Determining a Viable Protocol for the Derivatisation of Artemisinin into Dihydroartemisinin

A study commissioned through

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Introduction

More than 600 million people, most of them children living in Sub-Saharan Africa, face daily the threat of dying from malaria because effective treatments are not accessible to them. In many malaria stricken areas affordable medicines, such as chloroquine, sulfadoxine–pyrimethamine (SP) and mefloquine, which have been used for many years, are no longer effective, because the parasites have become resistant [19]. Since 2001, WHO has therefore recommended the switch to artemisinin-based combination therapies (ACTs), which provide a rapid and reliable cure with very few side effects [2]. Between 2001 and 2005, 56 countries have adopted these ACTs as first or second line treatment and 29 countries have started deploying them [20]. The main problem with the ACTs is their price. ACTs are 10 to 20 times more expensive than the old monotherapies, which puts them beyond the reach of many people and particularly the poorer section of the population. So there is an urgent need to bring down the costs. Once the price has been reduced, and/or donors can subsidise the ACTs to a point where they can be made available to all those who need them, demand will outstrip production by far, so additional production capacities will also be required.

In order to produce ACTs, *Artemisia annua*, the plant from which artemisinin comes, has to be grown and extracted. Breeding more productive plants and extracting them in a more efficient manner can bring down some of the costs, which at the moment constitute approximately between 25% and 35% of the total costs. Because of its poor oral availability, artemisinin is not used directly anymore but modified into so-called derivates like dihydroartemisinin, artesunate and artemether [1], adding another 20–40% to the costs, depending on the type of derivate chosen. Here too, some cost savings are possible. The remainder of the costs come from compounding and tabletting and from the companion drug chosen for the ACT. The combination dihydroartemisinin-piperaquine

is the cheapest option. It is, however, not yet on the official list of ACTs recommended for Africa. Costs could also be brought down by promoting the pharmaceutical production of antimalarials in those malaria-affected countries that do not yet produce their own malaria medicines, since labour there is cheaper and there could also be savings on logistics.

The study, which is presented here focuses on the production of dihydroartemisinin (DHA), the simplest derivate of artemisinin and the only one where derivatisation reduces significantly the cost of the treatment, since the reduction in the amount of active substance required for the treatment after derivatisation, a reduction of about 40%, outweighs by far the costs of this modification.

DHA was developed in China about thirty years ago. Since then, a variety of protocols have been developed to perform the derivatisation. Derivatisation is either done by companies who extract artemisinin or by pharmaceutical companies who produce ACTs. Each of these companies uses its own proprietary protocol and any new entrant into the market will either have to rely on competitors to do the derivatisation or has to do his own research to find a suitable protocol. This situation poses an obstacle, for instance, if newly established extraction companies in Africa are to supply pharmaceutical companies in Africa when neither of them have access to their own or an independent derivatisation unit.

Some protocols for the production of DHA have been described in the scientific literature, but most of these are not optimal for practical purposes. The aim of the study presented here is to provide a publicly accessible protocol that might be a suitable starting point for an economically and ecologically viable unit for the production DHA thus saving those who would like to enter this field some time and money in their pursuit. Since there is always room for improvement, suggestions from the scientific community to make this protocol even more efficient are invited. In order to facilitate such a collective effort, trials with alternatives that did not produce the desired results are also fully documented in this report.

Preliminary considerations

Choice of solvent and means of reduction

The aim of this work is to determine an economically and environmentally optimal protocol for the chemical modification of artemisinin to dihydroartemisinin. The choice between different approaches was made according to the following criteria: An approach that promised a higher yield was preferred over an approach with a lower yield, between two different agents the more effective agent was preferred over the less effective one and the cheaper agent was given preference over the more expensive agent and finally, if economic efficiency was the same, preference was given to the less toxic agent.

In the literature two methods of reduction are mentioned.

The first method proposes to convert artemisinin to dihydroartemisinin by reduction with sodium borohydride in methanol or ethanol at about 0° to 5°C [1- 11]. In the literature only minor differences in the conditions of the reaction are reported, but there are obvious differences in the workup.

The second method shows the reduction with DIBAL-H in dichloromethane at -78°C [12- 15]. The disadvantages of DIBAL-H are the smaller yield and the higher prices of both the solvent and the means of reduction.

In the literature THF is also used as a solvent in the reduction of artemisinin, but the use of THF was not considered here, because it is more toxic and expensive than methanol [9].

According to the considerations outlined above, sodium borohydride to be suspended in ethanol or methanol was selected as a base of the study.

Artemisinin   *Dihydroartemisinin*
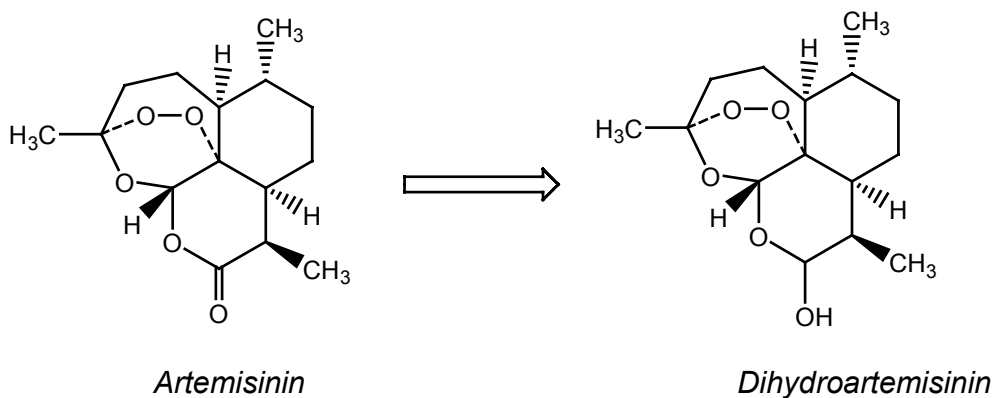
In order to decide between ethanol and methanol, the optimum solubility of artemisinin was investigated.

At room temperature, the concentration of a saturated solution (highest concentration of artemisinin) was shown to be approximately 0.5 g/ 10 ml for methanol and approximately 0.3 g/ 10 ml for ethanol.

Judging from these results, methanol is the better solvent and was therefore used in all the following steps. It should be mentioned that it takes a long time (about half a day) until the solution is saturated no matter whether artemisinin is added step by step to the methanol or all at once.

A higher saturation of the solution would be achieved by raising the temperature (40°- 50°C), but this attempt is of no actual use, because the reaction temperature is at 0° to 5°C and so the additional artemisinin in the solution would precipitate. In an effort to increase the solubility, the artemisinin was pulverised into smaller particles, but no change in solubility could be observed.

Point of departure

Among the protocols that use methanol and $NaBH_4$ the procedure described by Shrimali [1] et al. appeared to be the most promising, claiming a good yield while using the smallest amount of methanol. There are, however, some inconsistencies in this report:

According to Shrimali et al. 10 g of artemisinin should be dissolved in 40 ml of methanol, but in view of the above mentioned solubility tests this seems to be more than questionable. In addition, the small amount of $NaBH_4$ which he added, only 0.25 g, is doubtful as well. Even if, hypothetically, all four H-atoms would react with artemisinin, the amount of $NaBH_4$ involved would simply be too small. $NaBH_4$ also reacts (in parts) with the solvent methanol, and not only with artemisinin and therefore would not be completely available for the reaction of artemisinin to dihydroartemisinin. Experimentation was therefore required to determine the correct proportions.

Experimental section

General procedure

The experimental attempts focused on the following reaction:

Artemisinin suspended in methanol was cooled in an ice bath to about 0° to 5°C. To the cooled solution $NaBH_4$ was added step by step in small amounts over a period of 30 minutes. Afterwards, the solution was stirred vigorous for another hour. The reaction was monitored by TLC. Then the reaction mixture was neutralised (pH 5- 6) with 30% of a mixture of acetic acid/ methanol and evaporated to dryness under reduced pressure. The white residue was extracted with 50 ml ethyl acetate several times. The ethyl acetate extracts were combined, dried with Na_2SO_4, filtered and evaporated to dryness under reduced pressure.

The characterisation of the structure was made by NMR and HPLC.

First attempt

Artemisinin was completely dissolved in methanol at a constant temperature of about 0° to 5°C (table: entry 1, page 15). $NaBH_4$ was slowly added to the artemisinin over a period of 30 minutes until a ratio of 1:1 was reached. During this procedure gas developed and the temperature increased by 1-2 degrees.

To monitor the conversion, a TLC (CH_2Cl_2:MeOH= 20:0.5) was made after 1 hour. There was still artemisinin in the reaction mixture and therefore further amounts of $NaBH_4$ were gradually added. After 5 hours, the final ratio of artemisinin to $NaBH_4$ had increased to 1:3, but the TLC did not show any further reduction of artemisinin to dihydroartemisinin. The reaction mixture was neutralised with 30% of a mixture of acetic acid/ methanol and evaporated to dryness under reduced pressure. The white residue was extracted with 50 ml ethyl acetate five times. The combined ethyl acetate extracts were dried for 30 minutes with Na_2SO_4, filtered and evaporated to dryness under reduced pressure.

A yield of 90% was obtained.

The experiment showed that the long time taken for adding $NaBH_4$ to the reaction mixture was disadvantageous since artemisinin could be found unreacted in the product as shown in figure 1.

In the following tests all the $NaBH_4$ was added step by step in small amounts over a period of only 30 minutes, which led to better results.
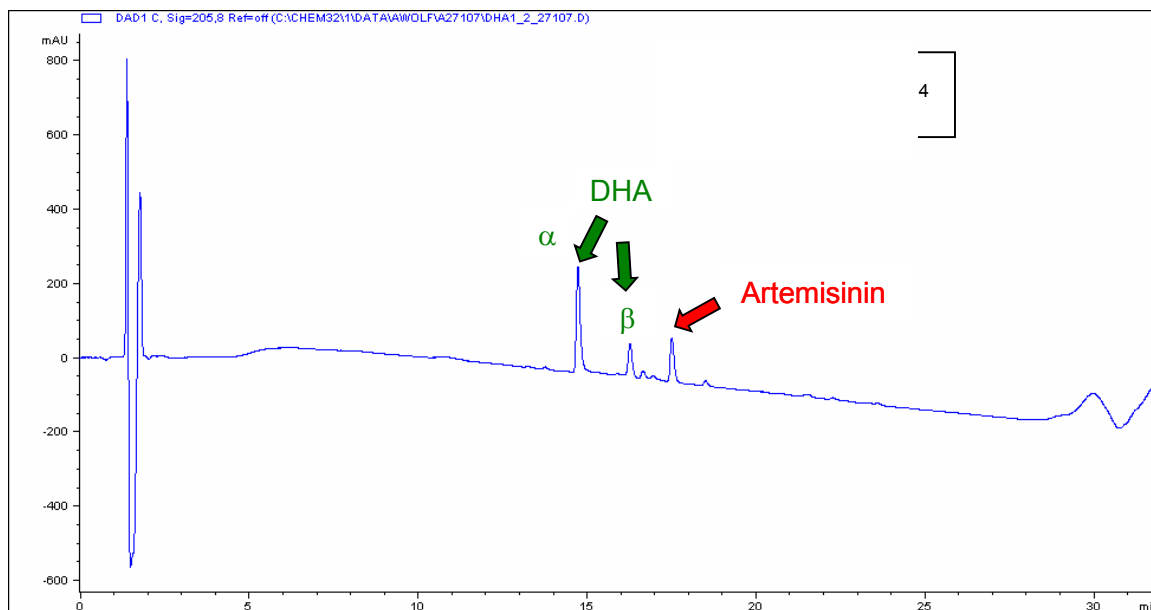
Figure 1: HPLC- curve of the first attempt

## Changes in the amount of artemisinin

In the first experiments an unsaturated solution of artemisinin in methanol was used. Although yields were satisfying, the amount of methanol used was more than expected.

The next consideration was to perform the reduction of artemisinin in a more concentrated suspension. The amount of artemisinin in methanol was enhanced from 3 g/ 40 ml up to 6.6 g/ 40 ml. The obstacle to raising the concentration of the suspension was a mechanical one. From 5 g/ 40 ml onwards, a magnetic stirrer was not sufficient, therefore in the work with higher concentrations a mechanical stirrer had to be employed.

Changes in the amount of NaBH$_4$

In the literature different amounts of NaBH$_4$ are mentioned for the reduction of artemisinin to dihydroartemisinin. Some of these are questionable, because it can be theoretically predicted that the amount of NaBH$_4$ is too small. During the investigation different ratios of artemisinin to NaBH$_4$, such as 1:2, 1:2.5 and 1:3, were tested.

There is no obvious difference in yield using ratios of 1:2.5 and 1:3; therefore the first-mentioned ratio is the better option.
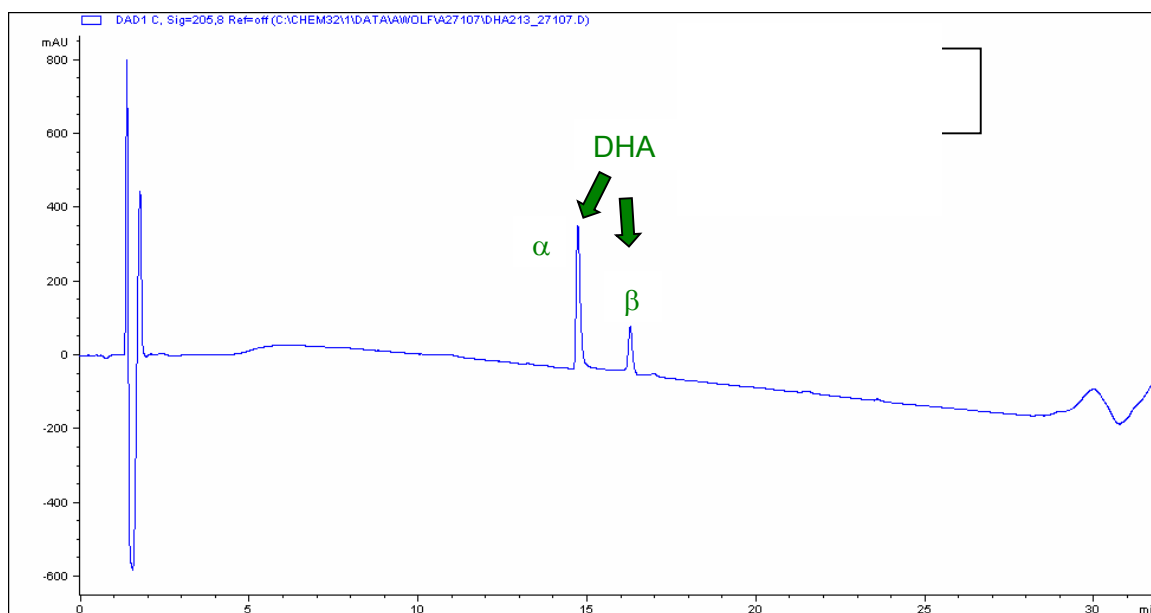
Figure 2: HPLC- curve of an optimised attempt

Two different types of $NaBH_4$ were used, powder and granulate, which both worked equally well. Due to health considerations granulate is the preferred option because less toxic dust emerges during handling. Furthermore, $NaBH_4$ granulate is more stable during storage.

Workup

In the literature two different workups are reported.

In most protocols, the reaction is stopped by neutralisation with acetic acid, the reaction mixture is evaporated, and the dihydroartemisinin in the residue extracted with ethyl acetate. The other method of workup is to precipitate the dihydroartemisinin with cold water after neutralizing the reaction mixture with acetic acid.

The whole purpose of the acid is to destroy the surplus $NaBH_4$ and alkalic impurities. It should be mentioned that dihydroartemisinin is sensitive to acid conditions, and thus the pH value should not fall below about 5 to 6.

The first method of workup has only one minor disadvantage: the large amount of ethyl acetate necessary for complete extraction of the dihydroartemisinin. The ethyl acetate can, however, be recycled by column-distillation.

The second option for the workup was tried as well. After precipitation, the water of crystallisation could only be removed completely by dissolving the precipitate in dichloromethane and thereafter evaporating it to dryness under reduced pressure. The yield was relatively low. The trial showed that with a high concentration of dihydroartemisinin in the reaction mixture, precipitation by adding cold water does not work in a satisfying way.

An additional possibility not mentioned in the literature is the use of hydrochloric acid instead of acetic acid for the neutralisation of the reaction mixture. By using hydrochloric acid, the surplus $NaBH_4$ should be destroyed, and the inorganic salts should not dissolve in the extraction agent (EtOAc) but should rather stay in the residue. It needs to be noted, however, that especially with hydrochloric acid, the acid has to be added very slowly in small amounts because this reaction is quite vigorous. In the first attempt, hydrochloric acid with the same pH value as the 30% acetic acid was used, a pH value of 2.

To obtain a reaction mixture with a pH value of 5 to 6, a larger amount of hydrochloric acid was needed compared to the quantity of acetic acid required. As a result, the destruction of excess $NaBH_4$ and alkalic impurities, which had been produced during the reaction, proceeded too slowly and incompletely.

In the next attempt, diluted hydrochloric acid (2N in H_2O) was used, which led to a satisfying result.

When working with hydrochloric acid, however, the product, dihydroartemisinin, became a brown instead of a white crystalline powder, which is obviously a disadvantage.

The conclusion from the above trials was that neutralisation with acetic acid and, after evaporation, the extraction with ethyl acetate is more advantageous.

Recrystallisation

Recrystallisation is only necessary if the dihydroartemisinin is to be used directly as a drug. If DHA is only the first step in the production of some other derivate, recrystallisation is not necessary.

In the literature two different solvents for the purification of dihydroartemisinin by recrystallisation are mentioned and both of them were tested.

Firstly, ethyl acetate/ hexane in a ratio of 1:3 and secondly, diisopropyl ether were used. Dihydroartemisinin is brought to suspension with the solvent and heated up to 80°-90°C. Afterwards small additional amounts of the corresponding solvent are added to the suspension and heated up to reflux again. In both cases no completely clear solution could be achieved, therefore the residue was eliminated by filtering with a heating funnel. The precipitation took place over night without action of light. Afterwards the

precipitate was filtered under suction and dried under reduced pressure. TLC analysis detected in both mother liquors remnants of dihydroartemisinin, which can be recuperated during recrystallisation of the next batch.

During the process of recrystallisation, only minor differences between the two solvents were noted, like the faster precipitation of dihydroartemisinin with diisopropyl ether compared to ethyl acetate/ hexane. Another small difference is the crystal form that is finer with diisopropyl ether than with ethyl acetate.

Since there were no obvious advantages in using diisopropyl ether while there are more problems in the handling of the substance during the production process, ethyl acetate/ hexane appeared to be the better choice as the solvent for recrystallisation.

Recycling of methanol

Most units working on the derivatisation of artemisinin apparently treat the methanol used in the reaction as a consumable. Recycling of the methanol would be of economical and ecological advantage.

When evaporated methanol was used a second time, the reaction was too vigorous, the temperature increased up to 20°C. The reason must have been the rapid destruction of $NaBH_4$, which did not react with the artemisinin anymore.

It was obvious that the evaporated methanol can not be re-used without clarification because impurities from the first reduction interfere with the following reduction. Theoretically, these impurities could be different forms of boric acid methyl esters.

There may be three different ways to clarify the used solvent: by addition of chemicals, by introduction of specifically designed polymers that filter out the impurities or by fractional distillation. Before any of these

processes can be introduced, however, a more precise knowledge of the target compounds would be necessary. The characterisation of these impurities proved difficult. HPLC and NMR were used to determine the nature of the waste products, but no satisfying answers could be achieved with the available equipment.

Some attempts of chemical clarification of the   methanol were nevertheless made:

At first, methanol was redistilled with a column at 80-90°C with the addition of NaOH. This base should saponify the esters into non volatile acids, which should then stay in the distillation flask. When the methanol that had been redistilled in this manner was re-used, the result remained unsatisfactory: The transformation of artemisinin to dihydroartemisinin remained incomplete, and the temperature increased too much.

To remedy this situation, a small amount of dextrose, which should built a complex with the ester, was added to the redistilled methanol and stirred over night. This attempt was also unsuccessful.

Another consideration was that maybe the problem of the reusability of methanol stemmed from a small amount of water therein and not the esters of borohydride. Therefore a 3Å molecular sieve was added to the redistilled methanol, but it also did not lead to a satisfying result.

The problem of the reusability of methanol could not be solved, and would require more detailed chemical analysis of the spent solvent.

Use of NaOMe

$NaBH_4$ does not only react with artemisinin but, to a limited extent, also with methanol. In order to suppress the hydrolysis of $NaBH_4$ in methanol, the use of NaOMe is recommended. The desired result of such an addition would be to reduce the amount of $NaBH_4$ required for the reaction and maybe even the amount of impurities in the spent solvent.

The test was started with a ratio of artemisinin to NaBH$_4$ of 1:1.5. The ratio of artemisinin to NaOMe was 1:0.02. The reaction conditions and the implementation were as usual. After 1 hour a TLC was made in order to monitor the conversion. There was still artemisinin in the reaction mixture as well as some by-products. Therefore another small amount of NaBH$_4$ was added to the reaction mixture and the ratio of artemisinin to NaBH$_4$ increased to 1:2. An hour later a TLC was performed, but no obvious differences could be seen, and the reaction was stopped.

The reduction remained incomplete, the amount of NaBH$_4$ needed for the reaction could not be reduced, and some by-products developed. A possible explanation could be the high sensitivity of artemisinin.

In order to analyse the incomplete conversion of artemisinin to dihydroartemisinin a HPLC was made. The result is shown below.

Figure 3: HPLC- curve of the test with NaOMe
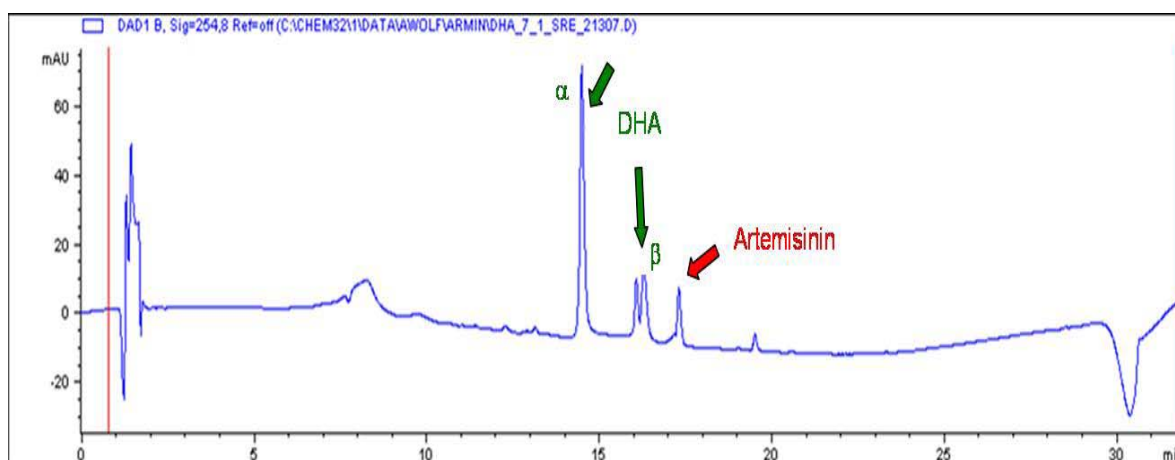
Review of the tests

Entry	Artemisinin	NaBH$_4$	MeOH	Ratio (Artemisinin/ NaBH$_4$)	Yield
1	1 g [a]	402 mg	40 ml	01:03	90%
2	1 g	268 mg	40 ml	01:02	91%
3	1 g	402 mg	40 ml	01:03	95%
4	1 g [b]	335 mg	40 ml	1:2.5	85%
5	1 g [c]	335 mg	40 ml	1:2.5	99%
6	2 g	670 mg	20 ml	1:2.5	95%
7	2 g [g]	670 mg	20 ml	1:2.5	0%
8	2 g [h]	670 mg	20 ml	1:2.5	0%
9	2 g [i]	670 mg	20 ml	1:2.5	0%
10	3 g	804 mg	40 ml	01:02	95%
11	3 g	1.2 g	40 ml	01:03	96%
12	4 g	1.6 g	40 ml	01:03	96%
13	4 g	1.3 g	40 ml	1:2.5	96%
14	4 g [c]	1.6 g	40 ml	1:2.5	90%
15	4 g [c]	1.6 g	40 ml	1:2.5	80%
16	4 g [d]	1.3 g	40 ml	1:2.5	0%
17	4 g [e]	1.3 g	40 ml	1:2.5	88%
18	4 g [e]	1.3 g	40 ml	1:2.5	96%
18	4.5 g	1.5 g	40 ml	1:2.5	93%
20	4.5 g	1.8 g	40 ml	01:03	93%
21	5.5 g	1.85 g	40 ml	1:2.5	94%
22	6.5 g [j]	2.2 g	60 ml	1:2.5	86%
23	6.6 g [f]	2.2 g	40 ml	1:2.5	95%
24	2 g [k]	537 mg	20 ml	01:02	---

[a] long reaction time

[b] precipitation with water

[c] workup with hydrochloric acid

[d] reused MeOH

[e] reused ethyl acetate

[f] mechanical stirrer

[g] redistilled MeOH

[h] redistilled MeOH+ dextrose

[i] redistilled MeOH+ molecular sieve

[j] big attempt with a magnetic stirrer

[k] attempt with NaOMe

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Results

The trials have led to a protocol, which produces a high yield of dihydroartemisinin, while the amount of methanol and the amount of $NaBH_4$ needed for the reduction could be reduced.

The acetic acid used to stop the reaction requires a somewhat tedious removal but works better than other alternatives tested.

The extracting agent, ethyl acetate, can be reused after a distillation with a column.

A still unsolved problem is the reusability of methanol. Some methods were tried but without success.

Detailed description of preferred option

Material	amount	mmol	Mr
Artemisinin	6.6 g	23.4 mmol	282.34
$NaBH_4$	2.2 g	58.4 mmol	37.83
methanol dest.	40 ml		

Apparatus:
  three- necked flask
  thermometer
  mechanical stirrer
  ice- bath
  venting

Implementation:

Artemisinin (6.6 g) is suspended in methanol (40 ml) and cooled in an ice bath to about 0° to 5°C. To the cooled suspension $NaBH_4$ (2.2 g) is added step by step in small amounts over a period of 30 minutes. Afterwards, the reaction mixture is stirred vigorously for another hour.

Note: In order to get a better distribution of artemisinin in methanol the reaction mixture should be stirred vigorously with a mechanical stirrer.

It should be mentioned that some gas develops, and the temperature increases 1-2 degrees as the $NaBH_4$ is added.

The reaction is monitored by TLC (CH_2Cl_2:MeOH= 20:0.5) to ensure a complete transformation.

> *Note:* The substances are detected by spraying with molybdatophosphoric acid and by subsequent heating with a heat gun.

Workup:

A mixture of 12 ml of acetic acid and 12 ml methanol (in total 30% volume in relationship to the solution) is prepared and added to the solution until the pH value of about 5 to 6 is reached to stop the reaction. Afterwards the neutralised reaction mixture is evaporated to dryness under reduced pressure and finally lyophilised.

> *Note:* Evaporating under high- vacuum is necessary to remove the acetic acid completely from the residue. With the evaporator available in the laboratory a faint smell of acetic acid remained on the product, although the quantity involved was below the level of detection by NMR.

The residue is extracted with 50 ml ethyl acetate several times (up to seven times) until no dihydroartemisinin can be found in the extracting agent. To control this, a TLC is made.

> *Note*: The ethyl acetate can be recycled by column-distillation. On an industrial scale the amount of extracting agent and the number of extractions required could be reduced by counter-current continuous extraction.

The combined ethyl acetate extracts are dried with Na_2SO_4 (about 15-20 g), filtered, and evaporated to dryness under reduced pressure (at the end with an oil pump).

> *Note:* The combined extracts are cloudy, and after drying with Na_2SO_4, they should be filtered until they appear transparent.

6.3 grams (95% yield), of a white, crystalline powder are gained, which is, according to NMR analysis, pure dihydroartemisinin (margin of error 1-2%).

The characterisation of the structure is made with NMR and HPLC.

Recrystallisation:

Recrystallisation is only necessary if the dihydroartemisinin is to be used directly as a drug. If it is only the first step in the production of some other derivates, re-crystallisation is not necessary.

Apparatus:
  round- bottom flask
  magnetic stirrer
  oil bath
  reflux condenser
  venting

Ethyl acetate/ hexane:

The product is suspended in hexane and heated up to reflux (80-90°C). Then ethyl acetate is added in small amounts to the suspension and heated up again to reflux. At the end the final ratio of ethyl acetate to hexane is 1:3, about 500 ml in total.

The solution was not completely clear and therefore filtered with a heating funnel. Afterwards, dihydroartemisinin is precipitated over night without action of light and then filtered under suction and dried under reduced pressure (73%; 4.6 g).

In the mother liquor there was still some dihydroartemisinin (15-20%; 0.95-1.3 g) that should be recovered when the next batch is recrystallised.

> *Note:* Reducing this large amount of solvent used for the recrystallisation is possible but is a technical rather than a chemical problem, which needs to be addressed during scale up.

Analytical results
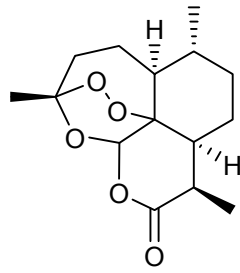
Synonyms: **Artemisinin**

Chemical formula: $C_{15}H_{22}O_5$

Molecular mass: 282. 34 g/mol

Appearance: white, crystalline powder
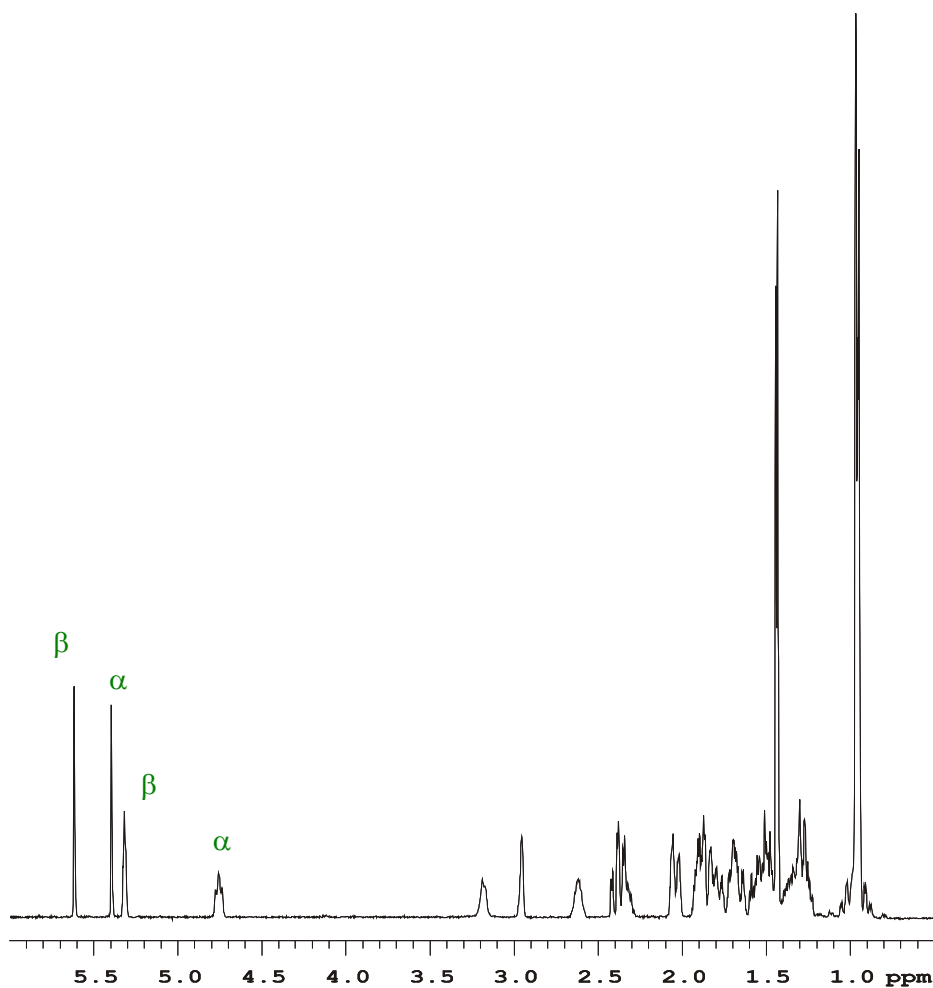
Rf- Value: 0.72 (CH_2Cl_2: MeOH = 20: 0.5)
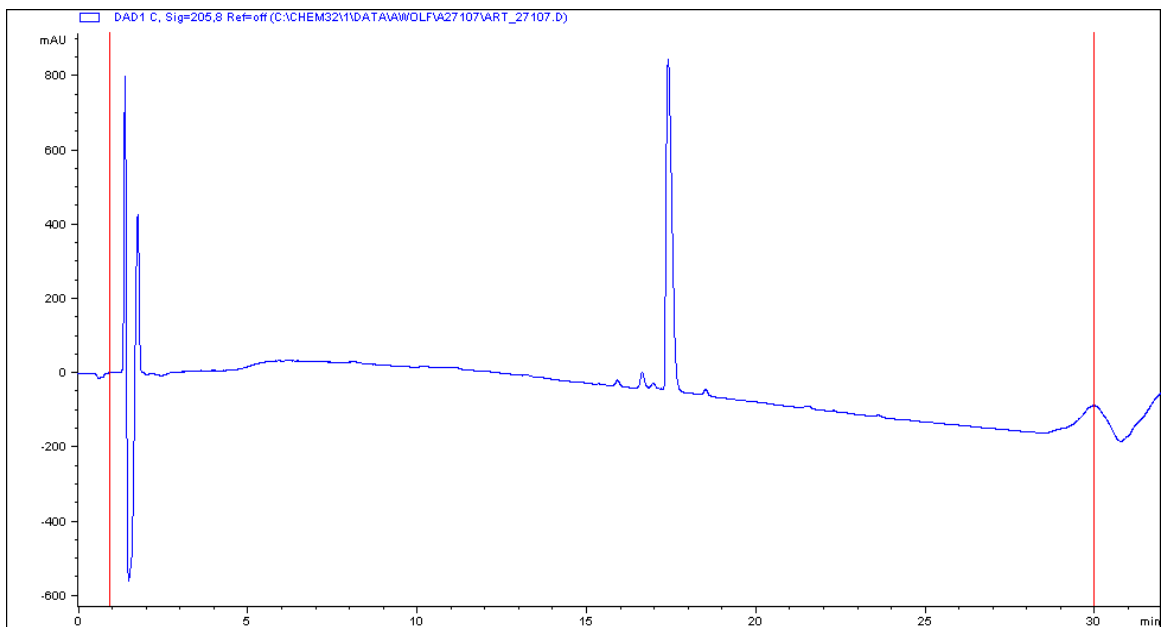
Figure 8: HPLC- curve from Artemisinin sample
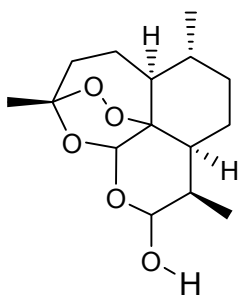
Synonyms:	**Dihydroartemsinin**
Chemical formula:	$C_{15}H_{24}O_5$
Molecular mass:	284. 35 g/mol
Appearance:	white, crystalline powder
Rf- Value:	0.51 (CH_2Cl_2: MeOH = 20: 0.5)
Melting point:	142°C (identical with dihydroartemisinin sample)
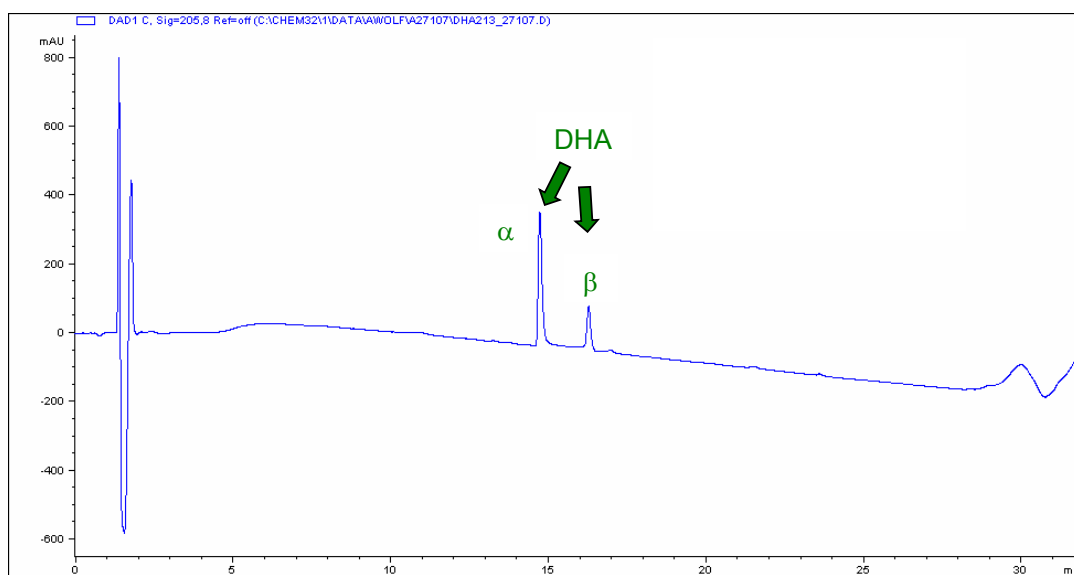
Figure 9: HPLC- curve of self- produced dihydroartemisinin

Figure 10: NMR of self- produced dihydroartemisinin

Methods of measurements

The dihydroartemisinin was characterised using two different methods.
At the beginning a NMR and a HPLC were made of the dihydroartemisinin which was sent from the Dang Quang Trading Company in Vietnam in order to make meaningful comparison to the self-produced dihydroartemisinin.

The solvent for the NMR measurements is chloroform D+ 0.03% TMS.
The HPLC measurements are made with a gradient of two solvents, water with 1% formic acid and acetonitrile with 1% formic acid and a flowrate of 0.3 ml/ min.
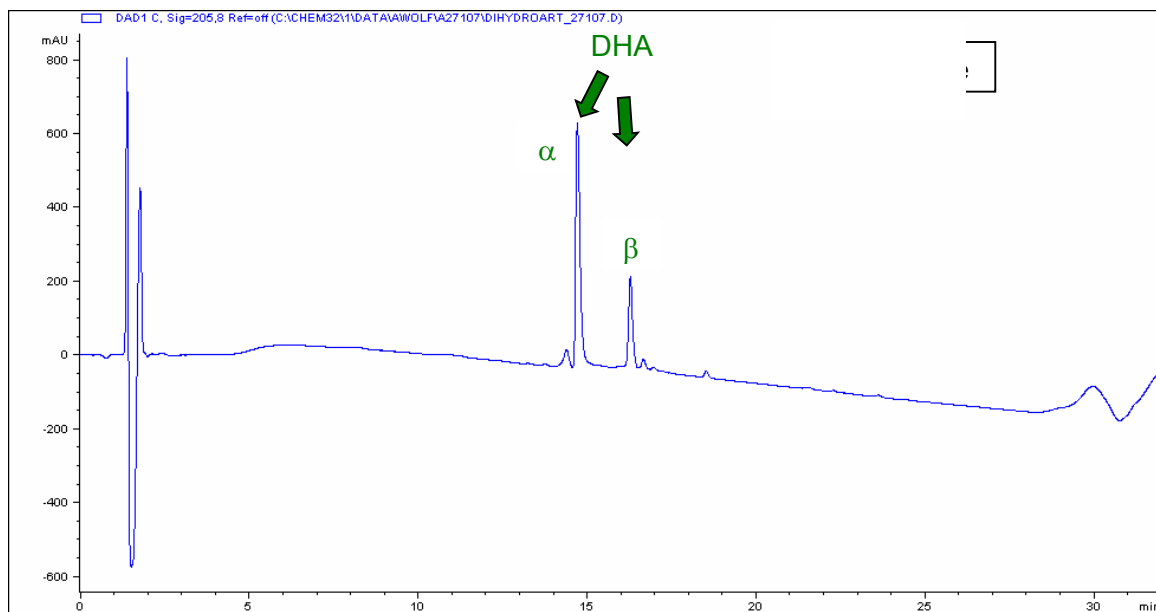
Figure 4: HPLC- curve of Dihydroartemisinin sample
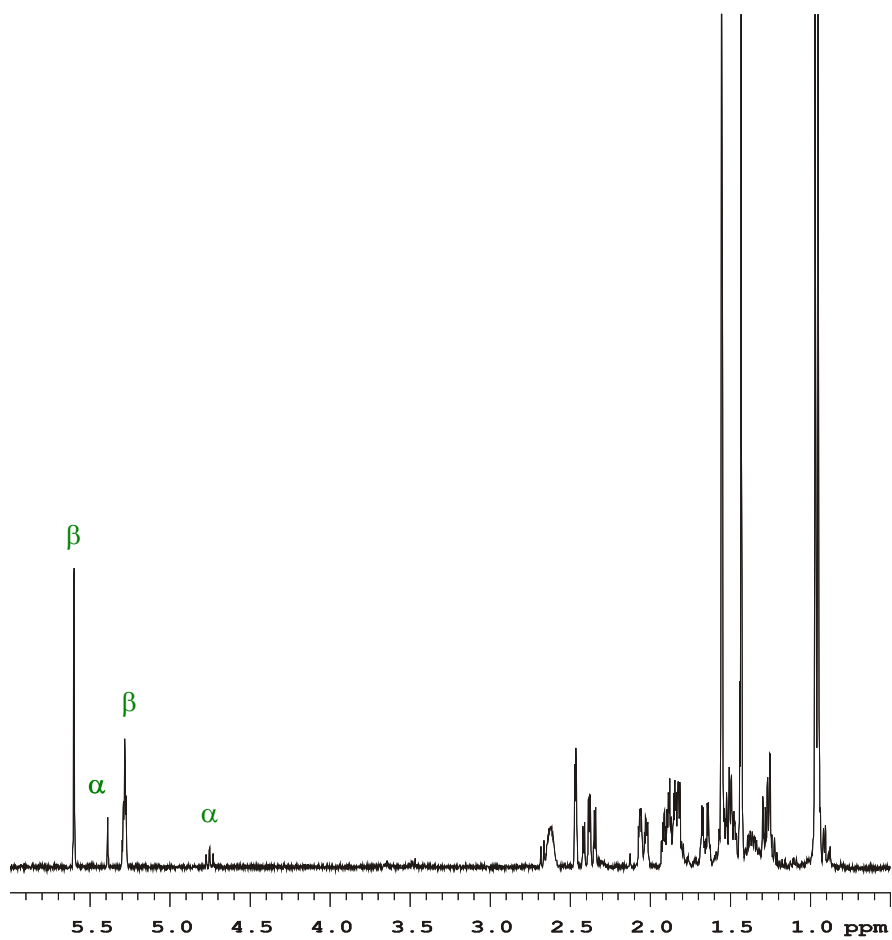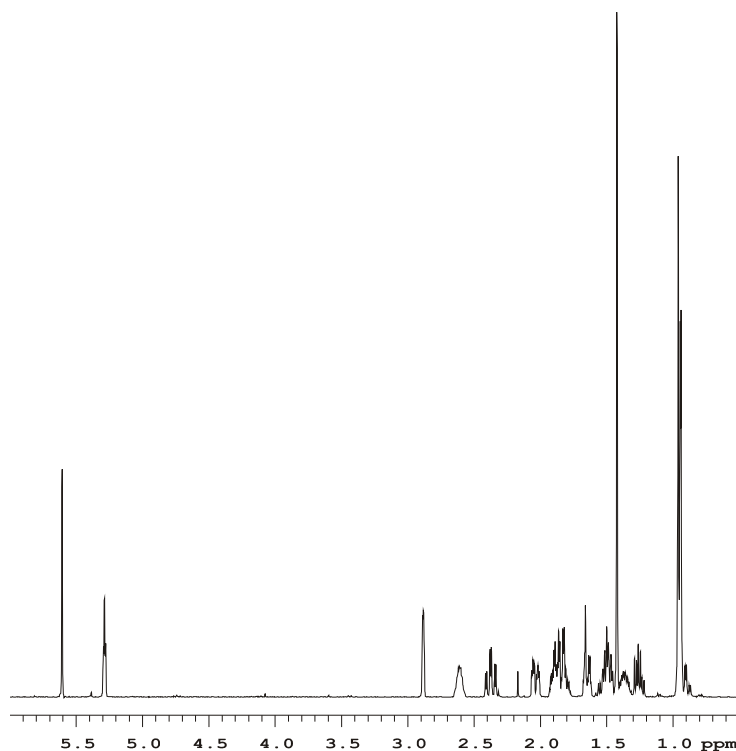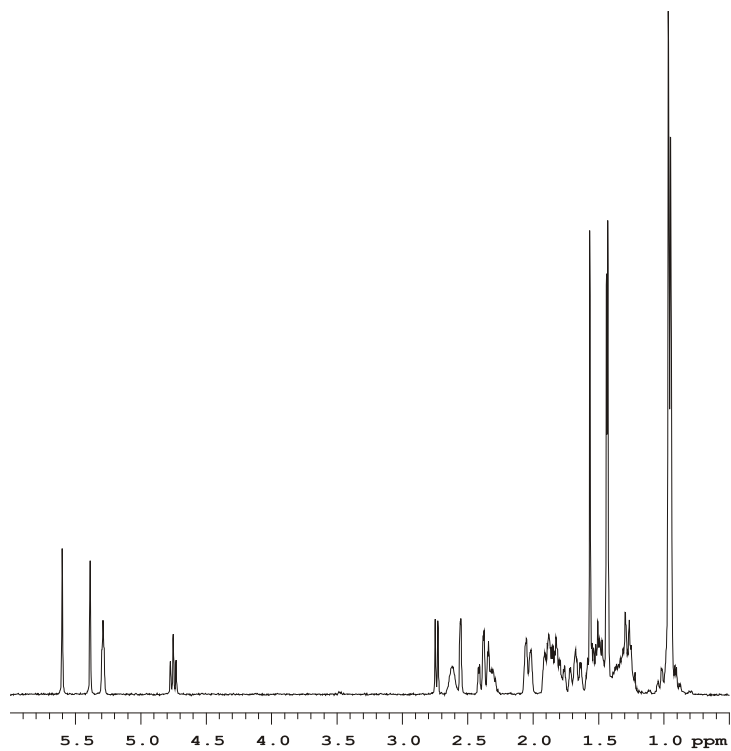

Figure 5: NMR of Dihydroartemisinin sample

There is a difference in the spectrum of NMR if the measurement is done immediately after preparing the sample (only β-dihydroartemisinin; figure 6) or after a longer period of time (e.g. 12 hrs; α:β- dihydroartemisinin=1:1; figure 7). The reason for that is the adjustment of equilibrium between the two isomers of dihydroartemisinin, which is dependent on the solvent.

HPLC was used as well for the characterisation of dihydroartemisinin showing two different peaks in the spectrum. The first possible explanation was that due to the addition of an acid during the measurement an open-chained lactone had developed, but an additional measurement without acid proved this assumption to be wrong. Another reason could be the availability of the two isomers of dihydroartemisinin, α- and β-dihydroartemisinin. To solve this issue, an NMR was made using the same solvent ratio that produced the peaks in the HPLC measurement (70% water and 30% acetonitrile). In this way it could be shown that the two isomers are present in a specific ratio of about 1:2.8 (α/β-dihydroartemisinin; shown in figure 9).

Figure 6: NMR of recrystallised dihydroartemisinin immediately after preparing the sample, β-dihydroartemisinin

Figure 7: NMR of re-crystallised dihydroartemisinin after 12 hours, α/β-dihydroartemisinin

Chemicals used

Acetic acid 30%, (Riedl- de Haën)

Acetonitrile, (Fluka)

Artemisinin, (Dang Quang Trading Company, Vietnam)

Aqua bi-destillata

Chloroform D+ 0.03% TMS, (Euro-top)

Dichloromethane, (Brenntag)

Dihydroartemisinin, (Dang Quang Trading Company, Vietnam)

Diisopropyl ether, (Fluka)

Ethanol 96%, (Brenntag)

Ethyl acetate dest., (Brenntag)

Formic acid, (Brenntag)

Hydrochloric acid, diluted (2 mol/ l) p.a., (Merck)

Methanol dest., (Brenntag)

Methanol redest.

Molybdophosphoric acid (Aldrich; in ethanol)

Sodium borohydride

  granulate, 10-40 mesh, 98%; (Sigma Aldrich)

  powder, 98%; (Sigma Aldrich)

Sodium sulphate anhydrous; (Merck)

Equipment used

Magnetic stirrer
  IKAMAG RCT
Mechanical stirrer
  Heidolph; typ RZR 1
NMR
  Variant Unity Inova 400 MHz
HPLC
  Analytic, RP
Agilent Zorbax SP- C18; 3.5 µm; 2.1x 150 mm with Guard Cartridge;
flow 0.3 ml
Diodes- Array- Detector; (Agilent)

Figure11: screenshot of the setup of the HPLC-method

Estimate of the costs

Cost chemicals for the derivatisation of 1 kg of artemisinin

	unit	quant	unit	quant	price USD p/unit	cost USD	recycling possible
Artemisinin	g	6.6	kg	1.00	350.00	350.00	no
Methanol (reaction)	ml	40	l	6.06	0.40	2.42	maybe
Methanol (neutralisation)	ml	12	l	1.81	0.40	0.72	maybe
Sodium Borohydride	g	2.2	kg	0.33	60.00	19.80	no
Acetic acid	ml	12	l	1.82	0.54	0.98	no
Ethyl acetate (workup)	ml	50	l	7.58	1.20	9.10	yes
Ethyl acetate (re-crystallisation)	ml	17	l	2.58	1.20	3.10	yes
Sodium sulphate	g	20	kg	3.03	0.10	0.30	no
Hexane (re-crystallisation)	ml	33	l	5.00	0.68	3.40	yes
sum						389.83	

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