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Examining [2,2′-bipyridyl]-3,3′-diol as a possible DNA model base pair

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Abstract

[2,2′-Bipyridyl]-3,3′-diol ($BP(OH)_2$) is known to undergo excited state intramolecular double proton transfer in solution. Steady state absorption and fluorescence spectra of $BP(OH)_2$ were studied here in solvents of varying polarity and hydrogen bonding capability, and in binary mixtures of *p*-dioxane/water in order to test its applicability as a model base pair. Unique absorption due to water solvation was observed in the region of 400–450 nm. Only the di-keto-tautomer fluoresces in all the solvents studied here. A large blue-shift in the fluorescence band due to intermolecular hydrogen bonding was observed in polar, protic solvents which increases with increasing solvent polarity. Results from the absorption spectra in the binary mixtures were fitted to a binding isotherm model and three water molecules were found to solvate each of the two hydrogen bonding centers in $BP(OH)_2$. Molecular dynamics simulations were performed for a dodecamer duplex DNA containing $BP(OH)_2$ as a model base pair in the center of the duplex. The results of the simulations indicate that $BP(OH)_2$ can serve as a good mimic of a natural base pair with no major perturbation to the helical structure's stability. One of the two hydrogen bonds in $BP(OH)_2$ resides in the major groove of the duplex DNA, whereas the other one is situated in the minor groove. The preferential stacking between the flanking base pairs of duplex DNA and its unique spectroscopic features in water make $BP(OH)_2$ a potential probe for tautomerization in DNA and a possible sensor for water accessibility in the major and minor grooves of DNA.

Keywords: [2,2′-Bipyridyl]-3,3′-diol; Binary mixtures; Absorption; Fluorescence; Molecular dynamics simulations

1. Introduction

The DNA structure was originally proposed by Watson and Crick to comprise a double helix [1]. Hydrogen bonding and stacking interactions maintain the DNA double helix structural pattern. The hydrogen bonding patterns of adenine:thymine (dA:dT) and cytosine:guanine (dC:dG) determine the sequence-specific base pairing in duplex DNA. The keto-amine tautomers are the dominant base pair forms which are responsible for high-sequence specificity in duplex DNA. Double-proton transfer reaction along two parallel hydrogen bonds joining the DNA chains could originate rare tautomers as shown in Fig. 1a [1–4]. Although these enol-imine rare tautomers are relatively unstable, their existence may be longer than the DNA unwinding and strand separation processes. This can cause a spontaneous or induced base mispairing in DNA [5]. If undetected by the DNA repair machinery, this violation of proper Watson–Crick pairing might cause an alteration of the genetic code leading to point mutations [1,6].

Despite its potential importance, tautomerization in DNA has not been well-characterized experimentally due to the inability to selectively initiate and follow a proton transfer reaction in a specific base pair without disturbing the other base pairs in the DNA polymer. Experiments directed at examining tautomerization usually involve stable base analogues that are believed to favor rare tautomers, such as O^6-methyguanine or N^6-methoxyadenine [5]. These studies are not ideal, because the base analogues are known to cause structural perturbations to the DNA duplex. On the other hand, a variety of molecules which have intramolecular hydrogen bonds can be studied as model base pairs. These molecules may be photoexcited to undergo proton transfer [7,8]. This process is known as Excited State Intramolecular Proton Transfer (ESIPT) (for a recent review see Ref. [9]). Molecules that can undergo ESIPT allow for the controlled initiation and subsequent time-dependent characterization of both the H-bond itself and the proton transfer process.

We have recently proposed one such molecule, 2-(2′-hydroxyphenyl)benzoxazole (HBO), as a structural mimic of

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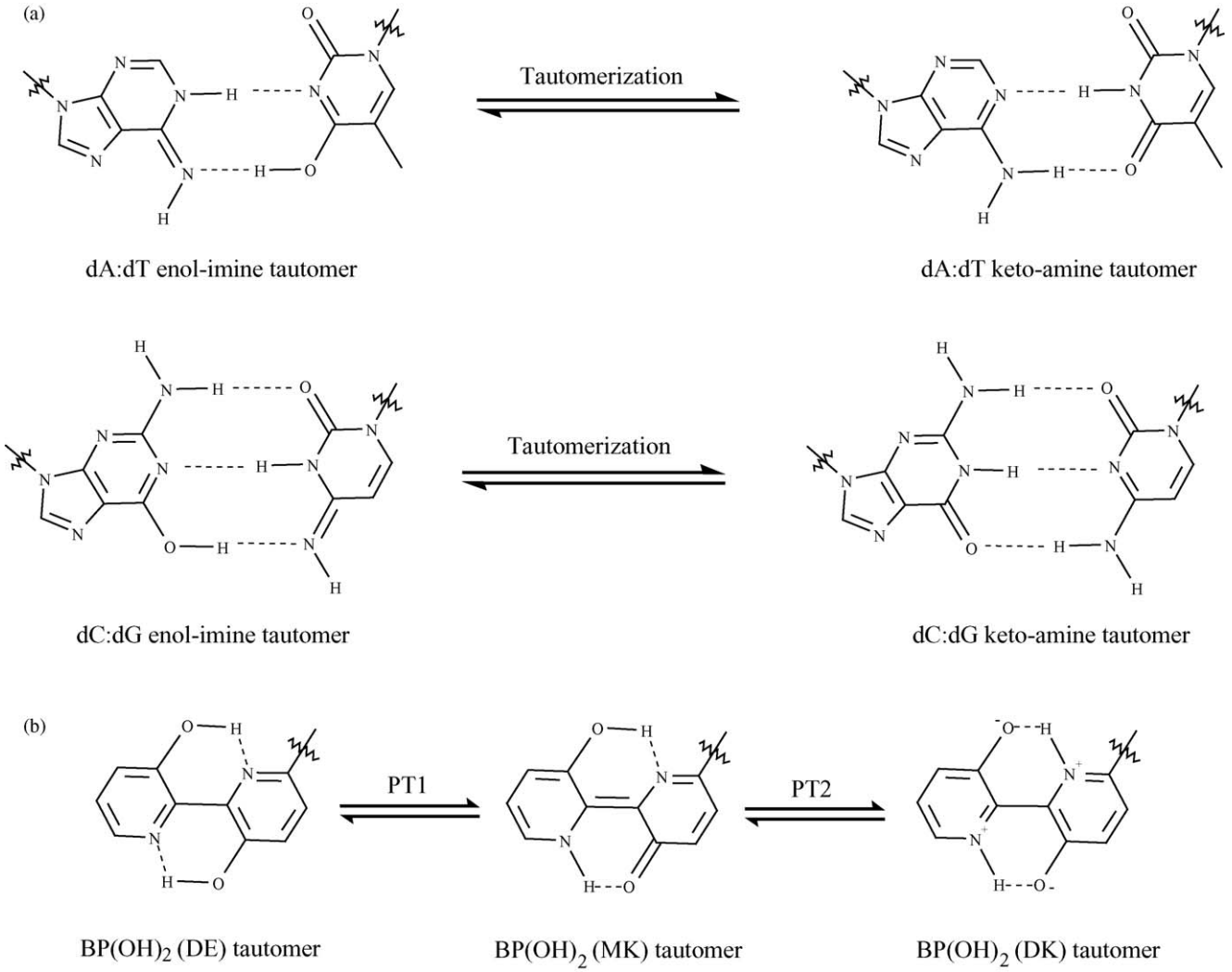


Fig. 1. (a) Tautomerization in natural base pairs. (b) Tautomerization in $BP(OH)_2$ model base pair after single proton transfer (PT1) and double proton transfer (PT2). The zigzag lines represent the sugar connections to the natural base pairs, and to the proposed C-atom in $BP(OH)_2$.

a DNA base pair for which tautomerization may be initiated at a defined time and position within duplex DNA [10–13]. We characterized HBO in time and frequency domains in solution [12,13] as well as incorporated in DNA [10,11,13]. In contrast to other model base pairs, such as dimers of 7-azaindole [14], HBO can be incorporated into duplex DNA in a way which aligns the hydrogen bond of HBO with the hydrogen bonds of the flanking bases [10]. Therefore, HBO may be used to probe the biologically relevant duplex environment. However, it is important to keep in mind the strengths and weaknesses of HBO as a model. Some structural and electronic properties of HBO in DNA are expected to be different from a Watson–Crick base pair due to the fact that the phenyl and benzoxazole rings of HBO are connected by one covalent and one H-bond, while the natural pyrimidine and purine rings are connected only by H-bonds. However, as a model of a DNA base pair, HBO has several advantages. HBO is easily incorporated into duplex DNA without a strong perturbation of the duplex [10]. Additionally, the change in magnitude and orientation of the HBO dipole moment upon phototautomerization is expected to be similar to that which occurs during natural tautomerization [10,11].

In an effort to narrow the gap between a model base pair and natural DNA base pairs in terms of structural similarities and properties, a new base pair model is proposed here. The [2,2′-bipyridyl]-3,3′-diol ($BP(OH)_2$) molecule (shown in Fig. 1b) is known to undergo an extremely fast Excited State Intramolecular Double Proton Transfer (ESIDPT) in solution [15]. As shown in Fig. 1b, $BP(OH)_2$ possesses two internal hydrogen bonds which makes it a better representation of a natural DNA base pair than HBO. These hydrogen bonds have been detected by NMR [16] and IR [17] spectroscopy. In solution at room temperature, $BP(OH)_2$ absorbs in the region of 350 nm yet fluoresces strongly in the green. If incorporated in duplex DNA, this allows for a selective photoinduced tautomerization in $BP(OH)_2$ without disturbing the duplex natural base pairs. Quantum yields of fluorescence in the order of 0.2–0.4 were observed in different solvents at room temperature with lifetimes of a few ns [15,18–21]. Comparison of the absorption and emission properties of $BP(OH)_2$ with related systems possessing only

one hydrogen bond reveals that the second hydroxyl group is essential to the observation of the strong green emission. This molecule is also planar in crystalline form [22] and is expected to retain its planarity in solutions of non-interacting solvents because of the two strong intramolecular hydrogen bonds. This planarity is expected to allow maximum interaction with flanking bases upon incorporation in duplex DNA, and minimum structural perturbation to the helical structure. Electro-optical absorption and emission and calculated excited state dipole moments show that the dipole moments of the di-enol (DE) and the di-keto (DK) tautomers are negligible, whereas it is 4.0–4.9 D for the mono-keto (MK) tautomer [23,24].

Over the past decade, a wealth of knowledge has been obtained about the dynamics of the ESIDPT in $BP(OH)_2$ due to the ability to resolve the ultrafast proton transfer dynamics in subpicoseond and femtosecond time regimes. Several experimental [25–29] and theoretical [30–33] studies have characterized the ultrafast dynamics in $BP(OH)_2$. Of central interest is whether the protons are transferred simultaneously (concerted process) or sequentially (two-step process). In the latter case, the MK tautomer form should appear as an intermediate. From the analysis of the fluorescence decays, it was concluded that the double proton transfer in photoexcited $BP(OH)_2$ occurs through both a concerted and a stepwise mechanism [25]. By careful selection of the excitation wavelength, an energy barrier was ascertained in the first stage of the stepwise mechanism which was estimated to be $\approx 600\,cm^{-1}$ [26]. In sol–gel glasses as a restricted matrix, the barrier height was estimated to be $\approx 300\,cm^{-1}$ [27].

The effect of solvent on the MK to DK proton transfer dynamics in $BP(OH)_2$ was studied in protic and aprotic solvents [28]. In aprotic solvents, no influence of the nature of the solvent, temperature and deuteration of the hydroxyl groups on the proton transfer dynamics was found. In protic solvents, however, the proton transfer time is found to change proportionally with the viscosity coefficient of the solvent. It was concluded that proton transfer is promoted by several intramolecular modes in aprotic solvents, whereas in protic solvents the effect of interactions of $BP(OH)_2$ with neighboring solvent molecules on the excited state potential energy surface play a crucial role in transferring the second proton. No tunneling mechanism was visible from the deuterium effect on the proton transfer dynamics. However, a slowing down in the second proton transfer rate was correlated to the higher viscosity of deuterated solvent as compared to its protonated analogue [28]. Anisotropy measurements show that the disappearance of the di-enol tautomers is a (quasi-)barrierless process [29].

In this paper, the $BP(OH)_2$ molecule is examined as a potential model for a natural base pair to study tautomerization in DNA. We examine the solvent effects on the absorption and fluorescence spectra of $BP(OH)_2$. The study is carried out in different solvents of varying polarity and hydrogen bonding capability, and in binary solvent mixtures of *p*-dioxane and water. *p*-dioxane and water are miscible in all proportions thus provide the opportunity to study the effect of a broad range of solvent polarity. Their mixtures show non-ideal behavior and are proposed as media to study probes in microenvironments similar to these encountered in vesicles and at interfaces [34]. Although *p*-dioxane consists of a set of two identical CH_2–O–CH_2 polar groups at a short distance from each other, its average dipole moment is almost zero due to the fact that the two groups oppose each other in the molecular configuration [35]. In that aspect, *p*-dioxane resembles the interior of the DNA environment in which local dipolar base pairs exist at short distances from each other. On the other hand, water is important as a natural solvent in biological systems and exists in the major and minor grooves of DNA. Combining the two solvents in a mixture, the role played by *p*-dioxane in protecting the solute molecule in the first solvation shell against the approach of proton donor species (water) resembles the stacking inside DNA which prevents the base pairs from exposure to water in the major and minor grooves of the duplex.

We then model $BP(OH)_2$ in a DNA duplex structure similar to that used for the HBO model [10], and we discuss the stability of $BP(OH)_2$ when it is incorporated into the duplex by molecular dynamics simulations. Finally, we discuss the unique spectroscopic features of $BP(OH)_2$ in water and how they can make the $BP(OH)_2$ molecule a potential probe to study tautomerization in DNA, and a possible water sensor in the DNA minor and major grooves.

2. Experimental and theoretical methods

$BP(OH)_2$ (98%) was obtained from Aldrich Chemical Co., Inc. and was used without further purification. All solvents (spectroscopy grade) were purchased from Aldrich. Millipore water was used. The concentration of $BP(OH)_2$ in solution was 10 μM. Absorption spectra were obtained with an HP 845x Diode Array spectrophotometer. Fluorescence spectra were recorded on a Shimadzu RF-5301 PC spectrofluorophotometer. In all the experiments, samples were contained in a 1 cm path length quartz cell and the measurements were performed at room temperature.

Geometry optimization and atomic charges (from electrostatic potential fitting) of $BP(OH)_2$ nucleotide were carried out using the GAMESS program [36] at the 6-31G* level of calculations. The crystal structure of $BP(OH)_2$ was used as the starting geometry [22]. The pyridyl rings of $BP(OH)_2$ remained coplanar as predicted by the crystal structure [22] and by other ab initio calculations [37,38]. The derived charges were then used for the $BP(OH)_2$ nucleotide in the molecular dynamics simulations. The latter was performed using the AMBER 8 program [39]. The Cheatham et al. force field [40,41] was used.

3. Results

3.1. Steady state spectra in different solvents

In order to fully interpret the effects of the biological environment on the spectroscopy of $BP(OH)_2$ as a model base pair it is important to understand the spectroscopy of $BP(OH)_2$ in a variety of simple solvent environments of varying polarity and hydrogen bonding capability.

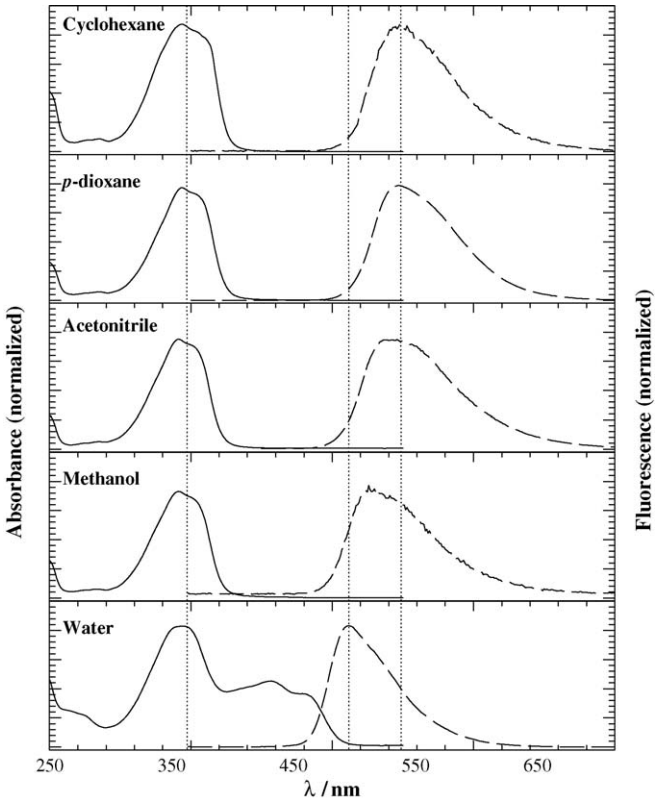


Fig. 2. Absorption (solid lines) and fluorescence (dashed lines) spectra of $BP(OH)_2$ dissolved in different solvents as indicated. The excitation wavelength was 344 nm.

The absorption and fluorescence spectra of $BP(OH)_2$ in different solvents are shown in Fig. 2 in the spectral region from 250 to 650 nm. The dielectric constants (ε) and the empirical parameters of solvent polarity (π^*) are shown in Table 1 for the solvents used here. A solvent such as *p*-dioxane, which appears to be non-polar according to its static dielectric constant ($\varepsilon = 2.21$), has a high solvent polarity parameter ($\pi^* = 0.55$). *p*-Dioxane has two CH_2–O–CH_2 groups opposite to each other which results in a net zero dipole moment. Hence, it is considered a non-dipolar solvent. However, *p*-dioxane exhibits a large quadrupole moment [35] which is reflected in the π^* parameter. In all the solvents used, the position of the absorption maximum of the DE tautomer is almost the same. This is explained by the absence of any measurable solvatochromic effect due to the very low dipole moment of the DE tautomer [23,24]. In water, the absorption spectrum shows two new peaks with maxima at 409 and 435 nm. The origin of these two peaks is due to the disruption of the intramolecular hydrogen bonds in $BP(OH)_2$ and the formation of intermolecular hydrogen bonds with water molecules (vide infra). In the fluorescence spectra, ESIDPT results in a highly fluorescent zwitterionic DK, with a large stokes shift (ca. 7500 cm^{-1} in aprotic solvents, Fig. 2). The fluorescence peak maxima are shown in Table 1 for each solvent. In aprotic solvents, the fluorescence peak position remains almost unchanged. In methanol, however, the fluorescence peak is blue-shifted by ca. 725 cm^{-1} from that in acetonitrile. Since methanol is less polar than acetonitrile, this blue-shift must derive from intermolecular hydrogen bonding interaction between $BP(OH)_2$ and methanol in the excited state potential energy surface as suggested by Marks et al. [28]. The blue-shift is more pronounced in water (ca. 1361 cm^{-1} from that in acetonitrile) due to the stronger hydrogen bonding character of water [42].

Table 1
Solvent parameters and fluorescence spectral maxima of $BP(OH)_2$

Solvent	Dielectric constant (ε) at 25 °C [42]	π^* [42]	Fluorescence spectral maximum (nm)
Cyclohexane	2.02	0.00	498
p-Dioxane	2.21	0.55	497
Acetonitrile	35.94	0.75	493
Methanol	32.66	0.60	476
Water	78.30	1.09	462

To further understand the effect of water on the steady state spectra of $BP(OH)_2$, we investigate next the spectral change in a binary mixture of *p*-dioxane/water. This mixture was chosen in order to follow the effect of changing the environment around the guest molecule from weakly polar, aproic (*p*-dioxane) to highly polar, protic (water) conditions. These solvent-induced environments resemble biological environments as mentioned earlier.

3.2. Steady state spectra in p-dioxane/water binary mixtures

The absorption spectra of $BP(OH)_2$ in the *p*-dioxane/water binary mixtures are shown in Fig. 3 for different water content. The spectra show a decrease in the absorbance intensity of the peak at 344 nm as water content increases with a concomitant

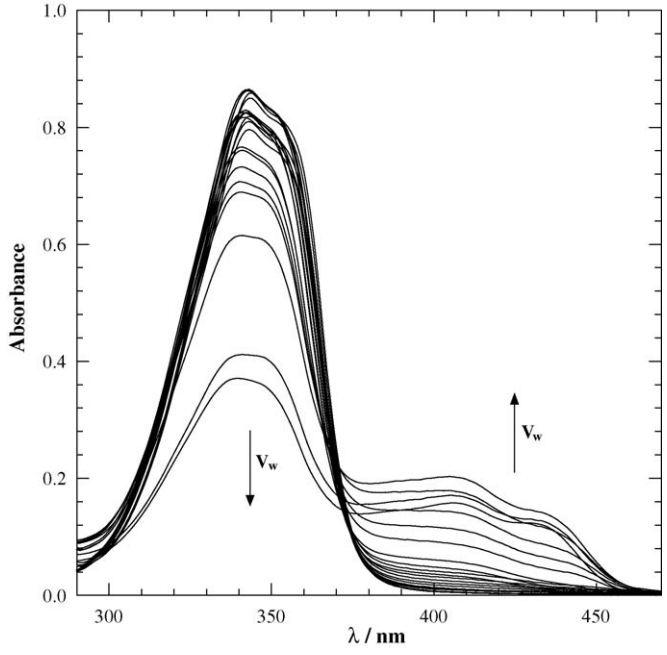


Fig. 3. Absorption spectra of $BP(OH)_2$ in *p*-dioxane/water binary mixtures. The arrows indicate increasing water content. The water volume fraction (V_w) ranged from 0 to 0.98.

appearance of new absorption in the region of 400–450 nm. A peak at 409 nm grows in intensity as water volume fraction (V_w) increases. As water content increases beyond a volume fraction of 0.65, a second peak emerges at 435 nm. An isosbestic point at 375 nm can be clearly seen up to a water volume fraction of 0.65. As the water volume fraction increases beyond 0.65, the absorption spectra no longer pass through the isosbestic point and the absorbance at 344 nm decreases dramatically. The existence of an isosbestic point is likely due to the presence of one equilibrium reaction between $BP(OH)_2$ and water, producing the $BP(OH)_2{:}(H_2O)_x$ complex as observed in other systems [43], where x is the number of water molecules in the complex. At water volume fractions higher than 0.65, another equilibrium starts to emerge due to more water molecules (y) being added to the above complex. These two equilibria are expressed as:

$$BP(OH)_2 + xH_2O \rightleftharpoons BP(OH)_2{:}\,(H_2O)_x \quad (1)$$

$$BP(OH)_2{:}\,(H_2O)_x + yH_2O \rightleftharpoons BP(OH)_2{:}\,(H_2O)_{x+y} \quad (2)$$

The molecular structure of $BP(OH)_2$ suggests that the first equilibrium is due to the solvation of one of the two hydrogen bonding centers in $BP(OH)_2$ by water, while the other equilibrium results in solvation of the second center.

For the above model of solvation to be valid, we should consider the following assumption. Increasing the water content changes the absorption spectra (and the fluorescence spectra, vide infra). This change is due to local solvating water molecules (x and y in Eqs. (1) and (2)) interacting with the hydrogen bonding centers. In this case, water molecules in the first solvation shell will only participate by certain x and y numbers and the rest of the water molecules will have the same effect as the bulk water molecules.

Solvation of $BP(OH)_2$ by water molecules may be represented by the following relationship:

$$\frac{[BS_n]}{[B]} = K_{eq}[S]^n \quad (3)$$

where $[BS_n]$ represents the concentration of solvated $BP(OH)_2$ by water molecules, [B] the concentration of unsolvated $BP(OH)_2$, [S] the water concentration, n represents the total number of water molecules in the first solvation shell involved in solvating the two hydrogen bonding centers ($x+y$), and K_{eq} is the total equilibrium constant. An expression relating the relative concentrations to the observed absorbance (A_{obs}), and to the absorbances of solvated $BP(OH)_2$ (A_{BS_n}) and unsolvated $BP(OH)_2$ (A_B) is given by [44]:

$$\frac{[BS_n]}{[B]} = \frac{A_{obs} - A_B}{A_{BS_n} - A_{obs}} \quad (4)$$

Combining Eqs. (3) and (4), one obtains the expression:

$$A_{obs} = \frac{A_B + K_{eq}A_{BS_n}[S]^n}{1 + K_{eq}[S]^n} \quad (5)$$

Eq. (5) (often referred to as "binding isotherm") can be used to describe the observed change in the absorbance as a function of water concentration. As shown in Fig. 3, the change in the peak intensity at 344 nm and the fact that there is no solvatochromic shift make it possible to apply Eq. (5) to describe this change as a function of water concentration. It should be mentioned here that if more than one species exist in the ground state in which they contribute to the overall intensity at 344 nm, this may complicate the quantitative analysis using Eq. (5) to extract the total number of solvating water molecules (n). Johansson et al. varied the temperature of a solution of $BP(OH)_2$ solvated in water [45]. As the temperature was elevated, they detected an increase in the peak intensity at 344 nm, a decrease in the intensity of the peaks at 400–450 nm, and the existence of an isosbestic point. They also measured only one magnitude and one direction of the transition dipole moments for these peaks. The authors indicated that the results are compatible with only one species of a given symmetry, the ground state of which has two minima. The presence of the two minima is due to interactions with the solvent molecules. Accordingly, the decrease in the peak intensity at 344 nm seems to be a good measure for how water solvates the $BP(OH)_2$ molecule.

A plot of the absorbance change at 344 nm as a function of water volume fraction shows asymptotic behavior at low water content which starts to fall off sharply at water volume fraction around 0.65. The data are shown in Fig. 4 along with the non-linear least-squares fit to Eq. (5). The best fit yields $n = 6.15$ which suggests that six water molecules are needed in order to solvate the $BP(OH)_2$ molecule. Giving the symmetrical geometry of $BP(OH)_2$, three water molecules can be assumed to solvate each hydrogen bonding center (i.e. $x = y = 3$ in Eqs. (1) and (2)). Using ab initio and semiempirical methods, Carballeira and Perez-Juste found that two water molecules interact with each

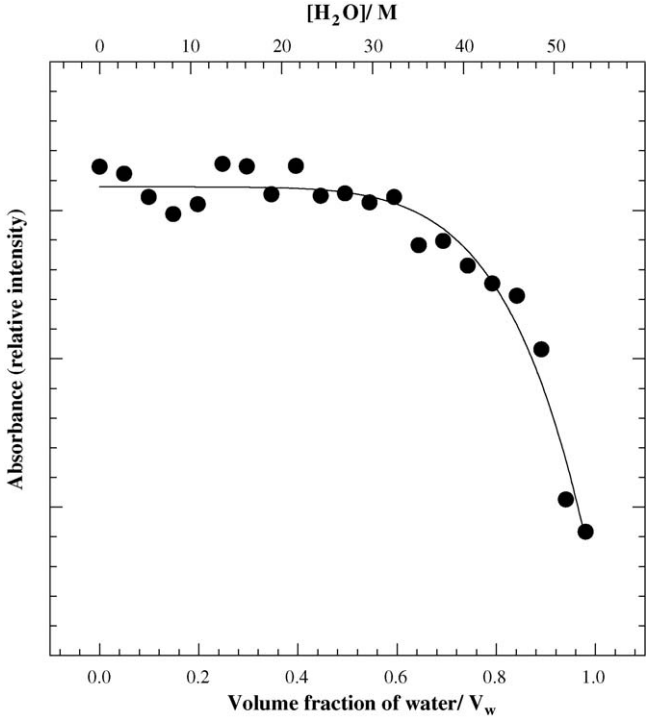


Fig. 4. Absorbance change at 344 nm for $BP(OH)_2$ as a function of water volume fraction (bottom axis) and water concentration (top axis) in the p-dioxane/water binary mixtures. The solid line is the best non-linear least-squares fit to Eq. (5).

hydrogen bonding center of $BP(OH)_2$ [38]. In other systems, the presence of water was found to disrupt the intramolecular hydrogen bond in the Piraxicam drug by forming a complex between Piraxicam and 2.5 ± 0.2 water molecules [43]. Lee et al. found that proton dissociation from 1- and 2-naphthol (with only one proton transfer center) depends on the presence of a specific water cluster composed of a minimum of four water molecules [46]. Similar results were also observed for 1-naphthol-2-sulfonic acid (possessing an intramolecular hydrogen bond) in which proton dissociation is suggested to be controlled by reorientational motions of the adjacent water molecules and requires a common $(H_2O)_{4\pm1}$ cluster as the proton acceptor [47].

The ability of *p*-dioxane/water binary mixture to donate hydrogen bonds is evaluated, using Kamlet–Taft parameter α, to be very poor for lower water concentrations [43,48,49]. Above 25 M concentration of water, this ability increases rapidly and approaches the behavior in pure water. This is manifested in Fig. 4 in which the sharp decline in the absorption intensity of the 344 peak starts at water concentration between 25 and 30 M.

Different levels of calculations show that solvation of $BP(OH)_2$ by water slightly stabilizes the DK tautomer with respect to the DE tautomer in the ground state [32,38]. This can be explained by the zwitterionic nature of the DK tautomer which is more favorable in polar solvents. The first solvation step represented by Eq. (1) is not expected to produce the MK tautomer. This is because of the absence of any solvatochromic shifts in the absorption spectra of the peak at 409 nm as a function of increasing water content. Hence, the two peaks at 409 and 435 nm observed here in water are due to the solvated DE tautomer or the solvated DK tautomer in the ground state.

The steady state fluorescence spectra were recorded for the same binary mixtures after excitation at 344 nm and are shown in Fig. 5. By increasing the water content, an increase in the fluorescence peak intensity is observed accompanied by a blue-shift. An isoemissive point at 512 nm exists in the range of water volume fractions from 0.1 to 0.65. The fluorescence spectral intensity decreases and the spectra no longer pass through the isoemissive point for volume fractions of water larger than 0.65, confirming the presence of one equilibrium at water volume fractions up to 0.65 and two equilibria at higher water content. The inset in Fig. 5 shows the change in the fluorescence peak intensity as a function of water volume fraction. As shown in Fig. 5, no new peaks were observed in the fluorescence spectra as the water content increases. Changing the excitation wavelength has no effect on the fluorescence band shape. This indicates the existence of one tautomer in the excited state which is the DK tautomer. From the time-resolved fluorescence measurements, Rurack et al. [50] showed that upon excitation (in neutral water) the different Franck–Condon tautomers undergo rapid transformation to produce the emissive DK tautomer. The existence of only one fluorescence peak indicates also that there are no anion products and that the protons stay intact with the $BP(OH)_2$ moiety [51]. This is in agreement with the one major decay (≥98%) parameter observed in the time resolved fluorescence measurements of $BP(OH)_2$ in water [50].

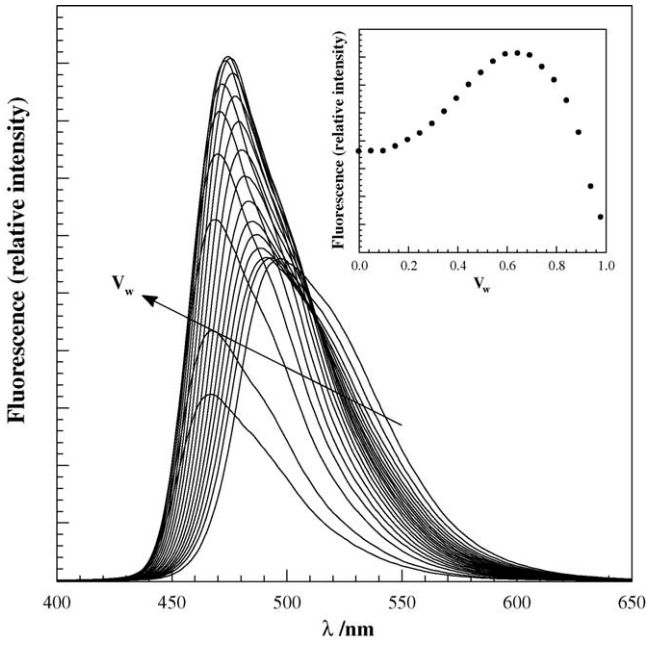


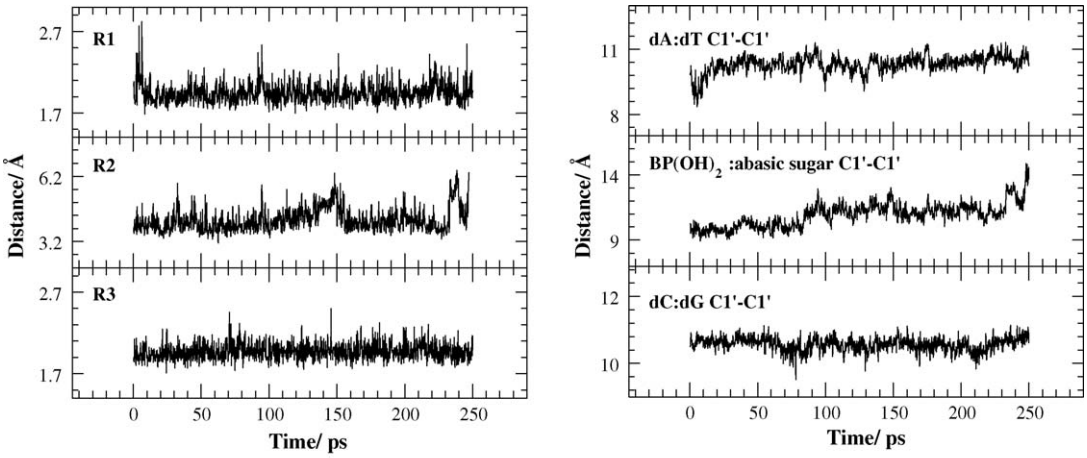
Fig. 5. Fluorescence spectra of $BP(OH)_2$ in *p*-dioxane/water binary mixtures. The arrow indicates increasing water content. The water volume fraction (V_w) ranged from 0 to 0.98. The inset shows the fluorescence peak intensity change of $BP(OH)_2$ as a function of increasing water volume fraction in the *p*-dioxane/water binary mixtures.

Volume fractions of water higher than 0.85 result in a slight decrease in the intensities of the absorption peaks at 409 and 435 nm (Fig. 3). This observation may be attributed to the formation of water aggregates around the $BP(OH)_2$ molecule. Similar effects were observed in the fluorescence spectra after excitation at 409 or 435 nm (data are not shown).

3.3. Molecular dynamics simulations

Modeling $BP(OH)_2$ inside a DNA duplex was performed by conducting molecular dynamics simulations using AMBER 8 program [39]. The DNA structure used in the simulations was similar to that reported for the HBO model [10], by replacing the HBO molecule by the $BP(OH)_2$ molecule. The resulted duplex structure was composed of the oligonucleotide "5′-CGTTTCXTTCTC", where X represents the position of the $BP(OH)_2$ molecule in the dodecamer. The complementary oligonucleotide contains an abasic site located at the position opposite to $BP(OH)_2$. Geometry optimization at the 6-31G* level was carried out from the starting conformation of the $BP(OH)_2$ nucleotide including the base and the sugar. Electrostatic potential fit to atomic centers was used to derive the charges. The atomic charges used for $BP(OH)_2$ nucleotide are included in the supplementary materials (Fig. S1). The derived charges for $BP(OH)_2$ were then used in the molecular dynamics simulations. The equilibrium bond and angle terms in simulations were based on the geometry optimized structure, with force constants approximated from the Cheatham et al. force field [40,41].

The duplex DNA was then solvated in a box of explicit water molecules with neutralizing sodium counter ions. The DNA



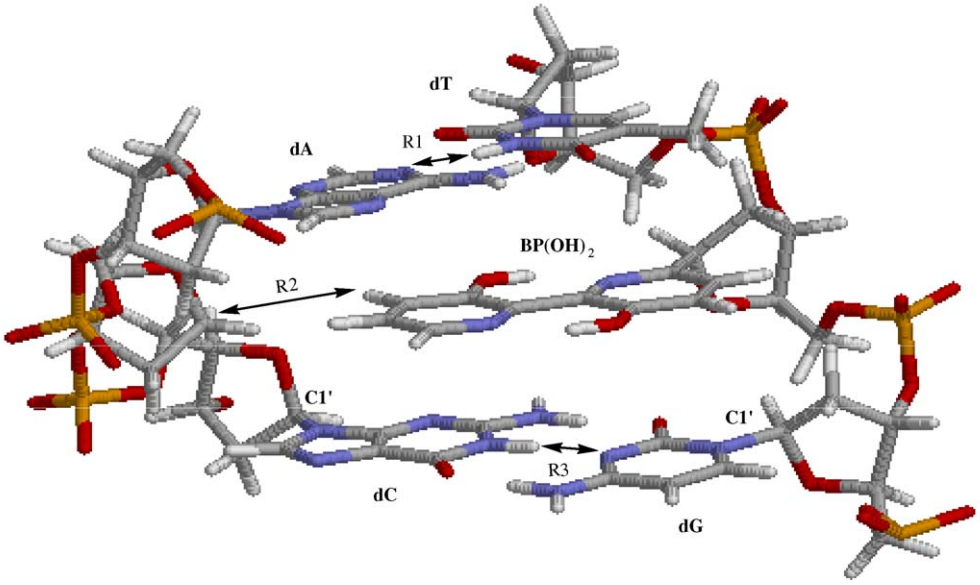


Fig. 6. Distance fluctuations in duplex DNA containing $BP(OH)_2$ paired opposite an abasic site over the final 250 ps of molecular dynamics simulations. Distances R1, R2, and R3 (upper left panel) correspond to those labeled in the figure. The C1′–C1′ distances (upper right panel) correspond to the distances between the C1′ sugar carbons at the model and at the flanking base pairs.

structure was first minimized, followed by water and sodium ion equilibration. A 2 ns constant volume production run was then executed at 300 K. The trajectories were stable and predict that the DNA remains in a double helix structure. Most importantly, all the base pairs, including those flanking $BP(OH)_2$, were not fluctional and remained H-bonded throughout the simulations. Fig. 6 shows the distance fluctuations of $BP(OH)_2$ from its paired abasic site and the fluctuations in the hydrogen bonding distances in the flanking base pairs over the final 250 ps of the simulations. $BP(OH)_2$ itself is seen to undergo limited fluctuations during simulations. The motion corresponds to slipping of $BP(OH)_2$ within the duplex and probably results from the absence of a covalent linkage between $BP(OH)_2$ and the abasic furan ring. Regardless, $BP(OH)_2$ remains intercalated throughout the simulations with its unconnected pyridine ring well stacked between the flanking bases of the opposite strand. The only other significant distortion which occurred in $BP(OH)_2$ is shown in the C1′–C1′ distance in Fig. 6 in which the duplex shows a slight (∼15%) widening of the minor groove. This is again due to the covalent linkage connecting $BP(OH)_2$ to only one strand. Overall, the simulations predict that the structure of duplex DNA containing the model base pair is not significantly perturbed and imply that $BP(OH)_2$ paired opposite an abasic site is a good mimic of a natural base pair. The average structure of duplex DNA over the entire 2 ns simulations is shown in Fig. 7 with the hydrogen atoms removed for clarity. In this structure, the long axis of the molecular plan of the $BP(OH)_2$ molecule makes an angle of 85° with respect to the helical long axis. The short axis of the $BP(OH)_2$ molecular plan is almost perpendicular to the helical long axis. This geometry positions one of the hydrogen bonds of $BP(OH)_2$ in the major groove and the other one in the minor groove of the DNA. The final 250 ps of the simulations for all the base pairs are included in the supplementary materials (Figs. S2 and S3).

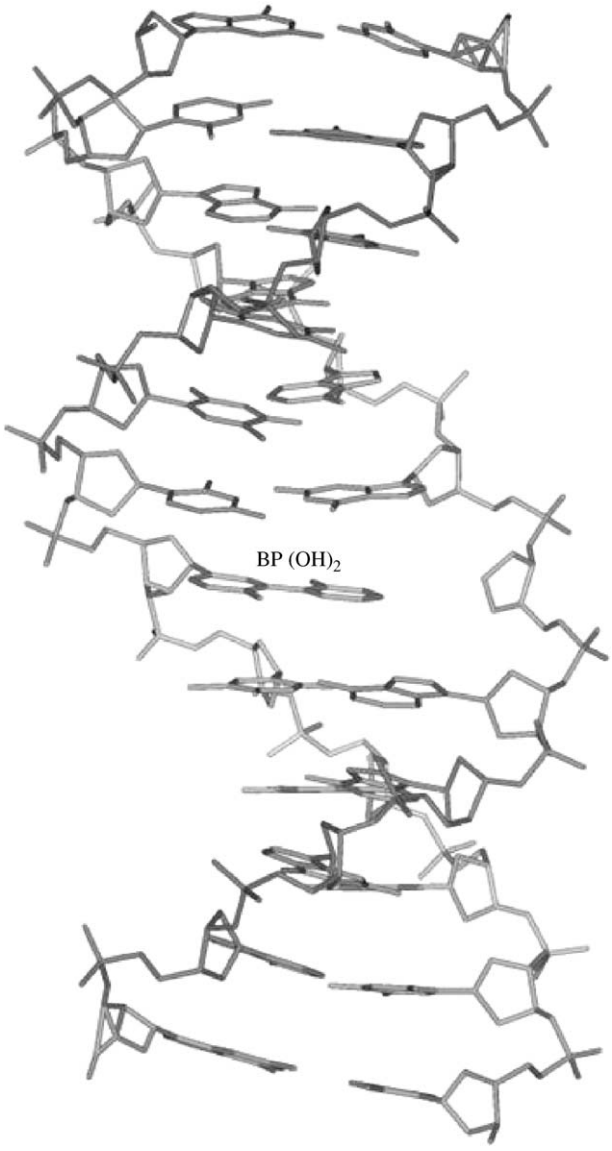


Fig. 7. The average structure of the dodecamer duplex containing the $BP(OH)_2$ model opposite an abasic site over the entire 2 ns of molecular dynamics simulations. Hydrogen atoms were removed for clarity.

4. Discussion

The excited state proton transfer reaction in $BP(OH)_2$ occurs along the H-bonds and was found to depend strongly on the coplanarity of the molecular system. By employing different levels of theoretical calculations, both the DE and DK tautomers were predicted to be planar in S_0 and S_1 states [30–32,38]. The deriving force of this reaction was reported to be due to a charge redistribution in the excited state in which the nitrogen heteroatoms become more positively charged and the oxygen atoms of the two hydroxyl groups become negatively charged, leading to a zwitterionic DK [38]. The new N^+–H···O^- hydrogen bonds in DK (see Fig. 1) are expected to restrain rotation around the inter-ring bond which keeps the molecular structure planar for the DK tautomer. Fig. 2 shows that, unlike in other intramolecular hydrogen bonded systems [12], no primary fluorescence was observed from the absorbing DE tautomer of $BP(OH)_2$ even in polar protic solvents. This observation, along with the coplanarity of the DK tautomer, explains the efficient fluorescence from the excited DK tautomer [15,18–21].

As a model base pair, $BP(OH)_2$ shows promise based on the following facts. $BP(OH)_2$ can be selectively excited in the presence of natural bases because its absorption bands occur at longer wavelengths. The molecular size of $BP(OH)_2$ is smaller than any combined natural base pairs. Thus, if $BP(OH)_2$ is incorporated in a DNA duplex from the carbon position shown in Fig. 1 pairing with an abasic site, only minimum perturbation is expected to the overall stability of the duplex as shown by the molecular dynamics simulations presented above. Also, the coplanarity of the $BP(OH)_2$ molecule discussed above is important in minimizing the effect of structural perturbation inside a DNA duplex, and is expected to stabilize the duplex helical structure by providing through-space interactions with the flanking bases. The two intramolecular hydrogen bonds in the $BP(OH)_2$ molecule make it a better mimic of a natural base pair than, for example, HBO [10]. Furthermore, the average structure of the simulations presented in Fig. 7 shows that the geometry of the $BP(OH)_2$ molecule in the duplex positions one hydrogen bond in the minor groove and the other one in the major groove, thus providing a good probing of the fluctional motion of DNA. The change in the dipole moment for only the MK tautomer may preferentially stabilize this form in a DNA duplex due to a selective interaction with the local dipoles of the flanking base pairs. Also, the structures of the hydrogen bonds in the DK tautomer and one of the two hydrogen bonds in the MK tautomer of $BP(OH)_2$ show a better alignment with those of the flanking base pairs than in the case of the DE tautomer (see Fig. 1). This may act as a probe of through-space interactions between the hydrogen bonding centers of the base pairs in DNA. In addition, the structure of the DK tautomer in the excited state resembles the ground state structure of the keto-amine natural base pair tautomers (Fig. 1). If through-space interactions dominate in duplex DNA, this may stabilize the DK tautomer in the excited state and eventually increase its lifetime as compared to its lifetime in solution. In the HBO model base pair, the keto lifetime in DNA increased 17-fold over the keto lifetime in *n*-hexane [11]. In the case of $BP(OH)_2$, the presence of two hydrogen bonds may induce more effect on the DK tautomer lifetime in DNA.

The spectral changes due to water solvation reveal important features for $BP(OH)_2$ as a model base pair. The absorption peaks due to water solvation in the ground state (see Fig. 2) may act as a probe of how $BP(OH)_2$ will orient itself inside DNA. Since the two peaks at 409 and 435 nm are due to water complexes with $BP(OH)_2$, these peaks can be used as a probe of the accessibility of the $BP(OH)_2$ molecule to water in the minor and major grooves. The difference in the absorption behavior after solvation of each hydrogen bonding center in $BP(OH)_2$ (Fig. 3) will also make it possible to distinguish water accessibility from one or both grooves in DNA. Furthermore, the large blue-shift (36 nm) in the fluorescence peak of the DK tautomer in going from a non-polar, aprotic solvent (cyclohexane) to a strongly polar, protic solvent (water) makes $BP(OH)_2$ a unique probe in biological systems using fluorescence measurements.

Finally, the size effect of the $BP(OH)_2$ molecule on the stability of DNA duplex deserves mentioning. From the molecular dynamics simulations presented here, no major perturbation is caused to the helical structure by the presence of $BP(OH)_2$. Increasing the molecular size by substituting a side chain in $BP(OH)_2$ is a possible mechanism to maximize interactions with the flanking bases. Borowicz et al. [52,53] reported that simple methyl substitution in one or more positions in $BP(OH)_2$ leaves the photophysics of the double-proton transfer mechanism almost unchanged.

5. Conclusions

The steady state absorption and fluorescence spectra of $BP(OH)_2$ were studied in solvents of varying polarity and hydrogen bonding capability. In all the solvents studied here, no solvatochromic shift was observed in the absorption spectra. In water, solvation of the hydrogen bonding centers of $BP(OH)_2$ results in two new absorption peaks in the red edge of the absorption spectrum. A blue-shift in fluorescence was only observed in polar, protic solvents due to intermolecular hydrogen bonding between $BP(OH)_2$ and the solvent. In *p*-dioxane/water binary mixtures, the intensities of the two new absorption peaks are enhanced and the fluorescence peak of the DK tautomer shows a blue-shift as the water content increases. The data were analyzed using a binding isotherm model. The analysis shows that six water molecules are needed to solvate the two hydrogen bonding centers in $BP(OH)_2$. Given the symmetrical structure of the $BP(OH)_2$ molecule, three water molecules solvate each center. Molecular dynamics simulations were performed for a duplex DNA containing $BP(OH)_2$ as a model base pair. The results of the simulations indicate that $BP(OH)_2$ is a good mimic of a natural base pair with no major perturbation to the stability of the helical structure. One of the two hydrogen bonds in $BP(OH)_2$ resides in the major groove of the duplex DNA, whereas the other one is situated in the minor groove. The unique spectroscopic features in water as a solvent make $BP(OH)_2$ a potential probe for tautomerization in DNA and a possible sensor for water accessibility in the major and minor grooves of DNA. It is anticipated that the work described here may serve as a stimulus to incorporate this model in DNA for such study.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jphotochem.2006.02.015.

References

[1] J.D. Watson, F.H.C. Crick, Nature 171 (1953) 964–967.
[2] M.D. Topal, J.R. Fresco, Nature 263 (1976) 285–289.
[3] J.W. Drake, R.H. Baltz, Annu. Rev. Biochem. 45 (1976) 11–37.
[4] N.K. Sinha, M.D. Haimes, J. Biol. Chem. 256 (1981) 10671–10683.
[5] H. Nishio, A. Ono, A. Matsuda, T. Ueda, Nucleic Acids Res. 20 (1992) 777–782;
P. Strazewski, C. Tamm, Angew. Chem., Int. Ed. 29 (1990) 36–57;
B. Singer, F. Chavez, M.F. Goodman, J.M. Essigmann, M.K. Dosanjh, Proc. Natl. Acad. Sci. U.S.A. 86 (1989) 8271–8274.
[6] J.W. Drake, The Molecular Basis of Mutation, San Francisco, Holden-Day, 1970;
C. Auerbach, Mutations Research. Problems, Results and Perspectives, Chapman and Hall, London, 1976;
H. Echols, M.F. Goodman, Annu. Rev. Biochem. 60 (1991) 477–511.
[7] S.M. Ormson, R.G. Brown, Prog. React. Kinet. 19 (1994) 45–91.
[8] D. Le Gourrierec, M.S. Ormson, R.G. Brown, Prog. React. Kinet. 19 (1994) 211–275.
[9] E.T.J. Nibbering, T. Elsaesser, Chem. Rev. 104 (2004) 1887–1914.
[10] A.K. Ogawa, O.K. Abou-Zied, V. Tsui, R. Jimenez, D.A. Case, F.E. Romesberg, J. Am. Chem. Soc. 122 (2000) 9917–9920.
[11] O.K. Abou-Zied, R. Jimenez, F.E. Romesberg, J. Am. Chem. Soc. 123 (2001) 4613–4614.
[12] O.K. Abou-Zied, R. Jimenez, E.H.Z. Thompson, D.P. Millar, F.E. Romesberg, J. Phys. Chem. A 106 (2002) 3665–3672.
[13] H. Wang, H. Zhang, O.K. Abou-Zied, C. Yu, F.E. Romesberg, M. Glasbeek, Chem. Phys. Lett. 367 (2003) 599–608.
[14] C.A. Taylor, M.A. El-Bayoumi, M. Kasha, Proc. Natl. Acad. Sci. U.S.A. 63 (1969) 253–260.
[15] H. Bulska, Chem. Phys. Lett. 98 (1983) 398–402.
[16] K. Rurack, R. Radeglia, Eur. J. Inorg. Chem. (2000) 2217–2282;
Ł. Kaczmarek, B. Zagrodzki, B. Kamieński, M. Pietrzak, W. Schilf, A. Leś, J. Mol. Struct. 553 (2000) 61–72;
J. Sitkowski, L. Stefaniak, Ł. Kaczmarek, G.A. Webb, J. Mol. Struct. 385 (1996) 65–67.
[17] P. Borowicz, A. Leś, L. Adamowicz, J. Waluk, Photochem. Photobiol. Sci. 4 (2005) 143–148.
[18] H. Bulska, A. Grabowska, Z.R. Grabowski, J. Lumin. 35 (1986) 189–197.
[19] J. Sepiol, H. Bulska, A. Grabowska, Chem. Phys. Lett. 140 (1987) 607–610.
[20] M. Kaschke, S. Rentsch, J. Opfermann, Laser Chem. 8 (1988) 377–384.
[21] J. Sepiol, A. Grabowska, H. Bulska, A. Mordzinski, F. Perez Salgado, R.P.H. Rettschnick, Chem. Phys. Lett. 163 (1989) 443–448.
[22] J. Lipkowski, A. Grabowska, J. Waluk, G. Calestani, B.A. Hess, J. Crystallogr. Spectrosc. Res. 22 (1992) 563–572.
[23] R. Wortmann, K. Elich, S. Lebus, W. Liptay, P. Borowicz, J. Phys. Chem. 96 (1992) 9724–9730.
[24] P. Borowicz, A. Grabowska, R. Wortmann, W. Liptay, J. Lumin. 52 (1992) 265–273.
[25] H. Zhang, P. van der Meulen, M. Glasbeek, Chem. Phys. Lett. 253 (1996) 97–102.
[26] D. Marks, P. Prosposito, H. Zhang, M. Glasbeek, Chem. Phys. Lett. 289 (1998) 535–540.
[27] P. Prosposito, D. Marks, H. Zhang, M. Glasbeek, J. Phys. Chem. A 102 (1998) 8894–8902.
[28] D. Marks, H. Zhang, M. Glasbeek, P. Borowicz, A. Grabowska, Chem. Phys. Lett. 275 (1997) 370–376.
[29] P. Toele, H. Zhang, M. Glasbeek, J. Phys. Chem. A 106 (2002) 3651–3658.
[30] V. Barone, C. Adamo, Chem. Phys. Lett. 241 (1995) 1–6.
[31] A.L. Sobolewski, L. Adamowicz, Chem. Phys. Lett. 252 (1996) 33–41.
[32] V. Barone, A. Palma, N. Sanna, Chem. Phys. Lett. 381 (2003) 451–457.
[33] R. Gelabert, M. Moreno, J.M. Lluch, Chem. Phys. Chem. 5 (2004) 1372–1378.
[34] M. Belletête, G. Lessard, G. Durocher, J. Lumin. 42 (1989) 337–347;
M. Belletête, M. Lachapelle, G. Durocher, J. Phys. Chem. 94 (1990) 5337–5341;
E.C.C. Melo, S.M. Costa, A.L. Maçanita, H. Santos, J. Colloid Interface Sci. 141 (1991) 439–453.

[35] P. Suppan, Photochem. Photobiol. A 50 (1990) 293–330;
P. Suppan, J. Lumin. 33 (1985) 29–32.
[36] M.W. Schmidt, K.K. Baldridge, J.A. Boatz, S.T. Elbert, M.S. Gordon, J.H. Jensen, S. Koseki, N. Matsunaga, K.A. Nguyen, S.J. Su, T.L. Windus, M. Dupuis, J.A. Montgomery, J. Comput. Chem. 14 (1993) 1347–1363.
[37] A. Mordzinski, K. Kownacki, A. Les, N.A. Oyler, L. Adamowicz, F.W. Langkilde, R. Wilbrandt, J. Phys. Chem. 98 (1994) 5212–5220.
[38] L. Carballeira, I. Perez-Juste, J. Mol. Struct. (Theochem.) 368 (1996) 17–25.
[39] D.A. Case, T.A. Darden, T.E. Cheatham, III, C.L. Simmerling, J. Wang, R.E. Duke, R. Luo, K.M. Merz, B. Wang, D.A. Pearlman, M. Crowley, S. Brozell, V. Tsui, H. Gohlke, J. Mongan, V. Hornak, G. Cui, P. Beroza, C. Schafmeister, J.W. Caldwell, W.S. Ross, P. A. Kollman, AMBER 8: Univeristy of California, San Francisco, 2004.
[40] T.E.I. Cheatham, P. Cieplak, P.A. Kollman, J. Biomol. Struct. Dyn. 16 (1999) 845–862.
[41] W.D. Cornell, P. Cieplak, C.I. Bayly, I.R. Gould, K.M.J. Merz, D.M. Ferguson, D.C. Spellmeyer, T. Fox, J.W. Caldwell, P.A. Kollman, J. Am. Chem. Soc. 117 (1995) 5179–5197.
[42] C. Reichardt, Solvents and Solvent Effects in Organic Chemistry, VCH, Weinheim, 1988.
[43] S.M. Andrade, M.B. Costa, Phys. Chem. Chem. Phys. 1 (1999) 4213–4218.
[44] K.A. Connors, Binding Constants. The Measurements of Molecular Complex Stability, Wiley, New York, 1987.
[45] L.B.-A. Johansson, L. Persson, H. Langhals, J. Chem. Soc., Faraday Trans. 92 (1996) 4909–4911.
[46] J. Lee, G.W. Robinson, S.P. Webb, L.A. Philips, L.H. Clark, J. Am. Chem. Soc. 108 (1986) 6538–6542;
J. Lee, J. Am. Chem. Soc. 111 (1989) 427–431.
[47] R. Krishnan, T.G. Fillingim, J. Lee, G.W. Robinson, J. Am. Chem. Soc. 112 (1990) 1353–1357.
[48] E. Casassas, G. Fonrodona, A. de Juan, J. Solution Chem. 21 (1992) 147–162.
[49] W.J. Cheong, P.W. Carr, Anal. Chem. 60 (1988) 820–826.
[50] K. Rurack, K. Hoffmann, W. Al-Soufi, U. Resch-Genger, J. Phys. Chem. B 106 (2002) 9744–9752.
[51] J. Lee, R.D. Griffin, G.W. Robinson, J. Chem. Phys. 82 (1985) 4920–4925;
L.M. Tolbert, L.C. Harvey, R.C. Lum, J. Phys. Chem. 97 (1993) 13335–13340.
[52] P. Borowicz, A. Grabowska, R. Wortmann, W. Liptay, J. Lumin. 52 (1992) 265–273.
[53] P. Borowicz, A. Grabowska, A. Leś, Ł. Kaczmarek, B. Zagrodzki, Chem. Phys. Lett. 291 (1998) 351–359.