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Fluorescent homodimer styrylcyanines: synthesis and spectral-luminescent studies in nucleic acids and protein complexes

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Abstract

A series of homodimer styrylcyanine dyes based on (*p*-dimethylaminostyryl)pyridinium, (*p*-dimethylaminostyryl)benzoxazolium, (*p*-dimethylaminostyryl)benzothiazolium, (*p*-dimethylaminostyryl)-1,3,3-trimethyl-3*H*-indolium residues were synthesized. Spectral-luminescent properties of the obtained homodimers in free state and in the presence of nucleic acids and BSA were studied. Homodimer styrylcyanines with the length of linkage group of 2 or more carbon atoms demonstrated a DNA-binding preference. Significant long-wave shifts of fluorescence and emission maxima of dyes with short linkage group could be explained by the interaction between chromophores due to the short distance between them, as it is the case for molecular aggregates. Homodimer dyes based on the (*p*-dimethylaminostyryl)pyridinium residue having linkage group of 5 or more carbon atoms interact with dsDNA with significant emission increase and could be used as DNA specific fluorescent probes.

Keywords: Homodimer styryl dyes; Nucleic acids; Fluorescent detection

1. Introduction

Styrylcyanines are known as excellent fluorophores that are used to probe the microviscosity of micelles, microemultions and lipid membrane [1]. Today styryl dyes are among the most sensitive probes for unspecific fluorescent detection of proteins in the presence of sodium dodecyl sulfate [2]. The styrylcyanine dye, 4-[4-dimethylamino)styryl]-1-methylpyridinium iodide (**F**, Fig. 1), was shown to be a minor groove binder with high affinity to double helical DNA [3]. Styryl dyes were used for DNA sequencing on gels [4]. Also, due to the capacity of styryl benzothiazolium dyes to penetrate through cell membranes, these dyes were proposed for the DNA visualization in fluorescent microscopy [5].

Earlier homodimer (*p*-dimethylaminostyryl)pyridinium styryls were synthesized and proposed as paper colorants [6], also (*p*-dimethylaminostyryl)benzothiazolium homodimers with the short linkage group were reported [7,8]. Recently, the spectral-luminescent properties of series of homodimer styryls containing (*p*-dimethylaminostyryl)pyridinium template with aliphatic linkage groups were studied in the presence of various biopolymers. We first proposed these homodimers as possible fluorescent probes for DNA detection [9]. It was shown that DNA-binding affinity of homodimers with linkage group length of 5 or 10 carbon atoms

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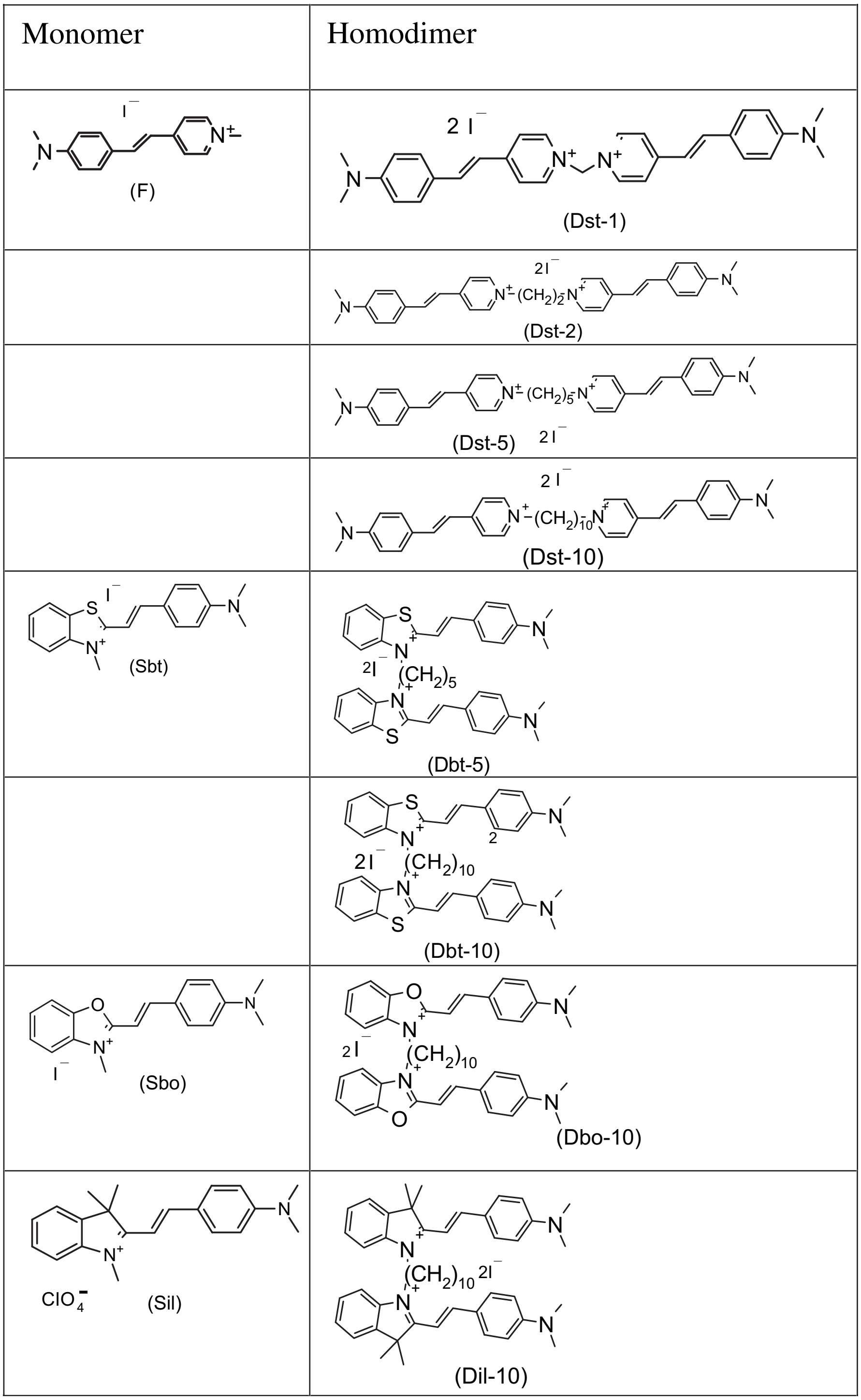


Fig. 1. Structures of monomer and homodimer styryl dyes.

significantly exceeds the affinity of parent monomer. Also, it was concluded that the homodimers with long linkage group could bind with dsDNA via different mechanisms than the parent monomer [9].

As a continuation of these studies, a series of homodimer dyes containing (*p*-dimethylaminostyryl)-benzoxazolium, (*p*-dimethylaminostyryl)benzothiazolium, and (*p*-dimethylaminostyryl)-1,3,3-trimethyl-3 *H*-indolium residues were synthesized. During the designing of novel homodimer styrylcyanines selective to nucleic acids, we used the linkage group with a length of 5 or 10 carbon atoms. Herein the synthetic protocols for (*p*-dimethylaminostyryl)pyridinium homodimers and novel styrylcyanine homodimers are presented. Spectral-luminescent characteristics of homodimers both unbound and in the presence of nucleic acids and BSA in comparison with corresponding monomer dyes are reported. Also, computer simulation aimed at the explanation of the nature of red shift observed for the absorption and emission maxima of dyes with short linkage group as compared to the parent monomer is carried out.

2. Results and discussion

2.1. Synthesis of homodimer styrylcyanines

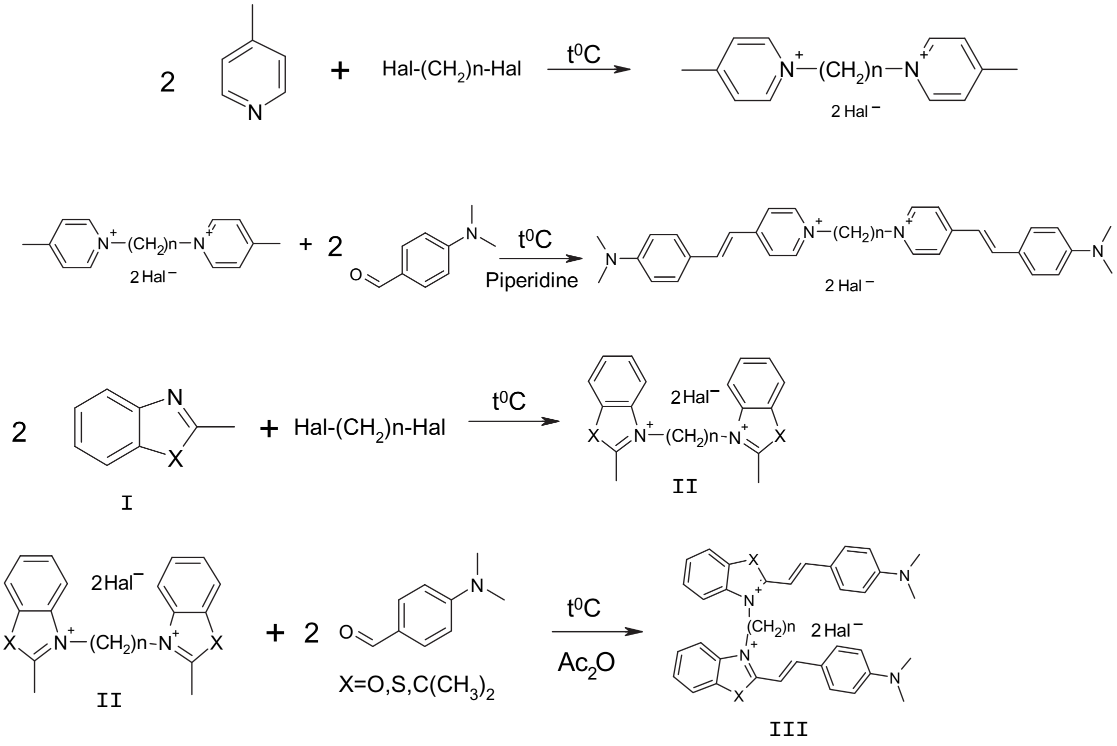
For the synthesis of homodimer styryl dyes, we used the reaction (Scheme 1). Structures of obtained dyes and their parent monomers are presented in Fig. 1. In the first stage, a quaternary salt was obtained by fusion of γ-picoline with corresponding dihalogenalkane (Protocol 1). To obtain dyes **Dst-2**, **Dst-5** and **Dst-10** a mixture of quaternary salt and corresponding aldehyde was boiled in *n*-butanol in the presence of piperidine (Protocol 3) similarly as described in Refs. [10,11]. However, under such conditions synthesis of dye **D-1** failed. This dye was obtained by the boiling of quaternary salt and *p*-dimethylaminobenzaldehyde in acetic anhydride (Protocol 4) [12].

To obtain quaternary salts, which are precursors for the dyes **Dbo-10**, **Dil-10**, **Dbt-10** and **Dbt-5**, corresponding heterocycle (2-methylbenzoxazole, 2,3,3-trimethylindolenine or 2-methylbenzothiazole) was boiled in dioxane with dihalogenalkane (Protocol 2). *p*-Dimethylaminobenzaldehyde was added to the quaternary salts and the mixture was boiled in acetic anhydride (Protocol 4) similarly as described in Ref. [12]. Parent dyes **F**, **Sid** and **Sib** were obtained according to Method 3 and the dyes **Sbt**, **Sbo** and **Sil** according to the Protocol 4. The structure of the dyes was confirmed with ^{1}H NMR and element analysis.

2.2. Spectral properties of free dyes

Spectral-luminescent characteristics of styryl dyes and corresponding homodimers in aqueous buffer are presented in Table 1. The studied dyes have wide band absorption spectra, which is typical for styryls, with a maxima located between 452 and 542 nm. Absorption maxima of homodimer styryls **Dst-5**, **Dst-10**, **Dbt-5**, **Dbt-10**, **Dbo-10** and **Dil-10** with long linkage group (5 or 10 carbon atoms) are shifted up to 35 nm to the long-wave region relative to the maxima of the parent monomers. For the (*p*-dimethylaminostyryl)pyridinium dyes with short linkage group (**Dst-1** and **Dst-2**) (1 and 2 carbon atoms) a larger bathochromic shift (90 and 47 nm, respectively) is observed (Fig. 2).

For both monomer and dimer dyes excitation spectra are shifted to the long-wave region by up to 40 nm relative to their absorption spectra. The maxima of emission spectra of dyes in aqueous buffer are located in the 554–645 nm range. Emission maxima of homodimer



Scheme 1. Synthesis of homodimer styryl dyes.

Table 1
Spectral-luminescent characteristics of monomer and homodimer styryl dyes

Name	Free dye				In DNA presence					In RNA presence					In BSA presence				
	λ_{abs}	λ_{ex}	λ_{em}	I	λ_{abs}	λ_{ex}	λ_{em}	I	I/I_0	λ_{abs}	λ_{ex}	λ_{em}	I	I/I_0	λ_{abs}	λ_{ex}	λ_{em}	I	I/I_0
F	452	460	607	3.2	455	495	606	24	7.4	453	510	611	33.6	10.5	451	470	595	4.8	1.5
Dst-l	542	550	640	0.6	569	580	645	1.7	2.9	574	550	570	2.3	4.00	545	555	623	2.3	4
Dst-2	499	510	645	1.2	507	540	640	20	16.7	500	564	656	10	8.3	499	510	620	3	2.5
Dst-5	460	480	614	2.2	483	480	600	296	134	470	510	614	33	15	469	490	590	23.9	10.8
Dst-10	460	465	609	2.8	480	490	600	341	121	479	510	612	73	25	470	490	583	53.2	19
Sbo	488	495	554	41	490	530	565	304	7.4	491	535	568	568	14	487	495	551	47.5	1.2
Dbo-10	467	500	557	3.9	478	520	563	207	53	478	530	570	33	8.4	469	520	557	116	30
SiI	535	545	589	14	537	560	596	159	11.4	538	570	600	178	12.8	537	550	589	36	2.6
Dil-10	509	550	595	5.3	515	550	600	267	50.7	521	550	607	40	7.6	512	550	600	71	13.4
Sbt	512	530	592	20	520	520	600	448	22.2	520	540	605	503	25	512	530	591	23.7	1.2
Dbt-5	501	536	600	3	506	550	606	74	24.6	497	555	607	21	7	501	540	592	15	5
Dbt-10	488	532	592	1.1	505	544	600	86	79	502	560	605	16	14.6	489	542	586	60	55

λ_{abs}, λ_{ex}, λ_{em}: maximum wavelengths of absorption, fluorescence excitation and emission spectra (nm); I_0: dye intrinsic fluorescence intensity (arbitrary units); I (DNA/RNA/BSA), dye fluorescence intensity in the presence of DNA/RNA/BSA (arbitrary units); I/I_0: enhancement of fluorescence intensity in DNA/RNA/BSA complexes.

styryls with long linkage group (styryls **Dst-5**, **Dst-10**, **Dbt-5**, **Dbt-10**, **Dbo-10** and **Dil-10**) are close to those of parent monomer, while for the dyes with short linkage group the emission maxima are bathochromically shifted to 35–39 nm relative to the maxima of free monomer. The dyes demonstrate moderate or large Stokes shift values (up to 160 nm for monomer **F**). Fluorescence intensity of studied styryl dyes is insignificant (quantum yield does not exceed 0.03), studied homodimers have a lower intrinsic emission than parent monomers.

2.3. Spectral properties of the dyes in the presence of DNA and RNA

Spectral-luminescent characteristics of studied dyes in DNA and RNA complexes are presented in Table 1. Absorption maxima of the studied dyes in nucleic acid containing solution are shifted to the long-wave region relative to the maxima of free styryls and are situated between 453 and 574 nm, but the shapes of absorption bands stay practically unchanged. As in the case of free dyes, in the presence of DNA the maxima of absorption spectra of (*p*-dimethylaminostyryl)pyridinium homodimers with short linkage group (**Dst-1** and **Dst-2**) are significantly shifted to the long-wave region relative to those of the parent monomer and homodimers with long linkage group (**Dst-5**, **Dst-10**) (Fig. 3).

For the majority of dyes in nucleic acid complexes excitation maxima are long-wave shifted relative to the absorption maxima (up to 44 nm). Emission maxima of the dyes in the presence of DNA/RNA are situated between 563 and 656 nm. Emission maxima of DNA and RNA complexes of styryl dyes are close to each other, while emission intensity enhancement in these complexes could significantly differ. The majority of the studied homodimer dyes demonstrated DNA preference, while for dimer with short linkage group, **Dst-1**,

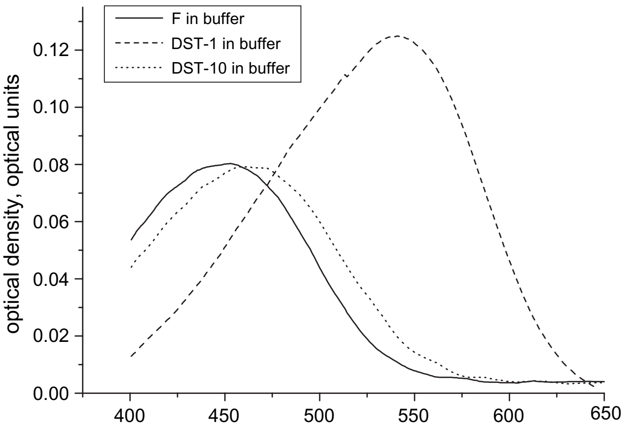


Fig. 2. Absorption spectra of **F**, **Dst-1**, and **Dst-10** in buffer.

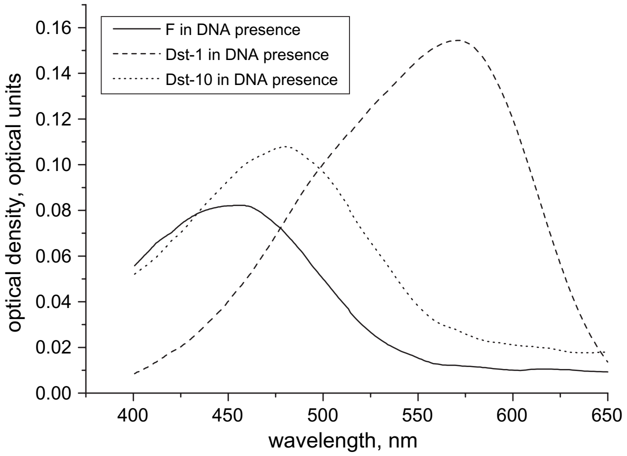


Fig. 3. Absorption spectra of **F**, **Dst-1**, and **Dst-10** in the presence of DNA.

and monomers, **F**, **Sbo**, **Sil**, and **Sbt**, small RNA preference is shown. Homodimer styrylcyanines increased their emission intensity in the presence of DNA by 2.9–134 times and in the presence of RNA by 4–25 times.

It is shown that emission intensities of (*p*-dimethylaminostyryl)pyridinium homodimers with long linkage group (**Dst-5**, **Dst-10**) in the presence of DNA significantly exceed those for parent monomer and for the dyes with short linkage group (**Dst-1**, **Dst-2**) (Fig. 4). Homodimers based on (*p*-dimethylaminostyryl)benzoxazolium, (*p*-dimethylaminostyryl)-1,3,3-trimethyl-3*H*-indolium and (*p*-dimethylaminostyryl)benzothiazolium residues (**Dbo**, **Dil**, **Dbt** dyes) demonstrate inverse behavior, fluorescence intensity of these dyes in the presence of DNA is less than that for parent monomers. However, values of emission intensity increasing (I/I_0) in the presence of DNA for all homodimers (except **Dst-1**) are higher than for corresponding monomers due to the very low intrinsic fluorescence of homodimers.

2.4. Spectral properties of the dyes in the presence of BSA

Characteristics of absorption, fluorescence excitation and fluorescence spectra of monomer and homodimer styryls are presented in Table 1. Maxima of absorption spectra of the dyes in BSA complexes are located between 451 and 545 nm. For studied dyes in the presence of BSA, positions of absorption bands are close to those in the spectra of free styryls. For presented dyes, except **Dbo-10**, excitation maxima are bathochromically shifted up to 53 nm relative to the absorption maxima.

Maxima of fluorescence spectra of the homodimer dyes in the presence of BSA are situated between 551 and 620 nm. In the presence of BSA, homodimer styryls enhance their fluorescence intensity by 1.2–55 times. It is necessary to note that for the majority of homodimers fluorescence intensity in the presence of BSA is higher than for corresponding monomers, also the value of emission intensity increasing (I/I_0) for all homodimer dyes exceeds that for monomers. However, fluorescence enhancement and intensity for almost all the dyes (with exception of **Dst-1**) in DNA complexes significantly surpass corresponding values for BSA complexes.

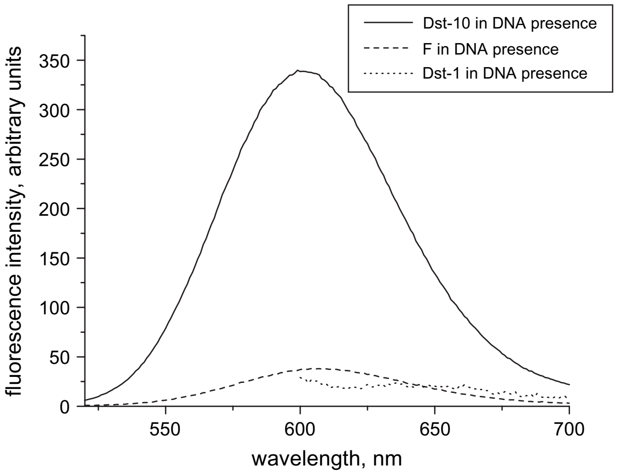


Fig. 4. Fluorescence spectra of styryl dyes **F**, **Dst-1**, and **Dst-10** in the presence of DNA.

2.5. Computer simulations of the dimer absorption spectra

As it was described above, absorption and emission maxima of dimers with short (1 to 2 carbon atoms) linkage group length (**Dst-1**, **Dst-2**) are significantly shifted to the long-wave region compared to that of parent monomer and dimers with long linkage groups (5 or more carbon atoms). We suggested that the nature of this shift is the interaction between the styryl chromophores in a dimer. For the dimers with short linkage groups (1 or 2 carbon atoms) because of small distance between the bound chromophores, a spectral effect similar to that of molecular aggregates is observed. To support this suggestion, we performed the computer simulation of the absorption spectra of the dye **F** and its dimers **Dst-1**, **Dst-2** and **Dst-5** (Fig. 5). The conformation of linkage group of dimer dyes was constructed according to the sp^3-hybridization for carbon atoms (C–C bond length, 1.54 Å; C–C–C angle, approximately 110 °).

The results of the calculation are presented in Table 2. For the monomer dye **F**, the calculated absorption wavelength value ($\lambda_{\text{calc}} = 458$ nm) is close to the experimentally obtained one ($\lambda_{\text{abs}} = 452$ nm). For the dimer dyes **Dst-1**, **Dst-2** and **Dst-5**, two values of absorption wavelength were obtained (λ^1_{calc}, λ^2_{calc}). This result could be explained by the splitting of lowest excited singlet electronic energy levels of dimer chromophores into two levels of different energy due to the chromophore interaction. This conclusion is supported

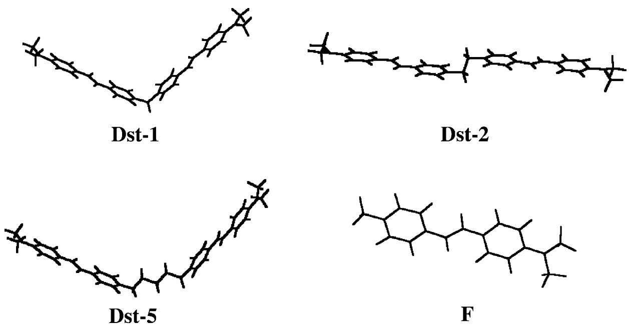


Fig. 5. The optimized geometry of the dye **F** molecule and constructed geometry of its dimers **Dst-1**, **Dst-2** and **Dst-5**, for which the absorption spectrum calculation was performed.

Table 2
Experimentally obtained wavelengths of absorption spectrum maxima (λ_{abs}), and the calculated wavelengths (λ^1_{calc}, λ^2_{calc}) and corresponding values of oscillator strength (f^1, f^2) of the S_0–S_1 transition of the dye **F** and its dimers **Dst-1**, **Dst-2** and **Dst-5**

Dye	λ_{abs}	λ^1_{calc}	f^1	λ^2_{calc}	f^2
F	452	458	1.7	–	–
Dst-1	542	527	2.4	457	1.3
Dst-2	499	517	3.2	472	0.06
Dst-5	460	485	1.9	468	1.2

by the results obtained for oscillator strength values (f^1 and f^2) for the calculated transitions. The oscillator strength value determines the transition intensity. It is seen from Table 2 that for the dyes **Dst-1** and **Dst-5**, for which the chromophores are nonparallel to another, both the values of f^1 and f^2 are comparable (2.4 and 1.3 for **Dst-1**, 1.9 and 1.2 for **Dst-5**, respectively). At the same time, for **Dst-2** its two chromophores are parallel and form the so-called "head to tail" conformation, which is typical for J-aggregates. According to the calculation results for **Dst-2** (Table 2), the long-wave transition ($f^1 = 3.2$) is much more intensive than the short-wave one. The oscillator strength of the short-wave transition ($f^2 = 0.06$) is two orders of magnitude less than f^1, and thus the short-wave transition could be considered as prohibited. The same effect, namely the manifestation of the most strongly long-wave shifted transition in the absorption spectrum, is observed in "head to tail" molecular aggregates (J-aggregates).

According to the calculation results (Table 2), the most intensive transition wavelength (λ^1_{calc}) of the dimer is shifted to the long-wave region as compared to the monomer, and for dimer with shorter linkage group the shift is larger than for the dimer with longer one (λ^1_{calc} is 527, 517 and 485 nm for **Dst-1**, **Dst-2** and **Dst-5**, respectively, while for **F** it is 458 nm). Thus the results of computer simulation (λ^1_{calc}) generally agree with the experimental results (λ_{abs}). The less intensive transition (λ^2_{calc}) is not clearly observed experimentally in the absorption spectra because the spectral bands are wide enough for more intensive band to overlap the less intensive one. The shift of the calculated dimer transition wavelength λ^1_{calc} as compared to monomer one is due to the interaction between chromophores in the dimer. This interaction is stronger for short linkage groups and rather weak for long ones, thus the wavelength shift for the dimers with short linkage groups is larger than for those with long linkage groups.

Thus, the computer simulation confirms the assumption that the interaction between the chromophores is the reason for the experimentally observed long-wave shift of the absorption maximum of dimers with the short linkage group as compared to the parent monomer.

3. Conclusions

1. A series of homodimer styryl dyes based on (*p*-dimethylaminostyryl)pyridinium, (*p*-dimethylaminostyryl)benzoxazolium, (*p*-dimethylaminostyryl)benzothiazolium, (*p*-dimethylaminostyryl)-1,3,3-trimethyl-3*H*-indolium residues have been synthesized and their spectral-luminescent properties in the presence of biopolymers were first studied.
2. Strong dependence of spectral-luminescent properties of homodimers on the length of the linkage group was shown. Absorption and emission maxima of the homodimers with short linkage group (**Dst-1**, **Dst-2**) are significantly shifted to the long-wave region relative to those of parent monomer and homodimers with long linkage group.
3. It was shown that for the obtained homodimer dyes the intrinsic fluorescence intensity is lower than that for parent monomers. Synthesized dimer dyes with the linkage group length of 2 or more carbon atoms demonstrated increased binding affinity to DNA. For parent monomers and for the dyes with shortest linkage group (1 carbon atom) slight RNA preference was shown.
4. DNA-binding affinity of (*p*-dimethylaminostyryl)pyridinium homodimers with long linkage group significantly exceeds that of parent monomer that is known as fluorescent dye for DNA analysis [3]. We consider that these homodimers could be used as sensitive, DNA specific probes.
5. Computer simulation of absorption spectra of the dimers was in agreement with the experimentally obtained results. The long-wave shift of absorption maxima for the dimers with short linkage group relative to the maximum of parent monomer is connected with the interaction between the dimer chromophores. The same effect is observed for molecular aggregates.

4. Experimental

The styryl dimer dyes were synthesized according to the procedures described below. Structures of obtained compounds were confirmed with ^{1}H NMR and elemental analysis.

4.1. Protocol 1. Preparation of quaternary salts of γ-picoline

A mixture of 0.011 mol γ-picoline and 0.005 mol dihalogenalkane was heated for 3 h at 120 °C. To the obtained alloy, 5 ml of isopropanol was added and then the mixture was refluxed for 3 h. After cooling, the product was filtered off and washed with diethyl ether. Quaternary salts were used without further purification.

4.2. Protocol 2. Preparation of quaternary salts of heterocycles

A mixture of 0.011 mol of compound (*I*) and 0.005 mol dihalogenalkane in 5 ml of dioxane was boiled for 6 h. Obtained salt was precipitated by diethyl ether and filtered off. Precipitate was washed with isopropanol and ether. Quaternary salts were used without further purification.

4.3. Protocol 3. Preparation of dyes ***Dst-2***, ***Dst-5***, ***Dst-10*** and ***F***

A mixture of 0.001 mol of quaternary salt, 0.002 mol of *p*-dimethylaminobenzaldehyde and 6 drops of piperidine in 5 ml of *n*-butanol was boiled for 4 h. Crystalline dye precipitate was filtered off, washed with isopropanol and ether and crystallized from DMF–methanol mixture.

4.4. Protocol 4. Preparation of dyes ***Dbo-10***, ***Dil-10***, ***Dbt-10***, ***Dbt-5***, ***Sbt***, ***Sbo*** and ***Sil***

A mixture of 0.001 mol of quaternary salt, 0.002 mol of *p*-dimethylaminobenzaldehyde in 3 ml of acetic anhydride was boiled for 10 min. Obtained dye was precipitated by diethyl ether and filtered off. Precipitate was washed with alcohol and crystallized from DMF–methanol mixture.

4.5. Spectral-luminescent studies

Absorption spectra were obtained with Specord M 40 spectrophotometer (Germany). Fluorescence excitation and emission spectra were obtained with a Cary Eclipse fluorescence spectrophotometer (Australia). All measurements were carried out at room temperature.

4.6. Preparation of working solutions

The dye stock solutions with concentration 2×10^{-3} M were prepared in DMFA. Concentration of dye (per chromophore) in working solutions was 5×10^{-6} M. A 0.05 M Tris–HCl (pH = 8.0) buffer was used for the measurements. Total DNA from chicken erythrocytes, total yeast RNA, and BSA were purchased from Sigma. The nucleic acids concentrations in the working solutions were 6×10^{-5} M (b.p.) for DNA and 1.2×10^{-4} M (b) for RNA. Used concentration of BSA in working solutions was 0.2 mg/ml.

4.7. Computer simulation methods

We are thankful to Prof. O.I. Tolmachev (Institute of Organic Chemistry, NASci of Ukraine, Kyiv, Ukraine) for providing the possibility to use HyperChem 5.01 program package.

The structure of the monomer dye **F** was optimized with the semi-empirical calculation method AM-1 from the program package HyperChem 5.01. The dimer dyes **Dst-1**, **Dst-2** and **Dst-5** were constructed by attaching two optimized **F** dyes by a linkage group. The linkage group was constructed with a C–C bond length of 1.54 Å and a C–C–C angle of approximately 110° (sp^3-hybridization for carbon atoms). The parameters (wavelength and oscillator strength) of the transition between highest occupied and lowest unoccupied electronic orbital were calculated with the semi-empirical method ZINDO/S. For construction of the excited state function with the method of configuration interaction, 16 single excited configurations (4×4 orbitals) were used.

Acknowledgements

We are thankful to Dr. O.D. Kachkovskiy for his help in computer simulation and for valuable discussion.

Appendix A. ^{1}H NMR spectra and results of elemental analysis of synthesized dyes

Appendix A1. 4-[2-(4-Dimethylaminophenyl)-1-ethenyl]-1-4-[2-(4-dimethylaminophenyl)-1-ethenyl]-1-pyridiniumylmethylpyridinium diiodide (*Dst-1*)

Yield: 60%; m.p.: 208–210 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 3.06 (12H, s), 6.83 (6H, m), 7.24 (2H, d, $J = 18.0$ Hz), 7.65 (4H, d, $J = 9.0$ Hz), 8.10 (2H, d, $J = 18.6$ Hz), 8.16 (4H, d, $J = 9.3$), 9.03 (4H, br d, $J = 5.0$ Hz). Anal. calcd. for $C_{31}H_{34}I_2N_4$: C, 51.97; H, 4.78; N, 7.82; I, 35.43. Found: C, 51.73; H, 4.81; N, 7.74; I, 35.50.

Appendix A2. 4-[2-(4-Dimethylaminophenyl)-1-ethenyl]-1-(2-4-[2-(4-dimethylaminophenyl)-1-ethenyl]-1-pyridiniumylethyl)pyridinium diperchloride (*Dst-2*)

Yield: 83%; m.p.: 233–235 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 3.05 (12H, s), 5.69 (2H, dd, $J_1 = 9.7$ Hz, $J_2 = 3.3$ Hz), 7.22 (2H, dd, $J_1 = 17.3$ Hz, $J_2 = 3.3$ Hz), 7.62 (4H, d, $J = 10.0$ Hz), 8.10 (6H, m), 8.90 (4H, d, $J = 7.7$ Hz). Anal. calcd. for $C_{32}H_{36}I_2N_4$: C, 52.62; H, 4.97; N, 7.67; I, 34.75. Found: C, 52.39; H, 4.95; N, 7.70; I, 34.67.

*Appendix A3. 4-[2-(4-Dimethylaminophenyl)-1-ethenyl]-1-(5-4-[2-(4-dimethylaminophenyl)-1-ethenyl]-1-pyridiniumylpentyl)pyridinium diiodide (**Dst-5**)*

Yield: 87%; m.p.: 285–286 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.23 (2H, m), 2.95 (4H, quint, $J = 7.3$ Hz), 3.02 (12H, s), 4.44 (4H, t, $J = 7.3$ Hz), 6.78 (4H, d, $J = 10.0$ Hz), 7.17 (2H, d, $J = 17.7$ Hz), 7.58 (4H, d, $J = 9.7$ Hz), 7.95 (2H, d, $J = 18.0$ Hz), 8.08 (4H, d, $J = 7.7$ Hz), 8.77 (4H, d, $J = 7.7$ Hz). Anal. calcd. for $C_{35}H_{42}I_2N_4$: C, 54.41; H, 5.48; N, 7.25; I, 32.85. Found: C, 54.52; H, 5.44; N, 7.16; I, 32.74.

*Appendix A4. 4-[2-(4-Dimethylaminophenyl)-1-ethenyl]-1-(10-4-[2-(4-dimethylaminophenyl)-1-ethenyl]-1-pyridiniumyldecyl)pyridinium diiodide (**Dst-10**)*

Yield: 88%; m.p.: 278–279 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.27 (12H, m), 1.88 (4H, t, $J = 6.7$ Hz), 3.03 (12H, s), 4.42 (4H, t, $J = 7.6$ Hz), 6.79 (4H, d, $J = 10.0$ Hz), 7.20 (2H, d, $J = 17.7$ Hz), 7.60 (4H, d, $J = 10.0$ Hz), 7.95 (2H, d, $J = 17.7$ Hz), 8.08 (4H, d, $J = 7.7$ Hz), 8.79 (4H, d, $J = 8.0$ Hz). Anal. calcd. for $C_{40}H_{52}I_2N_4$: C, 57.01; H, 6.22; N, 6.65; I, 30.12. Found: C, 56.94; H, 6.25; N, 6.59; I, 30.23.

*Appendix A5. 2-[2-(4-Dimethylaminophenyl)-1-ethenyl]-3-(5-2-[2-(4-dimethylaminophenyl)-1-ethenyl]-1,3-benzothiazolium-3-ylpentyl)-1,3-benzothiazolium diiodide (**Dbt-5**)*

Yield: 78%; m.p.: 266–268 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.50 (2H, m), 1.89 (4H, m), 3.14 (12H, s), 4.79 (4H, m), 6.82 (4H, d, $J = 9.7$ Hz), 7.52 (2H, d, $J = 16.7$ Hz), 7.68 (4H, m), 7.83 (4H, d, $J = 9.0$ Hz), 7.92 (2H, d, $J = 16.7$ Hz), 8.07 (2H, d, $J = 8.7$ Hz), 8.28 (2H, d, $J = 8.7$ Hz). Anal. calcd. for $C_{39}H_{42}I_2N_4S_2$: C, 52.95; H, 4.78; N, 6.33; I, 28.69. Found: C, 52.83; H, 4.80; N, 6.39; I, 28.73.

*Appendix A6. 2-[2-(4-Dimethylaminophenyl)-1-ethenyl]-3-(10-2-[2-(4-dimethylaminophenyl)-1-ethenyl]-1,3-benzoxazolium-3-yldecyl)-1,3-benzoxazolium diiodide (**Dbo-10**)*

Yield: 82%; m.p.: 127–129 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.35 (12H, m), 1.84 (4H, m), 3.12 (12H, s), 4.57 (4H, m), 6.85 (4H, d, $J = 9.7$ Hz), 7.35 (2H, d, $J = 17.0$ Hz), 7.67 (4H, m), 7.95 (8H, m), 8.25 (2H, d, $J = 17.0$ Hz). Anal. calcd. for $C_{44}H_{52}I_2N_4O_2$: C, 57.27; H, 5.68; N, 6.07; I, 27.51. Found: C, 57.21; H, 5.64; N, 5.59; I, 27.62.

*Appendix A7. 2-[2-(4-dimethylaminophenyl)-1-ethenyl]-3-(10-2-[2-(4-dimethylaminophenyl)-1-ethenyl]-1,3-benzothiazolium-3-yldecyl)-1,3-benzothiazolium diiodide (**Dbt-10**)*

Yield: 86%; m.p.: 237–239 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.32 (12H, m), 1.78 (4H, m), 3.08 (12H, s), 4.80 (4H, m), 6.80 (4H, d, $J = 10.0$ Hz), 7.58 (2H, d, $J = 16.0$ Hz), 7.70 (2H, t, $J = 8.3$ Hz), 7.80 (4H, t, $J = 8.0$ Hz), 7.91 (4H, d, $J = 8.7$ Hz), 8.11 (4H, m), 8.34 (2H, d, $J = 8.3$ Hz). Anal. calcd. for $C_{44}H_{52}I_2N_4S_2$: C, 55.35; H, 5.49; N, 5.87; I, 26.58. Found: C, 55.21; H, 5.45; N, 5.81; I, 26.61.

*Appendix A8. 2-[2-(4-Dimethylaminophenyl)-1-ethenyl]-1-(10-2-[2-(4-dimethylaminophenyl)-1-ethenyl]-3,3-dimethyl-3H-1-indoliumyldecyl)-3,3-dimethyl-3H-indolium diiodide (**Dil-10**)*

Yield: 81%; m.p.: 260–262 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.30 (12H, m), 1.76 (12H, s), 4.54 (4H, t, $J = 8.0$ Hz), 6.88 (4H, d, $J = 10.3$ Hz), 7.25 (2H, d, $J = 17.0$), 7.52 (4H, m), 7.71 (2H, d, $J = 8.7$ Hz), 7.80 (2H, d, $J = 10.0$ Hz), 8.10 (4H, d, $J = 10.0$ Hz), 8.35 (2H, d, $J = 16.7$ Hz). Anal. calcd. for $C_{50}H_{64}I_2N_4$: C, 61.60; H, 6.62; N, 5.75; I, 26.03. Found: C, 61.47; H, 6.65; N, 5.69; I, 26.14.

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