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[10] Integration of the initial spectrum proves the inclusion of one benzene molecule per dimer.

[11] The undimerized **3e** remains dissolved in toluene, while it precipitates from *p*-xylene.

[12] MD simulations in vacuo (Tripos force field, M. Clark, R. D. Cramer, N. van Opdenbusch, *J. Comp. Chem.* **1989**, *10*, 982 – 1012; 300 K, 200 ps) on dimers with included benzene led to the following cavity sizes (energy-minimized average structures) expressed by the distance of the two calix[4]arene mean planes defined by the four methylene carbons: **3d/3d** 10.0 Å, **3e/3e** 8.4 Å, **3d/3e** 9.8 Å. The hydrogen bonds characterized by the distance between nitrogen (N_I attached to the calixarene, N_{II} attached to the urea-residues) and the carbonyl oxygen are $N_I \cdots O = 2.55$ and 3.02 Å, $N_{II} \cdots O = 2.52$ and 2.98 Å in **3d/3e** and $N_I \cdots O = 2.62$, $N_{II} \cdots O = 2.48$ Å in **3d/3d**. Thus, the distinctly smaller cavity explains that homodimers **3e/3e** are not formed in benzene, while the slight difference in size between **3d/3d** and **3d/3e** together with less favorable hydrogen-bond distances might explain that also heterodimers are impossible with larger guests such as toluene or p-xylene.

[13] If in a control experiment [**3a**/C_6H_6/**3a**] is treated with C_6D_{12} or C_6H_{12} under the same conditions, the signal of included C_6H_6 (at $\delta = 3.88$ in C_6D_{12}) disappears completely, being replaced by a signal at $\delta = -1.44$ for included C_6H_{12} in the latter case.

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[15] This means a high value for the free energy of activation ΔG^{+}; compare the "constrictive binding" defined by Cram for (hemi)-carceplexes: "Container Molecules and Their Guests": D. J. Cram, J. M. Cram in *Monographs in Supramolecular Chemsitry* (Ed.: J. F. Stoddart), Royal Society of Chemistry, Cambridge, **1994**.

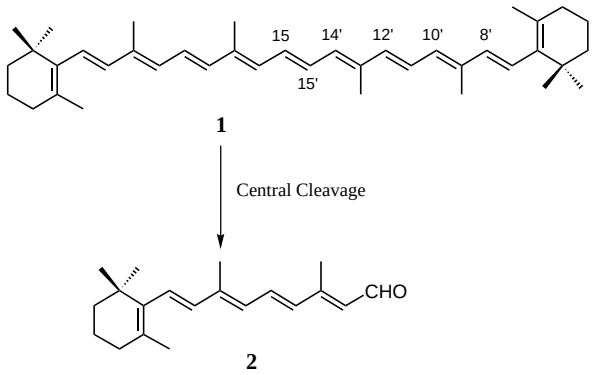
[16] These procedures would be analogous to the formation of rotaxanes by "slipping" or by "threading + capping".

A Supramolecular Enzyme Mimic That Catalyzes the 15,15′ Double Bond Scission of *β*,*β*-Carotene**

Richard R. French, Philipp Holzer, Michele G. Leuenberger, and Wolf-D. Woggon*

Dedicated to Professor Albert Eschenmoser on the occasion of his 75th birthday

The enzymes (carotene dioxygenases, CDOs) that cleave *β*,*β*-carotene **1** to provide retinal **2** as a precursor for retinol (vitamin A) are of significance to animal and human nutrition.[1] To date two modes of cleavage of **1** have been proposed: 1) the more recently discovered excentric cleavage which yields apocarotenals, which can be degraded to **2** by *β*-oxidation, and 2) the central cleavage of **1** which gives retinal **2** directly (Scheme 1).[2, 3] The CDO enzymes responsible for



Scheme 1.

catalyzing these reactions have been neither purified nor are their respective co-factors known. Results concerning central cleavage suggest that the enzyme involved places its active site's metal complex directly above the C(15)=C(15′) bond.[4, 5] The fact that this CDO controls the regiospecific cleavage of one C=C bond out of a possible six within the substrate is an intriguing and challenging one.

To mimic such a regioselective system the following strategy was employed. a) The synthesis of a receptor for **1** for which the binding constant K_a for **1** is orders of magnitude greater than that for retinal **2** was necessary in order to prevent product inhibition. b) The introduction of a reactive metal center which is capable of cleaving *E*-configured, conjugated double bonds to aldehydes. c) The use of a co-

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oxidant which is inert towards **1** in the absence of a catalytic metal center.

We have previously reported on a cyclodextrin-based synthetic receptor for **1** ($K_a = 2.4 \times 10^6\,\text{M}^{-1}$) in which two β-cyclodextrin units are linked through a free-base porphyrin.[6] As it was anticipated that the binding of **1** to the receptor would be enhanced by an increase in planarity of the porphyrin, as would be the case in the catalytic system, we have synthesized the corresponding porphyrinatozinc(II) complex **3** (Figure 1a). The binding constant K_a for the binding of

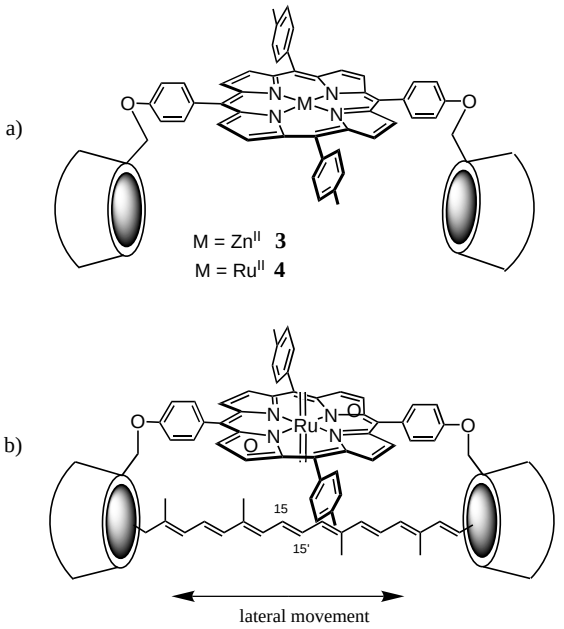
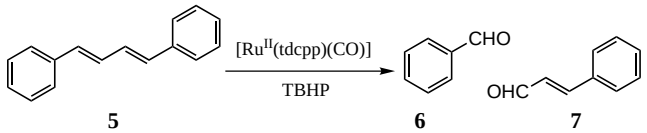


Figure 1. a) Structures of the receptors **3** and **4**. b) Schematic representation of the inclusion complex between **1** and **4**. The double-headed arrow denotes the possible lateral movement of **1** within the binding pocket.

1 to **3** was determined to be $K_a = 8.3 \times 10^6\,\text{M}^{-1}$ by using fluorescence spectroscopy.[6] This satisfied the first of our strategic criteria for mimicking the biological system as the binding constant for retinal **2** to β-cyclodextrin has been reported to be $3.6 \times 10^3\,\text{M}^{-1}$.[7]

With regard to the choice of a metalloporphyrin capable of cleaving double bonds, there was only one precedent in the literature: reaction of α-methylstyrene to give acetophenone in the presence of [Ru(tdfpp)] (tdfpp = tetrakis(2,6-difluorophenyl)porphyrin) and *tert*-butyl hydroperoxide (TBHP).[8] We have systematically studied the reactivity of *open-face* as well as *face-protected* ruthenium porphyrins towards substrates containing conjugated *E*-configured double bonds in the presence of TBHP. A representative example is the cleavage of *trans,trans*-1,4-diphenyl-1,3-butadiene (**5**) by Ru[tdcpp] (tdcpp = tetrakis(2,6-dichlorophenylporphyrin)) which gives benzaldehyde (**6**) and cinnamylaldehyde (**7**) (Scheme 2).[9] Importantly, the first step in the cleavage

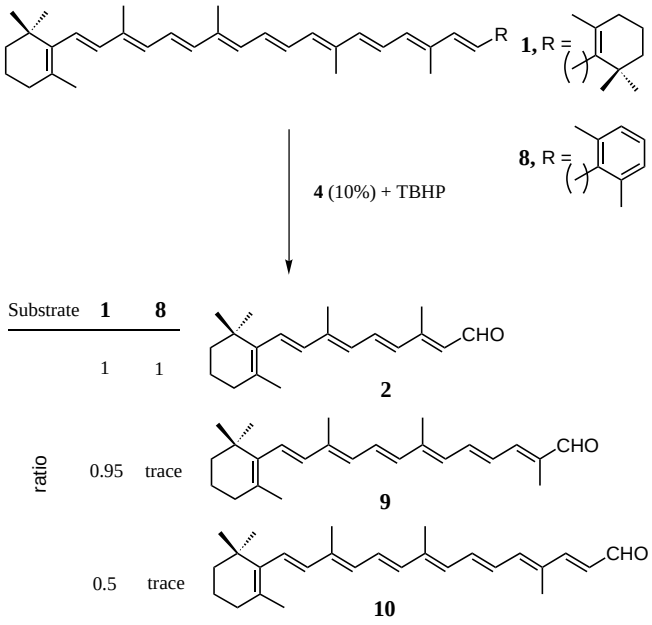


Scheme 2.

of double bonds is epoxide formation, which is followed by TBHP/ruthenium porphyrin mediated fragmentation to yield the products **6** and **7**. The clean reaction of various oxo–Ru porphyrins with the *E*-configured double bonds of **5** and **1** (see below) is novel and is in contrast to the reactivity of oxo–iron porphyrins[10] and oxo–manganese salene complexes[11] which exhibit a clear preference for *Z* double bonds. For the latter systems it has been suggested that the olefin approaches the metal oxo double bond in a "side-on" fashion. This interaction is impossible between **5** and Ru[tdcpp](O)$_2$ or between **1** and the dioxo complex of **4**. Since we also observed cleavage of *Z*-configured, conjugated olefins by ruthenium porphyrins exhibiting varying degrees of *face-protection*, we conclude that the Ru=O group attacks both *E* and *Z* double bonds "head-on".[9] Thus, there is no need to invoke different mechanisms for epoxide formation depending on the configuration of the olefin.[12] Finally, we have also tested the stability of β,β-carotene towards TBHP, and established that no derivatization or degradation took place within 24 h at room temperature. A further control experiment with [Ru(tpp)]/TBHP (tpp = tetraphenylporphyrin) and β,β-carotene in dichloromethane gave wholesale degradation of **1**, the complex mixture of products being impossible to characterize.

With the above prerequisites satisfied, the stage was set to employ the supramolecular system **4** (Figure 1a) to investigate the catalytic cleavage of β,β-carotene **1**.

Dimers **3** and **4** were obtained by the reaction of commercially available 6^A-O-(*p*-tosyl)-β-cyclodextrin[13] with the corresponding metal porphyrin in the presence of cesium carbonate (DMF, three days, HPLC control),[14] and the desired products were purified by preparative HPLC. For the catalytic cleavage of β,β-carotene **1**, a biphasic system was established in which **1** is extracted from a 9:1 mixture of hexane and chloroform into a water phase containing the dimer **4** (10 Mol-%) and TBHP (Scheme 3). The reaction products,



Scheme 3. Product ratios for oxidations of **1** and **8** with the catalyst **4**.

  0570-0833/00/3907-1268 $ 17.50+.50/0

released from the receptor, are then extracted into the organic phase, aliquots of which were subjected to HPLC conditions developed for the analysis of carotene dioxygenase enzyme studies.[15] The reaction products were identified by retention time (co-injection with authentic samples) and by their UV spectra; external calibration curves were used for the quantification. The ratio of reaction products is given in Scheme 3.

It is evident that **1** is not only cleaved at the central double bond but also at C(12′)=C(11′) to give 12′-apocarotenal **9** and at C(10′)=C(9′) to give 10′-apocarotenal **10**. The combined yield of aldehydes **2**, **9**, and **10** was 30%, which compares well with the efficiency of β,β-carotene 15,15′ dioxgenase which gives retinal **2** in 20–25%.[4] Most interestingly, the double bond in closest proximity to the central one in **1**, C(14′)=C(13′), remains untouched. We postulated that this phenomenon is related to interactions between the end-groups of **1** and the cyclodextrin moieties, such that a lateral movement of **1** within the binding pocket exposes only three double bonds to the reactive Ru=O group (Figure 1b). If this hypothesis is true, one would expect a carotenoid with one modified end-group to demonstrate a different selectivity in double-bond cleavage. Accordingly, we investigated the oxidation of synthetic carotenoid **8**,[16] a substrate of the native enzyme,[17] under identical conditions. The selectivity for C(15)=C(15′) cleavage is almost exclusive when one of the cyclohexene end-groups of β,β-carotene **1** is replaced by an *ortho*-dimethylphenyl group, supporting the relationship between substrate mobility and selectivity. This suggests that stronger hydrophobic interactions between the aromatic end-group of **8** and the β-cyclodextrin cavity are responsible for stabilizing the 1:1 inclusion complex with the central double bond under the reactive ruthenium center.

We have demonstrated the selective cleavage of carotenoids to provide retinal **2** with a supramoleclular enzyme mimic which shows an unusual reactivity towards olefins in the presence of TBHP. Work is currently underway to mimic the reactivity and selectivity of the enzymes responsible for excentric cleavage of β,β-carotene **1**.[18]

Experimental Section

Physical data for **4**. λ_{max} = 414 (100%), 534 (10%) 570 (4%); ^{1}H NMR (600 MHz, 25 °C, [D$_6$]DMSO): δ = 8.61 (d, 4H, H-2,8,12,18, J = 4.5 Hz), 8.56 (d, 4H, H-3,7,13,17, J = 4.5 Hz), 8.06 (m, 4H, H-2″,6″), 7.94 (m, 4H, H-3″,5″), 7.58 (m, 4H, H-2′,6′), 7.33 (m, 4H, H-3′,5′), 5.9–5.6 (br. m, 28H, 2OH), 5.0–4.8 (m, 14H, anomeric H), 4.7–4.4 (*m*, 12H, 1OH), 3.8–3.25 (m, 84H, β-CD (H-2,3,4,5,6,6′)), 2.65 (*s*, 6H, ArCH$_3$); MALDI-TOF-MS: *m*/*z*: 3007 [M^+], 3024 [M+H$_2$O]; HPLC (LiChrospher 100Rp-18 (5 μm), 250-4) R_t = 9.43 min (20–60% acetonitrile in 20 min, flow 1.5 mL min^{-1}).

General procedure for cleavage reactions: A 25-mL round-bottomed flask which had been purged with argon and fitted with an egg-shaped magnetic stir bar was charged with a solution of dimer **4** (2.3 mg, 10 Mol-%) in H$_2$O (1 mL). TBHP (30 μL of a 70% solution in water, 30 equiv with respect to **1**) was added. β,β-Carotene **1** (4 mg) was dissolved in hexane/chloroform (9/1; 10 mL) and added to the reaction flask to produce a biphasic system. The reaction system was closed and stirred vigorously to ensure good mixing of the two phases. At different times during the reaction, stirring was stopped to allow phase separation. Aliquots (20 μL) of the organic phase were taken and subjected to HPLC analysis (LiChrospher 100 Rp-18 5 μm, length × ID = 125 mm × 4.6 mm, 25 °C, 1 mL min^{-1}, gradient: acetonitrile:1% $NH_4OAc_{(aq)}$ (1:1) (100%) →acetonitrile:*i*PrOH (1:1) (100%) in 10 min, then acetonitrile:*i*PrOH (1:1) (100%) for 5 min, then acetonitrile:*i*PrOH (1:1) (100%) →acetonitrile:1% $NH_4OAc_{(aq)}$ (1:1) (100%) in 2 min). A diode array detector was used for the detection. R_t = 10.39 (**2**), R_t = 12.05 (**9**), R_t = 12.20 min (**10**). In all reactions, carotenoids **1** (R_t = 14.0 min) and **8** (R_t = 15.3 min) were completly consumed after 24 h.

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The Spatial Demand of Dendrimers: Deslipping of Rotaxanes**

Gosia M. Hübner, Guido Nachtsheim, Qian Yi Li, Christian Seel, and Fritz Vögtle*

Dendrimers are generally not rigid molecules because of the flexibility of their building blocks,[1] and this makes it difficult to compare their size with that of voluminous, but rather rigid "conventional" molecules. Recently we described the effect of slight changes in the size of wheels and stoppers

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