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Synthesis of a new β-naphthothiazole monomethine cyanine dye for the detection of DNA in aqueous solution

Reda M. El-Shishtawy*, Abdullah M. Asiri, Salem A. Basaif, T. Rashad Sobahi

Faculty of Science, Chemistry Department, King Abdul-Aziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia

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ABSTRACT

Novel monomethine cyanine dye (MC) derived from β-naphthothiazole and benzothiazole has been prepared and characterized by ^{1}H and ^{13}C NMR, FTIR, ESIMS, elemental analyses, absorption and fluorescence spectroscopy. The dye was conveniently synthesized by the condensation of two sulfate heterocyclic quaternary salts. The interaction between calf thymus DNA (ct-DNA) in tris(hydroxymethyl)aminomethane–HCl (Tris–HCl) aqueous buffer solution and MC has been studied with spectral fluorescence method. The binding constant value has been determined by fluorescence titration of MC with ct-DNA concentrations. The result obtained is consistent with an intercalative binding interaction between MC and ct-DNA. Compared with ethidium bromide (EB), MC showed a huge fluorescence enhancement upon mixing with ct-DNA.

1. Introduction

Manipulation at the molecular level of individual chemical and biological substances in solution is currently a challenging target in physics, chemistry, and biology. In particular, direct visualization of macromolecules such as DNA by staining with a suitable fluorescent dye is of great interest. Therefore, exploring new fluorescent dyes and expanding the tool box of currently available fluorescent probes for the monitoring of biological systems and processes is a challenge crucial to several research areas spanning from medical diagnostics to genomics and live cell metabolism [1]. In all these fields, new and more efficient analytical tools to characterize nucleic acids, proteins, and cells and the production of low cost, easy to manipulate systems for fast sample analysis and large-scale screening are highly required. In this rapidly developing context, fluorescent detection has become one the most exploited techniques owing to its sensitivity and noninvasiveness [2]. Conventionally, EB has been used for the detection of DNA, however its mutagenic effects pose some environmental concerns [3–5].

On the other hand, cyanine dyes are sensitive and more safe fluorescent probes and are widely used for the detection of nucleic acids [6–8]. In this way femtomoles of nucleic acids may be detected and selectively identified even in the presence of other biopolymers [9–12]. Cyanine dyes demonstrate a significant increase in fluorescence intensity upon binding to DNA [13], and the resulting high signal/noise ratio affords detection in a homogeneous medium [14–18]. The huge enhancement in fluorescence upon binding to DNA is believed to originate from the loss of mobility around the methine bridge between the two heterocyclic moieties [19]. There are three main modes of non-covalent interactions of these small molecules with DNA, intercalation, minor groove binding and electrostatic interaction of highly positively charged molecules with nucleotide phosphate backbone [14,20].

The structural differences between biological molecules results in a different modes of interaction with the same probe. Moreover, size of aromatic moiety as well as bulkiness of attached substituents and their ability to interact non-covalently with polynucleotide could also control intercalation ability as well as orientation of intercalated molecule [21,22]. Therefore, tailoring new molecular probes for the detection of biological targets is being attractive for many scientists. In a continuation of our interest in cyanine dyes suitable for the detection of DNA [23], we report here the synthesis of a new MC and its binding properties with ct-DNA.

2. Experimental

2.1. General

Ethidium bromide, calf thymus DNA of low molecular weight (1254 g/mol base pairs) and other reagents were of the highest purity available, purchased from Sigma–Aldrich Company and used as received. Solvents were of analytical grade. ^{1}H and ^{13}C

* Corresponding author. Permanent address: Department of Dyes, Dyeing and Textile Auxiliaries, Textile Research Division, National Research Centre, El-Behouth St. Dokki, Cairo, PO 12622, Egypt.
E-mail address: elshishtawy@hotmail.com (R.M. El-Shishtawy).

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NMR spectra were recorded in DMSO-d6 solutions on a Bruker Avance 600 MHz spectrometer. Infrared spectra were performed on a PerkinElmer spectrum 100 FTIR spectrometer. ESI mass spectra were recorded on a Bruker Esquire 6000. Elemental microanalyses were performed at the Cairo University Microanalytical Center. Melting points were determined in open capillary tubes in a Stuart Scientific melting point apparatus SMP3 and are uncorrected.

2.2. Absorption spectra

Absorption measurements were carried out at the MC concentration 1.3×10^{-6} in DMSO, 2×10^{-6} M in Tris–HCl buffer solution (50 mM, pH 7.4) and the ct-DNA concentration 6×10^{-5} M. For EB, the measurements were made at concentration 1×10^{-5} M in DMSO, 2×10^{-6} M in buffer and the ct-DNA concentration 4×10^{-5} M. Absorption spectra were measured on a UV-1650 PC Shimadzu spectrophotometer.

2.3. Fluorescence titration

Stock solutions of MC (1×10^{-5} M) and ct-DNA (5×10^{-4} M, moles of base pairs per liter) were prepared in DMSO and Tris–HCl buffer solution, respectively. Fluorescence titration with ct-DNA concentrations (0–180 μM) were made by mixing 5 ml of MC solution with Tris–HCl buffer solution without and with ct-DNA up to the meniscus of 25 ml volumetric flask for making the working solutions of MC (2×10^{-6} M). Fluorescence spectra were measured on a RF-5301 PC Shimadzu spectrofluorophotometer and are uncorrected.

2.4. Synthesis

2.4.1. (Z)-1-methyl-2-((3-methylbenzo[d]thiazol-2(3H) ylidene)methyl)naphtho[1,2-d]thiazol-1-ium methylsulfate

A mixture of 2-(methylthio)benzo[d]thiazole (1.99 g, 10 mmol), 2-methylnaphtho[1,2-d]thiazole (1.8 g, 10 mmol), and dimethyl sulfate (2.65 g, 21 mmol) was heated at 130 °C for 5 h until practically total conversion into the corresponding quaternary ammonium salts was achieved. The resulting mixture of solid salts was heated at 100–105 °C in dry MeOH/pyridine/triethyl amine solvent mixture (60 ml, 2:3:1, v/v) for 9 h. The mixture was concentrated in vacuo and the crude yellow product was filtered, washed with acetone and air-dried. Recrystallization from DMF affords the analytical pure product as a yellow powder (1.6 g, 34%). Mp 304–305 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) 3.41 (3H, s, OCH_3), 4.07 (3H, s, N–CH_3), 4.50 (3H, s, N–CH_3), 6.82 (1H, s, H-α), 7.48 (1H, t, J = 8.4 Hz, H-5′), 7.67 (1H, t, 8.4 Hz, H-6′), 7.72 (1H, t, J = 7.8 Hz, H-6), 7.78 (1H, t, J = 8.4 Hz, H-5), 7.87 (1H, d, J = 8.4 Hz, H-8), 8.05 (1H, d, J = 8.4 Hz, H-7′), 8.16 (1H, d, J = 7.8 Hz, H-4′), 8.20 (1H, d, J = 7.8 Hz, H-7), 8.25 (1H, d, J = 8.4 Hz, H-9), 8.83 (1H, d, J = 8.4 Hz, H-4); ^{13}C NMR (150.87 MHz, DMSO-d_6): d (ppm) 33.99 (N–CH_3), 39.97 (N–CH_3), 40.08 (OCH_3), 83.31 (C-α), 113.63 (C-4′), 119.69 (C-6′), 121.81(C-3a′), 122.61 (C-9), 122.80 (C-9a), 123.39 (C-5′), 124.61 (C-3a), 126.49 (C-5), 124.68 (C-6), 126.97 (C-7), 127.43 (C-4), 128.46 (C-8), 129.54 (C-7′), 133.55 (C-4a), 136.26 (C-7a), 140.73 (C-7a′), 161.17 (C-2′), 162.72 (C-2). IR ν (cm^{-1}): 3065, 2935, 1531, 1472, 1438, 1280, 1227, 1127, 1165, 1055, 840, 807, 737. UV/vis (DMSO): λ_{max} = 445 nm and ε_{max} = 60,000 M^{-1} cm^{-1}. MS (ESI): M$^+$; found: 361.0, $C_{21}H_{17}N_2S_2$ requires 361.0. Anal. Calc. For $C_{22}H_{20}N_2O_4S_3.1.5H_2O$: C; 52.88, H; 4.64, N; 5.61. Found. C; 52.42, H; 4.95, N; 6.19%.

3. Results and discussion

3.1. Synthesis

The classical structural criteria of small molecules for optimum DNA fit and interaction have to meet: a crescent shape that complements the helical DNA minor groove, recognition units (H-bond donors and acceptors) on the side of the molecule facing DNA, cationic centre at terminals of the molecules to enhance electrostatic interactions with the negatively charged phosphate groups on DNA, and hydrophobic character by an extended unfused heterocyclic structure to allow optimization of the compound for DNA minor groove interactions [24–26,21]. With this in mind, the synthesis of MC (Fig. 1) was made for the detection of ct-DNA as an alternative to the classical mutagenic and carcinogenic [23] EB stain (Fig. 1).

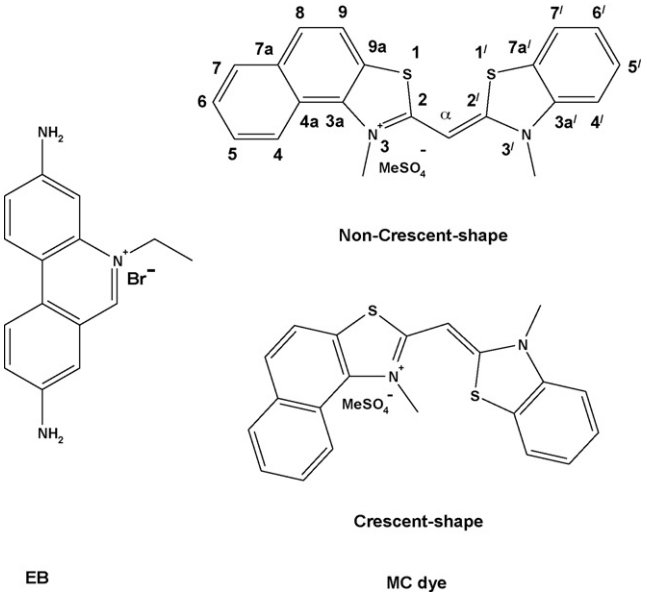


Fig. 1. Ethidium bromide and monomethine cyanine dyes used.

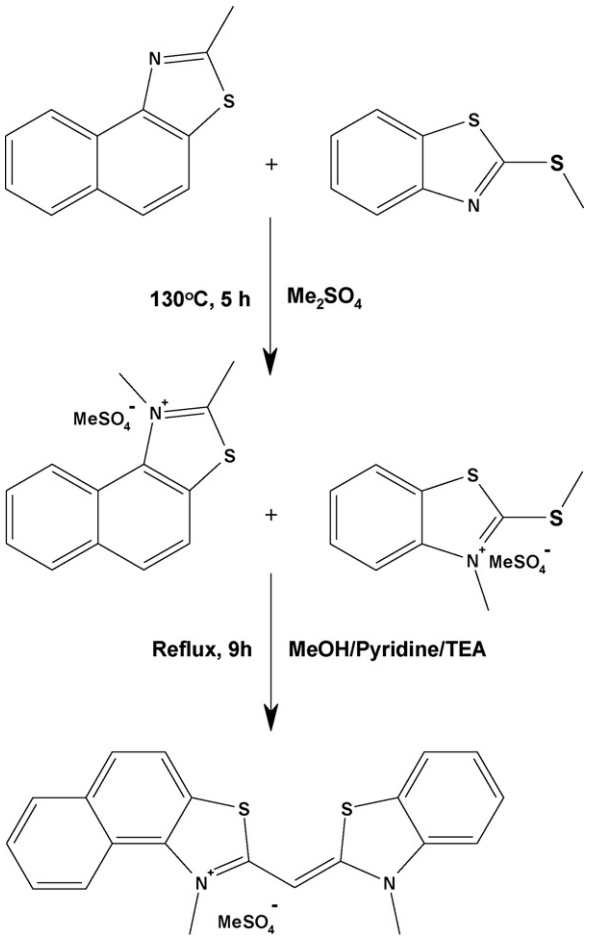
Monomethine cyanine dyes are generally synthesized by the reaction of two heterocyclic quaternary salts, one bearing a methyl group and the other a thioalkyl as a good nucleofugic leaving group, both in the 2-position in relation to the heteroaromatic ammonium salt. Deprotonation of the first quaternary salt by a base such as triethyl amine or pyridine gives the methylene derivative base, which acts as the nucleophilic reagent [27]. The synthetic route of MC is shown in Scheme 1.

N-Alkylation of a mixture (1:1 molar ratio) of 2-methylnaphtho [1,2-d]thiazole and 2-(methylthio)benzo[d]thiazole with dimethyl sulfate at 130 °C afforded the corresponding methylate salts as a viscous material, which was further used in the synthesis of MC without purification. Treatment of the methylate salts with a pyridine/triethylamine mixture (3:1 by volume) in MeOH at 100–105 °C gave MC as a yellow product in 34% yield. The structure of MC was evidenced by its ^{1}H NMR, ^{13}C NMR, FTIR, ESIMS, absorption and fluorescence spectroscopy

3.2. Absorption and fluorescence measurements

It is known that cyanine dyes tend to self-aggregate in solution [28,29], and since this process can influence the course of dye binding to a given substrate such as ct-DNA, a dilute solution of the dye in the range of 10^{-6} M has to be used. The absorption and fluorescence spectra of free MC in DMSO (1.3×10^{-6} M), Tris–HCl buffer (pH 7.4) (2×10^{-6} M) and in Tris–HCl buffer (2×10^{-6} M) containing ct-DNA (6×10^{-5} M) are presented in Fig. 2.

The absorption spectrum of MC (Fig. 2) in DMSO in the visible region exhibits one absorption band (with a maximum at 446 nm) and a shoulder at about 422 nm. The spectrum of MC in buffer solution in the visible region has a similar shape, and its maximum is



Scheme 1. Synthesis of monomethine cyanine dye.

blue shifted by 6 nm and its shoulder is also blue shifted by about 3 nm when compared to the maximum of the dye in DMSO. On the other hand, the absorption spectrum of MC in buffer solution containing ct-DNA in the visible region has also a similar shape with those of DMSO and buffer and its maximum is the same as in buffer, whereas its shoulder appears at about the same as in DMSO. This spectral similarity of MC in the above different media suggests that the observed spectrum corresponds to the absorption of the monomer dye molecules and the shoulders correspond to different vibronic transitions to the same electronic excited state [19]. Increasing the polarity of the solvent from DMSO to aqueous medium has led to a blue shift in the absorption spectra. This shift is most likely explained as follows; since MC is ionic dye, which exhibits a polar character in the ground state, hence the solvent molecules are oriented in such a way as required by the polar character of the MC molecule. During the transition, which occurs within a very small time interval, only the electrons have the time to change position. The excited MC molecules, in which the electric dipole has been weakened and has been reoriented, are now within a solvent cage that is no longer adopted to the electronic requirements of the excited molecule, since the solvent cage is suitable for the electronic distribution in the ground state molecule. Thus, a polar solvent creates a stabilizing solvent cage around this ionic dye in the ground state, but a destabilizing solvent cage for the excited state. The transition energy increases with increasing solvent polarity. An increase in solvent polarity results in a blue shift of the charge transfer band. In this context, hydrogen bonds created between water molecules in aqueous medium and MC molecules can reduce the delocalization of the lone pair of electrons of hetrocyclic nitrogen, which result in the observed solvatochromic behavior.

The fluorescence spectrum of MC (Fig. 2) in DMSO exhibits a weak fluorescence band with a maximum at 503 nm, which is blue shifted to 493 and 487 nm in buffer and buffer solution containing ct-DNA, respectively. This blue shift can be explained as above, in which the aqueous solvent stabilize the ground state and destabilize the excited state of MC molecules. It is worth noting that, the fluorescence of MC is largely enhanced upon binding with ct-DNA when compared with its fluorescence in the free state in both DMSO and buffer solutions. This fluorescence enhancement is attributable to the fact that on photoexcitation a lack of free rotation around the internuclear bridge makes isomerisation around the methine bond impossible [19,30], and subsequently nonradiative deactivation of the excited state is not possible causing the dye to fluoresce.

For comparison, the absorption and fluorescence spectra of EB (Fig. 3) were similarly measured in DMSO (1×10^{-5} M) and Tris–HCl buffer (pH 7.4) (2×10^{-6} M) and in Tris–HCl buffer (2×10^{-6} M) containing ct-DNA (4×10^{-5} M) and summarized in Table 1.

As can be seen, a similar spectral shape of absorption is also obtained for EB in DMSO, Tris–HCl buffer and in Tris–HCl buffer containing ct-DNA. This indicates that the observed spectrum corresponds to the absorption of the monomer dye molecules. Also, the blue shift observed in the absorption as well as in the fluorescence can be rationalized as in the case of MC since EB dye is also ionic with a positive charge as in the case of MC. However, the enhancement of the fluorescent intensity of EB was far weak relative to the

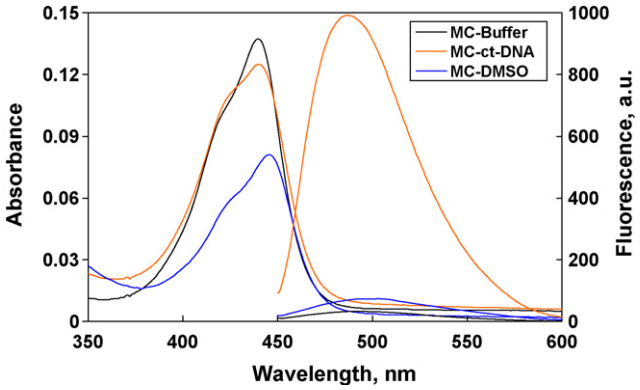


Fig. 2. Absorption and fluorescence spectra of MC dye in DMSO solution, in Tris–HCl buffer and in the presence of ct-DNA solution. The dye concentration used for the measurements was 1.3×10^{-6} M for DMSO, 2×10^{-6} M for buffer and DNA concentration was.

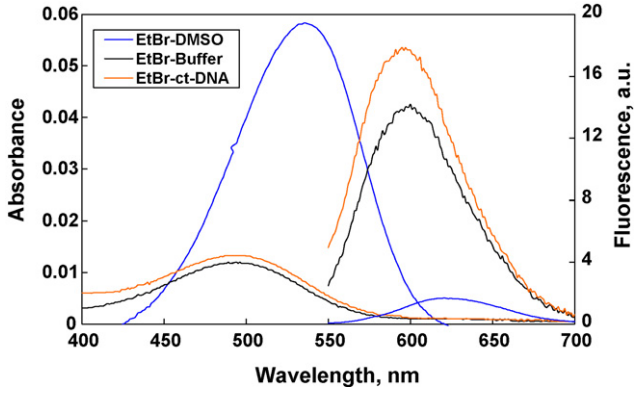


Fig. 3. Absorption and fluorescence spectra of EtBr dye in DMSO solution, in Tris–HCl buffer and in the presence of ct-DNA solution. The dye concentration used for the measurements was 1×10^{-5} M in DMSO, 2×10^{-6} M in buffer and DNA concentration was 4×10^{-5} M.

Table 1
Spectroscopic data of the investigated dyes.

Dye	ε (DMSO) (M^{-1} cm^{-1})	λ_{max} (DMSO) (nm)	ε (DNA) (M^{-1} cm^{1})	λ_{max}[a] (DNA) (nm)	λ_{ex}[b] (nm)	F_{em} (DMSO) (nm)	F_{em}[c] DNA (nm)	K_b[d] (M^{-1})	FE (%)[e]
MC	62,230	446	62,500	440	440	503	487	1.01×10^4	1914
EB	5830	535	6650	490	490	628	597	1.50×10^5	26

[a] Maximum wavelength of absorption of MC dye in Tris–HCl buffer solution containing ct-DNA.
[b] Excitation wavelength.
[c] Emmision wavelength.
[d] Data of K_b for EB is taken from Ref. [15].
[e] FE is the calculated fluorescence enhancement percent at the same ct-DNA base pairs/dye ratio (20) for both EB and MC dyes using this equation: $\%FE = ((I - I_0)/I_0) \times 100$ where I_0 and I are the fluorescence intensity in presence and absence of DNA, respectively.

case of MC as shown in Figs. 2 and 3 and Table 1. This result suggests the suitability of using MC as a fluorescent probe for the detection of ct-DNA in aqueous solution.

3.3. Fluorescence titration of MC with ct-DNA and binding constant

It has been reported that the binding constant of probes which bind to DNA by intercalation mode via hydrophobic, van der Waals and electrostatic forces usually do not exceed 10^7 M^{-1}. For example K_b values of acridine orange [31,32], EB [31,15] and thiazole orange [33] are equal to 3.1×10^4, 1.5×10^5 and 10^6 M^{-1}, respectively. However, complexes of groove-binding molecules such as netropsin and distamycin A stabilized by hydrogen bonds may have the binding constants values as large as 10^8–10^9 M^{-1} [34]. Since MC chemical structure (Fig. 1) reveals possibility for a crescent shape, hence it was of interest to find out whether MC probe is complexing with ct-DNA by intercalation or groove mode. For this purpose, fluorescence titration of MC (2×10^{-6} M) was conducted using different concentrations of ct-DNA.

The binding of MC with ct-DNA can be represented by the following equation [34];

$$\underset{C_f}{[Dye]} + \underset{C_{bs}}{[DNA]} \rightarrow \underset{C_b}{[Dye \cdot DNA]}$$

$$K_b = \frac{C_b}{C_f C_{bs}} \quad (1)$$

where C_b is the concentration of the MC molecules bound to ct-DNA, C_f the free MC concentration, C_{bs} the concentration of the available binding site of ct-DNA. We consider the number of available ct-DNA binding sites to be equal to the number of base pairs in the ct-DNA strand which are unoccupied by dye molecules. If C_{Dye} is the total concentration of MC in solution and $C_{ct\text{-}DNA}$ (ct-DNA concentration) significantly higher than C_{Dye}, we can consider C_b to be equal to $C_{ct\text{-}DNA}$ and then we can get Eqs. (2) and (3).

$$C_f = C_{Dye} - C_b \quad (2)$$

$$C_{bs} = C_{ct\text{-}DNA} \quad (3)$$

Using Eqs. (1)–(3) leads to Eq. (4).

$$K_b = \frac{C_b}{[C_{Dye} - C_b]C_{ct\text{-}DNA}} \quad (4)$$

Eq. (4) can be rewritten to give Eq. (5).

$$\frac{1}{K_b} = \frac{[C_{Dye} - C_b]C_{ct\text{-}DNA}}{C_b} \quad (5)$$

Dividing Eq. (5) by $C_{ct\text{-}DNA}$ leads to Eq. (6).

$$\frac{1}{C_{ct\text{-}DNA}K_b} = \frac{[C_{Dye} - C_b]C_{ct\text{-}DNA}}{C_b} \quad (6)$$

Eq. (6) leads to Eq. (7).

$$\frac{1}{C_{ct\text{-}DNA}K_b} = \frac{C_{Dye}}{C_b} - 1 \quad (7)$$

Upon MC binding with ct-DNA during the fluorescence titration, the fluorescence intensity I increases until it reaches its maximum value I_{max} when all C_{Dye} get completely bound with ct-DNA, assuming that there is only one mechanism of the dye ct-DNA interaction in this case and the intrinsic fluorescence of the free dye is negligible. Hence, the fraction of the bound dye as follows;

$$\frac{C_b}{C_{Dye}} = \frac{I}{I_{max}} \quad (8)$$

Thus, Eq. (7) can be rewritten in terms of the fluorescence intensity to give Eq. (9)

$$\frac{1}{C_{ct\text{-}DNA}K_b} = \frac{I_{max}}{I} - 1 \quad (9)$$

Eq. (9) can have the form of Eq. (10).

$$\frac{I_{max}}{I} = 1 + \frac{1}{C_{ct\text{-}DNA}K_b} \quad (10)$$

Dividing Eq. (10) by I_{max} leads to Eq. (11).

$$\frac{1}{I} = \frac{1}{I_{max}} + \frac{1}{C_{ct\text{-}DNA}I_{max}K_b} \quad (11)$$

A plot of $1/I$ versus $1/C_{ct\text{-}DNA}$ is expected to be linear with a slope of $1/I_{max}K_b$ and an intercept of $1/I_{max}$. As can be seen in Fig. 4 the linear fitting of Eq. (11) between the inverse of ct-DNA concentration and the inverse of the fluorescence intensity holds indeed and the estimated K_b value was equal to 1.01×10^4 M^{-1} as shown in Table 1. It is indicated (Table 1) that the binding constant K_b of MC is approx. 15-times lower of K_b of EB. On other hand, fluorescence enhancement is 74-times higher. It means that the ability of visualization of ct-DNA using MC dye is 74/15 = 4.933. Also, K_b value of MC suggests that MC forms a stable complex with ct-DNA and the order of 10^4 M^{-1} for K_b indicates that MC can be considered as a small molecule that interacts with ct-DNA by intercalation via a non-crescent shape (Fig. 1). A similar intercalation binding constants

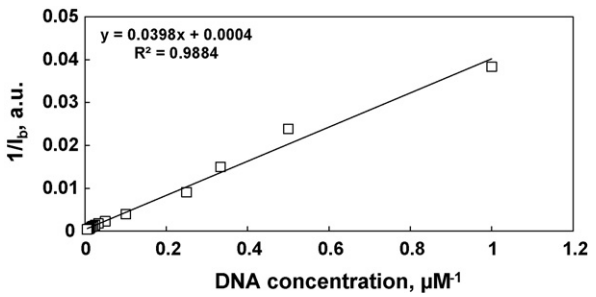


Fig. 4. Fluorescence titration of MC dye solution (2×10^{-6} M) as a function of the DNA concentrations.

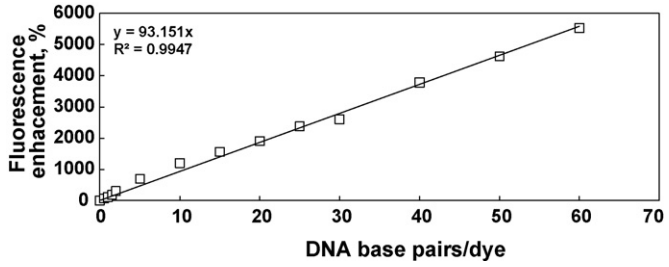


Fig. 5. Fluorescence titration of MC dye solution (2×10^{-6} M) as a function of the DNA base pairs/dye.

for other cyanine dyes has been reported [34]. The fluorescence enhancement per cent as a result of ct-DNA complexation is shown in Fig. 5. A linear increase of the fluorescence enhancement per cent with the ct-DNA concentration is observed for mixing ratios less than 60 ct-DNA b.p/dye. On increasing the b.p/dye ratio further, the linear relation started to increase in a less proportion in a curvature manner (data not shown) due the close saturation of MC molecules which means a presence of a limited number of molecules, which are not enough to bind completely a further increase in ct-DNA.

4. Conclusion

A new monomethine cyanine dye derived from β-naphthothiazole has been synthesized and fully spectroscopically characterized. The use of this dye as a probe for the detection of ct-DNA in aqueous solution has been studied with the absorption and fluorescence spectroscopy. The result indicates that MC dye would bind with ct-DNA by intercalation mode. The fluorescence enhancement upon ct-DNA binding was huge when compared with EB to suggest a potential replacement of the mutagenic EB stain for the detection of DNA.

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References

[1] (a) M. Sameiro, T. Gonçalves, Chem. Rev. 109 (2009) 190;
(b) L.D. Lavis, R.T. Raines, ACS Chem. Biol. 3 (2008) 142;
(c) S. Mann, Angew. Chem. Int. Ed. 47 (2008) 2;
(d) U. Resch-Ganger, M. Grabolle, S. Cavaliere-Jaricot, R. Nitschke, T. Nann, Nat. Methods 5 (2008) 763;
(e) A.K. Chen, Z. Cheng, M.A. Behlke, A. Tsourkas, Anal. Chem. 80 (2008) 7437;
(f) A. Simeonov, A.C. Jadhav, J. Thomas, Y. Wang, R. Huang, N.T. Southall, P. Shinn, J. Smith, C.P. Austin, D.S. Auld, J. Inglese, J. Med. Chem. 51 (2008) 2363;
(g) M.R. Longmire, M. Ogawa, Y. Hama, N. Kosaka, C.A.S. Regino, P.L. Choyke, H. Kobayashi, Bioconjugate Chem. 19 (2008) 1735;
(h) N. Johnsson, K. Johnsson, ACS Chem. Biol. 2 (2007) 31;
(i) A.A. Martí, S. Jockusch, N. Stevens, J. Ju, N.J. Turro, Acc. Chem. Res. 40 (2007) 402;
(l) R.C. Willis, Anal. Chem. (2007) 1785;
(m) Y. Hama, Y. Urano, Y. Koyama, M. Bernardo, P.L. Choyke, H. Kobayashi, Bioconjugate Chem. 17 (2006) 426;
(o) R. Yuste, Nat. Methods 2 (2005) 902;
(p) J.W. Lichtman, S.E. Fraser, Nat. Neurosci. 4 (2001) 1215;
(q) P.R. Selvin, Nat. Struct. Biol. 7 (2000) 730.
[2] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, 3rd ed., Springer, New York, 2006.
[3] G.J. Kantor, D.R. Hull, Biophys. J. 27 (1979) 359.
[4] P.A. Cerutti, Science 227 (1985) 375.
[5] R. Marks, Cancer 75 (1995) 607.
[6] R.P. Haugland, Handbook of Fluorescent Probes and Research Products, 9th ed., Molecular Probes, Eugene, OR, 2002.
[7] T.G. Deligeorgiev, Molecular probes based on cyanine dyes for nucleic acid research, in: S. Daehne, U. Resch-Genger, O.S. Wolfbeis (Eds.), Near-infrared Dyes for High Technology Applications (NATO ASI Series), Kluwer, Dordrecht, 1998, p. 125.
[8] T. Matsuzaki, T. Suzuki, K. Fujikura, K. Takata, Acta Histochem. Cytochem. 30 (1997) 309.
[9] M.A. Quesada, H.S. Rye, J.C. Gingrich, A.N. Glazer, R.A. Mathies, Biotechniques 10 (1991) 616.
[10] C. Schneeberger, P. Speiser, F. Kury, R. Zeillinger, PCR Methods Appl. 4 (1995) 234.
[11] U. Lieberwirth, J.K. Arden-Jacob, H. Drexhage, D.P. Herten, R. Műller, M. Neumann, A. Schultz, S. Siebert, G. Sagner, S. Klingel, M. Sauer, Wolfrum, J. Anal. Chem. 70 (1998) 4771.
[12] A.N. Glazer, R.A. Mathies, Curr. Opin. Biotechnol. 8 (1997) 94.
[13] L. Glavaš-Obrovac, I. Piantanida, S. Marczi, L. Mašić, I.I. Timcheva, T.G. Deligeorgiev, Bioorg. Med. Chem. 17 (2009) 4747, and references cited therein.
[14] H.S. Rye, S. Yue, D.E. Wemmer, M.A. Quesada, R.P. Haugland, R.A. Mathies, A.N. Glazer, Nucleic Acids Res. 20 (1992) 2803.
[15] H.S. Rye, J.M. Dabora, M.A. Quesada, R.A. Mathies, A.N. Glazer, Anal. Biochem. 208 (1993) 144.
[16] D.M. Schmidt, J.D. Ernst, Anal. Biochem. 232 (1995) 144.
[17] T. Ishiguro, J. Saitoh, H. Yawata, M. Otsuka, T. Inoue, Y. Sugiura, Nucleic Acids Res. 24 (1996) 4992.
[18] N. Svanvik, G. Westman, D. Wang, M. Kubista, Anal. Biochem. 281 (2000) 26.
[19] C. Carlsson, A. Larsson, M. Jonsson, B. Albinsson, B. Nordén, J. Phys. Chem. 98 (1994) 10313.
[20] M. Demeunynck, C. Bailly, W.D. Wilson (Eds.), DNA and RNA Binders, Wiley-VCH, Weinheim, 2002.
[21] C.R. Cantor, P.R. Schimmel, Biophysical Chemistry, WH Freeman and Co., San Francisco, 1980, pp 1109–1181.
[22] W.D. Wilson, L. Ratmeyer, M. Zhao, L. Strekowski, D. Boykin, Biochemistry 32 (1993) 4098.
[23] R.M. El-Shishtawy, C.R. Santos, I. Gonçalves, H. Marcelino, P. Almeida, Bioorg. Med. Chem. 15 (2007) 5537.
[24] S.A. Shaikh, S.R. Ahmed, B. Jayaram, Arch. Biochem. Biophys. 429 (2004) 81.
[25] J.B. Chaires, J. Ren, D. Hamelberg, A. Kumar, V. Pandya, D.W. Boykin, W.D. Wilson, J. Med. Chem. 47 (2004) 5729.
[26] T.A. Fairley, R.R. Tidwell, I. Donkor, N.A. Naiman, K.A. Ohemeng, R.J. Lombardy, J.A. Bentley, M. Cory, J. Med. Chem. 36 (1993) 1746.
[27] F.M. Hamer, in: A. Weissberger (Ed.), The Cyanine Dyes and Related Compounds—The Chemistry of Heterocyclic Compounds, vol. 18, Interscience, New York, 1971.
[28] T. Biver, A. De Biasi, F. Secco, M. Venturini, S. Yarmoluk, Biophys. J. 89 (2005) 374.
[29] W. West, S. Pearce, J. Phys. Chem. 69 (1965) 1894.
[30] M.Yu. Anikovsky, A.S. Tatikolov, L.A. Shvedova, V.A. Kuzmin, Russ. Chem. Bull. 50 (2001) 1190.
[31] D.J. Arndt-Jovin, T.M. Jovin, in: D.L. Taylor, Y.L. Wang (Eds.), Methods in Cell Biology, vol. 30, Academic Press, New York, 1989, pp. 417–448.
[32] K. Darzynkiewicz, J. Kapuscinski, in: M.R. Melamed, T. Lindmo, M.L. Mendelson (Eds.), Flow Cytometry and Sorting, 2nd ed., Wiley-Liss, New York, 1990, pp. 291–314.
[33] H.S. Rye, M.A. Quesada, K. Peck, R.A. Mathies, A.N. Glazer, Nucleic Acids Res. 19 (1991) 327.
[34] S.M. Yarmoluk, S.S. Lukashov, M.Yu. Losytskyy, B. Akerman, O.S. Kornyushyna, Spectrochim. Acta Part A 58 (2002) 3223, and references cited therein.