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ORIGINAL ARTICLE

Influence of Solvent Polarizability on the Keto-Enol Equilibrium in 4-[5-(naphthalen-1-ylmethyl) -1,3,4-thiadiazol-2-yl]benzene-1,3-diol

Arkadiusz Matwijczuk[1,4] · Andrzej Górecki[3] · Daniel Kamiński[2] · Beata Myśliwa-Kurdziel[4] · Leszek Fiedor[4] · Andrzej Niewiadomy[2] · Grzegorz P. Karwasz[5] · Mariusz Gagoś[6]

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Abstract This work presents spectroscopic studies of the keto–enol equilibrium induced by solvent polarizability in 4-[5-(naphthalen-1-ylmethyl)-1,3,4-thiadiazol-2-yl]benzene-1,3-diol a strong antiproliferative and anticancer thiadiazol derivative. Electronic absorption, steady state and time resolved fluorescence, and infrared spectroscopies were applied to investigate the keto and enol forms of this compound in a series of polar and non-polar solvents. The enol form dominates in polar solvents while, surprisingly, the keto form dominates in non-polar solvents with high average electric dipole polarizability e.g. *n*-alkenes. The electronic absorption spectrum of this derivative is more dependent on spatially averaged electric dipole polarizability of the solvent than on Kirkwood's correlation or on Lorenz-Lorenz electric polarizability. By analogy of *n*-alkanes to the alkyl parts of lipids, one can expect that the transformation of 1,3,4-thiadiazoles to the keto form may be facilitated in the hydrophobic core of the lipid membrane. Such a transition may be of great practical importance for the design of biologically active pharmaceutics, which are able to interact with the hydrophobic regions of cell membranes in a specific manner.

Keywords 1,3,4-thiadiazoles · Intramolecular proton transfer · Keto-enol tautomery · Molecular spectroscopy

✉ Arkadiusz Matwijczuk
arekmatwijczuk@gmail.com

✉ Mariusz Gagoś
mariusz.gagos@poczta.umcs.lublin.pl

[1] Department of Biophysics, University of Life Sciences in Lublin, 20-950 Lublin, Poland

[2] Department of Chemistry, University of Life Sciences in Lublin, 20-950 Lublin, Poland

[3] Department of Physical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

[4] Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland

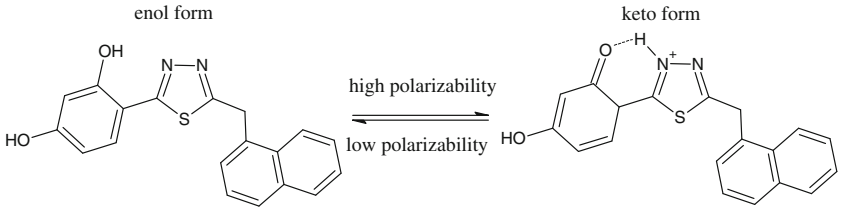
[5] Aleksander Jabłoński Institute of Physics, Nicolaus Copernicus University, 87-100 Toruń, Poland

[6] Department of Cell Biology, Institute of Biology and Biochemistry, Maria Curie-Skłodowska University, 20-033 Lublin, Poland

Introduction

1,3,4-thiadiazoles exhibit promising antiproliferative and anticancer properties [1–6], and as such, along with various similar systems, are recently a subject of intense research. However, the molecular mechanisms related to their biological activity are not entirely known. The electronic absorption and Fourier Transform Infrared Spectroscopy (FTIR) studies showed that the keto forms of these compounds are present in alkane solvents [7]. Also a new compound of this type, 4-[5-(naphthalen-1-ylmethyl)-1,3,4-thiadiazol-2-yl]benzene-1,3-diol (NTBD) shows keto-enol equilibrium which can be induced by e.g. solvent polarizability (not polarity) and temperature [8–10]. These two observed forms (keto and enol) of NTBD may differently interact with biological systems. Therefore, a detailed study of the environment polarizability on NTBD and other 1,3,4-thiadiazoles may help in understanding molecular mechanisms underlying its biological activity.

In a wide variety of molecules in which an intramolecular hydrogen bond may occur, the keto form is usually dominant in polar solvents. The enol form is favored in non-polar solvents, where it is stabilized by the intramolecular hydrogen



Scheme 1 Chemical structures of 4-[5-(naphthalen-1-ylmethyl)-1,3,4-thiadiazol-2-yl]benzene-1,3-diol (NTBD, enol and keto form)

bond [11]. Opposite effects were reported for some Schiff's bases, which have a more polarized keto form dominant in non-polar solvents [12, 13] or other systems [14]. In some cases, the rearrangement can be induced thermally and may lead to a change in the color (termochromism). For example, the enol form can be yellow while the keto one - colorless [11].

This work presents experimental an study on NTBD, a compound from the 1,3,4-thiadiazole family (Scheme 1) composed of a 1,3,4-thiadiazole ring bonded to the resorcine ring with different substituents in position 4 of the thiadiazole ring [15, 16]. In this study, the electronic absorption, steady state and time resolved fluorescence, and FTIR spectroscopies were applied to investigate the keto-enol equilibrium of NTBD in a series of polar and non-polar solvents. In the case of e.g. *n*--hexane or *n*-heptane non-polar solvents with high *spatially averaged electric dipole polarizability* (further abbreviated polarizability) values, the electronic absorption spectra exhibit a band with the maximum ~273 nm (related to the keto form) and a much weaker one with the maximum ~320 nm (typical for enol forms). In polar solvents, the band with the maximum at 273 nm decreases and a very intensive band with the maximum at ~320 nm appears. Similar to previous investigation of (2-(4-fluorophenylamino)-5-(2,4-dihydroxybenzeno)-1,3,4-thiadiazole) (FABT) [7], this study shows that also changes in the electronic absorption and fluorescence spectra of NTBD depend on the *electric dipole polarizability* of the solvent rather than on the dielectric constant-related Kirkwood's correlation ε ((ε-1)/(2ε + 1)) or on the refractive index-related Lorenz-Lorenz function n ($(n^2-1/n^2 + 2)$) [7]. Additionally, we show that the observed fluorescence lifetimes for NTBD also depend on the solvent polarizability.

Materials and Methods

Synthesis of NTBD 4-[5-(Naphthalen-1-ylmethyl)-1,3,4-thiadiazol-2-yl]benzene-1,3-diol (NTBD) with molecular weight of 334,39 g/mol used in this study (Scheme 1A) was synthesized in the Department of Chemistry of the University of Life Sciences in Lublin [4].

Table 1 Position of electronic absorption maxima compared to the average dipole molecular polarizability, dielectric constant ε, index of refraction *n*, dipole moment μ of the solvents [19]. The solvents are ordered following the rising value of the polarizability α

	Solvents		λ [nm]		α	ε	n	μ
			Enol	Keto	[10^{-24} cm^3]			[debye]
Polar	1	Water	329	–	1.45	80.1	1.3333	1.855
	2	Methanol	321	–	3.29	33	1.3265	1.700
	3	Acetonitrile	322	–	4.40	36.64	1.3416	3.925
	4	Ethanol	322	–	5.41	25.3	1.3594	1.690
	5	Acetone	326	–	6.33	21	1.3587	2.880
	6	DMSO	319	–	7.30	47.24	1.4773	3.960
	7	Propan-2-ol	324	–	7.61	20.18	1.3772	1.580
	8	Chloroform	327	–	9.50	4.81	1.4429	1.040
Non-polar	9	Pentane	325	274	9.99	1.84	1.3575	0.130
	10	Benzene	326	–	10.00	2.28	1.5011	0
	11	Cyclohexane	325	277	11.00	2.02	1.4262	0
	12	Tetrachloromethane	327	281	11.30	2.24	1.4631	0
	13	*n*-hexane	327	273	11.90	1.89	1.3723	0
	14	*n*-heptane	327	273	13.60	1.92	1.3876	0
	15	Octane	326	276	15.90	1.948	1.3947	0
	16	Undecane	325	274	21.03	1.997	1.4147	0
	17	Dodecane	326	274	22.75	2.012	1.4186	0

The product was purified by means of HPLC on a YMC C-30 column (250 mm, diameter of 4.6 mm). A mixture of acetonitrile:CH_3OH:H_2O (72:8:3 *v/v*) was applied as the mobile phase. The NTBD was recrystallized from 99.8 % methanol shortly before use. In order to remove methanol traces, the samples were dried under vacuum for 1.5 h. All solvents were purchased from Sigma-Aldrich Co.

Spectroscopic Methods Electronic absorption spectra were recorded at 23 °C on a double-beam UV-Vis spectrophotometer Cary 300 Bio (Varian, USA) equipped with a thermostatted cuvette holder with a 6 × 6 multicell Peltier block. The temperature was controlled with a thermocouple probe (Cary Series II, Varian, USA) placed directly in the quartz cuvette. The spectra were recorded from 200 to 600 nm. The concentration of NTBD used in the measurements was 3.4×10^{-3} M in *n*-heptane and 2.2×10^{-5} M in methanol. Samples were prepared by weighing out fixed amounts of compounds and dissolving them in a known volume of an appropriate solvent.

The fluorescence excitation and emission spectra were recorded on a Cary Eclipse spectrofluorometer (Varian, USA). NTBD was excited at 273 or 321 nm, fluorescence was recorded with 0.5 nm resolution, and the spectra were corrected for the lamp and photomultiplier spectral characteristics. The excitation and emission slits were set to 2 nm. All fluorescence spectra were measured in a 10 × 10 mm quartz cuvette.

The measurements of ATR-FTIR background-corrected spectra were carried out in solvents using a HATR Ge trough (45° cut, yielding 10 internal reflections) crystal plate for liquids and were recorded with a 670-IR spectrometer (Varian, USA). Typically, 25 scans were collected, Fourier-transformed, and averaged for each measurement. The IR absorption spectra at a resolution of one data point per 1 cm^{-1} were obtained in the region between 4000 and 400 cm^{-1}. The instrument was purged with argon for 40 min before and then during the measurements. The Ge crystal was cleaned with ultra-pure organic solvents (Sigma-Aldrich Co.). All experiments were carried out at 20 °C.

Time-correlated single photon counting (TCSPC) measurements were performed on a FluoroCube fluorometer (Horiba, France). Samples were excited with pulsed

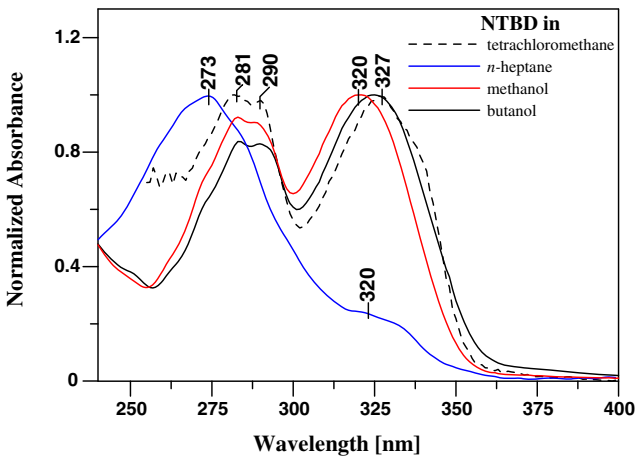


Fig. 1 Normalized electronic absorption spectra of NTBD dissolved in a series of organic solvents

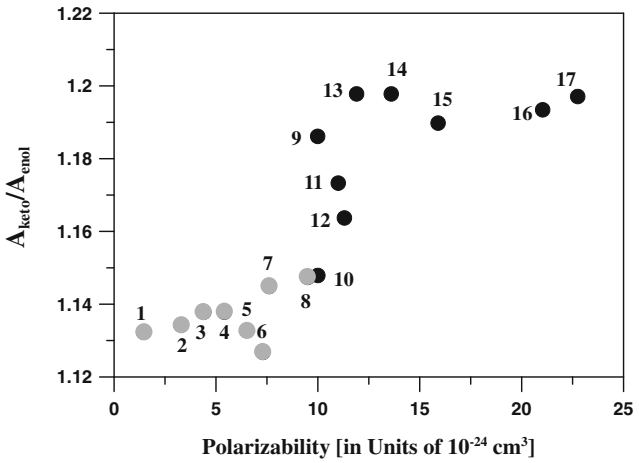


Fig. 2 The ratio of absorbance A_{keto}/A_{enol} (keto - absorbance maximum from 273 nm, enol - absorbance maximum from 325 nm) of NTBD vs. the average electric dipole polarizabilities α (in units of 10^{-24} cm^3). Numbers refer to the following solvents: *1* – water, *2* – methanol, *3* – acetonitrile, *4* – ethanol, *5* – acetone, *6* – DMSO, *7* – propan-2-ol, *8* – chloroform, *9* – pentane, *10* – benzene, *11* – cyclohexane, *12* – tetrachloromethane, *13* – *n*-hexane, *14* – *n*-heptane, *15* - *n*-octane, *16* – undecane, *17* – dodecane (see the text for details). *Grey circles* indicate polar solvents

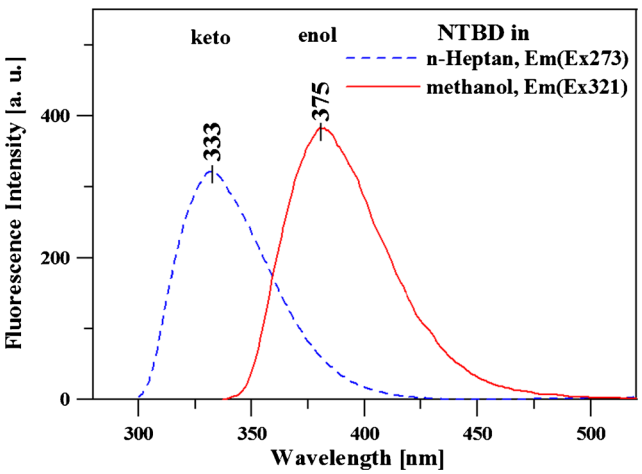


Fig. 3 Fluorescence emission spectra of NTBD in methanol and *n*-heptane. The samples were excited at 273 and 320 nm. In methanol, using 320 nm excitation, the emission maximum at 375 nm is typical of the enol form (*solid red line*), in *n*-heptane with excitation at 273 nm, the emission maximum at 273 nm is assigned to the keto form (*dashed blue line*)

NanoLED diodes at 294 nm (pulse duration of 700 ps) or with a laser diode NanoLED at 372 nm (pulse duration of 150 ps), both operated with a 1 MHz repetition rate. To avoid the pulse pile-up, the power of pulses was adjusted to an appropriate level using a neutral gradient filter. The fluorescence emission was recorded using a picosecond detector (IBH, JobinYvon, UK). The data station and the DAS 6 (JobinYvon (IBH, UK)) software were used for data acquisition and signal analysis. All fluorescence decays were measured in a 10 × 10 mm quartz cuvette, using an emitter band-pass filter with transmittance of 320–350 nm (excitation at 294 nm) or >420 nm (excitation at 372 nm). The excitation profiles, required for the deconvolution analysis, were measured without the emitter filters on a light scattering cuvette. All measurements were performed at 20 °C. Each fluorescence decay was analyzed with a multiexponential model given by the equation:

$$I_t = \sum_i \alpha_i \exp(^{-t}/_{\tau_i}) \quad (1)$$

where α_i and τ_i are the pre-exponential factor and decay time of component *i*, respectively.

Best-fit parameters were obtained by minimizing the reduced χ^2 value as well as residual distribution of experimental data.

Results and Discussion

To better understand the dependence of the keto-enol equilibrium in NTBD on solvent polarity, a set of spectrophotometric measurements was carried out. Overall, 17 solvents with various polarities were tested (see Table 1). Figure 1 presents the NTBD absorption spectra, normalized at the maximum, in two polar (methanol and butanol) and two non-polar (*n*-heptane and tetrachloromethane (CCl_4)) solvents. Two distinctive effects were observed along the series of the solvents used. The first one was observed in polar solvents as the appearance of an absorption band with the maximum at 325 nm. The measured intensities of this band were normalized and had a similar shape in all spectra. Compared to other polar solvents, this band was significantly broader in water (not presented). It shows a minor (several nm) red shift that correlates with the decreasing value of the dipole moment of the solvents (or with increasing dipole polarizability, Table 1).

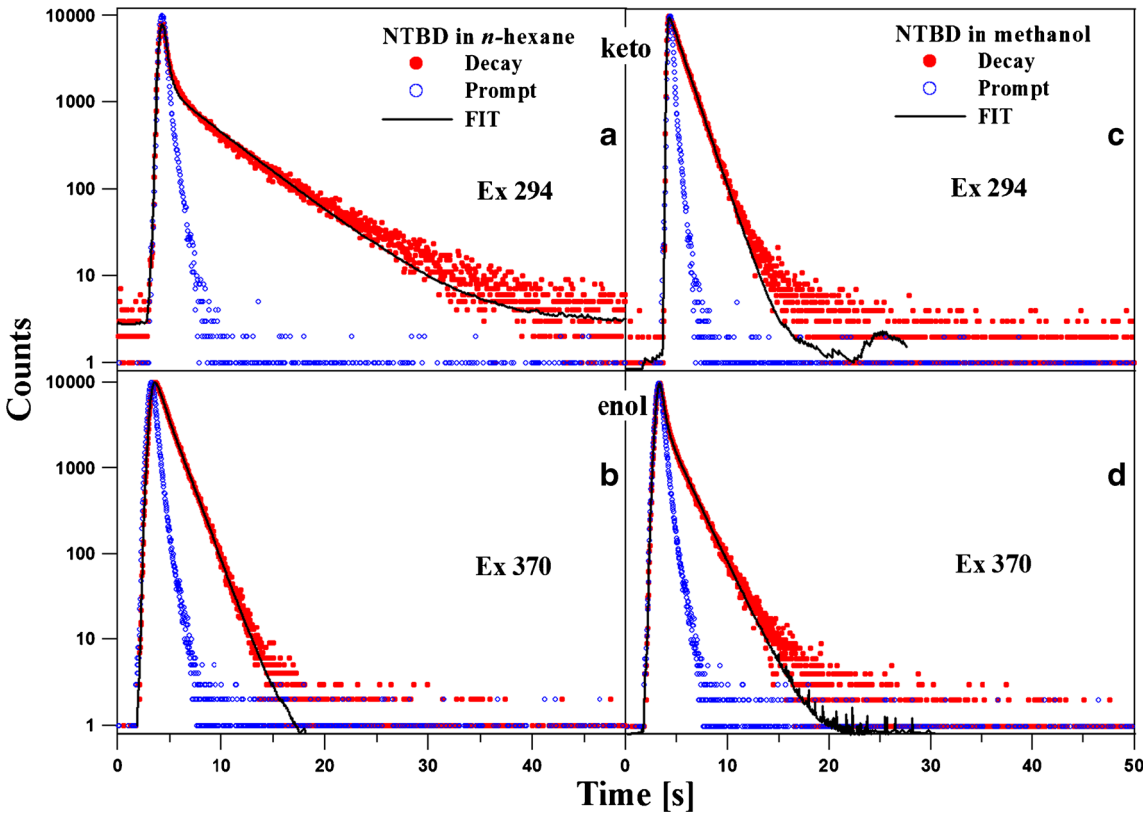


Fig. 4 Fluorescence lifetime measurements. The *dotted red curve* shows the decay of NTBD emission in *n*-hexane (Panels **a** and **c**) and in methanol (Panels **b** and **d**). *Black solid lines* are the exponential fits. The excitation pulse profiles are set up at 294 nm (Panel **a** and **b**) and 370 nm (Panels **c** and **d**), are shown as a *blue dotted curve*. The second component of fluorescence decay on panels **a** and **d** originates from the solvents

The second effect, which is a new band with the maximum 273 nm, takes place in non-polar solvents (with high polarizability, Table 1). The band with the maximum at 285 nm is characteristic for the naphthalene group in NTBD (Scheme 1). The maximum near 325 nm was still present, but its intensity was significantly lower, which suggested existence of two tautomeric forms of NTBD. Similar keto-enol equilibrium effects were reported for 2-(methyl-α-iminoethyl)-4,6-dichlorophenol in tetrachloromethane and the shifts of absorption bands depending on the solvent polarity were observed in a number of other solvents [12].

The positioning of the absorption maximum (at ~273 nm) together with the very low value of the molar extinction coefficient (~100 $M^{-1} \times cm^{-1}$) may correspond to the $n \rightarrow \pi^*$ electronic transition in the carbonyl group (Scheme 1) [17]. Moreover, the FTIR spectra confirm the presence of the band related to the carbonyl group in NTBD (see Fig. 6). These results indicate intramolecular proton transfer from the –OH group (in the *ortho* position) to the nitrogen atom in the 1,3,4-thiadiazole ring and formation of the keto form of NTBD (see Scheme 1B). Similar transitions have been observed in some other molecules [11, 13, 14].

As presented in Table 1, the absorption maxima at 273 and 328 nm show slight solvent *permanent dipole moment* dependence. Three alternative relationships between these absorption maxima and other solvent parameters were tested, namely, the solvent dielectric constant-related Kirkwood's polarity function (ε-1/2ε + 1), the solvent refractive index-related Lorenz-Lorenz (n^2-1/n^2 + 2) function [18–20], and *average electric dipole polarizability* α [19]. The shifts of the absorption maxima do not correlate with the first two parameters (not shown). Figure 2 presents the dependence of the ratio between the keto and enol absorption maxima on the *electric dipole polarizability*. It can be noticed that the intensity ratio between absorbance of the band from the maximum at ~325 nm to the one with the maximum at ~273 nm correlates well with the *average electric dipole polarizability* of the solvent.

On the basis of the relations presented in Fig. 2, it can be seen that the enol-to-keto transformation of the NTBD molecule occurs at a specific value α of the solvent, which is equal to 9×10^{-24} cm^3 (Table 1). For solvents with α close to this value, e.g. in *n*-hexane or *n*-heptane, both absorption maxima are observed. The band typical for the keto form appears in all solvents used but its intensity decreases as the value of α increases. The same effect was observed previously for FABT [7] where keto-enol transition appeared around 10×10^{-24} cm^3. This clearly shows a pure effect of the substituent on the keto-enol transformation in the function of solvent polarizability. The fluorescence spectra of NTBD in various solvents suggest the presence of two forms of NTBD. Figure 3 presents the NTBD fluorescence emission spectra in methanol and in *n*-heptane. The same excitation wavelengths were applied for both samples (273 and 321 nm). In methanol, when the excitation at 321 nm was used, an emission signal with the maximum at 375 nm was observed and assigned to the enol form. Also in *n*-heptane, the emission signal at 333 nm, typical for the keto form, was observed upon excitation at 273 nm. Similar effects were observed in our previous study [7].

Also the fluorescence lifetimes of NTBD (Panels A, B, C, D in Fig. 4) reflect the keto-enol equilibrium. The fluorescence decays were measured using the TCSPC technique and analyzed as either mono- or multiexponentials (Eq. 1). The experimental results clearly indicate two characteristics lifetimes for NTBD. The first one with a lifetime around 2.5 ns is related to the keto form while the other (~1 ns) to the enol form.

Two excitation wavelengths were applied in TCSPC experiments: 372 nm for the excitation of enol and 294 nm for the keto form. Upon excitation at 372 nm, the fluorescence lifetimes decreased from 2.2 to 1 ns, which correlates with the increasing solvent polarizability (Fig. 5b). When the 294 nm excitation was applied, the characteristic fluorescence lifetime substantially increased from 1.4 to ~6 ns along with the

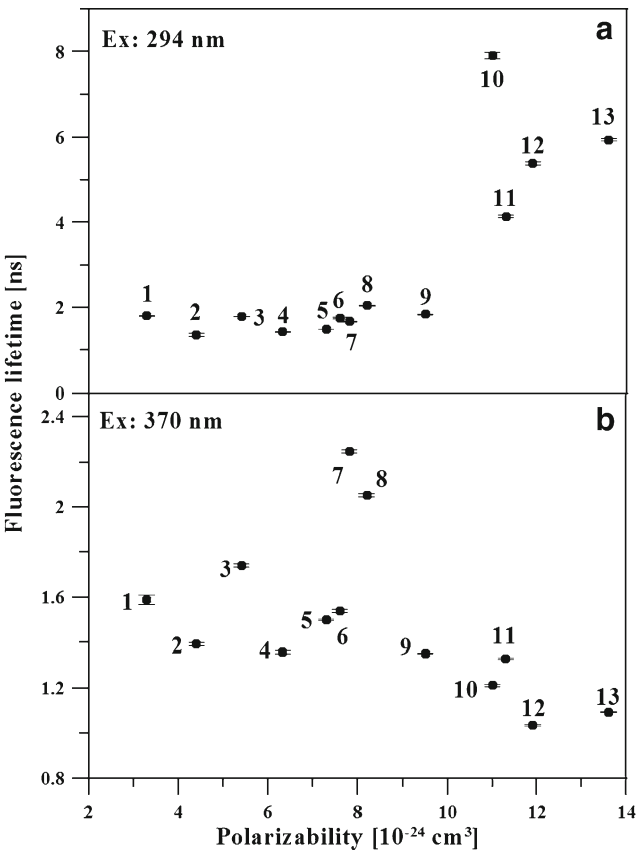


Fig. 5 The average fluorescence lifetimes of NTBD observed upon excitation at 372 nm (Panel **a**) and 294 nm (Panel **b**). The measurements were carried out using the TCSPC technique in various solvents: *1* - methanol, *2* - acetonitrile, *3* - ethanol, *4* - acetone, *5* - DMSO, *6* - propan-2-ol, *7* - DMF, *8* - butanol, *9* - chloroform, *10* - cyclohexane *11* - tetrachloromethane, *12* - *n*-hexane, *13* - *n*-heptane

Table 2 The fluorescence lifetimes in NTBD

	Fluorescence lifetime [ns]		
	Solvents	Excitation at 294 nm	Excitation at 372 nm
1.	Methanol	1803 ± 0003	1,589 ± 0,022
2.	Acetonitrile	1,365 ± 0,039	1,394 ± 0,008
3.	Ethanol	1,793 ± 0,003	1,742 ± 0,007
4.	DMSO	1,489 ± 0,005	1,501 ± 0,004
5.	Propan-2-ol	1,765 ± 0,019	1,540 ± 0,008
6.	DMF	1,676 ± 0,007	2,247 ± 0,007
7.	Butanol	2,079 ± 0,012	2,055 ± 0,007
8.	Chloroform	1,841 ± 0,009	1,352 ± 0,002
9.	Tetrachloromethane	4,148 ± 0,031	1,330 ± 0,004
10.	Cyclohexane	7,917 ± 0,076	1,021 ± 0,002
11.	*n*-hexane	5,392 ± 0,039	1,036 ± 0,002
12.	*n*-heptane	5,148 ± 0,025	1,094 ± 0,003

increase in the solvent polarizability (Fig. 5a). Notable changes in the fluorescence lifetime were observed for samples excited at 294 nm (keto form) when α was higher than 9×10^{-24} cm^3. In solvents with polarizability lower than 9×10^{-24} cm^3 (in which the enol form predominates), the fluorescence lifetimes were similar, about 2 ns for both excitations (Fig. 5a, b). This effect may be related to the excitation of the naphthalene group of NTBD (5 double conjugated bounds), which absorbs in the region of 275–295 nm, i.e. near the excitation wavelength (Fig. 1). The excitation at 370 nm resulted in a shorter fluorescence lifetime, probably due to the excitation of the resorcyl and thiadiazole rings (Fig. 4b, d). This difference in the absorption of chromophores is a well-known auxochromic effect (Table 2).

In order to gain deeper insight into the solvent polarizability-induced intramolecular proton transfer in NTBD, FTIR spectroscopy in the range from 1000 to 3600 cm^{-1} was used. The ATR-FTIR spectra of NTBD measured in tetrachloromethane and methanol are shown in Fig. 6. NTBD solutions were placed on a Ge crystal and the solvent was evaporated in a N_2 stream. Table 3 summarizes the positions of the main IR absorption bands of NTBD and their assignment in different solvents. The locations of particular absorption bands in the spectra were assigned based on the previously published data [11].

Additional information about the keto-enol equilibrium in NTBD was extracted from the FTIR spectra in the range 1550 and 1760 cm^{-1}, where the band near 1735 cm^{-1} coming from the carbonyl C=O group is present (Table 3). This band, with varying position and intensity, was observed in all the solvents. In methanol and other polar solvents, it (the C=O vibration group band) is present at 1726 cm^{-1} while in tetrachloromethane it shifts to 1737 cm^{-1} and gains in intensity. This band was observed, but not as intense, in another 1,3,4-thiadiazol [7]. In methanol, two other very narrow IR bands are present at 1627 cm^{-1} and 1594 cm^{-1}. They

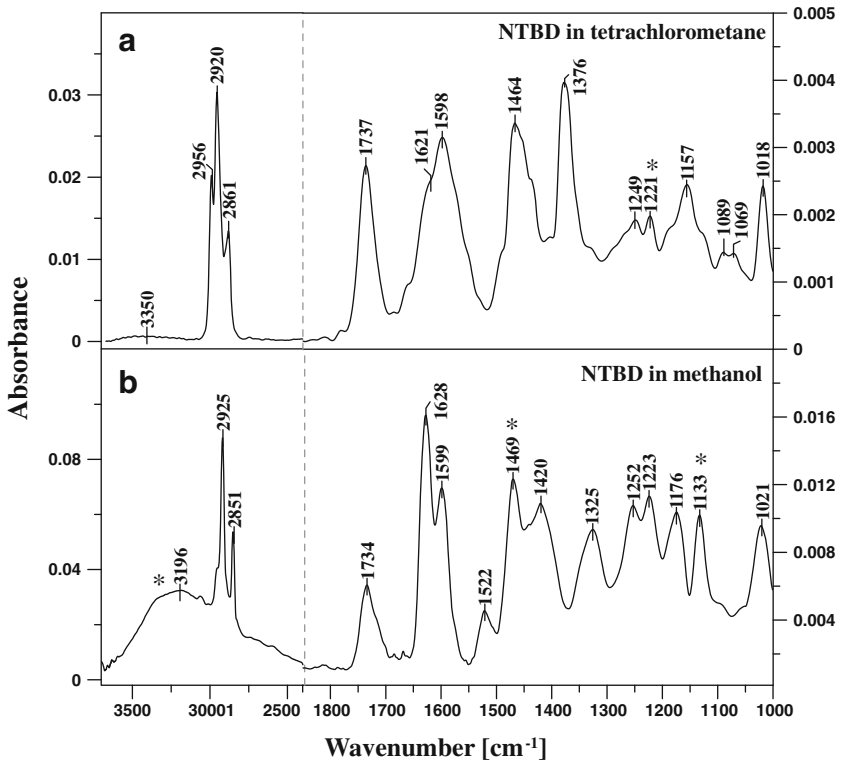


Fig. 6 ATR-FTIR absorption spectra of NTBD dissolved in CCl_4 (Panel **a**) and methanol (Panel **b**). The measurements were carried out from the solvents using a trough HATR Ge crystal plate for liquids. The *asterisk* denotes both the solvent and molecule band

Table 3 The position of FTIR vibrations in NTBD. The asterisk symbol denotes both the solvent and molecule band

Band position [cm^{-1}]		Vibration
Tetrachloromethane	Methanol	
3350	3145	ν (O-H)
	3066	
2956	2976	ν (O-H) + ν (C-H)
2920	2925	
2861	2851	
	2724	
	2591	
1737	1726	ν (C=O)
1621	–	
–	1627	ν (C=N)
1598	1595	
–	1523	
–	1511	ν (C=C)
1464	1470	
–	1415	δ (C-H) + ν (C=C)
1376	–	ν (N-H) + ν (C-H)
–	1325	ν (C-O)
1249	1249	
1221	1221	ν (C-N)
1157	1184	δ (C-C) in C-(C = O)-C + ν (C-C)
	1170	in $C_{10}H_8$*
–	1133	
1089	1089	ν (C-N) + ν (C-C) in $C_{10}H_8$*
1069	1047	
1018	–	

ν valence vibration; *δ* deformation; *s* symmetric; *as* asymmetric

$C_{10}H_8$* naphthalene ring

are related to C=N stretching vibrations present in the 1,3,4-thiadiazole ring in the enol form of NTBD (Scheme 1A). It has been observed that their intensity decreases with the increasing solvent polarizability [7]. In tetrachloromethane (Fig. 6), a wide band at 1598 cm^{-1} related mostly to one of the C=N groups is seen, as shown in Scheme 1.

To summarize, in solvents with low polarizability, two bands (C=N vibrations) in the enol form are observed (Scheme 1), while in those with high polarizability, the intensity of one of the bands decreases in favor of the band typical of the carbonyl group (the keto form, Scheme 1).

A similar effect to the one presented in Fig. 2 is shown in Fig. 7, which shows that the ratio between the 1737/1628 cm^{-1} intensities (keto-enol) correlates with the ratio between the respective forms of NTBD in various solvents. The A_{keto}/A_{enol} ratio is strongly dependent on solvent polarizability and, similarly to the relationship presented in Fig. 2, the transition from the enol to the keto form also occurs at α values near 10×10^{-24} cm^3.

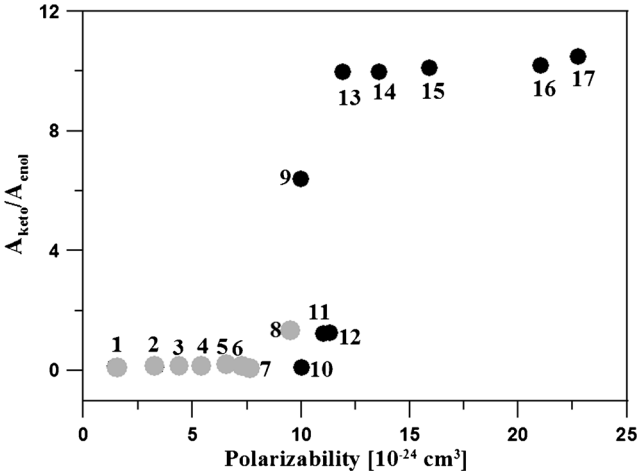


Fig. 7 The ratio of absorbance A_{keto}/A_{enol} (A_{keto} - absorbance maximum for the keto form from the region between 1720 and 1740 cm^{-1}, A_{enol} - absorbance maximum for the enol from the region between 1625 and 1632 cm^{-1}) of NTBD dependent on the average electric dipole polarizabilities (in units of 10^{-24} cm^3). Numbers refer to the following solvents: *1* - water, *2* - methanol, *3* - acetonitrile, *4* - ethanol, *5* - acetone, *6* - DMSO, *7* - propan-2-ol, *8* - chloroform, *9* - pentane, *10* - benzene, *11* - cyclohexane, *12* - tetrachloromethane, *13* - *n*-hexane, *14* - *n*-heptane, *15* - *n*-octane, *16* - undecane, *17* - dodecane. *Grey circles* indicate polar solvents

Conclusions

Similar to previously investigated FABT, solvents may also influence the intramolecular proton transfer such as the keto-enol equilibrium in NTBD (4-[5-(naphthalen-1-ylmethyl)-1,3,4-thiadiazol-2-yl]benzene-1,3-diol). Many studies discuss the solvent effects by comparing their electric polarities and correlating the shifts of the electronic absorption maxima to the solvent dipole moment. In the case of NTBD, the shifts of the corresponding bands and the tautomeric equilibrium are not related to solvent polarities but rather to the electric average dipole polarizabilities.

Molecules that form intramolecular hydrogen bonds are capable of keto-enol interconversion depending on the environment (solvent). In polar solvents, they usually adapt the solvated keto form, while in non-polar solvents their predominant enol form is often stabilized by an intramolecular hydrogen bond. An unusual opposite solvent effect is observed for 1,3,4-thiadiazoles, which in non-polar solvents occur as the more polarizable keto form, while in polar solvents they adapt the enolic conformation.

The strong dependence of the keto-enol equilibrium on solvent polarizability seems to be characteristic for the studied group of 1,3,4-thiadiazoles. Additionally, this equilibrium also depends on the substituent in the 1,3,4-thiadiazole molecule. From the biological point of view, the keto forms of 1,3,4-thiadiazoles may interact more efficiently with the hydrophobic part of cell membranes. This appears interesting not only from the theoretical point of view, but also because it may

provide a means to control interactions of 1,3,4-tiadiazoles with biological systems. The results presented in this work indicate a possibility of intramolecular proton transfer in a hydrophobic environment, such as lipid membranes or proteins, which is significant for understanding its activity in biological systems [21]. Therefore, these compounds, in particular NTBD, may find application as molecular sensors. e.g. in monitoring biological membranes.

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