The effect of the *p*-nitro group on the chemistry of phenylnitrene. A study *via* intramolecular trapping

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The photodecomposition of a pyrazolyl substituted *p*-nitrophenyl azide has been studied as an intramolecular model for the reactivity of the parent azide, largely used for photochemical labelling. The singlet nitrene is trapped by intramolecular cyclization onto the pyrazole nitrogen as well as by the low yield addition of intermolecular nucleophiles (EtOH, Et_2NH). Ring expansion to a didehydroazepine is absent. The triplet nitrene abstracts hydrogen (intermolecularly) only slightly more efficiently than the non-nitrated derivative, while it is rather efficiently reduced *via* electron transfer in the presence of amines. Hydrogen abstraction is efficient for the excited triplet nitrenes, as revealed by an intramolecular reaction.

Aryl azides have long been proved to be useful reagents for photochemical labelling.[1,2] Suitably substituted derivatives can be irradiated at a sufficiently long wavelength, thereby avoiding damage of the receptor. Aryl nitrenes are generated under these conditions and have been shown to be both highly reactive and selective. Their use for labelling and for other applications has preceded the definition of the mechanism, which is still a subject of investigation. It is now recognised that four intermediates are involved in the photodecomposition of phenyl azide and its derivatives, *viz*. singlet and triplet nitrene as well as the cyclic isomers, the benzoazirine and didehydroazepine.[3–7] However, the poor yield of isolated products obtained in many cases from the irradiation in solution of aryl azides and the considerably different sensitivity of the intermediates to the different spectroscopic techniques may hinder the determination of the main process under a given set of conditions.

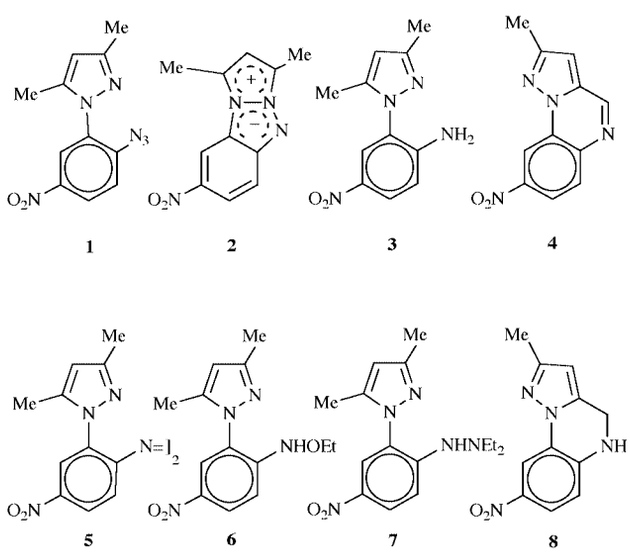
The most commonly used phenyl azides for photochemical labelling are nitro derivatives and this has led to extensive investigation of such compounds. Early studies proved that the lowest state of the nitrene is a triplet[8] and investigated the formation of transients in a matrix.[9,10] These were complemented by several spectroscopic and product studies.[11–15] The main conclusions are that triplet reactions, in particular dimerization to the azo compound, dominate. Hydrogen abstraction is ineffective, except with amines where an electron transfer path is involved. Contrary to most phenyl azides, no trapping product from the didehydroazepine is obtained from the 4-nitro- and very little from the 3-nitro derivative, possibly due to the short lifetime of this intermediate.[11,12] Likewise, no trapping of singlet nitrene has been observed, despite the expectation that the substituent increases the electrophilicity of this intermediate, except for a low (9%) yield of hydrazine when irradiating in *neat* diethylamine.

It is in a way disappointing that useful reagents for labelling give poor yields of trapping products in solution, and that their chemistry is dominated by triplet coupling to the azo compound, the typical reaction of stable triplet nitrenes. Several groups, including our own, have used intramolecular models for determining the reactivity of nitrenes,[16–19] and it may be that the results obtained from such compounds are indicative of the chemistry occurring in the complex with the receptor. Phenyl nitrenes bearing 3,5-dimethylpyrazolyl as a substituent in position 2 are useful models, since both electrophilic and radical based intramolecular reactions are expected to be facile.[16–18] Herein we report a study on the corresponding 5-nitro derivative.

Results

Room temperature photolysis

The photochemistry of 1-(2-azido-5-nitrophenyl)-3,5-dimethyl-1*H*-pyrazole (**1**) was studied in acetonitrile and in ethanol. In the former solvent, the main product from the decomposition of a 1×10^{-4} M solution of **1** was the heterocycle **2** accompanied by a small amount of the amine **3** and of the pyrazoloquinoxaline **4** (see Scheme 1 and Table 1). When a more



Scheme 1

concentrated solution (4×10^{-3} M) was photolysed with a more powerful lamp the azo derivative **5** became an important product, but the yield of product **2** was not significantly diminished.

Carrying out the photodecomposition in ethanol led to similar results, again with formation of the azo compound only in the higher concentration experiment, except for the fact that an additional product formed in low yield was detected, but not

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Table 1 Products from the irradiation of azide **1** at room temperature

Solvent	[1]	Lamp	Additive	% Yield of the products 2	3	4	5	6	7
MeCN	1×10^{-4}	[a]		37	4	6			
	4×10^{-3}	[b]		41	3	2	27		
	1×10^{-4}	[a]	EtOH, 1 M	38	4	4			
	4×10^{-3}	[b]	MK 9×10^{-3} M	5	38				
	1×10^{-4}	[a]	DEA 0.1 M	32	36				
EtOH	1×10^{-4}	[a]		45	15			*ca.* 5	
	4×10^{-3}	[b]		32	12		11	*ca.* 5	
	4×10^{-3}	[b]	MK 9×10^{-3} M		42		15		
	1×10^{-4}	[a]	DEA 0.1 M	36	31			*ca.* 5	*ca.* 1

[a] Low-pressure mercury arc (15 W) inserted below the cell. [b] Focused high-pressure mercury arc (200 W).

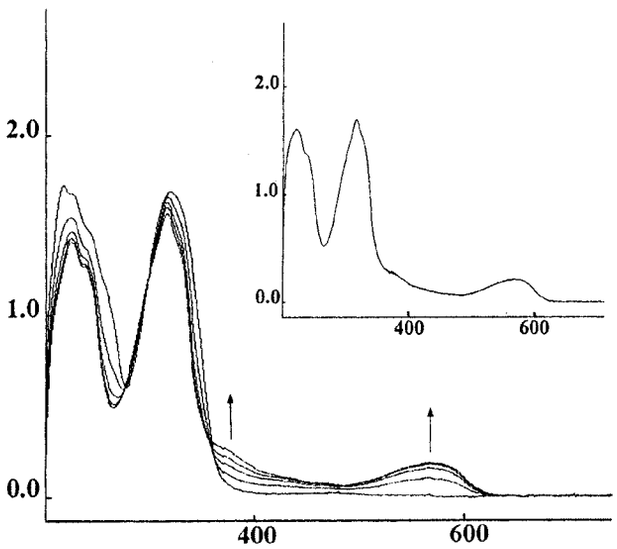


Fig. 1 Irradiation of azide **1** (1×10^4 M) in glassy ethanol at 90 K. Inset: spectrum after 8 min irradiation.

isolated. HPLC/mass examination suggested that this was the *O*-ethylhydroxylamine **6**. This product was not detected in MeCN containing 1 M ethanol.

Sensitization and trapping experiments

The decomposition of the azide was also performed in the presence of Michler's ketone as the sensitizer. As is clear from Table 1, amine **3** was by far the main product under these conditions, with only a small amount of heterocycle **2** in acetonitrile and none of it (or of product **6**) in ethanol.

Direct photolysis in the presence of 0.1 M diethylamine (DEA) in acetonitrile caused a large increase of the yield of amine **3**, the disappearance of quinoxaline **4** and only a minimal change in the yield of **2**. In ethanol the yield of amine was likewise increased, while the yields of compounds **2** and **6** were little affected and HPLC/mass analysis revealed a trace of an additional compound formed under these conditions, with a mass spectrum compatible with the structure of hydrazine **7**.

Photolysis in a glassy matrix

The photochemical decomposition of azide **1** was then performed in ethanol glass at 90 K. New absorption bands developed in the visible region (Fig. 1) finally giving a spectrum with maxima at 565 and 316 nm (see the inset). Conservation of the isosbestic points indicated that no secondary photoreactions occurred up to >70% azide conversion. This spectrum disappeared upon melting the matrix. Analysis of the photolyte showed that under these conditions the main product was the azo compound **5** accompanied by the amine **3** (Table 2).

Table 2 Products from the irradiation of azide **1** in a glassy ethanol matrix at 90 K

Conditions	% Yield of the products 2	3	5	8
fast heating		20	30	
slow heating	tr	66		
2nd irr., >450 nm		15	15	50

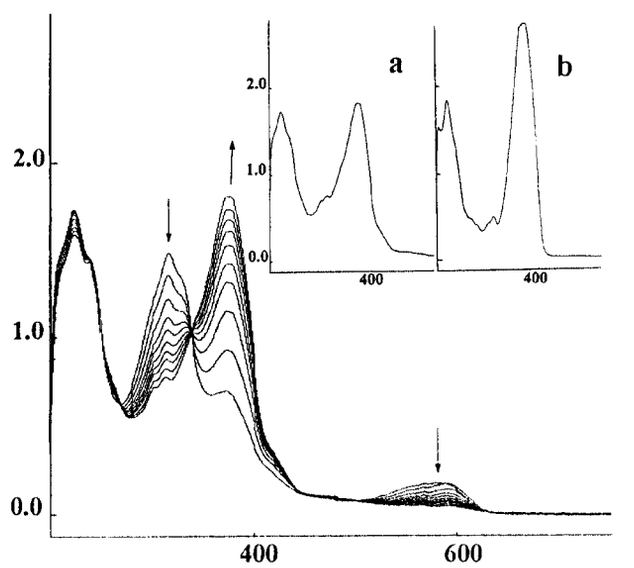


Fig. 2 Evolution at 100 K of the spectrum obtained as in Fig. 1. The initial spectrum has been obtained after 8 min irradiation at 90 K, followed by 3 h at 90 K and 1.5 h at 95 K then at 100 K. The following spectra have been taken after a 30 min interval each. Inset a: final spectrum. Inset b: amine **3** (1×10^4 M) in glassy ethanol at 90 K.

When the temperature was maintained at 90 K the above absorption was fairly stable (a 10% decrease in 3 h). Raising the temperature to 95 K caused a red shift of the longest wavelength absorption to 578 nm and a further raise at 100 K led both to a further red shift and to a measurable, though still slow, decomposition ($k_{obs} = 2.4 \times 10^{-3}$ min^{-1}). A new spectrum developed with an intensive maximum at 375 nm and very little absorption in the visible (Fig. 2). As can be seen from the inset, this was quite close to the spectrum of amine **3** in ethanol glass, and indeed chemical analysis showed that by far the main product under these conditions was the amine (except for a trace of **2**, explaining the absorption in the visible).

In another experiment the azide was first decomposed by irradiation at 254 nm in the glass and then a second irradiation was carried out at $\lambda > 455$ nm. Under these conditions the above spectrum was converted into a new spectrum with

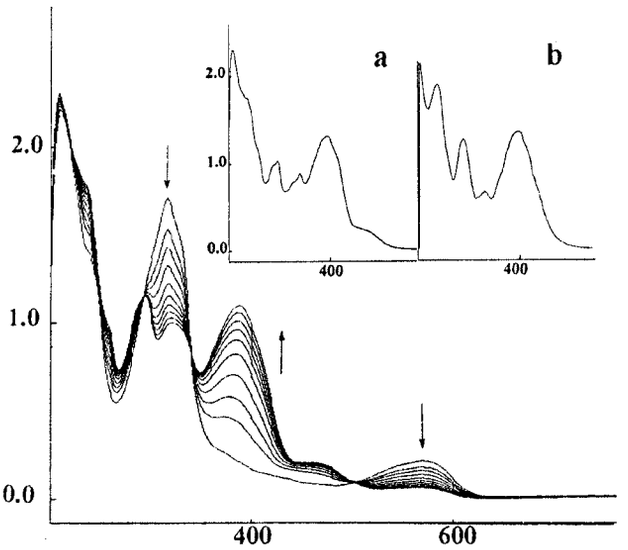


Fig. 3 Irradiation ($\lambda > 455$ nm) of the intermediate obtained as in Fig. 1 at 90 K. Each spectrum was taken after 12 min irradiation. Inset a: after 120 min irradiation and equilibration at 115 K (allowing the decomposition of the small amount of residual triplet). Inset b: spectrum of the HPLC fraction identified as dihydroquinoxaline **8**.

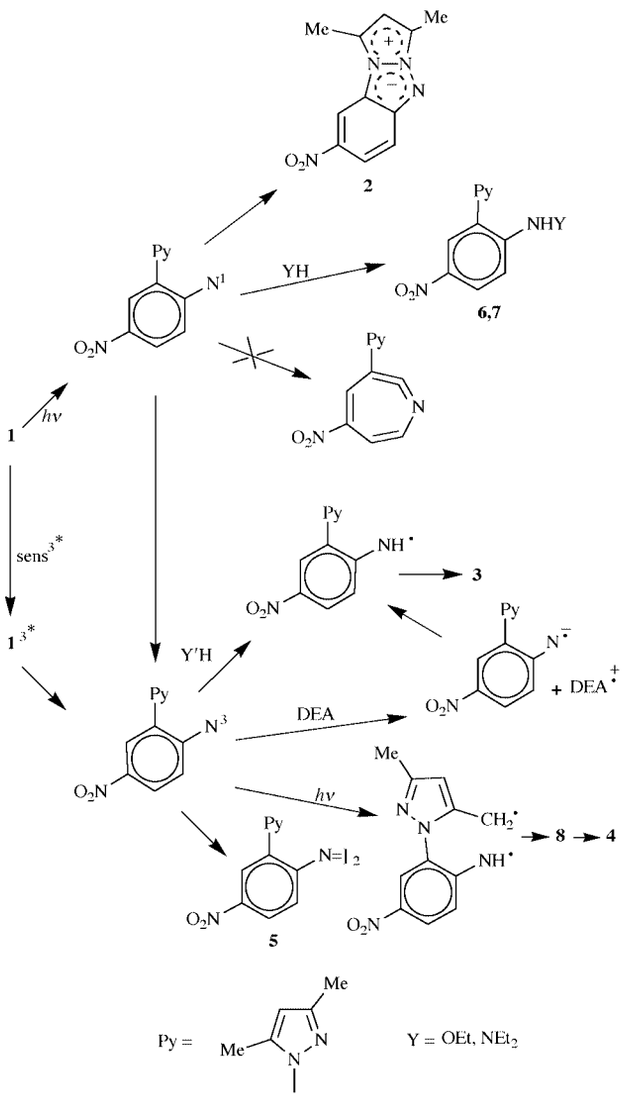
maxima at 386, 317 and 294 nm, again maintaining the isosbestic points (Fig. 3). A long irradiation time was required and after 120 min the conversion of the azide had reached 83% as judged from the residual absorption at 565 nm. Thawing the matrix and HPLC analysis of the sample showed that besides minor amounts of the amine and the azo derivative, a main product was formed which had a UV spectrum quite similar to that observed in the matrix after double irradiation (see inset). This could be identified by HPLC/mass spectroscopic analysis as the dihydropyrazoloquinoxaline **8**. On long standing in solution (several months), this product underwent spontaneous oxidation to quinoxaline **4**.

Discussion

The presence of a nucleophilic group (the pyrazole nitrogen) in azide **1** allows for intramolecular trapping of the singlet state of the corresponding nitrene. In fact, heterocycle **2** is the main product (yields ranging from 30 to 50%) from the photodecomposition of **1** in both MeCN and EtOH.

The success of intramolecular trapping contrasts with the poor yield of the corresponding intermolecular reaction. Even in the presence of an electron-withdrawing substituent such as the nitro group which makes the nitrene a stronger electrophile, intermolecular trapping remains a minor process. This occurs with a yield below 10%, *viz*. in the same order as that observed with *p*-nitrophenylnitrene, which, as mentioned in the introduction, gives 9% of the hydrazine in neat DEA (this nitrene can be trapped by aromatics, however).[20] It should be noted that other substituents allow a high yield of hydrazine to be obtained by irradiation in the presence of amines. This is the case for *p*-cyanophenyl azide,[11] where apparently electronic activation is operating, as well as with some 2,6-disubstituted phenylnitrenes, apparently due to a steric, not an electronic, effect since the result is independent from the nature of the substituent (same result with 2,6-dimethyl and 2,6-difluoro derivatives).[5] With the present nitrene, even a weak nucleophile such as ethanol is a good enough trap if used as the solvent (not at a lower concentration, up to 1 M in MeCN). The observed trapping is unspecific: in 0.1 M DEA in ethanol the main adduct is hydroxylamine **6** arising from addition of the solvent, with a much lower amount of hydrazine **7** incorporating the better nucleophile DEA. Thus, the main cause of the poor yield of intermolecular reactions must be the short lifetime of the singlet nitrene, rather than its intrinsic reactivity.

Consistent with this hypothesis, the typical reaction of singlet phenylnitrene, *viz*. the rearrangement to the dehydroazepine, is conspicuously absent with this derivative; should it be formed, it would be trapped by nucleophiles such as amines as is consistently observed with other azides,[3–7,21] including pyrazolyl derivatives[18b] (Scheme 2). Likewise, no aminoazepine



Scheme 2

was obtained in a previous study with the non-pyrazole bearing *p*-nitrophenylnitrene.[12] On the contrary, other electron-withdrawing substituted (*e.g.* polyfluoro) phenylnitrenes undergo ring enlargement as the main process (except, as mentioned above, with *o*,*o*′-disubstituted derivatives) and the corresponding aminoazepines are the main products in the presence of DEA.[21]

Thus, the simplest hypothesis is that ISC to the triplet state is faster in this case, and limits all other competing processes from the singlet except for the highly favoured cyclization to the heterocycle **2**. The ground triplet state of phenylnitrenes is known to be largely stabilised with respect to the singlet, but ISC is relatively slow (recently measured as 3×10^6 s^{-1} for parent phenylnitrene).[22] The data on the nitro substituted nitrene suggest that ISC is faster. There is evidence that polarization increases the rate of ISC in biradicaloid states.[23,24] In the present case, this may also be due to the fact that the triplet state is higher in energy, making ISC over a smaller gap faster. Support for this suggestion is the fact that the *reactivity*

of the triplet state is increased. In a hydrogen-donating solvent such as ethanol this is revealed by the significant yield of amine at room temperature. As one may expect, in an inert solvent and with a higher absorbed light flux the main reaction from the triplet state remains dimerization to the azo compound (27% in MeCN).

Reduction of the nitrene to amine **3** is effective under two conditions. The first one is triplet photosensitization of the azide decomposition. As shown in Table 1, Michler's ketone (MK, chosen because it allows selective irradiation) sensitization generates the triplet nitrene and this gives the amine (presumably *via* reduction by MK, see below) with a strong decrease in or elimination of the singlet nitrene products.

The second condition involves electron transfer. The experiments conducted in the presence of DEA show that the yield of singlet products is marginally affected (except for the formation of the trace of hydrazine **7**) and the yield of amine **3** is greatly increased. This fits with the previous suggestion by Schuster that the *p*-nitrophenylnitrene triplet state is a powerful electron acceptor,[12] and strengthens the evidence that a selective quenching of the triplet state is involved in the formation of the amine. The electron transfer–proton transfer path is a more effective mechanism for triplet state reduction than hydrogen abstraction (compare the yield of **3** in the presence of DEA and in neat ethanol). This suggests that Michler's ketone acts both as sensitizer and as reducing agent and this causes the higher yield of amine than of azo compound.

The situation changes at low temperature. First of all, the triplet can be observed in a glassy matrix. The spectrum measured at 90 K shows the characteristic intense absorption in the visible (565 nm, log ε *ca*. 3.4), which is red shifted with respect to the non-nitrated pyrazolylphenylnitrene (510 nm, log ε 3.3).[18] A similar spectrum has been observed in several simple phenyl azides,[25] although Harder *et al.* observed very little visible absorption during the photolysis of *p*-nitrophenyl azide in methyltetrahydrofuran glass.[15]

As expected from work with other phenylnitrenes,[4,6,15,18] only triplet chemistry takes place under these conditions, since the thermal barrier for accessing reactions on the singlet surface cannot be overcome. Heating of the matrix after irradiation diminishes the viscosity[18] and reproduces a situation similar to that studied by Schuster by laser photolysis of *p*-nitrophenyl azide[12] with a relatively high triplet nitrene concentration. Under this condition dimerization to the azo compound is the main reaction. On the other hand, maintaining the glass at 95–100 K for a long period of time allows hydrogen abstraction to take place before the matrix softens to a sufficient degree, and thus coupling of the triplet is unimportant. The reaction occurs as a pseudo first order process and cleanly leads to the amine as shown in Fig. 2, obviously through hydrogen abstraction from the solvent. Harder *et al.* reported an intermediate spectrum attributed to the iminyl radical in their matrix study of *p*-nitrophenyl azide.[15] However, in the present case we have no evidence for the building up of a significant concentration of an intermediate after the nitrene, although this may escape detection if, as appears to be the case in the study by Harder, it has a spectrum quite close to that of the amine. The nitro group somewhat increases the hydrogen atom abstraction rate, as judged by the fact that under matrix conditions the amine is practically the only product obtained, while the azo still competes (amine–azo ratio *ca*. 2) with the non-nitrated pyrazolylphenylnitrene under the same conditions.[18b]

In contrast to the various intermolecular channels observed for triplet nitrene both at high and low temperatures, the seemingly attractive intramolecular hydrogen abstraction reaction from the pyrazole methyl group is unimportant. The formation of quinoxaline is only a minor process. Apparently, the increase in the radical reactivity of the nitrene induced by the nitro group is not sufficient to make this reaction channel effective.

However, electronic excitation of the triplet state makes hydrogen abstraction effective. It has been suggested that the lowest excited state of triplet arylnitrenes corresponds to a $\pi \rightarrow n$ transition;[26] thus, the excited triplet can be likened to an iminyl radical and is expected to be a much better hydrogen abstractor. In the matrix such a process obviously takes place intramolecularly and leads to the dihydroquinoxaline **8**.

In conclusion, the data obtained with a model system demonstrate both singlet and triplet nitrene reactions through product studies. This reveals the possibility of trapping the singlet through an intramolecular reaction, while confirming that intermolecular trapping *in solution* is a marginal process. Likewise, nitro substitution only marginally increases the radical reactivity of the triplet. The utility of nitrophenyl azides for photochemical labelling of biomolecules is related to better complexation with the receptors, which would make the reaction more similar to the present model - rather than to an intrinsic change in the reactivity of the nitrene.

Experimental

General

Acetonitrile and 95% ethanol were spectroscopic grade solvents. Azide **1** was prepared and purified as previously reported.[18c] Photoproducts **2** to **5** were likewise previously reported.[18c] The photoreactions were monitored by HPLC. A Jasco PU 980 instrument with UV-975 detector was used, with a 25 cm × 4.6 mm Merck Purospher RP-18 LiChroCART 250-4 column (and a Purospher RP-18 LiChroCART 4-4 precolumn). Various water–acetonitrile mixtures were used as the eluent. HPLC/mass experiments (on a Finnigan LCQ instrument) were used for the detection of trace products (see below).

Room temperature irradiations

1×10^{-4} M Solutions of azide **1** in a 1 cm optical path cell (2 mL) were irradiated by means of a bifilar low-pressure mercury arc inserted below the cell (Helios Italquartz 15 W). More concentrated solutions (4×10^{-3} M) were irradiated by means of a focused high-pressure mercury arc (Osram HBO 200W/2; in the MK sensitized experiments a cutoff filter with $\lambda_{tr} > 350$ nm was inserted). The product distribution as determined by HPLC is reported in Table 1. Apart from the previously reported products **2**–**5**, in the experiments in ethanol HPLC revealed a further product, with a mass spectrum compatible with the structure of *1-(2-ethoxyamino-5-nitrophenyl)-3,5-dimethyl-1H-pyrazole* (**6**). T_r 13.2 min (1 to 1 MeCN–H_2O mixture, 0.5 ml min^{-1}). Mass spectrum: *m*/*z* 277 ($M^+ + 1$), 231 (base peak), 185 ($M^+ - EtOH$, $-NO_2$). In the irradiation in EtOH with 0.1 M DEA, a further trace peak was detected, with a spectrum compatible with the structure of *1-[2-(2,2-diethylhydrazino)-5-nitrophenyl]-3,5-dimethyl-1H-pyrazole* (**7**). T_r 18.5 min (1 to 1 MeCN–H_2O mixture, 0.5 ml min^{-1}). Mass spectrum: *m*/*z* 304 ($M^+ + 1$), 231 (base peak), 185 ($M^+ - Et_2NH$, $-NO_2$).

Low temperature experiments

1×10^{-4} M Solutions of the azide **1** in EtOH (2 mL) in a 1 cm optical path quartz cell with a quartz to glass graded seal were degassed by means of four freeze–degas–thaw cycles and sealed. The cell was inserted into an Oxford DN 1704 liquid nitrogen cryostat fitted with a calibrated ITC4 temperature controller and placed in a UV-vis Kontron Uvikon 941 spectrophotometer and irradiated from the bottom as above. Irradiation was discontinued when taking the spectra. In a typical experiment, the solution was equilibrated for 30 min at 90 K and then irradiated for 8 min. After this time the temperature was either gradually raised (see text and Fig. 2)

or allowed to quickly reach room temperature (within 30 min in the latter case). The solution was analyzed by HPLC.

In double irradiation experiments, samples irradiated at 254 nm as above were further irradiated by means of a focused high-pressure mercury arc (Osram 200 W/2) passed through a cutoff filter (λ_{tr} > 455 nm). The beam reached the cell through a side opening perpendicular to the analyzing beam. The sample was then rapidly heated to room temperature and analyzed as above. In such experiments a new peak was detected in HPLC, which exhibited a mass spectrum compatible with the structure of *2-methyl-8-nitro-4,5-dihydropyrazolo[1,5-a]quinoxaline* (**8**). T_r 12.0 min (1 to 1 MeCN–H_2O mixture). Mass spectrum: *m*/*z* 231 ($M^+ + 1$), 214 ($M^+ - NH_3$), 185 (base peak, $M^+ - NO_2$), 168 ($M^+ - NH_3 - NO_2$). Examination of a solution of **8** kept in the dark for 12 months showed that this was completely transformed into the rearomatized derivative **4**.

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