SYNLETT Spotlight 435

This feature focuses on a reagent chosen by a postgraduate, highlighting the uses and preparation of the reagent in current research

Cyanine Dyes

Compiled by Neil Norouzi

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Introduction

Cyanine dyes are highly conjugated, fluorescent molecules with absorption and emission wavelengths in the near infra-red region (700–900 nm). The simplest synthetic route to heptamethine cyanine dyes **1** (so-called because of the seven carbons in the conjugated backbone) was first described by Narayanan and Patonay who heated N-alkylated indolium salts **2** with 2-chloro-1-formyl-3-(hydroxyl methylene) (**3**) in a Vilsmeier-type reaction.¹ These heptamethine cyanine scaffolds can be readily modified through displacement of the labile chloride group by nucleophiles,^{2,3,4} resulting in fluorescent molecules with varying quantum yields, extinction coefficients, and fluorescence maxima. Conjugation to biomolecules is achieved through chlorine substitution by 3-(4-hydroxyphenyl) propionic acid. The resulting cyanine dye has a carboxylic acid moiety which can be coupled to an amine-containing compound via amide-bond formation. Enhanced aqueous solubility is typically achieved through sulfonation of the indole **2**. As biological tissue does not absorb strongly within the near infra-red window, cyanine fluorophores are ideal for in vivo optical imaging application,^{5,6,7} while clinically, indocyanine green has been used for over 25 years in fluorescence angiography and opthalmology (mouse $LD_{50} = 60 \text{ mg/kg}$).^{8,9}



Scheme 1 Synthesis of heptamethine cyanine dyes 1

Abstract

(A) Necrosis Detection

Necrotic tissue is found in a variety of disease states including cancer and sepsis¹⁰ where levels of extracellular DNA are increased due to dead or dying cells. Murthy et al. described a hybrid heptamethine (IR-786)–bisbenzimidazole (Hoechst 33258)¹¹ probe that accumulates in necrotic tissue by binding to extracellular DNA.² In vivo analysis in mice ischemia–reperfusion models confirmed probe accumulation in necrotic tissue.²

(B) pH Sensor

Nagano and co-workers synthesised a ratiometric, NIR heptamethine pH sensor. By using two excitation wavelengths (670 nm and 750 nm), the relative fluorescence intensities ($\lambda_{em} = 780$ nm) allowed pH values between 6 and 10 to be readily measured. Incubation of HeLa cells with the sensor resulted in staining of lysosomes and mitochondria with a demonstrable ability to monitor intracellular pH changes.⁴

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(C) Reactive Oxygen Species Detection

Uncontrolled reactive oxygen species (ROS) are implicated in several inflammatory disease states.¹² Nagano and co-workers reported the real-time analysis of ROS by linking two NIR cyanine dyes with different oxidation potentials.¹³ A turn on fluorescence signal was observed upon oxidation of the more susceptible cyanine dye as this removed the static quenching effect. A strong fluorescence signal was found after incubation with a variety of ROS such as the hydroxyl radical (OH) using Fenton's reagent and superoxide (O₂⁻⁻) generated from xanthene oxidase.¹³

(D) H₂S Molecule Sensor

Hydrogen sulfide is known to be an important gaseous signaling molecule and is key in the regulation of blood pressure.¹⁴ Zhang and co-workers developed a real-time NIR sensor for H_2S by incorporating 3-nitrophenol onto the heptamethine dye scaffold which resulted in photo-induced electron transfer (PET)¹⁵ and quenching of the cyanine dye fluorescence.³ This was liberated by nitro group reduction with hydrogen sulfide. Incubation with other reactive sulfide species such as glutathione and cysteine gave a far weaker fluorescence increase.



Bioaccumulation of metal ions such as silver can demonstrate adverse biological effects due to binding to functional groups such as thiols.¹⁶ Zheng, Jiang and co-workers developed a Ag⁺ sensor based on a heptamethine cyanine motif that contained an adenine moiety.¹⁷ Aggregation¹⁸ of the cyanine dye with increasing concentrations of Ag⁺ ions resulted in a fluorescence shift of 185 nm with a detection limit of 34 nM. High selectivity over other metal ions such as copper and iron was demonstrated.







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