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Two-Photon Chemistry from Upper Triplet States of Thymine

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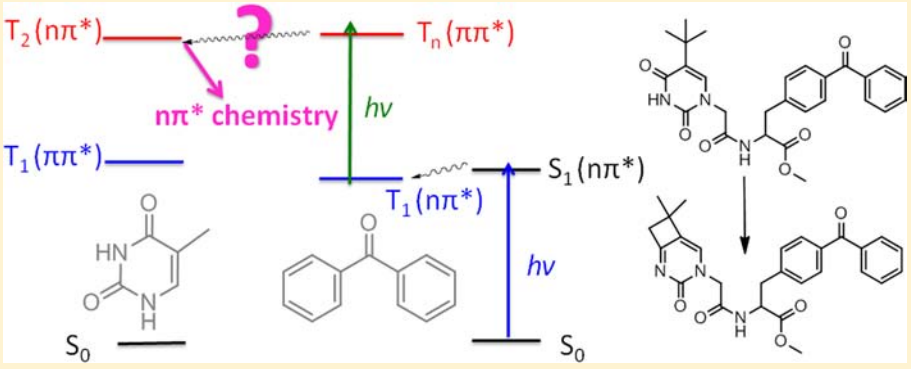
Supporting Information



ABSTRACT: Photolysis of the benzophenone chromophore by means of high energy laser pulses has been used as a tool to populate upper thymine-like triplet states via intramolecular sensitization. These species undergo characteristic nπ* triplet photoreactivity, as revealed by the Norrish–Yang photocyclization of 5-*tert*-butyluracil.

INTRODUCTION

Understanding the photochemistry of thymine (Thy) is essential to explain the processes involved in DNA damage. In this context, intensive effort has been devoted to develop complementary approaches based on the isolation and characterization of photoproducts, photophysical studies, and theoretical calculations.[1−4] As a result, involvement of the Thy triplet excited state, populated either intrinsically or through photosensitization, has been highlighted and closely related to the formation of dimeric lesions.[2] The well-known cyclobutane dimers are obtained through [2 + 2] photocycloaddition between the C5−C6 double bonds of two Thy bases, which can occur from both the lowest singlet and triplet excited states. A rare type of dimeric products are the 5-thyminyl-5,6-dihydrothymine adducts, the so-called spore photoproducts. They are assumed to arise from a Thy triplet excited state, based on steady-state photosensitization studies.[5,6] The (6-4) photoproducts (6-4PPs) are important lesions resulting from a Paternò–Büchi reaction between the C4 carbonyl group of one Thy and the C5−C6 double bond of another one, followed by oxetane ring opening. Interestingly, the 6-4PPs are exclusively formed upon direct UV irradiation, in sharp contrast with cyclobutane and spore photoproducts.

Absorption of UV light by Thy populates the singlet manifold;[7] subsequent intersystem crossing leads ultimately to the lowest triplet state (T_1), which is of ππ* nature. According to the generally accepted paradigm, 6-4PPs formation occurs from an excited singlet state, upon direct UVB irradiation.[8] This would be consistent with the unsuccessful photosensitization attempts; however, the Paternò–Büchi photocycloaddition is a characteristic nπ* triplet process, together with hydrogen abstraction and the Norrish family of photoreactions.[9]

Thus, it appears feasible that some of the assumed "singlet" Thy photoreactivity proceeds instead from an upper triplet excited state with the appropriate electronic configuration. Actually, the T_2 (nπ*) triplet of Thy is located ca. 35 kJ mol^{-1} below S_1 and can be efficiently reached by intersystem crossing.[10,11] The relatively large T_1−T_2 energy gap (ca. 100 kJ mol^{-1})[11,12] would open the channel for chemical reaction pathways from T_2, in competition with internal conversion to T_1 (Scheme 1).

With this background, the aim of the present work was to explore the photochemistry of Thy from upper nπ* triplet excited states, through the Norrish–Yang photocyclization of dyad **1a** (Scheme 2). This model compound contains a benzophenone (BP) chromophore and a 5-*tert*-butyluracil (*t*-Ura) moiety, covalently linked by an aliphatic amide spacer. The *t*-Ura substructure presents γ-hydrogens prone to be abstracted by the neighboring C4 carbonyl group. Indeed, UVC excitation of *t*-Ura gives rise to 1,2-dihydrocyclobuta[*d*]-pyrimidine-2-one after dehydration of a cyclobutanol intermediate.[13,14] To investigate a possible Norrish–Yang process from the T_2 (nπ*) state, triplet photosensitization is needed. In

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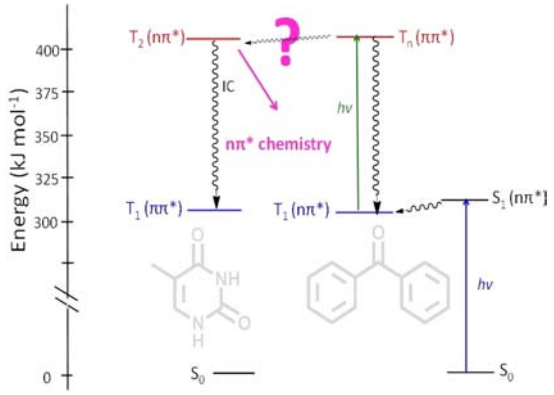
Scheme 1. Energy Diagram Showing the Key Triplet Excited States of the Thy and BP Chromophores



this approach, an experimental challenge is to find a sensitizer that can be selectively excited at wavelengths longer than 300 nm to reach a highly energetic triplet state (>400 kJ mol^{-1}).[11,12] In this context, BP is a good candidate since its T_n has an energy of 403 kJ mol^{-1} and can be generated by multiphoton excitation at 355 nm.[15,16] Nonetheless, the relatively short lifetime of this excited state (37 ps)[16] would require unrealistic high *t*-Ura concentrations for detectable intermolecular triplet−triplet energy transfer (TTET).[15,17−19] This problem could be circumvented in the analogous intramolecular process, as intended in the *t*-Ura-BP dyad **1a**.

EXPERIMENTAL SECTION

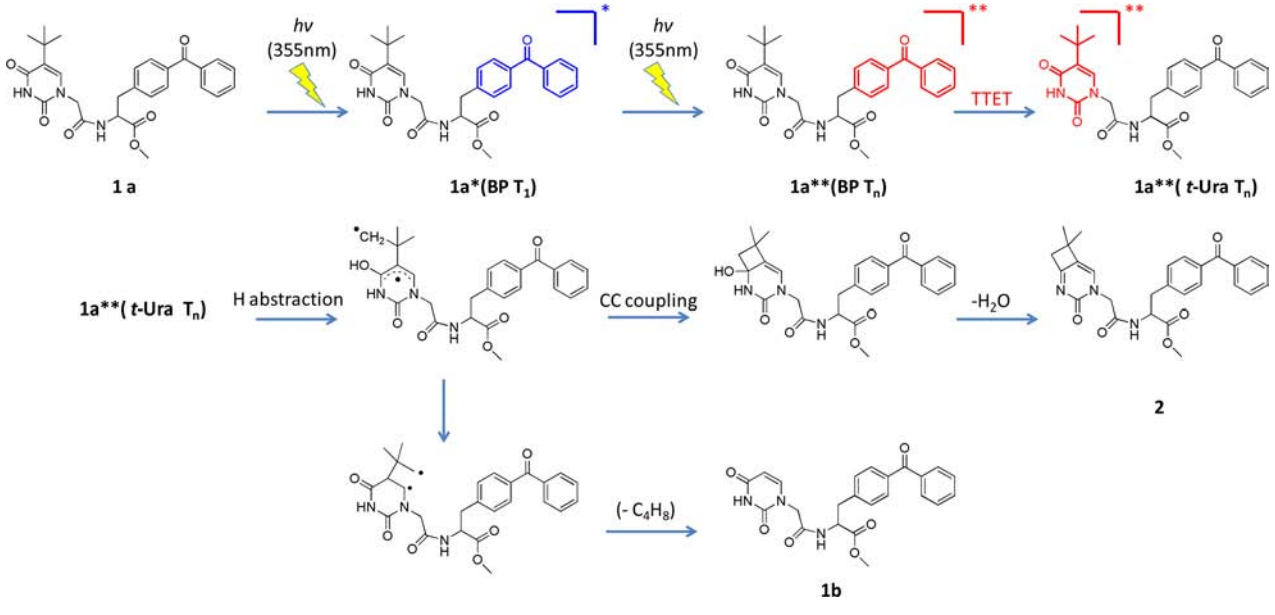
General. Methanol, acetonitrile (HPLC grade), chloroform, ethyl acetate, dimethylformamide, and dichloromethane were acquired from commercial sources. Sulfuric acid, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC), 4-dimethylaminopyridine (DMAP), *o*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyl uranium tetrafluoroborate (TBTU) and *N*,*N*-diisopropylethylamine (DIEA) were purchased from Sigma and used as received. 4-Benzoylphenylalanine was purchased from Bachem. A Bruker Avance 300 spectrometer was used for the NMR experiments.

Synthetic Details. *Synthesis of the Methyl Ester of 4-Benzoylphenylalanine.* To a stirred solution of 4-benzoylphenylalanine (1.0 g, 3.7 mmol) in 20 mL of MeOH was added 200 μL of H_2SO_4. The reaction mixture was refluxed overnight and concentrated to dryness. The residue was purified by silica gel column chromatography with $CHCl_3$/MeOH 9.8:0.2 as eluent, affording a yellow oil (0.7 g, 2.5 mmol). Yield: 68%; ^{1}H NMR (300 MHz, CD_3OD) δ 7.85−7.16 (m, 9H), 3.87−3.76 (m, 1H), 3.71 (s, 3H), 3.14 (dd, J = 13.5, 6.0 Hz, 1H), 3.03 (dd, J = 13.5, 7.1 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 198.2, 176.0, 144.0, 138.9, 137.3, 133.7, 131.3, 130.9, 130.5, 129.5, 56.5, 52.5 and 41.6; HRMS (ESI) m/z calcd for $C_{17}H_{17}NO_3$ [MH^+] 284.1287, found 284.1300.

*Synthesis of Methyl 3-(4-Benzoylphenyl)-2-[5-(tert-butyluracil-1-yl)acetamido]propanoate (**1a**).* 5-*tert*-Butyluracil-1-acetic acid was prepared and purified according to the previously reported procedures.[20,21] To a stirred solution of this compound (0.5 g, 2.2 mmol) in CH_2Cl_2 was added EDC (0.4 mL, 2.2 mmol) at 0 °C. The reaction mixture was stirred for 0.5 h at 0 °C. Then, the methyl ester of 4-benzoylphenylalanine (0.6 g, 2.2 mmol) and DMAP (0.1 equiv) dissolved in CH_2Cl_2 were added dropwise to the solution, at 0 °C. The reaction mixture was stirred overnight at room temperature. The solvent was evaporated under reduced pressure and the residue redissolved in EtOAc (20 mL). The organic layer was washed consecutively with 1 N HCl, 5% $NaHCO_3$, and brine. Then, the solution was dried over $MgSO_4$ and evaporated under reduced pressure. The final product was purified by silica gel column chromatography using EtOAc/hexane 8:2 as eluent. Compound **1a** was obtained as a white solid (0.7 g, 1.4 mmol). Yield: 65% ; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.98 (s, 1H), 7.79−7.43 (m, 7H), 7.24−7.12 (m, 2H), 6.98 (s, 1H), 4.90 (dt, J = 7.7, 6.0 Hz, 1H), 4.40 (d, J = 15.6 Hz, 1H), 4.26 (d, J = 15.6 Hz, 1H), 3.74 (s, 3H), 3.32−3.10 (m, 2H), 1.25 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.4, 171.5, 166.7, 162.7, 151.0, 140.7, 139.5, 137.6, 136.6, 132.6, 130.5, 130.1, 129.4, 128.5, 123.0, 53.5, 52.8, 50.9, 37.8, 33.1 and 28.8; HRMS (ESI) m/z calcd for $C_{27}H_{30}N_3O_6$ [MH^+] 492.2144, found 492.2135.

*Synthesis of Methyl 3-(4-Benzoylphenyl)-2-(2-uracil-1-acetamido)propanoate (**1b**).* Uracil-1-acetic acid was prepared and purified according to the previously reported procedures.[20,21] To a stirred solution of this compound (0.3 g, 1.8 mmol) in CH_2Cl_2 was added EDC (0.3 mL, 1.8 mmol) at 0 °C. The reaction mixture was stirred for 0.5 h at 0 °C. The methyl ester of 4-benzoylphenylalanine (0.5 g, 1.8 mmol) and DMAP (0.1 equiv) dissolved in CH_2Cl_2 were added dropwise to the solution, at 0 °C. The reaction mixture was stirred overnight at room temperature. The organic layer was washed

Scheme 2. Photochemical Steps Involved in Formation of Two-Photon Photoproducts upon Laser Irradiation of Dyad 1a



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consecutively with 1 N HCl, 5% $NaHCO_3$, and brine. Then, the solution was dried over $MgSO_4$ and evaporated under reduced pressure. The final product was purified by silica gel column chromatography using EtOAc/MeOH 100:1 as eluent. Compound **1b** was obtained as a white solid (0.1 g, 0.22 mmol). Yield 12%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78−7.45 (m, 8H), 7.22 (d, J = 8.1 Hz, 1H), 7.16 (d, J = 8.1 Hz, 1H), 5.71 (d, J = 6.5, 1H), 4.91 (m, 1H), 4.40 (d, J = 15.6 Hz, 1H), 4.23 (d, J = 15.6 Hz, 1H), 3.77 (s, 3H), 3.28 (dd, J = 6.0, 14.0 Hz, 1H), 3.16 (d, J = 4.0, 14.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.6, 171.7, 166.6, 164.0, 151.2, 145.3, 141.0, 137.4, 136.4, 132.6, 130.4, 130.1, 129.4, 128.4, 102.4, 53.4, 52.8, 50.3 and 37.7; HRMS (ESI) m/z calcd for $C_{23}H_{22}N_3O_6$ [MH^+] 436.1509, found 436.1490.

Synthesis of (7,7-Dimethyl-3-oxo-2,4-diazabicyclo[4.2.0]octa-1,5-dien-4-yl)acetic Acid. 5-*tert*-Butyluracil-1-acetic acid (0.2 g, 0.9 mmol) in acetonitrile (500 mL) was irradiated at 254 nm for 4 h with a low-pressure mercury lamp. The course of the reaction was monitored by UV−vis spectrophotometry. After 4 h, the new band absorbing at 315 nm corresponding to the pyrimidone chromophore reached its maximum absorbance. At this point, the irradiated solution was evaporated under vacuum. Thus, a yellow oil was obtained (0.2 g, 0.9 mmol) and subsequently used in the next synthetic step without further purification. Yield: 99%. The compound was characterized as its methyl ester. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.70 (s, 1H), 4.58 (s, 2H), 3.70 (s, 3H), 3.13 (s, 2H), 1.40 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 184.4, 171.2, 154.8, 142.3, 130.8, 54.8, 54.5, 52.6, 42.9 and 29.4; HRMS (ESI) m/z calcd for $C_{11}H_{15}N_2O_3$ [MH^+] 223.1075, found 223.1083.

*Synthesis of Methyl 3-(4-Benzoylphenyl)-2-[(7,7-dimethyl-3-oxo-2,4-diazabicyclo[4.2.0]octa-1,5-dien-4-yl)acetamido]propanoate (**2**).* To (7,7-dimethyl-3-oxo-2,4-diazabicyclo[4.2.0]octa-1,5-dien-4-yl)-acetic acid (0.2 g, 1.0 mmol) in 2 mL of anhydrous DMF was added a solution of the methyl ester of 4-benzoylphenylalanine (0.3 g, 1.0 mmol), TBTU (1.1 mmol), and DIEA (2.7 mmol) in 2 mL of anhydrous dimethylformamide. The reaction was stirred at room temperature for 2 h and subsequently quenched by addition of water (4 mL). Then, the reaction mixture was extracted with toluene (2 × 6 mL), washed with water (2 × 6 mL), dried over anhydrous magnesium sulfate, and evaporated. Further purification by silica gel column chromatography allowed obtaining 2 as yellowish oil (0.3 g, 0.7 mmol). Yield 69%; ^{1}H NMR (300 MHz, CD_3CN) δ 7.80−7.64 (m, 5H), 7.57 (t, J = 6.0 Hz, 2H), 7.37 (d, J = 9.0 Hz, 2H), 7.26 (s, 1H), 7.20 (d, J = 60 Hz, 1H), 4.80−4.73 (m, 1H), 4.43 (d, J = 15 Hz, 1H), 4.35 (d, J = 15 Hz, 1H), 3.70 (s, 3H), 3.25 (dd, J = 15.0, 6.0 Hz, 1H), 3.16−3.10 (m+s, 3H), 1.43 (s, 6H); ^{13}C NMR (75 MHz, CD_3CN) δ 195.6, 181.8, 171.0, 166.7, 157.7, 141.5, 138.8, 137.3, 135.7, 132.1, 129.6, 129.4, 129.2, 128.5, 128.1, 53.2, 52.9, 51.6, 49.9, 40.7, 36.7 and 25.7; HRMS (ESI) m/z calcd for $C_{27}H_{28}N_3O_5$ [MH^+] 474.2029, found 474.2023.

Instrumental Methods. *UPLC−MS/MS Analysis.* Liquid chromatography was performed on an ACQUITY UPLC system (Waters Corp.) with a conditioned autosampler at 4 °C. The separation was carried out on an ACQUITY UPLC BEH C18 column (50 mm × 2.1 mm i.d., 1.7 μm). The column temperature was maintained at 40 °C. The analysis was achieved using methanol/water (containing 0.01% formic acid) 35:65 v/v as the mobile phase with a flow rate of 0.5 mL min^{-1}. The injection volume was 1 μL. A Waters ACQUITY XevoQToF Spectrometer (Waters Corp.) was connected to the UPLC system via an electrospray ionization (ESI) interface. The ESI source was operated in positive ionization mode with the capillary voltage at 1.5 kV. The temperature of the source and desolvation was set at 110 and 300 °C, respectively. The cone and desolvation gas flows were 100 L h^{-1} and 800 L h^{-1}, respectively. The collision gas flow was 0.2 mL/min and collision energy of 15 or 18 V was applied. All data collected in Centroid mode were acquired using Masslynx software (Waters Corp.). Leucine-enkephalin was used as the lock mass generating an [MH^+] ion (m/z 556.2771) at a concentration of 500 pg mL^{-1} and flow rate of 50 μL min^{-1} to ensure accuracy during the MS analysis.

HPLC Analysis. A Varian system formed by a 9012Q pump and a photodiode array (Varian 9065) and equipped with a Mediterranean Sea C18 column (5 μm, 25 cm × 0.46 cm) was employed to analyze the irradiated samples. Acetonitrile/water-TFA 0.1% 40:60 was used as eluent with 0.7 mL of flow, and chromatograms were registered with a detection wavelength of 320 nm.

Laser Flash Photolysis. Pulsed Nd:YAG Laser system instrument using 355 nm as excitation wavelength, beam diameter of 0.6 cm, and pulse duration of 10 ns pulse^{-1} was used. The energy was set at 15 mJ pulse^{-1}. The apparatus consisted of the pulsed laser, the Xe lamp, a monochromator, and a photomultiplier. The output signal from the oscilloscope was transferred to a personal computer. Quartz cells of 1 cm optical path length were employed for the measurements.

Two-Color Two-Laser Flash Photolysis. Acetonitrile solutions of **1a** were purged with Ar in a quartz cell. The absorbance of the samples was adjusted to be 1.1 at 308 nm for a 1 cm path length. A XeCl excimer laser (308 nm, Lambda Physik, Lextra 50) and the second harmonics (532 nm) of a Nd:YAG laser (Lotis TII, LT-2137) were used as the excitation light source with a repetition rate of 1 Hz. The delay time between the 308 and 532 nm laser pulses was 100 ns. The laser beams were perpendicularly crossed at the center of the sample where the monitoring light from a Xe lamp with a cutoff filter (λ > 470 nm) passed to a detection system. The transient signal was analyzed by the least-squared best-fitting method. The intensities of the incident 308 and 532 nm laser pulses were 30 mJ pulse^{-1} and 132 mJ pulse^{-1}, respectively. Upon 308 nm laser pulsing the sample with the laser power, the absorbance change generated at 532 nm was 0.41 at 100 ns. For photoproduct analysis, 3600 dual laser pulses were beamed into the sample.

Sample Irradiations. Solutions of **1a** with an absorbance of 0.3 at 355 nm were prepared using acetonitrile as a solvent and bubbled with N_2, prior to irradiation in quartz cuvettes.

Multiphoton Irradiation. Excitation of the samples (300 shots) was performed using the laser apparatus described above setting the pulse energy at 40 mJ pulse^{-1}. A control experiment was run with energy per pulse of 15 mJ.

Steady-State Monochromatic Irradiation. A Microbeam system (model L-201) including a Xe lamp (150 W) equipped with a monochromator (model 101) was employed with this purpose. The samples were irradiated for 2.5 h.

*Quadratic Dependence of the Yield of **2** on Laser Dose.* Samples were irradiated (300 shots) using different laser pulses ranging from 12 to 41 mJ pulse^{-1}. The photomixtures were concentrated (50×) and submitted to HPLC analysis to establish **2** formation yield upon irradiation. This was accomplished by comparison with a calibration curve constructed by employing authentic samples of **2** at different concentrations.

RESULTS AND DISCUSSION

The UV−vis absorption spectrum of **1a** exhibits a band centered at ca. 260 nm and another one peaking at ca. 340 nm. Irradiation of the long wavelength band would lead to selective excitation of the BP chromophore. Laser flash photolysis of **1a** in acetonitrile at 355 nm showed the well-known BP triplet−triplet transient absorption spectrum, with two maxima centered at ca. 330 and 530 nm (Figure 1, top). No ketyl radical formation was observed (see Figure S9 of Supporting Information), which rules out an intramolecular hydrogen abstraction from the *tert*-butyl group by the BP triplet excited state. Under the same conditions, the triplet lifetime of **1a** was shorter than that of BP (1.4 μs vs 2.0 μs); the observed intramolecular quenching (k = 2 × 10^5 s^{-1}) reflects the interaction between the BP and *t*-Ura units of dyad **1a**. It is worth noting that high energy irradiation at 355 nm is appropriate to excite both the ground and the triplet excited state of BP (Figure 1, top). The molar absorption coefficient of the latter is 2 orders of magnitude larger, which would

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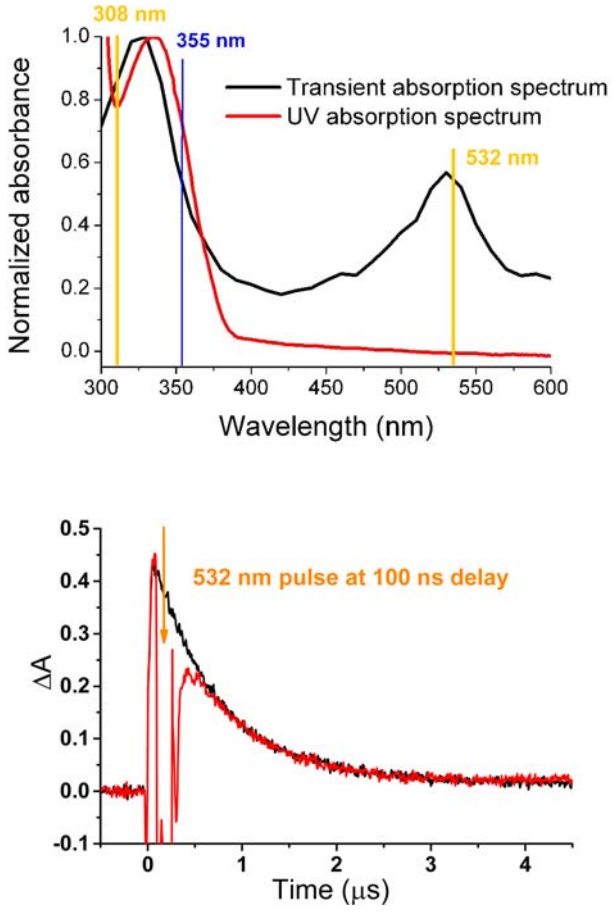

Figure 1. (Top) Normalized UV−vis and transient absorption spectra of **1a** (3×10^{-3} M in acetonitrile) upon laser flash photolysis at 355 nm. (Bottom) Kinetic traces of the transient absorption at 520 nm after the 308 nm laser (black line) and the two-color two-laser (308/532 nm, red line) photolysis of **1a**. The spike at 100 ns is due to the intense scattering of the 532 nm laser light.

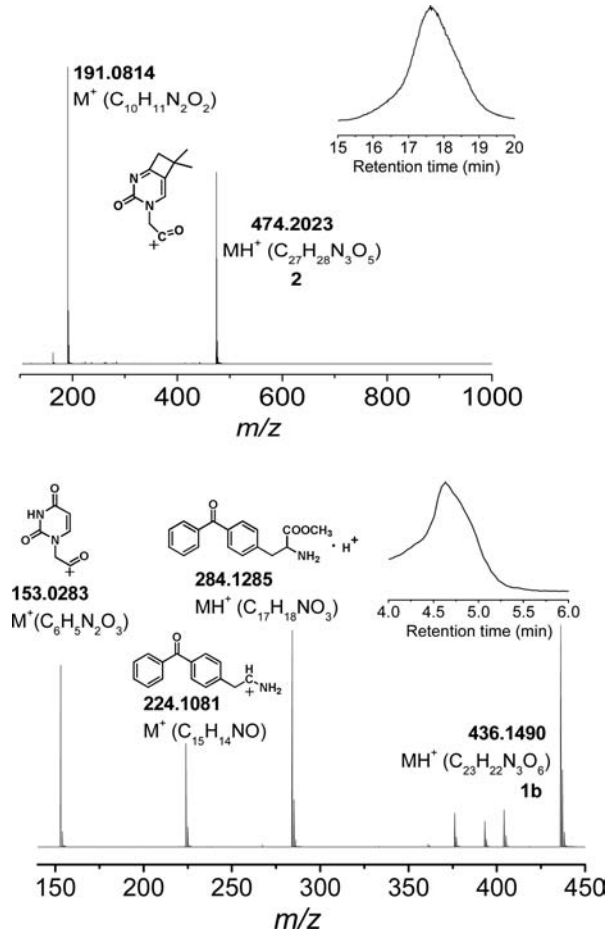

Figure 2. Ion trap mass spectra obtained from UPLC−MS/MS analysis of **1a** after irradiation with a 355 nm laser (300 pulses, 40 mJ $pulse^{-1}$). (Top) detection of photoproduct **2** (at 18 min elution time, collision energy 18 V, parent ion 474.20). Inset: chromatogram registered for the 474.20 → 191.08 transition. (Bottom) detection of photoproduct **1b** (at 4.7 min elution time, collision energy 15 V, parent ion 436.15). Inset: chromatogram registered for the 436.15 → 284.13 transition.

compensate for its lower instantaneous concentration. Thus, during the laser pulse both species should compete for absorption of the exciting light.[17,22]

For product studies, a N_2-deaerated solution of **1a** in acetonitrile (absorbance 0.3 at 355 nm) was submitted to multiphoton excitation using a Nd:YAG laser beam with an energy of 40 mJ per pulse.[23]

The photolyzate was concentrated and analyzed by UPLC coupled with tandem mass spectrometry. As shown in Figure 2, selected ion monitoring revealed the presence of a new compound with its molecular ion MH^+ at m/z 474.2023, corresponding to the formula $C_{27}H_{28}N_3O_5$. It was tentatively assigned to photoproduct **2** (Scheme 2), arising from a Norrish−Yang photocyclization, followed by dehydration of the cyclobutanol intermediate.[13,14] This assignment was unambiguously confirmed by the independent synthesis of **2**, which was achieved by coupling the methyl ester of 4-benzoylphenylalanine with (7,7-dimethyl-3-oxo-2,4-diazabicyclo[4.2.0]octa-1,5-dien-4-yl)acetic acid.

Remarkably, the retention time, the molecular ion, and the fragmentation pattern of the photoproduct were identical to those of the synthesized compound. The MS/MS spectrum showed the characteristic α-cleavage of amides and gave rise to the acyl cation $C_{10}H_{11}N_2O_2^+$ (m/z 191.0814).

The 1,4-biradical intermediate of the Norrish−Yang reaction, after tautomerization, could in principle undergo C−C splitting to afford the unsubstituted uracil **1b** (Scheme 2). As shown in Figure 2, bottom, selected ion monitoring also revealed the possible presence of this compound, with a molecular ion MH^+ at m/z 436.1490, corresponding to the expected formula $C_{23}H_{22}N_3O_6$. The assignment was confirmed by independent synthesis of **1b** through coupling of the methyl ester of 4-benzoylphenylalanine with uracil-1-acetic acid. Again, the retention time, the molecular ion, and the fragmentation pattern of the photoproduct were identical to those of the synthesized compound. The MS/MS spectrum showed ionic fragments compatible with the structure (Figure 2, bottom).

Typically the quantum yields for energy transfer from ultra short-lived upper triplet states are markedly low, due to the competition with efficient internal conversion affording T_1. For instance, the value for energy transfer from the T_n state of BP to solvent CCl_4 is as low as 0.0023.[15] In addition, the quantum yield of the Norrish−Yang cyclization from **1a****(***t*-Ura T_n**) is expectedly low, because the predominating decay pathway must be again internal conversion to the lowest lying triplet. Assuming a lifetime of ca. 50 ps for **1a****(***t*-Ura T_n**) and a

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reaction rate constant in the order of 10^8 M^{-1} s^{-1}, only 1% of this excited state would be productive. Accordingly, taking into account the efficiency of both intramolecular energy transfer and Norrish–Yang reaction, a rough estimation of ca. 10^{-5} seems reasonable for the overall quantum yield of the photoprocess.

As a matter of fact, when the amount of photoproduct obtained after irradiation with 355 nm laser pulses (40 mJ $pulse^{-1}$) was quantified by means of HPLC, using authentic compound **2** for constructing a calibration curve (see Supporting Information), a 0.003% chemical yield was determined. The number of pulses was limited to 300 in order to keep a low conversion and to minimize secondary photolysis of **2**. The amount of cleavage product **1b** was even lower; so it was just enough for UPLC–MS/MS detection and did not allow performing accurate quantitative measurements.

In agreement with the formation of **2** by a biphotonic process, when dyad **1a** was photolyzed at 355 nm by means of a xenon lamp coupled to a monochromator, not even traces of **2** could be observed in the photomixture. In addition, two blank experiments were run in which 5-*tert*-butyluracil-1-acetic acid was irradiated with the 355 nm laser (40 mJ $pulse^{-1}$) alone or in the presence of equimolar amounts of the methyl ester of *N*-acetyl-4-benzoylphenylalanine. No change was observed in the former case, demonstrating that BP photosensitization is indeed needed for the photoreaction to take place. Moreover, in the intermolecular case formation of the Norrish–Yang photoproduct was not observed (excluding the involvement of triplet–triplet annihilation), and cyclobutane dimers were not found.

An attempt was made to obtain kinetic evidence supporting the occurrence of biphotonic processes in the photolysis of **1a** by means of two-laser two-color experiments. Initial excitation was done at 308 nm (XeCl excimer laser), whereas photolysis of the resulting **1a***(**BP** T_1) transient was selectively achieved at 532 nm (Nd:YAG laser). Unfortunately, the results (Figure 1, bottom) did not show a clear irreversible bleaching of the trace, probably because of the low reaction quantum yield (see above) and the limitations imposed by the signal-to-noise ratio.

Product studies were also performed with the two-laser two-color setup, by multiple repetition of the 308/532 sequence. This led to similar results as those obtained with the 355 nm laser photolysis, although formation of **2** was somewhat enhanced, and detection of **1b** was improved.

The key piece of evidence unambiguously demonstrating that formation of photoproduct **2** occurs via a biphotonic process was obtained by monitoring its formation as a function of the laser energy. Samples of **1a** were irradiated using a range of pulse energies from 12 to 41 mJ. The photolyzates were submitted to HPLC analysis, and the yield of photoproduct **2** obtained in each case was quantified by comparison with the calibration curve (see Supporting Information). Figure 3 shows the log–log plot, which yields a slope of 1.91, thus confirming the biphotonic nature of the process.

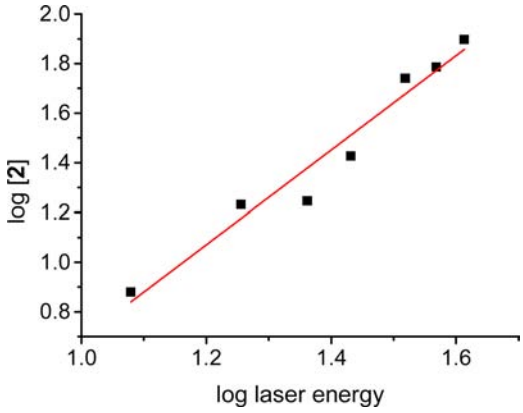

Figure 3. Log–log representation of photoproduct **2** concentration in the irradiated cuvettes (nM) versus laser energy (mJ $pulse^{-1}$).

CONCLUSIONS

In summary, biphotonic excitation of the benzophenone chromophore by means of high energy laser pulses leads to successful intramolecular sensitization, populating an upper thymine-like triplet state. The relatively long lifetime of this species opens the channel for nπ^* triplet chemistry. The feasibility of this concept has been demonstrated by the Norrish–Yang photocyclization of *tert*-butyluracil.

ASSOCIATED CONTENT

Supporting Information

Synthetic scheme of the dyads, ^1H and ^{13}C NMR of each compound, together with the HPLC calibration curve of photoproduct **2** and laser flash photolysis spectra of **1a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Cadet, J.; Mouret, S.; Ravanat, J.-L.; Douki, T. *Photochem. Photobiol.* **2012**, *88*, 1048–1065.
(2) Cuquerella, M. C.; Lhiaubet-Vallet, V.; Bosca, F.; Miranda, M. A. *Chem. Sci.* **2011**, *2*, 1219–1232.
(3) Cuquerella, M. C.; Lhiaubet-Vallet, V.; Cadet, J.; Miranda, M. A. *Acc. Chem. Res.* **2012**, *45*, 1558–1570.
(4) Heil, K.; Pearson, D.; Carell, T. *Chem. Soc. Rev.* **2011**, *40*, 4271–4278.
(5) Desnous, C.; Guillaume, D.; Clivio, P. *Chem. Rev.* **2010**, *110*, 1213–1232.
(6) Moysan, A.; Viari, A.; Vigny, P.; Voituriez, L.; Cadet, J.; Moustacchi, E.; Sage, E. *Biochemistry* **1991**, *30*, 7080–7088.
(7) Serrano-Andrés, L.; Merchán, M. *J. Photochem. Photobiol. C* **2009**, *10*, 21–32.
(8) Douki, T. *Photochem. Photobiol. Sci.* **2013**, *12*, 1286–1302.
(9) Turro, N. J.; Ramamurthy, V.; Scaiano, J. C. *Modern Molecular Photochemistry of Organic Molecules*; University Science Books: Herndon, VA, 2010.

(10) González-Luque, R.; Climent, T.; González-Ramírez, I.; Merchán, M.; Serrano-Andrés, L. *J. Chem. Theory Comput.* **2010**, *6*, 2103−2114.
(11) Harada, Y.; Okabe, C.; Kobayashi, T.; Suzuki, T.; Ichimura, T.; Nishi, N.; Xu, Y.-Z. *J. Phys. Chem. Lett.* **2010**, *1*, 480−484.
(12) Serrano-Pérez, J. J.; González-Luque, R.; Merchán, M.; Serrano-Andrés, L. *J. Phys. Chem. B* **2007**, *111*, 11880−11883.
(13) Basnak, I.; McKinnell, D.; Spencer, N.; Balkan, A.; R. Ashton, P.; T. Walker, R. *J. Chem. Soc., Perkin Trans. 1* **1997**, 121−126.
(14) McKinnell, D.; Basnak, I.; Hamor, T. A.; Spencer, N.; Ashton, P. R.; Walker, R. T. *Tetrahedron* **1996**, *52*, 10159−10168.
(15) Cai, X.; Sakamoto, M.; Hara, M.; Sugimoto, A.; Tojo, S.; Kawai, K.; Endo, M.; Fujitsuka, M.; Majima, T. *Photochem. Photobiol. Sci.* **2003**, *2*, 1209−1214.
(16) Cai, X.; Sakamoto, M.; Fujitsuka, M.; Majima, T. *Chem.—Eur. J.* **2005**, *11*, 6471−6477.
(17) McGimpsey, W. C.; Scaiano, J. C. *Chem. Phys. Lett.* **1987**, *138*, 13−17.
(18) Sakamoto, M.; Cai, X.; Hara, M.; Fujitsuka, M.; Majima, T. *J. Phys. Chem. A* **2005**, *109*, 4657−4661.
(19) Sakamoto, M.; Cai, X.; Hara, M.; Tojo, S.; Fujitsuka, M.; Majima, T. *J. Phys. Chem. A* **2004**, *108*, 10941−10948.
(20) Basnak, I.; Balkan, A.; Coe, P. L.; Walker, R. T. *Nucleosides Nucleotides* **1994**, *13*, 177−196.
(21) Butenandt, J.; Epple, R.; Wallenborn, E.-U.; Eker, A. P. M.; Gramlich, V.; Carell, T. *Chem.—Eur. J.* **2000**, *6*, 62−72.
(22) McGimpsey, W. G.; Scaiano, J. C. *J. Am. Chem. Soc.* **1989**, *111*, 335−340.
(23) Miranda, M. A.; Pérez-Prieto, J.; Font-Sanchis, E.; Scaiano, J. C. *Acc. Chem. Res.* **2001**, *34*, 717−726.