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Intramolecular Interactions in the Triplet Excited States of Benzophenone–Thymine Dyads

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Abstract: Time-resolved and product studies on the synthesized dyads **1** and **2** have provided evidence that the benzophenone-to-thymine orientation strongly influences intramolecular photophysical and photochemical processes. The prevailing reaction mechanism has been established as a Paterno–Büchi cycloaddition to give oxetanes **3**–**6**; however, the ability of benzophenone to achieve a formal hydrogen abstraction from the methyl group of thymidine has also been evidenced by the formation of photoproducts **7** and **8**. These processes have been observed only in the case of the *cisoid* dyad **1**. Adiabatic photochemical cycloreversion of the oxetane ring is achieved upon direct photolysis to give the starting dyad **1** in its excited triplet state. The photobiological implications of the above results are discussed with respect to benzophenone-photosensitized damage of thymidine.

Keywords: DNA damage · oxetanes · photochemistry · photosensitization · time-resolved spectroscopy

Introduction

Exposure of living organisms to solar radiation may induce lethal mutagenic and carcinogenic effects as a result of photochemical modifications of DNA. The cyclobutane pyrimidine dimers pyr<>pyr are formed as the most abundant mutagenic photoproducts.[1–3] Consequently, most research on DNA photodamage, mutagenesis, and repair has focused on the formation and repair of this particular type of damage. More recent studies have identified the pyrimidine (6–4) pyrimidone photoadducts, in which the 6-position of one base is bonded to the 4-position of the adjacent one, as very effective UV photoproducts that cause damaging mutations.[4–10] These adducts are considered to be formed from a photochemical Paterno–Büchi type cycloaddition involving the C5=C6 double bond of the 5′-pyrimidine and the C4 carbonyl group of the 3′-pyrimidine. The presumed oxetane is thought to undergo fast ring-opening and isomerization to the observed (6–4) photoproducts. In the last decade, it has been discovered that a protein can effect the photoreversal of (6–4) photoproducts.[11] Such a protein binds to the (6–4) lesion in the dark and, upon absorption of a photon, repairs the photoproduct to give the normal base forms.[12] Excitation of the enzyme–substrate complex causes cycloreversion by means of a photosensitized electron-transfer mechanism analogous to that reported for the pyr<>pyr DNA photolyase.[13,14] In this context, there are some examples in the literature on the synthesis of model systems designed to study the mechanism of photosensitized reactions involving DNA and proteins.[15]

At wavelengths longer than 290 nm, where DNA is not absorbing, the bulk of the photobiological effects is mediated by photosensitizers.[16–18] For instance, a number of nonsteroidal anti-inflammatory drugs (NSAIDs) are known to photosensitize DNA damage.[19–22] Hence, modified nucleosides containing key substructures present in drugs and nucleic acids can be relevant models to study the excited-state interactions and the primary photophysical/photochemical processes underlying drug-mediated photoreactions of DNA and their photobiological consequences.[23] In particular, they could help to gain some insight into the chemical

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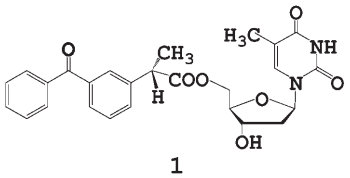
nature of the adducts formed in photosensitized DNA-drug systems, which is poorly understood.

Among NSAIDs, ketoprofen (KP, 2-[3-benzoylphenyl]-propionic acid) is a well-known benzophenone-derived photosensitizer. It produces both photoallergic and phototoxic effects,[24–28] and its photosensitizing properties toward biological targets have been widely investigated.[29–33] Specifically, KP induces photooxidative DNA damage (including strand breaks and base lesions) and photosensitizes the formation of cyclobutane thymine dimers.[34–37] These photobiological properties are attributable to the benzophenone (BP) substructure, as the parent compound BP photosensitizes similar DNA reactions.[29] Indeed, previous studies have shown that there is a strong intermolecular interaction between the excited BP chromophore and thymine derivatives,[38–41] including the free thymidine nucleoside[42] and a thymidine 5′-monophosphate.[43] Fast quenching of triplet BP by thymine derivatives has been observed and attributed to the occurrence of energy or electron transfer; however, recent evidence has been obtained which suggests that this quenching is mainly associated with a Paterno–Büchi cycloaddition leading to the formation of oxetanes.[44]

To gain a better understanding of this type of processes, two thymidine-derived nucleosides (compounds **1** and **2**) have been prepared in the present work by covalently attaching a benzophenone derivative, namely (*S*)-ketoprofen, to positions 5′ or 3′ of the sugar.



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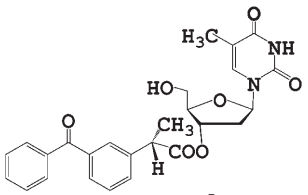

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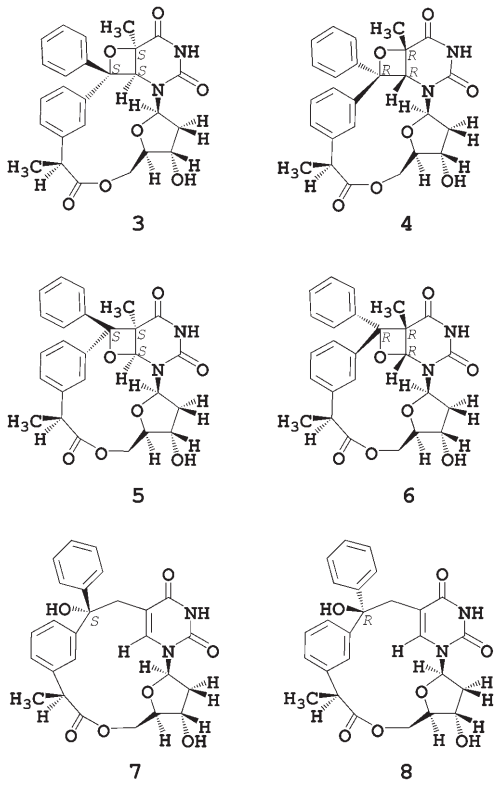
These dyads were designed as models to study the interaction between excited drugs and nucleic acids. Furthermore, they present a different spatial arrangement (*transoid* or *cisoid*) of the BP chromophore relative to the thymine (Thy) unit that could allow an investigation of the influence of the benzophenone-to-thymine orientation on the photophysical and photochemical properties. Analogous model systems based on *S*-KP have been used for the study of drug-photosensitized lipid peroxidation,[45] although in this case, *S*-KP was linked to a cyclohexa-1,4-diene moiety as a source of the doubly allylic hydrogens present in polyunsaturated fatty acids.

It will be shown that the predominating photoreaction pathway in the case of dyad **1** is actually a Paterno–Büchi cycloaddition to give a complex mixture of isomeric oxetanes. The repair of this model lesion can be easily achieved by direct photolysis that effects cycloreversion in a rare, adiabatic process[46] leading to the ground-state thymine plus the excited triplet state of the carbonyl moiety.

Results and Discussion

Photochemical reactivity of dyads 1 and 2: Steady-state irradiation of dyad **1** in acetonitrile, through Pyrex, led to the formation of several photoproducts; in contrast, irradiation of **2** gave only polymerization. The photoproducts were separated by semipreparative HPLC, and their structures were unambiguously determined by a complete assignment of the ^{1}H and ^{13}C NMR signals by the use of a combination of H,H (COSY) and H,C correlations (HSQC and HMBC). The main photoproducts were found to be oxetanes **3**–**6** (combined yield 52%). Minor amounts of cyclic compounds **7** and **8** (14%) were also obtained (Scheme 1).

The regiochemistry of **3** and **4** became evident from the ^{13}C chemical shifts of the bridgehead carbon atoms of the oxetane ring (δ_{CH} = 61.0/60.8 ppm and δ_{C} = 77.4/77.9 ppm); they are essentially coincident with those reported for related compounds.[41] In contrast, the regioisomeric oxetanes **5** and **6** displayed characteristic ^{13}C signals with



3 4 5 6 7 8

Scheme 1. Photoproducts resulting upon irradiation of **1** in acetonitrile.

Table 1. Selected proton NOE data for compound **3** in $CDCl_3$.

	Irradiation of:									
NOE observed [%]:	ArH	H1′	H6	H5′	H5′	H4′	C*H*-CH_3	H2″	H2′	CH-C*H*$_3$
Ar*H* (δ = 8.03 ppm)	–	0	6.67	0.57	0	0	1.39	0	0.47	0.61
H1′ (δ = 5.92 ppm)	0	–	0.73	0	0	1.41	0	3.64	0.33	0
H6 (δ = 5.36 ppm)	6.82	1.16	–	0.18	0	0.34	0	0	1.47	0
H5′ (δ = 4.70 ppm)	0.39	0	0.47	–	66.13	2.15	0	0	0	0
H5′ (δ = 4.46 ppm)	0.54	0	0	13.16	–	3.66	0	0	0	0
H3′ (δ = 4.40 ppm)	–	0.41	0	1.62	–	0	0	1.20	0	0.52
H4′ (δ = 4.10 ppm)	0	1.57	0	2.89	5.30	–	0	0	0	0
C*H*-CH_3 (δ = 3.96 ppm)	2.09	0	0	0	0	0	–	0	0	2.54
H2″ (δ = 2.20 ppm)	0	2.85	0	0	0	0	0	–	1.14	0.12
H2′ (δ = 1.80 ppm)	2.71	1.28	0	0	0	0	0	13.60	–	0
C–C*H*$_3$ (δ = 1.77 ppm)	–	0	1.29	–	0	0	0	0.27	0	0
CH-C*H*$_3$ (δ = 1.61 ppm)	0.39	0	0	0	0	0	0.97	0	0	–

Table 2. Selected proton NOE data for compound **4** in $CDCl_3$.

	Irradiation of:									
NOE observed [%]:	ArH	H1′	H6	H5′	H3′	H4′	C*H*-CH_3	H2″ + H2′	C–C*H*$_3$	CH-C*H*$_3$
Ar*H* (δ = 8.08 ppm)	–	0	3.60	0	0.56	0.38	0.85	0.29	0	0.50
H1′ (δ = 6.40 ppm)	0	–	0	0	0	1.69	0	2.02	0	0
H5′ (δ = 4.85 ppm)	0	0	–	–	7.90	1.85	0	0.53	0	0
H6 (δ = 4.74 ppm)	3.09	0	–	–	0.33	0	0	0	1.20	0
H5′ (δ = 4.45 ppm)	0	0	0.90	20.40	–	1.53	0	0	0	0
H3′ (δ = 4.38 ppm)	1.20	0	1.41	0	–	0	0	1.91	0	0
H4′ (δ = 3.97 ppm)	0	2.58	0	3.90	1.96	–	–	0.67	0	0.54
C*H*-CH_3 (δ = 3.87 ppm)	1.20	0	0	0	0	–	–	0	0	1.62
H2″ + H2′ (δ = 2.20 ppm)	0	3.80	5.99	0	2.30	–	0	–	0.30	–
C–C*H*$_3$ (δ = 1.75 ppm)	0	0	0.5	0	0	0	0	1.00	–	–
CH-C*H*$_3$ (δ = 1.58 ppm)	0.52	0	2.86	0	2.00	1.30	3.44	–	0	–

Table 3. Selected proton NOE data for compound **5** in $CDCl_3$.

	Irradiation of:			
NOE observed [%]:	H1′	H6	H5′	H4′
ArH (δ = 8.07 ppm)	0	5.10	2.57	0
H1′ (δ = 6.10 ppm)	–	0.56	0	0.14
H6 (δ = 5.70 ppm)	0.09	–	0.80	0
H5′ (δ = 4.68 ppm)	0	0	–	3.90
H3′ (δ = 4.66 ppm)	0	1.28	–	0
H5′ (δ = 4.33 ppm)	0	0	3.42	1.75
H4′ (δ = 4.02 ppm)	0.57	0	2.06	–
C*H*-CH_3 (δ = 3.93 ppm)	0	0	0	0
H2″ + H2′ (δ = 2.53 ppm)	2.98	0.62	1.59	1.38
CH-C*H*$_3$ (δ = 1.60 ppm)	0	0	0.34	0
C–C*H*$_3$ (δ = 1.07 ppm)	0	0.80	0	0

chemical shifts δ_{CH} = 79.8/81.9 ppm and δ_C = 57.8 ppm.[41] This assignment is also supported by the known α- and β-effects of the oxygen atom on the ^{13}C chemical shifts; detailed estimations based on these effects are given in the Supporting Information S10–S15.

The stereochemistry of **3–6** was basically assigned by means of NOE experiments (Tables 1–4). This assignment was fully confirmed for oxetane **4** by X-ray crystallography. For instance, upon irradiation of the proton of the oxetane (H6) remarkable NOE enhancements for H1′ were only observed for **3** and **5**. Moreover, a clear NOE interaction was found in the four compounds between H6 and the *ortho*-proton of the benzene ring that is integrated into the macrocycle. As a general rule, sizable NOE effects were observed between protons at a distances of less than 3–4 Å in the molecular models obtained upon MOPAC optimization of the geometries (see the structures in the Supporting Information, S19–S22). Overall, these data support the structural assignment of the isolated oxetanes as shown in Scheme 1.

The ^1H NMR spectra of the minor cyclic photoproducts **7** and **8** showed the disappearance of the thymine methyl group of **1** and the presence of two new diastereotopic methylene protons between δ = 3.3 and 3.6 ppm. In the ^{13}C NMR spectra, the most salient feature was the lack of the thymine methyl group and the ketone carbonyl, together with the appearance of new signals corresponding to the methylene carbon at $\delta \approx 41$ ppm and the quaternary carbon at $\delta \approx 79$ ppm.

Again, the stereochemistry was assigned by means of NOE experiments (Tables 5 and 6). The main interaction for discrimination purposes was found between the internal methylene proton of **7** (δ = 3.34 ppm) and the *ortho*-protons of the phenyl (C_6H_5) group at δ = 7.57 ppm. The rela-

Table 4. Selected proton NOE data for compound **6** in $CDCl_3$.

	Irradiation of:						
NOE observed [%]:	ArH	H1′	H6	H3′	H2′	CH-CH_3	C–CH_3
Ar*H* (δ = 8.57 ppm)	–	0	2.79	0	0	0	0
H1′ (δ = 6.41 ppm)	0	–	0	0	0	0	0
H6 (δ = 5.28 ppm)	2.27	0.13	–	1.83	0.97	0	1.23
H5′ (δ = 4.55 ppm)	0	0	0	2.22	0	0	0
H4′ (δ = 4.27 ppm)	0	0	0	–	0	0	0
H3′ (δ = 4.20 ppm)	0	0	3.07	–	2.04	0	0
C*H*-CH$_3$ (δ = 4.10 ppm)	1.03	0	0	0	0	1.28	0
H5′ (δ = 4.05 ppm)	0	0	0	0	0	0	0
H2″ (δ = 2.48 ppm)	0	1.68	0.47	0	7.57	0	0
H2′ (δ = 1.72 ppm)	0	0	0.84	1.21	–	0	0
CH-CH_3 (δ = 1.61 ppm)	0	0.18	0	0	0	–	0
C–CH_3 (δ = 1.17 ppm)	0	0.01	0.50	0	0	0	–

Table 5. Selected proton NOE data for compound **7** in CD_3OD.

	Irradiation of:						
NOE observed [%]:	H1′	H6	C(H)*H*	C(*H*)H	C*H*-CH$_3$	H2″	H2′
ArH (δ = 7.57 ppm, d)	0	0	0	1.45	0	0	0
(δ = 7.16 ppm, s)	0	1.69	0	2.21	6.25	0	1.01
H6 (δ = 6.54 ppm)	0	–	0	1.26	0	0	5.32
H1′ (δ = 6.10 ppm)	–	0	0	0	0	4.09	0
C(H)*H* (δ = 3.58 ppm)	0	0	–	6.32	0	0	0
C(*H*)H (δ = 3.34 ppm)	0	2.75	20.36	–	0	0	0
H2″ (δ = 1.76 ppm)	2.32	0	0	0	0	–	20.79
H2′ (δ = 0.77 ppm)	0	3.58	0	0	0	19.38	–

Table 6. Selected proton NOE data for compound **8** in CD_3OD.

	Irradiation of:			
NOE observed [%]:	H1′	H6	H2″	H2′
ArH (δ = 7.30 ppm, s)	0	1.52	0	0
H6 (δ = 6.67 ppm)	0	–	0	4.27
H1′ (δ = 6.14 ppm)	–	0	4.72	0
H5′ (δ = 4.31 ppm)	0	1.15	0	0
H2″ (δ = 2.12 ppm)	2.98	0	–	16.24
H2′ (δ = 1.73 ppm)	0	3.03	19.53	–

tive spatial arrangement of these protons, which explains the observed NOE effect, is shown in the Supporting Information (S23, top view). Unfortunately, the corresponding experiment could not be performed with the other stereoisomer (**8**) because its two methylene protons (internal and external) do not appear as separate signals in the ^{1}H NMR spectrum. An interesting feature observed for compound **7** was the upfield shift of its H2′ proton, which appeared at δ = 0.77 ppm. In the minimized models (see Supporting Information, S23 (bottom view) and S24) it becomes clear that this proton falls into the shielding region of the disubstituted aromatic ring only in the case of **7**. Accordingly, a clear NOE interaction was observed between H2′ and the aromatic singlet at δ = 7.2–7.3 ppm for compound **7**, but not for its stereoisomer **8**.

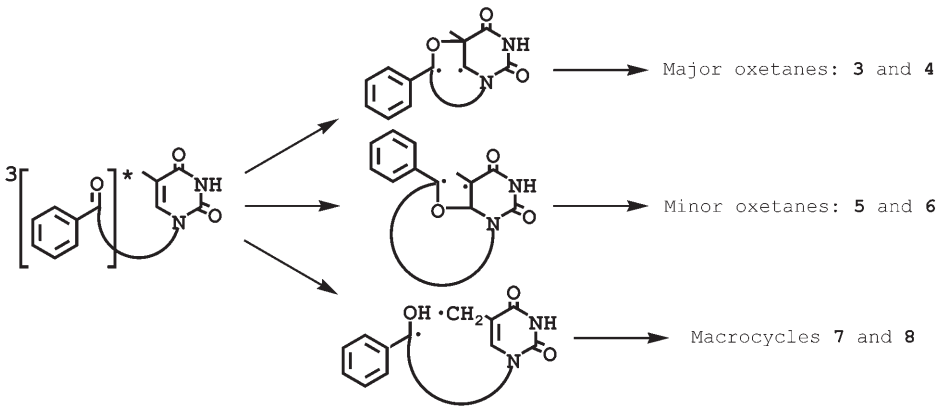
Oxetanes **3**–**6** are clearly formed by means of an intramolecular Paterno–Büchi cycloaddition between the carbonyl group of the benzophenone and the double bond of the thymine base. Intermolecular cycloadditions of this type have been reported for thymine derivatives.[38,41,44,47] The factors governing regioselectivity in oxetane formation are not clear; however, they are thought to include the stability of 1,4-biradical intermediates, steric effects, and hydrogen-bonding interactions. In the case of thymidine, the N,O-acetal regioisomers (analogous to **5** and **6**) are the major products by far. The enhanced formation of oxetanes with the O atom of the carbonyl linked to the C5 thymine position (as in **3** and **4**) starting from dyad **1** must be attributed to their lower ring strain. Here, the rigidity of the resulting macrocycle seems to play a key role.

On the other hand, the minor photoproducts **7** and **8** arise as a result of hydrogen abstraction by the benzophenone carbonyl from the methyl group of the thymine base. The resulting biradical intermediate would collapse upon intramolecular coupling with C–C bond formation. Hydrogen abstraction by benzophenone is well known.[48] Moreover, some products of the reported benzophenone-mediated photosensitization of thymidine can be rationalized by a formal hydrogen abstraction from the C5 methyl group [Eq. (1)].[42]

$$^3\mathrm{BP} + \mathrm{dThd} \rightarrow \mathrm{BPH}^{\cdot} + \mathrm{dThd}(-\mathrm{H})^{\cdot} \quad (1)$$

To sum up, the mechanisms taking place in the photoreactions of dyad **1** are shown in Scheme 2.

Photophysics of dyads 1 and 2: Transient absorbance data were recorded for the two dyads and compared with those obtained for *S*-KP as a reference compound. All experiments were performed in pure acetonitrile under an anaerobic atmosphere and with an excitation wavelength of 355 nm. In all cases, the typical triplet–triplet absorption spectrum of benzophenone was obtained,[49] with a maximum at $\lambda \approx 530$ nm (Figure 1 a). However, significant differences were found in the lifetimes. Thus, decay of the triplet state was markedly slower for *S*-KP (τ_{KP} = 1.3 μs) than for



Scheme 2. Simplified mechanisms of the intramolecular photoreaction between the two active moieties of dyad **1**.

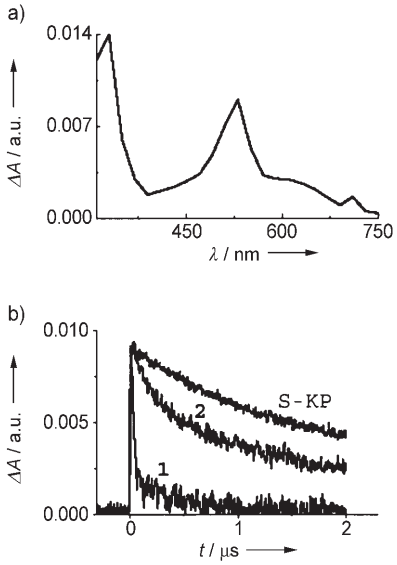


Figure 1. a) Transient spectrum obtained for dyad **1** in acetonitrile 35 ns after the laser pulse at 355 nm. Similar spectra were obtained for dyad **2** and *S*-KP. b) Decays of the triplet signal at 530 nm for *S*-KP and dyads **1** and **2**.

any of the dyads; this effect was specially noteworthy in the case of **1** ($\tau = 20$ ns), as shown in Figure 1 b.

The dramatically shorter lifetime of the excited triplet state in the *cisoid* dyad **1** is in excellent agreement with its higher reactivity, leading to the formation of oxetanes **3**–**6** and, to a lesser extent, to macrocycles **7** and **8**. None of the intermediate biradical species appearing in Scheme 2 were observed (presumably too short-lived).

Mechanism of benzophenone-photosensitized DNA damage at thymine sites: Formation of cyclobutane thymine dimers, which is one of the major DNA lesions, has been reported to occur upon BP and KP photosensitization.[36,37] The required triplet–triplet energy transfer, which would be thermodynamically disfavored, does not seem to play a major role in the intramolecular deactivation of BP triplets by Thy in the dyads. Such an energy transfer should be evidenced through the known triplet–triplet absorption of Thy at 370 nm, which was not detected in our experiments. Dimerization was not observed, probably owing to the inefficient intermolecular reaction between small amounts of triplet Thy and Thy units of other nucleoside in the ground state. In this context, the use of a BP-functionalized oligonucleotide with two (or more) adjacent Thy bases could increase the efficiency of this process by an enhanced intramolecular quenching process.

Thus, it cannot be completely ruled out that a small fraction of thermally activated BP triplets could achieve energy transfer to Thy in very low quantum yields (below the detection limits of the laser flash photolysis technique). This reaction pathway, although minor, could be of biological significance.[42] Besides, the triplet energy of the Thy base in DNA must be lower than that in the dyad; this would enhance the prospects of triplet–triplet energy transfer (and hence cyclobutane dimer formation) in the biomacromolecule.

It has been reported that BP and KP photosensitize oxidative damage to DNA. Although the guanine bases appear to be the preferred sites for this damage, the free dThd nucleoside has also been found to undergo BP-photosensitized oxidation. Electron transfer from dThd to triplet BP, followed by proton transfer, has been proposed as the operating mechanism. This would be equivalent to formal hydrogen abstraction for the thymine methyl group, with formation of an allylic radical as the key intermediate. Subsequent trapping by oxygen accounts for some of the dThd oxidation products. Although electron transfer from Thy to BP would be thermodynamically feasible ($\Delta G = -30\,\mathrm{kJ\,mol^{-1}}$), formation of the resulting radical ions was not observed in the laser flash photolysis experiments with the dyads. However, a significant amount of photoproducts arising from a formal intramolecular hydrogen abstraction (such as **7** and **8**) was obtained; this strongly supports the mechanism proposed in the literature[42] for the generation of oxidative dThd lesions. Nonetheless, oxidation of the Thy sites in DNA by this mechanism would be unlikely because the purine bases (such as guanine) have much lower oxidation potentials and would be much better electron donors toward the excited BP chromophore.[37]

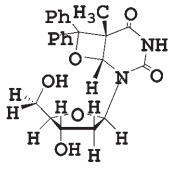
Overall, the results obtained by means of time-resolved and product studies on dyad **1** provide evidence that there is indeed a strong intramolecular interaction between the BP and Thy moieties in the triplet excited state. This is essentially attributable to a Paterno–Büchi photoreaction, in which the initial step is the formation of a new bond be-

tween the excited carbonyl oxygen and one of the Thy olefinic carbons to give oxetanes **3–6** as final products. Such products have been actually isolated and identified in the preparative irradiations.

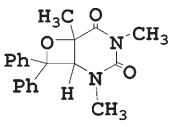
The question remains as to whether oxetane formation upon photolysis of KP in the presence of DNA might still be a possible reaction pathway. This possibility and its biological significance still need to be checked. In this context, it is interesting to mention that oxetanes are believed to be involved in the formation and photoenzymatic repair of the DNA damage associated with pyrimidine (6–4) pyrimidone photoproducts.[40] Here, photolyases play a key role by acting as electron donors to achieve the reductive oxetane cycloreversion to afford two repaired pyrimidine units.[40,50]

Adiabatic photochemical cycloreversion of oxetanes: In a preliminary communication, it was reported that the excited triplet state of the carbonyl compound, together with the ground state of the pyrimidine base, are generated upon laser irradiation ($\lambda = 266$ nm) of oxetanes derived from 1,3-dimethylthymine.[46] This is a rare and interesting case of adiabatic photochemical reaction whose mechanism needs to be clarified in more detail.

To gain further understanding of this type of oxetane cycloreversion, the direct photolysis of **3** (the major product obtained upon irradiation of dyad **1**) has been examined in the present work. Here, the strain release associated with cleavage of the macrocycle was expected to have a marked influence on the process. Hence, the oxetanes obtained from the intermolecular photochemical reaction of benzophenone with thymidine (BP-dThd)[44] and 1,3-dimethylthymine (BP-DMT)[46] were used for a comparison. All the experiments were performed in acetonitrile/water (4:1 v/v).



BP-dThd



BP-DMT

Prior to irradiation, the UV/Vis absorption spectra of the oxetanes showed a band in the $\lambda = 220$–230 nm region. The course of the photolysis was monitored by following the increase of the UV absorption at $\lambda = 254$ nm, which is typical of the carbonyl compound. The cycloreversion quantum yields were calculated from the slopes of the straight lines obtained when the absorbance at $\lambda = 254$ nm was plotted against the irradiation time (Figure 2a); the values were found to be 0.7 and 0.3 for **3** and BP-dThd, respectively, referenced to BP-DMT (photolysis quantum yield = 0.5).[46] Conversions were kept below 10% throughout the measurements.

In a similar way, in order to determine the quantum yield for the opposite process, which is the photolysis of dyad **1**,

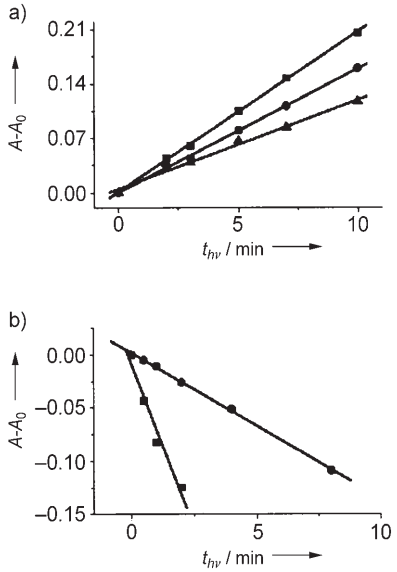


Figure 2. Plot of the absorbance of the carbonyl band at 254 nm against the irradiation time for a) oxetanes **3** (■), BP-dThd (▲) and BP-DMT (●) and, b) dyad **1** (●) and BP in 2-propanol (■).

an analogous experimental procedure was followed. In this case, the reference was a solution of BP in 2-propanol, with a limiting photoreduction quantum yield of 1.96. Both solutions were photolyzed under an anaerobic atmosphere, and the decrease of the carbonyl absorption at $\lambda = 254$ nm was followed. A photolysis quantum yield of 0.4 for dyad **1** was obtained as described above (Figure 2b), also at low conversions (<10%). Since 83% of all the photoproducts of **1** are oxetanes (compounds **3–6**, Scheme 1), the Paterno–Büchi quantum yield would be ≈0.3.

As expected for an adiabatic cycloreversion, the transient spectrum (Figure 3) resulting from laser flash photolysis ($\lambda = 266$ nm, 10 ns, 2–5 mJ per pulse) of oxetane **3** was identical to that obtained from **1** (Figure 1a), reliably assigned to the well-characterized triplet–triplet absorption of benzophenone ($\lambda_{max} = 530$ nm). This process was nearly quantitative, as indicated by the very high quantum yield (close to unity), measured from the transient absorbance at $\lambda = 530$ nm immediately after the laser pulse compared with that of benzophenone.

In the case of BP-DMT, a lower efficiency for this adiabatic process was previously reported (quantum yield ≈0.4).[46] This is qualitatively consistent with the results from preparative experiments (Figure 2a), in which **3** also clearly reacted faster than BP-DMT. Thus, the strain release associated with cleavage of the macrocyclic ring in **3** seems to play an important role in enhancing the reaction.

A simplified energy diagram which indicates that the adiabatic photochemical cycloreversion of oxetane **3** is energetically feasible is shown in Figure 4. The key intermediates are supposed to be the 1,4 biradicals (singlet and triplet) derived from C–C bond scission. Cleavage of the triplet biradical to afford the ground state carbonyl chromophore ap-

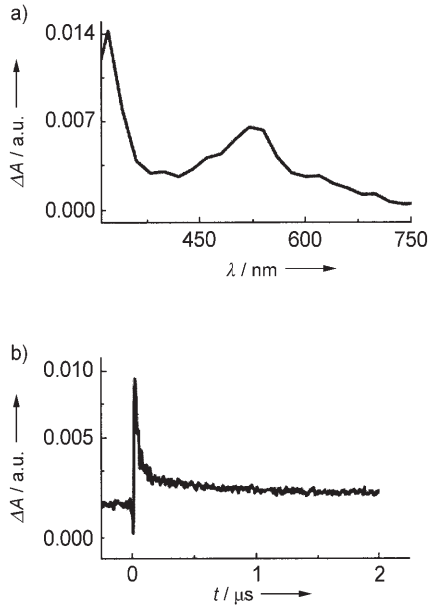


Figure 3. a) Transient spectrum obtained 100 ns after laser irradiation of oxetane **3** at 266 nm. b) Decay of the triplet signal at 530 nm.

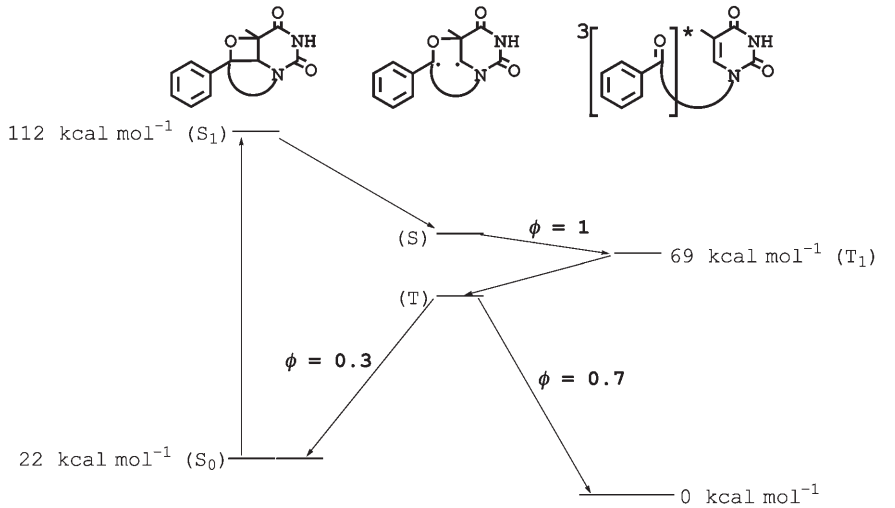


Figure 4. Energy diagram for the adiabatic photochemical processes as cycloreversion of thymine oxetane adduct **3** and cycloaddition of dyad **1**.

pears to be roughly twice as fast as its ring closure to the oxetane adducts.

Conclusion

The benzophenone-to-thymine orientation has a strong influence on the intramolecular photosensitization of thymidine by ketoprofen. The prevailing mechanism for such a process is a Paterno–Büchi cycloaddition to afford oxetanes **3**–**6**; however, the ability of benzophenone to achieve a formal hydrogen abstraction from the methyl group of thymidine has also been evidenced through formation of photoproducts **7** and **8**. These processes provide efficient deactivation pathways for the ketoprofen triplet excited state only in the case of the *cisoid* dyad **1**.

In addition, unambiguous experimental evidence has been obtained which supports the fact that the obtained macrocyclic oxetanes undergo an adiabatic photochemical cycloreversion under direct photolysis to yield the starting dyad (**1**) in its excited triplet state. This rare photochemical process is more efficient than the retro-Paterno–Büchi reaction of the oxetanes obtained from intermolecular photocycloaddition between thymine derivatives and benzophenone.

Experimental Section

Materials: (*S*)-Ketoprofen [(*S*)-2-(3-benzoylphenyl)propionic acid, (*S*)-KP], thymidine (dThd), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDAC) were obtained from commercial sources. Acetonitrile and methanol (HPLC grade) were used without further purification.

Analytical instrumentation: UV spectra were recorded on a UV/Vis scanning spectrophotometer with a slit width of 5 nm. NMR spectra were recorded with a 300 MHz instrument. Bruker Avance 400/500 spectrometers were used for NOE and two-dimensional NMR experiments; transient NOE effects were recorded with a mixing time of 500 ms. For HPLC/MS analyses, the API-ES positive ionization was used for mass detection and MeOH/H_2O (50:50) as the eluent for separation. The identity of the compounds was confirmed by fast-atom bombardment (FAB) and electronic impact (EI) recorded in a high-resolution mass spectrometer (HRMS).

Nanosecond laser-flash photolysis: A pulsed Nd:YAG laser was used for excitation at 355 or 266 nm. The single pulses had a duration of about 10 ns and the energy was about 10 and 4 mJ per pulse, respectively. A pulsed xenon lamp was employed as the detecting light source. The laser flash-photolysis apparatus consisted of a pulsed laser, a Xe lamp, a monochromator, and a photomultiplier tube (PMT) system. The output signal from the oscilloscope was fed to a personal computer. The substrate concentration was about 1.5 mM and ≈0.1 mM for irradiation at 355 nm and 266 nm, respectively. All solutions were deaerated by bubbling nitrogen. These values ensured an absorbance of ≈0.2 in the laser cell at the excitation wavelength.

Synthesis and characterization of the dyads: Compounds **1** and **2** were obtained by condensation of the photosensitizing benzophenone-containing drug (*S*)-KP with dThd and a carbodiimide (EDAC) as the activating agent. Both dyads were fully characterized by ^{1}H NMR, ^{13}C NMR, and mass spectrometry (see the spectra in the Supporting Information, S2 and S3).

Synthesis of compounds 1 and 2: Thymidine (dThd) (0.30 g, 1.23 mmol) and (*S*)-ketoprofen [(*S*)-KP] (0.27 g, 1.19 mmol) were dissolved in anhydrous pyridine (2 mL). EDAC (0.23 g, 1.19 mmol) was slowly added at 0 °C under constant stirring. When the addition was complete, the reaction mixture was kept at 0 °C for 2 h. After this time, the reaction mixture was concentrated under reduced pressure to remove the pyridine. The resulting viscous material was resuspended in CH_2Cl_2 (25 mL) and

washed with an aqueous solution of $NaHCO_3$ (1 M) to remove the unreacted dThd and (*S*)-KP. The organic phase was dried with anhydrous Na_2SO_4, and the solvent was evaporated to dryness under reduced pressure. The residue was submitted to column chromatography (silica gel, $CHCl_3/CH_3OH$ 10:1, v/v) to afford the corresponding 5′-ester **1** (55%) and 3′-ester **2** (16%).

*S-Ketoprofen, 5′-ester with thymidine (**1**)*: Yield 55%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.57 (br s, 1H, NH), 7.85–7.10 (m, 10H, Ar-H + HC=), 6.20 (t, J = 6.9 Hz, 1H, H1′), 4.55 (dd, J = 12.1, 4.0 Hz, 1H, H5′), 4.22 (dd, J = 12.1, 3.3 Hz, 1H, H5′), 4.20–4.10 (m, 2H, H3′ + H4′), 3.85 (q, J = 7.1 Hz, 1H, C*H*-CH_3), 3.02 (br s, 1H, OH), 2.25 (m, 1H, H2″), 1.90 (s, 3H, =C−CH_3), 1.74 (m, 1H, H2′), 1.58 ppm (d, J = 7.1 Hz, 3H, CH-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 196.4 (C), 174.0 (C), 164.0 (C), 150.4 (C), 140.6 (C), 138.0 (C), 137.0 (C), 135.2 (CH), 132.8 (CH), 131.3 (CH), 129.9 (CH), 129.3 (CH), 128.6 (CH), 128.5 (CH), 128.3 (CH), 110.9 (C), 85.1 (CH), 84.3 (CH), 71.2 (CH), 64.3 (CH_2), 45.4 (CH), 39.9 (CH_2), 18.4 (CH_3), 12.6 ppm (CH_3); HRMS (EI) calcd for: $C_{26}H_{26}O_7N_2$ 478.1740; found 478.1750.

*S-Ketoprofen, 3′-ester with thymidine (**2**)*: Yield 16%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.85 (br s, 1H, NH), 7.80–7.40 (m, 10H, Ar-H + HC=), 6.20 (dd, J = 8.2, 6.1 Hz, 1H, H1′), 5.40 (m, 1H, H3′), 3.95–3.75 (m, 4H, H4′ + 2×H5′ + C*H*-CH_3), 3.33 (br s, 1H, OH), 2.55–2.30 (m, 2H, H2′ + H2″), 1.85 (s, 3H, =C−CH_3), 1.56 ppm (d, J = 7.1 Hz, 3H, CH-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 196.0 (C), 173.7 (C), 163.8 (C), 150.5 (C), 140.2 (C), 138.0 (C), 137.2 (C), 136.6 (CH), 131.3 (CH), 130.2 (CH), 129.9 (CH), 129.3 (CH), 129.0 (CH), 128.7 (CH), 128.4 (CH), 111.3 (C), 86.6 (CH), 84.8 (CH), 75.2 (CH), 62.0 (CH_2), 45.0 (CH), 36.0 (CH_2), 18.3 (CH_3), 12.1 ppm (CH_3); HRMS (EI) calcd. for $C_{26}H_{26}O_7N_2$: 478.1740; found 478.1721.

Steady-state photolysis of 1 and 2 and characterization of the photoproducts

Steady-state photolysis of 1 and 2: A 2.63×10^{-3} M solution of the dyad (**1** or **2**) in acetonitrile was placed into a Pyrex tube surrounding a centrally positioned quartz cooling jacket containing a 125 W medium-pressure Hg lamp. The solution was degassed for 30 min with a stream of argon and then irradiated for 5.5 h. After evaporation of the solvent, the residue was submitted to reverse phase HPLC on a RP-18 column (water/acetonitrile (40:60), isocratic solvent). Six photoproducts were separated in the case of **1**. All of them were fully characterized by ^{1}H NMR, ^{13}C NMR, mass spectrometry and, in the case of oxetane **4**, x-ray diffraction (see the spectra in the Supporting Information, S4–S9). CCDC-266772 contains 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.

Identification of photoproducts:

*Oxetane **3***: Yield 19%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.03 (s, 1H, Ar-H), 7.40–6.90 (m, 9H, Ar-H + NH), 5.92 (dd, J = 9.4, 5.1 Hz, 1H, H1′), 5.36 (s,1H, H6), 4.70 (dd, J = 12.7, 1.4 Hz, 1H, H5′), 4.46 (dd, J = 12.7, 1.9 Hz, 1H, H5′), 4.40 (m, 1H, H3′), 4.10 (m, 1H, H4′), 3.93 (q, J = 7.2 Hz, 1H, C*H*-CH_3), 2.20 (m, 1H, H2″), 2.05 (br s, 1H, OH), 1.80 (m, 1H, H2′), 1.77 (s, 3H, C−CH_3), 1.61 ppm (d, J = 7.2 Hz, 3H, CH-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.1 (C), 170.0 (C), 150.6 (C), 144.4 (C), 139.4 (C), 137.6 (C), 129.1 (CH), 128.4 (CH), 128.3 (CH), 126.5 (CH), 126.2 (CH), 125.3 (CH), 124.9 (CH), 92.0 (C), 84.6 (CH), 83.6 (CH), 77.2 (C, peak observed in the long-range HC correlation), 70.7 (CH), 63.4 (CH_2), 61.0 (CH), 46.3 (CH), 40.7 (CH_2), 22.9 (CH_3), 19.1 ppm (CH_3); HRMS (FAB) calcd. for $C_{26}H_{26}O_7N_2$: 478.1740; found 478.1750.

*Oxetane **4***: Yield: 14%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.08 (s, 1H, Ar-H), 7.70–6.90 (m, 9H, Ar-H + NH), 6.38 (t, J = 7.5 Hz, 1H, H1′), 4.81 (dd, J = 12.3, 1.5 Hz, 1H, H5′), 4.74 (s, 1H, H6), 4.40 (dd, J = 12.3, 2.0 Hz, 1H, H5′), 4.35 (br s, 1H, H3′), 4.00–3.94 (m, 1H, H4′), 3.87 (q, J = 7.2 Hz, 1H, C*H*-CH_3), 2.20 (m, 2H, H2′ + H2″), 1.80 (br s, 1H, OH), 1.75 (s, 3H, C−CH_3), 1.55 ppm (d, J = 7.2 Hz, 3H, CH-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.6 (C), 168.8 (C), 151.2 (C), 145.8 (C), 139.4 (C), 138.6 (C), 128.9 (CH), 128.7 (CH), 128.2 (CH), 128.1 (CH), 125.1 (CH), 123.0 (CH), 121.8 (CH), 91.2 (C), 82.9 (CH), 82.0 (CH), 77.6 (C, peak observed in the long-range HC correlation), 70.0 (CH), 61.3 (CH_2), 60.8 (CH), 45.2 (CH), 37.1 (CH_2), 22.3 (CH_3), 17.8 ppm (CH_3); HRMS (FAB) calcd. for $C_{26}H_{26}O_7N_2$: 478.1740; found 478.1751.

*Oxetane **5***: Yield: 10%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.07 (s, 1H, Ar-H), 7.65–7.15 (m, 9H, Ar-H + NH), 6.10 (dd, J = 6.6, 4.2 Hz, 1H, H1′), 5.70 (s, 1H, H6), 4.68 (dd, J = 12.3, 2.4 Hz, 1H, H5′), 4.66 (m, 1H, H3′), 4.33 (dd, J = 12.3, 1.2 Hz, 1H, H5′), 4.02 (m, 1H, H4′), 3.93 (q, J = 7.2 Hz, 1H, C*H*-CH_3), 2.72 (br s, 1H, OH), 2.51 (m, 2H, H2′ + H2″), 1.60 (d, J = 7.2 Hz, 3H, CH-CH_3), 1.07 ppm (s, 3H, C−CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 175.6 (C), 170.6 (C), 150.5 (C), 140.7 (C), 139.4 (C), 138.9 (C), 129.2 (CH), 128.5 (CH), 128.3 (CH), 127.8 (CH), 127.6 (CH), 126.7 (CH), 125.0 (CH), 87.7 (C), 83.8 (CH), 83.1 (CH), 79.8 (CH), 68.9 (CH), 63.7 (CH_2), 57.8 (C), 47.5 (CH), 41.0 (CH_2), 20.1 (CH_3), 17.6 ppm (CH_3); HRMS (EI) calcd. for $C_{26}H_{26}O_7N_2$: 478.1740; found 478.1708.

*Oxetane **6***: Yield: 9%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.57 (s, 1H, Ar-H), 7.65–7.20 (m, 9H, Ar-H + NH), 6.40 (t, J = 6.7 Hz, 1H, H1′), 5.28 (s, 1H, H6), 4.50 (dd, J = 12.8, 2.7 Hz, 1H, H5′), 4.29 (m, 1H, H4′), 4.20 (m, 1H, H3′), 4.10 (q, J = 6.9 Hz, 1H, C*H*-CH_3), 4.05 (dd, J = 12.8, 7.5 Hz, 1H, H5′), 2.48 (m, 2H, H2″ + OH), 1.72 (m, 1H, H2′), 1.61 (d, J = 6.9 Hz, 3H, CH-CH_3), 1.17 ppm (s, 3H, C−CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 175.1 (C), 170.6 (C), 150.7 (C), 139.5 (C), 139.0 (C), 138.7 (C), 130.2 (CH), 129.3 (CH), 129.0 (CH), 128.5 (CH), 127.9 (CH), 126.3 (CH), 125.0 (CH), 86.8 (C), 84.8 (CH), 83.5 (CH), 81.9 (CH), 71.1 (CH), 64.3 (CH_2), 57.8 (C), 43.6 (CH), 40.6 (CH_2), 16.7 (CH_3), 14.0 ppm (CH_3); HRMS (EI) calcd for $C_{26}H_{26}O_7N_2$ 478.1740; found 478.1753.

*Compound **7***: Yield: 6%; ^{1}H NMR (300 MHz, CD_3OD): δ = 7.64–7.10 (m, 10H, Ar-H + NH), 6.54 (s, 1H, H6), 6.10 (dd, J = 9.3, 5.7 Hz, 1H, H1′), 4.80 (dd, J = 12.9, 1.8 Hz, 1H, H5′), 3.95–3.90 (m, 3H, H3′ + H4′ + H5′), 3.73 (q, J = 7.2 Hz, 1H, C*H*-CH_3), 3.58 (d, J = 14.1 Hz, 1H, CH_2), 3.34 (d, J = 14.1 Hz, 1H, CH_2), 1.76 (ddd, J = 13.5, 5.7, 1.5 Hz, 1H, H2″), 1.47 (d, J = 7.2 Hz, 3H, CH-CH_3), 0.77 ppm (ddd, J = 13.5, 9.3, 6.3 Hz, 1H, H2′); ^{13}C NMR (75 MHz, CD_3OD): δ = 175.7 (C), 167.2 (C), 151.6 (C), 149.1 (C), 147.8 (C), 141.6 (C), 138.9 (CH), 130.3 (CH), 130.0 (CH), 129.2 (CH), 128.1 (CH), 127.3 (CH), 125.7 (CH), 124.9 (CH), 111.7 (C), 86.7 (CH), 84.7 (CH), 79.7 (C), 71.5 (CH), 64.6 (CH_2), 46.7 (CH), 41.8 (CH_2), 37.6 (CH_2), 17.5 ppm (CH_3); HRMS (EI) calcd for $C_{26}H_{26}O_7N_2$ 478.1740; found 478.1753; HRMS (FAB) calcd. for $C_{26}H_{26}O_7N_2$ 478.1740; found 478.1760.

*Compound **8***: Yield: 8%; ^{1}H NMR (300 MHz, CD_3OD): δ = 7.60–7.10 (m, 10H, Ar-H + NH), 6.67 (s, 1H, H6), 6.14 (dd, J = 8.2, 6.0 Hz, 1H, H1′), 4.31 (dd, J = 12.3, 7.5 Hz, 1H, H5′), 4.10–3.90 (m, 3H, H3′ + H4′ + H5′), 3.80 (q, J = 6.9 Hz, 1H, C*H*-CH_3), 3.40 (m, 2H, CH_2), 2.12 (ddd, J = 13.8, 6.0, 2.7 Hz, 1H, H2″), 1.73 (ddd, J = 13.8, 8.2, 6.0 Hz, 1H, H2′) 1.39 ppm (d, J = 6.9 Hz, 3H, CH-CH_3); ^{13}C NMR (75 MHz, CD_3OD): δ = 176.1 (C), 167.7 (C), 151.6 (C), 149.1 (C), 147.3 (C), 142.0 (C), 140.0 (CH), 129.7 (CH), 129.0 (CH), 128.0 (CH), 127.4 (CH), 127.2 (CH), 126.2 (CH), 126.1 (CH), 111.7 (C), 85.6 (CH), 84.9 (CH), 79.6 (C), 71.5 (CH), 64.6 (CH_2), 46.6 (CH), 39.9 (CH_2), 39.6 (CH_2), 18.3 ppm (CH_3); HRMS (EI) calcd for $C_{26}H_{26}O_7N_2$ 478.1740; found 478.1739.

The stereochemistry of oxetanes **3**–**6** and reduced cyclic photoproducts **7** and **8** was assigned by means of nuclear Overhauser effect (NOE) experiments. On the other hand, the ^{1}H and ^{13}C signals were assigned by a combination of H,H (COSY) and H,C (edited HSQC) correlations, along with the NOE results. Quaternary carbons were assigned by long-range H,C correlation (HMBC).

Determination of the photolysis quantum yields: Solutions (A_{254} = 0.4) of the oxetanes **3**, BP-dThd, and BP-DMT in acetonitrile/water (4:1) under N_2 were irradiated at λ = 254 nm with a low-pressure Hg lamp. The UV absorption of the carbonyl band at λ = 254 nm was measured after different irradiation times (0, 2, 3, 5, 7, and 10 s). The same procedure was used for the irradiation of solutions ($A_{350}\approx0.05$) of dyad **1** or equimolar mixtures of BP with dThd and BP with DMT in acetonitrile/water (4:1) with a lamp emitting mainly at λ = 350 nm (Gaussian distribution).

The photolysis quantum yields were then obtained for each compound by plotting the absorbance at 254 nm against the irradiation time, from the slopes of each linear fitting. The quantum yield of 0.5 for the photolysis of oxetane BP-DMT in acetonitrile was used as a standard.

Acknowledgements

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