

PHOTOPHYSICAL AND OPTICAL PROBE PROPERTIES OF 1-(*p*-N,N-DIMETHYLAMINOPHENYL)-4-PHENYL- 2-METHYL-1*E*,3*E*-BUTADIENE

A. K. SINGH* and T. S. R. KRISHNA
Department of Chemistry, Indian Institute of Technology,
Powai, Bombay 400-076, India

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Abstract – A hitherto unknown diphenylbutadiene analog *viz.* 1-(*p*-N,N-dimethylaminophenyl)-4-phenyl-2-methyl-1*E*,3*E*-butadiene (**10**) has been prepared and its absorption, excitation, and fluorescent emission properties in different media including various organic solvents and aqueous bovine serum albumin (BSA) have been studied. For comparison, these properties have also been investigated for the parent diphenylbutadiene (**2**). Diene **10** exhibits solvent polarity/polarizability-sensitive fluorescence properties (λ_{\max} , Φ_f , τ_f , K_f , f). It also binds to the hydrophobic domains of aqueous bovine serum albumin (BSA) with a binding constant of $3.89 \times 10^4 \text{ M}^{-1}$. The relative fluorescence quantum yield of **10** increases, while, the fluorescence lifetime decreases with increasing concentration of BSA. The results highlight the polar character of the singlet excited state of diphenylpolyenes and the utility of **10** as fluorescence probe for studying microenvironments of organized assemblies and biological supramolecular structures.

INTRODUCTION

Light-induced geometrical isomerization of conjugated linear polyenes has been known to be an important photobiological process taking place in the opsin family of proteins.¹ Consequently, photochemistry, photophysics and spectroscopy of conjugated linear polyenes have been the subject matter of much interest in recent years.^{2–8} In this respect, α,ω -diphenyl polyenes [Ar (CH=CH)_{*n*}Ar, Fig. 1, **1**] have attracted a great deal of attention as models of the retinylidene polyenes and in recent years much efforts have been directed towards understanding the nature of the electronically excited state of these systems. Several excellent reviews covering the photochemistry, photophysics and spectroscopy of α,ω -diphenylpolyenes have appeared recently in literature.^{6–8} The nature of the electronically excited states of diphenylpolyenes has thus been extensively studied and it is believed that all-*trans*-1,4-diphenylbutadiene (all-*trans* DPB, **2**) has two lowest singlet states *viz.* $^1A_g^*$ and $^1B_u^*$ and the energy gap in solution is small (approx. 200 cm^{-1} with $^1B_u^*$ lying below $^1A_g^*$); however the actual order of the two lowest excited states has not been resolved adequately. Upon absorption of photons diphenylpolyenes produce 'planar' state which undergoes further twisting yielding the 'perpendicular species' from where isomerization products are obtained^{6–8}.

Irradiation of all-*trans*-DPB (**2**) in organic solvents gives *cis*, *trans*-DPB (**4**) as the predominant photoproduct.^{9,10} All-*trans*-DPB (**2**) shows fluorescence from

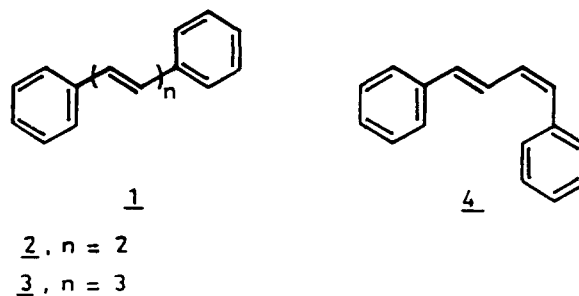


Figure 1. Chemical structure of α,ω -diphenylpolyenes.

low lying $^1B_u^*$ state in hydrocarbon solvents. Both, the photoisomerization as well as fluorescence of all-*trans*-DPB (**2**) are affected by substituents, and in attempts to better understand the nature of the excited state potential energy surfaces of linearly conjugated polyenes, the effects of substituents on diphenylpolyene photochemistry has been studied. It has been shown that addition of cyano substituents to all-*trans*-DPB (**2**) causes a change in the distribution of photoisomers.^{5,11} Further, the linear nature of diphenylpolyenes is useful for anisotropic fluorescence studies and such molecules, particularly, all-*trans*-diphenylhexatriene(**3**) and its derivatives are used as fluorescence probes to study the micropolarity inside the hydrophobic domains of the microheterogeneous media.

It is believed that planar B_u^* states have partial charge associated with them, and further considerable separation of charge may develop upon twisting to a perpendicular geometry. The development of charge as a function of twist angle in olefins containing carbons of

* To whom correspondence should be addressed.

different electronegativities causes a rapid increase in dipole moment as the perpendicular geometry is reached. This "sudden polarization effect" has been tested by theoretical^{5,12,13} as well as some experimental studies.¹⁴ However, experimental studies are limited and additional investigations are required to establish whether the perpendicular excited singlet states of linearly conjugated C=C chromophores are best described as nonionic or dipolar/zwitterionic states.

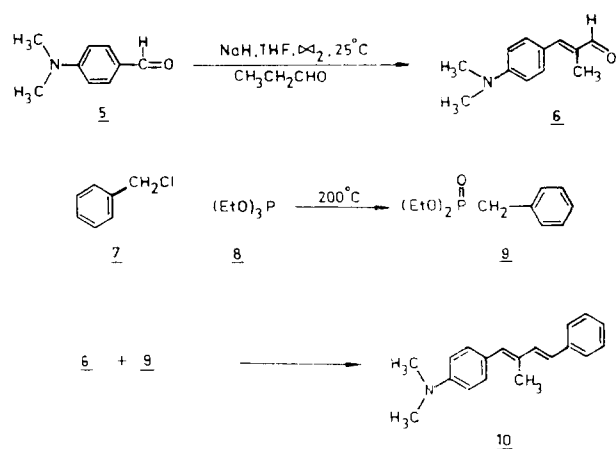
In view of the above we thought it desirable to study the singlet excited state properties of diphenylpolyenes bearing electron withdrawing/electron donating substituents for thermodynamic reasons and potentially steric substituents for kinetic reasons. Presence of such thermodynamic and/or kinetic controls in the diphenylpolyene chromophore can influence (stabilize/destabilize the excited state intermediate) the charge separation in the excited state and therefore photophysical studies on such molecules can help us to understand the nature of twisting of the C=C chromophore and sudden polarization effect in the electronically excited state. A polarized excited state can also be used to characterize the microenvironment of organized assemblies and complex biological structures.

In this paper we report absorption, excitation and fluorescent emission properties of a hitherto unknown diphenylpolyene namely, 1-(*p*-*N,N*-dimethylaminophenyl)-4-phenyl-2-methyl-1*E*,3*E*-butadiene (**10**) bearing a donor (*N,N*-dimethyl group) in one of the aromatic ring and an electron donating as well as potentially steric group (the methyl group) in the chain double bond.

MATERIALS AND METHODS

General Procedures. Starting materials and reagents for the preparation of **10** were obtained from Aldrich Chem. Co. U.S.A. Organic solvents from Spectrochem Bombay were of uv-vis grade. Uv-vis spectra were obtained on Beckman DU-6 spectrophotometer. IR spectra were recorded on Perkin-Elmer 625 Infrared spectrophotometer. ¹H NMR spectra were recorded on Varian 300 MHz NMR spectrometer using TMS as internal standard. 1*E*,3*E*-DPB (**2**) was obtained as previously described.¹⁵

Synthesis of 1-(*p*-*N,N*-dimethylaminophenyl)-4-phenyl-2-methyl-1*E*,3*E*-butadiene (10**).** *p*-*N,N*-Dimethylaminobenzaldehyde (**5**, 7.30 g, 0.05 mole) in dry THF was taken in a 2-neck round bottom flask under constant flow of dry N₂. To this was added sodium hydride (1g) and the mixture was stirred at room temperature for 1 hour. Propanaldehyde (3.5 ml) was then dropwise added to the stirring mixture. After additional stirring for 30 minutes, the reaction mixture was subjected to usual work-up and the organic layer was taken-up in ether. Ether was distilled out on a rotary evaporator to obtain 1-*p*-*N,N*-dimethylamino, α -methyl-cinnamaldehyde (**6**) in 44% yield.



Scheme

Benzyl phosphonate **9** was prepared by usual procedure by heating benzyl chloride and triethyl phosphite followed by distillation. Phosphonate **9** (1 mole equivalent) was stirred with sodium hydride (1.5 mole equivalent) in dry DMF under N₂ for 30 minute at ambient temperature. Aldehyde **6** (1 mole equivalent to the phosphonate) was added to the reaction mixture and stirring was continued for another hour. To the reaction mixture was added ice-cooled brine solution. The reaction mixture was taken-up in ether, washed with water and finally ether was removed on a rotavapour. The yellowish solid obtained was subjected to multiple crystallization from benzene to obtain pure **10** in 68% yield; m.p. 150-51°C; uv-vis(MeOH), 347 nm (ϵ , 2.75×10^4 mole⁻¹ cm⁻¹L); IR (KBr) ν_{\max} : 3100, 3000-2900, 1620, 1550, 1380, 860, 720 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ : 1.59 (3H, s), 2.98 (6H, s), 6.72 (2H, d, J = 9 Hz), 6.90 (1H, d, J = 16 Hz), 7.08 (1H, d, J = 16 Hz), 7.20 (1H, t), 7.24 (1H, s), 7.34 (2H, t), 7.42 (2H, d), 7.44 (2H, d, J = 9 Hz).

Fluorescence Studies. All samples were handled under protective red lamp and degassed prior to fluorescence measurements. The steady-state fluorescence studies were performed on Spex Fluolog instrument. Relative fluorescence quantum yields (Φ_f) were determined by standard procedures. The fluorescence lifetime (τ_f) measurements studies were carried out on SP-70 nanosecond fluorescence spectrofluorimeter (Applied Photophysics, U.K.) equipped with thyatron gated discharge lamp with strong lines from 300 nm to 400 nm with amplitude output of <1 ns and a photomultiplier tube type XP2020 Q having spectral range from 200 nm to 800 nm. The fluorescence lifetimes were calculated by DEC Minc computer using the software provided by the manufacturer.

Fluorescence rate constant, k_f , and the oscillator strength, f , were calculated using the following equations.¹⁶

$$k_f = 2.88 \times 10^9 \times n^2 \times (\bar{\nu}_1)^3 \times \epsilon \times (\bar{\nu}_0)^{-1} \times w,$$

$$f = 4.88 \times 10^9 \times \epsilon \times w$$

where ϵ is extinction coefficient, n is refractive index, $\bar{\nu}_1$ is

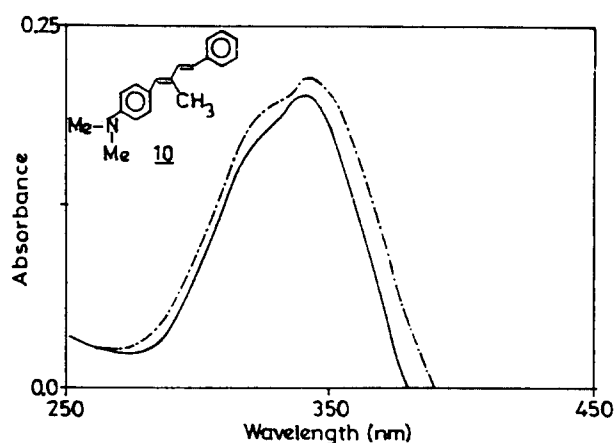


Figure 2. Absorption spectra of **10** in *n*-hexane (—) and in methanol (---).

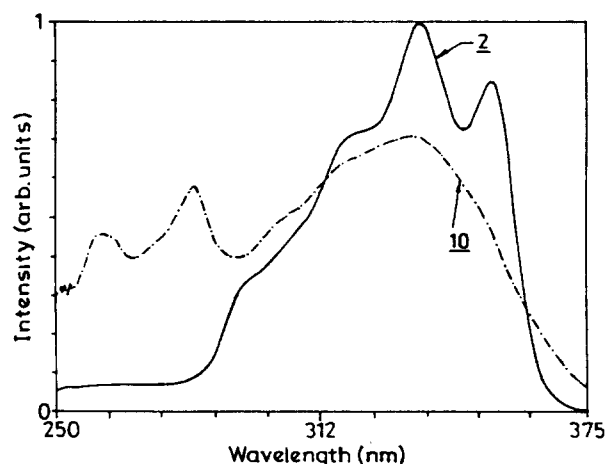


Figure 3. Excitation spectra of **2** (—) and **10** (---) in $100 \mu\text{M}$ aqueous BSA (emission λ_{max} for **2**: 403 nm; and for **10**: 422 nm).

fluorescence λ_{max} in cm^{-1} , $\bar{\nu}_a$ is absorption λ_{max} in cm^{-1} , w is width at half height of absorption spectrum. The binding constant, K , for the binding of fluoroprobe **10** to bovine serum albumin (BSA) and the corresponding fluorescence enhancements were calculated by the following equation.¹⁷

$$\Delta\Phi_{\text{R}}^{-1} = (1 + \Phi_{\text{a}}/\Phi_{\text{b}})^{-1} (1 + 1/K C_{\text{BSA}})^{-1}$$

$$\Delta\Phi_{\text{R}}^{-1} = (1 - \Phi/\Phi_{\text{a}})^{-1}$$

A plot Φ_{R}^{-1} vs. C_{BSA}^{-1} gives K .

RESULTS AND DISCUSSION

Diene **2** was synthesized by the usual known procedure.¹⁵ However, for the synthesis of diene **10** methods as outlined in the Scheme were employed. Both the

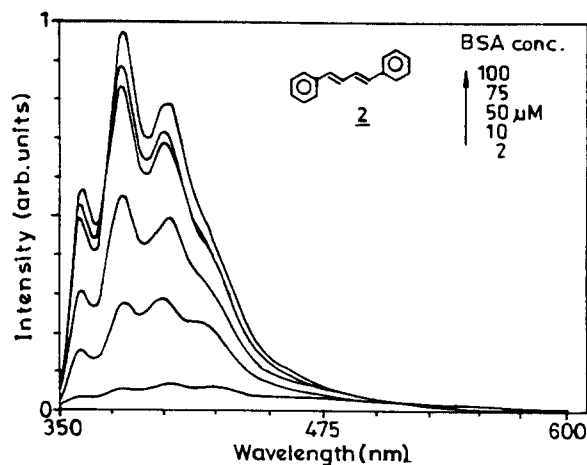


Figure 4. Fluorescence spectra of **2** in different concentrations of aqueous BSA; excitation λ_{max} : 330 nm. With increasing concentration (\uparrow) of BSA, the fluorescence of **2** increases.

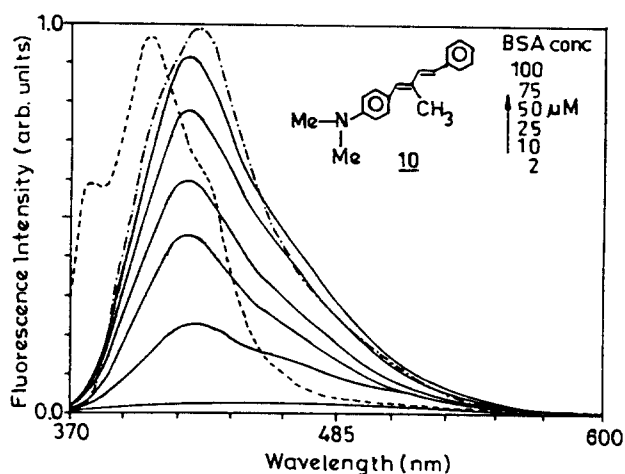


Figure 5. Fluorescence spectra (excitation λ_{max} : 350 nm) of **10** in *n*-hexane (---); methanol (-.-); and in different concentrations of aqueous BSA (—). With increasing concentrations (\uparrow) of BSA, the fluorescence of **10** is increased.

synthetic dienes showed expected physico-chemical characterization data. Table 1 summarizes some of the photophysically relevant spectroscopic data of **10** in organic solvents. Typical absorption spectra of **10** in *n*-hexane and methanol are presented in Fig. 2. In polar, aprotic solvent such as dimethylformamide **10** shows a red-shifted absorption band at 355 nm as compared to its absorption band at 341 nm in a nonpolar solvent like *n*-hexane. Similar absorption trends for **10** are seen in polarizable solvents like benzene in which it showed a band at 351 nm. The excitation spectrum (Fig. 3) of **2** in $100 \mu\text{M}$ aqueous BSA solution is characterized by peaks at 322, 336 and 354 nm (due to **2**) and a peak at 295 nm due to the protein. The excitation spectrum (Fig. 3) of **10** in $100 \mu\text{M}$ aqueous BSA solution is however characterized by a rather broad band with excitation maximum at 356 nm. The fluorescent emission spectra of **2** and **10**

Table 1. Summary of photophysical data for **10**.

Medium ^a	$\lambda_{\text{abs}}^{\text{max}}$ (nm)	$\lambda_{\text{em}}^{\text{b}}$ (nm)	Φ_{f} (± 0.01) ($\times 10^8 \text{ s}^{-1}$)	k_{f}	f
<i>n</i> -Hexane	341	400	0.032	4.55	0.68
Benzene	351	433	0.038	3.95	0.61
Dichloromethane	349	423	0.030	4.53	0.73
Methanol	342	430	0.034	3.54	0.70
Ethanol	345	425	0.035	4.42	0.78
Dimethylformamide	355	439	0.038	3.88	0.68

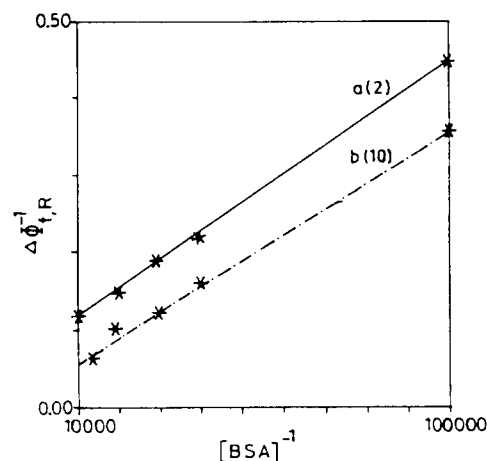
^aconcentration of **6**: $1.0 \times 10^{-5} \text{ M}$ ^b λ_{excit} : 340 – 356 nm

in different media are presented in Figs. 4 and 5 respectively. The fluorescence spectra of **2** in aqueous BSA is characterized by peaks at 362, 382 and 403 nm. Upon excitation at 350 nm, compound **10** in aqueous BSA exhibits fluorescence peak at 422 nm. As given in Table 1, the fluorescence maxima of **10** is found to vary with the polarity of the solvents. As compared to the structured fluorescence band of **2**, compound **10** shows a rather broad fluorescence band. The fluorescence quantum yield (Φ_{f}) of **10** which is 0.032 in *n*-hexane slightly increases in polar solvents. On the other hand, the fluorescence lifetime (τ_{f}) of **10** drastically decreases in polar solvents (ns : *n*-hexane, 0.99; tetrahydrofuran, 0.71; methanol, 0.58; and in acetonitrile, 0.45). The oscillator strength (f) is found to be high and compares well with the ' f ' of the parent diene **2**. These results indicate towards solvation dynamics-induced sensitivity of the fluorophore **10**.

The singlet excited state of **10** can have appreciable dipolar character and in polar solvents the twisted excited state gets further stabilized. The formation of excited species having perpendicular geometry is facilitated by the presence of polar solvents and stabilizing substituents. It is also apparent that, like in parent diphenylbutadiene **2**, the lowest singlet excited state in **10** can have B_{u} character. It is known that the B_{u} state has more ionic character due to a lower electron correlation resulting in an increased local electron density

Table 2. Fluorescence λ_{max} and lifetimes (τ_{f}) of **10** at various concentrations of aqueous BSA.

[BSA] (μM)	flu λ_{max} (nm)	τ_{f} (ns)	X^2
2	427,466	1.37	1.11
10	425	1.08	1.08
25	422	0.68	0.97
50	422	0.59	1.05
75	422	0.55	1.08
100	422	0.71	1.33

Figure 6. A plot of $\Phi_{\text{f,R}}^{-1}$ vs. C^{-1} [BSA] for (a): **2** (—); and (b): **10** (---).

and a higher degree of charge separation. Excited states of such character are most likely to get stabilized more by polar solvents and stabilizing substituents such as those present in **10**.

The solvent-sensitivity of the fluorescence of **10** prompted us to evaluate its properties as fluorescence-probe. For this purpose we studied the fluorescence properties of **10** in bovine serum albumin (BSA). It is found that the fluorescent emission intensity of **2** (at all λ_{max} i.e. 362, 382 and 403 nm) as well as **10** (at 422 nm) linearly increases with increase in BSA concentration as shown in Figs. 4 and 5 respectively. As compared to in an organic solvent, a fluorescence intensity enhancement of approximately 18 and 13 times respectively for diene **10** and **2** in aqueous BSA was determined. The nature of binding between the fluorophores **2** and **10** and BSA was further studied by determining the binding constants which is found to be $3.89 \times 10^4 \text{ M}^{-1}$ for **10** and $2.26 \times 10^4 \text{ M}^{-1}$ for **2**. A plot of relative fluorescence quantum yields (Φ_{f}) of **2** and **10** vs. BSA concentrations is presented in Fig.6. The fluorescence maxima (flu λ_{max}) and lifetimes (τ_{f}) of **10** at various concentrations of BSA are given in Table 2. The flu-

Table 3. A comparison of the binding constant and relative increase in fluorescence intensity of **10** and other probes when bound to aqueous BSA at 25°C.

Probe	Binding constant, K (M^{-1})	Fluorescence intensity relative increase
2	2.26×10^4	13
10	3.89×10^4	18
ANS	7.6×10^5	13
Prodan	1×10^5	3
Sub. Coumarin	30×10^4	10

ANS: Anilino naphthalene sulphonate; Prodan: 6-Propynyl-2-(dimethylamino) naphthalene; Sub. Coumarin: N,N-Dimethylamino-3-methyl coumarin.

orescence lifetime of **10** decreases as the concentration of BSA is increased.

The binding constant and fluorescence enhancement of **10** has also been compared with some well known fluorescence probes and the data are presented in Table 3. Due to their hydrophobic nature **2** and **10** are not soluble in aqueous medium and hence these are expected to bind to the hydrophobic domains of the BSA protein. Molecular mechanics calculations show that **10** has a linear shape with end-to-end length of 16 Å. The linear geometry of **10** combined with its interesting electronically excited singlet state characteristics can be useful in the studies of the microenvironment of organized assemblies and complex biological systems of lipid bilayer and biological membranes.

Overall, besides providing experimental evidence towards the involvement of polar excited states in the photoprocesses of linearly conjugated C=C chromophores, the present investigations provide new directions for the development of newer molecular frame-work of novel optical probes.

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