Cholesterol–diaryl ketone stereoisomeric dyads as models for "clean" type I and type II photooxygenation mechanisms†

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Received 22nd November 2007, Accepted 4th January 2008
First published as an Advance Article on the web 31st January 2008
DOI: 10.1039/b718068c

Cholesterol (Ch) is a major target for oxidative degradation in cell membranes, a process which can occur by two mechanisms: Type I (*via* free radicals) and Type II (mediated by $^1O_2$). In the present work, several dyads have been synthesized from β- and α-Ch and ketoprofen (KP) or tiaprofenic acid (TPA). Upon irradiation under anaerobic conditions, KP–α-Ch dyads were efficiently photolyzed, *via* intramolecular hydrogen abstraction from C-7. By contrast, KP–β-Ch, TPA–α-Ch, and TPA–β-Ch remained unchanged after prolonged irradiation. The transient absorption spectra of KP–α-Ch were assigned to the short-lived biradicals resulting from intramolecular hydrogen abstraction. Interestingly, the spectra and lifetimes obtained for the TPA-derived dyads were very similar to those of the TPA triplet excited state. For the KP–α-Ch dyads, generation of singlet oxygen was expectedly negligible. Conversely, for TPA–α-Ch a Φ_Δ value as high as 0.5 was determined. Thus, KP-based dyads are appropriate models for clean type I Ch oxidation, whereas the TPA derivatives are suitable systems for investigation of the purely type II process.

Introduction

It is well established that cholesterol (cholest-5-en-3β-ol, Ch), one of the most important structural components of cell membranes, is a major target for oxidative degradation, a process which can result in potentially pathological consequences.[1] Two mechanisms have been considered for Ch oxidation: Type I (*via* free radicals) and Type II (mediated by $^1O_2$). The former involves hydrogen abstraction from Ch and could be promoted, in principle, by UVA-irradiation in combination with photosensitizing agents.[1,2]

Ketoprofen (KP) has been recognized to be the strongest photosensitizer among non-steroidal anti-inflammatory drugs (NSAID).[3–5] This property is attributable to the benzophenone chromophore, whose lowest-lying triplet state is n,π* in nature;[6] accordingly, KP is a typical Type I photosensitizer[7] and is known to induce photoperoxidation of polyunsaturated fatty acids. In the course of model studies, we have found a significant stereodifferentiation in the intramolecular hydrogen abstraction from enantiomerically pure 1,4-cyclohexadienes (as simple sources of double allylic hydrogens) by (*S*)-KP, where chiral discrimination was demonstrated by measurement of the very short triplet lifetimes of the benzophenone chromophore (18–31 ns).[8,9]

Tiaprofenic acid (TPA) is also a benzophenone-derived NSAID with proven photosensitizing potential,[4,10,11] which contains the 2-benzoylthiophene chromophore. In contrast to KP, TPA has a π,π* lowest triplet excited-state,[6,12] and thereby the contribution of type II oxidation mechanism is enhanced.

In a preliminary communication we have reported the efficient and selective photogeneration of 7-allyl Ch radicals by intramolecular hydrogen abstraction in (*S*)- and (*R*)-KP–α-Ch model dyads.[13] Such dyads are reminiscent of Breslow's biomimetic systems constructed by esterification of cholestanol, androstanol and related compounds with achiral benzophenone derived carboxylic acids,[14] which were designed to study the remote photooxidation of steroids. This type of system was selected as a mimic of the cytochrome P-450 enzyme to achieve a selectivity dominated by the geometry of the catalyst–substrate complex. In this process, benzophenone removes the C-14 hydrogen, giving rise to an olefin and a mixture of cyclic products in the C or D rings. However, none of the employed systems contained a double bond in ring B (as Ch).[14] On the other hand, isolation and structural elucidation of the photoproducts was not attempted, and laser flash photolysis studies to investigate transient species such as triplets and biradicals were not performed. In the present work, we have completed these studies and extended them to the analogous excited state interactions between Ch and TPA. For this purpose, several ketoprofen–cholesterol (KP–Ch) and tiaprofenic acid–cholesterol (TPA–Ch) dyads have been synthesized through conjugation of β- and α-Ch with the corresponding NSAID (Scheme 1). Inclusion of KP–β-Ch and TPA–β-Ch in the series was done for comparison, as these dyads should not be reactive in the intramolecular process due to the extended relationship between the two active moieties, which prevents their close approach. Interestingly, singlet oxygen measurements by time-resolved near infrared emission have shown that the KP-based dyads are appropriate models for clean type I Ch oxidation, whereas the TPA-derivatives are suitable systems for the investigation of purely type II process.

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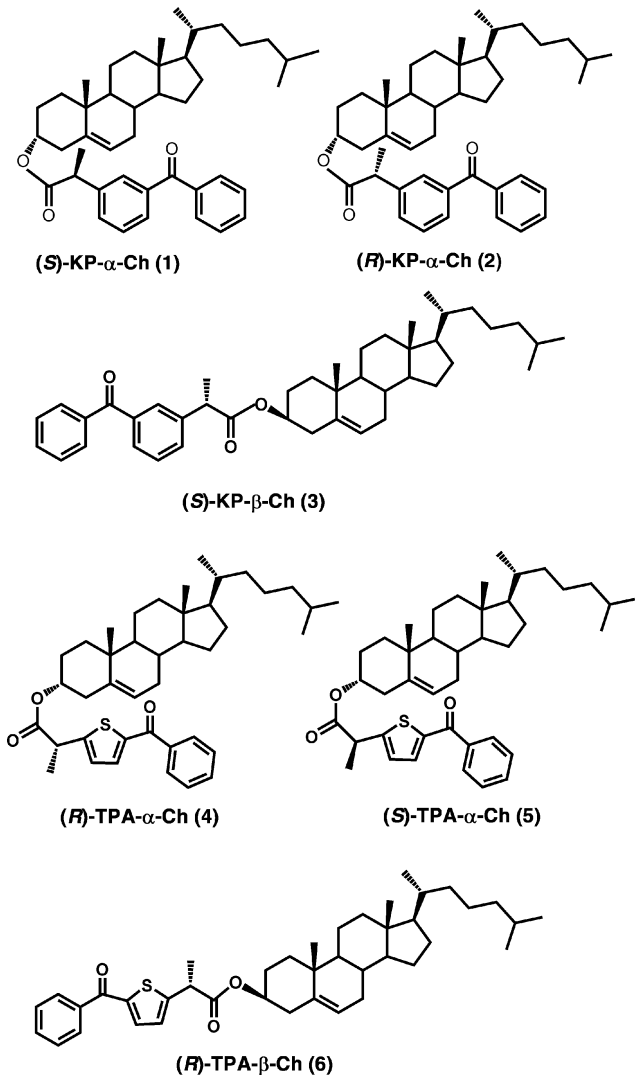
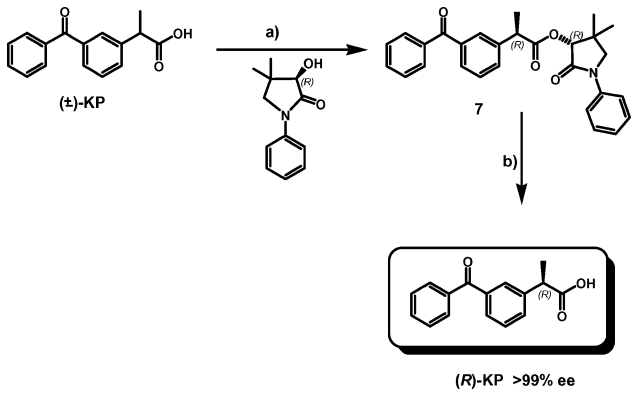
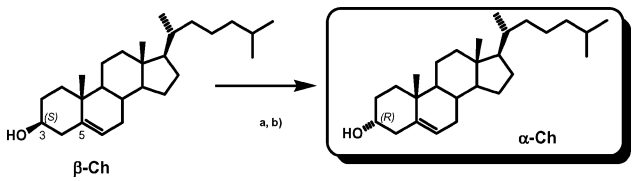
† Electronic supplementary information (ESI) available: Further experimental details and spectra. See DOI: 10.1039/b718068c



Scheme 1 Structures of dyads **1–6**.



Scheme 2 Synthesis of (*R*)-KP: a) PCl_5, CCl_4; b) AcOH, HCl 2 N.



Scheme 3 Synthesis of α-Ch: a) PPh_3, $ClCH_2CO_2H$, diisopropyl azodicarboxylate (DIAD), THF; b) MeOH, K_2CO_3.

Results and discussion

Synthesis of the dyads

The employed (*S*)-KP was commercially available, and its (*R*) enantiomer was obtained in two ways: i) for preparation of small amounts of compound suitable for photophysical and photochemical studies in the early stages, it was separated by chiral HPLC from the racemic mixture and ii) in order to obtain larger amounts of the compound for further studies, an asymmetric synthesis was carried out starting from racemic ketoprofen, using (*R*)-3-hydroxy-4,4-dimethyl-1-phenyl-2-pyrrolidinone as a chiral auxiliary;[15] this led to the corresponding ester **7** with high diastereoselectivity. Subsequent acidic hydrolysis under controlled conditions afforded (*R*)-KP with high enantioselectivity (Scheme 2). The (*R*) and (*S*) enantiomers of TPA were separated by chiral HPLC from the racemic mixture.

For the preparation of (*S*)-KP–α-Ch and (*R*)-KP–α-Ch as well as (*R*)-TPA–α-Ch and (*S*)-TPA–α-Ch inversion of the β-cholesterol configuration was accomplished by a Mitsunobu reaction (Scheme 3).[16]

Dyads of interest (**1–6**) were synthesized by esterification of α- or β-Ch with the appropriate arylpropionic acid following standard procedures.[8] All new compounds were fully characterized by NMR and MS data.

The esterification reaction between racemic TPA and α-Ch afforded the expected diastereomeric mixture; however, when crystallization from hexane–ethyl acetate was attempted only one of the stereoisomers precipitated (**4**). On the other hand, elimination of the solvent from the filtered solution gave rise to a viscous oil; its spectroscopic properties were identical to those of **5** obtained by direct esterification of (*S*)-TPA and α-Ch.

Steady-state photolysis under anaerobic conditions

Irradiations were performed with monochromatic light at λ_{max} = 266 nm in CH_2Cl_2 (*ca.* 10^{-5} M solutions), under inert atmosphere, and monitored by UV-spectrophotometry, following the decrease in the absorption at 266 nm. The irradiation wavelength was selected because it corresponded to the intersection of the UV-spectra of KP- and TPA-derived dyads at the same concentration (see, for example, Fig. 1A); in other words, the molar absorption coefficient of all dyads was the same at 266 nm. The results of the irradiations are shown in Fig. 1B.

In agreement with their different abilities to adopt a folded conformation allowing a close approach between the active moieties, dyads **1** and **2** were efficiently photolyzed while **3** remained almost unchanged after prolonged irradiation.[13] On the other hand, as shown in Fig. 1B, the KP-based dyads **1** and **2** were clearly more photoreactive than their TPA analogs **4** and **5**. Moreover, in the case of the photoreactive dyads **1** and **2** it can be observed that (*R*)-KP–α-Ch (**2**) was significantly more photoreactive than its (*S*)-stereoisomer **1**. Hence, as a general observation, the photoreactivity of the dyads was markedly

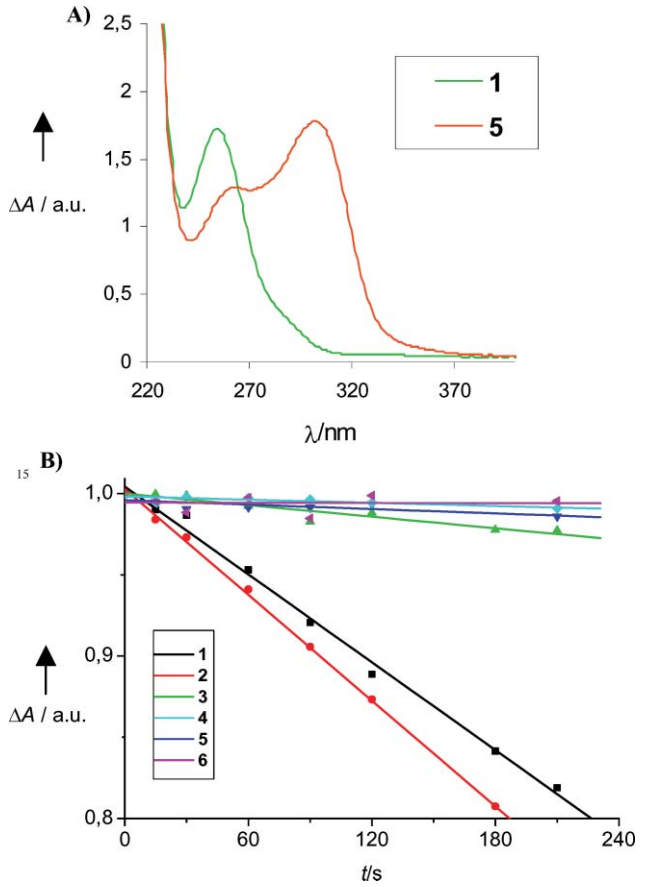
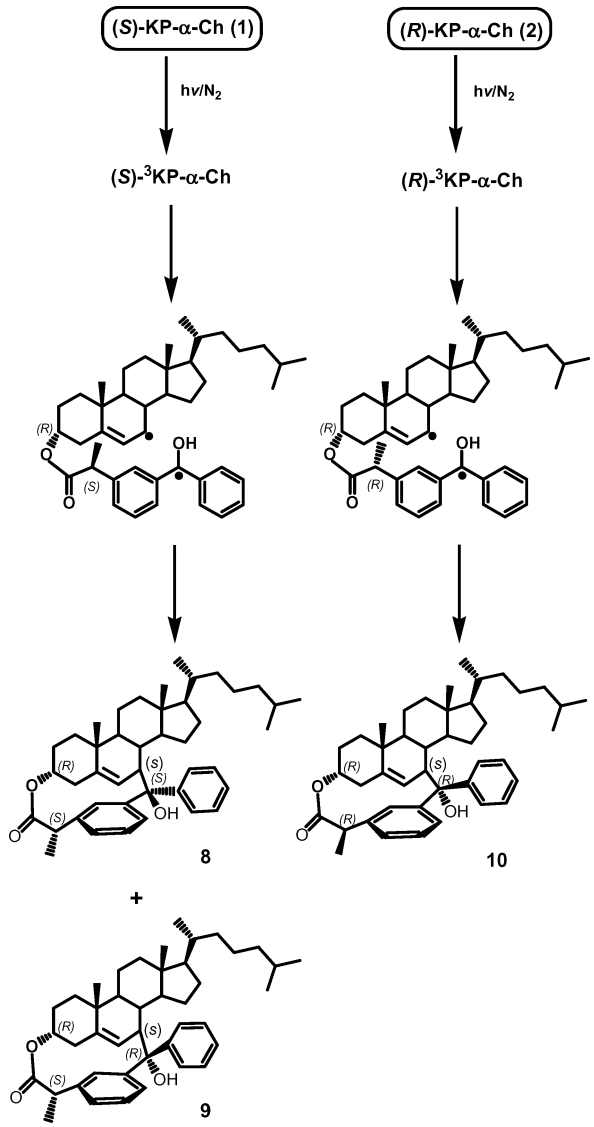


Fig. 1 A) UV-spectra of dyads **1** and **5** in CH_2Cl_2 (10^{-5} M solutions). B) Degradation of dyads (**1–6**) as a function of the irradiation time at 266 nm.

dependent on the electronic nature of the excited states (n,π* *vs.* π,π*) and on the configuration of the asymmetric carbons.

Photoproduct formation was investigated by steady-state irradiation of more concentrated (*ca.* 10^{-2} M) dichloromethane solutions under nitrogen, through Pyrex, with a 400 W medium pressure mercury lamp. Dyads **4** and **5** were markedly unreactive and did not give rise to any isolable photoproduct. This was in agreement with the lack of reactivity observed in the kinetic photodegradation experiments shown in Fig. 1B. Conversely, dyads **1** and **2** progressively disappeared upon irradiation, affording well-defined photoproducts. Their photomixtures were submitted to silica gel column chromatography, using hexane–ethyl acetate–dichloromethane (70 : 20 : 10 v/v/v) as eluent. Spectral analysis of the separated fractions revealed the formation of two diastereomeric compounds **8** and **9** from (*S*)-KP–α-Ch (**1**), while in the case of (*R*)-KP–α-Ch (**2**) only one photoproduct (**10**) was obtained (Scheme 4).[13]

Compounds **8–10** are the result of intramolecular hydrogen abstraction from the C-7 position of Ch and subsequent C–C coupling of the generated biradicals. It is interesting to note that this photocyclization occurs at the activated allylic position of the B ring, but not at the 4 allylic position of the A ring, obviously due to steric hindrance. The nonactivated C and D rings (where reaction was reported to occur in saturated benzophenone–steroid systems) remained unaffected.[14]



Scheme 4 Formation of products **8–10** upon photolysis of dyads **1** and **2**.

The structures of the photoproducts were unambiguously assigned on the basis of their NMR spectroscopic data (^{1}H, ^{13}C, DEPT, COSY 45, HMQC, NOEDIFF). Mass spectrometry (including high resolution measurements) supported the assignments. Due to the rigidity of the polycyclic skeletons, NOE experiments were essential to assign the stereochemistry of the new asymmetric carbons, generated upon C–C coupling of the two radical termini. The most significant interactions were observed between the *ortho*-protons of the phenyl group and the allylic protons at C-7.[13]

In view of the existing literature precedents on related steroid–benzophenone systems,[14] it appeared interesting to compare the circular dichroism (CD) spectra of the KP-derived dyads with those of the pure (*S*)- and (*R*)-KP enantiomers. In principle, the relative photoreactivities could be correlated with CD changes associated with ground state conformational differences.[14] Unfortunately, although clear CD spectra were obtained in all cases, no significant differences were found between the dyads (see supporting information).

Table 1 Photophysical parameters of dyads **1**, **2**, **4** and **5**

Parameter	(*S*)-KP–α-Ch (**1**)	(*R*)-KP–α-Ch (**2**)	(*R*)- or (*S*)-TPA–α-Ch (**4**, **5**)
τ_T/μs	0.010 ± 0.002	0.012 ± 0.002	5.500 ± 0.100
k_{iq}/s^{-1}	1.0×10^8	1.2×10^8	1.0×10^4
k_H/s^{-1}	0.8×10^8	1.0×10^8	$<1.0 \times 10^4$
k_π/s^{-1}	0.2×10^8	0.2×10^8	$<1.0 \times 10^4$
$\Phi_{\text{ketyl radical}}$	0.80 ± 0.02	0.80 ± 0.02	<0.01
$\tau_{\text{biradical}}$/μs	0.28 ± 0.01	0.22 ± 0.01	—

Transient spectroscopic studies

The transient absorption spectra obtained upon 355 nm excitation of (*S*)-KP–α-Ch (**1**) and (*R*)-KP–α-Ch (**2**), 20 ns after the laser pulse, were very similar; they did not correspond to their triplet excited states but to the corresponding biradicals.[13] This is shown in the insets in Fig. 2 for the case of (*S*)-KP–α-Ch (top) and (*R*)-KP–α-Ch (bottom). The assignment was based on the typical bands with maxima at *ca.* 340 nm and 545 nm (relative intensities *ca.* 5 : 1).[4,17,18] Interestingly, the lifetimes of the diastereomeric biradicals were significantly different, namely 280 ns for (*S*)-KP–α-Ch *vs.* 220 ns for (*R*)-KP–α-Ch (see Table 1). They were unaffected by the temperature and were quenched by oxygen (see Fig. 2) with k_q *ca.* 3.6×10^9 M^{-1} s^{-1}.

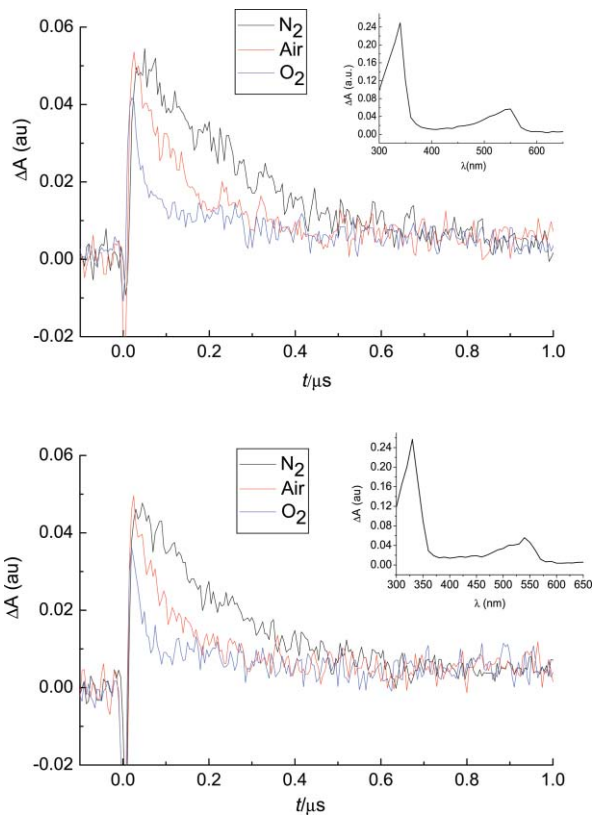


Fig. 2 Decays of the biradicals generated from dichloromethane solutions of dyads **1** (top) and **2** (bottom), upon laser flash photolysis under different conditions, monitored at 545 nm. The insets show the transient absorption spectra of the biradicals.

In view of the difficulty to achieve direct observation of the short-lived triplet excited states, another set of experiments were performed to obtain accurate values for the triplet lifetimes of (*S*)-KP–α-Ch and (*R*)-KP–α-Ch by the well-established energy transfer method, using naphthalene (NP) as acceptor.[8,19] Thus, laser flash photolysis of dyads **1** and **2** was performed at the excitation wavelength of 355 nm in the presence of increasing amounts of NP, and the triplet–triplet absorption of NP was observed and monitored at 415 nm.

When the reciprocal transient absorbance at 415 nm was plotted against the reciprocal of concentration of NP two straight lines were obtained [see Fig. 3 and eqn (1)].

$$1/A_{415} = a + a/(k_q \times \tau_T \times [\text{NP}]) \quad (1)$$

A_{415} is the absorbance of the triplet of NP at 415 nm, before significant decay takes place, k_q is the bimolecular rate constant for triplet quenching by NP, τ_T is the triplet lifetime of **1** or **2** in the absence of NP, and a is a constant. The Stern–Volmer parameters ($k_q \times \tau_T$) were obtained from the intercept-to-slope ratios [Fig. 3 and eqn (1)]. They were found to be 79 M^{-1} for **1** and 94 M^{-1} for **2**. On the other hand, the intermolecular k_q determined in dichloromethane for (*S*)-KP was 8×10^9 M^{-1} s^{-1}, which was assumed to be the same for the dyads. With these data, the values calculated for the triplet lifetimes of (*S*)-KP–α-Ch and (*R*)-KP–α-Ch were 10 ns and 12 ns respectively.

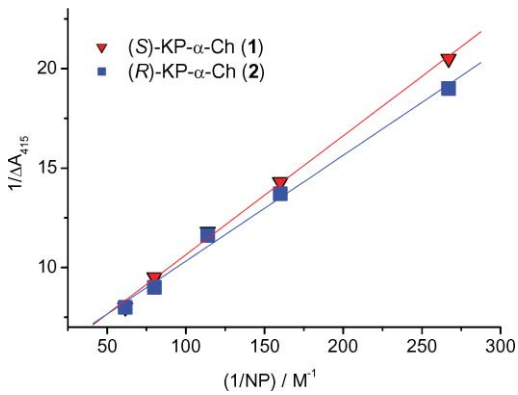


Fig. 3 Double reciprocal plot for quenching of dyads (*S*)-KP–α-Ch and (*R*)-KP–α-Ch triplet excited state by NP in CH_2Cl_2.

The intramolecular quenching rate constants (k_{iq}) were determined by using eqn (2), where τ_i are the lifetimes of the ketone triplets in compounds (*S*)-KP–α-Ch, (*R*)-KP–α-Ch and (*S*)-KP–β-Ch, while τ_0 is the (*S*)-KP triplet lifetime. The obtained values were used to calculate the rate constants for hydrogen abstraction (k_H) and for physical quenching by the π system (k_π), (see Table 1) using eqn (3) and (4).

$$k_{iq} = 1/\tau_i - 1/\tau_0 \quad (2)$$

$$k_H = k_{iq}\Phi_{\text{ketyl radical}} \quad (3)$$

$$k_{iq} = k_H + k_\pi \quad (4)$$

By contrast, the triplet excited state of the extended diastereoisomeric form **3** (Fig. 4A) was much longer-lived, so it was possible

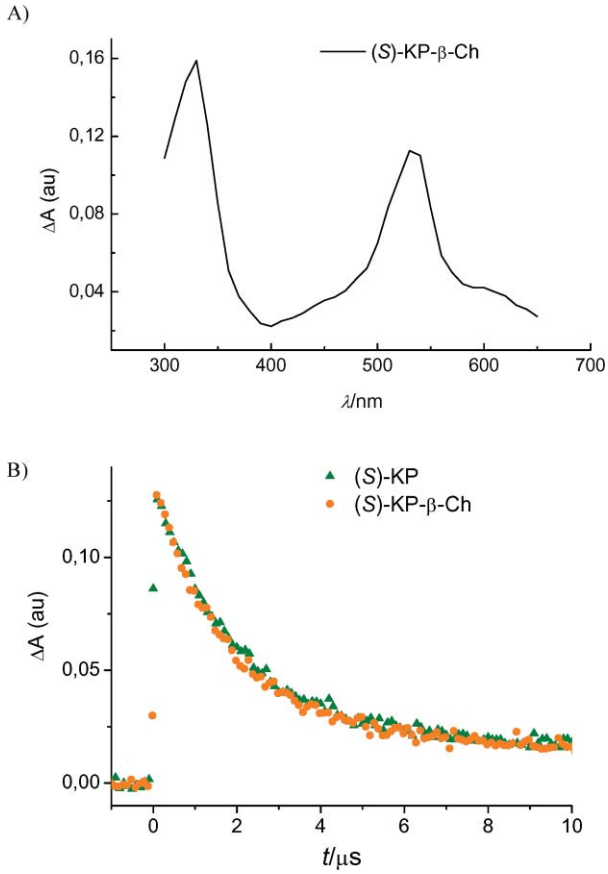


Fig. 4 A) Transient absorption spectrum for dyad **3**, 0.5 μs after the laser pulse (λ_{exc} = 355). B) Decays of the triplets generated from (*S*)-KP and dyad **3** monitored at 520 nm.

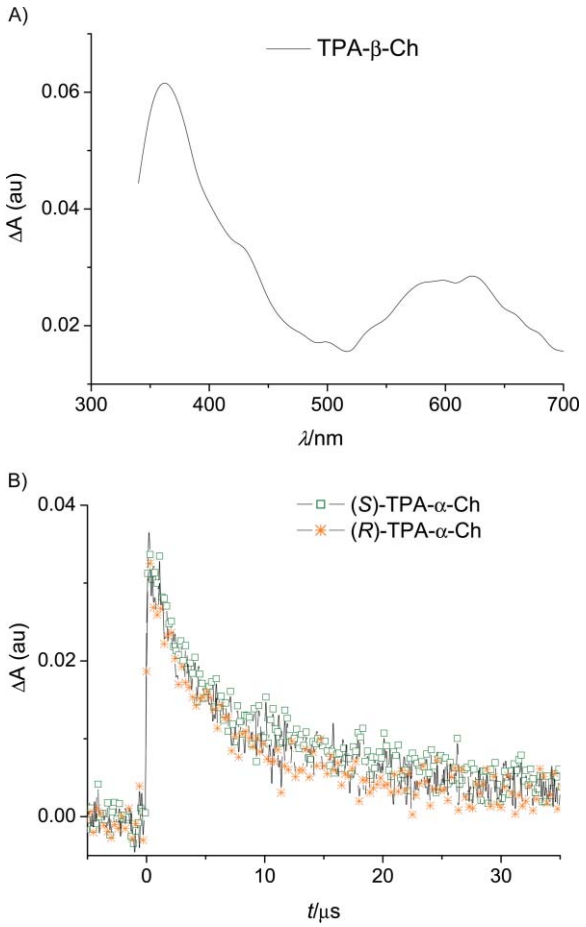


Fig. 5 A) Transient spectrum for dyad **6**, 1 μs after the laser pulse (λ_{exc} = 355 nm). B) Decays of the triplets generated from dyads **4** and **5**, monitored at 600 nm.

to record its absorption spectrum, which showed the typical T–T benzophenone bands with maxima at *ca.* 330 nm and 525 nm.[4,17,18] Direct determination of its lifetime (1.69 μs) showed that it was essentially coincident with that obtained for (*S*)-KP under the same conditions (1.70 μs, see Fig. 4B).

In this context, the transient absorption spectra obtained by laser flash photolysis experiments from all the tiaprofenic acid-derived dyads, (*S*)-TPA–α-Ch, (*R*)-TPA–α-Ch and TPA–β-Ch, were very similar to that described for the tiaprofenic acid triplet excited state (typical bands with maxima at *ca.* 360 nm and 600 nm and relative intensities of *ca.* 2 : 1).[4,12] For example, Fig. 5A shows the T–T absorption spectrum of TPA–β-Ch. In addition, from the decay traces of the triplets generated upon laser flash photolysis of **4** and **5** (Fig. 5B), it was observed that the triplet excited state lifetimes of the two dyads (*ca.* 5.5 μs) were also very close to each other.

Singlet oxygen quantum yields

Time-resolved near infrared emission studies were carried out on the dyads, in order to assess their ability to photosensitize the production of excited singlet molecular oxygen ($^1O_2$ or $^1\Delta_g$). Formation of this species was detected by time-resolved measurements of the luminescence at 1270 nm, in dichloromethane, using an appropriate diode as detector. Fig. 6 shows that, while dyads **1** and **2** produced negligible luminescence, the β isomer **3** gave rise

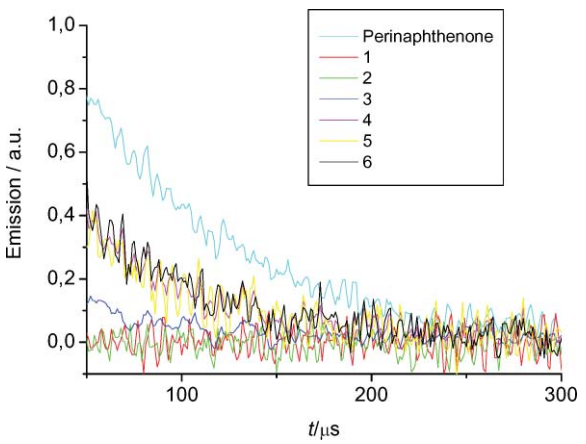


Fig. 6 Time-resolved emission at 1270 nm upon 308 nm excitation of dyads **1–6**, using perinaphthenone as standard for comparison.

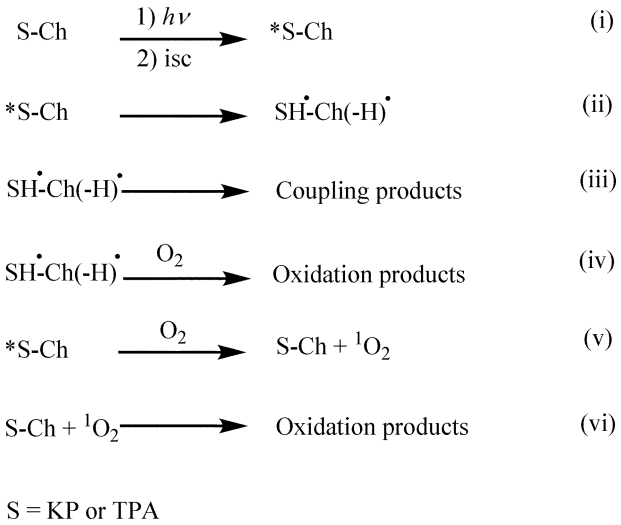
to singlet oxygen with a quantum yield (Φ_Δ) of 0.2, in the range of the values described for ketoprofen (KP) in organic solvents.[20] These results can be understood on the basis of the very fast intramolecular hydrogen abstraction process occurring in dyads **1** and **2**, which is not possible in **3**. On the other hand, the Φ_Δ for dyads **4–6** was *ca.* 0.5 in all cases, close to that described for tiaprofenic acid (TPA).[20]

As regards the singlet oxygen lifetime in dichloromethane, it was measured using perinaphthenone as standard and was 76 μs, in reasonable agreement with the literature.[21,22] Likewise, the lifetime obtained in the presence of dyads **4–6** was *ca.* 64 μs, reasonably close to the expected value.

Type I *vs.* Type II photooxidation mechanisms

The nature of the involved excited triplet states showed a dramatic influence on the photobehavior. Thus, when cholesterol was attached to KP (an nπ* diaryl ketone) the photoreactivity under anaerobic conditions was remarkable. This was clearly evident from the steady-state studies, where α-dyads **1** and **2** were efficiently photodegraded. Accordingly, in the laser flash photolysis experiments a very short triplet lifetime was estimated for the α-dyads **1** and **2** due to efficient photogeneration of 7-allyl Ch biradicals by intramolecular H abstraction. Thus, product studies as well as biradical detection support the high nπ* photoreactivity. By contrast, a quite different situation was observed for the photostable dyads derived from TPA, a diaryl ketone with ππ* character.

Likewise, the electronic configuration of the excited triplet state has a strong influence on the photooxidation mechanism. This can be conveniently discussed on the basis of Scheme 5, which summarizes all the relevant steps involved in the overall process. Thus, the Type I mechanism would involve intramolecular hydrogen abstraction (ii), followed by oxygen quenching of the resulting biradicals (iv). As stated above, the rate constants determined for step ii are *ca.* 10^8 s^{-1} for the KP–α-Ch dyads (nπ*) and lower than 10^4 s^{-1} for the TPA–α-Ch analogs (ππ*). Step (iv) was also very fast in the KP derivatives; its rate constant was nearly diffusion-controlled (k_q(iv) = 3.6×10^9 M^{-1} s^{-1}).



Scheme 5 Cholesterol oxidation mechanisms intramolecularly photosensitized by diaryl ketones.

Alternatively, the Type II mechanism would occur following steps (v) and (vi), becoming the only significant pathway in the case of TPA–α-Ch dyads. The rate constant for triplet quenching by oxygen was $k_q(v) = 0.7 \times 10^9$ M^{-1} s^{-1} for **3** and 0.6×10^9 M^{-1} s^{-1} for dyads **4–6**. As a result of such quenching, $^1O_2$ was actually produced with $\Phi_\Delta = 0.5$; its reaction with the dyads is shown in step vi.

Conclusions

The present work has demonstrated the different behavior between the α-dyads derived from KP (an nπ* diaryl ketone) and those derived from TPA (a heterocyclic analog with ππ* character). Thus, KP–α-Ch dyads are appropriate systems to generate biradicals by intramolecular hydrogen abstraction from the 7-allyl Ch position; in addition, in these dyads the generation of singlet oxygen is expectedly negligible. By contrast, the corresponding TPA–α-Ch dyads are unreactive *via* intramolecular hydrogen abstraction, but $^1O_2$ is produced with $\Phi_\Delta = 0.5$. Therefore, KP-based dyads are appropriate models for clean type I Ch oxidation, whereas the TPA-derivatives are suitable systems for investigation of the purely type II process.

Experimental

General

(*S*)-Ketoprofen [(*S*)-2-(3-benzoylphenyl)propionic acid, (*S*)-KP] and β-cholesterol [cholest-5-en-3β-ol, Ch] were commercially available. The preparation of (*R*)-ketoprofen and α-cholesterol is detailed in the Supporting Information. The two enantiomers of tiaprofenic acid [(*R*)-and (*S*)-2-(5-benzoylthiophen-2-yl)propionic acid, TPA) were obtained by chiral HPLC separation of the racemic mixture, using hexane–methyl *tert*-butyl ether–acetic acid (70 : 30 : 0.1 v/v/v) as the mobile phase; flow-rate 2 mL min^{-1}. Chromatographic HPLC separation was performed coupled with a chiral detector. Samples were injected onto a semipreparative column (Kromasil CHI-TBB). Irradiations with 266 nm light were carried out with the Xe lamp of a Photon Technology spectrofluorometer, equipped with a monochromator. The ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ as solvent at 300 or 500 and 75 MHz, respectively; chemical shifts are reported in ppm downfield from an internal solvent peak. Exact mass was obtained by fast atom bombardment (FAB) recorded in a VG Autospec high-resolution mass spectrometer (HRMS). All reactions were monitored by analytical TLC with silica gel 60 F_{254} (Merck) revealed with cerium ammonium sulfate–ammonium molybdate reagent. The residues were purified through silica gel 60 (0.063–0.2 mm).

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 ~10 ns duration and the energy was from 10 to 1 mJ per pulse. The laser flash photolysis apparatus consisted of the pulsed laser, the 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. All experiments were carried out at room temperature. The samples were dissolved in dichloromethane to have an absorbance *ca.* 0.05 at 355 nm. Solutions were deaerated by bubbling nitrogen (when specified). As naphthalene (NP) does not absorb at 355 nm, under these conditions more than 99% of the light was always absorbed by the dyads. The rate constants of triplet excited state quenching by oxygen, NP and cholesterol Ch were determined by the Stern–Volmer equation ($1/\tau = 1/\tau_0 + k$[Quencher]). Concentrations between 0.5 and 20 mM were used for NP, from 1 to 50 mM for Ch

and 1.27 mM and 0.27 mM for oxygen (concentrations of pure O_2 gas and air at room temperature, respectively). An energy transfer reaction from (*R* or *S*)-KP–α-Ch to NP was used to calculate the triplet state lifetimes of these dyads. These experiments were carried out using dichloromethane solutions of the (*R* or *S*)-KP–α-Ch dyads (0.5 mM and 2 mM–20 mm).

Singlet oxygen measurements

The luminescence (1270 nm) from singlet oxygen was detected by means of an Oriel 71614 germanium photodiode (5 mm^2) coupled to the laser photolysis cell in right-angle geometry. An excimer laser (LEXTRA50 Lambda Physik) was used for the excitation at 308 nm (laser excitation at 5 low-pulse energies for each molecule). A 5 mm thick (5 cm diameter) 1050 nm cut-off silicon filter and a 1270 nm interference filter were placed between the diode and the cell. The photodiode output current was amplified and fed into a TDS-640A Tektronix oscilloscope *via* a Co-linear 150 MHz, 20 dB amplifier. The output signal from the oscilloscope was transferred to a personal computer for study. Thus, the singlet oxygen quantum yield (Φ_Δ) of the dyads was determined in dichloromethane solutions using the same absorbance value (0.30) at 308 nm for each compound. A singlet oxygen quantum yield (Φ_Δ) of 0.95 for perinaphthenone in dichloromethane was used as standard.[23]

Synthesis of the dyads 4 and 5

To a cold solution of racemic 2-(5-benzoylthiophen-2-yl)-propanoic acid (100 mg, 0.38 mmol) in CH_2Cl_2 (1.5 mL), dicyclohexylcarbodiimide (DCC, 136 mg, 0.66 mmol) was added portionwise, and the mixture was stirred at 0 °C for 30 min. Then, a solution of α-cholesterol (131 mg, 0.34 mmol) and 4-dimethylaminopyridine (DMAP, 4 mg, 0.033 mmol) in CH_2Cl_2 (1.2 mL) was added, and stirring was continued for 8 h at the same temperature. The reaction mixture was filtered through a pad of Celite®, brine (5 mL) added to the filtrate, and the mixture was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were washed with water, the organic phase was dried over Na_2SO_4 and evaporated and further purified by column chromatography (eluent: hexane–dichloromethane–ethyl acetate 90 : 5 : 5 v/v/v) to yield a diastereomeric mixture of the corresponding esters. After crystallization of the above mixture from hexane–ethyl acetate (1 : 1 v/v), one of the stereoisomers precipitated as a white solid (88 mg, 0,14 mmol, 41%, **4**); the other isomer was obtained by elimination of the solvent from the filtered solutions as a colorless oil (79 mg, 0,12 mmol, 37%, **5**). Alternative synthesis of the corresponding ester from enantiomerically pure (*S*)-TPA obtained by chiral HPLC separation of the racemic mixture was used for stereochemical assignment.

Data for (*R*)-TPA–α-Ch (4) and (*S*)-TPA–α-Ch (5)

Compound 4. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.64 (s, 3H), 0.86 (d, J = 6.5 Hz, 6H), 0.88 (d, J = 6.5 Hz, 3H), 0.97 (s, 3H), 1.61 (d, J = 7.2 Hz, 3H), 0.90–1.99 (complex signal, 26 H), 2.20 (broad d, J = 15.0 Hz, 1H), 2.45 (broad d, J = 15.0 Hz, 1H), 3.99 (q, J = 7.2 Hz, 1H), 4.97 (m, 1H), 5.19 (broad d, J = 5.1 Hz, 1H), 7.00 (d, J = 3.9 Hz, 1H), 7.48–7.58 (m, 4H), 7.84 (d, J = 7.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 11.8, 18.7, 18.8, 19.0, 20.7, 22.6, 22.8, 23.8, 24.2, 26.0, 28.0, 28.2, 31.7, 31.8, 33.6, 35.7, 36.0, 36.1, 36.9, 39.5, 39.6, 42.0, 42.2, 50.0, 56.0, 56.5, 71.9, 122.5, 126.0, 128.3, 129.1, 132.1, 134.6, 137.7, 138.0, 142.0, 153.1, 171.7, 187.7. HRMS (FAB) $C_{41}H_{56}O_3S$ m/z calcd: 628.39502 [M^+]; found 628.39624.

Compound 5. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.64 (s, 3H), 0.86 (d, J = 6.5 Hz, 6H), 0.88 (d, J = 6.5 Hz, 3H), 0.96 (s, 3H), 1.60 (d, J = 7.2 Hz, 3H), 0.90–2.02 (complex signal, 26 H), 2.15 (broad d, J = 15.0 Hz, 1H), 2.40 (broad d, J = 15.0 Hz, 1H), 3.99 (q, J = 7.2 Hz, 1H), 4.97 (m, 1H), 5.08 (broad d, J = 4.8 Hz, 1H), 7.00 (d, J = 3.6 Hz, 1H), 7.47–7.57 (m, 4H), 7.84 (d, J = 6.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 11.8, 18.6, 18.7, 18.8, 20.7, 22.5, 22.8, 23.7, 24.1, 26.1, 28.0, 28.1, 31.6, 31.8, 33.6, 35.7, 36.0, 36.1, 36.8, 39.5, 39.6, 41.8, 42.2, 50.0, 56.0, 56.5, 71.8, 122.5, 125.9, 128.3, 129.0, 132.1, 134.5, 137.7, 138.0, 142.0, 153.1, 171.6, 187.6. HRMS (FAB) $C_{41}H_{56}O_3S$ m/z calcd: 628.39502 [M^+]; found 628.39381.

3β-Cholesteryl 2-(5-benzoylthienyl)propanoate (TPA–β-Ch, 6)

To a solution of racemic 2-(5-benzoylthien-2-yl)propanoyl chloride (*ca.* 100 mg, 0.36 mmol) in CH_2Cl_2 (15 mL), β-cholesterol (150 mg, 0.39 mmol) in CH_2Cl_2 (3 mL) was added dropwise, and the mixture was heated under reflux for 7 h. The reaction mixture was cooled to room temperature and then it was washed with water (3 × 10 mol) and brine (10 mol). The organic phase was dried over Na_2SO_4, evaporated and purified by column chromatography (eluent: hexane–dichloromethane–ethyl acetate 90 : 5 : 5 v/v/v) to give the corresponding ester **TPA–β-Ch** (189 mg, 0.30 mmol, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.67 (s, 3H), 0.84 (d, J = 6.6 Hz, 6H), 0.90 (d, J = 6.6 Hz, 3H), 1.01 (s, 3H), 1.60 (d, J = 7.2 Hz, 3H), 0.90–2.05 (complex signal, 26 H), 2.32 (m, 2H), 3.99 (q, J = 7.2 Hz, 1H), 4.66 (m, 1H), 5.37 (m, 1H), 7.02 (d, J = 3.9 Hz, 1H), 7.45–7.57 (m, 4H), 7.83 (d, J = 6.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 11.7, 18.7, 19.2, 19.3, 21.0, 22.5, 22.8, 23.8, 24.3, 28.0, 28.2, 31.8, 31.9, 35.8, 36.2, 36.6, 36.9, 37.8, 38.0, 39.5, 39.7, 41.9, 42.3, 50.0, 56.1, 56.7, 75.1, 122.8, 125.8, 128.3, 129.1, 132.1, 134.8, 138.0, 139.3, 142.2, 152.9, 171.8, 188.0. HRMS (FAB) $C_{41}H_{56}O_3S$ m/z calcd: 628.39502 [M^+]; found 628.39293.

Steady-state photolysis of the dyads 1, 2, 4 and 5

Deaerated dichloromethane (20 mL) solutions of (*S*)- or (*R*)-KP–α-Ch dyads and (*S*)- or (*R*)-TPA–α-Ch dyads (100 mg, 0.16 mmol) were irradiated through Pyrex with a 400 W medium pressure mercury lamp. Reactions were monitored by TLC and NMR, and only KP derived dyads (**1** and **2**) were found to be reactive. After 4 hours, the reaction mixtures were concentrated under reduced pressure and submitted to silica gel column chromatography, using hexane–ethyl acetate–dichloromethane (eluent: 70 : 20 : 10 v/v/v). This afforded the pure photoproducts **8–10**.[13]

Acknowledgements

Financial support from the Spanish Government (Grant CTQ2004–03811 and CTQ2007–67010), Generalitat Valenciana (GV06/099), Vicerrectorado de Investigación Desarrollo e Innovación de la UPV (PAID-06–06) and Juan de la Cierva contract to I. A., are gratefully acknowledged.

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