Godoy Ortega, G.; Rodríguez Muñiz, Gemma María; Lhiaubet, Virginie Lyria; Lorente, C.; Thomas, AH. (2025). Pterin-Thymidine Adducts: From Their Photochemical Synthesis to Their Photosensitizing Properties. The Journal of Physical Chemistry B. 129(13):3334-3344. https://doi.org/10.1021/acs.jpcb.5c00185

Pterin-Thymidine Adducts: From Their Photochemical Synthesis to Their Photosensitizing Properties

Gricelda Godoy-Ortega[a], Gemma M. Rodríguez-Muñiz[b], Virginie Lhiaubet-Vallet[b], Carolina Lorente[a,*], Andrés H. Thomas[a,*]

a *Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, CCT La Plata-CONICET, Diagonal 113 y 64, S/N, 1900, La Plata, Argentina.*

b *Instituto Universitario Mixto de Tecnología Química, Universitat Politècnica de València - Consejo Superior de Investigaciones Científicas, Avenida de los Naranjos s/n, 46022, Valencia, Spain.*

* Corresponding authors e-mails:
clorente@inifta.unlp.edu.ar (Carolina Lorente)
athomas@inifta.unlp.edu.ar (Andrés H. Thomas)

ABSTRACT

Pterin (Ptr) is the model compound of aromatic pterins, which are efficient photosensitizers present in human skin, able to oxidize biomolecules upon UVA irradiation. Photosensitization involves a chemical alteration of a biomolecule as a result of the initial absorption of radiation by another chemical species, the photosensitizer. Under anaerobic conditions Ptr reacts with thymine (T) to form photoadducts (T-Ptr). In this work we present a method to prepare and purify T-Ptr adducts, using 2'-deoxythymidine 5′-monophosphate (dTMP) and single stranded oligonucleotide 5′-d(TTTTT)-3´ (dT_5), and investigate their photosensitizing properties. Interestingly, Ptr moiety, when attached to T, retains its photophysical properties. The adduct dTMP-Ptr, upon excitation, forms singlet and triplet excited states, the latter being capable of transferring energy to dissolved O_2 and generate singlet oxygen, with an efficiency similar to Ptr. In air-equilibrated solutions, both dTMP-Ptr and dT_5-Ptr adducts are able to photosensitize the oxidation of tryptophan and 2′-deoxyguanosine 5′-monophosphate, two of the main targets of photosensitization in biological systems, with efficiencies close to that of free Ptr. The mechanisms involved in the oxidation of biomolecules can be either type I (electron transfer) or type II (singlet oxygen).

INTRODUCTION

Electromagnetic radiation induces chemical modifications to biological components present in living systems and participates in the generation of human skin cancers.[1] Most of the solar energy incidence on Earth's surface corresponds to ultraviolet A (UVA) (320–400 nm), visible (Vis) (400–700 nm) and infrared (>700 nm) radiations, which are poorly absorbed by the DNA, and, therefore, are responsible for only a low proportion of directly formed photolesions. However, UVA radiation is recognized as a class I carcinogen, and it mostly acts indirectly by means of photosensitized processes.[2,3] A photosensitized reaction is defined as a process by which a chemical change occurs in one compound, the substrate or target, as a result of the initial absorption of electromagnetic radiation by another compound, the photosensitizer (or referred to simply as sensitizer).[4] Different mechanisms have been described for photosensitization reactions of biomolecules, including type I and type II oxidations, triplet–triplet energy transfer and formation of photoadducts.[5,6]

Pterins are present in the human epidermis because 5,6,7,8-tetrahydrobiopterin (H_4Bip) is an essential cofactor in the hydroxylation of aromatic amino acids[7] and participates in the regulation of melanin biosynthesis.[8] Melanin is a pigment of skin and the main protection against electromagnetic radiation.[9] The H_4Bip metabolism is altered in vitiligo[10,11] producing oxidized or aromatic pterins that accumulate in the affected tissues, which additionally suffer from depigmentation due to the lack of melanin production. Aromatic pterins[12] are well known natural photosensitizers[13,14] and are able to photosensitize the oxidation of many biomolecules.[15,16] Pterin (Ptr), the parent unsubstituted compound of oxidized pterins (Figure 1), and the vitiligo-related pterin derivatives (biopterin, 6-formylpterin and 6-carboxypterin) are efficient photosensitizers of nucleotides.[15,17]

In fact, it is now well established that oxidized pterins in aqueous solution at neutral or acidic pH absorb in the UVA range (320-400 nm) (Figure 1) and photosensitize the degradation of nucleobases mainly *via* type I mechanism. The oxidation of purines and pyrimidines through this mechanism has been extensively studied.[18,19,20] All the nucleobases may undergo one-electron oxidation, according to their corresponding ionization potentials that follow the order: guanine (G) < adenine (A) < cytosine (C) ~ thymine (T).[21,22,23,24] G is thermodynamically the nucleobase most easily oxidized, and, as a consequence, it acts as the sink of positive charge migration and oxidatively generated lesions in double-stranded DNA.[25,26,27,28] Therefore, the amount of modified pyrimidine bases in a DNA molecule is lower than the total amount of pyrimidine bases that underwent electron transfer. However, this positive charge migration is not so efficient in single strand oligonucleotides.[26]

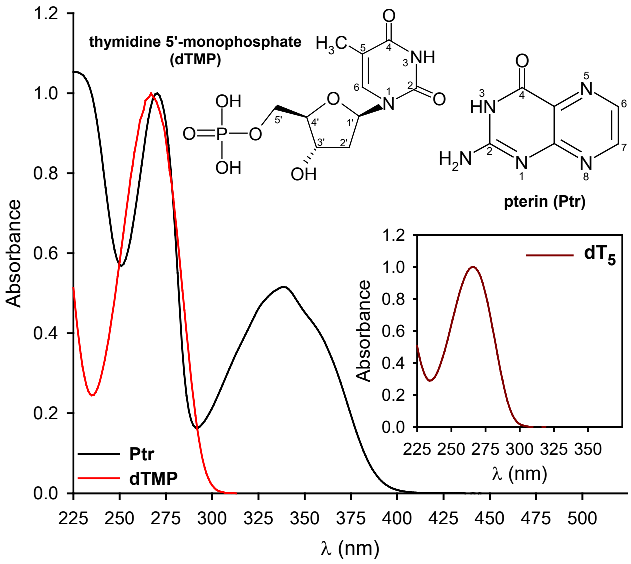


Figure 1. Normalized absorption spectra of Ptr, dTMP and dT₅ in air-equilibrated aqueous solutions at pH 5.5. Molecular structure of Ptr and dTMP.

Type I photosensitization of nucleobases by Ptr and other oxidized pterin derivatives starts with the electron transfer (or hydrogen abstraction) from the substrate (S) to the triplet excited state of Ptr (^{3}Ptr*), leading to the formation of the corresponding pair of radicals (Reaction 1). In the presence of O_2, the Ptr radical (Ptr$^{\bullet-}$ or Ptr(H)$^\bullet$) reduces O_2 to yield superoxide anion ($O_2^{\bullet-}$), together with regeneration of the photosensitizer (Reaction 2). Subsequently, $O_2^{\bullet-}$ leads to the formation of H_2O_2 (Reaction 3). Concerning the radical of the substrate (S$^{\bullet+}$ or S(-H)$^\bullet$), it can undergo many different reactions and eventually gives rise to stable oxidized products. In the case of thymine substrates, after deprotonation or hydration, peroxyl radicals (T-OO$^\bullet$) are formed (Reaction 4). These radicals then participate in a series of pathways (Reaction 5) that finally yield a family of oxygenated products. The oxidation of various compounds bearing the T moiety by different photosensitizers has been thoroughly investigated[18,29,30,31,32] These processes, using pterins as photosensitizers, have been studied for biomolecules containing G, A and T and other biomolecules under different experimental conditions.[16,33,34]

$$^3\text{Ptr}^* + \text{S} \longrightarrow \text{Ptr}^{\bullet-}/\text{PtrH}^\bullet + \text{S}^{\bullet+}/\text{S(-H)}^\bullet \qquad (1)$$

$$\text{Ptr}^{\bullet-}/\text{Ptr(H)}^\bullet + O_2 \longrightarrow \text{Ptr} + O_2^{\bullet-} \qquad (2)$$

$$2H^+ + 2\,O_2^{\bullet-} \longrightarrow H_2O_2 + O_2 \qquad (3)$$

$$\text{S}^{\bullet+}/\text{S(-H)}^\bullet + O_2 \longrightarrow \text{S-OO}^\bullet \qquad (4)$$

$$\text{S-OO}^\bullet \longrightarrow \longrightarrow \longrightarrow \text{S(ox)} \qquad (5)$$

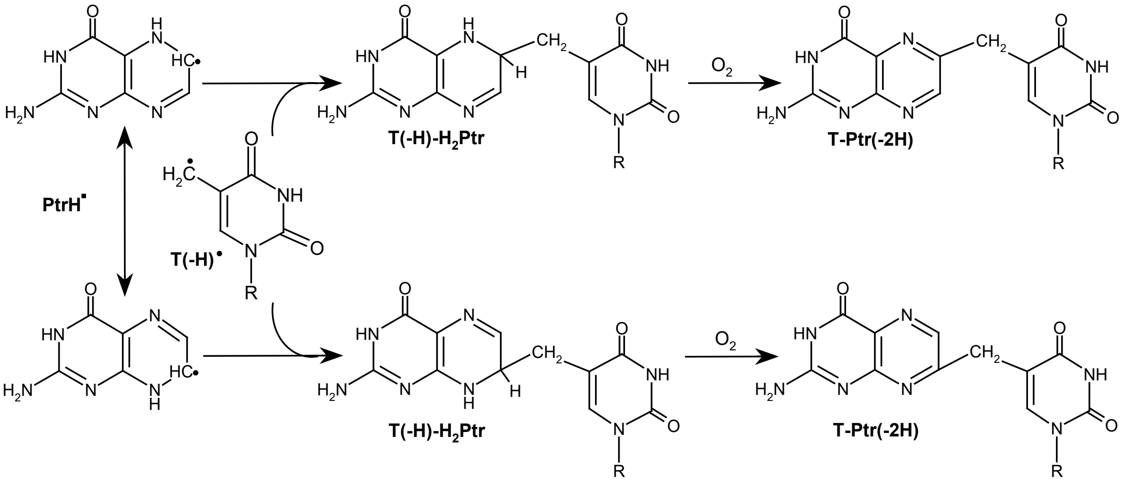
The behavior observed for the UVA-photosensitization of nucleotides with Ptr in the absence of O_2 is different. For purines, radicals are formed, but a recombination between Ptr and purine radicals takes place leading to the recovery of the reactants (Reaction 6).[35] In consequence, no consumption of the nucleotide is registered. However, in equivalent experimental conditions, it was observed that the pyrimidine T is consumed.[36] This process has been extensively studied using the nucleoside thymidine (dT), the nucleotide thymidine 5′-monophosphate (dTMP), the single stranded 5-mer with sequence 5′-d(TTTTT)-3′ (dT_5) and, also calf thymus DNA.[33,36,37] The reaction between $^3Ptr^*$ and dT or dTMP leads to the formation of covalent adducts (Reactions 7 and 8), where the photosensitizer moiety, covalently linked to the nucleobase, preserves its photophysical properties and its ability to photosensitize the production of $^1O_2$. The molecular mass of dT-Ptr and dTMP-Ptr adducts corresponds to [dT-Ptr(-2H)][37] and [dTMP-Ptr(-2H)],[33] respectively. This mechanism is possible only under anaerobic conditions because the presence of O_2 rapidly leads to the elimination of Ptr radicals (Reaction 2) and prevents Reaction 7.

$$\text{Ptr}^{\bullet -}/\text{PtrH}^{\bullet} + \text{S}^{\bullet +}/\text{S(-H)}^{\bullet} \xrightarrow{\qquad\qquad} \text{Ptr} + \text{S} \qquad (6)$$

$$\text{Ptr}^{\bullet -}/\text{PtrH}^{\bullet} + \text{S}^{\bullet +}/\text{S(-H)}^{\bullet} \longrightarrow \longrightarrow \longrightarrow \text{S(-H)-H}_2\text{Ptr} \qquad (7)$$

$$\text{S(-H)-H}_2\text{Ptr} \longrightarrow \xrightarrow{O_2} \longrightarrow \text{(S-Ptr)(-2H)} \qquad (8)$$

Although the detailed mechanism for the formation of T-Ptr adducts has not yet been fully elucidated, a rational hypothesis is that one-electron oxidation of T by $^3Ptr^*$ leads to the formation of the 5-(uracilyl)methyl radical ($T(-H)^{\bullet}$) together with Ptr radical, which subsequently combine to form a T-Ptr adduct where the pterin moiety is reduced (dTMP(-H)-H_2Ptr) (Reaction 7). As soon as O_2 is introduced in the solution, this precursor re-aromatizes to yield the stable product (T-Ptr)(-2H) (Reaction 8).[33] The chemical structures of the intermediates involved in this hypothetical mechanism are shown in Scheme 1. If the irradiation takes place in the presence of O_2, T-Ptr adducts are not detected, because, as explained before, both radicals are trapped by O_2. Adducts are only formed under anaerobic conditions, but since Reaction 7 competes with Reaction 6, the quantum yield of adduct formation is low.[33] Summarizing, to obtain T-Ptr adducts it is necessary to irradiate an O_2-free solution containing both reactants (Reaction 7) and, then, to introduce air to form the stable compound (Reaction 8) (Scheme 1).



Scheme 1. Mechanism proposed for the formation of the adduct T-Ptr. Ptr: pterin; T: isolated thymidine, dTMP, or thymine inserted into an oligonucleotide or DNA.

As abovementioned, the low ionization potential of G should avoid the formation of T-Ptr by reducing the formed T radicals. In this context, recent studies demonstrated that indeed G radicals can be obtained by electron transfer to T radicals (Reaction 9).[38] Remarkably, this process does not completely inhibit the formation of the dTMP-Ptr adduct, which is detected, albeit in smaller amounts than in the absence of 2′-deoxyguanosine 5′-monophosphate (dGMP), during the irradiation of an O_2-free solution containing dGMP, dTMP and Ptr.[38] This result is in agreement with the characterization of T-Ptr adducts in calf thymus DNA[36] and oligonucleotides containing G and T[38] and evidences that G cannot totally avoid the formation of the photoadducts.

$$G + T^{\bullet +}/T(\text{-}H)^{\bullet} \xrightarrow{\hspace{3cm}} G^{\bullet +}/G(\text{-}H)^{\bullet} + T \qquad (9)$$

The T-Ptr adducts retain the spectroscopic properties of the photosensitizer; *e.g.* an absorption band in the UVA region and similar fluorescence properties.[33,36,37] In addition, it was demonstrated that the adduct with dT is able to photosensitize de production of $^1O_2$. These features suggest that T-Ptr adducts might have photosensitizing properties, which would be of particular relevance since these adducts are derived from two endogenous components and can be inserted in oligonucleotides or DNA molecules. In this work, we present for the first time a method to prepare and purify dTMP-Ptr adducts and investigate their photosensitizing properties using small biomolecules as oxidizable substrates. In particular, we used dGMP and Trp, which are two of the main targets of photosensitization in biological systems, to compare the T-Ptr adducts photosensitizing efficiency with free Ptr and discuss the involved mechanisms.

EXPERIMENTAL SECTION

General

Pterin (Ptr) solutions were prepared using the solid reagent provided by Schircks Laboratories (Switzerland) to the highest purity available (>98%) without further purification. 2'-deoxythymidine 5′-monophosphate (dTMP), 2′-deoxyguanosine 5′- monophosphate (dGMP), single stranded oligonucleotide 5′-d (TTTTT)-3´ (dT_5), tryptophan (Trp) and phenalenone (PN) were provided by Sigma-Aldrich Laboratories and used without further purification.

The pH measurements were performed with a pH-meter sensION and a combined pH microelectrode when required. The pH of the aqueous solutions used was adjusted using small volumes of 2M HCl and NaOH solutions.

Steady-state Irradiation

Samples were irradiated using 3 Rayonet RPR 3500 lamps (Southern N.E.Ultraviolet Co.) with emission centered at 350 nm [bandwidth ~20 nm], arranged in a black box so that the cell containing the sample is surrounded by the lamps.

When anaerobiosis conditions were required, the samples were bubbled with argon (Linde, purity >99.998%), previously hydrated in a water trap to prevent the bubbled solution from concentrating. An argon-filled balloon was placed over the cell lid to maintain anaerobic conditions throughout the irradiation.

High-performance liquid chromatography (HPLC)

High-performance liquid chromatography (HPLC) was used for isolation of the T-Ptr adducts (preparative HPLC) and for analysis of the irradiated solutions (analytical HPLC).

Preparative HPLC. A Prominence equipment from Shimadzu (solvent delivery module LC-20AT, online degasser DGU-20A5, communications bus module CBM-20, auto sampler SIL-20 A HT, column oven CTO-10AS VP, PDA detector SPD-M20A and fluorescence detector RF- 20 A) was used to separate the photoadducts formed during the photosensitization assays. A Synergi Polar-RP column (ether-linked phenyl phase with polar endcapping, 150 × 4.6 µm, Phenomenex) was used for stationary phase. The mobile phases used were: (i) 93% ammonium acetate (NH_4Ac) 1 mM (pH= 6.8) and 7% acetonitrile for dT_5-Ptr adduct and (ii) 97% formic acid 10 mM (pH= 3.3) and 3% acetonitrile for dTMP-Ptr adduct. The flow rate was 1 mL/min.

Analytical HPLC. A Prominence equipment from Shimadzu (solvent delivery module LC-20AT, online degasser DGU-20A5, communications bus module CBM-20, auto sampler SIL-20 A HT, column oven CTO-10AS VP, PDA detector SPD-M20A and fluorescence detector RF- 20 A) was used to analyze the photosensitization assays. A Synergi Polar-RP column (ether-linked phenyl phase with endcapping, 150 × 4.6 µm, Phenomenex) was used for stationary phase. The mobile phases used were: 93% NH_4Ac 1 mM (pH=6.8) and 7% acetonitrile for dT_5-Ptr adduct and (ii) 97% formic acid 10 mM (pH= 3.3) and 3% acetonitrile for dTMP-Ptr adduct. The flow rate was 1 mL/min.

Fluorescence measurements

Steady-state and time-resolved fluorescence measurements were performed at room temperature using a single-photon-counting equipment FL3TCSPC-SP (Horiba Jobin Yvon).

Steady-state measurements. To obtain the fluorescence spectra, the sample solution in a quartz cell was irradiated with a CW 450W Xenon source through an excitation monochromator and the luminescence was registered at 90^o with respect to the incident beam, after passing through an emission monochromator, using a room-temperature R928P detector. Spectra were corrected for wavelength-dependent emission profiles with corrections factors supplied by the manufacturer and using the software FluorEssence^{TM} version 3.9 (Horiba Jobin Yvon).

Time-resolved experiments. A NanoLED source (maximum at 341 nm, ~ 1 ns pulse duration) was used for excitation, and the emitted photons, after passing through a monochromator, were detected by a TBX-04 detector and counted by a FluoroHub-B module. The selected counting time window for the measurements reported in this study was 0-200 ns.

Laser Flash Photolysis

Studies of triplet excited states and radicals. Laser flash photolysis experiments were run using as excitation set-up an optical parametric oscillator (OPO, EKSPLA NT342) pumped with the 3rd harmonic of a Nd:YAG laser (EKSPLA PS5062). Experiments were performed using λ_{exc}=340 nm as excitation wavelength, with a 5 ns pulse duration of 8 mJ/pulse. Detection system consists of a LP980 instrument (Edinburgh Instruments) with a pulsed Xenon lamp (150 W), a monochromator (TMS302-A, 150 grooves/mm grating) and a PMT (Hamamatsu Photonics). Absorbance of *ca.* 0.4 at the excitation wavelength was used, and measurements were performed using 1 cm pathway cuvettes under air, N_2 or O_2 atmosphere.

Singlet oxygen detection. Singlet oxygen luminescence was monitored at 1270 nm using a refrigerated NIR detector (Hamamatsu, voltage set at 800 V) cooled at -62.8 °C with a Peltier cell and coupled to the laser photolysis cell in right-angle geometry. The samples in D_2O were excited at λ_{exc}=355 nm with the 3rd harmonic of a Nd:YAG laser (L52137V LOTISTII) with a pulse energy of 9 mJ. 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. Measurements were performed under air atmosphere using 1 cm pathway cuvettes. Quantum yield of singlet oxygen (Φ_Δ) formation was determined by monitoring the phosphorescence at 1270 nm upon laser excitation of an optically matched solution (A= 0.35) of pterin and TMP-Ptr relative to a standard solution of PN.

RESULTS & DISCUSSION

Preparation of the pterin-thymidine adducts

Aqueous solutions containing pterin (Ptr) and a substrate containing thymine (2'-deoxythymidine 5′-monophosphate (dTMP) or single stranded oligonucleotide 5′-d-(TTTTT)-3′ (dT_5)) were exposed to UVA irradiation in anaerobic conditions (see Experimental Section). Under these experimental conditions, as can be inferred from the corresponding absorption spectra (Figure 1), Ptr was the only absorbing species, whereas dTMP and dT_5 did not absorb radiation. The photolysis was carried out at pH 5.5, where Ptr is present at more than 99% in its acid form (p*K*a(Ptr)= 7.9),[15] the predominant form in biological systems. The photochemical reactions were followed by UV/Vis spectrophotometry and HPLC with spectrophotometric and fluorescence detection.

For the generation of the dTMP-Ptr adduct, a Ptr concentration of 150 µM and a nucleotide concentration of 1 mM were used. This high nucleotide concentration, that can be reached due to the high solubility of nucleotides in H_2O, favors the formation of the adduct. The solutions were irradiated in a quartz round bottom flask for 4 hours with constant stirring, after which the solution was adjusted to pH 10.5 at which Ptr is more soluble, to avoid subsequent precipitation. In the case of the oligonucleotide, the procedure was the same, except: i) a concentration of 150 µM of dT_5 was used because it is not possible to reach higher concentrations and ii) the irradiation time was 80 min. Despite the lower concentration and the shorter irradiation time, a significant amount of dT_5-Ptr adduct was obtained since the process is more efficient for the oligonucleotide than for the free (or isolated) nucleotide.[36]

Once the adducts were generated, they were isolated by semipreparative HPLC, the purity of the solution was checked by analytical HPLC (Experimental Section) and then stored at 4°C in alkaline media until used in the photosensitization assays. For dTMP the adduct was well separated from both reactants and obtained as a pure compound, whereas for dT_5 the adduct could be separated from Ptr, but not from the remaining oligonucleotide and the resulting solutions contained both dT_5-Ptr adduct and dT_5 (Figure S1). The solutions were concentrated using a rotary evaporator or dried by lyophilization. Prior to any further experiment with the adducts, the purity of the solution was checked by analytical HPLC again, in particular to search for potential contamination by free Ptr. The adduct solution was discarded if the presence of free Ptr or compounds with spectral features (absorption and emission) compatible with aromatic pterins was detected.

Spectroscopic and photosensitizing properties of the adducts were investigated and the corresponding results will be presented and discussed in the following sections. The experiments were performed for dTMP-Ptr and dT_5-Ptr adducts. According to what was explained in this section, in the former case, pure solutions containing only the adduct were used, whereas in the latter case, solutions containing both the adduct and the remaining oligonucleotide were employed. It is also worth mentioning that the term dTMP-Ptr adduct refers to a mix of several isomers, corresponding to, at least, addition at two different positions of the pterin moiety (Scheme 1).[37] The term dT_5-Ptr adducts also correspond to a mix of compounds in which not only the position of binding on the pterin moiety can differ, but also the targeted T in sequence and/or number of Ptr units covalently attached to the oligonucleotide.

Absorption and emission

The absorption spectrum of the dTMP-Ptr adduct in aqueous solution at pH 6.0 showed the typical absorption bands of the neutral form of pterins, with maxima at approximately 270 and 340 nm (Table 1, Figure 2). This spectrum is similar to that registered for the adduct of the nucleoside (dT-Ptr).[37] The spectrum of dTMP-Ptr at pH 10.5 is different to that at pH 6.0 and shows the typical absorption bands of the anionic form of pterins, with maxima at approximately 250 and 360 nm (Table 1, Figure 2). This spectral behavior confirms that the ionizable group of the pterin moiety is free in the adduct, as suggested previously.[37] The absorption spectrum of the pure dT_5-Ptr could not be obtained because it could not be separated from dT_5 in chromatographic runs. However, the spectra of the dT_5-Ptr/dT_5 mixture, registered with the PDA detector of the HPLC equipment, showed an absorption band in the

UVA range corresponding to the pterin chromophore (Figure S2).

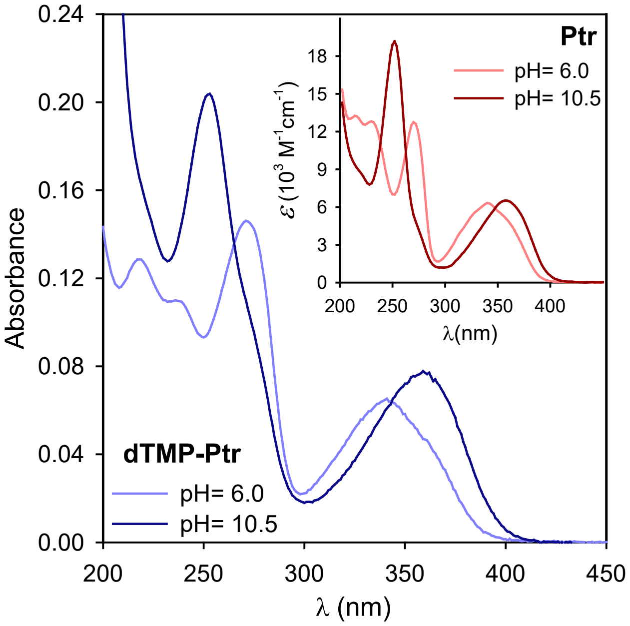


Figure 2. Absorption spectra of air-equilibrated aqueous solutions of dTMP-Ptr (11.5 μM) at pH 6.0 and 10.5). Inset: Absorption spectra of the acid (neutral) and basic (monoanionic form) of Ptr in aqueous solution.

The emission spectra of dTMP-Ptr, dT_5-Ptr and Ptr, registered by excitation into the low-energy pterin band (340 nm), were similar (Figure 3). The fluorescence quantum yields (Φ_F) were determined from the corrected fluorescence spectra using Equation 10:

$$\Phi_F = \Phi_F^R \frac{I/A}{I^R/A^R} \left(\frac{n^R}{n}\right)^2 \tag{10}$$

where *I* is the total fluorescence intensity, *A* is the absorbance at the excitation wavelength (kept below 0.10 to avoid inner filter effects), n is the refractive index of each solvent and the superscript R refers to the reference fluorophore. In our experiments Ptr in acidic aqueous solution (Φ_F= 0.3)[15¡Error! Marcador no definido.] was used as a reference. The obtained Φ_F value for dTMP-Ptr was close to that of Ptr (Table 1), which is in agreement with the Φ_F value reported for the adduct of Ptr with the nucleoside (dT-Ptr).[37] In contrast, this value is higher than that reported in Ref. 33, but this study determined in a solution containing both dTMP-Ptr adduct and free nucleotide, which suggest that the singlet excited state of the pterin moiety might have been quenched by the remaining dTMP that was at high concentration. In fact, the singlet excited states of acid forms of pterins are efficiently deactivated by nucleotides, with bimolecular quenching rate constants in the range of diffusion controlled limit.[39] The dT_5-Ptr adduct presents a lower Φ_F value (Table 1). Although in this case the adduct is not purified, the quenching by the remaining oligonucleotide should be negligible since its concentration is very low (about 40 μM). Therefore, the Φ_F value calculated for dT$_5$-Ptr is reliable.

Table 1. Photophysical properties of Ptr and adducts in air-equilibrated aqueous solutions. Wavelengths of absorption (λ_A) and fluorescence (λ_F) maxima, fluorescence quantum yields (Φ_F), fluorescence lifetimes (τ_F), pre-exponential factors (%α) and quantum yields of $^1O_2$ production (Φ_Δ). Measurements were carried out at pH 6.0. Fluorescence spectra were registered by excitation at 340 nm, τ_F values were calculated in decays registered after excitation at 341 nm (see Experimental Section).

	Ptr	**dTMP-Ptr**	**dT$_5$-Ptr**
Absorption			
λ_{A1} (nm)	270 ± 2	271 ± 2	--
λ_{A2} (nm)	340 ± 2	343 ± 2	341 ± 2
Fluorescence			
λ_F (nm)	450 ± 3	450 ± 3	451 ± 3
τ_{F1} (ns)	6.0 ± 0.5	6.0 ± 0.5	7.4 ± 0.5
%α_1	100	67	80
τ_{F2} (ns)	-	3.2 ± 0.5	3.1 ± 0.5
%α_2	-	33	20
Φ_F	0.33 ± 0.01	0.33 ± 0.03	0.20 ± 0.03
Triplet excited states			
τ_T (μs)	6.3 ± 0.5	4.5 ± 0.5	
Φ_Δ	0.18 ± 0.02	0.18 ± 0.05	--

Time-resolved studies of fluorescence were performed using excitation at 341 nm and fluorescence decays were analyzed at 450 nm for the three compounds (Ptr, dTMP-Ptr and dT$_5$-Ptr). For Ptr, as expected a first-order rate law was observed for the emission decays and a lifetime (τ_F) of 6.0 ns was obtained. This value is equal, within the experimental error, to that previously reported.[15] For the adducts the emission decays were clearly biexponential with a short-lived component of about 3 ns and a longer-lived component τ_F value similar to that of Ptr (Table 1). The pre-exponential factors of the fittings indicate that the short-lived ones are minor components. However, it is not clear why fluorescence of adducts present two components. One could speculate that they correspond to different isomers, but further analysis should be performed to confirm this hypothesis.

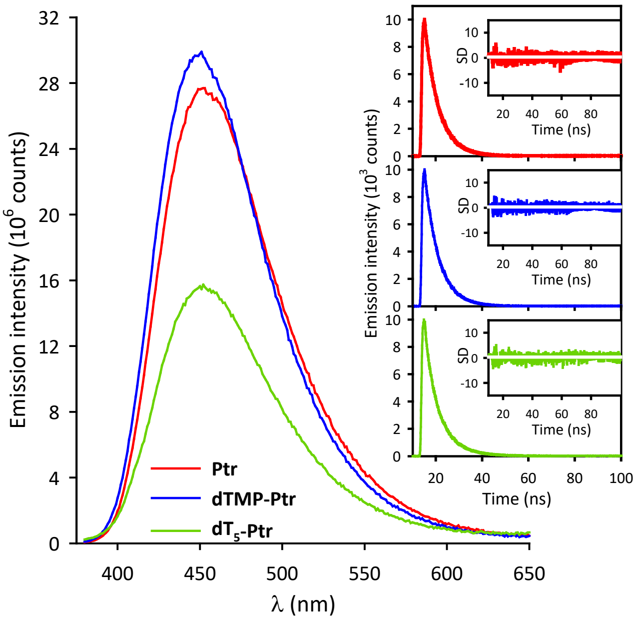


Figure 3. Corrected fluorescence spectra of air-equilibrated aqueous solutions of dTMP-Ptr, dT_5-Ptr and Ptr obtained by excitation at 340 nm. For comparative purposes, the three solutions presented the same absorbance at 340 nm. Insets: Emission decays recorded at 450 nm (λ_{exc}= 341 nm) and residual analysis; Ptr decays were fitted with a monoexponential function, whereas dTMP-Ptr and dT_5-Ptr decays were fitted with biexponential functions (Table 1).

The Triplet Excited States

The triplet excited state of dTMP-Ptr was investigated by laser flash photolysis (LFP) (Experimental Section) and the results were compared to control experiments performed using Ptr. Transient absorption spectrum obtained by excitation at 340 nm of N_2-saturated solutions (A_{355nm}= 0.3, pH 5.5) were registered at different times after the laser pulse. Results obtained for Ptr were similar to those previously reported and correspond to the differential transient absorption spectrum of the triplet excited state of the neutral form of Ptr (^{3}Ptr*) in aqueous solution.[35] The transient absorption spectra recorded for dTMP-Ptr were similar to those of Ptr obtained under the same experimental conditions (Figure 4), evidencing the formation of a triplet excited state (dTMP-^{3}Ptr*).

For both compounds the decays of the transient absorption bands at 400–600 nm were adjusted with mono-exponential fitting functions and the corresponding lifetimes (τ_T) were determined (Table 1). It is worth mentioning that the decay of ^{3}Ptr* has been reported to be bi-exponential with two components that correspond to the lactim (short-lived triplet) and the lactam (long-lived triplet) tautomers.[40] Under our experimental conditions, the short-lived component for Ptr, of about 0.3 μs, could not be detected and values obtained were similar to those previously reported for the long-lived component. The τ_T value calculated for the adduct was of the same order of magnitude as the value determined for Ptr (Table 1).

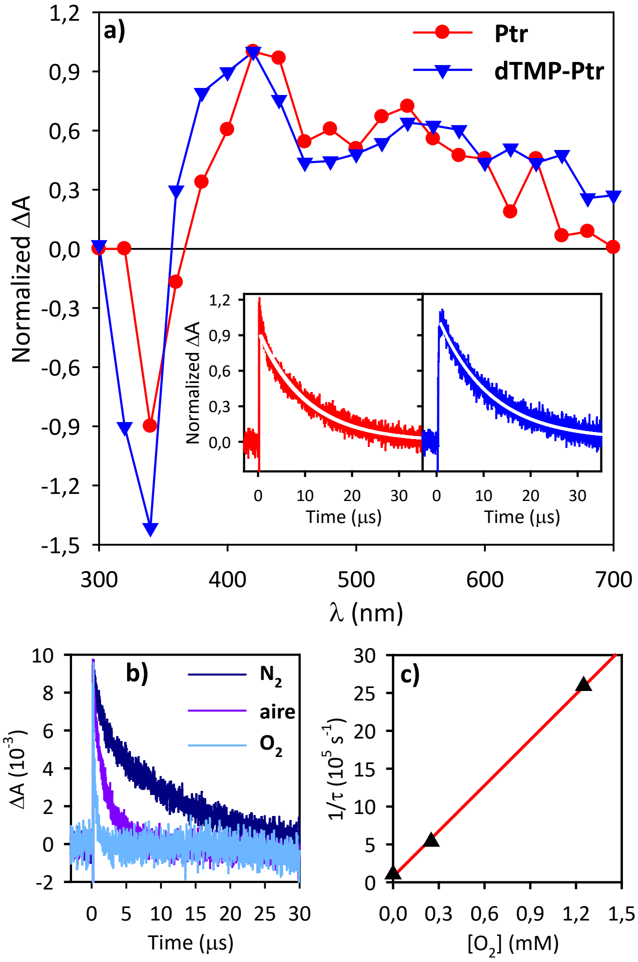


Figure 4. LFP experiments. a) Normalized differential transient absorption spectra recorded at 0.2 μs after the 340 nm laser pulse of N_2-saturated aqueous solutions of Ptr and dTMP-Ptr. Inset: Time dependence of the absorbance at 400 nm, with mono-exponential fitting. Experiments performed in Ar-saturated aqueous solutions. b) and c) quenching of the triplet excited state of the adduct dTMP-Ptr by dissolved O_2. b) Decays registered at 400 nm for different O_2 concentration. c) τ_T^{-1} vs. [O_2] and linear regression to determine the rate constant. Absorbance at 340 nm: 0.3, which corresponds to a concentration of the photosensitizers of about 60 μM, pH= 6.0.

Experiments carried out in air-equilibrated and O_2-saturated solutions demonstrated that dissolved O_2 increases the corresponding decay rates of the transient detected with dTMP-Ptr, which is expected for triplet excited states (Figures 4b and 4c). The corresponding bimolecular quenching rate constant (k_T^{O2}) was calculated by the Stern–Volmer analysis (Equation 11),

$$\frac{1}{\tau_T} = \frac{1}{\tau_T^0} + k_T^{O2}\,[O_2] \qquad (11)$$

where τ_T and τ_T^0 are the triplet lifetimes of in the presence and the absence of O_2, respectively. A value of 2.0 (±0.2)×10^9 $M^{-1}s^{-1}$ was obtained, which is in the range of diffusion-controlled limit and is a typical value expected for the quenching of a triplet excited state of a small organic compound by O_2 in aqueous solution. Moreover, a similar value has been reported for the quenching of $^3Ptr^*$ by O_2.[35]

Therefore, the information presented in Table 1 and Figure 4 confirms that the adduct, upon excitation, forms a triplet excited state with features similar to those described for Ptr. In consequence, the adduct should be able to photoinduce the generation of $^1O_2$.

To confirm this point, the phosphorescence of $^1O_2$ was registered using a NIR detector (Experimental Section). D_2O was used as a solvent to increase the lifetime of $^1O_2$ (τ_Δ) and then obtain more intense signals.[41,42] For D_2O solutions of dTMP-Ptr, a mono exponential decay was registered for the NIR emission at 1270 nm. The lifetime of 27 μs, is in agreement with the lifetime of $^1O_2$ (τ_Δ) in D_2O[43] (Figure 5). As a control experiment, the sample was bubbled with N_2, which resulted in the disappearance of the signal. These results, in connection with the fact that the triplet excited state is quenched by O_2, confirmed that upon UVA excitation the adduct is able to transfer energy to dissolved O_2 and generate $^1O_2$.

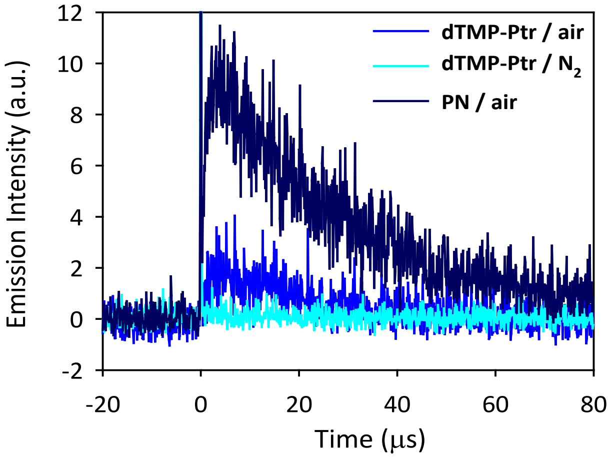


Figure 5. LFP experiments. Selected NIR emission decays of time-resolved $^1O_2$ experiments. The experiments were performed in air-equilibrated D_2O solutions (pD= 6.05) containing phenalenone (PN) or dTMP-Ptr at the same absorbance (A_{355}= 0.35) at the excitation wavelength (355 nm).

To determine the efficiency of this process, similar experiments were performed using phenalenone (PN) as a $^1O_2$ photosensitizer. Thus, D_2O solutions containing PN or dTMP-Ptr with the same absorbance at 355 nm (i.e., laser excitation wavelength) were analyzed. As expected, the signal of the NIR emission of $^1O_2$ generated by PN decayed mono-exponentially with a lifetime τ_Δ of ca 28 μs (Figure 5). The area under the curve phosphorescence intensity *vs.* time is proportional to the amount of $^1O_2$ generated. Therefore, the quantum yield of $^1O_2$ production of the adduct ($\emptyset_{\Delta}^{dTMP-Ptr}$) can be calculated with Equation 12.

$$\emptyset_{\Delta}^{dTMP-Ptr} = \frac{area(dTMP-Ptr)}{area(PN)}\, \emptyset_{\Delta}^{PN} \qquad (12)$$

Where $\emptyset_{\Delta}^{PN}$ is the quantum yield of $^1O_2$ production of PN and area(dTMP-Ptr) and area(PN) are the areas under the $^1O_2$ phosphorescence decay *vs.* time determined by integration for the adduct and PN, respectively. The $\emptyset_{\Delta}^{dTMP-Ptr}$ value was equal to the reported value for $\emptyset_{\Delta}^{Ptr}$ (Table 1), which means that the adduct is an efficient photosensitizer of $^1O_2$ and that the attachment of the Ptr moiety to the nucleotide does not affect its capability to transfer energy to dissolved O_2 and generate the reactive oxygen species.

This study could not be carried out for dT_5-Ptr because this adduct was obtained at much lower quantities than the corresponding dTMP adduct. Therefore, the signals recorded in both types of time resolved experiments (transient spectra and NIR emission) were too low and triplet excited states and $^1O_2$ could not be registered.

Photosensitizing properties

The results presented in the previous section suggest that T-Ptr adducts should have the capability to photosensitize the oxidation of biomolecules. To explore this point, we chose two compounds that are suitable for a simple and overall evaluation of the ability of a given compound to act as a photosensitizer in biological systems: tryptophan (Trp) and 2′-deoxyguanosine 5′-monophosphate (dGMP). These two model oxidizable biological targets were selected because: (i) they are representative targets of proteins and DNA,[6] respectively, sensitive to oxidations which affect the functionality of the biomolecules; (ii) they are able to be photosensitized through both type I and type II mechanisms;[6] and (iii) their photosensitized oxidations by pterins has already been thoroughly investigated;[16,34,44]. To compare the photosensitizing efficiencies of the adducts to Ptr, experiments were carried out using the free photosensitizer. Therefore, aqueous solutions (pH= 9.5) containing a given substrate (Trp or dGMP) and a given photosensitizer (Ptr, dTMP-Ptr or dT_5-Ptr) were exposed to UVA radiation for different times and then analyzed by HPLC. The experiments were performed in alkaline media to favor the production of $^1O_2$ ($\emptyset_{\Delta}^{Ptr}$ is higher for the anionic form of Ptr)[15] and its reaction with the substrates (the rate of the reaction of $^1O_2$ with dGMP is higher in alkaline media).[45] To discard direct photolysis of the substrates, in all cases control experiments in the absence of photosensitizers were performed.

For air-equilibrated solutions of dTMP-Ptr (6 μM) and Trp (50 μM) a significant decrease in the amino acid concentration was observed in a few minutes, whereas the adduct was not consumed in the process (Figure 6a). The rate of Trp consumption was similar to that observed using Ptr (6 μM) as the photosensitizer (Figure 6a). A control photolysis was performed under the same conditions, but in the absence of dTMP-Ptr or Ptr no photodegradation of Trp was observed, confirming that the adduct is able to photosensitize the oxidation of Trp. In the irradiated solutions several products were detected, among which a compound had an absorption spectrum and a retention time that matched with *N*-formylkynurenine (NFKyn), a typical product of the oxidation of Trp by $^1O_2$.[19,46] This result is in agreement with the production of $^1O_2$ by dTMP-Ptr (*vide supra*). With comparative purposes, a similar experiment was carried out using Ptr as the photosensitizer. As can be appreciated in Figure 6a, the rate of consumption of Trp was similar for both photosensitizers.

To further investigate if the adduct dTMP-Ptr is able to photosensitize the oxidation of other biomolecules, similar photolysis experiments were performed using dGMP as a substrate (Figure 6b). The results were similar to those observed using Trp, that is, dTMP-Ptr is able to photooxidize the substrate with efficiency comparable to that of Ptr.

Similar experiments performed using dT_5-Ptr as a photosensitizer and Trp as a substrate (Figure 7) showed that this adduct is also able to photoinduce the oxidation of the amino acid. It is worth mentioning that for comparative purposes, in the photolysis carried out with Ptr and dT_5-Ptr, the absorbance at 340 nm was the same. Using the extinction coefficient of Ptr at that wavelength, the concentration of Ptr (free or attached to dT_5) was 6 μM. Therefore, as mentioned above, although dT_5-Ptr could not be obtained pure and several adducts are possible, the results shown in Figure 7, evidence that the photosensitizing efficiencies of Ptr in the adduct and free are similar. This is a relevant result since the formation of triplet excited states in dT_5-Ptr, as explained before, could not been demonstrated by LFP experiments. The photosensitizing properties of Ptr attached to a nucleotide or to an oligonucleotide chain do not change, when evaluated in terms of efficiency to oxidize Trp. Again, a fast assessment of the products detected by HPLC revealed the presence of NFKyn, which confirms that dT_5-Ptr is able to photosensitize the formation of $^1O_2$.

According to previous studies, the photosensitized oxidations of both substrates by Ptr takes place mainly through type I mechanism; type II mechanism contributes, but to a lesser extent.[16] Therefore, it is reasonable to propose that the adduct can act as a type I photosensitizer, as well. Although the photosensitizing capacity of dTMP-Ptr via type II mechanism has been evidenced spectroscopically through the detection $^1O_2$, and analytically with the generation of NFKyn in the photosensitized oxidation of Trp, up to this point no data supporting the possibility of a type I mechanism has been shown.

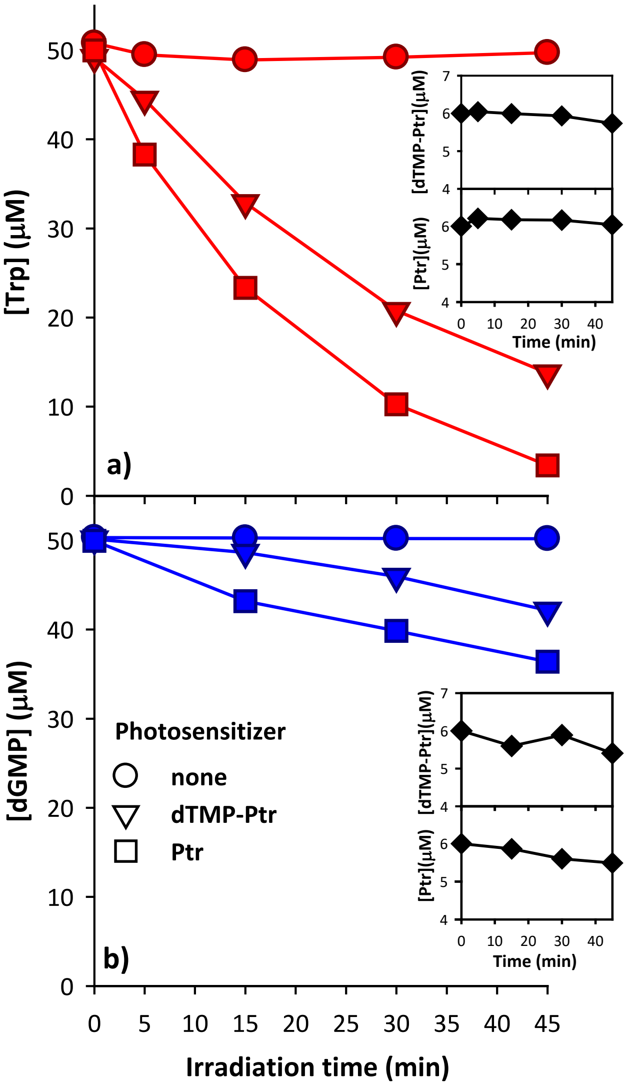


Figure 6. Photosensitization of a) Trp and b) dGMP by Ptr and dTMP-Ptr. Concentrations determined by HPLC analysis of solutions containing a given substrate (50 μM) and a given photosensitizer (6 μM) at different irradiation times. Control photolysis were carried out in the absence of photosensitizer. Experiments performed in air-equilibrated aqueous solutions at pH 9.5, using UVA radiation. Insets: time evolution of the concentration of the photosensitizers in the photosensitization experiments.

To explore type I mechanism, the interaction of the triplet excited state of dTMP-Ptr (dTMP-^{3}Ptr*) with dGMP was investigated by LFP. In these experiments we recorded the triplet transient decays at 430 nm at different dGMP concentrations (Figure 8a) and the τ_T values were determined with mono-exponential fittings. Then, Stern-Volmer analysis was carried out using Equation 13 (Inset Figure 8a).

$$\frac{\tau_T^0}{\tau_T} = 1 + K_D^T\,[Q] = 1 + k_q^T\,\tau_T^0\,[dGMP] \qquad (13)$$

where τ_T^0 and τ_T are the triplet lifetimes of dTMP-^{3}Ptr* in the absence and in the presence of quencher, respectively, K_D^T (= $k_q^T\tau_T^0$) is the Stern–Volmer constant for the quenching of ^{3}Ptr*, with k_q^T as the bimolecular quenching rate constant, and [dGMP] is the nucleotide concentration. This analysis returned a value of 1.2 (±0.2) × 10^9 $M^{-1}s^{-1}$ for k_q^T, which corresponds to a near diffusion-controlled quenching, thus providing direct evidence for the interaction of dTMP-^{3}Ptr* with dGMP.

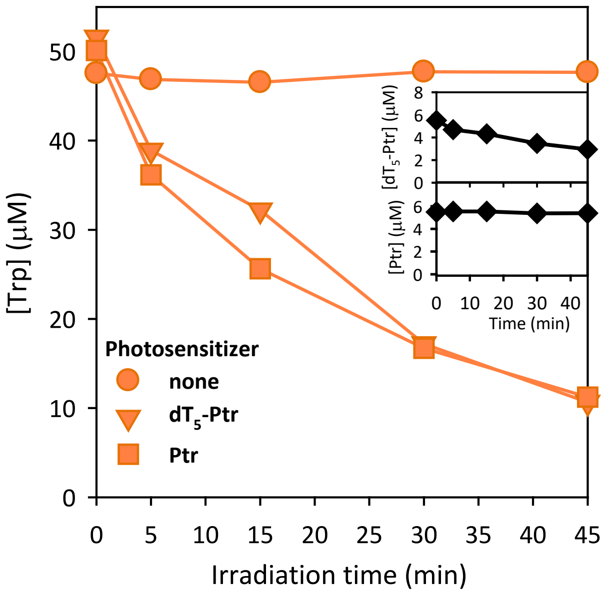


Figure 7. Photosensitization of Trp by Ptr and dT$_5$-Ptr. Concentrations determined by HPLC analysis of solutions containing Trp (50 µM) and a given photosensitizer (6 µM) at different irradiation times. Control photolysis were carried out in the absence of photosensitizer. Experiments performed in air-equilibrated aqueous solutions at pH 9.5, using UVA radiation. Insets: time evolution of the concentration of the photosensitizers.

Photosensitization through type I mechanism is initiated by an electron transfer from the nucleotide to dTMP-^{3}Ptr*. To analyze this mechanism in the context of this quenching study, we investigated the formation of the dGMP radical. We chose this radical because this species has been well characterized by LFP[47,48] and its formation by photosensitization with Ptr has been previously proven.[16,17,45] The formation of the dGMP radical can be monitored by the time evolution of the absorbance at 320 nm, which corresponds to the maximum of its typical narrow transient absorption band. The traces recorded at this wavelength followed first-order growth kinetics (Figure 8b). The lifetime value obtained for the formation of the dGMP radical was equal, within the experimental error, to that obtained for the decay of dTMP-^{3}Ptr* at the corresponding dGMP concentration. Consequently, the results confirm the formation of the dGMP radical by electron transfer from the nucleotide to the adduct.

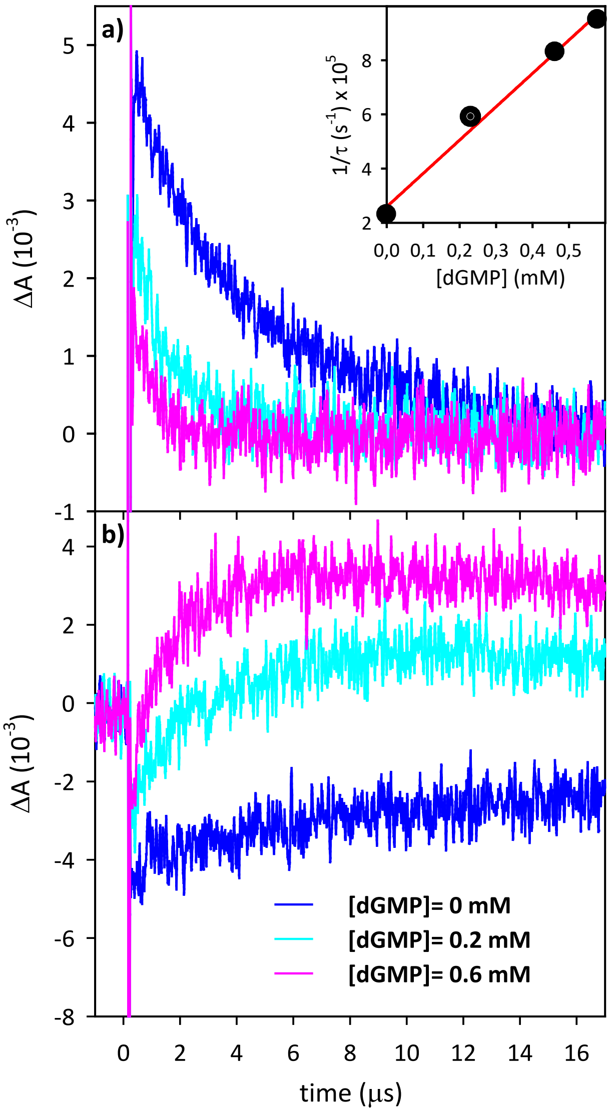


Figure 8. LFP experiments. a) Time dependence of the absorbance at 420 nm, maximum of the dTMP-^{3}Ptr* transient spectrum, at different dGMP concentrations; inset: Stern–Volmer plot of the quenching of dTMP-^{3}Ptr* by dGMP, τ_T values were calculated by mono-exponential fitting of the transient absorbance ΔA decay. b) Time dependence of the absorbance at 320 nm, maximum of the guanine radical transient spectrum, at various dGMP concentrations. Experiments performed in Ar-saturated aqueous solutions. [dTMP-Ptr]≅ 60 µM, pH= 6.0.

CONCLUSIONS

Aromatic pterins, and especially their parent unsubstituted compound pterin (Ptr), are efficient photosensitizers present in the human epidermis. Their activity a photooxidants upon UVA irradiation is well established for DNA, proteins, lipids or their elemental components. In this context, previous studies demonstrated that the reaction between the triplet excited state of Ptr ($^3Ptr^*$) and thymine (T), in the absence of O_2, leads to the formation of covalent adducts, in which the photosensitizer preserves its chemical structure. In this work T-Ptr adducts were synthesized, by irradiating aqueous solutions containing Ptr and a substrate containing T (2'-deoxythymidine 5′-monophosphate (dTMP) or single stranded oligonucleotide 5′-d(TTTTT)-3′ (dT_5)) with UVA in anaerobic conditions, and then purified by HPLC. As a result, dTMP-Ptr was obtained as a pure compound, whereas dT_5-Ptr could not be completely separated from the remaining oligonucleotide.

The emission spectra of dTMP-Ptr, dT_5-Ptr and Ptr are similar. The fluorescence quantum yield (Φ_F) of dTMP-Ptr is also comparable to that of Ptr, but the dT_5-Ptr adduct presents a lower Φ_F value. For the adducts, the emission decays are biexponential with a short-lived component of about 3 ns and a longer-lived component close to that of the monoexponential decay of Ptr. The adduct dTMP-Ptr, upon excitation, forms triplet excited state with features similar to those described for Ptr and is able to transfer energy to dissolved O_2 and generate $^1O_2$, with an efficiency similar to Ptr. For dT_5-Ptr, triplet excited state could not be investigated because this adduct is obtained at much lower concentration than the corresponding dTMP adduct.

In air-equilibrated solutions and under UVA irradiation, both dTMP-Ptr and dT_5-Ptr are able to photosensitize the oxidation of biomolecules, such as tryptophan and 2′-deoxyguanosine 5′-monophosphate, with efficiencies close to that of free Ptr. The adducts act as photosensitizers through both type I and type II mechanisms.

The results presented in this work show that Ptr covalently attached to T in nucleotides or oligonucleotides retains not only its spectroscopic features, but also its capability to act as an efficient photosensitizer of biomolecules. The formation *in vivo* of this type of photoadducts should not be discarded since pterins, under certain pathological situations, accumulate in tissues exposed to light and where the O_2 concentration can be very low. It is interesting to explore potential applications of these adducts as fluorescent markers and photosensitizers for PDT.

SUPPORTING INFORMATION

Chromatograms of the isolation of dTMP-Ptr and dT_5-Ptr adducts; absorption spectrum of the dT_5-Ptr/dT_5 isolated mixture.

ACKNOWLEDGEMENTS

The present work was partially supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Grants P-UE 2017 22920170100100CO, Grant PIP 2021-2023 GI 11220200100877CO), Agencia de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación (Agencia I+D+i, Grants PICT 2019–3416), Universidad Nacional de La Plata (UNLP, Grant 11/X840). The authors thank CONICET and CSIC for supporting their collaboration through a Project of International Cooperation (Programme 2023). Also, financial support from CSIC (i-COOP, ref COOPB22038) and the Spanish (project PID2021-128348NB-I00 funded by MCIN/AEI/ 10.13039/501100011033/and "FEDER a way of making Europe" and regional (CIAICO/2021/061) governments is acknowledged. G.G.O. thanks CONICET for doctoral research fellowship. Authors thank Jesuán J. Farías for the preparation of the standard of NFKyn. C.L. and A.H.T. are research members of CONICET. V.L.V. and G.M.R.M. are research members of CSIC.

REFERENCES

---

1 Cadet, J.; Douki, T. Formation of UV-induced DNA Damage Contributing to Skin Cancer Development. *Photochem. Photobiol. Sci.* **2018**, 17 (12), 1816-1841. DOI: 10.1039/c7pp00395a

2 El Ghissassi, F.; Baan, R.; Straif, K.; Grosse, Y.; Secretan, B.; Bouvard, V.; Benbrahim-Tallaa, L.; Guha, N.; Freeman, C.; Galichet, L. *et al.* Cogliano, V. A Review of Human Carcinogens--Part D: Radiation. *Lancet Oncol.* **2009**, 10 (8), 751-752. DOI: 10.1016/s1470-2045(09)70213-x

3 Cadet, J.; Douki, T. Oxidatively Generated Damage to DNA by UVA Radiation in Cells and Human Skin. *J. Invest. Dermatol.* **2011**, 131 (5), 1005-1007. DOI: 10.1038/jid.2011.51

4 Braslavsky, S. E. Glossary of Terms Used in Photochemistry, 3rd edition (IUPAC Recommendations 2006). *Pure App. Chem.* **2007**, 79 (3), 293-465. DOI: 10.1351/pac200779030293

5 Baptista, M.S.; Cadet, J.; Di Mascio, P.; Ghogare, A. A.; Greer, A.; Hamblin, M. R.; Lorente, C.; Nunez, S. C.; Ribeiro, M. S.; Thomas, A. H. *et al.* Type I and Type II Photosensitized Oxidation Reactions: Guidelines and Mechanistic Pathways. *Photochem. Photobiol.* **2017**, 93 (4), 912-919. DOI: 10.1111/php.12716

6 Baptista, M. S.; Cadet, J.; Greer, A.; Thomas, A. H. Photosensitization Reactions of

---

Biomolecules: Definition, Targets and Mechanisms. *Photochem. Photobiol.* **2021**, 97 (6), 1456-1483. DOI: 10.1111/php.13470

7 Ziegler, I. Production of Pteridines During Hematopoiesis and T-lymphocyte Proliferation: Potential Participation in the Control of Cytokine Signal Transmission. *Med. Res. Rev*. **1990**, 10 (1), 95-114. DOI: 10.1002/med.2610100104

8 Nichol, C. A.; Smith, G. K.; Duch, D. S. Biosynthesis and Metabolism of Tetrahydrobiopterin and Molybdopterin. *Annu. Rev. Biochem*. **1985**, 54, 729-764. DOI: 10.1146/annurev.bi.54.070185.003501

9 Schallreuter, K. U.; Wood, J. M.; Pittelkow, M. R.; Gütlich, M.; Lemke, K. R.; Rödl, W.; Swanson, N. N.; Hitzemann, K.; Ziegler, I. Regulation of Melanin Biosynthesis in the Human Epidermis by Tetrahydrobiopterin. *Science* **1994**, 263 (5152), 1444-1446. DOI: 10.1126/science.8128228

10 Schallreuter, K. U.; Moore, J.; Wood, J. M.; Beazley, W. D.; Peters, E. M.; Marles, L. K.; Behrens-Williams, S. C.; Dummer, R.; Blau, N.; Thöny, B. Epidermal H(2)O(2) Accumulation Alters Tetrahydrobiopterin (6BH4) Recycling in Vitiligo: Identification of a General Mechanism in Regulation of all 6BH4-Dependent Processes?. *J. Invest. Dermatol*. **2001**, 116 (1), 167-174. DOI: 10.1046/j.1523-1747.2001.00220.x

11 Glassman, S. J. Vitiligo, Reactive Oxygen Species and T-cells. *Clin. Sci. (Lond).* **2011**, 120 (3), 99-120. DOI: 10.1042/CS20090603

12 Buglak, A. A.; Kapitonova, M. A.; Vechtomova, Y. L.; Telegina, T. A. Insights into Molecular Structure of Pterins Suitable for Biomedical Applications. *Int. J. Mol. Sci.* **2022**, 23 (23), 15222. DOI: 10.3390/ijms232315222

13 Wondrak, G. T.; Jacobson, M. K.; Jacobson, E. L. Endogenous UVA-Photosensitizers: Mediators of Skin Photodamage and Novel Targets for Skin Photoprotection. *Photochem. Photobiol. Sci.* **2006**, 5 (2), 215-237. DOI: 10.1039/b504573h

14 Bastos, E. L.; Quina, F. H.; Baptista, M. S. Endogenous Photosensitizers in Human Skin. *Chem. Rev*. **2023**, 123 (16), 9720-9785. DOI: 10.1021/acs.chemrev.2c00787

15 Lorente, C.; Thomas, A. H. Photophysics and Photochemistry of Pterins in Aqueous Solution. *Acc. Chem. Res*. **2006,** 39 (6), 395-402. DOI: 10.1021/ar050151c

16 Lorente, C.; Serrano, M. P.; Vignoni, M.; Dántola, M. L.; Thomas, A. H. A Model to Understand Type I Oxidations of Biomolecules Photosensitized by Pterins. *J. Photochem. Photobiol.* **2021**, 7, 100045. DOI: 10.1016/j.jpap.2021.100045

17 Serrano, M. P.; Lorente, C.; Vieyra, F. E.; Borsarelli, C. D.; Thomas, A. H. Photosensitizing Properties of Biopterin and its Photoproducts Using 2'-Deoxyguanosine 5'-Monophosphate as

---

an Oxidizable Target. *Phys. Chem. Chem. Phys*. **2012**, 14 (33), 11657-11665. DOI: 10.1039/c2cp41476g

18 Cadet, J.; Wagner, J. R.; Shafirovich, V.; Geacintov, N. E. One-Electron Oxidation Reactions of Purine and Pyrimidine Bases in Cellular DNA. *Int. J. Radiat. Biol.* **2014**, 90 (6), 423-432. DOI: 10.3109/09553002.2013.877176

19 Di Mascio, P.; Martinez, G. R.; Miyamoto, S.; Ronsein, G. E.; Medeiros, M. H. G.; Cadet, J. Singlet Molecular Oxygen Reactions with Nucleic Acids, Lipids, and Proteins. *Chem. Rev.* **2019**, 119 (3), 2043-2086. DOI: 10.1021/acs.chemrev.8b00554

20 Cadet, J.; Douki, T.; Ravanat, J. L. Oxidatively Generated Damage to the Guanine Moiety of DNA: Mechanistic Aspects and Formation in Cells. *Acc. Chem. Res.* **2008**, 41 (8), 1075-1083. DOI: 10.1021/ar700245e

21 Steenken, S.; Jovanovic, S. V. How Easily Oxidizable Is DNA? One-Electron Reduction Potentials of Adenosine and Guanosine Radicals in Aqueous Solution. *J. Am. Chem. Soc.* **1997**, 119 (3), 617–618. DOI: 10.1021/ja962255b

22 Yanagawa, H.; Ogawa, Y.; Ueno, M. Redox Ribonucleosides. Isolation and Characterization of 5-Hydroxyuridine, 8-Hydroxyguanosine, and 8-Hydroxyadenosine from Torula Yeast RNA. *J. Biol. Chem.* **1992**, 267 (19), 13320-13326. DOI: 10.1016/S0021-9258(18)42213-2

23 Burrows, C. J.; Muller, J. G. Oxidative Nucleobase Modifications Leading to Strand Scission. *Chem. Rev.* **1998**, 98 (3), 1109-1152. DOI: 10.1021/cr960421s

24 Ravanat, J. L.; Douki, T.; Cadet, J. Direct and Indirect Effects of UV Radiation on DNA and its Components. *J. Photochem. Photobiol. B.* **2001**, 63 (1-3), 88-102. DOI: 10.1016/s1011-1344(01)00206-8

25 Melvin, T.; Cunniffe, S. M.; O'Neill, P.; Parker, A. W.; Roldan-Arjona, T. Guanine is the Target for Direct Ionisation Damage in DNA, as Detected Using Excision Enzymes. *Nucleic Acids Res*. **1998**, 26(21), 4935-4942. DOI: 10.1093/nar/26.21.4935

26 Giese, B. Long-Distance Charge Transport in DNA: The Hopping Mechanism. *Acc. Chem. Res*. **2000**, 33 (9), 631-636. DOI: 10.1021/ar990040b

27 Hall, D. B.; Holmlin, R. E.; Barton, J. K. Oxidative DNA Damage Through Long-Range Electron Transfer. *Nature.* **1996**, 382 (6593), 731-735. DOI: 10.1038/382731a0

28 Schuster, G. B. Long-Range Charge Transfer in DNA: Transient Structural Distortions Control the Distance Dependence. *Acc. Chem. Res*. **2000**, 33 (4), 253-260. DOI: 10.1021/ar980059z

29 Wagner, J. R.; van Lier, J. E.; Berger, M.; Cadet, J. Thymidine Hydroperoxides: Structural Assignment, Conformational Features, and Thermal Decomposition in Water. *J. Am. Chem. Soc.* **1994**, 116 (6), 2235-2242. DOI: 10.1021/ja00085a001

---

30 Cadet, J.; Wagner, J. R. Oxidatively Generated Base Damage to Cellular DNA by Hydroxyl Radical and One-Electron Oxidants: Similarities and Differences. *Arch. Biochem. Biophys*. **2014**, 557, 47-54. DOI: 10.1016/j.abb.2014.05.001

31 von Sonntag, C.; Schuchmann, H. P. The Elucidation of Peroxyl Radical Reactions in Aqueous Solution with the Help of Radiation-Chemical Methods. *Angew. Chem. Int. Ed. Engl*. **1991**, 30 (10), 1229-1253. DOI: 10.1002/anie.199112291

32 von Sonntag, C.; Schuchmann, H. P. Peroxyl Radicals in Aqueous Solutions. In: *The Chemistry of Free Radicals: Peroxyl Radicals*, Z. B. Alfassi (Ed.), John W and Sons, 1997, pp. 173-234.

33 Serrano, M. P.; Vignoni, M.; Lorente, C; Vicendo, P.; Oliveros, E.; Thomas, A. H. Thymidine Radical Formation Via One-Electron Transfer Oxidation Photoinduced by Pterin: Mechanism and Products Characterization. *Free Radic. Biol. Med*. **2016**, 96, 418-431. DOI: 10.1016/j.freeradbiomed.2016.04.196

34 Serrano, M. P.; Estébanez, S.; Vignoni, M.; Lorente, C.; Vicendo, P.; Oliveros, E.; Thomas, A. H. Photosensitized Oxidation of 2´-Deoxyguanosine 5´-Monophosphate: Mechanism of the Competitive Reactions and Product Characterization. *New J. Chem.* **2017**, 41 (15), 7273-7282. DOI: 10.1039/c7nj00739f

35 Serrano, M. P.; Lorente, C.; Borsarelli, C. D.; Thomas, A. H. Unraveling the Degradation Mechanism of Purine Nucleotides Photosensitized by Pterins: The Role of Charge-Transfer Steps. *Chemphyschem*. **2015**, 16 (10), 2244-2252. DOI: 10.1002/cphc.201500219

36 Estébanez, S.; Lorente, C.; Tosato, M. G.; Miranda, M. A.; Marín, M. L.; Lhiaubet-Vallet, V.; Thomas, A. H. Photochemical Formation of a Fluorescent Thymidine-Pterin Adduct in DNA. *Dyes Pigm*. **2019**, 160, 624-632. DOI: 10.1016/j.dyepig.2018.08.024

37 Estébanez, S.; Thomas, A. H.; Lorente, C. Deoxythymidine-Pterin Fluorescent Adduct Formation through a Photosensitized Process. *Chemphyschem*. **2018**, 19 (3), 300-306. DOI: 10.1002/cphc.201701101

38 Estébanez, S.; Rivera, A. M.; Neyra Recky, J. R.; Thomas, A. H.; Lhiaubet-Vallet, V.; Lorente, C. Pterin-Photosensitization of Thymine Under Anaerobic Conditions in the Presence of Guanine. *Free Radic. Biol. Med*. **2021**, 174, 321-328. DOI: 10.1016/j.freeradbiomed.2021.07.032

39 Petroselli, G.; Dántola, M. L.; Cabrerizo, F. M.; Lorente, C; Braun, A. M.; Oliveros, E.; Thomas, A. H. Quenching of the Fluorescence of Aromatic Pterins by Deoxynucleotides. *J. Phys. Chem. A.* **2009**, 113 (9), 1794-1799. DOI: 10.1021/jp8101496

40 Ledbetter, J. W.; Pfleiderer, W.; Freisheim, J. H. Photosensitized Reduction of L-Biopterin in the Active Ternary Complex of Dihydrofolate Reductase. *Photochem. Photobiol.* **1995**, 62 (1),

71-81. DOI: 10.1111/j.1751-1097.1995.tb05241.x

41 Wilkinson, F.; Helman, W. P.; Ross, A. B. Rate Constants for the Decay and Reactions of the Lowest Electronically Excited Singlet State of Molecular Oxygen in Solution. An Expanded and Revised Compilation. *J. Phys. Chem. Ref. Data* **1995**, 24 (2), 663-677. DOI: 10.1063/1.555965

42 Bregnhøj, M.; Westberg, M.; Minaev, B. F.; Ogilby, P. R. Singlet Oxygen Photophysics in Liquid Solvents: Converging on a Unified Picture. *Acc. Chem. Res*. **2017**, 50(8), 1920-1927. DOI: 10.1021/acs.accounts.7b00169

43 Matheson, I.B.C.; Lee, J.; King, A. D. The Lifetime of Singlet Oxygen ($^1\Delta_g$) in Heavy Water, a Revised Value. *Chem. Phys. Lett.* **1978**, 55 (1), 49-51. DOI: 10.1016/0009-2614(78)85129-x

44 Thomas, A. H.; Serrano, M. P.; Rahal, V.; Vicendo, P.; Claparols, C.; Oliveros, E.; Lorente, C. Tryptophan Oxidation Photosensitized by Pterin. *Free Radic. Biol. Med.* **2013**, 63, 467-475. DOI: 10.1016/j.freeradbiomed.2013.05.044

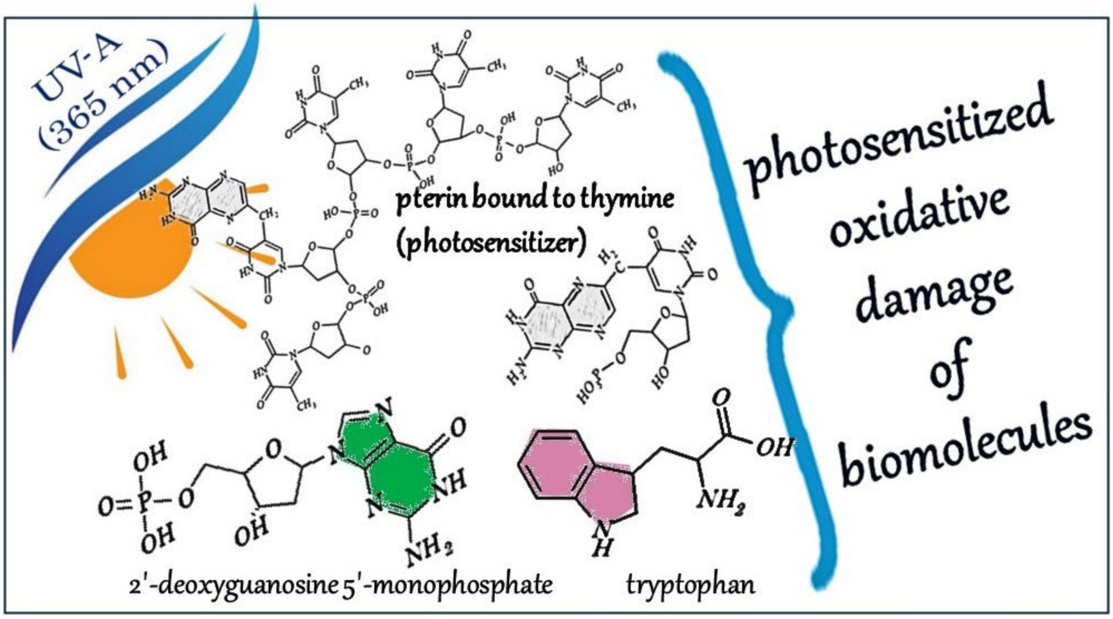
45 Petroselli, G.; Dántola, M. L.; Cabrerizo, F. M.; Capparelli, A. L.; Lorente, C.; Oliveros, E.; Thomas, A. H. Oxidation of 2'-Deoxyguanosine 5'-Monophosphate Photoinduced by Pterin: Type I versus Type II Mechanism. *J. Am. Chem. Soc*. **2008**, 130 (10), 3001-3011. DOI: 10.1021/ja075367j

46 Pattison, D. I.; Rahmanto, A. S.; Davies, M. J. Photooxidation of Proteins. *Photochem. Photobiol. Sci.* **2012**, 11 (1), 38-53. DOI: 10.1039/c1pp05164d

47 Rokhlenko, Y.; Geacintov, N. E.; Shafirovich, V. Lifetimes and Reaction Pathways of Guanine Radical Cations and Neutral Guanine Radicals in an Oligonucleotide in Aqueous Solutions. *J. Am. Chem. Soc.* **2012**, 134 (10), 4955-4962. DOI: 10.1021/ja212186w

48 Chatgilialoglu, C.; D’Angelantonio, M.; Kciuk, G.; Bobrowski, K. New Insights into the Reaction Paths of Hydroxyl Radicals with 2’-Deoxyguanosine. *Chem. Res. Toxicol*. **2011**, 24 (12), 2200-2206. DOI: 10.1021/tx2003245

TOC Graphic


UV-A
(365 nm)
pterin bound to thymine
(photosensitizer)
photosensitized oxidative damage of biomolecules
2'-deoxyguanosine 5'-monophosphate
tryptophan