

## Azido derivative of a hemicyanine dye with high sensitivity to visible light

Mikhail F. Budyka,<sup>\*a</sup> Natalya V. Biktimirova,<sup>a</sup> Tatyana N. Gavrishova<sup>a</sup> and Viacheslav I. Kozlovskii<sup>b</sup>

<sup>a</sup> Institute of Problems of Chemical Physics, Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russian Federation. Fax: +7 496 514 3244; e-mail: budyka@icp.ac.ru

<sup>b</sup> Institute of Energy Problems for Chemical Physics (Branch), Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russian Federation

DOI: 10.1016/j.mencom.2007.05.009

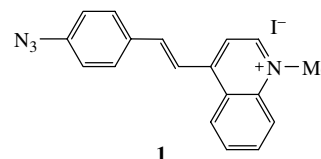
The synthesised (*E*)-1-methyl-4-(4-azidostyryl)quinolinium iodide has the quantum yield of photodissociation  $0.84 \pm 0.17$ , which is independent of excitation wavelength (365–485 nm) and dissolved oxygen.

Aromatic azides are widely used as photoaffinity labels,<sup>1</sup> reagents for nucleic acid modification<sup>2</sup> and photodynamic therapy agents.<sup>3</sup> To avoid the photodestruction of biological materials (proteins or polynucleotides) during a photolabeling experiment, azide should have an absorption band in the long-wavelength region and be photoactive, *i.e.*, decompose with high quantum yields ( $\varphi > 0.1$ ) upon irradiation in this spectral region. However, the spectral sensitivity of used azides is restricted by UV and short-wavelength visible regions,<sup>1,2</sup> whereas azides having absorption bands in the visible region (so-called azido dyes) loss light sensitivity, and their decomposition quantum yields drop below 0.01.<sup>4</sup> The exception is azido dyes, the derivatives of phenanthridine and acridine, which have quantum yields of 0.5–1.0 upon irradiation by short-wavelength visible light.<sup>2,5</sup> Note that all of the known azido dyes are positively charged aromatic systems.

The photoactivity of aromatic azides depends on the nature of the molecular orbital (MO) that is filled in the lowest excited singlet ( $S_1$ ) state.<sup>6</sup> If the antibonding  $\sigma_{NN^*}$  MO is filled, the azide is photoactive (photodissociation quantum yield  $\varphi > 0.1$ ). In turn, the filling of the  $\sigma_{NN^*}$  MO depends on the size and charge of the aromatic  $\pi$  system. When the size of the  $\pi$  system increases above a certain threshold, the  $\sigma_{NN^*}$  MO ceases to be filled in the  $S_1$  state, and the azide becomes photoinert ( $\varphi$  drops below 0.01). The threshold size is predicted to be 22 and 18  $\pi$  electrons for the neutral and positively charged azides, respectively.<sup>7</sup>

The  $\pi$  system of styrylquinoline comprises exactly 18 electrons. Thus, one could expect that, in azidohemicyanine dye **1**, which is a derivative of 4-styrylquinoline, the  $\sigma_{NN^*}$  MO would be filled in the  $S_1$  state. We carried out quantum-chemical calculations of azide **1** in the  $S_0$  and  $S_1$  states and defined the nature of the orbital that is filled in the  $S_1$  state.

Figure 1 shows the structure of the frontier orbitals for **1**, the highest occupied (HOMO) and the lowest unoccupied (LUMO) MOs in the ground  $S_0$  state, and the lowest (LSOMO) and the highest (HSOMO) semi-occupied MOs in the excited singlet ( $S_1$ ) state calculated using the PM3 method.<sup>†,8</sup> One can see that in the ground state the frontier MOs are  $\pi$ -type orbitals, HOMO being localised mainly on the styryl group and LUMO, on quinoline nucleus. In the  $S_0$  state, the  $\sigma_{NN}$  MO is 3.62 eV higher than LUMO, it is LUMO+7. However, in the  $S_1$  state, namely the  $\sigma_{NN}$  MO is filled, and this is a prerequisite for subsequent dissociation of the azido group.



Thus, calculations predict azido dye **1** to be photoactive, *i.e.*, it will decompose with a high quantum yield upon irradiation with visible light.

Taking into account the results of calculations, we synthesised (*E*)-1-methyl-4-(4-azidostyryl)quinolinium iodide **1** by methylation of corresponding (*E*)-4-(4-azidostyryl)quinoline and investigated its photochemical properties.<sup>‡</sup>

Azido dye **1** possesses a strong absorption band in the visible region of the spectrum,  $\lambda_{\max}$  ( $\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ) 417 nm (28900),

<sup>†</sup> The structures of azide **1** (without a counterion) in the  $S_0$  and  $S_1$  states were calculated with the full geometry optimization using the MOPAC 2002 program package. Excited state was calculated using configuration interaction (1225 configurations, 8 electrons at 7 MOs in the active space). The applicability of the PM3 method to the problem under discussion was demonstrated earlier.<sup>7</sup>

<sup>‡</sup> (*E*)-1-methyl-4-(4-azidostyryl)quinolinium iodide **1** [the reaction and the purification of azide **1** were carried out in the dark (under red light)]. MeI (0.5 ml) was added to a solution of (*E*)-4-(4-azidostyryl)quinoline (60 mg) in 5 ml of anhydrous acetonitrile. The mixture was heated at 40 °C for 5 h and then allowed to stand overnight at 20 °C. To a cooled mixture, 5 ml of light petroleum (bp 60–80 °C) was added, the resulting precipitate was filtered off and recrystallised from a mixture of acetonitrile and light petroleum to give **1** in 80% yield; mp 185 °C (decomp.). <sup>1</sup>H NMR (200 MHz, Bruker DPX-200, [<sup>2</sup>H<sub>6</sub>]DMSO)  $\delta$ : 9.4 (d, 1H, quinoline, *J* 9 Hz), 9.15 (d, 1H, quinoline, *J* 9 Hz), 7.9–8.7 (m, 8H, quinoline, C<sub>6</sub>H<sub>4</sub>, CH=CH), 7.25 (d, 2H, C<sub>6</sub>H<sub>4</sub>, *J* 8 Hz), 4.62 (s, 3H, Me). MS (ESI), *m/z*: 287.110 [M]<sup>+</sup>; calc. for C<sub>18</sub>H<sub>15</sub>N<sub>4</sub><sup>+</sup>: 287.130. IR (KBr,  $\nu/\text{cm}^{-1}$ ): 2127 (N<sub>3</sub>), 1593, 1567 (CH=CH), 1424, 1369 (Me), 837 (*p*-C<sub>6</sub>H<sub>4</sub>).

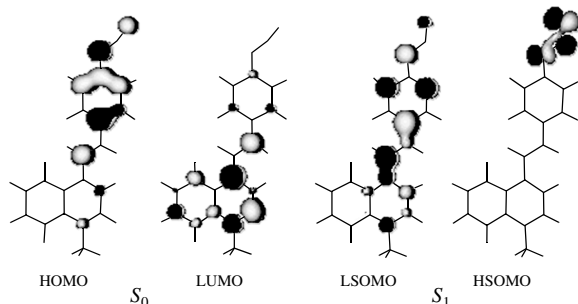
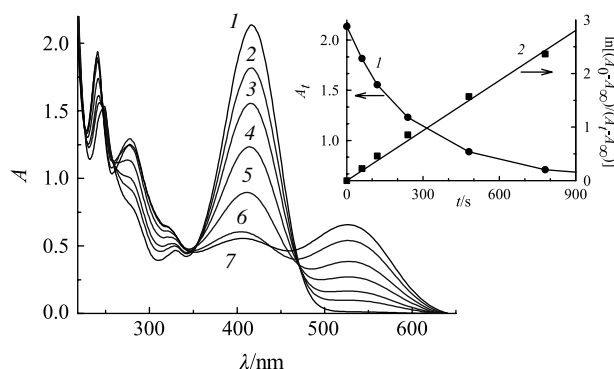


Figure 1 Structure of frontier MOs of **1** in the  $S_0$  and  $S_1$  states.



**Figure 2** Spectral changes upon irradiation of a  $7.4 \times 10^{-5}$  M solution of azidohemicyanine dye **1** in degassed EtOH with light at 485 nm, irradiation time: (1) 0, (2) 60, (3) 120, (4) 240, (5) 480, (6) 1380 and (7) 2900 s; light intensity,  $1.08 \times 10^{-9}$  einstein  $\text{cm}^{-2} \text{s}^{-1}$ . Insert: (1) kinetic curve at 417 nm and (2) its anamorphosis in the semilogarithmic coordinates.

tailing to 500 nm, and a less intense bands in the UV region, 332 nm (6200) and 249 nm (19650) (Figure 2, curve 1).

Upon irradiation within the long-wavelength absorption band, azide **1** effectively decomposes. As an example, Figure 2 shows spectral changes upon the irradiation of an azide solution with visible light at 485 nm in the absence of oxygen.<sup>§</sup> One can see that the reaction is uniform within the initial period of photolysis, which is demonstrated by isosbestic points at 354.1 and 472.5 nm (curves 1–4 in Figure 2). At this timepoint, in the reaction mixture there were two main components, as it was shown by electrospray mass spectrometry:<sup>¶</sup> the precursor azide, whose peak with  $m/z$  287.105 was maximal in the spectrum (100%), and the related primary amine, whose peak with  $m/z$  261.116 was 59% in intensity. All other peaks in the mass spectrum were by more than one order of magnitude less abundant. Thus, the absorption band at 520 nm, which appears in the spectrum during irradiation (Figure 2), can definitely be attributed to (*E*)-1-methyl-4-(4-aminostyryl)quinolinium iodide, which is practically the only reaction product at the initial period of photolysis in degassed solution.

During further irradiation, isosbestic points disappeared thus indicating the proceeding of secondary reactions. The following compounds were identified in the final reaction mixture by electrospray mass spectrometry: the primary amine, which remained the main reaction product,  $m/z$  261.113 (100%), corresponding azo compound,  $m/z$  259.099 ( $M^{2+}$ , double charged ion,  $M = 518$ , 62%), and hydrazo compound,  $m/z$  260.106 ( $M^{2+}$ , double charged ion,  $M = 520$ , 8%). There were also less intense peaks of unidentified products with  $m/z$  218.097 (15%), 229.593 ( $M^{2+}$ , double charged ion,  $M = 459$ , 14%), and 295.080 (15%), which could be secondary reaction products.

**Table 1** Photodissociation quantum yields for (*E*)-1-methyl-4-(4-azido-styryl)quinolinium iodide in EtOH (error  $\pm 20\%$ ).

Wavelength $\lambda_{\text{ex}}$ /nm	Quantum yield $\phi$
365	0.80, 0.86 <sup>a</sup>
479	0.89
485	0.85, 0.80 <sup>a</sup>

<sup>a</sup>Degassed solution.

<sup>§</sup> A mercury high-pressure lamp (DRSh-500) combined with glass filters to select the Hg line at 365 nm, ultrabright LED SDK-470, combined with a glass filter (GS-16) to select the 479 nm band and KGM-24-250 lamp, combined with glass and interference filters to select the 485 nm band, were used as photochemical light sources. Solutions in EtOH and quartz cuvettes with optical path 1 cm were used. Deoxygenation of the solution was carried out using a freeze–pump–thaw technique.

<sup>¶</sup> Electrospray mass spectra were obtained with the custom built high-resolution electrospray ionization orthogonal-injection time-of-flight mass spectrometer.<sup>9</sup>

Upon photolysis in the presence of oxygen, the ratio of amine, azo compound and hydrazo compound did not practically change (100, 62 and 8%, respectively), but additional oxygenated products were formed: corresponding nitroso,  $m/z$  275.098 (9%), nitro,  $m/z$  291.099 (4%), and azoxy,  $m/z$  267.099 ( $M^{2+}$ , double charged ion,  $M = 534$ , 6%) compounds. The same unidentified products as in the degassed solution were also observed with the same proportion.

Obviously, all identified products formation can be explained by the reactions of triplet nitrene.<sup>10</sup> The relatively low quantity of nitroso and nitro compounds is noteworthy. The charged nitrene, generated from azido dye, appears to be less reactive towards oxygen, as compared to a neutral analogue.<sup>††</sup>

The quantum yield of azido group photodissociation was measured by monitoring the decrease in the absorbance of azide at an absorption maximum (417 nm). Insert in Figure 2 shows the kinetics of absorbance changes at the azide band maximum and its anamorphosis in the semilogarithmic coordinates. The photodissociation quantum yields of azido dye **1** under various conditions of photolysis are summarised in Table 1.

One can see that, within the experimental accuracy, the quantum yield is equal to  $0.84 \pm 0.17$  and independent of both excitation wavelength (365–485 nm) and dissolved oxygen. The prediction that positively charged heteroaromatic azide with an aromatic  $\pi$  system of 18 electrons should be photoactive is corroborated.

Thus, we describe for the first time the quantum-chemical calculations, synthesis and photochemical properties of an azido derivative of a hemicyanine dye, which decomposes with a high quantum yield upon irradiation in the spectral region from UV to almost 500 nm. Azido dye **1** possesses the highest sensitivity to long-wavelength visible light.

This work was supported by the Russian Foundation for Basic Research (grant no. 03-03-32116).

## References

- (a) U. Henriksen, O. Buchardt and P. E. Nielsen, *J. Photochem. Photobiol. A: Chem.*, 1991, **57**, 331; (b) S. Q. Zhuo, S. Garrod, P. Miller and W. S. Allison, *J. Biol. Chem.*, 1992, **267**, 12916; (c) R. S. Pandurangi, R. R. Kuntz and W. A. Volkert, *Appl. Radiat. Isotopes*, 1995, **46**, 233; (d) M. J. Bouchet and M. Goeldner, *Photochem. Photobiol.*, 1997, **65**, 195.
- N. P. Gritsan, A. A. Koshkin, A. Yu. Denisov, Yu. Ya. Markushin, E. V. Cherepanova and A. V. Lebedev, *J. Photochem. Photobiol. B: Biol.*, 1997, **37**, 40.
- R. Rajagopalan, G. Cantrell, S. I. Achilefu, J. E. Bugai and R. B. Dorshow, *US Patent*, 0180864, 2004.
- M. F. Budyka, T. S. Zyubina and M. M. Kantor, *Zh. Fiz. Khim.*, 2000, **74**, 1115 (*Russ. J. Phys. Chem.*, 2000, **74**, 995).
- M. F. Budyka, N. V. Biktimirova and T. N. Gavrishova, *Khim. Vys. Energ.*, 2006, **40**, 208 [*High Energy Chem. (Engl. Transl.)*, 2006, **40**, 170].
- M. F. Budyka and T. S. Zyubina, *J. Mol. Struct. (Theochem)*, 1997, **419**, 191, and references therein.
- M. F. Budyka and I. V. Oshkin, *J. Mol. Struct. (Theochem)*, 2006, **759**, 137.
- (a) J. J. P. Stewart, *J. Comput. Chem.*, 1989, **10**, 208; (b) J. J. P. Stewart, *J. Comput. Chem.*, 1989, **10**, 221.
- A. F. Dodonov, V. I. Kozlovski, I. V. Soulimenkov, V. V. Raznikov, A. V. Loboda, Z. Zhen, T. Horwath and H. Wollnik, *Eur. J. Mass Spectrom.*, 2000, **6**, 481.
- Azides and Nitrenes. Reactivity and Utility*, ed. E. F. V. Scriven, Academic Press, New York, 1984.

Received: 31st October 2006; Com. 06/2807

<sup>††</sup> We undertook comparative photolysis of neutral (*E*)-4-(4-azido-styryl)-quinoline under the same conditions and found that in this case the final reaction mixture contained 60% nitroso and 40% nitro compounds.