Process Development for the Manufacture of the Antimalarial Amodiaquine Dihydrochloride Dihydrate

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Abstract

A robust process technology for the manufacture of the active pharmaceutical ingredient (API) amodiaquine dihydrochloride dihydrate (ADQ, **3**), an important antimalarial, is reported. The process consists of a three-step synthetic route that involves a Mannich reaction, condensation with 4,7-dichloroquinoline (DCQ, **5**) and rehydration. Additionally, a cost-competitive process for the production of DCQ (**5**) is also reported wherein DCQ (**5**) was prepared in four steps from *meta*-chloroaniline (**7**). 4-Amido-2-(diethylaminomethyl)phenol (**14**), DCQ (**5**), and ADQ (**3**) were obtained in yields of 92, 89 and 90% respectively.

Introduction

Malaria is still one of the leading causes of death worldwide, with an estimated 247 million cases and 619,000 deaths reported in 2021.[1] The main epidemic areas of malaria are distributed in Africa (96%), followed by Southeast Asia (SE Asia) (2%) and the Eastern Mediterranean Region (2%).[1] The World Health Organization (WHO) hopes to eliminate malaria in at least 35 additional countries (based on data from 2021) by 2030.[2]

Quinine (Figure 1, **1**) was the sole antimalarial drug used since its discovery in the 19th century, followed by chloroquine (**2**).[3] However, due to emerging chloroquine resistance, more antimalarial drugs such as amodiaquine (ADQ, **3**) were discovered.[4,5]

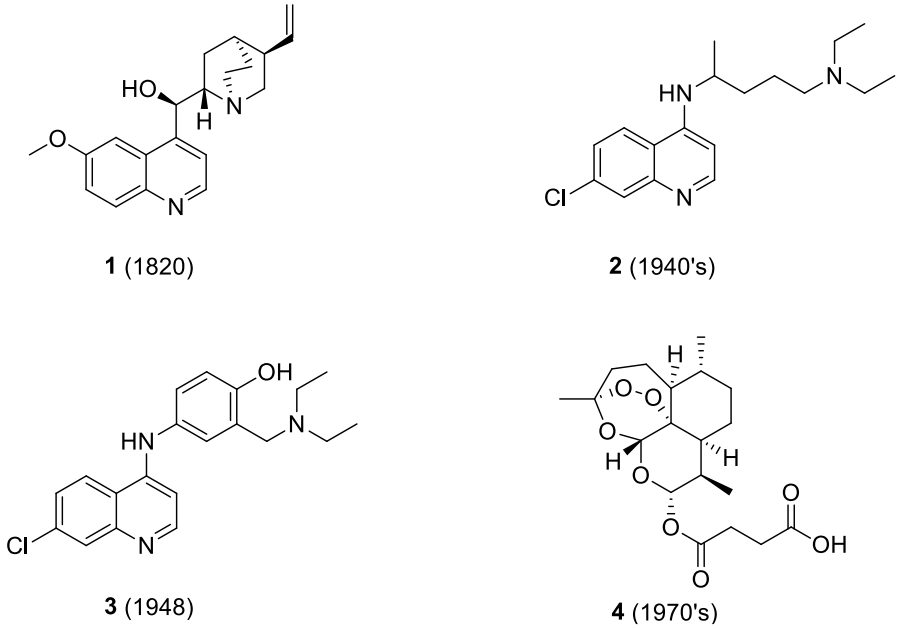


Figure 1: The structures of current antimalarial agents; quinine (**1**), chloroquine (**2**), amodiaquine (**3**) and artesunate (**4**).

ADQ (**3**), first discovered in 1948, is a 4-aminoquinoline antimalarial drug used (in base or acid form) as an alternative against chloroquine-resistant strains.[6-8] Due to the severe side effects from the sole use of ADQ (**3**), the World Health Organisation (WHO) has recommended the implementation of artemisinin-based combination therapy (ACT), which is the pairing of ADQ (**3**) with artemisinin derivatives such as artesunate (**4**) as a first-line of treatment for uncomplicated malaria.[9,10] A 2006 study on the use of ACT in a village in Uganda concluded that the use of ACT offered an important step forward for the treatment of malaria in Africa and that more extensive research into the development of a cost-effective ACT as well as co-formulations is a necessity.[11]

Despite the available antimalarial drugs such as amodiaquine (**3**), most of sub-Saharan Africa still lacks adequate access to good quality, affordable antimalarial drugs due to most active pharmaceutical ingredients (APIs) being imported from other countries. While published processes are available for ADQ and 4,7-DCQ, [9,10,12-20] each of these methods have limitations such as low yields, formation of impurities, the use of expensive solvents or hazardous solvents or more unit operations are required for production of Amodiaquine, which drives up the energy requirements.[9,21] Additionally, no methods are reported for the removal of impurities or for the exact determination of the crystal water molecules in ADQ.[12] These limitations make it difficult to produce ADQ (**3**) at a competitive price.

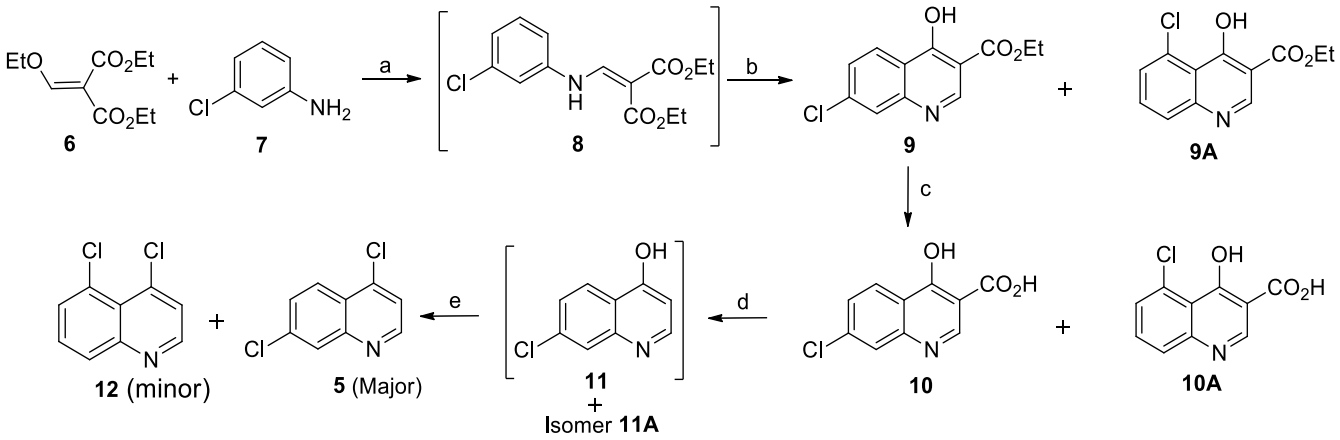
Process development entails the development, optimization and scale-up of a chemical synthetic route that can be transferred into a cost-effective, safe, and reproducible manufacturing process. The development comprises three stages: bench-scale, kilo-scale, and pilot-plant scale, with process validation at each stage.[22,23] During the initial stages, the most robust synthetic route is investigated, optimized and validated, followed by scale-up of the chosen route to kilogram scale in the Kilo lab. Key factors that are considered during each stage include the temperature of the reaction, reaction time, number of steps, product loss minimization, work-up and product isolation procedure, waste management and environmental impact, reproducibility and costs involved. When the above-mentioned are satisfied, the process gets transferred to the pilot plant where aspects such as scalability, safety, and quality are further evaluated. In each stage of development, total process cost is measured, which ultimately contributes to the total API product cost (material cost + conversion cost).[22,23]

Results and Discussion

4,7-Dichloroquinoline (**5**) is an important component of several antimalarial drugs[15,24] and is therefore a major driver of cost in the production of amodiaquine (**3**), as it accounts for over 40% of the raw material costs. Thus, a robust, cost-competitive process for the production of amodiaquine (**3**) would require preferred pricing from commercial suppliers. This, however, would be a temporal solution as there would be no internal control of costs, hence, an equally cost-competitive process for the manufacture of 4,7-dichloroquinoline (**5**) needed to be developed in-house for continuous raw materials supply without interruption during ADQ (**3**) production.

Preparation and Process Development of 4,7-Dichloroquinoline (5)

The process for the commercial production of 4,7-dichloroquinoline (**5**) was developed according to the methodology of Price and Roberts (Scheme 1),[15] which is centred on diethoxymethylene malonate (**6**) and *meta*-chloroaniline (**7**), with necessary modifications.



Scheme 1: Reagents and conditions: a) 100 °C, 2 h; b) DPE, 250 °C, 2 h; c) 10% aq. NaOH, 2 h, 10% aq. H_2SO_4; d) DPE, 250 °C, 2 h; e) 135 °C, $POCl_3$, 2 h.

The synthesis of 4,7-dichloroquinoline (**5**) commenced by the conjugate addition of diethoxymethylene malonate (**6**) and *meta*-chloroaniline (**7**) to afford the acrylate intermediate **8**, which, upon thermal cyclisation in diphenyl ether (DPE), afforded the quinoline ester **9** in good yields (90-96%). Hydrolysis of the ester (**9**) in aqueous sodium hydroxide to the quinoline acid (**10**) was achieved in essentially quantitative yields, while thermal decarboxylation and subsequent chlorination with $POCl_3$ gave the target product **5** in 81-90% yields. The GC-MS chromatogram (Figure 2) of the crude product showed an extra peak at a retention time of 13.13 minutes with a similar mass to that of 4,7-dichloroquinoline (**5**).[15] After isolation and characterisation using 1D and 2D NMR, the identity of the impurity was confirmed to be the 4,5-dichloroquinoline isomer (**12**, Figure 2).[20] The ^{1}H NMR spectrum of the isomer **12** displayed two doublets integrating for one proton each at δH 8.72 (d, *J* 4.68 Hz, 1H, H-2) and at δH 7.52 (d, *J* 4.68 Hz, 1H, H-3) for the protons on the B-ring. An ABX spin system was observed at δH 8.05 (dd, J_1 8.32, J_2 1.44 Hz, H-8); 7.67 (dd, J_1 7.56, J_2 1.42 Hz, 1H, H-6) and 7.60 (dd, J_1 7.96, J_2 7.96 Hz, 1H, H-7) for the A-ring aromatic protons. Additional distinguishing NMR correlations were observed in the COSY NMR spectrum, with proton-proton correlation between the three protons H-6, H-7 and H-8 confirming that they were adjacent to each other. The ortho-meta (7.56, 1.42 Hz) coupling constants for H-6, ortho-ortho (7.96, 7.96 Hz) coupling constants for H-7 and ortho-meta (8.32, 1.44 Hz) coupling constants for H-8 provide further confirmation for the arrangement of these protons.

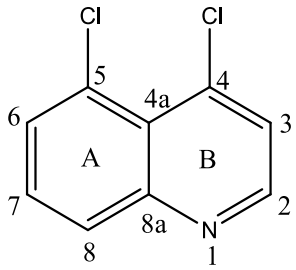


Figure 2: 4,5-Dichloroquinoline (**12**).

Attempted recrystallization in hexanes, as reported by Price and Roberts[15] afforded the pure product in moderate yields of 59-65% (Table 1, Entry 1). Several solvents and conditions were tried in order to improve the yields, with recrystallization in heptane resulting in a slight improvement in yields without compromising the purity (Entry 2). In contrast to the alkanes, OH-containing solvents such as ethanol and methanol resulted in a drastic decrease in yields (Entries 3 and 5). In an attempt to minimize the solubility of the product, 5% water was added to the recrystallization, this however showed an inverse relationship between purity and yields, as the purity significantly reduced from 99.5% to 86% and 90% for MeOH/H_2O and EtOH/H_2O respectively.

Table 1: Recrystallisation of crude 4,7-dichloroquinoline (**5**)

Entry	**Solvent**	**Yield (%)**	**Purity by GC (%)**
1	Hexanes	58-65	99.5
2	Heptane	60-67	99.5
3	EtOH	45-56	99.5
4	EtOH/H_2O	65-75	90
5	MeOH	51-60	99.5
6	MeOH/H_2O	67-73	86

Even though successful in its objective of removing the 4,5-dichloroquinoline isomer (**12**), the loss in yield and a need for an extra step outweighed the advantages. Another option to remove the isomer, would be to take advantage of solubility due to the difference in the acidity of the two chloroquinoline acid isomers during hydrolysis.

Acetic acid has been reported[25] as an excellent solvent for the selective precipitation for the isolation of the two quinoline acid isomers (**10** & **10A**), however, the harsh conditions (reflux) significantly reduce the selectivity, resulting in lower yields of the quinoline acid (**10**). Additionally, it is expensive and would require extra care in the plant due to its strong odour and harmful effects, thus, using acetic acid as the solvent for the elimination of the 4,5-isomer (**12**) would not be ideal on a commercial scale.

The reported pH for the precipitation of the quinoline acid (**10**) is Congo red (pH 5),[15] which upon decarboxylation and chlorination affords 4,7-dichloroquinoline (**5**) with 3-4% of the 4,5-dichloroquinoline isomer (**12**). In this study, we envisaged that, owing to the difference in acidity, the two isomers, or at least the majority thereof, would precipitate at different pH values, and thus be isolable (Figure 3). Upon hydrolysis of **9** with NaOH, the resulting mixture is basified to pH 8.2-8.4, instead of pH 4 as reported in the literature. The precipitate is then isolated by filtration and slurry-washed at pH 4 to remove any remaining sodium salt. The resulting quinoline acid is then subjected to decarboxylation and chlorination with $POCl_3$ to afford the target product 4,7-dichloroquinoline (**5**) in high purity.

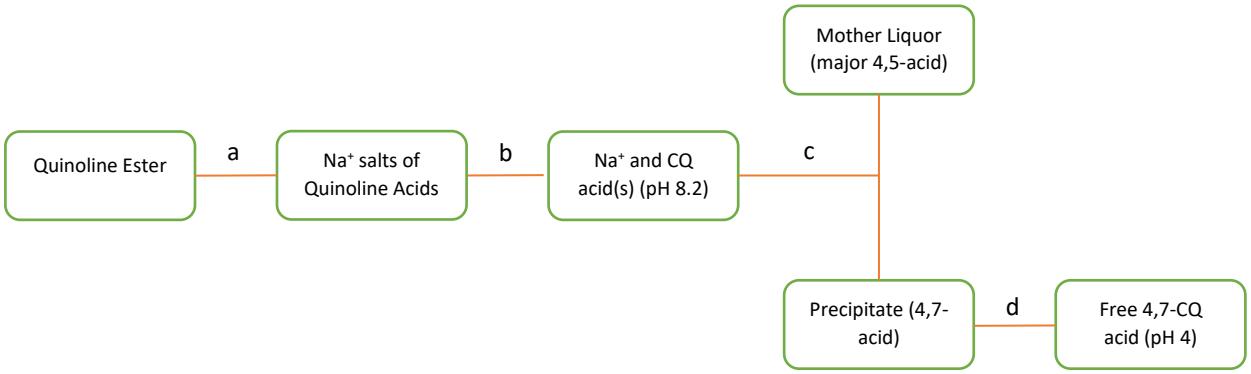


Figure 3: Fractional precipitation flow diagram consisting of a) Hydrolysis with NaOH; b) Neutralisation; c) Filtration; d) Slurry-wash at pH 4. (CQ – Chloroquinoline acid)

We began our investigation by precipitating the quinoline acid (**10**) at pH 6.5, which afforded the acid in almost quantitative yields (98%, Table 2, Entry 1). However, GC assay results indicated that the final product is contaminated with the isomer (Figure 4).

Nonetheless, as the pH was increased from 7.0 to 7.5, the isomer in the isolated product decreased (Entries 2 and 3). Similarly, at pH 8.0-8.10 (Entries 4 and 5), only trace amounts of the isomer were observed. At pH 8.2 and 8.5 (Entries 6 and 7), there was virtually no isomer

observed in the spectrum (Figure 5), affording the target product in 99.3% purity by GC assay. Although the yields decreased from 99% (Entry 1) to 90% (Entry 6), the fractional precipitation technique proved superior to solvent recrystallization where yield losses of up to 25% were observed. Additionally, this is a single-operation process and does not require extra solvent. Moreover, the reactions were first attempted at room temperature at which a thick heterogenous slurry formed and rendered the mixture difficult to stir. However, as temperature was increased to 45 °C, the mixture was homogenous, easy to stir and filter which ultimately afforded the desired purity.

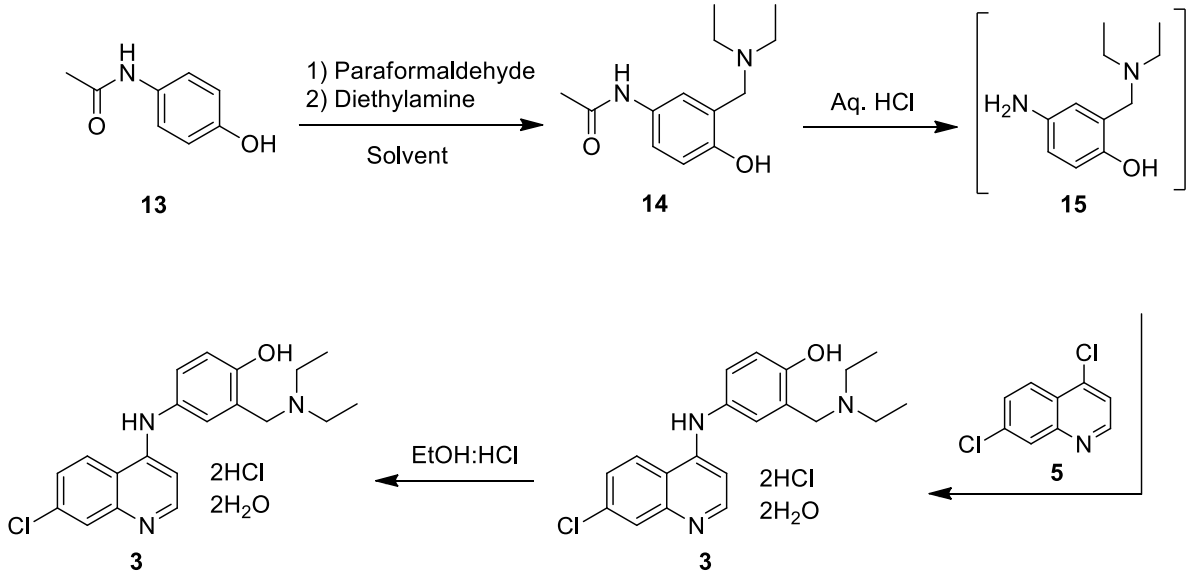
Table 2: Effect of changing pH on the purity of 4,7-DCQ (**5**)

Entry	**Temperature (°C)**	**pH**	**Yield of 5 (%)**	**Outcome observed in GC-MS**
1	45	6.5	99	Isomer observed
2	45	7.0	97	Isomer observed
3	45	7.5	95	Isomer observed
4	45	8.0	92	Traces of the isomer
5	45	8.10	92	Traces of the isomer
6	45	8.20	90	Single peak (Figure 5)
7	45	8.5	84	Single peak

The optimized process described above was demonstrated to be repeatable in more than 20 experiments varying in scale from 15 grams to 500 grams. A 4,7-DCQ yield of 81% and purity of more than 97% were achieved throughout. This process resulted in an overall yield of 68% of the correct quality 4,7-DCQ. Based on this, a material cost per kilogram for 4,7-DCQ was calculated as $24.17 versus a market price of $42.00/kg. The material margin of the process at current market prices is slightly better at 42% versus the target material margin of 37% specified in the scope of this project. That said, successful development of a cost-effective, competitive process for production of 4,7-dichloroquinoline (**5**) would mitigate the reliance on imports and provide a steady supply of this critical intermediate.

Preparation of Amodiaquine dihydrochloride dihydrate

The synthesis of amodiaquine dihydrochloride dihydrate (**3**) was performed as shown in Scheme 2 following the reported procedure by Burckhalter *et al*[12] with slight modifications. Amodiaquine dihydrochloride dihydrate (**3**) was prepared via a 4 step synthetic scheme involving a Mannich reaction, followed by hydrolysis of the amide group and a subsequent condensation with 4,7-dichloroquinoline (**5**). The key intermediate **14**, was prepared by subjecting 4-acetamidophenol (**13**) to a Mannich reaction with diethylamine (DEA) and paraformaldehyde in a solvent. Several reaction conditions were attempted before achieving a robust method to obtain the Mannich base (**14**) in desirable yields.



Scheme 2: Preparation of amodiaquine dihydrochloride dihydrate (**3**) from 4-acetamidophenol (**13**).

The first attempt followed the reported procedure where paraformaldehyde was reacted with DEA in methanol for 2 h at 40 °C to allow for the formation of the iminium ion, followed by the addition of 4-acetamidophenol (**13**) and stirring the reaction at 64 °C for 3 h (Table 3). However, TLC analysis showed incomplete conversion of **13** within 3 h, thus the reaction was continued for 24 h while monitoring progress at intervals of 2 h to afford the Mannich base (**14**) in a moderate yield of 60%. With the intention of reducing the reaction time, the reaction was repeated in methanol and 32% HCl, however, within 7 h, TLC analysis showed the formation of unidentifiable impurities. The following attempts ran the Mannich reaction in acetic acid at varying temperatures from 50 °C to 80 °C for 5-24 h. The reaction proceeded

well at lower temperature but, slowly. As the reaction temperature was increased, more impurities, which were attributed to the double-Mannich reaction,[5] were formed instead.

Table 3: Varying reaction conditions for the preparation of the Mannich base (**14**)

Entry	Solvent	Temperature (°C)	Time (h)	Yield (%)
1	Methanol	65	3	82
2	Methanol	60	24	60
3	Ethanol	78	15	Poor conversion
4	Isopropanol	85	24	87
5	Methanol + HCl	60	7 h	-
6	Isopropanol + *p*-TSA	85	24 h	61
7	AcOH	50/80	5-24 h	-
8	Toluene	85	15 h	95

The reaction was then attempted in ethanol at 78 °C, which, within 15 h had proceeded poorly. Isopropanol was the next solvent attempted at 85 °C, which showed improved yields of 87% after 24 h with minimal impurity formation. To catalyse the reaction, with the aim of reducing the reaction time, the reaction was repeated in isopropanol in the presence of *p*-toluenesulfonic acid (*p*-TSA) as catalyst (Entry 6). However, TLC analysis showed formation of more impurities than was observed in the earlier attempt (Entry 4) and the Mannich base (**14**) was obtained in reduced yields of 61% (versus 87%). It was clear at this point that the use of any acid promoted the formation of more impurities. The next attempt saw the reaction performed in toluene at 85 °C which afforded **14** in an excellent yield of 95% within 15 h. Moreover, to the best of our knowledge, a C-C bond formation Mannich reaction in toluene has not been reported in the literature previously.[26] Due to toluene's relative affordability and its ability to be recycled and reused, this contributes to cost cutting and ultimately renders our process competitively cheaper.

Having successfully developed the process for the preparation of the Mannich base (**14**), the next step was to synthesise the final product **3**. The synthesis of ADQ (**3**) was carried out in

two steps from the intermediate **14**, following the reported procedure which involved hydrolysis of the Mannich base followed by condensation with 4,7-DCQ (**5**) *in situ*.[12] As with the preparation of the Mannich base, several reaction conditions were examined to find a robust process for the preparation of ADQ (**3**).

Table 4: Reaction conditions for the synthesis of amodiaquine dihydrochloride dihydrate (**3**)

Entry	Hydrolysis conditions	Condensation conditions	Yield (%)
1	20% HCl, 80 °C, 4 h	EtOH, 24 h, 78 °C	43
2	32% HCl (9 mL), H_2O (9 mL), EtOH (7.4 mL), 3 h	3 h, 78 °C	10
3	32% HCl (9 mL), H_2O (9 mL), IPA (7.4 mL), 80 °C, 2.5 h	2 h, 80 °C	58
4	32% HCl (5 mL), H_2O (5 mL), 80 °C, 5 h	15 h, 80 °C	53
5	32% HCl, 80-85 °C, 4 h, H_2O	3 h, 80-85 °C	90

For the first attempt the Mannich base (**14**) was refluxed in 20% HCl for 4 h at 80 °C followed by distillation of the excess HCl, and then condensation of 4,7-DCQ (**5**) in ethanol for 24 h to give ADQ (**3**) in 43% yield. In addition to the low yield obtained, this process required extra energy to distil out water from the reaction, thus, it would not be practical during scale-up of the process. The next attempt involved refluxing the Mannich base (**14**) in a mixture of HCl/H_2O/solvent for 3-5 h, where the solvent was either ethanol or isopropanol, followed by condensation with 4,7-DCQ (**5**). Table 4 Entry 2 shows the reaction in ethanol produced a low yield of 10%, whereas isopropanol (Entry 3) resulted in an improved yield of 58%. When the same reaction conditions are attempted in the absence of an organic solvent (Entry 4), a yield of 53% was obtained. The next attempt involved subjecting the Mannich base (**14**) to hydrolysis in commercial grade HCl (32%) at 85 °C for 4 h to produce the amine (**15**), which after pH adjustment to 4, was condensed with 4,7-DCQ (**5**) *in situ* to afford the desired ADQ dihydrochloride dihydrate (**3**). Crude **3** was recrystallized from ethanol and rehydrated by refluxing in water followed by precipitation at cool conditions to obtain ADQ dihydrochloride dihydrate (**3**) in an excellent yield of 90% with USP quality. The HPLC chromatogram shows

only a single peak at a retention time between 5-6 minutes, proving the absence of starting material (Figures 6 & 7).

Once a robust synthetic route suitable for the manufacturing process was developed and optimized, the next step was to prove scalability and reproducibility. This was done by following the developed route on a 100-400 g and 5 kg scale at least three times (100-400g) as shown in Table 5, analysing the intermediates and products by GC-MS, IR, NMR, MP and HPLC. There were no significant changes required to the route on a 300 g scale, however, as the scale was increased to 5 kg, temperature and time became the major optimization points. The larger the reactor, the longer it took to heat up the reaction as required, therefore heating the reaction and subsequently cooling to adjust the pH after hydrolysis of the Mannich base (**14**), followed by condensation with 4,7-DCQ (**5**) at 90 °C was not feasible. It then became necessary to adjust the pH under hot conditions at 50 °C, which did not cause any problems or impurity formation despite our concerns.

The overall yield for the optimized process is 78%. Based on this the material cost for ADQ has been calculated as $16.91 per kilogram, resulting in a material margin of 58%. This is slightly better than the target margin of 57% defined in the scope of this project. Therefore, if the process developed in this project is to be commercialized and ADQ sold at an average market price of $38.80/kg, it will yield an acceptable raw material margin of 56%. Because of the improved technology developed by CPT, the selling price can be reduced by 13% to $33.80 and still yield an acceptable raw material margin of 50%. It can thus be concluded that CPT was successful in optimizing the known processes and lowered the required selling price by 13% at current low materials cost. In conclusion, 4,7-dichloroquinoline (**5**), 4-amido-2-(diethylaminomethyl)phenol (**14**) and amodiaquine dihydrochloride dihydrate (**3**) were synthesized on kilogram scale, with the current project resulting in the successful development of a robust, economically competitive, scalable and reproducible process that can be transferred to a commercial process for the manufacture of the antimalarial API amodiaquine dihydrochloride dihydrate (**3**).

Experimental

General Experimental procedure

All raw materials and solvents purchased were used without further purification. Thin Layer Chromatography (TLC) was performed on Macherey-Nagel 0.2 mm silica gel 60 F254 packed aluminium plates observed under UV light at 254 nm. The synthesised compounds were analysed by FTIR spectroscopy, NMR spectroscopy with the residual solvent peak as an internal reference (DMSO-d_6 = 2.50 and 39.5 ppm and $CDCl_3$ = 7.26 and 77.16 ppm for ^1H and ^{13}C NMR spectra respectively), and Gas Chromatography Mass Spectroscopy (GC-MS). The purity of the final product **3** was determined using High-Performance Liquid Chromatography (HPLC) on a Hitachi system equipped with a LUNA C18 column and a diode array detector set at 224 nm.

Thermal analyses of final ADQ products (**3**) were conducted using thermo-gravimetric analyses (TGA), the TGA-TA 5500 and differential scanning calorimetry (DSC), DSC-TA 2500, under a nitrogen atmosphere. The TGA and DSC thermograms were analysed by TRIOS 5.3.0.48151 version and Origin2018. Isothermal experiments were performed with a TRIOS 5.3.0.48151 version calorimeter with a nitrogen flow rate of 50 mL/min.

3-Carbethoxy-7-chloro-4-hydroxyquinoline (9)

52 g (0.240 mol, 1.1 equiv.) of diethoxymethylene malonate was added to *meta*-chloroaniline (30 g, 0.235 mol, 1.0 equiv.) and the reaction mixture was heated under stirring at 97-100 °C for 2 h. Under a nitrogen atmosphere, the warm acrylate was added dropwise into a hot solution of diphenyl ether (225 °C). The reaction mixture was stirred at 225 °C for 2 h followed by cooling to 50 °C. 100 mL of toluene was added to the semi-solid mass and the mixture was stirred well for 15 min. The product was filtered *in vacuo*, washed with toluene (2x 50 mL) and dried in the oven (40 °C) to afford **9** as a brown fluffy solid (54 g, 0.241 mol, 91%). mp = 294-296 °C (lit. = 295-297 °C).[27]

7-Chloro-4-hydroxyquinoline-3-carboxylic acid (10)

To a stirred solution of aq. NaOH (25%, 200 mL), the quinoline ester **9** (54 g, 0.241 mol, 1.0 equiv.) was added. The reaction mixture was heated at 95-97 °C for 2 h, during which all the ester dissolved to form a brown solution. The reaction mixture was cooled to room temperature and neutralised to pH 8.2 by slow addition of 10% aq. H_2SO_4 solution. The reaction mixture was heated at 45 °C for 1 h (maintaining pH 8.2). The precipitate was collected by filtration

while warm and washed with water (2x 200 mL). The filter cake was suspended in 250 mL water, stirred vigorously, and the pH adjusted to 4 by slow addition of 10% aq. H_2SO_4 solution. The quinoline acid was filtered *in vacuo*, bed-washed with 200 mL H_2O and dried in a vacuum oven (100 °C) overnight to afford **10** as a white powder (43 g, 0.120 mol, 89%). mp = 272-273°C (lit. = 273-274 °C).[27]

4,7-Dichloroquinoline (5)

To a stirred solution of diphenyl ether (200 mL) under a nitrogen atmosphere, quinoline acid (**10**, 42 g, 0.188 mol, 1.0 equiv.) was added. The reaction mixture was refluxed at 225-227 °C for 4 h, during which all the solid dissolved to form a light brown solution. The reaction mixture was cooled gradually to 30 °C and then 18.6 mL (1.1 equiv.) $POCl_3$ was added dropwise over 10 min. The reaction mixture was refluxed at 133-135 °C for 2 h, then cooled to 30 °C and 50 mL toluene was added. The organic layer was extracted three times at 80 °C with 100 mL of 10% aq. HCl. The combined aqueous layers were washed with 100 mL toluene and then chilled to 15 °C. The pH was adjusted to 0.5 with 25% aq. NaOH and the resulting brown precipitate was filtered off. The mother liquor was basified to pH 12.6 by slow addition of 25% aq. NaOH solution, the product was collected by filtration under vacuum and slurry-washed with 300 mL water. The product was filtered *in vacuo*, dried in an oven at 40 °C for 48 h to afford **5** as a cream white solid (33.4 g, 0.169 mol, 90%). mp = 84-85 °C (lit = 84-86 °C).[27] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (d, *J* 4.72 Hz, 1H, H-2); 8.15 (d, *J* 8.96 Hz, 1H, H-5); 8.10 (d, *J* 2.04 Hz, 1H, H-8); 7.57 (dd, J_1 8.96, J_2 2.08 Hz, 1H, H-6); 7.47 (d, *J* 4.72 Hz, 1H, H-3). HRMS *m/z* $[M+H]^+$ 197.040 (calculated for $C_9H_5Cl_2N$ 198.057).

4,5-Dichloroquinoline (12)

Isolated from the crude product by column chromatography, eluting with EtOAc/Hexanes (1:9 to 3:7 v/v) to afford the isomer **12** as a white crystalline solid. mp = 115.7-116.4 °C (lit = 116-117 °C).[20] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (d, *J* 4.68 Hz, 1H, H-2)); 8.05 (dd, J_1 8.32 J_2 1.44 Hz, 1H, H-8); 7.67 (dd, J_1 7.56 J_2 1.42 Hz, 1H, H-6); 7.60 (dd, J_1 7.96 J_2 7.96 Hz, 1H, H-7); 7.52 (d, *J* 4.68 Hz, 1H, H-3). HRMS *m/z* $[M+H]^+$ 199.039 (calculated for $C_9H_5Cl_2N$ 198.057).

4-Amido-2-(diethylaminomethyl)phenol (14)

To a solution of paraformaldehyde (11.92 g, 0.397 mol, 1.2 equiv.) in toluene (200 mL) was added diethylamine (30.2 g, 0.413 mol, 1.25 equiv.) dropwise. The mixture was stirred for 2 h

at 40 °C before adding 4-acetamidophenol (50 g, 0.331 mol, 1.0 equiv.) to the mixture followed by stirring for 15 h at 80-85 °C. The mixture was gradually cooled to room temperature and subsequently stirred for 2 h at 5-10 °C. The product was filtered *in vacuo*, washed with toluene (2x 50 mL) and water (50 mL) and dried in the oven at 40 °C to afford **14** (74 g, 0.314 mol, 95%) as a white powder: mp = 133,3- 135,5 °C (lit = 135 °C).[12] ^{1}H NMR (400 MHz, DMSO-d_6) 9.60 (s, 1H, NH); 7.26 (s, 1H, Ar-H); 7.23-7.21(d, *J* 8.29 Hz, 1H, Ar-H); 6.59-6.57 (d, 1H, *J* 8.84 Hz, Ar-H); 3.63 (s, 2H, C$\underline{H}_2$NEt$_2$); 1.22 (s, 3H, AcC$\underline{H}_3$); 0.99-0.97 (t, 6H, *J* 7.13 Hz, N(CH$_2$C$\underline{H}_3$)$_2$). HRMS *m/z* $[M+H]^+$ 237.186 (calculated for $C_{13}H_{21}N_2O_2$ 236.16).

Amodiaquine dihydrochloride dihydrate (3)

4-Amido-2-(diethylaminomethyl)phenol (**14,** 50 g, 0.212 mol, 1.0 equiv.) was added to a flask charged with 32% HCl (110 mL, 0.966 mol). The mixture was stirred for 15 min. at room temperature followed by reflux at 85 °C for 4 h. H_2O (200 mL) was added to the flask, the heating was turned off and the temperature allowed to cool to 50 °C. The pH of the mixture was adjusted to 4 using 25% aq. NaOH solution. 4,7-DCQ (**5**, 42 g, 0.212 mol, 1.0 equiv.) was added to the mixture. The mixture was then refluxed at 85 °C for 3 h followed by stirring the mixture at 5 °C for 2 h. The yellow product was collected by vacuum filtration and washed with water (2x 50 mL). The crude amodiaquine was kept under vacuum for 30 minutes after which it was refluxed in a 300 mL solution of EtOH/HCl (5:1 equiv.) for 2 h at 80 °C. The yellow product was then allowed to precipitate at 5-10 °C for 2 h at which time it was filtered *in vacuo*, washed with a 100 mL cold solution of EtOH/HCl (5:1 equiv.) and air-dried overnight. The product was then refluxed in water (2.5 mL/g) at 95 °C for 2 h followed by precipitation overnight at room temperature under stirring. The reaction mixture was cooled to 0-5 °C for 2 h. The product was filtered *in vacuo*, washed with cold water (2x 50 mL) and air-dried overnight before drying in the oven at 40 °C to obtain **3** (88.5 g, 0.190 mol, 90%) as a yellow solid. HPLC (C18) P_{HPLC} 100%, t_R 6.2 min. mp = 159-166 °C (lit. = 160°C).[12] Water content = 8% (USP standard = 7.0% - 9.0%). ^{1}H NMR (400 MHz, DMSO-d_6) 14.96 (br s, 1H, OH); 11.26 (s, 1H, NH); 10.93 (br s, 1H, NH); 10.41 (br s, 1H, NH); 8.97 (d, 1H, *J* 9.16 Hz, Ar-H); 8.47 (d, 1H, *J* 7.04 Hz, Ar-H); 8.22 (d, 1H, *J* 2.17 Hz, Ar-H); 7.83 (dd, 1H, J_1 2.06, J_2 2.06 Hz, Ar-H); 7.70 (d, 1H, *J* 2.55 Hz, Ar-H); 7.35 (dd, 1H, J_1 2.60, J_2 2.60 Hz, Ar-H); 7.24 (d, 1H, *J* 8.60 Hz, Ar-H); 6.85 (d, 1H, *J* 6.97 Hz, Ar-H); 4.24 (s, 2H, CH$_2$); 3.11 (s, 4H, 2x CH$_2$); 1.29 (s, 6H, 2x CH$_3$). HRMS *m/z* $[M+H]^+$ 356.189 (calculated for $C_{20}H_{22}ClN_3O$, 356.157).

HPLC method

The purity of the final product **3** was determined by High-Performance Liquid Chromatography (HPLC) using the LUNA C18 column on a Hitachi system equipped with a diode array detector set at 224 nm. The HPLC method followed that of the USP method for amodiaquine hydrochloride. Compounds were dissolved in water (15 mg/100 mL) and injected through a loop. Retention time (t_R) was obtained at a flow rate of 1.2 mL/min using an isocratic run of 78% eluent A (potassium phosphate buffer) and 22% eluent B (MeOH) for a period of 0 min. to 15 min. The purity of the sample was determined based on the pharmacopoeia standard by preparing two standard solutions (15 mg in 100 mL water), one with six injections and the other with two injections to determine standard recovery with acceptable criteria of 97-103. After different drying conditions were evaluated, the oven dried product (15 mg) was dissolved in 100 mL water and injected (10 µL) into the specified column with a runtime of 15 min.

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