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Reflections on carotenoid synthesis

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Abstract - This lecture will comprise a review of the state of the art of both academic and industrial aspects of carotenoid research: 1. The present research frontiers of carotenoid synthesis. 2. The future trends and opportunities in the field. 3. The resources needed to explore and go beyond these frontiers. The more important methodologies used in total and partial synthesis will be discussed, including catalysis and organometallic chemistry as well as technical realization, safety, ecology and economy of processes. Where appropriate, biological aspects, such as biosynthesis, biocatalysis, fixed enzymes and organisms, biotechnology and molecular biology will be dealt with. In this context, some more critical questions will be asked concerning e.g.: the future of chemical synthesis, the implementation of biocatalysis, the application of molecular biology to process development, etc. Moreover, the now historic visions and milestones that lead the way to some of the more important ROCHE carotenoid products will be discussed, including my personal views and reflections. Finally, some recent contributions from our laboratories will be presented.

INTRODUCTION

According to the Oxford Dictionary (ref. 1), one can read that reflection is defined as meditation, recollection of a thing or reflecting on a person.

Following this line of thought, a very beautiful oil painting by Edvard Munch, named "Summernight" (Inger at the beach) (ref. 2), in my opinion, best depicts the mood needed for reflecting on one of the most important topics treated in this Symposium.

On the other hand, one can take some time at the end of the day to go back over it in the mind, relishing what went wrong, fashioning it into a prelude for tomorrow, as to the Devil's New Dictionary (ref. 3).

Thus reflecting on the past three decades, I can say that not much went wrong; far from it. One of the highlights was the Trondheim Symposium in 1966 (ref. 4), a most memorable occasion. The section on Total Synthesis comprised lectures by O. Isler, B.C.L. Weedon, T. Kralt, U. Schwieter, J.D. Surmatis and the present author, who as a greenhorn in the field, had the privilege to present the first total synthesis of rhodoxanthin (ref. 5) and who cannot help reminiscing about those early days. From then on a series of very successful symposia took place at three-year intervals up to the present 10th Anniversary Symposium.

Listening to my favourite poet (ref. 6): "Drei Jahr ist eine kurze Zeit, und, Gott! das Feld ist gar zu weit.", it is obvious that I have to obey Voltaire's order: "If you wish to converse with me, confine your terms". Thus, I am forced to define the topics in the field of carotenoid synthesis I want to discuss with you: 1. The present research frontiers. 2. The future trends and opportunities and 3. The resources needed to cross these frontiers.

Of course, I shall include my personal views and reflections. So this presentation will not be a highly scientific progress report but rather an almost philosophical contemplation.

SYNTHESIS

Defining the term synthesis, we should not only consider total synthesis, but also partial and biomimetic synthesis, chemical catalysis, biocatalysis, fermentation and cell culture.

I agree that today romance in carotenoid synthesis has diminished - some say only nostalgic feelings have remained. In my opinion, however, fascination, beauty and even art in the field have increased. Or was it rather serendipity? If you define serendipity as an aptitude to make fortunate discoveries accidentally (ref. 1), then you have the definition of making an invention. But that is not enough! Vision is the headline of the present! Not illusion! We must set the milestones, if you define them as significant or important events in the history of a person or a thing (ref. 1).

Of course, it is an old concept. It was followed by Dr. O. Isler from the very beginning! He always let us have a share in his visions, his imaginative contemplations, which he finally realized! And he also set the

milestones for future activities. His philosophy was both simple and very modern - although over-emphasized today: You have to have a goal, you have to be original and creative, you have to submit your expertise and secure the resources. Of course, you have to supervise the results and, most importantly, realize them.

For realization, we need resources, and the methodologies at our disposal at present are the following: Wittig/Horner reactions, Julia/Otera reactions, Grignard/Nef reactions, Lindlar partial hydrogenations, oxidations, enol ether condensations, vinyl type organometallic additions (Schlosser, Duhamel), aldol condensations, hydrocarbonylations.

Apart from some interesting variations of the Julia type and organometallic reactions, I consider this state of the art totally inadequate for the future. Therefore, it is important for both academia and industry to invest heavily in the exploration of new, efficient, economic and ecologically acceptable methodologies.

Rhodoxanthin

As mentioned before, my first task at ROCHE was to make rhodoxanthin (Fig. 1), the red pigment of Taxus baccata L., one of the yew tree species, of the fish Tilapia, of several fungi and of bird feathers, readily available in g-amounts for testing. As a starting material I decided to use the so-called C_{18}-ketone which I had received from my colleague and friend U. Schwieter. Unfortunately, I was unable to realize my goal although I had exactly followed the instructions given in a former ROCHE patent (ref. 7).

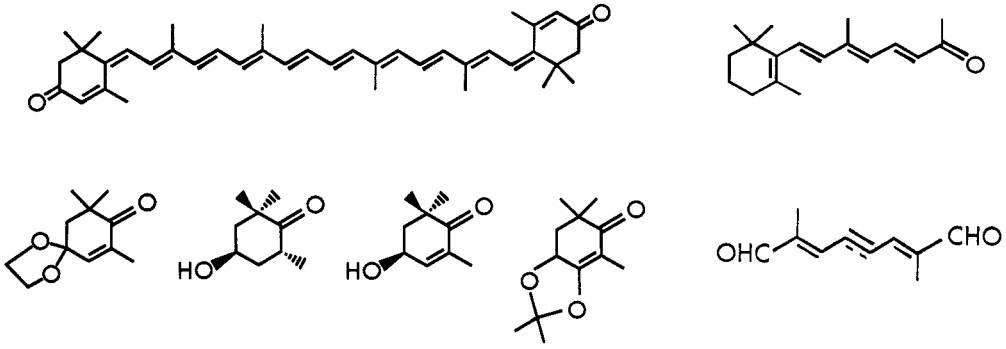

Fig. 1. Rhodoxanthin and some important building units

I was, however, consoled by John Dewey who stated that "we only think when we are confronted with a problem". I had not considered thoroughly enough the following three points: 1. How to prepare efficiently the desired end group beforehand, 2. how to choose and prepare a suitable central unit and 3. how to combine the end group with the central unit. However, we quickly reactivated an old concept, namely to use small cyclic building units which already possess the desired oxygen function together with the correct stereochemistry and optical purity. Thus for rhodoxanthin we chose the first C_9-unit and for zeaxanthin and astaxanthin the remaining three ones as will be discussed in the following sections (Fig. 1).

Of course suitable central units are required, and the most important one proved to be the C_{10}-dialdehyde (refs. 8-11) out of a choice of many others.

Finally, by employing suitable C_{14}- (ref. 5) and C_{15}-units (ref. 12), we succeeded in making multigram amounts of rhodoxanthin and even in developing an efficient technical synthesis (ref. 12). We were able to cross the borderline which encouraged us to proceed with more fastidious goals.

As expected, the (all-E)-isomer is not the only isomer existing (ref. 13). Most interestingly, Matsuno and coworkers (ref. 14) were able to characterize the (all-E)-, the (6Z)- and the (6Z,6'Z)-isomer from the fish Tilapia nilotica. Moreover, Schiedt and Bischof (ref. 15) were able to detect a 1:2:1-mixture of these isomers in egg yolk after administration of the (all-E)-compound to the laying hen.

Zeaxanthin

So we immediately set out to invent a procedure to convert rhodoxanthin into another important pigment, namely zeaxanthin (ref. 16). As expected, we obtained a 1:2:1-mixture of the (3R,3'R)-, (3R,3'S; meso)- and (3S,3'S)-isomers. It is important to note that we now have a method at hand to make two naturally occurring pigments by only a few consecutive steps.

Of course, when Columbus on his first visit to Cuba in 1492 was given a strange plant called maize (meaning "vegetable of life") by the native Arawaks (ref. 17), nobody cared about zeaxanthin and its stereochemical complexity. Not until 1969 when the absolute configuration of zeaxanthin was established by

Weedon and co-workers (ref. 18), and in 1983, when Rüttimann and co-workers (ref. 19) had shown that Zea mays and other plant sources exclusively contain the (3R,3'R)-isomer. However, in 1985, Schiedt and co-workers (ref. 20) and in 1986 Matsuno and co-workers (ref. 14,21) were able to detect all three optical isomers of zeaxanthin in rainbow trout, fish, shrimp and turtle. Thus, (3R,3'R)-, (3R,3'S;meso)- and (3S,3'S)-zeaxanthin are now proven to be natural products.

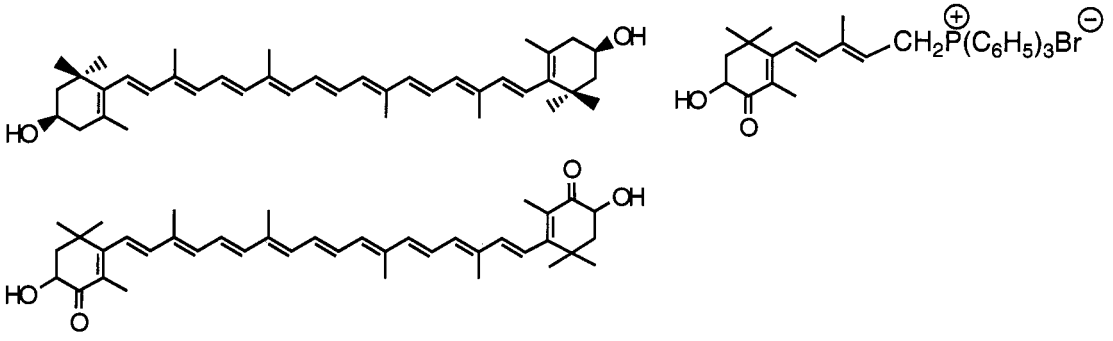

Fig. 2. Zeaxanthin, astaxanthin and an important C_{15}-building unit

In 1975, we were able to report the first total synthesis of (3R,3'R)-zeaxanthin (ref. 22), and shortly afterwards the total synthesis of the (3R,3'S;meso)- and (3S,3'S)-isomers was successfully completed (ref. 23).

Later, my colleague and friend E. Widmer and his team (ref. 24) succeeded in developing a five-step technical synthesis using the same original $C_{15} + C_{10} + C_{15} = C_{40}$ scheme but synthesizing the C_{15}-unit according to a new and very efficient procedure.

Today, in the mid-1990s, when reflecting on our early efforts, I am inclined to admit that an approach based on the biosynthesis of zeaxanthin might be a promising alternative. As was demonstrated by Britton and co-workers in 1977 (ref. 25-26), lycopene is smoothly cyclized to give ß-carotene which is then regio- and stereoselectively hydroxylated. The great potential of molecular biology in this area has already been recognized (refs. 27-29).

Astaxanthin

It was in the mid 1970s, when stereochemistry really claimed its right. We were forced to reflect on chirality, optical activity and symmetry, such as C_1, C_2 and S_2. Further, we were confronted with systems such as AA, ABC and ABBA. Zeaxanthin and astaxanthin both belong to the AA-system (ref. 30) whereas the ABC-system is true for lutein (ref. 31). The ABBA-system of tunaxanthin causes the existence of ten optical isomers, which were all synthesized more than ten years ago (ref. 31).

With regard to astaxanthin, a special history supports the development of this most interesting pigment. The exciting milestones that led to the commercialization of this carotenoid were already expertly reported at the Boston meeting in 1987 by Bernhard (ref. 32). Today I should like to add some personal views and reflections.

Thus I shall never forget a discussion with Dr. O. Isler in the early 1970s, when he exclaimed: "You should not forget, astaxanthin could become a business. But we need g-amounts for testing, and fast!" Confronted with this assignment, I first felt rather uneasy, but later we thought: if prawn were able to synthesize astaxanthin from ß-carotene (ref. 33), why should we not be able to do so without enzymes? In fact, astaxanthin was first prepared from crustaxanthin as early as 1967 by Leftwick and Weedon (ref. 34), and it was obvious to make use of their approach. So when Kienzle (ref. 35) and later Hodler (ref. 36) took over, they quickly succeeded in preparing astaxanthin in 7 % and 20 % yield, respectively.

Thus, the problem was solved rather quickly and the material provided for preliminary investigations. However, for making the multi-kg amounts required for extended studies, partial synthesis was not the method of choice. We had reached the borderline. Moreover, partial synthesis produced a 1:2:1-mixture of the (3S,3'S)-, (3S,3'R;meso)- and (3R,3'R)-isomers as could readily be detected by the remarkable HPLC-method developed by Vecchi and Müller (ref. 37).

We therefore decided to develop a total synthesis aiming at (3S,3'S)-astaxanthin corresponding to the product isolated from Haematococcus pluvialis (ref. 38). The establishment of the (3S,3'S)-configuration of astaxanthin in 1974 by Liaaen-Jensen and co-workers (ref. 38) represents one of the most important milestones in the history of this carotenoid (ref. 32). The solution to the synthetic problem was found rather quickly when Kienzle reactivated an important concept, namely the allylic rearrangement of a suitable C_{15}-intermediate (Fig. 3) to introduce the desired 4-oxo-group. Moreover, the statement often put forward by experts that astaxanthin "racemized" rather fast, was proved wrong by a deuterium-exchange experiment

as suggested by Schmid (ref. 39): Kienzle was able to show that no deuterium was incorporated α to the hydroxyl group. This finding was the starting signal for our activities, and in 1976 the first total synthesis of

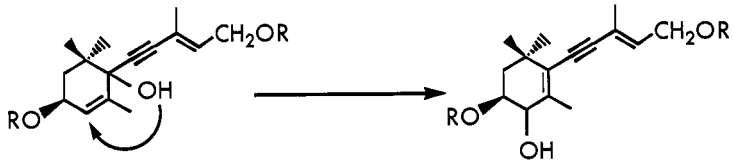

Fig. 3. Reactivated important concept: allylic rearrangement

(3S,3'S)-astaxanthin was reported (ref. 40).

Now all of a sudden dramatic news reached us from Trondheim (ref. 41) that lobster eggs not only contain (3S,3'S)-astaxanthin as expected but also considerable amounts of the (3S,3'R;meso)- and (3R,3'R)-isomer. This was the first finding of meso-astaxanthin and a meso-carotenoid in general in Nature (ref. 42). Shortly afterwards, Schiedt and co-workers reported that also in salmon a similar isomer distribution prevails (ref. 43).

These important findings served as basis for the development of a very efficient technical five-step synthesis of astaxanthin (the 1:2:1-mixture of the (3S,3'S)-, meso- and (3R,3'R)-isomers) by Widmer and co-workers (ref. 44) according to the same original $C_{15} + C_{10} + C_{15} = C_{40}$ scheme. Contrary to the first report (ref. 40), however, the racemic C_{15}-Wittig salt (Fig. 2) was synthesized following a new and very short route.

These efforts resulted in our sales product CAROPHYLL Pink which is now sold to fish farmers all over the world.

CRITICAL ISSUES

In this context, it is important to define the term Commercial Process. What are the criteria that have to be fulfilled? Of course, only few steps and unit operations are allowed, only advanced technology is applied, and process integrated environmental protection, regeneration and/or recycling of auxiliary chemicals, high purity products and economic competitiveness are guaranteed.

But what is the future of chemical synthesis? In my opinion, the future of chemical synthesis is bright not only in the carotenoid field. In fact, chemical R & D is one of the most important driving forces behind technical and economic progress.

What is the impact of catalysis? Some experts say: the future will be a catalytic one only, but they neglect definitions. As Berzelius said in 1836: A catalyst is a substance which facilitates a chemical reaction, and which itself undergoes no permanent change. Thus a catalyst can very well be used in equimolar amounts if it can be recycled.

The impact of organometallic chemistry will be great, and the present developments in the field are already quite encouraging.

Do we need patents? Of course we do, for both industrial and academic institutions. Otherwise any commercially applicable compound, process or formulation could be used by a competitor free of charge, which would mean a serious set-back of progress in numerous important research fields.

What is the correct definition for the term natural? Is it good for a product extracted from natural sources only? May we also include a biotechnically produced carotenoid? How about metabolites? The dilemma can be solved by accepting the definition recently suggested by Liaaen-Jensen (ref. 45): Carotenoids biosynthesized de novo from small carbon units and metabolic products of such carotenoids are considered as natural.

Consequently, the term nature-identical must equal natural, provided we deal with pure compounds possessing identical structures.

The use of (Z)-isomers is not yet quite clear - are they artifacts? What is an artifact? There are still some contradictory opinions left, but as pointed out by Liaaen-Jensen (ref. 45) there can be no doubt that well-defined (Z)-isomers, such as (9Z)- and (13Z)-ß-carotene are natural products. In order to be able to study the chemical and biological behaviour of these compounds more closely, we decided to make these compounds readily available by total synthesis (ref. 46). Surprisingly, the pure crystalline compounds proved to be quite stable.

Not quite unexpected, the relative provitamin A activity of these isomers compared to the (all-E)-compound proved to be rather low as determined by my colleague and friend H. Weiser (ref. 47). Thus (9Z)-ß-carotene exhibits only 25 %, the (13Z)-compound 42 % of the activity of (all-E)-ß-carotene. A low biopotency of (9Z)-ß-carotene has already been reported by Ben-Amotz and co-workers (ref. 48).

The (9Z)- and (13Z)-isomers of astaxanthin needed for registration purposes were prepared by Pd-catalysed isomerization of the (all-E)-compound followed by chromatography, HPLC-separation and

crystallization (ref. 49). This task proved much more difficult than with ß-carotene.

In the C_{20}-series, two important (Z)-isomers deserve mentioning: (13Z)- and (9Z)-retinoic acid. The first represents ROACCUTANE® (ROCHE) (ref. 50), the second is a very interesting substance that binds and activates the nuclear receptor RXRα (refs. 51-52). Both compounds can be considered as metabolites of ß-carotene (ref. 53).

A similar situation is observed with optical isomers. Until recently, (3R,3'R)- and (3R,3'S;meso)-astaxanthin (refs. 42-43) as well as (3S,3'S)- and (3R,3'S;meso)-zeaxanthin (refs. 14,20-21) were considered as unnatural. Today the question arises: why does nature produce enantiomers and meso-compounds? What is their function? For example, why does the human macula contain meso-zeaxanthin (ref. 54)? Does it exert a specific function?

To the customer, we have to offer the pure enantiomer, if nature produces only one, because the others might be inactive or harmful and therefore must be considered like any other impurity. The optical purity of the synthetic enantiomer must at least be the same as that of the natural compound, or better.

TRENDS AND OPPORTUNITIES

First of all, we have to improve the existing processes. Regeneration and/or recycling of auxiliary chemicals and solvents must be guaranteed, continuous performance and supercritical fluids employed.

We have to invent and develop new reactions and technologies whereby the reinforcement of basic research, especially chemical catalysis is absolutely necessary.

In biocatalysis, embedded immobilized enzymes and microorganisms will become the methodology of choice thus reducing the formation of useless biomass. Here the impact of molecular biology cannot be overemphasized, offering the use of genetically engineered hosts.

Concluding this chapter on new opportunities and methods, I should like to report on three recent chemical contributions from our laboratories, demonstrating that by skilful application of so-called classical methods significant progress can still be achieved:

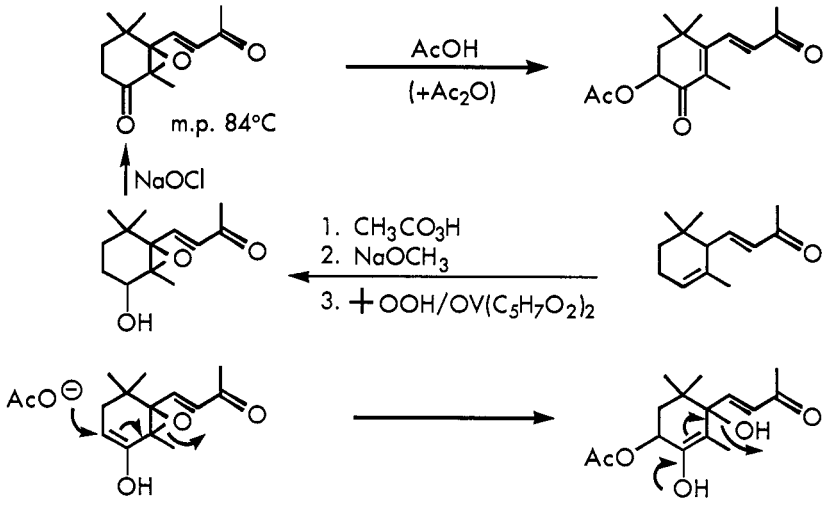

Fig. 4. C_{13}-Astaxanthin building unit

1. The hydroxy epoxide on the lower left (Fig. 4), readily obtained from α-ionone, was oxidized to 4-oxo-5,6-epoxy-ß-ionone, which on treatment with acetic acid/acetic anhydride smoothly afforded the desired C_{13}-building unit (ref. 55). Concerning the mechanism of this transformation, an enol of the type shown could be considered as an intermediate.

2. As a second example, an interesting synthesis of a new optically active C_{10}-unit, useful for the construction of crustaxanthin isomers, is presented: The hydroxy aldehyde on the upper left (Fig. 5) was readily epoxidized to give the corresponding epoxide which was smoothly transformed into the desired C_{10}-crustaxanthin unit (ref. 56).

Depending on the stereochemistry of the starting hydroxy aldehyde and the type of epoxidation, all optical isomers of crustaxanthin may be synthesized if desired.

3. Epoxidation of (3R)-3-hydroxy-ß-ionone (Fig. 6) gave a mixture of epoxy ketones which could be readily separated. Subsequent oxidation and epoxide ring opening then led to the pure enantiomeric (+)-(6S)- and (-)-(6R)-dehydrovomifoliols (ref. 57).

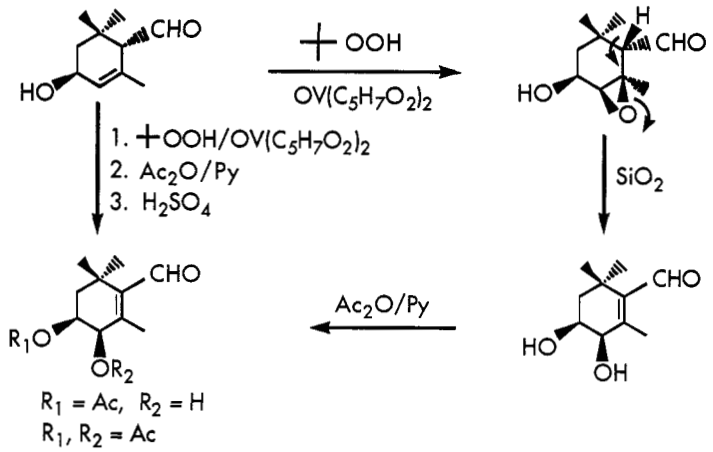

Fig. 5. Crustaxanthin building unit

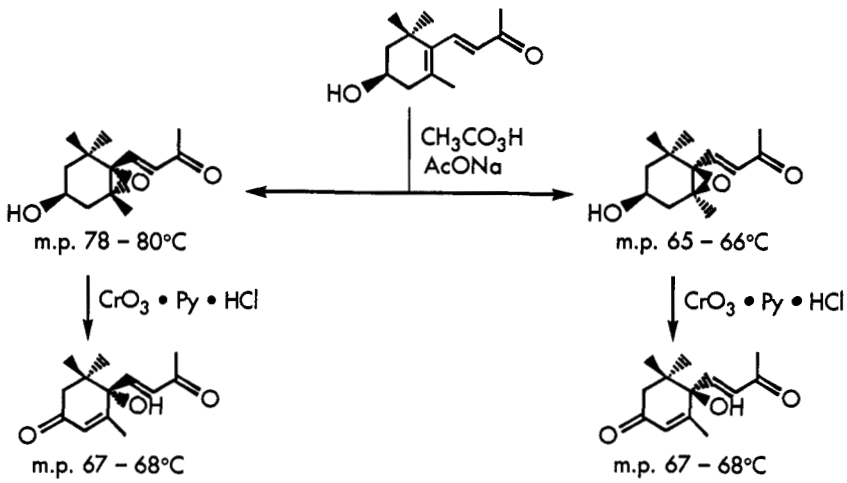

Fig. 6. Synthesis of (+)-(6S)- and (-)-(6R)-dehydrovomifoliol

KEY CHALLENGES

Some of the future key challenges in the field of chemical synthesis are shown by the following examples:

1. Olefination: Improvement of the Wittig type reactions regarding stereoselectivity, yield, recycling of triphenylphosphine oxide. Exploration of new reaction types: catalytic, stereoselective, no formation of triphenylphosphine oxide.
2. Vinylation/Ethinylation: Replacement of acetylene by ethylene. Catalytic, regioselective, e.g. with dicarbonyl compounds, such as 4-oxo-ß-ionone.
3. Hydrogenation: Improvement of catalytic partial and asymmetric hydrogenations, continuous and selective performance, e.g. with polyenyne systems, trisubstituted double bonds and carbonyl compounds.
4. Oxidation: Invention of new catalytic, regio- and stereoselective oxidation reactions, e.g. direct allylic oxidations, direct asymmetric hydroxylations.
5. (E/Z)-Isomerization: Development of new, catalytic, regio- and stereoselective isomerizations, e.g. kinetically controlled catalytic transformation of (all-E)-ß-carotene into (9Z)-ß-carotene, regioselective rearrangement of double bonds, e.g. α-ionone ⇌ ß-ionone, α-isophorone ⇌ ß-isophorone.

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