Selective Photoexcitation of the Thione and Thiol Forms of *N*-Hydroxypyridine-4(1*H*)-Thione: A Tautomeric Heteroaromatic System

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Received June 10, 1999. Revised Manuscript Received August 20, 1999

Abstract: The strong solvent-dependence of the tautomeric equilibrium exhibited by *N*-hydroxypyridine-4(1*H*)-thione (4-NHPT) and the distinct absorption properties of the thione and thiol tautomers offer the rare opportunity to investigate the photochemistry of each form independently. Laser flash photolysis studies have revealed that triplet state formation was the main photoprocess undergone by the thione tautomer of 4-NHPT in protic solvents ($\Phi_T = 0.90$, $\lambda_{exc} = 355$ nm), whereas pulsed excitation ($\lambda_{exc} = 308$ nm) of the thiol form in apolar media resulted in a less efficient intersystem crossing ($\Phi_T = 0.19$) accompanied by homolytic S−H bond cleavage ($\Phi_{S-H} = 0.24$). The latter process leads to the production of the *N*-oxy-4-pyridinethiyl radical (4-PyNOS•). In most organic solvents, however, the thione and thiol tautomers of 4-NHPT coexist. Under these conditions, the form absorbing the excitation light was observed to undergo the expected primary photochemistry followed by secondary processes involving reaction with the ground state of the nonexcited tautomer (i.e., electron transfer from the thione triplet state to the thiol form and addition of 4-PyNOS• to the carbon−sulfur double bond of the tautomeric thione). Contrary to the closely related *N*-hydroxypyridine-2(1*H*)-thione, 4-NHPT was not found to be a primary photochemical precursor of hydroxyl radicals (•OH) in organic solvents. The results obtained in this work are discussed in terms of structure/photoreactivity and with regard to the reported photobiological effects of *N*-hydroxypyridinethiones.

Introduction

Tautomerism of heteroaromatic compounds is thought to play a significant role in a number of biochemical processes such as proton transport, enzymatic catalysis, and spontaneous or induced mutagenesis. This has motivated numerous investigations devoted to the determination of tautomeric stabilities or aimed at understanding protomeric equilibria and photoinduced tautomerization.[1] Model molecules have been utilized in an attempt to simplify the study of these complex biological phenomena. In this respect, hydroxypyridine−pyridone systems and their sulfur analogues have often been considered as prototypes for tautomerism in nucleic acid bases and derivatives.[2] Both theoretical and experimental studies have demonstrated strong environmental effects on the tautomeric equilibrium and have led to the following consensus: the enol (thiol) species is favored in the gas phase or in nonpolar solvents, while the keto (thione) form predominates in polar media.[1a,2a] Tautomerization has long been known to take place by proton transfer. However, the exact mechanism depends on the isomeric substitution pattern of the system studied: 2-hydroxy- (and 2-mercapto-) pyridines were observed to undergo both dissociative (i.e., stepwise protonation and deprotonation) and nondissociative (i.e., intramolecular, dimeric, or solvent-mediated) proton transfer, whereas in the case of 4-hydroxy- (and 4-mercapto-) derivatives, the longer distance between donor and acceptor sites prohibits an intramolecular process and tautomerization can only proceed via a dissociative mechanism.[3]

In addition to these biologically relevant properties, hydroxypyridine−pyridone related systems, and in particular their sulfur analogues, have also been found to exhibit pharmacological as well as photodynamic activities.[4] Recently, the 2-isomer of *N*-hydroxypyridinethione (2-NHPT) has been the subject of considerable interest, initiated mainly by the discovery of its potential use as a photochemical source of hydroxyl radicals (•OH) suitable for both chemical and biological investigations.[5−9] The process giving rise to •OH is a photoinduced homolytic cleavage of the nitrogen−oxygen bond, as described by eq 1. A detailed laser flash photolysis study of 2-NHPT led us to conclude that (i) contrary to the initial belief, 2-NHPT cannot

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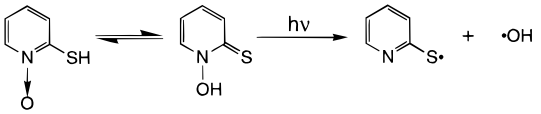
10.1021/ja991951j CCC: $18.00 © 1999 American Chemical Society
Published on Web 10/08/1999

be considered as a *selective* precursor of •OH since, in addition to N−O bond homolysis, it undergoes several other photoprocesses leading to the production of various highly reactive species and (ii) contribution from the thiol form is negligible, such that the observed photochemistry can be entirely attributed to the thione structure.[9]



(1)

In contrast, we report here that thione−thiol tautomerism (eq 2) plays a crucial role in the case of *N*-hydroxypyridine-4(1*H*)-thione (4-NHPT). Although only sparse literature is available on this compound, it was recently shown to cause DNA photodamage[6,7] and to induce photooxidation of purines,[10] with •OH proposed as the reactive species responsible for the biological effects. In the latter studies as well as in a recent investigation of the photochemistry of 4-NHPT,[11] the tautomerism was not taken into consideration. However, since 4-NHPT is soluble in both polar and apolar media, its localization in biological systems may vary, and consequently, the molecule may exist in the thione and/or thiol form(s), depending on its biological microenvironment.

(2)

In the present work, the photochemistry of each tautomer of 4-NHPT has been investigated by laser flash photolysis after careful selection of the experimental conditions (i.e., solvent and excitation wavelength). The results obtained reveal the importance of the role of the thione−thiol tautomerism on the photochemistry of 4-NHPT and the influence of the isomeric substitution pattern (i.e., 2- or 4-position) on the photochemical behavior of *N*-hydroxypyridinethiones. This study also points out that an accurate knowledge of the tautomeric structure absorbing the excitation light is essential for correct interpretation of the data.

Experimental Section

General. Ground-state absorption properties were studied using a Hewlett-Packard HP 8453 UV−visible spectrophotometer (Hewlett-Packard Company, Palo Alto, CA). Corrected steady-state emission and excitation spectra were recorded with a Fluoromax spectrofluorimeter (Spex Industries, Edison, NJ). Fluorescence quantum yields (Φ_f) were determined at an excitation wavelength of 300 or 360 nm as previously described,[12] with 2-aminopyridine ($\Phi_f = 0.60 \pm 0.05$ in 50 mM sulfuric acid[13]) and/or anthracene ($\Phi_f = 0.27 \pm 0.03$ in N_2-saturated ethanol[14]) as standard(s).

Materials. 4-NHPT was prepared as reported[15] using 4-chloropyridine *N*-oxide and thiourea, from Sigma-Aldrich, as starting materials. Recrystallization from ethanol led to yellow needles of 4-NHPT, the absorption spectrum of which displayed a maximum at 322 nm (ϵ = 18 600 M^{-1} cm^{-1}) in neutral phosphate buffer, in agreement with published data.[7] 2-NHPT was provided by Aldrich and recrystallized twice from aqueous ethanol before use.[9] 4-Mercaptopyridine **1**, the pyridine *N*-oxides **2a**−**c**, the thiophenols **3a**−**c,** and 4,4′-dipyridyl disulfide (Aldrithiol-4) **4** (see Figure 1) were also from Aldrich. All pyridine derivatives were stored as solids at −20 °C. Solution samples were prepared immediately before use and protected from light at all times. All other reagents were purchased from Sigma-Aldrich or Fisher Chemical and were of the highest purity available. Di-*tert*-butyl peroxide was percolated through activated alumina immediately prior to use. All *trans*-β-carotene was purified by recrystallization from benzene/methanol. Benzhydrol and benzophenone were recrystallized twice from aqueous ethanol and ethanol, respectively. Solvents were of spectroscopic grade from Fisher and used as received.

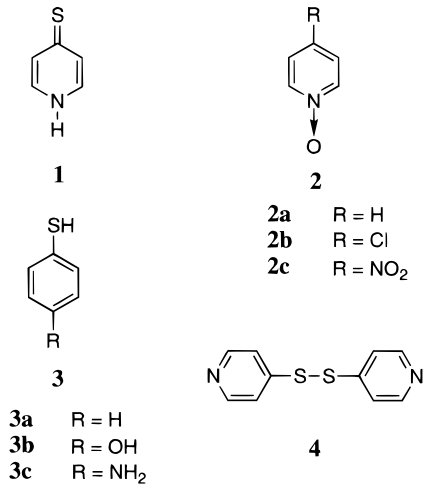


Figure 1. Chemical structures of 4-mercaptopyridine (**1**), pyridine *N*-oxides (**2**), thiophenols (**3**), and 4,4′-dipyridyl disulfide (**4**).

Laser Flash Photolysis. Details of the laser flash photolysis system have been provided elsewhere.[12,16] Laser excitation was carried out using the 266- or 355-nm harmonic of a Quantel YG660 Nd:YAG laser (8-ns pulse duration) or the 308-nm emission line from a Lambda Physik EMG 103 MSC XeCl excimer laser (12-ns pulse duration). For some of the experiments with the excimer laser, modified, laboratory-built data acquisition electronics were used. Briefly, the voltage on the PMT load resistor was preamplified by an operational amplifier and fed into a summing amplifier, where the DC offset voltage (backoff) was compensated by addition of an equal voltage of opposite sign. An active 3rd order Bessel low-pass filter (bandwidth of 4.5 MHz, corresponding to a rise time of 90 ns) was used to filter the DC-compensated signal, which was then captured by the digital oscilloscope. This arrangement greatly suppressed the interference of electromagnetic noise generated by the excimer laser.

Laser intensities (from 1 to 18 mJ cm^{-2} $pulse^{-1}$) and gases (O_2, air, or N_2) used for saturating the solutions are specified in the text or in the figure legends. Samples were contained in 10 × 10 mm^2 quartz cuvettes. Due to the photolability of 4-NHPT, flash photolysis data were predominantly acquired under continuous flow conditions, and a high flow rate was maintained to ensure irradiation of a completely fresh volume of sample with each laser pulse.[17] Quenching rate constants were measured using static cells, and the sample was submitted to a limited number of laser pulses. Quantum yields (Φ) were determined by comparative actinometry[18] using benzophenone (for which $\Phi_{T(BP)} = 1.0$[19] and $\epsilon_{T(BP)} = 7220$ $M^{-1}cm^{-1}$ at 530 nm[20] in deaerated benzene) as reference and calculated using eq 3, as previously described.[9]

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$$\Phi = \Phi_{T(BP)}\,(A/A_{T(BP)})(\epsilon_{T(BP)}/\epsilon) \quad (3)$$

The photoconductivity setup, which allows both optical and conductivity detection on the same sample, has been described in detail elsewhere.[21]

Hydroxyl Radical Formation. The consumption of *N*,*N*-dimethyl-4-nitrosoaniline (RNO) by chemical reaction with •OH can be followed by monitoring the bleaching of its characteristic 420-nm absorption band, and constitutes an indirect way to probe the photoinduced generation of •OH.[22]

Results

1. Thione−Thiol Tautomerism of 4-NHPT.[23] In aprotic, nonpolar solvents, such as methylene chloride (CH_2Cl_2), chloroform ($CHCl_3$), and benzene, the spectrum of the thiol form of 4-NHPT exhibits a single absorption maximum around 295 nm, as shown in Figure 2A. A linear Beer−Lambert plot was obtained in the measured range of concentrations from 5 to 500 μM (at 295 nm, $\epsilon = 18\,200$ M^{-1} cm^{-1} in CH_2Cl_2, 20 100 M^{-1} cm^{-1} in $CHCl_3$, and 17 600 M^{-1} cm^{-1} in benzene).[24] Excitation of 4-NHPT in these solvents leads to an emission spectrum with a maximum at 388 nm. Identical fluorescence emission spectra were recorded irrespective of the excitation wavelength used, which demonstrates the presence of only one fluorescing species. From the overlap of the emission and excitation (or absorption) spectra, the (0−0) S_1-S_0 energy of the thiol tautomer was estimated to be 334 kJ mol^{-1}. A fluorescence quantum yield of $\sim 2 \times 10^{-3}$ was determined in CH_2Cl_2 and $CHCl_3$, under air-saturated conditions.

On the other hand, the absorption spectrum recorded for low concentrations of 4-NHPT (≤ 5 μM) in polar, protic solvents, was found to display a single maximum at 330−340 nm. An example is shown in Figure 2A in the case of 2-propanol ($\lambda_{max} = 335$ nm, $\epsilon = 16\,000$ M^{-1} cm^{-1}). The fact that the composition of the tautomeric equilibrium is reversed by transfer from apolar to polar solvents has been established for hydroxypyridines−pyridones and related systems.[25] Therefore, the absorption band was assigned to the thione tautomer of 4-NHPT. Excitation of 4-NHPT, under these conditions, leads to an emission spectrum with a maximum at 450 nm. A (0−0) S_1-S_0 energy value of 293 kJ mol^{-1} and a very low fluorescence quantum yield ($\leq 1 \times 10^{-3}$) were determined, as described above.

In most solvents, however, the ground-state absorption spectrum of 4-NHPT exhibits two absorption bands with maxima at 295 nm and 340−360 nm, which were attributed to the thiol form and thione tautomer, respectively. The ratio between the two absorption bands, which reflects the tautomeric equilibrium mixture, was found to be solvent-dependent, as illustrated by Figure 2B. Selective excitation into these bands leads to different emission spectra displaying single maxima at 388 nm and 400−450 nm, respectively.

Consequently, by choosing the solvent and excitation wavelength, it is possible to *selectively* investigate the photochemistry of each one of the tautomeric structures of 4-NHPT, by itself in solution or in the presence of the other form. It should again be stressed that, in all cases, the use of a flow-through system was necessary for a correct interpretation of the photochemistry of 4-NHPT, as excitation under static conditions led to completely different results, due to secondary photochemistry of photoproducts.

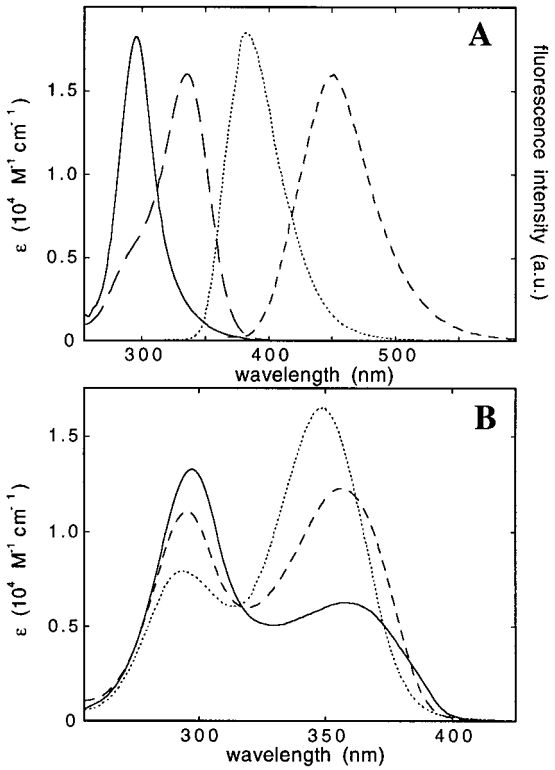

Figure 2. **(A)** Absorption spectra of the thiol and thione tautomers of 4-NHPT in CH_2Cl_2 (—) and 2-propanol (— — —), respectively and corresponding fluorescence emission spectra obtained in CH_2Cl_2 (•••) and 2-propanol (- - -) after excitation at 300 and 350 nm, respectively. **(B)** Ground-state absorption spectrum of 4-NHPT in ethyl acetate (—), acetonitrile (- - -) and methanol (•••).

2. Photochemistry of the Thiol Form of 4-NHPT. Figure 3 shows the time-dependent transient absorption spectrum recorded after excitation ($\lambda_{exc} = 308$ nm) of the thiol tautomer in CH_2Cl_2, under air-saturated conditions. Similar transient spectra were formed on photolysis of 4-NHPT in $CHCl_3$ and benzene.

The absorption bands with maxima at 360 and 710 nm, the weak shoulder at 420 nm and the negative band around 300 nm, all result from primary photoprocesses taking place within the pulse duration. The similarity in the second-order decays recorded at 360, 420, and 710 nm indicates that all these absorption bands originate from a single long-lived species. Under both aerated and deaerated conditions, the signals in the 280−330 nm region, were composed of an instantaneous depletion and a subsequent slow growth in absorbance, which follows second-order kinetics. A fast partial recovery with a rise time of 500 ns (see inset of Figure 3) was also detected in the absence of oxygen, suggesting that, in addition to the long-lived species, excitation of the thiol tautomer of 4-NHPT produces a short-lived, oxygen-sensitive intermediate.

The primary long-lived species absorbing at 360 and 710 nm decays via second-order kinetics in a radical-like manner and, based on numerous reports concerning photolysis of thiols,[26−28] a reasonable hypothesis for the generation of this intermediate is a photoinduced homolytic S−H bond cleavage of the thiol

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(24) Experimental errors on ϵ values are $\leq 5\%$.

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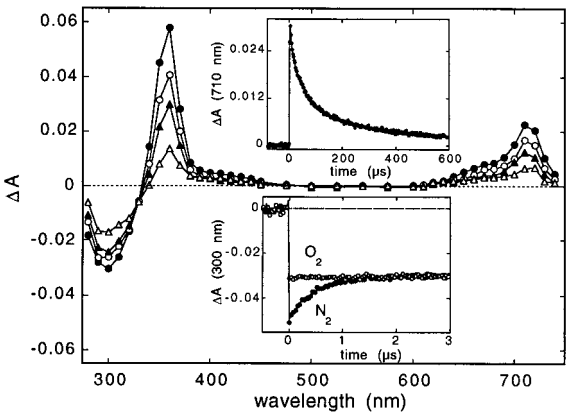
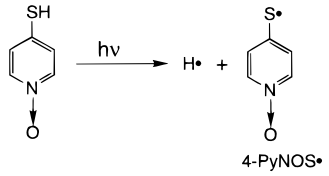


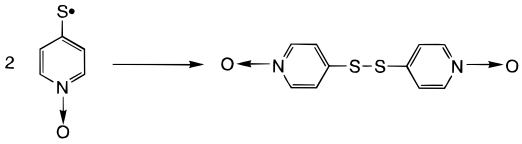
Figure 3. Time-dependent transient absorption spectrum recorded for the thiol tautomer of 4-NHPT in air-equilibrated CH_2Cl_2 5 μs (●), 25 μs (○), 50 μs (▲), and 150 μs (△) after flash photolysis (λ_{exc} = 308 nm, 8.5 mJ cm^{-2} pulse^{-1}). The absorbance of the solution was 0.3 at the excitation wavelength. The second-order decay measured at 710 nm is presented in one of the insets. The other inset displays the kinetic signals at 300 nm observed in the presence and the absence of oxygen.

tautomer of 4-NHPT, as described by eq 4. A similar mechanism was verified to take place upon pulsed excitation (λ_{exc} = 355 nm) of the structurally closely related 4-hydroxythiophenol (**3b**) in methanol and 4-aminothiophenol (**3c**) in CH_2Cl_2, producing the corresponding thiyl radicals, identified through their absorption maximum at 520[29] and 580 nm,[30] respectively.



(4)

Homolytic cleavage of the S−H bond in the thiol tautomer of 4-NHPT results in the formation of the *N*-oxy-4-pyridinethiyl radical (4-PyNOS•). The fact that the spectral profile of the long-lived species (see Figure 3) is similar to those reported for different 4-substituted benzenethiyl radicals[28,31,32] is a first argument in favor of S−H bond photohomolysis. Furthermore, the second-order decay of the long-lived species giving rise to a permanent product absorbing at λ < 330 nm (see Figure 3) is consistent with the recombination of 4-PyNOS• (eq 5) resulting in the formation of 4,4′-dithiobis(pyridine *N*-oxide), which has been identified as a photoproduct of 4-NHPT.[6]



(5)

To further confirm the hypothesis of homolytic S−H bond cleavage, 4-PyNOS• was generated independently using the known property of *tert*-butoxyl radicals (*t*-BuO•) to abstract hydrogen atoms,[33,34] especially from thiols.[35] Flash photolysis (λ_{exc} = 266 nm) of di-*tert*-butyl peroxide in O_2-saturated CH_2Cl_2 (which produces *t*-BuO•) carried out in the presence of 4-NHPT gave rise to a secondary transient species, the absorption spectrum of which was found to be identical to that produced by direct excitation (λ_{exc} = 308 nm) of the thiol tautomer of 4-NHPT in CH_2Cl_2, whereas the same experiment performed in O_2-saturated DMSO, under conditions where the thione was the predominant tautomer, did not. The rate constant for hydrogen abstraction from the thiol tautomer of 4-NHPT by *t*-BuO• was estimated[36] to be 3.5×10^9 M^{-1} s^{-1}. This value is higher than that reported in the case of hexanethiol (k_Q = 6.5 $\times 10^7$ M^{-1} s^{-1}),[35] which can be rationalized in terms of S−H bond strengths, known to be lower for aromatic thiols than for aliphatic thiols.[37] The same reactivity pattern was also reported in the case of aromatic and aliphatic alcohols.[38]

Determination of the quantum yield of homolytic S−H bond cleavage (Φ_{S-H}) undergone by the thiol form of 4-NHPT is complicated by the high intensity of the absorption band exhibited by 4-PyNOS• in the ground-state depletion region. However, an estimation of Φ_{S-H} was obtained indirectly. In CH_2Cl_2, the ratio, k_R/ϵ, between the recombination rate constant and the molar absorption coefficient of 4-PyNOS• at 710 nm, was determined to be (1.40 ± 0.25) $\times 10^6$ cm s^{-1} using the second-order decay of the 710-nm band. On the basis of the assumption that the rates of recombination of thiyl radicals are diffusion-controlled,[39] ϵ was calculated to be (11 000 ± 2 000) M^{-1} cm^{-1}, which is comparable to the molar absorption coefficients reported for similar 4-substituted benzenethiyl radicals.[31,40] Using this ϵ value, the quantum yield of photoproduction of 4-PyNOS• (which is equivalent to Φ_{S-H}) was estimated to be 0.24 by comparative actinometry.[41]

Typical reactions undergone by thiyl radicals include hydrogen atom abstraction, addition to conjugated and nonconjugated unsaturated bonds and electron transfer.[28,42,43] The reactivity of 4-PyNOS• was investigated by monitoring the decay of the 360 and 710 nm bands for several concentrations of potential scavengers. The quenching rate constants obtained in this way are reported in Table 1. They show that 4-PyNOS• exhibits a higher reactivity toward electron acceptors than toward hydrogen donors. It is also worth mentioning that the rate constants determined for addition reactions of 4-PyNOS• to 1,3-cyclohexadiene and methyl methacrylate are much lower than those measured in the case of the 2-pyridinethiyl radical, for which $k_Q = 2.6 \times 10^9$ and 1×10^8 M^{-1} s^{-1}, respectively.[9] This lower reactivity results from the radical stabilizing properties of the 4-pyridyl *N*-oxide group (vide infra).

Energy transfer experiments unambiguously identified the short-lived, oxygen-dependent species produced by the thiol tautomer of 4-NHPT as an excited triplet state. The transient absorption spectrum recorded after photolysis (λ_{exc} = 308 nm) of 4-NHPT in deaerated CH_2Cl_2 in the presence of perylene

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Table 1. Reactivity of the Main Transient Species Formed by Pulsed Excitation of the Thione and Thiol Tautomers of 4-NHPT in Organic Solvents

	k_Q (M^{-1} s^{-1})[b]	
quenchers[a]	4-PyNOS•[c]	^{3}(4-NHPT)* thione[d]
1,3-CHD	1.6×10^8	
MMA	$<5 \times 10^4$	
DABCO		1.8×10^6
TMB	4.6×10^9	2.0×10^7
MV^{2+}		1.2×10^{10}
α-tocopherol	4.9×10^6	
3b	6.0×10^7	2.0×10^7

[a] The abbreviations used in this table correspond to 1,3-cyclohexadiene (1,3-CHD), methyl methacrylate (MMA), 1,4-diazabicyclo[2,2,2]-octane (DABCO), 3,3′,5,5′-tetramethylbenzidine (TMB), and methyl viologen (MV^{2+}). [b] Unless otherwise stated, the rate constants were determined by monitoring the decay of the appropriate transient absorption for different substrate concentrations. [c] In CH_2Cl_2. [d] In N_2-saturated methanol.

exhibited an absorption band with a maximum around 490 nm, which is characteristic of ^{3}P*, the triplet state of perylene.[18] By monitoring the buildup corresponding to the sensitized formation of ^{3}P* at 490 nm for several concentrations of triplet energy acceptor (from 25 to 200 μM), the rate constant for energy transfer was found to be $(1.2 \pm 0.3) \times 10^{10}$ M^{-1} s^{-1}. Since a similar rate constant ($k_Q = 1.5 \times 10^{10}$ M^{-1} s^{-1}) was obtained by following the fast growth at 300 nm (see inset of Figure 3), this secondary process was assigned to ground-state recovery of 4-NHPT due to intersystem crossing ($T_1 \rightarrow S_0$) of the triplet state.

The fast recovery at 300 nm was used to estimate the quantum yield of triplet state formation, Φ_T, assuming that the thiol triplet state exhibits only negligible absorption at that wavelength. The amplitude of the recovery was monitored as a function of laser pulse intensity and a lower limit for Φ_T was found to be 0.12 from eq 3.[44] Φ_T was also determined by an indirect method based on the sensitized generation of the triplet state of perylene. In the presence of enough perylene (~250 μM) to trap all the triplet states produced by the thiol tautomer, the quantum yield of ^{3}P* formation is equivalent to Φ_T. The variations of the amplitude of the signal recorded at 490 nm were monitored as a function of laser intensity and, Φ_T determined by this method was found to be 0.19, using an ϵ_T value of 13 400 M^{-1} cm^{-1} at 490 nm, for ^{3}P*.[45]

3. Photochemistry of the Thione Form of 4-NHPT. Figure 4 presents the time-dependent transient absorption spectrum recorded after flash photolysis ($\lambda_{exc} = 355$ nm) of a solution of 4-NHPT (4 μM) in 2-propanol. Similar spectra were obtained by excitation of the thione tautomer of 4-NHPT in other protic solvents (e.g., methanol). Under O_2-saturated conditions, little transient absorption was observed except for the negative band around 330−335 nm, which corresponds to photochemical consumption of starting material. After the initial instantaneous depletion, the kinetic signals recorded at $\lambda < 360$ nm display no changes over several milliseconds, suggesting permanent bleaching of 4-NHPT and formation of stable photoproduct(s).

Under deaerated conditions, the transient absorption spectrum recorded immediately after the pulse, exhibits a broad absorption with a maximum at 600 nm and a shoulder around 400 nm, in addition to the negative band. Both absorptions follow identical decay kinetics (a lifetime of 7 μs was measured under the

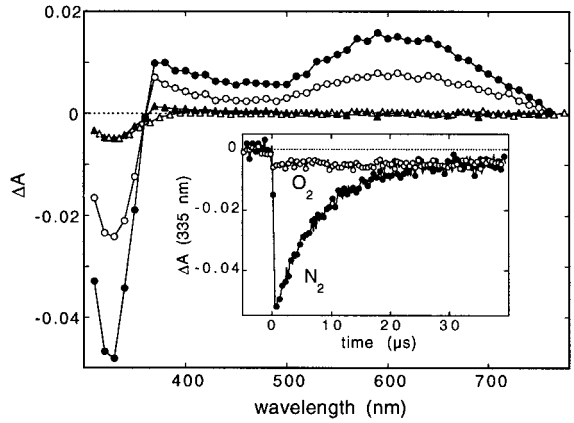


Figure 4. Transient absorption spectra recorded for 4-NHPT (~4 μM) in N_2-saturated 2-propanol 1 μs (●), 2 μs (○), and 14 μs (▲) after flash photolysis ($\lambda_{exc} = 355$ nm, 3.5 mJ cm^{-2} $pulse^{-1}$) and under O_2-saturated conditions 30 μs (△) after the pulse. The inset shows an example of kinetic signals produced at 335 nm by excitation ($\lambda_{exc} = 355$ nm) of a solution of 4-NHPT in 2-propanol (absorbance = 0.06 at 355 nm) in the presence (○) and absence (●) of O_2.

experimental conditions used to record the spectrum). The corresponding species was identified as the triplet state of the thione tautomer of 4-NHPT, through its efficient quenching by O_2 ($k_Q = 1.5 \times 10^9$ M^{-1} s^{-1}) and the observation of sensitized formation of the triplet state of merocyanine 540 at 660 nm[46] ($k_Q = 6.0 \times 10^9$ M^{-1} s^{-1}) and of perylene at 490 nm[18] ($k_Q = 1.5 \times 10^{10}$ M^{-1} s^{-1}). The lifetime of the triplet state of 4-NHPT was found to be highly dependent on the ground-state concentration. A plot of the observed decay constant against [4-NHPT] at 400 and 600 nm led to a bimolecular rate constant of 2.5×10^9 M^{-1} s^{-1}.[47] A relatively short lifetime and an efficient quenching by the ground-state are typical characteristics of thione triplets.[48,49]

The quantum yield of intersystem crossing (Φ_T) of the thione tautomer of 4-NHPT was estimated from the ground-state recovery at 335 nm, using the same approach as previously described in the case of the thiol form.[50] A lower limit for Φ_T was calculated to be 0.90 from eq 3.[51] Similarly, the quantum yield of permanent photobleaching was determined to be 0.06, under O_2- and N_2-saturated conditions. By direct comparison of the ΔA values of the recovery signals at 335 nm and the maximum absorption of the triplet state at 600 nm, ϵ_T, the molar absorption coefficient of the thione triplet was calculated to be 5200 M^{-1} cm^{-1} at 600 nm using eq 6.

$$\epsilon_T = \epsilon_{GS}(335\text{ nm})\ \Delta A(600\text{ nm})/\Delta A(335\text{ nm}) \qquad (6)$$

The thione triplet was found to react efficiently with both electron donors and acceptors but higher rate constants were measured for reactions with acceptors than donors (Table 1). Figure 5 shows the time-dependent transient absorption spectrum recorded after excitation ($\lambda_{exc} = 355$ nm) of the thione tautomer of 4-NHPT in deaerated methanol in the presence of methyl viologen (MV^{2+}). In addition to the absorption bands of $MV^{\bullet+}$

(44) In eq 3, an ϵ value of 18 200 M^{-1} cm^{-1} was used for the molar absorption coefficient of the ground-state of 4-NHPT in CH_2Cl_2.

(45) Carmichael, I.; Helman, W. P.; Hug, G. L. *J. Phys. Chem. Ref. Data* **1987**, *16*, 239.

(46) Redmond, R. W.; Srichai, M. B.; Bilitz, J. M.; Schlomer, D. D.; Krieg, M. *Photochem. Photobiol.* **1994**, *60*, 348.

(47) The concentration of 4-NHPT was varied from 1 to 120 μM.

(48) de Mayo, P. *Acc. Chem. Res.* **1976**, *9*, 52.

(49) Bhattacharyya, K.; Ramamurthy, V.; Das, P. K. *J. Phys. Chem.* **1987**, *91*, 5626.

(50) The triplet state of the thione tautomer of 4-NHPT is assumed to exhibit only negligible absorption at that wavelength.

(51) In eq 3, an ϵ value of 16 000 M^{-1} cm^{-1} was used for the molar absorption coefficient of the ground-state of 4-NHPT in 2-propanol.

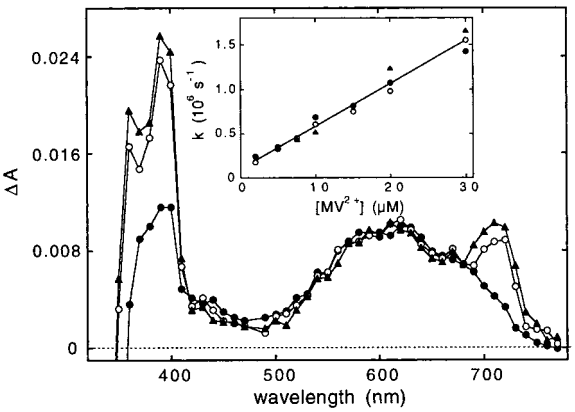
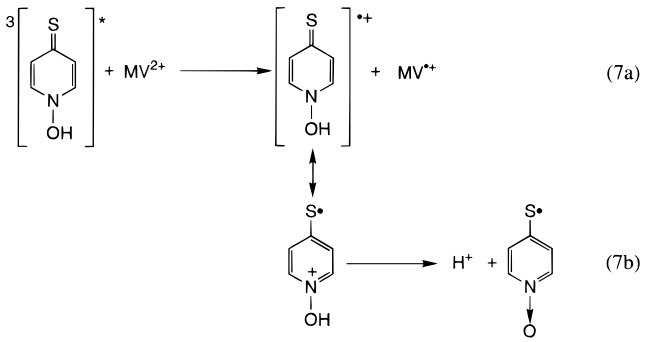


Figure 5. Time-dependent transient absorption spectrum obtained after pulsed excitation (λ_{exc} = 355 nm, 7 mJ cm^{-2} pulse^{-1}) of the thione tautomer of 4-NHPT in deaerated methanol in the presence of 50 μM of methyl viologen. The spectra were recorded 400 ns (●), 1 μs (○), and 2 μs (▲) after the pulse. The inset shows the variations of the time constants measured at 400 nm (●), 360 nm (○), and 720 nm (▲) as a function of MV^{2+} concentration.

(the reduced form of MV^{2+}) at 395 and 610 nm,[52] one observes the formation of two additional absorption maxima at 360 and 710 nm. The rise time of the latter absorption bands was found to be identical to that measured at 395 nm,[53] and the time constants of all the secondary growths were similarly affected by an increase in MV^{2+} concentration, as shown in the inset of Figure 5.

These results indicate that the species absorbing at 360 and 710 nm is produced concomitant to $MV^{\bullet+}$ via electron transfer. The similarity between the absorption spectrum of this species and that of 4-PyNOS• (see Figure 3) suggests that the radical cation $(4\text{-NHPT})^{\bullet+}$, which is expected to be formed in the electron-transfer reaction (eq 7a), undergoes a fast deprotonation leading to the formation of the neutral 4-PyNOS• (eq 7b).



An important question is whether 4-NHPT, similar to its structural isomer 2-NHPT, is a photochemical precursor of hydroxyl radicals in organic solvents. The formation of •OH was probed using *N,N*-dimethyl-4-nitrosoaniline (RNO).[54] Pulsed excitation (λ_{exc} = 355 nm) of 4-NHPT in O_2-saturated acetonitrile in the presence of RNO did not lead to any detectable bleaching at 420 nm on the time scales of flash photolysis experiments. This result implies that, contrary to what was

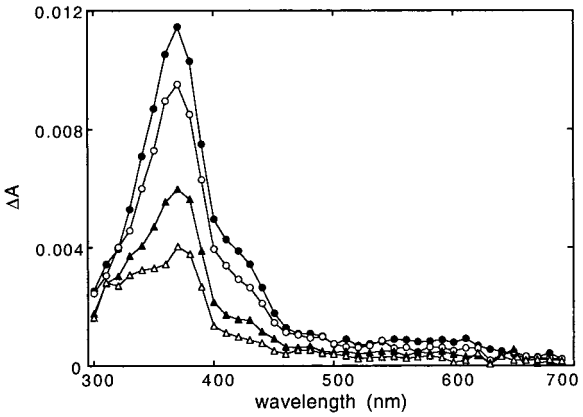


Figure 6. Absorption properties of the 4-pyridinethiyl radical produced by flash photolysis (λ_{exc} = 266 nm, 3 mJ cm^{-2} pulse^{-1}) of compound **4** in air-saturated acetonitrile. The spectra were recorded 5 μs (●), 10 μs (○), 30 μs (▲), and 70 μs (△) after the pulse.

observed for 2-NHPT,[9] singlet-state mediated N−O bond homolysis producing the hydroxyl and corresponding thiyl radicals (eq 1) does not take place in the case of the thione tautomer of 4-NHPT. This was further confirmed by experiments where the 4-pyridinethiyl radical was generated independently by 266-nm photolysis of 4,4′-dipyridyl disulfide (**4**) in air-saturated acetonitrile. Photoinduced homolytic cleavage of disulfide bonds to give sulfur-centered radicals has previously been observed in the case of diphenyl disulfide,[55] 2,2′-dipyridyl disulfide and 2,2′-dithiobis(pyridine *N*-oxide).[9,56] Under these conditions, the 4-pyridinethiyl radical exhibits an absorption band with a maximum at 370 nm, a shoulder around 420 nm and a weak, broad absorption at 550−600 nm (see Figure 6). It was found to be unaffected by the presence of O_2 but efficiently quenched by methyl methacrylate and 1,3-cyclohexadiene (known thiyl radicals scavengers).[55,57] The absence of this species on irradiation of the thione tautomer of 4-NHPT shows that N−O bond cleavage is not significant.

4. Photochemistry of Tautomeric Mixtures. In solvents where both tautomers coexist, the profile of the transient absorption spectra was observed to depend on the excitation wavelength used. In all cases, the tautomeric form absorbing the excitation light was found to undergo its expected primary photochemistry followed by secondary processes involving reaction with the ground state of the nonexcited tautomer.

The time-dependent transient absorption spectrum obtained by 308-nm irradiation of 4-NHPT in deaerated acetonitrile is presented on Figure 7A. It is similar to that produced by pulsed excitation (λ_{exc} = 308 nm) of the thiol tautomer in CH_2Cl_2 (see Figure 3). The only difference between the two transient spectra is a bleaching around 345 nm, in the spectral region corresponding to ground-state absorption of the thione form.[58]

The signal recorded at 345 nm was found to be a composite of two processes (i.e., decay of 4-PyNOS• and slow ground-state depletion) that follow the same first-order kinetics with a time constant of 80 μs. This indicates that, in this system, one of the deactivation pathways undergone by 4-PyNOS• is a chemical reaction involving the thione tautomer. On the basis of the known reactivity of the closely related 2-pyridinethiyl[9,57] and *N*-oxy-2-pyridinethiyl[9] radicals toward their thione precur-

(52) Farrington, J. A.; Ebert, M.; Land, E. J.; Fletcher, K. *Biochim. Biophys. Acta* **1973**, *314*, 372.

(53) Due to the similarity in the absorption properties of the thione triplet and $MV^{\bullet+}$ between 500 and 700 nm, one cannot clearly observe the decay of the triplet state and the formation of $MV^{\bullet+}$ in that spectral region.

(54) Due to the absorption of RNO at 355 nm, the highest RNO concentration which can be used in these experiments was determined to be 50 μM. Under these conditions, the lowest measurable quantum yield of •OH production was estimated to be 0.005.[21]

(55) Ito, O.; Matsuda, M. *J. Am. Chem. Soc.* **1979**, *101*, 5732.

(56) Alam, M. M.; Watanabe, A.; Ito, O. *J. Org. Chem.* **1995**, *60*, 3440.

(57) Aveline, B. M.; Kochevar, I. E.; Redmond, R. W. *J. Am. Chem. Soc.* **1995**, *117*, 9699.

(58) The absence of a transient absorption around 600 nm allows to rule out a direct excitation of the thione tautomer of 4-NHPT at 308 nm.

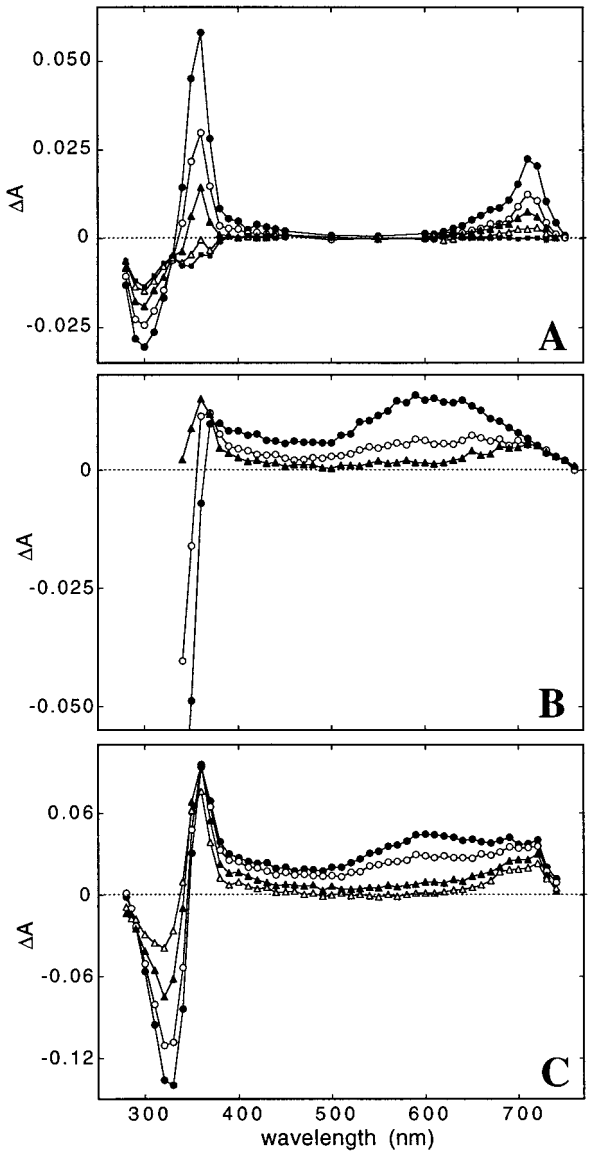
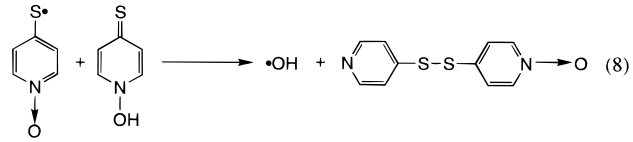
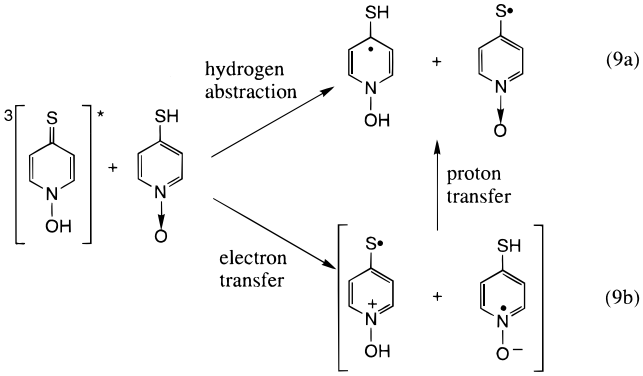


Figure 7. Time-dependent transient absorption spectra recorded for **(**A**)** 4-NHPT (18 μM) in N_2-saturated acetonitrile 5 μs (●), 50 μs (○), 100 μs (▲), 300 μs (△), and 600 μs (■) after excitation at 308 nm (8.5 mJ cm^{-2} pulse^{-1}) and for 4-NHPT (50 μM) in N_2-saturated methanol **(**B**)** 1 μs (●), 6 μs (○), and 14 μs (▲) after excitation at 355 nm (3 mJ cm^{-2} pulse^{-1}) and **(**C**)** 1 μs (●), 2 μs (○), 5 μs (▲), and 15 μs (△) after excitation at 308 nm (8.5 mJ cm^{-2} pulse^{-1}).

sors, the interaction between 4-PyNOS• and the thione form of 4-NHPT can be described by eq 8. Addition of 4-PyNOS• to the carbon−sulfur double bond[59] is then expected to give •OH (vide infra) and the unsymmetrical disulfide, which has been identified as a photoproduct of 4-NHPT.[6]

Figure 7B illustrates the case where the thione tautomer in deaerated methanol is selectively excited at 355 nm. Concomitant with the decay of the thione triplet state at 600 nm, a buildup of new absorption bands with maxima at 360 and 710 nm is observed; these bands are not formed in the presence of O_2 or 1,3-cyclohexadiene (triplet state quenchers) in solution. Furthermore, the decay at 600 nm and the growth at 360 nm have identical time constants and are similarly reduced with increasing [4-NHPT]. Since (i) quenching of structurally similar thione triplets by their ground-state precursor is known to proceed via physical deactivation[29,60] and (ii) formation of a secondary species absorbing at 360 and 710 nm is not observed when only the thione is present in solution, a thione triplet state/thione ground-state interaction can be ruled out, and reaction of the triplet state of the thione form of 4-NHPT presumably takes place instead with the thiol tautomer. The transient spectrum of the species produced by this reaction was found to be identical to that of 4-PyNOS• formed either by photoinduced homolytic S−H bond cleavage of the thiol tautomer (see Figure 3) or by electron transfer from the thione triplet state to methyl viologen and subsequent deprotonation of the radical cation, (4-NHPT)$^{\bullet+}$ (see Figure 5). At least two different mechanisms can be considered for the reaction between the thione triplet and thiol ground-state of 4-NHPT: abstraction of the thiol hydrogen atom (eq 9a) or electron-transfer involving the *N*-oxide part of the thiol molecule (eq 9b). Since photoconductivity experiments showed no detectable formation of secondary, charged species, the electron transfer reaction, if it takes place, must be immediately followed by proton transfer. Both mechanisms are therefore expected to give rise to the same neutral species, involving 4-PyNOS•.





To get some insight into the mechanism, we have studied the reactivity of the thione triplet of 4-NHPT toward relevant model molecules, namely thiophenols and pyridine *N*-oxides, with which the reaction occurs exclusively by hydrogen abstraction[42,61] and electron transfer,[62] respectively. Rate constants for the quenching of the triplet state of 4-NHPT by the thiophenols **3a**−**c** were determined to be 1−3.5 × 10^7 M^{-1} s^{-1}. The reactions were identified as hydrogen abstractions based on the observation of the characteristic absorptions of the corresponding thiyl radicals. Higher rate constants (k_Q = 5 × 10^8 − 5 × 10^9 M^{-1} s^{-1}) were measured for the electron transfer from the thione triplet state of 4-NHPT to the pyridine *N*-oxides **2a**−**c**. The transient absorption spectra recorded after pulsed excitation of 4-NHPT (λ_{exc} = 355 nm) in the presence of compounds **2a**, **2b,** and **2c** all show the formation of absorption

(59) On the basis of the ground-state absorption properties of 4-NHPT in acetonitrile (see Figure 2B), the thione concentration can be estimated to be equal to that of the thiol tautomer (i.e, ∼9 μM under the experimental conditions used). Since the lifetime of 4-PyNOS• was measured to be 80 μs at 360 and 710 nm, k_Q[thione] must be <12, 500 s^{-1}, which implies k_Q < 1.4 × 10^9 M^{-1} s^{-1}. This k_Q value is comparable to the reaction rate constants determined for the addition of similar thiyl radicals to their thione precursors (k_Q = (3−4) × 10^9 M^{-1} s^{-1}).[57]

(60) Bhattacharrya, K.; Das, P. K.; Ramamurthy, V.; Rao, V. P. *J. Chem. Soc., Faraday Trans. 2* **1986**, *82*, 135.

(61) Colle, T. H.; Lewis, E. S. *J. Am. Chem. Soc.* **1979**, *101*, 1810.

(62) Bakac, A.; Butkovic, V.; Espenson, J. H.; Lovric, J.; Orhanovic, M. *Inorg. Chem.* **1996**, *35*, 5168.

bands at 360 and 710 nm.[63] The higher reactivity displayed by ^{3}(4-NHPT)* toward pyridine *N*-oxides than thiophenols favors the hypothesis of an electron-transfer mechanism immediately followed by proton transfer (i.e., eq 9b).

Finally, Figure 7C shows an example where both tautomeric forms absorb the irradiation light. Such behavior was observed after pulsed excitation ($\lambda_{exc} = 308$ nm) of 4-NHPT in deaerated methanol. The spectral features suggest that the thione and thiol tautomeric structures undergo their own primary (mainly intersystem crossing and homolytic S−H bond cleavage, respectively) and secondary (electron transfer and addition, respectively) photochemistry. In this last case, which corresponds to a "nonselective" excitation of 4-NHPT, the profile of the transient absorption spectra was found to strongly depend on the experimental conditions used (i.e. solvent and λ_{exc}) and to reflect the relative absorbances of the thione and thiol tautomers at the excitation wavelength.

Discussion

To the best of our knowledge, 4-NHPT is one of the rare examples of a tautomeric heteroaromatic system for which the photochemistry of both its thione and thiol forms can be investigated in a specific manner. A comprehensive study of the photoprocesses undergone by 4-thiouridine is crucial to understand its central role in the photo-crosslinking of tRNA. However, identification of the tautomeric structures of the transient species and of the ground-state precursor is not straightforward and requires the use of analogues that do not tautomerize.[64] The same experimental approach was applied to the elucidation of the photochemistry of 6-mercaptopurine.[65] This compound, which is a metabolite of azathioprine (a common immunosuppressant for renal transplant recipients), is thought to be responsible for the side effect of severe skin cancer frequently associated with the drug.[65] In the case of 4-NHPT, the strong solvent dependence of its tautomeric equilibrium and the distinct absorption properties of its thione and thiol forms allow the investigation of the photochemistry of each tautomer to be carried out in an unambiguous manner, which makes the use of nontautomeric analogues unnecessary.

The photochemical behavior of the thione tautomer of 4-NHPT was found to be very different from that of 2-NHPT.[9] Similar to most thiones previously studied,[48,60,66] upon pulsed excitation ($\lambda_{exc} = 355$ nm, in 2-propanol), the 4-isomer undergoes a very efficient intersystem crossing ($\Phi_T = 0.90$) to a triplet state exhibiting properties (such as short lifetime, efficient quenching by the ground state, high reactivity toward both electron donors and acceptors) which are characteristics of thione triplet states.[29,67,68] On the other hand, photolysis of 2-NHPT under similar experimental conditions ($\lambda_{exc} = 355$ nm, in methanol) mainly causes homolytic N−O bond cleavage ($\Phi_{N-O} = 0.28$) and intersystem crossing, as a minor primary photoprocess ($\Phi_T = 0.04$).[9] Thus, contrary to 4-NHPT, 2-NHPT does not behave as a typical thione. Ortho (2-) substituents have long been known to have specific or "anomalous" effects on the properties of aromatic molecules in solution. As already mentioned, 2-isomers of hydroxypyridine−pyridone related systems can undergo hydrogen bonding either intra- or intermolecularly (with another molecule within a dimer or with a solvent molecule).[3] This hydrogen bonding, which does not take place to such a large extent in the case of 4-NHPT, decreases the π character of the carbon−sulfur double bond and therefore explains the unusual photochemistry of 2-NHPT. However, if the isomeric substitution pattern (4- vs 2-position) was the only factor responsible for the difference between the photochemistry of the thione tautomer of 4-NHPT and that of 2-NHPT, a similar situation would be expected to be observed for the structurally closely related pyridine-2(1*H*)-thione and pyridine-4(1*H*)-thione (compound **1**). A recent investigation of the photochemistry of these molecules by laser flash photolysis[29] has revealed an identical photochemical behavior characterized by a highly efficient intersystem crossing ($\Phi_T \geq 0.75$), in agreement with the thione structure of these compounds.[48,60,66] This suggests that, in addition to the isomeric substitution pattern, the nature of the substituent (*N*-hydroxy group in the case of 2-NHPT and 4-NHPT vs *N*-H group in the case of the pyridinethiones) must also play an important role.

Flash photolysis of the thiol tautomer of 4-NHPT ($\lambda_{exc} = 308$ nm, in CH_2Cl_2) was found to induce intersystem crossing ($\Phi_T = 0.19$) and homolytic S−H bond cleavage ($\Phi_{S-H} = 0.24$). S−H bond homolysis has long been recognized as the major photoprocess undergone by thiols.[26,27] Intersystem crossing, on the other hand, is not so typical for this type of molecule.[26,27] However, the thiol tautomer of 4-NHPT can also be described as a 4-substituted pyridine *N*-oxide, for which triplet state formation is known to be a typical photoprocess.[69−71] It is worth mentioning here that the photochemistry of *N*-oxide compounds has been intensively studied for the last two decades.[69] The interest mainly lies in the photochemical oxygen atom transfer reaction of heterocyclic *N*-oxides, which is considered as one of the best mechanistic model systems for biological oxidations catalyzed by monooxygenase enzymes. As far as the photodeoxygenation of heterocyclic *N*-oxides is concerned, various chemical mechanisms have been suggested in the literature and are still the subject of discussion.[69,72] In the case of the thiol tautomer of 4-NHPT, we have no experimental evidence which would allow us to conclude whether photodeoxygenation is significant in CH_2Cl_2, under flow-through irradiation conditions.

The *N*-oxy-4-pyridinethiyl radical (4-PyNOS•), generated by homolytic S−H bond cleavage of the thiol tautomer of 4-NHPT, was observed to be long-lived, compared to similar species such as the 2-pyridinethiyl[9] and *N*-oxy-2-pyridinethiyl[9,73] radicals. It was also found to exhibit a lower reactivity, in particular toward unsaturated systems. This behavior is readily explained by the fact that the 4-pyridyl *N*-oxide has been reported to be an excellent radical stabilizing group.[74] The potent stabilizing effect is attributed to spin delocalization, which imparts nitroxide radical character to the intermediate (eq 10).

Discrepancies exist between the present work and a recent report on the photochemistry of 4-NHPT,[11] where the molecule was assumed to exist exclusively in the thione form. The main

(63) (a) The different pyridine *N*-oxides used do not absorb light at 355 nm. (b) In these experiments, the concentration of 4-NHPT was ≤5 μM, so that only the thione tautomer was present in solution and secondary formation of the 360−710 nm absorbing species could not result from reaction with the thiol form.

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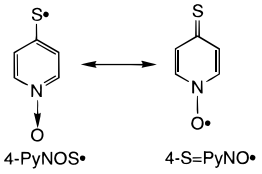
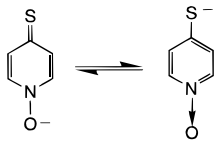


(10)

conclusions of that study are that upon pulsed excitation (λ_{exc} = 355 nm), 4-NHPT in tetrahydrofuran (THF)[75] or acetonitrile undergoes (i) intersystem crossing, (ii) homolytic N−O bond cleavage giving rise to the hydroxyl and 4-pyridinethiyl radicals, and (iii) O−H bond homolysis generating the nitroxide radical 4-S═PyNO• (see eq 10), which is reported to exhibit an absorption with a maximum around 720 nm. Differences in the experimental conditions may explain to some extent the different results obtained. The concentrations used in the present study were typically <50 μM for spectral measurements whereas they were much higher in the recent publication (200-400 μM). This may explain the lack of significant triplet-state absorption at 600 nm in their spectra as the thione triplet state has been shown here to display a high reactivity toward both the ground-state thione and the thiol tautomer and can therefore be expected to be efficiently quenched under high concentration conditions. Evidence of this quenching is provided by the transient absorption spectrum recorded in THF, which shows growth of an absorption at 720 nm, similar to that observed in our experiments on reaction of triplet thione with the thiol tautomer. As this reaction requires the presence of the thiol form and does *not* take place when only the thione tautomer exists in solution, we prefer to assign this band to the *N*-oxy-4-pyridinethiyl radical (4-PyNOS•) rather than to the nitroxide radical 4-S═PyNO•. This conclusion is supported by the fact that the thiol alone in solution gives rise to the same transient absorption spectrum resulting from homolytic S−H bond cleavage. Spectral comparisons are also made difficult by the different detection systems used. The recent study utilized a multichannel diode array system, which limits the observable spectral range to $\lambda \geq$ 400 nm, thereby truncating the spectra at the short wavelength end. Thus, the large absorption band of 4-PyNOS• at 360 nm was, for example, not detected. The apparent absorption band at 420 nm, which was assigned to the 4-pyridinethiyl radical (4-PyS•), may result from a sensitivity cutoff of the detection system around 400 nm. Additionally, the absence of RNO bleaching by chemical reaction with •OH allowed us to conclude that N−O bond homolysis, which would produce 4-PyS•, was not a significant primary photoprocess of 4-NHPT. Given the much higher concentrations used in the published study, the production of •OH may well arise from secondary reactions such as shown here in eq 8. Alternatively, the high photolability of 4-NHPT absolutely required continuous flow conditions in our experiments and the use of static samples may give rise to secondary photoproduct reactions if such precautions are not observed.

From a biological point of view, the most significant outcome of this work is that neither the thione nor the thiol tautomer of 4-NHPT was found to be a primary photochemical source of hydroxyl radicals in organic solvents. This result seems a priori in contradiction with reports of DNA damage[6,7] and oxidation of purines[10] induced by 4-NHPT via photochemical release of •OH. However as already mentioned, although hydroxyl radicals were not detected under the flow-through conditions used in this study, secondary processes such as eq 8, which are favored in static samples, can take place and provide •OH. Furthermore, the results presented here were obtained in organic solvents while irradiations of 4-NHPT in the presence of DNA[6,7] and purines[10] were carried out in more biologically relevant environments. In addition, in aqueous media, the 4-isomer exhibits an acid−base equilibrium with a reported pK_a of 3.82[15] and therefore exists as an anion (eq 11) under physiological conditions.



(11)

Studies are currently under way to get some insight into the photoprocesses undergone by 4-NHPT in neutral buffer solutions and verify whether the reported higher efficacy at inducing DNA photodamage exhibited by 4-NHPT, compared to 2-NHPT,[7] corresponds to a more efficient photochemical generation of hydroxyl radicals. Results from preliminary experiments seem to indicate that this is not the case, as the anionic form of 4-NHPT was not found to be a primary photochemical precursor of •OH.

Acknowledgment. We gratefully acknowledge Hans-Christian Lüdemann for his help in the photoconductivity experiments. We also thank Günther N. Grimm (Institute of Organic Chemistry, University of Würzburg, Germany) for suggestions concerning the synthesis of 4-NHPT and David McGarvey (Keele University, Department of Chemistry, Staffordshire, UK) for helpful discussions. This work was supported by NIH Grant RO1 CA68524 A and the MFEL program of the ONR under contract N00014-94-1-0927.

JA991951J

(75) The ground-state absorption spectrum of 4-NHPT in THF has been reported elsewhere.[11]