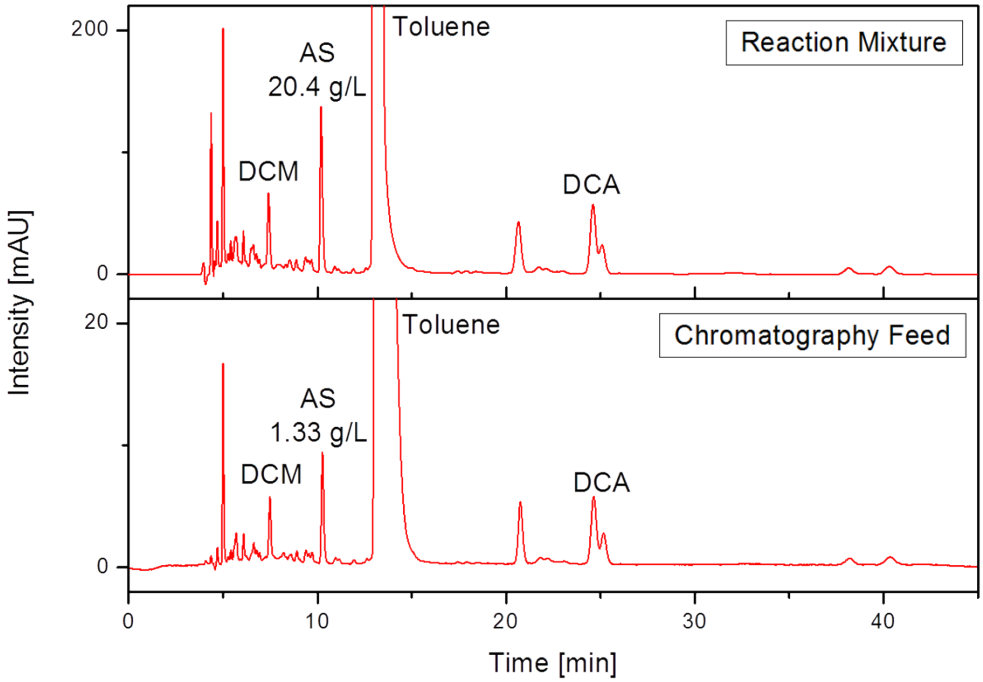


Figure S7: Chromatograms of the reaction mixture and the feed of multicolumn chromatography. AS: α-artesunate **7**, DCA: 9,10-dicyanoanthracene, DCM: dichloromethane.

Gradient Elution Chromatography: To isolate α-artesunate **7** using multicolumn chromatography, five steps of gradient elution were studied using one representative column (Table S2). The duration of each step was the same (7.5 min), so that this regime can be easily operated in a multicolumn unit equipped with five identical columns, allowing for continuous feeding. In the first elution step, most of nonpolar impurities, i.e. 9,10-dicyanoanthracene and artemisinin **3**, elute. Artesunate elutes in the second step. To collect target **7**, the column effluent was fractionated from 18.1 min to 23.0 min ("artesunate fraction"). To confirm that no α-artesunate **7** is lost in the other elution steps, the other column effluents were also collected and analyzed by HPLC.

Table S2: Elution steps of chromatography (identical for all columns).

Step	Composition [vol.%]	Flow-rate [mL/min]	Volume [ml]
Loading	reaction mixture/*n*-hexane = 10/90	4.00	30

1st elution	*n*-hexane/ethyl acetate	= 85/15	2.67	20
2nd elution	*n*-hexane/ethyl acetate	= 75/25	1.60	12
Rinsing	ethanol	= 100	1.60	12
Equilibration	*n*-hexane/ethyl acetate	= 85/15	1.60	12

To validate the process, six cycles were carried out in series collecting the artesunate and waste fractions. Figure S8 shows the chromatograms of six runs, which demonstate very good reproducibility.

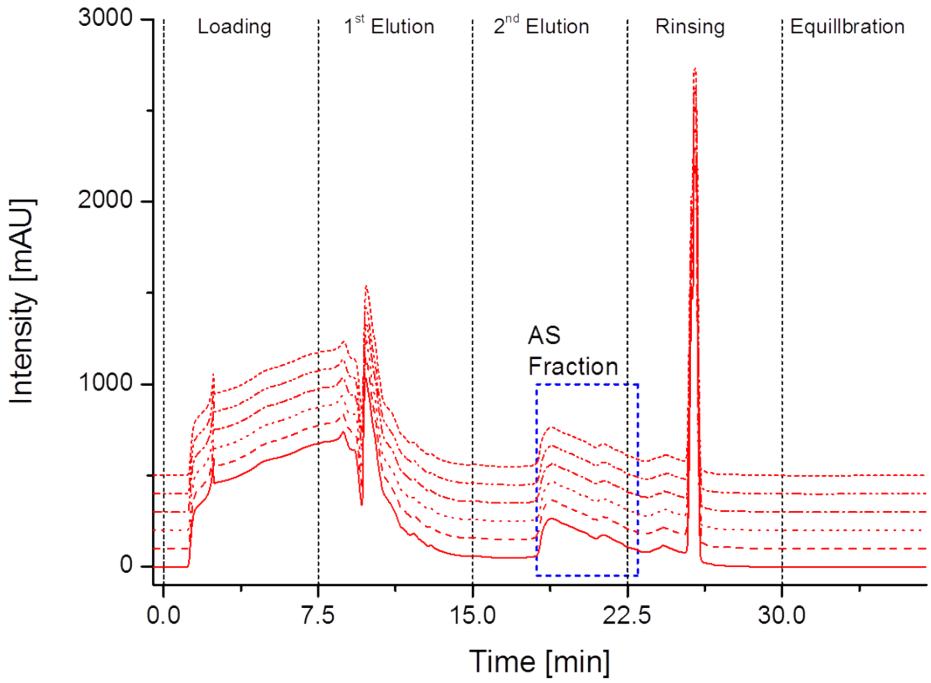


Figure S8: Chromatograms of the five-step gradient elution for α-artesunate **7** separation (overlap of six consecutive cycles).

Figure S9 shows the HPLC chromatograms of the artesunate fraction and the waste fraction. In the HPLC chromatogram of the feed, two major solvents of the reaction feed, dichloromethane and toluene were identified with artesunate and 9,10-dicyanoanthracene. In the HPLC chromatogram of the artesunate fraction, most of impurities and two major reaction mixture solvents were removed and only two distinct peaks of ethyl acetate and α-artesunate **7** were detected. In the waste fraction chromatogram, there is no distinct α-artesunate **7** peak. The

collected concentration of α-artesunate **7** was 4.26 g/L enriched by gradient elution and the recovery yield of α-artesunate **7** during the chromatographic separation was 100%. The original reaction mixture comprised mainly of toluene and dichloromethane, which are classified as toxic solvents. However, the solvent composition of artesunate fraction was changed prior to chromatography to a less toxic combination, *n*-hexane/ethyl acetate = 75/25 (v/v).[10]

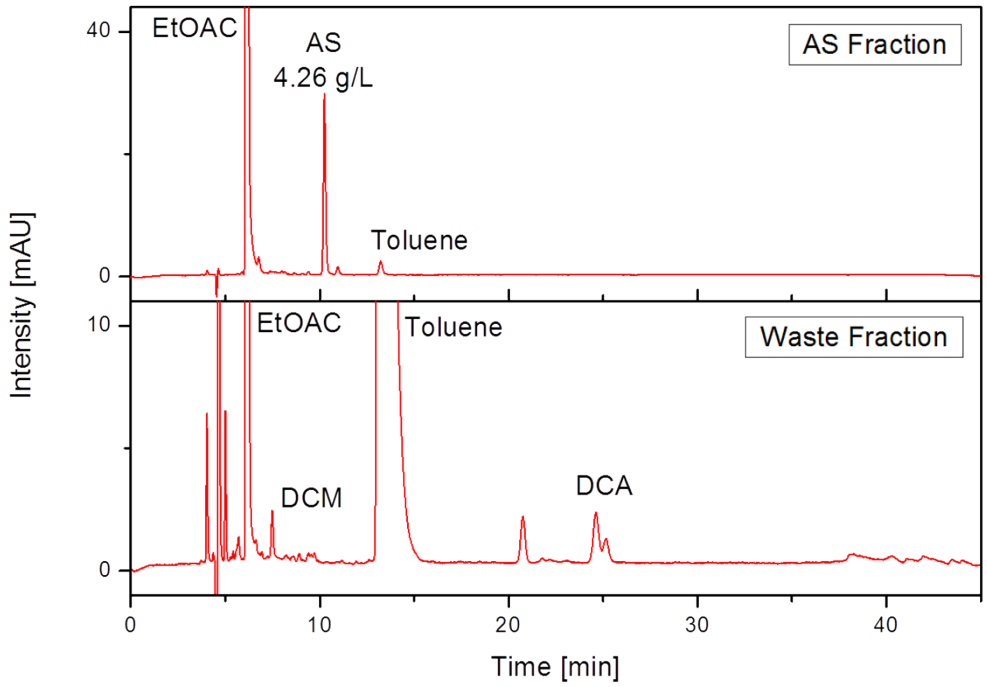


Figure S9: Comparisons of HPLC chromatograms for multicolumn chromatography fractions. AS: α-artesunate **7**, EtOAcEtOAc: ethyl acetate, DCM: dichloromethane, DCA: 9,10-dicyanoanthracene.

Crystallization: The purity of the α-artesunate **7** fraction collected in the chromatographic separation process was 92.3%. For further removal of remaining impurities in the artesunate fraction, just a single recrystallization step was required with *n*-hexane/ethyl acetate = 90/10 (v/v) as an anti-solvent. The obtained artesunate fraction was evaporated, and the residue was recrystallized. After filtering with a glass filter, the filtrate and recrystallized artesunate product were analyzed by HPLC. The concentration of artesunate in the filtrate anti-solvent was 0.46 g/L at ambient temperature. Recrystallized artesunate was dissolved in ethanol for HPLC analysis. Figure S10 shows the HPLC chromatograms of artesunate fractions before and after recrystallization. Two solvent peaks, ethyl acetate and toluene, were removed with small impurity

peaks around the artesunate peak. The purity of recrystallized artesunate was over 99.5% as determined by HPLC.

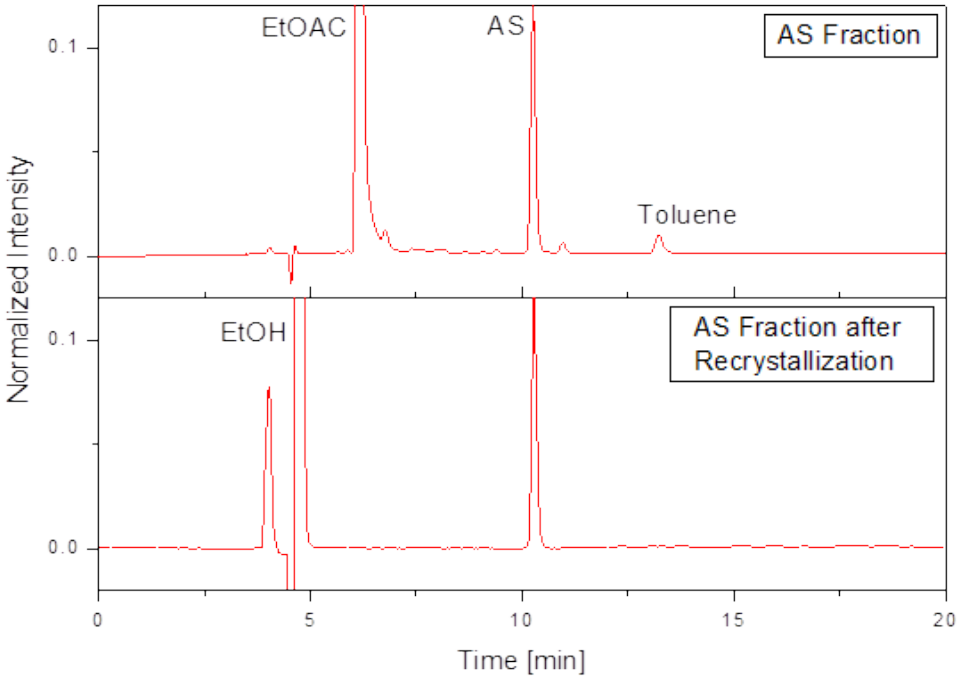


Figure S10: Comparisons of HPLC chromatograms prior to (top) and following recrystallization (bottom) of the artesunate fraction. AS: α-artesunate 7, EtOH: ethanol, EtOAC: ethyl acetate, DCM: dichloromethane, DCA: 9,10-dicyanoanthracene.

Continuous Separation of α-Artesunate: For the continuous production by the direct-connection of the reactor and separator, the decribed filtration step needs to be also continuous. Figure S16 illustrates two parallel filtration units. While one filtration unit is filtering the diluted reaction effluent, the other filtration unit is washed with ethanol to remove filtration residue that is freely soluble in ethanol. The recovery yield of artesunate during the dilution and filtration of reaction mixture is 65.2%. To build up the multicolumn chromatographic separator after filtration, five identical columns are used, (one column per each elution step) allowing for continuous feeding of the effluent of module 3 as shown in Figure S11. The artesunate fractions collected from the multicolumn separator can then be directly fed into a continuous crystallizer. Theoretical recovery yield of artesunate in the continuous crystallizer can reach 73% according to the concentration of α-artesunate **7** remaining in the anti-solvent and the concentration of artesunate product of the multicolumn separator. Since loss of artesunate can be avoided in the

chromatography step, the overall recovery yield of artesunate in the entire purification sequences is estimated to be 47.6% (Table S3).

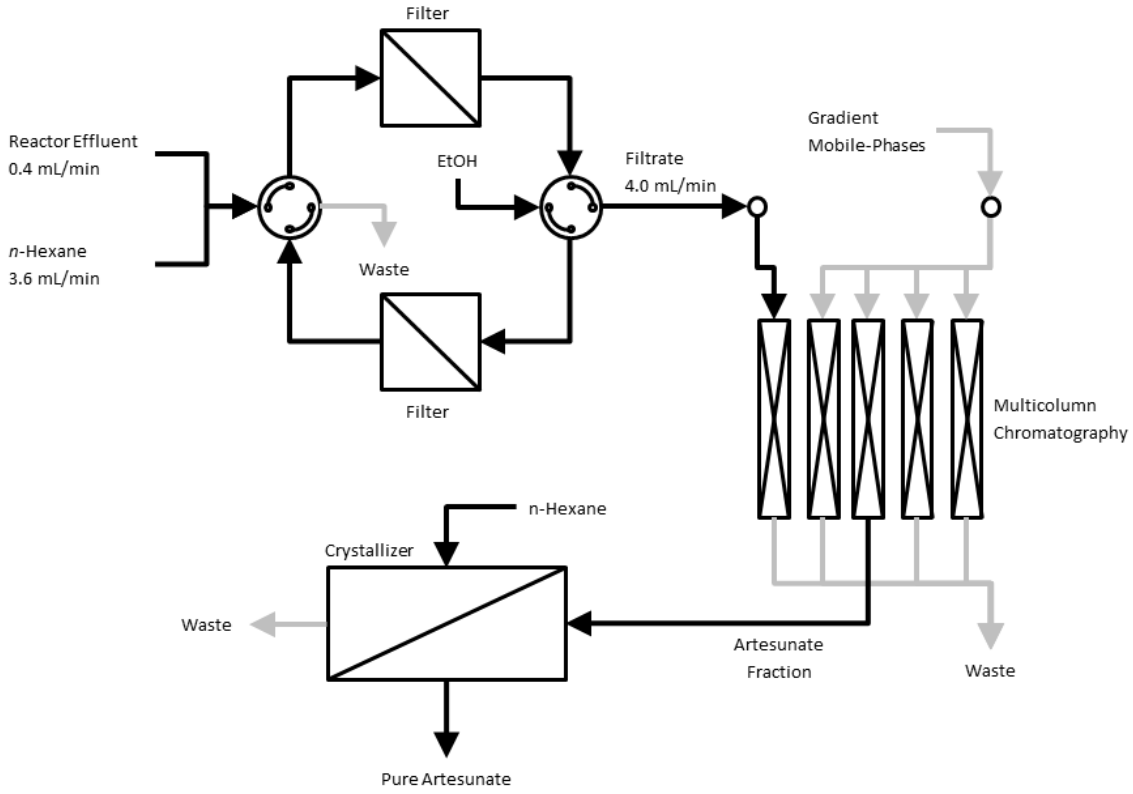


Figure S11: Schematic diagram of the purification sequence including filtration, multicolumn chromatography, and crystallization for the continuous provision of artesunate after Module 3. EtOH: ethanol.

Table S3: Results of the Overall α-Artesunate **7** Separation.

Filtration	Purity [%]	~40
	Yield [%]	65.2 (65.2)[a)]
Multicolumn Chromatography	Purity [%]	92.3
	Yield [%]	100 (65.2)[a)]
Crystallization	Purity [%]	> 99.5
	Yield [%]	73.0 (47.6)[a)]

[a)] Recovery yield of α-artesunate **7** with respect to the mass in the reactor effluent of module 3.

References:

1. X. Liu, and K. F. Jensen, *Green Chem.*, 2013, **15**, 1538.
2. J. G. Kralj, H. R. Sahoo, and K. F. Jensen, *Lab Chip*, 2007, **7**, 256.
3. a) D. Kopetzki, F. Lévesque, and P. H. Seeberger, *Chem. Eur. J.*, 2013, **19**, 5450. For our initial work using a medium-pressure Hg lamp, see: b) F. Lévesque and P. H. Seeberger, *Angew. Chem. Int. Ed.*, 2012, **51**, 1706.
4. C. Singh and P. Tiwari, *Tetrahedron Lett.*, 2002, **43**, 7235.
5. R. W. Stringham and D. S. Teager, *Org. Process Res. Dev.*, 2012, **16**, 764.
6. A. Brossi, *et al.*, *J. Med. Chem.*, 1988, **31**, 645.
7. R. K. Haynes, *et al.*, *Eur. J. Org. Chem.*, 2002, 113.
8. X.-d. Luo, J. J. C. Yeh, and A. Brossi, *Helv. Chim. Acta*, 1984, **67**, 1515.
9. R. J. S. Silva, *et al.*, *J. Chromatogr. A*, 2010, **1217**, 8257.
10. U.S. FDA, Guidance for Industry Q3C - Tables and List, Feb. 2012, Rev. 2 http://www.gmp-compliance.org/guidemgr/files/Q3CTABLES&LIST.PDF.