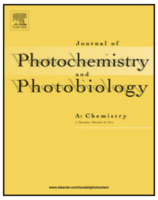
Journal of Photochemistry and Photobiology A: Chemistry 316 (2016) 31–36

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

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



# Novel indotricarbocyanine dyes covalently bonded to polyethylene glycol for theranostics

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ARTICLE INFO

*Article history:*
Received 30 April 2015
Received in revised form 4 October 2015
Accepted 10 October 2015
Available online 23 October 2015

*Keywords:*
Indotricarbocyanine dyes
Photodynamic therapy
HeLa cells
Photo and dark cytotoxicity

ABSTRACT

The synthesis methods for the indotricarbocyanine dyes covalently bonded to polyethylene glycols with varying degrees of polymerization have been developed. New indotricarbocyanine was synthesized in one step using the Mukayama reagent in polyethylene glycol with cesium fluoride as a basic catalyst. *In vivo* investigation of the phototherapeutic dye activity has shown selective recognition of malignant tissues, where accumulation of the dye was 4–5 times higher than in normal tissues. The necrotic area after local photoirradiation at the effective energy fluence 180 J/cm$^2$, $\lambda = 780$ nm was observed to a depth of 2.5 cm making the synthesized dyes very promising for further pharmaceutic research.

## 1. Introduction

Polymethine dyes attract much attention because of their potential applications in medicine and biology. Series of symmetrical polymethine dyes (ICG, Cy5.5, Cy7.0, IR783, *etc.*) containing a variety of the bis-N-substituted indole or benzindole moieties linked by linear conjugation with and without a fused substituted ring have been extensively used as reagents for *in vivo* tumor imaging [1–6]. In the last few years indotricarbocyanine dyes have shown themselves as effective imaging agents incorporated into the nanoparticles for cancer theranostics. [3,7,8].

At the present time the porphyrin-based photosensitizers (porphyrins, chlorines, phthalocyanines, bacteriochlorines) are generally used for photodynamic therapy (PDT). However, compared with cyanine dyes, most of the porphyrin-based photosensitizers have a lower absorption wavelength (650–700 nm) that is not optimal for deep penetration into tissues. Among the cyanine dyes, indotricarbocyanine dyes exhibit long-wavelength absorption in the region 700–800 nm. It is now established that light in the near IR spectral region possesses a maximal power of penetration into the majority of biological tissues [9,10]. Besides, with the use of cyanine dyes, one can combine optical imaging of tumors and phototherapy due to their high ($>10^5\,M^{-1}\,cm^{-1}$) molar extinction coefficient (higher than that of a porphyrin-based photosensitizer) and fluorescence in the "tissue transmission window". As shown previously, indotricarbocyanine dyes with a bromide anion demonstrate the photodynamic activity under hypoxic conditions [11]. This emphasizes the fact that indotricarbocyanine-based photosensitizers are an alternative for the traditional means of cancer cell damage. Modification of indotricarbocyanine dyes by biocompatible objects should lead to the increased tumor specificity. Because of this, indotricarbocyanine dyes are suggested as suitable photosensitizers for PDT.

Previously it has been reported [11–13] that some of the synthesized indotricarbocyanine dyes covalently bonded to monosaccharides demonstrate high photodynamic activity against a wide range of tumors. However, the limitation of these dyes was their insufficient water solubility for the effective intravenous injection. To increase water solubility of the dyes, tricarbocyanine covalently bonded to polyethylene glycols with varying degrees of polymerization was synthesized. In this way new dyes have a much greater solubility in water (higher than 10 mg/ml). Moreover, these compounds exhibit greater accumulation selectivity in tumors than in normal tissues and lower toxicity compared to the dyes modified by monosaccharides.

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http://dx.doi.org/10.1016/j.jphotochem.2015.10.008
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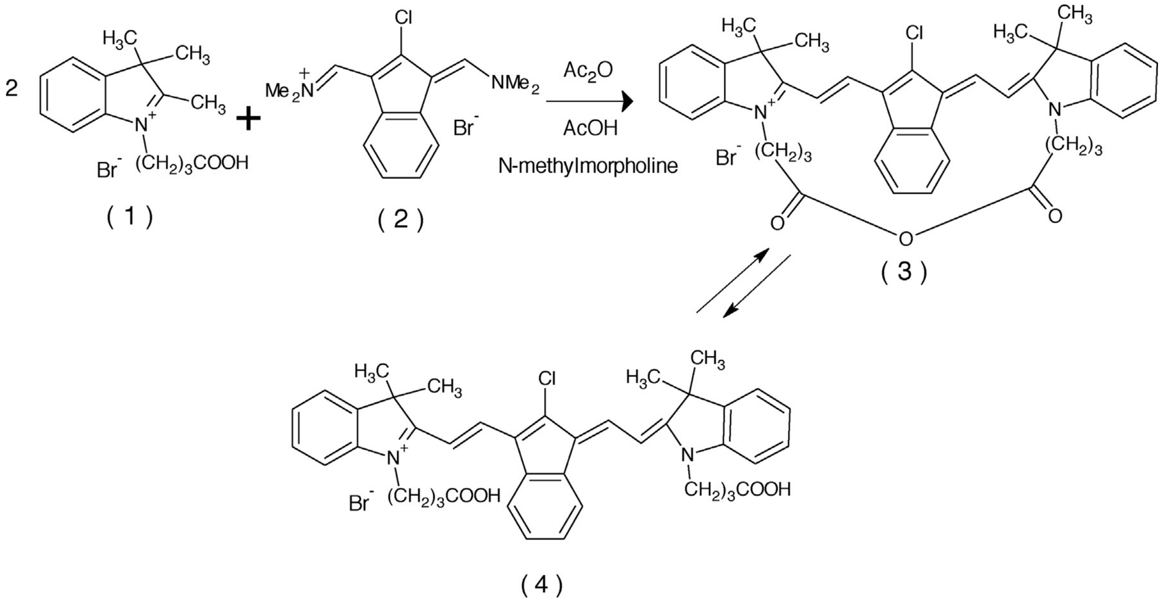


**Scheme 1.** Synthesis procedure for dyes **3**,**4**.

## 2. Synthesis

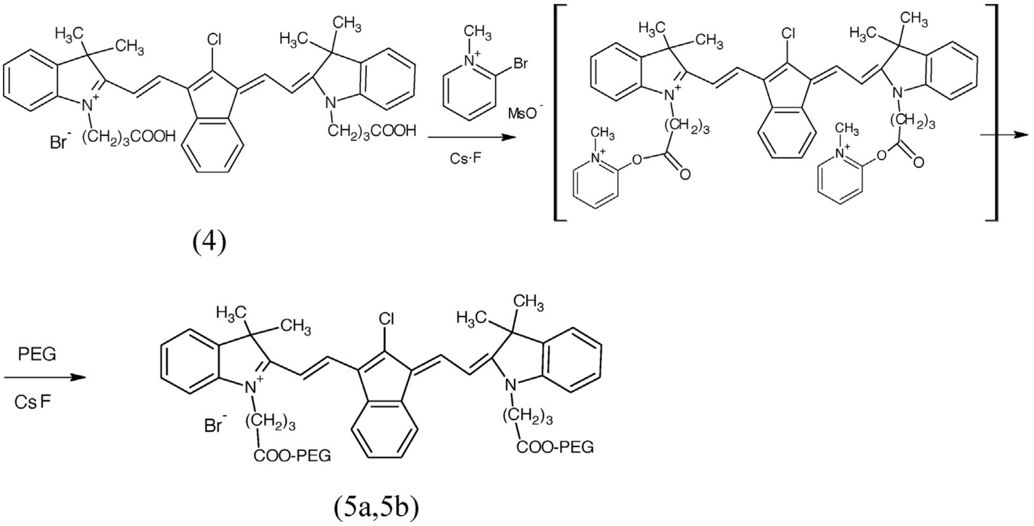
Synthesis of the compounds (**5a, 5b**) along with their precursors is illustrated by Schemes 1 and 2. Scheme 1 demonstrates the synthesis of compound **3** obtained by the condensation reaction of indolium salt **1** with salt **2** in acetic acid, acetic anhydride in the presence of *N*-methylmorpholine. Compound **3** is obtained in the anhydride form **3** and may be converted to the acid form **4** by treatment with water in the acidic medium. The existence of the anhydride form (3) is confirmed by the fact that there are no vibrations in the region 3550–3500 $cm^{-1}$ of an IR spectrum.The inverse transformation (**4**– **3**) may be realized by heating in 1,2-dichloroethane with equimolar azeotropic removal of water. It should be noted that compound **3** is much less soluble in 1,2-dichloroethane than compound **4**, that can effectively purify compound **3** from nonpolar organic impurities by the inverse transformation procedure.

Scheme 2 shows the process of synthesizing the cyanine-dye modified polyethylene glycols with the average molecular weight 300 and 400 g/mol. The reaction is carried out in a single step using the Mukayama reagent (1-methyl-2-bromopyridine methyl sulfonate) in the polyethylene glycol used both as a reactant and a solvent. Cesium fluoride was used as a basic catalyst.

Subsequent isolation of the dye was carried out by the addition of ethyl acetate. Salts of inorganic acids and 1-methyl-2-bromopyridine were separated by filtration after dissolving the cyanine dye in dichloroethane. The presence of a pure dye was confirmed by $^1$H NMR and MS. Dyes **5a** and **5b** in mass spectra are shown as multiple peaks corresponding to dye **3** bonded to polyethylene glycols with various degrees of polymerization.

## 3. Photophysical properties

A maximal absorption wavelength of the dye **5a** is observed at 723 nm in ethanol, at 733 nm in chloroform, and at 734 nm in biological structures (HeLa cells). It should be noted that the synthesized dyes have strong absorptions in the near infrared region coincident with a “phototherapeutic window” (700–900 nm). Maximum of a fluorescence spectrum of the dye in ethanol is located at $\lambda = 749$ nm and in chloroform — at $\lambda = 761$ nm



**Scheme 2.** Synthesis procedure for compounds **5a, 5b.**