Ruthenium Porphyrin-β-Cyclodextrin Complexes as Supramolecular Enzyme Models for Regioselective Cleavage of Carotenoids

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2006

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1 Introduction

1.1 Introduction to carotenoids

1.1.1 Occurrence and function

The term “carotenoid” encompasses a diverse family of naturally occurring pigment molecules belonging to the terpenoid class. Some of the most strikingly obvious and best known examples of the natural occurrence of carotenoids are provided by the yellow-orange colors of flowers (*e.g.*, sunflower, marigold), the orange-red colors of fruit (*e.g.*, tomato, orange), and the orange roots of carrots.

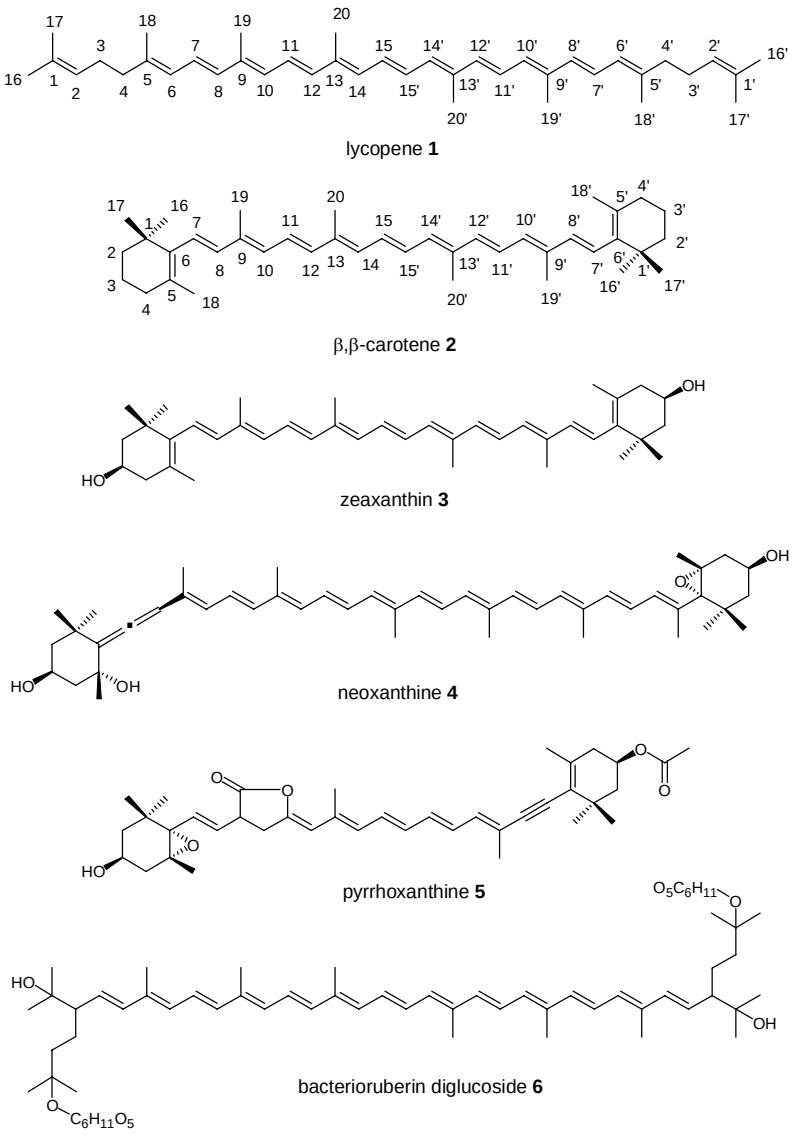


Figure 1 – Structure of lycopene **1**, β,β-carotene **2** and some structural diversities

To date, more than 600 naturally occurring carotenoids are known, all derived from the same basic C_{40} isoprenoid skeleton by modifications such as cyclization, substitution, elimination, addition, rearrangement and oxidation. The basic structure is illustrated by lycopene **1**, β,β-carotene **2** and the diversity of structures by further examples in *figure 1*. The remarkably long chain of conjugated double bonds explains the most evident properties: the intense color ranging from the yellow tones to the deep red.

Carotenoids are widely distributed in nature. They are commonly thought of as the pigments in plants such as fruits, flowers and vegetables, but in fact they also occur widely in microorganisms and animals. Apart from their occurrence in phototrophic bacteria, carotenoids are often responsible for the yellow, orange or red colors in non-phototrophic bacteria, yeasts and moulds. The importance of the carotenoids in these micro-organisms lies mainly in protection against damage by light and oxygen.

The greatest production of carotenoids occurs within the photosynthetic tissues of plants and algae. Here, carotenoids are found universally in the photosynthetic apparatus, though their presence is often masked by the green colour of chlorophyll and revealed only when chlorophyll is degraded as, for example, in autumn leaves. The annual natural production of carotenoids has been estimated to be in the order of 100 million tons, of which some single carotenoids contribute upwards of 10 million tons each.

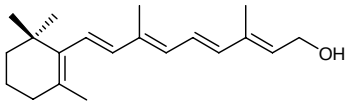
Animals appear to be incapable of biosynthesising carotenoids, but many animals are colored by carotenoids that originate from their diet. Some eye-catching examples are provided by bird feathers (*e.g.*, ketocarotenoids in the pink feathers of flamingos) and the skin and flesh of some fish (*e.g.*, salmon). Coloration by carotenoids is not normally seen in mammals, although humans ingesting large amounts of carotenoids, either as dietary supplements or as “tanning agents”, may show slight yellow or orange color in the skin.

Carotenoids do not only act as pigments. However, their biological relevance is based mainly on their role in the photosynthetic process with the double function of harvesting energy from light and protecting the reaction center from excess irradiation

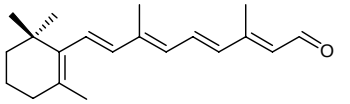
and unwanted side reactions (*e.g.*, with singlet oxygen). Carotenoids also fulfil the essential function as precursors of vitamin A in animals.[1]

1.1.2 Carotenoids and nutrition

The presence of a fat soluble principle, essential for life, was discovered in foods such as egg yolk and butter fat in the early part of the twentieth century. Fish oils proved to be the most important source of this factor, which was named vitamin A (retinol **7**). The link between vitamin A and carotenoids was established when it was shown that crystalline β,β-carotene possesses high vitamin A activity[1] and that, in the rat, β,β-carotene is metabolised to vitamin A which is stored in the liver.[2] In 1931, the structure of vitamin A was established by Karrer et al (*figure 2*).[3, 4]



retinol **7**



retinal **8**

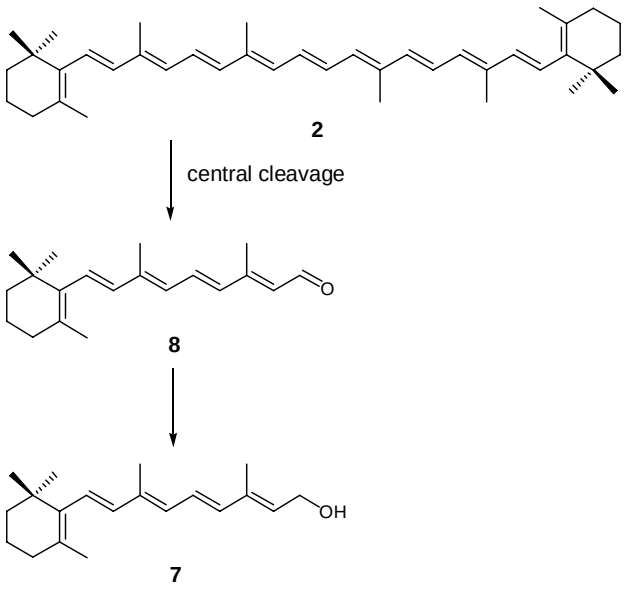
Figure 2 – Structure of vitamin A (retinol) and retinal

The most important nutritional role of carotenoids, particularly β,β-carotene **2**, is as pro-vitamin A. The involvement of vitamin A aldehyde-retinal **8**, as the chromophore of the visual pigment in the eye, is essential to the process of vision.[5] Vitamin A deficiency is still a major nutritional problem in many parts of the developing world where its consequences, xerophthalmia, blindness and premature death are still common, particularly in children. Vitamin A also has important systemic functions in maintaining growth, reproductive efficiency and plays a critical role in the maintenance of epithelial tissues by preventing their keratinisation. The importance of the latter effect has led to the synthesis of a wide range of related compounds, the retinoids, and to the evaluation of these substances for therapeutic use to treat skin problems such as acne, and also as cancer-prevention agents.[6-9]

Carotenoids are also important in the protection against disease *via* their role as antioxidants. Extreme sensitivity to light, seen in humans suffering from erythropoietic protoporphyria, an abnormality in porphyrin metabolism, has long been treated by the administration of β,β-carotene to prevent singlet oxygen formation. Most current research is focused on a proposed role for carotenoids as lipid antioxidants which are able to protect against oxidation and other destructive processes mediated by free radicals,[10-12] though more specific effects on the immune system are still under investigation.[13, 14]

1.1.3 Mechanistic aspects of the production of retinal from β,β-carotene: central cleavage or excentric cleavage

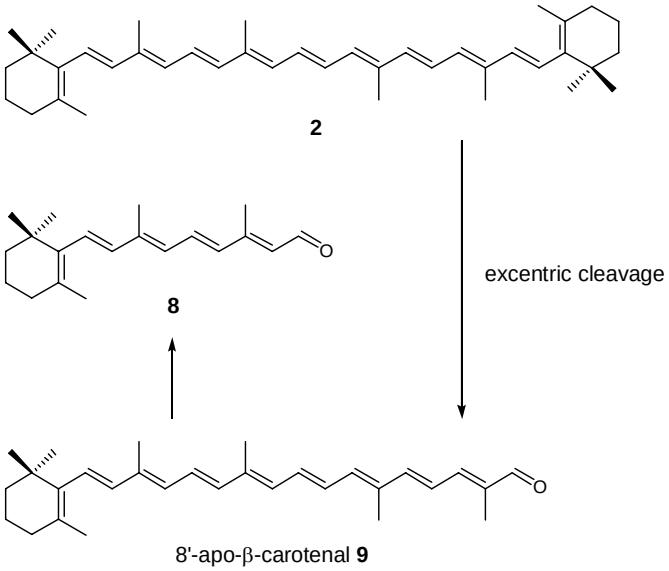
In 1930, Moore experimentally recognized the production of vitamin A,[2] the structure of which was not fully confirmed at that time, in vitamin A deficient rats fed with a β,β-carotene dietary suppliment. Later, the resemblance of the structure of vitamin A[3] to half of the β,β-carotene molecule suggested that retinol might have been formed from β,β-carotene by central cleavage, providing two molecules of retinal (*scheme 1*).



Scheme 1 - β,β-Carotene **2** as a precursor for vitamin A **7** (central cleavage pathway)

However, as early as 1960, Glover suggested an excentric cleavage pathway in which, as shown in *scheme 2*, the double bond next to the β-ring of β,β-carotene reacts first,

followed by sequential oxidative removal of fragments containing between two and five carbon atoms to provide 1 molecule of retinal.[15] Two observations supported this idea: i) the biological activity of β,β-carotene was always inferior when compared to that of retinol; ii) apo-β-carotenals also showed high biological activity.



Scheme 2 – Proposed excentric cleavage pathway

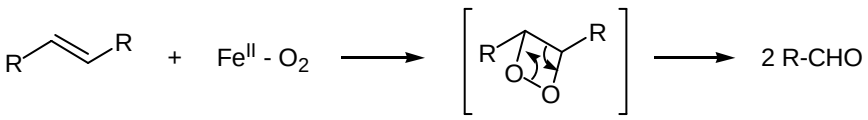
Before the discovery of the enzymatic activity *in vitro*, only speculative explanations were proposed for the mechanisms of the cleavage reactions. Two schools of thought have both been argued over the most important pathway to yield vitamin A: i) proceeding over the central cleavage producing two moles of retinal from each mole of β,β-carotene, or ii) if the excentric cleavage was biologically more relevant.

1.1.3.1 β,β-Carotene-15,15'-monooxygenase: the enzyme for the central cleavage of β,β–carotene

The *in vitro* central cleavage to retinal was independently reported in rat liver and intestine by two research groups in 1965.[16, 17] In both studies the production of other aldehydes, *e.g.*, apo-β-carotenals, was not observed. The activity was almost totally localized in the cytosolic fraction, with very little catalytic activity in the membranes. Only two years later, an enzyme which was responsible for the central cleavage was identified.[18] Although complete characterization was not possible at that time, this

enzyme was admitted into the Enzyme Handbook with the name β,β-carotene-15,15'-dioxygenase (CDO) under the E.C. number 1.13.11.21.

The features of the enzyme had already been investigated in 1965.[16] Molecular oxygen is required for the cleavage, and the presence of a metallic binding site was suspected due to the activity inhibition by iron complexing substances, such as EDTA, phenanthroline and α,α'-dipyridine. A mechanism, involving a [*2+2*] cycloaddition to the central bond to afford a dioxetan, which is further cleaved to two molecules of aldehyde, was postulated as shown in *scheme 3*.



Scheme 3 – Proposed mechanism of cleavage

The enzyme activity was shown to increase upon addition of thiols (mercapto ethanol, cystein) to the incubation mixture and was inhibited by SH-specific reagents (N-ethylmaleinimide, iodo acetamide, *p*-hydroxy mercury benzoic acid), indicating that the enzyme contains SH-groups essential for activity. Different detergents, lipid and bile salt were tested to mimic the conditions in the intestinal mucosa, a tissue from which the enzyme is largely expressed. These additives are probably important for the solubilization of the lipophilic substrate. The optimal pH for CDO was determined to be 7.7.

In 1969, the discovery of CDO's activity in other animals (*e. g.*, rabbit) was published again by Olson.[19] The sole product of the enzymatic assay was found to be retinal, confirming the central cleavage. During the course of this study, the enzyme activity towards apo-β-carotenals was also tested. Surprisingly, 10'-apo-β-carotenal **10** reacted 11 times faster than β,β-carotene, and the rate increased with decreasing length of the substrate. However, this result was not reproducible.

The distribution of enzyme activity in the different animals was the subject of a publication in 1972.[20] The cleavage of β,β-carotene had been reported as important in

herbivores (guinea pig, rabbit), normal for omnivores (tortoise, freshwater fish, chicken); the meat eaters showed low activity; and the cat showed no ability at all to produce vitamin A from carotenoids.

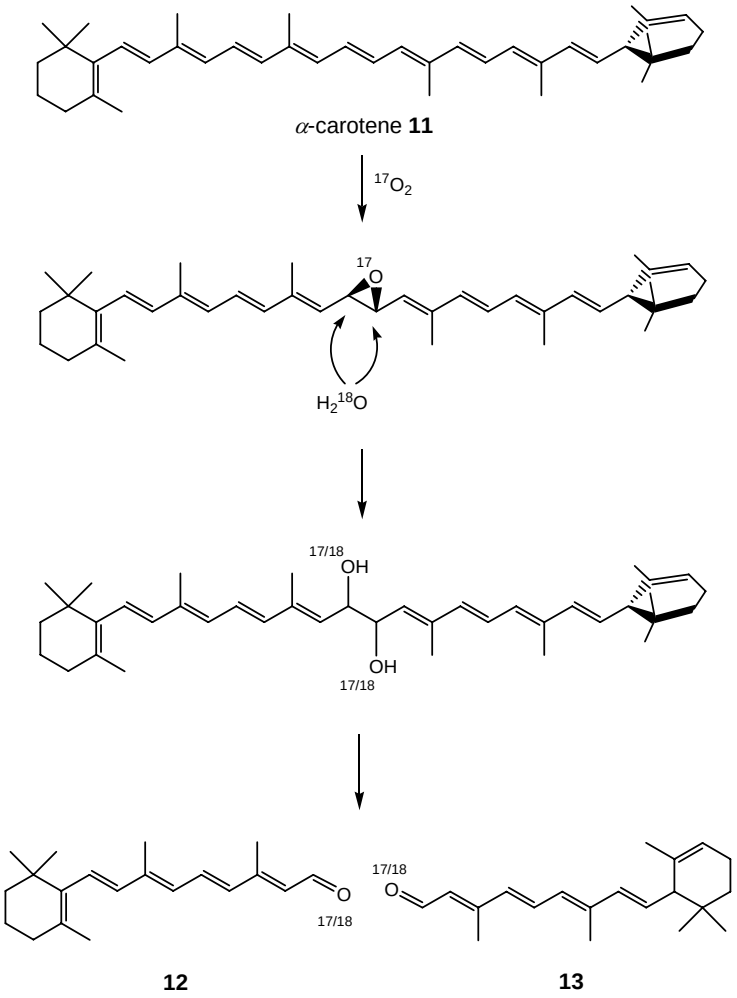
In 1989, Lackshman reported the production of retinal as the main product of a 45-60% acetone precipitation protein from the intestinal mucosa of rat and rabbit.[21] The postulated role of iron (II) in the catalysis was confirmed by increasing activity of the enzyme in the presence of 1mM $FeSO_4$. Traces of apo-β-carotenals were found in the same order of magnitude from incubation of β,β-carotene with the denaturated enzyme, excluding that these side products arose from an enzymatic reaction.

An interesting report appeared in 1994, including several aspects of the understanding of retinal production.[22] Cytosolic homogenate from guinea pig intestinal mucosa was found responsible for the production of retinal from β,β-carotene with a stoichiometric value between 1.5-2. Starting from [15,15'-^{14}C,^{3}H]-β,β-carotene, the same amount of ^{14}C and ^{3}H was detected in the metabolite, additionally supporting the central cleavage theory because an excentric cleavage, followed by β-oxidations, would result in the loss of the ^{3}H at C(15). Butylhydroxytoluene (BHT) was used in order to avoid oxidation of the cleavage products during extraction, and the procedure was shortened so that the products would not stay for a long time in solution.

The control of reaction stoichiometry, and perhaps definitive results about the central cleavage, were published in 1996.[23] Using cytosolic and membrane fractions of pork, Nagao and co-workers calculated a stoichiometric conversion of 1.88±0.08. By-products, originating from non-enzymatic reactions, were only found in trace quantities. The addition of formaldehyde, which could concur with retinal in the formation of Schiff-Base complex with protein, increased the rate of retinal isolated from 50% to 90%. The concentration of the biomolecule is important; working with 3.7mg of protein per ml (10 times higher than usual), the isolation of retinal decreased to 80%, despite the addition of formaldehyde. The ability to cleave 8'-apo-β-carotenal **9** was reported again, but only traces of 8'-apo-β-carotenal (< 2.1%) were cleaved to retinal.

In 2000, two research groups independently succeeded in cloning the key enzyme in vitamin A formation from chicken and the fruit fly *Drosophila melanogaster*.[24-26] For the first time, Woggon and Wirtz *et al*. established a purification protocol for the enzyme isolated from chicken intestinal mucosa, which led to the identification of the catalytically active protein.[24, 25] Sequencing and expression of the hexa-His-tagged protein in *Escherichia coli* and BHK (baby hamster kidney) cells gave, after affinity chromatography, a catalytically active, cytosolic enzyme (60.3 kDa), which cleaves β,β-carotene to retinal as the only product. Iron was identified as the only metal ion associated with the over-expressed protein in a 1:1 stoichiometry, and since a chromophore is absent in the protein, heme coordination and iron complexation by tyrosines can be excluded. Even without *X*-ray crystallography, it could be predicted that the active site contains a mononuclear iron complex, presumably consisting of histidine and carboxylic acid residues. Subsequently, the substrate specificity of the enzyme was investigated.[27] It was revealed that any deviation from the rodlike structure of β,β-carotene is not tolerated by the enzyme, implicating a rather rigid substrate binding pocket. *Via* hydrophobic interaction of amino acids with the methyl groups of β,β-carotene, the substrate is bound such that only the central double bond can be attacked. Substrates with only one functional group in either carotenoid end group are cleaved.

In 2001, a publication from the same group provided a strong evidence that oxidative cleavage at the central double bond is catalyzed in a monooxygenase mechanism *via* a transient carotene epoxide but not by a dioxygenase mechanism.[28] Incubation of α-carotene **11** with the enzyme in 85% $^{17}O_2$ and 95% $H_2{}^{18}O$ revealed equal enrichment of the ^{17}O- and ^{18}O-label in both derivatives of metabolites **12** and **13**. This result proves the incorporation of one ^{17}O atom of molecular oxygen and the concomitant incorporation of ^{18}O from labeled water (*scheme 4*). Experimental evidence accounts for a monooxygenase-type mechanism in which the first step is an epoxidation of the central double bond of **11**, followed by unselective ring opening by water and final diol cleavage to yield the aldehydes **12** and **13**. This sequence implies a high-valent non-heme oxoiron species as the reactive intermediate.



Scheme 4 – Enzymatic cleavage of α-carotene **11** in the presence of $^{17}O_2$ and $H_2{}^{18}O$[28]

Since the mechanism was suggested as monooxygenase process, a more appropriate term, BOC or BOC I (β,β-carotene-15,15'-monooxygenase), has been widely accepted instead of CDO. (for BOC II, please see next section)

Subsequently, BOC genes from mouse and human were identified and the recombinant proteins biochemically characterized in several laboratories.[29-32] Mammalian BOC catalyzed the cleavage of carotenoid substrates with at least one unsubstituted β-ionone ring, such as β,β-carotene and β-cryptoxanthin, and there was no observed cleavage of lycopene **1** and zeaxanthin **3**.[32] The K_m values for β,β-carotene were estimated to be in the range of 1-10μM.[26, 29, 30, 32] BOC exhibits a slightly alkaline pH optimum, and enzymatic activity is sensitive to chelating agents such as *o*-phenanthroline and α,α'-bipyridyl, indicating that it depends on ferrous iron. Thus, the purified recombinant

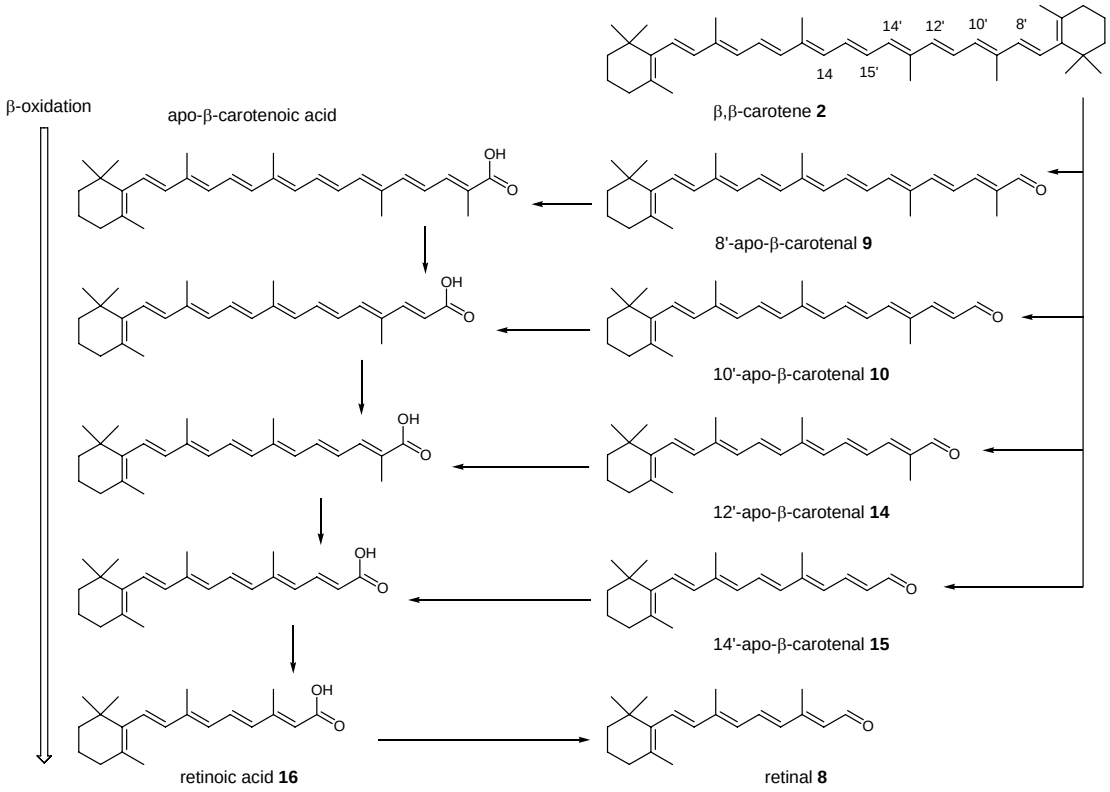
BOCs share biochemical properties which have already been described for the native BOCs.

1.1.3.2 Excentric cleavage

As early as 1960, Glover had proposed the excentric cleavage pathway of β,β-carotene as the most relevant biological mechanism for the production of vitamin A.[15] Comparison of the biological activity of retinal with β,β-carotene was a motive in supporting this theory. The pro-vitamin A activity of these two compounds was identical and it was therefore deduced that only one molecule of retinal is generated from each molecule of β,β-carotene, *i.e.*, the compound is cleaved excentrically. This observation constitutes the evidence for excentric cleavage followed by degradation of the product(s). However, today, this assumption must be rectified according to the bioavailability of carotenoids. The absorption of β,β-carotene and retinal in intestines do not proceed quantitatively and thus they can not be compared to each other. Also, the observed effect could be due to the formation of retinoic acid.

Glover compared the reactivity of the double bonds against chemical reagents. The central double bond was found to be less reactive, whereas, in contrast, the first double bond C(7)-C(8) of the polyene chain is easily cleaved. However, the relevance of this to an enzymatic reaction is questionable. The real support for this alternative metabolism would have been the *in vivo* detection of apo-β-carotenals, but this was never observed during the first investigation. Thus, it led to the conclusion that they are present in an active form, which is either water-soluble or tightly bound to the protein.

An excentric cleavage requires a second fragmentation reaction (or a series of shortenings). A formal β-oxidation was proposed by Glover. Analogous to fatty acid, the β-oxidation would cleave the double bond next to the acid function of an apo-β-carotenoic acid, derived from the oxidation of the corresponding aldehyde. This process should be stopped by the presence of the methyl group at C(13). (*scheme 5*)

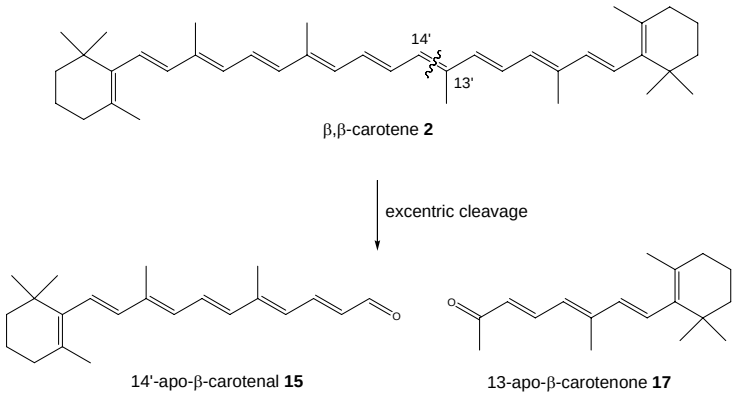


Scheme 5 – Possible metabolic pathways for β,β-carotene **2**

Direct evidence for the excentric pathway emerged from the *in vivo* isolation of apo-β-carotenals **9**, **10**, **14** from chicken intestine.[33] Sharma *et al*. suggested a double mechanism: i) central cleavage catalyzed by a cytosolic enzyme (BOC) and ii) excentric cleavage to apo-β-carotenals catalyzed by the membrane fraction. The total amount of apo-β-carotenals produced was less than detected for retinal. The difference was also explained by the dependence on cofactors. The presence of NAD^+ or $NADP^+$ did not influence the activity of BOC, on the other hand, the oxidative excentric cleavage was stimulated by these cofactors. Feeding rats with 8'-apo-β-carotenal **9** allowed the isolation of 8'-apo-, 10'-apo- and 12'-apo-β-carotenoic acid, thus supporting the theory of β-oxidation.

Within the last decade of the twentieth century, the most assiduous promoter of the excentric cleavage was the group of Krinsky.[34-38] Incubating β,β-carotene with homogenate from different tissues (liver, kidney, lungs and feet) of ape, human, ferret and rat, they detected a NAD^+-dependent production of apo-β-carotenals, retinal,

retinoic acid **16** and, for the first time, one shorter metabolite-13-apo-β-carotenone **17**.[34, 35] DTT (dithiothreitol) concentration strongly influenced the reaction, and SH-specific reagents like disulfiram could inhibit the cleavage. The studies were performed using a membrane fraction; pH optimum lying between 6.5 and 7 was definitively lower than that for the central cleavage. The β-oxidation degradation mechanism was suggested because of the time dependent decrease of 8'-apo-β-carotenal **9**, the metabolite that was the main product at the beginning of the reaction. The total production of apo-β-carotenals was 6-15 times higher than that of retinoids. The concomitant existence of 14'-apo-β-carotenal **15** and 13-apo-β-carotenone **17** (*scheme 6*) was the first direct evidence of an excentric cleavage.

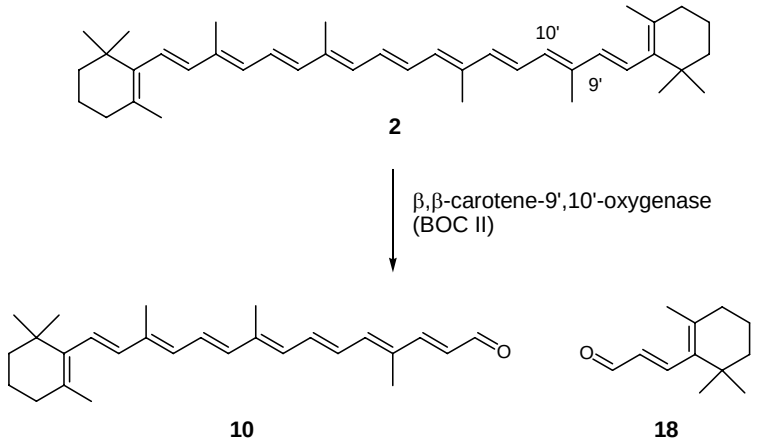


Scheme 6 – The excentric cleavage of β,β-carotene **2** at C(14')-C(13') double bond

Two further elegant publications validated the excentric pathway because retinoic acid was found in an incubation assay with β,β-carotene or apo-β-carotenoic acid as substrate, whereas the oxidation of retinal to the acid was inhibited by citral (an inhibitor of oxidation of retinal).[36, 38] The detection of retinoic acid among the metabolites means that the origin of these retinoids must be associated with an alternative metabolism for β,β-carotene, supporting the mechanism of the excentric cleavage followed by β-oxidations.

In 2001, direct evidence of excentric cleavage was reported by von Lintig and co-workers.[39] They identified a cDNA from mouse encoding a type of carotene oxygenase (532 amino acids) (β,β-carotene-9',10'-oxygenase, BOC II) which catalyzes exclusively the asymmetric oxidative cleavage of β,β-carotene at the C(9')-C(10')

double bond, resulting in the formation of 10'-apo-β-carotenal **10** and corresponding fragment-β-ionone **18** (*scheme 7*). Besides β,β-carotene, lycopene **1** is also oxidatively cleaved by this enzyme. The deduced amino acid sequence shares 39% sequence identity with the BOC I of mouse. Several highly conserved stretches of amino acids and six conserved histidines probably involved in binding the cofactor iron(II) were found, indicating that the encoded protein belongs to the same class of enzymes. Later, they also succeeded in cloning cDNAs encoding this new type of enzyme from human and zebrafish.

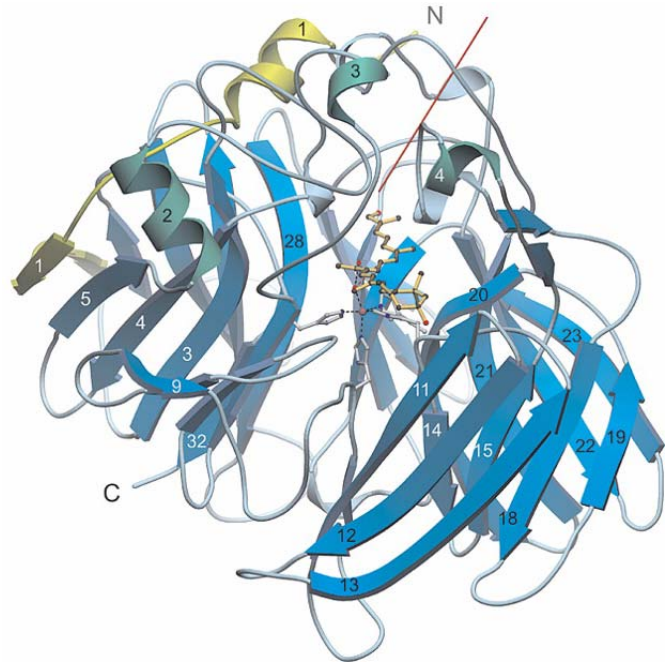


Scheme 7 – The excentric cleavage catalyzed by β,β-carotene-9'10'-oxygenase

In 2005, another striking result was reported by Al-Babili and co-workers.[40, 41] They characterized a novel carotenoid oxygenase (apocarotenoid-15,15'-oxygenase, ACO) from the cyanobacteria *Synechocystis* sp. PCC 6803.[40] Surprisingly, in contrast to the β-oxidation mechanism proposed by Glover, this enzyme could directly cleave apocarotenals to retinoids. Later, the crystal structure of enzyme was published.[41]

The ACO was expressed in *Escherichia coli* inclusion bodies and (re)natured with yields of ~ 2 mg purified enzyme per liter culture. It is activated upon addition of iron(II) ions. As shown in *figure 3A*, ACO consists of a seven-bladed β-propeller with four histidine residues at the propeller axis, holding the iron(II) ion and thus marking the active center. A tunnel, lined with numerous nonpolar residues, runs perpendicular to the propeller axis and extends to the active center (*figure 3B*).

A



B

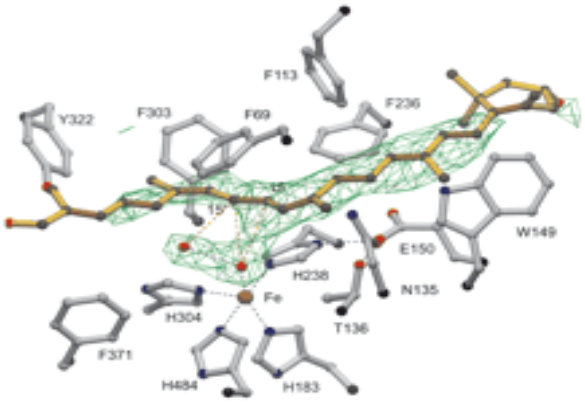
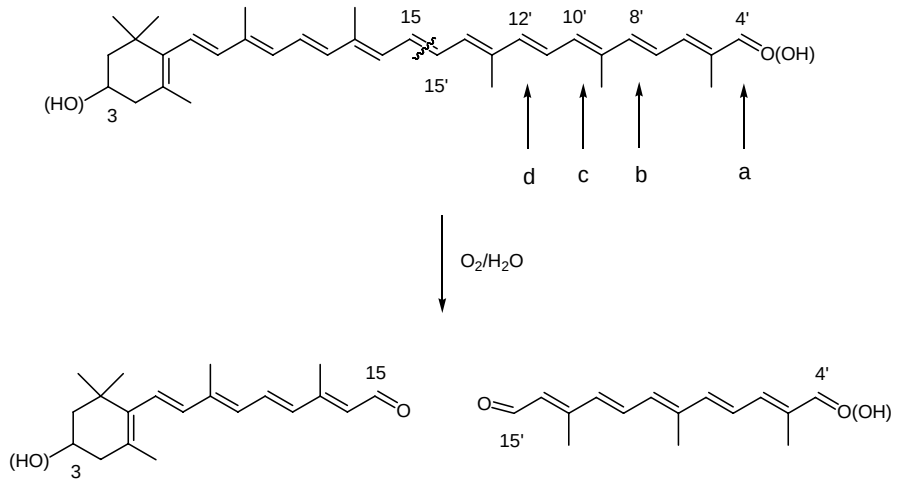


Figure 3 – Crystal structure of ACO (with substrate)[41]

Comparing the sequence of ACO with six other members of the carotenoid oxygenase family, the author presumed that the carotenoid oxygenase family shares a common chain fold and possess similar active center since the four active center histidines are strictly conserved and their environment is well conserved.

As shown in *scheme 8*, investigation of the substrate specificity of ACO revealed that only *all-trans* homologs *a*, *b*, *c* and *d* as alcohols or aldehydes with and without the 3-hydroxy group could be cleaved, consisting with the substrate fit of binding tunnel.



Scheme 8 – The enzymatic cleavage catalyzed by ACO

1.1.3.3 Summary

Following such intense research activity of the investigation of the metabolisms of carotenoids, finally, the debate has been settled as both pathways are demonstrated to be important in nature. Each pathway is used preferentially in mammalian β,β-carotene metabolism, depending on the specific tissue.

1.2 Supramolecular chemistry and enzyme mimics

1.2.1 What is supramolecular chemistry?

A precise definition of "*Supramolecular Chemistry*" is difficult to summarize. One of the foremost exponents of supramolecular chemistry, Jean-Marie Lehn, has described the term in the following manner:[42] *Supramolecular Chemistry can be defined as chemistry beyond the molecule, referring to the organised entities of higher complexity that result from the association of two or more chemical species held together by intermolecular forces.* However, this rather broad and loose description is nevertheless accurate.

Indeed, supramolecular chemistry does cover a broad area of scientific research and discovery. It is a highly interdisciplinary field, extending over organic chemistry, coordination chemistry, physical chemistry and the experimental/theoretical studies of interactions. Thus, supramolecular chemistry sits at the crossroads of physics, chemistry and biology, drawing on the physics of organised condensed matter and expanding, through "classical" chemistry, to the biological properties of very large molecular assemblies.

Lehn's decsription fails to give the appropriate weight to "*intermolecular forces"*. However, in a sense, it might be regarded as noncovalent interactions such as Coulomb forces, hydrophobic interactions and hydrogen bonding. These noncovalent interaction are the basis of many biological processes such as receptor-ligand binding, enzyme-substrate complex formation, antibody-hapten binding, and cell surface recognition. Accordingly, supramolecular chemistry has provided, and is likely to continue to provide, insight into these phenomena through the investigation of artificial synthesizable molecular assemblies.

1.2.2 Application of supramolecular systems to enzyme mimics

Enzymes and receptors are supramolecular systems by their very definition. It is therefore easy to conceive mimicking their behaviour with smaller, designed entities which incorporate the features (binding site and reactivity) necessary for the activity of the native enzyme. Such molecules are the targets of many supramolecular chemists and are often described as artificial enzymes or more accurately as enzyme mimics.

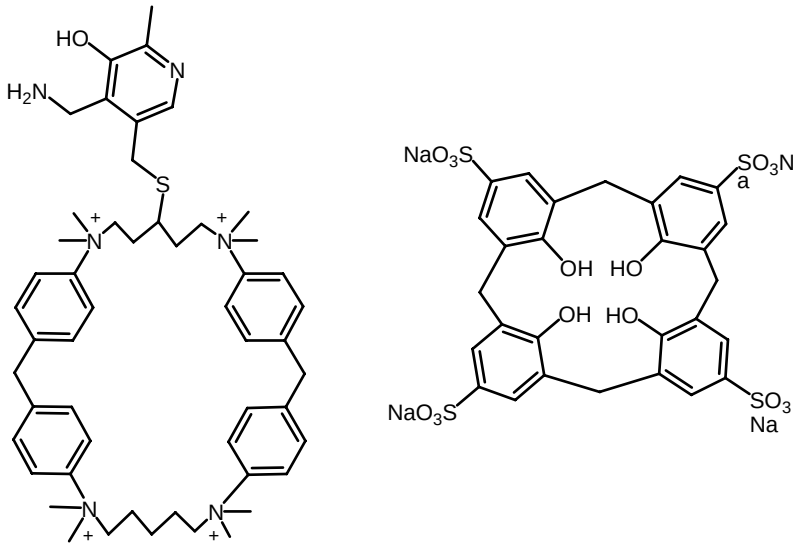


Figure 4 – A cyclophane based transaminase model (*left*)[43] and a calixarene based ribonuclease model (*right*)[44].

The first property that a potential enzyme mimic must have, is a binding domain. In a native enzyme this is provided by the tertiary structure of the protein, which generates a specific microenvironment for substrate binding and subsequent catalysis in aqueous media. To incorporate this property into a synthetic system, one needs to look towards macrocyclic compounds which exhibit guest-inclusion capability, such as cyclodextrins, cyclophanes and calixarenes. Among those, cyclodextrins seem to be the most important candidate for the following reasons.

i) They are seminatural products, produced from a renewable natural material, starch, by a relatively simple enzymatic conversion.

ii) They are produced in thousands of tons per year by environmentally friendly technologies. As a result, the prices have dropped to levels where they become acceptable for most industrial purposes.

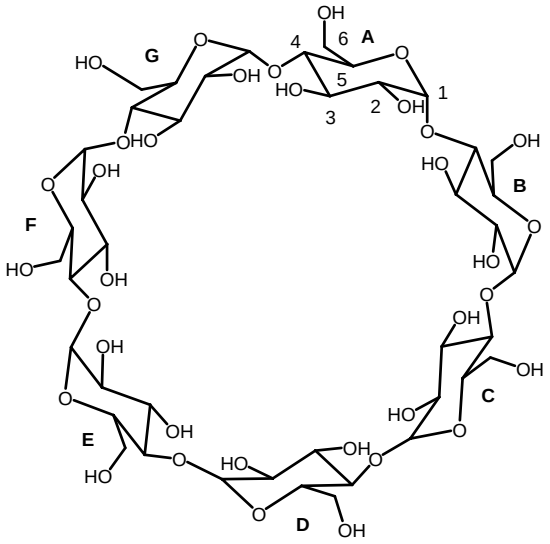
iii) Through their inclusion complex forming ability, important properties of the complexed substances can be modified significantly. This unprecedented "molecular encapsulation" is widely utilized in many industrial products, technologies, and analytical methods.

iv) Any toxic effect is of secondary character and can be eliminated by selecting the appropriate cyclodextrin type, derivative or mode of application, so they can be consumed by humans as ingredients of drugs, foods, or cosmetics.

1.2.3 The cyclodextrins

1.2.3.1 Chemical structures

The cyclodextrins (CDs) are a family of cyclic oligosaccharides comprising α-1,4-linked glucopyranose units. The family is made up of three major and well-known CDs, α–, β–, and γ-CD, containing six, seven and eight glucose subunits respectively (example as *figure 5*), and of rare minor cyclic oligosaccharides which contain up to fourteen such sugar residues.



β-cyclodextrin **19**

Figure 5 – The structure of β-cyclodextrin (β-CD) **19**. The glucose residues are labelled as A – G.

As a consequence of the $^4C_1$ conformation of the glucopyranose units, all secondary hydroxy groups are situated on one of the two edges of the ring, the so called secondary face. The seven primary hydroxy groups thus form the opposite primary face. The openings at the two faces are slightly different in size: the secondary opening is the larger. Thus the ring is more accurately a conical cylinder, which is frequently characterised as a doughnut or wreath shaped truncated cone. The cavity is lined by the C(3)-, C(5)-hydrogen atoms and the glucosidic oxygen bridges, lending it hydrophobic character in contrast to the hydrophilic nature of the outer surface. The nonbonding electron pairs of the glycosidic oxygen bridges are directed towards the inside of the cavity, producing a high electron density and lending to it some *Lewis* base character. The characteristic structural features and the dimensions of β-CD **19** are shown in

schematic form in *figure 6*. This schematic representation of **19** will be used to denote the full cyclodextrin structure.

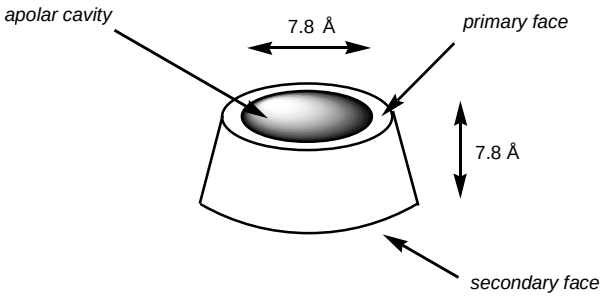


Figure 6 – Functional scheme of β-CD torus

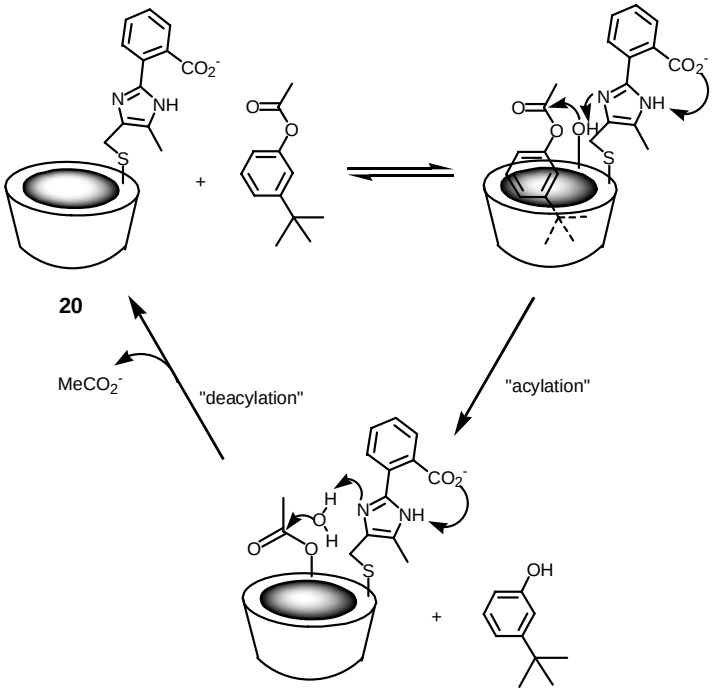
The C(2)-hydroxy group of one glucopyranoside unit can form a hydrogen bond with the C(3)-hydroxy group of the adjacent glucopyranose unit as the proton donor, lending C(2)-hydroxy group the most acidic (pKa ~ 12). In the β-CD molecule, a complete secondary belt is formed by these hydrogen bonds, so that β-CD is a rather ridig structure.

1.2.3.2 Cyclodextrin based enzyme mimics

CDs have been widely employed as the substrate-recognition moieties in enzyme models, mainly due to i) their high water solubility; ii) their ability to accommodate a wide variety of guest molecules; iii) their well defined molecular structure iv) the regular arrangement of the hydroxyl groups around the rim of the cavity allowing a variety of functional/catalytic residues to be introduced. All of these properties have made the molecular design of CD-based enzyme mimics successful and fruitful. For recent comprehensive reviews of this field see refs.[45-47]

Attachment of a simple catalytic group to a CD can afford interesting enzyme mimics. For instance, a system which has been mimicked by CDs is that of chymotrypsin. At the active site of serine proteases, carboxylate (aspartate), imidazole (histidine) and hydroxy (serine) residues form a triad charge relay system. It was proposed that a proton relay from the serine to histidine is accompanied by proton transfer from the histidine to aspartate, which is the key to the enormous catalytic activities of these enzymes. As a mimic of this charge relay system, the effect of 2-benzimidazoleacectic

acid on the α-CD catalyzed hydrolysis of *m*-tert-butylphenyl acetate was examined.[48] A 10 fold acceleration, ascribable to cooperation of the carboxylate, imidazole, and hydroxy residues of α-CD, was observed. Later, an even better mimic for the charge relay system, appended CD-derivative **20**, was reported (*scheme 9*).[49, 50] Both carboxylate and imidazole were attached to the secondary face of α-, β-, and γ-CDs. The kinetic parameters reported for ester hydrolysis by the β-CD based enzyme mimic are comparable with the values of chymotrypsin. The specificities are dependent on the nature of CDs. α- and β-CD based models are better than the γ-CD based system in phenyl ester hydrolysis, whereas the γ-CD system hydrolyses tryptophane ethyl ester faster than the other two. In the same way as chymotrypsin, the enzyme mimics show presteady-state acylation and steady-state deacylation and turnover.



Scheme 9 - Mechanism of ester hydrolysis by a chymotrypsin mimic (a modified CD involving the charge relay system of hydroxyl, imidazolyl, and carboxylate residues).[49, 50]

Alternatively, the serine-histidine-aspartate catalytic triad has been introduced as a tripeptide (Ser-His-Asp) to the primary face of β-CD.[51]

The models mentioned above, as in the enzyme they mimic, require the substrate to be bound before reaction and subsequently released. However, CDs bind typical substrates

in water with binding constants of ca. 10^4 M^{-1} or less (a recent exception is lithocholic acid, whose binding constant to β-CD exceeds 10^6 M^{-1}).[52] This binding is not as strong as that of most enzymes, which typically bind several substrate segments to give cooperative binding effects. Thus it is of interest to make artificial enzymes that use two or more CDs to bind substrates well. It would be expected that a substrate that binds to both CD cavities could have a binding constant exceeding 10^8 M^{-1}. Simple additivity of the binding free energies would lead to 10^8 M^{-1}, while the entropy advantage of the chelate effect should lead to an even larger binding constant than that.

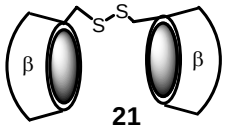
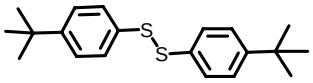
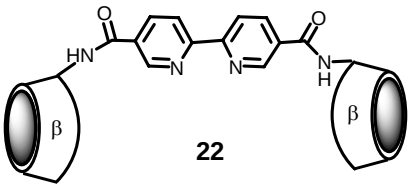
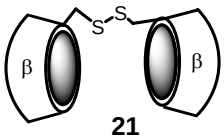
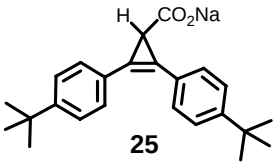
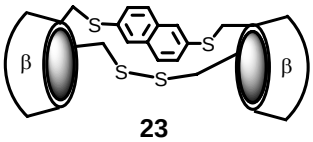
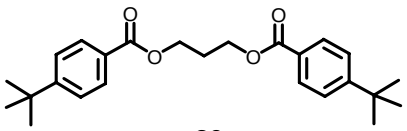
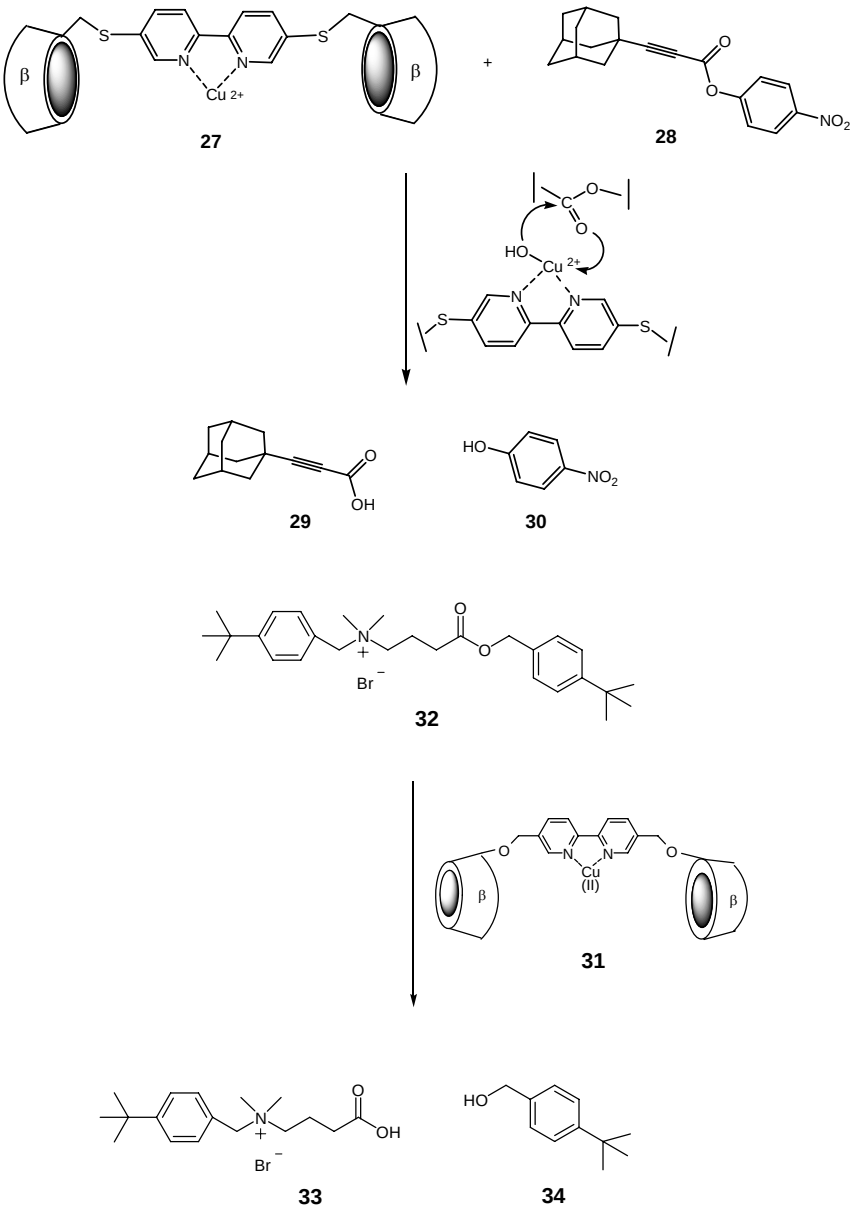
Host	Guest	Binding constant, K_a
 **21**	 **24**	1×10^6 M^{-1}
 **22**	tetra-aryl porphyrin	5×10^7 M^{-1}
 **21**	 **25**	3.5×10^8 M^{-1}
 **23**	 **26**	1×10^{10} M^{-1}

Table 1

It has been shown by Breslow *et al.*,[53] and more recently by Nolte *et al.*[54] that substrates bearing two hydrophobic segments can be bound very tightly by dimeric CD systems. It has also been shown that guests displaying the correct size and geometry can be bound with binding constants in the range of 10^6 - 10^8 M^{-1} with single linked

CD dimers (**21** and **22** in *table 1*) and up to 10^{10} M^{-1} with double linked systems such as **23** (*table 1*).[55] Heterodimers have also been made linking two different CDs[56, 57] and even CD tetramers.[58, 59]

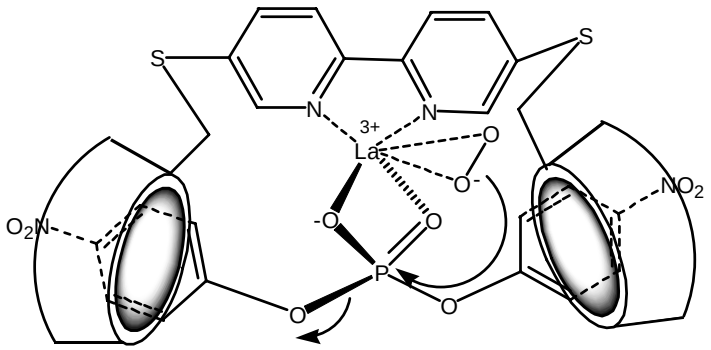
If a CD dimer has a catalytic group in the linker, one might observe strong catalysis in complexes where a substrate functional group is held directly above the catalytic linker group. An early example of this, reported by Breslow *et al.*[60, 61] is the esterase model **27** which contains a 2,2'-bipyridyl subunit as a chelation centre (*scheme 10*).



Scheme 10 – Esterase function of a metallo-CD-dimer.[60-62]

Substrate **28** was hydrolysed with at least 50 turnovers in water at pH 7, the products, acid **29** and nitrophenol **30**, being detected by gas chromatography. The catalyst **27** gives an acceleration of 2×10^5 fold over uncatalysed hydrolysis, but is not effective for unactivated alkyl esters. This challenge was overcome by an Cu(II) complex of a β-CD dimer **31** linked on the secondary faces, leading to 1000-fold acceleration for substrate **32**.[62]

The Cu(II) ion was later replaced by La(III) to provide a catalyst, **27**-La(III), for the cleavage of phosphate esters in the presence of H_2O_2 (*figure 7*).[63]



27-La (III) + substrate

Figure 7 – H_2O_2 mediated cleavage of phosphate esters by a La^{3+} modified CD-dimer.[63]

Breslow first mentioned the possibility of including two known catalytic residues, namely metallosalens and metalloporphyrins, into CD-dimers as early as 1994,[64] however, no results were published and this was purely a claim to the field. In 1996, the first publication regarding β-CD containing porphyrin and salen linkers appeared.[59] Accordingly, porphyrin linked β-CD derivatives **35**, **36** and **37**, and salen linked β-CD-dimer **38** were synthesized (*figure 8*).

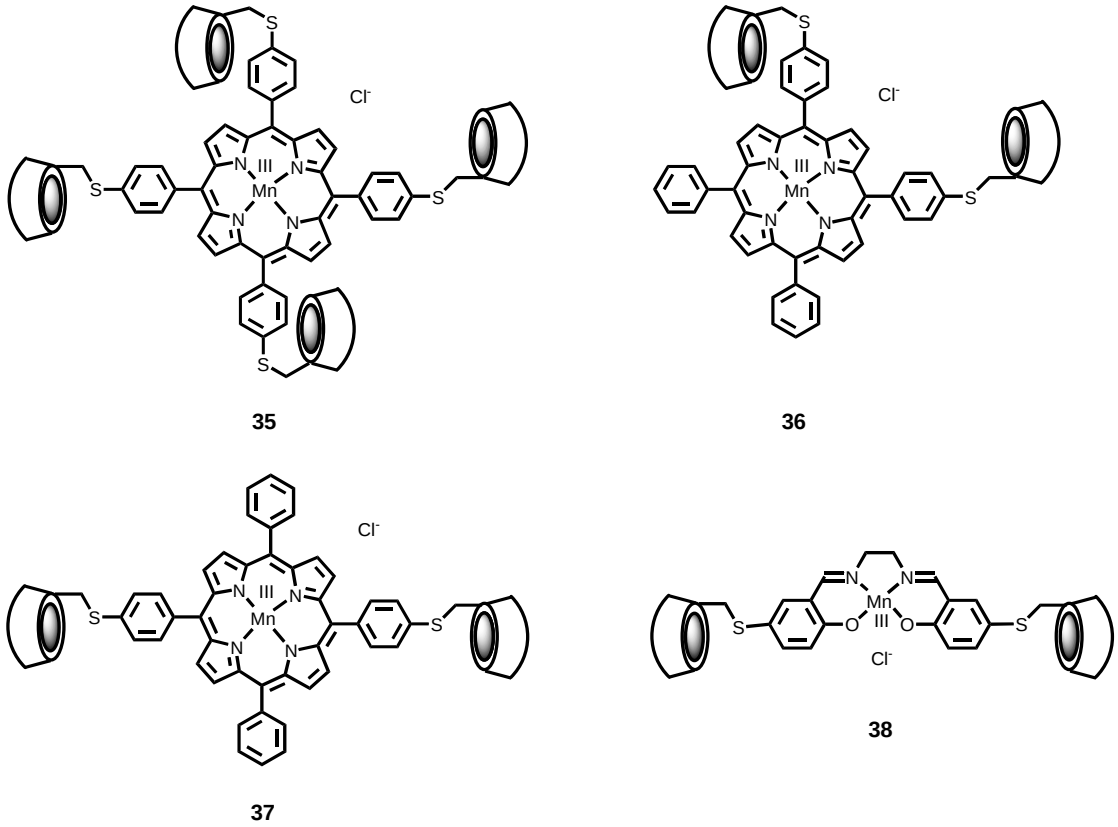
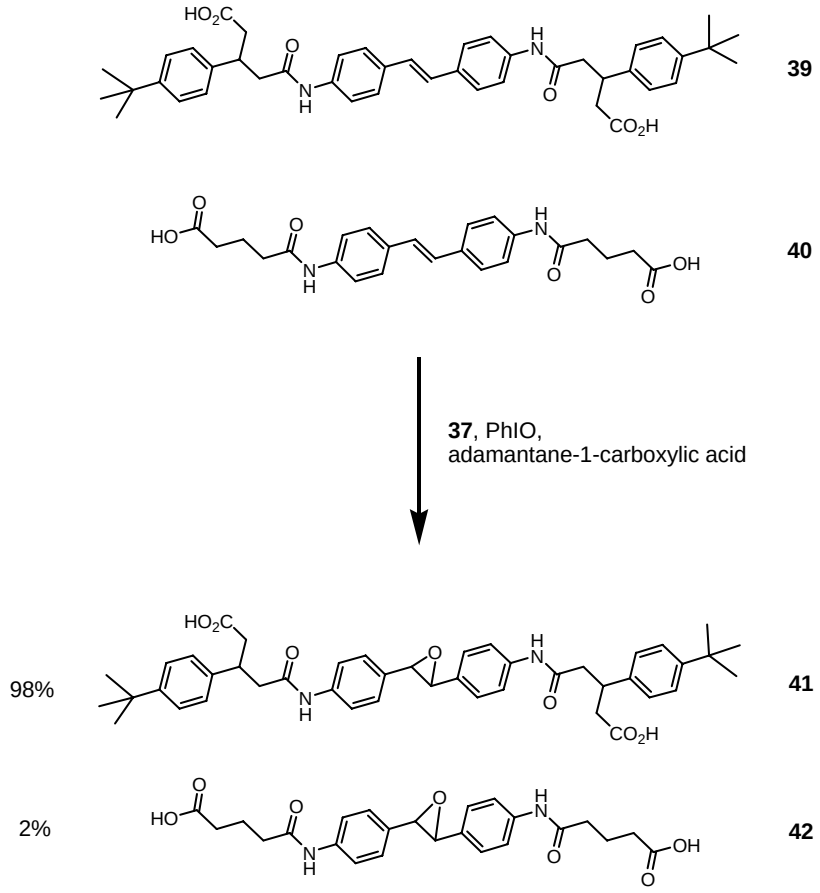


Figure 8 – The structures of β-CD dimers or tetramers containing porphyrin and salen linkers[59]

The porphyrin containing derivatives were used to selectively epoxidize stilbene derivative **39** in competition experiments with "worse fitting" stilbene **40** (*scheme 11*). Adamantane-1-carboxylic acid is used as a face protection agent. When the substrate is bound to the catalyst on one face of the porphyrin, there is the possibility that the oxo group goes to the other face and performs unselective epoxidation. The adamantane carboxylate could coordinate to that face and prevent such non-selective oxidation. It is large enough not to be tolerated on the same face as bound substrate, whereas experiments with acetate ion showed no such face selectivity and reduced the selectivity of the reaction.

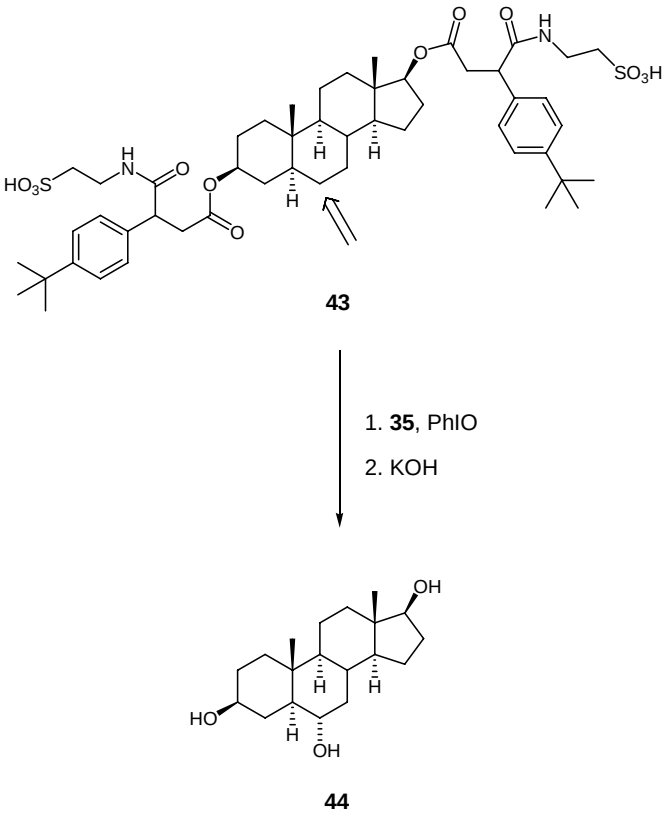


Scheme 11 – Competition epoxidation with β-CD-dimer **37**, selectivity was a factor of 50 higher for **39** over **40**.[59]

The epoxidations with **35** and **37** were run with up to 40 turnovers, although it was reported that the reaction slowed down after some time. This was due to product inhibition, such that the reaction product, epoxide **41**, can still bind with an appreciably high binding constant to the receptor. It can of course be expected if that the endgroups, as the major recognition sites of the substrate, have not been altered during the reaction and can thus show the same cooperative binding effects when present in epoxide **41**.

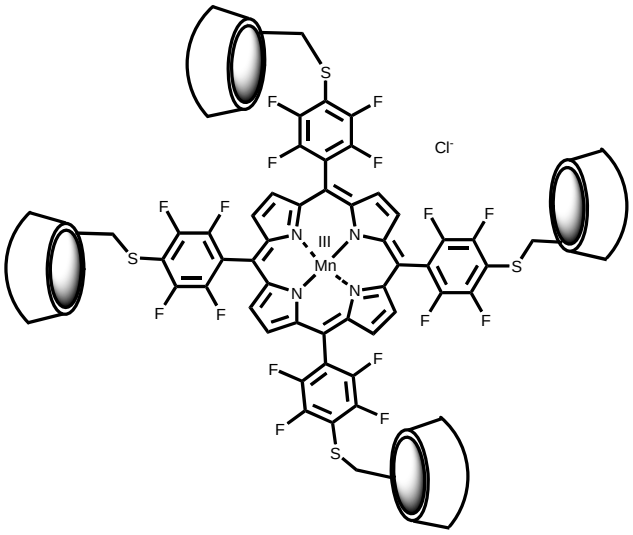
A further development in the regiospecific catalytic capabilities of porphyrin linked CD systems was the selective catalytic hydroxylation of a steroid derivative by a cytochrome P-450 enzyme mimic.[65-67] Thus, CD-tetramer **35** (10 mol%) was incubated with derivatised steroid **43** in water for 2h with PhIO as the oxygen source (*scheme 12*). 40% conversion of **43** was observed after ester hydrolysis and quantification of the triol **44**. The regioselectivity of the reaction was shown to be

complete. The reaction was also stereospecific, yielding only the equatorial C(6)-alcohol. The catalyst was capable of only 4 turnovers before being oxidatively destroyed.



Scheme 12 – Regioselective hydroxylation of steroid by a cytochrome P-450 mimic.[65, 66]

The system was later improved by increasing the stability and reactivity of the catalyst through introduction of fluorine to the free aromatic positions of the porphyrin linker, β-CD-tetramer **45** (*figure 9*).[67, 68] In contrast to 4 turnovers from catalyst **35**, **45** was used in 1 mol% and gave 100% conversion of **43** to **44** (95 turnovers). However, reaction with 0.1 mol% gave only 18.7% of product but with 187 turnovers, indicating that at the high conversion case there might be some inhibition by product binding to the catalyst.



45

Figure 9 – Improved P-450 mimic[68]

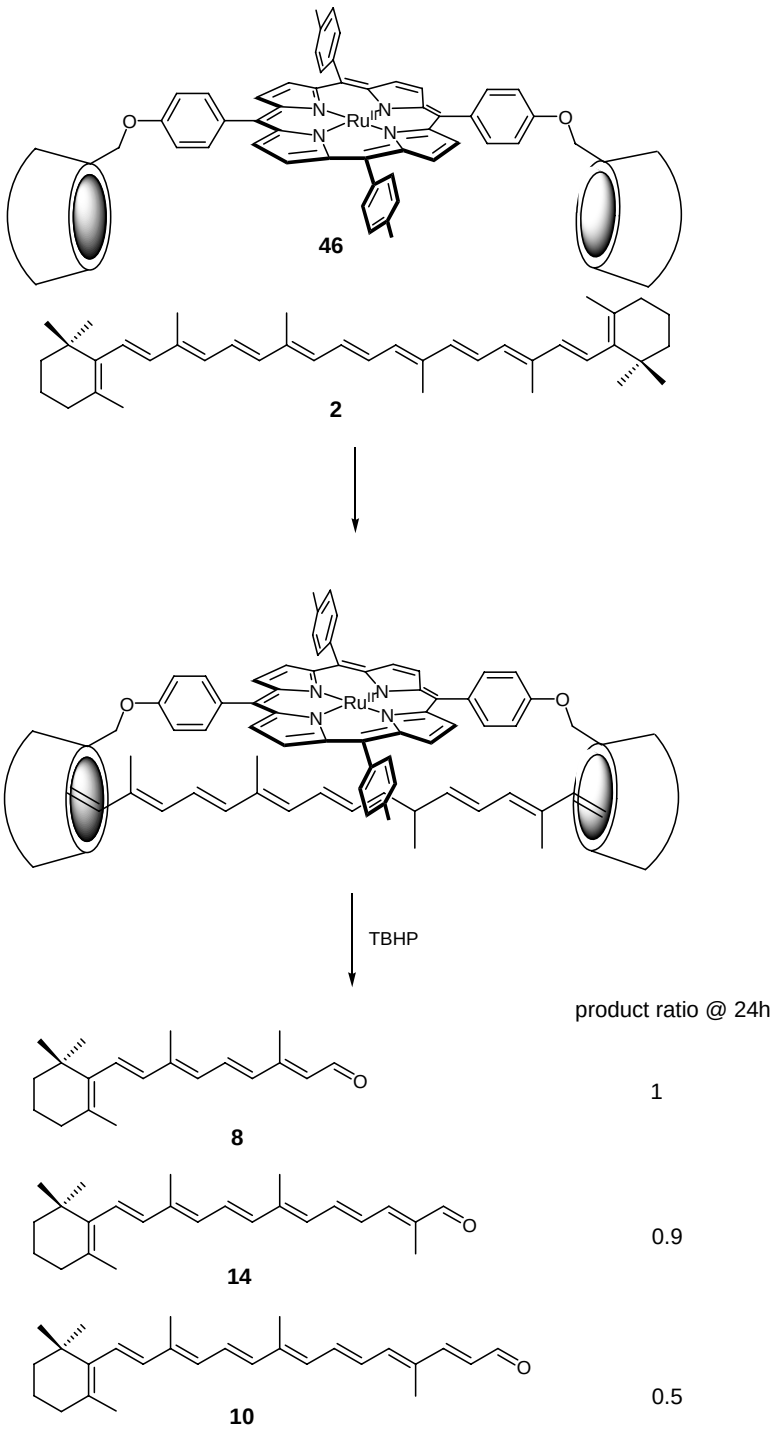
1.2.4 β-CD based enzyme mimic of β,β-carotene-15,15'-moooxygenase: the essential contributions from "Woggon group"[69-76]

Supramolecular systems, and especially CD based enzyme mimics, have been used to catalyse a range of reactions with good selectivity. The hydrophobic pocket of CDs is a good "artificial receptor" moiety, and has been shown in CD dimer/tetramer systems to be useful in the cooperative binding and subsequent geometric control of specific substrates. However, few of the current works on these more complicated enzyme mimics have been devoted to the conversion of natural substrates to biologically important metabolites.

As previously mentioned, Woggon *et al*. have been able to purify the enzyme, which is able to centrally cleave β,β-carotene, from chicken's intestinal mucosa. Further investigation showed that, in contrast to earlier belief, this enzyme is not a dioxygenase but operates by a monooxygenase mechanism in which the first step is an epoxidation of the central C(15)-C(15') double bond.

Parallel to efforts to purify and investigate the native protein, for the first time, they have developed a supramolecular system **46** which mimics the enzymatic cleavage of carotenoids (*scheme 13*).[69-73, 75, 76] This enzyme mimic consists of two β-CD moieties linked with a ruthenium porphyrin on the primary face. Each of the β-CDs is shown to be capable of binding one of the cyclohexenoid endgroups of β,β-carotene, leaving the porphyrin to span the polyene chain. Based on the *MOLOC* program calculation, it was estimated that approximately half of β,β-carotene would be included in the β-CD cavities and that the C(15)-C(15') double bond would be perfectly placed under a corresponding oxo-ruthenium complex.

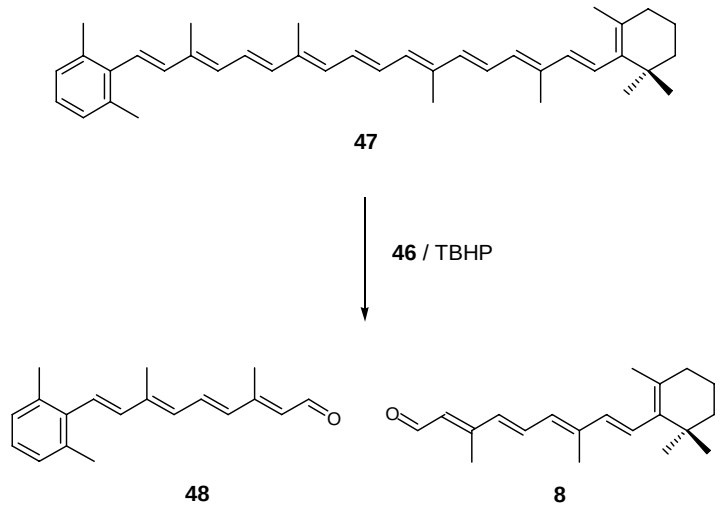
To apply such system to catalyse the cleavage of β,β-carotene, a biphasic system was established, in which β,β-carotene was extracted from a 9:1 mixture of hexane/ chloroform into the water phase containing the catalyst **46** and cooxidant *tert.*-butyl hydroperoxide (TBHP). The reaction products, released from the catalyst, were then extracted back to the organic phase, aliquots of which were subjected to HPLC measure without workup. It was reasonable that this kind of biphasic system could efficiently avoid the product-inhibition effect.



Scheme 13 – The proposal binding mode of **46** and **2**, and the cleavage result with TBHP as cooxidant in biphasic reaction system.[69-73, 75]

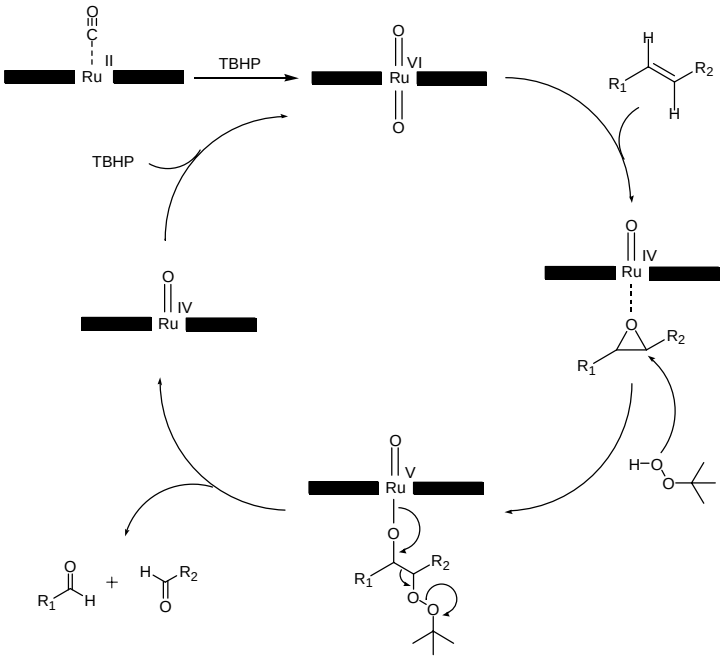
The results from cleavage catalyzed by **46**/TBHP showed that β,β-carotene is not only cleaved at the central double bond (~40%) but also at excentric double bonds to give 12'-apo-β-carotenal **14** and 10'-apo-β-carotenal **10**. It was suggested that lateral movement of β,β-carotene within the hydrophobic cavity of β-CD is responsible for the

unselective cleavage. Using 17-nor-ϕ,β-carotene **47** as substrate, the cleavage was indeed very regiospecific since only retinal and the corresponding fragment **48** were detected. (*scheme 14*) This suggested that the stronger hydrophobic interaction between the aromatic endgroup of **47** and β-CD cavity was responsible for stabilizing the 1:1 inclusion complex, leaving the central double bond under the reactive ruthenium center in contrast to β,β-carotene.



Scheme 14 – Selectively central cleavage of 17-nor-ϕ,β-carotene **47**[69-71, 73, 75]

It should be noted that the first step in the cleavage of the double bond is epoxide formation catalyzed by the active oxo-ruthenium porphyrin species, which is followed by ruthenium porphyrin/TBHP mediated fragmentation yielding the aldehydes as shown in *scheme 15*.[74, 76]



Scheme 15 - Catalytic cycle with TBHP as cooxidant[74, 76]

2 Aim of the project

To date, the debate between central or excentric cleavage of β,β-carotene should be settled as both pathways are important in nature. Each pathway is used preferentially in mammalian β, β-carotene metabolism, depending on the specific tissue.

However, the enzymes catalyzing the excentric cleavage of β,β-carotene and their mechanism of action have not been unambiguously characterised. As β,β-carotene-15,15'-monooxygenase, these enzymes also present a challenge to the synthetic chemist in the ability to attack a single double bond in a polyene substrate. The goal of this work was to prepare supramolecular enzyme mimics which are capable of binding β,β-carotene **2** and can selectively cleave the excentric double bonds to yield apocarotenals.

3 Results and Discussion

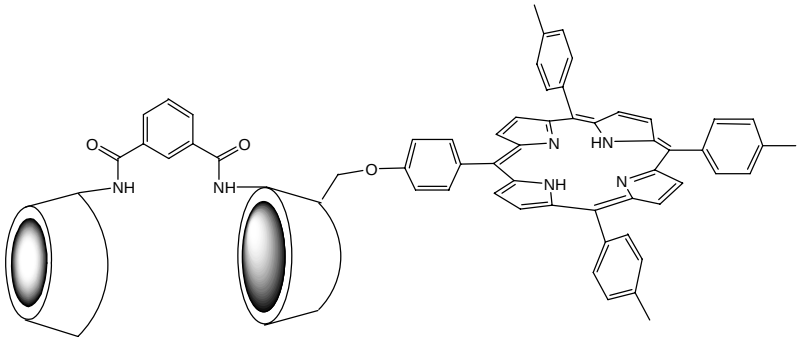
3.1 A rigidly linked Ru(II)-porphyrin-*bis*-β-CD complex 58, the first enzyme mimic for regioselective cleavage of carotenoids

3.1.1 Design of the Ru(II)-porphyrin-*bis*-β-CD complex 58

As mentioned before, a Ru(II)-porphyrin-*bis*-β-CD complex **46** has been developed in our group to mimic the enzymatic central cleavage of carotenoid.[69-72] In this structure, β-CD moieties act as hydrophobic hosts to bind two cyclohexenoid endgroups of carotenoid, leaving porphyrin to span the polyene chain. With TBHP as cooxidant, the central C(15)-C(15') double bond, which in a static view sits under the active ruthenium center, could be selectively cleaved. Encouraged by this good result, we wanted to construct a new supramolecular system to mimic the excentric cleavage of carotenoid.

Based on the binding mode of **46**/β,β-carotene **2**, for mimicking the excentric cleavage, it was easy to conceive that the ruthenium-porphyrin moiety could be retained as active catalytic center, whereas the binding mode of β,β-carotene to supramolecular host should be reconstructed. To achieve the excentric cleavage, one could envisage that an extended hydrophobic cavity, inside which β,β-carotene could be bound more deeply, would drive the excentric double bonds under the active ruthenium center of the porphyrin (*figure 10*).

A receptor model **49** has been suggested by *MOLOC* calculation to be an excellent candidate for binding β,β-carotene. It was shown that β,β-carotene could be bound into the longer cavity produced by a dimeric β-CD moiety which was linked on secondary face with a rigid 1,3-diamidephenyl spacer, leaving the porphyrin moiety to span the polyene chain over excentric double bonds (*figure 10*).[74]



49

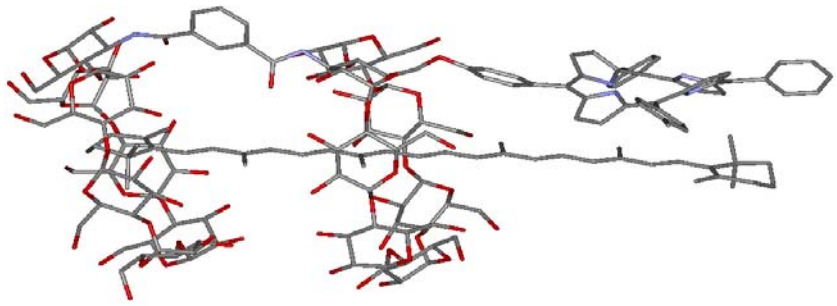
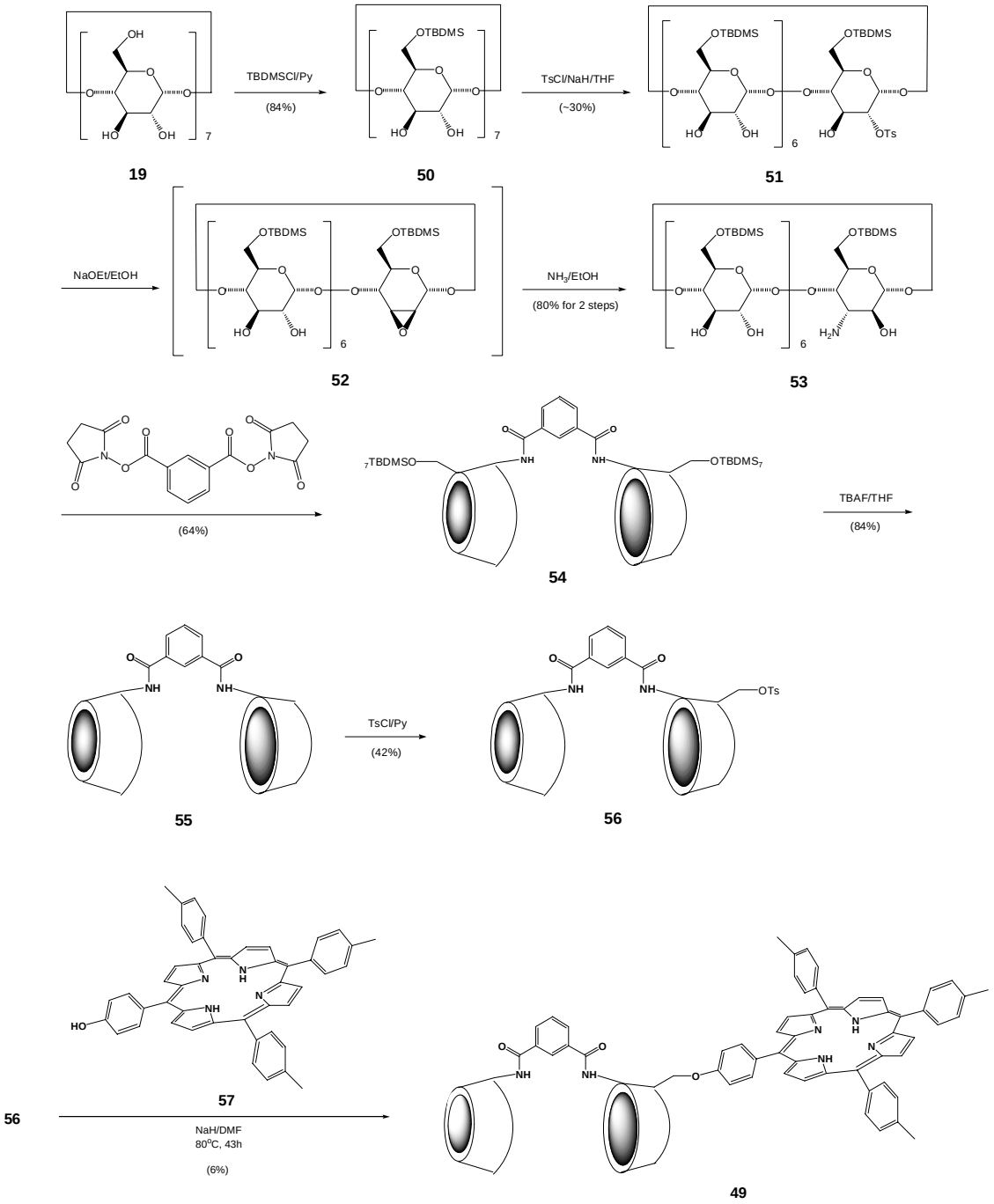


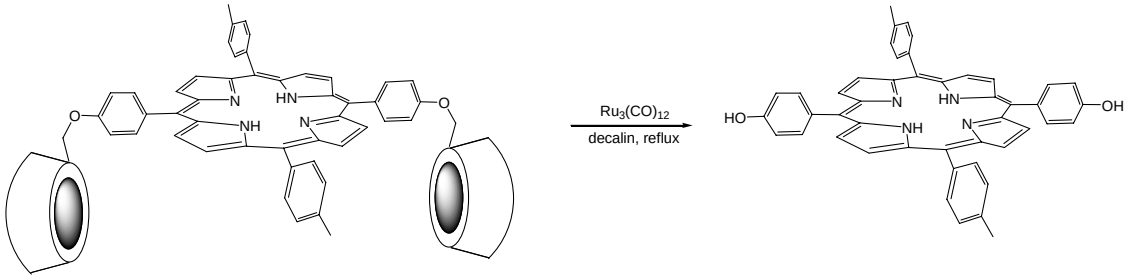
Figure 10 – Computer generated binding mode of **49**/β,β-carotene **2**

The synthesis of **49** was carried out by P. Holzer as part of his Ph. D. thesis (*scheme 16*).[74] Treatment of dry β-CD **19** with *tert.*-butyldimethylsilyl chloride (TBDMSCl) in dry pyridine furnished TBDMS-β-CD **50**. After mono-tosylation at C(2)-hydroxy group of **50**, the resulting β-CD **51** was refluxed in the solution of NaOEt in ethanol to provide the corresponding epoxide **52** as intermediate. Without work-up, the solution of **52** in ethanol was saturated with ammonia, and then refluxed to provide 3-deoxy-mono-amino-β-CD **53** in 80% yield calculated from **51**. Coupling of **53** with isophthalic di(*N*-hydroxysuccinimide)ester in refluxing THF furnished β-CD dimer **54** in 64% yield, followed by deprotection with TBAF. Mono-tosylation of **55** was carried out with equal equivalent of *p*-TsCl in pyridine to provide 6-mono-Ts-β-CD dimer **56** in 42% yield after purification by preparative HPLC. It should be noted that **56** was a mixture of isomers resulted from unselective mono-tosylation of primary hydroxyl groups. Further separation of isomers was difficult. It was postulated that the binding models of β,β-carotene towards isomers were not much different, so that the mixture of **56** was directly used for the next step without isolation. Treatment of porphyrin **57** with NaH and 2 equivalents of **56** in DMF furnished **49** in 6% isolated yield.



Scheme 16 – Synthesis of supramolecular receptor **49**

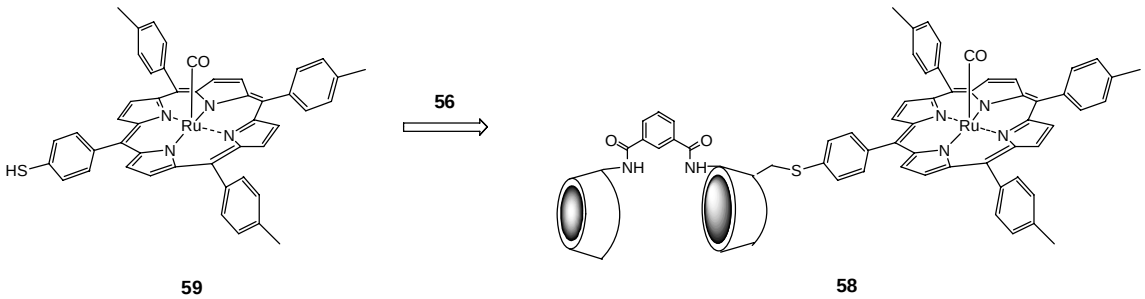
With the receptor in hand, it was logical to directly introduce ruthenium into **49** to obtain the corresponding ruthenium-porphyrin complex. However, in a similar case as shown in *scheme 17*, it was found that the β-CD moiety was degraded, *via* a ruthenium mediated decomposition, under the standard reaction condition required for insertion of ruthenium (excess ruthenium dodecacarbonyl, 190°C, 4h).[69]



Scheme 17 – Degradation of porphyrin-CD complex under standard ruthenium insertion reaction

Alternatively, one could conceive that the synthesis of the corresponding ruthenium complex of **49** may be achieved by coupling of **56** with Ru(II)-**57**. However, owing to the poor reactivity of **57**, this protocol was unreliable. Yield of coupling of **56** with **57** is only 6%. After introduction of ruthenium into **57**, NMR of the resulting Ru(II)-**57** showed that protons of phenol ring shifted to low field, suggesting its nucleophilicity would be less than that of **57**. According to this finding, it could be predicted that the yield of coupling reaction would be lower than 6% in the presence of ruthenium. Furthermore, since the preparation of **56** was difficult (4.6% overall yield from β-CD with 6-step sequence), use of a large excess of **56** to increase the yield of coupling was unaffordable.

In view of these problems, complex **58** has to be synthesized by using porphyrin **59** as precursor since thiophenol is a good nucleophile (*scheme 18*).



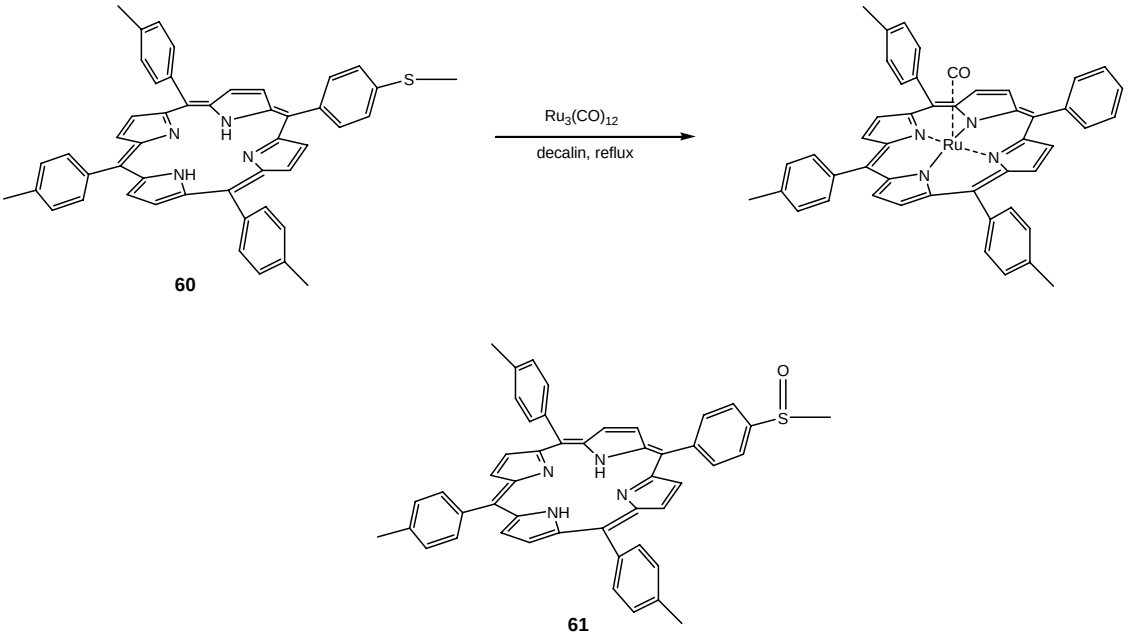
Scheme 18 – Synthesis of complex **58** from porphyrin **59**

3.1.2 Synthesis of Ru(II)-porphyrin-*bis*-β-CD complex 58

Since β-CD dimer **56** has been already prepared by P. Holzer (*scheme 16*), the crucial synthetic task of this project is the synthesis of Ru(II)-porphyrin **59** and the sequent coupling to obtain complex **58**.

3.1.2.1 The first attempt for synthesis of Ru(II)-porphyrin 59 *via Pummerer rearrangement*

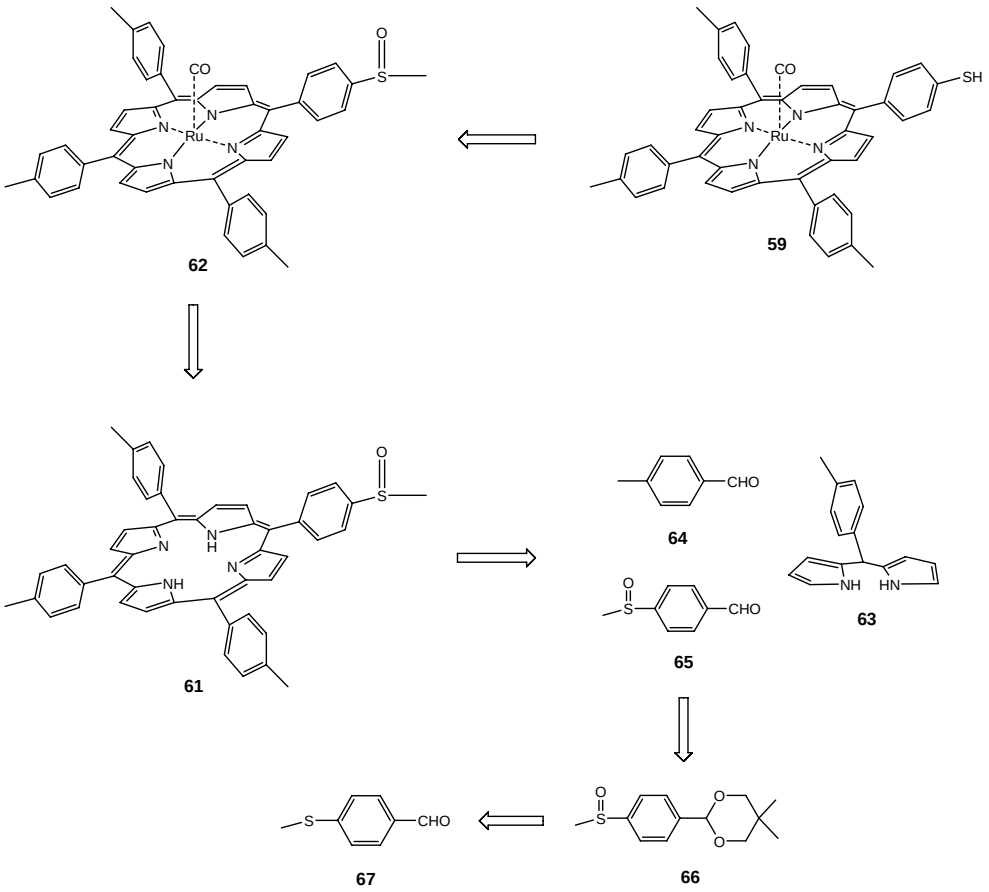
A previous investigation revealed that 5-methylthiophenyl-10,15,20-tri(methylphenyl)-porphyrin **60** was extremely labile under the standard condition for ruthenium insertion (*scheme 19*). The problem might arise from the binding of sulfur to ruthenium and consequent degradation.



Scheme 19

It was envisaged that the corresponding methylsulfoxy porphyrin **61** might be less likely to bind to ruthenium, and thus might survive under the harsh reaction condition. In addition, to the best of our knowledge, Pummerer rearrangement[77-79] is efficient for converting methylsulfoxy precursors to the corresponding thiol compounds under mild reaction conditions. Thus, porphyrin **61** was chosen as the precursor of **59**.

3.1.2.1.1 Retrosynthetic analysis



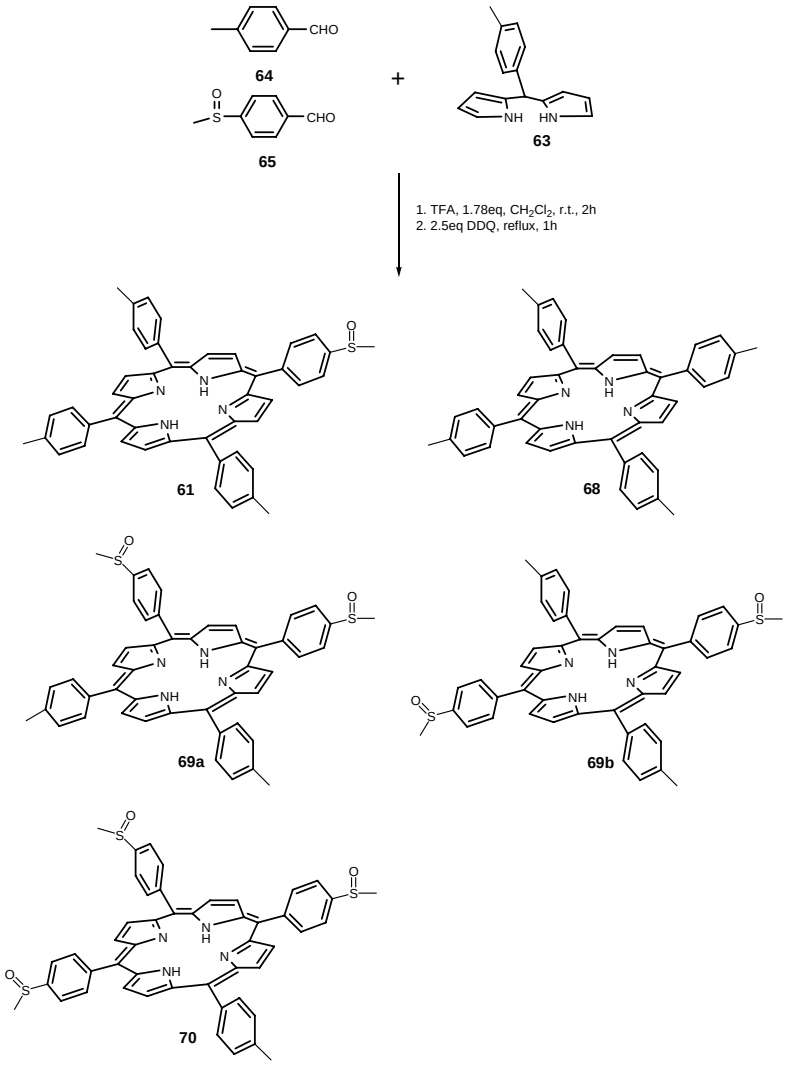
Scheme 20 – Retrosynthetic analysis of **59** with **61** as intermediate (***Pummerer rearrangement***)

As illustrated in *scheme 20*, **59** could be generated from porphyrin **62** *via* Pummerer rearrangement. In principle, **62** could be accessible from free base porphyrin **61** by insertion of ruthenium. The latter porphyrin **61** could be prepared from *p*-tolyldipyrromethane **63**, *p*-methylbenzaldehyde **64** and *p*-(methylsulfoxy)benzaldehyde **65** which could be accessible from *p*-(methylthio)benzaldehyde **67** by simple functional group manipulations.[80-82]

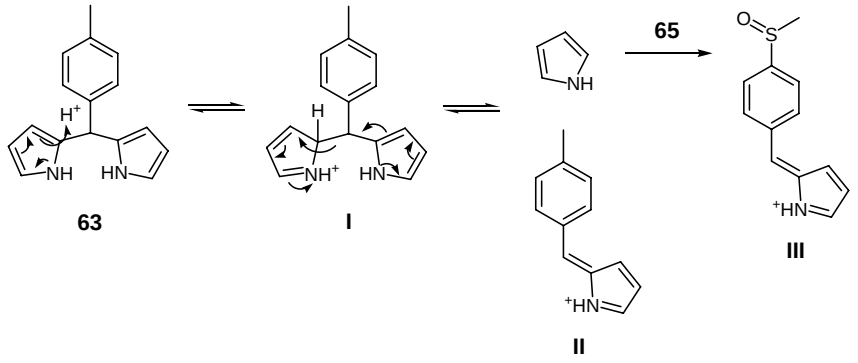
3.1.2.1.2 Synthesis

The synthesis of the porphyrin **61** was achieved by acid catalyzed condensation of *p*-tolyldipyrromethane **63**[83, 84] with *p*-methylbenzaldehyde **64** and *p*-(methylsulfoxy)-benzaldehyde **65**. Thus, treatment of a solution of **63**, **64** and **65** in CH_2Cl_2 with trifluoroacetic acid (TFA) at room temperature, followed by oxidation of the crude

porphyrinogen products with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ), afforded a mixture of porphyrins **61**, **68-70** (*scheme 21*).[85] This mixture was the result of competing acid catalyzed "scrambling" which occurs during the condensation. The proposed mechanism of this reaction is shown in *scheme 22*.[86] The reaction allows the production, from **63** *via* **I**, of rogue intermediate **III** which can then substitute for species **II** to produce a mixture of porphyrinic products. Column chromatography of the mixture afforded pure porphyrin **61** in 17% yield.

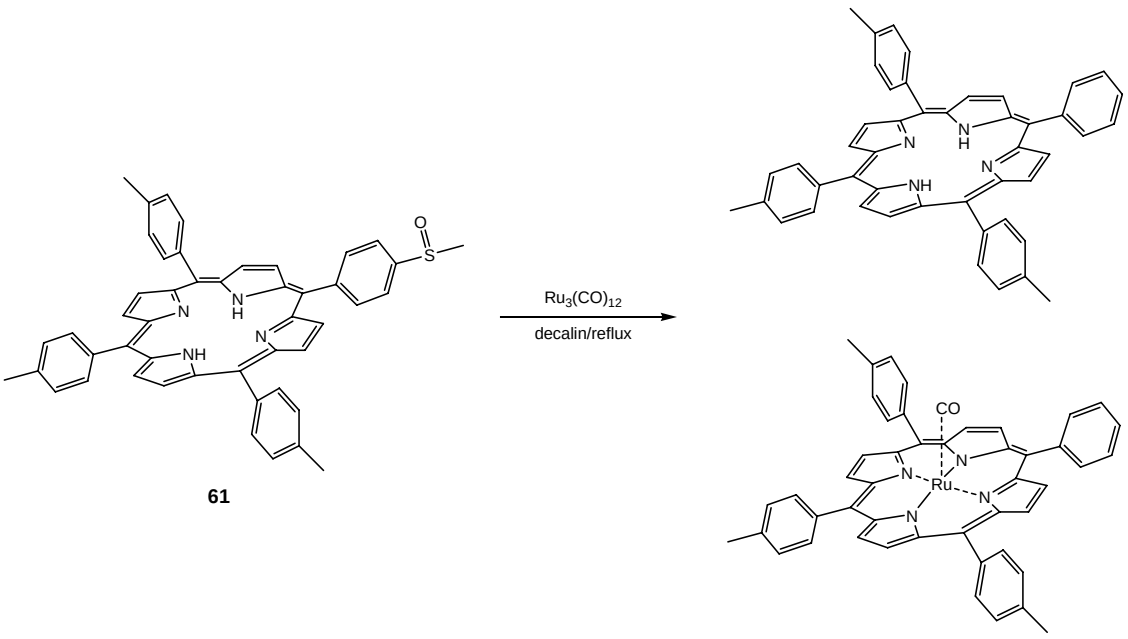


Scheme 21 - Porphyrin condensation reaction



Scheme 22 - Mechanism of the scrambling reaction which leads to porphyrin mixture

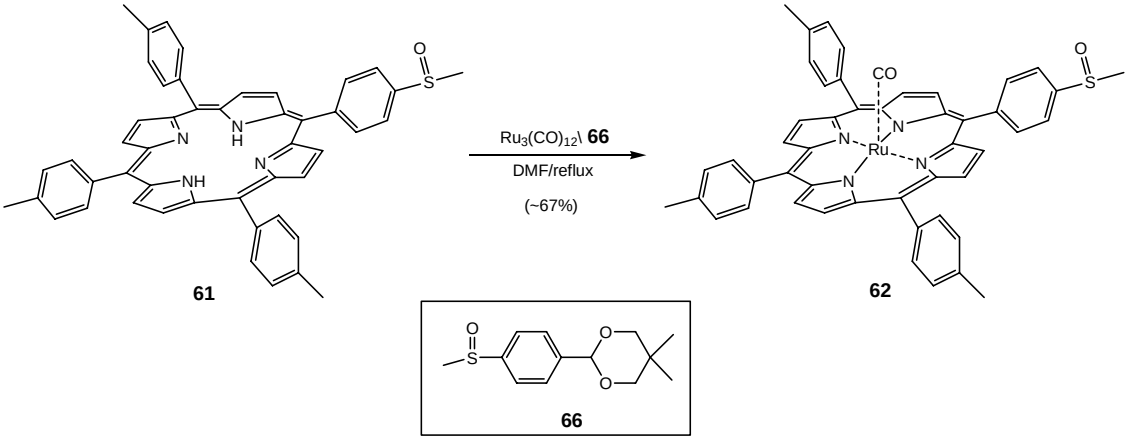
Attempt to introduce ruthenium into **61** under the standard condition was unsuccessful, leading to a mixture of porphyrins without methylsulfinyl group (*scheme 23*).



Scheme 23 – Attempt to insert ruthenium to **61** under standard condition

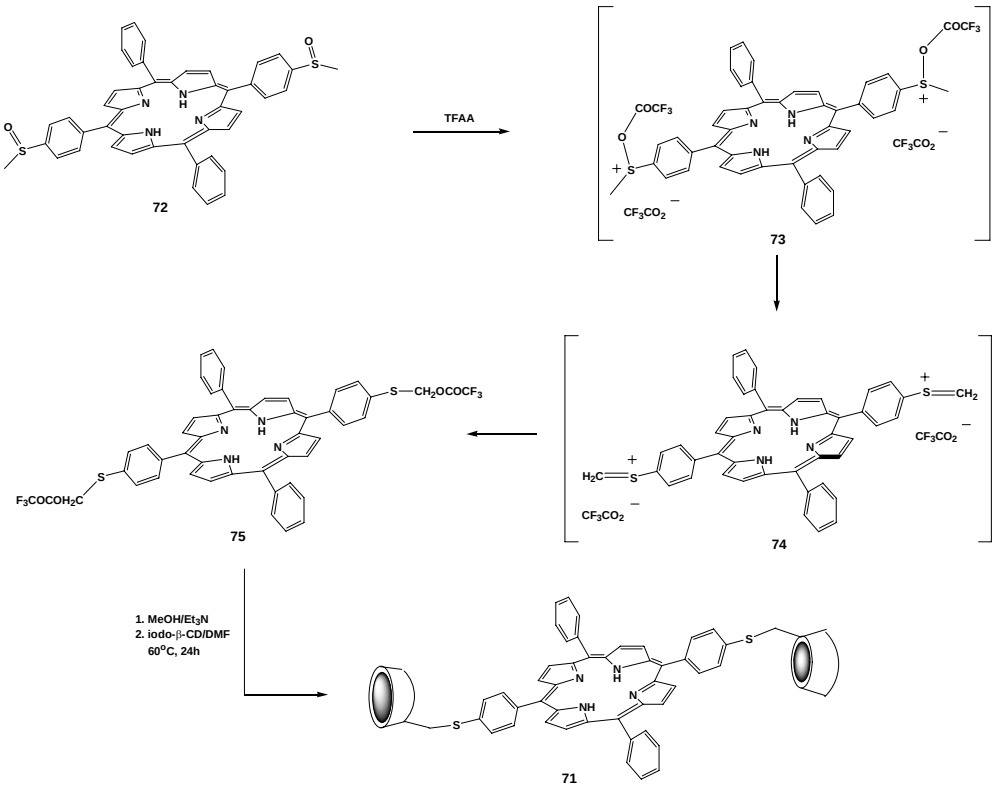
Further investigations on the reaction conditions revealed that the same result was obtained even in refluxing toluene. In contrast, porphyrin **61** survived without reaction at 90^{o}C. It was thus postulated that this undesired result could be due to the same reason suggested in the case of porphyrin **60** at high temperature.

At this stage, it was reasonably expected that the addition of another ligand of ruthenium in the reaction system would block the coordination of methylsulfoxy group and thus inhibit the ruthenium mediated degradation. To achieve this strategy, the ligand should possess appropriate binding constant to ruthenium, leading to a competitive inhibition. On the other hand, it should be easily removed by simple work-up. After a number of attempts, compound **66** was found to be an ideal scavenger. Using 12 equivalents of **66** in refluxing DMF furnished **62** in 67% isolated yield (*scheme 24*).



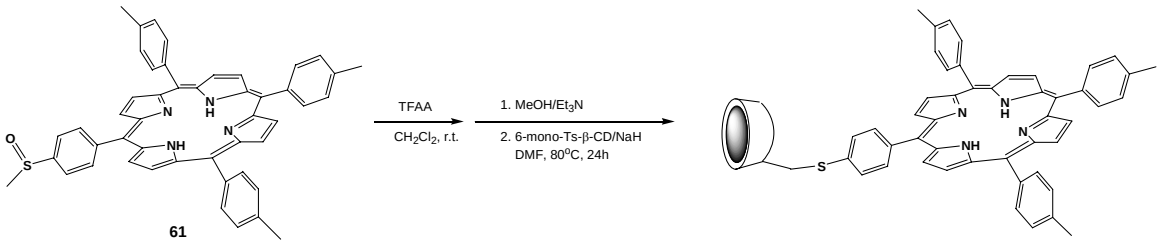
Scheme 24 – Sucessful insertion of ruthenium to porphyrin **61**

Once the precursor **62** was obtained, it was now required to convert to Ru(II)-porphyrin complex **59**. Breslow reported a similar procedure to produce a porphyrin-CD complex **71** *via* Pummerer rearrangement.[59] As illustrated in literature (*scheme 25*), treatment of porphyrin **72** with trifluoroacetic anhydride (TFAA) in CH_2Cl_2 provided a porphyrin dithioacetal acetate **75**, which was labile and used immediately for the next step. The corresponding *bis*-(*p*-thiophenyl)porphyrin could be generated from **75** *in situ* with MeOH/Et_3N, which was then directly coupled with 6-deoxy-mono-iodo-β-CD to furnish complex **71**.



Scheme 25 - Synthesis of complex **71** *via* Pummerer rearrangement[59]

Despite a number of attempts, **62** could not be converted directly to **59** or by means of "*in situ*" to **58**. Exposure of **62** to TFAA only led to decomposition judged by TLC and ESI-MS. In contrast, the free base porphyrin **61** could provide the corresponding coupling product verified by ESI-MS. (*scheme 26*)

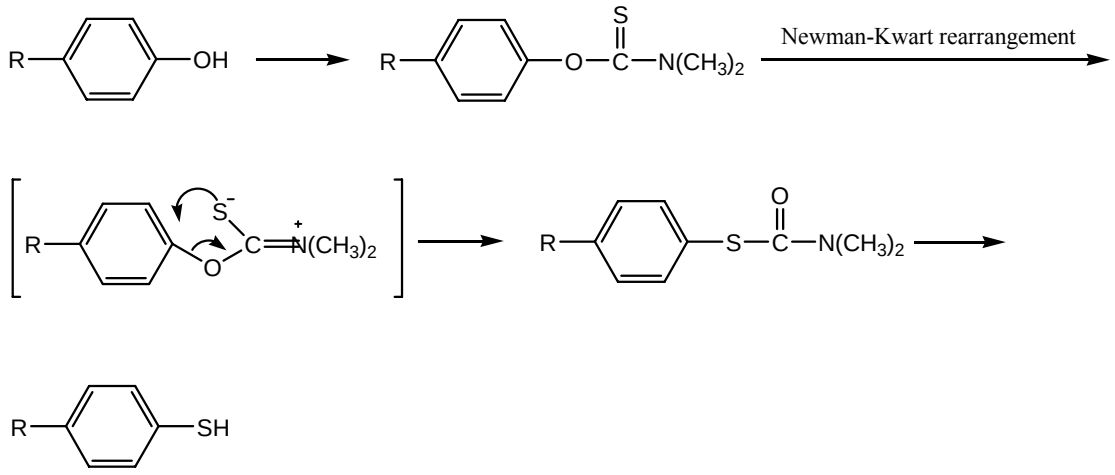


Scheme 26 – The conversion of free base porphyrin **61** to β-CD complex *via* procedure reported by Breslow.[59]

3.1.2.2 The second attempt for synthesis of Ru(II)-porphyrin 59 *via Newman-Kwart rearrangement*

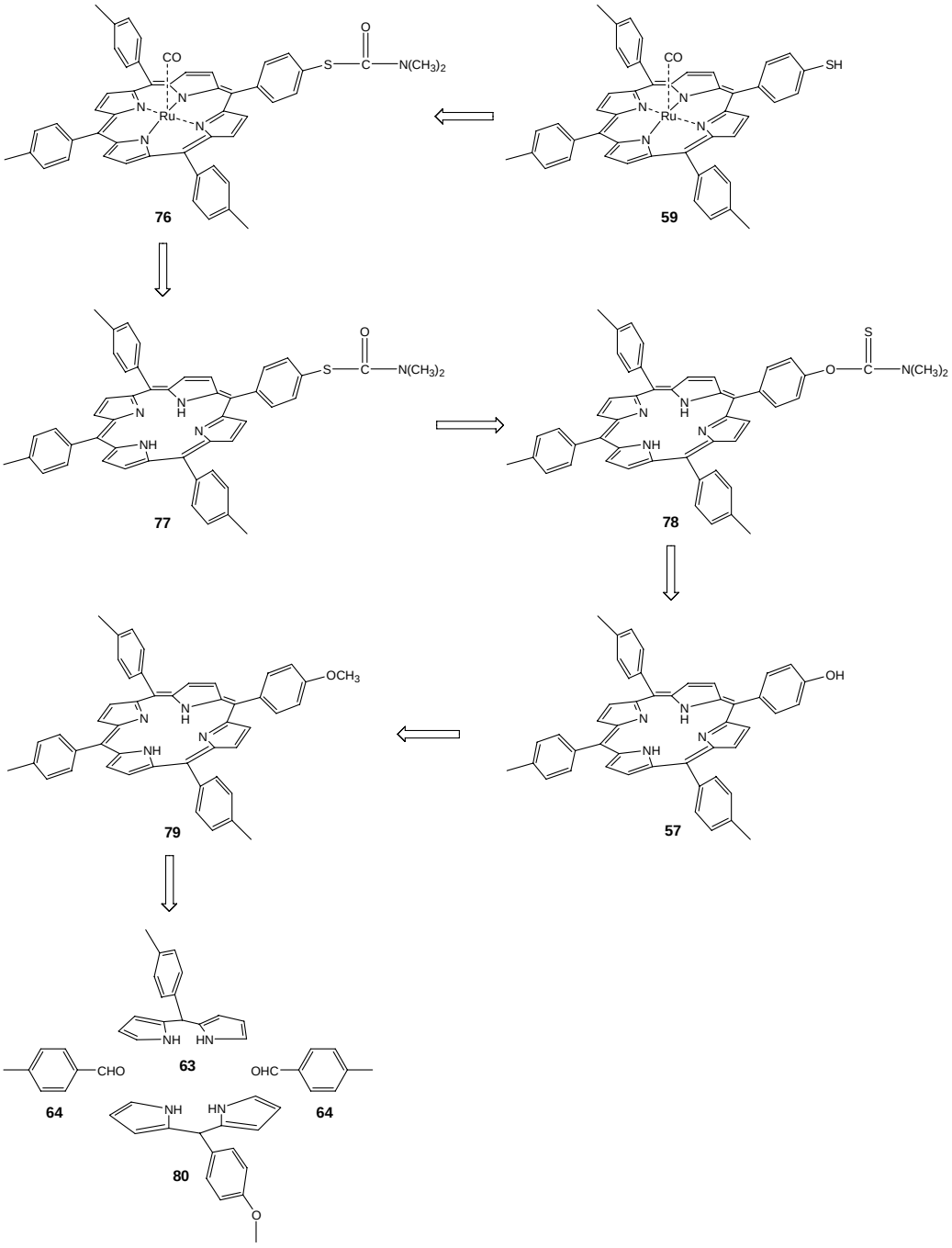
3.1.2.2.1 Retrosynthetic analysis

Because of the failure of the first strategy, the attention was turned to phenol substituted porphyrin **57**. It was known that Newman-Kwart rearrangement could efficiently convert phenol to thiophenol.[87] Thus, phenols are readily converted into the corresponding *O*-aryl dialkylthiocarbamates in high yield by treatment with dialkylthiocarbamyl chloride, which is then pyrolyzed to *S*-aryl dialkylcarbamates under "neat" reaction condition or in solvents with high boiling points. It was proposed that the pyrolysis involved intramolecular nucleophilic attack of the sulfur at the carbon holding the oxygen. The desired polarization is assisted by the dialkylamino group (*scheme 27*). In turn, *S*-aryl dialkylcarbamates could be hydrolysed to the corresponding aryl mercaptans easily.



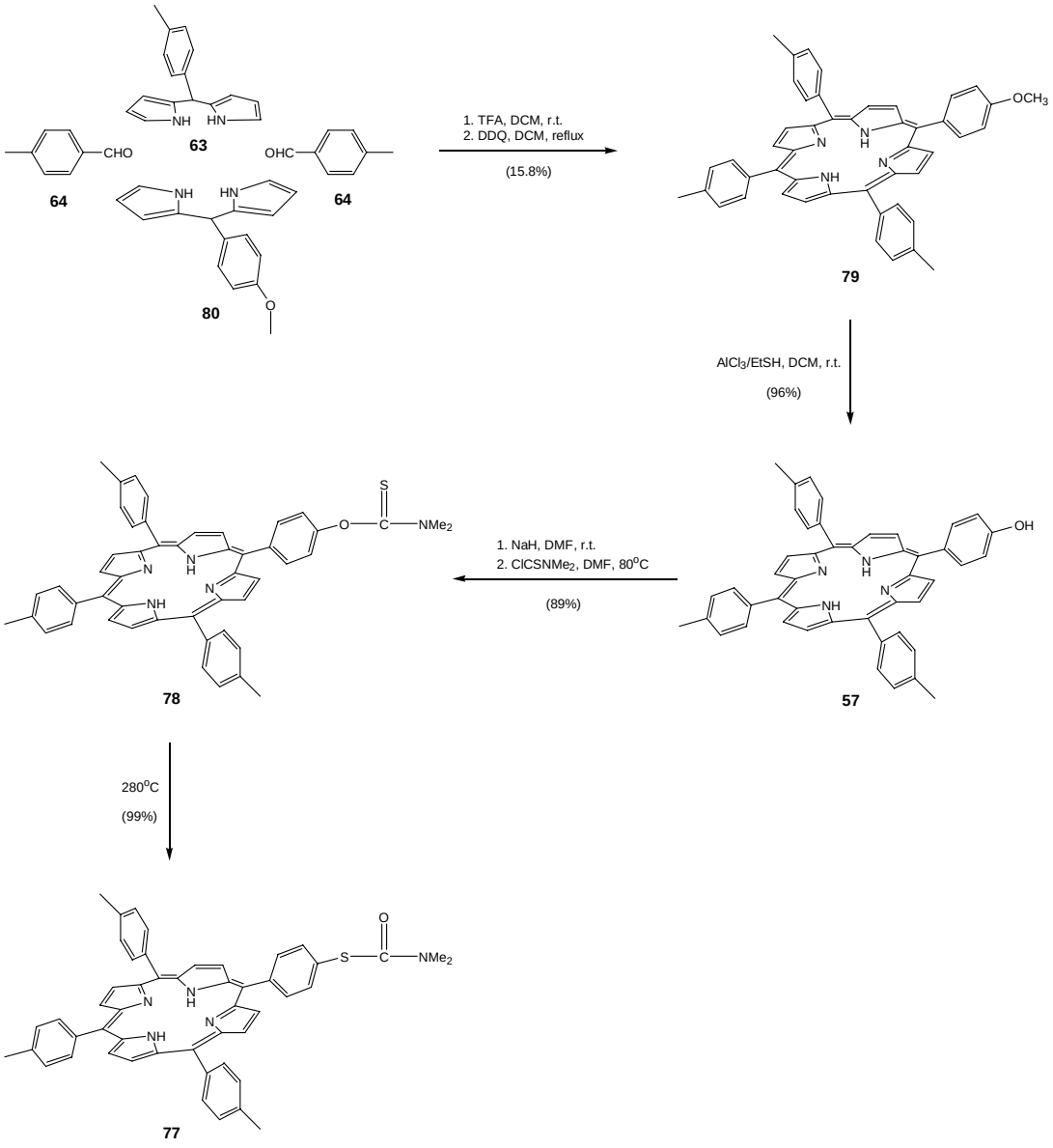
Scheme 27 - Mechanism of Newman-Kwart rearrangement

Therefore, a new strategy was planned to achieve the target porphyrin **59**. The retrosynthesis is outlined in *scheme 28*. The crucial thiophenol-Ru(II)-porphyrin **59** could arise from prophyrin **57** *via* the sequence of Newman-Kwart rearrangement, followed by insertion of ruthenium and subsequent deprotection. Porphyrin **57** should be accessible from *p*-tolyldipyrromethane **63**, anisyldipyrromenthane **80** and *p*-methylbenzaldehyde **64**, followed by cleavage of methoxy group.



Scheme 28 – Retrosynthetic analysis of **59** with **57** as intermediate (***Newman-Kwart rearrangement***)

3.1.2.2.2 Synthesis

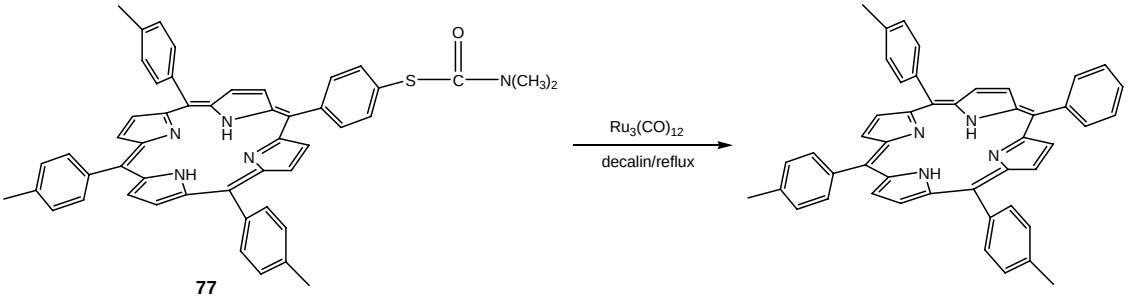


Scheme 29 – Synthesis of porphyrin **77** *via* Newman-Kwart rearrangement

Porphyrin **79** was achieved by acid catalysed condensation, followed by cleavage of the methoxy group.[69] Thus, treatment of a solution of **63**, **64** and **80**[83, 84] in CH_2Cl_2 with 3.56 equivalents of TFA at room temperature, followed by DDQ oxidation, gave a mixture of porphyrins. After column chromatography, the second band was collected and afforded porphyrin **79** in 15.8% yield, which was subsequently converted to **57** in 96% yield with large excess of $AlCl_3$/ethanethiol in CH_2Cl_2 at room temperature over night. In turn, after deprotonation with sodium hydride, porphyrin **57** was converted to

the corresponding *O*-aryl dimethylthiocarbamated porphyrin **78** in 89% yield by treatment with dimethylthiocarbamyl chloride in DMF at 80°C. By means of neat reaction at 280°C, **78** rearranged to *S*-aryl dimethylcarbamated porphyrin **77** quantitively (*scheme 29*).

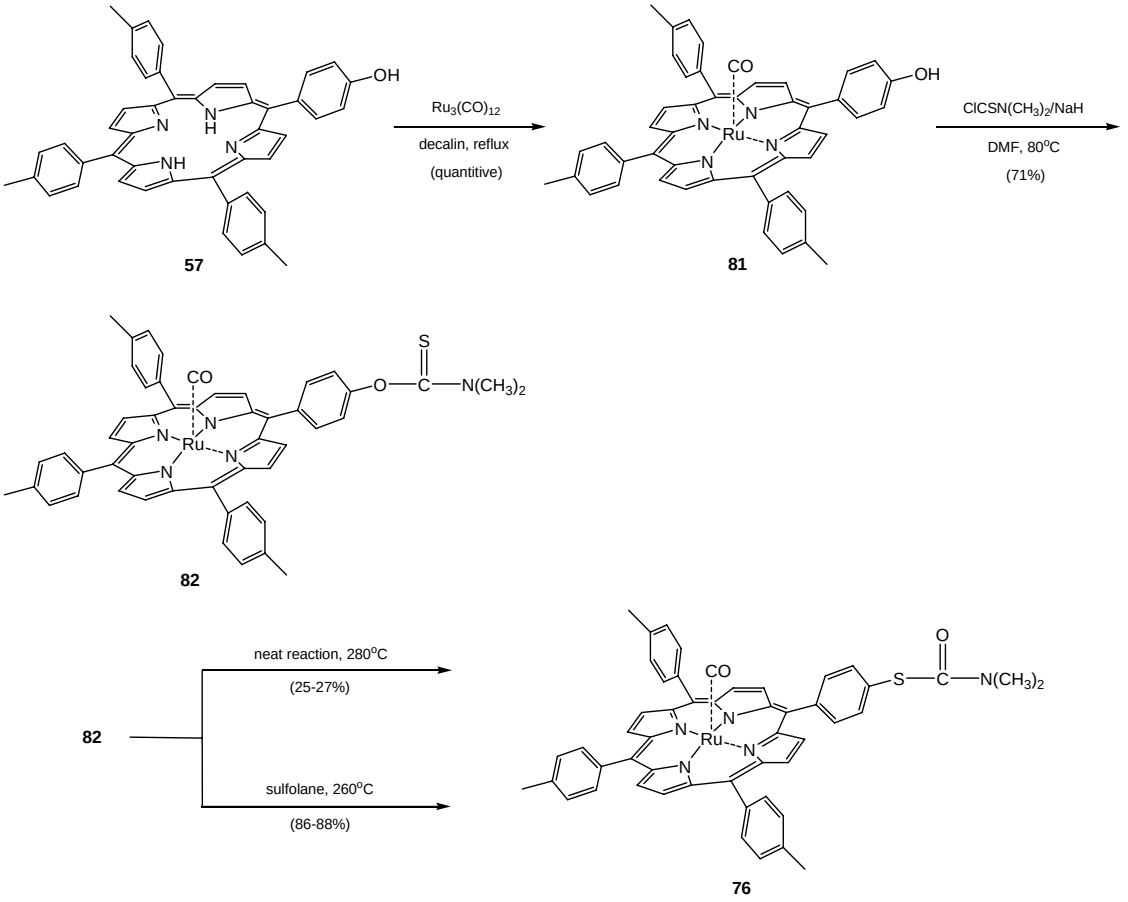
Unfortunately, attempts to introduce ruthenium into **77** under the standard condition only afforded the free base porphyrin as sole product (*scheme 30*). The similar methodology used for preparation of ruthenium porphyrin **62** also led to the same result.



Scheme 30 - The result of insertion of ruthenium into **77** under the standard condition

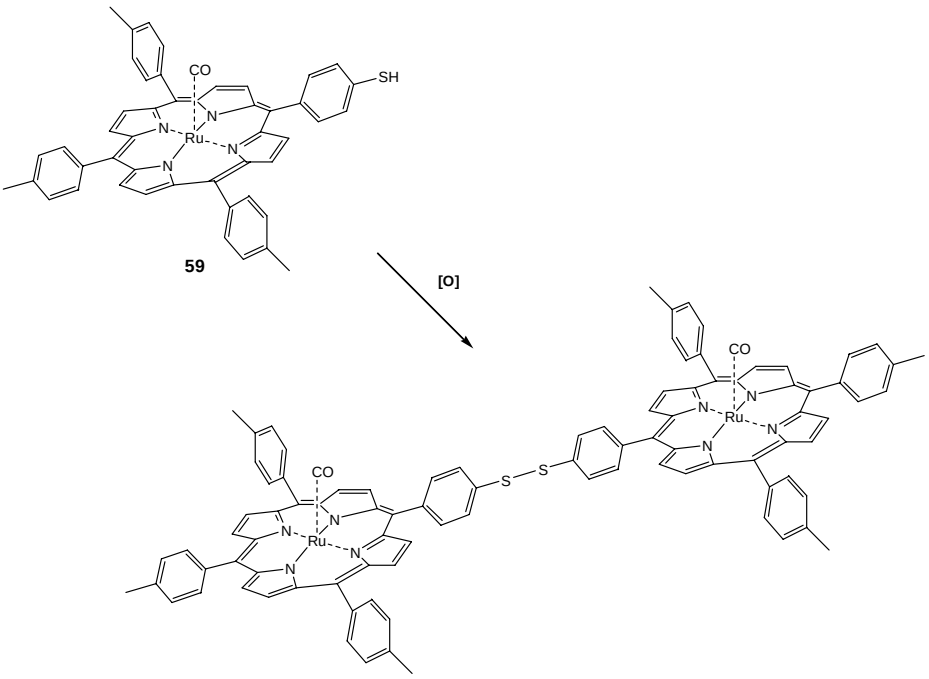
At this stage, based on the lesson learned from previous works, it was reasonable to suggest that the introduction of ruthenium should be done as early as possible to avoid the degradation of sulfur functional group mediated by ruthenium. Thus, the attention was turned to β-carbonyl [5-phenol-10,15,20-tri(methylphenyl)-porphyrinato] ruthenium **81** as the precursor of **59**.

Under the standard condition, phenol porphyrin **57** provided the corresponding ruthenium complex **81** quantitively. Treatment of **81** with sodium hydride in DMF, and then dimethyllthiocarbamyl chloride at 80°C provided dimethylthiocarbamated porphyrin **82** in 71% yield. The following Newmann-Kwart rearrangement was firstly tested under neat condition (280°C), leading to **76** (25-27%) along with unreacted **82** and a number of unidentified side products. Several solvents with boiling points within 120-180°C were screened to elicit the rearrangement but failed without reaction. Remarkably, using sulfolane as solvent finally provided a workable solution, furnishing the product of rearrangement, porphyrin **76**, in 86-88% yield (*scheme 31*).



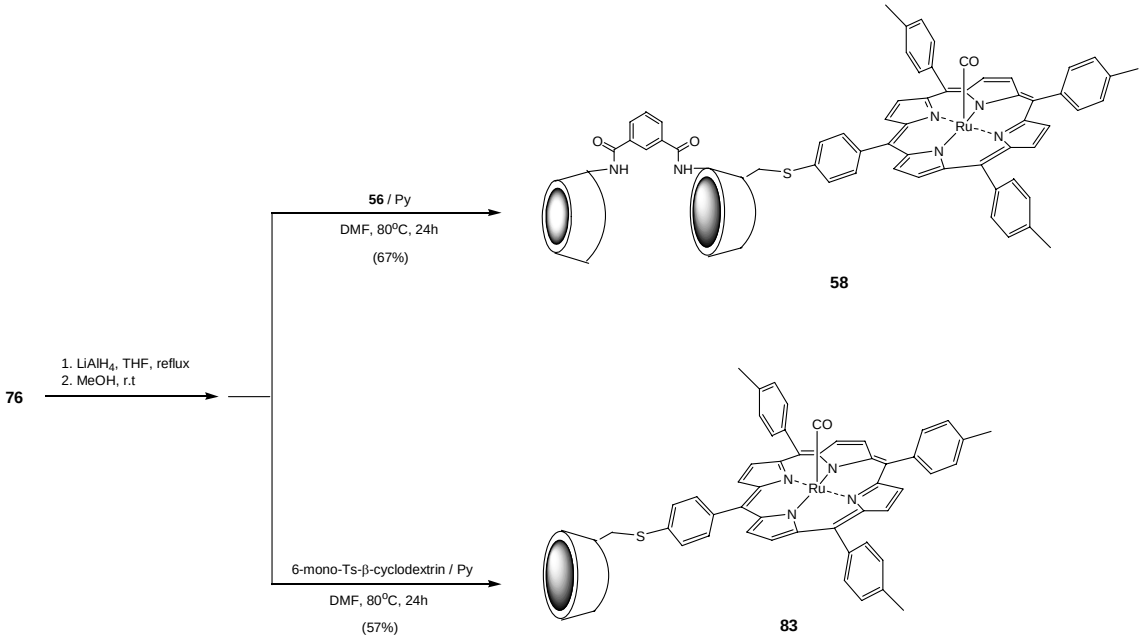
Scheme 31 – Preparation of porphyrin **76**

With the crucial precursor **76** in hand, it was now required to convert to porphyrin **59** for coupling with β-CD dimer **56**. Thus, porphyrin **76** was subjected to the reduction with $LiAlH_4$ to remove the dimethylcarbamate protecting group. Despite a number of attempts, Ru(II)-porphyrin **59** could not be obtained. Closer investigation suggested that **59** was extremely unstable to dioxygen. A sequent oxidation might happen to produce a dimeric porphyrin with disulfur linker even with traces of dioxygen, which was confirmed by ESI-MS (*scheme 32*). To this point, separation of **59** was impossible.



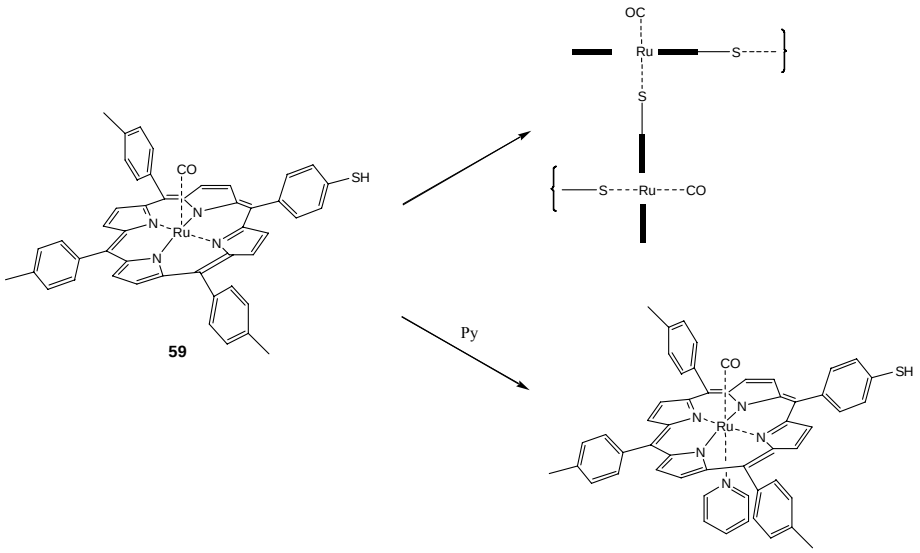
Scheme 32 – Oxidation of **59** with traces of oxygen

Eventually, an "*in situ*" procedure was developed to avoid separation of **59** as shown in *scheme 33*. Thus, porphyrin **76** was treated with 3 equivalents of $LiAlH_4$ in refluxing THF under argon to afford deprotonated **59**. Water and oxygen free methanol was added to quench the reaction, and then evaporated under *vacuo* at room temperature. The resulting residue was dissolved in a degassed solution of 2 equivalents of **56** in DMF together with 100 equivalents of pyridine. The reaction mixture was heated at 80^{o}C under argon for 24h. This procedure furnished the desired supramolecular complex **58** in 67% yield after purification by semi-preparative HPLC. The same procedure was also applied to produce a mono-β-CD complex **83** in 57% yield.



Scheme 33 – Preparation of supramolecular complex **58** and **83**

It should be noted that pyridine was used to avoid the self-assembling of **59** since coordination of thiol group to ruthenium would block the coupling (*scheme 34*).



Scheme 34 – Self-assembling of **59**

3.1.3 Reactivity of supramolecular complex 58/TBHP towards β,β-carotene

For the catalytic cleavage of β,β-carotene **2**, initial reactions were carried out under identical biphasic conditions as R. French reported.[69] Thus, a round bottomed flask which had been purged with argon and fitted with an appropriately sized egg shaped magnetic stir bar was charged with a solution of **58** in water (75nM/ml). TBHP (70% in water) (300 equivalents to **58**) was added. β,β-Carotene was added to the reaction flask as a solution in chloroform/hexane (1:9) (750nM/ml) to produce a biphasic system. The reaction was closed and stirred vigorously to ensure good mixing of the two phases at room temperature. At intervals, stirring was stopped to allow phase separation. Aliquots (20μl) of the organic phase were taken and subjected to HPLC analysis.

β,β-Carotene was fully consumed after 18h and six products of the reaction could be identified: 13-apo-β-carotenone **17** (peak *a*), retinal **8** (peak *b*), 14'-apo-β-carotenal **15** (peak *c*), 12'-apo-β-carotenal **14** (peak *d*), 10'-apo-β-carotenal **10** (peak *e*), 8'-apo-β-carotenal **9** (peak *f*, traces) by means of UV/Vis, LC-MS or co-injection with authentic standards. (*figure 11*) It should be noted that **15** and **17** were only verified by UV/Vis[35] and LC-MS but could not be quantified since the corresponding authentic standards were not available.

Upon quantification of the three "cleavage" products (**8**, **10**, **14**), the combined yield from the reaction at 10.5h (the time point when concentration of **10** and **14** reached maximum) was 8.5%, which is similar to the results from complex **46** (7%) as R. French reported.[69] The selectivity for the cleavage is similar to that of **46** as well. It should be noted that the yield of the reaction is based on the fact that one molecule of β,β-carotene would be cleaved to give two molecules of retinal, but the cleavage to apo-β-carotenals would only give one molecule of these products. But, for comparing with reported results of **46**, the selectivity is demonstrated as product ratio [C (apo-β-carotenal, nmol/ml)/C (retinal, nmol/ml)].

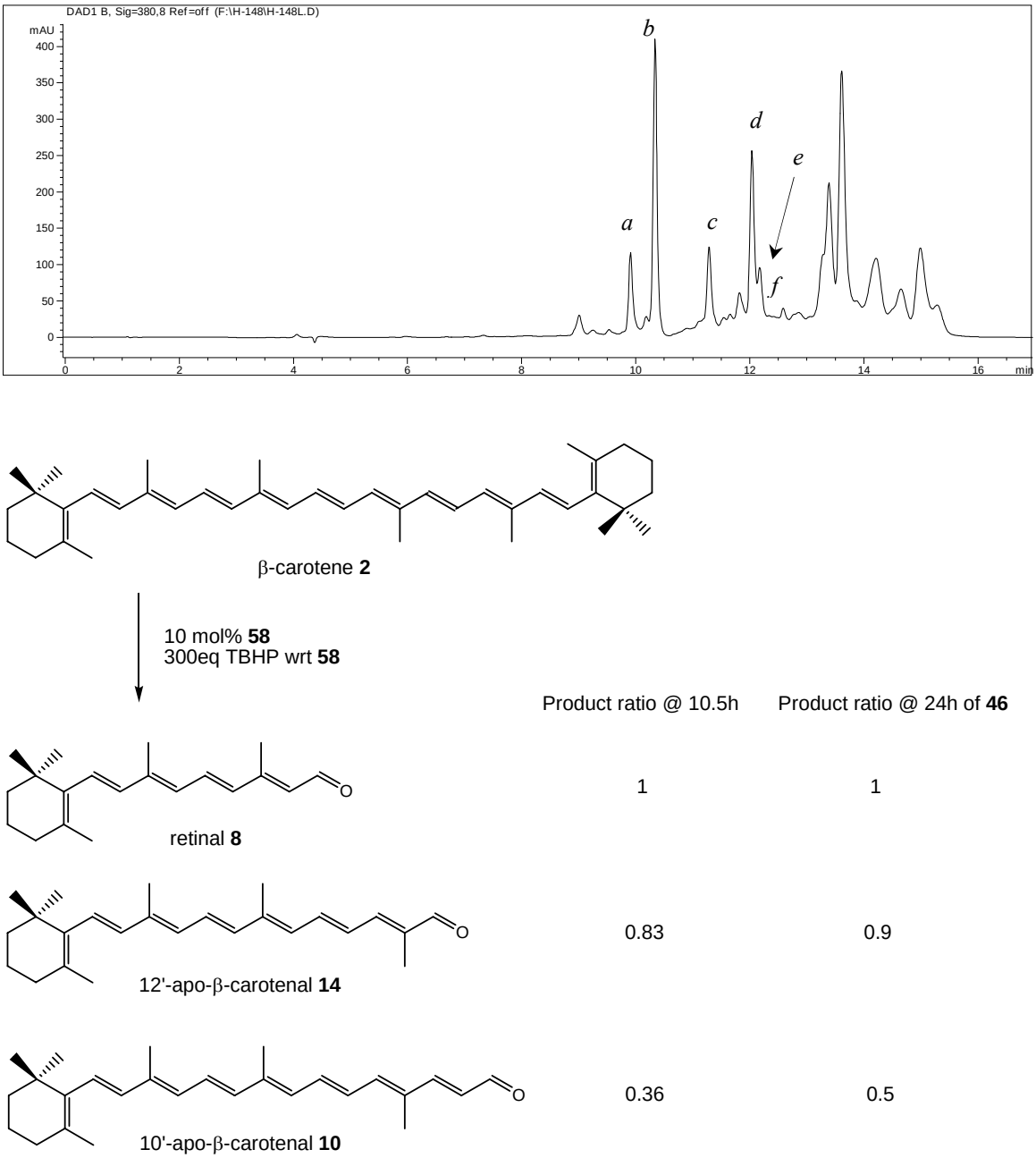


Figure 11 – HPLC chromatogram of reaction of **2** with 10% **58** after 10.5h (λ_{det} = 380nm) and product ratio (compared with the result of **46**)

For practical reasons, a modified procedure was developed. The solution of β,β-carotene (265nmol/ml) in hexane/chloroform (9:1) was added into the flash containing a solution of **58** (75nmol/ml, 30%) in water, to which 300 equivalents of TBHP (wrt **58**) had been added. The biphasic system was stirred vigorously at 31°C. Under this reaction condition, the combined yield (7.3%) of **8**, **10**, **14** and the cleavage selectivity

were similar to that obtained before (*figure 12*). However, the reaction time was reduced to 8h, making reaction control much easier.

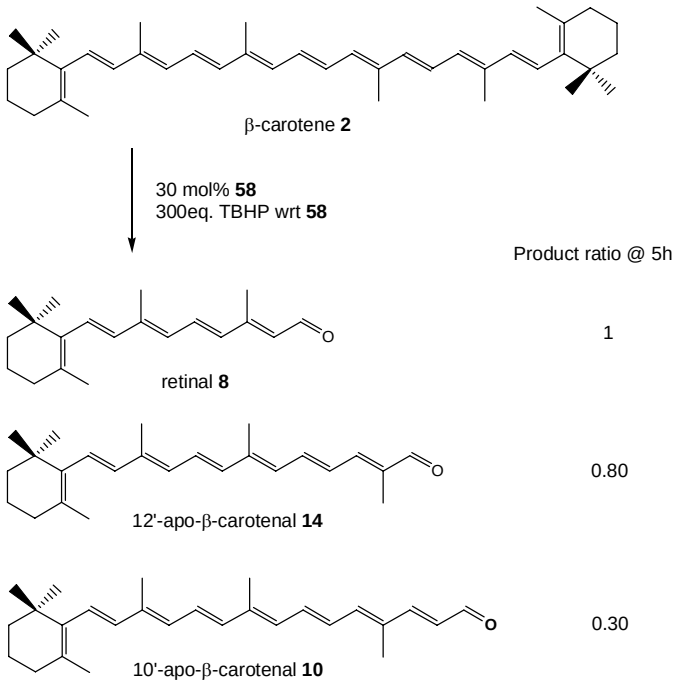


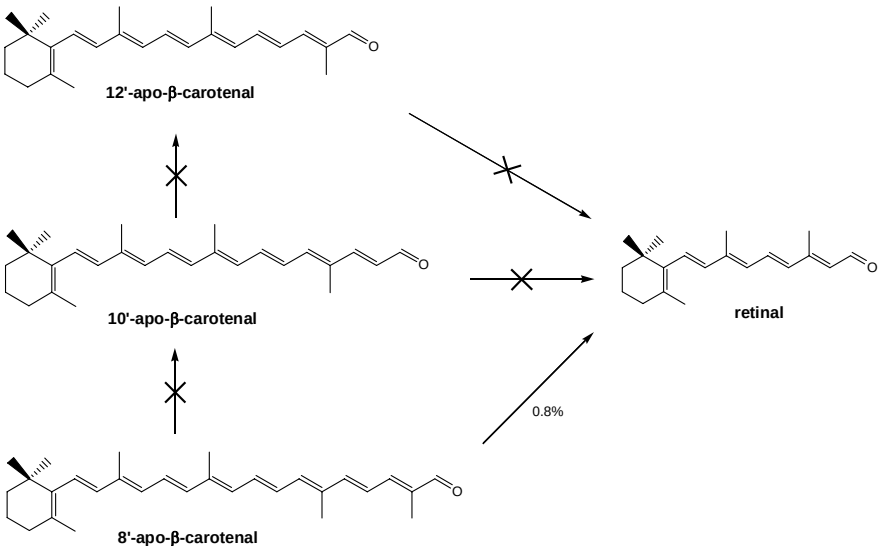
Figure 12 – Product ratio of reaction of **2** with 30% **58** at 31°C (λ_{det} = 380nm)

Suprisingly, the results shown in *figure 11* and *12* are very similar to those observed using complex **46**, the catalyst designed for the central cleavage of β,β-carotene (*scheme 13*). It was postulated that one of two phenomena, degradation of apo-β-carotenals to retinal and/or motion of β,β-carotene within the binding pocket, were occurring during the reaction to cause this anomalous result. These two postulates and the manner in which they were addressed will be discussed in the following section.

3.1.3.1 Degradation of apo-β-carotenals to retinal or mobility of binding?

3.1.3.1.1 Cleavage of apo-β-carotenals by complex 58/TBHP

During the catalytic cleavage reaction, it was observed that the apo-β-carotenals were sensitive to the reaction condition and they might be continuously degraded to retinal. Therefore, degradations of apo-β-carotenals by complex **58** was investigated under the identical modified procedure for cleavage of β,β-carotene (30% **58** at 31°C).



Scheme 35 – Degradations of apo-β-carotenals by complex **58**

Reactions of **58**/apo-β-carotenals with TBHP as cooxidant showed that: i) 8'-apo-β-carotenal could not be cleaved to 10'- and 12'-apo-β-carotenal, but to retinal in 0.8% yield; ii) 10'-apo-β-carotenal could not be cleaved to 12'-apo-β-carotenals, and both of them could not be degraded to retinal (*scheme 35*). Since only traces of 8'-apo-β-carotenal was produced during the cleavage reaction, the effect of conversion could be omitted.

3.1.3.1.2 Is β,β-carotene mobile when bound to the complex?

If β,β-carotene is mobile when bound to the complex, then this phenomenon must be related to the interaction between the endgroup of β,β-carotene and β-CD cavity, such that a lateral movement of β,β-carotene within the binding pocket would expose several double bonds to the reactive ruthenium center.[69] Thus, if the answer is "yes", one would expect a carotenoid with modified endgroup(s) to increase the binding constant to β-CD cavity would demonstrate a different selectivity in cleavage. For this purpose, a synthetic carotenoid **84**[88] with 2,6-dimethylphenyl as endgroups was chosen as substrate (*figure 13*). The hydrophobic interaction between the aromatic endgroup of **84** and β-CD cavity would be much stronger than that from cyclohexane endgroup of β,β-carotene, stabilizing the 1:1 inclusion complex as expected from the cleavage results of carotenoid **47** using catalyst **47**/TBHP (section 1.2.4).

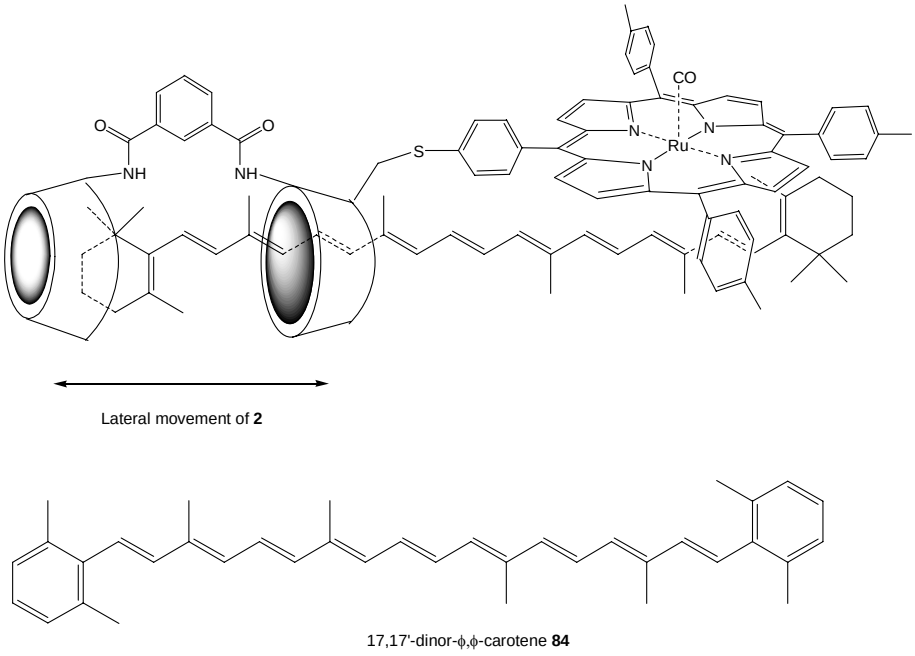
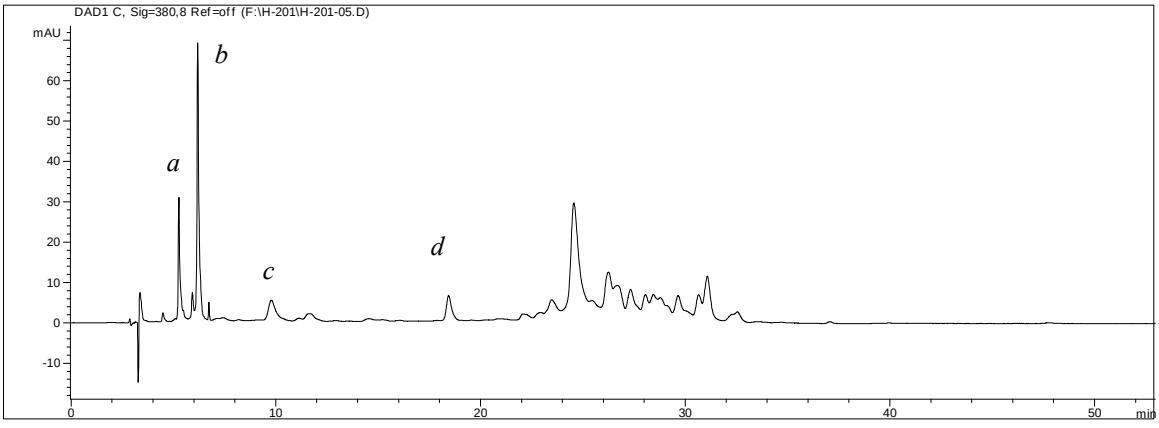


Figure 13 – Lateral movement of 2 in β-CD cavity and the structure of endgroup modified carotenoid **84**

Accordingly, the profile of synthetic carotenoid **84** was investigated under the identical condition (30% **58** at 31°C). The HPLC chromatogram (C-30) for this reaction is shown in *figure 14*. Four products were identified by means of UV/Vis: 13-apo-ϕ-carotenone **85** (peak *a*, R_t = 5.3min, λ_{max} = 340nm, further assignment see section 3.2.4 and 3.2.5), ϕ-retinal **86** (peak *b*, R_t = 6.2min, λ_{max} = 380nm)[69], 14'-apo-ϕ-carotenal **87** (peak *c*, R_t = 9.8min, λ_{max} = 405nm) and 12'-apo-ϕ-carotenal **88** (peak *d*, R_t = 18.5min, λ_{max} = 425nm). Surprisingly, the main product is ϕ-retinal **86** from central cleavage but not apocarotenals. Furthermore, a similar result was obtained with the mono-β-CD complex **83** as shown in *figure 14*.

A)



B)

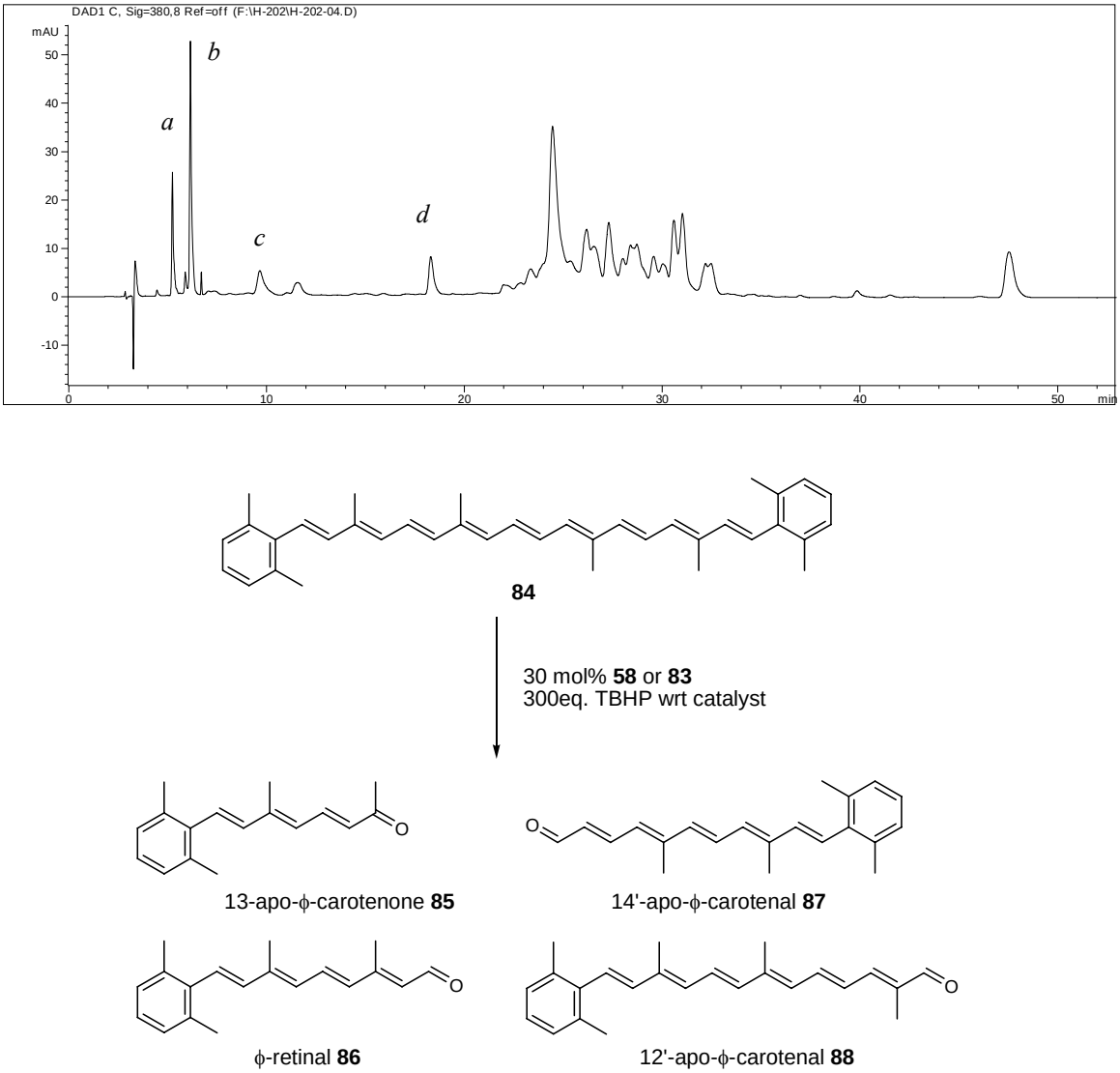


Figure 14 – HPLC chromatogram (C-30): A) 30% **58** at 31°C after 4h; B) 30% **83** at 31°C after 4h

Based on these results, it was suspected that only the first β-CD directly linked to the porphyrin was used for binding carotenoids. At this stage, two postulates were suggested (*figure 15*): i) the dimeric β-CD moiety does not provide a rigid, extended cavity due to the conformational flexibility of the linker and/or due to the small driving force for the dimethylphenyl group to slip from the first CD unit into the second one and ii) each β-CD unit binds a carotenoid molecule and that a 2:1 complex is produced.

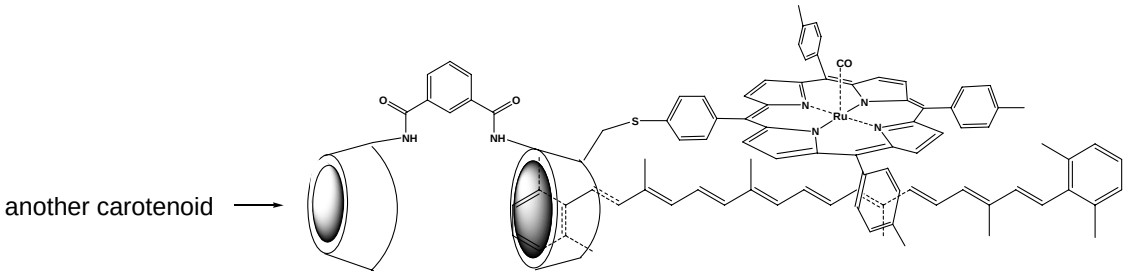
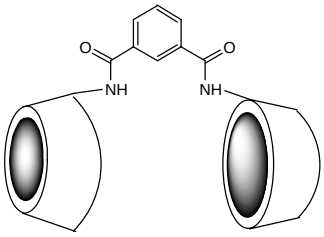


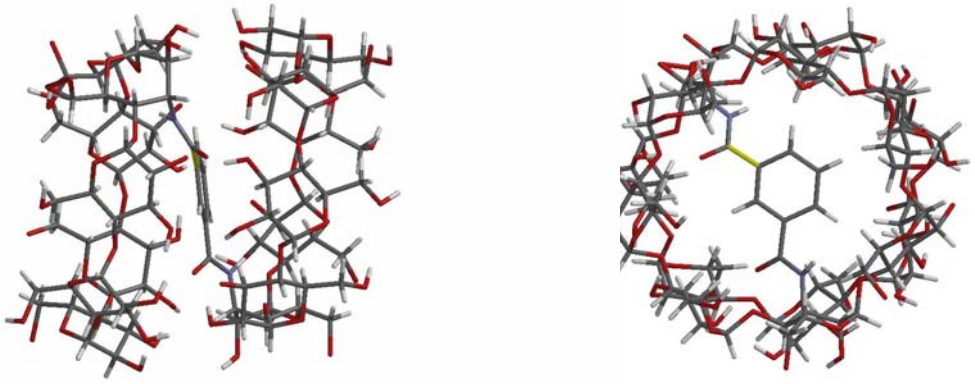
Figure 15 – Two possible binding modes of **58/84**

3.1.4 The crystal structure of the dimeric β-CD moiety 55

To date, no crystal structure of CD dimer linked *via* the secondary face has been reported. However, fortunately, a single crystal of the β-CD dimer **55** was obtained from water/acetone by means of *solvent diffusion*. The corresponding crystal structure is shown in *figure 16* (for details see experiment part).



55



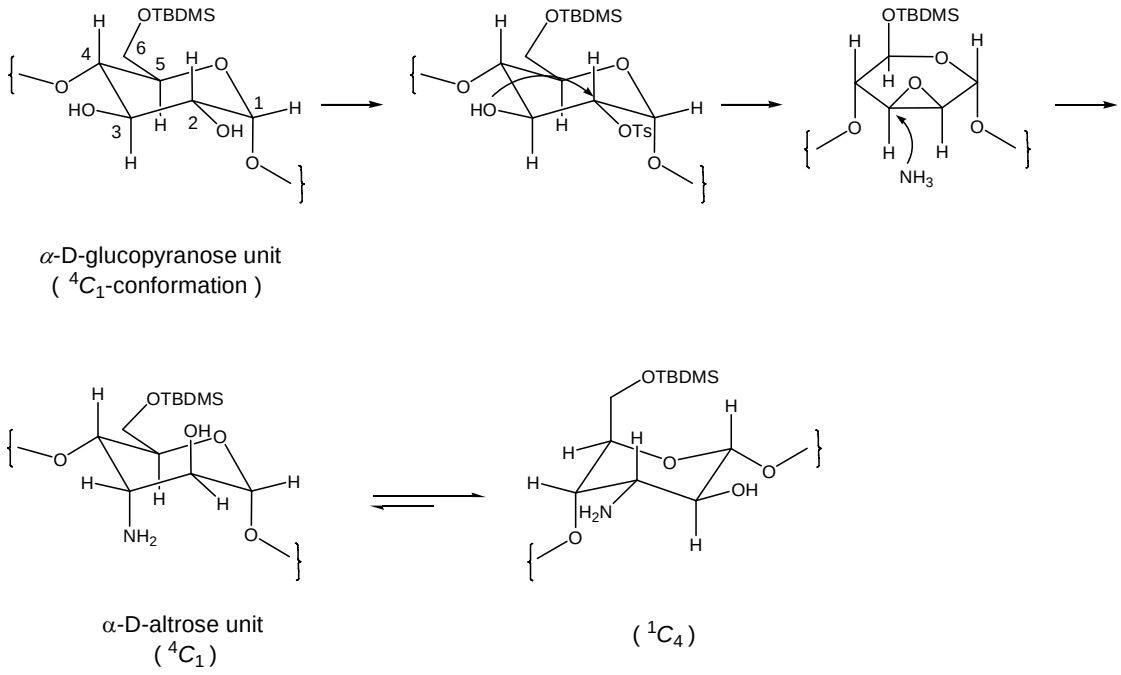
A B

Figure 16 – Crystal structure of **55** : A) side view B) top view from the primary face

Obviously, in the solid state the β-CDs of the dimer are perfectly aligned, forming an extended cavity which is organized by hydrogen-bonding network between two

secondary faces. But, unexpectedly the diamide linker is placed in the axis of the molecule between two β-CD cavities, preventing the binding of carotenoids by both hydrophobic cavities. As a consequence the ruthenium complex **58**/TBHP behaves in the same way as **46**/TBHP or **83**/TBHP with respct to the regioselective oxidation of carotenoids.

Actually, the structure is unusual. In the sequence of modification to prepare this dimer (*scheme 36*), the C(2)-tosylated unit of CD (α-D-glucopyranose, $^4C_1$-conformation) was first transformed into the 2,3-manno-epoxide, which was then subjected to a nucleophilic attack by ammonia, leading to the diaxial 1,2-amino alcohol. Since the resulting $^4C_1$ modified α-D-altrose unit is unfavourable, it usually flaps to the more stable $^1C_4$ conformation, in which both C(2)-OH and C(3)-NH_2 are in favourable equatorial position.[89]



Scheme 36 – The sequence for modification at C(3)

However, in this special case, the *X*-ray structure of **55** reveals that the modified α-altrose unit stays in less stable $^4C_1$ conformation. This conformation is essential for self-assembling the secondary faces since the resulting distance between these two β-CDs is appropriate for interaction by hydrogen-bonding. In addition, the formation of

hydrogen-bonding between oxygen atoms of the linker-amides and adjacent C(3)-OHs is another driving force. It could be concluded that the free energy gained by self-assembling in water is sufficient to compensate for the conversion of the altrose unit from $^{1}C_{4}$ to the less stable $^{4}C_{1}$ conformation.

3.2 A flexibly linked Ru(II)-porphyrin-*bis*-β-CD complex, the second enzyme mimic for the excentric cleavage of carotenoids

3.2.1 Design of a C_6-flexibly linked Ru(II)-porphyrin-*bis*-β-CD complex 89

From the first generation supramolecular complex **58** and corresponding references,[56, 89-93] it is now clear that, if the β-CD dimer is produced by C(3)-modification *via* 2,3-manno-epoxide, there is one of two phenomena which were not favourable for the binding of carotenoids:

i) If the modified altrose unit is in an unfavourable $^4C_1$ conformation, the entry of β-CD cavity is partially blocked since the amide group of the linker occupies an axial position and thus is directed towards the interior of the cavity. (*sheme 35*, $^4C_1$ conformation of altose)

ii) If the modified altrose unit is in a favourable $^1C_4$ conformation, the shape and size of the β-CD cavity changes to a distorted ellipsoid, more narrow than that of the unmodified β-CD and hence unfavourable for binding of carotenoids (example as shown in *figure 17*).[94]

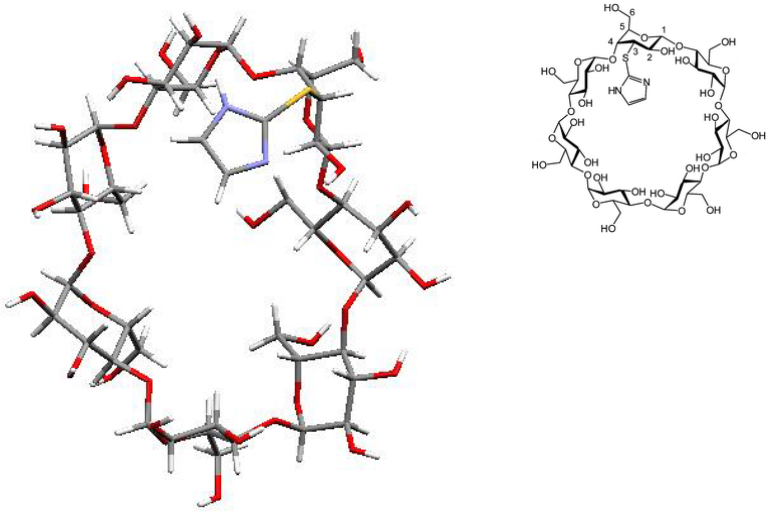
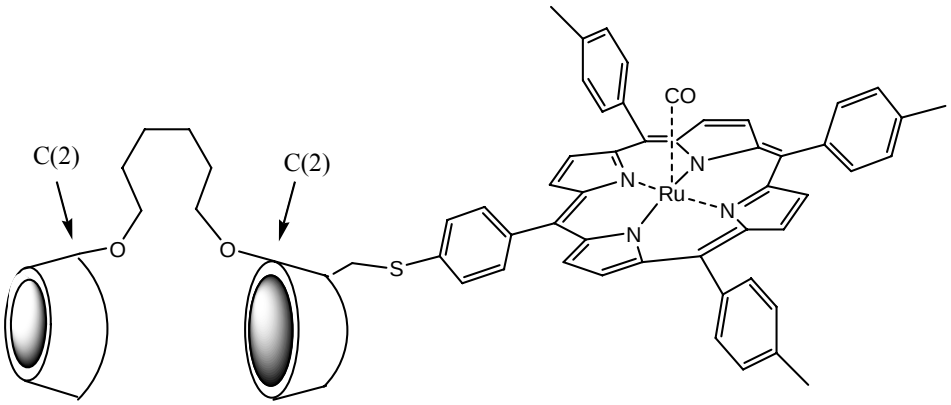


Figure 17 - mono[3-(2-Imidazolythio)]-*altro*-β-cyclodextrin (*right*) and the corresponding crystal structure (*middle*)[94]

Thus, to avoid these problems mentioned above, C_6-flexibly linked Ru(II)-porphyrin-*bis*-β-CD complex **89** was designed as shown in *figure 18*.



89

Figure 18 - Designed second generation enzyme mimic **89**

It has been known that C(2)-OH is the most acidic hydroxyl group of β-CD (*p*Ka ~ 12). Thus, direct introduction of linker into C(2) would not result in conformational changes of β-CD.[93, 95-98] On the other hand, since C(2)-OH is in an equatorial position, this flexible C_6 linker might stay outside the cavity. It might act as a loop,[99] and thus these two β-CDs of the dimer could self-assemble to produce an extended cavity *via* a hydrogen-bonding network on the secondary faces as suggested from the crystal structure of the β-CD dimer **55**.

3.2.2 Testing the principle

To the best of our knowledge, it was still possible that the flexible linker could be self-included into the cavity of β-CD in aqueous environment if it was long enough.[93, 98] To exclude this possibility, β-CD dimer **93** was prepared and then subjected to 2D NOE measurement (for details of synthesis, please see next section). In theory, if the linker was self-included inside the cavity, NOEs between protons of the linker and CD-C(3, 5)-Hs, which point into the cavity, should be observed.

The NMR spectra of dimer **93** in D_2O showed that dimer was symmetric. Examination of the coupling constants revealed that $J_{1,2}$ (3.6 Hz) and $J_{2,3}$ (9.9Hz) of the modified glucose unit (A) were similar to those of free β-CD **19**, suggesting the conformation of this unit was retained. Moreover, NOEs were not observed between the protons of the linker and protons at C(3, 5), indicating that the linker stayed outside the cavity as desired. (*figure 19*)

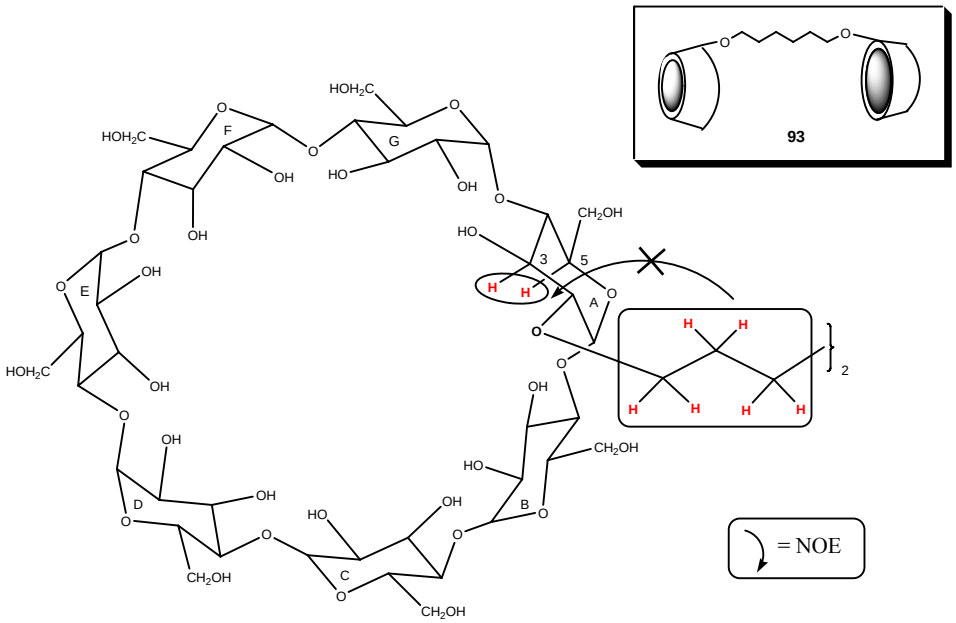
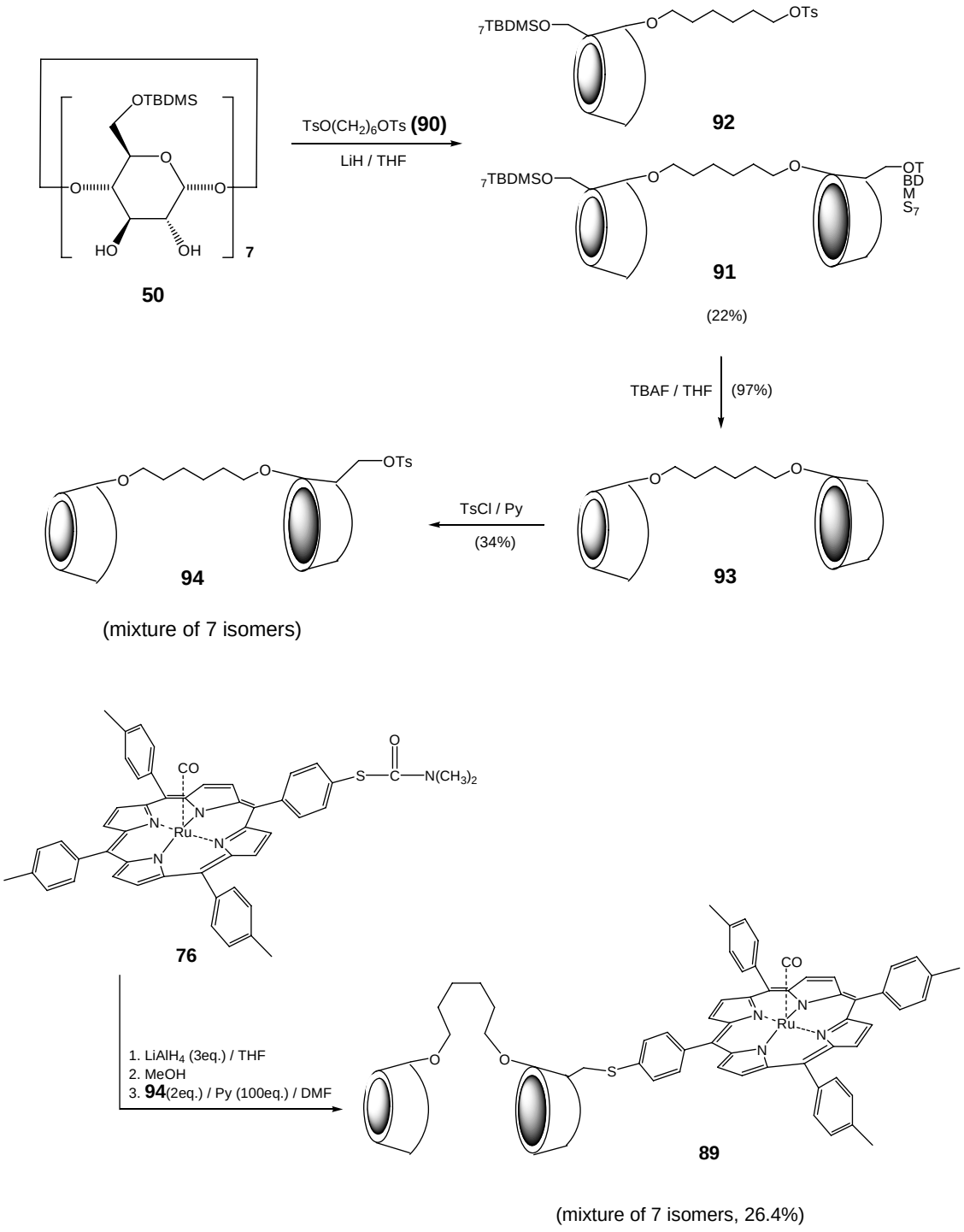


Figure 19 - Result of NOE measurement for β-CD dimer **93**

3.2.3 Synthesis of the flexibly linked Ru(II)-porphyrin-*bis*-β-CD complex 89

Complex **89** was synthesized as shown in *scheme 37*. TBDMS-β-CD **50** was selectively deprotonated at C(2)-OH with one equivalent of LiH in THF, and then reacted with tosylated diol **90**[100] in refluxing THF for 5 days.[101] After column chromatography, a mixture of dimer **92** and monomer **91** was obtained, but further separation by normal or reversed phase column chromatography was impossible. After a lot of attempts, eventually, the separation was carried out easily with column chromatography on charcoal (ethyl acetate/CH_2Cl_2 = 2:1) to furnish pure dimer **92** in 22% yield. After deprotection with TBAF in refluxing THF, free β-CD dimer **93** was obtained quantitively, which was mono-tosylated at the primary face with one equivalent of *p*-TsCl in dry pyridine at room temperature. It should be noted that the resulting mono-tosyl-β-CD dimer **94** is a mixture of isomers, generated from the unselective tosylation of one of the primary hydroxyl groups. This mixture could not be separated further and was directly used for the next step. Then, following the similar procedure used for preparation of complex **58**, compound **89** as a mixture of isomers was obtained in 26.4% yield, identified by UV/Vis (H_2O, λ_{max} = 412nm) and ESI-MS (3171 $[M+Na]^+$, 1597.3 $[M+2Na]^{2+}/2$, 1573.9 $[M-2H]^{2-}/2$).



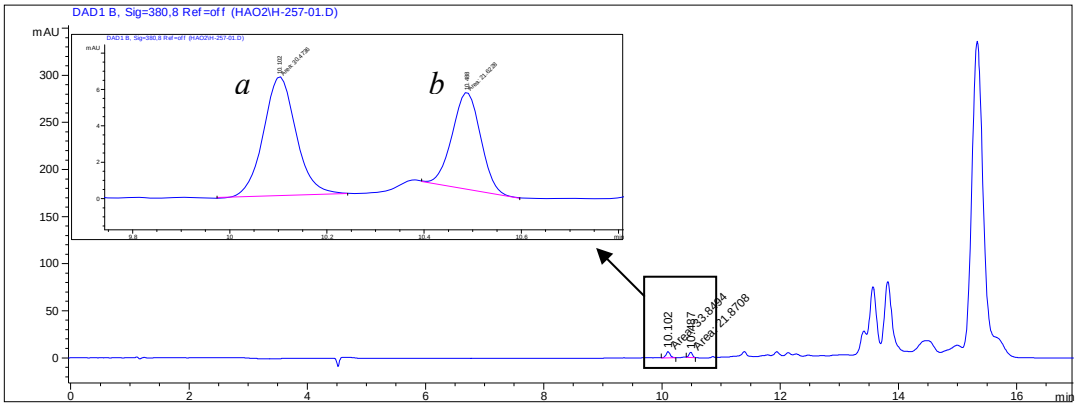
Scheme 37 - Synthesis of complex **89**

3.2.4 Reactivity of complex 89/TBHP towards carotenoids

For the catalytic cleavage, initial reaction was carried out with β,β-carotene **2** as substrate under the modified conditions described in section 3.1.3 (catalytic/substrate/TBHP = 1:3.5:300, @ 31°C). β,β-carotene was completely

consumed after 8h. As shown in *figure 20*, it was observed that the ratio of 13-apo-β-carotenone **17** (R_t = 10.1min)/retinal **8** (R_t = 10.5min) decreased over the reaction time, and the final result was similar to those with complex **58** as catalyst and β,β-carotene as substrate (*figure 11*).

A)



B)

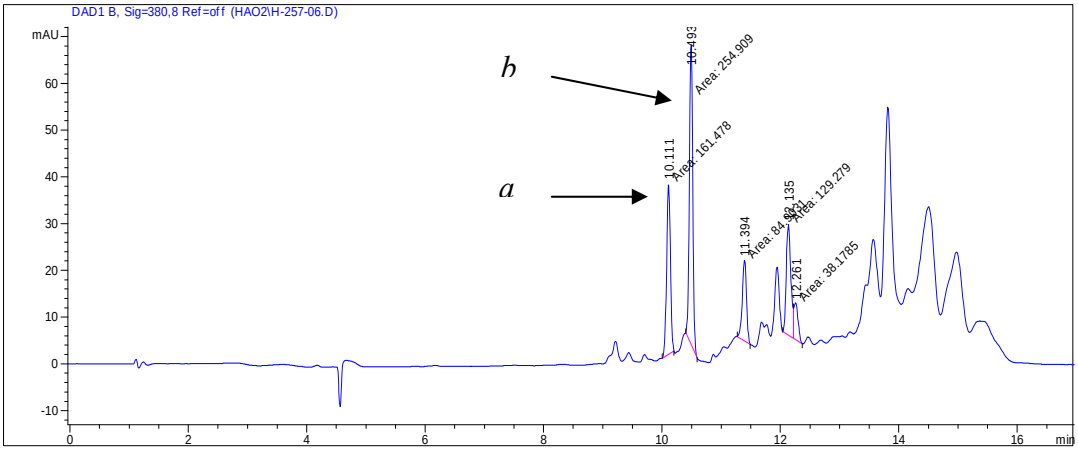
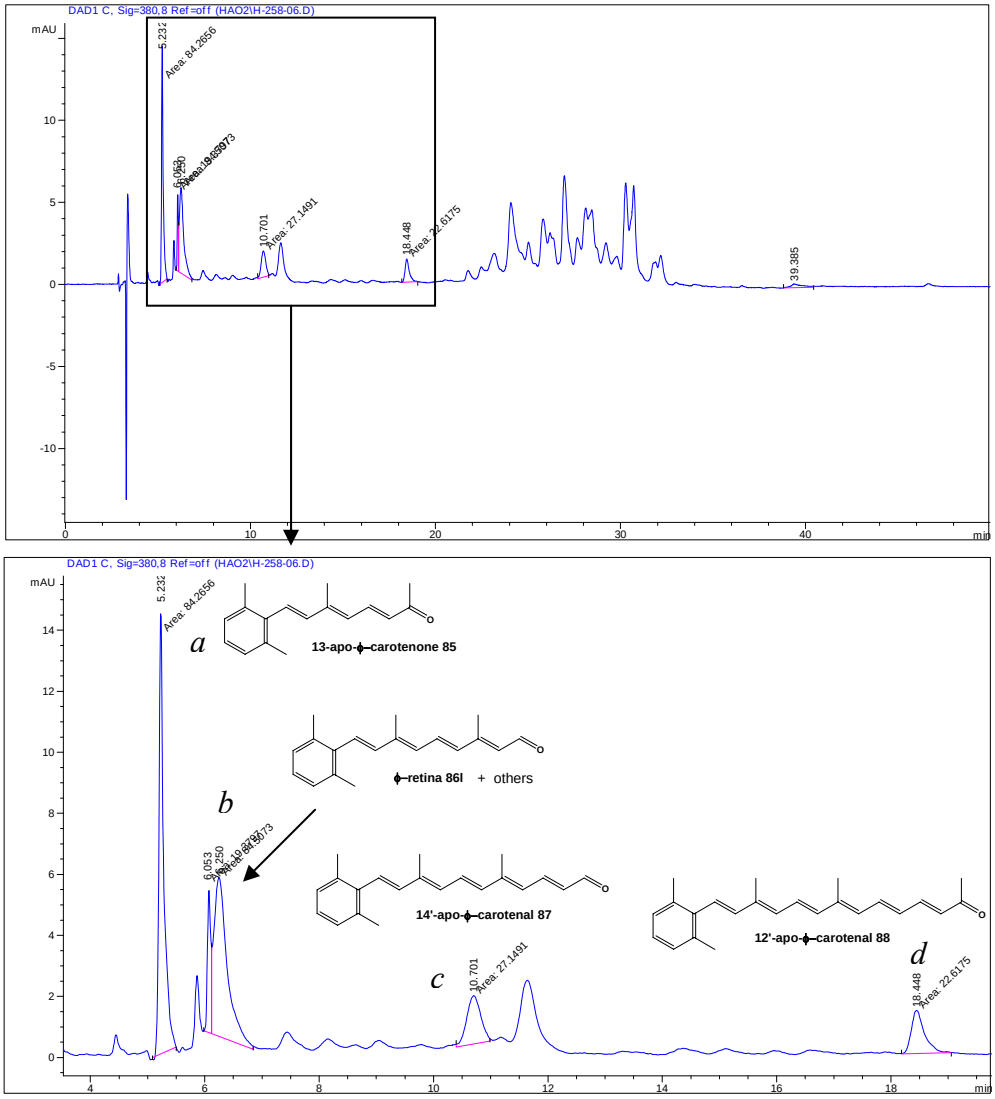


Figure 20 - A) HPLC chromatogram (RP-18) of reaction of **89**/**2** after 1h (λ_{det} = 380nm); B) HPLC chromatogram after 8h (λ_{det} = 380nm): Peak *a* and *b* are from 13-apo-β-carotenone **17** and retinal **8** respectively.

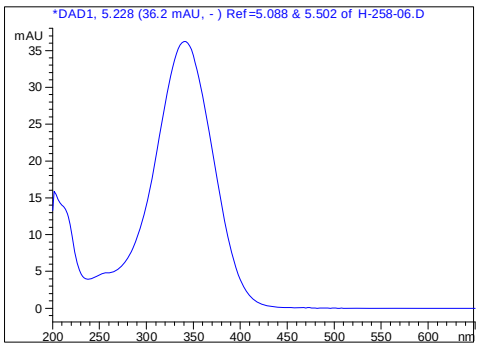
The cleavage was further examined with 17,17'-dinor-ϕ,ϕ-carotene **84** as substrate. As expected, the reaction time was reduced to 5h. Interestingly, a "de-centralized" cleavage was observed (*figure 21 and scheme 38*). The main peak *a* (R_t = 5.18min, λ_{max} = 340nm) was verified to be 13-apo-ϕ-carotenone **85** by co-injection with synthetic authentic standard (for synthesis of **85**, please see next section). Peak *c* and *d* were identified as 14'-apo-ϕ-carotenal **87** (R_t = 10.7min, λ_{max} = 402nm) and 12'-apo-ϕ-

carotenal **88** (R_t = 18.5min, λ_{max} = 425nm) according to their UV/Vis spectra. Peak *b* (R_t = 6.25min) was a mixture containing ϕ-retinal **86** (λ_{max} = 380nm).

A)



B)



C)

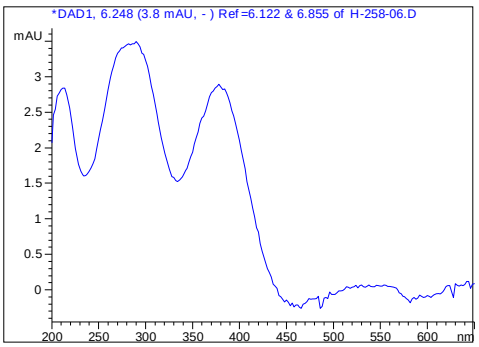
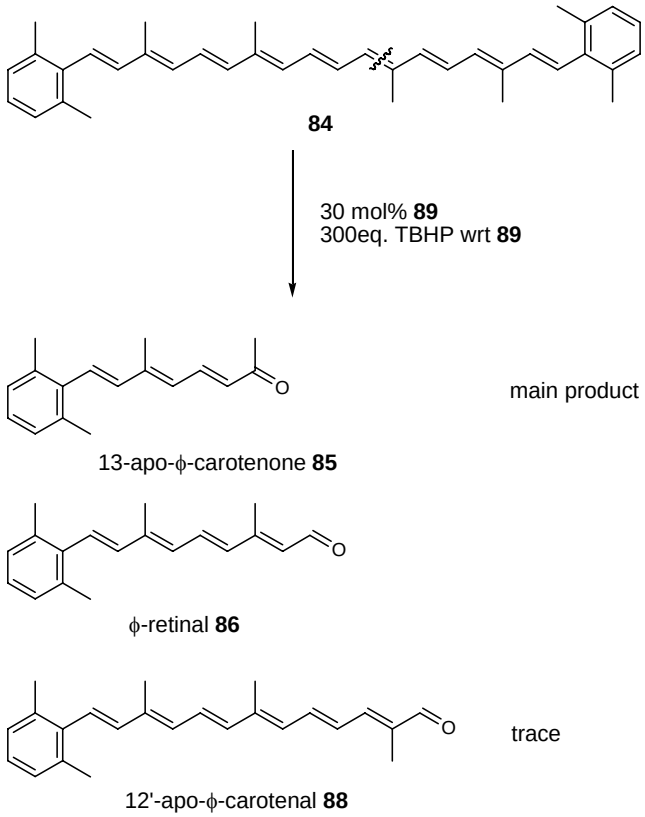


Figure 21 – A) HPLC chromatogram (C-30) of reaction of **89**/**84**/**TBHP** (λ_{det} = 380nm) after 5h; B) UV/Vis spectra of peak *a*; C) UV/Vis spectra of peak *b*



Scheme 38- Reaction of synthetic carotenoid **84** with complex **89** under the modified condition

However, in contrast to the first supramolecular complex **58**, the combined yield of cleavage products was extremely low. A similar result was obtained even if the ratio of catalyst to substrate (**84/89)** is 10:1. It was possible that the cleavage products were bound by **89** in aqueous phase as well as **84** since the endgroup with strong interaction with β-CD was not been altered, and then they were continuously degraded to some other small fragments. To exclude this possibility, $CHCl_3$/hexane (2:3) was used as organic phase for the cleavage reaction to increase the efficiency of product-extraction. Surprisingly, the substrate was completely consumed after 3h, but the yield of 13-apo-ϕ-carotenone **85** (*figure 22*) was similar to that with $CHCl_3$/hexane (1:9).

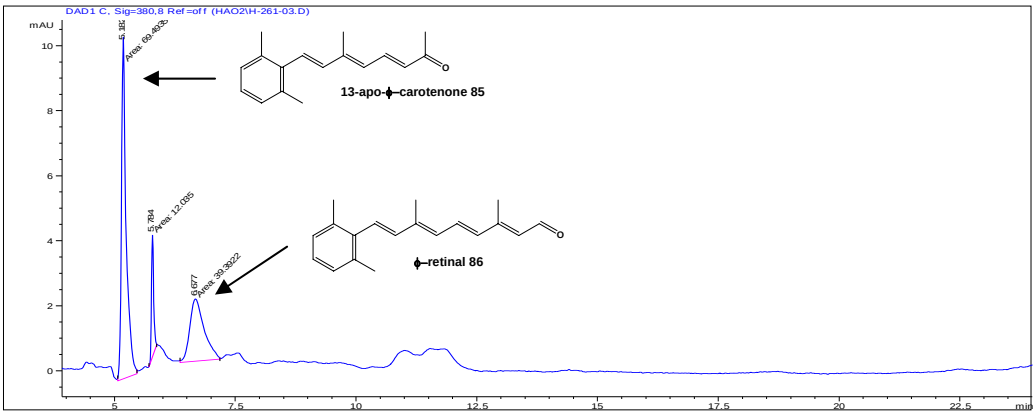
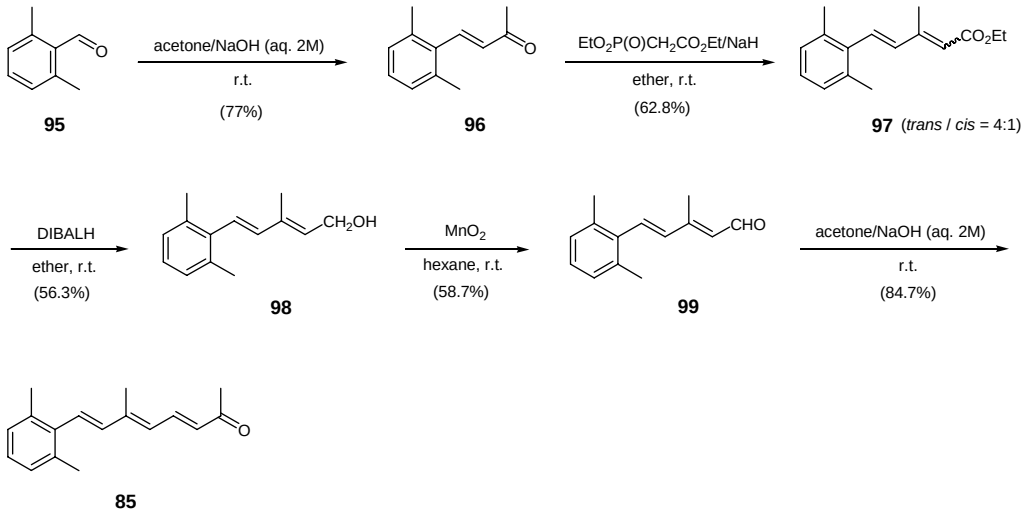


Figure 22 - HPLC chromatogram (C-30) of cleavage of **84** with $CHCl_3$/hexane (2/3) after 3h (λ_{det} = 380nm). Peak (R_t = 6.67min) was from ϕ-retinal **86** as UV/Vis suggested.

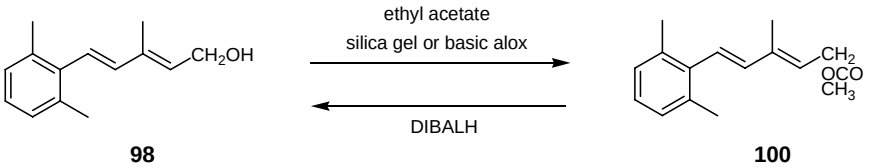
3.2.5 Synthesis of 13-apo-ϕ-carotenone 85



Scheme 39 - Synthesis of 13-apo-ϕ-carotenone **85**

As shown in *scheme 39*, 13-apo-ϕ-carotenone **85** was synthesized starting from 2,6-dimethylbenzaldehyde **95**.[88, 102, 103] Catalyzed by NaOH (aq. 2M), **95** reacted with acetone at room temperature to give ketone **96** in 77% yield.[88] The selective formation of (*E*)-**96** is due to steric hindrance of the aromatic methyl groups. Using *Wittig-Horner* conditions, ketone **96** was converted to the ester **97** obtained as a mixture of *EE*/*ZE* = 4:1 according to ^{1}H-NMR. Employing di(*iso*-butyl)aluminum hydride (DIBALH), ester **97** was reduced to the alcohol **98**.[103] After column chromatography, the *all-E* isomer

of **98** was obtained in 56.3% yield. It should be noted that ethyl acetate could not be used as solvent for column chromatography since alcohol **98** was acylated to the ester **100** catalyzed by silica gel or basic alox (*scheme 40*).



Scheme 40 - Acylation of alcohol **98** by ethyl acetate

Alcohol **98** was then oxidized with an excess of MnO_2 in hexane to furnish the corresponding (all-*E*)-aldehyde **99** in 58.7% yield, which was then converted to ketone **85** in 84.7% yield with acetone catalyzed by NaOH (2M). The all-*E* configuration of **85** was verified by ^{1}H-NMR and 2D COSY and NOE experiments as shown in *figure 23*.

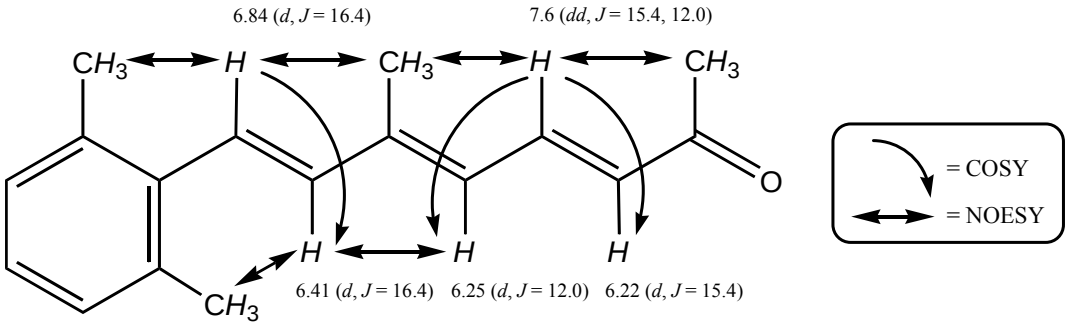


Figure 23 – The all-*E* configuration of **85** confirmed by NMR examinations

3.2.6 Argument of the cleavage results from complex 89

The cleavage of 17,17'-dinor-ϕ,ϕ-carotene **84** catalyzed by the enzyme mimic **89** is selective at C(13')-C(14') double bond of the polyene chain. However, the result is not as expected from our design (*figure 24*). In contrast to the results from complex **58** or **83**, the "de-centralized" cleavage corresponds to a shift of oxo-ruthenium attack by only 2-2.5Å, suggesting that the second β-CD unit does not participate in binding/orienting the substrate.

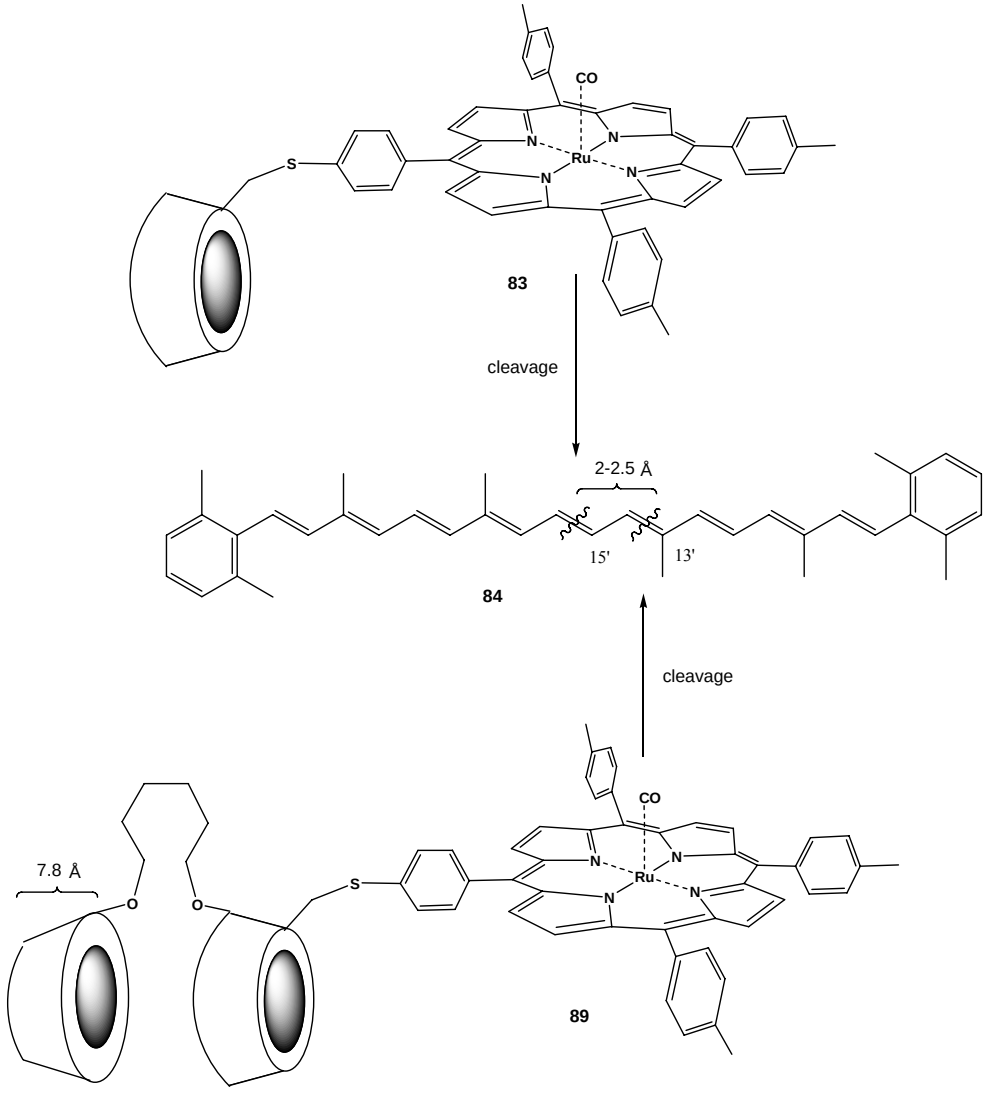


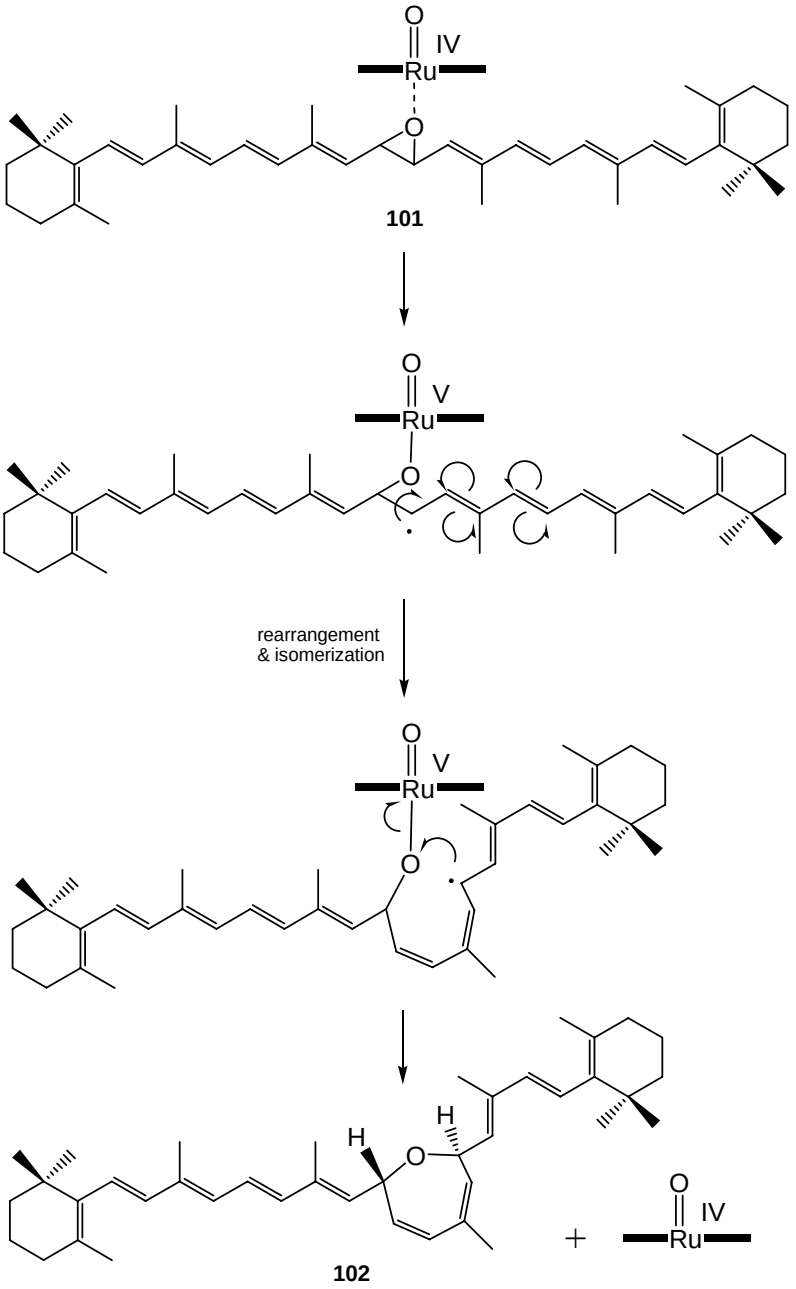
Figure 24

There may be, however, other reasons why **89** seemingly fails to show truly excentric cleavage. In order to discuss this aspect one has to consider two important facts. i) The

HPLC of the reaction mixture of **89**/TBHP/**84** (see *figure 21*/top) shows quite a number of peaks (R_t = 22-32min) displaying a retinyl chromophore (λ_{max} = 320-380nm), suggesting the presence of carotenoid-epoxides which have not been cleaved to aldehydes. This observation is related to ii) The mechanism of oxo-ruthenium/TBHP supported double bond cleavage (see *scheme 15*) comprises a fast epoxidation and a very slow ring opening fragmentation in which the ruthenium porphyrin must participate.[74, 76] This two-step mechanism poses a problem that was actually not considered when the supramolecular enzyme model for excentric cleavage was designed.

In fact we expect a "successful" system that the "initial" epoxide stays bound to oxo-ruthenium, does not move to expose another double bond for epoxidation or de-complex and has to be rebound for cleavage. Further, we anticipate that the cleavage of ruthenium-bound epoxide is insensitive to steric hindrance, *i.e.*, trisubstituted epoxides are cleaved with the same rates as disubstituted ones. However, for all these aspects a clear-cut knowledge is not available.

The situation is further complicated by the possibility that the "initial" epoxide may undergo ruthenium catalyzed reactions other than TBHP assisted ring opening and fragmentation. It was indeed shown by M. Kundu in our group (*scheme 41*) that the "central" epoxide of β,β-carotene **101** undergoes rearrangement/isomerization to the dihydro-oxepin **102** which displays a retinyl-like chromophore (λ_{max} = 332nm).[76] In these experiments it was demonstrated that it was impossible to isolate the central 15,15'-epoxy carotene. Accordingly the bulk of compounds visible in HPLC (*figure 21*) with long retention times probably comprise carotenoid epoxides and their rearrangement products.



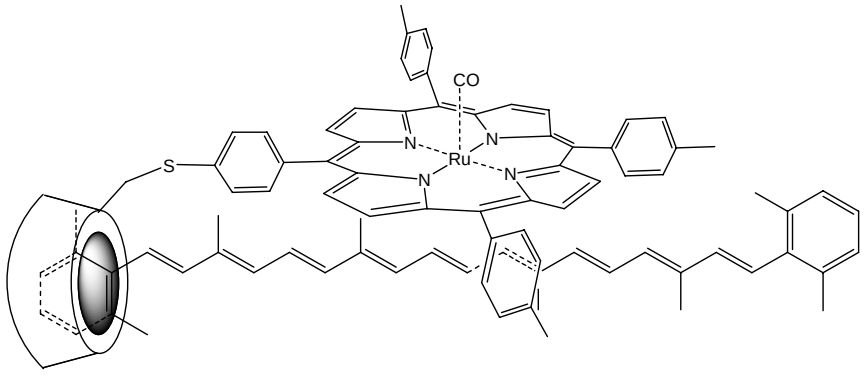
Scheme 41 – Ru-assisted transformation of the central epoxide **101** to the dihydro-oxepin **102**

In summary it looks that we asked too much from complex **89**. Obviously, for a "successful" excentric cleavage system the two reactions must be separated, *i. e.*, a complex must be found to catalyze epoxidation without performing fragmentation, then analysis of the epoxides provides the required information regarding regioselectivity. In a separate reaction, the mixture of epoxides can then be subjected to cleavage/fragmentation in order to obtain the aldehydes.

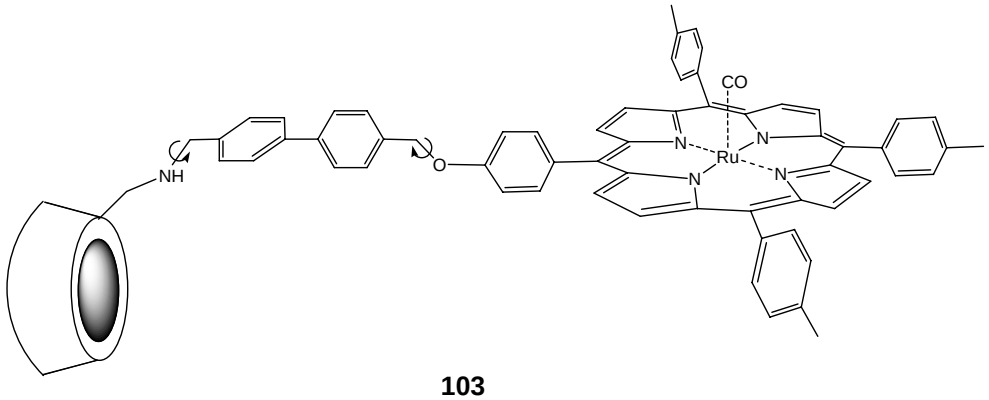
3.3 A Ru(II)-porphyrin-mono-β-CD complex, the third enzyme mimic for the excentric cleavage of carotenoids

3.3.1 Design of complex 103

As mentioned in section 3.1, it has been found that the mono-β-CD complex **83** could act as catalyst for the central cleavage of carotenoids. As shown in *figure 25*, the binding mode of **83**/**84** suggests that one of dimethylphenyl endgroups of carotenoid **84** binds inside the cavity of β-CD, leaving the C(15)-C(15') double bond exposed to the active ruthenium center.



83 / 84



103

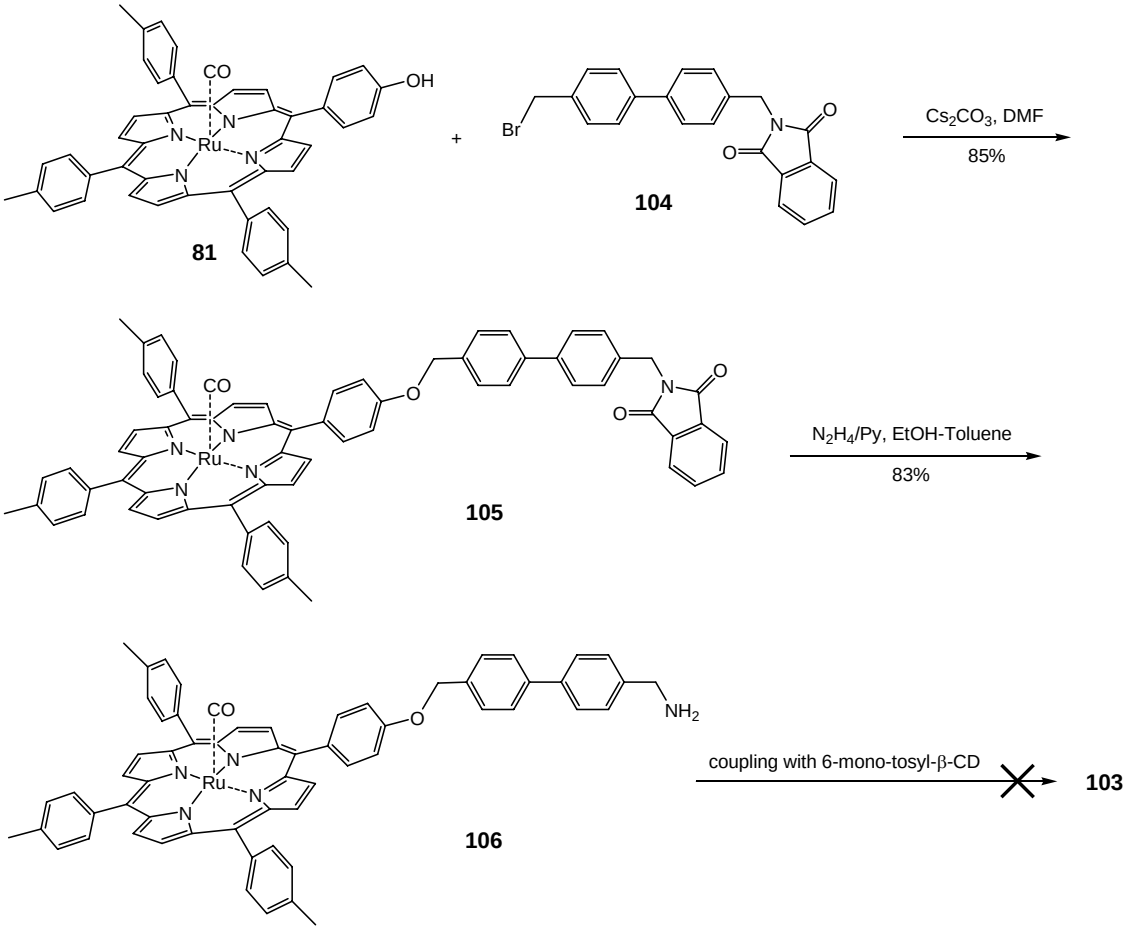
Figure 25 – Design of the third enzyme mimic **103**

Based on this hypothesis, it is reasonable to conceive that introduction of a rigid spacer between β-CD and the porphyrin moiety of complex **83** could make one of the excentric double bonds of **84** under active ruthenium center. Therefore, Ru(II)-porphyrin-mono-β-CD complex **103** was designed to mimic the excentric cleavage of carotenoids.

As shown in *figure 24*, β-CD and porphyrin moieties of **101** are linked by a biphenyl spacer. Due to free rotation at benzylic position, it is expected that complex **101** could self-adjust to the favourable conformation for the cleavage of carotenoids when the rod-like substrate binds to the β-CD receptor.

3.3.2 Synthesis of mono-β-CD complex 103

The first synthetic strategy was examined as shown in *scheme 42* below.

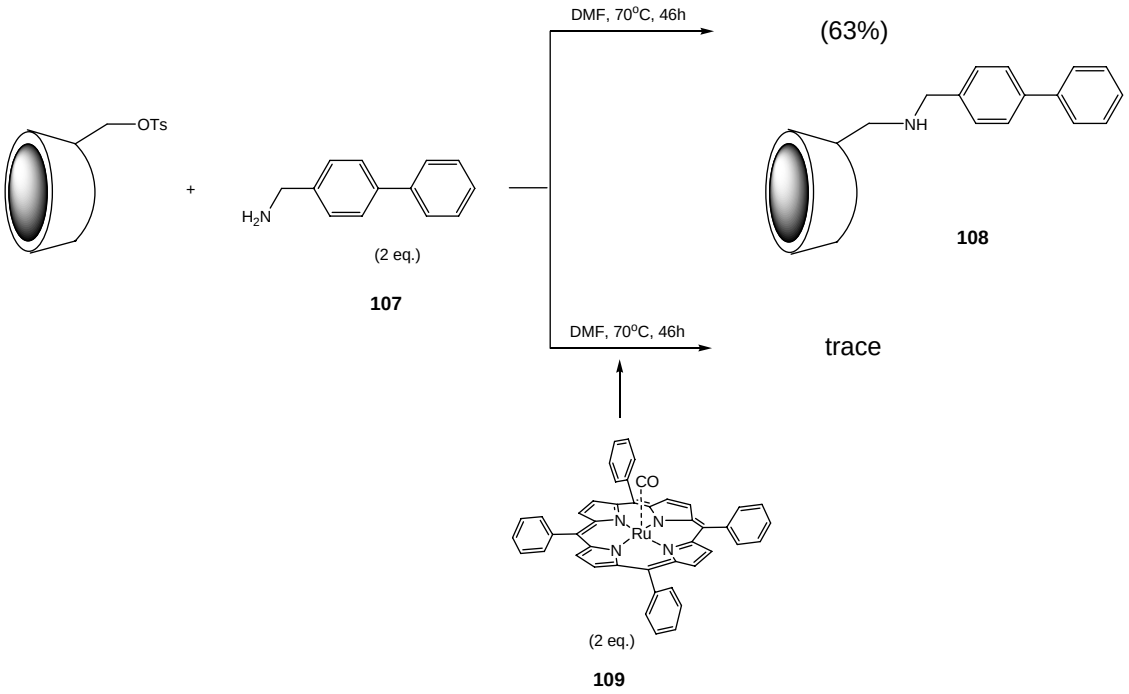


Scheme 42 - First synthetic strategy of complex **103**

Treatment of ruthenium porphyrin **81** with bromide **104** (prepared by S. Gabutti[104]) in the presence of Cs_2CO_3 as base in DMF afforded porphyrin **105** in 85.3% yield. Subsequent deprotection with hydrazine in EtOH/toluene (2:1) furnished free amino-porphyrin **106** in 82.9% yield. Pyridine was used to avoid coordination of the terminal

amino group to ruthenium. However, the reaction of porphyrin **106** with 6-mono-tosyl-β-CD did not afford the coupling product-complex **103**.

To investigate the reaction condition of coupling, a series of reactions between *p*-phenylbenzylamine **107** and 6-mono-tosyl-β-CD was examined, revealing that treatment of 6-mono-tosyl-CD with 2 equivalents of **107** in DMF for 46h at 70°C indeed provided the product **108** in 62% yield. (*scheme 43*)

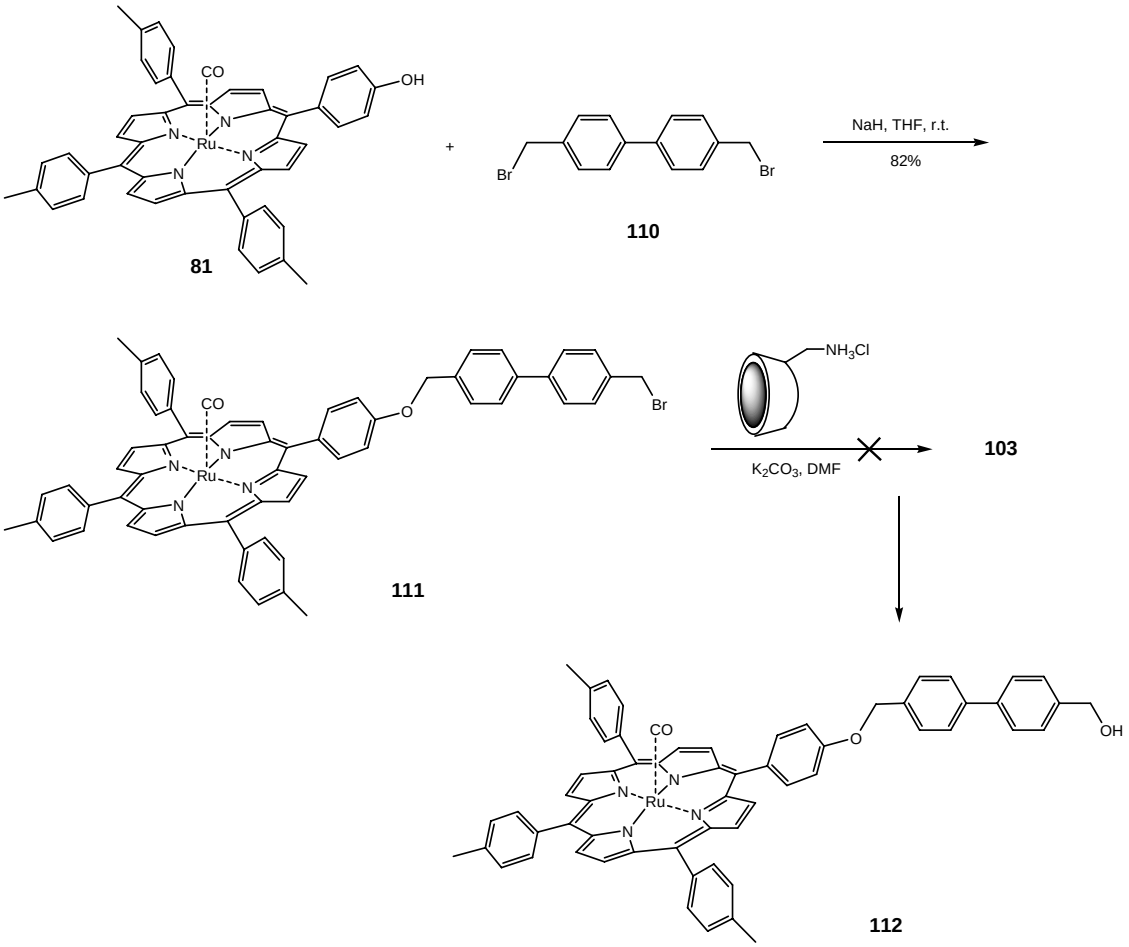


Scheme 43 – Results of mode reactions

However, under the same reaction condition, coupling of **106** with 6-mono-tosyl-β-CD did not give the desired product **103**, even if 100 equivalents of pyridine was used to try to exclude coordination of the amino group to ruthenium. This result was not understood till it was found that ruthenium porphyrin "inhibited" the coupling reaction: treatment of 6-mono-tosyl-CD with 2 equivalents of **107** in the presence of 2 equivalents of ruthenium *meso*-tetraphenylporphyrin **109** in DMF only provided traces of **108**. (*scheme 43*)

It was concluded that this "inhibition" was still due to the coordination of the amino group to ruthenium. Thus, a reversed strategy was investigated as shown in *scheme 44*

since, owing to steric hindrance, 6-deoxy-mono-amino-β-CD might not coordinate to ruthenium.



Scheme 44 - Second synthetic strategy of complex **103**

Treatment of **81** with excess of dibromide **110** (prepared by S. Gabutti[104]) at room temperature yielded benzylbromide **111**. However, the subsequent coupling reaction only provided benzylalcohol **112** probably due to hydrolysis of **111** by traces of water associated to β-CD.

3.3.3 Design and synthesis of an alternative mono-β-CD complex 113

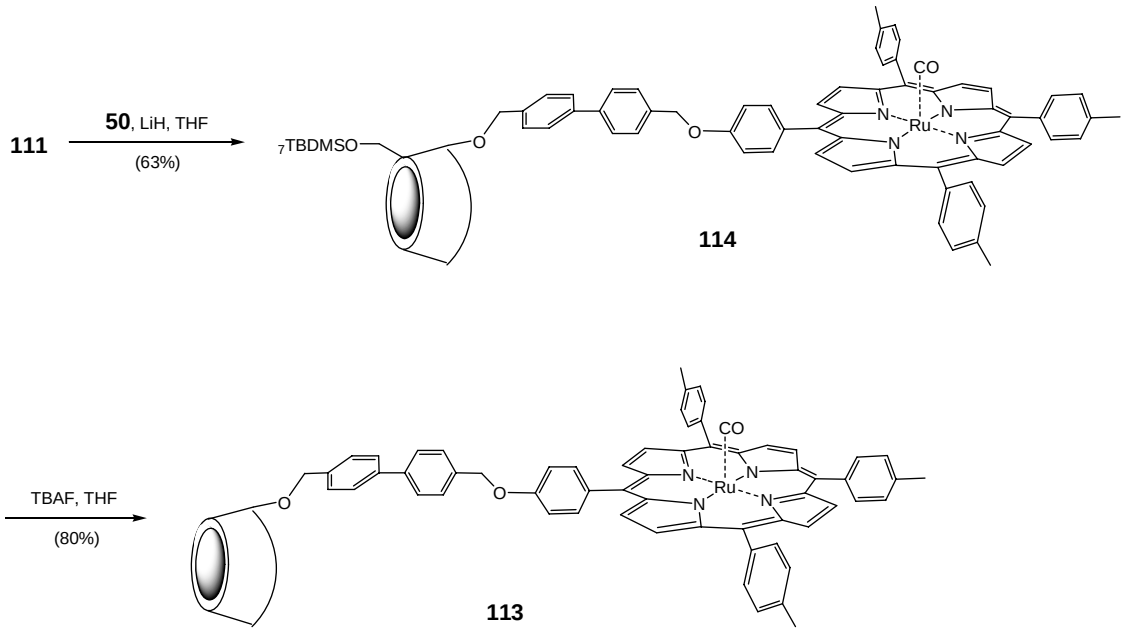
The synthetic problems to attach the ruthenium porphyrin moiety to the primary face of β-CD led us to consider an alternative complex **113**, in which the porphyrin moiety was linked to the secondary face of β-CD.

The synthetic strategy is outlined in scheme *45*. TBDMS-β-CD **50** was chosen as starting material because:

i) TBDMS-protecting groups crowded around the primary face are expected to block the entry of biphenyl group into the cavity of β-CD from the primary face.

ii) As mentioned before, C(2)-OH is the most acidic hydroxyl group, which can be selectively deprotonated to act as a strong nucleophile for coupling. Further, the possible deprotonation of several C(2)-OH groups prevents the biphenyl group to enter the cavity of β-CD from the secondary face.

Thus, TBDMS-β-CD **50** was deprotonated by 7 equivalents of LiH, and then reacted with 0.1 equivalent of bromoporphyrin **111** in refluxing THF for 40h under argon to afford protected complex **114** in 63.7% yield (calculated from **111**). During reaction, no alcohol **112** was observed. After deprotection with excess of TBAF in refluxing THF, the desired complex **113** was obtained in 80% yield.



Scheme 45 - Synthesis of complex **113**

3.3.4 Reactivity of supramolecular complex 113/TBHP towards carotenoids

Since complex **113** is insoluble in water, initial reactions were carried out in DMF. Thus, a solution of **113**, 17,17'-dinor-ϕ,ϕ-carotene **84** (10 eq.) and TBHP (300 eq.) in DMF was stirred at 31°C under argon. At intervals, aliquots of the reaction mixture were subjected to HPLC analysis (C-30). Surprisingly, the result turned out to be central cleavage as shown in *figure 26*.

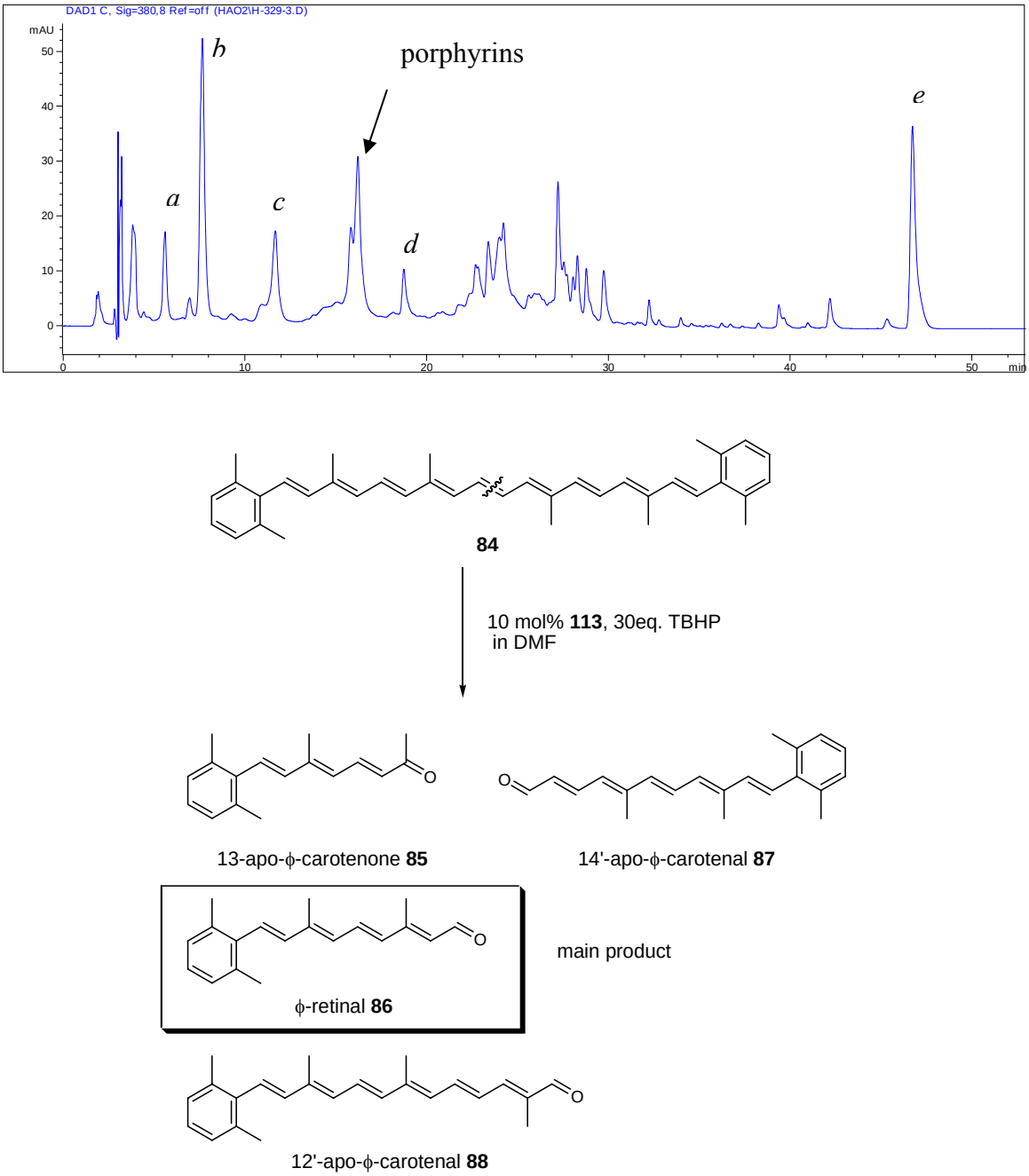
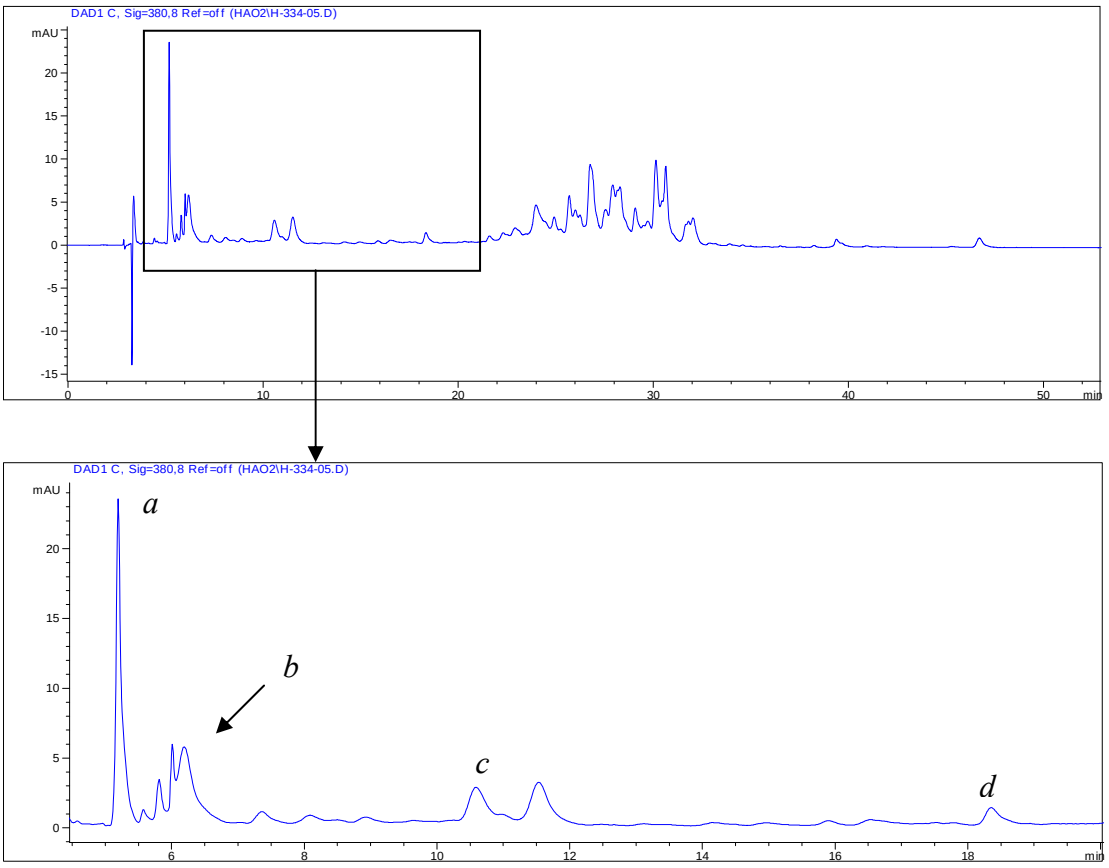


Figure 26 – HPLC chromatogram (C-30) of reaction of **84** with 10% **113** in DMF after 3h (λ_{det} = 380nm). Peak *a-e* are 13-apo-ϕ-carotenone **85**, ϕ-retinal **86,** 14'-apo-ϕ-carotenal **87**, 12'-apo-ϕ-carotenal **88** and substrate **84** respectively.

In contrast, a de-centralized cleavage was obtained under biphasic reaction conditions afterwards. It was found that MeOH could be a co-solvent to increase solubility of complex **113** in water. Thus, to a solution of complex **113** in MeOH/H_2O (1:9) was added the solution of carotenoid **84** in hexane/$CHCl_3$ (9:1) to produce a biphasic system. With TBHP (300 eq. to **113**) as cooxidant, this biphasic mixture was stirred vigorously at 31°C. The HPLC analysis is shown in *figure 27*.



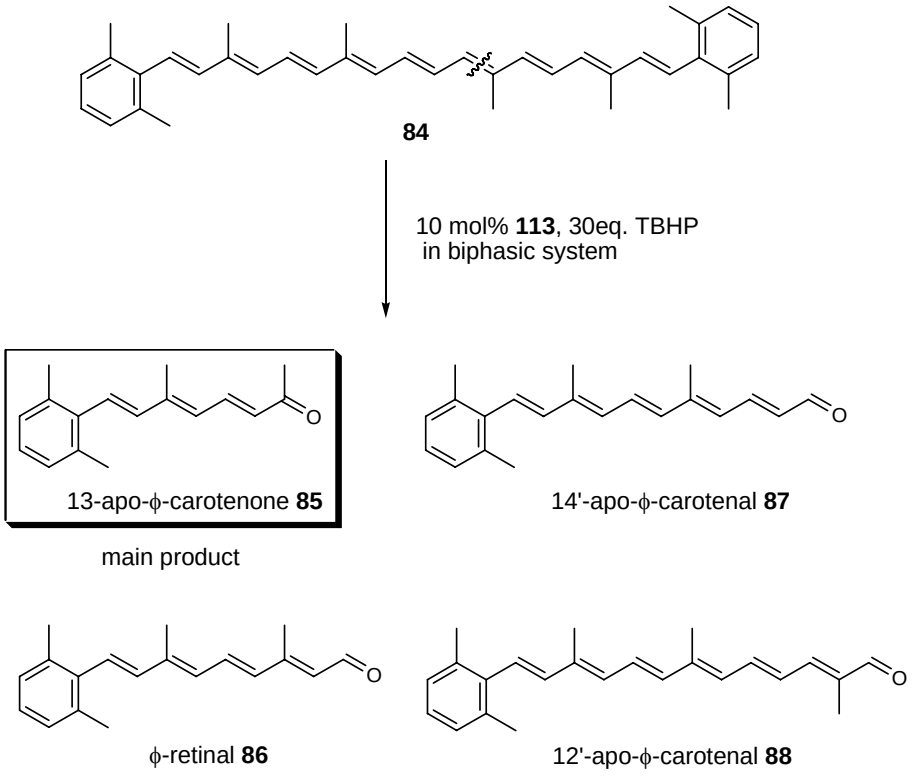
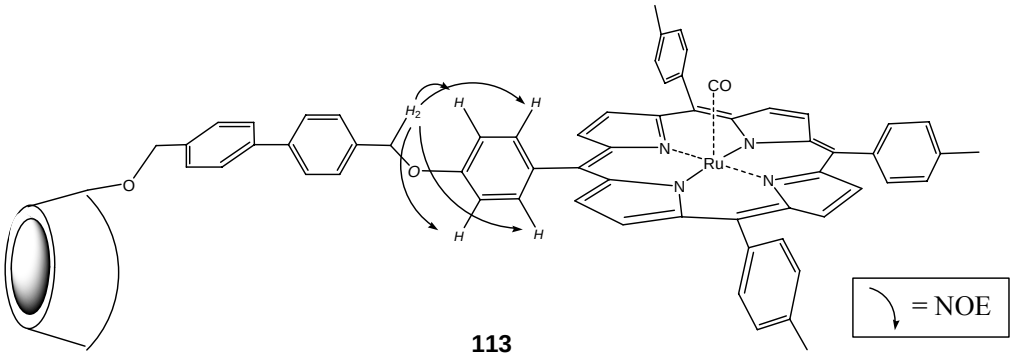
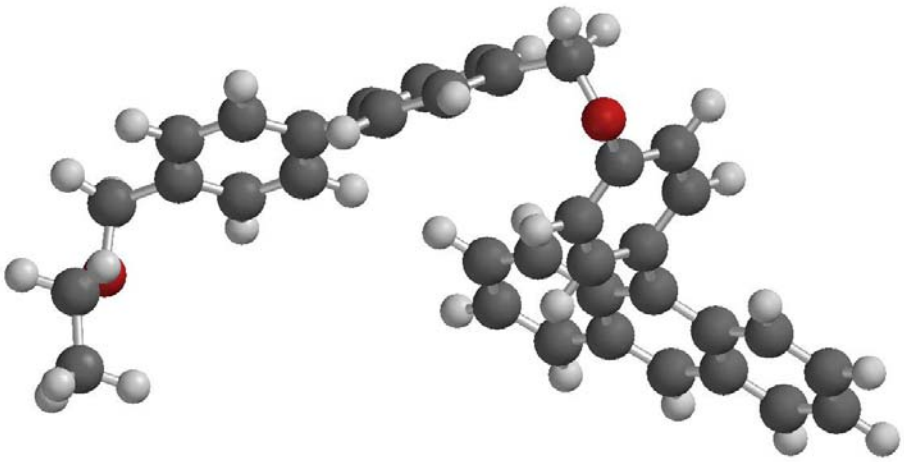


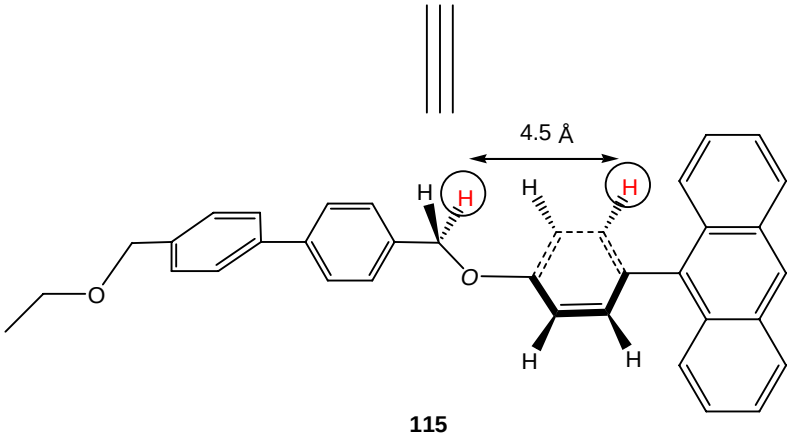
Figure 27 – HPLC chromatogram (C-30) of reaction of **84** with 10% **113** in biphasic system (λ_{det} = 380nm). Peak *a*, *c*, *d* are **85**, **87** and **88** respectively. Peak *b* is from **86** together with others.

These results revealed that the cleavage catalyzed by complex **113** is solvent dependent. The cleavage result obtained in DMF was suggested by NMR spectra of **113** in DMSO-D_6 (*figure 28*). NOEs were observed between the spacer and the *meta*-protons of the adjacent phenyl ring of porphyrin unit. It is suspected that, due to the rotation of C-O ether bond, spacer-porphyrin moiety of complex **113** stays in a partially folded but not a rodlike conformation in DMF, reducing the distance between ruthenium center (reactive site) and β-CD (substrate binding site) so that complex **113** acts as a catalyst for the central cleavage of carotenoids in DMF. Further, this hypothesis was confirmed by computational calculation of a model structure **115** with the *Titan* program, revealing that the distance between protons (marked in red) is 4.5 Å.



113





115

Figure 28 – NOEs between spacer and adjacent phenyl ring of porphyrin unit in **113** (DMSO-D_6) and computational calculation of **115**

4 Summary

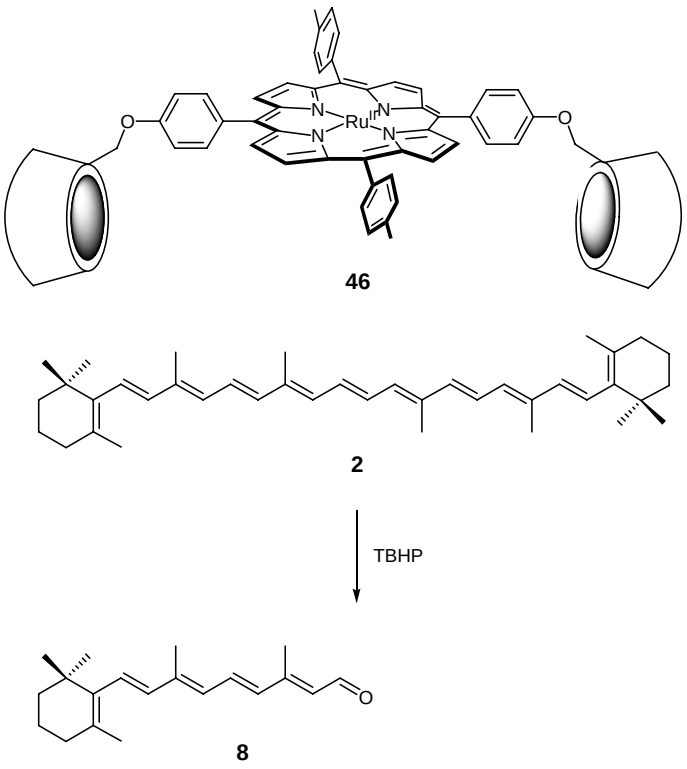
All naturally occurring vitamin A derives from enzymatic oxidative cleavage of β,β-carotene or other carotenoids with pro-vitamin A activity. Two metabolic pathways of β,β-carotene to retinal (vitamin A aldehyde) have been proposed: the central cleavage providing 2 mol of retinal, and the excentric cleavage yielding apo-β-carotenals first, which are subsequently transformed into retinal. Each pathway is used preferentially in mammalian β,β-carotene metabolism, depending on the specific tissue.

Due to their unusual reactivity to regioselectively cleave one double bond in a conjugated polyene of very hydrophobic substrates, these enzymes present a formidable challenge to chemists interested in enzyme catalysis.

Regarding the central cleavage of carotenoids, considerable progress has been accomplished both with respect to the identification/isolation of the proteins from various tissues/species and concerning the synthesis of structural remote enzyme models which effectively mimic the enzymatic reaction.

The excentric oxidative cleavage of carotenoids is not only significant to mammals but even more important in the plant kingdom providing metabolites used as fragrances and for defensive mechanism.

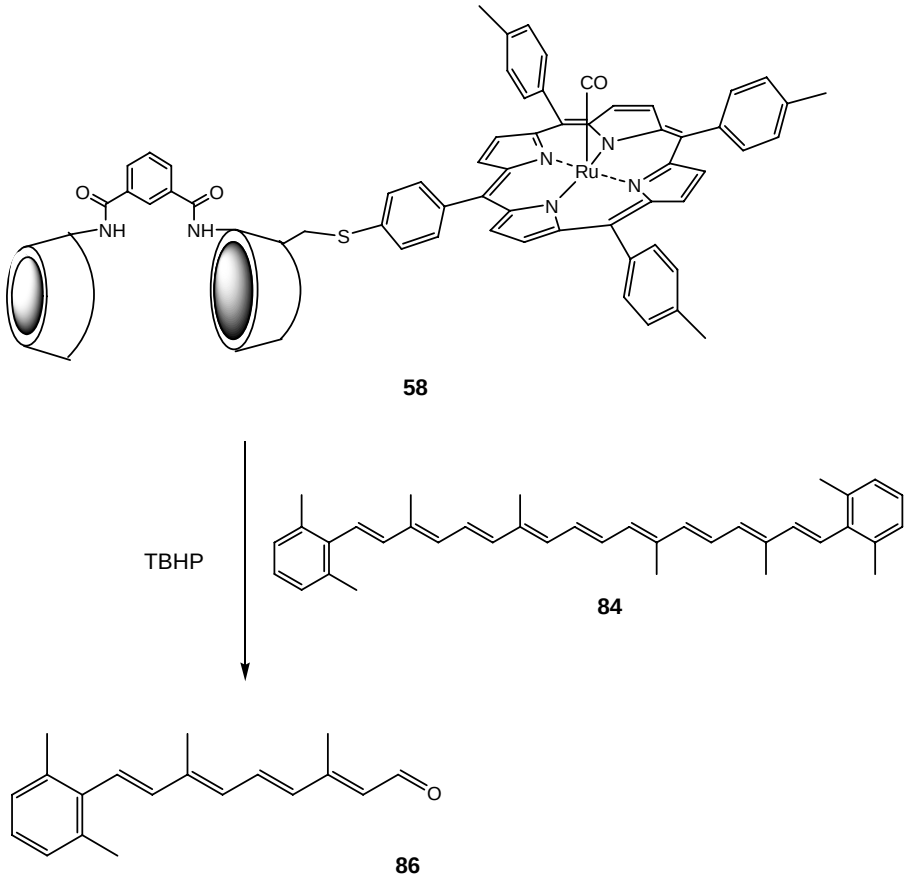
The latter research area is overall less advanced than the former in particular no enzyme mimics have been available at the outset of this thesis.



Scheme 46 – The central cleavage of β,β-carotene **2** to retinal **8** can be mediated by the β-CD based enzyme mimic **46**.

Encouraged by the success of complex **46** as an enzyme mimic for central cleavage of carotenoids (*scheme 46*), we decided to develop related supramolecular models to mimic the reactivity and selectivity of the enzymes catalyzing the excentric cleavage of carotenoids. Within this context, three β-CD linked ruthenium porphyrin complexes have been designed and synthesized.

The first generation enzyme mimic, complex **58**, designed by computer modelling, comprises a rigidly linked dimeric β-CD moiety as the substrate recognition site and a ruthenium porphyrin, attached to one of the primary faces of β-CDs, as site of reactivity.



Scheme 47 – The reactivity of **58**/TBHP towards 17,17-dinor-ϕ,ϕ-carotene **84**

However, the reactivity of complex **58** towards 17,17'-dinor-ϕ,ϕ-carotene **84** reveals that this supramolecular model actually mimics the central cleavage pathway (*scheme 47*), which is consistent with the crystal structure of β-CD dimer moiety obtained later (*figure 29*). Owing to the modification of β-CD *via* 2,3-manno-epoxide and intramolecular self-assembling by hydrogen-bonding network, the diamide linker sits between β-CDs, blocking the entrance of the substrate to the second β-CD unit.

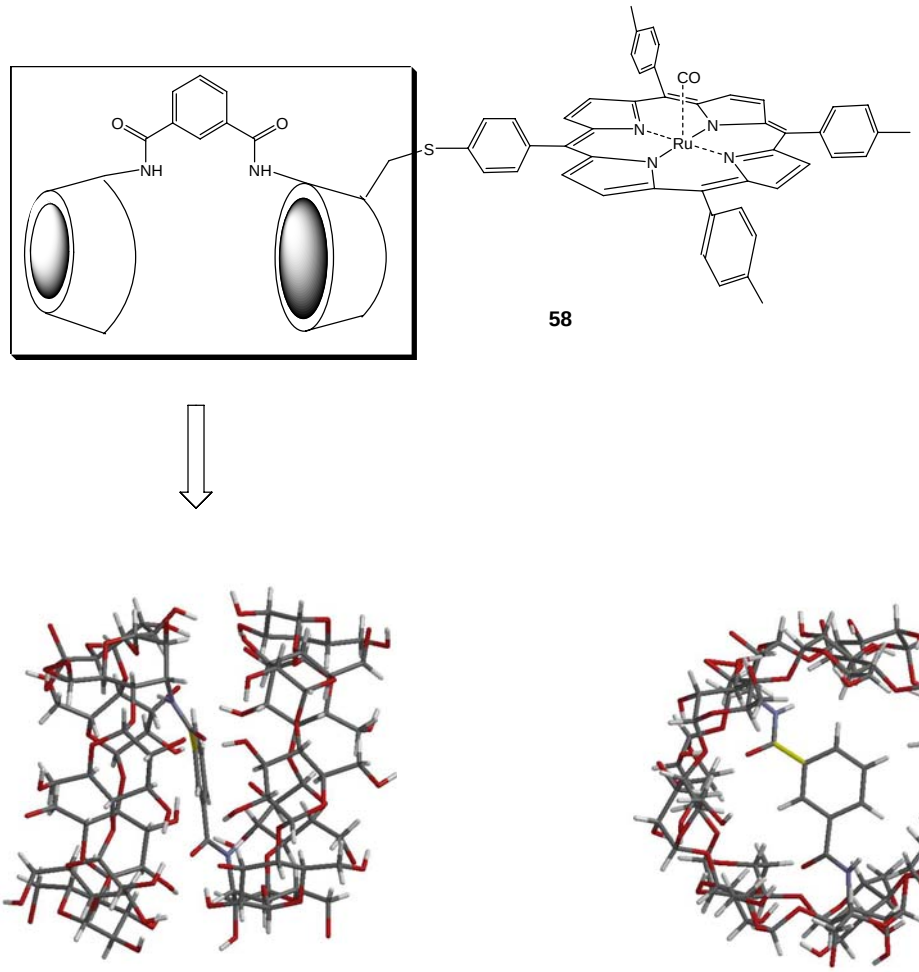
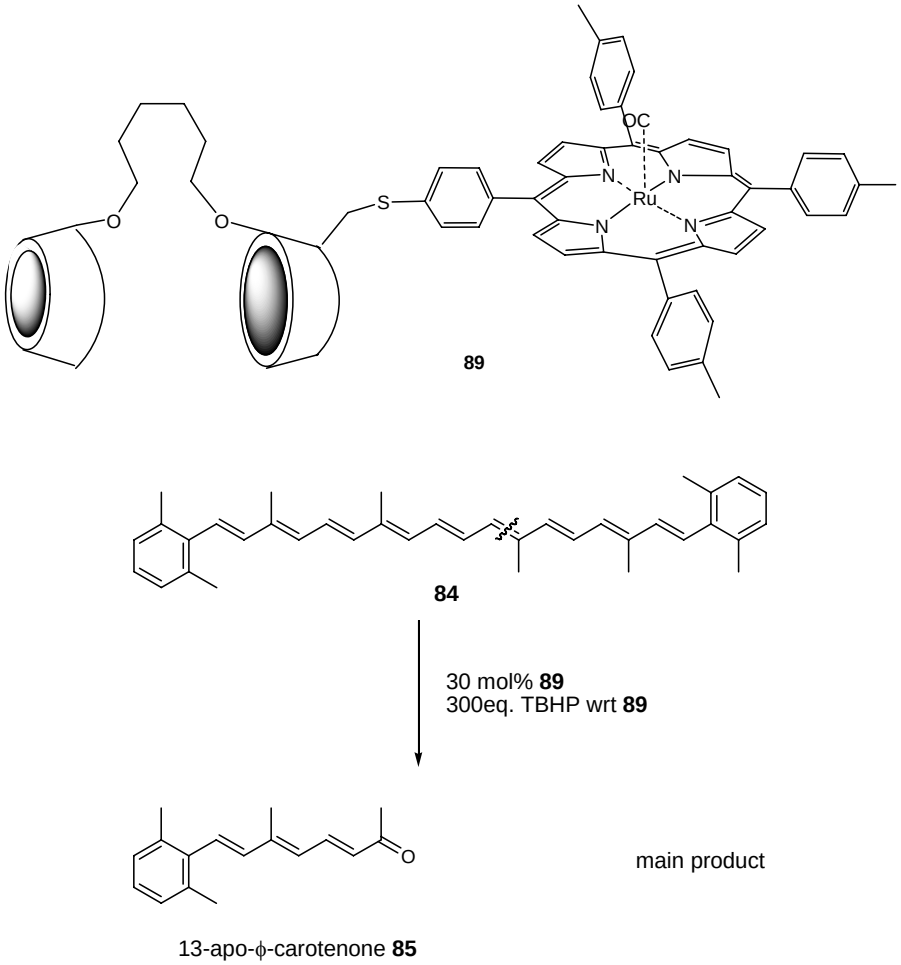


Figure 29 – Crystal structure of dimeric β-CD moiety of **58**

In order to avoid "capping" of β-CD by the linker, the second complex **89** was designed and synthesized (*scheme 48*). The dimeric β-CD moiety is linked by a C_6-flexible chain directly on C(2). It is conceived that the linker might act as a loop outside the dimeric β-CD moiety, holding β-CDs together *via* a hydrogen-bonding network to produce an extended cavity as the substrate binding site. The results with **89**/TBHP reveals that this complex cleaves carotenoids at C(13')-C(14') double bond, mimicking excentric cleavage to a certain extent.



Scheme 48 – Cleavage of carotenoid **84** to 13-apocarotenone **85** can be mediated by β-CD based enzyme mimic **89**

The fact that mono-β-CD complex **83** could mimic the central cleavage of carotenoids was then used to design and prepare complex **113** which contains a rigid biphenyl linker, attached to the secondary face of β-CD, to increase the distance between the β-CD unit and the ruthenium porphyrin moiety (*figure 30*).

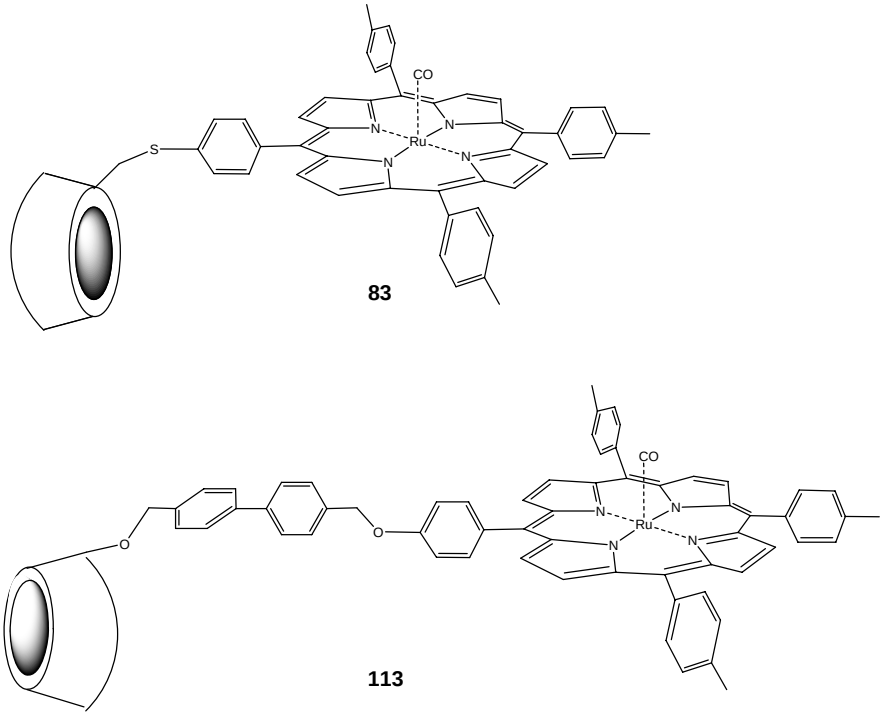


Figure 30 – Structures of complexes **83** and **113**

The reactivity of complex **113**/TBHP towards carotenoid **84** is solvent dependent. A central cleavage was observed in DMF, whereas an excentric cleavage at C(14')-C(13') double bond was observed in the biphasic system. NOE spectra and computational calculation suggests that complex **113** adopts a partial folded conformaion in DMF, reducing the distance between β-CD and active ruthenium center.

In conclusion the work presented here comprises the synthesis of several new monomeric and dimeric β-CD-ruthenium porphyrin complexes which in the presence of TBHP show the reactivity required to cleave conjugated double bond to aldehydes, depending on the relative orientation/distance of the substrate binding site (β-CD) to the reactive site (Ru=O). These complexes, binding carotenoids in a supramolecular fashion, cleave the symmetric polyolefins either at the central C(15)-C(15') or slightly excentric C(13')-C(14') double bond. As such, these complexes mimic to a certain extent both classes of enzymes which metabolize carotenoids in nature.

5 Experimental Part

5.1 General

Solvents and Reagents

Reagents were used as received from *Fluka AG,* and *Aldrich Chemie* unless otherwise stated. Chemicals of the quality *purum*, *purum p. a.* or >98% were used without further purification. Retinal **8**, 12'-apo-β-carotenal and 14'-apo-β-carotenal for HPLC reference were obtained as a gift from *F. Hoffman - La Roche AG*. 6-*O*-monotosyl-β-CD and 6-monodeoxy-6-monoamino-β-CD hydrochloride were purchased from *CycloLab Ltd* (Budapest, Hungary).

Solvents for chromatography and extractions were distilled prior to use. Dry dichloromethane (CH_2Cl_2) and dimethylformamide (DMF) and decalin were distilled from CaH_2, diethyl ether (Et_2O) and tetrahydrofuran (THF) from Na/benzophenone, MeOH from Mg, pyridine from KOH. All freshly dried solvents were stored over activated molecular sieves (4Å). Further solvents used for reactions corresponded to the quality *puriss p. a.*, *abs.*, *over Molecular Sieves* from *Fluka AG*. HPLC-grade solvents were purchased and used for analytical or semi-preparative RP-HPLC. Degassed solvents for reactions under oxygen-free condition were obtained by three freeze-pump-thaw cycles in high-vacuum.

For an inert atmosphere Argon 56 (< 4 ppm other gases) from *Carbagas AG* (Lenzburg, Switzerland) or Argon 6.0 (<1.5 ppm other gases) from *PanGas AG* (Dagmersellen, Switzerland) were used.

Materials and Instruments

Solvents were removed with a *Büchi* (Switzerland) rotary evaporator (Waterbath 461, Rotavapor RE 111 and Vauum Controller 168) and a MZ 2C membrane pump (*Vacuubrand*). For cooling a mixture of EtOH and water was kept at 0°C with a UKW 300 thermostat (*Vacuubrand*).

For weighing compounds and reagents Mettler (Switzerland) balances P1200 (>1g), AE 163 (<1g), and AX205 (<100mg) were used.

A high-vacuum pump D5E from *Trivac* (Köln, Germany) was used for drying compounds and degassing solvents.

Slow addition of reagents to a reaction mixture was achieved with a Precidor 5003 syringe pump (*Informs HT*, Switzerland).

For all non-aqueous reactions glassware was flame dried either under vacuum or argon overpressure, and the atmosphere was exchanged by three cycles of evacuating and flushing with argon.

Melting points (mp) were determined on a *büchi* 510 apparatus and are uncorrected. For the porphyrins and CDs, the melting points are > 250°C and were not determined.

Chromatographic Methods

Analytical thin layer chromatography (TLC) was performed on 0.25 mm precoated glass plates (5×10 cm, silica gel 60 F_{254}, *Merck AG*, Germany), 0.25mm precoated glass plates (5×10 cm, aluminium oxide 60 F_{254}, *Merck AG*, Germany), or on 0.25 mm precoated glass plates (5×10 cm, RP-18 F_{254s}, *Merck AG*, Germany). Compounds were detected at 254 nm (UV) or at 366 nm (fluorescence). For carbohydrates, compounds were visualized by *p*-anisaldehyde dip. *Description*: TLC (solvent): R_f.

For normal phase **column chromatography** silica gel 60 from *Merck* (0.040-0.063 mm, 230-400 mesh) or aluminum oxide 90 from *Merck* (standardized (activity II-III), 0.063-0.2 mm, 70-230 mesh) were used. For reversed phase column chromatography, RP-18 silica gel (fully endcapped) from *Fluka* (0.040-0.063mm, 230-400 mesh) was used. For ion-exchange column chromatography, Amberlite CG-50 from *Fluka* (weak acidic, H^+-Form, 100-200 mesh) was used. Flash chromatography was performed under pressure from N_2.

Analytical reversed phase HPLC (synthesis) was performed on LiChrospher® 100 RP-18 silica gel from *Merck* (5 μm particle size, 4×250 mm column) with nanopure water and HPLC-grade solvents. Analytical reversed HPLC (enzyme mimic reactions) was performed on LiChrospher® 100 RP-18 silica gel from *Bischoff* (Leonberg,

Germany) (5 μm particle size, 4.6×125 mm column) or YMC C30 from *YMC Europe GMBH* (Schermbeck, Germany) (3 μm particle size, 4.6×250 mm column) according to substrate. **Semi-preparative HPLC** was performed on LiChrospher® 100 RP-18 silica gel from *Merck* (10 μm particle size, 10×250 mm column). HPLC-System: 1) *Merck Hitachi* LaChrom system (analytical for synthesis or semi-preparative) with Solvent degasser L-7612, Pump L-7100, UV detector L-7400, Interface D-7000; 2) *Agilent* 1100 Series 1100 HPLC system (analytical for synthesis and enzyme mimic reaction) with Solvent degasser G1322A, BinPump G1312A, Autosampler G1313A, Thermostatic column housing G1316A, Diode array UV detector G1315B).

Spectroscopic Methods

Ultra violet-Visible absorption spectra (UV/Vis) were recorded on an *Agilent* 8453 Diode Array spectrophotometer using optical 110 QS *Hellma* cuvettes (10 mm light path). Description: UV/Vis (solvent): wavelength of maxima (λ_{max}) in nm (relative extinction coefficient in %).

Infrared spectra (IR) were measured on a *Perkin-Elmer* 1600 series FTIR spectrometer in KBr or on a *FTIR-8400S* from *SHIMADZU*. Description: IR (medium): wavenumbers of transmission maxima in cm^{-1}.

Electron impact mass spectra (EI-MS) and **fast atom bombardment mass spectra (FAB-MS)** were measured by *Dr. H. Nadig* on a *Finnigan MAT 95Q* spectrometer and *Finnigan MAT 8400* spectrometer in the mass spectrometry laboratory of the department. As matrix for FAB-MS *m*-nitro-benzenealcohol (NBA) or glycine (GLY) was used. **Electron spray ionization mass spectra (ESI-MS)** was record on a *Bruker* Esquire 3000^{plus}. For **matrix-assisted laser desorption/ionization mass spectra** in conjunction with **time of flight** mass analysis **(MALDI-TOF-MS)** a *Perspective Biosystems Vestec Mass Spectrometry Products* Voyager™ Elite Biospectrometry™ Research Station was used. As matrix for MALDI-TOF-MS, 0.1M of 2,5-dihydroxybenzoic acid in MeCN/H_2O/EtOH = 50:45:5 was used. Description: MS (solvent): mass peaks in *m/z* (relative intensity in %). Peaks with an intensity of less than 5% were not considered.

^{1}H-Nuclear magnetic resonance spectroscopy (^{1}H-NMR) was performed using either a *Bruker* av250 (250MHz), *Bruker* DPX-NMR (400MHz), *Bruker* DRX-500 (500MHz) or *Bruker* DRX-600 (600MHz) spectrometer. Solvents for NMR were obtained from *Cambridge Isotope Laboratories* (Andover, MA, USA). $CDCl_3$ was filtered through basic alumina prior to use. All spectra were recorded at room temperature. If necessary for the interpretation correlated spectra like COSY, TOCSY, NOESY and ROESY were recorded also. Descripition: ^{1}H-NMR (frequency, solvent): δ_H in ppm relative to TMS or residual solvent peaks (peak multiplicity: *s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet, *m* = multiplet, *br* = broad; coupling constants *J* in Hertz).

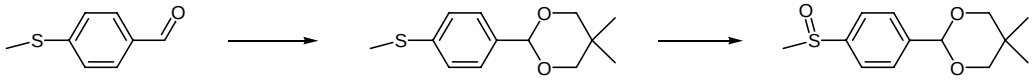
^{13}C-Nuclear magnetic resonance spectroscopy (^{13}C-NMR) was ^{1}H-decoupled and recorded on a *Bruker* DPX-NMR (100MHz), *Bruker* DRX-500 (125 MHz) spectrometer. For the assignment of carbons ATP, DEPT, HETCOR, HMQC and HMBC experiments were carried out if essential. Descripition: ^{13}C-NMR (frequency, solvent): δ_C in ppm relative to residual solvent peaks.

LC-MS analysis was preformed on LiChrospher® 100 RP-18 silica gel from *Bischoff* (Leonberg, Germany) (5 μm particle size, 4.6×125 mm column), using the combination of *Agilent* 1100 Series 1100 HPLC system and *Bruker* Esquire 3000^{plus} ESI-MS system.

Single crystal *X*-ray structures were determined by *Dr. Markus Neuburger* and *Dr. Silvia Schaffner* in the chemical crystallography laboratory of the department. Data collection was carried out on a *Nonius KappaCCD* diffractometer using the COLLECT software suite. The usual corrections were applied. No absorption correction was determined. The structures were solved by direct method using the program DIRDIF-99. Anisotropic least-squares refinement was carried out on all non-hydrogen atoms using the program CRYSTALS. Hydrogen atoms were in calculated positions.

5.2 Synthesis

2-[4-(Methylsulfoxy)phenyl]-5,5'-dimethyl-1,3-dioxane **66**[80]



To a solution of 4-(methylthio)benzaldehyde **67** (11.42g, 75mmol) and neopentyl glycol (8.59g, 82.5mmol) in toluene (150ml) was added *p*-toluenesulfonic acid (95mg, 0.5mmol). The resulting suspension was heated at reflux with a Dean-Stark trap for 2h. At room temperature, the reaction mixture was washed with saturated $NaHCO_3$ (aq.) and water, dried over Na_2SO_4 and evaporated in *vacuo* to afford crude product, which was recrystallized from hexane to give 2-[4-(methylthio)phenyl]-5,5'-dimethyl- 1,3-dioxane (16.2g, 91%) as white needle crystals.

mp: 73.8-74.8°C (Lit.[80] 74-75°C)
TLC (ethyl acetate/hexane =1:2 + 1% Et_3N): R_f= 0.75
^{1}H-NMR (400MHz, $CDCl_3$): 7.35 (AA'BB', 4H, Ar-*H*), 5.36 (*s*, 1H, ArC*H*), 3.75 (AB/2, *J* = 11.1, 2H, CH_2), 3.64 (AB/2, *J* = 11.1, 2H, CH_2), 2.48 (*s*, 3H, CH_3S), 1.29 (*s*, 3H, CH_3), 0.79 (*s*, 3H, CH_3)
^{13}C-NMR (100MHz, $CDCl_3$): 139.6, 135.6, 127.0, 126.9, 101.8, 78.1, 30.6, 23.5, 22.3, 16.3
EI-MS: 238.2 $[M]^{+\cdot}$ (44), 152.2 (100), 124.2 (20), 109.1 (14)

A solution of 2-[4-(methylthio)phenyl]-5,5'-dimethyl-1,3-dioxane (1g, 4.2mmol) in CH_2Cl_2 (8ml) was cooled to -15°C and stirred vigorously and a suspension of *m*-CPBA (1.2g, 4.7mmol) in CH_2Cl_2 (6ml) was added in portions. The mixture was stirred at 0°C for 2h. $Ca(OH)_2$ and Na_2SO_4 were added and stirring was continued for 1h at room temperature. After filtration and evaporation, the resulting colorless oil was purified by column chromatography (SiO_2, ethyl acetate/hexane = 2:1) to give the title compound (0.9g, 85%) as white needle crystals.

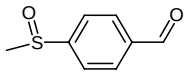
mp: 116.9-117.4°C (Lit.[80] 116-117°C)
TLC (ethyl acetate): R_f= 0.4

^{1}H-NMR (400MHz, $CDCl_3$): 7.7-7.64 (*m*, 4H, Ar-*H*), 5.44 (*s*, 1H, ArC*H*), 3.78 (AB/2, *J* = 11.1, 2H, CH_2), 3.67 (AB/2, *J* = 11.1, 2H, CH_2), 2.69 (*s*, 3H, CH_3S), 1.29 (*s*, 3H, CH_3), 0.81 (*s*, 3H, CH_3)

^{13}C-NMR (100MHz, $CDCl_3$): 146.7, 141.9, 127.7, 123.8, 101.2, 78.1, 44.5, 30.7, 23.4, 22.2

EI-MS: 254 $[M]^{+\cdot}$ (100), 239 (68), 168 (50), 153 (37), 124 (50), 69 (27), 41 (16)

4-(Methylsulfoxy)benzaldehyde **65**



To a suspension of acetal **66** (0.63g, 2.5mmol) in water (2ml) was added TFA (6.5ml). The resulting solution was stirred overnight at room temperature and then poured into water (40ml) and extracted with toluene. The combined extractions were washed with water and evaporated in *vacuo*. The residue was purified by column chromatography (SiO_2, ethyl acetate) to give the title compound (0.34g, 80%) as white solid.

mp: 87.4-88.4°C (Lit.: 81-82°C,[82] 85-86°C[81])

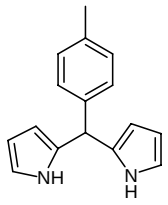
TLC (ethyl acetate): R_f = 0.29

^{1}H-NMR (400MHz, $CDCl_3$): 10.1 (*s*, 1H, C*H*O), 7.94 (AA'BB", 4H, Ar-*H*), 2.8 (*s*, 3H, CH_3)

^{13}C-NMR (100MHz, $CDCl_3$): 191.5, 152.9, 138.6, 130.8, 124.6, 44.2

EI-MS: 168 $[M]^{+\cdot}$ (100), 153 (43), 125 (15), 121 (10), 97 (9), 77 (9)

p-Tolyldipyrromethane **63**[83, 84]



To a solution of *p*-tolylaldehyde (1.85ml, 15.6mmol) in pyrrole (27ml, 390mmol) was added TFA (0.12ml, 1.56mmol). The reaction was stirred at room temperature in dark

under argon for 4.5h and then quenched with saturated $NaHCO_3$ (aq., 50ml). The resulting mixture was extracted with CH_2Cl_2 and the combined extractions were washed with brine, dried over Na_2SO_4 and evaporated in *vacuo* to afford a dark oil. Column chromatography (SiO_2, ethyl acetate/hexane = 1:2 + 1% Et_3N) gave crude product, which was recrystallized from ethyl acetate/hexane to afford the title compound (2.3g, 62%) as prey solid.

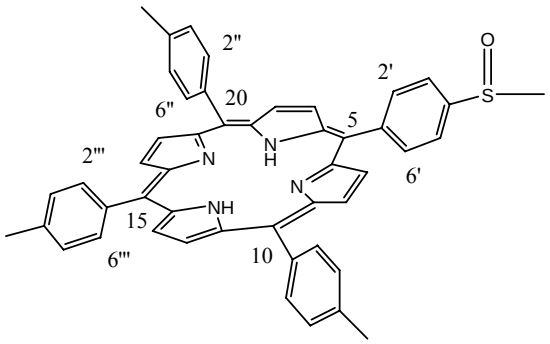
TLC (ethyl acetate/hexane = 1:2): R_f= 0.38

^{1}H-NMR (400MHz, $CDCl_3$): 7.9 (*brs*, 2H, N*H*), 7.12 (*m*, 4H, Ar-*H*), 6.69 (*m*, 2H, pyro-H_2), 6.15 (*m*, 2H, pyro-H_4), 5.92 (*m*, 2H, pyro-H_3), 5.44 (*s*, 1H, meso-*H*), 2.33 (*s*, 3H, CH_3)

^{13}C-NMR (100MHz, $CDCl_3$): 139.5, 137, 133.1, 129.7, 128.7, 117.5, 108.8, 107.5, 44, 21.4

ESI-MS (CH_2Cl_2/MeOH): 259.0 $[M+Na]^+$

[5-(*p*-Methylsulfoxy)phenyl-10,15,20-tri(*p*-methylphenyl)]porphyrin **61**



To a solution of **63** (200mg, 0.85mmol), **65** (71mg, 0.42mmol) and *p*-tolylaldehyde (51mg, 0.42mmol) in CH_2Cl_2 (80ml) was added TFA (0.12ml, 1.5mmol). The reaction was stirred for 2h at room temperature under argon and then the solution of DDQ (300mg, 1.32mmol) in THF (3ml) was added. The resulting mixture was heated at reflux for 1h. The reaction mixture was filtered through silica gel (CH_2Cl_2+1%Et_3N, then CH_2Cl_2/MeOH = 2:1) to afford the crude product (second band), which was further purified by column chromatography (basic alox, CH_2Cl_2) to give the title compound (43.1mg, 14.2%) as purple solid.

TLC (basic alox, CH_2Cl_2): R_f= 0.25

UV/Vis (MeOH, λ): 415 (100, *Soret*), 513 (4)

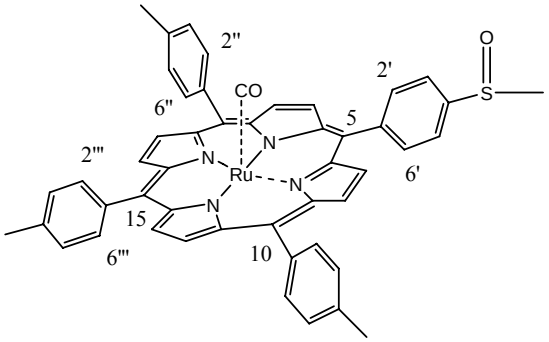
^{1}H-NMR (500MHz, $CDCl_3$): 8.90 (*d*, *J* = 4.8, 2H, C(2, 8)-H), 8.88 (*s*, 4H, C(12, 13, 17, 18)-H), 8.76 (*d*, *J* = 4.8, 2H, C(3, 7)-H), 8.38 (AA'BB', *J* = 8.3, 2H, C(2', 6')-H), 8.10 (*d*, 6H, C(2'', 6'', 2''', 6''')-H), 8.03 (AA'BB', *J* = 8.1, 2H, C(3', 5')-H), 7.56 (*d*, 6H, C(3'', 5'', 3''', 5''')-H), 3.05 (*s*, 3H, C*H*$_3$S), 2.71 (*s*, 9H, ArC*H*$_3$), -2.75 (*s*, 2H, N*H*)

^{13}C-NMR (125MHz, $CDCl_3$): 145.59 (C(1')), 145.23 (C(4')), 139.27 (C(1''')), 139.23 (C(1'')), 137.60 (C(4'')), 137.58 (C(4''')), 135.41 (C(2', 6')), 134.65 (C(2'', 6'', 2''', 6''')), 132-130 (*brs*, C(2, 3, 7, 8, 12, 13, 17, 18)), 127.61 (C(3'', 5'')), 127.59 (C(3''', 5''')), 122.17 (C(3', 5')), 120.91 (C(15)), 120.61 (C(10, 20)), 117.81 (C(5)), 44.19 (*C*H$_3$S), 21.67 (Ar*C*H$_3$)

MALDI-TOF-MS: 718.9 [M], 702.9 [M-16]

ESI-MS (CH_2Cl_2/MeOH): 741.0 $[M+Na]^+$, 719.0 $[M+H]^+$, 717.2 $[M-H]^-$

β-Carbonyl [5-(*p*-methylsulfoxyphenyl)-10,15,20-tri(*p*-methylphenyl)-porphyrinato] ruthenium **62**



To a solution of porphyrin **61** (43.9mg, 0.061mmol) and acetal **66** (0.196mg, 0.733mmol) in DMF (25ml, degassed) was added trirutheniumdodecacarbonyl (78.1mg, 0.122mmol). The reaction was heated at reflux for 3h under argon. After evaporation of DMF in high *vacuo*, the resulting residue was purified by column chromatography (SiO_2, CH_2Cl_2/acetone = 20:1) to give the title compound (33.6mg, 67%) as red solid.

TLC (CH_2Cl_2/MeOH = 20:1): R_f= 0.29

UV/Vis (CH_2Cl_2, λ): 414 (100, *Soret*), 531 (10)

IR (neat, ν/cm^{-1}): 1932.5 (νCO), 1008.7 [Ru (II) oxidation state marker band]

HPLC (LiChrospher® 100 RP-18 (5µm) 250×4, 80% MeOH to 100% MeOH in 13min, then @ 100% MeOH till 30min, flow 1ml/min, λ_{det} = 413nm, T = 40°C): R_t = 15.29min

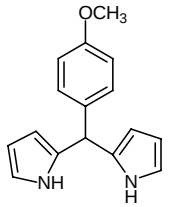
^{1}H-NMR (500MHz, $CDCl_3$/MeOH-D_4): 8.63 (*d*, *J* = 4.9, 2H, porphyrin-H), 8.61 (*s*, 4H, porphyrin-H), 8.48 (*d*, *J* = 4.9, 2H, porphyrin-H), 8.32 (*d*, *J* = 7.6, 1H, C(2' or 6')-H), 8.25 (*d*, *J* = 7.6, 1H, C(2' or 6')-H), 8.01 (*m*, 3H, C(2'', 2''' or 6'', 6''')), 7.95-7.89 (*m*, 5H, C(3', 5') and C(2'', 2''' or 6'', 6''')-H), 7.47-7.43 (*m*, 6H, C(3'', 5'', 3''', 5''')-H), 3.0 (*s*, 3H, CH_3S), 2.61 (*s*, 9H, ArCH_3)

^{13}C-NMR (125MHz, $CDCl_3$/MeOH-D_4): 180.71 (CO), 146.41 (C(1')), 144.27/144.19/144.07/143.20 (C(1, 4, 6, 9, 11, 14, 16, 19)), 143.49 (C(4')), 139.54 (C(1''')), 139.51 (C(1'')), 136.99 (C(4'')), 136.97 (C(4''')), 135.11/134.85 (C(2', 6')), 134.24/133.77 (2'', 2''' and 6'', 6''')), 132.0/131.74/131.63/130.74 (C(2, 3, 7, 8, 12, 13, 17, 18)), 127.33/127.12 (C(3'', 3''' and 5'', 5''')), 122.29/121.75 (C(3', 5')), 122.04 (C(10, 15, 20)), 119.30 (C(5)), 43.52 (*C*H_3S), 21.4 (Ar*C*H_3)

MALDI-TOF-MS: 818.2 [M-CO], 802.1 [M-CO-16]

ESI-MS (CH_2Cl_2/MeOH): 869.03 $[M+Na]^+$

5-(*p*-Methoxyphenyl)dipyrromenane **80**[83, 84]



To a solution of ansialdehyde (3.4ml, 28.8mmol) in pyrrol (50ml, 720mmol) was added TFA (0.23ml, 2.88mmol). The reaction was stirred under argon at room temperature in dark for 30min and then quenched with saturated $NaHCO_3$ (aq., 80ml). The resulting mixture was extracted with ethyl acetate. The combined extractions were washed with brine, dried over Na_2SO_4 and evaporated in *vacuo* to afford a dark oil. Column chromatography (SiO_2, ethyl acetate/hexane = 1:2 + 1% Et_3N) gave crude product as orange oil, which was recrystallized from ethyl acetate/hexane to afford the title compound (5.4g, 74%) as prey solid.

mp: 97-98°C (Lit.[84] 99°C)

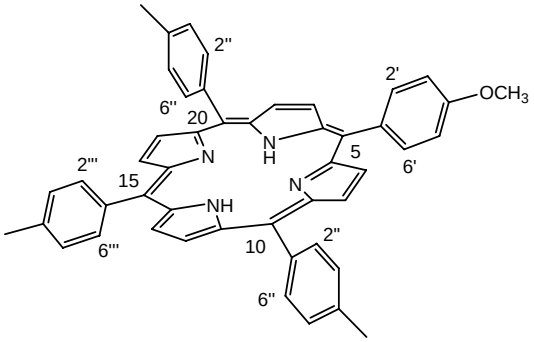
TLC (ethyl acetate/hexane = 1:2): R_f = 0.44

^{1}H-NMR (400MHz, $CDCl_3$): 7.90 (*brs*, 2H, N*H*), 7.13 (AA'BB', 2H, C(3', 5')-H), 6.86 (AA'BB', 2H, C(2', 6')-H), 6.69 (*m*, 2H, pyro-H_2), 6.16 (*dd*, *J* = 5.8, 2.8, 2H, pyro-H_3), 5.91 (*m*, 2H, pyro-H_4), 5.43 (*s*, 1H, ArC*H*), 3.80 (*s*, 3H, ArCH_3)

^{13}C-NMR (100MHz, $CDCl_3$): 158.7, 134.3, 133, 129.5, 117.2, 114.1, 108.5, 107.2, 55.4, 43.3

ESI-MS (CH_2Cl_2/MeOH): 253.0 $[M+H]^+$, 275.0 $[M+Na]^+$, 251.2 $[M-H]^-$

5-(*p*-Methoxyphenyl)-10,15,20-tri(*p*-methylphenyl)-porphyrin **79**



To a solution of **63** (100mg, 0.42mmol), **80** (107mg, 0.42mmol) and *p*-tolylaldehyde (102mg, 0.85mmol) in CH_2Cl_2 (85ml) was added TFA (0.120ml, 1.51mmol). The reaction was stirred for 1h at room temperature under argon and then the solution of DDQ (480mg, 2.1mmol) in THF (5ml) was added. The resulting mixture was heated at reflux for 1h and Et_3N (0.85ml) was added. The mixture was directly filtered through silica gel (CH_2Cl_2+1%Et_3N) to afford a mixture of porphyrins, which was separated by column chromatography (SiO_2, CH_2Cl_2/hexane = 2:1, second band) to give the title compound (45.8mg, 15.8%) as purple solid.

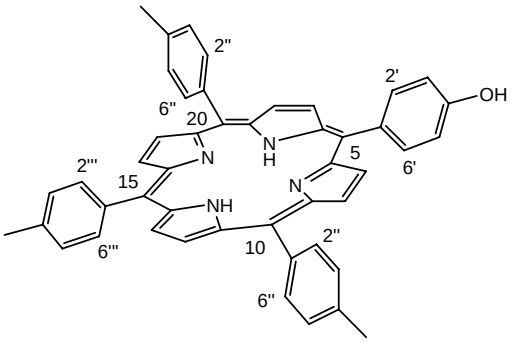
TLC (CH_2Cl_2/hexane = 3:1): R_f = 0.51

UV/Vis (CH_2Cl_2, λ): 420 (100, *Soret*), 517 (5)

^{1}H-NMR (500MHz, $CDCl_3$): 8.86 (*d*, 8H, porphyrin-*H*), 8.13 (AA'BB', *J* = 8.4, 2H, C(2', 6')-H), 8.10 (*d*, 6H, C(2'', 6'', 2''', 6''')-H), 7.56 (*d*, 6H, C(3'', 5'', 3''', 5''')-H), 7.29 (AA'BB', *J* = 8.4, 2H, C(3', 5')-H), 4.10 (*s*, 3H, OCH_3), 2.71 (*s*, 9H, ArCH_3), -2.76 (*s*, 2H, porphyrin-N*H*)

^{13}C-NMR (500MHz, $CDCl_3$): 159.50 (C(4')), 139.45 (C(1'', 1''')), 137.45 (C(4'', 4''')), 135.72 (C(2', 6')), 134.80 (C(1')), 134.66 (C(2'', 6'' and 2''', 6''')), 131 (*brs*, C(1, 2, 3, 4, 6,7, 8, 9, 11 12, 13, 14, 16, 17, 18, 19)), 127.55 (C(3'', 5'' and 3''', 5''')), 120.22 (C(10, 20)), 120.18 (C(15)), 119.88 (C(5)), 55.71 (O*C*H_3), 21.68 (Ar*C*H_3)
ESI-MS (CH_2Cl_2/MeOH): 687.2 $[M+H]^+$

5-(*p*-Hydroxyphenyl)-10,15,20-tri(*p*-methylphenyl)-porphyrin **57**

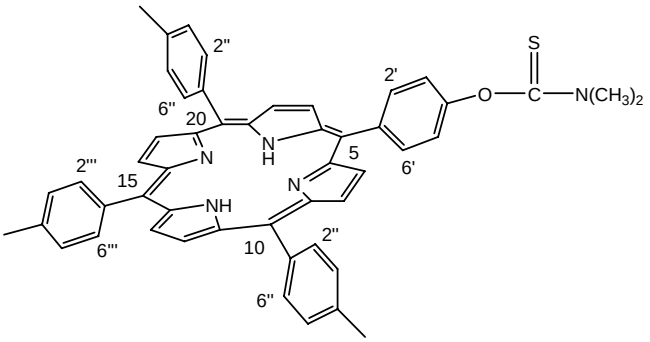


To a solution of porphyrin **79** (266.7mg, 0.297mmol) in CH_2Cl_2 (21.2ml) was added ethanthiol (21.2ml, 286mmol) followed by $AlCl_3$ (2.12g, 15.87mmol). The reaction was stirred at room temperature overnight and then quenched by the slow addition of saturated $NaHCO_3$ (aq., 200ml). The biphasic mixture was extracted with 4 portions of CH_2Cl_2 and then the combined extractions were washed with brine, dried over Na_2SO_4 and evaporated in *vacuo*. The resulting residue was purified by column chromatography (SiO_2, CH_2Cl_2+1% Et_3N and then ethyl acetate/hexane = 2:1) to give the title compound (251mg, 96%) as purple solid.

TLC (ethyl acetate/hexane = 1:2): R_f = 0.45
UV/Vis (CH_2Cl_2, λ): 419 (100, *Soret*), 517 (4)
HPLC (LiChrospher® 100 RP-18 (5µm) 250×4, 80% MeOH to 100% MeOH in 10min, then @ 100% MeOH till 30min, flow 1ml/min, λ_{det} = 420nm, T = 40°C): R_t = 20.99min
^{1}H-NMR (500MHz, $CDCl_3$): 8.86 (*m*, 8H, porphyrin-H), 8.10 (*d*, 6H, C(2'', 6'', 2''', 6''')-H), 8.06 (AA'BB', *J* = 8.5, 2H, C(2', 6')-H)), 7.55 (*d*, 6H, C(3'', 5'', 3''', 5''')-H), 7.16 (AA'BB', *J* = 8.5, 2H, C(3', 5')-H), 2.71 (*s*, 9H, ArCH_3), -2.76 (*s*, 2H, porphyrin-N*H*)

^{13}C-NMR (500MHz, $CDCl_3$): 155.50 (C(4')), 139.43 (C(1'', 1''')), 137.46 (C(4'', 4''')), 135.82 (C(2', 6')), 134.95 (C(1')), 134.66 (C(2'', 2''', 6''', 6''')), 131.2 (*brs*, C(2, 3, 7, 8, 12, 13, 17, 18)), 127.55 (C(3'', 3''', 5'', 5''')), 120.25 (C(10, 20)), 120.23 (C(5)), 119.71 (C(5)), 113.78 (C(3', 5')), 21. 67 (Ar*C*H_3)
ESI-MS (CH_2Cl_2/MeOH): 673.2 $[M+H]^+$, 671.4 $[M-H]^-$

5-(*p*-*O*-dimethylthiocarbamylphenyl)-10,15,20-tri(*p*-methylphenyl)-porphyrin **78**

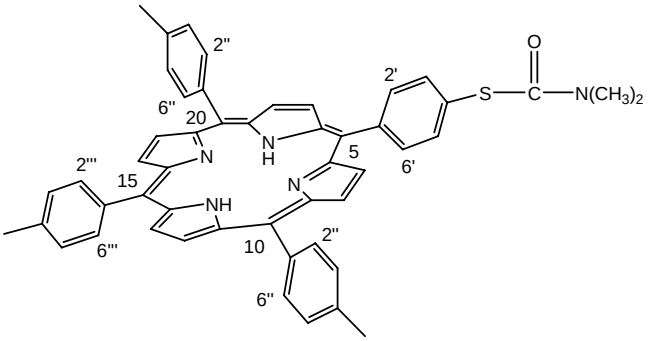


To a solution of porphyrin **57** (5mg, 7.44μmol) in dry DMF (1ml) was added NaH (60% in oil, 0.9mg, 22.32μmol). The resulting suspension was stirred vigorously at room temperature for 15min and then a solution of dimethylthiocarbamylphenyl chloride (1.1mg, 8.27μmol) in DMF (1ml) was added. The reaction was heated at 80°C for 2h and then quenched with water (5ml). The biphasic mixture was extracted with ethyl acetate and the combined extractions were washed with 1M NaOH (aq.), brine, dried over Na_2SO_4 and evaporated in *vacuo*. Purification by column chromatography (SiO_2, ethyl acetate/hexane = 1:4) gave the title compound (5.03mg, 89%) as purple solid.

TLC (ethyl acetate/hexane = 1:3): R_f = 0.37
UV/Vis (CH_2Cl_2 + 1% Et_3N, λ): 419 (100, *Soret*), 516 (4)
^{1}H-NMR (400MHz, $CDCl_3$): 8.9 (*d*, *J* = 4.8, 2H, porphyrin-H), 8.88 (*d*, *J* = 4.8, 2H, porphyrin-H), 8.86 (*s*, 4H, porphyrin-H), 8.23 (AA'BB', *J* = 8.3, 2H, C(2', 6')-H), 8.10 (*d*, 6H, C(2'', 6'', 2''', 6''')-H), 7.56 (*d*, 6H, C(3'', 5'', 3''', 5''')-H), 7.46 (AA'BB', *J* = 8.3, 2H, C(3', 5')-H), 3.60 (*s*, 3H, NCH_3), 3.53 (*s*, 3H, NCH_3), 2.71 (*s*, 9H, ArCH_3), -2.76 (*s*, 2H, porphyrin-N*H*)
MALDI-TOF-MS: 760.3 [M+1]

ESI-MS (CH_2Cl_2/MeOH): 760 $[M+H]^+$, 782 $[M+Na]^+$

5-(*p*-*S*-dimethylcarbamylphenyl)-10,15,20-tri(*p*-methylphenyl)-porphyrin **77**



A flask which had been purged with argon was charged with porphyrin **78** (5mg, 6.62μmol). It was heated in a boiling glycerin-bath for 30min. Column chromatography (SiO_2, CH_2Cl_2/hexane = 3:1 to 4:1) gave the title compound (4.97mg, 99%) as purple solid.

TLC (ethyl acetate/hexane = 1:2): R_f = 0.48

UV/Vis (CH_2Cl_2 + 1% Et_3N, λ): 419 (100, *Soret*), 516 (4)

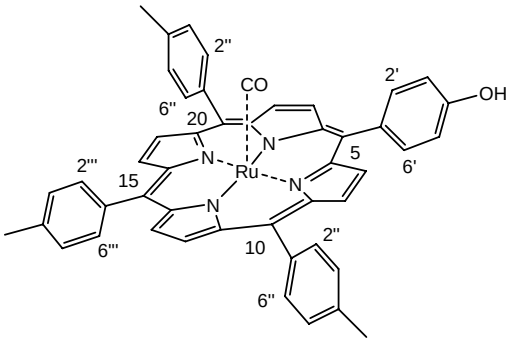
^{1}H-NMR (500MHz, $CDCl_3$): 8.90-8.88 (*m*, 8H, porphyrin-H), 8.26 (AA'BB', *J* = 8.2, 2H, C(2', 6')-H), 8.12 (*d*, 6H, C(2'', 6'', 2''', 6''')-H), 7.90 (AA'BB', *J* = 8.2, 2H, C(3', 5')-H), 7.57 (*m*, 6H, C(3'', 5'', 3''', 5''')-H), 3.25 (*s*, 3H, NC*H*$_3$), 3.19 (*s*, 3H, NC*H*$_3$), 2.72 (*s*, 6H, ArC*H*$_3$), 2.71 (*s*, 3H, ArC*H*$_3$), -2.77 (*s*, 2H, porphyrin-N*H*)

^{13}C-NMR (125MHz, $CDCl_3$): 167.18 (C=O), 143.31 (C(1')), 139.40 (C(1''')), 139.35 (C(1'')), 137.50 (C(4'')), 137.48 (C(4''')), 135.15 (C(2', 6')), 134.66 (C(2'', 6'', 2''', 6''')), 133.90 (C(3', 5')), 131 (*brs*, C(2, 3, 7, 8, 12, 13, 17, 18)), 128.57 (C(4')), 127.58 (C(3'', 5'')), 127.55 (C(3''', 5''')), 120.55 (C(15)), 120.41 (C(10, 20)), 118.85 (C(5)), 37.25 (N*C*H$_3$), 21.68 (Ar*C*H$_3$)

MALDI-TOF-MS: 759.9 [M+1]

ESI-MS (CH_2Cl_2/MeOH): 760 $[M+H]^+$, 781.9 $[M+Na]^+$

β-Carbonyl [5-(*p*-hydroxyphenyl)-10,15,20-tri(*p*-methylphenyl)-porphyrinato] ruthenium **81**



To a suspension of porphyrin **57** (178mg, 0.265mmol) in dry, degassed decalin (90ml) was added tririutheniumdodecacarbonyl (254mg, 0.40mmol). The reaction was heated under reflux for 4h in argon. Decalin was removed in high *vacuo* and the resulting crude product was purified by column chromatography (SiO_2, hexane/ethyl acetate = 3:1) to give the title compound (201.6mg, 95%) as red solid.

TLC (ethyl acetate/hexane = 1:2): R_f= 0.35

UV/Vis (CH_2Cl_2, λ): 413 (100, *Soret*), 530 (11)

IR (KBr, ν/cm^{-1}): 1941.2 (νCO), 1008.4 [Ru (II) oxidation state marker band]

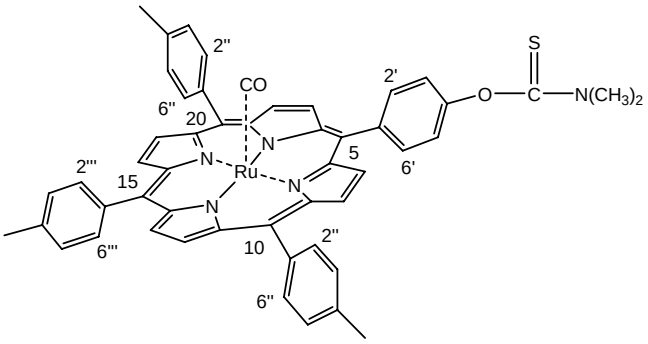
HPLC (LiChrospher® 100 RP-18 (5μm) 250×4, 80% MeOH to 100% MeOH in 13min, then @ 100% MeOH till 30min, flow 1ml/min, λ_{det} = 413nm, T = 40°C): R_t = 14.59min

^{1}H-NMR (500MHz, $CDCl_3$/MeOH-D_4): 8.69 (*d*, *J* = 4.9, 2H, porphyrin-H), 8.66 (*d*, 2H, porphyrin-H), 8.65 (*s*, 4H, porphyrin-H), 8.07 (*dd*, *J* = 7.6, 1.8, 3H, C(2'', 2''' or 6'', 6''')-H), 8.01 (*dd*, *J* = 8.2, 2.2, 1H, C(2' or 6')-H), 7.96 (*dd*, *J* = 7.6, 1.8, 3H, C(2'', 2''' or 6'', 6''')-H), 7.92 (*dd, J* = 8.2, 2.2, 1H, C(2' or 6')-H), 7.49 (*m*, 6H, C(3'', 5'', 3''', 5''')-H), 7.14 (*m*, 2H, C(3', 5')-H), 2.66 (*s*, 9H, ArCH_3)

^{13}C-NMR (125MHz, $CDCl_3$/MeOH-D_4): 181.0 (CO), 156.1 (C(4')), 144.3 (C(1, 9)), 144.02 (C(4, 6)), 144.0 (C(11, 14, 16, 19)), 139.6 (C(1'', 1''')), 136.9 (C(4'', 4''')), 135.4/134.9 (C(2', 6')), 134.3/133.7 (C(2'', 6'', 2''', 6''')), 134.2 (C(1')), 131.54/131.47 (C(2, 3, 7, 8, 12, 13, 17, 18)), 127.3/127.1 (C(3'', 5'', 3''', 5''')), 121.7/121.6 (C(5,10,15,20)), 113.4/113.3 (C(3', 5')), 21.5 (ArCH_3)

ESI-MS (CH_2Cl_2/MeOH): 823.0 $[M+Na]^+$, 800.0 $[M]^+$

β-Carbonyl [5-(*p*-*O*-dimethylthiocarbamylphenyl)-10,15,20-tri(*p*-methylphenyl)-porphyrinato] ruthenium **82**



To a solution of porphyrin **81** (50mg, 0.062mmol) in dry, degassed DMF (5ml) was added NaH (60% in oil, 5.1mg, 0.125mmol). The suspension was stirred vigorously at room temperature for 30min and then dimethylthiocarbamylphenyl chloride (15.7mg, 0.125mmol) was added. The reaction was heated at 80°C in argon for 3h and then quenched with saturated $NaHCO_3$ (aq. 10ml). The mixture was extracted with CH_2Cl_2 and the combined extractions were washed with brine, dried over Na_2SO_4 and evaporated in *vacuo*. Column chromatography (SiO_2, CH_2Cl_2) gave the title compound (39.2mg, 71%) as red solid together with reclaimed porphyrin **81** (14mg, 28%).

TLC (CH_2Cl_2): R_f = 0.38

UV/Vis (CH_2Cl_2, λ): 413 (100, *Soret*), 529 (10)

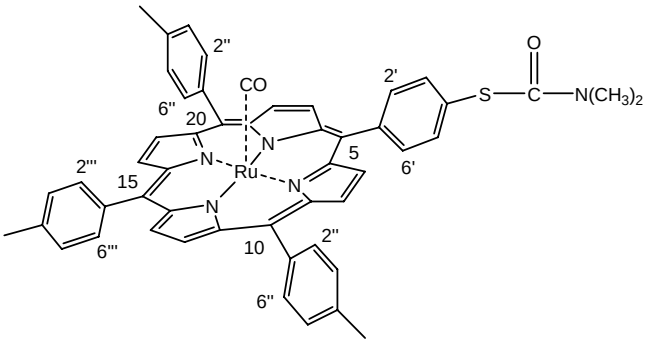
HPLC (LiChrospher® 100 RP-18 (5μm) 250×4, 80% MeOH to 100% MeOH in 13min, then @ 100% MeOH till 30min, flow 1ml/min, λ_{det} = 413nm, T = 40°C): R_t = 15.86min

^{1}H-NMR (400MHz, $CDCl_3$): 8.68-8.64 (*m*, 8H, porphyrin-H), 8.22 (*m*, 1H, C(2' or 6')-H), 8.10 (*m*, 4H, C(2' or 6')-H and C(2'', 2''' or 6'', 6''')-H), 7.99 (*m*, 3H, C(2'', 2''' or 6'', 6''')-H), 7.53-7.49 (*m*, 6H, C(3'', 5'', 3''', 5''')-H), 7.41-7.38 (*m*, 2H, C(3', 5')-H), 3.58 (*s*, 3H, NCH_3), 3.51 (*s*, 3H, NCH_3), 2.68 (*s*, 9H, ArCH_3)

MALDI-TOF-MS: 859.04 [M-CO]

ESI-MS (CH_2Cl_2/MeOH): 910.1 $[M+Na]^+$

β-Carbonyl [5-(*p*-*S*-dimethylcarbamylphenyl)-10,15,20-tri(*p*-methylphenyl)-porphyrinato] ruthenium **76**



Method 1: A flask which had been purged with argon was charged with porphyrin **82** (13.8mg, 15.6μmol). It was heated in a boiling glycerin-bath for 50min. (reaction should be controlled by HPLC) Column chromatography (SiO_2, CH_2Cl_2 and then CH_2Cl_2/acetone = 100:1) gave the title compound (3.5mg, 25%) as red solid.

Method 2: The solution of porphyrin **82** (29.5mg, 33.3μmol) in dry, degassed sulfolane (4ml) was heated in a boiling glycerin-bath under argon for 35min. (reaction should be controlled by HPLC) After evaporation of sulfolane in high *vacuo*, the resulting residue was dissolved in CH_2Cl_2/hexane (1:1) (50ml) and washed with water to remove residual sulfolane. Column chromatography (SiO_2, CH_2Cl_2 and then ethyl acetate/hexane = 1:3) gave the title compound (25.6mg, 87%) as red solid.

TLC (ethyl acetate/hexane = 1:2): R_f = 0.28

UV/Vis (CH_2Cl_2, λ): 413 (100, *Soret*), 529 (10)

IR (neat, ν/cm^{-1}): 1924.8 (νCO), 1006.8 [Ru (II) oxidation state marker band]

HPLC (LiChrospher® 100 RP-18 (5μm) 250×4, 90% MeOH to 100% MeOH in 15min, then @ 100% MeOH till 30min, flow 1ml/min, λ_{det} = 413nm, T = 30°C): R_t = 14.35min (This method was also used for reaction control. R_t (porphyrin **82**) = 13.27min)

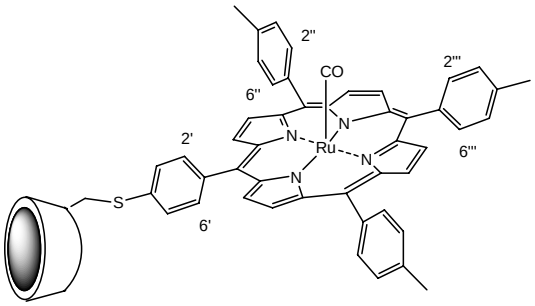
^{1}H-NMR (500MHz, $CDCl_3$/MeOH): 8.58 (*m*, 8H, porphyrin-H), 8.14 (*dd*, *J* = 7.8, 1.9, 1H, C(2' or 6')-H), 8.04 (*dd*, *J* = 7.8, 1.9, 1H, C(2' or 6')-H), 8.0-7.98 (*m*, 3H, C(2'', 2''' or 6'', 6''')-H), 7.90-7.88 (*m*, 3H, C(2'', 2''' or 6'', 6''')-H), 7.75 (*dd*, *J* = 7.8, 1.8, 1H, C(3' or 5')-H), 7.72 (*dd*, *J* = 7.8, 1.8, 1H, C(3' or 5')-H), 7.44-7.40 (*m*, 6H, C(3'', 5'', 3''', 5''')-H), 3.16 (*s*, 3H, NCH_3), 3.06 (*s*, 3H, NCH_3), 2.59 (*s*, 9H, ArCH_3)

^{13}C-NMR (125MHz, $CDCl_3$/MeOH): 180.81 (CO), 167.94 (C=O), 144.11/144.023/144.017/143.49 (C(1, 4, 6, 9, 11, 14, 16, 19)), 143.87 (C(1')), 139.63 (1''')), 139.61 (1'')), 136.87 (C(4'', 4''')), 134.75/134.41 (C(2', 6')), 134.21/133.75 (C(2'', 6'', 2''', 6''')), 133.60/133.56 (C(3', 5')), 131.68/131.51/131.47/131.24 (C(2, 3, 7, 8, 12, 13, 17, 18)), 127.48 (C(4')), 127.26 (C(3'' or 5'')), 127.24 (C(3''' or 5''')), 127.06 (C(3'' or 5'')), 127.04 (C(3''' or 5''')), 121.95 (C(15)), 121.81 (C(10, 20)), 120.27 (C(5)), 37.06 (NCH_3), 21.34 ($ArCH_3$)

MALDI-TOF-MS: 858.91 [M-CO]

ESI-MS (CH_2Cl_2/MeOH): 910.1 $[M+Na]^+$, 918.3 $[M+CH_3O]^-$

Ruthenium porphyrin-β-CD complex **83**



To a solution of porphyrin **76** (9.42mg, 10.6μmol) in dry, degassed THF (2ml) was added $LiAlH_4$ (1M in THF, 31.9μl) at 0°C. The reaction mixture was then heated at reflux in argon for 2h. At room temperature, dry and degassed MeOH (1ml) was added to quench the reaction. After evaporation of solvent in *vacuo* under argon at room temperature, to the resulting residue was added the solution of 6-mono-tosyl-β-CD (27.4mg, 21.3μmol) and pyridine (85.5μl, 1.05mmol) in dry, degassed DMF (2ml). The reaction mixture was heated at 70°C for 24h in argon and then quenched with saturated NH_4Cl (aq.) at room temperature. After removal of the solvent in *vacuo*, the resulting crude product was purified by semi-preparative HPLC (LiChrospher® 100 RP-18 (10 μm) 250×10, 60% MeOH to 90% MeOH in 15min, then to 100% MeOH in 10min, @ 100% MeOH for 10min, flow 5ml/min, λ_{det} = 412nm, T = 40°C) to give the title compound (11.7mg, 56.8%) as a red solid.

TLC (RP-18, MeOH/H_2O = 9:1): R_f = 0.17

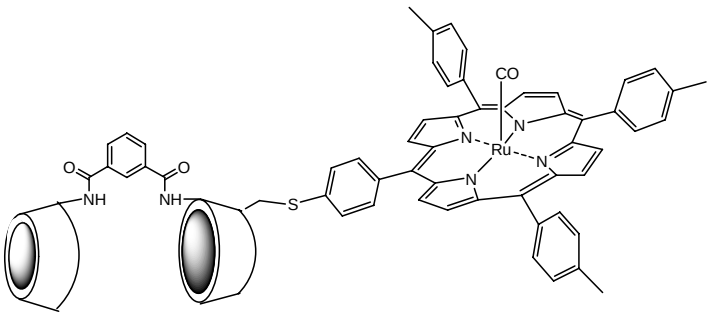
UV/Vis (MeOH, λ): 410 (100, *Soret*), 530 (9)

HPLC (LiChrospher® 100 RP-18 (5μm) 250×4, 60% MeOH to 90% MeOH in 15min, then to 100% MeOH in 10min, @ 100% MeOH for 10min, flow 1ml/min, λ_{det} = 412nm, T = 40°C): R_t = 19.91min

^{1}H-NMR (600MHz, DMSO-D_6): 8.61-8.56 (*m*, 8H, prophyrin-H), 8.07 (*m*, 4H, C(2', 2'', 2''' or 6', 6'', 6''')-H), 7.95 (*m*, 4H, C(2', 2'', 2''' or 6', 6'', 6''')-H), 7.75 (*m*, 2H, C(3', 5')-H), 7.58 (*m*, 6H, C(3'', 3''' and 5'', 5''')-H), 5.86-5.74 (*m*, 14H, CD-C(2, 3)-OH), 5.02-4.81 (*m*, 7H, CD-C(1)-H), 4.61-4.36 (*m*, 6H, CD-C(6)-OH), 3.8-3.35 (*m*, heavily overlapped together with H_2O), 2.65 (*s*, 9H, ArCH_3)

ESI-MS (MeOH): 1955.3 $[M+Na]^+$, 989.2 $[M+2Na]^{2+}/2$, 1931.4 $[M-H]^-$

Ruthenium porphyrin-*bis*-β-CD complex **58**



To a solution of porphyrin **76** (6.52mg, 7.4μmol) in dry, degassed THF (2ml) was added $LiAlH_4$ (1M in THF, 22.1μl) at 0°C. The reaction mixture was heated at reflux in argon for 2h. At room temperature, dry and degassed MeOH (1ml) was added to quench the reaction. After evaporation of solvent in *vacuo* under argon at room temperature, to the resulting residue was added the solution of 6-mono-tosyl-β-CD dimer **56** (37.52mg, 14.7μmol) and pyridine (59.2μl, 0.74mmol) in dry, degassed DMF (2ml). The reaction mixture was heated at 70°C in argon for 24h and then quenched with saturated NH_4Cl (aq.). After removal of the solvent in *vacuo*, the resulting crude product was purified by semi-preparative HPLC (LiChrospher® 100 RP-18 (10 μm) 250×10, 60% MeOH to 90% MeOH in 15min, then to 100% MeOH in 10min, @ 100% MeOH for 10min, flow 5ml/min, λ_{det} = 412nm, T = 40°C) to give the title compound (15.7mg, 68%) as red solid.

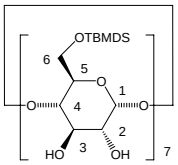
TLC (RP-18, MeOH/H_2O = 10:1): R_f = 0.49

UV/Vis (MeOH, λ): 410 (100, *Soret*), 530 (8)

HPLC (LiChrospher® 100 RP-18 (5μm) 250×4, 60% MeOH to 90% MeOH in 15min, then to 100% MeOH in 10min, @ 100% MeOH for 10min, flow 1ml/min, λ_{det} = 412nm, T = 40°C): R_t = 19.69min

ESI-MS (MeOH/H_2O): 1620.3 $[M+2Na]^{2+}/2$, 1596.3 $[M-2H]^{2-}/2$

Heptakis(6-*O*-*tert*.-butyldimethylsilyl)-β-CD **50**[105]



To a solution of dry β-CD (1g, 0.88mmol) in dry pyridine (20ml) was added the solution of TBDMSCl (1.19g, 7.5mmol) in pyridine (10ml) dropwise by syringe over 90min at 0°C. The reaction mixture was stirred at room temperature for 20h and then poured into ice-water and stirred vigorously for 10min. The resulting precipitates were filtered, washed with ice-water and dissolved in ethyl acetate. The organic solution was washed with 1M HCl (aq.), saturated $NaHCO_3$ (aq.) and then brine, dried over Na_2SO_4 and evaporated in *vacuo*. The resulting crude product was purified by column chromatography (SiO_2, ethyl acetate/EtOH/H_2O = 50:4:2) to give the title compound (1.31g, 76.9%) as white solid.

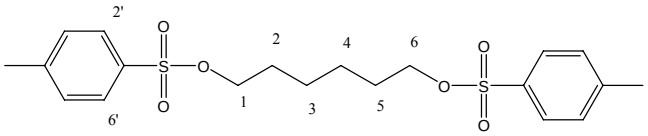
TLC (ethyl acetate/EtOH/H_2O = 50:7:4): R_f= 0.38

^{1}H-NMR (400MHz, Toluene-D8): 7.25 (*s*, 7H, C(3)-O*H*), 5.83 (*s*, 7H, C(2)-O*H*), 4.93 (*d*, *J* = 3.3, 7H, C(1)-H), 4.31 (*t*, *J* = 9.1, 7H, C(3)-H), 4.07 (*dd*, *J* = 11, 3, 7H, C(6*b*)-H), 3.88 (*d*, *J* = 11, 7H, C(6*a*)-H), 3.78 (*d*, *J* = 9.5, 7H, C(5)-H), 3.72 (*dd*, *J* = 9.6, 3.5, 7H, C(2)-H), 3.63 (*t*, *J* = 9.1, 7H, C(4)-H), 1.02 (*s*, 63H, CH_3C), 0.2-0.18 (42H, CH_3Si)

^{13}C-NMR (100MHz, Toluene-D_8): 102.8 (C(1)), 82.69 (C(4)), 74.36 (C(2)), 74.13 (C(3)), 73.02 (C(5)), 62.49 (C(6)), 26.26 (*C*H_3C), 18.71 (CH_3*C*), -4.66 (*C*H_3Si), -4.84 (*C*H_3Si)

ESI-MS (CH_2Cl_2/MeOH): 1956.1 $[M+Na]^+$, 989.7 $[M+2Na]^{2+}/2$

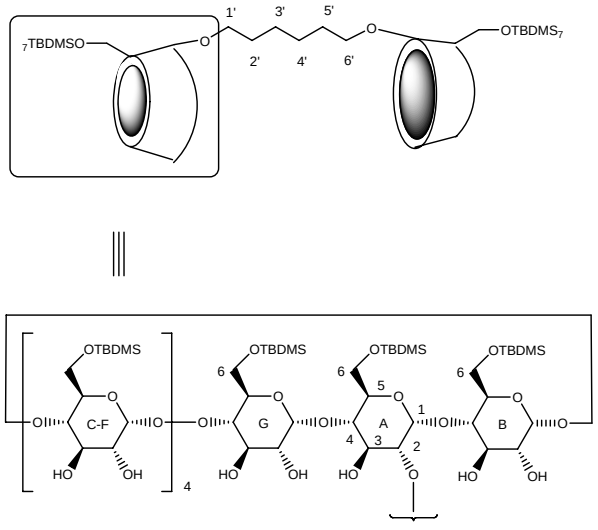
1,6-*Bis*[(*p*-tolylsulfonyl)oxy]hexane **90**[100]



To a solution of 1,6-hexanediol (3.26g, 27.3mmol) in dry pyridine (200ml) was added *p*-tosyl chloride (31g, 0.159mol) in portions over a period of 1h at 0°C. The resulting suspension was stirred at 4°C overnight. The reaction mixture was poured into ice-water (1L) and concentrated HCl (50ml) to give a white solid, which was purified by column chromatography (SiO_2, ethyl acetate/hexane = 1:3) to give the title compound (8.3g, 71.3%) as white solid.

mp: 72-73°C (Lit. [100]: 70-71.5°C)
TLC (ethyl acetate/hexane = 1:2): R_f = 0.22
^{1}H-NMR (400MHz, $CDCl_3$): 7.78 (AA'BB', *J* = 8.3, 4H, C(2', 6')-H), 7.35 (AA'BB', *J* = 8.3, 4H, C(3', 5')-H), 3.98 (*t*, *J* = 6.3, 4H, C(1, 6)-H), 2.45 (*s*, 6H, ArCH_3), 1.61-1.57 (*m*, 4H, C(2, 5)-H), 1.29-1.25 (*m*, 4H, C(3, 4)-H)
^{13}C-NMR (100MHz, $CDCl_3$): 145.2 (C(1')), 133.5 (C(4')), 130.3 (C(2', 6')), 128.3 (C(3', 5')), 70.7 (C(1, 6)), 29.0 (C(2, 5)), 25.2 (C(3, 4)), 22.1 (Ar*C*H_3)
ESI-MS (CH_2Cl_2/MeOH): 875 $[2M+Na]^+$, 449 $[M+Na]^+$

1,6-Bis[[heptakis(6-*O*-*tert*-butyldimethylsilyl)-β-CD]-2-oxy]hexane **91**



To a solution of dry TBDMS-β-CD **50** (9g, 4.65mmol) in dry THF (120ml) was added LiH (55.4mg, 6.62mmol). The mixture was stirred for 1h at room temperature and then heated under reflux for 2h. A solution of **90** (0.79g, 1.86mmol) in THF (40ml) was added and heating under reflux was continued for 5 days. The solvent was removed in *vacuo* and ethyl acetate was added. The solution was washed with saturated $NaHCO_3$ (aq.), brine, dried over Na_2SO_4 and evaporated in *vacuo*. The resulting crude product was purified by column chromatography (SiO_2, ethyl acetate/EtOH/H_2O = 50:4:2) followed by charcoal column chromatography (ethyl acetate/CH_2Cl_2 = 2:1) to give the title compound (2g, 27.2%) as white solid.

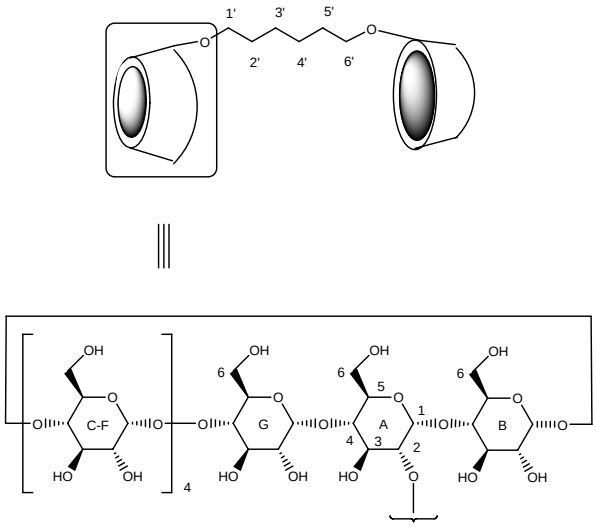
TLC (ethyl acetate/EtOH/H_2O = 50:7:4): R_f= 0.30

^{1}H-NMR (500MHz, $CDCl_3$): 6.2 (*brs*, 2H, C(3_A)-O*H*), 5.56-5.3 (*m*, C(2)-O*H*), 5.19 (*brs*, 2H, C(2_G)-O*H*), 5.1 (*brs*, 2H, C(2_B)-O*H*), 4.99 (*d*, *J* = 3, 2H, C(1_A)-H), 4.92 (*d*, *J* = 3.3, 2H, C(1_B)-H), 4.91-4.88 (*m*, 10H, C(1)-H), 4.54 (*brs*, 2H, C(3_B)-O*H*), 4.2-3.9 (*m*, 26H, C(3)-14H, C(6)-10H, C(1', 6')-2H), 3.8-3.5 (*m*, 58H, C(2)-12H, C(4)-12H, C(5)-14H, C(6)-18H, C(1', 6')-2H), 3.36 (*m*, 2H, C(4_A)-H), 3.16 (*dd*, *J* = 9.6, 3, 2H, C(2_A)-H), 1.85 (*m*, 2H, C(3', 4')-H), 1.60 (*m*, 2H, C(2', 5')-H), 1.46 (*m*, 2H, C(2', 5')-H), 1.08 (*m*, 2H, C(3', 4')-H), 0.88-0.86 (*m*, 126H, C*H*$_3$C), 0.03-0.02 (*m*, 84H, C*H*$_3$Si)

^{13}C-NMR (125MHz, $CDCl_3$): 102.66 (C(1_B)), 102.27 (C(1_G)), 102.02, 101.98, 101.87, 99.89 (C(1_A)), 83.38 (C(4_A)), 81.94, 81.84, 81.68, 81.23, 79.45 (C(4_B)), 77.11, 74.0 (C(2_B)), 73.79, 73.70, 73.53 (C(3_A)), 73.46, 73.40, 73.33, 73.20, 72.84 (C(5_B)), 72.52, 72.50, 72.43, 72.25, 72.05, 71.52 (C(1', 6')), 71.45 (C(3_B)), 62.25 (C(6_A)), 61.73, 61.68, 61.64, 61.57, 61.48, 61.24, 30.03 (C(2', 5')), 25.92, 25.90, 25.80, 23.46 (C(3', 4')), 18.38, 18.28, 18.15, -5.04, -5.08, -5.21, -5.23, -5.35

ESI-MS (CH_2Cl_2/MeOH): 1997.7 $[M+2Na]^{2+}/2$, 1973.8 $[M-2H]^{2-}/2$

1,6-Bis[(β-CD)-2-oxy]hexane **93**



To a solution of protected dimer **91** (1.65g, 0.42mmol) in THF (50ml) was added TBAF (1M in THF, 8.2mmol). The reaction was heated under reflux for 18h. The solvent was removed in *vacuo* and the residue was dissolved in water. Purification by amberlite CG-50 ion-exchange resin (weak acid, H^+-form, 100-200mesh, H_2O/*t*-butanol = 10:1) followed by reversed phase column chromatography (RP-18, gradient elution with H_2O/MeOH=5:1 and then 2:1) gave the title compound (951mg, 97%) as white solid.

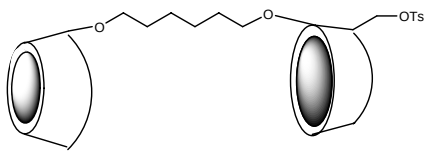
TLC (ethyl acetate/*i*-PrOH/NH_3(aq., 33%)/H_2O =7:7:5:4): R_f= 0.04

^{1}H-NMR (500MHz, D_2O): 5.17 (*d*, *J* = 3.6, 2H, C(1_A)-H), 5.06 (*m*, 12H, C(1)-H), 4.03-3.55 (*m*, 86H, C(2, 3, 4, 5, 6, 1', 6')-H), 3.47 (*dd*, *J* = 9.9, 3.6, 2H, C(2_A)-H), 1.62 (*m*, 4H, C(2', 5')-H), 1.38 (*m*, 4H, C(3', 4')-H)

^{13}C-NMR (125MHz, D_2O): 102.58, 102.52, 102.46, 102.43, 100.75 (C(1_A)), 82.12 (C(4_B)), 81.94 (C(4_A)), 81.80, 81.75, 81.72, 80.81(C(2_A)), 73.69, 73.19 (C(1', 6')), 73.0 (C(3_A)), 72.68, 72.66, 72.47, 72.41, 72.28, 72.19, 60.88, 29.41 (C(2', 5')), 25.25 (C(3', 4'))

ESI-MS (MeOH/H_2O): 2373.9 $[M+Na]^+$, 1198.4 $[M+2Na]^{2+}/2$, 2350 $[M-H]^-$, 1174.5 $[M-2H]^{2-}/2$

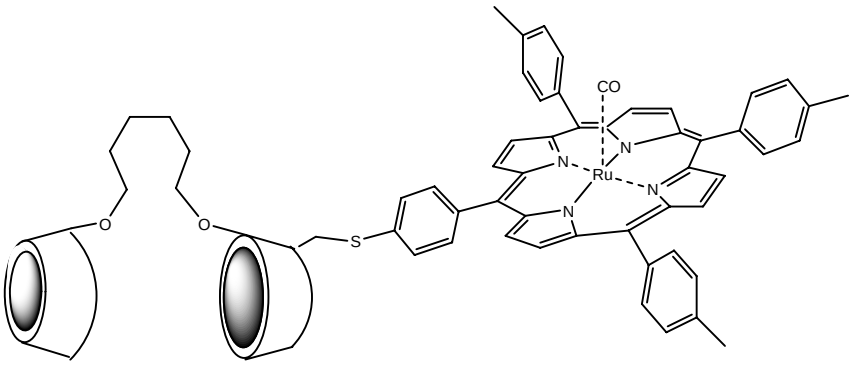
6-*O*-Mono-tosyl-β-CD dimer **94**



To a solution of β-CD dimer **93** (308mg, 0.131mmol) in dry pyridine (14ml) was added *p*-tosyl chloride (28mg, 0.144mmol) at 0°C. The reaction was stirred for 1h at 0°C and then for 5h at room temperature. Water (3ml) was added to quench the reaction. Solvents were removed in *vacuo* and the resulting foam was stirred vigorously in acetone (100ml). The resulting white precipitate was collected and purified by reversed phase column chromatography (RP-18, gradient elution with 10% MeCN and then 15% MeCN) to afford the title compound (112mg, 34%) as white solid together with reclaimed educt **93** (163.5mg, 53%)

TLC (RP-18, MeCN/H_2O=3:10): R_f= 0.33
HPLC (LiChrospher® 100 RP-18 (5μm) 250×4, 20% MeCN, flow = 1ml/min, λ_{det} = 220nm, T=40°C): R_t = 2.64min
ESI-MS (MeOH/H_2O): 2527.8 $[M+Na]^+$, 1274.9 $[M+2Na]^{2+}/2$, 2503.9 $[M-H]^-$, 1251.4 $[M-2H]^{2-}/2$

Ruthenium porphyrin-*bis*-β-CD complex **89**

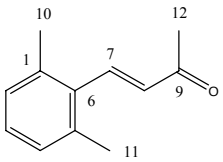


To a solution of porphyrin **76** (6.84mg, 7.7μmol) in dry, degassed THF (2ml) was added $LiAlH_4$ (1M in THF, 23.1μl) at 0°C. The reaction mixture was heated under

reflux in argon for 2h. At room temperature, dry and degassed MeOH (2ml) was added to quench the reaction. After evaporation of solvent in *vacuo* under argon at room temperature, to the resulting residue was added the solution of 6-mono-tosyl-β-CD dimer **94** (38.7mg, 15.4μmol) and pyridine (62.1ul, 0.77mmol) in dry, degassed DMF (2ml). The reaction mixture was heated at 70°C for 21h in argon. After removal of the solvent in *vacuo*, the resulting crude product was purified by reversed phase column chromatography (RP-18, gradient elution with MeOH/H_2O = 1:1, then 10:1) to give the title compound (6.4mg, 26.4%) as red solid.

TLC (RP-18, MeOH/H_2O=10:1): R_f= 0.41
HPLC (LiChrospher® 100 RP-18 (5μm) 250×4, 60% MeOH to 90% MeOH in 15min, then to 100% MeOH in 10min, @100% MeOH for 10min, flow = 1ml/min, λ_{det} = 412nm, T = 40°C): R_t = 18.64min
UV/Vis (H_2O, λ): 412 (100, *Soret*), 533 (11)
ESI-MS (MeOH/H_2O): 3171 $[M+Na]^+$, 1597.3 $[M+2Na]^{2+}/2$, 1573.9 $[M-2H]^{2-}/2$, 1048.7 $[M-3H]^{3-}/3$

(*E*)-4-(2,6-dimethylphenyl)3-buten-2-one **96**[88]

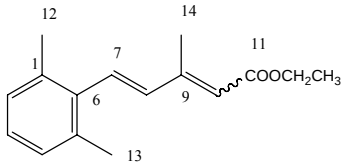


To a solution of 2,6-dimethylbenzaldehyde (3.98g, 28.8mmol) in acetone (36ml) was added 2M NaOH (aq., 4.8ml) over 1h at 0°C. The mixture was stirred for 3h at 0°C and then for 18h at room temperature. The reaction solution was then poured into aqueous H_2SO_4 (2M, 80ml) and extracted with CH_2Cl_2. The combined extractions were washed with saturated $NaHCO_3$ (aq.), brine, dried over Na_2SO_4 and evaporated in *vacuo* at room temperature. Column chromatography (SiO_2, ethyl acetate/hexane = 1:20) gave the title compound (3.98g, 77%) as yellowish oil.

TLC (ethyl acetate/hexane = 1:9): R_f= 0.23
UV/Vis (hexane, λ): 216 (100), 276 (78)

^{1}H-NMR (400MHz, $CDCl_3$): 7.68 (*d*, *J* = 16.7, 1H, (C7)-H), 7.2-7.1 (*m*, 3H, C(2, 3, 4)-H), 6.34 (*d*, *J* = 16.7, 1H, C(8)-H), 2.4 (*s*, 3H, C(12)-H), 2.35 (*s*, 6H, C(10,11)-H)
^{13}C-NMR (100MHz, $CDCl_3$): 198.4 (C(9)), 142 (C(7)), 136.58 (C(1, 5)), 133.95 (C(6)), 133.02 (C(8)), 128.46 (C(3)), 128.31 (C(2), C(4)), 27.5 (C(12)), 21.05 (C(10, 11))
EI-MS: 174.1 $[M]^{+\cdot}$ (10), 159.1 (100), 115.1 (19), 91.1 (10)
ESI-MS (CH_2Cl_2/MeOH): 175.0 $[M+H]^+$, 197.0 $[M+Na]^+$, 370.9 $[2M+Na]^+$

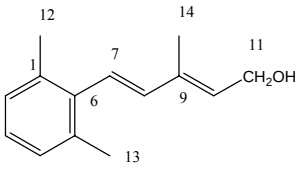
Ethyl 3-methyl-5-(2,6-dimethylphenyl)penta-2,4-dienoate **97**



To a suspension of NaH (60 % in mineral oil, 155mg, 3.9mmol) in dry ether (2ml) was added a solution of triethylphosphonoacetate (0.77g, 3.36mmol) in dry ether (3ml) dropwise at room temperature. After being stirred for 2h at room temperature, the mixture was added with a solution of **96** (540mg, 3mmol) in ether (4ml) and reacted at room temperature overnight. The next morning additional triethylphosphonoacetaten (0.24g, 1.0mmol) was reacted with NaH (46.5mg) and added to the reaction. On the next day, the reaction was quenched with water and the aqueous phase was extracted with ether. The combined extractions were washed with water, dried over Na_2SO_4 and evaporated in *vacuo* at room temperature. Column chromatography (SiO_2, ethyl acetate/hexane = 1:20) gave the title compound (470.4mg, 62.8%) as yellowish oil. (^{1}H-NMR: 80% all-*E*).

TLC (ethyl acetate/hexane = 1:9): R_f = 0.42
^{1}H-NMR (all-*E* isomer) (400MHz, $CDCl_3$): 7.1-7.03 (*m*, 3H, C(2, 3, 4)-H), 6.98 (*d*, *J* = 16.2, 1H, C(7)-H), 6.32 (*d*, *J* = 16.2, 1H, C(8)-H), 5.8 (*s*, 1H, C(10)-H), 4.20 (*q*, *J* = 7, 2H, CH_2CH_3), 2.43 (*d*, *J* = 1.3, 3H, C(14)-H), 2.3 (*s*, 6H, Ar*H*), 1.3(*t*, *J* = 7, 3H, CH_2CH_3)
ESI-MS (CH_2Cl_2/MeOH): 267.1 $[M+Na]^+$

(all-*E*)-3-methyl-5-(2,6-dimethylphenyl)penta-2,4-dien-1-ol **98**



To a solution of **97** (637mg, 2.61mmol) in dry ether (20ml) at 0°C was added DIBALH (1M in hexane, 5.2ml). After being stirred for 5h at room temperature, the reaction was diluted with ether (20ml) and quenched by careful addition of brine at 0°C, followed by dropwise addition of 3M HCl (aq. 10ml). The mixture was extracted with ether and the combined extractions were washed with saturated $NaHCO_3$ (aq.), brine, dried over Na_2SO_4 and evaporated in *vacuo* at room temperature. Column chromatography (basic alox, hexane/TBME=2:1) gave title compound (297mg, 56.3%) as yellowish oil.

TLC (basic alox, ethyl acetate/hexane = 1:2): R_f = 0.26

UV/Vis (CH_2Cl_2, λ): 269 (100), 229 (80)

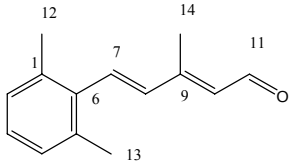
IR (neat, ν/cm^{-1}): 3317 (*brs*), 2916.17, 1465.8

^{1}H-NMR (400MHz, $CDCl_3$): 7.05 (*s*, 3H, C(2, 3, 4)-H), 6.57 (*d*, *J* = 16.4, 1H, C(7)-H), 6.28 (*d*, *J* = 16.4, 1H, C(8)-H), 5.71 (*t*, *J* = 6.8, 1H, C(10)-H), 4.35 (*d*, *J* = 6.8, 2H, C(11)-H), 2.3 (*s*, 6H, Ar*H*), 1.95 (*s*, 3H, C(14)-H)

^{13}C-NMR (100MHz, $CDCl_3$): 138.47 (C(8)), 137.5 (C(6)), 136.9 (C(9)), 136.4 (C(1), C(5)), 130.6 (C(10)), 128.21 (C(2, 4)), 126.95/126.7 (C(3, 7)), 59.9 (C11)), 21.41 (C(12, 13)), 12.9 (C(14))

EI-MS: 202.1 $[M]^{+\cdot}$ (48), 171.1 (46), 157.1 (100), 119.1 (52)

(all-*E*)-3-methyl-5-(2,6-dimethylphenyl)penta-2,4-dienal **99**



To a solution of **98** (577mg, 2.9mmol) in hexane (80ml) was added activated MnO_2 (5.5g, 58mmol). The reaction mixture was stirred at room temperature for 2h. MnO_2

was filtered off and washed with CH_2Cl_2. The combined filtrate was concentrated in *vacuo* at room temperature. Purification by column chromatography (SiO_2, hexane/TBME = 4:1) to give the title compound (335.5mg, 58.7%) as yellowish oil.

TLC (ethyl acetate/hexane = 1:2): R_f = 0.46

UV/Vis (CH_2Cl_2, λ): 313 (100), 250 (shoulder, 38)

IR (neat, ν/cm^{-1}): 1658.7, 1612.4, 1465.8, 1380.9

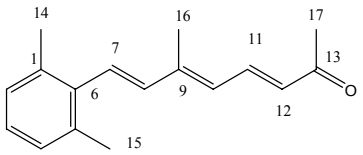
HPLC (YMC C30 (3μm) 250×4.6, @MeOH/H_2O (9:1) (100%) for 5min, to MeOH/TBME (7:3) (100%) in 30min, then @MeOH/TBME (7:3) for 17min, flow: 1ml/min, λ_{det} = 313nm, T = 25°C): R_t = 4.55

^{1}H-NMR (400MHz, $CDCl_3$): 10.19 (*d*, *J* = 8.1, 1H, C(11)-H), 7.15 (*d*, *J* = 16.5, 1H, C(7)-H), 7.12-7.02 (*m*, 3H, C(2, 3, 4)-H), 6.44 (*d*, *J* = 16.5, 1H, C(8)-H), 6.01 (*d*, *J* = 8.1, 1H, C(10)-H), 2.42 (*d*, *J* = 1.4, 3H, C(14)-H), 2.3 (*s*, 6H, C(12, 13)-H)

^{13}C-NMR (100MHz, $CDCl_3$): 191.7 (C(11)), 154.5 (C(9)), 137.4 (C(8)), 136.6 (C(1, 5)), 136 (C(6)), 134.7 (C(7)), 130.3 (C(10)), 128.6 (C(2, 4)), 128.1 (C(3)), 21.4 (C(12, 13)), 13.4 (C(14))

EI-MS: 200.1 $[M]^{+\cdot}$ (58), 185.1 (49), 157.0 (100), 142.1 (88), 115.1 (57)

(all-*E*) 6-methyl-8-(2,6-dimethylphenyl)octa-3,5,7-trien-2-one **85**



To a solution of aldehyde **99** (18.7mg, 0.093mmol) in acetone (1ml) was added 2M NaOH (aq., 14.3μl) at 0°C. The reaction solution was stirred for 3h at 0°C, 18h at room temperature and then diluted with ether (10ml). The organic layer was washed with brine, dried over Na_2SO_4 and evaporated in *vacuo* at room temperature. Column chromatography (SiO_2, hexane/TBME = 4:1) gave the title compound (19mg, 84.7%) as yellowish oil.

TLC (hexane/TBME = 2:1): R_f = 0.34

UV/Vis (CH_2Cl_2, λ): 340 (100)

IR (neat, ν/cm^{-1}): 1658.7, 1589.2

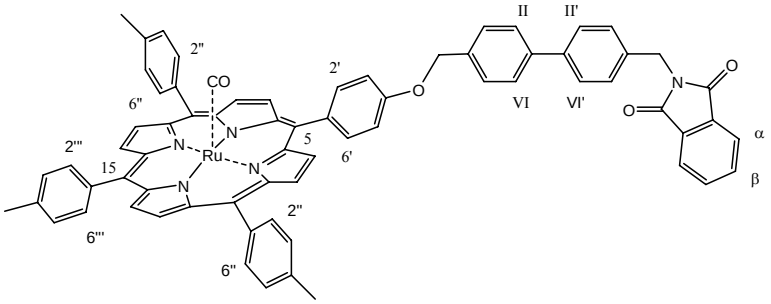
HPLC (YMC C30 (3μm) 250×4.6, @MeOH/H_2O (9:1) (100%) for 5min, to MeOH/TBME (7:3) (100%) in 30min, then @MeOH/TBME (7:3) for 17min, flow 1ml/min, λ_{det} = 340nm, T = 25^{o}C): R_t = 5.28

^{1}H-NMR (400MHz, $CDCl_3$): 7.6 (*dd*, *J* = 15.4, 12.0, 1H, C(11)-H), 7.1-7.0 (*m*, 3H, C(2,3,4)-H), 6.84 (*d*, *J* = 16.4, 1H, C(7)-H), 6.41 (*d*, *J* = 16.4, 1H, C(8)-H), 6.25 (*d*, *J* = 12.0, 1H, C(10)-H), 6.22 (*d*, *J* = 15.4, 1H, C(12)-H), 2.33 (*s*, 6H, C(14, 15)-H), 2.32 (*s*, 3H, C(17)-H), 2.17 (*d*, *J* = 1.4, 3H, C(16)-H)

^{13}C-NMR (100MHz, $CDCl_3$): 198.6 (C(13)), 145 (C(9)), 139 (C(11)), 138 (C(8)), 136.5 (C(6)), 136.2 (C(1, 5)), 130.19 (C(7)), 130.13 (C(12), 129.2 (C(10)), 128.1 (C(2, 4)), 127.2 (C(3)), 28 (C(17)), 21.2 (C(14, 15)), 13.3 (C(16))

EI-MS: 240.2 $[M]^{+\cdot}$ (21), 225.1 (51), 207.1 (27), 197.1 (100), 182.1 (69), 43 (32)

β-Carbonyl [5-*p*-(((4'-(1,3-dioxo-1,3-dihydro-isoindol-2-ylmethyl)biphenyl)-4-yl) methoxyphenyl)-10,15,20-tri(*p*-methylphenyl)-porphyrinato] ruthenium **105**



The suspension of Cs_2CO_3 (169mg, 0.52mmol) and phenol porphyrin **81** (40.5mg, 0.051mmol) in dry, deagssed DMF (3ml) was stirred at room temperature for 2h, then the solution of 2-(4'-bromomethyl-biphenyl-4-yl-methyl)-isoindole-1,3-dione **104** (105.6mg, 0.260mmol) in dry, degassed DMF (6ml) was added. The reaction mixture was heated at 80^{o}C in argon for 3.5h and then diluted with ethyl acetate. The resulting organic layer was washed with water, dried over Na_2SO_4 and concentrated in *vacuo*. The crude product was purified by column chromatography (SiO_2, ethyl acetate/hexane = 1:3) to give the title compound (48.63mg, 85%) as red solid.

TLC (ethyl acetate/hexane=1:2): R_f= 0.33

UV/Vis (CH_2Cl_2, λ): 413 (100, *Soret*), 530 (11)

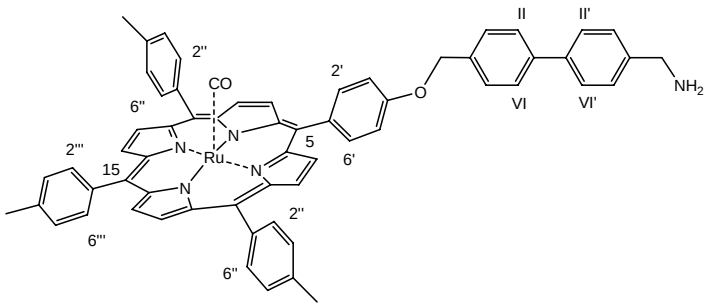
IR (neat, ν/cm^{-1}): 1934.5 (CO), 1716.5 (νC=O), 1008.7 [Ru (II) oxidation state marker band]

^{1}H-NMR (500MHz, $CDCl_3$/MeOH-D_4): 8.71 (*d*, *J* = 4.8, 2H, porphyrin-H), 8.692 (*d*, 2H, porphyrin-H), 8.687 (*s*, 4H, porphyrin-H), 8.14 (*dd*, *J* = 8.3, 2.3, 1H, C(2' or 6')-H), 8.10 (*dd*, *J* = 7.6, 1.9, 3H, C(2'', 2''' or 6'', 6''')-H), 8.04 (*dd*, *J* = 8.3, 2.3, 1H, C(2' or 6')-H), 8.0 (*dd*, *J* = 7.6, 1.9, 3H, C(2'', 2''' or 6'', 6''')-H), 7.85 (*dd*, *J* =5.5, 3, 2H, phthalimide-α-H), 7.73 (*dd*, *J* = 5.5, 3, 2H, phthalimide-β-H), 7.69 (*s*, 4H, C(II, III, V, VI)-H), 7.62 (AA'BB', *J* = 8.2, 2H, C(II', VI')-H), 7.56-7.50 (*m*, 8H, C(3'', 5'', 3''', 5''')-H and C(III', V')-H), 7.37 (*dd*, *J* = 8.3, 2.7, 1H, C(3' or 5')-H), 7.33 (*dd*, *J* = 8.3, 2.7, 1H, C(3' or 5')-H), 5.38 (*s*, 2H, O*C*H_2), 4.92 (*s*, 2H, *C*H_2N), 2.69 (*s*, 9H, Ar*C*H_3)

^{13}C-NMR (125MHz, $CDCl_3$/MeOH-D_4): 207.0 (could be *C*O), 168.17 (N*C*O), 158.26 (C(4')), 144.15/143.99 (C(1, 4, 6, 9, 11, 14, 16, 19)), 140.52/140.38 (C(I, I')), 139.73 (could be C(1')), 139.56 (C(1'', 1''')), 136.83 (C(4'', 4''')), 136.15 (C(IV)), 135.43/134.78 (C(2', 6')), 135.31 (C(IV')), 134.23/133.69 (C(2'', 6'' and 2''', 6''')), 134.06 (phthalimide-β-C), 132.02 (*C*CON), 131.51/131.48/131.43 (C(2, 3, 7, 8, 12, 13, 17, 18)), 129.07 (C(III', V')), 128.23 (C(III, V)), 127.42/127.36 (C(II, VI and II', VI')), 127.27/127.03 (C(3'', 3''' and 5'', 5''')), 123.38 (phthalimide-α-C), 121.71 (C(10, 20)), 121.69 (C(15)), 121.25 (C(5)), 112.87/112.69 (C(3', 5')), 70.03 (Ar*C*H_2O), 41.27 (Ar*C*H_2N), 21.42 (Ar*C*H_3)

ESI-MS (CH_2Cl_2/MeOH): 1147.8 $[M+Na]^+$, 1124.9$[M]^+$

β-Carbonyl [5-*p*-(((4'-amino-biphenyl)-4-yl)methoxyphenyl)-10,15,20- tri(*p*-methylphenyl)-porphyrinato] ruthenium **106**



The solution of porphyrin **105** (21.4mg, 0.019mmol) and hydrazine monohydrate (0.2ml, 4mmol) in EtOH/toluene (2:1, 8ml, degassed) was heated under reflux in argon for 3h. At room temperature, the reaction mixture was diluted with ethyl acetate, and then washed with water, dried over Na_2SO_4 concentrated in *vacuo*. The resulting crude product was dissolved in CH_2Cl_2/pyridine to give a solution, which was subjected to column chromatography (SiO_2, CH_2Cl_2/MeOH=20:1 + 1% Et_3N) to give the title compound (15.7mg, 83%) as red solid.

TLC (CH_2Cl_2/MeOH=5:1 + 1% Et_3N): R_f= 0.53

UV/Vis (CH_2Cl_2, λ): 415 (100, *Soret*), 535 (8)

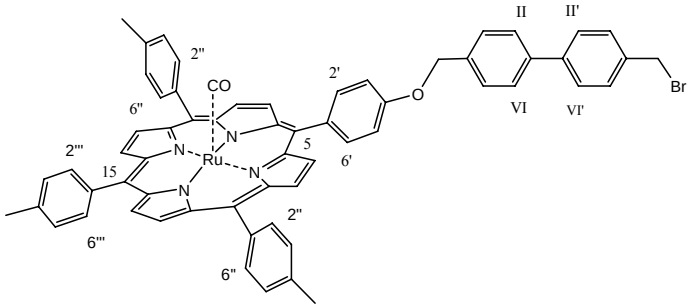
IR (neat, ν/cm^{-1}): 1940 (CO), 1006.8 [Ru (II) oxidation state marker band]

^{1}H-NMR (500MHz, $CDCl_3$/pyridine-D_5): 8.72 (*d*, *J* = 4.8, 2H, porphyrin-H), 8.70 (*d*, *J* = 4.8, 2H, porphyrin-H), 8.69 (*s*, 4H, porphyrin-H), 8.12 (*dd*, J = 8.3, 2.3, 1H, C(2' or 6')-H), 8.08 (*m*, 3H, C(2'', 2''' or 6'', 6''')-H), 8.01 (*dd*, *J* = 8.3, 2.3, 1H, C(2' or 6')-H), 7.96 (*m*, 3H, C(2'', 2''' or 6'', 6''')-H), 7.72-7.68 (*m*, 4H, biphenyl-H), 7.63 (*m*, 2H, C(II', VI")-H), 7.50-7.43 (*m*, 8H, C(3'', 3''', 5'', 5''')-H and C(III', V')-H), 7.36 (*dd*, *J* = 8.3, 2.3, 1H, C(3' or 5')-H), 7.32 (*dd*, *J* = 8.3, 2.3, 1H, C(3' or 5')-H), 5.34 (*s*, 2H, ArOCH_2), 3.94 (*s*, 2H, C$H_2$$NH_2$), 2.64 (*s*, 9H, ArC$H_3$)

^{13}C-NMR (125MHz, $CDCl_3$/pyridine-D_5): 179.6 (CO), 157.8 (C(4')), 143.3/143.2/143.15/143.14 (C(1, 4, 6, 9, 11, 14, 16, 19)), 141.5 (C(IV')), 140.2 (C(I)), 139.1 (C(1'', 1''')), 138.7 (C(I')), 136.2 (C(4'', 4''')), 135.5 (C(IV)), 135.3 (C(1')), 134.7/134.4 (C(2', 6')), 133.6/133.3 (C(2'', 6'', 2''', 6''')), 131.21/131.18/131.14 (C(2, 3, 7, 8, 12, 13, 17, 18)), 127.7 (C(III, V)), 127.2 (C(III', V')), 126.72/126.68 (C(II, VI, II', VI')), 126.64/126.47 (C(3'', 5'', 3''', 5''')), 120.99 (C(10, 20)), 120.97 (C(15)), 120.6 (C(5)), 112.3/112.2 (C(3', 5')), 69.4 (ArO*C*H_2), 45.4 (Ar*C*$H_2$$NH_2$), 20.8 (Ar*C*$H_3$)

ESI-MS (CH_2Cl_2/MeOH): 1017.9 $[M+Na]^+$, 995.9 $[M+1]^+$

β-Carbonyl [5-*p*-(((4'-bromo-biphenyl)-4-yl)-methoxyphenyl)-10,15,20-tri(*p*-methyl phenyl)-porphyrinato] ruthenium **111**



To a solution of phenol porphyrin **81** (24.9mg, 0.031mmol) in dry, degassed THF (2ml) was added NaH (60% in oil, 2.5mg, 0.062mmol). The resulting suspension was stirred at room temperature in argon for 18h, then the degassed solution of dibromide **110** (52.8mg, 0.155mmol) in THF (3ml) was added at 0°C. After being stirred at room temperature in argon for 18h, the reaction mixture was diluted with ethyl acetate (30ml) and quenched with slow addition of ice-water. The organic layer was separated, washed with water, dried over Na_2SO_4 and concentrated in *vacuo*. The crude product was purified by column chromatography (SiO_2, ethyl acetate/hexane = 1:4) to give the title compound (26.9mg, 82%) as red solid.

TLC (ethyl acetate/hexane=1:2): R_f= 0.43

UV/Vis (CH_2Cl_2, λ): 413 (100, *Soret*), 530 (10)

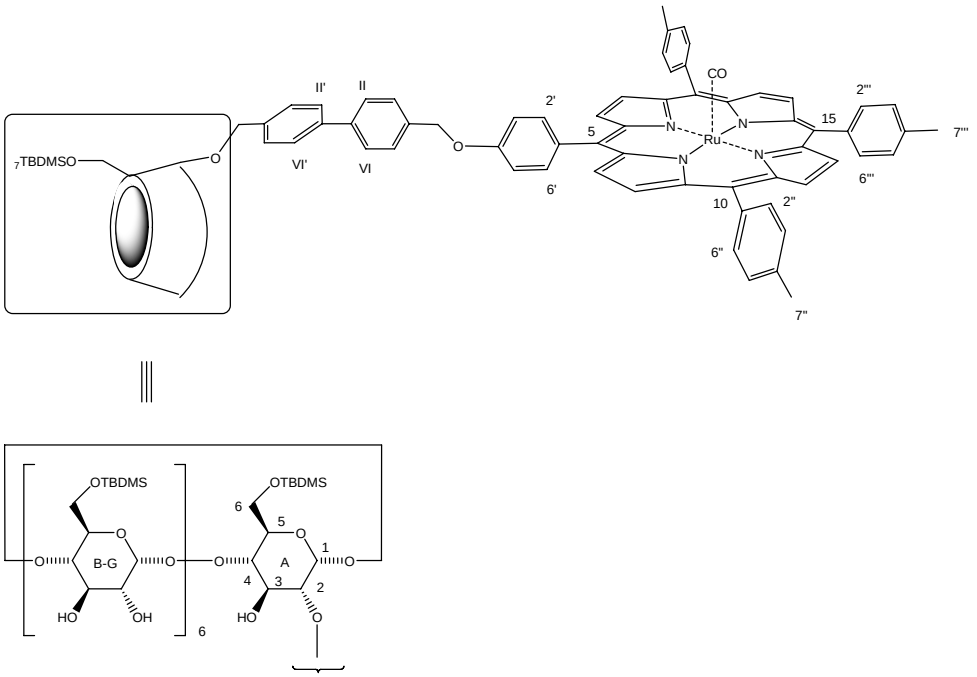
IR (neat, ν/cm^{-1}): 1928.7 (CO), 1006.8 [Ru (II) oxidation state marker band]

^{1}H-NMR (500MHz, $CDCl_3$/MeOH-D_4): 8.64-8.58 (*m*, 8H, porphyrin-H), 8.07 (*dd*, *J* = 8.2, 2.3, 1H, C(2' or 6')-H), 8.03 (*m*, 3H, C(2'' , 2''' or 6'', 6''')-H), 7.97 (*dd*, *J* = 8.2, 2.3, 1H, C(2' or 6')-H), 7.92 (*m*, 3H, C(2'', 2''' or 6'', 6''')-H), 7.64 (*m*, 4H, C(II, III, V, VI)-H), 7.58 (AA'BB', *J* = 8.2, 2H, C(II', VI")-H), 7.47-7.44 (*m*, 8H, C(3'', 3''', 5'', 5''')-H and C(III', V')-H), 7.3 (*dd*, *J* = 8.3, 2.3, 1H, C(3' or 5')-H), 7.26 (*dd*, 1H, H-C(3' or 5')), 5.32 (*s*, 2H, ArOCH_2), 4.51 (*s*, 2H, CH_2Br), 2.62 (*s*, 9H, ArCH_3)

^{13}C-NMR (125MHz, $CDCl_3$/MeOH-D_4): 181 (*C*O), 158.27 (C(4')), 144.19/144.04 (C(1, 4, 6, 9, 11, 14, 16, 19)), 140.97 (C(I')), 140.31 (C(I)), 139.69 (C(1'', 1''')), 137.0 (C(IV')), 136.88 (C(4'', 4''')), , 136.47 (C(IV)), 135.50 (C(1')), 135.36/134.87 (C(2', 6')), 134.27/133.78 (C(2'', 2''', 6'', 6''')), 131.52/131.49/131.42 (C(2, 3, 7, 8, 12, 13, 17, 18)), 129.59 (C(III', V')), 128.34 (C(III, V)), 127.55 (C(II', VI')), 127.42 (C(II,

VI)), 127.30/127.09 (C(3'', 3'', 5'', 5''')), 121.74 (C(10, 15, 20)), 121.25 (C(5)), 112.96/112.77 (C(3', 5')), 70.10 (ArO*C*H_2), 33.35 (*C*H_2Br), 21.42 (Ar*C*H_3)
ESI-MS (CH_2Cl_2/MeOH): 1080.7 $[M+Na]^+$, 1088.9 $[M+MeO]^-$

Protected ruthenium porphyrin-mono-β-CD complex **114**



To a solution of TBDMS-β-CD **50** (520.5mg, 0,27mmol) in dry, deagssed THF (4ml) was added LiH (15.8mg, 1.9mmol). The suspension was stirred at room temperature for 1h and then heated under reflux for 2h in argon. At room temperature, the degassed solution of mono-bromo-porphyrin **111** (28.5mg, 0.027mmol) in THF (6ml) was added. The resulting reaction mixture was heated under reflux in argon for 40h. At 0°C, the reaction was diluted with ethyl acetate, followed by slow addition of saturated NH_4Cl (aq.). The organic layer was separated, washed with water, dried over Na_2SO_4 and concentrated in *vacuo*. The residue was separated by column chromatography (SiO_2, ethyl acetate/EtOH/H_2O = 50:2:1) to afford the title compound (49.9mg, 64%) as red solid.

TLC (ethyl acetate/EtOH/H_2O=50:7:4): R_f= 0.48
UV/Vis (MeOH, λ): 410 (100, *Soret*), 530 (8)

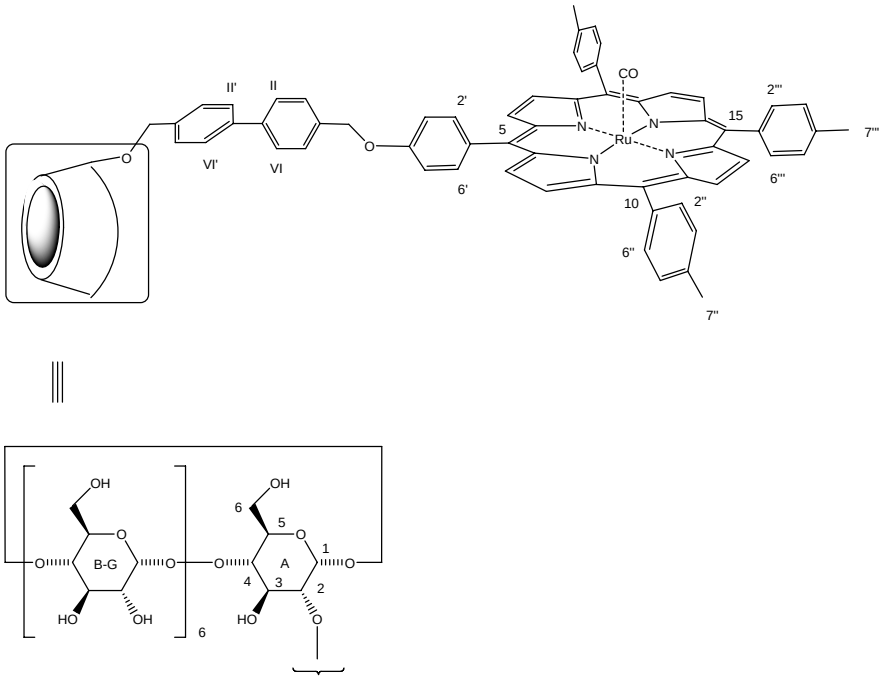
IR (neat, ν/cm^{-1}): 1930.6 (CO), 1035.7 (νC-O), 1006.8 [Ru (II) oxidation state marker band]

^{1}H-NMR (600MHz, toluene-D_8/MeOH-D_4): 8.92 (*d*, *J* = 4.8, 2H, C(3, 7)-H), 8.89 (*d*, *J* = 4.8, 2H, C(2, 8)-H), 8.86 (*s*, 4H, C(12, 13, 17, 18)-H), 8.13 (*dd*, *J* = 8.2, 2.3, 1H, C(2' or 6')-H), 8.11-8.05 (*m*, 7H, C(2', 2'', 2''' or 6', 6'', 6''')-H), 7.63 (*m*, 4H, C(II, VI, II', VI')-H), 7.60 (*d*, *J* = 8.3, C(III, V)-H), 7.54 (*d*, *J* = 8.3, C(III', V')-H), 7.35 (*m*, 3H, C(3'', 5'' or 3''', 5''')-H), 7.31 (*m*, 3H, C(3'', 5'' or 3''', 5''')-H), 7.25 (*dd*, *J* = 8.3, 2.8, 1H, C(3' or 5')-H), 7.18 (*dd*, *J* = 8.3, 2.8, 1H, C(3' or 5')-H, 5.10 (*m*, 7H, CD-C(1_{B-G})-H and CD-O-C*H*$_2$), 5.0 (*s*, 2H, Ar-O-C*H*$_2$-Ar), 4.97 (*d*, *J* = 3, 1H, CD-C(1_A)-H), 4.86 (*d*, 1H, CD-O-C*H*$_2$), 4.48 (*t*, *J* = 9.1, 1H, CD-C(3_A)-H), 4.3-3.57 (Heavily overlapped together with MeOH): 4.08 (CD-C(6_A)-H), 3.86 (CD-C(6_A)-H), 3.81 (CD-C(5_A)-H), 3.71 (CD-C(4_A)-H), 3.57 (CD-C(2_A)-H), 2.45 (*s*, 6H, C(7'')-H), 2.44 (*s*, 3H, C(7''')-H), 0.97-0.94 (m, 63H, C*H*$_3$C), 0.17-0.10 (m, 42H, C*H*$_3$Si)

^{13}C-NMR (125MHz, toluene-D_8/MeOH-D_4): 181.6 (CO), 159.0 (C(4')), 145.1, 144.9 (C(1, 9, 11, 14, 16, 19)), 141.3 (C(I')), 140.8 (C(I)), 140.6 (C(1'', 1''')), 137.1 (C(IV, IV')), 137.0 (C(4'', 4''')), 136.1 (C(1')), 136.0/135.4 (C(2', 6')), 134.9/134.4 (2'', 6'', 2''', 6''')), 132.1 (C(2, 3, 7, 8, 12, 13, 17, 18)), 130.1 (C(III', V')), 128.5 (III, V)), 127.9/127.5 (C(3'', 5'', 3''', 5''')), 127.6 (C(II, VI, II', VI')), 122.4 (C(10, 15, 20)), 122.1 (C(5)), 113.4/113.2 (C(3', 5')), 103.7/103.45/103.4/103.2 (could be CD-C(1_{B-G})), 101.9 (CD-C(1_A)), 82.4 (CD-C(4_A)), 82.3/82.1/82.0 (could be CD-C(4_{B-G})), 80.3 (CD -C(2_A)), 74.3 (CD-O-*C*H$_2$), 74.1, 73.9, 73.2 [73.3 = (CD-C(3_A)) was overlapped inside], 73.0 (CD-C(5_A)), 70.4, 70.1 (Ar-O-*C*H$_2$-Ar), 62.6 (CD-C(6)), 26.2 (*C*H$_3$C), 21.4 (Ar*C*H$_3$), 18.7 (CH$_3$*C*), -4.71/-4.77/-4.85 (*C*H$_3$Si)

ESI-MS (MeOH): 2934.8 [M+Na]$^+$, 1478.6 [M+2Na]$^+$/2, 2911.1 [M-H]$^-$

Ruthenium porphyrin-β-CD complex **113**



To a solution of protected ruthenium porphyrin-mono-β-CD complex **114** (7.4mg, 2.6μmol) in dry, degassed THF (2ml) was added TBAF (1M in THF, 25μl, 25μmol). The reaction solution was heated under reflux in argon for 19h. After evaporation of solvent in *vacuo*, the resulting residue was treated with Amberlite CG-50 (weakly acidic, H^+-form, 100-200 mesh, MeOH) to removed excess of TBAF, then purified by reversed phase column chromatography (RP-18, MeOH/H_2O = 20:1) to afford the title compound (4.3mg, 80%) as red solid.

TLC (ethyl acetate/*i*-PrOH/NH_3 (aq., 33%)/H_2O = 7:7:5:4): R_f = 0.51; (RP-18, MeOH/H_2O = 20:1): R_f= 0.43

UV/Vis (MeOH, λ): 410 (100, *Soret*), 530 (8)

HPLC (LiChrospher® 100 RP-18 (5μm) 250×4, 80% MeOH to 100% MeOH in 5min, @100% MeOH for 15min, flow = 1ml/min, λ_{det} = 412nm, T=40°C): R_t = 7.83min

^{1}H-NMR (500MHz, DMSO-D_6/D_2O): 8.59 (*d*, *J* = 4.8, 2H, porphyrin-H), 8.56 (*d*, *J* = 4.8, 2H, porphyrin-H), 8.55 (*s*, 4H, porphyrin-H), 8.1-8.08 (*m*, 1H, C(2' or 6')-H), 8.04-8.02 (*m*, 3H, C(2'', 2''' or 6'', 6''')-H), 7.98-7.96 (*m*, 1H, C(2' or 6')-H), 7.93-7.92 (*m*, 3H, C(2'', 2''' or 6'', 6''')-H), 7.8 (*d*, *J* = 8.2, 2H, C(II, VI)-H), 7.75-7.71 (*m*, 4H, C(III, V, II', VI')-H), 7.59-7.55 (*m*, 6H, C(3'', 5''', 3'', 5''')-H), 7.52 (*d*, *J* = 8.2,

2H, C(III', V')-H), 7.45-7.39 (*m*, 2H, C(3', 5')-H), 5.39 (*s*, 2H, Ar-O-CH_2-Ar), 4.94 (*d*, J = 3, CD-C(1_A)-H), 4.89-4.80 (*m*, 8H, CD-C($H1_{B-G}$)-H and CD-O-CH_2), 3.7-3.28 (heavily overlapped together with H_2O), 2.63 (*s*, 9H, ArCH_3)

ESI-MS (MeOH): 2135.5 $[M+Na]^+$, 1079.3 $[M+2Na]^{2+}/2$, 2111.0 $[M-H]^-$, 1054.9 $[M-2H]^{2-}/2$

5.3 Enzyme mimic reaction

Gereral procedure for the cleavage reactions: A round bottomed flask. which had been purged with argon and fitted with an appropriately sized egg shaped magnetic stir bar, was charged with a solution of catalyst in H_2O or MeOH/H_2O (1:9) according to the property of catalyst. TBHP (70% in water) (300 eq. to catalyst) was added. β,β-carotene or synthetic carotenoid (10 or 3.5 eq. to catalyst) was added to the reaction flask as a solution in chloroform/hexane (9:1) to produce a biphasic system. The reaction was closed, heated in 31-32^{o}C water bath and stirred vigorously to ensure good mixing of two phases. At time points during the reaction, stirring was stopped to allow phase separation. Aliquots (20μl) of the organic phase were taken and subjected to HPLC analysis.

HPLC methods:

1. For β,β-carotene **2** as substrate

   LiChrospher 100 RP-18 (5μm, length × ID = 125mm × 4.6mm), 25°C, 1ml/min, gradient: acetonitrile/1% $NH_4OAc_{(aq)}$ (1:1) to acetonitrile/*i*-PrOH (1:1) in 10min, @ acetonitrile/*i*-PrOH (1:1) for 5min, then acetonitrile/*i*-PrOH (1:1) to acetonitrile/1% $NH_4OAc_{(aq)}$ (1:1) in 2min. Detection was by diode array detector (λ_{det} = 340nm, 380nm, 405nm, 426nm, 450nm). R_t retinal = 10.39min, R_t 12'-apo = 12.05min, R_t 10'-apo = 12.20min. Quantification was by means of external curves.

   For LC-MS analysis, the same column was used: 25^{o}C, 0.3ml/min, gradient: [acetonitrile/0.05% $NH_4OAc_{(aq)}$ (1:1)/acetonitrile/*i*-PrOH (1:1)] (1:1) to acetonitrile/*i*-PrOH (1:1) in 20min, @ acetonitrile/*i*-PrOH (1:1) for 20min, then acetonitrile/*i*-PrOH (1:1) to acetonitrile/1% $NH_4OAc_{(aq)}$ (1:1) in 2min. Temperature of ESI-MS was 300^{o}C.

2. For 17,17'-dinor-ϕ,ϕ-carotene **84** as substrate

   YMC C30 (3 μm particle size, 4.6×250 mm), 25°C, 1ml/min, gradient: @ MeOH/H_2O (9:1) for 5min, then to MeOH/TBME (7:3) in 30min, @ MeOH/TBME (7:3) for 17min. Detection was by diode array detector (λ_{det} = 340nm, 380nm, 405nm, 426nm, 450nm). R_t retinal = 8.88min, R_t 12'-apo = 19.85min. Quantification was by means of external curves.

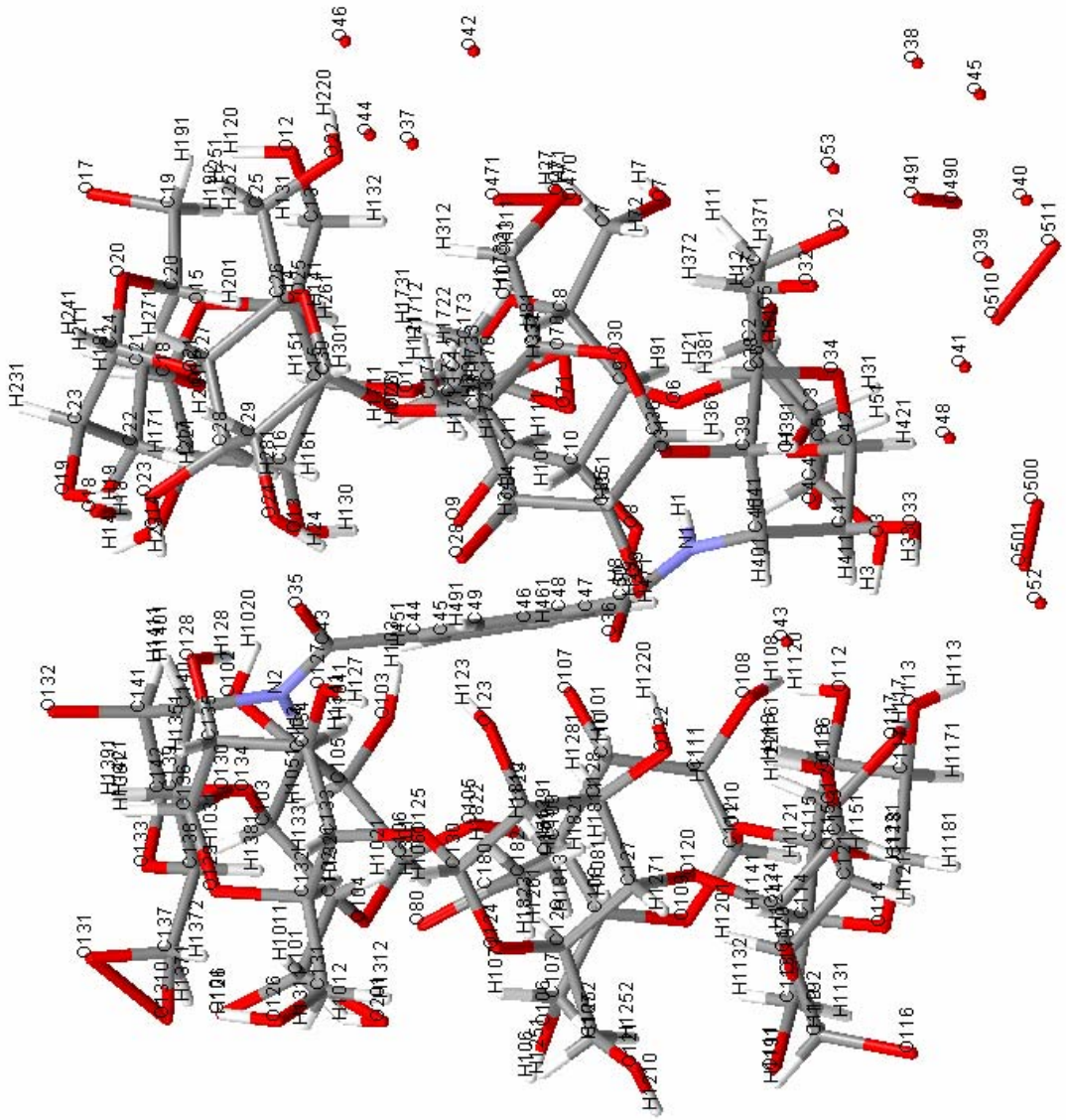
5.4 Detailed parameters of *X*-ray structure

Table 2 - Crystal and Structure Refinement Data for Compound **55**

Compound	**55**
Formula	$C_{92}H_{144}N_2O_{70}$
Composition	$C_{92}H_{146}N_2O_{70} \cdot 1.5\ C_3H_6O \cdot 15.5\ H_2O$
FW	2721.14
Crystal size (mm)	0.24×0.24×0.15
Morphology	plate
Cryst color	colorless
Wavelength (Å)	0.71073
Crystal system	monoclinic
Space group	$P2_1$
T, K	173
a/Å	17.5185(2)
b/Å	19.6595(2)
c/Å	18.5663(2)
α, deg	90.00
β, deg	99.0029(7)
γ, deg	90.00
V, Å^3	6315.55(12)
Z	2
θ (min, max)	2.944, 27.504
h, k, l (min, max)	(-22,18), (-25,24), (-22,24)
no. of refln measured	19224
no. of unique reflns	15477
no. of parameters	1774
ρ_{calc} (g/cm^3)	1.431
μ, mm^{-1}	0.129
F(000)	2860
R indexes	R1 = 0.0516[a]
[$I > 3.00\sigma(I)$]	wR2 = 0.0608[b]
R (all data)	R1 = 0.0704
	wR2 = 0.0774
GoF	1.0828

[a] $R = \sum(Fo - Fc)/\sum(Fo)$. [b] $wR2 = \{\sum[w(Fo^2 - Fc^2)^2]/\sum[w(Fo^2)^2]\}^{1/2}$.

Structure (*mercury view*) and atoms coordination for compound 55



Atom	X/ Y/ Z/ Uiso
C1	0.6889(2)/ 0.5501(3)/ 0.6980(3)/ 0.0895
C2	0.68588(18)/ 0.5898(2)/ 0.7667(2)/ 0.0575
C3	0.76177(17)/ 0.59164(17)/ 0.8187(2)/ 0.0420
C4	0.75166(17)/ 0.62419(16)/ 0.89046(19)/ 0.0392
C5	0.6841(2)/ 0.59323(17)/ 0.9210(3)/ 0.0530
C6	0.6123(2)/ 0.59046(17)/ 0.8648(3)/ 0.0547
C7	0.4488(2)/ 0.60524(18)/ 0.74514(17)/ 0.0450
C8	0.45861(19)/ 0.67446(16)/ 0.78035(17)/ 0.0383
C9	0.50997(16)/ 0.67643(15)/ 0.85458(17)/ 0.0340

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C10     0.51213(18)/ 0.74701(15)/ 0.88711(15)/ 0.0343
C11     0.43140(19)/ 0.77232(16)/ 0.88852(17)/ 0.0385
C12     0.38201(18)/ 0.76569(16)/ 0.81350(17)/ 0.0368
C13     0.2932(3)/ 0.7753(2)/ 0.6331(2)/ 0.0586
C14     0.34707(19)/ 0.83573(18)/ 0.64304(17)/ 0.0420
C15     0.36529(19)/ 0.85757(17)/ 0.72281(17)/ 0.0389
C16     0.4096(2)/ 0.92427(17)/ 0.73198(18)/ 0.0481
C17     0.3680(2)/ 0.97810(18)/ 0.68069(19)/ 0.0463
C18     0.35022(18)/ 0.95106(18)/ 0.60384(18)/ 0.0412
C19     0.36724(19)/ 0.86793(19)/ 0.43534(19)/ 0.0453
C20     0.43721(17)/ 0.91271(17)/ 0.45371(17)/ 0.0372
C21     0.42590(17)/ 0.96925(17)/ 0.50586(18)/ 0.0392
C22     0.49311(18)/ 1.01937(17)/ 0.5163(2)/ 0.0437
C23     0.50287(19)/ 1.04531(18)/ 0.4410(2)/ 0.0460
C24     0.51319(17)/ 0.98711(17)/ 0.39075(18)/ 0.0403
C25     0.58552(19)/ 0.8318(2)/ 0.3183(2)/ 0.0502
C26     0.65377(17)/ 0.86928(19)/ 0.36036(17)/ 0.0411
C27     0.63918(16)/ 0.94511(18)/ 0.36833(16)/ 0.0375
C28     0.71260(17)/ 0.98230(17)/ 0.40161(17)/ 0.0380
C29     0.77753(17)/ 0.9655(2)/ 0.35924(17)/ 0.0432
C30     0.78753(17)/ 0.8895(2)/ 0.35364(16)/ 0.0450
C31     0.79709(19)/ 0.7165(2)/ 0.3849(2)/ 0.0530
C32     0.84386(18)/ 0.74680(19)/ 0.45300(18)/ 0.0435
C33     0.87248(17)/ 0.81791(19)/ 0.43932(17)/ 0.0416
C34     0.92450(18)/ 0.84402(18)/ 0.50707(18)/ 0.0416
C35     0.98871(17)/ 0.79379(19)/ 0.53414(17)/ 0.0419
C36     0.95668(17)/ 0.72211(18)/ 0.53930(17)/ 0.0401
C37     0.8493(2)/ 0.5804(2)/ 0.5728(2)/ 0.0516
C38     0.86254(17)/ 0.61974(18)/ 0.64423(18)/ 0.0406
C39     0.93063(17)/ 0.66923(17)/ 0.65137(17)/ 0.0382
C40     0.94492(16)/ 0.70263(16)/ 0.72678(17)/ 0.0360
C41     0.95269(17)/ 0.64735(17)/ 0.78584(18)/ 0.0398
C42     0.88511(17)/ 0.59759(17)/ 0.77300(18)/ 0.0387
C43     0.70729(19)/ 1.01258(17)/ 0.6784(2)/ 0.0425
C44     0.7312(2)/ 0.95164(17)/ 0.7240(2)/ 0.0458
C45     0.6926(3)/ 0.9312(2)/ 0.7802(3)/ 0.0701
C46     0.7145(3)/ 0.8728(2)/ 0.8194(3)/ 0.0809
C47     0.7779(3)/ 0.83420(19)/ 0.8033(3)/ 0.0666
C48     0.8172(2)/ 0.85575(18)/ 0.74785(19)/ 0.0455
C49     0.7946(2)/ 0.91357(17)/ 0.70903(18)/ 0.0428
C50     0.8854(2)/ 0.81640(17)/ 0.72873(18)/ 0.0410
C101    0.7110(2)/ 1.1904(3)/ 0.9611(2)/ 0.0655
C102    0.6635(2)/ 1.1266(2)/ 0.9564(2)/ 0.0521
C103    0.5925(2)/ 1.12738(18)/ 0.89736(18)/ 0.0442
C104    0.5417(2)/ 1.06507(16)/ 0.90073(17)/ 0.0405
C105    0.5258(2)/ 1.05237(18)/ 0.97910(19)/ 0.0464
C106    0.6010(2)/ 1.05623(19)/ 1.03304(19)/ 0.0506
C107    0.7761(2)/ 1.04193(19)/ 1.1408(2)/ 0.0500
C108    0.7585(2)/ 0.97214(19)/ 1.11164(18)/ 0.0469
C109    0.6739(2)/ 0.95654(18)/ 1.08173(17)/ 0.0445
C110    0.6658(2)/ 0.8844(2)/ 1.0529(2)/ 0.0553
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C111 0.7014(2)/ 0.83462(19)/ 1.11122(19)/ 0.0477
C112 0.78305(19)/ 0.85632(18)/ 1.14194(16)/ 0.0417
C113 0.9725(3)/ 0.9126(2)/ 1.1690(2)/ 0.0669
C114 0.9675(2)/ 0.86270(18)/ 1.10709(18)/ 0.0453
C115 0.90011(19)/ 0.81436(17)/ 1.10007(16)/ 0.0401
C116 0.90171(19)/ 0.76656(17)/ 1.03657(18)/ 0.0406
C117 0.97955(19)/ 0.73036(16)/ 1.04365(17)/ 0.0394
C118 1.04587(19)/ 0.78103(16)/ 1.05800(17)/ 0.0379
C119 1.1679(5)/ 0.9303(3)/ 1.0514(3)/ 0.1526
C120 1.1380(3)/ 0.9074(2)/ 0.97311(19)/ 0.0666
C121 1.1206(2)/ 0.83140(16)/ 0.97164(17)/ 0.0415
C122 1.11495(18)/ 0.80369(15)/ 0.89421(17)/ 0.0372
C123 1.18826(19)/ 0.82245(18)/ 0.86377(19)/ 0.0437
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C125 1.1820(4)/ 1.0773(2)/ 0.8524(3)/ 0.0878
C126 1.1327(2)/ 1.04844(18)/ 0.7847(2)/ 0.0495
C127 1.15557(19)/ 0.97640(18)/ 0.76702(18)/ 0.0434
C128 1.11130(18)/ 0.95170(17)/0.69465(17)/ 0.0396
C129 1.11890(19)/ 1.0048(2)/ 0.63649(19)/ 0.0515
C130 1.0940(2)/ 1.0744(2)/ 0.6590(2)/ 0.0539
C131 1.0033(2)/ 1.2115(2)/ 0.7398(2)/ 0.0565
C132 0.9410(2)/ 1.1710(2)/ 0.6929(2)/ 0.0502
C133 0.96621(19)/ 1.1272(2)/ 0.63447(19)/ 0.0476
C134 0.8970(2)/ 1.0937(2)/ 0.5901(2)/ 0.0511
C135 0.8361(2)/ 1.1448(2)/ 0.5602(2)/ 0.0502
C136 0.81818(19)/ 1.1924(2)/ 0.6203(2)/ 0.0492
C137 0.7760(3)/ 1.2696(2)/ 0.7740(4)/ 0.0809/
C138 0.7227(2)/ 1.20821(18)/ 0.7638(2)/ 0.0507
C139 0.70983(19)/ 1.18263(17)/ 0.6854(2)/ 0.0445
C140 0.64832(19)/ 1.12655(17)/ 0.67355(19)/ 0.0413
C141 0.57569(19)/ 1.15146(18)/ 0.7015(2)/ 0.0445
C142 0.59339(19)/ 1.18071(17)/ 0.7779(2)/ 0.0461
C170 0.6458(3)/ 0.7736(3)/ 0.6221(3)/ 0.0953
C171 0.6683(5)/ 0.8218(3)/ 0.5677(3)/ 0.1201
C172 0.5829(5)/ 0.8002(6)/ 0.6578(5)/ 0.0908
C173 0.5678(6)/ 0.7604(7)/ 0.6302(8)/ 0.1243
C180 0.8778(7)/ 1.0431(6)/ 0.8934(6)/ 0.1135
C181 0.8529(9)/ 0.9863(9)/ 0.9343(8)/ 0.1259
C182 0.9388(6)/ 1.0220(8)/ 0.8560(7)/ 0.1092
N1 0.88207(15)/ 0.74895(14)/ 0.73804(14)/ 0.0386
N2 0.67588(16)/ 1.06368(14)/ 0.71116(15)/ 0.0399
O1 0.81746(12)/ 0.63208(12)/ 0.78693(12)/ 0.0405
O2 0.71418(18)/ 0.4827(2)/ 0.7123(3)/ 0.1173
O3 0.82076(14)/ 0.61501(13)/ 0.93972(14)/ 0.0478
O4 0.66963(16)/ 0.62852(14)/ 0.98440(18)/ 0.0614
O5 0.62741(14)/ 0.55747(15)/ 0.8016(2)/ 0.0745
O6 0.58783(12)/ 0.65899(12)/ 0.84717(15)/ 0.0454
O7 0.42276(15)/ 0.55452(12)/ 0.79033(13)/ 0.0475
O8 0.55421(14)/ 0.74341(12)/ 0.95973(12)/ 0.0453
O9 0.43308(16)/ 0.84203(13)/ 0.90986(14)/ 0.0535
O10 0.38228(12)/ 0.69792(11)/ 0.78833(12)/ 0.0391

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O11     0.41457(13)/ 0.80932(11)/ 0.76474(12)/ 0.0393
O12     0.2771(2)/ 0.75562(17)/ 0.55974(17)/ 0.0869
O13     0.4150(2)/ 0.94879(13)/ 0.80443(14)/ 0.0652
O14     0.41389(17)/ 1.03789(12)/ 0.68281(15)/ 0.0543
O15     0.30794(12)/ 0.89037(13)/ 0.60090(12)/ 0.0441
O16     0.42068(12)/ 0.94140(12)/ 0.57690(12)/ 0.0404
O17     0.29896(15)/ 0.90688(19)/ 0.41317(19)/ 0.0772
O18     0.47694(15)/ 1.07576(13)/ 0.55928(15)/ 0.0534
O19     0.56383(15)/ 1.09333(13)/ 0.44445(17)/ 0.0570
O20     0.45075(12)/ 0.94194(12)/ 0.38506(12)/ 0.0400
O21     0.58408(11)/ 0.95389(12)/ 0.41712(12)/ 0.0378
O22     0.59653(16)/ 0.76033(16)/ 0.3249(2)/ 0.0745
O23     0.69620(13)/ 1.05371(13)/ 0.40000(14)/ 0.0494
O24     0.84723(12)/ 0.99873(15)/ 0.39242(13)/ 0.0521
O25     0.71787(12)/ 0.85937(14)/ 0.32000(11)/ 0.0450
O26     0.80912(12)/ 0.86481(13)/ 0.42593(11)/ 0.0406
O27     0.76681(15)/ 0.65057(15)/ 0.39866(16)/ 0.0615
O28     0.95654(15)/ 0.90838(15)/ 0.49005(16)/ 0.0587
O29     1.03057(13)/ 0.81441(14)/ 0.60238(12)/ 0.0464
O30     0.90924(13)/ 0.70285(13)/ 0.47360(12)/ 0.0452
O31     0.91422(12)/ 0.72207(12)/ 0.59829(11)/ 0.0394
O32     0.91854(17)/ 0.54819(16)/ 0.56000(17)/ 0.0681
O33     1.02030(12)/ 0.60816(12)/ 0.78314(15)/ 0.0464
O34     0.87522(12)/ 0.57018(12)/ 0.70173(12)/ 0.0413
O35     0.71664(16)/ 1.01374(13)/ 0.61324(14)/ 0.0520
O36     0.93892(15)/ 0.84641(13)/ 0.70685(14)/ 0.0521
O37     0.16077(16)/ 0.66332(14)/ 0.75417(16)/ 0.0576
O38     0.8260(2)/ 0.3636(2)/ 0.5194(2)/ 0.0900
O39     0.8540(2)/ 0.41805(18)/ 0.7406(3)/ 0.1136
O40     0.9422(2)/ 0.3710(2)/ 0.6378(2)/ 0.0923
O41     1.02360(17)/ 0.49915(16)/ 0.67175(18)/ 0.0675
O42     0.67804(18)/ 0.62847(15)/ 0.26710(18)/ 0.0723
O43     0.59758(19)/ 0.70597(16)/ 1.15273(19)/ 0.0722
O44     0.4195(3)/ 0.7113(2)/ 0.4847(2)/ 0.1026
O45     0.5152(3)/ 0.3090(2)/ 0.8718(2)/ 0.1104
O46     0.1526(3)/ 0.6562(3)/ 0.6090(2)/ 0.1052
O48     0.6525(3)/ 0.5082(3)/ 1.0495(3)/ 0.0471
O52     1.0490(5)/ 0.5676(4)/ 0.9346(5)/ 0.1034
O53     0.5108(4)/ 0.4385(4)/ 0.8233(8)/ 0.1345
O70     0.6690(6)/ 0.7152(4)/ 0.6310(5)/ 0.1209
O71     0.7009(5)/ 0.7424(4)/ 0.6622(5)/ 0.1123
O80     0.8497(6)/ 1.1014(6)/ 0.8951(6)/ 0.1326
O101    0.6675(3)/ 1.2559(2)/ 0.9705(3)/ 0.0525
O102    0.47142(15)/ 1.07586(12)/ 0.85350(13)/ 0.0481
O103    0.49120(19)/ 0.98896(14)/ 0.98476(15)/ 0.0628
O104    0.63976(16)/ 1.11744(13)/ 1.02622(13)/ 0.0520
O105    0.64724(16)/ 0.99974(13)/ 1.02049(13)/ 0.0546
O106    0.73272(15)/ 1.05780(13)/ 1.19761(14)/ 0.0516
O107    0.58534(16)/ 0.86888(13)/ 1.03070(16)/ 0.0611
O108    0.70355(15)/ 0.76764(13)/ 1.08189(14)/ 0.0498
O109    0.78450(15)/ 0.92354(13)/ 1.16814(12)/ 0.0483
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O110    0.82810(13)/ 0.84935(12)/ 1.08569(11)/ 0.0425
O111    1.0303(3)/ 0.9626(2)/ 1.1671(3)/ 0.1252
O112    0.84215(14)/ 0.71647(14)/ 1.03461(14)/ 0.0514
O113    0.98734(13)/ 0.69263(12)/ 0.97919(13)/ 0.0435
O114    1.03802(13)/ 0.82366(12)/ 1.11797(11)/ 0.0417
O115    1.04737(14)/ 0.81949(11)/ 0.99393(11)/ 0.0410
O116    1.2431(3)/ 0.8867(3)/ 1.0806(2)/ 0.1219
O117    1.10513(14)/ 0.73194(12)/ 0.89370(13)/ 0.0457
O118    1.18759(14)/ 0.79891(13)/ 0.79236(13)/ 0.0475
O119    1.20512(19)/ 0.92164(14)/ 0.94032(14)/ 0.0665
O120    1.13851(12)/ 0.93029(11)/ 0.82203(11)/ 0.0383
O121    1.2610(2)/ 1.07311(18)/ 0.8476(2)/ 0.1090
O122    1.14343(13)/ 0.88881(13)/ 0.67908(12)/ 0.0462
O123    1.07555(18)/ 0.98370(17)/ 0.56878(15)/ 0.0727
O124    1.14129(14)/ 1.09274(13)/ 0.72484(16)/ 0.0562
O125    1.01592(13)/ 1.07215(14)/ 0.66665(14)/ 0.0521
O126    1.03991(15)/ 1.25865(15)/ 0.69918(16)/ 0.0584
O127    0.91997(17)/ 1.0573(2)/ 0.53008(19)/ 0.0808
O128    0.76734(14)/ 1.11300(16)/ 0.52578(14)/ 0.0569
O129    0.88657(14)/ 1.22126(13)/ 0.65861(14)/ 0.0521
O130    0.77866(13)/ 1.15466(12)/ 0.66759(13)/ 0.0452
O131    0.7497(3)/ 1.3210(2)/ 0.7365(3)/ 0.0675
O132    0.54110(15)/ 1.20554(15)/ 0.65567(15)/ 0.0572
O133    0.65069(14)/ 1.23108(12)/ 0.78385(17)/ 0.0544
O134    0.61667(14)/ 1.12608(11)/ 0.82667(12)/ 0.0427
O201    0.7880(4)/ 1.1679(3)/ 1.0060(4)/ 0.0762
O470    0.6677(5)/ 0.6341(5)/ 0.5011(5)/ 0.0981
O471    0.6396(4)/ 0.6811(4)/ 0.4611(4)/ 0.0669
O490    0.6524(6)/ 0.3898(4)/ 0.8344(6)/ 0.0901
O491    0.6567(5)/ 0.4130(5)/ 0.7942(4)/ 0.0812
O500    0.9133(5)/ 0.5086(4)/ 0.9579(5)/ 0.0848
O501    0.9575(3)/ 0.5509(3)/ 0.9655(3)/ 0.0589
O510    0.6469(4)/ 0.4193(3)/ 0.9821(3)/ 0.0669
O511    0.6293(5)/ 0.3453(6)/ 0.9767(4)/ 0.1005
O1310   0.7832(4)/ 1.3047(4)/ 0.8282(4)/ 0.0547
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H3      0.8279/ 0.6476/ 0.9745/ 0.0500
H7      0.3746/ 0.5541/ 0.7984/ 0.0500
H8      0.5673/ 0.7805/ 0.9837/ 0.0500
H14     0.3831/ 1.0712/ 0.6924/ 0.0500
H18     0.4635/ 1.0561/ 0.5998/ 0.0500
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H24     0.8703/ 0.9853/ 0.4358/ 0.0500
H27     0.7225/ 0.6480/ 0.4184/ 0.0500
H29     0.9982/ 0.8192/ 0.6346/ 0.0500
H33     1.0633/ 0.6300/ 0.7767/ 0.0500
H103    0.4844/ 0.9583/ 0.9504/ 0.0500
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H1822   0.9226/ 1.0285/ 0.8026/ 0.1320
H1823   0.9865/ 1.0500/ 0.8727/ 0.1320
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7 Curriculum vitae

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Personal

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Sex:	Male	Marital Status:	Married to Qian Zhang, GM. Assistant in Boehler Thyssen (Shanghai) Trading Co., Ltd., Shanghai, China; One daughter

Education

2002-present: University of Basel, Basel, Switzerland.
Ph.D. student
Research advisor: Prof. Wolf-D. Woggon
Research Project: Ruthenium porphyrin-β-cyclodextrin complexes as supermolecular enzyme models for regioselective cleavage of carotenoids.

1996-2000: School of Pharmacy, Shanghai Medical University, Shanghai, China.
Degree awarded: Master degree.
Research advisor: Prof. Ren Wen
Research Projects: 1. Study of indole derivatives as estrogen antagonists; 2. Synthesis of new 5-HT_{2A} ligands--4-piperidinyl sulfur ether and sulfone derivatives.

1991-1995 Shanghai Medical University, Shanghai, China.
Degree awarded: B.S.
Research advisor (1995): Prof. Ren Wen (School of Pharmacy, Shanghai Medical University)
Practical Project: synthesis of β-carboline derivatives.

1988-1991 Jingye High School, Shanghai, China

Professional Experience

2000-2002 Lecturer, Department of Medicinal Chemistry, School of Pharmacy, Shanghai Medical University, Shanghai, China

1995-2000 Teaching assistant, Department of Medicinal Chemistry, School of Pharmacy, Shanghai Medical University, Shanghai, China

Publications

Hao Wang, Wolf-D. Woggon. **Enzyme mimics for the excentric cleavage of carotenoids.** *in prep*.

Hao Wang, Ren Wen, Lei Huang, Robert B. Innis, Ping-Zhong Tan. **Synthesis and biological activities of new 5-HT_{2A} selective ligands--*N*-substituted piperidinyl-4-phenylthioether and sulfone derivatives.** *Acta. Pharmaceutica Sinica*. (2001), 36(4), 274-277

Hao Wang, Ren Wen, Wei-Qun Jiang, Xiao-Chun Dong, Lei Huang, Ning Li, Lin Cao. **Synthesis of several 3-oxamoyl-2-phenylindole derivatives and their inhibition ability on cultured rat luteal cell growth.** *J. Chin. Pharmaceutic Sciences*. (2001), 10(1), 49-51

Zhi-Gang Lin, Hao Wang, Wei-Qun Jiang, Ren Wen. **A new synthesis of 4-amino-β-carboline derivatives.** *Chin. J. Med. Chem*. (2001), 11(3), 157-159

Experimental Skills

Practical experience in NMR, ESI-MS, MALDI-TOF-MS, HPLC, Ion Exchange Resin, UV-VIS, FTIR and Working in Glove Box.
Well-experienced in Metallorganic Chemistry; Polyenl- and Cyclodextrin Chemistry.

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Aug. 31, 2006

8 Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, dass ich meine Dissertation selbständig und nur mit den angegebenen Hilfsmitteln angefertigt habe. Ich erkläre ausserdem, dass ich diese Dissertation an keiner anderen Fakultät eingereicht habe.

Basel, den 31. August 2006

Hao Wang