Dyes and Pigments 90 (2011) 201–210

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

Review

The synthesis, structure and spectral properties of new long-wavelength benzodipyrroleninium-based bis-styryl dyes

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ARTICLE INFO

Article history:
Received 19 August 2010
Received in revised form
2 December 2010
Accepted 7 December 2010
Available online 15 December 2010

Keywords:
Bis-styryl dyes
Benzodipyrrolenine
Biscyanine dyes
Synthesis
Molecular structure
Spectral properties

ABSTRACT

Novel bis-styryl dyes based on a quaternized centrosymmetrical benzodipyrrolenine moiety were synthesized by reaction of a quaternized benzodipyrrolenine with two equivalents of 4-dimethylaminobenzaldehyde, 4-dimethylaminocinnamoic aldehyde or 4-(3,5-diphenyl-4,5-dihydro-1*H*-1-pyrazolyl) benzaldehyde. The molecular structures and spectral properties of these dyes were investigated using PM3 and PPP CI quantum chemical simulations and compared with biscyanines and parent “monomeric” styryl dyes. Conjugation of two uniform “monomeric” styryl chromophores to form a bis-chromophoric system was found to cause a pronounced red-shift (~100 nm) of the absorption and emission maxima, an increase of the extinction coefficients but also a conformational rigidization of the molecules. This results in better vibrationally resolved absorption and emission bands and, in general, in increased quantum yields. Bis-styryl dyes absorb and emit in the red and near-IR spectral region (643–812 nm). They have high extinction coefficients (≤133,000 M^{-1} cm^{-1}), large Stokes’ shifts (up to 55 nm in chloroform and 90 nm in methanol), and quantum yields in chloroform ≤10%, whereas polar solvents decrease quantum yields. Despite their symmetrical structures these bis-styryl dyes exhibit spectral properties in solvents of different polarity that are more resemble those of unsymmetrical “monomeric” styryls.

1. Introduction

Red and near-infrared absorbing and emitting polymethine dyes are currently of considerable technical interest due to their tremendous potential for practical applications such as optical recording techniques [1,2], non-linear optics [3,4], fluorescent probes for biological research and biomedical assays particularly for binding with nucleic acids [5–8] and proteins [9], tumor imaging and photodynamic therapy [10–12]. Molecular structures and spectral properties of the compounds containing a polymethine chromophore system were thoroughly investigated [13–16]. A promising approach to design longer-wavelength dyes with advantageous properties consists in the conjugation of two parent “monomeric” polymethine chromophores in one molecule to bis-polymethine dyes [17], which until now have only been sparsely investigated.

Recently we reported the synthesis, molecular structures and spectral properties of a series of bis-trimethine cyanine dyes **1a**–**1e** (Scheme 1) all containing a centrosymmetrical benzodipyrrolene moiety and variable terminal heterocyclic end-groups [18]. Conjugation of two uniform “monomeric” trimethine chromophores to form a bis-trimethine molecule was found to cause splitting of the long-wavelength absorption band of the parent “monomeric” dye into two bands, which was in good agreement with Kiprianov’s theory [17]. One of these new bands was red-shifted while the other was blue-shifted compared to the position of the original long-wavelength absorption maximum in the parent dye. Also, in the example of these dyes it was demonstrated that fusion of two trimethine chromophores into one biscyanine molecule leads not only to a substantial red-shift of the absorption and emission bands but also to a noticeable increase of the extinction coefficients and fluorescence quantum yields, which is extremely important for many practical applications.

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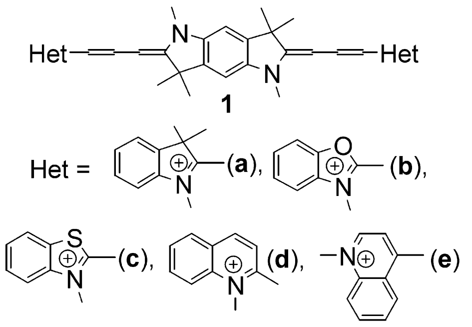
doi:10.1016/j.dyepig.2010.12.004



Scheme 1.

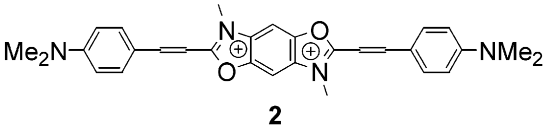
Despite the large variety of biscyanine dyes that were synthesized and investigated [17,19–23], there is a pronounced lack of data for bis-styryl dyes. Similar to cyanines, styryls belong to a subclass of polymethine dyes bearing a positive charge delocalized within the polymethine chain; however, contrary to cyanines which contain an odd number of methine groups, styryl dyes have an even number of these groups in the polymethine chain. These classes of dyes are very different in the regards to their molecular and electronic structures and spectral properties [24–26]. While a number of styryl dyes with a single polymethine chromophore and various heterocyclic and aromatic end-groups are described in the literature [16 and refs. cited therein], the only centrosymmetrical bis-styryl dye that was previously reported is the benzodioxazole-based dye **2** (Scheme 2) [21]. The absorption spectrum of this dye shows only one intense long-wavelength band, which was red-shifted by 86 nm as compared to the parent "monomeric" dye. The molecular structure and fluorescence properties of dye **2** were not investigated. Bis-styryl dyes with a centrosymmetrical benzodipyrroleninium moiety instead of a benzodioxazole central bridge have not been described, although just indolenine based polymethines are of main practical value due to their advantageous photophysical properties such as higher photo stability [26–28]. Thus, the majority of commercially available long-wavelength polymethine dyes such as **Cy** series (GE Healthcare), **Alexa Fluor** dyes (Life Technologies), **DyLight** dyes (*Dyomics*), **IRDyes** (*Li-Cor Biosciences*), **Seta** and **Square** dyes (*SETA Biomedicals*) are all based on indolenine or benzoindolenine heterocyclic end-groups [28].

This paper is aimed at describing the synthesis of a series of bis-styryl dyes **3a**–**3c** (Scheme 3) containing a benzodipyrroleninium moiety as the central bridging group and further to investigate the molecular structures and spectral properties of these dyes and to compare them to the "monomeric" styryls **4a**–**4c** and bis-trimethines **1a**–**1e** (Scheme 1).

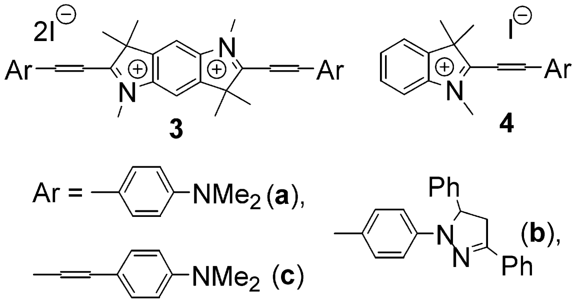
2. Experimental

2.1. General

Chemical reactions were monitored by TLC (Silica Gel 60 F_{254} TLC Plates, Merck). ^{1}H NMR spectra were measured on a *Varian Mercury-VX-200* (200 MHz) spectrometer in DMSO-d_6 using TMS as an internal standard.



Scheme 2.



Scheme 3.

Absorption and emission spectra were measured in chloroform and methanol at room temperature in a standard 1-cm quartz cells. The dye concentrations for the spectral measurements were in the range of $1.0–3.0 \times 10^{-7}$ M.

Absorption spectra were recorded on a *PerkinElmer Lambda 35* UV/Vis spectrophotometer. Absorption maxima were obtained with an accuracy of 0.5 nm. For measurement of the extinction coefficients (ε), each dye (7–10 mg) was dissolved in 50 mL methanol, the stock solution was diluted (1:2000) and the absorbance (A) was measured in a 5-cm standard quartz cell. The extinction coefficients were calculated according to the Lambert–Beer's law. The ε of each dye was independently measured several times and the average value was taken. The reproducibility for determining the extinction coefficients was about 1000 M^{-1} cm^{-1}.

Emission spectra and quantum yields (Φ_F) were taken on a *Varian Cary Eclipse* spectrofluorometer. The spectra were corrected. Emission maxima were obtained with an accuracy of 0.5 nm. For the determination of the quantum yields of dyes **1a** and **3a**–**3c**, the integrated relative intensities of the dyes were measured against **Cy5** (*GE Healthcare*) in water as the reference. To avoid aggregation the absorbances of the bis-styryl dyes solutions at the excitation wavelength (610 nm) were between 0.12 and 0.18 measured in a 5-cm cell. The emission spectra of the solutions were recorded and the absolute quantum yields (Φ_F) were determined as described in [29] using **Cy5** ($\Phi_F = 27\%$ [30]) as the reference dye for bis-styryl dyes. Rhodamine B ($\Phi_F = 97\%$ in ethanol [31]) was used as the reference dye to determine the quantum yields of trimethines **4a**–**4c**. The quantum yield of each dye was independently measured 3–4 times and the average value was calculated. The reproducibility was no worse than 5%.

Quantum chemical semi-empirical simulations were carried out by the PM3 method using standard parameterization [32,33]. Full geometry optimization of the ground state was undertaken. PPP CI calculations [34,35] were done for the PM3 optimized geometries using the following parameters: $\beta_{CN} = -1.9$ eV, $\beta_{CC} = -2.1$ eV, $E_N = -21.22$ eV, $\gamma_{NN} = 12.98$ eV [36].

2-(1,1,3-trimethyl-2,3-dihydro-1*H*-2-indenyliden) acetaldehyde (Fisher's aldehyde) and 4-dimethylaminobenzaldehyde were purchased from Sigma–Aldrich, 3-(4-dimethylaminophenyl) acrylaldehyde was from Merck. 4-(3,5-diphenyl-4,5-dihydro-1*H*-1-pyrazolyl) benzaldehyde was synthesized according to [37]. 2-(3-1,3,3,5,7,7-hexamethyl-6-[3-(1,3,3-trimethyl-3*H*-2-indoliumyl)-2-propenylidene]-1,2,3,5,6,7-hexahydropyrrolo[2,3-*f*]indol-2-yliden-1-propenyl)-1,3,3-trimethyl-3*H*-indolium (**1**a) was synthesized according to [18]. Other chemicals, reagents and solvents were from Merck.

2.2. Synthesis

2.2.1. 1,2,3,3,5,6,7,7-Octamethyl-3,7-dihydropyrrolo[2,3-f] indolediium diiodide (5) [38]

0.45 g of benzodipyrrolenine [38] was refluxed in large excess of methyl iodide for 7 h. The reaction mixture was diluted with ether;

the obtained precipitate was filtered off, washed with ether and dissolved in chloroform. The salt **5** was filtered off and washed with chloroform. Yield: 0.45 g (47%). ^{1}H NMR (200 MHz, DMSO-d$_6$, ppm): δ 8.53 (2H, s, arom H), 4.01 (6H, s, N—CH$_3$), 2.82 (6H, s, 2,2-CH$_3$), 1.59 (12H, s, $C(C\underline{H}_3)_2$). Anal. Calcd. for $C_{18}H_{26}N_2I_2$: C, 41.24; H, 4.95; N, 5.34%. Found: C, 41.64; H, 5.07; N, 5.74%.

2.2.2. *2,6-Di[2-(4-dimethylaminophenyl)-1-ethenyl]-1,3,3,5,7,7-hexamethyl-3,7-dihydropyrrolo[2,3-f]indolediium diiodide (**3a**)*

A mixture of 100 mg (0.2 mmol) of benzodipyrrolenine quaternary salt **5** and 60 mg (0.42 mmol) of 4-dimethylaminobenzaldehyde in 5 mL of acetic anhydride was refluxed for 40 min. After cooling the reaction mixture was diluted with ether, the precipitate was filtered, washed with ether and column purified (Silica gel 60, 20% methanol—chloroform) to yield 70 mg (47%) of **3a**; mp. 263–265 °C ^{1}H NMR (200 MHz, DMSO-d$_6$, ppm): δ 8.34 (2H, d, J = 15.5 Hz, ethylene), 8.21 (2H, s, dipyrrolenine arom H), 8.09 (4H, d, J = 8.9 Hz, arom H), 7.25 (2H, d, J = 15.4 Hz, ethylene), 6.91 (4H, d, J = 8.9 Hz, arom H), 4.01 (6H, s, N$^+$CH$_3$), 3.19 (12H, s, N(CH$_3$)$_2$), 1.81 (12H, s, C(CH$_3$)$_2$). Anal. Calcd. for $C_{36}H_{44}N_4I_2$: C, 54.92; H, 5.59; N, 7.12%. Found: C, 54.85; H, 5.70; N, 6.91%.

2.2.3. *2,6-Di-2-[4-(3,5-diphenyl-4,5-dihydro-1H-1-pyrazolyl)phenyl]-1-ethenyl-1,3,3,5,7,7-hexamethyl-3,7-dihydropyrrolo[2,3-f]indolediium diiodide (**3b**)*

A mixture of 100 mg (0.2 mmol) of benzodipyrrolenine quaternary salt **5** and 140 mg (0.4 mmol) of 4-(3,5-diphenyl-4,5-dihydro-1*H*-1-pyrazolyl)benzaldehyde [37] in 5 mL of acetic anhydride was refluxed for 30 min. After cooling the reaction mixture was diluted with ether, filtered, washed with ether and column purified (Silica gel 60, 10% methanol—chloroform) to give 70 mg (45%) of **3b**; mp. 297–299 °C ^{1}H NMR (200 MHz, DMSO-d$_6$, ppm): δ 8.33 (2H, d, J = 15 Hz, ethylene), 8.29 (2H, s, dipyrrolenine arom H), 8.09 (4H, d, J = 8.1 Hz, arom H), 8.2–7.3 (20H, m, arom. H and 2H ethylene), 7.19 (4H, d, J = 8.3 Hz, arom H), 5.93–5.85(4H, m, pyrazoline ring), 4.03(6H, s, N$^+$CH$_3$ and 2H, pyrazoline ring), 1.80(12H, s, C(CH$_3$)$_2$). Anal. Calcd. for $C_{62}H_{58}N_6I_2$: C, 65.21; H, 5.08; N, 7.36%. Found: C, 65.32; H, 5.24; N, 6.96%.

2.2.4. *2,6-Di[4-(4-dimethylaminophenyl)-1,3-butadienyl]-1,3,3,5,7,7-hexamethyl-3,7-dihydropyrrolo[2,3-f]indolediium diiodide (**3c**)*

96 mg (0.18 mmol) of benzodipyrrolenine quaternary salt **5** and 95 mg (0.54 mmol) of 3-(4-dimethylaminophenyl) acrylaldehyde were dissolved in 5 mL of acetic anhydride and the mixture was refluxed for 1 h. After cooling, the reaction mixture was diluted with benzene, filtered, washed with benzene and dried. Yield 90 mg (60%); mp. 320–323 °C ^{1}H NMR (200 MHz, DMSO-d$_6$, ppm): δ 8.34 (2H, t, J = 14.7 Hz, CH), 8.25 (2H, s, dipyrrolenine arom H), 7.78 (2H, d, J = 14.7 Hz, CH), 7.61 (4H, d, J = 8.8 Hz, arom H), 7.33 (2H, t, J = 14.7 Hz, CH), 6.94 (2H, t, J = 15 Hz, CH), 6.85 (4H, d, J = 8.8 Hz, arom H), 3.91 (6H, s, N$^+$CH_3), 3.11 (12H, s, N(CH_3)$_2$), 1.78 (12H, s, indolenine C(CH$_3$)$_2$). Anal. Calcd. for $C_{40}H_{48}N_4I_2$: C, 57.24; H, 5.72; N, 6.68%. Found: C, 57.38; H, 5.59; N, 6.75%.

2.2.5. *General procedure for synthesis of styryl dyes **4a**–**4c***

A mixture of 0.30 mmol of 1,2,3,3-tetramethylindolenium iodide **6** and 0.32 mmol of corresponding aldehyde was refluxed in 10 mL of acetic anhydride for 1 h. The reaction mixture was poured into water and the solvent was removed under reduced pressure by a rotary evaporator. The residue was re-crystallized from ethanol.

2.2.5.1. *2-(4-Dimethylaminophenyl)-1-ethenyl]-1,3,3-trimethyl-3H-indolium iodide (**4a**).*

Yield 82%; mp. 175–177 °C ^{1}H NMR (200 MHz, DMSO-d$_6$, ppm): δ 8.31 (1H, d, J = 16 Hz, ethylene), 8.07 (2H, d, J = 9 Hz, arom H), 7.83–7.62 (4H, m, indolenine), 7.25 (1H, d, J = 15.7 Hz, ethylene), 6.87 (2H, d, J = 8.5 Hz, arom H), 3.96 (3H, s, N$^+$CH$_3$), 3.19 (6H, s, N(CH$_3$)$_2$), 1.74 (6H, s, C(CH$_3$)$_2$). Anal. Calcd. for $C_{21}H_{25}N_2I$: C, 58.29; H, 5.78; N, 6.48%. Found: C, 58.17; H, 5.62; N, 6.53%.

2.2.5.2. *2-(E)-2-[4-(3,5-diphenyl-4,5-dihydro-1H-1-pyrazolyl)phenyl]-1-ethenyl-1,3,3-trimethyl-3H-indolium iodide (**4b**).*

Yield 80%; mp. 245 °C ^{1}H NMR (200 MHz, DMSO-d$_6$, ppm): δ 8.29 (12H, d, J = 15.4 Hz, ethylene), 8.04 (2H, d, J = 8.1 Hz, arom H), 7.9–7.2 (15H, m, arom. H and 1H ethylene), 7.17 (2H, d, J = 8.4 Hz, arom H), 5.92–5.80 (2H, m, pyrazoline ring), 4.03(1H, d, pyrazoline ring), 3.96(3H, s, N$^+$CH$_3$), 1.75(6H, s, C(CH$_3$)$_2$). Anal. Calcd. for $C_{34}H_{32}N_3I$: C, 66.94; H, 5.25; N, 6.89%. Found: C, 66.90; H, 5.34; N, 6.96%.

2.2.5.3. *2-[(1E,3E)-4-(4-dimethylaminophenyl)-1,3-butadienyl]-1,3,3-trimethyl-3H-indolium iodide (**4c**).*

Yield 75%; mp. 240 °C ^{1}H NMR (200 MHz, DMSO-d$_6$, ppm): δ 8.31 (1H, t, 15 Hz, CH), 7.85–7.45 (7H, m, indolenine, arom., ethylene), 7.29 (1H, t, J = 14.7 Hz, CH), 6.95 (1H, d, J = 14.7 Hz, CH), 3.89 (3H, s, N$^+$CH$_3$), 3.08 (6H, s, N(CH$_3$)$_2$), 1.71 (6H, s, C(CH$_3$)$_2$). Anal. Calcd. for $C_{23}H_{27}N_2I$: C, 60.21; H, 5.89; N, 6.11%. Found: C, 60.35; H, 5.71; N, 6.19%.

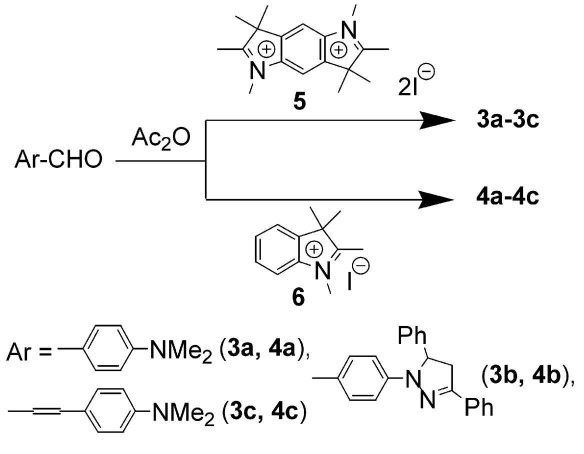
3. Results and discussion

3.1. *Synthesis*

Bis-styryl dyes **3a**–**3c** were synthesized in good yields (45–60%) *via* condensation of benzodipyrrolenine di-quaternary salt **5** [38] with two equivalents of 4-dimethylaminobenzaldehyde, 4-(3,5-diphenyl-4,5-dihydro-1*H*-1-pyrazolyl)benzaldehyde [37] and 4-dimethylaminocinnamoic aldehyde, respectively, in acetic anhydride under reflux (Scheme 4). The formation of undesirable mono-condensation product was avoided by addition of an excess of aldehyde. The “monomeric” styryl dyes **4a**–**4c** were obtained under the same reaction conditions with quaternized indolenine **6** as the starting material.

3.2. *Molecular structures*

The structures of bis-styryls **3a**–**3c** and parent styryls **4a**–**4c** were confirmed by ^{1}H NMR data. Similarly to bis-trimethine dyes **1a**–**1e** [18], the spin–spin coupling constant (J) of the methine hydrogens are in the range of 13.5–15.0 Hz indicating that the polymethine chain in both series **3a**–**3c** and **4a**–**4c** is in all-*trans* conformation. This conformation seems not to depend on the



Scheme 4.

terminal end-groups (**1**a, **3a**, **3b**, **4a**, **4b**) or the chain length (dimethines **3a** and **4a** or tetramethines **3c** and **4c**). The conformational analysis using the quantum chemical semi-empirical method PM3 [32,33] indicates a global minimum of the potential energy just for this all-trans form. In this conformation the angle between principle axes of the two uniform parent chromophores in bis-styryl molecules is close to 180°. The conformation of the terminal dimethylamino groups in dyes **3a**, **3c**, **4a** and **4c** ensures their strong conjugation with the main chromophore π–electron system. The 1,3-diphenylpyrazolinyl moiety in dyes **3b** and **4b** has a planar form and is also well-conjugated with the polymethine chromophore. The 5-phenyl ring is out of plane with the pyrazoline ring and therefore not in conjugation with the main chromophore system.

Importantly, quantum chemical simulations show that the minimal energy barrier of the internal rotation in bis-styryl molecules **3a**–**3c** is higher compared to that of the parent styryl dyes **4a**–**4c** by a factor of about 1.5–1.7 (Fig. 1, Table 1). Thus the minimal rotational barrier for the "monomeric" styryl dye **4a** corresponding to the rotation around the single bond connected to indolenine moiety (ϕ_1) is 5.57 kcal/mol while for the bis-styryl **3a** this barrier is 9.37 kcal/mol and corresponds to the rotation around the single bonds connected to the dimethylamino group (ϕ_6). The rotational energy for the benzodipyrroneninium ($\Delta H^{\neq}(\phi_1)$) and dimethylaminophenyl ($\Delta H^{\neq}(\phi_5)$) moieties in the bis-styryl **3a** are also about twice as high as compared to $\Delta H^{\neq}(\phi_5)$ and $\Delta H^{\neq}(\phi_6)$ of the parent styryl **4a**. Thus, the "dimerization" of styryl **4a** to **3a** causes a pronounced increase of the three rotational barriers $\Delta H^{\neq}(\phi_1)$, $\Delta H^{\neq}(\phi_5)$ and $\Delta H^{\neq}(\phi_6)$ by 7.47 kcal/mol, 6.78 kcal/mol and 3.75 kcal/mol, respectively. The rotation around ϕ_2 in both **3a** and **4a** is not probable due to the high $\Delta H^{\neq}$.

A similar increase in $\Delta H^{\neq}$ is also observed for dyes **3b**, **4b** and **3c**, **4c** (Table 1). The minimal rotational barrier in these molecules corresponds to the rotation around pyrazoline moiety and dimethylamino group, respectively.

Based on this result one can conclude that the conjugation of two parent styryl chromophores to a bis-styryl system results in an increase of the conformational rigidity: the molecule containing two conjugated chromophores becomes less flexible and statistically more planar.

Importantly such a correlation is not observed in the series of bis-trimethine dyes **1a**–**1e**. The minimal rotational energy barriers in the bis-trimethine **1a** (7.82 kcal/mol and 9.00 kcal/mol) are even less than for the parent trimethine dye **7** (13.31 kcal/mol and 13.55 kcal/mol) (Table 1). Contrary to styryl dyes, the "dimerization" of trimethine **7** to **1a** decreases the $\Delta H^{\neq}$ of ϕ_2 and ϕ_5 by 5.73 and 4.31 kcal/mol but increases the $\Delta H^{\neq}$ of ϕ_1 and ϕ_4 by 5.02 and 5.68 kcal/mol, respectively, which does not cause a pronounced change of the overall conformational flexibility of the molecule.

Table 1
Rotational energy barriers ($\Delta H^{\neq}$) in polymethines (**4a**–**4c**, **7**) and bis-polymethines (**1a, 3a**–**3c**).

Dye	$\Delta H^{\neq}(\phi_1)$ [kcal/mol]	$\Delta H^{\neq}(\phi_2)$ [kcal/mol]	$\Delta H^{\neq}(\phi_3)$ [kcal/mol]	$\Delta H^{\neq}(\phi_4)$ [kcal/mol]	$\Delta H^{\neq}(\phi_5)$ [kcal/mol]	$\Delta H^{\neq}(\phi_6)$ [kcal/mol]
1a	18.33	7.82	–	19.23	9.00	–
3a	13.04	>35	–	–	13.92	9.37
3b	11.50	>35	–	–	11.12	9.01
3c	10.85	7.87	10.25	9.11	8.31	6.49
4a	5.57	>35	–	–	7.14	5.62
4b	7.45	>35	–	–	7.15	5.54
4c	7.32	15.60	6.73	15.55	5.30	4.28
7	13.31	13.55	–	13.55	13.31	–

The rotational barriers decrease in the order of **3a** > **3b** > **3c**, which results in increase of the conformational flexibility of the molecules.

The PM3-calculated energy barriers of the internal rotation and the electron density distributions evidence that both bis-styryl and parent styryl dyes exist mostly in the canonical (contributing) form **A** with a positive charge on the indolenine or benzodipyrrolenium moieties (Fig. 1). Because of this the delocalized positive charge in bis-styryl molecules **3a**–**3c** is shifted from the terminal nitrogens (q_{Term}) to the central benzodipyrrolenine nitrogens (q_{Centr}), which results in a positive $\Delta q = q_{\text{Centr}} - q_{\text{Term}}$ (Table 2). At the same time opposite effect (negative Δq) was observed for bis-trimethine dyes such as **1**a, where a positive charge predominated on the terminal heterocyclic end-groups [18].

Remarkably, bis-styryl molecules have lower polarity compared to parent styryls: the Δq values in **3a**–**3c** are about 4–5.5 times lower than those for **4a**–**4c**. This should cause less pronounced solvation effects in bis-styryls compared to parent styryls.

The quantum chemical simulations of the electron density distribution are in good agreement with ^{1}H NMR data: similar to bis-trimethine dyes [18] we found clear correlations between the hydrogen chemical shifts (δ) for the bis-styryl molecules and the calculated charges (q) on nitrogens of central and terminal moieties

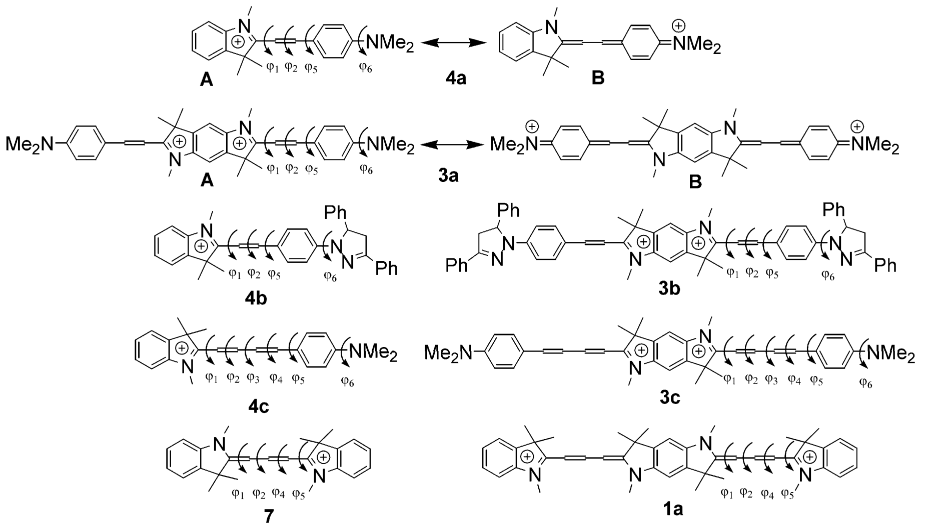
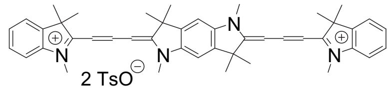
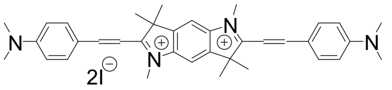
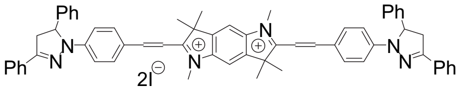
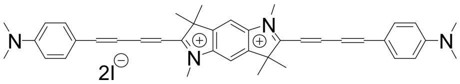
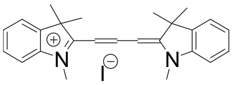
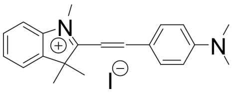
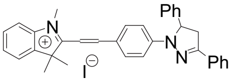
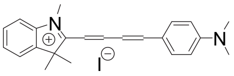


Fig. 1. Resonance forms and internal rotation angles in polymethine and bis-polymethine molecules.

Table 2
The PM3-calculated electron charges on nitrogen atoms of the central (q_{Centr}) and terminal (q_{Term}) moieties of dyes **1a**, **3a**–**3c**, **4a**–**4c**, **7** and the experimental chemical shifts of methyl hydrogen atoms at nitrogens of the central (δ_{Centr}) and terminal (δ_{Term}) moieties.

Molecule	Dye	q_{Term}	q_{Centr}	Δq	δ_{Term} [ppm]	δ_{Centr} [ppm]	$\Delta\delta$ [ppm]
1a		+0.392	+0.226	−0.166	3.69	3.65	−0.04
3a		+0.187	+0.263	+0.076	3.19	4.01	+0.82
3b		+0.198	+0.248	+0.050	–	4.03	–
3c		+0.173	+0.235	+0.062	3.10	3.90	+0.80
7		+0.303	+0.303	0.0			
4a		+0.090	+0.390	+0.300	3.19	3.96	+0.77
4b		+0.104	+0.377	+0.273	–	3.96	–
4c		+0.065	+0.380	+0.315	3.08	3.89	+0.81

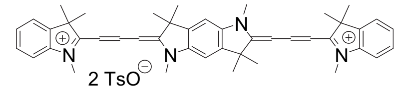
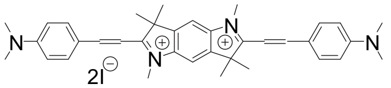
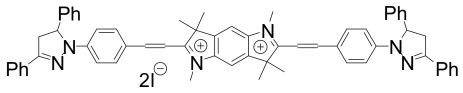
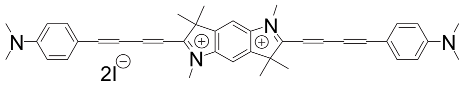
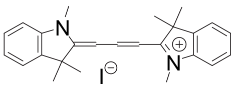
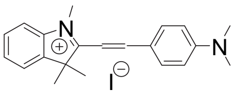
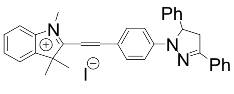
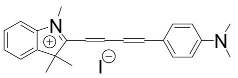
as well as between Δδ and Δq values. The proton signals of the methyl groups at the central benzodipyrrolene nitrogens (δ_{Centr}) of dye **1**a are observed at higher magnetic fields as compared to those of the methyl hydrogens at the terminal indolenine nitrogens (δ_{Term}), showing a negative Δδ while positive Δδ's were found for bis-styryl dyes **3a** and **3c** (Table 2). The aromatic hydrogen signals of the benzodipyrroleninium (**3a**–**3c**) can be found at lower magnetic fields (8.21, 8.29 and 8.24 ppm, respectively) showing higher positive charge on the central moiety as compared to the biscyanine **1**a (δ 7.85 ppm). The different pattern of the charge distribution in the bis-styryl molecules **3a**–**3c** compared to bis-cyanine **1**a is probably due to the different charge alteration on the polymethine chains: bis-styryls have the even number of methine groups while biscyanines have an odd number. The nature of the different charge distribution in conventional ("monomeric") cyanine and styryl dyes was discussed elsewhere [24–26].

3.3. *Spectral properties*

The spectral characteristics of the investigated bis-styryl dyes **3a**–**3c** and parent styryls **4a**–**4c** measured in chloroform and methanol are given in Table 3 while the representative absorption and emission spectra are shown in Figs. 2 and 3. For an analysis of the correlations between the spectral properties and the molecular structures we simulated the spatial and electronic structures of these molecules and calculated the electron transitions under excitation. The simulations were carried out using semi-empirical quantum chemical methods PM3 CI [32,33] and PPP CI [34–36].

The absorption spectral shapes of bis-styryls were found to be in good agreement with Kiprianov's theory [17]: because of the linear combination of two parent chromophores only one intense, long-wavelength absorption band, red-shifted by 90–110 nm compared to that of parent "monomeric" styryl dyes is observed. Quantum chemical simulations evidence that, similarly to the bis-trimethines **1a**–**1e** [18] this absorption is based on an $S_0 \rightarrow S_1{}^*$ electron transition corresponding to the superposition of two configurations: a larger HOMO → LUMO contribution and a smaller (HOMO − 1) → (LUMO + 1) contribution (Fig. 4). The transition $S_0 \rightarrow S_1{}^*$ is localized mostly within the two polymethine chains, the nitrogen atoms of dimethylamino groups (dyes **3a**, **3c**) or pyrazoline nitrogens (dye **3b**) and the central benzodipyrroleninium nitrogens. The structures of boundary orbitals, localization and polarization of the

Table 3
Spectral characteristics of bis-polymethine and parent polymethine dyes.

	Dye	Solvent	λ_{max} (Abs) [nm]	ε [M^{-1} cm^{-1}]	λ_{max} (Em) [nm]	Φ_F [%]	$\Delta\nu_{st}$[cm^{-1}]
1a		$CHCl_3$	658		677	18.8	430
		MeOH	644	250,000	664	10.4	460
3a		$CHCl_3$	655		683	9.2	630
		MeOH	643	133,000	680	1.2	830
3b		$CHCl_3$	697		736	10.0	760
		MeOH	675	80,000	737	1.7	1250
3c		$CHCl_3$	757		812	2.4	900
		MeOH	720	100,000	810	1.0	1540
7		$CHCl_3$	550		570	4.0	640
		MeOH	545	134,000	568	3.0	740
4a		$CHCl_3$	563		592	1.7	870
		MeOH	545	83,200	590	0.9	1400
4b		$CHCl_3$	600		655	7.4	1400
		MeOH	568	73,000	659	3.0	2530
4c		$CHCl_3$	645		697	5.5	1160
		MeOH	605	70,700	697	2.5	2130

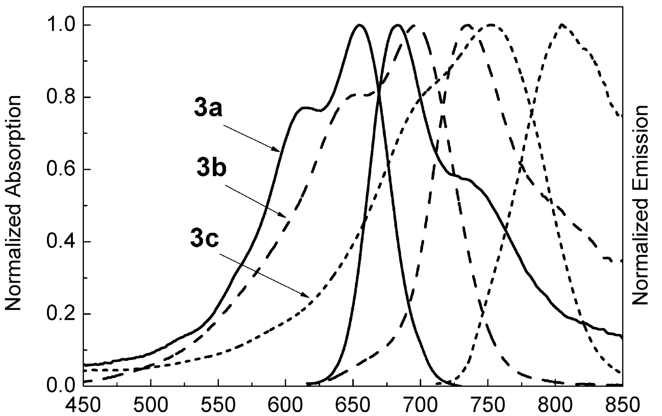


Fig. 2. Normalized absorption and emission spectra of bis-styryl dyes **3a** (solid line), **3b** (dash line) and **3c** (short dash line) in chloroform.

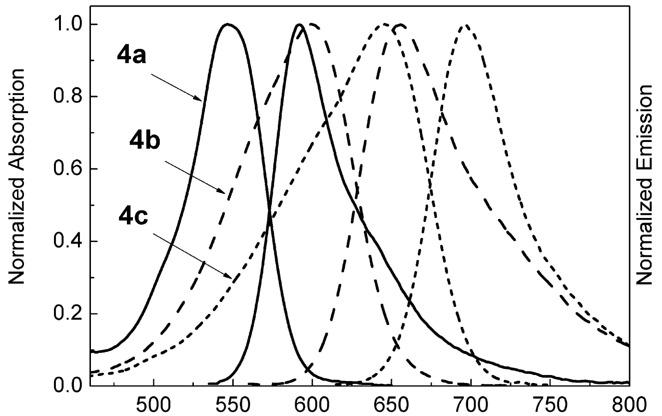


Fig. 3. Absorption and emission spectra of parent styryl dyes **4a** (solid line), **4b** (dash line) and **4c** (short dash line) in chloroform.

electron transition $S_0 \rightarrow S_1^*$ in the biscyanines **3a**–**3c** are also very similar to those in bis-trimethines **1a**–**1e** and the parent styryl molecules **4a**–**4c** (Fig. 5).

The long-wavelength absorption and emission maxima of bis-polymethine dyes **1a** and **3a**–**3c** are between 643–757 nm and 664–812 nm, respectively. The terminal end-groups strongly influence the $S_0 \rightarrow S_1^*$ transition energy and the electron distribution in both the ground and the first excited states. In general, these maxima show a pronounced red-shift in the order of **1a** ≈ **3a** < **3b** < **3c**, which is in good agreement with the quantum chemical simulations showing a decrease of the $S_0 \rightarrow S_1^*$ energy in the same order. The only exception was found for the absorption maxima of **1a** and **3a** that were almost identical. An extension of the polymethine chains in bis-styryl dye **3a** by one vinylene group to form dye **3c** causes a significant red-shift of the absorption maxima in chloroform and methanol by 1970 cm^{-1} and 1780 cm^{-1}, respectively, and an even more pronounced red-shift of the emission maxima (2220 cm^{-1} and 2300 cm^{-1}). Remarkably, in the parent styryls **4a** and **4c** these shifts are even higher: 2260 cm^{-1} in chloroform and 1820 cm^{-1} in methanol for the absorption and 2550 cm^{-1} and 2600 cm^{-1}, respectively, for the emission, which can be attributed to the above discussed, more rigid structure ($\Delta H^{\neq}$ values in Table 1) and lower polarity (Δq values in Table 2) of bis-styryl molecules compared to "monomeric" styryls.

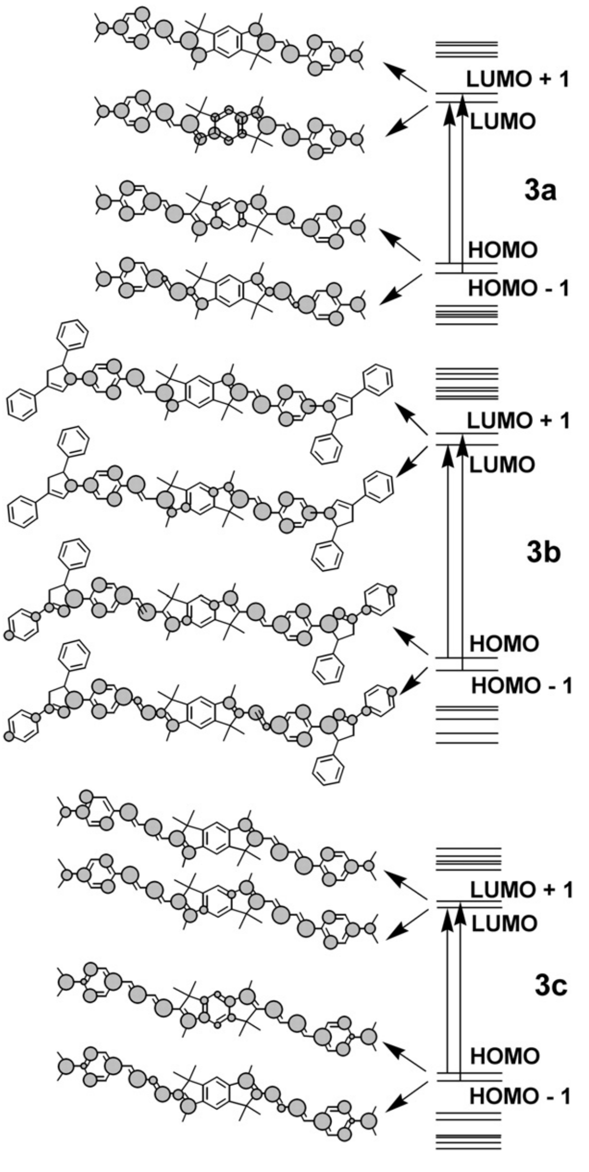


Fig. 4. Structure of the boundary orbitals in molecules **3a**, **3b** and **3c** obtained by PM3 CI method.

The extinction coefficients (ε) of bis-styryl dyes are always higher compared to parent styryls (Table 2), however, these values are lower than those for bis-trimethine cyanines such as **7** described in [18]. The parent styryls also have lower extinction coefficients compared to the "monomeric" trimethines. An extension of the conjugated chain in both bis-styryl **3a** and "monomeric" styryl **4a** by a vinylene group to form vinylogs **3c** and **4c** decreases the extinction coefficients. The substitution of the dimethylamino group in **3a** and **3c** with a pyrazoline moiety (dyes **3b** and **4b**) also reduces the ε values. Such effect of initially increasing and later decreasing extinction coefficients upon extension of the conjugated chain in polymethine dyes was already discussed in the example of monomeric polymethines elsewhere [13]. It was found to be connected with an increase of the conformational flexibility of their molecules, which is also in good agreement with our data ($\Delta H^{\neq}$ values in Table 1).

The Stokes' shifts ($\Delta\nu_{St}$) noticeably increase in the same order as the red-shifts of the absorption and emission bands (**1a** < **3a** < **3b** < **3c**) and this increase becomes even more pronounced in polar solvents (e.g. methanol compared to chloroform). Typically the Stokes' shifts in methanol are higher compared to those in chloroform due to a substantial blue-shift of the absorption maxima. The increase of the Stokes' shift was found to correlate well with the absolute difference between the calculated electron charges on the nitrogens of the central and terminal moieties (Δq) but also with the ^1H NMR Δδ values in Table 2. Such a correlation evidences that the Stokes' energy losses originate not only from intramolecular conformational motion but are also due to intermolecular rearrangements between the solvated dye and solvent molecules in the excited state.

In general, an increase of the Stokes' shift is observed when the dye molecule polarity and/or solvent polarity increase. Thus, the centrosymmetrical trimethine **7** with zero dipole moment and $\Delta q = 0$ exhibits a minimal $\Delta\nu_{St}$ (Table 3), but the $\Delta\nu_{St}$ increases simultaneously with the dye polarity for styryls **4a**–**4c**. Remarkably, bis-polymethines **1a** and **3a**–**3c**, which are centrosymmetrical and virtually have zero dipole moments also demonstrate a strong correlation of the Stokes' shifts with the Δq and Δδ values. This correlation can be explained by the fact that the bis-styryl molecules consist of two uniformly unsymmetrical chromophores that behave as relatively independent polar moieties (Fig. 5) and therefore the Stokes' energy losses correlate with the Δq of each of these chomophore systems. Nevertheless, the Stokes' shifts of the bis-polymethines are always smaller compared to the parent "monomeric" dyes, which is due to their more rigid structure

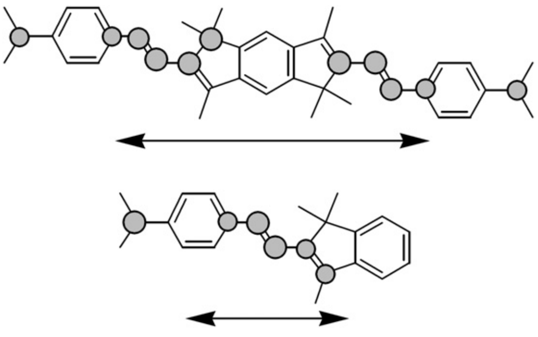


Fig. 5. Localization and polarization of electron transition $S_0 \rightarrow S_1^*$ in bis-styryl **3a** and parent styryl **4a** (PPP CI method).

Table 4
The electron charges on the nitrogen atoms of the central (q_{Centr}) and terminal (q_{Term}) moieties of dyes **1a** and **3a**–**3c** in the ground and first excited states (PPP CI method).

Molecule	Ground state			First excited state		
	q_{Centr}	q_{Term}	Δq	q^*_{Centr}	q^*_{Term}	Δq^*
1a	+0.180	+0.514	+0.334	+0.205	+0.500	+0.295
3a	+0.466	+0.348	−0.118	+0.488	+0.414	−0.074
3b	+0.482	+0.180	−0.302	+0.480	+0.232	−0.248
3c	+0.451	+0.322	−0.129	+0.477	+0.372	−0.105

(higher $\Delta H^{\neq}$) and lower polarity (lower Δq). A similar correlation between $\Delta\nu_{St}$, Δq and $\Delta\delta$ was also reported for bis-trimethines **1a**–**1e** [18].

Solvation effects affecting the absorption and emission maxima, Stokes' shifts, vibrational structures of the spectral bands, and the quantum yields can be understood from an analysis of the electron density distribution in the ground and first excited states. The PM3- and PPP-calculated positive charge (q) in the ground state of bis-styryl dyes **3a**–**3c** is localized mostly on the nitrogen atoms of the central benzodipyrroleninium moiety (Tables 2 and 4), which is in good agreement with the ^{1}H NMR data in Table 2.

As can be seen from Table 4 under excitation the q values in **3a**–**3c** increase (negative electronic charges decrease) at both the central and terminal nitrogen atoms, however, the increase is more pronounced for the terminal nitrogens ($q^*_{Term} - q_{Term}$) than for the central nitrogens ($q^*_{Centr} - q_{Centr}$). As a result, the difference between the charges on the terminal and central nitrogens in the excited state (Δq^*) is less compared to that in the ground state (Δq), and is responsible for the less pronounced alteration of the electronic charges in the excited state. The same pattern of the charge redistribution under excitation was observed for bis-trimethines **1a**–**1e** with the exception that positive charge predominates on the terminal nitrogens [18]. The ΔP values ($\Delta P = P^* - P_0$) in Table 5 evidence that the bond orders (P) in bis-styryls **3a**–**3c** and bis-trimethines **1a**–**1e** also become smoother in the excited state (P^*): the bond orders of double bonds reduce under excitation while the single bonds orders remain almost unchanged. The electron density redistribution upon excitation in bis-styryls **3a**–**3c** increases the contribution of the resonance form **B** in the excited state (Fig. 1).

The smoothened alteration of the electron density (electronic charges and bond orders) in the excited state compared to the ground state causes a negative solvatochromic but negligible solvatofluorochromic effects. The spectral maxima of bis-styryl dyes **3a**–**3c** measured in methanol are blue-shifted as compared to chloroform by 11–26 nm in the absorption but this shift does not exceed 3 nm in the emission (Table 3).

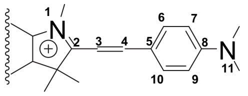
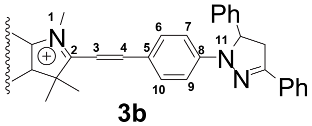
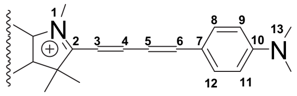
Similar correlations between the electron density distribution in the ground and excited states and the solvation effects in the absorption and emission spectra were also observed for bis-trimethines **1a**–**1e** [18].

The vibrational structures of the absorption and emission bands of cyanine dyes is known to smoothen with increased conjugated chain length (trimethines < pentamethines < heptamethines) [13] while the spectra of styryl dyes are always smoother compared to cyanines, which is mostly due to low energy barriers for the internal rotations of the polymethine chain and/or the terminal end-groups [14–16]. Surprisingly, the smoother vibrational structures in the spectra of parent styryl dyes **4a**–**4c** (Fig. 3) is easier to identify upon an increase of the conjugation chain length in bis-styryl dyes **3a**–**3c** (Fig. 2). This effect can be attributed to the above-mentioned increase of the conformational rigidity (Table 1) and decrease of polarity (Table 2) of bis-styryl molecules **3a**–**3c** as compared to "monomeric" styryls **4a**–**4c**. It is worth mentioning that this effect is not observed for the bis-trimethine **1a** and the parent trimethine **7**: both dyes exhibit well-resolved vibrational structures, which can be explained by the higher rotational energies of styryls **1a** and **7** compared to bis-styryls **3a**–**3c**.

The vibrational structures of the absorption and emission bands of bis-styryl dyes become more pronounced in the same order as the increase of the rotational barriers $\Delta H^{\neq}$: **3c < 3b < 3a**. The vibrational structures, which are very well identified in chloroform, becomes less pronounced but still well-defined in higher polar solvents such as methanol (Fig. 6).

The quantum yield (Φ_F) in both low-polar (chloroform) and polar (methanol) solvents increases in the order: **3c** < **3a** < **3b** < **1a**. Typically the Φ_F's of bis-styryl dyes are lower compared to bis-trimethines. Due to the higher excited state polarity of bis-styryls as compared to bis-trimethines (Δq^* in Table 2) the difference in Φ_F is even more pronounced in a polar solvent.

Table 5
The bond orders in molecules **3a**–**3c** in the ground (P_0) and first excited (P^*) states (PPP CI method).

Bond	 **3a**			 **3b**			 **3c**		
	P_0	P^*	ΔP	P_0	P^*	ΔP	P_0	P^*	ΔP
1–2	0.602	0.544	−0.058	0.620	0.560	−0.060	0.582	0.543	−0.039
2–3	0.587	0.582	−0.005	0.564	0.578	+0.014	0.615	0.608	−0.006
3–4	0.684	0.661	−0.023	0.712	0.679	−0.033	0.645	0.631	−0.015
4–5	0.545	0.533	−0.012	0.509	0.510	+0.001	0.612	0.602	−0.010
5–6	0.526	0.514	−0.012	0.552	0.539	−0.013	0.683	0.664	−0.019
6–7	0.754	0.757	+0.003	0.722	0.729	+0.007	0.533	0.531	−0.002
7–8	0.529	0.513	−0.016	0.594	0.575	−0.019	0.533	0.521	−0.012
8–9	0.530	0.515	−0.015	0.595	0.577	−0.018	0.747	0.752	+0.005
9–10	0.751	0.753	+0.002	0.720	0.726	+0.006	0.540	0.527	−0.013
10–5	0.528	0.517	−0.011	0.552	0.540	−0.012	–	–	–
8–11	0.577	0.593	+0.016	0.351	0.371	+0.020	–	–	–
10–11	–	–	–	–	–	–	0.542	0.528	−0.013
11–12	–	–	–	–	–	–	0.744	0.748	+0.005
12–7	–	–	–	–	–	–	0.536	0.525	−0.011
10–13	–	–	–	–	–	–	0.554	0.568	+0.014

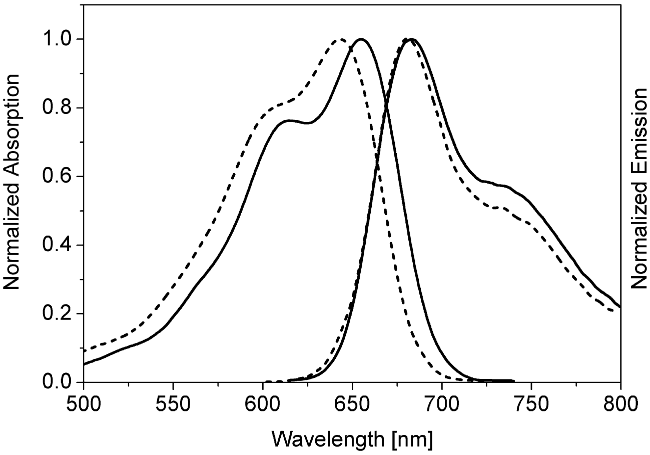


Fig. 6. Absorption and emission spectra of bis-styryl dyes **3a** in chloroform (solid line) and methanol (dash line).

The quantum yield of bis-styryl dye **3a** is higher compared to parent styryl **4a** (Table 3) by factor of 5.4 in chloroform and 1.3 in methanol. The more pronounced increase of Φ_F in chloroform compared to methanol upon dimerization of **4a** to **3a** evidences that the increase is mostly due to an increase of conformational rigidity but not a decrease of molecular polarity. A similar increase of Φ_F (by factor of ~5) is observed for the bis-trimethine **1a** compared to parent trimethine **7**. This increase is not so much attributed to molecular rigidity but a decrease in *cis-trans*-photoisomerization tendency, which was already described for the benzodithiazole-based bis-trimethines [39,40]. The Φ_F of the bis-styryl **3b** in chloroform is also higher than for the parent dye **4b** but it substantially decreases in methanol.

An extension of the polymethine chain in monomeric styryl **4a** by a vinylene group to form **4c** causes a substantial increase in Φ_F (Table 3). At the same time the longest-wavelength bis-styryl **3c** exhibits a smaller quantum yields compared to both the parent styryl **4c** and the bis-styryl **3a**. More likely this is due to an increased radiationless transition caused by a vibrationally induced internal conversion, which is known to substantially increase in the spectral range above 700 nm [13].

An increase of the solvent polarity noticeably decreases the quantum yields of bis-styryl and bis-trimethine dyes, which is due to a well-known effect of quenching of polar dye molecules in polar solvents. The quantum yields of **1a** and **3a**–**3c** correlate well with the Stokes' shifts: the larger the Stokes' shift the lower the quantum yield. The same effects were observed in the series of bis-trimethine dyes **1a**–**1e** [18].

4. Conclusions

Novel bis-styryl dyes were synthesized in satisfactory yields (45–60%) *via* the reaction of quaternized benzodipyrrolenine with two equivalents of an aromatic aldehyde. This approach opens up a convenient way to produce a series of bis-styryls with different end-groups.

Extension of the conjugated styryl dye chains by fusion of two uniform "monomeric" styryl chromophores to a bis-chromophoric molecular system causes a pronounced conformational rigidization, which is a key parameter affecting the spectral properties of these dyes. In contrast to other known polymethine and biscyanine dyes, the "dimerization" of monomeric styryls to bis-styryl dyes results in better identified vibrational structures of the absorption and emission bands.

The synthesized bis-styryl dyes absorb at 643–757 nm with high extinction coefficients (up to 133,000 M^{-1} cm^{-1}), have relatively large Stokes' shifts (up to 55 nm in chloroform and 90 nm in methanol), and emit at 680–812 nm. Despite their symmetrical structures, bis-styryls behave similarly to unsymmetrical "monomeric" styryl dyes but their absorption and emission maxima are red-shifted by about 100 nm, which is important for many practical applications. Comparable to biscyanine dyes they are more sensitive to the solvent polarity showing negative solvatochromism and reduced fluorescence in polar solvents. These characteristics of bisstyryl dyes demonstrate their potential as biomedical probes for proteins, lipids and cells.

Acknowledgement

This work was supported by the National Academy of Sciences of Ukraine, project No. 0110U000488. The authors would like to thank Dr. Vladimir Musatov (SSI "Institute for Single Crystals" of the National Academy of Sciences of Ukraine) for the ^{1}H NMR measurements.

References

[1] Berneth H, Bruder F. Optical data carrier comprising a cyanine dye as light-absorbent compound in the information layer. US 2005042407 (B2); 2005.
[2] Metz H, Mura J. Lightfast cyanine dye compositions for optical data recording. WO2005023939; 2005.
[3] Sibbett W, Taylor JR. Passive mode locking in the near infrared. IEEE J Quantum Electr 1984;20(2):108–10.
[4] Schäfer FP. Dye lasers. 3rd ed. Berlin: Springer–Verlag; 1990.
[5] Haugland R. Handbook of fluorescent probes and research products. 9th ed. USA: Molecular Probes; 2002.
[6] Gadjev NI, Deligeorgiev TG, Kim SH. Preparation of monomethine cyanine dyes as noncovalent labels for nucleic acids. Dyes Pigm 1999;40(2–3):181–6.
[7] Gadjev NI, Deligeorgiev TG, Timcheva II, Maximova VA. Synthesis and properties of YOYO-1-type homodimeric monomethine cyanine dyes as noncovalent nucleic acid labels. Dyes Pigm 2003;57(2):161–4.
[8] Deligeorgiev TG, Gadjev NI, Vasilev AA, Maximova VA, Timcheva II, Katerinopoulos HE, et al. Synthesis and properties of novel asymmetric monomethine cyanine dyes as non-covalent labels for nucleic acids. Dyes Pigm 2007;75(2):466–73.
[9] Yarmoluk SM, Kryvorotenko DV, Balanda AO, Losytskyy MY, Kovalska VB. Proteins and cyanine dyes. Part III. Synthesis and spectroscopic studies of benzothiazolo-4-[1,2,6-trimethylpyridinium] monomethine cyanine dyes for fluorescent detection of bovine serum albumin in solutions. Dyes Pigm 2001;51(1):41–9.
[10] Gomer C. Preclinical examination of first and second generation photosensitizers used in photodynamic therapy. Photochem Photobiol 1991;54(6):1093–107.
[11] Redmond RW, Srichai MB, Bllitz JM, Schlomer DD, Kreig M. Merocyanine dyes: effect of structural modifications on photophysical properties and biological activity. Photochem Photobiol 1994;60(4):348–55.
[12] Pandey RK, James N, Chen Y, Dobhal MP. Cyanine dye-based compounds for tumor imaging with and without photodynamic therapy. Top Heterocycl Chem 2008;14:41–74.
[13] Ishchenko AA. Structure and spectral-luminescent properties of polymethine dyes [In Russian]. Kiev: Naukova dumka; 1994.
[14] Az Al-Ansari I. Interaction of solvent with the ground and excited state of 2- and 4-[4-(dimethylamino)styryl]-1-alkylpyridinium iodides: an absorption and fluorescence study. Bull Soc Chim France 1997;134:593–9.
[15] Hebert P, Baldacchino G. Photochemistry of an unsymmetrical polymethine-cyanine dye; solute–solvent interactions and relaxation dynamics of LDS 751. J Photochem Photobiol A Chem 1994;84(1):45–55.
[16] Paczkowski J, Kabatc J, Jedrzejewska B. Polymethine dyes as fluorescent probes and visible-light photoinitiators for free radical polymerization. Top Heterocycl Chem 2008;14:183–220.
[17] Kiprianov AI. Absorption spectra of organic dyes with two conjugated chromophores. Russ Chem Rev 1971;40(7):594–607.
[18] Klochko OP, Fedyunyayeva IA, Khabuseva SU, Semenova OM, Terpetschnig EA, Patsenker LD. Benzodipyrrolenine-based biscyanine dyes: synthesis, molecular structure and spectroscopic characterization. Dyes Pigm 2010;85(1–2):7–15.
[19] Kiprianov AI, Verbovskaya TM, Mushkalo IL. Cyanine dyes with two conjugated chromophores. Russ J Org Chem 1967;3(11):2036–41 [in Russian].
[20] Mihailenko FA, Boguslavskaya AN. Cyanine dyes with two conjugated chromophores. X Ukr Khim Zh 1969;35(9):943–7 [Russ Ed].
[21] Kiprianov AI, Fridman SG. Cyanine dyes with two conjugated chromophores. VI. Russ J Org Chem 1968;4(4):696–703 [In Russian].

[22] Mihailenko FA, Mushkalo IL, Boguslavskaya AN. Vinilene homologues of biscarbocyanines with conjugated chromophores. Ukr Khim Zh 1974;40(3): 253–8 [Russ Ed].

[23] Mihailenko FA, Boguslavskaya AN, Kiprianov AI. Biscyanines from isomeric benzodipyrrolenines. Chem Heter Comp 1971;7(5):578–80.

[24] Dähne S. Color and constitution: one hundred years of research. Science 1978;199(4334):1163–7.

[25] Baraldi I, Brancolini G. Solvent influence on absorption and fluorescence spectra of merocyanine dyes: a theoretical and experimental study. Chem Phys 2003;288(2–3):309–25.

[26] Henary M, Mojzych M. Stability and reactivity of polymethine dyes in solution. Top Heterocycl Chem 2008;14:221–38.

[27] Ernst LA, Gupta RK, Mujumdar RB, Waggoner AS. Cyanine dye labeling reagents for sulfhydryl groups. Cytometry 1989;10(1):3–10.

[28] Patsenker LD, Tatarets AL, Terpetschnig EA. Long-wavelength probes and labels based on cyanines and squaraines. In: Demchenko A, Wolfbeis OS, editors. Advanced fluorescence reporters in chemistry and biology I, vol. 8. Springer Verlag; 2010. 390 pp., P.65–104.

[29] Parker CA. Photoluminescence of solutions. Amsterdam: Elsevier; 1968.

[30] Mujumdar RB, Ernst LA, Mujumdar SR, Lewis CJ, Waggoner AS. Cyanine dye labeling reagents: sulfoindocyanine succinimidyl esters. Bioconjug Chem 1993;4(2):105–11.

[31] Therenin AN. Photonics of dyes molecules and related organic compounds [In Russia]. Leningrad: Nauka; 1967.

[32] Stewart JJP. Optimization of parameters for semiempirical methods I. Method J Comput Chem 1989;10(2):209–20.

[33] Stewart JJP. Optimization of parameters for semiempirical methods II. Applications. J Comput Chem 1989;10(2):221–32.

[34] Pariser R, Parr RG. A semi-empirical theory of the electronic spectra and electronic structure of complex unsaturated molecules. I. J Chem Phys 1953;21(3):466–71.

[35] Pople GA. Electron interaction in unsaturated hydrocarbons. Trans Faraday Soc 1953;49(12):1375–85.

[36] Fabian J, Zahradnic R. PPP-Berechnung zum Vinylenschift symmetrischer Farbstoff. Wiss Z Thechn Univ (Dresden) 1977;26(2):315–23.

[37] Kutulya LA, Shevchenko AE, Surov Yu N. Structures of the products of formylation of 1,3,5-triphenyl- and 1,5 -diphenyl-3-stryl-2-pyrazolines. Chem Heter Comp 1975;11:215–7.

[38] Mihailenko FA, Boguslavskaya AN. Synthesis of isomeric benzodipyrrolenines and their derivatives. Chem Heter Comp 1971;7(5):574–7.

[39] Borisevich YuYe, Kuz'min VA, Mihailenko FA, Dyadyusha GG. Triplet stages of biscyanines dyes. Dokl Akad Nauk SSSR 1976;228(2):373 [In Russian].

[40] Kuz'min VA, Borisevich YuYe, Dyadyusha GG, Mihailenko FA. Effect of triplet levels splitting of biscyanines dyes. Dokl Akad Nauk SSSR 1976;229(1):131 [In Russian].