Photocatalysis

DOI: 10.1002/anie.201409146

LED-Illuminated NMR Studies of Flavin-Catalyzed Photooxidations Reveal Solvent Control of the Electron-Transfer Mechanism**

*Christian Feldmeier, Hanna Bartling, Kathrin Magerl, and Ruth M. Gschwind**

Abstract: *Mechanistic insights into chemical photocatalysis are mainly the domain of UV/Vis spectroscopy, because NMR spectroscopy has been limited by the type of illumination so far. An improved LED-based illumination device can be used to obtain NMR reaction profiles of photocatalytic reactions under synthetic conditions and perform both photo-CIDNP and intermediate studies. Flavin-catalyzed photooxidations of alcohols show the potential of this setup. After identical initial photoreaction steps the stabilization of a downstream intermediate is the key to the further reaction mechanism and the reactivity. As a chemical photocatalyst flavin can act either as a one- or a two-electron mediator when the stability of the zwitterionic radical pair is moldulated in different solvents. This demonstrates the importance of downstream intermediates and NMR-accessible complementary information in photocatalytic reactions and suggests the control of photoorganic reactions by solvent effects.*

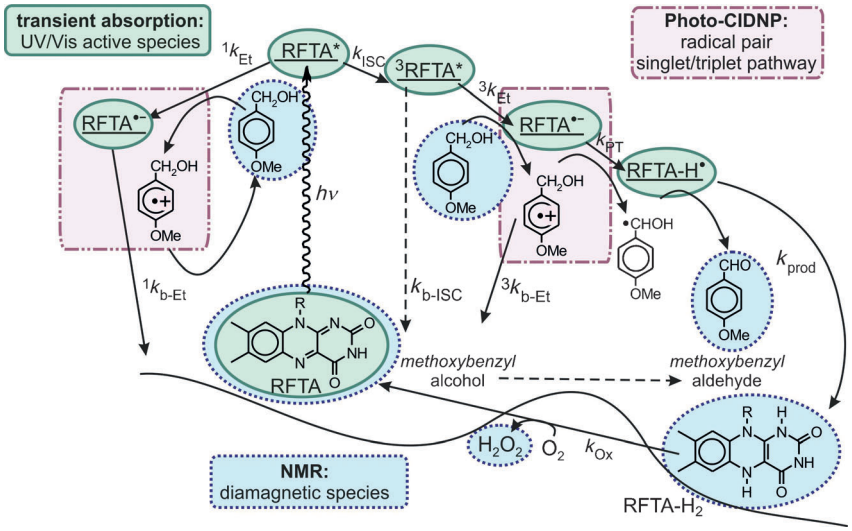

Figure 1. NMR complements UV/Vis spectroscopy with regards to structure information and time resolution in photocatalytic reactions. In RFTA-catalyzed photooxidations of alcohols, the initial excited states and radicals are accessible by UV/Vis spectroscopy (green).[2] LED illumination for in situ NMR spectroscopy provides reaction profiles of substrates, products, and diamagnetic states of the photocatalyst (blue) and information about transient radical pairs via CIDNP (purple).

Chemical photocatalysis has developed into a booming field with various synthetic applications.[1] Mechanistic insights into photocatalytic reactions are so far mainly the domain of UV/Vis spectroscopy due to its ability to characterize even excited states of the photocatalysts with ultrafast time resolution and high sensitivity (demonstrated for the mechanism of a flavin-catalyzed photooxidation in Figure 1[2]). However, even the most elaborate UV/Vis study is limited by the principle drawbacks of this method. Thus, UV/Vis focuses on the initial reaction steps of the photocatalyst but can gain only little information about the structures, aggregation trends, solvent effects, and substrates. Also the investigation of entire reaction pathways and especially the effects of downstream intermediates is difficult. These structural aspects and timescales are the classical domain of NMR spectroscopy, which in turn is a very slow and insensitive method and in many aspects complementary to UV/Vis spectroscopy. NMR spectroscopy can directly detect diamagnetic intermediates but neither excited states nor most of the radical species (Figure 1 blue highlights), which is reflected in many elaborate NMR studies of photoreactions, for example, in solid-state NMR spectroscopy,[3] protein folding,[4] organometallic photochemistry,[5] and photocatalysis.[6] Through the use of modern NMR photo-CIDNP techniques (CIDNP = chemically induced dynamic nuclear polarization)[7] with laser illumination, also transient radical pairs in photochemical reactions can be studied indirectly (purple frames in Figure 1).[4a,b] However, the detection and interpretation of CIDNP effects in closed catalytic cycles, typical for photocatalysis, are difficult.[8] Moreover, the high light intensity of a laser often leads to fast degradation of the photocatalyst. As a result the detection of whole reaction profiles including both catalyst and substrate/product is difficult with laser setups and the results often are not comparable to synthetic applications using lower light intensities.

Recently, we developed an LED illumination device for NMR spectroscopic investigations and were able to increase

[*] Dr. C. Feldmeier, H. Bartling, Prof. Dr. R. M. Gschwind
Institut für Organische Chemie, Universität Regensburg
93040 Regensburg (Germany)
E-mail: ruth.gschwind@ur.de
K. Magerl
Institut für Physikalische und Theoretische Chemie
Universität Regensburg (Germany)

[**] We thank GRK Chemical Photocatalysis for financial support and Prof. Dick and Prof. Riedle for many useful discussions.
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201409146.

the light intensity inside the sample by a factor of ca. 800.[9] This LED device can be used in a pulsed and in a continuous-wave mode and facilitates time-resolved photo-CIDNP as well as the detection of entire reaction pathways. Therefore, with this LED setup now the full potential of NMR spectroscopy can be applied to study the mechanisms of photocatalytic reactions. This device now can be used to investigate the relevance of downstream intermediates and solvent effects in photocatalysis as well as the informative value of NMR reaction profiles for mechanistic studies, which is well known for organocatalytic reactions.[10]

For the present study the flavin-catalyzed photooxidation of benzyl alcohols[2,11] was chosen (Figure 1). Flavins are able to catalyze a huge variety of different biochemical reactions in various proteins; their photoreactions in proteins are well investigated[4a,12] and flavins are known to produce CIDNP effects.[7b] Flavins can act as both one-electron and two-electron mediators in redox reactions[13] by modulating the stability of the semiquinone radical in the protein.[13b,c,14] Furthermore, flavin derivatives are successfully used as photocatalysts[1a,11a,13e] for the oxidation of various substrates,[1a,15] but to our knowledge the modification of the electron-transfer properties of flavin in synthetic applications has never been reported so far.

The flavin-catalyzed photooxidation of benzylic alcohol was the subject of an in-depth mechanistic UV/Vis study (Figure 1).[2] After excitation of the photocatalyst by blue light, two electrons and two protons are transferred from methoxybenzyl alcohol (MBA) to riboflavin tetraacetate (RFTA) yielding methoxybenzyl aldehyde (MBAld) and reduced RFTA (RFTA-H_2). The reduced form of flavin is referred to as RFTA-H_2 hereafter, although the spectra indicate a mixture of RFTA-H_2 and RFTA-H^- as expected for a CD_3CN/D_2O mixture. Subsequently RFTA is recovered by reaction with oxygen leading to H_2O_2. The UV/Vis study revealed the flavin triplet state as the key intermediate for a productive electron transfer and a subsequent protonation of the flavin radical anion.[2] The strong solvent dependence of the yields and reaction rates[11a] (high yields, fast reaction in CD_3CN/D_2O; low yields, slow reaction in CD_3CN) remained unclear. In previous mechanistic NMR studies, the interruption of catalytic cycles, for example, by omitting substrates, has proven to be a successful strategy for the stabilization and NMR detection of otherwise inaccessible intermediates.[10a–c,16] Therefore, oxygen was excluded to interrupt the catalytic cycle of flavin, preventing the reoxidation of the reduced flavin species and allowing their characterization by combined NMR and UV/Vis studies (Supporting Information (SI), Chapter B). In addition, the stoichiometry of the product and the reduced flavin species can be monitored and CIDNP effects are not cancelled by closing the cycle. As solvent both pure CD_3CN and CD_3CN/D_2O (1:1) were chosen to elucidate the influence of solvent, particularly with regard to the flavin oxidation state and the associated role of flavin as a one- or two-electron acceptor in photooxidations.

First, the reduced flavin species (semiquinone or hydroquinone) was studied in pure CD_3CN. Figure 2c shows ^1H NMR spectra of the reaction mixture in deoxygenated CD_3CN before illumination and after 2 h of illumination time. With continued illumination the amount of the product methoxybenzyl aldehyde (MBAld, black) increases, while the amount of the oxidized flavin (RFTA, blue) decreases and a complete new set of signals (green) appears, which was assigned to the two-electron reduced hydroquinone form of flavin (RFTA-H_2) (SI, Chapter 2). The linewidths of all the signals are extremely small even during the reaction, indicating a very short lifetime of the proposed transient flavin semiquinone radicals (Figure 2c). Photo-CIDNP measurements[7a,b,8,17] on this sample proved that this reaction nevertheless involves radical pairs. The opposite signs of the CIDNP phases of the oxidized and the reduced flavin signals (Figure 2g; SI, Chapter 11) are in line with the initial reaction mechanism proposed by UV studies in CD_3CN/D_2O:[2] First an electron transfer occurs from MBA to RFTA. The subsequent spin-correlated zwitterionic radical pair in the singlet state recombines and results in photo-CIDNP effects in the set of RFTA signals. In the case of intersystem crossing the radical pair in the triplet state yields the product and causes the photo-CIDNP polarizations in the set of RFTA-H_2 signals. Thus, the combined information from ^1H NMR spectra and CIDNP effects show that also in pure CD_3CN the product is formed from the triplet states of the zwitterionic radical pairs. However, the absolute amount of these radicals is very low and their lifetime extremely short.

Next, entire reaction profiles were investigated revealing the relative stoichiometry of the involved species. Figure 2e shows the reaction profile in deoxygenated pure CD_3CN. Upon illumination RFTA is directly reduced to RFTA-H_2 on the NMR timescale without the semiquinone radical altering the kinetics. The lack of oxygen prevents reoxidation and the concentration of RFTA-H_2 increases at a rate corresponding to the decrease of RFTA. In addition, the product MBAld is initially formed at the same rate as the reduced flavin. This reveals the requirement of one molecule of RFTA for the oxidation of one molecule of MBA and proves a formal two-electron process in pure CD_3CN. The reaction proceeds very slowly indeed, as observed by synthetic investigations[11a] and shows yields of only about 50% after 4 h.

The combined information obtained from ^1H NMR spectra, CIDNP effects, and reaction kinetics shows that the triplet state of the flavin semiquinone radical is decisive for the product formation, but its lifetime extends beyond the NMR timescale affecting neither the NMR spectra nor the associated kinetics. That means that the formation of zwitterionic radical pairs in pure CD_3CN is energetically unfavorable to such an extent[18] that a formal two-electron process is observed on the NMR timescale. Furthermore, the significant CIDNP effects in combination with slow reaction rates and low yields indicate the presence of zwitterionic radical contact ion pairs allowing for an effective intersystem crossing and a fast electron backtransfer in the singlet state. To sum up, the spectra and the reaction kinetics show a formal two-electron process in pure CD_3CN with the insufficient formation and stabilization of the zwitterionic radical pair as most probable rate-limiting step.

With deoxygenated CD_3CN/D_2O (1:1) as the solvent the character of the dominating reduced flavin species changes drastically. Upon illumination the signals of RFTA rapidly

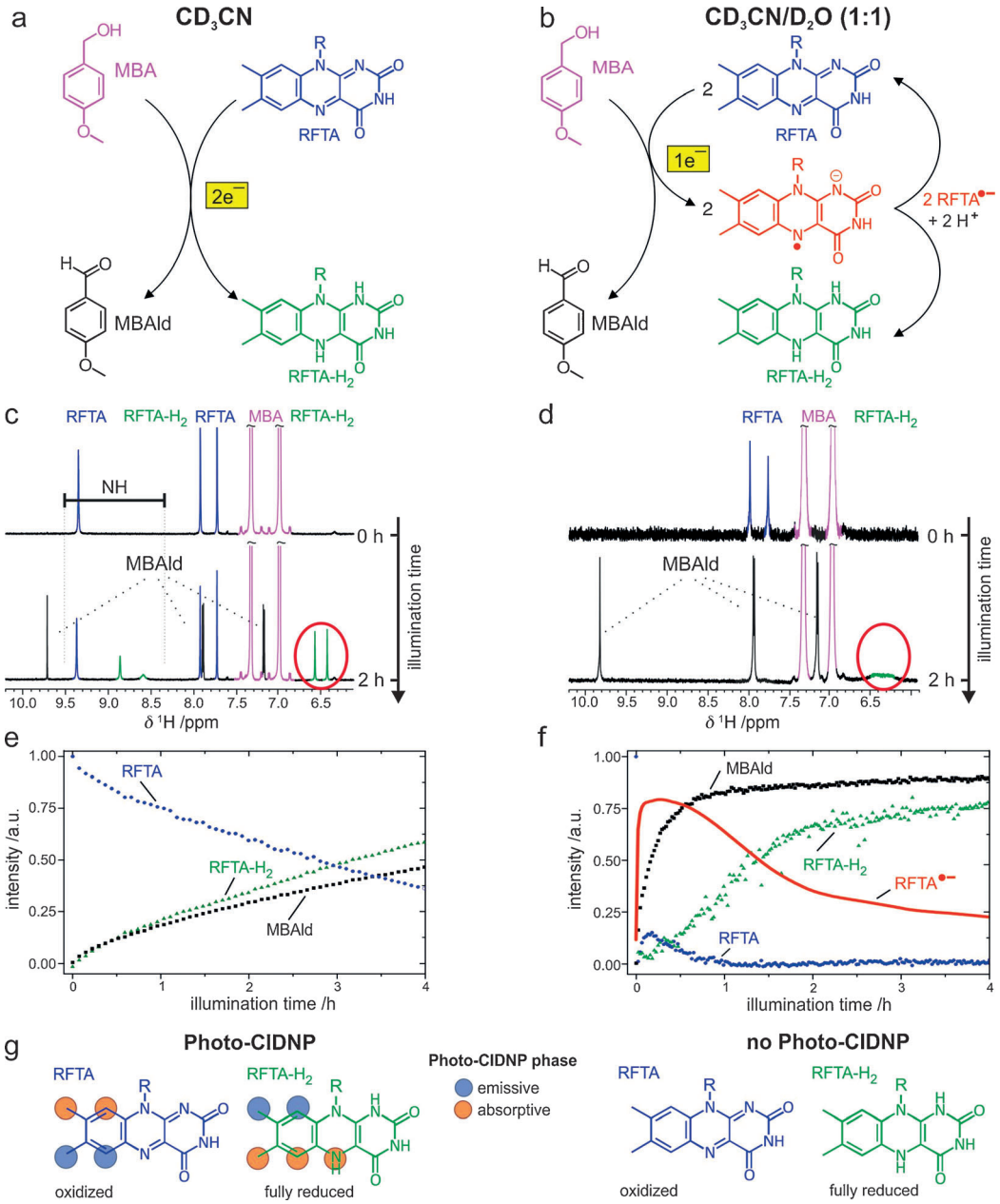

Figure 2. NMR reaction profiles reveal that chemical photooxidations by flavin can be switched between one- and two-electron-transfer mechanisms by solvent properties. Oxygen exclusion interrupts the catalytic cycle of flavin and allows for the identification of the reductive pathway: a formal two-electron transfer in CD_3CN (a) and a one-electron transfer in CD_3CN/D_2O (1:1) (b). Selected ^1H NMR spectra recorded before and during the reaction show no line broadening of any signal in CD_3CN (c), whereas in CD_3CN/D_2O (1:1) (d) both detectable flavin species show broad linewidths indicating chemical exchange with a flavin radical. The corresponding reaction profile in CD_3CN (e) shows a slow formal two-electron-transfer mechanism on the NMR timescale. In CD_3CN/D_2O (1:1) (f) fast product formation, high amounts of the anionic semiquinone radical $RFTA^{\bullet-}$ as intermediate (smoothened curve for details see SI), and delayed formation of the twofold reduced flavin RFTA-H_2 is observed indicating a one-electron-transfer process. Photo-CIDNP polarizations detected in the oxidized and fully reduced flavin species (g) in CD_3CN in combination with the results from recent UV/Vis studies[2] in CD_3CN/D_2O (1:1) show that the product formation via triplet zwitterionic radical pairs is identical in the two solvents. However, the NMR reaction profiles reveal that the stabilization of these downstream intermediates controls the further reaction mechanism.

decrease and broaden, whereas those of the starting material, product, and solvent remain sharp (Figure 2d). After a time delay RFTA-H_2 is formed showing also broad signals (Figure 2d). The signals for RFTA and (less pronounced) RFTA-H_2 show the typical distance dependence of the linewidths expected for a chemical exchange with a semiquinone radical, which was also previously described for flavin mononucleotide (FMN) ring systems in deoxygenated solution.[19] The line broadening is most pronounced for the protons of the isoalloxazine ring system; the signals of the side chain show reduced line broadening with increasing distance from the radical (SI, Chapter 3). After the light was turned off, the product formation stops immediately and the broadened lines remain for hours. Upon addition of oxygen the flavin signals recover to sharp lines indicating the reoxidation of the semiquinone radicals to RFTA (SI, Chapter 4). In contrast to experiments in CD_3CN no photo-CIDNP polarizations are detected in deoxygenated CD_3CN/D_2O (1:1) (SI, Chapters 5 and 11).

The change of the reaction profiles in CD_3CN/D_2O reveals the role of the semiquinone radicals in the reaction mechanism (Figure 2f). First, the product formation is strongly accelerated (50% yield after 10 min compared to 4 h in CD_3CN); second, the relative stoichiometries of RFTA, RFTA-H_2, and the product MBAld change drastically. While MBAld is rapidly formed, the concentration of RFTA drops close to zero and RFTA-H_2 increases with a significant time delay and at a reduced rate. This reaction profile is typical for the presence

of a stabilized reaction intermediate in the mechanism. The line broadening of the NMR signals reveal flavin semiquinone radicals as intermediate species (see above and Figure 2 f). The high amount of flavin radical intermediates and the concurrent immediate oxidation of the alcohol to the aldehyde impressively show that RFTA acts in CD_3CN/D_2O as a one-electron oxidation agent for the oxidation of MBA. Under synthetic conditions, that is, with varying amounts of oxygen, similar line broadening of the flavin resonances is observed in CD_3CN/D_2O as well as identical reaction profiles after the full consumption of oxygen (SI, Chapters B, 6, and 7). This confirms that the one-electron mechanism is generally valid in CD_3CN/D_2O.

The semiquinone radical intermediate was identified as the anionic semiquinone radical $RFTA^{\bullet-}$ by UV/Vis spectroscopy, which has proven effective for the detection and characterization of flavin semiquinone radicals[2,20] (SI, Chapter B). The dominant occurrence of $RFTA^{\bullet-}$ and the immediate oxidation of the alcohol without a radical intermediate detectable on the NMR timescale (SI, Chapters 7 and 9) clearly indicate that flavin acts as a one-electron oxidizing agent and that two flavin molecules are necessary for the complete oxidation of the alcohol. The resulting $RFTA^{\bullet-}$ radicals have significantly longer lifetimes in the presence of oxygen, which is a strong hint that in the presence of oxygen mainly $RFTA^{\bullet-}$ is reoxidized to RFTA. Previous diffusion-ordered spectroscopy (DOSY) experiments with RFTA and derivatives in CD_3CN, D_2O, and CD_3CN/D_2O (1:1) combined with reactivity studies indicated not only a significant amount of RFTA dimers in CD_3CN/D_2O (1:1) (aggregation number 1.7) but also an increasing reactivity and decreasing stability of the flavin photocatalysts with reduction of the aggregation.[11a] Therefore, monomeric RFTA molecules are assumed to be the reactive catalytic species. The dimer is proposed to be important only for the disproportionnation process under the exclusion of oxygen (SI, Chapter B).

Based on this NMR study including reaction profiles and CIDNP effects in combination with the results of a time-resolved UV/Vis study[2] we propose an extended mechanism for the flavin-catalyzed photooxidation of methoxybenzyl alcohol. The time-resolved UV/Vis study in CD_3CN/D_2O (1:1) and the CIDNP studies in pure CD_3CN show that the initial photoexcitation steps and the formation of the excited triplet state as the key intermediate for the product formation are identical in these solvent systems. However, starting from the electron-transfer step creating a zwitterionic radical pair, the solvent interactions determine the further mechanistic pathway. In CD_3CN/D_2O (1:1) the radical counterions are stabilized and separated, leading to a fast one-electron oxdiation process. In pure CD_3CN the formation energy of the charge separation is much higher. The resulting zwitterions form contact ion pairs and facilitate an effective electron backtransfer after intersystem crossing. Both effects lead to a slow reaction with a formal two-electron-transfer mechanism on the NMR timescale.

In conclusion, the new LED setup, which made both NMR reaction profiles and CIDNP studies possible, led to new insights into the reaction mechanism of photocatalytic reactions. The new findings are not only complementary to the results of UV/Vis studies but crucial for the understanding of the mechanism. Downstream intermediates, especially ion pairs, and processes accessible on the NMR timescale can be decisive for the mechanistic pathway of photoreactions. Thus, this study shows that the control of the one- versus two-electron processes of flavin and potentially also other photocatalysts is possible without any protein but by just making use of solvent and solvation properties.

Received: September 16, 2014
Published online: December 2, 2014

Keywords: flavin · NMR spectroscopy · photocatalysis · solvent effects · UV/Vis spectroscopy

[1] a) B. König, S. Kümmel, R. Cibulka in *Chemical Photocatalysis* (Ed.: B. König), de Gruyter, Berlin, **2013**, pp. 45 – 66; b) J. W. Tucker, C. R. J. Stephenson, *J. Org. Chem.* **2012**, *77*, 1617 – 1622; c) C. K. Prier, D. A. Rankic, D. W. C. MacMillan, *Chem. Rev.* **2013**, *113*, 5322 – 5363; d) T. P. Yoon, M. A. Ischay, J. Du, *Nat. Chem.* **2010**, *2*, 527 – 532.

[2] U. Megerle, M. Wenninger, R.-J. Kutta, R. Lechner, B. König, B. Dick, E. Riedle, *Phys. Chem. Chem. Phys.* **2011**, *13*, 8869 – 8880.

[3] a) S.-J. Hwang, C. Petucci, D. Raftery, *J. Am. Chem. Soc.* **1998**, *120*, 4388 – 4397; b) S. Pilkenton, S.-J. Hwang, D. Raftery, *J. Phys. Chem. B* **1999**, *103*, 11152 – 11160.

[4] a) J. Wirmer, T. Kühn, H. Schwalbe, *Angew. Chem. Int. Ed.* **2001**, *40*, 4248 – 4251; *Angew. Chem.* **2001**, *113*, 4378 – 4381; b) T. Kühn, H. Schwalbe, *J. Am. Chem. Soc.* **2000**, *122*, 6169 – 6174; c) K. H. Mok, T. Nagashima, I. J. Day, J. A. Jones, C. J. V. Jones, C. M. Dobson, P. J. Hore, *J. Am. Chem. Soc.* **2003**, *125*, 12484 – 12492.

[5] G. E. Ball in *Spectroscopic Properties of Inorganic and Organometallic Compounds*: *Techniques*, *Materials and Applications*, *Vol. 41*, The Royal Society of Chemistry, London, **2010**, pp. 262 – 287.

[6] A. Mills, C. O'Rourke, *Catal. Today* **2014**, *230*, 256 – 264.

[7] a) M. Goez in *Adv. Photochem.* (Eds.: D. C. Neckers, D. H. Volman, G. von Bünau), Wiley, New York, **1997**; b) J. Hore, R. W. Broadhurst, *Prog. Nucl. Magn. Reson. Spectrosc.* **1993**, *25*, 345 – 402; c) H. Hayashi in *Introduction to Dynamic Spin Chemistry*: *Magnetic Field Effects upon Chemical and Biochemical Reactions*, World Scientific, Singapore, **2004**; d) L. Kuhn in *Top. Curr. Chem.*, Springer, Berlin/Heidelberg, **2013**, pp. 1 – 72; e) N. J. Turro, V. Ramamurthy, J. C. Scaiano, *Modern Molecular Photochemistry of Organic Molecules*, University Science Books, Sausalito **2010**.

[8] M. Goez in *Adv. Photochem.* (Eds.: C. Douglas, D. H. Neckers, G. V. B. Volman), Wiley, New York, **2007**, pp. 63 – 163.

[9] C. Feldmeier, H. Bartling, E. Riedle, R. M. Gschwind, *J. Magn. Reson.* **2013**, *232*, 39 – 44.

[10] a) M. B. Schmid, K. Zeitler, R. M. Gschwind, *Angew. Chem. Int. Ed.* **2010**, *49*, 4997 – 5003; *Angew. Chem.* **2010**, *122*, 5117 – 5123; b) M. B. Schmid, K. Zeitler, R. M. Gschwind, *J. Am. Chem. Soc.* **2011**, *133*, 7065 – 7074; c) M. B. Schmid, K. Zeitler, R. M. Gschwind, *J. Org. Chem.* **2011**, *76*, 3005 – 3015; d) D. G. Blackmond, *Angew. Chem. Int. Ed.* **2005**, *44*, 4302 – 4320; *Angew. Chem.* **2005**, *117*, 4374 – 4393.

[11] a) J. Dadová, S. Kümmel, C. Feldmeier, J. Cibulková, R. Pažout, J. Maixner, R. M. Gschwind, B. König, R. Cibulka, *Chem. Eur. J.* **2013**, *19*, 1066 – 1075; b) R. Lechner, S. Kümmel, B. König, *Photochem. Photobiol. Sci.* **2010**, *9*, 1367 – 1377; c) J. Svoboda, H. Schmaderer, B. König, *Chem. Eur. J.* **2008**, *14*, 1854 – 1865.

[12] G. Richter, S. Weber, W. Römisch, A. Bacher, M. Fischer, W. Eisenreich, *J. Am. Chem. Soc.* **2005**, *127*, 17245 – 17252.
[13] a) R. Miura, *Chem. Rec.* **2001**, *1*, 183 – 194; b) A. M. Edwards in *Fla-vins*: *Photochemistry and Photobiology* (Eds.: E. Silva, A. M. Edwards), **2006**, pp. 1 – 11; c) V. Massey, *Biochem. Soc. Trans.* **2000**, *28*, 283 – 296; d) M. W. Fraaije, A. Mattevi, *Trends Biochem. Sci.* **2000**, *25*, 126 – 132; e) G. de Gonzalo, M. W. Fraaije, *ChemCatChem* **2013**, *5*, 403 – 415.
[14] S. Ghisla, V. Massey, *Biochem. J.* **1986**, *239*, 1 – 12.
[15] a) K. Tatsumi, H. Ichikawa, S. Wada, *J. Contam. Hydrol.* **1992**, *9*, 207 – 219; b) C. B. Martin, M.-L. Tsao, C. M. Hadad, M. S. Platz, *J. Am. Chem. Soc.* **2002**, *124*, 7226 – 7234; c) E. Silva, A. M. Edwards, D. Pacheco, *J. Nutr. Biochem.* **1999**, *10*, 181 – 185; d) W. A. Massad, Y. Barbieri, M. Romero, N. A. García, *Photochem. Photobiol.* **2008**, *84*, 1201 – 1208; e) J. M. Kim, M. A. Bogdan, P. S. Mariano, *J. Am. Chem. Soc.* **1993**, *115*, 10591 – 10595.
[16] a) T. Gärtner, N. Yoshikai, M. Neumeier, E. Nakamura, R. M. Gschwind, *Chem. Commun.* **2010**, *46*, 4625 – 4626; b) K. Schober, H. Zhang, R. M. Gschwind, *J. Am. Chem. Soc.* **2008**, *130*, 12310 – 12317; c) M. Neumeier, R. M. Gschwind, *J. Am. Chem. Soc.* **2014**, *136*, 5765 – 5772.
[17] a) M. Goez, *ChemInform* **2010**, *41*; b) M. Goez in *Annu. Reports NMR Spectrosc.*, *Vol. 66* (Ed.: A. W. Graham), Academic Press, **2009**, pp. 77 – 147.
[18] J. C. Scaiano, D. J. Lougnot, *J. Phys. Chem.* **1984**, *88*, 3379 – 3382.
[19] a) D. D. Macmurchie, R. J. Cushley, *Can. J. Chem.* **1978**, *56*, 1045 – 1051; b) P. R. Andrews, M. D. Fenn, *Aust. J. Chem.* **1975**, *28*, 1609 – 1612.
[20] M. Sakai, H. Takahashi, *J. Mol. Struct.* **1996**, *379*, 9 – 18.

