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Orientation-dependent quenching of the triplet excited 6-thiopurine by nucleobases

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ABSTRACT

Nanosecond transient absorption and steady-state photochemical studies showed that interactions of the nucleic acid bases: uracil, thymine (5-methyluracil), 5-ethyluracil and adenine with triplet excited 9-substituted 6-thiopurine chromophore (TP) is influenced by the mutual orientation of the heterocyclic rings. In an aqueous solution containing a mixture of monomers, where no restrictions are imposed on ring-to-ring orientation, all the nucleobases quench the T_1 state of 9-propyl-6-thiopurine (PTP) with similarly large rate constants ($k_q^{inter} \sim 10^9\ M^{-1}\ s^{-1}$). Steady-state irradiation of TP in the presence of uridine and adenosine led to adducts formed via [2 + 2] cycloaddition of C=S to the olefinic fragments of the nucleobases. In newly synthesized dyads composed of trimethylene-linked TP and nucleobase pairs, only thymine and 5-ethyluracil reduced the TP-like T_1 state lifetime ($k_q^{intra} \sim 5 \times 10^6\ s^{-1}$). The relative orientations of the 6-thiopurine and nucleobase rings in the dyads are limited by the spacer. The length of the trimethylene chain does not allow for a close approach of the reactive centers for [2 + 2] photocycloadditions. In steady-state irradiation only the dyads containing thymine or 5-ethyluracil are photoreactive, and they form intramolecular cyclophane-type products albeit with low quantum yields ($\phi \leq 6 \times 10^{-3}$). The structure of the photoproducts can be rationalized by assuming an initial H atom abstraction from the 5-alkyl group at the C(5) position of the uracil ring by an excited thiocarbonyl group. The preferential reversal of biradicals formed from [2 + 2] photocycladditon and from H atom abstraction has been suggested as mechanisms responsible for quenching of the TP T_1 state by nucleobases.

1. Introduction

Utilizations of 6-thiopurine for structural studies of nucleic acids [1–3] and as a potential phototherapeutic agent [4,5] explore its photochemical reactivity in polynucleotides labeled with this modified, sulfur containing purine base. The 6-thiopurine chromophore absorbs at longer-wavelengths than do common, non-sulfur containing purine and pyrimidine nucleic acid bases. The thiocarbonyl group containing purine is therefore the primary excitation target upon UV-A irradiation of the biopolymeric systems containing it. Despite the importance of the photoreactions initiated by excited 6-thiopurine, the electronically excited states of the compound in such assembled systems have not been hitherto observed directly, and the dynamics of their depopulation have not been determined. The spectral, photophysical and photochemical studies have been confined to monomeric species: 6-thiopurine [6] in organic solvents, 6-thiopurine 2′,3′,5′-tri-O-acetylriboside (TI) in acetonitrile and in aqueous solution [7,8]. It has been found that the S_2 (π,π^*) state directly formed by irradiation within an intense absorption band (e.g.: TI in CH_3CN λ_{max} = 325 nm, ε_{max} = 22,500 $M^{-1}\ cm^{-1}$ [7]) undergoes efficient and fast relaxation to the lowest T_1 (π,π^*) state ($\phi_T = 1$ [6]).

Extrapolations of the photophysical characteristics of a monomer to a polymeric system may not be, however, appropriate. Indeed, a number of early as well as recent papers on the photophysics of non-modified bases containing dinucleotides, oligonucleotides and more structurally complicated related systems have demonstrated that the deactivation channels of the singlet excited state reached directly by the light absorption are altered in oligomers. This is due to the interactions such as base–base stacking in the polymer and, possibly, to the formation of H-bonded base pairs in nucleic acids duplexes [9–12]. As a consequence, the electronic nature and reactivity of the excited state involved in photochemistry may be changed upon incorporation of the monomer into a biomacromolecule [13]. The results of the very recent femtosecond time-resolved studies of several

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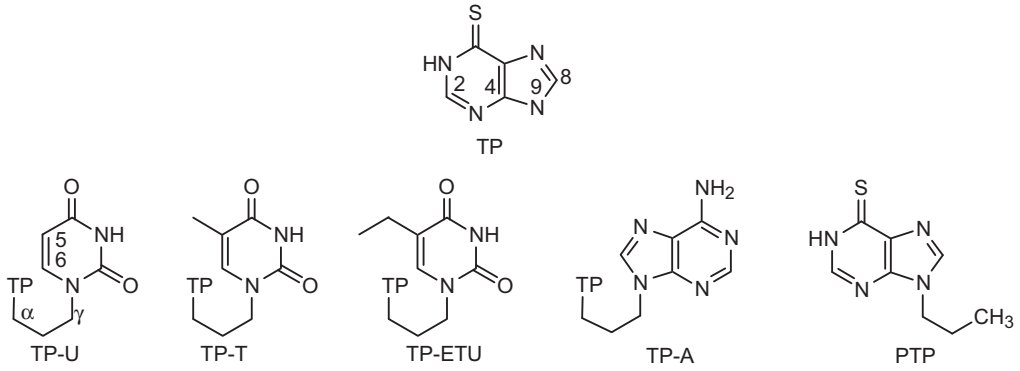


Chart 1. Structures of the compounds and atom numbering.

diribonucleoside monophosphates ApA, ApG, ApC, ApU, and CpG have been considered as evidence for the formation of singlet exciplexes with lifetimes much longer (ps timescale) than those of the singlet excited states of the monomers (fs timescale) [11]. Because of the fundamental relevance of the photophysics of nucleic acid to an understanding of the mechanism of the UV induced photodamage in living systems, most studies have been performed on shorter or longer biopolymer fragments composed of canonical purine and pyrimidine nucleosides. The photophysics of oligonucleotides containing thiocarbonyl derivatives of the nucleobases have received very little attention. To our knowledge, only a single report, concerning the photophysics of tRNA's containing the 4-thiouracil riboside (TUrd), has appeared [14]. The T_1 (π,π^*) excited state of TUrd is the photoreactive state both in photoreactions of tRNA and in solutions of the monomers. The estimated intersystem crossing yield for TUrd in tRNA is ~1/3 of that determined for monomeric 4-thiouridine ($\phi_T = 1$ [2]). The triplet lifetime of TUrd increases when it is in tRNA as compared to the free monomer in solution. The rigidity of the structure has been suggested as a possible reason for the ϕ_T decrease and the T_1 state lifetime lengthening when TUrd is inserted into tRNA [14].

In this paper we report the results on our study of a series of a newly synthesized TP-nucleobase dyads using nanosecond laser flash photolysis (LFP) and steady-state photochemistry. The dyads are simple models of dinucleotides, and they are composed of covalently linked 6-thiopurine and a common nucleobase (uracil, thymine, adenine) or 5-ethyluracil, a system of relevance with respect to its potent antiviral properties [15]. The structures of the compounds and the acronyms used throughout this paper are presented in Chart 1. All studies were performed in biologically important aqueous media. The results obtained for the dinucleotide models are discussed in relation to the monomeric chromophores.

2. Experimental

2.1. Materials

Synthesis of 9-propyl-6-thiopurine (PTP), TP-T and 1-propyluracil, 1-propylthymine, 1-propyl-5-ethyluracil, 9-propyladenine has been described previously [12,16]. TP-U, TP-ETU and TP-A were synthesized by the procedure analogous to that described for TP-T [16]. The details of the isolation and purification procedure, and selected spectral data for the dyads are presented in Supplementary data. The site of the attachment of the trimethylene spacer to the N-9 position of the 6-thiopurine ring was unequivocally proven by the observation of the long-range ^1H–^{13}C coupling between the 6-thiopurine α-methylene proton and the purine ring C4 atom in the ^1H–^{13}C HMBC spectrum.

2.2. Methods

2.2.1. Instrumentation

The UV absorption spectra were recorded with a Cary 300 Bio (Varian) spectrophotometer. The NMR spectra were measured at 400 MHz (^1H) with Bruker AVANCE II or at 600 MHz (^1H) with Bruker AVANCE spectrometers. The chemical shifts (δ) are in ppm relative to TMS. High-resolution MS spectra (MALDI) were measured on Q-Tof spectrometer. HPLC chromatography was performed on a Waters 600 E instrument equipped with a Waters 991 Photodiode Array UV–VIS detector (PDD) using a reversed phase column: Waters X-Terra RP18 (5 μm; 4.6 mm × 150 mm) for analytical runs and Waters X-Terra RP18 (7 μm; 7.8 mm × 150 mm) for preparative separations. A gradient of CH_3CN in H_2O was used as an eluent. Water was doubly distilled and purified using Simplicity Ultrapure Water System (Millipore).

2.2.2. Nanosecond laser flash photolysis

The nanosecond laser flash photolysis setup and its data acquisition system have been described in detail [17]. All experiments were carried out in a rectangular quartz optical cell (1 cm × 1 cm) using argon-saturated solutions in a phosphate buffer (0.01 M, pH 5.8). The samples were excited with 1–5 mJ laser pulses at λ = 355 nm.

2.2.3. Analytical scale irradiation and quantum yield determination

Solutions (2.6 mL) of TP-U, TP-T, TP-ETU and TP-A ($c \sim 1 \times 10^{-4}$ M) in phosphate buffer were placed in a 1 cm × 1 cm UV cell and deoxygenated by bubbling with argon. Irradiations were performed at λ = 351 nm with an argon ion laser (Innova) equipped with a home-built selector based on a double Pellin–Brocka prism. For quantum yield determinations, the samples were irradiated with the λ = 313 nm line, isolated from a high-pressure mercury lamp. Irradiation was continued to a low substrate conversion, and concentration changes were determined by HPLC analyses. Uranyl oxalate actinometry was used [18].

2.2.4. Preparative irradiations

The compound TP-T (15 mg) was dissolved in water (~500 mL), and the solution was irradiated in portions (80 mL) with a 150-W high pressure immersion mercury lamp through a pyrex filter under an argon atmosphere. Irradiation was continued to ca. 60% conversion of the substrate. The irradiated solutions were collected and concentrated under reduced pressure. An analogous procedure was used in preparative irradiation of TP-ETU. The compound HP-CH_2-U, the single product formed from TP-T, was isolated by preparative HPLC using 5% aq. CH_3CN in the isocratic mode (10 min), followed by gradient (5% aq. $CH_3CN \rightarrow$ (15 min)

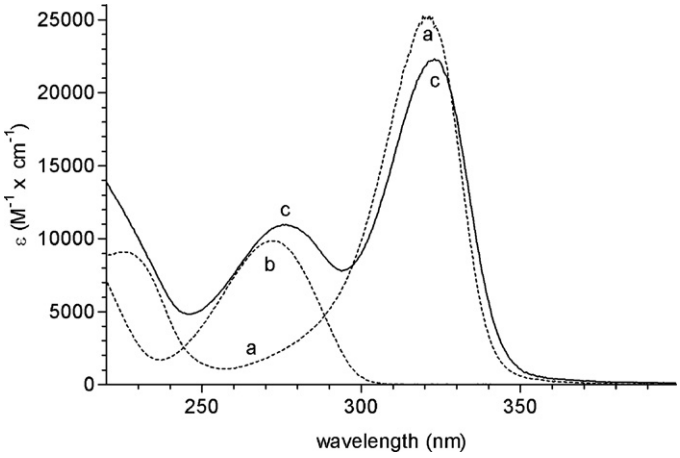


Fig. 1. The UV absorption spectrum of (a) PTP, (b) 1-propylthymine, (c) TP-T in 0.01 M phosphate buffer (pH 5.8).

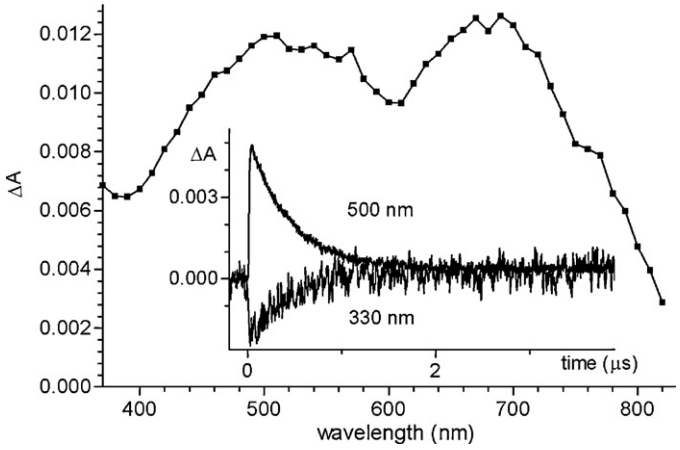


Fig. 2. The transient absorption spectrum of PTP ($c = 6.2 \times 10^{-4}$ M) in argon saturated phosphate buffer, $\lambda_{exc} = 355$ nm. Inset: kinetics of PTP ($c = 3.2 \times 10^{-4}$ M) 500 nm-absorption decay ($\tau = 440$ ns) and 330 nm-growth ($\tau = 450$ ns) measured in argon-saturated buffer solution (pH 5.8).

28% CH_3CN). The compound HP-CH(CH_3)-U, the dominant photoproduct obtained from TP-ETU, was separated from the mixture by two step HPLC chromatography using a gradient (5% aq. $CH_3CN \rightarrow$ (15 min) 28% CH_3CN) and finally an isocratic elution with 5% aq. CH_3CN.

Photoproduct HP-CH_2-U isolated yield 70%; UV (H_2O) $\lambda_{max} = 276$ nm $\varepsilon_{max} = 11{,}000\ M^{-1}\ cm^{-1}$; HR-MS (Maldi) calcd for $C_{13}H_{13}N_6O_3$ ($M-H^+$) 301.1049, found 301.1061; NMR chemical shifts of ^1H and ^{13}C in Section 3.3.

Photoproduct HP-CH(CH_3)-U isolated (non-optimized) yield 40%; UV (H_2O) $\lambda_{max} = 276$ nm; HR-MS (Maldi) calcd for $C_{14}H_{15}N_6O_3$ ($M-H^+$) 315.1206, found 315.1217. NMR chemical shifts of ^1H and ^{13}C in signals in Section 3.3.

3. Results and discussion

3.1. Ground state absorption

The UV absorption spectra of the TP-T dyad chosen as an example, and its monomeric components: PTP and 1-propylthymine, all measured in buffered aqueous solution (pH 5.8) are presented in Fig. 1. Under these pH conditions, 6-thiopurine and the nucleobase residues in their ground states (S_0) exist exclusively in their neutral form ($pK_a = 7.8$ for PTP from spectrophotometric titration, pK_a nucleobases [19]). The TP-T dyad absorption maxima are slightly red-shifted ($\leq$3 nm), and its overall absorption intensity is weaker than sum of the components (PTP + 1-propylthymine). The same conclusion was reached from the quantitative inspection of the UV absorption spectra of the remaining dyads and their components. A wide variety of dinucleosides and trimethylene bridged base-base pairs have been shown previously to exhibit similar UV absorption properties in aqueous solution. The changes in the UV spectra of the bichromophoric compounds with respect to the sum of their components have been usually expressed by hypochromism (%H) and were related to the vertical base–base interaction (stacking), which perturbs the electronic transitions [12,13]. It may be therefore anticipated that in the ground state (S_0) a fraction of TP-U, TP-T, TP-A and TP-ETU dyads (%H values = 7.5–11.5%, Table 1, Supplementary data) exists in a folded molecular conformation in which the TP and the nucleobase are stacked, i.e. the heterocyclic rings of the bases are in parallel planes. It should be noticed that %H values do not describe quantitatively the contribution of the folded conformation in a conformational equilibrium in the S_0 state.

3.2. Nanosecond laser flash photolysis

The laser flash photolysis experiments have been performed in the first place for PTP. The transient absorption spectrum obtained for PTP in argon-saturated solution is presented in Fig. 2.

In the region 370–800 nm the spectrum features two bands with maximum at 520 nm and 680 nm. The transient absorption extends to shorter wavelengths overlapping the absorption of PTP in its S_0 state because bleaching of the ground state signal could be observed only below $\lambda \leq 330$ nm, where the absorption of PTP is strong. The shapes of the transient absorption bands do not depend on the delay time, the PTP concentration and the power (1–5 mJ) of the 355 nm laser pulse. The transient absorption decays monoexponentially, and the decay kinetics do not depend on the monitoring wavelengths. The single species responsible for the observed transient spectra was identified as the T_1 excited state of PTP. This assignment was based on the experimentally observed quenching by oxygen

Table 1
Photophysical and photochemical properties of the T_1 state of PTP and dyads in buffer solution.

Comp.	τ_T^0 (ns)[a]	$k_{sq} \times 10^{-9}$ ($M^{-1}\ s^{-1}$)[a]	$\phi_T \times \varepsilon_T$ ($M^{-1}\ cm^{-1}$)[b]	$k_q^{inter} \times 10^{-9}$ ($M^{-1}\ s^{-1}$)[c]		$k_q^{intra} \times 10^{-6}$ (s^{-1})[d]
PTP	1160 ± 50	4.4 ± 0.2	2800	–		–
TP-U	1090 ± 40	3.5 ± 0.2	2700	1.0	0.8 (Urd [8])	–
TP-T	150 ± 10	3.5 ± 0.3	2800	1.5		5.9
TP-ETU	200 ± 10	3.5 ± 0.2	2800	1.5		4.0
TP-A	1180 ± 40	1.9 ± 0.4	2700	1.3	1.2 (Ado [8])	–

[a] From S-V plots of $1/\tau$ vs. concentration of the solutions; concentration range: PTP $c = (4.2–12.3) \times 10^{-4}$ M, TP-U and TP-T $c = (1–4) \times 10^{-4}$ M, TP-A $c = (2–5) \times 10^{-4}$ M, τ_T^0 for TP-ETU calculated from $1/\tau = (1/\tau^0) + k_{sq} \times c_{TP\text{-}ETU}$, using lifetime ($\tau_T$) from measurement at single concentration ($c = 3.8 \times 10^{-4}$ M, $\tau = 160$ ns) and assuming k_{sq} (TP-ETU) = k_{sq} (TP-U).

[b] From Eq. (1), ε_T at $\lambda = 500$ nm.

[c] From S-V plots of $1/\tau$ vs. concentration of 1-propyl derivatives of U, T, ETU, 9-methyl adenine, PTP $c = 4.2 \times 10^{-4}$ M.

[d] Rate constant for intramolecular quenching from Eq. (2).

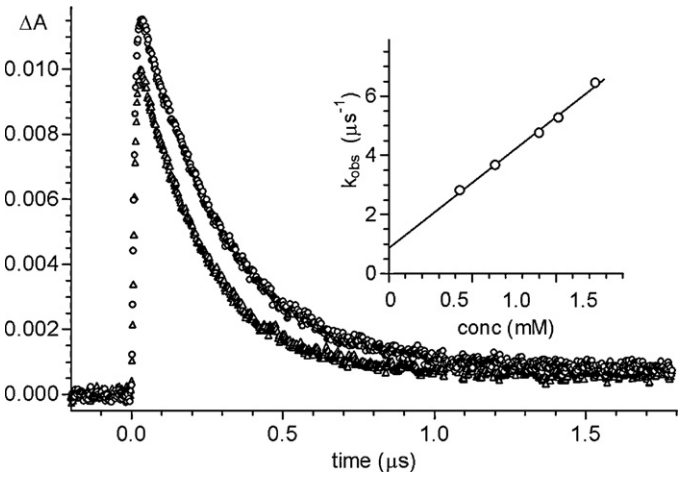


Fig. 3. Kinetic traces of the 500-nm absorbance recorded for argon-saturated and air-equilibrated solution of PTP ($c = 6.2 \times 10^{-4}$ M) in phosphate buffer, $\tau = 280$ ns in argon, $\tau = 220$ ns in air. Inset: the Stern-Volmer plot of k_{obs} (500 nm) vs. PTP concentration in argon-saturated buffer solution.

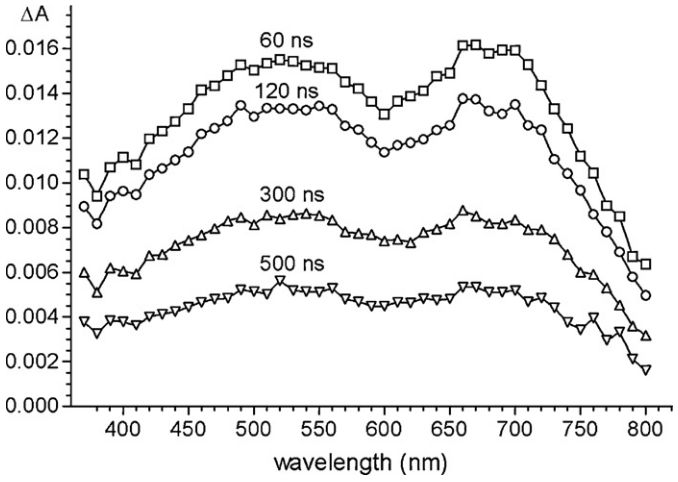


Fig. 4. The transient absorption spectrum of TP-U ($c = 3.9 \times 10^{-4}$ M) in argon saturated phosphate buffer, $\lambda_{exc} = 355$ nm recorded at various delay time.

(Fig. 3) and on the dependence of the transient absorption decay on the concentration of the compound (self-quenching) (Fig. 3 inset).

The rate constant for the quenching of the transient species by oxygen, $k_{O_2} \approx 4 \times 10^9\ M^{-1}\ s^{-1}$ was estimated from the experimental data and the oxygen concentration in aqueous solution ($c_{O_2} = 2.9 \times 10^{-4}$ M [18]). The large value of k_{O2}, approaching the diffusion rate constant ($k_{diff} = 6.5 \times 10^9\ M^{-1}\ s^{-1}$ in water) is characteristic for the quenching of the T_1 state of thiocarbonyl compounds by molecular oxygen [6,7,20]. The 500-nm absorption decays and 330-nm absorption grows to $\Delta A = 0$ with rate constants identical within experimental error. The plot shown as inset in Fig. 2 evidences that the T_1 state returns the S_0 state of PTP quantitatively.

Using benzophenone as a reference compound, the value of $\phi_T \times \varepsilon_T$ (Table 1) were determined from the relation

$$\phi_T \times \varepsilon_T = \left(\frac{A}{A^{REF}}\right) \times \phi_T^{REF} \times \varepsilon_T^{REF} \quad (1)$$

where ϕ_T and ε_T are the quantum yield of triplet formation and the triplet-triplet absorption coefficient, respectively, of PTP. The ϕ_T^{REF} and ε_T^{REF} refer to the triplet yield and extinction coefficient, respectively, of benzophenone in acetonitrile ($\phi_T^{BP} = 1$; $\varepsilon_T^{BP} = 6500\ M^{-1}\ cm^{-1}$ at 520 nm [18,21]).

The concentration-independent T_1 state lifetime (τ_T^0) and the self-quenching rate constant (k_{sq}) for PTP were determined from kinetic measurements at $\lambda = 520$ nm performed for a series of solutions of PTP at concentrations in the range of $(4.2–12.3) \times 10^{-4}$ M. The experimental data were analyzed according to the Stern–Volmer formalism, and the results are presented in Table 1. The value of τ_T^0 and k_{sq} were obtained, respectively, from the inverse intercept and the slope of the observed linear plot of the decay rate constant vs. the PTP concentration (Fig. 3 inset).

In the presence of N-1 propyl substituted uracil, thymine, 5-ethyluracil and N-9-propyladenine, the decay of the PTP triplet-triplet absorption was accelerated. The products resulting from the PTP T_1 state quenching could not be observed in these LFP experiments. The transient absorption decay can be satisfactorily described by a single exponential function irrespective of the monitoring wavelengths within the 370–800 nm spectral range. Kinetic measurements have been performed at $\lambda = 520$ nm using solutions containing PTP (4.0×10^{-4} M) and various concentrations of the N-alkylated nucleobases. The intermolecular quenching rate constants (k_q^{inter}) determined from Stern–Volmer analysis of the results obtained from LFP experiments are presented in Table 1.

In general, the values of bimolecular rate constants ($k_q^{inter} \sim 10^9\ M^{-1}\ s^{-1}$) are similar for all of the N-alkylated nucleobases under study, and these measured rate constants are very close to those determined previously from the quenching of the 6-thiopurine riboside T_1 state by uridine (Urd) and adenosine (Ado) in buffer solution ($0.8 \times 10^9\ M^{-1}s^{-1}$ Urd, $1.2 \times 10^9\ M^{-1}s^{-1}$ Ado [8]).

The transient spectra obtained upon 355 nm excitation of the dyads TP-T, TP-U, TP-ETU, and TP-A resemble that obtained for PTP, both with respect to the location of the band maxima and the band shapes. The spectrum of TP-U, as a typical example, is presented in Fig. 4.

The analysis of kinetic curves, at the short-wavelength (520 nm) and long-wavelength (700 nm) absorption bands, gave, within experimental error, the same lifetime, indicating that only one transient was present. The similarity of the transient spectra of the dyads and PTP suggests that the identities of the observed transient species are the same in all cases and that they are associated with T_1 excited state of 6-thiopurine chromophore. Measurements of the 500 nm absorption decay rate constants as a function of the concentration of the dyads, using the Stern–Volmer formalism, gave the intrinsic lifetimes (τ_T^0) and the self-quenching rate constants (k_{sq}) for the T_1 excited states of the dyads. The obtained results are presented in Table 1. It is worth noting that the $\phi_T \times \varepsilon_T$ values determined for the dyads (Table 1) are the same as that found for PTP. This can be interpreted as an indication that the singlet excited state obtained by direct light absorption either by the folded conformation or other, non-stacked conformation of the TP-nucleobase dyads relaxed to the 6-thiopurine T_1 state with equal efficiency as that of the PTP monomer. An alternative explanation would be that, in the conformational equilibria of the dyads in their S_0 states, the population of their stacked form is small.

As shown in Table 1, the intrinsic lifetimes of the T_1 states (τ_T^0) obtained for TP-U and TP-A are very similar to that for the monomer PTP. This is contrasted by the significant shortening of τ_T^0 found for the dyads TP-T and TP-ETU where the 6-thiopurine T_1 states were quenched selectively by the 5-methyl- and 5-ethyl substituents containing T and ETU neighbours. The first-order rate constants for intramolecular quenching of the 6-thiopurine T_1 states by T and ETU (k_q^{intra}) in the dyads TP-T and TP-ETU were calculated from the relation:

$$k_q^{intra} = \frac{1}{\tau_T^0}(\text{dyad}) - \frac{1}{\tau_T^0}(\text{PTP}) \quad (2)$$

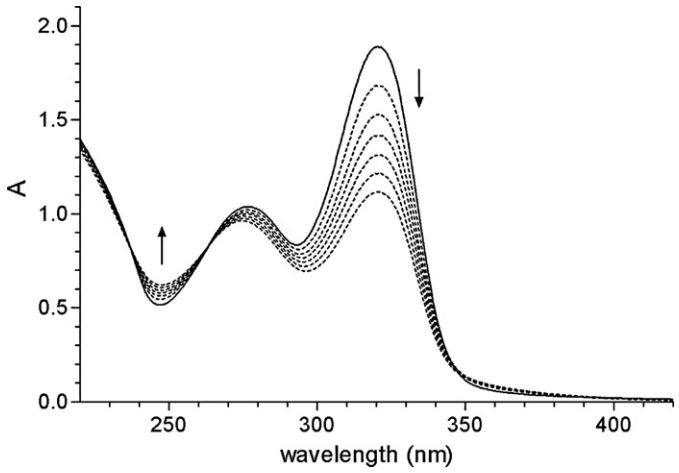


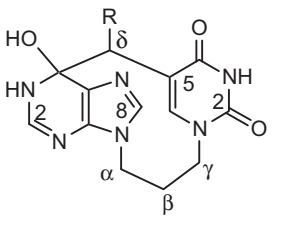
Fig. 5. Changes in the absorption spectra of the argon-saturated solution of TP-T ($c = 0.85 \times 10^{-4}$ M) in phosphate buffer (pH 5.8) before (solid line) and after irradiation (dotted lines) at $\lambda = 330$ nm; spectra measured every 0.5 h.

where τ_T^0 (dyad) is the intrinsic T_1 lifetime of TP-T or TP-ETU. The values are included in Table 1.

3.3. Stationary photochemistry

Dilute solutions ($1–1.5 \times 10^{-4}$ M) of the dyads TP-U, TP-A, TP-T and TP-ETU in argon-saturated phosphate buffer (pH 5.8) were irradiated with 350 nm light. The progress of the reaction was monitored by UV spectroscopy and by HPLC. The uracil and adenine containing dyads TP-U and TP-A were stable even under prolonged irradiation ($\phi_R < 5 \times 10^{-4}$). In contrast, the dyads containing 5-alkyluracil: thymine and 5-ethyluracil undergo photoreaction with quantum yields $\phi_R = 0.001$ (TP-T) and $\phi_R = 0.006$ (TP-ETU). The formation of the photoproducts caused a gradual decrease in the absorbance of the long-wavelength absorption band characteristic of 6-thiopurine residue (Fig. 5).

The photochemical reaction of TP-T was quenched by KI. The Stern–Volmer quenching constant determined for TP-T solution ($c = 1 \times 10^{-4}$ M) equals $K = k_q \times \tau = 544.5\ M^{-1}$. The lifetime of the photochemically reactive precursor, $\tau = 147$ ns, was calculated by substituting $k_q = 3.7 \times 10^9\ M^{-1}\ s^{-1}$ for the quenching of the T_1 state of PTP by iodide anions determined in a LFP experiment. The lifetime of the reactive state is very similar to that of the T_1 state (141 ns) calculated from the data in Table 1 for a 1×10^{-4} M solution of TP-T proving that the photoproducts arise from the excited triplet state. A single photoproduct (HP-CH_2-U) in the case of TP-T and one predominate product (HP-CH(CH_3)-U) in the case of TP-ETU were detected by chromatographic analysis (HPLC) of the photolysed solutions (Fig. 1 Supplementary data). The structures of the photoproducts are presented in Chart 2.



R = H HP-CH_2-U

R = CH_3 HP-CH(CH_3)-U

Chart 2. Structures of the photoproducts.

The photoproducts are chiral compounds due to the presence of asymmetric atoms: a purine C-6 bearing the hydroxyl group in HP-CH_2-U, and, additionally, a carbon atom C-δ in HP-CH(CH_3)-U molecules. The products were isolated by preparative HPLC of the photolysed solution as a racemate (CD spectrum did not show the Cotton effect) and a 3:1 mixture of diasteroisomers, respectively. The main diastereoisomer of HP-CH(CH_3)-U was separated from the mixture of diastereoisomers by repeated high-performance chromatography.

The structure of the photoproducts was determined on the basis of the results of spectroscopic studies. The UV spectra of HP-CH_2-U and HP-CH(CH_3)-U show a single broad absorption band with a maximum at 276 nm indicating the lack of the thiocarbonyl chromophore (Fig. 1b, Supplementary data). The exact mass of the ($M–H^+$) ion observed in Maldi HR-MS spectra of the photoproducts was 16 amu less than for that of the ($M–H^+$) ion of the respective substrate. This is consistent with the replacement of sulfur by an oxygen atom. The presence of a hydroxyl substituent in the photoproducts results from a dark reaction: –SH → –OH, occurring on the minute-time scale. There are several reported examples of such transformation in aqueous solution of related compounds containing thiol group attached to C(sp^3), e.g.: transformation of 4-mercapto-3-methyl-3,4-dihydrouracil into stable, isolated 4-hydroxy-3-methyl-3,4-dihydrouracil derivative [22]. However, it should be pointed out that 3,4-dihydro-4-mercaptouracil residue [2], which possesses N(3)-H adjacent to C(4)-SH undergoes hydrogen sulfide elimination instead of >C-SH → >C-OH substitution. The stability of the photoproducts HP-CH_2-U and HP-CH(CH_3)-U, and their thiolated precursors towards elimination reaction (H_2O or H_2S, respectively) may be likely due to steric constrains imposed by both methylene bridge linking purine C(6) and uracil C(5) atoms, and trimethylene bridge connecting purine N(9) and uracil N(1) atoms (Chart 2).

The structure of cyclophane-type photoproducts containing uracil (U) and a 6-hydroxy-1,6-dihydropurine ring (HP) linked by both trimethylene and methylene chains was unambiguously established on the basis of the NMR data. The assignments of all ^1H and ^{13}C signals were performed on the basis of chemical shifts and $^1H–^1H$ COSY, $^1H–^{13}C$ HSQC and $^1H–^{13}C$ HMBC spectra. The chemical shifts and signal assignments are presented in Table 2.

3.4. Mechanism of intramolecular and intermolecular quenching of 6-thiopurine

The results presented above demonstrate the diverse ability of nucleobases to act as quenchers of the triplet excited 6-thiopurine moiety in intramolecular and intermolecular processes. The bimolecular rate constants (k_q^{inter}) for quenching of PTP by N-propyl derivatives of U, T, ETU and A were all similarly large, amounting ca. 1/6 of the diffusion-controlled rate constant (Table 1). In contrast, in an intramolecular process, in dyads, only T and ETU were effective quenchers. Unfortunately, the mechanisms responsible for the quenching in both inter- and intramolecular systems could not be unequivocally established because the products of the quenching could not be observed by transient absorption measurements. However, simple energetic considerations preclude a triplet–triplet energy-transfer mechanism because the energies of the T_1 states of common nucleobases and their derivatives (e.g. ribosides) are higher by ≥5 kcal/mol than the triplet-state energies of the 6-thiopurine nucleoside or 9-methyl-6-thiopurine [7,23,24].

Important conclusions concerning the mechanism of the quenching can be drawn from stationary photochemical studies and stereochemical considerations. We have demonstrated recently that the T_1 state of the nucleoside thioinosine (TI, 6-thiopurine riboside) reacts in an intermolecular process with

Table 2
^{1}H and ^{13}C NMR chemical shifts[a] (in ppm) of the photoproducts and dyad TP-ETU.

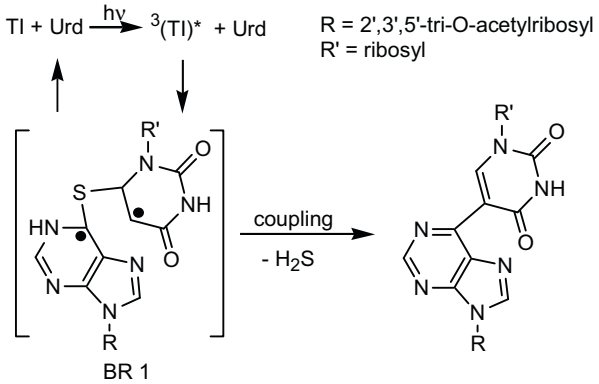
H atom	HP-CH_2-U	HP-CH(CH_3)-U	TP-ETU
P8	7.36 (s, 1)	7.76 (s, 1)	9.34 (s, 1)
P2	6.97 (s, 1)	7.03 (s, 1)	8.42 (s, 1)
U6	4.84 (s, 1)	5.08 (s, 1)	7.37 (s, 1)
γH	4.25 and 3.13 (2m, total 2)	4.28 and 3.17 (2m, total 2)	4.03 (m, 2)
αH	4.13 (m, 2)	4.21 (m, 2)	4.66 (m, 2)
δH	2.80 and 2.35 (2d, total 2)	2.80 (q, 1)	2.45 (q, 2)
βH	2.14 (m, 2)	2.20 (m, 2)	2.56 (m, 2)
δCH_3	–	1.45 (d, 3)	1.19 (t, 3)

C atom	HP-CH_2-U[b]	HP-CH(CH_3)-U[b]	TP-ETU
U4	167.0	165.7	168.4
U2	152.4	151.5	154.3
U6	148.9	148.8	143.4
P2	146.9	147.1	150.7
P4	141.8	140.2	143.9
P8	137.2	136.2	142.3
P5	120.2	119.9	128.4
U5	107.8	109.1	120.7
P6	88.1	87.6	174.8
γC	46.6	46.1	47.5
αC	43.7	43.5	46.0
δC	39.4	47.9	21.17
βC	26.8	26.5	30.2
δCH_3	–	8.31	12.7

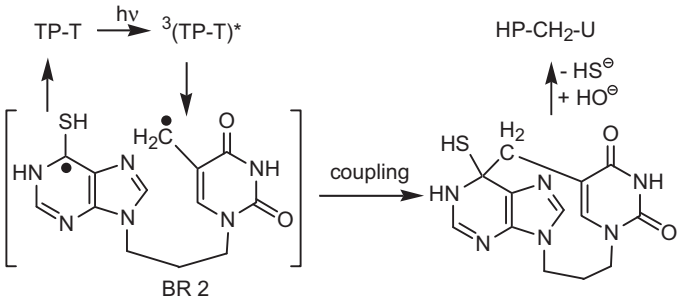
[a] Spectra of photoproducts measured in D_2O at ambient temperature (HP-CH_2-U) or at 10 °C (HP-CH(CH_3)-U), spectra of TP-ETU in TFA at ambient temperature.
[b] Chemical shifts of carbon atoms in photoproducts were determined from HSQC and HMBC spectra.

uridine (Urd, uracil riboside) and with adenosine (Ado, adenine riboside) via [2+2] cycloadditions of the C=S group of TI to the olefinic fragments of Urd and Ado [8]. The structure of the identified stable adduct obtained from Urd is presented, as an example, in Scheme 1. The inefficiency of the formation of the photoadducts could be ascribed to a preferential reversal of the initially formed biradicals (e.g.: BR 1, Scheme 1) to generate the reactants [8].

The formation of BR1 requires the close approach of the reactive molecular fragments within the TI T_1 state lifetime. In solutions containing monomers: TI (and PTP) and each of the nucleobase derivative (Urd, Ado, 1-propyl derivative of U, T, ETU, 9-propyl adenine), there was apparently no obvious restriction in the relative orientation of the 6-thiopurine-nucleobase rings, and the required spatial alignment of the reactive centers was attainable. In the dyads TP-U, TP-T, TP-ETU, TP-A, the relative orientations of 6-thiopurine and nucleobase rings are limited by the length of the trimethylene spacer. Inspection of ball and stick molecular models shows that the spacer is too short to allow for the approach of the



Scheme 1.



Scheme 2.

reactive double bonds of nucleobases C(5)=C(6) in U, N(7)=C(8) in A into close proximity of the thiocarbonyl group. This would explain the inefficiency of uracil and adenine to quench the T_1 excited state of the dyads TP-U and TP-A.

The length of the trimethylene chain is however sufficient to allow for the methyl and ethyl substituents at carbon atom C(5) of the T and ETU to approach closely the C=S group. The H atom abstraction from these alkyl groups by an excited thiocarbonyl group to form an intramolecular biradical can be postulated as the quenching mechanism. Similar biradical formed by H atom abstraction by an excited 4-thiopyrimidine from the methyl group of thymine was previously postulated as an intermediate in formation of some products isolated from irradiated aqueous solution of dinucleoside phosphates or methylphosphonates [2,22]. It is important to emphasize that no permanent photochemistry was observed upon excitation of the TP-T and TP-ETU dyads at λ = 355 nm in LFP experiments. This indicates that the major process undergone by the biradicals, e.g. BR 2 from TP-T presented in Scheme 2, was reversion to the substrates. The suggested mechanism of the quenching process in the dyads is confirmed by the observed selectivity of quenching (only T and ETU act as quenchers), by the structures of isolated photoproducts HP-CH_2-U and HP-CH(CH_3)-U (Chart 2) and by the rather low quantum yields of their formation. It is interesting to note that main photoproduct isolated by Woisard et al. [3] from irradiated aqueous solution of thymidylyl-(5′-3′)-2′-deoxy-6-thioinosine resulted from (2+2) cycloaddition of 6-thioinosine C=S and thymine C(5)=C(6) bonds. However sugar-phosphate spacer is longer than trimethylene bridge in the dyads TP-U, TP-T and TP-ETU, and it is of sufficient length to allow dinucleoside phosphate molecule to adopt a conformation favorable for the intramolecular (2+2) photocycloaddition [3].

Hydrogen atom abstraction has been found to be an important deactivation pathway of the triplet excited states of some ketones and thioketones (e.g. benzophenone [25–28], aralkylthiones [29]) either of n,π^* configurations or of π,π^* configurations (e.g. 2-benzoylthiophene [30]). The pair of radicals produced in the intermolecular processes and the biradicals formed by intramolecular H abstractions in some of these systems decay predominately or in some cases, almost exclusively, by back hydrogen transfer [27–30]. A reversion of the 1,4-biradical intermediates initially formed in the photocycloaddition reactions between olefins and the triplet excited thiones [31,32] or ketones [33] are usually the key argument explaining the inefficiencies in photoproduct formation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jphotochem.2010.09.018.

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