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Dyes and Pigments 50 (2001) 21–28

DYES and PIGMENTS

www.elsevier.com/locate/dyepig

Interaction of cyanine dyes with nucleic acids — XXVII: synthesis and spectral properties of novel homodi- and homotrimeric monomethine cyanine dyes

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Received 20 December 2000; received in revised form 21 March 2001; accepted 12 April 2001

Abstract

Three new methods of preparation based on reactions of pyrylium salts with primary amines, acylation of polyamines with activated carboxyl derivatives of cyanine dyes, diisocyanate with aminoderivatives of cyanine dyes were used to obtain fluorescent homodi- and, for the first time, homotrimer cyanine dyes. Spectral-luminescent properties of synthesized dyes and their nucleic acid complexes were studied.

Keywords: Synthesis; Homo-*n*-meric cyanine dyes; Nucleic acids; Fluorescence

1. Introduction

Recent years have witnessed increasing interest in the application of highly fluorescent homodimeric monomethine cyanine dyes in the detection of double-stranded DNA (dsDNA) in agarose gel electrophoresis, capillary gel electrophoresis, and fluorescence microscopy [1].

In 1992 Rye et al. [2] first described two novel, highly fluorescent dimeric asymmetric cyanine dyes namely the thiazole orange dimer (**TOTO**; 1, 1′-(4,4,7,7-tetramethyl-4,7-diazaundecamethylene)-bis-4-[3-methyl-2,e-dihydro-(benzo-1,3-thiazole)-2-methylidene]-quinolinium tetraiodide) and the oxazole yellow dimer (**YOYO**; an analog of **TOTO** with a benzo-1,3-oxazole instead of the benzo-1,3-thiazole). Both **TOTO** and **YOYO** bind very tightly to dsDNA and form complexes with dsDNA which remain stable during electrophoresis. Cyanine dimers significantly enhanced their fluorescence on binding to dsDNA 1100-fold for **TOTO** and 3200-fold for **YOYO** [2,3].

In the homodimeric cyanine dyes described thus far, an aminoalkyl linkage [4,5] of the spermine type connected the chromophores. Attention has mainly been paid to the influence of the structure and nature of a chromophore itself on the spectral properties of the homodimere-DNA complex [6]. However, such studies were unable to elucidate the influence of the nature, rigidity and composition of the link group on the properties of the complexes. The method of alkylation of the *N,N,N',N'*-tetramethyldiaminoalkane by halogenoderivatives of dyes, which is

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0143-7208/01/$ - see front matter
PII: S0143-7208(01)00023-7

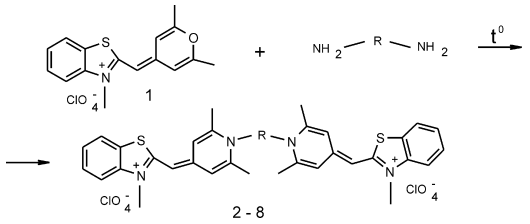
widely applied in the synthesis of dimer derivatives, does not provide homo-*n*-mers linkages which are substantially different in chemical composition and structure [2]. Thus, our attention has turned to ways of obtaining homodimeric cyanine with linking groups that differ in both chemical nature and structure.

In this paper we present three synthetic procedures for the preparation of novel homodi- and homotrimeric monomethine cyanines. The spectral properties of the dyes prepared and their complexes with DNA and RNA as well as the analysis of the bridging group's influence on the complexes are studied.

2. Results and discussion

The reaction of pyrylocyanine [**Cyan 39 (1)**] with various amines, which was described previously [7], is carried out in boiling DMF and gives sufficient yields. Using this method, benzothiazolo-4-[2,6- dimethylpyridine] homodimers with bridged linkages of various length and chemical nature (**2–8**) were obtained (Scheme 1). In the case of diamines with a chain length less than 5 carbon atoms the reaction provides pure monocyanine derivatives or mixtures of monomeric and dimeric derivatives.

The second method used in the preparation of the novel fluorescent homodimeric cyanines is based on the acylation of di- and triamines with dyes containing the carboxyl group (1-(5-carboxypentyl)-2,6-dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)pyridinium perchlorate (**9**)) in the presence of *N,N*-dicyclohexylcarbo diimide (DCC) or *N,N*-carbonyldiimidazole (CDI)



Scheme 1. Rs are given in Table 3.

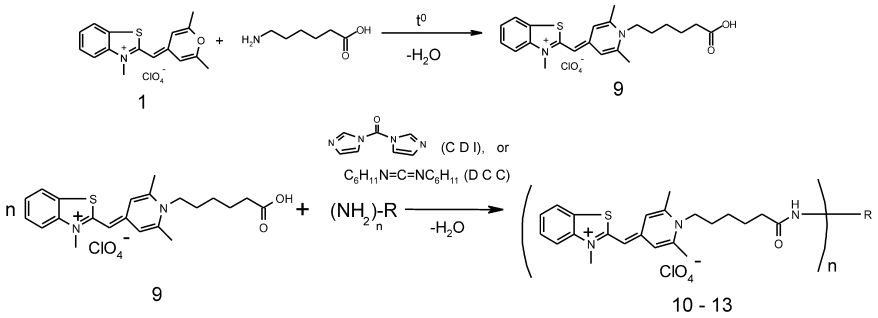
(Scheme 2). In this way, novel homotrimer cyanines (**11,12**) were synthesized.

We applied a third method for the synthesis of a homodimeric dye with rather rigid (in the configuration meaning) linkage (**15**). This comprised the use of the reaction of isocyanates with amines (Scheme 3). Through the acylation of paraphenylenediamine by the **9** derivative in the presence of CDI, we obtained compound **14**. The dye **15** by obtained with the reaction of the derivative **14** with the biphenylbiisocyanate.

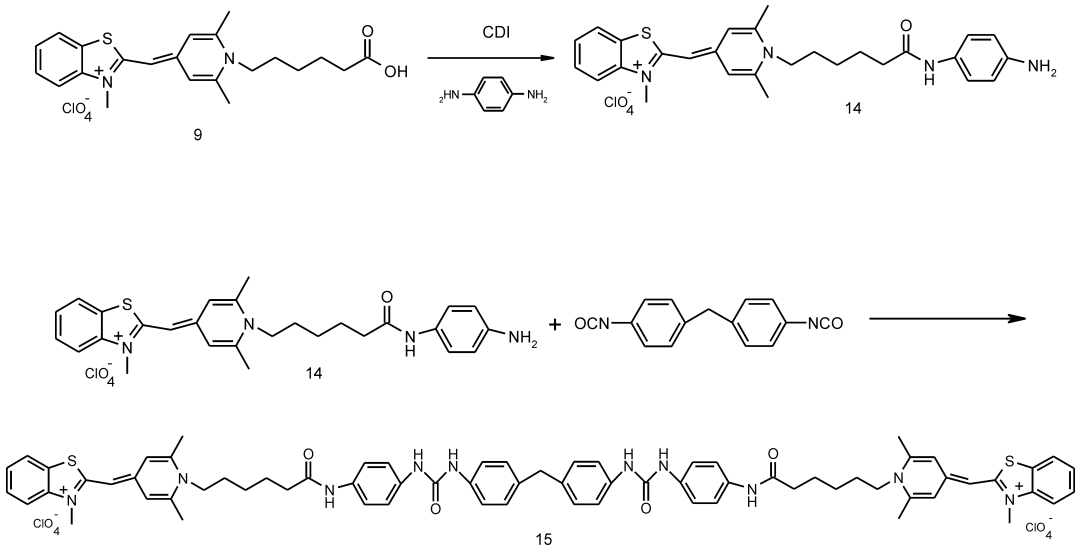
Characteristics of the absorption spectra of free homodimer dyes and their complexes with nucleic acids are presented in Table 1. The monomer monomethiyne cyanine dye Cyan 40 is presented for comparison purposes [8]. Spectral studies have shown that the synthesized homodi- and homotrimers have an increased capacity to form aggregates in aqueous solution as compared to the parent monomeric dye [9]. The single molecule band (λ_{mol}) was present in the absorption spectra of a solution of the dye in DMF for all of the dyes studied except **4**. Additional blueshifted bands caused by the formation of the H-aggregates [409–417 nm (λ_1) and 421–434 nm (λ_2)] appeared in the spectra of both the homodi- and homotrimers in aqueous buffer (Tris–HCl, pH = 7.5) in contrast to **Cyan 40** [9]. Clearly, the tendency of the cyanine dyes to aggregate can be attributed the conjugation of the dyes. The presence of nucleic acids destroys the aggregates, which results in the redistribution of the intensities of the various aggregation bands and the corresponding increase in intensity of the monomeric band [9].

The fluorescent spectra of the dyes in DMF, buffer and their nucleic acid complexes are presented in Table 2. All of the dyes studied displayed relatively weak fluorescence intensity in the unbound state, but form highly fluorescent complexes both with DNA and RNA (Table 2). Fluorescent maxima (λ_{em}) of the homo-*n*-meric dyes in buffer were between 515 and 591 nm. Excitation spectra studies have shown that these maxima represent the emission of dye aggregates of a different structure [9].

Fluorescnece maxima of the dyes bound to nucleic acid were between 482 and 495 nm for DNA, and between 482 and 498 nm for RNA complexes. The observed fluorescence enhance-



Scheme 2. Rs are given in Table 3.



Scheme 3.

ment for the *n*-mer cyanines at the wavelength of dye–nucleic acids complexes emission (near 485 nm) amounted to about 1000 times for the dye–DNA complexes and 400 times for the dye–RNA complexes (Table 2).

More detailed studies of the spectral-luminescent properties of the cyanine dyes and the correlation between the structure of the linking groups and the properties of the dyes as probes for nucleic acids are under investigation. We suggest that the aggregation processes that are observed for the homodi- and homotrimer cyanine dyes significantly increase the bound-to-free dye fluorescence-intensity ratio [10]; thus, the sensitivity of the homo-*n*-meric cyanines to the presence of nucleic acids is increased. It is considered that the presented novel dyes can be successfully used as fluorescent probes for nucleic acid detection.

3. Experimental

Melting points of the obtained compounds were determined in capillaries without correction. The ^{1}H NMR spectra were recorded in DMSO-d_6 using a Varian 300 MHz instrument. The absorption

Table 1
Characteristics of absorption spectra of homodimer cyanine dyes in free state and in complexes with nucleic acids[a]

Dye	DMF λ_{abs} (nm)	Buffer λ_{abs} (nm)			DNA λ_{abs} (nm)			RNA λ_{abs} (nm)		
	λ_{mol}	λ_1	λ_2	λ_{mol}	λ_1	λ_2	λ_{mol}	λ_1	λ_2	λ_{mol}
2	446	–	425	451	401	424	447	410	431	450
3	445	417	434	454	402	420	448	402	419	450
4	445	–	421	451	–	424	451	–	423	451
5	445	411	427	448	403	430	455	400	433	451
6	444	–	431	450	–	428	455	402	438	452
7	442	410	423	453	401	429	450	411	429	445
8	443	410	426	453	–	427	449	415	–	445
10	445	405	425	452	404	432	454	–	427	452
11	443	410	428	450	404	434	447	411	425	448
12	443	409	422	449	403	431	447	–	428	444
13	443	410	431	440	409	434	447	–	433	440
15	444	411	431	452	409	434	448	409	433	449
Cyan 40	441	–	–	436	–	–	437	–	–	437

[a] λ_1, λ_2, λ_{mol}, wavelengths of absorption maxima which belongs correspondingly to the first aggregate, second aggregate and monomeric bands.

Table 2
Characteristics of fluorescent properties of homodimeric cyanine dyes in free state and in the complexes with nucleic acids[a]

Dye	DMF λ_{em} (nm)	DMF I_0 (a.u.)	Buffer λ_{em} (nm)	Buffer I_0 (a.u.) on λ_{em}	Buffer I_0 (a.u.) on 485 nm	DNA λ_{em} (nm)	DNA I (a.u.)	RNA λ_{em} (nm)	RNA I (a.u.)
2	482	0.306	515	0.064	0.05	485	6	485	11.7
3	485	0.300	591	0.330	0.05	482	4.7	484	17.6
4	487	0.224	554	0.084	0.05	496	6.1	498	3.1
5	482	0.302	531	0.240	0.12	495	32.1	489	12.2
6	485	0.312	540	0.245	0.13	493	12.2	493	49.3
7	482	0.336	578	0.365	0.10	484	108	485	40.3
8	481	0.301	575	0.225	0.08	484	60.1	486	18.3
10	487	0.598	570	0.395	0.15	483	83.6	483	50.3
11	486	0.318	565	0.398	0.06	485	50.8	487	24.4
12	483	0.298	552	0.350	0.10	484	64.5	486	23.1
13	483	0.303	580	0.176	0.08	483	43.8	484	16.5
15	482	0.306	530	0.120	0.10	484	20.5	485	11.0
Cyan 40	480	0.286	478	0.245	–	480	59.8	482	67.3

[a] a.u., Arbitrary units; I_0, free dye emission intensity; I, complexed with nucleic acid dye emission intensity.

spectra were recorded in DMF on a Specord M-40 UV/VIS spectrophotometer. Nucleic acids and dye solutions were prepared and spectroscopic measurements were carried out as described previously [9]. DMF and ethanol were purchased from "Sigma". Fluorescence spectra were recorded on a Hitachi 850 fluorescent spectrophotometer.

3.1. Preparation of homodimeric monomethine cyanine dyes by Scheme 1

Cyan 39 (1) (2.25 mM) and 1 mM of primary diamine in 5 ml DMF were refluxed for 2–3 h, after which 10–15 ml of alcohol was added and the reaction mixture was cooled to room temperature.

The precipitated dyes were filtered and recrystalized from the mixture (1:2) of DMF with alcohol. Melting points and yields of the Series 1 dyes are given in Table 3.

The dyes obtained are following:

*3.1.1. 1-{5-[2,6-Dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)-1-pyridiniumyl] pentyl}-2,6-dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)pyridinium diperchlorate (**2**)*

The ^{1}H NMR data: 1.56 (*s*, 2H), 1.80 (*m*, 4H); 2.70 (*s*, 12H); 3.68 (*s*, 6H); 4.20 (broad *t*, 4H); 6.03 (*s*, 2H); 7.23 (*m*, 6H); 7.53 (*m*, 4H); 7.83 (*d*, 2H, 8.2 Hz).

*3.1.2. 1-{6-[2,6-Dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)-1-pyridiniumyl] hexyl}-2,6-dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)pyridinium diperchlorate (**3**)*

The ^{1}H NMR data: 1.51 (*s*, 4H); 1.76 (*m*, 4H); 2.69 (*s*, 12H); 3.68 (*s*, 6H); 4.18 (broad *t*, 4H); 6.03 (*s*, 2H); 7.23 (*m*, 6H); 7.53 (*m*, 4H); 7.85 (*d*, 2H, 8.6 Hz).

*3.1.3. 1-(2-{2-[2,6-Dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)-1-pyridiniumyl]ethoxy}ethyl)-2,6-dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)pyridinium diperchlorate (**4**)*

The ^{1}H NMR data: 2.56 (*s*, 12H); 3.51 (*s*, 6H); 3.83 (*m*, 4H); 4.42 (broad *t*, 4H); 5.94 (*s*, 2H); 7.08 (*m*, 6H); 7.28 (*m*, 2H); 7.38 (*m*, 2H); 7.73 (*d*, 2 H, 8.4 Hz).

*3.1.4. 1-(3-{3-[2,6-Dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)-1-pyridiniumyl]propylamino}propyl)-2,6-dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)pyridinium diperchlorate (**5**)*

The ^{1}H NMR data: 1.88 (broad *m*, 4H); 2.50 (broad *s*, 4H); 2.67 (broad *s*.); 3.65 (broad *s*, 6H); 4.24(broad *t*, 4H); 5.96 (broad *s*, 2H); 7.15 (broad *m*, 6H); 7.47 (broad m, 4H); 7.79 (broad **d**, 2 H, 8.7 Hz).

*3.1.5. 1-[2-(2-{2-[2,6-Dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)-1-pyridiniumyl]ethylamino}ethylamino)ethyl]-2,6-dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)pyridinium diperchlorate (**6**)*

The ^{1}H NMR data: 2.68 (broad *s*, 12H); 3.56 (broad *m*, 8H); 3.65 (broad *s*, 6H); 4.25 (broad *t*, 4H); 5.97 (broad *s*, 2H); 7.16 (broad *m*, 6H); 7.51 (broad *m*, 4H); 7.84 (broad *d*, 2H, 8.7 Hz).

3.1.6. 1-[6-(4-{6-[2,6-Dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)-1-pyridiniumyl]hexanoyl}piperazino)-6-oxohexyl]-2,6-dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzo thiazol-2-ylidenmethyl)pyridinium diperchlorate (**7**)

The ^{1}H NMR data: 1.42 (*m*, 4H); 1.59 (broad *m*, 4H); 1.74 (broad *m*, 4H); 2.37 (broad *t*, 4H); 2.68 (*s*, 12H); 3.47 (broad *d*, 4H, 7.5Hz); 3.67 (*s*, 6H); 4.14 (broad *t*, 4H); 6.02 (*s*, 2H); 7.22 (*m*, 6H); 7.53 (*m*, 4H); 7.86 (*d*, 2 H, 8.5 Hz).

*3.1.7. 1-{12-[2,6-Dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)-1-pyridiniumyl]dodecyl}-2,6-dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl) pyridinium diperchlorate (**8**)*

The ^{1}H NMR data: 1.23 (*m*, 10H); 1.54 (*s*, 6H); 1.78 (*m*, 4H); 2.72 (*s*, 12H); 3.71 (*s*, 6H); 4.21 (broad *t*, 4H); 6.05 (*s*, 2H); 7.27 (*m*, 6H); 7.57 (*m*, 4H); 7.82 (*d*, 2H, 8.4 Hz).

3.2. Preparation of of homo-n-meric monomethine cyanine dyes according to Scheme 2

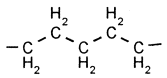
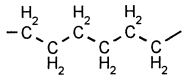
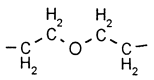
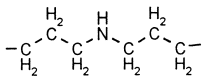
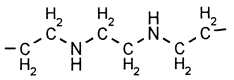
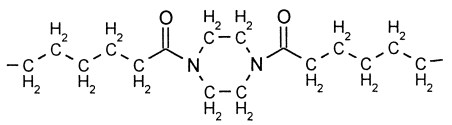
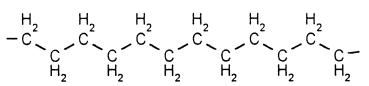
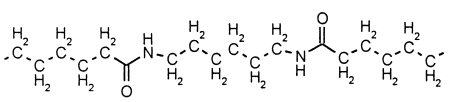
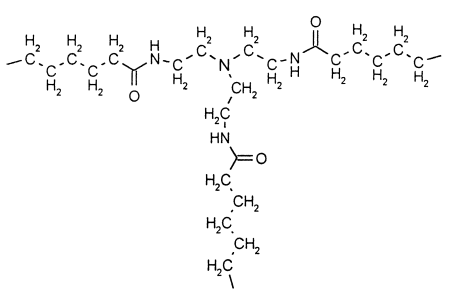
*3.2.1. 1-(5-Carboxypentyl)-2,6-dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)pyridinium perchlorate (**9**)*

0.131 g (1 mM) of ε-aminocaproic acid and 0.277 g (0.75 mM) of the **Cyan 39** were dissolved in 5 ml of DMF. The reaction mixture was refluxed for 30 min and the solvent was then evaporated in vacuum and 5 ml of ethanol was added. The precipitate was filtered, washed with water and air-dried.

*3.2.2. The homo-n-meric monomethine cyanine dyes **10, 12, 13***

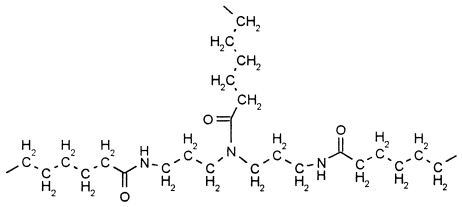
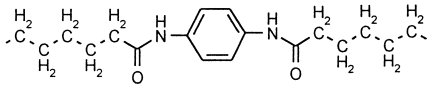
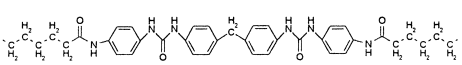
A mixture of 0.145 g (0.3 mM) of the dye **9**, 0.203 g (1.5 mM) *N*-hydroxybenzotriazole and

Table 3
Molecular formula, melting points and yields of the dyes

Dye	Structure of linker	Molecular formula	Yield (%)	mp (°C)
2		$C_{37}H_{42}N_4O_8S_2Cl_2$	55	310–313[a]
3		$C_{38}H_{44}N_4O_8S_2Cl_2$	61	312–315[a]
4		$C_{36}H_{40}N_4O_9S_2Cl_2$	40	247–250[a]
5		$C_{38}H_{45}N_5O_8S_2Cl_2$	43	274–277
6		$C_{38}H_{46}N_6O_8S_2Cl_2$	45	257–261[a]
7		$C_{48}H_{60}N_6O_{10}S_2Cl_2$	65	287–289
8		$C_{44}H_{56}N_4O_8S_2Cl_2$	62	272–274
10		$C_{50}H_{66}N_6O_{10}S_2Cl_2$	56	130–134
11		$C_{72}H_{93}N_{10}O_{15}S_3Cl_3$	65	154–157

(*continued on next page*)

Table 3 (*continued*)

Dye	Structure of linker	Molecular formula	Yield (%)	mp (°C)
12		$C_{72}H_{92}N_9O_{15}S_3Cl_3$	59	144–145
13		$C_{50}H_{58}N_6O_{10}S_2Cl_2$	61	278–280[a]
15		$C_{71}H_{76}N_{10}O_{12}S_2Cl_2$	63	315–317[a]

[a] Melts with decomposition.

0.310 g (1.5 mM) of *N,N′*-dicyclohexylcarbodiimide in 5 ml DMF was stirred for 24 h at 0°C. Then 0,1 mM of the polyamine added and the reaction mixture was stirred for 24 h at room temperature, which was followed by water addition. The precipitate was filtered and recrystallized from the mixture of DMF with alcohol (1:2). Melting points and yields of the dyes obtained are given in Table 3.

The dyes obtained are following:

*3.2.3. 1-[5-(6-{5-[2,6-Dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)-1-pyridiniumyl] pentylcarboxamido}hexylcarbamoyl) pentyl]-2,6-dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)pyridinium diperchlorate (**10**)*

The ^{1}H NMR data: 1.30–1.35 (*m*, 4H); 1.38 (broad *m*, 4H); 1.45–1.50 (*m*, 4H); 1.59 (broad *m*, 4H); 1.70 (broad *m*, 4H); 2.08 (*t*, 4H, 6.3 Hz); 2.67 (*s*, 12H); 2.99 (*d.d*, 4H, 6.4Hz); 3.68 (*s*, 6H); 4.15 (*t*, 4H, 7.2 Hz); 6.03 (*s*, 2H); 7.22 (*s*, 4H); 7.27 (*t*, 2H, 7.0 Hz); 7.54 (*m*, 4H); 7.73 (broad *s*, 2H); 7.86 (*d*, 2H, 7.2 Hz).

*3.2.4. 1-[5-Di(3-{5-[2,6-dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)-1-pyridiniumyl]pentylcarboxamido}propyl) carbamoylpentyl]-2,6-dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)pyridinium triperchlorate (**12**)*

The ^{1}H NMR data: 1.38 (broad *m*, 6H); 1.60 (broad *m*, 6H); 1.70 (broad *m*, 6H); 2.13 (*t*, 6H, 6.3 Hz); 2.55 (*m*, + DMSO, 6H); 2.65 (*s*, 18H); 3.1 (broad *m*, 6H); 3.64 (*s*, 9H); 4.11 (broad *m*, 6H); 5.96 (*s*, 3H); 7.17 (*s*, 6H); 7.23 (*t*, 3H, 7.1Hz); 7.49 (*m*, 6H); 7.72 (broad *s*, 3H); 7.82 (*d*, 3H, 7.2 Hz).

*3.2.5. 1-[5-(4-5-[2,6-Dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)-1-pyridiniumyl]pentylcarboxamidophenylcarbamoyl) pentyl]-2,6-dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)pyridinium (**13**)*

The ^{1}H NMR data: 1.45 (*m*, 4H); 1.66 (*m*, 8H); 2.31 (broad *t*, 4H); 2.68 (*s*, 12H); 3.68 (*s*, 6H); 4.18 (broad *t*, 4H); 6.03 (*s*, 2H); 7.23 (*m*, 6H); 7.52 (*m*, 4H); 7.86 (*d*, 2H, 8.7 Hz); 9.78 (*s*, 2H).

3.3. Preparation of the 1-{5-[2-di(2-{5-[2,6-dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)-1-pyridiniumyl]pentylcarboxamido}ethyl)aminoethylcarbamoyl]pentyl}-2,6-dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)pyridinium triperchlorate (**11**)

Dye **9** (0.242 g, 0.5 mM) and 0.081g (0.5 mM) of *N,N*-carbonyldiimidazole were dissolved in 5 ml of anhydrous DMF at ~40 °C. Then 0.022 ml (0.15 mM) of tris-(2-aminoethyl)amine was added and mixture left overnight at room temperature, after which water was added. The precipitate was filtered and recrystallized from the mixture of DMF with alcohol (1:2). Melting point and yield of the dye **11** are given in Table 3.

The ^{1}H NMR data: 1.41 (broad *m*, 6H); 1.59–1.70 (broad *m*, 16H); 2.11 (broad *m*, 4H); 2.30 (broad *t*, 2H); 2.63 (*s*, 18H); 3.03 (broad *m*, 2H); 3.07 (broad *m*, 2H); 3.25 (broad *m*, 4H); 3.60 (*s*, 3H); 3.63 (*s*, 6H); 4.10 (broad *m*, 6H); 5.90 (*s*, 1H); 6.92 (*s*, 1H); 5.94 (*s*, 1H); 7.11 (*s*, 4H); 7.14 (*s*, 2H); 7.22 (*m*, 3H); 7.46 (*m*, 6H); 7.79 (*m*, 3H); 7.87 (broad *m*, 2H).

3.4. Preparation of homo-n-meric monomethine cyanine dyes by Scheme 3

3.4.1. 1-[5-(4-Aminophenylcarbamoyl)pentyl]-2,6-dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)pyridinium perchloride (**14**)

Dye **9** (0.242 g, 0.5 mM) and 0.081 g (0.5 mM) of *N,N*-carbonyldiimidazole were dissolved in 5 ml of anhydrous DMF at ~40 °C and then 0.059 g (0.55 mM) of 1,4-phenylenediamine was added. The mixture was left overnight at room temperature, which was followed by water addition. The precipitate was filtered and recrystallized from the mixture of DMF with alcohol (1:2).

3.4.2. Bis-(4-[4-(6-{2,6-dimethyl-4-(3-methyl-2,3-dihydro-1,3-benzothiazol-2-ylidenmethyl)pyridiniumyl}hexanoyl)aminophenyl]carbamoyl aminophenyl)methane diperchlorate (**15**)

Dye **14** (0.286 g, 0.5 mM) and 0.050 g (0.2 mM) of 4,4′-methylenebis(phenyl isocyanate) were dissolved in 5 ml of anhydrous DMF and left overnight at room temperature, after which water was added. The precipitate was filtered and recrystallized from the mixture of DMF with alcohol (1:2). Melting point and yield of the dye **15** are given in Table 3.

The ^{1}H NMR data: 1.46 (*m*, 4H); 1.76 (*m*, 8H); 2.32 (broad *t*, 4H); 2.68 (*s*, 12H); 3.68 (*s*, 6H); 3.81 (*s*, 2H); 4.19 (broad *t*, 4H); 6.03 (*s*, 2H); 7.09 (*d*, 4H, 9.2 Hz); 7.23 (*m*, 6H); 7.35 (*d*, 4H, 9.7 Hz); 7.49 (*d*, 4H, 9.7 Hz); 7.52 (*m*, 6H); 7.84 (*d*, 4H, 9.2 Hz); 8.63 (broad *s*, 4H); 9.73 (broad *s*, 2H).

Acknowledgements

This work was sponsored by the United States Department of Energy (USDOE), Washington, DC, with funding from the Initiatives for Proliferation Prevention (IPP) Program, and was performed under the terms and conditions of Material Support Agreement No. B507077 between the Institute for Molecular Biology and Genetics (IMBG), Kyiv, Ukraine, and the University of California (UC), Lawrence Livermore National Laboratory (LLNL), Livermore, CA, USA.

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