Probing Nature By Chemical Synthesis

Karl Gademann

Laboratorium für Organische Chemie der ETH Zürich

1. The Power of Chemical Synthesis

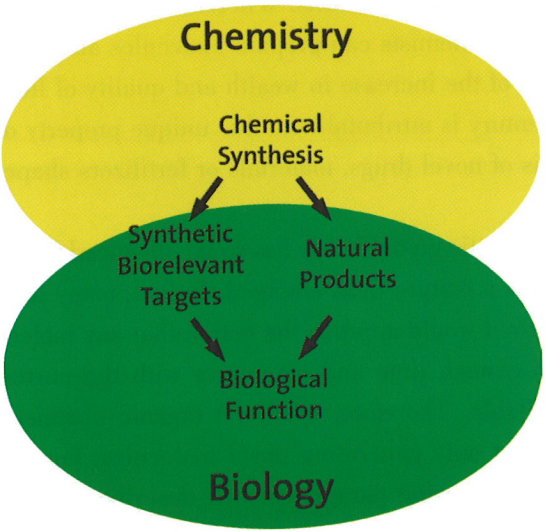
Chemistry as a field can be defined by its powerful ability to create its own objects of study by synthesis. Whereas an astronomer cannot synthesize a galaxy, chemists can prepare molecules and study their properties. Much of the increase in wealth and quality of life of mankind in the last century is attributable to this unique property of chemistry. The synthesis of novel drugs, materials or fertilizers shaped our world as it is today.

Nowadays, synthetic methodology has so far advanced that a perception of chemistry as a mature field emerged. In fact, many leading researchers in the field would approve the notion that any molecule "can be made" given enough time and manpower with the current synthetic methods available. Therefore, synthetic organic chemistry faces the challenge of not only generating novel molecules, but ***rather novel function.*** The following paragraphs will describe our own efforts in generating novel functions based on biologically validated molecules as starting points.

2. Chemical Biology as Research Strategy

In nowadays science, one could argue that the most fundamental discoveries will emerge at the interfaces of different fields. The research focus of our group is concentrated on the interface of chemistry and biology. We use a variety of techniques from the core of chemistry such organic synthesis, combined with physical tools such as spectroscopy, biochemical tools and computational simulations in order to study a fundamental problem in biology. This approach has sometimes been referred to as chemical biology, which can be defined as studying living systems with chemical methods and synthetic molecules. Such biorelevant intermediates are used to probe fundamental mechanisms in nature and to generate and stimulate biological function.

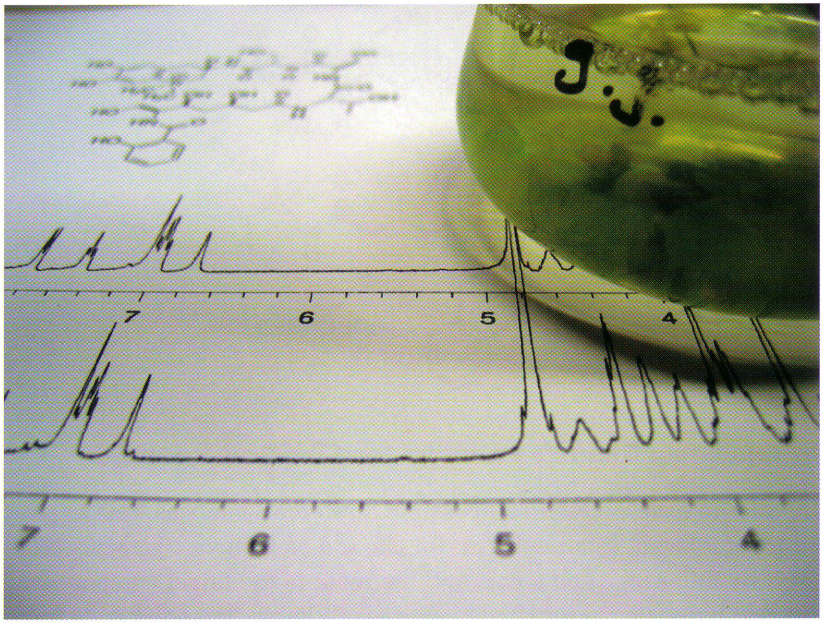
Figure 1. Fundamental mechanisms in biology can be probed by chemical synthesis.



3. The Mystery of Plankton Iron Mining

Plankton blooms are one of the largest dynamic biological phenomena that can be observed on earth. These massive accumulations of algae can span up to several hundred kilometers and are easily visible from space. The phenomenal growth of these organisms has been linked to the bioavailability of iron in water. Iron can become a growth-limiting nutrient in the open ocean due to its very low solubility. Therefore the growth of these blue-green algae can be directly stimulated for example by iron fertilization. Interestingly, the molecular mechanism of cyanobacterial iron acquisition is rather unknown. Using the chemical biology approach outlined above, we are investigating how cyanobacteria acquire, transport and store iron in order tow grow. As cyanobacteria constitute a major part of the prokaryotic phytoplankton and thus form part of the basis of the food chain, the understanding how such organisms grow is of central importance to the global ecosystem. Moreover, as these organisms constitute major sinks in the global CO2 cycle, cyanobacterial growth has been linked to global climate changes.

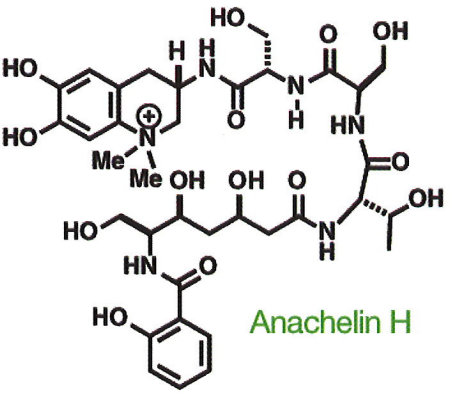
Figure 2. A culture of *Anabaena cylindrica* (top right) standing on spectra of the natural product and synthetic anachelin.



4. Anachelin – A Key Molecule For Blue-Green Algae

A key substance in this fundamental process in nature is *anachelin*, which is produced by the cyanobacterium *Anabaena cylindrica*. This natural product was first isolated in a collaborative effort of German and British chemists, which succeeded in the elaboration of its constitution. Later, a Japanese group isolated the same compound together with several related congeners. In all these investigations, several questions about this natural product remained unanswered. The structure of anachelin (the configuration, constitution and conformation) could not be fully established at that time. More importantly, the biological proof whether this substance acts indeed as a cyanobacterial growth factor is yet to be given. Last, questions about its biomechanism and mode of action remained unanswered as well. All these challenges provided the classic incentive for total synthesis, and therefore we initiated a research program directed to address these questions.

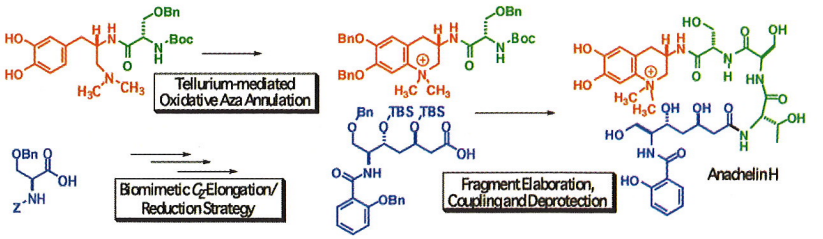
Figure 3. Anachelin H is a key substance in A. *cylindrica*. The correct stereochemistry was determined by total synthesis of this natural product.



5. Emulating Nature's Pathways for Chemical Synthesis

In order to have access to all possible diastereoisomers as well as possible biorelevant intermediates, we chose a *biomimetic* synthesis strategy. By following such a concept, the route to the target compound is devised based on a postulated biogenesis. Whereas the polyketide fragment in blue (Figure 4) probably emanates from a non-ribosomal peptide synthetase/polyketide synthase pathway, the alkaloid fragment itself is unprecedented in other natural products. Based on the biosynthetic hypothesis outlined below, we developed a Tellurium-mediated aza annulation as well as biomimetic C_2-elongation/reduction strategy to have access to both alkaloid and polyketide fragments.

Figure 4. Emulating nature's pathways for organic synthesis leads to a concise route to anachelin.

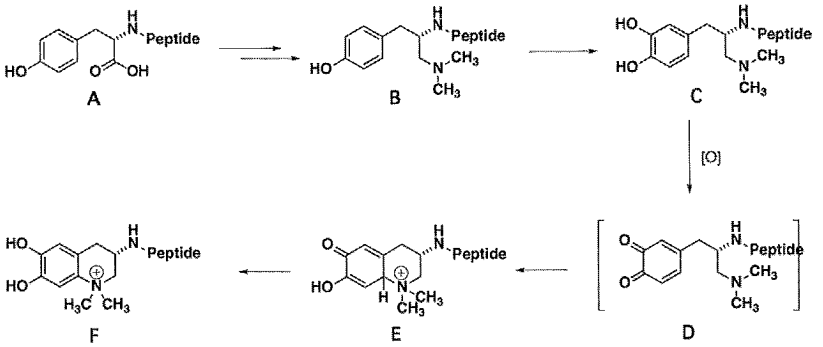


The fragments were then merged with a dipeptide fragment and, after final coupling/deprotection steps, the target compound could be isolated. Based on this biomimetic strategy, a stereodivergent route to the polyketide was carried out, which resulted in the total synthesis of anachelin.

6. The Cyanobacterial Crafting of Anachelin Investigated

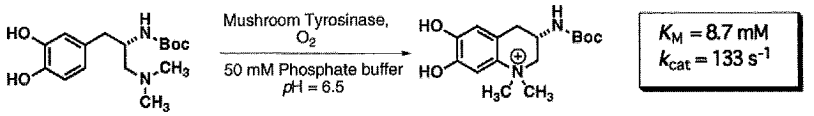
The alkaloid chromophore of anachelin is structurally unprecedented in other natural products, and the biosynthesis of anachelin is unknown. We proposed a hypothesis (Figure 5) for the biogenesis of this compound related to the well-known pathway of melanin pigment formation: A C-terminal bound tyrosine **A** residue is reductively aminated and methylated to give **B**. This compound is then first hydroxylated (to **C**) and then further oxidized to the quinone. An intramolecular 1,4 addition then furnishes, after tautomerization of **E**, the anachelin chromophore **F.**

Figure 5. A biogenetic hypothesis for the anachelin chromophore.



In order to corroborate this hypothesis in the chemical laboratory, we designed in vitro experiments involving the enzyme tyrosinase. Indeed, we were pleased to find that this enzyme is able to convert a model substrate to the anachelin chromophore. Kinetic analysis of the reaction revealed Michaelis-Menten kinetics with the catalytic efficiency decrease only about a factor of 30 to the natural substrate.

Figure 6. Designed *in vitro* experiments corroborating the biogenetic proposal.



These results are striking, as they provide further support for the biogenetic hypothesis outlined above. Further experiments, including *in vivo* feeding experiments and whole cell transformations are currently carried out.

7. Leveraging Scientific Knowledge – Targeting Hemochromatosis

All these investigations so far were directed towards the understanding of a fundamental mechanism in nature. In fact, the results already allow for a characterization of the molecular mechanism of cyanobacterial iron mining. Moreover, this basic research can be leveraged and applied to develop both medical and technological applications. In particular, a research program funded by the Swiss National Science Foundation focuses on the application of the gained knowledge towards finding a molecular solution to treat *hemochromatosis.*

Hemochromatosis is one of the most prevalent genetic diseases in Switzerland, where 1 out of 200 adults are thought to be affected. The disease results in iron overload, which causes oxidative stress to tissue resulting in organ damage and decrease life expectancy. However, the disease is poorly known and diagnosed, because its symptoms are easily overlooked. In fall 2004, federal counselor Pascal Couchepin publicly announced that he suffered from hemochromatosis, thus helping to educate people about this disease.

The current medical treatment of choice is phlebotomy, i.e. removal of blood from patients. This therapy can cause patient compliance problems and is also not possible for people with weak medical conditions. In addition, for people who suffer from iron overload due to blood transfusions, phlebotomy is not an option. These people are currently treated with Desferal®, which needs to be administered for several hours for several days, therefore patient compliance is a great problem. An

orally available drug (a pill) which would lead to decreased iron levels is therefore highly desirable.
In the context of the SNF sponsored research program, we are currently applying the basic knowledge gained in the context of cyanobacterial iron acquisition to the identification of molecules suitable for iron chelation. Already at this stage, several interesting molecules have been identified and are currently being evaluated.

8. Conclusion

This research demonstrates how a multi-disciplinary problem solving approach including total synthesis, biochemistry, spectroscopy and computational chemistry can address fundamental problems in biology. Combining these tools, we were able to investigate and study a basic mechanism in nature such as cyanobacterial iron mining. This knowledge might provide valuable insight, because of the central role of cyanobacteria in the global ecosystem. Moreover, CO_2 consumption by the blue-green algae is thought to significantly regulate the global climate and the temperature on earth.
This research also demonstrates how basic knowledge can be leveraged and applied to problems of technological and medical importance. In particular, the work directed towards the identification of orally available molecules for therapy of hemochromatosis and related iron overload diseases showcases the application of basic science in medicine.

9. Acknowledgements

I would like to express my gratitude again to the Latsis Foundation; it's a great honor and pleasure to receive such an award. Moreover, financial support by Prof. Dr. Erick Carreira is kindly acknowledged, as is support from the Swiss National Science Foundation and the Roche Research Foundation. Last but not least, I would like to thank my wife and my family for their love and strong support.