Efficient and Selective Photogeneration of Cholesterol-Derived Radicals by Intramolecular Hydrogen Abstraction in Model Dyads

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Received July 27, 2006

ABSTRACT
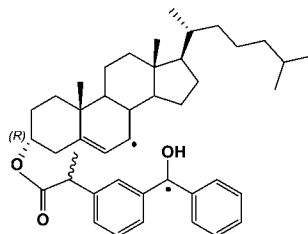
Three model dyads have been synthesized by esterification of β- and α-cholesterol (Ch) with (S)- and/or (R)-ketoprofen (Kp). The α-dyads are efficient photogenerators of the 7-allyl Ch radicals by intramolecular H abstraction. Subsequent cyclization via C–C coupling occurs in a stereoselective way.

Cholesterol (cholest-5-en-3β-ol, Ch) is one of the structural components of cell membranes, where it accounts for a substantial part of the total lipids and is an important target for oxidative damage.[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]

Benzophenone (Bz) is a typical Type I photosensitizer;[3]

however, Bz-photosensitized Ch oxidation has not been previously reported, despite its potential mechanistic interest. Moreover, ketoprofen (Kp) is a nonsteroidal antiinflammatory drug containing the Bz chromophore.[4–6] It is known to induce photoperoxidation of polyunsaturated fatty acids, but no attention has been paid to its excited-state interaction with Ch.

In the present work, we have undertaken a steady-state and time-resolved study on the intramolecular abstraction of the Ch allylic hydrogens by excited Kp, which would be

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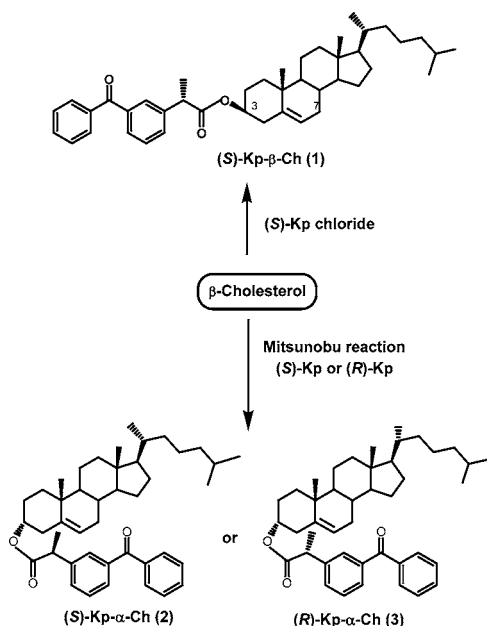
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the key step in radical-mediated (Type I) Ch oxidation. For this purpose, several ketoprofen—cholesterol (Kp—Ch) dyads have been synthesized through conjugation of β- and α-Ch with (S)- and (R)-Kp (Scheme 1). Inclusion of Kp-β-Ch in

(S)-Kp-β-Ch (1)

(S)-Kp chloride

β-Cholesterol

Mitsunobu reaction
(S)-Kp or (R)-Kp

or

(S)-Kp-α-Ch (2)   (R)-Kp-α-Ch (3)

the series was done for comparison, as this dyad should not be reactive in the intramolecular hydrogen abstraction process due to the extended relationship between the two active moieties, which prevents their close approach.

These Kp—Ch dyads are reminiscent of Breslow's biomimetic systems constructed by esterification of cholestanol, androstanol, and related compounds with Bz-derived carboxylic acids,[7] which were designed to study the remote photooxidation of steroids. In this process, Bz 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).[7] 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.

We have previously reported a significant stereodifferentiation in the intramolecular hydrogen abstraction from enantiomerically pure 1,4-cyclohexadienes by (S)-Kp, where chiral discrimination was demonstrated by measurement of the very short triplet lifetimes of the Bz chromophore (18−31 ns).[8,9] Besides, diastereodifferentiation was also found in the quenching of (S)-Kp triplet states by chiral tetrahydrofuran deriva-

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tives as H donors.[10] However, stereodifferentiation in the biradical lifetimes related to photoproduct formation remains to be investigated. Here, it will be shown that the biradicals formed upon intramolecular hydrogen abstraction in the selected Kp—Ch dyads can be detected by laser flash photolysis; their lifetimes exhibit significant stereodifferentiation, which can be correlated with the observed stereoselectivity in the formation of the photoproducts resulting from C—C coupling.

The employed (S)-Kp was commercially available, and its (R) enantiomer was obtained by chiral HPLC separation of the racemic mixture. Preparation of the (S)-Kp-β-Ch dyad (**1**) was achieved following standard esterification procedures,[8] whereas (R)-Kp- and (S)-Kp-α-Ch dyads (**2** and **3**) were synthesized by a Mitsunobu reaction (Scheme 1).[11] Once prepared, the Kp—Ch dyads were submitted to steady-state and laser flash photolysis studies.

Irradiations were performed at λ_{max} = 350 nm (Gaussian distribution) in dichloromethane (ca. 10^{-5} M solutions), under an inert atmosphere, and were monitored by UV spectrophotometry following the disappearance of the typical π,π^* benzophenone absorption band at 254 nm (see Figure 1).[8,10]
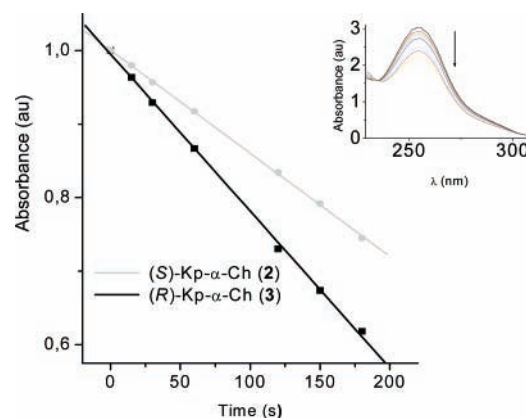

Figure 1. Decrease of the absorbance at λ_{254} for dyads **2** and **3** as a function of the irradiation time. Inset: UV spectrum of a dichloromethane solution of **2** after increasing irradiation times.

In agreement with their different abilities to adopt a folded conformation allowing a close approach between the two active moieties, dyads **2** and **3** were photolyzed in a few minutes while **1** remained unchanged after prolonged irradiation. Moreover, as shown in Figure 1, (R)-Kp-α-Ch (**3**) was clearly more photoreactive than its epimer (S)-Kp-α-Ch (**2**). Hence, the photoreactivity of Kp—Ch dyads was markedly configuration dependent.

Photoproduct formation was investigated by steady-state irradiation of more concentrated (ca. 10^{-2} M) dichloromethane solutions of dyads **2** and **3** under nitrogen, through Pyrex, with a 400 W medium-pressure mercury lamp. The photomixtures were submitted to silica gel column chromatography, using hexane/ethyl acetate/dichloromethane (70:20:10 v/v/v) as eluent, which afforded the pure photoproducts. Spectral analysis revealed formation of two diastereomeric compounds
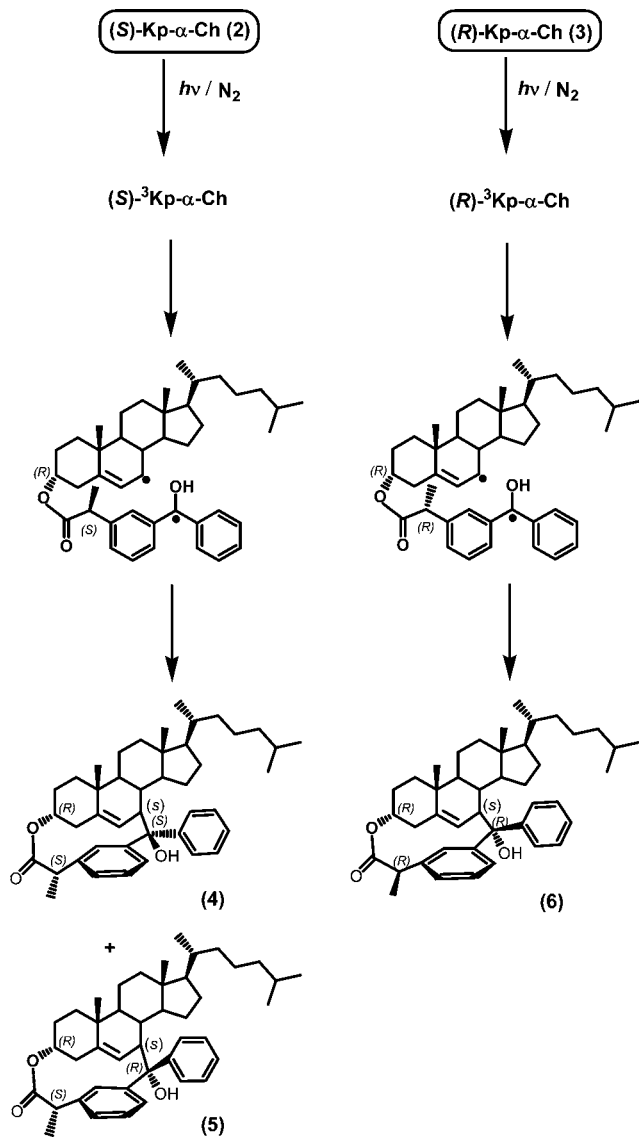
4 and **5** from (*S*)-Kp-α-Ch (**2**), whereas in the case of (*R*)-Kp-α-Ch (**3**), only one photoproduct (**6**) was obtained (Scheme 2).

Scheme 2. Simplified Mechanism Explaining the Formation of Products **4**−**6** upon Photolysis of Dyads **2** and **3**

Products **4**−**6** 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 Breslow's systems) remain unaffected in the case of Ch.

The structures of the photoproducts were unambiguously assigned on the basis of their NMR spectroscopic data (¹H, ¹³C, DEPT, COSY 45, HMQC, NOEDIFF). Mass spectrometry (including high-resolution measurements) supported the assignments. Because of 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 (see Figure 2). More details are given in the Supporting Information.
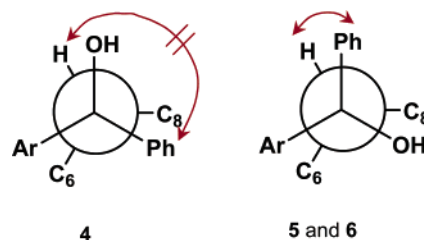

Figure 2. Diagnostic NOE interactions in compounds **4**−**6**.

The obtained results clearly show that Kp−Ch dyads exhibit a significant differentiation in their photoreactivity and give rise to macrocyclic products derived from intermediate biradicals in a diastereoselective manner. Hence, it appeared interesting to perform a laser flash photolysis study on (*S*)-Kp-β-Ch (**1**), (*S*)-Kp-α-Ch (**2**), and (*R*)-Kp-α-Ch (**3**), to clarify the role of the chiral discrimination in the early steps of the intramolecular hydrogen abstraction process. The experiments were carried out in dichloromethane solutions, under nitrogen, using 355 nm as the excitation wavelength. For dyad **1**, the typical triplet−triplet absorption of the benzophenone chromophore (λ_{max} = 325 and 525 nm)[12,13] was observed 20 ns after the laser pulse (Figure 3A); its

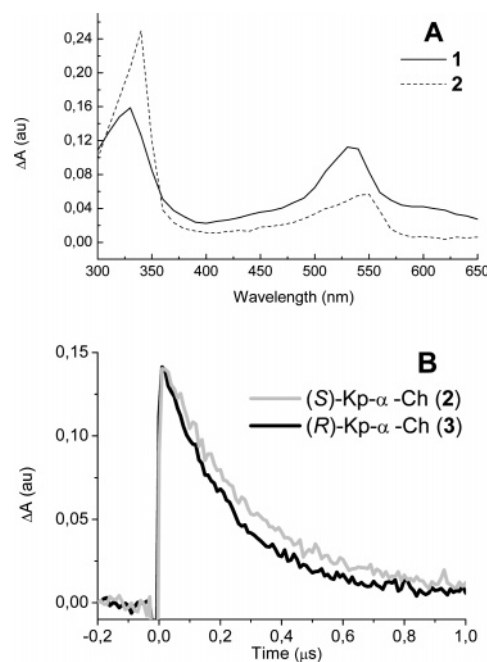**Figure 3.** (A) Transient spectra obtained for dyads **1** and **2** in CH₂-Cl₂ 20 ns after the laser pulse at 355 nm. (B) Decays of the biradicals generated from dyads **2** and **3**, monitored at 330 nm.

lifetime (1.69 μs) was very similar to that obtained for (S)-Kp (1.70 μs). By contrast, the transient absorption spectra obtained for dyads **2** and **3** under the same conditions did not correspond to their triplet excited states but instead to the corresponding biradicals. A safe assignment of these transient species was based on the typical bands with maxima at ca. 330 and 545 nm and on their relative intensities ($\epsilon_{545}/\epsilon_{330}$, ca. 0.17).[5,12] Figure 3A shows the biradical generated from **2**; a similar spectrum was obtained in the case of **3**.

The decay kinetics of these transient intermediates was monitored at 330 nm, and the biradical lifetimes found for dyads **2** and **3** were 280 and 220 ns, respectively (Figure 3B and Table 1). As expected for related 1-hydroxy-1,*n* biradi-

Table 1. Photophysical Parameters of Dyads **1−3**

parameters	(S)-Kp-β-Ch (**1**)	(S)-Kp-α-Ch (**2**)	(R)-Kp-α-Ch (**3**)
τ_T (μs)	1.690	0.010	0.012
k_{iq} (s^{-1})[a]	$<1.0 \times 10^4$	1.0×10^8	1.2×10^8
k_H (s^{-1}),[b] (%)		0.8×10^8	1.0×10^8
k_π (s^{-1}),[b] (%)		0.2×10^8	0.2×10^8
$\Phi_{\text{ketyl radical}}$[c]	0.2	0.8 (0.7)[d]	0.8 (0.7)[d]
$\tau_{\text{biradical}}$ (μs)		0.28 (0.55)[d]	0.22 (0.49)[d]

[a] The intramolecular quenching rate constants of (S)-Kp-α-Ch and (R)-Kp-α-Ch were obtained by using the equation $k_{iq} = 1/\tau_i - 1/\tau_0$, where τ_i is the lifetimes of the ketone triplets in compounds **2** and **3** and τ_0 is the (S)-Kp triplet lifetime (1.7 μs). [b] The rate constants for hydrogen abstraction and physical quenching by the π system were obtained using the following equations: $k_H = k_{iq} \times \Phi_{\text{ketyl radical}}$ and $k_{iq} = k_H + k_\pi$. [c] The quantum yield of ketyl radical formation was determined by the comparative method, using Bz as the standard. [d] In the presence of 1.2 M pyridine.

cals, the lifetimes were significantly longer in the presence of added pyridine as a Lewis base[14,15] (see Table 1); however, they were not affected by the temperature and were quenched by oxygen very quickly (rate constant of ca. 3.6×10^9 M^{-1}

s^{-1}). All together, the above facts are consistent with intersystem crossing concurrent with product formation as the key factor determining biradical lifetimes[14] in the case of **2** and **3**. The photophysical data obtained for dyads **1−3** correlated well with the results from the steady-state studies.

Thus, photolysis of **2** and **3** gave rise to photocyclization products arising from the reaction of the Bz-derived triplet excited states with the 7-allylic position of the cholesterol skeleton by an intramolecular hydrogen abstraction process (Scheme 2). The more reactive (R)-Kp-α-Ch system in the steady-state photolysis (Figure 1) produced also the shorter-lived biradical in the laser flash photolysis experiments (Figure 3B and Table 1). Besides, the nonreactive triplet excited state of dyad **1** was actually detected, whereas the T−T absorption of **2** and **3** was not clearly identified due to the short triplet lifetimes (10−12 ns, as revealed by indirect measurements based on energy transfer to naphthalene).[9,16]

In conclusion, the Kp−Ch dyads studied in the present work seem to be appropriate model systems to generate the allylic 7-Ch radical in an efficient and selective fashion. Oxygen quenching of the Bz-derived triplets would only be expected for (S)-Kp-β-Ch, in view of its sufficiently long lifetime; by contrast, this process would not compete with the very fast intramolecular H abstraction in (S)- and (R)-Kp-α-Ch. The biradicals generated from the last two compounds, however, live long enough to be quenched by oxygen, to give the 7- hydroperoxide and the 7-keto compounds. Thus, dyad **1** would be a suitable model to study Type II Ch photooxidation, whereas dyads **2** and **3** appear to be excellent systems to investigate clean Ch photooxidation via the Type I mechanism. Systematic work is currently being performed in our group to explore the potential of this concept.

Supporting Information Available: Spectroscopic data and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

OL061854C

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