

## Structure and electronic absorption spectra of cyanine dyes – derivatives of tetrazolo- and triazoloisindole

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The electronic structure and absorption spectra of cyanine dyes – tetrazoloisindole derivatives and triazoloisindole were calculated. It was shown that these dyes, in terms of their electronic structure, are trimethine cyanine, although formally they are monomethine cyanine. The electron donation of the tetrazoloisindole and triazoloisindole residues was determined on the Ilchenko scale, which allows them to quantitatively quantify their Bruker basicity in comparison with the most known heterocyclic end groups of cyanine dyes.

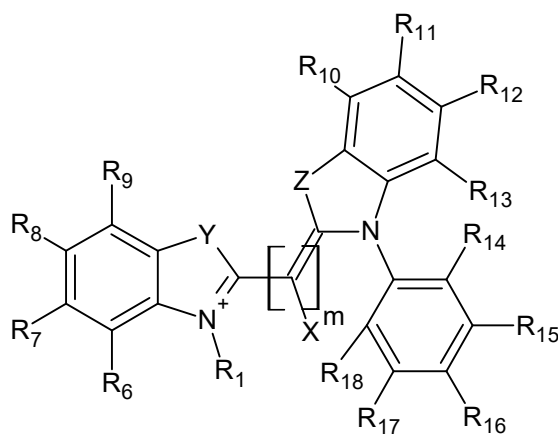
### Introduction

Cyanine dyes have found a wide practical use during the last century and they are still generating a significant and increasing interest over the very recent years. Indeed their applications in biochemistry and medicine are becoming numerous, especially as they show a peculiar ability to act as effective marker of biomolecules [1-10].

Thus, dyes exhibiting improved properties such as enhanced water solubility, higher extinction coefficients, photo- and structure stabilities have been developed for optical labeling determination of amino acids, proteins, antibodies, nucleic acids, DNA, RNA, polymers, drugs, etc. [3].

In particular, the fluorescent dyes with general structure shown in Fig.1 invented several years ago can be used as markers for the analysis of the majority of clinical, biological, biochemical or chemical substances, eg cells, antibodies, proteins, hormones, nucleic acids acids,

oligonucleotides, natural or modified nucleotides, carbohydrates [4].



**Figure 1.** The general structure for fluorescent dyes, where  $m = 1,3,5,7$  or  $9$ ;  $X = H$ , halogen, alkyloxy, aryloxy, alkylthio, arylthio, amino, substituted amino, aryl, alkyl groups under certain conditions;  $Y = O$ , substituted N, S, Se etc.;  $Z = O$ , substituted N, S, Se, etc.;  $R_1 =$  aryl, alkyl groups;  $R_6-18 =$  carboxy, sulfonate, sulfinate, sulfoxide, sulfone, sulfonamide, hydroxy, amino etc..

Some dyes can interact with DNA, forming fluorescent conjugates. Thus, six carbocyanine dyes were synthesized and their

interaction with DNA was investigated. Also they can be used as fluorescent markers in molecular biology [5].

Some dyes can be used as fluorescent markers which are sensitive to acids or alkalis, so can be used in intracellular media [6].

It is necessary to distinguish dyes that are used to produce biosensors for the detection of changes in bonding, conformation or post-translational modification of the target molecule [7].

Indolinenpentamethine dyes, their derivatives and salts containing the reaction groups at the 3<sup>rd</sup> and 5<sup>th</sup> positions of the indolenine ring are also equally important. Such dyes are more sensitive for determining the hybridization of nucleic acids than most of the known fluorescent markers [8].

The use of cyanine dyes in the analytical field is also promising. An interesting example is the use for detection of lithium ions when investigating the spectral characteristics of the heptamethine cyanine dye (JCM-15C5) crown ether and its quenching by lithium ion [9]. Studies have shown that Li<sup>+</sup>-ion selectively quenches the fluorescence intensity of JCM-15C5 by the static quenching mechanism.

Carbocyanine dyes absorbing and fluorescing in the near-IR range are increasingly being used as labels, probes,

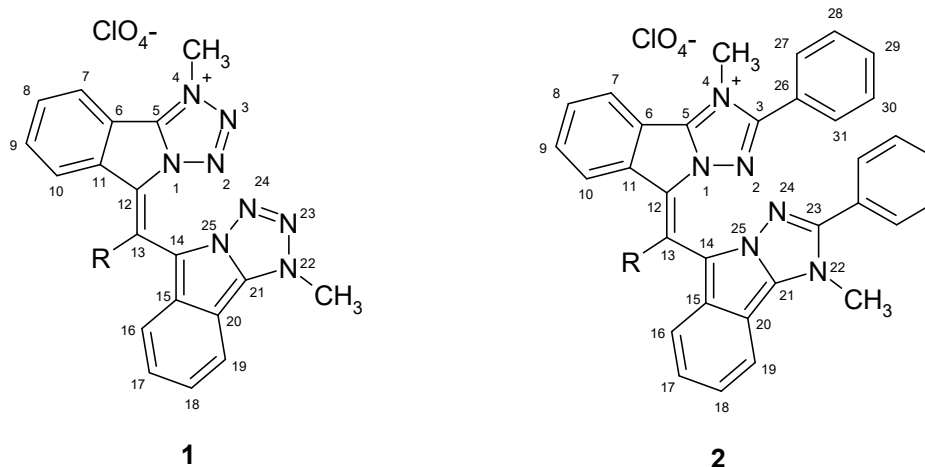
imaging agents or markers in the analytical, biological and medical fields [10].

Thus, new possibilities of using cyanine dyes require careful study of the electronic structure and factors that can influence it.

Synthesized by us [11, 12] cyanine dyes - derivatives of tetrazoloisoindole **1** and triazoloisoindole **2** (R = Alk, Ar, Het) are similar to few dyes depicted in Fig. 1., where m = 1. Our dyes had unusually high logarithms of extinction as for monomethine cyanine. Taking into account the traditions of the famous Ukrainian Kiprianov-Babichev school on the study of the dependence of absorption spectra on the structure of cyanine dyes [13–15], we have studied the electronic structure and absorption spectra of dyes **1** and **2**.

## Results and discussion

We carried out a quantum-chemical calculation of the electronic structure and spectral data of the dye molecule **1** and **2**, where R = H (Figure 2) by the Pariser-Parr-Pople (PPP) method, which is considered as most suitable for studying the electronic structure and absorption spectra of cyanine dyes. Calculations were carried out in program MOPAC.



**Figure 2.** Numbering of atoms in the molecule of cyanine dyes - derivatives of tetrazoloisindole **1** and triazoloisindole **2**.

As parameters for the PPP calculations, the ionization potentials of the carbon and nitrogen atoms used in the work [16]:  $I_C=11.16$  eV,  $I_{N^-}=I_{N^+}=18.50$  eV,  $I_N=14.12$  eV; single-center integrals of the interaction of  $p$ -electrons:  $\gamma_{CC}=-6.20$  eV,  $\gamma_{NN}=-7.20$  eV, and to calculate the two-center integrals of the interaction of  $p$ -electrons the Mataga-Nishimoto equation was used [17]. It was considered that the influence of the substituents on the parameters and results of calculations is not significant. The lengths of bonds and valence angles in the molecule were taken as standard, only the valence angle of the *meso*-carbon atom was increased to  $130^\circ$ , as found in [18]. As in [16], the configuration interaction of four single-cavity configurations (KB 2x2) was taken into

account when calculating the dye absorption maxima. For dyes **1** and **2**, absorption maxima of 557 nm and 559 nm of the long-wavelength absorption band were found, which were close to those found experimentally (557 nm [19, 20] and 568 nm [20, 21], respectively), and the oscillator strengths  $f$ :  $f = 0.531$  and  $0.587$ . The strength of the oscillators characterizes the intensity of absorption bands, and they can be compared with area under the absorption band, so can be indirectly compared with the absorption extinction of these dyes ( $\lg \epsilon = 4.86$  [19] for dye **1**). Table 1 shows  $\pi$ -charges on atoms ( $q_r$ ) and orders of  $\pi$ -bonds ( $p_{rs}$ ) in the main ( $S_0$ ) and in the first excited state ( $S_1$ ).

Table 1.  $\pi$ -charges on atoms ( $q_r$ ) and orders of  $\pi$ -bonds ( $p_{rs}$ ) in the main ( $S_0$ ) and in the first excited state ( $S_1$ ) for dyes **1** and **2**, where R=H.

Dye 1

Atoms	$q_r$		Bonds	$p_{rs}$	
	$S_0$	$S_1$		$S_0$	$S_1$
1	0.669	0.611	1, 2	0.499	0.457
2	-0.308	-0.305	1, 5	0.551	0.491
3	-0.221	-0.210	1, 12	0.420	0.437
4	0.631	0.627	2, 3	0.656	0.671
5	-0.053	-0.078	3, 4	0.598	0.589
6	-0.101	-0.106	4, 5	0.562	0.552
7	0.006	0.024	5, 6	0.439	0.451
8	0.004	0.040	6, 7	0.581	0.586
9	0.040	0.015	6, 11	0.561	0.525
10	-0.008	0.060	7, 8	0.709	0.681
11	-0.023	-0.068	8, 9	0.619	0.622
12	-0.196	-0.025	9, 10	0.705	0.687
13	0.117	-0.169	10, 11	0.586	0.573
			11, 12	0.447	0.479
			12, 13	0.621	0.552

Dye 2

Atoms	$q_r$		Bonds	$p_{rs}$	
	$S_0$	$S_1$		$S_0$	$S_1$
1	0.643	0.586	1, 2	0.412	0.370
2	-0.469	-0.482	1, 5	0.594	0.525
3	0.101	0.112	1, 12	0.420	0.451
4	0.495	0.499	2, 3	0.639	0.649
5	-0.079	-0.091	3, 4	0.584	0.570
6	-0.097	-0.106	3, 26	0.352	0.356
7	-0.001	0.017	4, 5	0.483	0.483
8	-0.001	0.029	5, 6	0.449	0.460
9	0.030	0.007	6, 7	0.577	0.580
10	-0.008	0.056	6, 11	0.557	0.523
11	-0.029	-0.076	7, 8	0.712	0.686
12	-0.190	-0.024	8, 9	0.616	0.621

13	0.116	-0.168	9, 10	0.708	0.686
26	-0.042	-0.041	10, 11	0.582	0.579
27	0.021	0.023	11, 12	0.453	0.471
28	0.008	0.008	12, 13	0.620	0.553
29	0.041	0.044	26, 27	0.622	0.621
30	0.017	0.017	26, 31	0.621	0.619
31	0.003	0.005	27, 28	0.675	0.676
			28, 29	0.661	0.661
			29, 30	0.659	0.659
			30, 31	0.677	0.677

In the ground state of the dyes, a significant positive  $\pi$ -charge (0.116-0.117  $e$ ) is found in the *meso*-position at carbon atom, which correlates with the results of [18], and when excited, the electron density increases and the  $\pi$ -charge becomes negative (-0.168÷-0.169  $e$ ).

The molecules of dyes **1** and **2**, R = H belong to the symmetry group  $C_{2v}$ . Their higher occupied molecular orbitals (HOMO) refer to the representation  $A_2$  of this group, that is, asymmetric with respect to the symmetry plane  $\sigma_v$  ( $xz$ ), and the lowest unoccupied molecular orbitals (LUMO) refer to the representation  $B_1$ , that is, symmetric with respect to the symmetry plane  $\sigma_v$  ( $xz$ ). Accordingly, the electronic transition between them  $A_2 \times B_1 = B_2$  will be allowed and polarized along the long axis of the chromophore ( $y$  axis), that is, the transition  $M$  will be directed along the  $y$  axis and correspond to the intense absorption band, that coincides with calculations and experiment.

An analogous symmetry of HOMO and LUMO is observed in the class of dyes A according to the classification proposed in [22], which divides symmetrical compounds into two classes: A and B, depending on which type,  ${}^1A_1$ - ${}^1B_{1a}$  or  ${}^1A_1$ - ${}^1B_{1b}$ , belongs to the main absorption band. Class A, in particular, includes classical trimethine cyanine dyes (carbocyanines) [22], for example, 2-quinoline derivatives. Therefore, it can be concluded that the dyes **1** and **2**, which are considered in the presented paper, are of the same electronic structure as the ordinary carbocyanines, although formally it

is monomethine cyanines. It is known, that the value of the absorption maximum and extinction usually increase from monomethine to trimethine cyanines. This can be observed, for example, for the classical mono- and trimethine cyanines of the benzothiazole series, where for *bis*-[3-methylbenzthiazole-2]-monomethine cyanine iodide  $\lambda_{\max} = 422$  nm,  $\lg \epsilon = 4.91$ , for *bis*-[3-methylbenzthiazole-2]-tetramethine cyanine iodide  $\lambda_{\max} = 558$  nm,  $\lg \epsilon = 5.12$  [23].

This identity allows us to assume that the effect of the substituents in the *meso*-position of the carbocyaninium and in the *meso*-position of the dyes **1** and **2** will be the same, for example, the electron-withdrawing substituents in these positions will effect the bathochromic effect in accordance with the Förster-Dewar-Knott rule (FDK rule) [24–26], which was confirmed by the results of [11, 12].

In search of further analogies, we determined the electron donation  $D$  (another name - basicity of Brooker [27–29], i.e. the ability of heterocyclic nuclei to give  $\pi$ -electrons in the general  $\pi$ -electron dye system) on the Ilchenko scale using the method described in [30, 31]. This method makes possible to quantify  $D$  by means of an empirical equation, establishes a relationship between the absolute value of the difference of the electron donation  $|\Delta D|$  and the deviation  $\Delta \lambda$  (the difference between the absorption maximum of an asymmetric dye and the arithmetic mean of the absorption maxima of two corresponding symmetric dyes):  $\Delta \lambda = 36|\Delta D|^2 - 6|\Delta D|^3$  (1) [30]. The results are shown in Table 2.

Table 2. Calculation of *D* tetrazoloisindole **3** and triazoloisindole **4** residues according to the Ilchenko scale, where  $\lambda_{\max}$  is the experimental value of the absorption maxima of the corresponding asymmetric dyes,  $(\lambda_{\max 1} + \lambda_{\max 2})/2$  and is the arithmetic mean of the experimental values of the absorption maxima of the corresponding symmetrical dyes [19, 21, 30],  $\Delta\lambda$  - deviation,  $|\Delta D|$  - the absolute value of the difference in electron donation calculated from the empirical equation (1) from [30],  $D_I$  - electron-donation of the corresponding heterocyclic residues according to the Ilchenko scale [30],  $D_{\text{tet}}$  - calculated electron donation of **3**,  $D_{\text{tr}}$  - calculated electron donation of **4**.

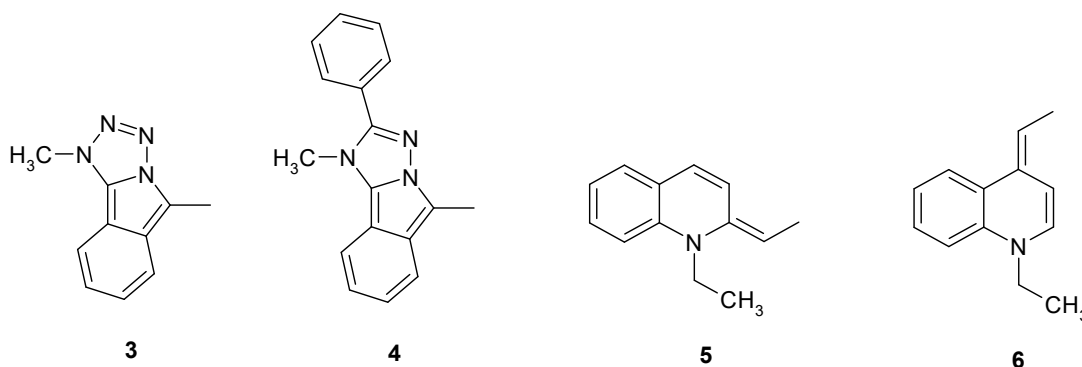
Asymmetric Dyes	$\lambda_{\max}$ , nm [19]	$\lambda_{\max 1}$ , nm [19]	$\lambda_{\max 2}$ , nm [30]	$(\lambda_{\max 1} + \lambda_{\max 2})/2$ , nm	$\Delta\lambda$ , nm	$ \Delta D $	$D_I$	$D_{\text{tet}}$	$D_{\text{tr}}$ average
	539	557	548	553	14	0.647	1.0	1.647	1.68
	516	557	485	521	5	0.38	1.45	1.83	
	555	557	559	558	3	0.275	1.50	1.775	
	580	557	605	581	1	0.162	1.78	1.618	
	539	557	540	549	10	0.537	2.20	1.663	
	521	557	610	584	63	1.525	0	1.525	

Asymmetric Dyes	$\lambda_{\max}$ , nm [21]	$\lambda_{\max 1}$ , nm [21]	$\lambda_{\max 2}$ , nm [30]	$(\lambda_{\max 1} + \lambda_{\max 2})/2$ , nm	$\Delta\lambda$ , nm	$ \Delta D $	$D_I$	$D_{\text{tr}}$	$D_{\text{tr}}$ average
	526	568	548	558	10	0.553	1.0	1.553	2.16
	512	568	485	527	15	0.674	1.45	2.124	

	550	568	559	564	14	0.647	1.50	2.147
	603	568	605	587	17	0.772	1.78	2.552
	544	568	540	554	10	0.553	2.20	2.753
	505	568	610	589	84	1.833	0	1.833

It turned out that for **3** the electron donation  $D = 1.68$  is close to the value  $D =$

1.78 [30] for the 2-quinoline residue **5**, and for **4**  $D = 2.16$  is close to the value  $D = 2.02$  [30] for the 4-quinoline residue **6** (Fig. 3).



**Figure 3.** Tetrazoloisindolium **3**, triazoloisindolium **4**, 2-quinoline **5** and 4-quinoline **6** heterocyclic residues.

Thus, the replacement of carbon atoms in the heterocyclic residue by more electronegative nitrogen atoms naturally decreases the electron donation of the heterocyclic residues in the dyes, and a certain chemical similarity to the 2-quinoline

residue **5** can be expected from the tetrazoloisindole residue **3**, and from the triazoloisindole residue **4** with 4-quinoline residue **6**.

## Conclusions

The electronic structure and absorption spectra of cyanine dyes **1** and **2** (R = H) were calculated. It is shown that dyes **1** and **2** in their electronic structure belongs to trimethine cyanines, although formally it is monomethine cyanine. The electron donation of the tetrazoloisindole and triazoloisindole residues was determined on the Ilchenko scale, which allows them to quantitatively quantify their Bruker basicity in comparison with the most known heterocyclic end groups of cyanine dyes.

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