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ORIGINAL PAPER

Preparation and Spectral Characterization of Fluorescence Probes Based on 4-*N*,*N*-Dimethylamino Benzoic Acid and Sterically Hindered Amines

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Abstract The adducts of simple chromophore 4-*N,N*-dimethylamino benzoic acid with 2,2,6,6-tetrametyl-4-hydroxy- or 4-amino-piperidine were examined as fluorescence probes (spin double sensors) to monitor radical processes. The links in the adducts were either an ester or amide group, and the sterically hindered amines were in the form of -NH, -NO• and –NOR. The spectral properties of the three related derivatives (esters or amides) were quite similar. The maxima of the absorption spectra were in the range of 295–315 nm, and the maximum of fluorescence was located in the range of 330–360 nm, depending on the polarity of the solvent. In polar solvents, a red-shifted fluorescence band at 460–475 nm was observed. The fluorescence of these derivatives was rather weak as compared to anthracene under the same conditions. The Stokes shift was large, as high as 6,000 cm^{-1}, indicating the formation of a twisted intramolecular charge transfer (TICT) state. No large differences in Stokes shifts were observed in polymer matrices of poly (methyl methacrylate), polystyrene and poly(vinyl chloride). The extent of intramolecular quenching was expressed as Φ_{NX}/Φ_{NO} (X=H, NOR) and was in the range of 1–3 in solution and as high as 8 in polymer matrices. The low efficiency of intramolecular quenching limits the application of these new adducts as fluorescence probes for the monitoring of radical processes in solution but favors their application in polymer matrices.

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Introduction

Various fluorescence probes with different structures are used to monitor processes in chemistry and especially biology, where monitoring is required in different media, including micelles, membranes, solid amorphous matrices and other complex mixtures [1–5]. The spectral parameter of choice for monitoring is fluorescence, because it exhibits a strong dependence on medium. The advantages of fluorescence include high sensitivity, simple detection, easy quantitative evaluation of the selected chromophore and a distinct medium effect. Simple chromophores like substituted aromatic hydrocarbons are widely used for this purpose.

The simplest chromophore of this type is a molecule having strong electron donor-acceptor substituents. A typical example is a benzene ring substituted with a strong electron donating group like dimethylamino, and opposite to this group, an electron withdrawing group such as a nitrile, carbonyl, carboxyl or ester group. A typical representative of this class of compounds is 4-(*N,N*-dimethylamino)benzonitrile. Often, these compounds exhibit dual emission – normal (mirror-like fluorescence to the absorption) or local (LE) and red-shifted fluorescence due to intramolecular charge transfer (ICT) or twisted intramolecular charge transfer fluorescence (TICT). This type of excited state is non-emissive but photochemically active.

Since the late 1950s, considerable attention has been given to the photophysical properties of derivatives of dimethylamino benzoic acid and dimethylaminobenzonitrile, due to appearance of unusual dual fluorescence in polar solvents

[6]. These studies have focused on the influence of the electron donor or electron acceptor substituents as well as the polarity of the medium and concentration of the chromophore.

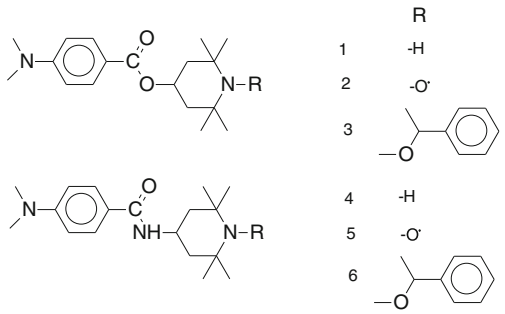
Since the introduction of the TICT state by Grabowski and Rettig [7, 8] there have been many studies with the aim of confirming or disproving this phenomenon, especially with compounds having a benzene ring with electron donating and withdrawing groups. A spectral study of methyl-4-(*N,N*-dimethylaminobenzoate) in a variety solvents revealed that normal fluorescence is observed in most solvents, but the red-shifted fluorescence in acetonitrile has been ascribed to an excited dimer [9]. Even more complex is the spectral behavior of aminosalicylates like methyl-2-hydroxy-4-dimethylaminobenzoate, which exhibits three competitive emissions, including normal fluorescence, TICT state fluorescence and intramolecular proton-transfer fluorescence [10]. Extensive studies on jet-cooled aniline derivatives confirmed the existence of a monomolecular mechanism involving the adiabatic formation of a fluorescent TICT state induced by water solvent molecules and a mechanism involving self-cluster formation that induces excimer-like behavior [11]. A study confirmed that, in the presence of colloidal SiO_2, the formation of an excited TICT state is enhanced by hydrogen bonding between the carboxylic group of dimethylamino benzoic acid (DMABA) and colloidal SiO_2 [12]. A similar enhancement of fluorescence of DMABA was also observed with DMABA absorbed on NaY zeolites due to the formation of hydrogen bonding [13]. Systematic investigation of the CT dual fluorescence of sodium p-dialkylaminobenzoates in ionic micelles demonstrated that the CT process is affected by the electric field at the ionic micelle-water interface and that the CT emission is enhanced at higher electric field [14]. Contrary to its ICT emission, that of DMABA at the sodium bis(2-ethylhexyl) sulfosuccinate (AOT) reverse micelle-water pool interface is much weaker [15]. The same probe, DMABA, exhibited concentration-dependent dual fluorescence in chloroform. It was concluded that the ICT process and related ICT fluorescence are facilitated by intramolecular H-bonding mediated by excited state proton transfer and that this effect is weaker than that of solvent polarity [16]. For series of substituted phenyl p-dimethylaminobenzoates, it was observed that the CT emission in the same solvent shifts to lower energy (red-shift) with increasing electron-withdrawing ability of the substituent on the phenyl ring, whereas the locally excited (LE) shows no change. This shift was decreased in polar solvents [17]. When the dimethylamino substituent was changed to phenylamino, the ICT character of the emissive state of 4-(*N*-phenylamino) benzoic acid showed a single band emission with a sluggish response to solvent polarity. The ICT state occurring in this probe is due to the *N*-phenyl/amino conjugation effect [18]. CT dual fluorescence was observed for a series of p-*N,N*-dimethylaminobenzoates with alkyl substituents of various lengths. The fluorescence intensity ratio of the CT band to the local band was decreased with increasing carbon number in the range from 1 to 3 and then slightly increased with longer alkyl chains. Conversely, this ratio for longer alkyl substituents is increased in ionic micelles [19].

Stable, free aminoxyl radicals are widely utilized in various fields of chemical and biochemical science, mainly as probes to monitor radical reactions [20–25]. An important role for nitroxide free radicals in biological systems is spin labeling for the exploration of protein structure, dynamics and function [26–28]. Another use employs their ability to easily react with free radicals and significant stability in biological environments to follow oxidative damage in biological systems. In this case, reaction of nitroxide with radicals leads to the formation of diamagnetic *N*-hydroxylamines or *N*-alkoxyamines, which exhibit increased fluorescence if the reduced species exhibits fluorescence. In another method, a fluorophore is attached to a nitrone used for spin trapping [29]. In polymer chemistry, stable aminoxyl radicals are used as mediating radicals in radical polymerization, which provides control of the molecular weight and molecular weight distribution (polydispersity) of the polymer as well as the possibility of preparing designed polymers [30–34]. Moreover, the adducts of aminoxyls with fluorescence probes can provide a deeper understanding of radical polymerizations by using sensitive fluorescence spectroscopy [35].

Over the past decades, sterically hindered amines (HAS), especially derivatives of 2,2,6,6-tetramethylpiperidine, have become the most effective catalysts and stabilizers, especially in the preparation of polyolefins [36–38]. Their stabilization action is based on the formation and regeneration of *N*-oxyl radicals.

A significant amount of attention is often directed at the initial stage (the so-called induction period) of polymer photodegradation. In these studies, fluorescence spectroscopy was utilized in thermooxidative studies of polypropylene [39, 40]. Fluorophores attached to HAS in the form of fluorophore-spacer-receptor were used as fluorescence probes to exploit the changes of probe fluorescence intensity during the polymer degradation process. To find the ideal system, various fluorophore-spacer-HAS systems were synthesized and spectrally characterized [23–25, 41–46].

In this paper, we used the simple chromophore 4-dimethylaminobenzoic acid (DMABA) and prepared corresponding esters and amides with 4-amino- and 4-hydroxy-2,2,6,6-tetramethyl piperidine, where the sterically hindered centers were in the form of the parent amine, *N*-oxyl or *N*-alkoxy group (Scheme 1). Detailed spectral characterization was performed in both solution and polymer matrices.



Scheme 1 Structure of the studied compounds

Experimental

Synthesis

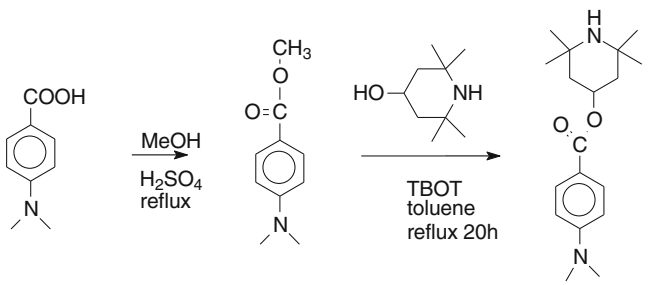
The synthesis of the ester derivatives shown in Scheme 1 was performed according to Schemes 2, 3 and 4. To prepare the required compounds, methyl 4-*N,N*-dimethylamino benzoate and 4-*N,N*-dimethylamino benzoic acid chloride were synthesized. The NMR chemical shifts and FTIR absorption bands of the prepared derivatives are summarized in the Supplementary material.

Methyl 4-N,N-Dimethylamino Benzoate

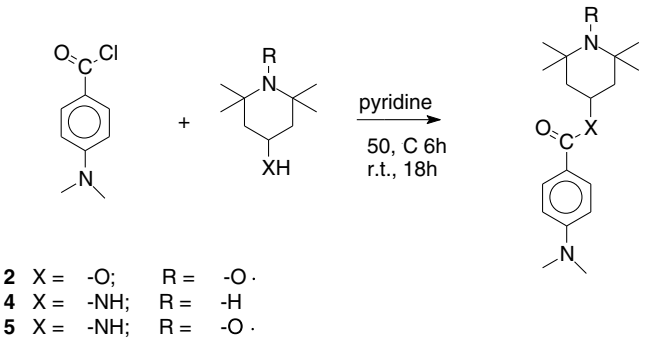
The methyl ester was prepared by refluxing 4-*N,N*-dimethylamino benzoic acid in methanol in the presence of sulfuric acid as a catalyst. Molecular sieves were added to absorb the water by-product and shift the reaction toward the formation of the methyl ester. The crude methyl ester was obtained after evaporation of the solvent following neutralization of the acid with sodium carbonate. The pure product was crystallized from ethanol.

4-N,N-Dimethylamino Benzoic Acid Chloride

4-*N,N*-dimethylamino benzoic acid chloride was synthesized from 4-*N,N*-dimethylamino benzoic acid by reaction with oxalyl chloride [47] or $SOCl_2$ [48] and was used without further purification.



Scheme 2 Synthesis of derivative 1



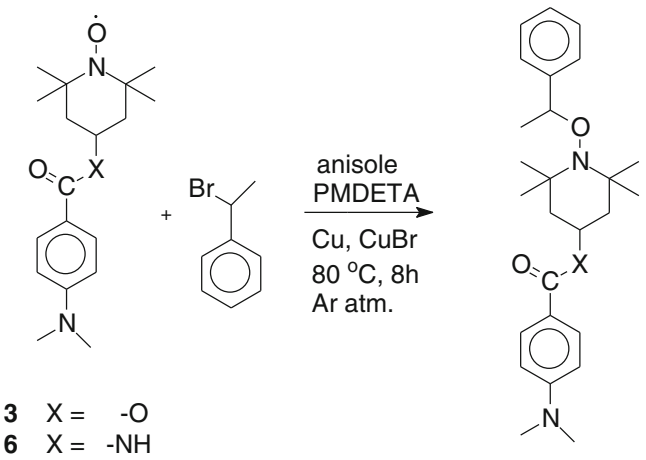
Scheme 3 Synthesis of derivatives 2, 4 and 5

1-(2,2,6,6-Tetramethyl Piperidine-4-yl)-4-N,N-Dimethylaminobenzoate (1) (Scheme 2)

The synthesis of 1 was performed through the catalytic reaction of methyl-4-*N,N*-dimethylamino benzoate (1 g, 5.6 mmol) with 2,2,6,6-tetramethyl-4-hydroxypiperidine (TEMPOL) (0.704 g, 5.6 mmol) in dry toluene over 20 h at reflux with a liquid-liquid separator (a decanter) using tetrabutyl orthotitanate (TBOT) as the catalyst. The solvent was evaporated on a rotary evaporator, and the solid residue was separated on a silica column using ethyl acetate, methylene chloride and methanol as the eluents. Brownish crystals of 1 with a melting point of 144–145 °C were obtained from the elution solution in 26 % yield (0.46 g). The product was analyzed by HPLC-MS, FTIR and NMR spectroscopy.

1-(1-Oxy-2,2,6,6-Tetramethyl Piperidine-4-yl)-4-N,N-Dimethylamino Benzoate (2)

The synthesis of 2 was performed according to the method described by Vamecq et al. [49] by dropwise addition of a solution of acyl chloride in dry pyridine (20 ml) to a



Scheme 4 Synthesis of derivatives 3 and 6

solution of 1-oxy-2,2,6,6-tetramethyl 4-hydroxypiperidine (3 g; 0.018 mol) in dry THF (20 ml) and dry pyridine (15 ml). The resulting mixture was stirred under argon atmosphere at 50 °C for 6 h and at room temperature for 18 h. The solvent was evaporated, and the solid residue was separated on a silica column using methylene chloride and a mixture of ethyl acetate and *i*-hexane as eluents. Orange crystals of 2 with a melting point of 134–137 °C were obtained from the elution solution in 23 % yield (1.3 g). The product was analyzed by HPLC-MS, FTIR and ^{1}H NMR spectroscopy in the presence of pentafluorophenyl hydrazine as well as by ESR (Fig. 1).

1-((1-Phenylethoxy)-2,2,6,6-Tetramethylpiperidine-4-yl)-4-N,N-Dimethylamino Benzoate (3)

Compound 2 (0.384 g, 1.2 mmol), bromoethyl benzene (0.148 g, 0.8 mmol), PMDETA (pentamethyldiethylene triamine) (0.244 g 1.4 mmol) and anisole (15 ml) were added to a Schlenk flask, which was deareated with three freeze-pump-thaw cycles and then filled with argon. Next, CuBr (0.058 g, 0.4 mmol) and Cu (0.076 g, 1.2 mmol) were quickly added to the reaction mixture, which was stirred at 80 °C for 18 h. After cooling to room temperature, the volume was reduced, and the residue was separated on basic alumina (Al_2O_3) using THF as an eluent to remove the copper complex. The solvent was evaporated, and the solid residue was separated on a silica column using gradient elution with *i*-hexane/ethyl acetate mixtures, beginning with *i*-hexane. White crystals of 3 with a melting point of 80–82 °C were obtained in 62 % yield. The product was analyzed by HPLC-MS, FTIR and NMR spectroscopy.

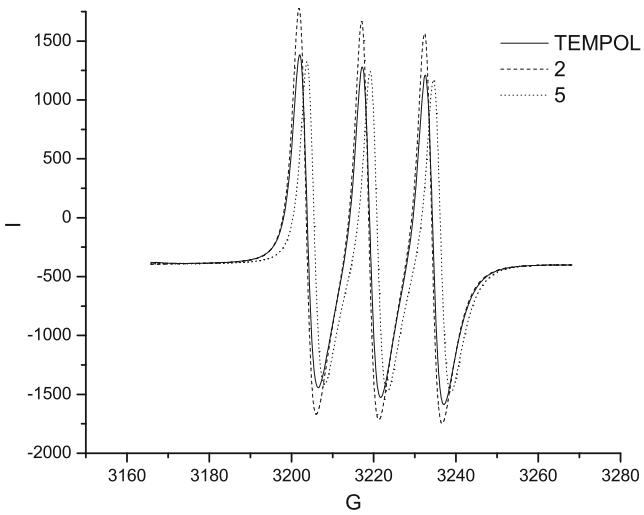


Fig. 1 ESR spectra of 2 and 5 compared with TEMPOL in benzene solution (c=10^{-3} mol L^{-1})

1-(2,2,6,6-Tetramethyl Piperidine-4-yl)-4-N,N-Dimethylamino Benzamide (4). (Scheme 3)

The synthesis of 4 was performed according to Vamecq et al. [49] by the dropwise addition of a solution of acyl chloride in dry pyridine (20 ml) to a solution of 2,2,6,6-tertamethyl 4-aminopiperidine (1.1 ml, 6.04 mmol) in dry THF (20 ml) and dry pyridine (15 ml). The reaction mixture was stirred under argon at 50 °C for 6 h and at room temperature for 18 h. The reaction mixture was quenched with 100 ml of 1 M hydrochloric acid (with ice), and the solvents were evaporated. The resulting yellow viscous liquid was separated on a silica column using ethyl acetate and methanol as eluents. The methanol fraction was condensed, dissolved in water, neutralized with sodium carbonate solution and extracted with methylene chloride. White crystals of 4 with a melting point of 59–61 °C were obtained from methylene chloride in 68 % yield (1.25 g). The product was analyzed by HPLC-MS, FTIR and NMR spectroscopy.

1-(1-Oxy-2,2,6,6-Tetramethylpiperidine-4-yl)-4-N,N-Dimethylamino Benzamide (5)

The synthesis of 5 was performed by the dropwise addition of a solution of acyl chloride in dry pyridine (10 ml) to a solution of 1-oxy-2,2,6,6-tetramethyl 4-aminopiperidine in dry THF (20 ml) and dry pyridine (15 ml). The reaction mixture was stirred under an argon atmosphere at 50 °C for 6 h and at room temperature for 18 h. The solvent was evaporated, and the solid residue was separated on a silica column using gradient elution with *i*-hexane/ethyl acetate mixtures, beginning with *i*-hexane. Orange crystals of 5 with a melting point of 204–212 °C were obtained in 21 % yield. The product was analyzed by HPLC-MS, FTIR and ^{1}H NMR spectroscopy (in the presence of pentafluorophenyl hydrazine). Additionally, the ESR spectrum was compared to that of TEMPO (Fig. 1).

1-((1-Phenylethoxy)-2,2,6,6-Tetramethylpiperidine-4-yl)-4-N,N-Dimethylamino Benzamide (6)

Compound 5 (0.382 g, 1.2 mmol), bromoethyl benzene (0.148 g, 0.8 mmol), PMDETA (pentamethyldiethylene triamine) (0.244 g 1.4 mmol) and anisole (15 ml) were added to a Schlenk flask, which was deareated with three freeze-pump-thaw cycles and filled with argon. Next, CuBr (0.058 g, 0.4 mmol) and Cu (0.076 g, 1.2 mmol) were quickly added to the reaction mixture, which was stirred at 80 °C for 18 h. After cooling to room temperature, the volume was reduced on a rotary evaporator and the residue was separated on basic alumina (Al_2O_3) using THF as an eluent to remove the copper complex. The solvent was evaporated, and the solid residue was separated on a silica column using gradient elution with *i*-hexane/ethyl acetate mixtures, beginning with a 5/1 mixture

(v/v). White crystals of 6 with a melting point of 164–167 °C were obtained in 31 % yield. The product was analyzed by HPLC-MS, FTIR and NMR spectroscopy.

Preparation of Polymer Films

Polymer films doped with fluorescence probes were prepared by casting from solution. Films of polystyrene (PS) (Chemische Werke Huels, Germany) and poly(methyl methacrylate) (PMMA) (Diacon, ICI, England) were prepared by casting 0.002 or 0.02 mg of probe in 1 ml of a chloroform solution of polymer (5 g/100 ml) on a glass plate (25×40 mm). To ensure slow evaporation of the solvent, the glass plates were covered with Petri dishes. Films of poly(vinylchloride) (PVC) (Neralit, Spolana Neratovice s.e., CR) were prepared in a similar fashion by casting from tetrahydrofuran solution (5 g/100 ml). The concentration of the probe was 0.002 and 0.02 mol kg^{-1}.

The quantum yields in solution and of the doped polymer films were determined relative to anthracene in the respective medium according to relation (1) [50]:

$$\Phi_F = \Phi_F^S \frac{\int_0^\infty I_F(\nu)d\nu}{\int_0^\infty I_F^S(\nu)d\nu}\left(\frac{1-10^{-A^S}}{1-10^{-A}}\right), \quad (1)$$

where Φ_F^S is the quantum yield of anthracene, which was assumed to be 0.25 for a non-polar medium, the integrals $\int_0^\infty I_F(\nu)d\nu$ and $\int_0^\infty I_F^S(\nu)d\nu$ are the areas under the emission curves of the probe and standard, respectively, and A and A^S are absorptions of the probe and standard at the excitation wavelength. Anthracene was excited at λ_{ex} 355–365 nm, depending on the medium.

The quantum yield of doped polymer films was determined with anthracene as the standard in the respective medium using the quantum yield of anthracene in cyclohexane 0.25 [51]. The quantum yields of anthracene fluorescence in different media, which were determined by comparison with the fluorescence of anthracene in cyclohexane, were found to be 0.20 in methanol and acetonitrile, and 0.11 in chloroform. In polymer matrices, the quantum yields were assumed to be 0.20 in PMMA, 0.16 in PS and 0.10 in PVC. The quantum yields in the solutions and films were corrected to different absorptions at the excitation wavelength [50]. The fluorescence spectra were taken using excitation at the maximum of the longest wavelength absorption band.

The fluorescence lifetime measurements were performed on a LIF 200 (Lasertechnik Ltd., Berlin, F.R.G.), which operates as a stroboscope. The excitation source was a nitrogen laser emitting at 337 nm, and the emission was selected by a cut-off filter. The output signal of the boxcar integrator was digitized and transferred to a PC using a home-made program. The fluorescence decay curves were evaluated by a simple phase plane method [52] using J. Snyder's program based on Ref. [53]. The standard deviation $G^{1/2}=\Sigma((I_{exp} - I_{calc})^2/n)^{1/2}$, where I_{exp} and I_{calc} are the intensity of the experimental and calculated emissions, respectively, was used to judge whether the decay was mono-exponential. The decay curve was assumed to be mono-exponential when $G^{1/2}$ was less than 5 %. Alternatively, the fitting of fluorescence decay curves for a bi-exponential decay model was performed using an adapted FluoFit MatLab package [54].

Results and Discussion

Synthesis of Probes

Although 4-*N,N*-dimethylamino benzoic acid is a simple molecule, its reactivity is strongly influenced by the presence of the electron donating dimethylamino group. A series of simple alkyl ester derivatives of 4-*N,N*-dimethylamino benzoic acid were prepared by refluxing the acid in the corresponding alcohol [19]. The substituted phenylamino benzoates were obtained by reaction of 4-*N,N*-dimethylamino benzoic acid with substituted phenols in the presence of $POCl_3$ [17]. For the synthesis of alkanolamine esters or benzamides, reaction of 4-*N,N*-dimethylamino benzoyl chloride with the corresponding alkanolamines or amines was applied [55].

The synthesis of 2,2,6,6-tetramethyl piperidine (TMP) ester and amide derivatives was influenced by both the reactivity of the acid and the reactivity of the 4-hydroxy- or 4-amino group of the piperidine. Due to the lower reactivity of TMP-type compounds, their synthesis through reaction of the corresponding 4-hydroxy- or 4-amino derivative with an ester was not successful. For synthesis of the ester derivatives (1–3), two possible methods were examined. In the first method, the synthesis was done through a re-esterification reaction from the methyl ester (Scheme 2). This method is relatively well suited for the synthesis of TMP esters. The 1-oxy-4-hydroxy-TMP exhibited lower reactivity in the re-esterification reaction than the parent amine. Therefore, the second method was employed for the preparation of its ester derivative (2). In this method, the 4-hydroxy-TMP derivative was reacted with 4-*N,N*-dimethylamino benzoyl chloride to give the corresponding ester (Scheme 3). Similarly, the TMP amide derivatives (4 and 5) were prepared by the reaction of the 4-amino-TMP compounds with 4-*N,N*-dimethylamino benzoyl chloride (Scheme 3). In Fig. 1, the ESR spectra of derivatives 2 and 5 are compared to TEMPOL as a model compound. The shapes (typical triplet due to interaction of an electron with a nitrogen atom) of the observed ESR

signals were similar to TEMPOL, indicating that derivatives 2 and 5 possessed the same type of paramagnetic center. The comparable integral intensities of the signals also confirmed the purity of these radical derivatives.

Synthesis of the alkoxyamine derivatives (3, 6) was carried out by reaction of the corresponding *N*-oxy derivative with bromoethyl benzene in the presence of Cu and CuBr. Using this method, a 60 % yield of ester (3) and 30 % yield of amide (6) were achieved. Usually, *N*-alkoxyamines can be prepared in a two-step synthesis by the radical reaction of *N*-oxyl with vinyl (styrene) derivatives and subsequent reduction with $NaBH_4$ [56, 57]. However, the synthesis of derivatives 3 and 6 employing the approach given in the literature [57, 58] was unsuccessful. Less than 5 % yield was obtained for 3, together with side-products.

Spectral Measurements

The comprehensive spectral characteristics of the fluorescence probes based on 4-*N,N*-dimethylaminobenzoic acid were performed in solvents of various polarities as well as in several polymer matrices.

All compounds exhibited strong absorption bands in the near-UV region around 300 nm. The longest wavelength absorption band was influenced by the solvent polarity. It was shifted bathochromically 10 to 15 nm with increasing polarity from cyclohexane to methanol (Fig. 2, Table 1), independent of the oxidation state of the sterically hindered nitrogen. There was a sign of vibrational structure in this absorption band in non-polar cyclohexane (Fig. 2). The effect of the surrounding environment on this absorption band was more pronounced in liquid solvents than in polymer matrices (Table 2). This red shift of the absorption maxima with environment polarity corresponded to a strongly mixed $n\pi/\pi\pi^*$ transition, with the latter dominating as the lowest state [55].

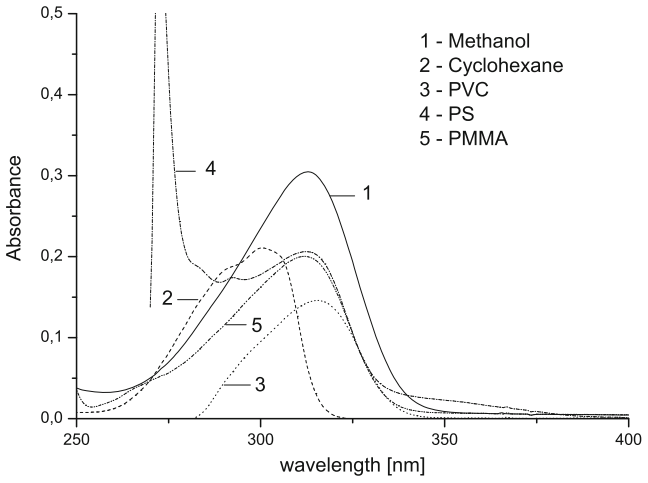


Fig. 2 UV absorption spectra of 1-(2,2,6,6-tetramethylpiperidin-4-yl)-4-*N,N*-dimethylaminobenzoate (1) in various media

The high molar decadic extinction coefficient (>4.1) indicated a strongly allowed transition that was not significantly influenced by solvent polarity [55]. The absorption maxima of the amide derivatives (4, 5, 6) in all of the studied media showed a larger red-shift as compared to the esters (1, 2, 3) (Tables 1 and 2, Figs. 3 and 4, S1a-d and S2a-c). This was due to more prevalent electron donation and conjugation in the case of the amides, as observed by Allen et al [55].

The fluorescence values of the parent amine and alkoxy derivatives of 1-(R-2,2,6,6-tetramethylpiperidin-4-yl)-4-*N,N*-dimethylamino benzoate, expressed as quantum yields in non-polar and polar solvents as well as polymer matrices, were rather low (Tables 1 and 2). The Stokes shifts of these derivatives indicated a difference in geometry between the ground and excited states. In cyclohexane solution, the studied derivatives exhibited one broad emission band with a maximum at 330–340 nm (Table 1a, Fig. 3 and Fig. S1a). The maxima of fluorescence of the benzamide derivatives (4, 5, 6) were red-shifted in comparison to the ester derivatives (1, 2, 3). The hindered amine moiety may have been protonated in the parent amine (1, 4), oxidized form (2, 5) or substituted N-O-R form (3, 6). The presence of a free radical center usually leads to intramolecular quenching, which is caused by an increase in the rate of radiationless processes due to the paramagnetism of the free electron at the sterically hindered nitrogen position [20–23, 41–46]. The extent of intra-molecular fluorescence quenching was expressed as the ratio of fluorescence quantum yields Φ_{NX}/Φ_{NO} of the parent amine NH or N-O-R species and the oxidized N-O$^{\bullet}$ species (Table 1). Comparison of the observed Φ_{NX}/Φ_{NO} ratios indicated that the fluorescence behavior of the studied derivatives was influenced by the type of chemical bond between the chromophore and nitroxide. For the series of ester derivatives (1, 2, 3), Φ_{NX}/Φ_{NO} values around 2.5 corresponded to relatively low intramolecular quenching (Table 1). Φ_{NX}/Φ_{NO} values of as high as 1.5 for the amide derivatives indicated poorly efficient or no intramolecular fluorescence quenching (Table 1). It should be noted that the solubility of 4 in cyclohexane was very low. Therefore, the quantum yield of 4 in cyclohexane might have a large error.

In the fluorescence spectra of benzoate derivatives 1–3 in methanol, acetonitrile and chloroform, broad emission bands with maxima at 350–360 nm were observed. For amide derivatives 4–6, a second broad fluorescence band with a maximum at 460–475 nm was found (Fig. 3 and Fig. S1) in methanol solution. This band was not detected in cyclohexane. On the other hand, in acetonitrile and chloroform, dual fluorescence was observed for the ester derivatives too (Fig. 3 and Fig. S1c,d). The fluorescence intensities of the studied compounds in polar methanol, acetonitrile and chloroform were approximately two orders of magnitude lower compared to non-polar cyclohexane (Fig. 3). The values of Φ_{NX}/Φ_{NO} observed for both the ester and amide derivatives in MeOH were approximately 1, which indicated a low efficiency of

Table 1 Spectral characteristics of derivatives of 1-(R-2,2,6,6-tetramethylpiperidin-4-yl)-4-*N,N*-dimethylamino benzoate (1, 2, 3) or benzamide (4, 5, 6) in solvents

Sample[a]	λ^{A}_{max} [b] nm	log ε[b] mol^{-1} L cm^{-1}	$\lambda^{F}_{max.}$ [c] nm	$\Delta\nu$[e] cm^{-1}	Φ_r(A) [f]	Φ_{NX}/Φ_{NO} (X=H or O-R)	
a/ cyclohexane							
1	300	4.324	332	3212	0.158	2.5	
2	303	4.494	334	3063	0.064	–	
3	300	4.344	332	3212	0.173	2.7	
4	284	4.275	336	5449	0.043	1.0	
5	286	4.359	338	5379	0.0529	–	
6	284	4.369	336	5449	0.079	1.5	
b/ methanol							
1	313	4.484	340; 357	3937	0.0015	1.2	
2	311	4.608	339;355	3985	0.0013	–	
3	310	4.391	338, 357	4247	0.0023	1.8	
4	305	4.347	359; 471	4931	0.0054	0.5	
5	304	4.390	358; 465	4961	0.0099	–	
6	303	4.356	358,470	5070	0.0111	1.1	
c/Acetonitrile							
1	309	4.466	346; 481	3460	0.029	2.6	
2	311	4.559	360; 485	4376	0.011	–	
3	310	4.495	358; 474	4325	0.029	2.6	
4	293	4.347	355; 450	5960	0.069	3.0	
5	296	4.458	339; 454	4285	0.023	–	
6	293	4.441	355; 452	5960	0.066	2.9	
d/chloroform							
							τ^{h} ns
1	314	4.513	353; 426	3502	0.036	0.82	1.40
2	304	4.374	356; 427	4789	0.044	–	1.20
3	309	4.450	350; 417	3791	0.039	0.98	0.96
4	293	4.459	361; 425	6429	0.004	0.57	3.10
5	297	4.515	378; 421	7187	0.007	–	1.97
6	295	4.517	378; 421	7471	0.005	0.71	2.66

[a] Structure of sample according to Scheme 1. [b] Maximum of the longest wavelength absorption band. [c] Log of the molar extinction coefficient. [d] Maximum of the fluorescence band. [e] Stokes shift in cm^{-1}. [f] Relative quantum yield to anthracene. Absolute quantum yield might be estimated taking Φ=0.25 for anthracene in non polar cyclohexane. [g] Extent of intramolecular quenching. [h] Life-time in ns calculated by fitting to mono-exponential or bi-exponential

intramolecular quenching. The values of Φ_{NX}/Φ_{NO} in acetonitrile were in the range of 2.6–3.0 (Table 1a). Such dual fluorescence of the benzene derivatives with strong electron donating groups stems from the twisted intramolecular charge transfer (TICT) state, as suggested by Grabowski [7] and Rettig [8]. This state depends on the conformational freedom of the dimethylamino group [58]. The solvent plays a decisive role in stabilizing the highly dipolar TICT state with partial or complete electron transfer. Therefore, it is formed in polar media only [59]. Similar dual fluorescence was observed for 4-*N,N*-dimethyl amino benzoic acid and some of its esters in polar solutions [16, 17, 19].

As mentioned above, the fluorescence intensity of the studied derivatives in methanol was very low in comparison to cyclohexane. The addition of different concentrations of methanol to a cyclohexane solution of derivative 6 showed an effective quenching of fluorescence by increasing the methanol concentration to >0.5 mol L^{-1}. As shown in Fig. 5, quenching of the fluorescence of 6 with methanol did not obey the Stern – Volmer equation. The observed nonlinear dependence can be interpreted as a preferential solvation of the dipolar ground state by polar solvent molecules [60]. As shown in Fig. 6, the observed solvatation effect was higher in the case of amide derivatives compared to ester derivatives.

An unusual result was observed upon comparison of the fluorescence intensity of the amide derivatives (4, 5 and 6) in methanol. Instead of the expected decrease of fluorescence intensity in the case of N-oxyl derivative 5 by

Table 2 Spectral characteristics of derivatives of 1-(R-2,2,6,6-tetramethylpiperidin-4-yl)-4-N,N-dimethylamino benzoate (1, 2, 3) or benzamide (4, 5, 6) in polymer matrices

Sample[a]	λ^{A}_{max} [b] nm	log ε[b] mol^{-1} L cm^{-1}	$\lambda^{F}_{max.}$ [c] nm	$\Delta\nu$[e] cm^{-1}	Φ_r(A)[f]	Φ_{NX}/Φ_{NO} (X=H or O-R)	τ[h] ns
a/ Polystyrene (PS)							
1	312	4.314	344	2981	0.065	2.0	2.61
2	311	4.46	343	2999	0.033	–	0.8(79 %) 3.9(21 %)
3	308	4.31	339	2969	0.071	2.2	2.20
4	296	4.024	357; 382	5772	0.092	1.7	3.60
5	297	4.205	356; 382	5580	0.055	–	0.9(68 %) 3.8(32 %)
6	292	4.099	347	5428	0.088	1.6	2.0
b/ Polymethylmethacrylate (PMMA)							
1	312	4.31	358; 411	4118	0.445	8.1	2.20
2	311	4.421	353	3825	0.055	–	0.3(89 %) 3.3(11 %)
3	307	4.464	349	3920	0.425	7.8	2.90
4	298	4.219	351	5067	0.099	5.8	2.70
5	298	4.258	354	5308	0.017	–	0.7(78 %) 3.9(22 %)
6	291	4.374	358	6431	0.056	3.3	2.60
c/ Poly(vinylchloride) (PVC)							
1	315	4.164	365	4348	0.254	2.5	2.40
2	314	4.239	370	4820	0.103	–	0.13 (81 %) 2.48 (19 %)
3	311	4.404	357	4143	0.150	1.5	2.5
4	306	4.109	371	5941	0.249	3.1	2.4
5	301	4.274	370	6195	0.080	–	1.90
6	299	4.417	372	6563	0.11	1.4	2.0

[a] Structure of sample according to Scheme 1. [b] Maximum of the longest wavelength absorption band. [c] Log of the molar extinction coefficient. [d] Maximum of the fluorescence band. [e] Stokes shift in cm^{-1}. [f] Relative quantum yield to anthracene. Absolute quantum yield might be estimated taking Φ=0.25 for anthracene in non polar cyclohexane. [g] Extent of intramolecular quenching where NR is either NH (parent amine) or OR (alkoxy). [h] Life-time in ns calculated by fitting to mono-exponential or bi-exponential

intramolecular quenching, a higher intensity of fluorescence than in the case of parent amine 4 was observed. We compared the fluorescence of N-oxyl derivatives 2 and 5 and alkoxyamine derivatives 3 and 6 with and without an equimolar concentration of pentafluorophenyl hydrazine (PFFH) (reduction agent for aminooxyl radicals). The fluorescence spectra of derivatives 3 and 6 were not influenced by the addition of PFFH. The fluorescence intensity of derivative 2 increased as expected through reduction of the paramagnetic center. Surprisingly, the fluorescence intensity of derivative 5 decreased to the level observed for derivatives of the parent amine of amide type 4 upon the addition of an

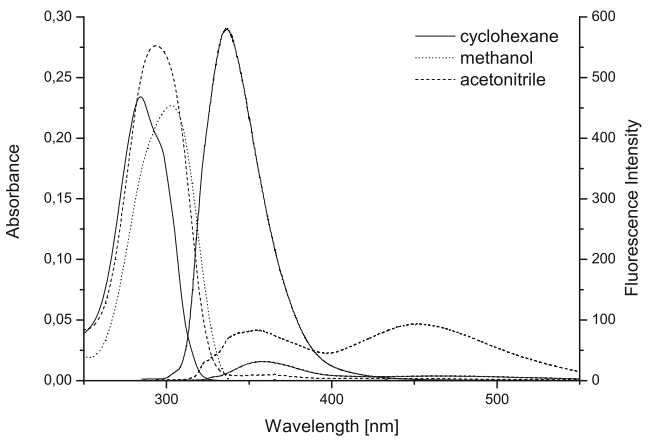


Fig. 3 UV absorption and fluorescence emission spectra of 6 in cyclohexane, methanol and acetonitrile solution (c=10^{-5} mol L^{-1})

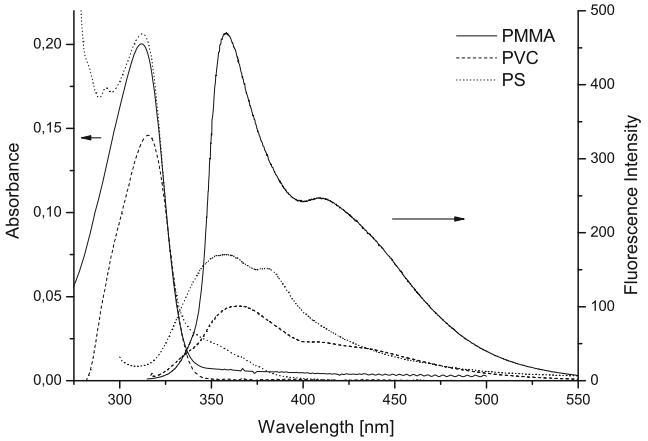


Fig. 4 UV absorption and fluorescence emission spectra of 1 in PMMA, PVC and PS matrices (c=0,002 mol kg^{-1})

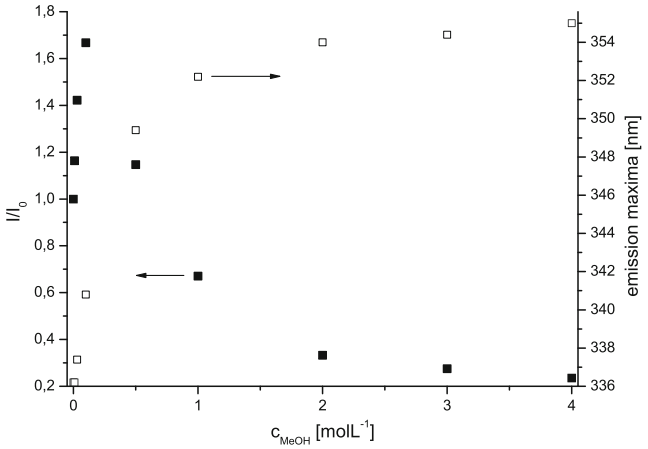


Fig. 5 Quenching of fluorescence and shift of emission maxima of derivative 6 in cyclohexane (c=10^{-5} mol L^{-1}) by addition of methanol

equimolar concentration of PFFH (Figs. S3, S4). Therefore, the fluorescence of 4 was strongly influenced by solute – solvent interactions in methanol solution. These types of interactions were more efficient than in the case of *N*-oxyl derivative 5, where intramolecular quenching should be operating. The primary pathway of these interactions could be through intermolecular hydrogen bonding with methanol. The possibility of methanol forming hydrogen bonds with the piperidine NH group is lower in the case of N-oxyl derivative 5 than in the parent amine or hydroxylamine (formed after reduction of N-oxy radical with PFFH). The dependence of fluorescence properties of the amide-type derivatives was also evident from a comparison of the results observed in protic methanol and aprotic acetonitrile. In acetonitrile, the CT emission was more intense than in methanol, where the locally excited state is more favorable,

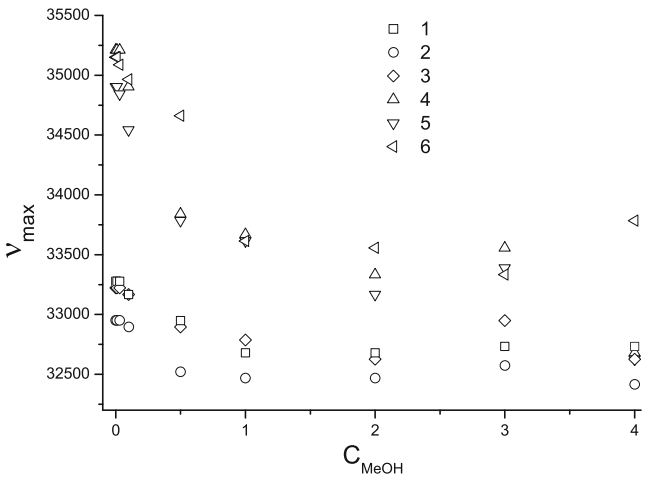


Fig. 6 Dependence of the position of absorption maxima of the derivatives 1-(1-R-2,2,6,6-tetramethylpiperidin -4-yl)-4-*N,N*-dimethylaminobenzoate (1, 2, 3) and –benzamide (4, 5, 6) in cyclohexane, (c=10^{-5} mol L^{-1}) in cyclohexane) on added methanol

as is evident in Fig. 3 (see also Figs. S1b and S1c in supplementary material).

In the PS, PMMA and PVC matrices, the studied derivatives exhibited broad fluorescence bands with maxima between 345 and 375 nm, depending on the derivative and polymer matrix (Fig. 4, Table 2). In some cases, dual fluorescence similar to that in methanol solution was observed. The twisted intramolecular charge transfer (TICT) fluorescence in polymer matrices was less intense than in polar methanol or acetonitrile due to the frozen rotation of the dimethylamino moiety in the rigid polymer.

Comparison of the fluorescence quantum yield of derivatives 1, 2 and 3 (Φ_{NX}/Φ_{NO} ratio) in PMMA showed more efficient intramolecular fluorescence quenching due to the presence of the N-oxyl group than was observed in cyclohexane and methanol (Table 2b). An effective intramolecular fluorescence quenching by the N-oxyl radical in PMMA versus the solution state was also observed for derivative 5 in comparison to parent amine 4, as well as N-O-R derivative 6 (Table 2). A similar trend was observed in the PVC and PS matrices. In these cases, the quenching effect was lower than in PMMA (Table 2). Intramolecular energy transfer (quenching) is more pronounced in polymer matrices, while the TICT state plays an important role in the spectral characterization of the studied probes in polar solutions. Solid polymer matrices do not allow free rotation of the dimethylamino group; thus, CT states do not play such an important role in the spectral properties of the studied probes in polymer matrices.

The lifetimes of fluorescence of the esters and amides were determined in chloroform and polymer matrices. The lifetimes of derivatives 1–6 in chloroform were in the range of 1–4 ns, and intermolecular quenching in 2 and 5 due to the paramagnetic center did not influence the fluorescence decay. Contrary to what was observed in the PS and PMMA matrices, the decay of fluorescence of 2 and 5 was biexponential, with a short lifetime of less than 1 ns. In PVC, the fluorescence decay was monoexponential, as in chloroform. Because the lifetimes of derivatives 1–6 were short, we were unable to perform more detailed analyses.

Conclusion

Probes based on intramolecular quenching of the fluorescence of both the chromophore and paramagnetic center using the 4-*N,N*-dimethylaminophenyl chromophore with an ester or amide linkage to sterically hindered amines did not exhibit large operating ranges, especially in polar solutions. For amide derivatives 4 and 5, an opposite effect was observed due to the strong hydrogen bonding of 4 with methanol, which is a more efficient quenching agent than the paramagnetic group (N-O). A larger operation range of

intramolecular quenching of as high as 8 was observed in polar matrices for the ester derivatives compared to the amide series. Therefore, these probes might be useful for following radical processes in rigid polar media but within a limited range.

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References

1. Lakowicz JR (1999) Principles of fluorescence spectroscopy, 2nd edn. Kluwer Academic/Plenum Publisher, New York
2. Valuer B (2001) Molecular fluorescence: principles and applications. Wiley-VCH Verlag GmbH, Weinheim
3. Albani JR (2005) Structure and dynamics of macromoleules; absorption and fluorescence studies. Elsevier, Amsterdam
4. Winnik FM, Regismond STA (1996) Fluorescence methods in the study of the interaction of surfactant with polymers. Colloid Surface Physicochem Eng Aspects 118:1–39. doi:10.1016/0927-7757(96)03733-8
5. Capek I (2002) Fate of excited probes in micellar systems. Adv Colloid Interface Sci 97:91–149. doi:10.1016/S0001-8686(01)00049-5
6. Lippert E, Luder W, Boos H(1962) In: Mangini A (ed) Advances in molecular spectroscopy Pergamon, Oxford pp 443
7. Grabowski ZR, Rotkiewicz K, Siemiarczuk A, Cowley DJ, Baumann W (1979) Twisted Intramolecular Charge Transfer States (TICT) - new class of excited-states with a full charge separation. Nouv J Chim 3:443–454
8. Rettig W (1986) Charge separation in excited states of decoupled systems—TICT compounds and implications regarding the development of new laser dyes and the primary process of vision and photosynthesis. Angew Chem Intern Ed 25:971–988. doi:10.1002/anie.198609711
9. Revill JAT, Brown RG (1992) Excimer versus TICT state formation in polar solutions of methyl 4-(N, N-demethylamino)benzoate. Chem Phys Lett 188:433–438. doi:10.1016/0009-2614(92)80843-Z
10. Gormin D, Kasha M (1988) Triple fluorescence in aminosalicylates. Modulaton of normal proton transfer, and twisted intramolecular charge transfer (TICT) fluorescence by physical and chemical perturbations. Chem Phys Lett 153:574–576. doi:10.1016/0009-2614(88)85263-1
11. Dendonder-Lardeux C, Jouvet C, Martrenchard S, Solgadi D, Mc Combie J, Howells HD, Palmers TF, Subaric-Leitis A, Monte C, Rettig W, Zimmermann P (1995) Photochemistry in jet cooled aniline derivatives. Chem Phys 191:271–287. doi:10.1016/0301-0104(94)00334-7
12. Kim Y, Cheon HW, Yoon M, Song NW, Kim D (1997) SiO2 Colloidal effects on the twisted intramolecular charge transfer of p-N,N-dimethylaminobenzoic acid in acetonitrile. Chem Phys Lett 264:673–679. doi:10.1016/S0009-2614(96)01388-7
13. Kim Y, Lee BI, Yoon M (1998) Excited-state intramolecular charge transfer of p-N,N-dimethylaminobenzoic acid in Y zeolites: hydrogen bonding effects. Chem Phys Lett 286:466–472. doi:10.1016/S0009-2614(98)00125-0
14. Jiang Y-B, Wang X-J, Jin M-G, Lin L-R (1999) The effect of micelle-water interface electric field on the intramolecular charge transfer within ionic micelles. Dual fluorescence of sodium p-dialkylaminobenzoates in cetylamonium micelles. J Photochem Photobiol Chem 126:125–133. doi:10.1016/S1010-6030(99)00100-8
15. Jiang Y-B, Jin M-G (2000) Intramolecular charge transfer at reverse micelle-water pool interface: p-N, N- dimethylaminobenzoic acid in AOT/cyclohexane/water reverse micelle. Spectrochim Acta Part A 56:623–627. doi:10.1016/S1386-1425(99)00171-7
16. Jiang Y-B (1999) Concentration dependent dual fluorescence of 4-(N, N-dimethylamino) bnzoic acid in chloroform. Spectrochim Acta Part A 55:833–837. doi:10.1016/S1386-1425(98)00229-7
17. Huang W, Zhang X, Ma L-H, Wang Ch-J, Jiang Y-B (2002) Intramolecular charge transfer dual fluorescence of substituted-phenyl p-dimethylaminobenzoates with comparable electron acceptors. Chem Phys Lett 352:401–407. doi:10.1016/S0009-2614(01)01491-9
18. Ma L-H, Chen Z-B, Jiang Y-B (2003) Intramolecular charge transfer with 4(N-ohenylamino)benzoic acid. The N-phenyl conjugation effect. Chem Phys Lett 372:104–113. doi:10.1016/S0009-2614(03)00372-5
19. Zhang C-H, Chen Z-B, Jiang Y-B (2004) Intramolecular charge transfer dual fluorescence p-methylaminobenzoates. Spectrochim Acta Part A 60:2729–2732. doi:10.1016/j.saa.2004.01.011
20. Blough NV, Simpson DJ (1988) Chemically mediated fluorescence yield switching in nitroxide –fluorophore adducts: optical sensors of radical/redox reaction. J Am Chem Soc 110:1915–1917. doi:10.1021/ja00214a041
21. Gerlock JL, Zacmanidis PJ, Bauer DR, Simpson DJ, Blough NV, Solmeen IT (1990) Fluorescence detection of free radicals by nitroxide scavenging. Free Rad Res Communs 10:119–121
22. Török M, Hideg K, Dux L, Horváth LI (1997) Accessibility of protein sulfhydryl groups to nitroxyl spin labels. J Mol Struct 408/409:177–180. doi:10.1016/S0022-2860(96)09634-2
23. Hrdlovič P, Chmela Š, Búcsiová L (1996) Spectral characteristics of bifunctional fluorescence probe based on naphthalene: comparison in solution and in polymer matrix. Chem Papers 50:271–278
24. Hrdlovič P, Chmela Š (1997) Spectral characteristics of multifunctional probes based on pyrene in solution and in polymer matrices. J PhotochemPhotobiol A Chem 105:83–88. doi:10.1016/S1010-6030(97)00008-7
25. Búcsiová L, Hrdlovič P, Chmela Š (2001) Spectral characteristics of fluorescence probes based on pyrene in solution and in polymer matrix. J Photochem Photobiol A Chem 143:59–68. doi:10.1016/S1010-6030(01)00483-X
26. Hrdlovič P, Chmela Š, Danko M (1998) Spectral characteristics and photochemical stability of fluorescence probes based on 1,8-naphthaleneimide in solution and in polymer matrix. JPhotochem Photobiol A Chem 112:197–203. doi:10.1016/S1010-6030(97)00277-3
27. Hrdlovič P, Danko M, Chmela Š (2002) Spectral properties and photolytical stability of anthracene-hindered amine probes: influence of the medium. J Photochem Photobiol A Chem 149:207–216. doi:10.1016/S1010-6030(02)00011-4
28. Hrdlovič P, Kollár J, Chmela Š (2004) Novel fluorescence probe based on pyrene and piperazine; spectral properties in solution and in polymer matrices. J Photochem Photobiol A Chem 163:289–296. doi:10.1016/j.jphotochem.2003.12.002
29. Hrdlovič P, Chmela Š (1998) Spectral and photostabilizing characteristics of adducts of aromatic hydrocarbons and hindered amine stabilizers. Polym Degrad Stab 61:177–182. doi:10.1016/S0141-3910(97)00067-0

30. Bucsiová L, Chmela Š, Hrdlovič P (2001) Preparation, photochemical stability and stabilising efficiency of adducts of pyrene and hindered amine stabilisers in iPP matrix. Polym Degrad Stab 71:135–145. doi:10.1016/S0141-3910(00)00164-6
31. Danko M, Chmela Š, Hrdlovič P (2003) Photochemical stability and photostabilizing efficiency of anthracene/hindered amine stabilizer probes in polymer matrices. Polym Degrad Stab 79:333–343. doi:10.1016/S0141-3910(02)00297-5
32. Hawker CJ (1997) Living free radical polymerization: a unique technique for the preparation of controlled macromolecular architecture. Acc Chem Res 30:373–382. doi:10.1021/ar960248m
33. Matyjaszewski K (1998) Controlled radical polymerization, Vol. 685. Matyjaszewski K (ed) ACS, Washington, p 2
34. Georges MK, Veregin RPN, Kazmaier PM, Hamer GK (1994) Polydispersity polystyrene by a free-radical polymerization process-rate enhancement. Macromolecules 27:7228–7229. doi:10.1021/ma00102a039
35. Li I, Howell BA, Matyjaszewski K, Shigemoto T, Smith PB, Priddy DB (1995) Kinetics of decomposition of 2,2,6,6-tetramethyl-1-(1-phenylethoxy)piperidine and its implications on nitroxyl-mediated styrene polymerization. Macromolecules 28:6692–6693. doi:10.1021/ma00123a044
36. Fukuda T, Terauchi T, Goto A, Tsujii Y, Miyamoto T, Shimizu Y (1996) Well-defined block copolymers comprising styrene − acrylonitrile random copolymer sequences synthesized by "living" radical polymerization. Macromolecules 29:3050–3052. doi:10.1021/ma951792y
37. Bálint J, Kiss V, Egri G, Kálai T, Demeter Á, Balog M, Fogassy E, Hideg K (2004) Kinetic resolution of 1-oxyl-3-hydroxymethyl-2,2,5,5- tetramethylpyrrolidine derivatives by lipase-catalyzed enantiomer selective acylation. Tetrahedron-Asymmetry 15:671–679. doi:10.1016/j.tetasy.2003.12.026
38. Essman M, Sár PC, Hideg K, Marsh D (1993) Iodoacetamide, indanedione, and chloromercuric spin label reagents with derivatized nitroxide rings as ESR Reporter Groups for Protein Conformation and Dynamics. Anal Biochem 213:336–348. doi:10.1006/abio.1993.1430
39. Qi C, Kusnetzow AK, Hideg K, Price EA, Haworth IS, Qin PZ (2007) Nanometer distance measurements in RNA using site-directed spin labeling. Biophys J 93:2110–2117. doi:10.1529/biophysj.107.109439
40. Kálai T, Hideg É, Vass I, Hideg K (1998) Double (Fluorescent and Spin) sensors for detection of reactive oxygen species in the thylakoid membrane. Free Radical Biol Med 24:649–652. doi:10.1016/S0891-5849(97)00339-0
41. Chmela Š, Hrčková L (2009) Nitroxide mediated styrene radical polymerization using a fluorescence marked mediator. Eur Polym J 45:2580–2586. doi:10.1016/j.eurpolymj.2009.06.019
42. Step EN, Turro NJ, Klemchuk PP, Gande ME (1995) Model studies on the mechanism of hals stabilization. Angew Makromol Chem 232:65–83. doi:10.1002/apmc.1995.052320105
43. Chirinos-Padrón AJ (1989) Mechanistic aspects of polymer photostabilization. J Photochem Photobiol A Chem 49:1–39. doi:10.1016/1010-6030(89)87103-5
44. Pospıšil J (1991) Photo-oxidation degradation of polymers: causes, consequences and protection. Chem Listy 85:904–923
45. Micallef AS, Blinco JP, George GA, Reid DA, Rizzardo E, Thang SH, Bottle SE (2005) The application of a novel profluorescent nitroxide to monitor thermo-oxidative degradation of polypropylene Polym. Degrad Stab 89:427–435. doi:10.1016/j.polymdegradstab.2005.01.030
46. Fairfull-Smith KE, Blinco JP, Keddie DJ, George GA, Bottle SE (2008) A novel profluorescent dinitroxide for imaging polypropylene degradation. Macromolecules 41:1577–1580. doi:10.1021/ma701944p
47. Carver FJ, Hunter CA, Livingstone DJ, McCabe JF, Seward EM (2002) Substituent effects on edge-to-face aromatic interactions. Chem Europ J 8:2847–2859. doi:10.1002/1521-3765(20020703)8:13<2847::AID-CHEM2847>3.0.CO;2-M
48. Takashi H, Yoshihiko I, Kiyotaka K, Shinsaku N, Kazuko I et al (1990) The effects of new cytochalasins from phomopsis sp. and the derivatives on cellular structure and actin polymerization. Chem Pharm Bull 38:971–974
49. Vamecq J, Lambert D, Poupaert JH, Masereel B, Stables J (1998) Anticonvulsant activity and interactions with neuronal voltage-dependent sodium channel of analogues of ameltolide. J Med Chem 41:3307–3313. doi:10.1021/jm9608772
50. Kawski A, Kubicki A, Kuklinski B, Gryczynski I (1993) Unusual absorption and fluorescence properties of 1,6-diphenyl-1,3,5-hexatriene in poly(vinyl alcohol) film. J Photochem Photobiol A Chem 71:161–167. doi:10.1016/1010-6030(93)85068-J
51. Birks JB (1968) Photophysics of aromatic molecules. Willey-Interscience a Division of John Wiley and Sons Ltd, New York, London, Toronto, Sidney, Ch 4. p 121–127
52. Adamson AW, Demas JN (1971) Evaluation of photoluminescence lifetimes. J Phys Chem 75:2463–2466. doi:10.1021/j100685a009
53. Demas JN (1973) Excited state lifetime measurements. Appendix E. Academic, New York, p 245
54. Enderlein J, Erdmann R (1997) Fast fitting of multi-exponential decay curves. Opt Commun 134:371–378. doi:10.1016/S0030-4018(96)00384-7
55. Allen NS, Edge M, Sethi S, Catalina F, Corrales T (2000) Photochemistry and photo-induced co-synergistic polymerization activites of novel N,N-dimethylaminobenzoates and benzamides. J Photochem Photobiology A Chem 137:169–176. doi:10.1016/S1010-6030(00)00364-6
56. Danko M, Chmela Š, Hrdlovič P (2006) Synthesis, photochemical stability and photo-stabilizing efficiency of probes based on benzothioxanthene chromophore and hindered amine stabilizer. Polym Degrad Stab 91:1045–1051. doi:10.1016/j.polymdegradstab.2005.07.009
57. Kollár J, Hrdlovič P, Chmela Š (2008) Synthesis and spectral characteristics of di-substituted 1,8-naphthalimides; bi-radical formation. J Photochem Photobiology A Chem 195:64–71. doi:10.1016/j.jphotochem.2007.09.008
58. Rettig W, Lapouyade R (1994) Fluorescent probes based on twisted intramolecular charge transfer (TICT) states and other adiabatic photoreactions. In: Lakowicz JR (ed) Topics in fluorescence spectroscopy, Vol. 4.: probe design and chemical sensing. Plenum Press, New York, pp 109–149
59. Kaholek M, Hrdlovič P (1999) Characteristics of the excited states of 3-substituted coumarin derivatives and transfer of electronic energy to N-oxyl radicals. J Photochem Photobiol A Chem 127:45–55. doi:10.1016/S1010-6030(99)00133-1
60. Köhler G, Rechthaler K, Rotkiewicz K, Rettig W (1996) Formation and stabilization of twisted intramolecular charge transfer states in binary mixed solvents. Chem Phys 207:85–101. doi:10.1016/0301-0104(96)00054-7