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Novel synthetic approach to near-infrared heptamethine cyanine dyes and spectroscopic characterization in presence of biological molecules

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ABSTRACT

Two near-infrared symmetric heptamethine cyanine dyes, containing integrated chlorosubstituted cyclohexenyl ring were obtained via a novel synthetic approach, using mild conditions at room temperature. UV–vis and fluorescence properties of the dyes were evaluated in organic solutions varying solvent polarity. Further spectral studies illustrating their application as potential labels for biological molecules, were reported in the presence of deoxyribonucleic acid (DNA), bovine serum albumin (BSA), and phospholipids (PC). The dyes undergo H-type intramolecular complexation between the two cyanine subunits when free in buffer. Absorption studies show that binding of cyanines to DNA, BSA and lipid membranes leads to the red shift in the H-aggregates peak with appearance of monomeric and dimeric dye species pointing to the disaggregation of the dyes under study in the presence of biological molecules. The fact that the decrease of liposome-induced cyanine aggregation was more pronounced in the zwitterionic phosphatidylcholine bilayer, suggests that the disruption of highly organized molecular arrangements are most likely electrostatically-controlled.

1. Introduction

Among a wide variety of fluorescent dyes currently used in biomedical research and industry, considerable attention is given to near-infrared (NIR) cyanine probes, which have found numerous analytical applications in enzymatic and immuno-assays [1,2], drug displacement studies [3], DNA sequencing [4] and cancer targeting applications [5], as optical imaging agents, labels and stains [6–8], to name only a few. Undeniably, NIR cyanine dyes have been extensively used due to their advantageous photophysical properties, namely absorption and fluorescence spectrum in the NIR region (670–1100 nm). Another essential feature of cyanine dyes is very high polarizability of the π-electron system along the polymethine group in the ground state, which give rise to strong dispersion forces between two cyanine molecules in solution [9]. These dispersion forces are thought to control the formation of extended aggregates of cyanines in solutions [10–12]. Since cyanine dyes undergo self-organization, they have been found to be an invaluable tool used to study molecular organization in proteomics [13], optics [14] and nanoscience [15].

Depending on the angle of molecular slippage, α, self-association of cyanine dyes results in the formation of molecular aggregates of different structures, known as H-and J-aggregates [16,17]. Large molecular slippage ($\alpha < 32^\circ$) led to the self-organization of "brickwork" arrangements (J-aggregates), while small one ($\alpha > 32^\circ$) is characterized by "card-pack" structures (H-aggregates). Dictated by the geometry of the molecules within the aggregate, H- and J-aggregation patterns possess unique electronic and spectroscopic properties and can be easily identified by spectroscopic methods. According to Kasha [18] exciton model for molecular aggregates, the interactions between neighboring transition dipoles of tightly packed molecules generates a splitting of the excited state into excitonic levels that are shared between all the molecules within arrangement. When two dipoles are face-to-face (H-aggregates), the energy of the allowed state corresponding to in phase transition dipoles is increased by repulsive electrostatic interactions between the transition dipoles [18]. In contrast, the lower exciton level is observed for molecules with end-to-end packing (J-aggregates), which is manifested in red shift in the absorption spectrum.

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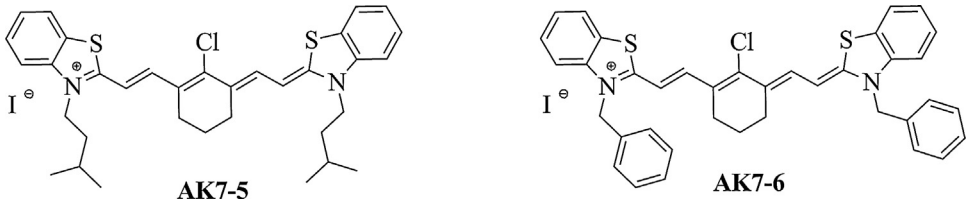


Fig. 1. Studied dyes **AK7**.

Trends of cyanine dye aggregation are well described now, and it has been found that the structure of the dye, particularly the presence of substituents in heterocyclic residues and the N-alkyl chain length, directly dictates the type of aggregates formed [11,19,20]. The other factors contributing to the formation of highly organized molecular arrangements are probe concentration [21], solvent polarity [22], temperature [23], presence of polyelectrolytes [24,25], polymers [26], Langmuir–Blodgett films [19], etc. The self-association have been shown to be inhibited also by high ionic strength since at high salt concentration the aggregates are more energetically stable then the monomeric dye due to the formation of contact ion pairs between the cationic dyes and counterions [10]. An additional agent affecting the formation of ordered nanoassemblies of noncovalently coupled probes is surfactants [11,27,28]. Numerous studies indicate that the addition of surfactants can provoke the aggregation of dyes, in certain cases changing the morphology of arrangements [11,28]. Recently, it was concluded that the presence of biomolecules, such as proteins and nucleic acids, have a significant impact on aggregation properties of organic dye molecules [15,20,21,29–31]. Specifically, DNA was shown to serve as a template for the growth of helical arrays of cyanine dyes [15,21,29]. The assembly of these supramolecular structures occurs by a cooperative, chain-growth mechanism and is essentially promoted by DNA [15,29]. The role of proteins as scaffold for cyanine aggregation or disaggregation was also described in a number of works [20,31].

The aim of the present paper was threefold: (i) to utilize a novel synthetic approach to NIR heptamethine cyanine dyes containing integrated chloro-substituted cyclohexenyl ring (Fig. 1), (ii) to investigate photophysical properties of the cyanines in organic solvents with varying polarity and (iii) to examine the impact of DNA, bovine serum albumin (BSA) and liposomes (PC) on aggregation properties of the examined dyes.

2. Materials and methods

2.1. Materials

Unless otherwise stated, reagents and solvents used in the synthesis and analysis of the dyes were obtained from Sigma–Aldrich, Organica Feinchemie GmbH Wolfen, Alfa-Aesar and Deutero GmbH, as commercial products of analytical grade and used in the synthesis of all target products without further purification. The solvents used for the spectrophotometric and spectrofluorimetric analyses were supplied by Macron Fine Chemicals TM. Double stranded deoxyribonucleic acid from salmon testes (DNA), bovine serum albumin (BSA), 2-Oleoyl-1-palmitoyl-*sn*-*glycero*-3-phosphocholine (PC) and Tris–HCl were purchased from Sigma.

2.2. Analysis methods and equipment

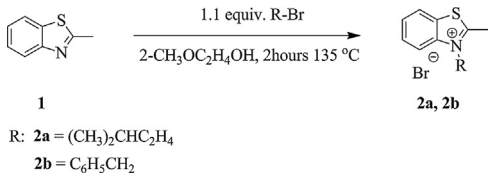
All products including dyes and intermediates were characterized using various spectroscopic techniques. Recrystallization from methanol was carried out as a main method of purification in order to obtain analytical samples of the target products. NMR spectra were recorded on Brucker Avance III HD, 500 MHz instrument (^{1}H NMR 500 MHz, ^{13}C NMR 125 MHz) in DMSO-d_6 at 25 °C. The Coupling constants J were expressed in Hz. ESI mass spectra of both positive and negative ionization modes were recorded on triple quadrupole mass spectrometer Agilent 6410 coupled with HPLC Agilent 1200 Series. Melting point temperatures were determined on a Kofler bench and are uncorrected. UV–vis spectra of the dyes were recorded in various solvents of spectroscopic grade on a Cecil Aurius CE 3021 UV–vis spectrophotometer at room temperature. The corresponding emission spectra were recorded on Perkin Elmer LS45 spectrofluorimeter at room temperature using 10-mm path-length quartz fluorescence cuvettes. The dye stock solutions (1 mM) were prepared in DMSO, which were diluted with the corresponding organic solvent. UV–visible spectra of heptamethine dyes in the presence of DNA, BSA and liposomes were measured in the range 500–900 nm with Cecil Aurius CE 3021 UV–vis spectrophotometer at room temperature using 10-mm path-length quartz cuvettes.

2.3. Synthesis of intermediates ***2a***, ***2b***

2-methyl benzothiazole **1** (1.27 mL, 10 mmol) and a small excess of the corresponding alkylating reagent R-Br (11 mmol) were dissolved in 2-methoxyethanol (2 mL). The reaction mixture was heated at reflux for 2 h. The reaction outcome was monitored by TLC. After the reaction was completed, and the reaction mixture was cooled down to room temperature, N-quaternary product **2** was precipitated with diethyl ether (20 mL). The precipitate formed was filtered off, washed with diethyl ether, and stored in a desiccator (Scheme 1). Purification of the benzothiazolium salts was held by recrystallization from methanol.

3-Isopentyl-2-methylbenzo[d]thiazol-3-ium bromide (**2a**): (pale brown solid), yield = 29%, m.p. = 190–193 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 1.00 (6H, d, J 6.6, 2x CH_3— CH), 1.71–1.76 (2H, m, CH_2), 1.86 (1H, sept., CH), 3.27 (3H, s, CH_3), 4.72–4.76 (2H, m, CH_2—N), 7.78–7.82 (1H, m, ArH), 7.88–7.91 (1H, m, ArH), 8.31 (1H, d, J 8.5, ArH), 8.53 (1H, d, J 8.2, ArH); ^{13}C NMR (DMSO-d_6, 125 MHz) δ/ppm: 17.4; 22.6; 26.0; 36.5; 48.4; 117.2; 125.3; 128.4; 129.6; 129.8; 141.0; 177.3.

3-Benzyl-2-methylbenzo[d]thiazol-3-ium bromide (**2b**): (pale pink solid), yield = 72%, m.p. = 190–193 °C; m.p. = 246–249 °C, lit. m. p. = 255–256 °C [33]; ^{1}H NMR (DMSO-d_6, 500 MHz) δ/ppm: 3.30 (3H, s, CH_3), 6.13 (2H, s, CH_2—N), 7.33-7.42 (5H, m, 5x ArH), 7.78–7.86 (2H, m, 2x ArH), 7.23 (1H, d, J 8.3, ArH), 8.55 (5H, m, 5x ArH); ^{13}C NMR (DMSO-d_6, 125 MHz) δ/ppm: 17.9; 52.4; 117.6; 125.4; 127.5; 127.5; 128.7; 129.0; 129.6; 129.7; 130.0; 133.3; 141.4; 178.9.



Scheme 1. Synthesis of N-quaternary 2-methylbenzothiazolium salts **2a, 2b**.