Probing Lipid Peroxidation by Using Linoleic Acid and Benzophenone

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Abstract: A thorough mechanistic study has been performed on the reaction between benzophenone (BZP) and a series of 1,4-dienes, including 1,4-cyclohexadiene (CHD), 1,4-dihydro-2-methylbenzoic acid (MBA), 1,4dihydro-1,2-dimethylbenzoic acid (DMBA) and linoleic acid (LA). A combination of steady-state photolysis, laser flash photolysis (LFP), and photochemically induced dynamic nuclear polarization (photo-CIDNP) have been used. Irradiation of BZP and CHD led to a cross-coupled sensitizer-diene product, together with 6, 7, and 8. With MBA and DMBA as hydrogen donors, photoproducts arising from cross-coupling of sensitizer and diene radicals were found; compound 7 was also obtained, but 6 and o-toluic acid were

Introduction

Peroxidation of polyunsaturated fatty acids plays a crucial role in many oxidative processes and is responsible for food deterioration and numerous diseases, including Alzheimer's disease, atherosclerosis, and cancer rheumatoid arthritis.^[1] It is generally accepted that a key step in lipid peroxidation (type I mechanism) is hydrogen abstraction of the allylic hydrogen atoms from fatty acids by reactive free radicals, such as hydroxyl, alkyl, alkoxyl, peroxyl radicals, and carbonyl

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only isolated in the irradiation of BZP with MBA. Triplet lifetimes were determined in the absence and in the presence of several diene concentrations. All three model compounds showed similar reactivity ($k_q \approx 10^8 \,\mathrm{M^{-1}\,s^{-1}}$) towards triplet excited BZP. Partly reversible hydrogen abstraction of the allylic hydrogen atoms of CHD, MBA, and DMBA was also detected by photo-CIDNP on different timescales. Polarizations of the diamagnetic products were in full agreement with the results derived from LFP. Finally, LA also un-

Keywords: dienes • hydrogen abstraction • photochemistry • photolysis • transient absorption spectroscopy derwent partly reversible hydrogen abstraction during photoreaction with BZP. Subsequent hydrogen transfer between primary radicals led to conjugated derivatives of LA. The unpaired electron spin population in linoleyl radical (LA') was predominantly found on H(1-5) protons. To date, LA-related radicals were only reported upon hydrogen transfer from highly substituted model compounds by steady-state EPR spectroscopy. Herein, we have experimentally established the formation of LA' and shown that it converts into two dominating conjugated isomers on the millisecond timescale. Such processes are at the basis of alterations of membrane structures caused by oxidative stress.

triplets.^[2] After oxygen trapping, the fatty acid free-radical forms hydroperoxide, which in turn can initiate the peroxidation chain cycle through the generation of hydroxyl and alkoxyl radicals.^[3]

The reaction of the benzophenone (BZP) triplet excited state with allylic hydrogen atoms has attracted considerable interest during recent decades. Hydrogen abstraction and π quenching have been found to be the most important deactivation processes.^[4]

Herein, we build up the knowledge of lipid peroxidation by starting with 1,4-cyclohexadiene (CHD) and its derivatives as simple models and then move on to linoleic acid (LA), which serves as a realistic paradigm for lipid peroxidation. Studies performed by combining HPLC and laser flash photolysis (LFP) on tiaprofenic acid, which is a benzophenone-derived, nonsteroidal anti-inflammatory drug, have demonstrated that LA photoperoxidation is mediated by both type I and type II mechanisms through hydrogen abstraction and singlet oxygen production, respectively.^[5] Irradiation of BZP in the presence of 1,4-cyclohexadienes (CHDs) as models for LA leads to a complex mixture of photoproducts arising from hydrogen abstraction, via triplet radical pairs.^[6] Interestingly, with enantiomerically pure BZP--CHD model dyads, a significant stereodifferentation



has been found in the intramolecular hydrogen-abstraction process, as revealed by the triplet lifetimes.^[7]

In addition to LFP and photoproduct studies, chemically induced dynamic nuclear polarization (CIDNP) is a powerful mechanistic tool for the study of free-radical reactions.^[8] Thus, CIDNP allows reactions involving radical/radical ion pairs, which initially reside in a spin-correlated state, to be followed selectively. To form a product from the two free radicals, their electron spins have to form an overall singlet. Since the nuclear spin states, observable by NMR spectroscopy, are coupled to the spin states of the electron, electronspin alterations can be detected by NMR spectroscopy. The consequence of the spin-selected reaction pathways is a non-Boltzmann population of spin states within "cage" and "escape" products, resulting in enhanced absorption (EA, "positive peaks") or emission (EE, "negative peaks") in the NMR spectrum.^[9] Accordingly, CIDNP allows very shortlived intermediates to be detected at very low concentrations due to large enhancement factors.

The intensity of such polarized NMR signals of the products mirrors, for example, the spin distribution in the intermediate radicals. Thus, CIDNP provides information about initially formed radicals (with lifetimes of a few ns) and products formed selectively by radical pathways.

With this background, the aim of the present work was to perform a thorough mechanistic study on the reaction between BZP and a series of 1,4-dienes, including CHD, 1,4dihydro-2-methylbenzoic acid (MBA), 1,4-dihydro-1,2-dimethylbenzoic acid (DMBA), and LA. All of these dienes contain double allylic hydrogen atoms and may be suitable to provide conclusive evidence for the generation and behavior of caged radical pairs. For this purpose, a combination of steady-state photolysis, LFP, and photo-CIDNP appeared to be ideal.^[10]



Results and Discussion

Steady-state photolysis: Irradiations were performed at $\lambda_{max} = 350$ nm (Gaussian distribution) in acetonitrile under an argon atmosphere. They were monitored by UV spectro-photometry following the disappearance of the typical π - π * BZP absorption band at 254 nm. As shown in Figure 1a, BZP reacts with CHD in a few minutes under anaerobic conditions. Similar spectra were obtained for the irradiations



Figure 1. Photoreactivity of BZP in the presence of different dienes; a) UV spectra of a solution of BZP $(1.0 \times 10^{-4} \text{ M})$ and CHD $(1.5 \times 10^{-3} \text{ M})$ in acetonitrile after increasing irradiation times (t=0, 1, 3, 5, 10 min)under argon. b) Decrease in the absorbance at λ_{254} for solutions of BZP with CHD (**n**), MBA (**A**), and DMBA (**O**) as a function of the irradiation time.

of BZP with MBA and DMBA, although BZP was clearly more photoreactive in the presence of CHD (Figure 1b).

The photochemical reaction of BZP with CHD has been previously studied. Using LFP, Scaiano and Encinas observed quenching of the benzophenone triplet excited state (³BZP*) by CHD to produce the corresponding radical pairs.^[4a] Furthermore, we found that irradiation of oxygenated solutions of methanol containing BZP and CHD led to a complex mixture.^[6] Analysis of the mixture by HPLC, GC-MS, and ¹H NMR spectroscopy showed the formation of different photoproducts. They were tentatively identified on the basis of their MS data; however, isolation and structural elucidation was not attempted. On the other hand, no reports have been found about the photochemical reaction of BZP with MBA and DMBA.

Thus, to investigate formation of the photoproducts, steady-state irradiation of concentrated acetonitrile solutions containing BZP and the different dienes was carried out under nitrogen, through Pyrex, with a 400 W medium-pressure mercury lamp.

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The complex photomixture obtained by irradiation of BZP and CHD was analyzed by GC-MS and then submitted to silica gel column chromatography. Spectral analysis of the separated fractions revealed the formation of the cross-coupled sensitizer-diene product 1 (ca. 17%), together with the reduced (6, ca. 13%) and dimeric (7, ca. 4%) forms of the sensitizer (Scheme 1). Careful GC-MS analysis of the mix-



Scheme 1. Product formation in the photoreaction of BZP with CHD, MBA, and DMBA.

ture also revealed the presence of benzene (8) and several other minor products obtained by dimerization and further oxidation of the cyclohexadienyl radical. The structure of compound 1 was unambiguously assigned on the basis of NMR spectroscopic data (1 H, 13 C). The structures of 6 and 7 were assigned by comparison with pure compounds.

Likewise, irradiation of BZP in the presence of MBA led to a complex photomixture that was resolved by reversephase HPLC. The photoproducts arising from cross-coupling of sensitizer and diene radicals (2, 3) were obtained with yields of 6.3 and 13%, respectively (Scheme 1). Similar results were obtained by irradiation of BZP with DMBA; in this case, compounds 4 (15%) and 5 (28%) were isolated as the major photoproducts. Furthermore, compound 7 was obtained in both irradiations (17–23%). On the other hand, compound 6 (23%) and *o*-toluic acid (9, 30%) were only isolated from the photomixture obtained from the irradiation of BZP with MBA.

The structures of photoproducts **2–5** were unambiguously assigned based on their NMR spectroscopic data (¹H, ¹³C) and IR spectra. Mass spectrometry supported the assignments. X-ray analysis of crystals obtained from compounds **2** and **4** (Figure 2) clearly supported the *cis* configuration of these photoproducts.



Figure 2. Crystal structures of photoproducts 2 (top) and 4 (bottom). Ellipsoids are drawn at the 50% probability level.

Transient absorption spectroscopy: To investigate the mechanism involved in the photoreactions of BZP with CHD, MBA, and DMBA, LFP studies were performed on deareated solutions in acetonitrile, using 355 nm laser excitation (Nd:YAG). In all cases, the characteristic spectrum of the ketone triplet excited state (³BZP*; λ_{max} =330 and 525 nm)^[2c] was observed immediately after the laser pulse. This species was clearly quenched by added CHD, MBA, or DMBA (Figure 3).

The triplet lifetimes were determined in the absence and in the presence of several quencher concentrations. The rate constants, k_q , were obtained from Stern–Volmer plots (Figure 3, insets). They were found to be 1.2×10^8 , 1.1×10^8 , and $8.8 \times 10^7 \text{ m}^{-1} \text{ s}^{-1}$ for CHD, MBA, and DMBA, respectively; these values are in reasonable agreement with the value of $2.9 \times 10^8 \text{ m}^{-1} \text{ s}^{-1}$ reported for the BZP/CHD system in benzene.^[4a]

On the other hand, transient absorption spectra obtained upon excitation at 355 nm of a deareated acetonitrile solution containing BZP $(3.5 \times 10^{-3} \text{ M})$ and a large excess of



Figure 3. Normalized ${}^{3}\text{BZP}*$ decay traces at 525 nm in the presence of increasing amounts (0.25–6 mM) of CHD (a), MBA (b), and DMBA (c) after laser excitation at 355 nm. Insets: Stern–Volmer plots of ${}^{3}\text{BZP}*$ quenching by CHD, MBA, and DMBA.

CHD $(2 \times 10^{-1} \text{ m})$ are shown in Figure 4. Under these conditions, only the ketyl radical (BZPH, λ_{max} =545 nm) was detected, even at short delay times after the laser pulse.

Photo-CIDNP: To bridge the gap between steady-state photolysis and the time-resolved investigation, we performed photo-CIDNP experiments with CHD, MBA, DMBA, and LA using BZP as the hydrogen-abstracting agent in



Figure 4. Transient absorption spectra for BZP $(3.5 \times 10^{-3} \text{ M})$ and CHD $(2.0 \times 10^{-1} \text{ M})$ in acetonitrile obtained 0.4 (**n**), 1.0 (**o**), 4.0 (**A**), 10.5 (**v**), 21.5 (**•**), 32.2 (**4**), and 38.3 µs (hexagon) after the laser pulse (355 nm).

[D₃]acetonitrile. A Nd:YAG laser at 355 nm and a Hg–Xe high-pressure lamp were used as the irradiation sources.

Model systems CHD, MBA, and DMBA: Figure 5a shows the ¹H NMR spectrum of the (non-irradiated) mixture (1/1) of CHD/BZP as the reference and the ¹H CIDNP spectra



Figure 5. ¹H NMR (a) and CIDNP spectra obtained after photolysis by a Nd:YAG laser (1 μ s) (b) and Hg–Xe high-pressure lamp (300 ms) (c). All experiments were performed with BZP/CHD in [D₃]acetonitrile at room temperature.

obtained after its photolysis by a laser and a Hg–Xe lamp (Figure 5b and c, respectively) representing different timescales. The ¹H NMR spectrum (Figure 5a) presents an overlay of the signals from the two parent compounds. The background-free (Figure 5b, see the Experimental Section) ¹H CIDNP spectrum taken 1 μ s after (time delay to the center of the radio frequency (RF) pulse) the laser pulse (8 ns) reveals resonances in enhanced emission and absorption.

After this short delay between irradiation and the observing RF pulse, predominantly geminate ("cage") radical pairs led to the formation of CIDNP effects.

The most prominent polarized signals (enhanced emission) were observed for H(1) (δ =2.64 ppm) and H(2) (δ = 5.68 ppm) of CHD, indicating that hydrogen abstraction by the triplet excited state of BZP from CHD was reversible at this short timescale. Although H(1) and H(2) are equivalent



Figure 6. Calculated (B3LYP/ TZVP) ¹H hfcs (in gauss) of protons in the CHD[•] radical.

in CHD, they do not retain their equivalence in CHD'. In this case, an average value of the coupling constants can be used to predict the sign of CIDNP polarization. Calculations (DFT) shown in Figure 6 indicate that H(1a,b) and H-(2a,b) in CHD' possess averaged isotropic hyperfine coupling constants (hfcs) with opposite signs, that is, $hfc_{H(1a,b)} =$ $(hfc_{H(1a)} \times 2 + hfc_{H(1b)})/3 = +$ 87.7 G, $hfc_{H(2a,b)} = (hfc_{H(1a)} \times 2 +$

 $hfc_{H(2b)}/4 = -2.8 \text{ G.}$ According

to Kaptein's rules,^[11] this should lead to diverging CIDNP polarizations after the reversible hydrogen abstraction.

However, in the present case, we most likely observe a violation of Kaptein's rules due to the large $hfc_{H(1a,b)}$ and small $hfc_{H(2a,b)}^{[12]}$ in the CHD' radical.

Apart from the geminate polarizations of the parent CHD, a number of additional polarized CIDNP signals are detected. The emissive resonances at $\delta = 4.15$ and 5.90 ppm, together with the enhanced absorption at $\delta = 5.75$ ppm, are assigned to H(1), H(3), and H(2) of tertiary alcohol 1, which is the coupling product of the radicals formed by hydrogen atom transfer between CHD' and BZPH', respectively. The opposite signs of the polarizations of H(2) and H(3) of 1 are in agreement with Kaptein's rules and DFT calculations. Polarization of CH and OH protons of corresponding 6 are found at $\delta = 5.80$ and 3.80 ppm. As the result of hydrogen transfer from CHD' to the ketyl radical, we observed a polarized signal attributed to 8 at $\delta = 7.37$ ppm (enhanced emission). Thus, compounds 6 and 1 are produced in cages on the timescale of this experiment. Compound 7, which might be formed by coupling of two ketyl radicals, is silent in ¹H CIDNP due to the small hfcs of the phenyl protons in the ketyl radicals.

Figure 5c shows the ¹H CIDNP spectrum of a CHD/BZP mixture acquired 300 ms after the flash of a Hg–Xe high-pressure lamp. While indicating an almost identical number of polarized signals, this spectrum possesses quite a different polarization pattern from that of Figure 5b. Most of the polarizations appear in opposite directions than those of the 1 µs laser-CIDNP spectrum (Figure 5b). This is due to the much longer delay time (300 ms) between the lamp flash and the observing RF pulse. On this timescale, signals of "escape" products dominate. According to Kaptein's rules, cage and escape products must carry opposite polarizations. Thus, products that can be formed by both geminate and



Scheme 2. Product formation in the photoreaction of BZP and CHD as determined by ¹H CIDNP.

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Figure 7. ¹H NMR (a) and CIDNP spectra obtained after photolysis by a Hg–Xe high-pressure lamp (b). All experiments were performed with BZP/CHD in $[D_3]$ acetonitrile at room temperature.

The reactivity of MBA and DMBA essentially reflects that of CHD. Since the reactivity of the escape channel is attenuated by steric factors, a smaller amount of follow-up products can be established for MBA and DMBA. Compounds 2, 3, 4, and 5 as well as 6 were observed being polarized in CIDNP spectra. The ¹H CIDNP spectra recorded after photoexcitation of mixtures of MBA and DMBA are presented in the Supporting Information.

Linoleic acid (LA): The principal reaction routes established for the simple models CHD, MBA, and DMBA are the basis for gaining insight into the reactivity of LA. Unexpectedly, ¹H CIDNP experiments with mixtures of BZP and LA, using the laser, did not yield sufficiently intense signals to allow clear-cut analysis. However, the application of the high-pressure Hg–Xe lamp as the light source led to distinguishable CIDNP polarizations. It has to be considered that both geminate and escape polarizations may appear in the spectrum.^[13] Under these conditions, geminate and escape polarizations may be cancelled by each other to a certain extent. Moreover, nuclear spin relaxation does not allow efficient saturation of the background NMR spectroscopy signals after 300 ms, thus complicating analysis of the spectrum.

To avoid misinterpretations, we concentrate on new, additionally emerging signals. Figure 7 shows ¹H NMR and CIDNP spectra of LA in the presence of BZP. Theoretical (DFT) calculations predict that predominant spin population in the linoleyl radical LA⁺ resides at the penta-1,4-diene moiety mirrored by the resonances of H(1)–H(5) in the CIDNP spectra (Scheme 3). Therefore, products formed via LA⁺ in the CIDNP spectrum must inherit polarizations



trans-LA

Scheme 3. Product formation in the photoreaction of BZP and LA as determined by ¹H CIDNP.

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based on H(1)–H(5). Indeed, the multiplet at $\delta = 6.31$ ppm, appearing in enhanced absorption, can be straightforwardly assigned to the vinylic H(3) protons of both isomers of conjugated derivatives of LA (CLA1, CLA2). Additionally, polarizations of the protons H(2) and H(4) of CLA1 of CLA2, respectively, appear at $\delta = 5.82$ ppm. Those conjugated acids are formed by hydrogen abstraction of one of the double allylic hydrogen atoms H(3) of LA by the triplet excited state of BZP. Reverse hydrogen transfer, that is, hydrogen transfer from the ketyl radical (H(1) of BZPH') to LA' leads to the formation of a conjugated diene system. This is in agreement with DFT calculations and EPR spectroscopy experiments.^[14] The background CIDNP spectrum indicates strong polarization of H(1)-H(5) of parent LA, which points to the reversibility of hydrogen abstraction: H(1) of the ketyl radical is transferred back to regenerate parent LA as well as its trans isomer. The signal corresponding to trans-LA is found at $\delta = 5.25$ ppm (Figure 7b). The triplet at $\delta = 4.51$ ppm (J= 9.9 Hz) is attributed to the tertiary proton of LA-BZP, produced by coupling of primary ketyl and LA' radicals. The mechanism of the photoreaction of LA with BZP is presented in Scheme 3.

LA also undergoes partly reversible hydrogen abstraction during photoreaction with BZP. Follow-up hydrogen transfer between primary radicals leads to the formation of conjugated derivatives of LA. On the other side, reverse hydrogen transition can direct *cis-trans* isomerization of the parent LA. The unpaired electron spin population in LA[•] is predominantly located on protons H(1)-H(5).

Conclusion

The photoreaction between BZP and model systems CHD, MBA, and DMBA proceeds by hydrogen abstraction of the allylic hydrogen by the triplet excited state of BZP. In neat acetonitrile, all three model compounds show similar reactivity towards ³BZP*, the lifetimes of which in the presence of dienes were found to be in good agreement with previous studies on CHD in benzene.^[4a]

Partly reversible hydrogen abstraction of the allylic hydrogen atoms of CHD, MBA, and DMBA was also detected by photo-CIDNP on different timescales. CIDNP polarizations of the diamagnetic products were in a full agreement with the results derived by LFP.

Similar processes have been found for LA, yet LA-related radicals were only reported when generated by hydrogen transfer from highly substituted model compounds, allowing their observation by steady-state EPR spectroscopy.^[14] Herein, we have experimentally established the formation of LA[•] and shown that LA[•] converts into two dominating conjugated isomers on the ms timescale. Such processes are the basis of alterations of membrane structures caused by oxidative stress.

Experimental Section

General: BZP, CHD, and MBA were commercially available, whereas DMBA was synthesized through Birch reduction and subsequent methylation of **9**. The ¹H and ¹³C NMR spectra were recorded at 300 and 75 MHz, respectively, on a Bruker AC-300 spectrometer in CDCl₃; chemical shifts are reported in ppm downfield from an internal solvent signal. GC-MS analysis was performed on an Agilent mass selective detector coupled to an HP GC system. High-resolution mass spectra (EI, FAB) were recorded on a VG Auto Spec Fisons spectrometer instrument. All reactions were monitored by analytical TLC with silica gel 60 F254 (Merck), revealed with cerium ammonium sulfate/ammonium molybdate reagent. Column chromatogaphy was performed on silica gel 60 (0.063–0.2 mm) and HPLC was carried out by using a C-18, Kromasil column. **General irradiation procedure**

Procedure A: Solutions of CHD, MBA or DMBA $(1.5 \times 10^{-3} \text{ M})$ and BZP $(1.0 \times 10^{-4} \text{ M})$ in acetonitrile were irradiated in a multilamp photoreactor at $\lambda_{max} = 350 \text{ nm}$ (Gaussian distribution) under an argon atmosphere. They were monitored by UV spectrophotometry, following the disappearance of the absorption band at 254 nm

Procedure B: Deaerated solutions of CHD, MBA or DMBA $(1.3 \times 10^{-1} \text{ M})$ and BZP $(3.3 \times 10^{-2} \text{ M})$ in acetonitrile were irradiated through Pyrex with a 400 W medium-pressure mercury lamp. Reactions were monitored by TLC and NMR spectroscopy. After 10 h, the reaction mixtures were concentrated under reduced pressure and submitted to chromatography. The CHD and BZP reaction mixture was purified by silica gel column chromatography, using hexane/ethyl acetate (90:10 v/v) as the eluent. The reaction mixtures obtained by irradiation of MBA or DMBA and BZP were separated by reverse-phase HPLC chromatography, using acetonitrile/water/acetic acid (70:29.7:0.3 v/v/v) as the eluent.

Laser flash photolysis measurements: A pulsed Nd:YAG laser (SL404G-10 Spectrum Laser Systems) was used for the excitation at 355 nm. The single pulses were about 10 ns in duration and the energy was 16 mJ per pulse. The laser flash photolysis apparatus consisted of the pulsed laser, a Xe lamp, a monochromator, and a photomultiplier made up of a tube, housing, and power supply. The output signal from the oscilloscope was transferred to a personal computer for data analysis. All experiments were carried out at room temperature. The sensitizer (BZP) dissolved in acetonitrile (3.5 mM) had an absorbance of about 0.3 at 355 nm. Solutions were deaerated by bubbling nitrogen through the solution. The rate constants of triplet excited-state quenching by CHD, MBA, and DMBA were determined by the Stern–Volmer equation $(1/\tau = 1/\tau_0 + k_q)$ [Quencher]). Concentrations between 0.25 and 6.00 mm were used for CHD, MBA, and DMBA.

Photo-CIDNP experiments: ¹H NMR and CIDNP spectra were recorded on a 200 MHz Bruker AVANCE DPX spectrometer. Irradiation was carried out by using a frequency-tripled Spectra Physics Nd:YAG INDI laser (355 nm, ca. 40 mJ per pulse, ca. 10 ns) and a Hamamatsu (Japan) Hg–Xe lamp (SP4, L8252 lamp, 150 W, 300 ms). The following pulse sequence was used: presaturation laser/lamp flash–30°–RF–detection pulse (2.2 µs)–free induction decay. The concentrations of BZP, CHD, MBA, DMBA, and LA were 0.01 M. The hyperfine coupling constants (hfc) of the free radicals were calculated by using the Gaussian 03 package.^[15] All calculations (geometry optimizations and single-point calculations) were conducted at the B3LYP^[16] level of theory with the basis set TZVP.^[17]

Compound 1: ¹H NMR (300 MHz; CDCl₃, TMS): δ = 7.6–7.1 (m, 10H), 5.9 (m, 2H), 5.5 (m, 2H), 4.1 (m, 1H), 2.7 ppm (m, 2H); ¹³C NMR (75 MHz; CDCl₃, TMS): δ = 146.1, 133.0–125.0, 79.5, 44.4, 27.2 ppm; HRMS: *m*/*z* calcd for C₁₉H₁₉O: 263.14359 [*M* H]⁺; found: 263.14379.

Compound 2: ¹H NMR (300 MHz; CDCl₃, TMS): δ = 7.6–7.1 (m, 10H), 5.8 (m, 1 H), 5.6 (m, 1 H), 5.3 (m, 1 H), 4.1 (m, 1 H), 3.6 (m, 1 H), 1.6 ppm (s, 3 H); ¹³C NMR (75 MHz; CDCl₃, TMS): δ = 175.2, 144.7, 144.3, 127.3, 127.3, 127.0, 125.7, 125.6, 124.6, 124.5, 121.5, 78.5, 47.7, 44.4, 21.2 ppm; HRMS: *m*/*z* calcd for C₂₁H₁₈O₂: 302.13068 [*M*–18]⁺; found: 302.12991.

Compound 3: ¹H NMR (300 MHz; CDCl₃): δ = 7.6–7.1 (m, 10H), 5.9 (m, 1H), 5.7 (m, 1H), 5.5 (m, 1H), 4.1 (m, 1H), 3.6 (m, 1H), 1.7 ppm (s, 3H); ¹³C NMR (75 MHz; CDCl₃): δ =177.4, 145.4, 145.3, 133.1, 128.4,

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127.8, 126.8, 126.7, 125.7, 125.5, 125.4, 122.7, 79.5, 47.4, 44.7, 22.2 ppm; HRMS: m/z calcd for C₂₁H₂₀O₃: 320.14124 [*M*]⁺; found: 320.14007.

Compound 4: M.p. 186–188 °C (from CH₃CN); ¹H NMR (300 MHz; CDCl₃): δ =7.6–7.2 (m, 10H), 5.7 (m, 1H), 5.6 (m, 1H), 5.4 (m, 1H), 4.1 (m, 1H), 1.7 (s, 3H), 1.4 ppm (s, 3H); ¹³C NMR (75 MHz; CDCl₃): δ = 177.6, 145.6, 145.3, 137.8, 132.4, 128.4, 128.4, 126.7, 126.7, 126.0, 125.6, 125.5, 122.0, 80.2, 48.7, 45.1, 22.9, 19.9 ppm; HRMS: *m*/*z* calcd for C₂₂H₂₃O₃: 335.16472 [*M*+H]⁺; found: 335.16509.

Compound 5: ¹H NMR (300 MHz; CDCl₃): δ = 7.6–7.2 (m, 10 H), 5.7 (m, 1H), 5.6 (m, 1H), 5.4 (m, 1H), 4.2 (m, 1H), 1.7 (s, 3H), 1.3 ppm (s, 3H); ¹³C NMR (75 MHz; CDCl₃): δ = 179.4, 145.4, 145.2, 137.7, 132.5, 128.3, 126.8, 126.7, 125.7, 125.6, 125.5, 121.8, 79.2, 48.0, 45.0, 23.9, 20.0 ppm; HRMS: *m*/*z* calcd for C₂₂H₂₂O₃: 334.15689 [*M*]⁺; found: 334.15627.

X-ray crystallography: X-ray structures were measured on a Bruker Appe-II CCD instrument. CCDC-819279 (2) and 819278 (4) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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- a) A. W. Girotti, *Photochem. Photobiol.* **1990**, *51*, 497–509; b) A. Sevanian, F. Ursini, *Free Radical Biol. Med.* **2000**, *29*, 306–311; c) T. J. Montine, M. D. Neely, J. F. Quinn, M. F. Beal, W. R. Markesbery, L. J. Roberts, J. D. Morrow, *Free Radical Biol. Med.* **2002**, *33*, 620–626; d) F. Shahidi, Y. Zhong, *Chem. Soc. Rev.* **2010**, *39*, 4067–4079.
- [2] a) N. A. Porter, *Methods Enzymol.* 1984, 105, 273–282; b) N. A. Porter, Acc. Chem. Res. 1986, 19, 262–268; c) F. Bosca, M. Miranda, J. Photochem. Photobiol. B 1998, 43, 1–26.
- [3] A. C. Velosa, W. J. Baader, C. V. Stevani, C. M. Mano, E. J. H. Bechara, *Chem. Res. Toxicol.* 2007, 20, 1162–1169.
- [4] a) M. V. Encinas, J. C. Scaiano, J. Am. Chem. Soc. 1981, 103, 6393–6397; b) W. M. Nau, F. L. Cozens, J. C. Scaiano, J. Am. Chem. Soc. 1996, 118, 2275–2282; c) W. Adam, J. N. Moorthy, W. M. Nau, J. C. Scaiano, J. Org. Chem. 1997, 62, 8082–8090.
- [5] F. Boscá, M. A. Miranda, I. M. Morera, A. Samadi, J. Photochem. Photobiol. B 2000, 58, 1–5.

- [6] A. Samadi, L. A. Martínez, M. A. Miranda, I. M. Morera, *Photo-chem. Photobiol.* 2001, 73, 359–365.
- [7] a) M. A. Miranda, L. A. Martínez, A. Samadi, F. Boscá, I. M. Morera, *Chem. Commun.* 2002, 280–281; b) F. Boscá, I. Andreu, I. M. Morera, A. Samadi, M. A. Miranda, *Chem. Commun.* 2003, 1592.
- [8] a) M. Goez, I. Frisch, J. Phys. Chem. A 2002, 106, 8079–8084; b) M. Goez, G. Eckert, Phys. Chem. Chem. Phys. 2006, 8, 5294; c) H. D. Roth, Photochem. Photobiol. Sci. 2008, 7, 540–546; d) D. Neshchadin, R. Levinn, G. Gescheidt, S. N. Batchelor, Chem. Eur. J. 2010, 16, 7008–7016.
- [9] a) G. L. Closs, Adv. Magn. Reson. 1974, 7, 157–229; b) K. M. M. Salikhov, Yu. N. Sagdeev, R. Z. Buchachenko, Studies in Physical and Theoretical Chemistry, Vol. 22: Spin Polarization and Magnetic Effects in Radical Reactions, Elsevier, Amsterdam, 1984, p. 419.
- [10] M. Goez, Concepts Magn. Reson. 1995, 7, 69-86.
- [11] a) G. L. Closs, J. Am. Chem. Soc. 1969, 91, 4552–4554; b) R. Kaptein, Chem. Phys. Lett. 1969, 4, 214–216.
- [12] K. Salikhov, Chem. Phys. 1982, 64, 371-379.
- [13] M. Goez, Chem. Phys. Lett. 1992, 188, 451-456.
- [14] H. Kitaguchi, K. Ohkubo, S. Ogo, S. Fukuzumi, J. Am. Chem. Soc. 2005, 127, 6605–6609.
- [15] Gaussian 03, Revision C.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, Gaussian, Inc., Wallingford CT, 2004.
- [16] a) A. D. Becke, J. Chem. Phys. 1993, 98, 5648; b) P. J. Stephens, F. J. Devlin, C. F. Chabalowski, M. J. Frisch, J. Phys. Chem. 1994, 98, 11623–11627.
- [17] A. Schäfer, C. Huber, R. Ahlrichs, J. Chem. Phys. 1994, 100, 5829.

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