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

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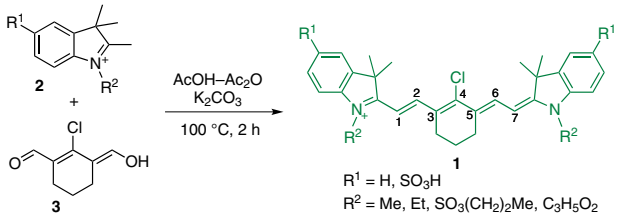
Introduction

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.

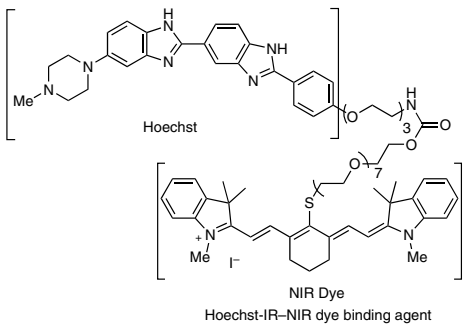
The resulting cyanine dye has a carboxylic acid moiety which can be coupled to an amine-containing compound via amide-bond formation. Enhanced aqueous solubility is typically achieved through sulfonation of the indole **2**. As biological tissue does not absorb strongly within the near infra-red window, 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 mg/kg).[8,9]



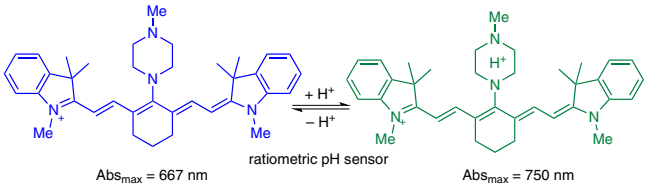
Scheme 1 Synthesis of heptamethine cyanine dyes **1**

Abstract

(A) *Necrosis Detection*
Necrotic tissue is found in a variety of disease states including cancer and sepsis[10] 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 (λ_{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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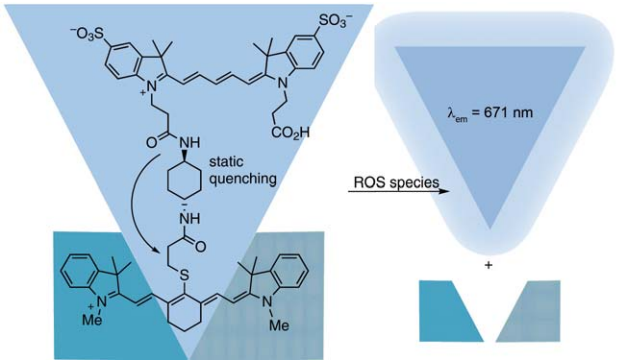
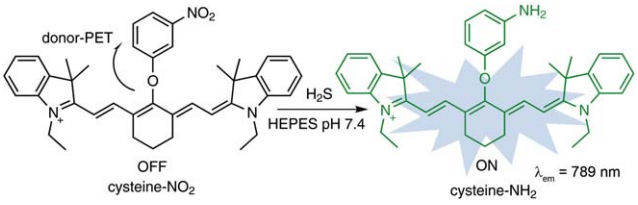
(C) *Reactive Oxygen Species Detection*

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 ($O_2^{\cdot-}$) generated from xanthene oxidase.[13]



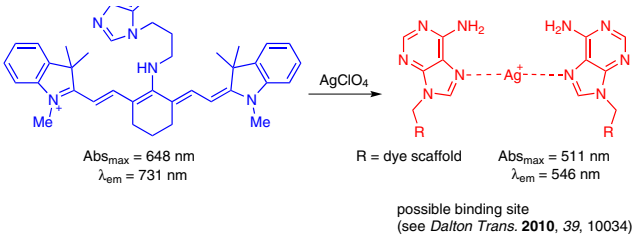
(D) *H_2S Molecule Sensor*

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.



(E) *Silver Sensor*

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] Aggregation[18] 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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