Dyes and Pigments 130 (2016) 122–128

Contents lists available at ScienceDirect

Dyes and Pigments

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

Novel asymmetric monomethine cyanine dyes derived from sulfobetaine benzothiazolium moiety as potential fluorescent dyes for non-covalent labeling of DNA

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ARTICLE INFO

Article history:
Received 28 January 2016
Received in revised form
11 March 2016
Accepted 13 March 2016
Available online 16 March 2016

Keywords:
Monomethine cyanine dyes
Fluorescence
DNA markers
Binding parameters

ABSTRACT

Four novel monomethine cyanine dyes derived from a condensation reaction between N-quaternary 2-thiomethylbenzothiazolic, and 4-methylquinolinuim choromophores have been synthesized and characterized by ^{1}H NMR, APT carbon NMR, ESI mass spectrometry, IR, absorption and fluorescence spectroscopy. The main synthetic approach for the target cyanines involves variations on both the substituent on the aromatic ring of the benzothiazolium derivative, as well as the size of the component yielding the additional positive charge linked to the quinolinium moiety. The interaction between novel fluorophores and dsDNA has been studied. The dyes were found to have negligible fluorescence in the buffer solution, but exhibited a significant emission increase upon binding to dsDNA. The binding parameters of cyanine dyes have been determined by fluorescence titration using the McGhee & von Hippel site-excluded model. The results obtained are consistent with an intercalative binding mode between cyanines and DNA.

1. Introduction

The design of extrinsic fluorescent probes and labels for quantitative sensing, rapid visualization and characterization of nucleic acids has been attracting considerable attention in recent years and dictating by the very weak intrinsic emission of DNA and RNA. A wide range of fluorophores, including Hoechst 33258 [1], styrylcyanine dye DSMI [2], acridine orange [3], DAPI (4',6-diamidino-2-phenylindole) [4], ethidium bromide [5] have been employed for DNA identification. However, the presented above dyes are generally highly emissive in solution thereby creating a strong background signal. Moreover, the applicability of some commercially available dyes, like ethidium bromide, which is considered as being mutagenic, is complicated by some environmental concerns [6,7]. Therefore, significant research efforts are being directed at the developing and investigating new fluorescent compounds. To date the majority of fluorescent agents commonly used for DNA recognition belongs to the cyanine group. Due to their excellent staining properties, cyanine dyes are generally used for sizing and purification of DNA fragments [8,9], fluorescent microscopy applications [10], DNA damage detection [11], microarray-based expression analysis [12], DNA sequencing [13,14], DNA intercalation bioanalytical assays as well as for staining of nucleic acids in electrophoresis [8,15–18]. The applicability of this class of fluorophores is based on the fact that these dyes display high affinity for nucleic acids double strands and the huge enhancement in emission upon binding to DNA. The nature of sharp increase in fluorescence is supposed to originate from the loss of mobility around the methine bridge between the two heterocyclic moieties as a result of the cyanine - DNA interaction [19].

Cyanine dyes are photosensitive compounds possessing two quaternized, nitrogen-containing, heterocyclic structures, which are linked through a polymethine bridge [20]. This dual structure of cyanine molecules gives the impetus for three predominant non-covalent binding modes: i) intercalation between adjacent base pairs, ii) minor groove binding, and iii) electrostatic interaction of highly positively charged molecules with nucleotide phosphate

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http://dx.doi.org/10.1016/j.dyepig.2016.03.021
0143-7208/

backbone. The direct mode of the cyanine-DNA binding substantially vary with the minor change of the heterocyclic moieties in cyanine dye structure, multiplicity of the methine bridge, as well as with charge and bulkiness of attached substituents [21,22]. The presence of methine bridge, possessing conformational flexibility, allows the cyanine molecule to adjust to the DNA groove. It has been shown, that the majority of groove binders associate with DNA helix through forming hydrogen bonds to the base pairs, stabilizing in such a manner structure of the dye-DNA complex structure [23]. Besides, cyanine dyes devoid of hydrogen bond donors have been found bind to the minor groove also as dimers [24]. However, the numerous studies indicate that monomethine cyanine dyes, being a planar aromatic ring system predominantly belong to the group of intercalators [17,25]. This type of binding results from the incorporation of a dye planar aromatic moiety between the DNA base pairs, followed by unwinding and lengthening of the DNA helix.

During the last decades we have investigated novel derivatives of monomethine cyanine dyes based mainly on thiazole orange and oxazole yellow chromophores for non-covalent labeling of DNA [17,21]. In a continuation of our interest in DNA reporter molecules, herein we directed our efforts towards the synthesis and evaluating the DNA-binding ability of four novel monomethine cyanine dyes differing by their structure and spectroscopic behavior. Our goals was twofold: i) to synthesize monomethine cyanines varying the substituent on the aromatic ring of the benzothiazolium derivative, as well as the size of the component yielding the additional positive charge linked to the quinolinium moiety; ii) using fluorescence spectroscopy technique to assess the sensitivity of novel monomethine dyes to dsDNA. We have shown that novel fluorophores associate strongly with dsDNA presumably by intercalation binding mode.

2. Materials and methods

2.1. Materials

All starting materials and solvents required for the synthesis of the cyanine dyes were purchased from Sigma–Aldrich, Organica Feinchemie GmbH Wolfen, Fluka, Alfa-Aesar, TCI Europe, Deutero GmbH. Compounds 6a and 6b were commercially purchased from Organica Feinchemie GmbH Wolfen, and used without any further purification. The solvents used for the spectrophotometric and spectrofluorimetric analyses were purchased form Macron Fine Chemicals TM. Salmon testes DNA was obtained from Sigma–Aldrich. Tris–HCl and EDTA were obtained from Sigma (St.Louis, MO, USA). All other starting materials and solvents were commercial products of analytical grade and were used without further purification.

2.2. Analysis methods and equipment

The title monomethinecyanine dyes were purified by recrystalisation from methanol and their chemical structures were evaluated by ^{1}H NMR and APT-NMR spectra recorded on a Brucker Avance III HD 500 MHz, Bruker Avance 300 and Bruker Avance 600 MHz in DMSO-d_6 at 25 °C. The chemical shifts were reported in ppm in δ-values with respect to tetramethylsilane (TMS), or the deuterated solvent peak as an internal reference. Coupling constants J are expressed in Hz. IR spectra were recorder on Specord 85 ER in nujol. ESI mass spectra were obtained on TSQ Quantum Access Max Masspectrometer – Triple Quadrupol in methanol/water (80:20% v/v). The melting points were determined on a Kofler bench and are uncorrected. Absorption spectra were recorded on a Cecil Aurius CE 3021 UV–Vis spectrophotometer at room temperature. Fluorescence measurements were performed in 10 mM Tris–HCl, 0.5 mM EDTA buffer, pH 7.4 at room temperature using 10-mm path-length quartz cuvettes in a Perkin Elmer LS45 fluorescence spectrometer.

2.3. Preparation of intermediates *3a*, *3b*

A large excess of 1,3-dibromopropane **2** (15.29 mL, 0.15 mol) was added dropwise to N-methyl pyrrolidine **1a** (3.12 mL, 0.03 mol) or N-methyl piperidine **1b** (3.65 mL, 0.03 mol) and the reaction vessel was stored in a dark place [26,27] for 48 hours (Scheme 1). The precipitated products **3a**, **3b** were collected by filtration and stored in a desiccator. Their structures were evaluated by ^{1}H NMR and ^{13}C NMR spectra.

2.4. Preparation of N-quaternary 4-methyl quinolinium derivatives *5a*, *5b*

4-methylquinoline **4** (1.45 mL, 11 mmol) was mixed with the corresponding mono N-quaternary salt **3a** or **3b**, and the reaction mixture was heated to boiling point for 15 minutes with stirring in 2-methoxyethanol (10 mL) (Scheme 2). The products were isolated as semi-solids, and used without further purification in the next step yielding the target monomethine cyanine dyes.

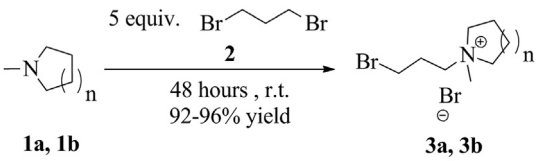
2.5. Synthesis of the cyanine dyes

In a reaction vessel equipped with magnetic stirrer, equimolar amounts of intermediate **5a** or **5b** (3 mmol) and **6a** or **6b** (3 mmol) respectively were dissolved in 10 mL methanol. N-Ethyl-diisopropylamine (0.52 mL, 3 mmol) was added dropwise, and the reaction mixture was vigorously stirred at room temperature for 2 hours (Scheme 3), followed by addition of diethyl ether (20 mL) in order to precipitate the dyes.

The reactions were held in a well ventilated hood, due to the evolution of methyl mercaptan. Upon completion of the reaction, the product was dissolved in methanol and transferred to a beaker containing 200 mL of an aqueous solution of potassium iodide. The precipitated dye was suction filtered and air dried. The corresponding yields and melting point temperatures of the dyes are given in Table 1.

1-(3-bromopropyl)-1-methylpyrrolidin-1-ium bromide (3a); (white solid), yield = 92%, m.p. = 173–175 °C, lit. m.p. = 177–179 °C [28]; ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 2.06–2.12 (4H, m, 2 × CH_2), 2.28–2.34 (2H, m, CH_2), 3.03 (3H, s, CH_3–N), 3.45–3.49 (4H, m, 2 × CH_2), 3.53–3.59 (4H, m, 2 × CH_2), 3.60 (2H, t, J 6.5, CH_2-N); ^{13}C NMR (DMSO-d_6, 125 MHz) δ/ppm: 21.5; 26.9; 31.2; 48.1; 62.1; 64.1.

1-(3-bromopropyl)-1-methylpiperidin-1-ium bromide (3b); (white solid), yield = 96%, m.p. = 196–199 °C, lit. m.p. = 190–193 °C [28]; ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 1.52–1.57 (4H, m, 2 × CH_2), 1.77–1.80 (4H, m, 2 × CH_2), 2.24–2.30 (2H, m, CH_2), 3.05 (3H, s, CH_3-N), 3.36–3.39 (4H, m, 2 × CH_2), 3.46–3.49 (4H, m, 2 × CH_2), 3.61 (2H, t, J 6.4, CH_2–N); ^{13}C NMR (DMSO-d_6, 125 MHz) δ/



1a, 1b **3a, 3b**

n: **1a**, **3a** = 1 / **1b**, **3b** = 2

Scheme 1. Preparation of intermediates **3a** and **3b**.