



Synthesis, spectral properties of cell-permeant dimethine cyanine dyes and their application as fluorescent probes in living cell imaging and flow cytometry



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ABSTRACT

A series of dimethine cyanine dyes, used as fluorescent probes, were synthesized under microwave irradiation, and identified by ¹H NMR, IR, elemental analysis and HRMS. The investigation of their spectral properties in phosphate-buffer saline (PBS) showed that the absorption and emission maxima of the dyes were in the region 370–480 nm and 471–569 nm, respectively. The properties of the dyes as fluorescent probes for living cells imaging and flow cytometry were investigated. The results showed that dyes **1**, **2**, **8** or **9** could penetrate an intact cell membrane, stained the cell nuclear and exhibited bright fluorescence. Little background interference, low cell cytotoxicity and little photobleaching were showed during the imaging tests. The dyes **1**, **8** and **9** could be applied in flow cytometry and dye **1**/PI, dye **8**/PI or dye **9**/PI couple could be proposed as double staining agents to measure sperm cell viability. Thus the dyes represented the cell-permeant fluorescent probes.

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1. Introduction

In the present, the fluorescence technology has become the most sensitive and easily available method to study intermolecular interactions and the transcriptional dynamics of the cell nucleus [1,2]. Especially, the fluorescent imaging technology and flow cytometry have been recognized to have extremely valuable applications for biologic and medicinal diagnostic study [3,4]. For example, micro-flow imaging, an instrument comprising a flow cell, and a microscope-based imaging system has become a standard for the determination of size distributions of therapeutic proteins [5,6]. Generally, flow cytometric measurements are possible by means of both scattering and fluorescence signals in order to collect cell parameters [7]. The scattering signals can be applied to distinguish between different cell types and structures [8], but it is not always correlated to cell nature due to cell

complexity. The fluorescence signals have been widely used since the use of dyes in single or multiple analyses provides additional information about cell structure or functionality with more efficient and less time-consuming way [9,10]. However, the living cell imaging technology and flow cytometry are often limited by the available commercially fluorescent probes, which are rare and generally non cell-permeant etc. Thus, it is necessary to synthesize small organic fluorescent probes with cell-permeant, non-toxic, tolerated by living cells, and photostable properties [11].

Methine cyanine dyes, due to their wide-color range, high molar extinction coefficients, high fluorescence quantum yields and attractive spectral-luminescent characteristics for biomedical applications, have been used in biomedical field [12–18]. Dimethine cyanine dyes are one of the important branches of methine cyanine dyes [19,20]. Having stability, good affinity for biological structure like DNA/RNA [21–26] and increased fluorescent intensity when bound to the biological molecule, they have been applied in some biomedical fields, including gel staining [27], cell imaging [11], which present great potential to be developed into biological fluorescent probes. In a preceding paper, we reported the synthesis of a series of dimethine cyanine dyes with indole nucleus, and

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investigated both experimentally and theoretically the absorption and fluorescence properties of these dyes, and their fluorescence maxima were located at 513–549 nm in methanol [28]. We also synthesized and studied a series of dimethine cyanine dyes as fluorescent dyes for DNA or BSA detection, and it was found that significant enhancement of the fluorescent quantum yield was observed for all the dyes in the presence of DNA [29]. Present work is a continuation of researches aimed on the search of cell-permeant dimethine cyanine dyes with attractive spectral-luminescent characteristics for biomedical applications, particularly for the usage as fluorescent probes in living cells imaging technology and flow cytometry.

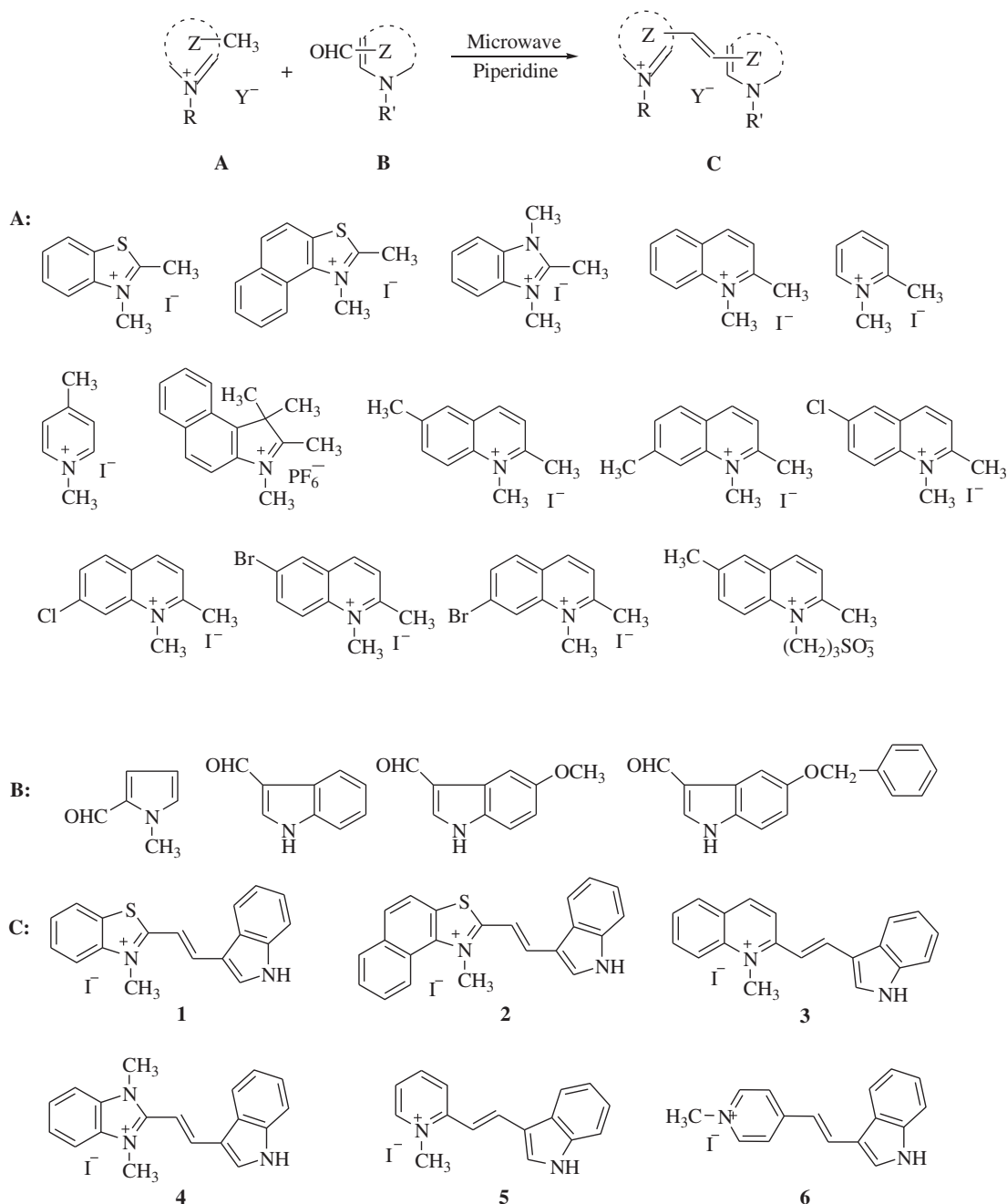
Our previous efforts have been devoted to designing, tuning the optical properties of cyanine dyes as well as searching some cyanine dyes with spectral-luminescent performance for

biomedical applications [30–33], and it is found that most of cyanine dyes with indole nucleus show excellent performance in biological field. Therefore, the objective of this study is to prepare a series of dimethine cyanine dyes with indole nucleus by a microwave-assisted method, and investigate their optical spectra in PBS buffer, then explore the potential applications of the prepared dimethine cyanine dyes (Scheme 1) as fluorescent probes in living cell imaging technology and flow cytometry.

2. Experimental

2.1. Measurements

Melting points were taken on an XT-4 micromelting apparatus and uncorrected. Elemental analyses were performed with a Vario



Scheme 1. Synthesis of dimethine cyanine dyes.

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