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Interaction of cyanine dyes with nucleic acids. XX. New methods for the preparation of fluorescent probes based on benzothiazol-4-[2,6-dimethylpyridinium] cyanine dyes

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Abstract

Two new methods of preparing fluorescent dyes containing affinity-modifying groups for nucleic acid detection are proposed. One method of preparation is based on the reaction of the pyrylium monomethine cyanine dye 2-(2,6-dimethyl-pyran-4-ilidenmethyl)-3-methyl-benzothiazolium perchlorate with primary amines. The second method of preparation involves the amidation of carboxy substituted dyes with appropriate amines. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Synthesis; Monomethine cyanine dyes; Nucleic acids; Fluorescence

1. Introduction

Fluorescent monomethine cyanine dyes are very promising for the development of new spectral approaches to covalent labeling and detection of nucleic acids [1]. These dyes are increasingly used in fluorescent microscopy, DNA sequencing [2], staining nucleic acids in gel electrophoresis [3].

As a rule, the reaction of quaternary salts of heterocyclic 2- or 4-alkylthio compound with a quaternary salt, which have a reactive methyl group, in the presence of basic reagent is used for the preparation of monomethine dye [4]. More recently Deligeorgiev et al. [5] proposed a novel method for preparation of symmetric and asymetric monomethine cyanine dyes. Quaternary salts of heterocyclic 2- or 4-methyl compound and *N*-heterocyclic 2- or 4-sulfobetainic compounds were reacted by simple melting or by boiling in different solvents in the absence of basic compound [5].

Recently, we first used 4-[(1-methylbenzothiazolyliliden-2)methyl]-1,2,6-trimehtylpyridinium perchlorate (**Cyan 40**) as fluorescent dye for the nucleic acids staining in solutions and agarose gels [6]. **Cyan 40** was first prepared by Kelemen et al. in 1962 by the reaction of 2-(2,6-dimethyl-pyran-4-ilidenmethyl)-3-methyl-benzothiazolium perchlorate (**Cyan 39**) with methylamine [7]. We improved the mentioned reaction and used it widely for the development of new fluorescent probes for nucleic acid detection [8] and for the cyanine dye conjugation with aminoalkyloligonucleotides [9]. In this work the reaction of primary amines with the pyryl-

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ium salt was taken as principle for the design of new class of fluorescent probes with the "affinity-modifying groups". We mean groups which are not able to cause steric hindrances to the direct interaction of the dye's nucleus (intercalation or groove binding) with nucleic acid, but are able of non-covalent binding with mentioned biopolymers and in this way to affect the stability of dye–nucleic acid complexes.

Besides, to synthesize the probes with affinity-modifying groups we used the reaction of acylation of aliphatic and aromatic amines with cyanines containing carboxyl group which was previously bridged through a linker to monomethine dye [10]. Firstly, we used this method for the synthesis of homodi- and homotrimeric cyanine dyes [11,12]. Here we report this approach to the development of the fluorescent probes with affinity-modifying groups.

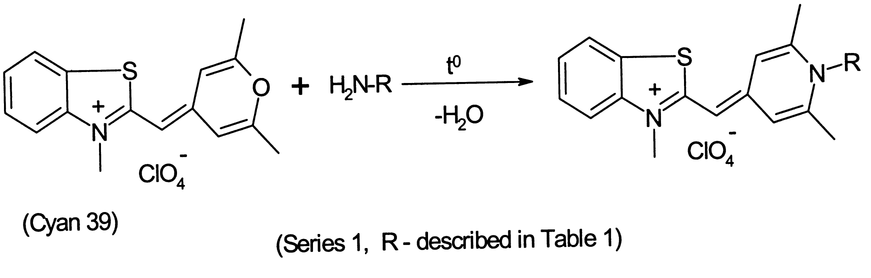
2. Results and discussion

Monomethine cyanines are weakly fluorescent in the free state but show strong fluorescence upon binding to DNA or RNA, and some of these dyes are the most sensitive nucleic acid stains currently available [1,13]. The strong enhancement of the dye molecule fluorescence results from decreased rotational mobility around the internuclear bridge between the two aromatic ring systems of the dye chromophore [14]. There are some models of the possible dye fixation in the presence of nucleic acid [7,15,16]. As a rule, "intercalation models" of binding of monomethine cyanines to DNA implies the insertion of dye with a conjugated ring system into the interior of the helix where it is nestled between the adjacent DNA base pairs.

The aim of the research was to design dyes with affinity-modifying groups that can increase their interactions with nucleic acids and stabilize the complexes. For this purpose, two chemical approaches to the obtaining of cyanine monomethine pyridinum dyes were developed.

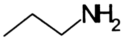
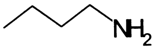
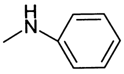
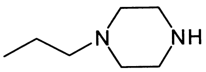
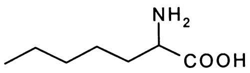
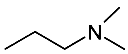
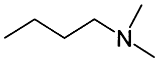
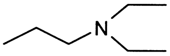
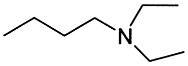
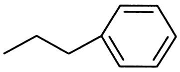
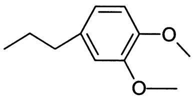
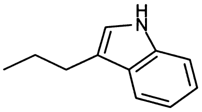
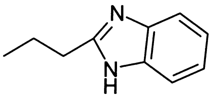
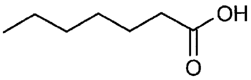
Previously, the reaction of pyrylocyanine (**Cyan 39**) only with aniline and methylamine was described [6]. We studied this reaction with different amines and developed the general procedure of the reaction. It was carried out in boiling DMF and gave sufficient yields of the derivatives (Scheme 1, Table 1). In the case of diamines ethylendiamine and 1,3-diaminopropane the reaction yields pure monocyanine derivatives. The lengthening of the carbon chain up to the 5 atoms led to the formation of the mixture of the reaction products. But for the amines that contain 6 or more atoms in the carbon chain pure biscyanines were obtained [17]. The attempts to obtain pyridinium derivative of cyanine dyes using the similar reactions for α-aminoacids failed (data not presented). In the case of lysine only ε-aminogroup reacted and the **D-4** compound was formed with rather high yield. This observation showed that the reaction of pyrilocyanine **Cyan 39** was sensitive to the steric hindrances in amines.

Amines with various substituents were used in the reaction with pyrilocyanine **Cyan 39**. We have shown that the presence of phenylhydrazine (**D-14**), hydroxyl (**D-6**, **9**, **20**), carboxyl (**D-7**) and secondary amino groups (**D-11**) in amine did not hinder the obtaining of derivatives with the good yield. Obtained compounds with amino and carboxyl groups were convenient intermediates for the



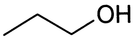
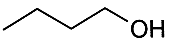
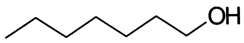
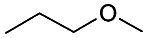
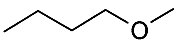
Scheme 1.

Table 1
Structures and some physico-chemical data for dyes (Series 1)

Dye	R	λ_{max} (nm)	M.p. (°C)	Yield (%)	Molecular formula	CHN analysis (%)					
						C		H		N	
						Found	Calc.	Found	Calc.	Found	Calc.
D-12		446	261–263 p	85	$C_{18}H_{22}ClN_3O_4S$	52.1	52.49	5.2	5.38	10.3	10.20
D-13		445	265–267 p	87	$C_{19}H_{24}ClN_3O_4S$	52.9	53.04	5.5	5.53	10.2	10.03
D-14		446	268–270 p	93	$C_{22}H_{22}ClN_3O_4S$	57.4	57.45	4.6	4.82	9.0	9.14
D-11		446	252–253	86	$C_{22}H_{29}ClN_4O_4S$	54.6	54.93	6.2	6.08	11.4	11.65
D-4		444	229–230	93	$C_{22}H_{28}ClN_3O_6S$	52.9	53.06	5.7	5.67	8.3	8.44
D-5		446	226–227	95	$C_{20}H_{26}ClN_3O_4S$	54.4	54.60	5.7	5.96	9.6	9.55
D-24		444	234–236 p	88	$C_{21}H_{28}ClN_3O_4S$	55.5	55.56	6.3	6.22	9.3	9.26
D-3		446	223–224 p	95	$C_{22}H_{30}ClN_3O_4S$	5.6	56.46	6.6	6.46	9.0	8.98
D-27		445	245–247 p	92	$C_{23}H_{32}ClN_3O_4S$	56.9	57.31	6.8	6.69	8.8	8.72
D-10		446	285–287 p	96	$C_{24}H_{25}ClN_2O_4S$	60.7	60.95	5.1	5.33	5.8	5.92
D-2		447	246–247 p	94	$C_{26}H_{29}ClN_2O_6S$	58.2	58.59	5.3	5.48	5.1	5.26
D-8		446	289–290 p	78	$C_{26}H_{26}ClN_3O_4S$	60.2	60.69	5.2	5.12	8.1	8.21
D-1		448	220–221 p	91	$C_{25}H_{25}ClN_4O_4S$	58.4	58.53	4.9	4.91	11.0	10.92
D-7		444	219–220 p	88	$C_{22}H_{27}ClN_2O_6S$	54.3	54.71	5.3	5.63	5.9	5.80

(continued on next page)

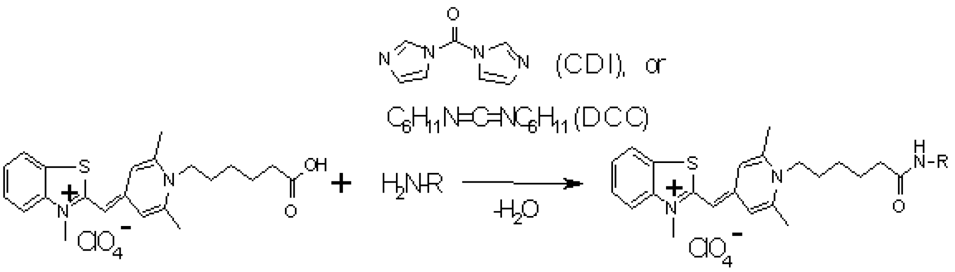
Table 1 (*continued*)

Dye	R	λ_{max} (nm)	M.p. (°C)	Yield (%)	Molecular formula	CHN analysis (%) C Found	C Calc.	H Found	H Calc.	N Found	N Calc.
D-9		444	281–282 p	90	$C_{18}H_{21}ClN_2O_5S$	52.2	52.36	5.2	5.13	6.8	6.78
D-6		444	246–247	92	$C_{19}H_{23}ClN_2O_5S$	53.1	53.46	5.5	5.43	6.5	6.56
D-20		446	250–252 p	95	$C_{22}H_{29}ClN_2O_5S$	56.3	56.34	6.3	6.23	5.8	5.97
D-25		445	245–247 p	96	$C_{19}H_{23}ClN_2O_5S$	53.3	53.46	5.5	5.43	6.6	6.56
D-26		444	221–223 p	94	$C_{20}H_{25}ClN_2O_5S$	54.4	54.48	5.8	5.71	6.4	6.35

following design of novel specific fluorescent probes, including homodimeric and homotrimeric dyes [18].

The second method of the novel fluorescent cyanines preparing is based on the acylation of aromatic and aliphatic amines by the dyes containing carboxyl groups with the use of coupling reagent *N,N'*-carbonyldiimidazole (CDI) (Scheme 2, Table 2). We proposed the convenient general procedure of the preparation and purification of compounds. The reaction was carried out successfully and with high yields. Besides, by this procedure, there is no need in protective groups for the indole cycle, hydroxyl groups and aromatic diamine. We could not synthesize the derivative of 9-aminoacridine using the mentioned procedure, so to obtain the 9-aminoacridine derivative we used a more active reagent — *N,N'*-dicyclohexylcarbodiimide (DCC) — in the presence of 1-hydroxybenzotriazole.

Spectral-luminescent properties of synthesized dyes in non-bound state and in complexes with nucleic acids were studied. It was shown that the incorporation of the of hydroxyl group into the dye's structure leads to the increase of the dye's fluorescence when complexed with DNA [8]. The presence of aminoacrydine template leads to the significant decrease of the emission of dye in non-bound state [19] and thus to the increase in the dye

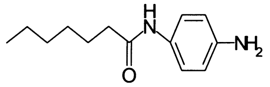
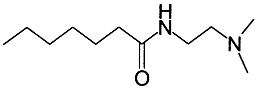
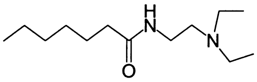
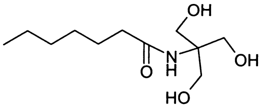
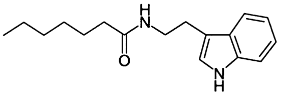
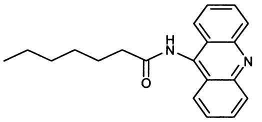


(D-7)

(Series 2, R - described in Table 2)

Scheme 2.

Table 2
Structures and some physico-chemical data for dyes (Series 2)

Dye	R	λ_{max} (nm)	M.p. (°C)	Yield (%)	Molecular formula	CHN analysis (%)					
						C		H		N	
						Found	Calc.	Found	Calc.	Found	Calc.
D-23		447	133–135 dec.	89	$C_{28}H_{33}ClN_4O_5S$	63.7	63.77	5.2	5.35	8.6	8.50
D-21		444	134–136	90	$C_{26}H_{37}ClN_4O_5S$	52.9	53.28	6.2	6.19	7.3	7.17
D-18		446	151–153	92	$C_{28}H_{41}ClN_4O_5S$	58.3	58.68	5.7	5.80	9.8	9.78
D-19		445	191–193	68	$C_{26}H_{36}ClN_3O_8S$	56.3	56.46	6.8	6.74	10.2	10.13
D-16		445	210–212	87	$C_{32}H_{37}ClN_4O_5S$	53.1	53.46	5.3	5.43	6.4	6.56
D-17		359, 447	165–167	65	$C_{35}H_{35}ClN_4O_5S$	54.2	54.58	5.8	5.71	6.4	6.35

sensitivity as a probe for the nucleic acids detection. Analysis of the data obtained shows that the **D-17**, **D-19** dyes are perspective as fluorescent probes for nucleic acids detection.

3. Experimental

Melting points of the obtained compounds were determined in capillaries without correction. The absorption spectra were recorded in DMF on Specord M-40 UV/vis spectrophotometer (Germany). The ^{1}H NMR spectra were recorded in DMSO-d_6 using a Varian (300 MHz) instrument; coupling constants are quoted in Hz.

3.1. Preparation of dyes from the Series 1

To the 0.75 mmol (0.277 g) of the **Cyan 39** dye in 5 ml DMF 1 mmol of primary amine was added. The reaction mixture was refluxed during 10 min. Then solvent was evaporated in vacuum and 5 ml of ethanol was added. The precipitate was filtered, washed with water and air dried.

Melting points, λ_{max}, yields and analysis of dyes from Series 1 are given in Table 1. The NMR data for the dyes are presented in the Appendix.

3.2. Preparation of dyes from the Series 2

To the 0.5 mmol (0.241 g) of the **D-7** dye in 1 ml DMF at 40°C 0.55 mmol (0.089 g) of CDl was added. After 5 min solution of 0.5 mmol of amine in DMF was added and stored during 1 h in the cause of aliphatic amines or during 24 h for aromatic amine. Then solvent was evaporated in vacuum. The dyes were crystallized from ethanol. The precipitate was filtered, washed with water and air dried.

Melting points, λ_{max}, yields and analysis of dyes from Series 2 are given in Table 2. The NMR data for the dyes are presented in the Appendix.

*3.3. Preparations of the **D-17** dye*

To the 0.5 mmol (0.241 g) of the **D-7** dye in 7 ml DMF at 0°C 0.5 mmol (0.097 g) of 9-aminoacrydine, 1 mmol (0.206 g) DCC, and 0.5 mmol (0.068 g) 1-hydroxybenzotriazole was added. The reaction mixture was stirred at this temperature during 24 h. Solution was heated to the room temperature and filtered. Then solvent was evaporated in vacuum. The dye was crystallized from ethanol. The precipitate was filtered, washed with water and air dried.

Melting point, λ_{max}, yield and analysis of the **D-17** dye are given in Table 2. The ^{1}H NMR data for the dye are presented in the Appendix.

Appendix 1. 1H NMR spectra of synthesized dyes

D-1. 2-1-[2-(1*H*-Benzo[*d*]imidazol-2-yl)ethyl]-2,6-di-methyl-1,4-dihydro-4-pyridinylidenmethyl-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 2.68 (6H, s), 3.37 (2H, t, J = 6.9), 3.69 (3H, s), 4.67 (2H, t, J = 7.1), 6.03 (1H, s), 7.07 (1H, s), 7.20 (4H, m), 7.49 (4H, m), 7.83 (1H, t, J = 6.9).

D-2. 2-[1-(3,4-Dimethoxyphenethyl)-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl]-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 2.63 (6H, s), 3.02 (2H, t, J = 6.9), 3.70 (6H, s), 3.73 (3H, s), 4.40 (2H, t, J = 7.2), 6.05 (1H, s), 6.81 (2H, m), 6.91 (1H, d, J = 7.5), 7.23 (2H, s), 7.29 (1H, t, J = 7.8), 7.55 (2H, m), 7.86 (1H, d, J = 7.8).

D-3. 2-[1-(2-Diethylaminoethyl)-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl]-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (CDCl$_3$) 0.88 (6H, t, J = 8.3), 2.52 (4H, q, J = 8.2), 2.72 (8H, m), 3.69 (3H, s), 4.28 (2H, t, J = 7.3), 6.05 (1H, s), 7.24 (2H, s), 7.28 (1H, t, J = 7.3), 7.55 (2H, m), 7.86 (1H, d, J = 7.7).

D-4. 2-[1-(5-Amino-5-carboxypentyl)-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl]-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 1.51 (2H, m, br), 1.73 (4H, m, br), 3.20 (1H, m, br), 3.67 (3H, s), 4.16 (2H, t, br), 6.03 (1H, s), 6.68 (6H, s), 7.22 (2H, s), 7.26 (1H, t, J = 7.2), 7.51 (2H, m), 7.82 (1H, d, J = 7.5).

D-5. 2-[1-(2-Dimethylaminoethyl)-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl]-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 2.25 (6H, s), 2.65 (2H, t, J = 7.3), 2.70 (6H, s), 6.69 (3H, s), 6.04 (1H, s), 7.22 (2H, s), 7.28 (1H, t, J = 7.2), 7.54 (2H, m), 7.85 (1H, d, J = 7.87).

D-6. 2-[1-(3-Hydroxypropyl)-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl]-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 1.88 (2H, m, br), 2.69 (6H, s), 3.55 (2H, m, br), 3.68 (3H, s), 4.28 (2H, t, J = 6.9), 4.81 (1H, t, br), 6.03 (1H, s), 7.23 (2H, s), 7.27 (1H, t, J = 7.8), 7.53 (2H, m), 7.87 (1H, d, J = 7.2).

D-7. 2-[1-(5-Carboxypentyl)-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl]-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 1.43 (2H, quint, J = 7.2), 1.60 (2H, quint, J = 7.2), 1.72 (2H, quint, J = 7.1), 2.25 (1H, t, J = 7.3), 2.67 (6H, s), 3.67 (3H, s), 4.16 (2H, t, J = 6.8), 6.02 (1H, s), 7.19 (2H, s), 7.26 (1H, t, J = 7.2), 7.50 (2H, m), 7.83 (1H, d, J = 7.8).

D-8. 2-1-[2-(1*H*-3-Indolyl)ethyl]-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 2.66 (6H, s,), 3.21 (2H, t, J = 7.0), 3.65 (3H, s), 4.45 (2H, t, J = 7.2), 6.05 (1H, s), 7.01 (1H, t, J = 6.9), 7.10 (1H, t, J = 7.0), 7.21 (4H, m), 7.38 (1H, d, J = 7.5), 7.54 (3H, m), 7.85 (1H, d, J = 7.8), 10.98 (1H, s).

D-9. 2-[1-(2-Hydroxyethyl)-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl]-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 2.68 (6H, s), 3.67 (3H, s), 3.79 (2H, q, J = 7.1), 4.34 (2H, t, J = 6.9), 5.25 (1H, t, J = 7.4), 6.02 (1H, s), 7.20 (2H, s), 7.27 (1H, t, J = 7.2), 7.53 (2H, m), 7.86 (1H, d, J = 7.8).

D-10. 2-(2,6-Dimethyl-1-phenethyl-1,4-dihydro-4-pyridinylidenmethyl)-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 2.67 (6H, s), 3.10 (2H, t, J = 6.9), 3.70 (3H,s), 4.41 (2H, m), 6.05 (1H, s), 7.23 (2H, s), 7.32 (6H, m), 7.56 (2H, m), 7.87 (1H, d, J = 7.8).

D-11. 2-[2,6-Dimethyl-1-(2-piperazinoethyl)-1,4-dihydro-4-pyridinylidenmethyl]-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 2.70 (10H, m, br), 2.71 (2H, t, J = 7.3), 3.11 (4H, m, br), 3.69 (3H, s), 4.31 (2H, t, J= 6.9), 6.04 (1H, s), 7.22 (2H, s), 7.29 (1H, t, J=7.3), 7.55 (2H, m), 7.86 (1H, d, J=7.8).

D-12. 2-[1-(2-Aminoethyl)-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl]-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 2.70 (6H, s), 2.91 (2H, t, J=5.0), 3.68 (3H, s), 4.19 (2H, t, J=7.8), 6.03 (1H, s), 7.22 (2H, s), 7.27 (1H, t, J=6.7), 7.53 (2H, m), 7.84 (1H, d, J=7.7).

D-13. 2-[1-(3-Aminopropyl)-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl]-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 1.87 (2H, quint, J=5.2), 2.68 (6H, s), 2.81 (2H, t, J=6.0), 3.67 (3H, s), 4.26 (2H, t, J=6.8), 6.01 (1H, s), 7.21 (2H, s), 7.29 (1H, t, J=6.9), 7.51 (2H, m), 7.84 (1H, d, J=7.8).

D-14. 2-(1-Anilino-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl)-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 2.46 (6H, s), 3.77 (3H, s), 6.20 (1H, s), 6.56 (2H, d, J=7.2), 6.95 (2H, t, J=7.2), 7.29-7.37 (5H, m), 7.56 (1H, t, J=7.7), 7.67 (1H, d, J=7.7), 7.92 (1H, d, J=7.7), 9.87 (1H, s).

D-16. 2-(1-5-[2-(1*H*-3-Indolyl)ethylcarbamoyl]-pentyl-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl)-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 1.39 (2H, m), 1.59 (2H, quint, J=6.9), 1.71 (2H, m), 2.10 (2H, t, J=6.8), 2.60 (6H, s), 2.80 (2H, t, J=6.8) 3.17 (3H, s), 3.35 (2H, dd, J=6.4), 4.14 (2H, t, J=7.2), 6.00 (1H, s), 6.97 (1H, t, J=7.1), 7.00 (1H, t, J=7.1), 7.14 (1H, s), 7.20 (2H, s) 7.27 (1H, t, J=7.1), 7.31 (1H, d, J=7.0) 7.53 (3H, m), 7.85 (1H, d, J=7.2), 7.92 (1H, t, br), 10.79 (1H, s).

D-17. 2-1-[5-(9-Acridinylcarbamoyl)pentyl]-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 1.60 (2H, m), 1.87 (4H, m), 2.72 (8H, m), 3.68 (3H, s), 4.26 (2H, t, J=7.2), 6.00 (1H, s), 7.24 (2H, s), 7.28 (1H, t, J=7.2), 7.55 (2H, m), 7.63 (2H, t, J=7.3), 7.86 (3H, m), 8.12 (2H, d, J=8.0), 8.19 (2H, d, J=8.0), 10.63 (1H, s).

D-18. 2-1-[5-(2-Diethylaminoethylcarbamoyl)-pentyl]-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 0.94 (6H, t, J=6.8), 1.39 (2H, m), 1.57 (2H, quint, J=6.9), 1.72 (2H, m), 2.10 (2H, t, J=6.3), 2.45 (6H, m), 2.64 (6H, s), 3.08 (2H, d t, br), 3.67 (3H, s), 4.15 (2H, t, J=7.2), 6.00 (1H, s), 6.50 (6H, s), 7.20 (2H, s), 7.27 (1H, t, J=7.2), 7.50 (2H, m), 7.6 (1H, b s), 7.81 (1H, d, J=7.2).

D-19. 2-(1-5-[2-Hydroxy-1,1-di(hydroxymethyl)-ethylcarbamoyl]pentyl-2-methyl-1,4-dihydro-4-pyridinylidenmethyl)-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 1.42 (2H, m), 1.60 (2H, quint, J=6.9), 1.73 (2H, m), 2.20 (2H, t, J=6.3), 2.68 (6H, s), 3.55 (6H, s), 3.68 (3H, s), 4.16 (2H, t, J=7.2), 4.35 (3H, s, br), 6.00 (1H, s), 7.00 (1H, s, br), 7.22 (2H, s), 7.27 (1H, t, J=6.9), 7.51 (2H, m), 7.82 (1H, d, J=7.4).

D-20. 2-[1-(6-Hydroxyhexyl)-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl]-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 1.42 (2H, m), 1.60 (2H, quint, J=6.9), 1.73 (2H, m), 2.20 (2H, t, J=6.3), 2.68 (6H, s), 3.55 (6H, s), 3.68 (3H, s), 4.16 (2H, t, J=7.2), 4.35 (3H, s, br), 6.00 (1H, s), 7.00 (1H, s, br), 7.22 (2H, s), 7.27 (1H, t, J=6.9), 7.51 (2H, m), 7.82 (1H, d, J=7.4).

D-21. 2-1-[5-(2-Dimethylaminoethylcarbamoyl)-pentyl]-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 1.39 (2H, m), 1.59 (2H, quint, J=6.9), 1.71 (2H, m), 2.14 (8H, m), 2.27 (2H, t, J=6.3), 2.67 (6H, s), 3.15 (2H, d t, br), 3.67 (3H, s), 4.16 (2H, t, J=7.1), 6.00 (1H, s), 7.21 (2H, s), 7.26 (1H, t, J=7.0), 7.50 (2H, m), 7.60 (1H, s, br), 7.84 (1H, d, J=8.0).

D-23. 2-1-[5-(4-Aminophenylcarbamoyl)pentyl]-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 1.45 (2H, m), 1.66 (2H, quint, J=6.9), 1.78 (2H, m), 2.27 (2H, t, J=6.3), 2.67 (6H, s), 3.67 (3H, s), 4.17 (2H, t, J=7.2), 4.82 (2H, s, br), 6.01 (1H, s), 6.47 (2H, d, J=8.0), 7.21 (2H, s), 7.29 (1H, t, J=7.2), 7.53 (4H, m), 7.86 (1H, d, J=7.2), 9.43 (1H, s).

D-24. 2-[1-(3-Dimethylaminopropyl)-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl]-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 1.88 (2H, quint, J=5.1), 2.18 (6H, s), 2.34 (2H, t, J=6.0), 2.69 (6H, s), 3.68 (3H, s), 4.22 (2H, t, J= 7.6), 6.02 (1H, s), 7.21 (2H, s), 7.27 (1H, t, J=8.0), 7.52 (2H, m), 7.85 (1H, d, J=8.1).

D-25. 2-[1-(2-Methoxyethyl)-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl]-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 2.67

(6H, s), 3.26 (3H, s), 3.68 (3H, s), 3.73 (2H, t, $J = 5.2$), 4.46 (2H, t, $J = 5.2$), 6.03 (1H, s), 7.21 (2H, s), 7.28 (1H, t, $J = 7.5$), 7.54 (2H, m), 7.85 (1H, d, $J = 8.0$).

D-26. 2-[1-(3-Methoxypropyl)-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl]-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 1.98 (2H, quint, $J = 5.1$), 2.67 (6H, s), 3.25 (3H, s), 3.46 (2H, t, $J = 5.9$), 3.63 (3H, s), 4.25 (2H, t, $J = 7.6$), 6.02 (1H, s), 7.21 (2H, s), 7.27 (1H, t, $J = 7.5$), 7.52 (2H, m), 7.85 (1H, d, $J = 8.0$).

D-27. 2-[1-(3-Diethylaminopropyl)-2,6-dimethyl-1,4-dihydro-4-pyridinylidenmethyl]-3-methyl-1,3-benzothiazol-3-ium perchloride. δ_H (DMSO-d_6) 0.98 (6H, t, $J = 8.0$), 1.83 (2H, quint, $J = 5.1$), 2.50 (6H, m), 2.69 (6H, s), 3.68 (3H, s), 4.22 (2H, t, $J = 7.9$), 6.03 (1H, s), 7.23 (2H, s), 7.27 (1H, t, $J = 7.6$), 2.01 (2H, m), 7.85 (1H, d, $J = 7.8$).

References

[1] Haughland RP. Molecular probes. Handbook of fluorescent probes and research chemicals. Eugene, OR: Molecular Probes Inc, 1996, 679..

[2] Ju J, Glazer A, Mathies R. Energy transfer primer: a new fluorescent labeling paradigm for DNA sequencing and analysis. Nature Medicine 1996;2:246–9.

[3] Rye H, Quesada M, Peck K, Glazer A. High-sensitive two-colour detection of double-stranded DNA with a confocal fluorescence gel scanner using ethidium homodimer and thiazole orange. Nucleic Acids Res 1990;19:333–72.

[4] Hamer F. The cyanine dyes and related compounds. New York: Interscience Publisher, 1964, pp. 58–59.

[5] Deligeorgiev T, Zaneva D, Kim S, Sabnis M. Preparation of monomethine cyanine dyes for nucleic acid detection. Dyes and Pigments 1998;37(3):205–11.

[6] Yarmoluk S, Kovalska V, Kovtun Y, Interaction of cyanine dyes with nucleic acids V. Towards model of "half intercalation" of monomethine cyanine dyes into double-stranded nucleic acids. Biopolymers and Cell 1999; 15(1):75–83.

[7] Kelåmen J, Wizinger R. Helvetica Chåmica Acta 1962; 45:1908–17.

[8] Kryvorotenko D, Kovalska V, Yarmoluk S. Interaction of cyanine dyes with nucleic acids. 10. Synthesis and spectral properties of novel benzothiazo-4-[2,6-dimethylpyridinium] monomethyne cyanine dyes. Biopolymers and Cell 2000;16(2):145–52.

[9] Yarmoluk S, Kostenko O, Dubey I. Labeling of oligonucleotides with pyrylium cyanine dyes. Bioorganic & Medicinal Letters 2000;10(19):2201–4.

[10] Yarmoluk S, Kryvorotenko D, Gerasymchuk Yu. Interaction of cyanine dyes with nucleic acids. 5. Synthesis and spectroscopic properties of thiazole orange–amino acids conjugates. Biopolymers and Cell 1999;15(3):247–51.

[11] Yarmoluk S, Kocheshev I, Kovalska V. Synthesis and spectral properties of novel fluorescent homotrimer cyanine dyes. Proc. 8th European Conf on Spectroscopy of Biological Molecules, post-deadline papers. 1999:19–20.

[12] Kocheshev I, Kovalska V, Yarmoluk S. Synthesis and spectral properties of novel fluorescent homotrimere cyanine dyes. Proc. 8th European Conf. on Spectroscopy of Biological Molecules. 1999:623-4.

[13] Lee G, Chen C, Chiu A. Thiazole Orange: a new dye for reticulocyte analysis. Cytometry 1986;7:508–17.

[14] Carrlsson C, Larsson A, Jonsson M, et al. Optical and photophysical properties of the Oxazol Yellow DNA probes YO and YOYO. J Phys Chem 1994;98:10313–21.

[15] Jacobsen J, Pedersen J, Hansen L, Wemmer D. Site selective bis-intercalation of a homodimeric thiazole orange dye in DNA oligonucleotides. Nucleic Acids Res 1995; 23:753–60.

[16] Rye H, Glazer A. Interaction of dimeric intercalating dyes with single-stranded DNA. Nucleic Acids Res 1995; 23:1215–22.

[17] Kocheshev I, Kovalska V, Yarmoluk S. Interaction of cyanine dyes with nucleic acids. 11. Synthesis and spectral properties of novel 2,6-dimethyl-4-[(3-methyl-1,3-benzothiazol-3-ium-2-yl)methylene]-1,4-dihydro-1-pyridinium homodimer cyanine dyes. Biopolymers and Cell 1999; 15(5):449–55.

[18] Kocheshev I, Kryvorotenko D, Yarmoluk S. Biopolymers and Cell, in press.

[19] Kryvorotenko D, Kovalska V, Yarmoluk S. Interaction of cyanine dyes with nucleic acids. 16. Novel fluorescent benzothiazol-4-[2,6-dimethylpyridinium] monomethyne cyanine dyes. Biopolymers and Cell 2000;16(4):320–6.