

# Spectral and Photophysical Studies of Substituted Indigo Derivatives in Their Keto Forms

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The spectral and photophysical properties of indigo derivatives with di-, tetra-, and hexa-substitution in their neutral (keto) form are investigated in solution. The study comprises absorption and emission spectra, together with quantitative measurements of quantum yields of fluorescence ( $\phi_F$ ) and singlet oxygen formation ( $\phi_{\Delta}$ ) and fluorescence lifetimes. The energy difference between the HOMO and LUMO orbitals is dependent on the degree (number of groups) and relative position of substitution. The  $\phi_F$  and  $\phi_{\Delta}$  values were found to be very low  $\leq 10^{-3}$ . Because of the absence of transient triplet–triplet signal, the intersystem crossing yields ( $\phi_T$ ) were estimated by assuming that all the triplet states formed give rise to singlet oxygen formation, that is,  $\phi_{\Delta} \approx \phi_T$ . It was then possible from  $\phi_{IC} = 1 - \phi_F - \phi_T$  to estimate the  $S_1 \sim \sim \rightarrow S_0$  internal conversion yields and thus, with the other data, to determine the rate constants for all decay processes. From these, several conclusions are drawn. Firstly, the radiationless rate constants,  $k_{NR}$ , clearly dominate over the radiative rate constants,  $k_F$  (and processes). Secondly, the main deactivation channel for the compounds in their keto form is the radiationless  $S_1 \sim \sim \rightarrow S_0$  internal conversion process. Finally, although the changes are relatively small, internal conversion yield seems to be independent of the overall pattern of substitution. A more detailed investigation of the decay profiles with collection at the blue and red emission of the fluorescence band of indigo and one di-substituted indigo reveals the decays to be bi-exponential and that at longer emission wavelengths these appear to be associated with both rise and decay times indicating that two excited species exist, which is consistent with a keto-excited form giving rise (by fast proton transfer) to the enol-form of indigo. Evidence is presented which supports the idea that intramolecular (and possibly some intermolecular) proton transfer can explain the high efficiency of internal conversion in indigo.

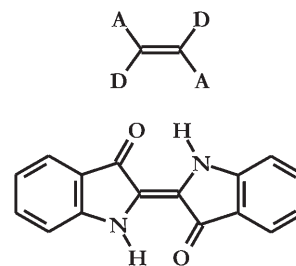
stants,  $k_{NR}$ , clearly dominate over the radiative rate constants,  $k_F$  (and processes). Secondly, the main deactivation channel for the compounds in their keto form is the radiationless  $S_1 \sim \sim \rightarrow S_0$  internal conversion process. Finally, although the changes are relatively small, internal conversion yield seems to be independent of the overall pattern of substitution. A more detailed investigation of the decay profiles with collection at the blue and red emission of the fluorescence band of indigo and one di-substituted indigo reveals the decays to be bi-exponential and that at longer emission wavelengths these appear to be associated with both rise and decay times indicating that two excited species exist, which is consistent with a keto-excited form giving rise (by fast proton transfer) to the enol-form of indigo. Evidence is presented which supports the idea that intramolecular (and possibly some intermolecular) proton transfer can explain the high efficiency of internal conversion in indigo.

## 1. Introduction

The natural indigo plant source woad, *Isatis tinctoria*, was known throughout Europe and the Middle East as far back as the time of the ancient Egyptians, who used it to dye the cloth wrappings applied for the mummies.<sup>[1]</sup> As a molecule, indigo has an almost mythical status, and several illustrative examples of its “impact” in the history of mankind can be identified. From the Jewish “sacred blue”, the “tecklet”, through the use of woad by Napoleon Bonaparte’s army to dye the fabric for their uniforms, up to its continuing use today in “blue jeans”, indigo is the dye that has given all of these their magnificent blue color.

The photophysics of indigo and related molecules has been recently investigated.<sup>[2]</sup> Indigo has been shown to owe its high bathochromicity (shift to longer wavelengths) to the special orientation of the electron donor (–NH) and acceptor (–C=O) groups, see Scheme 1. Depending on the nature of the substitution (electron-donating or withdrawing) the relative energy of the HOMO and LUMO will be affected through the donor and acceptor group characteristics. As will be shown and discussed herein, the donor and acceptor groups can be influenced by peripheral substitution at the indigo benzene rings.

In addition to its peculiar bathochromicity, indigo is also one of the most stable dyes used technologically. In fact, this



Scheme 1. Structure and chromophoric system of indigo.

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stability can be considered to be one of the properties that has led to its mystic character and longevity as a molecule. The dyeing process depends on interactions with oxygen as also, presumably, does the fading of indigo. In the latter respect, there are some old reports where the fading of indigo was first investigated. These studies, dated from more than 80 years ago report that isatin (a molecule whose structure mirrors one half of the indigo molecule with one C=O in the place of the C=C bond) is the major degradation product, which, according to the authors, indicated the oxidative nature of the reaction.<sup>[3]</sup> Later solution studies seemed to confirm this and provided evidence for a self-sensitized photo-oxidation reaction involving singlet oxygen. More recently Novotna and co-workers studied the degradation of indigo in dichloromethane solution and isatin appeared as the major degradation product, yet no systematic study of oxygen's influence on the reaction mechanism has been given.<sup>[4]</sup> With indigo and its derivatives, the role of oxygen is also of primordial importance in the process of textile dyeing. The dyeing process with a vat dye, such as indigo, involves the reduction of the colored form of the dye (insoluble in water) followed by oxidation with atmospheric oxygen.<sup>[1,2]</sup> This is in contrary to the premise that oxidation by oxygen is the active participant in indigo degradation. In textiles and paintings, that is, in solid matrixes, indigo is reported to be stable (or photostable), whereas the degradation is found in solution. Moreover, recent studies on some of the more highly substituted indigo dyes also considered in the present work have shown them to be powerful sensors for ozone determination, showing both the versatility of these compounds and also the urgent need of further investigations on the interaction of indigo with oxygen.<sup>[5]</sup> The efficiency of triplet state and singlet oxygen formation by indigo is thus an important parameter.

We report the effect of substitution on the spectral and photophysical properties of several indigo derivatives. Of particular relevance is, what we believe to be, the first report of singlet-oxygen yields for indigo and for these new derivatives. As shown previously, intersystem crossing from upper-excited singlet to triplet states is not a favored mechanism for the keto forms of indigo, Tyrian purple, indirubin, and indigocarmine.<sup>[2]</sup> In the present study  $\phi_{\Delta}$  determinations provide a way to estimate indirectly singlet-triplet intersystem crossing yields for these compounds.

## Experimental Section

The indigo derivatives were synthesized by procedures described elsewhere.<sup>[6]</sup> A short description of the synthetic procedures and characterizations is described below.

**Family 1.** 4,4'-Dibromoindigo [2-(4-bromo-1,3-dihydro-3-oxo-2H-indol-2-ylidene)-1,2-dihydro-3H-4-bromoindol-3-one; **1**]: The dye **1** was prepared from 6-bromo-2-nitrobenzaldehyde, acetone and KOH as described for **9** in ref. [7]. For analytical data of **1** see ref. [8].

4,4'-Dichloroindigo [2-(4-chloro-1,3-dihydro-3-oxo-2H-indol-2-ylidene)-1,2-dihydro-3H-4-chloroindol-3-one; **2**]: The dye **2** was pre-

pared from 6-chloro-2-nitrobenzaldehyde, acetone and KOH as described for **9** in ref. [6]. For analytical data of **2** see ref. [8].

6,6'-Difluoroindigo [2-(6-fluoro-1,3-dihydro-3-oxo-2H-indol-2-ylidene)-1,2-dihydro-3H-6-fluoroindol-3-one; **3**]: The dye **3** was prepared from 4-fluoro-2-nitrobenzaldehyde, acetone and KOH as described for **9** in ref. [6]. For analytical data of **3** see ref. [8].

6,6'-Dimethoxyindigo [2-(6-methoxy-1,3-dihydro-3-oxo-2H-indol-2-ylidene)-1,2-dihydro-3H-6-methoxyindol-3-one; **4**]: The dye **4** was prepared from 4-methoxy-2-nitrobenzaldehyde, acetone and KOH as described for **9** in ref. [6]. For analytical data of **4** see ref. [8].

**Family 2.** 7,7'-Dimethoxy-4,4'-dinyloxyindigotin [2-(7-methoxy-4-nonyloxy-1,3-dihydro-3-oxo-2H-indol-2-ylidene)-1,2-dihydro-3H-7-methoxy-4-nonyloxyindol-3-one; **5**]: Compound **5** was prepared from 3-methoxy-2-nitro-6-nonyloxybenzaldehyde and acetone.<sup>[6]</sup>

5,5',6,6'-Tetramethoxyindigo [2-(5,6-dimethoxy-1,3-dihydro-3-oxo-2H-indol-2-ylidene)-1,2-dihydro-3H-5,6-dimethoxyindol-3-one; **6**]: The dye **6** was prepared from 4,5-dimethoxy-2-nitrobenzaldehyde, acetone, and KOH.<sup>[7]</sup>

**Family 3.** 5,5'-Diamino-4,4'-dihexyloxy-7,7'-dimethoxyindigotin [2-(5-amino-4-hexyloxy-7-methoxy-5-nitro-1,3-dihydro-3-oxo-2H-indol-2-ylidene)-1,2-dihydro-3H-5-amino-4-hexyloxy-7-methoxyindol-3-one; **7**]: Compound **7** was prepared by reduction of 4,4'-dihexyloxy-7,7'-dimethoxy-5,5'-dinitroindigotin (from 2-hexyloxy-5-methoxy-3,6-dinitrobenzaldehyde, acetone, and KOH) with sodium dithionite ( $\text{Na}_2\text{S}_2\text{O}_4$ ).<sup>[6]</sup>

5,5'-Diamino-4,4'-dibutoxy-7,7'-dimethoxyindigotin [2-(5-amino-4-butoxy-7-methoxy-5-nitro-1,3-dihydro-3-oxo-2H-indol-2-ylidene)-1,2-dihydro-3H-5-amino-4-butoxy-7-methoxyindol-3-one; **8**]: Compound **8** was prepared by treatment of 4,4'-dibutoxy-7,7'-dimethoxy-5,5'-dinitroindigotin (from 2-butoxy-5-methoxy-3,6-dinitrobenzaldehyde, acetone, and KOH) with sodium dithionite ( $\text{Na}_2\text{S}_2\text{O}_4$ ).<sup>[6]</sup>

4,4'-Dibutoxy-7,7'-dimethoxy-5,5'-dinitroindigotin [2-(4-butoxy-7-methoxy-5-nitro-1,3-dihydro-3-oxo-2H-indol-2-ylidene)-1,2-dihydro-3H-4-butoxy-7-methoxy-5-nitroindol-3-one; **9**]: Compound **9** was prepared from 2-butoxy-5-methoxy-3,6-dinitrobenzaldehyde and acetone/KOH.<sup>[6]</sup>

Indigo, indirubin, indigocarmine, and Tyrian purple were obtained as previously reported.<sup>[2]</sup> The solvents used, benzene, dioxane and *N,N*-dimethylformamide (DMF), were of spectroscopic or equivalent grade. Deuterated water was purchased from Aldrich and stored under Ar or N<sub>2</sub> inert atmosphere in a freezer. Prior to use it was left for 30 min at room temperature.

Absorption and fluorescence spectra were recorded on Shimadzu UV-2100 and Horiba-Jovin-Yvon Spex Fluorog 3-2.2 spectrophotometers, respectively. Fluorescence spectra were corrected for the wavelength response of the system. The fluorescence quantum yields of the compounds were determined using indigo ( $\phi_{\text{F}} = 0.0023$  in DMF).<sup>[2]</sup>

The singlet oxygen yields ( $\phi_{\Delta}$ ) and lifetimes of the indigo derivatives were obtained by measuring the phosphorescence emission of O<sub>2</sub> (at 1270 nm) in air-equilibrated dimethylformamide (DMF) with a germanium diode detector and a frequency doubled pulse (532 nm) from a Nd:YAG laser. The quantum yield of the singlet oxygen formation was obtained by comparison of the initial emission intensity (signal, in mV, extrapolated to zero time) for solutions optically matched at the excitation wavelength ( $A_{532} \approx 0.2$ ) with TPP taken as standard using  $\phi_{\Delta} = 0.28$ . The  $\phi_{\Delta}$  value for TPP in

DMF was obtained with the same procedure, taking as reference TPP in benzene ( $\phi_{\Delta} = 0.62$ ).

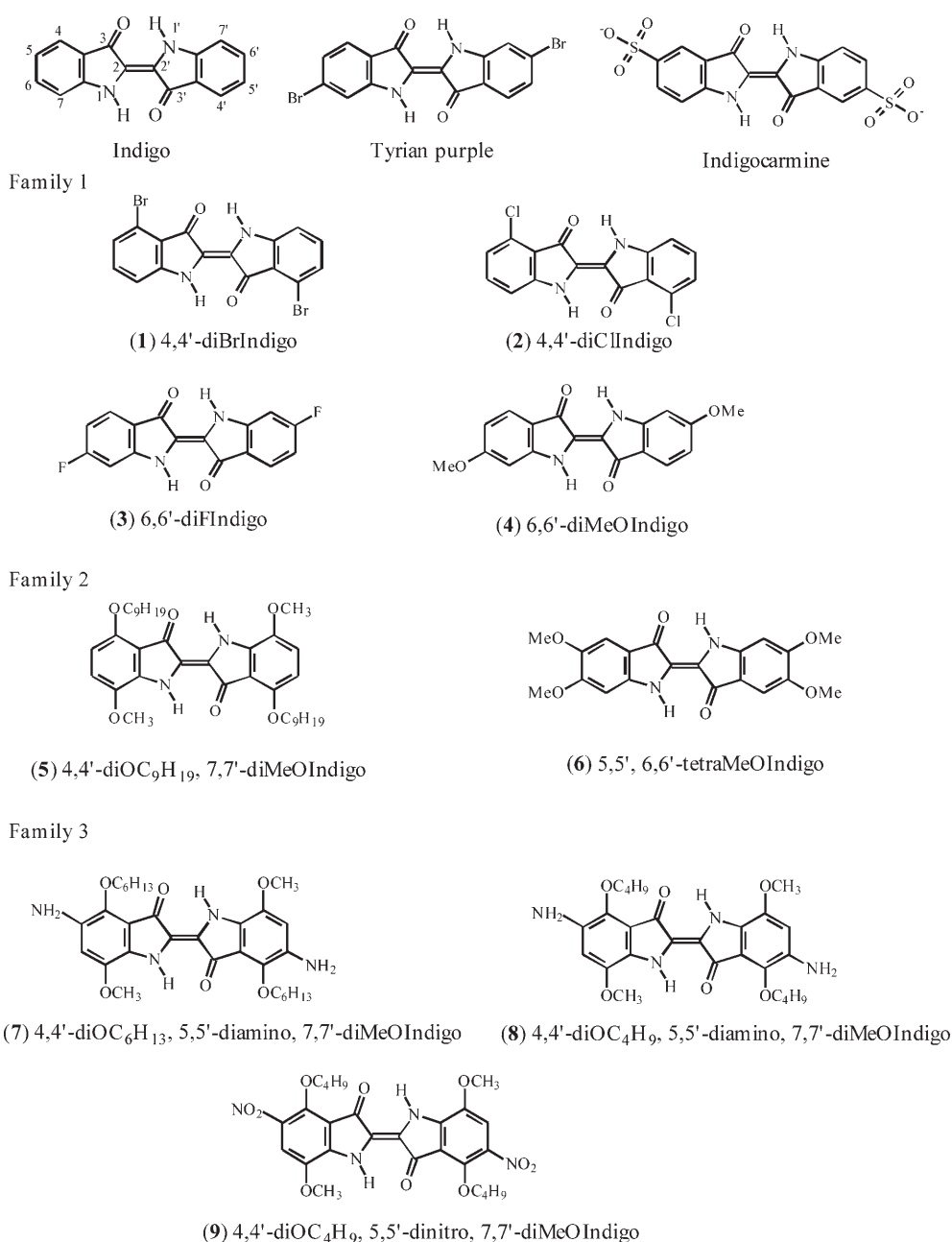
The fluorescence decays of the compounds were obtained with picosecond resolution on an apparatus described elsewhere<sup>[9]</sup> and were analyzed using the method of modulating functions implemented by Striker.<sup>[10]</sup>

## 2. Results

The structures and acronyms for the compounds studied are divided into three families, depending on the degree of substitution (di-, tetra-, or hexa-substitution), and are depicted in Scheme 2. Numbering is given for indigo.

Figure 1 shows the absorption, fluorescence emission and excitation spectra for selected compounds from Scheme 1. Data for indigo and Tyrian purple have previously been published.<sup>[2]</sup> There is a perfect match between the absorption and fluorescence excitation spectra for the longest wavelength absorption, which attests to the high purity of the compounds. This is of particular relevance with indigo derivatives, since, due to the low fluorescence yields (generally with  $\phi_f$  values  $\leq 10^{-3}$ ) artifacts from impurities can dominate photo-physical properties.

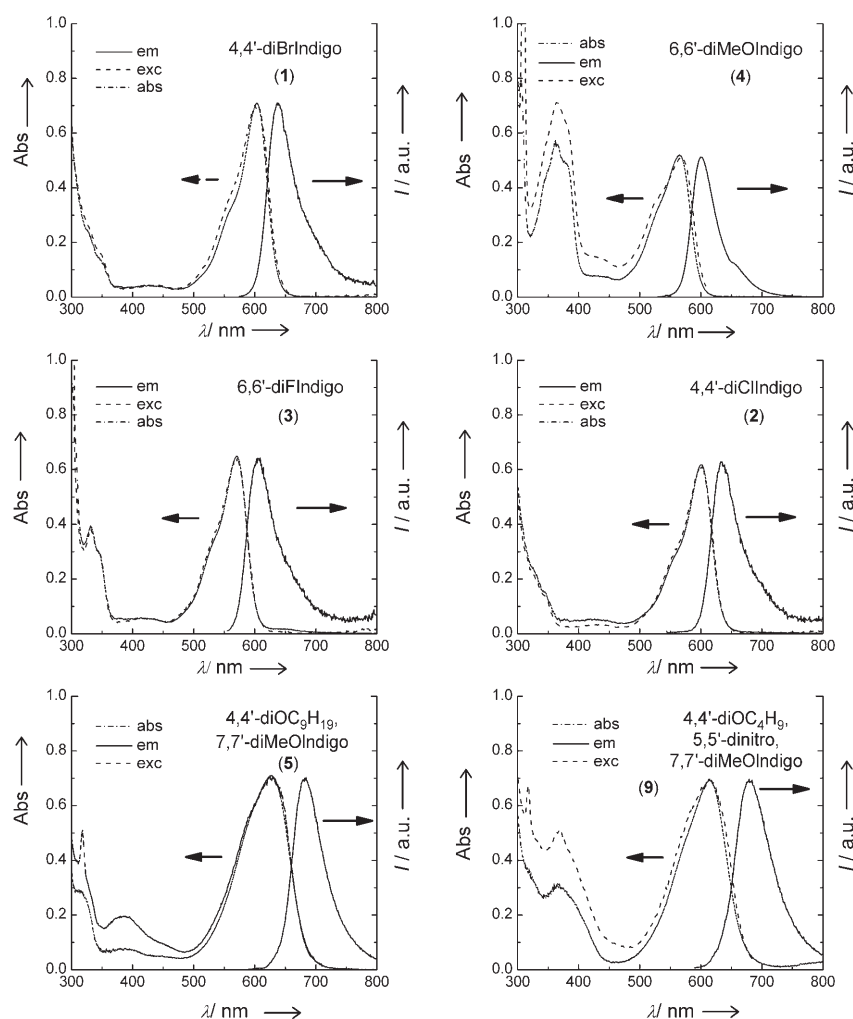
Singlet oxygen formation quantum yields were obtained following photolysis of aerated *N,N'*-dimethylformamide (DMF) solutions of the indigo derivatives. Excitations with laser pulses from a frequency doubled Nd:YAG laser (@532 nm) led to the characteristic phosphorescence signal of singlet oxygen at  $\approx 1270$  nm. The corresponding  $\phi_{\Delta}$  values were determined by plotting the initial phosphorescence intensity (at 1270 nm) as a function of the laser dose and comparing the slope with that obtained with tetraphenylporphyrin (TPP) in DMF as standard (see experimental for further details). Illustrative plots are given in Figure 2, and confirm good linearity over the intensity range studied. Data for all the compounds can be found in Table 2.



Scheme 2. Structures and acronyms for the studied substituted indigo derivatives.

## 3. Discussion

In Figure 1, the clear mirror-image relationship between the absorption and emission spectra displayed by the di-substituted compounds (Family 1) can be noted, whereas with the compounds representative of the Families 2 and 3, that is tetra- and hexa-substituted compounds respectively, this relationship vanishes. This suggests that the bulky groups influence the nature of the structure(s) in the ground-state. It can be seen in Figure 1 that, in contrast with spectra of all the di-substituted compounds, the absorption spectra for the tetra-substituted 4,4'-diOC<sub>9</sub>H<sub>19</sub>, 7,7'-diMeOIndigo, compound 5, and



**Figure 1.** Absorption, fluorescence emission, and excitation spectra (normalized) for representative Family 1, 2 and 3 compounds (Scheme 2) in dioxane at  $T = 293$  K in their keto forms.

hexa-substituted 4,4'-diOC<sub>4</sub>H<sub>9</sub>, 5,5'-dinitro,7,7'-diMeOIndigo, compound **9**, are broad, whereas the corresponding emission spectra are clearly much sharper. These latter compounds also present the highest Stokes shifts ( $\Delta_{SS}$ , seen as the difference between the absorption and emission maxima in Table 1). In addition, and also in contrast with the spectra of the di-substituted compounds (Family 1), the excitation spectra only partially match the absorption spectra for compounds **5** and **9** in the regions of the high energy tail of the first absorption spectra and the (presumably) second transition located with maxima at  $\approx 380$  nm. The above results strongly suggest non-planarity of compounds **5** and **9** because of the presence of the alkyl groups. In addition, the absence of a total match of the fluorescence excitation with the absorption band for **5** and **9** suggests that there may be an ensemble of conformers present. In the excited state the relative population of the more twisted conformers is decreased and thus the wide distribution of var-

**Table 1.** Spectral data including absorption ( $\lambda_{\text{abs}}^{\text{max}}$ ) and emission ( $\lambda_{\text{fluo}}^{\text{max}}$ ) wavelength maxima, extinction coefficients ( $\epsilon_s$ ) and Stokes shift values ( $\Delta_{SS}$ ) for keto forms of indigo Family 1,2, and 3 compounds in dioxane (Dx), benzene (Bz) and dimethylformamide (DMF),  $T = 293$  K.

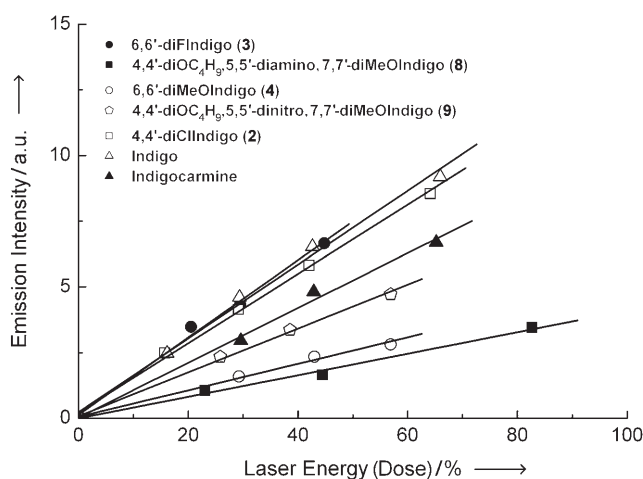
Compound	$\lambda_{\text{abs}}^{\text{max}}$ [nm]			$\epsilon_s$ [ $\text{M}^{-1} \text{cm}^{-1}$ ]	$\lambda_{\text{fluo}}^{\text{max}}$ [nm]	$\Delta_{SS}$ [ $\text{cm}^{-1}$ ]
	Dx	Bz	DMF			
indigo <sup>[a]</sup>	–	–	610	22 140	653	1080
Tyrian purple <sup>[a]</sup>	–	–	601	12 680	640	1014
indigocarmine <sup>[a]</sup>	–	–	618	8080	661	1052
Family 1						
4,4'-diBrIndigo ( <b>1</b> )	602	605	616	25 771	637	913
4,4'-diClIndigo ( <b>2</b> )	600	600	613	19 942	633	869
6,6'-diFlIndigo ( <b>3</b> )	570	580	580	19 070	605	1015
6,6'-diMeOIndigo ( <b>4</b> )	566	565	570	15 394	610	1274
Family 2						
4,4'-diOC <sub>9</sub> H <sub>19</sub> ,7,7'-diMeOIndigo ( <b>5</b> )	629	635	638	17 074	680	1192
5,5', 6,6'-tetraMeOIndigo ( <b>6</b> )	588	585	596	7963	ND <sup>[b]</sup>	–
Family 3						
4,4'-diOC <sub>6</sub> H <sub>13</sub> ,5,5'-diamino, 7,7'-diMeOIndigo ( <b>7</b> )	732	735	748	10 248	ND <sup>[b]</sup>	–
4,4'-diOC <sub>4</sub> H <sub>9</sub> ,5,5'-diamino, 7,7'-diMeOIndigo ( <b>8</b> )	730	735	742	10 800 <sup>[c]</sup> 3567 6230 <sup>[c]</sup>	ND <sup>[b]</sup>	–
4,4'-diOC <sub>4</sub> H <sub>9</sub> ,5,5'-dinitro, 7,7'-diMeOIndigo ( <b>9</b> )	615	615	620	18 064	680	1554

[a] Data in DMF taken from ref. [2]. [b] Emission could not be detected with our current experimental conditions. [c] Values in benzene.

**Table 2.** Photophysical properties for the keto forms of the Indigo Family 1, 2, and 3 compounds in dioxane at  $T = 293$  K.

Compound	$\phi_F^{[d]}$	$\tau_F$ [ns]	$\phi_A \approx \phi_T$	$\phi_C$	$k_F$ [ns <sup>-1</sup> ]	$k_{NR}$ [ns <sup>-1</sup> ]	$k_{IC}$ [ns <sup>-1</sup> ]	$k_{ISC}$ [ns <sup>-1</sup> ]
indigo <sup>[a]</sup>	0.0023	0.14	0.00117	0.996	0.0164	7.12	7.12	0.0084
Tyrian purple <sup>[a]</sup>	0.0071	0.323	0.000552	0.992	0.022	3.07	3.07	0.0017
indigocarmine <sup>[a]</sup>	0.0015	0.110	0.0008	0.998	0.0136	9.08	9.07	0.0073
Family 1								
4,4'-diBrIndigo (1)	0.00462	0.150	0.00235	0.992	0.0308	6.64	6.62	0.0157
4,4'-diClIndigo (2)	0.00406	0.134	0.001	0.994	0.0303	7.43	7.42	0.0075
6,6'-diFIndigo(3)	0.00169	0.068	0.00113	0.997	0.0248	14.68	14.66	0.0166
6,6'-diMeOIndigo (4)	0.000337	0.015 <sup>[b]</sup>	0.0005	0.999	0.0225	66.64	66.61	0.0333
Family 2								
4,4'-diOC <sub>9</sub> H <sub>19</sub> ,7,7'-diMeOIndigo (5)	0.000175	0.034	0.00117	0.999	0.0051	29.23	29.2	0.0034
5,5', 6,6'-tetraMeOIndigo (6)	ND <sup>[c]</sup>	–	0.000186	0.999	–	–	–	–
Family 3								
4,4'-diOC <sub>6</sub> H <sub>13</sub> ,5,5'-diamino, 7,7'-diMeOIndigo (7)	ND <sup>[c]</sup>	–	0.0012	0.998	–	–	–	–
4,4'-diOC <sub>4</sub> H <sub>9</sub> ,5,5'-diamino, 7,7'-diMeOIndigo (8)	ND <sup>[c]</sup>	–	0.0003	0.999	–	–	–	–
4,4'-diOC <sub>4</sub> H <sub>9</sub> ,5,5'-dinitro, 7,7'-diMeOIndigo (9)	0.00011	0.032 <sup>[b]</sup>	0.00083	0.968	0.00342	31.25	31.22	0.0260

[a] Data in DMF taken from ref. [2], with the exception of  $\phi_A \approx \phi_T$  values (this work) and thus the split of the radiationless rate constants into  $k_{IC}$  and  $k_{ISC}$ . [b] Value determined in benzene. [c] Emission could not be detected with our current experimental conditions, that is,  $\phi_F \leq 10^{-5}$ . [d] Average values from three independent determinations with associated errors varying from 1–10%.



**Figure 2.** Plots of initial phosphorescence of singlet oxygen at 1270 nm as function of laser energy for the indigo derivatives in DMF at  $T = 293$  K.

iously twisted conformers in  $S_0$  has been replaced by a narrow distribution of only slightly twisted conformers in  $S_1$ . That is, in  $S_1$  the excited state is expected to show a single conformer, in contrast to the situation for the ground state. Recently, ab initio methods were applied to study indigo derivatives suggesting that some of the alkoxy derivatives considered might be nonplanar (or present different conformations) in  $S_0$ .<sup>[11]</sup> This seems to constitute an additional and independent finding that validates what is experimentally observed here; it also shows that substitution in peripheral positions of indigo can induce deviations from planarity similar to those observed when the substitution is made in the nitrogen atoms.<sup>[12]</sup>

In Table 1, the spectral data for Family 1 compounds are presented for three solvents of different polarity. Although some shifts in the maxima are observed with solvent polarity, this

does not seem to be particularly significant. Nevertheless a brief comment on the solvent dependence is useful since these well-known polar molecules seem to respond to the polarity of the media.<sup>[6,12]</sup> If one takes the dielectric constant of the media ( $\epsilon$ ) as indicative of the solvent polarity, one can observe that in general the absorption wavelength maxima red-shifts with the increase in the  $\epsilon$  value [ $\epsilon^{25^\circ\text{C}}(\text{dioxane}) = 2.2$ ;  $\epsilon^{25^\circ\text{C}}(\text{benzene}) = 2.3$ ;  $\epsilon^{25^\circ\text{C}}(\text{DMF}) = 36.7$ ]. However, the compounds do not respond identically to this property which indicates differences in the polarities of the molecules. This means that a greater polarity of the solvent induces a red-shift of the polar molecules (stabilizing the LUMO) whereas in the case of the presumably less polar molecules (3, 4, and 9) this effect is not observed. A detailed analysis of the dependence on polarity of the current studied molecules is however beyond the scope of the present study. A more important effect is that substitution in the Tyrian purple-like compounds [6,6'-diFIndigo (3) and 6,6'-diMeOIndigo (4)] blue-shifts the absorption spectra maxima by 20–30 nm (see Table 1) relative to Tyrian purple itself.

The large bathochromicity presented by indigo was described by Klessinger and Luetke as an H-chromophore.<sup>[13]</sup> Later on Klessinger used the principle of captodative substitution in order to explain “the unusual color” of indigo.<sup>[14]</sup> More recently ab initio studies have confirmed that in fact the chromophoric unit is indeed centered on the H-chromophore.<sup>[11,15]</sup> In a simplified description, based on MO-orbital theory (and illustrated in Scheme 1), the  $\pi$ -electron system of the chromophoric system of indigo is assigned as coming from two identical subsystems (one radical bonding center and one radical antibonding center giving rise to two  $\pi$  MOs), which are connected through the central C=C bond, and are substituted by two donor (NH or D) and two acceptor (C=O or A) groups.<sup>[14]</sup> If the energy splitting ( $\Delta E$ ) between these two MOs is small, they will

be the HOMO and LUMO orbitals of the system and the difference between them is responsible for the absorption of light of longest wavelength.<sup>[14]</sup> Depending on the nature of the substitution (electron-donating or withdrawing) the relative energy of these two MOs will be affected through the characteristics of the donor and acceptor groups. From Table 1, it is clear that in general the gradual introduction of varying numbers of substituents increases the wavelength maxima of the three families in the order  $1 < 2 < 3$ , corresponding to a decreased  $\Delta E$  between the HOMO and LUMO orbitals. However, this should be considered as a trend rather than a rule since, for example, compound **4**, when compared with Tyrian purple, displays (in the same solvent: DMF) a 31 nm blue-shift whereas with the tetra-substituted compound **6** the blue-shift is only of 5 nm, see Table 1. This suggests that the position, and not simply the number of substituent groups, also determines the relative position of the HOMO and LUMO orbitals.

Particularly marked red-shifts are seen with compounds 4,4'-diOC<sub>6</sub>H<sub>13</sub>,5,5'-diamino,7,7'-diMeOIndigo (**7**) and 4,4'-diOC<sub>4</sub>H<sub>9</sub>,5,5'-diamino,7,7'-diMeOIndigo (**8**) which possess the electron donating -NH<sub>2</sub> group (which adds electron density to the  $\pi$ -system), in contrast with the strongly deactivating group (electron withdrawing) NO<sub>2</sub> group in 4,4'-diOC<sub>4</sub>H<sub>9</sub>,5,5'-dinitro,7,7'-diMeOIndigo (**9**) which removes electron density from the  $\pi$ -system and blue-shifts the maxima. This behavior is reasonable, since electron withdrawing groups are known to lower the energy of a molecular orbital whereas electron donating groups increase it. The observation that the hexa-substituted compound **9** possesses a 18 nm blue-shift relative to, for example, the tetra-substituted compound **5** (Table 1) is in line with the above ideas regarding the effects of both the number of substituents and their relative positions in determining the  $\Delta E$  splitting.

It is particularly interesting with these systems that the substitution occurs at the peripheral positions of the indigo ring, that is, outside the central core (C=C with D and A groups), showing that this can also strongly influence the spectroscopic properties of these compounds. Moreover, this also has a strong influence on other physical properties, such as the solubility. While indigo and its derivatives are almost insoluble, the new 5,5'-diamino-substituted compounds (compounds **7** and **8**) show a high solubility.

It is also necessary to briefly comment on the molar extinction coefficients given in Table 1. It is worth noting that while the values for all the compounds indicate strongly allowed transitions, in some cases the values are much lower ( $\approx 5000 \text{ M}^{-1} \text{ cm}^{-1}$ ) than in the other compounds ( $\approx 20000 \text{ M}^{-1} \text{ cm}^{-1}$ ). Whilst there is no simple explanation for this observation, the results are reproducible (each value is in general the average of various independent measurements). It is possible that in part this arises from the presence of non-chromophoric groups in the molecules, which affect the molecular weight (and hence molar concentration) but do not influence the actual transition.

The electronic conversion between the triplet and singlet states of molecular oxygen by direct excitation is forbidden by spin conservation laws and therefore has a very low probabili-

ty. However, by interaction with other excited molecules, triplet molecular oxygen can be converted into singlet oxygen (<sup>1</sup>O<sub>2</sub>). This sensitization is usually produced by energy transfer from the long-lived state of the sensitizer: the triplet state (the so-called Type II mechanism), although in some cases the lowest singlet excited state may be involved. The efficiency of sensitized <sup>1</sup>O<sub>2</sub> formation is defined as the quantum yield for singlet oxygen formation:  $\phi_{\Delta}$ . We have measured singlet oxygen quantum yields for all the compounds in DMF solution. From these, quantum yields for triplet formation were estimated, assuming 100% efficiency for the Type II sensitization. Although this assumption has proved valid for a number of systems, including conjugated organic polymers, it is not necessarily true, therefore these values must be considered as lower limits.<sup>[16]</sup>

Table 2 presents photophysical data for the studied indigo families in dioxane. For the Family 1 compounds, substitution in the 4,4' positions induces the highest fluorescence quantum yield and  $\tau_{\text{F}}$  values. Substitution in 6,6' strongly decreases the  $\phi_{\text{F}}$  and  $\tau_{\text{F}}$  values relative to Tyrian purple. With the dimethoxylated **4**, this decreases by more than an order of magnitude. Although, because of the very low values, caution is needed in looking for trends in the triplet quantum yields, almost all the substituted derivatives have higher values than the compound Tyrian purple (see Table 2). The exception is again the **4** which has  $\phi_{\Delta}$  and  $\phi_{\text{T}}$  values identical to Tyrian purple.

Moreover, a substitution by either chlorine or bromine in the 4,4' positions results in an increase of  $\phi_{\Delta}$  and  $\phi_{\text{T}}$  (180–425%), large increase in  $k_{\text{ISC}}$  (700–900%), doubling of the  $k_{\text{IC}}$  values and essentially no change in the  $k_{\text{F}}$  values [0.022 ns<sup>-1</sup> for Tyrian purple vs 0.03 ns<sup>-1</sup> for 4,4'-diBrindigo (**1**) and 4,4'-diClIndigo (**2**)]. The overall effect of substituting either chlorine or bromine at the 4,4' positions of indigo can be explained by an increase in spin-orbit coupling as a consequence of the classical heavy-atom effect.<sup>[17]</sup> However, it is relevant to compare these compounds with Tyrian purple, which has dibromo substitution at the 6,6' positions, and whose triplet quantum yield is slightly lower than the 6,6'-difluoro compound **3**. The heavy-atom effect is partly a through-space effect, and this feature, which is observed with the 4,4' derivatives but not with the 6,6' ones, means that the 4,4' substituents must be close to the chromophore. This provides strong support for the model of Klessinger, involving excitations in molecular orbitals based around the central double bond.<sup>[14]</sup>

Within the di-substituted compounds (which includes Tyrian purple and indigocarmine), the di-substitution in the 6,6' positions with bromine (Tyrian purple) and methoxy groups (compound **4**) leads to smaller  $\phi_{\Delta}$  values; while the 6,6' substitution with fluorine groups (compound **3**) and bromine at the 4,4' positions (**1**) leads to the highest  $\phi_{\Delta} (\approx \phi_{\text{T}})$ . These results support the idea that spin-orbit coupling in these positions is negligible, as seen by the lack of any correlation with the spin-orbit coupling constant ( $\zeta$ , the spin-orbital operator  $H_{\text{SO}} = \zeta \cdot \vec{L} \cdot \vec{S}$ ). With these systems, inductive or other effects which destabilize the indigo chromophoric unit may be dominant.

With the two tetra-substituted compounds (Family 2) it seems clear that the substitution of methoxy groups in the 5,5'

and 6,6' diagonal positions, compound **6**, leads to one order of magnitude lower  $\phi_{\Delta}(\approx\phi_{\tau})$  compared with the substitutions in the 4,4' and 7,7' positions, although in this last case two of the substituents are more bulky. This might suggest a poor contact between the indigo donor compound **5** and the sensitized molecular oxygen.

The  $k_{\text{f}}$  values for all of Family 1 compounds are fairly constant ( $0.02\text{--}0.03\text{ ns}^{-1}$ ). These natural fluorescence lifetimes ( $\tau^{\circ}$ ) of 30–50 ns are typical values for allowed  $\pi,\pi^*$  transitions. We can also see in Table 1 that the molar extinction coefficient values are typical for such allowed transitions.

The above results are all consistent with a model of excited state decay in indigo derivatives proceeding  $>99\%$  *via* internal conversion. Note also that from Table 2 the sum of the nonradiative rate constants ( $k_{\text{NR}}$ ) is effectively identical to the  $k_{\text{IC}}$  values, clearly showing this as the dominant decay process.

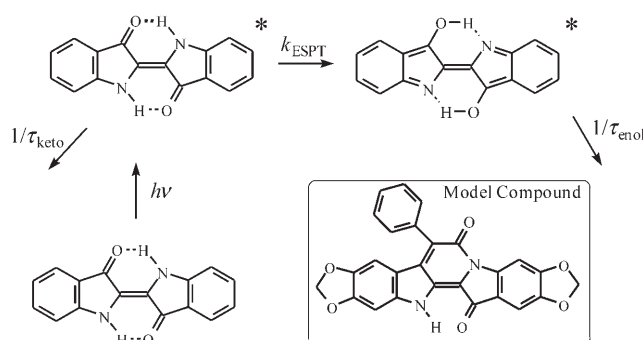
For all the compounds studied, the central  $\text{HN}=\text{C}=\text{C}=\text{O}$  core is present. Here, hydrogen bonds between adjacent carbonyl and N–H groups, keep the molecule in a stable trans planar configuration thus preventing *cis*–*trans* isomerization.<sup>[18,19]</sup> In the excited state, fast proton transfer between the NH and C=O groups seems to compete with fluorescence and intersystem crossing leading to the enhancement of the internal conversion deactivation channel. There are several literature examples where intra- and/or intermolecular proton transfer in the excited states leads to very low fluorescence yields, usually involving hydroxyl and carbonyl groups. For example, with the flavylium cation form of anthocyanins, adiabatic proton transfer (to water), which occurs in 6–7 ps, was found to be the predominant process responsible for its weak fluorescence,<sup>[20]</sup> whilst in the classical example of 3-hydroxyflavone, intramolecular proton transfer occurs in 250 fs.<sup>[21,22]</sup>

With fluorescent, intramolecular proton transfer systems followed by steady-state techniques, the initial and final forms normally correspond to independent emissive bands.<sup>[22]</sup> Some notable exceptions are 3-hydroxyflavone<sup>[21,23]</sup> (in the absence of hydrogen-bond impurities) and a hydroxyphenylbenzothiazole derivative which has in common a single emission band with a Stokes shift greater than 200 nm.<sup>[24]</sup> This arises because the absorbing species is not the same as the emitting species. In the first case,<sup>[21]</sup> the proton migrates from a hydroxy to a carbonyl group whereas in the latter from a hydroxyl to an aromatic nitrogen.<sup>[24]</sup> One other possibility arises when the fluorescence band is the result of two emissive bands that strongly overlap. One way to obtain additional information on these processes is to collect fluorescence decays at different regions of the band.<sup>[25]</sup> The relative population of each species will give rise to different contributions in the decay profiles whose contributions will be given by the pre-exponential factors associated with the decay times.

To follow these possibilities of proton transfer in indigo, further experiments were made by observing the emission decay profiles of indigo itself at the short wavelength onset of the emission spectrum (620 nm) and at longer wavelengths (650 nm). At both emission wavelengths the decays were fitted with double exponential decay laws. The decays in Table 1 are not, in fact, strictly single exponentials. A more careful analysis

of the decays of indigo and some of its derivatives seems to reveal these to follow double-exponential behavior. However, others keep their single exponential decay character. One of these is compound **4**, which has a very short decay time (15 ps) possibly suggesting an intrinsic property of the compound or that the rising component is in the fs time regime and therefore could not be seen with our current time-resolution. The data in Table 2 report the decay time value with (generally) an additional shorter rise-time value (16–20 ps).

The time profiles of fluorescence collected at the two emission wavelengths (620 and 650 nm for example) show a long decay-time (values displayed in Table 2 are consistent with the major fluorescence contribution of the decay) and a second shorter time which appears as a decay-time at the onset of the emission spectrum (620 nm) and as a rise-time at longer wavelengths (650 nm). We should note that in indigo the wavelength maximum is approximately 610 nm, see Table 1. This is consistent with a two-state model in the excited state where one of the species is being formed in the excited state (whose emission contribution predominates at longer wavelengths) from the decay of the other (whose emission contribution is superior at shorter wavelengths). With the indigo-like systems, these results strongly suggest that the excited enol-form is produced at the expense of the initially generated keto form, see Scheme 3. We note that other groups, using femtosecond



**Scheme 3.** Scheme illustrating the excited state proton transfer kinetics in indigo.

transient absorption spectroscopy, have found decay profiles for indigocarmine with 3–4 decay components (which are apparently solvent dependent) in the ps/fs time domain, including some with rising components.<sup>[26]</sup>

According to Scheme 3, the rate constant for excited state proton transfer (ESPT) can be determined through Equation (1)<sup>[25]</sup>

$$k_{\text{ESPT}} = \frac{1}{\tau_2} - \frac{1}{\tau_{\text{keto}}} \quad (1)$$

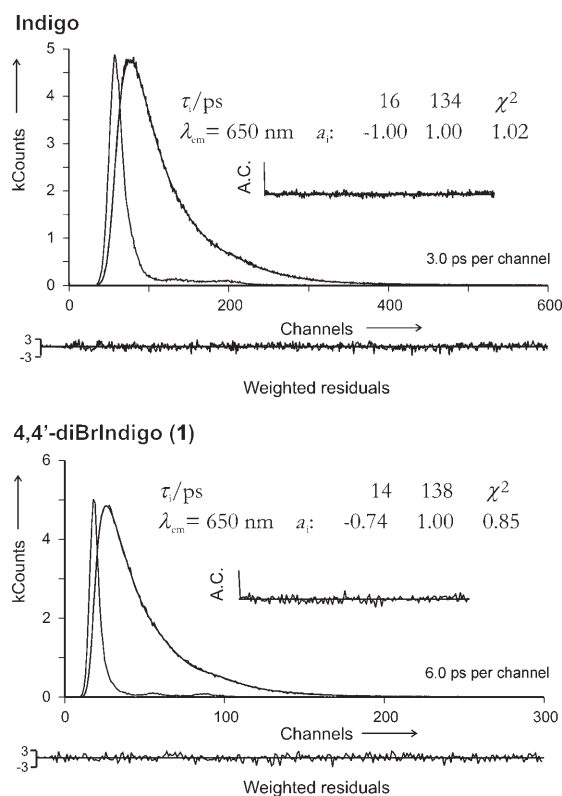
where  $\tau_{\text{keto}}$  is the decay time of indigo (or indigo related compound) in the absence of other deactivation processes, that is, in the present case, in the absence of the ESPT. This lifetime can be independently obtained with a model compound in

which this process is blocked. *N,N'*-substituted indigo derivatives were considered, however for these compounds it is known that *cis-trans* photoisomerization is present and thus this process leads to an additional deactivation process not present in indigo.<sup>[12,27]</sup> Therefore the *N,N'*-substituted indigo dyes are discarded as model compounds. To circumvent this problem we have made use of a model compound possessing a rigid molecular structure (with a moiety identical to indigo), where intramolecular proton transfer is allowed between adjacent carbonyl and NH groups but where isomerization is blocked (see structure in Scheme 3).<sup>[28]</sup> A decay time of 152 ps is found for the *N*-methylated analogue of this model compound which is also similar to the mono-exponential decay value reported for indigo (140 ps in Table 2 and ref. [2]). Therefore by using this lifetime value  $\tau_{\text{keto}}$  ( $\approx 152$  ps) and from the data in Figure 3 and Equation (1), we obtain values for this process of  $5.56 \times 10^{10} \text{ s}^{-1}$  for indigo and  $6.49 \times 10^{10} \text{ s}^{-1}$  for **1**. These are, however, preliminary results and a more detailed study is in progress.

If proton transfer from the NH to the C=O group in the excited state is the main factor controlling the dominant internal conversion over other  $S_1$  decay pathways, then deuteration, by addition of deuterated water ( $D_2O$ ) in 2% volume to the

system (deuteration effect), would affect the observed photo-physical parameters. Adding  $D_2O$  readily exchanges the amino hydrogen atom with deuterium. However, as pointed out by other groups in studies with indigocarmine, this effect is promoted either by intra- or intermolecular hydrogen bonding.<sup>[26]</sup> If H-bonding is involved in the deactivation process, the addition of  $D_2O$  would cause a dynamic effect. To check this hypothesis we have performed this experiment with compound **3** and compared the fluorescence quantum yield and emission spectra in dioxane and dioxane: $D_2O$  (2%). There is a threefold increase of the  $\phi_F$  value from 0.00169 (Table 1) to 0.0055, confirming the presence of the deuterium effect with these indigo derivatives. Although no shift is observed in the absorption spectra, a red-shift of approximately 6 nm is observed in the broad emission maximum when going from dioxane to the dioxane-deuterated water mixture, which may be associated with changes in polarity. Detailed studies on polarity effects on model systems are in progress.

In summary, we believe that indigo and its derivatives are examples where intramolecular (or intermolecular<sup>[26]</sup>) proton transfer is involved in a highly efficient reaction between NH and C=O groups. This can be seen through the photophysical parameters, where internal conversion dominates  $S_1$  deactivation,<sup>[2]</sup> by their significant Stokes shifts (indicating different absorbing and emissive species) as well as by the deeper analysis of the decay profiles particularly in the red region of the emission where a rising component associated with the formation of the enol form of indigo is observed. Moreover, as previously reported for indigo and the derivatives Tyrian purple, indirubin, and indigocarmine,<sup>[2]</sup> and as will be reported subsequently for the present compounds, the fluorescence quantum yields for their reduced (leuco) forms increase by two orders of magnitude and, as a consequence, the rate constants for radiative and radiationless processes become competitive. This is due to the absence of the competitive proton transfer mechanism, since with the leuco forms the oxygen becomes negatively charged ( $C=O \rightarrow C-O^-$ ).<sup>[2,29]</sup> In addition, with the leuco form since there is the possibility of free rotation around the C–C central bond, rotational isomerism is also allowed, which is known to increase the fluorescence yields in indigo related compounds. Further, when proton transfer and *cis-trans* isomerization are blocked in related indigo derivatives, the fluorescence and intersystem crossing yields, increase drastically at the expense of internal conversion.<sup>[28]</sup> However, we note alternative interpretations claiming that blocking the proton transfer still gives rise to identical behavior,<sup>[30]</sup> or that with indigocarmine the effect involves intermolecular hydrogen bonding with solvent.<sup>[26]</sup> Thus, an unambiguous mechanism is still needed. However, we believe that the present results give further insight to the understanding of indigo's high photostability and, by confirming low yields of singlet oxygen formation ( $\phi_{\Delta} \leq 10^{-3}$ ), suggest that the degradation of indigo is unlikely to occur through interactions with sensitized molecular oxygen (type II). These do not, however, exclude other mechanisms involving oxygen.



**Figure 3.** Fluorescence decays of indigo (top panel) in DMF and of 4,4'-diBrIndigo (**1**) in dioxane at  $T = 293$  K collected at 650 nm (long-wavelength tail of the emission band). In the case of indigo the excitation was at 441 nm whereas for **1** at 397 nm. Note that the wavelength emission maximum is 610 and 616 nm for indigo and **1**, respectively, see Table 1. Shown as insets are the decay times ( $\tau$ /ns), pre-exponential factors ( $a_i$ ), and chi-squared values ( $\chi^2$ ). Also shown are auto-correlation functions (A.C.) and the weighted residuals for a better judgment of the quality of the fits.



## 4. Conclusions

In this work, we report spectroscopic and photophysical properties of a series of substituted indigo derivatives. Their color shifts from the characteristic indigo blue but their photophysical properties are similar to those of the parent compound, with internal conversion being the major deactivation channel of the singlet excited state. In agreement with the absence of transient triplet absorption on flash photolysis (with a few exceptions),<sup>[2]</sup> we obtain very low sensitized singlet oxygen yields ( $\phi_{\Delta}$ ) for indigo and several of its derivatives, indicating inefficient singlet oxygen sensitization and confirming the very low  $S_1 \sim \rightarrow T_1$  intersystem crossing probability. In addition, from the study of deuterium isotope effects, the results also strongly support the idea, in agreement with earlier suggestions, that either intra- or intermolecular proton transfer plays a crucial role in the radiationless internal conversion pathway.

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**Keywords:** dyes · emission spectroscopy · fluorescence · indigo · photochemistry

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