NPR

RSCPublishing

Highlight

Synthetic Chemistry Fuels Interdisciplinary Approaches to the Production of Artemisinin

Cite this: DOI: 10.1039/xoxxoooooox

Michael A. Corsello and Neil K. Garg

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

www.rsc.org/

In the developing world, multi-drug resistant malaria caused by the parasite *Plasmodium falciparum* is an epidemic that claims the lives of 1–3 million people per year. Artemisinin, a naturally occurring small molecule that has seen little resistance from malarial parasites, is a valuable weapon in the fight against this disease. Several easily accessible artemisinin derivatives, including artesunate and artemether, display potent antimalarial activity against drug-resistant malaria strains; however, the global supply of artemisinin from natural sources alone remains highly inconsistent and unreliable. As a result, several approaches to artemisinin production have been developed, spanning areas such as total synthesis, flow chemistry, synthetic biology, and semi-synthesis. This review highlights achievements in all areas, in addition to the interplay between synthetic biology and synthetic chemistry that has fueled the recent industrial-scale production of artemisinin.

Introduction

With effective control of mosquito populations and access to medical care, malarial infection has become a rare concern for citizens of developed countries. In the developing world, this is not yet the case. Malaria, specifically cerebral malaria, caused by the parasite *Plasmodium falciparum*, claims the lives of 1–3 million people per year in developing countries, many of whom are children under 5 years of age.[1] With the onset of resistance to current medications in these areas, such as the readily available and commonly used chloroquine (**2**), there is an urgent need for access to effective medications.[2] The discovery of artemisinin (**3**), also known as qinghaosu, and its derivatives met this need and transformed the landscape of modern anti-malarial therapy, as these compounds remain effective against multi-drug resistant *P. falciparum*, with minimal report of resistance and efforts to control resistance.[3–4]

Artemisinin was first isolated in 1971 in China as part of a government-backed initiative to discover anti-malarial drugs for North Vietnamese troops during the Vietnam War.[5,6] The discovery was guided by knowledge from traditional Chinese medicine and was communicated in 1979.[7] Artemisinin is a structurally fascinating natural product containing a complex tetracyclic core. Embedded within this core is a unique endoperoxide bridge. Furthermore, artemisinin contains seven stereocenters, six of which are contiguous, and an acetal linkage between a seven-membered and six-membered ring.

Artemisinin and derivatives are believed to operate via a different mode of action than the quinine-derived compounds, and while the exact nature of the mechanism of action is currently unknown, the endoperoxide bridge is believed to be involved.[8–9] The potencies of artemisinin and derivatives are also impressive, each with low nanomolar EC_{50} values.[3] Several derivatives have become frontline drugs due to better activity and bioavailability than the parent compound.[10] Artesunate (**4**) and artemether (**5**) are the drugs most commonly recommended by the World Health Organization (WHO) for treatment of severe malaria, often in combination with other anti-malarial drugs with different modes of action.[3] This approach, called artemisinin-based combination therapy (ACT), allows for reduced treatment time, diminished parasite recrudescence, and lower probability of resistance development. Artemisinin combination therapies are currently the gold standard for malarial therapy. Interestingly, artemisinin and derivatives have shown promising anti-cancer properties, and research to harness these properties is underway.[10]

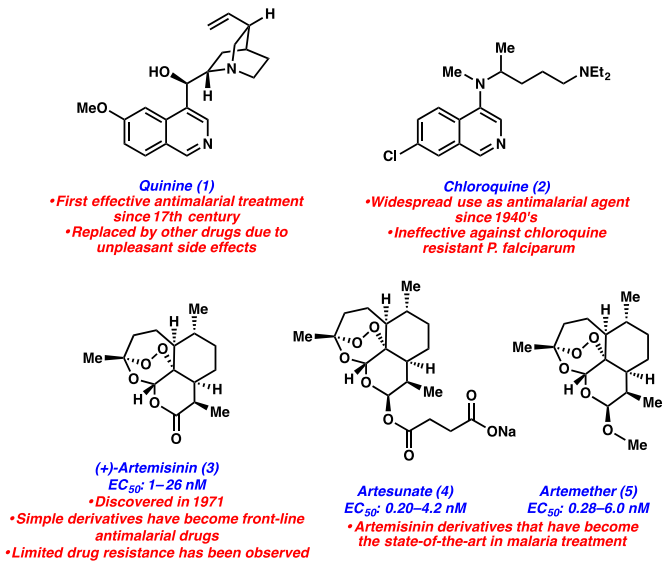

Figure 1 Structures of past and present front-line antimalarial drugs.

Access to these life-saving medicines is unfortunately limited where it is most needed. This is largely due to the high variability of the price of artemisinin and the inability of those in developing countries to afford the drug.[6,11] Currently, the primary method for the production of artemisinin is isolation from the plant *Artemisia annua*, which contains varying amounts of the compound.[12] It takes 12 to 18 months to obtain the compound from the plant and the recovery yield is typically below 1 wt%.[1,13] Environmental and economic factors render the amount of artemisinin obtained through this method unpredictable. As a result, supplying enough artemisinin to meet the needs of developing countries using only the isolation method is exceedingly challenging. Production of this compound has been a topic of interest for several years and, fortunately, there have been many efforts from different arenas of science to solve the supply problem.[14–15] These areas include total synthesis, biosynthesis & semi-synthesis, continuous flow chemistry, and genetic modification of *A. annua*.

Figure 2 Current production of artemisinin relies on isolation from *Artemisia annua*. Many factors limit the production of artemisinin from natural sources, including harvesting time and recovery yield. It takes approximately 12 to 18 months to obtain artemisinin by this method. Furthermore, the recovery yield is less than 1% and depends heavily on temperature, humidity, and soil type, which causes the price of artemisinin to be highly variable. Image from: *Science*, 2008, *320*, 330.

This review highlights the vast impact of synthetic chemistry on approaches to solving the problem of artemisinin production, including examples from total synthesis, synthetic biology, and semi-synthesis, including efforts using continuous flow chemistry. Diverse as all these approaches may be, it is most remarkable that they all converge in their final steps based on the bond disconnection strategy discovered during the first total synthesis of artemisinin by Schmid and Hofheinz in 1982.[16] This final assembly, which is presumably also the biosynthetic strategy, sets the stage for the concise assembly of the complex oxygenated core of the natural product.

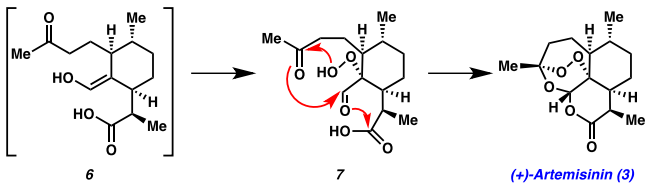

Figure 3 General synthetic strategy to build artemisinin. In the first synthesis of artemisinin by Schmid and Hofheinz, a photooxidation and acid-mediated ring closure sequence was employed to construct its oxygenated core. This strategy has heavily influenced many subsequent synthetic and semi-synthetic endeavors toward artemisinin production.

Total synthesis of artemisinin

Given the fascinating structure and the remarkable biological activity, it is not surprising that artemisinin is a popular synthetic target and there have been many total syntheses.[19–17] The details of many of these syntheses have been elegantly reviewed elsewhere and will not be included in this review.[18,19] Rather, the focus will be on the bond disconnection strategy developed from the first total synthesis of artemisinin and its subsequent use in other synthetic efforts.

In 1983, Schmid and Hofheinz disclosed the first total synthesis of artemisinin~~, demonstrating the remarkable ability to construct such a uniquely complex molecule via total synthesis~~.[19] Starting from (–)-isopulegol (**8**), a key intermediate **9** was rapidly forged in 11 steps. This compound **9** was poised to undergo a photooxygenation and acid-mediated ring closing sequence to deliver artemisinin (**3**). This landmark 13-step synthesis set the precedent for an efficient method to assemble the highly oxygenated core.

Yadav and coworkers reported total syntheses of artemisinin in 2003 and 2010.[26,27] Their most recent and expedient synthesis of artemisinin started from commercially available (*R*)-(+)-citronellal (**10**) and built key intermediate **11** using an 8-step process. Though structurally distinct from intermediate **9** from the work of Schmid and Hofheinz, this precursor **11** underwent a familiar photooxygenation and acid-

mediated ring closure. This approach demonstrated the utility of the bond disconnection strategy formulated in the first artemisinin synthesis.

In 2012, the Cook group published a remarkably concise and efficient synthesis of artemisinin.[28] The synthesis proceeded in just 6 steps ~~(5 pots)~~ from readily available cyclohexenone (**12**) and required only 3 chromatographic events. The key intermediate, built in 5 steps, was again subjected to a similar photooxygenation and ring closure sequence. In contrast to other approaches, the Cook synthesis does not rely on a naturally derived terpene as the starting material and source of chirality, but introduces the chirality using asymmetric catalysis. With the low step count and minimal chromatographic purifications, the Cook synthesis shows progress toward a total synthesis that may ultimately lend itself to the large-scale production of the natural product. Although the achievements in artemisinin total synthesis have been impressive, industrial manufacturing of the compound strictly via total synthesis has not yet been possible.

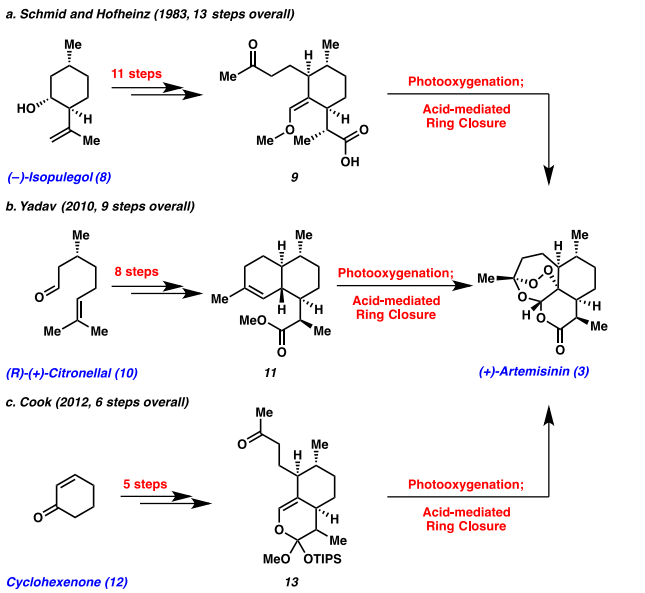

Figure 4 Select total syntheses of artemisinin from commercially available materials. a) Schmid and Hofheinz accomplished the first total synthesis of artemisinin from (–)-isopulegol in 13 steps. b) Yadav and coworkers reported a 9-step synthesis in 2010 beginning from (*R*)-(+)-citronellal, resulting in the shortest synthesis at the time. c) In 2012, Cook and coworkers reported a remarkably concise 6-step synthesis, which is the shortest synthesis of artemisinin to date.

Initial semi-synthetic efforts to produce artemisinin

Although it was shown in the 1980s that artemisinin could be accessed by total synthesis, the routes to the desired compound were not viable for significant production, as access to the carbon skeleton and the subsequent oxygenations lacked the necessary efficiency. In 1989, Acton and Roth disclosed a semi-synthesis of the molecule from another constituent of *Artemisia annua*, called artemisinic acid (**14**), a conversion that is used by the plant and is the inspiration for the efficient and scalable syntheses that are available today.[20–21] The route involved reduction of the artemisinic acid (**14**) using nickel boride to afford dihydroartemisinic acid (**15**). Hydroperoxide **16** was then formed from the singlet oxygen ene reaction of **15**. Exposure of **16** to acid initiated ring closure delivering artemisinin (**3**) in 30% overall yield.

This semi-synthetic route was not suitable for industrial-scale manufacturing, although many lessons were learned that would later facilitate the production of artemisinin. Most importantly, the study by Acton and Roth showed that easy access to artemisinic acid could result in an efficient process to make artemisinin, but only if the diastereoselective hydrogenation, the singlet oxygenation, and the subsequent oxygenation/rearrangement could be implemented in an industrial environment. The photooxidation step would be especially challenging, as it would require specialized equipment for large-scale synthesis. Furthermore, side reactions promoted by the free acid in the molecule impacted the overall yield of this process. Nevertheless, Acton and Roth discovered a very elegant, essentially biomimetic strategy, but it would require significant industrial optimization ~~industrialization effort~~ to become viable.

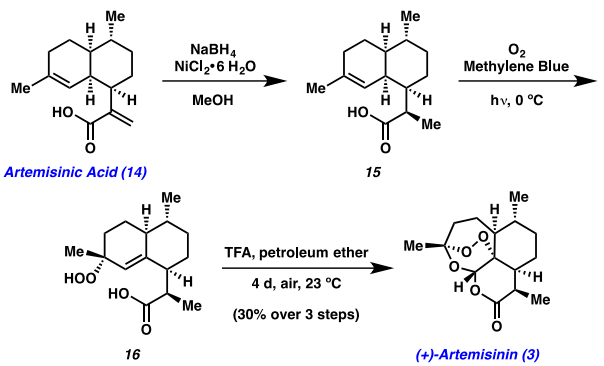

Figure 5 Semi-synthesis of artemisinin from artemisinic acid. In 1989, Acton and Roth reported an efficient semi-synthesis of artemisinin from artemisinic acid, a more abundant constituent of *Artemisia annua*. Isolation of this compound and direct conversion to artemisinin represents an effective strategy toward production of this antimalarial agent.

Unprecedented biosynthetic approach to artemisinin production

In the early 2000s, collaboration between the laboratory of Keasling at the University of California, Berkeley, Amyris Inc., and PATH, with funding from the Bill and Melinda Gates Foundation, led the development of the semi-synthetic artemisinin project.[22] In 2003, Keasling and coworkers reported a salient approach to the production of artemisinin using a combination of synthetic biology and chemistry.[23] Guided by the biosynthesis of artemisinin in *A. annua*[24], their initial notion was to produce terpenoid intermediates along the artemisinin

biosynthetic pathway using microbial fermentation with subsequent chemical elaboration to artemisinin. They initially reported the production of amorphadiene (**19**), a biosynthetic precursor to artemisinin, in *E. coli.* by engineering the bacterium to produce amorphadiene. Improvements to this process eventually resulted in the production of 25 g/L of amorphadiene.[25] Glucose (**17**) was chosen as the carbon source due its low cost and facile conversion to acetyl-CoA and ultimately farnesyl pyrophosphate (**18**), via the mevalonate pathway. It is this sesquiterpene precursor **18** that is then acted upon by the terpene synthase, amorphadiene synthase to provide amorphadiene. The Keasling lab and Amyris also investigated the production of isoprenoid precursors in the yeast *Saccharomyces cerevisiae*[26] and ultimately found that switching the host organism to an appropriate strain of *S. cerevisiae* could increase yield of amorphadiene to 40 g per L.[27] Amorphadiene (**19**) was originally chosen as a suitable semi-synthetic precursor as it was found that the conversion of farnesyl pyrophosphate (**18**) to amorphadiene (**19**) is the first committed step in the artemisinin biosynthesis.[38,28,29] However, identification of the oxidase enzymes capable of converting amorphadiene (**19**) to artemisinic acid (**14**)[40,30,31] allowed the target of microbial fermentation to change as it was previously demonstrated that artemisinic acid is an excellent synthetic precursor to artemisinin. After extensive optimization of the fermentation process, artemisinic acid could be produced in yeast at an impressive titre of 25 g per L.[45] This work provided a striking route to artemisinic acid, which was designed to be operationally simple and affordable. Formation of **14** provides a remarkable example of the use of synthetic biology to efficiently build complex structures.

With a robust microbial fermentation process in hand, the Keasling lab and Amyris took the now readily available artemisinic acid and used it as a starting point for semi-synthetic access to artemisinin, thus improving upon known semi-syntheses.[31–34,45] The initial reduction in their process involved hydrogenation of artemisinic acid (**14**) using Wilkinson's catalyst and delivered a 9:1 diastereomeric ratio of products favoring the required dihydroartemisinic acid (**15**) in 99% yield. Esterification of the acid using oxalyl chloride and catalytic DMF delivered ester **21**. The formation of the ester was pursued in order to minimize side reactions caused by the acid in subsequent reactions. Singlet oxygen ene reaction was then effected by decomposition of hydrogen peroxide under Aubry conditions.[32] This method is useful for large-scale reactions using singlet oxygen[33] as it obviates the need for photochemical reactions, where scale-up can be challenging.[34] Hock cleavage and acid-mediated ring closure delivers artemisinin (**3**) in 19% overall yield. Although the yield is not optimal, the route demonstrates a notable improvement for the chemical production of artemisinin from artemisinic acid. The selectivity of the first step was greatly improved, side reactions were avoided by installation of the ester, and scalable conditions were used for the final step. Most importantly, the microbial production of artemisinic acid offers a sustainable source of this precursor, which contains the complete carbon skeleton with the correct stereochemistry of artemisinin. Full details of the semi-synthetic artemisinin project have recently been reviewed.[36]

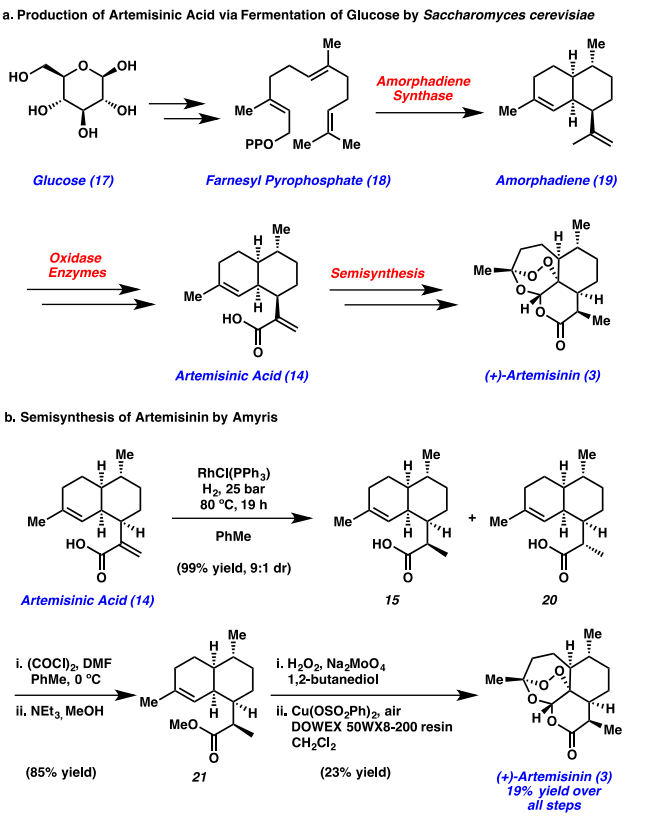

Figure 6 Interdisciplinary approach to the production of artemisinin. The Keasling laboratory and Amyris have developed an efficient fermentation process for the production of artemisinic acid and a scalable semi-synthesis of artemisinin.

Continuous-flow chemical strategy

The synthetic strategy first described by Acton and Roth and later made synthetically viable by Amyris was further improved in 2012 by Seeberger and coworkers. Seeberger's team realized the benefits of flow reactions for photochemical processes and disclosed an elegant semi-synthetic approach to artemisinin.[35] First, dihydroartemisinic acid (**15**) was obtained via the reduction of artemisinic acid (**14**), which can be produced by microbial fermentation. Dihydroartemisinic acid could also be isolated from the mother liquor resulting from a crystallization step in the isolation of artemisinin from *A. annua*.[36] This waste stream contains a significant quantity of dihydroartemisinic acid that can be isolated after simple basic extraction and used directly in the continuous flow setup without purification. As mentioned previously, the conversion of dihydroartemisinic acid to artemisinin through batch processes was not scalable prior to 2013,[40] which limited the production of the important anti-malarial compound by this method. The flow chemical method was expected to be an inexpensive, operationally simpler alternative because isolation

or purification of intermediates is not required. The process involves a singlet oxygen ene reaction of dihydroartemisinic acid (**15**), followed by Hock cleavage and a subsequent oxidation/acid-mediated ring closure sequence. Remarkably, these reactions are all done in one continuous process affording artemisinin (**3**) in 46% overall yield from dihydroartemisinic acid (**15**). As light can only travel a certain distance through solution before being absorbed by a photosensitizer, large reaction vessels have serious physical limitations in photochemical reactions.[50] The Seeberger group addressed this problem by wrapping a tube, through which the reaction mixture flowed, around a lamp ensuring high absorption of light.[37] Further benefits of flow chemical methods have been described in detail elsewhere.[38] At the reported level of scalability, it is estimated that 165 grams of artemisinin could be provided per day by flow chemical methods using the current equipment. Attempts to further scale this process are underway.[39] This impressive continuous flow method has the potential to contribute an intriguing technological platform for the production of artemisinin.

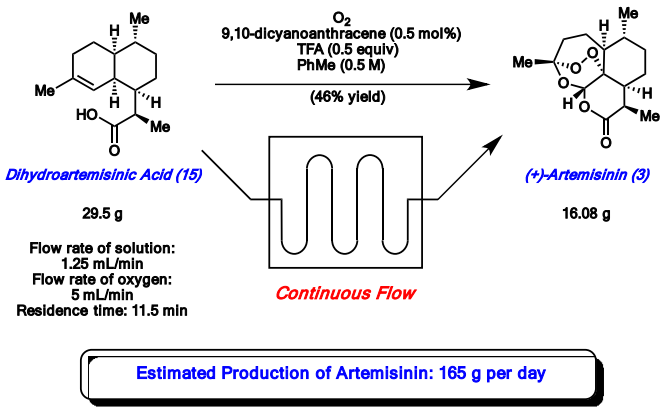

Figure 7 Semi-synthesis of artemisinin using continuous flow. The Seeberger group has recently developed a continuous flow approach to the production of artemisinin from dihydroartemisinic acid. This precursor can be isolated in high quantity from *A. annua* and can be converted in one operation via continuous flow to artemisinin.

Successful Large-Scale Production

In 2013, Sanofi announced the large-scale production of artemisinin (**3**) from microbially derived artemisinic acid (**14**) at its Garessio site in Italy using the technology discovered by Amyris.[40] The semi-synthetic transformations of artemisinic acid to artemisinin are related to those first described by Acton and Roth over 25 years ago and mimic what is assumed to happen in *A. annua*. In 2014, Sanofi described the technical details of their highly optimized semi-synthesis.[41] Related to the approach described by Amyris[45], the first step consisted of transition metal-catalyzed diastereoselective hydrogenation of artemisinic acid (**14**) to dihydroartemisinic acid (**15**). The reaction used by Amyris provided **15** in near quantitative yield with good diastereoselectivity (9:1 in favor of **15**). Sanofi improved upon this process by first switching from Wilkinson's catalyst to a ruthenium catalyst, $RuCl_2$[(*R*)-dtbm-Segphos](DMF)$_2$. This switch to a reagent-controlled process solved two problems: First, high loading and cost of Wilkinson's catalyst warranted a cheaper approach due to the cost of rhodium. Second, by using a chiral ligand, the diastereoselectivity of the reaction was improved to 19:1 favoring **15**. The conditions developed by Sanofi also featured reduction of the temperature in the reaction from 80 ºC to 25 ºC, reduction in the time from 19 hours to 5–6 hours, and the use of methanol as a green solvent alternative to toluene, all while drastically improving the s/c ratio from 100/1 to over 4000/1. All of these factors constitute an industrially viable hydrogenation process. In addition to the traditional transition metal-catalyzed hydrogenation, Sanofi has developed a large-scale diastereoselective diimide reduction of artemisinic acid (**14**).[42] The diimide was prepared *in situ* by reacting air with hydrazine in an IPA solution. This reagent effected a highly diastereoselective reduction of artemisinic acid (**14**) to dihydroartemisinic acid (**15**). The mechanistic details of this reaction have been described by Castro, Eisenstein, and researchers at Sanofi.[43] It is noteworthy that hydrogenation is achieved by bubbling air into this reaction rather than hydrogen. As hydrazine/oxygen mixtures serve as rocket fuel, it is remarkable that this process was safely performed on large scale.[56] Subsequently, dihydroartemisinic acid (**15**) was converted to mixed anhydride **22** in quantitative yield. Mixed anhydride **22** served as the precursor for the oxygenation/acid-mediated ring closure sequence. Despite the tremendous challenges associated with performing photochemical processes on large-scale, Sanofi employed a singlet oxygen ene reaction involving light, air and tetraphenylporphyrin (TPP). TPP is a readily available and highly effective sensitizer for intersystem crossing from triplet oxygen to singlet oxygen.[55] The mechanism is believed to proceed successively via a singlet oxygen ene reaction, Hock cleavage of the resultant hydroperoxide, additional oxygenation, and ring closure. This impressive semi-synthesis proceeds in an overall 55% yield of crystalline artemisinin (**3**) and is one of few large-scale photochemical reactions practiced industrially.

The route developed by Sanofi provides an average batch size of 370 kilograms of artemisinin and, most importantly, meets the WHO quality and purity requirements for a drug.[44] In 2013, Sanofi was able to produce 35 tons of artemisinin for further derivativization and implementation into the clinic. It is expected that 60 tons of artemisinin will be accessible by the end of 2014 via this method, which has become a sustainable supplement to direct isolation from *A. annua*.

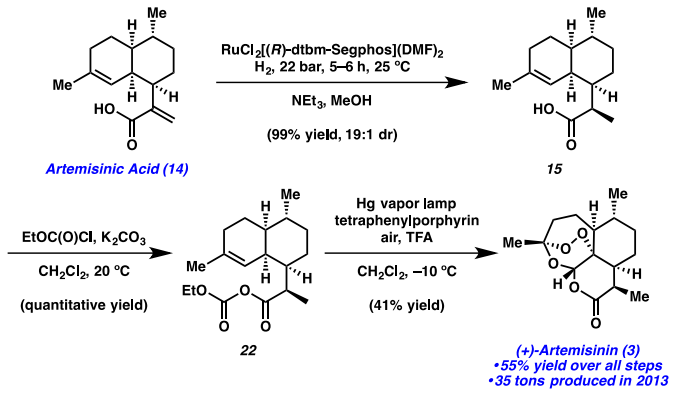

Figure 8 Improved semi-synthetic process by Sanofi. In collaboration with the Keasling laboratory and Amyris, Sanofi improved the previous semi-synthetic route to artemisinin. Using this fully implemented industrial process, 35 tons of artemisinin were produced in 2013 and 60 tons are expected in 2014. This process, coupled with isolation from *A. annua*, provides a promising means to address the artemisinin supply problem. Image of pilot plant provided by Sanofi.

Conclusions

In developing countries, multi-drug resistant malaria is an ~~severe~~ epidemic that claims the lives of 1–3 million people per year. However, the naturally occurring small molecule artemisinin and its derivatives can be used to treat this deadly illness. As isolation of artemisinin from natural sources is subject to environmental and economic fluctuation, great efforts in total synthesis, synthetic biology, semi-synthesis, and continuous flow chemistry have been undertaken. The synthetic strategy for the synthesis of artemisinin first developed by Schmid and Hofheinz, as well as the pioneering study by Acton and Roth on the biosynthetic mechanism, have given direction to many of the most promising approaches available today, including the brilliant approaches demonstrated by Yadav, Cook, Seeberger, and others.

The synthetic biological and semi-synthetic approaches to artemisinin have had a tremendous impact on the artemisinin supply problem. The efforts of the Keasling laboratory and Amyris led to a practical means to access large quantities of artemisinic acid using synthetic biology. This process, coupled with an efficient chemical conversion of artemisinic acid to artemisinin using homogeneous catalysis ~~(or organocatalysis)~~ and photochemistry, rendered a robust and scalable approach to artemisinin production. The industrial implementation at Sanofi has already produced 35 tons of artemisinin in 2013, and is thus able to reliably supplement the quantities obtained from extractive sources. This collaborative project could also provide a sustainable platform for analog synthesis, which could address recent development of artemisinin resistance.[45] The combination of synthetic biology and chemical synthesis serves as an icon for interdisciplinary science as a tool to solve world health problems~~, as does the no-profit, no-loss business model which enables the best access possible for those who need this life-saving medicine the most.~~

Acknowledgements

We thank Dr. Kai Rossen (Sanofi) and Professor Yi Tang (UCLA) for editing and providing valuable feedback for this review. M.A.C. acknowledges the NSF for a graduate research fellowship (DGE-1144087).

Notes and references

[a] Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095, United States

[1] V. Hale, J. D. Keasling, N. Renninger and T. T. Diagana, *Am. J. Trop. Med. Hyg.*, 2007, **77**, 198–202.
[2] T. E. Wellems and C. V. Plowe, *J. Infect. Dis.*, 2001, **184**, 770–776.
[3] *Guidelines for the treatment of malaria,* 2nd edn, World Health Organization, Geneva, 2010.
[4] F. Ariey, B. Witkowski, C. Amaratunga, J. Beghain, A.-C. Langlois, N. Khim, S. Kim, V. Duru, C. Bouchier, L. Ma, P. Lim, R. Leang, S. Duong, S. Sreng, S. Suon, C. M. Chuor, D. M. Bout, S. Ménard, W. O. Rogers, B. Genton, T. Fandeur, O. Miotto, P. Ringwald, J. Le Bras, A. Berry, J.-C. Barale, R. M. Fairhurst, F. Benoit-Vical, O. Mercereau-Puijalon and D. Ménard, *Nature,* 2014, **505**, 50–55.
[5] N. J. White, *Science*, 2008, **320**, 330–334.
[6] Y. Tu, *Nat. Med.*, 2011, **17**, 1217–1220.
[7] Qinghaosu Antimalaria Coordinating Research Group, *Chin. Med. J. (Engl.)*, 1979, **92**, 811–816.
[8] S. R. Meshnick, *Int. J. Parasitol.*, 2002, **32**, 1655–1660.
[9] N. Edikpo, S. Ghasi, A. Elias and N. Oguanobi, *Mol. Cell. Pharmacol.*, 2013, **5**, 75–89.
[10] M. P. Crespo-Ortiz and M. Q. Wei, *J. Biomed. Biotechnol.*, 2012, vol. 2012, Article ID 247597, 18 pages.
[11] M. Enserink, *Science*, 2005, **307**, 33.
[12] T. E. Wallaart, N. Pras, A. C. Beekman and W. J. Quax, *Planta Med.*, 2000, **66**, 57–62.
[13] D. C. Jain, A. K. Mathur, M. M. Gupta, A. K. Singh, R. K. Verma, A. P. Gupta and S. Kumar, *Phytochemistry*, 1996, **43**, 993–1001.
[14] M. Z. Abdin, M. Israr, R. U. Rehman and S. K. Jain, *Planta Med.* 2003,

69, 289–299.
[15] M. Farhi, E. Marhevka, J. Ben-Ari, A. Algamas-Dimantov, Z. Liang, V. Zeevi, O. Edelbaum, B. Spitzer-Rimon, H. Abeliovich, B. Schwartz, T. Tzfira, A. Vainstein, *Nat. Biotechnol.* 2011, **29**, 1072–1074.
[16] G. Schmid and W. Hofheinz, *J. Am. Chem. Soc.* 1983, **105**, 624–625.
[17] C. Zhu and S. P. Cook, *J. Am. Chem. Soc.*, 2012, **134**, 13577–13579.
[18] W.-S. Zhou, *Pure Appl. Chem.*, 1986, **58**, 817−824.
[19] S. P. Cook, *Synlett*, 2014, **25**, 751–759.
[20] N. Acton and R. J. Roth, *J. Nat. Prod.*, 1989, **52**, 1183−1185.
[21] J. Kong, Y. Yang, W. Wang, K. Cheng and P. Zhu, *RSC Adv.*, 2013, **3**, 7622–7641.
[22] C. J. Paddon and J. D. Keasling, *Nature Rev. Microbiol.*, 2014, **12**, 355–367.
[23] V. J. Martin, D. J. Pitera, S. T. Withers, J. D. Newman and J. D. Keasling, *Nat. Biotechnol.*, 2003, **21**, 796–802.
[24] G. D. Brown, *Molecules*, 2010, **15**, 7603–7698.
[25] H. Tsuruta, C. J. Paddon, D. Eng, J. R. Lenihan, T. Horning, L. C. Anthony, R. Regentin, J. D. Keasling, N. S. Renninger and J. D. Newman, *PLoS ONE*, 2009, **4**, e4489.
[26] D.-K. Ro, E. M. Paradise, M. Ouellet, K. J. Fisher, K. L. Newman, J. M. Ndungu, K. A. Ho, R. A. Eachus, T. S. Ham, J. Kirby, M. C. Y. Chang, S. T. Withers, Y. Shiba, R. Sarpong, J. D. Keasling, *Nature*, 2006, **440**, 940–943.
[27] P. J. Westfall, D. J. Pitera, J. R. Lenihan, D. Eng, F. X. Woolard, R. Regentin, T. Horning, H. Tsuruta, D. J. Melis, A. Owens, S. Fickes, D. Diola, K. R. Benjamin, J. D. Keasling, M. D. Leavell, D. J. McPhee, N. S. Renninger, J. D. Newman and C. J. Paddon, *Proc. Natl. Acad. Sci.*, 2012, **109**, E111–E118.
[28] H. J. Bouwmeester, T. E. Wallaart, M. H. A. Janssen, B. van Loo, B. J. M. Jansen, M. A. Posthumus, C. O. Schmidt, J.-W. De Kraker, W. A. König and M. C. R. Franssen, *Phytochemistry*, 1999, **52**, 843–854.
[29] P. Mercke, M. Bengtsson, H. J. Bouwmeester, M. A. Posthumus and P. E. Brodelius, *Arch. Biochem. Biophys.*, 2000, **381**, 173–180.
[30] K. H. Teoh, D. R. Polichuk, D. W. Reed and P. S. Covello, *Botany*, 2009, **87**, 635–642.
[31] C. J. Paddon, P. J. Westfall, D. J. Pitera, K. Benjamin, K. Fisher, D. McPhee, M. D. Leavell, A. Tai, A. Main, D. Eng, D. R. Polichuk, K. H. Teoh, D. W. Reed, T. Treynor, J. Lenihan, H. Jiang, M. Fleck, S. Bajad, G. Dang, D. Dengrove, D. Diola, G. Dorin, K. W. Ellens, S. Fickes, J. Galazzo, S. P. Gaucher, T. Geistlinger, R. Henry, M. Hepp, T. Horning, T. Iqbal, L. Kizer, B. Lieu, D. Melis, N. Moss, R. Regentin, S. Secrest, H. Tsuruta, R. Vazquez, L. F. Westblade, L. Xu, M. Yu, Y. Zhang, L. Zhao, J. Lievense, P. S. Covello, J. D. Keasling, K. K. Reiling, N. S. Renninger and J. D. Newman, *Nature*, 2013, **496**, 528–532.
[32] J.-M. Aubry, B. Cazin and F. Duprat, *J. Org. Chem.*, 1989, **54**, 726–728.
[33] P. L. Alsters, W. Jary, V. Nardello-Rataj and J.-M. Aubry, *Org. Process Res. Dev.*, 2010, **14**, 259–262.
[34] D. Ravelli, S. Protti, P. Neri, M. Fagnonia and A. Albini, *Green Chem.*, 2011, **13**, 1876–1884.
[35] F. Lévesque and P. H. Seeberger, *Angew. Chem. Int. Ed.*, 2012, **51**, 1706–1709.
[36] D. Kopetzki, F. Lévesque and P. H. Seeberger, *Chem. Eur. J.*, 2013, **19**, 5450–5456.
[37] F. Lévesque and P. H. Seeberger, *Org. Lett.*, 2011, **13**, 5008–5011.
[38] D. T. McQuade and P. H. Seeberger, *J. Org. Chem.*, 2013, **78**, 6384–6389.
[39] R. Mundy, Malaria hopes rise as chemists produce cheap artemisinin. http://www.scidev.net/global/disease/news/malaria-hopes-rise-as-chemists-produce-cheap-artemisinin.html (accessed April 2014).
[40] Sanofi and PATH announce the Launch of Large-scale Production of Semisynthetic Artemisinin against Malaria, http://en.sanofi.com/Images/32474_20130411_ARTEMISININE_en.pdf, (accessed April 2014).
[41] J. Turconi, F. Griolet, R. Guevel, G. Oddon, R. Villa, A. Geatti, M. Hvala, K. Rossen, R. Göller and A. Burgard, *Org. Process Res. Dev.*, 2014, **18**, 417–422.
[42] M. P. Feth, K. Rossen and A. Burgard, *Org. Process Res. Dev.*, 2013, **17**, 282−293.
[43] B. Castro, R. Chaudret, G. Ricci, M. Kurz, P. Ochsenbein, G. Kretzschmar, V. Kraft, K. Rossen and O. Eisenstein, *J. Org. Chem.*, 2014, **79**, 5939–5947.
[44] Acceptance of non-plant-derived-artemisinin by WHO, Prequalification of Medicines Programme (PQP), http://apps.who.int/prequal/info_press/documents/PQ_non-plant_derived_artemisinin_1.pdf, (accessed April 2014).
[45] E. A. Ashley, M. Dhorda, R. M. Fairhurst, C. Amaratunga, P. Lim, S. Suon, S. Sreng, J. M. Anderson, S. Mao, B. Sam, C. Sopha, C. M. Chuor, C. Nguon, S. Sovannaroth, S. Pukrittayakamee, P. Jittamala, K. Chotivanich, K. Chutasmit, C. Suchatsoonthorn, R. Runcharoen, T. T. Hien, N. T. Thuy-Nhien, N. V. Thanh, N. H. Phu, Y. Htut, K.-T. Han, K. H. Aye, O. A. Mokuolu, R. R. Olaosebikan, O. O. Folaranmi, M. Mayxay, M. Khanthavong, B. Hongvanthong, P. N. Newton, M. A. Onyamboko, C. I. Fanello, A. K. Tshefu, N. Mishra, N. Valecha, A. P. Phyo, F. Nosten, P. Yi, R. Tripura, S. Borrmann, M. Bashraheil, J. Peshu, M. A. Faiz, A. Ghose, M. A. Hossain, R. Samad, M. R. Rahman, M. M. Hasan, A. Islam, O. Miotto, R. Amato, B. MacInnis, J. Stalker, D. P. Kwiatkowski, Z. Bozdech, A. Jeeyapant, P. Y. Cheah, T. Sakulthaew, J. Chalk, B. Intharabut, K. Silamut, S. J. Lee, B. Vihokhern, C. Kunasol, M. Imwong, J. Tarning, W. J. Taylor, S. Yeung, C. J. Woodrow, J. A. Flegg, D. Das, J. Smith, M. Venkatesan, C. V. Plowe, K. Stepniewska, P. J. Guerin, A. M. Dondorp, N. P. Day and N. J. White, *N. Engl. J. Med.*, 2014, **371**, 411–423.